

## Impacts of pesticides and vinasse on the composition and functional diversity of aquatic macroinvertebrates exposed in a mesocosm system

Carina Pereira Cotta<sup>a</sup>, Thandy Junio da Silva Pinto<sup>b,\*</sup>, Diego Ferreira Gomes<sup>a</sup>, Allan Pretti Ogura<sup>a</sup>, Laís Conceição Menezes da Silva<sup>a,c</sup>, Janaina Braga do Carmo<sup>d</sup>, Cassiana Carolina Montagner<sup>b</sup>, Evaldo Luiz Gaeta Espíndola<sup>a</sup>, Raquel Aparecida Moreira<sup>a,e</sup>

<sup>a</sup> Laboratory of Ecotoxicology and Applied Ecology (LEEA), Department of Hydraulics and Sanitation (SHS), Postgraduate Degree in Environmental Engineering Sciences (PPG-SEA), São Carlos School of Engineering (EESC), University of São Paulo, Av. Trabalhador São Carlense, 400, 13.560-970 São Carlos, SP Brazil

<sup>b</sup> Institute of Chemistry, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil

<sup>c</sup> Laboratory of Microbial Processes & Biodiversity, Federal University of São Carlos (UFSCar), São Carlos, São Paulo, Brazil

<sup>d</sup> Department of Physics, Chemistry and Mathematics, Federal University of São Carlos (UFSCar), Rodovia João Leme dos Santos, SP-264, km 110, Sorocaba, São Paulo 18052-780, Brazil

<sup>e</sup> Department of Basic Sciences (ZAB), Faculty of Animal Science and Food Engineering (FZEA) at the University of São Paulo (USP), Av. Duque de Caxias Norte, 225, 13635-900 Pirassununga, Brazil

### ARTICLE INFO

#### Keywords:

Functional feeding groups  
Herbicide  
Invertebrates  
Insecticide  
Tropical species  
Stillage

### ABSTRACT

Modern agriculture plays a significant role in the global economy. However, the continuous use of pesticides and fertilizers raises concerns about aquatic pollution and its effects on indigenous organisms. Thus, this study investigated the impact of the pesticides Regent® 800 WG (active ingredient - a.i. fipronil) and DMA® 806 BR (a.i. 2,4-D), as well as sugarcane vinasse, an ethanol industry byproduct applied in sugarcane crops as fertilizer, individually and in mixtures, on the functional diversity of benthic macroinvertebrates at a mesocosm scale. For that, open-air mesocosms were used, in which the effects of contaminants were evaluated over time (1, 7, 14, 28, 75, and 150 days after contamination). The macroinvertebrate community was monitored using colonization structures, while physicochemical parameters, metals, and pesticides were analyzed throughout the experiment. In the control and 2,4-D-treated mesocosms, collectors, predators, and scrapers were the predominant organisms. In mesocosms contaminated with fipronil and pesticide mixture, predators such as Odonata and Tanytopodinae disappeared after contamination. In the pesticide and vinasse mixture treatment, collectors, filter feeders, and scrapers predominated over time. Functional richness and diversity were reduced in the pesticide mixture. Contamination by the mixture of pesticides and vinasse reduced functional diversity, due to the disappearance of predators and tolerant organisms. Therefore, the functionality of the macroinvertebrate fauna may be affected by the application of these contaminants in areas close to sugarcane cultivation. These effects represent a risk to the balance of aquatic ecosystems, compromising trophic chains, ecological functions, and the overall health of the aquatic ecosystem.

### 1. Introduction

In recent decades, the use of pesticides has increased significantly worldwide, driven primarily by the modernization of agriculture and the pursuit of higher productivity (Santos and Bernardes, 2018). Brazil, in particular, has become the largest consumer of pesticides, with approximately 755,489 tons of active ingredients in 2023 (BRASIL, 2025), and 663 new pesticide formulations registered in 2024, the highest number ever recorded (BRASIL, 2025). In this context, 2,

4-dichlorophenoxyacetic acid (2,4-D) and fipronil stand out as highly consumed pesticides (Cotta et al., 2023), which are primarily, but not exclusively, applied on crops such as sugarcane (Goulart et al., 2020).

The herbicide 2,4-D is one of the most commonly used active ingredients in monocultures, acting as a plant growth inhibitor, particularly in eudicots (Marcato et al., 2017). It induces changes in the cell wall, increasing its plasticity, and abnormally stimulates protein and ethylene production. These processes trigger uncontrolled cell division and compromise the vascular tissues of plants ((US EPA, 2005); Islam

\* Correspondence author at. Institute of Chemistry, University of Campinas, R. Zeferino Vaz - Barão Geraldo, 13083-970 Campinas, SP Brazil.

E-mail address: [thandyjuniosilva@gmail.com](mailto:thandyjuniosilva@gmail.com) (T.J.S. Pinto).

<https://doi.org/10.1016/j.aquatox.2026.107724>

Received 17 August 2025; Received in revised form 13 January 2026; Accepted 17 January 2026

Available online 18 January 2026

0166-445X/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

et al., 2018). 2,4-D exhibits low biodegradability in aquatic environments and can persist for long periods (Chingombe et al., 2006). It has been detected in water bodies worldwide at concentrations ranging from 0.009 to 2.44  $\mu\text{g L}^{-1}$  (CETESB, 2018; Ferreira et al., 2022). Fipronil, on the other hand, is a highly hazardous insecticide with strong activity, systemic action, and a broad spectrum of pest control (Gonçalves et al., 2022). It primarily acts by inhibiting the neurotransmitter gamma-aminobutyric acid (GABA) (Singh et al., 2021). Fipronil has been detected in various freshwater environments worldwide, ranging from 0.006 to 26.2  $\mu\text{g L}^{-1}$  (Bradley et al., 2017; Cuenca et al., 2022). In Brazil, both fipronil and 2,4-D have also been identified in various water bodies in different states of the country, at concentrations ranging from 0.1 to 445  $\mu\text{g L}^{-1}$  and from 1.14 to 366  $\mu\text{g L}^{-1}$  (Marchesan et al., 2010; Pinheiro et al., 2010; CETESB, 2018, CETESB, 2021, Panis et al., 2022), exceeding national and international guidelines for the protection of aquatic life (CONAMA, 2005; US EPA, 2015).

In addition to the application of pesticides in crops, the generation of sugarcane vinasse is another significant environmental concern associated with ethanol production. Vinasse is a byproduct of the manufacturing process with a high pollution potential (Azevedo-Santos et al., 2024). It is estimated that for every liter of ethanol produced, approximately 14 liters of vinasse are generated (Espana-Gamboa et al., 2012). Vinasse has been used as an alternative fertilizer in sugarcane monocultures due to its high nutrient content, such as nitrogen and phosphorus, which contribute to enriching the soil (Baffa et al., 2009). Its chemical composition is predominantly water (94-97%), along with high concentrations of organic matter, potassium, calcium, magnesium (Wadt, 2008; Ferreira, 2009), and other metals (Cotta et al., 2023). Vinasse composition can vary depending on the specific alcohol production process employed (Godoi et al., 2019; Santos et al., 2019).

Pesticides and other compounds can reach aquatic ecosystems (Kumar et al., 2023), whether through surface runoff, leaching, volatilization, drift, and other transport pathways (Stehle and Schulz, 2015; Freitas et al., 2024). Vinasse, in turn, can also negatively impact the aquatic biota (Cotta et al., 2023; Azevedo-Santos et al., 2024), causing significant harm to aquatic ecosystems (Rulli et al., 2023). There is growing evidence that the presence of these contaminants in aquatic environments leads to severe adverse effects, including alterations in reproduction (Pinto et al., 2021), predation (Ruggiero et al., 2024), growth (Saraiva et al., 2018), behavior ((Silva et al., 2015); Freitas et al., 2019), and alterations in antioxidant defense systems, resulting in oxidative damage (Da Silva et al., 2024); in more extreme cases, exposure can lead to organism mortality (Botelho et al., 2012; Silva et al., 2021). Over the long term, these impacts can compromise the population dynamics of aquatic ecosystems (Azevedo-Santos et al., 2024).

The classification of aquatic macroinvertebrates into functional feeding groups (FFGs) is based on their morpho-behavioral characteristics, which are directly related to how these organisms obtain and process food. This approach is an important tool for characterizing ecosystems and assessing the impacts they experience (Cummins et al., 2005). In addition to the direct effects on organisms, water pollution from pesticides and other contaminants, such as vinasse, can indirectly alter the type and/or availability of food resources (Kefford et al., 2024; Schafer et al., 2012)). This results in the elimination and/or favoring of certain functional groups, with consequent impacts on functional richness and diversity and population balance (Duan et al., 2011).

In a previous study, Cotta et al. (2023) described the impacts of the pesticides fipronil and 2,4-D, and vinasse, both individually and in combination, on macroinvertebrate communities by using colonization structures in outdoor mesocosms. They exposed the communities to environmentally realistic concentrations of these compounds for 1, 7, 14, 28, 75, and 150 days after contamination, observing changes in community structure such as shifts in dominance, exclusion of groups, and reduction in richness. However, what remains unknown is whether and how these community shifts responses mean in terms of ecosystem functions, i.e., the FFGs reorganization and/or compensations of

functional diversity over time and in the different contamination scenarios. In the present study, we addressed this gap by processing these taxonomic data into FFG composition and diversity of benthic macroinvertebrates across the contamination exposure and stressors combination. Based on this, the pesticides' modes of action, and the pathways of resources, we formulated the following four mechanistic hypotheses: (i) the herbicide 2,4-D directly impacts vegetal scrapers, limiting their energy intake by affecting their main food source (periphyton/macrophyte biofilms) through its auxin-mimicking (hormone-disrupting) mechanism of action; (ii) fipronil is expected to reduce the insect abundance through its direct action on insect GABA/GluCl receptors (neurotoxicity), followed by effects on predators and collector-gatherer composition that are composed mainly by insects or depend on invertebrate detritus; (iii) vinasse alone, due to its high organic content, favours collector-gatherer, increasing fine particulate organic matter (FPOM) and detrital resources; however, the high oxygen demand can simultaneously filter out sensitive taxa, selecting tolerant species; and (iv) mixture settings are expected to promote a depletion of functional diversity through additive/synergistic toxicity and changes in resource/oxygen content and pH level, intensifying shifts in FFG composition relative to exposures to a single stress factor.

## 2. Materials and methods

### 2.1. Experimental design

The mesocosm experiments were conducted at the Center for Water Resources and Environmental Studies - CHREA (22°01'22"S, 43°57'38"W), located at the University of São Paulo (USP), in the Southeastern region of Brazil. The mesocosms were set up in non-toxic polypropylene tanks with a capacity of 1500 L, a depth of 0.83 m, and a top diameter of 1.75 m (Fig. 1). To minimize thermal variations, each unit was partially buried (0.6 m below ground level). The substrate was prepared with a 20 cm layer of natural oxisol collected *in situ* (detailed physicochemical properties described in Figueirêdo et al. (2020), over which well water was added to reach a 60 cm water column. The biotic community was established by inoculating sediments from uncontaminated mesocosms previously colonized at CRHEA (Moreira et al., 2017), complemented with aquatic macrophytes (*Salvinia auriculata*, *Pistia stratiotes*, *Eichhornia crassipes*, and *Myriophyllum aquaticum*), including the fauna associated with their root systems, as well as zooplankton and phytoplankton samples collected from the Broa Reservoir (Itirapina, São Paulo). The mesocosms remained in a stabilization phase for six months before treatment application, allowing natural colonization by benthic and aerial macroinvertebrates. We did not perform taxonomic monitoring during stabilization; instead, we inferred community stabilization based on (i) the prolonged duration without disturbance, which greatly exceeds the 4-8 weeks colonization windows commonly used for macroinvertebrates on artificial substrates and in stream/pond mesocosms (US EPA, 1999; CALEPA, 2005; Bartkowska et al., 2023), (ii) compliance with outdoor mesocosm guidelines that emphasize stable abiotic conditions before exposure (OECD, 2006), (iii) our experiment also had controls, that could be used to infer the level of variability in communities, and (iv) we sampled twice prior to contamination across all treatments to indicate baseline responses in mesocosms. One week before contamination, water quality parameters (temperature, dissolved oxygen, pH, and conductivity) were measured in all tanks to confirm consistency between tanks; values are presented in Table S1.

