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Article

Vermicompost in the Phytoremediation of Copper by Black Oats (*Avena strigosa* SCHREB, 1771*)*, in the Leaching of Nutrients and Heavy Metals in a Sandy Soil

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RESUMO
Fungicidas cúpricos são aplicados em videiras para prevenção e controle de doenças fúngicas, porém, podem contaminar o solo comr cobre, o que demanda técnicas como a fitorremediação para a solução deste problema. O objetivo deste estudo foi avaliar doses de vermicomposto na fitorremediação do cobre por *Avena strigosa*, e na lixiviação em solo arenoso. As plantas de *A. strigosa* foram cultivadas em vasos na casa de vegetação, em um solo arenoso de vinhedo contaminado com 25 mg kg^{-1} de cobre. Os tratamentos constituíram-se das doses de vermicomposto equivalentes a 0, 5, 10, 20 e 40 ton ha^{-1}. Lixiviações foram induzidas a cada 15 dias após a semeadura. No florescimento, foram determinadas massa seca, atividade de enzimas do estresse oxidativo, teores de nutrientes e metais pesados. O vermicomposto reduziu a fitotoxidade de cobre, e na dose de 10 ton ha^{-1} aumentou a produção de massa seca e o acúmulo de cobre, em comparação ao solo sem vermicomposto. Na dose de 10 ton ha^{-1} o lixiviado apresentou concentrações de cobre, zinco, manganês e fósforo inferiores às doses de 20 e 40 ton ha^{-1}. Elevadas doses de vermicomposto não devem ser utilizadas na fitorremediação de solos arenosos devido ao risco de contaminação subsuperficial por metais pesados.
Palavras-chave: contaminação; vinhedo; adubo orgânico; aveia preta; biorremediação.

ABSTRACT
Copper-based fungicides are applied to grapevines for the prevention and control of fungal diseases; however, they can contaminate the soil with copper, necessitating techniques such as phytoremediation to address this issue. The aim of this study was to evaluate vermicompost doses in the phytoremediation of copper by *Avena strigosa* and the leaching of metals in sandy soil. *Avena strigosa* plants were grown in pots in a greenhouse, in sandy vineyard soil contaminated with 25 mg kg^{-1} of copper. The treatments consisted of vermicompost doses equivalent to 0, 5, 10, 20, and 40 ton ha^{-1}. Leaching was induced every 15 days after sowing. At flowering, dry mass, oxidative stress enzyme activity, and nutrient and heavy metal contents were determined. Vermicompost reduced copper phytotoxicity, and at the dose of 10 ton ha^{-1}, it increased dry mass production and copper accumulation, compared to the soil without vermicompost. At the 10 ton ha^{-1} dose, the leachate showed concentrations of copper, zinc, manganese, and phosphorus lower than

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those at the 20 and 40 ton ha^{-1} doses. High doses of vermicompost should not be used in the phytoremediation of sandy soils due to the risk of subsurface contamination by heavy metals.
Keywords: contamination; vineyard; organic fertilizer; black oat; bioremediation.

Introduction

Soil contamination with copper (Cu) occurs in several countries due to mining activities, improper waste disposal, the addition of fertilizers, and the application of phytosanitary products (Campillo-Cora et al. 2019; Chileshe et al. 2020; Covre et al. 2022). In vineyard areas, this contamination is amplified by the successive use of high doses of copper fungicides, such as Bordeaux mixture ($Ca(OH)_2+CuSO_4$) (Morsch et al. 2024). This problem has been widely reported in the wine-growing regions of Campanha Gaúcha, in the far south of Brazil (Silva et al. 2022b), where the predominantly sandy soils in this region have a low CEC (Cation Exchange Capacity), low clay and organic matter content, favoring the mobility of Cu and increasing its phytotoxic potential for plants and the environment (Morsch et al. 2024).

Cu is an essential micronutrient for living organisms, as it acts as an enzymatic cofactor in essential processes such as photosynthesis, respiration and the electron transport chain, as well as composing defense genes and structural proteins (Kumar et al. 2021; Mir et al. 2021a). However, an excess of this metal in the soil can compromise plant growth and productivity (Mir et al. 2021a), causing destabilization of the cell membrane, reduced photosynthesis and altered enzyme activity, which can lead to plant death (Shabbir et al. 2020). In vineyards, when present in high concentrations, Cu can cause morphological and physiological changes in both vines and ground cover plants (De Conti et al. 2019). Studies indicate that uncontaminated agricultural soils have natural Cu levels between 2 and 20 mg kg^{-1}, while values above 30 mg kg^{-1} in sandy soils are already considered contaminants and can negatively affect vegetation (De Conti et al., 2018; Morsch et al., 2024). In vineyards in southern Brazil, levels higher than 35 mg kg^{-1} have been detected, highlighting the need for remediation strategies (Schwalbert et al., 2021).

Phytoremediation is a promising strategy for reducing the availability and mobility of Cu in contaminated soils, allowing it to be extracted or stabilized by plants adapted to these environments (Marques et al. 2023). However, even phytoremediating species can face growth difficulties in highly contaminated soils, making it necessary to apply mitigating materials that support plant establishment and reduce metal toxicity. Vermicompost, for example, has been used for this purpose due to its high organic matter content, which is capable of complexing heavy metals and modifying their availability in the soil, stimulating biological activity and promoting plant growth (Santana et al. 2018; Rangel et al. 2023). Vermicompost can also reduce the mobility of heavy metals in the soil due to the presence of humic substances and negatively charged functional groups, such as carboxylic acids, phenolic and alcoholic hydroxyls, which form complexes with heavy metals from the soil (Wang et al. 2018).

The leaching of chemical elements is a determining factor in assessing the efficiency of phytoremediation, especially in sandy soils, where the mobility of nutrients and heavy metals is high due to the soil's low CEC and greater permeability (Bolan et al., 2021). The addition of vermicompost can directly influence this process, as it contains substances such as humic and fulvic acids, which can both immobilize heavy metals and increase their solubility and leaching (Hamedi et al., 2021). Studies show that leached copper can be transported in the form of soluble complexes with dissolved organic matter, increasing the risk of groundwater contamination (Filipović et al., 2023). In addition, phosphorus (P), often present in high concentrations in vermicompost, can be mobilized in the soil, intensifying the risk of eutrophication of water bodies (Jordán et al., 2020). Elements such as zinc (Zn) and manganese (Mn) can also become more mobile due to the mineralization of organic matter, leading to losses of essential nutrients for plants and possible environmental toxicity (Facco et al., 2023). Thus, evaluating the leaching of Cu, P, Zn and Mn is essential to understand the impacts of applying vermicompost to contaminated soils and ensure that this phytoremediation strategy does not pose additional environmental risks (Shrestha et al., 2019).

The use of vermicompost as an ameliorator in the phytoremediation of Cu-contaminated soils is a topic that still needs further study, especially regarding to the leaching of heavy metals in sandy soils. The hypothesis of this study is that vermicompost reduces the phytotoxic effects of Cu, increases plant growth and does not promote the leaching of metals and phosphorus from the contaminated soil. Given this context, the aim of this study was to verify the effect of increasing doses of vermicompost on the phytoremediation of Cu by black oats and on the leaching of nutrients and heavy metals in a sandy vineyard soil.

