

A comparative study of the acute toxicity of the herbicide atrazine to cladocerans *Daphnia magna*, *Ceriodaphnia silvestrii* and *Macrothrix flabelligera*

Um estudo comparativo da toxicidade aguda do herbicida atrazina aos cladóceros *Daphnia magna*, *Ceriodaphnia silvestrii* e *Macrothrix flabelligera*

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Abstract: Aim: In this study we compared the sensitivity of three species of Cladocera, *Daphnia magna*, *Ceriodaphnia silvestrii* and *Macrothrix flabelligera*, to the commercial product of the herbicide Atrazine, the Atrazine Atanor 50 SC[®] (500 g/L), widely used on crops in Brazil. **Methods:** Acute toxicity tests were performed at the nominal atrazine concentrations 2.25, 4.5, 9.0, 18.0, 36 and 72 mg L⁻¹, on *C. silvestrii* and *M. flabelligera* and at 2.25, 4.5, 9.0, 18.0, 36, 72 and 144 mg L⁻¹ on *D. magna*. The range of concentrations tested was established in a series of preliminary tests. **Results:** The toxicity tests showed that the two species naturally occurring in water bodies in Brazil were more susceptible than *Daphnia magna*. The effective concentrations of Atrazine Atanor 50 SC[®] (EC₅₀-48 h) to the species *M. flabelligera*, *C. silvestrii* and *D. magna* were 12.37 ± 2.67 mg L⁻¹, 14.30 ± 1.55 mg L⁻¹ and 50.41 ± 2.64 mg L⁻¹, respectively. Furthermore, when EC₅₀ observed here for *M. flabelligera* and *C. silvestrii* were compared with published values of EC₅₀ or LC₅₀ (mg L⁻¹) for various aquatic organisms exposed to atrazine, it was seen that these two cladocerans were the most sensitive to the herbicide. **Conclusions:** Considering these results and the broad distribution of *C. silvestrii* and *M. flabelligera* in tropical and subtropical regions, it is concluded that these native species would be valuable test organisms in ecotoxicological tests, for the monitoring of toxic substances in tropical freshwaters.

Keywords: Cladocera, Ecotoxicology, Atrazine Atanor 50 SC[®], pesticide.

Resumo: Objetivo: Neste estudo comparamos a sensibilidade de três espécies de cladóceros, *Daphnia magna*, *Ceriodaphnia silvestrii* e *Macrothrix flabelligera* quando expostas ao herbicida Atrazina Atanor 50 SC[®] (500 g/L), amplamente utilizado em plantações no Brasil. **Métodos:** Foram realizados testes de toxicidade aguda das concentrações nominais de atrazina nos valores de 2,25; 4,5; 9,0; 18,0; 36 e 72 mg L⁻¹ para *C. silvestrii* e *M. flabelligera* e de 2,25; 4,5; 9,0; 18,0; 36; 72 e 144 mg L⁻¹ para *D. magna*. Estas faixas de concentrações foram estabelecidas em testes preliminares. **Resultados:** Os testes de toxicidade mostraram que as duas espécies que ocorrem naturalmente nos corpos d'água do Brasil foram mais sensíveis do que *Daphnia magna*. As concentrações efetivas de Atrazina Atanor 50 SC[®] (CE₅₀-48 h) para *M. flabelligera*, *C. silvestrii* e *D. magna*, foram 12.37 ± 2.67 mg L⁻¹, 14.30 ± 1.55 mg L⁻¹ e 50.41 ± 2.64 mg L⁻¹, respectivamente. Comparando-se os valores de CE₅₀ da atrazina obtidos para *M. flabelligera* e *C. silvestrii* com valores de CE₅₀ ou CL₅₀ (mg L⁻¹) disponíveis na literatura para diferentes organismos aquáticos observa-se que os cladóceros testados foram mais sensíveis ao herbicida do que os demais. **Conclusões:** Com base nestes resultados e considerando-se a ampla distribuição de *C. silvestrii* e *M. flabelligera* em regiões tropicais e subtropicais, conclui-se que estas espécies nativas são importantes organismos-teste a serem utilizados em ensaios ecotoxicológicos para o monitoramento de toxicidade nas águas doces tropicais.

Palavras-chave: Cladocera, Ecotoxicologia, Atrazina Atanor 50 SC[®], pesticida.

1. Introduction

The number of synthetic chemical substances has greatly expanded over the last few decades. Large quantities of toxic chemicals are released into the environment, contaminating air, soil and water (Bonaventura and Johnson, 1997). Among the numerous chemicals that reach bodies of freshwater, pesticides have particularly serious effects on the aquatic biota and can lead to great losses in biodiversity (Grützmacher et al., 2008; Aguilar-Alberola and Mesquita-Joanes, 2012).

