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Research Article

Ecotoxicological Assessment of Ibuprofen in Environmental Relevance Models: Impact on *Artemia salina* and *Medicago Sativa*

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Abstract

Ibuprofen, one of the most widely used non-steroidal anti-inflammatory drugs, has proven effective in treating various human conditions. However, its persistence in aquatic environments has raised concerns about its ecotoxicological effects. This study assesses the effects of ibuprofen on both aquatic and terrestrial organisms, focusing specifically on seed germination in *Medicago sativa* and acute toxicity in *Artemia salina*. The goal is to better understand the environmental impact of ibuprofen and contribute to the development of stricter regulations regarding emerging pollutants. Germination and radicle elongation tests were performed on *Medicago sativa* seeds exposed to different concentrations of ibuprofen (10, 100, and 1000 µg/mL) and colchicine. Seeds were treated in Petri dishes and observed at 24 and 48 hours to measure germination rates and root elongation. Additionally, an acute toxicity test was conducted on *Artemia salina* with ibuprofen and potassium dichromate, assessing mortality after 24 hours of exposure. Germination Test: At low ibuprofen concentrations (10 and 100 µg/mL), germination rates increased, whereas at high concentrations (1000 µg/mL), there was a significant inhibition. Colchicine treatment promoted a 95% germination rate at 48 hours, showing a positive effect on seedling growth. Radicle Elongation Assay: Seeds treated with ibuprofen at low concentrations (10 and 100 µg/mL) showed greater radicle elongation, while the 1000 µg/mL concentration caused significant inhibition. In contrast, colchicine inhibited root growth, consistent with its known effect on microtubule polymerization and cell division. Acute Toxicity Test with *Artemia salina*: A dose-response analysis revealed that potassium dichromate was significantly more toxic than ibuprofen, with an LD50 of 16.4 µg/mL compared to 254.2 µg/mL for ibuprofen. This indicates that potassium dichromate is more potent in inducing mortality in *Artemia salina*. The study demonstrates that ibuprofen has hormetic effects on *Medicago sativa*, promoting germination and root elongation at low concentrations but inhibiting these processes at higher concentrations. Additionally, potassium dichromate exhibited greater toxicity than ibuprofen. These results underscore the importance of evaluating the environmental impact of pharmaceuticals and the need for stronger regulations on pharmaceutical pollutants.

Keywords

Ibuprofen, Ecotoxicology, Emerging Pollutants, Seed Germination, Acute Toxicity

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1. Introduction

Ibuprofen, one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) worldwide, has proven effective in the treatment of various human conditions. However, its presence in the environment has raised concerns about its potential ecotoxicological effects. Its high-water solubility and persistence in aquatic systems, along with the growing concern about emerging pollutants, have driven research on its impact on aquatic and terrestrial ecosystems [1, 2]. Despite the widespread use of ibuprofen, its potential effects on aquatic and terrestrial biota are still not fully understood [3, 4].

In this context, the ecotoxicological assessment of ibuprofen is crucial in determining its environmental relevance and the risks associated with its release into the environment. In recent years, there has been an increased focus on evaluating the effects of emerging pollutants, such as pharmaceutical products, on aquatic and terrestrial organisms [3, 5]. Using relevant environmental assessment models, such as *Artemia salina* and plant seed growth and germination, provides a more comprehensive approach to ecological impacts, covering different trophic levels and environments. These models offer an effective means to assess ibuprofen's effects on aquatic organisms and plant species, allowing the prediction of potential long-term ecological risks [6, 7].

The study of the ecotoxicological effects of ibuprofen on aquatic and terrestrial organisms has a significant impact on legislation and environmental management policies. The growing concern over emerging pollutants, such as pharmaceutical products, has driven the need to review and strengthen environmental regulations. Research with standardized ecotoxicological tests [8] could promote the creation of stricter regulations on the release of these compounds into the environment. Despite the utility of these drugs in human health, their persistent presence in water bodies and accumulation in organisms may represent a significant risk to biodiversity and ecosystem functioning [3].

Ibuprofen concentrations in water bodies and soil vary considerably depending on the region and type of environmental sample. In wastewater, concentrations range from 0.004 to 603μg/L across different countries (Bosnia, China, Croatia, Greece, Herzegovina, Korea, Serbia, Sweden, Switzerland, and the UK), with higher values reported in countries like Pakistan (703-1673μg/L) and South Africa (45μg/L) [9-11]. In surface waters, average concentrations range from 0.98 to 1417μg/L (Canada and China, respectively) [9, 12]. In sludge and soils, concentrations also show significant variability, ranging from 0.009μg/kg in South Africa to 6064μg/kg in Pakistan [13]. Soil samples showed ibuprofen concentrations ranging from 321 to 610μg/kg, while agricultural soils irrigated with ibuprofen-contaminated wastewater had a concentration of 0.213μg/L [14, 15]. Groundwater concentrations of ibuprofen in Europe ranged from 3ng/L to 395ng/L [9]. Additionally, ibuprofen transformation products are detected in wastewater treatment plants at concentrations up to 7768ng/L [16].

