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EVALUATION OF THE DISTRIBUTION OF THE ACCESSIBLE COPPER FRACTION IN THE PLUM PLANTATION

Mariana RUSU[1], Irina Gabriela CARA[1], Manuela FILIP[1], D. ȚOPA[1],G. JITĂREANU[1]

[1] ”Ion Ionescu de la Brad” University of Life Sciences Iasi
Corresponding author: mrusu@uaiasi.ro

Abstract: *Contamination of soils with heavy metals represented and represents one of the most important problems for the natural function of ecosystems, but also for human health. Widespread use of Cu-based fungicides has led to increased Cu concentration in soils. The present work reveal a topical topic regarding food safety and consists in evaluating the accessible fraction of Cu that can persist in the soil and later can be translocated in fruits. In order to obtain sustainable and efficient productions, against the backdrop of severe climate changes and the negative effects on the orchard system, the environment and human society, the most suitable protection technologies were optimized within the Adamachi farm - Iasi University of Life Sciences (IULS). In order to maintain the diversity of fruit tree species and ensure their longevity in terms of productivity, as well as the proper maintenance of the plum orchard, in order to reduce the contamination of the fruit tree ecosystem with polluting substances, antifungal treatments based on Cu20% were carried out in the recommended doses, followed by a study of field to determine the total Cu concentration and its transfer from the soil to the plum leaves, from two cultivars, Record and Centenary. Soil samples were analyzed pre-harvest and taken at two depths (0-20 cm and 20-40 cm), analyzing the concentration of total Cu in aqua regia and accessible Cu in a solution of 1n ammonium acetate and EDTA 0.01n using atomic absorption spectrometry – AAS, in the flame atomization variant (ContrAA 700, Analytik Jena). According to the analyses, the total Cu content shows high values (114.22 mg Kg^{-1}), exceeding the maximum admissible limit (≤ 100 mg Kg^{-1}), but not the limit of intervention thresholds. The supply status of the soil in accessible Cu shows high values (> 3 mg Kg^{-1}) with the possibility of translocation in the plant and leaching on the soil profile. The higher concentrations of heavy metal were found at the soil surface, in 0 – 20 cm soil horizon, but below the maximum safety limits based on World Health Organization (WHO) guidelines. Therefore, to prevent soil pollution with heavy metals, it is recommended to use them as rationally as possible and apply them at the most suitable times.*

Key words: plum orchard, soil, copper, concentration, heavy metals

INTRODUCTION

Heavy metal toxicity has become an important component of contemporary pathology (Burducea et al, 2022).

Vital processes of accumulation and transformation of substances take place in the soil, and at the contact of the roots with the soil solution and its solid part, processes of absorption and exchange of substances take place, processes that constitute the basis of mineral nutrition of plants (Jităreanu et al, 2021).

Copper is widely used and is the most common fungicide used in the protection of fruit trees for a very long time.

Bordeaux mixture contains various hazardous metals other than Cu and therefore its application may also result in increased metals in the soil of fruit orchards (Mirlean et al., 2005) reported that a significant accumulation of Zn in addition to Cu was found in orchard soils with long-term application of Bordeaux mixture. To assess the environmental risk of Cu contamination of orchard soils, it is essential to understand the key factors and processes that determine the mobility and bioavailability of Cu in soil.

The mobility and bioavailability of soil elements depend primarily on their chemical speciation. Various soil extraction methods have been developed and used to fractionate Cu and deduce their possible chemical species in fruit orchards. Consequently, it has been suggested that humus (Topa et al, 2021) and Fe oxyhydroxides are the major soil constituents for Cu retention in orchard soils.

Contamination of orchards with heavy metals can pose serious threats to public health due to their accumulation in human food chains as a result of uptake by fruit trees from contaminated soils. Anthropogenic causes of heavy metal contamination in orchard soils include irrigation of contaminated water and intensive application of insecticides and pesticides (Cara et al, 2021).

Furthermore, the metals found in the soil are absorbed by the plants along the phenological stages to later end up in the fruit (soil-plant-human transfer or soil-plant-animal-human transfer).

Consequently, it is vital to regularly monitor the amount of heavy metals in soil. Secondly, the pollution level should be assessed and efficient solutions should be developed in the affected areas.

