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Use of Cu Fungicides in Vineyards and Olive Groves

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1. Introduction

Losses caused by pests, diseases and weeds on all agriculture crops in Europe are considerably heavy (28.8 %). They can be reduced in different ways: by law regulations, professional set up of orchards, breading less sensitive or resistant crops, different technical measures of production, mechanical, physical, biological and chemical measures. The use of pesticides to control microbial, fungal and insect plant pests has long been a feature of conventional agricultural practice and their use has made it possible to increase crop yields and food production. Many of these pesticides have toxic effects that are not confined to their target species. Their application may have negative impact on organisms that benefit a wider agro ecosystem and their use may result in an increased accumulation of heavy metals in the soil. Even if just in traces, heavy metals are the primary sign of soil and groundwater contamination. There are various causes that lead to the pollution of agricultural soils and the problem of soil contamination with heavy metals is a central and current issue in modern ecology.

Fungicide use is the most important component of pest and disease control programs in vine and olive production systems. This is because some fungal diseases have a potential to destroy horticultural crops and make them unsalable. The practical and economic problems for producers are more acute in organic production systems than in the conventional ones, because the use of fungicides in organic production is much more limited. Whilst several synthetic active ingredients are available in the conventional production, these are not allowed in organic agriculture, except for certain copper products, the use of which is considered to be traditional organic practice. In most countries copper fungicides can be used in organic crop production.

Copper fungicides have been used in pome and stone fruit orchards and vineyards for more than 100 years. The most common fungal diseases controlled by copper fungicides in vineyards are *Plasmopara viticola* (B. and C.) Berl. and De Toni and *Phomopsis viticola* Sacc. Copper fungicides such as Bordeaux mixture (a complex of copper sulphate and lime) has been used in viticulture as a plant protection product against the stated fungal diseases since the 18th century. This was the first fungicide to be used on a large scale worldwide. Even today, the only fungicides allowed under organic standards and effective against *Plasmopara viticola* are based on copper hydroxide and copper sulphate. Moreover, other copper compounds have been introduced, including copper carbonate, copper ammonium carbonate, copper hydroxide, copper oxide, copper oxychloride, copper oxychloride sulphate, etc. However, their long-term application and subsequent wash-off from the treated plants have resulted into an extensive copper accumulation in vineyard soils. According to the information gathered to date, a long-term use of copper fungicides in viticulture results in the ingression of significant quantities of copper, which remain in the surface soil layer at 0 - 0.2 m, as has been verified by a number of researchers. The bulk of copper accumulated in leaves and soil after the treatment of the vine with copper fungicides returns to the surface layer of soil through tillage or the biological cycle. Copper can simultaneously be both a micronutrient and a toxic element, depending on its concentration in the soil. In the soil copper is bound to organic matter, to Fe and Mn oxides, adsorbed to clay surface, it is present in the matrix of primary silicate minerals, in secondary minerals or within amorphous matter. The sum of it all can be defined as total copper in soil. Determination of the total content of metals in soils is an important step in estimating the hazards to the vital roles of soil in the ecosystem, and also in comparison with the quality standards in terms of the effects of pollution and sustainability of the system. From the ecotoxicological aspect, it is equally important to determine the bioavailability of copper accumulated in vineyards. Copper availability to biota and its mobility are the most important factors for soil environment. Copper bioavailability is influenced not only by physical and chemical properties of the soil, but also by environmental factors such as climate, biological population, and type and source of contaminants. Copper is toxic for soil organisms and plants, expecialy copper contents as high as those reported in vineyard soils. Even low concentrations of copper in soil may result in long-term effects including reduced microbial and earthworm activity and subsequent loss of fertility. Humans are exposed to copper from many sources. 75 to 99% of total copper intake is from food. Possible undesirable effects of copper fungicides on the health of workers exposed to the chemicals and consumers of crop products treated with them are a major concern. In humans, acute ingestion of copper sulphate may cause gastrointestinal injury, haemolysis, methemoglobinemia, hepatorenal failure, shock, or even death.

In olive orchards, olive leaf spot disease is caused by fungus *Spilocea oleaginea* Cast. Today, olive leaf spot is a significant and serious problem in almost all our olive orchards, including those with organic production. It adversely affects fertility of infected trees, and its recurrence year after year causes degradation of whole olive trees, particularly the young ones. Olive leaf spot is readily controlled by copper fungicides. For effective olive protection, several applications are necessary in one year. Concentration of applied copper fungicides must be strictly under control because of possible copper residues in olive fruits and consequently in oil, which is restricted by law. In years with particularly warm and rainy autumns, one treatment with copper fungicides in autumn is not enough, but it is necessary to perform at least three treatments with copper fungicides. Undoubtedly, increasing the number of treatments in autumn renders it impossible to fully observe all the regulations. On the other hand, if the regulations are fully observed, the question arises whether it is actually possible to adequately protect olive groves against this unpleasant and rising disease at all.

2. Copper fungicides in vineyards and olive groves

The contamination of agricultural soils with inorganic (copper-based) and organic pesticides, including their residues, presents a major environmental and toxicological concern. Agricultural soils are particularly exposed to excessive contamination by heavy metals, the reasons being traffic, households and anthropogenic impact. Anthropogenic impact is especially conspicuous in vineyard soils, orchards and gardens. The use of copper fungicides is the most important component of disease control programs in vine and olive production systems. This is because fungal diseases, such as *Plasmopara viticola*, *Phomopsis viticola* and *Spilocea oleaginea*, have the potential to destroy vineyards and olive orchards. Due to a devasting and lasting effect fungal diseases can have on horticultural crops, the use of copper fungicides is considered best practice in preventative agrochemical spray programs (McConnell et al., 2003). This involves many fungicide applications in the course of one year. A regular use of fungicides can potentially carry a risk to the environment, particulaty if residues are retained in soil or transferred into water (Kookana et al., 1998; Wightwick & Allison, 2007; Komarek et al., 2010). Concern has been caused by the long-term use of copper fungicides, which can result in an accumulation of copper in the surface layer of the soil (Wightwick et al., 2006; Komarek et al., 2010). This has a negativ effect on soil organisms and carries a potential risk to long-term fertility of the soil (Wightwick et al., 2008; Komarek et al., 2010). Regarding the environmental and toxicological hazards associated with the extensive use of fungicides, the choice of fungicides should be performed carefully according to the physico-chemical properties of the soils and climatic and hydrogeological characteristics of the physico-growing regions. In vine and olive growing production systems, a balance needs to be found between controlling fungal disease risks on crops and protecting agro ecosystems. Copper residues resulting from copper fungicide applications in vineyards and olive orchards have affected the key soil health indicators. To protect soil health, alternatives to copper for disease control will need to be developed, along with remedial technologies to reduce copper contamination in soils. Contamination with metals and organic pollutants, together with erosion and tillage, reduces the quality of the soils and poses a serious environmental and toxicological threat. Vineyard soils are usually highly degraded soils in terms of biochemical properties (Miguens et al., 2007) and are therefore more suspectible to contamination. During the last few decades, some European vineyards have been abandoned, mostly those situated on steep slopes, wich has led to intensive soil erosion and subsequent dispersion of the pollutants into the environment (Novoa-Munoz et al., 2007; Fernandez-Calvino et al., 2008).

Ever since the 18th century copper fungicides have been used in viticulture as plant-protecting preparations against fungal diseases (Merry et al., 1983). As in the past, so too today, a part of the fungicides used for the protection of vines are based on copper as active ingredient. In Croatia, the permission for the application in viticulture, for the control of fungal diseases, has been given to the following preparations: copper sulfate, AI - copper (I) oxide, AI – copper oxychloride, AI - copper hydroxide, AI - copper-hydroxide-calcium sulfate complex, AI - copper-hydroxide - calcium-chloride complex, AI – combination of copper and organic fungicides, and AI – combination of copper and mineral oils.

