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Integrated ecosystem models (soil-water) to analyze pesticide toxicity to aquatic organisms at two different temperature conditions

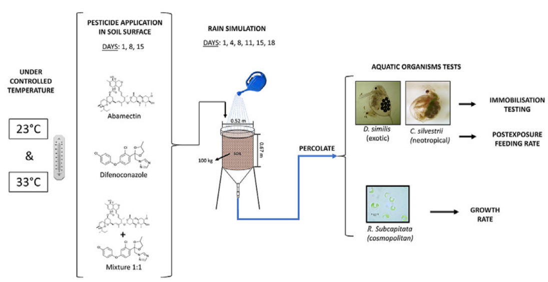
Livia de Figueirêdo Pitombeira [a,*], Danillo B. Athayde [a], Michiel A. Daam [b], Glauce Guerra [c], Paulo José Duarte-Neto [c], Hugo Sarmento [d], Evaldo L.G. Espíndola [a]

[a] PPG-SEA and NEEA/CRHEA/SHS, São Carlos School of Engineering, University of São Paulo, Av. Trabalhador São Carlense, 400, 13.560-970, São Carlos, Brazil
[b] CENSE, Department of Environmental Sciences and Engineering, Faculty of Sciences and Technology, New University of Lisbon, Quinta da Torre, 2829-516, Caparica, Portugal
[c] PPGBEA, Department of Statistics and Informatics, Rural Federal University of Pernambuco, R. Dom Manoel de Medeiros, s/n, Dois Irmãos, 52171900, Recife, Brazil
[d] Laboratory of Microbial Processes and Biodiversity, Department of Hydrobiology, Federal University of São Carlos (UFSCar), 13565-905, São Carlos, Brazil

HIGHLIGHTS

- Mesocosm was used to evaluate pesticide toxicity.
- Kraft 36 EC® is more toxic than Score 250 EC® for *C. silvestrii* and *D. similis.*
- Different temperatures were tested aiming at real analysis conditions.
- Native species was more sensitive at 33 °C than at 23 °C to compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

In order to increase the knowledge about pesticides considering the soil-water interaction, ecosystem models (mesoscosms) were used to analyze the of leachate on the immobility and feeding rate of the cladocerans, *Ceriodaphnia silvestrii* and *D. similis* and algae *Raphidocelis subcapitata*, at two different temperatures. Mesocosm were filled with natural soil (latosolo) that were contaminated with insecticide/acaricide Kraft 36 EC® and fungicide Score 250 EC®, using the recommended concentration for strawberry crops (10.8 g abamectin/ha and 20 g difenoconazole/ha). Pesticides were applied once (hand sprayers) and the precipitation was simulated twice a week (Days 1, 4, 8, 11, 15 and 18). The mesocosm were kept in a room with a controlled temperature (23 and 33 °C) and photoperiod (12h light/12h dark). The Kraft 36 EC® insecticide showed toxicity for both species of cladocerans tested, with effects on immobility and feeding rate, both at 23 and 33 °C. Score 250 EC® showed to be toxic only for the experiments that analyzed the immobility of *C. silvestrii* at 23 °C and the feeding of *D. smilis* at 33 °C, demonstrating that the effects are species-specific and related to the temperature at which they are tested. While for species *R. subcapitata* there was an effect only for mixture treatments of the pesticides analyzed at both temperatures. Thereby, zooplanktonic organisms may be at risk when exposed to this

* Corresponding author. NEEA/CRHEA, São Carlos School of Engineering, University of São Paulo, Av. Trabalhador São Carlense, 400, 13.560-970, São Carlos, Brazil.

E-mail address: livia.pitombeira@gmail.com (L. de Figueirêdo Pitombeira).

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compound even after percolating in a soil column, which could lead to effects on the entire aquatic trophic chain and that temperature can influence the organism response to the contaminant.

1. Introduction

Brazil is the largest consumer of pesticides in the world (Pignati et al., 2017), consuming the equivalent of ~20% of all those used worldwide (Fernandes et al., 2020). Despite Brazil being the world's largest consumer of these products, there are few studies evaluating the toxicity of these compounds in a tropical environment." Current data do not reflect the reality of the field since climatic conditions (especially temperature, rainy and humid conditions), as well as agricultural practices that determine the fate and effects of pesticides in these environments (Beck et al., 2018; Daam et al., 2019; Oliveira et al., 2018).

In practice, farmers usually do not follow guidelines recommended by manufacturers of the products, leading to the excessive use of pesticides in addition to often making mixtures of compounds (Gentil et al., 2019; Mottes et al., 2014; Pouokam et al., 2017; Settle et al., 2014). As a result, pesticides have been found in the most diverse tropical environments, from underground water (Sorensen et al., 2015), water and river sediment (Cornejo et al., 2019; Nguyen et al., 2019), in marine environments (Alava and Ross, 2018) and even in the air (Langenbach et al., 2017) and contaminating both target and nontarget organisms.

Associated with this lack of data in tropical environments, studies that consider realistic environmental conditions (such as temperature and natural soil) are even more scarce (Pitombeira de Figueirêdo et al., 2020), and available studies usually only consider one ecosystem (i.e., either only a terrestrial or surface water ecosystem). However, once applied to the soil, pesticides can reach the water bodies through percolation and runoff processes, especially in tropical environments with intense rainfall (Chrétien et al., 2017; Dores et al., 2016; Moreira et al., 2010). Soils exposed to higher rainfall intensities leads to a high probability of pesticide movement into surface and groundwater because they have a higher hydraulic conductivity (Bloomfield et al., 2006). Also, since soil property varies with type and water content of soil (Donga et al., 2020), it directly influences the toxicity of these compounds since higher soil water content will result in an increased degradation rate of pesticides as already proved in the literature (Pérez-Lucas et al., 2019).

Currently, studies evaluating pesticides toxicity are concentrated in isolated environments, such as surface water, soil, or even sediment, and there are still few studies that evaluate the toxicity in percolates or groundwater (Carriquiriborde et al., 2014; Machiwal et al., 2018). However, some studies already determine presence for several pesticides in these environments (El Alfy and Faraj, 2017; Navarrete et al., 2018; Polanco-Rodríguez et al., 2020), demonstrating the need for studies that take into account the soil/water interaction. Mesoscosms have been used successfully around the world in studies aimed at a more realistic and integrative approach aiming to evaluated both direct and indirect effects on organisms, thus allowing a better understanding of the impacts of pesticides on organisms, as well as their populations and communities (Kobashi et al., 2017; Szöcs et al., 2015)

In view of this, the aim of the present study was to evaluate the toxicity of leachate using an ecosystem model (mesocosm) contaminated with two pesticides used in strawberry culture, the insecticide/acaricide Kraft 36 EC® (a.i. abamectin) and the fungicide Score 250 EC® (a.i. difenoconazole), isolated and in binary mixture, to aquatic organisms (*Ceriodaphnia silvestrii, Daphnia similis* and *Raphidocelis subcapitata*), at two different temperatures (23 and 33 °C), simulating realistic field conditions.

