

Manuscript Number: ATMENV-D-17-01701R2

Title: PM10 Oxidative Potential at a Central Mediterranean Site: Association with Chemical Composition and Meteorological Parameters.

Article Type: Research Paper

Keywords: Oxidative stress; PM10 particulate matter; Dithiothreitol assay; Ascorbic acid assay; PM10 chemical composition.

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Abstract: An extended study on the oxidative potential (OP) of PM10 particles collected from December 2014 to October 2015 at a peninsular site of the Central Mediterranean basin has been performed. PM10 particles have been selected to better account for all different aged/fresh particle types. Two acellular assays, i.e., the dithiothreitol (DTT) and ascorbic acid (AA) methods, were used to measure the OP of PM10 particles chemically speciated by more than 40 species.

DTT and AA assays provide close mean values of volume normalized OPV responses, with similar variability range, i.e., mean OPDTTV = 0.24 ± 0.12 nmolDTT min⁻¹ m⁻³ and mean OPAAV = 0.29 ± 0.18 nmolAA min⁻¹ m⁻³. Also mass normalized OPm responses are similar for both assays, with mean value close to 0.008 nmol min⁻¹ µg⁻¹.

The measured OPDTTV and OPAAV are correlated with several inorganic species, namely ions and metals, and with organic/elemental carbon. The discrimination of the data according seasonality, i.e., Autumn-Winter (AW, October-March) and Spring-Summer (SS, April-September) days, shows a clear seasonal trend of correlation coefficients. In AW, OPDTTV is strongly correlated with nss-K⁺ and nss-Ca²⁺, in addition to Ba, Cd, Ce, Cr, Cu, Fe, and Mn (traffic-related metals) and with EC, OC, and POC associated with a traffic exhaust source and/or with the combustion including biomass-burning source. Otherwise, OPDTTV of SS samples is correlated only with NH₄⁺, Cu, EC, OC, and POC.

The OPAAV of AW samples is well correlated with Ba, Ce, Cr, Cu, Fe, Mn, nss-K⁺, EC, OC, and POC, which are related with traffic and/or combustion emissions. Conversely, in SS, OPAAV is mainly correlated with NH₄⁺, nss-K⁺, nss-Mg²⁺, nss-Ca²⁺, nss-SO₄²⁻, Cu, Mn, P, Pb, and oxalate, that are species related to secondary aerosols and resuspended soil from vehicular traffic and/or transported Saharan dust.

These findings point the importance of both organic components and transition metals to PM oxidative properties, and also suggest that synergistic/antagonistic interactions and cross-correlations between the PM redox-active components are likely responsible for the seasonal variation of the AA and DTT assay response. The inter-correlation among all analyzed species has been investigated to explain contrasting results

and the negative correlations between OP values and some chemical species.



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Manuscript No: **ATMENV-D-17-01701R1**

To the Editor of
Atmospheric Environment

Dear Editor,

with the present letter we intend to submit our manuscript to publication on your journal Atmospheric Environment:

PM₁₀ Oxidative Potential at a Central Mediterranean Site: Association with Chemical Composition and Meteorological Parameters

Maria Chiara Pietrogrande, Maria Rita Perrone, Francesco Manarini, Salvatore Romano, Roberto Udisti, Silvia Becagli

As requested by the Editor, the previously revised manuscript has been further revised following the minor revisions suggested by the Reviewer I.

In particular:

- the novelty of the paper has been described in detail by adding several sentences in different parts of the paper,
- the details on volume and mass of the PM samples as well as on procedure of the OP assays have been added.

All revisions are clearly marked in the electronic version of the manuscript “Highlighted Manuscript”, where changes are tracked in **red**.

Therefore, we strongly hope that our paper can be accepted for publication on your journal.

Sincerely yours,

Ferrara, June 6, 2018

Prof. M. Chiara Pietrogrande

Dear Reviewer #1,

thanks for having acknowledged our effort in improving the manuscript quality based on your suggestions and for the additional comments reported below.

To allow a further improvement of the paper we changed the Manuscript following your suggestions and obtained the 2nd Revised Manuscript, where changes are highlighted in red in the marked copy.

General Comments

“The reviewer acknowledges the author's effort in improving the manuscript quality. Though the authors have made efforts in addressing the points raised during earlier reviewing process, but in reviewer's opinion they still remain unanswered. For example, Novelty of Work. The authors' statement of 'extended study of PM10 samples at the site' itself is a novelty, is not an appropriate statement. While the authors incorporated some text in the Introduction section of the revised manuscript, they don't either reflect or highlight them in discussion part. Another instance, as in lines 297-301 in support of comparative difference between PM2.5 and coarse PM-induced oxidative potential, the authors just tried to describe the results from literature instead of identifying the possible relation between their work and that of others and thus, I assume, failed to establish the novelty. “

A minor revision is suggested to the authors to further improve the quality of manuscript, which could award a publication in the journal.

To fulfil your general comments several changes and addition have been made in different parts of the paper, i.e., abstract, discussion and conclusions. In particular:

Lines 27-31 of the paper abstract have been replaced with the following ones:

“An extended study on the oxidative potential (OP) of PM10 particles collected from December 2014 to October 2015 at a peninsular site of the Central Mediterranean basin has been performed. PM10 particles have been selected to better account for all different aged/fresh particle types. Two acellular assays, i.e., the dithiothreitol (DTT) and ascorbic acid (AA) methods, were used to measure the OP of PM10 particles chemically speciated by more than 40 species.”

Then, the following sentence has been added at the end of the abstract (Lines 49-54):

“The inter-correlation among all analyzed species has been investigated to explain contrasting results and the negative correlations between OP values and some chemical species. “

Then, the following sentences have been added in the Result section:

Line 309: *“This is consistent with the location of the study site, which is in a suburban site of the flat Salento's peninsula, in the Central Mediterranean, away from large sources of local pollution.”*

Line 325: *“In this study, OP responses were measured for PM10 samples collected for a long time series over a full year, to give insight into OP seasonal trend to be related with meteorological/atmospheric conditions and particle chemical composition. The daily trend of OPV and OPm values for the 53 samples is shown in Fig. 2a-2b, in addition with that of the PM10 mass concentration (Fig. 2c).”*

Line 346: *“ Such a large variability may be likely ascribed to the specific characteristics of the study site, where both PM concentration and composition day-by-day change, because of the impact of long-range transport from the surrounding anthropogenic and desert regions, and the*

Mediterranean Sea. (Perrone et al., 2014a, 2014b, 2016; Becagli et al., 2017; Chirizzi et al., 2017). This represents a paper peculiarity, since most of the previous studies were mainly performed at sites impacted by specific quite constant pollution sources, e.g. traffic sites, underground train stations, farms (Mugica et al., 2009; Cheung et al., 2010; Godri et al., 2011; Boogaard et al., 2012; Janssen et al., 2014; Waked et al., 2014; Hellack et al., 2015; Pant et al., 2015; Calas et al., 2017; Weber et al., 2018).

Finally the novelty of the paper was further highlighted by adding the following sentences in Section 4 of the submitted paper:

at the beginning of the section (lines 508-518): *“An extended study on the PM10 induced oxidative potential using the DTT and AA assays has been presented in the paper. The long PM10 collection duration – ten months – and the detailed chemical speciation of the samples – more than 40 species – made it possible to highlight the day-by-day variability and seasonal evolution of OP and relate them with the PM chemical properties. A marked temporal variability was found, that represents a peculiarity of the study site, characterized by different aged/fresh aerosol types delivered by the long-range transport from the surroundings, i.e., anthropogenic and desert areas and the Mediterranean Sea.*

On this basis, the following conclusions can be drawn:

- *The OP_{DTT} and OP_{AA} responses from the two acellular assays are very similar in mean values and variability range, but differ in the association with PM10 chemical composition, as well as in seasonality of such association.”*

Line 524: *“OP_{AA}_v of AW samples was strongly positively correlated with the main tracers of traffic and/or combustion emissions.”*

Line 528: *“All the species highly correlated with OP_{DTT}_v and OP_{AA}_v values were also significantly inter-correlated, suggesting that cross-correlations could be responsible for the association of some of the above reported PM₁₀ redox-active components. This last result highlights that it is important to look for cross-correlations between the different species to properly identify the redox active species related to OP as well as to explain contrasting results reported in literature. In fact, we found that the negative correlations observed on AW and SS between the OP_{DTT}_v and OP_{AA}_v values and Na⁺ and Cl⁻, respectively, could be due to some of the PM redox-active components affecting the AA and DTT assay responses, which were negatively correlated with Na⁺ and/or Cl⁻, respectively.”*

Specific Comments:

Line 37: the full form of 'AW' and 'SS' are still missing at its first instance.