The number of treatments with copper fungicides is estimated to be extremely high, 8-14, (Gracanin, 1947; Flores Velez, 1996; M. Romic et al., 2001). One author presents data on as many as thirty treatments with copper fungicides a year to protect vineyards from deseases. Today, however, a satisfactory protection can be achieved in 4-6 treatments (Cvjetkovic, 1996). This presents not only economy, but also a significant ecological achievement. In their research some authors state that from 2 to 5 $kgha^{-1}$ of copper is introduced in one vegetativ year (Besnard et al., 2001; M. Romic et al., 2001). Some authors state that from 0 to 7,5 $kgha^{-1}$ of copper is introduced in a year (Parat et al., 2002), while others report data showing that twenty years ago up to 15 $kgha^{-1}$ of copper were put in vineyard soils in a year (Delas & Juste, 1975). As mentioned above, the highest total copper concentration is retained in the surface layer of soil, specifically up to 15 cm of depth. In terms of environmental protection, the question of toxic effect of the accumulated total copper is raised. Hence, in the past ten or so years, numerous studies have been carried out on the concentration of total copper in vineyard soils after a long-term application of copper fungicides. Some results of maximum recorded concentrations of total copper in surface layers of vineyard soils in the world are as follows: South Italy - 75 $mgkg^{-1}$ and North Italy – 297 $mgkg^{-1}$ (Deluisa et al., 1996); Greece – 100 $mgkg^{-1}$ (Parat et al., 2002); Moldova – 230 $mgkg^{-1}$ (Delas & Juste, 1975); Australia – 250 $mgkg^{-1}$ (Pietrzak & McPhail, 2004); South France – 250 $mgkg^{-1}$ (Brun et al., 1998); Bordeaux (France) – 1500 $mgkg^{-1}$ (Flores Velez et al., 1996); South Brazil – 3200 $mgkg^{-1}$ (Mirlean et al., 2007); India – 131 $mgkg^{-1}$ (Prasad et al., 1984); New Zealand – 304 $mgkg^{-1}$ (Morgan & Taylor, 2004).

Arable land usually contains between 5 and 30 $mgCukg^{-1}$ of soil, while in treated vineyards the total copper concentration can range even from 100 to 1500 $mgkg^{-1}$ (Drouineau & Mazoyer, 1962; Delas, 1963; Geoffrion, 1975; Deluisa et al., 1996; Flores Velez et al., 1996; Besnard et al., 1999). In more recent research, some authors state that total copper concentrations in vineyard soils are much lower (200 to 500 $mgkg^{-1}$) (Brun et al., 2003).

The total copper concentration in the north-western Croatia ranges from 5 to 248 $mgkg^{-1}$ with the median of 26 $mgkg^{-1}$. Concentrations higher than the permitted values have been recorded in two sampling locations and are most probably of the anthropogenic origin. High values have been measured in soils subjected to intensive wine-growing, i.e. in soils contaminated by copper sulfate (Miko et al., 2000). The stated author indicates that the total copper concentration in the analysed soils of carbonate terrains range from 6 to 923 $mgkg^{-1}$.

Research results of foreign authors also indicate an increase in the total copper concentration in soil after a long-term application of copper fungicides in viticulture. In the last century, the total copper concentrations of 1500 to 3000 $kgha^{-1}$ were the result of plant-protecting preparations used in viticulture (Geoffrion, 1975). Furthermore, the author presents supporting results of his own research, ranging from 870 to 1870 $kgha^{-1}$ of total copper in vineyard soils under research. Results of several studies show that the applied copper remains in the soil, as it binds tightly to organic matter, clay minerals or to Fe, Al and Mn oxides (Stevenson & Cole 1999). Results of vineyard soils research in eastern China showed an increase of 34.2 $mgkg^{-1}$ in total copper concentration in the surface layer of the soils, after a ten-year application of Bordeaux mixture (Li, 1994). Research of vineyard soils of the French part of the Mediterranean, established that total copper concentrations vary from 31 $mgkg^{-1}$ to 250 $mgkg^{-1}$ compared to the soils of woodland areas, where total copper concentrations ranged from 14 $mgkg^{-1}$ to 29 $mgkg^{-1}$ (Brun et al., 1998). In more recent research, some authors state that total copper concentrations in arable land range from 5 to 30 $mgkg^{-1}$, while in most vineyards they amount to 200 to 500 $mgkg^{-1}$, which they bring into connection with the use of copper fungicides (Brun et al., 2003). Research of agricultural soils of central and eastern Giorgia established values of total copper concentrations five to ten times higher than the ones permitted (the highest value amounted to 1023 $mgkg^{-1}$). By comparison to other profiles, the authors concluded that the results obtained were also connected with the application of copper sulfates used in viticulture (Narimanidze & Bruckner, 1999).

As a consequence of soil erosion, total copper gets run off the surfaces under research, and consequently the leaching of copper into deeper layers of soil is limited (Besnard et al., 2001). A high concentration of copper can be run off by surface waters in very contaminated soils. This has been substantiated by the research of many other authors (Albaladejo et al., 1995; Ribolzi et al., 2002).

The relatively long resistence time of copper in top soils, largely related to the high affinity of copper for organic matter and hydrous oxides, means that long-term accumulation of copper is likely. The accumulation of copper in top soils also corresponds to the zone in the soil profile of greatist bilogical activity. Detrimental affects o elevated copper concentrations upon mycorrhizal associations (Georgieva et al., 2002), microbial populations and function (Dumestre et al., 1999) and a range of mesofauna (Paoletti et al., 1998) have been documented.

Besides vineyards, copper fungicides have also been used in hop fields (Schramel et al., 2000; Komarek et al., 2009), apple (W. Li et al., 2005), avocado orchards (Van Zweiten et al., 2004) and during the cultivation of tomatoes and potatoes (Adriano, 2001).

2.1 Behavior of copper in vineyard and olive grove soils

The levels of copper in soil averagely vary from 5 $mgkg^{-1}$ to 50 $mgkg^{-1}$. Copper belongs to a group of heavy metals which are adsorbed tightly onto soil colloids, binding to them as Cu2+ cation. The stated metal derives from primary minerals, where it is found in a univalent form, and after the disintegration it oxidizes in Cu^{2+}. In the soil it forms very stable and complex compounds with organic acids, semi-disintegrated or humified organic matter. Thus bound, it is sparsely accessible to plants and therefore its deficiency occurs more often in very humified soils, due to the "organic" fixation. In the research of the mobility of copper within the soil profile it has been concluded that the translocation of the total copper in soil occurs in both directions, specifically in the form of Cu complex, usually with amino acids, resulting in significant amounts being contained in plant roots (Vukadinovic & Loncaric, 1998).

Copper in soil is found: bound to organic matter of the soil, adsorbed to clay surface, bound to Fe and Mn oxides, present in the structure of primary silicate minerals, present in secondary minerals or within amorphous matter. The sum of all the above can be defined as total copper in soil (Chaignon et al., 2002; Parat et al., 2002). Such strong sorption/complexation properties make it one of the least mobile metals in soils. However, metals of antropogenic origin present in general a greater mobility in soil comparatively to a natural origin where the metals are strongly associated with soil components (Baize, 1997).

Copper is bound to the adsorption soil complex in the form of Cu^{2+} or CuOH. Since this bond is very tight, plants have difficulties using this part of copper. For the removal of copper ions from the adsorption complex, H^+ ions are the most effective. Furthermore, the amount of total copper in soil depends primarily on the parental rocks from which the soil has developed. An important source of copper is the mineral chalcopyrite ($CuFeS_2$). In the forms of CuS or Cu_2S it is found in marshy soils. Acidic rocks such as granite contain around 10-100 $mgkg^{-1}$ of Cu, while basic rocks contain a somewhat higher amount. Hardly soluble phosphates, carbonates and copper sulfides can also be found in the soil. At a higher content of organic matter, an intensified nutrient fixation occurs. Accessibility of the copper thus bound varies, sometimes the bond is so tight that plants are not able to use copper, while sometimes they are. Deficiency of total copper in soil most often occurs precisely in soils rich in organic matter (Anic, 1973). The same author states that apart from the pH reaction of the soil, the concentration of Al ions and calcium in soil is important as well. Although a very low concentration of copper is needed for it to function in the soil, it is still often deficient. The surplus of total copper is more frequent in acidic soils, as well as in orchards and vineyards after a long-term application of Bordeaux mixture ($Ca(OH)_2$ + $CuSO_4$) and other plant-protecting preparations based on copper as the active ingredient. Due to its poor mobility, total copper is accumulated in the surface layer. Although the accumulation results in a higher concentration of total copper, toxicity usually does not occur, as it quickly transforms in forms less accessible to the plant.

