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BILLINGS RESERVOIR (BRAZIL): CHEMICAL STUDIES ON WATER AND CHEMICAL AND MORPHOLOGICAL STUDIES ON ZEBRAFISH GILLS*

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ABSTRACT

Previous study of this research group revealed that water from the Billings reservoir (Brazil) intended for human use (water supply and seafood) has microbiological contamination and causes lethality and brain and behavioral impairments in zebrafish. The objective of this study was to understand what have induced these impairments in the animal model. Chemical analyses on water samples from Rio Pequeno (RP), Rio Grande (RG), and製Bororé (BO) rivers, as well as chemical and morphological analyses on zebrafish gills exposed to those waters were performed. Waters samples from RP, RG, and BO presented high levels of phosphorus. BO water and fish gills exposed to this water presented high levels of nitrogen. RG water caused potassium contamination in gills. Phosphorus, nitrogen, and potassium are indicators of anthropogenic pollution. RG water and fish gills exposed to this water presented low levels of calcium, which might be an indication of chemical imbalance that could lead to health problems in aquatic animals. RG and BO waters resulted in zirconium contamination in gills. BO water induced respiratory lamellae thickening in the gills, which may be the underlying mechanism for the observed hypoxia. In conclusion, behavioral, brain, and respiratory defects observed previously were induced by chemical and morphological disturbances due to anthropogenic pollution in the Billings reservoir.

Keywords: *Danio rerio*; drinking water; exposure; water quality; water supply.

REPRESA BILLINGS (BRASIL): ESTUDOS QUÍMICOS NA ÁGUA E ESTUDOS QUÍMICOS E MORFOLÓGICOS NAS BRÂNQUIAS DE ZEBRAFISH

RESUMO

Estudos prévios deste grupo de pesquisa revelaram que a água da represa Billings (Brasil) utilizada para consumo humano apresenta contaminação microbiológica e induz danos comportamentais, encefálicos e letalidade em zebrafish. O objetivo deste estudo foi entender o que induziu esses danos no modelo animal. Foram realizadas análises químicas em amostras de água do Rio Pequeno (RP), do Rio Grande (RG) e de Bororé (BO), além de análises químicas e morfológicas nas brânquias de zebrafish expostos a estas águas. Amostras de água do RP, do RG e de BO apresentaram altos níveis de fósforo. Águas de BO e as brânquias dos zebrafish expostos a estas águas apresentaram altos níveis de nitrogênio. Águas do RG resultaram em contaminação por potássio nas brânquias. Fósforo, nitrogênio e potássio são indicadores de poluição antropogênica. Águas do RG e as brânquias dos zebrafish expostos a estas águas apresentaram baixos níveis de cálcio, um indicativo de problemas na saúde animal. Águas do RG e de BO resultaram em contaminação por zircônio nas brânquias. Águas de BO induziram espessamento das lamelas respiratórias das brânquias, que deve ser o mecanismo subjacente para a hipóxia observada. Conclui-se que os danos comportamentais, encefálicos e respiratórios observados anteriormente foram induzidos por distúrbios químicos e morfológicos em decorrência de poluição antropogênica na represa Billings.

Palavras-chave: qualidade da água; abastecimento de água; *Danio rerio*; exposição.

INTRODUCTION

The Billings reservoir is located in the metropolitan area of São Paulo, Brazil, and is the biggest reservoir of the state (Hortellani et al., 2013). Rio Grande (RG), Rio Pequeno (RP), Capivari, Pedra Branca, Taquacetuba, Bororé (BO), Cocaia, and Alvarenga rivers are the arms that belong to the narrow central body of the Billings reservoir (Rodrigues et al., 2010). Nowadays, activities such as energy generation, recreation, amateur and commercial fishing, and water supply are commonly performed in the Billings reservoir (Gemelgo et al., 2009; Wengrat and Bicudo, 2011).

