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Flumioxazin-Based Herbicide-Induced Stress in Raphidocelis subcapitata and Impact on Zooplankton Feeding Behavior

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Abstract

The widespread use of herbicides has unknown and unexplored consequences for the aquatic ecosystem; herbicides based on the active ingredient flumioxazin have been used effectively for agriculture to control broad-leaved weeds. In this sense, this study investigated the direct effects of the flumioxazin-based herbicide on the microalgae *Raphidocelis subcapitata* and the impact of stressed algae for feed zooplankton organisms *Ceriodaphnia silvestrii* and *Daphnia magna*. The results revealed that the microalgae showed high sensitivity to the herbicide, showing phytotoxicity from 2.726 μg L^{-1} with the effective concentration of 50% (EC_{50}) of 4.57 μg L^{-1}. Carbohydrate accumulation was also observed in response to the presence of flumioxazin-based herbicide. Furthermore, the filtration and ingestion rates of cladocerans in response to microalgae contaminated with the herbicide were found to be altered and specific to each species. The differences in body size and individual dietary needs among the species resulted in variations in the amount of phytoplankton ingested. However, at the EC_{50} found for microalgae, the ingestion of cladocerans was not altered, suggesting adequate adaptation to the available food. However, a reduction in the filtration rate was observed for *D. magna*. These results emphasize the importance of considering the different effects of herbicides on different trophic levels of aquatic ecosystems, showing that the feeding of microcrustaceans can be a crucial factor in determining the impact of these products chemicals throughout the ecosystem as the stress experienced by algae at the lower trophic level can propagate at the higher trophic levels.

1 INTRODUCTION

Chemical pollution resulting from pesticide use is one of the major challenges faced by phytoplankton and zooplankton in aquatic ecosystems, making it essential to understand their interactions and the effects these substances have on these organisms. Herbicides, one of the main types of pesticides, are widely used for weed control in agricultural fields (Mohd Nizam et al. 2023). Following their application to target plants, residues can reach water bodies through runoff or leaching processes, causing potential contamination and threatening the integrity of ecosystems (Cosgrove et al. 2019; Mojiri et al. 2020). These compounds, when interacting with aquatic organisms, can directly impact primary producers, such as microalgae, causing changes in their growth and biochemical composition. Indirectly, they also influence primary consumers, like microcrustaceans, by altering the quality and availability of their main food sources (Fayaz et al 2024; Jia et al, 2024). Thus, understanding these effects, including at physiological and behavioral levels within lower trophic levels, is essential to assess the potential for contaminant transfer along the food chain and the associated ecological risks.

Flumioxazin-based herbicides are among the most widely used in the agricultural sector. They are crucial for controlling a wide variety of weeds (Ando et al. 2017), including *Bidens pilosa*, *Pomoea grandifolia, Commelina benghalensis, Galinsoga parviflora, Digitaria horizontalis* and *Euphorbia heterophylla* (Saballos et al. 2024). Flumioxazin is classified in the group of N-phenyl-phthalamides and its mechanism of action in target plants involves the inhibition of protoporphyrinogen oxidase (protox), an enzyme responsible for the synthesis of chlorophyll that catalyzes the oxidation of protoporphyrinogen

IX to protoporphyrin IX (Saladin et al. 2003) According to the package insert for the commercial product Flumioxazin 500 SC®, the substance can be applied to different types of crops including Potato, Coffee, Sugarcane, Onion, forest species (Eucalyptus and Pine), Beans and Soybeans. Since this herbicide is applied in the field to a wide variety of crops, it is essential to be concerned about the impacts it may cause to non-target individuals present in the ecosystem.

In aquatic ecosystems, microalgae play a fundamental role in maintaining water quality and sustaining healthy aquatic ecosystems by being the main producers in these environments and the base of the entire food chain. The green microalgae *Raphidocelis subcapitata*, a species recognized worldwide as a model for aquatic ecotoxicology, plays an important role in primary production and nutrient cycling in aquatic systems (Alho et al. 2019). Microalgae are also a vital source of food for aquatic organisms, such as zooplankton. They also play a crucial role in maintaining oxygen balance in the water and significantly impact the overall quality of the aquatic habitat (Fallahi et al. 2021). However, studies have shown that microalgae are highly sensitive to pesticides (Dorigo et al. 2004; Magnusson et al. 2010; Turemis et al. 2018) and exposure to these compounds chemicals can result in various effects such as growth inhibition, changes in photosynthesis and biomolecules and cellular disturbances. These impacts on microalgae can have repercussions throughout the aquatic trophic chain, as changes in the growth and biochemical composition of these species can compromise the quality of food available to primary consumers, such as zooplankton. Changes in the health and availability of microalgae can decrease the efficiency of energy and nutrient transfer to higher trophic levels, thus affecting the ecological balance of the aquatic environment. Furthermore, the toxic effects that pesticides have on microalgae suggest that prolonged exposure to these chemical compounds can cause substantial structural and functional modifications in the ecosystem.

In this context, cladocerans such as *Daphnia magna* and *Ceriodaphnia silvestrii*, occupying the second trophic level as primary consumers, play a crucial role in the regulation of trophic levels and energy transfer within aquatic ecosystems. Relying on a diet that includes microalgae and organic matter present in the water, these organisms become susceptible to both direct and indirect effects of herbicides like flumioxazin introduced into the environment. Exposure to contaminated microalgae may impact their filtration and ingestion rates, consequently affecting their viability and reproductive capacity, which could influence the overall stability and dynamics of aquatic ecosystems.

Ecotoxicity and food inhibition bioassays on model organisms such as *R. subcapitat*a and cladocerans are essential tools for detecting lethal and sublethal effects on the aquatic environment and are considered reliable and fast. Food inhibition tests, in particular, are considered to be indicators of effects on the most sensitive levels of the organism, covering physiological aspects and biochemicals, as they provide early results (Agatz et al. 2013). These tests are particularly relevant due to their ability to assess ingestion and filtration, behavioral and physiological processes exhibited by zooplankton species when subjected to a diversity of diets (Lürling and Van der Grinten 2003; Lurling and Beekman 2006). Filtration or purification rates indicate the amount of water filtered for the collection of food particles, while

ingestion rates refer to the simple quantity of these particles collected from a food suspension at a designated time interval (Davis and Gobler 2011; Kâ et al. 2012).

Given the above, this study aimed to evaluate the ecotoxicity of commercial herbicide containing flumioxazin on the green microalga *R. subcapitata*, for the determination of the effective concentration (EC_{50}) and, based on this information, using the contaminated microalgae via the dietary route, to investigate the filtration and ingestion rates in standardized cladocerans for toxicity tests, *D. magna* and *C. silvestrii.* This approach is relevant because the feeding mechanism in question is common in natural environments, where the introduction of contaminants often occurs and can play a critical functional role in trophic chains. With this, we can provide valuable insights into the ecotoxicity of this herbicide and its impacts on organisms contributing to a comprehensive assessment of their potential to affect aquatic ecosystems.

