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Original Article

Phytotoxicity of pulp industry sludge in *Festuca arundinacea* and *Triticum aestivum* in different soils[1]

Fitotoxicidade de lodo da indústria de celulose em *Festuca arundinacea* e *Triticum aestivum* em diferentes solos

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HIGHLIGHTS:

Pulp industry sludge has the potential to promote plant growth.
Festuca arundinacea *seeds showed high germination index (> 80%), indicating that the sludge is not phytotoxic.*
Growth responses varied between soil types, highlighting the importance of soil characteristics for sludge applications.

ABSTRACT: The use of alternative waste products as organic soil amendments has become increasingly important for researchers and the industrial sector, as they promote environmental protection and sustainable development. The objective of this study was to assess phytotoxicity of different pulp industry sludge rates applied to two soils as soil amendment, based on their effects on seed germination and radicle elongation of tall fescue (*Festuca arundinacea*) and common wheat (*Triticum aestivum*). The pulp industry sludge rates applied were 1.31, 1.57, 2.61, 3.27, 3.92, and 5.23 Mg ha^{-1}, based on soil liming requirements. Absorbent paper and soil without sludge application were used as control treatments. The bioassay was conducted by sowing 20 seeds in 15 g of soil, which were maintained in a germination chamber at 25 °C for 7 days (*T. aestivum*) and 14 days (*F. arundinacea*) with a 12-hour photoperiod. Relative germination percentage, relative root growth, germination index, normalized residual germination, and normalized residual root elongation were evaluated. The results highlight the potential of using pulp industry sludge as an organic soil amendment, as it showed significant improvements in germination and root elongation for *F. arundinacea*. Although phytotoxicity was observed in *T. aestivum* seeds at lower sludge rates, the toxic effect decreased at higher rates, indicating that the use of pulp industry sludge is promising for agricultural and environmental applications.

Key words: soil amendment, germination, radicle elongation, wheat

RESUMO: A utilização de alternativas de resíduos, como os corretivos orgânicos, está a tornar-se cada vez mais importante tanto para os investigadores como para o setor industrial, a fim de promover o cuidado ambiental e o desenvolvimento sustentável. O objetivo desta pesquisa foi examinar a existência de fitotoxicidade em dois solos após a aplicação de doses de lodo da indústria de celulose, utilizado como corretivo, avaliando seu impacto na germinação e no alongamento da radícula em sementes de *Festuca arundinacea* e trigo comum (*Triticum aestivum)*. As doses de lodo da indústria de celulose foram equivalentes a 1,31, 1,57, 2,61, 3,27, 3,92 e 5,23 t ha^{-1} e estabelecidas com base na necessidade de calagem do solo. Os tratamentos de controle foram utilizados papel absorvente e solo sem aplicação de lodo. O bioensaio foi avaliado colocando 20 sementes em 15 g de solo. Utilizou-se câmara de germinação com fotoperíodo durante 7 dias no caso do trigo e 14 dias para *F. arundinacea,* a 25 °C. Foram calculados a porcentagem relativa de germinação, o crescimento relativo das raízes, o índice de germinação, a germinação residual normalizada e o alongamento residual normalizado das raízes. Os resultados destacam o potencial do lodo como condicionador orgânico evidenciando melhorias significativas na germinação e no alongamento radicular da *F. arundinacea*. Apesar da fitotoxicidade observada na semente de trigo em doses mais baixas, seu efeito foi reduzido em doses mais altas, sugerindo um possível uso promissor do lodo em aplicações agrícolas e ambientais.

Palavras-chave: condicionador de solo, germinação, alongamento de raiz, trigo

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Introduction

Pulp production generates large quantities of waste sludge, which poses environmental challenges for its safe and sustainable disposal (Barros & Marisquirena, 2023). Thus, this issue should be comprehensively addressed to achieve a balance between industrial development and environmental sustainability. The agricultural use of pulp industry sludge is one of the alternatives, as it has several significant benefits to soil and the environment. These sludges contain various essential nutrients, including nitrogen, phosphorus and potassium, that can enrich the soil and improve its fertility (Aravena et al., 2007). In addition, it can be used to correct soil acidity, being an alternative to agricultural lime (Maciel et al., 2015).

The use of pulp sludges can reduce the dependency on chemical soil amendments and fertilizers, contributing to environmental and economic sustainability of agriculture (Aravena et al., 2007). However, this material may contain other organic or inorganic components that may negatively affect plant development (Granato et al., 2004). Prior analysis and evaluation are essential to determine the potential effectiveness of pulp industry sludges as soil amendments. Bioassays are a key tool in this process, as they provide data on the viability and environmental safety of these sludges.

