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Fate and toxicity of 2,4-D and fipronil in mesocosm systems

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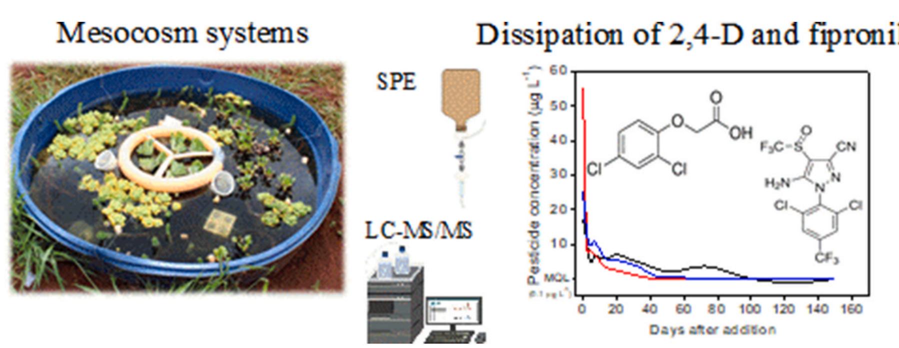
HIGHLIGHTS

- Dissipation of pesticides in mesocosm systems was influenced by treatments.
- Degradation products were quantified in the mesocosm samples after the application.
- Deposition of pesticides in the sediment was observed.
- The risk assessment in a more representative environmental scenario was carried out.
- Significant toxicological effects were observed in the different organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

2,4-D and fipronil are among Brazil's most used pesticides. The presence of these substances in surface waters is a concern for the aquatic ecosystem health. Thus, understanding the behavior of these substances under environmentally relevant conditions is essential for an effective risk assessment. This study aimed to determine the degradation profiles of 2,4-D and fipronil after controlled application in aquatic mesocosm systems under influencing factors such as environmental aspects and vinasse application, evaluate pesticide dissipation at the water-sediment interface, and perform an environmental risk assessment in water and sediment compartments. Mesocosm systems were divided into six different treatments, namely: control (C), vinasse application (V), 2,4-D application (D), fipronil application (F), mixture of 2,4-D and fipronil application (M), and mixture of 2,4-D and fipronil with vinasse application (MV). Pesticide application was performed according to typical Brazilian sugarcane management procedures, and the experimental systems were monitored for 150 days. Pesticide dissipation kinetics was modeled using first-order reaction models. The estimated half-life times of 2,4-D were 18.2 days for individual application, 50.2 days for combined application, and 9.6 days for combined application with vinasse. For fipronil, the respective half-life times were 11.7, 13.8, and 24.5 days. The dynamics of pesticides in surface waters resulted in the deposition of these compounds in the sediment. Also, fipronil transformation products fipronil-sulfide and fipronil-sulfone were quantified in water 21 days after pesticide application. Finally,

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performed risk assessments showed significant potential risk to environmental health, with RQ values for 2,4-D up to 1359 in freshwater and 98 in sediment, and RQ values for fipronil up to 22,078 in freshwater and 2582 in sediment.

1. Introduction

As one of the world leaders in the production of agricultural products, Brazil has high levels of pesticide use and, consequently, elevated levels of pesticides and their decomposition products present the environment. The herbicide 2,4-D and the insecticide fipronil are among Brazil's most used pesticides in sugarcane crops. In 2020, 2,4-D was the second most-used herbicide in the country, while fipronil was the seventh most-used insecticide (IBAMA, 2022; Moutinho et al., 2020). These pesticides and their decomposition products contaminate surface waters and present a risk to the health of aquatic ecosystems.

The physicochemical properties of 2,4-D and fipronil differ and impact the environmental fate of these substances. 2,4-D is an acidic ionizable organic compound (pKa: 2.73) from the phenoxy family with high water solubility (24,300 mg L^{-1} at 20 °C) and a low tendency to adsorb to soil and sediment (Koc = 20 to 280) (Buerge et al., 2020; PPDB, 2021a). The main degradation routes of 2,4-D in the environment are via oxidation, reduction, photolysis, and hydrolysis, resulting in the formation of several transformation products (1,2,4-benzenetriol, 4-chlorophenol, 2,4-dichlorophenol, among others) (Montgomery, 2007). Fipronil is a non-ionizable polar organic compound from the phenylpyrazole family with low water solubility (3.78 mg L^{-1} at 20 °C) and a high tendency to adsorb to soil and sediment (K_{oc} = 825) (Bonmatin et al., 2015; PPDB, 2021b). Environmental degradation of fipronil occurs via several mechanisms (reduction, oxidation, hydrolysis, among others). It generates transformation products including fipronil-sulfide, fipronil-sulfone, fipronil-desulfinyl, and fipronil-amide. (Kaur et al., 2015; Tomazini et al., 2021).

The continuous or inappropriate application of pesticides can result in harmful effects on the environment as a result of contamination of different environmental matrices (Kalsi and Kaur, 2019; Silva et al., 2018). 2,4-D and fipronil have been detected in the environment in several regions of Brazil, mainly in water bodies close to agricultural areas. Albuquerque et al. (2016) compiled data on pesticide occurrence in freshwater in Brazil and concluded that fipronil was the most frequently detected pesticide among 254 samples (54%) at concentrations ranging from 0.05 to 26.2 μg L^{-1}. In the State of São Paulo, fipronil and 2,4-D were quantified in 62% and 20% of surface freshwater samples monitored between 2015 and 2016, respectively, at concentrations above national and international aquatic life protection criteria (CETESB, 2021). In addition, studies reported the occurrence of fipronil and 2,4-D in water bodies in Brazil at concentrations ranging from 0.1 to 445 μg L^{-1} and 1.14–366 μg L^{-1}, respectively (Marchesan et al., 2010; Pinheiro et al., 2010). Due to the occurrence of 2,4-D and fipronil in the environment, often simultaneously, there are several adverse effects on organisms of different trophic levels reported in the literature based on studies carried out in the laboratory and in the field (Freitas et al., 2022, 2019; Moreira et al., 2021, 2020b, 2020a; da Silva Pinto et al., 2021a–d, 2022, 2023; Silberschmidt Freitas et al., 2022; Silva et al., 2020; Triques et al., 2021).

In addition to the use of pesticides, the use of fertilizers helps to increase the productivity of plantations. Vinasse is a brownish liquid by-product from the fermentation of sugarcane juice during the production of ethanol. It is composed of water, organic matter, and minerals (potassium, calcium, magnesium, and sulfur) and has an acidic pH and high levels of Biochemical Oxygen Demand (BOD) (da Silva et al., 2007). Although it has polluting potential, this by-product has been used as fertilizer in the cultivation of sugarcane, partially replacing the use of mineral fertilizers, thus reducing the costs involved in crop management. The adequate application of vinasse, considering the physicochemical properties of the soil, allows an increase in the absorption of nutrients by the plants, increasing the productivity of sugarcane crops (da Silva et al., 2007). However, its improper application can harm the environment due to the leaching of metals from the soil, soil contamination, nutrient imbalance, and surface water contamination (Chitolina and Harder, 2020; da Silva et al., 2007).

