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FIRST EVALUATION OF GENOTOXICITY OF MACAÉ (RJ) ESTUARINE WATERS

Primeira avaliação da genotoxicidade das águas do estuário de Macaé (RJ)

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ABSTRACT

Low levels of contamination were detected recently on Macaé estuarine waters showing effects in aquatic organisms at different levels. Nonetheless, genotoxicity was not yet evaluated for these waters. A preliminary study was performed using zebrafish Danio rerio *by exposing this species to Macaé River estuarine waters. DNA and chromosome damage was evaluated by the comet assay, after 24h and 48h of exposure; and by the micronucleus test, after 72 h and seven days of exposure. The results showed DNA damage (% Tail DNA) 5.1-fold higher in fish exposed to estuarine waters than in controls after 48 h of exposure; and significantly higher number of micronuclei/thousand (10-12) in fish exposed to estuarine waters than in controls after three and seven days of exposure. The results of the present study, although preliminary, evidenced genotoxicity of the estuarine waters from the Macaé River, indicating that genotoxicity was at levels to induce DNA and chromosome damage in adult fish.*

***Key words**: Macaé River,* Danio rerio*, Comet Assay, Micronuclei Test, Brazil.*

RESUMO

Níveis baixos de contaminação foram detectados recentemente nas águas estuarinas de Macaé, cujos efeitos crônicos foram descritos em alguns organismos aquáticos em níveis diferentes. No entanto, genotoxicidade ainda não foi avaliada para estas águas. Um estudo

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preliminar foi realizado utilizando o peixe-zebra *Danio rerio* e expondo o peixe às águas estuarinas do Rio Macaé. O dano no DNA e nos cromossomos foi avaliado pelo ensaio do cometa após 24h e 48h de exposição e pelo teste do micronúcleo após 72 h e sete dias de exposição. Os resultados mostraram dano no DNA (% DNA na cauda) 5,1 vezes maior em peixes expostos à água do estuário que nos indivíduos controles após 48 h de exposição; e significativamente maior número de micronúcleos (10-12/1000) nos peixes expostos à água do estuário que nos controles após três e sete dias de exposição. Os resultados do presente estudo, ainda que preliminares, evidenciam genotoxicidade das águas estuarinas do Rio Macaé, indicando que a genotoxicidade está em níveis capazes de induzir dano no DNA e chromossomos de peixes adultos.

Palavras-chaves: Rio Macaé, *Danio rerio*, Ensaio do cometa, Teste do micronúcleo, Brasil.

INTRODUCTION

Estuaries usually receive a wide range of materials produced by natural and anthropogenic sources. At the Macaé River basin and estuary (State of Rio de Janeiro), urbanization has increased during the last decade due to petroleum exploration activities at Campos Basin. Nowadays, the Macaé River estuary receives untreated domestic sewage mainly from almost 200,000 inhabitants of the Macaé municipality. Other pollution activities such as the urban runoff, solid waste disposal, agriculture and animal husbandry were potential sources of contaminants to the estuary. Molisani *et al*., (2013a) estimated that anthropogenic sources were responsible for 62% of the total emission of Zn (29 t/year), 73% of Cu (7.0 t/year), 53% of Pb (7.9 t/year) and 57% of Cd (3.7 t/year) to the Macaé River basin and estuary; and thus, exceeding the loads from natural sources such as soil runoff and atmospheric deposition. In addition, other activities such as navigation and urbanization have increased the presence of oil derivatives and compounds such as polycyclic aromatic hydrocarbons (PAHs) in the estuary. As a consequence, high PAH levels on mussel from the estuary were found, ranging from 2,876-6,101 µg/kg, which were typical of polluted areas (Santiago *et al*., 2016). Furthermore, many other biological effects have been found in the estuary, such as the catfish protective enzymatic responses to PAHs from the estuarine waters and sediments (Berenger, 2013); high concentrations of Cr in mussels (Santiago *et al*., 2016); fish abnormalities during embryonic development and juvenile growth (Nascimento, 2015); and mortality of amphipods exposed to estuarine sediments (Molisani *et al*., 2013a). Some metals and PAHs may be genotoxic at certain levels (Jha, 2004), therefore, our aim was to evaluate the levels of genotoxicity that these waters may cause in the known animal model. Genotoxicity tests are very sensitive end-points that affect genetic material at very low levels of contaminants, and allow to find effects in low sensitive species or at higher ontogenetic levels such as adults, which may not be detected with less sensible markers. Therefore, the evaluation of genotoxicity is important to predict the extend of the effects in the local aquatic community.