Population growth and illegal industrial sewage discharge (especially by automotive and metallurgical industries) contributed to increasing water pollution of the Billings reservoir (Gemelgo et al., 2009; Hortellani et al., 2013). Moreover, the deviation of the Pinheiros and Tietê rivers (Sao Paulo, Brazil) to the Billings reservoir to increase the hydroelectric power have increased the pollution of its waters (Wengrat and Bicudo, 2011). Nevertheless, RG and Taquacetuba arms of the Billings reservoir are used for water supply of 1.6 million people, with the possibility of supplying more than 4.5 million people (Wengrat and Bicudo, 2011; SABESP, 2015b).

In the years 2014-2015 occurred the greatest water collapse ever recorded in the São Paulo state (Sousa and Silva, 2014; Dobrovolski and Rattis, 2015). The Cantareira System, the largest system for the collection and treatment of water in the metropolitan area of São Paulo, used for water supply of 8.8 million people, almost dried up. Low levels of rainfall (Climatempo, 2015; Dobrovolski and Rattis, 2015), increase in water consumption (population growth), loss of about one third of the drinking water between the Basic Sanitation Company (SABESP, Sao Paulo, Brazil) and the households (SABESP, 2016), as well as soil sealing with asphalt were some of the factors that contributed for the hydric collapse, resulting in a shortage of drinking water for the population (Sousa and Silva, 2014).

A more efficient management plan for water supply in the metropolitan area of São Paulo Region is badly needed. The intensification of use of the Billings reservoir is being planned (Brasil, 2015). Billings reservoir is considered to have ten times the capacity as a water reservoir than the Cantareira system and is pointed out by non-governmental organizations as a relevant alternative for water supply of the area (SABESP, 2015a).

The water from three areas of the Billings reservoir intended for human use, as the RP, RG, and BO rivers was previously studied by Leme et al. (2018). RP has been considered an area for water resource (Brasil, 2015). RG is currently used for water supply. BO river is used for commercial/amateur fishing. This study revealed that water samples from these three areas present relevant microbiological contamination for heterotrophic bacteria and *Escherichia coli*. When a zebrafish experimental model was exposed to these RP water samples, it was observed induction of exploratory and locomotor impairments and anxiety-like effects in fish. Water from the BO river induces changes in fish behavior related to hypoxia and alarm reactions. Zebrafish also presents astrogliosis. RG and BO water increase zebrafish lethality rates. Thus, it has been shown that water from RP, RG, and BO rivers have microbiological contaminants, behavior impairments, astrogliosis, and increased lethality in zebrafish (Leme et al., 2018). However, the mechanisms involved in the behavioral and brain impairments found in the animal model previously reported are still unknown.

Zebrafish has been used as an experimental model for chemical toxicology (Hill et al., 2005), including neurotoxicity testing and brain disorders understanding (Bailey et al., 2013; Kalueff et al., 2014), as well as for translational neuroscience, e.g., to understand human physiological biomarkers, neural pathways, and pathological processes (Kalueff et al., 2013, 2014).

Therefore, the objective of the present study was to investigate several chemical parameters of water quality from RP, RG, and BO rivers and relate to the chemical and morphological analyses in zebrafish gills after their exposure to this contaminated water. The present findings may contribute to the Basic Sanitation Company to draw up better action plans for water treatment and distribution.

MATERIAL AND METHODS

Ethics statement

The animals used in this study were maintained in accordance with the guidelines of the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (CEUA-UNIP Permit Number: 374/15). These guidelines are similar to those of the National Institutes of Health, USA.

Billings reservoir water samples

Water samples from RP, RG, and BO (Billings reservoir) were collected in three different areas intended for human use (water and seafood). A detailed map describing the exact sampling sites and additional information is found in the previous study (Leme et al., 2018). One water sample from each area was collected during the rainy season (December); 12-15 m deep.

Procedures of water collection were based on the Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2005). Water was collected in sterile polyethylene 5-liter containers (for fish exposure) and sterile conical tubes containing 1.8% sodium thiosulphate solution (for chemical analysis). Immediately, samples were transferred to UNIP laboratory under light protection and controlled temperature (4 ± 2°C).