The experiment followed a randomized design with six treatments and three replicates each: untreated control (C); treatment with the herbicide DMA® 806BR (active ingredient 2,4-D, Dow AgroSciences Industrial Ltda) (D); treatment with the insecticide Regent® 800WG (active ingredient fipronil, BASF AS) (F); treatment with vinasse (V); mixture of 2,4-D and fipronil (M); and mixture of 2,4-D, fipronil, and vinasse (MV). The target concentrations were calculated based on the recommended doses for the application in sugarcane (pre-emergence



Fig. 1. Colonization structures (A); overview of outdoor mesocosms (B); zoomed-in view of a mesocosm contaminated with the pesticide mixture (C).

stage), considering a total drift scenario in which 100% of the compounds would reach the aquatic surface, as described by Pinto et al. (2021a). The recommended pesticide doses are 3.5 L ha<sup>-1</sup> of DMA® 806BR (equivalent to 2.35 kg of active ingredient ha<sup>-1</sup>) and 500 g ha<sup>-1</sup> of Regent® 800WG (400 g of active ingredient ha<sup>-1</sup>). Therefore, considering the volume of the experimental units, 678 mg of 2,4-D (*i.e.*, 1 mL of the commercial product) and 96 mg of fipronil (*i.e.*, 120 mg of the commercial product) were added per mesocosm. Each mesocosm contaminated with vinasse received 20 L, as per regional recommendations (Pinto et al., 2021a). The expected nominal concentrations were 447 µg L<sup>-1</sup> for 2,4-D, 64 µg L<sup>-1</sup> for fipronil, and 1.3% (v/v) for vinasse for both the individual and mixture treatments.

## 2.2. Collection and analysis of the community of benthic macroinvertebrates

The experiments with benthic macroinvertebrates were carried out by Cotta et al. (2023), which describes the methodological procedures for community sampling and characterization, as well as the effects of pesticides and vinasse pollution on the composition and ecological indices of macroinvertebrate assemblages. Experiments were conducted between October 2018 and April 2019 to evaluate the benthic macroinvertebrate community, using standardized colonization structures with natural substrate and dry biomass of *S. auriculata* (Fig. 1). In each mesocosm, 16 structures were installed 50 days prior to the first sampling to allow natural colonization. Sampling occurred eight times: two before (T-7 and T-1 days) and six after contamination (T1, T7, T14, T28, T75, and T150 days). At each collection, two structures per mesocosm were removed and preserved in ethanol 70% for laboratory analysis.

In parallel with the biological collections, water sampling for chemical analysis was conducted at the same periods. For this, sterile glass bottles (1L) were used for pesticide quantification, polyethylene bottles (1L) for physicochemical and nutrient analyses, as well as additional samples collected on the contamination day specifically for metal analysis. The physicochemical parameters of the water, including temperature, pH, dissolved oxygen, turbidity, and electrical conductivity, were measured *in situ* using a multiparameter probe (HORIBA U-10), while chlorophyll *a* was quantified using a portable fluorimeter (Turner Designs Aquafluor).

## 2.3. Composition and functional diversity

Data on the taxonomic composition of the macroinvertebrate community were obtained in Cotta et al. (2023). The authors identified the fauna to the family level, except for the classification of Chironomidae into subfamily and Oligochaeta into subclass. Based on taxonomic identification, in the present study, the families were categorized into Functional Feeding Groups (FFGs) according to the way in which organisms obtain food. The FFGs were: (i) collector-gatherers (CG), (ii) filter-feeders (FF), (iii) filter-collectors (FC), (iv) scrapers (Sc), (v) shredders (Sh), and (vi) predators (Pr). This classification was based on

the keys established in previous studies (Cummins et al., 2005; Damani-Ambarita et al., 2016; Ramírez & Gutiérrez-Fonseca, 2014) and considered the predominant feeding strategies of each taxon, as detailed in Table 4 (results section). Accordingly, individuals from Chironominae, Orthocladinae, Leptophlebiidae, and Culicidae were classified as CG, whereas Ceratopogonidae, Sciomyzidae, Tabanidae, Corixidae, and Muscidae were classified as Pr. For the other families, the classification was consistent with the cited references. All organisms from Hydrophilidae and Dryopidae were in the adult stage and were classified as CG and Sc, respectively.

## 2.4. Chemical analysis

The analyses of the physicochemical and biological variables of the water, as well as the determination of metals and pesticides in the sediments, were carried out in synchronization with the collection schedule of the previously established colonization structures. For water analyses, specific and validated analytical methodologies were adopted for each parameter. Water hardness was determined by titration according to the ABNT NBR 12621 standard (1995), while total dissolved phosphorus was quantified by spectrometry following the method described by Andersen (1976). Ammonium ion concentrations were analyzed as per Hansen and Koroleff (2007), and nitrite and nitrate levels were determined according to Mackereth et al. (1978). Total nitrogen was assessed following standardized APHA methodology (APHA, 1995), and turbidity was measured using a spectrophotometer (manufacturer: model Hach DR 2000).

The quantification of pesticides in water samples was performed using liquid chromatography coupled with mass spectrometry (LC-MS/MS), following a validated protocol by Goulart et al. (2020). The limit of quantification (LOQ) of the method for 2,4-D and fipronil was 5.0 ng/L and 0.1 ng/L, respectively. In parallel, metal concentrations in both the pure vinasse used for contamination and the water samples were determined by atomic absorption spectrometry using AA 240FS VARIAN equipment, as per APHA guidelines (APHA, 2018). Metals were assessed in unfiltered samples; thus, values are reported as total metals. Organic quality parameters included the determination of biochemical oxygen demand (BOD) through a 5-day incubation method and chemical oxygen demand (COD) using the closed reflux technique (APHA, 2018), the latter performed on pure vinasse aliquots.

## 2.5. Data analysis

To evaluate the effects of pesticides and vinasse on the functional diversity of the benthic community, mixed model analysis of variance (ANOVA) was employed, considering a repeated measures design. In this approach, the different experimental treatments were included as between-subject effects, while the sampling times were treated as within-subject effects (repeated measures). The sphericity assumption was verified using Mauchly's test. In cases where this assumption was not met, the Greenhouse-Geisser correction was applied to adjust the

degrees of freedom for temporal analyses. Additionally, the interaction between treatments and time was assessed by multiple group comparisons using Tukey's post-hoc test, with a significance level established at  $\alpha = 0.05$ . Analyses were carried out in software Jamovi (version 2.3.21.0). For the physicochemical parameters measured in the mesocosms, multivariate differences among treatments and sampling times were evaluated using PERMANOVA, based on Euclidean distances calculated from standardized physicochemical variables (z-score). The model included the factors treatment, time, and their interaction, with significance assessed using 999 permutations. All analyses were conducted in RStudio (version 2026.01.0) using R software (version 4.5.2) and the vegan package (Oksanen et al., 2022). A principal components analysis (PCA) was used to visualize the multivariate patterns detected by the PERMANOVA, as the analysis was based on Euclidean distances derived from standardized physicochemical variables. The PCA was performed using CANOCO software (version 4.5).

### 3. Results and discussion

#### 3.1. Description of contaminants

##### 3.1.1. Vinasse

The vinasse used was characterized by low pH (3.91), high concentrations of BOD (46500 mg L<sup>-1</sup>) and COD (107000 mg L<sup>-1</sup>), as well as high levels of phosphorus (150 mg L<sup>-1</sup>) and total nitrogen (639 mg L<sup>-1</sup>), electrical conductivity (8.42 mS cm<sup>-1</sup>), and hardness (3500 mg CaCO<sub>3</sub> L<sup>-1</sup>). The physicochemical parameters of the raw vinasse applied to the mesocosms are given in Table 1.

Vinasse enters aquatic ecosystems through diffuse pathways (Gunkel et al., 2007) and represents a major environmental concern (Fuess and Garcia, 2014), as it has a high polluting potential, being up to one hundred times more harmful than domestic sewage (Christofoletti et al., 2013). This occurs due to the characteristics of vinasse, a dark brown effluent characterized by high concentrations of organic compounds, various ions, including metals, in addition to high electrical conductivity, COD, and BOD, and reduced pH (Pinto et al., 2021; Piffer et al., 2022; Pinto et al., 2023). Vinasse composition has the potential to significantly alter several physicochemical parameters of water, as

**Table 1**  
Physicochemical characterization of pure vinasse adapted from Pinto et al. (2021).

Parameter	Concentration
pH	3.91
Electrical conductivity (mS cm <sup>-1</sup> )	8.42
Biological oxygen demand - BOD (mg L <sup>-1</sup> )	46.500
Chemical oxygen demand - COD (mg L <sup>-1</sup> )	107.000
Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	3.500
Total nitrogen (mg L <sup>-1</sup> )	639
Total phosphorus (mg L <sup>-1</sup> )	150
Ammonium (mg L <sup>-1</sup> )	53
Chromium - Cr (mg L <sup>-1</sup> )	0.207
Copper - Cu (mg L <sup>-1</sup> )	3
Lead - Pb (mg L <sup>-1</sup> )	0.340
Manganese -Mn (mg L <sup>-1</sup> )	6.24
Iron - Fe (mg L <sup>-1</sup> )	247
Nickel - Ni (mg L <sup>-1</sup> )	0.183
Cadmium - Cd (mg L <sup>-1</sup> )	0.025
Zinc - Zn (mg L <sup>-1</sup> )	1.23
Cobalt - Co (mg L <sup>-1</sup> )	0.219
Lithium - Li (mg L <sup>-1</sup> )	0.031
Magnesium - Mg (mg L <sup>-1</sup> )	391
Antimony - Sb (mg L <sup>-1</sup> )	0.530
Aluminium - Al (mg L <sup>-1</sup> )	500
Barium - Ba (mg L <sup>-1</sup> )	2
Calcium - Ca (mg L <sup>-1</sup> )	1.140
Sodium - Na (mg L <sup>-1</sup> )	60
Potassium - K (mg L <sup>-1</sup> )	3.360

Source: adapted from Pinto et al. (2021).

observed in this study, with an increase in metal concentrations, a decrease in pH, and increases in electrical conductivity, phosphorus, and ammonium concentrations. Such changes can trigger eutrophication processes in receiving water bodies (Hoarau et al., 2018), leading to acute toxicity for different organisms (Fraga et al., 2024) or even the death of organisms at different trophic levels (Moran-Salazar et al., 2016; Cotta et al., 2023; Pinto et al., 2023). The composition of raw vinasse can present great variability, as Coelho et al. (2018) observed extremely high concentrations of metals, such as: 2.660 mg L<sup>-1</sup> of potassium (K), 31.300 mg L<sup>-1</sup> of sodium (Na), 527.0 mg L<sup>-1</sup> of calcium (Ca), 269.0 mg L<sup>-1</sup> of magnesium (Mg), 6.920 mg L<sup>-1</sup> of iron (Fe) and 2.390 mg L<sup>-1</sup> of zinc (Zn). Similarly, other studies also identified high metal contents in different vinasse samples, with values ranging from 1.600 to 3.400 mg L<sup>-1</sup> of K, 162 to 264 mg L<sup>-1</sup> of Mg, 670 to 3.160 mg L<sup>-1</sup> of Ca, 8.6 to 114 mg L<sup>-1</sup> of Na, 1.2 to 1.7 mg L<sup>-1</sup> of Zn, 0.35 to 0.8 mg L<sup>-1</sup> of copper (Cu), and 0.04 to 3.6 mg L<sup>-1</sup> of chromium (Cr) (Christofoletti et al., 2013; Correia et al., 2017; Reis and Hu, 2017).