The full explanation of 'AW' and 'SS' acronyms was added (line 38 of the revised version).

Lines 182-194: While the authors have explained the details of PM sample (like volume & PM mass in OP assays) in the Response sheet, they failed to reflect the same in the manuscript.

The Authors acknowledge the omission, then the following sentences were added at the line 198 of the revised manuscript:

“In both assays, 30 μl of 100 mM antioxidant solution were used, which contain 300 nmoles, corresponding to 46 μg of DDT or AA, and added to the reaction solution obtained from extraction of ¼ of each PM₁₀ filter (from collecting 55 m³ of ambient air). By considering that the investigated

filters had a mean PM₁₀ mass concentration of 33 ±13 (μg m⁻³), each sample contain on mean 454 ±179 μg of PM₁₀.”

A new sentence was also added at line 212:

“The obtained OP responses were then normalized to air collected volume, i.e. volume-normalized OP_V (nmol min⁻¹ m⁻³) and to PM10 sampled mass, i.e., mass-normalized OP_m (nmol min⁻¹ μg⁻¹).

Lines 182-201: It's still unclear what are the 'standards' used for DTT and AA assay to prepare a linear standard fitting?

Indeed, the concentration versus time plot used to compute the linear fitting is based on the different concentrations of the DTT and AA solutions originally added to the sample (100 nmol), which decrease during the reaction course as a consequence of their oxidation in the DTT or AA assay. To better explain this point, the following sentences were changed at the line 203 of the revised manuscript:

“The kinetics of DTT or AA oxidation was followed by measuring the decrease of DTT or AA concentrations added to the sample (100 nmol) over the reaction course. The DTT or AA depletion rate (nmol^{DTT} min⁻¹ and nmol^{AA} min⁻¹, respectively) was computed as the slope of the straight line obtained by linear fitting of five experimental points of the DTT or AA concentration at different reaction times (5, 10, 15, 25, 40 minutes).”

PM₁₀ Oxidative Potential at a Central Mediterranean Site: Association with Chemical Composition and Meteorological Parameters

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Highlights

- Oxidative potential is assessed for PM₁₀ collected at a Central Mediterranean Site.
- Two cell-free assays yield similar OP^{DTT} and OP^{AA} responses.
- Association of volume normalized OP^{DTT} and OP^{AA} with PM chemical components varies with seasons.
- Metals and primary organic carbon are the main responsible for PM-induced OP.

Keywords

Oxidative potential; PM₁₀ particulate matter; Dithiothreitol assay; Ascorbic acid assay; PM₁₀ chemical composition.

Abstract

An extended study on the oxidative potential (OP) of PM₁₀ particles collected from December 2014 to October 2015 at a peninsular site of the Central Mediterranean basin has been performed. PM₁₀ particles have been selected to better account for all different aged/fresh particle types. Two acellular assays, i.e., the dithiothreitol (DTT) and ascorbic acid (AA) methods, were used to measure the OP of PM₁₀ particles chemically speciated by more than 40 species.

DTT and AA assays provide close mean values of volume normalized OP_v responses, with similar variability range, i.e., mean OP^{DTT}_v = 0.24±0.12 nmol^{DTT} min⁻¹ m⁻³ and mean OP^{AA}_v = 0.29±0.18 nmol^{AA} min⁻¹ m⁻³. Also mass normalized OP_m responses are similar for both assays, with mean value close to 0.008 nmol min⁻¹ μg⁻¹.

The measured OP^{DTT}_v and OP^{AA}_v are correlated with several inorganic species, namely ions and metals, and with organic/elemental carbon. The discrimination of the data according seasonality, i.e., Autumn-Winter (AW, October–March) and Spring-Summer (SS, April–September) days, shows a clear seasonal trend of correlation coefficients. In AW, OP^{DTT}_v is strongly correlated with

40 nss-K⁺ and nss-Ca²⁺, in addition to Ba, Cd, Ce, Cr, Cu, Fe, and Mn (traffic-related metals) and with
41 EC, OC, and POC associated with a traffic exhaust source and/or with the combustion including
42 biomass-burning source. Otherwise, OP^{DTT}_v of SS samples is correlated only with NH₄⁺, Cu, EC,
43 OC, and POC.

44 The OP^{AA}_v of AW samples is well correlated with Ba, Ce, Cr, Cu, Fe, Mn, nss-K⁺, EC, OC, and
45 POC, which are related with traffic and/or combustion emissions. Conversely, in SS, OP^{AA}_v is
46 mainly correlated with NH₄⁺, nss-K⁺, nss-Mg²⁺, nss-Ca²⁺, nss-SO₄²⁻, Cu, Mn, P, Pb, and oxalate,
47 that are species related to secondary aerosols and resuspended soil from vehicular traffic and/or
48 transported Saharan dust.

49 These findings point the importance of both organic components and transition metals to PM
50 oxidative properties, and also suggest that synergistic/antagonistic interactions and cross-
51 correlations between the PM redox-active components are likely responsible for the seasonal
52 variation of the AA and DTT assay response. The inter-correlation among all analyzed species has
53 been investigated to explain contrasting results and the negative correlations between OP values and
54 some chemical species.

55

56 **Capsule**

57 Cell-free methods assessed the oxidative potential of particulate matter collected at a Central
58 Mediterranean Site and the results were correlated with PM₁₀ chemical composition.

59

60 1. Introduction

61
62 Numerous epidemiological and toxicological studies have shown a relationship between ambient
63 particulate matter (PM) exposure and adverse health effects in humans, mainly respiratory and
64 cardiovascular diseases (Hetland et al., 2004; Delfino et al., 2005; Ghio et al., 2012; Pöschl and
65 Shiraiwa, 2015). The most accredited pathophysiological mechanisms proposed so far involve the
66 production of reactive oxygen species (ROS), which can activate a number of redox sensitive
67 signaling pathways, triggering a cascade of events associated with inflammation and potential cell
68 apoptosis (Borm et al., 2007; Akhtar et al., 2010; Lodovici et al., 2011; Ghio et al., 2012; Delfino et
69 al., 2013; Quintana et al., 2015). Recognizing the importance of this ROS generation step, oxidative
70 potential (OP) has been defined, as the capability of particles to deplete physiological antioxidants
71 (reductants) and generate ROS. OP has been proposed as a more biologically relevant metric than
72 bulk PM mass concentration to represent the integrated effects from multiple toxic components in
73 PM (Ayres et al., 2008; Hedayat et al., 2014; Janssen et al., 2015).

74 Among the variety of procedures that have been developed to quantify different aspects of PM-
75 induced oxidative stress, *in vitro* acellular methods have the advantages of requiring less-controlled
76 environments in comparison with cellular assays and providing faster readouts allowing high
77 throughput measurements of the PM oxidative potential. They are based on different principles to
78 represent a surrogate of a plausible mechanism of PM ROS generation at the interface of the
79 air/respiratory system. They include oxidation of endogenously generated protective antioxidants,
80 e.g., dithiothreitol (DTT assay, Cho et al., 2005; Charrier and Anastasio, 2012; Fang et al., 2016),
81 glutathione (GSH, Godri et al., 2011), ascorbic acid (AA, Mudway et al., 2004; Ayres et al., 2008),
82 synthetic RTLf (Szigeti et al., 2016) and hydroxyl radical generation in the presence of H₂O₂ (Jung
83 et al., 2006; Vidrio et al., 2009).

84 In this study, two common acellular techniques based on low-cost spectrophotometric UV-Vis
85 measurements were used. One is the dithiothreitol (DTT) assay, where DTT acts as a surrogate for
86 biological reducing agents owing to its two sulfhydryl groups (Cho et al., 2005; Li et al., 2009;
87 Charrier and Anastasio, 2012; Hedayat et al., 2014; Janssen et al., 2014; Verma et al., 2015;
88 Charrier et al., 2015; Shirmohammadi, et al., 2017; Xiong et al., 2017). The other is the ascorbic
89 acid assay (AA) based on oxidation of ascorbate, that is a physiological antioxidant found in lung
90 fluid (Mudway et al., 2004; Ayres et al., 2008; Fang et al., 2016).

91 The objective of this study is to investigate the oxidative potential of PM₁₀ particles collected at a
92 peninsular site, which may be considered as representative of coastal sites of the Central
93 Mediterranean area (Fig. 1), away from large sources of local pollution (e.g. Perrone et al., 2013).

