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Vermicompost Modulates Copper Tolerance and Accumulation in Chives (*Allium schoenoprasum* L.)

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Abstract Copper (Cu) contamination compromises soil quality and crop development and may pose risks to food safety at elevated concentrations. In this study, the effects of increasing Cu doses in soil (0–800 mg dm^{-3}) on the growth, root biomass, root morphology, and Cu accumulation in *Allium schoenoprasum* L. and the influence of vermicompost, were evaluated. After 30 days of soil incubation, available Cu concentration in the soil increased proportionally with the applied dose, reaching 163.8 mg dm^{-3} at a dose of 800 mg dm^{-3}. Vermicompost contained 43.2 mg Cu kg^{-1}, contributing to an increase of approximately 1.295 mg Cu dm^{-3} in the soil upon application. Compared with that in the control treatment, a high Cu dose (800 mg dm^{-3}) reduced the root dry biomass by 93.9% and decreased the root length and surface area, indicating phytotoxicity. Compared with a dose of 200 mg dm^{-3} but no amendment, vermicompost increased the root dry biomass by 143.1%. Across treatments, Cu accumulation decreased in the order of roots > bulbs > leaves. The maximum concentration reached 236.20 mg kg^{-1} in the roots (200 mg dm^{-3} + vermicompost), exceeded 70 mg kg^{-1} in the bulbs (800 mg dm^{-3}), and reached 9.90 mg kg^{-1} in the leaves (800 mg dm^{-3}). At a dose of 200 mg dm^{-3}, vermicompost increased the total plant Cu content by 62.8%, whereas no increase was observed at a dose of 800 mg dm^{-3}. These results demonstrate that the addition of vermicompost modulated Cu tolerance and tissue accumulation in *Allium schoenoprasum* L., with beneficial effects at a dose of 200 mg dm^{-3} but no mitigation of phytotoxicity at a dose of 800 mg dm^{-3}.

Keywords Amaryllidaceae · Root length · Phytotoxicity · Soil organic matter · Heavy metals

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1 Introduction

The degree of copper (Cu) accumulation in agricultural soils has increased in recent decades, thereby amplifying the risk of phytotoxicity and trace metal entry into the food chain (Gupta et al., 2019). Heavy metals are metallic elements with a relative density greater than 4 g cm^{-3} (Abubakar, 2024). Cu is considered a strategic metal within the contemporary industrial context, occupying the third position in terms of global economic importance behind only iron and aluminum (Rouhani et al., 2024). However, although Cu is an essential micronutrient for plants and humans, its excessive accumulation in soils can impair plant growth, disrupt the root morphology, and compromise crop productivity, and concentrations greater than 20 μg g^{-1} may cause toxic effects in humans (Abubakar, 2024).

Cu ore mining and processing generate significant environmental impacts, mainly because of the large volume of tailings resulting from extraction. As highlighted by Rouhani et al. (2024), to obtain a single ton of Cu, it is necessary to extract and crush more than 150 tons of ore, thus demonstrating the magnitude of environmental degradation associated with this activity. The continuous input of Cu into agricultural soils through mining residues, sewage sludge, fertilizers, and animal manure may lead to its accumulation above natural background levels, thereby increasing the risk of phytotoxicity and food chain transfer.

Allium schoenoprasum L. is an herbaceous, perennial species belonging to the Amaryllidaceae family (Algharib et al., 2021). The genus *Allium* comprises more than 700 identified species, several of which are widely consumed as vegetables, herbs, and spices (Čepulienė et al., 2024). Chives are cultivated globally and represent economically relevant culinary herbs that are particularly valued for their leaves, which are directly consumed fresh or minimally processed.

Owing to their direct consumption and short production cycle, culinary herbs are particularly susceptible to heavy metal accumulation, which may pose food safety concerns. Recent studies have emphasized that trace elements can accumulate in leafy vegetables and herbs to reach levels above permissible limits when cultivated in contaminated soils, representing a potential risk to consumers (Gupta et al., 2019; Qadr & Amin, 2025; Romero-Crespo et al., 2023). Because chives are frequently consumed fresh (albeit in small portions), even moderate Cu accumulation in edible tissues may contribute to dietary exposure. Therefore, understanding Cu partitioning among roots, bulbs, and leaves is essential not only from a physiological perspective but also from a food safety assessment perspective.

Plant species with potential applications in phytoremediation strategies have received increasing research interest. Although the tolerance of *Allium* species to abiotic stresses has been investigated, information on Cu toxicity responses in *Allium schoenoprasum* L. remains limited, particularly under combined organic amendment conditions. This species exhibits morphological and physiological hardiness characteristics, such as tolerance to low temperatures and adaptation to diverse soil conditions (Čepulienė et al., 2024), which suggests potential flexibility under metal stress.

Studies have indicated that the application of vermicompost derived from organic waste can improve the performance of plant species exposed to heavy metals by modifying metal bioavailability and stimulating root development (Aransiola et al., 2024; Nepal et al., 2024). Vermicomposting involving the use of earthworms (*Eisenia fetida*) is an efficient strategy for recycling organic residues and improving soil physicochemical and biological properties (Poornima et al., 2024; Yadav et al., 2023). In metal-contaminated soils, the added vermicompost may either immobilize or mobilize Cu depending on its composition, thereby influencing plant uptake and tolerance mechanisms.

To date, no studies have explicitly aimed to evaluate the combined effect of increasing Cu toxicity levels and vermicompost application on the growth, root morphology, and Cu accumulation in *Allium schoenoprasum* L., which represents a clear gap in the literature, particularly with respect to the dual relevance of chives as culinary herbs and as potential species that can tolerate metal stress.

We hypothesized that the addition of vermicompost alters soil Cu availability and thereby modulates plant tolerance and Cu accumulation in *Allium schoenoprasum* L. Within this context, this study aimed to evaluate the effects of increasing soil Cu doses on the growth, root morphology, and Cu accumulation in *Allium schoenoprasum* L. and to determine whether vermicompost application affects plant tolerance and Cu distribution in tissues.

2 Materials and Methods

2.1 Growth Conditions and Experimental Design

The experiment was conducted in a greenhouse at the Educational Production Unit (UEP)–Plant Crops, located at the IFSULDEMINAS Farm School, Inconfidentes Campus, in Inconfidentes, Minas Gerais (22°18′50.572″ S, 46°19′52.024″ W; at an altitude of 845 m). The local climate is subtropical, with dry winter and mild summer seasons and an average annual temperature of 18.0 °C, according to the Köppen classification (Inconfidentes 2024). During the experimental period, the average air temperature reached 21.7 °C, and the average relative humidity reached 80.6%. These data were obtained from the meteorological station closest to the experimental site (code V0527), located in Águas de Lindóia, São Paulo, belonging to the National Institute of Meteorology (INMET, 2025).

The experimental soil was a hapludox. Granulometric analysis revealed contents of 18.0% clay, 41.7% silt, and 40.3% sand, indicating that this soil can be classified as loam-textured soil. After sieving, planting fertilization was conducted equally across all the treatments, comprising 0.555 g dm^{-3} of P, 0.055 g dm^{-3} of S, 0.025 g dm^{-3} of N, 0.088 g dm^{-3} of Ca, 0.043 g dm^{-3} of K, and 0.004 g dm^{-3} of B. The applied fertilizers were single superphosphate, urea, and potassium chloride. For acidity correction, limestone with a total relative neutralizing power of 80% (38% Ca and 10% Mg) was employed at a concentration of 1.25 dm^{-3}, following the recommendations described in Aguiar et al. (2014) for this crop. The fertilizer material, limestone, and copper sulfate (applied as a Cu source in the treatments) were macerated and mixed with a portion of the soil for subsequent homogenization in each plot.

Twelve treatments were evaluated, encompassing six Cu concentrations (0, 50, 100, 200, 400, and 800 mg dm^{-3}), applied in the presence or absence of vermicompost. In the treatments with vermicompost, a dose equivalent to 60 t ha^{-1} of the material (dry basis) produced by earthworms (*Eisenia foetida*) from the decomposition of cured bovine manure (5 worm units per kg of bovine manure) was incorporated over a period of six months. The dose of dried vermicompost was equivalent to 3% (m/v) of the bulk soil. Thus, the treatments were denoted 0 + verm, 50 + verm, 100 + verm, 200 + verm, 400 + verm, and 800 + verm (with vermicompost) and 0, 50, 100, 200, 400, and 800 mg dm^{-3} (without vermicompost), respectively.

