



Characterisation of the correlations between oxidative potential and in vitro biological effects of PM₁₀ at three sites in the central Mediterranean

Maria Rachele Guascito^{a,b}, Maria Giulia Lionetto^a, Franco Mazzotta^c, Marianna Conte^d,
 Maria Elena Giordano^a, Roberto Caricato^a, Anna Rita De Bartolomeo^a, Adelaide Dinoi^b,
 Daniela Cesari^b, Eva Merico^b, Laura Mazzotta^c, Daniele Contini^{b,*}

^a Department of Environmental and Biological Sciences and Technologies (DISTEBA), University of Salento, Lecce 73100, Italy

^b Institute of Atmospheric Sciences and Climate, ISAC-CNR, Str. Prv. Lecce-Monteroni km 1.2, 73100 Lecce, Italy

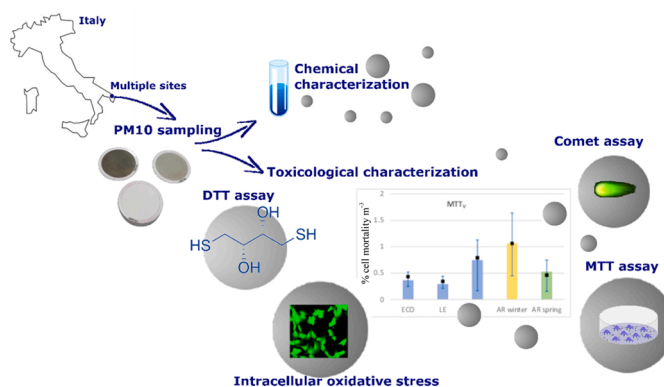
^c Studio Effemme Chimica Applicata, s.r.l. Via Pio XII, 73018 Squinzano, Italy

^d Laboratory for Observations and Analyses of Earth and Climate, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile (ENEA), 00123 Roma, Italy

HIGHLIGHTS

- Acellular and intracellular oxidative stress indicators are strongly influenced by combustion sources.
- Lower-than-average intrinsic toxicity was observed during the advection of African dust.
- Acellular OP^{DTT} may not fully represent the intracellular oxidative stress at all sites and conditions.
- Cytotoxicity correlated with both the OP^{DTT} and the OSGC_V at two sites out of three.
- Genotoxicity obtained via the Comet assay was well correlated with cytotoxicity at all sites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: John D Atkinson

Keywords:

PM₁₀
 Oxidative potential
 Cytotoxicity
 Intracellular oxidative stress
 Genotoxicity
 MTT

ABSTRACT

Atmospheric particulate matter (PM) is one of the major risks for global health. The exact mechanisms of toxicity are still not completely understood leading to contrasting results when different toxicity metrics are compared. In this work, PM₁₀ was collected at three sites for the determination of acellular oxidative potential (OP), intracellular oxidative stress (OSGC), cytotoxicity (MTT assay), and genotoxicity (Comet assay). The in vitro tests were done on the A549 cell line. The objective was to investigate the correlations among acellular and intracellular toxicity indicators, the variability among the sites, and how these correlations were influenced by the main sources by using PMF receptor model coupled with MLR. The OP^{DTT}, OSGC_V, and cytotoxicity were strongly influenced by combustion sources. Advection of African dust led to lower-than-average intrinsic toxicity indicators. OP^{DTT} and OSGC_V showed site-dependent correlations suggesting that acellular OP may not be fully representative of the intracellular oxidative stress at all sites and conditions. Cytotoxicity correlated with both

* Corresponding author.

E-mail address: d.contini@isac.cnr.it (D. Contini).

<https://doi.org/10.1016/j.jhazmat.2023.130872>

Received 3 August 2022; Received in revised form 9 December 2022; Accepted 24 January 2023

Available online 25 January 2023

0304-3894/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

OP_V^{DTT} and $OSGC_V$ at two sites out of three and the strength of the correlation was larger with $OSGC_V$. Genotoxicity was correlated with cytotoxicity at all sites and correlated with both, OP_V^{DTT} and $OSGC_V$, at two sites out of three. Results suggest that several toxicity indicators are useful to gain a global picture of the potential health effects of PM.

1. Introduction

Exposure to atmospheric pollutants has adverse health effects leading to increased health care costs, morbidity, and premature deaths and constituting an economic and societal challenge for policymakers [113, 33,34,5,62,74,88]. Atmospheric pollution, both in outdoor and in indoor environments, is the largest environmental risk to global health [72]. Among the different atmospheric pollutants, particulate matter (PM) is considered one of the leading risk factors for human health globally, potentially causing several million deaths per year [21,61]. PM is a complex mixture of components having large spatial and temporal variabilities of its physical-chemical properties [3]. Different properties of PM could induce several diversified biological responses leading to negative health effects [110]. However, the exact mechanisms of toxicity and the causal relationships between exposure to PM and pathological effects are still not completely understood leading to contrasting results when different toxicological indicators are compared and to uncertainties in the choice of the metrics that could better represent health effects at different sites and conditions [1,107,58,62,97].

Several studies suggested that the oxidative potential (OP) of PM, defined as its ability to generate oxidative stress in biological systems, could be an integrated metric to assess the risks of several health effects induced by PM exposure [109]. This metric could be more effective than the sole PM mass concentration, bringing information on health-relevant components of PM [29,49,51,63,67,96]. This led to the development and use of several acellular approaches to determine the oxidative potential of PM [49]. The most widely used are the DTT (dithiothreitol) assay, mainly sensitive to organic compounds (including water-soluble organic carbon and secondary organic aerosol), hulis, and quinones [17,18,2,6]; the AA (ascorbic acid) assay mainly sensitive to transition metals [69,86,92]; the DCFH (dichlorofluorescein) and the EPR electron paramagnetic resonance methods [6,49]. The recent work, based on data collected in Europe, of Daellenbach et al. [31] showed that the contributions of sources to PM mass concentrations and to OP are significantly different suggesting that mitigation strategies aimed at reducing PM concentration alone could be not efficient in reducing oxidative potential. Thereby, if the oxidative potential is linked to major health effects, it would be advisable to implement reduction strategies aimed at specific sources rather than at the total PM mass concentration.

The link between oxidative stress and health effects remains uncertain [103,105,106,6] and contrasting results were obtained when the acellular determination of OP was compared to intracellular ROS generation or to in vitro and in vivo determination of biological endpoints [64,65,75,97]. Moreover, specific OP detection could not give a clear picture of all ROS and, recently, Jiang et al. [60] put in evidence the importance of taking into account both PM-bound ROS and PM-induced ROS, as well as their interactions. Additional studies are needed to gain further knowledge regarding the association of acellular and intracellular oxidative potential with the outcomes of different in vitro biological effects and how these associations are influenced by PM sources and by the physical and chemical properties of PM.

This study aims to improve knowledge regarding the metrics of toxicity of PM_{10} and the potential relationships between the acellular endpoints and the health-related intracellular outcomes using data collected at three different sites. The intrinsic capacity of PM to oxidize target molecules by generating ROS in presence of oxygen has been associated to some chemical components of PM able to catalyse redox reactions in biological systems (i.e. active redox cycling catalysts, such

as quinones or quinone-type compounds and transition metals). This capacity of PM to oxidize target molecules, defined PM oxidative potential (OP), in this paper was assessed by the acellular DTT assay [20]. PM_{10} induced cytotoxicity, intracellular oxidative stress and genotoxicity were chosen as health-related cellular outcomes for this study. Cytotoxicity was assessed by the MTT test as reduction of cell viability [39,55]. It represents a general cellular toxic outcome, and provides an integrated endpoint of the multiple toxic effects that PM can produce at the cellular level, taking into account the possible synergistic, additive, and antagonistic effects that all the PM components can overall exert on the cells. The intracellular oxidative stress was measured by the ROS-sensitive fluorescent probe CM- H_2 DCFDA in order to assess the PM_{10} induces alteration in the intracellular redox status of the cell which in turn can jeopardize cell functioning and survival [4]. Oxidative stress is known to be a major cause of DNA damage such as base alterations, base loss, and DNA strand breaks [45]. Therefore, in a subset of samples, randomly chosen in the whole sample set, the study was deepened with the measurement of the PM_{10} induced genotoxic effects, assessed by the comet assay, which is the gold standard for the measurement of single/double strand breaks in DNA [22]. Specific objectives were to assess: (i) whether the acellular PM_{10} oxidative potential (measured using the DTT assay) was correlated with intracellular oxidative stress; (ii) whether acellular and intracellular endpoints were correlated with the major components of PM_{10} to gain information regarding the influence of PM_{10} sources on toxicity; (iii) the correlation among acellular and cellular outcomes and if these correlations were site-dependent. Cellular tests were performed on A549 cell line, representative of the Alveolar Type II pneumocytes of the human lung [41], which was often used as a cellular model to estimate adverse health effects of PM [65, 73, 102,111] A549 cell line was exposed to aqueous extracts of sampled PM_{10} for simulating the physiological exposure conditions at the level of respiratory epithelium, where the surface of the respiratory epithelial cells is covered by a thin fluid layer in which inhaled PM_{10} dissolves. There are several studies that use source apportionment to quantitatively investigate the contributions of different PM sources to acellular OP [13,16, 17,71]. However, this approach is used in a limited number of studies for evaluation of the contributions of sources to cellular toxicity outcomes [104,108]. In other studies the influence of specific sources on cellular toxicity indicators was investigated such as road dust [95], biomass burning [98], open burning sources [103], pyrotechnic smokes [32]. In other cases, qualitative indications of the role of sources on cellular toxicity were obtained by correlation with chemical components of PM tracers/indicators of specific sources [57,65,66,97]. To the best of our knowledge, this is the first work dealing with source apportionment of both acellular and cellular toxicity indicators at multiple sites. This approach, together with the analysis of correlations between acellular and intracellular toxicity indicators of PM, gives insights into the role of sources and how they influence site-dependency of correlations among different biological assays providing information useful to better understand toxicity metrics and to plan future mitigation strategies for health-related effects of particle pollution.

2. Methods

2.1. Description of the measurement sites

PM_{10} samples were collected at three different sites located in south-eastern Italy (Fig. S5, supplementary information) during specific measurement campaigns.

The first site was the Environmental-Climate Observatory (ECO) of Lecce (40°20' 8" N; 18°07'28" E), a regional station of the Global Atmosphere Watch (GAW-WMO program), with instruments placed at about 12 m above the ground. It is an urban background site located at the University Campus at about 4.5 km SW of the urban area of Lecce. It is interested by medium and long range transport of dust and pollution, including biomass burning for domestic heating and agricultural activities. The local anthropogenic emissions are limited to activities inside the Campus and vehicular traffic in some roads located nearby [25,36,50].

The second site (LE) had urban characteristics and it was at the centre of the town of Lecce (40°21'22" N; 18°10' 02" E) which has about 100,000 inhabitants. The instruments were located on the roof of an University building at about 14 m above the ground and facing a four-lane road with traffic reaching up to 2400 vehicles h⁻¹ [23; 24]. This site was located at about 4.3 km north-east of the ECO observatory.

The third site (AR) was in the centre of the municipality of Aradeo (40°07'47" N; 18°07'56" E) with about 10,000 inhabitants. The site can be classified as an urban background site potentially influenced by the local urban activities, by biomass burning and agricultural activities, and by the nearby industrial activities with a cement production plant located at about 7.2 km in the north-east direction. Instruments were located on the roof of the city Hall at about 14 m above the ground. AR site was located at 22.9 km south of the ECO observatory and there was no previously available data, in the scientific literature, regarding PM concentrations or composition at this location.

