An evaluation of the ecotoxicity of surface waters of Pernambuco Rivers (Brazil)

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,

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ABSTRACT

The contamination of continental aquatic ecosystems has become a major concern for society, and there is currently a vast literature on the impacts caused by different types of contaminants on these ecosystems. In Brazil, despite the importance that water resources play for the country's development, the quality and quantity of river waters have been increasingly affected by the disordered occupation of the hydrographic basins. In the present work, water samples from two hydrographic basins of Brazilian rivers were analyzed to evaluate the water quality and ecotoxicity status. Results of surface waters from GL-1 Botafogo basin, i.e., sampling points P9 and ETAB2, showed toxic effects. A significant increase in zebrafish (*Danio rerio*) embryo mortality and development anomalies, as well as a decrease in the hatching rate and larvae total length were observed for the ETAB2 sample. In sample P9, a significant increase in the rate of embryonic development anomalies at 48 hpf and in the larvae total length at 96 hpf were observed. Results from Bita and Utinga basin, i.e., sampling points U1, U2, U3, ETAS1 and ETAS2, showed similar significant alterations in zebrafish embryo development, hatching rate, and larvae length. Overall, this study further supports the use of zebrafish embryo bioassays as a fast-high throughput approach for screening the toxicity of water samples, evidencing the potential impacts of organism's exposure on these ecosystems.

Keywords: Ecotoxicity, surface waters, embryo bioassays, zebrafish (*Danio rerio*), environmental contaminants.

RESUMO

A contaminação de ecossistemas aquáticos continentais tem se tornado uma grande preocupação para a sociedade, existindo, atualmente, uma vasta literatura sobre os impactos causados por diferentes tipos de contaminantes nesses ecossistemas. No Brasil, apesar da importância que os recursos hídricos desempenham para o desenvolvimento do país, a qualidade e a quantidade das águas dos rios têm sido cada vez mais afetadas pela ocupação desordenada das bacias hidrográficas. No presente trabalho, amostras de água de duas bacias hidrográficas de rios no Brasil foram analisadas de modo a avaliar a qualidade da água e o seu potencial ecotoxicológico. Os resultados dos ensaios com as amostras de água superficial da bacia do Botafogo, ou seja, os pontos de amostragem P9 e ETAB2, evidenciaram efeitos tóxicos. Um aumento significativo na mortalidade embrionária e anomalias de desenvolvimento do peixe-zebra (*Danio rerio*), bem como uma diminuição na taxa de eclosão e comprimento total das larvas foram observados para a amostra ETAB2. Na amostra P9, foi observado um aumento significativo na taxa de anomalias de desenvolvimento embrionário a 48 hpf e no comprimento total das larvas a 96 hpf. Os resultados para a bacia de Bita e Utinga, ou seja, pontos de amostragem U1, U2, U3, ETAS1 e ETAS2, mostraram alterações significativas similares no desenvolvimento embrionário, taxa de eclosão e comprimento das larvas de peixe-zebra. Globalmente, este estudo suporta o uso de bioensaios com embriões de peixe-zebra como uma abordagem rápida e de alto rendimento para rastrear a toxicidade de amostras de água, evidenciando os potenciais impactos da exposição dos organismos nesses ecossistemas.

Palavras-chave: Ecotoxicidade, águas superficiais, bioensaios com embriões, peixe-zebra (*Danio rerio*), contaminantes ambientais.

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ABBREVIATIONS AND ACRONYMS

APAC	Pernambuco Water and Climate Agency
BOGA	Bioterium of Aquatic Organisms of CIIMAR
CIIMAR	Interdisciplinary Centre of Marine and Environmental Research
COMPESA	Pernambuco Company for Sanitation
CONAMA	Brazil National Environment Council
CPRH	Pernambuco State Environmental Agency
DBPs	Disinfection By-products
DO	Dissolved oxygen
EC'S	Emerging contaminants
FET	Fish embryo acute toxicity test
GL-1/GL-2	Group of Small Coastal Basins
hpf	hours post-fertilization
NORMAN	Network of reference laboratories, research centers and related organizations for monitoring of emerging environmental substances
OCDE	Organization for Economic Co-operation and Development.
RAS	Recirculating Aquaculture Systems
SUAPE	Port and Industrial Complex of Pernambuco
US EPA	United States Environmental Protection Agency

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1. INTRODUCTION

1.1 Water quality

The use of water by the human society accrues from their personal needs, economic and social activities. In this context, quality is a fundamental aspect, especially when referring to water main uses, as for human supply. Water quality is defined in terms of its chemical, physical, and biological contents. The water quality of rivers and lakes changes with seasons and geographic areas, even when there is no pollution present. Guidelines for water quality provide basic scientific information about quality parameters and ecologically relevant toxicological threshold values to protect specific water uses (Lawson, 2011).

The utilization of water resources has suffered significant restrictions as consequence of the natural and anthropogenic impacts on rivers, which alter the quality and quantity of available water. (Souza *et al.*, 2014; Medeiros *et al.*, 2016). Human activities, resulting from land use and occupation, and industrial activities are the main factors responsible for water pollution. These actions generate effluents with a diversity of pollutants, compromising continental water bodies (rivers, lakes, and reservoirs), which constitute their final destination. In fact, these effluents comprise about 50% of the planet's polluting load, requiring special attention regarding environmental legislation (Sodré *et al.*, 2007). In effect, contamination of aquatic matrices has occurred in many water supplies, turning it a great challenge for the producing systems and water distributors, as well as for public health (Carvalho Filho, 2019).

The evaluation of water quality is traditionally focused on the determination of nutrients, microbiological tests, and the concentration of priority pollutants, such as: pesticides, hydrocarbons, and industrial chemical compounds. However, with the advance of analytical techniques, it was possible to evidence a series of other organic contaminants present in surface and wastewaters, occurring in concentrations of the order of ng/L to µg/L (Pal *et al.* 2014; Groselli, 2016).

1.2 Water quality in Brazil

Brazil has a privileged position regarding the availability of water resources; about 12% of the planet's fresh water is contained in Brazilian territory, however, these resources are distributed irregularly across the country (Americo-Pinheiro, *et al.*, 2017). Further, despite the importance that water resources yield for the country's regional development, the quality and quantity of river waters have been increasingly affected by the disorderly occupation of river basins (Rodgher *et al.*, 2005; Medeiros *et al.*, 2016).

In Brazil, regulatory standards have been established, aiming to: i) preserve, improve and recover environmental quality; ii) ensure conditions for socio-economic development, and iii) protect the dignity of human life (Silva, 2015). Brazilian environmental laws require toxicity analyzes to be carried out, in conjunction with physico-chemical analysis, to address water quality. Due to their geographical and topographic characteristics, in Brazil, the river basins (reference unit adopted for monitoring surface water quality) are generally defined as the area where water is captured (drainage) for a main river and its tributaries (CPRH, 2017). In chapter IV of Resolution no. 357/430 of the National Environment Council (CONAMA), regarding the conditions and standards for the discharge of effluents, it is established in § 1 and 2 of article 34 that the effluent shall not cause or have the potential to cause toxic effects to aquatic organisms in the receiving body and that the toxicity criteria should be based on results of standardized ecotoxicological tests using aquatic organisms (Costa *et al.*, 2008).

Bearing in mind Brazilian great demand of water for urban supply and the aim to monitor the region´s water quality, as well as generating information to subsidize water resources policies with the government, the adequate assessment of surface waters quality and contamination is indispensable.

1.3 Chemical assessment

1.3.1 Water physico-chemical parameters

Important physical and chemical parameters influencing the aquatic ecosystems are temperature, pH, salinity, dissolved oxygen (DO), and carbon dioxide. Others include total suspended and dissolved solids, total alkalinity and acidity, and heavy metal contaminants. These parameters are the limiting factors for the survival of aquatic organisms (flora and fauna) (Lawson, 2011). The temperature affects the metabolic activity, growth, feeding, reproduction, distribution, and migratory behaviors of aquatic organisms (Odum, 1971; Largler *et al*., 1977; Boyd, 1979; Suski *et al*., 2006; Lawson, 2011). Hydrogen ion concentration or pH, as one of the vital environmental characteristics, influences the survival, metabolism, physiology, and growth of aquatic organisms. pH may be affected by total alkalinity and the acidity of the bottom sediments, run off from surrounding rocks and water discharges (Lawson, 2011). On the other hand, the DO affects the solubility and availability of nutrients. Its low levels can result in alterations of the oxidation state of substances, from the oxidized to the reduced form, thereby increasing the levels of toxic metabolites. (Lawson, 2011). Dissolved carbon dioxide in the aquatic environment, an important parameter in primary production and phytoplankton biomass, increases with decreased DO (Lawson, 2011).