Material and Methods

Soil and vermicompost

The soil classified as Acrisol (IUSS Working Group WRB 2015) was collected at a depth of 0-20 cm from a vineyard in Campanha Gaúcha, in the town of Santana do Livramento, Rio Grande do Sul, Brazil (30°48'27"S and 55°22'42"W). The soil was air-dried, sieved through a 2 mm mesh, analyzed according to Table 1 and then the pH was corrected to 6.0, as recommended by the CQFS 2016, with calcium carbonate, according to technical recommendations for growing oats. The soil was then incubated with around 150 kg of soil for 30 days with humidity controlled at 70% of field capacity by adding distilled water when necessary. Contamination of the soil with 25 mg $kg^{-1}$of Cu (Brunetto et al., 2023) was carried out in the laboratory by adding copper sulphate (33%) and copper chloride (66%), followed by incubation for 30 days, adding up to 60 days of soil incubation from the start of the process. Previous studies have quantified Cu levels of ~35 mg Cu kg^{-1}Mehlich$^{(-1)}$in sandy soils from vineyards in the Campanha Gaúcha region (Schwalbert et al. 2021; Brunetto et al. 2023; Morsch et al. 2024) and reported toxicity in cover crops between the rows of vineyards (Miotto et al. 2017). Based on this information, it was decided to use a Mehlich^{-1}content of 25 mg Cu kg^{-1}, higher than the natural values observed in most Brazilian soils, but close to the Cu levels observed in vineyard soils in this region.

The vermicompost was produced from an organic compost containing 70% (v/v) rice husk and 30% (v/v) dairy cattle manure (Table 1; Vione et al., 2018). The composting process was carried out over 60 days, with frequent turning and moistening by means of manual watering in order to keep the water content between 40 and 60%. Once the mesophilic temperature had stabilized, the organic compost was sent to the vermicomposting process, which took place in covered masonry beds. 1,718 adult earthworms (0.2 m^3 of compost) of the *Eisenia andrei* Bouché (1972) species were inoculated. Vermicomposting took place for 150 days at a gravimetric humidity of ~80%.

Plant cultivation and experimental design

The black oats (*Avena strigosa* Schreb, 1771) were grown in a greenhouse, in pots made from 2-liter PET bottles, with the neck facing downwards, making it easier to collect the leachate. 1.5 kg of copper-contaminated soil was added to . The experimental design was entirely randomized, with five treatments, corresponding to doses of 0, 5, 10, 20 and 40 tons $ha^{-1}$of vermicompost and five replications. Thus, each pot received 0, 3.75, 7.5, 15 or 30 g of vermicompost, respectively. In the treatment without vermicompost (dose 0), the soil received 7 mg $kg^{-1}$of P and 8.8 mg $kg_{(}^{-1})$ of K via a solution of $KH_2PO_4$to allow the production of sufficient dry mass for the analyses (CQFS 2016). Sowing was carried out at the beginning of April, with seven seeds per pot, leaving three seedlings after thinning. Soil moisture was adjusted daily by weighing the pots and adding distilled water. The crop was harvested at flowering, 52 days after emergence.

Leachate collection and analysis

To collect the leachate, around 300 mL of distilled water was added per pot, which caused the soil to become saturated. For 2 hours, all the leachate was collected in bottles placed below the pots planted with black oats. The procedure was carried out on days 01, 15, 30, 45 and 52 after sowing. The leachate was stored at 4 °C until the pH, Cu, Zn, Fe and Mn contents were determined using an atomic absorption spectrophotometer (932 AA, GBC, Australia) and P by colorimetry (Murphy and Riley 1962).

Antioxidative enzymes

At 50 days after emergence, a whole leaf from each plant was collected, immediately placed in a container with liquid N_2 and then stored in an ultrafreezer (-80 °C) to determine the activity of the enzymes superoxide dismutase (SOD) and non-specific peroxidases (POD) (Zeraik et al. 2008). The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined according to Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of the photoreduction of nitro blue tetrazolium (NBT 50%) (Beauchamp & Fridovich 1971). The activity of non-specific peroxidases (POD, EC 1.11.1.7) was determined according to Zeraik et al. (2008), using guaiacol as a substrate and a molar extinction coefficient of 26.6 mmol L 1cm$^{(-1)}$ $^{(}$Chance & Maehley 1955). A POD unit is defined as the amount of enzyme that catalyzes the conversion of guaiacol and hydrogen peroxide to form 1 *µmol* of tetraguaiacol min $^{-1}$mL $^{(-1)}$) of extract at 470 nm (SF325NM, Bel Engineering, Italy).

Dry mass and chemical analysis of plants and soil

The plants were collected at flowering and the roots were separated manually, washed in running water, in a 0.02 mol L^{-1}EDTA solution and finally in distilled water. The aerial part and roots were dried in an air-circulating oven at 65°C until constant mass. The contents of Cu, P, K, Mg, Fe and Zn in the aerial part and Cu in the root were determined using an atomic absorption spectrophotometer (932 AA, GBC, Australia) after nitric-perchloric digestion (Teixeira et al., 2017). The N content in the aerial part was determined using the Kjeldahl^{-1}method (Bremner & Mulvaney 1982) after sulfuric digestion of the tissues. The accumulated Cu content (metal content × biomass) in the aerial part (E) and roots was also determined. The Cu, Zn, Fe, Mn, P and K contents of the soil were extracted using Mehlich-3. K was determined using a flame photometer (DM-62, DIGIMED, Brazil), P by colorimetry and the others using an atomic absorption spectrophotometer.

Statistical analysis

The results were subjected to analysis of variance (ANOVA) and the means were compared using the Tukey test ($p < 0.05$) and regression analysis in the R software (R Core Team 2020). Finally, the main component analysis (PCA) was carried out and the correlation matrix between the parameters evaluated was constructed. Spearmann's linear correlation coefficients were calculated for the matrix, with a 5% significance level.

Results

The vermicompost used as a fertilizer for growing black oats had characteristics that allowed it to be added to the soil, in accordance with the minimum parameters established by Brazilian legislation (Brazil 2020) (Table

1). The high P and K contents of this organic fertilizer stand out, which consequently caused significant increases in the soil, as well as the Mg and Mn contents (Table 1). However, the concentrations of available Cu, Zn and Fe in the soil did not differ as the doses of vermicompost added to the soil increased in the evaluation carried out after the black oats were grown (Cu $p=0.139$ and Zn $p=0.544$).

Table 1. Vermicompost and soil characteristics at the start of the experiment and after cultivation with *Avena strigosa* in soil contaminated with 25 mg kg^{-1} of Cu and vermicompost doses of 0, 5, 10, 20 and 40 ton ha^{-1}.

Parameter	Vermicompost	Starting soil	Soil after cultivation (dose, t ha^{-1})				
			0	5	10	20	40
pH	7.2	6.8	6.6ns	6.5 ns	7.1ns	7.1ns	6.8ns
M.O. (%)	n.a.	1.20	n.d	n.d	n.d	n.d	n.d
C.O. (g kg^{-1}) [1]	170	n.d	n.d	n.d	n.d	n.d	n.d
Total N (%)	1.1	n.d	n.d	n.d	n.d	n.d	n.d
Clay (%)	n.a.	12	n.d	n.d	n.d	n.d	n.d
Sand (%)	n.a.	54	n.d	n.d	n.d	n.d	n.d
$Al^{(3+)}$ (cmolc L^{-1})	nd	0.10	n.d	n.d	n.d	n.d	n.d
CTC	n.a.	4.90	n.d	n.d	n.d	n.d	n.d
P (g kg^{-1}) [1]	4.00	n.d	5.53d	10.83cd	12.86c	21.45b	36.28a
K (g kg^{-1}) [1]	4.00	n.d	22.60b	22.00b	22.20b	24.00b	32.20a
Ca cmolc L^{-1}	0.48	2.07	n.d	n.d	n.d	n.d	n.d
Mg (mg kg^{-1})	1.20	60.70	111.34c	134.83c	140.27c	184.36b	259.77a
Cu (mg kg-1)	131.50	24.80	35.20ns	36.10 ns	35.10 ns	34.77 ns	35.10 ns
Zn (mg kg-1)	64.90	7.90	13.95ns	11.44 ns	15.73 ns	13.57 ns	16.12 ns
Fe (mg kg-1)	34.50	n.d	18.03ns	18.90 ns	19.08 ns	19.31 ns	18.73 ns
Mn (mg kg-1)	201.60	n.d	99.03ab	94.74b	98.87ab	97.36ab	104.67a

n.a.: not determined.

