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RESEARCH ARTICLE

Sugarcane vinasse provokes acute and chronic responses and bioaccumulation of metals in benthic macroinvertebrates

Priscille Dreux Fraga[1] · Gabriele Verônica de Mello Gabriel[2] · Janaina Braga do Carmo[2] · Evaldo Luiz Gaeta Espindola[1,3] · Thandy Junio da Silva Pinto[1,4]

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Abstract

Brazil is a major producer of sugarcane bioethanol, which has raised concerns about its environmental impact. The industrial process for obtaining ethanol generates a by-product with a high pollution potential called vinasse. If vinasse reaches watercourses, it may affect the biological communities, such as the aquatic macroinvertebrates, which include species sensitive to environmental contamination. Thus, this study evaluated the ecotoxicological effects of sugarcane vinasse on tropical benthic macroinvertebrates (*Allonais inaequalis*, *Chironomus sancticaroli*, *Strandesia trispinosa*, and *Hyalella meinerti*). The study was divided into three phases. First, acute toxicity tests were carried out with the four species. The species *A. inaequalis* (average LC_{50} = 0.460% confidence interval, CI 0.380–0.540%) was more sensitive to vinasse than *C. sancticaroli* (LC_{50} 0.721%, CI 0.565–0.878%), *H. meinerti* (EC_{50} 0.781%, CI 0.637–0.925%), and *S. trispinosa* (EC_{50} 1.283%, CI 1.045–1.522%). In the second phase, the consequences of chronic exposure to vinasse were assessed in the two more sensitive species. Impairments in reproduction and population growth rates for *A. inaequalis* and on the development, metamorphosis, and body growth of *C. sancticaroli* larvae occurred. Finally, the bioaccumulation of metals after chronic exposure was determined in the third phase. Vinasse provoked decreases in the body residue of the essential metals Zn and Mn and the accumulation of Cd, Pb, and Cr with the potential for biomagnification throughout the food webs. Low concentrations of vinasse (below 1%) provoked lethal and sublethal effects on benthic organisms, with several cascade effects on aquatic environments, given the ecological importance of this group in freshwater and terrestrial ecosystems.

Keywords Ethanol · Growth · industrial waste · Organic load · Reproduction · Stillage · Tropical species

Introduction

The Industrial Revolution increased the world's energy dependence, with emphasis on the use of fossil fuels, which presents elevated costs of transport and production, besides the high rate of carbon emission into the atmosphere, as well as the depletion of the fossil reserves (Goldemberg and Lucon 2007). Thus, new energy alternatives have started to gain attention, and global interest in bioenergy production has grown in the last decades (Duarah et al. 2022). This is the case with the production of sugarcane ethanol. Brazil is a great producer of ethanol due to this extensive territory, water availability, and favorable solar radiation, which provides the existence of large monocultures (Martinelli and Filoso 2008). In the 2022/2023 Brazilian harvest, around 610 million tons of sugarcane were produced, with a total volume of ethanol obtained of 27.4 billion liters (CONAB 2023). Thus, discussions regarding agricultural

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✉ Priscille Dreux Fraga
pri.dreux@gmail.com

[1] São Carlos Engineering School (EESC), Center of Water Resources and Environmental Studies (CRHEA), Nucleus of Ecotoxicology and Applied Ecology (NEEA), University of São Paulo, Av. Trabalhador São Carlense, 400, São Carlos 13560-970, Brazil

[2] Federal University of São Carlos (UFSCar), Department of Physics, Chemistry, and Mathematics, Rodovia João Leme Dos Santos, SP-264, Km 110, Sorocaba, São Paulo 18052-780, Brazil

[3] Post-Graduate Program in Environmental Engineering Sciences, EESC/CRHEA, NEEA, University of São Paulo, Av. Trabalhador São Carlense, 400, São Carlos 13560-970, Brazil

[4] Analytical Chemistry Department, Institute of Chemistry, University of Campinas, Campinas, São Paulo, Brazil

and industrial production of ethanol are crucial, mainly about the environmental impacts arising from the large-scale production.

During the distillation process of fermentation liquor to obtain ethanol, a liquid by-product with a high potential for pollution called vinasse is produced (Silva et al. 2007). For each liter of ethanol obtained, 8 to 18 L of vinasse are generated (Parnaudeau et al. 2008). Thus, considering ethanol production in Brazil, billions of liters of vinasse are produced annually. This compound has acidic characteristics (pH 3.5–5), with the appearance of a dark brown paste and an unpleasant odor, which contains a high organic and metal load (Christofoletti et al. 2013). Consequently, ethanol-producing industries need a destination for this residue. The fertirrigation of sugarcane fields is adopted as the main alternative in Brazil, focusing on the use of potassium as a nutrient source (Moraes et al. 2014). Due to its high organic load and nutrient concentration, such as potassium, calcium, and nitrogen, fertirrigation can be beneficial for crop growth (Botelho et al. 2012). On the other hand, there are still doubts about the use of vinasse for this purpose due to impacts on soil and the possibility of contamination of water resources by leaching, percolation, and surface runoff processes (Santana and Fernandes Machado 2008; Silva et al. 2007). In addition, the improper disposal of vinasse during fertirrigation activities and accidents during storage and transport may imply a direct entry into aquatic ecosystems (Martinelli and Filoso 2008). When vinasse reaches freshwater environments, it can disrupt biological communities, leading to imbalances in the aquatic environment. This disruption can reduce productivity, biodiversity, nutrient cycling, and the dynamics of food webs (McBride et al. 2011).

Benthic macroinvertebrates, such as the insect *Chironomus sancticaroli* (Diptera: Chironomidae), the Oligochaeta *Allonais inaequalis* (Haplotaxida: Naididae), the amphipod *Hyalella meinerti* (Amphipoda: Hyalellidae), and the ostracod *Strandesia trispinosa* (Podocopida: Cyprididae) can be used as bioindicators of environmental stress, mainly because their life cycles include sediment, water column, and, in the case of *C. sancticaroli*, terrestrial ecosystems in the adult stage (De Castro-Català et al. 2016; Silva et al. 2007). These organisms compose the basis of the food chain. They are essential in the secondary productivity in freshwater and the transfer of energy and nutrients to higher trophic levels, influencing the stability and resilience of ecological systems. Thus, the loss of these species by increased mortality and impairments in biomass and population growth imply damage to ecosystem services (Chagnon et al. 2015). In addition, benthic organisms can bioaccumulate metals via different exposure routes (contaminated water and sediment) with risks of biomagnification throughout the food chain (Leppänen 1995). Vinasse has several metals in its composition with the potential for bioaccumulation by exposed organisms (Christofoletti et al. 2013; Coelho et al. 2018; España-Gamboa et al. 2011).

Given the substantial production of sugarcane vinasse, its pollution potential, and its widespread use as a fertilizer in sugarcane cultivation, it is crucial to assess its impact on aquatic organisms. Moreover, there exists a notable knowledge gap concerning the ecotoxicological effects of vinasse on freshwater species. To address this, the present study evaluated (i) the implications of acute exposure to vinasse on *C. sancticaroli*, *A. inaequalis*, *H. meinerti*, and *S. trispinosa* species; (ii) the subchronic responses on the survival, development, and growth of *C. sancticaroli* and chronic implication for the reproduction and biomass of *A. inaequalis*; and (iii) the bioaccumulation of metals after subchronic and chronic exposure in both species. The results described will bring new discussions about the environmental implications of potential contamination of freshwater ecosystems caused by fertirrigation practices in sugarcane management.

Materials and methods

Test organisms

The test organisms were obtained from cultures kept at the Nucleus of Ecotoxicology and Applied Ecology (NEEA) in the Center for Water Resource and Environmental Studies (CRHEA). The species *C. sancticaroli* and *A. inaequalis* were maintained according to the procedure described in Pinto et al. (2021b) and Corbi et al. (2015) in plastic trays filled with culturing water (pH 7.0–7.5, electrical conductivity 50.5 ± 1.4 µS cm^{-1}, and hardness 12–16 mg $CaCO_3$ L^{-1}) and artificial sediment (fine sand washed and burned at 550 °C for 4 h) in a proportion of 4:1 (water: sediment), with constant aeration. Food (Tetramin® suspension 5 g L^{-1}) was provided ad libitum each other day to *C. sancticaroli* and one time a week to *A. inaequalis*. *H. meinerti* was kept according to the Brazilian normative NBR 15470 (2013) in a glass aquarium filled with 4 L of culturing water (pH 7.0–7.5, electrical conductivity 150–160 µS cm^{-1}, and hardness 40–48 mg $CaCO_3$ L^{-1}), containing the macrophytes *Egeria densa* and *Myriophyllum aquaticum* as food supply and substrate, and constant aeration. Organisms were fed ad libitum with compound food (1:1, Tetramin® suspension 10 g L^{-1} and biological yeast 5 mg mL^{-1}) each other day. The species *S. trispinosa* was obtained from outdoor cultures (see Pinto et al. 2021a) and acclimated in the laboratory 10 days before experiments. For that, the natural water was replaced with culturing water under the same conditions as *H. meinerti.* Decomposition leaves from the outdoor cultures were provided as food resources. All cultures were kept at a room temperature of 25 ± 1 °C and a daily cycle of 12:12 (light:

dark). For the toxicity tests with *C. sancticaroli*, the organisms were obtained from egg masses from the cultures, and *A. inaequalis* was chosen by the body size of 7.8 ± 0.37 mm (Rocha et al. 2018) as the species reproduces by bipartition (Armendáriz 1999). *S. trispinosa* was also chosen by the body size of 0.93 ± 0.08 mm (Rocha et al. 2018). *H. meinerti* juveniles (1–7 days) were separated from cultures and kept in a new glass aquarium until they reached the age of 7–14 days before the toxicity tests.

