



Study of the Impact of Copper on Soil Organisms and Tomato Quality

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ABSTRACT

Background: The aim of our work is to assess the impact of copper-contaminated soil on tomato fruit and soil organisms. Excessive use of copper-based pesticides has many consequences for soil biology and food quality.

Methods: Our study was carried out in a section of a tomato plot in the Mostaganem region of northern Algeria. The plot was contaminated with copper, at different doses D1 (10 mg Cu Kg⁻¹), D2 (100 mg Cu Kg⁻¹), D3 (200 mg Cu Kg⁻¹) and D4 (300 mg Cu Kg⁻¹) in separate blocks. The concentrations of copper and carbohydrates and the homogeneity of the wells were assessed in order to determine the quality of the tomatoes in relation to the levels of soil contamination. Furthermore, the respiratory activity of the microorganisms and the feeding activity of the soil fauna were assessed.

Result: The highest copper concentrations were 1.5±0.17 mg Cu Kg⁻¹ and 2.05±0.3 mg Cu Kg⁻¹ in the sites contaminated by the highest doses D3 and D4 and the highest carbohydrate concentrations were 53.03±1.38 g kg⁻¹ and 61.15±0.65 g kg⁻¹ in these two sites. Microbial respiration values were lowest in D3, with 8.3.10⁻⁴ ± 3.6.10⁻⁴ mg CO₂ g⁻¹ h⁻¹ and in D4, with 1.5.10⁻³ ± 1.4.10⁻⁴ mg CO₂ g⁻¹ h⁻¹. In addition, wildlife feeding activity was low at both sites, with values of 27.56±17.77 % for the D3 site and 37.5±17.48 % for the D4 site. Our study has shown that copper contamination of soils affects the biological activities of soil organisms.

Key words: Bait lamina, Copper, Microbial respiration, Pesticide, Tomato.

INTRODUCTION

Tomato production often faces challenges related to pests and diseases, necessitating the use of pesticides to protect crops (Nechadi *et al.*, 2002; Nishant and Upadhyay, 2016; Son *et al.*, 2018). Among these pesticides copper is widely used due to its antimicrobial and fungicidal properties (Harkat *et al.*, 2021).

Copper has been used extensively as a fungicide for over a century (Karimi *et al.*, 2021). However, prolonged exposure to copper can lead to an accumulation of this metal in the fruit, posing toxicity risks for consumers, as plants are capable of absorbing copper and accumulating it in their tissues (Sbartai *et al.*, 2012). Furthermore, excessive accumulation of copper in the soil can alter its biological quality, as copper accumulates in the first layers of the soil (10 cm), which are home to a wide range of organisms (earthworms, fungi, bacteria, *etc.*) (Crowther *et al.*, 2019; Jiang *et al.*, 2007). Although copper can be effective in protecting tomatoes, it is essential to understand its effect on the soil and the fruit in order to minimise undesirable impacts on the environment and human health. The study of the impact of pollutants on the biological quality of the environment must provide information on the state of organisms living in the soil, hence the need to use bioindicators (Belhaouari *et al.*, 2014; Kadri *et al.*, 2023; Traiche *et al.*, 2018). Furthermore, the study of the impact of pollutants on foodstuffs must provide information on both their nutritional quality and the health risks of bioaccumulation (Faure and Reymermier, 2021; Mohanapriya *et al.*, 2024). Our work has two objectives: (i) to assess tomato quality by evaluating copper bioaccumulation, carbohydrate concentration and tomato homogeneity after

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the application of copper in pesticide form in an experimental field located in the region of Mostaganem in Algeria; (ii) to assess the impact of copper on soil biology using two bioindicators, the feeding activity of soil-dwelling organisms using the Bait-lamina test and the respiration of soil microorganisms via their production of CO₂.

