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The influence of heavy metals on toxicogenetic damage in a Brazilian tropical river

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HIGHLIGHTS

- Poti river carries polluted water.
- Poti river water exhibited genotoxic effects in *Oreochromis niloticus.*
- Heavy metal-induced significant toxic effects.

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ABSTRACT

Anthropogenic activities in tropical rivers favor the eutrophication process, which causes increased concentration of heavy metals. The presence and bioaccumulation of metals are directly related to the presence of genotoxic damage in aquatic organisms. Thus, we evaluated the presence of heavy metals (Fe, Zn, Cr, Cu and Al) and performed toxicogenetic tests in surface (S) and bottom (B) of water samples of the Poti river (Piaui/Brazil). Cytotoxicity and genotoxicity tests were performed in *Allium cepa*, and micronucleus (MN) and comet assay were performed in *Oreochromis niloticus.* The chemical analysis showed concentrations above the limit for Cu, Cr, Fe and Al according to Brazilian laws, characterizing anthropogenic disturbance in this aquatic environment. Toxicogenetic analysis presented significant cytotoxic, mutagenic and genotoxic effects in different exposure times and water layers (S and B), especially alterations in mitotic spindle defects, MN formations, nuclear bud and DNA strand breaks. Correlations between Fe and cytotoxicity, and Al and mutagenicity were statistically significant and point out to the participation of heavy metals in genotoxic damage. Therefore, Poti river water samples presented toxicogenetic effects on all bioindicators analyzed, which are most likely related to heavy metals pollution.

1. Introduction

All living organisms are interacting with the aquatic environment, and environment degradation by human activities may cause DNA damage in these aquatic organisms (Akinboro et al., 2011; Nunes et al., 2011). Changes in the rate of cell division and/or

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DNA structure are harmful to the cells, which can interfere with vital processes such as DNA replication and gene transcription. In addition, these alterations may also cause gene mutations and chromosomal aberrations that contribute to cancer development and cell death (Ossana et al., 2013). The detection of pollutants in aquatic environments, and their likely effects on organisms are important for studying the impact on animals, plants and especially human population (Leme and Marin-Morales, 2009).

Heavy metals are potentially genotoxic and carcinogenic, and are known as oxidative stress inducers, stimulating the production of reactive oxygen species (ROS), which cause DNA damage and cell death (Lushchak, 2011). Heavy metals bioaccumulate in the environment and may increase the risk of various degenerative diseases, including cancer (Beyersmann and Hartiwig, 2008). The presence of high concentrations of heavy metals is related to the drastic reduction of water quality and mainly related to human activities (Kumar et al., 2015).

The complexity of the pollutants in environmental samples demands a multitude of genotoxicity tests, with increasing simplicity, sensitivity, and affordability (Tabrez et al., 2011). In this sense, to evaluate toxicogenetics effects of complex mixtures from river water samples, ecotoxicological tests (cytotoxicity, genotoxicity and mutagenicity) are carried out in microorganisms, animal cells and plants, alone or combined (Žegura et al., 2009; Mazzeo et al., 2013).

In aquatic environments, fishes are often used as biological indicators of water quality, and biomonitors for the presence of metals and pollutants (Torres de Lemos et al., 2007). Fishes also provide information of pollutants' bioavailability that contribute to the process of biomagnification (metals) and the risks for human health, since is part of human diet. Data from bioassays using fishes have shown good correlation with genotoxicity in human cells exposed to mutagens (Marcon et al., 2010).

Plants are also excellent biological systems, because they are good bioindicators of toxicity, with high sensitivity to detect cytotoxic and mutagenic agents through different genetic mechanisms, including point mutations and chromosomal aberrations. The impact of the mutagens in these tests is indicated by the inhibition in the growth of root and shoot (Siddiqui et al., 2011a, b). The species *Allium Cepa* (2n = 16) is one of the best systems for evaluating cytotoxicity and mutagenicity of environmental substances (Leme and Marin-Morales, 2009), and is widely used in monitoring the effect of pollutants, including heavy metals, cyanotoxins and hydrophilic and lipophilic chemicals (Bianchi et al., 2011).

The Poti river, a tropical and shallow river from the semi-arid region of Brazil, has lentic characteristics, and is stratified mainly during the dry season. The river runs through the urban area of Teresina (Piauí, Brazil), and has suffered direct consequences from city development, mostly because of an incomplete sewage system. Low levels of sewerage coverage impair the river by increasing the pollutant load, especially during the dry season, reducing the water quality index (WQI) as a result of its artificial eutrophication (Silva et al., 2010).

Fast urbanization and industrialization have resulted in the tremendous release of xenobiotic compounds into the environment (Tabrez and Ahmad, 2011). Thus, it is suggested to carry out studies to detect the presence of metallic pollutants and other chemical and biological contaminants in the aquatic environment and their possible toxicogenetics effects. In this sense, this study aimed to evaluate genotoxic, mutagenic and cytotoxic effects in water samples of the Poti river within the urban area of Teresina, and correlate the possible genetic damage to metallic pollutants, including aluminum (Al), copper (Cu), chromium (Cr), iron (Fe) and zinc (Zn).

2. Materials and methods

2.1. Study area and collection points

The Poti river is located in northeast of Brazil, and its source flows from the state of Ceará to Piauí with its mouth in the city of Teresina (Piauí, Brazil), where it find its affluent, the Parnaiba River. Poti River is classified according to the Brazilian laws (CONAMA, 2005), as class 2 river (intended for human consumption, agriculture, recreation and fishing activity). The three sampling sites selected for the river are equidistant from each other, within 26 km of urban area (Fig. 1). Point 1 (P1) is located at 5°6′53.16″S and 42°43′52.81″W; Point 2 (P2) is located at 5° 3′51.50″S and 42°48′24.82″W and point 3 (P3) is located at 5°2′2.76″S and 42°49′48.57″ W, approximately 1000 m from its mouth. The P1 is upstream the urban area of Teresina, being influenced only by rural communities and sand and rock extractors; P2 is located within the city, which represents the intermediate zone of the city, and is influenced by almost two thirds of the urban area; and P3 is located downstream the city, and has suffered influence of dredging and horticulture activities. Each point was evaluated for both surface (S) and bottom (B) water sampling.

Water samples used as controls (positive and negative) for testing *Oreochromis niloticus* and *Allium cepa* were from fish farming tanks and dechlorinated water, respectively. Collection of water samples were in September 2014, during dry season (hottest period of the year) (Oliveira and Silva, 2014).

