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Inflammation response, oxidative stress and DNA damage caused by urban air pollution exposure increase in the lack of DNA repair XPC protein

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ABSTRACT

Air pollution represents a considerable threat to health worldwide. The São Paulo Metropolitan area, in Brazil, has a unique composition of atmospheric pollutants with a population of nearly 20 million people and 9 million passenger cars. It is long known that exposure to particulate matter less than 2.5 μm ($PM_{2.5}$) can cause various health effects such as DNA damage. One of the most versatile defense mechanisms against the accumulation of DNA damage is the nucleotide excision repair (NER), which includes XPC protein. However, the mechanisms by which NER protects against adverse health effects related to air pollution are largely unknown. We hypothesized that reduction of XPC activity may contribute to inflammation response, oxidative stress and DNA damage after $PM_{2.5}$ exposure. To address these important questions, XPC knockout and wild type mice were exposed to $PM_{2.5}$ using the Harvard Ambient Particle concentrator. Results from one-single exposure have shown a significant increase in the levels of anti-ICAM, IL-1β, and TNF-α in the polluted group when compared to the filtered air group. Continued chronic $PM_{2.5}$ exposure increased levels of carbonylated proteins, especially in the lung of XPC mice, probably as a consequence of oxidative stress. As a response to DNA damage, XPC mice lungs exhibit increased γ-H2AX, followed by severe atypical hyperplasia. Emissions from vehicles are composed of hazardous substances, with polycyclic aromatic hydrocarbons (PAHs) and metals being most frequently cited as the major contributors to negative health impacts. This analysis showed that benzo[b]fluoranthene, 2-nitrofluorene and 9,10-anthraquinone were the most abundant PAHs and derivatives. Taken together, these findings demonstrate the participation of XPC protein, and NER pathway, in the protection of mice against the carcinogenic potential of air pollution. This implicates that DNA is damaged directly (forming adducts) or indirectly (Reactive Oxygen Species) by the various compounds detected in urban $PM_{2.5}$.

Abbreviations: BaPE, Benzo[a]pyrene equivalent index; BPDE, B(a)P diol epoxide; CR, Carcinogenic risks; EC_{inh}, Exposure concentration; HAPC, Harvard/U.S. Environmental Protection Agency Ambient Particle Concentrator; HQ, Hazard quotient; ICAM-1, Intercellular Adhesion Molecule-1; LCR, Lung cancer risk; LPS, Lipopolysaccharides; NER, Nucleotide excision repair; nitro-PAHs, nitrated-PAHs; oxy-PAHs, oxygenated-PAHs; PM, Particulate matter; $PM_{2.5}$, PM less than 2.5 μm; PAHs, Polycyclic aromatic hydrocarbons; ROS, Reactive oxygen species; SPMA, São Paulo Metropolitan Area; UR, Unit risk; XPA, Xeroderma pigmentosum group A; XPC, Xeroderma pigmentosum group C; WHO, World Health Organization; 8-oxodGuo, 8-hydroxydeoxyguanosine.

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1. Introduction

Millions of people die every year from diseases caused by exposure to air pollution, particularly associated with large urban conglomerates (Landrigan et al., 2017). The story is similar in many of the world's megacities, where the health effects range from increased hospital admissions and emergency room visits (Rajak and Chattopadhyay, 2019). According to the World Health Organization (WHO), 9 out of 10 people around the world breathe air containing high levels of pollution and ambient air pollution alone has caused approximately 4.2 million premature deaths globally (Cohen et al., 2017; WHO, 2018a). In particular, the São Paulo Metropolitan Area (SPMA), located in the southeast of Brazil, has a population of about 20 million inhabitants and 9 million passenger cars (Brito et al., 2018) with increased risk of hospitalizations due to respiratory and cardiovascular diseases associated with air pollution (Gouveia et al., 2017).

Aerosol particles are a mix of solid and liquid components, that have a complex chemical composition and these components have a major health impact (Burnett et al., 2018). The health risk that comes from inhalation of ambient particulate matter (PM) is due to the toxicity of the components and particle size (Adams et al., 2015). Although a wide array of carcinogens and toxic compounds are part of urban pollution (Velali et al., 2018; Wang et al., 2017), the identification of the most relevant among them, concerning to human health impacts as well as their actual pathway, remains highly elusive, preventing effective mitigation and abatement strategies.

According to the Global Burden of Disease Study exposure to PM less than 2.5 μm ($PM_{2.5}$) is the fifth leading risk factor for death worldwide (Cohen et al., 2017). Although PM has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2016), little is known about the exact mechanisms by which these particles from air pollution may contribute to the cancer risk.

Toxicological studies suggest PM-induced oxidant production, including reactive oxygen species (ROS), as a possible mechanism by which PM exposure results in adverse health effects (Bates et al., 2015; González-Flecha, 2004). These particles contain chemical species such as polycyclic aromatic hydrocarbons (PAHs) (Brito et al., 2013) and transition metals that are capable of ROS generation after inhalation (Park et al., 2018). Moreover, reactive PAHs intermediates might induce bulky DNA adducts and can cause oxidative DNA damage (Abbas et al., 2011; Pardo et al., 2018).

In fact, people are increasingly suffering from air pollution exposure, an important risk factor for lung cancer (Fajersztajn et al., 2013). Cancer development is certainly promoted by the accumulation of somatic DNA damage and mutagenesis (Mavragani et al., 2017; Moraes et al., 2012). One of the most sophisticated defense mechanisms against mutations and damage accumulation is the nucleotide excision repair (NER), that among others contains the proteins Xeroderma pigmentosum group A (XPA) and the Xeroderma pigmentosum group C (XPC) (Scharer, 2013). Interestingly, Melis et al. (2008) suggested that XPC-deficient mice are more sensitive to oxidative DNA-damaging agents in the lung compared with the XPA-deficient mice and wild-type controls. NER-deficient mice have also been shown to have a higher incidence of lung tumors when exposed to benzo[a]pyrene (BaP), a typical representative compound of mutagenic and/or carcinogenic PAHs (John et al., 2012). More recently, Zhou and collaborators (2019) have demonstrated that XPC mice exposed to chronic cigarette smoke developed lung tumors through impaired repair of oxidatively generated DNA. There is growing evidence that compounds present in the PM from air pollution such as heavy metals, aldehydes, and BaP can greatly inhibit NER (Mehta et al., 2008). These findings led us to investigate if the reduction of NER capacity affects how ambient PM contributes to negative effects on health.

To address these important questions, we used the Harvard/U.S. Environmental Protection Agency Ambient Particle Concentrator (HAPC). The HAPC provides a combination of an experimental model taking into account the dynamics of pollutants in real ambient air with the controlled exposure of animals lacking XPC protein of NER pathway. The following parameters were evaluated: (a) inflammatory response using *in vivo* imaging and immunohistochemical analysis; (b) oxidative stress by quantification of carbonylated proteins; (c) detection of γ-H2AX foci used as a DNA damage biomarker; (d) histopathological lesions in the lungs and (e) characterization of the chemical composition of ambient PM by identifying and quantifying PAHs, nitrated-PAHs (nitro-PAHs), oxygenated-PAHs (oxy-PAHs) and trace elements from PM. To our knowledge, this study provides the first results compelling evidence that the *in vivo* induction of inflammatory effects, DNA damage and oxidative stress by inhalable ambient $PM_{2.5}$ are controlled by the NER pathway.

2. Material and methods

2.1. Animal exposure

This study was approved by the Ethical Committee for Research of the São Paulo University Medical School (Agreement number 173/14). All experiments were also compliant with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

To be closer to realistic exposure conditions, the exposures were conducted using the HAPC, an equipment developed by the Harvard School of Public Health designed to deliver concentrated ambient fine particles for conducting animals. The HAPC is able to concentrate $PM_{2.5}$ up to 30 times, separating particles from gases by particle inertia using virtual impaction. It has two chambers, one receiving the air concentrated with particles and the other receiving the ambient air (Falcão de Oliveira et al., 2018). This $PM_{2.5}$ passes through the equipment and is collected without altering its physical–chemical properties (Lawrence et al., 2004). The HAPC was operated on a series of animal exposure experiments and $PM_{2.5}$ concentrations, exposure duration, temperature and humidity were monitored in real-time using a particulate monitor (DataRam4, Thermo Fisher Scientific). Animals were exposed daily to an accumulated dose (concentration vs time of exposure) of 600 μg m^{-3} $PM_{2.5}$ for 1 h, which corresponds to an average concentration of 25 μg m^{-3} $PM_{2.5}$ in 24 h, according to the concentration established by the WHO.