Addressing pharmaceutical pollution could also lead to the development of more detailed risk assessment protocols for pharmaceutical products to assess their environmental impact before commercialization. Current studies suggest that more rigorous ecotoxicological testing should be incorporated in early stages of new drug development [17].

This study employed ecotoxicological models to analyze the potential effects of ibuprofen on key organisms such as *Artemia salina* and the early stages of plant life cycles in *Medicago sativa*, which are essential for ecosystem stability. Through these assays, we aim to expand our understanding of the environmental toxicity of pharmaceutical products and promote the development of more effective and sustainable environmental management strategies.

2. Materials and Methods

2.1. Pre-germination Considerations for *Medicago Sativa*

Seeds were purchased from Rancho Los Molinos®, lot K474, Morelos, Mexico. Before the test, a careful inspection was performed to select seeds and seed coats that were undamaged, ensuring a high germination rate. The seeds were then washed with neutral detergent and distilled water to remove any pollutants, additives that might affect embryo viability, dormancy-promoting hormones, and substances preventing fungal growth.

2.2. Germination Test

For the seed germination test, Petri dishes (75 ×15mm) and filter paper were used as the substrate. The seeds (n = 10, in quadruplicate) were hydrated with 1% DMSO in water (vehicle), 1000μg/mL of colchicine (COL, Sigma®), and ibuprofen (IBP, commercial tablet isolated) at concentrations of 10, 100, and 1000μg/mL. The seeds were then incubated in a controlled incubator at room temperature (26.3-31.4 ℃) under artificial white light. Radicle emergence was recorded and photographed at 24 and 48 hours using a FinePix XP120 camera (FUJIFILM®), with the camera positioned 10cm from the Petri dish. The images were analyzed to count the number of germinated seeds at different time points.

2.3. Radicle Elongation Assay

For the radicle elongation assay, Petri dishes (75 ×15mm) and filter paper were used as the substrate. The seeds (n = 10, in triplicate) were initially hydrated with purified water

(0.1mL/cm²) and incubated for 24 hours in a controlled incubator at room temperature (26.3-31.4 ℃) under artificial white light. After 24 hours, the seeds were photographed and the initial radicle length was quantified using the histogram function of GIMP v2 software.

Subsequently, the seeds were treated with 1% DMSO in water (vehicle), COL at 1000μg/mL, and IBP at concentrations of 10, 100, and 1000μg/mL. After 24 and 48 hours of exposure, the seeds were photographed again. Radicle elongation was quantified by measuring the area in pixels using the GIMP v2 software [18].

2.4. *Artemia Salina* Acute Toxicity Assay

The acute toxicity of ibuprofen and potassium dichromate on *Artemia salina* was evaluated through a 24-hour exposure study. The compounds were prepared in 1% DMSO [19] with 30% saline solution, covering a logarithmic concentration range from 10,000 μg/mL to 0.0001 μg/mL (log10 10,000 = 4 to log10 0.0001 = -4). Artemia cysts were hatched under controlled conditions (25 ℃ ± 2 ℃) with continuous aeration and white light for 24 hours. Post-hatching, the nauplii were fed with baker's yeast for 2 hours, after which the medium was changed. Ten nauplii were then exposed to the test concentrations in individual test tubes. The control group received 1% DMSO in saline solution. Mortality was assessed 24 hours after observation by noting the movement of the nauplii. This experimental design ensures reproducibility by controlling factors such as temperature, aeration, light, feeding, and compound concentrations, providing a reliable foundation for aquatic toxicology studies [20].

2.5. Statistics Methods

Germination data were analyzed using one-way ANOVA to compare the means of five treatment groups at 24 and 48 hours. Tukey's multiple comparison test was applied between means. Statistical significance was set at $p < 0.05$. The effect size was assessed using R^2. Data were analyzed with GraphPad Prism v5.1 software.

Radicle elongation data were analyzed using one-way ANOVA to compare treatment groups at 0, 24, and 48 hours. Bartlett's test was applied to assess variance homogeneity. For significant ANOVA results, post hoc pairwise comparisons were performed using Bonferroni's test (0 hours) and Dunnett's test (24 and 48 hours), with DMSO 1% as the control group. Descriptive statistics (mean ± standard error) and 95% confidence intervals were calculated. Statistical significance was set at $p < 0.05$, and all analyses were conducted using GraphPad Prism v5.01.

For the *Artemia salina* test, dose-response curves for IBP and $K_2Cr_2O_7$ were analyzed using GraphPad Prism v5.01. The data were fitted to a log (lethal dose) vs. response — Variable slope model, commonly used for dose-response analysis. The model assumes a sigmoidal dose-response curve with a variable slope. All parameters were reported with standard errors and 95% confidence intervals. Model fit was assessed using R^2 values, where values close to 1 indicate a good fit, and precision was evaluated using the standard error of residuals (Sy.x).

