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Original Article

Toxicological evaluation of rice paddy wastewater on the germination of zea mays

Evaluación toxicológica de aguas residuales de arroceras en el proceso germinativo de zea mays

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Spanish version

Abstract

Objective: To evaluate the toxicity effect of irrigation water from rice crops on the germination process of Zea mays seeds.
Methodology: A quantitative approach with a descriptive and experimental design was used. Water samples were collected from two rice cultivation systems in the department of Norte de Santander and subjected to physicochemical analysis. Field germination tests and in vitro bioassays were also conducted to evaluate phytotoxic effects.
Results: The physicochemical analyses revealed differences in water quality between the evaluated systems, suggesting varying levels of biodegradability and the presence of persistent organic compounds. No acute phytotoxic effects were detected in the field germination tests, with a 100% germination rate achieved in the wastewater treatments, while the positive control showed total inhibition. Differential responses were observed in the in vitro bioassays, with biostimulatory effects in one system and inhibitory effects in the other. Statistical analysis showed significant differences between treatments ($p < 0.05$).
Conclusions: Wastewater from rice farming systems can produce sublethal physiological effects under controlled conditions. Its use as an irrigation alternative for crops such as maize could be viable in the short term, provided it comes from systems with low agrochemical loads.

Keywords: agricultural wastewater, phytotoxicity, germination, Zea mays**.**

Resumen

Objetivo: Evaluar el efecto de toxicidad de las aguas de riego provenientes de cultivos de arroz en el proceso germinativo de semillas de Zea mays.
Metodología: Se empleó un enfoque cuantitativo con diseño descriptivo y experimental. Las muestras de agua fueron recolectadas en dos sistemas de cultivo de arroz del departamento de Norte de Santander y sometidas a análisis fisicoquímicos. Además, se realizaron ensayos de germinación en campo y bioensayos in vitro para evaluar efectos fitotóxicos.
Resultados: Los análisis fisicoquímicos evidenciaron diferencias en la calidad del agua entre los sistemas evaluados, sugiriendo distintos niveles de biodegradabilidad y presencia de compuestos orgánicos persistentes. En los ensayos de germinación en campo no se detectaron efectos fitotóxicos agudos, alcanzándose una tasa de germinación del 100 % en los tratamientos con aguas residuales, mientras que el control positivo presentó inhibición total. En los bioensayos in vitro se observaron respuestas diferenciadas, con efectos bioestimulantes en un sistema y efectos inhibitorios en el otro. El análisis estadístico mostró diferencias significativas entre tratamientos ($p < 0,05$).
Conclusiones: Las aguas residuales de sistemas arroceros pueden generar efectos fisiológicos subletales en condiciones controladas. Su uso como alternativa de riego para cultivos como el maíz podría ser viable a corto plazo, siempre que provengan de sistemas con baja carga de agroquímicos.

Palabras clave: Aguas residuales agrícolas, fitotoxicidad, germinación, Zea mays.

Contribution to the literature
This study provides toxicological data on the effects of herbicides on maize germination processes. Through the use of bioassays, it enables the monitoring of potential impacts of herbicides used in rice cultivation on maize germination.

The most relevant results include:
The most significant findings of the research highlight the toxicological effects on maize germination caused by high concentrations of herbicides used in rice paddies, which are carried away through the wastewater generated there and subsequently used for irrigation water that could have a toxicological effect on the germination processes of various crops.

These results contribute to the following:
This research makes contributions in the scientific, environmental, and methodological fields.
From a scientific perspective, it explains how agrochemicals dissolved in rice paddy water alter germination processes in Zea mays seeds. From an environmental perspective, it helps determine whether irrigation water from rice paddies is suitable for irrigation or, conversely, could have a negative effect on crops, thereby enabling the prediction and prevention of yield failures in corn crops. From a methodological perspective, bioassays help establish the use of corn germination as an indicator for calculating the Germination Index and the Percentage of Phytotoxicity.

