



Evaluation of transgenerational effects caused by metals as environmental pollutants in *Daphnia magna*

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Abstract The present study aimed to evaluate the acute and chronic toxicity of environmentally relevant concentrations of metals (Mn, Al, Fe, and Pb) in *Daphnia magna* and the generational transposition of reproductive and morphological damages. The effective concentration for 10% of the organisms from each metal was obtained by the acute toxicity test (96 hours); then, another five concentrations lower than this one were defined for the chronic experimentation (21 days), in which the number of neonates generated by each individual was checked daily. At the end of the exposition, the lengths and number of morphological damages were recorded in each adult daphnid. During this, the molt generated on the 14th and 21st days were collected and cultivated for posterior evaluation of the same parameters. Alterations in the reproductive performance were observed in the organisms exposed to manganese and aluminum (4.0 and 0.5 mg L⁻¹, respectively). Organisms exposed to aluminum (0.05 mg L⁻¹) and iron (0.27 mg L⁻¹) showed a reduction in body length. It is

also noteworthy that the molt of these adults and their respective offspring also presented reproductive alterations, especially the molt from the 14th day of lead exposure (0.02 mg L⁻¹) and the 21st day of manganese exposure (4.0 mg L⁻¹). Such effects allow us to conclude that environments polluted by metals can reduce the ability of the species to maintain themselves in the ecosystem. In addition, there is a need to increase the control and monitoring of metals, such as aluminum, which present risks even in low concentrations.

Keywords Ecotoxicology · Microcrustaceans · Metal contamination · Reproductive toxicity

Introduction

Water pollution from erroneous anthropogenic actions favors the presence of metals and other contaminants in water (Kassim et al. 2011). Once released, the metals can deposit in the sediment or be available in the water column (Simpson and Spadaro 2016), damaging the quality of soil, water, and all surrounding ecosystem. In addition, geogenic sources also contribute to the occurrence of these elements in groundwater and surface water (Martin et al. 2015; Winkel et al. 2008). However, they cause concern due to their persistence, toxicity, and bioaccumulation ability in living aquatic organisms (Ahmed et al. 2015; Islam et al. 2015), including plants (Salawu et al. 2018).

Among the anthropogenic polluting sources responsible for the occurrence of metals in water bodies, mining stands out (Li et al. 2015), and discharge of effluents

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without previous treatment (Chen et al. 2015) and the excessive use of chemical fertilizers and pesticides in agriculture (Eqani et al. 2012), and for this reason, it has been the subject of several studies that evaluate the toxicity in humans (Cerrillos et al. 2019; Deshommes et al. 2016; Myers et al. 2009), fish (Gao et al. 2019; Marins et al. 2019; Rodrigues et al. 2020; Yin et al. 2018), and rodents (Asser et al. 2019; Cobbina et al. 2015a, b; Kumar et al. 2009; Wang et al. 2018; Zhai et al. 2017), but less frequently in *Daphnia* sp. (Araujo et al. 2019; Cui et al. 2018; Zimmermann et al. 2017).

Consequently, the enrichment of heavy metals in wildlife through the food chain poses potential threats to humans (Lei et al. 2016). In addition, it should be noted that most current water potability systems in developing countries are insufficient for the full removal of these compounds, characterizing as an environmental and public health problem. Knowing that measuring only the total metal concentrations in water or sediment is an insufficient way to accurately assess their impact (de Miguel et al. 2005; Wu et al. 2016), to understand how these substances behave at different levels can be the best way to understand their biological risk.

Aluminum (Al), iron (Fe), manganese (Mn), and lead (Pb) are among the main environmental pollutants of freshwater (Castro et al. 2018; Dalzochio et al. 2018; Kakoi et al. 2016; Marsidi et al. 2018), and generally have their effects associated with neurotoxicity (Altenhofen et al. 2017; Guiney et al. 2017; Wang et al. 2016, 2018), with very few studies addressing reproductive aspects of toxicity caused by such substances. But some authors already consider some metals as endocrine disruptors, especially because they interfere with steroid receptors; for example, cadmium, barium, and chromium have shown estrogenic activity in estrogen receptors (Choe et al. 2003), while nickel, lead, and mercury induced the expression of genes regulated by estrogen and receptor progesterone in MCF-7 cells (Martin et al. 2003). For these reasons, some authors recognize these substances as metalloestrogens (Paschoalini et al. 2019; Safe 2003).

Some evidence indicates mechanisms of action that affect reproductive impairment through these substances on aquatic organisms, for example, in fish exposure to mercury, causing oxidative stress in the gonads and disruption of the transcription of hypothalamic-pituitary-gonadal (HPG) axis genes of zebrafish (Guchhait et al. 2018), and modulating the aromatase gene expression altering normal development of oocytes in *Trichogaster*

fasciata (Zhang et al. 2016), or in microcrustacean exposure to cadmium, lead, and mercury, affecting the transcriptional modulation of the genes encoding antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) that are involved in cellular defense system protection against xenobiotics and consequently the induction of oxidative stress in *Daphnia magna* (Kim et al. 2017).

Either way, endocrine disruption can occur at different physiological levels (hormonal secretion, hormonal interaction, hormonal excretion) (Rodríguez et al. 2007), consequently causing problems for future generations. Even so, in aquatic species, there is a lack of information about the transgenerational toxicity of these metals (Mn, Al, Fe, and Pb) (Chen et al. 2017; Reis et al. 2018), that is, effects on the progeny from parents exposed at critical stages of development (Yang et al. 2013). It is noteworthy that studies that address effects on parents are important for ecotoxicology; however, the demonstrations of effects on offspring are fundamental, as they link what is commonly studied (parents) to what can represent a significant ecological effect, that is, the molts' aptitude to sensitivity to induced stressor (Reátegui-Zirena et al. 2017).