2. Materials and methods

For this study, two pesticides commonly applied in strawberry cultivation (Novelli et al., 2012; Nunes et al., 2016; Pitombeira de Figueirêdo et al, 2019, 2020; Sanches et al., 2017a) were used in the experiments. The insecticide/acaricide Kraft 36 EC® (36 g/L) that has abamectin as its active ingredient, a compound from the family of avermectins. It is commonly used in the control of the mite *Tetranychus urticae*, is able to be reapplied by spray drift every 7 days diluted in water. Score 250 EC® is a systemic fungicide (250 g/L), a.i. difenoconazole, used to combat the species *Mycosphaerella fragariae*, and can be reapplied every 14 days also in aqueous solution with spray drift, as recommended in the product instructions provided by the manufacturers (10.8 g abamectin/ha and 20 g difenoconazole/ha).

2.1. Mesocosm experiment

Aiming at integrating soil/water, mesocosm filled with 100 kg natural soil (latosolo, 11.1% organic matter; cation exchange capacity (CEC) 3.52 (cmolc/kg); 35% clay, 21% silt, 22% fine sand, 20% medium sand, 2% coarse sand) in a conical tank that allowed the percolation of the leachate were used (Fig. 1). There were three replicates for each treatment: Kraft (K), Score (S), Kraft + Score (K + S) and a control, therefore totaling 12 mesocosm. The precipitations were simulated twice a week (days 1, 4, 8, 11, 15 and 18), the first precipitation was 35L (to soak the soil) and the others 5L

Fig. 1. Mesocosm structure emphasizing soil-water interface, mainly focusing on soil-water transfer via leaching.

with the same water used to prepare the culture medium of the test organisms, that is, uncontaminated water. Pesticides were applied (hand sprayers) once a week and the concentration used was the recommended dosage for strawberry crop (10.8 g abamectin/ha and 20 g difenoconazole/ha). For the application, in the isolated treatments, the solutions were diluted using 1 L Milli-Q® water (4.8 μl Kraft 36 EC® and 1.3 μl Score 250 EC®), while for mixtures, the same pesticides amounts were diluted in 0.5 L Milli-Q® water, thus adding 1 L of solution for contamination. In the controls, only Mili-Q® water (1L) was applied. The simulators were kept in temperature controlled room at either 23 or 33 °C and a photoperiod (12h light/12h dark). For a more detailed account of the methodology, see Pitombeira de Figueirêdo et al. (2020).

2.2. Test and culture organisms

Organisms used in all experiments, i.e. the cladocerans *C. silvestrii* (native) and *D. similis* (exotic and widely used in toxicity tests) and the microalgae *R. subcapitata* (broad geographic distribution), were cultured at the NEEA/CRHEA laboratories - EESC/USP. Each species was maintained and cultured following standards defined by the Brazilian Association of Technical Standards (ABNT) (ABNT, 2018, 2017, 2016). The same standards were used to conduct toxicity tests for these species on each precipitation day (days 1, 4, 8, 11, 15 and 18), so the toxicity tests were conducted six times. In addition, a toxicity test was carried out with reference substances for each species (KCl − *C. silvestrii* and NaCl − *D. similis*). In addition to the acute toxicity tests (cladocerans) and chronic effects (algae) of the pesticides, post-exposure tests were performed with *D. similis* and *C. silvestrii* that survived the acute test, through evaluation of changes in the feeding rate. This was done following the protocol developed by McWilliam and Baird (2002), by evaluating the 4h consumption rate of 10 cladocerans in 15 mL ASTM medium (ASTM, 1980) containing 4.6×10^5 mL *R. subcapitata* cells.

2.3. Chemical analyses

Concentration of stock solutions were verified using an HPLC-DAD (Agilent 1200 series), with C18 column (4.6 × 250 mm, 5 μm) and isocratic mobile phase (acetonitrile, 1% water with acetic acid 95.5%), an injection volume of 20 mL and flow rate of 1 mL/min, which was injected three times. Abamectin was detected and measured at 246 nm (with retention times, RT of 3.6 min), whereas difenoconazole at 220 nm (RT = 7.3 min), with limits of detection and quantification of 0.05 mg/L and 0.16 mg/L for abamectin and 0.01 mg/L and 0.03 mg/L for difenoconazole, respectively (Moreira et al., 2017; Sanches et al., 2017b).

In the leachate test physicochemical parameters were analyzed, including the parameters recommended by the standards for the tested species (pH-Micronal B374 potentiometer, dissolved oxygen-YSI 55 25FT, conductivity- Orion model 145, turbidity and total dissolved solids - Orion model 145 - direct reading on equipment), hardness and nutrient concentrations (nitrogen and total phosphorus, nitrate, nitrite, ammonium ion, silicate, total dissolved phosphorus and inorganic phosphorus) contained in the samples (APHA, 1995; Golterman et al., 1978; Koroleff, 1976; Mackereth et al., 1979)

2.4. Statistical analyses

Initially, to reduce dimensionality of the physical-chemical variables (k = 15), a principal component analysis (PCA) was performed based on the variance and covariance matrix of the scaled data obtained from the analysis of the leachate during the events that simulated the rain. Retained principal components (PCs) were determined based on the scree plot of the respective eigenvalues. The retained components were used as covariables in the Generalized Mixed Linear Model (GLMM), in which the analyzed treatments (Kraft, Score, Kraft + Score and Control) were used as explanatory variables, as well as the two temperatures tested (23 °C and 33 °C) and their interactions. Rain was added as a random effect.

The choice of the best model was made using theAkaike Information Criterion (AIC), corrected for a small sample size (AICc). The significance of each of those explanatory variable were based on a hierarchical procedure, conducted from the full model and compared it with simpler models, first taking off the random effect, followed by Principal Components, interaction temperature-treatment, and by each factor (Table 1). The best model was that one with the lowest AIC, and differences between models were considered when ΔAIC was more than two units (Burnham and Anderson, 2002; Hobbs and Hilborn, 2006).

The response variables for the cladoceran models were: immobility (acute test), modeled using a binomial probability distribution and logit link function; feed rate (post exposure test) and algal cell growth were considered to have a gamma distribution and a log link function. The data variability, as well as the structure of the models used was verified by means of the residual diagnosis. PCA was performed using the standard princomp function and GLMM was carried out using (R Core Team, 2018) and the “lme4″ package (Bates et al., 2015), considering the control group as a reference treatment at 23 °C and a 5% significance level.

3. Results

The first three PCs were retained and accounted for 75% of the total variance (Figure S1) PC1 (50%) was strongly correlated with nitrite, nitrate, ammonium ion, total dissolved phosphate, pH, conductivity, salinity, hardness, total dissolved solids and turbidity. In turn, PC2 (14.7%) was correlated with silicate, total phosphorus and total nitrogen. Finally, PC3 (10%) was represented only by dissolved oxygen (Fig. 2).