The Water Directive of the European Commission establishes classification criteria for good ecological status in rivers. Table 1 shows the maximum values for the main physical-chemical parameters.

Table 1. Maximum water allowable values of nitrates, phosphates, ammonia, pH, and dissolved oxygen (DO)

Parameters	Acceptable values
Nitrates	≤ 25 mg/L
Phosphate	≤ 0.10 mg/L
Ammonia	≤ 1.0 mg/L
pH	6-9
DO	≥ 5 mg/L

Source: Ministry Environment-Portugal, 2009.

Also, Brazilian legislation establishes classification criteria for water quality. Brazilian waters classified as Class II are waters that can be destined to: a) the supply for human consumption, after conventional treatment; b) the protection of aquatic communities; c) the recreation of primary contact, such as swimming, water skiing and diving; d) irrigation of vegetables, fruit plants and parks, gardens, sports and leisure fields, with which the public may come into direct contact; and e) aquaculture and fishing). Table 2 shows parameters and conditions of water quality within class II.

Table 2. Maximum water allowable values of nitrites, nitrates, pH, and dissolved oxygen (DO) from CONAMA Nº 357/430 - Water quality.

Parameters	Acceptable values
Nitrites	≤ 25 mg/L
Nitrates	10 mg/L
pH	6-9
OD	> 6 mg/L

Source: CONAMA Resolution 357/430, Brazil, 2011

1.3.2 Emerging contaminants

The emerging contaminants (ECs) are particularly important among the organic pollutants. ECs such as: algae and cyanobacterial toxins, flame retardants, insecticides, chemical additives, personal care products, drugs of abuse, and pharmaceutical compounds, stand out (NORMAN). The ECs can reach the natural environments through runoff from rural areas, or even through the supply of treated or untreated wastewater effluents (Ferrey *et al.*, 2018; Freitas, 2018). For example, one of the main contributions of pharmacological ECs to aquatic ecosystems comes from the effluents from wastewater treatment plants (WWTP), due to the fact that the majoritary of the applied treatments at these facilities

cannot eliminate these contaminants (Farré *et al.*, 2018). Although ECs exist, typically, in low concentrations in surface waters, there is a growing concern about them, due to the evidence of biological effects at sub-lethal concentrations (Pal *et al.*, 2014). Furthermore, in addition to the possibility of interaction with other substances, once in the aquatic ecosystems, ECs are subjected to biotransformation processes, which may generate by-products with greater toxic potential than the parent compounds (Ferrey *et al.*, 2018; Freitas, 2018).

1.3.3 Heavy metals

Among inorganic pollutants, heavy metals are particularly relevant, differing from organic pollutants since they are not subjected to biodegradation processes. In the aquatic environment, metals occur in different forms, such as: i) dissolved in an ionic form, ii) linked to organic and inorganic complexes, iii) associated with particles with different sizes or molecular weights (e.g., inorganic or organic colloids), iv) retained in the sediment, or v) incorporated into the biota (Salomons and Förstner, 1984). Generally, metals are present in the aquatic ecosystems in low concentrations, due to soil erosion, causing no harmful effects to the health of the organisms. However, metals can be released from suspended particles and sediments into the water, leading to additional contamination of the aquatic environment, potential reaching toxic levels (Nabinger, 2017). In fact, although some metals have biological functions (in low concentrations), heavy metals are generally not metabolizable, which means that they remain in a cumulative character along the food chain (bioaccumulation), which can cause toxicity, according to increased concentration and exposure time (Pellegrini *et al*., 1999; Wang and Slack, 2000; Albuquerque, 2017). The evaluation of the concentration of heavy metals in the environment has been the focus of several studies, due to the characteristics of some elements, which can cause adverse effects on the vital systems of organisms, resulting, for example, in changes in enzymatic processes, accumulation in tissues, or even changes in density, community structure and composition of species and populations (Wang and Slack, 2000). According to the US Environmental Protection Agency (US EPA), arsenic, cadmium, mercury and lead are the heavy metals which are most toxic to the environment and humans (Govind and Madhuri, 2014).

1.4 Biological assessment

1.4.1 Ecotoxicology

The term ecotoxicology was first coined in 1969 by the Professor R. Truhaut, who defined it as “a science describing the toxic effects of various agents on living organisms, especially

on populations and communities within ecosystems". The essence of ecotoxicology lies in two main areas: a study of the environment with origins in the science of ecology; and a study of the interactions of toxic chemicals with individual living organisms – the science of toxicology (Connell *et al.* 2009).

The role of ecotoxicology for the better understanding of the effects of pollutants on organisms is highlighted, and, in particular, that of aquatic ecotoxicology, since water bodies are the main receptors of pollutants, either by indirect or direct discharge (Costa *et al.*, 2008; Araújo & Souza 2013). For a better understanding of the impacts of pollutants on living beings, it is important to note that studies with traditional physico-chemical analyses are unable to distinguish between inert substances and substances that affect biological systems. Contaminant analysis of water can be an element of integrated monitoring and assessment, where chemical and biological effects measurements are combined, to assess potential harm to living resources. The role of chemical measurements in integrated monitoring programs is to help in the identification of the chemical causes responsible for the observed biological effects (Soares *et al.*, 2018).

Ecotoxicological bioassays consist of the exposure of test organisms in controlled conditions to matrices (e.g. water, sediment) whose toxicity is intended to assess, either in the laboratory or under field conditions, and the measurement of ecologically-relevant quantitative responses (Martinez-Haro *et al.*, 2015; Soares *et al.*, 2018). Toxicity tests are important to assess the potential impact and environmental risk of pollutants, since chemical analyses alone do not allow this type of evaluation. In fact, biological effects methods, namely bioassays and biomarkers, are proposed to contribute to the smooth integration of the chemical and biological information, allowing a better understanding of cause-effect relationships (European Commission, 2009, 2010).

1.4.2 Zebrafish (*Danio rerio*) embryo bioassays

Since the embryonic development allows to better capture chemical toxicity, due to the many biological process occurring, embryo bioassays have been proposed as an alternative to juveniles/adults and long life cycle animal tests (Van Leeuwen *et al.*, 1985; Versonnen and Janssen, 2004). Also, the European Directive - European Directive 2010/63 / EU (EU, 2010) - requires the application of the 3R's principle in the use of animals for scientific purposes: replacement, reduction, and refinement, emphasizing the urgency to develop validated alternative methods. In this context, embryonic bioassays are increasingly used as an alternative to standardized toxicity tests with juveniles and adults, minimizing the number of live organisms used and complying with the statutes that regulate the use of animals in trials (Alves, 2017).

Further, pollutant induced behavioral modifications may serve as a more subtle readout for ecological and physiological fitness (Gerhardt 2007; Gaaied et al. 2020). Behavioral analysis can be considered as a robust and sensitive tool for detecting sub-lethal effects of chemical on fish (Kane et al. 2004; Eissa et al. 2010; Gaaied et al. 2020).

For an organism to be considered as a biological model it must have technical advantages that facilitate the study of the biological effects, processes and mechanisms involved in an experimental test context (Silva, 2016). Zebrafish (*Danio rerio*) is a validated and standardized model species for ecotoxicological studies (Busquet *et al.*, 2014). Zebrafish has a number of attributes that make it particularly suitable to experimental manipulation: it is a small, robust fish, easy to maintain in laboratory conditions; it can breeds all year round; generation time is short, typically 3-4 months; eggs are large relative to other fish (0.7 mm in diameter at fertilization) and optically transparent. Furthermore, fertilization is external so live embryos are accessible to manipulation and can be monitored through all developmental stages under a dissecting microscope. Also, development is rapid, with precursors to all major organs developed within 36 hpf and larvae displaying food seeking and active avoidance behaviour within five days post-fertilization, i.e. 2-3 days after hatching (Spence *et al.*, 2008).

