

Original Article

## Cytogenotoxicity of Soil Contaminated with an Atrazine-Based Herbicide Under Laboratory Conditions: Bioassays with *Allium cepa*

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### Abstract

Atrazine is a widely used herbicide for weed control in crops such as corn, sugarcane, and sorghum. However, it can have adverse effects on non-target organisms. This study aimed to evaluate the cytogenotoxic effects of a Red-Yellow Latosol contaminated with atrazine under laboratory conditions. Concentrations exceeding the recommended field doses (2 mg/kg to 1024 mg/kg) were tested, simulating excessive herbicide use and contamination from effluent disposal, such as wash water from spraying tanks and improper disposal of pesticide containers — major point sources of agricultural soil contamination. *Allium cepa* bioassays were conducted. Soil contaminated with 16, 32, 64, 128, 256, and 512 mg/kg of atrazine reduced cell viability. Only the 8 mg/kg concentration significantly decreased the mitotic index. Conversely, except for the 32 and 128 mg/kg treatments, all other concentrations increased the frequency of aberrant cells compared to the control soil. Atrazine is poorly adsorbed in soils with low clay content and moderate to low organic matter content, which increases its bioavailability and, consequently, its cytogenotoxic potential. Therefore, implementing mechanisms capable of retaining and treating effluents with high atrazine concentrations before soil disposal is strongly recommended.

**Keywords:** Cell cycle, Cell viability, Chromosomal aberrations, Pesticide.

### INTRODUCTION

Brazil is globally recognized as one of the leading consumers of pesticides. In 2022, approximately 800,000 tons of active ingredients were sold in the country, with herbicides accounting for 61.49% of total sales. Among these, atrazine stood out as a key active ingredient, with 47,663.24 tons sold (IBAMA, 2022).

Atrazine, a member of the triazine chemical group, is a widely used herbicide for weed control in crops such as corn, sorghum, and sugarcane. Its mode of action involves binding to the plastoquinone site in photosystem II, disrupting electron transport and consequently inhibiting photosynthesis (Roman *et al.*, 2005; Bibi *et al.*, 2019).

The soil acts as a significant reservoir for atrazine, and its half-life ranges from 1.4 to 108 days,

depending on environmental conditions (Kim *et al.*, 2021). Although atrazine is agronomically effective in target crop systems, its persistence in the environment raises concerns about unintended toxicity. Several studies have demonstrated that atrazine can be harmful to non-target plant species, which often lack the enzymatic mechanisms required for detoxification, leading to disruptions in cell division, chromosomal stability, and overall plant development (El-Ghamery *et al.*, 2000; Srisvatava & Mishra, 2009; Kumar & Srivastava, 2015; Ventura-Camargo & Marin-Morales, 2016; Felisbino *et al.*, 2018; Bibi *et al.*, 2019; Souza *et al.*, 2023; Deng *et al.*, 2024). In addition to plants, atrazine also poses risks to soil microorganisms (Deng *et al.*, 2024) and soil invertebrates (Lammertyn *et al.*, 2021; Deng *et al.*, 2024).

Due to its moderate to high mobility in soil, atrazine has been detected in surface and groundwater at

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concentrations exceeding safety thresholds for aquatic life and human health (Battaglin *et al.*, 2000; Moreira *et al.*, 2012; Vonberg *et al.*, 2014; Dias *et al.*, 2018; Kim *et al.*, 2021; Urseler *et al.*, 2022). In this sense, atrazine caused acute and chronic toxicity in microcrustaceans (Souza *et al.*, 2023), histopathological, biochemical, and physiological changes (Mela *et al.*, 2013), cytogenotoxicity and DNA damage in fish (Cavas *et al.*, 2011; Deng *et al.*, 2024), and endocrine dysfunction, neurotoxicity, digestive disorders, birth defects (Deng *et al.*, 2024) and reproductive dysfunction in humans (Guimarães-Ervilha *et al.*, 2025).

The primary sources of point contamination in soil include accidental spills during pesticide preparation and the improper disposal of containers (Osborne *et al.*, 2015; Xu *et al.*, 2021). In China, for example, 62% of farmers dispose of packaging directly in crop fields or water bodies, increasing the release of pesticide residues into the environment (Xu *et al.*, 2021).

Another source of soil contamination is the direct discharge of effluents from spray tank washings (Higginbotham *et al.*, 1999; Helweg *et al.*, 2002; Osborne *et al.*, 2015; Xu *et al.*, 2021). Many farmers reuse water from equipment washing to maximize pesticide use in cultivation areas, a practice considered inappropriate (Bernardi *et al.*, 2018) due to the increased amount of pesticide applied (Damalas *et al.*, 2008; Osborne *et al.*, 2015). When present at high concentrations in agricultural soils, atrazine and its residues can inhibit crop growth and bioaccumulate, potentially transferring through the food chain (Singh *et al.*, 2018; Houjayfa *et al.*, 2020; Lima *et al.*, 2022).