These bioassays allow for the simulation of interactions between sludge and soil components, revealing beneficial or adverse effects before large-scale application. Furthermore, bioassays can assist in identifying optimal rates and necessary conditions to maximize soil acidity correction, ensuring the sustainable and responsible management of these industrial wastes. Additionally, bioassays allow for the detection of toxic substances and laboratory evaluation of phytotoxicity to plant materials, which may cause adverse consequences to crop development; phytotoxicity analysis is an important biological test and a common and crucial practice for evaluating effects that may inhibit seed germination or plant growth (Zhang et al., 2021; Niu et al., 2021).

Phytotoxicity assays based on seed germination and radicle elongation can be conducted using various species, including economically important plants, which are easily accessible and present a fast growth (Fletcher et al., 1985). The use of agricultural species in phytotoxicity assays has numerous advantages compared to those that use animals or algae. The advantages of using plant seeds include their dormant state (dried or dehydrated), which allows them to remain viable under adverse conditions, and their ability to undergo rapid changes in metabolism, nutrient transport, and cell division when exposed to favorable conditions (Mayer & Polsakoff-Mayer, 1982). These immediate responses to environmental conditions make seeds ideal organisms to serve as environmental sentinels (Wang, 1991).

Verifications of suitability of planting media for germination tests generally involves the use of seeds from species known to be sensitive to toxic substances, including *Festuca rubra* and *Hordeum vulgare* (Don et al., 2013). Poaceae species of the genus *Festuca* have shown highly contrasting reactions to phytotoxicity: *F. rubra* is a forage species highly sensitivity to phytotoxic compounds during germination and seedling stages (Don et al., 2013), whereas *F. arundinacea* is a perennial winter forage grass, widely used as livestock feed in intensive systems, such as dairy farming.

In this context, the objective of this study was to assess phytotoxicity of different pulp industry sludge rates applied to two soils as soil amendment, based on their effects on seed germination and radicle elongation of tall fescue (*Festuca arundinacea* cv. Carapé) and common wheat (*Triticum aestivum*).

Material and Methods

The study was conducted at the Soil Physics Laboratory of the South Central Regional Technological Institute of the Technological University of Uruguay, in Durazno, Uruguay (-33.38718524176408 latitude, -56.514919676477334 longitude, and 90 m altitude). The two soils used were classified according to the USDA, EMBRAPA, and FAO classification systems as: Alfisols (USDA-NRCS, 2014), Planossolos (Santos, 2018), and Eutric Luvic Cambisol and Dystric Luvic Cambisol (WRB, 2014). The pulp industry sludge rates applied were equivalent to 1.31, 1.57, 2.61, 3.27, 3.92, and 5.23 Mg ha^{-1}, based on the soil liming requirements (50, 75, 100, 125, 150, and 200%, respectively), estimated considering an increase in base saturation to 100 and a 50.28% soil acidity neutralization by the sludge. Chemical characteristics of the soils and the pulp industry sludge used are shown in Tables 1 and 2, respectively.

The soils with different sludge rates were incubated for 170 days prior to the establishment of bioassays. Soil acidity neutralization was tested by incubating a 1 kg air-dried, sieved (2 mm) sample of each soil type for each treatment. Samples were placed into transparent polyethylene bags with a gas exchange tube, containing the different sludge rates (without temperature control). Sludge was manually mixed with the soil in each sample before closing the bag and then mixed manually weekly. Distilled water was applied through the tube of each experimental unit until soil moisture reached 60 to 70% of field capacity.

A bioassay was conducted using samples from each treatment to evaluate the biological effects of sludge application to soils. Germination bioassays were performed according to the methodology proposed by Zucconi et al. (1981): 15 g of soil were placed in Petri dishes, and 20 seeds of tall fescue (*Festuca arundinacea* cv. Carapé) or wheat (*Triticum aestivum*) were sown per Petri dish, with three replications. Samples were compared to a control with absorbent paper and a control with soil without sludge application. Moisture was maintained between 60 and 70% of field capacity using with distilled water.

The Petri dishes were incubated in a germination chamber (Tecnal TE-406/1) for seven days (*T. aestivum* seeds) and 14 days (*F. arundinacea* seeds) under a 12-hour photoperiod, constant temperature of 25 °C, and 75% relative air humidity.