Acute toxicity test

Acute toxicity tests were performed under the same temperature (25 ± 1 °C) and light conditions (12-h light: 12-h dark) for all cultures. Preliminary tests were carried out to establish the dilutions used in the acute experiments. A summary of the test conditions with all species is presented as supplementary material (Table S1). The test solutions were obtained by diluting the raw vinasse using the respective culturing water. Laboratory controls were prepared with culture water only.

The bioassays with *A. inaequalis* and *S. trispinosa* were made in 50-mL non-toxic plastic bottles (Copaza®) containing 10 mL of test solution, without substrate and feeding (Rocha et al. 2018). The organisms were exposed to five concentrations of crude vinasse: 0.125, 0.25, 0.50, 1.0, and 2.0% for *A. inaequalis* and 1.0, 1.2, 1.4, 1.6, and 1.8% for *S. trispinosa*. Four replicates were prepared for each of the concentrations that were tested, and six individuals of *A. inaequalis* (7.8 ± 0.37 mm) and five individuals of *S. trispinosa* (0.93 ± 0.08 mm) were placed by replicate. The experiments lasted 48 h for *S. trispinosa* and 96 h for *A. inaequalis*, evaluating the immobility and mortality of the organisms, respectively.

The tests with *C. sancticaroli* were carried out in 400-mL non-toxic plastic bottles (Copaza®) containing 240 mL of the test solution and 60 g of artificial sediment. Five concentrations of vinasse were prepared (0.4, 0.6, 0.8, 1.0, and 1.2%) besides the laboratory control. Four replicates containing six larvae each (7 to 8 days old) were prepared in each concentration, adapting the methodology described in the OECD (2011) guideline. At the experiment's end (96 h), larvae mortality was assessed. The experiments with *H. meinerti* were carried out in 400-mL non-toxic plastic bottles (Copaza®) containing 200 mL of test solution and a nylon screen (4 cm × 15 cm, 0.25 mesh-size) as substrate. Five concentrations of vinasse were prepared (0.6, 0.8, 1.0, 1.2, 1.4%) with four replicates containing ten organisms (7 to 14 days old) each (NBR 15470 2013). The acute tests lasted 96 h, assessing the individuals' immobility.

Food was provided at the beginning and middle of the experiments with *C. sancticaroli* (0.62 mg TetraMin® larvae^{-1} day^{-1}) and *H. meinerti* (0.025 mL compound food organism^{-1}). The acute experiments with the four species were repeated three times with different populations. The dissolved oxygen and temperature (YSI55-25 ft), pH (Micronal B374), and electrical conductivity (Oriom 145) were measured at the beginning and end of the tests. The criteria for validity were mortality/immobility in the control group below 10% in all species (OECD 2011; Pinto et al. 2021c).

Subchronic and chronic toxicity test

The bioassays were performed with five concentrations of vinasse for the species *C. sancticaroli* (0.041, 0.081, 0.163, 0.325, and 0.65%) and *A. inaequalis* (0.025, 0.05, 0.10, 0.20 and 0.40%) besides of the untreated control (culturing water). The higher concentration of vinasse for each species was selected based on their respective CL_{10} value obtained on acute tests. These two species were selected due to their sensitivity to vinasse (see the "Acute tests" section). A summary of the conditions used in the experiments is presented as supplementary material (Table S2).

The bioassays with both species were made in 40-mL non-toxic plastic bottles containing 60 g of the substrate (fine sand) and 240 mL of test solution. Eight replicates containing ten organisms each (larvae of *C. sancticaroli* 4 days old and individuals of 7.8 ± 0.37 mm for *A. inaequalis*) were prepared per test concentration. The tests lasted 8 and 10 days for *C. sancticaroli* (subchronic exposure) and *A. inaequalis* (chronic exposure). Food was provided every other day (0.62 mg TetraMin® organism^{-1} day^{-1}). The dissolved oxygen, pH, and electrical conductivity were measured at the beginning and end of the experiments. Besides, the concentration of ammonium (Hansen and Koroleff 1999) was determined at the experiment's end. The tests were validated for a maximum mortality of 20% for *C. sancticaroli* and population doubling for *A. inaequalis* in the control treatment (Pinto et al. 2021a). The experiments with both species were repeated three times with different populations.

At the end of the experiment with *C. sancticaroli*, the number of alive larvae, pupae, and adults was registered. The larvae were anesthetized in phenoxyethanol and photographed with graph paper. The larval body length was measured using the free software *Kinovea* 0.8.15 (https://www.kinovea.org/) (Pinto et al. 2021a, b, c). For *A. inaequalis*, the number of alive organisms was counted after 10 days for reproduction determination. After that, the individuals of both species were kept in clean water for 24 h for gut cleansing and further determination of metal bioaccumulation.

Analyses of crude vinasse

Physical–chemical and biological characterization of crude vinasse used in the present study was performed. The biochemical oxygen demand (BOD) was measured by the

oxygen consumption during a 5-day incubation time. The chemical oxygen demand (COD) was analyzed by the closed reflux method (APHA 2018). Furthermore, the pH (pH-meter micronal B374), electrical conductivity, salinity, total dissolved solids (conductivity meter Oriom 145), hardness (ABNT NBR 1995), total nitrogen (APHA 2018), nitrite, nitrate, and ammonium ion (Hansen and Koroleff 1999; Mackereth et al. 1978), total and dissolved phosphorus, and inorganic phosphate (Andersen 1976) were analyzed. Additionally, an aliquot was taken for metal analyses.

Chemical analyses of metals

The preserved organisms were dried (60 °C) until constant weight to determine the dry biomass (0.01 mg). The metals were extracted by acid digestion with 5 mL of HNO_3:H_2O_2 mixture (2:1 v:v) in a water bath at 80 °C for 4 h. Afterwards, the sample volume was completed up to 10 mL with HNO_3 (2 M). The blank was carried out with the acid and peroxide mixture and completed up to 10 mL (Afridi et al. 2006).

In addition, samples from the test solutions were taken at the beginning of each chronic and subchronic experiment. The samples and crude vinasse aliquot were prepared for chemical analysis by acid digestion (USEPA 1992). Analytical blanks were prepared following the same procedures. Metals were quantified by microwave plasma atomic emission spectroscopy (MP-AES 4200, Agilent Technologies). For the analytical calibration curves, multielement standard solutions (Agilent Technologies®) were utilized, except for aluminum (Sigma-Aldrich®) and potassium (SpecSol®). The limits of detection (LOD) and quantification (LOQ) were obtained according to the method described in Hage and Carr (2012).

Data analysis

In the acute tests, lethal and effect concentrations for 50%, 20%, and 10% of organisms were estimated by non-linear regression with a logistic model. The responses in the subchronic and chronic bioassays and metal residuals in the organisms were compared with the control group by one-way ANOVA, followed by Dunnett's post hoc test. Data normality and homogeneity of variances were determined using the Shapiro–Wilk and Levene tests, respectively. In the case of non-compliance with the parametric requirements, the non-parametric test of Kruskal–Wallis was used. The Ash Free Dry Weight of *C. sancticaroli* (AFDW) was estimated based on the body length of the larvae, following the method described by Pinto et al. (2021a, b, c). The presented results are the average of the three repetitions of acute and chronic assays. All analyses were performed in the Statistica 7.0 software (StatSoft 2004) with a confidence interval of 95% ($p < 0.05$).