When plants absorb the metals it is recommended to keep the quantities under strict control in order to prevent cell damage which is potentially caused by heavy metals presence. Once the metals cross plasma membrane, metal ions are tied to either chelates or to other agents. Plants can generally resist heavy metal uptake until a critical toxicity level is reached. Subsequently, it leads to an inhibition in root growth, smaller surfaces of the leaves, delayed overall growth. It is self-evident that the symptoms appear in function of the heavy metal concentration, exposure time to elevated doses and ultimately the species' own tolerance to metals.

Furthemore, accumulation of heavy metals in humans is a real twenty-first century concern and it might be traced through the ingestion of contaminated food.

The present work reveal a topical topic regarding food safety and consists in evaluating the accessible fraction of Cu that can persist in the soil and later can be translocated in plum fruits.

In order to obtain sustainable and efficient productions, against the backdrop of severe climate changes and the negative effects on the orchard system, the environment and human society, the most suitable protection technologies were optimized within the Adamachi farm - Iasi University of Life Sciences (IULS).

In order to maintain the diversity of fruit tree species and ensure their longevity in terms of productivity, as well as the proper maintenance of the plum orchard, in order to reduce the contamination of the fruit tree ecosystem with polluting substances, antifungal treatments based on Cu20% were carried out in the recommended doses, followed by a study of field to determine the total Cu concentration and its transfer from the soil to the plum leaves, from two cultivars, Record and Centenary.

MATERIAL AND METHOD

The scientific research took place in the V.Adamachi horticultural farm of the Didactic Station belonging to the University of Life Sciences in Iasi. The farm is part of the cadastral territory of the municipality of Iasi and is bounded by the following neighbors:

- to the NE: Iasi Municipality
- to SE: IULS
-to the S: Iasi Botanical Garden
- at SW: SC. Vinicom Iasi
-to N: Agricultural School Group V. Adamachi Iasi

The studied area is part of the Hilly Plain of Jijia, the geographical subunit of the Hilly Plain of Jijia-Bahlui. This vast sculptural geomorphological subunit of the Moldavian Plateau, with a predominance in the geological substratum of large rock drillings, little resistant to the action of erosive factors, is characterized by a relief of hills, hills and low plateaus. These heights are framed by wide valleys, with energy up to 100-150 m, thus the researched area is located at altitudes of 100-150 m.

Figure 1 – Location of the research area

The study took place on the above mentioned horticultural farm, in a plum orchard (*Prunus domestica* L.) on a chambic chernozem type soil, with loamy-clay texture, and medium to good fertility. The soil texture was determined by Bouyoucos' methodology (Sandoval et al. 2011).

The plum orchard was established in autumn 2014. The plot concerned is found on the upper creek Podgoriilor (on the left). The area is characterized by a strongly anthropological landscape. The altitude of the studied area varies between 80 and 95 meters. The plant has values ranging from 7 to 10% and it has a mainly western exposure.

The climate of the farm is temperate continental, the average annual temperature is about 10° C and the average annual precipitation is 518 millimeters in 2021 (according to the weather station from Iaşi Institute for Research in Agriculture and Environment). The growing conditions for *Prunus domestica* L. in 2022 were: monthly average temperature (°C), solar irradiance (W/m²), vapor pressure deficit (kPa) *(figure 2*), relative humidity (%), precipitation (mm), humidity at leaf surface (min) and Delta T (°C) (*figure 3*).

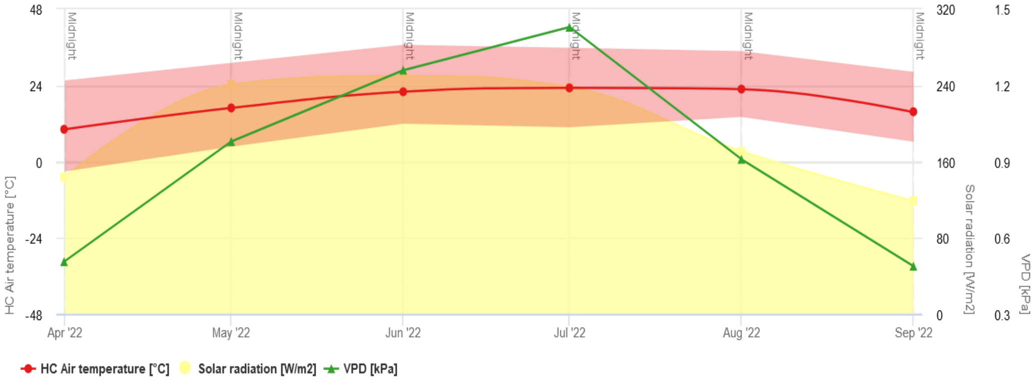

Figure 2 – Dates of the growth period (months)