The laboratory control for all tested species had their premises accepted, according to the standard for each species, for the performance of the tests at both temperatures, with survival above 90% for *C. silvestrii* and *D. similis* and growth rates were >1 over 96h test duration for *R. subcapitata* (Fig. 3). Tests carried out with reference substance for *C. silvestrii* presented averages (±standard deviation) of 1.34 (±0.23) g $NaCl.L^{-1}$ for 2016 and 1.21 ± (0.25) g $NaCl.L^{-1}$ for 2017, while *D. smilis* had averages (±standard deviation) of 611.05 (±87.62) mg $KCl.L^{-1}$ and 611.68 (±74.70) mg $KCl.L^{-1}$ for tests performed also with reference substances in 2016 and 2017, respectively. In *Ceriodaphnia* and *daphnia* test, individuals that were unable to swim within 10s after gentle agitation of test vial were considered immobilized.

When analyzing *C. silvestrii*'s immobility, it was determined that the best model to describe the data was the complete model that showed that all explanatory variables are important to the model (Table S1) and the binomial probability distribution and logit link function were considered appropriate (Table S1 and Figure S2). Regarding the coefficients of the best model, significant p-values were obtained for all variables analyzed (Fig. 4A; Table S2), except for treatments based on the fungicide Score 250 EC® at 33 °C. In addition to this, the effect of dissolved oxygen (PC3) was not significative (p = 0.10). For the immobility of *D. similis*, the best model was also the complete model, with appropriate probability distribution and link function (Table S3 and Figure S4). Significant differences were observed for Kraft 36 EC® and K + S treatments (in

Table 1
Comparison of results of the models used in GLMM (Generalized Linear Mixed Model). Explanatory variables: temperature (23 and 33 °C), treatments (control − without contamination; K- Kraft 36 EC®; S- Score 250 EC®; K + S -mixture Kraft 36 EC® and Score 250 EC®), physico-chemical parameters grouped into main components (PC_1, PC_2 and PC_3) and interaction temperature and treatment. Rain: random effect. +: factor presence in the model. *NA*: factor absence in the model.

(Intercept)	Temperature	Treatments	PC_1	PC_2	PC_3	Interaction (temperature): (treatment)	Random effect
Complete	+	+	+	+	+	+	+
m1	+	+	+	+	+	+	*NA*
m2	+	+	+	+	*NA*	+	+
m3	+	+	+	*NA*	+	+	+
m4	+	+	*NA*	+	+	+	+
m5	+	+	+	+	+	*NA*	+
m6	+	+	*NA*	*NA*	*NA*	+	+

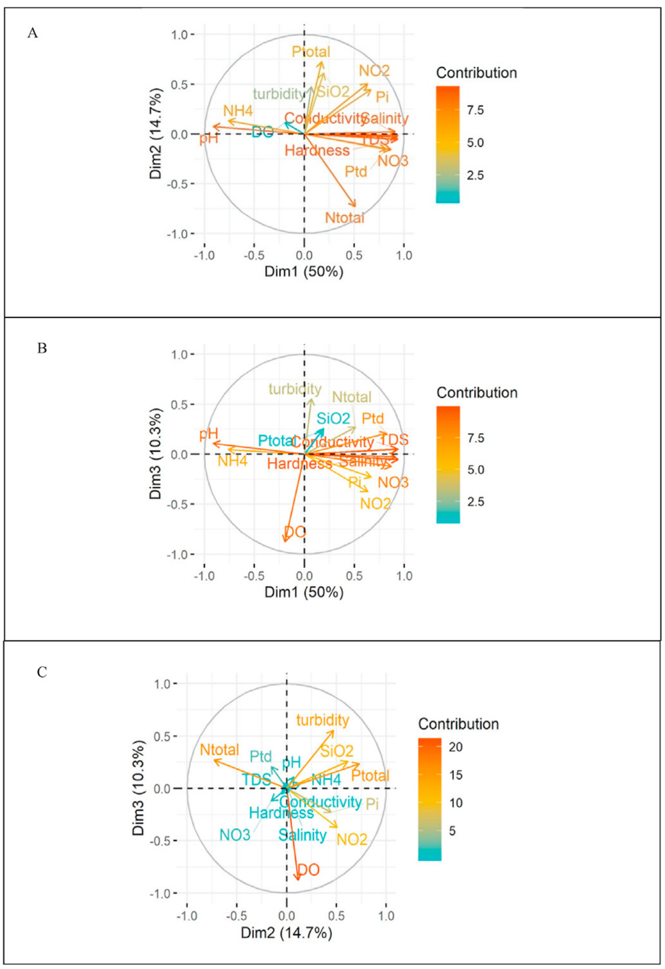

Fig. 2. Principal component analysis (PCA) representing the distribution of the analyzed physico-chemical parameters of the leachate obtained during the simulation of the six rains in the mesocosms. Legend: NO_2=Nitrite; NO_3=Nitrate; NH_4^+=Ammonium ion; SiO_2 = silicate total; PTD = dissolved phosphorus; Pi = Inorganic phosphate; Ntotal = Total nitrogen; Ptotal = Phosphorus total; pH; DO = Dissolved oxygen; Conductivity; Salinity; Hardness; TDS = Total dissolved solids; turbidity. A:PC_1 x P_2, B: PC_1 x PC_3 and C: PC_2 x PC_3.

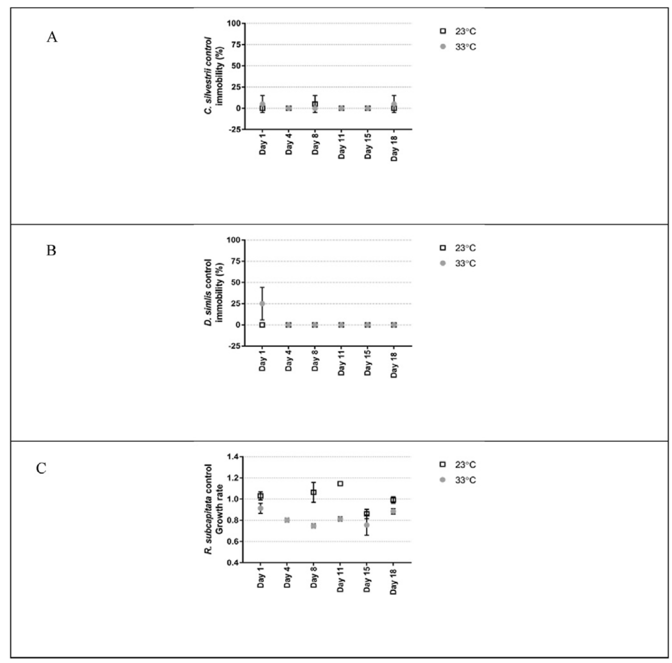

Fig. 3. Laboratory control (mean ± standard deviation) of A- *Ceriodaphnia silvestrii* immobility, B- *Daphnia similis* immobility and C- *Raphidocelis subcapitata* growth rate, during days 1, 4, 8, 11, 15 and 18 of the experiments, realized at 23 and 33 °C.

23 and 33 °C) and all physical chemical parameters (Fig. 3B; Table S4). These data show that acaricide Kraft 36 EC® is the most toxic treatment for both tested organisms.

