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MANAGEMENT OF COPPER-CONTAMINATED SOILS: FEASIBILITY ANALYSIS OF PHYTOREMEDIATION WITH *Cyperus rotundus* L.

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Abstract

Copper is among eight essential micronutrients for plant growth. Like other metals, due to human activity in the Anthropocene, it behaves as an important pollutant in the biosphere. In this context, this study aims to identify the potential of the plant species *Cyperus rotundus* L. (nutgrass) in the treatment of copper-contaminated soils to observe: the reduction in the amount of copper in the soil after experiment, difference in fresh and dry mass and chemical parameters such as electrical conductivity (EC) and soil hydrogen potential (pH). According to the parameters analyzed, nutgrass demonstrated significantly greater efficiency in phytoextraction when copper levels in the soil were at higher doses. This adaptive response indicates that the plant has a high capacity for copper accumulation in conditions of high contamination, which makes its use viable as an effective strategy for decontaminating soils saturated with this metal. Therefore, nutsedge proves to be a promising candidate for use in the phytoremediation technique, particularly in areas where copper contamination reaches critical levels.

Keywords: soil conservation; clean energy; metal prospecting; analytical techniques.

GERENCIAMENTO DE SOLOS CONTAMINADOS POR COBRE: ANÁLISE DA VIABILIDADE DE FITORREMEDIAÇÃO COM *Cyperus rotundus* L.

Resumo

Cobre está entre oito micronutrientes essenciais para o crescimento vegetal. Assim como os demais metais, em função da atividade humana no Antropoceno, comporta-se como um poluente importante na biosfera. Nesse contexto, este estudo tem como objetivo identificar o potencial da espécie vegetal *Cyperus rotundus* L. (tiririca) no tratamento de solos contaminados por cobre a observar: a redução da quantidade de cobre no solo após experimento, diferença da massa fresca e seca e parâmetros químicos como condutividade elétrica (CE) e potencial hidrogeniônico do solo (pH). De acordo com os parâmetros analisados, a tiririca demonstrou uma eficiência

significativamente maior na fitoextração quando os níveis de cobre no solo estavam em doses mais elevadas. Essa resposta adaptativa indica que a planta possui uma alta capacidade de acumulação de cobre em condições de elevada contaminação, o que viabiliza sua utilização como uma estratégia eficaz para a descontaminação de solos saturados com esse metal. Portanto, a tiririca se revela uma candidata promissora para ser utilizada na técnica de fitorremediação, particularmente em áreas onde a contaminação por cobre atinge níveis críticos.
Palavras chave: conservação dos solos; energia limpa; prospecção de metais; técnicas analíticas.

1. Introduction

Anthropogenic activities have become determining factors in the biogeochemical cycle of heavy metals. Mobilizing metals such as copper (Cu), cadmium (Cd), lead (Pb), mercury (Hg), nickel (Ni), chromium (Cr), and zinc (Zn) into the biosphere, these are responsible for physiological mechanisms, environmental pollution, and toxicity to living organisms, even at low concentrations (Sodré; Lenzi; Costa, 2001).

Among the heavy metals associated with soil contamination, copper stands out as an essential element for plants and other organisms (Festa; Thiele, 2011). This essentiality is evidenced by the fact that copper is among the eight fundamental micronutrients for plant growth and development, playing roles in metabolism as an activator or component of important enzymes involved in oxidation and reduction processes (Nazir; Hussain; Fariduddin, 2019; Raven; Eichhorn, 2018). However, copper has shifted from being a key essential micronutrient in soils to becoming a toxic agent (Poggere *et al*., 2023).

When the presence of this micronutrient exceeds 200 $mg.kg^{-1}$, it indicates the need for intervention in agricultural areas (Brazil, 2009). This situation is mainly a consequence of mining activities and the use of copper-based fungicides in agriculture (Carneiro; Siqueira; Moreira, 2002). Therefore, there arises a need to improve techniques capable of decontaminating soils that should be both efficient and low-cost. These qualities have become essential, given that conventional techniques are expensive and negatively impact the soil microbiota (Mendonça *et al*., 2021).

