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Generation and photogeneration of hydroxyl radicals and singlet oxygen by particulate matter and its inorganic components

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ABSTRACT

Polluted air containing particulate matter (PM) is a huge environmental problem in the city of Kraków, Poland. PM causes numerous negative health effects including infection of the respiration system and cardiovascular disorders. The development of autoimmune disorders can be facilitated by redox active transition metals due to the generation of reactive oxygen species (ROS) and induction of oxidative stress, which results in lipid peroxidation, protein oxidation and severe oxidative modification of DNA. Our goal was to study ROS generation (in particular, $HO^{\bullet}$ and $^1O_2$) in the presence of PM collected *via* different procedures at different locations in Kraków. Scanning electron microscopy analysis confirmed that in all collected samples, aggregates of small grains could be observed. Cold plasma treatment was used to remove parts of the organic matter and to uncover inorganic compounds which become more relevant following plasma treatment. All samples were photoactive and showed hydroxyl radical generation. Singlet oxygen production studies indicated that during plasma treatment, unsaturated organic species are produced that intensify the generation of $^1O_2$. Generation of hydroxyl radicals and singlet oxygen *in vitro* was confirmed using human epithelial cell line A549.

1. Introduction

Air pollution is one of the most serious environmental problems the world is facing today. It strongly affects our health and quality of life. It was proven that air pollution increases hospital admissions [1–3], respiratory problems [4–6], intensifies cardiovascular [7–10] and lung disorders [11–14], promotes premature mortality [15–17] and affects autoimmune diseases [18]. Particulate matter (PM) is one of the main contributors to air pollution and includes heterogeneous particles, both solid and liquid droplets, suspended in air [19]. Sources of PM can be natural (dust storms, forest fires, sea spray), but also anthropogenic (combustion of coal and other fuels, industrial waste, brakes and tires abrasion, *etc.*) [20]. Particulate matter can be divided into three groups based on its size: (i) PM_{10} – particles with aerodynamic diameter smaller than 10 μm, (ii) $PM_{2.5}$ – particles with aerodynamic diameter smaller than 2.5 μm, and (iii) PM_1 – particles with aerodynamic diameter smaller than 1 μm [19]. Due to the fact that smaller particles can reach deeper parts of the human respiratory system, PM_1 causes more acute effects than $PM_{2.5}$ [21]. Particles with aerodynamic diameter smaller than 2.5 μm are able to enter the alveolar region of lungs causing irritation, which can then trigger inflammation [22]. Inhaled particles containing transition metal compounds can induce oxidation of proteins and lipids as well as DNA damage. This may promote increasing inflammation states [23–26]. Oxidative stress may be a result of soluble transition metal compounds present in PM. Transition metal ions can be transferred to body fluids in two ways: (i) in rain or fog droplets and inhaled as such by humans, or (ii) particles containing transition metals can be inhaled and then solubilized in body fluids. The ions originating from iron, copper and chromium PM, were hardly studied in terms of ROS production [27–30]. Recently, a significant interest in the impact of

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organic components of PM on oxidative stress, mainly PAHs (polycyclic aromatic hydrocarbons) and quinones [31–33], was observed. Transition metal compounds present in PM can enhance the detrimental influence of organic pollutants making the overall effect stronger than that of the separate components [34,35].

The main goal of this study was to investigate reactive species generation in the presence of PM collected in Kraków. Oxygen plasma treatment (PT) was adopted to decrease the organic carbon content in the PM, which enabled us to observe the difference between the original PM and the inorganic fraction of PM. Experiments with non-cellular systems were performed in suspension, both in the dark and after irradiation, to follow thermal and photo-induced hydroxyl radical formation. ROS generation was later also followed *in vitro* using the A549 cell line.

2. Material and methods

2.1. Materials

Samples of $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial were provided by the Voivodship Inspectorate for Environmental Protection (VEIP) in Kraków. They were collected on quartz fiber filters (diam. 47 mm, pore size 2.2 μm) according to procedures of the Voivodship Inspectorate for Environmental Protection in Kraków. The air quality assessment in Poland is carried out based on relevant legal acts that define the air monitoring system, define the scope and method of testing, determine the minimum number of stations as well as assessment methods and criteria. The relevant laws and regulations can be found on the website ⟨http://monitoring.krakow.pios.gov.pl/ocena-jakosci-powietrza⟩.
$PM_{2.5}$ Urban.A was collected in an area closely located to crowded streets in the city center, whereas $PM_{2.5}$ Industrial was collected in the neighborhood of power plants and steel works. Sample of PM Urban.B was collected in the city center, next to crowded streets by a custom designed system that is a variation of typical sample collectors. In this system, air was sucked by a pump (*ca.* 30 m^3/h) through 16 PTFE filters (diam. 47 mm, pore size 2.2 μm) placed in a self-designed Plexiglas cassette. All filters were held with metal rings in fixed positions in the cassette. This cassette was connected tightly to the pump which sucked air from outside through the filters and allowed the collection of PM on the filters. Filters were changed every week. The system did not have a size-separation unit, so particles of different size were collected simultaneously.

Terephthalic acid was purchased from Aldrich (98%), deuterated methanol from Merck, sodium hydroxide and methanol from POCh.

2.2. Particulate matter extraction and plasma treatment (PT) procedure

Collected filters were immersed in methanol and sonicated for 1 min in a water-bath sonicator (SONIC-5, Polsonic, 620 W). Subsequently, the PM was dried overnight at 60 °C (Pol-Eko-Aparatura Sp. J.). The Plasma Zepto system (Diener Electronic GmbH) was used for the removal of organic compounds from the PM. This method was used in our earlier work [36] and was applied to Kraków PM. We used different times of plasma treatment in the case of all PM samples, from 2 h up to 15 h, for PM collected by our custom designed system. Plasma treated samples of PM are encoded later in the text as PT. The content of carbon, hydrogen, nitrogen and sulfur in these samples was measured by elemental analysis (Elementar, Vario Micro Cube).

2.3. Morphology and elemental composition analyses

Scanning electron microscopy (SEM) technique was used to evaluate particle shape, morphology and chemical composition of collected dusts (TESCAN Vega3 LMU microscope equipped with a LaB_6 cathode and EDS detector, Oxford Instruments, X-act, SDD 10 mm^2). EDS mapping was performed on whole photos collected during SEM analysis in order to assess the distribution of elements and evaluate their homogeneity in the tested samples.

