RESEARCH ARTICLE

Environmentally realistic concentrations of eprinomectin induce phytotoxic and genotoxic effects in *Allium cepa*

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Abstract

Eprinomectin, a veterinary drug within the family of avermectins, is widely used in the agricultural sector to combat a variety of parasites, mainly nematodes. However, only 10% of the drug is metabolized in the organism, so large quantities of the drug are released into the environment through urine and/or feces. Soil is the first and main environmental compartment to be contaminated by it, and nontargeted organisms can be affected. Thus, the present study aims to evaluate the phytotoxicity (through the evaluation of germination, root development, and germination speed) and genotoxicity (through an assessment of the induction of micronuclei and chromosomal aberrations) of eprinomectin. For the analyses, *Allium cepa* seeds were germinated in soil contaminated with a range of concentrations of eprinomectin: from 0.5 to 62.5 µg/g for the genotoxicity test and from 0.5 to 128.0 µg/g for the phytotoxicity test. The results showed that seed germination was not affected, but root development was affected at concentrations of 0.5 µg/g, 1.0 µg/g, 4.0 µg/g, 8.0 µg/g, 64.0 µg/g, and 128.0 µg/g, and germination speed was significantly changed at concentrations of 1.0 µg/g, 4.0 µg/g, 16.0 µg/g, 32.0 µg/g, and 64.0 µg/g. Significant differences in the mitotic index and genotoxicity index were observed only at concentrations of 2.5 µg/g and 12.5 µg/g, respectively. Only the 0.5 µg/g concentration did not show significant induction of micronuclei in the meristematic cells, but the damage observed at other concentrations did not persist in F_1 cells. According to the results, eprinomectin is both phytotoxic and genotoxic, so the release of eprinomectin into the environment should be minimized.

Introduction

Antiparasitic drugs such as avermectin have been widely used in veterinary medicine to combat both ecto- and endo-parasites (Seelanan et al. 2006), and their antiviral activity against a broad range of viruses, including dengue (Tay et al. 2013) and SARS-CoV-2 (Caly et al., 2012), has been studied. The avermectin family comprises a complex of chemically related agents produced by the actinomycete *Streptomyces avermitilis* and includes a variety of drugs, such as abamectin, doramectin, ivermectin, emamectin, and eprinomectin (Burg et al. 1979). Among these, abamectin and ivermectin have been used the longest. The most recently developed drug, eprinomectin, has shown satisfactory results at low doses (Giannetti et al., 2011).

Eprinomectin (4″-epi-acetylamino-4″-deoxy-avermectin B1) was developed to reduce the risk to human health in the mid-1990s by Shoop et al. (1996). It can be used to treat livestock at any stage of life and has much lower levels of milk residues than other avermectins. However, similar to other medicines, only a small percentage of the eprinomectin administered is metabolized by animals. Approximately, 90% is released into the environment through feces or urine without modification (Merck 1996). As a result, the drug has been found in soil, the initial environmental compartment affected, and in surface and groundwater (Jensen et al. 2003; Kolar et al. 2008).

Pharmaceutical drugs are an important group of chemical contaminants since they are developed to produce biological

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effects at low doses. Due to the high level of conservation of features within vertebrate taxa, both human and veterinary medicines may produce effects in nontargeted organisms (Celander et al. 2011). Therefore, it is important to evaluate the effects of these substances on the environment to determine if their presence will affect organisms living within that ecosystem. Eprinomectin, as a recently developed drug, has not been thoroughly studied in regard to its possible effects on nontargeted organisms.

The concentration of avermectins in environmental compartments initially depends on the method of application, the dose applied, the frequency of dosing, and the number of treated animals in a given area (Kovecses and Marcogliese 2005). According to Oliveira-Ferreira et al. (2016), these drugs can be found in the soil in the µg/g range. When a single animal is treated with a recommended dose of topically formulated avermectin, concentrations of 18.5 µg/g dry weight can be detected 2 days posttreatment on a single dung pile, and even after 28 days, avermectin is still being excreted (Herd, 1996). Weather conditions are one of the key driving factors involved in avermectin degradation (Erzen et al., 2005), and consequently, the half-life of drugs in the avermectin family varies significantly under different field conditions (Bai and Ogbourne 2016). According to Kovecses and Marcogliese (2005), the half-life of avermectins in soil can vary from 91–217 days outdoors in winter to 7–14 days outdoors in summer. Regarding eprinomectin, its half-life in soil is approximately 64 days, according to the Merck Company (1996). Avermectins can remain in the environment for a considerable period of time, and their high binding affinity for soils and other organic matter might result in their environmental accumulation (Halley et al. 1989), so it is important to assess a wide concentration range of avermectin that could be present in soil.

The use of higher plants, also known as vascular plants (Akeroyd and Synge 1992), in the assessment of environmental impact is widely recommended by international institutions such as the United Nations Environmental Program (UNEP), the World Health Organization (WHO), the US Environmental Protection Agency (USEPA), and the International Organization for Standardization (ISO 18763:2016). *Allium cepa* represents one of the main plants used in ecotoxicological tests since it can demonstrate the ability of different substances to alter DNA (Leme and Marin-Morales, 2009), germination, and the initial development of an organism (Fiskejö 1985). Notably, it has been used for the detection of contamination caused by pesticides (Souza et al. 2017; Verma and Srivastava 2018; Fioresi et al. 2020; Mota et al. 2022), medicinal drugs (Gupta et al. 2020; Das et al. 2021; Eckert et al. 2022), wastewater (Yadav et al. 2019; Furtado et al. 2020; Sharma et al. 2021), and sewage sludge (Sommaggio et al. 2018; Abreu-Junior et al. 2019; Moraes Cunha Gonçalves et al., 2020).

Ecotoxicological studies testing the effects of veterinary drugs on plants are scarce, and the few studies that have been reported described experiments that were performed in vitro. Furthermore, they did not involve the exposure of plants to contaminated soil and used concentrations unlikely to be found in the environment. In addition, studies conducted in vitro need to be verified, as bioavailability in soil can greatly differ from in vitro conditions (Jjemba 2002). According to these studies, avermectins do not negatively affect plants (Vokřál et al. 2019). Fully understanding the phytotoxicity of these compounds is important since plant sensitivity to drugs may reduce biodiversity in certain areas (Bártíková et al., 2016).

Thus, this study aimed to evaluate the phytotoxic and genotoxic effects of eprinomectin in vivo using the bioindicator organism *A. cepa* in the concentration range from 0.5 to 62.5 µg/g for the genotoxicity test and from 0.5 to 128.0 µg/g for the phytotoxicity test. Phytotoxic analyses were carried out using parameters such as the germination index, initial root development, and germination speed index. Genotoxic analyses were performed by analyzing chromosomal and nuclear aberrations and detecting the presence of micronuclei (MN) in cells within the meristematic region of plants and the presence of MN in F_1 cells.

