Urban $PM_{2.5}$ oxidative potential: Importance of chemical species and comparison of two spectrophotometric cell-free assays☆

Marco Visentin

Antonella Pagnoni

Elena Sarti

Maria Chiara Pietrogrande*

mpc@unife.it

Department of Chemical and Pharmaceutical Sciences, Via Fossato di Mortara 17/19, 44121 Ferrara, Italy

*Corresponding author.

☆This paper has been recommended for acceptance by David Carpenter.

Abstract

Oxidative potential (OP) of particulate matter (PM) − defined as the capacity of PM to oxidize target molecules generating reactive oxygen species (ROS) − has been proposed as a more health relevant metric than PM mass. In this study two cell-free methods were used to assess the OP of PM filters collected at an urban site and to evaluate correlation with PM mass and PM composition.

Among the different assays existing, two inexpensive and user-friendly methods were used both based on spectrophotometric measurements of depletion rate of target reagents oxidized by redox-active species present in PM. One assay measures the consumption of dithiothreitol (OP_{DTT}) and the other the ascorbate (OP_{AA}).

Although both assays respond to the same redox-active species, i.e., quinones and transition metals, no correlations were found between OP_{DTT} and OP_{AA} responses to compounds standard solutions as well as to ambient samples. When expressed in relation to air volume, OP_{DTT} m^{-3} strongly correlates with $PM_{2.5}$ mass whereas no correlation was found for OP_{AA} m^{-3} with $PM_{2.5.}$ When expressed on mass basis, both OP_{DTT} μg^{-1} and OP_{AA} μg^{-1} show a strong dependence on the sample composition, with higher OP for summer samples. OP_{DTT} m^{-3} were highly correlated with the determined metals (Cu, Zn, Cr, Fe, Ni, Mn) whereas OP_{AA} m^{-3} showed only moderate correlation with Cu and Mn.

Thus, the two assays could potentially provide complementary information on oxidative potential characteristic of PM. Consequently, the combination of the two approaches can strengthen each other in giving insight into the contribution of chemical composition to oxidative properties of PM, which can subsequently be used to study health effects.

Inexpensive cell-free methods assessed the oxidative potential of particulate matter collected at an urban site and the results were correlated with chemical composition.

Keywords: Oxidative stress; Reactive oxygen species; Dithiothreitol; Ascorbic acid; Particulate matter

1 Introduction

Numerous studies have linked exposure to airborne particulate matter to a wide range of adverse health end points, including, but not limited to, cardiovascular diseases, respiratory problems, and adverse neurodevelopmental effects [Delfino et al., 2005, Ghio and Madden, 2012, Lodovici and Bigagli 2011, MohanKumar et al., 2008].

In most cases, effects were linked to PM mass concentration whereas evidences indicate that PM sources and constituents are more closely linked to the induction of toxic responses [Borm et al., 2007]. In fact, much of the ambient particle mass consists of low toxicity components such as chlorides, sulphates and nitrates, while relatively tiny masses of transition metals and organic species may make a major contribution in worsening human health [Mudway et al., 2011].

The mechanisms of PM related to health effects are still incompletely understood but an emerging hypothesis is that such toxic effects are mediated by inflammatory responses originated from PM-induced oxidative activity, leading to the generation of reactive oxygen species upon the interaction of PM with epithelial cells and macrophages [Squadrito et al., 2001]. Oxidative stress results when the generation of ROS, or free radicals, exceeds the available antioxidant defences [Janssen et al., 2014]. Inflammation is initially a protective mechanism which removes the injurious stimuli and produces ROS able to induce cell killing. In the early phase of inflammation, oxidant stress does not directly cause cell damage and can induce the transcription of stress defence genes including antioxidant genes. This preconditioning effect of ROS enhances the resistance against future inflammatory oxidant stress and promotes the initiation of tissue repair processes. The additional release of cell contents amplifies the inflammatory process and consequently can induce tissue injury [Kelly 2003].

ROS are families of compounds containing oxygen radicals and/or strong non-radical oxidative agents, including hydroxyl ($^{\cdot}OH$), hydroperoxyl ($^{\cdot}HO_2$), superoxide ($^{\cdot-}O_2$), organic peroxy radicals and hydrogen peroxide (H_2O_2). Free radicals are potentially very dangerous since they can react indiscriminately with neighbouring molecules. Within the affected cells, ROS are formed through the reduction of oxygen by biological reducing agents such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). These agents react with the redox-active chemical species with the catalytic assistance from electron transfer reactions [Dellinger et al., 2001, Squadrito et al., 2001]. This process has the potential to both deplete antioxidant species in the cell and generate reactive oxygen species, contributing to induction of oxidative stress.

Furthermore, it has been found that ROS are present in the atmosphere on respirable particles to which we are exposed [Hasson and Paulson 2003, Venkatachari et al., 2007].

The overall oxidative capacity of PM has been proposed as a metric that is more closely related to biological responses to particles exposure and thus could be more informative than PM mass alone [Borm et al., 2007]. Oxidative potential is an attractive measure because it integrates various biologically relevant properties, including size, surface and chemical composition.

A few chemical components of ambient PM have been identified to catalyze redox reactions in biological systems, as active redox cycling catalysts, such as quinones or quinone-type compounds and transition metals (e.g., Fe, Mn, Cu, V, and Ni) [Charrier and Anastasio 2012, Kelly 2003, Lin and Yu 2011].

Several methods to provide the rapid read out of the oxidative potential have been developed. The only analytical method that provides direct quantification of radical species in the samples is electron spin resonance (ESR). However, ESR is an expensive and complicated instrument which has low sensitivity due to low steady state concentration and short radical's lifetimes [Hedayat et al., 2014]. Other assays are based on the capacity of PM suspensions to oxidize target antioxidants selected to simulate the respiratory tract lining fluids (RTLFs), which represent the first physical interface encountered by inhaled materials. The original method used an antioxidant mixture containing ascorbate (vitamin C), urate and reduced glutathione that were found present in RTLFs at high concentrations [Ayres et al., 2008, Zielinski et al., 1999]. A simplified version based on an only ascorbate solution has also been developed [Mudway et al., 2011, Janssen et al., 2014, Hedayat et al., 2014]. Another common assay is based on dithiothreitol (DTT), a strong reducing agent that simulates cellular reducing species in the biological systems [Charrier and Anastasio 2012, Cho et al., 2005, Janssen et al., 2014, Yang et al., 2014].

Some studies make use of fluorescent (2′,7′-dichlorofluorescin diacetate, profluorescent nitroxide probes, dihydrorhodamine) or chemiluminescent (acridinium ester) reagents which emit after chemical reactions with ROS [Hedayat et al., 2014, Yang et al., 2014].