Plants receive copper mostly in ionic form, and it returns into the soil through harvest residues; this is why surface horizons (15-20 cm) are often richer in total copper than deeper mineral layers (Gracanin, 1947). This has been confirmed by the research of other authors as well (Ribolzi et al., 2002). According to the stated author, total copper is particularly deficient in some heath, acidic mineral and many marshy soils in Europe, America, Australia, the Filipines… The author notes that Maquenne and Demoussy conducted research of French heath soils containing the lowest concentration of total copper, as low as 2 mg in 1 kg of soil. He also reports data on the presence of total copper in the main types of soil in the former states of the USSR. It was thus established that red soils abound the most in total copper, and are followed by chernozemic soils and finally by very podzolic sandy soils, while in the peat and marshy soils the concentration of total copper oscillates.

In sandy soils more than 3% of total copper concentration is accounted for by exchangeable copper, of which exchangeable copper bound to nitrates accounts for 1%, and free Cu accounts for 2-9%. The rest of exchangeable copper is bound to organic matter in the soil (Temminghoff et al., 1994).

The research led the stated authors to the conclusion that the concentration of total copper is inversely proportional to the soil depth (up to 60 cm). They noted the highest concentration level of total copper in the surface layer, at 0 to 3 cm of depth (Besnard et al., 2001).

2.2 Bioavailability of copper in vineyerd and olive grove soils

Factors affecting the distribution and migration of heavy metals in the profiles of agricultural soils are as follows:

- soil type, despite its morphogenetic features being disrupted by tillage
- change in the sequence of genetic horizons and active depth of the profile
- amount of organic matter and pH reaction
- consumption process and accumulation of clay, Fe and Mn oxide
- whether the element is of geogenous, pedogenous or anthropogenous origin.

The process of intake of copper through plant roots is an active process and it is believed that a specific transmitter exists. In terms of intake, it is competed by Mn, Fe and Zn, and it has also been noted that a good supply in plants of nitrogen and phosphorous often results in copper deficiency (Vukadinovic & Loncaric, 1998). The stated authors point out that the availability of total copper is considerably influenced by pH reaction of the soil, and that its availability increases with the acidity. Total copper binds more tightly to organic matter of the soil than other micronutrients (e.g. Zn^{2+}, Mn^{2+}), which is why Cu-organic complexes play an important role in copper mobility regulation and availability in the soil (M. Romic & D. Romic, 1998). The stated authors point out that in fluvial and alluvial soil the redistribution of copper within fractions occurs relatively quickly, it is not retained in the exchangeable fraction, which considerably decreases the risk of its mobility and inclusion into the food chain. Apart from organic matter, soil carbonates proved to be another important factor controlling copper mobility in soils. Activity of copper in calcareous soils is to a great extent controlled by the surface precipitation of CuCO3 (Besnard et al., 2001; Ponizovsky et al., 2007). This is especially important in alkaline soils containing high concentrations of carbonates, which is the case for many vineyards. Results of the research on the relation between pH reaction of the soil and availability of total copper show that increase in the pH value of the soil causes increase in the amount of the bound copper in soil, reducing its mobility in the process (McLaren & Crowford, 1973). A finding that some authors point out as one of the greatest is that increase in bioavailable copper concentration is proportional to the increase in pH reaction of the acidic soils rhizosphere (Chaignon et al., 2002; Parat et al., 2002). A high total copper concentration in calcareous soils is caused by deficiency of other heavy metals, such as Fe and Zn, due to their antagonistic interrelation (Chaignon et al., 2001). Equal results were obtained in their research of carbonate soils. They conclude that plants take in copper, as well as other metals (Cd), more intensely from contaminated soils poor in iron and zinc.

Many authors have also researched the correlation between total copper and other metals in soils. In the research of agricultural soils in Zagreb and the surrounding area, it has been established that the content of lead shows a good correlation with the content of copper, zinc and cadmium ($r>0.43$) (M. Romic & D. Romic, 1998). The same results were obtained by some foreign authors while doing research on soils in Georgia (Narimanidze & Bruckner, 1999).

Total copper has a high positive correlation with the amount of organic matter in soil, while total zinc does not show correlation with any of the soil features, nor with the elements encompassed by the research (Cu, Fe, Ni, Cd, Cr) (M. Romic, 2002) .

Iron is the main factor responsible for the accumulation of total copper in the clay fraction of the soil (Parat et al., 2002).

2.3 Physiological role of copper in plants

In 1931 Anna L. Sommer established that copper was one of the first microelements found to be essential for the growth and development of plants. (Anic, 1973).

Physiological role of copper is very important, as it is an integral part or activator of many enzymes which participate in oxidation processes. It affects protein synthesis, it stabilizes chlorophyll molecules and participates in the synthesis of anthocyanins. It is included in the structure of plastocianin, cyto-chrom-oxidase c (transport of electrons), phenol oxidase (oxidation of phenol into quinone), lactase and phenolase, hidroxilase (translocation of phenyl alanine into tyrosine), oxygenase, ascorbic acid oxidase, superoxyde-dismuthase, several amino-oxidase, galacto-oxidase etc. Copper has a distinct affinity to protein structure, and consequently 70% of copper in plants is bound to proteins in chloroplasts, where they act as stabilisers, especially of chlorophyll. Furthermore, it has a significant role in the metabolism of nitrogen compounds, as it regulates the binding of ammonium keto acids, affects the synthesis of nucleic acids, bacterial leg-hemoglobin, the metabolism of carbohydrates, the formation of pollen and plant fertility, it increases resistance to low temperatures etc. (Vukadinovic & Loncaric, 1998).

The authors point out that copper toxicity is manifested with the reduced growth of roots and shoots, with the chlorosis of older leaves and dark-red margin necrosis.

The critical lower limit of this element ranges from 1μgCug^{-1} to 5 μgCug^{-1} of dry matter, and depends on many factors, such as: variety, plant organ, plant development level. The upper toxicity limit of the stated element in the leaf ranges from 20 μgCug^{-1} to 30 μgCug^{-1} of dry matter (Marschner, 1995). The author adds that increased copper concentrations in the plant are connected with the application of plant-protecting products, giving as example vineyards and copper fungicides used for disease control.

The highest total copper concentrations are found at the depth of up to 15 cm (Brun et al., 1998), in which soil layer plant roots are found, therefore the authors conclude that plants are directly exposed to high levels of copper contamination. The same authors indicate that Cu concentrations in roots are a good indicator of Cu bioavailability in soils (Brun, et al., 2001; Chopin, et al., 2008). However, this time-consuming approach is not suitable for routine analyses. Furthermore, it should be pointed out that Cu uptake by roots is species-dependent and influenced by root type and size (Brun, et al., 2008; Chopin, et al., 2008).

2.4 Copper content in grapes, must and wine

Considering the high total copper concentrations accumulated in the surface layer of vineyard soils, due to a long-term use of plant-protecting products based on copper as active ingredient, many questions arise. One of them is whether increased concentrations of the above-mentioned are found in grapes, must and wine, and, if so, whether they can at all be connected to the concentrations found in soil. The answer to this question is comprehensive and deserves a separate research. Since this is not the subject mater of this paper, only some literature data will be briefly presented. The purpose of this subtitle is to give a wider introduction into the issue of copper contamination of soils, as well as to give an insight into the possible consequences of this problem on grapes, must and wine, i.e. on food products. It is important to point out the origin of copper in wine, coming from three sources, which are as follows: plant-protecting products based on copper as active ingredient, winemaking equipment in cellars, and addition of Cu-salt for the elimination of H_2S from wine. The highest concentration of copper taken in from soil is accumulated in the plant root (Chaignon et al., 2002).