For the tests carried out with the surviving organisms of the acute test (post exposure), the best model for *C. silvestrii* was model 6 (Table S1 and Figure S3), that is, there was no significant relationship between the physicochemical parameters and the response variables (Table S2). Significant p-values (Fig. 5A) were found for organisms exposed to Kraft 36 EC® (at 23 and 33 °C). The increase in temperature when evaluated in isolation also presented a significant p-value, therefore interfering in the feeding rate of these organisms.

The results for the post exposure test with *D. similis* (Table S3) and for the test with *R. subcapitata* (Table S5) found that the best model proposed was the complete model (Figure S5 and S6, respectively) when comparing both AICc values and residue distribution for tested models, demonstrating a good adherence to the data. However, for post-exposure testing with *D. similis*, show significant effects ($p < 0.05$) with organisms exposed to isolated pesticides (K and S), at both temperatures tested (Fig. 5B), as well as for components three (Table S4).

For the algae test, significant effects were observed for the physicochemical parameters, except for dissolved oxygen, as well as for temperatures when analyzed as an isolated factor (Table S6). For the analyzed treatments, significant differences were found for the pesticide mixture at 23 and 33 °C, as well as for the fungicide when applied isolated (S) at 33 °C (Fig. 6). However, for Score 250 EC® the graph shows that the effect for this compound is positive for this species, producing a slight increase in the *R. subcapitata* growth rate.

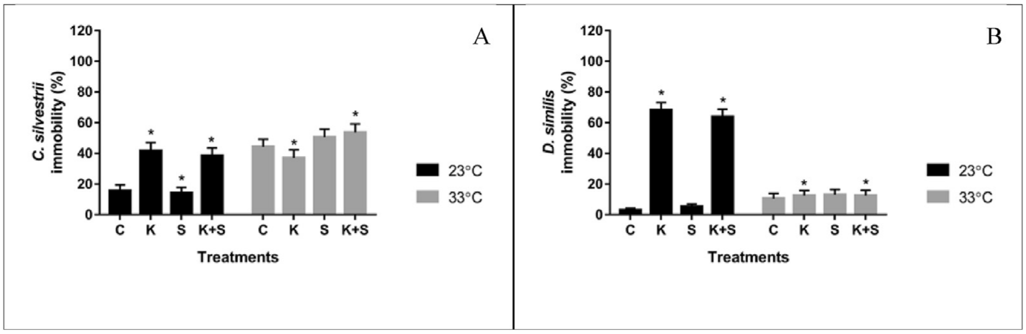

Fig. 4. Immobility (mean ± standard deviation) of *Ceriodaphnia silvestrii* (A) and *Daphnia similis* (B) when exposed to C – control (without contamination), Kraft 36 EC® (K), Score 250 EC® (S) and mixture Kraft 36 EC® and Score 250 EC® (K + S). Experiments conducted at 23 and 33 °C. *p < 0.05 compared by the GLMM (Generalized linear mixed model).

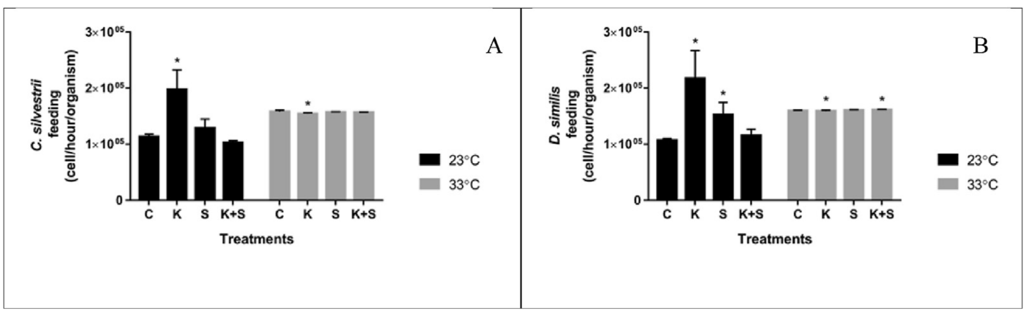

Fig. 5. Feeding rate (mean ± standard deviation) of *Ceriodaphnia silvestrii* (A) and *Daphnia similis* (B) when exposed to C – control (without contamination), Kraft 36 EC® (K), Score 250 EC® (S) and mixture Kraft 36 EC® and Score 250 EC® (K + S). Experiments conducted at 23 and 33 °C. *p < 0.05 compared by GLMM (Generalized linear mixed model).

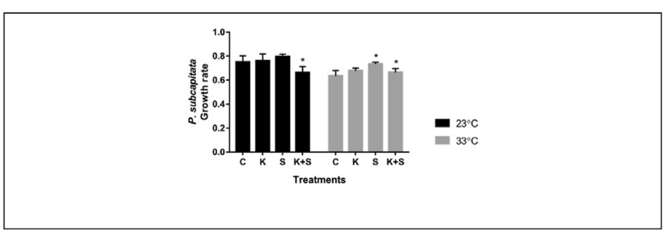

Fig. 6. Growth rate (mean ± standard deviation) of *Raphidocelis subcapitata* when exposed to C – control (without contamination), Kraft 36 EC® (K), Score 250 EC® (S) and mixture Kraft 36 EC® and Score 250 EC® (K + S). Experiments conducted at 23 and 33 °C.

4. Discussion

Among the analyzed organisms, it was observed that the cladoceran species (*D. similis* and *C. silvestrii*) were more sensitive to the tested compounds than the analyzed algae species (*R. subcapitata*). This is in agreement with the data reported by Ccanccapa et al. (2016), who evaluated the effects of 50 pesticides on species of three trophic levels (algae - unknown species, daphnia - *D. magna* and fish - *Oncorhynchus mykiss*) and demionstrated that *D. magna* was the most sensitive species among the tested organisms. However, in the same experiment it was identified that the pesticides of the azole class (class the fungicide Score 250 EC® belongs to) showed toxicity to algae while in our study no effects of the fungicide for *R. subcapitata* were identified. Relatively low toxicity (as compared to crustaceans) of triazole fungicides (tebuconazole and tetraconazole) to *R. subcapitata* was also shown recently in the literature by Marinho et al. (2020). Fletcher et al. (2000) relates this low toxicity of triazoles to the fact that this group of pesticides has the potential to protect photosynthetic organisms from stress, since they increase the defenses of antioxidant systems.

This tolerance of the species *R. subcapitata* to pesticides that are not in the herbicide class, such as insecticides, has also been reported by Silva et al. (2018). In this study, the authors found an EC_{50} value of 4067 (2781–7882) μg/L for the insecticide chlorpyrifos, while for the herbicide terbuthylazine the EC_{50} value was 65 (46–100) μg/L. The specific mode of action of herbicides, commonly related to photosystems or plant enzymes (Sherwani et al., 2015), justifies the high toxicity of these compounds to autotrophic organisms, showing a clear relationship between sensitive species and the herbicide's mode of action. Regarding fungicides, effects were observed in green algae species, such as *R. subcapitata*, mainly in compounds related to biosynthesis pathway for ergosterol, such as fenpropidin and fenpropimorph, which demonstrated high toxicity to this species (Coors et al., 2018). This would happen, according to the authors, to the fact that in the synthesis of sterols, including ergosterol, in several phyla, a large number of different enzymes is necessary.