Materials and methods

Materials

Seeds of *A. cepa* from the same batch, variety (Baia periform), without the addition of chemical agents, were acquired from the Isla Sementes Company. Eprinomectin (CAS 123997-26-2) at 96% purity was purchased from Sigma-Aldrich. The soil used in the present study was collected in the region of Campinas, São Paulo state, Brazil (22° 24′ 36″ S/47° 33′ 36″ W) and was characterized as oxisol. The soil physicochemical properties and the results of the granulometry analysis performed according to NBR7181 (2016) are summarized in Tables 1 and 2. The soil was dried at room temperature and sieved (mesh = 5 mm). The water-holding capacity (WHC) of the soil was calculated based on ISO 16387 (2013) and OECD 232 (2016); a tube with filter paper was filled with soil to a depth of 5 cm and placed on a rack in a water bath. The tube was gradually submerged until the water level rose above the top of the soil but below the upper edge of the tube. The soil sample was left in the water for approximately 3 h. Afterward, the tube containing the soil sample was placed for a period of 2 h on wet, finely ground quartz sand for draining. The soil sample was then weighed, dried at 105 °C, and reweighed. The following formula was used to calculate the WHC:

Table 1 Physicochemical analysis of soil sample used in the experiments

Parameters	Results	Error[1]	MAV[2] (mg/kg)	Method	Reference
Arsenic mg/kg	6.850	0.097	15.0	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Barium mg/kg	19.98	0.18	120	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Cadmium mg/kg	< 0.153	0.0023	1.3	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Calcium mg/kg	857	40	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Lead mg/kg	17.70	0.5	72	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Copper mg/kg	23.52	0.26	60	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Chromium mg/kg	24.94	0.29	75	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Magnesium mg/kg	377	19	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Mercury mg/kg	< 0.010	0.00033	0.5	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Molybdenum mg/kg	2.500	0.043	5	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Nickel mg/kg	5.820	0.074	30	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Organic matter (g/dm^3)	20		-	Ashing at 440 °C	NBR 13600: 1996
pH	5.4	0.76	-	Potentiometric	USEPA 9045D (2004)
Selenium mg/kg	< 0.255	0.016	1.2	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Sodium mg/kg	131.0	5.1	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Zinc mg/kg	24.87	0.48	86	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Nitric nitrogen mg/kg	225.2	2.7	-	Ion chromatography	USEPA 300.1 (1993)
Sulfur mg/kg	8.02	0.18	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Potassium mg/kg	308	12	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Ammoniacal nitrogen mg NH^-_3N/kg	62.3	1.4	-	Titulometric	-
Kjeldahl nitrogen mg NH^-_3N/kg	186	4	-	Titulometric	Baird et al. (2017)
Phosphorus mg/kg	871	42	-	Plasma spectrometry	USEPA 3050B (1996)/USEPA 6010C (2007)
Nitrite mg/kg	< 0.030	0.00059	-	Ion chromatography	USEPA 300.1 (1993)
Total organic carbon % p/p	7.82	-	-	-	-
Electrical conductivity dS/m	0.623	-	-	-	-

[1]Confidence level of 95%. [2]*MAV*, maximum allowed values according to CETESB 045/2014/E/C/I (2014)

$$WHC = \frac{m_S - m_T - m_D}{m_D} \times 100$$

where

WHC   is the water-holding capacity as a percentage of dry mass, %.

m_S is the combined mass of the water-saturated substrate, the tube, and the filter paper.

m_T is the tare (mass of tube plus mass of filter paper).

m_D is the dry mass of the substrate.

Table 2 Granulometry of the soil sample used in the experiments

Texture	Diameter(mm)	(%)
Clay	< 0.002	7.59
Silt	0.002–0.06	16.4899
Fine sand	0.06–0.2	74.3000
Medium sand	0.2–0.6	1.1400
Coarse sand	0.6–2.0	0.4400
Fine gravel	2.0–6.0	0.0000
Medium gravel	6–20	0.0000
Thick gravel	20–60	0.0000

The soil was then contaminated with a stock solution containing eprinomectin diluted in ethanol.

Experimental procedures

The soil was contaminated with increasing doses of eprinomectin that corresponded to concentrations in which the drug has been found within the environment. Soil contamination occurred 1 day prior to the experiments so that the solvent used to create the stock solution could completely evaporate. After being contaminated, the soil was homogenized and dried for 24 h in a fume hood.

For all of the experiments, a control treatment containing soil and solvent (SolvC) was carried out to compare it with the negative control (NC), which contained soil mixed with ultrapure water, to statistically analyze any significant differences. If no difference was observed, we concluded that the solvent did not affect the parameters tested. On the day of each experiment, soil moisture was adjusted using ultrapure water to maintain 70% of the maximum WHC, as recommended by the USEPA (1996).

Assessment of genotoxicity

Genotoxicity was analyzed using four different concentrations of eprinomectin ($C1_{Gen}$ = 0.5 µg/g, $C2_{Gen}$ = 2.5 µg/g, $C3_{Gen}$ = 12.5 µg/g, and $C4_{Gen}$ = 62.5 µg/g). NC and SolvC conditions were also included, as was a positive control (PC) which consisted of soil mixed with the herbicide trifluralin (Fernandes et al., 2007). Each treatment consisted of 90 g of dry soil distributed between 3 Petri dishes (30 g per dish), so 45 µg of eprinomectin was added to treatment C1, 225 µg of eprinomectin to treatment C2, 1124 µg to treatment C3, and 5625 µg to treatment C4. To achieve the desired concentration for each treatment, a stock solution containing 37.5 mL of solvent and 7020 µg (7.02 mg) of eprinomectin was made, and the stock solution (quantities listed below) was added to 90 g of soil. Each quantity of stock solution was diluted in solvent to allow

for the homogenization of the soil and, consequently, an effective dispersal of the eprinomectin into the soil sample.

- $C1_{Gen}$: 0.24 mL stock solution + 28.56 mL solvent = 45 µg of eprinomectin in 90 g of soil
- $C2_{Gen}$: 1.2 mL stock solution + 27.6 mL solvent = 225 µg of eprinomectin in 90 g of soil
- $C3_{Gen}$: 6.0 mL stock solution + 22.8 mL solvent = 1123 µg of eprinomectin in 90 g of soil
- $C4_{Gen}$: 28.8 mL stock solution + 0 mL solvent = 5500 µg of eprinomectin in 90 g of soil

After contamination, the soil was dried for 24 h to allow for the complete evaporation of solvent. On the day of the experiment, the soil of each treatment was moistened with 28.8 mL (70% WHC) of ultrapure water, and fifty seeds of *A. cepa* were randomly placed within each Petri dish (90 mm × 1.5 mm).

Petri dishes were then arranged randomly in a BOD incubator at 22 ± 2 °C with a 12:12 h light:dark photoperiod (Souza et al. 2017).