Copper is found in rigid parts of the cluster (seeds, skin, stem, etc.), and through processing it enters must and wine (Radovanovic, 1970). The same author presents data on copper concentration in must, which ranges from 0.2 mgL^{-1} to 4.0 mgL^{-1} (an average of 2.0 mgL^{-1}), and on copper concentration in wine, which ranges from 0.1 mgL^{-1} to 5.0 mgL^{-1}. Must always contains a relatively high copper concentration, around 5.0 mgL^{-1} (Ribereau-Gayon et al., 2000). The authors come to a conclusion that the largest part of copper in must originates from copper fungicides (copper-sulfate) used in vineyards for disease control. The same conclusion has been reached by some other researchers (Puig-Deu et al., 1994). The above-mentioned authors report that new wines contain as little as 0.3 mgL^{-1} to 0.4 mgL^{-1}, which is significantly less than the maximum level of copper concentration in wine permitted by the EU regulations, which amounts to 1.0 mgL^{-1}. Croatia has the same permitted level of copper concentration, prescribed by the Wine Act (Croatian Official Gazette, 96/1996) and the related regulations. Copper concentrations in Californian wines are as follows: white wines 0.13 mgL^{-1}, rose wines 0.16 mgL^{-1}, red wines 0.17 mgL^{-1}, and sparkling wines 0.07 mgL^{-1} (Boulton et al., 1996). According to the results of some research, wine contains from 30 μgL^{-1} to 1500 μgL^{-1} of copper, which is an average of 0.2 mgL^{-1} (Margalit, 1996). The author points out that copper concentrations of around 4.0 mgL^{-1} are dangerous for wine, and states that wine with concentrations from 0.5 $mgCuL^{-1}$ to 1.0 $mgCuL^{-1}$ is safe. Copper concentration in wine of 1.0 mgL^{-1} is detected by the senses (Zoecklein et al., 1995).

2.5 Contamination of different types of vineyard soils with copper in this part of Mediterranean region

2.5.1 Different types of vineyard soils

Vine is a very old field crop, native of the Mediterranean countries. Its beginnings date from the times of the Ancient Egypt, Greece and Rome. In the time of the Roman Empire cultivation of vine spread fast to Croatia (Licul and Premuzic, 1993.). From the time between the two World Wars until the middle of the last century, conditions for the development of viticulture were not very favorable. It was not until after that period that a rapid development of viticulture started, through the introduction of new varieties and rootstocks, and through the work on the clonal selection and hybridisation. Viticulture is a very important agricultural field in our country, as it makes more than a tenth of the overall agricultural production value. Its importance is also evident from the fact that, apart from the wine-growers whose aim is production for the market, there are tens of thousands of other wine-growers, who are either subsistence wine-growers or they grow vine as a hobby in small vineyards which are not even listed (Mirosevic, 1996).

This part of Mediterranean region consists of a number of sub-regions, while each sub-region comprises a number of vineyard areas. Following an extensive analysis of the region, sampling was made at four different locations, which had been selected by taking into account differences between respective types of soil typical in the area under research. Areas with anthropogenic colluvial soils were selected as first location. The secound location comprised areas with anthropogenic soils on flysch, areas with anthropogenic soils on terra rossa were selected for third location, while anthropogenic terrace soils on cretaceous limestones were selected as fourth location. The age of the vineyards (40 - 70 years old) had an important role in the selection, due to long-term use of copper fungicides. In order to establish the so-called "background" concentration, woodland soils were selected, as no copper-based plant-protection products had ever been used on them.

Anthropogenic colluvial soils, on flysch and terra rossa, were made by the work of man – by land clearing, digging, terracing, and fertilisation, with the aim to increase their fertility and to protect them from erosion. Viticultural area with anthropogenic terrace soils is defined as hilly and rolling. The parent rock consists of deposits of cretaceous formation, specifically limestone, particularly developed in coastal areas of wine-growing hills. In the past, this area was relatively poorly populated, man's influence was minimal, and only an insignificant amount of land was cultivated. In the course of historic development the number of inhabitants increased, and so did the land cultivation, and people started making terraces.

Characteristics of anthropogenic colluvial soils vary greatly depending on the origin of quaternary deposit, depth, properties of substrate onto which colluvium is deposited, as well as hydrological and geomorphological and lithological conditions of the location in which colluvium is accumulated (Males et al., 1998). On the basis of the results of chemical analyses, it has been determined that the anthropogenic colluvial soils are alkaline reactions (pH 7.30-7.73), the alkalinity increasing with depth, although insignificantly. This type of soils are highly carbonate (26.4-76.8%)and limy (5.79-20.07%) and poorly humic (1.10-11.12%). It has been established that they are moderately to well-supplied with nitrogen. Anthropogenic colluvial soils have shown poor to good supply level of potassium, while they are very poorly to poorly-supplied with phosphorus. Loam texture dominates in these soils. The soils are skeletal, their water capacity is low, while the air capacity is high (Vitanovic et al., 2010a).

Significant variations of the properties of anthropogenic soils on flysch are a result of the lithological complexity of flysch (Males et al., 1998). Anthropogenic soils on flysch are alkaline reactions (pH 7.16-7.44) and their alkalinity increases with depth, although insignificantly. These soils are very carbonate (8.2-62.0%), very limy (3.24-29.98%) and poorly humic (1.84-5.71%). In the researched soils the supply level of nitrogen is good, the supply level of potassium is poor to good, while phosphorous is very poorly to poorly present.

This type of soil shows great variability of the mechanical composition. It has been established that these soils are as skeletal as anthropogenic colluvial soils. Generally, chemical and hydrophysical properties of this type of soil are totally converse in comparison to other soils (Vitanovic et al., 2010a).

Anthropogenic soils on terra rossa are deep, with a specific red color (Males et al., 1998). This type of soil has alkaline reaction (pH 6.81-7.26) which increases with depth. Anthropogenic soils on terra rossa are, unlike the above described soil types, less carbonate (0.4-26.0%). Moreover, they have lower content of total lime (0-9.16%), i.e. they are not limy. However, the analysis has determined that they are considerably humic (1.63-11.47%) and well-supplied with total nitrogen, potassium and phosphorous. The textures are clay loam and silty clay loam. It has been determined that these soils are very skeletal. Generally, this type of soil, in addition to a heavy texture composition, has favorable hydrophysical properties (Vitanovic et al., 2010a).

A characteristic of antropogenic terrace soils is a shallow A-horizon of small-grained soil, located above the parent rocks (Males et al., 1998). Anthropogenic terrace soils are alkaline reactions (pH 6.77-7.29) and their alkalinity increases with depth, however, they have a weaker alkaline reaction than the above described soils. These soils have a low content of total carbonates (1.2-48.0%) and active lime (0-25.56%), but they are very highly humic (3.50-11.24%). They are very rich in nitrogen and very well-supplied with potassium and phosphorous, the content of which increases with depth. An analysis of the mechanical composition indicates that the soils are comprised of loam and silt loam. The soils are skeletal, mainly medium gravelly to cobbly, the water capacity is low, while their air capacity is high. Their water permeability is high. The structure of soils in these vineyards is mainly stable and well-defined (Vitanovic et al., 2010a).

2.5.2 Copper introduction by copper fungicides and total copper content in different types of vineyard soils

Bordeaux mixture, copper-hydroxide-Ca-chloride complex and copper (I) oxide are most frequently used copper fungicides in this part of Mediterranean region. Each of them contains different quantities of active ingredients, and is applied in different concentrations. The total quantity of copper introduced into one hectare of vineyards during one vegetative year has been calculated. The result shows that every vegetative year 2.90 kgCuha^{-1} is introduced in antropogenic colluvial soil, antropogenic soil on flysch and on terra rossa. Copper fungicides used in antropogenic terrace soils on cretaceous limestones introduce 4.20 kgCuha^{-1} every vegetative year (Vitanovic et al., 2010a).