2.4. Detection of hydroxyl radicals in a non-cellular system

The hydroxylation reaction of terephthalic acid (TA) was used for hydroxyl radicals generation monitoring [37]. Hydroxyterephthalic acid is a highly fluorescent product of this reaction and its concentration is proportional to the hydroxyl radicals produced during irradiation. All examined samples were suspended in a terephthalate solution (3 mM TA, dissolved in 10 mM NaOH) to a concentration of 0.55 mg/mL and placed in a quartz cylindrical cuvette (5 cm diam., 1 cm optical path, 16 mL volume). A Xenon lamp (150 W, Instytut Fotonowy) coupled to a water-cooling system was applied for irradiation of PM suspensions. For irradiation in the UV-Vis range (320–650 nm), a 320 nm cut-off filter and a 10 cm thick copper sulfate solution filter (0.1 M in water) were used, and for the UV-Vis-NIR range (320–950 nm) a 10 cm thick water filter instead of copper sulfate solution was used. Experiments were also performed in the dark under magnetic stirring. The average irradiation was kept at the level of 0.85 mW cm^{-2}. After every 60 min of irradiation, 1 mL of sample was taken and filtered through MCE filters (pore size of 0.22 μm). The fluorescence intensity of the generated product of the reaction was monitored by recording emission spectra in the range of 350–500 nm (λ_{max} = 425 nm) upon excitation at 315 nm using a Perkin-Elmer LS-55 spectrofluorimeter.

2.5. Singlet oxygen generation in a non-cellular system

Detection of singlet oxygen was performed according to the procedure described by Buchalska et al. [38]. All PM samples were suspended in deuterated methanol (0.1 g/dm^3) in a quartz 3 mL cuvette equipped with a rubber septum. Five minutes of oxygen bubbling was applied directly before the measurements. Fluorolog-3 spectrofluorimeter (Horiba Jobin Yvon) was used for spectra collection. Suspensions were excited continuously at 290 nm with the monochromatized light of the xenon lamp of the spectrometer. Phosphorescence spectra of singlet oxygen were recorded by the iHR320 imaging spectrometer with a NIR photomultiplier tube and with an InP/InGaAsP photocathode (Hamamatsu) (average of 150 scans) [36].

2.6. Covering plates with particulate matter samples

For the *in vitro* experiments, 96-wells plates were covered with PM. Different concentrations of PM Urban.B in cold methanol were added to the wells. Plates were kept under a laminar flow hood for methanol evaporation and were then directly used for *in vitro* experiments.

2.7. Cell culture conditions

Type II human alveolar-like epithelial cell line A549 was used in the biological studies. Cells were routinely cultivated in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37 °C in a humidified incubator in a 5% CO_2 atmosphere.

2.8. Evaluation of reactive oxygen species production by A549 cells

To estimate the production of singlet oxygen in the cells, the singlet oxygen sensor green (SOSG, ThermoFisher Scientific) was used. Aminophenyl fluorescein (APF, ENZO) was used to determine the production of hydroxyl radicals, hypochlorite or peroxynitrite induced in the cell culture after exposure to PM Urban.B.

A549 cells were seeded on prepared 96 well plates (covered with PM Urban.B) with a density of 3×10^4 cells per cm^2. After 24 h of exposure, sensing agents were added (5 μM SOSG and 5 μM APF) and incubated for 30 min at 37 °C. After the staining, the ROS indicators were washed two

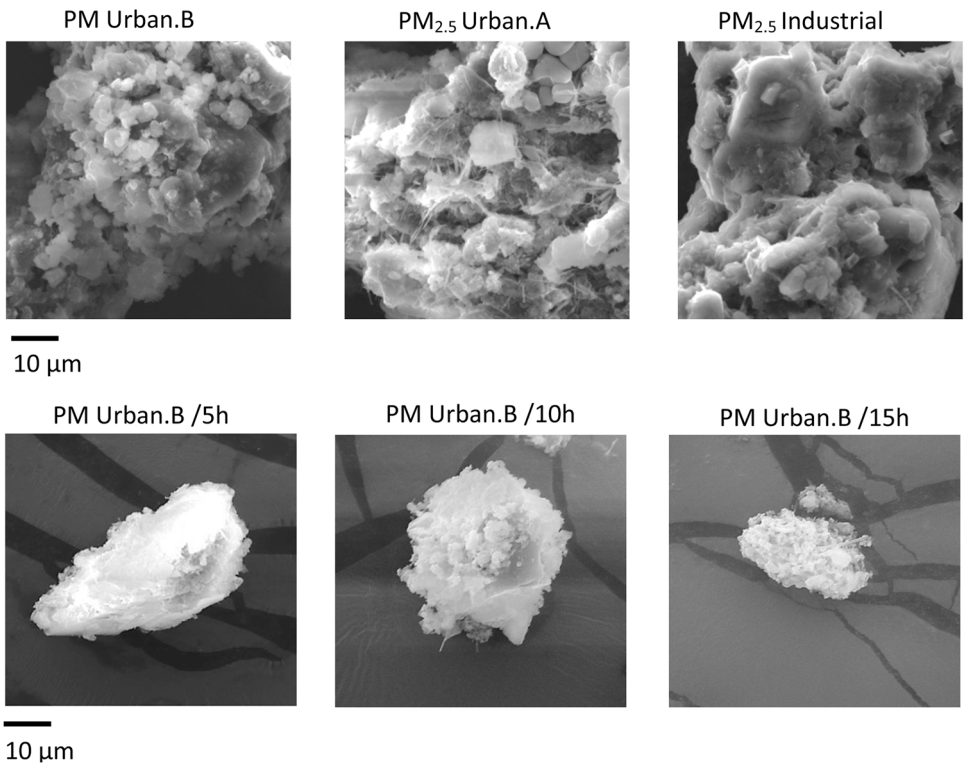

Fig. 1. Comparison of PM collected in different locations in Kraków and PM Urban.B after 5, 10 and 15 h of plasma treatment.

times with PBS. Tecan Infinite 200 plate reader was used to record the fluorescent signal of the cell at 535 nm (excitation wavelength 485 nm). The results are presented as a relative increase of the fluorescent signal of the treated cells compared to the untreated control. The experiments were performed in three replicates, each one repeated six times. The mean values ± standard error of mean (SEM) were computed.

To avoid false positive results, appropriate controls containing ROS indicators and PM samples (without cells) were used. SOSG was used up to 400 μg/cm^2 of PM Urban.B, while APF induced a false positive signal at the concentration of PM higher than 100 μg/cm^2.