The increasing world population and corresponding demand for food has encouraged the expansion of agribusiness and motivated the use of large quantities of herbicides on crops, to decrease damage caused by weeds and thus boost productivity. The herbicide atrazine, which is an inhibitor of photosynthesis, is also an artificial compound classified as a potential endocrine disruptor that may act by stimulating the activity of the aromatase enzyme that converts testosterone to estrogen (Sanderson et al., 2000). In the tropics, owing to its relatively free mobility in the soil and the frequently torrential rains in the summer, most of the atrazine applied to crops is leached out and, consequently, residues are found in various environments, especially in surface water and groundwater (Gilliom et al., 2006; Wightwick and Allinson, 2007).

Cladocerans are an important component of the zooplankton. They consume phytoplankton and are, in turn, heavily preyed upon by larval fish (Dettmers and Stein, 1992). The use of cladocerans in toxicity tests has many advantages, including their ease of handling, the possibility of obtaining clones by parthenogenesis, their short life cycle and high sensitivity to toxicants. These characteristics make them suitable test organisms for bioassays (Adema, 1978; Trayler and Davis, 1996). The cladoceran most commonly used in aquatic ecotoxicology is undoubtedly *Daphnia magna* (Baillieul and Blust, 1999; Biesinger and Christensen, 1972; Lilius et al., 1995; Winner and Farrell, 1976). However, the natural geographical distribution of this species is restricted to areas of northern high and mid-latitudes (Mitchell et al., 2004). The use of such temperate species in tropical regions for ecotoxicological assessments seems inappropriate, since the toxic responses of organisms to chemicals are often species-specific.

Acute toxicity of the herbicide atrazine to several other species belonging to different taxonomical groups can be found in the literature in relation to

mortality, expressed as LC_{50} of atrazine: 17.1 mg L⁻¹ to the cladoceran *Pseudosida ramosa* (Freitas and Rocha, 2011); 33.0 mg L⁻¹ to the amphipod *Hyalella azteca* (Wan et al., 2006); 36.7 mg L⁻¹ to the anostracean *Thamnocephalus platyurus* (Palma et al., 2008); 18.9 mg L⁻¹ to the amphibian *Rana catesbeiana* (Taylor et al., 1991); over 480 mg L⁻¹ (Wan et al. 2006) and 48.0 mg L⁻¹ to the fish *Oncorhynchus kisutch* (Wan et al., 2006). In relation to the other parameters as growth inhibition after 72 hours of exposure a LC_{50} of 0.196 mg L⁻¹ of atrazine was found to the algae *Raphidocelis subcapitata*, according to Pérez et al. (2011). In relation to luminescence inhibition to the bacterium *Vibrio fischeri* Palma et al. (2008) obtained LC_{50} values of 69.4 mg L⁻¹.

With this study we intend to extend the knowledge particularly regarding the effects of atrazine upon non-target freshwater invertebrates, evaluating its acute toxicity effects on two Neotropical native species occurring in Brazil.

Thus, in the present study we used the cladocerans *Daphnia magna*, *Ceriodaphnia silvestrii* and *Macrothrix flabelligera* to test the acute effects of the commercial product Atrazine Atanor 50 SC[®], with the goal of determining the acute toxicity of atrazine to cladocerans and comparing the results obtained for *Daphnia magna* with those for species *Ceriodaphnia silvestrii* and *Macrothrix flabelligera* occurring in Brazilian freshwaters, leading to a discussion of the importance of using native species in ecotoxicological monitoring.

2. Material and Methods

2.1. Organism and culture conditions

The cladoceran *Daphnia magna* is an exotic planktonic microcrustacean widely distributed in the northern hemisphere, measuring 3 to 6 mm in length (ABNT, 2004; Koivisto, 1995). The species *Ceriodaphnia silvestrii* is a native planktonic microcrustacean, found in Brazil and Argentina, which reaches just 0.8 to 0.9 mm in length (Fonseca and Rocha, 2004) *Macrothrix flabelligera* is a non-planktonic microcrustacean isolated in Australia and Brazil, 1.0 mm long (Güntzel et al., 2004).

Ceriodaphnia silvestrii was originally isolated from Broa Reservoir, Brotas, SP. *Daphnia magna* was obtained from the Ecotoxicology Laboratory of the Center for Nuclear Energy in Agriculture (CENA), Piracicaba SP and kept in stock cultures for more than 5 years in the laboratory of Ecotoxicology at the Federal University of São Carlos. *Macrothrix*

flabelligera was isolated from experimental tanks of 10.000 L capacity, maintained at the Aquaculture Station of the Hydrobiology Department at the Federal University of São Carlos, São Carlos, SP.