Soil in pesticide preparation areas tends to exhibit higher concentrations of pesticides than in other agricultural areas (Osborne *et al.*, 2015). De Wilde *et al.* (2007) reported atrazine residue concentrations in specific farm areas in the United States ranging from 0.1 to 3,562 mg/L at pesticide mixing and loading sites, 0.8 to 1,090 mg/L at container storage locations, and 1.2 to 61 mg/L in equipment wash areas. Studies on pesticide levels in Brazilian soils are scarce (Fernandes *et al.*, 2020). Only one study shows that in Brazilian soils, atrazine concentrations range from 0.0021 to 0.2528 mg/kg (Pinheiro *et al.*, 2011). In contrast, agricultural soils in China have reported concentrations ranging from 1.86 to 1,100 mg/kg (Liu *et al.*, 2020).

Bioassays using model plants, such as *Allium cepa* (onion), are recommended for assessing the adverse effects of herbicides (Felisbino *et al.*, 2018; Camilo-Cotrim *et al.*, 2022; Souza *et al.*, 2023) and serve as an effective tool for evaluating soil quality, contributing to the protection of surface and groundwater resources. Specifically, *A. cepa* has been widely used to analyze cell viability (Ghosh *et al.*, 2015; Ghosh *et al.*,

2016; Huang *et al.*, 2022), investigate cell proliferation, and identify mitotic and chromosomal abnormalities (Leme & Marin-Morales, 2009; Aragão *et al.*, 2024). These analyses are important because there is no safe exposure limit for cytotoxic, genotoxic, and mutagenic substances, which can lead to genetic instability (Souza *et al.*, 2024). The *A. cepa* assay for mitotic and chromosomal abnormalities is highly correlated with carcinogenicity tests in rodents (Rank & Nielsen, 1994). For this reason, it is considered a viable alternative to animal experimentation (Camilo-Cotrim *et al.*, 2022).

Given these considerations, this study analyzed the cytogenotoxic effects of soil contaminated with different concentrations of atrazine using *A. cepa* bioassays. Concentrations exceeding the recommended field doses were tested, simulating excessive pesticide use and contamination from spray tank washings and improper disposal of pesticide containers.

## MATERIALS AND METHODS

### Soil

The Red-Yellow Latosols are found in extensive areas throughout the Brazilian territory and are widely used for agriculture and livestock farming (Almeida *et al.*, 2021). Samples of Red-Yellow Latosol were collected from the A horizon at a depth of 0–20 cm at Sítio Jaqueira Agroecologia, located in the municipality of Alegre, Espírito Santo, Brazil (Latitude 20°45'49" S, Longitude 41°31'57" W). The collected soil was homogenized, sieved through a 2 mm mesh, and stored at room temperature. The site follows agroecological practices; therefore, there is no history of pesticide application.

The following physical and chemical properties of the soil were determined at the Soil Laboratory of the Federal University of Espírito Santo: pH (soil-to-water ratio of 1:2.5); P (Mehlich-1 extractant, colorimetric determination); K and Na (Mehlich-1 extractant, flame photometry determination); Ca and Mg (1 mol/L KCl extractant, atomic absorption spectrometry determination); Al (1 mol/L KCl extractant, titrimetric determination); H + Al (0.5 mol/L calcium acetate extractant at pH 7, titrimetric determination); sum of exchangeable bases, effective cation exchange capacity, total cation exchange capacity, base saturation index, aluminum saturation index; Fe, Cu, Zn, and Mn (Mehlich-1 extractant, atomic absorption spectrometry determination); organic matter (wet oxidation of carbon using potassium dichromate in an acidic H<sub>2</sub>SO<sub>4</sub> medium); remaining P (0.01 mol/L CaCl<sub>2</sub> solution containing 60 mg/L P); and particle size distribution (agitation at 50 rpm for 16 hours using a Wagner-type shaker, chemical dispersion with 0.1 mol/L NaOH, and determination of

silt and clay fractions by pipette method). The physicochemical characterization of the soil is shown in Table 1.

**Table 1.** Physicochemical properties of the soil

	Unity	Result
Coarse sandy	%	52.53
Fine sandy	%	7.48
Silt	%	8.25
Clay	%	31.74
Texture	-	Medium texture
pH (H <sub>2</sub> O)	-	5.46
P	mg/dm <sup>3</sup>	3.40
Na	mg/dm <sup>3</sup>	1.50
K	mg/dm <sup>3</sup>	79.20
Ca	cmolc/dm <sup>3</sup>	2.91
Mg	cmolc/dm <sup>3</sup>	1.09
Al	cmolc/dm <sup>3</sup>	0.12
H + Al	cmolc/dm <sup>3</sup>	3.58
Sum of bases	cmolc/dm <sup>3</sup>	4.21
Effective cation exchange capacity	cmolc/dm <sup>3</sup>	4.33
Cation exchange capacity (pH 7.0)	cmolc/dm <sup>3</sup>	7.79
Base saturation index	%	54.05
Aluminum saturation index	%	2.77
Fe	mg/dm <sup>3</sup>	106.20
Cu	mg/dm <sup>3</sup>	2.40
Zn	mg/dm <sup>3</sup>	7.70
Mn	mg/dm <sup>3</sup>	67.10
B	mg/dm <sup>3</sup>	0.27
Organic matter	g/kg	26.89
Remaining phosphorus	mg/L	52.20

## Atrazine

The commercial formulation used was Herbitrin

500 BR (Adama), containing 500 g/L (50.0% w/v) of atrazine (6-chloro-N<sup>2</sup>-ethyl-N<sup>4</sup>-isopropyl-1,3,5-triazine-2,4-diamin e) and 598.80 g/L (59.8% w/v) of other ingredients.