3. Results and Discussion

3.1. Germination Test

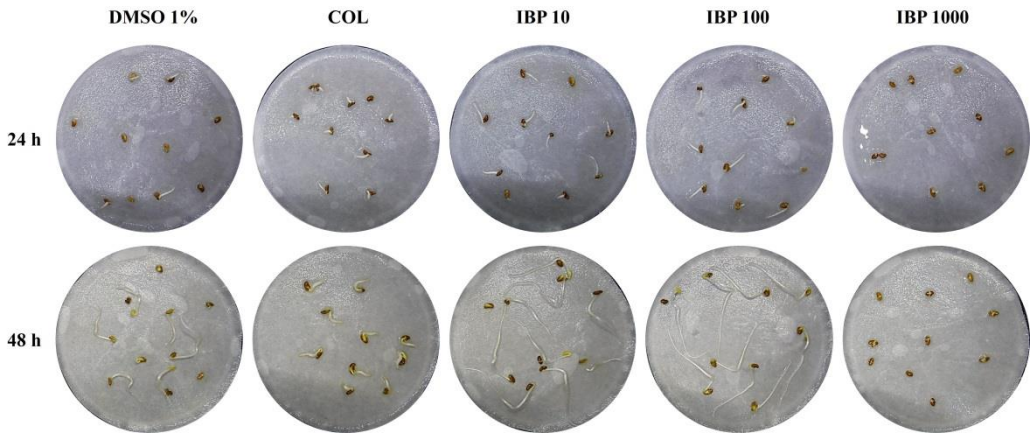

Figure 1. *Effect of 1% DMSO, COL (1000μg/mL), and ibuprofen (10, 100, and 1000 μg/mL) on seed germination at 24 and 48 hours. Representative images of seed germination under different treatment conditions are shown. Seeds were treated with 1% DMSO (vehicle control), COL at a concentration of 1000μg/mL, or ibuprofen (IBP) at concentrations of 10, 100, and 1000 μg/mL.*

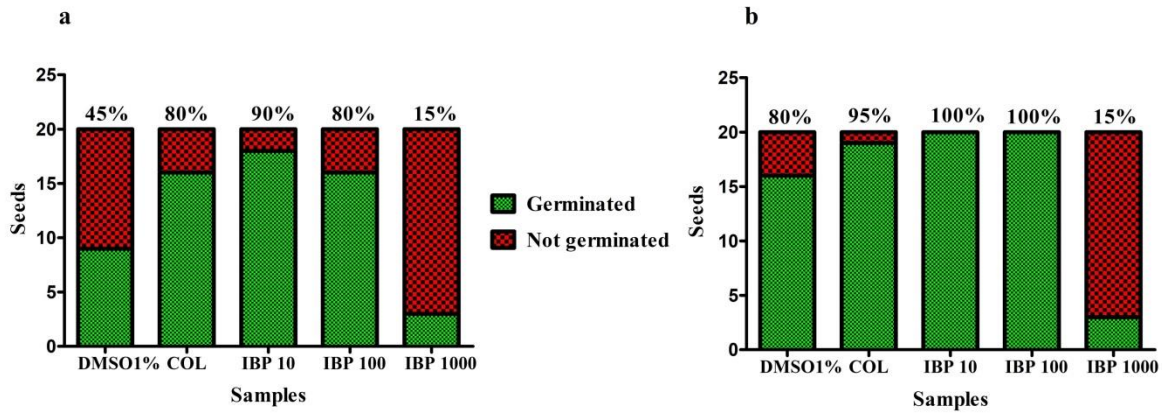

Figure 2. *Germination rates of Medicago sativa seeds at 24 hours (Panel a) and 48 hours (Panel b). The R^2 values for the 24-hour and 48-hour data were 0.9377 and 0.9621, respectively. Data represents the number of germinated and non-germinated seeds out of a total of 40 seeds per treatment group. Statistical analysis was performed using one-way ANOVA, with Tukey's post-hoc test for multiple comparisons.*

The effects of various treatments on the germination of *Medicago sativa* seeds were evaluated at 24 and 48 hours after treatment (Figure 1). Germination percentages were recorded and analyzed statistically using ANOVA, followed by Tukey's post-hoc test for pairwise comparisons (Figure 2).

After 24 hours of treatment, the control group treated with the vehicle (DMSO 1%) exhibited a germination rate of 45%, which increased to 80% at 48 hours, reflecting a natural progression in seed germination over time. Statistically, a significant difference was found between the DMSO 1% and other treatments ($p < 0.0001$), with IBP at 1000μg/mL showing the most significant inhibitory effect. IBP 1000 exhibited a significantly lower germination rate of 15%, which did not change over the 24 hours ($p < 0.001$).

The treatment with colchicine (1000μg/mL) resulted in a marked increase in germination, reaching 80% at 24 hours and 95% at 48 hours. In this study, COL significantly increased germination compared to the control group ($p < 0.0001$).

Germination under the influence of IBP showed concentration- and time-dependent effects. At 10 μg/mL, IBP induced a high germination rate of 90% at 24 hours, which increased to 100% by 48 hours, suggesting a promotive effect at low concentrations (hormesis). Similarly, at 100 μg/mL, germination rose from 80% at 24 hours to 100% at 48 hours, demonstrating a consistent positive response over time. Statistical analysis showed significant differences between IBP 1000 and both IBP 10 and IBP 100 ($p < 0.05$), with IBP 1000 exhibiting a much lower germination rate, consistent with its toxic effects.