The sensitivity of zooplankton species to pesticides, as shown in our study, was also reported Cruzeiro et al. (2017) for the species *Artemia salina* and *D. magna*. When they analyzed environmental samples from the Douro estuary in which there were 54 different pesticides, they found *D. magna* was extremely sensitive to this mixture of compounds, with mortalities 3- to 4-fold higher than for artemia. For Kraft 36 EC® acaricides, when constructing the species sensitivity distribution curves (SSDs), Moreira et al. (2017) reported that *D. similis* is the most sensitive among the other aquatic invertebrates, whereas for the 250 EC® fungicide Score, *D. magna* showed the highest sensitivity. Analyzing the immobility averages for the two studied pesticides, it can be observed that at 23 °C the toxic effect is very similar for both species with Kraft 36 EC®, isolated and in mixture, and more toxic than Score 250 EC® isolated.

However, at 33 °C it is possible to identify a greater sensitivity of *C. silvestrii* when compared to *D. similis*, for all treatments, with high immobility even for the isolated Score 250 EC®.

The high sensitivity to pesticides of *C. silvestrii* compared to other crustaceans was also shown by Mansano et al. (2018), who identified *C. silvestrii* as the most sensitive, followed by *D. magna* when compared with five other crustaceans (*Tigriopus japonicus, Artemia salina, Hydroides elegans, Aiptasia* sp. and *Balanus amphitrite*) exposed to the pesticides diuron and carbofuran. Tropical species were also more sensitive when compared to temperate species, according to Pham and Bui (2018), when comparing the sensitivity of *D. magna* and *D. lumholtzi* to the insecticide diazinon. Moreira et al. (2016) also identified a greater sensitivity of tropical species of rotifers exposed to fungicides when compared with temperate species. According to authors, such as Daam and Rico (2016), using native species to assess pesticide risks is essential, as it reduces the uncertainty in the extrapolation of toxicity data, since in tropical countries it is very common to use data from temperate regions for those assessments (Raymundo et al., 2019).

In addition to the immobility criterion, *C. silvestrii* and *D. similis* also showed they were susceptible in the two temperatures tested for the acaricide Kraft 36 EC® regarding their ability to feed. An effect for *D. similis* exposed to Score 250 EC® alone at both 23 and 33 °C was also observed. The reduction in the feed rate of organisms exposed to pesticides was also reported in the literature as an important endpoint since this reduction can have consequences for the energy balance and for the reproduction of species (Agatz et al., 2013; Agatz and Brown, 2013; Arias et al., 2020; Barata et al., 2008). In addition, the reduction in the organism's feeding rate has been shown to be negatively related to growth and reproduction, leading to a reduction in the number of organisms at the population level (Ribeiro et al., 2014). This was demonstrated by the study by Araujo et al. (2019) who saw a decrease in the rate of population increase (r) in two monophyletic species of *Daphnia* exposed to lead and mancozeb.

Authors such as Sancho et al. (2009) consider the feeding rate as a more sensitive parameter when compared to other sublethal parameters such as, glycogen, lipid, protein, and caloric content. However, Salesa et al. (2017) did not identify any effect on feeding of *D. magna* when exposed to gemfibrozil at two different temperatures (22 and 28 °C). Comparing the effects of the fungicide mancozeb to *D. magna* and *D. similis,* Araujo et al. (2019) found a decrease in the feeding rate of *D. similis* at concentrations above 0.9 mg mancozeb/L, whereas for *D. magna* there was no effect of feeding rate at the concentrations tested (0.15, 0.37 and 0.52 mg/L), thus demonstrating a greater sensitivity of the tropical species to the compound. Increased feeding rates after removal of the stressor have also been observed previously, which have been explained as a compensation mechanism for the energy loss related with the stress (Ferreira et al., 2008; McMahon and Rigler, 1965). This is in agreement with the increased feeding rate that was observed for both species and at both temperatures after exposure to Kraft in the present study (Fig. 5).

According to Cerezer et al. (2020), higher temperatures may have an indirect effect on the response of organisms to chemical compounds, limiting their defense capacity, which could reduce their harmful effects at cellular level. Moreover, under these conditions, increases in organic molecule intake may occur, increasing their toxic potential, accelerating metabolism and expending energy reserves. Effects were observed in the study by Jacquin et al. (2019), with the fish *Carassius auratus*, exposed to realistic conditions with rivers close to agricultural areas of France, contaminated with mixtures of herbicides of different modes of action. They found that the increase of 22 °C to 32 °C intensified the harmful effect at the molecular and cellular levels. Laetz et al. (2014) found similar results converging to this behavior, in which the increase in neurotoxicity in juvenile silver salmon caused by the mixture of pesticides is directly related to the increase in temperature.

5. Conclusions

Among the aquatic species analyzed, the cladocerans *C. silvestrii* and *D. similis* proved to be sensitive especially to the insecticide Kraft 36 EC®, as already reported in previous study (Moreira et al., 2020). For the two temperatures tested (23 and 33 °C) effects on immobility as well as the feeding rate were found, demonstrating that zooplanktonic organisms may be at risk when exposed to this compound even after percolating in a soil column, which could lead to effects on the entire aquatic trophic chain. However, the *R. subcapitata* algae was sensitive only to pesticide mixtures when tested under these methodological conditions.

Author statement

Livia Pitombeira de Figueirêdo – Conceptualization; Methodology; Validation; Writing – original draft; Writing – review & editing. Danillo B. Athayde - Methodology, Michiel A. Daam - Conceptualization; Writing – review & editing. Glauce da Silva Guerra- Methodology. Paulo José Duarte-Neto - Methodology, Hugo Sarmento - Writing – review & editing, Evaldo L.G. Espíndola – Conceptualization; Resources; Supervision

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.129422.

References

ABNT, 2018. Associação Brasileira de Normas Técnicas. NBR 12648- Ecotoxicologia aquática - Toxicidade crônica - Método de ensaio com algas (Chlorophyceae) https://doi.org/01.080.10;13.220.99.

ABNT, 2017. Associação Brasileira de Normas Técnicas. NBR 13373 - Ecotoxicologia aquática - Toxicidade crônica - Método de ensaio com Ceriodaphnia spp (Crustacea, Cladrocera).

ABNT, 2016. Associação Brasileira de Normas Técnicas. NBR 12713 - Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com Daphnia spp (Crustacea, Cladocera).

Agatz, A., Brown, C.D., 2013. Evidence for links between feeding inhibition, population characteristics, and sensitivity to acute toxicity for daphnia magna.