The prepared soil and soil–vermicompost mixture (after limestone, fertilizers, and copper were added) were transferred to 32-cell trays with a capacity of 250 mL each. The cells exhibited dimensions of 6 × 6 × 13 cm (width × length × height), with a conical shape. The soil remained in the tray for 30 days, and the field capacity was maintained at 70%. A total of 20 seeds of Totem chives (*Allium schoenoprasum* L.) (Sakata Sudamerica®) were subsequently sown per plot. A high plant density was employed to ensure that all the plots contained sufficient dry matter and root production to perform all the proposed analyses. The plants remained in the soil and received periodic irrigation (with respect to the field capacity) for 115 days after sowing. Foliar fertilization with a nitrogen source was conducted starting 30 days after sowing, with weekly applications until one week before the harvest. The nitrogen source was urea, at a concentration of 0.5 g L^{-1} N, with an application of approximately 20 mL per plot in each fertigation event.

The experiments were conducted in a completely randomized design (CRD) with 12 treatments and 16 replications, totaling 192 experimental units. Each experimental unit comprised a tray cell with approximately 250 mL of substrate (soil or soil + vermicompost) and 20 chive (*Allium schoenoprasum* L.) seedlings.

2.2 Chemical Analysis of Vermicompost and Soil

Before the chive seeds were sown, a compost sample was collected, and its levels of organic matter (OM), macro- (N, P, K, Mg, Ca, and S) and micronutrients (Cu, Fe, Zn, Mn, and B), pH, total organic carbon, and moisture were quantified. The samples were dried at 65 °C before extraction. Nutrients, except for N, were extracted using nitric-perchloric digestion and quantified through the use of colorimetry (P and B), atomic absorption spectrometry (K, Ca, Mg, Cu, Fe, Mn and Zn), and turbidimetry (S). Total nitrogen was determined with the Kjeldahl method (Malavolta et al., 1997). Organic carbon was determined using the Walkley and Black method (Nelson e Sommers 1996). The pH was determined in 0.01 mol

L^{-1} $CaCl_2$, and the moisture content was determined through the gravimetric method.

Dry soil or soil + vermicompost were analyzed at three time points, namely, 1) at the beginning (0 days) before preparation; 2) after 30 days of incubation of fertilized soil under the addition of vermicompost and/various doses of Cu; and 3) after 40 days of incubation, i.e., 85 days after sowing, totaling 115 days from day 0. The following parameters were evaluated: pH; P (Mehlich-1); exchangeable K, C and Mg; OM; exchangeable Al^{3+}; sum of bases (SB); and base saturation (V). The analyses were performed via the use of the methods described in Teixeira et al. (2017).

The analytical results correspond to a homogenized composite sample prepared from all the replications and thus represent a single representative value for each parameter. The soil samples were analyzed at three time points, namely, 0 days (original soil), 30 days (30 days after the application of Cu, vermicompost, fertilizers and limestone) and 115 days (after the harvest).

2.3 Biometric and Biomass Measurements

At 115 days (on the harvest day), the plants were separated into three parts (leaves, bulbs, and roots). The number of bulbs and leaves per plot was counted. The length was measured with a ruler, and the diameter of the five largest leaves of each sample unit was measured with a digital caliper. Leaves and bulbs were separated and weighed to determine fresh matter. The samples were then dried in a forced-air oven (model MA035, Marconi, Piracicaba, Brazil) at 65 ± 2 °C, after which the dry matter content was determined. All measurements were performed using an analytical balance (model AY220, Shimadzu, Kyoto, Japan). The contents of fresh and dry matter in the leaves, bulbs, and roots were obtained from all the materials collected from each plot (cell) and are expressed in g per tray.

The roots from each plot were subsequently washed in running water to remove adhering soil and then fixed in 70% ethanol for subsequent morphological analysis using the WinRHIZO imaging system.

2.4 Root Morphology

Morphological root analysis was performed using WinRhizoArabidopsis software, version 2022 (WinRHIZO™, Regent Instruments Inc., Québec City, QC, Canada), coupled to an LA2400 scanner (Epson America Inc., Long Beach, CA, USA) equipped with an additional light unit (TPU). The following parameters were determined: total root length (cm), surface area (cm^2), average diameter (mm), and root volume (cm^3). Moreover, the root length was analyzed by diameter class (0 to 4.5 mm) using the same software.

2.5 Macronutrient Content in Plants

Leaf, bulb and root dry matter were subsequently ground in a Willey mill (model TE-650, Tecnal, Piracicaba, SP, Brazil) and sent for determination of the macronutrient content in plant tissues (leaves, bulbs and roots), according to the recommendations of Malavolta et al. (1997). Nitrogen was determined via sulfur digestion followed by distillation (Kjeldahl method). With respect to the other nutrients, the samples were subjected to nitric-perchloric digestion. The concentrations of calcium, magnesium, potassium, and copper were determined using high-sensitivity atomic emission spectrometry (MP-AES 4200, Agilent Technologies, Santa Clara, CA, USA). The phosphorus and sulfur levels were determined via spectrophotometry (model 600S, FEMTO Indústria e Comércio de Instrumentos Ltda., São Paulo, SP, Brazil).

2.6 Data Analysis

For all parameters analyzed, means and corresponding standard errors (SE) were calculated. The data were subjected to statistical analysis using analysis of variance (ANOVA) and the Scott−Knott test, thereby adopting a significance level of 5% ($p \leq 0.05$), with the aid of Sisvar software (version 4.3; Universidade Federal de Lavras, Lavras, Minas Gerais). The Scott–Knott procedure was adopted to facilitate objective grouping of multiple treatment means into statistically distinct and nonoverlapping clusters, thereby increasing interpretability in the experiments with several treatment combinations. Linear and nonlinear regression analyses of the data, obtained as a function of the Cu dose, were also performed. Models were chosen on the basis of their accuracy, which was evaluated via the Willmott index of agreement (dw). Adjustments were made in Microsoft Excel, with

the aid of the generalized reduced gradient (GRG2) method (Lasdon et al., 1978), and the adjustments were evaluated on the basis of ANOVA and the coefficient of determination (R^2). The mathematical equations of the models are described in the Supplementary Material (Online Resources).

3 Results

3.1 Chemical Analysis of Vermicompost and Soil

The chemical composition of vermicompost, which was applied as an organic conditioner in the treatments, was analyzed, and the results are summarized in Table 1, confirming its value as an organic conditioner, with a pH close to neutral (6.5 in $CaCl_2$).

The OM content in vermicompost was high (134 g kg^{-1}). Moreover, high values of total nitrogen (14.4 g kg^{-1}), total phosphorus (6.8 g kg^{-1}), and potassium (3.5 g kg^{-1}) were observed. In addition, an excellent C/N ratio of 9.3 was obtained (Table 1). The applied vermicompost exhibited a low concentration of Cu (43.2 mg kg^{-1}), which resulted in the application of 2.6 kg Cu ha^{-1} (1.295 mg Cu dm^{-3}).

To establish the experimental context, the results of initial chemical characterization of the soil, performed before treatment application (day zero), are provided in Tables S1 and S2 (Online Resources). The soil exhibited a pH of 5.29, an OM content of 1.86 dag dm^{-3}, and an initial Cu content of 1.9 mg dm^{-3}.

After 30 days of incubation, the soil pH increased from 6.25 to 7.21, which was accompanied by an increase in SB, which reached 6.50 cmol dm^{-3} in the vermicompost treatments (Table S2, Online Resources). The cation exchange capacity (CEC) also increased, reaching 7.74 cmol dm^{-3} in the 400 mg Cu dm^{-3} treatment in the presence of vermicompost (Table S2, Online Resources). The phosphorus concentrations ranged from 22.5 to 53.2 mg dm^{-3}, and the potassium concentration reached 205.3 mg dm^{-3}. The iron concentrations varied between 39.8 and 53.1 mg dm^{-3}, and the zinc concentrations ranged from 3.3 to 7.3 mg dm^{-3}, both of which increased when associated with vermicompost (Tables S1 and S2, Online Resources).