Preparation of the slides of A. cepa

After 5 days of exposure, the roots were collected and fixed in Carnoy 3:1 (ethanol:acetic acid, v/v) for 12 h. To prepare slides, roots were hydrolyzed in 1 M HCl at 60 °C for 10 min and then submitted to a Schiff reagent for 2 h (Mello and Vidal 1978). Both the meristematic region and the F_1 region were sectioned and arranged on slides. To enhance staining and improve cell spreading, a drop of 2% acetic carmine acid was added, and then the material was covered by a coverslip and lightly macerated. Coverslips were removed with liquid nitrogen, and the permanent slides were mounted with Entellan®. The slides were analyzed under an optical microscope at a magnification of 1000×. Fifteen slides were prepared per Petri dish, totaling 45 slides per treatment.

Evaluation of genotoxicity in meristematic cells of A. cepa

Genotoxicity in meristematic cells was evaluated according to Souza et al. (2017). Approximately, 15,000 meristematic cells were analyzed per treatment. Cytotoxicity was evaluated based on the mitotic index (MI) according to the formula:

$$\text{MI} = \frac{number\ of\ cells\ in\ division}{total\ number\ of\ cells\ observed} \times 100$$

Genotoxicity was analyzed based on the number of cells carrying chromosomal and nuclear aberrations, such as nuclear buds, anaphasic and telophasic bridges, and chromosomal losses and breaks. The genotoxicity index (GenI) was calculated using the formula:

$$GenI = \frac{number\ of\ cells\ with\ chromosomal\ aberrations\ +\ nuclear\ buds}{number\ of\ cells\ observed} \times 100$$

The frequency of micronuclei (FMN) was determined by assessing the number of cells containing MN:

$$FMN = \frac{number\ of\ cells\ with\ MN}{number\ of\ cells\ observed} \times 100$$

The presence of MN can be used as a biomarker for genotoxicity and chromosomal instability-related events. When this damage is not repaired, it has the capacity to produce mutagenic effects.

Evaluation of cells with micronuclei in the F_1 region of A. cepa

Approximately, 15,000 cells from the F_1 region of each treatment were analyzed to confirm findings indicating that eprinomectin has the capacity to produce mutagenic effects. The number of cells carrying MN was counted, and the FMN was determined.

Assessment of phytotoxicity

For the evaluation of phytotoxicity, nine different concentrations of eprinomectin were used: $C1_{Phyto}$ = 0.5 μg/g, $C2_{Phyto}$ = 1.0 μg/g, $C3_{Phyto}$ = 2.0 μg/g, $C4_{Phyto}$ = 4.0 μg/g, $C5_{Phyto}$ = 8.0 μg/g, $C6_{Phyto}$ = 16.0 μg/g, $C7_{Phyto}$ = 32.0 μg/g, $C8_{Phyto}$ = 64.0 μg/g, and $C9_{Phyto}$ = 128.0 μg/g.

Each treatment consisted of 150 g of dry soil divided into 5 Petri dishes (30 g per dish). Soil contamination was carried out in the same way as for the genotoxicity test but adjusted to 150 g of soil.

The NC consisted of soil and ultrapure water, and the PC contained a mixture of soil and zinc sulfate heptahydrate (0.05 M). A SolvC condition was also performed. Thirty seeds of *A. cepa* were randomly placed in each Petri dish (90 mm × 1.5 mm), and then, the dishes were arranged in a BOD incubator at 22 ± 2 °C for 96 h (Palmieri et al. 2014). After periods of 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 h, the number of germinated seeds was assessed.

To calculate the germination speed index (GSI), the following formula was used:

$$GSI = (N_1 \times 1) + (N_2 - N_1) \times \tfrac{1}{2} + (N_3 - N_2) \times \tfrac{1}{3} + (N_y - (N_{y-1})) \times \tfrac{1}{y}$$

where Ny is the number of seeds germinated in a given period (Palmieri et al. 2014). After 96 h, root lengths were measured to determine initial development.

The percentage of germinated seeds was also calculated:

$$\% = \frac{number\ of\ germinated\ seeds}{total\ of\ seeds\ distributed} \times 100$$

Statistical analyses

Statistical analyses were performed using the IBM SPSS Statistics 22 program. For each analysis, normality and homogeneity tests were performed using the Shapiro–Wilk test and Levene test, respectively. When the data showed a normal and homogeneous distribution, parametric tests (*t*-tests) were performed at a probability level of 5% ($p < 0.05$); data with nonnormal and nonhomogeneous distributions were assessed using a nonparametric test (Kruskal–Wallis/Dunn test) at a probability level of 5%.

Results

Phytotoxicity

According to a statistical assessment of the data, all phytotoxic parameters were homogeneously and normally distributed, and none differed significantly in the NC and SolvC conditions.

The germination rate remained constant throughout all treatment groups, differing significantly only in regard to the PC ($p = 0.000$) (Table 3). Initial root development was affected at most concentrations of eprinomectin when compared with NC. Only treatments $C3_{Phyto}$, $C6_{Phyto}$, and $C7_{Phyto}$ did not show significant reductions in relation to the NC. Seeds of the PC did not reach a sufficient length to be measured (Table 3).

Table 3 Mean values and standard deviations for phytotoxicity parameters analyzed in *A. cepa* roots after exposure to eprinomectin

Treatments	Germinated seeds(%)	GSI	Root growth(cm)
NC	94.0 ± 0.4	5.5 ± 0.36	1.66 ± 0.51
SolvC	93.0 ± 0.0	5.2 ± 0.39	1.57 ± 0.43
PC	40.0 ± 3.9*	1.3 ± 0.46*	0.00
C1$_{Phyto}$	92.5 ± 2.5	5.2 ± 0.84	1.47 ± 0.40*
C2$_{Phyto}$	91.0 ± 1.1	5.0 ± 0.22*	1.51 ± 0.47*
C3$_{Phyto}$	96.5 ± 1.2	5.3 ± 0.16	1.55 ± 0.49
C4$_{Phyto}$	96.0 ± 0.8	4.6 ± 0.25*	1.41 ± 0.38*
C5$_{Phyto}$	92.5 ± 1.3	4.9 ± 0.50	1.49 ± 0.44*
C6$_{Phyto}$	89.0 ± 2.4	4.9 ± 0.28*	1.47 ± 0.55
C7$_{Phyto}$	89.0 ± 2.3	4.7 ± 0.35*	1.58 ± 0.45
C8$_{Phyto}$	89.0 ± 1.6	4.7 ± 0.35*	1.48 ± 0.39*
C9$_{Phyto}$	94.0 ± 0.8	5.1 ± 0.24	1.39 ± 0.35*

GSI, germination speed index; *NC*, negative control; *SolvC*, solvent control; *PC*, positive control; *C1*, 0.5 μg/g; *C2*, 1.0 μg/g; *C3*, 2.0 μg/g; *C4*, 4.0 μg/g; *C5*, 8.0 μg/g; *C6*, 16.0 μg/g; *C7*, 32 μg/g; *C8*, 64 μg/g; *C9*, 128 μg/g. **p* < 0.05

The majority of the treatments caused delayed germination of seeds when compared to the NC. Significant differences in the speed of germination relative to the NC were observed for the PC ($p = 0.000$), C2$_{Phyto}$ ($p = 0.027$), C4$_{Phyto}$ ($p = 0.003$), C6$_{Phyto}$ ($p = 0.027$), C7$_{Phyto}$ ($p = 0.012$), and C8$_{Phyto}$ ($p = 0.010$) treatment groups (Table 3).