In addition to its low cost, the use of vinasse as a fertilizer intensified in Brazil after its water dumping ban in the 1980s (Chitolina and Harder, 2020; da Silva Pinto et al., 2021b). However, its presence in Brazilian water bodies due to improper disposal or its use in the fertigation process is not uncommon (Chitolina and Harder, 2020; da Silva et al., 2007). Several studies report toxic effects on biodiversity when exposed to vinasse (Girotto et al., 2022; Ogura et al., 2022;da Silva Pinto et al., 2021a,d, 2022, 2023; Portruneli et al., 2021; Silberschmidt Freitas et al., 2022; Silva et al., 2020). Also, vinasse can modify the physicochemical properties of water (pH, content of organic matter, and nutrients) and, consequently, influence the environmental behavior of other contaminants. Thus, studies that evaluate the effects related to the presence of vinasse in the environment after its use as a fertilizer in sugarcane cultivation are still necessary.

To study the dynamics of pesticides in the environment, the use of artificial aquatic systems called mesocosms allows the simulation of the effects of stressors on the structure and function of the environment, providing more realistic results of biota exposure and environmental behavior of contaminants such as transformation, dissipation, and transport (Bejarano et al., 2005; Finnegan et al., 2018; da Silva Pinto et al., 2023; Silberschmidt Freitas et al., 2022). These structures represent simple food chains in lentic systems, analogous to water bodies in several Brazilian landscapes. Mesocosms are used as surrogate ecosystems and reduce uncertainty in the extrapolation of laboratory bioassays to real environmental effects, making it possible to obtain more complete data for the assessment of the environmental risk of pesticides (Beuter et al., 2019; Finnegan et al., 2018; Goulart et al., 2020).

Bejarano et al. (2005) conducted studies in modular estuarine salt marsh mesocosms to evaluate atrazine effects in meiobenthos. Beuter et al. (2019) evaluated carbaryl adverse effects in macroinvertebrate communities employing lotic mesocosm systems, and Finnegan et al. (2018) used freshwater mesocosm systems to assess the impact of thiamethoxican on multiple trophic levels (phytoplankton, zooplankton, and macroinvertebrates). However, there are no reports in the literature of studies in mesocosm systems that assess the dynamics, persistence, and ecotoxicological effects of 2,4-D and fipronil, either applied individually or in combination, after controlled application according to conventional Brazilian management practices.

This study aimed to determine the degradation profile of 2,4-D and fipronil after controlled application in the semi-field using aquatic mesocosm systems and their dissipation at the water-sediment interface. Also, this study aimed to evaluate the influence of vinasse application on pesticide fate and persistence. Finally, this study aimed to perform an environmental risk assessment by comparing the pesticide occurrence data with the predicted no-effect concentration (PNEC) and with different endpoints observed after exposure of organisms in different trophic levels.

The results obtained in this study provided unprecedented data in more realistic contamination scenarios, which helped predict the fate and persistence of 2,4-D and fipronil in mesocosm systems exposed to conventional Brazilian sugarcane management practices. Also, this study allowed the evaluation of vinasse and abiotic factors (wind, solar radiation, temperature, nutrients) on the environmental behavior of pesticides and the assessment of the environmental risk for aquatic life organisms, thus allowing the prediction of ecotoxicological effects resulting from contamination exposure.

2. Materials and methods

2.1. Reagents and materials

The internal standard 2,4-D (ring $^{13}C^{6}$) 100 μg mL^{-1} in acetonitrile was obtained from Cambridge Isotope Laboratories Inc. (Massachusetts, USA). The internal standard fipronil-(pyrazole-$^{13}C_3$, cyano-^{13}C) (99%) and the standards of fipronil (97.9%), fipronil sulfide (99%), fipronil sulfone (99%) and 2,4-D (99.9%) were purchased from Merck (Darmstadt, Germany). Dichloromethane reagent grade was purchased from Merck (Darmstadt, Germany). Methanol reagent grade and acetonitrile reagent grade were acquired from J. T. Baker (Phillipsburg, USA). Sulfuric acid ACS grade was purchased from Synth (São Paulo, Brazil) and ammonium hydroxide was obtained from Fluka Analytical (Buchs, Switzerland). Ultrapure water was obtained from a Synergy Water Purification System from Millipore (Burlington, USA). Regent 800 WG (a.i. fipronil) was purchased from BASF (Ludwigshafen am Rhein, Germany) and DMA 806 BR (a.i. 2,4-D) was purchased from Dow Chemical Company (Midland, USA). 500 mg HLB Oasis cartridges were purchased from Waters Corporation (Milford, USA), glass microfiber filters (47 mm diameter, grade 13400) were purchased from Sartorius (Gottingen, Germany), and hydrophobic PTFE syringe filters (0.45 μm) were purchased from Analítica (São Paulo, Brazil).

Individual stock solutions (400 μg mL^{-1}) were prepared in methanol for 2,4-D, fipronil, fipronil-sulfide, fipronil-sulfone, and fipronil-(pyrazole-$^{13}C_3$, cyano-^{13}C). The stock solution (10 μg mL^{-1}) for 2,4-D (ring $^{13}C_6$) was prepared in acetonitrile. All stock solutions were stored in amber glass bottles at −4 °C until needed (Goulart et al., 2020).

2.2. Experimental design

The mesocosm systems were assembled at the Center for Water Resources and Environmental Studies/University of São Paulo (CRHEA/USP, Itirapina-SP, Brazil), as described in da Silva Pinto et al. (2021a). 20 systems were built using polypropylene tanks with a capacity of 1500 L (1.75 m diameter x 0.83 m depth). Each structure was individually buried 0.6 m below ground level. 0.2 m of natural soil (Oxisol) collected from the same area was used as sediment (physicochemical properties described in Figueirêdo et al., 2020). The structures were filled with artesian well water (depth of 0.6 m). Macrophytes and planktonic communities (phytoplankton and zooplankton) were added. The communities were collected in the Lobo reservoir (Itirapina-SP, Brazil). An acclimation period of 6 months was used to stabilize the biological communities in the mesocosms.

The mesocosm systems were distributed into six different treatments: 5 control mesocosms without pesticide application (C); 3 mesocosms with vinasse application and without pesticide application (vinasse physicochemical properties are described in da Silva Pinto et al. (2021a) (V); 3 mesocosms with DMA 806 BR (a.i. 2,4-D) application (D); 3 mesocosms with Regent 800 WG (a.i. fipronil) application (F); 3 mesocosms with a mixture of DMA 806 BR and Regent 800 WG application (M); and 3 mesocosms with vinasse application and with a mixture of Regent 800 WG and DMA 806 BR application (MV). The concentrated solutions of the commercial pesticide products were prepared in well water, considering the size of the mesocosms and the recommended application rate for sugarcane cultivation for 2,4-D (3.5 L of DMA 806 BR ha^{-1} or 2.5 kg of a.i. ha^{-1}) and fipronil (500 g of Regent 800 WG ha^{-1} or 400 g of a.i. ha^{-1}) (MAPA, 2018). Pesticides were applied by spraying, resulting in a nominal concentration of 447 μg L^{-1} for 2,4-D and 64 μg L^{-1} for fipronil. In the mesocosms with vinasse treatment, 20 L of vinasse was applied, corresponding to a nominal concentration of 1.3% (v/v), according to the dose used by local farmers.

