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# 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 Emett 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 mantained 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* ($10^5$ 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 ($NaAsO_3$) and sodium arsenate ($NaAsO_4$) from Sigma-Aldrich, 99% purity. The solutions Fe III were prepared using ferric chloride ($FeCl_3$) 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-Karber 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:

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

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:

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

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

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).

**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 | *D. similis* | *C. silvestrii* |
|---|---|---|
| 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 Biossays | *D. similis* | | *C. silvestrii* | |
|---|---|---|---|---|
| | 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* |

| | *D. similis* x *C. silvestrii* | |
|---|---|---|
| | H | Z |
| As V | 1.18 | 1.28* |
| As III | 1.18 | 1.02 |

* Significant Z values

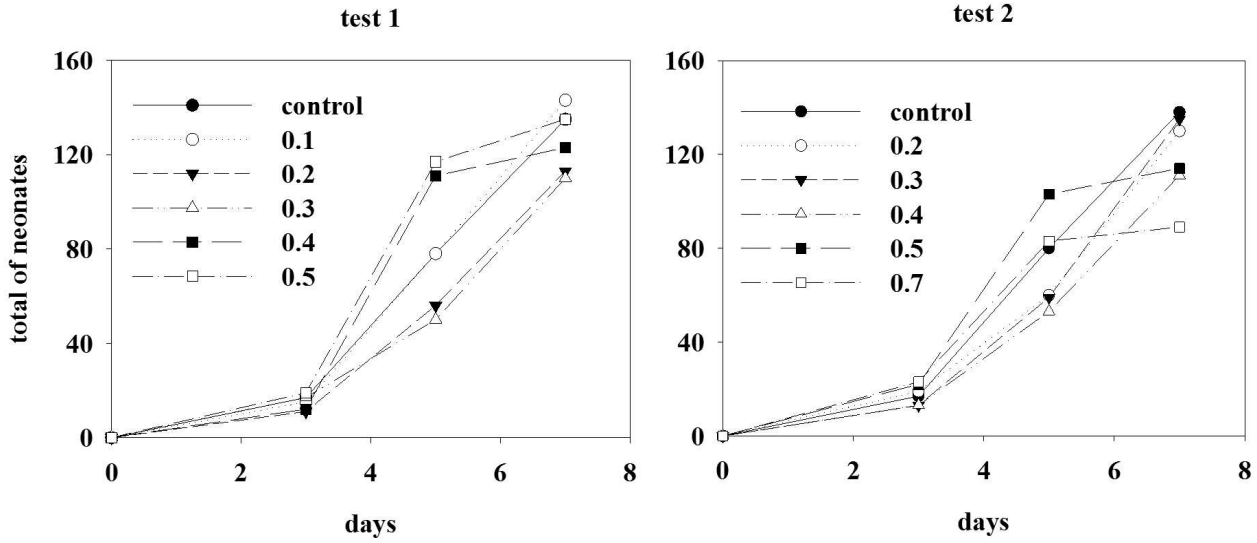


**Figure 1 -** Total neonates of *C. silvestrii* in chronic arsenate toxicity tests.

**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 *Hyalella 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 $FeCl_3$ and $Fe_3O_4$ isolates did not cause toxicity to the epithelium of *Tetrahymena pyriformis*. However, $Fe_3O_4$ 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

