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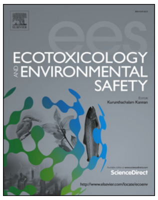

Effects of zinc addition to a copper-contaminated vineyard soil on sorption of Zn by soil and plant physiological responses

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ABSTRACT

The occurrence of high levels of Cu in vineyard soils is often the result of intensive use of fungicides for the preventive control of foliar diseases and can cause toxicity to plants. Nowadays many grape growers in Southern Brazil have replaced Cu-based with Zn-based products. The aim of the study was to evaluate whether the increase in Zn concentration in a soil with high Cu contents can interfere with the dynamics of these elements, and if this increase in Zn may cause toxicity to maize (*Zea mays* L.). Soil samples were collected in two areas, one in a vineyard with more than 30 years of cultivation and high concentration of Cu and the other on a natural grassland area adjacent to the vineyard. Different doses of Cu and Zn were added to the soil, and the adsorption isotherms were built following the Langmuir's model. In a second experiment, the vineyard soil was spiked with different Zn concentrations (0, 30, 60, 90, 180, and 270 mg Zn kg^{-1}) in 3 kg pots where maize was grown in a greenhouse for 35 days. When Cu and Zn were added together, there was a reduction in the quantities adsorbed, especially for Zn. Zn addition decreased the total plant dry matter and specific leaf mass. Furthermore, with the increase in the activity of catalase, an activation of the antioxidant system was observed. However, the system was not sufficiently effective to reverse the stress levels imposed on soil, especially in plants grown in the highest doses of Zn. At doses higher than 90 Zn mg kg^{-1} in the Cu-contaminated vineyard soil, maize plants were no longer able to activate the protection mechanism and suffered from metal stress, resulting in suppressed dry matter yields due to impaired functioning of the photosynthetic apparatus and changes in the enzymatic activity of plants. Replacement of Cu- by Zn-based fungicides to avoid Cu toxicity has resulted in soil vineyards contaminated with these metals and damaging of plant photosynthetic apparatus and enzyme activity.

1. Introduction

Viticulture is an activity in expansion in Brazil, especially in the state of Rio Grande do Sul, in southern Brazil, where there are currently about 50,000 ha of vines, representing approximately 62% of the vineyards area in the country (IBGE, 2012). For control of foliar diseases, the viticulture of southern Brazil passes through frequent applications of copper (Cu)-based fungicides, which often leads to increased Cu concentration in the soil, up to levels that are toxic to plants. As an alternative, some producers began using zinc (Zn)-based fungicides, resulting in the occurrence of high levels of both elements in vineyard soils (Mackie et al., 2012; Fernández-Calviño et al., 2010, 2012; Brunetto et al., 2014; Miotto et al., 2014). However, recently, it has been observed that young vines transplanted into anciently eradicated vineyard soils with high concentrations of Cu and Zn had a slower growth rate, leaf chlorosis and accumulated both elements in the roots (Miotto et al., 2014).

Even though Cu and Zn are plant micronutrients, performing structural and enzymatic activation functions (Pilon et al., 2006; Kabata-Pendias, 2011), but they might have also toxic effects if present in too high concentrations. In fact, plants grown in soils with high concentrations of Cu and Zn can accumulate these

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metals in their tissues (Kabata-Pendias, 2011), resulting in negative biochemical and physiological responses that vary according to the plant organ, the concentration, and the tissue tolerance (Mateos-Naranjo et al., 2013).

The absorption of excessive Cu and Zn can cause oxidative stress due to the imbalance between the antioxidant responses and increased production of reactive oxygen species (ROS) (Girotto et al., 2013). By means of Fenton's reaction, the redox cycle between $Cu^{2+}$and Cu^{+} catalyzes the production of hydroxyl radicals (OH^{-}) from superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2), increasing the harmful effects of these reactive species (Briat and Lebrun, 1999). Zinc, in turn, may generate oxidative stress through interference with the antioxidant defense system of plants (Gratão et al., 2005).

The high Cu content in plants also may inhibit growth by interfering in important cellular processes such as membrane permeability, alterations in the structure of chromatin, protein synthesis, and the activity of enzymes involved in photosynthesis and respiration (Yruela, 2005; Gratão et al., 2005). Increases of Zn in the plant may affect the photosynthetic efficiency by inhibiting chlorophyll biosynthesis and decreasing carbon assimilation (Chen et al., 2008; Dhir et al., 2008), potentially reducing rooting capacity and even causing leaf chlorosis (Castiglione et al., 2007), actions that contributes to the inhibition of plant growth (Girotto et al., 2013). Furthermore, Zn toxicity may compromise membrane permeability, the electron transport chain (De Magalhães et al., 2004), and absorption and redistribution of nutrients (Jiang and Wang, 2008; Wang et al., 2009).

The accumulation of Cu and Zn, but especially their availability within the rhizosphere, depends on the competitive interaction for the same adsorption sites, as well as on the interactions with other chemical species present in the medium (Pérez-Novo et al., 2009, 2011). Therefore, the sorption kinetics of these heavy metals in the soil is most likely different when added simultaneously than when added alone (Arias et al., 2006). Studies on sorption of kinetics of these metals added simultaneously or alone can provided valuable information to predict if application of Zn-based fungicides will enhance desorption of Cu by soil or if soil adsorption sites that have been occupied previously by Cu will result in higher availability of Zn.