MATERIALS AND METHODS

Study site

The study was carried out in a section of a tomato plot in the Mostaganem region (Algeria). The soil section of the tomato plot studied was contaminated with copper, with different doses applied in three separate blocks (Fig 1). The randomization of replication was conducted in 3 blocks. Each block was subjected to 4 doses of copper and a control

(Control T: 0 mg Cu Kg⁻¹, D1: 10 mg Cu Kg⁻¹, D2: 100 mg Cu Kg⁻¹, D3: 200 mg Cu Kg⁻¹ and D4: 300 mg Cu Kg⁻¹) (Kadri *et al.*, 2023). The soil used was characterized (Table 1). Our study consisted of two complementary parts: analysis of tomato quality and soil biology.

Tomato quality was studied using three parameters: phenotypic criteria, carbohydrate analysis in tomato fruit and copper analysis in tomato fruit. Soil quality was studied using two bioindicators, the activity of microbial respiration and the feeding activity of the soil mesofauna.

The laboratory analyses were carried out in the Biotechnology Laboratory Applied to Agriculture and Environmental Preservation, Higher School of Agronomy in Mostaganem, during the period 2023 and 2024.

Tomato quality

Phenotypic criteria

According to the International Standards for Fruits and Vegetables guidelines (OECD, 2019), an assessment of the homogeneity of tomatoes was conducted for samples harvested from different soils with varying degrees of contamination. This evaluation is based on standardized criteria that ensure the fruits are comparable in terms of physical characteristics and quality, thereby allowing for the analysis of the impact of contamination on their development.

Total sugars analysis of tomato fruit

The total sugars in tomato fruit were analyzed using the protocol described by (Nielsen, 2017). The operation began with the extraction of carbohydrates. The colour complex was then formed and after the calibration curve had been prepared, the absorbance of the samples was read using a spectrophotometer at a wavelength of 490 nm.

Analysis of copper in tomato fruit

In order to determine the level of copper bioaccumulation in tomato fruit, we mineralised the fruit using the method of (Jones and Benton, 2017). A test sample of 1 g of plant material was dried at 70°C for 48 h, crushed and calcined in a muffle furnace at 450°C for 4 hours. The ash was mineralised with aqua regia (25% HNO₃ and 75% HCl) in a hot plate at 90°C for 75 minutes. The copper content of the fruit was then determined by atomic absorption spectrometry (240FS AA, SpectreAA).

Biological quality of the soil

Respiratory activity of microorganisms

Microbial respiration of the soil was measured using the method (ISO 16072:2002). 20 grams of soil was incubated in a centrifuge tube suspended in a flask into which 20 ml of sodium hydroxide solution had been pipetted. The flask was sealed and incubated for 24 h in a temperature-controlled room (22 ± 1°C). The CO₂ produced was absorbed into the sodium hydroxide solution. After titration of the solution not consumed, the quantity of CO₂ produced by the microorganisms was calculated.

Feeding activity of fauna

The feeding activity of soil fauna was measured using the Bait lamina method (ISO 18311:2016). PVC bars measuring 12 cm with 16 holes each spaced 5 mm apart were used. The cavities were filled with a paste specially designed to assess biological activity (Cellulose, Agar-Agar, dry plant debris). A total of 216 Bait lamina were placed in the site (12 Bait lamina × 3 replicates × 3 blocks). The interpretation of the results is based on the total average consumption of

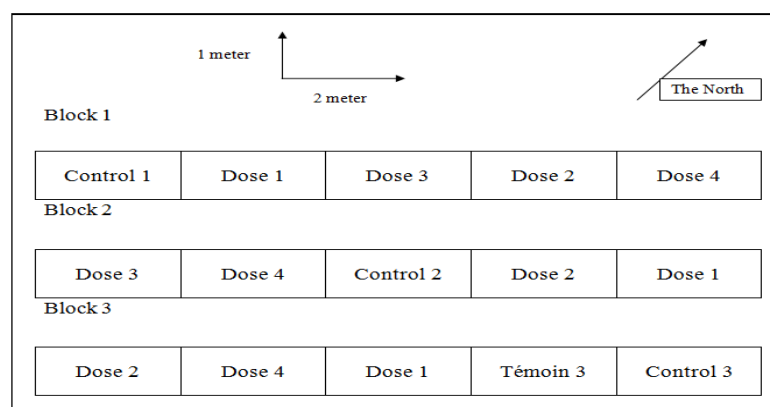


Fig 1: Field experimental plan.

