Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v39i3.27787

Influence of natural radon and metal contamination on surface water quality from a Brazilian Semiarid Region

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ABSTRACT. The Brazilian semiarid region presents some adverse environmental conditions for the settled population such as a restricted water availability and the presence of radon and metal natural sources that can contaminate the water reservoirs and consequently become a concern for human health. The present study evaluated the water quality of the Riacho das Cachoeiras Dam located in the urban area of Lajes Pintadas (state of Rio Grande do Norte, Brazil) as source for human consumption. An analysis of Physicochemical parameters, heavy metal content and Radon in water samples was performed along with the assessment of the water mutagenic potential through Micronucleus Test (MN) on *Tradescantia pallida* and *Oreochromis niloticus*. The content of metals in water for Al, Cd, and Ni were above water quality guidelines for human consumption. Moreover, high levels of Pb along with dissolved Radon were found. An acute and chronic mutagenic water capability was observed. These findings demonstrated that the water quality is unsuitable for human consumption due to the presence of high levels of contaminants mainly from geogenic origin and its deleterious effect on living systems.

Keywords: radiation, lead, mutagenicity, micronucleus, geogenic contamination.

Influência da contaminação natural pelo radônio e metais na qualidade das águas superficiais da Região Semiárida Brasileira

RESUMO. A região semiárida brasileira apresenta condições ambientais adversas para população local, como a escassez na disponibilidade de água e a presença de fontes naturais de radônio e de metais que podem contaminar os reservatórios de água e, consequentemente, tornar-se um problema de saúde humana. O estudo avaliou a qualidade da água do Açude do Riacho das Cachoeiras localizado na área urbana de Lajes Pintadas (Rio Grande do Norte, Brasil) como fonte para o consumo humano. Foram realizadas análises físico-químicas, de conteúdo de metais e de radônio em amostras de água em conjunto com a avaliação do potencial mutagênico da água por meio do teste de micronúcleos (MN) em *Tradescantia pallida* e *Oreochromis niloticus*. Os metais Al, Cd, e Ni estavam acima das diretrizes de qualidade da água para o consumo humano. Além disso, foram encontrados altos níveis de Pb dissolvido, juntamente com o radônio. Observou-se um potencial de indução de efeito mutagênico nas amostras de água testadas, tanto na exposição aguda quanto na crônica. Estes resultados demonstram que a qualidade da água do Açude está imprópria para o consumo humano devido à presença de elevados níveis de contaminantes de origem geogênica, com capacidade de exercer efeito deletério sobre os organismos.

Palavras-chave: radiação, chumbo, mutagenicidade, micronúcleos, contaminação geogênica.

Introduction

The Brazilian semiarid region covers a surface of one million of Km^2, 12% of the national territory, where more than 22 million of inhabitants face an adverse environmental scenario due to water scarcity (IBGE, 2016). Low rainfall rate along with long periods of drought led to build water reservoirs (dams), aimed to counteract the hydric needs of the population (INSA, 2014). Apart from that, some geochemical caracteristics (e.g. the presence of some earth's crust constituents) can negatively affect water quality by lixiviation, run off and leaching processes turningthe available water a potential vector for dissemination of toxic substances. Water potability monitoring of these reservoirs involves the assessment of general characteristics such as microbiological and physicochemical parameters without considering certain aspects of the involved environment that could become a toxicological human health concern (CONAMA, 2005). The terrestrial crust from the Brazilian semiarid territory has one of the most important reservoirs of uranium worldwide (IAEA, 2009). The presence of crystalline (metamorphic and granitic) rocks in the region can lead to enrich water sources with uranium and/or its by-products such as Radon (^{222}Rn) an Lead (Pb) (CPRM, 2013). These uranium by-products have some toxicological characteristics to be taken into account. The former is an unstable gaseous radionucleide catalogued as a proven human carcinogen (IARC, 2009). Radon is considered the second cause of lung cancer worldwide (IAEA, 2009; WHO, 2009). The second, besides to promote a variety of toxicological effects on exposed population (e.g., cognitive dysfunction, neurobehavioral disorders, renal damage, hypertension) has been catalogued as a probable human carcinogen (IARC, 2009; Beyersmann & Hartwig, 2008; Martínez, Alvez, & Barbieri, 2013).