We used wild type and XPC-knockout mice that were originally gently provided by Dr. Jan H.J. Hoeijmakers, from Erasmus Medical Center, Rotterdam, Netherlands. The generation and characterization of XPC mice were previously described (Cheo et al., 1997). Two experimental groups were utilized with five animals each: filtered and polluted air. To evaluate the acute inflammatory response, 20 animals (ten XPC and ten wild type mice) were submitted to one-single exposure (one day). For the acute experiments, we added three XPC mice exposed to nebulized lipopolysaccharides (LPS) as a positive control to our *in vivo* imaging analysis. Afterward, 40 animals were exposed to inhaled $PM_{2.5}$ for 6 months (5 days/week). In the chronic inhalation study, half of the animals were analyzed for potential oxidative stress and the other half was used for DNA damage and histopathological analysis.

2.2. In vivo imaging of Intercellular adhesion molecule-1 (ICAM-1) targeted nanoparticles

Mice were submitted to one-single exposure with an accumulated dose of 600 μg m^{-3} $PM_{2.5}$ during 1 h. As a positive control, mice were exposed to nebulized LPS according to de Souza Xavier Costa et al. (2017). The final nebulization protocol consisted of 5 mL of LPS solution at 1.5 mg/mL (Lipopolysaccharides from Escherichia coli 0111: B4 –Sigma-Aldrich) during 15 min. After 24 h, we injected intravenously anti-ICAM-1 targeted nanoparticles labeled with the lipophilic fluorophore DiIC18(5); 1,1′-dioctadecyl-3,3,3′,3′- tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD) (Thermo Fisher Scientific,

Waltham, MA, USA) 2 h prior to imaging, conducted using IVIS spectrum (Excitation 640 nm, emission 680 nm). Image analysis was performed with Living Image 4.0 (PerkinElmer) software, and fluorescence intensity was quantified by measuring the radiance within our determined region of interest, i.e. the animal's chest.

2.3. Immunohistochemical analysis

Lung tissue sections were immunostained using anti-TNF-α (Santa Cruz Biotechnology–sc-1348, Santa Cruz, CA, USA) and anti-IL-1β (Santa Cruz – sc-7884) antibodies following the protocol performed by de Souza Xavier Costa et al. (2017). The software Image-Pro® Plus version 4.5 (Media Cybernetics – Silver Spring MD, USA) was used for quantitative analysis. The results are expressed in the proportion of positively stained tissue area per total lung tissue area.

2.4. Quantification of carbonylated proteins

Carbonylated proteins are a well-used marker for oxidative stress (Suzuki et al., 2010). Mice lungs were harvested after experiments and lysed with lysis buffer (50 mM Tris, pH 7.5, 20 mM NaCl, 1 mM $MgCl_2$, 0.1% SDS, protease inhibitor Cocktail Set II and III, Calbiochem, Merck, White House Station, NJ, USA) with the addition of benzonase (0.25 U/μl, Novagen, Merck, Millipore) during 40 min. The cells were then centrifuged for 10 min at 14,000 rpm at 4 °C. Total protein quantification was performed using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Protein denaturation was performed as previously standardized (Cortat et al., 2013). Bolt® 4–12% Bis-Tris Plus SDS polyacrylamide gel (Life Technologies) was used to separate 40 μg of total protein sample, and iBlot® Gel Transfer Stacks Nitrocellulose kit (Life Technologies) was used during the transfer step of proteins to the membrane. The membrane was blocked with 5 mg/ml milk for 1 h and incubated overnight with DNPH antibody (1:1000, Sigma-Aldrich, St. Louis, MI, USA), followed by PBS wash and 1 h incubation at room temperature with rabbit peroxidase antibody (1:2000, Sigma-Aldrich). Membranes were revealed with Luminata™ Forte Western HRP substrate (Millipore Corporation, Billerica, MA, USA) in Universal Hood III Gel Doc equipment (Bio-Rad, Hercules, CA, USA).

2.5. γ-H2AX as a DNA damage biomarker

A variety of factors known to have a causal relationship with lung carcinogenesis are also implicated in the phosphorylated form of the histone variant H2AX (**γ**-H2AX), a key molecule of the DNA-damage response system (Matthaios et al., 2013). DNA damage was thus evaluated by the detection of **γ**-H2AX. After deparaffinization slides were hydrated for 5 min in graded alcohol series (100%, 95%, 70% and H_2O) and incubated in EDTA for 50 min at 95 °C. Slides were then incubated with Ab mix (TBS (1%), BSA (4%) and Tween20 (0.02%)) for 20 min at RT, followed by treatment overnight at 4 °C with anti- **γ**-H2AX (Millipore) diluted 1:200 in Ab mix. Then, slides were washed twice in TBS (1%) and finally incubated for 1 h at RT with 1:500 goat anti-mouse HRP-conjugated secondary antibody (Sigma-Aldrich). After dehydration of tissue sections through graded alcohol series, slides were mounted with Entellan and cover slides. Visual counting was performed to quantify the foci number per cell to each treatment (filtered and polluted air exposed to XPC and wild type mice) using a Zeiss microscope Bloom. The results were expressed as a percentage of the number of γ-H2AX-positive cells over the total number of cells (2000 cells).

2.6. Descriptive analysis of histopathological lesions

Mice were euthanized and lungs were fixed in 4% buffered paraformaldehyde solution and embedded in paraffin. Five-micrometer thick sections were prepared and stained with hematoxylin-eosin H&E. Briefly, the histological sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, and washed in running water. The sections were immersed in Harris' hematoxylin for two minutes, washed in running water, rinsed in distilled water, stained in an aqueous solution of eosin, dehydrated in ascending concentrations of ethanol and mounted with Entellan™ (Merck) mounting media. Semi-quantitative analyses of lesions were categorized based upon previously published diagnostic criteria (Renne et al., 2009) and we used the following graduation: grade 0 (absent), 1 (hyperplasia), 2 (moderate atypical hyperplasia) and 3 (severe atypical hyperplasia). The diagnosis of bronchiolar epithelial hyperplasia was based on the absence of central connective tissue stalk resulting in an undulating, rugose epithelial surface, and irregular arrangement of cell layers. Moderate atypical hyperplasia was characterized by pleomorphic basal cells on the presence of mild nuclear atypia. Finally, lung tissue that showed more diffuse and severe nuclear atypia (hyperchromatic and pleomorphic nuclei) with atypical bronchiolar cells extending into adjacent alveolar spaces was classified as severe atypical hyperplasia.

2.7. Site description and sample collection

The experiments were carried out at the Faculty of Medicine of the University of São Paulo School situated near downtown São Paulo and close to several main crossroads, a region characterized by heavy traffic (Akinaga et al., 2009). $PM_{2.5}$ samples were collected for a period of 24 h using a high volume filter sampler (flow rate of 1.3 $m^3.min^{-1}$) and were deployed near to the HAPC (equipment where the animals were exposed). Prior to sampling, quartz fiber filters (20 cm × 25 cm, Millipore, USA) were pre-cleaned by heating in an oven at 800 °C for 8 h, equilibrated at room temperature and weighed in a microbalance before and after the sampling, in order to estimate the $PM_{2.5}$ concentration. A total of 18 samples were collected between August/2015 and February/2016. After sampling, the filters were weighed, wrapped in aluminum foil and stored at 4 °C in the refrigerator. Finally, $PM_{2.5}$ samples were used for chemical analysis such as PAHs, nitro-PAHs, oxy-PAHs, and trace elements.

2.8. Analytical methods

2.8.1. Polycyclic aromatic hydrocarbons and derivatives

PAHs and their derivatives were determined at the Institute of Chemistry at the Federal University of Bahia, Brazil. The filters were extracted for 23 min using an ultrasonic bath (in 4.2 cm^2 punches) with a 500 μL solution (18% of acetonitrile in dichloromethane), employing extraction devices (Whatman Mini™ UniPrep Filters, Whatman, USA). The extracts were analyzed in a gas chromatograph with high-resolution mass spectrometer detection (GC–MS) (Santos et al., 2016).

The United States Environmental Protection Agency priority PAHs mixture standard was purchased from Supelco (EPA 610, 2000 μg mL^{-1} each species in methanol:dichloromethane): acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, indeno[1,2,3-cd]pyrene (InP), dibenzo[ah]anthracene (DBA) and benzo[ghi]perylene (BPe). Individual standards of 1000 μg mL^{-1} perylene (Per), 50 μg mL^{-1} coronene (Cor) were purchased from Sigma-Aldrich. Naphthalene was purchased from Supelco (Nap, 2000 μg mL^{-1}) (St Louis, USA). Quinones investigated in this study were purchased from Fluka (St. Louis, USA): 1,4-benzoquinone (1,4-BQ), 9,10-phenanthraquinone (9,10-PQ), 9,10-anthraquinone (9,10-AQ), 1,2-naphthoquinone (1,2-NQ) and 1,4-naphthoquinone (1,4-NQ). Benzo(e)pyrene (BeP) was quantified using the same calibration curve as BaP, as both species have similar fragmentation patterns in the mass spectrometer (Robbat and Wilton, 2014).