2 MATERIALS AND METHODS

2.1 Study models

The green microalga *Raphidocelis subcapitata* was obtained from the algae culture kept at the Limnology Laboratory of the Federal University of Alfenas/MG. Algae maintenance was performed weekly and 1-liter Erlenmeyer flasks containing 500 mL of L.C. Oligo culture medium (Afnor 1980) were used. Before being used, the L.C. Oligo growing medium was subjected to sterilization in an autoclave at 121°C for 20 minutes. Cultivation conditions were maintained at a temperature of 23 ± 2°C, lighting of 6800 lux, with a photoperiod of 12 hours of light and 12 hours of darkness. The vials were shaken daily to prevent decantation of the cells. Healthy algae in the logarithmic exponential growth phase were used to conduct the contaminant exposure test.

The zooplankton species *Ceriodaphnia silvestrii* was collected in the natural environment by using a 68 µm mesh zooplankton net, in the dammed portion of the São Tomé River Furnas Reservoir (21^{o}S 2751 46^{o}W 0002), located in the municipality of Alfenas - MG. In the laboratory, the organisms were sorted, identified, and transferred to glass beakers with a capacity of 1000 mL, containing water collected from their reservoir. After a period of adaptation to laboratory conditions, the organisms were submitted to the acclimatization process to the cultivation maintenance water or water reconstituted. The species acclimatized in the laboratory was cultivated under the conditions of cultivation established by ABNT NBR 13373:2022. In turn, the species *Daphnia magna* was acquired through online trading and acclimatized and cultivated in the laboratory following the guidelines of ABNT NBR 12713:2022.

2.2 Test solutions

The test solutions were prepared by diluting the commercial product Sumysin 500 SC® containing 500 g/L of the active ingredient flumioxazine in distilled water and then in the L.C. Oligo culture media. The test concentrations for the inhibition test with the green microalga *R. subcapitata* were determined

based on the EC_{50} (0.852 µg L^{-1}) previously calculated and presented in the PPDB (Pesticide Properties DataBase) databases for *R. subcapitata* from the flumioxazin active ingredient per se. The following doses of the active ingredient of flumioxazin were tested and 3.2 was the multiplication factor used between them: CN: negative control; T1: 0.0832 µg L^{-1}; T2: 0.266 µg L^{-1}; T3: 0.852 µg L^{-1}; T4: 2.726 µg L^{-1}; T5: 8.724 µg L^{-1}; T6: 27.918 µg L^{-1}; and T7: 89.338 µg L^{-1}.

2.3 Green microalgae growth inhibition test

Green microalgae growth inhibition test the test was carried out following the NF EN ISO 8692 (2012) standard, containing 6 replicates per concentration. Each replicate was represented by a 100 mL glass test tube containing 30 mL of the test solutions and an initial cell density of $1x10^4$ cells mL^{-1}. The tests were conducted in an air-conditioned room at 23 ± 2°C, illuminated with an intensity of 6800 Lux, and periodic manual shaking at each sampling. Sampling for the determination of cell density was performed every 24 hours, and the cell density of the microalgae *R. subcapitata* was determined using Fuchs Rosenthal counting chambers. For this, 0.5 mL of each sample was collected and fixed with 0.5 mL of 4% formaldehyde for subsequent processing counting. Specific growth rates were calculated based on abundance considering their exponential growth phase from 48 to 96 h, where the cell growth rate is the specific growth was calculated according to the following equation:

$$r = [Ln (N2) - Ln (N1)] / Dt$$

Where, r is the intrinsic growth rate, N1 is the population size at the beginning of a time interval, N2 is the population size at the end of the time interval and Dt is the temporal variation.

From the results obtained, the EC50, the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were calculated and then proceeded with the filtration rate experiment with zooplanktonic organisms.

2.4 Biochemical analysis of the microalgae: Total carbohydrates

The total carbohydrate content was analyzed following the methodology described in (Albalasmeh et al. 2013). The method is a colorimetric reaction with sulfuric acid that quantifies the total intracellular carbohydrates present in the microalgae. A new growth cycle of the microalgae was initiated with concentrations covering a spectrum that included the previous results of the inhibition tests with *R. subcapitata*, being the NOEC (0.852 µg L^{-1}), the Effective Concentration 50% (EC_{50}, 4.57 µg L^{-1}) and a third concentration (8.724 µg L^{-1}) that showed a statistically significant difference with the control treatment in terms of microalgae cell growth and, from this exposure of the microalgae to the concentrations of total carbohydrates were analyzed. Samples from each treatment were collected (30 mL) at 96 hours of exposure and added to test tubes. The tubes were centrifuged (BL-206/1 FANEM®) for 10 minutes at 1600 rpm. After centrifugation, the supernatant was discarded and the pellet was removed adding 1 mL of distilled water. Then, the samples were frozen until the final analysis.

For the analysis, the samples were thawed and shaken to homogenize the cells. Then, 3 mL of concentrated sulfuric acid were added to each sample and stirred for 3 minutes and then placed in an ice bath to be cooled. After cooling, each sample was transferred to a 2 mL quartz cuvette, and the absorbance was read on a spectrophotometer at 315 nm (Biospectro® SP-220 spectrophotometer). A calibration curve was constructed following the same definitions of microalgae and using a glucose standard.

2.5 Experimental design of the filtration rate and ingestion tests with *C. silvestrii* and *D. magna*

Evaluations of filtration and ingestion of the green microalga *R. subcapitata* contaminated with a herbicide containing flumioxazin were carried out with the cladocerans *C. silvestrii* and *D. magna*. These tests exposed four non-oviparous adults to the contaminated microalgae per repetition (with 4 repetitions). The total duration of the experiments was 3 hours, including an initial period of 30 minutes for the fasting of the organisms, during which no food was provided. The organisms were kept in a temperature-controlled room at 23+/-2°C, illuminated by artificial light with a light intensity of 1000 Lux. During the experiments, the organisms were placed in acrylic containers with 30 mL of reconstituted water and an algal suspension with a concentration of $5x10^4$ cell mL^{-1} for each level of concentration of the herbicide. The specific concentrations of the herbicide tested were previously determined using the green microalgae growth inhibition test, resulting in EC_{50} values, a concentration considered safe for the environment, and a third concentration considered toxic to the microalgae. After 96 hours of microalgae growth in each herbicide's concentration, the samples were centrifuged at 1600 revolutions per minute (rpm) and resuspended in deionized water. This procedure was carried out in order to provide exclusively contaminated food for zooplankton organisms.