Table 1: Physico-chemical characteristics of the soil.

Soil caractéristiques	pH	redox potential (mV)	Total carbon (%)	Water retention (%)	Sand (%)	Clay (%)	Silt (%) capacity (meq 100g ⁻¹)	Cation exchange (%)	total limestone	Copper (mg Cu Kg ⁻¹)
	8.33±0.12	-79±7.0	18.60±0.32	23.23±2.45	94.4±1.5	2.8±0.7	2.8±0.9	14.89±3.34	59±2	13.18±1.69

the paste in the bait lamina strips in the sites studied after one month's exposure.

Statistical analysis

As part of this study, we carried out in-depth statistical analyses to assess the relationship between the different variables. These analyses were carried out using Origin Pro 2019b software, a scientific data analysis tool renowned for its accuracy and versatility. The software's advanced features enabled the use of Duncan's test for detailed comparisons among groups, while facilitating the visualization and interpretation of complex data essential to this research (OriginLab Corporation, 2019).

RESULTS AND DISCUSSION

Tomato quality

Phenotypic criteria

After harvesting the tomato fruit from the three blocks on our site, the results of the assessment of the homogeneity of the tomato fruit were recorded. A homogeneity of colour was observed in fruits from both contaminated and in control soils. The homogeneity classes ranged from Extra to Class I (Table 2).

Tomato fruits from the control soil exhibit similar shapes and colors to those harvested from contaminated soils (D1, D2, D3 and D4).

Concentration of total sugars in tomato fruit

The fruits analysed showed significant variations in terms of total sugar content. The fruit with the highest carbohydrate concentration was at D4 (the most contaminated soil), with $61.15 \pm 0.65 \text{ g kg}^{-1}$. In contrast, the lowest concentration was $48.74 \pm 0.49 \text{ g kg}^{-1}$ in fruit from the control soil T.

In contaminated soils, an increase in the total sugars concentration of tomato fruits is observed in relation to the level of copper contamination (Fig 2).

Copper levels in tomato fruit

According to the histogram (Fig 3), the fruit analysed showed a gradient of copper contamination, with the highest copper concentration being $2.05 \pm 0.3 \text{ mg Cu Kg}^{-1}$ in the fruit from soil D4 (the most contaminated soil). The lowest copper concentration was $0.115 \pm 0.02 \text{ mg Cu Kg}^{-1}$ in fruit from the control soil T. Copper levels in tomato fruits increase in parallel with the increasing degree of soil contamination with copper.

Table 2: Tomato fruit homogeneity table (OECD, 2019).

Class	Homogeneity of the fruit
Extra	The 5% tolerance covers all defects in shape and colour
Class I	The 10% tolerance covers all defects in shape and colour
Class II	The 10% tolerance covers all malformations and serious skin and colour defects.

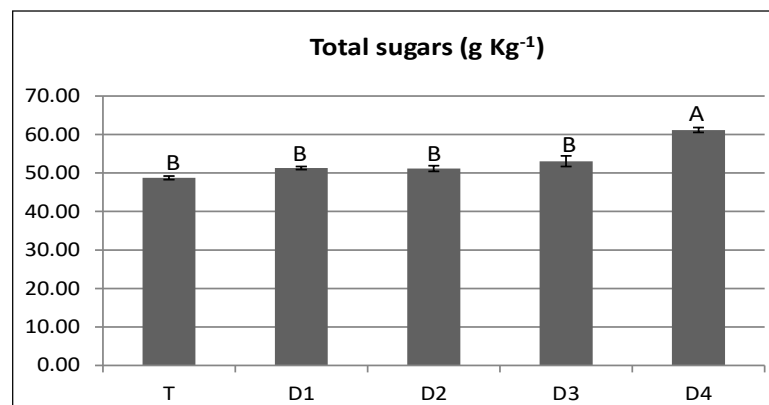


Fig 2: Concentrations of total sugars in tomato fruit. For each site, different letters indicate significant differences (Duncan's test, $p < 0.05$).