At 48 hours, the ANOVA again revealed significant differences among the treatments ($p < 0.0001$). The IBP 1000 treatment remained the most inhibitory, with a germination rate of only 15%, as observed at both 24 and 48 hours. IBP 10 and IBP 100 demonstrated similar effects, with germination rates of 100% at 48 hours. The colchicine treatment showed the highest germination rate at 95%, significantly different from both DMSO 1% and IBP 1000 ($p < 0.001$).

Polyploidy, a genetic condition where plants possess more than two complete sets of chromosomes, can be artificially induced through treatments such as colchicine. This phenomenon is known to improve various agronomic traits, including increased cell size, which promotes higher yields and more robust growth. Polyploid plants exhibit greater resistance to abiotic stress (drought, salinity, extreme temperatures) and enhanced tolerance to diseases and pests due to an increase in the production of bioactive compounds. Additionally, polyploidy improves plant competitiveness by increasing vigor and growth, while also enhancing fruit size and quality, as well as post-harvest resistance [21].

The effects of colchicine in promoting germination, observed at both 24 and 48 hours, are consistent with previous studies that highlight colchicine's ability to induce polyploidy and enhance seed vigor in various plant species. Colchicine's interference with mitotic spindle formation likely results alteration of cellular division and growth processes, ultimately leading to enhanced seedling establishment [18, 22]. Thus, colchicine-induced polyploidy is a key mechanism driving improved seedling vigor, contributing to better plant establishment and growth under various conditions.

Ibuprofen, a compound known to influence plant hormonal signaling [23], demonstrated a concentration-dependent effect on germination. At lower concentrations (10 μg/mL and 100 μg/mL, IBP promoted germination, likely through its interaction with hormonal pathways such as jasmonic acid or abscisic acid, which are known to regulate seed germination. However, at the higher concentration of IBP 1000, a significant inhibitory effect was observed, suggesting potential cellular toxicity or interference with essential metabolic processes required for early seed development. The phenomenon of hormesis may explain the observed effects of IBP, particularly at lower concentrations (10 μg/mL and 100 μg/mL. At these concentrations, ibuprofen promoted germination. However, at higher concentrations (IBP 1000), a strong inhibitory effect was observed, supporting the idea of hormesis, where low doses have beneficial effects and high doses are toxic [24]. Previous studies on various plant species, includ-

ing *Allium schoenoprasum* and *Lactuca sativa*, have also reported similar concentration-dependent phytotoxic effects of ibuprofen [25].

DMSO 1%, while serving as the control, showed a typical rate of germination for untreated seeds, which increased over time, as expected. The statistically significant differences observed between DMSO 1% and the treatments involving IBP 10, IBP 100, and colchicine suggest that these compounds influence germination through mechanisms that are both time- and concentration-dependent.

3.2. Radicle Elongation Assay

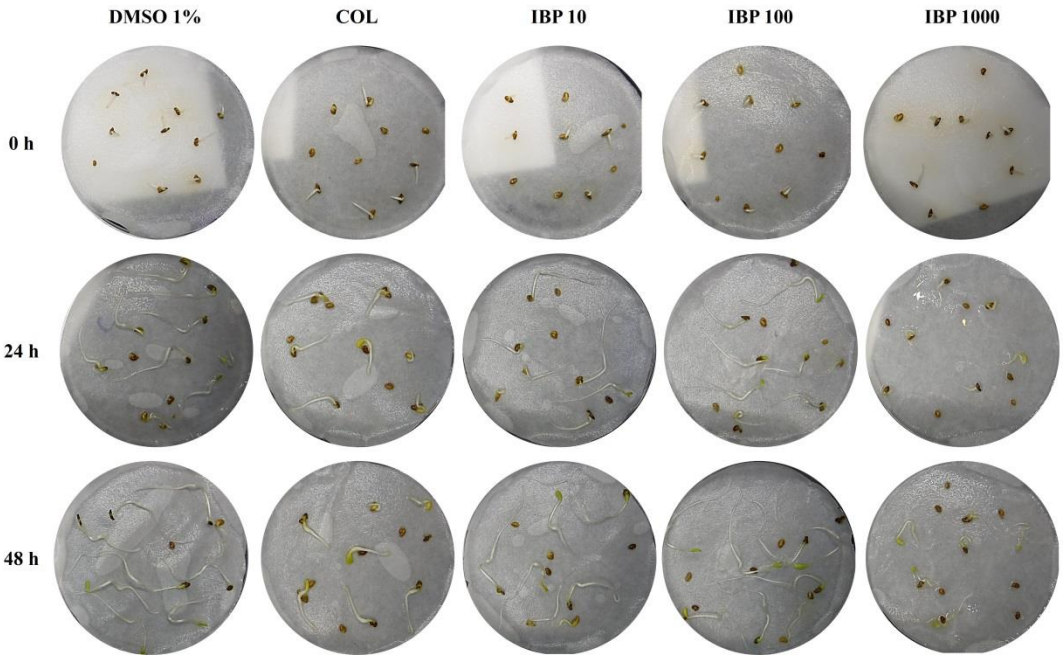

Figure 3. *Root elongation of Medicago sativa seedlings after exposure to 1% DMSO, COL (1000µg/mL), and ibuprofen (10, 100, and 1000 µg/mL). Seedlings of Medicago sativa were germinated for 24 hours in commercially purified water and subsequently exposed to either 1% DMSO (vehicle control), COL at 1000µg/mL, or IBP at 10, 100, or 1000 µg/mL. Representative images were captured at 24 and 48 hours after treatment to assess root elongation.*