2.2. Sampling campaigns and instruments used

Daily PM₁₀ samples (starting from midnight) were collected simultaneously on three different substrates using different collocated samplers. Specifically, an automatic low-volume sampler at 38.3 L min⁻¹ (Zambelli Explorer Plus) was used to collect samples on PTFE filters (Whatman, 47 mm in diameter); a medium volume sampler operating at 200 L min⁻¹ (TCR-Tecora Echo Hi-Vol) was used to collect PM₁₀ particles on glass fibre and quartz fibre filters (102 mm in diameter); a third low-volume (38.3 L min⁻¹) sampler was used to collect PM₁₀ particles on quartz fibre filters (Whatman, 47 mm in diameter) pre-fired at 700 °C for 2 h before sampling to eliminate eventual contaminations. The third sampler was a dual channel automatic sampler (SWAM, Fai Instruments srl) with determination of concentration through β-ray attenuation at ECO site, and a Bravo HPLUS (TCR Tecora) sampler at LE and AR sites.

The three samplers were collocated, close to each other, at the different sites during the three campaigns. Quartz filters were used for the determination of chemical composition and for acellular DTT assay; PTFE filters were used for in vitro toxicological tests. The first campaign was performed at ECO site between 19/02/2019 and 29/04/2019 (39 sampling days), the second campaign was performed at LE site between 07/06/2019 and 22/08/2019 (30 sampling days), and the third campaign was performed at AR site between 17/12/2019 and 13/06/2020 (47 sampling days).

2.3. Determination of PM₁₀ concentrations and carbon content

Gravimetric determination, according to UNI EN 12341 (2014) method, of PM₁₀ on low-volume quartz and Teflon filters was done by weighing the filters with a microbalance (Sartorius Cubis, model MSx 6.6 S, ± 1 µg resolution). For medium volume filters, a different microbalance (Sartorius BP 211D, ± 10 µg resolution) was used. More details are reported in Section S1 (supplementary information).

Carbon content, separated in organic (OC) and elemental carbon (EC), was determined in each low-volume sampled quartz filter, using a Sunset laboratory carbon analyser (Sunset Laboratory Inc., Tigard OR, USA). The methodology followed was the thermo-optical transmittance (TOT) approach for charring carbon correction with the EUSAAR2 protocol [8]. More details are reported in Section S1 (supplementary

information) and in previous studies done in this area [11,70].

2.4. Determination of chemical composition

Medium volume filters were used for the determination of the main metals and water-soluble ions. Each filter was divided into four quarters, three of them were used for chemical analysis, while the remaining was kept for further replicas.

The first quarter was used for the determination of elemental concentrations via ICP-MS analysis (PerkinElmer NexION 1000 and NexION 300x). Elements quantified were As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Ti, V, and Zn. The second quarter was analyzed for the determination of water-soluble ions (SO₄²⁻, NO₃⁻, K⁺, Cl⁻, Br⁻) using the ion chromatography (ICS1100, Thermo Scientific). The third quarter was analyzed for the determination of ammonium using the UV-VIS spectrometry indophenol method. A Sigma Aldrich 1000 mg L⁻¹ standard solution was used to prepare a calibration together with the Shimadzu UV-1900i spectrophotometer. More details on the methodology used are reported in Section S2 (supplementary information).

Final concentrations were calculated by subtracting values found on field blank filters and a concentration was quantified if, after blank subtraction, was larger than the variability (i.e. standard deviation) of the blanks.

2.5. Determination of oxidative potential

Oxidative potential (OP) was determined using the DTT (Dithiothreitol) acellular assay on the water-soluble fractions of PM₁₀ extracted from ¼ of each low-volume quartz filter. The approach, described in more detail in Section S3 (supplementary information) was the same used in Cho et al. [20]. The strong reducing agent DTT is oxidized to form disulphides when electrons are transferred from DTT to molecular oxygen through the redox reactions accelerated by PM catalytic components (Fig. S1). The rate of DTT consumption is related to the concentration of the catalytically redox-active species in the PM, under conditions of DTT excess, and it was quantitatively determined with spectrophotometric analyses. OP values were calculated by subtracting the average value obtained in field blanks and expressed normalised in volume of sampled air (i.e. OP_V^{DTT}). Uncertainty on OP^{DTT} was determined by performing replicates and, on average, it was ~10%.

2.6. Cell viability measurement by MTT assay

Cell viability was determined using the MTT test on A549 cells, based on the production of formazan crystals that accumulate within healthy cells and are released after the treatment with DMSO [55,94]. The assay measures cellular metabolic activity as an indicator of cell viability. The viable cells contain mitochondrial NAD(P)H-dependent oxidoreductase enzymes which reduce a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan that accumulate within healthy cells. The insoluble formazan crystals are dissolved with DMSO and the resulting coloured solution is spectrophotometrically analysed by measuring absorbance at 570 nm (EON, BioTek Instruments, Winooski, VT, USA). Cell viability inhibition, representing the cytotoxic effect able to reduce the mitochondrial activity of cells, was evaluated in relative terms as the net effect of PM₁₀ (i.e. subtracting field blanks). Six replicates per sample were carried out to assess the uncertainties that ranged between 5% and 10%. More details are reported in Section S4 and Fig. S2 (supplementary information).

2.7. Determination of intracellular oxidative stress

The intracellular oxidative stress (OSGC) was determined using the cell-permeant probe sensitive to reactive species 5-(and-6)-chloromethyl-20,70-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA) (Ex/Em: 492–495/517–527 nm) (Thermo Fisher Scientific,

Waltham, MA, USA). When the probe reaches the intracellular compartment, it loses the acetate group, which is removed by intracellular esterase, and undergoes hydrolysis to form the DCFH carboxylate anion, which is trapped inside the cell. DCFH oxidation by intracellular oxidants species produces the fluorescent product DCF [40]. Fluorescence intensity was measured using the Synergy™ (BioTek Instruments, Inc., Winooski, VT, USA) multi-mode microplate reader. The results were expressed as a relative variation of the fluorescence intensity of the negative control (untreated cells). More details of the methodology are reported in Section S5 and Fig. S3 (supplementary information) and in Giordano et al. [44].

2.8. Genotoxicity assessment via Comet assay

The comet assay was applied to a subset of samples randomly chosen in the datasets. The assay was based on the migration of damaged DNA fragments due to an electric field, while undamaged DNA remains within the confines of the nucleus. DNA damage is assessed by the evaluation of the DNA “comet” tail shape and migration pattern [22,41]. The DNA damage was quantified as the percentage of DNA in the tail, after subtraction of the value measured in cells exposed to aqueous extracts of field blank value (used as control). More details are given in Section S6 and Fig. S4 (supplementary information).

2.9. Statistical analysis and source apportionment

Average, median, standard deviations and inter-quartile range (25th – 75th percentiles) were calculated for the different variables. Statistical significant differences were evaluated using the T-test with a threshold of the p-value set at 5% (p-value 0.05).

The EPA PMF5 (Positive Matrix Factorization) receptor model was applied to identify the number of sources, their chemical profiles, and their contributions to the measured PM₁₀ concentrations. PMF is widely used worldwide for source apportionment of particulate matter being able to operate when limited information on the natural and anthropogenic sources acting at the measurement site are available [7,54]. Considering that the available number of samples at each site is relatively limited, it has been decided to use a single input dataset obtained pooling together the measurements at the different sites to improve robustness of the results. This is an approach already used in previous studies [3,27]. The input variables were classified using both, the Signal-to-Noise (S/N) criteria [76] and the percentage of data above the detection limits [3]. Some constraints were applied on the base solution in order to improve the separation between factors profiles [43] and bootstrap method [77] was used to estimate uncertainty in final PMF results. Additional details on PMF configuration are reported in Section S7 (supplementary information).

A multi-linear regression (MLR) analysis was done between the daily contributions to PM₁₀ of the different sources estimated by the model PMF (i.e. the independent variable) and the daily measured toxicity indicators (i.e. either OP_V^{DTT}, OSGC_V, or MTT_V). This allowed to determine the contribution of each source identified by the PMF to the measured toxicity indicators. The fitting β coefficients (i.e. the slopes) of MLR was obtained using the XLSTAT tool imposing the intercept equal to zero. The slopes represent the intrinsic contributions of each source to the specific toxicological indicator used as independent variable. The approach is the same already used in a previous works [13,66,68].

3. Results and discussion

3.1. Concentration and chemical composition

PM₁₀ concentrations at the three sites and the chemical compositions are compared in Table S1 (supplementary information) in terms of averages and standard deviations. It has to be mentioned that the sampling was not simultaneous so that some differences found could be due to

seasonal changes that are present in this area with typical winter PM₁₀ concentrations larger than those observed in summer [12,15]. In addition, the measurement campaign at AR site was done in 2020 and, in this year, Italy was subjected to a national lockdown and a series of limitations to the movement of people due to COVID-19 pandemic [19,28]. Measurements were not taken during lockdown but interested (partially) the period after lockdown, when the restart of activities was slow and there are some indications that this could have slightly reduced concentrations at least in this region of Italy [37].

The comparison of the urban LE site with the suburban ECO site shows similar PM₁₀ concentrations and this was expected from a previous study on PM_{2.5} [10] but showing a different composition. Specifically, a larger EC concentration and a slightly lower OC was observed at LE site compatible with increased emissions from traffic road in the urban environment. The average OC/EC ratio could give some indication of the role of different combustions sources. Typical values between 3.5 and 9, not strongly depending on particle concentrations, were observed in regional background sites of Europe [9] and of Italy [35,91]. Previous studies indicate that biomass burning emissions are characterised by values of OC/EC higher than those observed in road fuel and in heavy fuel (like that of shipping) combustion emissions [112,14,90]. Average OC/EC ratios were 5.5 at ECO and 4.5 at LE suggesting a larger contribution of road traffic at LE site. Cr, Cu, and Mo were also slightly larger being elements associated to particles from traffic emissions. Instead, the differences observed in nitrate (lower at LE compared to ECO site) and in sulphate (higher at LE compared to ECO) were likely induced by seasonal changes. This because nitrate is characterised by thermal instability and it has, typically, lower concentrations in summer compared to winter and spring, while sulphate has the opposite trend [82,87,26]. The AR site was characterised by the largest PM₁₀ concentrations, due essentially to high values during the winter period (Fig. 1) accompanied by the largest average values of OC and EC, again mainly driven by the winter period. The average OC/EC ratio at AR site was 9.6, significantly (t-Test, p < 0.05) higher than those observed at the other sites suggesting a larger contribution of PM₁₀ originating from biomass burning. This aspect was also supported by the largest concentrations of K⁺, associable with biomass burning, observed at AR site compared to the other sites. Pearson correlation coefficients between OC and EC increase moving from 0.6 at the urban (LE) site to 0.92 (at ECO site) and 0.96 (at AR site). This happened because the urban site was influenced by several sources characterised by different primary OC/EC ratios, instead at the ECO and AR sites, the time series of OC and EC were likely influenced by biomass burning and modulated by meteorology and micrometeorology, increasing in this way the correlation between the two chemical species. This behaviour was observed also in other studies [10,38].

3.2. Acellular and intracellular oxidative potential

The average values and the standard deviations of the acellular OP^{DTT} normalised in volume (OP_V^{DTT}) and in mass (OP_M^{DTT}) were reported in Table 1 together with the values of the intracellular oxidative stress. The latter was reported as % variation of CM-H₂DCFDA fluorescence (indicated as OSGC), normalised in volume (OSGC_V), and in mass (OSGC_M). Fig. 2 compares the statistics of OP_V^{DTT} and OSGC_V at the three sites and for the two seasons at the AR site.