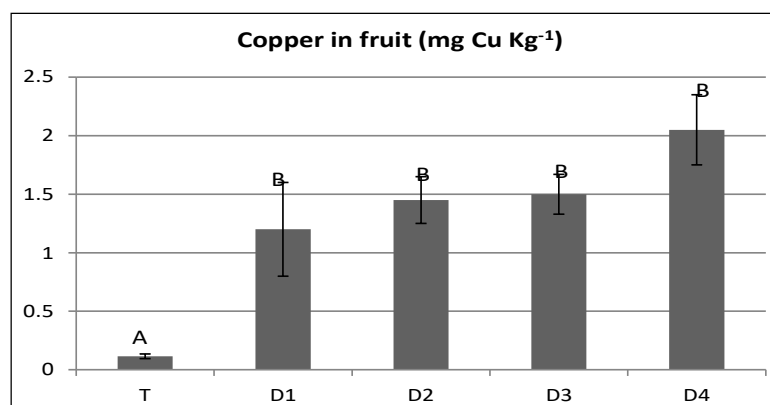


Fig 3: Copper dose in tomato fruit. For each site, different letters indicate significant differences (Duncan's test, $p < 0.05$).

Biological quality of soil

Microbial respiration

Our results show that the value of microbial respiration in the contaminated soils is lower than in the control soil T, with the difference becoming significant at soils D3 and D4 (Fig 4). Microbial respiration reached its highest level in the control soil T, which was not treated with copper, with a measured rate of $5.1 \cdot 10^{-3} \pm 3.6 \cdot 10^{-3} \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$. The lowest respiration $8.31 \cdot 10^{-4} \pm 3.6 \cdot 10^{-4} \text{ mg CO}_2 \text{ g}^{-1} \text{ h}^{-1}$, is recorded in the most highly polluted soil D4.

Feeding activity of fauna

Our results show that the feeding activity of fauna is lower in contaminated soils compared to the control soil, with the difference becoming significant at sites D2, D3 and D4 (Fig 5). The highest feeding activity, at $64 \pm 15.88\%$, was observed in the control soil T, a minimally contaminated soil left untreated with copper. The biological activity of microorganisms decreases with increasing copper levels in the soil.

Impact of copper on tomatoes

The results of our research show that tomato fruit from plants grown in contaminated soils have phenotypic characteristics similar to those of fruit from control soils (Table 3). Other authors have shown that copper treatments have no effect on fruit colour, fruit shape and stalk tip colour (Namdev *et al.*, 2017). Copper is an essential element for tomatoes, playing an important biological role in protein production and is involved in plant respiration (in the krebs cycle). It is also linked to chlorophyll performance (Stevens *et al.*, 2018). Tomato tolerance to copper can be attributed to its metal complexing ability. The intracellular presence of organic ligands within tomatoes ensures complexation and therefore detoxification of many metal ions (Tremel-Schaub and Feix, 2005). We observed an increase in the concentration of total sugars in the fruit as the copper concentration increased. This positive correlation suggests that copper-induced stress influences carbohydrate metabolism in the plant. Previous studies, such as López-Vargas *et al.* (2018), have shown that the application of Cu

Table 3: Results of phenotypic criteria for tomato fruits.

Repetition	T	D1	D2	D3	D4
Homogeneity of tomato fruit	Extra	Extra	Class I	Extra	Class I