Graphical Abstract

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Introduction

Water is an essential resource for agriculture, and its availability is crucial in intensive irrigation systems, such as rice cultivation. However, agriculture faces increasingly complex challenges, such as water scarcity and deteriorating water quality. These problems are linked to the overexploitation of aquifers, climate change, and the intensive use of agrochemicals (1). This situation has led to a search for alternatives that optimize water use, such as the reuse of wastewater for agricultural irrigation. While this practice offers benefits in terms of availability and cost, it also carries environmental and health risks (2). Rice cultivation (Oryza sativa) is characterized by high water demand and the routine use of fertilizers, herbicides, and other chemicals. As a result, agricultural effluents are generated that may contain high concentrations of salts, organic matter, metals, and agrochemical residues. These elements can alter the physicochemical properties of the soil and the water bodies into which they are discharged (3). The accumulation of these contaminants not only harms agricultural productivity but also poses a risk to aquatic ecosystems and the sustainability of irrigation systems. In this context, germination is a crucial stage in the life cycle of plants. It not only marks the beginning of the crop but also influences its growth and yield over time. Exposure of seeds to contaminated irrigation water can affect key physiological processes, cause osmotic and oxidative stress, and lead to changes in the growth of both the roots and the aboveground parts of seedlings. This is due to a loss of turgor, which results in a reduction in cell volume and an increase in solutes, leading to stunted growth (4). Recent studies have revealed that wastewater can negatively affect germination rates, interfere with root elongation, and increase indicators of cellular stress, such as hydrogen peroxide production and electrolyte leakage. All of this can lead to abnormal plant development and even plant death (5), (6). Zea mays (maize) is widely recognized as a bioindicator in environmental toxicity studies, especially for evaluating herbicides and water quality. This is mainly due to its high sensitivity during the early stages of development (7). This characteristic makes it possible to identify sublethal effects of contaminants in reused water, which might not be detected in analyses that focus solely on physicochemical aspects (8-9). Furthermore, it has been documented that maize's response to factors such as salinity and the presence of toxic compounds reliably reflects alterations in the plants' physiological and biochemical mechanisms. Although the use of wastewater in agriculture has increased, there are still few studies that combine the physicochemical characterization of this water with biological assessments of the germination process, both under controlled laboratory conditions and in real-world agricultural settings. This lack of knowledge hinders our understanding of how contaminants in irrigation water affect early plant growth. This, in turn, hinders the ability to make informed decisions for sustainable water management. Therefore, the objective of this study was to evaluate how wastewater from rice crops affects the germination of Zea may.

Materials and methods

Study Design

The experimental phase focused on evaluating the toxicological effect of rice paddy wastewater on the germination and early growth of Zea mays through controlled experiments. Wastewater

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samples were collected from two rice-growing systems located in the municipalities of El Zulia (Rice Farm A; 7°55′22″N, 72°35′43″W) and Santiago (Rice Farm B; 7°53′46.1″N, 72°41′59.8″W), in the department of Norte de Santander, Colombia. Sampling was conducted during the active spraying season, which is considered the time of highest pollutant load in the water column. In each system, composite sampling was performed by collecting aliquots at five different points in the water column, which were then combined to form a representative sample. Rice Field A was treated with the herbicide atrazine, while Rice Field B was treated with the herbicide propanil.

Sample Preservation

Samples were collected in accordance with the guidelines established in *Standard Methods for the Examination of Water and Wastewater*. They were stored in pre-prepared polyethylene containers and kept at 4 °C until analysis in the laboratory, which was performed within six hours of collection.

Physicochemical Characterization

The following water quality parameters were determined: chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), dissolved oxygen, pH, electrical conductivity, turbidity, alkalinity, acidity, total and calcium hardness, chlorides, and total suspended solids. All determinations were performed in accordance with the standardized methods described in Standard Methods, using volumetric, potentiometric, and gravimetric techniques, depending on the parameter being evaluated.

Study Area and Soil Sampling

Soil samples were collected from two experimental plots located in the villages of Las Violetas (plot 1; 7°55′58″N, 72°47′26″W) and Boyacá (plot 2; 7°55′13″N, 72°48′08″W), in the municipality of Gramalote, Norte de Santander, Colombia. Sampling was conducted randomly within the area designated for planting. At each site, ten samples were collected at a depth of 20 cm, corresponding to the zone where root activity of the maize crop is most intense and where there is the greatest interaction with irrigation water. The samples were mixed to create a representative sample from each site.

Physicochemical Characterization of the Soil

Soil samples were analyzed both before and after the irrigation period, with the aim of establishing baseline conditions and evaluating any changes that might result from the use of wastewater. The analyses were conducted in duplicate and included measurements of organic matter, texture, pH, and electrical conductivity. Organic matter was determined using the Walkley and Black method. To assess texture, the Bouyoucos hydrometer method was applied. In addition, pH and electrical conductivity were measured in a 1:1 soil-water suspension.

Sowing Zea mays (corn) Seeds

Zea mays seeds of the Puyita variety were used; this variety is known for its adaptation to temperate climates. All seeds came from the same certified commercial lot, ensuring that they are genetically and physiologically homogeneous. These seeds were selected for their uniform size, with an

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average diameter of 8 to 10 mm per seed. In addition, they are mostly oval in shape, although some are slightly flattened. Sowing was carried out in two experimental plots, each measuring 1 × 4 m (individual area of 4 m²), located in the villages of Las Violetas and Boyacá, in the municipality of Gramalote, Norte de Santander, Colombia. The direct-seeding method was used, placing the seeds at a depth of approximately 4 cm and ensuring uniform spacing between plants and rows, as suggested by (10).