The OP^{DTT} values are compatible with previous observations at the ECO site where seasonal means of OP_V^{DTT} of PM₁₀ between 0.2 (spring) and 0.5 (autumn) nmol min⁻¹ m⁻³ were observed in a previous study [43]. Comparable values were also observed in other sites in southern Italy [13,81,83], while slightly larger mean values have been observed in northern Italy [101,84,85]. Statistically significant differences (t-Test, p < 0.05) were observed between ECO and LE sites and between winter and spring at the AR site, with the mean winter value significantly larger compared to that of spring. Previous study in this area by means of source apportionment [43] and correlation of OP_V^{DTT} with concentration

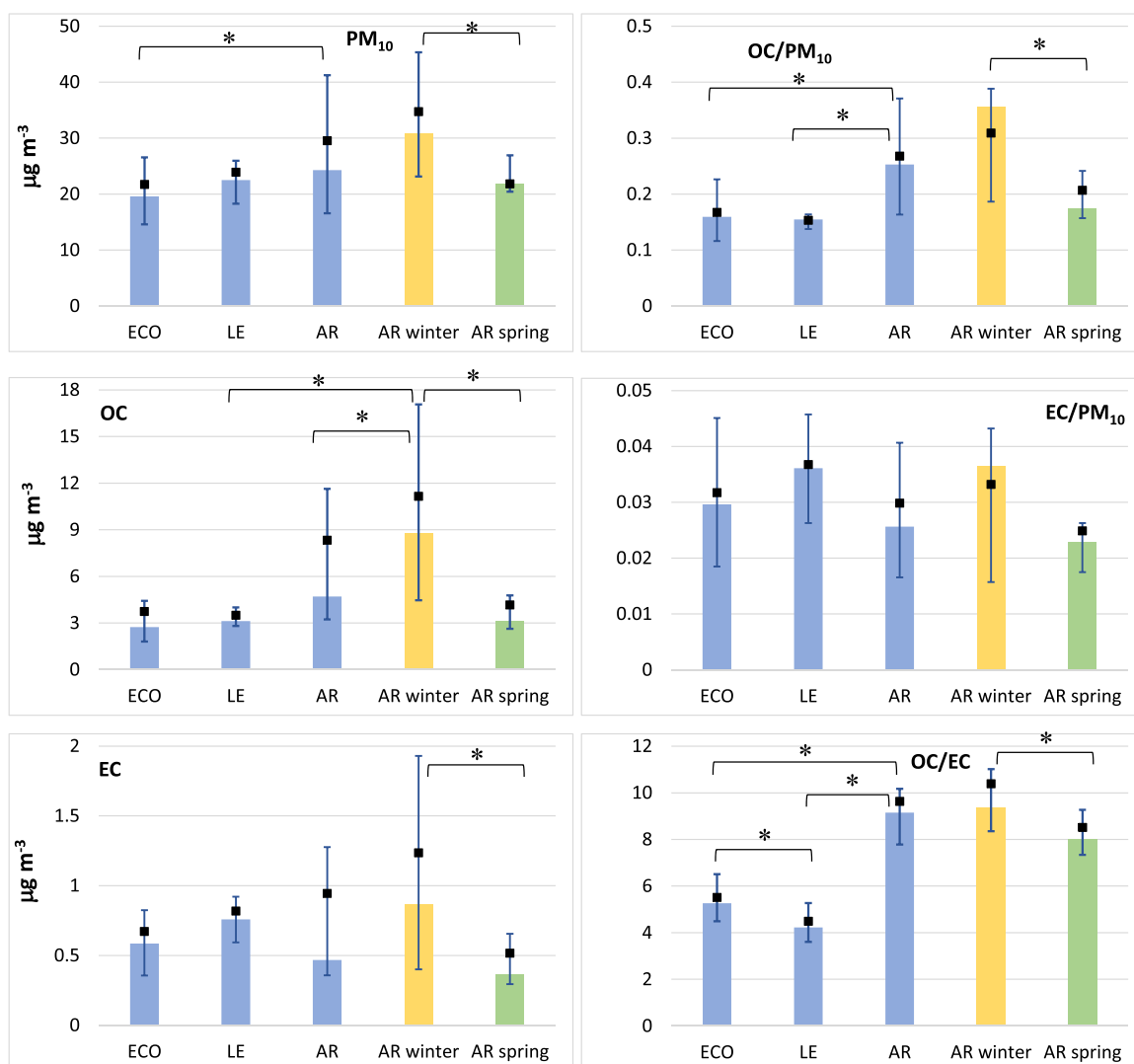


Fig. 1. Median (bars) and average (marks) concentrations of PM_{10} , OC, and EC (left panels). OC/PM_{10} , EC/PM_{10} , and OC/EC ratio are reported (right panels). Error bars represent the interquartile range (25th – 75th percentiles). Data for the AR site are presented also separately for winter and spring periods. (*) Indicates a difference statistically significant ($p < 0.05$).

Table 1

Average and standard deviation (in parenthesis) of the different acellular and in vitro tests at the three sites normalised in volume and in mass.

	ECO	LE	AR
OP_V^{DTT}	0.30	0.24	0.29
$nmol\ min^{-1}\ m^{-3}$	(0.14)	(0.11)	(0.16)
OP_M^{DTT}	14.6	10.1	10.4
$pmol\ min^{-1}\ \mu g^{-1}$	(5.0)	(2.6)	(3.5)
OSGC	30.6	22.8	28.5
% fluor. variability	(16.9)	(13.9)	(28.3)
$OSGC_V$	0.59	0.42	0.53
% fluor. variability m^{-3}	(0.38)	(0.25)	(0.54)
$OSGC_M$	4.41	3.25	3.31
% fluor. var. μg^{-1}	(2.30)	(2.41)	(2.94)
MTT	23.1	18.8	42.2
% cell mortality	(14.7)	(8.8)	(32.9)
MTT_V	0.43	0.34	0.79
% cell mortality m^{-3}	(0.32)	(0.16)	(0.63)
MTT_M	3.48	2.70	5.56
% cell mortality μg^{-1}	(1.87)	(1.69)	(3.99)

of organic tracers in PM [83] suggested that this trend is likely due to the contributions of combustion sources (biomass burning and vehicle exhaust) that are larger during the cold months compared to the warm ones. Results obtained here (Fig. 3) show that intracellular oxidative stress ($OSGC_V$) presents a pattern comparable to the acellular OP_V^{DTT} with a statistically significant difference between ECO and LE sites and a seasonal trend at AR site with a winter mean value larger than the mean value in spring, even if in this case the difference is not statistically significant because of the large standard deviation.

To investigate the role of combustion sources, a correlation analysis was done (Fig. 3) between OP_V^{DTT} and $OSGC_V$ with the content of TC at the three sites. Fig. 3 also shows the correlation of the acellular OP_V^{DTT} with the cellular analysis of ROS ($OSGC_V$). The marks in red represent the days characterised by African dust advection. These cases were identified via the same approach used by Conte et al. [25] using different information: (i) the concentrations of elements associated with crustal dust (Fe, Mn); (ii) the three-day back-trajectories calculated using the NOAA HYSPLIT model [93] for three arrival heights (200, 500, and 1000 m) at the coordinates of the observatory at midnight; (iii) the daily forecast of African dust transport in the BSCDREAM8b model (www.bsc.es); (iv) the satellite images (MODIS) and EARLINET network data (when available). Results for the AR site were presented separately for

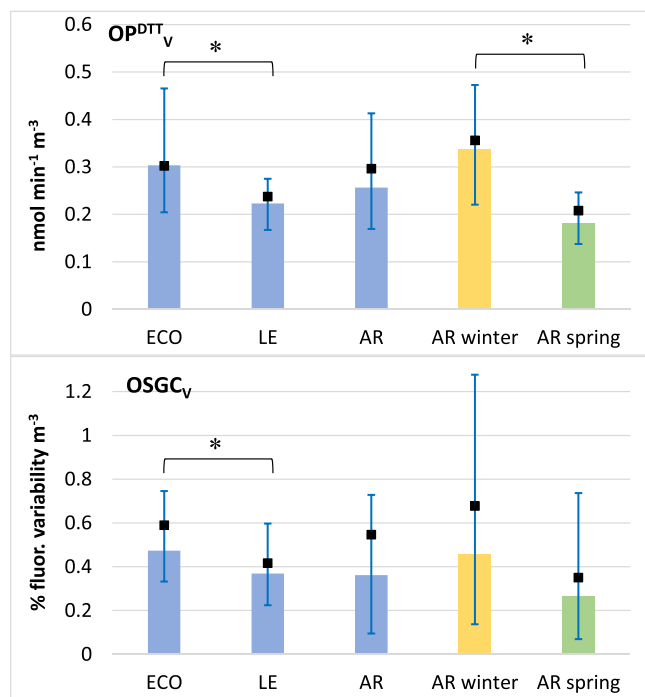


Fig. 2. Median (bars) and average (marks) values of OP_V^{DTT} (top) and $OSGC_V$ (bottom). Error bars represent the interquartile (25th – 75th percentiles) range. Statistics for the AR site are also presented separately for the winter and spring periods. (*) Indicates a difference statistically significant ($p < 0.05$).

the winter (black marks) and spring (green marks) periods.

The OP_V^{DTT} was well correlated with TC at all sites and similar statistically significant correlations were also observed with OC and EC separately (not shown). This is a consequence of the relevant role of combustion sources such as biomass burning and vehicles exhaust to OP_V^{DTT} as observed in source apportionment studies [43]. Park et al. [79] found that higher toxicity is associated with combustion rather than non-combustion aerosol. Correlations of $OSGC_V$ and TC were weaker compared to OP_V^{DTT} and statistically significant only for the ECO and AR sites. At the urban LE site, the simultaneous contributions of different anthropogenic sources and the lack of biomass burning (due to the summer measurement period) led to a negligible correlation. The correlations of OP_V^{DTT} and $OSGC_V$ were site-dependent, being statistically significant at ECO and AR site and negligible at the urban LE site. This suggests that the determination of acellular oxidative potential via DTT assay may not be fully representative of the intracellular oxidative stress at all sites and conditions. Crobeddu et al. [30] found a low but statistically significant correlation between intracellular ROS production (on NCI-H292 cell line) and OP_V^{DTT} induced by urban $PM_{2.5}$ collected in Paris. Tuet et al. [97] found seasonal dependent correlations among the induced intracellular oxidative stress and the OP_V^{DTT} of $PM_{2.5}$ collected at different sites of the greater Atlanta area with negligible correlation in winter and significant correlation in summer. An analysis of oxidative potential and inflammatory impacts in Beijing (China) suggested large differences in the contributions of different PM sources to ROS variability at central and peri-urban sites [66].

The mean intrinsic OP_M^{DTT} during dust advection were $6.7 (\pm 2.5 \text{ dev. st.}) \text{ pmol min}^{-1} \mu\text{g}^{-1}$ and $7.3 (\pm 0.9 \text{ dev. st.}) \text{ pmol min}^{-1} \mu\text{g}^{-1}$ at ECO and AR sites, respectively. These values are lower than the mean values at the sites (Table 1) confirming that African dust has a lower-than-average intrinsic oxidative potential as already observed by Chirizzi et al. [18]. Results here confirm that this holds also for intracellular determination of oxidative stress with mean intrinsic $OSGC_M$ equal to $2.9 (\pm 1.5 \text{ dev. st.}) \%$ fluorescence variability μg^{-1} and $0.67 (\pm 0.74 \text{ dev. st.}) \%$ fluorescence variability μg^{-1} at ECO and AR sites,

respectively.

3.3. Results of cytotoxicity via MTT assay

Cytotoxicity obtained by the MTT assay at the three sites was compared in Table 1 and in Fig. 4 showing statistically significantly larger values at the AR site compared to the other two sites. The mean values at ECO and LE sites were comparable. Cytotoxicity during winter was significantly larger than that during spring at AR site. For comparison, the MTT reduction of viability of A549 cells exposed for 24 h to PM_{10} samples collected at different sites in Catalonia (Spain) ranged from negative values (i.e. no effect) to 34% [89]. MTT reduction activity of RAW264.7 macrophages after 16 h of incubation with $PM_{2.5}$ or $PM_{10-2.5}$ samples reached 60% at different sites in Netherlands [94]. Huang et al. [56] found A549 cells mortality in the range 26.8–46.9% by MTT assay due to $PM_{2.5}$ collected in Guangzhou (China).