Germination, germination speed index, and radicle and epicotyl growth were evaluated. Relative germination percentage (RGP, Eq. 1), relative radicle growth (RRG, Eq. 2), and germination index (GI, Eq. 3) were calculated following the methodology described by Tiquia (2000). The interpretation of

Table 1. Mean and standard deviation of chemical attributes of the two selected soils: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Variables	Soil A	Soil B	Unit
	Mean	Mean	
pH (KCl)	4.12 ± 0.04	3.94 ± 0.05	
pH (H_2O)	5.29 ± 0.05	5.13 ± 0.06	mg kg^{-1} or mg dm^{-3}
Phosphorus (P)	2.67 ± 0.58	1.00 ± 0.00	
Nitrate (NO_3-N)	6.67 ± 0.58	5.00 ± 2.65	
Nitrogen (N)	1372.60 ± 44.41	963.77 ± 21.74	mg kg^{-1}
Organic matter	2.37 ± 0.32	2.63 ± 0.12	%
Organic carbon	1.47 ± 0.15	1.63 ± 0.06	
Calcium (Ca)	3.80 ± 0.10	3.73 ± 0.12	
Sodium (Na)	0.06 ± 0.01	0.08 ± 0.00	
Magnesium (Mg)	0.70 ± 0.00	0,87 ± 0.06	
Potassium (K)	0.11 ± 0.01	0.27 ± 0.01	meq 100 g^{-1}
Aluminum (Al)	0.16 ± 0.01	0.18 ± 0.02	
Titratable acidity	1.31 ± 0.07	1.29 ± 0.09	
Cation exchange capacity	6.00 ± 0.10	6.23 ± 0.12	

Table 2. Mean and standard deviation of chemical attributes of the pulp industry sludge

Variables	Mean	Unit
pH (H_2O)	8.8	
Aluminum (Al)	0.64 ± 0.02	
Calcium (Ca)	21.9 ± 0.2	
Iron (Fe)	0.32 ± 0.01	
Phosphorus (P)	0.80 ± 0.01	
Magnesium (Mg)	1.05 ± 0.02	% (m/m)
Manganese (Mn)	0.11 ± 0.01	
Potassium (K)	0.21 ± 0.01	
Sodium (Na)	0.25 ± 0.01	
Arsenic (As)	1.93 ± 0.08	
Cadmium (Cd)	0.04 ± 0.01	
Lead (Pb)	6.4 ± 0.1	
Copper (Cu)	4.6 ± 0.4	
Chromium (Cr)	13.6 ± 0.3	μg g^{-1}
Mercury (Hg)	<0.01	
Nickel (Ni)	26.7 ± 0.9	
Zinc (Zn)	<0.5	

the germination index was established as follows: germination indices below 50% indicate high material phytotoxicity, between 50% and 80% indicate moderate phytotoxicity, and above 80% indicate that the material does not exhibit phytotoxicity (Emino & Warman, 2004).

$$RGP(\%) = \frac{\text{N. germinated seeds with the problem sample}}{\text{N. germinated seeds in the control}} \times 10 \quad (1)$$

$$RRG(\%) = \frac{\text{Mean radicle length with the test sample}}{\text{Mean radicle length in the control}} \quad (2)$$

$$GI(\%) = \frac{RGP \times RRG}{100} \quad (3)$$

Additionally, normalized residual germination (NRG, Eq. 4) and normalized residual radicle elongation (NRE, Eq. 5) were calculated. Bagur-González et al. (2011) established phytotoxicities from -1 to > 0 for NRG and NRE into the following categories: 0 to -0.25 = low phytotoxicity; -0.25 to -0.5 = moderate phytotoxicity; -0.5 to -0.75 = high phytotoxicity; -0.75 to -1.0 = very high phytotoxicity; and NRG or NRE > 0 indicate radicle growth or hormesis.

$$NRG = \frac{\text{Germ} \times \text{Germ control}}{\text{Germ control}} \quad (4)$$

$$NRE = \frac{\text{Elong} \times \text{Elong control}}{\text{Elong control}} \quad (5)$$

A completely randomized experimental design with three replicates was used. Percentage data were transformed according to the formulas (angular transformation) indicated by Montgomery (2011), prior to statistical analyses. Germination percentages were analyzed using generalized linear mixed model in the R software (R Core Team, 2007). Data were subjected to analysis of variance (ANOVA) and Dunnett's test ($p \leq 0.05$) was used to compare means of experimental groups with a control group. Additionally, the data were subjected to ANOVA (F-test) followed by Tukey's test ($p \leq 0.05$) to compare means. Interactions between soil types and sludge rates were assessed through linear, polynomial, and other regression models. The best-fitting model was displayed in graphs. Statistical analyses were performed using SAS® 24 and InfoStat® (Di Rienzo et al., 2015).