Results and discussion

Vinasse characterization

Table 1 presents the physical–chemical and biological characterization of the crude vinasse used in the experiments. Vinasse composition depends on the sugarcane harvest, the fermentation, and the distillation conditions adopted (España-Gamboa et al. 2011). Therefore, the composition varies across different studies, as shown in Table 1. The crude vinasse used in the present study had high polluting potential, mainly compared with the other studies, denoted by the high electrical conductivity and concentration of phosphorus, nitrogen, potassium, calcium, aluminum, magnesium, and other metals, in addition to a high organic load (BOD and COD).

Acute tests

Table 2 shows the average lethal or effect concentrations for *C. sancticaroli*, *A. inaequalis*, *S. trispinosa*, and *H. meinerti*. Survival of all species was higher than 90% in the control groups, and the experiments were valid. At the beginning and end of the tests, increases in the vinasse concentrations reduced pH and dissolved oxygen (DO) and increased the electrical conductivity. At the end of acute toxicity tests, DO was below 6 mg L^{-1} (Tables S3 till S14).

In the present study, *A. inaequalis* was more sensitive to vinasse than *C. sancticaroli*, followed by *H. meinerti* and *S. trispinosa*. The LC_{50}-96 h value to *A. inaequalis* was also below the reported for the microcrustaceans *Ceriodaphnia dubia* (EC_{50}-48 h 0.67%) and *Daphnia magna* (EC_{50}-48 h 0.80%), and the fish *Danio rerio* (LC_{50}-96 h 2.62%) (Botelho et al. 2012). In the same way, all species in the present study had lower lethal values than the microcrustaceans *D. magna* (EC_{50}-48 h 3.6%) and *D. similis* (EC_{50}-48 h 2.2%) (Ferreira et al. 2011). However, as discussed previously, the characteristics of vinasse are variable, and the results are not directly comparable.

Both cited studies used methods to treat vinasse and compared the toxicity before and after treatment. After adjusting the pH values from 4 to 7, Botelho et al. (2012) observed a reduction in toxicity with increases in the EC_{50} up to 4.5-times (final EC_{50}-48 h 2.99%) for *C. dubia*, 7-times (EC_{50}-48 h 5.62%) for *D. magna*, and 3.2-times (LC_{50}-96 h 8.34%) for *D. rerio*. After vinasse treatment with the fungus *Pleurotus sajor-caju*, Ferreira et al. (2011) noticed a reduction of the toxicity for *D. magna* 18-times (final EC_{50}-48 h 66%) and *D. similis* 9.5-times (EC_{50}-48 h 21%). These

Table 1 Physical–chemical parameters of the crude vinasse used in the present study and comparison with sugarcane vinasse used in other ecotoxicological studies from the literature

Parameters	Unity	Present study	Christofoletti et al. (2013)	Coelho et al. (2018)	Correia et al. (2017)	Ferreira et al. (2011)	Garcia et al. (2017)
pH	-	3.91	3.9	4.14	4.37	3.9	4.6
Electrical conductivity	$\mu S\ cm^{-1}$	8420	-	-	15,110	8630	8294
Salinity	%	4.5	-	-	-	-	-
Total dissolved solids	$mg\ L^{-1}$	4450		-	-	152,126	-
Hardness	$mg\ CaCO_3\ L^{-1}$	3500	2493	-	276	-	3390.4
Total nitrogen	$mg\ L^{-1}$	639	-	-	171	70	234.1
Total phosphorus	$mg\ L^{-1}$	149,670	190	11,600	207	-	-
Nitrate	$mg\ L^{-1}$	9.3	-	-	1.49	-	-
Nitrite	$mg\ L^{-1}$	0.2	-	-	0.033	-	-
Ammonium ion	$mg\ L^{-1}$	52.6	-	-	< LOQ	-	10,920
Inorganic phosphate	$mg\ L^{-1}$	82.9	-	-	-	200	5518
Total dissolved phosphorus	$mg\ L^{-1}$	84.9	-	-	-	-	-
BOD*	$mg\ L^{-1}$	46,500	5046	5850	7941	11,300	13,394.3
COD*	$mg\ L^{-1}$	107,000	13,380	12,533	25,225	42,000	31,723.2
Chromium (Cr)	$mg\ L^{-1}$	0.207	0.04	0.014	3.56	-	-
Copper (Cu)	$mg\ L^{-1}$	3	0.35	0.042	0.76	-	-
Lead (Pb)	$mg\ L^{-1}$	0.34	-	< LOQ	< LOQ	-	-
Manganese (Mn)	$mg\ L^{-1}$	6.24	-	2.170	-	-	-
Nickel (Ni)	$mg\ L^{-1}$	0.183	0.03	0.017	< LOQ	-	-
Cadmium (Cd)	$mg\ L^{-1}$	0.025	-	-	< LOQ	-	-
Zinc (Zn)	$mg\ L^{-1}$	1.225	1.66	2.39	< LOQ	-	-
Cobalt (Co)	$mg\ L^{-1}$	0.219	-	0.012	-	-	-
Strontium (Sr)	$mg\ L^{-1}$	1.518	-	1.550	-	-	-
Molybdenum (Mo)	$mg\ L^{-1}$	0.080	0.008	< LOQ	< LOQ	-	-
Lithium (Li)	$mg\ L^{-1}$	0.031	-		-	-	-
Magnesium (Mg)	$mg\ L^{-1}$	391	237	269	264	290	321,250
Antimony (Sb)	$mg\ L^{-1}$	0.53	-	-	-	-	-
Aluminum (Al)	$mg\ L^{-1}$	499.9	-	0.580	-	-	-
Barium (Ba)	$mg\ L^{-1}$	1.95	-	0.920	-	-	-
Calcium (Ca)	$mg\ L^{-1}$	1140	719	527	671	460	828
Sodium (Na)	$mg\ L^{-1}$	60	-	31.300	114	-	13,570
Potassium (K)	$mg\ L^{-1}$	3360	2056	2660	3401	2300	3276

**BOD* biochemical oxygen demand, *COD* chemical oxygen demand

Table 2 Lethal (LC) and effect (EC) concentrations (mean and confidence intervals) for the acute toxicity tests with *C. sancticaroli*, *A. inaequalis*, *S. trispinosa*, and *H. meinerti*

	C. sancticaroli (96 h)	*A. inaequalis* (96 h)		*S. trispinosa* (48 h)	*H. meinerti* (96 h)
LC_{50} (%)	0.721 (0.565–0.878)	0.460 (0.380–0.540)	EC_{50} (%)	1.283 (1.045–1.522)	0.781 (0.637–0.925)
LC_{20} (%)	0.652 (0.466–0.838)	0.395 (0.263–0.528)	EC_{20} (%)	1.085 (0.918–1.251)	0.651 (0.533–0.769)
LC_{10} (%)	0.615 (0.412–0.818)	0.363 (0.198–0.529)	EC_{10} (%)	0.982 (0.850–1.115)	0.585 (0.480–0.691)

results show that the crude vinasse characteristics, including its acidity, influence the survival of exposed organisms. Vinasse toxicity is possibly caused by the metals and low pH (Garcia et al. 2017). Besides physiological effects, the low pH may increase the availability of metals for exposed organisms (Eggleton and Thomas 2004). Also, acute toxicity (96 h) is reported in the fish *Oreochromis niloticus* at dilutions between 5 and 10% (Correia et al. 2017; Marinho et al.

2014). Coelho et al. (2018) observed toxicity for the same species post-exposure to crude and leached vinasse at 2.5%. Despite these results, the ecotoxicological effects caused by vinasse on aquatic species are still little explored in the literature. Thus, these results help understand the potential implications to biological communities in freshwater environments contaminated by vinasse, showing that low dilutions cause high mortality rates.

Subchronic and chronic tests

The physical–chemical water parameters (pH, electrical conductivity, and DO) evaluated in the bioassays are presented as supplementary material (Tables S15 till S20). The pH values and electrical conductivity increased between the beginning and end of the tests. At the end of the assays, pH was higher than 6.5, and ammonium levels were lower than 2 mg L^{-1} for experiments with both species. For *C. sancticaroli*, the DO values strongly decreased from the beginning (7.2 ± 0.2 mg L^{-1}) to the end of the tests (2.8 ± 0.7 mg L^{-1}) at the higher concentration (0.65%). For the other treatments, values remained higher than 4 mg L^{-1}. Oppositely, no significant decreases in DO occur for experiments with *A. inaequalis*, and concentrations were higher than 6 mg L^{-1}. Artificial aeration was not introduced as the decrease in oxygen levels is considered a stressor related to vinasse pollution (Gunkel et al. 2007; Pinto et al. 2021a, b, c).