##### 3.1.2. Pesticides

Table 2 shows the concentrations of fipronil and 2,4-D quantified in the mesocosms throughout the experiment after the contamination. In the control mesocosms, trace concentrations of 2,4-D and fipronil and its metabolites were detected over time; however, these values were negligible compared with those observed in the contaminated treatments. This was attributed to low-level cross-contamination during the sampling of water, sediment, and fauna. In mesocosms containing 2,4-D, decreases in concentrations were observed over time, and fipronil was not detected above the limit of quantification. In the fipronil-treated mesocosms, concentrations of the parent insecticide decreased throughout the experiment, reaching the lowest levels at the end of the exposure period (T150). Concentrations of fipronil-sulfide and fipronil-sulfone remained below the limit of quantification until T14; however, both metabolites were quantified at subsequent sampling times and then gradually decreased until T150. In mesocosms exposed to both mixtures (M and MV), 2,4-D and fipronil were detected at all sampling dates, decreasing over time until T150. In these treatments, fipronil-sulfide concentrations were below the limit of quantification until T7, and were higher at later dates, as well as for fipronil-sulfone in the MV mesocosms. In pesticide mixture mesocosms, concentrations above the limit of quantification were observed at T1 and T7.

Water quality parameters directly influenced the half-life of fipronil, with emphasis on the treatment that included vinasse mixed with pesticides (MV). Goulart et al. (2024) investigated the pesticides degradation in the same mesocosms and estimated the half-life of fipronil as 11.7 days for fipronil-only treatment, 13.8 days in the pesticide mixture treatment, and 24.5 days in the combined pesticide and vinasse treatment. Although the fipronil concentrations in MV were initially lower than those in other treatments, its value was still 4 µg L<sup>-1</sup> at T150, while fipronil reached 0.001 and 0.1 µg L<sup>-1</sup> in control and fipronil-only treatments, respectively. The reduction in the degradation rate observed in the pesticide-vinasse treatment can be attributed to the pH modification promoted by vinasse, which reached values of up to 4.62 at T1. Fipronil is known to exhibit greater stability in aquatic environments with acidic to neutral pH, reducing its potential for hydrolysis (Qu et al., 2016; Singh et al., 2021). This observation has also been reported for other compounds, such as the insecticide malathion, as increases in pH and sorption to sediment mitigated its toxicity in aquatic systems (Brogan and Relyea, 2014; Bray et al., 2021). Vinasse caused a tenfold increase in water turbidity, rising from values below 5 NTU before application to greater than 100 NTU after contamination in vinasse-treated mesocosms, and then decreasing over time until T150 (Table S1). This increase reduced the penetration of solar radiation into the system, directly affecting the photodegradation process of fipronil, which is a key driver for this compound (Bonmatin et al., 2015). Therefore, the higher turbidity in the vinasse treatments limited this process, since dissipation rates depend on both the depth of light

**Table 2**

Mean concentrations of pesticides over time ( $\mu\text{g L}^{-1}$ ). The acronyms correspond to the molecules fipronil (F), fipronil sulfide (Fd), and fipronil sulfone (Fn). The treatments are control (C), 2,4-D (D), fipronil (F), mixture of pesticides (M), vinasse (V), and mixture of pesticides with vinasse (MV). LOQ = Limit of Quantification.

	C	F	Fd	Fn	D	F	Fd	Fn	D	F	Fd	Fn
T-7	< LOQ	0.0006 ± 0.00007	< LOQ	< LOQ	0.018 ± 0.009	0.0003 ± 0.00003	< LOQ	< LOQ	< LOQ	0.0006 ± 0.00002	< LOQ	< LOQ
T1	0.0954 ± 0.16	0.006 ± 0.013	< LOQ	< LOQ	1385 ± 268.41	< LOQ	< LOQ	< LOQ	< LOQ	42.54 ± 11.22	< LOQ	< LOQ
T7	0.1964 ± 0.084	0.0044 ± 0.001	< LOQ	0.0004 ± 0.0002	522.53 ± 54.44	< LOQ	< LOQ	< LOQ	< LOQ	7.96 ± 1.04	< LOQ	< LOQ
T14	0.1348 ± 0.04	0.0047 ± 0.002	< LOQ	0.0007 ± 0.00001	375.57 ± 30.22	< LOQ	< LOQ	< LOQ	< LOQ	3.23 ± 1.14	< LOQ	< LOQ
T21	0.062 ± 0.052	0.009 ± 0.014	0.0002 ± 0.0006	0.0014 ± 0.002	484.86 ± 102.39	< LOQ	< LOQ	< LOQ	< LOQ	3.52 ± 1.24	0.065 ± 0.028	0.1683 ± 0.090
T75	0.0121 ± 0.003	0.0007 ± 0.00	0.0001 ± 0	0.0002 ± 0.0001	122.14 ± 78.85	< LOQ	< LOQ	< LOQ	0.0313 ± 0.007	0.0998 ± 0.0852	0.0023 ± 0.0012	0.0027 ± 0.015
T150	0.0137 ± 0.0104	0.0002 ± 0.0002	< LOQ	0.00004 ± 0.00005	6.87 ± 10.85	< LOQ	< LOQ	< LOQ	0.0102 ± 0.0123	0.0027 ± 0.0019	0.0022 ± 0.0011	0.0039 ± 0.0025
	<b>M</b>	<b>V</b>	<b>MV</b>									
	<b>D</b>	<b>F</b>	<b>Fd</b>	<b>Fn</b>	<b>D</b>	<b>F</b>	<b>Fd</b>	<b>Fn</b>	<b>D</b>	<b>F</b>	<b>Fd</b>	<b>Fn</b>
T-7	< LOQ	0.0005 ± 0.000004	< LOQ	< LOQ	< LOQ	0.0005 ± 0.00007	< LOQ	< LOQ	< LOQ	0.0006 ± 0.00007	< LOQ	< LOQ
T1	1168 ± 438.68	38.39 ± 0.39	< LOQ	< LOQ	< LOQ	0.449 ± 0.36	0.0012 ± 0.0005	0.0045 ± 0.002	1658 ± 1735	16.94 ± 0.34	< LOQ	< LOQ
T7	488.74 ± 20.25	10.69 ± 0.12	< LOQ	0.12 ± 0.20	< LOQ	< LOQ	< LOQ	< LOQ	318.54 ± 28.28	6.56 ± 1.37	< LOQ	< LOQ
T14	401.88 ± 65.67	5.69 ± 0.3	< LOQ	< LOQ	0.093	0.0148	< LOQ	< LOQ	208.6	6.0	< LOQ	< LOQ
T21	539.73 ± 27.60	5.27 ± 0.13	0.080 ± 0.013	0.1367 ± 0.018	0.148	0.006	< LOQ	< LOQ	233.6	7	0.050	0.060
T75	239.55 ± 34.679	0.1 ± 0.072	0.0131 ± 0.0134	0.0132 ± 0.011	0.1861 ± 0	0.0042 ± 0	< LOQ	< LOQ	0.7	3.5	0.3054	0.1081
T150	91.3288 ± 15.8386	0.0243 ± 0.0088	0.0265 ± 0.0183	0.038 ± 0.027	< LOQ	< LOQ	< LOQ	< LOQ	0.055	0.0877	0.1356	0.0428

penetration and the turbidity of the water column. As fipronil has a moderate to strong affinity for organic matter (organic carbon partition coefficient – Koc from 825 to 6863), it is expected to adsorb to suspended solids and sediment (Kim et al., 2025). This tendency was previously confirmed by Goulart et al. (2024), who showed that vinasse increased the organic matter content of sediment samples and consequently favored the sorption of fipronil in that compartment.

In the mesocosms, 2,4-D has half-life that can vary from 10 to 50 days (Goulart et al., 2024). In this study, its behavior was the opposite of that of fipronil, with greater degradation observed in the MV treatment. In the 2,4-D-alone treatment, concentrations at the end of the experiment (T150) were  $6.87 \pm 10.85 \mu\text{g L}^{-1}$ , whereas in the MV treatment, values were significantly lower, reaching  $0.055 \mu\text{g L}^{-1}$  at the same time point. This difference from the other treatments may stem from vinasse's high loads of organic matter and nutrients, which create favorable conditions for biofilm development and microbial activity capable of accelerating pesticide degradation (Jote, 2019; Katagi, 2010; Ordaz-Guillén et al., 2014).

### 3.1.3. Metals

Metal concentrations in the water analysed on the day of mesocosm contamination are presented in Table 3. Vinasse application increased metal levels in the water, with a tenfold increase in manganese and a twentyfold increase in potassium compared with control mesocosms ( $p < 0.05$ ). In addition, concentrations of barium, cobalt, copper, and zinc were also significantly higher than those in the control treatment ( $p < 0.05$ ). This increase can be attributed to the fact that vinasse presents high concentrations of metals in its composition, as observed in item 4.1 of this work and reported by other authors in the literature (Christofoletti et al., 2013; Pinto et al., 2023). This condition may represent a negative impact on aquatic ecosystems, since the characteristic acidity of vinasse tends to increase the bioavailability of metals, favoring their assimilation by exposed organisms and increasing their toxicity (Eggleton and Thomas, 2004; Botelho et al., 2012). Since metals

**Table 3**

Concentrations (mean ± SE) of metals in water ( $\text{mg L}^{-1}$ ) of the control (C), 2,4-D (D), fipronil (F), pesticide mixture (M), vinasse (V), and pesticide mixture with vinasse (MV) mesocosms in samples collected on the day of contamination. Asterisks (\*) indicate differences from the mesocosm control.

	C	D	F	M	V	MV
Aluminum	1.4 ± 0.7	4.9 ± 2.9*	5.2 ± 3.8*	3.7 ± 1.1	3.3 ± 1.0	3.4 ± 0.3
Barium	0.004 ± 0.002	0.01 ± 0.004	0.05 ± 0.02	0.02 ± 0.01*	0.03 ± 0.002*	0.03 ± 0.01*
Cadmium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cobalt	< LOQ	< LOQ	< LOQ	0.005 ± 0.01	0.04 ± 0.01*	0.06 ± 0.01*
Chromium	< LOQ	0.0005 ± 0.0001	0.0005 ± 0.0005	< LOQ	0.001 ± 0.001*	0.003 ± 0.00
Copper	0.02 ± 0.005	0.04 ± 0.01*	0.03 ± 0.01*	0.04 ± 0.004*	0.05 ± 0.002*	0.06 ± 0.01*
Lead	0.02 ± 0.01	0.02 ± 0.003	0.02 ± 0.02	< LOQ	0.02 ± 0.001	0.01 ± 0.003
Manganese	0.09 ± 0.03	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.9 ± 0.5*	0.8 ± 0.1*
Nickel	0.003 ± 0.004	0.001 ± 0.001	0.002 ± 0.002	0.002 ± 0.004	0.001 ± 0.002	0.01 ± 0.02
Potassium	3.3 ± 0.6	4.5 ± 0.4	4.6 ± 1.2	4.9 ± 1.4	62.2 ± 3.1*	65.1 ± 3.7*
Zinc	0.009 ± 0.01	0.004 ± 0.004	0.03 ± 0.01	0.05 ± 0.05	0.06 ± 0.01*	0.1 ± 0.07*

LOQ (Limit of Quantification) being  $0.008 \text{ mg L}^{-1}$  for Cadmium,  $0.009 \text{ mg L}^{-1}$  for Cobalt,  $0.0006 \text{ mg L}^{-1}$  for Chromium and  $0.008 \text{ mg L}^{-1}$  for Lead.

are not subject to microbial or chemical degradation, they tend to persist in the water column or become deposited in the sediment (Briffa et al., 2020). Several metals can bioaccumulate within organisms and may even be biomagnified through food webs ((Fraga et al., 2024); Mustapha

et al., 2025). The interaction between vinasse and metals can trigger acute and chronic toxicity, affecting biological parameters such as the population growth rate of *Allonais inaequalis* and the body development of *Chironomus sancticarioli* (Fraga et al., 2024). Similarly, Pinto et al. (2023) demonstrated that exposure to water contaminated with a mixture of vinasse and pesticide (MV) resulted in the mortality of *C. sancticarioli*, even 75 days after the contamination event.