MATERIAL AND METHODS

Water samples were taken from one point at the Macaé River in May 2014 during low tide from surface waters near the mouth of the estuary (22°22'11.8"S; 41°46'33.9"W) (Figure 1)

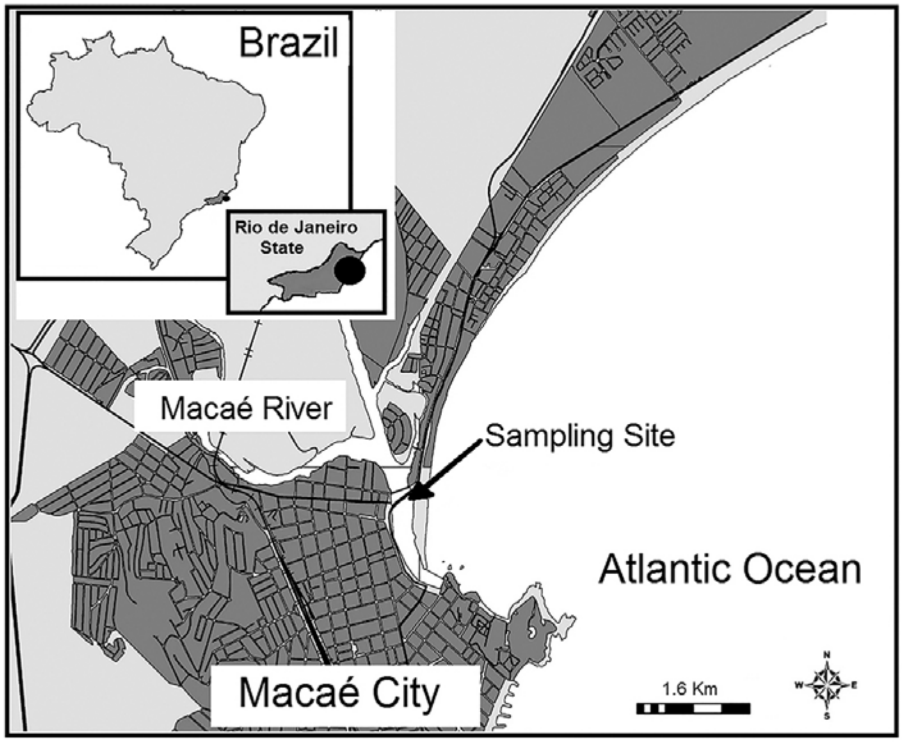

Figure 1 - Sampling site (arrow) at the estuarine region of the Macaé River, Rio de Janeiro state.

and used immediately for the experiment. The salinity was 0.6 at the time of collection and was maintained at 26 ± 1˚C during the assays. A total of forty-eight individuals of *Danio rerio* (Hamilton, 1822), purchased from an aquarium store, were accommodated in 10 L aquaria and maintained for seven days on filtered and sterilized tap water with constant aeration and fed daily with commercial food (Alcon, MEP 200 complex). Twenty-four animals were transferred to a new aquarium with fresh filtered and sterilized tap water (control unit) and the other 24 were submitted to the freshly collected estuarine water (treated unit). Temperature and aeration were maintained, but feeding was suspended during all the experiment. Six individuals were collected from the experimental units exposed to estuarine waters at each time (24 h, 48 h, 72 h and 168 h), and an other six per time were simultaneously collected from the control unit. Those collected at 24 h and 48 h were used for the Comet assay and those collected at 72 h and 168 h were used for the micronuclei test. Animals were sedated in a 0.3 g/L benzocaine bath before blood sampling. The comet assay followed the standard protocol with modifications described by Bücker & Conceição (2012). The comets (nucleoids) of erythrocytes were visualized under epifluorescent Olympus microscope using the DNA-specific stain DAPI (4′,6′-diamino-2-phenylindole hydrocloride). Approximately one hundred comets per individual have their percentage of DNA in the tail (% tail DNA) measured by the Comet Score™ Software, v.1.5 (TriTek Corporation), and it was obtained a mean value per individual. Then, the mean and standard error from individual means were obtained for each treatment and time. The induction factor (IF) was also calculated in accordance to Štrut *et al*. (2011). Micronuclei (MN) test followed the standard procedure with modifications described by Bücker & Conceição (2012). For counting MN, the smear was stained with 0.007% acridine orange and visualized under epifluorescent microscope. The mean number of MN/thousand was obtained for each treatment and time. Micronucleus were recognized by their characteristic structure, which are rounded, have color and texture similar to the nucleus, with a diameter ranging from 1/16 to 1/3 of the diameter of the erythrocyte nucleus. Damage obtained at different units and time were compared using the non-parametric Kruskal-Wallis test (a = 0.05) performed by the statistical package STATISTICA, V. 7 (Statsoft Inc.).