At the end of the chive cultivation period (115 days), the soil pH remained high, at 7.23, and an SB of up to 4.64 cmol dm^{-3} and a CEC of up to 10.01 cmol dm^{-3} were observed. Compared with that during the previous period, the phosphorus concentrations fluctuated between 9.9 and 37.7 mg dm^{-3}, and the potassium concentration decreased, ranging from 46.7 to 94.5 mg dm^{-3}. The OM content slightly varied, stabilizing between 0.93 and 1.86 dag dm^{-3}. Among the micronutrients, the iron contents ranged from 23.1 to 32.3 mg dm^{-3}, and the zinc contents varied between 1.7 and 8.4 mg dm^{-3}. Other parameters, such as exchangeable aluminum (Al), potential acidity (H + Al), base saturation (V), aluminum saturation (m), residual phosphorus (P-rem), the calcium/magnesium ratio, and the magnesium/potassium ratio, are provided in Table S3, Online Resources.

3.2 Copper content in Soil and Plant Tissues

The available Cu levels increased as a function of the Cu dose 30 days after the application of Cu and other fertilizers and soil amendments. In soil fertilized with vermicompost, a response similar to that in the Gaussian model was observed (Table S4, Online Resources), which indicates that at doses of 0, 50, and 100 mg dm^{-3}, there were no significant increases. However, higher doses resulted in a notable increase, with an estimated peak of 182.3 mg dm^{-3} at 357.9 mg dm^{-3}. In the soil without vermicompost, the response of available Cu at 30 days was exponential, with a notable increase even at the lowest doses, with a maximum estimated value of 169.9 mg dm^{-3} (Table S5, Online Resources). In the samples evaluated after 115 days, the maximum value of available Cu reached 210.0 mg dm^{-3} in soil fertilized with

Table 1 Chemical composition of the vermicompost employed as an organic conditioner

pH ($CaCl_2$)	N (Kjeldahl) g kg^{-1}	P	K	S	Ca	Mg	CO*	C/N*	Zn mg kg^{-1}	Fe	Mn	Cu	B
6.5	14.4	6.8	3.5	1.0	8.7	2.8	134	9.3	192	16,853	354	43.2	38

*Organic carbon (CO); carbon/nitrogen ratio

vermicompost and 229.3 mg dm^{-3} in soil without the addition of vermicompost.

With respect to the Cu level in the plant, Cu preferentially accumulated in the roots, followed by the bulbs and leaves (Fig. 2). The plant response in the soil without vermicompost as a function of the Cu dose was linear in the bulbs and leaves (Table S6, Online Resources) and exponential in the roots (Table S7, Online Resources). In the soil fertilized with vermicompost, the responses of the Cu content in the different plant parts fit the Gaussian model, except for the Cu content in the roots, where the fit was not significant (Table S5, Online Resources). In the bulbs, the maximum estimated Cu accumulation reached 1399.9 mg kg^{-1}, which occurred at a dose of 2501 mg dm^{-3}, i.e., a dose much greater than the highest dose employed in this study. Notably, this finding indicates that the peak phase was not reached in this experiment, with a slight increase observed in the accumulated level and a significant increase only at 800 mg dm^{-3}, which resulted in the accumulation of 38.9 mg Cu kg^{-1}. In the soil without vermicompost, a similar trend was observed. Notably, a significant increase was already observed at a dose of 400 mg dm^{-3} and was much more notable, reaching a value of 73.3 mg kg^{-1}. The increase in Cu in the roots occurred at lower doses and peaked at a value of 384.5 mg dm^{-3}.

At time zero (0 days), the concentration of available Cu in the soil (Fig. 1) was constant in all the treatments, with a value of 1.90 mg dm^{-3}. After 30 days under the application of Cu (at various doses), in which the soil was maintained at the field capacity with respect to irrigation without cultivation, the Cu concentration in the soil proportionally increased with the dose. After 30 days of incubation, the soil Cu concentrations ranged from 17.8 to 163.8 mg dm^{-3} in the treatments without vermicompost addition, corresponding to concentrations of 17.8, 36.2, 80.8, 102.6, 157.4, and 161.7 mg dm^{-3} in the 0, 50, 100, 200, 400 and 800 mg Cu dm^{-3} treatments, respectively. In the treatments where vermicompost was applied, the values were 86.2, 62.7, 86.9, 123.9, 156.7 and 163.8 mg dm^{-3} for the same Cu doses. In general, the presence of vermicompost resulted in higher soil Cu concentrations (Fig. 1).

After 115 days (after 85 days of chive cultivation), a reduction in the available Cu concentration was observed in the 0, 50, 100, 200, and 400 mg dm^{-3} treatments, regardless of vermicompost addition (Fig. 1). However, only the 800 mg Cu dm^{-3} treatment resulted in an increase in the Cu concentration at 115 days, reaching values of 197.5 and 186.8 mg dm^{-3} Cu in the presence and absence of vermicompost, respectively.

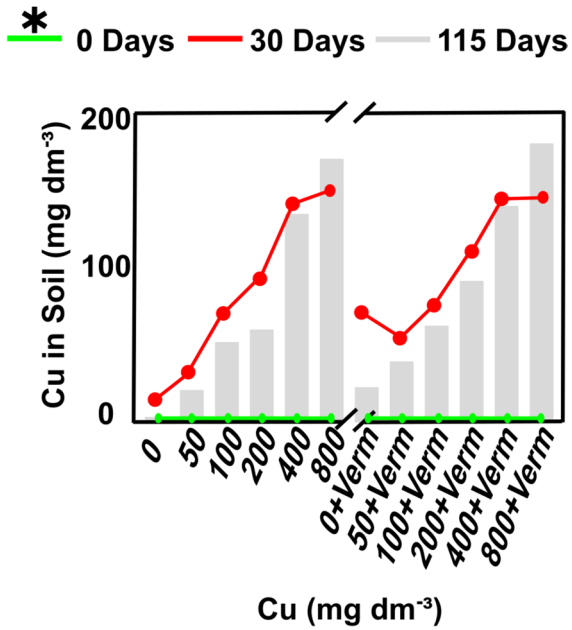


Fig. 1 Available copper levels in the soil throughout the experiment (at the start (0 days), at 30 days, and at the end (115 days)) under the application of copper at different doses (Cu: 0, 50, 100, 200, 400 and 800 mg dm^{-3}), with or without vermicompost (verm) addition. *Initial soil Cu concentrations measured after soil sampling and prior to the experimental treatments (original soil Cu levels)

In the leaf tissue (Fig. 2A), the highest degree of Cu accumulation occurred in the 800 mg dm^{-3} treatment, followed by the 800 mg dm^{-3} treatment combined with vermicompost, with values of 9.90 and 9.00 mg kg^{-1}, respectively. Compared with the treatments where vermicompost was applied, the 100, 200, and 400 mg Cu dm^{-3} treatments resulted in greater Cu accumulation levels in the leaves. In the bulbs (Fig. 2B), the 800 Cu mg dm^{-3} treatment resulted in the highest accumulation level, exceeding 70 mg kg^{-1}, followed by the 800 + verm treatment.

The highest degree of Cu accumulation was observed in the roots (Fig. 2C), and the highest content was observed in the 200 mg dm^{-3} + vermicompost (236.20 mg kg^{-1}) treatment, followed by the 800 mg dm^{-3} (197.2 mg kg^{-1}) treatment. When the

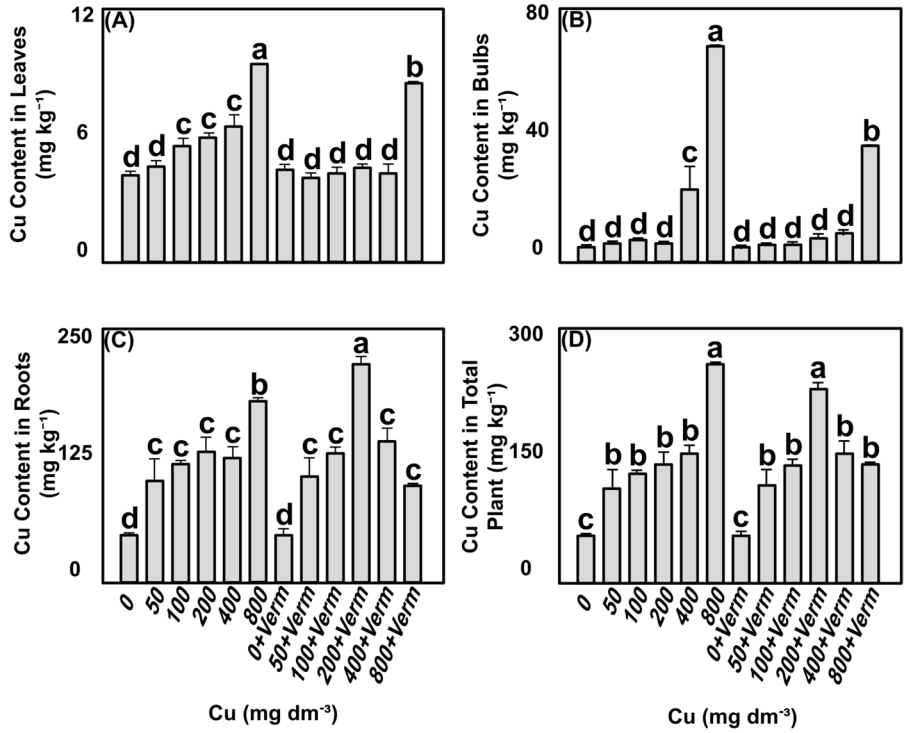


Fig. 2 Copper (Cu) content in the tissues of chive plants (*Allium schoenoprasum* L), i.e., (**A**) leaves, (**B**) bulbs, (**C**) roots and (**D**) total (leaves, bulbs and roots), exposed to different Cu concentrations applied to the soil. Means between treatments followed by the same letter do not significantly differ on the basis of the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications ($n = 16$). Verm = vermicompost