Application of Irrigation Treatments

The experiment was conducted using a completely randomized design (CRD), focusing on a single study factor: the type of water used for irrigation. Four treatments were evaluated: (I) wastewater from Rice Mill A, (II) wastewater from Rice Mill B, (III) a negative control using clean water, and (IV) a positive control consisting of the application of glyphosate at a concentration of 480 g/L (11). The treatments were randomly assigned to separate furrows within each experimental plot and evaluated at two locations (the villages of Las Violetas and Boyacá), which were considered independent experimental sites. At each location, five seedlings were planted per treatment, and these were considered individual experimental units. No replicates were implemented within each location; however, when both locations were considered together, a total of ten seedlings per treatment (n = 10) was obtained, for an overall total of 40 seedlings in the experiment. Each seedling received 5 mL of the corresponding treatment, applied twice daily over a 20-day period, directly at the base of the plant to simulate localized exposure. During the experimental period, the growth and overall condition of the seedlings were monitored. The variables analyzed included germination rate, seedling length, and physiological changes.

Acute toxicity bioassays with Zea mays (Preparation of herbicide solutions)

Wastewater samples were diluted in distilled water following a volume-to-volume (% v/v) dilution scheme, with concentrations in accordance with the methodology proposed by (12): 75, 50, 25, 10, and 1%, with a final volume of 100 mL for each dilution. All solutions were prepared in volumetric flasks and mixed manually for one minute. Dilutions of the herbicides propanil and atrazine were then prepared. Propanil (480 g/L, commercial formulation) was initially diluted in a range of 1 to 75%. However, at the 1% concentration, complete inhibition occurred, so the concentrations were adjusted to a range between 0.2 and 1%. For the dilution calculation, a final volume of 50 mL was ensured for each dilution. Atrazine (800 g/kg, solid formulation) was prepared from a 25 g/L standard solution, equivalent to the dose used in agriculture (5 kg in 200 L). From this solution, dilutions were prepared at 75%, 50%, 25%, 10%, and 1%, with a final volume of 100 mL, using distilled water as the solvent.

Germination Bioassay

The germination bioassay was based on the methodology described by (13), with some modifications, in which 5 mL of each dilution was placed in Petri dishes using sterile cotton as a substrate. Twenty Zea mays seeds were distributed in each dish. The dishes were incubated for 5 days in the dark at a temperature of 22 ± 2 °C, and maintenance watering was performed on the third day. A negative control (distilled water) and a positive inhibition control (undiluted wastewater

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(100% v/v) and herbicides at their maximum concentration) were used. Once the incubation period was complete, the length of the radicle was measured in millimeters, and the percentage of growth and the percentage of inhibition were calculated using Equations 1 and 2.

$$\%\ growth = \frac{average\ .dilution\ ratios}{averege.negative\ control\ lengths} \quad \text{Ec (1)}$$

$$\%\ inhibition = 100\% - \%\ growth \quad \text{Ec (2)}$$

Cytogenetic Analysis

To assess cellular damage, the methodology described by (14) was followed. Zea mays root tips were used; these were immersed in hydrochloric acid for 20 minutes to help break down the tissue. They were then washed with distilled water and stained with orcein acetate for 45 minutes. The stained samples were placed on microscope slides and subsequently observed under a microscope to identify any possible alterations in cell division.

Statistical Analysis

The data on root length obtained in each experimental replicate were organized and processed in spreadsheets. To this end, a 2k factorial design was used, with two different wastewater treatments and three replicates of each; a one-way analysis of variance (ANOVA) was applied, with a significance level of α = 0.05, to analyze the differences between the treatments (wastewater dilutions, negative control, and positive control).

Results and discussion

Physicochemical Characterization of Irrigation Water

Table 1 below shows the physicochemical parameters measured in the wastewater from two rice-growing systems. These parameters are key to assessing water quality and its impact on agricultural processes, especially during germination.

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Table 1.Results of the physicochemical characterization of wastewater from rice mills.

Variables	Units	Rice Field A (El Zulia)	Rice Field B (Santiago)
Chemical Oxygen Demand (COD)	mg/L O_2	64.0	76.8
Biochemical Oxygen Demand (BOD_5)	mg/L O_2	60.0	22.0
Dissolved oxygen	mg OD/l	6.8	5.2
pH	Unidades de pH	7.0	7.2
Conductivity	µS	0.70	0.1
Turbidity	NTU	16.5	699
Alkalinity	mg/L $CaCO_3$	34.0	26.0
Total acidity	mg/L $CaCO_3$	9.5	4.5
Calcium hardness	mg/L $CaCO_3$	85.0	50.0
Total hardness	mg/L $CaCO_3$	123.0	126.0
Chlorides	mg/L Cl	16.8	23.3
Total suspended solids	mg SST/L	0.025	0.0