Chironomus sancticaroli

Survival of *C. sancticaroli* in the control group was $92.08 \pm 6.88\%$, and the tests were validated. No effects on survival occurred for none of the vinasse concentrations after 8-day exposure ($p > 0.05$, Fig. 1). Regarding larval growth, no effects occurred for organisms exposed to low vinasse concentrations of 0.041 to 0.325% ($p > 0.05$, Fig. 2). In the intermediate concentration (0.163%), organisms accelerated the development and metamorphosis by increasing the number of pupae ($28.61 \pm 20.51\%$) compared with the control ($9.72 \pm 8.67\%$, $p < 0.001$, Fig. 1). For the other concentrations, no differences occurred in the number of pupae formed. Sibley et al. (1997) noticed that the midge *C. tentans* start the metamorphosis when larvae achieve minimal biomass (0.5 mg), thus indicating a minimal growth that initiates the pupation. Moreover, studies have reported that chironomid larvae can identify the contamination by metals and adopt different strategies to escape from these contaminants (Belowitz et al. 2014; De Haas et al. 2006; Wentsel et al. 1977). Thus, the advancement of metamorphosis in the intermediate concentration (0.163%) can be associated with an avoidance response from the contamination since the larvae had sufficient growth conditions to start pupation.

No differences occurred in the number of adults (midges) between control and treatments from 0.041 to 0.325% ($p > 0.05$). On the other hand, exposure to the higher concentration of vinasse (0.65%) decreased the number of pupae and adults compared to the control group ($p < 0.01$, Fig. 1), thus indicating a delay in larval development. Similar effects occurred for *C. sancticaroli* larvae, which presented delays in development when exposed to antimony metal, the insecticide fipronil, and phenanthrene hydrocarbon (Moraes et al. 2014; Pinto et al. 2021b; Richardi et al. 2018). The body length of the fourth instar larvae of *C. sancticaroli* ranges between 9.37 to 15.1 mm, while for the third instar, the values are 5.12 to 6.60 mm (Fonseca and Rocha 2004). The body length of the organisms from control and low concentrations (0.041 to 0.325%) is within the fourth instar ranges. In contrast, the larvae exposed to 0.65% presented

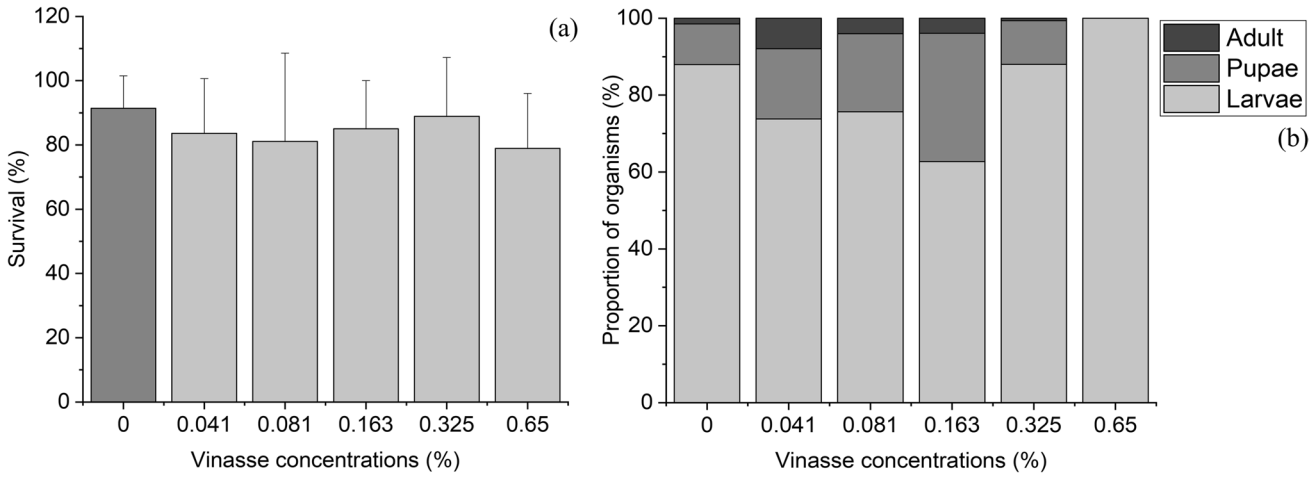

Fig. 1 Survival (mean ± SD) of *C. sancticaroli* in the 8-day subchronic tests (**a**) and percentage of larvae, pupae, and adults alive in the experiment ends (**b**). The number of pupae was increased at 0.163%, and the number of pupae and adults was decreased at 0.65% ($p < 0.05$)

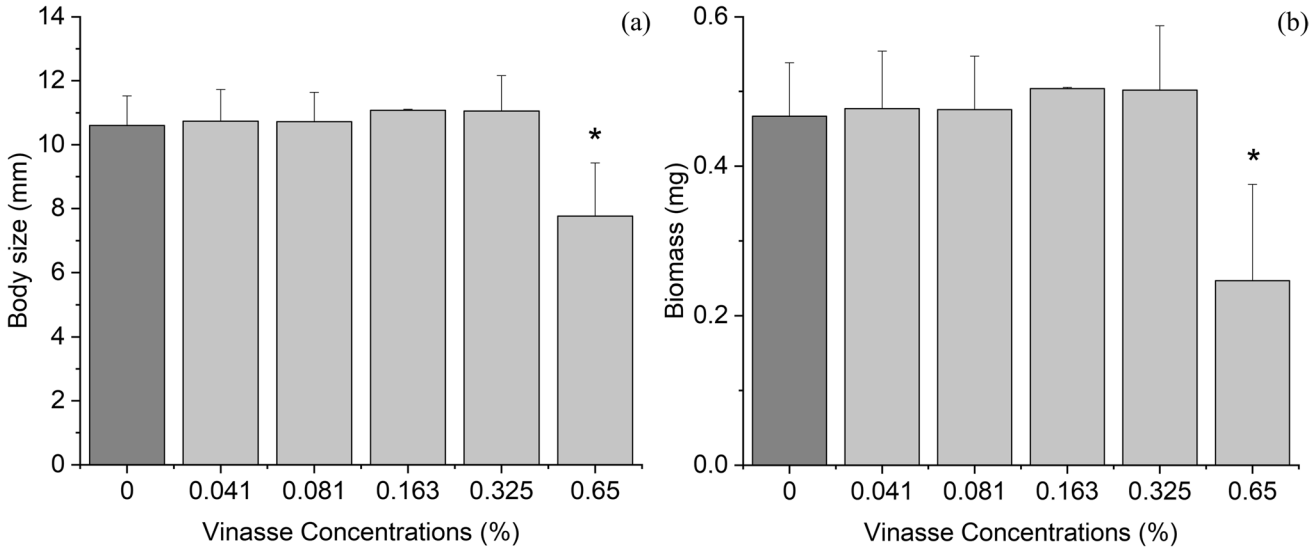

Fig. 2 Body size (**a**) and ash-free dry weight (**b**) of *C. sancticaroli* larvae (mean ± SD) after 8-day exposure to vinasse. Values statistically different from the control ($p < 0.05$) are indicated by asterisks (*)

a lower body length, reinforcing the hypothesis of delays in development.

In the same way as for development, a decrease of 33% in the body length (7.76 ± 1.66 mm) and 50% in biomass (0.24 ± 0.13 mg, $p < 0.05$, Fig. 2) after exposure to 0.65% of vinasse occurred. In a study with four species of chironomids (*C. riparius*, *C. prasinus*, *C. tentans*, and *C. plumosus*), most of the consumed food was converted into biomass during the larvae growth phase (Péry et al. 2005). However, organisms exposed to stressors have less energy available for growth than non-stressed individuals, implying less biomass acquisition and shorter body length (Liber et al. 1996), as observed in the present study. Exposure to chemical stressors triggers severe population declines that can be inferred from the growth of the early stages of life (Liber et al. 1996). Reductions in larval biomass in chironomids are associated with a proportional decline in emergence and reproduction, which leads to a smaller number of larvae in the next generation, thus reducing the population size with risks of local extinction (Sibley et al. 1997).

Allonais inaequalis

Figure 3 shows the reproduction and population growth of *A. inaequalis* post-10-day exposure. Organisms from intermediated concentrations (0.05 and 0.1%) presented increases in the final number of organisms and population growth rate ($p < 0.05$). The increment was about 27% and 19%, respectively. Contrarily, the reproduction of organisms exposed to the highest vinasse concentration (0.4%) was 68% lower than the control, with the reduction in population growth rates reaching 80% ($p < 0.05$).