For the feeding inhibition tests, four replicates of the control contain only reconstituted water and algae cells. At the beginning and the the end of the experiment, the control groups were fixed with a solution of formaldehyde a 4% to allow subsequent counting of the number of algae cells using a Fuchs Rosenthal chamber, under a Zeiss microscope at 40x magnification, to monitor algal growth throughout the experimental period. For all treatments, samples were collected at the beginning and end of the experiment and were also fixed with a 4% formaldehyde solution, so that cell density could be counted before and after. After filtration and ingestion of the algae by the organisms. Also, at the start of the tests, measurements were taken of variables such as pH, electrical conductivity, temperature, hardness water, and dissolved oxygen concentration, to verify the characteristics of the reconstituted water used for dilutions.

Filtration rates were calculated using the algal cell count method and the following equation by Peters (1984) was used:

F = V (ln C0-ln Ct) / (tN), where:

F = filtration rate (mL individual^{-1} hour^{-1}); V = volume of the sample in the container test; N = number of individuals in the flask; t = duration of the experiment in hours; C0 = initial concentration of the algae and Ct = final concentration of the algae in the containers experimental.

Ingestion rates were expressed as the average number of algal cells ingested by cladocerans (cels ind^{-1} hour^{-1}) throughout the experiment. For the calculations the following equation, proposed by Paffenhöfer (1971), was used:

I = V (C0-Ct) / (tN), where:

I = The ingestion rate (cels ind^{-1} hour^{-1}); V = volume of the sample in the test container; C0 = initial concentration of the algal suspension and Ct = final concentration of the algal suspension in the experimental containers; t = duration of the experiment (in hours); N (number of cladocerans).

2.6 Statistical analysis

Each laboratory test was analyzed and significant differences between treatments and the respective controls were tested using one-way ANOVA followed by Dunnett's post-hoc test. This analysis allowed the calculation of growth inhibition concentration (EC_{50}) values, the determination of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Prior to the analyses, the normality of the data was verified using the Shapiro-Wilk tests. Statistical analyses were performed using Minitab 17 software and graphs were constructed using GraphPad Prism (version 8).

3 RESULTS

3.1 Inhibition of green microalgae - *R. subcapitata*

Microalgae growth patterns were distinct within the tested doses, with notable differences observed in treatment 4 (2.726 µg L^{-1}). Significant reductions were noticed in both the growth curve and the rate of growth. The growth curve graphs (Fig. 1) showed that at the highest flumioxazin concentrations, the microalgae inhibited their growth. The microalgae demonstrated a healthy growth pattern up to treatment 3 (0.852 µg L^{-1}), remaining the same as the control treatment.

The growth rate analysis (Fig. 2) corroborated these findings, revealing significant drops in the microalgae's ability to proliferate from treatment 4. This transition point marked a substantial change in growth conditions, even concentrations above the reference EC_{50}, indicating a dose response dependent on flumioxazin. The results of the experiment allowed the determination of key parameters describing the response of microalgae to flumioxazin, such as EC_{50}, the NOEC and LOEC. The 50% Effective Concentration was identified as 4.57 µg L^{-1}. This value represents the concentration at which we observed a 50% reduction in the growth of microalgae. The LOEC was determined to be 2.726 µg L^{-1}. Concentrations equal to or higher than this value showed significant adverse effects on the growth of the microalgae during the experimental period. This result is crucial for establishing exposure that

minimizes harmful impacts on aquatic ecosystems. NOEC, provides important information for establishing safe exposure levels for organisms to the substance and was identified as 0.852 μg L^{-1}. Concentrations below this threshold do not showed significant observable effects on microalgae growth.

3.2 Total carbohydrates of the microalgae

The evaluation of the total carbohydrate content (Fig. 3) in the microalgae in response to the concentrations of flumioxazin revealed significant patterns compared to the control, showing a dose-dependent response with an increase in total carbohydrate content. For all concentrations, the herbicide showed a clear influence on the carbohydrate contente with a 1.8-fold increase at 0.852 μg L^{-1} 2.3 times at 4.57 μg L^{-1} and 2.5 times in 8.724 μg L^{-1}. The dose-dependent response observed highlights the complexity of the interactions between microalgae and flumioxazin.

3.3 Filtration rate and ingestion in *C. silvestrii* and *D. magna*

The concentrations established in the microalgae toxicity tests (0.852 μg L^{-1}, 4.57 μg L^{-1}, and 8.724 μg L^{-1}) were applied to determine the rate of filtration and ingestion in *C. silvestrii* and *D. magna*. The results showed different responses between the species, indicating differential sensitivity to the presence of the flumioxazin-based herbicide. For *C. silvestrii*, a significant increase in filtration and ingestion rates was observed in response to the concentration of 8.724 μg L^{-1} and remained the same as the control in the other treatments (Fig. 4). By contrast, in *D. magna*, the results were divergent. The filtration rate was significantly reduced both at a concentration of 4.57 μg L^{-1} and 8.724 μg L^{-1} (Fig. 5), indicating a marked sensitivity to these concentrations of the contaminated microalgae with flumioxazin. At the same time, the ingestion rate decreased by 8.724 μg L^{-1}.

4 DISCUSSIONS

The flumioxazin-based herbicide proved to be a substantial inhibitor of the cell growth of *R. subcapitata* in this study, when 2.726 μg L^{-1} of flumioxazin present in the microalgae cells reduced their proliferation, corroborating results described by Geoffroy et al. (2004) presented for the species, *Scenedesmus obliquus* and Ando et al. (2017) who observed inhibition in the growth of *Pseudokirchneriella subcapitata* and *Synechococcus* sp. when testing the herbicide. This consistency in the inhibitory effects highlights the robustness of the impact of flumioxazin on different microalgae species. However, it is recognized that contamination of primary producers constitutes a threat to subsequent trophic levels in aquatic ecosystems (Hill et al. 2010), which can occur due to stress, exposure, or feeding. Microcrustaceans, for example, play an essential role in the food web, and disturbances at this trophic level have the potential to negatively affect both the lower and higher trophic levels, causing an environmental imbalance (Malzahn et al. 2010). This interconnection highlights the vulnerability of aquatic ecosystems in the face of pesticide contamination.

Microalgae, representing the first trophic level, not only inhibit growth, respond to flumioxazin-induced stress by accumulating carbohydrates in their cells, using them as a source of energy. In a study with the

herbicide pendimentalin, an increase in carbohydrate molecules was also observed in the species from the green microalga Protosiphon botryoides (Shabana et al. 2001). This increase in carbohydrates may indicate an adaptive strategy of the microalgae in an attempt to preserve its growth rate under the adverse conditions caused by the herbicide. Thus, in stressful situations, the synthesis of carbohydrates can be favored as a strategy for storing carbon with a view to future use, when the environmental conditions become (Markou et al. 2012). On the other hand, it can also indicate failures in the realization of the photosynthesis (Shabana et al. 2001). Furthermore, one study indicates the potential for accumulation of flumioxazin in the cells of *P. subcapitata*, which, although low, is not insignificant, and is directly associated with the lipophilicity of the chemical compound (Ando et al. 2017).