In our study, we chose *Daphnia magna* as a model organism, which are freshwater zooplankton that play a key role in the food chain, because they transfer biomass and lipid-like macronutrients from primary producers to higher trophic levels (Ferain et al. 2018). Studies evaluating transgenerational effects in this species generally demonstrate *Daphnia's* ability to alter even its normal biological behavior; for example, the offspring of parents exposed to predation are born with a mother-induced defense (Agrawal et al. 1999), or mothers cultivated in poor conditions can produce more resistant offspring (Mitchell and Read 2005). Other authors even report the transposition of genotoxic damage to the offspring of parents exposed to uranium (Plaire et al. 2013) and gamma radiation (Parisot et al. 2015). So, these transgenerational effects are critical for the survival of organisms under normal or unfavorable and unpredictable conditions (Plautz et al. 2013), especially because, if the stressor experienced by the offspring differs from that experienced by the parents, a new and potentially suitable phenotype can be expressed in these individuals, being the phenotypic variability increased in this generation of offspring (Bayadev 2005; Colombo et al. 2014).

Therefore, the aim of our study was to evaluate the toxicity and generational transposition of reproductive and morphological damage caused by metals, commonly found as water pollutants, in *Daphnia magna* after acute and chronic exposure.

Materials and methods

Metals

The metals used in the present study for acute and chronic toxicity were aluminum, lead, iron, and manganese, which have worldwide environmental occurrence (Deshommes et al. 2016; Marsidi et al. 2018; Panhwar et al. 2016). The concentrations used, as well as the reference values provided by the Brazilian legislation, can be observed in Table 1. The chemical reagents used for the exposures were aluminum chloride (LabSynth®), lead acetate (LabSynth®), ferric chloride (LabSynth®), and manganese chloride (LabSynth®). The concentrations of each experimental group were prepared from stock solutions of each metal and were confirmed using a bench spectrophotometer (HANNA®).

Model organism

The organisms used were originated from *Daphnia magna* clones grown at the Feevale University Ecotoxicology laboratory under standard conditions for approximately 3 years. The cultivated water used comes from reverse osmosis and then reconstituted with salts, as recommended by NBR 12.713 (ABNT 2016), with pH and hardness adjusted between 7.0 and 8.0 and between

175 and 225 mg L⁻¹ CaCO₃, respectively, and previous aeration of at least 12 h. The clones used as reproductive matrices were kept in batches of up to 25 adults per liter, in a 1000 mL container, with diffused light (16 h photoperiod of light) and temperature ranging from 18 to 22 °C in a modified BOD incubator. For food, the green alga *Desmodesmus subspicatus* Chodat, 1942 was used, providing the amount of approximately 10⁶ cells mL⁻¹ per adult organism every 48 h. For acute and chronic toxicity tests, neonates aged less than 24 h old were obtained from females with an average of 20 days old.

Acute toxicity assay

For acute toxicity assay, *Daphnia magna* neonates were exposed (n = 20 per group, 4 replicates with 5 organisms in 50 mL) to dilutions prepared with different concentrations of metals to be tested for 48 h. During this period, the organisms were kept at 18 to 22 °C without food (ABNT 2016). At the end of the exposure, the number of immobile organisms was observed, aiming to calculate the effective concentration for 10% of the organisms (EC₁₀), that is, the concentration responsible for the acute effect in 10% of the animals. This concentration was used as the maximum concentration in the chronic toxicity assay, aiming to maintain an adequate number of organisms throughout the 21 days of exposure.

Chronic toxicity assay

From the EC₁₀ of each metal of the organisms, lower concentrations were established for the chronic test. Briefly, 10 female neonates were exposed at these concentrations for 21 days, and in this trial each neonate

Table 1 Concentrations used for each metal in both exposures (acute and chronic) with their respective value provided in legislation

	Metal	Concentrations used (mg L ⁻¹)	Legislation (mg L ⁻¹)*
Acute exposure	Manganese	0.50, 1.50, 3.00, 9.00, 15.0	0.5
	Aluminum	0.025, 0.05, 0.10, 0.15, 0.20	0.2
	Iron	0.05, 0.15, 0.30, 0.50, 0.80	5.0
	Lead	0.01, 0.08, 0.10, 0.15, 0.20	0.03
Chronic exposure	Manganese	0.25, 0.50, 1.00, 2.00, 4.00	0.5
	Aluminum	0.025, 0.05, 0.10, 0.15, 0.20	0.2
	Iron	0.01, 0.03, 0.065, 0.13, 0.27	5.0
	Lead	0.01, 0.02, 0.03, 0.04, 0.05	0.03

*Resolution 357 refers to Class I freshwater, established by the National Environmental Council (CONAMA) in 2005

was kept alone in 50 mL beakers. Renewal of the dilutions from chronic experiments was performed every 48 h. During the experiment, each individual was daily observed, recording the date of first hatching and the daily number of newborns generated per female. At the end of the experiment, images of each female were captured by optical microscopy ($\times 40$ magnification) (Olympus IX73) in order to verify possible morphological changes (carapace damage, shortening of the digestive tract, and caudal spine shortening) and also to measure the body length with the aid of ImageJ® software. For the data on morphological damages, the percentage of changes cited in each evaluated group was calculated, for later statistical evaluation. During the 21 days of experiments, the molts generated on the 14th and 21st days were collected and kept in the laboratory, in standard culture medium, but not exposed to metals, also for 21 days, to record the number of offspring generated by this brood and also to verify the possible morphological alterations and their respective lengths (same protocols previously cited). The feeding of the organisms used in the chronic experiment, as well as their respective generations that were collected, occurred with the *Desmodesmus subspicatus* algae every 48 h, as already described.

