Evaluation of Metal Contents in Correlation with Phytosanitary Treatments at Vineyard

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The present work aims with the evaluation of copper, manganese and zinc concentrations (mobile forms) from vineyard soil before and after phytosanitary treatment with Curzate Manox and Dithane M-45 compounds, during and after remanence period. Different vineyard soils types were collected at 0-20 cm and 20-40 cm depths. Flame Atomic Absorption Spectroscopy (FAAS) method was used for measurements of the micronutrients. The soil samples were analyzed after 5 and 21 days after treatment application. Since copper is mainly accumulates in the upper layer following fungicidal sprays application, high levels of copper concentrations are obtained. The soil samples exhibits different behavior in terms of manganese and zinc contents. Manganese and zinc levels are classified as medium in the beginning of the experiment (Mn-M_0 and Zn-M_0), whereas these levels increased in the soil samples (at moments M_1- 5 days and M_2- 21 days after treatment). This behavior can be due to the Mancozeb decomposition, knowing that Mancozeb decomposes in the pH range 5-9 and it remains short time into the soil.

Keywords: dithiocarbamates, Mancozeb, vineyard, copper, manganese, zinc

Due to its significant economic impact, nowadays, the wine sector is considerably developed all over the world. The vineyard soils contribute through its minerals contents to the healthy growing of the wine grapes. In recent decades, soil chemistry has been the attention of numerous research studies [1-3]. Furthermore, the metals contents of vineyard soils are an important environmental issue that can influence the quality of the plant products. Various environmental factors (e.g. region, type of vineyard soil, temperature, precipitation, wind, etc.) as well as the anthropogenic constituents (e.g. fertilizers, metal-based pesticides, municipal wastes, wine-making technology and storage, etc.) can influence the wine sector [3-5].

Accumulation of micronutrients and macronutrients in soil represents an important aspect for the plant nutrition, which can come from the mineral fertilizers [6] and pesticides residues [7].

Investigation of metals concentrations in vineyard soil and wine has a great interest considering that the pollution due to pesticide application may contribute to negative effects on human health [8]. Many researchers carried out extensive studies on vineyard soil [9-14] and assessment of metallic profile of wines [15-24].

Copper is considered one of most important micronutrient for plant and it plays an essential role in the metabolic processes. It can exist in two oxidation states (Cu^{2+}, Cu^{+}) in the environmental cell and it well adsorbed into the soil layers exhibiting a higher mobility [25-27]. Calin et al. [15] studied the cooper content in various types of wines and the copper level was below 1 mg/L, limit set by OIV [28].

Zinc is also a crucial nutrient for plants and it is often deficient in the natural soils. Its concentration ranges from 40 to 120 mg/ kg and the addition of commercial Zn- based pesticides and fungicides contribute to its accumulation into the soils, achieving considerably higher concentration. The increase in zinc concentration in the soils over the optimum level can poison the soils and inhibits the plant growth [29, 30].

Among copper and zinc, manganese fractions enhance the quality of the soils. It has a significant role in the synthesis of chlorophyll and it is present in soil as exchangeable Mn or Mn oxide [31, 32]. The investigations of the effect of pH and its association with the metals content were studied by Voegelin et al. [33], which reported high mobility of Zn in slightly acidic soils. Scannavino et al. [34] found a significant pH gradient between the top and bottom layers of the soil, showing that top layer is more acidic than the bottom ones.

The treatment with pesticides results in increased of metals concentration in soils. Fungicides compounds, namely dithiocarbamates are widely used for soil treatment in order to control the microbial plant diseases. The levels concentration of different metals (e.g. Mn, Zn) increase after the application of the pesticides on the soils [35]. The behavior of fungicides type-carbamates in soils was studied and no significant inhibitory effect of fungicides on soil was observed, but the color changes of the soil was observed after treatments, indicating that soil microorganisms become adapted to carbamates after repeated applications [36]. However, Wang et al. [37] suggest that carbamates could temporarily reduce the soil bacterial community after repeated applications.