In this context, the phytoremediation technique, which involves the use of plants to degrade, immobilize, extract, contain, or mobilize soil contaminants, becomes a viable solution for managing soils contaminated by copper (USEPA, 2000). Phytoextraction and phytostabilization are among the best-known mechanisms for remediating this contaminant. Both have gained prominence, but phytoextraction stands out due to the possibility of using certain plants known as hyperaccumulators. These plants absorb and tolerate extremely high levels of contaminants, most commonly heavy metals (Brady; Weil, 2013; Mendonça *et al*., 2021).

Therefore, this study aims to identify the ability of the plant species *Cyperus rotundus* L. (nutgrass) to phytoextract copper from contaminated soils, observing parameters such as shoot biomass, electrical conductivity (EC), and soil pH.

2. Materials and Methods

2.1 Soil collection, nutgrass replication and experiment setup

The experiment was conducted at the Environmental Sciences Laboratory of the Universidade Federal Rural do Rio de Janeiro - Três Rios Institute. On the thirteenth day of november 2023, soil was collected in the municipality of Chiador - MG, with geographic coordinates of latitude 22°5’9.44”S; longitude 43°8'36.79”W. The soil collected for use in the experiment is classified as Dystrophic Red Argisol (Minas Gerais, 2001). On the same day and at the same location, tubers of the plant species *Cyperus rotundus* L. were also collected (Figure 1-A).

After collection, 400 grams of soil (air-dried soil samples) were placed in one-liter plastic pots (Figure 1-B), previously weighed on a Shimadzu analytical balance (MODEL BL3200H). The pots were not perforated to prevent loss of the contaminant solution. Then, four tubers were planted in each pot, and the soil was watered with distilled water as needed. The plants remained in these conditions for ten days to grow before soil contamination (Figure 1-C).

Figure 1. Soil distribution in plastic pots in A. Separation of tubers in B, and growth stage of seedlings after 10 days in C.

A) B) C)

On November 23rd, 2023, the experiment was set up. Copper was applied as a pure copper sulfate solution ($CuSO_4 \cdot 5H_2O$) for analysis. However, stoichiometric calculations were necessary to determine the amount of purified copper required for the study. The stoichiometry yielded the following copper values for the treatments: 6.66 mg, 66.56 mg, 666.06 mg. Simultaneously, the soil was analyzed and found to already contain copper, which required adding this amount to the calculated doses for contamination. This made it possible to determine the total amount of copper in each treatment.

2.2 Experimental Design

The experimental design used was a completely randomized block design, in a 4 x 3 factorial scheme, with 4 treatments and 3 replicates, as follows: T1 = 600 mg Cu^{++}; T2 = 606.66 mg Cu^{++}; T3 = 666.56 mg Cu^{++}; T4 = 1,266.06 mg Cu^{++}.

2.3 Collection and Analysis

On November 30th, 2023, seven days after contamination, the experiment collection was conducted. The analysis of the aerial part was performed by counting the number of tillers in each pot, as well as measuring their length. Next, the aerial part was collected by cutting the tillers near the roots, and then the fresh mass was weighed. Afterward, the aerial part was placed in a drying oven, SolidSteel brand (MODEL SSDC110L), for sixty minutes at 65°C to obtain the dry mass, which was then weighed again. It should be noted that all data obtained from the aerial part were recorded in Microsoft Excel® 2022 for further analysis.

For the analysis of copper content, the methodology proposed by Giesbrecht (1979) for the photometric determination of copper concentration was used, with adaptations. Initially, the standard curve of Cu^{++} was constructed based on the relationship between increasing concentrations of copper in $mol \cdot L^{-1}$ and absorbance in the IL-592 UV-VIS SPECTROPHOTOMETER model, under a wavelength range of 800 nanometers (nm). The data obtained were processed using Microsoft Excel® 2022 software. The resulting equation is presented below (Equation I), with R^2 = 0.9997.

$$\mathbf{y=0.0819x + 0.0003} \qquad \text{(Equation I)}$$

Table 1. Standard curve of Cu^{++} for spectrophotometric analysis. Source: Personal Archive, 2023.