Fig. S2 (supplementary information) reports the correlation of MTT_V with PM_{10} and TC measured at the three sites. Correlations with PM_{10} are relatively small at all sites, instead, statistically significant correlations were observed with TC at ECO and AR site and no correlation was observed at the LE site in summer. This suggests that combustion sources play a relevant role in determining cytotoxicity. Advection of African dust leads to samples having lower-than-average cytotoxicity confirming that this natural source not only has low intrinsic oxidative potential but also lower MTT reduction on A549 cells. There are not several studies on the cytotoxicity of the African dust, the results here are in agreement with the analysis done by Faraji et al. [39], that observed that particles related to dust storms caused less toxic effects, as determined by MTT on human peripheral blood mononuclear cells (PMBCs), compared to other high pollution events. Similarly, low intrinsic toxicity was found for Arizona desert dust compared to other sources [79]. Cytotoxicity values (expressed as a relative decrease of viable cells compared to control) were observed for water-soluble PM in the range from 2% to 67% (winter) and from zero (no effect) to 50% (summer) [78]. A similar seasonal trend was observed in Milan (Italy) with larger cytotoxicity during winter [47,48]. Gualtieri et al. [46] showed that cytotoxicity of winter $PM_{2.5}$ in Milan likely derived from its oxidative potential. Instead, cell viability reduction two times higher in summer PM samples compared to winter ones ($27 \pm 5\%$ and $14 \pm 5\%$, respectively) were reported for Milan [80]. Similar seasonality with larger cytotoxicity, and cell mortality up to 60% by MTT assay, induced by PM in summer was observed by Happo et al. [52] for samples collected in Kuopio (Finland). No significant differences in cytotoxicity of water soluble PM_{10} (up to 34%) was observed in Catalonia (Spain) according to the season [89].

Fig. 5 shows the correlation of cytotoxicity with both the OP_V^{DTT} and the $OSGC_V$. Statistically significant correlations were observed at the ECO and AR sites, and a negligible correlation was observed at the urban LE site in summer. At all sites, the strength of the correlation was larger with intracellular oxidative stress compared to acellular oxidative potential. These results suggest that biomass burning and combustion sources likely affect the ability of PM to produce ROS, and are also responsible for decreased mitochondrial functionality. Literature data on the association between in vitro ROS-activity and the in vitro or in vivo toxicity of PM are relatively limited [62,94,99]. Statistically significant correlations between intrinsic DTT redox activity and MTT cytotoxicity of PM were obtained at two sites in Thessaloniki (Greece) with a stronger correlation during the winter period [99]. Steenhof et al. [94] also found statistically significant correlation between OP_V^{DTT} and in vitro toxicity evaluated in murine macrophages (RAW264.7 cells). Garza et al. [42] found a qualitative correlation between A549 cell deaths (by MTT assay) and intracellular ROS production suggesting that cytotoxicity can be indicative of ROS production. Park et al. [79] found a moderate correlation between the overall toxicity score and intrinsic OP_M^{DTT} of particulate matter from different sources collected in Korea. This is because it was found that OP_V^{DTT} varies significantly even with

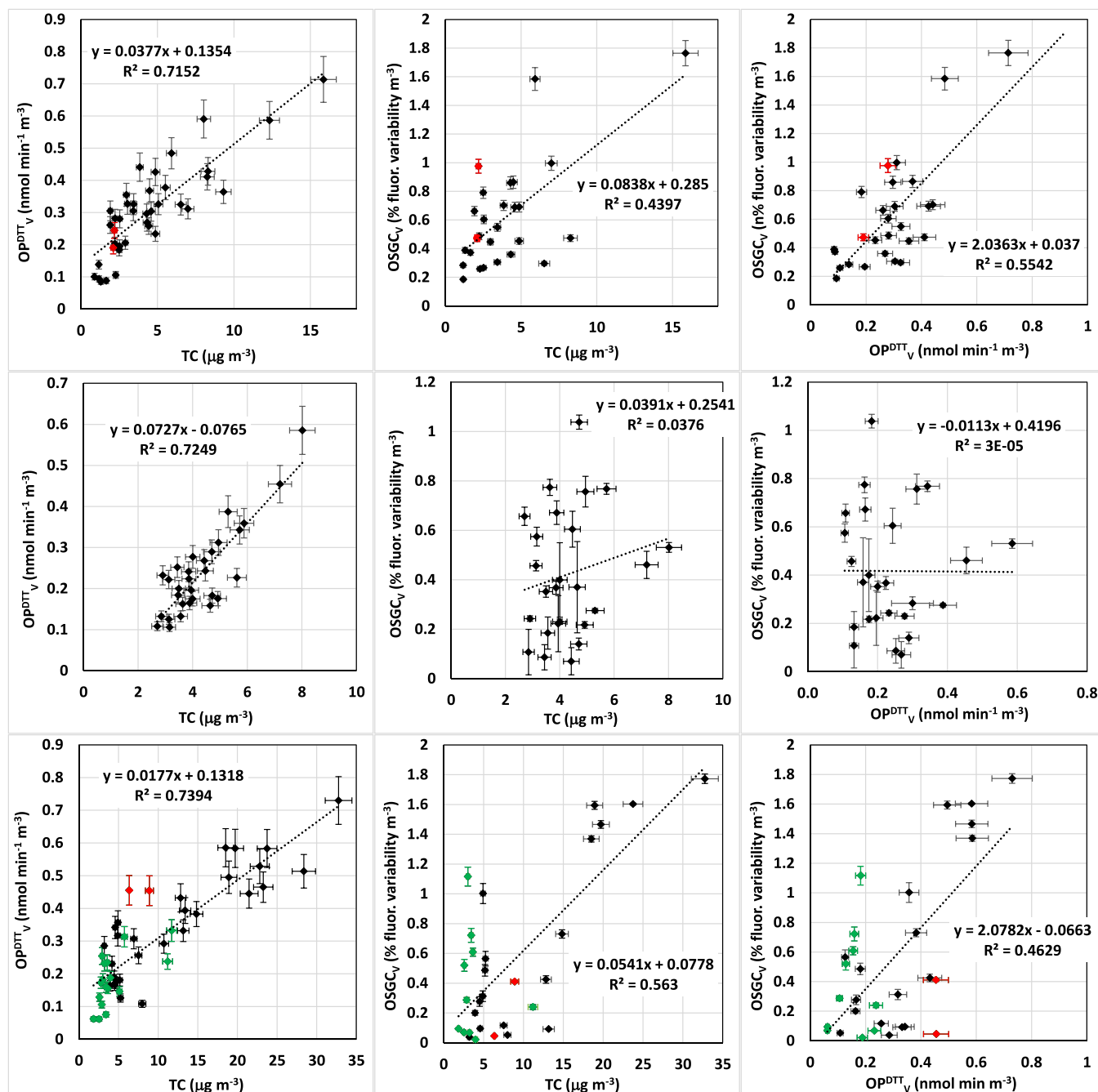


Fig. 3. Correlation between OPV^{DDT} and TC (left), between $OSGC_v$ and TC (centre), and between OPV^{DDT} and $OSGC_v$ (right) at the three sites, from top to bottom: ECO, LE, and AR. Red marks indicate events of African dust advection. Green marks represent data collected during the spring period at the AR site.

similar $PM_{2.5}$ concentrations according to the contributions of different sources.

3.4. Results of genotoxicity via Comet assay

Genotoxicity results gave mean values of 15.2% (st.dev. 15.4%) of DNA in tail at the ECO site, 16.7% (st.dev. 13.0%) at the LE site, and 29.8% (st.dev. 20.0%) at the AR site. For comparison, the genotoxicity found in the aqueous extracts of $PM_{0.49}$ in Thessaloniki (Greece) showed the largest values (45–61% DNA in tail) in wintertime samples from the traffic site as well as by a summertime sample from the urban background site [100]. The values found here are significantly higher than genotoxicity of aqueous extracts of PM_{10} in Catalonia (Spain), which exhibited values below 4% of DNA in tail [89]. The genotoxicity of

size-fractionated samples collected at an urban site in Leeds (UK) showed that fine particulate matter had the greatest ability to produce DNA damage with values ranging from 10% to ~40% of DNA in the tail [53].

Fig. 6 shows the correlation of comet assay results normalised by volume with OPV^{DDT} , $OSGC_v$, and MTT_v . Genotoxicity correlated with cytotoxicity at all sites. Cell viability reduction was also found positively correlated with Comet assay results in Perrone et al. [80]. Velali et al. [99] found a positive correlation even if not statistically significant and no correlation was found in the results of Roig et al. [89]. Genotoxicity was found to correlate with both acellular OPV^{DDT} and intracellular oxidative stress at ECO and AR sites but no significant correlation was observed at the urban site in summer. This suggests that combustions sources such as biomass burning play a relevant role in determining both

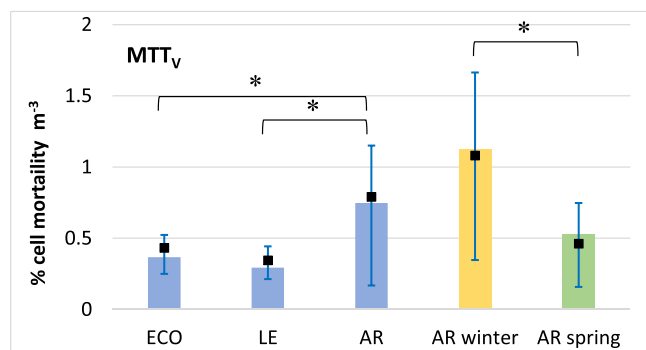


Fig. 4. Median (bars) and average (marks) values of MTT_V . Error bars represent the interquartile (25th – 75th percentiles) range. Statistics for the AR site are presented also separately for winter and spring periods. (*) Indicates a difference statistically significant ($p < 0.05$).

genotoxicity and oxidative stress. The strength of the correlation was comparable for acellular OP and intracellular OSG_{CV} . For comparison, Velali et al. [100] found that wintertime PM from an urban background site in Thessaloniki (Greece) provoked a 1.7-time higher intrinsic DNA damage than wintertime PM from an urban traffic site, suggesting that biomass burning emissions may have a larger ability to induce DNA damage compared to traffic emissions. Jiang et al. [59] found larger genotoxicity of PM_{10} samples collected at Wuhan (China) with percentages of DNA in the tail up to 50% during winter compared to the summer period with a similar seasonality of ROS production.

3.5. Results of source apportionment

The best PMF5 solution was obtained using the six factors shown in Fig. S7 (supplementary information). The first factor was characterised by OC, EC, and K^+ and it was interpreted as the biomass burning source. The second factor was characterised by EC, Cu, Sb, and to a lower extent by EC; this was interpreted as traffic and road dust source. The third factor was characterised by several metals including Cr, Ni, and Pb and it was interpreted as an industrial contribution. A similar factor was already observed in this area in previous work [12]. It is a source giving low contribution ($\sim 1 \mu g m^{-3}$) to measured PM_{10} . The fourth factor, characterised mainly by sulphate and V, was interpreted as secondary sulphate source. The fifth factor was characterised by nitrate and, to a lower extent, by sulphate, chloride, and carbon and it was interpreted as a mixed regional and nitrate source.

The application of MLR allowed to link the sources found with PMF to the different toxicological indicators (i.e. OP_V^{DTT} , OSG_{CV} , and MTT_V). The intrinsic contributions of the different sources is reported in Table S2.

The relative contributions of the different sources to PM_{10} and to the three toxicological indicators at the different sites are reported in Fig. 7. Industrial source gave comparable contributions to PM_{10} and to toxicity indicators at all sites, that were relatively low relatively low coherently with the classifications of the sites that are not significantly influenced by local industrial emissions. However, it is interesting to observe that industrial source had higher contributions to cellular oxidative stress compared to acellular OP_V^{DTT} and to MTT_V at all sites. The opposite was observed for marine source that had negligible contribution to OSG_{CV} but detectable contributions to OP_V^{DTT} and to MTT_V even if lower, in relative terms, compared to the contribution of this source to PM_{10} . Non-negligible contributions of sea spray to OP_V^{DTT} were observed also in other studies [17,43] and it could be due to the presence of biogenic component in marine emissions and to shipping pollution transported with sea salt [16]. Sulphate contributed to the toxicity indicators OP_V^{DTT} and OSG_{CV} less than to the PM_{10} in relative terms and had a negligible contributions MTT_V at all sites. This suggests that this source has a low

intrinsic toxicity, and does not contribute to cytotoxicity, in agreement with the results of Xu et al. (2022). The largest contributions of this source were observed at the LE site during the summer campaign because sulphate formation is favoured compared to nitrate in the warm season [43]. The contribution of biomass burning was observed at all sites with the largest value found at AR site as it was inferred by the analysis of the carbon content of PM_{10} . It is known that this source has strong influence on both acellular OP_V^{DTT} [13], on cellular oxidative stress [98], and on cytotoxicity [99]. The additional information obtained here is that, on average, biomass burning had the largest intrinsic contribution to MTT_V compared to the other toxicity indicators, suggesting that this could be a better toxicity metric to investigate the health-related effects of this source. The opposite seasonality of sulphate and biomass burning sources could induce different seasonal trends on the studied metric as observed also in other studies [57,97].