Genotoxicity

According to the Levene and Shapiro–Wilk tests, the assessment of GenI and FMN (both meristematic and F$_1$ regions) produced data that demonstrated nonnormal and nonhomogeneous distributions. Assessment of MI produced data that were characterized as having a normal and homogeneous distribution. There was no statistically significant difference between NC and SolvC for any of the genotoxicity parameters analyzed.

Regarding meristematic cells, a significant decrease in MI was observed only for treatment C2$_{Gen}$ ($p = 0.012$) (Table 4). Analyses of chromosomal and nuclear aberrations (GenI) revealed that the C3$_{Gen}$ ($p = 0.042$) group was the only one which showed statistically significant damage (Table 4), such as chromosomal loss (Fig. 1A), chromosomal breakage (Fig. 1B), and nuclear bud (Fig. 1C). Induction of MN (FMN) (Fig. 1D) was significant regarding treatments C2$_{Gen}$ ($p = 0.012$), C3$_{Gen}$ ($p = 0.037$), and C4$_{Gen}$ ($p = 0.035$) (Table 4).

An assessment of the FMN of the cells of the F$_1$ region revealed no significant increase in MN formation (Table 4).

Discussion

The use of *A. cepa* as a bioindicator organism allows for the analysis of several parameters to determine whether different substances released into the environment produce toxic effects. The tests allow researchers to determine whether a substance is toxic through the assessment of seed germination and root growth (Palmieri et al. 2014) and, at the cellular level, allow for the evaluation of the ability of toxic substances to interact and damage DNA (Leme and Marin-Morales, 2009).

Parameters such as seed germination and root growth, used to determine phytotoxicity, have been gaining popularity in recent years (Ruttkay-Nedecky et al., 2017; Pinho et al. 2017; Delerue et al. 2019; Garcia et al. 2019). The inhibition of seed germination is considered a lethal effect if it is a result of embryo death (Sobrero and Ronco 2004), while root length analysis is considered a sensitive indicator for plant development (Ratsch and Johndro 1986). A number of studies have been carried out using these parameters for the evaluation of several potentially toxic substances, such as agro-industrial waste (Luo et al. 2018), treated water (Priac et al. 2017), and metals (Gharebaghi et al. 2017); however, data of this nature are limited or nonexistent regarding the use of avermectins.

Previous reports have shown that avermectins do not have a negative effect on plants (Dybas 1989; Bloom and Matheson, 1993; Halley et al. 1993), but detailed parameters used to reach such a conclusion were not provided by these studies. Moreover, previous reports have also shown that the penetration of these substances into plant bodies, either directly through the leaf surface or through absorption from contaminated soils, was insignificant because it was believed that avermectins were rapidly photodegraded (Halley et al. 1993; Mckellar 1997). However, a recent study concluded that plants grown close to feces contaminated with ivermectin had a high internal concentration of the drug, and, therefore, the capacity of plants to absorb significant quantities of these drugs was demonstrated (Iglesias et al. 2018).

According to our data, eprinomectin is not able to make the embryo nonviable, since all concentrations of the eprinomectin treatment germinated at levels similar to the NC. However, the eprinomectin treatment did alter the initial development and root growth of the species since a reduction in the lengths of roots of treated plants was observable even at low concentrations of the drug. The results herein regarding the phytotoxic effects of

Table 4 Mean values and standard deviations for genotoxicity parameters analyzed in *A. cepa* roots after exposure to eprinomectin

Treatments	MI	GenI	MNF	
			Meristematic cells	F$_1$ cells
NC	7.71 ± 3.27	0.09 ± 0.23	0.22 ± 0.35	0.42 ± 0.46
SolvC	6.40 ± 3.20	0.07 ± 0.25	0.18 ± 0.48	0.54 ± 0.67
CP	7.54 ± 3.98	2.25 ± 2.12*	2.65 ± 2.28*	1.41 ± 1.25
C1$_{Gen}$	6.89 ± 3.12	0.16 ± 0.28	0.31 ± 0.70	0.43 ± 0.45
C2$_{Gen}$	5.91 ± 3.34*	0.09 ± 0.18	0.45 ± 0.47*	0.66 ± 0.87
C3$_{Gen}$	6.59 ± 3.66	0.16 ± 0.21*	0.46 ± 0.60*	0.58 ± 0.93
C4$_{Gen}$	7.43 ± 3.36	0.08 ± 0.17	0.45 ± 0.54*	0.62 ± 0.68

MI, mitotic index; *GenI*, genotoxicity index; *MNF*, micronuclei frequency; *NC*, negative control; *SolvC*, solvent control; *PC*, positive control; *C1*, 0.5 µg/g; *C2*, 2.5 µg/g; *C3*, 12.5 µg/g; *C4*, 62.5 µg/g. *$p < 0.05$
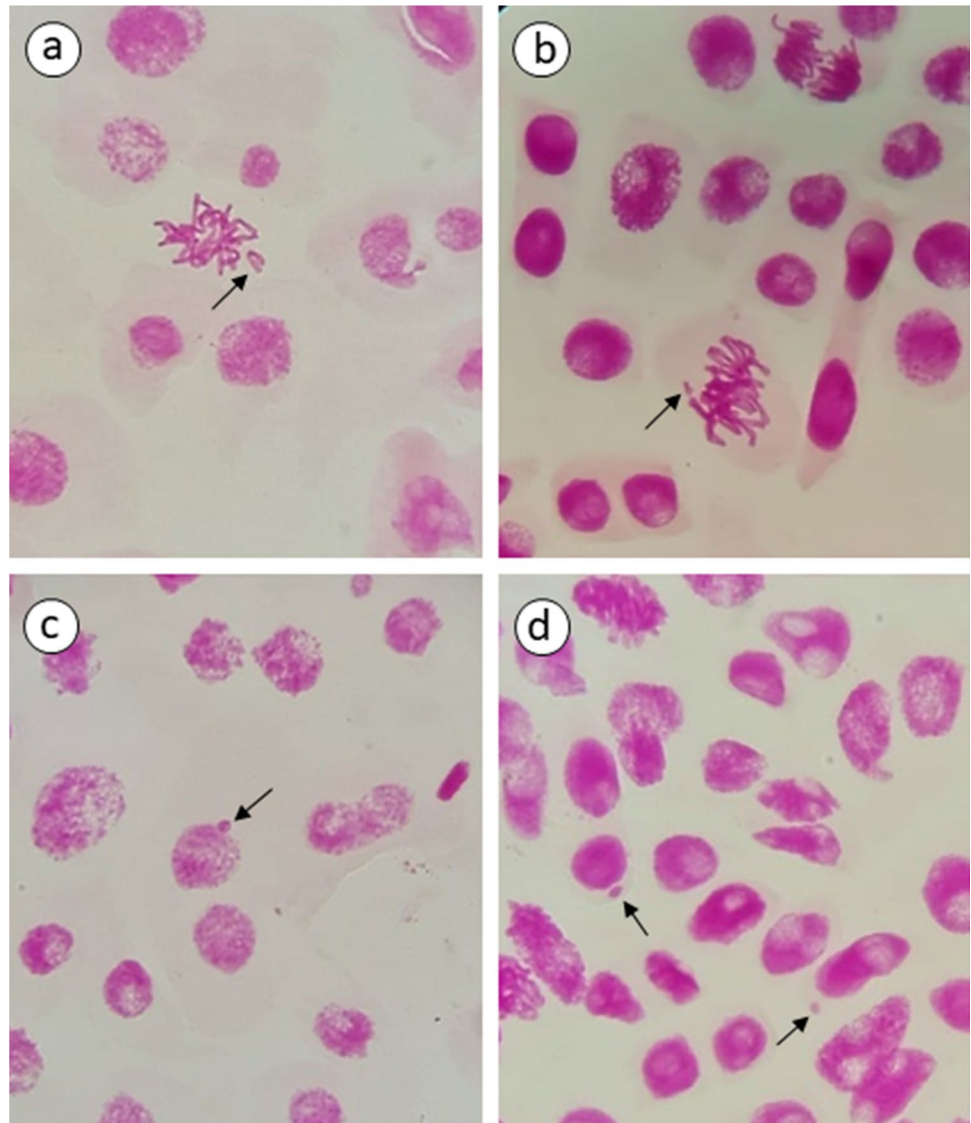
eprinomectin corroborate the results determined by Vokřál et al. (2019) and Ahmed (2014) for other avermectins. No effect on seed germination was observed in *Sinapis alba* after exposure to ivermectin (Vokřál et al., 2019) or in *A. cepa* after exposure to a mixture of abamectin and emamectin benzoate. Root growth, however, was reduced even at low concentrations in *S. alba* and in concentrations ranging from 0.6 to 1.0 mL/L in *A. cepa*.