The total copper concentrations in anthropogenic colluvial soils ranged between 70.50 mgkg^{-1} and 181.62 mgkg^{-1}, while concentrations from 21.85 mgkg^{-1} to 49.05 mgkg^{-1} were recorded in control areas. Anthropogenic soils on flysch contained from 163.68 mgCukg^{-1} to 302.05 mgCukg^{-1}, while the concentrations of this metal in control areas ranged from 44.42 mgkg^{-1} to 124.77 mgkg^{-1}. The total copper concentrations in antropogenic soils on terra rossa ranged from 113.46 mgkg^{-1} to 252.89 mgkg^{-1} in vineyard soils, whereas in control areas they ranged from 52.03 mgkg^{-1} to 290.11 mgkg^{-1}. Anthropogenic terrace soils on cretaceous limestones contained from 138.79 mgkg^{-1} to 625.79 mgkg^{-1}. Concentrations of this metal in control area soils varied from 45.94 mgkg^{-1} to 140.01 mgkg^{-1}. What is evident from the above-mentioned data is that total copper concentrations in all types of vineyard soils were higher than in the control soils. According to the research results, vineyard soils of this part of the Mediteranean contain from 70.50 mgkg^{-1} to 625.79 mgkg^{-1} of total copper, while concentrations of the metal in control areas are quite lower (21.85 mgkg^{-1} - 290.11 mgkg^{-1}) (Vitanovic et al., 2010a; 2010b).Results of total copper concentrations in all researched soils show a significant difference in concentrations of total copper between the vineyard and control areas. Significantly higher concentrations of this metal were identified in vineyard soils. Based on the obtained results, it can be concluded that total copper accumulates in the surface layer of vineyard soils due to long-term use of copper fungicides. The results of total copper concentrations in various types of researched soils also indicate a considerable difference in total copper concentrations (with 95% certainty) between colluvial anthropogenic soils and anthropogenic terrace soils on cretaceous limestones. Significantly higher concentrations of this metal were identified in anthropogenic terrace soils on cretaceous limestones. The reason for higher concentrations of total copper in heavier soils can be found in stronger bonding of copper with particles of heavier soils. In such soils copper is known to leach more slowly and in lesser quantities into lower layers, while its leaching is more excessive and faster in lighter soils. There are no significant differences in concentrations of the metal under research between anthropogenic soils on flysch and terra rossa and other researched anthropogenic soils (Vitanovic et al., 2010a; 2010b).

Considering the average concentrations of the metal under research, anthropogenic colluvial soils and anthropogenic soils of terra rossa were contaminated with copper, while anthropogenic soils on flysch and anthropogenic terrace soils on cretaceous limestones were polluted with researched metal. Human environment (food, drink, air) is either directly or indirectly connected with soil. Its quality is directly dependant on soil properties. The results of this research are very important, since they are the basis for defining soils as polluted, which will irreversibly remain such, and contaminated, in which the contamination can still be reduced (Vitanovic et al., 2010a).

2.6 Olive leaf spot - The biggest problem in most Mediterranean olive groves

Olive leaf spot, also known as peacock spot, is caused by the fungus *Spilocaea oleaginea*, which attacks the olive exclusively. The Frenchman Castagne described the disease for the first time as early as 1845, under the name *Cycloconium oleaginum*. Today, it is present throughout the world, in all olive-growing regions. The disease is common worldwide and causes serious problems in cooler olive-growing regions, with yield losses estimated to be as high as 20% (Wilson & Miller, 1949). Experts regard it as one of the most widespread and dangerous olive fungal diseases (Obonar et al., 2008). It has been known in this part of the Adriatic coast since the 8th century.

Since the disease most often attacks leaves, it is on them that the symptoms are most conspicuous (Photo 1).

Photo 1. Olive leaf spot

2.6.1 Symptoms and damages of disease

The infected leaves have spots on them which resemble spots on peacock's tail feathers, hence the name peacock spot. In the initial phase of infection dark-green oily spots, difficult to detect, appear on the upper leaf surface. The spots gradually turn yellow, with a discernible yellow-brown halo around them. In this later phase of infection the spots are very conspicuous. As the disease develops, the spots turn dark-brown, their number increases, and they cover more and more surface of the infected leaves. In the final phase of infection the leaf skin detaches from the epidermis, causing the spots to turn white (Photo 2). In rare cases, the symptoms of the disease also occur on the undersides of leaves, as well as on stems and fruits.

Photo 2. Symptoms of the disease

Damage caused by olive leaf spot is manifested in yield reduction of infected trees, as a consequence of a great loss in leaf mass. The fungus penetrates below the leaf epidermis, spreading into and in between cells. It feeds itself and releases toxins which disturb the creation of starch, necessary for normal plant functions, which consequently leads to the falling off of the sick leaves. Since the leaves fall after the flower buds have been formed, they become insufficient for an adequate diet of flowers, which consequently remain under grown and do not bear fruit. In the case of a serious autumn and subsequently a serious spring infection, leaves fall off massively. Consequently, the trees become partially or completely defoliated. In older olive grove reoccurrence of the disease for several consecutive years can cause defoliation and destruction of individual trees (Photo 3). The damage is greater if the disease spreads in a young olive grove, as then all olive trees can be destroyed (Miller, 1949; Azeri, 1993; Katalinic et al., 2009). Fruit infection can cause unacceptable blemishes on table olives, and when it occurs on oil-producing cultivars, infection may cause a delay in ripening and a decrease in oil yields (Verona & Gambogi, 1964).

2.7 Use of copper fungicides against olive leaf spot and possible copper traces in olive oil

Application of copper-based fungicides is the main method of olive leaf spot control throughout olive-growing regions of the world (Teviotdale et al., 1989; Graniti, 1993; Katalinic et al. 2009). For effective olive protection, several applications of copper fungicides are necessary in one year. For a long time it was thought that olive leaf spot infection could be prevented by two applications of copper preparations, i.e. by one treatment in autumn (September - October) and another one in spring (March - April). The autumn treatment, before the rains begin, is necessary because the rainy period creates conditions for primary infections. This reduces the possibility of spring infections by 87% to 95% (Obonar et al., 2008; Katalinic et al. 2009). Timing of the fungicide applications is vital for effective control of olive leaf spot (Graniti, 1993). In the Mediterranean region, fungicides are usually applied before the onset of the main infection periods, which often coincide with the main shoot-growth seasons (spring and/or autumn) (Prota, 1995). In organic production, for protection of olives against olive leaf spot only the use of copper fungicides is allowed. Concentration of applied preparations must be strictly under control because of possible copper residues in olive fruits and consequently in oil, which is restricted by law. In years with particularly warm and rainy autumns, one treatment with copper fungicides in autumn is not enough, but at least three treatments with copper fungicides are necessary. However, here a problem is encountered. To control olive leaf spot, preparations with increased concentrations are used, and in Croatia treatments with copper fungicides are allowed up to 56 days prior to harvest. In countries members of the EU copper fungicides are allowed up to 21 days prior to harvest. Under local conditions, harvest begins in early November, which means that control should be completed as early as the beginning of September. In years with the aforementioned conditions this is not possible, due to great quantities of precipitation and high temperatures during the whole autumn period, which is optimal for the development and spreading of the disease. The stated conditions require an increased number of treatments. However, in olive groves with organic production the application of only 3 kg of copper per hectare is permitted per year (Croatian Official gazette, 12/2001, 91/2001). The law also provides the maximum quantity allowed in table olives and olive oils, which is 0.40 $mgCukg^{-1}$. Undoubtedly, increasing the number of treatments in autumn renders it impossible to fully observe all the regulations. On the other hand, if the regulations are fully observed, the question arises whether it is actually possible to adequately protect olive groves against this unpleasant and spreading disease at all. In Mediterranean regions, such as Italy and Spain, three fungicide applications (end of winter, end of spring and late autumn) are recommended (Graniti, 1993). In Californian olive groves a single spray of copper-sulphate (Bordeaux mixture) gave satisfactory control of olive leaf spot when applied prior to rains in autumn or early winter (Wilson & Miller, 1949). Some authors also reported that one annual application of copper-based fungicides in late autumn, before the rainy period, effectively controlled olive leaf spot under low disease attack in Californian olive-growing region (Teviotdale et al., 1989). In New Zealand olive groves, multiple applications of copper and/or systemic fungicides are commonly used annually, but often fail to provide effective control, resulting in very high disease levels in some regions (MacDonald et al., 2000).