3. Results and discussion

3.1. Scanning electron microscopy analyses

Scanning electron microscopy images of dusts from Kraków do not show any significant differences in morphology. Samples collected near the Aleje Street ($PM_{2.5}$ Urban.A, PM Urban.B) are characterized by particles similar in size and surface morphology, while dust from an industrial area consists of aggregates with a smoother surface (Fig. 1). In the case of $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial, dust was collected on quartz fiber filters and the extraction procedure resulted in contamination of PM with some fibers from the filters. Sonication from this type of filter is difficult even when methanol is used as a solvent. Longer sonication times caused damage of the filters such that the $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial samples contained more quartz fibers, which could not be removed even after syringe filtration through filters with 5 μm pores, since the quartz fibers were smaller than the filter pores. The same effect was observed by other groups working with quartz fiber filters [39,40]. In the case of PM collected by us (PM Urban.B), huge aggregates of particles were observed before plasma treatment. After removing the organic part from PM, the observed aggregates appeared smaller, composed of many smaller grains. The longer the plasma treatment time, the smaller the grains. This type of morphology can point to easy separation of small particles by ultra-sonication to obtain smaller particles than the original PM Urban.B. In PT PM, rougher surfaces of aggregates were observed.

PM Urban.B contains particles with a larger diameter than 2.5 μm (Fig. S1). During collection of PM, particles aggregate on the filter and create large conglomerates composed of small particles. For this sample, there is plenty of sulfur that can prove the presence of sulfates in the sample. The contents of oxygen and sodium were also high. Other elements, like aluminum, silicon, calcium and iron are distributed homogeneously, with more intense signals for some specific particles. The EDX analysis shows that this sample contains some calcium sulfate, potassium sulfate and silicon compounds, very likely silica and aluminosilicates.

The $PM_{2.5}$ Urban.A sample was collected in the city center close to one of the most crowded streets in Kraków (Fig. S2). Carbon is among the most common elements in this sample. Silicon is equally distributed within the whole sample, including fibers from the quartz filters used during the collection of the sample, as evidenced by elongated shapes of these particles. This PM contains also oxygen, sodium and calcium, as well as silicates of magnesium and calcium. Transition metals, such as manganese, iron, copper and chromium, were found in significantly smaller amounts.

The last type of sample was $PM_{2.5}$ collected in the industrial zone – $PM_{2.5}$ Industrial (Fig. S3). It was also collected on quartz fiber filters, so the needles of SiO_2 could be observed in the EDS images. In this sample the content of carbon is moderate. In places with high carbon concentration, also a high content of oxygen was found. Large aggregates or crystals of inorganic salts, including Na_2SO_4 and $MgSO_4$, were observed. The remaining elements are present in lower concentrations and are distributed homogenously over the whole sample. Oxygen, sulfur and sodium are the most abundant elements in this sample and can indicate the existence of Na_2SO_4. A relatively high content of potassium and chlorine may indicate the presence of KCl or other salts of these elements. However, in this sample, we did not find high quantities of transition metal compounds.

In plasma treated sample of PM Urban.B (Fig. S4) an efficient

Table 1
Content of carbon, hydrogen, nitrogen and sulfur in collected and plasma treated samples/all data presented in %/.

	$PM_{2.5}$ Urban.A	$PM_{2.5}$ industrial	PM Urban.B	PM Urban.B/5 h	PM Urban.B/10 h	PM Urban.B/15 h
Carbon	44.00 ± 0.07	42.22 ± 2.82	43.57 ± 0.95	14.88 ± 0.74	9.55 ± 1.34	6.54 ± 0.20
Hydrogen	5.18 ± 0.12	5.53 ± 0.20	4.84 ± 0.03	3.12 ± 0.05	2.62 ± 0.04	2.48 ± 0.03
Nitrogen	6.83 ± 0.41	6.94 ± 1.13	7.14 ± 0.05	6.91 ± 0.07	6.72 ± 0.17	6.32 ± 0.03
Sulfur	2.62 ± 0.16	2.60 ± 0.38	3.30 ± 0.12	12.48 ± 0.40	15.24 ± 0.71	15.49 ± 0.12

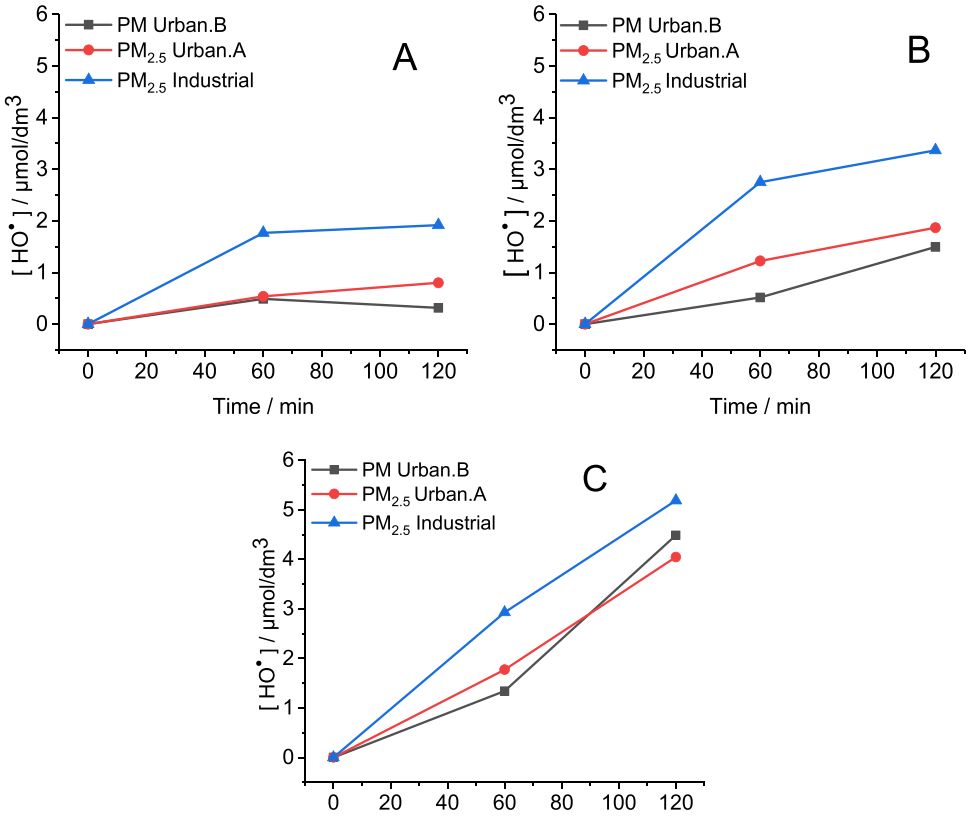

Fig. 2. Hydroxyl radical concentration produced in the presence of PM Urban.B, $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial in the dark (A), during irradiation with UV-Vis (B), and UV-Vis-NIR light (C).