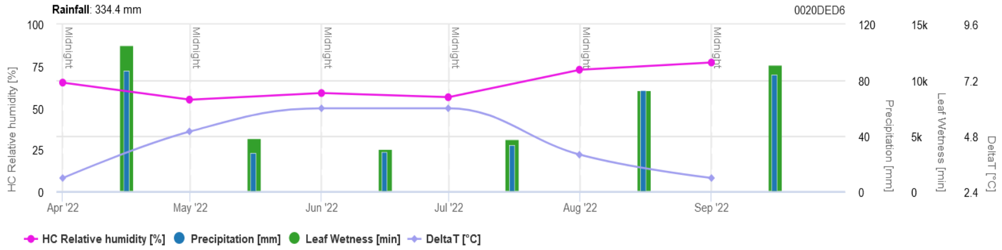

Figure 3 – Dates of the growth period (months)

PLANT AND SOIL SAMPLING, PREPARATION AND LABORATORY STUDIES

Plant Samples

The vegetation period when plum leaves were collected was between July to early August (pre-harvest). It was conducted by taking a random sample from 10 different trees. The leaves were healthy and mature collected from among the annual sprouts of each variety covered in the research (Record and Centenary).

Laboratory internship. The splint samples were incontinently delivered to the laboratory, and they were irrigated by short absorption in distilled water. Washing was performed in order to remove the substances performing from treatment against conditions, pests and to avoid any clinging dust and soil patches.

Latterly, according to Tariq FS et al., 2016 and Samsuri AW et al., 2019, the samples were dried at room temperature for 48 hours and placed in the roaster for 72 hours until full drying. Dry leaves were diced using a factory shop. 1 ±0.0001 g from each sample was counted. It was digested with 2 mL of HNO_3 65% at 95 ° C for 30 twinkles on a hot plate, also another mL of HNO_3 65 was added to each sample and they were hotted for 2 hours to a temperature of 95 ° C ± 5 °C (Tariq FS, 2021). The excerpt was cooled and latterly filtered through quantitative sludge paper and brought into 50 mL holders with distilled water.

Soil Samples

Fieldwork. Soil samples matched the leaf harvesting area. Sampling was done at intervals of two depths: 0 – 20 cm; 20 – 40 cm, with agrochemical probes. Each partial sample was introduced into a box, therefore the average sample was constituted. Average agrochemical samples packed in wooden boxes were sent to the laboratory.

Laboratory internship. CU TOTAL in royal water. Before disaggregation with aqua regia, the soil was prepared according to ISO 11464. The dry sample, mortared to sizes <150 µm, weighed (3 ±0.0001 g soil) into Teflon microwave vials (Speedwave) and extracted with a mixture of HCl 37% (d=1.18 g/cm^3) and HNO_3 65% (d= 1.42 g/cm^3) by maintaining for 16 h at room temperature, followed by boiling under reflux for 2 h. The extract to be clarified and brought to volume with nitric acid.

Steps for determination the Cu content accessible from the soil, they found in : preparation of the samples , they were dried, mortared and weighed 5 ±0.0001 g soil in a plastic bottle and added 50 cm ^{3} extraction solution (CH_3 $COONH_4$ 1n and EDTA-H_2O 01n, pH=7) .

Other chemical soil properties were analyzed: pH, phosphorus and potassium content, microelements (Zn, Cu Fe, Mn), organic carbon, organic matter content were determined following Sadzawka et al. (2006). The total and soluble soil Cu were determined according to Zagal and Sadzawka (2007) and Stuckey et al. (2008), respectively, and the data presented in Table 2.

Measurement of concentrations in soil/leaf samples

Prior to the preparation of soil and leaf samples, all glassware was carefully cleaned by immersion in warm 10% HNO_3 for a minimum of 6 h after which they were rinsed with ultrapure water and stored under appropriate conditions to avoid any contamination.

In each series of analyses, control extractions were performed using the same procedure, with the same reagents, but without the soil/leaf sample.

The concentrations of the analyzed samples were quantified with atomic absorption spectrometry (AAS ContrAA 700, Analytik Jena) with a flame atomizer system (table 1 and figure 4).