### 3.2. Physicochemical variables of water

PERMANOVA analysis revealed significant effects of treatment, time, and their interaction on the set of physicochemical variables evaluated ( $p < 0.001$  for all terms). Treatment explained 28.0% of the total variance ( $R^2 = 0.280$ ), whereas time accounted for 15.9% ( $R^2 = 0.160$ ). The treatment  $\times$  time interaction showed the highest explanatory power ( $R^2 = 0.326$ ), indicating that physicochemical responses to the treatments varied over time and followed distinct trajectories under each experimental condition. Due to the significant interaction, the large number of possible comparisons, and the unbalanced number of replicates among groups, pairwise contrasts exhibited low statistical power and no significant differences after correction for multiple comparisons ( $p > 0.05$ ). Therefore, the interpretation of experimental effects was primarily based on the global PERMANOVA model together with the patterns revealed by PCA ordination.

In the PCA analysis of water variables, the first axis represented 74%

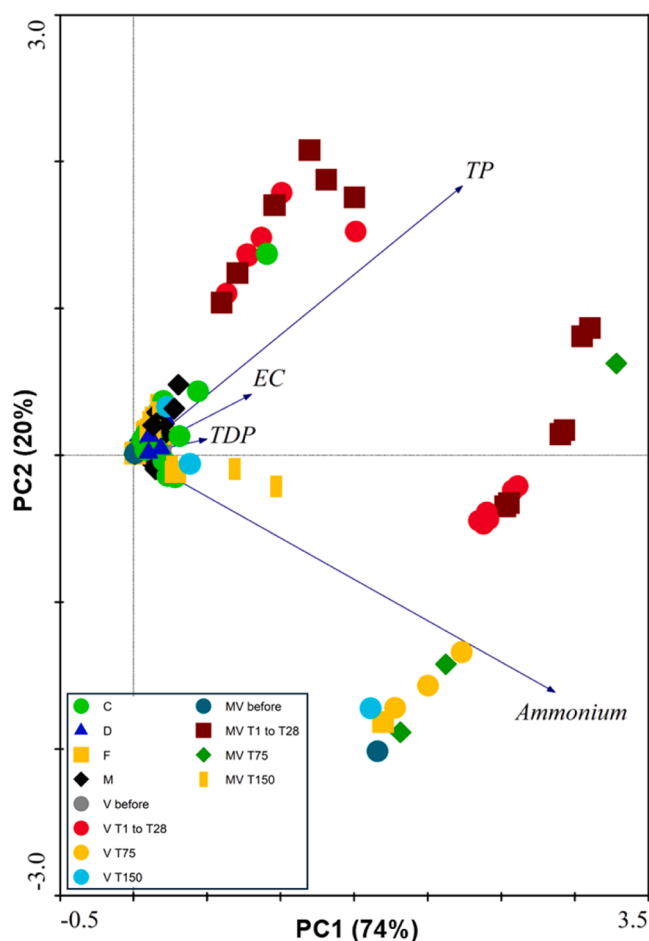


Fig. 2. Principal Component Analysis (PCA) of the physicochemical variables of water from the mesocosms under different treatments and sampling times. C = Control; D = 2,4-D; F = Fipronil; M = Fipronil and 2,4-D; V = Vinasse; MV = Fipronil, 2,4-D, and Vinasse. TP = total phosphorus, EC = electrical conductivity, and TDP = total dissolved phosphorus.

of the variance, while the second axis represented 20%. In the diagram (Fig. 2), the mesocosms that were not treated with vinasse were grouped regardless of the sampling time, indicating that water parameters were consistent between treatments these treatments and control over time. In the mesocosms treated with vinasse (V and MV), there was a clear separation from the other treatments, including the control. The concentration of total and dissolved ammonium and phosphorus and electrical conductivity were strongly and directly associated with the vinasse points (Fig. 2). These parameters increased in vinasse-treated mesocosms after contamination (Table S1), resulting from a direct process of vinasse addition which, due to its composition, led to an increase in ammonia and dissolved phosphorus. The remaining parameters were not displayed in the diagram because they showed a low contribution to the separation among points. Nevertheless, a marked decrease in water pH was observed following vinasse addition, indicating the development of a more acidic environment in vinasse-treated mesocosms from T1 to T14 ( $pH < 5.5$ ), with values returning to above 6.5 at T21, T75, and T150.

This study showed a reduction in dissolved oxygen concentrations in all treatments, which could be explained by the organic matter degradation process; decomposing bacteria preferentially use dissolved oxygen, resulting in a decrease in its availability for organisms of greater biological complexity (Von Sperling, 1996). This reduction may be associated with the progressive mortality of macrophytes present in the mesocosms, which led to the accumulation of organic matter in the system and is considered the main factor responsible for the drop in oxygen levels in non-vinasse-treated mesocosms (Pinto et al., 2021). However, even more pronounced changes were observed in the treatments that received vinasse (see Table S1), which presented significantly lower dissolved oxygen concentrations. These changes can be largely attributed to the high organic matter content of the vinasse (Table 1), which promotes the proliferation of decomposing microorganisms and leads to oxygen depletion (Azevedo-Santos et al., 2024). The decomposing activity, associated with the significant supply of nutrients from the vinasse, increased electrical conductivity of water and stimulated the growth of algae (Aguiar et al., 2011; Rulli et al., 2023). This process justifies the increases observed in the levels of chlorophyll a and b, especially in the vinasse-treatments mesocosms.

### 3.3. Benthic macroinvertebrate community

#### 3.3.1. Composition of the community

A total of 19 families distributed in seven orders and the subclass Oligochaeta were found in the mesocosms throughout the experiment (Table 4 and Table 5), belonging to the families Libellulidae and Coenagrionidae (Order Odonata), Planorbidae (Order Pulmonata), Hydrophilidae, Haliplidae and Dryopidae (Order Coleoptera), Leptophlebiidae and Caenidae (Order Ephemeroptera), Physidae (Order Basommatophora), Corixidae and Belostomatidae (Order Hemiptera) and Phoridae, Ephydriidae, Sciomyzidae, Tabanidae, Muscidae, Culicidae, Ceratopogonidae (Order Diptera), and the subfamilies Chironominae and Tanytopodinae (Family Chironomidae) also belonging to the Order Diptera (Table 4 and Table 5). These families total 3066 identified organisms, of which the largest number was found in the control (966 organisms) (Table 4).

#### 3.3.2. Community functional diversity

The organisms were classified according to the functional feeding groups, and the results obtained can be seen in Fig. 3. No temporal differences were detected for total abundance (ANOVA,  $F = 1.767$  and  $p = 0.125$ ). Considering the abundance of functional groups individually, no differences were found between treatments. No temporal differences were found in the abundance of predators, collector-gatherers, and scrapers (ANOVA,  $F = 1.333; 1.854$  and  $0.685$ ,  $p > 0.05$ ). No shredders were found in any sample throughout the study.

Regarding recovery patterns, our results indicate that the benthic

**Table 4**

Mean number of benthic macroinvertebrate families in the control (C), 2,4-D (D) and fipronil mesocosms over the time of 7 days (T-7) and 1-day (T-1) before contamination and 1 (T1), 7 (T7), 14 (T14), 28 (T28), 75 (T75) and 150 (T150) days after contamination. The classification of Functional Food Groups (FFG) was separated into collector-gatherers (CG), filter-feeders (FF), filter-collectors (FC), scrapers (SC), shredders (SH), and predators (PR).

Order	Family	FFG	C								D								F							
			T-7	T-1	T1	T7	T14	T21	T75	T150	T-7	T-1	T1	T7	T14	T21	T75	T150	T-7	T-1	T1	T7	T14	T21	T75	T150
Odonata	Libellulidae	PR	2	1	4	1	2	2	5	4	-	1	-	2	3	-	2	4	1	-	2	-	-	-	-	-
	Coenagrionidae	PR	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pulmonata	Planorbidae	SC	7	6	3	2	9	8	23	8	-	-	1	1	2	1	34	1	-	12	9	3	6	14	22	2
Diptera	Phoridae	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Ephydriidae	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Sciomyzidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	
	Tabanidae	PR	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
	Muscidae	PR	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Culicidae	CG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Ceratopogonidae	PR	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Chironominae	CG	145	34	25	12	-	2	25	107	27	-	3	12	3	8	41	39	13	17	-	-	-	8	154	
	Tanypodinae	PR	13	8	-	11	2	1	-	1	3	-	4	2	-	-	-	1	7	1	-	-	-	-	-	
	Orthoclaeniidae	CG	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
Coleoptera	Hydrophilidae***	FC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Halplidae**	**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Dryopidae****	SC	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Ephemeroptera	Leptophlebiidae	CG	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Caenidae	CG	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Basommatophora	Physidae	SC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Hemiptera	Corixidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Belostomatidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
	Oligochaeta*	CG	2	7	7	12	77	68	79	234	14	3	26	29	62	9	35	55	3	-	22	14	7	69	17	11

\* The Oligochaeta classification at the subclass level.

\*\* Functional Food Classification not identified by Functional Food Group.

\*\*\* All organisms were in the adult stage and were classified as collectors-gatherers.

\*\*\*\* All organisms were in the adult stage and were classified as scrapers.

**Table 5**

Mean number of benthic macroinvertebrate families in the control (C), 2,4-D (D) and fipronil mesocosms over the time of 7 days (T-7) and 1 day (T-1) before contamination and 1 (T1), 7 (T7), 14 (T14), 28 (T28), 75 (T75) and 150 (T150) days after contamination. The classification of Functional Food Groups (FFG) was separated into collector-gatherers (CG), filter-feeders (FF), filter-collectors (FC), scrapers (SC), shredders (SH), and predators (PR). (continuation).

