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Original Article

Ecotoxicity of Rice Husk Ash in Copper-Contaminated Soils Using Plant Bioassays

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Abstract

Rice husk ash (RHA) is a by-product generated from the controlled combustion of rice husks, offering potential applications in agriculture. This study evaluates the ecotoxicological effects of RHA on copper-contaminated soils, particularly vineyard soil, which presents high levels of copper accumulation due to fungicide use. Three soil types—vineyard soil (V), native field soil (NF), and tropical artificial soil (TAS)—were analyzed under different RHA concentrations (5%, 15%, 25%, 50%, and 100%). Two model plant species, lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*), were used in germination assays to assess toxicity levels. The vineyard soil presented 34.19 mg/kg of copper (Cu), exceeding the quality reference value (11 mg/kg) established by environmental regulations, while Native Field soil contained only 0.81 mg/kg of Cu. Germination index (GI) and normalized residual germination percentage index (NGI) indicated a moderate toxicity (-0.34%) for lettuce at 5% RHA, while toxicity levels were lower at 25% and 50% RHA. Root growth inhibition was observed in lettuce at 6.16% and 6.03% for 25% and 50% RHA, respectively. Arugula showed higher sensitivity to RHA concentrations, with root growth inhibition reaching 72.89% in vineyard soil and 60.78%, 63.04%, and 72.38% at 5%, 15%, and 25% RHA, respectively. The findings provide ecotoxicological evidence on the short-term effects of RHA in copper-contaminated soils and reinforce the importance of cautious application to avoid phytotoxicity. While RHA may be non-toxic under certain conditions, further research is needed to determine its long-term safety and potential benefits in agro-industrial residue management.

Keywords: Copper Contamination, Germination Index, Rice Husk Ash, Phytotoxicity.

INTRODUCTION

The rice (*Oryza sativa* L) is one of the most consumed cereals in the world. According to the National Supply Company (CONAB), rice husk is one of the most abundant agricultural by-products. Rice husk is described as a protective layer that forms during grain development, with low density and high volume, representing, on average, 20% of the total weight of rice (Walter et al., 2008). Its composition is 50% cellulose, 30% lignin, and 95 - 98% inorganic residues rich in hydrated amorphous silica (Silva *et al.*, 2008).

Due to its high volume and low biodegradability, rice husk management poses an environmental and economic challenge. In this case, one of the most effective alternatives is controlled burning, considering that rice husk has a high calorific value, having a volatile content of 74% and about 12.8% ash, which indicates that it can be a good fuel (Armesto *et al.*, 2002). Its combustion, in addition to significantly reducing the volume of the waste, generates heat, which can be used as an alternative source for grain drying or thermoelectric generation, reducing the use of fossil fuels (Capeletto & Zanchi de Moura, 2014). By burning rice husks, rice husk ash (RHA) is produced, a material with valuable properties for agriculture, standing out for its ability to correct soil acidity, increase water retention, and provide essential nutrients for plant development (Pinto *et al.*, 2009; Masulili *et al.*, 2010).

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The use of RHA in soils has gained increasing attention, especially in agricultural areas where sustainable resource management is key. Research indicates that this compound can act as a corrective for the soil and improve its physical, chemical, and biological characteristics, contributing to decreasing soil density and increasing porosity, in addition to raising pH and nutrient availability (Pauletto *et al*., 1990; Karmakar *et al*., 2009). Yin *et al.* (2022) concluded that adding CA to acid soil improved its properties, favored the growth of cotton seedlings, and could replace commercial substrates. Applying rice husk ash (RHA) to pastures reduced acidity and increased nutrient availability, improving soil structure (Martins Filho *et al*., 2020).

Despite its potential benefits, the use of RHA in soils should be approached with caution. High concentrations can compromise the germination and initial growth of plants, in addition to intensifying the toxicity of soils already impacted, such as those contaminated by heavy metals. In wine-growing soils, for example, the accumulated presence of copper due to the use of fungicides is a critical factor that requires careful approaches (FEPAM, 2014).

Given this scenario, the research in question analyzes the ecotoxicological effects of the use of RHA in different types of soil and different concentrations, using two model plant species: lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*). Soils with varied characteristics, including vineyard, native field (NF), and tropical artificial soil (TAS), at different concentrations of RHA, were analyzed. The germination, root development, and initial growth rates of the plants were also evaluated, allowing an understanding of the interactions between the residue and the various substrates.

Thus, the research seeks to evaluate the effects of the addition of RHA in copper-contaminated soil on ecotoxicity through germination assays. Thus, this study contributes to the expansion of knowledge about the responsible management of agro-industrial residues and the promotion of more sustainable agricultural practices.