RESULTS

DNA damage as the % tail DNA after the first 24 h of exposure was not significantly different from the control. Only after 48 h of exposure, it was possible to detect significantly higher levels of DNA damage in individuals exposed to the estuarine water of the Macaé River compared to the controls (KW-H (1, 12) = 8.3, p = 0.0039) (Figure 2a). The number of MN/thousand was significantly higher in fish exposed to the estuarine waters than the controls, which was evident after the 72 h of exposition (KW-H (1, 12) = 8.45, p = 0.0036) and after the seven days of exposition (KW-H (1, 12) = 8.67, p = 0.0032) (Figure 2b). The induction factors showed increased values with time (Figure 3), obtained from % tail DNA for 24 h and 48 h and from micronuclei/1,000 for 3 and 7 days of exposition.

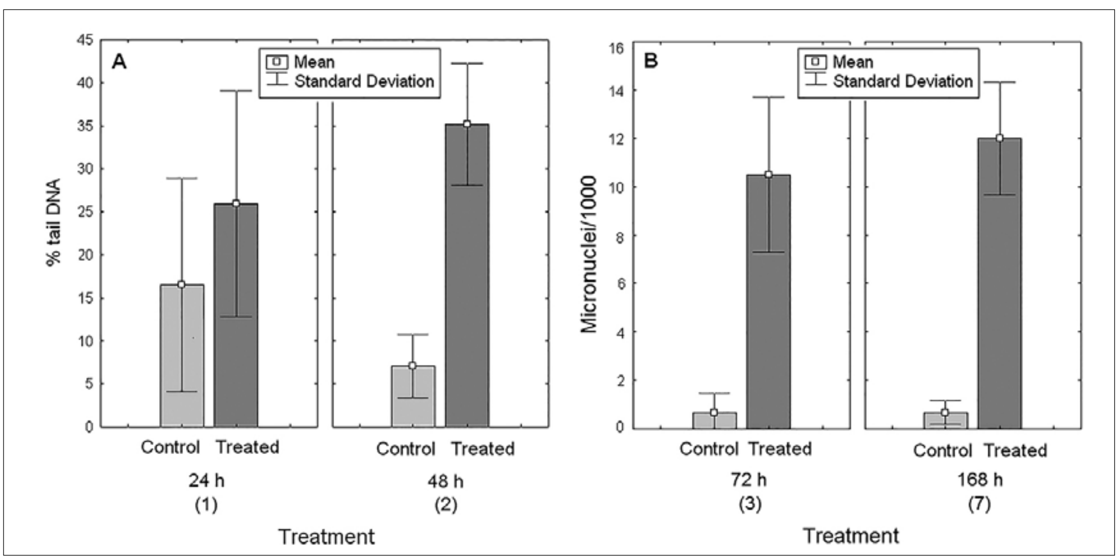

Figure 2 - DNA and chromosome damage observed in the zebrafish *Danio rerio* after exposed to control and estuarine waters. A) % comet tail DNA; B) Micronuclei/1000 cels per fish.

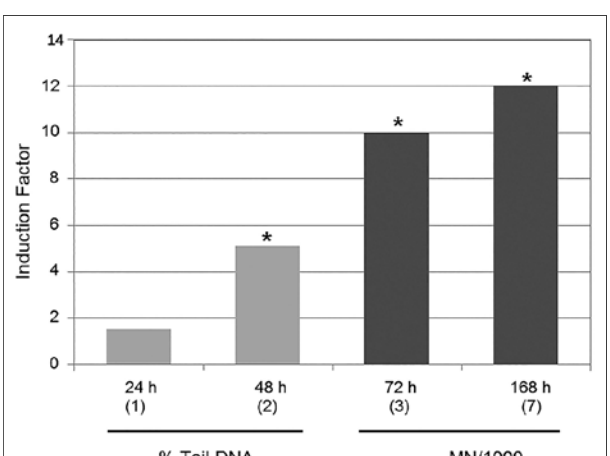

Figure 3 - Induction factor (how many times higher was the damage in the treated units in relation to the controls) for different period of times as evaluated by the comet assay (24 h and 48 h) and by the micronuclei test (3 and 7 days).

DISCUSSION

Genotoxicity tests have proven to be sensitive enough for detecting the effects of xenobiotic on aquatic organisms (Jha, 2004; Ohe *et al*., 2004; Smit *et al*., 2009). Aquatic organisms such as fish have been used as sentinels to monitor contamination with genotoxic chemicals (Lemos *et al*., 2007; Domingos *et al*., 2009) and Zebrafish used in the present study has been recently evaluated as potential *in vivo* model for genotoxicity (Chakravarthy *et al*., 2014).