*Means followed by the same letter in the line do not differ by Tukey's test with $p<0.05$. [ns]Not significant.

Source: Elaborated by the authors

Plant growth and copper concentration

The addition of vermicompost to the soil increased the dry mass of the aerial part and roots of the black oat plants compared to mineral fertilization ($p<0.05$; Figure 1A and Figure 1B). These increases were up to 50% in the aerial part and in the roots. The Cu content in the aerial part and roots of the black oats did not change in the presence of the different doses of vermicompost ($p_{(aerial\ part)} = 0.187$; $p_{root}=0.09$; Figure 1C and Figure 1D). However, compared to the treatment without vermicompost (V0), the addition of vermicompost to the soil increased phytoextraction (dry mass x Cu content in the tissue) by up to 45% in the aerial part (Figure 1E).

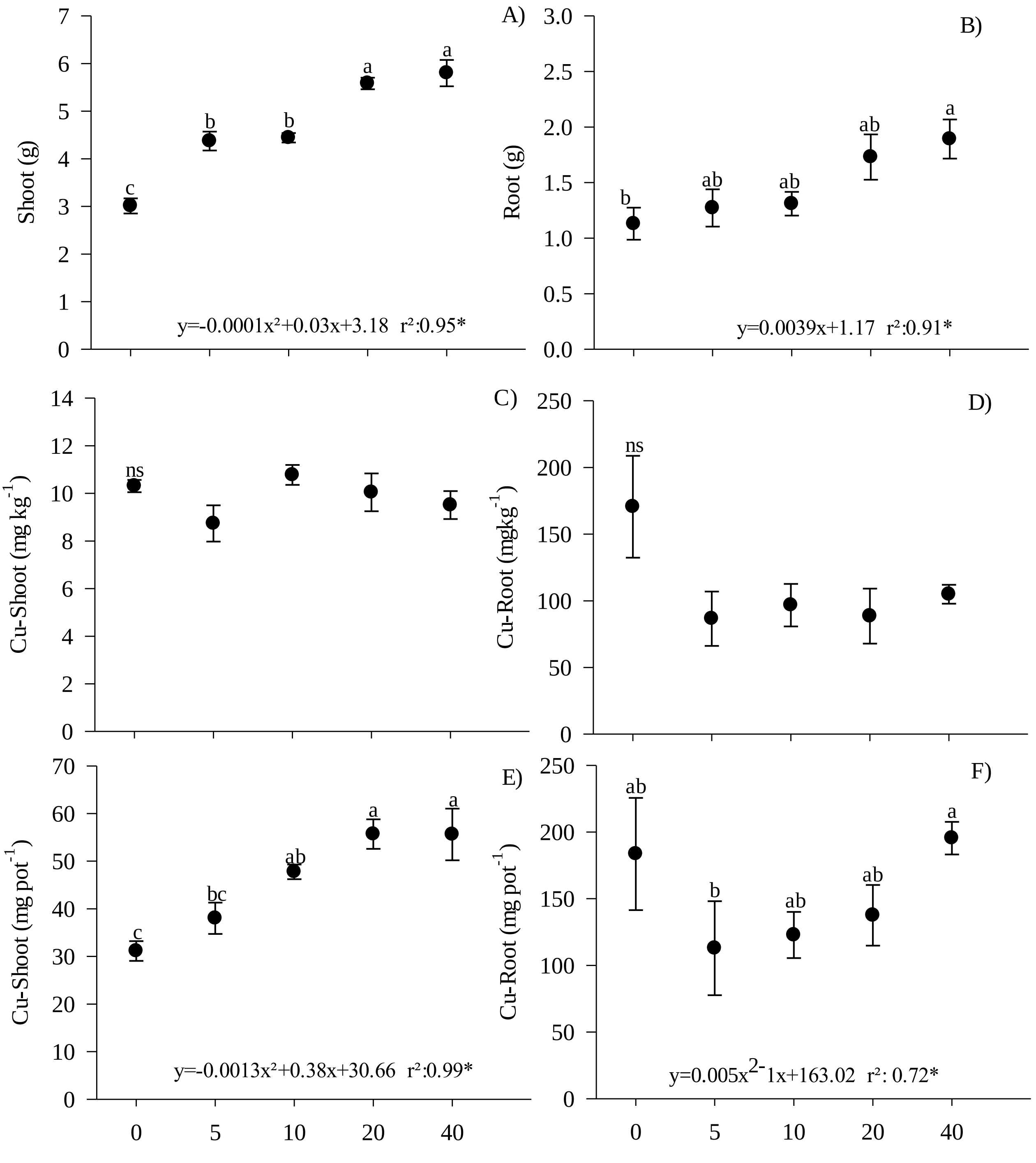

Figure 1 - Dry mass of aerial part (A) and roots (B), Cu content in aerial part (C) and roots (D), and accumulated Cu content (metal content × biomass) in aerial part (E) and roots (F) of *Avena strigosa* grown in sandy soil after contamination with 25 mg kg^{-1}of Cu and addition of vermicompost in doses equivalent to 0, 5, 10, 20 and 40 ton ha^{-1}. Averages followed by the same letter do not differ by the *Tukey* test with *p*< 0.05. * significant with *p*<0.05. ns: not significant.

Source: Elaborated by the authors

Activity of oxidative stress enzymes

The black oat plants that received 20 and 40 tons ha^{-1}of vermicompost showed higher activity of the enzyme superoxide dismutase (SOD) in the leaves, on average 43% higher than the other treatments (Figure

2A). However, higher activities of non-specific peroxidases (POD) were observed in the leaves of plants grown at doses of 10 and 20 tons ha$^{(-1)}$ (Figure 2B).

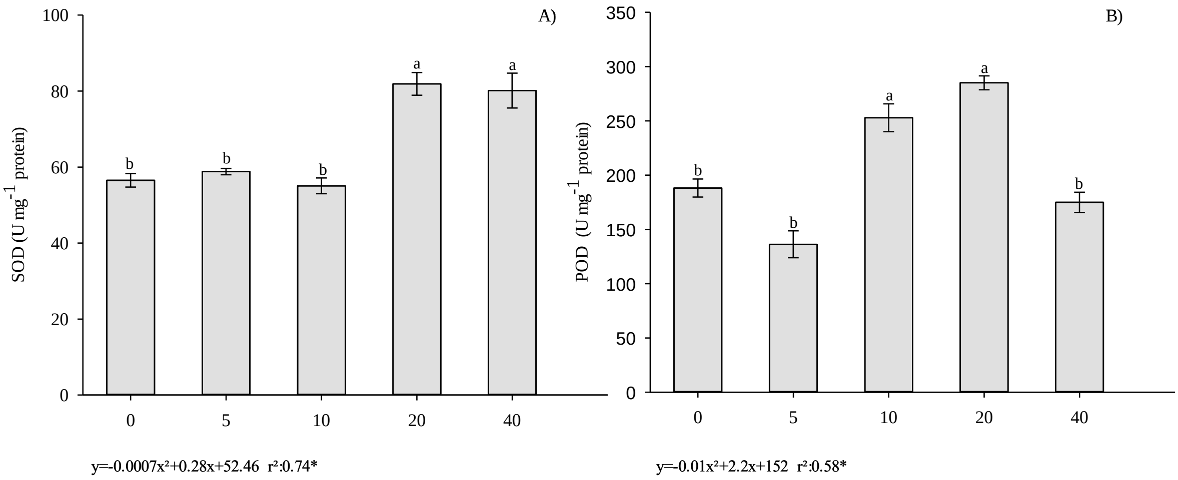

Figure 2. Activity of the enzymes superoxide dismutase (SOD) and non-specific peroxidases (POD) in leaves of *Avena strigosa* grown in sandy soil after contamination with 25 mg kg^{-1}of Cu and addition of vermicompost in doses equivalent to 0, 5, 10, 20 and 40 ton ha^{-1}. *significant at $p<0.05$. ns: not significant. Averages followed by the same letter do not differ by the *Tukey* test with $p< 0.05$. Source: Elaborated by the authors