Figure 1. Peixe zebra (*Danio rerio*). Fonte: https://www.noldus.com/blog/testing-pcbs-toxicity-behavior-zebrafish

1.5 Objectives

In this work a holistic approach, combining chemical and biological assessments, was utilized to assess the quality and ecotoxicity status of surface waters of two hydrographic basins of rivers in the state of Pernambuco, Brazil. We expect by this, to contribute globally to the improvement of the ecotoxicological risk assessment of environmental contaminants, while at the same time support with scientific knowledge to the establishment and improvement of Pernambuco regulatory environmental measures, as generating information to subsidize water resources policies with the local government due to the strategic importance of Pernambuco dams for urban and industrial supply in the region.
Specific objectives include:

1. To evaluate the water physico-chemical parameters of water samples from two hydrographic basins of rivers in the state of Pernambuco, Brazil;
2. Assess biological effects of exposure to the surface waters samples of the two referred hydrographic basins, through the application of zebrafish embryo bioassays;
3. To evaluate the presence/concentration of ECs and heavy metals in the same water samples, inferring a cause-effect relationships.

2. MATERIALS AND METHODS

2.1. Site selection and sampling

2.1.1. Study area

Located in the northeast of Brazil, the state of Pernambuco has thirteen hydrographic basins that are part of the management of water resources. The study area includes two groups of Coastal Rivers Basins, specifically the Botafogo River Basin (Group of Small Coastal Basins - GL-1), located in the northern region of the state of Pernambuco, and the Utinga and Bita River Basin (Group of Small Coastal Basins – GL-2), which is located in the southern region of the State of Pernambuco (Figure 2). The regions for the study were chosen based on their importance for water urban supply, considering that they are regions with a history of contamination and presence of chemical industries, sanitary landfills, and sugar-alcohol mills.

Figure 2. State of Pernambuco, Brazil, GL1 and GL2 River Basin groups. http://www.apac.pe.gov.br/monitoramento/.

2.1.1.1. GL1 Botafogo river basin

According to the Pernambuco Water and Climate Agency – APAC, the Botafogo river basin is part of the GL-1 and is one of the main basins of this group. The Botafogo River, with about 51 km, has the largest extension basin, in addition to being the most important for the supply of water to the Metropolitan Region of Recife. The Botafogo reservoir is the only one in the GL-1 group, with a maximum capacity above 1 million m^3. Eleven samples were collected (P1, P2, P3, P4, P5, P6, P7, P8, P9-Botafogo river; ETAb1, ETAb2-entrance and exit of Botafogo water treatment plant, respectively) (Figure 3) along the GL-1 basin.

Figure 3. Collection points at GL-1 group - Botafogo. Google earth.

Upstream of P6 and downstream of P7 points, there is a chemical industry for chlorine and soda that uses mercury in the electrolysis process. This industry has been installed in the region since the 1970s. Therefore, there are studies that report cases of contamination by mercury in river sediments in this region. Points P8 and P9 are located upstream and downstream, respectively, of an industrial landfill that receives hazardous waste.

2.1.1.2 GL2 Bita e Utinga river basin

Bita and Utinga are two important dams of the GL-2 basin group that supply the Industrial Complex of Suape and the cities Ipojuca and Cabo de Santo Agostinho. These dams receive water from important rivers in the region. In summer, the Bita dam receives water reinforcement from the Ipojuca river, to ensure water supply during the dry season. The Ipojuca River, one of the most important rivers in the State of Pernambuco, receives wastewater and industrial effluents from sugar-alcohol mills. The water collected from the Bita and Utinga dams is sent to a treatment plant operated by Sanitation Company of Pernambuco (COMPESA). Nine samples were collected (IP1, IP2- Ipojuca river; B2, B3-Bita river; U1, U2, U3-Utinga river; ETAS1, ETAS2-entrance and exit of the SUAPE water treatment plant, respectively) in the region that covers the GL-2 basin group (Figure 4).

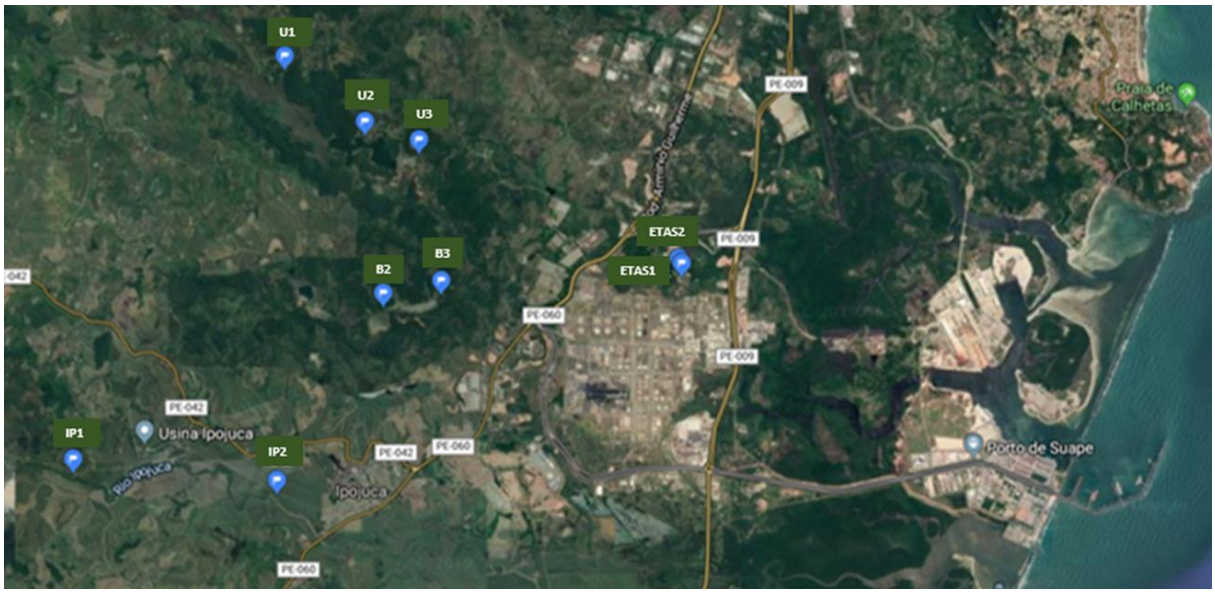

Figure 4. Collection points at GL-2 group – Bita and Utinga. Google earth.

2.1.2 Sample collection

The samples were collected in partnership with the technicians of the laboratory of the State Environmental Agency – CPRH, in December 2019 (summer). At each sampling site the coordinates of the place were taken. Each water sample was collected and stored into 900 mL inert bottles. After collection, the samples were transported in ice and stored at -80 °C, until analysis. For heavy metals analysis, an additional 60 mL of surface water was collected. Blanks were made with Milli-Q water (60 mL). The samples and blanks were filtered through 0.45 µm filters in the CPRH laboratory. After filtration, all samples and blanks (MilliQ water) were acidified with 1% HNO_3, stored in 50 mL falcon tubes and frozen at -80 °C.

2.1.3 Physico-chemical parameters

Part of the collected samples were used (in replicate) to measure the following physico-chemical parameters: ammonium, nitrates, nitrites, pH and DO (Table 3). DO was measured with portable meter Hach HQ40d and pH was measured with Sartorius Basic pH meter PB-11-11. Ammonium, nitrates and nitrites were measured with a Palintest Photometer 7500 measuring equipment and respective reagents of analysis. Previously, the water was filtered, with a vacuum filtration system, through a whatman TM (0.45µm) microfiber glass filter (GE Healthcare Life Sciences) to remove particulate matter. pH correction, when necessary, was performed with a solution of 0.10 M NaOH.

2.1.4 Chemical analysis

This work will be complemented with the chemical evaluation of the collected samples. Chemical analysis will be carried out to detect different ECs and heavy metals in the water

samples, considering the high anthropogenic pressure on domestic and industry activities at the places of collection.

Due to the situation that occurred in this year of 2020 facing the pandemic, there were delays with this assessment. Therefore, the results presented will not include chemical assessments.

2.2 Zebrafish (*Danio rerio*) embryo bioassays

2.2.1. Adult zebrafish

The zebrafish stock was kept at 28 °C ± 1 ºC with dechlorinated and aerated water in a recirculation system, with mechanical and biological filters, under a photoperiod of 14:10 h (light/dark). The animals were fed ad *libitum* twice daily with a commercial diet of Tetramin fish (Tetra, Melle, Germany) (Soares, 2009). Animals were maintained at the Bioterium of Aquatic Organisms (BOGA-CIIMAR) which complies to the guidelines of the General Direction of Veterinary of Portugal (Law decree 113/2013), based on the European animal's directive 2010/63/EU.

2.2.2 Reproduction

One day before reproduction, in the late afternoon, zebrafish females and males (in a proportion of 1: 2) were placed in spawning tanks, with a net bottom, to prevent predation of eggs by the parents, covered with glass balls to stimulate spawning. The breeders were kept under the same temperature and photoperiod conditions as the stock animals. As zebrafish is an early spawner, in the next morning the breeders were removed 30 min after the start of the light period and the eggs were collected and cleaned.