Based on the flumioxazin concentrations selected for the tests with the microalgae, three doses were specifically chosen to evaluate the effects on the filtration rate and ingestion of the cladocerans. These concentrations covered a spectrum that included a level considered safe for the environment (0.852 µg L^{-1}), an Effective Concentration 50% (EC_{50}, 4.57 µg L^{-1}), and a third concentration (8.724 µg L^{-1}) which showed a difference statistically significant concerning the control treatment in terms of cell growth of the microalgae. For both species, *C. silvestrii* and *D. magna*, both the filtration rate and ingestion rate were adequate at the given concentration as a safe level for exposure of the organisms in this study. It was also possible to observed that there were species-specific differences in these filtration and ingestion rates in relation to the amount ingested at this safety dose, a characteristic also found by (Pérez-Moralez and Sarma 2014) when testing the feeding on different zooplankton and observing differences related to the species body size and food requirements.

At the EC_{50} concentration of 4.57 µg L^{-1} and the dose of toxic effect for the microalgae, *D. magna* exhibited a significant reduction in both the filtration rate and the ingestion. *D. magna* can show different feeding behaviors in the face of food quality (Rodrigues et al. 2021). The feeding of organisms zooplankton can be interrupted in situations where the food comes from a different source inadequate or due to the toxicity of algal cells (Rey et al. 2001; Orlowicz 2012). This response may be related to the high levels of carbohydrates and contamination by the herbicide present in the microalgae. Furthermore, it is assumed that a rejection by *D. magna* to the contaminated algal cells, according to observed previously in the studies of McMahon and Rigler (1963) involving *D. magna* and *Chlorella* sp. These results and observations suggest that *D. magna*, when encountering cells contaminated algae, can manifest a rejection mechanism (Rey et al. 2001), increased by intestinal receptors, a behavior that may be associated with evolutionary adaptations to avoid eating potentially harmful foods or toxic.

Concerning the responses observed in *C. silvestrii*, a stimulus was noted in the rate of filtration and ingestion of the highest dose of flumioxazin present in the microalgae. This increase may can probably be attributed to a phenomenon known as hormesis, a stimulating effect observed in response to exposures to moderately toxic doses, as observed by other researchers in studies with these cladocerans (Muyssen et al. 2005; Castelhano Gebara et al. 2021). Compared to *D. magna*, the species *C. silvestrii* demonstrated greater food tolerance to the herbicide, suggesting that in a contaminated natural environment with the product, it could easily become dominant in the face of stress. It is

recognized that pesticides tend to induce the dominance of smaller species of zooplankton and, consequently, a reduction in the average body size of individuals in the community (Hanazato 2001).

These observations reinforce the importance of understanding specific interactions between zooplankton and food sources, especially in environments subject to contamination. By understanding how zooplankton responds to changes in their food sources, such as the contamination of microalgae by flumioxazin, we identified the possible transfer of energy in the trophic chain. In this dynamic, energy is transferred from the primary producers, such as microalgae, to the primary consumers, such as the zooplankton, and subsequently to secondary consumers such as fish. This transfer occurs as organisms consume others to obtain energy, and the energy thus acquired is used to sustain their metabolic activities and is also transferred along the food chain, as organisms are consumed by other predators. The efficiency of this energy transfer directly influences the structure and functioning of aquatic ecosystems, affecting the abundance and species diversity and, consequently, the stability and health of these environments.

5 CONCLUSIONS

The microalgae showed significant sensitivity, showing phytotoxicity at a dose of 2.726 μg L^{-1} and accumulating carbohydrates in response to the presence of the flumioxazine-based herbicide at the concentrations evaluated, including the 50% effective concentration (EC_{50}) of 4.57 μg L^{-1}. These results indicated the impactful response of the microalgae to exposure to flumioxazine, highlighting the usefulness of the bioassay as a sensitive tool in evaluating the effects of the herbicide on this aquatic organism. The filtration and ingestion rates of the cladocerans in response to the microalgae contaminated with the herbicide were specific to each species. The body size and individual food requirements of each organism, *D. magna* or *C. silvestrii*, resulted in differences in the amounts ingested. For both cladoceran species evaluated, ingestion was not altered at the 50% effective concentration found for the microalgae, suggesting adequate adaptation to the available food. The filtration rate was reduced when the algae offered to *D. magna* was exposed to the EC_{50} concentration, 4.57 μg L^{-1}. These findings highlight the importance of considering the specific responses of different organisms in ecotoxicological studies, especially when evaluating the influence of herbicides in the aquatic environment.

Declarations

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CRediT authorship contribution statement

Tamires de Freitas Oliveira: Conceptualization, Formal analysis, Methodology, Project administration, Data curation, Writing – original draft, Writing – review & editing. **Larissa Fonseca Andrade Vieira:** Conceptualization, Formal analysis, Project administration, Writing – original draft, Methodology, Data curation, Writing – review & editing.

Conflicts of interest: The authors declare that they are not aware of any conflicts of financial interest or personal relationships that could have influenced the work reported in this article at the time of its production.

Data availability

Data will be made available on request.

References

1. AFNOR, Association Française Normalisation., 1980. Norme experimentale. T90-304. Essais deseaux Determination de l'inhibition de *Scenedesmus subspicatus* par une substance, Paris, France.
2. Agatz A, Cole TA, Preuss TG, et al (2013) Feeding inhibition explains effects of imidacloprid on the growth, maturation, reproduction, and survival of Daphnia magna. Environ Sci Technol 47:2909–2917. https://doi.org/10.1021/es304784t
3. Albalasmeh AA, Berhe AA, Ghezzehei TA (2013) A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydr Polym 97:253–261. https://doi.org/10.1016/j.carbpol.2013.04.072
4. Alho L de OG, Gebara RC, Paina K de A, et al (2019) Responses of Raphidocelis subcapitata exposed to Cd and Pb: Mechanisms of toxicity assessed by multiple endpoints. Ecotoxicol Environ Saf 169:950–959. https://doi.org/10.1016/j.ecoenv.2018.11.087
5. Ando D, Fujisawa T, Katagi T (2017) Fate of Flumioxazin in Aquatic Plants: Two Algae (Pseudokirchneriella subcapitata, Synechococcus sp.), Duckweed (Lemna sp.), and Water Milfoil (Myriophyllum elatinoides). J Agric Food Chem 65:8813–8822. https://doi.org/10.1021/acs.jafc.7b03355
6. Castelhano Gebara R, de Oliveira Gonçalves Alho L, Bruno de Abreu C, et al (2021) Toxicity and Risk Assessment of Zinc and Aluminum Mixtures to Ceriodaphnia silvestrii (Crustacea: Cladocera). John Wiley & Sons, Ltd
7. Cosgrove S, Jefferson B, Jarvis P (2019) Pesticide removal from drinking water sources by adsorption: a review. Environ Technol Rev 8:1–24. https://doi.org/10.1080/21622515.2019.1593514
8. Davis TW, Gobler CJ (2011) Grazing by mesozooplankton and microzooplankton on toxic and non-toxic strains of Microcystis in the Transquaking River, a tributary of Chesapeake Bay. J Plankton Res