The physicochemical parameters of mesocosm water samples (electrical conductivity, dissolved oxygen, turbidity, hardness, and pH) were monitored during sampling using the ProDSS Multiparameter Digital Water Quality Meter (YSI, Yellow Springs, USA). In addition, total nitrogen, total phosphorus, inorganic phosphate, nitrite, nitrate, and ammonium concentration levels were determined as described by da Silva Pinto et al. (2021a).

2.3. Sample collection and sample preparation

Water samples were collected from the subsurface layer in 1 L amber glass bottles as described in Goulart et al. (2020). All samples were filtered in a laboratory-made 20–500 μm mesh Nylon filter, vacuum-filtered using a glass microfiber filter, and refrigerated at 4 °C until extraction. Except for the V and MV treatments, all samples were run in monoplicates for each mesocosm system (IS1, IS2, and IS3). Considering the limitations in sample preparation due to the presence of vinasse, a composite sample (CS) consisting of a mixture of individual mesocosm samples was analyzed for each treatment. The samples were collected 7 days before application, then 2 h and 2, 4, 7, 14, 21, 31, 45, 75, 101 and 150 days after application.

Sediment samples were collected using a core sampler (diameter of 5.0 cm) as described in Goulart et al. (2023). For each mesocosm, three aliquots were successively sampled and then combined. All samples were dried under ambient conditions, granulometrically separated with a 1.0 mm particle size sieve, stored in plastic containers, and refrigerated at 4 °C until extraction. All samples were run in monoplicates for each mesocosm system. The samples were collected 8 days before application, then 2, 7, 14, 21, 75, 85, and 150 days after application.

Water samples (500 mL) were extracted by solid-phase extraction (SPE) (Goulart et al., 2020). Briefly, 500 mg HLB Oasis cartridges were conditioned with 5 mL of methanol and 5 mL of ultrapure water. Then, the samples were passed at a flow rate of 7 mL min^{-1}. Loaded cartridges were dried under vacuum for 10 min, and analytes were eluted from the cartridges with 4 mL of methanol and 4 mL of acetonitrile. The extracts were reduced to dryness with nitrogen gas and brought to a final volume of 500 μL using water:methanol 70:30 (v/v). Final extracts were filtered with a hydrophobic PTFE syringe filter.

Sediment samples (10 g) were extracted by solid-liquid extraction (SLE) (Goulart et al., 2023). Briefly, the samples were acidified with 1 mL of sulfuric acid 0.1 mol L^{-1}. Extraction was performed in two cycles using 20 mL of dichloromethane per cycle. Samples were homogenized in a Genius-3 IKA Vortex mixer (IKA, Staufen, Germany) for 1 min, sonicated in a Q9.5/40A ultrasonic bath (Eco-Sonics, Indaiatuba, Brazil) for 10 min, and separated in an MPW-351 centrifuge (MPW Med. Instruments, Warsaw, Poland) at 4000 rpm for 10 min. The organic phase was collected at the end of each cycle. After both cycles, the aliquots were combined, reduced to dryness with nitrogen gas, and brought to a final volume of 1500 μL using water:methanol 70:30 (v/v). Final extracts were filtered with a hydrophobic PTFE syringe filter.

Validation studies showed that both sample preparation procedures are under the required standards. All complementary information is fully described in Goulart et al. (2020, 2023).

2.4. Chemical analysis

Target compounds were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 1200 liquid chromatograph coupled with an Agilent 6410B triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA) with electrospray ionization (ESI), as described in Goulart et al. (2020).

The instrumental limits of detection (IDLs) were 0.5 μg L^{-1} for 2,4-D and 0.05 μg L^{-1} for fipronil, fipronil-sulfide, and fipronil sulfone, whereas the instrumental limits of quantification (IQLs) were 1.0 μg L^{-1} for 2,4-D and 0.1 μg L^{-1} for fipronil, fipronil-sulfide, and fipronil sulfone. Validation studies showed that the analytical method is under the required standards. All complementary information is fully described in

Goulart et al. (2020).

2.5. Data analysis

2.5.1. Water quality parameters

Temporal and between treatments changes in the physicochemical water parameters were assessed using the Principal Response Curve (PRC). A Redundancy Analysis was applied to the time-series data of mesocosms. The analysis significance ($p < 0.05$) was attested by the Monte Carlo permutation test using 499 permutations. Significant deviations of each treatment relative to control mesocosms in each period were assessed by Generalized Linear Models (GLM) using the Gaussian family with identity-link function in software R (version 3.6.0, 2009) applying RStudio (version February 1, 1335, 2019). The confidence level was 95% ($p < 0.05$).

2.5.2. Pesticide dissipation kinetics

Pesticide dissipation kinetics were modeled using the integrated rate laws for zeroth-, first-, and second-order reactions. Three evaluation metrics were employed to evaluate the performance of the linear regression models: the coefficient of determination (R^2), the normalized mean absolute error (NMAE), and the normalized root-mean-square error (NRMSE). Regression models were built for 2,4-D and fipronil for each mesocosm system with pesticide application (F or D, M, and MV treatments). Next, half-live times ($t_{1/2}$) were calculated using the best-suited models. The statistical differences between the half-life of 2,4-D and fipronil in the different mesocosm systems were assessed by the Student's t-test for the comparison of two means.

2.6. Environmental risk assessment and ecotoxicological tests

Risk assessment was performed using the standard approach based on the risk quotient (RQ), dividing the Measured Environmental Concentration (MEC) by the respective water quality criteria (WQC). RQ values greater than 1 indicate a potential risk to water and sediment organisms. Lowest Predicted No-Effect Concentration (LPNEC) values for chronic exposure were adopted as WQC values and retrieved from the NORMAN Ecotoxicology Database (NORMAN, 2023).

Concomitantly to the evaluation of pesticide dissipation in the mesocosm systems after controlled application in the semi-field, ecotoxicological tests were carried out to assess the impact of water contamination by 2,4-D and fipronil individually, in combination, and in combination with vinasse application. Acute and chronic toxicity tests were carried out *in situ* and the laboratory with water collected from mesocosm systems, using organisms from different trophic levels (Girotto et al., 2022; Ogura et al., 2022; da Silva Pinto et al., 2021a,d, 2022, 2023; Portruneli et al., 2021; Silberschmidt Freitas et al., 2022; Silva et al., 2020). Thus, in addition to the risk quotient approach, the data of pesticides obtained in this study were associated with the various effects observed in the ecotoxicological tests.