2.2.3 Fish embryo acute toxicity (FET) test

The exposure method was performed according to the OCDE guidelines for the testing of chemicals nº 236 – Fish Embryo Acute Toxicity (FET) test (OCDE, 2013). Newly fertilized zebrafish eggs (n=20) were selected, under a stereomicroscope, and exposed to the water samples (2 mL, renewed daily) in 24-well polystyrene plates (1 egg/well), with four internal controls (exposure to fresh de-chlorinated clean water). In addition to the exposure plates, a control plate was provided with all embryos kept in maintenance water, as mentioned above. The 24-wells plates were incubated at 26 ± 1 °C under the same photoperiod conditions as the zebrafish stock. Every 24 hours, up to four apical observations were recorded as indicators of lethality (coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and lack of heartbeat). Additionally, embryonic malformations were also registered at each time point (24, 48, 72 and 96 hpf).

At the end of the test (96 hpf) sensorimotor reflexes were analyzed (96hpf), by gently touching each larva with a micropipette tip, in the head and in the tail, to register either positive (immediate swimming) or negative responses (no movement upon touch). This procedure was repeated 4-times for each larva (alternating head and tail touches and allowing 30 s rest per larva between each individual touch) to calculate the proportion of positive responses. Posteriorly, the larvae were maintained for 30 minutes at 4ºCfor transitory anesthesia and length measure. The total larvae length was measured from the tip of the face to the tip of the tail. All observations were made under an inverted microscope (Nikon Eclipse TS100) equipped with a digital camera (Nikon D5-Fi2) and a microscope camera controller (Nikon's Digital Sight DS-U3). Each assay was performed twice as previously described [20 sampling points * 2 (40 assays) with an n=40 (4 replicates with 5 embryos per plate *2) for each water sample]. For samples that presented non-standard physico-chemical parameters and significant effects on embryos, the respective physico-chemical parameter was corrected to normal values and a new test was performed. Parameter correction was performed for one sampling point (ETAB2), therefore two additional tests were performed.

2.3 Statistical analysis

Statistical analysis was made in the STATISCA 13.0 program. The data were tested for normality (Kolmogorov-Smirmov) and homogeneity of variances (Cochran C test). The mortality rate, embryonic development anomalies and hatching rate were analyzed by a factorial ANOVA with treatments and hours of embryonic development as independent variables. Post-hoc comparisons were performed using the Tukey HSD multiple comparison test. An ANOVA-one way was used to analyze the total larvae length and sensorimotor reflexes. The level of significance was defined as $p < 0.05$.

3. RESULTS

3.1. Physico-chemical parameters

Table 3 shows the values of the physico-chemical parameters of the surface waters samples at distinct sampling points along Botafogo and Bita and Utinga reservoirs.

Table 3. Water physico-chemical parameters: pH, dissolved oxygen (DO), ammonium (NH_4+), nitrite (NO_2^-) and nitrate (NO_3^-).

Sample	Coordinates	pH	DO (mg/L)	NH_4^+ (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)
P1	-7.844368, -35.035430	6.74	7.57	0.08	0.02	0.63
P2	-7.836819, -35.034153	6.80	7.55	0.07	0.02	0.84
P3	-7.834897, -35.033153	6.11	7.82	0.30	0.00	0.62
P4	-7.816845, -35.017317	6.48	8.20	0.39	0.00	0.61
P5	-7.811278, -35.002927	6.74	7.68	0.05	0.04	0.72
P6	-7.790460, -34.981458	6.71	7.98	0.03	0.01	<<
P7	-7.786303, -34.972655	6.83	7.50	0.01	0.03	<<
P8	-7.708560, -34.950790	5.71	7.79	0.10	0.04	<<
P9	-7.704126, -34.937256	6.31	7.56	0.01	0.01	1.70
ETAB1	-7.853600, -34.938606	6.66	7.42	0.03	0.02	<<
ETAB2	-7.853127, -34.937782	4.13	7.44	0.18	0.00	<<
IP1	-8.396580, -35.102005	6.48	8.13	<<	0.00	0.82
IP2	-8.399541, -35.073031	6.01	7.21	0.01	0.00	0.77
B2	-8.373229, -35.057920	7.10	7.08	0.17	0.00	0.61
B3	-8.371478, -35.049734	6.22	7.66	0.01	0.00	0.86
U1	-8.339792, -35.071964	6.31	7.92	0.09	0.01	0.67
U2	-8.349017, -35.060484	6.22	7.84	0.18	0.02	0.72
U3	-8.351554, -35.052888	6.60	8.08	0.25	0.02	0.41
ETAS1	-8.368243, -35.015866	6.79	7.48	0.06	0.02	0.68
ETAS2	-8.368927, -35.015538	6.12	7.16	0.13	0.01	0.61

All samples showed DO, NO2- and NO3- values within the established by CONAMA (DO ≥ 6 mg/L, NO2- <10 mg/L, NO3- ≤ 25 mg/L) and recommend for zebrafish (DO ≥ 6 mg/L, NO2- <1 mg/L, NO3- ≤ 48 mg/L) (Schneider et al., 2009; OCDE, 2013, CONAMA 357/2005). The P3, P8, P9, ETAB2, IP2, B3, U1, U2 and ETAS2 samples showed a pH below the recommended pH for maintenance of zebrafish (pH 6.5-8.5) (OCDE, 2013). Zebrafish is a robust species and pH toxic values are reported as < 5.9 and > 8.1 (Engeszer et al., 2007). With exception of ETAB2 (pH= 4.13), in the tests performed with these samples, there was no signs of mortality or development toxic effects in zebrafish embryos. Therefore, and as pH values were above the reported toxic values for zebrafish, pH corrections for these samples was not performed. The ETAB2 sample showed significant toxicity in exposed embryos, therefore, the pH correction was performed, with the objective of verifying in the toxic effect was caused by a low pH value. (Engeszer et al., 2007).

Early reported research on the toxic effects of ammonia to fish implicated NH3 as being toxic in values above 0.2 mg/L, and NH4+ being considered nontoxic, due to the fact that most biological membranes are permeable to un-ionized ammonia (NH3) but relatively impermeable to ammonium ions (NH4+). Toxicity, expressed as total ammonia (the sum of NH3 and NH4+) in the environment, increases with water pH as the proportion of NH3 increases. The pH of the ammonia/ammonium reaction is around 9.5 and varies with ionic strength, pressure, and temperature. (Thurston et al. 1981; Randall and Tsui, 2002; Pereria & Mercante, 2018). All samples showed low values of NH4+ and pH values below 9.5, therefore corrections were not made

3.2 Zebrafish (*Danio rerio*) embryo bioassay

3.2.1 GL-1 Botafogo basin

3.2.1.1 Cumulative mortality rate

Cumulative mortality rates of embryos at different stages of embryonic development are shown in Figure 5. Between 24- and 96- hpf, the mortality rate ranged from 2.5 to 40%, within the different samples. ETAB2 sample was significantly different from control at all stages of zebrafish development ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test). Cumulative mortality rates were of 3.75 and 12.5% and 27.5 and 40% for control and ETAB2 at 8 and 96 hpf, respectively. There were no statistical significance differences among the other treatments and hours of development ($p \geq 0.05$; factorial ANOVA).

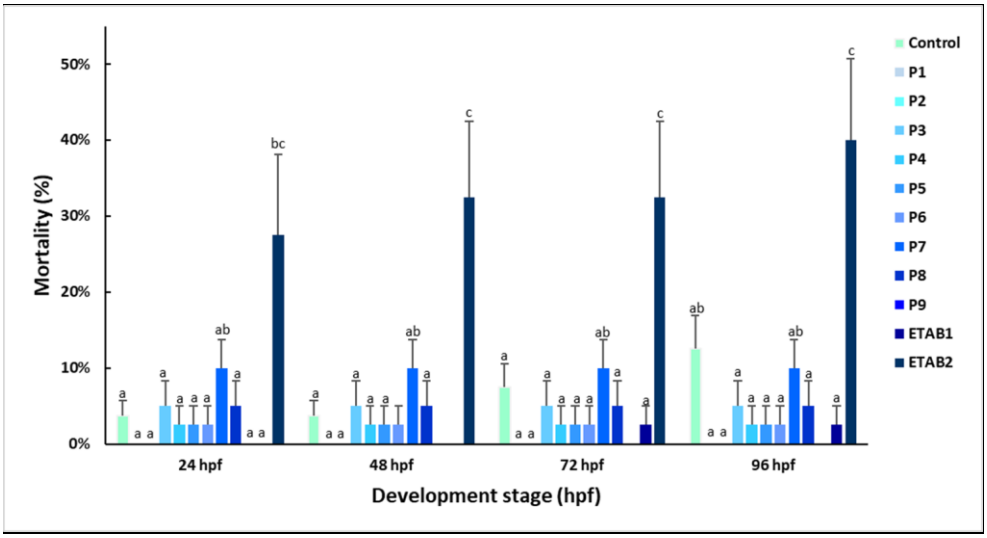

Figure 5. Cumulative mortality rate (%) at different embryonic development stages (24, 48, 72 and 96 hpf) of zebrafish (*Danio rerio*) exposed to river surface water samples and control. Values are presented as mean ± standard error. Different letters indicate significant differences between treatments ($p < 0.01$, factorial ANOVA, followed by Tukey HSD multiple comparison test).