The nitro-PAHs certified standard solutions SRM 2264 (aromatic hydrocarbons nitrated in methylene chloride I) and SRM 2265 (polycyclic aromatic hydrocarbons nitrated in methylene chloride II) were

purchased from the National Institute of Standards and Technology (NIST, USA). Both certified solutions contained 1-nitronaphthalene (1-NNap), 2-nitronaphthalene (2-NNap), 1-methyl-4-nitronaphthalene (1-Methyl-4-NNap), 1-methyl-5-nitronaphthalene (1-Methyl-5-NNap), 1-methyl-6-nitronaphthalene (1-Methyl-6-NNap), 2-methyl-4-nitronaphthalene (2-Methyl-4-NNap), 2-nitrobiphenyl (2-NBP), 3-nitrobiphenyl (3-NBP), 4-nitrobiphenyl (4-NBP), 5-nitroacenaphthene (5-NAce), 2-nitrofluorene (2-NFlu), 2-nitrophenanthrene (2-NPhe), 3-nitrophenanthrene (3-NPhe), 9-nitrophenanthrene (9-NPhe), 2-nitroanthracene (2-NAnt), 9-nitroanthracene (9-NAnt), 2-nitrofluoranthene (2-NFlt), 3-nitrofluoranthene (3-NFlt), 1-nitropyrene (1-NPyr), 2-nitropyrene (2-NPyr), 4-nitropyrene (4-NPyr), 6-nitrochrysene (6-NChr), 7-nitrobenz[a]anthracene (7-NBaA), 3-nitrobenzanthrone (3-NBA), 6-nitrobenzo[a]pyrene (6-NBaP), 1-nitrobenzo[e]pyrene (1-NBeP), and 3-nitrobenzo[e]pyrene (3-NBeP).

2.8.2. Elements determination

For the determination of the elements, the filters underwent extraction with a microwave digestion system (MLS-1200 mega, Milestone Inc., Italy) similarly as in Pereira et al. (2017). Part of the filter was separated for the analysis, cut in small pieces and inserted in PTFE vessels with 0.2 mL of HF, 4 mL of HNO_3 and 2 mL of H_2O_2 (all products by Merck), these vessels underwent three-stage digestion within a microwave digester (250 W, for 5 min; 400 W, for 5 min; and 600 W for 2 min). After this procedure, the extracts were filtered with PTFE syringe filters (0.45 μm), diluted 8 times, and stored at 4 °C. The trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS) in triplicates; the ICP-MS standards were purchased from High-Purity Standards (USA) and used for calibration.

2.8.3. Health risk assessment of metals and PAHs

The exposure concentration (EC_{inh}) was calculated to understand the risks of exposure to metals in $PM_{2.5}$ by inhalation (Hu et al., 2012; US EPA, 2009) (Equation (1)). *C* is the metal concentration in $PM_{2.5}$, *ET* is the exposure time (24 h/day), *EF* is the exposure frequency (extrapolating to 365 days a year in this study), *ED* is the exposure duration (6 years for children and 24 years for adults), AT_n for non-carcinogens was equal to *ED* × 365 days × 24 h/day and for carcinogens equal to 75.5 years (considering the life expectancy in Brazil in 2015 of 75.5 years (IBGE, 2016)) × 365 days/year × 24 h:

$$ECinh = C \times (ET \times EF \times ED)/ATn \quad (1)$$

The non-carcinogenic risk was assessed with the hazard quotient (HQ). HQ and carcinogenic risks (CR) by metals inhalation in $PM_{2.5}$ were calculated according to the following equations (Hu et al., 2012; US EPA, 2009); *RfCi* is the inhalation reference concentration (mg m^{-3}) and *IUR* is the inhalation unit risk ((μg m^{-3})$^{-1}$):

$$HQ = ECinh/\left(RfCi \times 1000\ \ \mu g\ \ mg^{-1}\right) \quad (2)$$

$$CR = IUR \times ECinh \quad (3)$$

The benzo[a]pyrene equivalent index (BaPE) was calculated to evaluate the carcinogenicity due to exposure to PAHs (Equation (4)). Values above 1 ng m^{-3} represent an increased cancer risk (Vasconcellos et al., 2011; Yassaa et al., 2001):

$$BaPE = ([BaA] \times 0.06) + ([BbF] \times 0.07) + ([BkF] \times 0.07) + ([BaP] \times 1) + ([DBA] \times 0.6) + ([InP] \times 0.08) \quad (4)$$

The carcinogenic potentials for exposure to PAHs and nitro-PAHs (BaP-TEQ) were calculated with Equations (5) and (6) (de Oliveira Galvão et al., 2018;Jung et al., 2010):

$$BaP - TEQ(PAHs) = ([BaA] \times 0.1) + ([Chr] \times 0.01) + ([BbF] \times 0.1) + ([BkF] \times 0.1) + ([BaP] \times 1) + ([InP] \times 0.1) + ([DBA] \times 5) + ([BPe] \times 0.01) \quad (5)$$

$$BaP - TEQ(nitroPAHs) = ([1NPyr] \times 0.1) + ([4NPyr] \times 0.1) + ([6NChr] \times 10) \quad (6)$$

Lung cancer risk (LCR) from exposure to particulate PAHs was estimated by multiplying BaP-TEQ by the unit risk (UR) established by the WHO (8.7×10^{-5} (ng m^{-3})$^{-1}$) (de Oliveira Alves et al., 2015; WHO, 2000).

2.9. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 15.0 and Prism 5, GraphPad Software Inc. Statistical significance was assessed using one-way ANOVA followed by a Bonferroni test or Dunnett's test. The Pearson coefficients were calculated, and they were employed to determine how much the values of the variables are linearly correlated. The mean differences and coefficients (*R*) were considered statistically significant at $p \leq 0.05$.

3. Results and discussions

3.1. $PM_{2.5}$ promotes a stronger inflammatory response in XPC mice in comparison to wild type mice

The expression of ICAM-1, the predominant cell adhesion protein in the lung epithelium and endothelium, plays an important role in the inflammatory response as shown in previous studies (Hu et al., 2008; McMillan et al., 2011). Since $PM_{2.5}$ is known to induce an inflammatory response (Gruzieva et al., 2017; Yuan et al., 2019), we investigated whether the effects of these inhalable particles are related to the NER pathway. After a one-single exposure, results showed a significantly stronger fluorescent signal for anti-ICAM in both XPC and wild type mice of the polluted air group than the observed in the filtered air group in real-time (Fig. 1A and 1B). In parallel, animals were subjected to an acute stressor LPS and as expected, we obtained a higher inflammatory effect compared with filtered air in XPC animals (Fig. 1C). Furthermore, among the animals exposed to ambient pollution, XPC mice have shown a stronger inflammatory response compared to wild type mice (Fig. 1D). These data demonstrate that exposure to ambient air pollution in São Paulo promotes acute inflammatory responses in mice, especially in the lack of XPC protein.

Evaluating these same animals, immunohistochemical analysis confirmed that $PM_{2.5}$ exposure from São Paulo strongly induced the secretion of IL-1β and TNF-α in the lung of XPC mice (Fig. 2). Furthermore, XPC and wild type mice exposed to $PM_{2.5}$ had a significantly higher increase of TNF-α and IL-1β levels in the lungs than the untreated group. IL-1β is produced rapidly by macrophages in response to inflammatory stimuli, inducing different immune cellular and molecular mechanisms that include the expression and synthesis of adhesion molecules (e.g. ICAM-1) (Saber et al., 2006). The inflammatory response after ambient $PM_{2.5}$ exposure has also been reported in the lungs of rats (Li et al., 2015). Different classes of pollutants can interact synergistically regarding toxicity in air samples. In a previous study in São Paulo, the acute exposure to low doses of $PM_{2.5}$ was able to induce inflammation (increased inflammatory cytokines as IL-6 and TNF-α) and oxidative damage to the lung of mice (Riva et al., 2011). The wide variety of components, including chemical and biological fractions found in PM, such as PAHs (Vogel et al., 2020) and endotoxin (Behbod et al., 2013), has been associated with inflammation effects. Therefore, our data demonstrate that exposure to ambient air pollution in São Paulo promotes acute inflammatory responses in mice, especially in mice lacking XPC protein.