33:415–430. https://doi.org/10.1093/plankt/fbq109

9. Dorigo U, Bourrain X, Bérard A, Leboulanger C (2004) Seasonal changes in the sensitivity of river microalgae to atrazine and isoproturon along a contamination gradient. Sci Total Environ 318:101–114. https://doi.org/10.1016/S0048-9697(03)00398-X
10. Fallahi A, Rezvani F, Asgharnejad H, et al (2021) Interactions of microalgae-bacteria consortia for nutrient removal from wastewater: A review. Chemosphere 272:129878. https://doi.org/10.1016/j.chemosphere.2021.129878
11. Geoffroy L, Frankart C, Eullaffroy P (2004) Comparison of different physiological parameter responses in Lemna minor and Scenedesmus obliquus exposed to herbicide flumioxazin. Environ Pollut 131:233–241. https://doi.org/10.1016/j.envpol.2004.02.021
12. Hanazato T (2001) Pesticide effects on freshwater zooplankton: An ecological perspective. Environ Pollut 112:1–10. https://doi.org/10.1016/S0269-7491(00)00110-X
13. Hill WR, Ryon MG, Smith JG, et al (2010) The role of periphyton in mediating the effects of pollution in a stream ecosystem. Environ Manage 45:563–576. https://doi.org/10.1007/s00267-010-9425-2
14. Kâ S, Mendoza-Vera JM, Bouvy M, et al (2012) Can tropical freshwater zooplankton graze efficiently on cyanobacteria? Hydrobiologia 679:119–138. https://doi.org/10.1007/s10750-011-0860-8
15. Lurling M, Beekman W (2006) Influence of food-type on the population growth rate of teh rotifer Brachionus calyciflorus in short-chronic assays. Acta Zool Sin 52:70–78
16. Lürling M, Van der Grinten E (2003) Life-history characteristics of Daphnia exposed to dissolved microcystin-LR and to the cyanobacterium Microcystis aeruginosa with and without microcystins. Environ Toxicol Chem 22:1281–1287. https://doi.org/10.1897/1551-5028(2003)022<1281:LHCODE>2.0.CO;2
17. Magnusson M, Heimann K, Quayle P, Negri AP (2010) Additive toxicity of herbicide mixtures and comparative sensitivity of tropical benthic microalgae. Mar Pollut Bull 60:1978–1987. https://doi.org/10.1016/j.marpolbul.2010.07.031
18. Malzahn AM, Hantzsche F, Schoo KL, et al (2010) Differential effects of nutrient-limited primary production on primary, secondary or tertiary consumers. Oecologia 162:35–48. https://doi.org/10.1007/s00442-009-1458-y
19. Markou G, Angelidaki I, Georgakakis D (2012) Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Appl Microbiol Biotechnol 96:631–645. https://doi.org/10.1007/s00253-012-4398-0
20. McMahon JW, Rigler FH (1963) Mechanisms Regulating the Feeding Rate of Daphnia Magna Straus. Can J Zool 41:321–332. https://doi.org/10.1139/z63-027
21. Mohd Nizam SN, Haji Baharudin NS, Ahmad H (2023) Application of pesticide in paddy fields: a Southeast Asia case study review. Environ Geochem Health 45:5557–5577. https://doi.org/10.1007/s10653-023-01668-8
22. Mojiri A, Zhou JL, Robinson B, et al (2020) Pesticides in aquatic environments and their removal by adsorption methods. Chemosphere 253:126646.

https://doi.org/10.1016/j.chemosphere.2020.126646

23. Muyssen BTA, Bossuyt BTA, Janssen CR (2005) Inter- and intra-species variation in acute zinc tolerance of field-collected cladoceran populations. Chemosphere 61:1159–1167. https://doi.org/10.1016/j.chemosphere.2005.02.076
24. Orlowicz E (2012) Filtering efficiency and feeding mechanisms of Daphnia pulex on Microcystis aeruginosa and Nannochloropsis. Mar Biol Commons 15
25. Paffenhöfer GA (1971) Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod Calanus helgolandicus. Mar Biol 11:286–298. https://doi.org/10.1007/BF00401275
26. Pérez-Moralez A, Sarma SSS (2014) Feeding and filtration rates of zooplankton (rotifers and cladocerans) fed toxic cyanobacterium (Microcystis aeruginosa). J Environ Biol 35:1061–1066
27. Rey C, Harris R, Irigoien X, et al (2001) Influence of algal diet on growth and ingestion of Calanus helgolandicus nauplii. Mar Ecol Prog Ser 216:151–165. https://doi.org/10.3354/meps216151
28. Rodrigues S, Pinto I, Martins F, et al (2021) Can biochemical endpoints improve the sensitivity of the biomonitoring strategy using bioassays with standard species, for water quality evaluation? Ecotoxicol Environ Saf 215:112151. https://doi.org/10.1016/j.ecoenv.2021.112151
29. Saballos AI, Brooks MD, Tranel PJ, Williams MM (2024) Mapping of flumioxazin tolerance in a snap bean diversity panel leads to the discovery of a master genomic region controlling multiple stress resistance genes. Front Plant Sci 15:. https://doi.org/10.3389/fpls.2024.1404889
30. Saladin G, Clément C, Magné C (2003) Stress effects of flumioxazin herbicide on grapevine (Vitis vinifera L.) grown in vitro. Plant Cell Rep 21:1221–1227. https://doi.org/10.1007/s00299-003-0658-x
31. Shabana EF, Battah MG, Kobbia IA, Eladel HM (2001) Effect of pendimethalin on growth and photosynthetic activity of Protosiphon botryoides in different nutrient states. Ecotoxicol Environ Saf 49:106–110. https://doi.org/10.1006/eesa.2000.1942
32. Turemis M, Silletti S, Pezzotti G, et al (2018) Optical biosensor based on the microalga-paramecium symbiosis for improved marine monitoring. Sensors Actuators, B Chem 270:424–432. https://doi.org/10.1016/j.snb.2018.04.111