MATERIAL AND METHODS

Rice husk ash (RHA) was collected at a rice processing mill located in the city of Cachoeira do Sul, in the state of Rio Grande do Sul (RS). Three types of soil were used: vineyard soil (V), collected from the municipality of Santana do Livramento (RS), predominantly classified as Ultisols, with associations of Litholic Neosols and Chromic Alisols with grayish Ultisols; native field soil (NF), collected from an adjacent area with low anthropogenic impact; and tropical artificial soil (TAS), composed of 70% sand, 20% kaolin, and 10% coconut fiber powder, used as a contaminant-free control substrate.

The trials were carried out with two plant species, lettuce (*Lactuca sativa*) and rocket (*Eruca sativa*). The concentrations of CCA were 100%, 50%, 25%, 15%, and 5% in 3 repetitions, as well as a negative control (100% soil) and a positive control (100% soil and 1% zinc sulphate added). The maximum water retention capacity (MWRC) of 60% was used. This represents the largest volume of water that the soil can retain against the force of gravity, filling all the available pores, especially the micropores. Therefore, the retention capacity was determined for each type of soil. Each container received 20 ml of soil/grey, together with 10 seeds of the plant species. The samples were kept under temperature control in an incubator at 22 °C for 5 days.

After the end of the incubation period, seed germination was evaluated, and radicle and hypocotyl measurements were performed. Based on these measurements, the following indices were calculated: germination index (GI), normalized residual germination percentage index (NGI), normalized residual radical percentage elongation index (NEI), relative radicle growth (RRG) and relative seed germination percentage (RGP).

The calculations of these indices and the classification of toxicity were based on the study of Bagur-González *et al*. (2010), who evaluated the toxicity using the bioassay with *Lactuca sativa* and categorized the normalized residual germination percentage (NGI) and normalized residual radical elongation (NEI) indices into different toxicity levels: (a) 0 to -0.25 indicates low toxicity, (b) -0.25 to -0.5 represents moderate toxicity, (c) -0.5 to -0.75 reflects high toxicity, and (d) -0.75 to -1 corresponds to very high toxicity. Values of NEI greater than 0 suggest stimulation of seed growth, a phenomenon known as hormesis.

These categories were used to interpret the effects of RHA concentrations on different soil types and plant species (lettuce and arugula). In addition, qualitative comparisons were made between the results obtained in native, vineyard, and artificial soils (TAS). Descriptive statistics were performed on the data to obtain the mean radicle and hypocotyl length values. To evaluate the data, the native grassland soil and vineyard soil were used as negative controls.

To further validate these findings, statistical tests were applied to assess the significance of observed differences. The data were initially tested for normality using the Shapiro-Wilk test. To ensure the suitability of the analysis of variance (ANOVA), data were transformed using Box-Cox when necessary. After verifying normality, one-way ANOVA was applied to assess significant differences among treatments. When significant differences were detected ($p < 0.05$), Tukey's multiple comparisons test was performed to identify which treatments exhibited statistically significant differences.

All statistical analyses were conducted using Python, employing the scipy library for ANOVA and statsmodels for the Tukey test. The development and execution of the code were assisted by ChatGPT-4 (OpenAI) to optimize and automate the statistical processing. However, all analyses and interpretations were rigorously reviewed by a researcher to ensure accuracy and methodological consistency.

RESULTS AND DISCUSSION

The soils used in the experiment showed distinct characteristics concerning the copper content, with the vineyard soil presenting 34.19 mg/kg of Cu, exceeding the Quality Reference Value (QRV) of 11 mg/kg established by the State Foundation for Environmental Protection (FEPAM) of Rio Grande do Sul. These reference values were defined in 2014 through FEPAM Ordinance No. 85/2014 and apply to nine chemical elements naturally occurring in the state's geomorphological and geological provinces, including copper. This threshold reflects the impact of decades of use of copper-based fungicides. The native grassland soil, with only 0.81 mg/kg de Cu, was used as a negative control due to its low contamination by this metal.

Variations in germination and root growth indices in lettuce were observed, depending on the rice husk ash concentration and soil type (Table 1). In the native field soil, the normalized germination indexes (NGI) were higher than those of the vineyard soil, while in the soil with the addition of 5% RHA, the toxicity was moderate (-0.34%). At concentrations of 25% and 50% of ash, the NGI values were reduced (-0.02% and -0.08%, respectively), indicating less impact under these conditions (Table 2).