3.2.1.2 Embryonic development anomalies

Abnormal development (%) in embryos (Figure 6) was recorded within each sample during the different embryonic development stages (24, 48, 72 and 96 hpf). At 24 hpf there was a significant increase in embryo anomalies at the ETB2 sample in comparison with the control group ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test)). At 48 hpf a significant increment in the percentage of embryo anomalies was also observed at the P9 sample in comparison with control ($p < 0.01$) The percentage of development anomalies, at 48 hpf, ranged from 1.56% for the control group to 27.5% and 100% for the P9 and ETAB2 samples, respectively. Afterwards, there was a reduction in the percentage of anomalies until the end of the test for the P9 sample whereas for ETAB2 the percentage of development anomalies (100%) was maintained until the end of the test (96 hpf). Furthermore, ETAB2 was significantly different from all other treatments at all zebrafish embryonic development stages. The main development anomalies found included: edema of the yolk-sac and heart, folded tails, and spine curvatures (Figure 7). No statistically significant differences were found for the other treatments in comparison with control at the different stages of embryonic development ($p \geq 0.05$; factorial ANOVA).

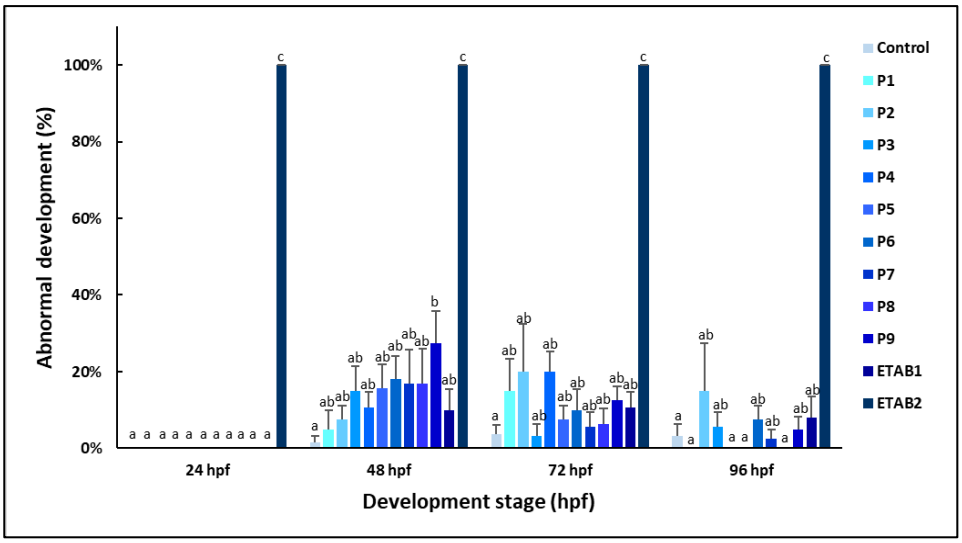

Figure 6. Development anomalies (%) at different embryonic development stages (24, 48, 72 and 96 hpf) of zebrafish (*Danio rerio*) exposed to river surface water samples and control. Values presented as mean ± standard error. Different letters indicate significant differences between treatments ($p < 0.01$, factorial ANOVA, followed by Tukey HSD multiple comparison test).

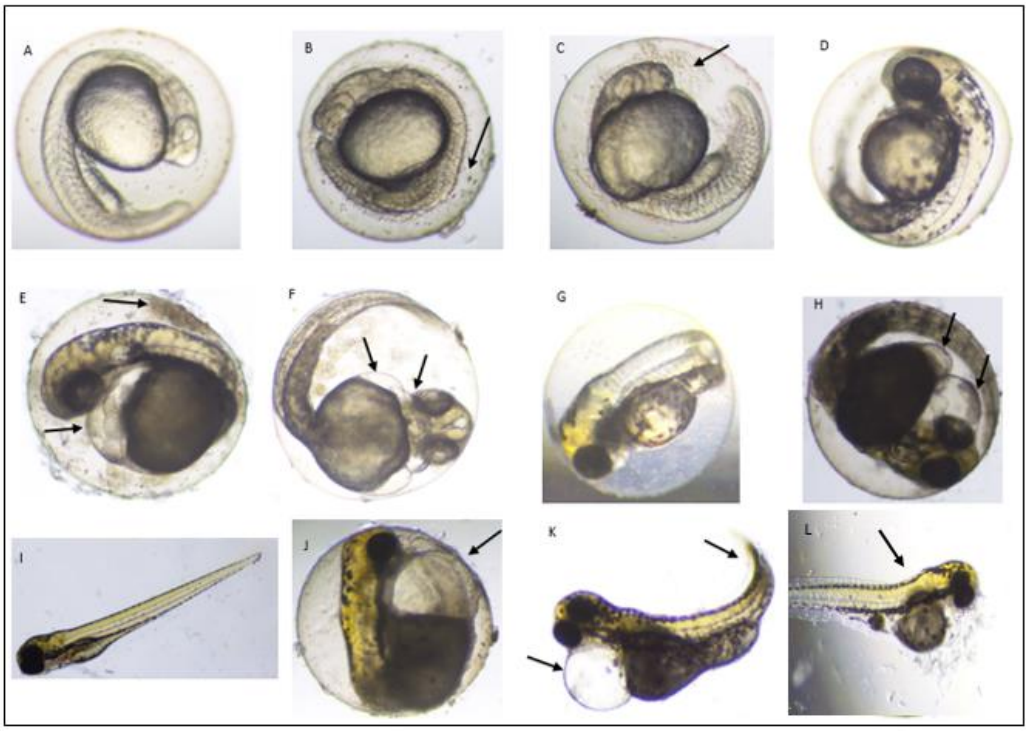

Figure 7. Development anomalies of zebrafish (*Danio rerio*) exposed to water samples of Botafogo Basin. (A) control at 24 hpf; (B) and (C) ETAB2 at 24 hpf, dispersed yolk; (D) control at 48 hpf; (E) and (F) ETAB2 at 48 hpf with dispersed yolk, pericardial and yolk-sac edema; (G) control at 72 hpf; (H) ETAB2 at 72 hpf, pericardial and yolk-sac edema; (I) control at 96 hpf; (J) ETAB2 at 96 hpf, embryo with pericardial edema; (K) ETAB2 at 96 hpf, larva with pericardial edema and folded tail; (L) ETAB2 at 96 hpf, larva with spine curvature. The arrows indicate anomalies localization.

3.2.1.3 Hatching rate

Figure 8 represents the hatching rates (%) of embryos at 72 and 96 hpf. At 72 and 96 hpf there was a statistically significant decrease in the hatching ate at the ETAB2 sample in comparison with the control group ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test). At 96 hpf hatching rate was of 10.83% for the ETAB2 sample while all embryos were hatched at the control group and the others treating samples.