Results and Discussion

Tables 3 and 4 show the results of analysis of variance (ANOVA) and Dunnett's test for data of epicotyl growth and radicle elongation for *Triticum aestivum* seeds as a function of

Table 3. Analysis of variance and Dunnett's test for epicotyl growth of *Triticum aestivum* as a function of applying different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Source	DF	Adjusted SS	Adjusted MS	F Value	p Value
			Soil A		
Treatment	6	13.13	2.188	1.38	0.290
Error	14	22.26	1.590		
Total	20	35.39			
			Soil B		
Treatment	6	8.943	1.490	0.95	0.493
Error	14	22.027	1.573		
Total	20	30.970			

DF - Degrees of Freedom; Adjusted SS - Adjusted Sum of Squares; Adjusted MS - Adjusted Mean Square; Dunnett's test ($p \leq 0.05$)

Table 4. Analysis of variance and Dunnett's test for radicle elongation of *Triticum aestivum* as a function of applying different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Source	DF	Adjusted SS	Adjusted MS	F Value	p Value
			Soil A		
Treatment	6	5.206	0.8676	0.58	0.743
Error	14	21.047	1.5033		
Total	20	26.252			
			Soil B		
Treatment	6	2.939	0.4898	0.76	0.612
Error	14	9.013	0.6438		
Total	20	11.952			

DF - Degrees of Freedom; Adjusted SS - Adjusted Sum of Squares; Adjusted MS - Adjusted Mean Square; Dunnett's test ($p \leq 0.05$)

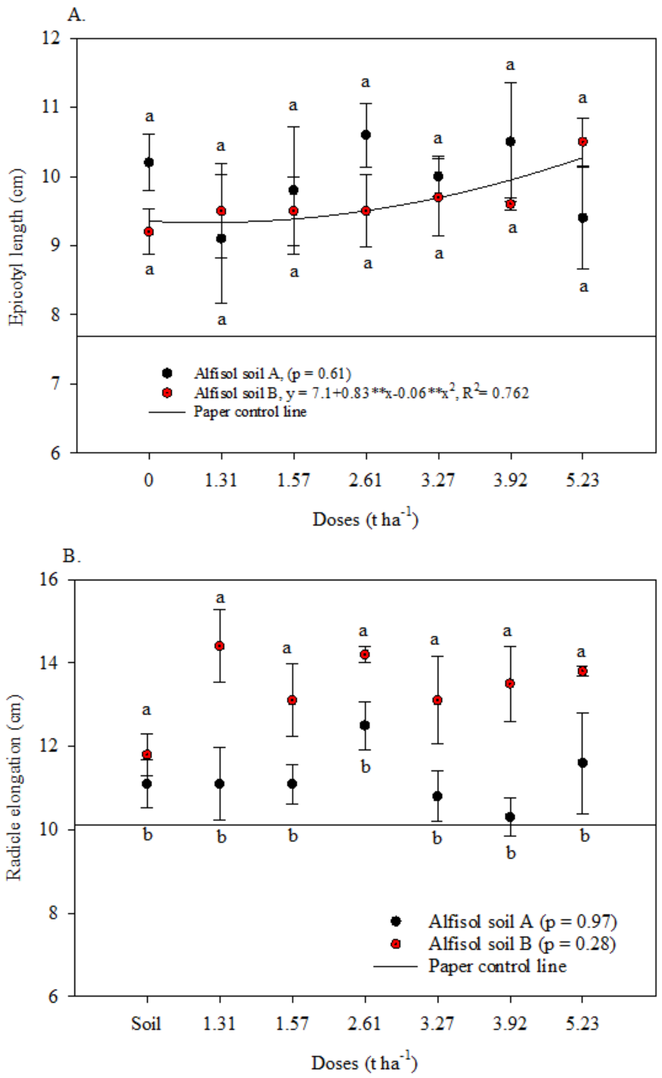
applying different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B). The epicotyl growth for *T. aestivum* seeds grown in Soils A and B was not significantly affected by sludge rates. The ANOVA results showed that the effects of treatments were similar for radicle elongation of *T. aestivum* in Soils A and B.

Mean epicotyl growth and radicle elongation of *T. aestivum* under application of pulp industry sludge rates in Soils A and B are shown in Figure 1. The longest and shortest lengths were 12.06 cm in Soil A at a sludge rate of 3.92 Mg ha^{-1} and 6.93 cm in the control treatment, respectively. Coefficients of determination were 0.1 for Soil A (considered low) and 0.76 for Soil B (considered high). All sludge rates resulted in greater epicotyl growth than the control (absorbent paper) and soil without sludge application. The coefficients of determination were similar for radicle elongation.

Analysis of variance and Tukey's test showed no statistically significant effect of the interaction between factors (soil type and pulp industry sludge rates) on epicotyl growth of *T. aestivum*. The statistical analysis showed a significant effect of the soil type factor on radicle elongation (Figure 1B). *T. aestivum* seeds exhibited the longest roots (15.8 cm) when applying a sludge rate of 1.31 Mg ha^{-1} to Soil B; the smallest radicle elongation (9.1 cm) was found for the control. Coefficients of determination were 0.02 (MSE = 0.68) and 0.28 (MSE = 0.81) for Soils A and B, respectively. Thus, a greater epicotyl and root development was found in soils with application of pulp industry sludge, indicating that it improved soil physical and chemical properties, promoting the growth and establishment of *T. aestivum* seedlings. Other studies have also used wheat plants as bioindicators (Gianelli et al., 2011; Castillo et al., 2020; Lallana et al., 2022).