The ANOVA revealed significant differences in radicle and hypocotyl length for lettuce ($p < 0.05$). The Tukey test confirmed that 100% RHA significantly differed from Vineyard Soil in both radicle and hypocotyl length ($p < 0.05$), indicating an influence of high RHA concentrations on seedling development. No significant differences were detected among other treatments (Table 1). Lettuce root growth was inhibited by 6.16% and 6.03% at concentrations of 25% and 50% of ash, respectively, and by 12.25% in native field soil. In the TAS and 100% RHA substrate, germination and root growth were the highest, presenting the best performances among all the conditions tested.

In the experiments carried out, arugula (*Eruca sativa*) was more sensitive to soil conditions and RHA concentrations, as evidenced by the NGI and NEI indexes. In vineyard soil, toxicity levels were moderate to high (NGI: -0.25 to -0.72) at 15% to 50% RHA, leading to reduced germination and radicle elongation (Tables 3 and 4). The ANOVA confirmed significant differences among treatments ($p < 0.05$), with the Tukey test indicating that 100% RHA was significantly different from nearly all treatments for radicle length ($p < 0.05$), emphasizing its strong impact (Table 3). Additionally, 50% RHA differed significantly from Vineyard Soil. The inhibition of root growth was 72.89% in the vineyard soil without ash addition, while in the concentrations of 5%, 15%, and 25% of ash, the values were 60.78%, 63.04%, and 72.38%, respectively.

Artificial soils, such as TAS (tropical artificial soil) and substrate with 100% ash, showed the best results for both species tested, showing greater germination and initial development (Tables 1 and 3). In TAS, the NGI and NEI indices indicated positive or neutral effects, especially at concentrations of 100% RHA, which provided high germination and superior root elongation when compared to the other treatments (NGI: 0.45; NEI: 0.57). This performance can be attributed to the increased water retention and the absence of contaminants in these substrates. In general, rice husk ash (RHA) showed potential to partially attenuate vineyard soil toxicity, especially at intermediate concentrations (25%-50 %).

The dosage limit depends on the effects of RHA on soil attributes. It can increase pH and nutrient availability (Silva *et al*., 2008; Pinto *et al*., 2009; Sandrini, 2010), as well as it can affect soil water physical attributes. The magnitude of these effects depends on the characteristics of the RHA itself and the soil, the dosage of the RHA, and the interaction between the soil and the RHA. The increase in pH is more immediate and comprehensive, as it affects several other attributes and processes in the soil, and because of this, it can serve as an initial guide to establish the dosage limit of a given RHA that a soil can receive.

Substrates are the means for the development of the plant's root system. Many of them need a good substrate composition to provide nutritional material, which depends on the type of species, soil conditions, and climate to meet the needs of the crop. It is the mixtures that make up the substrates that define the nutritional value of their composition, and their percentages concerning their pH and consistency should be studied. Substrates with RHA and organic waste improved the growth and productivity of cherry tomatoes, highlighting RHA as a sustainable alternative (Soldateli *et al.*, 2020). The application of RHA to home-grown tomatoes increased yields, provided nutrients, and reduced costs, making it an effective alternative to liming (Mini, 2024).

Table 1. Germination results for lettuce (*Lactuca sativa*) in vineyard soil with different concentrations of rice husk ash (RHA), using native field soil as the negative control for index calculations.

Sample	AHL (mm)	ARL (mm)	IRG (%)	Germ. (%)	RGP (%)	RRG (%)	GI (%)	NGI (%)	NEI (%)
Native field	20.60 ± 5.35ab	14.53 ± 6.01a	-	76.67	-	-	-	-	-
TAS	22.47 ± 10.81ab	19.35 ± 8.54a	-33.20	88	73.33	133.20	97.68	0.15	0.33
Vineyard	18.20 ± 6.03b	16.56 ± 7.05a	-13.96	83.33	83.33	113.96	94.97	0.09	0.14
5% RHA	21.35 ± 7.46 ab	16.93 ± 6.36a	-16.50	55.17	53.33	116.50	62.14	-0.28	0.17
15% RHA	24.84 ± 5.80 a	17.55 ± 7.03a	-20.80	92.86	86.67	120.80	104.69	0.21	0.21
25% RHA	20.45 ± 8.12ab	15.54 ± 4.62a	-6.94	81.48	73.33	106.94	78.43	0.06	0.07
50% RHA	21.71 ± 6.35ab	15.56 ± 6.07a	-7.09	100	100	107.09	107.09	0.30	0.07
100% RHA	25.16 ± 5.48a	25.34 ± 5.99b	-74.40	100	100	174.40	174.40	0.30	0.74
PC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: **TAS** = Tropical Artificial Soil; **AHL** = Average hypocotyl length; **ARL** = Average radicle length; **IRG** = Inhibition of radicle growth; **Germ.** = Germination; **RGP** = Relative seed germination percentage; **RRG** = Relative radicle growth; **GI** = Germination index; **NGI** = Normalized residual germination percentage index; **NEI** = Normalized residual radical percent elongation index; **PC** = Positive control. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Table 2. Germination results for lettuce (*Lactuca sativa*) in vineyard soil with different concentrations of rice husk ash (RHA), using vineyard soil as the negative control for index calculations.