3. Results and discussion

3.1. Water quality parameters

Physicochemical water parameters for the mesocosms are presented in Table S1. Fig. 1 shows the PRC curve with the physicochemical water parameters measured throughout the experimental period. The PRC results are displayed in a diagram in which the time was plotted on the horizontal axis, the treatments on the vertical axis, and the water parameter's weights were displayed in a separate diagram. In this diagram, high positive weights denoted similar responses to the PRC response, and negative weights indicated inverse responses. The PRC analysis and the diagram interpretation are best described in Van den Brink et al. (1999).

According to the Monte-Carlo permutation test (499 permutations), the analysis was significant ($p = 0.002$ and $F = 130.3$). In the PRC, 24% of all variation in physicochemical data can be attributed to the time and 56% to treatments. The remainder (residues) is associated with variability that the dataset cannot explain (20%).

Significant deviations were detected between treatments that received vinasse (V and MV) and control mesocosms from T1 to T75. In the mesocosms with vinasse, pH and dissolved oxygen presented negative weights, indicating an inverse response denoted by water acidification and decreases in oxygen levels close to anoxic conditions. Other physicochemical properties in these systems showed the same deviation trend after application, as indicated by the PCR. Thus, increases in these parameters' values occurred after application, provoking alterations in water quality associated with an ionic and organic load in the aquatic system. These variations can be justified by the characteristics of the crude vinasse, which resulted in changes in water quality parameters (da Silva Pinto et al., 2023).

There were no differences over time between the control mesocosms (C) and the F, D, and M treatments ($p > 0.05$), thus demonstrating that pesticide application at the tested concentrations did not alter the water parameters throughout time.

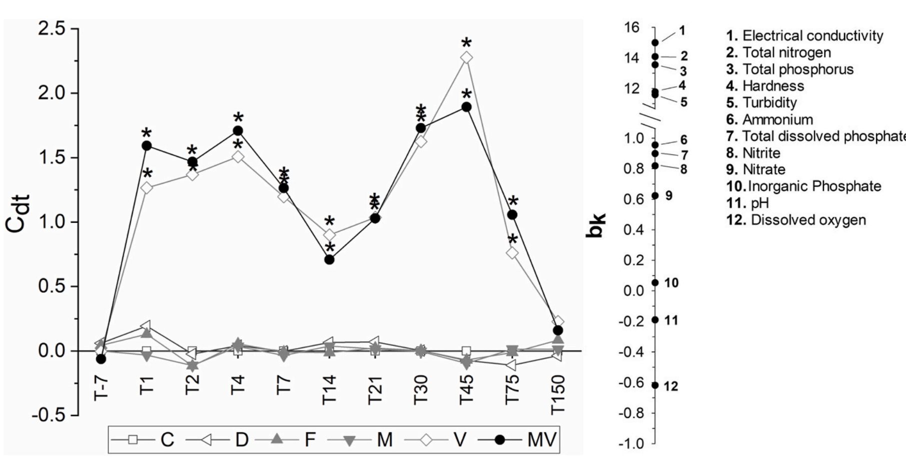

Fig. 1. Principal response curves (PRC) showing the physicochemical water parameters measured throughout the experimental period (from 7 days before application (T-7) to 150 days after application (T150)) in different mesocosm systems. Physicochemical water parameter weights are provided on the right axis. Asterisks indicate a significant deviation of treatment relative to control mesocosms at specific sampling days ($p < 0.05$). C: control; D: 2,4-D application; F: fipronil application; M: mixture of fipronil and 2,4-D application; V: vinasse application; MV: mixture of 2,4-D and fipronil with vinasse application.

3.2. Pesticide fate in the water compartment

Mean concentrations of 2,4-D and fipronil in the six treatments measured 2 h after pesticide application differed significantly from the nominal concentrations, ranging from 665 to 1656 μg L^{-1} and 17 to 56 μg L^{-1}, respectively. This variation is probably due to the impossibility of homogenizing the mesocosms in order not to cause sediment disturbances. Similar variations between nominal and quantified concentrations were observed by Lobson et al. (2018) when assessing the fate of the insecticide thiamethoxam in mesocosms. Pesticide concentrations measured 2 days after application were closer to nominal concentrations, meaning the mesocosms needed a period for their complete homogenization. Thus, the first sampling point (2 h) was not considered in the analysis of kinetic dissipation models.

Pesticide residues were detected in the mesocosm systems before application in concentration levels of ng L^{-1}. Trace-level contamination of both pesticides was also quantified throughout the experiment. 150 days after the pesticide application, residual concentrations of 2,4-D and fipronil ranged from 55 ng L^{-1} to 105 μg L^{-1} and 1 to 88 ng L^{-1}, respectively (Figs. 2 and 3). Fipronil dissipation resulted in fipronil-sulfide and fipronil-sulfone formation, quantified in water samples 21 days after pesticide application.

The performance of kinetic dissipation models for zeroth-, first-, and second-order reactions are shown in Table S2 for 2,4-D and Table S3 for fipronil. For both individual pesticide application (D or F treatment) and combined pesticide application (M treatment), one linear regression model was built for each mesocosm system individually as independent systems (IS1, IS2, and IS3), and a fourth linear regression model was built using the three mesocosm systems as replicates (Rep). For the MV treatment, only one linear regression model (CS) was built due to the adoption of composite sampling and, thus, the inexistence of replicate analysis.

The metric R^2 is a dimensionless score between 0 and 1 that measures how well a statistical model predicts an outcome, *i.e.*, it expresses the proportion of variance in the dependent variable explained by the statistical model. Thus, the greater the R^2 is, the better the regression model fits the observed data. MAE and RMSE are two convenient metrics that express the accuracy of the model in the units of the dependent variable. The MAE metric represents the average of the absolute differences between prediction and observation, *i.e.*, it measures the average magnitude of the residuals. In contrast, the RMSE metric represents the average deviation of the differences between prediction and observation, *i.e.*, it measures the average deviation of the residuals. Thus, the lower the MAE and RMSE are, the higher is the accuracy of the regression model. However, such metrics do not perform well if comparing model fits for different response variables or if the response variable is somehow modified, such is the case for first- (ln-transformed) and second-order (reciprocal-transformed) reaction models. Yet, the lack of comparability can be overcome if the metrics are brought on the same scale or otherwise standardized. Thus, the normalized mean absolute error (NMAE) and the normalized root-mean-square error (NRMSE) were adopted for model comparison. The metrics were normalized using the standard deviation of the observed responses (s_{yobs}).

The R^2, NMAE, and NRMSE metrics in Table S2 reveal that the first-order reaction is the most suitable model for 2,4-D in individual application (D) and combined application with vinasse (MV), whereas the second-order reaction seemed the most appropriate model for combined application (M). However, the differences between the first- and second-order reaction model metrics are slim, which would allow the adoption of the first-order reaction model for 2,4-D in combined application (M). For fipronil, the first-order reaction model was the most suitable for individual application (F), combined application (M), and combined application with vinasse (MV) (Table S3).