Data analysis

From the results obtained after the mortality and immobility readings in the acute toxicity assay, the EC_{10} was calculated with the aid of the ICPIN software by the linear interpolation method. In the chronic toxicity assay, data obtained through the registration of neonates generated by female and body length were tested for data normality through the Kolmogorov-Smirnov test and subsequently submitted to parametric (one-way ANOVA and Tukey) and non-parametric (Kruskal-Wallis and Dunn) tests when appropriate, with the aid of GraphPad Prism 6 software. Results were considered statistically significant when $p < 0.05$.

Results

Acute toxicity assay

When conducting the acute experiment with aluminum, the concentrations used did not cause any kind of lethality or immobility to the organisms during 48 h of the test

(in order to confirm this result, the acute experiment was repeated), allowing the use of the same concentrations for the chronic experiment. In contrast, the other acute tests allowed us to establish the EC_{10} for manganese, iron, and lead, as can be seen in Table 2.

Chronic toxicity assay: reproductive and length parameters

Over the 21 days of chronic experimentation with each metal, the number of neonates generated per female was recorded, as can be seen in Fig. 1(A₁–A₄), and details the cumulative number of offspring produced at different concentrations of each experiment. Microcrustaceans exposed to manganese 4.0 mg L⁻¹ and aluminum 0.05 mg L⁻¹ showed a significant reduction in the cumulative number of generated neonates compared to the control group ($p = 0.02$ and $p = 0.005$, respectively). Organisms exposed to iron and lead showed no significant changes in relation to control ($p = 0.27$ and $p = 0.28$, respectively). During chronic assay with aluminum, 100% lethality of organisms exposed to the highest concentrations occurred after 72 h (0.15 and 0.20 mg L⁻¹) and 06 days (0.1 mg L⁻¹), remaining only the organisms exposed to 0.05 and 0.025 mg L⁻¹.

At the end of the exposure period, each *D. magna* was measured (Fig. 1(B₁–B₄)), and it was observed that organisms exposed to aluminum 0.05 mg L⁻¹ and iron 0.27 mg L⁻¹ showed a significant reduction in the corporal length in relation to the control group ($p = 0.0002$ and $p = 0.0003$, respectively).

Transgenerational evaluate

The molts that were generated during the chronic experiment, on the 14th and 21st day, by females exposed to different metals were cultured in the laboratory under the same conditions for 21 days. For these organisms,

Table 2 EC_{10} calculated after acute exposure of *Daphnia magna* to metals

Metals	EC_{10} (mg L ⁻¹)
Lead	0.053
Iron	0.27
Manganese	3.86

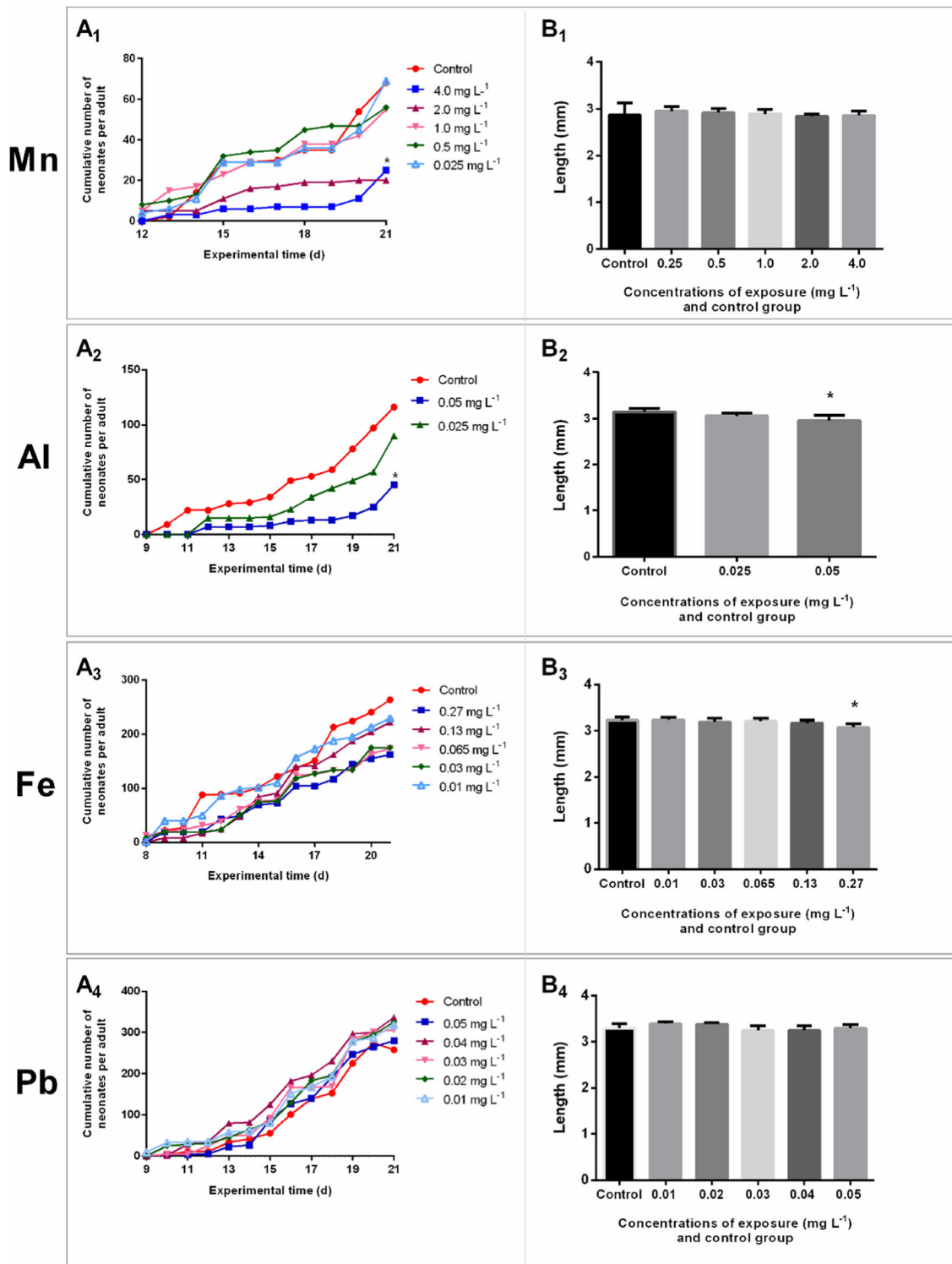


Fig. 1 (A₁–A₄) Cumulative number of newborns generated by females during the 21-day chronic toxicity assay. Asterisks represent a statistical difference in relation to the control group. (B₁–B₄)