The regional and nitrate source had the largest contribution to both PM_{10} and toxicity indicators at ECO site and its weight decreased at the two urban sites. This source contributed to toxicity indicators more than to PM_{10} , in relative terms suggesting its relevant role in determining both, the oxidative stress and the cytotoxicity. This could be due to the association of this source with secondary organics and to secondary nitrate likely coming from gaseous precursors emitted by combustion sources [13,104]. Fig. 7 shows that the traffic and road dust contribution was lowest at the ECO site and increased at the two urban sites. It is interesting to observe that this source had comparable contributions to PM_{10} and to toxicity indicators at the ECO and AR sites, instead, at the urban LE site the contribution to toxicity is enhanced compared to that to PM_{10} . This happens for both acellular and intracellular test analysed suggesting that fresh traffic emissions at urban sites could have a greater toxicity potential compared to that observed at background sites. Higher production of intracellular ROS due to road dust at urban sites compared to urban background was observed also in different megacities in China [95]. This, together with the opposite spatial variability of regional and nitrate aerosol, could induces different spatial variabilities of the studied metrics when background and urban sites are compared as observed also in other studies [57, 104].

It is interesting to observe that at ECO and AR sites there is a single dominant source for all the three toxicity indicators: regional and nitrate at ECO and biomass burning at AR and this lead to good correlations among acellular and intracellular toxicity indicators. Instead, at LE site the comparable influences of different sources (traffic and road dust, biomass burning, sulphate) having different intrinsic contributions to the observed metrics lead to weak or negligible correlations.

4. Concluding remarks

The acellular oxidative potential obtained by DTT assay (OP_V^{DTT}), the intracellular oxidative stress (OSG_{CV}) of A549 cell line, and the cytotoxicity estimated by the MTT assay are strongly influenced by combustion sources such as biomass burning and vehicle exhaust. Instead, lower-than-average intrinsic (i.e. normalised in mass) values OP_M^{DTT} , OSG_M , and MTT_M were observed during advection of African dust. The relevant role of combustion sources is the most likely driver of the seasonal trend of the toxicity indicators with higher toxicity and oxidative potential observed during winter compared to the spring period.

The analysis of the correlations of OP_V^{DTT} and OSG_{CV} furnished site-dependent results, being statistically significant at ECO and AR sites and negligible at the urban LE site. This suggests that the oxidative potential measured via the acellular DTT assay may not be fully representative of the intracellular oxidative stress at all sites and conditions.

Cytotoxicity showed a statistically significant correlation with both the OP_V^{DTT} and the intracellular oxidative stress at the ECO and AR sites, however the strength of these correlations was site-dependent with negligible values observed at the urban LE site in summer. At all sites, the strength of the correlation was larger with intracellular oxidative

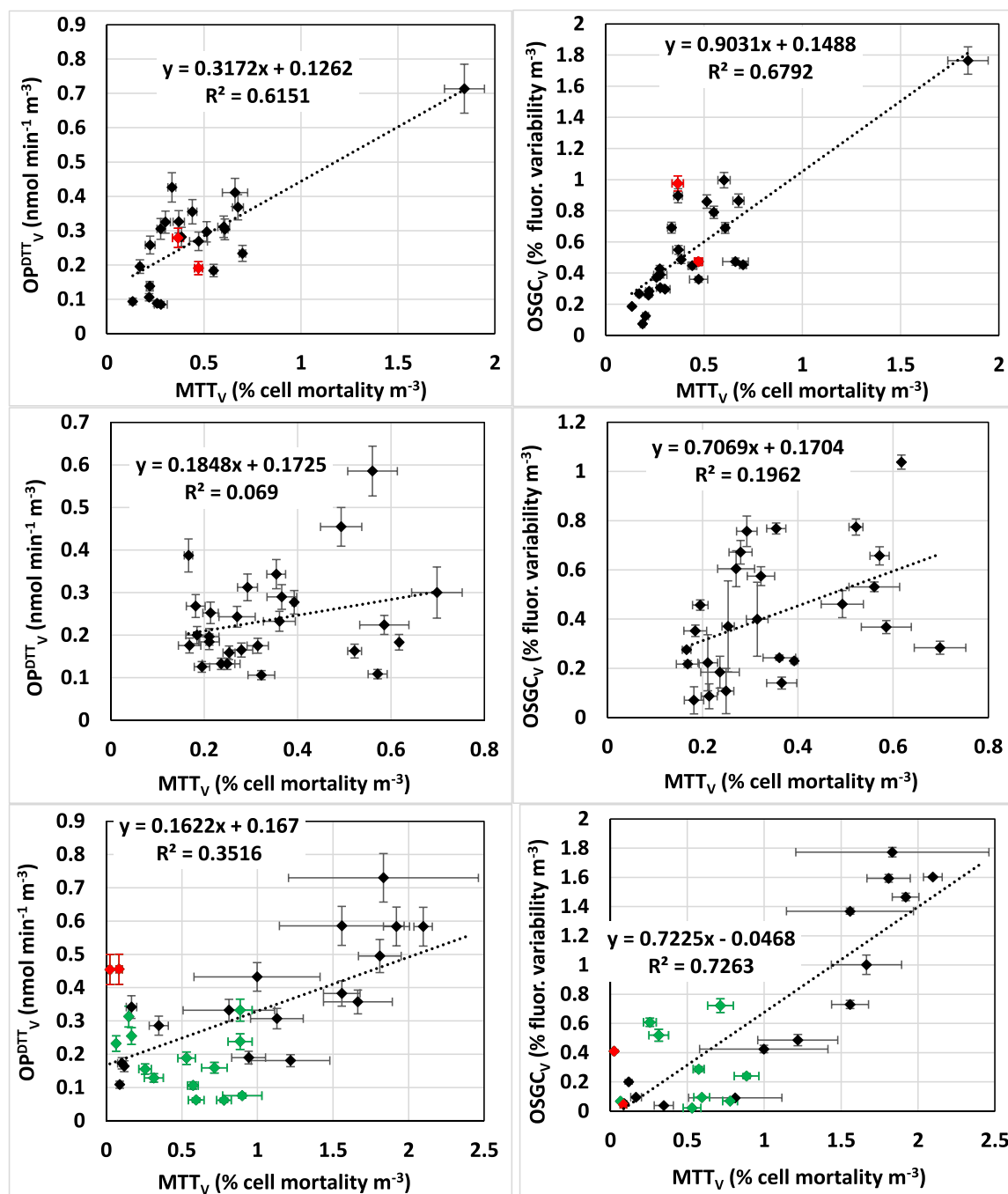


Fig. 5. Correlation between MTT_V and OP_V^{DIT} (left), and between MTT_V and $OSGC_V$ (right) at the three sites, from top to bottom: ECO, LE, and AR. Red marks represent cases with long-range dust advection. Green marks represent cases of measurement in spring 2020 at the end of COVID-19 lockdown period.

stress compared to the acellular oxidative potential.

Genotoxicity obtained via the Comet assay was well correlated with cytotoxicity at all sites suggesting that similar sources influence both Comet and MTT results. Statistically significant correlations of genotoxicity were observed with both acellular oxidative potential and intracellular oxidative stress at the ECO and AR sites, however, a negligible correlation was observed at the urban LE site in summer. This trend was likely due to the influence of the combustion sources, especially biomass burning, that play an important role in both oxidative stress and genotoxicity.

Results of the source apportionment obtained with the PMF-MLR approach showed that biomass burning, traffic and road dust, and regional and nitrate sources have relevant intrinsic contributions to the

three toxicity indicators (OP_V^{DIT} , $OSGC_V$, and MTT_V) with average relative contributions larger than those to PM_{10} . Instead, sulphate and marine sources had larger contribution to PM_{10} compared to the contributions to the different health-related indicators. Marine aerosol did not contribute to intracellular oxidative stress, however, non-negligible contributions were associated to OP_V^{DIT} and MTT_V likely due to the biogenic content and to the shipping emissions transported with sea salt. Sulphate did not contribute to cytotoxicity measured by the MTT assay, however, non-negligible contributions were observed for OP_V^{DIT} and $OSGC_V$. On average, biomass burning had the largest intrinsic contribution to MTT_V compared to the other indicators, being MTT_V a metric more suitable to characterise health impact of this source. Considering that both biomass burning and sulphate have strong and

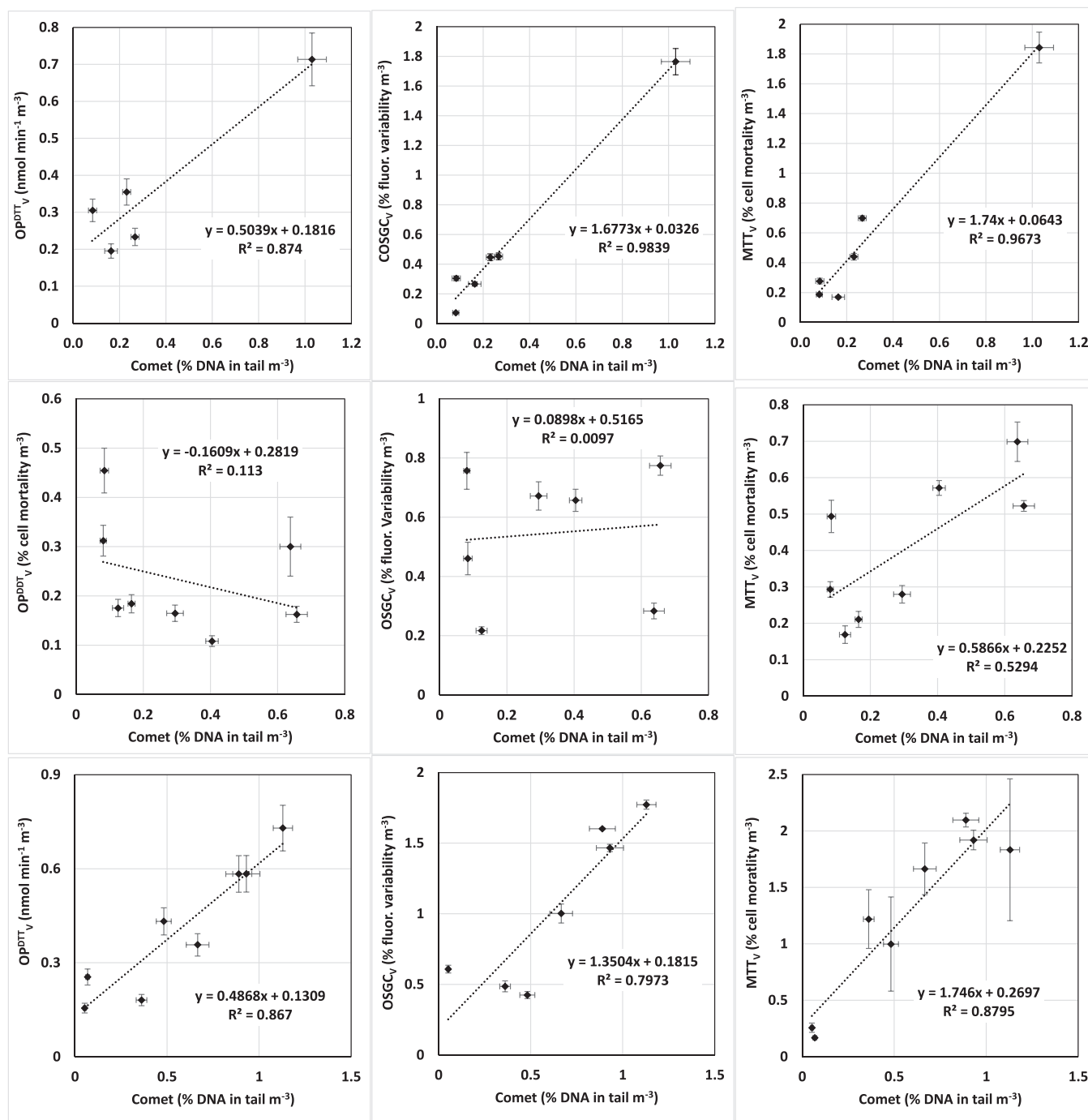


Fig. 6. Correlation between genotoxicity with OPDT_V (left), with OSGC_V (centre), and with MTT_V (right) at the three sites, from top to bottom: ECO, LE, and AR.

opposite seasonality, they could induce different seasonal trends in the different toxicity indicators. Regional and nitrate source, rich in secondary organic aerosol and nitrates, had relevant influence on both acellular and intracellular metrics, however, its role was more evident at the urban background site. Traffic and road dust source influenced all toxicity indicators, however, its intrinsic contribution was more relevant for fresh emissions in the urban area compared to the urban background, thereby having a spatial variability opposite to that of the regional and nitrate source.