Gungor et al. (2010) demonstrated an association between LPS-induced pulmonary inflammation and inhibition of NER in the lungs of mice. Another interesting result showed that the DNA repair gene XPC

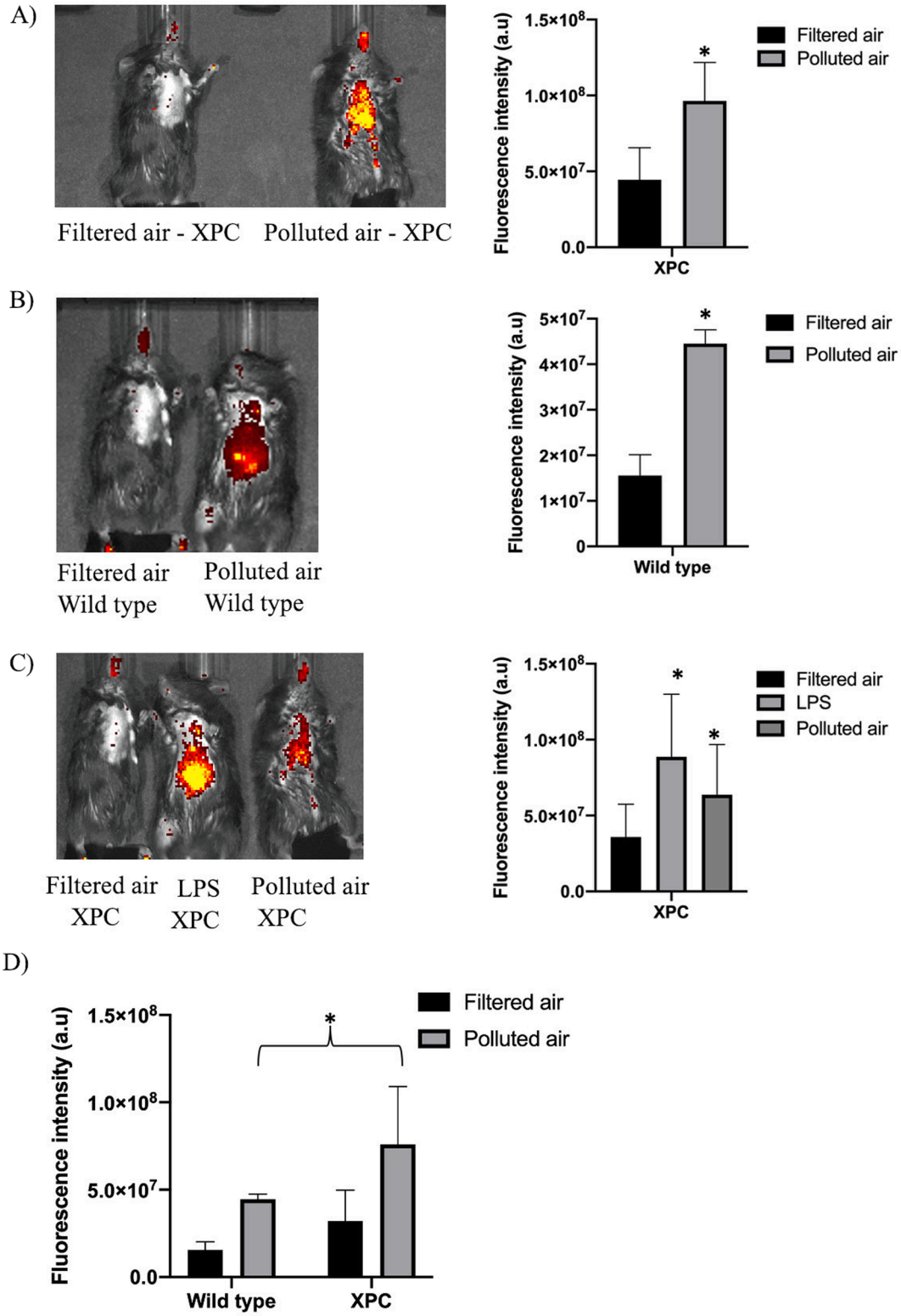

Fig. 1. Inflammatory responses in XPC mice exposed to polluted air. Representative image and fluorescence intensity of ICAM-1 targeted nanoparticles labeled with DiD (excitation 640 nm, emission 680 nm) in XPC knockout and wild type mice exposed to ambient air pollution in São Paulo, Brazil. (A) XPC animals after filtered air and $PM_{2.5}$ exposure. (B) Wild type animals after filtered air and $PM_{2.5}$ exposure. (C) XPC animals after filtered air, lipopolysaccharide (LPS) and $PM_{2.5}$ exposure. (D) Comparative analysis between wild type and XPC animals after $PM_{2.5}$ exposure. *$p < 0.05$ statistically significant according to Dunnett's test or Bonferroni test.

may play an important role in the air pollution-induced pathogenesis of the inflammatory disease bronchitis in children, indicating that DNA damage repaired by NER is associated with lung inflammation (Ghosh et al., 2016). Here, we confirmed that inhalable $PM_{2.5}$ increases inflammatory responses in mice, particularly in the absence of XPC protein. Thus, unrepaired DNA damage may represent a significant contributing factor to the development of respiratory diseases.

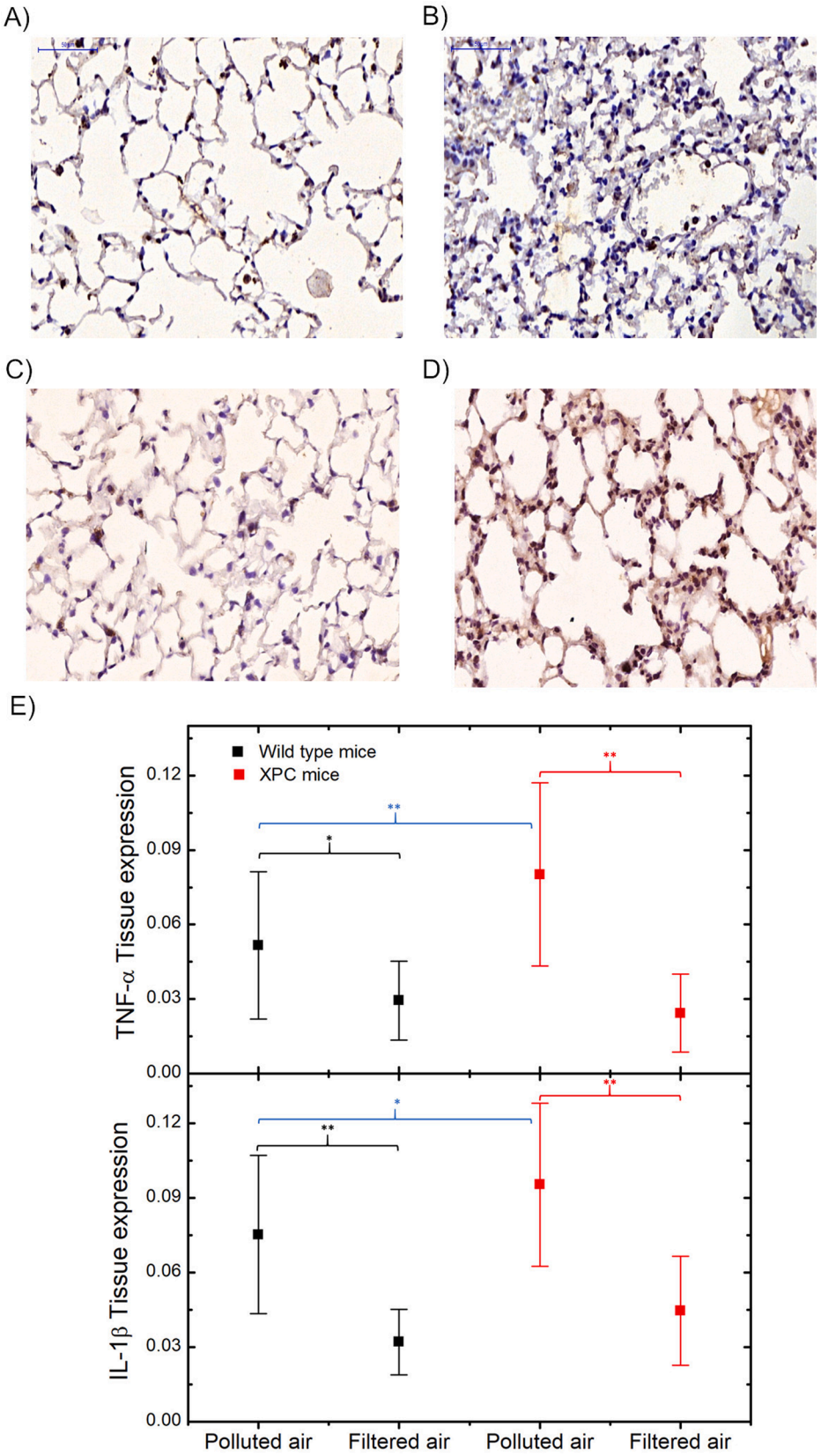

Fig. 2. Increase of IL-1β and TNF-α in the lung of wild type and XPC knockout mice after filtered and $PM_{2.5}$ exposure. Representative images of immunohistochemical expression of TNF- α (A and B, filtered and polluted air groups, respectively) and IL-1β (C and D, filtered and polluted air groups, respectively) in the lungs of XPC mice. (E) Quantitative results of TNF- α and IL-1β in lung tissue. *$p < 0.05$ and **$p < 0.001$ statistically significant according to Bonferroni test.