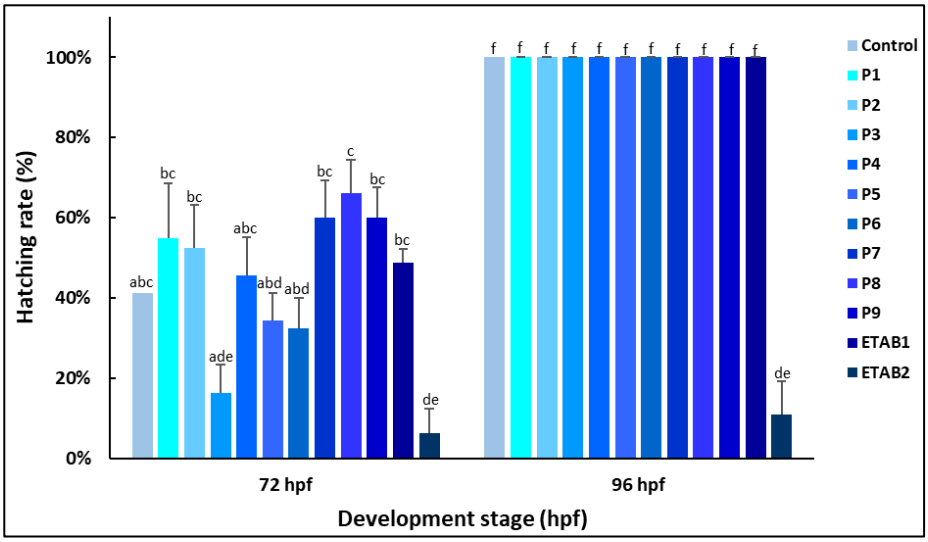

Figure 8. Hatching rate (%) of zebrafish (Danio rerio) at 72 and 96 hpf exposed to river surface water samples and control. Values are presented as mean ± standard error. The letters indicate significant differences between treatments ($p < 0.01$; factorial ANOVA followed by the Tukey HSD multiple comparison test).

3.2.1.4 Total larvae length

The total average length of zebrafish larvae at 96 hpf is shown in Table 4. There was a significant increment in larvae length at the P9 sample and a significant decrease for ETAB2 sample in comparison with all the other treatments ($p < 0.01$; ANOVA one-way, followed by the Tukey HSD multiple comparison test).

Table 4. Total average length (µm) of zebrafish (*Danio rerio*) larvae at 96 hpf exposed to river surface water samples of Botafogo basin and control group.

	Average total lenght
Control	3734,626 ± 13,31
P1	3741,450 ± 17,13
P2	3762,691 ± 13,57
P3	3734,273 ± 16,52
P4	3790,658 ± 12,76
P5	3716,145 ± 18,66
P6	3712,610 ± 18,59
P7	3758,385 ± 17,55
P8	3760,308 ± 17,67
P9	3860,568 ± 14,31*
ETAB1	3760,970 ± 20,08
ETAB2	2483,644 ± 66,66*

* $p < 0.01$

3.2.1.5 Sensorimotor reflexes

Positive head and tail touch responses (% total) of zebrafish larvae (96 hpf) showed significant alterations between the control group (100%) and the ETAB2 sample (15%) ($p < 0.01$; ANOVA one-way, followed by the Tukey HSD multiple comparison test). For the others treatment there were no significant alterations in comparison with the control group ($p \geq 0.05$; ANOVA one-way).

3.2.1.6 ETAB2 test with pH correction

ETAB2 sample presented a low pH value, which was posteriorly correct (from 4.13 to 7.0) to evaluate if the observed toxicity was due to pH imbalance. Therefore, tests were performed with the corrected pH, ETAB2 cor, to compare with results from the ETAB2 sample. As there were no statistically significant differences between the controls of the tests performed with the ETAB2 and the ETAB2 cor samples ($p > 0.05$; factorial ANOVA), these were grouped. Cumulative mortality rates of embryos at different stages of embryonic development are shown in Figure 9 (a). Cumulative mortality rate was unaltered at the ETAB2 cor sample in comparison with control at all the stages of embryonic development ($p \geq 0.05$; factorial ANOVA). On the other hand, there was a statistically significant increase in mortality for ETBA2 sample, at all stages of development, compared to the control group ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test). At 24 hpf there was a significant increase in embryo development anomalies for the ETAB2 (100%) sample in comparison with the control group (0%) ($p < 0.01$; factorial ANOVA followed by

Tukey HSD multiple comparison test) while at 48, 72 and 96 hpf, this increment was statistically significant for both samples (ETAB2 and ETAB2 cor) in comparison with control (Figure 9 (b)). At 96 hpf the percentage of embryo anomalies were control (5,62%), ETAB2 (100%), ETAB2 cor (65%). The main development anomalies found included: edema of the yolk-sac and heart, folded tails, and spine curvatures. At 72 hpf there were no statistically significant differences in the hatching rates of the tested samples and the control ($p \geq 0.05$; factorial ANOVA) (data not shown). A significant decrease in the hatching rate was found at 96 hpf for both ETAB2 and ETAB2 cor sample in comparison to the control group ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test) (data not shown). Hatching rates were of 11%, 47.5% and 100% for the ETAB2, ETB2 cor and control groups, respectively.

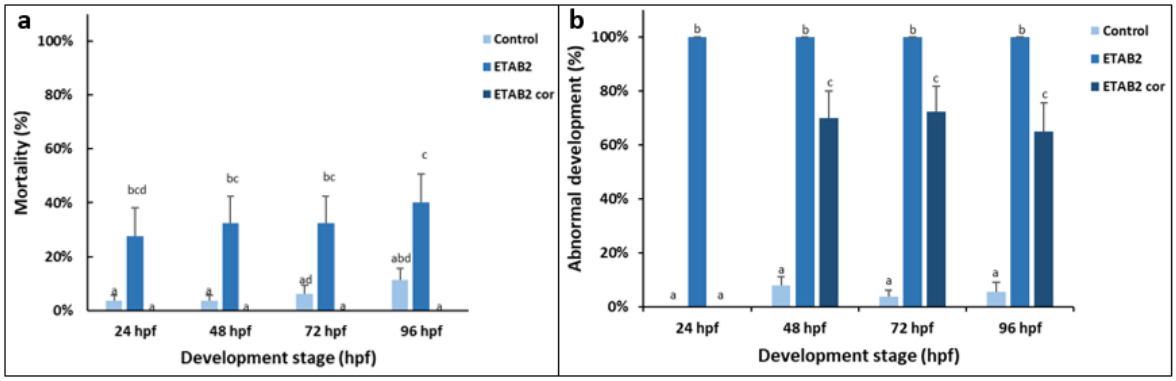

Figure 9. Cumulative mortality rate (%) (a) and abnormal development rate (%) (b) at different embryonic development stages (24, 48, 72 and 96 hpf) of zebrafish (*Danio rerio*) exposed to river surface water samples and control. Values are presented as mean ± standard error. The letters indicate significant differences between treatments ($p < 0.01$; factorial ANOVA followed by the Tukey HSD multiple comparison test).

The total average length of zebrafish larvae at 96 hpf is shown in Table 5. There was a significant decrease in larvae length at ETAB2 and ETAB2 cor samples in comparison with the control group ($p < 0.01$; ANOVA one-way, followed by the Tukey HSD multiple comparison test).

Table 5. Total average length (µm) of zebrafish (*Danio rerio*) larvae at 96 hpf exposed to river surface water ETAB2 and ETAB2 cor samples and control groups.

	Medium lenght growth
Control	3724,383 ± 16,74
ETAB2	2483,644 ± 66,66*
ETAB2cor	3521,809 ± 20,83*

* $p < 0.01$

Positive head and tail touch responses (% total) of zebrafish larvae (96 hpf) showed significant differences for the ETAB2 and ETAB2 cor samples in comparison with the control group ($p < 0.01$; ANOVA one-way) (Figure 10).

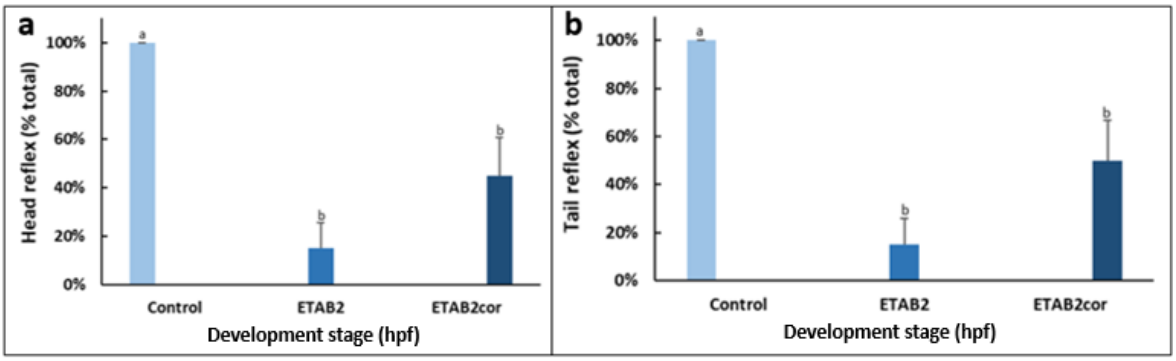

Figure 10. Positive head and tail touch responses (% total) of zebrafish larvae at 96 hpf. Values are presented as mean ± standard error. The letters indicate significant differences between treatments ($p < 0.01$; ANOVA one-way followed by the Tukey HSD multiple comparison test).