This study is focused on two cell-free assays commonly used to assess the oxidative capacity of $PM_{2.5}$ samples: dithiothreitol and ascorbic acid assays. In both cases, the redox-active species present in PM oxidize the reagents and the oxidative potential is determined as the rate of reagent depletion measured with spectrophotometric techniques as user-friendly, direct and inexpensive tools. Most of the current literature indicates that the two assays respond differently to the various redox-active species. In particular, the DTT assay is known to be strongly sensitive to organic species, such as polycyclic aromatic hydrocarbons (PAHs) and quinones [Charrier and Anastasio 2012, Cho et al., 2005, Chung et al., 2006, Li et al., 2009], and only recently, it was found by some authors sensitive also to laboratory solutions of transition metal ions, such as Cu (II) and Zn (II), [Charrier and Anastasio 2012, Lin and Yu 2011]. It is well known that the presence of transition metals promotes oxidation reaction of ascorbic acid [Ayres et al., 2008, Buettner and Jurkiewicz 1996, Xu and Jordan 1990], but also quinones have been discovered able to oxidize ascorbic acid [Mudway et al., 2011, Roginski et al., 1999].

Given the limited comparative information on the different methods to measure OP, the primary objective of this study was to compare the two assays by measuring their responses to standard solutions of some quinones, PAHs, oxo-PAHs and metals commonly present in environmental particulate matter. The aim was to highlight different sensitivity to the ROS generating compounds and better understand the effects of redox-active chemical species in ambient PM.

The second step of the current study was to assess the oxidative potential of real-world urban $PM_{2.5}$ samples collected in Northern Italy that is recognized as one of the most worrying air pollution situations in Europe, where high anthropogenic emissions and meteorological factors may cause air pollution episodes and serious risks for human health [Pietrogrande et al., 2016]. The relationship of OP with the PM mass and

chemical composition was investigated.

Although some field studies have investigated OP from different locations, i.e., Germany, the Netherlands, Los Angeles [Cho et al., 2005, Mudway et al., 2011, Saffari et al., 2015, Verma et al., 2015], at our knowledge only one study has been recently published concerning Northern Italy [Perrone et al., 2016]. In addition, most of the papers have generally focused on a specific procedure to evaluate OP, while only few compared different measurement methods.

2 Materials and methods

2.1 Standards and reagents

15 compounds representative of different chemical classes of catalytically redox-active species were considered for this study: 4 quinones–namely 9,10-phenantrenequinone (9,10-PNQ), 1,2-naphthoquinone (1,2-NPQ), 1,4-naphthoquinone (1,4-NPQ) and anthraquinone−3 PAHs (naphthalene, phenanthrene and anthracene), 2 oxo-PAH (1,8-naphthalic anhydride and xanthone) and 6 transition metals, i.e., copper (II), manganese (II), Nickel (II), chromium (III), zinc (II) and iron (III).

Individual standard stock solutions were prepared for each analyte by weighting pure standards (Acros Organics, Sigma Aldrich, Dr. Ehrenstorfer, Carlo Erba Reagenti) at a concentration of 10^{-2} M using acetonitrile (for quinones, PAHs and oxo-PAHs) or MilliQ water for metal ions as solvent. The solutions were stored in amber glass vials in the dark at −20 °C.

DTT solution was prepared at a concentration of 10 mM in a 0.1 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4) at pH 7.4. AA solution was made at the same concentration as DTT in MilliQ water. Aqueous solutions of the reagents are unstable at room temperature and DTT solution is also sensible to light, thus they were preserved in amber glass vials in the dark and at −20 °C.

To reduce the background oxidation of DTT and AA in the blank, the phosphate buffer was treated with a cation exchange resin (Chelex 100 resin, sodium form, Sigma Aldrich) to remove trace metals, mainly transition metals, which are commonly, recognize as redox-active species. The resin was poured into an acid-rinsed glass chromatography column that had a permanent glass frit to contain the Chelex. The phosphate solution was allowed to drip through the resin at 4 °C and the resulting treated phosphate buffer was collected into a clean, acid washed, Teflon (PTFE) bottle [Charrier and Anastasio 2012]. Chelex-resin treated buffer has been used in all the experimental work.

2.2 DTT assay

Dithiothreitol is a strong reducing agent which forms six membered ring with an internal disulphide bond when oxidized. The assay is based on a two-step reaction. In the first step, redox active compounds oxidize DTT to its disulphide form which donates an electron to dissolved molecular oxygen, forming superoxide anion [Kumagai et al., 2002]. Superoxides can subsequently comproportionates to hydrogen peroxide and oxygen. Such a redox cycle catalysed by redox-active species (Fig. 1a) is similar to cycles that occur in living cells [Li et al., 2009]. The rate of DTT-disulphide formation is proportional to the concentration of redox-active species in the sample when DTT is added in excess in the reaction environment [Cho et al., 2005]. In the second step, the remaining DTT is reacted with DTNB (5,5′-Dithiobis(2-nitrobenzoic acid), Ellman's Reagent) to generate DTT-disulphide and 2-nitro-5-thiobenzoic (TNB) that is the “coloured” species measured spectrophotometrically, with a strong absorbance in the Vis region (Fig. 1b).

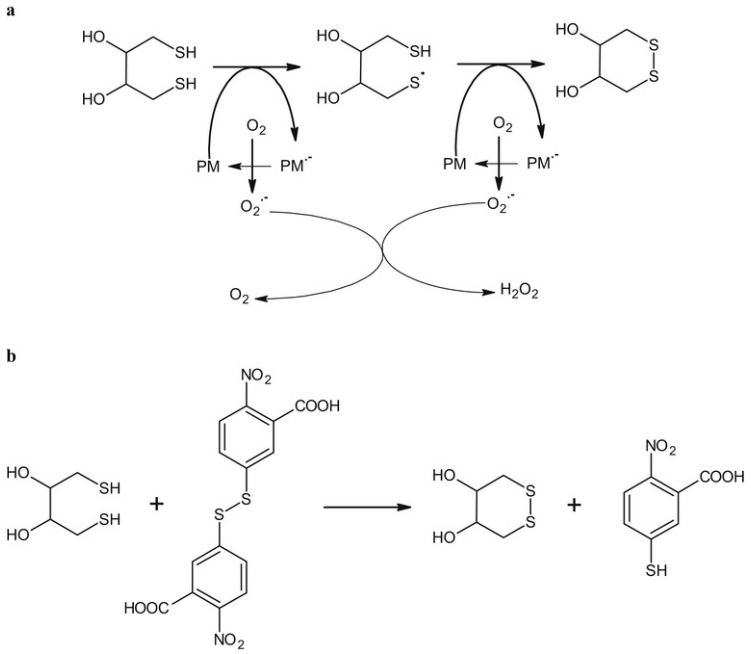


Fig. 1 a) Dithiothreitol (DTT) oxidation by redox active species in PM with subsequently ROS formation. b) DTT reaction with 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB).