2.2. Chemical analysis water

Water samples were collected manually, in triplicates, at 25 cm from the surface (S) and near the bottom (B) of each point. For sampling, it was used polyethylene bottles (500 ml) for surface collections and Van Dorn bottle with horizontal flow (5 L) for bottom collections. Immediately after collection, samples were stored and chilled to 4 °C, transported and analyzed, within 24 h, in order to evaluate the levels of iron (Fe) (mg/l), zinc (Zn) (mg/L), copper (Cu) (mg/L) and chromium (Cr) (mg/L) through flame atomic absorption spectrophotometry (according to APHA et al., 2005). Water samples were acidified and, subsequently, subjected to acid digestion and concentration for flame atomic absorption spectrophotometer (Varian-AA50B model) analysis. Aluminum (Al) (mg/L) quantification was determined by the Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). Water samples from local fish farming tanks were used as control (CO). The sum of all metals' concentrations by point and layers were considered as accumulated metals (Marcon et al., 2010).

2.3. Bioassay O. niloticus

Specimens of *Oreochromis niloticus* from local fish farming tanks with approximately same weight (300 g), size (15–20 cm) and age (2 months), were used for the comet assays (genotoxicity), micronucleus (mutagenicity) and nuclear/cellular abnormalities (cytotoxicity) tests. Fishes were acclimated (29 ± 2° C, pH 7.8 ± 0.3) in 350 L tank (Duarte et al., 2012) and subsequently transferred to aquarium filled with Poti river waters.

A total of 24 tanks of 15 L were used, where each aquarium received one fish, and each point/layer was measured in triplicates (NC, P1S, P1B, P2S, P2B, P3S, P3B and PC). One third of the aquarium water was renewed daily, during the 6 days of exposure time (144 h). Water from fish farming tanks was used for negative (NC) and positive (PC) controls. Cyclophosphamide (4 mg/L), injected intraperitoneally and below the pectoral fin, was the cytotoxic,

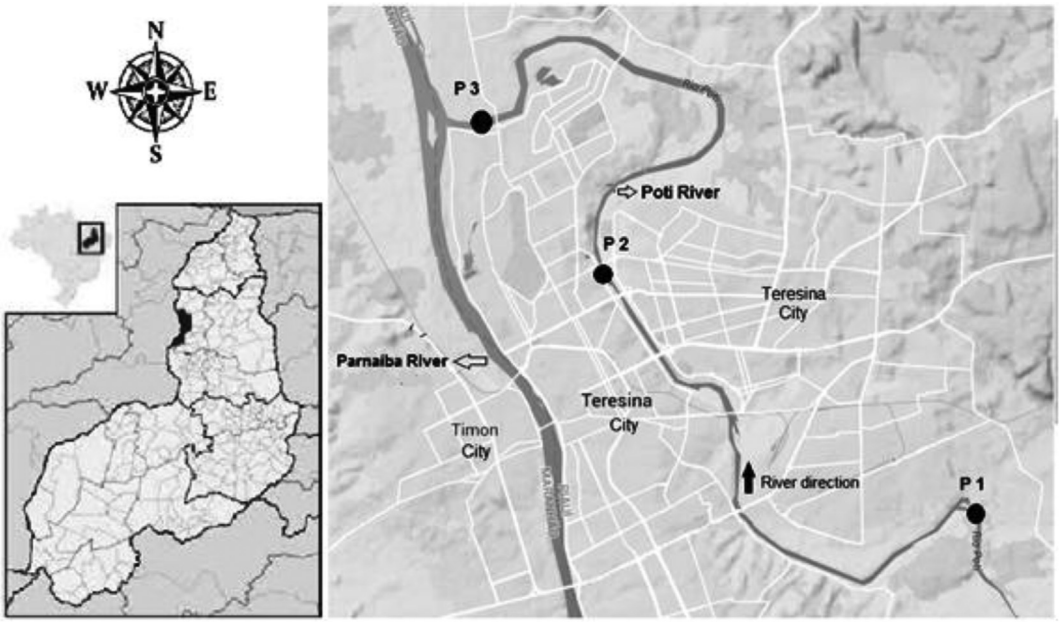

Fig. 1. Location of collection points (1, 2 and 3) in area of study of the basin of the river Poti, Teresina, Piauí, Brazil.

genotoxic and mutagenic agent used as positive control for *Oreochromis niloticus* testing (Bolognesi and Hayashi, 2011).

After exposure times for every toxicogenetic test, 0.5 ml of blood from each fish was collected by gill puncturing with heparinized syringes. After puncture, each specimen was immediately returned to aquarium for continuing the experiment. After the last gill puncture, the specimens were euthanized with anesthetics according to the Ethics Committee for the Use of Experimental Animals/Brazil. This study was approved by the Ethics in Animal Experimentation of Federal University of Piauí Committee (No. 103/14).

2.4. Comet assay

The comet assay was performed after 24 and 72 h of water exposure, according to the protocol of Singh et al. (1988) with some modifications. Briefly, 5 μL of gill blood was mixed with 100 μL of low melting agarose (0.5%) at 37 °C. Then, the mixture was spread on a slide, previously coated with agarose (1.5%) and covered with a coverslip. After polymerization (drying in refrigerator for 10 min), the coverslips were removed. Slides were then protected from light and vertically immersed into a cold lysis solution (89% stock lysis solution, 1% Triton X-100, 10% DMSO, pH 10; stock lysis solution 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10) for at least 12 h. The slides were subjected to electrophoresis in alkaline solution (1 mM EDTA, 300 mM NaOH, pH > 13) under the following conditions: 20 V (0.74 V cm−1), 300 mA, 20 min in a low light environment and followed by neutralization with Tris-HCl (0.4 M, pH 7.5 for 15 min); washing with ice-cold distilled water and air dried. Finally, the slides were fixed with cold absolute methanol for 3 min, air dried, and stained with silver nitrate. Photomicrography was carried out using an optical microscope with 400× magnification to count classes of damages: class 0 (C0, genetic material without damage or intact); class 1 (C1, mild damage), class 2 (C2, moderate damage), class 3 (C3, severe damage) and class 4 (C4, maximum damage). In order to determine the damage index (DI), the total score for the sample gel was between 0 and 400 arbitrary units, and was defined as: DI: 0 × (C0) + 1 × (C1) + 2 × (C2) + 3 × (C3) + 4 × (C4). Each image was scored according to the extent of DNA migration based on a visual analysis of 100 cells in each slide. Damage frequency (DF) analysis, varying from 0 to 100 (%), was define as: DF = 100 - C0, in which C0 represents the number of class 0 (C0) cells out of 100 cells evaluated. We analyzed the damage index (DI) and damage frequency (DF) in 300 cells per fish (900 cells per group).

2.5. Micronucleus test (MN) and nuclear morphological changes (NMC)

MN tests were carried out after periods of three days (72 h) to 6 days (144 h) of water exposure, according to the protocol of Souza and Fontanetti (2006). Smear slides with 50 μL of gill blood, air dried, were fixed in absolute methanol for 10 min and stained with 10% giemsa. For each fish (specimen) three slides were prepared, and a total of 3000 cells were analyzed using an optical microscope under 1000× magnification. Mutagenicity was determined by the frequency of micronuclei and nuclear buds; and cytotoxicity was determined by the frequency of nuclear morphological changes (blebbed, lobed and notched) and binucleate cells of fish erythrocytes.