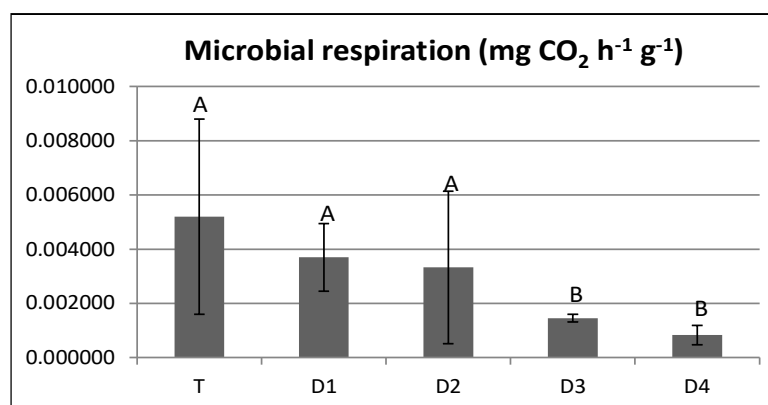


Fig 4: Microbial respiration at the site. For each site, different letters indicate significant differences (Duncan's test, $p < 0.05$).

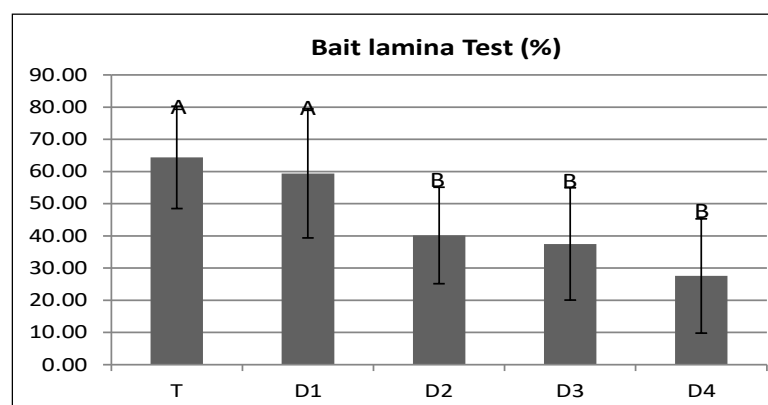


Fig 5: Feeding activity of soil fauna (Bait Lamina). For each site, different letters indicate significant differences (Duncan's test, $p < 0.05$).

nanoparticles leads to the transformation of organic acids into simple sugars in tomatoes. In addition, excess copper affects enzymes involved in carbohydrate metabolic cycles (Singh *et al.*, 2007), leading to carbohydrate accumulation in fruit (Benguennouna *et al.*, 2023). Oxidative stress caused by high doses of copper pesticide can activate certain metabolic pathways in plant cells. In response to this stress, plants increase the production of antioxidant compounds, including carbohydrates that act as antioxidant molecules, neutralising free radicals and thus contributing to protection against oxidation (Liao *et al.*, 2015; Mengome *et al.*, 2014; Kang *et al.*, 2015).

The concentration of copper in the fruit from the four soils differed significantly from the control soil. According to our results, copper concentrations in tomatoes do not exceed the acceptable daily intake (ADI) of 0.15 kg kg⁻¹ (Anses, 2018), equivalent to a daily intake of 9 mg Cu kg⁻¹ (0.15 × 60 kg average weight). They increase as soil contamination increases, with high copper concentrations in fruit reaching 2.05±0.3 mg Cu kg⁻¹ in the most contaminated soil (D4).

Tomatoes can accumulate copper in various organs, such as leaves and stems (Tremel-Schaub and Feix, 2005). They also accumulate metals in the roots (Mazhoudi *et al.*, 1997). In fact, metals are absorbed by the roots and transported to the various organs and fruits *via* the elaborated sap. Several factors can increase the uptake of metals by the roots, in particular those linked to the soil (natural concentration, pH, Eh, CEC), climate (temperature, humidity) and the plant (influence of the roots on the soil, influence of micro-organisms), can increase the uptake of metals by the roots (Tremel-Schaub and Feix, 2005).

Some authors have pointed out that there is no correlation between the concentration of total copper in the soil and the concentration of the metal in tomato fruit, stems or leaves (Badilla-Ohlbaum *et al.*, 2001).

Impact of copper on soil biology

Biological indicators are used to analyse the relationship between copper accumulation in the soil and the biological quality of the contaminated soil (Bispo *et al.*, 2009; Sheramati and Varma, 2015).

