

Salomão José Cohin de Pinho

Estudo da viabilidade do cladóceros tropical  
*Macrothrix elegans* Sars, 1901 (Cladocera,  
Macrothricidae) como organismo-teste em  
ensaios ecotoxicológicos.

Salvador  
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Orientador: Prof. Dr. Eduardo Mendes  
da Silva.

Co-orientadora: Dr<sup>a</sup>. Carla Chastinet

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Dedico este trabalho à minha  
esposa, amiga e companheira,  
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## 1 **Introdução geral**

2

3 A água é fundamental para o desenvolvimento humano, entretanto, a sua qualidade pode ser  
4 influenciada por muitas das atividades humanas (industrial, agro-pastoril, urbanização),  
5 perturbando a condição biológica de ecossistemas aquáticos e sendo prejudicial para a saúde  
6 humana (Primack & Rodrigues, 2002; Durusoy & Kambur, 2003). A degradação na qualidade da  
7 água gera incertezas em relação ao custo-benefício desses avanços, devido à criação de novas  
8 substâncias químicas lançadas no ambiente a cada dia, sem sequer saber quais efeitos essas  
9 substâncias terão no ambiente e no ser humano (Esteves, 1998; Espíndola *et al.*, 2000; Knie &  
10 Lopes, 2004).

11

12 O desenvolvimento da sociedade atual veio seguido de uma série de dependências, trazendo, em  
13 conjunto, efeitos diretos e indiretos ao meio ambiente e ao próprio ser humano, muitos deles  
14 irreversíveis, em todo o planeta (Moss,1980; Esteves, 1998; Espíndola *et al.*, 2000; Chastinet,  
15 2002; Knie & Lopes, 2004).

16

17 Algumas das formas mais comuns de degradação deste ecossistema são os despejos de efluentes  
18 em rios, lançamento de esgoto tratado de forma ineficiente, dragagem, construções de  
19 hidrelétricas e a mineração, que causa a liberação de metais para o ambiente (Lewis et al., 1999).

20

21 Cada vez mais, o homem sofre as conseqüências do uso inadequado dos recursos naturais, sendo  
22 que um dos mais preocupantes atualmente é a contaminação da água por metais, que além de  
23 causar graves doenças, também afeta de forma geral os organismos aquáticos, reduzindo a  
24 capacidade dessas comunidades de se reproduzir e manter a espécie no local, gerando um grave  
25 desequilíbrio (Krane,1999; Brooks, 2004).

1  
2 Atualmente, uma ferramenta muito utilizada para avaliar os riscos e perigos que o ambiente corre  
3 é a análises de risco ecológico (ARE) (CEO, 2003), onde a integração dessa ARE à avaliação de  
4 risco à saúde humana segue como a abordagem mais atual e poderosa (Pereira *et al.*, 2004). Esta  
5 avaliação ocorre em três etapas, (i) a identificação e formulação do problema que gera dois  
6 produtos (i.i) determinação dos *endpoints* (alvos fisiológicos) e (i.ii) modelos conceituais), (ii) a  
7 fase de análise e (iii) a fase de caracterização do risco (U.S. EPA, 1998).

8  
9 A utilização de espécies de alta relevância ecológica, representatividade e função ecológica, para  
10 esses ecossistemas, possibilita à Ecotoxicologia tropical maior autonomia, deixando de ser  
11 apenas uma extensão da Ecotoxicologia desenvolvida nos países temperados, visto que são  
12 ecossistemas com características bem diferenciadas (Ricklefs, 2003; Zagatto & Bertoletti 2006).

13  
14 Atualmente, existe uma grande preocupação em relação a estes impactos no ambiente aquático,  
15 fazendo com que uma série de medidas sejam tomadas, dentre elas resoluções  
16 (CONAMA357/05, por exemplo) e normas visando proteger e preservar este ambiente  
17 (Espíndola *et al.*, 2000; Knie & Lopes, 2004). No entanto, para as regiões de clima tropical, em  
18 termos de ensaios ecotoxicológicos, ainda existe uma dependência muito grande dos países de  
19 clima temperado, principalmente no Brasil, fazendo uso de espécies exóticas, reduzindo a  
20 relevância ecológica dos resultados obtidos (Zagatto & Bertoletti 2006). Sendo assim, é de suma  
21 importância o estudo de organismos que sejam representativos e endêmicos de regiões com  
22 clima tropical. O presente trabalho visa avaliar a viabilidade do cladóceros tropical *Macrothrix*  
23 *elegans* como potencial organismo-teste em ensaios ecotoxicológicos em ambientes tropicais.