Figures

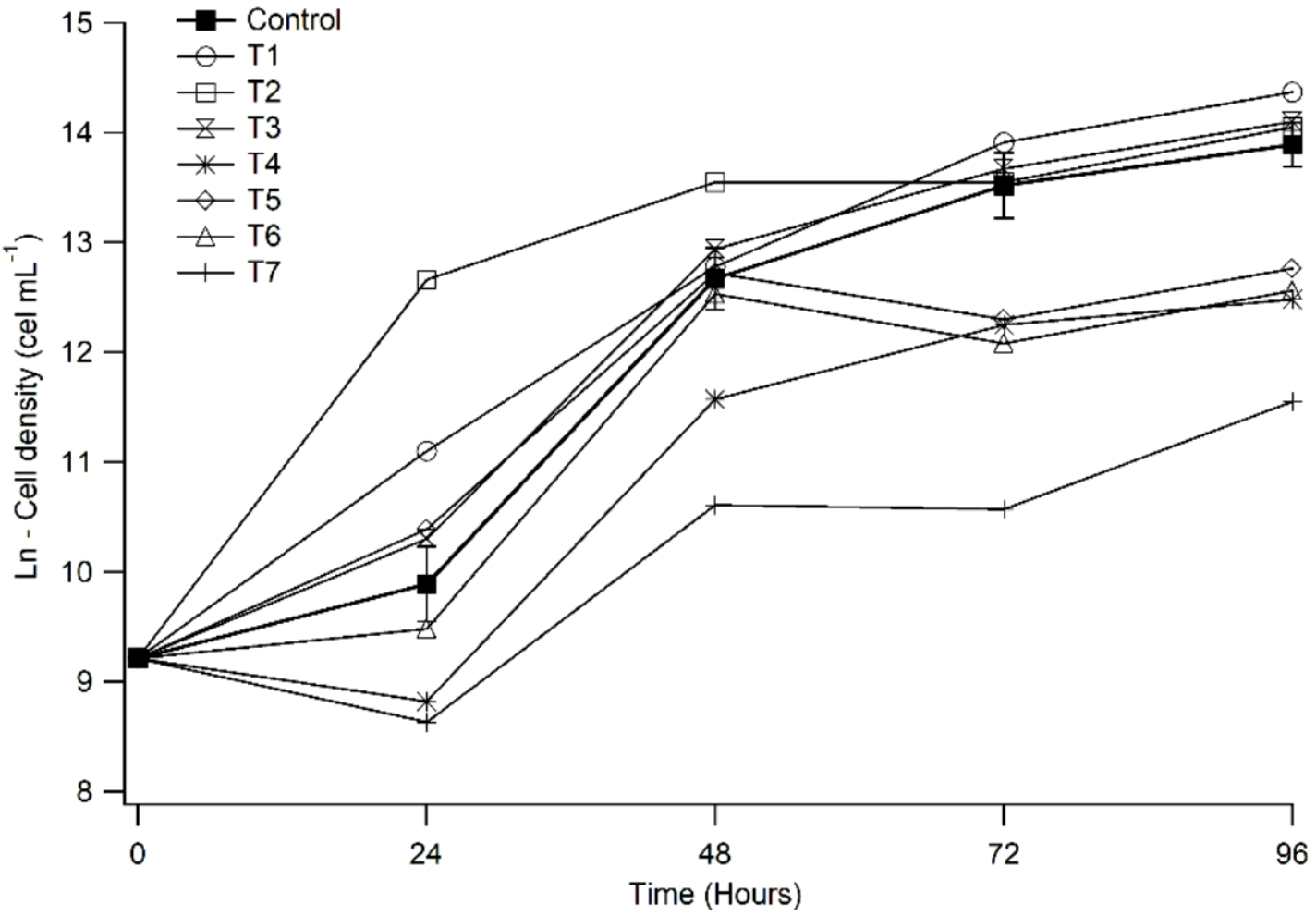


Figure 1

Growth parameter (Ln of cell density number (cell mL^{-1}) from *Raphidocelis subcapitata* tested after exposure to the herbicide based on flumioxazine. Legend: CN: negative control; T1: 0,0832 µg L^{-1}; T2: 0,266 µg L^{-1}; T3: 0,852 µg L^{-1}; T4: 2,726 µg L^{-1}; T5: 8,724 µg L^{-1}; T6: 27,918 µg L^{-1} and T7: 89,338 µg L^{-1}.