Leachate

On the first date (one day after the experiment was set up) when the induced leaching of elements from the soil was carried out by adding excess water, copper leaching was low, at less than 300 μg L$^{(-1)}$ (Figure 3a). In the second evaluation (at 15 days), the concentration of Cu in the leachate reached its highest value, although there were no statistical differences between the treatments. In this collection there was a linear increase in the concentration of copper in the leachate between the 0 and 20 t ha^{-1}doses and at this dose the maximum value of copper in the leachate quantified throughout the experiment was observed, which was 2,924 μg L^{-1}. Increasing the dose of vermicompost to 40 t ha^{-1}did not increase leaching, but kept it high (2,032 μg L$^{-1)}$. In the last evaluation (52 days) there was a statistically significant linear increase in the concentration of Cu in the leachate as the dose of vermicompost increased. However, all values were lower than those observed at 15 days (average of 395.6 μg L$^{-1)}$. In the treatment without vermicompost, the total leached copper content was 3,103 μg L^{-1}, while in the doses of 20 and 40 t ha^{-1}these values were 5,360 and 4,777 μg L^{-1}, respectively.

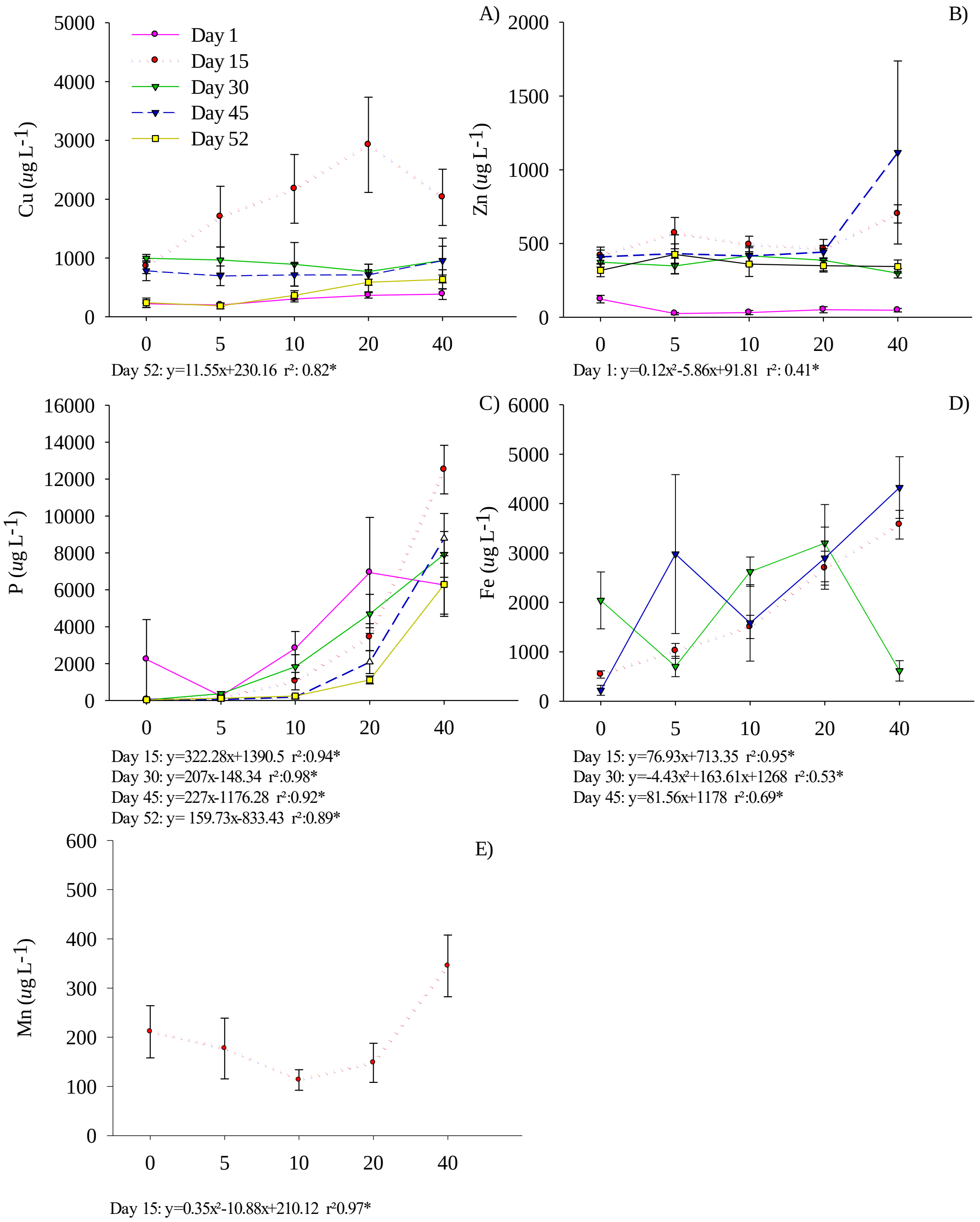

Figure 3: Content of Cu (A), Zn (B), P (C), Fe (D) and Mn (E) in the leachate collected at 01, 15, 30, 45 and 52 days after sowing *Avena strigosa* in sandy soil contaminated with 25 mg kg^{-1}of Cu and with doses of 0, 5, 10, 20 and 40 tons ha^{-1}of vermicompost. * significant at p<0.05. ns: not significant. Averages followed by the same letter do not differ by the *Tukey* test with p< 0.05. * significant with p<0.05. ns: not significant. Source: Elaborated by the authors

The concentration of zinc in the leachate was low in the evaluation carried out on the first day after the experiment was set up in all treatments (average of 280 μg L^{-1}) (Figure 3B). However, concentrations increased in the following evaluations. At 15 days, the average concentration was 490 μg L^{-1}, with no statistical difference between the treatments, but with an upward trend in response to the increase in the vermicompost dose. In the evaluation carried out at 52 days, there was a non-significant increase ($p=0.339$) in the concentration of Zn in the leachate from the soil that received the highest dose, reaching 552 μg L^{-1}.

The concentration of P in the leachate increased in response to the increase in the dose of vermicompost added to the soil (Figure 3C). In the first evaluation, the highest value (6,670 μg L^{-1}) was observed at a dose of 20 tons ha^{-1}, although there were no statistical differences between the treatments. In the different samples, there was a statistically significant increase in the concentration of P in the leachate as a result of increasing doses of vermicompost. At the highest dose (40 ton ha^{-1}), the total concentration of phosphorus in the leachate was 40,520 μg L^{-1}, while at the lowest dose (5 ton ha^{-1}), it was 1,966 μg L^{-1}, lower than in the control (2,340 μg L^{-1}), which had a higher concentration due to the application of mineral phosphate fertilizer in this treatment.