The cultures of *D. magna* were kept in an incubator at a controlled temperature of $22 \pm 2^\circ\text{C}$ and with a 12h light: 12h dark photoperiod. While *C. silvestrii* and *M. flabelligera* were kept at $25 \pm 2^\circ\text{C}$, with a 16h light: 8h dark photoperiod.

C. silvestrii and *M. flabelligera* were cultured in reconstituted soft water, prepared according to ABNT (2005) with the following characteristics: pH 7.0 to 7.8, hardness between 40 and 48 mg $\text{CaCO}_3 \text{ L}^{-1}$ and electrical conductivity $160 \mu\text{S cm}^{-1}$. These two species were kept in 2L beakers holding up to 140 individuals.

D. magna was cultured in an artificial culture medium of high hardness (ASTM, 1980), as follows: pH 8.0 to 8.6 and hardness between 180 and 220 mg $\text{CaCO}_3 \text{ L}^{-1}$. This species was maintained in beakers of 2 L holding up to 50 individuals.

Cultures of *C. silvestrii* and *M. flabelligera* were fed with a suspension of the alga *Raphidocelis subcapitata*, grown in CHU-12 medium (Müller, 1972) to a final density of $1 \times 10^5 \text{ cells mL}^{-1}$, and a feed composed of yeast and fish meal (fermented in the ratio 1:1) at a concentration of 1 mL L^{-1} (ABNT, 2004). The *D. magna* cultures were fed as described for *C. silvestrii* and *M. flabelligera*, but with twice the quantity of algal suspension ($2 \times 10^5 \text{ cells mL}^{-1}$).

The cultures were renewed three times a week, when the water and the food were changed. The health and sensitivity of the test organisms were checked monthly, by performing acute toxicity tests with the reference substance sodium chloride for *C. silvestrii* and potassium dichromate for *D. magna* and *M. flabelligera*.

2.2. Acute toxicity tests

The atrazine herbicide used, Atanor 50 SC® (500 g/L), contained 50% of the active ingredient. This commercial product (soluble in water) was diluted directly in reconstituted water for the tests. The test procedure conformed to the Brazilian Standard Method (ABNT, 2004). The acute toxicity tests consisted in exposing 5 neonates, aged less than 24 hours, in replicate, to a series of nominal concentrations of atrazine (2.25; 4.5; 9.0; 18.0; 36 and 72 mg L^{-1} for *C. silvestrii* and *M. flabelligera* and 2.25; 4.5; 9.0; 18.0; 36; 72 and 144.0 mg L^{-1} for *D. magna*) in 10 ml test solution. The range of concentrations tested was established in a series of

preliminary tests. The concentrations were prepared immediately before the tests.

The toxicity tests lasted 48 hours and were performed in cell culture plates with 6 wells (TPP®). The control was reconstituted water. Four replicates were done for the control and for each tested concentration of atrazine. The experiments were kept at the same temperature as the stock cultures, without food or light. At the beginning of the tests, the variables pH, conductivity, temperature and hardness were measured in the test-solutions; these variables were measured again at the end of the test (except hardness) for those to which there was not a sufficient volume.

2.3. Data treatment and statistical analysis

After the period of exposure (48 hours), the motionless organisms were counted. If the percentage of motionless organisms in the control exceeded 10%, the test would be considered invalid; however, this did not occur in this experiment. The results were expressed as EC_{50} (48-h) – effective average concentration that causes an acute effect on 50% of the organisms in the exposure time (ABNT, 2004), with a confidence interval of 95%, using the statistical program Trimmed Spearman – Karber (Hamilton et al., 1977).

The sensitivity of the three cladoceran species exposed to the herbicide atrazine was evaluated from their performance in three test runs. The results were shown as box-plot graphs in which the upper and lower confidence limits approximately correspond to plus or minus two standard deviations for the mean EC_{50} 48-h values obtained.

3. Results

During the acute toxicity tests for *C. silvestrii* and *M. flabelligera*, the average pH measured in the test solutions remained within the range from 7.0 to 7.7 and did not vary by more than 1.0 unit in any test, the temperature varied between 24.3 and 25.1°C, the electrical conductivity between 152.6 and 159.2 $\mu\text{S cm}^{-1}$ and the water hardness between 40 and 48 mg $\text{CaCO}_3 \text{ L}^{-1}$. For the *D. magna* tests the pH value stayed within the range 8.1 to 8.5 and did not vary more than 0.5 unit during the test; the temperature varied between 21.6 and 22.9°C, the electrical conductivity 502 to 552 $\mu\text{S cm}^{-1}$ and the initial hardness varied from 180 to 188 mg $\text{CaCO}_3 \text{ L}^{-1}$. Thus, the toxicity tests fulfilled the validity criteria specified in the guidelines of the Brazilian Association of Technical Standards (ABNT, 2005) for *C. silvestrii* and of the Organization for

Economic Cooperation and Development (OECD, 2004) for *D. magna*. At the end of the tests, the mortality in the controls never exceeded 10%, as recommended in the OECD guidelines.