removal of organic compounds was successful, since the carbon content dropped below the detection limit. The 15 h plasma treated sample (Fig. S4) mainly consists of calcium and sulfur compounds (gypsum), as well as noticeable amounts of aluminum and silicon, suggesting the presence of aluminosilicates. Among the more interesting redox active and biologically significant elements, only iron was detected. Plasma treatment resulted in removal of some organic components and revealed some inorganic compounds that became more exposed after plasma treatment.

Since SEM/EDS analysis cannot provide information about the carbon and hydrogen content, elemental analysis of all collected samples was performed. The results are presented in Table 1.

3.2. Detection of hydroxyl radicals

Fig. 2 shows the results for $HO^{\bullet}$ generation in the dark (A) and after irradiation (B, C) in the presence of the three PM samples collected in Kraków. All samples are active in the dark. Dark activity of the samples can stem from a significantly longer lifetime of ROS adsorbed on the surface of PM. The dark activity of the samples confirms, that photogeneration of ROS before inhalation should not be neglected in the whole chain of reactions involving PM and ROS. The most active sample comes from the industrial zone ($PM_{2.5}$ Industrial), whereas $HO^{\bullet}$ generation by the samples from the city center ($PM_{2.5}$ Urban.A and PM Urban. B) is moderate. The production of $HO^{\bullet}$ slightly increases for all samples upon irradiation with UV-Vis (320–650 nm) light. The highest photoactivity was observed for the sample from the industrial area, while the lowest photoactivity was observed for PM Urban.B. Among PM samples collected in the city center, more $HO^{\bullet}$ radicals were produced for samples of $PM_{2.5}$ collected on quartz fiber filters. Experiments performed under UV-Vis-NIR (320–950 nm) light confirmed that upon irradiation the PM samples show the highest photoactivity. The highest efficiency of hydroxyl radical generation was observed for the industrial PM sample, while PM Urban.B and $PM_{2.5}$ Urban.A samples showed a similar photoactivity.

Fig. 3 presents the results of hydroxyl radical detection in the presence of PM samples after 10 h of plasma treatment. In dark experiments, only PM Urban.B/10 h PT sample was not active, whereas samples from VIEP ($PM_{2.5}$ Urban.A, $PM_{2.5}$ Industrial) revealed the same thermal activity (Fig. 3A). Analogously, upon irradiation with UV-Vis light, $PM_{2.5}$ Urban.A/10 h PT and $PM_{2.5}$ Industrial/10 h PT appeared the most photoactive samples showing almost the same activity, whereas hydroxyl radical generation in the presence of PM Urban.B/10 h PT showed a negligible activity during 2 h of irradiation (Fig. 3B). Upon irradiation with UV-Vis-NIR light, a pronounced difference between all PM samples could be observed. Samples from Urban.A and Industrial places were very active, the $HO^{\bullet}$ concentration was even higher than for samples before plasma treatment, whereas PM Urban.B showed a very

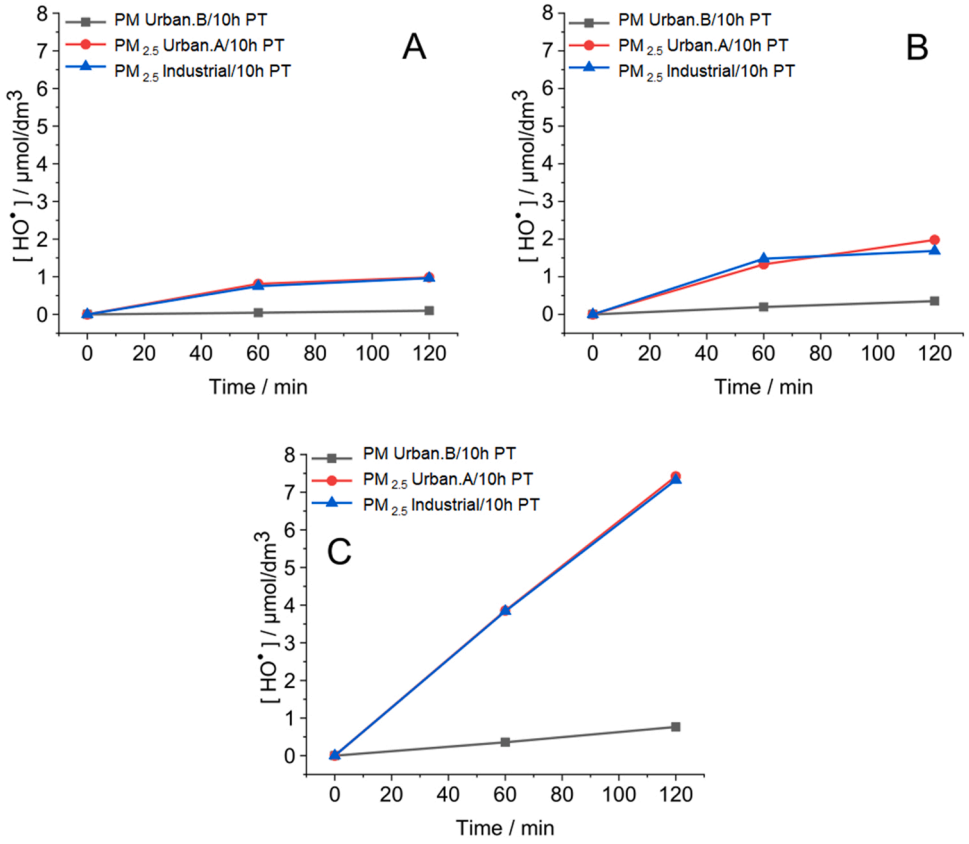

Fig. 3. Hydroxyl radical concentration generated in the presence of PM Urban.B, $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial after 10 h of plasma treatment in the dark (A), during irradiation with UV-Vis (B), and UV-Vis-NIR (C) light.

poor photoactivity compared to other tested samples (Fig. 3C). Differences in $HO^{\bullet}$ generation by these samples can result from differences in sample collection and preparation. $PM_{2.5}$ Urban.A and $PM_{2.5}$ Industrial were collected in very small amounts by the Voivodship Environmental Protection Inspectorate in Kraków for their air pollution analysis. After extraction the dust was not ground. Due to large enough quantities of the PM Urban.B samples, this dust was ground in an agate mortar that resulted in crushing particles into smaller pieces. Organic compounds are easier to remove from smaller particles due to the larger surface area, whereas cold plasma treatment is more efficient in the case of larger surface areas. This could be the reason for the difference in the results, since the carbon content in samples from the VIEP contained much more organic matter than samples from PM Urban.B, which can influence the production of $HO^{\bullet}$.