2.6. Allium cepa test

In order to characterize the toxicogenetic potential of river water, the *A. cepa* test was conducted according to Panda and Achary (2014). Initially, a variety of bulbs (Bay Periforme) from the same lot were germinated in distilled water for 48 h at room temperature in laboratory. After germination, the roots were exposed to Poti water samples for 24, 48, 72 h. After each ET, roots were harvested and fixed in ethanol-acetic acid (3:1) for 24 h. After fixation, the roots were rinsed with distilled water and hydrolyzed in 1 M HCl at 60 °C for 10 min and stained with 2% acetic orcein.

Mutagenicity was assessed by analysing 1000 cells for chromosomal aberrations and micronuclei formation in meristematic cells of *A. cepa*. The cytotoxic potential was determined by the mitotic index (MI), which was defined as the number of dividing cells per 1000 cells of each bulb. Analyses were performed under an optical microscope with 400× magnification. Five bulbs were analyzed for each collection site. The positive control (PC) used for

Allium cepa test was copper sulfate 0.0006 mg/ml diluted in distilled water.

2.7. Statistical analysis

The concentrations of metals, for points and layers, compared to the negative control (NC) were analyzed by ANOVA with post Fisher test (LSD). Cytotoxic, genotoxic and mutagenic data were analyzed by Nested RM-MANOVA test, with post Fisher test (LSD), and all points per layer were compared among themselves and controls. Pearson's correlation was performed among cytotoxic, genotoxic and mutagenic data of metal concentrations in order to identify which variables are associated with each type of genetic damage. All statistical analyzes were performed using the software STATISTIC 8.0 (StatSoft, Inc., USA). Differences at $p < 0.05$, $p \leq 0.001$ were considered statistically significant for all analysis.

3. Results

3.1. Chemical analysis of water

The concentration of Zn and Al from P1 and P2 compared to NC showed significant differences ($p < 0.05$). P2 presented high concentrations of Cu, compared to other points. No significant differences were found for Fe and Cr values. Considering accumulated metals, P2 was significantly different compared to NC and P3.

There were no significant differences between layers (S and B) for the evaluated metals in all considered points. However, analyzing each point (P1, P2, P3), it was identified significant differences in P1S, with higher amount of Al and lower Zn concentrations when compared to P1B. P2S presented higher Zn and lower Fe concentrations, as well as accumulated metals compared to P2B. P3S showed more Al concentrations than P3B. In addition, it was observed that Cu (all points), Fe (P2B) and Al (P1S and B) concentrations were above the values allowed by the Brazilian legislation (CONAMA-357/2005) for class 2 rivers (Table 1).

3.2. DNA damage in blood cells of Oreochromis niloticus

The damage index (DI) and the damage frequency (DF) from *O. niloticus* blood cells, for all points (P1, P2 and P3), were significantly higher when compared to the negative control (NC), and statistically lower than the positive control (PC). These data indicate that water samples of the Poti river can induce genotoxic damage in the ecosystem species, because of the significant increased levels of damage index and damage frequency at 24 h and 72 h (ET). Samples from the P3 were more genotoxic than P2. There were no genotoxic differences between layers (surface and bottom) at any point of the river (Table 2, Fig. 2).

3.3. Mutagenicity and cytotoxicity in blood cells of Oreochromis niloticus

It was observed significant mutagenicity (micronuclei and nuclear buds) in blood cells of *O. niloticus* only in P1B when compared to NC. Higher mutagenicity was found in P1compared to P2 and P3. There were no significant differences between layers (surface and bottom) at any point of the river, excepted higher mutagenicity in P1B compared to P1S. In relation to cytotoxicity (binucleate cells and nuclear abnormalities), greater cytotoxicity was observed in P2 when compared to NC ($p < 0.05$). However, when considering the points per layer, P2B and P3B were cytotoxic compared to NC. The P2 and P3 bottom water samples are more cytotoxic than their respective surface point. There was no mutagenic or cytotoxic differences between different exposure times (72 h or 144 h) (Table 3).

3.4. Cytotoxicity and mutagenicity in Allium cepa cells

In meristematic cells of *Allium cepa*, significant cytotoxicity was observed in P2 and P3 compared to the NC. P2 and P3 were more cytotoxic than P1 ($p < 0.05$). P2B and P3S were as cytotoxic as PC. It was also observed mutagenicity (CA − chromosomal abnormalities) in P1, P3 and P2S compared to NC. P3 was more mutagenic than P2 ($p < 0.05$). Cytotoxicity and mutagenicity increased with exposure times (24 h, 48 h and 72 h). There were no significant differences in cytotoxicity and mutagenicity between layers (surface and bottom). However, mutagenicity of P1S was significantly higher than P1B and P3B was higher than P3S (Table 4).

3.5. Correlation between metals and genetic damage

Heavy metal concentrations were statistically correlated with cytotoxicity and mutagenicity in the *A. cepa* and *O. niloticus* test. Cytotoxicity was positively correlated with Fe dosage in both *A. cepa* ($r = 0.4763$, $p = 0.046$) and *O. niloticus* ($r = 0.814$, $p = 0.031$) (Fig. 3). Mutagenicity was positively correlated with Al dosage in *O. niloticus* ($r = 0.6897$, $p = 0.002$) (Fig. 3). There was no significant correlation between genotoxicity and water metal concentrations by the comet assay.

4. Discussion

Usually, metals are present in small quantities in aquatic environments, but can be discharged in significant quantities by human activities, including urban sewage, industrial, agricultural and mining activities (Marcon et al., 2010; Manzano et al., 2015). High incidence of metals are related to the mechanisms of superoxide radical formation, hydroxyl radical, and, eventually, the production of mutagenic and carcinogenic malon-dialdehyde (MDA), 4-

Table 1
Concentrations of metals (mean ± standard deviation) in water samples collected at three different points of Poti River (Teresina, PI), and control in September 2014.

