



Arsenic toxicity to cladocerans isolated and associated with iron: implications for aquatic environments

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ABSTRACT

Arsenic is an ametal ubiquitous in nature and known by its high toxicity. Many studies have tried to elucidate the arsenic metabolism in the cell and its impact to plants, animals and human health. In aqueous phase, inorganic arsenic is more common and its oxidation state (As III and As V) depends on physical and chemical environmental conditions. The aim of this study was to evaluate toxicity of arsenic to *Daphnia similis* and *Ceriodaphnia silvestrii*, isolated and associated with iron. The results showed differences in toxicity of As III and As V to both species. Effective concentration (EC50) mean values were 0.45 mg L⁻¹ (As III) and 0.54 mg L⁻¹ (As V) for *D. similis*, and 0.44 mg L⁻¹ (As III) and 0.69 mg L⁻¹ (As V) for *C. silvestrii*. However, As V IC25 mean value was 0.59 mg L⁻¹, indicating that *C. silvestrii* has mechanisms to reduce arsenic toxicity. On the other hand, when associated with iron at 0.02 and 2.00 mg L⁻¹, EC50 values decreased for *D. similis* (0.34 and 0.38 mg L⁻¹) as well as *C. silvestrii* (0.37 and 0.37 mg L⁻¹), showing synergistic effect of these substances.

Key words: aquatic ecotoxicology, metals, acute and chronic effects, *D. similis*, *C. silvestrii*.

INTRODUCTION

Arsenic is an extremely toxic ametal widely distributed in nature. It is associated with ores of metals such as copper, lead and gold (Oreland and Stolz 2003), adsorbed to oxides and hydroxides of iron and manganese (Meng et al. 2002, Sarifuzzaman et al. 2007, Zeng et al. 2008) or organic matter (Redman et al. 2002, Wang and Mulligan 2006). Arsenic contamination has been

strongly related to mining activities (Sharma and Sohn 2009).

Arsenic pentavalent is predominant in aquatic and aerobic environments. The trivalent form, is more toxic, as demonstrated by Styblo et al. (2000) evaluating human hepatocytes, epidermal keratinocytes, bronchial epithelial cells and urinary bladder cells. However, it is usually present in anoxic environments with low oxidation potential, like groundwater (Oreland and Stolz 2003, Borba et al. 2000, Borba et al. 2004, Sharma and Sohn 2009).

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Several studies have tried to identify this ametal toxicity, isolated or associated with other metals, to aquatic and terrestrial organisms (Styblo et al. 2000, Lyn Patrick 2003, Levy et al. 2005, Norwood et al. 2007, Liao et al. 2008, Fikirdeşici et al. 2012, Miao et al. 2012, Zhang et al. 2013, Zou et al. 2013). Furthermore, mitigation and remediation actions in the environment have been used in processes involving precipitation or adsorption of this ametal mainly to oxides and hydroxides iron (Meng et al. 2002, Zaw and Emmett 2002, Fernandes-Machado and Miotto-Bigatão 2007).

Arsenic metabolism plays a key role in its toxicity and involves the reduction to trivalent form, followed by methylation. Yin et al. (2011) evaluated arsenic metabolism in ciliate *Tetrahymena thermophila*, and observed, after 48 h of exposure, that the dissolved arsenate (As V) was converted into arsenite (As III) and methylated forms.

Although, the methylation of inorganic trivalent arsenic is associated with cellular detoxification mechanisms, methylated forms may be more cytotoxic, with inhibitory genotoxicity for some enzymes (Thomas et al. 2001, Styblo et al. 2000, Wang et al. 2002) damaging the DNA, which can be enhanced associated with iron ions (Fe^{2+}) (Ahmad et al. 2002). Arsenic has high potential to bioaccumulate in animals and vegetables (Liao et al. 2008, Miao et al. 2012), exposed to man through ingestion of contaminated food.

Thereby, evaluating arsenic toxicity to sensitive aquatic organisms is critical to preserve these ecosystems and to prevent contamination and bioaccumulation in humans. Such evaluations, using an ecotoxicological approach, allow assessment of direct arsenic effects isolated or associated with other substances.