In the next experiments PM samples collected by our custom sampling system were considered since a sufficient quantity of these samples was available to perform all experiments. The sample was treated with a low temperature oxygen plasma to remove organics from the material. The original carbon content in PM Urban.B was around 43%, after 5 h of plasma treatment it decreased to 15%, whereas after 10 and 15 h of plasma treatment this content further decreased to 9.5% and 6.5%, respectively. Only the original sample was active in the dark, the remaining samples generated negligible amounts of $HO^{\bullet}$ (Fig. 4A). This observation may suggest that organic components are responsible for hydroxyl radical generation as the result of dark reactions. Upon UV-Vis irradiation a high photoactivity was observed only for PM Urban.B before plasma treatment (Fig. 4B). Samples after different times of plasma treatment are similarly photoactive after 60 min irradiation; only after 120 min of irradiation small differences are noticeable. The hydroxyl radical production in the presence of PM Urban.B after 5 h of plasma treatment is a bit higher than in the presence of similar samples treated for 10 and 15 h. Fig. 4C presents the results of experiments involving UV-Vis-NIR irradiation, which are more diverse than the previous ones. Original PM Urban.B sample containing organic matter was still the most photoactive sample, PM Urban.B /5 h PT is the second most photoactive sample, with almost the same efficiency of $HO^{\bullet}$ production as PM Urban.B after 60 min. PM samples after 10 and 15 h of plasma treatment showed the lowest photoactivity in this experiment (under all tested irradiation conditions). It suggests that plasma treatment longer than 10 h does not affect the photoactivity of particulate matter, and the remaining carbon content in these samples can be attributed to soot or different non-oxidizable organic carbon species which cannot be removed by plasma treatment and do not show the photoactivity towards hydroxyl radicals formation.

3.3. Detection of singlet oxygen

The phosphorescence of singlet oxygen ($^1O_2$) was subsequently measured in the presence of all samples of PM collected in Kraków. Among all, the most photoactive sample in the production of singlet oxygen was $PM_{2.5}$ Urban.A, and the next was PM Urban.B collected by our custom designed system, whereas the third sample $PM_{2.5}$ from the industrial area was totally inactive (Fig. 5A). Both photoactive samples were collected at almost the same location in the city center where the main source of air pollution comes from car engines. Afterwards, samples were treated with plasma for 2 h. $^1O_2$ generation decreased only for the $PM_{2.5}$ Urban.A sample, since for PM Urban.B it remained almost at the same level, whereas in the case of $PM_{2.5}$ from the industrial area it increased 7 times (Fig. 5B).

In order to account for these results, PM Urban.B samples were treated with plasma for 2, 5, 10 and 15 h (Fig. 6). Longer plasma treatment leads to a more efficient removal (combustion) of organic components, resulting in a lower efficiency of singlet oxygen production. In this experiment samples after 2 h of plasma treatment generated

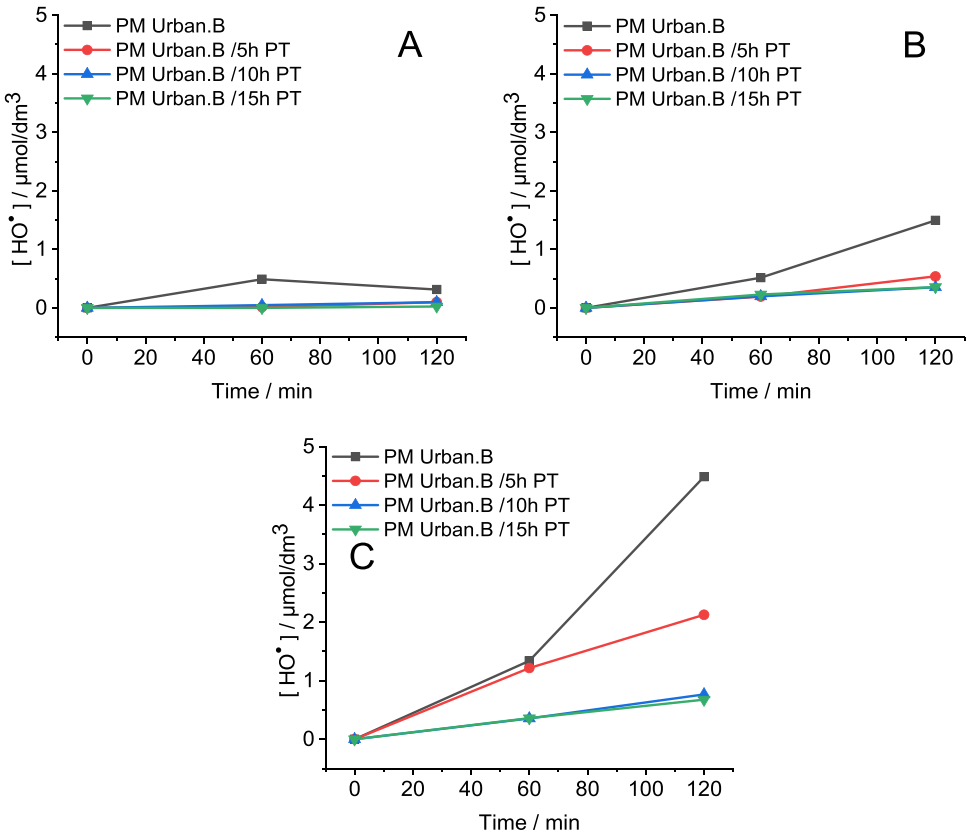

Fig. 4. Hydroxyl radical concentration produced in the presence of PM Urban.B and after 5, 10 and 15 h of plasma treatment in the dark (A), during irradiation with UV-Vis (B), and UV-Vis-NIR (C) light.