alt-text: Fig. 1

The experimental procedure proposed by Charrier and Anastasio (2012) was followed. Briefly, a small volume (<30 µl) of stock solution of the compound of interest was introduced into an amber vial containing 3 mL~~l~~ of phosphate buffer at pH 7.4 and the obtained solution was heated at 37 °C using a dry bath. Once the temperature reached the desired value, 30 µl of the DTT solution 10 mM were added to the vial, i.e., time zero. At defined times, a 0.50 mL aliquot of the reaction mixture was removed and added to 0.50 mL of 10% trichloroacetic acid to cease the reaction. After that all solutions at fixed time points were collected, 50 µL of a 10.0 mM DTNB solution in phosphate buffer at pH 7.4 were added, well mixed, and allowed to react for 2 min. Then 2.0 mL of 0.40 M Tris-HCl buffer at pH 8.9 with 20 mM of EDTA were added. It is important to increase pH value because the protonated form of TNB (Fig. 1b) shows only a slight absorbance in contrast with the mercaptide ion (TNB~~2-~~ ~~2-~~, thiol group pKa = 4.53 at 25 °C) which has a higher absorbance (ε = 14,150~~150~~ M^{-1} cm^{-1} at 412 nm) [Li et al., 2009]. DTNB was added before the Tris buffer, as suggested by Charrier, to ensure that the sample remains quenched until DTT has reacted with DTNB, even if the reaction between DTNB and DTT is very fast [Charrier and Anastasio 2012, Li et al., 2009].

The reactions were performed at pH 7.4 and 37 °C to simulate biological conditions that normally occur in human body. All the reactions were performed in dark vials covered with aluminum foil and stored in the dark when not in use, since both DTT and TNB are sensitive to light.

TNB was quantified in a UV-~~-~~Vis spectrophotometer (Varian Cary 50) using a 1 cm path length optical PS (polystyrene) cell.

2.3 AA assay

The AA assay uses ascorbic acid as a simplified model of the synthetic respiratory tract lining fluids: ascorbic acid is oxidized to dehydroascorbic acid while redox active species in PM are reduced. These reduced species can then transfer an electron to oxygen molecules promoting the formation of ROS (Fig. 2) [Mudway et al., 2011, Zielinski et al., 1999].

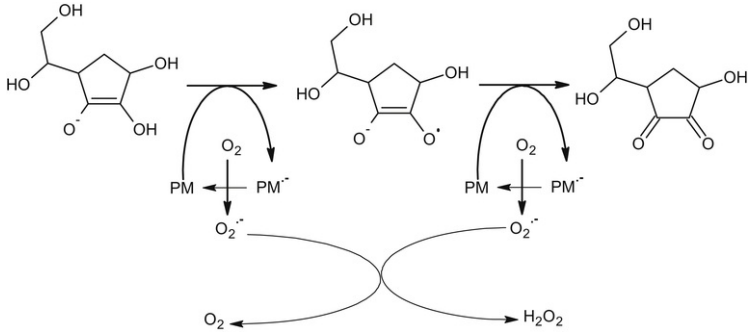


Fig. 2 Ascorbic acid (AA) oxidation by redox active species in PM with subsequently ROS formation.

alt-text: Fig. 2

AA assay was performed by adapting to a classical spectrophotometer the literature protocols that use 96 well plates equipped with a plate reader [Janssen et al., 2014, Mudway et al., 2011, Yang et al., 2014]. In our case, the reaction occurred directly into the spectrophotometric cuvette. Briefly, an aliquot of the stock solutions of analytes was diluted into 3 mL of phosphate buffer and incubated in the spectrophotometric quartz cuvette at 37 °C. After adding 30 µl of ascorbic acid solution 10 mM, the absorption was measured at 265 nm at known time intervals. At pH 7.4 the vitamin C is present almost totally as ascorbate ion that has a molar extinction coefficient of about 14,500 M^{-1} cm^{-1} at the considered wavelength [Buettner and Jurkiewicz 1996].

2.4 Analysis of ambient particulate matter

2.4.1 Filter storage and extraction

Samples of $PM_{2.5}$ were collected onto quartz filter (Whatman, 47 mm diameter, low volume sampler, ≈55 m^3 day^{-1}) in different seasons from an urban site in Bologna, a city of about 400,000 inhabitants in Northern Italy. Additional information about sampling can be found in Pietrogrande et al. (2016). The $PM_{2.5}$ filters were stored in a freezer (−20 °C) in the dark prior to use.

A quarter of the filter was extracted for 15 min in an ultrasonic bath using 10 mL of the Chelex treated phosphate buffer 0.1 M at pH 7.4. The extract was then filtered on a regenerate cellulose syringe filter (13 mm, 0.22 µm, Kinesis) to remove the suspended solid particles. Then, 3 mL of the solution were submitted to each analysis.

2.4.2 Chemical analysis for transition metals

$PM_{2.5}$ quartz filters were submitted to chemical analysis for water soluble metals that may play a role in ROS generation (Cu, Mn, Ni, Cr, Zn and Fe).

Briefly, one half of the filter was extracted in 10 mL of Chelex treated phosphate buffer; the solution was then filtered on regenerated cellulose syringe filter and finally submitted to the analysis.

The determinations were performed by Graphite Furnace Atomic Absorption Spectroscopy, GF-AAS (Perkin-Elmer Analyst 800) equipped with Zeeman background correction and hollow cathode lamps (Lumina lamp, Perkin-Elmer) radiation source for iron (wavelength 248.3 nm), manganese (wavelength 279.5 nm), copper (wavelength 324.8 nm), zinc (wavelength 213.9 nm), chromium (wavelength 357.9 nm) and nickel (wavelength 232.0 nm).

The aqueous standard solutions for calibration curves were prepared by dilution of monoelement solutions certified to 1000 ± 2 mg l^{-1} in 2% HNO_3 of Mn, Fe, Zn, Ni, Cr (Fluka, Trace Cert) and Cu (Carlo Erba). $Mg(NO_3)_2$ and Pd (Atomic Absorption Modifier Solution, Perkin-Elmer) were added as modifiers for Fe, Mn, Cu and Cr determination. When necessary, samples were diluted with ultrapure water to obtain concentrations within the linear range of responses.