Chemical analyses of water samples

The chemical analyses were performed in duplicate up to 24h after each water collection at LABORTECHNIC and CRIRELAB (Sao Paulo, Brazil). The following chemicals (following their respective standardized method) were measured (Eaton et al., 2005): (1) ammonia (NH_3), SM4500-NH_3-C (titrimetric method); (2) cadmium (total), SM3111B (direct air acetylene flame method); (3) calcium, SM3111B (direct air acetylene flame method); (4) chromium (total), SM3111B (direct air acetylene flame method); (5) cobalt (total), SM3111B (direct air acetylene flame method); (6) copper (dissolved), SM3111B (direct air acetylene flame method); (7) iron (total), SM3111B (direct air acetylene flame method); (8) magnesium, SM3111B (direct air acetylene flame method); (9) manganese (total), SM3111B (direct air acetylene flame method); (10) nitrate, SM4500-NO_3 (nitrate electrode method); (11) nitrite, SM4500-NO_2-B (colorimetric method);

(12) nitrogen (total), SM4500-NORG (Kjeldahl methods); (13) phosphorus (inorganic, dissolved), SM4500-P (colorimetric method); (14 and 15) phosphorus (total and total dissolved), SM4500-P (colorimetric method); and (16) zinc (total), SM3111B (direct air acetylene flame method). In addition to the chemical analysis of RP, RG, and BO samples, water samples from UNIP lab were used for comparation (control group, dechlorinated water from SABESP, Sao Paulo, Brazil).

Animals

Thirty-two adult zebrafish (*Danio rerio*) of 3-4 months and 4-5 cm were used for the animal model. They were obtained from a certified breeder (Okani Uwataira-Piscicultura, Sao Paulo, Brazil) and were transferred and acclimated using standard protocols. Housing conditions, zebrafish chow, and maintenance conditions were based on the previous study (Leme et al., 2018).

Chemical and morphological analyses of zebrafish gills

The zebrafish from each group (RP, RG, BO, and control, dechlorinated water from SABESP, Sao Paulo, Brazil, n = 8/group) were maintained together in glass aquariums (20 x 20 x 20 cm, one aquarium per group, aerated by air compressors) with the corresponding water samples. All zebrafish were exposed to such waters for 96h. The gills of the zebrafish (n = 8/group) were collected after spontaneous death (daily observation) or after up to 96h-exposure to each water sample. The gills were evaluated by scanning electron microscopy (SEM) under 10-kV working conditions and by energy-dispersive X-ray spectroscopy (EDX, JSM 6510, JEOL, Tokyo, Japan) as previously reported (Dalboni et al., 2019). The same procedure was used for both SEM and EDX analyses. Briefly, the gills were fixed in 10% buffered formaldehyde for 12h, washed and dehydrated in an increasing alcoholic graduation. The gills were then processed to the critical point with hexametildisilazane (HMDS) and filtered. After complete drying, the samples were assembled on stubs using a conductive tape and then metalized with gold. The metallization (Desk V, Denton Vacuum, Corporate Headquarters Denton Vacuum, Moorestown, NJ, USA) of non-conductive biological samples increases the surface conductivity after the thin layer of gold deposition on the reentrances and prominences of the surface of the samples. Both gills from each zebrafish (from the four groups) were qualitatively blindly evaluated by a pathologist for morphology; and quantitatively evaluated for the percentage of atom of chemical element per particle. The EDX methodology tracks for all known chemical elements. In the case of the samples in the present study, the following elements were found and studied: aluminum, calcium, carbon, niobium, nitrogen, oxygen, potassium, silicon, and zirconium. At least four fields of view were evaluated for each gill; the mean was used for analysis to avoid overestimated statistical results.

Statistical analysis

Homogeneity and normality were verified using the Bartlett's and Shapiro-Wilk tests. One-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test were used to compare parametric data of the chemical analyses in gills. The results are expressed as the mean ± standard error (SE). In all cases, the results were considered significant at $p < 0.05$.