Cladocerans are in the transition zone in aquatic food chains, between producers and secondary consumers, and can be considered essential to transfer metals through the trophic chain (Tsui and Wang 2007), being used in ecotoxicological

studies about arsenic toxicity (Yu and Wang 2002, Fikirdeşici et al. 2012, Miao et al. 2012).

In this context, the present study aimed to evaluate the potential of arsenic toxicity for two species of cladocerans, *Daphnia similis* and *Ceriodaphnia silvestrii*. Three experiments were carried out: (1) evaluation of arsenate and arsenite EC50 to cladocerans; (2) evaluation of arsenate IC25 to *C. silvestrii* and viability assessment of the neonates, considering that surface water contains predominantly the pentavalent form; and (3) evaluation of arsenate EC50 in presence of Fe (III) in the concentrations normally observed in surface waters in the Iron Quadrangle, Minas Gerais, Brazil, where gold mines are located (Borba et al. 2000).

MATERIALS AND METHODS

CULTIVATION AND SENSITIVITY OF CLADOCERANS

Acute toxicity tests were conducted with *D. similis* and *C. silvestrii* and chronic toxicity tests were conducted with *C. silvestrii*. Cladocerans were maintained in glass vats at densities of 30 ind.L⁻¹ and 21 ± 1 °C for *D. similis* and 100 ind.L⁻¹ and 25 ± 1 °C for *C. silvestrii* in 12 h photoperiod. The water used for cultivation came from a natural spring situated in Belo Horizonte, MG. The test-organisms were fed with a compound of fish food and yeast (1 ml.L⁻¹) and Chlorophyceae algae-*Raphidocelis subcapitata* (10⁵ cel ml⁻¹), according to ABNT protocols (2009; 2010). Sensitivity tests were conducted monthly using sodium chloride (NaCl) as reference substance at concentrations of 1.2, 1.4, 1.6, 1.8 and 2.0 mg L⁻¹ for *C. silvestrii* and 1.4, 1.7, 2.0, 2.3 and 2.6 mg L⁻¹ for *D. similis*. All tests followed standardized protocols (ABNT 2009, 2010).

AS AND FE TEST SOLUTIONS

The stock solutions were prepared at concentration of 20 g L⁻¹ and were subsequently diluted to 200 mg

L⁻¹ using distilled water before testing. The solutions of As III and V were prepared by dissolving sodium arsenite (NaAsO₃) and sodium arsenate (NaAsO₄) from Sigma-Aldrich, 99% purity. The solutions Fe III were prepared using ferric chloride (FeCl₃) from Merck, 99% purity.

DETERMINATION OF ARSENIC TEST CONCENTRATIONS

The test concentrations were calculated in logarithmic series, considering the range between the highest concentration, where no effect was observed, and the lowest concentration, in which 100% immobility was observed.

DETERMINATION OF EC50 OF AS III AND V

Acute toxicity tests with As III and V were conducted, using three replicates containing five neonates aged 6 to 24 h, exposed to 10 ml of the test solution in atoxic polypropylene recipients, maintained in the dark for 48 h without food. Six concentrations of As were defined for the tests: 0.2, 0.3, 0.4, 0.5, 0.7 and 1.0 mg L⁻¹ for As III, and 0.3, 0.4, 0.5, 0.7, 1.0 and 1.3 mg L⁻¹ for As V. Each test included a control consisting of water used for cultivation. Data evaluation was done through the Trimmed Spearman-Kärber program (Hamilton et al. 1977) used to calculate the concentration that causes 50% immobilization of organisms (EC50, 48 h).

At least five toxicity tests were conducted with each species. For validation, only tests with less than 10% immobilization in the control treatment were considered. For tests using a mixture of As V and Fe, the fixed concentrations of iron were 0.02, 0.2 and 2.0 mg L⁻¹.

DETERMINATION OF IC25 OF AS V AND VIABILITY ASSESSMENT OF NEONATES

For chronic toxicity tests, ten specimens of *C. silvestrii* were exposed individually in 20 ml of the test solution in atoxic polypropylene recipients for 7 days. The organisms were maintained at

photoperiod of 12 hours, 25 ± 1 °C and fed at the beginning of the experiments and every monitoring. During the experiments, pH and dissolved oxygen were monitored.