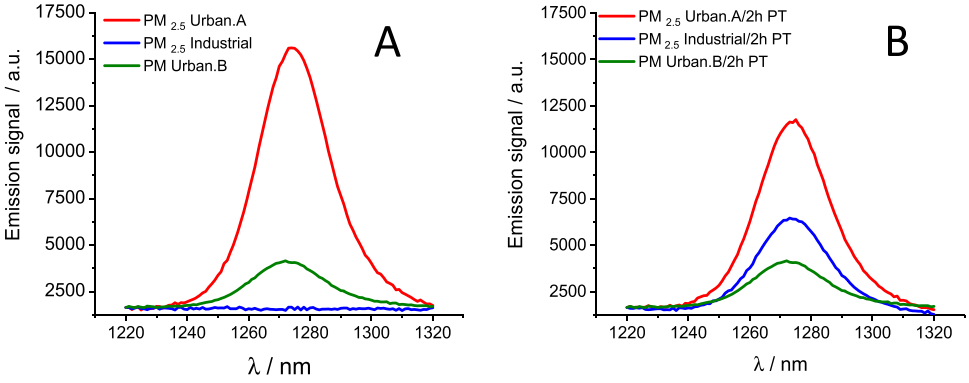

Fig. 5. Phosphorescence of singlet oxygen photo-generated in the presence of $PM_{2.5}$ Urban.A, $PM_{2.5}$ Industrial and PM Urban.B before (A) and after 2 h of plasma treatment (B).

more singlet oxygen than the original PM Urban.B sample. This is consistent with our earlier results where PM collected in Kraków and treated by plasma for 2 h gave a higher phosphorescence signal of singlet oxygen [36]. This is related to the generation of unsaturated organic compounds which intensify the generation of singlet oxygen. A significant decrease in singlet oxygen generation was observed for samples after 5 h of plasma treatment, where the phosphorescence of $^1O_2$ dropped 3 fold. Interestingly, PMs after 10 and 15 h of plasma treatment showed a similar photoactivity in terms of singlet oxygen formation, which proves that 10 h of plasma treatment removes organics that affect ROS photogeneration. This furthermore, proves that a longer plasma treatment does not change the photoactivity of the collected PM samples.

3.4. Reactive oxygen species generation by A549 cells

To evaluate the production of reactive oxygen species upon contact with PM Urban.B, A549 cells were exposed to plates covered with PM. Such a way of seeding was chosen in order to ensure excellent contact between A549 cells and particulate matter without limiting the cells to collect nutrients from the medium [41]. The selected concentrations of PM and way of exposure did not induce significant toxicity in A549 cells. Reactions of fluorescent sensing agents with ROS generated by PM in the absence of cells were performed in order to avoid false positive results that arose from oxidation of SOSG and APF by PM. It was possible to evaluate ROS production by SOSG up to 400 µg/cm^2 of PM and APF only up to 100 µg/cm^2.

24 h of incubation of PM Urban.B induced oxidative stress in A549

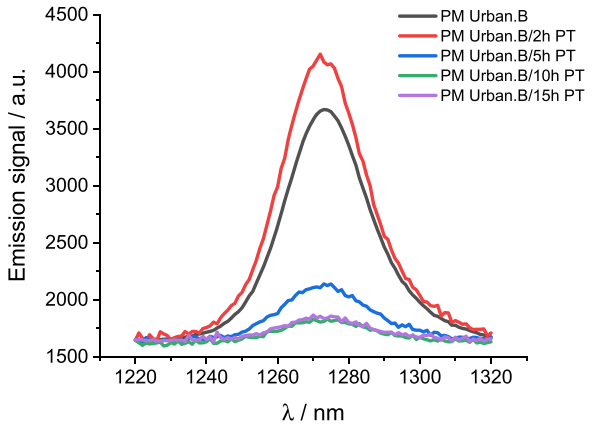

Fig. 6. Phosphorescence of singlet oxygen photo-generated in the presence of PM Urban.B and after 2, 5, 10 and 15 h of plasma treatment.

cells. Fluorescent assays confirmed concentration-dependent production of both singlet oxygen and hydroxyl radicals inside the cells at concentrations higher than 6 μg/cm^2 (Fig. 7). Comparing the results of induced ROS production to a standardized sample of air pollutions (SRM 1648a, NIST, USA) [41] it is evident that PM Urban.B induced a smaller effect on A549 cells, confirming the influence of PM composition on the efficiency of ROS production.

4. Conclusion

Air pollution returns to Kraków every autumn and comprises one of the most important problems for the city. Starting from September 1, 2019 the heating of houses based on coal and wood has been declared as illegal. Due to the confirmed effect of PM on environmental health issues, it is important to understand the impact of PM on oxidative stress mechanisms, especially ROS generation, which heavily affect human health.

PM (including $PM_{2.5}$) was collected on filters, extracted and dried to powders. They form huge agglomerates composed of many small particles. Suspending PM powder in water and ultra-sonication can help to break-up these aggregates. From the analyzes of the morphology of samples from different places in Kraków, it can be concluded that PM with a higher content of organic components has a smoother surface, whereas PM with reduced carbon content has a rougher surface. Moreover, oxidation of organics exposes inorganic components, which become more freely available. In experiments with terephthalic acid, all samples were active in the dark, which may be accounted for by the thermal-activity of PM as described in our earlier work [36]. Upon irradiation with UV-Vis and UV-Vis-NIR light, the photoactivity of all samples was observed, whereby $PM_{2.5}$ from the industrial area appeared to be the most photoactive. After 10 h of plasma treatment, PM Urban.B became the least photoactive sample. This loss of photoactivity is associated with the sample preparation before the experiment, since grinding of the sample decreased the particle size of PM (breaking up agglomerates), increased the available surface area and therefore facilitated oxidation and removal of organics during plasma treatment.

Studies on PM Urban.B after different times of plasma treatment showed that generation of hydroxyl radicals in the dark was observed only for the original PM Urban.B sample. This suggests that the organic part of the PM is responsible for its thermal activity. Upon irradiation within the range of 320–650 nm, the photoactivity decreased with increasing time of plasma treatment, but samples after 10 and 15 h of plasma treatment showed a similar, low efficiency of $HO^{\bullet}$ generation. A similar situation occurred in the case of 320–950 nm irradiation. 10 h of plasma treatment was sufficient to oxidize carbon containing compounds that can be easily removed from the PM. All remaining organic matter apparently does not affect the photoactivity.