3.2. Carbonylated protein: an oxidative stress marker

To more comprehensively delineate the different responses related to the NER pathway, we next sought to investigate if $PM_{2.5}$ exposure can cause oxidative stress in the lungs of animals. For this, we quantified the levels of carbonylated proteins, which is a well-known marker for oxidative damage to proteins and reflects cellular damage induced by multiple forms of ROS (Fedorova et al., 2014). The results of this analysis clearly showed that long exposure to $PM_{2.5}$ increased levels of carbonylated proteins in the lung of XPC compared to wild type mice (Fig. 3).

Interestingly, previous studies demonstrated the vulnerability of DNA repair mechanism to inactivation by protein oxidation, which has implications for carcinogenesis and other diseases (McAdam et al., 2016; Moreno et al., 2019). Our study demonstrates that air pollution exposure causes extensive protein oxidation, a marker for increased oxidative stress, in lung cells lacking XPC protein. This effect may be induced by direct inhalation of ROS found in the PM from SPMA or by the presence of inorganic and organic compounds that are capable of generating ROS after inhalation. This result suggests that the DNA repair gene XPC may play an important role in protecting cells from air pollution and from the development of diseases associated with oxidative stress.

3.3. $PM_{2.5}$-induced histone H2AX phosphorylation persists in the lack of DNA repair XPC protein

The compiled evidence supports that various levels of PM exposure induce significant DNA damage in humans, animals and other experimental systems (DeMarini, 2013; International Agency for Research on Cancer, 2016). To further examine the DNA damage, wild type and XPC mice were exposed to inhaled $PM_{2.5}$ for 6 months using HAPC. As revealed in lung tissues by immunochemistry analysis, increased levels of γ-H2AX were found in both XPC and wild animals of the polluted air group when compared to the filtered air group ($p < 0.0001$). Interestingly, a massive increase of 2-fold of γ-H2AX positive nuclei was observed in the polluted air group of XPC mice in comparison with wild type mice ($p < 0.0001$) (Fig. 4). This indicates the important role of XPC in the repair of DNA lesions by various compounds detected in urban $PM_{2.5}$.

Multiple mechanisms are involved in DNA damage (e.g. formation of bulky adducts, strand breaks, oxidatively damaged DNA, induction or alteration of DNA repair pathways and others). Gualtieri et al. (2011), for example, reported that Milan $PM_{2.5}$ induces DNA damage in human bronchial epithelial cells as observed by increased phosphorylation of ATM, Chk2 and γ-H2AX. It also promotes the ROS formation and increased levels of 8-hydroxydeoxyguanosine (8-oxodGuo), suggesting that the particles could cause oxidatively induced DNA damage. Furthermore, DNA damage directly caused by PM has mainly been associated with PAHs and metal content (Veerappan et al., 2019). In this context, Lepers et al. (2014) demonstrated the ability of PM samples from industrial, urban or rural areas, to generate bulky DNA adducts in human bronchial epithelial cells. Our present data suggest the ability of inhaled $PM_{2.5}$ to induce DNA damage directly (forming adducts by PAHs and metals found in $PM_{2.5}$ samples which we will expound on further in section 3.5.2) or indirectly (by ROS).

Regarding the NER pathway, Mehta et al. (2008) reported that urban PM greatly inhibits NER for ultraviolet light and B(a)P diol epoxide (BPDE) induced DNA damage in human lung cells. These effects were associated with PM components such as heavy metals and aldehyde that can directly modify repair proteins and DNA; ROS and secondary ROS products and by direct modifications of DNA replication proteins due to these compounds in the PM. Additionally, Holcomb et al.(2016) found that the abundance of XPC protein, which is required for functional NER, is significantly reduced by treatment with cigarette smoke condensate, increasing the persistence of DNA lesions in human lung cells. Following this key role of the NER pathway, our results showed that $PM_{2.5}$-induced DNA damage mainly in the lack of DNA repair XPC protein, suggesting that the carcinogenicity of urban PM may act through its effect on suppression of DNA repair.

3.4. Histopathological lesions of the lung tissue

Given clearly increased DNA damage, we evaluated the impact of the XPC protein and NER pathway, on $PM_{2.5}$-induced lung preneoplastic lesions. Histological examination of the tissues under a light microscope revealed epithelial changes including hyperplasia and an increase in precancerous lesions in both wild type and XPC mice (Fig. 5). Bronchiolar epithelial hyperplasia (Fig. 5A) was more pronounced in wild type mice (Fig. 5E). There was no difference in the relative distribution of moderate atypical hyperplasia (Fig. 5B) between XPC and wild type animals (Fig. 5E). However, severe atypical hyperplasia (Fig. 5C and D) was clearly more significative in XPC mice compared to wild type mice (Fig. 5E). This demonstrates that the lack of DNA repair XPC protein accelerates the development of lesions with more diffuse and severe

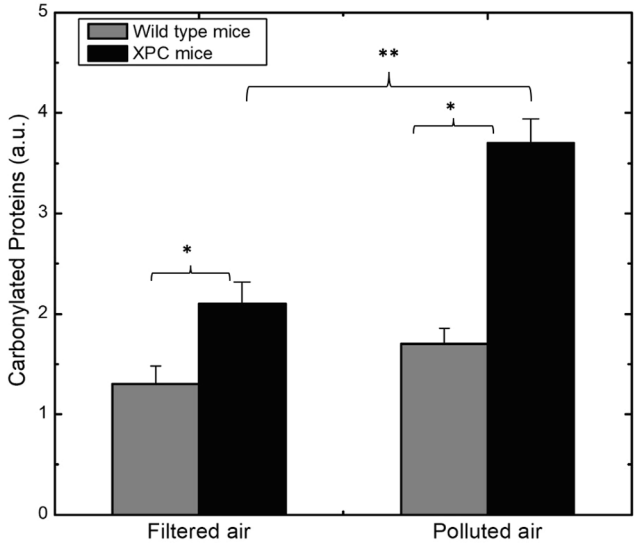

Fig. 3. XPC mice have increased levels of carbonylated protein after air pollution exposure. Quantification of carbonylated proteins in the lung of wild type and XPC knockout mice after filtered and $PM_{2.5}$ exposure. *$p < 0.01$ and **$p < 0.0001$ statistically significant according to Bonferroni test.

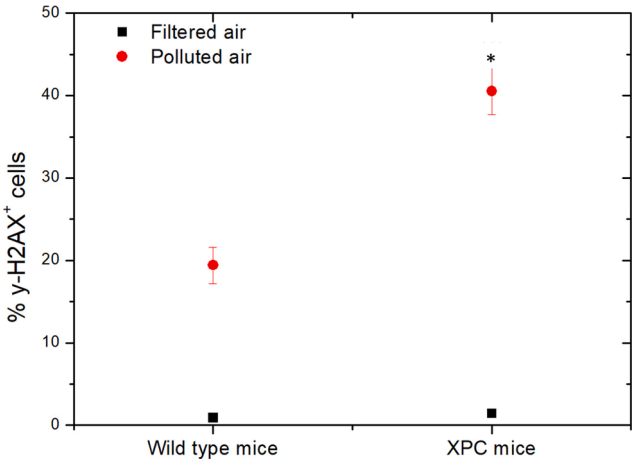

Fig. 4. XPC mice show persistent γ-H2AX after $PM_{2.5}$ exposure. * $p < 0.0001$ statistically significant between wild type and XPC mice in the polluted air group according to Bonferroni test.