In sites where a single source dominated the contributions to the three metrics investigated, such as ECO and AR, a good correlation among acellular and intracellular metrics was observed. Instead, at the urban LE site the comparable influences of different sources (traffic and

road dust, biomass burning, sulphate) having different intrinsic contributions to the observed metrics lead to weak or negligible correlations. Therefore, this study suggests that it could be useful to investigate simultaneously several toxicity indicators to gain a global picture of the potential health effects of atmospheric aerosol at a specific site. It is also advisable to perform further studies and researches, with analysis of additional compounds and longer measurement periods at different sites, to improve the understanding of spatial and seasonal variabilities of toxicity indicators.

Author contributions

D. Contini, M.R. Guascito, M.G. Lionetto, M. Conte conceptualized

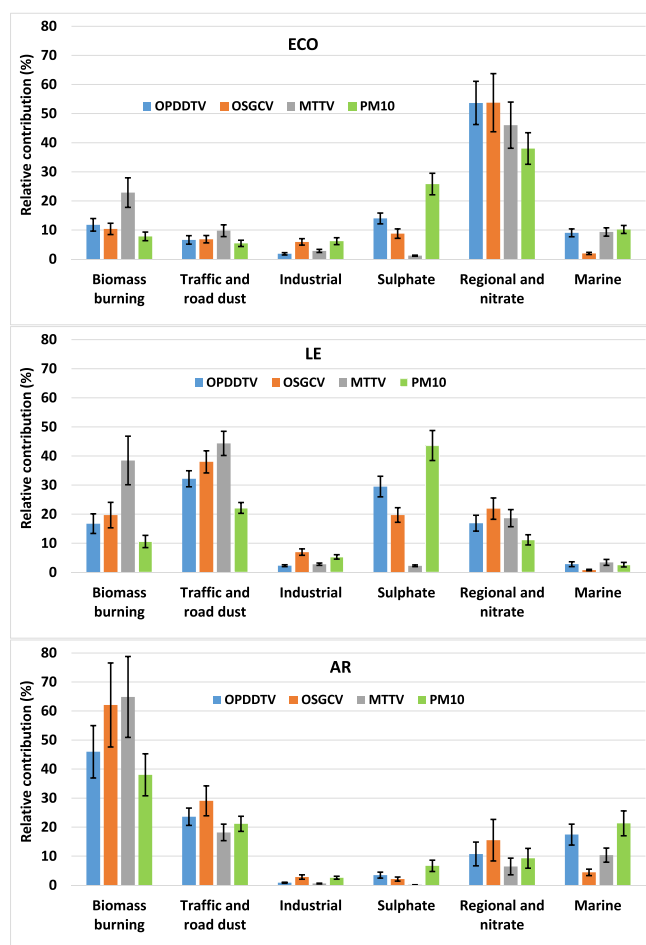


Fig. 7. Average relative contributions of the different source to PM_{10} , $OPDDTV$, $OSGCV$, and $MTTV$ obtained by the PMF-MLR approach at the three sites. Errors bars represent the standard errors.

the study design; D. Cesari, E. Merico, A. Dinoi, and M. Conte collected samples and performed analysis of carbon content; F. Mazzotta and L. Mazzotta performed chemical analysis; A.R. De Bartolomeo performed acellular determination of oxidative potential; M. E. Giordano and R. Caricato performed biological assays. All authors collaborated to the interpretation of results, wrote, read, commented, and approved the final manuscript.

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.

Data Availability

Data will be made available on request.

Acknowledgements

This research was funded by project PAPER (Paper Analyzer for Particulate Exposure Risk), funded within POR Puglia FESR-FSE 2014-2020—Asse prioritario 1—Azione 1.6—Bando Innonetwork—Aiuti a sostegno delle attività di R&S, grant number PH3B166. Authors wish to thank Dr. Franco Papadia (Aradeo Municipality) and Mr. Lucio Perrone (University of Salento) for their help in logistics and for hosting the instruments during the measurement campaigns.

Environmental implication

Exposure to atmospheric pollutants has adverse health effects. Among atmospheric pollutants, particulate matter (PM) is one of the leading environmental risk factors for human health and could be considered hazardous material. The exact mechanisms of toxicity are still not completely understood, and contrasting results were obtained when comparing acellular and intracellular toxicity indicators. This study aims at improving current knowledge on this topic by investigating the correlation among chemical compositions of PM_{10} , its oxidative potential and outcomes of biological tests on exposed A549 cells in three different sites. This will lead to a better understanding of the role of different PM_{10} natural and anthropogenic sources on toxicity and of the site-dependent behaviour of in vitro biological outcomes.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.130872](https://doi.org/10.1016/j.jhazmat.2023.130872).

References

- [1] Ali, M.U., Lin, S., Yousaf, B., Abbas, Q., Ahmed, M., Munir, M., et al., 2020. Pollution characteristics, mechanism of toxicity and health effects of the ultrafine particles in the indoor environment: Current status and future perspectives. *Crit Rev Environ Sci* 52, 436–473.
- [2] Altuwaijiri, A., Pirhadi, M., Kalafy, M., Alharbi, B., Sioutas, C., 2022. Impact of different sources on the oxidative potential of ambient particulate matter PM_{10} in Riyadh, Saudi Arabia: A focus on dust emissions. *Sci. Total Environ.* 806, 150590.
- [3] Amato, F., Alastuey, A., Karanasiou, A., Lucarelli, F., Nava, S., Calzolari, G., et al., 2016. AIRUSE-LIFEC: A harmonized PM speciation and source apportionment in five southern European cities. *Atmos Chem Phys* 16, 3289–3309.
- [4] Ayres, J.G., Borm, P., Cassee, F.R., Castranova, V., Donaldson, K., Ghio, A., et al., 2008. Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—A Workshop report and consensus statement. *Inhal Toxicol* 20, 75–99.
- [5] Basagaña, X., Jacquemin, B., Karanasiou, A., Ostro, B., Querol, X., Agis, D., et al., 2015. Short-term effects of particulate matter constituents on daily hospitalizations and mortality in five South-European cities: Results from the MED-PARTICLES project (on behalf of the MED-PARTICLES Study group). *Environ Int* 75, 151–158.
- [6] Bates, J.T., Fang, T., Verma, V., Zeng, L., Weber, R.J., Tolbert, P.E., et al., 2019. Review of acellular assays of ambient particulate matter oxidative potential: methods and relationships with composition, sources, and health effects. *Environ Sci Technol* 53, 4003–4019.
- [7] Belis, C.A., Pernigotti, D., Pirovano, G., Faveo, O., Jaffrezzo, J.L., Kuenen, J., et al., 2020. Evaluation of receptor and chemical transport models for PM_{10} source apportionment. *Atmos Environ X* 5, 100053.
- [8] Cavalli, F., Viana, M., Yttri, K.E., Genberg, J., Putaud, J.P., 2010. Toward a standardised thermal-optical protocol for measuring atmospheric organic and elemental carbon: the EUSAAR protocol. *Atmos Meas Tech* 3, 79–89.
- [9] Cavalli, F., Alastuey, A., Areskou, H., Ceburnis, D., Cech, J., Genberg, J., et al., 2016. A European aerosol phenomenology—4: harmonized concentrations of carbonaceous aerosol at 10 regional background sites across Europe. *Atmos Environ* 144, 133–145.
- [10] Cesari, D., Donato, A., Conte, M., Merico, E., Giangreco, A., Giangreco, F., et al., 2016. An inter-comparison of $PM_{2.5}$ at urban and urban background sites: chemical characterization and source apportionment. *Atmos Res* 174–175, 106–119.
- [11] Cesari, D., Merico, E., Dinoi, A., Marinoni, A., Bonasoni, P., Contini, D., 2018. Seasonal variability of carbonaceous aerosols in an urban background area in Southern Italy. *Atmos Res* 200, 97–108.
- [12] Cesari, D., Benedetto, De, Bonasoni, G.E., Busetto, P., Dinoi, M., Merico, A., et al., 2018. Seasonal variability of $PM_{2.5}$ and PM_{10} composition and sources in an urban background site in Southern Italy. *Sci Total Environ* 612, 202–213.
- [13] Cesari, D., Merico, E., Grasso, F.M., Decesari, S., Belosi, F., Manarini, F., et al., 2019. Source Apportionment of $PM_{2.5}$ and of its Oxidative Potential in an Industrial Suburban Site in South Italy. *Atmosphere* 10 (12), 758.
- [14] Cesari, D., Merico, E., Dinoi, A., Gambaro, A., Morabito, E., Gregoris, E., et al., 2020. An inter-comparison of size segregated carbonaceous aerosol collected by low-volume impactor in the port-cities of Venice (Italy) and Rijeka (Croatia). *Atmos Pollut Res* 11 (10), 1705–1714.
- [15] Cesari, D., Merico, E., Grasso, F.M., Dinoi, A., Conte, M., Genga, A., et al., 2021. Analysis of the contribution to PM_{10} concentrations of the largest coal-fired power plant of Italy in four different sites. *Atmos Pollut Res* 12 (8), 101135.
- [16] Chen, K., Xu, J., Famiyeh, L., Sun, Y., Ji, D., Xu, H., et al., 2022. Chemical constituents, driving factors, and source apportionment of oxidative potential of