The family Naididae, which includes the *A. inaequalis* species, can alternate between sexual and asexual reproduction, distinguishing this family from most aquatic oligochaetes (Erséus et al. 2017; Parish 1981). Asexual reproduction occurs where populations can self-perpetuate from the individual fragmentation (partition) and are predominantly among this family. Sexual reproduction mainly occurs when individuals are exposed to stressors. Thus, the asexual reproduction pattern allows an individual to generate a new offspring every few days and is associated with water quality, food availability, and temperature (Erséus et al. 2017; Parish 1981). In this way, the increased population growth rates in the intermediate concentrations were associated with higher food availability once vinasse had a high organic matter load. On the other hand, the stress caused to *A. inaequalis* by exposure to the highest concentration (0.4%) may have triggered a reduction in the fragmentation rate and, consequently, an alteration in the reproduction of organisms. Besides, organisms exposed to this concentration presented increased biomass (3.6 times) compared with control ($p < 0.05$, Fig. 3). To other treatments, no effects occurred for this parameter ($p > 0.05$).

To the best of our knowledge, no effects related to vinasse exposure are described in the literature on the reproduction of aquatic oligochaetes. On the other way, Alves et al. (2015) describe the toxic effects of distinct doses of sugarcane vinasse (25 to 294 mL Kg^{-1} DW of soil) on the soil oligochaetes *Eisenia andrei* (Lumbricidae) and *Enchytraeus crypticus* (Enchytraeidae). Exposure to vinasse impaired the reproduction of both species on natural soils, beyond the reduction of body growth and avoidance behavior of *E. andrei*, unlike the present study, where the reduced

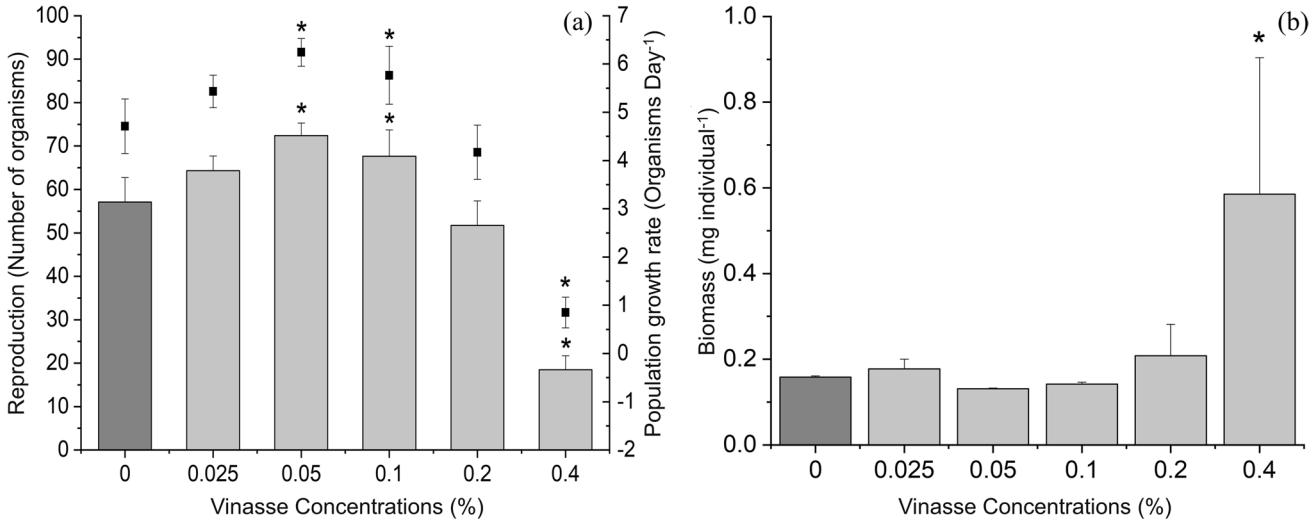

Fig. 3 Reproduction and population growth rate (mean ± SD) of *A. inaequalis* post chronic exposure (**a**) and biomass of organisms (**b**). Values statistically different from the control ($p < 0.05$) are indicated by asterisks (*)

reproductive rates of *A. inaequalis* were followed by increases in biomass. However, as previously discussed, the *A. inaequalis* species presents specific reproductive strategies. We hypothesized that exposure to 0.4% of vinasse inhibits asexual reproduction, and organisms invest their energy in the body's growth for sexual maturation. However, post-10-day exposure, probably no sexual reproduction occurred once the naidids died soon after laying the cocoons (Learner et al. 1978). Besides, some individuals presented asexual reproduction once new organisms were found at 0.4% at the end of exposure. Further investigations can determine if the increases in body growth in these conditions are due to the sexual maturation of individuals or associated with inhibitions in asexual reproduction.

In addition, other studies have reported that the exposure of nematode *Caenorhabditis elegans* to metals zinc, aluminum, and titanium reduced its reproductive capability (Wang et al. 2009). Also, Vranken and Heip (1986) observed a significant reduction in fecundity and reproduction of a marine nematode *Diplolaimella* spec 1 (non-described species) when exposed to copper and lead. These metals are part of the chemical composition of vinasse; however, because of their mixture complexity, it is impossible to determine which one may be related to the effects observed in the present study.

Metals bioaccumulation

Table 3 shows the results for the metals quantified in the test solutions and the body residue of *C. sancticaroli* larvae. The concentration of Mn increases as the concentration of vinasse rises in the test solutions and decreases in the tissue residue of larvae exposed to 0.325% compared to control larvae ($p < 0.05$). The metal Zn was quantified only in the highest concentrations of vinasse (0.163 to 0.65%). Regarding the tissue residue, decreases occurred in the organisms exposed from 0.081 to 0.65% ($p < 0.05$). The concentration of Zn in organisms decreased according to the vinasse concentrations rising in test solutions.

The Cd concentration was above the quantification limit in control and all vinasse dilutions. Significative accumulation occurred on larvae exposed to 0.325 and 0.65% ($p < 0.05$, Table 3). Pb increased according to the vinasse concentrations rises and was accumulated in larvae tissue on treatments from 0.163 to 0.65% ($p < 0.05$). Besides, Pb was quantified in organisms from 0.081%; however, due to the high variability between replicates, no statistical differences were detected. In the same way, no differences in tissue residue of exposed larvae occurred for Cu, Ni, and Cr compared with control organisms ($p > 0.05$, Table 3).

Table 4 shows the result for the metals quantified on *A. inaequalis* tissue residue and vinasse concentrations. Zn was not detectable on test solutions until 0.2% and, as observed for *C. sancticaroli*, exposure to 0.1% reduced Zn tissue residue in *A. inaequalis*. Bioaccumulation of Cd and Cr in tissue residue occurred in organisms exposed to 0.1% vinasse ($p < 0.05$). No alterations occurred for Mn, Cu, Ni, and Pb ($p > 0.05$).

Manganese is an essential trace element with biochemical and cellular functions (Santamaria and Sulsky 2010). Ben-Shahar (2018) observed that Mn directly affects multiple molecular and physiological processes mainly associated with insect development and behavior. The present study has shown decreases in Mn levels at 0.325% for *C. sancticaroli*, which may imply effects on the organisms. The reduction in levels of Zn in *C. sancticaroli* and *A. inaequalis* was an unexpected response. It was expected that increases

Table 3 Concentration of metals in test solutions and tissue residue of *C. sancticaroli* larvae (mean ± SD) post-8-day exposure. Asterisks (*) and bold values denote differences in body residue from control ($p < 0.05$)

	Vinasse concentrations (%)	Mn	Zn	Cu	Ni	Cd	Pb	Cr
Test solution (μg L^{-1})	**0**	<LOQ	<LOQ	2.0 ± 0.4	1.9 ± 0.8	<LOQ	<LOQ	<LOQ
	0.041	2.8 ± 0.0	<LOQ	4.6 ± 0.9	<LOQ	<LOQ	2.9 ± 3.2	<LOQ
	0.081	4.4 ± 0.5	<LOQ	4.0 ± 0.8	<LOQ	<LOQ	1.1 ± 1.9	<LOQ
	0.163	24.6 ± 0.8	91.0 ± 3.2	47.8 ± 2.1	<LOQ	<LOQ	6.3 ± 3.2	2.3 ± 0.4
	0.325	19.2 ± 1.3	209.2 ± 4.3	36.4 ± 0.8	<LOQ	<LOQ	7.5 ± 3.2	0.7 ± 0.5
	0.65	42.8 ± 0.7	115.2 ± 2.8	56.4 ± 1.3	<LOQ	<LOQ	8.7 ± 2.8	2.1 ± 0.0
Tissue residue (μg g DW^{-1})	**0**	3.7 ± 0.6	32.5 ± 4.8	4.6 ± 1.1	0.6 ± 0.3	<LOQ	<LOQ	<LOQ
	0.041	3.3 ± 1.0	25.2 ± 1.9	6.0 ± 0.1	1.1 ± 0.1	0.1 ± 0.1	<LOQ	<LOQ
	0.081	4.0 ± 1.1	**18.6 ± 4.4***	5.8 ± 1.8	0.8 ± 0.5	<LOQ	1.1 ± 1.9	<LOQ
	0.163	2.9 ± 0.7	**15.8 ± 3.2***	4.4 ± 1.4	0.5 ± 0.2	0.1 ± 0.1	**2.5 ± 0.2***	<LOQ
	0.325	**2.4 ± 0.5***	**15.2 ± 5.8***	3.2 ± 0.8	0.7 ± 0.4	**0.1 ± 0.0***	**2.0 ± 0.4***	<LOQ
	0.65	2.5 ± 0.4	**12.8 ± 3.6***	6.6 ± 1.9	0.9 ± 0.4	**0.2 ± 0.0***	**2.5 ± 0.5***	<LOQ