Despite the scientific evidence of uranium by-products toxicity little is known about these elements as potential human health concern in Brazil, (Bonotto, 2014; Santos & Bonotto, 2014) and in areas with restricted accessibility to water such as the Brazilian northeast no reports were found in the scientific literature yet.

In this context, due to the complexity of the resulting toxic mixture to be taken into account, additional analytical approaches to determine the toxicological effect of the substance/s present in water samples can be performed. Mutagenicity tests have been applied to evaluate different pollutants and contaminants in freshwater ecosystems (Misík, et al., 2011; Vincent-Huber, Heas-Moisan, & Munschy, 2012). Micronuclei (MN) test is one of the most used assays in different models to determine chromosomal damage caused by the presence of compounds and mixture of substances (Misík, et al., 2011; Garcia et al., 2011; Hoshina, Angelis, & Marin-Morales, 2008). *Tradescantia pallida* plants have been widely used to perform micronuclei test provingto be an excellent biondicator for environmental quality assessment (Garcia et al., 2011; Leal, Crispim, Frota, Kelecom, & Silva, 2008; Thewes, Endres Júnior, & Droste, 2011). In the same way, the use of *Oreochromis niloticus* fish due to its capability of adaptation to different environmental conditions, have also been extensively used in freshwater reservoirs to perform this mutagenic assay (Hoshina et al., 2008; Carvalho, Bernusso, Araújo, Espíndola, & Fernandes, 2012; Soliman & Ibrahim, 2012). Facing a probably natural contamination due to the geological characteristics of the Brazilian semiarid region, the adverse climatic conditions that lead to restrict the water availability for the population and the absence of studies of water quality of the Riacho das Cachoeiras Dam, a strategic regional water reservoir, the present study aimed to evaluate water quality for human consumption considering i) the assessment of radon and metal contamination and ii) the evaluation of mutagenic effect using micronuclei tests in *Tradescantia pallida* and *Oreochromis niloticus*..

Material and methods

Area of study and sampling points

The city of Lajes Pintadas is located in the Brazilian semiarid region, 135 km away from the capital of Rio Grande do Norte. According to the 2010 census, the city has a population of 4614 inhabitants (IBGE, 2016). The Riacho das Cachoeiras Dam (6,000,000 m^3 of capacity) is a rain water reservoir located within the city limits and is used for aquaculture, irrigation, recreation, fishing activities and as drinking water source.

As sampling points for collecting water samples in the dam, three georeferenced points were chosen, indicated as P1 (6° 8' 30.08" S, 36° 6' 54.39" W), P2 (6° 8' 37.26" S, 36° 6' 55.78" W) and P3 (6° 8' 41.53" S, 36° 7' 0.46" W) (Figure 1).

Sample collection

The sample collection was performed on December 2011. It consisted in the collection of 1 liter of surface water taken at 1-meter deep per sampling point from the Riacho das Cachoeiras Dam collected in polyethylene flasks, previously soaked in 20% v/v nitric acid, rinsed with distilled water and dried. No conservatives were used and the samples were kept frozen at -20°C until analysis, in accordance to the *American Public Health Association* (APHA; AWWA; WPCF, 2005).

Fish sampling was performed by handicraft capture. The specimens for control group were obtained from an aquarium and acclimatized in chlorine-free water for a period of 120 days before the experimental assay. The use of fishes in this research was conducted in accordance with the National guidelines for animal welfare.