On days 01 and 52, the Fe concentrations were below the limit detectable by the methodology used. At 15 and 45 days, there was a linear increase in the concentration of Fe in the leachate, especially at the higher doses of vermicompost (20 and 40 tons ha^{-1}; Figure 3D). The total amount of Fe in the leachate was around 95% higher in the 20 and 40 ton ha^{-1}doses of vermicompost compared to the control. Mn was not detected on the first leaching date. On the second date, the highest concentration occurred in the leachate from the highest dose of vermicompost (40 ton $ha^{-1)}$, where an increase of approximately 200 μg $L^{-1}$was observed in relation to the average of the other treatments (Figure 3E).

Principal Component Analysis

PCA explained 56.4% of the variability in the data (Figure 4). Most of the variation in the original data set (35.3%) was explained by the first component (Factor 1), which was mainly associated to the dry mass of roots and aerial part; K, P and Mg in the soil; K and Mn in the tissue; Cu and P contents in the leachate; and SOD and POD activity. These variables were related to the higher doses of vermicompost (20 and 40 tons $ha^{-1)}$, which increased plant growth and attenuated the toxic effects of Cu through greater nutritional input and/or by increasing the activity of antioxidative enzymes. SOD activity showed a positive correlation with P content in the tissue (ϱ: 0.62) and a negative correlation with Mn content (ϱ:-0.70). The higher nutrient levels in the soil (K, Mg and P) at the higher doses showed a positive correlation with better P nutrition (Pshoot) and plant biomass production (Root and Shoot; Figure 5). On the other hand, these treatments showed the highest levels of Cu and P in the leachate, indicating a possible risk of contamination. The second component of the PCA explained 20.3% of the variation in the data set and was related to the levels of Cu, Fe, Mn and Zn in the soil and N, Fe, Zn and Cu in the tissue. The high Cu content in the soil and tissue resulted in reduced plant growth ($\varrho_{root\ \&\ Cu}$: -0.59; $\varrho_{shoot\ \&\ Cu}$: -0.37; $\varrho_{root\ \&\ Cushoot}$: -0.24; $\varrho_{(shoot\ \&\ Curoot):}$-0.42; $\varrho_{(shoot\ \&\ Cushoot)\ :}$-0.22; $\varrho_{(root\ \&\ Cushoot)\ :}$-0.46). The treatments with the lowest doses (5 and 10 tons ha^{-1}) of vermicompost showed a relation with these variables. The Mn content in the aerial part of the plant was the only variable related to the treatment without adding vermicompost.

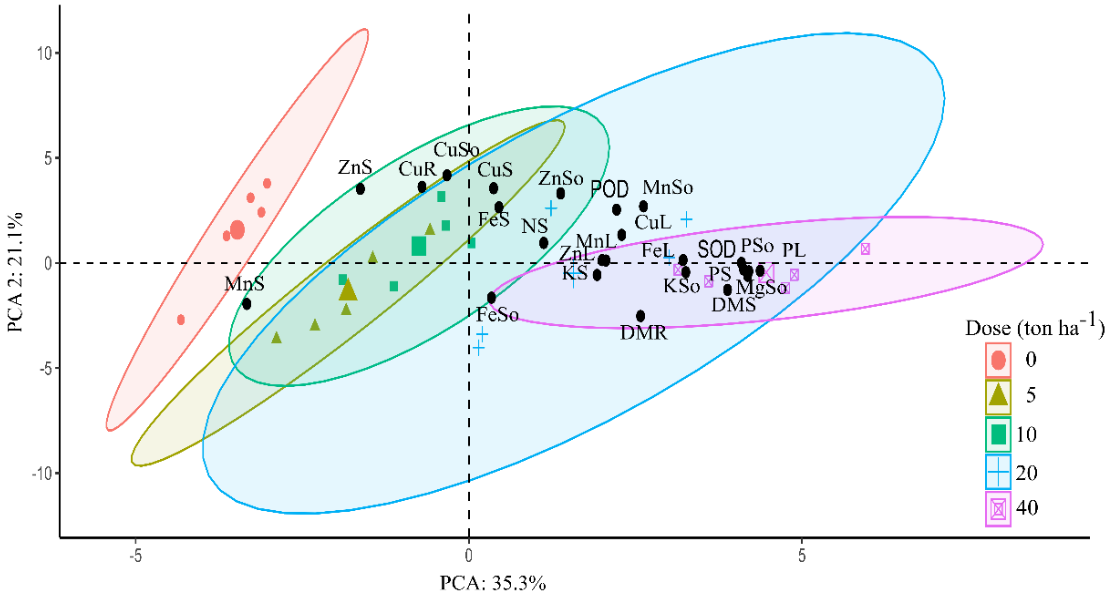

Figure 4. Principal component analysis considering&Principal component analysis considering the variables dry mass of aerial part (DMS), root (DMR), content of copper (CuS), zinc (ZnS), iron (FeS), manganese (MnS), nitrogen (NS), potassium (KS) in aerial part, copper in root (CuR), activity of peroxidase (POD) and superoxide dismutase (SOD) in leaf tissue, levels of copper (CuSo), zinc (ZnSo), iron (MnSo), manganese (MnSo), phosphorus (PSo) and potassium (KSo) in the soil, concentrations of Cu (CuL), Zn (ZnL), P (PL), Mn (MnL) and total Fe (FeL) in the leachate and the vermicompost doses of 0, 5, 10, 20 and 40 tons ha^{-1}. * significant with $p<0.05$. ns: not significant. Source: Elaborated by the authors

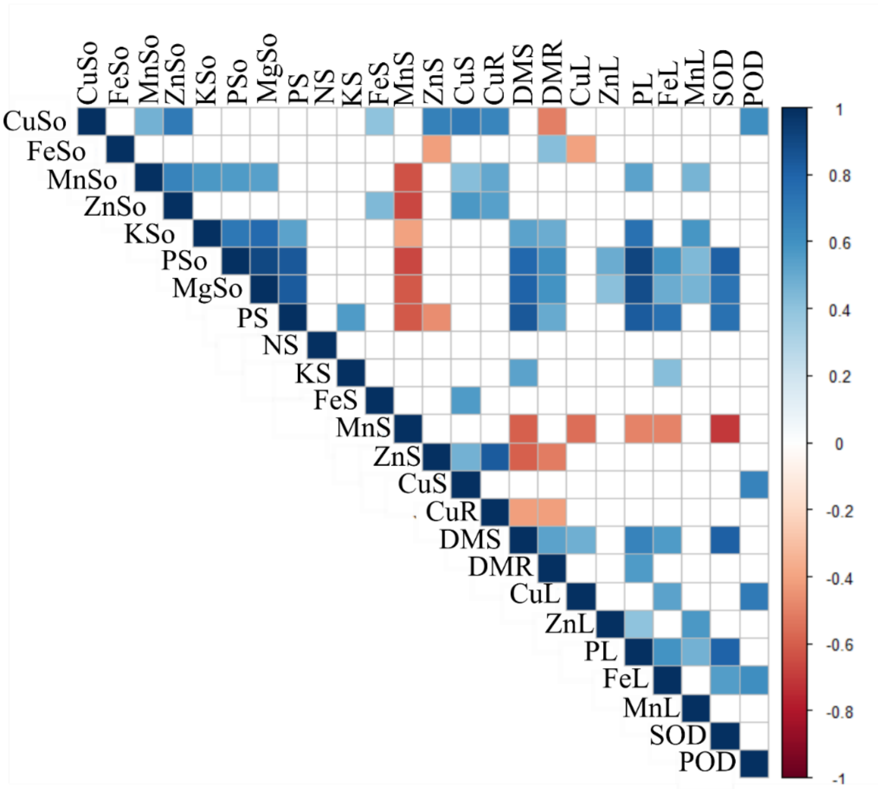

Figure 5 *Spearman*'s correlation matrix considering plant, soil and leachate variables and vermicompost doses of 0, 5, 10, 20 and 40 tons ha^{-1}. Blank squares (not filled in) are not significant. Source: Elaborated by the authors

Discussion

Black oats are commonly used as a ground cover plant in vineyards, including as a phytostabilizer for soils contaminated with copper (Trentin et al. 2022). However, when there is an excess of Cu, there can be a reduction in the photosynthetic rate and an alteration in enzyme activity, which results in growth inhibition, as well as other effects that can lead to plant death (Shabbir et al. 2020; Mir et al. 2021a). Tissue contents above 30 mg Cu kg^{-1}can cause physiological stress and hinder plant development (Kumar et al., 2021). De Conti et al. (2018) observed that from a soil content of 31.5 mg $kg^{-1}$of Cu (extracted by EDTA), black oat plants reduced growth and dry mass production.