Aliquots of 20 µL of standard solutions and sample extracts were injected into the graphite tube at room temperature, then two steps of drying were performed: the first ramped for 10 s–100 °C and held for 30 s, the second ramped for 15 s–130 °C and held for 30 s. After drying, the pyrolysis and atomization were operated at specific temperatures determined for each element under study. Finally, the graphite tube was cleaned by raising the temperature from atomization value to cleaning temperature in 1 s and held for 3 s; the internal argon gas flow rate was 250 mL min^{-1}.

Three readings were taken for each calibration standard and samples.

The detection limit of each analyzed element was calculated by using Statistical procedures reported in “IUPAC Compendium of Analytical Nomenclature (Definitive Rules, 1997) [Inczedy et al., 1998]”.

2.5 Data analysis

The depletion rates of DTT or AA were determined by linear fitting of five experimental points of the reagents concentration versus time (5, 10, 15, 25, 40 min) plot. In general, good linearity was found over the entire experiment duration, with the exception of few experiments where the last point of the kinetic study (40 min) was rejected due to the linearity loss.

For both methods, the reagents blank was determined by performing the assays on a 100 μM reagents (DTT or AA) solution in buffer. Similarly, blank filter response was determined by measuring the depletion rates of DTT or AA on the extract of a quarter of blank quartz filters. Sample and blank assays were run in duplicate. The average reagent blank and filter blank responses were subtracted from the depletion rates of standard solutions and real PM samples, respectively.

The results were expressed as μM min^{-1} of DTT and AA depletion, i.e., OP_{DTT} and OP_{AA}, respectively. Regarding real samples, reagents depletion rates were converted into relative quantities related to sampled air volume (nmol of DTT or AA min^{-1} m^{-3}) or to the collected PM mass (nmol of DTT or AA min^{-1} μg^{-1}). OP expressed on air volume basis is the primary metric of toxicological interest since it represents the amount of inhaled air and thus the effective human exposure to oxidative stress.

3 Results and discussion

3.1 Assays of individual organic species and transition metals

3.1.1 DTT assay

The first goal of this study was to identify species that mostly contribute to DTT oxidation in PM samples. The test was applied to 15 individual chemical compounds commonly present in ambient PM, i.e., quinones, PAHs, oxo-PAHs and dissolved transition metals [Connell et al., 2006, Li et al., 2009, Walgraeve et al., 2010]. (Table 1). The obtained data show that most of the tested quinones elicit oxidative effect, since the measured depletion rates were much higher than the reagent blanks. In particular, 9,10-PNQ is the most reactive species (depletion rate = 3.2 μM min^{-1} for the 0.17 μM diluted solution), followed by 1,2-NPQ and 1,4-NPQ (depletion rate = 5.2 and 2.2 μM min^{-1}, respectively). In contrast, anthraquinone showed only a very slight redox activity (depletion rate = 0.1 μM min^{-1}).

Table 1 DTT assay response to stock solutions.

alt-text: Table 1

	Conc. (μM)	Depletion rate (μM min^{-1})
9,10-PQN	0.17	3.22
1,2-NPQ	1.0	5.20
1,4-NPQ	1.0	2.21
Anthraquinone	1.0	0.11
Cu^{2+}	1.0	1.05
Mn^{2+}	1.0	0.92
Ni^{2+}	1.0	0.40
Cr^{3+}	1.0	0.33
Zn^{2+}	1.0	0.28
Fe^{3+}	1.0	0.28

Other investigated species were two oxo-PAHs found in PM samples in the investigated area, i.e., 1,8-naphthalic anhydride and xanthone. Though their molecular formulas are similar to the considered quinones, they did not cause measurable DTT oxidation. In addition to the aforementioned organic species, 3 PAHs were considered. They correspond to the not oxidized homologs of the tested quinones and caused no loss of DTT during the tests.

This finding agrees with other studies that indicate PAHs as not redox active species in the DTT assay even if the assay response could sometimes be correlated with PAHs concentration. This is likely due to the reactions PAHs can undergo; in fact, they can be oxidized to more polar compounds including quinones which have demonstrate a very strong redox activity [Charrier and Anastasio 2012, Cho et al., 2005].

Metal ions activity in DTT assay is still an unclear topic due to the conflicting results published in literature [Ayres et al., 2008, Charrier and Anastasio 2012, Perrone et al., 2016]. Thus, we also tested six soluble transition metals (Table 1). All the considered metals showed redox activity, even with a DTT depletion rate lower than that found for quinones (excluding anthraquinone): Cu (II) and Mn (II) were the most reactive (depletion rate ≈1.0 μM min^{-1}) followed by Ni (II), Cr (II), Zn (II) and Fe (III) (depletion rate ≈0.3 μM/min). These results are consistent with the findings of Lin and Yu (2011) and Charrier and Anastasio (2012) that also observed the highest DTT loss for Cu (II) followed by the other cations with the same sequence of relative reactivity.

3.1.2 AA assay

The sensitivity of the AA assay to redox-active species was investigated by measuring the rate of AA consumption for the same standard solutions of 15 individual compounds submitted to the DTT assay (Table 2).

Table 2 Ascorbate assay response to stock solutions.

alt-text: Table 2

	Conc. (μM)	Depletion rate (μM min^{-1})
1,2-NPQ	0.17	1.37
1,4-NPQ	1.0	0.64
9,10-PQN	1.0	0.40
Cu^{2+}	0.17	3.52
Cr^{3+}	1.0	1.02
Fe^{3+}	1.0	0.76
Zn^{2+}	1.0	0.65
Ni^{2+}	1.0	0.48
Mn^{2+}	1.0	0.07

Transition metals are in general the most redox active species, with Cu^{2+} by far the most reactive ion (depletion rate ≈ 3.5 μM min^{-1} for the 0.17 μM diluted solution) followed by Cr^{3+} (depletion rate ≈ 1.0 μM min^{-1}), Fe^{3+}, Zn^{2+} and Ni^{2+} (depletion rate ≈ 0.6 μM min^{-1}). An exception is Mn^{2+} that showed a depletion rate close to blank values.

Among the investigated quinones, 1,2-NPQ showed the highest activity (depletion rate ≈ 1.4 μM min^{-1} for the 0.17 μM diluted solution) followed by 1,4-NPQ and 9,10-PNQ with a redox capacity similar to metals (depletion rate ≈ 0.5 μM min^{-1}). Anthraquinone did not cause measurable ascorbate oxidation. As for DTT assay, PAHs and oxo-PAHs showed no redox activity.