Olive leaf spot is a disease which has caused death of an increasing number of olive groves in almost all olive-growing areas in recent years. Urged by this problem we set up an experiment in order to find out if several autumn applications of copper fungicides would leave copper residues in olive oil. The experiment was conducted in 2009 and 2010 in a 25-year-old olive grove. In both years olives were treated 3 times in the autumn, every 15 days, with copper fungicides (copper-oxychloride) in the concentration of 0.3%. Copper-oxychloride is permitted for use even in organic production. Due to its good qualities it is especially used for the protection of quality vine varieties, and its application in olive-growing is of a particular importance. Due to its short waiting period (21 days from last spray to harvest), in European countries it is used to fight olive leaf spot in all the applications. Each year of the research 12 olive trees were randomly selected, 4 trees for each treatment combination (four trees – one treatment, four trees – two treatments and four trees – three treatments). The first treatment was performed on 15th September 2009 on all the 12 trees. The second treatment was done on 1st October 2009 on 8 trees, and the third was performed on 15th October 2009 on the last 4 trees. The treatments in 2010 were performed in the same way, on 14th September, 1st October and 15th October 2010. After the harvest, the fruits were not washed and were processed in a mini oil mill of the type Abencor mc2 – water bath with adjusted conditions (temp. < 27 ^{o}C and kneading time around 40mins, centrifuge 3000 r.p.m.). The copper residues in olive oil were determined by flame atomic absorption spectrometry (SpectrAA Varian 220). The results of this research show that in both years no traces of copper above the permitted limit (0.13 - 0.26 $mgkg^{-1}$) were found in any of the samples.

3. Conclusion

Owing to the importance of fungal diseases in the production of grapes, olives and olive oil, as well as to a great number of people engaged in the stated productions, it is necessary to continually improve their knowledge of fungal diseases and measures of their control. The importance of this knowledge is also accentuated by the fact that numerous chemical products of higher or lower toxicity for people and environment are used in the protection of vines and olives. It is, therefore, necessary to have a particularly good knowledge of the qualities of products for the protection of the stated crops, as well as of the correct application procedures. Even if just in traces, heavy metals are the primary sign of the soil and groundwater contamination. Polluted agricultural soils present a serious agro-environmental concern. Soil health is fundamental for the sustainability of agriculture. The benefits of having healthy soils are numerous. In healthy soils microorganisms play a major role, especially in nutrient cycling, making many nutrients available to plants. Contamination of vineyards and olive groves with copper compromises soil health and opposes the ideals of IPM and organic production. Copper fungicides have been used in vineyards and olive groves for protection from different fungal diseases for a long time. Many researchers indicate that the use of copper fungicides has led to an increase of total copper in surface layer of vineyard soils. Therefore, the selection of fungicides should be performed carefully, bearing in mind agricultural practices, physico-chemical characteristics of the soils and climatic and hydrogeological regimes in individual vine and olive growing regions. Pest control such as IPM and pest management in organic production, which manage pest damage with the least possible hazard to humans and the environment, should be encouraged. Our research has shown that application of copper fungicides to vineyards has resulted in the accumulation of copper residues in surface soils. Concentrations of up to 625.79 mg/kg were found in antropogenic terrace soil on cretaceous limestones. The reason of significant quantities of heavy metals remaining in the surface layer of vineyard soils lies in the long-term use of copper fungicides and the number of treatments per one vegetative year.The research has shown that repeated treatment of olive groves with copper fungicides in the period prior to the harvest is very efficient in olive leaf spot control. The large number of treatments has been efficient in the control of the disease, so that after processing of the treated olives no traces of copper in oil above the permitted limits were found.

On the basis of all the research and findings to date, as the positive so too the negative ones, on the use of copper fungicides in viticulture and olive-growing, it will be necessary in the future to supplement the knowledge of all the agricultural producers and thus enhance the efficiency of plant-protection measures and reduce the threat for people, useful organisms and environment.

4. References

Adriano, D.C. (2001). Trace element sin the terrestrial environments: biogeochemestry, bioavilability, and risks of metals. Springer-Verlag, New Yourk-Berlin-Heidelberg-Tokyo

Albaladejo, J.; Ortiz, R.; Guillen, F.; Alvarez, J.; Martinez-Mena, M. & Castillo, V. (1995). Erodibility of agricultural soils in the semiarid mediterranean area of Spain. Arid Soil Reserch and Rehabilitation. Vol. 9. (n.d. 1995), pp. 219-226, ISSN 0890-3069

Anic, J. (1973). Ishrana bilja. Faculty of Agriculture, Zagreb, Croatia

Azeri, T. (1993). Research on olive leaf spot, olive knot and Verticillium wilt of olive in Turkey. EPPO Bulletin, Vol. 23, (n.d. 1993), pp. 437-440, ISSN 0250-8052

Baize, D. (January 1997). Teneurs en elements traces metalliques dans les sols (France). INRA Editions, ISNB 978-2-7380-0747-6, Paris, France

Besnard, E.; Chenu, C. & Robert, M. (1999). Distribution of copper in vineyard soils, as influenced by organic amendments. Symposium J09-Environmental Pollution and Natural Backgrounds. In: Journal of Conference Abstracts Cambridge Publications. 01.02.2010, Available from
http://www.the-conference.com/JConfAbs/4/564.html

Besnard, E.; Chenu, C. & Robert, M. (2001). Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyards soils. Environmental Pollutions. Vol. 112, No. 3, (n.d. 2001), pp. 329-337, ISSN 0269-7491

Boulton, R.B. (1996). Principle and practices of vinemaking, The Chapman & Hall, ISBN 9781441951908, University of California, Davis, USA

Brun, L.A.; Maillet, J.; Richarte, J.; Herrmann, P. & Rémy, J.C. (1998). Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. Environmental Pollution. Vol. 102, No. 2-3, (n.d. 1998), pp. 151-161, ISSN 0269-7491

Brun, L.A.; Maillet, J.; Hinsinger, P. & Pepin, M. (2001) Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environmental Pollution. Vol. 111, (n.d. 2001), pp. 293-303, ISSN 0269-7491

Brun, L.A.; Le Corff, J. & Maillet, J. (2003). Effects of elevated soil copper on phenology, growth and reproduction of five ruderal plant species. Environmental Pollution. Vol. 122, No. 3, (n.d. 2003), pp. 361-368, ISSN 0269-7491

Chaignon, V.; Sanchez-Neira, I.; Jaillard, B. & Hinsinger, P. (2001). Relation between copper phytoavailablity and chemical properties of 29 contaminated soils from a vine-growing area. Proceedings of the 6th International Conference on the Biogeochemistry of Trace Elaments, Guelph, Ontario, Canada July-August 2001

Chaignon, V.; Sanchez-Neira, I.; Herrmann, P.; Jaillard, B. & Hinsinger, P. (2002). Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. Environmental Pollution. Vol. 123, (n.d. 2002), pp. 229-228, ISSN 0269-7491

Chopin, E.I.B.; Marin, B.; Mkoungafoko, R.; Rigaux, A., Hopgood, M.J. & Delannoy, E. (2008). Factors affecting distribution and mobility of trace elements (Cu, Pb, Zn) in a perennial grapevine (Vitis vinifera L.) in the Chapmagne region of France. Environmental Pollution. Vol. 156, (n.d. 2008), pp. 1092-1098, ISSN 0269-7491

Croatian Official Gazette (1996). Regulations on Wine Production, No. 96/1996

Croatian Official Gazette (2001). Organic Agriculture and Organic Food Production Act, No. 12/2001

Croatian Official Gazette (2001). Regulations on Organic Plant Production, No. 91/2001