Cu content in the plant as a whole was analyzed (Fig. 2D), the 800 mg Cu dm^{-3} treatment and the 200 mg Cu dm^{-3} + vermicompost treatment yielded the highest total Cu contents, reaching 280.40 and 247.98 mg kg^{-1}, respectively.

3.3 Root Morphology

As shown in Fig. 3A and B, the root length and total volume decreased in the treatments with high copper doses, especially at a dose of 800 mg dm^{-3}, both with and without vermicompost. However, with the application of vermicompost at levels of 200 and 400 mg dm^{-3}, the total root length was greater than that in the treatments without vermicompost. The root surface area followed a similar trend, although the addition of vermicompost resulted in larger areas at doses of 100, 200, and 400 mg Cu dm^{-3} (Fig. 3B). The average root diameter (Fig. 3D) increased only at the highest dose compared with that at all the other doses.

The length of very fine roots and fine roots decreased with increasing copper dose, but the addition of vermicompost at levels of 200 and 400 mg dm^{-3} resulted in larger average values (Fig. 4A). The surface area and volume of very fine roots and fine roots responded to only the addition of vermicompost at a level of 400 mg dm^{-3} (Fig. 4B and C, respectively). Compared with the treatments without Cu addition, the root thickness, length, surface area, and volume were greater in the 100, 200, and 400 mg Cu dm^{-3} treatments (Fig. 4).

3.4 Biometric Measurements

With or without vermicompost addition, the leaf length was significantly smaller at a dose of 800 mg dm^{-3} (Fig. 5A). The same result was obtained for the leaf diameter. However, the addition of vermicompost contributed to increased diameters in the 0 + verm, 100 + verm, 200 + verm and 400 + verm treatments.

As shown in Fig. 5C, compared with the treatments without vermicompost, the number of leaves per plant was greater in the treatments with vermicompost, especially at intermediate Cu doses (200 and 400 mg dm^{-3}). From a dose of 100 mg dm^{-3} copper onward, the number of bulbs decreased (Fig. 5D). However, except for the 800 + verm treatment, vermicompost application yielded a larger number

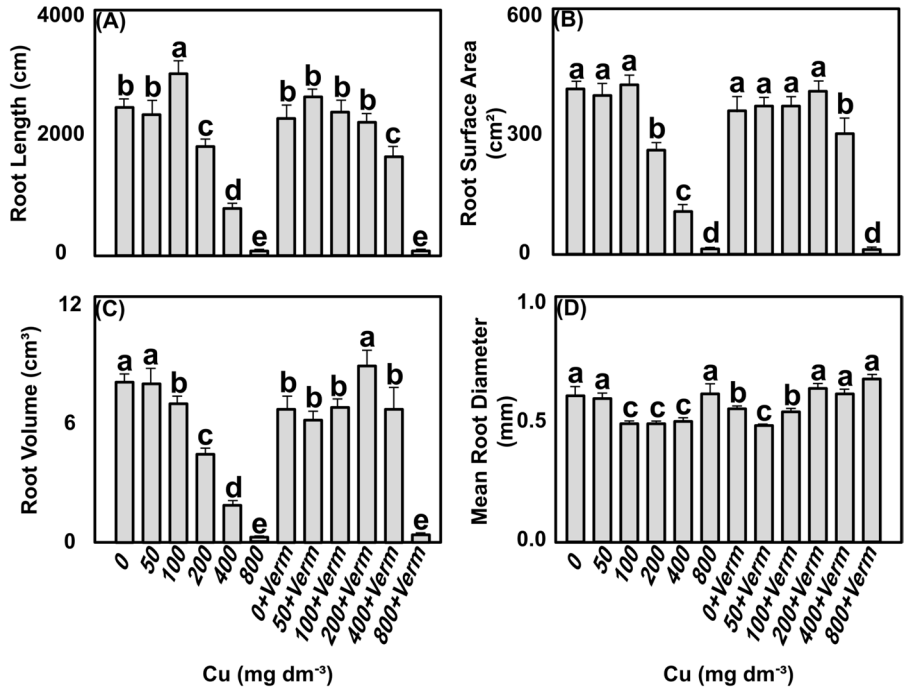


Fig. 3 **A** Total root length (cm), (**B**) root surface area (cm^2), (**C**) root volume (cm^3) and (**D**) average root diameter (mm) of chives (*Allium schoenoprasum* L.) exposed to different Cu concentrations applied to the soil. Means between treatments followed by the same letter do not significantly differ on the basis of the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications ($n = 16$). Verm = vermicompost

of bulbs. This beneficial effect was greater in the 400 + verm treatment.

As shown in Fig. 6A, the amount of fresh leaf matter decreased at the highest copper doses (200, 400, and 800 mg dm^{-3}) in the absence of vermicompost. However, the addition of vermicompost decreased this effect, leading to partial recovery of fresh leaf matter, except at the highest copper dose, where the performance remained lower. The results of dry matter analysis revealed differences between the treatments that matched the difference in the leaf fresh weight.

The content of bulb dry matter was lower at a dose of 800 mg dm^{-3} with or without vermicompost (Fig. 6C). However, the use of vermicompost at levels of 200 and 400 mg dm^{-3} resulted in a greater content of bulb dry matter. A dose of 800 mg dm^{-3} (with and without vermicompost) significantly decreased the root dry biomass (Fig. 6D), but vermicompost addition increased the root biomass at doses of 200 and 400 mg dm^{-3}.

The nitrogen (N) content in the leaves was greater in response to the addition of 50 mg dm^{-3} Cu without vermicompost and in response to the addition of 50, 100, and 800 mg dm^{-3} Cu with vermicompost (Table 2). With respect to phosphorus (P), higher levels were observed in the vermicompost treatments at doses of 100 and 200 mg dm^{-3}. The potassium (K) levels were relatively low in the 800 + verm treatment, followed by those in the 0, 50, 800, and 400 + verm treatments. The foliar calcium (Ca) levels were high at doses of 0 and 50 mg/L, and the magnesium (Mg) levels were high in the treatments with 0, 50, and 100 mg/L. The application of Cu at doses above 200 mg dm^{-3} to the soil, regardless of the presence of vermicompost, reduced the levels of these elements (Table 2). Sulfur (S) levels responded positively to vermicompost addition, especially at higher Cu doses. The highest values were observed at doses of 200, 400, and 800 mg dm^{-3} with vermicompost (4.06, 3.57, and 3.66 g kg^{-1}, respectively).

There were no differences between treatments in terms of the N content in the bulbs (Table 3). With respect to P, the highest values were observed in the 0 mg dm^{-3} treatment and the 0 mg dm^{-3} + vermicompost (0, 50, 100, and 200 + vermicompost) treatments, with levels ranging from 1.20 to 1.27 g kg^{-1}. Conversely, higher Cu doses (≥ 400 mg dm^{-3}) caused a decrease in P levels. The K levels were greater in the 400 mg dm^{-3}, 800 mg dm^{-3}, 100 + verm, and 800 + verm treatments than in the other treatments, and the Ca content was highest in the 800 + verm treatment.