24



1 **Conclusão geral**

2

3 Após todos os experimentos com as substâncias de referência, *M. elegans* demonstrou ser  
4 sensível a ambas, apresentando um coeficiente de variação baixo, estando este apto a ser  
5 utilizado como organismo-teste para ambientes contaminados por cádmio.

6

7 *M. elegans* demonstrou ter um grande potencial para ser utilizado em ensaios ecotoxicológicos  
8 dos mais variados tipos, mesmo quando comparado outros cladóceros com ensaios já  
9 padronizados. No entanto, ainda é necessário que sejam realizados mais investigações para que  
10 este cladóceros possa ser incluído em normas de padronização.

11

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17

18

1 **MACROTHRIX ELEGANS SARS, 1901 (CLADOCERA, MACROTHRICIDAE): A**  
2 **VIALE ALTERNATIVE FOR ECOTOXICOLOGICAL TESTS IN TROPICAL**  
3 **FRESHWATER**

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36

## Abstract

The current study investigates the suitability of a tropical cladoceran species, *Macrothrix elegans* Sars (1901) for toxicity testing in ecotoxicological tests at 25°C involving two reference substances, cadmium chloride (CdCl<sub>2</sub>) both in natural and artificial media, and potassium chloride (KCl) in natural medium. The work provides a comparison among other cladoceran species from tropical and temperate environments, and discusses the potential of *M. elegans* as a bioindicator of stress in tropical waters. An experiment on the animal's life-span has been included to assess its reproductive potential. On average, at 25°C, *M. elegans* lives 38.2 ± 4.4 days and each female produces 211.8±30.4 neonates. The criterion of toxicity adopted in the bioassays was mortality/immobility using neonates up to 24-h old. The CdCl<sub>2</sub> 48-h EC<sub>50</sub> mean values and their confidence intervals were 0.035 mg.l<sup>-1</sup> [0.020-0.050 mg.l<sup>-1</sup>] in natural medium, and 0.018 mg.l<sup>-1</sup> [0.012-0.024 mg.l<sup>-1</sup>] in the artificial medium. The KCl 48-h EC<sub>50</sub> mean value (natural medium only) was 749.08 mg.l<sup>-1</sup> [625.32-872.76 mg.l<sup>-1</sup>]. The work demonstrates the great potential of *M. elegans* as a test organism in ecotoxicological tests involving these substances in tropical freshwater ecosystems.

**KEY WORDS:** Cladoceran; Life-table; Ecotoxicological tests; EC<sub>50</sub>; Cadmium chloride; Potassium chloride.

## 1 **1- INTRODUCTION**

2

3 Aquatic ecosystems present high biodiversity in comparison to terrestrial ecosystems (Ricklefs,  
4 2004), however due to the attraction human settlements feel for water resources, aquatic  
5 ecosystems are severely endangered, as far as biodiversity is concerned (Moss,1980; Esteves,  
6 1998; Espíndola et al., 2000; Chastinet, 2002; Knie & Lopes, 2004). Loss of biodiversity is  
7 mainly caused by habitat reduction, alteration or destruction, introduction of foreign species and  
8 pollution (Primack & Rodrigues, 2002; Ricklefs, 2004). As a result of industrial discharges in  
9 water bodies, many aquatic organisms are frequently exposed to complex mixtures (Brooks et  
10 al., 2004), sometimes of unknown composition (Knie & Lopes, 2004), that may reduce the  
11 natural ability of populations to remain adaptable and productive in their ecosystems (Krane et  
12 al., 1999; da Silva et al., 2000).

13

14 A variety of test organisms and endpoints have been used to assess the relative toxicity of a  
15 certain pollutant or mixture (Rand *et al.*, 1995). Mortality is usually the most applied response  
16 criterion and acute tests are still of great importance for legal reasons to control the emission of  
17 toxic substances in the environment (Moss, 1980; Abel & Axiak, 1991), in spite of its low  
18 ecological relevance (Gray, 1989; Lacher Jr. & Goldstein, 1997). Invertebrates have been largely  
19 used in the assessment of pollutant-induced stress in aquatic ecosystems (Oliveira-Neto & Botta-  
20 Paschoal, 2002; Sarma *et al.*, 2005). In addition to their biological and genetic characteristics and  
21 pivotal position in the food chain, these organisms are also sensitive to a variety of pollutants  
22 normally found in aquatic environments (Lagadic & Caquet 1998; Kast-Hutchenson *et al.*, 2001;  
23 Chastinet, 2002). Among aquatic invertebrates, cladocerans play an important role in the  
24 ecotoxicological assessment of chemicals in freshwater ecosystems, as they are easily  
25 maintained in laboratory at low costs, showing also a low genetic variability in laboratory