The COD/BOD_5 ratio at Rice Mill A was 1.06, suggesting that the organic matter is highly biodegradable. When values are below 2, this typically indicates that the wastewater contains mainly compounds that degrade easily and has a low concentration of refractory substances (15). This behavior is consistent with a neutral pH of 7.0 and a dissolved oxygen level of 6.8 mg/L, both within the recommended ranges for agricultural use (16). In contrast, Rice Field B showed a COD/BOD_5 ratio of 3.49, indicating the presence of organic matter that is poorly biodegradable, possibly related to compounds that tend to persist (17). Although herbicides and their metabolites were not measured, this behavior resembles that observed in rice-growing systems during advanced stages of cultivation. During these phases, post-emergence herbicides such as propanil are used; their transformation products, such as 3,4-dichloroaniline, tend to be more persistent in the environment (18). The high turbidity observed in Rice Field B (699 NTU) supports this interpretation, as it suggests a high concentration of fine particles and organic matter in the water. These solids help agrochemicals and organic matter adhere to colloids, which reduces their bioavailability and delays their degradation (19). Although electrical conductivity and chloride levels did not exceed critical limits for irrigation, the combination of high turbidity and low biodegradability could increase the risk of sublethal effects during germination. The differences observed between the two systems are not due solely to wastewater quality, but also to how agriculture is managed and the stage of the crop cycle. Water from land under preparation is generally more biodegradable, while water generated during active crop growth tends to have a higher load of persistent compounds and suspended solids.

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Physicochemical Characterization of the Soil Before and After Irrigation (table 2)

Table 2. shows the results of the physicochemical characterization of the soil before and after irrigation with water from rice fields.

Initials

Variables	Units	Lot 1 (vereda las violetas)	Lot 2 (vereda Boyacá)	Glyphosate sample
Organic matter	mg O_2/L	4.4	3.8	
Texture	-	Sandy loam	Sandy loam	
pH	Units de pH	3.1	5.4	
Conductivity	µS	0.1	0.3	
		Finals		
Organic matter	mg O_2/L	10.2%	4.6%	5.1%
Texture	-	Sandy loam	Sandy loam	Sandy loam
pH	Units de pH	3.7	5.4	5.3
Conductivity	µS	0.31	1.5	1.6

Before irrigation, both soil types exhibited sandy loam and sndy clay loam textures, with moderate levels of organic matter—characteristics common in agricultural soils. After applying the treatments, an increase in organic matter was observed, especially in Plot 1, which recorded a 10.25% increase, and to a lesser extent in Plot 2, with a 4.95% increase. This increase indicates the transport of organic compounds—both dissolved and particulate—associated with runoff and plant residues. This phenomenon has previously been reported in flood irrigation systems (20). Soil pH showed a moderate increase in both plots, suggesting a slight trend toward less acidic conditions. This behavior is consistent with studies indicating that constant irrigation and flooding promote redox processes and the incorporation of basic cations, which help to partially neutralize soil acidity (21); The magnitude of the change also indicates that the soil has a high buffering capacity, which helps prevent sudden changes in acidity. Conductivity showed a slight increase in soils irrigated with surface water from rice-growing systems, suggesting a moderate incorporation of salts and nutrients, without posing a risk of salinization. In contrast, the glyphosate treatment showed the highest conductivity levels, indicating localized accumulation of the herbicide. This is consistent with its ability to adsorb onto mineral particles and organic matter (22). A comparison between the initial and final soil descriptions revealed differences in the proportions of sand, silt, and clay. However, these changes do not indicate an actual alteration in soil texture, but rather a superficial redistribution of the finer fractions, mainly due to the effect of irrigation. Therefore, overall, soil texture remained constant throughout the study.

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Effect of Irrigation Treatments on Zea mays (Field)

In both plots, irrigation treatments with wastewater from rice crops (Rice Field A and Rice Field B) achieved a 100% germination rate, which is comparable to that of the negative control. In contrast, the positive control with glyphosate completely inhibited germination, confirming the sensitivity of the assay and the validity of the experimental design. The absence of acute phytotoxic effects during germination indicates that the use of this wastewater does not impair the initial establishment of the crop under field conditions. This behavior could be related to a biostimulant effect of the irrigation water, due to the presence of organic matter and nutrients—such as nitrogen and phosphorus—available in forms that plants can easily assimilate (23). The average lengths of the seedlings were similar between the rice-water treatments and the negative control. In Plot 1, the values ranged from 23 to 24 cm, while in Plot 2, they ranged from 32 to 34 cm. The observed differences were not statistically significant and indicate a uniform response of the crop, suggesting that soil conditions did not affect initial growth. This is consistent with maize's remarkable adaptability during the germination stage (24). The absence of adverse effects in the field, despite the previously reported physicochemical differences in the water, can be explained by the soil's natural attenuation processes. These include sorption, physical retention, biodegradation, and dilution, which help reduce the bioavailability of potentially toxic compounds (25). In this context, the soil acts as a buffer that protects seedlings from direct exposure during the early stages of their development. However, the difference between these results and those from laboratory tests—where water from Arrocera B showed inhibitory effects—suggests that sublethal or chronic effects may exist that do not manifest during short periods of exposure in the field. Recent studies suggest that prolonged exposure to mixtures of agrochemicals can cause oxidative stress, genetic damage, and changes in the soil microbiota. These effects do not always manifest during germination but can influence later stages of plant growth (26). The findings indicate that the use of wastewater from rice crops could be a viable alternative for the short-term irrigation of Zea mays, as it does not cause immediate phytotoxic effects and also provides nutrients to the system. However, it is essential to emphasize the importance of evaluating long-term effects. This involves considering complete crop cycles, the accumulation

Toxicity of Rice Farming Wastewater to Zea mays (in vitro assays)

The results of toxicity bioassays conducted with wastewater from two rice-growing systems are shown in Figure 1. For rice fields A and B, Table 3 describes the percentage of growth and the percentage of inhibition in Zea mays seeds. Different dilutions affect radicle length.