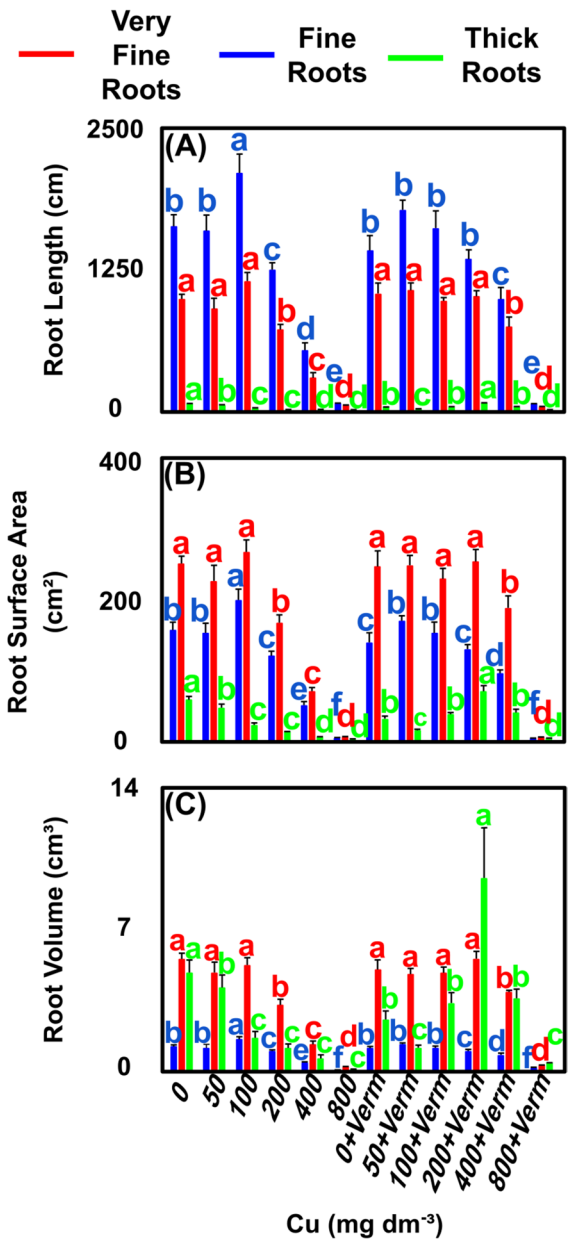


Fig. 4 **A** Total root length (cm), (**B**) root surface area (cm^2) and (**C**) root volume (cm^3). The blue bars indicate very fine roots, the red bars indicate fine roots, and the green bars indicate thick roots of chives (*Allium schoenoprasum* L.) exposed to different Cu concentrations applied to the soil. Means between treatments followed by the same letter do not significantly differ on the basis of the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications ($n = 16$). Verm = vermicompost

The Mg content did not differ among treatments, and the 800 + verm treatment yielded the highest average S content in the bulbs (Table 3). The lowest N content in the roots occurred in the 400 + verm treatment, followed by the 200 + verm, 400 + verm, 0.50 mg dm^{-3} and 100 mg dm^{-3} treatments. The P content was high in virtually all the treatments where Cu and vermicompost were applied, except the 0 + verm treatment.

The K and Mg contents in the roots were significantly lower at a dose of 800 mg dm^{-3} without vermicompost addition. The 800 mg dm^{-3}, 50 + verm, and 800 + verm treatments yielded higher Ca contents than those in the other treatments. The highest S content in the roots occurred in the 800 + verm treatment (Table 3).

4 Discussion

The application of copper sulfate at increasing doses to the soil progressively increased the Cu content after 30 days of incubation. The treatments where vermicompost was applied generally resulted in higher concentrations of available Cu, which can be explained by the Cu content of 43.2 mg kg^{-1} present in the vermicompost itself. For example, in the 0 mg dm^{-3} + vermicompost treatment, the Cu concentration reached 86.20 mg dm^{-3} at 30 days, whereas the value reached only 17.80 mg dm^{-3} in the same treatment without vermicompost.

These results highlight the dual role of vermicompost in the soil–plant system. On the one hand, vermicompost increases soil fertility through increases in OM, CEC, and nutrient availability levels. On the other hand, it contributes additional Cu to the soil (approximately 2.6 kg ha^{-1} or 1.295 mg Cu dm^{-3}on the basis of its concentration and the applied dose), clearly increasing the baseline soil Cu level even in the treatments without the addition of copper sulfate. The Cu present in vermicompost is associated with the bovine manure employed as the primary substrate. In dairy production systems, Cu supplementation in feed is common, and residual Cu is frequently detected in cattle manure and derived composted materials (Nicholson et al., 2003). In Brazil, cattle manure is among the most accessible and widely applied organic residues in agriculture, which justifies its use in this study but also explains

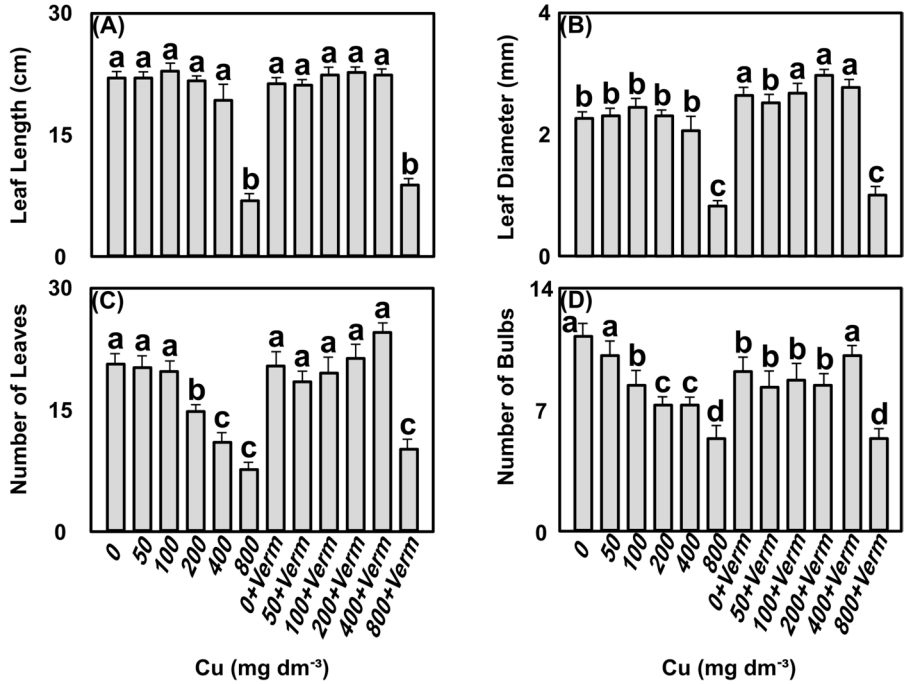


Fig. 5 **A** Leaf length (cm), (**B**) leaf diameter (mm), (**C**) number of leaves, and (**D**) number of bulbs of chives (*Allium schoenoprasum* L.) exposed to different Cu concentrations applied to the soil. Means between treatments followed by the same letter do not significantly differ on the basis of the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications ($n = 16$). Verm = vermicompost

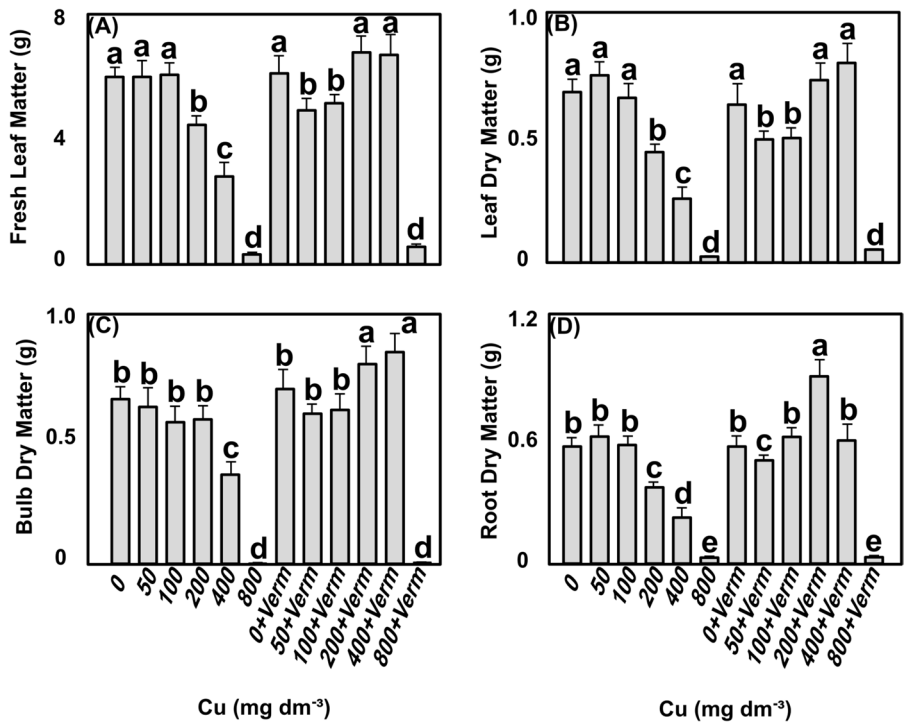


Fig. 6 **A** Leaf fresh matter, (**B**) leaf dry matter, (**C**) bulb dry matter and (**D**) root dry matter of chives (*Allium schoenoprasum* L.) exposed to different Cu concentrations applied to the soil. Means between treatments followed by the same letter do not significantly differ on the basis of the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications ($n = 16$). Verm = vermicompost

the increased initial soil Cu content observed in the 0 mg dm^{-3} + vermicompost treatment. Although OM-mediated complexation modulates Cu bioavailability, vermicompost cannot be regarded solely as a mitigating agent since it simultaneously increases the total Cu input to the system and influences the baseline Cu availability level.