1 cultures (parthenogenesis), and are susceptible to a variety of toxic chemicals (Buikema &  
2 Cairns, 1980; Maciorowski & Clarke, 1980). Moreover, cladocerans represent an important link  
3 for the transfer of energy between primary producers and the secondary and tertiary consumers  
4 (Moss, 1980; Sarma *et al.*, 2005). According to Gray (1989) and Chapman (2002b) the choice of  
5 an organism for using in ecotoxicological tests should be based on ecological parameters,  
6 considering that different species may exhibit different levels of tolerance to a pollutant, and that  
7 tolerance may also vary among individuals of the same species. Ecologically speaking, this is of  
8 extreme importance, as several ecotoxicological tests using aquatic temperate organisms, such as  
9 *Daphnia magna* and *Ceriodaphnia dubia* are largely used in the tropics. (CETESB, 1998;  
10 ABNT, 2005; Beatrici, *et al.*, 2006).

11  
12 Tropical and temperate ecosystems differ in many aspects. Generally, tropical ecosystems  
13 present specific challenges for research and conservation due to their inherent ecological  
14 characteristics (Lacher & Goldstein, 1997). Variations in parameters such as luminosity,  
15 temperature, food quality, competition and predation, together with the complex trophic  
16 relationships among organisms (Ricklefs, 2004) are essentially important to understand the  
17 natural restrictions of ecotoxicological tests in tropical aquatic ecosystems. The strategies of life  
18 adopted by tropical and temperate cladocerans suffer direct influence of biotic factors (predation,  
19 intra- and inter- specific competition) and abiotic (temperature, luminosity, and oxygen  
20 saturation), although temperature seems to be the most significant factor (Sarma, 2005). Despite  
21 the tropics shelter about 75% of the biodiversity on the planet, and are under process of  
22 economical, social, urban, and industrial expansion thus, naturally requiring more ecological  
23 attention, the largest amount of ecotoxicological tests, however, have been carried out in  
24 temperate climate (Lacher Jr. & Goldstein, 1997). In this way, it may be unsuitable to apply a  
25 standardised bioassay with an exotic species in detriment of finding a local and sensitive species,

1 which would certainly provide more realistic and ecologically relevant results (Gray, 1989).  
2 Therefore, it is necessary the standardisation of ecotoxicological tests using adequate tropical  
3 organisms (Gray, 1989; Zagatto & Bertolotti 2006). On the other hand, legal instruments require  
4 the attainment of ecotoxicological tests standardised by institutions internationally recognised. In  
5 this sense, there is a great demand towards the development of tests involving tropical  
6 organisms.

7  
8 The present work examines the biology, laboratory cultures, and the suitability of the tropical  
9 cladoceran *Macrothrix elegans* Sars (1901) in ecotoxicological tests. The study aims to  
10 determine the potential of this animal as a test organism investigating its sensitiveness to two  
11 reference substances (cadmium chloride and potassium chloride) tested in natural and artificial  
12 media, thus demonstrating its viability as a test-organism of pollution in tropical freshwater  
13 ecosystems.

14 |



## 1 2- MATERIALS AND METHODS

2

3 *M. elegans* was collected from a dam at the Capivari Creek (12°38'24"S; 39°04'25"W) in a  
4 district of Cruz das Almas (Ba, Brasil). The animals have been cultured in the laboratory since  
5 year 2000 and maintained in water from the Capivari Creek (Andrade, 2003) until 2004.  
6 Thereafter, *M. elegans* has been cultured in water from the Jauá Lake (12°49'12"S; 38°13'11"W)  
7 located in (Camaçari, Ba) which has been used as a reference water in other studies (e.g. da Silva  
8 *et al.*, 2000; Abdon, 2005; Araújo *et al.*, 2006) thus, justifying its choice. The water was  
9 collected monthly, left in the dark to age for about one month, and filtered through a 10- $\mu$ m net  
10 before use.

11

12 In the laboratory, *M. elegans* organisms were maintained in 600-ml glass vessels containing 450  
13 ml of medium. The temperature was set at 25°C $\pm$ 1°C along with a photoperiod of 16:8 h (light:  
14 dark). Approximately 50 individuals were placed in each vessel, feeding (daily) on an algae  
15 (*Pseudokirchneriella subcaptata*) suspension with a final density of 10<sup>6</sup> cells ml<sup>-1</sup>.day<sup>-1</sup>. Filtered  
16 water from Jauá Lake (no adjustments) was used as natural medium. The ASTM hard water  
17 medium (ASTM, 1980) was diluted at 50% in ultra pure water (USFilter) to obtain the artificial  
18 medium (1:1). Once a day, all neonates were removed from each culture flask, until the sixth  
19 brood, when the adults were then discarded, and new cultures of *M. elegans* started from  
20 neonates of the sixth generation using the procedure of Chastinet (2002) slightly modified. Every  
21 three-day, all animals were transferred to a clean flask holding fresh medium and algal  
22 suspension at the pre-established density of cells.