Based on the active ingredient concentration in the commercial product, a stock solution was prepared, followed by the preparation of test solutions. Distilled water was used as the dilution medium. The tested concentrations exceeded the recommended field dose (1.25 mg/kg for corn cultivation), simulating the discharge of effluents from the washing of empty pesticide containers and spray tanks. These concentrations were established using a multiplication factor of 2×, in accordance with EPA OPPTS 850.4200 guidelines (USEPA, 1996): 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 mg/kg.

## *A. cepa*

*A. cepa* (2n = 16) seeds of the Baia Periforme variety, free from pesticide treatment, were purchased from ISLA<sup>®</sup>.

## Bioassay setup

The soil's maximum water holding capacity (WHC) was determined according to Niva and Brown (2019) and adjusted to 60%. The soil was contaminated following the methodology of Carniel and Segat (2018), homogenized, and distributed in Petri dishes (100 × 15 mm). Each dish was filled with 40 g of soil, and 20 *A. cepa* seeds were placed in each dish. The control group consisted of soil moistened only with distilled water. The assay was performed in triplicate.

## Cell viability

Evans blue dye was used to assess cell viability. *A. cepa* roots were stained with an aqueous solution of 0.25% w/v Evans blue for 20 minutes, then washed with distilled water until excess dye was removed. Some roots were photographed under a Leica EZ4HD stereomicroscope. Additionally, five 1 cm root segments were suspended in 4 mL of N,N-dimethylformamide and then soaked for 2 hours at room temperature. The samples were then centrifuged at 3000 rpm for 5 minutes to extract the dye from the cells. The absorbance of the extract was measured using a spectrophotometer (IL-226-nm, Kazuaki) at 600 nm, following the methods of Ghosh *et al.* (2015, 2016) and Huang *et al.* (2022).

## Cytogenotoxicity

Root tips approximately 1.5 cm long were collected and fixed in ethanol-acetic acid (3:1, v/v), then

collected and fixed in ethanol-acetic acid (3:1, v/v), then stored in a refrigerator. After washing with distilled water to remove excess fixative, the roots were hydrolyzed in 5N HCl for 20 minutes at room temperature, followed by another wash in distilled water. Next, the meristematic region of the roots was sectioned, stained with 2% orcein for 10 minutes, covered with a coverslip, and macerated. Ten slides per treatment were analyzed, totaling 5000 cells (500 cells/slide), observed under a Nikon Eclipse E200 optical microscope at 400× magnification. The evaluated parameters included the mitotic index (MI), phase index (PI), and the induction of cell abnormalities (CA), calculated using the following formulas:

$$MI (\%) = \frac{\text{number of cells in mitosis}}{\text{total numbers of cells}} \times 100$$

$$PI (\%) = \frac{\text{number of cells in specific mitotic phase}}{\text{total number of cells in mitosis}} \times 100$$

$$AC (\%) = \frac{\text{number of cells with abnormalities}}{\text{total of cells}} \times 100$$

Images of the main alterations found were recorded using a Leica DM500 binocular microscope with a Leica ICC50 HD camera (Leica Microsystems) attached.

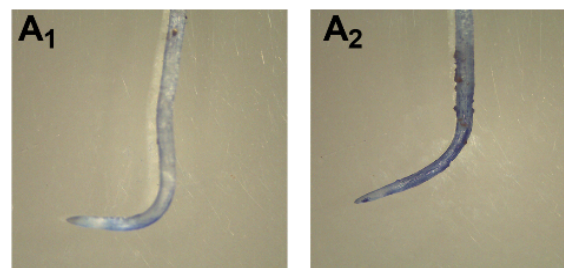
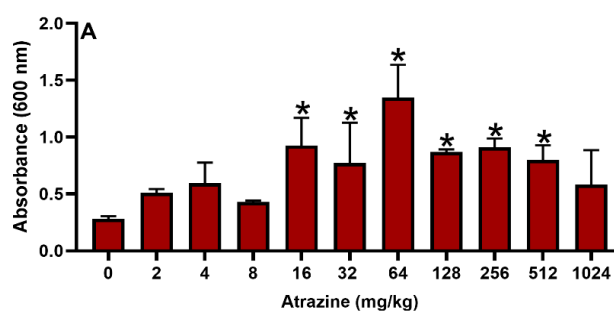
### Statistical Analysis

The data were subjected to analysis of variance, and comparisons between treatments and the control were performed using the Dunnett test ( $p < 0.05$ ). When the assumptions for parametric analysis were not met, the non-parametric Kruskal-Wallis test was applied ( $p < 0.05$ ). All analyses were conducted using GraphPad Prism software version 10.