Analysis of variance and Dunnett's test evaluating epicotyl growth and radicle elongation of *Festuca arundinacea* as a function of applying different pulp industry sludge rates to two Soils A and B are shown in Tables 5 and 6. The treatments had no statistically significant effects on epicotyl growth and radicle elongation of *F. arundinacea* grown in Soils A and B.

Mean epicotyl growth and radicle elongation of *F. arundinacea* grown under application of pulp industry sludge rates to Soils A and B are shown in Figure 2. *F. arundinacea* seeds showed the longest epicotyl growth (11.15 cm) when grown in Soil A with application of 3.92 Mg ha^{-1} of sludge. The shortest epicotyl growth (4.6 cm) was found for the control.



**Significant at $p \leq 0.01$ by F test. Means followed by different letters significantl by the Tukey's test ($p \leq 0.05$)

Figure 1. Epicotyl length (A) and radicle elongationlengths of *Triticum aestivum* as a function of applying diffpulp industry sludge rates to two soil types: Eutric LCambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Table 5. Analysis of variance and Dunnett's test for epicotyl growth of *Festuca arundinacea* as a function of applying different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Source	DF	Adjusted SS	Adjusted MS	F Value	p Value
			Soil A		
Treatment	6	2.758	0.4597	1.00	0.463
Error	14	6.433	0.4595		
Total	20	9.191			
			Soil B		
Treatment	6	2.892	0.4821	0.27	0.941
Error	14	24.740	1.7671		
Total	20	27.632			

DF - Degrees of Freedom; Adjusted SS - Adjusted Sum of Squares; Adjusted MS - Adjusted Mean Square; Dunnett's test ($p \leq 0.05$)

Coefficients of determination were 0.05 (Soil A) and 0.02 (Soil B). Mean epicotyl growth of all treatments were higher than those found for the control (absorbent paper) and the soil without sludge application. *F. arundinacea* seeds had the longest roots (9.23 cm) when grown in Soil A at a sludge rate of 5.23 Mg ha^{-1}; the lowest mean (4.3 cm) was found for the

Table 6. Analysis of variance and Dunnett's test for root length of *Festuca arundinacea* as a function of applying different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Source	DF	Adjusted SS	Adjusted MS	F Value	p Value
			Soil A		
Treatment	6	7.003	1.167	0.46	0.830
Error	14	35.887	2.563		
Total	20	42.890			
			Soil B		
Treatment	6	1.638	0.2730	1.20	0.361
Error	14	3.180	0.2271		
Total	20	4.818			

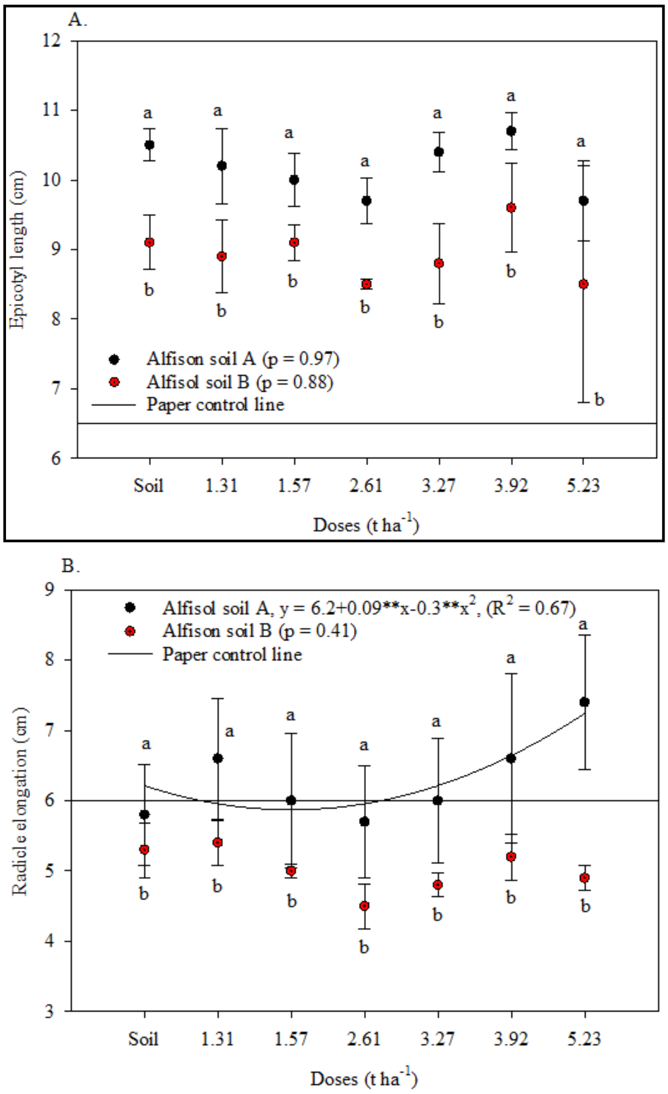
DF - Degrees of Freedom; Adjusted SS - Adjusted Sum of Squares; Adjusted MS - Adjusted Mean Square; Dunnett's test ($p \leq 0.05$)