Table 2 Macronutrients in the leaves of chive plants (*Allium schoenoprasum* L.) grown in soil treated with different copper doses (Cu: 0, 50, 100, 200, 400 and 800 mg dm^{-3}) with or without vermicompost addition

Means between treatments followed by the same letter do not significantly differ according to the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications

Cu treatments (mg dm^{-3})	N	P	K	Ca	Mg	S
	g kg^{-1}					
0	16.55 b	1.14 c	12.12 b	16.45 a	2.68 a	2.31 c
50	19.58 a	1.05 c	11.56 b	17.64 a	2.92 a	2.61 b
100	17.22 b	1.30 c	14.74 a	14.51 b	2.73 a	2.89 b
200	16.52 b	1.17 c	14.95 a	13.12 b	2.47 b	2.25 c
400	15.40 b	1.07 c	14.65 a	11.14 c	1.,96 c	2.26 c
800	16.80 b	0.67 d	12.19 b	14.44 b	2.31 b	2.40 c
0 + verm	16.54 b	1.49 b	14.33 a	10.27 c	2.32 b	2.29 c
50 + verm	18.01 a	1.57 b	13.50 a	10.32 c	2.48 b	1.99 c
100 + verm	17.97 a	1.75 a	13.12 a	8.64 d	2.31 b	2.22 c
200 + verm	15.43 b	1.81 a	14.96 a	10.18 c	2.20 c	4.06 a
400 + verm	15.21 b	1.12 c	11.47 b	8.12 d	1.66 c	3.57 a
800 + verm	18.82 a	0.79 d	8.79 c	11.01 c	1.98 c	3.66 a

Table 3 Macronutrients in the bulbs and roots of chive plants (*Allium schoenoprasum* L.) grown in soil treated with different copper doses (Cu: 0, 50, 100, 200, 400 and 800 mg dm^{-3}) with or without vermicompost addition

Treatments Cu (mg dm^{-3})	N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
	Plant bulbs (g kg^{-1})						Plant roots (g kg^{-1})					
0	6.54 a	1.12 a	6.25 b	2.96 b	0.79 a	1.32 d	15.98 b	0.89 b	11,80 b	5.42 b	2.97 a	1.72 d
50	7.51 a	1.00 b	4.79 b	2.55 b	0.75 a	1.51 d	16.21 b	1.07 a	10,97 b	5.76 b	3.18 a	1.91 d
100	7.63 a	0.98 b	6.13 b	2.51 b	0.71 a	1.23 d	16.28 b	0.88 b	11,55 b	5.27 b	2.90 a	1.65 d
200	6.68 a	0.91 b	5.30 b	1.65 c	0.59 a	0.94 d	17.90 a	1.02 a	12,09 b	5.70 b	2.73 a	1.73 d
400	6.79 a	0.91 b	7.65 a	2.60 b	0.73 a	1.37 d	17.21 a	0.85 b	11,23 b	4.83 b	2.08 b	1.74 d
800	6.80 a	0.74 b	7.86 a	3.84 a	0.90 a	2.82 b	17.34 a	0,85 b	7,48 c	7.37 a	1.53 c	2.25 c
0 + verm	6.18 a	1.22 a	6.17 b	2.13 c	0.74 a	1.60 d	16.87 a	0,96 b	15,37 a	6.09 b	3.07 a	1.52 d
50 + verm	6.93 a	1.27 a	6.02 b	1.76 c	0.69 a	1.51 d	18.26 a	1,14 a	16,81 a	6.98 a	3.57 a	1.70 d
100 + verm	5.60 a	1.20 a	7.63 a	2.13 c	0.81 a	1.40 d	16.97 a	1,16 a	13,06 b	5.43 b	3.01 a	1.78 d
200 + verm	6.09 a	1.23 a	6.39 b	2.09 c	0.77 a	1.88 c	15.57 b	1,10 a	11,73 b	5.15 b	3.16 a	2.14 c
400 + verm	7.43 a	1.01 b	5.97 b	1.95 c	0.68 a	2.17 c	13.84 c	1,20 a	11.97 b	5.00 b	3.11 a	2.85 b
800 + verm	6.38 a	0.66 b	7.57 a	2.79 b	0.81 a	3.59 a	15.22 b	1,16 a	10.08 b	7.26 a	1.94 b	3.66 a

Means between treatments followed by the same letter do not significantly differ according to the Scott−Knott test at the 5% probability level ($p \leq 0.05$). Each value indicates the mean ± standard error of 16 replications

After 115 days (after 85 days of chive cultivation), the available Cu concentration decreased in the 0, 50, 100, 200, and 400 mg dm^{-3} treatments, regardless of vermicompost addition. This decrease can be attributed to Cu absorption by plants during the growing cycle, as well as Cu adsorption onto the surface of soil colloids, complexation with OM, or possible leaching (Kumar et al., 2021).

Additionally, the application of vermicompost significantly influenced the physicochemical properties of the soil. Throughout the experiment, vermicompost altered the CEC, pH, OM content, and nutrient availability. Studies have indicated that vermicompost enhances soil fertility by increasing the CEC and stabilizing the soil pH near neutrality, thus optimizing nutrient dynamics (Lazzaretti et al., 2024; Kureljušić et al., 2024). Furthermore, vermicompost is rich in macro- and micronutrients, including N, P, K, Ca, Mg, Fe, Zn, Cu, and Mn, whose concentrations may increase during vermicomposting because of mineralization (Esmaielpour et al., 2020; Abawari, 2022; Talaat & Abdel-Salam, 2024).

Among the soil modifications promoted by vermicompost, the increase in OM plays a central role

in regulating Cu behavior in the soil–plant system. The OM content increased from 19.2 to 22.3 g dm^{-3} in soils without and with vermicompost, respectively, after 30 days of incubation. Cu exhibits high affinity for organic ligands and can be complexed by humified fractions, thereby reducing the activity of free Cu^{2+} ions in the soil solution (Pan et al., 2023). Therefore, the OM present in vermicompost modulates Cu bioavailability and mobility (Lazzaretti et al., 2024; Li et al., 2024). Although the total Cu levels may vary, only a small fraction (1–20%) is typically bioavailable, while most remains associated with organic complexes (Kumer et al., 2021).

However, Cu complexation by OM does not necessarily suggest complete immobilization. Depending on the concentration and speciation, organometallic complexes may either reduce or facilitate Cu uptake within a physiologically tolerable range. This dynamic behavior helps explain why, at intermediate doses (particularly 200 mg dm^{-3}), vermicompost favored Cu entry into the roots, whereas at higher doses (800 mg dm^{-3}), its influence on root accumulation was relatively smaller. Under excessive Cu levels, binding sites and regulatory processes may approach saturation, thus limiting the capacity of OM to further modulate the uptake intensity.

The presence of vermicompost decreased the overall level of Cu accumulation in the shoots, even though it contributed approximately 2.6 kg ha^{-1} of Cu to the soil on the basis of its concentration and the applied dose. These findings indicate that the increase in OM and CEC levels promoted a buffering effect, stabilizing Cu in less mobile fractions while still facilitating controlled interactions with the root system at moderate doses. By providing functional groups such as carboxyl, phenolic, and hydroxyl groups that can bind Cu, vermicompost promotes the formation of relatively stable complexes that regulate, rather than completely inhibit, Cu mobility.