## RESULTS

### Cell viability

The membrane stability of *A. cepa* root cells after exposure to soil contaminated with different concentrations of atrazine was assessed using the Evans blue dye technique. The results of the assay are presented in Figure 1. *A. cepa* roots exposed to soil contaminated with atrazine at concentrations of 16, 32, 64, 128, 256, and 512 mg/kg absorbed more dye than those exposed to the control soil (Figure 1A), indicating the cytotoxicity of these treatments. In contrast, the soil contaminated with 1024 mg/kg did not induce a significant response compared to the control soil (Figure 1A).



**Figure 1.** Effect of soil contaminated with different atrazine concentrations on the viability of *A. cepa* root cells. (A) Quantitative analysis indicating that concentrations ranging from 16 to 512 mg/kg induced a higher frequency of cell damage compared to the control soil. (A1 – A2) Qualitative analysis, showing a root from the control group (A1) and one exposed to soil contaminated with 128 mg/kg of atrazine (A2). Notice the blue-stained areas in the root exposed to the herbicide, indicating the presence of dead cells. Results are presented as mean  $\pm$  standard deviation. \*Denotes a statistically significant difference compared to the control soil ( $p < 0.05$ ).

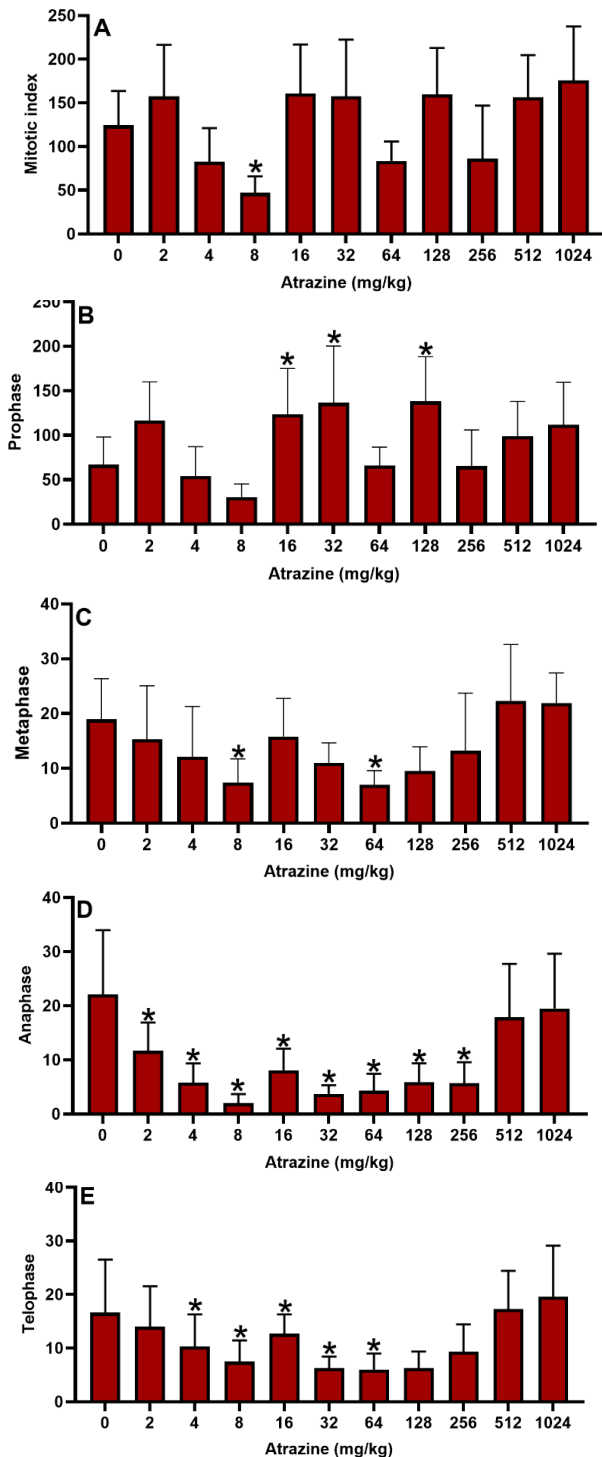
### Cytogenotoxicity

The cytogenotoxicity of soil contaminated with varying concentrations of atrazine was evaluated using the mitotic index, phase index, and mitotic and chromosomal alteration indices. The analysis of the mitotic index showed that only the soil contaminated with 8 mg/kg of atrazine significantly reduced the proportion of dividing cells by 37.7% compared to the uncontaminated soil (Figure 2A).