When possible, it is important to select the same reaction order for comparative purposes. Also, the half-life time estimation for the first-order reaction model is not dependent on the initial concentration of the reactant. In contrast, the zeroth-order reaction model is directly proportional to the initial concentration of the reactant, whereas the second-order reaction model is inversely proportional to it. Thus, adopting the first-order reaction model also facilitates the comparison of estimated half-live times with available literature. Therefore, the dissipation kinetics for fipronil and 2,4-D in all treatments (D or F, M, and MV) were assessed using the first-order reaction model since obtained evaluated metrics are considered satisfactory in dissipation studies (Kalsi and Kaur, 2019; Lobson et al., 2018; Simonin, 2016). Next, the half-life times for fipronil and 2,4-D (Table 1) were estimated for each treatment.

Table 1 shows that, in general, the half-life times obtained from the models for each mesocosm as independent systems are very close to the obtained values from the models built using the three mesocosm systems as replicates, demonstrating great mesocosm replicability. The estimated half-life times of 2,4-D were 18.2 days for individual application, 50.2 days for combined application, and 9.6 days for combined application with vinasse. For fipronil, the respective half-life times were 11.7, 13.8, and 24.6 days.

The Student's t-test for the comparison of two means ($\alpha = 0.05$) was used to assess the difference in half-live times between treatments of individual application (D or F) and combined application (M) using the three values obtained from each individual system. Due to adopting a composite sample, the half-life times obtained for the MV treatment were not included in the statistical analysis. The obtained p-value for 2,4-D (p-value = 0.0022) indicates that the null hypothesis of equality must be rejected, and there is a statistically significant difference between half-live times for 2,4-D when applied individually and in

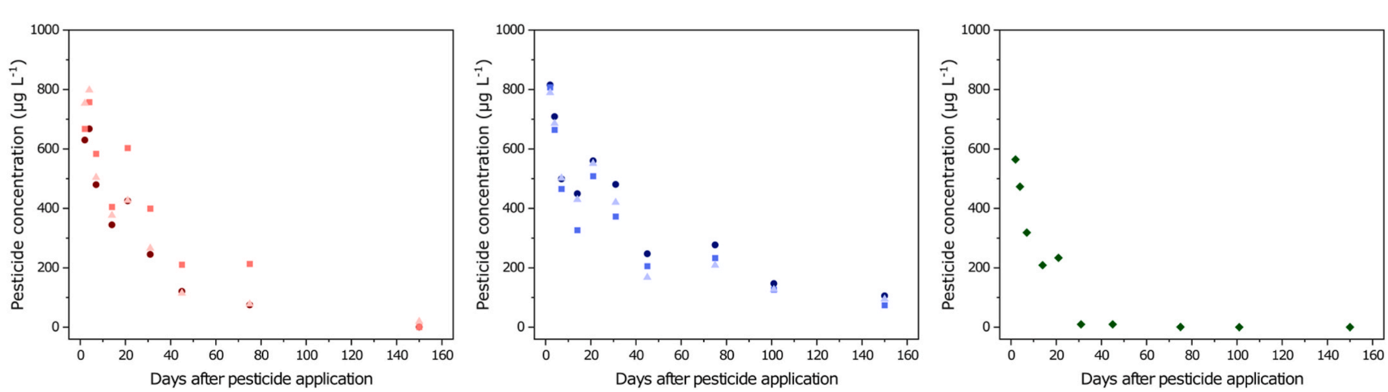

Fig. 2. 2,4-D concentration (μg L^{-1}) in the water compartment (2–150 days after pesticide application) for treatments D (red), M (blue), and MV (green) for independent mesocosm systems IS1 (dots), IS2 (squares), IS3 (triangles), and CS (diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

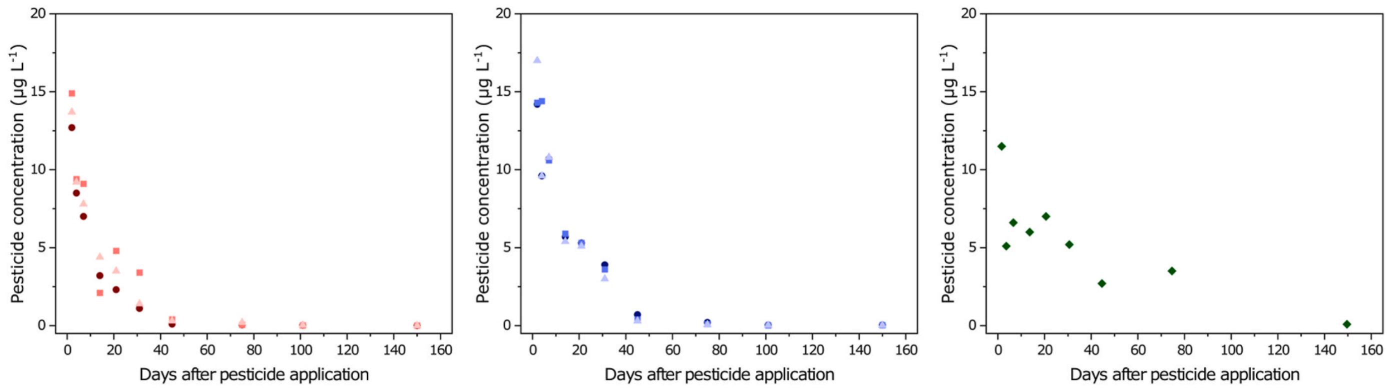

Fig. 3. Fipronil concentration (μg L^{-1}) in the water compartment (2–150 days after pesticide application) for treatments F (red), M (blue), and MV (green) for independent mesocosm systems IS1 (dots), IS2 (squares), IS3 (triangles), and CS (diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Rate constant (k) ($days^{-1}$) and standard deviation (s_k) ($days^{-1}$) for first-order reaction models and half-life times ($t_{1/2}$) (days) and standard deviation ($s_{t1/2}$) (days) for 2,4-D and fipronil in individual application (D or F), combined application (M), and combined application with vinasse (MV).