Our study showed a clear decrease in microbial respiration in soils heavily contaminated with copper, particularly in sections D3 and D4. In site D4, where the copper concentration applied to the soil reached 300 mg Cu Kg⁻¹, microbial respiration was 8 10⁻⁴± 3 10⁻⁴ mg CO₂ g⁻¹ h⁻¹, a reduction of 84.31% compared with the control soil, which showed respiration of 5.1 10⁻³±3.6 10⁻³ mg CO₂ g⁻¹ h⁻¹. Microbial respiration decreased by up to 58% for copper accumulations exceeding 300 mg Cu kg⁻¹ (Soler-Rovira *et al.*, 2013). Microbial respiration, which is sensitive to metal contamination, is considered to be an indicator of anthropogenic disturbance (Schloter *et al.*, 2003). High soil respiration may reflect good soil quality (Criquet, 2013).

Other researchers have indicated that soil micro-organisms, such as bacteria and fungi, may be sensitive

to high concentrations of copper, which may influence their respiration activity. In wine-growing soils, a 40% reduction in microbial respiration was observed for copper levels ranging from between 50 mg Cu kg⁻¹ and 100 mg Cu kg⁻¹ (Karimi *et al.*, 2021). Furthermore, heavy metal pollution leads to widespread “metallization” of the environment, negatively impacting soil (Vinogradov and Zubkova, 2022). Our study reveals a negative correlation between copper accumulation in the soil and microbial respiration. These results are in line with several studies showing the impact of metal pollution on soil microbial activity (Austruy *et al.*, 2016; Bahn *et al.*, 2016). Many researchers have established that certain metals, such as Cu, Al, Ag, Cd, Sn and Hg, are toxic to microorganisms (Desaunay, 2011).

With regard to the bioindicator Bait Lamina, we have observed a decrease in the feeding activity of soil organisms at several contaminated sites. Other authors have observed a decrease in the feeding activity of soil organisms in response to metal pollution (André *et al.*, 2009; Filzek *et al.*, 2004).

On sites polluted by metals, the abundance of detritus feeders is greatly reduced due to the high toxicity of the soil. Consequently, there is a large difference between controlled and polluted sites (Vorobeichik *et al.*, 2021).

Other studies have reported that copper has a negative impact on earthworms. Abundance is greatly reduced on the polluted site, but they have not completely disappeared (Vorobeichik *et al.*, 2021).

Our results indicate a negative correlation between the Bait lamina test and copper concentration in the soil, as also observed by Pesce *et al.* (2020). Several studies have demonstrated the negative impact of copper on protozoa (Ekelund *et al.*, 2003), enchytrata (Ruyters *et al.*, 2013) and springtails (Amorim *et al.*, 2005).

Collembola and enchytrata reproduction decreased by 50% after the application of 400 and 1895 kg Cu ha⁻¹ yr⁻¹, respectively, equivalent to 100 and 473.75 mg Cu kg⁻¹ yr⁻¹. Earthworm biomass was reduced by 15% following the application of 200 kg Cu ha⁻¹ yr⁻¹, equivalent to 50 mg Cu kg⁻¹ (Karimi *et al.*, 2021).

Although copper is an essential element for many soil organisms, at high concentrations it can become toxic, like many other chemical elements (Sheramati and Varma, 2015). This toxicity can lead to a reduction in the feeding activity of soil organisms.

CONCLUSION

The aim of our study was to assess the impact of copper-induced soil contamination on the quality of tomato fruit grown in contaminated soils and on soil organisms. The results of our work showed that the high use of copper in tomato cultivation does not have a negative effect on fruit quality. However, our results showed that excessive use of copper does have adverse effects on soil organisms. Our study contributes to a better understanding of the effects of copper-based pesticides on soil biology and tomato quality.

A healthy agrosystem must ensure both good quality crops and good soil biology. Our work paves the way for further research to determine the concentrations of copper that can be used in tomato cultivation without damaging soil biology.

Conflict of interest

All authors declare that they have no conflict of interest.

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