$CuSO_4.5H_2O$ 0.10 $mol.L^{-1}$ (mL)	H_2O distilled (mL)	Final concentration 0.10 $mol.L^{-1}$	Absorbance reading at 800 nm
0	10	0.00	0.000
1	9	0.01	0.122
2	8	0.02	0.235
4	6	0.04	0.464
6	4	0.06	0.715
8	2	0.08	0.925
10	0	0.10	1.169

Subsequently, 10 g of soil were weighed and diluted in 100 mL of distilled water to obtain the soil solution, which was then filtered through filter paper for analysis. The filtrate was placed in cuvettes for absorbance reading on the spectrophotometer. The absorbance values obtained were inserted into the equation derived from the standard curve, allowing the determination of the copper concentration in $mol \cdot L^{-1}$. To calculate the amount of copper present in 10 g of soil, the following formula was used:

COPPER IN (10 g): CC x 0.1 x 63.546 x 1,000 (Formula I)

Where:

CC: Copper concentration found in equation I, in ($mol \cdot L^{-1}$);

0.1: Volume of the solution;

63.546: Atomic weight of copper (g/mol);

1,000: Conversion factor to adjust the units.

After determining the amount of copper in the 10 gram sample, formula (II) was applied to estimate the residual copper present in the soil after the experiment:

$$CC_{(400g)} = \frac{400\,\mathrm{g} \times X\,\mathrm{mg}}{10\,\mathrm{g}}$$

(Formula II)

Where:

400 g: Amount of soil in the pot;

X mg: Copper found using Formula I;

10 g: Amount of soil used initially to determine the copper content..

Finally, the soil solution also had its pH and electrical conductivity analyzed using an Even pH meter, model PHS-3E, and a Tecnopon conductivity meter model mCA 150, both calibrated on the day of the analyses.

2.4 Statistical analyses

The sampled data were subjected to analysis of variance (ANOVA) at a significance level of ($p \leq 0.05$), followed by Tukey's test, using BioEstat software, version 5.0.

3. Results and discussion

3.1 Copper removal potential of nutgrass

According (Marchiol *et al*., 2004) ; Nascimento e Xing (2006), the plant species used in the phytoremediation technique must exhibit characteristics such as the ability to hyperaccumulate extracted metals, tolerance to high metal concentrations in the soil, rapid growth and high biomass production, and ease of harvest. These characteristics align with those displayed by the species used in this study. Furthermore, Mendonça *et al*. (2021) observed the significant potential of the plant species *Cyperus rotundus* L. in copper absorbtion, even though its contact with the contaminant had been for a brief period. The results of the residual copper analyses in the containers of each treatment, after seven days of exposure of nutgrass to copper, are presented in Table 2 below.

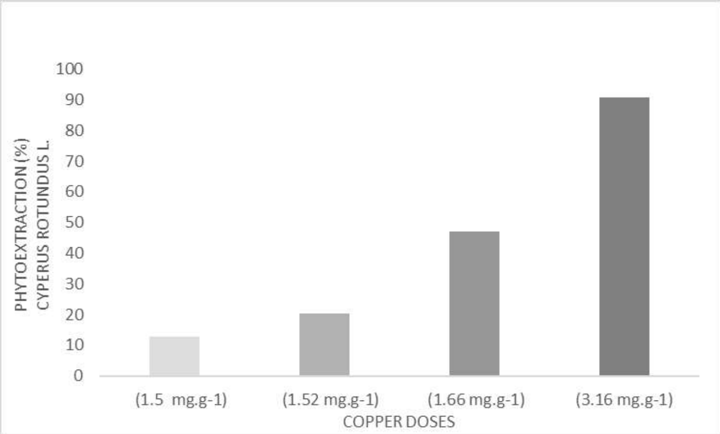
Table 2. Means of the amount of copper phytoextracted by nutgrass in each of the four treatments evaluated. The values presented correspond to the average of the three replicates performed for each treatment. Source: Personal Archive, 2023.

Treatments	Residual copper after experiment (mg)	Phytoextracted copper (mg)
1 (1.5 $mg.g^{-1}$)	524	76
2 (1.52 $mg.g^{-1}$)	483	123
3 (1.66 $mg.g^{-1}$)	354	313
4 (3.16 $mg.g^{-1}$)	118	1.148

Among the proposed treatments, a significant statistical difference was observed between treatments T1 and T3 ($p < 0.05$), T1 and T4 ($p < 0.01$), T2 and T3 ($p < 0.05$), T2 and T4 ($p < 0.01$), and T3 and T4 ($p < 0.01$), with the species *Cyperus rotundus* L. proving to be more efficient in copper removal in treatments with higher doses.