Pesticide	Treatment	Model	k ($days^{-1}$)	s_k ($days^{-1}$)	$t_{1/2}$ (days)	$s_{t1/2}$ (days)
2,4-D	D	IS1	0.0458	0.0035	15.14	1.15
		IS2	0.0437	0.0057	15.87	2.07
		IS3	0.0249	0.0023	27.87	2.57
		Mean of ISs	–	–	*19.63*	*7.14*
		Rep	0.0381	0.0030	18.19	1.43
	M	IS1	0.0130	0.0015	53.20	6.05
		IS2	0.0144	0.0018	48.19	5.91
		IS3	0.0140	0.0020	49.41	7.07
		Mean of ISs	–	–	*50.26*	*2.61*
		Rep	0.0138	0.0010	50.18	360
	MV	CS	0.0719	0.0084	9.63	1.13
Fipronil	F	IS1	0.0632	0.0044	10.96	0.76
		IS2	0.0625	0.0043	11.10	0.77
		IS3	0.0526	0.0031	13.17	0.78
		Mean of ISs	–	–	*11.74*	*1.24*
		Rep	0.0594	0.0025	11.66	0.50
	M	IS1	0.0452	0.0048	15.33	1.63
		IS2	0.0535	0.0066	12.96	1.61
		IS3	0.0517	0.0064	13.40	1.67
		Mean of ISs	–	–	*13.90*	*1.26*
		Rep	0.0501	0.0033	13.83	0.92
	MV	CS	0.0283	0.0039	24.51	3.34

IS: independent mesocosm system (model built individually for each mesocosm as an independent system); Rep: replicates (model built using the three mesocosm systems as replicates); CS: composite sample. $s_{t1/2}$ values for IS1, IS2, IS3, and Rep were calculated using error propagation. $s_{t1/2}$ values for Mean of ISs were calculated using the standard deviation statistic.

combination with fipronil. When applied in combination, the 2,4-D dissipation rate was slower than when applied individually. Such behavior may be related to the synergistic effect that influenced chemical/biological degradation processes, resulting in a slower dissipation (Roselló-Márquez et al., 2019). In contrast, the obtained p-value for fipronil (p-value = 0.1023) indicates that the null hypothesis must not be rejected, and there is no statistically significant difference between half-live times for fipronil when applied individually and in combination with 2,4-D.

Despite the lack of replication of the MV treatment, a qualitative analysis shows that 2,4-D half-life time decreased with vinasse application. This effect was observed since vinasse has a high organic matter and nutrient content, which may have favored microbial degradation in these systems (Abate Jote, 2019; Ordaz-Guillén et al., 2014). In contrast, fipronil half-life time increased with vinasse application, which may be associated with the increase in turbidity in MV treatments (Table S1) and, consequently, a decrease in the penetration depth of the solar radiation compared to mesocosms without the addition of vinasse. Under field conditions, fipronil undergoes degradation by solar radiation, but the dissipation rate depends on the radiation's depth of incidence and the water body's turbidity (Bonmatin et al., 2015).

The dissipation of organic pollutants in aquatic systems is influenced by several different processes (photolysis, chemical and biological degradation, sediment/water interface interaction, plant uptake, bioaccumulation, volatilization, and others) that interfere with their persistence and fate in the environment (Durães et al., 2018). In the aquatic environment, 2,4-D has a half-life of 10 to 50 days and is generally found in its anionic form. Anion decomposition can occur through hydrolysis, photolysis, and aerobic and anaerobic microbial degradation, and the dissipation rate depends on pH, temperature, dissolved oxygen, and nutrient levels (Abate Jote, 2019). The major transformation products of 2,4-D in the aquatic environment are 2-chlorohydroquinone, 2,4-dichlorophenol, and 1,2,4-benzentriol, which are formed via aerobic biodegradation, anaerobic degradation, and photolysis, respectively (Abate Jote, 2019; Islam et al., 2018; Ordaz-Guillén et al., 2014).

In contrast, the half-life of fipronil varies from 9 h to 220 days in the aquatic environment and depends on the characteristics of the water body (Bonmatin et al., 2015; PPDB, 2021b; Singh et al., 2021; Tingle et al., 2003). Fipronil's major degradation pathways include reduction, oxidation, photolysis, and hydrolysis. Such pathways mainly yield the transformation products fipronil-sulfide, fipronil-sulfone, fipronil-desulfinyl, and fipronil-amide, respectively (Tomazini et al., 2021).

In the mesocosms with vinasse application (MV), the highest concentrations of fipronil-sulfone and fipronil-sulfide were detected after 45 and 73 days of fipronil application. In the mesocosms without vinasse application (F and M), the maximum concentrations of degradation products were detected 31 days after contamination. This result corroborates with the fact that insecticide dissipation was slower in MV systems than in F and M treatments. In addition to the already mentioned degradation mechanisms, due to the presence of macrophytes and different organisms, the absorption of pesticides by plants and bioaccumulation are processes involved in the dissipation of 2,4-D and fipronil in mesocosm systems.

3.3. Pesticide fate in the sediment compartment

The dynamics of pesticides in surface waters resulted in the deposition of 2,4-D (Fig. 4) and fipronil (Fig. 5) in the sediment, which influences their bioavailability and possible adverse effects on benthic organisms (Islam et al., 2018). The maximum concentrations of 2,4-D in the different treatments (48, 106, and 228 μg kg^{-1}) were observed in the sediment samples collected 2 and 7 days after pesticide application. A trend of decreasing 2,4-D concentrations was observed 14 days after its application, ranging from 1 to 84 μg kg^{-1}. After this period, 2,4-D concentration was close to the IQL and lower than the IQL 150 days after its application. Fipronil was quantified in higher concentrations in sediment samples collected 7, 14, and 21 days after its application (33, 44, and 14 μg kg^{-1}, respectively). Vinasse application increased the organic matter content in the sediment, favoring the sorption of fipronil in that compartment. 21 days after its application, a trend of decreasing fipronil concentrations was observed, with concentrations ranging from 0.1 to 10 μg kg^{-1}.

2,4-D concentration levels in the sediment compartment were higher than fipronil, despite 2,4-D being more soluble in water and having a lower sorption capacity to the sediment (lower K_{oc}) than fipronil. This result may be related to the concentration of 2,4-D dissolved in water since the initial concentration of 2,4-D was higher than that of fipronil.

Fipronil-sulfide and fipronil-sulfone transformation products were also monitored and quantified in sediment samples collected 7 and 14 days after fipronil application. The concentrations of fipronil-sulfide and fipronil-sulfone ranged from 0.2 to 23 μg kg^{-1} and 0.1–14 μg kg^{-1}, respectively. The detection of fipronil transformation products may be associated with the biological degradation of fipronil in the sediment and/or the deposition of the transformation products formed in the aqueous medium (Demcheck and Skrobialowski, 2003). The maximum concentrations of fipronil-sulfide and fipronil-sulfone were quantified in the sediment of the mesocosms in MV treatments. Vinasse application increased the content of organic matter and nutrients, which may have favored the microbial degradation of fipronil in the sediment or the sorption of fipronil-sulfide and fipronil-sulfone dissolved in water.

3.4. Environmental risk assessment and ecotoxicological tests

LPNEC values retrieved from the NORMAN Ecotoxicology Database for 2,4-D, fipronil, fipronil-sulfide, and fipronil-sulfone for chronic exposure are displayed in Table S4 for freshwater and sediment (NORMAN, 2023). Risk assessment was performed only in the mesocosms with pesticide application (D or F, M, and MV).