RESULTS

Table 1 shows the chemical analyses of RP, RG, BO, and control waters. Ammonia, cadmium, chromium, cobalt, copper, iron, manganese, nitrate, nitrite, and zinc presented values within the limits of the reference values (Brasil, 2005, 2011; USEPA, 2018) for all the four water samples. Although calcium has no reference values, RG water presented low levels of calcium in relation to control water (26% less). Although nitrogen has no reference values, RP, RG, and BO waters presented high levels (compared to control water). BO water presented high levels of nitrogen (39% more than control water). Phosphorus (total) presented values above those established by the reference values in samples from all studied areas of the Billings reservoir. Control sample were within the limits of the reference values. Thus, the water samples from the three studied areas of the Billings reservoir presented chemical variations compared to control water, including low levels of calcium in RG water, high levels of nitrogen in BO water, and high levels of phosphorus in all studied areas of the Billings reservoir.

Figure 1 shows the gills of zebrafish which were exposed to RP, RG, and BO water samples and were evaluated by SEM. Waters from BO induced respiratory lamellae (L) thickening in zebrafish gills. Control water as well as waters from RP and RG did not induce any apparent morphological injury in the gills of the zebrafish.

Figure 2 shows the percentage of atom of chemical elements per particle that were detected by EDX in gills of zebrafish which were exposed to RP, RG, and BO water samples. Calcium ($F(3/23) = 3.68$, $p = 0.0267$) and nitrogen ($F(3/27) = 4.21$, $p = 0.0145$) levels were affected by Billings reservoir water samples exposure. Specifically, RG water decreased calcium levels in the gills of the zebrafish, compared with the control group. BO water increased nitrogen levels in the gills of the zebrafish, compared with the other three groups.

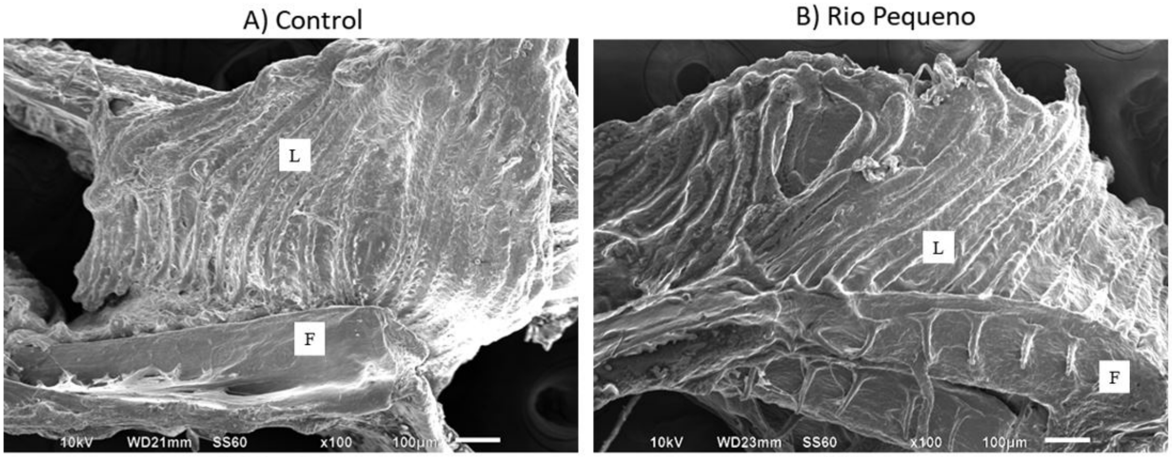
The other chemical elements that were detected by EDX were found to be similar among the four groups: carbon, oxygen, niobium, aluminum, and silicon (Figure 2 C). In relation to potassium and zirconium, only the exposure to RG water resulted in detectable potassium values in the zebrafish gills (0.81 ± 0.27% of atom per particle). In the same way, only the exposure to RG and BO waters resulted in detectable zirconium values in the zebrafish gills (1.41 ± 0.31 and 1.76 ± 0.25% of atom per particle, respectively).

Thus, the gills of the zebrafish exposed to Billings reservoir water samples (RG e BO) presented chemical variations compared to control water, including low levels of calcium after RG water exposure, high levels of nitrogen after BO water exposure, as well as potassium presence after RG water exposure, and zirconium presence after RG and BO water exposure.