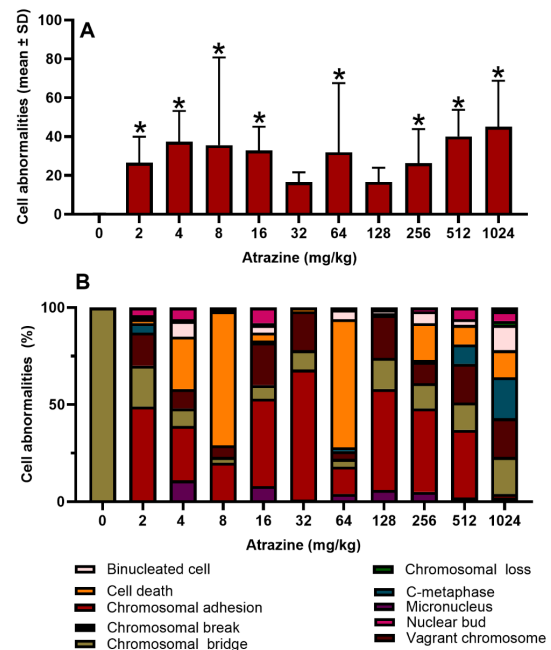
Concentrations of 16, 32, and 128 mg/kg caused a significant increase in the percentage of cells in prophase (Figure 2B). Meanwhile, treatments with 8 and 64 mg/kg of atrazine significantly reduced the frequency of cells in metaphase compared to the control soil (Figure 2C). Concentrations of 2 to 256 mg/kg reduced the frequency of cells in anaphase, making this the phase where atrazine caused the most significant effect, followed by telophase. Concentrations of 8 to 64 mg/kg reduced the percentage of cells in the final phase of mitosis.

Considering the frequency of cells with mitotic and chromosomal abnormalities, it can be observed that, with the exception of soils contaminated with atrazine at 32 and 128 mg/kg, the tested concentrations induced an increase in the number of aberrant cells compared to the control (Figure 3A). The main alterations induced by atrazine action were chromosomal adhesion, nuclear bud,

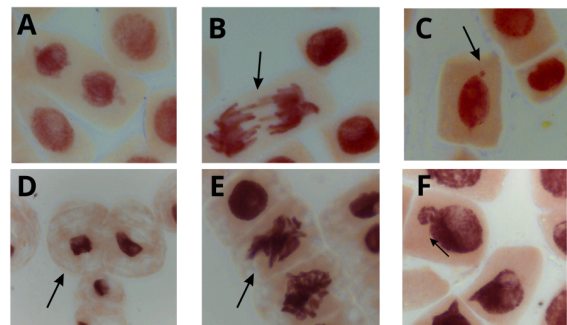
binucleated cells, c-metaphase, vagrant chromosomes, micronuclei, cell death, chromosomal loss, chromosomal bridge, and chromosomal break. Figure 3B shows the distribution of these alterations by treatment. Chromosomal adhesion was the most observed alteration (Figure 3B). Figure 4 presents illustrative micrographs showing the main chromosomal abnormalities identified in root cells of *Allium cepa* after exposure to soil contaminated with atrazine.



**Figure 2.** Effects of atrazine on the mitotic index (A) and phase index (B-E) of the *A. cepa* root meristem. Results are presented as mean  $\pm$  standard deviation. \*Denotes a statistically significant difference compared to the control soil ( $p < 0.05$ ).



**Figure 3.** (A) Frequency of mitotic and chromosomal abnormalities in *A. cepa* root meristem cells after exposure to atrazine-contaminated soil. (B) Distribution of specific observed alterations. In (A), results are presented as mean  $\pm$  standard deviation, and in (B), as a percentage. \*Denotes a statistically significant difference compared to the control soil ( $p < 0.05$ ).



**Figure 4.** Mitotic and chromosomal abnormalities observed in the root meristem of *A. cepa* after treatment with soil contaminated with different concentrations of the herbicide atrazine. (A) Vagrant chromosomes. (B) Chromosomal bridge. (C) Micronucleus. (D) Nuclear condensation, a hallmark of cells undergoing cell death. (E) Metaphase with adherence. (F) Nuclear bud.

Treatments with soil contaminated with 2, 4, 8, 16, 32, 128, 256, 512, 1024 mg/kg of atrazine induced a higher frequency of chromosomal adhesions than the control soil. Soils contaminated with 2, 4, 128, 256, 512, and 1024 mg/kg of atrazine induced a higher frequency of chromosomal bridges. Atrazine at 2, 4, 16, 32, 128, 512, and 1024 mg/kg induced a higher frequency of vagrant chromosomes compared to the control soil (Table 2).

Soil containing 8 mg/kg of atrazine induced a higher frequency of cell death. Treatments with soil contaminated with 4, 16, and 512 mg/kg of atrazine resulted in a higher frequency of nuclear buds compared to the control soil. Soil with 16 mg/kg of atrazine induced a higher frequency of micronuclei. Treatments with soil contaminated with 4, 64, and 1024 mg/kg of atrazine

induced a higher frequency of binucleated cells compared to the control soil (Table 2).

None of the concentrations of atrazine induced a higher frequency of chromosomal losses or chromosomal breaks than the control (Table 2).