23

24 A life-table experiment was carried out to examine biological and reproductive aspects of *M.*  
25 *elegans* (Shrivastava *et al.*, 1999). A single neonate (up to 24-h old) was placed individually in

1 each of the 11 150-ml glass jars each holding 80 ml of the natural medium (filtered water from  
2 Jauá Lake). The number of neonates produced per adult day<sup>-1</sup> was counted, and the longevity of  
3 each animal was recorded. These data was then used to build a life-table. The animals were then  
4 submitted to the same experimental conditions of temperature, photoperiod, and feeding  
5 described above for the stock cultures. The neonates were removed and counted daily, and the  
6 number of broods per individual recorded. The trial was ended after 44 days following the death  
7 of the last *M. elegans* individual.

8  
9 Ultra pure water (USFilter) was used for dilution throughout the experiments, excepting for  
10 those involving natural medium. The *Daphnia similis* standard bioassay (ABNT, 1993) and the  
11 work by Araújo (2005) with some modifications were used as reference in all ecotoxicological  
12 tests and only animals up to 24-h old were used for testing. A CdCl<sub>2</sub>-stock solution at 80 mg l<sup>-1</sup>  
13 and 10 g l<sup>-1</sup> for KCl was prepared and kept at 4°C until required. All tests were carried out in 50-  
14 ml glass flasks containing 40 ml of the test solution. In all 11 tests, four replicates, each holding  
15 five animals were used per each of the six concentrations tested per assay. Experimental controls  
16 (four replicates each) were set to account for the quality of the natural and artificial media. All  
17 tests were performed with neonates of daily controlled broods obtained from the stock cultures.  
18 The cadmium 48-h EC<sub>50</sub> for *M. elegans* was determined in eleven tests for animals tested in  
19 natural medium (N=11) and in seven tests for animals in the artificial medium (N=7). The KCl  
20 48-h EC<sub>50</sub> was determined in nine tests only in natural medium (N=9). The pH and conductivity  
21 | in the test vessels were measured at the beginning of each assay. Mortality/immobility was the  
22 | endpoint adopted in all tests and under these conditions, the dissolved oxygen was not a limiting  
23 | factor for testing (Abdon, 2005). The tests lasted for a 48-h period when the number of living  
24 | organisms were recorded. Animals immobilised for a period of at least 15 consecutive seconds  
25 | were assumed as dead.

1  
2 EC<sub>50</sub> mean values and their 95% confidence limits were calculated using the Probit analysis.  
3 Significant statistic differences among CdCl<sub>2</sub> and KCl 48-h EC<sub>50</sub> values obtained in this study  
4 for *M. elegans* and values for other organisms in comparison with *M. elegans*, were determined  
5 applying the Student *t*-test (Zar, 1996). The coefficients of variation were also calculated for the  
6 reference substances to evaluate the precision level of the results (Zagatto & Bertolotti, 2006).

7

1 **3- RESULTS AND DISCUSSION**

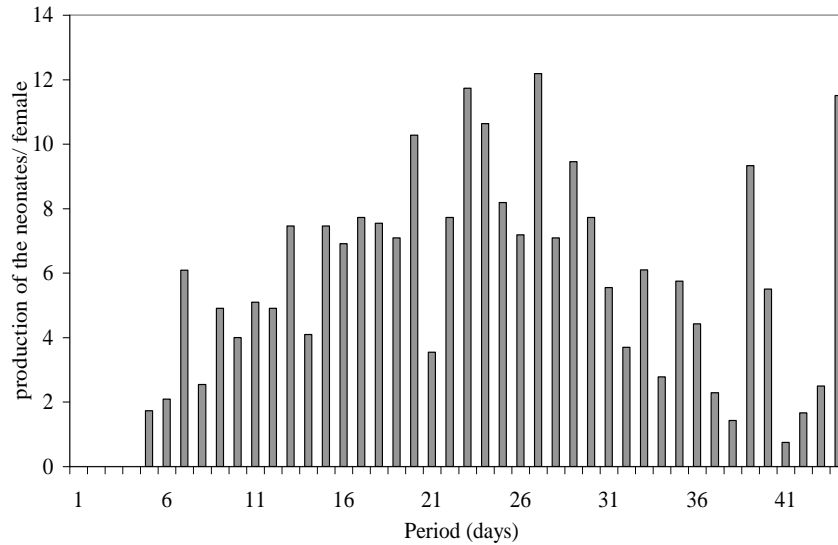
2

3 Tests aiming to define adequate species of cladocerans to assess pollution in tropical freshwater  
4 ecosystems are still in a crescent demand. At the moment, only a test with *Ceriodaphnia*  
5 *silvestrii* has been included in the Brazilian norm (NBR 13713, 2005).