Collection points	Fe mg/ml	Al mg/ml	Zn mg/mL	Cr mg/mL	Cu mg/mL
NC	0.010 ± 0.001	0.001 ± 0.002	0.007 ± 0.005	0.003 ± 0.003	0.001 ± 0.001
P1S	0.010 ± 0.001	0.200 ± 0.010[a,b]	0.040 ± 0.001[b]	0.01 ± 0.003[b]	0.015 ± 0.005[a]
P1B	0.310 ± 0.002[a,b]	0.130 ± 0.060[a,b]	0.075 ± 0.015[b]	0.016 ± 0.005[b]	0.050 ± 0.001[a]
P2S	0.040 ± 0.001	0.180 ± 0.029[a,b]	0.190 ± 0.001[a,b]	0.006 ± 0.003	0.080 ± 0.050[a,b]
P2B	0.630 ± 0.460[a,b]	0.067 ± 0.029[b]	0.035 ± 0.005[b]	0.058 ± 0.005[a,b]	0.070 ± 0.000[a,b]
P3S	0.31 ± 0.001[a,b]	0.13 ± 0.029[a,b]	0.040 ± 0.020[b]	0.018 ± 0.004[b]	0.030 ± 0.000[a]
P3B	0.040 ± 0.001	0.17 ± 0.029[a,b]	0.040 ± 0.001[b]	0.064 ± 0.001[a,b]	0.045 ± 0.005[a]
MPV	**0.3**	**0,1**	**0.18**	**0.05**	**0.009**

[a] Values that exceed the permitted by Brazilian law, in accordance with Resolution 357 (CONAMA, 2005); MPV: maximum permitted value.
[b] Statistically significant values when compared to the negative control (NC), $p < 0.05$; Fe - iron; Al - aluminum; Zn - zinc; Cr - chromium; Cu - copper; CO - control; P1S - point 1 surface; P1B - point 1 bottom; P2S - point 2 surface; P2B - point 2 bottom; P3S - point 3 surface; P3B - point 3 bottom.

Table 2
Damage index (DI) and damage frequency (DF) (mean ± standard deviation) from Comet assay in cell erythrocytes *Oreochromis niloticus* by sampling points and the layers Poti River (Teresina PI).

Point/Layers	24 h		72 h	
	ID	FD	ID	FD
NC	76.9 ± 14.8	47.7 ± 7.1	85.7 ± 6.0	53.4 ± 3.0
P1S	153.9 ± 23.9[a,b]	71.2 ± 11.5[a,b]	143.9 ± 12.5[a,b]	69.7 ± 2.7[a,b]
P1B	143.1 ± 35.1[a,b]	66.0 ± 9.2[a,b]	156.7 ± 9.2[a,b]	71.2 ± 3.1[a,b]
P2S	127.0 ± 26.7[a,b]	65.1 ± 7.0[a,b]	157.1 ± 31.0[a,b]	69.7 ± 6.2[a,b]
P2B	147.5 ± 17.7[a,b]	67.5 ± 5.2[a,b]	128.4 ± 23.5[a,b]	64.2 ± 7.0[a,b]
P3S	145.6 ± 19.4[a,b]	63.0 ± 6.8[a,b]	158.6 ± 17.8[a,b]	72.4 ± 5.5[a,b]
P3B	154.4 ± 22.6[a,b]	70.9 ± 4.8[a,b]	176.4 ± 13.1[a,b]	77.0 ± 4.3[a,b]
PC	276.1 ± 22.5[a]	89.2 ± 3.7[a]	286.3 ± 15.9[a]	90.4 ± 3.0[a]

[a] Statistically significant when compared to the negative control (NC).
[b] Statistically significant when compared to the positive control (PC) ($p < 0.05$); P1S - point 1 surface; P1B - point 1 bottom; P2S - point 2 surface; P2B - point 2 bottom; P3S - point 3 surface; P3B - point 3 bottom.

Table 3
Levels of mutagenicity (Mut) (micronuclei and nuclear buds) and Cytotoxicity (cito) (binucleate cells and nuclear abnormalities) (mean ± standard deviation) from erythrocyte cell *Oreochromis niloticus* by points and sampling strata river Poti (Teresina, PI).

Point/Layers	72 h		144 h	
	Mut	Cito	Mut	Cito
NC	8.5 ± 3.1	2.4 ± 1.4	5.1 ± 2.2	1.8 ± 1.1
P1S	9.0 ± 5.0	1.6 ± 1.4	8.1 ± 2.4	5.9 ± 3.6
P1B	20.6 ± 6.4[a,b]	3.8 ± 2.1	10.1 ± 2.3[a,b]	3.2 ± 2.2
P2S	5.1 ± 2.9	2.3 ± 2.2	4.8 ± 2.4	3.2 ± 2.1
P2B	7.7 ± 1.6	6.9 ± 3.1[a,b]	5.9 ± 2.6	5.1 ± 3.0[a,b]
P3S	14.6 ± 6.7	3.2 ± 2.8	3.5 ± 1.8	1.9 ± 1.1
P3B	11.3 ± 4.9	5.0 ± 3.0[a,b]	2.1 ± 2.0	4.0 ± 1.8[a,b]
PC	27.1 ± 7.4[a]	14.7 ± 3.2[a]	30.9 ± 5.6[a]	9.3 ± 3.4[a]

[a] Statistically significant when compared to the negative control (NC).
[b] Statistically significant when compared to the positive control (PC) ($p < 0.05$); P1S - point 1 surface; P1B - point 1 bottom; P2S - point 2 surface; P2B - point 2 bottom; P3S - point 3 surface; P3B - point 3 bottom; PC - positive control.

hydroxynonenal (HNE), and exocyclic DNA adducts (Jomova and Valko, 2011).

All points of the Poti River, both surface and bottom, especially P2, showed high copper concentrations. Toxicogenetic potential of this metal has been reported in cell lines of hamsters (Grillo et al., 2009), bacterial strains (Siddiqui et al., 2011a, b), plant cells (Wasi et al., 2013) and animal cells (Erbe et al., 2011). Sousa et al. (2017a, b) found copper values above the limits recommended by environmental laws. They have found that Cu, Fe, Cr, Al and Zn cause toxigenetic damages in both *Oreochromis niloticus e Allium cepa* (Sousa et al., 2017a, 2017b).

Toxic metals exert their toxicity in a number of ways, including displacement of essential metals from their normal binding sites on biological molecules (e.g., cadmium compete with zinc), inhibition of enzymatic functioning and disruption of nucleic acid structure (Jomova and Valko, 2011; Tabrez and Ahmad, 2011). The toxic effects of Copper, for instance, were described in various aquatic organisms, including fishes (Klauck et al., 2013). Rau et al. (2004) showed that fish cells are more sensitive to copper than mammalian cells. These data corroborated with the study of Arkhipchuk and Garanko (2005) in blood erythrocytes of *Carassius auratus gibelio*, which showed an increase in micronucleus formation and nuclear abnormalities after Copper exposure.

Copper is thought to contribute to significant toxicogenetic alterations observed, since it modifies the activity of antioxidant enzymes, which induces and aggravates oxidative stress (Lushchak, 2011). Fish exposed to copper have shown increased primary and secondary oxidative enzyme activities as reported by Hansen et al. (2006). In addition, other studies have shown that copper increases cytotoxicity and ROS production, resulting in increased DNA strand breaks (Bopp et al., 2008).

