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Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent

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Abstract

Micronuclei and nuclear alterations tests were performed on erythrocytes of *Oreochromis niloticus* (Perciformes, Cichlidae) in order to evaluate the water quality from Paraíba do Sul river, in an area affected by effluents from an oil shale processing plant, located in the city of São José dos Campos, Brazil-SP. Water samples were collected on 2004 May and August (dry season) and on 2004 November and 2005 January (rain season), in three distinct sites, comprising 12 samples. It was possible to detect substances of clastogenic and/or aneugenic potential, as well as cytotoxic substances, chiefly at the point corresponding to the drainage of oil shale plant wastes along the river. The highest incidence of micronuclei and nuclear alterations was detected during May and August, whereas the results obtained in November and January were insignificant. This work shows that the effluent treatment provided by the oil shale plant was not fully efficient to minimize the effect of cytotoxic and mutagenic substances in the test organism surveyed.

Keywords: Micronuclei test; *Oreochromis niloticus*; Mutagenicity; Biomonitoring; Oil

1. Introduction

Micronuclei can be formed by the interaction of chemical, physical or biological agents with nongenomic structures, such as the mitotic spindle and the kinetochore, promoting disturbances in the mitotic machinery and, consequently, failures on chromosomal segregation. These agents can also affect the DNA directly, generating chromosomal breakages, which might be visualized and quantified through micronuclei tests.

The micronucleus test has been performed to evaluate the environmental mutagenicity in ecosystem bioindicators. Consequently, in order to evaluate the quality of aquatic environments, this test has been carried out in several organisms, such as mussels [1] and, especially, fish [2].

It represents an important tool for the environmental diagnosis of areas under human influence, by detecting mutagenic agents responsible for genetic instability increases and, utmost, for some neoplasic transformation [3].

During micronuclei analyses, some authors have observed the occurrence of other nuclear abnormalities, suggesting that they must be taken into consideration along conventional micronuclei analysis [4–7]. Such

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abnormalities are related to cell division failures, cell death processes, and to genotoxicity and/or mutagenicity [8–10]. In fish, some of these nuclear alterations have been reported after exposure to chemical agents or polluted waters [11].

The formation of morphological alterations in the nuclear envelope described by Carrasco et al. [12] as blebbed (nuclei that present a relatively small evagination from the envelope, which seems to contain euchromatin), lobed (nuclei presenting evaginations larger than those from blebbed nuclei) and notched (nuclei present a remarkable notch containing nuclear material) have also been reported in fish erythrocytes, as a consequence of exposure to environmental and chemical contaminants of genotoxic, mutagenic or carcinogenic action, although the mechanisms responsible for such abnormalities have not been described yet [13–15].

In the present work, micronucleus and nuclear abnormalities tests in erythrocytes of *Oreochromis niloticus* (Perciformes, Cichlidae) were performed to diagnose the water quality of Paraíba do Sul river in an area that receives refinery oil effluent after treatment (São José dos Campos-SP, Brazil). The petroleum refinery produces gasoline, GLP, diesel oil, fuel oils, asphalt, kerosene oil, naphtha, sulfur and solvents [16]. The main substances present in this effluent are polycyclic aromatic hydrocarbons (PAHs), phenols and heavy metals [17]. The sewage treatment process involves flocculation, flotation, aerated tanks, activated sludge, biodisk and clarifier tanks.

The environmental diagnosis of this area is quite necessary, inasmuch as such effluents may contain harmful substances, deleterious to the genetic material of living organisms exposed to such waters, being able to affect human health as well. Besides, taking into consideration that seasonable variations may affect the concentration of pollutants in the water, as a result of natural and human conditions, such as changes in the water volume related to the rainfall index [18], we also aimed to evaluate the putative influence of season on the biologic results herein presented.

2. Material and methods

2.1. Biological material

The test-organism used was *O. niloticus* (Perciformes, Cichlidae), popularly known as Nile tilapia. Eighty individuals with a mean size of 15 cm were analyzed in order to avoid intra-specific differences related to either fish size or age. The specimens, reared on fish culture farms (UNESP, São José do Rio Preto campus-SP), were brought to the Laboratory of Water Toxicity at UNESP, Rio Claro Campus-SP, where they were acclimated in aerated and filtrated tanks for 1 week, at a mean temperature of 23 °C.

2.2. Studied area and water collection

Water samples were collected along Paraíba do Sul river, in the city of São José dos Campos-SP, Brazil (Fig. 1), in an area affected by a petroleum refinery. The collecting procedure was performed in May, August and November (2004) and in January (2005). Three collection sites, 500 m apart from each other, were established: site 1 (S1): upstream the waste discharge; site 2 (S2): located at the point where the wastes from

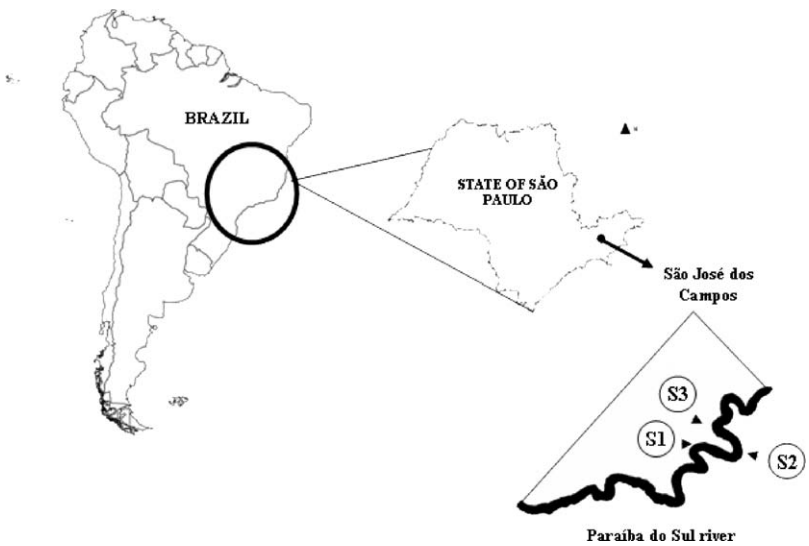

Fig. 1. São José dos Campos and collection sites in Paraiba do Sul river, in an area affected by an oil shale processing plant. S1: upstream the waste discharge; S2: located at the point where the wastes from the oil shale plant are released; S3: downstream the waste discharge.