In the case of singlet oxygen production, the highest signal was observed in the presence of $PM_{2.5}$ Urban.A, while no signal was detected for $PM_{2.5}$ Industrial. After 2 h of plasma treatment a decrease in singlet oxygen production for $PM_{2.5}$ Urban.A and an increase for $PM_{2.5}$ Industrial were observed. Studies on PM after different times of plasma treatment, led us to conclude that during plasma treatment some of the unsaturated organic compounds can be generated, which intensify the generation of singlet oxygen. After longer treatment times, carbonaceous compounds are oxidized and removed, which leads to a lower efficiency of singlet oxygen generation. Concluding, both thermal and photoinduced generation of reactive oxygen species can be attributed mainly to organic components of urban PM.

In vitro studies using A549 cell line confirmed the production of hydroxyl radicals and singlet oxygen in lung epithelial cells upon contact with the studied PM.

CRediT authorship contribution statement

Magdalena Mikrut: Investigation, Validation, Writing – original draft. **Olga Mazuryk:** Investigation, Writing – original draft. **Wojciech Macyk:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Rudi van Eldik:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Grażyna Stochel:** Conceptualization, Writing – original draft.

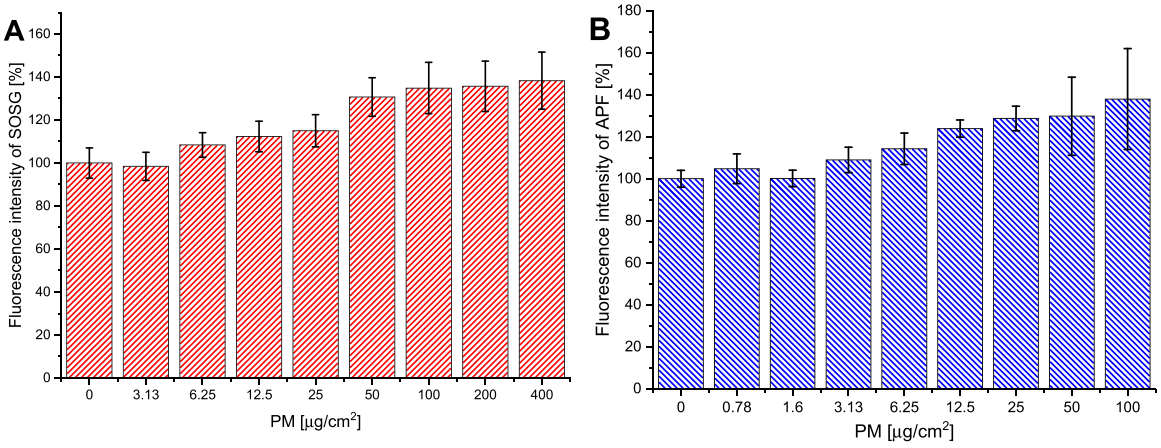

Fig. 7. Production of singlet oxygen (A) and hydroxyl radical (B) in A549 cells after 24 h incubation with different concentrations of PM Urban.B. The results are representative of six independent experiments performed in triplicate and are expressed as mean ± SEM (error bars) of replicates.

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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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2021.106478.