The values of EC_{50} - 48h and the respective 95% confidence intervals (CI), for the three species, of the commercial herbicide atrazine Atanor 50 SC[®] were calculated separately for each one of the three tests of each species. The EC_{50} data, CI and standard deviations are presented in Table 1. The sensitivity of *D. magna* was between 45.12 and 55.69 mg L⁻¹, with an average of 50.41 mg L⁻¹; of *C. silvestrii* between 11.20 and 17.39 mg L⁻¹, with an average of 14.30 mg L⁻¹, and of *M. flabelligera* between 7.02 and 17.71 mg L⁻¹, with an average of 12.37 mg L⁻¹ (Figure 1).

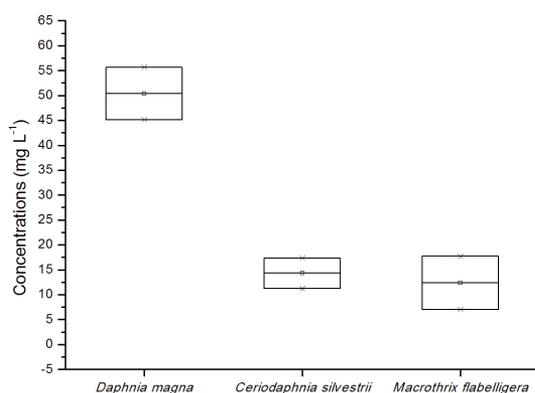


Figure 1. Box-plots representing mean EC_{50} - 48h sensitivity values \pm 2 standard deviations of *Daphnia magna*, *Ceriodaphnia silvestrii* and *Macrothrix flabelligera* to the atrazine Atanor 50 SC[®] based on three acute toxicity tests.

Table 1. Mean values for EC_{50} - 48h of atrazine and 95% confidence intervals (inside parenthesis) for the cladocerans *Ceriodaphnia silvestrii*, *Daphnia magna* and *Macrothrix flabelligera* based on the results of three tests performed plus the estimated mean and standard deviation.

Test Number	<i>Daphnia magna</i> (mg L ⁻¹)	<i>Ceriodaphnia silvestrii</i> (mg L ⁻¹)	<i>Macrothrix flabelligera</i> (mg L ⁻¹)
1	48.60 (36.13 - 65.39)	14.60 (11.69 - 18.24)	11.15 (8.18 - 15.20)
2	53.44 (35.31 - 80.88)	12.62 (10.33 - 15.43)	15.43 (11.96 - 19.91)
3	49.18 (38.40 - 62.99)	15.67 (12.60 - 19.49)	10.52 (8.06 - 13.73)
X \pm SD	50.41 \pm 2.64	14.30 \pm 1.55	12.37 \pm 2.67

Table 2. Acute toxicity values (EC_{50}) from the present study and from literature for some species of Cladocera exposed to atrazine.

Test organism	Parameters	EC_{50} (mg L ⁻¹)	Reference
<i>Ceriodaphnia dubia</i>	Immobilization - 48 h	14.6	Foster et al. (1998)
<i>Ceriodaphnia silvestrii</i>	Immobilization - 48 h	14.3	Present study
<i>Daphnia carinata</i>	Immobilization - 48 h	24.6	Phyu et al. (2004)
<i>Daphnia magna</i>	Immobilization - 48 h	50.4	Present study
	Immobilization - 48 h	35.5	Palma et al. (2008)
<i>Daphnia pulex</i>	Immobilization - 48 h	36.5	Hartman and Martin (1985)
<i>Macrothrix flabelligera</i>	Immobilization - 48 h	12.4	Present study

4. Discussion

According to the results, *M. flabelligera* and *C. silvestrii* showed greater sensitivity to the herbicide Atrazine Atanor SC[®] than *D. magna*, since the value of EC_{50} - 48h for *M. flabelligera* was fourfold and *C. silvestrii* threefold lower than that for *D. magna* (See Figure 1).

The values of EC_{50} - 48h of atrazine found in the literature for the cladocerans most often used as test-organisms in temperate regions were 24.6 mg L⁻¹ (Phyu et al., 2004), 26.9 mg L⁻¹ (Macek et al., 1976, cited in Freitas and Rocha, 2012) and 36.5 mg L⁻¹ (Hartman and Martin, 1985), for *Daphnia carinata*, *D. magna* and *D. pulex*, respectively. Thus, the values of EC_{50} - 48h for the native species (*Ceriodaphnia silvestrii* and *Macrothrix flabelligera*) are also lower than those reported for *Daphnia magna* as well as for other cladoceran species as can be observed in Table 2.