Solutions were completely renewed during each monitoring, conducted on alternate days. Neonates produced were counted and removed and the adults kept were fed with the diet used for culturing. At the end of the experiment, body lengths were measured by means of a ruler coupled to an optical microscope. The Inhibition Concentration (IC25) was calculated using ICPIN Program (2.0) (Norberg-King 1993). ANOVA and Tukey tests were used to compare differences of body lengths and reproduction between the treatments.

The neonates of the last reproduction of all replicates were divided into two groups, one group being maintained for 4 days, exposed to the same test conditions and another group in the cultivation water. At the end, their body lengths were measured to evaluate the persistence of toxic effects of arsenic.

COMPARISON OF EC50 VALUES

The EC50 (48h) means were compared using the formula endorsed by the USEPA (1985) and obtained from Costa et al. (2014) for calculating the magnitude parameter:

$$G = \sqrt{\left[\text{LOG} \left(\frac{HL(1)}{EC50(1)} \right) \right]^2 + \left[\text{LOG} \left(\frac{HL(2)}{EC50(2)} \right) \right]^2} \quad (\text{eq. 1})$$

where G is the magnitude parameter, HS is the upper limit of the confidence interval obtained by EC50 in 48h and the numbers 1 and 2 refer to the different tests. After G was calculated, H and Z were also calculated using the following equations:

$$H = 10^G \quad (\text{eq. 2})$$

$$Z = \frac{\text{HigherEC50}}{\text{LowerEC50}} \quad (\text{eq. 3})$$

where Z and H are comparison parameters and Higher and Lower EC50 refer to compared EC50 values. The difference between EC50 means were considered significant when $Z > H$.

RESULTS

Arsenate (As V) was less toxic than arsenite (As III), for both species and *C. silvestrii* was less sensitive than *D. similis*. The EC50 values for *D. similis* ranged from 0.47 to 0.67 mg L⁻¹ (As V) and from 0.34 to 0.52 mg L⁻¹ (As III), with EC50 mean values of 0.54 mg L⁻¹ (As V) and 0.45 mg L⁻¹ (As III). For *C. silvestrii*, EC50 values ranged from 0.47 to 0.96 mg L⁻¹ (As V) and from 0.41 to 0.48 mg L⁻¹ (As III), with mean values of 0.69 and 0.44 mg.L⁻¹, respectively. The toxicity of arsenate increased significantly in the presence of iron, except for *D. similis* at a concentration 0.2 mg L⁻¹ (Tables I and II).

Regarding chronic toxicity tests with As V, reduction in reproduction was observed at concentrations of 0.4 mg L⁻¹ and above it ($p < 0.05$). Furthermore, concentrations from 0.4 to 0.7 mg L⁻¹, showed a greater increase of neonates in the second reproduction, with a decrease in the third one (Fig. 1), resulting in a higher IC25 (Table III).