Aluminium was directly related to mutagenicity of the Poti river, both in animal testing system and plant. Aluminium toxicity was more prevalent in P1, and is probably derived from mining wash activities next to the region. The formation of aluminum sulphate is very common in water treatment processes that increase the concentration of this metal in the environment (Braga et al., 2002).

Aluminium is classified as an environmental micro contaminant because when combined to chloride it forms aluminum chloride ($AlCl_3$) that is highly toxic (Berthon, 2002). Aluminium is a highly cytotoxic to plants (Ternjej et al., 2010), with the most important physiological consequence being the interruption of root growth and changes in root morphology (Radić et al., 2010). Voutsinas et al. (1997) suggested that the cytoskeleton is the target structure of Al, because this metal acts on tubulins polymerization and, consequently, on the formation of the mitotic spindle, which may be associated with high CA presented in this study. Moreover, several cellular alterations were also reported in *A. cepa*, where Al has been related to interference in cell division kinetics, promoting chromosomal adhesion and nuclear fragmentation (Panda and Achary, 2014), chromosomal breaks, and MN formation (Tabrez and Ahmad, 2011).

Iron (Fe) was found in all sampling points of the Poti River, with

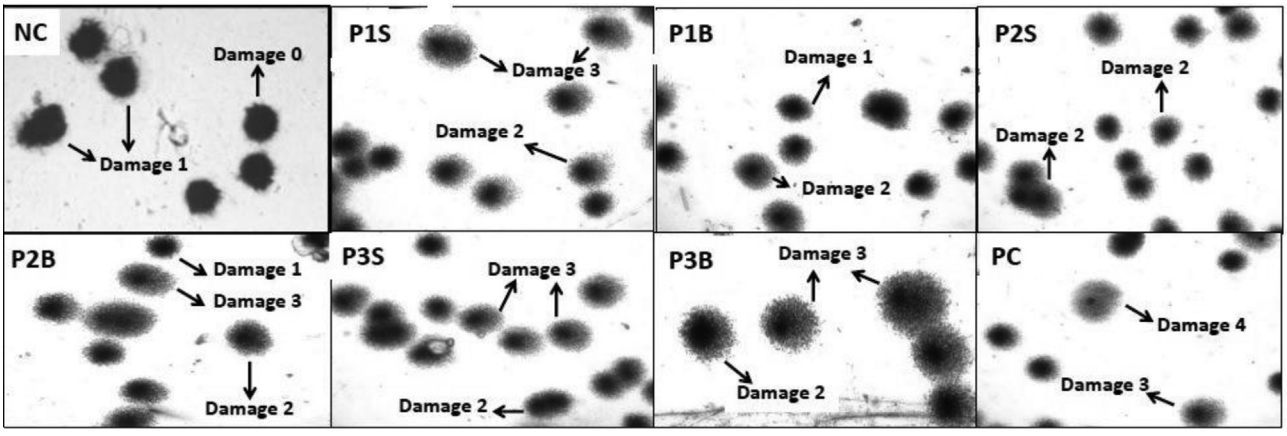

Fig. 2. Photomicrographic profile of fish blood cells with genotoxic damage after being exposed to Poti river water samples from different points and extracts.

Table 4
Mutagenicity Levels (Mut) (CA - chromosome abnormalities) and Cytotoxicity (MI - mitotic index) (mean ± standard deviation) from root meristem of Allium cepa, by points and sampling strata of the Poti river (Teresina PI).

Point/Layers	24 h		48 h		72 h	
	MI	AC	MI	AC	MI	AC
NC	19.49 ± 58.1	9.9 ± 5.3	13.70 ± 30.2	15.6 ± 8.0	18.75 ± 43.6	10.0 ± 7.6
P1S	18.82 ± 59.0[b]	30.2 ± 10.6[a]	12.91 ± 31.3	27.0 ± 8.1[a,b]	17.87 ± 44.1	30.9 ± 8.6[a,b]
P1B	20.95 ± 43.4[b]	21.3 ± 9.8[a,b]	10.26 ± 15.7	26.1 ± 7.9[a,b]	16.24 ± 20.6	23.9 ± 12.6[ab]
P2S	8.85 ± 8.7[a]	18.7 ± 5.9[ab]	12.59 ± 26.7[b]	28.1 ± 8.9[ab]	10.83 ± 45.7[a]	30.3 ± 8.9[a,b]
P2B	7.56 ± 22.3[a]	22.2 ± 8.4[b]	7.9 ± 24.9[a]	16.1 ± 8.2	8.19 ± 38.6[a]	16.4 ± 10.2
P3S	11.21 ± 60.4[a]	28.5 ± 9.9[a]	9.74 ± 12.4[a]	19.6 ± 5.3[b]	9.26 ± 17.2 [a]	28.3 ± 14.6[a,b]
P3B	10.52 ± 45.9[a]	20.2 ± 7.6a[b]	10.87 ± 25.8[a]	31.1 ± 11.3[a,b]	9.95 ± 19.1[a]	36.6 ± 10.4[a,b]
PC	8.51 ± 19.0[a]	34.1 ± 11.6[a]	8.21 ± 14.4[a]	45.5 ± 8.5[a]	7.64 ± 11.7[a]	50.4 ± 8.4[a]

[a] Statistically significant when compared to the negative control (NC).
[b] Statistically significant when compared to the positive control (PC); ($p < 0.05$); P1S - point 1 surface; P1B - point 1 bottom; P2S - point 2 surface; P2B - point 2 bottom; P3S - point 3 surface; P3B - point 3 bottom; PC - positive control.

higher concentration at the P2 bottom. Cytotoxicity in animal and plant systems was directly related to iron concentrations. This metal may originate from different sources, including natural erosion of rocks containing iron ore, meteorites (Kroschwitz, 1995), anthropogenic actions such as mining, smelting, welding, metal polishing and fuel blending (ILO, 1997), or by fertilizers used in farming (Sharma et al., 2000), as well as municipal and industrial sewage effluents (Klauck et al., 2013).

Fish absorb iron from the water by gills or food absorption (Bury et al., 2003), and this metal is essential in many vital processes. However, because of its ability to undergo reduction and oxidation, it cans generate free radicals (Lushchak, 2011). The Poty River during the dry season is stratified because of the absence of current and water imprisonment at its mouth. This condition, favors increased concentration of reactive species of Fe due to little water movement that make dispersion more difficult, especially with high environmental temperatures (35-40 °C) (Oliveira et al., 2010).