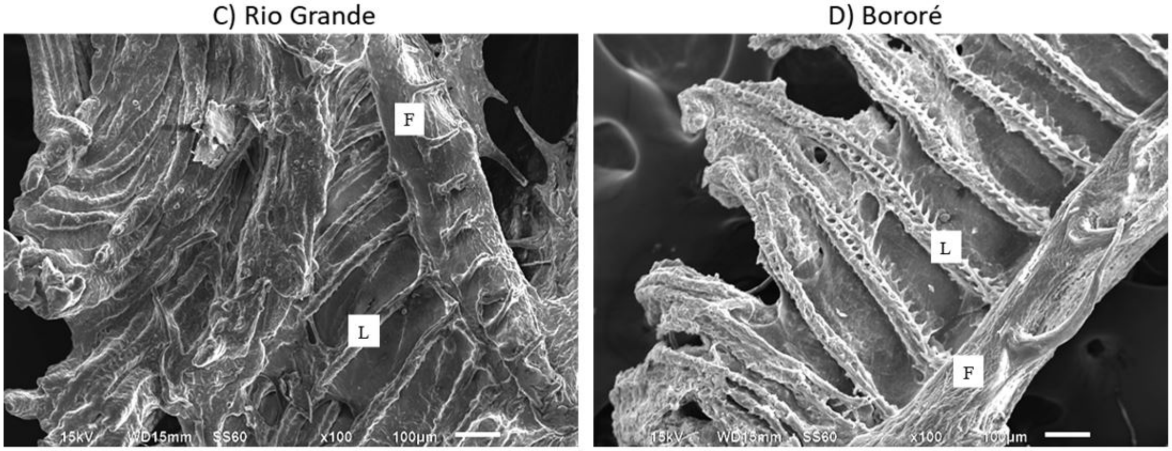
Table 1. Chemical analyses of Rio Pequeno (RP), Rio Grande (RG), and Bororé (BO) - Billings reservoir - water samples and water of control group (dechlorinated water from SABESP).

Variable	Control	RP	RG	BO	MCL#
Ammonia (mg L^{-1})	<0.41	0.44	0.49	0.52	1.50
Cadmium total (mg L^{-1})	<0.0013	<0.0013	<0.0013	<0.0013	0.005
Calcium (mg L^{-1})	15.24	19.33	11.27	18.37	no limit listed
Chromium total (mg L^{-1})	<0.019	<0.019	<0.019	<0.019	0.10
Cobalt total (mg L^{-1})	<0.001	0.004	0.003	0.005	0.2
Copper dissolved (mg L^{-1})	<0.03	<0.03	0.04	<0.03	2.0
Iron total (mg L^{-1})	<0.08	0.08	0.09	0.17	0.30
Magnesium (mg L^{-1})	1.3	2.22	1.41	2.29	no limit listed
Manganese total (mg L^{-1})	<0.02	0.02	0.02	0.03	0.05
Nitrate (mg L^{-1})	<1.0	1.3	1.4	1.5	10.0
Nitrite (mg L^{-1})	<1.0	<1.0	<1.0	<1.0	1.0
Nitrogen total (mg L^{-1})	<0.41	0.46	0.52	0.57	no limit listed
Phosphorus inorganic, dissolved (mg L^{-1})	<0.02	0.03	0.05	0.06	no limit listed
Phosphorus total, dissolved (mg L^{-1})	<0.02	0.04	0.06	0.07	no limit listed
Phosphorus total (mg L^{-1})	<0.02	0.07*	0.11*	0.13*	0.02
Zinc total (mg L^{-1})	0.01	0.13	0.25	0.03	5.0

maximum contaminant level: the highest level of a contaminant that is allowed in drinking water, which is based on reference values found at the resolutions of the United States Environmental Protection Agency (USEPA, 2018), CONAMA number 357 (Brasil, 2005), MS number 2914 (Brasil, 2011), and Standard Methods (Eaton et al., 2005); * above reference values.


A) Control

B) Rio Pequeno


C) Rio Grande

D) Bororé

Figure 1. Scanning electron microscopy in gills. Effects of exposure to (B) Rio Pequeno, (C) Rio Grande, and (D) Bororé (Billings reservoir), and (A) control water samples on gills of adult zebrafish evaluated by scanning electron microscopy. Photomicrographs of gills arches (F) and gills filaments and respiratory lamellae (L) of zebrafish (n = 8 per group).