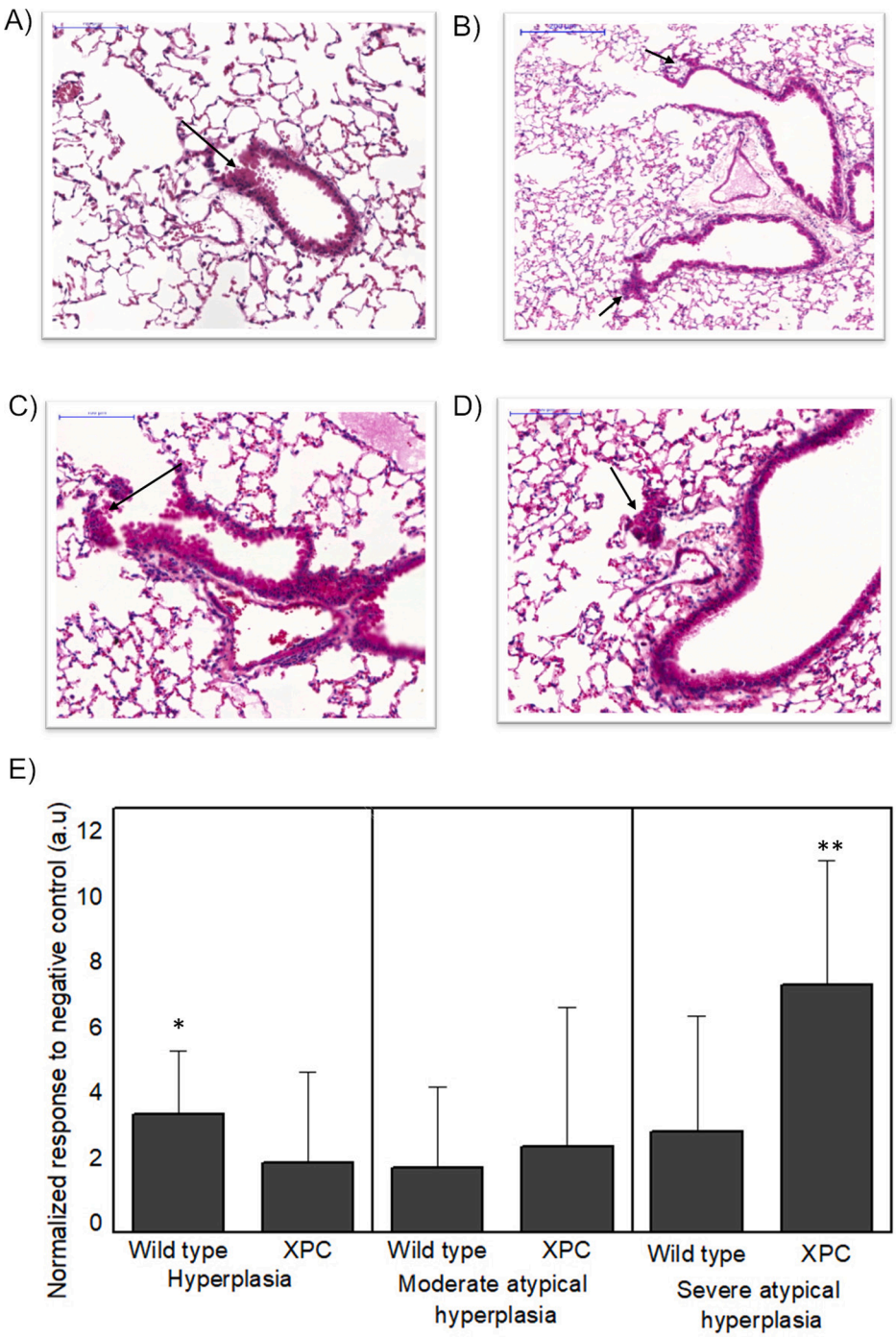

Fig. 5. Air pollution exposure induce lesions mainly in XPC mice. Representative images of hematoxylin and eosin (H&E) stained lung epithelial cells showing hyperplasia (A) and preneoplastic lesions as moderate atypical hyperplasia (B) and severe atypical hyperplasia (C, D) in XPC mice exposed to $PM_{2.5}$ for 6 months. Graph (E) showing a number of lesions counted in the lung of wild-type and XPC mice. The results were expressed in relation to a negative control (filtered air). *$p < 0.05$ and **$p < 0.001$ statistically significant according to Bonferroni test.

cellular atypia, which might progress to adenocarcinoma.

In this context, only a few studies have assessed the occurrence of cancer in animals exposed directly to air pollution by inhalation (International Agency for Research on Cancer, 2016). In particular, a study showed the presence of greater atypia in tumors developed in animals exposed to air pollution in São Paulo induced by urethane (Reymão et al., 1997). Another study in which mice were injected subcutaneously with organic particles extracted from air pollution, showed increased incidence of fibrosarcomas and pulmonary adenoma or adenocarcinoma (Epstein et al., 1979).

Here, we present for the first time the important of role of the NER pathway in the protection of mice against the carcinogenic potential of inhaled $PM_{2.5}$. Similarly, a recent study reported that XPC mice exposed during 9 months to cigarette smoke showed precancerous changes, including bronchiolar epithelial, some with focal dysplastic features, and early bronchoalveolar carcinoma (Zhou et al., 2019). It is interesting to note that Shen et al. (2005) found that persons with ''at-risk'' genotypes for both RAD23B and XPC had a significantly higher risk of lung cancer, showing that genetic variation in the NER pathway would play an important role in lung carcinogenesis in this specific population.

In general, no tumors were observed on the lung surface by macroscopic examination, except in a single XPC mice in the polluted group. Considering the results of our histological analysis, we believe that continued exposure for a period higher than 6 months, would probably allow for the development of lung tumors on gross examination in XPC mice exposed to air pollution. Further studies focusing on the role of XPC in $PM_{2.5}$-lung cancer development using molecular markers are therefore strongly encouraged.

3.5. $PM_{2.5}$ speciation and health impacts

Remarkably, it is recognized that not only the particle size but also the chemical composition from PM can influence its harmful effects on health (Kelly and Fussell, 2012). In this regard, we performed $PM_{2.5}$ speciation to check if vehicular emissions are predominant in our site as well as to identify the organic and inorganic compounds that may be associated with the inflammatory effects, oxidative stress and DNA damage observed in mice lungs.

3.5.1. Chemical characterization of $PM_{2.5}$ and its toxicity

In this study, ambient $PM_{2.5}$ presented a wide range of concentrations, varying between 10 and 45 μg m^{-3}, with an average of 28 μg m^{-3}, similar to the observed (30 μg m^{-3}) in a previous extensive study at the Butantã Campus of the University of São Paulo, located in a vast green area, yet near important expressways (Pereira et al., 2017). This average $PM_{2.5}$ concentration was comparable to the yearly $PM_{2.5}$ concentration observed previously for other Latin American metropolises (Santiago, 29 μg m^{-3}, 2016; and Mexico City, 22 μg m^{-3}, 2016). Nevertheless, this concentration is higher than the observed in some European (Berlin, 16 μg m^{-3}, 2016; and London, 12 μg m^{-3}, 2016) and North American metropolitan areas (New York, 7 μg m^{-3}, 2016; and Los Angeles 12 μg m^{-3}, 2016) (WHO, 2018b). In general, the Latin American largest cities present worst air quality than similar European and North American ones, since they lack stronger regulations concerning vehicular pollutant emissions (Lanzaco et al., 2017; Pereira et al., 2019).

The average concentration of total PAHs was 14 ng m^{-3}. The most dominant individual PAHs were BbF and Phe, followed by BeP (1.9, 1.9, and 1.4 ng m^{-3}, respectively) (Fig. 6A). The average BaP concentration in this study was 0.9 ng m^{-3} (Fig. 6A), comparable to the observed in a heavy traffic site in Rio de Janeiro (Ramos De Rainho et al., 2013). BaP in São Paulo presented higher average concentration than the observed in an extensive study in Cordoba, Argentina (a range of 0.12–0.46 ng m^{-3}), although lower than in a previous intensive study performed in an industrial area in Mexico City (4 ng m^{-3}) (Amarillo and Carreras, 2016; Guzmán-Torres et al., 2009). Contrastingly, a previous study performed in an intense biomass burning period in the Amazon, BaP presented a much lower average concentration (0.18 ng m^{-3}) (de Oliveira Alves et al., 2015).

Besides BaP, several other PAHs such as Chr, BbF, BkF and DBA have been found to be carcinogenic in experimental animals after inhalation or intratracheal ingestion (International Agency for Research on Cancer, 2016). Moreover, BbF has carcinogenic properties (Ravindra et al., 2008) and also was able to cause several DNA adducts in the lungs of rats (Ross et al., 1992). PAHs metabolites that form DNA adducts or ROS, such as 8-oxodGuo, may also contribute to oxidative DNA damage. Regarding lung cancer risk, mice exposed to inhaled $PM_{2.5}$ from São Paulo (same HAPC used in this study) had increased levels of 8-oxodGuo in the lung tissue (Falcão de Oliveira et al., 2018). It has been shown that, in humans, the oxidative damage-related gene OGG1 modulates risks for lung cancer due to exposure to PAHs from air pollution (Cruz et al., 2017; Lan et al., 2004).