control. Coefficients of determination were 0.67 for Soil A and 0.45 (MSE = 0.07) for Soil B.

The application of pulp industry sludge as a soil amendment resulted in varying effects on the growth of *F. arundinacea* and *T. aestivum* across the different soil types and sludge rates. Some sludge rates positively affected seedling growth, especially in root and epicotyl growth, but the benefits were not uniform across species or soil types. Overall, sludge treatments improved soil fertility, which may be attributed to the addition of organic matter and nutrients, such as nitrogen and phosphorus. However, the response to sludge application was rate-dependent, with optimal growth at moderate application rates. Excessive sludge rates, mainly exceeding 150% of soil liming requirements, did not enhance plant growth and may have led to nutrient imbalances or other adverse effects, such as soil compaction or reduced aeration. The different responses of *F. arundinacea* and *T. aestivum* to sludge application indicate species-specific physiological and morphological adaptations. *F. arundinacea*, a perennial grass, overall, exhibited a more robust response to sludge application, mainly in terms of root development, which is crucial for plant establishment and resilience in less fertile soils. The increased root length with moderate sludge application rates indicates improved nutrient uptake and soil exploration capacity, which are essential for sustainable growth under suboptimal conditions.

T. aestivum is an annual crop with different root architecture; it showed less pronounced improvements in growth at similar sludge rates. This is probably due to its different nutrient requirements or shorter growth cycle, which may prevent the full utilization of nutrients slowly released from the sludge. These findings indicate that sludge can be beneficial for both species, but *F. arundinacea* may be better suitable to explore the long-term benefits of this organic amendment. The interaction between soil type and sludge application was a critical factor for determining plant growth.

ANOVA and Tukey's test results indicated that the soil type significantly affected the effectiveness of sludge treatments (Figure 2). Soils with higher organic matter content or better structure may provide a more conducive environment for the sludge to enhance plant growth. Contrastingly, soils with poor initial fertility or more physically challenging conditions may require alternative management strategies to fully benefit from sludge. Soil A (Eutric Luvic Cambisol) may have had lower organic matter content or higher compaction, making the positive effects of sludge on root and epicotyl growth more evident, probably due to improvements in soil structure and nutrient availability.



**Significant at $p \leq 0.01$ by F test. Means followed by different letters significantly differ by the Tukey's test ($p \leq 0.05$)

Figure 2. Mean epicotyl length (A) and radicle elongation (B) for *Festuca arundinacea* cv. Carapé seeds grown under application of different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

Conversely, Soil B (Dystric Luvic Cambisol) was potentially more fertile or better structured, showing less significant improvements, indicating that the benefits of sludge applications may be more pronounced in marginal soils where health interventions are most needed. These findings indicate that moderate sludge application rates, mainly 100 to 150% of soil liming requirements, provide the best balance between improving soil fertility and avoiding potential negative effects such as nutrient imbalances or soil degradation. These rates may have enhanced plant growth, mainly for *F. arundinacea*, without risks associated with higher sludge rates. Moreover, despite the sludge application at these rates was beneficial, the lack of significant differences at higher rates indicates little advantages in exceeding the recommended levels.

These results have important implications for sustainable agriculture, mainly in the context of using industrial by-products as soil amendments. The positive response of *F.*

arundinacea, especially when grown in less fertile soils, indicates that the sludge can be an effective tool for improving pasture establishment and yield in marginal soils. Despite the benefits were less pronounced for *T. aestivum*, the use of sludge is a viable option for enhancing soil fertility, especially for systems focused on reducing dependence on synthetic fertilizers. Alvarez-Campos & Evanylo (2019) used *F. arundinacea* plants as bioindicator, with the N fertilizer equivalency method and several chemical, organic, and biological tests for quantifying available nitrogen and organic N mineralization of biosolids in the plants.