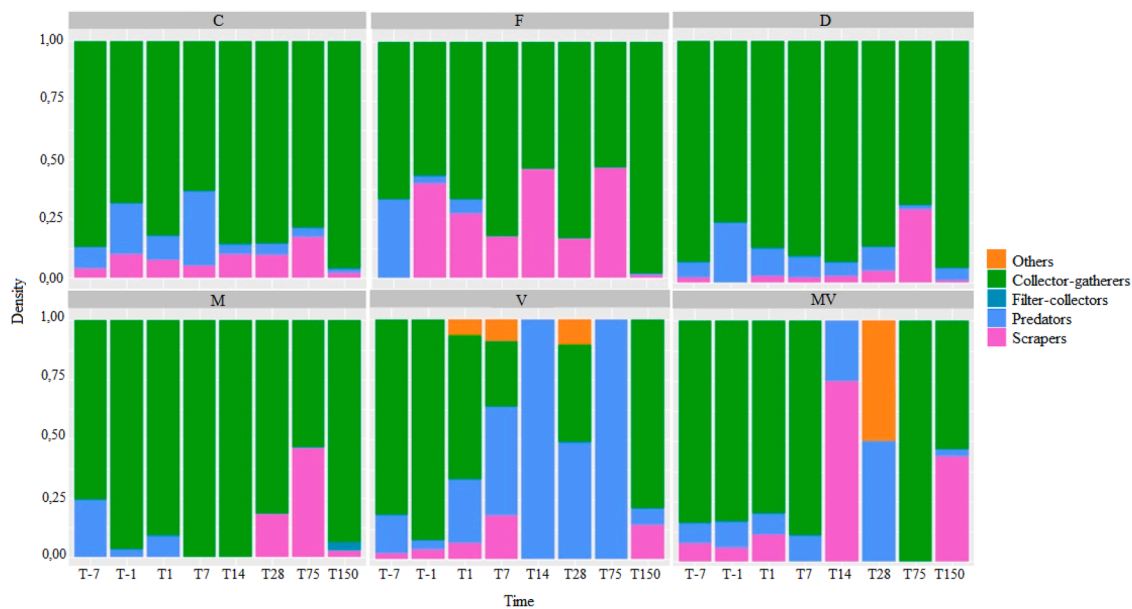
Order	Family	FFG	V									M					MV									
			T-7	T-1	T1	T7	T14	T21	T75	T150	T-7	T-1	T1	T7	T14	T21	T75	T150	T-7	T-1	T1	T7	T14	T21	T75	T150
Odonata	Libellulidae	PR	-	-	-	4	3	4	1	-	2	-	1	-	-	-	-	-	1	1	1	1	2	-	-	-
	Coenagrionidae	PR	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pulmonata	Planorbidae	SC	2	2	1	2	-	-	-	2	-	-	-	-	-	13	127	5	9	6	4	-	1	-	-	14
Diptera	Phoridae	**	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	
	Ephydriidae	**	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1-	-	-	
	Sciomyzidae	PR	-	-	-	-	1	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	-	-	
	Tabanidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
	Muscidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Culicidae	CG	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
	Ceratopogonidae	PR	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
	Chironominae	CG	61	26	9	3	-	1	-	11	18	27	2	-	-	-	7	89	94	77	24	5	-	38	14	
	Tanypodinae	PR	12	1	3	-	-	-	-	-	5	1	2	-	-	-	-	-	7	11	2	1	-	-	1	
	Orthoclaadiinae	CG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	
Coleoptera	Hydrophilidae***	FC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	7	-	-	-	-	-	-	-	
	Halplidae**	**	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Dryopidae****	SC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Ephemeroptera	Leptophlebiidae	CG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Caenidae	CG	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
Basommatophora	Physidae	SC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
Hemiptera	Corixidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	
	Belostomatidae	PR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Oligochaeta*	CG	3	2-	-	-	-	-	-	-	7	1	28	1-	1-	58	14-	81	3	4	3	3	-	-	3	

\* The Oligochaetata classification at the subclass level.

\*\* Functional Food Classification not identified by Functional Food Group.

\*\*\* All organisms were in the adult stage and were classified as collectors-gatherers.

\*\*\*\* All organisms were in the adult stage and were classified as scrapers.



**Fig. 3.** Functional diversity of benthic macroinvertebrates for the treatments control (C), 2,4-D (D), fipronil (F), pesticide mixture (M), vinasse (V), and pesticide mixture with vinasse (MV) during a period of 7 days (T-7) and 1-day (T-1) before contamination and 1 (T1), 7 (T7), 14 (T14), 28 (T28), 75 (T75), and 150 (T150) days after contamination.

macroinvertebrate community did not show full functional recovery by the end of the 150-day experiment. Although some functional groups reappeared at later sampling times (e.g., scrapers in treatment M after T28), key groups such as predators remained absent in treatments contaminated with fipronil and pesticide mixtures until T150. Similarly, collectors and scrapers showed unstable or incomplete recovery in vinasse and vinasse–pesticide treatments. These patterns suggest limited resilience and indicate that chronic exposure to pesticides and vinasse can lead to long-lasting functional impairments, preventing the re-establishment of community structure.

In the control, a significant number of collectors-gatherers can be observed, followed by predators and scrapers (Fig. 3). In the mesocosms treated with 2,4-D, collecting-gathering organisms were also found, and this classification was the most abundant, as well as predators and scrapers. In mesocosms treated with fipronil and pesticide mixture, predators were observed before contamination, in T-7 and T-1, and shortly after contamination in T1. After this period, predators disappeared in these two treatments and remained absent for 150 days after contamination. In these treatments, the organisms belonging to the order Odonata and the subfamily Tanypodinae, which are predators, disappeared during these periods, explaining the responses observed with the predators. For treatment F, only collectors-gatherers were found in T7 and T14, specifically due to organisms of the subclass Oligochaeta. Filter collectors were observed in the pesticide mixture treatment due to the appearance of the Hydrophilidae family. In this same treatment, the scrapers appeared in the samples from T28 onwards.

In the vinasse treatment, collectors and scrapers in T14 and T75 disappeared, and only predators were found in these treatments due to the presence of organisms from the Libellulidae, Sciomyzidae, and Ceratopogonidae families. The same happened for mesocosms treated with the mixture of vinasse and pesticides in T75 when only collectors-gatherers were found in the samples, specifically those belonging to the subfamily Chironominae. In T7, the scrapers were not found, and in T14, the collector-gatherers disappeared, increasing the relative abundance of scrapers. Finally, changes were observed between treatments for richness and functional diversity (mixed models ANOVA,  $F = 2.565$  and  $p < 0.05$ ), with a decrease of these parameters in the mesocosms M ( $p < 0.05$ ) about the control.

In treatment control, as in the other mesocosms before contamination, a significant number of collector-gatherers were observed, followed by predators and scrapers. The greater dominance of collectors-gatherers occurred mainly due to the presence of organisms from the subfamily Chironominae and subclass Oligochaeta. Collectors-gatherers are organisms that have mouthparts modified to collect fine (0.05–1 mm) and ultrafine (0.0005–0.05mm) organic matter that accumulates at the bottom of the water body, on the sediment, or other deposit regions of organic matter (Ramírez and Gutiérrez-Fonseca, 2014). This group of organisms is generally abundant in different types of environments, preserved and/or disturbed, mainly in lentic water bodies or regions of deposit (backwater) in lotic environments, where normally there is a greater deposition of organic matter, presenting an important role, both in the cycling of matter and energy and in secondary productivity within aquatic environments (Berg and Henthall, 1992; Damanik-Ambarita et al., 2016).

Oligochaeta and Chironominae include organisms tolerant to high levels of organic pollution, mainly due to morphological and physiological adaptive characteristics that allow these organisms to survive even in oxygen-poor environments (Statzner and Béche, 2010). However, as previously described, no organisms from either group were observed at T14 in the vinasse and vinasse–pesticide mixture treatments, or at T75 in the vinasse treatment alone, due to the factors discussed above, indicating the disappearance of collector-gatherers in these mesocosms. This, in turn, rejects our third hypothesis, since the collectors did not benefit from the increased organic content caused by the presence of vinasse. Although vinasse is rich in organic matter and, theoretically, could favor collectors, our results indicate that this functional group did not benefit from exposure to vinasse. This unexpected response can be explained by the high organic load and toxicity of vinasse, which can lead to oxygen depletion and physiological stress, limiting the ability of collector organisms to exploit increased food resources. Furthermore, the observed increase in chlorophyll a and b does not necessarily translate into higher food quality or accessibility, since organic enrichment can promote the growth of unpalatable algae and microbial biofilms. These effects are likely to occur due to combined exposure to pesticides, which can impair feeding activity, mobility, and survival of collector and scraper taxa. Therefore, the rejection of hypothesis (iii) suggests a decoupling between resource availability and

functional group performance under conditions of high chemical stress.

Due to the decrease in collectors-gatherers in vinasse-treated mesocosms, there was an increase in the relative abundance of predators and, to a lesser extent, of scrapers. Predators are those organisms that capture and consume live prey (Merritt and Cummins, 1996) and are represented in these treatments by the larvae of the order Odonata. Odonata larvae play an important role in the dynamics of the aquatic ecosystem (Costa et al., 2006), especially in lentic systems, are relevant predators in the freshwater food web, and serve as food for higher trophic levels (Arnqvist & Johansson, 1998). In addition to the disappearance of other functional groups, another factor associated with the increased abundance of predators is the increased availability of prey due to the proliferation of Phoridae, Ephydriidae, and Sciomyzidae fly larvae in treatments contaminated by vinasse (Corbet, 1999).

The elimination of insects in treatments contaminated by fipronil alone and mixed with 2,4-D resulted in the disappearance of predators. This partially confirms hypothesis (ii), as the abundance of scavengers was affected by fipronil, whereas no such effect was observed for collectors. Furthermore, hypothesis (i) can be rejected, since scrapers were not directly affected in the 2,4-D-contaminated treatments. The decline and disappearance of predators can cause major changes in ecosystem functions, as they are responsible for transferring energy between trophic levels and have the potential to control the populations of other organisms (Oberndorfer et al., 1984; Cooper et al., 1990). This observation is especially important because it highlights the lethal effects that can occur in top trophic populations, which are directly associated with the action of applied pesticides. Fipronil, in turn, has neurotoxic characteristics (Xu et al., 2018; Mamboungou et al., 2024), which can compromise the locomotion and predation capacity of exposed organisms (Ruggiero et al., 2024), further aggravating the imbalance in the energy flow of the ecosystem. Such an impact may justify the disappearance of predators and, consequently, explain the increase in the relative abundance of organisms from the collector-gatherer and scraper functional guilds.

Scraper organisms were the third most predominant functional group throughout the experiment. Scrapers play the trophic roles of herbivores, detritivores, and generalists, as they use different feeding strategies at the same time or during different life stages. Scraping biofilms, composed of algae, bacteria, fungi, animals, and debris adhered to different substrates, make up the various microhabitats in aquatic environments (Rawer-Jost et al., 2000). Despite an increase in the values of chlorophyll *a* and *b* parameters, associated with the increase in algae concentration, in the treatments containing vinasse, the collectors and scrapers disappeared in these mesocosms in some periods. In this case, an increase in the availability of food resources did not reflect, indirectly, in favoring this functional group, probably due to the high toxicity of vinasse and its mixture with pesticides. Organic pollution generally harms scraper fauna, leading to a decrease and/or disappearance of this functional group (Duan et al., 2011; Helson and Williams, 2013). The disappearance of collectors and scrapers can unbalance the aquatic ecosystem because, when consuming algae, these individuals exert an important effect on the population control of primary producers (Ramírez and Gutiérrez-Fonseca, 2014), on the consumption of productive biomass, and exert a strong influence on the algae species composition (Feminella and Hawkins, 1995).

Functional richness and diversity decreased because of exposure to the pesticide mixture, a pattern similar to that observed by Hou et al. (2025), confirming hypothesis (iv). This reinforces the risks associated with the occurrence of these two compounds in water bodies, both for the structure and functioning of these environments (Rumschlag et al., 2020), since changes in population dynamics can, in the long term, cause profound modifications in different ecosystem services, such as changes in the ecosystem's energy flow (Muturi et al., 2017), which can result in more severe impacts, such as eutrophication (Onyango et al., 2024).