Sample	AHL (mm)	ARL (mm)	IRG (%)	Germ. (%)	RGP (%)	RRG (%)	GI (%)	NGI (%)	NEI (%)
Vineyard	18.20 ± 6.03	16.56 ± 8.44	-	83.33	-	-	-	-	-
TAS	22.47 ± 10.81	19.35 ± 11.07	-16.88	88	73.33	116.88	85.71	0.06	0.17
Native field	20.60 ± 5.35	14.53 ± 7.97	12.25	76.67	76.67	87.75	67.27	-0.08	-0.12
5% RHA	21.35 ± 7.46	16.93 ± 9.68	-2.23	55.17	53.33	102.23	54.52	-0.34	0.02
15% RHA	24.84 ± 5.80	17.55 ± 8.46	-6	96.30	86.67	106	91.86	0.16	0.06
25% RHA	20.45 ± 8.12	15.54 ± 7.74	6.16	81.48	73.33	93.84	68.82	-0.02	-0.06
50% RHA	21.71 ± 6.35	15.56 ± 6.39	6.03	100	100	93.97	93.97	0.20	-0.06
100% RHA	25.16 ± 5.48	25.34 ± 6.09	-53.03	100	100	153.03	153.03	0.20	0.53
PC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: **TAS** = Tropical Artificial Soil; **AHL** = Average hypocotyl length; **ARL** = Average radicle length; **IRG** = Inhibition of radicle growth; **Germ.** = Germination; **RGP** = Relative seed germination percentage; **RRG** = Relative radicle growth; **GI** = Germination index; **NGI** = Normalized residual germination percentage index; **NEI** = Normalized residual radical percent elongation index; **PC** = Positive control. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Table 3. Germination results for arugula (*Eruca sativa*) in vineyard soil with different concentrations of rice husk ash (RHA), using native field soil as the negative control for index calculations.

Sample	AHL (mm)	ARL (mm)	IRG (%)	Germ. (%)	RGP (%)	RRG (%)	GI (%)	NGI (%)	NEI (%)
Native field	24.34 ± 9.07ab	15.71 ± 6.25a	-	82.14	-	-	-	-	-
TAS	34.36 ± 9.64c	24.73 ± 10.18b	-57.43	97	100	157.43	157.43	0.18	0.57
Vineyard	17.00 ± 6.07ad	4.26 ± 1.46c	72.89	66.67	71.43	27.11	19.37	-0.19	-0.73
5% RHA	16.34 ± 6.19d	6.16 ± 4.29c	60.78	83.33	89.29	39.22	35.02	0.01	-0.61
15% RHA	22.62 ± 8.74abd	5.81 ± 2.50c	63.04	70	75	36.96	27.72	-0.15	-0.63
25% RHA	20.58 ± 7.69abd	4.34 ± 2.41c	72.38	80	85.71	27.62	23.68	-0.03	-0.72
50% RHA	34.70 ± 11.16c	16.82 ± 6.14ab	-7.10	70	75	107.10	80.32	-0.15	0.07
100% RHA	26.76 ± 8.90bc	28.21 ± 12.91ab	-79.63	50	107.14	179.63	192.46	-0.39	0.80
PC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: **TAS** = Tropical Artificial Soil; **AHL** = Average hypocotyl length; **ARL** = Average radicle length; **IRG** = Inhibition of radicle growth; **Germ.** = Germination; **RGP** = Relative seed germination percentage; **RRG** = Relative radicle growth; **GI** = Germination index; **NGI** = Normalized residual germination percentage index; **NEI** = Normalized residual radical percent elongation index; **PC** = Positive control. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Table 4. Germination results for arugula (*Eruca sativa*) in vineyard soil with different concentrations of rice husk ash (RHA), using vineyard soil as the negative control for index calculations.