Length measured at the end of 21 days of exposure to different metals (Data are expressed as mean and standard deviation and asterisks indicate significant difference from control, $p < 0.05$)

the accumulated offspring per female was also recorded and can be observed in Fig. 2.

In the organisms collected on the 14th day of the chronic assay, a significant difference in *D. magna* exposed to iron 0.03 mg L^{-1} and lead 0.02 mg L^{-1} in relation to the control group ($p = 0.009$ and $p = 0.008$) was observed, presenting a reduction and increase, respectively, in the cumulated offspring. In contrast, for organisms collected on the 21st day of the chronic assay, only *D. magna* exposed to manganese 4.0 mg L^{-1} differed from control ($p < 0.0001$), showing an increase in the number of accumulated offspring.

Regarding the measured body length (Fig. 3), neonates collected on the 14th day of exposure of mothers exposed to manganese 0.5 mg L^{-1} (presenting a reduction in the body length), aluminum 0.025 mg L^{-1} (increase in length), iron (0.27 and 0.03 mg L^{-1}) (reduction in length), and lead 0.02 mg L^{-1} (increase in length) differed significantly from control after 21 days of observation ($p = 0.01, 0.0009, 0.0004, 0.0009, \text{ and } 0.016$, respectively). For newborns collected on the 21st day, maternal exposure to manganese 2.0 mg L^{-1} (showing increase in length), 1.0 mg L^{-1} , and 0.25 mg L^{-1} (reduction in length), all iron concentrations presenting an increase in the body length (except 0.03 mg L^{-1}), and all lead concentrations, with many variations in the body length, resulted in significant differences in the size of the generated newborns, even after 21 days of observation, indicating that prolonged exposure may result in further damage to consecutive offspring.

A comparative table (Table 3) between the concentration established by the current Brazilian legislation and the minimum effect concentration, i.e., the lowest concentration used in the experiment that resulted in some change in the mothers or offspring of the respective mothers, is presented. Briefly, it can be observed that the full chronic exposure of mothers (21 days) to the lowest concentrations of metals used resulted in the largest morphological effects of body length observed in offspring (except for aluminum), confirming that prolonged exposure to metals, even at concentrations lower than those established by law, may result in generational transposition of damage. The exception is aluminum, which proved to be more toxic than the other metals studied and caused lethality in the organisms at the beginning of the chronic experiment (72 h) even in low concentrations.

Morphological analysis

Despite reproductive and size changes, no major morphological changes were observed in the mothers of the chronic experiment and their respective molts (14th and 21st) over the 21 days of observation. Figure 4 exemplifies the changes that were sought, namely caudal spine shortening, digestive tract shortening, and carapace deformations. It is noteworthy that the differences were sought between the groups but only within the same experimental period (mothers exposed to 21 days, neonates collected on the 14th day, or neonates collected on the 21st day), and are shown in the same graph as an illustration. The p values of statistical comparisons can be seen in the Table 4.

Discussion

Changes that organisms show after prolonged exposure to different contaminants can vary from resistance/tolerance to increased sensitivity, both leading to problems in the management of the species (Kimberly and Salice 2015), because under stressful conditions the organisms generally use different strategies that increase survival and reproductive success, changing mainly their reproduction and growth rates (Guan and Wang 2006). In the metallic environments, these compensations serve to keep functions at a basic level; that is, the organisms injured by the stressor dedicate energy to improve the effects of stressors and, as a result, have less energy available for growth and reproduction (Kimberly and Salice 2015). However, as previously mentioned, organisms can also increase their tolerance to the environment, by increasing the expression of the metallothionein protein, for example, which is known to increase the resistance of different animal species to metals (Beg et al. 2015; Amiard et al. 2006; Wang and Rainbow 2010).

Interferences caused by heavy metals in the reproductive system of microcrustaceans are reported by some authors; Pérez and Hoang (2018) report alterations in the embryonic development of *D. magna* after cadmium exposure and cadmium and zinc mixture exposure. Differing from our findings, Araujo et al. (2019) report reduced reproductive performance of *D. magna* exposed to lead nitrate; however, the same authors describe a higher sensitivity of *D. similis* compared to *D. magna* for lead ecotoxicological evaluation,