Environ. Sci. Technol. 47, 9461–9469. https://doi.org/10.1021/es401428h.
Agatz, A., Cole, T.A., Preuss, T.G., Zimmer, E., Brown, C.D., 2013. Feeding inhibition explains E ff ects of imidacloprid on the growth, maturation, reproduction, and survival of Daphnia magna. Environ. Sci. Technol. 47, 2909–2917. https://doi.org/10.1021/es304784t.
Alava, J.J., Ross, P.S., 2018. Pollutants in tropical marine mammals of the Galápagos Islands, Ecuador: an ecotoxicological quest to the last Eden. Marine Mammal Ecotoxicology: Impacts of Multiple Stressors on Population Health. https://doi.org/10.1016/B978-0-12-812144-3.00008-5. Elsevier Inc.
APHA, 1995. Standard Methods for the Examination of Water and Wastewater, nineteenth ed. Standard Methods for the Examination of Water and Wastewater.
Araujo, G.S., Pinheiro, C., Pestana, J.L.T., Soares, A.M.V.M., Abessa, D.M.S., Loureiro, S., 2019. Toxicity of lead and mancozeb differs in two monophyletic Daphnia species. Ecotoxicol. Environ. Saf. 178, 230–238. https://doi.org/10.1016/j.ecoenv.2019.03.095.
Arias, M., Bonetto, C., Mugni, H., 2020. Sublethal effects on Simocephalus vetulus (Cladocera: daphnidae) of pulse exposures of cypermethrin. Ecotoxicol. Environ. Saf. 196, 110546. https://doi.org/10.1016/j.ecoenv.2020.110546.
ASTM, A.S. for T. and M., 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macro- Invertebrates and Amphibians.
Barata, C., Alañon, P., Gutierrez-Alonso, S., Riva, M.C., Fernández, C., Tarazona, J.V., 2008. A Daphnia magna feeding bioassay as a cost effective and ecological relevant sublethal toxicity test for Environmental Risk Assessment of toxic effluents. Sci. Total Environ. 405, 78–86. https://doi.org/10.1016/j.scitotenv.2008.06.028.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67. https://doi.org/10.18637/jss.v067.i01.
Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future köppen-geiger climate classification maps at 1-km resolution. Scientific Data 5, 1–12. https://doi.org/10.1038/sdata.2018.214.
Bloomfield, J.P., Williams, R.J., Gooddy, D.C., Cape, J.N., Guha, P., 2006. Impacts of climate change on the fate and behaviour of pesticides in surface and groundwater-a UK perspective. Sci. Total Environ. 369, 163–177. https://doi.org/10.1016/j.scitotenv.2006.05.019.
Information and likelihood theory: a basis for model selection and inference. In: Burnham, K.P., Anderson, D.R. (Eds.), 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer New York, New York, NY, pp. 49–97. https://doi.org/10.1007/978-0-387-22456-5_2.
Carriquiriborde, P., Mirabella, P., Waichman, A., Solomon, K., Van den Brink, P.J., Maund, S., 2014. Aquatic risk assessment of pesticides in Latin America. Integrated Environ. Assess. Manag. 10, 539–542. https://doi.org/10.1002/ieam.1561.
Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the ebro river basin: occurrence and risk assessment. Environ. Pollut. 211, 414–424. https://doi.org/10.1016/j.envpol.2015.12.059.
Cerezer, C., Marins, A.T., Cerezer, F.O., Severo, E.S., Leitemperger, J.W., Grubel Bandeira, N.M., Zanella, R., Loro, V.L., Santos, S., 2020. Influence of pesticides and abiotic conditions on biochemical biomarkers in Aegla aff. longirostri (crustacea, anomura): implications for conservation. Ecotoxicol. Environ. Saf. 203, 110982. https://doi.org/10.1016/j.ecoenv.2020.110982.
Chrétien, F., Giroux, I., Thériault, G., Gagnon, P., Corriveau, J., 2017. Surface runoff and subsurface tile drain losses of neonicotinoids and companion herbicides at edge-of-field. Environ. Pollut. 224, 255–264. https://doi.org/10.1016/j.envpol.2017.02.002.
Coors, A., Vollmar, P., Sacher, F., Kehrer, A., 2018. Is there synergistic interaction between fungicides inhibiting different enzymes in the ergosterol biosynthesis pathway in toxicity tests with the green alga Raphidocelis subcapitata? Ecotoxicology 27, 936–944. https://doi.org/10.1007/s10646-018-1917-5.
Cornejo, A., Tonin, A.M., Checa, B., Tuñon, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PloS One 14, 1–17. https://doi.org/10.1371/journal.pone.0220528.
Cruzeiro, Catarina, Amaral, Sofia, Rocha, Eduardo, Rocha, Maria João, 2017. Determination of 54 pesticides in waters of the Iberian Douro River estuary and risk assessment of environmentally relevant mixtures using theoretical approaches and Artemia salina and Daphnia magna bioassays. Ecotoxicol. Environ. Saf. 145 (Junne), 126–134. https://doi.org/10.1016/j.ecoenv.2017.07.010.
Daam, M.A., Chelinho, S., Niemeyer, J.C., Owojori, O.J., De Silva, P.M.C.S., Sousa, J.P., van Gestel, C.A.M., Römbke, J., 2019. Environmental risk assessment of pesticides in tropical terrestrial ecosystems: test procedures, current status and future perspectives. Ecotoxicol. Environ. Saf. 181, 534–547. https://doi.org/10.1016/j.ecoenv.2019.06.038.
Daam, M.A., Rico, A., 2016. Freshwater shrimps as sensitive test species for the risk assessment of pesticides in the tropics. Environ. Sci. Pollut. Control Ser. 25, 13235–13243. https://doi.org/10.1007/s11356-016-7451-1.
Donga, T.K., Meadow, R., Sitaula, B.K., Eklo, O.M., 2020. Impact of climate variability on the use and exposure of pesticides in sugarcane production in Malawi. In: Singh, B.R., Safalaoh, A., Amuri, N.A., Eik, L.O., Sitaula, B.K., Lal, R. (Eds.), Climate Impacts on Agricultural and Natural Resource Sustainability in Africa. Springer International Publishing, Cham, pp. 159–173. https://doi.org/10.1007/978-3-030-37537-9_9.
Dores, E.F.G.C., Spadotto, C.A., Weber, O.L.S., Dalla Villa, R., Vecchiato, A.B., Pinto, A.A., 2016. Environmental behavior of chlorpyrifos and endosulfan in a tropical soil in central Brazil. J. Agric. Food Chem. 64, 3942–3948. https://doi.org/10.1021/acs.jafc.5b04508.