6

7 *M. elegans* is a small tropical cladoceran which the total body length ranges from 605 to 1015  
8  $\mu\text{m}$  (Kotov, 2004). Hence, taking into account the epibenthic behaviour of *M. elegans*, these  
9 animals may be potentially used in a larger range of ecotoxicological tests than daphnids, for  
10 example, which the habits are naturally restricted to the water column. In general, these animals  
11 are associated with the macrophytes of the margins of lakes and rivers, occurring sporadically as  
12 planktonic (Elmoor-Loureiro, 1997). At laboratory conditions, *M. elegans* presented in natural  
13 medium an average life-span of  $38.2 \pm 4.4$  days with a maximum longevity of 44 days, and  
14 primiparous between the 5<sup>th</sup> and 6<sup>th</sup> day of life (Figure 1). On average, each female produced  
15  $211.8 \pm 30.4$  neonates, and the maximum number of neonates per female was 262 thus, showing  
16 the high reproductive capacity of *M. elegans* in 44 days. In addition, this tropical cladoceran  
17 presents: (i) parthenogenetic reproduction allowing small genetic variation among individuals,  
18 (ii) large number of neonates per brood, (iii) short life cycle, (iv) the cultures demand small  
19 areas, and (v) the animals are easily maintained in the laboratory at low costs. These  
20 characteristics are usually some of the requirements for the use of particular specie in  
21 ecotoxicological tests (Buikema & Cairns, 1980; Gray, 1989).

22

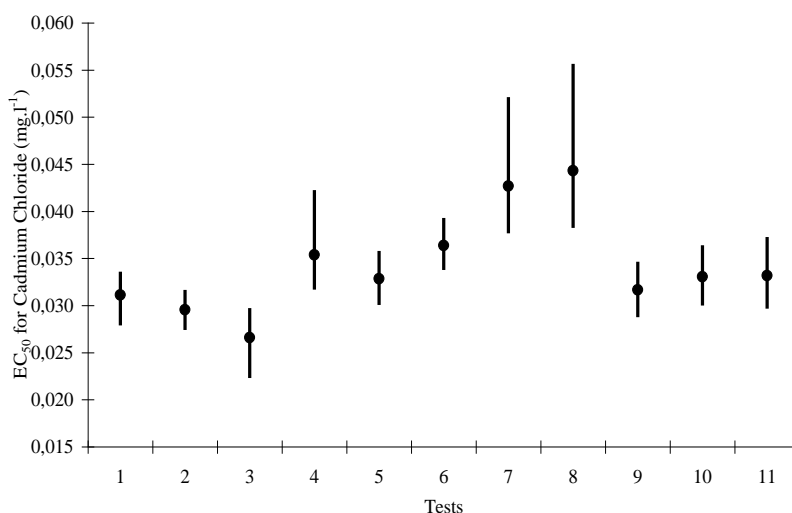


1  
 2 Figura 1: Average of neonates production for female along a life cycle of *M. elegans* in natural  
 3 medium.

4  
 5 *M. elegans* is a common neotropical species, widely distributed from Argentina to Mexico,  
 6 normally found in lakes and ponds (Kotov *et al.*, 2004). Araújo (2005) employed *M. elegans* to  
 7 monitor a metal-contaminated acidified pond (pH 3.1) in a district of Camaçari (Ba, Brazil),  
 8 comparing with results from fingerlings of *Poecilia reticulata* Peters (1859). The cladoceran  
 9 bioassay produced excellent results and there was no significant statistic difference between the  
 10 sensitivity levels obtained for *P. reticulata* and *M. elegans*. Saro *et al.* (2006) studied the same  
 11 pond carrying out an *in situ* microcosm test to compare the re-colonisation ability of *M. elegans*  
 12 in relation to three other tropical cladocerans (*Ceriodaphnia silvestrii*, *C. cornuta* and *Latonopsis*  
 13 *australis*). The authors verified that *M. elegans* was able to re-colonise the studied area in a more  
 14 efficient way than the other three species, thus showing its ability as a suitable biomonitor.