Zinc also influenced P2S and P1B in the Poti River. Although it

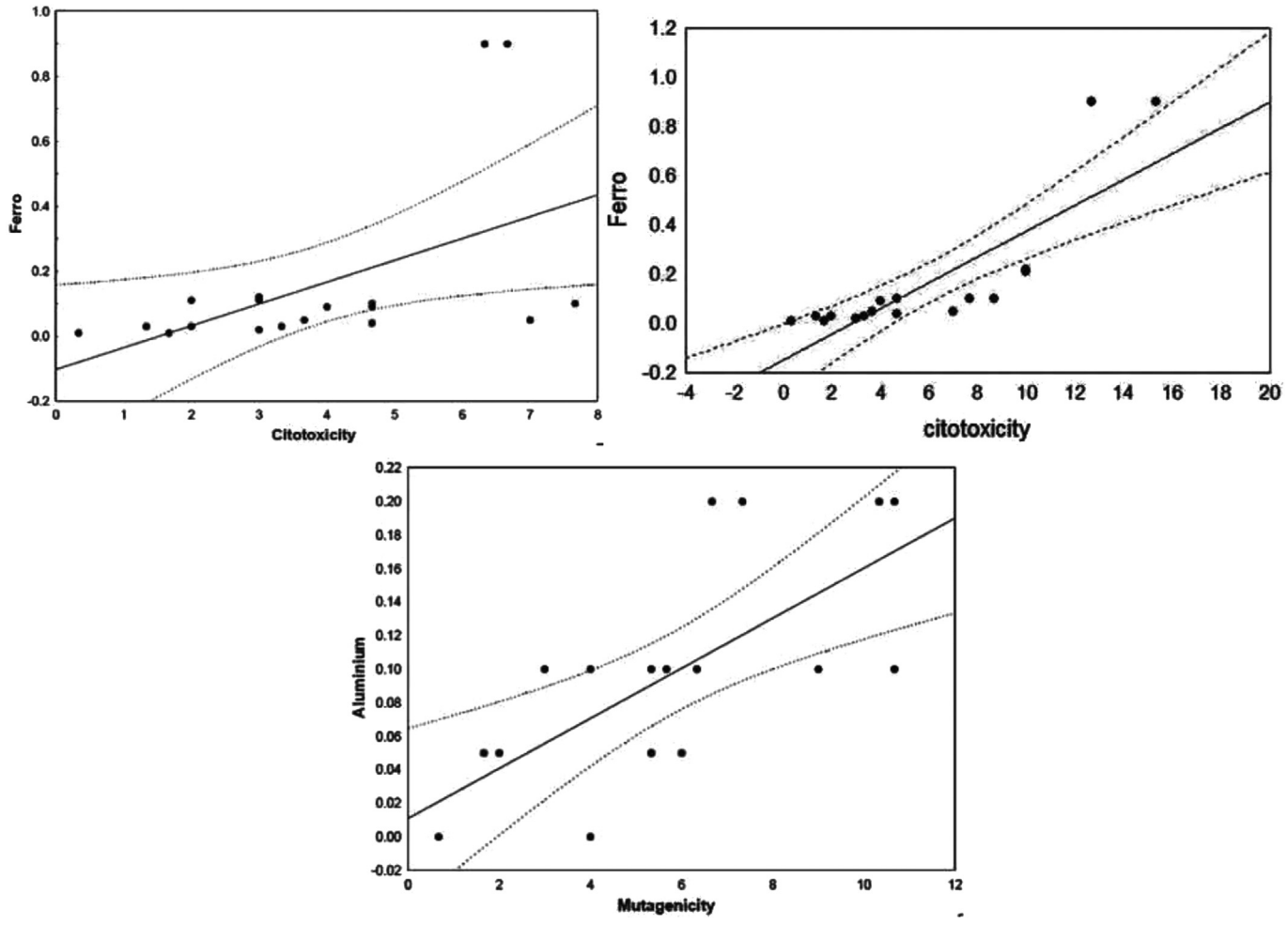

Fig. 3. Correlation between metals and genetic damage observed in the water of the Poti river (Teresina, PI).

was not directly correlated to toxicogenetic damage, these points had higher genotoxic, mutagenic and cytotoxic effects probably because Zn can be associated with other toxic heavy metals, such as cadmium and lead (Pb). In addition, Zn negatively influences Cu bioavailability and alters the metabolism of Fe, an essential component of DNA repair proteins and cell maintenance (Segura-Munoz et al., 2003).

Chromium (Cr) was found increased in P1S, where high levels of genotoxic and mutagenic damage occurred. Cr is an element with variable valency, which can enter into the Haber-Weiss reaction, resulting in the production of free radicals (OH). Moreover, Cr participates in reversible oxidation reactions that have been linked to ROS production (Lushchak, 2011). Thus, this metal may have contributed to toxicogenetic damages found in Poti River waters, since in its hexavalent form (Cr^{6+}), which is toxic at higher concentrations, is related to the development of genotoxic and carcinogenic lesions (Klauck et al., 2013; Tabrez et al., 2014). Additionally, in vivo and cell culture studies have also demonstrated an increased incidence of neoplastic transformation and tumor formation as a result of Cr exposure (Nickens et al., 2010; Zhitkovich, 2011).

Hexavalent chromium (Cr^{6+}) easily crosses the cell membranes by facilitated diffusion, and when participating in chemical reactions, it cans generate ROS and cellular lipoperoxidation (Marcon et al., 2010). Fish exposed to high doses of Cr^{6+} produce DNA-protein crosslinks (DPX) in erythrocytes, increasing the possibilities of genotoxic and mutagenic alterations (Lushchak et al., 2008; Kuykendall et al., 2006). In addition, Cr with residual Zn, arising from industrial or domestic effluents have shown mutagenic effects on meristematic cells of *A. cepa*, evidenced by the high frequency of chromosomal abnormalities, cell adhesion, micronuclei, multinucleated cells, chromosomal and chromatids breaks, bridges and chromosome fragments (Leme and Marin-Morales, 2009; Masood and Malik, 2013).

Heavy metals are known to have toxic effects and cause DNA damages (Korpinen et al., 2012). The presence of metallic elements in the Poti River waters may be one of the main factors that increased toxicogenetic damages in exposed plant and animal cells as documented in studies of similar aquatic environments. The presence of Cu, Fe, Al, Cr and Zn are related to direct or indirect interference of enzymatic processes and DNA repair in exposed organisms (Bianchi et al., 2011; Monteiro et al., 2011).

The presence of metals in waters due to natural or anthropogenic actions (Oliva et al., 2012) represent a significant source of environmental and human contamination, since metals are potentially genotoxic and carcinogenic. High Metal concentrations can be bioaccumulated and biofragmented, which lead to oxidative damage to exposed organisms, resulting in environmental mutagenicity and occurrence of various degenerative diseases, such as cancer (Wasi et al., 2013; Tabrez et al., 2014).

5. Conclusion

This study demonstrated cytotoxic, genotoxic and mutagenic effects of waters from the urban area of the Poti River. Chemical analysis revealed the presence of human pollution through the high concentrations of heavy metals found. Therefore, additional research is needed to better characterize the damage caused by each metal, and more importantly, governmental measures should be taken to minimize environmental impacts in this river located in the northeast region of Brazil.

Conflict of interest

None declared.