El Alfy, M., Faraj, T., 2017. Spatial distribution and health risk assessment for groundwater contamination from intensive pesticide use in arid areas. Environ. Geochem. Health 39, 231–253. https://doi.org/10.1007/s10653-016-9825-1.
Fernandes, C.L.F., Volcaõ, L.M., Ramires, P.F., Moura, R.R. De, Da Silva Júnior, F.M.R., 2020. Distribution of pesticides in agricultural and urban soils of Brazil: a critical review. Environ. Sci.: Processes and Impacts 22, 256–270. https://doi.org/10.1039/c9em00433e.
Ferreira, A.L.G., Loureiro, S., Soares, A.M.V.M., 2008. Toxicity prediction of binary combinations of cadmium, carbendazim and low dissolved oxygen on Daphnia magna. Aquat. Toxicol. 89, 28–39.
Fletcher, R.A., Gilley, A., Sankhla, N., Davis, T.D., 2000. Triazoles as plant growth regulators and stress protectants. In: Janick, J. (Ed.), Horticultural Reviews. John Wiley & Sons, pp. 55–138. https://doi.org/10.1002/9780470650776.ch3.
Gentil, C., Fantke, P., Mottes, C., Basset-Mens, C., 2019. Challenges and ways forward in pesticide emission and toxicity characterization modeling for tropical conditions. Int. J. Life Cycle Assess. https://doi.org/10.1007/s11367-019-01685-9.
Golterman, H.L., Golterman, H.L., Clymo, R.S., Ohnstad, M.A.M., 1978. Methods for Physical and Chemical Analysis of Fresh Waters, Handbooks (International Biological Programme). Blackwell Scientific.
Hobbs, N.T., Hilborn, R., 2006. Alternatives to statistical hypothesis testing in ecology: a guide to self teaching. Ecol. Appl. 16, 5–19. https://doi.org/10.1890/04-0645.
Jacquin, L., Gandar, A., Aguirre-Smith, M., Perrault, A., Hénaff, M.Le, Jong, L.De., Paris-Palacios, S., Laffaille, P., Jean, S., 2019. High temperature aggravates the effects of pesticides in goldfish. Ecotoxicol. Environ. Saf. 172, 255–264. https://doi.org/10.1016/j.ecoenv.2019.01.085.
Kobashi, K., Harada, T., Adachi, Y., Mori, M., Ihara, M., Hayasaka, D., 2017. Comparative ecotoxicity of imidacloprid and dinotefuran to aquatic insects in rice mesocosms. Ecotoxicol. Environ. Saf. 138, 122–129. https://doi.org/10.1016/j.ecoenv.2016.12.025.
Koroleff, F., 1976. Determination of nutrients: 2. Ammonia. In: Grasshoff, K. (Ed.), Methods of Seawater Analysis, pp. 126–133.
Laetz, C.A., Baldwin, D.H., Hebert, V.R., Stark, J.D., Scholz, N.L., 2014. Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon. Aquat. Toxicol. 146, 38–44. https://doi.org/10.1016/j.aquatox.2013.10.022.
Langenbach, T., Mano, D., Campos, M.M., Cunha, A.L.M.C., De Campos, T.M.P., 2017. Pesticide dispersion by spraying under tropical conditions. J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes 52, 843–849. https://doi.org/10.1080/03601234.2017.1359040.
Machiwal, D., Jha, M.K., Singh, V.P., Mohan, C., 2018. Assessment and mapping of groundwater vulnerability to pollution: current status and challenges. Earth Sci. Rev. 185, 901–927. https://doi.org/10.1016/j.earscirev.2018.08.009.
Mackereth, F.J.H., Heron, J., Talling, J.F., 1979. Water analysis: some revised methods for limnologists. Q. Rev. Biol. 54, 203. https://doi.org/10.1086/411247.
Mansano, A.S., Moreira, R.A., Dornfeld, H.C., Diniz, L.G.R., Vieira, E.M., Daam, M.A., Rocha, O., Seleghim, M.H.R., 2018. Acute and chronic toxicity of diuron and carbofuran to the neotropical cladoceran Ceriodaphnia silvestrii. Environ. Sci. Pollut. Control Ser. 25, 13335–13346. https://doi.org/10.1007/s11356-016-8274-9.
Marinho, M. da C., Diogo, B.S., Lage, O.M., Antunes, S.C., 2020. Ecotoxicological evaluation of fungicides used in viticulture in non-target organisms. Environ. Sci. Pollut. Control Ser. https://doi.org/10.1007/s11356-020-10245-w.
McMahon, J.W., Rigler, F.H., 1965. Feeding rate of Daphnia magna straus in different foods labeled with radioactive phosphorus. Limnol. Oceanogr. 10, 105–113. https://doi.org/10.4319/lo.1965.10.1.0105.
McWilliam, R.A., Baird, D.J., 2002. Application of postexposure feeding depression bioassays with *Daphnia magna* for assessment of toxic effluents in rivers. Environ. Toxicol. Chem. 21, 1462–1468. https://doi.org/10.1002/etc.5620210718.
Moreira, R.A., da Silva Mansano, A., Rocha, O., Daam, M.A., 2016. The use of rotifers as test species in the aquatic effect assessment of pesticides in the tropics. Hydrobiologia 773. https://doi.org/10.1007/s10750-016-2675-0.
Moreira, R.A., Daam, M.A., Vieira, B.H., Sanches, A.L.M., Reghini, M.V., da Silva Mansano, A., de Freitas, E.C., Espindola, E.L.G., Rocha, O., 2017. Toxicity of abamectin and difenoconazole mixtures to a Neotropical cladoceran after simulated run-off and spray drift exposure. Aquat. Toxicol. 185, 58–66. https://doi.org/10.1016/j.aquatox.2017.02.001.
Moreira, R.A., de Araujo, G.S., Silva, A.R.R.G., Daam, M.A., Rocha, O., Soares, A.M.V.M., Loureiro, S., 2020. Effects of abamectin-based and difenoconazole-based formulations and their mixtures in Daphnia magna: a multiple endpoint approach. Ecotoxicology. https://doi.org/10.1007/s10646-020-02218-z.
Moreira, S.M., Moreira-Santos, M., Rendón-von Osten, J., da Silva, E.M., Ribeiro, R., Guilhermino, L., Soares, A.M.V.M., 2010. Ecotoxicological tools for the tropics: sublethal assays with fish to evaluate edge-of-field pesticide runoff toxicity. Ecotoxicol. Environ. Saf. 73, 893–899. https://doi.org/10.1016/j.ecoenv.2010.04.007.
Mottes, C., Lesueur-Jannoyer, M., Le Bail, M., Malézieux, E., 2014. Pesticide transfer models in crop and watershed systems: a review. Agron. Sustain. Dev. 34, 229–250. https://doi.org/10.1007/s13593-013-0176-3.
Navarrete, I.A., Tee, K.A.M., Unson, J.R.S., Hallare, A.V., 2018. Organochlorine pesticide residues in surface water and groundwater along Pampanga River, Philippines. Environ. Monit. Assess. 190 https://doi.org/10.1007/s10661-018-6680-9.
Nguyen, T.X., Nguyen, B.T., Tran, H.T.T., Mai, H., Duong, T.T., Bach, Q.V., 2019. Seasonal, spatial variation, and potential sources of organochlorine pesticides in