Table 1

Optimal instrumental parameters for Cu determination by ContrAA 700

Parameters	**Setting**
Relative sensitivity	100%
C_2H_2 flow rate	250 L h^{-1}
N_2O flow rate	340 L h^{-1}
Burner height	6 mm
Pixels for quantification	5 (CP±1)
Injection time	5.0 s
Measurement time	3.0 s
Delay time	12.0 s
Wash time	20.0 s

Figure 4 - Atomic absorption spectrometry with a flame atomizer system

Data Analysis

Statistical data was reused using the IBM SPSS v20 software (IBM Corp, Armonk, NY, USA) and results were presented as means/- standard errors.

RESULTS AND DISCUSSIONS

The data referring to the influence of the samples depth on copper concentrations is shown in the Table 2.

The goal was to maintain the diversity of fruit tree species and ensure their longevity in terms of productivity, as well as the proper maintenance of the plum orchard, in order to reduce the contamination of the fruit tree ecosystem with polluting substances, antifungal treatments based on Cu were carried out in the recommended doses.

Table 2

Selective chemical soil properties

VARIETIES	Depth (cm)	pH	P (mg/kg)	K (mg/kg)	C_{org} (%)	Humus (%)	Zn (mg/kg)	$B_{soluble}$ (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
RECORD	0-20	7.2	96.7	409.5	3.04	5.24	1.87	0.38	21.78	26.15
	20-40	6.8	104.3	432.2	1.93	3.32	2.54	0.42	20.56	32.4
CENTENAR	0-20	7.1	78.3	414.3	2.57	4.43	2.38	0.32	22.63	28.3
	20-40	7.0	70.1	397.7	1.64	2.83	1.65	0.44	24.80	30.4

From a statistical perspective, significant results were recorded in all experimental variants (table 3).

Table 3

Distribution of copper in soil/ leaves

VARIETIES	Depth (cm)	Cu total (%)	Cu accesible (mg/kg)	Cu soluble (mg/kg)	Leaves (%)
RECORD	0-20 cm	114.22 ±9.5 ns	12.66±0.62 ns	0.53±0.01 a	8,13±0.08
	20-40 cm	103.18±9.08 ns	10.14±1.18 ns	0.37±0 c	
CENTENAR	0-20 cm	102.11±7.99 ns	11.04±1.34 ns	0.36±0 c	6,87±0.11
	20-40 cm	108.65±14.72 ns	12.13±0.69 ns	0,42±0 b	

*Values associated with different letters are significantly different according to Tukey's test at $p < 0.05$; ns - not significant; *- $p < 0.05$; nd - not detected; ** - WHO/FAO, 2021*

Subsequently, the use of copper-based fungicides increased the total concentration of copper in the soil exceeding the maximum permissible limit (≤100 mg Kg-1), but not the limit of intervention thresholds (according to WHO/FAO regulations as for 2021).

Varying amounts of copper are found in soil. In the case of the Record variety, in the superficial horizon (0-20 cm) it is richer in copper (114.22 mg/kg) than in the 0-20 cm depth, due to bioaccumulation processes. Most of the surface layer copper is in the form of ions adsorbed to clay or organic matter particles, in the form of insoluble salts, as soluble salts and as minerals. In the soil solution, the concentration of Cu ions is very low, 98% of it being complexed with organic substances.

They regulate the mobility and accessibility of copper for plants. The availability of copper for plants is conditioned by factors such as: organic matter; Soil pH and the presence of other metal ions in the soil solution. The supply status of the soil in accessible Cu shows high values (≥ 3 mg ppm) with the possibility of translocation in the plant and leaching on the soil profile. Although they present high values, they do not exceed the alert lines, so as to represent toxicity.The retention of Cu in the soil increases proportionally with the content of organic matter, and the reaction of the soil influences the behavior of copper.The absorption and translocation of copper in plants is related to the pH of the soil (much more active in an acidic environment). Its concentration decreases as the pH increases.Regarding the influence of plant variety on copper concentrations in plum leaves, they do not exceed the permissible limits for copper in plants (WHO, 2021).

CONCLUSIONS

The results revealed levels of total concentrations above the maximum allowed limit (≤100 mg Kg-1), but below the limit of intervention thresholds, regardless of the depth from which the sample was taken, highlighting a prudent management of the studied area. The supply status of the soil in accessible Cu shows high values (> 3 mg Kg-1), with the possibility of translocation in the plant and leaching on the soil profile.

Therefore, to prevent soil pollution with heavy metals, their rational use and application at the most appropriate times is recommended.

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