94 The Mediterranean basin is one of the Earth regions where the aerosol climate effects are maximum
95 and is foreseen as a hotspot for regional climate warming (Marmer and Langmann, 2005). The
96 composition of the Mediterranean PM originates from contribution of different aerosol types
97 because of the long-range transport from the surrounding regions: desert dust from the Sahara
98 desert, polluted particles from urban and industrial areas of North and East Europe, marine aerosols
99 from the Mediterranean itself or transported from the Atlantic ocean, and biomass burning particles,
100 often produced from forest fires, mainly during the summer (Perrone et al., 2014a, 2014b, 2016;
101 Becagli et al., 2017; Chirizzi et al., 2017). PM₁₀ particles have been sampled to better account for
102 all different aged/fresh aerosol types and a detailed chemical speciation was performed, concerning
103 more than 40 species. The chemical speciation includes the major ambient PM constituents
104 identified so far to be associated with the OP activity. They are transition metals (e.g., Fe, Cu, Cr),
105 organic carbon (OC), aromatic organic compounds (e.g., quinones), and water-soluble HULIS
106 (humic-like-substance) compounds (e.g., Lin, 2011; Janssen et al., 2014; Verma et al., 2015; Saffari
107 et al., 2015; Fang et al., 2017).

108 The PM₁₀ oxidative potential was measured with both the ascorbic acid and dithiothreitol assays
109 and the obtained responses were compared to investigate the chemical specificity of the two
110 methods. The investigation of the seasonal dependence of the OP responses on PM chemical
111 properties on spring-summer and autumn-winter represents another significant issue of this study.
112 Recently, the OP of PM_{2.5} and PM₁₀ samples collected at the study site has been investigated by
113 Chirizzi et al. (2017) using the DTT assay. Their study focused on the comparison of results
114 obtained during Sahara dust events with cases not influenced by Saharan dust, and with cases
115 referring to samples having high carbon content, due to relevant contributions from combustion
116 sources (mainly road traffic and biomass burning).

117 Here, a more extended study is reported concerning PM₁₀ samples collected for a long time series
118 over a full year, chemically characterized by a wide set of components, affected by different particle
119 types/sources and measuring OP values with both AA and DTT assays.

120 Although the data regarding the oxidative potential of PM₁₀ sources is still scarce, coarse aerosol
121 has been found linked to adverse health effects, i.e., inducing hemolysis and DNA degradation and
122 pro-inflammatory cytokines in vitro (Hetland et al., 2004; Osornio-Vargas et al., 2011; Janssen et
123 al., 2014; Quintana-Belmares et al., 2015). A significant fraction of the coarse PM species are
124 associated with mechanically generated aerosols, such as mineral and fugitive dust and with
125 tire/brake wear components from traffic and construction activities in urban environments (Hellack
126 et al., 2015; Pant et al., 2015; Fang et al., 2017). These components can induce ROS formation as
127 solid particles binding to a cell surface or entering the cell, or as “carrier” of more toxic species

128 such as metals and surface-sorbed PAHs (Schoonen et al., 2006; Schoonen et al., 2006; Hu et al.,
129 2008; Mugica et al., 2009; Osornio-Vargas et al., 2011; Boogaard et al., 2012; Fang et al., 2017).

130

131 **2. Materials and Methods**

132

133 *2.1. Sampling Site*

134

135 PM₁₀ samples were collected on the roof (~10 m above ground level) of the Mathematics and
136 Physics Department of the University of Salento, from December 2014 until October 2015. Seven
137 samples were collected per month, on average along one week for month, with few exceptions
138 because of technical reasons. The monitoring site is located in a suburban site (40.3°N; 18.1°E) of
139 the flat Salento's peninsula, in the Central Mediterranean (Fig. 1) and represents an ideal site for
140 monitoring aerosol of different types/sources (e.g., Basart et al., 2009; Perrone et al., 2013, 2014b,
141 2016; Mallet et al., 2016).

142 A low volume (2.3m³ h⁻¹) HYDRA-FAI sampler was used to collect 24-hour PM₁₀ samples on 47-
143 mm-diameter preheated filters (PALLFLEX, Tissuquartz). The filters were conditioned for 48 hours
144 (25°C and 50% humidity) before and after sampling and PM₁₀ mass concentrations were
145 determined by the gravimetric method. Uncertainties on mass concentrations are lower than 5%.
146 The PM loaded filters were divided in four punches for the determination of inorganic ions and
147 methanesulfonate, metals, organic and elemental carbon, and the oxidative potential.

148 Measurements from a local meteorological station at about 15 m above ground level were used to
149 characterize temperature, relative humidity, and rain during the PM₁₀ sampling time. The incoming
150 solar radiation was monitored by a LI200X pyranometer, operating within the 400-1100 nm spectral
151 range and located at about 10 m from the ground level. SO₂ and NO₂ mass concentrations were
152 obtained from the Arnesano monitoring station of the Regional Air Quality Agency (ARPA Puglia),
153 located ~ 500 m away from the monitoring site of this study.

154

155 *2.2. Ions, Metals, and Organic and Elemental Carbon Analyses in PM₁₀ samples*

156

157 Loaded as well as blank filters were submitted to different analyses to characterize the chemical
158 composition of the PM₁₀ collected particles by using the methods described in detail in Perrone et
159 al. (2014a).

160 Three Ion Chromatography fed by Gilson 222-XL auto sampler were used to quantify anions (Cl⁻,
161 NO₂⁻, NO₃⁻, SO₄²⁻, MSA⁻, oxalate, acetate, glycolate, propanoate, formate, and pyruvate) and cations

162 (Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺). Cations were determined with isocratic elution of 11.3 mM H₂SO₄ at
163 1.5 ml min⁻¹ by Dionex CS12A separation column. Details on ions determination are reported in
164 Perrone et al. (2014a, 2014b). A Varian 720-ES Inductively Coupled Plasma Atomic Emission
165 Spectrometer equipped with an ultrasonic nebulizer was used to determine the mass concentration
166 of Al, Ba, Cd, Ce, Co, Cr, Cu, Fe, La, Mn, Mo, Ni, P, Pb, Sr, Ti, V, and Zn. The Sunset Carbon
167 Analyzer Instrument with the EUSAAR-2 temperature program protocol was used to determine EC
168 and OC mass concentrations, with a measure uncertainty of 10% and 6% for EC and OC,
169 respectively (Cavalli et al., 2010).

170

171 *2.3. Assessment of the PM oxidative potential*

172

173 Oxidative Potential of the collected samples was assessed with both dithiothreitol (OP^{DTT} response)
174 and ascorbic acid (OP^{AA} response) acellular methods. OP response is measured as the antioxidant
175 depletion rate of known quantity of DTT and AA, following the experimental procedure described
176 elsewhere (Visentin et al., 2016).

177 Briefly, both the assays were performed on 3 mL of the aqueous extract of a quarter of the sampled
178 filters (extracted for 15 minutes in an ultrasonic bath using 10 mL of 0.1 M buffer at pH 7.4). The
179 extract was filtered on a regenerate cellulose syringe filter (13 mm, 0.22 μm, Kinesis) to remove the
180 suspended solid particles and then introduced into an amber vial at a constant temperature of 37°C
181 using a dry bath. Both the assays were performed under the biological relevant temperature of 37°C
182 and pH of 7.4. Spectrophotometric measurements were performed in a UV-Vis spectrophotometer
183 (Jasco V-730, JASCO EUROPE s.r.l.) with a 1 cm path length optical cell. Polystyrene and quartz
184 cuvette were used for DTT and AA assays, respectively.

185 In the DTT assay 30 μl of the 10 mM DTT solution was added to the sample (i.e., time zero) and
186 the rate of DTT depletion (OP_{DTT}) measured as following. At defined times, a 0.50 mL aliquot of
187 the reaction mixture was removed and the reaction stopped with trichloroacetic acid (0.50 mL of 10
188 %). Then, the remaining DTT is reacted with DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)) to
189 generate DTT-disulphide and 2-nitro-5-thiobenzoic acid (TNB): 50 μL of the DTNB solution (10
190 mM concentration in phosphate buffer at pH 7.4) were added to each aliquots and well mixed. After
191 two minutes to allow complete reaction, pH was increased to pH 8.9 by adding 2.0 mL of Tris-HCl
192 buffer (0.40 M at pH 8.9 with 20 mM of EDTA) to form the mercaptide ion (TNB²⁻), which has a
193 high absorbance ($\epsilon = 14150 \text{ M}^{-1}\text{cm}^{-1}$ at 412 nm) (Charrier and Anastasio, 2012; Li et al., 2009).

194 In the AA assay 30 μl of the 10 mM AA solution was added to the sample (i.e., time zero). Then,
195 the rate of AA depletion (OP_{AA}) was followed directly in the spectrophotometric cuvette by

196 measuring absorption of the ascorbate ion at 265 nm at known time intervals ($\epsilon = 14500 \text{ M}^{-1}\text{cm}^{-1}$ at
197 pH 7.4) (Mudway et al., 2004; Ayres et al., 2008).