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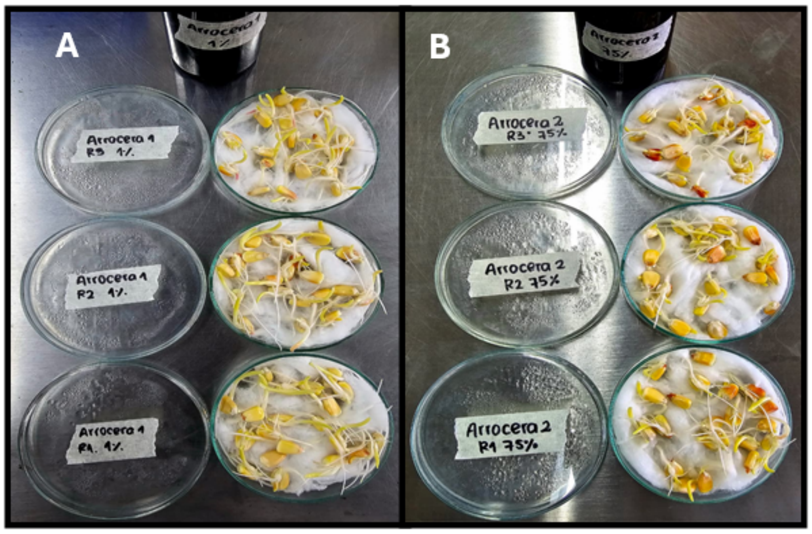

Figure 1. Final in vitro germination in water from Rice Fields A and B.

Table 3. Results of the Toxicity Test on Wastewater from Rice Mills A and B

Treatment	Repetitions	Average Length (cm)	DMS in cm	% growth	% inhibition	Treatment
1%	5.8			162.22	-62.22	1%
	6.8	6.57				
	6.9					
10%	5.8	6.42		158.44	-58.44	10%
	6.7					
	6.8					
25%	5.7	6.29		155.35	-55.35	25%
	6.4					
	6.7					
50%	5.4	5.53		136.63	-36.63	50%
	5.5					
	5.7					
75%	4.6	4.73	0.78	116.67	-16.67	75%
	4.7					
	4.8					
Control negativo	6.4	6.95		-	-	Control negativo
	7.1					
	7.2					
Control positivo	3.8	4.05		-	-	Control positivo
	4.0					
	4.0					

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Repetitions	Average Length (cm)	DMS in cm	% growth	% inhibition
5.1	5.23		85.53	14.47
5.5				
5.0				
5.0	5.15		118.48	-18.48
5.3		0.28		
5.0				
4.8	4.96		81.14	18.86
4.9				
5.1				
4.7	4.78		78.20	21.80
4.8				
4.7				
4.4	4.55		74.33	25.67
4.5				
4.4				
6.0	6.115			-
6.3				
6.1				
3.9	4.35			
4.7				
4.4				

The average variability of the rootlets is shown in Figure 2, which depicts the growth of rice paddy A treated with wastewater and the different herbicide treatments applied. The figure shows greater root growth in the treatments applied to rice paddy A compared to rice paddy B, with a minimum significant difference (MSD) of 0.78 cm in the first treatment, in contrast to rice field B, which had an MSD of 0.28 cm, as shown in Table 1.

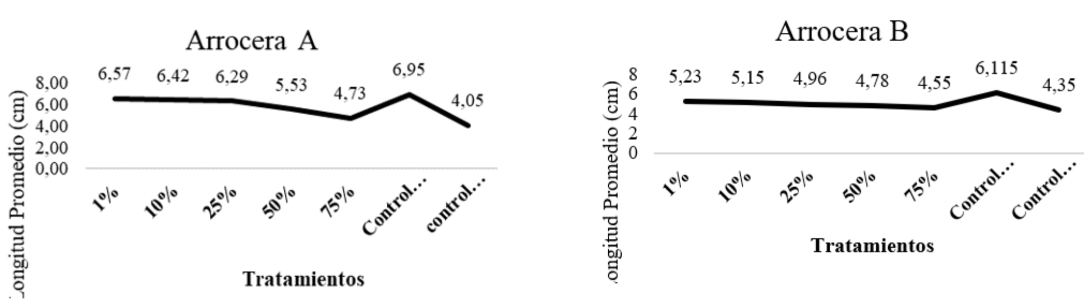

Figure 2. Average radicle length in seeds exposed to wastewater from rice fields A and B