- ambient fine particulate matter in a Port City in East China. *J Hazard Mater* 440, 129864.
- [17] Cheng, Y., Ma, Y., Dong, B., Qiu, X., Hu, D., 2021. Pollutants from primary sources dominate the oxidative potential of water-soluble PM_{2.5} in Hong Kong in terms of dithiothreitol (DTT) consumption and hydroxyl radical production. *J Hazard Mater* 405 (124218).
- [18] Chirizzi, D., Cesari, D., Guascito, M.R., Dinoi, A., Giotta, L., Donato, A., et al., 2017. Influence of Saharan dust outbreaks and carbon content on oxidative potential of water-soluble fractions of PM_{2.5} and PM₁₀. *Atmos Environ* 163 (1–8).
- [19] Chirizzi, D., Conte, M., Feltracco, M., Dinoi, A., Gregoris, E., Barbaro, E., et al., 2021. SARS-CoV-2 concentrations and virus-laden aerosol size distributions in outdoor air in north and south of Italy. *Environ Int* 146, 106255.
- [20] Cho, A.K., Sioutas, C., Miguel, A.H., Kumagai, Y., Schmitz, D.A., Singh, M., et al., 2005. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ Res* 99, 40–47.
- [21] Cohen, A.J., Brauer, M., Burnett, R., Anderson, H.R., Frostad, J., Estep, K., et al., 2017. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet* 389, 1907–1918.
- [22] Collins, A.R., 2003. The Comet Assay—principles, applications and limitations. *Methods Mol Biol* 203, 163–167.
- [23] Conte, M., Donato, A., Contini, D., 2018. Characterisation of particle size distributions and corresponding size-segregated turbulent fluxes simultaneously with CO₂ exchange in an urban area. *Sci Total Environ* 622–623, 1067–1078.
- [24] Conte, M., Contini, D., 2019. Size-resolved particle emission factors of vehicular traffic derived from urban eddy covariance measurements. *Environ Pollut* 251, 830–838.
- [25] Conte, M., Merico, E., Cesari, D., Dinoi, A., Grasso, F.M., Donato, A., et al., 2020. Long-term characterisation of African dust advection in south-eastern Italy: Influence on fine and coarse particle concentrations, size distributions, and carbon content. *Atmos Res* 233, 104690.
- [26] Contini, D., Cesari, D., Genga, A., Siciliano, M., Ielpo, P., Guascito, M.R., et al., 2014. Source apportionment of size-segregated atmospheric particles based on the major water-soluble components in Lecce (Italy). *Sci Total Environ* 472, 248–261.
- [27] Contini, D., Cesari, D., Conte, M., Donato, A., 2016. Application of PMF and CMB receptor models for the evaluation of the contribution of a large coal-fired power plant to PM₁₀ concentrations. *Sci Total Environ* 560–561, 131–140.
- [28] Contini, D., Costabile, F., 2020. Does air pollution influence COVID-19 outbreaks? *Atmosphere* 11, 377.
- [29] Costabile, F., Gualtieri, M., Canepari, S., Tranfo, G., Consales, C., Grollino, M.G., et al., 2019. Evidence of association between aerosol properties and in-vitro cellular oxidative response to PM₁, oxidative potential of PM_{2.5}, a biomarker of RNA oxidation, and its dependency on combustion sources. *Atmos Environ* 213 (444–455).
- [30] Crobeddu, B., Aragao-Santiago, L., Bui, L.C., Boland, S., Squiban, A.B., 2017. Oxidative potential of particulate matter 2.5 as predictive indicator of cellular stress. *Environ Pollut* 230, 125–133.
- [31] Daellenbach, K.R., Uzu, G., Jiang, J., Cassagnes, L.-E., Leni, Z., Vlachou, A., et al., 2020. Sources of particulate-matter air pollution and its oxidative potential in Europe. *Nature* 2020 587, 414–419.
- [32] de Lagarde, V.M., Rogez-Florent, T., Cazier, F., Dewaele, D., Cazier-Dennin, F., Ollivier, A., et al., 2022. Oxidative potential and in vitro toxicity of particles generated by pyrotechnic smokes in human small airway epithelial cells. *Ecotoxicol Environ Saf* 239, 113637.
- [33] Delfino, R.J., Sioutas, C., Malik, S., 2005. Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health. *Environ Health Perspect* 113 (8), 934–946.
- [34] Delfino, R.J., Staimer, N., Tjoa, T., Gillen, D.L., Schauer, J.J., Shafer, M.M., 2013. Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. *J Expo Sci Environ Epidemiol* 23 (5), 466–473.
- [35] Dinoi, A., Cesari, D., Marinoni, A., Bonasoni, P., Riccio, A., Chianese, E., et al., 2017. Inter-comparison of carbon content in PM_{2.5} and PM₁₀ collected at five measurement sites in Southern Italy. *Atmosphere* 8 (12), 243.
- [36] Dinoi, A., Weinhold, K., Wiedensohler, A., Contini, D., 2021. Study of new particle formation events in southern Italy. *Atmos Environ* 244, 117920.
- [37] Dinoi, A., Gulli, D., Ammoscato, I., Calidonna, C.R., Contini, D., 2021. Impact of the coronavirus pandemic lockdown on atmospheric nanoparticle concentrations in two sites of Southern Italy. *Atmosphere* 12 (3), 352.
- [38] Escudero, M., Viana, M., Querol, X., Alastuey, A., Díez Hernández, P., García Dos Santos, S., et al., 2015. Industrial sources of primary and secondary organic aerosols in two urban environments in Spain. *Environ Sci Pollut Res* 22, 10413–10424.
- [39] Faraji, M., Nodehi, R.N., Naddafi, K., Pourpak, Z., Alizadeh, Z., Rezaei, S., et al., 2018. Cytotoxicity of airborne particulate matter (PM₁₀) from dust storm and inversion conditions assessed by MTT assay. *J Air Pollut Health* 3 (3), 135–142.
- [40] Forman, H.J., Augusto, O., Brigelius-Flohe, R., Dennery, P.A., Kalyanaram, B., Ischiropoulos, H., et al., 2015. Even free radicals should follow some rules: a Guide to free radical research terminology and methodology. *Free Radic Biol Med* 78, 233–235.
- [41] Foster, K.A., Oster, C.G., Mayer, M.M., Avery, M.L., Audus, K.L., 1998. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. *Exp Cell Res* 243, 359–366.
- [42] Garza, K.M., Soto, K.F., Murr, L.E., 2008. Cytotoxicity and reactive oxygen species generation from aggregated carbon and carbonaceous nanoparticulate materials. *Int J Nanomed* 3 (1), 83–94.
- [43] Giannossa, L.C., Cesari, D., Merico, E., Dinoi, A., Mangone, A., Guascito, M.R., et al., 2022. Inter-annual variability of source contributions to PM₁₀, PM_{2.5}, and oxidative potential in an urban background site in the central Mediterranean. *J Environ Manag* 319 (115752).
- [44] Giordano, M.E., Caricato, R., Lionetto, M.G., 2020. Concentration dependence of the antioxidant and prooxidant activity of Trolox in HeLa cells: involvement in the induction of apoptotic volume decrease. *Antioxidants* (9), 1058.
- [45] Gonzalez-Hunt, C.P., Wadhwa, M., Sanders, L.H., 2018. DNA damage by oxidative stress: measurement strategies for two genomes. *Curr Opin Toxicol* 7, 87–94.
- [46] Gualtieri, M., Mantecca, P., Corvaja, V., Longhin, E., Perrone, M.G., Bolzacchini, E., et al., 2009. Winter fine particulate matter from Milan induces morphological and functional alterations in human pulmonary epithelial cells (A549). *Toxicol Lett* 188, 52–62.
- [47] Gualtieri, M., Øvrevik, J., Holme, J.A., Perrone, M.G., Bolzacchini, E., Schwarze, P.E., et al., 2010. Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells. *Toxicol Vitro* 24, 29–39.
- [48] Gualtieri, M., Longhin, E., Mattioli, M., Mantecca, P., Tinaglia, V., Mangano, E., et al., 2012. Gene expression profiling of A549 cells exposed to Milan PM_{2.5}. *Toxicol Lett* 209, 136–145.
- [49] Guo, H., Jin, L., Huang, S., 2020. Effect of PM characterization on PM oxidative potential by acellular assays: a review. *Rev Environ Health* 35 (4), 461–470.
- [50] Guascito, M.R., Cesari, D., Chirizzi, D., Genga, A., Contini, D., 2015. XPS surface chemical characterization of atmospheric particles of different sizes. *Atmos Environ* 116, 146–154.
- [51] Guascito, M.R., Pietrogrande, M.C., Decesari, S., Contini, D., 2021. Oxidative potential of atmospheric aerosols. *Atmosphere* 12 (5), 531.
- [52] Happonen, M., Markkanen, A., Markkanen, P., Jalava, P., Kuusipalo, K., Leskinen, A., et al., 2013. Seasonal variation in the toxicological properties of size-segregated indoor and outdoor air particulate matter. *Toxicol Vitro* 27, 1550–1561.
- [53] Healey, K., Lingard, J.J.N., Tomlin, A.S., Hughes, A., White, K.L.M., Wild, C.P., et al., 2005. Genotoxicity of size-fractionated samples of urban particulate matter. *Environ Mol Mutagen* 45, 380–387.
- [54] Hopke, P.K., Dai, Q., Li, L., Feng, Y., 2020. Global review of recent source apportionments for airborne particulate matter. *Sci Total Environ* 740, 140091.
- [55] Hsiao, W.W., Mo, Z.Y., Fang, M., Shi, X.M., Wang, F., 2000. Cytotoxicity of PM_{2.5} and PM_{2.5-10} ambient air pollutants assessed by the MTT and the Comet assays. *Mutat Res Gen Toxicol Environ* 471 (1), 44–55.
- [56] Huang, M., Kang, Y., Wang, W., Chan, C.Y., Wang, X., Wong, M.H., 2015. Potential cytotoxicity of water-soluble fraction of dust and particulate matters and relation to metal(loid)s based on three human cell lines. *Chemosphere* 135, 61–66.
- [57] Jaén, C., Villasclaras, P., Fernández, P., Grimalt, J.O., Udina, M., Bedia, C., et al., 2021. Source apportionment and toxicity of PM in urban, sub-urban, and rural air quality network stations in Catalonia. *Atmosphere* 12 (6), 744.
- [58] Jia, Y.Y., Wang, Q., Liu, T., 2017. Toxicity research of PM_{2.5} compositions in Vitro. *Int J Environ Res Public Health* 14 (232).
- [59] Jiang, L., Dai, H., Sun, Q., Geng, C., Yang, Y., Wu, T., et al., 2011. Ambient particulate matter on DNA damage in HepG2 cells. *Toxicol Ind Health* 27 (1), 87–95.
- [60] Jiang, H., Ahmed, C.M.S., Canchola, A., Chen, Y.J., Lin, Y.H., 2019. Use of dithiothreitol assay to evaluate the oxidative potential of atmospheric aerosols. *Atmosphere* 10, 571.
- [61] Lelieveld, J., Evans, J.S., Fnais, M., Giannadaki, D., Pozzer, A., 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 525, 367.
- [62] Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., et al., 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ Health Perspect* 111, 455–460.
- [63] Li, J., Li, J., Wang, G., Ho, K.F., Han, J., Dai, W., et al., 2022. In-vitro oxidative potential and inflammatory response of ambient PM_{2.5} in a rural region of Northwest China: Association with chemical compositions and source contribution. *Environ Res* 205 (112466).
- [64] Lionetto, M.G., Guascito, M.R., Caricato, R., Giordano, M.E., De Bartolomeo, A.R., Romano, M.P., et al., 2019. Correlation of oxidative potential with ecotoxicological and cytotoxicological potential of PM₁₀ at an urban background site in Italy. *Atmosphere* 10, 733.
- [65] Lionetto, M.G., Guascito, M.R., Giordano, M.E., Caricato, R., De Bartolomeo, A.R., Romano, M.P., et al., 2021. Oxidative potential, cytotoxicity, and intracellular oxidative stress generating capacity of PM₁₀: a case study in South of Italy. *Atmosphere* 12 (4), 464.
- [66] Liu, Q., Baumgartner, J., Zhang, Y., Liu, Y., Sun, Y., Zhang, M., 2014. Oxidative potential and inflammatory impacts of source apportioned ambient air pollution in Beijing. *Environ Sci Technol* 2014 (48), 12920–12929.
- [67] Liu, W., Xu, Y., Liu, W., Liu, Q., Yu, S., Liu, Y., et al., 2018. Oxidative potential of ambient PM_{2.5} in the coastal cities of the Bohai Sea, northern China: Seasonal variation and source apportionment. *Environ Pollut* 236, 514–528.
- [68] Liu, Y., Yang, Z., Liu, Q., Qi, X., Qu, J., Zhang, S., et al., 2021. Study on chemical components and sources of PM_{2.5} during heavy air pollution periods at a suburban site in Beijing of China. *Atmos. Pollut Res* 12, 188–199.
- [69] Massimi, L., Ristorini, M., Simonetti, G., Frezzini, M.A., Astolfi, M.L., Silvia, Canepari, 2020. Spatial mapping and size distribution of oxidative