198 In both assays, 30 μl of 100 mM antioxidant solution were used, which contain 300 nmoles,
199 corresponding to 46 μg of DDT or AA, and added to the reaction solution obtained from extraction
200 of $\frac{1}{4}$ of each PM_{10} filter (from collecting 55 m^3 of ambient air). By considering that the investigated
201 filters had a mean PM_{10} mass concentration of $33 \pm 13 (\mu\text{g m}^{-3})$, each sample contain average 454
202 $\pm 179 \mu\text{g}$ of PM_{10} .

203 The kinetics of DTT or AA oxidation was followed by measuring the decrease of DTT or AA
204 concentrations added to the sample (100 nmol) over the reaction course. The DTT or AA depletion
205 rate ($\text{nmol}^{\text{DTT}} \text{ min}^{-1}$ and $\text{nmol}^{\text{AA}} \text{ min}^{-1}$, respectively) was computed as the slope of the straight line
206 obtained by linear fitting of five experimental points of the DTT or AA concentration at different
207 reaction times (5, 10, 15, 25, 40 minutes). In general, a good linearity of the concentration-time
208 relationship was found with correlation coefficient $R \geq 0.98$ (Visentin et al., 2016). For both
209 methods, blank filter response was determined by measuring the depletion rates of DTT or AA on
210 the extract of a quarter of blank quartz filters. Sample and blank assays were run in duplicate. The
211 average filter blank responses were subtracted from the depletion rates of real PM samples.

212 The obtained OP responses were then normalized to air collected volume, i.e. volume-normalized
213 $\text{OP}_V (\text{nmol min}^{-1} \text{ m}^{-3})$ and to PM_{10} sampled mass, i.e., mass-normalized $\text{OP}_m (\text{nmol min}^{-1} \mu\text{g}^{-1})$.

214

215 **3. Results**

216

217 Meteorological data and concentrations of chemical species are reported in Table 1. For each
218 parameter, the mean values and the corresponding standard deviations (SD) were computed for all
219 the 53 monitoring days of this study and for autumn-winter (AW, December-March, 28 days) and
220 spring-summer (SS, April-October, 25 days) data, separately. The t-test was applied to the mean
221 AW and SS values to assess their statistical difference at a significance level of 0.05 (values marked
222 by * in Table 1).

223

224 *3.1. Meteorological data and gases pollutants*

225

226 Measured rainfall (Rain), relative humidity (RH), temperature (T) and solar irradiance values (Table
227 1) are characterized by marked seasonal differences ($p < 0.05$). In general, the measured values are
228 in satisfactory accordance, within ± 1 SD, with the data observed in the previous years 2008-2009
229 and 2011-2012 (Perrone et al. 2014a and 2014b, respectively).

230 Concentration of gas pollutants SO₂ and NO₂ are also given in Table 1, reported as overall and AW
231 and SS mean values.

232

233 3.2. PM₁₀ mass concentration and chemical composition

234

235 PM₁₀ mass show nearly constant values throughout the year ($33 \pm 13 \mu\text{g m}^{-3}$, Table 1), in
236 accordance with previous studies (Perrone et al., 2011, 2013, 2014a, 2016). This may be related to
237 the weak dependence of the planetary boundary layer (PBL) depth on seasons in the study area (De
238 Tomasi et al., 2011).

239 K⁺, Mg²⁺, Ca²⁺, and SO₄²⁻ mass concentrations have been splitted into sea-salt (ss-) and non-sea-
240 salt (nss-) mass concentrations to infer the anthropogenic contributions. Assuming that all measured
241 Na⁺ ions have a marine origin, the sea salt contribution to K⁺, Mg²⁺, Ca²⁺, and SO₄²⁻ has been
242 estimated as measured Na⁺ times 0.038, 0.119, 0.038, and 0.242, respectively (Pio et al., 2007).
243 Then, the nss-K⁺, nss-Mg²⁺, nss-Ca²⁺, and nss-SO₄²⁻ mass concentrations have been calculated by
244 subtracting the sea salt contribution to K⁺, Mg²⁺, Ca²⁺, and SO₄²⁻ mass concentration, respectively.
245 Note that the ss-K⁺, ss-Mg²⁺, ss-Ca²⁺, and ss-SO₄²⁻ mass concentrations do not significantly vary
246 with seasons, because of the week dependence on seasons of Na⁺. Conversely, the nss-K⁺, nss-
247 Mg²⁺, nss-Ca²⁺, and nss-SO₄²⁻ mass concentrations are significantly affected by the seasons
248 (significant difference reported in Table 1). The mean nss-K⁺ mass concentration is greater in AW
249 than in SS, which is consistent with the higher contribution of the biomass-burning source related to
250 the residential heating in the colder season (Perrone et al., 2013, 2014b). On the contrary, nss-Mg²⁺
251 and nss-Ca²⁺ concentrations are greater in SS than in AW, as a consequence of the lack of rainy
252 days and some outbreaks of Sahara dust, as they derive from the natural and anthropogenic dust
253 resuspension over the Mediterranean area. In addition, the nss-SO₄²⁻ mass concentration is greater
254 in SS than in AW, as it is partly related to the enhanced photochemistry and low air mass
255 renovation occurring in SS over the Mediterranean region (Perrone et al., 2014a).

256 Tested metals account on average 1.5% of the PM₁₀ mass, with the most abundant Al and Fe, which
257 are mainly associated with crustal matter (Perrone et al., 2014a), accounting on average 1% of PM₁₀
258 mass. In general, the metal mass is slightly greater in SS than in AW, with significant difference for
259 Al, Cd, Cr, Mn, Ni, Ti and V (marked values in Table 1).

260 Concerning organic components, OC and EC are the main components of PM₁₀, as they totally
261 account nearly 30% of PM₁₀ mass, with a clear seasonal trend. In fact, the contributions of OC and
262 EC to PM₁₀ mass are 26% and 10%, respectively, in colder season and 21% and 6% in warmer
263 months, respectively. The discrimination between emissions – EC from carbonaceous matter

264 burning and primary OC (POC) – and secondary processes – secondary organic carbon (SOC) –
265 can be attempted using the OC/EC ratio (Pio et al., 2011). In the study a mean OC/EC value of
266 3.3 ± 1.0 was found, representing SOC enriched environments (e.g., Sandrini et al., 2014).
267 Furthermore, the SOC and POC contributions were quantitatively calculated using the common
268 approach based on the minimum value of the OC/EC mass ratio $(OC/EC)_m$ (Pio et al., 2011; Day et
269 al., 2015). A $(OC/EC)_m$ value of 2.1 was computed, which is characteristic of a suburban area, with
270 lower pollution levels, in comparison with the low $(OC/EC)_m$ ratios (≤ 1) usually found in large
271 cities. Regional and long range transport of non-local SOC have been found to favor the increase of
272 the $(OC/EC)_m$ ratio (Pio et al., 2011) and have likely contributed at the study site (Perrone et al.,
273 2013, 2014a). The computed POC data show a clear season trend with significantly greater values
274 in AW than in SS, following the same seasonality of EC (difference reported in Table 1). In
275 contrast, SOC and OC values are characterized by only a weak season dependence (Table 1). We
276 believe that the good agreement between the seasonal trend of POC and EC, in addition with the
277 uniformity of SOC concentrations, likely support the POC and SOC estimations of this study.
278 The methanesulfonate ion (MSA^-) is determined as a product of the atmosphere oxidation of the
279 gaseous dimethylsulfide produced in seawater. It is greater in SS than in AW, as it is representative
280 of the biogenic activity of the Mediterranean Sea (Perrone et al., 2014a). MSA^- concentration
281 accounts for 0.2 and 0.02% of PM_{10} mass in SS and AW, respectively.
282 Finally, the composition of water soluble OC was investigated in detail by measuring oxalate,
283 acetate, glycolate, propanoate, formate, and pyruvate ions (Table 1). The tested WSOC species
284 represent on average 0.9 and 3.7% of the PM_{10} and OC mass concentration, respectively. Oxalate is
285 the dominating species, with concentration on average nearly 10 time greater than those of the other
286 WSOC species. It shows a clear seasonality with higher level in SS compared with those in AW.
287 Conversely, acetate, formate, and pyruvate show larger concentrations in AW than in SS.