LOQ limit of quantification of 0.001 μg L^{-1} to Mn, 0.004 μg L^{-1} to Zn, 0.0004 μg L^{-1} to Ni, 0.012 μg L^{-1} to Cd, 0.007 μg L^{-1} to Pb, and 0.002 μg L^{-1} to Cr

Table 4 Concentration of metals in test solutions and tissue residue of *A. inaequalis* (mean ± SD) post-10-day exposure. Asterisks (*) and bold values denote differences in body residue from control ($p < 0.05$)

	Concentration (%)	Mn	Zn	Cu	Ni	Cd	Pb	Cr
Test solutions (μg L^{-1})	**0**	<LOQ	<LOQ	2.0 ± 0.4	1.9 ± 0.8	<LOQ	<LOQ	<LOQ
	0.025	<LOQ	<LOQ	1.8 ± 1.3	1.1 ± 0.5	<LOQ	<LOQ	0.1 ± 0.0
	0.05	2.0 ± 0.4	<LOQ	3.0 ± 0.8	1.9 ± 0.8	<LOQ	<LOQ	0.1 ± 0.0
	0.1	7.2 ± 0.5	<LOQ	23.2 ± 2.0	13.7 ± 1.4	<LOQ	<LOQ	1.1 ± 0.0
	0.2	20.2 ± 0.5	504.4 ± 4.9	42.2 ± 1.0	4.9 ± 0.4	<LOQ	<LOQ	1.3 ± 0.4
	0.4	25.0 ± 1.5	135.0 ± 1.1	40.4 ± 0.8	3.7 ± 0.0	<LOQ	<LOQ	1.9 ± 0.4
Tissue residue (μg g DW^{-1})	**0**	0.8 ± 0.4	34.7 ± 1.5	4.5 ± 0.8	0.8 ± 0.2	<LOQ	<LOQ	<LOQ
	0.025	1.1 ± 1.1	29.9 ± 3.5	3.9 ± 0.1	0.6 ± 0.2	<LOQ	<LOQ	<LOQ
	0.05	1.2 ± 1.6	39.7 ± 24.0	6.5 ± 4.2	2.1 ± 2.3	0.2 ± 0.2	<LOQ	<LOQ
	0.1	0.8 ± 0.6	**25.9 ± 2.5***	3.3 ± 0.2	1.0 ± 0.7	<LOQ	<LOQ	<LOQ
	0.2	0.7 ± 0.5	30.1 ± 3.8	4.0 ± 0.7	0.7 ± 0.2	**0.1 ± 0.0***	<LOQ	**0.05 ± 0.1***
	0.4	0.5 ± 0.1	23.0 ± 7.7	3.1 ± 1.0	0.6 ± 0.0	<LOQ	<LOQ	<LOQ

LOQ limit of quantification of 0.001 μg L^{-1} to Mn, 0.004 μg L^{-1} to Zn, 0.012 μg L^{-1} to Cd, 0.007 μg L^{-1} to Pb, and 0.002 μg L^{-1} to Cr

in this metal concentration should provoke increases in uptake rates and accumulation in tissues until a critical accumulated body concentration as observed by Lobo et al. (2021) for the aquatic oligochaetes *Branchiura sowerbyi* and *Tubifex tubifex*. However, this relationship depends on metal availability (Rainbow and Luoma 2011). Thus, despite the increases in Zn concentration in the solutions, the metal may not be bioavailable to organisms. In this way, further studies may elucidate the mechanisms associated with these responses. Zn ions act in catalytic and structural roles in enzymes, providing a molecular basis for numerous biological functions (Maret 2005). In a study with *Drosophila melanogaster* flies, the depletion of zinc concentrations affected female fertility (Missirlis 2021), indicating possible negative implications for *C. sancticaroli* reproduction.

According to Craig et al. (1999), the metal Cd is chemically close to Zn, and its ions act on biochemical reactions and can change the activity of many hormones. However, increases in Cd may imply toxic responses in organisms. On the other hand, low doses of Pb already provoke toxicity in invertebrates (Shuhaimi-Othman et al. 2012). The exposure of the dipteran *Calliphora vicinato* to Cd and Pb induced malformation on flies and a delay of 18 and 24 h, respectively, on the average emerging time (Shulman et al. 2017). In the same way, the present study evidenced delays in the metamorphosis of *C. sancticaroli*, marked by decreases in the number of pupae and adults. The organism accumulated both metals in the concentration (0.65%) where these effects occurred.

Levels of Zn, Cu, Pb, and Cd in collector-gatherer macroinvertebrates, such as chironomids and oligochaetes, are

usually found in direct proportion to their concentrations in sediment or water (Goodyear and McNeill 1999). Thus, these results demonstrate that the metal residues in organisms are directly influenced by environmental contamination. Chiba et al. (2011) verified that the areas with the highest concentrations of Cu, Ni, and Zn had the lowest richness and diversity of benthic macroinvertebrates, such as Chironomidae, Tubificidae, Elmidae, and Ceratopogonidae. In the same way, Bian et al. (2016) observed negative correlations between heavy metal levels on benthic communities' diversity in monitored rivers. The authors concluded that the main pollutants in the sediments were Cd, Cu, and Pb.

The maintenance of benthic macroinvertebrates is fundamental for aquatic ecosystems once they make up a large proportion of the biodiversity and are an essential part of the energy and nutrients transferred for higher trophic levels (Chagnon et al. 2015). The bioaccumulation of metals by this group provokes ecological risks to aquatic and terrestrial environments. As evidence of this process, Rubio-Franchini and Rico-Martínez (2011) and Croteau et al. (2005) reported that Pb and Cd can be biomagnified through freshwater food webs until the top predators. Because of that, the metal contamination was not restricted to low trophic levels. The authors conclude that the lack of monitoring of this kind of pollution may increase the vulnerability of all aquatic food chains. Therefore, the results described in the present study indicate risks of biomagnification along with food webs with cascading effects on the aquatic and terrestrial ecosystems.

Implications and general remarks

Several technologies are available to manage the sugarcane vinasse, which include the concentration to reduce the volume, animal food production, incineration, biological conversion to biogas production, and application in agriculture by fertirrigation (Fuess et al. 2017). The direct or indirect release of vinasse into water bodies was prohibited in Brazil at the end of the 1970s (Brazil 1978). After this prohibition, fertirrigation was the only approach used in the country (Fuess et al. 2017). Brazil does not have specific legislation establishing safe distances from water bodies for the vinasse application, and it is up to the States to define their requirements. The State of São Paulo, the largest producer of sugarcane in Brazil (Ogura et al. 2022), for example, has a specific law that establishes minimum distances from water bodies for fertirrigation (CETESB 2015). However, most other producing states have not yet defined these requirements (Filho and Araujo 2016). In this way, fertirrigation practices may imply risks of indirect contamination by vinasse. Besides, illegal disposal, accidents in vinasse storage and transport, and improper disposal may provoke aquatic pollution by this compound (Martinelli and Filoso 2008). In line with this, Gunkel et al. (2007) reported evidence of contamination by sugarcane vinasse in the Ipojuca River in the northeast region of Brazil. The authors observed decreases in dissolved oxygen and increases in BOD and COD downstream of an ethanol mill and a sugarcane crop area that receives vinasse by fertirrigation. In this way, it is possible to assume that vinasse may reach aquatic environments, and the results reported throughout this manuscript point to potential risks to freshwater environments.

Conclusion

The sugarcane vinasse exhibited high toxicity to all test species at concentrations below 1.5%. Additionally, subchronic exposure adversely affected the development and growth of *C. sancticaroli* and the reproduction of *A. inaequalis*. Sugarcane vinasse boasts a complex composition, comprising various metals, nutrients, and organic compounds. Therefore, identifying these elements is crucial for comprehending this toxicity. Essential metals decreased in the exposed individuals, while toxic metals accumulated. This highlights the risk of biomagnification within food webs, with cascading effects on ecosystems. Despite the ethanol industry's significance and the substantial volume of vinasse produced, there is limited information in the literature regarding its impact on aquatic species. Hence, these results expand the discussion concerning the risks associated with fertigation practices.