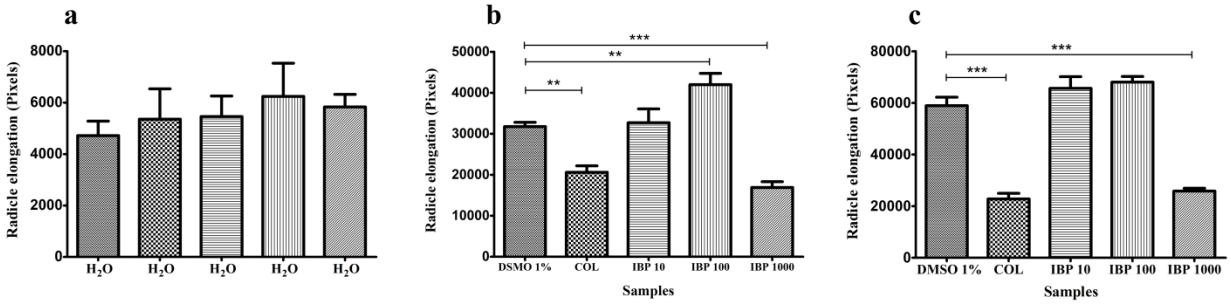

Figure 4. *Quantification of root elongation in Medicago sativa seedlings treated with DMSO, COL, and ibuprofen. Panel a) Shows the root size in pixels after 24 hours of germination, with an ANOVA followed by Bonferroni's test applied to assess significant differences between treatments. Panel b) Displays root elongation at 24 hours of exposure, analyzed using ANOVA with Dunnett's test. Panel c) Represents root elongation at 48 hours of exposure, also evaluated using ANOVA and Dunnett's test. Asterisks in the graphs indicate statistically significant differences between treatments based on the statistical tests performed.*

The results obtained from the radicle elongation assay in *Medicago sativa* indicate significant differences in growth depending on the treatment applied. Representative images illustrating the progression of root development at 0 hours (prior to treatment), and at 24- and 48-hours post-treatment are shown in Figure 3. Quantitative analysis of root growth was performed by measuring the radicle area (in pixels) from images taken 24 and 48 hours after treatment. The mean

radicle area per treatment group was calculated and is presented as the average ± standard error. These data are shown in Figure 4. Initially, the radicle length, measured in pixels, was homogeneous across samples, suggesting no considerable initial variability in seed size.

The elongation of radicles from *Medicago sativa* seeds was assessed after treatment with DMSO 1% (vehicle), colchicine (COL, 1000μg/mL), and IBP at concentrations of 10, 100, and 1000 μg/mL. Statistical analysis was performed using one-way ANOVA, Bartlett's test for homogeneity of variances, and post hoc multiple comparison tests at 0, 24, and 48 hours.

No significant differences in radicle elongation were observed between the treatment groups at time 0 ($p = 0.8209$), indicating that none of the treatments had an immediate effect on radicle growth. Bartlett's test revealed significant differences in variances between the groups ($p = 0.0191$), indicating that the data distribution exhibited variability.

At 24 hours, significant differences in radicle elongation were detected between the treatments ($p < 0.0001$), with post hoc analyses confirming that: DMSO 1% (control) exhibited significant radicle growth, reaching 31,746 ± 3,309 pixels ($p = 0.313$ compared to the 0-hour control). COL inhibited radicle growth significantly, with a value of 20,606 ± 5,007 pixels ($p = 0.003$), consistent with its known role as a microtubule polymerization inhibitor that affects cell division and elongation [18].

IBP at concentrations of 100 μg/mL promoted the greatest radicle growth (41,988 ± 8,706 pixels) ($p < 0.0001$ compared to DMSO 1%), while IBP 1000 μg/mL drastically reduced elongation (16,904 ± 4,484 pixels) ($p < 0.0001$ compared to DMSO 1%), indicating a potential cytotoxic or inhibitory effect at high concentrations, as has been observed in other plants exposed to NSAIDs [26].

The observed trends at 24 hours suggest that ibuprofen at low concentrations may enhance growth, while high concentrations inhibit it.