Of particular note is that increasing attention is being paid to nitro and oxy-PAHs in this study. Some of these compounds have shown to be more mutagenic and carcinogenic than unsubstituted PAHs (de Oliveira Galvão et al., 2018; Wei et al., 2012). The 2-NFlu and 9,10-AQ presented the highest average concentrations among the nitro and oxy-PAHs (1.0 and 1.0 ng m^{-3}, respectively) (Fig. 6B). 2-NFlu is known as a carcinogenic nitro-PAH and is emitted by diesel exhausts, together with the nitropyrenes (Draper, 1986; Fujimoto et al., 2003) and 9,10-AQ, which come from primary emissions and secondary formation (Pereira et al., 2017). Mutagenic tests have been widely used to detect the effects of ambient nitrated compounds (Durant et al., 1996; Fujimoto et al., 2003) and the presence of 2-NFlu induced mutations in the *Salmonella*/microsome assay (Suzuki et al., 1997), in mouse lymphoma L5178Y $Tk^{+/-}$ (Oberly et al., 1996) and rat epithelial intestinal IEC-17 (Glatt et al., 1990) cells.

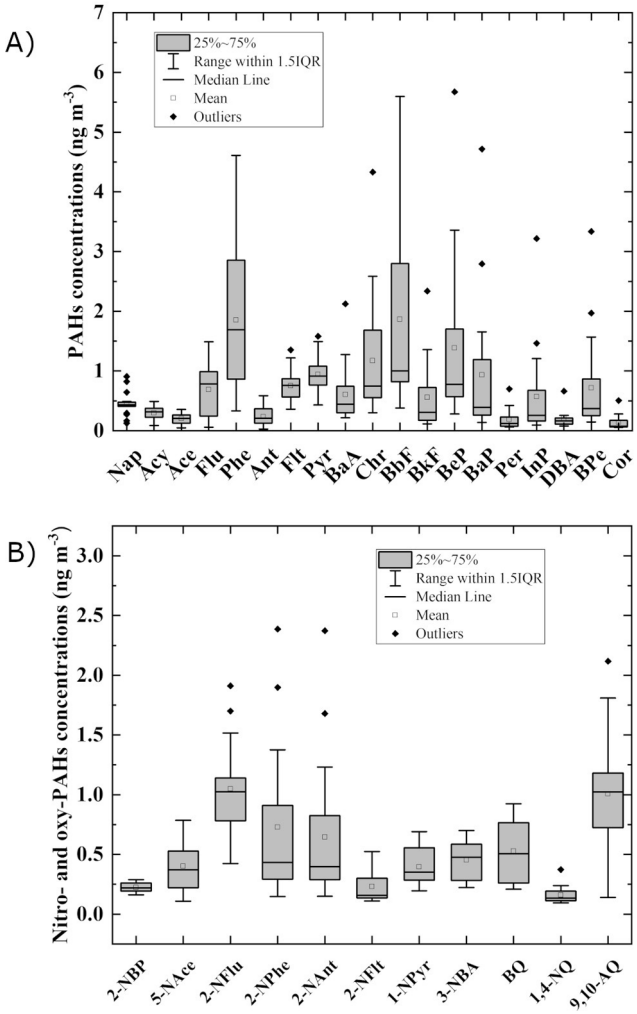

Fig. 6. Chemical composition of the $PM_{2.5}$ from São Paulo city. (A) total PAHs and (B) nitrated and oxygenated-PAHs derivatives concentrations in the $PM_{2.5}$ from São Paulo city.

Another finding indicates that personal air exposure to 9,10-AQ and individual PAHs originating from traffic emissions are responsible for the increase of oxidatively generated DNA damage in humans (Wei et al., 2010) and pro-inflammatory responses (Shang et al., 2013). In this study, 9,10-AQ average concentration was slightly higher than BaP concentration. Similarly, in traffic-related PM, the levels of this quinone are greater than that of BaP (Eiguren-Fernandez et al., 2008; Oda et al., 2001). In addition, a powerful mutagenic aromatic found in the airborne particles is 3-NBA. Note that in the present study the average concentration of 3-NBA was 0.45 ng m^{-3} (Fig. 6B) and the maximum concentration was 0.7 ng m^{-3}. The mutagenicity and genotoxicity of 3-NBA were found in different bacterial and mammalian assay systems (Arlt, 2005; Phousongphouang et al., 2000). It has also been found to induce DNA adducts and lung tumors in experimental animals (Nagy et al., 2005).

Concerning crustal elements analysis, Ca, Al and Mg, were observed in relatively high concentrations (1902, 1006, and 770 ng m^{-3}, respectively) (Fig. 7). Consistent with previous studies, this characterization is attributed to resuspended soil dust due to the heavy vehicular traffic on roads close to the sampling site (Pereira et al., 2017; Vasconcellos et al., 2007). The elements concentrations were, generally, higher than observed in $PM_{2.5}$ collected in Argentinean cities of Buenos Aires, and Cordoba, although toxic As and Cd presented higher average

concentrations in the latter city (São Paulo: 1.3 and 0.6 ng m^{-3}; and Cordoba: 2.7 and 12 ng m^{-3}, respectively) (Gómez et al., 2017; Lanzaco et al., 2017).

Exposure to carcinogenic metals such Ni, Co, As and Cd poses serious public health risks and these compounds were identified here (average concentration: 39.5, 0.08, 1.3 and 0.6 ng m^{-3}, respectively) (Fig. 7A and B). Moreover, transition metal ions can lead to ROS formation via Fenton-like reactions which may cause oxidative damage to biological macromolecules such as DNA (Valko et al., 2016). In this study, Fe was among the most abundant species and can be linked to oxidative stress by generating ROS (Charrier and Anastasio, 2015; Verma et al., 2009). Synergistic interaction between Fe and soot can lead to biological responses, in previous studies the exposure was accompanied by the elevation of IL-1β, indicating oxidative stress (Zhou et al., 2003). Another study in São Paulo city (2013–2014), showed that the abundance of Cu and Zn in PM water-soluble extracts were associated with oxidative and genotoxic effects (Palacio et al., 2016).

3.5.2. *Chemical components in PM linked with NER pathway*

Atmospheric PM is known to contain toxic compounds such as PAHs (Kim et al., 2013). DNA binding of these compounds and the associated effects that occur as a result is considered the major mechanism of PAHs-induced mutagenesis and carcinogenesis (Jarvis et al., 2014). One of the main functions of the NER pathway is the repair of bulky DNA adducts induced endogenously as well as by exogenous sources, such as PAHs found in air pollution. If not effectively repaired, these DNA adducts could result in mutations during DNA replication and may eventually lead to neoplastic transformation (Braithwaite et al., 1998; Cai et al., 2012).

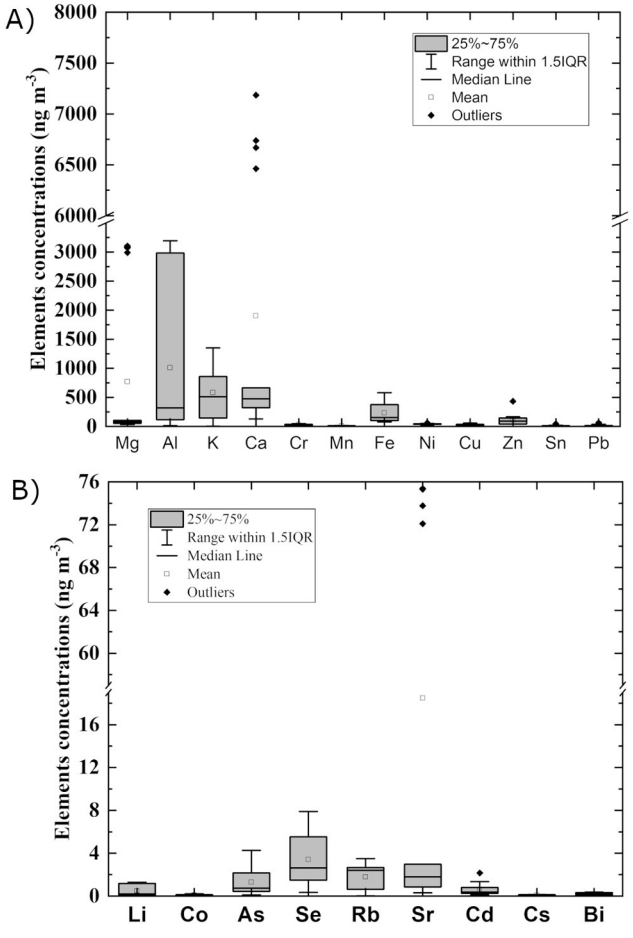

Fig. 7. Distribution of major metals (A) and minor (B) metals concentrations in the São Paulo city.