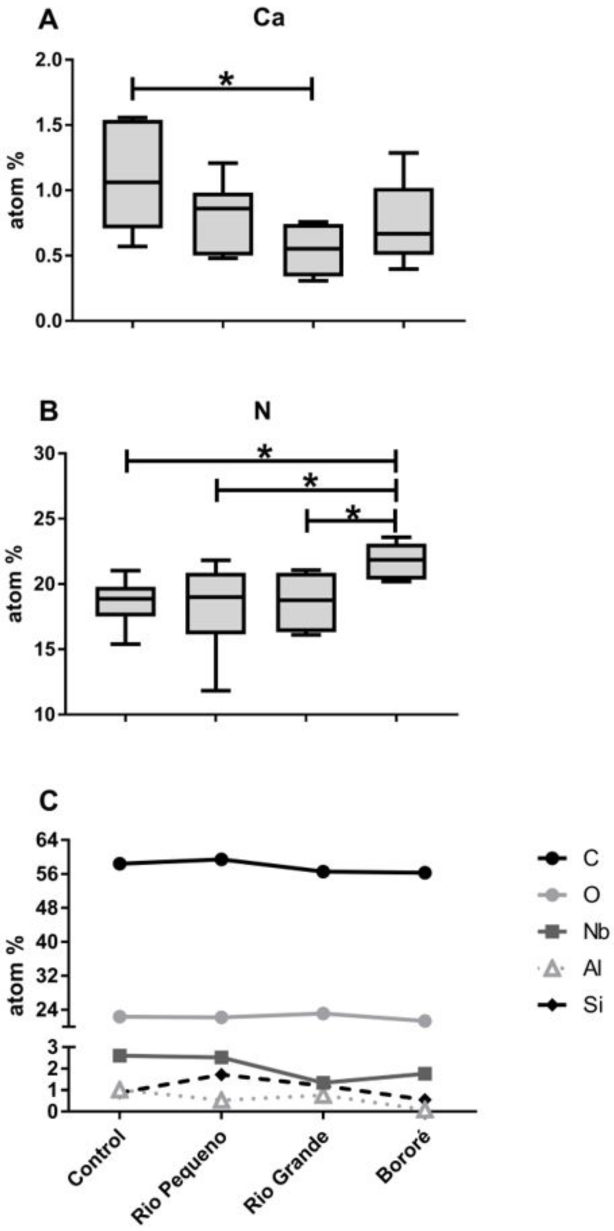


Figure 2. Effects of water samples from the rivers Rio Pequeno, Rio Grande, and Bororé (Billings reservoir) on gills of adult zebrafish evaluated by energy-dispersive X-ray spectroscopy (n = 4-8 per group). (A) Calcium (Ca); (B) nitrogen (N); and (C), carbon (C), oxygen (O), niobium (Nb), aluminum (Al), and silicon (Si). * p <0.05 (one-way ANOVA followed by the Newman-Keuls test). (A) and (B): the data are expressed as the mean, median, minimum, and maximal values (box splot); (C) the data are expressed as the mean.

DISCUSSION

It has been shown that Billings reservoir water samples (RP, RG, and BO), which serves for human consumption, present microbiological contamination and induce several brain and behavioral impairments in zebrafish, as well as an increase in zebrafish lethality (Leme et al., 2018). In the present study, chemical analyses on these water samples, as well as chemical and morphological analyses on gills of zebrafish exposed to those waters were performed to understand the mechanisms involved with the impairments found in the animal model.

The water samples from RP, RG, and BO rivers presented high levels of phosphorus. It is known that wastewater from urban and industrial sewage are import sources of phosphorus (Huang et al., 2018; Liu et al., 2018). The use of phosphorus detergent as well as phosphate fertilizers are also major causes for the high total phosphorus content in water reservoirs (Liu et al., 2018). Indeed, Billings reservoir receives industrial and domestic sewage (Gemelgo et al., 2009; Hortellani et al., 2013). Phosphorus is stored in water in soluble state, which combines with other compounds, changing into insoluble magnesium salt, calcium salt, and ferric salt. When phytoplankton consumes phosphorus, the deposited form of phosphorus is progressively released, changing the phosphorus balance. Phosphorus is the limiting factor of phytoplankton in the tributaries (Liu et al., 2018). The present findings of high levels of phosphorus in Billings reservoir were largely attributed to the overuse of anthropogenic phosphorus within the watershed.