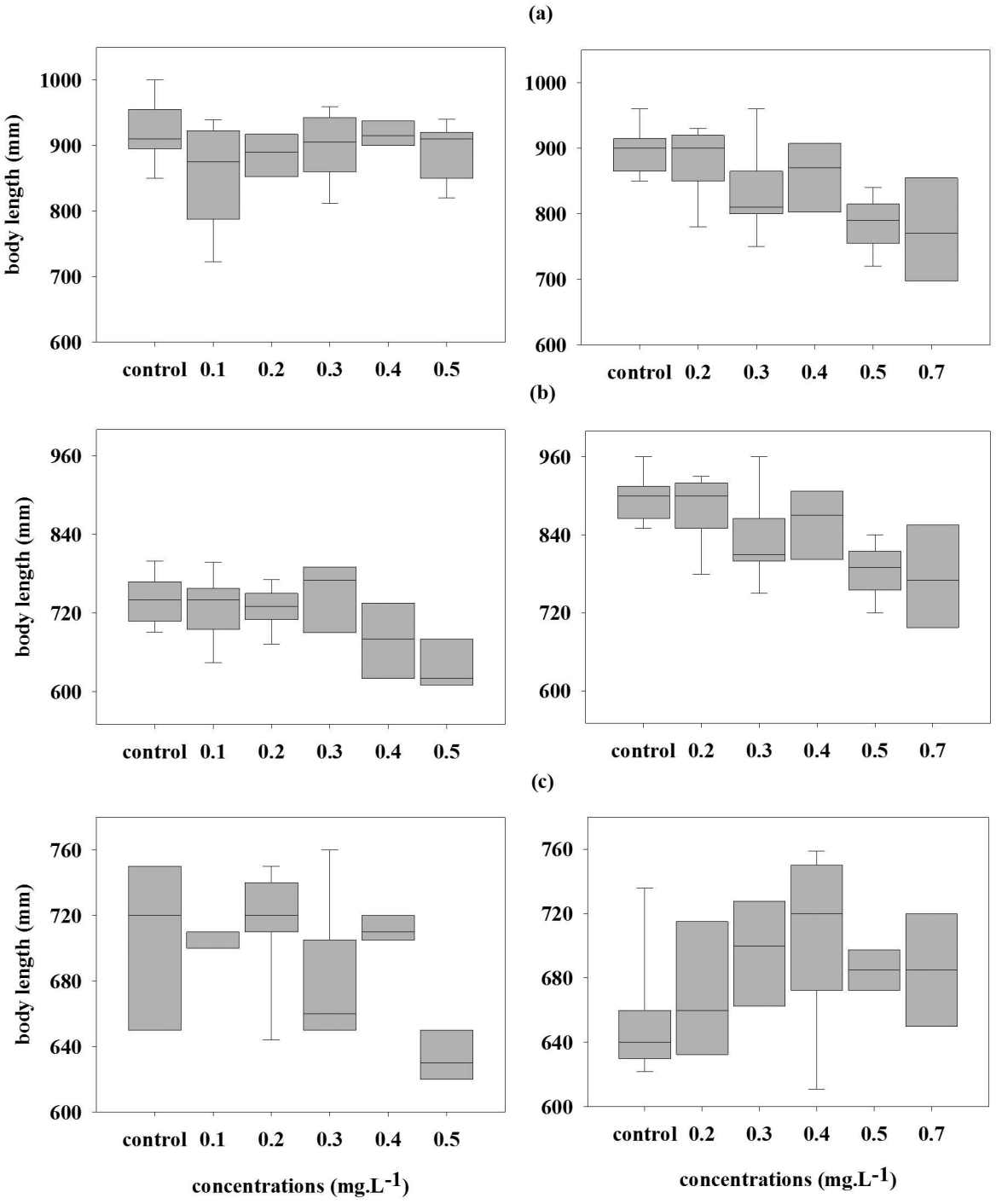


**Figure 2 -** Body length of adults of *C. silvestrii* after seven days **(a)** and neonates after four days maintained in arsenate solutions **(b)** and in natural water **(c)**. Test 1 (Left panel) and test 2 (right panel).

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%). Muyssen 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.*

## REFERENCES

ABNT - Associação Brasileira de Normas Técnicas. 2009. Ecotoxicologia aquática – Toxicidade aguda – Método de ensaio com *Daphnia* spp. (Cladocera, Crustacea). NBR 12713, p. 23.

ABNT - Associação Brasileira de Normas Técnicas. 2010. Ecotoxicologia aquática – Toxicidade crônica – Método de ensaio com *Ceriodaphnia* spp. (Crustacea, Cladocera). Errata 1: 2014. NBR 13373, p. 18.

Ahmad S, Kitchin KT and Cullen WR. 2002. Plasmid DNA damage caused by methylated arsenicals, ascorbic acid and human liver ferritin. Toxicol Lett 133: 47-57.

Amiard JC, Amiard-Triquet C, Barka S, Pellerin J and Rainbowd PS. 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76: 160-202.

Bodar CWM, Sluis I, Montfort JCP, Voogt PA and Zandee DI. 1990. Cadmium resistance in *Daphnia magna.* Aquat Toxicol 16: 33-40.

Borba RP, Figueiredo BR and Cavalcanti JA. 2004. Arsênio na água subterrânea em Ouro Preto e Mariana,

Quadrilátero Ferrífero (MG). R Esc Minas Ouro Preto 57(1): 45-51.

BORBA RP, FIGUEIREDO BR, RAWLINS B AND MATSCHULLAT J. 2000. Arsenic in water and sediment in the iron quadrangle, state of Minas Gerais, Brazil. R Bras Geoci 30: 558-561.