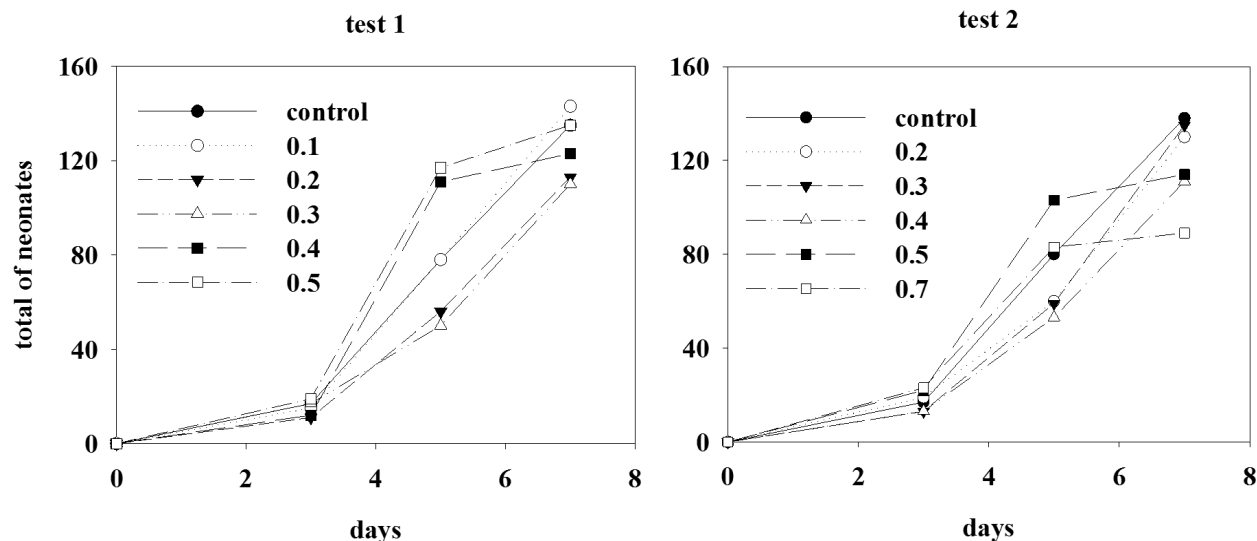


Figure 1 - Total neonates of *C. silvestrii* in chronic arsenate toxicity tests.

TABLE I
EC50 mean values (mg L⁻¹) and 95% confidence intervals (in parentheses) for *C. silvestrii* and *D. similis* for As III and As V isolated and associated with Fe.

Group tests	<i>D. similis</i>	<i>C. silvestrii</i>
As V	0.54 (0.48 - 0.61)	0.69 (0.63 - 0.77)
As III	0.45 (0.41 - 0.50)	0.44 (0.39 - 0.50)
As V + 0.02 Fe III	0.34 (0.31 - 0.39)	0.37 (0.34 - 0.41)
As V + 0.20 Fe III	0.66 (0.58 - 0.77)	0.50 (0.44 - 0.57)
As V + 2.00 Fe III	0.38 (0.31 - 0.46)	0.37 (0.30 - 0.47)

TABLE II
Parameters H and Z obtained from EC50 – 48 h mean values for As III and As V and As V associated with Fe for *D. similis* and *C. silvestrii*.

Pairs of Bioassays	<i>D. similis</i>		<i>C. silvestrii</i>	
	H	Z	H	Z
As V/AsIII	1.17	1.20*	1.18	1.57*
As V/As V + 0.02 Fe III	1.20	1.59*	1.24	2.03*
As V/As V + 0.2 Fe III	1.22	1.22	1.19	1.38*
As V/As V + 2 Fe III	1.25	1.42*	1.30	1.86*
	<i>D. similis</i> x <i>C. silvestrii</i>			
	H	Z		
As V	1.18	1.28*		
As III	1.18	1.02		

* Significant Z values

TABLE III
Mean values (n = 10) and standard deviations of neonates
in two tests carried out at different concentrations of As V
(mg L⁻¹) and their corresponding IC25 values.

As V Concentrations	Test 1	Test 2
Control	16.00 ± 3.67	13.80 ± 2.74
0.1	14.33 ± 4.85	-
0.2	11.30 ± 4.22	14.33 ± 3.35
0.3	11.00 ± 3.59	13.50 ± 2.32
0.4	12.30 ± 2.83	11.10 ± 3.60
0.5	13.44 ± 5.41	11.40 ± 1.96
0.7	-	9.11 ± 4.86
IC25	0.61	0.57

There was significant growth reduction in adults ($p < 0.002$) only at concentrations of 0.5 and 0.7 mg L⁻¹. Moreover, neonates maintained in the solution for 4 days showed significant body reduction ($p < 0.05$) (Fig. 2).

DISCUSSION

The sensitivity of *D. similis* was significantly higher than of *C. silvestrii* for arsenate. Immobility occurred abruptly in concentrations from 0.4 to 0.7 mg L⁻¹, for both forms of arsenic. These results corroborate data obtained by Norwood et al. (2007) who observed abrupt mortality of *Hyaella azteca* exposed to pentavalent form of arsenic.

The toxicity of arsenate is justified once this ametal can replace anion phosphates interfering in the metabolism of the organism, promoting, for example, depletion of ATP in the cell (Hughes 2002). Levy et al. (2005) found arsenate toxicity reduction for two algal species by adding phosphate in the solution. The As-V EC50 obtained for both species, corroborated with responses obtained by Fikirdeşici et al. (2012) who found EC50 of 0.5 mg L⁻¹ As-V for *Daphnia magna*. In the same study, the authors observed increased toxicity of arsenic when associated with cadmium, an extremely toxic metal.