**Table 2.** Frequency of chromosomal alterations induced by atrazine-contaminated soil in *A. cepa* cells

Atrazine (mg/kg)	CA	CB	VC	C-M	CD	CL	NB	MN	BC	B
0	0	0.1±0.31	0	0	0	0	0	0	0	0
2	12.8±6.9*	5.3±3.09*	4.6±4.3*	1.5±2.4 1	0.6±1.89	0.1±0.31	1.2±1.03	0	0.4±0.69	0
4	10.7±6.49*	3.4±2.11*	3.9±3.90*	0	10±14.49	0.1±0.31	2.6±2.31*	3.8±6.39	2.8±3.67*	0
8	7.3±4.52*	1±0.81	2±1.99	0	24.5±48.34*	0	0.3±0.67	0.1±0.31	0.4±0.69	0
16	14.9±6.11*	2.2±1.31	7.1±4.01*	0.5±0.7 0	1.2±3.79	0.3±0.48	2.5±2.22*	2.8±3.11*	1.1±1.85	0.3±0.67
32	10.6±3.71*	1.9±1.19	3.3±2.26*	0	0.3±0.94	0	0.1±0.31	0.2±0.42	0.1±0.31	0
64	4.4±2.59	1.2±1.13	1.2±1.31	0.7±1.1 5	20.9±34.47	0	0.2±0.42	1.4±2.45	1.4±1.17*	0.1±0.31
128	8.6±4.52*	2.7±1.88*	3.7±1.76*	0.1±0.3 1	0	0.2±0.63	0	0.9±1.28	0.3±0.48	0.1±0.31
256	11.2±9.10*	3.3±2.45*	2.8±2.44	0.2±0.6 3	4.9±13.51	0.1±0.31	0.6±1.34	1.4±2.17	1.8±2.85	0
512	14±9.01*	5.8±4.34*	6.8±2.97*	3.8±3.9 9*	3.8±7.56	0	2.4±1.42*	1±0.94	1.1±1.28*	0.2±0.42
1024	8.4±4.14*	8.3±5.12*	7±2.82*	6.8±5.6 7*	4.49±15.49	0.6±1.26	1.7±2.75	0.6±1.26	6.4±9.37	0.4±0.84

**Note:** CA: chromosomal adhesion. CB: chromosomal bridge. VC: vagrant chromosome. C-M: c-metaphase. CD: cell death. CL: chromosomal loss. NB: nuclear bud. MN: micronuclei. BC: binucleated cells. B: chromosomal break. \* Denotes statistical difference in relation to the control ( $p < 0.05$ ).

## DISCUSSION

The stability of *A. cepa* root cell membranes was analyzed using the Evans blue dye technique after exposure to atrazine-contaminated soil. Cytotoxic effects were observed at atrazine concentrations ranging from 16 to 512 mg/kg. The plasma membrane plays a crucial role in a cell's ability to withstand changes in environmental conditions and can, therefore, be used as a biomarker to assess damage induced by xenobiotics (Vijayaraghavareddy *et al.*, 2017).

The Evans blue dye is capable of penetrating broken or destabilized cellular membranes. Thus, when plant cells are exposed to agents that affect membrane integrity, an increase in the number of cells absorbing the dye is observed, compared to non-exposed cells. In

contrast, cells with intact membranes do not absorb Evans blue (Ghosh *et al.*, 2015; Vijayaraghavareddy *et al.*, 2017).

Membrane damage results primarily from lipid peroxidation caused by the production of reactive oxygen species (ROS). In fact, atrazine is a of photosystem II inhibitor. The primary target of atrazine in plants is the Qb protein. By binding to this protein, atrazine inhibits electron transfer in photosystem II, leading to an increased production of ROS, primarily singlet oxygen. The accumulation of ROS results in cellular damage, compromising cell integrity and membrane function through lipid peroxidation and protein oxidation (Ivanov *et al.*, 2005; Roman *et al.*, 2005; Erinle *et al.*, 2016). In this sense, Bibi *et al.* (2019) demonstrated that membrane damage caused by atrazine results primarily from lipid

peroxidation induced by the production of ROS, which led to reduced viability in root and leaf cells of *Zea mays* (corn).

Cells under stress conditions can adapt. High concentrations of atrazine could activate antioxidant defense mechanisms, reducing toxic effects on the membrane. This could be a possible explanation for the result obtained at the concentration of 1024 mg/kg.

The cytogenotoxic activity of atrazine has been also attributed to ROS, which can cause significant damage to various macromolecules, including DNA and RNA (Song *et al.*, 2009). In this study, the analysis of the mitotic index showed that only the soil contaminated with 8 mg/kg of atrazine was mitodepressive. The mitotic index provides indirect information about the checkpoints of the cell cycle necessary to maintain genetic material integrity during cell division. It is a parameter used to measure xenobiotic cytotoxicity, and its decline can be related to genotoxicity, as it results from delays in the G1/S or G2/M transition due to genetic material damage or failures in the mitotic machinery necessary for cell division (Graña, 2018).