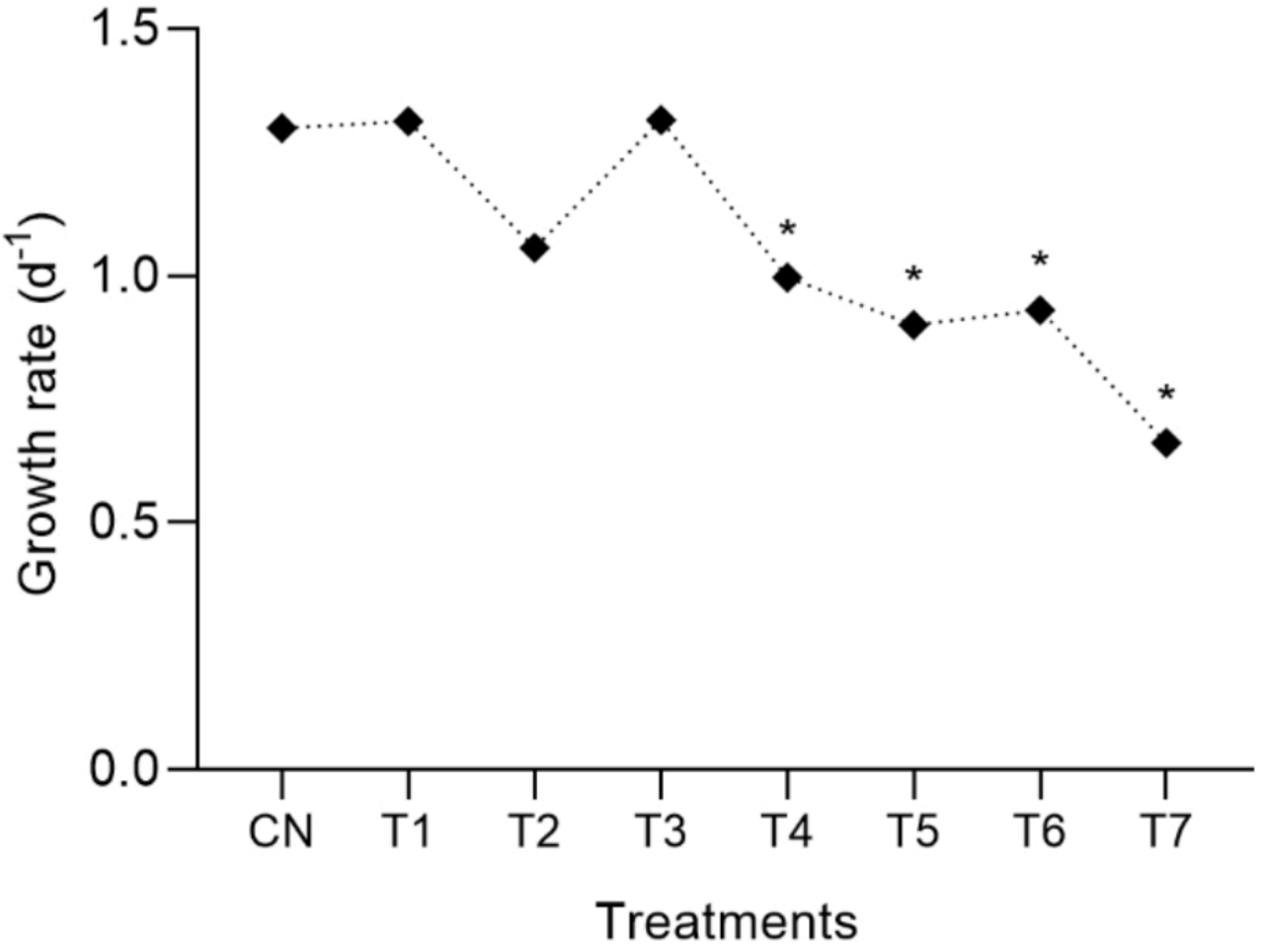


Figure 2

Growth parameter (Growth rate per day (d^{-1}) from *Raphidocelis subcapitata* tested after exposure to the flumioxazin-based herbicide. Legend: Legend: CN: negative control; T1: 0,0832 µg L^{-1}; T2: 0,266 µg L^{-1}; T3: 0,852 µg L^{-1}; T4: 2,726 µg L^{-1}; T5: 8,724 µg L^{-1}; T6: 27,918 µg L^{-1} and T7: 89,338 µg L^{-1}. The symbol * indicates a difference (Dunnet $p<0.05$) compared to the control treatment. The bars represent the standard deviation of the mean.

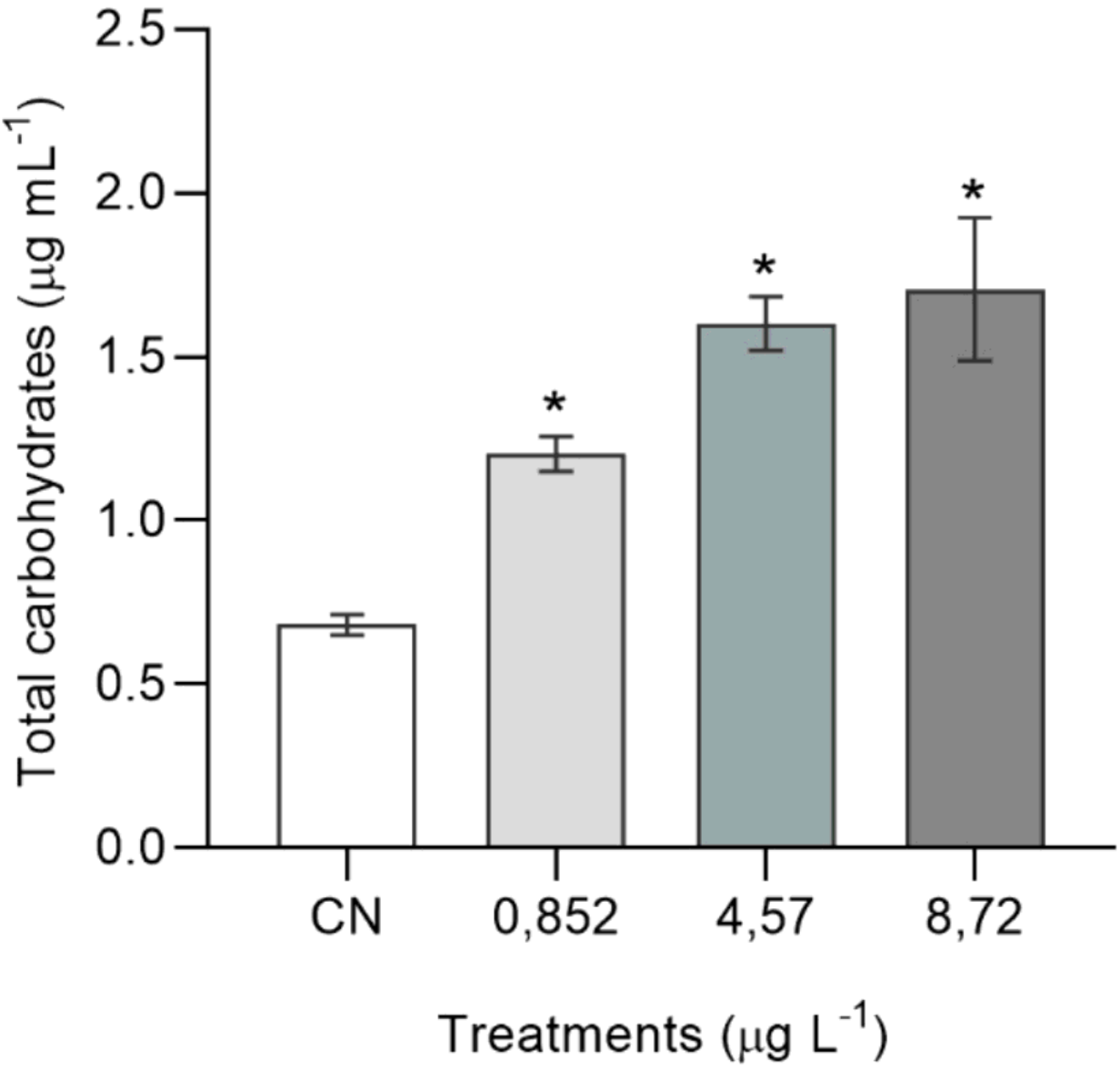


Figure 3

Total carbohydrate content of the microalga *Raphidocelis subcapitata* quantified after 96 hours of exposure to the flumioxazin-based herbicide. Legend: CN: negative control; T1: 0,0832 µg L^{-1}; T2: 0,266 µg L^{-1}; T3: 0,852 µg L^{-1}; T4: 2,726 µg L^{-1}; T5: 8,724 µg L^{-1}; T6: 27,918 µg L^{-1}; e T7: 89,338 µg L^{-1}. The symbol * indicates a difference (Dunnet $p<0.05$) compared to the control treatment. The bars represent the standard deviation of the mean.