15  
 16 The mean value obtained for the cadmium 48-h EC<sub>50</sub> test with *M. elegans* in natural medium was  
 17 0.035 mg.l<sup>-1</sup> [0.020-0.050 mg.l<sup>-1</sup>] (Figure 2). The pH and conductivity varied from 6.45 to 7.25  
 18 and from 100 to 250 μS.cm<sup>-1</sup>, respectively. In general, the water quality of a natural medium is

1 more susceptible to alterations than that of an artificial medium, which is produced and  
2 maintained under laboratory controlled conditions. Therefore, the use of natural media in  
3 ecotoxicological tests should be carefully thought about (Knie & Lopes, 2004). Nevertheless, the  
4 coefficient of variation in the tests using natural medium was only 15.4%, demonstrating a  
5 negligible interference of the medium and a good reproducibility of results. Abdon (2005) used  
6 the same natural medium, laboratory conditions, and general procedures to assess the toxicity of  
7 cadmium ( $\text{CdCl}_2$ ) to the tropical cladoceran *C. cornuta* obtaining a 48-h  $\text{EC}_{50}$  value at 0.103  
8  $\text{mg.l}^{-1}$  [0.030-0.141  $\text{mg.l}^{-1}$ ].

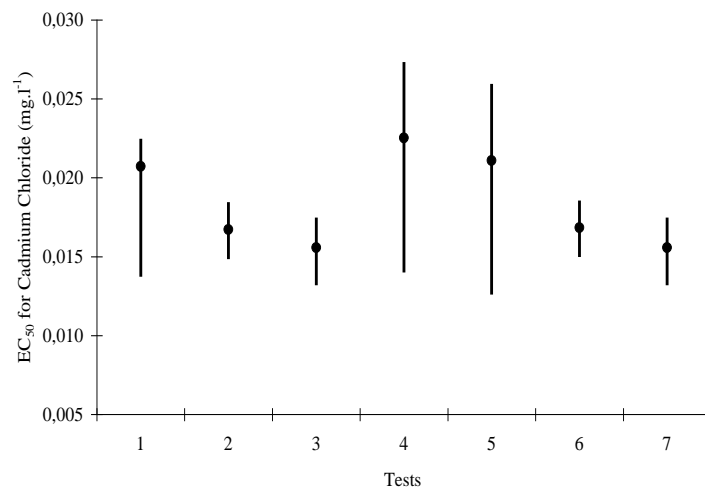


10  
11 Figure 2: Cadmium toxicity ( $\text{CdCl}_2$ ) to neonates of *M. elegans* exposed in natural medium at  
12  $25^\circ\text{C}$ .

13  
14 Despite the recommendation of the ISO standard (6341: 1989), that the ASTM medium being  
15 used as an appropriate medium for cladoceran cultures, this artificial medium without diluting  
16 was not well adequate for the growth of *M. elegans*. Nevertheless, once diluted in ultra pure  
17 water at a ratio (1:1) the animals presented a satisfactory growth. In our toxicity tests, *M. elegans*  
18 revealed a higher sensibility to cadmium when exposed to this metal in artificial medium -  
19 cadmium 48-h  $\text{EC}_{50}$  at 0.018  $\text{mg.l}^{-1}$  [0.012-0.024  $\text{mg.l}^{-1}$ ] (Figure 3). The pH and conductivity in

1 the artificial medium varied from 7.0 to 7.7 and from 220 to 320  $\mu\text{S}\cdot\text{cm}^{-1}$ , respectively.  
2 According to the results obtained here in artificial medium, *M. elegans* showed to be more  
3 sensitive toward Cd than *C. silvestrii*, which presented a 48-h  $\text{LC}_{50}$  mean of  $0.062 \text{ mg}\cdot\text{l}^{-1}$  [ $0.016$ -  
4  $0.110 \text{ mg}\cdot\text{l}^{-1}$ ] (Oliveira-Neto & Botta-Paschoal, 2002) and *D. magna* (48-h  $\text{LC}_{50}$  at  $0.033 \text{ mg}\cdot\text{l}^{-1}$   
5 (Schuytema *et al.* 1984). Furthermore, the Cd 48-h  $\text{LC}_{50}$  obtained for *C. silvestrii* was also  
6 determined in an artificial medium and was significantly different from that obtained here for *M.*  
7 *elegans* with a coefficient of variation of only 15.8% ( $p < 0.0001$ ). These results reinforce the  
8 great potential of *M. elegans* as test organism in the assessment of pollutant-induced stress in  
9 tropical freshwater ecosystems.

10



11

12 Figura 3: Cadmium toxicity ( $\text{CdCl}_2$ ) to neonates of *M. elegans* exposed in artificial medium at  
13  $25^{\circ}\text{C}$ .