water and sediment in the lower reaches of the dong nai river system in vietnam. Arch. Environ. Contam. Toxicol. 77, 514–526. https://doi.org/10.1007/s00244-019-00653-6.
Novelli, A., Vieira, B.H., Cordeiro, D., Cappelini, L.T., Vieira, E.M., Espindola, E.L., 2012. Lethal effects of abamectin on the aquatic organisms Daphnia similis, Chironomus xanthus and Danio rerio. Chemosphere 86, 36–40. https://doi.org/10.1016/j.chemosphere.2011.08.047.
Nunes, M.E.T., Daam, M.A., Espíndola, E.L.G., 2016. Survival, morphology and reproduction of Eisenia andrei (Annelida, Oligochaeta) as affected by Vertimec®18 EC (a.i. abamectin) in tests performed under tropical conditions. Appl. Soil Ecol. 100, 18–26. https://doi.org/10.1016/j.apsoil.2015.11.023.
Oliveira, V.B. de M., Bianchi, M. de O., Espíndola, E.L.G., 2018. Hazard assessment of the pesticides Kraft 36 EC and Score in a tropical natural soil using an ecotoxicological test battery. Environ. Toxicol. Chem. 9999, 1–6. https://doi.org/10.1002/etc.4056.
Pérez-Lucas, G., Vela, N., El Aatik, A., Navarro, S., 2019. Environmental risk of groundwater pollution by pesticide leaching through the soil profile. In: Pesticides - Use and Misuse and Their Impact in the Environment. IntechOpen, pp. 1–27. https://doi.org/10.5772/intechopen.82418.
Pham, Thanh Luu, Bui, Ha Manh, 2018. Comparison of Diazinon Toxicity to Temperate and Tropical Freshwater Daphnia Species. J. Chem. https://doi.org/10.1155/2018/9217815.
Pignati, W.A., Lima, F.A.N., de Se Lara, S.S. de, Correa, M.L.M., Barbosa, J.R., Leão, L.H. da C., Pignatti, M.G., 2017. Spatial distribution of pesticide use in Brazil: a strategy for Health Surveillance. Ciência Saúde Coletiva 22, 3281–3293. https://doi.org/10.1590/1413-812320172210.17742017.
Pitombeira de Figueirêdo, L., Athayde, D.B., Daam, M.A., van Gestel, C.A.M., Guerra, G. da S., Duarte-Neto, P.J., Espíndola, E.L.G., 2020. Impact of temperature on the toxicity of Kraft 36 EC® (a.s. abamectin) and Score 250 EC® (a.s. difenoconazole) to soil organisms under realistic environmental exposure scenarios. Ecotoxicol. Environ. Saf. 194, 110446. https://doi.org/10.1016/j.ecoenv.2020.110446.
Pitombeira de Figueirêdo, L., Daam, M.A., Mainardi, G., Mari, J., Espíndola, E.L.G., Gestel, C.A.M., Van Roelofs, D., 2019. The use of gene expression to unravel the single and mixture toxicity of abamectin and difenoconazole on survival and reproduction of the. Environ. Pollut. 244, 342–350. https://doi.org/10.1016/j.envpol.2018.10.077.
Polanco-Rodríguez, A.G., Arcega-Cabrera, F., Araujo-León, J.A., Lamas-Cosío, E., 2020. Organochlorine pesticides and potentially toxic elements in groundwater from a protected reserve in the maya region of hopelchen, Mexico. Bull. Environ. Contam. Toxicol. 104, 568–574. https://doi.org/10.1007/s00128-020-02848-3.
Pouokam, G.B., Album, W.L., Ndikontar, A.S., Sidatt, M.E.H., 2017. A pilot study in Cameroon to understand safe uses of pesticides in agriculture, risk factors for farmers' exposure and management of accidental cases. Toxics 5, 1–15. https://doi.org/10.3390/toxics5040030.
R Core Team, 2018. R: A Language and Environment for Statistical Computing.
Raymundo, L.B., Rocha, O., Moreira, R.A., Miguel, M., Daam, M.A., 2019. Sensitivity of tropical cladocerans to chlorpyrifos and other insecticides as compared to their temperate counterparts. Chemosphere 220, 937–942. https://doi.org/10.1016/j.chemosphere.2019.01.005.
Ribeiro, F., Gallego-Urrea, J.A., Jurkschat, K., Crossley, A., Hassellöv, M., Taylor, C., Soares, A.M.V.M., Loureiro, S., 2014. Silver nanoparticles and silver nitrate induce high toxicity to Pseudokirchneriella subcapitata, Daphnia magna and Danio rerio. Science of the Total Environment 466– 467, 232–241. https://doi.org/10.1016/j.scitotenv.2013.06.101.
Salesa, B., Ferrando, M.D., Villarroel, M.J., Sancho, E., 2017. Effect of the lipid regulator Gemfibrozil in the Cladocera Daphnia magna at different temperatures. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering 52, 228–234. https://doi.org/10.1080/10934529.2016.1246937.
Sanches, A.L.M., Horvath, B., Vanderlei, M., Aparecida, R., Cristina, E., Espíndola, E.L.G., Daam, M.A., 2017a. Single and mixture toxicity of abamectin and difenoconazole to adult zebra fi sh (Danio rerio). Chemosphere 188, 582–587. https://doi.org/10.1016/j.chemosphere.2017.09.027.
Sanches, A.L.M., Vieira, B.H., Reghini, M.V., Moreira, R.A., Freitas, E.C., Espíndola, E.L.G., Daam, M.A., 2017b. Single and mixture toxicity of abamectin and difenoconazole to adult zebrafish (Danio rerio). Chemosphere 188. https://doi.org/10.1016/j.chemosphere.2017.09.027.
Sancho, E., Villarroel, M.J., Andreu, E., Ferrando, M.D., 2009. Disturbances in energy metabolism of Daphnia magna after exposure to tebuconazole. Chemosphere 74, 1171–1178. https://doi.org/10.1016/j.chemosphere.2008.11.076.
Settle, W., Soumaré, M., Sarr, M., Garba, M.H., Poisot, A.S., 2014. Reducing pesticide risks to farming communities: cotton farmer field schools in Mali. Phil. Trans. Biol. Sci. 369 https://doi.org/10.1098/rstb.2012.0277.
Sherwani, S.I., Arif, I.A., Khan, H.A., 2015. Modes of action of different classes of herbicides. In: Herbicides, Physiology of Action and Safety. InTech, p. 13. https://doi.org/10.5772/61779.
Silva, E., Martins, C., Pereira, A.S., Loureiro, S., Cerejeira, M.J., 2018. Toxicity prediction and assessment of an environmentally realistic pesticide mixture to Daphnia magna and Raphidocelis subcapitata. Ecotoxicology 1–12. https://doi.org/10.1007/s10646-018-1938-0.
Sorensen, J.P.R., Lapworth, D.J., Nkhuwa, D.C.W., Stuart, M.E., Gooddy, D.C., Bell, R.A., Chirwa, M., Kabika, J., Liemisa, M., Chibesa, M., Pedley, S., 2015. Emerging contaminants in urban groundwater sources in Africa. Water Res. 72, 51–63. https://doi.org/10.1016/j.watres.2014.08.002.
Szöcs, E., Van den Brink, P.J., Lagadic, L., Caquet, T., Roucaute, M., Auber, A., Bayona, Y., Liess, M., Ebke, P., Ippolito, A., ter Braak, C.J.F., Brock, T.C.M., Schäfer, R.B., 2015. Analysing chemical-induced changes in macroinvertebrate communities in aquatic mesocosm experiments: a comparison of methods. Ecotoxicology 24, 760–769. https://doi.org/10.1007/s10646-015-1421-0.