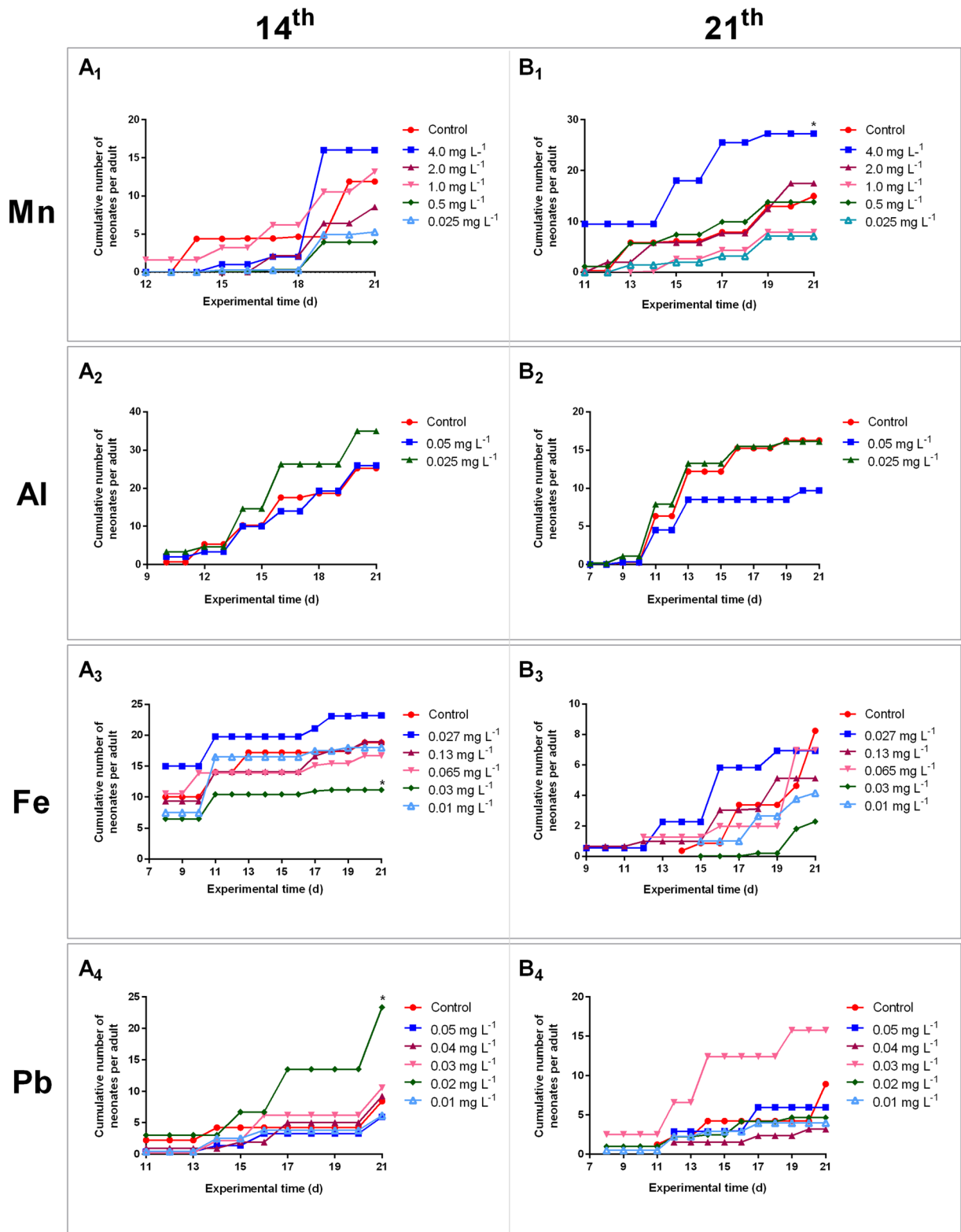


Fig. 2 Cumulative number of newborns generated by molt (collected at mothers' 14th and 21st exposure to different metals) over 21 days of observation. Asterisks represent a statistical difference in relation to the control group (* $p < 0.05$ and ** $p < 0.0001$)