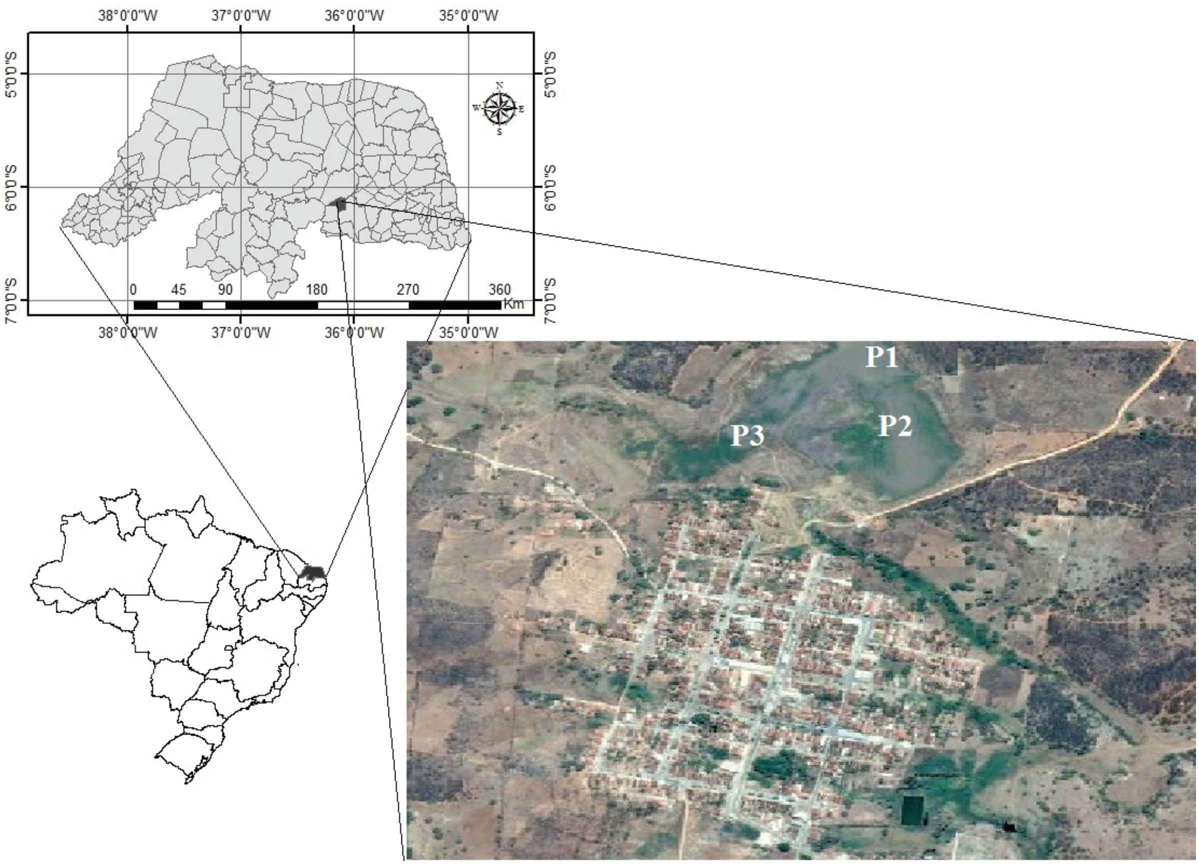

Figure 1. Geographic location of Lajes Pintadas (Rio Grande do Norte, Brazil), the Riacho das Cachoeiras dam and the sampling points. Adapted from Google Earth.

Water samples analysis

Physicochemical analysis:

For the physicochemical analysis of water, a multiparameter equipment for *in situ* measurements (In-Situ inc, model Troll 9000 Pro XP) was used. The parameters measured were pH, turbidity, temperature, conductivity and dissolved oxygen. The results obtained were compared with the national guideline for water quality for human consumption (CONAMA, 2005).