Within this context, organic amendments function as modulators of metal speciation and rhizosphere chemistry, thereby influencing whether Cu remains in exchangeable forms, soluble complexes, or more stable bound fractions. Therefore, under the conditions of this study, the increase in OM and associated complexation reactions represents a key pathway through which vermicompost modulates Cu bioavailability according to the soil Cu concentration, thereby increasing tolerance at intermediate doses but yielding limited regulatory capacity under excessive doses. In addition to these chemical mechanisms, vermicompost can stimulate microbial activity in the rhizosphere, which may in turn influence Cu dynamics through processes such as organic ligand production, redox transformations, and competition for binding sites (Gupta et al., 2019). Although microbial parameters were not evaluated in this study, the interaction between OM and soil microbiota likely contributed to the observed modulation of Cu availability and plant response levels.

With respect to the copper content in chive tissues, the accumulation pattern sequentially decreased in the roots, bulbs, and leaves (roots > bulbs > leaves). Species of the genus *Allium* sp. (particularly chives) exhibit structural differentiation in their vegetative tissues, such as leaves (green), bulbs (white), and roots. Each of these compartments can exhibit physiological and phytochemical variations (Dai et al., 2024), depending on the species, which renders individualized assessments of these structures necessary in the face of copper stress.

This pattern is consistent with the role of roots as the main barrier and retention compartment for Cu. According to Kumar et al. (2021), plants frequently restrict Cu transport toward aerial tissues to avoid phytotoxicity, thereby immobilizing the metal in cell walls, apoplasts, and root vacuoles. This greater Cu retention in chive roots occurs once COPT (CopperTransporters) and P-typeATPases (HMA) transporters are highly expressed in the roots, where they participate in both metal absorption and compartmentalization processes (Xu et al., 2024). In plants, nicotianamine (NA), a low-molecular-weight ligand, influences the detoxification and transport of metals such as copper, thereby influencing their distribution to avoid phytotoxicity in more sensitive tissues (Seregin & Kozhevnikova, 2023). Various exudates, such as organic and amino acids, released by roots can also cause the complexation of copper ions in the rhizosphere, thereby decreasing their mobility and bioavailability and consequently restricting their translocation to shoots (Wu et al., 2025).

Although Cu accumulates to a lesser extent than it does in the roots, the bulbs constitute the second-largest compartment. Bulbs function as storage organs that are rich in vacuoles and can store Cu

associated with phytochelatins, glutathione, and metallothioneins (Kumar et al., 2021; Xu et al., 2024). Thus, bulbs can function as additional filters, receiving some of the metal translocated via the xylem but still maintaining a compartmentalization pattern that minimizes mobilization to leaves and reduces damage to meristematic tissues (Czarnek et al., 2024; Hossain et al., 2012).

Copper accumulation in bulbs can pose risks to human health when these organs are consumed as food (Naseem et al., 2024). However, the species evaluated in this study does not produce edible bulbs. However, 9.90 mg kg^{-1} of Cu can accumulate in chive leaves. The literature indicates that foliar Cu concentrations between 5 and 30 mg kg^{-1} are considered adequate for plant tissues (Moura et al., 2021). According to the Food and Agriculture Organization (FAO)/World Health Organization (FAO/WHO, 2011), the maximum permissible limit for Cu in vegetables is 40 mg kg^{-1} (fresh weight basis). Thus, the concentration of 9.90 mg kg^{-1} observed in chive leaves in this study remains well below the FAO/WHO maximum permissible limit and within the physiological range reported for plants, indicating no immediate concern with respect to food safety, especially considering its use as a condiment (Nepal et al., 2024).

Food safety is a primary concern, and the results obtained for chives (*Allium schoenoprasum* L.) corroborate their viability for consumption. Studies on other species of the genus *Allium*, such as garlic (*Allium sativum* L.), have reinforced that integrated management practices, such as the use of compost and inorganic fertilizers, can significantly improve bulb quality, including the contents of ascorbic acid and pyruvic acid, without exceeding the safety limits for heavy metals (Tanveer et al., 2025). In *Allium schoenoprasum* L., the accumulation of potentially toxic elements was not observed, which highlights the safety of associated plant materials for human consumption (Vuković et al., 2023).

Studies on other *Allium* species have revealed that certain metals, such as Pb and Cd, notably accumulate in bulbs, whereas others, such as Cu, are more concentrated in roots and leaves and only reach the bulbs at high concentrations in the soil (Czarnek et al., 2024; Opriș et al., 2024). In accordance with the investigations of Wang et al. (2020) for chives (*Allium schoenoprasum* L.), when the same cultivation period as that adopted in this study was employed, the plants accumulated higher levels of Cu in the roots than in the other organs. The authors also reported that compared with lower doses, Cu doses equal to or greater than 300 mg kg^{-1} led to greater Cu accumulation in plant tissues.

Negative effects on shoot growth were already observed starting at a dose of 200 mg dm^{-3} but with more notable effects at a dose of 800 mg dm^{-3}. The reduction in the leaf diameter and the number of bulb and leaf parts demonstrate plant sensitivity to copper, which, at increased concentrations in the leaves, can lead to toxic effects. Copper in the shoots causes damage to the photosynthetic apparatus because of its ability to induce oxidative stress, which compromises the integrity of cell membranes and inhibits the activity of enzymes that are essential for photosynthesis (Kumar et al., 2021). Excess copper can lead to the generation of reactive oxygen species (ROS), resulting in lipid peroxidation and, consequently, affecting the efficiency of the photosynthetic machinery (Ren et al., 2022). The interference of Cu in electron transport systems and protein denaturation in the photosynthetic complex are additional mechanisms that result in reduced plant yields (Huang et al., 2022).

The root morphology was also affected by the increase in copper dose, with the highest dose (800 mg dm^{-3}) drastically decreasing the total root length, total surface area, and total root volume. Copper can impair the activity of root meristems (subapical regions), thereby directly affecting cell division at the root tips, which explains the observed morphological changes. Furthermore, fine roots and very fine roots are more susceptible to copper inhibition, as these tissues contain metabolically active and highly sensitive cells. As reported by Marques et al. (2018), the root morphology is a sensitive indicator of copper stress and is characterized by reduced root length and surface area, which directly reflects impaired shoot development, including a decreased leaf area, a reduced number of leaves, and decreased shoot growth. Similarly, in cotton seedlings, high Cu concentrations affect the total root length, surface area, and volume (Wu et al., 2025). The combined reduction in root system architecture and foliar development observed at a dose of 800 mg dm^{-3} clearly indicates severe copper toxicity under these conditions.

Vermicompost did not reduce the toxic effect of Cu on plants at a dose of 800 mg dm^{-3}. However,

its addition was effective at intermediate doses (200 + verm and 400 + verm), reducing phytotoxic effects and increasing plant tolerance. At these doses, vermicompost increased shoot and root biomass levels, benefiting both aerial organs and underground organs (bulbs and roots) directly exposed to the stressor metal. The application of vermicompost at appropriate doses has been consistently associated with improved growth and increased biomass in various plant species, including in soils contaminated by metals (Abawari, 2022; Ramazanoglu, 2024; Talaat & Abdel-Salam, 2024).

Mechanistically, this response is closely linked to modifications in the root architecture under stress. At doses of 200 and 400 mg dm^{-3}, vermicompost stimulated root development, increasing the total length, surface area, and volume of very fine, fine, and thick roots. Fine roots are particularly important for water and nutrient absorption, whereas thick roots contribute to anchorage and structural stability. A more extensive root system under Cu exposure indicates morphological adaptation, thereby enhancing soil exploration and increasing the capacity for Cu exclusion, sequestration, or compartmentalization within root tissues. Tolerance can be increased by adsorption to cell walls and Cu complexation, which limit its translocation to the shoot (Marques et al., 2019). The presence of longer and finer roots under Cu stress is considered an indicator of lower physiological disturbance and increased adaptation (Facco et al., 2023). Thus, the stimulation of root architecture by vermicompost contributes directly to the ability of the plant to manage copper stress.