The increased toxicity of arsenic in the presence of iron, demonstrates the risk of contamination in

aquatic environments naturally rich with this metal, as found in the Iron Quadrangle in Minas Gerais, Brazil, where arsenic concentrations in surface waters reached up to 160 mg L⁻¹ (Borba et al. 2000). Furthermore, the use of iron as coagulant for arsenic, especially as salts, may have an opposite effect to the expected one. Zou et al. (2013) verified that both FeCl₃ and Fe₃O₄ isolates did not cause toxicity to the epithelium of *Tetrahymena pyriformis*. However, Fe₃O₄ increased arsenate toxicity after long-term exposure by reducing arsenate (V) to arsenite (III).

Even though iron is an important nutrient, in excess, it can be extremely toxic, especially by promoting the fenton reaction and release of hydroxyl ions (Bury and Grosell 2003). In addition, iron in excess can be accumulated in the cell nucleus promoting oxidative conditions that cause DNA damage (Meneghini 1997, Emerit et al. 2001).

An alternative to the use of iron salts could be the adsorption to organic compounds as proposed by Fagundes et al. (2008), who used a complex of iron-chitosan (III) for the removal of arsenate from surface waters, besides many other low-cost adsorbents as dry plants, red mud, fly ash and zeolites (Chiban et al. 2012). Furthermore considering the chitin constitution of cladocerans carapace, the toxicity of arsenic may be enhanced by adsorption of this ametal to exoskeleton of test organisms, and iron could increase this adsorption, although some authors associate the metal adsorption to limestone from carapace (Tsui and Wang 2007).

Carney et al. (1986) assessed cadmium uptake in *Daphnia magna* and found that most of this metal was adsorbed to the shell bodies, and tolerance was related to molting. Yu and Wang (2002) observed that 50% of cadmium and zinc uptake by *Daphnia magna* was adsorbed to the exoskeleton. The same was observed by Robinson et al. (2003) who found cadmium accumulation in shells of *Daphnia magna* and *Ceriodaphnia dubia*, both species congeneric

with ours. These authors found a strong and positive correlation between adsorption saturation time and concentration of cadmium.

On the other hand, organic matter may be more effective in reducing toxicity instead of iron coagulants, since mortality of *C. silvestrii* did not