Metal analysis:

Metal analysis in water samples (Al, Cd, Co, Cu, Pb, Cr, Fe, Mn, Ni, Ag, and Zn) was performed by atomic absorption spectrophotometry. as described in APHA; AWWA; WPCF, (2005). Brieffly 4 ml of concentrated nitric acid was added into 250 ml of sample and then evaporated to near 10 ml in a hot plate. Then 2.5 ml of clolhidric acid were added and the mixture heated for 30 min. Then dried and reconstituted in a volumetric flask to 50 ml using deionized water.

The equipment used was a flame atomic absorption spectrophotometer (Varian model 50B). Every sample was measured by triplicate. Calibration standards along with spiked deionized water aliquots (used as internal quality control) were prepared with reagents (Specsol) traceable to The Institute of Standards and Technology (NIST).

Analysis of Radon:

Radon quantification was conducted by emanometry, by long period electret method using a RADELEC equipment (L-PERM ®) as described by Kotrappa (2008). Measurement was performed after five minutes of instrument stabilization. Every sample was measured by triplicate and an instrument sensitivity quality control was performed using a reference zero electret after every sample batch measured.

Mutagenicity analysis

Micronuclei tests in *Tradescantia pallida* (Trad-MCN):

Trad-MCNwas performed following the protocol described by Ma (1981). Briefly, young inflorescences were collected and then packed and put in hydroponic systems through three stages: (i) adaptation, (ii) exposure (12 hours), and (iii) recovery. For positive and negative control, a solution of 0.2% formaldehyde and Hoagland solution during the three stages were used respectively. After the hydroponic stage, the buttons containing the anthers were placed in fixative solution (acid acetic and ethylic alcohol 1:3) for 48 hours, and then stained with 2% acetic carmine. Ten slides were assayed and a total of 3000 cells in the tetrad stage per sample/control were analyzed using a 1000 x oil-immersion lens.

Micronucleus assays in *O. niloticus* erythrocytes:

Micronucleus assays in *Oreochromis niloticus* was performed following the protocol described by Çavas & Ergene-Gözükara (2005). Twenty-four fish: 12 fishes from Riacho da Cachoeiras dam and 12 control fishes were used.

Briefly, the mutagenicity test was carried out in blood samples obtained by gill puncture using heparinized syringes. After collection, three blood smears were prepared per animal. Then the slides were fixed with ethanol P. A. and stained with 10% Giemsa. A total of 1000 erythrocytes per animal were assessed using a 1000 x oil-immersion lens.

Statistical analysis

Statistical analysis was performed using SPSS 20. The results obtained of physicochemical, metal and radon analysis were evaluated by descriptive analysis and compared with the guideline of water quality for human consumption (CONAMA, 2005; EPA, 1994). Mutagenicity effect in *Tradescantia pallida* was statistically analyzed by comparison with control groups using Dunnett's t-test and for *O. niloticus* erythrocytes Mann-Whitney U test.

Results and discussion

The present study evaluated the water quality from the Riacho das Cachoeiras Dam taking into account potential natural contaminants presents in the environment and not included in conventional evaluation of water potability.

Analysis of water

Some raw physicochemical characteristics of water from the Riacho das Cachoeiras dam are shown in Table 1. Turbidity, pH, and dissolved oxygen were within normal levels. Nevertheless, a high conductivity in all sampled points was observed (CONAMA, 2005; CETESB, 2009). The former parameters describe a non-eutrhofic environment. The latter, can be interpreted as result of the low filled level and a concentration of mineral substances coming from the lixiviation and run off proceses from earth's crust.

Table 1. Physicochemical characteristics of water samples from the Riacho das Cachoeiras dam

Variable	Guideline	P1	P2	P3
pH	6.0 - 9.0	8.56	8.59	8.58
Turbidity /NTU	40	14.9	12.1	13.6
Temperature (°C)	-	26.0	25.7	26.0
Conductivity (μS cm^{-1})	100[a]	4135*	4156*	4179*
Disolved oxigen (mg L^{-1})	>6	10.5	9.5	9.6
Depth (m)	-	1.9	3.1	1.9

NTU = Nephelometric Turbidity Units. All the results were in accordance with the national guideline (CONAMA, 2005, CETESB, 2009). * Above reference value.