3.1.3 Inter-assay comparisons

Although both assays respond to the same redox-active species, the comparison between the obtained results clearly shows that they display different sensitivity, being the DDT assay more sensitive to quinones and the AA mainly to Cu^{2+} and 1,2-NPQ. Consequently, no correlation was found between OP_{DTT} and OP_{AA} values of the same solutions ($r < 0.4$). Thus, the two assays could potentially provide complementary information on oxidative potential characteristic of PM. Consequently, the combination of the two approaches can strengthen each other in giving insight into the contribution of chemical composition to oxidative properties of PM, which can subsequently be used to study health effects.

3.2 OP of ambient particulate matter

Both assays were applied to 10 real $PM_{2.5}$ samples to assess their oxidative potential. Five of the investigated filters were sampled during summertime and show low PM mass loading (≤28 μg m^{-3}), as it was typically found in Po valley during the warm seasons [Pietrogrande et al., 2016]. The other samples were collected in the cold season and show PM mass values ranging from 40 to 77 μg m^{-3}, that are usual in the region as a consequence of the stagnant atmospheric conditions and the strong emissions from anthropogenic sources yielding pollutant accumulation [Perrone et al., 2016, Pietrogrande et al., 2016].

The OP values measured by the DTT assay (OP_{DTT}) ranged from a minimum of ≈0.4 μM min^{-1} to a maximum of ≈2.4 μM min^{-1} while the responses of the AA assay were from 0.2 μM min^{-1} to 0.9 μM min^{-1} (OP_{AA}).

Even if the values obtained with the two assays are similar, no correlation was found between them, as revealed by the Pearson correlation coefficient ($r < 0.4$).

This behavior is in agreement with the results above reported for the standard solutions and with literature data, although few inter-assay comparisons have been published so far. Only a low correlation between OP_{DTT} and OP_{AA} was found by Janssen et al. (2014) and Yang et al. (2014) (Spearman correlation 0.4–0.6) for $PM_{2.5}$ samples from an urban background site and traffic site in the Netherlands and by Mudway et al. (2011) from samples collected from a kerbside station in London. OP_{AA} results were found better correlated with OP_{ESR} data (Spearman correlation ≈ 0.9, Janssen et al. (2014) and Yang et al. (2014)).

3.2.1 Correlations between measured OP and $PM_{2.5}$ composition

The responses of both assays were converted into relative quantities related to the sampled air volume (nmol of DTT or AA min^{-1} m^{-3}) as a metric of toxicological interest. The obtained OP_{DTT} values ranged from a minimum of ≈0.3 nmol min^{-1} m^{-3} to a maximum of ≈1.7 nmol min^{-1} m^{-3}, while OP_{AA} values from 0.2 to 0.6 nmol min^{-1} m^{-3} were obtained with the AA assay (Fig. 3). Similar low OP_{AA} values, less than 1 nmol min^{-1} m^{-3}, were found by Fang et al. (2016) at urban sites in southeastern US, by using experimental conditions close to those reported in the present work, among the widely different procedures reported by other authors [Yang et al., 2014].

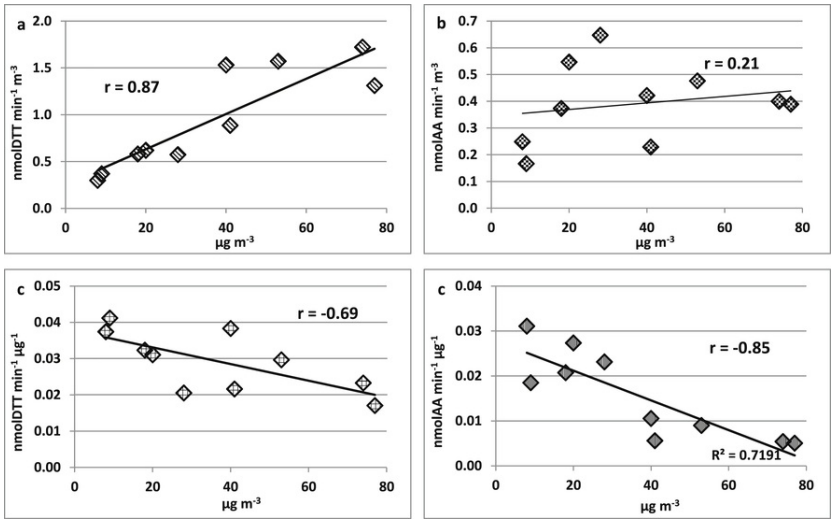


Fig. 3 Response of DTT (a, c) and AA (b, d) assays of real samples with different PM concentrations. Assay results are expressed on volume basis (nmol min^{-1} m^{-3}: a, b) and on mass basis (nmol min^{-1} $μg^{-1}$: c, d). Relationship of the assay response with PM concentration is described by the best linear fitting line and the corresponding Pearson coefficient (r).

alt-text: Fig. 3

These values were related with $PM_{2.5}$ mass concentration in order to give insight into the sensitivity of the investigated assays to the chemical properties of PM. The OP_{DTT} m^{-3} values strongly correlate with $PM_{2.5}$ mass (Pearson coefficient r = 0.87, Fig. 3) indicating a linear increase of oxidative activity with $PM_{2.5}$ mass while no correlation was found for OP_{AA} m^{-3} with $PM_{2.5}$ (r = 0.21, Fig. 3). This result suggests that OP_{DTT} may be considered an extensive property of $PM_{2.5}$ related to its amount, while OP_{AA} is mainly an intensive property, more related to its composition than to its mass. This can be visually shown by the plot in Fig. 3. Concerning the DTT assay, a strong increase of nearly three times was observed for OP_{DTT} from the less loaded samples (<28 μg m^{-3}) to the more charged ones (>28 μg m^{-3}), i.e., from average values of 0.5–1.5 nmol min^{-1} m^{-3} (Fig. 3a). On the contrary, the OP_{AA} response is nearly constant for the two groups of samples, i.e., ≈0.4 nmol min^{-1} m^{-3} (with maximum values obtained in warm period) nearly independent of the largely different mass loading (Fig. 3c). These findings are in line with previous studies that reported

that OP_{DTT} is in general well-correlated (Spearman correlation up to 0.9) with the PM amount, in contrast with OP_{AA} that shows poor correlation [Janssen et al., 2014, Yang et al., 2014]. In addition, the season trend found in this study agrees with that recently reported by Fang et al. [Fang et al., 2016], where higher OP_{DTT} value was measured in winter while maximum OP_{AA} was in summer/fall period at an urban site in Atlanta.