The characteristics of the vermicompost used in this research were within the limits set by international organizations (Brinton 2000; CPHEEO 2016). This organic fertilizer has already shown potential as a mitigator of excess Cu in the soil, especially in soils with acid pH, low levels of organic matter and sandy soils (Santana et al. 2018; Ehiomogue 2023). In a study using vermicompost in acidic soils, Liu et al. (2019) observed a reduction in the availability of heavy metals. Wang et al. (2024) tested the combined application of vermicompost with steel waste in an acid mining soil by copper sulphide and found that the mixture effectively reduced the bioavailability of Cu in the soil, increasing the effectiveness of phytoremediation by the accumulation of copper in *Lolium perene*.

This study showed that vermicompost increased nutrient levels in the soil and promoted greater plant growth, reducing the phytotoxic effects of Cu, especially in the higher doses (Figure 1; 20 and 40 ton $ha^{-1)}$, where plant biomass was higher than in the absence of vermicompost. In addition, the lower activity of the enzymes SOD and POD in the lower dose treatments shows that there was an improvement in the plant's biochemical defenses against excess Cu. The increase in the amount of biomass also resulted in a significant increase in the total accumulated Cu content in the black oat tissues (dry mass x Cu content in the tissue). Similar results were described by Silva et al. (2022a) in black oats grown in soils contaminated by heavy metals, under fertilization with pig manure. The authors observed greater biomass production and phytoextraction of Cu, Zn, Ni and Pb, without phytotoxic damage to the plant (Silva et al. 2022a).

The activity of antioxidant enzymes, such as SOD and POD, increased proportionally to the increase in nutrient content in the plant tissue, as observed in the higher doses of vermicompost (Figure 2; Shahkolaie et al. 2020). However, when plant biomass is related to Cu absorption, there is a proportional relationship between the reactivity of these enzymes and the accumulated Cu content. The accumulation of Cu in the leaves characterizes oxidative stress by increasing the formation of reactive oxygen species, which consequently unbalances the production of enzymes (Li et al. 2019b; Shahkolaie et al. 2020; Mir et al. 2021b). SOD is the first enzyme in the cellular detoxification process, as it rapidly converts superoxide radicals into $H_{(2)}$ $_{O2,}$which are subsequently degraded by the activity of POD (Shahkolaie et al. 2020; Schwalbert et al. 2021).

In the treatments with the lowest doses of vermicompost, the plants had higher levels of Mn and Zn in the aerial part, which decreased significantly as the dose increased (Supplementary material). The accumulation of these metals can indicate potential damage to the plant's metabolism (De Conti et al. 2019; Zeng et al. 2019), which in this study was corrected by the addition of vermicompost.

The use of high doses of vermicompost (20 and 40 tons ha^{-1}), despite promoting plant growth, increased the concentration of P, Cu, Zn, Mn and Fe in the leachate (Figure 3). This indicates that using this amount of vermicompost could result in contamination of subsurface water. Investigations into the leaching of heavy metals in sandy soils are essential to assess the mobility of these elements in the soil profile following the application of organic ameliorants (Fang et al. 2017). The binding of copper or copper-containing molecules to soluble C added to the soil by vermicompost can increase the leaching of this metal. In addition, the soil that has received the vermicompost tends to have greater porosity and hydraulic conductivity, which results in greater leaching (Bagheri et al. 2021).

According to Brazilian regulations, concentrations of Cu above 2,000 μg L^{-1}indicate groundwater improper for human consumption (CONAMA 2008). Some of the values found in this investigation exceed the established limits, which would indicate potential environmental damage, but more studies are needed to be carried out, especially considering undeformed soil samples, as the maintenance of soil structure has a direct influence on the flow of water in the soil and the leaching of chemical elements into the profile.

The linear increase in vermicompost doses is reflected in the increase in Cu concentration in the soil. This can be explained by the high concentration of this metal in the vermicompost (131.5 mg kg^{-1}; Table 1). Dairy cattle usually have their diet supplemented with Cu, Zn and Mn, and these elements, in excess, are eliminated in the feces, becoming part of the vermicompost (Daniel et al. 2023). Cipoleta et al. (2019) found increased leaching of Cu, above the limit recommended by legislation, in the highest doses of leonardite, a type of humus produced from fossilized peat. Filipović et al. (2023) correlated high concentrations of Cu in leachate from vineyard soils with high concentrations of dissolved organic carbon.

The leached levels of Zn, Fe and Mn (Figure 3) also increased, quadratically for Zn and Mn, and linearly for Fe. However, Zn remained within the limits recommended by the legislation. For Fe, Mn and Zn, the Brazilian legislation on groundwater for human consumption sets limits of 300 μg L^{-1}, 100 μg $L^{-1}$and 5,000 μg $L^{(-1)}$, respectively (CONAMA 2008). The Fe values observed (Figure 3E) were higher than those required by Brazilian legislation, especially in the higher doses of vermicompost, reaching a value of 8.514 μg L^{-1}. The Mn value observed at 15 days at a dose of 40 tons ha^{-1} also exceeded the legal limit. The same situation was observed by Facco et al (2023), who found that the addition of vermicompost led to an increase in Mn in the soil solution when compared to the control treatment.

It was observed that the higher doses of vermicompost had higher P leaching (Figure 6), demonstrating the impossibility of using them on sandy soil. Excess P can result in the transport of other ions linked to phosphate from surface runoff or percolation in the soil profile (Jordán et al. 2020).

High doses of vermicompost (20 tons ha^{-1}or more) promoted greater plant growth, but resulted in increased leaching of nutrients and heavy metals, indicating that the best option for this type of soil is an intermediate dose of vermicompost, close to 10 tons ha^{-1}. At this dose, there was satisfactory growth of the aerial part and root of the plant, above that observed in the treatment with mineral fertilizer and accumulation of Cu in the root and aerial part very similar to that observed in the highest dose of vermicompost. According to Shrestha et al (2019), due to the high risk of surface and groundwater pollution resulting from the leaching of metals and nutrients, the use of organic compounds for phytoremediation must consider the effects on plant growth and, likewise, the concentration of nutrients that can be leached. Vermicompost, even at low doses, can be effective in phytoremediating plant growth in soils contaminated with Cu. Santana et al (2015) observed that low doses of grape pomace vermicompost (equivalent to 20 mg $kg^{-1}$of P) favoured the phytostabilization of Cu by *Canavalia ensiformis* plants in sandy soil.

As observed in this study, the best application dose was 10 tons/ha, as it promoted the growth of black oats without causing significant leaching of the components present in the vermicompost. This approach could be adopted in other vineyards or in similar areas similar from this study.

For future studies, it is recommended to test other mitigants associated with vermicompost, such as biochar, for example, analyzing not only the mixture between the two, but also the ideal doses and the association with arbuscular mycorrhizal fungi. In addition, field research is recommended to validate the results under real conditions and assess the long-term effects. Studies involving the use of other plant species with phytoremediation potential are also recommended.