288

289 *3.3. Oxidative potential*

290

291 Oxidative Potential (OP) was measured for all 53 PM_{10} samples with both assays: the reagent
292 depletion rate (OP^{DTT} : $nmol^{DTT} min^{-1}$ and OP^{AA} : $nmol^{AA} min^{-1}$) was normalized by the volume of
293 sampled air (OP^{DTT}_V and OP^{AA}_V expressed as $nmol min^{-1} m^{-3}$) as an exposure metrics accounting
294 for inhaled air. In addition, OP^{DTT} and OP^{AA} were normalized by the PM_{10} mass (OP^{DTT}_m and
295 OP^{AA}_m expressed as $nmol min^{-1} \mu g^{-1}$) to point out the intrinsic ability of the particles to deplete
296 physically relevant antioxidants (Table 2). DTT and AA assays provide similar OP_V values within
297 the same variability range. In fact, the mean OP^{DTT}_V value is $0.24\pm 0.12 nmol^{DTT} min^{-1} m^{-3}$, ranging

298 from 0.02 to 0.53 $\text{nmol}^{\text{DTT}} \text{min}^{-1} \text{m}^{-3}$. The mean $\text{OP}^{\text{AA}}_{\text{V}}$ value is $0.29 \pm 0.18 \text{ nmol}^{\text{AA}} \text{min}^{-1} \text{m}^{-3}$,
299 ranging from 0.03 to 0.69 $\text{nmol}^{\text{AA}} \text{min}^{-1} \text{m}^{-3}$. OP_{m} responses, which are intensive parameters, also
300 show the same variability range for both assays: the $\text{OP}^{\text{DTT}}_{\text{m}}$ mean value is $0.008 \pm 0.004 \text{ nmol}^{\text{DTT}}$
301 $\text{min}^{-1} \mu\text{g}^{-1}$ and the $\text{OP}^{\text{AA}}_{\text{m}}$ mean value is $0.009 \pm 0.006 \text{ nmol}^{\text{AA}} \text{min}^{-1} \mu\text{g}^{-1}$ (Table 2). The measured
302 $\text{OP}^{\text{DTT}}_{\text{V}}$ values are in good agreement with the data reported by Chirizzi et al. (2017) for the same
303 site namely mean $\text{OP}^{\text{DTT}}_{\text{V}}$ value of $0.35 \pm 0.13 \text{ nmol}^{\text{DTT}} \text{min}^{-1} \text{m}^{-3}$ and mean $\text{OP}^{\text{DTT}}_{\text{m}}$ of 0.013 ± 0.005
304 $\text{nmol}^{\text{DTT}} \text{min}^{-1} \mu\text{g}^{-1}$ (PM_{10} samples with total carbon concentrations comparable with the yearly
305 average values).

306 Overall, our measurements of both DTT and AA activity are in the lower end of the typical range
307 observed for ambient particles at other locations, i.e., $\sim 0.2\text{-}2 \text{ nmol}^{\text{DTT}} \text{min}^{-1} \text{m}^{-3}$ and $\sim 0.3\text{-}4 \text{ nmol}^{\text{AA}}$
308 $\text{min}^{-1} \text{m}^{-3}$) (Janssen et al., 2014; Perrone M.G. et al., 2016; Calas et al., 2017; Weber et al., 2018).
309 This is consistent with the location of the study site, which is in a suburban site of the flat Salento's
310 peninsula, in the Central Mediterranean, away from large sources of local pollution. Janssen et al.
311 (2014) assessed OP^{AA} and OP^{DTT} of PM_{10} samples collected from different sites in the Netherlands
312 and found that the OP responses of both assays varied significantly from site to site, because of the
313 change of the particle properties, as the PM_{10} absorbance measurements and the mass
314 concentrations revealed. More specifically, they found markedly higher OP values at the
315 underground train station compared to other outdoor sites. The PM_{10} OP^{AA} values measured from
316 samples collected at urban background, farm, and stop&go sites varied within the variability ranges
317 of this study. Conversely, the corresponding PM_{10} OP^{DTT} values were on average 3-5 times greater
318 than the ones of this study. PM_{10} mass concentrations were within the variability range of the ones
319 of this study.

320 It is noteworthy that, although up to date OP has been more widely measured in $\text{PM}_{2.5}$ than in PM_{10}
321 samples, high correlation was found between OP data in $\text{PM}_{2.5}$ and PM_{10} using different assays. In
322 general, significantly lower $\text{OP}^{\text{DTT}}_{\text{m}}$ values have been found for PM_{10} compared with $\text{PM}_{2.5}$,
323 whereas very similar $\text{OP}^{\text{AA}}_{\text{m}}$ values have been measured for PM_{10} and $\text{PM}_{2.5}$ (Godri et al., 2011;
324 Boogaard et al., 2012; Janssen et al., 2014).

325 In this study, OP responses were measured for PM_{10} samples collected for a long time series over a
326 full year, to give insight into OP seasonal trend to be related with meteorological/atmospheric
327 conditions and particle chemical composition. The daily trend of OP_{V} and OP_{m} values for the 53
328 samples is shown in Fig. 2a-2b, in addition with that of the PM_{10} mass concentration (Fig. 2c). Data
329 below the detection limit have not been reported in Fig. 2a-2b. Figures 2a and 2c reveal that high
330 OP_{V} values are associated with high PM_{10} mass concentrations, indicating that the OP_{V} responses
331 are extensive parameters dependent on PM_{10} mass concentrations. This is described by the good

332 linear correlation of both OP^{DTT}_V and OP^{AA}_V with PM_{10} mass: the Pearson correlation coefficients
333 ($n=53$) are 0.67 ($p < 0.001$) and 0.46 ($p < 0.001$) for OP^{DTT}_V and OP^{AA}_V , respectively. This is in
334 good agreement with the results of Janssen's study (Janssen et al., 2014), that found Spearman
335 correlation coefficients between OP^{DTT}_V and OP^{AA}_V and PM_{10} equal to 0.75 ($p < 0.01$) and 0.47 ($p <$
336 0.05), respectively. Consistently, OP_m responses, show a small variability range through the
337 investigated period for both assays (Fig. 2b).

338 In general, a significant correlation was found between OP^{DTT}_V and OP^{AA}_V ($r= 0.52$, $p < 0.01$, Table
339 3), suggesting that DTT and AA assays have similar associations with the chemical species in the
340 studied PM samples. This is in agreement with some studies (e.g., Mudway et al. 2004; Janssen et
341 al. 2014; Yang et al. 2014), but in contrast with others reporting different sensitivity of the two
342 assays towards the same redox-active species (Fang et al, 2016; Szigeti et al., 2016; Visentin et al.,
343 2016).

344 A detailed inspection of Figures 2a, b reveals that individual OP^{DTT}_V and OP^{AA}_V values vary largely
345 day by day with different trend for the same PM samples, although the similarity of the mean values
346 and the overall good correlation of the data. Such a large variability may be likely ascribed to the
347 specific characteristics of the study site, where both PM concentration and composition day-by-day
348 change, because of the impact of long-range transport from the surrounding anthropogenic and
349 desert regions, and the Mediterranean Sea. (Perrone et al., 2014a, 2014b, 2016; Becagli et al., 2017;
350 Chirizzi et al., 2017). This represents a paper peculiarity, since most of the previous studies were
351 mainly performed at sites impacted by specific quite constant pollution sources, e.g. traffic sites,
352 underground train stations, farms (Mugica et al., 2009; Cheung et al., 2010; Godri et al., 2011;
353 Boogaard et al., 2012; Janssen et al., 2014; Waked et al., 2014; Hellack et al., 2015; Pant et al.,
354 2015; Calas et al., 2017; Weber et al., 2018).

355

356 *3.4. Seasonal variability of Oxidative Potential*

357

358 The seasonal evolution of extrinsic OP_V and intrinsic OP_m activity has been investigated in detail by
359 separately computing the mean values of the AW and SS periods and applying t statistics ($p < 0.05$).
360 Table 2 provides the mean $OP^{DTT}_{V,m}$ and $OP^{AA}_{V,m}$ values for AW and SS days and shows that they
361 are nearly the same in the two seasons. This is in contrast with the finding of Calas et al. (2017) that
362 investigated the seasonal variation of OP^{DTT}_V and OP^{AA}_V values for PM_{10} samples from the city of
363 Chamonix (France). They observed significant differences between cold (late November to late
364 February) and warm (late May to late September) periods. More specifically, the OP^{DTT}_V was two
365 times higher during the cold period compared with the warm period, whilst the OP^{AA}_V was 7.1

366 times higher in the cold period than in the warm period. However, they also found that the PM₁₀
367 mass concentration was 2.6 time higher in the cold than in the warm period, despite the results of
368 this study. The above reported contrasting results likely suggest a seasonal variation of the PM
369 redox-active components dependent on the monitoring site. In general, similar OP^{DTT}_v and OP^{AA}_v
370 seasonality with increase in cold season has been observed for ambient PM_{2.5} samples and has been
371 related to the higher PM_{2.5} mass concentration in comparison with spring-summer samples (Verma
372 et al., 2015; Saffari et al., 2015; Perrone M.G. et al., 2016; Visentin et al., 2016; Fang et al., 2016;
373 Szigeti et al., 2016).