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Author contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by P.D.F., G.V.d.M.G., and T.J.S.P.. P.D.F. wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Afridi HI, Kazi TG, Jamali MK, Kazi GH, Arain MB, Jalbani N, Shar GQ, Sarfaraz RA (2006) Evaluation of toxic metals in biological samples (scalp hair, blood and urine) of steel mill workers by electrothermal atomic absorption spectrometry. Toxicol Ind Health 22:381–393. https://doi.org/10.1177/0748233706073420

Alves PRL, Natal-da-Luz T, Sousa JP, Cardoso EJBN (2015) Ecotoxicological characterization of sugarcane vinasses when applied to tropical soils. Sci Total Environ 526:222–232. https://doi.org/10.1016/j.scitotenv.2015.03.150

Andersen JM (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

APHA (2018) Standard methods for the examination of water and wastewater. Pharmabooks, Washington, D.C.

Armendáriz LC (1999) Dinámica poblacional de *Allonais lairdi* (Oligochaeta, Naididae) en Los Talas, Provincia de Buenos Aires. Ecol Austral 9:20–27. https://ojs.ecologiaaustral.com.ar/index.php/Ecologia_Austral/article/view/1606. Acessed 05-14-2020

Belowitz R, Leonard EM, O'Donnell MJ (2014) Effects of exposure to high concentrations of waterborne Tl on K and Tl concentrations in *Chironomus riparius* larvae. Comp Biochem Physiol Part - C: Toxicol Pharmacol 166:59–64. https://doi.org/10.1016/j.cbpc.2014.07.003

Ben-Shahar Y (2018) The impact of environmental Mn exposure on insect biology. Front Genet 9:1–7. https://doi.org/10.3389/fgene.2018.00070

Bian B, Zhou Y, Fang BB (2016) Distribution of heavy metals and benthic macroinvertebrates: impacts from typical inflow river sediments in the Taihu Basin, China. Ecol Indic 69:348–359. https://doi.org/10.1016/j.ecolind.2016.04.048

Botelho RG, Tornisielo VL, de Olinda RA, Maranho LA, Machado-Neto L (2012) Acute toxicity of sugarcane vinasse to aquatic organisms before and after pH adjustment. Toxicol Environ Chem 94:2035–2045. https://doi.org/10.1080/02772248.2012.738516

Brasil (1978) Ministério de Estado do Interior. Portaria/GM Nº 323 - de 29 de Novembro de 1978. http://extwprlegs1.fao.org/docs/pdf/bra25137.pdf. Accessed 29 August 2022.

Castro-Català N, Kuzmanovic M, Roig N, Sierra J, Ginebreda A, Barceló D, Pérez S, Petrovic M, Picó Y, Schuhmacher M, Muñoz I (2016) Ecotoxicity of sediments in rivers: invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools. Sci Total Environ 540:297–306. https://doi.org/10.1016/j.scitotenv.2015.06.071

CETESB (2015) Norma Técnica P4.231: Vinhaça – Critérios e procedimentos para aplicação no solo agrícola, 3rd ed. Companhia Ambiental do Estado de São Paulo, São Paulo

Chagnon M, Kreutzweiser D, Mitchell EAD, Morrissey CA, Noome DA, Van der Sluijs JP (2015) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res 22:119–134. https://doi.org/10.1007/s11356-014-3277-x

Chiba W, Passerini M, Tundisi J (2011) Metal contamination in benthic macroinvertebrates in a sub-basin in the southeast of Brazil. Braz J Biol 71:391–399. https://doi.org/10.1590/S1519-69842011000300008

Christofoletti CA, Escher JP, Correia JE, Marinho JFU, Fontanetti CS (2013) Sugarcane vinasse: environmental implications of its use. Waste Manag 33:2752–2761. https://doi.org/10.1016/j.wasman.2013.09.005

Coelho MPM, Correia JE, Vasques LI, Marcato ACC, Guedes TA, Soto MA, Basso JB, Kiang C, Fontanetti CS (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

CONAB (2023) Produção de cana-de-açúcar e subprodutos (4º Levantamento – Safra 2022/23). https://www.conab.gov.br/info-agro/safras/cana/boletim-da-safra-de-cana-de-acucar. Acessed 08 October 2023

Corbi JJ, Gorni GR, Correa RC (2015) An evaluation of *Allonais inaequalis* Stephenson, 1911 (Oligochaeta: Naididae) as a toxicity test organism. Ecotoxicol Environ Cont 10:7–11. https://doi.org/10.5132/eec.2015.01.02

Correia JE, Christofoletti CA, Marcato ACC, Marinho JFU, Fontanetti CS (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

Craig A, Hare L, Tessier A (1999) Experimental evidence for cadmium uptake via calcium channels in the aquatic insect *Chironomus staegeri*. Aquat Toxicol 44:255–262. https://doi.org/10.1016/S0166-445X(98)00086-1

Croteau MN, Luoma SN, Stewart AR (2005) Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol Oceanogr 50:1511–1519. https://doi.org/10.4319/lo.2005.50.5.1511

De Haas EM, Wagner C, Koelmans AA, Kraak MHS, Admiraal W (2006) Habitat selection by chironomid larvae: fast growth requires fast food. J Anim Ecology 75:148–155. https://doi.org/10.1111/j.1365-2656.2005.01030.x

Duarah P, Haldar D, Patel AK, Dong CD, Singhania RR, Purkait MK (2022) A review on global perspectives of sustainable development in bioenergy generation. Bioresour Technol 348:126791. https://doi.org/10.1016/j.biortech.2022.126791

Eggleton J, Thomas KV (2004) A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ Int 30:973–980. https://doi.org/10.1016/j.envint.2004.03.001

Erséus C, Envall I, De Wit P, Gustavsson LM (2017) Molecular data reveal a tropical freshwater origin of Naidinae (Annelida, Clitellata, Naididae). Mol Phylogenet Evol 115:115–127. https://doi.org/10.1016/j.ympev.2017.07.016

España-Gamboa E, Mijangos-Cortes J, Barahona-Perez L, Domingez-Maldonado J, Hernández-Zarate G, Alzate-Gaviria L (2011) Vinasses: characterization and treatments. Waste Manag Res 29:1235–1250. https://doi.org/10.1177/0734242X10387313

Ferreira RLF, Aguiar MM, Messias TG, Pompeu GB, Queijeiro Lopez AM, Silva DP, Monteiro RT (2011) Evaluation of sugar-cane vinasse treated with *Pleurotus sajor-caju* utilizing aquatic organisms as toxicological indicators. Ecotoxicol Environ Saf 74:132–137. https://doi.org/10.1016/j.ecoenv.2010.08.042

Filho FEF, Araujo GJF (2016) Normativos federais e estaduais reguladores da destinação da vinhaça no Brasil: uma proposta de nova abordagem. Rev Adm Cont Econ Fund 7:146–160. https://doi.org/10.13059/racef.v7i3.390

Fonseca AL, Rocha O (2004) Laboratory cultures of the native species *Chironomus xantus* Repel, 1939 (Diptera-Chironomidae). Acta Limnol 16:153–161

Fuess LT, Rodrigues IJ, Garcia ML (2017) Fertirrigation with sugarcane vinasse: foreseeing potential impacts on soil and water resources through vinasse characterization. J Environ Sci Health A 52:1063–1072. https://doi.org/10.1080/10934529.2017.1338892

Garcia CFH, Souza RB, Souza CP, Christofoletti CA, Fontanetti CS (2017) Toxicity of two effluents from agricultural activity: comparing the genotoxicity of sugar cane and orange vinasse.