Although eprinomectin did not completely inhibit seed germination, the germination process was affected by delayed root development, as demonstrated by the reduction in germination speed. Unlike other xenobiotics, there is no information in the scientific literature concerning the action of avermectins in the physiology of plants. For example, it has previously been established that heavy metals can alter the distribution of auxin, a phytohormone responsible for several aspects of plant growth and development (Bücker-Neto et al. 2017). But, for avermectins, further studies must

be performed to clarify their mechanisms of action on plant cells. Regarding the phytotoxicity of eprinomectin, lethal effects were not observed, but the drug affected root development and the speed of germination, which demonstrated its significant potential to harm the species.

The decreased root growth and delayed germination speed caused by eprinomectin may be due to its potential mito-depressive effects, as demonstrated by assessment of the MI. In our experiments, MI was reduced after treatment with all concentrations of eprinomectin, and the reduction was statistically significant in C2_Gen. This parameter is used as an indicator of cytotoxicity and can be determined by the increase or decrease in the number of cells in the division process. Thus, a reduction in MI relative to the control indicates that differences are derived from the chemical effect of the substance, which results in the inhibition of the growth and development of the organism (Leme and Marin-Morales

2009). Mito-depressive effects have already been reported regarding the reduced elongation of *A. cepa* roots exposed to the herbicide imazethapyr (Liman et al. 2015), metals (Gupta et al. 2018), and mining waste (Andrade-Vieira et al., 2017).

Along with MI analysis, the *A. cepa* test allows for the evaluation of other cellular parameters, such as the formation of chromosomal and nuclear aberrations in meristematic cells and MN induction, both in meristematic and F_1 cells (Leme and Marin-Morales 2009). In meristematic cells, chromosomal aberrations demonstrate the genotoxic effects of substances, whereas MN induction is an indicator of chromosomal instability (Souza et al. 2017).

A significant increase in the number of aberrations was observed posttreatment with $C3_{Gen}$ within the meristematic cells. The principal effects included the formation of chromosome losses (Fig. 1A), chromosomal breakage (Fig. 1B), and nuclear buds (Fig. 1C). According to Fernandes et al. (2007), the presence of nuclear buds occurs as a result of the elimination of excessive genetic material derived from the polyploidization process. Chromosome losses indicate that eprinomectin may also act on the mitotic spindle of the cell. During cell division, an interruption or malformation of the mitotic spindle may produce inappropriate chromosome segregation when daughter cells are formed (Andrade-Vieira et al. 2012; Freitas et al. 2016), resulting in chromosome loss and other abnormalities, such as lagging chromosomes, chromosome stickiness, and disturbed anaphases (Tkalec et al. 2009).

The formation of MN, which can occur spontaneously or due to exposure to contaminants, is strongly related to the occurrence of lost and broken chromosomes (Heddle et al. 1983; Ma and Xu 1986). Since our data demonstrated both of these types of chromosomal aberrations, the induction of MN was expected, and it was observed significantly within the meristematic cells for treatments $C2_{Gen}$, $C3_{Gen}$, and $C4_{Gen}$ (Fig. 1D).

However, in cells from the F_1 region, the presence of MN was not significantly different. The analysis of the cells of this region is important because it allows for the evaluation of whether damage present in cells of the meristematic region was repaired and therefore not transferred to daughter cells. According to Ma et al. (1995), after mitotic division within the meristematic region, DNA damage from the region could be visualized as MN in the F_1 region. As there was no significant MN increase in this region, these results suggest that the cell repair system was able to neutralize the damage caused by eprinomectin. To confirm this evidence, recovery tests should be conducted in future studies.

The mechanism of action of avermectin in plant cells remains unknown, but our results indicate that eprinomectin may interact with DNA, causing nuclear abnormalities and chromosomal breaks. Additionally, eprinomectin may act on cellular components required for cell division, which could cause chromosomal losses. Based on these results, eprinomectin acts as a clastogenic

and aneugenic compound. The clastogenic effects are related to chromosomal breaks, and the aneugenic mode of action occurs as a consequence of failures in chromosomal attachment to the mitotic spindle (Leme and Marin-Morales 2009), herein observed as the loss of chromosomes.

Clastogenic alterations can be explained by the increased level of intracellular reactive oxygen species (ROS) generated by avermectins (Li et al. 2013; Zhang et al. 2017). When produced in abundance, ROS can lead to oxidative stress, which results in DNA damage (Schins and Knaapen 2007). According to Yi et al. (2007), ROS can also damage purine and pyrimidine bases and the deoxyribose unit in DNA, generating chromosomal breaks.