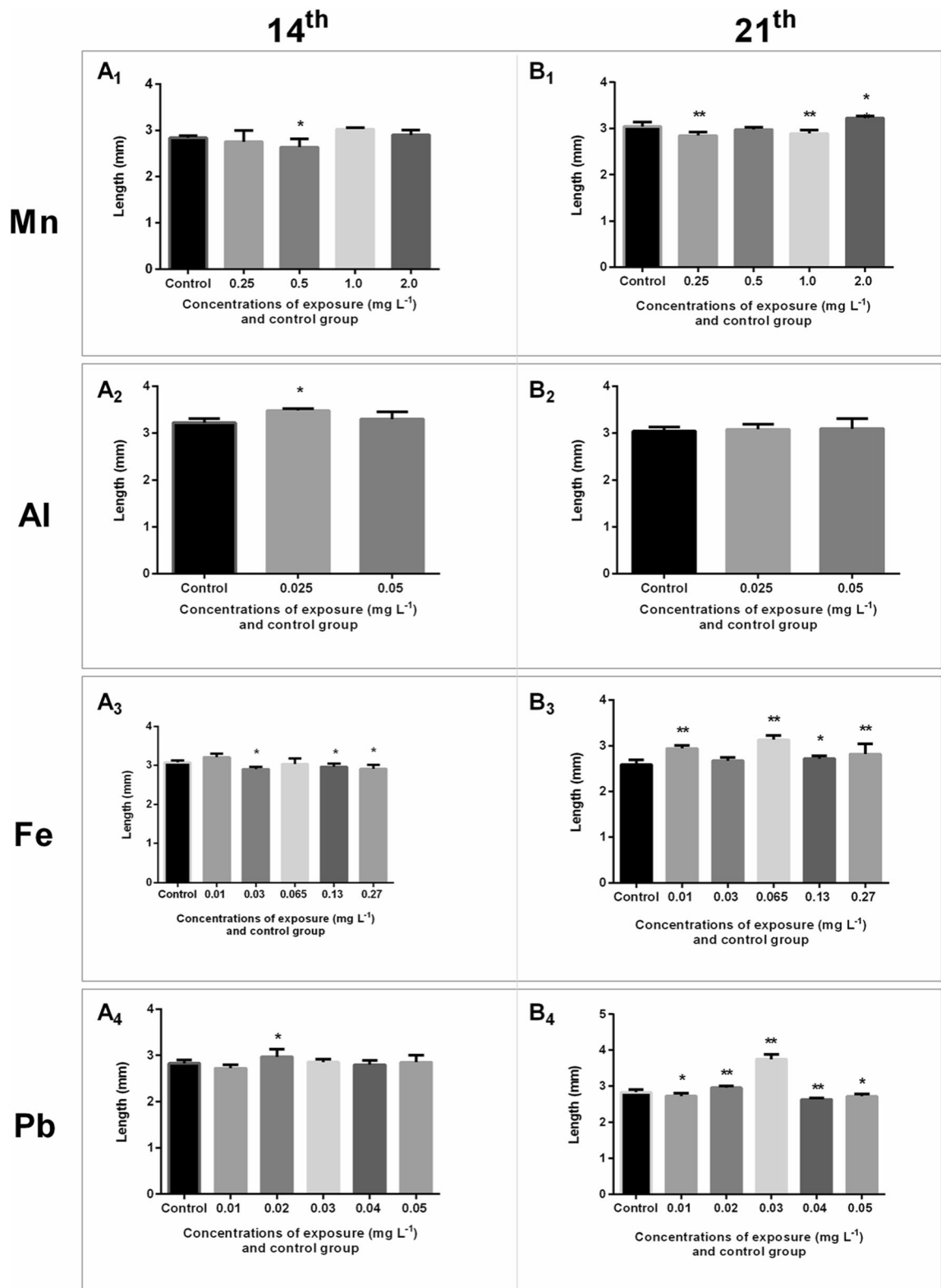


Fig. 3 Cumulative number of newborns generated by molt (collected at mothers' 14th and 21st exposure to different metals) over 21 days of observation. Data are expressed as mean and standard deviation (* $p < 0.05$ and ** $p < 0.0001$)

Table 3 Comparison between reference concentration established by the current Brazilian legislation and the minimum effect concentration in the present study

Metals	Brazilian legislation* (mg L ⁻¹)	Minimum concentration that causes effect (mg L ⁻¹), effect
Aluminum	0.2	0.10–100% lethality after 06 days of exposition.
Lead	0.033	0.01—Neonates from mothers exposed (21 days) at this concentration showed reduced body length.
Iron	5.0	0.01—Neonates from mothers exposed (21 days) at this concentration showed increased body length.
Manganese	0.5	0.25—Neonates from mothers exposed (21 days) at this concentration showed reduced body length.

*Resolution 357 refers to Class I freshwater, established by the National Environmental Council (CONAMA) in 2005

which may justify the non-reproducibility of data obtained from different studies with *D. magna*. Regaldo et al. (2014) also report reproductive changes in *D. magna* after exposure to copper, lead, and chromium. However, to date, we are unaware of studies reporting exposure to iron, aluminum, and manganese. In addition, no previous study has chronicled offspring generated by the organisms studied, as presented here. And, although no significant differences were observed in the morphological analysis, the mothers' exposure to metals resulted in reproductive and body length changes in the organisms as well as their respective offspring, indicating that the damage caused was transferred to subsequent generations, even when the concentrations used were lower than those established by law.

The alterations found in our study for the length of daphnids, in real environmental situations, could bring problems for the species, since species with reduced size end up becoming more vulnerable to predators (Lynch 1980). Therefore, we can affirm that exposures to low concentrations of Al and Fe (0.27 and 0.05 mg L⁻¹ respectively) during the growth of daphnids would result in a greater exposure to predators in the environment. However, it has already been described that reproduction occurs regardless of this growth inhibition or not, but with the brood quality altered (Skinner 1985; LeBlanc and Mclachlan 2009). There are reports that daphnids in the presence of a stressor produce fewer eggs, but of larger size, rich in lipids, which may even be more resistant (Knops et al. 2001). The alterations in growth rate can also be affected by changes in the intestinal microbiota of *D. magna* (Akbar et al. 2020), and it is worth mentioning that, although there is still no correlation in this species, in others there are already reports of changes in the intestinal microbiota caused by manganese (Ding

et al. 2020), iron (Fang et al. 2018), lead (Xing et al. 2019), and also aluminum (Alexandrov et al. 2020). So, we can assume that all changes in size induced by direct or mother exposure to metals can be considered toxicological responses of the species.

Manganese and iron can be classified as essential metals, which in low concentrations are essential for life, whereas aluminum and lead are considered non-essential, which have no known function in the body (Chang et al. 1996; Gebara et al. 2020; WHO/FAO/IAEA 1996). This fact may justify our finding of greater "tolerance" of daphnids to manganese and iron, since they endured exposures to higher concentrations in comparison to aluminum and lead, and it also goes according to the literature (Okamoto et al. 2014). Especially, since non-essential metals can cause toxicity even at low concentrations (Menon et al. 2016). For crustaceans, it is already known that there are several possible destinations for a metal: binding to metallothionein, transport to mitochondria, accumulation by lysosomes, transfer to the endoplasmic reticulum, or discharge back into the blood (Ahearn and Zhuang 1996; Ahearn et al. 2004), and the same is described for different animals (Banday et al. 2020; Frank et al. 2008; Monteiro et al. 2019), but all can assist in the imbalance of metallic homeostasis, and manifest through DNA damage, gene expression, and increased oxidative stress (Menon et al. 2016).