Freitas and Rocha (2011) tested the acute toxicity of atrazine (99% pure) on a species of tropical cladoceran *Pseudosida ramosa* and found LC_{50} to be 17.1 mg L⁻¹, also lower than the one recorded for the cladocerans usually used as test-organisms. However this value is also higher than the mean values recorded for the native species from our study, *Macrothrix flabelligera* (12.4 mg L⁻¹) and *Ceriodaphnia silvestrii* (14.3 mg L⁻¹).

Direct comparisons of LC_{50} or EC_{50} values measured with several different freshwater invertebrates are somewhat hard to interpret because laboratory culture conditions usually differ with

regard to photoperiod, temperature, exposure time and composition of culture media. In the case of atrazine, it is known that their toxicities can increase with increasing temperature (Folt et al., 1999; Mayasich et al., 1986). Therefore, differences in LC_{50} or EC_{50} values should be treated with caution when intrinsic differences in sensitivity are being compared among cladoceran species. Another important issue is the variation in the hardness of the reconstituted water used to culture the test species; thus, for *C. silvestrii* and *M. flabelligera*, the hardness was within the range stipulated in the ABNT (2005) guidelines for *C. silvestrii* (40 to 48 mg $CaCO_3$ L^{-1}), much lower than the value used for *D. magna* (180 to 220 mg $CaCO_3$ L^{-1}). Therefore, further studies of the sensitivity of *C. silvestrii* and *M. flabelligera* are needed with respect to variations in the hardness of the water; in particular testing with the same toxic compound, should be done at high hardness, close to the standard values recommended by OECD (2004) for *D. magna*.

It is expected that if it was possible to test both species at the same water hardness, their sensitivity would probably become closer, since increasing water hardness usually decreases toxicity (Aragão and Araújo, 2008). These tests would nevertheless require the previous and gradual adaptation of each species to a range of hardness that is outside of the optimum in their tolerance curve.

In this study, the objective was to compare the susceptibility of the cladoceran species under the test conditions recommended in the test protocols. When these protocols are used to establish the maximum allowed levels of substances in local water quality criteria, comparisons performed under local conditions are valid. For example, in the tropical countries temperatures that vary from 23 to 27°C are recommended for the toxicity tests, (ABNT, 2010), while in temperate countries, protocols recommend a temperature varying from 18 to 22°C (OECD 2004, 2008). The maximum allowed levels for atrazine in a tropical country, established with the use of a temperate cladoceran species protocol, could therefore underestimate the toxicity of this chemical product to the tropical biota. Table 2 displays the reported values of EC_{50} (mg L^{-1}) for cladocerans exposed to the herbicide atrazine. Comparing the EC_{50} - 48h values for atrazine obtained for *M. flabelligera* and *C. silvestrii* with those for other species, it can be seen that on the whole these two native species tested in the present study are more sensitive to atrazine than the cladoceran species tested in other studies.

The state of São Paulo is the greatest consumer of agrochemicals in Brazil (Armas et al., 2007). Over the years, triazines, and especially atrazine, have been used in great quantities in sugarcane plantations (Armas et al., 2005). In the sub-basin of the Corumbataí River (Piracicaba River basin), which encompasses a region of intensive sugarcane planting, atrazine concentrations that varied between 0.6 and 2.7 μg L^{-1} were found during the rainy summer (Armas et al., 2007). In Brazil, both the National Council for the Environment (CONAMA, 2005) and Ministry of Health– Brazil (2004) recommend a maximum level for atrazine of 2 μg L^{-1} in surface waters, in order to protect human health and aquatic life.

Although the actual concentrations found in Corumbataí River are above the maximum level permitted by law, they are about three to five orders of magnitude below the acute toxic level established in this study for *D. magna*, *C. silvestrii* and *M. flabelligera*. Apparently these species, in particular those occurring in Brazilian water bodies (*M. flabelligera* and *C. silvestrii*), would be protected from the acute effects of this herbicide. However, the indirect effects of atrazine on zooplankton organisms, such as alterations in the food sources should also be considered. As the primary mode of action of atrazine is the inhibition of photosynthesis, the direct effects of this compound in the water bodies occur mainly in the algal community (Dewey, 1986).