Ecotoxicol Environ Saf 142:216–221. https://doi.org/10.1016/j.ecoenv.2017.03.053
Goldemberg J, Lucon O (2007) Energy and environment in Brazil. Estud Av 21:7–20. https://doi.org/10.1590/S0103-40142007000100003
Goodyear KL, McNeill S (1999) Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: a review. Sci Total Environ 229:1–19. https://doi.org/10.1016/S0048-9697(99)00051-0
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
Hage DS, Carr JD (2012) Química analítica e análise quantitativa, 1a edn. Pearson, São Paulo
Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds) Methods of seawater analysis, 3rd edn. Wiley, New York, pp 159–228
Learner M, Lochhead G, Hughes B (1978) A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. Freshw Biol 8:357–375. https://doi.org/10.1111/j.1365-2427.1978.tb01457.x
Leppänen M (1995) The role of feeding behaviour in bioaccumulation of organic chemicals in benthic organisms. Ann Zool Fenn 32:247–255. https://www.jstor.org/stable/23735696. Acessed 05-14-2020
Liber K, Call DJ, Dawson TD, Whiteman FW, Dillon TM (1996) Effects of *Chironomus tentans* larval growth retardation on adult emergence and ovipositing success: implications for interpreting freshwater sediment bioassays. Hydrobiologia 323:155–167. https://doi.org/10.1007/BF00007844
Lobo H, Méndez-Fernández L, Martínez-Madrid M, Rodriguez P, Daam MA, Espíndola ELG (2021) Bioaccumulation and chronic toxicity of arsenic and zinc in the aquatic oligochaetes *Branchiura sowerbyi* and *Tubifex tubifex* (Annelida, Clitellata). Aquat Toxicol 239:105955. https://doi.org/10.1016/j.aquatox.2021.105955
Mackereth F, Heron J, Talling JS (1978) Water analysis: some revised methods for limnologists. Freshwater Biological Association, Cumbria
Maret W (2005) Zinc coordination environments in proteins determine zinc functions. J Trace Elem Med Biol 19:7–12. https://doi.org/10.1016/j.jtemb.2005.02.003
Marinho JFU, Correia JE, Marcato ACC, Pedro-Escher J, Fontanetti CS (2014) Sugar cane vinasse in water bodies: impact assessed by liver histopathology in tilapia. Ecotoxicol Environ Saf 110:239–245. https://doi.org/10.1016/j.ecoenv.2014.09.010
Martinelli LA, Filoso S (2008) Expansion of sugarcane ethanol production in Brazil: environmental and social challenges. Ecol Appl 18:885–898. https://doi.org/10.1890/07-1813.1
McBride AC, Dale VH, Baskaran LM, Downing ME, Eaton LM, Efroymson RA, Garten CT, Kline KL, Jager HI, Mulholland PJ, Parish ES, Schweizer PE, Storey JM (2011) Indicators to support environmental sustainability of bioenergy systems. Ecol Indic 11:1277–1289. https://doi.org/10.1016/j.ecolind.2011.01.010
Missirlis F (2021) Regulation and biological function of metal ions in Drosophila. Curr Opin Insect Sci 47:18–24. https://doi.org/10.1016/j.cois.2021.02.002
Moraes BS, Junqueira TL, Pavanello LG, Cavalett O, Mantelatto PE, Bonomi A, Zaiat M (2014) Anaerobic digestion of vinasse from sugarcane biorefineries in Brazil from energy, environmental, and economic perspectives: profit or expense? Appl Energy 113:825–835. https://doi.org/10.1016/j.apenergy.2013.07.018
NBR 12621 (1995) Waters - Determination of total hardness by EDTA-Na titrimetric method - Method of test. Associação Brasileira de Normas Técnicas, Rio de Janeiro
NBR 15470 (2013) Ecotoxicologia aquática — Toxicidade aguda e crônica — Método de ensaio com *Hyalella* spp (Amphipoda) em sedimentos. Associação Brasileira de Normas Técnicas, Rio de Janeiro
Ogura AP, Silva AC, Castro GB, Espíndola ELG, Silva AL (2022) An overview of the sugarcane expansion in the state of São Paulo (Brazil) over the last two decades and its environmental impacts. Sustain Prod Consum 32:66–75. https://doi.org/10.1016/j.spc.2022.04.010
Organization for Economic Co-operation and Development (2011) Test no. 235: *Chironomus* sp., acute immobilization test. OECD publishing. https://doi.org/10.1787/9789264122383-en
Parish J (1981) Reproductive ecology of Naididae (Oligochaeta). Hydrobiologia 83:115–123. https://doi.org/10.1007/BF02187156
Parnaudeau V, Condom N, Oliver R, Cazevieille P, Recous S (2008) Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. Bioresour Technol 99:1553–1562. https://doi.org/10.1016/j.biortech.2007.04.012
Péry ARR, Mons R, Garric J (2005) Modelling of the life cycle of *Chironomus* species using an energy-based model. Chemosphere 59:247–253. https://doi.org/10.1016/j.chemosphere.2004.11.083
Pinto TJS, Freitas JS, Moreira RA, Silva LCM, Yoshii MPC, Lopes LFP, Goulart BV, Vanderlei MR, Athayde DB, Fraga PD, Ogura AP, Schiesari L, Montagner CC, Daam MA, Espindola ELG (2021) Functional responses of *Hyalella meinerti* after exposure to environmentally realistic concentrations of 2,4-D, fipronil, and vinasse (individually and in mixture). Aquat Toxicol 231:105712. https://doi.org/10.1016/j.aquatox.2020.105712
Pinto TJS, Moreira RA, Silva LCM, Yoshii MPC, Goulart BV, Fraga PD, Montagner CC, Daam MA, Espindola ELG (2021) Impact of 2,4-D and fipronil on the tropical midge *Chironomus sancticaroli* (Diptera: Chironomidae). Aquat Toxicol 231:105712. https://doi.org/10.1016/j.aquatox.2020.105712
Pinto TJS, Moreira RA, Silva LCM, Yoshii MPC, Goulart BV, Fraga PD, Rolim VLS, Montagner CC, Daam MA, Espindola ELG (2021) Toxicity of fipronil and 2,4-D formulations (alone and in a mixture) to the tropical amphipod *Hyalella meinerti*. Environ Sci Pollut 28:38308–38321. https://doi.org/10.1007/s11356-021-13296-9
Rainbow PS, Luoma SN (2011) Metal toxicity, uptake and bioaccumulation in aquatic invertebrates—Modelling zinc in crustaceans. Aquat Toxicol 105:455–465. https://doi.org/10.1016/j.aquatox.2011.08.001
Richardi VS, Vicentini M, Morais GS, Rebechi D, da Silva TA, Fávaro LF, Navarro-Silva MA (2018) Effects of phenanthrene on different levels of biological organization in larvae of the sediment-dwelling invertebrate *Chironomus sancticaroli* (Diptera: Chironomidae). Environ Pollut 242:277–287. https://doi.org/10.1016/j.envpol.2018.06.091
Rubio-Franchini I, Rico-Martínez R (2011) Evidence of lead biomagnification in invertebrate predators from laboratory and field experiments. Environ Pollut 159:1831–1835. https://doi.org/10.1016/j.envpol.2011.03.021
Santamaria AB, Sulsky SI (2010) Risk assessment of an essential element: manganese. J Toxicol Environ Health Part A 73:128–155. https://doi.org/10.1080/15287390903337118
Santana VS, Fernandes Machado NRC (2008) Photocatalytic degradation of the vinasse under solar radiation. Catal Today 13:606–610. https://doi.org/10.1016/j.cattod.2007.12.131
Shuhaimi-Othman M, Nadzifah Y, Umirah NS, Ahmad AK (2012) Toxicity of Metals to an Aquatic Worm, *Nais elinguis* (Oligochaeta, Naididae). Res J Environ Toxicol 6:122–132. https://doi.org/10.3923/rjet.2012.122.132

Shulman MV, Pakhomov OY, Brygadyrenko VV (2017) Effect of lead and cadmium ions upon the pupariation and morphological changes in *Calliphora vicina* (Diptera, Calliphoridae). Oecologica 44:28–37. https://doi.org/10.1515/foecol-2017-0004

Sibley PK, Benoit DA, Ankley GT (1997) The significance of growth in *Chironomus tentans* sediment toxicity tests: relationship to reproduction and demographic endpoints. Environ Toxicol Chem 16:336–345. https://doi.org/10.1002/etc.5620160232

Silva MAS, Griebeler NP, Borges LC (2007) Uso de vinhaça e impactos nas propriedades do solo e lençol freático. Rev Bras Eng Agric Ambient 11:108–114. https://doi.org/10.1590/S1415-43662007000100014

USEPA (1992) Method 3005A: Acid digestion of waters for total recoverable or dissolved metals for analysis by FLAA or ICP Spectroscopy. Environmental Protection Agency, Washington, D.C, Office of Research and Development, U.S

Vranken G, Heip C (1986) Toxicity of copper, mercury and lead to a marine nematode. Mar Pollut Bull 17:453–457. https://doi.org/10.1016/0025-326X(86)90834-9

Wang H, Wick RL, Xing B (2009) Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode *Caenorhabditis elegans*. Environ Pollut 157:1171–1177. https://doi.org/10.1016/j.envpol.2008.11.004

Wentsel R, McIntosh A, McCafferty WP, Atchison G, Anderson V (1977) Avoidance response of Midge Larvae (*Chironomus Tentans*) to sediments containing heavy metals. Hydrobiologia 55:171–175. https://doi.org/10.1007/BF00021058

Wilkie AC, Riedesel KJ, Owens JM (2000) Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 19:63–102. https://doi.org/10.1016/S0961-9534(00)00017-9