BO water as well as the gills of the zebrafish exposed to BO water presented high levels of nitrogen. BO water samples and gills samples, respectively, presented 39% and 17% more nitrogen than their control samples. There are several possible nitrogen sources in the reservoirs, including urban domestic sewage, industrial wastewater discharges, agricultural nitrogen fertilizer, native soil organic, atmospheric nitrogen, and animal feedlots (Nestler et al., 2011). Therefore, nitrogen as well as phosphorus is considered a chemical marker of pollution (Liu et al., 2018). Excessive nitrogen discharge induces severe environmental problems, such as eutrophication and anoxia, also affecting human health (Zheng et al., 2017). High nitrogen, nitrites, and/or nitrates concentration in drinking water are related with the manifestation of cancer, diabetes, methemoglobinemia, thyroid disorders, and spontaneous abortions (Gao et al., 2012; Zheng et al., 2017). Thus, the uptake of nitrogen by zebrafish gills following exposure to polluted BO water may contribute to the brain and behavioral impairments found in zebrafish in (Leme et al., 2018) experiments.

RG water resulted in potassium contamination in the zebrafish gills. Together with phosphorus and nitrogen, potassium is also considered an indicator of anthropogenic pollution (Niagolova et al., 2005; Liu et al., 2018). Potassium fertilizers usage for agricultural activity as well as urban activity are import sources for the high potassium content in water reservoirs (Wayland et al., 2003). RG water was polluted, and the presence of polluting agents such as phosphorus and potassium seemed to be harmful to the brain and behavior of zebrafish exposed to those waters, as shown by (Leme et al., 2018).

RG water as well as the gills of the zebrafish exposed to RG water presented low levels of calcium. RG water samples and gills samples, respectively, presented 26% and 52% less calcium than their control samples. Calcium is a macronutrient present in the vertebrate body, which has several biological functions, such as regulation nerve impulses transmission, regulation of cell permeability, regulation of muscle contraction and myocardium activity, bone development, and blood clotting (Quattrini et al., 2016). Frequent calcium intake is considered vital for skeletal health and other non-skeletal body systems, such as the nervous system, muscle, and blood system (Heaney, 2006), as well as to improve acid-base balance in the body (Roux et al., 2004). High-calcium mineral waters are valuable dietary sources of calcium and are prescribed as good low-calorie calcium supplements (Bacciottini et al., 2004). Incidentally, regular use of water rich in calcium improves spine mineral concentrations (Costi et al., 2014) and femoral bone (Aptel et al., 1999) densities, and it has a protecting effect for gastric cancer (Yang et al., 1998) and breast cancer (Yang et al., 2000), and for low birth weight (Yang et al., 2002). Similarly, calcium-deficient water is considered a great risk to aquatic life (van Dam et al., 2010). Thus, the small amount of calcium found in RG water samples and in the gills of the zebrafish exposed to RG appear to be responsible, at least in part, for the brain and behavioral impairments found in zebrafish (Leme et al., 2018).

However, it is important to mention that some South-American continental aquatic ecosystems present low levels of calcium (Esteves, 2011) and that this is not necessarily an evidence of aquatic disturb. The present results based its conclusions on comparison with a control group to understand the experimental model. For additional conclusions about water and fish health, additional studies should be proposed.

Although control and RP water exposure did not result in any detectable zirconium level, zirconium was detected in gills of zebrafish exposed to RG and BO waters. In fact, it has been previously shown that neither control nor RP water exposure substantially affect brain and behavior of zebrafish, i.e., no biological effect. On the other hand, RG and BO waters induced behavioral impairments, astrogliosis, and increased the lethality rates of zebrafish (Leme et al., 2018). Zirconium is a transition metal with both stable and radioactive isotopes and resembles titanium in physical-chemical properties (Ghosh et al., 1992). Because of its valuable properties, such as corrosion-resistance, hardness, and permeability to neutrons, zirconium is extensively and increasing used in several chemical industry processes and in nuclear reactors (Shahid et al., 2013). Thereat, zirconium is nowadays more found in soil and water (Dou et al., 2011; Shahid et al., 2013), being considered an environmental pollutant associated with anthropogenic activities (Abollino et al., 2002). The present findings of zirconium in gills of zebrafish exposed to RG and BO waters reinforce the severity in Billings reservoir pollution. Moreover, zirconium may contribute to the brain and behavioral impairments previously found (Leme et al., 2018).