References

[1] Y. Tao, S. Mi, S. Zhou, S. Wang, X. Xie, Air pollution and hospital admissions for respiratory diseases in Lanzhou, China, Environ. Pollut. 185 (2014) 196–201.
[2] R. Lall, K. Ito, G.D. Thurston, Distributed lag analyses of daily hospital admissions and source-apportioned fine particle air pollution, Environ. Health Perspect. 119 (2010) 455–460.
[3] D.W. Dockery, D.Q. Rich, P.G. Goodman, L. Clancy, P. Ohman-Strickland, P. George, T. Kotlov, Effect of air pollution control on mortality and hospital admissions in Ireland, Res. Rep. (Health Eff. Inst.) (2013) 3–109.
[4] M. Guarnieri, J.R. Balmes, Outdoor air pollution and asthma, Lancet 383 (2014) 1581–1592.
[5] Z.J. Andersen, M. Hvidberg, S.S. Jensen, M. Ketzel, S. Loft, M. Sørensen, A. Tjønneland, K. Overvad, O. Raaschou-Nielsen, Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: a cohort study, Am. J. Respir. Crit. Care Med. 183 (2011) 455–461.
[6] S. Li, G. Williams, B. Jalaludin, P. Baker, Panel studies of air pollution on children's lung function and respiratory symptoms: a literature review, J. Asthma 49 (2012) 895–910.
[7] R.D. Brook, S. Rajagopalan, C.A. Pope III, J.R. Brook, A. Bhatnagar, A.V. Diez-Roux, F. Holguin, Y. Hong, R.V. Luepker, M.A. Mittleman, Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association, Circulation 121 (2010) 2331–2378.
[8] D.E. Newby, P.M. Mannucci, G.S. Tell, A.A. Baccarelli, R.D. Brook, K. Donaldson, F. Forastiere, M. Franchini, O.H. Franco, I. Graham, Expert position paper on air pollution and cardiovascular disease, Eur. Heart J. 36 (2014) 83–93.
[9] B.J. Lee, B. Kim, K. Lee, Air pollution exposure and cardiovascular disease, Toxicol. Res. 30 (2014) 71–75.
[10] B.A. Franklin, R. Brook, C.A. Pope III, Air pollution and cardiovascular disease, Curr. Probl. Cardiol. 40 (2015) 207–238.
[11] O. Raaschou-Nielsen, Z.J. Andersen, R. Beelen, E. Samoli, M. Stafoggia, G. Weinmayr, B. Hoffmann, P. Fischer, M.J. Nieuwenhuijsen, B. Brunekreef, Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE), Lancet Oncol. 14 (2013) 813–822.
[12] M. Adam, T. Schikowski, A.E. Carsin, Y. Cai, B. Jacquemin, M. Sanchez, A. Vierkötter, A. Marcon, D. Keidel, D. Sugiri, Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis, Eur. Respir. J. 45 (2015) 38–50.
[13] E.S. Schultz, O. Gruzieva, T. Bellander, M. Bottai, J. Hallberg, I. Kull, M. Svartengren, E. Melén, G. Pershagen, Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study, Am. J. Respir. Crit. Care Med. 186 (2012) 1286–1291.
[14] D. Ierodiakonou, A. Zanobetti, B.A. Coull, S. Melly, D.S. Postma, H.M. Boezen, J. M. Vonk, P.V. Williams, G.G. Shapiro, E.F. McKone, Ambient air pollution, lung function, and airway responsiveness in asthmatic children, J. Allergy Clin. Immunol. 137 (2016) 390–399.
[15] J. Lelieveld, J.S. Evans, M. Fnais, D. Giannadaki, A. Pozzer, The contribution of outdoor air pollution sources to premature mortality on a global scale, Nature 525 (2015) 367–371.
[16] S.D. Ghude, D. Chate, C. Jena, G. Beig, R. Kumar, M. Barth, G. Pfister, S. Fadnavis, P. Pithani, Premature mortality in India due to PM2.5 and ozone exposure, Geophys. Res. Lett. 43 (2016) 4650–4658.
[17] R. Xie, C.E. Sabel, X. Lu, W. Zhu, H. Kan, C.P. Nielsen, H. Wang, Long-term trend and spatial pattern of PM2.5 induced premature mortality in China, Environ. Int. 97 (2016) 180–186.
[18] A. Gawda, G. Majka, B. Nowak, J. Marcinkiewicz, Air pollution, oxidative stress, and exacerbation of autoimmune diseases, Cent. Eur. J. Immunol. 42 (2017) 305–312.
[19] WHO. Health Effects of Particulate Matter. Policy Implications for Countries in Eastern Europe, Caucasus and Central Asia, World Health Organization, 2013.
[20] K.H. Kim, E. Kabir, S. Kabir, A review on the human health impact of airborne particulate matter, Environ. Int. 74 (2015) 136–143.
[21] A. Zwozdziak, I. Sówka, E. Willak-Janc, J. Zwozdziak, K. Kwiecińska, W. Balińska-Miśkiewicz, Influence of PM 1 and PM 2.5 on lung function parameters in healthy schoolchildren—a panel study, Environ. Sci. Pollut. Res. 23 (2016) 23892–23901.
[22] Y. Li, K. Rittenhouse-Olson, W.L. Scheider, L. Mu, Effect of particulate matter air pollution on C-reactive protein: a review of epidemiologic studies, Rev. Environ. Health 27 (2012) 133–149.
[23] C.-H. Lai, C.-N. Lee, K.-J. Bai, Y.-L. Yang, K.-J. Chuang, S.-M. Wu, H.-C. Chuang, Protein oxidation and degradation caused by particulate matter, Sci. Rep. 6 (2016) 33727.
[24] K. Hanzalova, P. Rossner Jr., R.J. Sram, Oxidative damage induced by carcinogenic polycyclic aromatic hydrocarbons and organic extracts from urban air particulate matter, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 696 (2010) 114–121.
[25] X. Liu, Z. Meng, Effects of airborne fine particulate matter on antioxidant capacity and lipid peroxidation in multiple organs of rats, Inhal. Toxicol. 17 (2005) 467–473.
[26] P.S. Vinzents, P. Møller, M. Sørensen, L.E. Knudsen, O. Hertel, F.P. Jensen, B. Schibye, S. Loft, Personal exposure to ultrafine particles and oxidative DNA damage, Environ. Health Perspect. 113 (2005) 1485–1490.
[27] L.M. Gaetke, C.K. Chow, Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology 189 (2003) 147–163.
[28] S.J. Stohs, D. Bagchi, Oxidative mechanisms in the toxicity of metal ions, Free Radic. Biol. Med. 18 (1995) 321–336.
[29] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Oxford University Press, USA, 2015.
[30] S. Hippeli, E.F. Elstner, Transition metal ion-catalyzed oxygen activation during pathogenic processes, FEBS Lett. 443 (1999) 1–7.
[31] M.Y. Chung, R.A. Lazaro, D. Lim, J. Jackson, J. Lyon, D. Rendulic, A.S. Hasson, Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts, Environ. Sci. Technol. 40 (2006) 4880–4886.
[32] Y. Wei, I.-K. Han, M. Hu, M. Shao, J.J. Zhang, X. Tang, Personal exposure to particulate PAHs and anthraquinone and oxidative DNA damages in humans, Chemosphere 81 (2010) 1280–1285.
[33] S.M. Oh, H.R. Kim, Y.J. Park, S.Y. Lee, K.H. Chung, Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells), Mutat. Res. Genet. Toxicol. Environ. Mutagen. 723 (2011) 142–151.
[34] A. Valavanidis, K. Fiotakis, E. Bakeas, T. Vlahogianni, Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter, Redox Rep. 10 (2005) 37–51.
[35] Y. Li, P. Kuppusamy, J.L. Zweier, M.A. Trush, ESR evidence for the generation of reactive oxygen species from the copper-mediated oxidation of the benzene metabolite, hydroquinone: role in DNA damage, Chem. Biol. Interact. 94 (1995) 101–120.
[36] M. Mikrut, A. Regiel-Futyra, W. Macyk, G. Stochel, R. Van Eldik, Generation of hydroxyl radicals and singlet oxygen by particulate matter and its inorganic components, Environ. Pollut. 238 (2018) 638–646.
[37] K.-i. Ishibashi, A. Fujishima, T. Watanabe, K. Hashimoto, Detection of active oxidative species in TiO_2 photocatalysis using the fluorescence technique, Electrochem. Commun. 2 (2000) 207–210.
[38] M. Buchalska, M. Kobielusz, A. Matuszek, M. Pacia, S. Wojtyła, W. Macyk, On oxygen activation at rutile-and anatase-TiO_2, ACS Catal. 5 (2015) 7424–7431.
[39] A. Yang, A. Jedynska, B. Hellack, I. Kooter, G. Hoek, B. Brunekreef, T. A. Kuhlbusch, F.R. Cassee, N.A. Janssen, Measurement of the oxidative potential of PM2.5 and its constituents: the effect of extraction solvent and filter type, Atmos. Environ. 83 (2014) 35–42.
[40] K. Bein, A. Wexler, A high-efficiency, low-bias method for extracting particulate matter from filter and impactor substrates, Atmos. Environ. 90 (2014) 87–95.
[41] O. Mazuryk, G. Stochel, M. Brindell, Variations in reactive oxygen species generation by urban airborne particulate matter in lung epithelial cells — impact of inorganic fraction, Front. Chem. 8 (2020), 581752.