3.2.2 GL-2 Bita e Utinga basin

3.2.2.1 Cumulative mortality rate

There were no statistically significant differences among the different treatments and hours of development in comparison with the control group ($p \geq 0.05$; factorial ANOVA) (data not shown).

3.2.2.2 Embryonic development anomalies

Abnormal development (%) in embryos, shown in Figure 11, was recorded within each sample during the different embryonic development stages (24, 48, 72 and 96 hpf). At 24 hpf there were no statistically significant differences between the different treatments ($p \geq 0.05$; factorial ANOVA). At 48 hpf there was a significant difference ($p < 0.05$; factorial ANOVA followed by Tukey HSD multiple comparison test) in the percentage of abnormal development anomalies between the samples U1 (30%), U2 (40%), U3 (37.5%), ETAS1 (35%), ETAS2 (55%) and the control group (8.75%). Afterwards, there was a reduction in the percentage of anomalies until the end of the test, without significant differences between treatment groups treatments (72 and 96 hpf) ($p \geq 0.05$; factorial ANOVA) The main abnormalities found included: edema of the yolk-sac and pericardial edema (Figure 12).

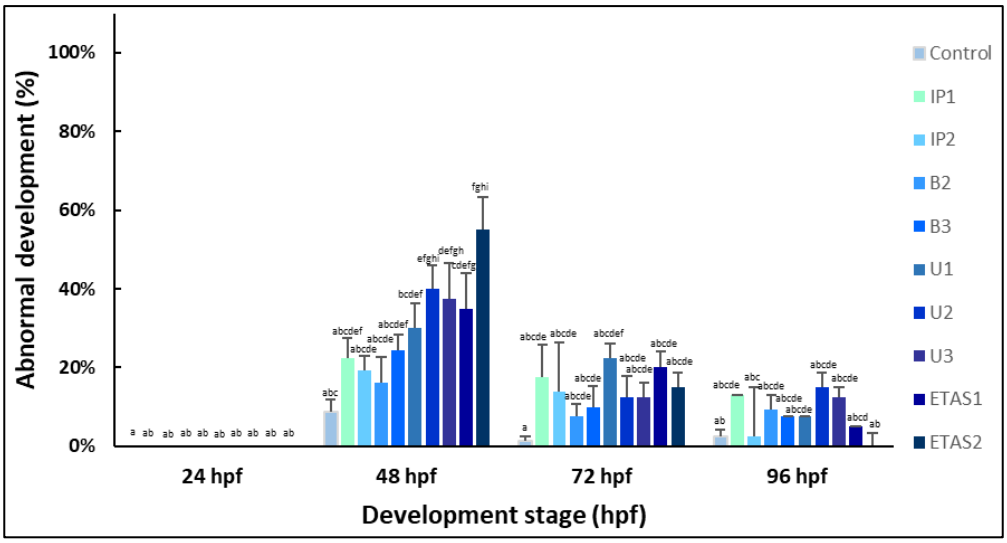

Figure 11. Development anomalies (%) at different embryonic development stages (24, 48, 72 and 96 hpf) of zebrafish (*Danio rerio*) exposed to river surface water samples and control. Values presented as mean ± standard error. Different letters indicate significant differences between treatments ($p < 0.05$, factorial ANOVA, followed by Tukey HSD multiple comparison test).

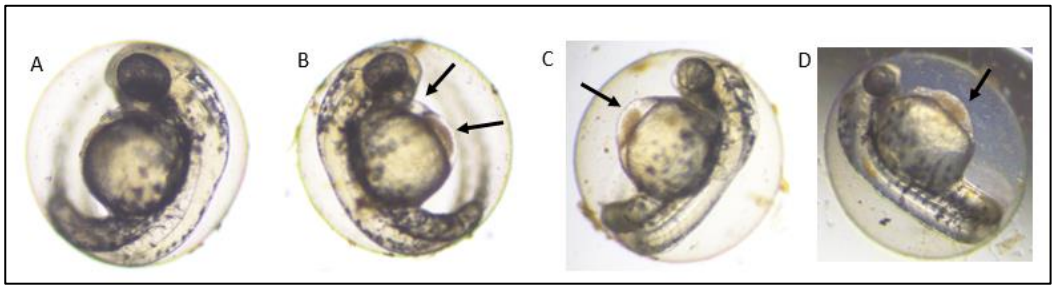

Figure 12. Development anomalies of zebrafish (*Danio rerio*) exposed to water samples of Bita and Utinga basin. (A) control at 48 hpf; (B) ETAS2 sample at 48 hpf, pericardial and yolk-sac edema; (C) and (D) ETAS2 sample at 48 hpf, yolk-sac edema. The arrows indicate embryo anomalies localization.

3.2.2.3 Hatching rate

At 72 hpf there was a statistically significant increase in the hatching rate at the U1, U2, U3, ETAS1 and ETAS2 samples in comparison with the control group ($p < 0.01$; factorial ANOVA followed by Tukey HSD multiple comparison test). At 96 hpf all embryos in the control group and samples were hatched (Figure 13).

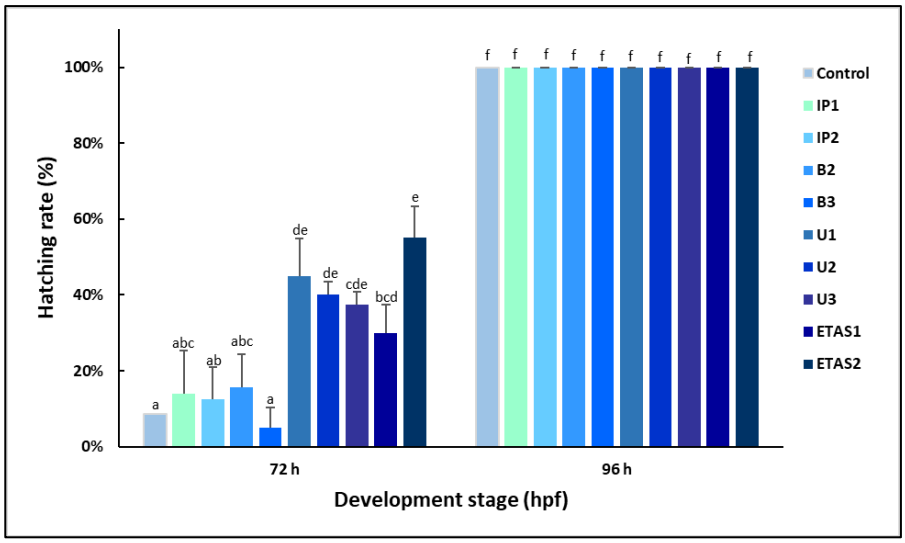

Figure 13. Hatching rate (%) of zebrafish (*Danio rerio*) at 72 and 96 hpf exposed to river surface water samples and control. Values are presented as mean ± standard error. The letters indicate significant differences between treatments ($p < 0.01$; factorial ANOVA followed by the Tukey HSD multiple comparison test).

3.2.2.4 Total larvae length

The total average length of zebrafish larvae (96 hpf) is shown in Table 6. There was a significant larvae length increment at U1, U2, U3, ETAS1, ETAS2 treatments in comparison with the control ($p < 0.01$; ANOVA one-way, followed by the Tukey HSD multiple comparison test).

Table 6. Total average length (µm) of zebrafish (*Danio rerio*) larvae (96 hpf) exposed to river surface water samples of Bita and Utinga basin and control group.

	Average total lenght
Control	3738,504 ± 15,97
IP1	3794,007 ± 11,78
IP2	3742,096 ± 19,54
B2	3798,065 ± 19,86
B3	3755,370 ± 19,14
U1	3834,548 ± 18,08*
U2	3849,657 ± 33,53*
U3	3860,320 ± 18,42*
ETAS1	3831,122 ± 18,91*
ETAS2	3864,355 ± 20,46*

*P<0.01

3.2.2.5 Sensorimotor reflexes

Positive head and tail touch responses (% total) of zebrafish larvae (96 hpf) showed no significant alterations between the different samples and the control group ($p \geq 0.05$; ANOVA one-way).

4. DISCUSSION

Brazilian quality and quantity of river waters have been increasingly affected by anthropogenic contamination (Rodgher et al., 2005; Medeiros et al., 2016). This study seeks to assess the overall quality of surface waters of two selected hydrographic basins of Pernambuco (Brazil) and to increase the information on the impact of environmental contaminants in these aquatic ecosystems, using zebrafish embryo bioassays.