374

375 *3.5. Association of OP_{DTT} and OP_{AA} activity with meteorological parameters, gas pollutants,* 376 *and PM chemical species*

377

378 The role of meteorological properties (rain, RH, T, and Solar Irradiance), gas pollutants (NO₂ and
379 SO₂) and PM chemical components on the oxidation activity was identified and apportioned by
380 computing the linear regression between the OP response and the measured ambient parameters.
381 The regression was conducted on the volume normalized levels of the OP^{DTT} and OP^{AA} values for
382 the whole data set (total) and for the samples collected in AW and SS, respectively. The calculated
383 Pearson's correlation coefficients are given in Table 3.

384 No significant correlation of OP^{AA}_v and OP^{DTT}_v is found with rain and solar irradiance. These
385 results may indicate that the OP response of both assays is not significantly sensitive to particle's
386 wet depositions and to particles whose formation is favoured by high solar irradiance (Seinfeld and
387 Pandis, 2006). Conversely, Perrone M.G. et al. (2016) found that OP^{DTT}_v showed significant
388 correlation with solar irradiance by testing OP values of total suspended particle samples collected
389 at two traffic sites of the Milan city centre (Italy). They assumed that the observed positive
390 correlation could be considered as a proxy for high concentrations of secondary oxidizing organics.
391 We found that OP^{AA}_v shows a positive correlation ($r=0.67$, $p<0.001$) with T in SS, when T values
392 are higher at the study site. Conversely, OP^{DTT}_v shows a positive correlation with RH ($r=0.42$, $p <$
393 0.05) only in AW, when RH values are higher at the study site. These results may likely indicate
394 that high OP^{AA}_v and OP^{DTT}_v values are likely associated with particles whose formation is favoured
395 by high temperature and RH values, respectively (Seinfeld and Pandis, 2006).

396 No significant correlation of OP^{AA}_v and OP^{DTT}_v with SO₂ is found in our study, as shown in Table
397 3. Sulphur dioxide is an acidic gas primarily produced from the combustion of fossil fuel
398 containing sulphur, or from the oxidation of naturally occurring sulphur gases (e.g. volcanic
399 emissions). Multiphase or heterogeneous oxidation of SO₂ have been suggested to potentially play

400 an important role in the atmospheric formation of oxygenated aerosols (Seinfeld and Pandis, 2006),
401 which are expected to be a good source of OP change. The findings of this study could indicate
402 either that the OP activity of both assays was not significantly sensitive to sulphate and sulphate
403 coated particles and/or that on average SO₂ was responsible for the quenching of the hydroxyl
404 radical (OH[•]) generation in the analysed PM₁₀ samples. In fact, Xu et al. (2015) showed that the
405 DTT activity over EC was decreased in the process of SO₂ reduction, with the consumption of
406 oxidative active sites. To our knowledge, no data have been reported in literature on the relationship
407 between SO₂ and the PM-induced oxidative stress, even if it is one of the most monitored gasses in
408 air quality studies. The result of this study is consistent with a recent study that showed the weak
409 health relevance of SO₂ (Pöschl and Shiraiwa, 2015).

410 OP^{AA}_v and OP^{DTT}_v show on average a positive correlation with NO₂ (Table 3) that is a product of
411 atmospheric photochemistry with strong adverse health effect (Janssen et al., 2014; Pöschl and
412 Shiraiwa, 2015). Janssen et al. (2014) found that OP^{DTT}_v and OP^{AA}_v of PM₁₀ samples are correlated
413 with NO₂ ($p < 0.01$) in accordance with the results of this study. In particular, OP^{DTT}_v showed higher
414 correlation for the PM₁₀ fraction than for the PM_{2.5} fraction. In contrast, Perrone M.G. (2016) found
415 for TSP samples that OP^{DTT}_v was negatively correlated with NO₂. Hellack et al. (2015) quantified
416 the traffic-related oxidative potential of PM₁₀ samples by measuring the OH[•] generation ability and
417 found no significant correlation of OH[•] with NO₂. The above reported contrasting results may be
418 likely explained by a quenching effect of PM components on the OH[•] generation and/or cross-
419 correlations between some redox active species and NO₂ concentrations.

420 The contribution of specific PM₁₀ components to oxidative potential was investigated by applying
421 simple linear regression (Pearson's correlation coefficients in Table 3). Overall, both OP^{DTT}_v and
422 OP^{AA}_v responses are broadly correlated with several inorganic species at significance level $p <$
423 0.05, namely ions, such as NH₄⁺, nss-K⁺, nss-Mg²⁺, nss-Ca²⁺, and NO₃⁻, metals, such as Ba, Cd, Ce,
424 Cr, Cu, Fe, Mn, and Pb, and organic components, i.e., OC, POC, EC and WSOC. Similar results are
425 reported by Calas et al. (2017) concerning OP responses and chemical composition of PM₁₀
426 samples of the city of Chamonix (France).

427 The discrimination of the data according seasonality, i.e., AW and SS days, clearly shows a
428 seasonal trend of the correlation coefficients (Table 3), suggesting a seasonal variation of the PM
429 redox-active components affecting the AA and DTT assay response. In autumn-winter, OP^{DTT}_v
430 values are highly correlated ($p < 0.01$) with nss-K⁺, nss-Ca²⁺, Ba, Cd, Ce, Cr, Cu, Fe, Mn, OC, EC,
431 POC, and all the investigated WSOC. These species are the main markers of traffic and/or
432 combustion emissions, which have been found the sources mainly responsible of the adverse effects
433 on human health (Cheung et al., 2010; Godri et al., 2011; Boogaard et al., 2012; Waked et al., 2014;

434 Calas et al., 2017). In fact, Ba, Ce, Cr, Cu, Fe, and Mn are mainly emitted into the atmosphere from
435 the wear from brake lining and tires (Waked et al., 2014; Pant et al., 2015). nss-Ca²⁺ may also
436 represent a traffic tracer, being associated with CaCO₃ from resuspended dust (e.g, Waked et al.,
437 2014). EC, OC, and POC components can be associated with the traffic exhaust source (Viana et
438 al., 2006) and/or with the combustion, including biomass-burning source. The contribution of this
439 last source is also suggested by the strong correlation of OP^{DTT}_v with nss-K⁺ (Waked et al., 2014).
440 Perrone et al. (2013) found for PM_{2.5} samples that OC, EC, and nss-K⁺ were combustion tracers,
441 including the biomass burning source at the study site. The shown dominant contribution of PM₁₀
442 carbon components to OP^{DTT}_v is consistent with literature data, although only few papers have been
443 published to date on coarse PM (Hu et al., 2008; Janssen et al., 2014; Pant et al., 2015; Chirizzi et
444 al., 2017). Table 3 also suggests that the OC contribution to OP^{DTT}_v was mainly due to POC,
445 because of the weak correlation OP^{DTT}_v with SOC.

446 The inter-correlation among the analysed species is investigated in detail, to better highlight
447 similarity in emission sources and/or secondary processes affecting the OP response. The
448 correlation coefficients among the analysed species are reported in Tables 4a and 4b for AW and
449 SS, respectively (positive *r* values significant at *p* < 0.05 for a two-tailed test are reported in bold).
450 Data of Table 4a show that all the species highly correlated with OP^{DTT}_v values are also
451 significantly inter-correlated. Therefore, cross-correlations could be responsible for association of
452 some of the above reported PM₁₀ redox-active components (Table 3).

453 In spring-summer OP^{DTT}_v values are highly correlated (*p* < 0.01) only with NH₄⁺, Cu, OC, EC, and
454 POC. Therefore, the contribution to OP^{DTT}_v of primary carbon components and Cu in ambient PM₁₀
455 samples is also observed in SS. Calas et al. (2017) also found that NH₄⁺ drives OP^{DTT}_v, in
456 accordance with the results of this study. The high correlation of NH₄⁺ with nss-SO₄²⁻, oxalate, Ni,
457 and V (Table 4b) indicates that NH₄⁺ was associated with a secondary sulfate source (e.g. Waked et
458 al., 2014; Calzolari et al., 2015). NH₄⁺ and nss-SO₄²⁻ are markers of secondary photochemical
459 formation. Their contribution is greater in SS than in AW at the study site (Table 1), because of the
460 enhanced photochemistry, which favours the formation of secondary aerosols, and the prevalent
461 stagnant conditions. The sulphate source has been found health relevant by Pöschl and Shiraiwa
462 (2015). Table 4b shows that Cu, OC, EC, and POC are highly inter-correlated in SS, but their
463 correlation with nss-K⁺, nss-Ca²⁺, Cd, Ce, Cr, Fe, Mn, and WSOC, respectively, is rather weak in
464 SS, in contrast to the AW findings. These last results suggest that the main sources of Cu, OC, EC,
465 and POC likely change from AW to SS and/or that the prevalent stagnant conditions occurring in
466 SS over the Mediterranean basin favor the particle's aging.