The purpose of our research was to evaluate copper, manganese and zinc concentrations (mobile forms) from vineyard soil before and after phytosanitary treatment with Curzate Manox and Dithane M-45, during and after remanence period.

Experimental part

Material and methods

Description of the area

The experimental site used for soils collection is located in Prahova County, Romania. The common soils are Afuz Ali and Coarna Neagra, The region is characterized by a favorable climatic conditions (sun, heat, water), which are vital to the healthy growth and development of grapevines during the growing seasons. The average annual

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temperature is 11.3 °C and the recorded mean annual precipitation is 642 mm.

Soil sampling

Soils were collected from the target location at depths of 0-20 cm and 20-40 cm and were subsequently tested at laboratory scale. Prior to the analysis, the samples from each soil layer were dried at room temperature for 24 h, finely grounded and sieved.

Laboratory experiment

AfuzAli and Coarna Neagra vineyard soils were subjected to laboratory experiments. The tests are designed to simulate the real conditions. Two cylindrical glass tubes (length = 30 cm, diameter = 5 cm) situated in a vertical position were filled with soils. One tube was filled with Afuz Ali 20-40 cm in the down part of the tube, followed by same soil at depth of 0-20 cm soil in the upper part, simulating the depth of soil in the real conditions. Coarna Neagra soils were operated following same procedure as is described above. In the next step, a volume of 10 mL pesticide solution was poured into the tubes filled with soils. After a time of 5 days from the pesticides application, a volume of 50 mL deionized water was poured into the tubes filled with treated soil, simulating the rain in real conditions. The water that passed through the tubes was collected and tested, imitating in this way, the leachate water of soils. After a period of 21 days from treatment, a new volume of 50 mL deionized water was poured onto the soil located in the tubes and the resulted leachate water was collected and analyzed. These two periods are denoted in this study as M_1 and M_2, respectively, specifically for Cu, Mn and Zn levels.

An amount of 200 g soil samples (taken from each vineyard category: AfuzAli and Coarna Neagra and collected from different depths) were mixed with a volume of 20 mL solution of pesticides and dried at room temperature. The soil samples were taken after 5 and 21 days from the treatment application. The purpose of analyzing the sample after 21 days is to understand the long-term behavior of the pesticides and their effect on the soils. The samples of 10 g from both Afuz Ali and Coarna Neagra vineyard soils were subjected to laboratory experiments.

Phytosanitary treatment applied to soil samples

The applied phytosanitary treatment consists of Curzate Manox /Dithane M-45 mixture with ratio = 50/50. Since the starting materials used for treatment of soils contain copper, manganese and zinc, these pesticides were chosen for experiments. Furthermore, Curzate Manox contains 50 wt% copper oxychloride and 18 wt% Mancozeb, whereas Dithane M/45 has 80 wt% Mancozeb.

Analyses

Soil samples were treated with a volume of 50 mL extractive solution consisting of 0.01 M ethylene-diaminetetraacetic acid (EDTA) and 1 N ammonium acetate at pH of 7 [38, 39]. The soil-solution mixture was stirred for 2 h and filtered off. The solutions were stored in polyethylene bottles and glassware was cleaned by soaking in 10 % v/v HNO_3 for 24 h and rinsing several times with deionized water.

The concentrations of Cu, Zn and Mn in the obtained soil-solution mixtures were measured by Flame Atomic Absorption Spectroscopy (FAAS). The experiments were performed with a Varian AA240 FS instrument using air-acetylene flame.