Cvjetkovic, B. (1996). Plamenjaca vinove loze. Croatian Journal of Plant Protection. Vol. 5, pp. 241-244, ISNN 1332-9545

Delas, J. (1963). La toxicité du cuivre accumulé dans les sols. Agrochimica. Vol. 7, (n.d. 1963), pp. 258-288, ISSN 0002-1857

Delas, J. & Juste, C. (1975). Quelques problemes poses par les sols viticoles acides. Connaissance de la Vigne et du Vin. Vol. 2, (n.d. 1975), pp. 67-80, ISSN 0010-597X

Deluisa, A.; Giandon, P.; Aichner, M.; Bortolami, P.; Bruna, L.; Lupetti, A.; Nardelli, F. & Stringari, G. (1996). Copper pollution in Italian vineyard soils. Communications in Soil Science and Plant Analysis. Vol. 27, (n.d. 1996), pp. 1537-1548, ISSN 1532-2416

Drouineau, G. & Mazoyer, R. (1962). Contribution á l'étude de la toxicité du cuivre dans les sols. Annales Agronomiques. Vol. 13, No. 1, (n.d. 1962), pp. 31-53, ISSN 0003-3839

Dumestre, A.; Sauve, S.; McBride, M.; Baveye P. & Berthelin, J. (1999). Copper speciation and microbial activity in long term contaminated soils. Archives of Environmental Contamination and Toxicology, Vol. 36, (n.d. 1999), pp. 124-131, ISSN 0090-4341

Fernandez-Calvino, D.; Pateiro-Moure, M.; Lopez-Periago, E.; Arias-Estevez, M. & Novoa-Munoz, J.C. (2008). Copper distribution and acid-base mobilization in vineyard soils and sediments from Galicia (NW Spain). European Journal of Soil Science, Vol. 59, (n.d. 2008), pp. 315-326, ISSN 1351-0754

Flores Velez, L.M.; Ducaroir, J.; Jaunet, A.M. & Robert, M. (1996). Study of the distribution of copper in an acid sandy vineyard soil by three different methods. European Journal of Soil Science. Vol. 47, (December 1996), pp. 523-532, ISSN 1351-0754

Geoffrion, R. (1975). L'altération des terres á vigne par une longue répétition des traitements a base de cuivre et de soufre. Phytoma; Défense des Cultures. Vol. 267, (n.d. 1975), pp. 14-16, ISSN 0370-2723

Georgieva S.S.; McGrath, S.P.; Hooper D.J. & Chambers, B.S. (2002). Nematode communities under stress: the long term effects of heavy metals in soil treated with sewage sludge. Applied Soil Ecology, Vol. 20, (n.d. 2002), pp. 27-42, ISSN 0929-1393

Gracanin, M. (1947). Pedologija, II dio - Fiziologija bilja. Poljoprivredni nakladni zavod, Zagreb, Croatia

Graniti, A. (1993). Olive scab: review. EPPO Bulletin, Vol. 23, (n.d. 1993), pp. 774-777, ISSN 0250-8052

Katalinic, M.; Kacic, S. & Vitanovic, E. (2009). Stetnici i bolesti masline (first edition). Agroknjiga, ISBN 9789537246198, Split, Croatia

Komarek, M.; Vanek, A.; Chrastny, V.; Szakova, J.; Kubova, K. & Drahota, P. (2009). Retention of copper originating from different fungicides in contrasting soil types. Journal of Hazardous Materials. Vol. 166, (n.d. 2009), pp. 1395-1402, ISSN 0304-3894

Komarek, M.; Cadkova, E.; Chrastny, V.; Bordas, F. & Bollinger, J.C. (2010). Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. Environment International. Vol. 36, (n.d. 2010), pp. 138 – 151, ISSN 0160-4120

Kookana, RS.; Baskaran, S. & Naidu, R. (1998). Pesticide fate and behaviour in Australian soils in relation to contamination and management of soil and water: a review. Australian Journal of Soil Research. Vol. 36, No. 5, (n.d. 1998), pp. 715 – 764, ISSN 1838-675X

Li, R.N. (1994). Effect of long-term applications of copper on soil and grape copper (Vitis vinifera). Canadian Journal of Soil Science. Vol. 74, No. 3, (August 1994), pp. 345-347, ISSN 0008-4271

Li, W.; Zhang, M. & Shu, H. (2005). Distribution and fractionation of copper in soils of apple orchards. Environmental Science and Pollution. Vol. 12, (n.d. 2005), pp. 168-172, ISSN 0944-1344

Licul, R. & Premuzic, D. (1993). Praktično vinogradarstvo i podrumarstvo. Nakladni zavod Znanje, ISBN 953-6124-09-2, Zagreb, Croatia

MacDonald, A.J.; Walter, M.; Trought, M.; Frampton, C.M. & Burnip, G. (2000). Survy of olive leaf spot in New Zealand. New Zealand Plant Protection, Vol. 53, (n.d. 2000), pp. 126-132, ISSN 1175-9003

Males, P.; Milos, B.; Pezo, I. & Bubic, J. (1998). Selection of grape rootstock for Kaštela fields, Proceedings of the Kaštela - Croatia cradle, Kastel Stari, Croatia, September-October 1998

Margalit, Y. (January 1996). Concept in wine chemestry, Wine Appreciation Guild, ISBN 978-0932664914, San Francisco, USA

Marschner, H. (1995). Mineral nutrition of higher plants, Academic Press INC., ISNB 012473541X, San Diego, CA, USA

Mc Laren, R.G. & Crawford, D.V. (1973). Studies on soil copper. II. The specific adsorption of copper by soils. Journa of Soil Science. Vol. 24, (n.d. 1973), pp. 443-452, ISSN 1365-2389

McConnell, S.; Wightwick, A.; Smith, T. & Porteous, C. (2003). Code of Environmental Best Practice for Viticulture – Sunraysia region. Environmental best practices Volume 1 – Environmental best practices. Department of Primary Industries. ISBN 1-74106-638-7, Victoria, Australia

Merry R.H.; Tiller, K.G. & Alston, A.M. (1983). Accumulation of copper, lead and arsenic in some Australian orchard soils. Australian Journal of Soil Research, Vol. 21, (n.d. 1983), pp. 549-561, ISSN 1838-675X

Miguens, T.; Leiros, C.; Gil-Sotres, F. & Trasar-Cepeda, C. (2007). Biochemical properties of vineyard soils in Galicia, Spain. Science of Total Environment. Vol. 378, (n.d. 2007), pp. 218-222, ISSN 0048-9697

Miko, S.; Peh, Z.; Halamic, J.; Prohic, E.; Galovic, L. & Sparica, M. (2000). Geochemical mapping in Croatia - state of the art. Proceedings of Secound Croatian Geology Congress, Cavtat, Croatia, May 2000

Miller, H.N. (1949). Development of the leaf spot fungus in the olive leaf. Phytopathology, Vol. 39, (n.d. 1949), pp. 403-410, ISSN 0031-949X

Mirlean, N.; Roisenberg, A. & Chies, J.O. (2007). Metal contamination of vineyard soils in wet subtropics (southern Brazil). Environmental Pollution. Vol. 149, No. 1, (n.d. 2007), pp. 10-17, ISSN 0269-7491

Mirosevic, N. (1996). Vinogradarstvo (first edition). Nakladni zavod Globus, ISBN 953-167-084-6, Zagreb, Croatia

Morgan, R.K. & Taylor, E. (2004). Copper accumulation in vineyard soils in New Zealand. Journal of Environmental Sciences. Vol. 1, No. 2, (n.d. 2004), pp. 139-167, ISSN 1001-0742

Narimanidze, E. & Bruckner, H. (1999). Survey on the metal contamination of agricultural soil in Georgia. Land Degradation & Development. Vol. 10, No. 5, (September-October 1999), pp. 467-588, ISSN 1085-3278

Novoa-Munoz, J.C.; Queijeiro, J.M.G.; Blanco-Ward, D.; Alvarez-Olleros, C.; Martinez-Cortizas, A. & Garcia-Rodeja, E. (2007). Total copper content and its distribution in acid vineyard soils developed from granitic rocks. Science of Total Environment. Vol. 378, (n.d. 2007), pp. 23-27, ISSN 0048-9697