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References

Akinboro, A., Mohammed, K., Rathnasamy, S., Muniandy, V.R., 2011. Genotoxicity assessment of water samples from the sungai dua river in pulau pinang, Malaysia, using the Allium cepa test. Trop. Life Sci. Res. 22, 23–35.
APHA, AWWA, WPCF, 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed. American Public Health Association, Washington, DC.
Arkhipchuk, V.V., Garanko, N.N., 2005. Using the nuclear biomarker and the micronucleus test on in vivo fish fin cells. Ecotoxicol. Environ. Saf. 62, 42–52.
Berthon, G., 2002. Aluminium speciation in relation to aluminium bioavailability, metabolism and toxicity. Chem. Rev. 228, 319–341.
Beyersmann, D., Hartiwig, A., 2008. Carcinogenic metal compounds: recent insight intomolecular and celullar mechanisms. Arch. Toxicol. 82, 493–512.
Bianchi, J., Espindola, E.L.G., Marin-Morales, M.A., 2011. Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents. Ecotoxicol. Environ. Saf. 74, 826–833.
Bolognesi, C., Hayashi, M., 2011. Micronucleus assay in aquatic animals. Mutagenesis 26, 205–213.
Bopp, S.K., Abicht, H.K., Knauer, K., 2008. Copper-induced oxidative stress in rainbow trout gill cells. Aquat. Toxicol. 86, 197–204.
Braga, B., Hespanhol, I., Conejo, J.G.L., Barros, M.T.L., Spencer, M., Porto, M., Nucci, N., Eiger, S., 2002. O meio aquático. São Paulo: Escola Politécnica da Universidade de São Paulo, pp. 72–87.
Bury, N.R., Walker, P.A., Glover, C.N., 2003. Nutritive metal uptake in teleost fish. J. Exp. Biol. 206, 11–23.
CONAMA (Conselho Nacional do Meio Ambiente), 2005. Resolução Nº 357. Ministério Do Meio Ambiente, MMA. Brasília. Distrito Federal. http://www.mma.gov.br. (Accessed 5 January 2015).
Duarte, I.D., Dias, M.C., David, J.A.O., Matsumoto, S.T., 2012. A qualidade da água da Lagoa Jacuném (Espírito Santo, Brasil) em relação a aspectos genotóxicos e mutagênicos, mensurados respectivamente pelo ensaio do cometa e teste do micronúcleo em peixes da espécie *Oreochromis niloticus*. Rev. Bras. Bioci., Porto Alegre 10 (2), 211–219.
Erbe, M.C.L., Ramsdorf, W.A., Vicari, T., Cestari, M.M., 2011. Toxicity evaluation of water samples collected near a hospital waste landfill through bioassays of genotoxicity piscine micronucleus test and comet assay in fish Astyanax and ecotoxicity *Vibrio fischeri* and *Daphnia magna*. Ecotoxicology 20, 320–328.
Grillo, C.A., Reigosa, M.A., Mele, M.F.L., 2009. Effects of copper ions released from metallic copper on CHO-K1 cells. Mutant. Res. 672, 45–50.
Hansen, B.H., Rømma, S., Søfteland, L.I., Olsvik, P.A., Andersen, R.A., 2006. Induction and activity of oxidative stress-related proteins during waterborne Cu-exposure in brown trout (Salmo truta). Chemosphere 65, 1707–1714.
ILO, 1997. Geneve. International Labour Organization. Encyclopaedia of Occupational Health and Safety. Metals: Chemical Properties an Toxicity, 4th. ed., vol. 3 63.1-63.68.
Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and humandisease. Toxicology 283, 65–87.
Klauck, C.R., Rodrigues, M.A.R., Silva, L.B., 2013. Toxicological evaluation of landfill leachate using plant (*Allium cepa*) and fish (*Leporinus obtusidens*) bioassays. Waste Manag. Res. 31, 1148–1153.
Korpinen, S., Meski, L., Andersen, J.H., Laamanen, M., 2012. Human pressures and their potential impact on the Baltic Sea ecosystem. Ecol. Indic. 15, 105–114.
Kroschwitz, J.I., 1995. Kirk-othmer Encyclopedia of Chemical Technology, fourth ed. John Wiley & Sons, New York, p. 14.
Kumar, K.S., Dahms, H.U., Won, E.J., Lee, J.S., Shin, K.H., 2015. Microalgae - a promisingtoolforheavymetalremediation. Ecotoxicol. Environ. Saf. 113, 329–352.
Kuykendall, J.R., Miller, K.L., Mellinger, K.N., Cain, A.V., 2006. Waterborne and dietary hexavalent chromium exposure causes DNA-protein crosslinK (DPX) formation in erythrocytes of largemouth bass (Micropterus salmoides). Aquat. Toxicol. 78, 27–31.
Leme, D.M., Marin-Morales, M.A., 2009. Allium cepa test in environmental monitoring: a review on its application. Mutat. Res. 682, 71–81.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.
Lushchak, O.V., Kubrak, O.I., Nykorak, M.Z., Storey, K.B., Lushchak, V.I., 2008. The effect of potassium dichromate on free radical processes in goldfish: possible protective role of glutathione. Aquat. Toxicol. 87, 108–114.
Manzano, B.C., Roberto, M.M., Hoshina, M.M., Menegário, A.A., Marin-Morales, M.A., 2015. Evaluation of the genotoxicity of waters impacted by domestic and industrial effluents of a highly industrialized region of São Paulo State, Brazil, by the comet assay in HTC cells. Environ. Sci. Pollut. Res. 22, 1399–1407.
Marcon, A.E., Ferreira, D.M., Moura, M.F.V., Campos, T.F.C., Amaral, V.S., Agnez-Lima, L.F., Medeiros, S.B.R., 2010. Genotoxic analysis in aquatic environment