It is known that fish are widely used in toxicity tests, as they can metabolize, concentrate, and store pollutants, thus presenting potential characteristics in the etiology of several diseases and mutagenesis processes (Martinez-Sales et al., 2015). In fact, heavy metals act as endocrine disruptors in fish (Ismail et al., 2017). Aluminum, cadmium, and copper can interfere with estrogenic hormones in fish, being potentially toxic to the reproductive system, resulting in reduced reproductive success (Rodrigues et al., 2019). Moreover, numerous deformities, including in the spine, are attributed to heavy metals such as cadmium and chromium (Sisinno and Oliveira-Filho, 2013). Another example occurs after acute ammonia poisoning, when fish presents hyperventilation, hyperexcitability, seizures, loss of balance, coma, and death (Twitchen and Eddy, 1994). This acute toxicity is mostly due to its effect on the central nervous system. Subsequently, all biological functions such as osmoregulation, respiration, and excretion are rapidly affected (Person Le Ruyet et al., 1998). Lastly, it is documented that zirconium (oxide nanoparticles) induces developmental toxicity in zebrafish embryos, including mortality, hatching delay, and malformations (Karthiga et al., 2019).

The morphological analysis by SEM of the gills of zebrafish which were exposed to Billings reservoir water samples revealed that BO waters induced respiratory lamellae thickening. The tissue lesions were mainly of first degree, which are related to reversible functional impairments in the gills. Direct contact of the gills with water facilitates the interaction with toxic substances, being considered a primary target organ for toxicity. It has been previously shown that specifically BO waters, but not RP neither RG water, induced behavioral impairments in zebrafish correlated to respiratory injuries and hypoxia. BO water decreased bottom time and increased surfacing time of zebrafish (Leme et al., 2018), which are parameters considered as indicatives of distress, such as toxicity or hypoxia (Kalueff et al., 2013). Indeed, within 8h and 24h after experimentally induced-hypoxia, the gills became dramatically affected, including expansion of lamellar respiratory surface area, elongation of respiratory lamellae, filament epithelial thickness reduction, and reduction in epithelial water-blood diffusion distance (Matey et al., 2008). Therefore, the present findings of morphological injuries in the gills of zebrafish exposed to BO waters morphologically explain that BO waters induced hypoxia in the zebrafish.

CONCLUSIONS

The study of the waters from Billings reservoir currently used for water supply and fishing revealed that these areas present several pollution indicators. The water samples from RP, RG, and BO rivers presented high levels of phosphorus. BO water and the gills of the zebrafish exposed to BO water presented high levels of nitrogen. RG water resulted in potassium contamination in the zebrafish gills. Phosphorus, nitrogen, and potassium are considered indicators of anthropogenic pollution and were associated with the previous data of microbiological contamination in these areas. RG water and fish gills exposed to this water presented low levels of calcium, which might be an indication of chemical imbalance that could lead to health problems in aquatic animals. Both RG and BO waters resulted in zirconium contamination in the zebrafish gills. Finally, BO waters induced respiratory lamellae thickening in the gills of zebrafish, revealing that it induced hypoxia in the zebrafish. The results obtained in this study (chemical analysis of the water and chemical and morphological analysis of the gills) reveals that the combination of behavioral, brain, respiratory, and morphological effects observed in zebrafish analyzed were induced by chemical disturbances in water due to anthropogenic pollution in the Billings reservoir. The governmental authorities should adopt more rigorous control policies for the disposal of sewage in the Billings reservoir as well as they should adopt specific and effective water treatment techniques to the chemical elements that were revealed to be affected in the present study.

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