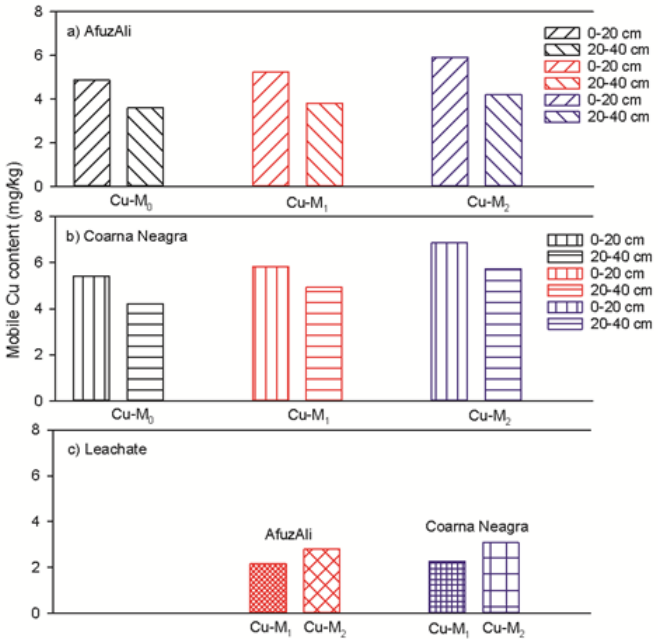
For *p*H measurements, the soil samples from both soil layers were mixed with deionized water and a solid: liquid ratio of 1:2.5 (w/v) was used [40]. The product was stirred for 2 h before analysis. In order to determine the *p*H, an amount of 10 g dried soil samples were taken at both time intervals (5 and 21 days), mixed with deionized water and stirred for 2 h. The *p*H of the resulted solutions was measured using a glass electrode through the potentiometric method.

Results and discussion

Copper is one of the investigated element in wine-growing area. The concentrations of copper in non-treated (fresh) vineyard soils samples range from 3.6 to 5.4 mg/kg and it depends on the depth, as shown in Figure 1. Furthermore, slightly increase in Cu contents was observed for the soil samples analyzed at the time of 5 days and 21 days after the treatment application. The maximum level of copper found in our experiments was 6.8 mg/kg (for Coarna Neagra soil at 0-20 cm depth, Figure 1b).

In case of 20-40 cm depth, it was observed that the copper concentrations are lower for both types of soils and all target moments (i.e., M_0, M_1 and M_2).

Figure 1c shows the opposite trend for the leachate waters from both soils. The mobile form of Cu increases with the exposure time to pesticides, suggesting the copper accumulation in leachate water of soils.



Cu-M_0 – mobile copper content of the beginning of the experiment (after soil sampling)
Cu-M_1 – mobile copper content at moment 5 days after phytosanitary treatment
Cu-M_2 – mobile copper content at moment 21 days after phytosanitary treatment

Fig. 1. The profiles of mobile form of copper for soil samples collected at 0-20 cm and 20-40 cm: a) AfuzAli and b) Coarna Neagra before and after phytosanitary treatment with solution of Curzate Manox /Dithane M-45 (ratio = 50/50); c) Leachate AfuzAli and Coarna Neagra after treatment. The averaged results of three tests are presented

Considering that the vineyard soils have been treated for many years with copper-based compounds, the obtained levels of copper concentrations are high. According to Lacatusu [39, 41], levels of copper (mobile form) more than 1.5 mg/kg are attributed to high concentrations. Similar results were obtained by Calin et