Regarding aneugenic action, there is some evidence that suggests that ivermectin can disrupt spindle formation in invertebrate organisms. In *Caenorhabditis elegans*, ivermectin can alter the expression of dyneins and kinesins, two proteins that play important roles in mitosis and meiosis, since they are associated with meiotic and mitotic spindle poles (Ballesteros et al. 2016); in *Onchocerca volvulus* and *Haemonchus contortus*, ivermectin changes the frequency of β-tubulin alleles (Eng et al. 2006), a structural protein of microtubules (Downing and Nogales 1998). Additionally, for *H. contortus*, ivermectin can bind to and alter the tubulin polymerization equilibrium, which can lead to mitotic arrest, as demonstrated by Ashraf et al. (2015). The aneugenic action of eprinomectin can also explain the reduction in MI, since mito-depressive effects may be related to the inhibition of microtubule formation or arresting of the 24-h cycle of *A. cepa* at the G1 and G2 phases, impaired nucleoprotein synthesis, and reduced levels of ATP that provide energy for spindle elongation, microtubule dynamics, and chromosomal movement (Türkoğlu 2012).

Studies testing the effects of avermectins on cells of vertebrate and invertebrate organisms have already shown that these substances may interact with DNA, corroborating our results. In these studies, low concentrations of avermectins were also responsible for damaging genetic material, as observed by Shen et al. (2011) and Al-Sarar et al. (2015), in silkworm hemocytes and in cell culture (CHO_{k1}), respectively. In bovine peripheral lymphocytes, Anchordoquy et al. (2019) observed an increased presence of MN and level of nuclear buds after treatment with 20 ng/mL, 40 ng/mL, and 60 ng/mL doramectin. The production of effects at low concentrations of these compounds is expected since veterinary drugs are designed to produce biological effects at low doses (Arnold et al. 2013). It is important to note that these studies were not performed in the soil and, therefore, were not able to evaluate the effects of the combination of soil and avermectins.

The physical characteristics of soil, such as pH, carbon content, and grain size, may interfere with the bioavailability of xenobiotics and alter the toxicity of the substances (Moreira et al. 2019). Additionally, interpretation

of the results obtained with plant bioassays in contaminated soils is complex as a result of the variety of factors that can influence the results (Delerue et al. 2019). According to the physicochemical analysis (Table 1), the presence of contaminants in soil, such as metals, was below the maximum allowed by Brazilian legislation, suggesting that the observed results were caused exclusively by eprinomectin. Information regarding the role of pH on the sorption/desorption of eprinomectin in soil is absent in the scientific literature, but it is known that eprinomectin will be adsorbed to the soil material at neutral pH; the degree of this adsorption will be determined by the physicochemical properties of the soil (Vássilis et al. 2016). Granulometry analysis (Table 2) showed that the soil used in this study presented low amounts of clay minerals and organic matter, characteristics that could contribute to a higher level of desorption, indicating toxic risk to the environment (Vássilis et al. 2016), as observed for *A. cepa*.

The results presented here suggest that eprinomectin has phytotoxic and genotoxic effects on *A. cepa*. The phytotoxicity was indicated by observed delays in the initial development of the plants and reduced germination speed. Genotoxicity was revealed by results demonstrating the clastogenic and aneugenic effects of the drug by showing its ability to interact with DNA and the mitotic spindle, inducing the formation of chromosomal and nuclear aberrations and MN. It is important to note that these effects were observed even at low concentrations, in accordance with recent studies assessing drugs belonging to the same family. Since limited information regarding the effects of avermectins, mainly eprinomectin, on plants is available, it is necessary to perform further investigations, such as recovery tests, to better understand the mechanisms of action responsible for producing the effects observed here. Generally, the release of eprinomectin into the environment should be minimized.

Author contribution RBS designed, carried out, and analyzed the experiments with support from CPS. The manuscript was written by RBS with input from all authors. JRG supervised the project. All authors read and approved the final manuscript.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Ahmed FAW (2014) Cytotoxic and genotoxic potency screening of WIDE-SPEC pesticide on *Allium cepa* L. root meristem cells. Journal of Natural Sciences Research 4:100–109

Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI (2015) Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid o CHO K1 cells. Environ Sci Pollut Res 22:17041–17052

Akeroyd J, Synge H (1992) In: Groombridge B (ed) Higher plant diversity. Global Biodiversity. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-2282-5_8

Anchordoquy JM, Anchordoquy JP, Nikoloff N, Gambaro R, Padula G, Seoane A, Furnus C (2019) Doramectin induced cytotoxic and genotoxic effects on bovine peripheral lymphocytes and cumulus cells in vitro. J Environ Sci Health B 54:147–154

Andrade-Vieira LF, Ferreira MFS Bernardes, PM Oliveira, WBS (2012) Toxicidade de Agrotóxicos: uma abordagem citogenética e molecular. In: Pratissoli D et al. (Org). Tópicos especiais em produção vegetal III, 1st edn. Alegre, UFES, 39-79 (in Portuguese)

Andrade-Vieira LF, Palmieri MJ, Davide LC (2017) Effects of long exposure to spent potliner on seeds, root tips, and meristematic cells of *Allium cepa* L. Environ Monit Assess 189:1–7

Arnold KE, Boxall AB, Brown AR, Cuthbert RJ, Gaw S, Hutchinson TH, Shore RF (2013) Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. Biol Lett. https://doi.org/10.1098/rsbl.2013.0492

Ashraf S, Beech RN, Hancock MA, Prichard RK (2015) Ivermectin binds to *Haemonchus contortus* tubulins and promotes stability of microtubules. Int J Parasitol 45:647–654

Bai SH, Ogbourne S (2016) Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. Chemosphere 154:204–214

Baird RB, Eaton AD, Rice EW (2017) Standard methods for the examination of water and wastewater, 23ed, American Public Health Association

Ballesteros C, Tritten L, O'Neill M, Burkman E, Zaky WI, Xia J, Geary TG (2016) The effects of ivermectin on Brugiamalayi females *in vitro*: a transcriptomic approach. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0004929

Bártíková H, Podlipná R, Skálová L (2016) Veterinary drugs in the environment and their toxicity to plants. Chemosphere 144:2290–2301

Bloom RA, Matheson JC III (1993) Environmental assessment of avermectins by the US Food and Drug Administration. Vet Parasitol 48:281–294

Bücker-Neto L, Paiva ALS, Machado RD, Arenhart RA, Margis-Pinheiro M (2017) Interactions between plant hormones and heavy metals responses. Genet Mol Biol 40:373–386

Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, Hartman R, Tunac JB (1979) Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob Agents Chemother 15:361–367

Caly L, Wagstaff KM, Jans DA (2012) Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? Antivir Res 95:202–206

Celande MC, Goldstone JV, Denslow ND, Iguchi T, Kille P, Meyerhoff RD, Wheeler JR (2011) Species extrapolation for the 21st century. Environ Toxicol Chem 30:52–63

CETESB 045/2014/E/C/I (2014) Valores orientadores para solo e água no estado de São Paulo. https://cetesb.sp.gov.br/solo/wp-conte nt/uploads/sites/18/2014/12/valores-orientadores-nov-2014.pdf Accessed 16 December 2019 (in Portuguese)

Das D, Mitra PK, Gupta S (2021) Evaluation of cytotoxicity induced by the anti-cancerous drugs doxorubicin and erlotinib in *Allium cepa* assay for eco-safety monitoring. Cytologia 86:195–199