3.4.1. Water compartment

The risk assessment performed in the water compartment (Fig. S1) indicated a potential risk to biodiversity for all compounds. From the 70 analyzed samples (10 sampling times (2, 4, 7, 14, 21, 31, 45, 75, 101, 150 days), 3 treatments with pesticide application (D or F, M, MV), and 3 independent mesocosm systems for each treatment except for the composite sample for MV), 2,4-D presented 64 (91%) samples with RQ values > 1, whereas fipronil presented 69 (99%). Also, fipronil-sulfide and fipronil-sulfone presented 31 (44%) and 32 (46%) samples with RQ values > 1. 2,4-D RQ values varied from 0.07 to 1,359, whereas fipronil RQ values varied from 1.3 to 22,078. Finally, fipronil-sulfide and fipronil-sulfone RQ values varied from 0.08 to 25 and 0.08 to 35, respectively. However, it is important to note that fipronil-sulfide and fipronil-sulfone recovery during method validation were unsatisfactory (Goulart et al., 2020). Thus, risk assessment for the fipronil transformation products must be considered preliminary.

3.4.2. Sediment compartment

The risk assessment performed in the sediment compartment (Fig. S2) indicated a potential risk to biodiversity for all compounds. From the 63 analyzed samples (7 sampling times (2, 7, 14, 21, 75, 85, 150 days), 3 treatments with pesticide application (D or F, M, MV), and 3 independent mesocosm systems for each treatment), 2,4-D presented 31 (49%) samples with RQ values > 1, whereas fipronil presented 51 (81%). Also, fipronil-sulfide and fipronil-sulfone presented 39 (62%) and 40 (63%) samples with RQ values > 1. 2,4-D RQ values varied from 0.4 to 98, whereas fipronil RQ values varied from 12 to 2582. Finally, fipronil-sulfide and fipronil-sulfone RQ values varied from 0.09 to 68 and 0.8 to 104, respectively.

3.4.3. Ecotoxicological tests

The toxicological effects observed after *in situ* and laboratory exposure were integrated into the pesticide dissipation profiles presented in Figs. 6 and 7.

da Silva Pinto et al. (2021a) assessed the functional responses of the amphipod *Hyalella meinerti* on acute (0–96 h after application) and chronic (7–14 days post) *in-situ* assays, as well as chronic laboratory tests by using water sampled 30 and 75 days post-application by the pesticides and vinasse. In the *in-situ* tests, 100% mortality was reported after exposure to mesocosms treated with fipronil, vinasse, and both mixtures. Although 2,4-D did not cause acute toxicity, it affected reproduction in chronic exposure. Regarding the laboratory assays, 100% lethality persisted after exposure to water sampled 30 days post-application in fipronil and mixture treatments. Also, exposure to 2,4-D-contaminated water reduced the number of juveniles produced. After 75 days, exposure to samples from fipronil and pesticide mixture mesocosms impaired sexual behavior and reproduction. The high mortality persisted in pesticides with vinasse-treated mesocosms, revealing the toxic potential of this mixture. Throughout time, exposure to

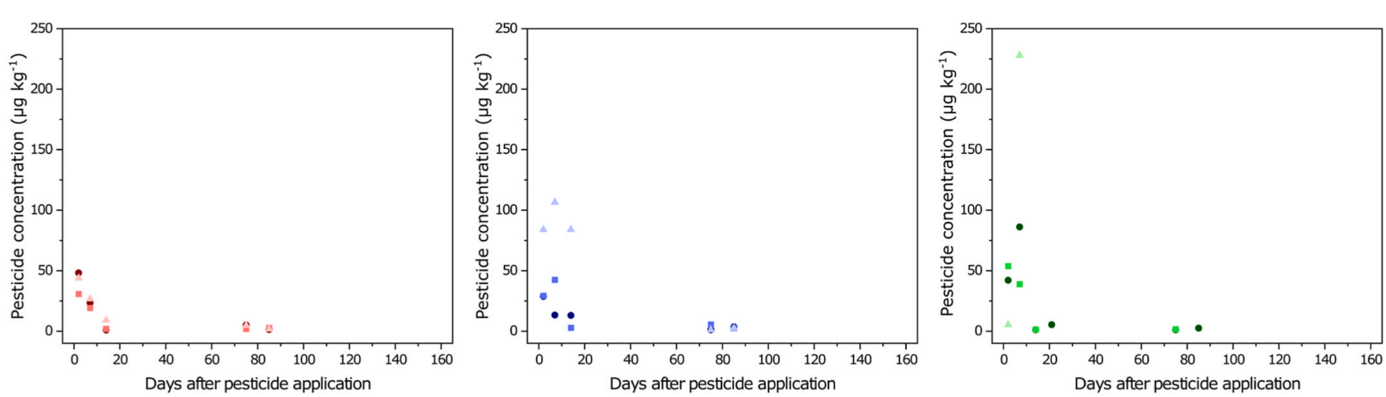

Fig. 4. 2,4-D concentration (μg kg^{-1}) in the sediment compartment (2–150 days after pesticide application) for treatments D (red), M (blue), and MV (green) for independent mesocosm systems IS1 (dots), IS2 (squares), and IS3 (triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

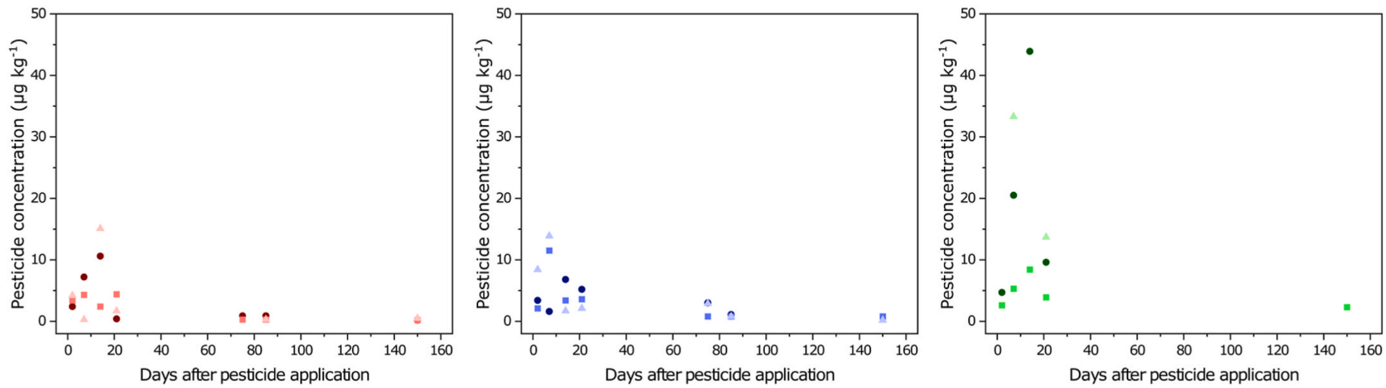

Fig. 5. Fipronil concentration (μg kg^{-1}) in the sediment compartment (2–150 days after pesticide application) for treatments F (red), M (blue), and MV (green) for independent mesocosm systems IS1 (dots), IS2 (squares), and IS3 (triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