under influence of cyanobacteria, metal and radioactivity. Chemosphere 81, 773–780.
Masood, F., Malik, A., 2013. Mutagenicity and genotoxicity assessment of industrial wastewaters. Environ. Sci. Pollut. Res. 20, 7386–7397.
Mazzeo, D.E.C., Matsumoto, S.T., Levy, C.E., Angelis, D.F., Marin-Morales, M.A., 2013. Application of micronucleus test and comet assay to evaluate BTEX biodegradation. Chemosphere 90, 1030–1036.
Monteiro, V., Cavalcante, D.G.S.M., Vilela, M.B.F.A., Sofia, S.H., Martinez, C.B.R., 2011. In vivo and in vitro exposures for the evaluation of the genotoxic effects of lead on the Neotropical freshwater fish *Prochilodus lineatus*. Aquat. Toxicol. 104, 291–298.
Nickens, K.P., Patierno, S.R., Ceryak, S., 2010. Chromium genotoxicity: a double-edgedsword. Chem. Biol. Interact. 188, 276–288.
Nunes, E.A., Lemos, C.T., Gavronski, L., Moreira, T.N., Oliveira, N.C.D., Silva, J., 2011. Genotoxic assessment on river water using different biological systems. Chemosphe 84, 47–53.
Oliva, M., Vicente, J.J., Gravato, C., Guilhermino, L., Galindo-Rianõ, M.D., 2012. Oxidative stress biomarkers in Senegal sole, Solea senegalensis, to assess the impact of heavy metal pollution in a Huelva estuary (SWSpain): seasonal and spatial variation. Ecotoxicol. Environ. Saf. 75, 151–162.
Oliveira, L.N., Silva, C.E., 2014. Qualidade da água do rio poty e suas implicações para atividade de lazer em Teresina-PI. Rev. Equador (UFPI) 3, 128–147.
Oliveira, C.N., Campus, V.P., Medeiros, Y.D.P., 2010. Avaliação e identificação de parâmetros importantes para a qualidade de corpos d'água no semiárido Baiano. Estudo de caso: bacia hidrográfica do rio Salitre. Quim. Nova 33 (5), 1059–1066.
Ossana, N.A., Castane, P.M., Salibian, A., 2013. Use of lithobates catesbeianus tadpoles in a multiple biomarker approach for the assessment of water quality of the reconquista river (Argentina). Arch. Environ. Contam. Toxicol. 65, 486–497.
Panda, B.B., Achary, V.M.M., 2014. Mitogen-activated protein kinase signal transduction and DNA repair network are involved in aluminum-induced DNA damage and adaptive response in root cells of *Allium cepa* L. Front. Plant Sci. 5, 1–10.
Radić, S., Stipaničev, D., Vujčić, V., Rajčić, M.M., Širac, S., Pevalek-Kozlina, B., 2010. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. Sci. Total Environ. 408, 1228–1233.
Rau, M.A., Whitaker, J., Freedman, J.H., Di Giulio, R.T., 2004. Differential susceptibility of fish and rat liver cells to oxidative stress and cytotoxicity upon exposure to prooxidants. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 137, 335–342.
Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S., 2012. Standard Methods for the Examination of Water and Wastewater, 22ed. American Public Health Association, American Water Works Association, Water Environment Federation, p. 1496.
Segura-Munoz, S.I., Trevilato, T.B.M., Takayanagui, A.M.M., Hering, S.E., Cupo, P., 2003. Metales pesados en água de bebederos de presión. Arch. Latinoam. Nutr. 53 (1), 48–53.
Sharma, V.K., Rhdy, K.B., Cargill, J.C., Tacker, M.E., Vazquez, F.G., 2000. Cases and solutions: metals and grain size distribution in soil of the middle Rio Grande basin, Texas, USA. Environ. Geol. 39 (6), 698–704.
Siddiqui, A.H., Tabrez, S., Ahmad, M., 2011a. Short term in vitro and in vivo genotoxicity testing systems for some water bodies of northern India. Environ. Monit. Assess. 180, 87–95.
Siddiqui, A.H., Tabrez, S., Ahmad, M., 2011b. Validation of plant based bioassays for the toxicity testing of Indian waters. Environ. Monit. Assess. 179, 241–253.
Silva, C.E., Almeida, K.S., Monteiro, P.B.C.L., 2010. Indice de qualidade da água do Rio Poty-Teresina-PI. ABES, X Simpósio Ítalo-Brasileiro Eng. Ambient. IV-011 7.
Singh, N.P., Mccoy, M.T., Tice, R.R., Scheider, E.L., 1988. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175 (1), 184–191.
Sousa, J.M.C., Peron, A.P., Silva, F.C.C., Dantas, E.B.S., Lima, A.M.V., Oliveira, V.A., Matos, L.A., Paz, M.F.C.J., Alencar, M.V.O.B., Islam, M.T., Melo-Cavalcante, A.A.C., Bonecker, C.C., Júlio Jr., H.F., 2017a. Physico-chemical and genotoxicity analysis of Guaribas river water in the Northeast Brazil. Chemosphere 177, 334e338.
Sousa, J.M.C., Peron, A.P., Sousa, L.S., Holanda, M.M., Silva, F.C.C., Lima, A.M.V., Oliveira, V.A., Lima, L.H.G.M., Matos, L.A., Dantas, S.M.M.M., Aguiar, R.P.S., Islam, M.T., Melo-Cavalcante, A.A.C., Bonecker, C.C., Júlio Jr., H.F., 2017b. Cytotoxicity and genotoxicity of Guaribas river water (Piauí, Brazil), influenced by anthropogenic action. Environ. Monit. Assess. 189, 301.
Souza, T.S., Fontanetti, C.S., 2006. Micronucleus test and observation of nuclear abnormalities in erythrocytes of Nile tilapia exposed to Waters affected by refinery effluent. Mutat. Res. 605, 87–93.
Tabrez, S., Ahmad, M., 2011. Oxidative stress mediated genotoxicity of wastewaters collected from two different stations in northern India. Mutat. Res. 726 (1), 15–20.
Tabrez, S., Shakil, S., Urooj, M., Damanhouri, G.A., Abuzenadah, A.M., Ahmad, M., 2011. Genotoxicity testing and biomarker studies on surface waters: An overview of the techniques and their efficacies. J. Environ. Sci. Health, Part C 29, 250–275.
Tabrez, S., Priyadarshini, M., Priyamvada, S., Khan, M.S., Na, A., Zaidi, S.K., 2014. Gene–environment interactions in heavy metal and pesticide carcinogenesis. Mutat. Res. 760, 1–9.
Torres de Lemos, C., Rodel, P.M., Terra, N.R., Oliveira, C.D., Erdtmann, B., 2007. River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol. Environ. Saf. 66, 391–401.
Ternjej, I., Mihaljevic, Z., Stankovic, I., Kerovec, M., Sipos, L., Zeljezic, D., et al., 2010. Estimation of DNA integrity in blood cells of eastern mosquitofish (*Gambusia holbrooki*) inhabiting an aluminium-polluted water environment: an alkaline Comet Assay study. Arch. Environ. Contam. Toxicol. 59, 182–193.
Voutsinas, G., Zarani, F., Kappas, A., 1997. The effect of environmental aneuploidy-inducing agents on the microtubule architecture of mitotic meristematic root cells in Hordeum vulgare. Cell. Biol. Int. 21, 411–418.
Wasi, S., Tabrez, S., Ahmad, M., 2013. Toxicological effects of major environmental pollutants: an overview. Environ. Monit. Assess. 185, 2585–2593.
Žegura, B., Heath, E., Černoša, A., Filipič, M., 2009. Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. Chemosphere 75, 1453–1460.
Zhitkovich, A., 2011. Chromium in drinking water: sources, metabolism, and cancerrisks. Chem. Res. Toxicol. 24, 1617–1629.