## 5. Conclusion

The main effects observed in this study were: (I) - vinasse contamination provoked alterations in water quality parameters, including changes in nutrient concentrations, among other ions; (II) - The presence of vinasse influenced the degradation rate of the pesticides, increasing the persistence of fipronil and a decrease in the degradation time of 2,4-D; (III) - The concentrations of metals in the water were mainly attributed to the presence of vinasse; (IV) - The changes mentioned above were reflected in the functional characteristics of the communities, and the treatments with 2,4-D, a greater occurrence of collector-gatherers and a drastic reduction of scrapers were observed, which were which were recorded at time T75. (V) - In the treatments with fipronil, a total absence of predators was observed at all times sampled after contamination; (VI) - In the treatments with pesticide mixture, there was a disappearance of predators; on the other hand, in the treatment with vinasse alone, the dominance of predators was observed, especially at times T14 and T75, accompanied by a reduction in collector-gatherers; (VII) - In the combined treatment of pesticides and vinasse, dominance of collector-gatherers was observed until time T7, followed by evident changes in the functional composition at subsequent times, which reinforces the joint influence of all pollutants on the structure and functional diversity of the community. Therefore, contamination by pesticides and vinasse had significant negative impacts on the functional diversity of macroinvertebrate communities, reducing the variety of ecological functions performed by these species. This can result in an impoverishment of essential ecological functions, compromising the health of aquatic ecosystems and their ability to provide ecosystem services.

## Ethical statement

No specific ethical approval was required for the use of invertebrates.

## Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Funding

Financial support was provided by São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP) (process number 2015/18790-3). C.P. Cotta would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). L.C.M. Silva thanks the National Council for Scientific and Technological Development for the doctoral scholarship (grant n. 140336/2017-6). R. A. Moreira, T.J.S. Pinto, A.P. Ogura, and D.F. Gomes would like to thank FAPESP for the postdoctoral grants (Process numbers: 2017/24126-4, 2022/14293-9, 2021/14789-1, and 2024/08039-8, respectively).

## Originality Statement

The authors confirm that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

## CRediT authorship contribution statement

**Carina Pereira Cotta:** Writing – original draft, Methodology, Investigation, Formal analysis. **Thandy Junio da Silva Pinto:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Diego Ferreira Gomes:** Writing – review & editing. **Allan Pretti Ogura:** Investigation. **Laís Conceição Menezes da Silva:** Investigation. **Janaina Braga do Carmo:** Resources, Methodology. **Cassiana Carolina Montagner:** Methodology, Formal analysis. **Evaldo**

**Luiz Gaeta Espíndola:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Raquel Aparecida Moreira:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

### 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.

### Acknowledgements

Financial support was provided by São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP) (process number 2015/18790-3). C.P. Cotta would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). L.C.M. Silva thanks the National Council for Scientific and Technological Development for the doctoral scholarship (grant n. 140336/2017-6). R. A. Moreira, T.J.S. Pinto, A.P. Ogura, and D.F. Gomes would like to thank FAPESP for the postdoctoral grants (Process numbers: 2017/24126-4, 2022/14293-9, 2021/14789-1, and 2024/08039-8, respectively).

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.aquatox.2026.107724](https://doi.org/10.1016/j.aquatox.2026.107724).

### Data availability

Data will be made available on request.

### References

- ABNT NBR 12621, 1995. Waters - Determination of total hardness by EDTA-Na titrimetric method - Method of test.
- Aguilar, V.M.C., Neto, J.A.B., Rangel, C.M., 2011. Eutrophication and hypoxia in four streams discharging in Guanabara Bay, RJ, Brazil, a case study. *Mar. Pollut. Bull.* 62 (8), 1915–1919. <https://doi.org/10.1016/j.marpolbul.2011.04.035>.
- Andersen, J.M., 1976. An ignition method for determination of total phosphorus in lake sediments. *Water. Res.* 10, 329–331. [https://doi.org/10.1016/0043-1354\(76\)90175-5](https://doi.org/10.1016/0043-1354(76)90175-5).
- APHA, 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. DC, Washington.
- APHA, 2018. Standard Methods for the Examination of Water and Wastewater. American Public Health Association.
- Arnqvist, G., Johansson, F., 1998. Ontogenetic reaction norms of predator-induced defensive morphology in dragonfly larvae. *Ecology*. 79 (6), 1847–1858. [https://doi.org/10.1890/0012-9658\(1998\)079\[1847:ORNOPI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[1847:ORNOPI]2.0.CO;2).
- Azevedo-Santos, V.M., Fernandes, J.A., de Souza Andrade, G., de Moraes, P.M., Magurran, A.E., Pelicice, F.M., Giarrizzo, T., 2024. An overview of vinasse pollution in aquatic ecosystems in Brazil. *Environ. Manage* 74 (6), 1037–1044. <https://doi.org/10.1007/s00267-024-01999-x>.
- Baffa, D.C.F., Freitas, R.G.De, do, R.P.C., 2009. O uso da vinhaça na cultura da cana de açúcar. *Nucleus*. 1 (2). ISSN 1982-2278.
- Bartkowska, A., Mieczan, T., Plaska, W., Bartkowska, A., Mieczan, T., Plaska, W., 2023. Colonization of Artificial Substrates by Invertebrate Macrofauna in a River Ecosystem—Implications for Forensic Entomology. *Int. J. Environ. Res. Public Health* 20. <https://doi.org/10.3390/ijerph20042834>.
- Berg, M.B., Hellenthal, R.A., 1992. The role of Chironomidae in energy flow of a lotic ecosystem. *Netherl. Journ. Aqua. Ecol.* 26 (2), 471–476. <https://doi.org/10.1007/BF02255277>.
- Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res.* 22, 35–67. <https://doi.org/10.1007/s11356-014-3332-7>.
- Botelho, R.G., Tornisielo, V.L., Olinda, R.A., Maranhão, L.A., Machado-Neto, L., 2012. Toxicidade aguda da vinhaça de cana-de-açúcar para organismos aquáticos antes e após ajuste de pH. *Toxicol. Environ. Chem.* 94 (10), 2035–2045. <https://doi.org/10.1080/02772248.2012.738516>.
- Bradley, P.M., Journey, C.A., Romanok, K.M., Barber, L.B., Buxton, H.T., Foreman, W.T., Villeneuve, D.L., 2017. Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in US streams. *Environ. Sci. Technol.* 51 (9), 4792–4802. <https://doi.org/10.1021/acs.est.7b00012>.
- BRASIL. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). Relatórios de comercialização de agrotóxicos. Disponível em: <https://www.gov.br/ibama/pt-br/assuntos/quimicos-e-biologicos/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos>. Accessed: 31 mar. 2025.
- BRASIL. Ministério da Agricultura e Pecuária (MAPA). Informações técnicas sobre agrotóxicos. Disponível em: <https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/agrotoxicos/informacoes-tecnicas>. Accessed: 31 mar. 2025.
- Bray, J., Miranda, A., Keely-Smith, A., Kaserzon, S., Elisei, G., Chou, A., Nichols, S.J., Thompson, R., Nugegoda, D., Kefford, B.J., 2021. Sub-organism (acetylcholinesterase activity), population (survival) and chemical concentration responses reinforce mechanisms of antagonism associated with malathion toxicity. *Sci. Total Environ.* 778, 146087. <https://doi.org/10.1016/j.scitotenv.2021.146087>.
- Briffa, J., Sinagra, E., Blundell, R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 6 (9). <https://doi.org/10.1016/j.heliyon.2020.e04691>, 1-26.
- Brogan, W.R., Relyea, R.A., 2014. A new mechanism of macrophyte mitigation: How submerged plants reduce malathion's acute toxicity to aquatic animals. *Chemosphere* 108, 405–410. <https://doi.org/10.1016/j.chemosphere.2014.02.041>.
- CALEPA, C.E.P.A., 2005. Benthic Macroinvertebrate Colonization of Artificial Substrates in Agriculture-dominated: Waterways of the Lower Sacramento River Watershed Surface Water Ambient Monitoring Program (SWAMP) Lower Sacramento River Watershed. URL [https://www.waterboards.ca.gov/water\\_issues/programs/swamp/docs/reglrbts/rb5\\_sacrwr\\_benthic.pdf](https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/reglrbts/rb5_sacrwr_benthic.pdf) (accessed 11.6.25).
- CETESB, C.A.D.S.P., 2018. Qualidade das águas interiores no estado de São Paulo 2017. <https://cetesb.sp.gov.br/aguas-interiores/wp-content/uploads/sites/12/2018/06/Relat%C3%B3rio-de-Qualidade-das-%C3%81guas-Interiores-no-Estado-de-S%C3%A3o-Paulo-2017.pdf> (accessed 11.5.25).
- CETESB, C.A.D.S.P., 2021. Diagnóstico da contaminação de águas superficiais, subterrâneas e sedimentos por agrotóxicos: relatório final. URL [http://cetesb.sp.gov.br/publicacoes-relatorios/>ISBN 978-65-5577-026-1](http://cetesb.sp.gov.br/publicacoes-relatorios/>ISBN%20978-65-5577-026-1) (accessed 11.5.25) ISBN 978-65-5577-026-1.
- Chingombe, P., Saha, B., Wakeman, R.J., 2006. Effect of surface modification of an engineered activated carbon on the sorption of 2,4-dichlorophenoxy acetic acid and benazolin from water. *J. Colloid. Interface Sci.* 297, 434–442. <https://doi.org/10.1016/j.jcis.2005.10.054>.
- Christofoletti, C.A., Escher, J.P., Correia, J.E., Marinho, J.F.U., 2013. Sugarcane vinasse: Environmental implications of its use. *Waste Manag.* 33, 2752–2761. <https://doi.org/10.1016/j.wasman.2013.09.005>.
- Coelho, M.P.M., Correia, J.E., Vasques, L.I., de Castro Marcato, A.C., de Andrade Guedes, T., Soto, M.A., Fontanetti, C.S., 2018. Toxicity evaluation of leached of sugarcane vinasse: Histopathology and immunostaining of cellular stress protein. *Ecotoxicol. Environ. Saf.* 165, 367–375. <https://doi.org/10.1016/j.ecoenv.2018.08.099>.
- CONAMA, 2005. RESOLUÇÃO N° 357, DE 17 DE MARÇO DE 2005. URL <https://www.legisweb.com.br/legislacao/?id=102255> (accessed 11.5.25).
- Cooper, S.D., Walde, S.J., Peckarsky, B.L., 1990. Prey exchange rates and the impact of predators on prey populations in streams. *Ecology*. 71, 1503–1514. <https://doi.org/10.2307/1938287>.
- Corbet, P.S., 1999. Dragonflies: Behaviour and ecology of Odonata, 64. Harley Books, Martins, p. 830.
- Correia, J.E., Christofoletti, C.A., Marcato, A.C.C., Marinho, J.F.U., Fontanetti, C.S., 2017. Histopathological analysis of tilapia gills (*Oreochromis niloticus* Linnaeus, 1758) exposed to sugarcane vinasse. *Ecotoxicol. Environ. Saf.* 135, 319–326. <https://doi.org/10.1016/j.ecoenv.2016.10.004>.
- Costa, C., Ide, S., Simonka, C.E., 2006. Insetos imaturos - metamorfose e identificação. Holos, Ribeirão Preto, p. 249.
- Cotta, C.P., Pinto, T.J., Yoshii, M.P.C., Silva, L.C., Ogura, A.P., Gabriel, G.V.M., Moreira, R.A., 2023. Exposure to fipronil, 2, 4-D and vinasse influences macroinvertebrate assemblage structure: An experimental mesocosm approach. *Sci. Total Environ.* 888, 164259. <https://doi.org/10.1016/j.scitotenv.2023.164259>.
- Cuenca, A.L.R., Simonato, J.D., Meletti, P.C., 2022. Acute exposure of embryo, larvae and adults of Danio rerio to fipronil commercial formulation reveals effects on development and motor control. *Ecotoxicology*. 31, 114–123. <https://doi.org/10.1007/s10646-021-02497-0>.
- Cummins, K.W., Merritt, R.W., Andrade, P.C.N., 2005. The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. *Neotrop. Fau. Environ.* 40 (1), 69–89. <https://doi.org/10.1080/01650520400025720>.
- Da Silva, A.P., Hernández, H.V.P., Comelli, C.L., Portugal, M.A.G., Delavy, F.M., de Souza, T.L., de Castilhos Ghisi, N., 2024. Meta-analytical review of antioxidant mechanisms responses in animals exposed to herbicide 2, 4-D herbicide. *Sci. Total Environ.* 888, 171680. <https://doi.org/10.1016/j.scitotenv.2024.171680>.
- Damanik-Ambarita, M.N., Lock, K., Boets, P., Everaert, G., Nguyen, T.H.T., Forio, M.A.E., Musonge, P.L.S., Suhareva, N., Bennetsen, E., Landuyt, D., Dominguez-Granda, L., Goethals, P.L.M., 2016. Ecological water quality analysis of the Guayas River basin (Ecuador) based on macroinvertebrates indices. *Limnologia* 57, 27–59. <https://doi.org/10.1016/j.limno.2016.01.001>.
- Duan, X.H., Wang, Z.Y., Xu, M.Z., 2011. Effects of fluvial processes and human activities on stream macro-invertebrates. *Internat. Journ. Sed. Res.* 26 (4), 416–430. [https://doi.org/10.1016/S1001-6279\(12\)60002-X](https://doi.org/10.1016/S1001-6279(12)60002-X).
- Eggleton, J., Thomas, K.V., 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environ. Int.* 30 (7), 973–980. <https://doi.org/10.1016/j.envint.2004.03.001>.
- Espana-Gamboa, E.L., Mijangos-Cortés, J.O., Hernández-Zárate, G., Maldonado, J.A.D., Alzate-Gaviria, L.M., 2012. Methane production by treating vinasses from hydrous ethanol using a modified UASB reactor. *Biotechnol. Biofuels*. 5, 1–9. <https://doi.org/10.1186/1754-6834-5-82>.