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In the samples from Rice Plant A, an increase in the average radicle length was observed in all dilutions evaluated (1–75%) compared to the negative control. Growth rates exceeding 100% and negative inhibition values indicate a hormetic response, characterized by biological stimulation at low concentrations of a stressor (27), (28). This effect is often related to the presence of residual nutrients and easily biodegradable organic compounds. These elements can drive metabolic processes related to cellular respiration and root growth. Recent studies have shown that crops exposed to agricultural water with moderate levels of organic matter and low concentrations of pesticides exhibit similar behaviors, indicating a biostimulatory effect during the early stages of plant development (29). In contrast, bioassays conducted with wastewater from Rice Mill B showed a concentration-dependent pattern of inhibition. Figure 3 shows progressive reductions in radicle length as the proportion of wastewater increased. The 50% and 75% dilutions exhibited the highest levels of inhibition, suggesting that toxic compounds remain in the environment. This behavior is consistent with the use of post-emergence herbicides such as propanil. Its metabolite, 3,4-dichloroaniline (3,4-DCA), is more persistent and toxic than the original molecule (30), (31). Studies such as those in (32) have shown that, in flooded rice cultivation systems, a considerable portion of 3,4-DCA can become trapped in the soil as a residue that cannot be extracted, which could prolong its persistence in the environment and increase its toxicity. It is worth noting that these compounds can accumulate in agricultural water bodies due to the various ways in which they degrade, which depend on microbial and photochemical processes that occur at different rates (33). The inhibitory pattern observed in Zea mays is consistent with the physiological effects reported for propanil and its metabolites. These compounds affect mitochondrial respiration, electron transport, and cell division in meristematic tissues (34). The differences between the two rice cultivation systems show that, even within the same production model, the chemical composition of wastewater can vary considerably, which in turn leads to very different physiological responses. While the water from Rice Farm A exhibited a biostimulatory effect at low concentrations, the water from Rice Farm B revealed inhibitory effects that could manifest as sublethal or chronic impacts, which are not always evident in short-term field germination trials.

Toxicity of Reference Herbicides (Atrazine and Propanil) in Zea mays

To contextualize the effects observed in wastewater from rice crops, bioassays were conducted using herbicides commonly applied to rice crops, such as atrazine and propanil, which successfully induced germination, as shown in Figure 3 below.

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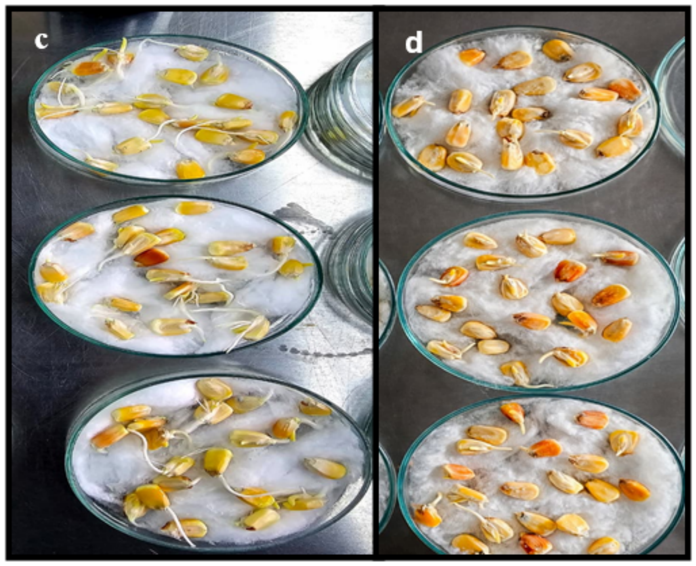
Figure 3. Final in vitro germination rates for the herbicides atrazine (c) and propanil (d).



However, the results regarding the impact on the germination process differed for each herbicide across the various treatments, as shown in Table 4 below, revealing a significant difference of 0.5 cm between the two rice paddies, with this herbicide having a greater impact on the second rice paddy.

Table 4. Results of the toxicity test for the herbicides atrazine and propanil

Treatment	Repetitions	Average Length (cm)	DMS in cm	% growth	% inhibition	Treatment	Repetitions	Average Length (cm)
1%	5.8			162.22	-62.22	1%	5.1	5.23
	6.8	6.57					5.5	
	6.9						5.0	
10%	5.8	6.42		158.44	-58.44	10%	5.0	5.15
	6.7						5.3	
	6.8						5.0	
25%	5.7	6.29		155.35	-55.35	25%	4.8	4.96
	6.4						4.9	
	6.7						5.1	
50%	5.4	5.53		136.63	-36.63	50%	4.7	4.78
	5.5						4.8	
	5.7						4.7	
75%	4.6	4.73	0.78	116.67	-16.67	75%	4.4	4.55
	4.7						4.5	
	4.8						4.4	
Control negativo	6.4	6.95		-	-	Control negativo	6.0	6.115
	7.1						6.3	
	7.2						6.1	
Control positivo	3.8	4.05		-	-	Control positivo	3.9	4.35
	4.0						4.7	
	4.0						4.4	

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DMS in cm	% growth	% inhibition
	85.53	14.47
0.28	118.48	-18.48
	81.14	18.86
	78.20	21.80
	74.33	25.67
		-

The application of these two herbicides resulted in reduced root growth, with the root treated with atrazine showing greater growth length; it achieved a growth of 3.0 cm with the 1% treatment, compared to propanil, which reached only 2.4 cm with the same treatment. Figure 4 shows the variability in root length according to the different treatments applied to the seed with the two herbicides, indicating that antagonism depends on the dose used during the process (35).