Germination index (GI) data for *F. arundinacea* and *T. aestivum* seeds grown under application of different pulp industry sludge rates to Soils A and B are shown in Figure 3. The statistical analysis showed no significant interaction effect between sludge rates and soil types. The highest GI was 145% at a sludge rate of 3.27 Mg ha^{-1}, while the highest mean was 130.17% at a rate of 1.31 Mg ha^{-1}, both in Soil B. In Soil A, the highest GI and mean GI were 108.86 and 94.49% at sludge rates of 2.61 and 5.23 Mg ha^{-1}, respectively.

GI combines relative seed germination and relative radicle growth, serving as an indicator of the effect of factors that can promote or inhibit germination. GI incorporates percentages of germinated seeds and radicle lengths during bioassays, providing a comprehensive view of seed germination potential under specific conditions.

The mean GI values found (54.98 to 70.28%) indicate that the evaluated sludge rates resulted in a moderate phytotoxicity to *T. aestivum* seeds, except for the 5.23 Mg ha^{-1} rate, which resulted in a germination rate higher than 80%. GI in the control treatments, without sludge application, reached 104.43 and 103.58%. The highest GI found for *F. arundinacea* seeds was 131.40% at a sludge rate of 1.31 Mg ha^{-1}, while the highest mean GI was 110.63% at a rate of 3.27 Mg ha^{-1}, in Soil B. In Soil A, the highest GI and highest mean GI were 130.20 and 113.73%, respectively, at a sludge rate of 5.23 Mg ha^{-1}. GI in the control treatments, without sludge application, reached 87.57 and 104.97%. The GI found for *Festuca arundinacea* seeds exceeded 80% at all evaluated sludge rates and soils, indicating that pulp industry sludge can promote growth with no phytotoxicity for germination at 170 days after application to the soil.

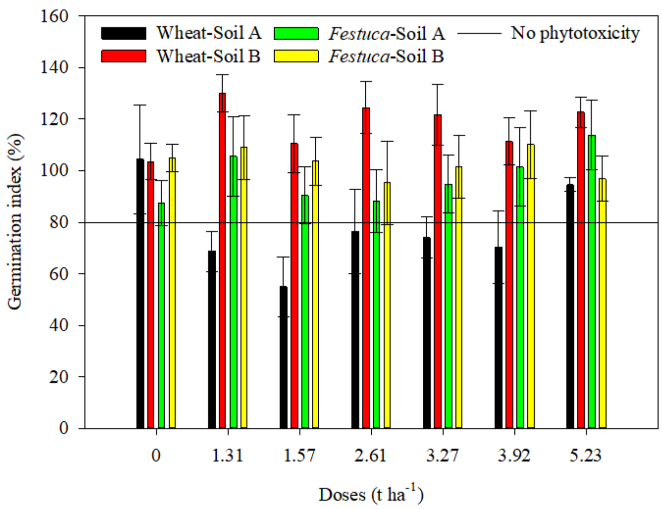


Figure 3. Germination index (GI; %) for *Festuca arundinacea* cv. Carapé and *Triticum aestivum* seeds grown under application of different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

The normalized residual germination (NRG) and normalized residual radicle elongation (NRE) for *F. arundinacea* and *T. aestivum* seeds grown under application of different pulp industry sludge rates to Soils A and B are shown in Figure 4. These results were similar to those obtained for GI. The statistical analysis showed no significant interaction or significant differences. According to NRG for *T. aestivum* seeds grown in Soil A, rates of 1.31, 1.57, 2.61, 3.27, and 3.92 Mg ha^{-1} resulted in moderate phytotoxicity, while 5.23 Mg ha^{-1} caused low phytotoxicity. However, the sludge rates caused low phytotoxicity in the control without sludge application. All sludge rates resulted in NRE > 0, indicating hormesis. *F. arundinacea* seeds presented NRG > 0, and the NRE denoted low phytotoxicity at rates of 1.57, 2.61 and 3.27 Mg ha^{-1}, but with no significant difference from the control without sludge application.