In order to give clearer insight into the possible effect of PM chemical composition on OP assays response, the obtained results were also expressed as relative quantity on $PM_{2.5}$ mass basis, i.e., as DTT or AA nmol min^{-1} μg^{-1} (Fig. 3b,d). Such values show a negative correlation with $PM_{2.5}$ concentration for both assays (Pearson coefficient r ≈ 0.7 for DTT and ≈ 0.85 for AA), suggesting a strong dependence of the PM oxidative potential on the sample composition. The negative correlation indicates that in general the less concentrated filters (samples with PM mass <28 μg m^{-3}) exhibit the higher OP. This pattern is particularly evident for the AA response, since OP_{AA} for the summer diluted samples is nearly three times higher than that of the more concentrated samples (mean values 0.02 and 0.07 nmol min^{-1} μg^{-1}, respectively, Fig. 3d). This may be related to the difference in chemical composition of the samples according to seasonality, with PM collected in warm season characterized by higher abundance of strong oxidized species, such as quinones. This explanation is supported by recent studies that point out that the secondary organic aerosol produced by photochemical aging may promote ROS formation and activity, as confirmed by chamber experiments under controlled laboratory conditions or by ambient PM in various cities of the world [McWhinney et al., 2013, Antiñolo et al., 2015, Saffari et al., 2015, Perrone et al., 2016].

The seasonal trend is not well-defined for OP values measured with the DTT assay, since OP_{DTT} shows similar values in warm and cold seasons, i.e., mean values 0.03 nmol min^{-1} μg^{-1}, Fig. 3b). This result confirm the aforementioned difference between DTT and AA assays that may be ascribed to the different activity of the chemical species present in PM in DTT and ascorbic acid oxidation.

3.2.2 Correlations between measured OP and $PM_{2.5}$ metal content

Among the species that mostly contribute to the oxidative potential of real world PM samples, water-soluble transition metals were investigated in this study, in particular Cu, Zn, Cr, Fe, Mn and Ni as metals commonly found in ambient PM [Charrier and Anastasio 2012, Connell et al., 2006, Vidrio et al., 2009, Walgraeve et al., 2010]. In fact, it is known that such ions stimulate the production of radicals, particularly via the Fenton reaction, which involves the reduction of H_2O_2 by a transition metal ion [Ayres et al., 2008, Buettner and Jurkiewicz 1996, Cho et al., 2005]. The studied filters were submitted to atomic absorption spectroscopy analysis and the concentrations of metal ions were determined (Table 3, ng m^{-3} for iron, from 6 to 21 ng m^{-3} for zinc and even always lower than 7 ng m^{-3} for the other metals. Such low metals concentration is in line with those previously found by some of the authors in the same area (Sarti et al., 2015). This may be a likely reason of the low OP_{AA} values measured, in particular related to the low concentration of cupper, from 2 to 7 ng m^{-3}, that displays the highest sensitivity to the AA assay. The reported data show that the metal concentration in air sampled volume (ng m^{-3}) increases with the PM mass concentration, while the relative contribution of metals to particles mass (ng μg^{-1}) increased from winter to summer, i.e., mean total ion concentrations from 1.2 to 5.1 ng μg^{-1}). In order to investigate the contribution of specific ions to DTT and AA oxidation activity, the OP_{DTT} and OP_{AA} responses were correlated with the measured ions concentration by computing the Pearson's coefficients (Table 4a, where all data are related to air volume, OP_{DTT} m^{-3} and OP_{AA} m^{-3} values). The OP_{DTT} m^{-3} values were well correlated with each of the determined metals (r = 0.84–0.95) and incredibly strongly with the total metals concentration (r = 0.98). Otherwise, the OP_{AA} m^{-3} data show a moderate correlation only for Cu and Mn (r ≈ 0.7), but no correlation with the other ions as well as with the total metals concentration (r = 0.49). These findings are consistent with the results of other recent studies that highlight that AA response is strongly correlated with Cu concentration and OP_{DTT} shows poorer correlation with levels of different aerosol species [Janssen et al., 2014, Fang et al., 2016].

Table 3 Metals concentration in analysed $PM_{2.5}$ filters.

alt-text: Table 3

PM concentration	Cu		Zn		Cr		Fe		Ni	Mn		Tot metals	
μg m^{-3}	ng m^{-3}	ng μg^{-1}	ng m^{-3}	ng μg^{-1}	ng m^{-3}	ng μg^{-1}	ng m^{-3}	ng μg^{-1}		ng m^{-3}	ng μg^{-1}	ng m^{-3}	ng μg^{-1}
8	2.0	0.25	6.2	0.78	2.4	0.30	29.1	3.64		1.3	0.16	41.0	5.13
9	1.9	0.21	9.5	1.05	2.8	0.31	25.0	2.78		2.1	0.23	41.3	4.59
18	2.6	0.15	10.8	0.60	4.7	0.26	28.1	1.56		2.6	0.15	48.8	2.71
20	4.8	0.24	7.9	0.40	4.8	0.24	31.0	1.55		4.4	0.22	53.0	2.65
41	2.8	0.07	11.5	0.28	9.2	0.22	37.5	0.91		2.1	0.05	63.0	1.54
53	6.1	0.12	21.3	0.40	20.4	0.38	39.3	0.74		7.6	0.14	94.8	1.79
74	7.5	0.10	16.1	0.22	25.5	0.34	39.3	0.53		5.5	0.07	93.8	1.27

77	4.9	0.06	13.1	0.17	25.6	0.33	42.9	0.56		4.6	0.06	91.1	1.18

Table 4 Pearson correlation coefficients among determined parameters. a) results expressed on volume basis, b) results expressed on mass basis.

alt-text: Table 4

a			
r	OP_{DTT} m^{-3}	OP_{AA} m^{-3}	PM ng m^{-3}
OP_{DTT} m^{-3}			
OP_{AA} m^{-3}	0.48		
PM	0.87	0.21	
Cu	0.90	0.72	0.79
Zn	0.88	0.39	0.71
Cr	0.95	0.40	0.98
Fe	0.88	0.39	0.94
Mn	0.84	0.75	0.67
Tot metals	0.98	0.49	0.95

b			
r	OP_{DTT} μg^{-1}	OP_{AA} μg^{-1}	PM ng m^{-3}
OP_{DTT} μg^{-1}			
OP_{AA} μg^{-1}	0.77		
PM	−0.69	−0.85	
Cu	0.84	0.95	−0.82
Zn	0.94	0.64	−0.83
Cr	−0.05	−0.38	0.51
Fe	0.84	0.82	−0.85
Mn	0.88	0.77	−0.78
Tot metals	0.90	0.82	−0.86

To give deeper insight into the effect of chemical composition, the correlation with metals concentration was investigated also for OP data based on µg of sampled PM (Table 4b, where all data are related to PM mass, ng μg^{-1}, OP_{DTT} μg^{-1} and OP_{AA} μg^{-1} values). For both the assays, these relative responses show in general good correlation with the relative concentrations of all the investigated metals ($r \geq 0.8$) with the exception of chromium ($r \leq 0.4$).