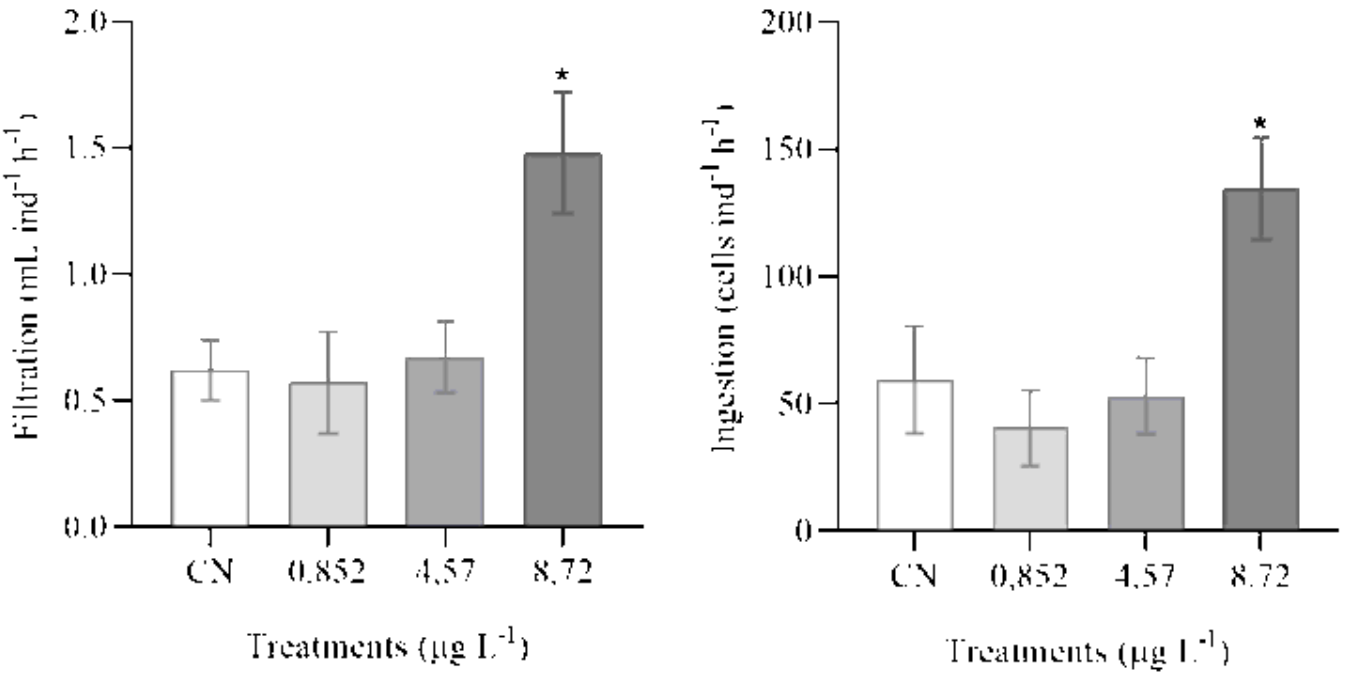


Figure 4

Filtration rates (mL ind^{-1} h^{-1}) and ingestion (cells ind^{-1} h^{-1}) of *Ceriodaphnia silvestrii* exposed to feed contaminated with flumioxazin-based herbicide for a period of 3 hours. Legend: The symbol * indicates a statistical difference (Dunnet $p<0.05$) in relation to the control treatment. The bars represent the standard deviation of the mean.

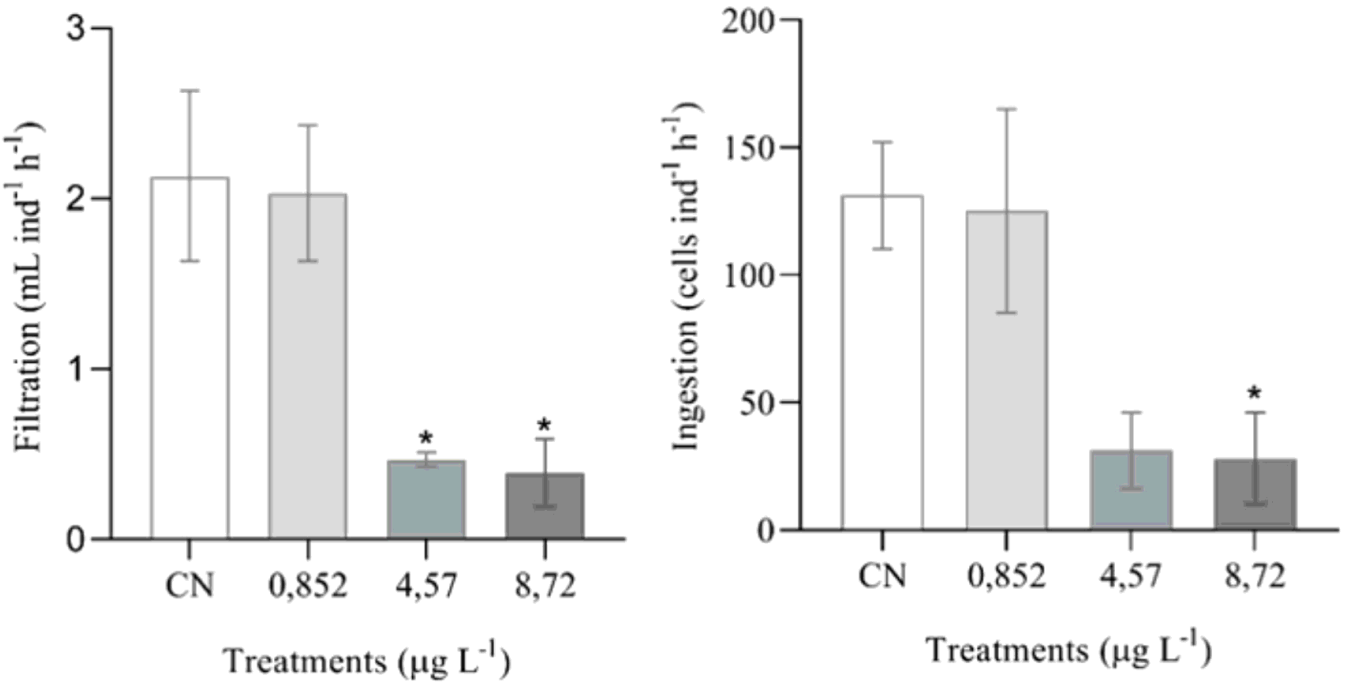


Figure 5

Filtration rates (mL ind^{-1} h^{-1}) and ingestion (cells ind^{-1} h^{-1}) of *Daphnia magna* exposed to feed contaminated with flumioxazin-based herbicide by 3-hour period. Legend: The symbol * indicates a statistical difference (Dunnet $p<0.05$) in relation to the control treatment. The bars represent the standard deviation of the mean.