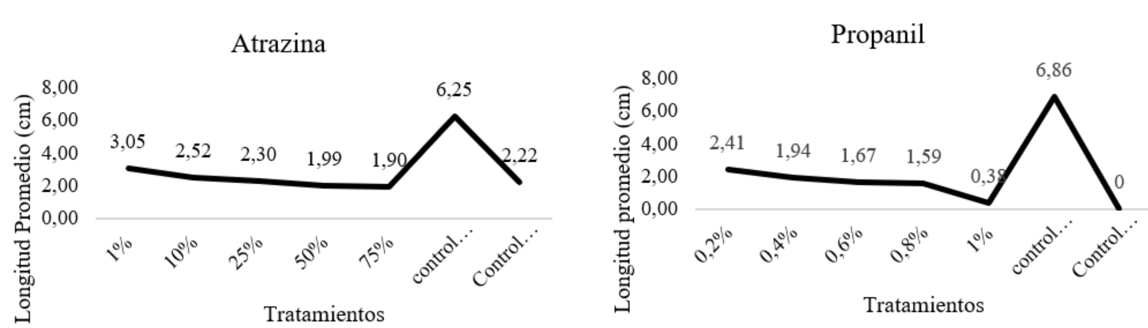

Figure 4. Average radicle length in seeds exposed to the herbicides atrazine and propanil.

Atrazine exhibited a moderate but consistent inhibitory effect on the root growth of Zea mays, with inhibition percentages ranging from 51% to 69% at the various concentrations evaluated. This behavior is consistent with its role as a Photosystem II inhibitor, which reduces the available energy required for cell elongation and the initial growth of seedlings. In contrast, propanil exhibited a

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highly phytotoxic effect, with inhibition rates reaching nearly 94% even at low concentrations, indicating a marked response compared to atrazine. This behavior is consistent with previous research indicating that propanil affects mitochondrial respiration, cell elongation, and protein synthesis in growing tissues. Furthermore, its effect is intensified by its major metabolite, 3,4-dichloroaniline (3,4-DCA) (36). Figure 4 below shows the in vitro process involving these herbicides. Studies have shown that metabolites derived from herbicides can cause oxidative stress by generating reactive oxygen species (ROS), which particularly affects meristematic tissues during germination (37), (38). Although plants have their own detoxification mechanisms, such as glycosylation carried out by UDP-glycosyltransferases, these mechanisms are sometimes insufficient. This occurs when toxin concentrations are very high or when the compounds bind to particles in the soil or water, making their removal difficult (39), (40), (41). Taken together, the results obtained with pure herbicides allow for the establishment of a toxicological framework that helps interpret the effects observed in wastewater from rice crops. In particular, the inhibitory pattern detected in the water from Rice Farm B appears to be related to the presence of propanil or its metabolites. On the other hand, the less severe effects observed in other samples deviate from the typical behavior of highly phytotoxic herbicides, such as propanil.

Cytogenetic Analysis

Exposure of Zea mays to herbicides did not cause any notable inhibition of germination or early radicle growth. No visible cytogenetic alterations, such as chromosomal aberrations, micronuclei, or anaphase bridges, were found; thus, mitotic division was observed, as shown in Figure 5 below.

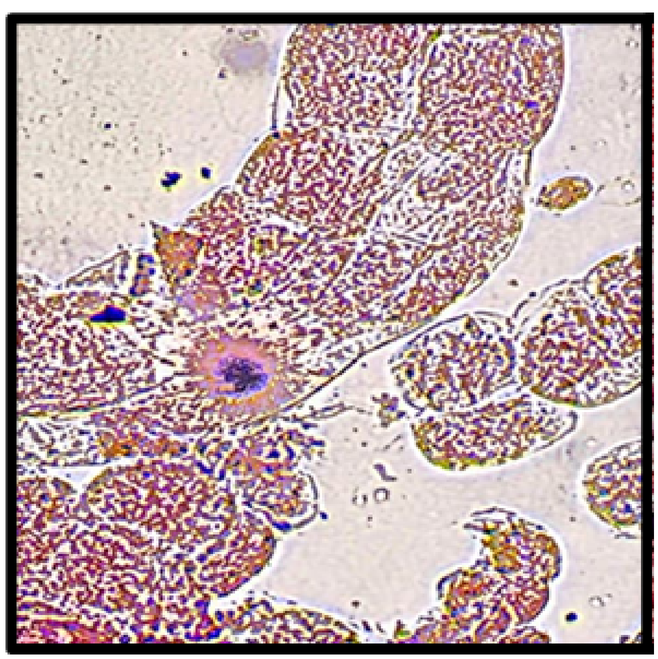

Figure 5.Células en división celular

This result demonstrates that there are significant physiological effects, but no evidence of detectable genetic damage has been found under the experimental conditions evaluated. Therefore, from a physiological standpoint, growth inhibition may be related to the disruption of