467 The high correlation ($p < 0.01$) in AW of OP^{AA}_V with $nss-K^+$, Ba, Ce, Cr, Cu, Fe, Mn, OC, EC, POC,
468 and acetate, glycolate, formate, and the high correlation among the species (Table 4a), allow
469 inferring that the OP^{AA}_V responses are mainly effected by the same species related to traffic and/or
470 combustion sources as the OP^{DTT}_V assay. Calas et al. (2017) also reported similar results. As
471 mentioned, PM_{10} traffic related redox active metals, i.e., Ce, Cr, Cu, Fe, Mn, play a predominant
472 role in driving ROS production, as found by other authors (Godri et al., 2010; Janssen et al., 2014).
473 The OP^{AA}_V responses measured in spring-summer are correlated with fewer PM_{10} chemical
474 components than in AW. In fact, OP^{AA}_V values are correlated with NH_4^+ , $nss-K^+$, $nss-Mg^{2+}$, $nss-$
475 Ca^{2+} , $nss-SO_4^{2-}$, Cu, Mn, P, Pb, and oxalate in SS. The strong inter-correlation ($p < 0.01$, Table 4b)
476 between NH_4^+ , $nss-SO_4^{2-}$, and oxalate suggests that these ions are associated with the common
477 ammonium sulphate source, as mentioned. The correlation of OP^{AA}_V with $nss-K^+$, $nss-Mg^{2+}$ and
478 $nss-Ca^{2+}$, Cu, Mn, P, and Pb, may be related to the resuspended soil source, which is driven either
479 by the vehicular traffic and/or by the long-range transport of Sahara dust particles (e.g. Calzolari et
480 al., 2015). The high contribution of this source to OP in SS months may be explained by the lack of
481 rainfall and the prevalence of stagnant conditions that favour the re-entrainment of road dust, which
482 also is a source of metals. To date, contrasting data have been found on negative health effect of
483 Saharan dust, as Perez et al. (2008) found that Saharan dust outbreaks, which daily increased PM_{10-}
484 $_{2.5}$ of $10 \mu g/m^3$, enhanced daily mortality by 8.4 compared with 1.4%, during non-Saharan dust
485 days. Otherwise, a recent paper of Chirizzi et al. (2017) in the study area indicates that Saharan dust
486 transported from Africa has lower intrinsic OP^{DTT}_m values with respect to the other sources acting
487 on the area (for water-soluble fraction).

488 Note from Table 3 that in SS OP^{AA}_V is weakly correlated with OC, EC, and POC, respectively, in
489 contrast to the AW results. Moreover, Table 3 shows that the correlation coefficient of OP^{DTT}_V with
490 OC, EC, and POC, respectively, decreases from AW to SS. As mentioned, Xu et al. (2015) showed
491 that the oxidation of SO_2 consumed active sites on O_3 -aged BC particles, which resulted in reduced
492 oxidative potential. Consequently, the DTT activity decreased with increasing sulphate production,
493 according to Xu et al. (2015). Note that the sulphate production is greater in SS than in AW (Table
494 2) and also that the SS stagnant conditions may favour the aging of carbonaceous particles at the
495 study site. Consequently, the SS decrease of active sites on carbonaceous particles is likely
496 responsible for the weaker correlation of OP^{DTT}_V and OP^{AA}_V with carbonaceous particles found in
497 SS. Such a hypothesis is also supported by the weak correlation in SS of OC and EC with some PM
498 species linked to combustion and/or traffic sources.

499 Table 3 also shows that negative correlations are found between OP values and some chemical
500 species. Both OP^{DTT}_V and OP^{AA}_V are negatively correlated with Na^+ and Cl^- , respectively, in AW

501 (Table 3) and Table 4a shows that the PM redox-active components affecting the AA and DTT
502 assay responses in AW, are negatively correlated with both Na^+ and Cl^- , respectively. These last
503 results further show that it is important looking for cross-correlations to better identify redox active
504 species as well as to explain contrasting results.

505

506 4. Conclusions

507

508 An extended study on the PM_{10} induced oxidative potential using the DTT and AA assays has been
509 presented in the paper. The long PM_{10} collection duration – ten months – and the detailed chemical
510 speciation of the samples – more than 40 species – made it possible to highlight the day-by-day
511 variability and seasonal evolution of OP and relate them with the PM chemical properties. A
512 marked temporal variability was found, that represents a peculiarity of the study site, characterized
513 by different aged/fresh aerosol types delivered by the long-range transport from the surroundings,
514 i.e., anthropogenic and desert areas and the Mediterranean Sea.

515 On this basis, the following conclusions can be drawn:

516 • The OP^{DTT} and OP^{AA} responses from the two acellular assays are very similar in mean values and
517 variability range, but differ in the association with PM_{10} chemical composition, as well as in
518 seasonality of such association. This information, combined with inter-correlation between the
519 investigated chemical species, made possible to identify sources and atmospheric processes
520 responsible for the PM oxidative potential.

521 • $\text{OP}^{\text{DTT}}_{\text{v}}$ of AW samples was well correlated with tracers of the resuspended reacted dust source,
522 vehicle-related metals, and EC and OC associated with combustion sources, while the $\text{OP}^{\text{DTT}}_{\text{v}}$ of SS
523 samples was correlated only with NH_4^+ , Cu, EC and POC.

524 • $\text{OP}^{\text{AA}}_{\text{v}}$ of AW samples was strongly positively correlated with the main tracers of traffic and/or
525 combustion emissions. Conversely, in SS, $\text{OP}^{\text{AA}}_{\text{v}}$ was mainly correlated with species related with
526 secondary aerosol (i.e., nss-SO_4^{2-} and oxalate) and resuspended soil from vehicular traffic and/or
527 long-range transport of Sahara dust (i.e., nss-Mg^{2+} , nss-Ca^{2+} , nss-SO_4^{2-} , Cu, Mn, P, Pb).

528 • All the species highly correlated with $\text{OP}^{\text{DTT}}_{\text{v}}$ and $\text{OP}^{\text{AA}}_{\text{v}}$ values were also significantly inter-
529 correlated, suggesting that cross-correlations could be responsible for the association of some of the
530 above reported PM_{10} redox-active components. This last result highlights that it is important to look
531 for cross-correlations between the different species to properly identify the redox active species
532 related to OP as well as to explain contrasting results reported in literature. In fact, we found that
533 the negative correlations observed on AW and SS between the $\text{OP}^{\text{DTT}}_{\text{v}}$ and $\text{OP}^{\text{AA}}_{\text{v}}$ values and Na^+

534 and Cl^- , respectively, could be due to some of the PM redox-active components affecting the AA
535 and DTT assay responses, which were negatively correlated with Na^+ and/or Cl^- , respectively.
536 Overall, this study has provided insight into the relative contribution of toxicologically-relevant
537 components in the Central Mediterranean. It is clear that further studies are required to elucidate the
538 association between the response of acellular models and in vivo interactions with cellular/tissue
539 matrix of biological systems, through which PM can elicit oxidative stress. Anyway, the obtained
540 information are vital in designing more straight-forward approaches to air quality management and
541 emission control technologies focused on specific components and sources.

542

543 **Acknowledgments**

544

545 S. Romano has carried out this work with the support of a PhD fellowship from the Mathematics
546 and Physics Department of the Salento University. The financial support for EARLINET in the
547 ACTRIS Research Infrastructure Project by the European Union's Horizon 2020 research and
548 innovation programme under grant agreement n. 654169 and previously under grant agreement n.
549 262254 in the 7th Framework Programme (FP7/2007-2013) is gratefully acknowledged.

550 Authors thank Dr. I. Bertoli from Department of Chemical and Pharmaceutical Sciences of
551 University of Ferrara for experimental work concerning OP measurements and P. Martano from
552 ISAC-CNR for providing solar irradiance measurements.

553

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823 **Figure captions**

824

825 **Figure 1.** Geographical location of the monitoring site (full dot) in the Mediterranean basin.

826

827 **Figure 2.** Temporal evolution of responses of DTT and AA assays and PM₁₀ concentration during
828 the study periods. Black bars: responses of DTT assay, OP^{DTT}; white bars: responses of AA assay,
829 OP^{AA}; gray bars: PM₁₀ mass concentration. 2a) daily evolution of the volume normalized OP_v
830 values, 2b) daily evolution of the mass normalized OP_m values, 2c) daily evolution of the PM₁₀
831 mass concentration.