Delerue F, Masfaraud JF, Lascourrèges JF, Atteia O (2019) A multi-site approach to investigate the role of toxicity and confounding factors on plant bioassay results. Chemosphere 219:482–492

Downing KH, Nogales E (1998) Tubulin and microtubule structure. Curr Opin Cell Biol 10:16–22

Dybas RA (1989) Abamectin use in crop protection. In *Ivermectin and abamectin*. Springer, New York, NY, pp 287-310

Eckert GL, Smaniotto TÂ, Dartora N, de Pelegrin CMG, Baroni S (2022) The chemical composition of different leaf extracts of *Lantana fucata* Lindl. influences its cytotoxic potential: a study using the *Allium cepa* model. J Ethnopharmacol:115003

Eng JKL, Blackhall WJ, Osei-Atweneboana MY, Bourguinat C, Galazzo D, Beech RN, Prichard RK (2006) Ivermectin selection on β-tubulin: evidence in *Onchocerca volvulus* and *Haemonchus contortus*. Mol Biochem Parasitol 150:229–235

Erzen NK, Kolar L, Flajs VC, Kužner J, Marc I, Pogačnik M (2005) Degradation of abamectin and doramectin on sheep grazed pasture. Ecotoxicology 14:627–635

Fernandes TC, Mazzeo DEC, Marin-Morales MA (2007) Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pestic Biochem Physiol 88:252–259

Fioresi VS, de Cássia Ribeiro Vieira B, de Campos JMS, da Silva Souza T (2020) Cytogenotoxic activity of the pesticides imidacloprid and iprodione on *Allium cepa* root meristem. Environ Sci Pollut Res 27:28066–28076

Fiskejö G (1985) The *Allium* test as a standard in environmental monitoring. Hereditas 102:99–112

Freitas AS, Cunha IMF, Andrade-Vieira LF, Techio VH (2016) Effect of SPL (spent pot liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in *Lactuca sativa* L. Ecotoxicol Environ Saf 124:426–434

Furtado AO, Almeida IV, Almeida ACC, Zotesso JP, Tavares CRG, Vicentini VEP (2020) Evaluation of hospital laundry effluents treated by advanced oxidation processes and their cytotoxic effects on *Allium cepa* L. Environ Monit Assess 192:1–8

Garcia CFH, Souza RB, de Souza CP, Fontanetti CS (2019) Effluent from citrus industry: toxic parameters of orange vinasse. Water Air Soil Pollut 230:201

Gharebaghi A, Haghighi AMH, Arouiee H (2017) Effect of cadmium on seed germination and earlier basil (*Ocimum basilicum* L. and *Ocimum basilicum* var. purpurescens) seedling growth. Trakia J Sci 1:1–4

Giannetti L, Giorgi A, Necci F, Ferretti G, Buiarelli F, Neri B (2011) Validation study on avermectine residues in foodstuffs. Anal Chim Acta 700:11–15

Gupta K, Mishra K, Srivastava S, Kumar A (2018) Cytotoxic assessment of chromium and arsenic using chromosomal behavior of root meristem in *Allium cepa* L. Bull Environ Contam Toxicol 100:803–808

Gupta S, Das D, Mitra PK, Halder S, Datta AK (2020) Assessment of cytotoxicity induced by hazardous chemotherapeutic drugs cyclophosphamide and 5-fluorouracil in *Allium cepa* assay for ecological safety. Cytologia 85:151–155

Halley BA, Jacob TA, Lu AYH (1989) The environmental impact of the use of ivermectin: environmental effects and fate. Chemosphere 18:1543–1563

Halley BA, VandenHeuvel WJ, Wislocki PG (1993) Environmental effects of the usage of avermectins in livestock. Vet Parasitol 48:109–125

Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF (1983) The induction of micronuclei as a measure of genotoxicity: a report of the US Environmental Protection Agency Gene-Tox Program. Mutation Res/Rev Gen Toxicol 123:61–118

Herd R (1996) Persistence of ivermectin in plasma and faeces following treatment of cows with ivermectin sustained release, pour-on or injectable formulations. Int J Parasitol 26:1087–1093

Iglesias LE, Saumell C, Sagüés F, Sallovitz JM, Lifschitz AL (2018) Ivermectin dissipation and movement from feces to soil under field conditions. J Environ Sci Health B 53:42–48

ISO 16387 (2013) Soil quality- effects of contaminants on Echytraeidae (*Enchytraeus* sp) – determination of effects on reproduction.

ISO 18763 (2016) Soil quality – determination of the toxic effects of pollutants on germination and early growth of higher plants.

Jensen J, Krogh PH, Sverdrup LE (2003) Effects of the antibacterial agents tiamulin, olanquindox and metronidazole and the anthelmintic ivermectin on the soil invertebrate species *Folsomia fimetaria* (Collembola) and *Enchytraeus crypticus* (Enchytraeidae). Chemosphere 50:437–443

Jjemba PK (2002) The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. *Agric Ecosyst Environ* 93:267–278

Kolar L, Eržen NK, Hogerwerf L, van Gestel CA (2008) Toxicity of abamectin and doramectin to soil invertebrates. Environ Pollut 151:182–189

Kovecses J, Marcogliese DJ (2005) Avermectins: potential environmental risks and impacts on freshwater ecosystems in Quebec. Environment Canada, Quebec Region, Environmental Conservation, St. Lawrence Centre

Leme DM, Marin-Morales MA (2009) *Allium cepa* test in environmental monitoring: a review on its application. Mutation Res/Rev Mutation Res 682:71-81

Li S, Li M, Cui Y, Wang X (2013) Avermectin exposure induces apoptosis in king pigeon brain neurons. Pestic Biochem Physiol 107:177–187

Luo Y, Liang J, Zeng G, Chen M, Mo D, Li G, Zhang D (2018) Seed germination test for toxicity evaluation of compost: its roles, problems and prospects. Waste Manag 71:109–114

Liman R, Ciğerci İH, Öztürk NS (2015) Determination of genotoxic effects of Imazethapyr herbicide in Allium cepa root cells by mitotic activity, chromosome aberration, and comet assay. Pestic Biochem Physiol 118:38–42

Ma TH, Xu Z (1986) Validation of a new protocol of the allium-micronucleus test for clastogens. In Environmental Mutagenesis. Wiley, New York, pp 49-49

Ma TH, Xu Z, Xu C, McConnel H, Rabago EV, Arreola GA (1995) The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. Mutat Res 334:185–195

McKellar QA (1997) Ecotoxicology and residues of anthelmintic compounds. Vet Parasitol 72:413–435

Mello MLS, Vidal BC (1978) A Reação de Feulgen. Ciência Cult. (in Portuguese)

Merck Company (1996) Ivomec Eprinex (eprinomectin) pour-on for beef and dairy cattle: environmental assessment. Report NADA 141-079EA, NJ, USA

Moraes Cunha Gonçalves M, de Almeida Lopes AC, Gomes RLF, de Melo WJ, Araujo ASF, Pinheiro JB, Marin-Morales MA (2020) Phytotoxicity and cytogenotoxicity of composted tannery sludge. Environ Sci Pollut Res 27:34495–34502

Moreira CG, De Carvalho TS, De Oliveira C, De Abreu LB, De Castro ACS, Ribeiro PG, Guilherme LRG (2019) Ecological risk assessment of cerium for tropical agroecosystems. Chemosphere 221:124–131

Mota TFM, Sampaio AR, Vasconcelos MW, de Castilhos Ghisi N (2022) *Allium cepa* test vs. insecticides: a scientometric and meta-analytical review. Environ Sci Pollut Res 29:42678–42691

NBR7181 (2016) Solo- análise granulométrica (in Portuguese)

NBR 13600 (1996) Solo- Determinação do teor de matéria orgânica por queima a 440 °C – Método de ensaio (in Portuguese)

OECD 232 (2016) OECD guidelines for testing chemicals - collembolan reproduction test in soil.