No significant difference was observed between treatments 1 and 2. This can be attributed to the small variation in copper doses applied between these treatments, which, not being sufficiently distinct, did not induce a significant increase in copper uptake by the plant. On the other hand, the absence of an excess of this element available in the soil may have been a limiting factor for greater metal absorption in these treatments. Figure 2 shows the copper removal efficiency by the plant in each treatment, expressed as a percentage.

Figure 2. Copper extraction. Source: Personal Archive, 2023.



In the natural environment, copper is present in soils in various forms, such as free ions, complexes dissolved in the soil solution, cation exchange sites of clays and organic matter, co-precipitated in oxides, as well as incorporated in biological residues and living organisms (Jatav *et al*., 2020). Absorption predominantly occurs in the active form of Cu^{2+}, however, copper uptake and transformation depend on several factors, such as the plant species in question, soil concentration, bioavailability, environmental conditions, and especially soil pH. In general, plants are unable to establish an upper limit for the absorption of this micronutrient and end up accumulating it (Silva; Vitti; Trevizam, 2007). As observed in the treatment with 3.16 $mg \cdot g^{-1}$ of copper, the nutgrass phytoextracted an average of 90.7% of the copper that was available in the soil. This is feasible due to the plant's ability to redistribute the absorbed copper and store it in other parts with the help of various transporters such as ATPase, ZIP, NRAMP, and COPT (Kumar *et al*., 2021). In other words, as the plant does not restrict the uptake of this micronutrient through its roots. Moreover, the higher its availability in the soil, the greater the absorption will be. With the aid of transporters, the absorbed copper is redistributed and stored in specific parts of the plant body, reflecting its hyperaccumulator characteristic.

3.2 Shoot Biomass

Static analyses did not reveal significant differences in shoot biomass ($p > 0.05$). The mean number of tillers ranged from 4 to 6 units. Fresh weight ranged from 1.09 to 5.34 grams, and dry weight varied from 0.3 to 0.66 grams. It means that, after analyzing the relationship between fresh and dry weight of the shoot biomass, it was found that 87.39% of the composition was water. Thus, there was no significant difference in the shoot biomass among the treatments, suggesting that, although copper was almost entirely absorbed in the treatment with the highest copper dose, it is believed that it was predominantly stored in the roots. Oliva *et al*. (2010) observed preferential accumulation of copper in the roots of *Erica andevalensis*, a phenomenon that has already been observed in other plants growing in copper-contaminated soils.

3.3 pH and EC

Table 3 presents the pH and electrical conductivity parameters obtained from soil solution analyses. In treatment 4, with 3.16 mg·g^{-1} of copper, the pH was 7.43, indicating a neutral/alkaline condition and representing the lowest pH found among the treatments.

Table 3. Mean values of pH and EC data obtained after the experiment. Source: Personal Archive, 2023.

Treatments		pH	Electrical Conductivity (mS.cm^{-1})	Temperature (ºC)
1	(1.5 mg.g^{-1})	9.99	112.3	22.4
2	(1.52 mg.g^{-1})	11.29	93.4	22.0
3	(1.66 mg.g^{-1})	10.10	160.0	21.2
4	(3.16 mg.g^{-1})	7.43	563.0	22.0

Copper absorption by the roots involves the activation of H^+-ATPases and acidification of the soil solution (Silva *et al*., 2022). The data indicate that the treatment with the highest copper absorption by the plants resulted in a decrease in pH, increases in the electrical conductivity of the soil solution, and lower shoots mass values after sampling. Meanwhile, electrical conductivity reached its peak in this same treatment.

4. Conclusion

Based on the analyses conducted and the results obtained, it is concluded that the plant species *Cyperus rotundus* L. (nutgrass) has potential for the phytoremediation of copper-contaminated soil, given that the brief contact of the plant with the contaminant demonstrated susceptibility to the absorption of this heavy metal.

Thus, nutgrass showed significantly higher efficiency in phytoextraction when copper levels in the soil were at higher doses. This adaptive response indicates that the plant has a high capacity for copper accumulation under conditions of elevated contamination, making it a viable strategy for the decontamination of soils saturated with this metal. Therefore, nutgrass emerges as a promising candidate for use in phytoremediation techniques, particularly in areas where copper contamination reaches critical levels.

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