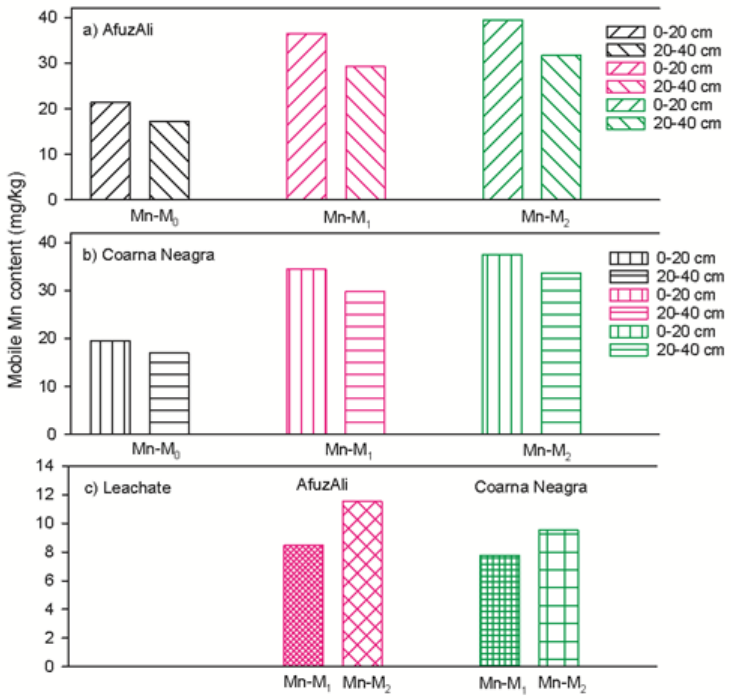


Fig. 2. The profiles of mobile form of zinc for soil samples collected at 0-20 cm and 20-40 cm: a) AfuzAli and b) Coarna Neagra before and after phytosanitary treatment with solution of Curzate Manox /Dithane M-45 (ratio = 50/50); c) Leachate AfuzAli and Coarna Neagra after treatment. The averaged results of three tests are presented

Mn-M₀ – mobile manganese content of the beginning of the experiment (after soil sampling)

Mn-M₁ – mobile manganese content 5 days after phytosanitary treatment

Mn-M₂ – mobile manganese content 21 days after phytosanitary treatment

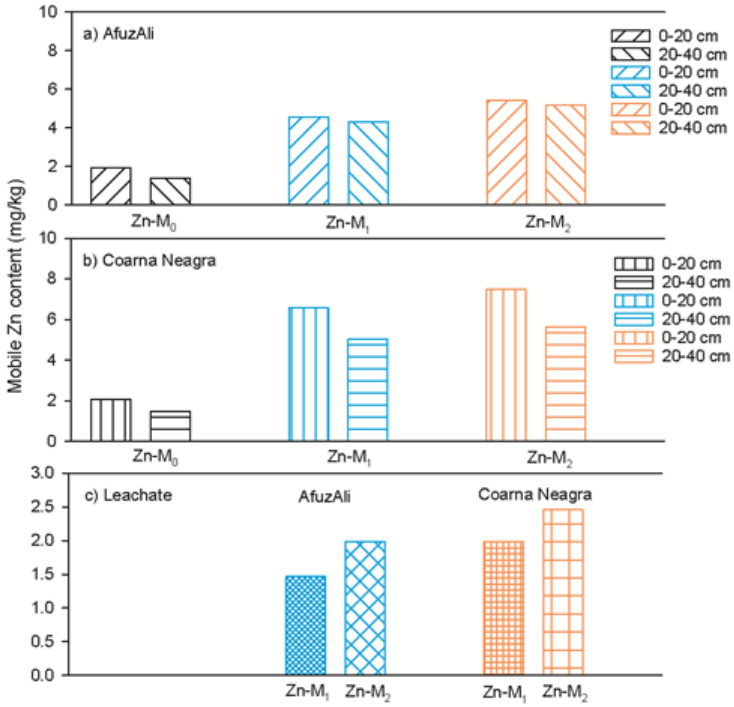


Fig. 3. The profiles of mobile form of zinc for soil samples collected at 0-20 cm and 20-40 cm: a) AfuzAli and b) Coarna Neagra before and after phytosanitary treatment with solution of Curzate Manox /Dithane M-45 (ratio = 50/50); c) Leachate AfuzAli and Coarna Neagra after treatment. The averages results of three tests are presented

Zn-M₀ – mobile zinc content of the beginning of the experiment (after soil sampling)

Zn-M₁ – mobile zinc content 5 days after phytosanitary treatment

Zn-M₂ – mobile zinc content 21 days after phytosanitary treatment

al. [9]. Copper is mainly accumulates in the upper layer following fungicidal sprays application [42]. This behavior was also observed in our study.

Since Zn and Mn are essential elements for grapevine growth, the behavior of Mn and Zn for studied samples is presented in this work. Figures 2 and 3 show the levels of Mn and Zn in vineyard soils. Mn concentrations range between 16.9-39.5 mg/kg (for soils) and 7.8-11.5 mg/kg (for leachate waters), whereas the content of Zn is significantly lower than Mn, ranging between 1.4-7.5 mg/kg for soil samples.