With exception of P8 and ETAB2, all samples evidenced pH, DO, NO2- and NO3- values within the Brazilian federal legislation (CONAMA 2005). P8 and ETAB2 samples showed pH values below the recommended; while for P8 sample pH value (5.71) was slight below the recommend (pH = 6-9), the ETAB2 sample showed a very low pH (4.13).

Among the samples of the GL-1 Botafogo basin a significant increase in mortality was observed for the ETAB2 sample at all stages of zebrafish development. Furthermore, a significant increase in the percentage of development anomalies in embryos exposed to the P9 sample at 48 hpf (27.5%) and the ETAB2 sample at all stages of zebrafish development was observed in comparison with control group. ETAB2 sample also evidenced a significant decrease in the hatching rate at 72 hpf and 96 hpf. Differences in larvae total length were also observed. While a significant increase in larvae length was observed for the P9 sample, a significant length decrease was observed for the ETAB2 sample. Sensorimotor reflexes were decreased by ETAB2 exposure.

The P9 sample was collected in an area downstream of an industrial landfill, which receives effluents directly into the water body, which may cause water contamination. Landfill leachate contains a mixture of many chemical compounds originated from the various disposed materials and/or resulting from biotic and abiotic processes in the system. These compounds are usual toxic and resistant to environmental degradation and can constitute a potential risk to the quality of receiving water bodies, when leachates are released into the environment (Oman & Hynning, 1993; Gotvajn, 2009). There is a considerable number of studies reported in the literature for metals concentrations from full-scale landfills, test cells, and laboratory works. Heavy metals, which are commonly found in high concentrations in landfill leachate include iron, manganese, zinc, chromium, lead, copper, and cadmium. These constitute a potential source of pollution for ground water, surface water and reservoirs (Christensen et al., 2001, 1994; Kjeldsen & Christophersen, 2001; Revans et al., 1999; Reinhart & Grosh, 1998; Robinson, 1995; Aziz et al., 2004). Fraser et al. (2000) investigated the formation of copper complexes in the leachate from an active urban landfill and found that some of the complexes formed were toxic to zebrafish embryos. Johnson and Sloman (2007) observed toxic effects of copper on zebrafish

embryos development, including an inhibitory effect on hatching. Length of individuals were recorded across treatments at 72 hpf and elevated copper was found to slow development.

Exposure to nickel in early life stages of zebrafish leads to morphological alterations, avoidance response impairment and locomotor deficits (Nabinger et al., 2018). Similar zebrafish toxic effects were observed in this work. Nevertheless, a chemical characterization of this water body is not reported in the literature and further chemical water analysis is needed to make a cause-effect relationship.

The ETAB2 sample was collected at the exit of the water treatment plant called ETA Botafogo, which is responsible for the treatment of the water that suppliesseveral cities in the region. As previously referred, the ETAB2 sample showed pH values (pH = 4.13) below the reported as toxic for zebrafish (pH < 5.9) (Engeszer, et al., 2013). On this regard, a pH correction was made to the ETAB2 sample - ETAB2cor- to reach a pH= 7. Embryos exposed to the ETAB2cor sample did not evidenced alterations in the mortality rate. However, embryo anomalies were observed in embryos exposed to the ETAB2 cor sample at 48, 72 and 96 hpf compared to the control. Exposure to the ETAB2 sample also resulted in a significant decrease in the hatching rate and total larvae length at 96 hpf compared to the control. Alterations in larvae sensorimotor reflexes, translated in a significant decrease in head and tail positive response of ETAB2 cor exposed larvae in comparison with the control group. Notwithstanding, ETAB2 cor exposure effects in zebrafish development were similar to the ones resulting from ETAB2 exposure, although in lesser extent. These results suggest that the observed effects in zebrafish embryo and larvae development may be partially caused by low pH, but not totally accounted for it. It is reported that during water chemical treatment in water treatment plants, the water can react with disinfection and coagulant agents forming emerging compounds, such as disinfection by-products (DBPs) (Chaves *et al*., 2019). Chaves *et al*. (2020), assessed the toxicity of non-chemically regulated DBPs using zebrafish embryo bioassays and reported that, globally, unregulated tested DBPs exhibited greater toxicity than the regulated ones. Reported effects of zebrafish embryo exposure to DBPs were yolk sac edema, pericardia edema, scoliosis or caudal malformations (Chaves *et al*., 2020). According COMPESA (organism responsible for the operation of the ETA Botafogo), as part of the water treatment, chlorine and liquid sulfate are added for disinfection. Chlorine and liquid sulfate can react with other compounds present in the water originating DBPs (Chaves *et al*., 2019). Therefore, we can hypothesize that zebrafish embryo toxicity observed for the ETAB2 sample may result from the presence of DBPs. Chlorine is more toxic and germicidal at lower pH (Kent *et al*., 2014), which would allow to explain the higher extent of observed effects at the ETAB2 sample compared the ETAB2 cor sample. For further corroboration of this hypothesis, chemical water analysis is needed.

Concerning the surface waters of GL-2 Bita and Utinga Basin, samples U1, U2, U3, B2, ETAS1 and ETAS2 evidenced NH4+ values above the suitable for zebrafish, nervertheless, NH4+values were low and this compound as been reported as non-toxic (Thurston *et al*. 1981; Randall & Tsui, 2002).

Regarding these basin samples, there were no significant effects on the mortality of exposed zebrafish embryos. Nevertheless, there was a significant increase in the percentage of development anomalies in embryos exposed to U1, U2, U3, ETAS1 and ETAS2 at 48 hpf. These same samples showed a significant decrease in the hatching rate at 72 hpf. Effects in embryo development and hatching rate were rescued at 96 hpf.

Some studies indicate the ability of zebrafish embryos to reverse anomalies, especially in the case of edemas, where the embryo can absorb the edema. At 96 hpf a significant increase in larvae length was observed at these samples in comparison with control group.

The samples U1, U2 and U3 were collected in the Utinga reservoir, at the beginning (U1), in the middle (U2) and at the water collection point of the reservoir for urban water supply (U3). The sample ETAS2 was collected at the entrance of the water treatment plant ETA SUAPE, which receives water from all points at the Bita and Utinga reservoir. Utinga reservoir receive water from rivers in the region, which receive wastewater and industrial effluents. Also, the Utinga reservoir is close to sugar cane plantations. Contamination by pesticides is reported near these mills and plantations. Tesolin *et al*. (2014) observed toxic effects of some herbicides used in the cultivation of sugar cane in zebrafish embryos, including edema, decreased heart rate, delayed embryonic development and yolk sac absorption. It is reported that zebrafish embryos exposure to 50 µg/l DDT, the hatching rate was 2 to 3 times lower than in embryos exposed to 0.05 µg/l DDT (Njiwa *et al*., 2004). Due to the transport capacity of the herbicides in the aquatic environment, it is also highlighted that these contaminants can represent a risk at different trophic levels, even far from the sugar cane cultivation areas. Also, many pesticides have been identified to impact the reproductive health and survival of aquatic organisms, some of which have been classified as ECs (Gray *et al*., 2001). Due to the localization of the sampling points (near chemical industries, sugar-alcohol mills, sugar cane plantation), one cannot exclude that the causes of the reported effects in the present study are related with the combined effects of complex mixtures.

The ETAS2 sample was collected at exit of the ETA SUAPE, which is responsible for the treatment and distribution of water to several cities in the region, including the SUAPE Port and Industrial Complex. As referred above, according COMPESA, water treatment in SUAPE ETA is made with chlorine and sulfate, which can react with other compounds present in the water forming DBPs. Thus, we can hypothesize that toxic effects resultant

from exposure to the ETBA2 sample may be originated by the presence of DBPs in the ETAS2 sample and/or the chlorine and sulfate. Moreover, to further corroborate the above advance hypotheses, water chemical analysis will be carried out to detect the existence and or concentration of different contaminants in the samples.

5. CONCLUSION

In this study the surface waters of two hydrographic basins of rivers in the state of Pernambuco, Brazil, were sampled for analysis. Most of these rivers receive urban and industrial effluents from multiple sources. These preliminary results allowed to prioritize water samples that should go through a more detailed characterization, thus supporting the use of zebrafish embryo bioassays as a fast, high throughput approach for screening the toxicity of water samples, evidencing their ecotoxicological status.

A chemical analysis of the collected samples will be performed to support the results found in this study.

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