In many cases, native tropical species have been more sensitive to many compounds than many non-native temperate species, as it was observed for sodium and potassium by Freitas and Rocha, 2011 and for pesticides (diazinon and methyl parathion) and metals (chromium and mercury) by Do Hong et al. (2004). However, many water quality criteria in tropical regions are based on ecotoxicological tests with non-native species. This may lead to errors of interpretation when the maximum permitted levels for each particular toxic substance are established. To establish a realistic profile of the toxicity of the herbicide atrazine, we suggest that tests with a number of other native species belonging to zooplankton and also to other communities should be performed, using concentration ranges found in tropical freshwaters in order to get more realistic insights on the unintentional side-effects of herbicides on non-target freshwater microbiota.

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References

- ADEMA, DMM. 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia*, vol. 59, p. 125-134. <http://dx.doi.org/10.1007/BF00020773>
- AGUILAR-ALBEROLA, JA. and MESQUITA-JOANES, F. 2012. Acute Toxicity Tests with Cadmium, Lead, Sodium Dodecyl Sulfate, and *Bacillus thuringiensis* on a Temporary Pond Ostracod. *International Rev Hydrobiologia*, vol. 97, no. 4, p. 375-388. <http://dx.doi.org/10.1002/iroh.201211497>
- American Society for Testing and Materials - ASTM. 1980. *E-790-80*: Standard practice for conducting acute toxicity test with fishes, macroinvertebrates and amphibians. Philadelphia: ASTM.
- ARAGÃO, MA. and ARAÚJO, PA. 2008. Métodos de ensaio de toxicidade com organismos aquáticos. In ZAGATTO, PA. and BERTOLETTI, E., orgs. *Ecotoxicologia aquática: princípios e aplicações*. São Carlos: RiMa. p. 117-152.
- ARMAS, ED., ROSIM-MONTEIRO, RT., AMÂNCIO, AV., CORREA, RML. and GUERCIO, MA. 2005. Uso de agrotóxicos em cana-de-açúcar na bacia do Rio Corumbataí e o risco de poluição hídrica. *Química Nova*, vol. 28, no. 6, p. 975-982. <http://dx.doi.org/10.1590/S0100-40422005000600008>
- ARMAS, ED., ROSIM-MONTEIRO, RT., ANTUNES, PM., SANTOS, MAPF., CAMARGO, PB. and ABAKERLI, RB. 2007. Diagnóstico espaço-temporal da ocorrência de herbicidas nas águas superficiais e sedimentos do Rio Corumbataí e principais afluentes. *Química Nova*, vol. 30, no. 5, p. 1119-1127. <http://dx.doi.org/10.1590/S0100-40422007000500013>
- Associação Brasileira de Normas Técnicas - ABNT. 2004. *NBR 12713*. Ecotoxicologia aquática - Toxicidade Aguda - Método de ensaio com *Daphnia* spp. (Cladocera, Crustacea). Rio de Janeiro: ABNT. p. 21.
- Associação Brasileira de Normas Técnicas - ABNT. 2005. *NBR 13373*: Ecotoxicologia aquática - Toxicidade crônica - Método de ensaio com *Ceriodaphnia* spp. (Crustacea, Cladocera). Rio de Janeiro: ABNT. p. 15.
- Associação Brasileira de Normas Técnicas - ABNT. 2010. *NBR 13373*: Ecotoxicologia aquática-Toxicidade crônica-Método de ensaio com *Ceriodaphnia* spp (Crustacea, Cladocera). Rio de Janeiro: ABNT. p. 18.
- BAILLIEUL, M. and BLUST, R. 1999. Analysis of the swimming velocity of cadmium-stressed *Daphnia magna*. *Aquatic Toxicology*, vol. 44, p. 245-254. [http://dx.doi.org/10.1016/S0166-445X\(98\)00080-0](http://dx.doi.org/10.1016/S0166-445X(98)00080-0)
- BIESINGER, KE. and CHRISTENSEN, GM. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. *Journal of the Fisheries Research Board of Canada*, vol. 29, no. 12, p. 1691-1700. <http://dx.doi.org/10.1139/f72-269>
- BONAVENTURA, C. and JOHNSON, FM. 1997. Healthy environments for healthy people: bioremediation today and tomorrow. *Environmental Health Perspectives*, vol. 105, no. 1, p. 5-20. PMID:9114274 PMCID:PMC1470315.
- Brazil. Ministry of Health. 2004. Decree nº 518. Lays down procedures and responsibilities for the control and monitoring of the quality of water for human consumption and its standard of drinkability, and related matters. *Diário Oficial da República Federativa do Brasil*.
- Conselho Nacional do Meio Ambiente - CONAMA. 2005. CONAMA resolution 357. Pronounces on the classification of water bodies and environmental directives in their respect, and also lays down conditions and Standards of effluent discharge, and other related questions. *Diário Oficial da República Federativa do Brasil*, mar.
- DETTMERS, JM. and STEIN, RA. 1992. Food consumption by larval gizzard shad: zooplankton effects and implications for reservoir communities. *Transactions of the American Fisheries Society*, vol. 121, p. 494-507. [http://dx.doi.org/10.1577/1548-8659\(1992\)121<0494:FCBLGS>2.3.CO;2](http://dx.doi.org/10.1577/1548-8659(1992)121<0494:FCBLGS>2.3.CO;2)
- DEWEY, SL. 1986. Effects of the herbicide atrazine on aquatic insect community structure and emergence. *Ecology*, vol. 67, no. 1, p. 148-162. <http://dx.doi.org/10.2307/1938513>
- DO HONG, LC., BECKER-VAN SLOOTEN, K. and TARRADELLAS, J. 2004. Tropical ecotoxicity testing with *Ceriodaphnia cornuta*. *Environmental Toxicology*, vol. 19, p. 497-504. PMID:15352266. <http://dx.doi.org/10.1002/tox.20055>
- FOLT, CL., CHEN, CY., MOORE, MV. and BURNAFORD, J. 1999. Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, vol. 44, p. 864-877. http://dx.doi.org/10.4319/lo.1999.44.3_part_2.0864
- FONSECA, AL. and ROCHA, O. 2004. The life cycle of *Ceriodaphnia silvestrii* Daday, 1902, a Neotropical endemic species (Crustacea, Cladocera, Daphnidae). *Acta Limnologica Brasiliensis*, vol. 16, p. 319-328.
- FOSTER, S., THOMAS, M. and KORTH, W. 1998. Laboratory-derived acute toxicity of selected pesticides to *Ceriodaphnia dubia*. *Australasian Journal of Ecotoxicology*, vol. 4, p. 53-59.
- FREITAS, EC. and ROCHA, O. 2011. Acute toxicity tests with the tropical cladoceran *Pseudosida ramosa*:

- the importance of using native species as test organisms. *Archives of Environmental Contamination and Toxicology*, vol. 60, p. 241-249. PMID:20464548. <http://dx.doi.org/10.1007/s00244-010-9541-2>
- FREITAS, EC. and ROCHA, O. 2012. Acute and chronic effects of atrazine and sodium dodecyl sulfate on the tropical freshwater cladoceran *Pseudosida ramosa*. *Ecotoxicology*, vol. 21, p. 1347-1357. PMID:22434152. <http://dx.doi.org/10.1007/s10646-012-0888-1>
- GILLIOM, RJ., BARBASH, JE., CRAWFORD, CG., HAMILTON, PA., MARTIN, JD., NAKAGAKI, N., NOWELL, LH., SCOTT, JC., STACKELBERG, PE., THELIN, GP. and WOLOCK, DM., 2006. *The quality of our nation's waters. Pesticides in the nation's streams and ground water, 1992-2001*. Reston: US Geological Survey.
- GRÜTZMACHER, DD., GRÜTZMACHER, AD., AGOSTINETTO, D., LOECK, AE., ROMAN, R., PEIXOTO, SC. and ZANELLA, R., 2008. Monitoramento de agrotóxicos em dois mananciais hídricos no sul do Brasil. *Revista Brasileira de Engenharia Agrícola e Ambiental*, vol. 12, no. 6, p. 632-637. <http://dx.doi.org/10.1590/S1415-43662008000600010>
- GÜNTZEL, AM., MATSUMURA-TUNDISI, T. and ROCHA, O. 2004. *Macrothrix flabelligera*, a newly-recorded Cladocera Macrothricidae in Brazilian freshwaters. *Brazilian Journal of Biology*, vol. 64, no. 2, p. 221-226. PMID:15462294. <http://dx.doi.org/10.1590/S1519-69842004000200006>
- HAMILTON, MA., RUSSO, RC. and THURFTON, RB. 1977. Trimmed Spearman-Kärber method for estimating median lethal concentration in toxicity bioassays. *Environmental Science and Technology*, v. 11, no. 7, p. 714-719. <http://dx.doi.org/10.1021/es60130a004>
- HARTMAN, NK. and MARTIN, DB. 1985. Effects of four agricultural pesticides on *Daphnia pulex*, *Lemma minor* and *Potamogeton pectinatus*. *Bulletin of Environmental Contamination and Toxicology*, vol. 5, p. 646-651.
- KOIVISTO, S. 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests? *Environmental Pollution*, vol. 90, no. 2, p. 263-267. [http://dx.doi.org/10.1016/0269-7491\(95\)00029-Q](http://dx.doi.org/10.1016/0269-7491(95)00029-Q)
- LILIUS, H., HASTBACKA, T. and ISOMAA, B. 1995. A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. *Environmental Toxicology and Chemistry*, vol. 14, p. 2085-2088.
- MAYASICH, JM., KARLANDER, EP. and TERLIZZI JUNIOR, DE. 1986. Growth responses of *Nannochloris oculata* Droop and *Phaeodactylum tricornutum* Bohlin to the herbicide atrazine as influenced by light intensity and temperature. *Aquatic Toxicology*, vol. 8, p. 175-184. [http://dx.doi.org/10.1016/0166-445X\(86\)90063-9](http://dx.doi.org/10.1016/0166-445X(86)90063-9)
- MITCHELL, SE., HALVES, J. and LAMPERT, W. 2004. Coexistence of similar genotypes of *Daphnia magna* in intermittent populations: response to thermal stress. *Oikos*, vol. 106, no. 3, p. 469-478. <http://dx.doi.org/10.1111/j.0030-1299.2004.13113.x>
- MÜLLER, H. 1972. Wachstum und phosphatbedarf von *Nitzschia actinastroides* (Lemn.) v. Goor in statischer und homokontinuierlicher kultur unter phosphatlimitierung. *Archiv für Hydrobiologie Supplement*, vol. 38, p. 399-484.
- Organization for Economic Cooperation and Development - OECD. 2004. *Guideline for testing of chemicals. Daphnia sp., acute immobilization test*. OECD 202. Paris: OECD.
- Organization for Economic Cooperation and Development - OECD. 2008. *Guidelines for testing of chemicals. Daphnia magna reproduction test*. OECD 211. Paris OECD.
- PALMA, P., PALMA, VL., FERNANDES, RM., SOARES, AMVM. and BARBOSA, IR. 2008. Acute toxicity of atrazine, endosulfan sulphate and chlorpyrifos to *Vibrio fischeri*, *Thamnocephalus platyurus* and *Daphnia magna*, relative to their concentrations in surface waters from the Alentejo region of Portugal. *Bulletin of Environmental Contamination and Toxicology*, vol. 81, no. 5, p. 485-489. PMID:18777155. <http://dx.doi.org/10.1007/s00128-008-9517-3>
- PÉREZ, J., DOMINGUES, I., SOARES, AM. and LOUREIRO, S. 2011. Growth rate of *Pseudokirchneriella subcapitata* exposed to herbicides found in surface waters in the Alqueva reservoir (Portugal): a bottom-up approach using binary mixtures. *Ecotoxicology*, vol. 20, no. 6, p. 1167-1175. PMID:21448621. <http://dx.doi.org/10.1007/s10646-011-0661-x>
- PHYU, YL., WARNE, MJ. and LIM, RP. 2004. Toxicity of atrazine and molinate to the cladoceran *Daphnia carinata* and the effect of river water and bottom sediment on their bioavailability. *Archives of Environmental Contamination and Toxicology*, vol. 46, p. 308-315. PMID:15195802.
- SANDERSON, JT., SEINEN, W., GIESY, JP. and VAN DEN BERG, M. 2000. 2-chloro- S-triazine herbicides induce aromatase (CYP-19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity. *Toxicological Sciences*, vol. 54, p. 121-127.
- TAYLOR, EJ., MAUND, SJ. and PASCOE, D. 1991. Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta:Diptera) and *Gammarus pulex* (L.) (Crustacea: Amphipoda). *Archives of Environmental Contamination and Toxicology*, vol. 21, no. 3, p. 371-6. PMID:1719943. <http://dx.doi.org/10.1007/BF01060358>