BURY N AND GROSELL M. 2003. Iron acquisition by teleost fish. Comp Biochem Phys C 135: 97-105.

CARNEY GC, SHORE P AND CHANDRA H. 1986. The uptake of cadmium from a dietary and soluble source by the crustacean *Daphnia magna.* Environ Res 39: 290-298.

CHIBAN M, ZERBET M, CARJA G AND SINAN F. 2012. Application of low-cost adsorbents for arsenic removal: a review. J Environ Chem Ecotoxicol 4(5): 91-102.

COSTA BVM, YOGUI GT AND SOUZA-SANTOS LP. 2014. Acute toxicity of tributyltin on the marine copepod *Tisbe biminiensis*. Braz J Oceanogr 62(1): 65-69.

COUSINS RJ. 1983. Metallothionein - Aspects related to copper and zinc metabolism. J Inherit Metab Dis 6(1): 15-21.

EMERIT J, BEAUMONT C AND TRIVIN F. 2001. Iron metabolism, free radicals, and oxidative injury. Biomed Pharmacother 55(6): 333-339.

FAGUNDES T, BACHMANN AWL, TOMAZ HSO, RODRIGUES CA. 2008. Adsorção de arsênio (V) pela quitosana ferro – III reticulada. Quim Nova 31(6): 1305-1309.

FERNANDES-MACHADO NRCF AND MIOTTO-BIGATÃO DMM. 2007. Utilização de zeólitas sintetizadas a partir de xisto retortado na remoção de arsênio em águas contaminadas. Quim Nova 30(5): 1108-1114.

FIKIRDEŞICI S, ALTINDAĞ A AND OZDEMIR E. 2012. Investigation of acute toxicity of cadmium-arsenic mixtures to *Daphnia magna* with toxic units approach. Turk J Zool 36(4): 543-550.

GUAN R AND WANG WX. 2006. Multigenerational cadmium acclimation and biokinetics in *Daphnia magna*. Environ Pollut 141: 343-352.

HAMILTON MA, RUSSO RC AND THURFTON RB. 1977. Trimmed Spearman-Karber methods for estimating median lethal concentration in toxicity biossay. Environ Sci Technol 11: 714-719.

HUGHES MF. 2002. Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133: 1-16.

KÄGI JHR AND SCHÄFFER A. 1990. Biochemistry of metallothioneint. Biochemistry-US 27(23): 8509-8515.

LAM IKS AND WANG WX. 2006. Transgenerational retention and maternal transfer of selenium in *Daphnia magna.* Environ Toxicol Chem 25(9): 2519-2525.

LEVY JL, STAUBER JL, ADAMS M, MAHER W, KIRBY JK AND JOLLEY DF. 2005. Toxicity, biotransformation, and mode of action of arsenic in two freshwater microalgae (*Chlorella* sp. and *Monoraphidium arcuatum*). Environ Toxicol Chem 24(10): 2630-2639.

LIAO CM, JAU SF, CHEN WY, LIN CM, JOU LJ, LIU CW, LIAO VHC AND CHANG FJ. 2008. Acute toxicity and bioaccumulation of arsenic in freshwater clam *Corbicula fluminea.* Environ Toxicol 23(6): 702-711.

LYN PATRICK ND. 2003. Toxic metals and antioxidants: Part. II. The role of antioxidants in arsenic and cadmium toxicity. Altern Med Rev 8(2): 106-128.

MENEGHINI R. 1997. Iron homeostasis, oxidative stress, and DNA damage. Free Radical Bio Med 23(5): 783-792.

MENG X, KORFIATIS GP, BANG S AND BANG KW. 2002. Combined effects of anions on arsenic removal by iron hydroxides. Toxicol Lett 133: 103-111.

MIAO AJ, WANG NX, YANG LY AND WANG WX. 2012. Accumulation kinetics of arsenic in *Daphnia magna* under different phosphorus and food density regimes. Environ Toxicol Chem 31(6): 1283-1291.

MUYSSEN BTA AND JANSSEN CR. 2002. Accumulation and regulation of zinc in *Daphnia magna*: links with homeostasis and toxicity. Arch Environ Con Tox 43: 492-496.

NORBERG-KING TJ. 1993. A linear interpolation method for subletal toxicity: the inhibition concentration (Icp) approach. Version 2.0. National Effluent Toxicity Assessment Centre. Technical Report, p. 3-93.