At 48 hours, similar patterns were observed, with significant differences between treatments ($p < 0.0001$). Post hoc analyses revealed: that DMSO 1% (control) maintained consistent radicle elongation, reaching 58,956 ± 10,339 pixels ($p = 0.128$ compared to 24 hours), indicating sustained growth. COL continued to inhibit growth, with a value of 22,781 ± 6,952 pixels ($p < 0.0001$ compared to DMSO 1%). IBP at 10 μg/mL (65,641 ± 14,346 pixels) and 100 μg/mL (68,061 ± 6,883 pixels) promoted significantly greater growth than the control ($p < 0.0001$ for both), supporting the hypothesis that ibuprofen at low concentrations may enhance growth by modulating signaling pathways related to cell division and elongation, as suggested by previous studies [27]. IBP at 1000 μg/mL (25,856 ± 3,460 pixels) resulted in severe growth inhibition ($p < 0.0001$ compared to DMSO 1%), which may be linked to disruption of cellular homeostasis or direct toxic effects.

The maximum concentration of ibuprofen (IBP) found in soils is 610μg/kg [26] (approximately 0.61μg/mL), which is much lower than the 1000μg/mL concentration that caused significant inhibition of *Medicago sativa* germination and elongation in the study. However, even at lower concentrations, IBP can have notable ecological impacts, particularly when soils are exposed to continuous or cumulative doses from pollution sources such as wastewater or contaminated agricultural water. The fact that IBP at 1000μg/mL significantly inhibits germination (reducing the rate to only 15%) suggests that even much lower concentrations in soil could still impact biodiversity and plant growth in agricultural or aquatic ecosystems. In *Medicago sativa*, lower IBP concentrations (10 and 100μg/mL) promoted germination, pointing to a potential hormesis effect, where low doses of a compound are beneficial, while higher doses are toxic. This phenomenon could be relevant in soils with moderate IBP concentrations, where low doses may unexpectedly stimulate growth or germination, but higher concentrations could completely inhibit these processes.

From an ecological perspective, the inhibition of germination and growth in species like *Medicago sativa*, a forage plant and model species in agronomic research, could have cascading effects on the food chain and overall ecosystem health. Plants that fail to germinate or grow properly also impact organisms that rely on them for food and other ecosystem services. In soils contaminated with IBP, competition among species may shift, with certain plants thriving while others, potentially more sensitive to pollutants, decline or vanish. Furthermore, IBP could disrupt soil microbiota, as some studies have shown that pharmaceuticals can alter soil microbial composition [25], affecting essential processes like organic matter decomposition and nutrient cycling. Such changes could reduce soil fertility, ultimately affecting agricultural productivity. Therefore, while IBP concentrations in soils may not be excessively high, their continuous presence and accumulation could result in long-term ecological consequences, underscoring the need to monitor and regulate emerging contaminants in both terrestrial and aquatic ecosystems.

3.3. *Artemia Salina* Acute Toxicity Assay

The toxicity of two chemical compounds, ibuprofen and potassium dichromate, was evaluated with respect to their effects on the mortality of *Artemia salina* in acute toxicity assays (Figure 5). Using logistic regression analysis, the concentrations that induced a 50% probability of mortality were estimated for both compounds.

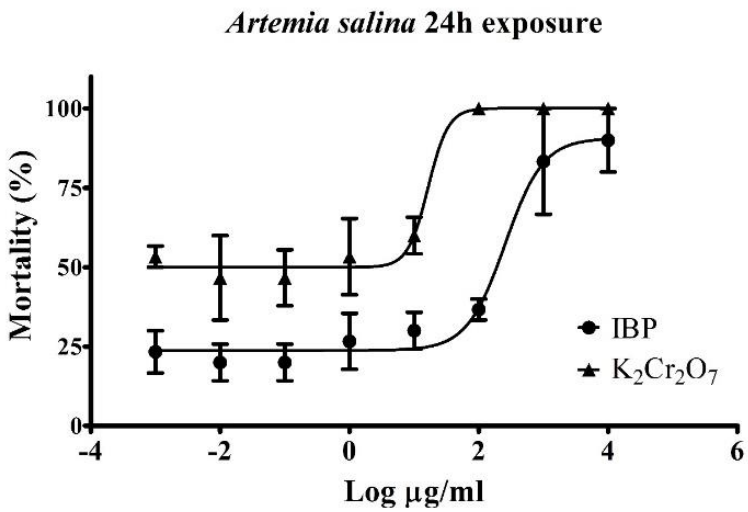

Figure 5. *Sigmoidal dose-response curves for IBP and $K_2Cr_2O_7$ following 24 hours of exposure. Mortality was measured as a percentage of total response, and concentrations are expressed in µg/mL. The curves were generated using the log (inhibitor) vs. response — Variable slope model, and the data illustrate the potency and sensitivity of each compound in inducing mortality.*

Table 1 presents the dose-response parameters and statistical fit for both IBP and $K_2Cr_2O_7$. The table includes key values such as LD_{50}, HillSlope, $LogLD_{50}$, and Span, as well as their respective standard errors and 95% confidence intervals.