Some studies have examined how the single addition of either Cu or Zn affects a variety of plant physiological processes (Jain et al., 2010; Cambrollé et al., 2012; Mateos-Naranjo et al., 2013). Other studies, using nutrient solution culture, reported the interaction effects between the two heavy metals in the antioxidant defense system of plants (Upadhyay and Panda, 2010). There are also reports of the interaction between Cu and Zn in triggering antioxidant defense mechanism of plants grown in soils rich in organic matter and clay content contaminated with both elements due to successive pig slurry applications that are high in Zn and Cu (Girotto et al., 2013). However, only few studies focused on the interaction between Cu and Zn and the effects on plants in vineyard soils characterized by a high accumulation of both elements.

Recently, plants have been used to transfer toxic metals from soils to shoots to clean up metal-polluted soils (Surriya et al., 2015). This practice is known as phytoremediation, and it is only feasible when the produced biomass can be valorized. In this regard, maize (*Zea mays* L.) has been widely used for this purpose, because it is an alternative of risk reduction and generation of an alternative income for agriculture (Meers et al., 2010). Moreover, maize fulfills the criteria of having a high biomass, a rapid growth rate, and heavy metal tolerance (Gupta et al., 2009; Girotto et al., 2013). According to Wuana and Okieimen (2010), the potential use of maize in phytoextraction technology is advocated especially for developing countries with scarce funds available for environmental restoration. Besides, maize can be grown in consortium with vineyards in heavy metals contaminated soils, being an interesting alternative income for winegrowers from southern Brazil.

Therefore, the objective of this study was to evaluate whether the increase in Zn concentration in a Cu contaminated vineyard soil causes additional plant toxicity symptoms to maize (*Zea mays* L.). Copper and Zn might interfere with each other in the soil-plant system having synergistic, antagonistic, or competitive effects further enhancing or reducing their toxicities. The plant biomass thereby was monitored together with the biochemical and physiological parameters to assess Zn and Cu toxicity symptoms.

2. Material and methods

2.1. Soil characterization

Soil samples were collected in two Typic Hapludalf (Soil Survey Staff, 2006). Both soil samples were collected from a depth of 0.00-0.15 m in November 2011. Subsequently, the soil samples were air dried, milled, and stored. The physicochemical characteristics of these two soils are presented in the Supplementary Material. Both soils presented about 90% sand and 5% clay. One soil was cultivated with grapes (*Vitis vinifera* L.) in Santana do Livramento (30 °47′34.5″S e 55 °22′5.5″W), state of Rio Grande do Sul, in southern Brazil, with a history of over 30 years of continuing applications of Cu-based fungicides for the preventive control of foliar diseases. This vineyard soil presented 120.8 and 18.7 mg kg^{-1} of available Cu and Zn extracted by EDTA, soil pH 5.7, and 18.5 g kg^{-1} of soil organic matter. The other was from an adjacent (30 °47′23.5"S e 55 °22′7.0″W), non-anthropic, natural grassland field. This soil had naturally low concentration of Cu and Zn (0.7 and 0.9 mg kg^{-1} extracted by EDTA, respectively), soil pH 5.2, and 9.0 g kg^{-1} of soil organic matter. Girotto et al. (2014) evaluated the soil Cu-forms in the same study area and they found 4 and 3% in the exchangeable form, 20% and 67% in the mineral fraction, 14% and 19% in the organic fraction, 62% and 11% in the residual fraction, in the soil under natural vegetation and the 30-years vineyard soil, respectively.

2.2. Experiment 1 – adsorption of Cu and Zn in a Cu-contaminated vineyard soil

To set the Zn treatment doses to be applied in Experiment 2, the Cu and Zn adsorption capacities were evaluated for the vineyard soil, following the protocols described in Casagrande et al. (2004). Samples of 0.5 g of air-dried soil were weighed (in triplicate) and added to 15 mL centrifuge tubes. Three sets of centrifuge tubes with soil samples were prepared. In the first one, we added 10 mL of 0.0025 mol L^{-1} $Ca(NO_3)_2$ solution containing 0; 2.5; 5.0; 10.0; 20.0; 40.0 or 60.0 mg L^{-1} Cu (corresponding to 0, 39, 79, 157, 315, 629 and 944 mmol L^{-1} Cu, respectively). In the second one, we added 10 mL of 0.0025 mol L^{-1} $Ca(NO_3)_2$ solution containing 0; 2.5; 5.0; 10.0; 20.0; 40.0 or 60.0 mg L^{-1} Zn (corresponding to 0, 38, 76, 153, 306, 612 and 918 mmol L^{-1} Zn, respectively). Finally, in the third one, we added 10 mL of 0.0025 mol L^{-1} $Ca(NO_3)_2$ solution containing 0; 2.5; 5.0; 10.0; 20.0; 40.0 or 60.0 mg L^{-1} of Cu and Zn to estimate the competitive adsorption buffer where both elements were added together. Merck Certipur 1.000 mg L^{-1} standard solutions were used to obtain the doses of Cu and Zn. After stirring for 16 h in an end-over-end shaker, the samples were centrifuged at 960 × *g* for 15 min, and the concentrations of Cu and Zn in the supernatant were determined by flame atomic absorption spectrometry (AAS). The soil adsorption capacity was estimated by subtracting the amount in solution from that initially added in the equilibrium solution. The adsorption isotherms were