14

15 According to Buikema & Cairns (1980) and Orr *et al.* (1990) a safe procedure to assure the  
16 quality of a certain culture aimed to produce healthy specimens for a reliable experimental  
17 control in ecotoxicological tests, consists in testing a reference substance which is expected to  
18 affect the organism independently of small variations in the composition of the medium used in  
19 the tests. Hence, results obtained by different laboratories following a pre-established

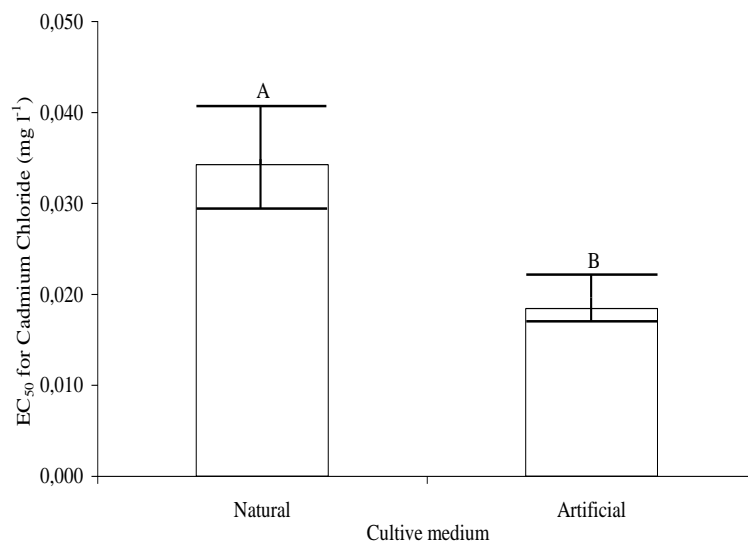
1 methodology may be more easily compared allowing ecotoxicological tests to be standardised in  
2 due time (Soares & Calow, 1993; Knie & Lopes, 2004). Boluda *et al.*, (2002) and  
3 Manusadžianas *et al.*, (2003) comment that variation in results of toxicity tests due to chemical  
4 interactions (other pollutants or experimental medium) may amplify or reduce the toxic effects of  
5 some pollutants, such as the case of chelanting agents, for example, which toxicity may be  
6 influenced by dissolved organic matter and water hardness. Knie & Lopes (2004) argue that  
7 there is a controversy involving the use of natural and artificial medium in ecotoxicological tests,  
8 although it seems agreeable that both are important according to specific situations. Artificial  
9 medium are of great important for the standardisation of ecotoxicological tests. On the other  
10 hand, tests involving natural medium may produce results of great ecological relevance, as they  
11 resembles more the environmental reality of the study area.

12  
13 Natural and artificial media used in this work presented a difference between their dissolved  
14 organic matter content, with larger concentration in the natural media. Conversely, the hardness,  
15 measured as  $\text{CaCO}_3$ , is much higher in the artificial medium ( $80 \text{ mg.l}^{-1}$ ) than in the natural  
16 medium ( $20 \text{ mg.l}^{-1}$ ). Our results, regarding the toxicity of cadmium to *M. elegans* shows that this  
17 tropical cladoceran is significantly more susceptible to cadmium in artificial medium (48-h  $\text{EC}_{50}$   
18 at  $0.018 \text{ mg l}^{-1}$ ) than in natural medium (48-h  $\text{EC}_{50}$  at  $0.035 \text{ mg l}^{-1}$ ) ( $p < 0.0001$ ) (Figure 4).  
19 Similarly, Penttinen *et al.* (1998) found that the sensibility of *Daphnia magna* towards Cd is  
20 higher in artificial medium (48-h  $\text{LC}_{50}$  at  $0.037 \text{ mg.l}^{-1}$ ) than in natural medium (48-h  $\text{LC}_{50}$  at  
21  $0.058 \text{ mg.l}^{-1}$ ). A possible explanation for the higher toxicity of cadmium to *M. elegans* recorded  
22 in this work in the artificial medium is the large contents of dissolved organic matter  $19.54 \text{ }\mu\text{g.l}^{-1}$   
23 (De Santana, 2004) and humid acids in the natural medium, which may have reduced the toxicity  
24 of Cd to the animals tested in the natural medium. The presence of organic compounds in the  
25 artificial medium was almost negligible. Akkanen & Kukkonen (2001) found a direct



1 relationship between increasing values of water hardness and reduced levels of complexation of  
2 cadmium by dissolved organic matter due to a preference in binding between ions of calcium and  
3 cadmium. The water hardness in our toxicity tests was four-fold higher in the artificial medium  
4 than in the natural water. Nevertheless, this difference did not seem to be enough to suppress the  
5 affinity of the dissolved organic matter and humic acids to the metal which presumably was  
6 responsible for the reduction in the bioavailability of cadmium and thus, its toxicity to the  
7 cladocerans in the natural medium (Rand *et al* 1995; Lewis *et al.* 1999; Kalis *et al.* 2006).