At the beginning of the experiment (Mn-M_0 and Zn-M_0), manganese and zinc levels are classified as medium, according to Lacatusu [40, 42]. After the treatment, at the periods of Mn- and Zn- M_1 and Mn- and Zn- M_2, manganese and zinc levels increased in the soil samples. This can be explained through the Mancozeb decomposition, considering that Mancozeb decomposes in the pH range 5-9 and it has low persistence in soil, with half-life of 1-7 days [43].

The impact of *p*H on the vineyard soils started to be recently investigated in wineyard research area. *p*H is a

No.	Sample	pH at M_0	pH at M_1	pH at M_2
1	Afuzali 0 - 20 cm	6.15	5.87	6.27
2	Afuzali 20 - 40 cm	6.24	6.09	6.32
3	Leachate Afuzali	-	6.18	5.97
4	Coarna Neagra 0 - 20 cm	6.31	6.07	6.19
5	Coarna Neagra 20 - 40 cm	6.19	5.96	6.23
6	Leachate Coarna Neagra	-	6.03	6.16

Table 1
*p*H VALUES (AS AVERAGE) OF SOIL SAMPLES BEFORE AND AFTER PHYTOSANITARY TREATMENT WITH SOLUTION OF CURZATE MANOX /DITHANE M-45 (RATIO = 50/50)

M_0 – the beginning of the experiment (after soil sampling)

M_1 – 5 days after phytosanitary treatment

M_2 –21 days after phytosanitary treatment

crucial parameter and it is related with the mobility of the microelements. The profiles of *p*H for analyzed soils samples collected at different depths are depicted in Table 1. Overall, the results show that the soils are moderately acidic with a tendency towards neutral. There is observed a slightly variations in *p*H with the nature of the soil, which will not strongly affect the microbial communities.

Conclusions

The present work aims with the evaluation of microelement contents (e.g. copper, manganese and zinc) from vineyard soils before and after phytosanitary treatment with Curzate Manox and Dithane M-45 compounds. Different types of vineyard soils were collected at depths of 0-20 cm and 20-40 cm. Flame Atomic Absorption Spectroscopy (FAAS) method was used for measurements of the micronutrients, whereas potentiometric method was applied for *p*H measurements. The soil samples were analyzed at the period of 5 and 21 days after treatment application.

Since copper is mainly accumulates in the upper layer, increased copper levels were found. The copper levels in non-treated soils samples were ranging range from 3.6 to 5.4 mg/kg, while the maximum copper concentration of treated soils founded in our work was 6.8 mg/kg (for Coarna Neagra 0-20 cm).

The soil samples exhibits different behavior in terms of manganese and zinc contents. Manganese and zinc levels are classified as medium in the beginning of the experiment (time M_0), whereas these levels increased in the soil samples (at moments M_1- 5 days and M_2- 21 days after treatment). It is known that Mancozeb decomposes in the pH range of 5-9 and it remains short time into the soil and the resulting behavior in our study can be due to the Mancozeb decomposition.

Higher Mn concentration values were found in our soils before and after treatment application compared other metals (e.g. Cu and Zn) and the averaged Mn level were roughly 5 times more than Cu level.

The content of Zn in the analyzed soil samples was considerably lower, ranging between 1.4-7.5 mg/kg.

The study of leachate waters of soils was performed in the present work and the content of Cu, Mn and Zn increase from M_1 to M_2 for both AfuzAli and Coarna Neagra. After 21 days from treatment application (M_2), Zn exhibits the lowest level in the leachate waters (e.g. 1.9 mg/kg), whereas the highest concentration is observed for Mn (e.g. 11.52 mg/kg) for AfuzAli.

Regarding *p*H measurements, the analyzed soils are moderately acidic towards neutral and a slightly variations in *p*H with the nature of the soil is observed.

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Manuscript received:19.12.2016