- TRAYLER, KM. and DAVIS, JA. 1996. Sensitivity of *Daphnia carinata* sensu lato to the insect growth regulator, pyriproxyfen. *Ecotoxicology and Environmental Safety*, vol. 33, no. 2, p. 154-156. PMID:8723752. <http://dx.doi.org/10.1006/eesa.1996.0019>
- WAN, MT., BUDAY, C., SCHROEDER, G., KUO, J. and PASTERNAK, J. 2006. Toxicity to *Daphnia magna*, *Hyalella azteca*, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, *Oncorhynchus tshawytscha*, and *Rana catesbeiana* of Atrazine, Metolachlor, Simazine, and Their Formulated Products. *Bulletin of Environmental Contamination and Toxicology*, vol. 76, p. 52-58. PMID:16404660. <http://dx.doi.org/10.1007/s00128-005-0888-4>
- WIGHTWICK, A. and ALLINSON, G. 2007. Pesticide residues in Victorian waterways: a review. *Australasian Journal of Ecotoxicology*, vol. 3, p. 91-112.
- WINNER, RW. and FARREL, MP. 1976. Acute and Chronic Toxicity of Copper to four Species of *Daphnia*. *Journal of the Fisheries Research Board of Canada*, vol. 33, p. 1685-1691. <http://dx.doi.org/10.1139/f76-215>

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