Table 1. *Dose-response parameters and statistical fit for ibuprofen (IBP) and potassium dichromate ($K_2Cr_2O_7$).*

Parameter	IBP	K_2 Cr_2 O_7
Bottom (Min. Value)	23.79 (±3.66)	49.99 (±3.42)
Top (Max. Value)	90.67 (±8.70)	100.1 (±4.83)
Log(LD_{50})	2.405 (±0.25)	1.215 (±0.90)
LD_{50}	254.2 µg/mL	16.40 µg/mL
HillSlope	1.475 (±0.76)	2.803 (±11.62)
Span	66.88 (±9.57)	50.11 (±5.98)
R^2	0.8142	0.8250
Sy.x	13.92	11.83

Bottom and Top: The minimum and maximum response values for IBP and $K_2Cr_2O_7$ were 23.79 and 90.67, and 49.99 and 100.1, respectively. These values show a reliable fit for both compounds, with a slight difference in response range. Log(LD_{50}): IBP had a Log(LD50) of 2.405, while $K_2Cr_2O_7$ had a lower value of 1.215, indicating that $K_2Cr_2O_7$ is more potent. LD_{50} (Concentration in µg/mL): IBP's LD_{50} was 254.2 µg/mL, whereas $K_2Cr_2O_7$ had a much lower LD_{50} of 16.40 µg/mL, confirming $K_2Cr_2O_7$'s higher potency. HillSlope: IBP's HillSlope was 1.475, and $K_2Cr_2O_7$'s was 2.803, suggesting $K_2Cr_2O_7$ induces a sharper, more sensitive dose-response. Span (Response Range): IBP's Span (66.88) was broader than $K_2Cr_2O_7$'s (50.11), indicating IBP affects a wider range of responses. R^2 (Goodness of Fit): Both compounds showed strong model fits with R^2 values of 0.8142 (IBP) and 0.8250 ($K_2Cr_2O_7$), suggesting high data reliability. Sy.x (Standard Error): $K_2Cr_2O_7$ had a lower Sy.x (11.83) compared to IBP (13.92), indicating a better fit for $K_2Cr_2O_7$. P-value: Both compounds had a highly significant P-value (3.214e-09), indicating strong statistical significance in their effects.

The dose-response analysis for IBP and $K_2Cr_2O_7$ revealed notable differences in their potency and sensitivity. $K_2Cr_2O_7$ exhibited a significantly lower LD_{50} value compared to IBP, indicating that it is more potent in inhibiting the biological response. Additionally, $K_2Cr_2O_7$ displayed a steeper HillSlope, suggesting a more sensitive and sharper dose-response relationship. This enhanced sensitivity implies that small changes in the concentration of $K_2Cr_2O_7$ produce more substantial effects, which may be indicative of a more precise mechanism of action.

In contrast, IBP showed a broader response range (Span), indicating a more extensive effect across a wider concentration range. While both compounds demonstrated strong fits to the dose-response models, as evidenced by high R^2 values and statistically significant p-values, the confidence intervals for some parameters, particularly $LogLD_{50}$ and HillSlope for $K_2Cr_2O_7$, were broader, reflecting a greater uncertainty in the estimation of its dose-response characteristics.

Despite these uncertainties, the overall goodness of fit for both models were satisfactory, and the precision of the estimations, as indicated by the standard error of residuals, was reasonable. These findings suggest that both compounds exhibit significant dose-dependent effects, with $K_2Cr_2O_7$ being more potent and exhibiting a more sensitive response compared to IBP. However,

further studies with a broader range of concentrations and biological assays may be needed to refine these estimates and better understand the underlying mechanisms of action.

In the study conducted with *Artemia salina*, the LD_{50} of ibuprofen was found to be 254.2 µg/mL. This determination is crucial for evaluating the toxicity of ibuprofen in aquatic organisms. The LD_{50} reflects the concentration of the contaminant that causes the death of half the exposed organisms and serves as an indicator of its toxicological potential in aquatic environments.

When comparing this LD_{50} with the concentrations of ibuprofen found in bodies of water, in many regions, the concentrations of ibuprofen in water are significantly lower. For instance, it has been reported that in wastewater from various regions worldwide, ibuprofen concentrations range from 0.004 to 603µg/L. The highest concentration found in surface water was 1417µg/L [26]. Converting these concentrations to µg/mL, the highest recorded concentration (1417µg/L) corresponds to approximately 1.417µg/mL, which is significantly lower than the LD50 of 254.2 µg/mL for *Artemia salina*. However, it is important to note that even at lower concentrations, ibuprofen could have sublethal effects on these organisms. Moreover, the persistent presence of ibuprofen in bodies of water could lead to cumulative effects, as aquatic organisms might be exposed to sublethal concentrations chronically. This prolonged exposure could alter species physiology and modify trophic relationships within aquatic ecosystems, even in the absence of immediate death.

Additionally, the toxicity of pharmaceutical pollutants is not limited to direct effects on individual organisms, but can also alter the water microbiota, affecting key biogeochemical processes like nutrient cycling and organic matter decomposition. This alteration of the microbiota could further reduce the productivity of the aquatic ecosystem.