Obonar, F.O.; Jaspers, M.V.; Jones, E.E. & Walter, M. (2008). Greenhouse and field evaluation of fungicides for control of olive leaf spot in New Zealand. Crop Protection, Vol. 27, No. 10, (October 2008), pp. 1335-1342, ISSN 0261-2194

Paoletti, M.G.; Sommaggio, D.; Favretto, M.R.; Petruzzelli, G.; Pezzarossa, B. & Barbafieri M. (1998). Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. Applied Soil Ecology, Vol. 10, (n.d. 1998), pp. 137-150, ISSN 0929-1393

Parat, C.; Chaussod, R.; Leveque, J.; Dousset, S. & Andreux, F. (2002). The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron. European Journal of Soil Science. Vol. 53, No. 4, (December 2002), pp. 663-669, ISSN 1351-0754

Pietrzak, U. & McPhail, D.C. (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geodarma. Vol. 122, (n.d. 2004), pp. 151-166, ISSN 0016-7061

Ponizovsky, A.A.; Allen, H.E. & Ackerman, A.J. (2007). Copper activity in soil solutions of calcereous soils. Environmental Pollution. Vol. 145, (n.d. 2007), pp. 1-6, ISSN 0269-7491

Prasad, B.R.; Basavaiah, S.; Subba Rao, A. & Subba Rao, I.V. (1984). Forms of copper in soils of grape orchards. Journal of the Indian Society of Soil Science. Vol. 32, (n.d. 1984), pp. 318-322, ISSN 0019-638X

Prota, U. (1995). Le malattiedell'olivo. Informatore Fitopatologico, Vol. 45, (n.d. 1995), pp. 16-26, ISSN 0020-0735

Puig-Deu, M.; Lamuela-Raventos, R.M.; Buxaderas, S. & Torre-Boronat, C. (1994). Determination of copper and iron in must: Comparison of wet and dry ashing. American Journal of Enology and Viticulture. Vol. 45, No. 1., (n.d. 1994), pp. 25-28, ISSN 002-9254

Radovanovic, V. (1970). Tehnologija vina, Građevinska knjiga, Beograd, Serbia

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A. & Dubourdieu, D. (2000). The Chemistry of Wine Stabilization and Treatments. In: Handbook of Enology, Volume 2, 86-97, John Wiley & Sons Ltd., ISBN 987-0-470-01037-2, Chichester, England

Ribolzi, O.; Valles, V.; Gomez, L. & Voltz, M. (2002). Speciation ond origin of particulate copper in runoff water from a Mediterranean vineyard catchment. Environmental Pollution. Vol. 117, No. 2, (August 2002), pp. 261-271, ISSN 0269-7491

Romic, M. & Romić, D. (1998). Sadržaj olova kadmija, cinka i bakra u poljoprivrednim tlima Zagreba i okolice. Agriculturae Conspectus Scientificus. Vol. 63, No. 3, (n.d. 1998), pp. 147-154, ISSN 1331-7768

Romic, M.; Romic, D. & Kraljickovic, J. (2001). Bakar u vinogradarskim tlima, In: Land management and soil protection for future generations, Z. Ratz, (Ed.), 1-77, Croatian Society of Soil Science, Zagreb, Croatia

Romic, M. (2002). Sadržaj, oblici i preraspodjela imisije teških kovina u poljoprivrednim tlima šireg područja Zagreba. PhD disertation. Faculty of Agriculture University of Zagreb, Zagreb, Croatia.

Schramel, O.; Michalke, B. & Kettrup, A. (2000). Study of copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. Science of Total Environment. Vol. 263, (n.d. 2000), pp. 11-22, ISSN 0048-9697

Stevenson, F.J. & Cole, M.A. (March 22. 1999). Cycle of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients , John Wiley & Sons, ISNB 978-0471320715, New York, USA

Temminghoff, E.J.M.; Vanderzee, S.E.A.T.M. & Keizer, M.G. (1994). The influence of pH on the desorption and speciation of copper in a sandy soil. Soil Science. Vol. 158, No. 6, (December 1994), pp. 398-408, ISSN 0038-075X

Treviotdale, B.L.; Sibbett, G.S. & Harper, D.H. (1989). Control of olive leaf spot by copper fungicides. Journal of Applied Agricurtural Research, Vol. 4, (n.d. 1989), pp. 185-189, ISSN 2006-750X

Van Zwieten, L.; Rust, J.; Kingston, T.; Merrington, G. & Morris, S. (2004). Influence of copper fungicide residues on occurrence of earthworms in avocado orchard soils. Sciencie of Total Environment. Vol. 329, (n.d. 2004), pp. 29-41, ISSN 0048-9697

Verona, O. & Gambogi, P. (1964). On the characteristics of oil produced by olives attacked by Cycloconium oleaginum. Agricoltura Itaiana., Vol. 64, (n.d. 1964), pp. 1135-1139

Vitanovic, E.; Vidacek, Z.; Katalinic, M.; Kacic, S. & Milos, B. (2010a). Copper in surface layer of Croatian vineyard soils. Journal of Food Agriculture and Environment, Vol. 8, No. 1, (January 2010), pp. 268-275, ISSN 1459-0255

Vitanovic, E.; Katalinic, M.; Kacic, S.; Zanetic, M.; Milos, B. & Radinovic, S. (2010b). The content and correlation of copper, iron and zinc in the surface layer of the Croatian vineyard soils. Journal of Food, Agriculture and Environment, Vol. 8, No. 3&4, (July-October 2010), pp. 1134-1140, ISSN 1459-0255

Vukadinovic, V. & Loncaric, Z. (1998). Ishrana bilja. Faculty of Agriculture, ISBN953-6331-04-7, Osijek, Croatia

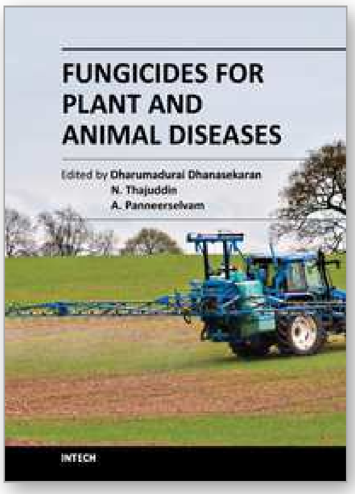
Wightwick, A.M.; Mollah, M.; Smith, J. & MacGregor A. (2006). Sampling considerations for surveying copper concentrations in Australian vineyard soils. Australian Journal o Soil Research, Vol. 44, No. 7, (October 2006), pp. 711-717, ISSN 1838-675X

Wightwick, A.M. & Allinson, G. (2007). Pesticide residues in Victorian waterways: a review. Australian Journal of Ecotoxicology. Vol. 13, (n.d. 2007), pp. 91 – 112, ISSN 1323-3475

Wightwick, A.M.; Mollah, M.R.; Partington, D.L. & Allison, G. (2008). Copper fungicide residues in Australian vineyard soils. Journal of Agricultural and Food Chemestry. Vol. 56, (2008), pp. 2457-246, ISSN 0021-8561

Wilson, E.E. & Miller H.N. (1949). Olive leaf spot and its control with fungicides. Hilgardia, Vol. 19, (n.d. 1949), pp. 1-24, ISSN 0073-2230

Zoecklein, B.W. (December 31. 1995). Wine analysis and production. Springer, ISBN 978-0412982415, University of California, Davis, USA



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A fungicide is a chemical pesticide compound that kills or inhibits the growth of fungi. In agriculture, fungicide is used to control fungi that threaten to destroy or compromise crops. Fungicides for Plant and Animal Diseases is a book that has been written to present the most significant advances in disciplines related to fungicides. This book comprises of 14 chapters considering the application of fungicides in the control and management of fungal diseases, which will be very helpful to the undergraduate and postgraduate students, researchers, teachers of microbiology, biotechnology, agriculture and horticulture.

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