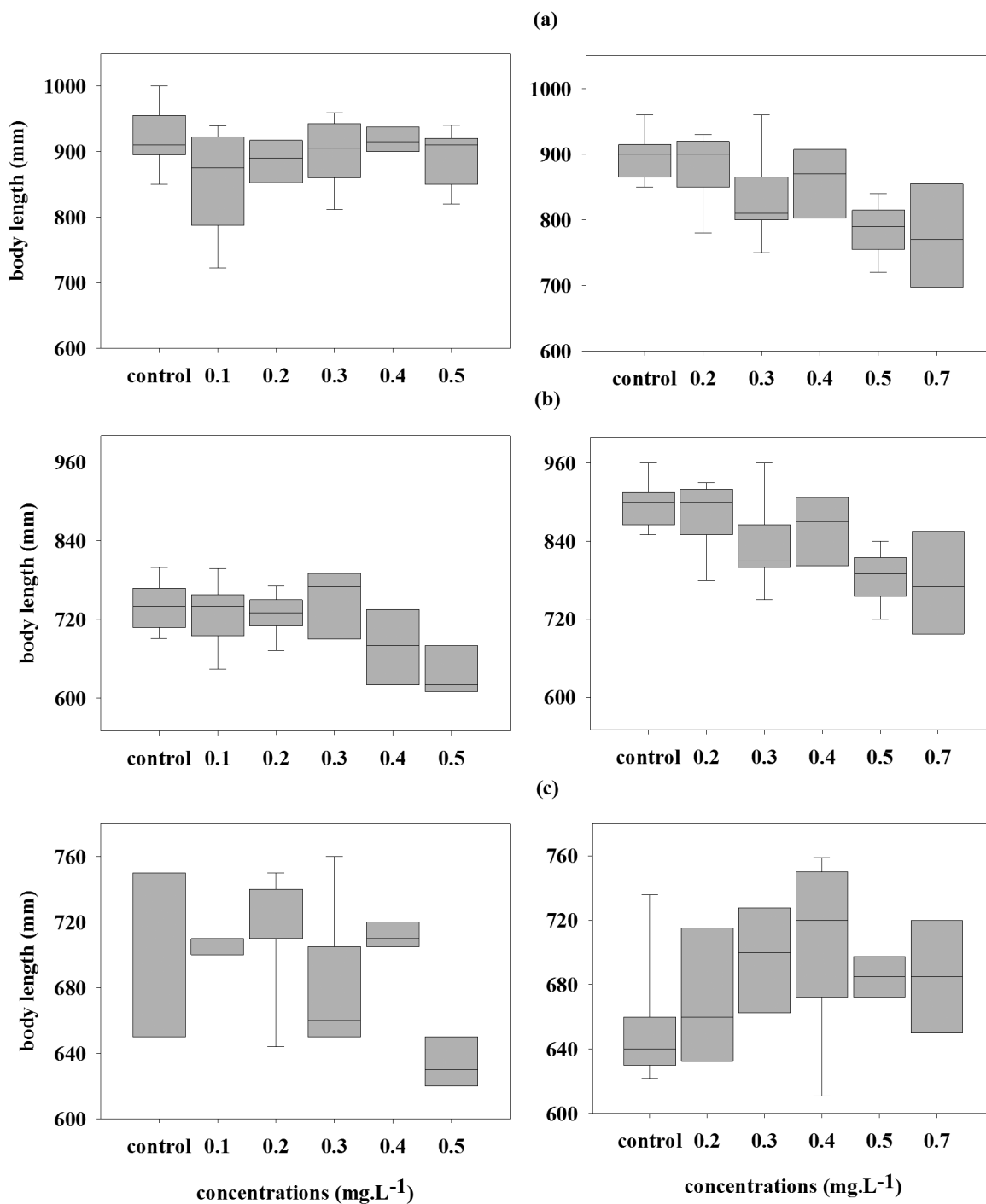


Figure 2 - Body length of adults of *C. silvestrii* after seven days (a) and neonates after four days maintained in arsenate solutions (b) and in natural water (c). Test 1 (Left panel) and test 2 (right panel).

exceed the 10%, value permitted for the control, and IC25 was higher than EC50. Reduced susceptibility of this organism may be related to food.

In natural environments, organic matter can oxidize arsenite (As III) to arsenate (As V) or chelate this ametal reducing its toxicity (Redman et al. 2002). However, organic matter, derived from the diet, may not have an important role as a chelator, considering the organisms of these tests, which are filter feeders. Furthermore, filtration activity can be changed in the presence of food, reducing passage times of both food and ametal, reducing their absorption by this test organism (Tsui and Wang 2007), the hypothesis more plausible for the present study.

Moreover, a favorable environment, rich in nutrients, could promote the formation of detoxifying agents, such as metallothionein, binding to arsenic ions, which makes this ametal unavailable (Miao et al. 2012). For many authors, metallothioneins are produced in situations of stress, such as contamination by metals, and play a key role in reducing toxicity of selenium, cadmium, zinc, copper, silver and arsenic among others (Cousins 1983, Kägi and Schäffer 1990, Roesijadi 1992, Amiard et al. 2006, Tsui and Wang 2007, Nordberg and Nordberg 2009, Miao et al. 2012). Bodar et al. (1990) reported a temporary cadmium tolerance in *Daphnia magna* by the production of metallothionein.

The reduction of metals assimilation in Cladocera can also occur through excretion, molting, and even by transfer from mother to neonate (Tsui and Wang 2007). Miao et al. (2012) found that arsenate bioaccumulation in *D. magna* was reduced under high food availability and there was loss of arsenic incorporated by ecdysis (4%), excretion (12%) and transfer to the neonate (30%). Muysen and Janssen (2002) observed in *D. magna*, exposed to zinc, that 38% of the accumulated metal was adsorbed by the carapaces and most metal loss occurred due to molting.

The greater number of neonates observed in the second generation (Fig. 1) at higher concentrations may be related to a trade-off when the number of neonates produced is proportional to maternal transfer of arsenic. Lam and Wang (2006) found that the production of neonates by *D. magna* was reduced together with the reduction of arsenic concentration in maternal cladocerans.