Anyway, metals are potentially toxic to the DNA of many organisms and are capable of inducing the production of reactive oxygen species (ROS) that can overwhelm the antioxidant defenses of the cells and result as a mechanism for oxidative stress, especially in long-term exposure (Ercal et al. 2001; Kim et al. 2017). Therefore, as already mentioned, the fact that progenies from newborns of mothers exposed for 21 days to Pb and Fe were able to normalize the production of

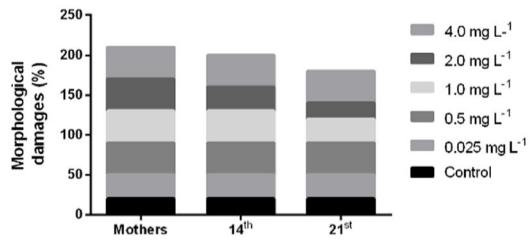
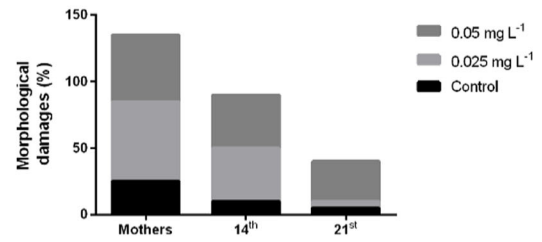
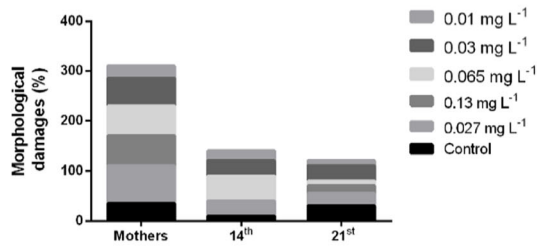
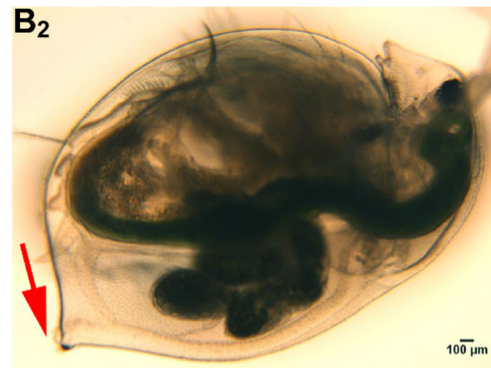
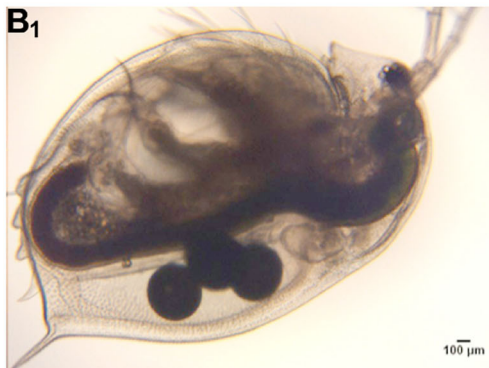
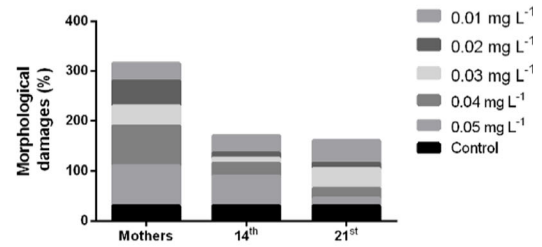
A₁ Manganese**A₂ Aluminum****A₃ Iron****A₄ Lead**

Fig. 4 (A₁–A₄) Graphs of means demonstrating that there was no significant difference in morphological analysis between groups from the same experimental period (mothers exposed to 21 days, offspring collected on the 14th day, or offspring collected on the 21st day) for manganese (A₁), aluminum (A₂), iron (A₃), or lead

(A₄). (B₁–B₄) *Daphnia magna* with normal morphology (B₁); caudal spine shortening, represented by red arrow (B₂); digestive tract shortening, represented by black arrow (B₃); and carapace deformation, shown by blue arrow (B₄)

Table 4 Morphological *p* value ($\alpha = 5\%$) analysis of statistical comparisons between groups in each experimental period (one-way ANOVA)

	Manganese	Aluminum	Iron	Lead
Mothers	0.701	0.430	0.765	0.778
Offspring (14th)	0.330	0.488	0.860	0.808
Offspring (21st)	0.600	0.552	0.941	0.965

neonates that had been deregulated in progeny of molt from mothers exposed for 14 days to these substances may be associated with the increased antioxidant defense system that activates detoxification mechanisms (Guan and Wang 2004), suggesting an adaptive response of this species against toxicity (Kim et al. 2017; Massarin et al. 2010). These results demonstrated that, when returning to the clean culture medium, *D. magna* is able to regulate its reproduction pattern, but even if the defense system acts, repairing the damage, other effects may increase (Ercal et al. 2001), as the repair process is subject to errors, corroborating the increase in body length changes of offspring of the newborns that were collected on the 21st day of exposure of mothers to metals.

Such adaptations to changes in the environment occur due to maternal effects, whereby mothers transmit information about environmental variability to the next generation, thus influencing their children's adaptive phenotypic responses or not (Enserink et al. 1993; Krylov and Osipova 2019; Mousseau and Fox 1998). In other words, Marshall and Uller (2007) state that maternal effects occur when the mother's phenotype or the environment she experiences influences her children's phenotype, in addition to the direct effect of the transmitted genes. The environments to which mothers are exposed can lead to either an increase or decrease in offspring, as well as changes in the size of their children (Lampert 1993), corroborating with our study, where the exposure of mothers to different metals resulted in different responses observed in the molts and their respective offspring.

In other aquatic organisms such as fish, metals in general are associated with numerous neurological, hepatic, renal, intestinal, and even genetic changes (Altenhofen et al. 2017; Coppo et al. 2018; Rodrigues et al. 2018, 2020; Wang et al. 2016); however, few studies address reproductive changes caused by these substances, as can be seen in a recent review (Rodrigues

et al. 2019). Gárriz et al. (2018) report morphological effects on the testis of *Odontesthes bonariensis* treated with cadmium, copper, and chromium, such as testicular fibrosis and pyknotic cells. With the metals worked in this article, studies are further restricted; Kida et al. (2016) report increased levels of testosterone and 17β estradiol in *Astyanax altiparanae* species sharply exposed to aluminum and manganese, respectively (0.5 mg L^{-1}).