NORDBERG M AND NORDBERG GF. 2009. Metallothioneins: historical development and overview. Met Ions Life Sci 5: 1-29.

NORWOOD WP, BORGMANN U AND DIXON DG. 2007. Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. Environ Pollut 147: 262-272.

ORELAND RS AND STOLZ JF. 2003. The Ecology of Arsenic. Science 300: 939-943.

REDMAN A, MACALADY DL AND AHMANN D. 2002. Natural organic matter affects arsenic speciation and sorption onto hematite. Environ Sci Technol 36: 2889-2896.

ROBINSON KA, BAIRDA DJ AND WRONAB FJ. 2003. Surface metal adsorption on zooplankton carapaces: implications for exposure and effects in consumer organisms. Environ Pollut 122: 159-167.

ROESIJADI G. 1992. Metallothioneis in metal regulation and toxicity in aquatic animals. Aquat Toxicol 22: 81-114.

SANCHEZ M, ANDREU-MOLINER E AND FERRANDO MD. 2004. Laboratory investigation into the development of resistance of *Daphnia magna* to the herbicide molinate. Ecotox Environ Safe 59: 316-323.

SARIFUZZAMAN M, SIDDIQUE MNA, FERDOUSI FK, AHMED E, ALAM AMS AND ULLAH SM. 2007. Studies of arsenic mobilization with iron, manganese and copper in borehole sediments of the River Padma. Pak J Anal Environ Chem 8(1 & 2): 91-95.

SHARMA VK AND SOHN M. 2009. Aquatic arsenic: toxicity, speciation, transformations, and remediation. Environ Int 35: 743-759.

STYBLO M, DEL RAZO LM, VEJA L, GERMOLEC DR, LECLUYSE EL, HAMILTON GA, REED W, WANG C, CULLEN WR AND TOMAS DJ. 2000. Comparative of toxicity of trivalent and pentavalent inorganic and methilated arsenicals in rat and human cells. Arch Toxicol 74: 289-299.

THOMAS DJ, STYBLO M AND LIN S. 2001. The cellular metabolism and systemic toxicity of arsenic. Toxicol Appl Pharm 176: 127-144.

TSUI MKT AND WANG WX. 2004a. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna.* Environ Sci Technol 38: 808-816.

TSUI MKT AND WANG WX. 2004b. Maternal transfer efficiency and transgenerational toxicity of methylmercury in *Daphnia magna.* Environ Toxicol Chem 23(6): 1504-1511.

TSUI MKT AND WANG WX. 2007. Biokinetics and tolerance development of toxic metals in *Daphnia magna.* Environ Toxicol Chem 26: 1023-1032.

USEPA - UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organims, 3th ed., EPA-600/4-85-013. Washington, DC. Office of Water.

WANG JP, QI L, MOORE MR AND NG JC. 2002. A review of animal models for the study of arsenic carcinogenesis. Toxicol Lett 133: 17-31.

WANG S AND MULLIGAN CN. 2006. Effect of natural organic matter on arsenic release from soils and sediments into groundwater. Environ Geochem Hlth 28: 197-214.

WARD TJ AND ROBINSON WE. 2005. Evolution of cadmium resistance in *Daphnia magna.* Environ Toxicol Chem 24(9): 2341-2349.

YIN XX, ZHANG YY, YANG J AND ZHU YG. 2011. Rapid biotransformation of arsenic by a model protozoan *Tetrahymena thermophila*. Environ Pollut 159: 837-840.

YU RQ AND WANG WX. 2002. Kinetic uptake of bioavailable cadmium, selenium, and zinc by *Daphnia magna.* Environ Toxicol Chem 21(11): 2348-2355.

ZAW M AND EMETT MT. 2002. Arsenic removal from water using advanced oxidation processes. Toxicol Lett 133: 113-118.

ZENG H, FISHER B AND GIAMMAR DE. 2008. Individual and competitive adsorption of arsenate and phosphate to a high-surface-area iron oxide-based sorbent. Environ Sci Technol 42: 147-152.

ZHANG J, DING T AND ZHANG C. 2013. Biosorption and toxicity responses to arsenite (As[III]) in *Scenedesmus quadricauda*. Chemosphere 92(9): 1077-1084.

ZOU XY, XU B, YU CP AND ZHANG HW. 2013. Combined toxicity of ferroferric oxide nanoparticles and arsenic to the ciliated protozoa *Tetrahymena pyriformis*. Aquat Toxicol 134-135: 6-73.