Low values of DNA damage may be observed as a response of fish acclimatization period. DNA damage observed in this study in the control at the first 24 h may be explained by this situation, considering that the damage was reduced to 6.0% after 48 hour. Longer acclimatization periods will always contribute to a more homogeneous response (Obernier & Baldwin, 2006).

DNA damage observed in the zebrafish exposed to the estuarine waters of the Macaé River, which was 5.1 fold higher than the control after 48 h of exposure (comet assay), suggests the presence of genotoxic substances. The number of micronuclei found in this study (> 10 MN/thousand) is higher than the values observed for clams at the Guaíba hydrographic basin, which showed values between 4.5 to 6.9 MN/thousand at sites contaminated by urban effluents, metals and agriculture activities (Villela *et al*., 2007) and similar to values found in fish species (9.0 to 11 MN/thousand) located close to sources of contamination by industrial and urban discharges at Paranaguá estuary (Paraná state) (Domingos *et al*., 2009).

Among possible genotoxic compounds, we can expect the influence of individual chemicals such as metals, PAHs and other contaminants as well as their possible synergic effects. As demonstrated, Pb enrichment in estuarine sediments (28 – 52 µg/g) compared to the natural levels, represented by the rock Pb content (range of 13 – 23 µg/g) was attributed to anthropogenic sources such as phosphate fertilizers and agricultural lime correctors, cattle feed supplement, pesticides, atmospheric fallout, solid waste disposal, burning and banned persistent sources such as paintings and gasoline (Molisani *et al*., 2015). Lead has been considered carcinogen and responsible for disturbing DNA repair process at low and non-cytotoxic concentrations (Bal *et al*., 2011; Hartwig, 1995).

Polycyclic aromatic hydrocarbons (PAHs) were also observed in the estuary, presenting dual conditions with concentrations typical of pristine areas or polluted coastal environment (Berenger, 2013). This condition is illustrated by the total PAHs in waters which ranged from no detectable concentrations to 120 ng/g, being this maximum value also measured at Guanabara Bay (Rio de Janeiro state), an area which is considered chronically contaminated by oil derivatives (Berenger, 2013; Wagener *et al*., 2012). In addition, bottom sediments of the estuary also displayed low total PAH concentrations (maximum of 70 ng/g), but with the presence of PAH compounds such as pyrene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene (Molisani *et al*., 2013b) that are described as carcinogenic PAHs and proven to be genotoxic (Hamoutene *et al*., 2002; Jha, 2004; Lemos *et al*., 2007; Weber *et al*., 2013). As a consequence of oil derivative presence in the estuary, high levels of such compounds in mussel from the estuary were found (2,876-6,101 µg/kg) (Santiago *et al*., 2016), values close to those reported in 2007 for mussel tissue from Guanabara Bay (4,000–6,000 µg/kg) by Francioni *et al*. (2007). Along the Macaé coast, PAH concentrations in mussel from Santana Island have increased in the last 10 years from 75 µg/kg to 120 µg/kg, exemplifying the temporal increasing concentration of such compounds in the coast.

The presence of PAHs was also confirmed by the higher induction of ethoxyresorufin-O-deethylase (EROD) activities in cathfish *Genidens genidens* following higher PAH concentrations in the estuarine waters, suggested fish responsiveness to oil presence, mainly due to the fact that such EROD values were similar to the magnitude measured in fish during a 24-hr exposure to diesel oil in the laboratory (Berenger, 2013). Finally, the chemical composition of estuarine sediments was assessed and compared to sediment quality guidelines from international (TEL and PEL) and Brazilian coastal areas (Molisani *et al*., 2013b). From comparison to the threshold effect level (TEL) and probable effect level (PEL) proposed by MacDonald *et al*. (1996), metals and PAHs in sediments from the Macaé river estuary were below of such levels and should not be associated to adverse biological effects. However, when compared to the Brazilian sediment quality guidelines proposed by Choueri *et al*. (2009), the results indicated moderate toxicity of these estuarine sediments based on exceeded of maximum Zn, Cr, Cu, Ni, Pb, V and PAH concentrations for some sites and sampling periods (Molisani *et al*., 2013b).

The results of the present study, although preliminary, evidenced genotoxicity of the estuarine waters from the Macaé River, indicating that genotoxicity was at levels to induce DNA and chromosome damage in adult fish.

Since the comet assay has been found to be one of the most sensitive end-point for oil contamination in aquatic organisms (Smit *et al.*, 2009) and probably for other source of pollution (Vincent-Huber *et al*., 2012; Osman, 2014), genotoxicity is gaining popularity as an additional tool for assessing environmental pollution at a multi-biomarker approach (Domingues *et al*., 2009; Goswami *et al*., 2014; Costa *et al*., 2014; Osman, 2014).

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