Tsui and Wang (2004a) also observed in *D. magna* that the losses due to maternal transfer of mercury ranged from 11 to 15% for inorganic form and from 32 to 41% for organic form of this metal. The same authors, evaluating only the organic form of mercury, found that maternal transfer is the second most important mechanism of loss of this metal (Tsui and Wang 2004b).

Thus, maternal transfer may be a strategy for reducing toxicity to adult individuals, representing an alternative route for disposal of contaminants (Tsui and Wang 2007). *Daphnia magna*, for example, has low capacity for arsenate bio-magnification but high rate of maternal transfer (Miao et al. 2012). In the control and As V concentrations lower than 0.4 mg L⁻¹, there was no significant difference between the number of neonates produced by the second and third generations.

Regarding body growth, the reduction occurred in levels of 0.5 and 0.7 mg L⁻¹, contrasting with high production of neonates, corroborating a trade-off on which the growth reduction compensates gain in reproduction. Guan and Wang (2006), evaluating the tolerance of *D. magna* exposed to cadmium observed the same effect of trade-off. In aquatic communities, this phenomenon is extremely important since it ensures the continuity of generations in stress environments.

In the present study, the growth of the neonates was reduced only when kept in a stress situation (arsenic solutions at concentrations of 0.5 and 0.7 mg L⁻¹). When these neonates were kept in clean water, their growth was not significantly different from the control. These results corroborate the

study of Miao et al. (2012) who observed efficient mechanisms for loss of arsenic in *D. magna*. Thus, the impact of arsenic contamination in cladocerans can be reversed after immobilization of this ametal in aquatic environments.

Moreover, compared to the control, the increase in growth of the neonates maintained in 0.3 mg L^{-1} of arsenate, suggested that these individuals may have developed resistance to this ametal. Sanchez et al. (2004) found increased resistance and tolerance in neonates of *D. magna* whose parents were exposed to zinc.

Ward and Robinson (2005) observed increased resistance in neonates of *D. magna* whose parents were previously exposed to cadmium. However, these authors observed loss of genetic variability in the cadmium resistant populations and increased sensitivity to other contaminants such as phenol.

CONCLUSIONS

Arsenite showed higher toxicity than its pentavalent form, although this toxicity was lower than expected according to the literature. Arsenic toxicity to *D. similis* and to *C. silvestrii* increased in the presence of iron. These results demonstrated the need for monitoring and reducing arsenic in aquatic environments, especially in places where the geological matrix is composed of iron ore. Reproductive and growth parameters were less affected than survival, indicating that, similar to *D. magna*, these organisms present efficient mechanisms for decontamination. However, further studies with biochemical biomarkers are needed to confirm it.

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RESUMO

Arsênio é um ametal amplamente distribuído na natureza e é distribuído e conhecido por sua elevada toxicidade. Muitos estudos tentaram elucidar o metabolismo do arsênio na célula e seu impacto para plantas, animais e à saúde humana. Em meio aquoso, arsênio inorgânico é mais comum e seu estado de oxidação (As III e As V) depende de condições físicas e químicas do ambiente. O objetivo deste estudo foi avaliar a toxicidade do arsênio a *Daphnia similis* e *Ceriodaphnia silvestrii*, isolado e associado a ferro. Os resultados demonstraram diferenças na toxicidade de As III e As V para ambas as espécies. Os valores médios da Concentração efetiva (CE50) foram 0.45 mg L^{-1} (As III) e 0.54 mg L^{-1} (As V) para *D. similis*, e 0.44 mg L^{-1} (As III) e 0.69 mg L^{-1} (As V) para *C. silvestrii*. Entretanto, o valor médio da CI25 para As V foi 0.59 mg L^{-1} , indicando que *C. silvestrii* possui mecanismos para reduzir toxicidade de arsênio. Por outro lado, quando associado a ferro nas concentrações de 0.02 e 2.00 mg L^{-1} , os valores de CE50 decresceram tanto para *D. similis* (0.34 e 0.38 mg L^{-1}) quanto para *C. silvestrii* (0.37 e 0.37 mg L^{-1}), demonstrando efeitos sinérgicos entre as substâncias.

Palavras-chave: ecotoxicologia aquática, metais, efeitos agudos e crônicos, *D. similis*, *C. silvestrii*.

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