Oliveira-Ferreira F, Rodrigues-Silva C, Rath S (2016) On-line solid-phase extraction-ultra high performance liquid chromatography-tandem mass spectrometry for the determination of avermectins and milbemycin in soils. J Chromatogr A 1471:118–125

Palmieri MJ, Luber J, Andrade-Vieira LF, Davide LC (2014) Cytotoxic and phytotoxic effects of the main chemical components of spent pot-liner: a comparative approach. Mutation Res/Gen Toxicol Environ Mutagen 763:30–35

Pinho IA, Lopes DV, Martins RC, Quina MJ (2017) Phytotoxicity assessment of olive mill solid wastes and the influence of phenolic compounds. Chemosphere 185:258–267

Priac A, Badot PM, Crini G (2017) Treated wastewater phytotoxicity assessment using *Lactuca sativa*: focus on germination and root elongation test parameters. Comptes rendus biologies 340:188–194

Ratsch HC, Johndro D (1986) Comparative toxicity of six test chemicals to lettuce using two root elongation test methods. Environ Monit Assess 6:267–276

Ruttkay-Nedecky B, Krystofova O, Nejdl L, Adam V (2017) Nanoparticles based on essential metals and their phytotoxicity. J Nanobiotechnol 15:33

Sharma P, Purchase D, Chandra R (2021) Residual pollutants in treated pulp paper mill wastewater and their phytotoxicity and cytotoxicity in *Allium cepa*. Environ Geochem Health 43:2143–2164

Seelanan P, Srisa-art M, Petsom A, Nhujak T (2006) Determination of avermectins in commercial formulations using microemulsion electrokinetic chromatography. Anal Chim Acta 570:8–14

Schins RP, Knaapen AM (2007) Genotoxicity of poorly soluble particles. Inhal Toxicol 19:189–198

Shen W, Zhao X, Wang Q, Niu B, Liu Y, He L, Weng H, Meng Z, Chen Y (2011) Genotoxicity evaluation of low doses of avermectin to hemocytes of silkworm (*Bombyx mori*) and response of gene expression to DNA damage. Pestic Biochem Physiol 101:159–164

Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H, Skelly BJ (1996) Eprinomectin: a novel avermectin for use as a topical endectocide for cattle. Int J Parasitol 26:1237–1242

Sobrero MV, Ronco A. ensayo de toxicidad aguda consemillas de lechuga (*Lactuca sativa* L.) (2004) In: Castillo G (ed) Ensayos toxicologicos y métodos de evaluación de callidad de aguas. Estandarización, intercalibración, reultados y aplicaciones. IDRC/IMTA, Canada, pp. 71-79 (in Spanish)

Sommaggio LRD, Mazzeo DEC, Levy CE, Marin-Morales MA (2018) Ecotoxicological and microbiological assessment of sewage sludge associated with sugarcane bagasse. Ecotoxicol Environ Saf 147:550–557

Souza RB, de Souza CP, Bueno OC, Fontanetti CS (2017) Genotoxicity evaluation of two metallic-insecticides using *Allium cepa* and *Tradescantia pallida*: a new alternative against leaf-cutting ants. Chemosphere 168:1093–1099

Tay MYF, Fraser JE, Chan WKK, Moreland NJ, Rathore AP, Wang C, Jans DA (2013) Nuclear localization of dengue virus (DENV) 1–4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin. Antivir Res 99:301–306

Tkalec M, Malarić K, Pavlica M, Pevalek-Kozlina B, Vidaković-Cifrek Ž (2009) Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of *Allium cepa* L. Mutation Res/Gen Toxicol Environ Mutagen 672:76–81

Türkoğlu Ş (2012) Determination of genotoxic effects of chlorfenvinphos and fenbuconazole in *Allium cepa* root cells by mitotic activity, chromosome aberration, DNA content, and comet assay. PesticBiochem Phys 103:224–230

USEPA 9045D (2004) Soil and waste pH

USEPA 3050B (1996) Acid digestion of sediments, sludges, and soils.

USEPA 300.1 (1993) Determination of inorganic anions in drinking water by ion chromatography.

USEPA 6010C (2007) Inductively coupled plasma-atomic emission spectrometry.

Vássilis LD, George BC, Charalampos PG, Athina PV, Xanthippos KN (2016) Mobility of pharmaceutical compounds in the terrestrial environment: adsorption kinetics of the macrocyclic lactone eprinomectin in soils. Chemosphere 144:1201–1206

Verma S, Srivastava A (2018) Morphotoxicity and cytogenotoxicity of pendimethalin in the test plant *Allium cepa* L.-A biomarker based study. Chemosphere 206:248–254

Vokřál I, Michaela Š, Radka P, Jiří L, Lukáš P, Dominika S, Katerina L, Barbora S, Lenka (2019) Ivermectin environmental impact: excretion profile in sheep and phytotoxic effect in *Sinapis alba*. Ecotoxicol Environ Saf 169:944-949

Yadav A, Raj A, Purchase D, Ferreira LFR, Saratale GD, Bharagava RN (2019) Phytotoxicity, cytotoxicity and genotoxicity evaluation of organic and inorganic pollutants rich tannery wastewater from a common effluent treatment plant (CETP) in Unnao district, India using *Vigna radiata* and *Allium cepa*. Chemosphere 224:324–332

Yi H, Wu L, Jiang L (2007) Genotoxicity of arsenic evaluated by Allium-root micronucleus assay. Sci Total Environ 383:232–236

Zhang Y, Wu J, Xu W, Gao J, Cao H, Yang M, Wang B, Hao Y, Tao L (2017) Cytotoxic effects of avermectin on human HepG2 cells in vitro bioassays. Environ Pollut 220:1127–1137

Abreu-Junior CH, de Lima Brossi MJ, Monteiro RT, Cardoso PHS, da Silva MT, Nogueira TAR, Capra GF (2019) Effects of sewage sludge application on unfertile tropical soils evaluated by multiple approaches: a field experiment in a commercial Eucalyptus plantation. Sci Total Environ 655:1457–1467