- potential of particulate matter released by spatially disaggregated sources. *Environ Poll* 266 (3), 115271.
- [70] Merico, E., Cesari, D., Dinoi, A., Gambaro, A., Barbaro, E., Guascito, M.R., et al., 2019. Inter-comparison of carbon content in PM10 and PM2.5 measured with two thermo-optical protocols on samples collected in a Mediterranean site. *Environ Sci Pollut Res* 26, 29334–29350.
- [71] Mousavi, A., Sowlat, M.H., Hasheminassab, S., Polidori, A., Shafer, M.M., Schauer, J.J., et al., 2019. Impact of emissions from the Ports of Los Angeles and Long Beach on the oxidative potential of ambient PM_{0.25} measured across the Los Angeles County. *Sci Total Environ* 651, 638–647.
- [72] Mudway, I.S., Kelly, F.J., Holgate, S.T., 2020. Oxidative stress in air pollution research. *Free Radic Biol Med* 151, 2–6.
- [73] Niu, X., Wang, Y., Ho, S.S.H., Chuang, H.C., Sun, J., Qu, L., et al., 2021. Characterization of organic aerosols in PM1 and their cytotoxicity in an urban roadside area in Hong Kong. *Chemosphere* 263, 128239.
- [74] Ostro, B., Malig, B., Hasheminassab, S., Berger, K., Chang, E., Sioutas, C., 2016. Associations of source-specific fine particulate matter with Emergency Department Visits in California. *Am J Epidemiol* 184, 450–459.
- [75] Ovrevik, J., 2019. Oxidative potential versus biological effects: a review on the relevance of cell-free/abiotic assays as predictors of toxicity from airborne particulate matter. *Int J Mol Sci* 20, 4772.
- [76] Paatero, P., Hopke, P.K., 2003. Discarding or down weighting high-noise variables in factor analytic models. *Anal Chim Acta* 490, 277–289.
- [77] Paatero, P., Eberly, S., Brown, S.G., Norris, G.A., 2014. Methods for estimating uncertainty in factor analytic solutions. *Atmos Meas Tech* 7 (3), 781–797.
- [78] Papachristou, E., Velali, E., Pantazaki, A.A., Choli-Papadopoulou, Th, Besis, A, Tsolakidou, A, Voutsas, D., Samara, C., 2013. The provoked cytotoxicity of airborne particulate matter (PM) of small size on human lung cell line MRC-5. In: 64th Congress Hellenic Society of Biochemistry & Molecular Biology. 6–8, Athens, Greece, Abstracts Vol. 59, p. 101 (P123).
- [79] Park, M., Joo, H.S., Lee, K., Jang, M., Kim, S.D., Kim, I., et al., 2018. Differential toxicities of fine particulate matters from various sources. *Sci Rep* 8, 17007.
- [80] Perrone, M.G., Gualtieri, M., Ferrero, L., Porto, C.L., Udisti, R., Bolzacchini, E., et al., 2010. Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan. *Chemosphere* 78, 1368–1377.
- [81] Perrone, M.R., Bertoli, I., Romano, S., Russo, M., Rispoli, G., Pietrogrande, M.C., 2019. PM2.5 and PM10 oxidative potential at a Central Mediterranean Site: Contrasts between dithiothreitol—And ascorbic acid—Measured values in relation with particle size and chemical composition. *Atmos Environ* 210, 143–155.
- [82] Pey, J., Pérez, N., Castillo, S., Viana, M., Moreno, T., Pandolfi, M., et al., 2009. Geochemistry of regional background aerosols in the Western Mediterranean. *Atmos Res* 94, 422–435.
- [83] Pietrogrande, M.C., Dalpiaz, C., Dell’Anna, R., Lazzeri, P., Manarini, F., Visentin, M., et al., 2018. Chemical composition and oxidative potential of atmospheric coarse particles at an industrial and urban background site in the alpine region of northern Italy. *Atmos Environ* 191, 340–350.
- [84] Pietrogrande, M.C., Bertoli, I., Manarini, F., Russo, M., 2019. Ascorbate assay as a measure of oxidative potential for ambient particles: Evidence for the importance of cell-free surrogate lung fluid composition. *Atmos Environ* 211, 103–112.
- [85] Pietrogrande, M.C., Russo, M., Zagatti, E., 2019. Review of PM oxidative potential measured with acellular assays in urban and rural sites across Italy. *Atmosphere* 10, 626.
- [86] Pietrogrande, M.C., Bertoli, I., Clauser, G., Dalpiaz, C., Dell’Anna, R., Lazzeri, P., et al., 2021. Chemical composition and oxidative potential of atmospheric particles heavily impacted by residential wood burning in the alpine region of northern Italy. *Atmos Environ* 253, 118360.
- [87] Querol, X., Alastuey, A., Moreno, T., Viana, M.M., Castillo, S., Pey, J., et al., 2008. Spatial and temporal variations in airborne particulate matter (PM10 and PM2.5) across Spain 1999–2005. *Atmos Environ* 42, 3964–3979.
- [88] Rivas, I., Vicens, L., Basagaña, X., Tobías, A., Katsouyanni, K., Walton, H., et al., 2021. Associations between sources of particle number and mortality in four European cities. *Environ Int* 155, 106662.
- [89] Roig, N., Sierra, J., Rovira, J., Schuhmacher, M., Domingo, J.L., Nadal, M., 2013. *In vitro* tests to assess toxic effects of airborne PM10 samples. Correlation with metals and chlorinated dioxins and furans. *Sci Total Environ* 443, 791–797.
- [90] Salam, A., Bauer, H., Kassin, K., Ullah, S.M., Puxbaum, H., 2003. Aerosol chemical characteristics of a mega-city in southeast asia (dhaka—Bangladesh). *Atmos Environ* 37 (18), 2517–2528.
- [91] Sandrini, S., Fuzzi, S., Piazzalunga, A., Prati, P., Bonasoni, P., Cavalli, F., et al., 2014. Spatial and seasonal variability of carbonaceous aerosol across Italy. *Atmos Environ* 99, 587–598.
- [92] Shahpoury, P., Zhang, Z.W., Arangio, A., Celo, V., Dabek-Zlotorzynska, E., Harner, T., et al., 2021. The influence of chemical composition, aerosol acidity, and metal dissolution on the oxidative potential of fine particulate matter and redox potential of the lung lining fluid. *Environ Int* 148, 106343.
- [93] Stein, A.F., Draxler, R.R., Rolph, G.D., Stunder, B.J.B., Cohen, M.D., Ngan, F., 2015. NOAA’s HYSPLIT atmospheric transport and dispersion modeling system. *Bull Am Meteorol Soc* 96 (12), 2059–2077.
- [94] Steenhof, M., Gosens, I., Strak, M., Godri, K.J., Hoek, G., Cassee, F.R., et al., 2011. In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential—the RAPTES project. Part Fibre Toxicol 8 (26). <https://doi.org/10.1186/1743-8977-8-26>.
- [95] Sun, J., Yu, J., Shen, Z., Niu, X., Wang, D., Wang, X., et al., 2021. Oxidative stress-inducing effects of various urban PM2.5 road dust on human lung epithelial cells among 10 Chinese megacities. *Ecotoxicol Environ Saf* 224 (112680).
- [96] Traina, G., Bolzacchini, E., Bonini, M., Contini, D., Mantecca, P., Caimmi, S.M.E., et al., 2022. Role of air pollutants mediated oxidative stress in respiratory Diseases. *Pediatr Allergy Immunol* 33 (Suppl. 27), 38–40.
- [97] Tuet, W.Y., Fok, S., Verma, V., Rodriguez, M.S.T., Grosberg, A., Champion, J.A., et al., 2016. Dose-dependent intracellular reactive oxygen and nitrogen species (ROS/RNS) production from particulate matter exposure: comparison to oxidative potential and chemical composition. *Atmos Environ* 144, 335–344.
- [98] Tuet, W.Y., Liu, F., de Oliveira Alves, N., Fok, S., Artaxo, P., Vasconcellos, P., et al., 2019. *Environ Sci Technol Lett* 6 (3), 126–132.
- [99] Velali, E., Papachristou, E., Pantazaki, A., Choli-Papadopoulou, T., Planou, S., Kouras, A., et al., 2016. Redox activity and in vitro bioactivity of the water-soluble fraction of urban particulate matter in relation to particle size and chemical composition. *Environ Pollut* 208, 774–786.
- [100] Velali, E., Papachristou, E., Pantazaki, A., Choli-Papadopoulou, T., Argyrou, N., Tsourouktsoglou, T., et al., 2016. Cytotoxicity and genotoxicity induced in vitro by solvent-extractable organic matter of size-segregated urban particulate matter. *Environ Pollut* 218, 1350–1362.
- [101] Visentin, M., Pagnoni, A., Sarti, E., Pietrogrande, M.C., 2016. Urban PM2.5 oxidative potential: Importance of chemical species and comparison of two spectrophotometric cell-free assays. *Environ Pollut* 219, 72–79.
- [102] Wang, B., Li, K., Jin, W., Lu, Y., Zhang, Y., Shen, G., et al., 2013. Properties and inflammatory effects of various size fractions of ambient particulate matter from Beijing on A549 and J774A.1 cells. *Environ Sci Technol* 47, 10583–10590.
- [103] Wang, J., Niu, X., Sun, J., Zhang, Y., Zhang, T., Shen, Z., et al., 2020. Source profiles of PM2.5 emitted from four typical open burning sources and its cytotoxicity to vascular smooth muscle cells. *Sci Total Environ* 715 (136949).
- [104] Wang, Y., Puthussery, J.V., Yu, H., Liu, Y., Salana, S., Verma, V., 2022. Sources of cellular oxidative potential of water-soluble fine ambient particulate matter in the Midwestern United States. *J Hazard Mater* 425, 127777.
- [105] Weichenthal, S., Lavigne, E., Evans, G., Pollitt, K., Burnett, R.T., 2016. Ambient PM 2.5 and risk of emergency room visits for myocardial infarction: impact of regional PM 2.5 oxidative potential: a case-crossover study. *J Environ Health* 15, 46.
- [106] Xiao, G.G., Wang, M., Li, N., Loo, J.A., Nel, A.E., 2003. Use of proteomics to demonstrate a hierarchical oxidative stress response to diesel exhaust particle chemicals in a macrophage cell line. *J Biol Chem* 278, 50781–50790.
- [107] Xie, W., You, J., Zhi, C., Li, L., 2021. The toxicity of ambient fine particulate matter (PM2.5) to vascular endothelial cells. *J Appl Toxicol* 41, 713–723.
- [108] Xu, F., Shi, X., Qiu, X., Jiang, X., Yanhua, Fang, Junxia, Wang, et al., 2020. Investigation of the chemical components of ambient fine particulate matter (PM2.5) associated with in vitro cellular responses to oxidative stress and inflammation. *Environ Int* 136 (105475).
- [109] Yang, A., Janssen, N.A., Brunekreef, B., Cassee, F.R., Hoek, G., Gehring, U., 2016. Children’s respiratory health and oxidative potential of PM2.5: The PIAMA birth cohort study. *J Occup Environ Med* 73, 154–160.
- [110] Yang, Z., Liu, Q., Liu, Y., Qi, X., Wang, X., 2021. Cell cycle arrest of human bronchial epithelial cells modulated by differences in chemical components of particulate matter. *RSC Adv* 11, 10582.
- [111] Yi, S., Zhang, F., Qu, F., Ding, W., 2012. Water-insoluble fraction of airborne particulate matter (PM10) induces oxidative stress in human lung epithelial A549 cells. *Environ Toxicol* 29, 226–233.
- [112] Zhang, N., Cao, J., Liu, S., Zhao, Z.Z., Xu, H., Xiao, S., 2014. Chemical composition and sources of PM2.5 and TSP collected at Qinghai Lake during summertime. *Atmos Res* 138 (213–222.).
- [113] Zhou, J., Ito, K., Lall, R., Lippmann, M., Thurston, G., 2011. Time-series analysis of mortality effects of fine particulate matter components in Detroit and Seattle. *Environ Health Perspect* 119, 461–466.