8



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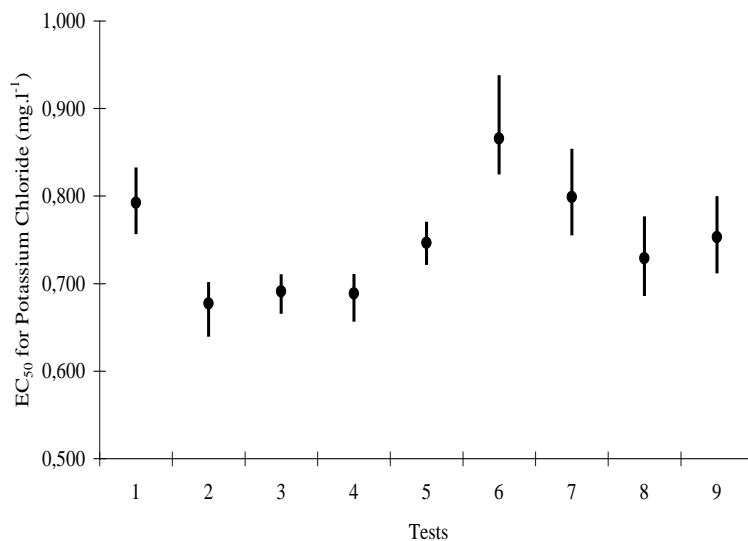
10 Figura 4: Toxicity of cadmium ( $\text{CdCl}_2$ ) to neonates of *M. elegans* exposed at  $25^\circ\text{C}$  in natural  
11 medium and artificial medium. Mean 48-h  $\text{EC}_{50}$  values and their 95% confidence limits (bars).

12

13 The tests to assess the toxicity of potassium chloride to the tropical cladoceran *M. elegans* were  
14 carried out in natural medium only. The mean value obtained for the KCl 48-h  $\text{EC}_{50}$  was at  
15  $749.08 \text{ mg.l}^{-1}$  [ $625.32\text{--}872.76 \text{ mg.l}^{-1}$ ] with a coefficient of variation of 8.2%, indicating a low  
16 variability and high precision of the results (Figure 5). The pH values varied between 6.63 and  
17 7.00 while the conductivity ranged from  $96 \mu\text{S.cm}^{-1}$  in the controls, which showed no mortality,  
18 to  $1900 \mu\text{S.cm}^{-1}$  in the highest concentration tested. Comparing the results obtained in this work

1 for the toxicity of KCl to *M. elegans* with toxicity data determined for *D. magna* (48-h LC<sub>50</sub> at  
2 660 mg.l<sup>-1</sup>) and *C. dubia* (48-h LC<sub>50</sub> at 630 mg l<sup>-1</sup>) (OECD, 2001), it is noticed that the values are  
3 reasonably close to each other.

4



5

6 Figura 5: Toxicity of potassium (KCl) to neonates of *M. elegans* exposed in natural medium at  
7 25<sup>0</sup>C. Values of 48-h EC<sub>50</sub> and their 95% confidence limits (bars).

8

9

10

1 **4- CONCLUSIONS**

2

3 The study showed clearly that the tropical cladoceran *M. elegans* is highly sensitive towards Cd  
4 either exposed in natural or artificial medium, even when compared with toxicity levels obtained  
5 for other tropical or temperate cladocerans in standardised tests. The variation in susceptibility  
6 levels observed between the two media tested may be attributed to differences in their  
7 composition. The higher organic matter content in the natural medium may have suppressed the  
8 cadmium toxicity to *M. elegans*. The results obtained either in the natural or artificial medium,  
9 however, were highly reproducible. *M. elegans* showed a great advantage as the animals are well  
10 adaptable to both, the natural and artificial medium thus, facilitating the standardisation of tests  
11 with this tropical cladoceran. *M. elegans* was also sensitive to potassium chloride and could be  
12 used in the quality control of cultures, particularly considering that these animals do not produce  
13 toxic residues, thus avoiding risks to researchers and the environment. The epibenthic behaviour  
14 of this tropical cladoceran may represent an advantage over other species considering that these  
15 animals could be potentially used in toxicity tests for the evaluation of the interface sediment-  
16 water. Overall, the tropical cladoceran, *M. elegans*, displays a great potential as a test organism  
17 in ecotoxicological tests. Nevertheless, more studies are required providing data on the  
18 characteristics of this animals and its sensibility to other potential toxic substances.

19

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21

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