4. Conclusions

In this study, the effects of colchicine and ibuprofen on *Medicago sativa* seed germination and radicle elongation were evaluated, revealing concentration- and time-dependent responses. Colchicine significantly promoted seed germination, while ibuprofen exhibited hormetic effects, enhancing germination at lower concentrations (10 and 100 µg/mL) but inhibiting it at higher concentrations (1000 µg/mL). These findings align with previous research on the hormetic nature of certain compounds, suggesting that IBP may influence seed germination through modulation of hormonal pathways.

For radicle elongation, ibuprofen at low concentrations (10 and 100 µg/mL) promoted growth, while higher concentrations (1000 µg/mL) led to significant inhibition, supporting the hypothesis of concentration-dependent toxicity. Conversely, colchicine inhibited radicle elongation, consistent with its known effects on microtubule polymerization and cell division.

The results from the dose-response in *Artemia salina* analysis demonstrate that $K_2Cr_2O_7$ is significantly more potent than IBP. The LD_{50} for $K_2Cr_2O_7$ was 16.40 µg/mL, which is much lower than IBP's LD_{50} of 254.2 µg/mL, indicating that $K_2Cr_2O_7$ requires much lower concentrations to achieve a 50% inhibition of the response.

Although the results of this study focus on acute toxicity assessments, specifically regarding the inhibition of *Medicago sativa* germination and root elongation, as well as the response of *Artemia salina* to varying ibuprofen concentrations, it is essential to acknowledge that sublethal, cumulative, and chronic effects of ibuprofen must also be considered in environmental risk assessments. Sublethal concentrations of ibuprofen, even when they do not cause immediate inhibition or mortality, can disrupt fundamental biological processes.

Abbreviations

NSAID	Non-steroidal Anti-inflammatory Drugs
DMSO	Dimethyl Sulfoxide
COL	Colchicine
ANOVA	Analysis of Variance
IBP	Ibuprofen
Log(LD_{50})	Logarithm of the Median Lethal Dose
LD_{50}	Median Lethal Dose
HillSlope	Hill Slope (Steepness of the Dose-response Curve)
Span	Difference Between the Minimum and Maximum Response
R^2	Coefficient of Determination
Sy.x	Standard Error of the Estimate (Standard Error of Regression)
P-value	Probability Value (Statistical Significance Indicator)

Author Contributions

María del Mar Cogollo-Urzola: Formal Analysis, Investigation, Visualization

Irma Catalina Coral-Bonia: Formal Analysis, Investigation

Edgar Fernando Peña-Torres: Validation, Writing – review & editing

Omar Aristeo Peña-Morán: Conceptualization, Methodology, Resources, Writing – original draft

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

María del Mar Cogollo-Urzola is a Pharmacy Technician graduate from the University of Córdoba (Colombia). In 2023, she conducted research during an academic exchange at the Universidad Autónoma del Estado de Quintana Roo (Mexico). Her areas of experience include pharmaceutical sciences, environmental toxicology, seed germination assays, and aquatic ecotoxicology.

Irma Catalina Coral-Bonia, a graduate of the Bachelor's degree in Pharmacy from the Universidad Autónoma del Estado de Quintana Roo, has research experience in cell culture, as well as in the search for bioactive compounds of natural origin with cytotoxic potential. She also has expertise in the isolation and quantification of active ingredients, as well as in the standardization of *in vitro* bioevaluation models.

Omar Aristeo Peña-Morán is a graduate of the Bachelor's, Master's, and Doctoral programs in Pharmacy from the Universidad Autónoma del Estado de Morelos. He is currently a full-time Professor and Researcher at the Department of Pharmaceutical Sciences in the Division of Health Sciences at the Universidad Aútonoma del Estado de Quintana Roo. His research lines include the search and design of bioactive compounds with cytotoxic potential from plant sources, utilizing computer-assisted methods. Additionally, he is currently focused on the ecotoxicological evaluation of pharmaceuticals in environmental models, exploring the impact of drugs on ecosystems and their potential environmental hazards.

Edgar Fernando Peña-Torres is a full-time professor at the Universidad del Caribe in the Gastronomy Program. He holds a degree in Biochemical Engineering from the Institute of Technology of Mazatlán (2010), and both a Master of Science and a Ph.D from the Research Center for Food and Development (CIAD), with a focus on animal science and technology. His research lines include food science and technology, meat products, phytochemicals, quality control, statistics, and experimental design. He has been teaching since 2014 and is currently a Level 1 member of the National System of Researchers (SNI) in Mexico.

Research Field

María del Mar Cogollo-Urzola: Pharmaceutical sciences, environmental toxicology, seed germination assays, and aquatic ecotoxicology.

Irma Catalina Coral-Bonia: Cell culture, natural product screening, cytotoxicity testing, bioactive compound isolation, active ingredient quantification, in vitro assay standardization, pharmacognosy, natural product chemistry.

Edgar Fernando Peña-Torres: Food science and technology, meat product development, phytochemical analysis, food quality control, experimental design in food, statistical analysis in research, nutritional biochemistry, functional foods research.

Omar Aristeo Peña Morán: Plant-based drug discovery, cytotoxic compound design, molecular docking simulations, ecotoxicological risk assessment, pharmaceutical environmental impact, in silico drug modeling, environmental toxicology, natural product pharmacology.