The general conclusion from these results suggests that the transition metals are the PM chemical components that mainly control the DTT oxidation while other chemical species, in addition to metals, affect the AA oxidation. This topic is still controversial: even if some literature data highlight that DTT assay is most sensitive to organic species [Ayres et al., 2008, Cho et al., 2005], interactions between metal ions and DTT have proven to be somewhat complex [Charrier and Anastasio 2012, Lin and Yu 2011].

4 Conclusions

The results of this study show that both the evaluated methods are user-friendly and inexpensive assays with suitable performance to assess the oxidative potential of atmospheric aerosol samples.

The comparative study of stock solutions of different redox-active species showed that the two methods have different sensibility to the same molecules, with DTT assay more sensible to three of the tested quinones (9,10-phenanthrenequinone 1,2 and 1,4-naphthoquinone) and AA assay exhibiting high response to Cu ions and 1,2-NPQ and minor but not negligible sensitivity to the other quinones and metals tested.

Such a difference between the two assays was also confirmed by the application to real world PM samples. The DTT response was mostly correlated with the PM mass while the AA data were strongly related to chemical composition, as suggested by results seasonality with summertime samples revealing higher redox activity.

As a general conclusion, our study suggests that, among the chemical species present in PM samples, the transition metals control the DTT oxidation while other chemical species, in addition to metals, contribute to the AA oxidation. The question is still open and further studies will be needed to give insight on the specific contribution of redox-active components to PM oxidative potential.

Moreover, data presented here underline that even small amount of some species that act as catalysers could give important effects on OP. This stresses once again the importance of an in-depth chemical speciation of particulate matter.

Acknowledgements

The authors thank personnel of the Regional Agency for Prevention, Environment and Energy of Emilia Romagna region (ARPAE-ER) for their support in providing the PM filters.

References

Antiñolo M., Willis M.D., Zhou S. and Abbatt J.P.D., Connecting the oxidation of soot to its redox cycling abilities, *Nat. Commun.* **6**, 2015.

Ayres J.G., Borm P., Cassee F.R., Castranova V., Donaldson K., Ghio A., Harrison R.M., Hider R., Kelly F., Kooter I.M., Marano F., Maynard R.L., Mudway I., Nel A., Sioutas C., Smith S., Baeza-Squiban A., Cho A., Duggan S. and Froines J., Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential—A workshop report and consensus statement, *Inhal. Toxicol.* **20**, 2008, 75–99.

Borm P.J.A., Kelly F., Künzli N., Schins R.P.F. and Donaldson K., Oxidant generation by particulate matter: from biologically effective dose to a promising, novel metric, *Occup. Environ. Med.* **64**, 2007, 73–74.

Buettner G.R. and Jurkiewicz B.A., Catalytic metals, ascorbate and free radicals: combinations to avoid, *Radiat. Res.* **145**, 1996, 532–541.

Charrier J.G. and Anastasio C., On dithiothreitol (DTT) as a measure of oxidative potential for ambient particles: evidence for the importance of soluble transition metals, *Atmos. Chem. Phys.* **12**, 2012, 11317–11350.

Cho A.K., Sioutas C., Miguel A.H., Kumagai Y., Schmitz D.A., Singh M., Eiguren-Fernandez A. and Froines J.R., Redox activity of airborne particulate matter at different sites in the Los Angeles Basin, *Environ. Res.* **99**, 2005, 40–47.

Chung M.Y., Lazaro R.A., Lim D., Jackson J., Lyon J., Rendulic D. and Hassom A.S., Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts, *Environ. Sci. Technol.* **40**, 2006, 4880–4886.

Connell D.P., Winter S.E., Conrad V.B., Kim M. and Crist K.C., The Steubenville Comprehensive Air Monitoring Program (SCAMP): concentrations and solubilities of $PM_{2.5}$ trace elements and their implications for source apportionment and health research, *J. Air Waste Manag.* **56**, 2006, 1750–1766.

Delfino R.J., Sioutas C. and Malik S., Potential role of ultrafine particles in associations between airborne particle mass and cardiovascular health, *Environ. Health Persp~~ect~~.* **113**, 2005, 934–946.

Dellinger B., Pryor W.A., Cueto R., Squadrito G.L., Hegde V. and Deutsch W.A., Role of free radicals in the toxicity of airborne fine particulate matter, *Chem. Res. Toxicol.* **14**, 2001, 1371–1377.

Fang T., Verma V., Bates J.T., Abrams J., Klein M., Strickland M.J., Sarnat S.E., Chang H.H., Mulholland J.A., Tolbert P.E., Russell A.G. and Weber R.J., Oxidative potential of ambient water-soluble $PM_{2.5}$ in the southeastern United States: contrasts in sources and health associations between ascorbic acid (AA) and dithiothreitol (DTT) assays, *Atmos. Chem. Phys.* **16**, 2016, 3865–3879.

Ghio Carraway M.S. and Madden M.C., Composition of air pollution particles and oxidative stress in cells, tissues, and living system, *J. Toxicol.Environ. Health B Crit. Rev.* **15**, 2012, 1–21.

Hasson A.S. and Paulson S.E., An investigation of the relationship between gas-phase and aerosol-borne hydroperoxides in urban air, *J. Aerosol Sci.* **34**, 2003, 459–468.

Hedayat F., Stevanovic S., Miljevic B., Bottle S. and Ristovski Z.D., Review-evaluating the molecular assays for measuring the oxidative potential of particulate matter, *Chem. Ind. Chem. Eng. Q* **21**, 2014, 201–210.

Inczedy J., Lengyel T. and Ure A.M., Compendium of analytical nomenclature, IUPAC Definitive Rules, third ed., 1998, Blackwell Scientific Publications; Oxford.

Janssen N.A.H., Yang A., Strak M., Steenhof M., Hellack B., Gerlofs-Nijland M.E., Kuhlbusch T., Kelly F., Harrison R., Brunekreef B., Hoek G. and Cassee F., Oxidative potential of particulate matter collected at sites with different source characteristics, *Sci. Total Environ.* **472**, 2014, 572–581.

Kelly F.J., Oxidative stress: its role in air pollution and adverse health effects, *Occup. Environ. Med.* **60**, 2003, 612–616.

Kumagai Y., Koide S., Taguchi K., Endo A., Nakai Y., Yoshikawa T. and Shimojo N., Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles, *Chem. Res. Toxicol.* **15**, 2002, 483–489.