- Feminella, J.W., Hawkins, C.P., 1995. Interactions between stream herbivores and periphyton. *J. N. Am. Benthol. Soc.* 14 (4), 465–509. <https://doi.org/10.2307/1467536>.
- Ferreira, T.P., Bauerfeldt, G.F., Castro, R.N., Magalhães, V.S., Alves, M.C., Scott, F.B., Cid, Y.P., 2022. Determination of fipronil and fipronil-sulfone in surface waters of the Guandu River Basin by high-performance liquid chromatography with mass spectrometry. *Bull. Environ. Contam. Toxicol.* 1–9. <https://doi.org/10.1007/s00128-021-03369-3>.
- Ferreira, L.F.R., 2009. Fungi biodegradation of vinasse from industrial sugarcane processing. University of São Paulo—ESALQ, p. 2009. Thesis (PhD).
- Figueiredo, L.P., Athayde, D.B., Daam, M.A., van Gestel, C.A.M., Duarte-Neto, P.J., Guerra, G.S., Espíndola, E.L.G., 2020. Impact of temperature on the toxicity of Kraft 36 EC® (a.s. abamectin) and Score 250 EC® (a.s. difenoconazole) to soil organisms under realistic environmental exposure scenarios. *Ecotoxicol. Environ. Saf.* 194, 110446. <https://doi.org/10.1016/j.ecoenv.2020.110446>.
- Fraga, P.D., de Mello Gabriel, G.V., do Carmo, J.B., Espíndola, E.L.G., Pinto, T.J.D.S., 2024. Sugarcane vinasse provokes acute and chronic responses and bioaccumulation of metals in benthic macroinvertebrates. *Environ. Sci. Pollut. Res.* 31 (3), 4067–4079. <https://doi.org/10.1007/s11356-023-31446-z>.
- Freitas, J.S., Giroto, L., Goulart, B.V., Alho, L.D.O.G., Gebara, R.C., Montagner, C.C., Espíndola, E.L.G., 2019. Effects of 2, 4-D-based herbicide (DMA® 806) on sensitivity, respiration rates, energy reserves and behavior of tadpoles. *Ecotoxicol. Environ. Saf.* 182, 109446. <https://doi.org/10.1016/j.ecoenv.2019.109446>.
- Freitas, I.B.F., Duarte-Neto, P.J., Sorigotto, L.R., et al., 2024. Effects of pasture intensification and sugarcane cultivation on non-target species: A realistic evaluation in pesticide-contaminated mesocosms. *Sci. Total Environ.* 922, 171425. <https://doi.org/10.1016/j.scitotenv.2024.171425>.
- Fuess, L.T., Garcia, M.L., 2014. Implications of stillage land disposal: a critical review on the impacts of fertigation. *J. Environ. Manage.* 145, 210–229. <https://doi.org/10.1016/j.jenvman.2014.07.003>.
- Godoi, L.A.G., Camilotti, P.R., Bernardes, A.N., Sanchez, B.L.S., Torres, A.P.R., da Conceição Gomes, A., Botta, L.S., 2019. Seasonal variation of the organic and inorganic composition of sugarcane vinasse: main implications for its environmental uses. *Environ. Sci. Pollut. Res.* 26 (28), 29267–29282. <https://doi.org/10.1007/s11356-019-06019-8>.
- Gonçalves, S., Vasconcelos, M.W., Mota, T.F.M., Lopes, J.M.H., Guimaraes, L.J., Miglioranza, K.S.B., Ghisi, N., de, C., 2022. Identifying global trends and gaps in research on pesticide fipronil: a scientometric review. *Environ. Sci. Pollut. Res.* 29, 79111–79125. <https://doi.org/10.1007/s11356-022-21135-8>.
- Goulart, B.V., Vizioli, B.D.C., Espíndola, E.L.G., Montagner, C.C., 2020. Matrix effect challenges to quantify 2,4-D and fipronil in aquatic systems. *Environ. Monit. Asses.* 192 (12), 1–12. <https://doi.org/10.1007/s10661-020-08776-3>.
- Goulart, B.V., Vizioli, B.D.C., da Silva Pinto, T.J., Freitas, J.S., Moreira, R.A., Da Silva, L.C.M., Montagner, C.C., 2024. Fate and toxicity of 2, 4-D and fipronil in mesocosm systems. *Chemosphere* 346, 140569. <https://doi.org/10.1016/j.chemosphere.2023.140569>.
- Gunkel, G., Kosmol, J., Sobral, M., Rohn, H., Montenegro, S., Aureliano, J., 2007. Sugar cane industry as a source of water pollution—Case study on the situation in Ipojuca River, Pernambuco, Brazil. *Water. Air. Soil. Pollut.* 180, 261–269. <https://doi.org/10.1007/s11270-006-9268-x>.
- Hansen, H.P., Koroleff, F., 2007. Determination of nutrients. *Methods of Seawater Analysis*. John Wiley & Sons, Ltd, pp. 159–228.
- Helson, J.E., Williams, D.D., 2013. Development of a macroinvertebrate multimetric index for the assessment of low-land streams in the neotropics. *Ecol. Indic.* 29, 167–178. <https://doi.org/10.1016/j.ecolind.2012.12.030>. Elsevier BV.
- Hoarau, J., Caro, Y., Grondin, L., Petit, T., 2018. Sugarcane vinasse processing: Toward a status shift from waste to valuable resource. A review. *J. Water. Process. Eng.* 24, 11–25. <https://doi.org/10.1016/j.jwpe.2018.05.003>.
- Hou, L., Xiong, W., Chen, M., Xu, J., Johnson, A.C., Zhan, A., Jin, X., 2025. Pesticide pollution reduces the functional diversity of macroinvertebrates in urban aquatic ecosystems. *Environ. Sci. Technol.* 59 (17), 8568–8577. <https://doi.org/10.1021/acs.est.5c01093>.
- Islam, F., Wang, J., Farooq, M.A., Khan, M.S.S., Xu, L., Zhu, J., Zhao, M., Munos, S., Li, Q. X., Zhou, W., 2018. Potential impact of the herbicide 2,4-dichlorophenoxyacetic acid on human and ecosystems. *Environ. Int.* 111, 332–351. <https://doi.org/10.1016/j.envint.2017.10.020>.
- Jote, C.A., 2019. A review of 2, 4-D environmental fate, persistence and toxicity effects on living organisms. *Organ. Medic. Chem. Int. J.* 9, 22–32. <https://doi.org/10.19080/OMCIJ.2019.09.555755>.
- Katagi, T., 2010. Bioconcentration, Bioaccumulation, and Metabolism of Pesticides in Aquatic Organisms. *Reviews of Environmental Contamination and Toxicology*. Springer, New York, NY, pp. 1–132. [https://doi.org/10.1007/978-1-4419-1440-8\\_1](https://doi.org/10.1007/978-1-4419-1440-8_1).
- Kefford, B.J., Brooks, A.J., Nichols, S.J., Bray, J.P., 2024. Macroinvertebrate community and leaf litter breakdown measures lack concordance associated with singular or multiple stressors. *Sci. Total Environ.* 953, 176082. <https://doi.org/10.1016/j.scitotenv.2024.176082>.
- Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., 2025. PubChem 2025 update. *Nucleic. Acids. Res.* 53 (D1), D1516–D1525. <https://doi.org/10.1093/nar/gkaf1059>.
- Kumar, P., Kumar, R., Thakur, K., Mahajan, D., Brar, B., Sharma, D., Kumar, S., Sharma, A.K., 2023. Impact of pesticides application on aquatic ecosystem and biodiversity: a review. *Biology Bulletin* 50 (6), 1362–1375. <https://doi.org/10.1134/S1062359023601386>.
- Mackereth, F.J.H., Heron, J., Talling, J.F., 1978. Water analysis: Some revised methods for limnologists. *Freshwater Biological Association, Ambleside, Cumbria*.
- Mambougou, J., Fernandes, É.K.K., Vieira, L.G., Rocha, T.L., 2024. Hazardous fipronil insecticide effects on aquatic animals' health: Historical review and trends. *Sci. Total Environ.*, 176334. <https://doi.org/10.1016/j.scitotenv.2024.176334>.
- Marcato, A.C.C., Souza, C.P., Fontanetti, C.S., 2017. Herbicide 2,4-D: A Review of Toxicity on Non-Target Organisms. *Water Air Soil Pollut* 228, 120. <https://doi.org/10.1007/s11270-017-3301-0>.
- Marchesan, E., Sartori, G.M.S., Avila, L.A.de, Machado, S.L.de O., Zanella, R., Primel, E. G., Macedo, V.R.M., Marchezan, M.G., 2010. Resíduos de agrotóxicos na água de rios da Depressão Central do Estado do Rio Grande do Sul. *Brasil. Ciência Rural.* 40, 1053–1059. <https://doi.org/10.1590/S0103-84782010005000078>.
- Merritt, R.W., Cummins, K.W., 1996. *An introduction to the aquatic insects of North America*, 3rd ed. Kendall/Hunt Publishing Company, Iowa - USA.
- Moran-Salazar, R.G., Sanchez-Lizarraga, A.L., Rodriguez-Campos, J., Davila-Vazquez, G., Marino-Marmolejo, E.N., Dendooven, L., Contreras-Ramos, S.M., 2016. Utilization of vinasses as soil amendment: consequences and perspectives. *Springerplus.* 5 (1), 1007. <https://doi.org/10.1186/s40064-016-2410-3>.
- Moreira, R.A., Daam, M.A., Vieira, B.H., Sanches, A.L.M., Reghini, M.V., Mansano, A.S., de Freitas, E.C., Espíndola, E.L.G., Rocha, O., 2017. Toxicity of abamectin and difenoconazole mixtures to a neotropical cladoceran after simulated run-off and spray drift exposure. *Aquat. Toxicol.* 185, 58–66. <https://doi.org/10.1016/j.aquatox.2017.02.001>.
- Mustapha, L.S., Obayomi, O.V., Obayomi, K.S., 2025. A comprehensive review on potential heavy metals in the environment: Persistence, bioaccumulation, ecotoxicology, and agricultural impacts. *Ecolog. Front.* 1–16. <https://doi.org/10.1016/j.ecofro.2025.10009>.
- Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K., Kim, C.H., 2017. Effect of pesticides on microbial communities in container aquatic habitats. *Sci. Rep.* 7 (1), 44565. <https://doi.org/10.1038/srep44565>.
- Oberdorfer, R.Y., McArthur, J.V., Barnes, J.R., Dixon, J., 1984. The effect of invertebrate predators on leaf litter processing in an alpine stream. *Ecol* 65 (4), 1325–1331. <https://doi.org/10.2307/1938337>.
- OECD, O. for E.C. and D., 2006. Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms) [WWW Document]. OECD. URL [https://www.oecd.org/en/publications/guidance-document-on-simulated-freshwater-lentic-field-tests-outdoor-microcosms-and-mesocosms\\_bb366ec4-en.html](https://www.oecd.org/en/publications/guidance-document-on-simulated-freshwater-lentic-field-tests-outdoor-microcosms-and-mesocosms_bb366ec4-en.html) (accessed 11.6.25).
- Oksanen, J., Simpson, G.L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solyomos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlenn, D., Ouellette, M.H., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C.J.F., Weedon, J., 2022. vegan: Community Ecology Package. R package version 2.6-4. <https://CRAN.R-project.org/package=vegan>.
- Onyango, J., van Bruggen, J.J.A., Kitaka, N., Simateka, J., Irvine, K., 2024. Effects of combined nutrient and pesticide exposure on algal biomass, and *Daphnia magna* abundance. *Environ. Syst. Res. (Heidelb)* 13 (1), 2005. <https://doi.org/10.1186/s40068-023-00326-3>.
- Ordaz-Guillén, Y., Galíndez-Mayer, C.J., Ruiz-Ordaz, N., Juárez-Ramírez, C., Santoyo-Tepole, F., Ramos-Monroy, O., 2014. Evaluating the degradation of the herbicides picloram and 2,4-D in a compartmentalized reactive biobarrier with internal liquid recirculation. *Environ. Sci. Pollut. Res.* 21, 8765–8773. <https://doi.org/10.1007/s11356-014-2809-8>.
- Panis, C., Kawassaki, A.C.B., Crestani, A.P.J., Pasotto, C.R., Bortoloti, D.S., Vicentini, G. E., Lucio, L.C., Ferreira, M.O., Prates, R.T.C., Vieira, V.K., Goboardi, S.C., Candiotti, L.Z.P., 2022. Evidence on Human Exposure to Pesticides and the Occurrence of Health Hazards in the Brazilian Population: A Systematic Review. *Front. Public Health* 9. <https://doi.org/10.3389/fpubh.2021.787438>.
- Piffer, M.A., Oliveira, C.A., Bovio-Winkler, P., Eng, F., Etchebehere, C., Zaiat, M., Fuess, L.T., 2022. Sulfate-and pH-driven metabolic flexibility in sugarcane vinasse dark fermentation stimulates biohydrogen evolution, sulfidogenesis or homoacetogenesis. *Int. J. Hydrogen. Energy* 47 (73), 31202–31222. <https://doi.org/10.1016/j.ijhydene.2022.07.039>.
- Pinheiro, A., Silva, M., Kraisch, R., 2010. Presença de pesticidas em águas superficiais e subterrâneas na bacia do Itajá. *SC. REGA* 7, 17–26. <https://doi.org/10.21168/regav7n2.p17-26>.
- Pinto, T.J.S., Freitas, J.S., Moreira, R.A., Silva, L.C.M., Yoshii, M.P.C., Lopes, L.F., de, P., Goulart, B.V., Vanderlei, M.R., Athayde, D.B., Fraga, P.D., Ogura, A.P., Schiesari, L., Montagner, C.C., Daam, M.A., Espíndola, E.L.G., 2021. Functional responses of *Hyalella meinerti* after exposure to environmentally realistic concentrations of 2,4-D, fipronil, and vinasse (individually and in mixture). *Aquat. Toxic.* 231, 105712. <https://doi.org/10.1016/j.aquatox.2020.105712>.
- Pinto, T.J.S., Moreira, R.A., Schmidt Freitas, J.S., da Silva, L.C.M., Yoshii, M.P.C., de Palma Lopes, L.F., Espíndola, E.L.G., 2023. Responses of *Chironomus sancticaroli* to the simulation of environmental contamination by sugarcane management practices: water and sediment toxicity. *Sci. Total Environ.* 857, 159643. <https://doi.org/10.1016/j.scitotenv.2022.159643>.
- Qu, H., Ma, R.X., Liu, D.H., Gao, J., Wang, F., Zhou, Z.Q., Wang, P., 2016. Environmental behavior of the chiral insecticide fipronil: enantioselective toxicity, distribution and transformation in aquatic ecosystem. *Water. Res.* 105, 138–146. <https://doi.org/10.1016/j.watres.2016.08.063>.
- Ramírez, A., Gutiérrez-Fonseca, P.E., Rio, R., Juan, S., Rico, P., 2014. Functional feeding groups of aquatic insect families in Latin America: a critical analysis and review of existing literature. *Rev. Bio. Trop.* 62 (S2), 155–167.
- Rawer-Jost, C., Böhrer, J., Blank, J., Rahmann, H., 2000. Macroinvertebrate functional feeding group methods in ecological assessment. *Hydrobiologia* 422, 225–232. <https://doi.org/10.1023/A:1017078401734>.