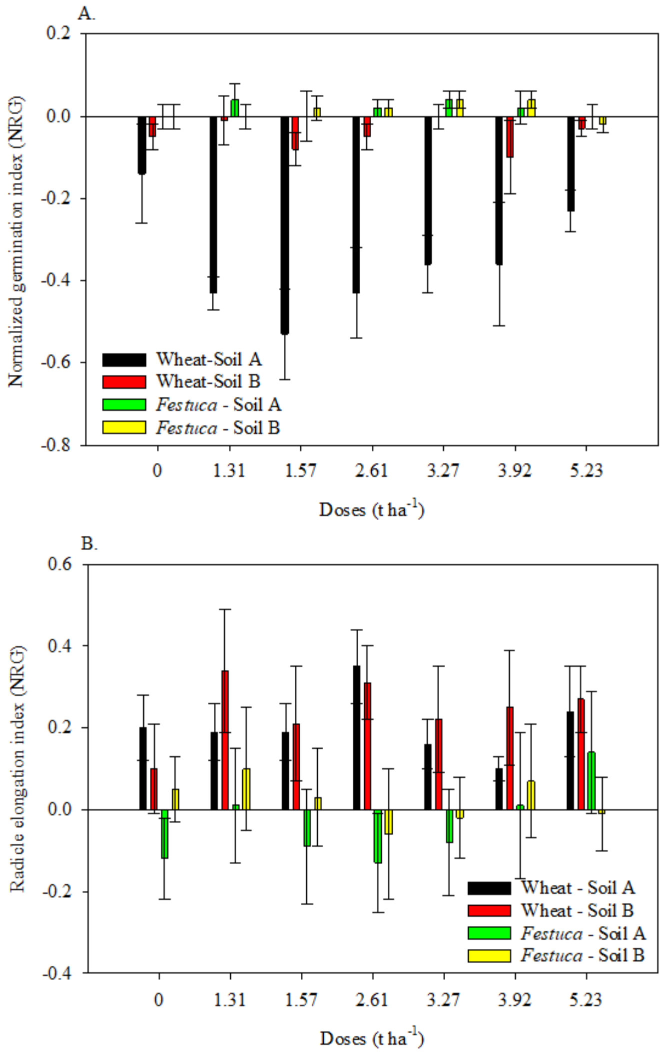


Figure 4. Normalized germination index (A) and radicle elongation index (B) for *Festuca arundinacea* cv. Carapé and *Triticum aestivum* seeds grown under application of different pulp industry sludge rates to two soil types: Eutric Luvic Cambisol (Soil A) and Dystric Luvic Cambisol (Soil B)

NRG found for *T. aestivum* seeds grown in Soil B denoted low phytotoxicity for all sludge rates and the control. The results showed NRE > 0, indicating no phytotoxicity. *F. arundinacea* seeds showed NRG > 0 for all rates, and NRE > 0 for rates of 2.61, 3.27, and 5.23 Mg ha^{-1}, denoting low phytotoxicity.

According to Escalante-Campos et al. (2012), in a heavy metal exposure bioassay, both root and shoot growth of *Festuca rubra* seedlings were reduced in the presence of cadmium; however, for *Axonopus affinis*, a stimulatory effect was observed on both coleoptile and radicle elongation, leading to the conclusion that seeds inoculated with the rhizobacterium *Pseudomonas* sp. Sp7D in both *A. affinis* and *F. rubra* exhibited growth-promoting potential in germination and seedling growth; this effect was more pronounced on *F.* rubra; moreover, this bacterium strain showed protective potential against cadmium in both species.

Furthermore, Soto et al. (2022) studied the salinity tolerance of *F. arundinacea* and concluded that the association of this species with a wild, safe endophytic fungus did not confer salinity tolerance to the wild population or cv. Taita, respectively, as no advantages were found in any of the germination-related variables. Additionally, Alvarez-Campos & Evanylo (2019) reported that correlations between N uptake by *F. arundinacea* and soil N tests showed that soil NO3–N and 7-AI were the best indicators of biosolid N availability in urban soils. However, the relatively low correlations between soil N indicators and N uptake by *F. arundinacea* were likely due to low residual N, high clay content, and potentially low microbial activity in the anthropogenic soil with low organic matter content. In a recent study, Liu et al. (2023) investigated the allelopathic effects of root exudates from *Zelkova schneideriana* on the growth of various crops, including *F. arundinacea*.

Root exudates, containing 39 identified compounds, inhibited growth at higher concentrations but promoted it at lower ones. *F. arundinacea* showed the least inhibition, making it a potential candidate for intercropping with *Z. schneideriana*. Franceschi et al. (2019) conducted a bioassay on *Cucumis sativus* plants and found leaching of the herbicide picloram in a dystrophic Red Yellow Latosol, but the leaching intensity decreased down the soil profile, regardless of limestone application. They concluded that picloram showed a high leaching rate under all studied conditions, limiting the increase in picloram leaching at rates below 768 g ha^{-1}.

Conclusions

1. *Festuca arundinacea* (tall fescue) seeds showed increased germination and root elongation for all evaluated pulp industry sludge rates at 170 days after application to soils.

2. Although *Triticum aestivum* (wheat) seeds demonstrated certain levels of phytotoxicity at the applied pulp industry sludge rates, these effects decreased at higher rates (around 5 Mg ha^{-1}).

3. Considering the lack of significant phytotoxicity towards *F. arundinacea* and *T. aestivum* seeds, pulp industry sludge emerges as a viable organic soil amendment alternative for agricultural and environmental applications.

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