Conclusion

The vermicompost dose of 10 ton $ha^{-1}$is a strategy for increasing the biomass production of black oats in phytoremediation programs for sandy soils contaminated with copper. In addition, it does not result in high leaching of nutrients and heavy metals into the soil profile. On the other hand, doses of vermicompost greater than 20 tons ha^{-1}, despite providing even greater growth for the black oat plants, result in high levels of copper, zinc, manganese, and phosphorus leaching into the soil profile, and should not be used in the phytoremediation of sandy soils.

References

Bagheri H, Zare Abyaneh H, Izady A 2021. Nutrient and colloid leaching from un-amended versus vermicompost-amended soil. *Soil and Tillage Research* 213: 105092. https://doi.org/10.1016/j.still.2021.105092

Beauchamp C, Fridovich, I 1971. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Analytical Biochemistry* 44: 276-287. http://dx.doi.org/10.1016/0003-2697(71)90370-8

Brazil 2020. Normative Instruction No. 61, of July 8, 2020. Establishes the rules on definitions, requirements, specifications, guarantees, tolerances, registration, packaging and labeling of organic fertilizers and biofertilizers intended for agriculture.

Bremner JM, Mulvaney, CS 1982. Nitrogen-Total. In: *Methods of soil analysis. Part 2. Chemical and microbiological properties*, Page, A.L., Miller, R.H. & Keeney, D.R. Eds., American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin, 595-624.

Brinton WF 2000. Compost quality standards and guidelines, Final Report by Woods End Research Laboratories for the New York State Association of Recyclers.

Brunetto G, Marques ACR, Trentin E, Sete PB, Soares CRFS, Ferreira PAA, Melo GWB, Zalamena J, da Silva LOS, Marchezan C, da Silva ICB, dos Santos JPJ, Morsch L 2023. Arbuscular mycorrhizal fungi inoculation as strategy to mitigate copper toxicity in young field-grown vines. *Ciência e Técnica Vitivinícola* 38: 60-66. https://doi.org/10.1051/ctv/ctv20233801060

Campillo-Cora C, Fernández-Calviño D, Pérez-Rodríguez P, Fernández-Sanjurjo MJ, Núñez-Delgado A, Álvarez-Rodríguez E, Arias-Estévez M, Nóvoa-Muñoz JC 2019. Copper and zinc in rhizospheric soil of wild plants growing in long-term acid vineyard soils. Insights on availability and metal remediation. *Science of The Total Environment* 672: 389-99. https://doi.org/10.1016/j.scitotenv.2019.03.301

Chance B, Maehly AC 1955. Assay of Catalase and Peroxidase. *Methods in Enzymology* 2: 764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8

Chileshe MN, Syampungani S, Sandell Festin E, Tigabu M, Daneshvar A, Odén PC 2020. Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. *Journal of Forestry Research* 31(4): 1283-1293. https://doi.org/10.1007/s11676-019-00921-0

Cipoleta NS, Simões da Silva LF, Lopes-Assad MLRC 2019. Use of organic materials to attenuate copper contamination of Bordeaux mixture. *Ambiência* 15(2): 289-307.

CONAMA 2008. National Environment Council. Resolution no. 396, of April 3, 2008. *Federal Official Gazette* 66: 64-68.

Covre WP, Carvalho CS, Campos ML, Rodrigues MTL, Silva CF, Fernandes AR, Alleoni LRF. 2022. Impact of copper mining wastes in the Amazon: Properties and risks to environment and human health. *Journal of Hazardous Materials* 421: 126688. https://doi.org/10.1016/j.jhazmat.2021.126688

Soil Chemistry and Fertility Commission (CQFS). 2016. *Manual de calagem e adubação para os estados do Rio Grande do Sul e Santa Catarina*, 11. ed., Porto Alegre: Sociedade Brasileira de Ciência do Solo-Núcleo Regional Sul, 376 p.

CPHEEO 2016. *Municipal Solid Waste Management Manual Part II: The manual*, Central Public Health and Environmental Engineering Organization (CPHEEO) Ministry of Urban Development.

Daniel JB, Brugger D, van der Drift S, van der Merwe D, Kendall N, Windisch W, Doelman J, Martín-Tereso J 2023. Zinc, copper, and manganese homeostasis and potential trace metal accumulation in dairy cows: longitudinal study from late lactation to subsequent mid-lactation. *The Journal of Nutrition* 153(4): 1008-1018. https://doi.org/10.1016/j.tjnut.2023.02.022.

De Conti L, Ceretta CA, Tiecher TL, da Silva LOS, Tassinari A, Somavilla LM, Mimmo T, Cesco S, Brunetto G2018. Growth and chemical changes in the rhizosphere of black oat (*Avena strigosa*) grown in soils contaminated with copper. *Ecotoxicology and Environmental Safety* 163: 19-27. https://doi.org/10.1016/j.ecoenv.2018.07.045.

De Conti L, Ceretta CA, Melo GWB, Tiecher TL, Silva LOS, Garlet LP, Mimmo T, Cesco S, Brunetto 2019. Intercropping of young grapevines with native grasses for phytoremediation of Cu-contaminated soils. *Chemosphere* 216: 147-156. https://doi.org/10.1016/j.chemosphere.2018.10.134.

Ehiomogue P 2023. Vermicompost remediation of contaminated soil: a mini review. Annals of the Faculty of Engineering Hunedoara-International *Journal of Engineering* 21(3): 153-160.

Facco DB, Trentin E, Drescher GL, Hammerschmitt RK, Ceretta CA, da Silva LS, Brunetto G, Ferreira PAA 2023. Chemical speciation of copper and manganese in soil solution and young grapevine growth in copper contaminated soil with amendment application. *Pedosphere* 33(3): 496-507. https://doi.org/10.1016/j.pedsph.2022.06.060.

Fang W, Delapp RC, Kosson DS, van der Sloot HA, Liu J 2017. Release of heavy metals during long-term land application of sewage sludge compost: Percolation leaching tests with repeated additions of compost. *Chemosphere* 169: 271-280. https://doi.org/10.1016/j.chemosphere.2016.11.086.

Filipović L, Defterdarović J, Chen R, Krevh V, Gerke HH, Baumgartl T, Kovač Z, Ondrašek G, Ružičić S, He H, Dusek J, Filipović V 2023. Leached copper correlation with dissolved organic carbon in sloped vineyard soil. *Water* 15(4): 800. https://doi.org/10.3390/w15040800.

Giannopolitis CN, Ries SK 1977. Superoxide dismutase I. Occurrence in higher plants. *Plant Physiology* 59: 309-314.

Hamedi A, Zarabi M, Mahdavi S 2021. Comparative study on the effect of common ions on Zn2+ and Cu2+ adsorption by cattle manure vermicompost (VC) and VC-amended soil. *Communications in Soil Science and Plant Analysis* 52(22): 2821-2836. https://doi.org/10.1080/00103624.2021.1966438

IUSS Working Group WRB. 2015. *World reference base for soil resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps*. World Soil Resources Reports No. 106. FAO, Rome. https://www.fao.org/3/i3794en/I3794en.pdf

Jordán MM, Almendro-Candel MB, Navarro-Pedreño J, Pardo F, García-Sánchez E, Bech J 2020. Bioavailability, mobility and leaching of phosphorus in a Mediterranean agricultural soil (ne Spain) amended with different doses of biosolids. *Environmental Geochemistry and Health*: 1-8. https://doi.org/10.1007/s10653-020-00772-3

Kumar V, Pandita S, Sidhu GPS, Sharma A, Khanna K, Kaur P, Bali AS, Setia R 2021. Copper bioavailability, uptake, toxicity and tolerance in plants: a comprehensive review. *Chemosphere* 262: 127810. https://doi.org/10.1016/j.chemosphere.2020.127810