In Table 2, is presented the concentration of the 11 metals analyzed. Al, Cd, Ni and Pb, were above the national guideline (CONAMA, 2005).

The presence of metals into a particular environment can be the result for the ocurrence of natural and/or anthropogenic sources. The lack of industrial activity near the dam and the absence of waste drainage from the Lajes Pintadas city into the water reservoir, discarded these anthropic contamination sources. Other activities such as the use of fertilizers or other domestic products could be considered as as low-probability and then neglected as potential contributors to the metal levels observed (Iqbal, Tirmizi, & Shah, 2013; Iqbal & Shah, 2011). Natural sources can strongly explain the levels of metal found. The stored water exclusively comes from rain. Moreover, the granitic composition of the surface earth crust, contribute to reject the possibility of water transference from other source such as a contaminated phreatic layer. Therefore, in this particular scenario the leaching process of minerals from hearth crust is the most probable source of those elements found (CPRM, 2014; Carvalho et al., 2012).

A particular high level of lead was established. This element is the terminal product of uranium degradation (Robertson, Allen, Laney, & Curnow, 2013). The lead contamination could be explained in addition to minerals lixiviation process, as result of the uranium natural degradation present in the studied region (CPRM, 2014; Geras'kin et al., 2007; Motoki, Campos, Fonseca, & Motoki, 2012). Nevertheless, such speculation needs to be verified through a future isotopic analysis aiming to understand the sources of lead involved (Cheng & Hu, 2010).

The hazard characterization of water samples for human consumption through Radon content is shown in Table 3. Mean Radon level in water samples was quite constant in all the dam and significantly high (expressed as mean ± SD): 60.0±7.3 Bq L^{-1} five-seven times higher than the EPA Guideline of 11 Bq L^{-1}. The Uranium-rich pegmatitic rocks described in the region explains the natural origin of radon (CPRM, 2014).

Table 2. Levels of metals (mg L^{-1}) in water samples from the Riacho das Cachoeiras dam.

Metal	P1	P2	P3	CONAMA	LOD
Al	0.229 ± 0.050[a]	0.225 ± 0.020[a]	0.240 ± 0.042[a]	0.200	<0.050
Cd	0.012 ± 0.004[a]	0.014 ± 0.003[a]	0.013 ± 0.002[a]	0.010	<0.010
Co	0.119 ± 0.005	0.121 ± 0.021	0.136 ± 0.003	0.200	<0.030
Cu	<0.010	<0.010	<0.010	0.013	<0.010
Pb	0.220 ± 0.030[a]	0.300 ± 0.000[a]	0.215 ± 0.027[a]	0.033	<0.030
Cr	0.013 ± 0.004	0.023 ± 0.002	0.018 ± 0.002	0.050	<0.010
Fe	1.974 ± 0.174	0.877 ± 0.033	1.575 ± 0.202	5.000	<0.010
Mn	0.038 ± 0.014	0.037 ± 0.012	0.037 ± 0.010	0.500	<0.010
Ni	0.078 ± 0.011[a]	0.122 ± 0.004[a]	0.108 ± 0.006[a]	0.025	<0.030
Ag	<0.030	<0.030	<0.030	0.050	<0.030
Zn	0.035 ± 0.005	0.035 ± 0.001	0.041 ± 0.001	5.000	<0.010

Results represented as mean ± SD obtained through the analysis of every point sampled per triplicated. Comparison of the results with the reference value of water quality: (a) Above national guidelines. LOD = Limit of detection of the method applied.

