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Genotoxic effect of inhaled ambient particulate matter

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In recent years great importance has been given to the adverse effects of particulate matter (PM) in health, and several epidemiological studies correlating the airborne particles with pulmonary injury have been carried out [1, 2]. The adverse effect of short- and long-term expositions to PM can, indeed, cause a variety of effects, from minor effects on the respiratory system to serious oncogenic effects that can lead to precocious death and are considered one of the top environmental public health concerns [3, 4]. Some cytogenetic techniques, as micronucleus assay, allow to detect chromosome breakage and loss by measuring the formation of micronuclei and proved to be a good tool in the evaluation of genotoxic damage induced by PM [5, 6] . Thus genotoxic effects can be evaluated by micronucleus test in order to identify a gradient of potential exposure and to assess environmental monitoring. However, cross investigations involving biomarkers of genotoxicity and electron microscopy PM-induced tracheal and lung damages in natural populations are not common.

Two study areas characterized by different concentrations of inhalable particles were selected, Lourinhã for the lowest and Paio Pires for the highest. *Mus spretus* (Algerian Mouse) were captured in polluted and unpolluted similar biotic areas. Slides were prepared from the bone marrow to estimate the frequency of micronuclei in polychromatic erythrocytes. The trachea and lung were removed, fixed and processed for histopathological observations under light and electron microscopy. The bronchoalveolar lavage fluid (BALF) was also analysed to evaluate the percentage of particles deposited in the respiratory tract. Statistical analysis using Mann-Whitney test was performed ($p < 0.05$). Ten animals were captured from each locality. The frequency of micronucleated polychromatic erythrocytes (MNPCEs) per 1000 polychromatic erythrocytes (PCEs) is shown in Figure 1. On average, the number of micronuclei increased with the level of pollution, i.e., was lower in Lourinhã (1.10 ± 1.29) and higher in Paio Pires (4.18 ± 2.23). There were significant differences between the two regions.

Light and electron microscopy studies combined with X-ray microanalysis are under way to detect and characterize the PM accumulating in the lungs (Figure 2).

Comparing the concentrations of PM_{10} from the polluted area with the classes of air quality index, we confirm that these concentrations are usually high. In this study and in concordance with the above fact, we observed that the animals captured in the polluted area present a significantly higher percentage of anomalies than the control group, which is also in agreement with the literature cited. So, the significant differences between sampling sites indicate that the animals are subjected to different intensities of genotoxic agents.

In this work we used *Mus spretus* as bioindicator species of the effects that environmental pollution, specifically PM, may have on health, being possible, within certain limits, to transfer this data to humans. Therefore, our results may provide valuable insight into the health risks posed by urban and

industrial particles, thus, alerting us of the need to use biomarkers of effects, exposure and susceptibility and to establish preventing measures before we are faced with the social burden and economic cost of PM pollution induced disease.

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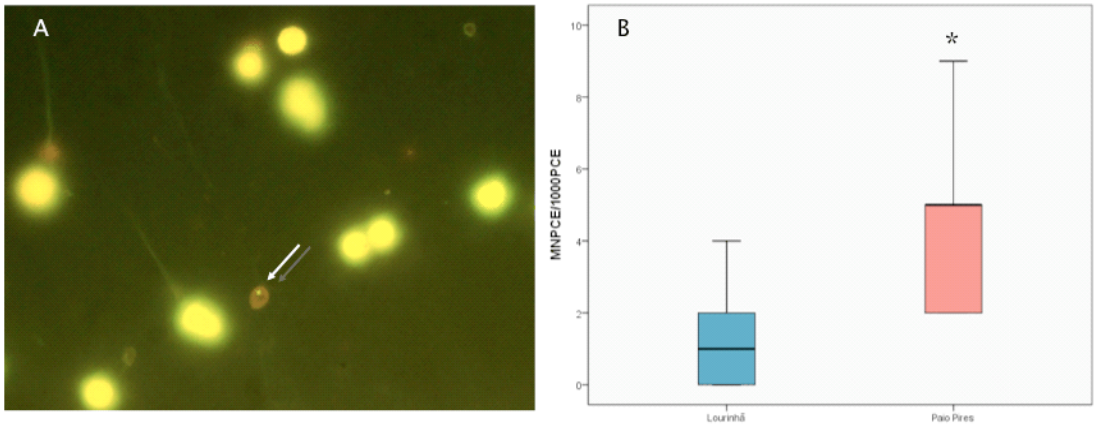

Figure 1. (A) Acridine orange-stained micronucleus (arrow) in a polychromatic erythrocyte; (B) Box plot of the number of micronuclei according to capture site. MNPCE/1000PCE, micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes; * statistically different from Lourinhã ($p < 0.05$).

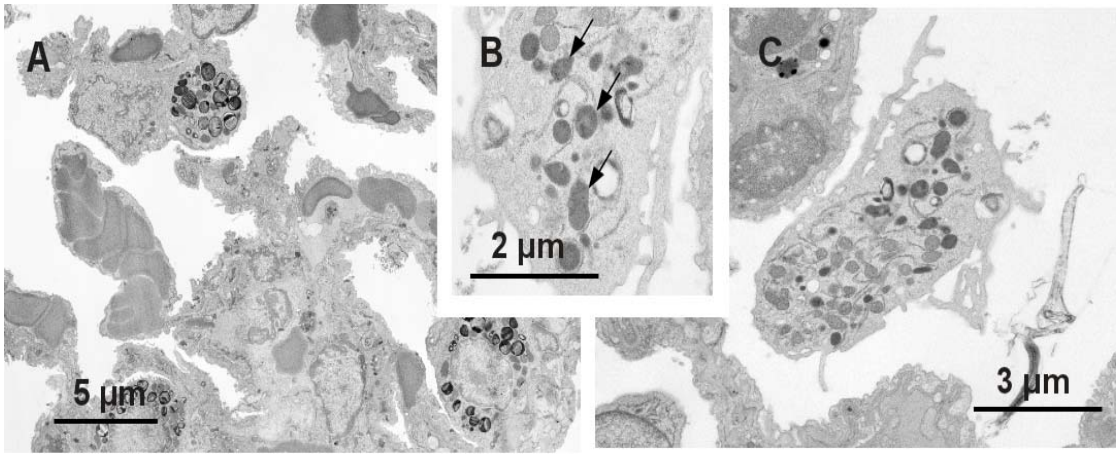

Figure 2. Preliminary transmission electron microscopy observations of lung tissue (Fig. 4A) reveals the presence of lisossome-filled (arrows) macrophages (Figs. 4B and 4C) that may contain ingested PM. Search for the PM is being carried out.

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