Li Q., Wyatt A. and Kamens R.M., Oxidant generation and toxicity enhancement of aged-diesel exhaust, *Atmos. Environ.* **43**, 2009, 1037–1042.

Lin P. and Yu J.Z., Generation of reactive oxygen species mediated by humic-like substances in atmospheric aerosols, *Environ. Sci. Technol.* **45**, 2011, 10362–10368.

Lodovici M. and Bigagli E., Oxidative stress and air pollution exposure, *J. Toxicol* 2011, article ID 487074.

McWhinney R.D., Zhou S. and Abbatt J.P.D., Naphthalene SOA: redox activity and naphthoquinone gas-particle partitioning, *Atmos. Chem. Phys.* **13**, 2013, 9731–9744.

MohanKumar S.M.J., Campbell A., Block M. and Veronesi B., Particulate matter, oxidative stress and neurotoxicity, *Neurotoxicology* **29**, 2008, 479–488.

Mudway I.S., Fuller G., Green D., Dunster C. and Kelly F.J., Quantifying the London specific component of PM_{10} oxidative activity, *U. K. Dep. Environ. Food Rural Aff.* 2011 (Change U.K. Dep. Environ. Food Rural Aff. 2011 with Report DEFRA, Department Environmental, Food and Rural Affairs, UK, 2011 (http://uk-ait.defra.gov.uk/library/reports?report_id=649)).

Perrone M.G., Zhou J., Malandrino M., Sangiorgi G., Rizzi C., Ferrero L., Dommen J. and Bolzacchini E., PM chemical composition and oxidative potential of the soluble fraction of particles at two sites in the urban area of Milan, Northern Italy, *Atmos. Environ.* **128**, 2016, 104–113.

Pietrogrande M.C., Bacco D., Ferrari S., Ricciardelli I., Scotto F., Trentini A. and Visentin M., Characteristics and major sources of carbonaceous aerosols in $PM_{2.5}$ in Emilia Romagna Region (Northern Italy) from four-year observations, *Sci. Total. Environ.* **553**, 2016, 172–183.

Roginski V.A., Barsukova T.K. and Stegmann H.B., Kinetics of redox interaction between substitutes quinones and ascorbate under aerobic conditions, *Chem. Bio. Interact.* **121**, 1999, 177–197.

Saffari A., Hasheminassab S., Wang D., Shafer M.M., Schauer J.J. and Sioutas C., Impact of primary and secondary organic sources on the oxidative potential of quasi-ultrafine particles (PM0.25) at three contrasting locations in the Los Angeles Basin, *Atmos. Environ.* **120**, 2015, 286–296.

Sarti E., Pasti L., Rossi M., Ascanelli M., Pagnoni A., Trombini M. and Remelli M., The composition of PM1 and $PM_{2.5}$ samples, metals and their water soluble fractions in the Bologna area (Italy), *Atmos. Pollut. Res.* **6**, 2015, 708–718.

Squadrito G.L., Cueto R., Dellinger B. and Pryor W.A., Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter, *Free Radic. Bio. Med.* **31**, 2001, 1132–1138.

Venkatachari P., Hopke P.K., Brune W.H., Ren X., Lesher R., Mao J. and Mitchell M., Characterization of wintertime reactive oxygen species concentrations in Flushing, New York, *Aerosol Sci. Tech.* **41**, 2007, 97–111.

Verma V., Fang T., Xu L., Peltier R.E., Russel A.G., Ng N.L. and Weber R.J., Organic aerosols associated with the generation of reactive oxygen species (ROS) by water-soluble $PM_{2.5}$, *Environ. Sci. Technol.* **49**, 2015, 4646–4656.

Vidrio E., Phuah C.H., Dillner A.M. and Anastasio C., Generation of hydroxyl radicals from ambient fine particles in a surrogate lung fluid solution, *Environ. Sci. Technol.* **43**, 2009, 922–927.

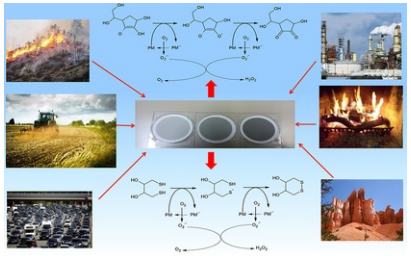
Walgraeve C., Demeestere K., Dewulf J., Zimmermann R. and Van Langenhove H., Oxygenated polycyclic aromatic hydrocarbons in atmospheric particulate matter: molecular characterization and occurrence, *Atmos. Environ.* **44**, 2010, 1831–1846.

Xu J. and Jordan R.B., Kinetics and mechanism of the reaction of aqueous copper(II) with ascorbic acid, *Inorg. Chem.* **29**, 1990, 2933–2936.

Yang A., Jedynska A., Hellack B., Kooter I., Hoek G., Brunekreef B., Kuhlbusch T.A.J., Cassee F.R. and Janssen N.A.H., Measurement of the oxidative potential of $PM_{2.5}$ and its constituents: the effect of extraction solvent and filter type, *Atmos. Environ.* **83**, 2014, 35–42.

Zielinski H., Mudway I.S., Bérubé K.A., Murphy S., Richards R. and Kelly F.J., Modeling the interactions of particulates with epithelial lining fluid antioxidants, *Am. J. Physiol.* **277**, 1999, L719–L726.

Graphical abstract



alt-text: Image 1

Highlights

- Oxidative potential was assessed of PM collected at an urban site in different seasons.
- We examined two cell-free assays for the determination of oxidative potential (OP) of PM.
- OP_{DTT} and OP_{AA} showed different sensitivity to the same chemical species.
- OP_{DTT} is strongly correlated with PM concentration in the atmosphere.
- Summertime samples show higher relative responses.
- Results suggest that metals are the main responsible for oxidative stress of PM. (Delete the first point and change the second point in: We assessed the oxidative potential (OP) of PM collected at an urban site using two cell-free assays.)

Queries and Answers

Query: Highlights should consist of 3 to 5 bullet points. There are “6” bullet points provided. Please edit the highlights to meet the requirement.
Answer: See instruct

Query: The citation Ghio et al, 2012 has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.
Answer: The correction made is right

Query: please check the journal title in the reference Delfino et al., 2005
Answer: *Persp.*

Query: Please provide the volume number or issue number or page range or article number for the bibliography in Ref(s). Mudway et al., 2011.
Answer: See instruct

Query: Please confirm that given names and surnames have been identified correctly.
Answer: Yes

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact j.miranda@elsevier.com immediately prior to returning your corrections.
Answer: Yes