The main source of exposure considered in risk assessment due to ^{222}Rn exposure has been inhaled air (IAEA, 2009; WHO, 2009). Risk management in radon contaminated areas includes preventive costless actions against radon exposure (through inhaled air) such as house aeration (WHO, 2009). Especially in warm regions such as the case of the Brazilian northeast, this action is naturally performed helping to reduce and/or prevent the exposure. Unlike, water from Uranium-rich regions can become a constant source to radon exposure (Geras'kin et al., 2007; Al Zabadi, Musmar, Issa, Dwaikat, & Saffarini, 2012; Murat Saç et al., 2014) especially where the accessibility to water supply is restricted as the case of Lajes Pintadas city. In this context, the importance to understand water as source of exposure is enhanced. The results obtained in this work, revealed by the first time radon water contamination describing the health hazard related to the natural radioactivity present in the Riacho das Cachoeira dam.

Table 3. Radon content in water samples from the Riacho das Cachoeiras dam.

Sample point	EPA, 1994	N	Radon (Bq L^{-1})
P1	11 Bq L^{-1}	8	61.8 ± 8.7
P2	11 Bq L^{-1}	9	60.7 ± 7.5
P3	11 Bq L^{-1}	9	60.4 ± 7.4

Results represented as mean ± SD. N = number of samples analyzed per point.
* Results over the EPA guideline of 11Bq L^{-1} (300 pCi L^{-1}).

Mutagenic analysis

Mutagenic assays have been very useful tools for detecting genetic damage caused by exposure to xenobiotics in plants, animals and humans (Garcia et al., 2011; Hoshina et al., 2008; Fenech et al., 2011).

Trad-MCN

Trad-MCN, have been used for testing a wide range of chemicals and complex mixtures of substances from environmental origin (Misík et al., 2011; Garcia et al., 2011; Thewes et al., 2008).

Mutagenic effect of water samples from Riacho das Cachoeira dam using Trad-MCN test is represented in Figure 2. A significant mutagenic effect was observed in all the samples tested, representing between 50 and 70% of the effect observed in the positive control group and more than six times higher than the effect observed in the negative control group.

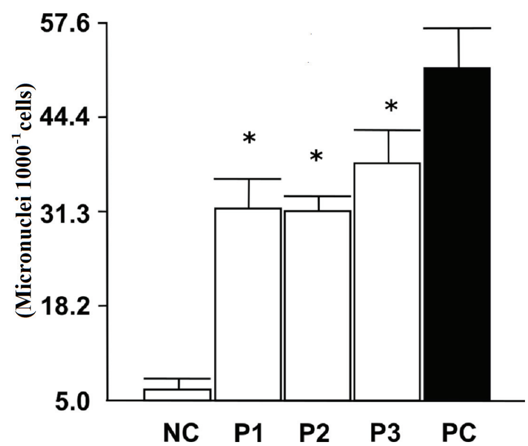

Figure 2. Analysis of mutagenic effect of water samples from Riacho das Cachoeira dam using Trad-MCN test. NC = negative control; PC = positive control. Results represented as mean ± SD. * Statistical difference, Dunnet test (P <0.001).

Oreochromis niloticus MN

Oreochromis niloticus has been widely used for analysis of chromosomal damage in impacted environments (Hoshina et al., 2008; Soliman & Ibrahim, 2012; Salvagni, Ternus, & Fuentefria, 2011). The Micronuclei frequency found in *O. niloticus* erythrocytes from Riacho das Cachoeira dam is represented in Figure 3.

A significant increment in micronuclei frequency in *O. niloticus* erythrocytes from Riacho das Cachoeira dam was observed, reinforcing the Trad-MCN results.

The mutagenic effect observed in the species analyzed lead to reach additional observations. By one hand Trad-MCN was the most sensitive species that lead to detect acute mutagenic effect of the toxic/s compound/s present in water from Riacho das Cachoeiras Dam in a short period of exposure (12 hours of exposure). Moreover, the frequency of micronuclei in *O. niloticus* erythrocytes describes a chronic effect due to the exposure to mutagenic substance/s. Nevertheless, it is needed to highlight that the negative conditions represented by a high conductivity could influence the elicited effect observed.