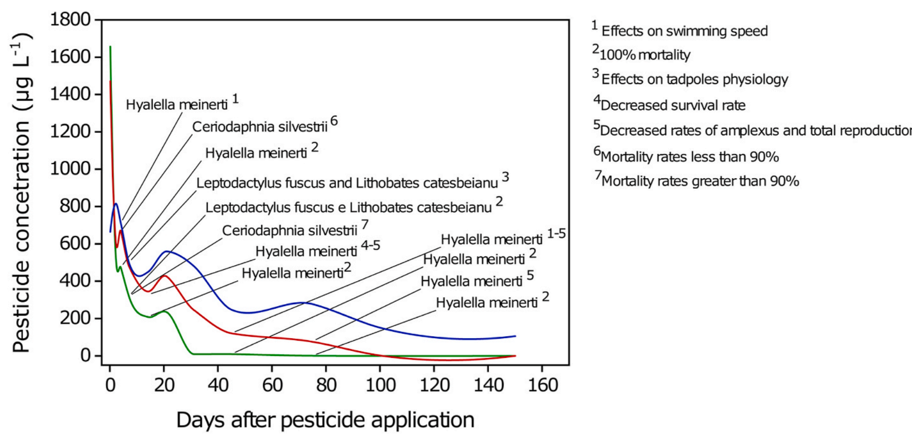

Fig. 6. 2,4-D concentration in mesocosm systems (2 h–150 days after pesticide application) associated with ecotoxicological effects on organisms of different trophic levels for treatments D (red), M (blue), and MV (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

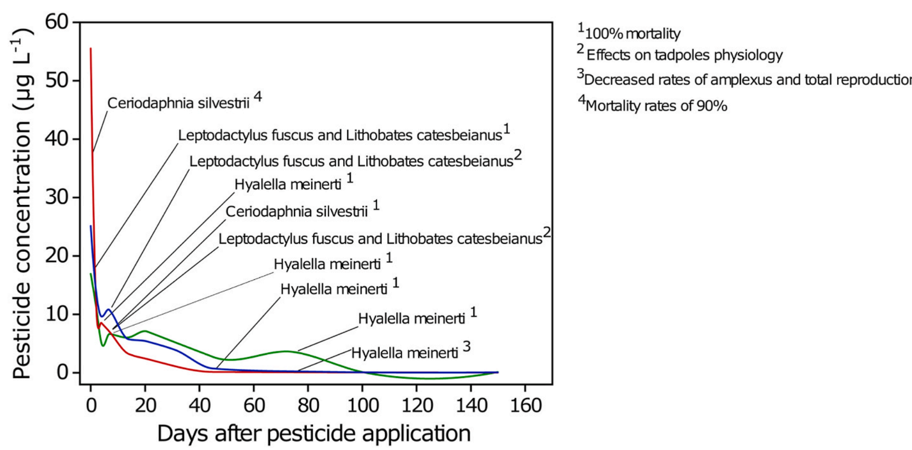

Fig. 7. Fipronil concentration in mesocosm systems (2 h–150 days after pesticide application) associated with ecotoxicological effects on organisms of different trophic levels for treatments F (red), M (blue), and MV (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contaminated treatments altered the swimming behavior of surviving organisms, impairing the ability to escape from predators and forage.

Freitas et al. (2022) studied the biochemical responses in tadpoles of the native species *Leptodactylus fuscus* and the nonnative species *Lithobates catesbeianus.* Organisms were caged in the mesocosms before pesticide application, and the tests lasted seven days. All organisms confined in mesocosms treated with vinasse alone and mixed with pesticides died soon after application (approximately 1 h). Tadpoles from treatments receiving pesticides alone had no lethal responses; however, the levels of antioxidant activity- and β-esterase- associated enzymes were altered, in addition to neurotoxicity and changes in lipid content. The authors verified that the treatment of the pesticide mixture induced most of the sublethal responses in the tadpoles. Also, the native species presented higher sensitivity to fipronil than the nonnative species.

Effects of pesticide application were also studied (*in-situ* and laboratory assays) with the native species *Ceriodaphnia silvestrii* by Silva et al. (2020). Fipronil, vinasse, and both mixtures provoked high mortality rates even after 21 days from application in acute *in-situ* assays. In chronic experiments, reproduction was impaired in all treatments where organisms survived throughout the experiment, and only 2,4-D did not reduce the population growth rates. An additive effect was observed in the mixture of pesticides and vinasse. The authors concluded that fipronil, vinasse, and both mixtures cause high toxicity even in the recommended doses with risks to aquatic ecosystems in the edge-of-fields.

In addition to the effects assessed on the aquatic organisms, the phytotoxicity of terrestrial plants was studied. Ogura et al. (2022) evaluated the effects of irrigation with water collected from the mesocosms (2 h, 14 days, and 30 days after application) on germination and growth of *Eruca sativa* L. The results indicated high phytotoxicity for 2, 4-D since it caused complete growth inhibition of irrigated plants even in low doses (0.2 μg L^{-1}). Conversely, no deleterious responses were reported after irrigation with water from treatments with fipronil and vinasse alone. 30 days after pesticide application, the effects of 2,4-D decreased dramatically due to a significant decrease in concentration. In addition, irrigation with 2,4-D-treated water inhibited the growth of *Phaseolus vulgaris* L. and *Zea mays* L., especially the root stretching. Finally, both species' growth decreased after irrigation with water from vinasse-treated mesocosms.

4. Conclusions

The dissipation kinetics of fipronil and 2,4-D in the water compartment were fitted to the first-order kinetics model and were influenced by the mesocosms' chemical composition, the compounds' physicochemical properties, and environmental factors. In the mesocosms where vinasse was applied, pesticide dissipation showed significant differences compared to systems without vinasse application. The dynamics of pesticides in surface waters resulted in the deposition of these compounds in the sediment. The transformation products fipronil-sulfide and fipronil-sulfone were quantified in the mesocosm water samples 21 days after the application of fipronil. The conducted risk assessment showed a potential risk to environmental health. RQ values for 2,4-D were up to 1359 in freshwater and 98 in sediment, whereas RQ values for fipronil were up to 22,078 and 2,582, respectively. Finally, significant toxicological effects were observed in the organisms of different trophic levels.

CRediT authorship contribution statement

Bianca Veloso Goulart: Investigation, Data curation, Writing – original draft, Methodology, Formal analysis. **Beatriz De Caroli Vizioli:** Formal analysis, Investigation, Methodology, Writing - original draft. **Thandy Junio da Silva Pinto:** Methodology. **Juliane Silberschmidt Freitas:** Methodology. **Raquel Aparecida Moreira:** Methodology. **Laís Conceiçao Menezes da Silva:** Methodology. **Maria Paula Cardoso Yoshii:** Methodology. **Laís Fernanda de Palma Lopes:** Methodology. **Allan Pretti Ogura:** Methodology. **Theodore Burdick Henry:** Writing – review & editing. **Evaldo Luiz Gaeta Espindola:** Methodology, Writing – review & editing. **Cassiana Carolina Montagner:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140569.

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