Atrazine also modified the phase index. The cell cycle advancement depends on the successful completion of each previous step. An increase in the proportion of cells in a specific phase suggests that they are taking longer than usual to complete it and progress. Therefore, the elevated percentage of cells in prophase indicates a blockage at this stage, possibly due to DNA repair failures or inhibition of tubulin polymerization, preventing mitotic spindle formation (Türkoglu, 2012; Grondona *et al.*, 2018; Souza *et al.*, 2023). The increase in cells in prophase and the subsequent decrease in cells in later stages of the cell cycle have been previously reported as an effect of atrazine in the root meristem cells of *A. cepa* and *Vicia faba* (El-Ghamery *et al.*, 2000; Souza *et al.*, 2023).

Cells damaged by genotoxic agents and not repaired can be observed under microscopy. Soil contaminated with atrazine resulted in a high frequency of aberrant cells. Among all treatments, chromosomal adhesion was the most frequently observed alteration. Metaphases with chromosomal adhesion and c-metaphases are related to aneugenicity due to dysfunctions in chromosomal condensation and mitotic spindle formation (Fiskesjö, 1985; Felisbino *et al.*, 2018). These alterations halt the cell cycle and lead to cell death (Fiskesjö, 1985). The high frequency of these alterations may explain the decline of cells in anaphase, an effect induced by most of the treatments.

Binucleated cells and chromosomal losses also suggest aneugenicity, as they result from cytokinesis inhibition (Bonciu *et al.*, 2018) and failures in mitotic spindle formation and/or its connection with kinetochore

proteins, respectively. Meanwhile, vagrant chromosomes move faster than the rest of the chromosomal set toward the cellular pole and lead to an unequal distribution of chromosomes between the daughter cells (Sabeen *et al.*, 2020; Macar *et al.*, 2023).

The formation of anaphase-telophase bridges can derive from terminal chromosomal breaks, followed by chromosomal adhesion (El-Ghamery *et al.*, 2000; Fioresi *et al.*, 2020). Additionally, chromosomal bridges may result from dysfunctions in telomeric proteins. As the cell cycle progresses, these bridges tend to generate chromosomal breaks, an indicator of clastogenic effects (Fiskesjö, 1985) and micronuclei, which originate from damage, breaks, and chromosomal losses induced in parent cells (Fioresi *et al.*, 2020). Nuclear buds result from amplified genetic material or chromosomal breaks and losses, similar to micronuclei (Lindberg *et al.*, 2007).

Although intermediate atrazine concentrations induced reductions in mitotic and phase indices and increased cell death (measured by Evans blue technique), the highest concentrations (512 and 1024 mg/kg) did not demonstrate these cytotoxic markers. However, they did lead to a significant increase in chromosomal abnormalities. This apparent discrepancy may reflect a non-monotonic response, as reported in other studies with atrazine (Brodeur *et al.*, 2013; Marcus & Fiumera, 2016). This means that the effect of atrazine does not consistently increase with increasing concentration; instead, the relationship can be non-linear, with effects observed at low concentrations that differ from those at higher concentrations (Brodeur *et al.*, 2013; Marcus & Fiumera, 2016). At high concentrations, cells may fail to activate adequate checkpoint responses or repair mechanisms, allowing damaged cells to continue through mitosis, which leads to genotoxic manifestation without necessarily reducing cell proliferation or viability at the population level. This could also indicate a shift from cytostatic to genotoxic predominance of the effect, with cells no longer halting division despite DNA or spindle damage.

Some studies have reported the toxic and cytogenotoxic effects of atrazine in various test systems (Cavas, 2011; Mela *et al.*, 2013; Lammertyn *et al.*, 2021; Souza *et al.*, 2023). Specifically, in plant studies, it has been reported that atrazine causes a decline in the mitotic index and an increase in the frequency of mitotic and chromosomal aberrations in *A. cepa* (El-Ghamery *et al.*, 2000; Srisvatava & Mishra, 2009; Ventura-Camargo & Marin-Morales, 2016; Felisbino *et al.*, 2018; Souza *et al.*, 2023), *V. faba* (El-Ghamery *et al.*, 2000; Srisvatava & Mishra, 2009), and *Fagopyrum esculentum* (Kumar & Srivastava, 2015), corroborating our data. Additionally, El-Ghamery *et al.* (2000) showed that atrazine reduced DNA and RNA content in the root meristem cells of *A.*

*cepa* and *V. faba*. Souza *et al.* (2023) reported that the herbicide altered the distribution of cells in the G1, S, and G2 phases and caused cell death in *A. cepa*. Bibi *et al.* (2019) reported that atrazine adversely affected the germination and growth of *Z. mays* seedlings by inducing oxidative stress, which causes increased cell damage and decreased cell viability, as well as impairing the concentration of light-harvesting pigments. In these studies, the exposure method used was direct contact, meaning the plants, bulbs or seeds, were exposed to aqueous solutions of atrazine (El-Ghamery *et al.*, 2000; Ventura-Camargo & Marin-Morales, 2016; Souza *et al.*, 2023), or sand was used as a substrate (Bibi *et al.*, 2019).