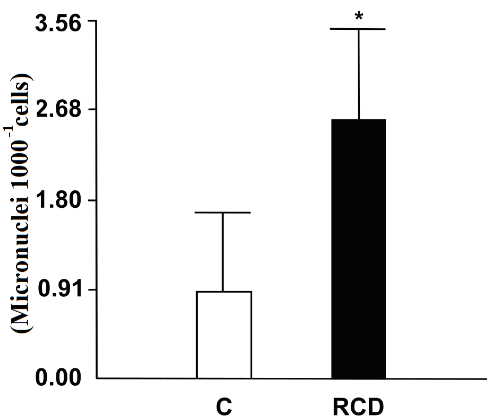

Figure 3. Micronuclei Frequency in erythrocytes of *O. niloticus* from Riacho das Cachoeira dam. C = control fish; RCD = Riacho das Cachoeiras dam fishes. Results represented as mean ± SD. * Statistical difference, Mann–Whitney U test ($P < 0.001$).

A variety of studies indicate the presence of heavy metals in complex environmental mixtures such as water samples from different natural sources as inducers of mutations in the genetic material (Patel, Lynch, Ruff, & Reynolds, 2012). Al, Cd, Ni and Pb have been widely linked with genotoxic and mutagenic effect (Bal, Protas, & Kasprzak, 2011; Lima et al., 2011). The Lead levels found in this work were seven fold the guideline value considered. Lead toxicity has been widely *in vivo* and *in vitro* studied. This metal is related to indirect mechanisms of genotoxicity through the alteration of cell functioning by mean the production of free radicals and the inhibition of DNA repair (García-Lestón, et al., 2010; Kasten-Jolly & Lawrence, 2014).

Radon ionizing radiation can directly affect the genetic material getting as consequences mutations, chromosome aberrations, sister chromatic exchange and micronuclei induction, or indirectly by the generation of reactive oxygen species, contributing to the mutagenic effect observed (Robertson et al., 2013; UNSCEAR, 2000).

Nevertheless, the role of the contaminants taken into account in this work and their relationship with the mutagenic effect observed is difficult to understand. By one hand the complexity of the composition of toxicological interest substances could lead to a synergic effect between them (UNSCEAR, 2000). On the other hand, in the case of heavy metals on aquatic compartments they can suffer physical and chemical modifications such as quelational effect along with alteration due to colloidal formation processes, affecting their bioavailability and consequently the expected toxicological effect (Mager, Esbaugh, Brix, Ryan, & Grosell, 2011; Rodgher & Espíndola, 2008). Three previous studies performed in the Brazilian northeast for this group, revealed mutagenic effect of water pollution/contamination from another reservoir (Lucrecia Dam and Extremoz lake) through Trad-MCN and micronuclei test in *O. niloticus* erythrocytes (Garcia et al., 2011; Barbosa, Cabral, Ferreira, Agnez-Lima, & Medeiros, 2010; Marcon, et al., 2010). In all cases an increased micronuclei frequency was observed in a magnitude significatively lower than the observed effect in this work. Is interesting to note that metal content was quite similar among the three works and lead levels were within the considered guideline level. These observations lead to hypothesize that the increased mutagenic damage observed was directly related to an additive or synergic mutagenic effect mainly due to the presence of a high radon and lead concentrations in the water of the Riacho das Cachoeira dam (García-Lestón et al., 2010).

Conclusion

The water quality of Riacho das Cachoeiras dam is affected for the presence of toxic substances, mainly due to high levels of lead and Radon, characterizing the source of water as not safe for human consumption. The mutagenic effects observed in two species confirm the unhealthy water for living systems and describe a hazardous source for the population of Lajes Pintadas City. Currently, this research group is performing a risk assessment aiming to understand role of natural radioactivity with the incidence of illness (mainly the high rate of oropharynx, stomach and lung cancers) observed in the population of Lajes Pintadas City.

Acknowledgements

The authors are grateful for the financial support in this research to: *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

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Received on May 11, 2015.
Accepted on May 2, 2017.