Aluminum and lead are considered metal estrogens because of their ability to interfere with the action of estrogen hormones (Safe 2003), by blocking or decreasing the binding of endogenous estrogens to their receptors and acting as agonists by interacting with ligand binding domains at estrogen receptors (Darbre 2006). In addition, the abnormal increase in the number of spermatogonia in fish may be related to constant exposure to metals capable of mimicking the action of 17β estradiol, such as lead and aluminum (Georgescu et al. 2011). In contrast, when there is an increase in the number of germ cells but a reduction in sperm, and consequently a reduction in reproductive performance, there may be disturbances in androgen production and signaling, affecting final spermatogenesis steps (Chaube et al. 2010). This type of behavior was reported by Paschoalini et al. (2019) in *Prochilodus argenteus* fish from a place contaminated by different heavy metals. The authors also defend the idea that metals can positively or negatively alter the reproductive success of fish. As we cannot assume that the mechanisms responsible for the alterations in the number of *D. magna* offspring studied are equivalent to the alterations found in fish or humans, studies that evaluate the pathways involved in the reproductive process in the species are necessary, and in order to refine all species reports of reproductive toxicity with the species.

Furthermore, it can be observed that the number of neonates generated by the mothers of the control groups of chronic exposure to metals differs from the number of neonates generated by this molt when followed for 21 days, perhaps because, although reproduction in the species occurs by parthenogenesis, as a classic study of the area (Guisande 1993) reveals, as the population density increases, there is a reduction in the sexual maturation age of the females, consequently resulting in a greater number of newborns generated during the experimental

period. What we mean is that the females used for chronic exposures are the result of lots where the organisms are kept inside a beaker in a small population, and their reproductive behavior cannot be compared with the reproductive behavior of their molt, which has origin of females kept in isolation. Moreover, there are reports that the temporal social isolation, as well as agglomeration, can lead to the ephippium formation by *Daphnia* (Gerber et al. 2018). Although it was not observed in our study, this corroborates with data from the literature that indicates that the environmental determination of sex involves the sensory transduction of environmental stimuli, in addition to the subsequent modification of hormonal pathways, in order to influence the sex of the offspring (Camp et al. 2019; Devlin and Nagahama 2002; Korpelainen 1989).

Finally, among the tested metals, the aluminum with the highest toxicity stands out, since, when performing the acute test, as recommended by Brazilian legislation, none of the tested concentrations caused lethality or immobility to the organisms; however, 24 h after the acute experimentation period, that is, after 72 h, we obtained 100% lethality of organisms exposed to the highest concentrations (0.15 and 0.20 mg L⁻¹). It is noteworthy that such values are only twice as high as allowed by the legislation, being well below the values found in some localities, which often exceed up to ten times higher (Bianchi et al. 2019; Dalzochio et al. 2018; Gurgel et al. 2016; Machado et al. 2017; Matos et al. 2017; Rodrigues et al. 2016; Souza et al. 2016).

The effective concentrations for 10% of the organisms obtained in the present study, for manganese, iron, and lead, are above the allowed values by the legislation, but also represent the reality of many Brazilian localities, such as Rio Grande do Sul, Sinos River, Paraná, Alto do Iguaçu River, and Minas Gerais, Pardo River, where the values of iron, manganese, and lead reach 2.0 mg L⁻¹, 7.84 mg L⁻¹, and 0.86 mg L⁻¹, respectively (Alves et al. 2014; Bianchi et al. 2019; Brito et al. 2018). It is also noteworthy, as shown in Table 2, that the four metals studied caused progeny damage to mothers chronically exposed to concentrations lower than those allowed by the law, noting that acute toxicity tests do not reflect well the risk that these contaminants pose to aquatic biota.

So, the findings point to the need for studies to assess the chronic toxicity of substances, as they do come close

to the conditions to which different organisms are exposed daily in the ecosystem, and as in the case of aluminum, for example, it was found that acute tests can provide mistaken answers about the actual toxicity of substances. In addition, lethality and immobility parameters commonly evaluated in acute toxicity tests do not fully reflect the effects caused by certain contaminants. The present study sets precedents for future work to evaluate whether acute exposure, as well as chronic exposure, to these and other metals is capable of interfering with the quality of generated offspring, as well as a biochemical evaluation of such organisms.

Conclusion

Brazilian and worldwide legislations establish reference limits for the occurrence of metals in water. However, watercourses that meet these limits are rare; generally, the values quantified by the studies are much higher than the allowed ones. Therefore, in our study, we evaluated the concentrations of environmental polluting metals which represent both general legislation and environmental reality. All metals (Al, Fe, Mn, and Pb) were able to cause some effect, whether in organisms chronically exposed to substances or their respective offspring. Besides, aluminum, which is strongly present in many types of soil, therefore also in surface waters, and even in drinking water in developing countries, generated the absolute lethality of organisms in less than 7 days of exposure in concentrations lower than permitted by the Brazilian legislation for surface water.

Transposing the results obtained to a systematic reality, the metals, capable of altering the reproductive normality and the morphology of the daphnids in our study, could interfere in the maintenance of the species in the ecosystem, as well as alter their intake by predators, and so on.

Our study warns the need to review global standards for testing toxicity samples with the species (*Daphnia magna*), since the acute test does not seem to accurately demonstrate the risk that substances offer to organisms, in addition to the need to increase the control and the monitoring of domestic and industrial waste containing such substances since they offer risks even in low concentrations. The study also sets precedents for investigating the mechanisms responsible for the generational transposition of damage caused by metals.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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