There are few studies that have examined the exposure of plants to atrazine in soil. In this sense, Song *et al.* (2014) investigated the effects of atrazine-contaminated soil on the activity of antioxidative enzymes - superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POD) - in *V. faba* roots. The study found that SOD activity was stimulated at a concentration of 2.5 mg/kg but inhibited at 5 and 10 mg/kg. Additionally, CAT and POD activities were generally suppressed at 10 mg/kg. DNA damage, assessed through the comet assay, increased following exposure to different atrazine concentrations (2.5, 5 and 10 mg/kg of soil) over time (7, 14, 21 and 28 days). However, the study did not present or discuss the characteristics of the soil used in the experiments.

The behavior of herbicides in the soil is influenced by both the physicochemical properties of the active ingredient and the physical, chemical, and mineralogical characteristics of the soil, particularly texture, pH, and organic matter. These factors play a crucial role in determining the mobility of the pesticide, its bioavailability, and toxicity (Roman *et al.*, 2005; Christofolletti *et al.*, 2009; Bandeira *et al.*, 2020).

Medium-textured soils, such as loamy sand-clay soils employed in this study, have a balanced composition of sand, silt, and clay. They combine some of the best properties of sandy soils (good drainage and aeration) and clayey soils (water and nutrient retention capacity), making them highly favorable for agriculture. Due to the significant sand content, these soils tend to have higher permeability, facilitating the leaching of atrazine into deeper layers, especially in areas with intense rainfall (Carvalho *et al.*, 2024).

The soil analyzed in this study had a moderate organic matter content (26.89 g/kg) and pH of 5.46 (moderate acidity), that is ideal for most crops (Prezotti & Guarçoni, 2013). The soil adsorption properties of photosystem inhibitors depend on soil pH and organic matter content. Atrazine adsorption increases with soil acidity and organic matter levels (Cerqueira *et al.*, 2005; Roman *et al.*, 2005). Additionally, if the soil has low

organic matter content, degradation may be slower (De Marco *et al.*, 1992; Roman *et al.*, 2005), therefore the toxicity is increased. In this sense, De Simone *et al.* (1992) demonstrated that no significant increase in micronucleated cells was observed in *V. faba* plants grown in forest soil with high organic matter content. In contrast, atrazine induced micronuclei only in the root tips of plants grown in soil low in organic matter (agricultural soil).

Concerning the soil acidity, soils with a pH below 7, atrazine can absorb hydrogen ions from the soil solution, acquiring a positive charge. This positive charge enhances the attraction between the atrazine molecule and the negatively charged soil colloids, leading to stronger adsorption (Manuchehri & Arnall, 2018).

In addition to these key parameters, several other physical and chemical properties were analyzed and presented in Table 1, including macro- and micronutrients (e.g., Ca, Mg, K, Fe, Mn, Zn). These elements are commonly assessed to determine soil fertility, as they are essential for plant growth and metabolism. We compared the macro and micronutrients data obtained with literature reference (Prezotte & Guarçoni, 2013). Since no nutrient imbalances were identified, that would indicate secondary stress, these parameters were not further explored in the discussion, as they are not expected to interfere with the herbicide's bioavailability or cytogenotoxic effects in *Allium cepa*.

In summary, the results indicate a moderate to low capacity for atrazine adsorption in the studied soil, which favors its bioavailability and, consequently, its toxicity and cytogenotoxicity.

Although the focus of this study was on atrazine as the active ingredient, it is important to note that the commercial product used contains approximately 60% of other, unspecified components. These so-called inert ingredients (often surfactants, solvents, or adjuvants) are typically not disclosed by the manufacturer, which limits our ability to evaluate their individual contributions to the observed effects. However, their presence may influence the toxicity profile of the formulation, potentially enhancing the bioavailability, uptake, or cellular impact of pesticides (Rodrigues *et al.*, 2019).

Despite this limitation, the use of the commercial formulation in ecotoxicological research is highly relevant, as it more accurately reflects real-world exposure scenarios encountered in agricultural environments. Farmers apply formulations, not pure active ingredients, and thus, assessing the biological effects of these mixtures provides ecologically meaningful data (Renaud *et al.*, 2018; Bandeira *et al.*, 2020). This approach aligns with growing concerns in environmental toxicology regarding the underestimated toxicity of co-formulants and the need for regulatory

assessments to consider whole formulations rather than isolated compounds.

We acknowledge this limitation in our interpretation and highlight the need for future studies that compare the effects of commercial formulations with those of technical-grade atrazine to disentangle better to disentangle the contributions of individual components better.

### CONCLUSION

The results of this study demonstrate that exposure to soil contaminated with atrazine caused significant cytotoxic and genotoxic effects in *A. cepa*, as evidenced by the reduction in the mitotic index, increased frequency of chromosomal aberrations, and decreased cell viability. The genotoxic effects of atrazine on plants have significant ecological implications. As plants form the foundation of terrestrial ecosystems, genetic alterations induced by atrazine can affect plant diversity, reproduction, and resilience. These results, highlighting the importance of mechanisms that can effectively retain and treat effluents with high concentrations of atrazine, which are disposed of in the soil, thereby reducing their adverse effects on plants and the environment as a whole.

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