Sample	AHL (mm)	ARL (mm)	IRG (%)	Germ. (%)	RGP (%)	RRG (%)	GI (%)	NGI (%)	NEI (%)
Vineyard	17.00 ± 6.07	4.26 ± 1.46	-	66.67	-	-	-	-	-
TAS	34.36 ± 9.64	24.73 ± 10.37	-480.61	97	93.33	580.61	541.90	0.45	4.81
Native field	26.59 ± 9.07	17.27 ± 6.25	-305.42	93.33	66.67	405.42	270.28	0.40	3.05
5% RHA	16.34 ± 6.19	6.16 ± 4.29	-44.65	83.33	83.33	144.65	120.54	0.25	0.45
15% RHA	22.62 ± 8.74	5.81 ± 2.50	-36.32	70	70	136.32	95.42	0.05	0.36
25% RHA	20.58 ± 7.69	4.34 ± 2.41	-1.88	80	80	101.88	81.51	0.20	0.02
50% RHA	34.70 ± 8.77	16.82 ± 6.08	-295	70	70	395	276.50	0.05	2.95
100% RHA	26.73 ± 8.90	28.21 ± 12.91	-562.52	50	100	662.52	662.52	-0.25	5.63
PC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: **TAS** = Tropical Artificial Soil; **AHL** = Average hypocotyl length; **ARL** = Average radicle length; **IRG** = Inhibition of radicle growth; **Germ.** = Germination; **RGP** = Relative seed germination percentage; **RRG** = Relative radicle growth; **GI** = Germination index; **NGI** = Normalized residual germination percentage index; **NEI** = Normalized residual radical percent elongation index; **PC** = Positive control. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

The RHA residue can be used as a source of nutrients and as a conditioner of the physical characteristics of degraded soils, especially in small farms (Pauletto *et al*., 1990). In studies on the effect of RHA on soil physical properties, Karmakar *et al*. (2009) observed that the soil density decreased with the application of this industrial waste. Masulili *et al*. (2010), studying the effect of carbonized rice husk and RHA, observed that with the application of these residues in the soil, there was a reduction in density and resistance to penetration and an increase in total porosity and water availability.

According to Reichard (2004), the water balance is defined as the sum of the amounts of water that enter and leave an element of soil volume and, in a given time interval, the result is the net amount of water that remains in it. From an agronomic point of view, water balance is essential, as it determines the water conditions under which a crop develops. The use of RHA increased water retention and soybean yields, as well as correcting acidity and supplying nutrients to the soil (Stracke *et al.*, 2020).

The absorption, distribution, and accumulation of heavy metals, including Cu and Zn, in plants are carried out through membrane transporters, which, in turn, are essential for the processes of homeostasis and tolerance or detoxification of these metals (Arbaoui *et al.*, 2014). Metal transporters in plants are present in the plasma membrane of cells of the epidermis and root cortex, including root hairs, or even in other plant organs, and also in the membranes of cell organelles, such as the vacuole (Grotz & Guerinot, 2006; Marschner, 2012; Arbaoui *et al.*, 2014).

According to Haag and Minami (1988), Arugula has a high copper extraction capacity. However, there is a gap in the scientific literature regarding the response of this vegetable to the increasing supply of copper, which may be a determining factor in increasing its productivity. This knowledge is essential to better understand the interaction between the plant and the micronutrient in agricultural systems. In addition, Bandeira *et al*. (2022) observed that the behavior of zinc in arugula presented statistically significant differences concerning the other vegetables evaluated, with higher levels of this metal compared to the other elements analyzed. These results reinforce the importance of further studies on the relationship of arugula with specific metals, considering its potential as a bioindicator and the implications for food and environmental security.

CONCLUSION

This study demonstrated that the addition of rice husk ash (RHA) to soils can result in phytotoxic effects on seed germination and early seedling development, with the intensity of the effects varying according to soil type and RHA concentration. The vineyard soil, which had high copper levels, showed greater toxicity, especially for arugula, suggesting that the interaction between RHA and contaminated substrates may exacerbate adverse effects on plant development. Although this study did not include pre- and post-treatment analyses of soil properties typically required to characterize agronomic soil amendments, it provides valuable ecotoxicological evidence regarding the short-term effects of RHA addition on plant development. These findings contribute to a better understanding of the interactions between RHA and different soil types.

These findings contribute to a better understanding of the interactions between RHA and different soil conditions and highlight the need for cautious evaluation of its use. Further research is required to assess the long-term effects and potential agronomic benefits of RHA, including its impact on soil properties and its safe application in diverse agricultural contexts.

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CREDIT AUTHOR STATEMENT

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