Li Q, Chen H-H, Qi Y-P, Ye X, Yang L-T, Huang Z-R, Chen L-S 2019. Excess copper effects on growth, uptake of water and nutrients, carbohydrates, and PSII photochemistry revealed by OJIP transients in Citrus seedlings. *Environmental Science and Pollution Research* 26(29): 30188-30205. https://doi.org/10.1007/s11356-019-06170-2

Liu B, Wu C, Pan P, Fu Y, He Z, Wu L, Li Q 2019. Remediation effectiveness of vermicompost for a potentially toxic metal-contaminated tropical acidic soil in China. *Ecotoxicology and Environmental Safety* 182: 109394. https://doi.org/10.1016/j.ecoenv.2019.109394

Marques ACR, Tiecher TL, Brunetto G, Vendruscolo D, De Conti L, Ambrosini VG, Miotto A, Rosa DJ, Silva ICB, Trentin E, Ferreira PAA, Jacques RJS, Pescador R, Comin JJ, Ceretta CAC, Melo GWB, Parent L-É 2023. Phytoremediation of Cu-contaminated vineyard soils in Brazil: A compendium of Brazilian pot studies. *Journal of Environmental Quality* 52(5): 1024-1036. https://doi.org/10.1002/jeq2.20503

Miotto A, Ceretta CAC, Girotto E, Trentin G, Kaminski J, De Conti L, Moreno T, Elena B, Brunetto G 2017. Copper accumulation and availability in sandy, acid, vineyard soils. *Communications in Soil Science and Plant Analysis* 48(10): 1167-1183. https://doi.org/10.1080/00103624.2017.1341908

Mir AR, Pichtel J, Hayat S 2021a. Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals* 34(4): 737-759. https://doi.org/10.1007/s10534-021-00306-z

Mir AR, Alam P, Hayat S 2021b. Effect of different levels of soil applied copper on the morpho-physiological, photochemical, and antioxidant system of *Brassica juncea*. *Journal of Soil Science and Plant Nutrition* 21: 3477-3492.

Morsch L, Marques ACR, Trentin E, Oliveira FN, Andreolli T, Barbosa JGP, Ferreira MM, Moura-Bueno JM, Comin JJ, Loss A, Lourenzi CR, Brunetto G2024. Diversity and botanical composition of native species in the

Pampa biome in vineyards cultivated on soils with high levels of copper, zinc and manganese and phytoremediation potential. *Chemosphere* 349: 140819. https://doi.org/10.1016/j.chemosphere.2023.140819

Murphy J, Riley JPA 1962. Modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 27: 31-36.

Rangel TS, Santana NA, Jacques RJS, Ramos RF, Scheid DL, Koppe E, Tabaldi LA, Silveira AO 2023. Organic fertilization and mycorrhization increase copper phytoremediation by *Canavalia ensiformis* in a sandy soil. *Environmental Science & Pollution Research* 30(26): 68271-68289. https://doi.org/10.1007/s11356-023-27126-7

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at: http://www.r-project.org/

Santana NA, Ferreira PAA, Soriani HH, Brunetto G, Nicoloso FT, Antoniolli ZI, Jacques RJS2015. Interaction between arbuscular mycorrhizal fungi and vermicompost on copper phytoremediation in a sandy soil. *Applied Soil Ecology* 96: 172-182. https://doi.org/10.1016/j.apsoil.2015.08.001

Santana NA, Rabuscke CM, Soares VB, Soriani HH, Nicoloso FT, Jacques RJS2018. Vermicompost dose and mycorrhization determine the efficiency of copper phytoremediation by *Canavalia ensiformis*. *Environmental Science and Pollution Research* 25: 12663-12677. https://doi.org/10.1007/s11356-018-1533-1

Schwalbert R, Stefanello LO, Schwalbert RA, Tarouco CP, Drescher GL, Trentin E, Tassinari A, Silva IB da, Brunetto G, Nicoloso FT 2021. Soil tillage affects soybean growth and promotes heavy metal accumulation in seeds. *Ecotoxicology and Environmental Safety* 216: 112191. https://doi.org/10.1016/j.ecoenv.2021.112191

Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S, Natasha, Murtaza G, Dumat C, Shahid M 2020. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere* 259: 127436. https://doi.org/10.1016/j.chemosphere.2020.127436

Shahkolaie SS, Baranimotlagh M, Dordipour E, Khormali F2020. Effects of inorganic & organic amendments on physiological parameters and antioxidant enzymes activities in *Zea mays* L. from a cadmium-contaminated calcareous soil. *South African Journal of Botany* 128: 132-140. https://doi.org/10.1016/j.sajb.2019.10.007

Shrestha P, Bellitürk K, Görres JH 2019. Phytoremediation of heavy metal-contaminated soil by switchgrass: A comparative study utilizing different composts and coir fiber on pollution remediation, plant productivity, & nutrient leaching. *International Journal of Environmental Research and Public Health* 6(7): 1261. https://doi.org/10.3390/ijerph16071261

Silva JAF, García AC, Lima AS, Souza CCB. Amaral Sobrinho NMB 2022a. Effect of short-term pig slurry amendment of soil on humified organic matter and its relationship with the dynamics of heavy metals and metals uptake by plants. *Journal of Environmental Science and Health, Part A* 57(11): 958-969. https://doi.org/10.1080/10934529.2022.2132795

Silva ICB, Marques ACR, Quadros FF, Sans G, Soares VM, De Conti L, Ceretta CA, Ferreira PAA, Toselli M, Brunetto G 2022b. Spatial variation of herbaceous cover species community in Cu-contaminated vineyards in

Pampa biome. *Environmental Science and Pollution Research* 27: 13348-13359. https://doi.org/10.1007/s11356-022-21738-1

Teixeira PC, Donagemma GK, Fontana A, Teixeira W G 2017. Manual of soil analysis methods.

Trentin E, Cesco S, Pii Y, Valentinuzzi F, Celletti S, Feil SB, Alzate Zuluaga MY, Ferreira PAA, Ricachenevsky FK, Stefanello LO, De Conti L, Brunetto G, Mimmo T 2022. Plant species and pH dependent responses to copper toxicity. *Environmental and Experimental Botany* 196: 104791. https://doi.org/10.1016/j.envexpbot.2022.104791

Vione EL, Silva LS, Cargnelutti Filho A, Aita NT, de Freitas de Morais A, Kokkonen da Silva AA 2018. Chemical characterization of composts and vermicomposts produced with rice husks and animal waste. *Revista Ceres* 65: 65-73.

Wang Y, Xu YA, Li D, Tang BC, Man SL, Jia YF, Xu H 2018. Vermicompost and biochar as bio-conditioners to immobilize heavy metal & improve soil fertility on cadmium contaminated soil under acid rain stress. *Science of the Total Environment* 621: 1057-1065. https://doi.org/10.1016/j.scitotenv.2017.10.121

Wang X, Xue J, He M, Qi H, Wang S2024. The effects of vermicompost and steel slag amendments on the physicochemical properties and bacterial community structure of acidic soil containing copper sulfide mines. *Applied Sciences* 14(3): 1289. https://doi.org/10.3390/app14031289

Zeng Q, Ling Q, Wu J, Yang Z, Liu R, Qi Y 2019. Excess copper-induced changes in antioxidative enzyme activity, mineral nutrient uptake and translocation in sugarcane seedlings. *Bulletin of Environmental Contamination and Toxicology* 103(6): 834-840. https://doi.org/10.1007/s00128-019-02735-6

Zeraik AE, Souza FS, Fatibello-Filho O, Leite OD 2008. Development of a spot test for monitoring peroxidase activity in a purification procedure. *Química Nova* 31: 731-734. https://doi.org/10.1590/S0100-40422008000400003