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key metabolic processes, such as cell elongation, mitochondrial respiration, and the production of moderate levels of reactive oxygen species (ROS), all of which may limit progression toward effective mitotic activity. Previous research has shown that propanil affects photosynthesis, oxygen uptake in mitochondria, and phosphate esterification in plant tissues (42), (43). The absence of chromosomal aberrations suggests that the cells did not reach a mitotic state active enough to exhibit visible genetic damage, or that the herbicide acts primarily by blocking processes that occur before mitosis begins. This behavior is consistent with reports indicating that a reduction in the mitotic index is a classic sign of cytotoxicity. According to (44), the inhibition of cell division can occur before structural damage to DNA is detected.

Figure 5 shows the normal process of cell division, indicating that the effects of the herbicides at these concentrations were not significant; however, this may vary from one seed population to another or from one herbicide to another, in agreement with (45), who suggest that toxicity would not occur because there is no impact on the process of cell division. Studies such as those in (46) have shown that propanil has genotoxic effects in species such as Lens culinaris and Allium cepa, in which micronuclei and mitotic aberrations were observed after exposures ranging from 2 to 12 mg/L. These differences highlight that factors such as plant species, herbicide concentration, exposure time, and the sensitivity of the bioassay influence the results. Table 6.

Statistical Analysis

Table 6. Analysis of Variance in Rice Production A y B

Source of the variations	Sum of Squares	Degrees of freedom	Mean of the Mean Squares	F	Probability	Critical value for F
Analysis of Variance in Rice Production A						
Among groups	20.7	6	3.4	6.7	0.0	2.8
Within the groups	7.2	14.	0.5			
Total	27.9	20				
Analysis of Variance in Rice Production B						
Among groups	5.9	6	0.9	3.21	0.0	2.8
Within the groups	4.3	14	0.3			
Total	10.3	20				

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The analysis of variance (ANOVA) for Rice Variety A, with the presence of the herbicide atrazine, revealed statistically significant differences among the treatments ($F(6,14) = 6.70$; $p = 0.0017$). This indicates different responses in the root elongation of Zea mays depending on the various dilutions and controls applied. These findings are consistent with recent research indicating that atrazine may have sublethal effects on root growth and other physiological processes, even at low concentrations. Similarly, in Rice Field B, where the influence of the herbicide propanil was observed, the ANOVA revealed significant differences among the treatments ($F(6,14) = 3.21$; $p = 0.0339$). This confirms that biological responses are not homogeneous, as evidenced by different responses in the root elongation of Zea mays. This pattern is consistent with previous reports indicating that propanil can cause significant physiological effects without necessarily causing detectable toxicity (47). Overall, the results show that wastewater from both rice mills has an impact on the root growth of Zea mays. At high concentrations, the effect was primarily inhibitory, likely due to the presence of herbicides and other agrochemicals. In contrast, lower concentrations produced varied responses, including slight increases in root length. The p-values, which are less than 0.05 in both statistical analyses, confirm that the wastewater has distinct physiological effects on Zea mays, regardless of the type of herbicide that predominates at each rice mill.

Conclusion

The differences between the laboratory and field trials conducted showed that germination alone is not sufficient to assess wastewater quality, as long-term sublethal effects could arise, demonstrating that the quality of water from rice fields varies depending on the crop stage and management practices, causing different physiological responses in Zea mays and variable effects on the soil; however, controlling the accumulation of these types of compounds in wastewater from rice paddies could be a viable option for its reuse, without causing phytotoxic effects, since they act as carriers of organic matter and salts without significantly altering the soil's structure or texture, thereby also preventing health risks associated with the accumulation of these compounds, and, in turn, serve as a sustainable alternative in agriculture.

CrediT authorship contribution statement

Conceptualization - Ideas: María Del Pilar Gutiérrez Orejuela, César Darío Peñaranda Medina, Nelson Alfonso Vega Contreras. Formal analysis: María Del Pilar Gutiérrez Orejuela, César Darío Peñaranda Medina, Nelson Alfonso Vega Contreras . Investigation: María Del Pilar Gutiérrez Orejuela, César Darío Peñaranda Medina, Nelson Alfonso Vega Contreras . Methodology: María Del Pilar Gutiérrez Orejuela, César Darío Peñaranda Medina, Nelson Alfonso Vega Contreras . Project Management: Nelson Alfonso Vega Contreras . Resources: María Del Pilar Gutiérrez Orejuela, César Darío Peñaranda Medina. Software: Yulia Ivanova, Julián David Castellanos. Supervision: Nelson Alfonso Vega Contreras. Validation: Nelson Alfonso Vega Contreras. Writing - original draft - Preparation: María Del Pilar Gutiérrez Orejuela, Nelson Alfonso Vega Contreras. Writing - revision and editing -Preparation: YMaría Del Pilar Gutiérrez Orejuela, Nelson Alfonso Vega Contreras.

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