- Reis, C.E.R., Hu, B., 2017. Vinasse from sugarcane ethanol production: better treatment or better utilization? *Front. Energy Res.* <https://doi.org/10.3389/fenrg.2017.00007>.
- Ruggiero, K.L.F., da Silva Pinto, T.J., Gomes, D.F., Dias, M.A., Montagner, C.C., Rocha, O., Moreira, R.A., 2024. Ecological Implications on Aquatic Food Webs Due to Effects of Pesticides on Invertebrate Predators in a Neotropical Region. *Arch. Environ. Contam. Toxicol.* 86 (2), 112–124. <https://doi.org/10.1007/s00244-024-01052-2>.
- Rulli, M.M., Del Gobbo, L.M., Colin, V.L., 2023. Harmful effects of sugarcane vinasse on water bodies: conventional remediation technologies. *Green Sustainable Process for Chemical and Environmental Engineering and Science*. Elsevier, pp. 375–394. <https://doi.org/10.1016/B978-0-323-95183-8.00016-0>.
- Rumschlag, S.L., Mahon, M.B., Hoverman, J.T., Raffel, T.R., Carrick, H.J., Hudson, P.J., Rohr, J.R., 2020. Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. *Nat. Commun.* 11 (1), 6333. <https://doi.org/10.1038/s41467-020-20192-2>.
- Santos, M.B.H., Bernardes, M.B.J., 2018. O uso dos agrotóxicos no Brasil: suas implicações e desafio. *Espaço em Revista* 20 (2), 29–42. <https://doi.org/10.5216/er.v20i2.57946>.
- Santos, P.S., Zaiat, M., do Nascimento, C.A.O., Fuess, L.T., 2019. Does sugarcane vinasse composition variability affect the bioenergy yield in anaerobic systems? A dual kinetic-energetic assessment. *J. Clean. Prod.* 240, 118005. <https://doi.org/10.1016/j.jclepro.2019.118005>.
- Saraiva, A.S., Sarmiento, R.A., Golovko, O., Randak, T., Pestana, J.L., Soares, A.M., 2018. Lethal and sub-lethal effects of cyproconazole on freshwater organisms: a case study with *Chironomus riparius* and *Dugesia tigrina*. *Environ. Sci. Pollut. Res.* 25, 12169–12176. <https://doi.org/10.1007/s11356-017-1180-y>.
- Schäfer, R.B., von der Ohe, P.C., Rasmussen, J., Kefford, B.J., Beketov, M.A., Schulz, R., Liess, M., 2012. Thresholds for the Effects of Pesticides on Invertebrate Communities and Leaf Breakdown in Stream Ecosystems. *Environ. Sci. Technol.* 46, 5134–5142. <https://doi.org/10.1021/es2039882>.
- Silva, A.R.R., Cardoso, D.N., Cruz, A., Lourenço, J., Mendo, S., Soares, A.M.V.M., Loureiro, S., 2015. Ecotoxicity and genotoxicity of a binary combination of triclosan and carbendazim to *Daphnia magna*. *Eco. Environ. Safet.* 115, 279–290. <https://doi.org/10.1016/j.ecoenv.2015.02.022>.
- Silva, L.C.M., Moreira, R.A., Pinto, T.J.S., Vanderlei, M.R., Athayde, D.B., Lopes, L.F.P., Ogura, A.P., Yoshii, M.P.C., Freitas, J.S., Montagner, C.C., Goulart, B.V., Schiesari, L., Daam, M.A., Espíndola, E.L.G., 2021. Lethal and sublethal toxicity of pesticides and vinasse used in sugarcane cultivation to *Ceriodaphnia silvestrii* (Crustacea: Cladocera). *Aquat. Toxicol.* 241, 106017. <https://doi.org/10.1016/j.aquatox.2021.106017>.
- Singh, N.S., Sharma, R., Singh, S.K., Singh, D.K., 2021. A comprehensive review of environmental fate and degradation of fipronil and its toxic metabolites. *Environ. Res.* 199, 111316. <https://doi.org/10.1016/j.envres.2021.111316>.
- Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshw. Biol.* 55, 80–119. <https://doi.org/10.1111/j.1365-2427.2009.02369.x>.
- Stehle, S., Schulz, R., 2015. Agricultural insecticides threaten surface waters at the global scale. *Proceed. Nation. Acad. Sci.* 112 (18), 5750–5755. <https://doi.org/10.1073/pnas.1500232112>.
- US EPA, U.S. environmental P.A., 1999. *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish (No. Second Edition)*. Office of Water, Washington, DC.
- US EPA, U.S. environmental P.A., 2005. Reregistration Eligibility Decision for 2,4-D. Washington, DC.
- US EPA, U.S. environmental P.A., 2015. *Aquatic Life Benchmarks and Ecological Risk Assessments for Registered Pesticides* [WWW Document]. URL <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk> (accessed 11.5.25).
- Von Sperling, M., 1996. *Introdução à qualidade das águas e ao tratamento de esgotos*, 1. Editora UFMG.
- Wadt, L.C., 2008. *Cultivo de Pleurotus spp. em vinhaça visando à produção de biomassa e exopolissacarídeos*. Dissertação (Mestrado em Ciências). Universidade de São Paulo, Piracicaba, 2008.
- Xu, H., Liu, X., Jia, Y., Dong, F., Xu, J., Wu, X., Zheng, Y., 2018. Fipronil-induced toxic effects in zebrafish (*Danio rerio*) larvae by using digital gene expression profiling. *Sci. Total Environ.* 639, 550–559. <https://doi.org/10.1016/j.scitotenv.2018.05.159>.