Extensive efforts are being addressed in order to contribute to a better understanding of the mechanisms of $PM_{2.5}$-induced lung toxicity. However, few studies have investigated the association between PM from air pollution and NER pathway. A previous work indicated that the DNA repair gene XPC may serve an essential function in the pathogenesis of $PM_{2.5}$-induced inflammatory disease bronchitis (Ghosh et al., 2016). Another study provided important insights into the molecular mechanism of 3-NBA-mutagenesis and in particular, showing that adenine adducts formed were efficiently repaired by the NER pathway (Kawanishi et al., 2013; Nishida et al., 2008). Specifically, BaP is metabolized into a highly reactive metabolite BPDE. BPDE-DNA adducts are recognized and repaired by NER pathway. Interestingly, it has been reported that mice deficient in the NER gene XPA have elevated sensitivity to BaP induction of lung tumors (Ide et al., 2000). Our analysis highlights that the peak BaP concentration was 4.7 ng m^{-3}, which exceeds the European target values of 1.0 ng m^{-3} and probably contributed to various adverse health effects observed here in the NER-deficient XPC mice.

$PM_{2.5}$ is a complex mixture and in addition to organic compounds, metals also are thought to be responsible for the toxicity of these particles. The carcinogenicity of Ni, Co, As and Cd has long been recognized and these metals have been shown to inhibit the removal of DNA damage by the NER pathway, by disrupting several steps of the repair process (Hartwig and Schwerdtle, 2002; Kopera et al., 2004). Previous studies have also demonstrated that lower doses of these metals in human lung cells are able to modify the expression of a wide variety of genes, including inflammatory cytokines, kinases and DNA repair proteins (Andrew et al., 2003; Yih et al., 2002).

Thus, our findings indicated that PAHs and their derivatives as well as metals could be responsible for the effects displayed in this study. Since those compounds are frequently found in the urban polluted air, an impaired removal of DNA adducts can lead to persistent DNA damage and thus increase their carcinogenic risk.

3.6. *Health risks from organic and inorganic $PM_{2.5}$ compounds*

The BaPE index was adopted as a tool to quantify the aerosol carcinogenicity and the average BaPE was 1.3 ng m^{-3} (0.2–6.1 ng m^{-3}); values above 1.0 ng m^{-3} represent an increased cancer risk (Vasconcellos et al., 2011). The average is lower than previously observed in extensive studies of $PM_{2.5}$ in Butantã University Campus in 2014 (2.4 ng m^{-3}), a year that presented low precipitation and high pollution levels (Pereira et al., 2017). The value is similar to the observed in a relatively polluted period in the city of Cordoba, Argentina (autumn, 1.24 ng m^{-3}) (Carreras et al., 2013), while in a study performed in the urban area of Guangzhou (China) the BaPE index reached values as high as 21.69 ng m^{-3} (Bi et al., 2003). The LCR values for the exposure to PAHs and nitro-PAHs were respectively 2×10^{-4} and 1.6×10^{-4}; values higher than 10^{-4} represent a high risk (Chen and Liao, 2006; de Oliveira Galvão et al., 2018). The sum of the LCR for exposure to PAHs and nitro-PAHs was of 3.6×10^{-4}, with the PAHs representing 56% of the potential and nitro-PAHs, 44%. The excess lifetime cancer cases per 100,000 exposed people were 36 if both PAHs and nitro-PAHs are considered, and 16 if only nitro-PAHs are considered, higher than the observed in an intense biomass burning period in the Amazon (7.3) (de Oliveira Galvão et al., 2018).

To understand the associated risks of exposure to metallic elements in $PM_{2.5}$ by inhalation, the carcinogenic risks (ECR_{child} and ECR_{adult}) were calculated similarly as in Hu et al. (2012) and US EPA (2009) (Table 1). The minimal threshold of ECR is established as 10^{-6} (below this value the risk is considered as negligible) and the maximum is of 10^{-4} (above that, considered as serious) (Ramírez et al., 2020; USEPA, 2009, 2001); all values of ECR_{child} and ECR_{adult} for metals in the present study were below the limit of 10^{-4}, although ECR_{adult} levels were above 10^{-6} for Ni and As (3×10^{-6}, and 1.7×10^{-6}, respectively). It is

alarming the high Ni contribution, reaching more than half of the total ECR_{child} and ECR_{adult} values; in a previous study in Beijing (China), with $PM_{2.5}$ collected in days of haze fog, the risk levels for Ni were lower (3.61×10^{-8} and 1.64×10^{-7} for ECR_{child} and ECR_{adult}, respectively) (Gao and Ji, 2018). The ECR_{child} and ECR_{adult} sums were of 1.4×10^{-6} and 5.7×10^{-6}, respectively; for a population of one million individuals, more than one child and five adults may present cancer due to lifetime exposure to these species, higher than in a study performed for Bogotá's PM_{10}, when it was estimated to three adults (Ramírez et al., 2020).

The non-carcinogenic risk was evaluated using HQ and the sum of HQs was 1.1, exceeding the safe level of 1. Ni also presented a higher HQ level than the other species, representing 70% of the sum; in a previous study performed in Edmonton (Canada), with $PM_{2.5}$ collected from 2009 to 2015, the total HQ was below 0.1 (Bari and Kindzierski, 2016) while the total HQ for a $PM_{2.5}$ study in Nanjing (China) was 2.96 (Hu et al., 2012).

4. Conclusions

Combined with atmospheric chemistry analysis, this study reveals the important role of XPC protein in the protection against carcinogenic potential of urban air pollution. The results showed that exposure to $PM_{2.5}$ from São Paulo promotes acute inflammatory responses in mice, especially in the absence of XPC protein. After continued $PM_{2.5}$ exposure, there is a significant increase in the levels of carbonylated proteins in the lung of XPC mice, probably as a consequence of oxidative stress. As a response to DNA damage, XPC mice lungs exhibit increased γ-H2AX, followed by severe atypical hyperplasia. Given their real atmosphere exposure, lungs are particularly vulnerable to different compounds such as total, nitro and oxy-PAHs as well as metallic elements. These compounds and other genotoxic chemicals into $PM_{2.5}$ can generate ROS, which might lead to oxidatively generated DNA damage. Concerning $PM_{2.5}$ speciation, which reinforces the dominant source of vehicular emissions in SPMA, the analysis showed that BbF, 2-NFlu and 9,10-AQ were the most abundant organic compounds identified and probably contributed to the inflammation, oxidative stress and DNA damage effects by direct and/or indirect mechanisms. Furthermore, there is a high potential risk of cancer development as a result of PAHs and metals exposure. Altogether, these results demonstrate the participation of XPC protein, and NER pathway, in the protection of mice against the carcinogenic potential of $PM_{2.5}$. This implicates that DNA is damaged directly (forming adducts) or indirectly (ROS) by the various compounds detected in urban $PM_{2.5}$. Thus, this work provides data clearly linking ambient air pollution with the induction of DNA damage, normally handled by DNA repair (such as NER), which are implicated in the mechanisms of lung carcinogenesis.

CRediT authorship contribution statement

Nilmara Oliveira Alves: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Guilherme Martins Pereira:** Methodology, Formal analysis, Investigation, Writing - review & editing. **Marlise Di Domenico:** Methodology, Investigation, Writing - review & editing. **Giovanna Costanzo:** Methodology, Investigation. **Sarah Benevenuto:** Methodology, Investigation, Writing - review & editing. **Adriana M. Oliveira Fonoff:** Methodology, Investigation. **Natália Souza Xavier Costa:** Methodology, Investigation. **Gabriel Ribeiro Júnior:** Methodology, Investigation. **Gustavo Satoru Kajitani:** Methodology, Investigation, Writing - review & editing. **Natália Cestari Moreno:** Methodology, Investigation, Writing - review & editing. **Wesley Fotoran:** Methodology, Investigation. **Janaína Iannicelli Torres:** Methodology, Investigation. **Jailson Bittencourt Andrade:** Methodology, Resources. **Mariana Matera Veras:** Methodology, Validation, Investigation. **Paulo Artaxo:** Methodology, Investigation, Resources, Writing - review & editing. **Carlos Frederico Martins Menck:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Funding acquisition. **Pérola Vasconcellos:** Methodology, Investigation, Resources, Writing - review & editing. **Paulo Saldiva:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Table 1
Carcinogenic and non-carcinogenic risks by inhalation of metals in the São Paulo city.

	ECR_{child}	ECR_{adult}	HQ
Mn	–	–	0.18
Co	5.9×10^{-8}	2.4×10^{-7}	0.01
Ni	7.5×10^{-7}	3.0×10^{-6}	0.79
As	4.4×10^{-7}	1.7×10^{-6}	0.09
Cd	8.5×10^{-8}	3.4×10^{-7}	0.06
Pb	9.3×10^{-8}	3.7×10^{-7}	–
Sum	1.4×10^{-6}	5.7×10^{-6}	1.1

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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