In addition to its ability to modulate the morphology, vermicompost modified the Cu distribution within the plant. In the absence of vermicompost, increasing soil Cu resulted in a clear dose-dependent increase in foliar accumulation. However, at moderate doses (particularly at 200 mg dm^{-3}), vermicompost reduced the Cu concentration in the leaves but increased Cu retention in the roots. This shift suggests preferential immobilization in the root system. The presence of humic and fulvic substances in vermicompost can generate soluble and insoluble Cu-organic complexes, thus influencing mobility and transport (Kumar et al., 2021). These complexes may decrease Cu availability for xylem loading while favoring retention in the roots, thereby deviating from the linear dose−response pattern observed in the leaves. In addition, the Cu retained in the roots may be stabilized in different chemical forms, including inorganic, water-soluble, and fractions bound to pectates, proteins, phosphates, and oxalates, which further restrict its translocation to aerial tissues.

In the roots, which represent the main compartment for Cu accumulation, this effect was particularly notable. At a dose of 200 mg dm^{-3} combined with vermicompost addition, the total plant Cu values were comparable to those observed at a dose of 800 mg dm^{-3} without vermicompost addition, with significantly greater root accumulation. These findings indicate that vermicompost can increase Cu retention in roots at moderate doses, even while reducing its translocation to shoots. One possible explanation for the response at a dose of 200 mg dm^{-3} is that vermicompost may increase Cu bioavailability within a physiologically manageable range. Kumar et al. (2021) reported that Cu complexation with OM can either reduce or increase mobility depending on chemical speciation and saturation. Thus, OM may have formed soluble Cu−organic complexes that enhanced root uptake while still promoting partial immobilization. Since vermicompost is rich in OM (Abawari, 2022), it can interact with Cu ions, thereby forming chelates that modulate absorption depending on the soil and plant conditions (Ramazanoglu, 2024).

Xu et al. (2024) reported that COPT1/2/5, HMA5, YSLs, and ZIPs are transporters involved in Cu uptake and translocation. Because the addition of vermicompost modified the soil pH, CEC, and OM content, a more favorable rhizosphere environment may have been created, influencing transporter activity. However, under an excessive Cu dose (800 mg dm^{-3}), these regulatory and complexation mechanisms likely became saturated, which explains why vermicompost could not prevent severe toxicity at this concentration.

Finally, OM can improve plant physiological responses in contaminated soils by reducing oxidative stress (Lazzaretti et al., 2024; Lai et al., 2025). Metal complexation reduces ROS formation and lipid peroxidation (Kumar et al., 2021; Ren et al., 2022). Organic amendments can also increase the activity of antioxidant enzymes and nonenzymatic antioxidants such as ascorbic acid (AsA) and glutathione (GSH) (Wu et al., 2025; Zhou et al., 2023), which

mitigate metal-induced oxidative stress (Alves et al., 2023; Zhou et al., 2023). Thus, by promoting a healthier rhizosphere and modulating Cu mobility and oxidative balance, vermicompost increases tolerance at moderate Cu doses, whereas at excessive concentrations (800 mg dm^{-3}), detoxification mechanisms are overwhelmed.

High Cu concentrations generally reduce the absorption of several macronutrients (P, Ca, K, and Mg) through direct root damage, increased permeability/cell death, and changes in transporter function (Kumar et al., 2021). The phosphorus content in chives decreased in the treatments with high doses of Cu (800 mg dm^{-3}), regardless of the addition of vermicompost. However, the addition of vermicompost increased P levels at intermediate doses. Phosphorus is among the elements most affected by excess Cu, as Cu stress decreases P influx activity in roots and alters the expression of P transporters (Xu et al., 2024; Feil et al., 2020).

The solubilization of phosphorus by organic acids released during microbial decomposition is an important mechanism driven by the increased microbial activity facilitated by vermicompost, especially in calcareous soils (Ramazanoglu, 2024). Furthermore, in onions, biochar inoculated with phosphate-solubilizing bacteria (PSB) has been demonstrated to increase the total P content in bulbs, highlighting the ability of these biofertilizers to mobilize and make P available in the soil (Blanco-Vargas et al., 2022). In onion crops (*Allium cepa* L.), the addition of compost also resulted in increased P levels in the leaf tissue (Showler, 2022).

A notable reduction in foliar Ca and Mg concentrations was observed at the highest Cu dose, particularly in the 800 mg dm^{-3} treatment combined with vermicompost. This decrease may be explained by competitive interactions among divalent cations, as excessive Cu^{2+} can interfere with Ca^{2+} and Mg^{2+} uptake through shared transport systems in the roots (Xu et al., 2024). Moreover, a high Cu dose may disrupt membrane integrity and xylem loading, thereby impairing the translocation of these nutrients to the shoots. Because Ca transport depends notably on transpiration-driven xylem flow, any Cu-induced root damage or physiological disturbance may limit its movement to the leaves. These combined mechanisms likely contributed to the nutritional imbalance observed under severe Cu stress.

With respect to sulfur (S), an increase in this element was observed in chive leaves at high copper doses in the soil and when vermicompost was added. Notably, little copper was translocated to the plant shoots. However, the response of the roots differed, as more metal accumulated. The treatments that yielded the greatest accumulation of Cu (200 + verm and 800 mg dm^{-3}) resulted in reduced sulfur levels. This finding can be explained by the fact that excess copper in the roots in these treatments activates response mechanisms that prioritize antioxidant compounds such as thiolates, glutathione, and enzymes involved in S metabolism. This process can reduce the levels of detectable free sulfur (Lacerda et al., 2025).

These findings indicate that the influence of vermicompost on copper tolerance in chives is dose dependent. Severe phytotoxicity at a dose of 800 mg Cu dm^{-3}, characterized by reductions in growth, biomass, and root integrity, could not be effectively mitigated, suggesting a limited buffering capacity under an excessive Cu supply. In contrast, at intermediate doses (200 and 400 mg dm^{-3}), vermicompost improved plant performance, particularly through enhanced biomass production and stimulation of the root architecture, which likely supported more efficient soil exploration and stress management.

Certain limitations should be acknowledged. The experiment was conducted under controlled conditions using a relatively small substrate volume (250 mL) with high plant density, a common approach in chive cultivation, but one that may influence root–soil interactions compared with larger containers or field conditions. In addition, the intrinsic Cu content in vermicompost contributed to initial soil Cu levels after incubation, thus complicating direct comparisons among treatments. Finally, the absence of repeated harvest cycles or field validation limits the extrapolation of the obtained findings to long-term agronomic or environmental scenarios.

Overall, the results of this study demonstrated that vermicompost can regulate Cu availability and improve plant performance under moderate contamination, whereas under an excessive Cu supply, its buffering capacity is restricted. These findings reinforce the importance of considering both metal dose and soil amendment characteristics when evaluating management strategies for Cu-affected soils.

5 Conclusion

Vermicompost modulated copper tolerance and accumulation in chives in a dose-dependent manner. Under a high Cu dose (800 mg dm^{-3}), phytotoxic effects prevailed, and vermicompost did not mitigate growth reduction or tissue Cu accumulation. At a moderate Cu dose (200 mg dm^{-3}), however, vermicompost enhanced plant growth and stimulated root development but increased Cu accumulation primarily in the roots.

Vermicompost also increased the baseline soil Cu content because of its intrinsic metal content, which should be considered in soil management and data interpretation. Although the Cu level in edible tissues increased with increasing soil Cu dose, the values remained below the maximum permissible limits under the conditions evaluated.

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Author's Contributions **Conceptualization:** Luciano Almeida Lima and Thiago Corrêa de Souza; **methodology:** Luciano Almeida Lima, Lucas Boscov Braos, Thiago Corrêa de Souza, and Daniele Maria Marques; **formal analysis:** Luciano Almeida Lima; **investigation:** Luciano Almeida Lima and Beatriz Bárbara Aparecida Pinto; **data curation:** Luciano Almeida Lima and Beatriz Bárbara Aparecida Pinto; **visualization:** Luciano Almeida Lima and Daniele Maria Marques; **writing–original draft:** Luciano Almeida Lima; **validation:** Luciano Almeida Lima and Thiago Corrêa de Souza; **supervision:** Thiago Corrêa de Souza and Lucas Boscov Braos; **resources:** Thiago Corrêa de Souza; **writing–review and editing:** Lucas Boscov Braos, Thiago Corrêa de Souza and Daniele Maria Marques; **project administration:** Thiago Corrêa de Souza; **funding acquisition:** Thiago Corrêa de Souza. All the authors have read and approved the final manuscript.

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Data Availability All the data supporting the findings of this study are available in the paper and its Supplementary Information (available in a spreadsheet).

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Competing interests The authors declare no competing interests.

Clinical Trial Number Not applicable.

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