832 **Table 1.** Measured values of meteorological parameters and species concentrations: mean and
833 standard deviation values were computed for the whole monitoring period (All Days) and for
834 autumn-winter (AW, December-March, 28 days) and spring-summer (SS, May-October, 25 days),
835 separately. The mass concentration of chemical species is in ng m^{-3} , unless differently specified.
836 The confidence interval (IC) of AW and SS mean values is also given in the table: significant
837 ($p<0.05$) difference between the seasons are marked by *.
838

	Species	All Days		AW Days			SS Days		
		Mean	SD	Mean	SD	IC	Mean	SD	IC
Meteo parameters	Rain (mm)	0.5	2.0	0.8*	2.0	0.9	0.1*	0.5	0.2
	RH (%)	75	11	80	11	4	69	8	3
	Temperature ($^{\circ}\text{C}$)	15	8	8*	3	1	22*	3	1
	Solar Irradiance (Wm^{-2})	185	98	113*	46	18	266*	74	30
Gas pollutant	NO_2 ($\mu\text{g m}^{-3}$)	31	16	39*	16	6	21*	8	3
	SO_2 ($\mu\text{g m}^{-3}$)	2.8	1.4	2.0	1.6	0.6	2.9	1.1	0.4
PM mass concentration	PM_{10} ($\mu\text{g m}^{-3}$)	33	13	36	15	6	29	10	4
PM chemical species	Na^+	647	374	602	440	174	698	283	114
	NH_4^+	478	274	446	289	114	514	258	104
	K^+	457	334	605*	385	152	290*	146	59
	ss- K^+	25	14	23	17	7	26	11	4
	nss- K^+	432	341	582*	394	156	264*	148	60
	Mg^{2+}	146	68	117*	51	20	178*	71	29
	ss- Mg^{2+}	77	44	71	51	20	83	33	13
	nss- Mg^{2+}	69	58	45*	38	15	96*	64	26
	Ca^{2+}	671	468	381*	245	97	997*	445	180
	ss- Ca^{2+}	25	14	23	17	7	26	11	4
	nss- Ca^{2+}	647	468	358*	251	99	970*	445	180
	Cl^-	326	440	426	548	217	214	239	96
	NO_2^-	53	8	53	8	-	-	-	-
	NO_3^-	2349	1799	2511	1801	712	2167	1817	734
	SO_4^{2-}	3145	1623	2341*	1137	450	4045*	1631	659
	ss- SO_4^{2-}	157	91	146	106	42	169	69	28
	nss- SO_4^{2-}	2988	1631	2196*	1134	449	3876*	1663	672
	F^-	0.9	1.8	0.4	0.5	0.2	1.4	2.3	0.9
	MSA^-	29	34	7*	4	2	54*	36	14
	Al	123	131	41*	47	19	212*	134	54
	Ba	6	3	6	4	1	5	2	0.7
	Cd	0.26	0.3	0.4*	0.3	0.1	0.1*	0.1	0.05
	Ce	0.15	0.06	0.15	0.06	0.02	0.15	0.06	0.02
	Co	0.4	0.7	0.4	0.9	0.3	0.4	0.5	0.2
	Cr	1.9	0.7	1.5*	0.5	0.2	2.3*	0.6	0.3
	Cu	8	5	10	6	2	7	3	1
	Fe	248	118	220	122	48	279	106	43
	La	0.07	0.05	0.04	0.03	0.01	0.09	0.05	0.02
	Mn	4.4	2.5	3.2*	1.3	0.5	5.9*	2.7	1.1
	Mo	2.6	1.7	2.9	1.7	0.7	2.3	1.6	0.65
	Ni	3.2	1.6	2.6*	1.1	0.4	3.9*	1.8	0.6
	P	27	15	17*	7	3	39*	14	6
Pb	14	35	19	46	18	8	12	5	
Sr	2.0	1.2	1.6	0.8	0.3	2.4	1.4	0.5	
Ti	4.1	3.9	1.5*	1.3	0.5	6.9*	4.0	1.6	
V	3.8	3.8	1.7*	1.0	0.4	6.2*	4.4	1.8	
Zn	54	29	60	32	13	48	23	9	
OC ($\mu\text{g m}^{-3}$)	7.9	5.3	9.4	6.4	2.5	6.1	3.3	1.3	
EC ($\mu\text{g m}^{-3}$)	2.7	2.1	3.6*	2.5	1.0	1.7*	0.7	0.3	
OC/EC	3.3	1.0	2.9	0.7	0.3	3.7	1.2	0.5	
SOC ($\mu\text{g m}^{-3}$)	2.1	2.1	1.7	1.9	0.7	2.5	2.3	0.9	
POC ($\mu\text{g m}^{-3}$)	5.8	4.5	7.7*	5.4	2.1	3.7*	1.5	0.6	
Water Soluble OC	Oxalate	217	100	168*	75	30	272*	97	39
	Acetate	20	15	30*	16	6	11*	6	2
	Glycolate	23	10	20	10	4	25	10	4
	Propionate	3	1.7	3.1	1.3	0.5	8.1	-	-
	Formate	28	12	32*	13	5	22*	8	3
	Pyruvate	9.3	2.4	10.2*	2.3	0.9	7.6*	1.7	0.7
	Undetermined Mass	11101	7141	14200*	6854	2711	7629*	5822	2352

839 **Table 2.** Oxidative Potential (OP) values measured with DTT (OP^{DTT}) and AA assays (OP^{AA}):
 840 mean and standard deviation values were computed for the whole monitoring period (all days) and
 841 for autumn-winter (AW, December-March, 28 days) and spring-summer (SS, May-October, 25
 842 days), separately. The confidence interval (IC) of AW and SS mean values is also given in the table:
 843 significant ($p < 0.05$) difference between the seasons are marked by *.
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Oxidative Potential	All Days		AW Days			SS Days		
	Mean	SD	Mean	SD	IC	Mean	SD	IC
OP^{DTT}_v ($nmol^{DTT} \text{ min}^{-1} \text{ m}^{-3}$)	0.24	0.12	0.26	0.15	0.06	0.22	0.09	0.04
OP^{AA}_v ($nmol^{AA} \text{ min}^{-1} \text{ m}^{-3}$)	0.29	0.18	0.33	0.17	0.07	0.24	0.19	0.07
OP^{DTT}_m ($nmol^{DTT} \text{ min}^{-1} \mu\text{g}^{-1}$)	0.008	0.004	0.007	0.003	0.001	0.008	0.004	0.002
OP^{AA}_m ($nmol^{AA} \text{ min}^{-1} \mu\text{g}^{-1}$)	0.009	0.006	0.010	0.005	0.002	0.008	0.007	0.003

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853 **Table 3.** Correlation between OP^{DTT}_V and OP^{AA}_V values with measured parameters: Pearson
 854 correlation coefficients (r) computed for the whole monitoring period (all days, 53 data) and for
 855 autumn-winter (AW, 28 data) and spring-summer (SS, 25 data), separately. For a two tailed test ***
 856 indicate excellent correlation ($p < 0.01$), **denotes values with $p < 0.02$, and * denotes values with
 857 $p < 0.05$.

Species		All days		AW days		SS days	
		OP^{DTT}_V	OP^{AA}_V	OP^{DTT}_V	OP^{AA}_V	OP^{DTT}_V	OP^{AA}_V
Meteo parameters	Rain	-0.25	-0.01	-0.32	-0.04	-0.28	-0.26
	RH	0.34	0.18	0.42*	0.31	-0.02	-0.23
	Temperature	-0.19	-0.12	-0.33	-0.12	0.35	0.67***
	Solar Irradiance	-0.13	-0.12	0.16	-0.06	-0.13	0.25
Gas pollutant	NO ₂	0.55 ***	0.29*	0.63 ***	0.23	0.28	-0.08
	SO ₂	-0.12	-0.25	-0.18	-0.34	0.05	-0.11
PM mass concentration	PM ₁₀	0.67 ***	0.46***	0.81***	0.51***	0.26	0.34
PM chemical species	Na ⁺	-0.51	-0.28	-0.6	-0.38*	-0.16	-0.08
	NH ₄ ⁺	0.20	0.07	0.15	-0.23	0.40*	0.49**
	nss-K ⁺	0.73 ***	0.50***	0.86***	0.53***	0.24	0.42*
	nss-Mg ²⁺	0.15	0.22	0.37	0.24	0.22	0.47**
	nss-Ca ²⁺	0.12	0.14	0.50***	0.30	0.24	0.49**
	Cl ⁻	-0.34	-0.17	-0.40*	-0.14	-0.43	-0.56
	NO ₂ ⁻	-0.27	-0.95	-0.27	-0.95***	---	---
	NO ₃ ⁻	0.39***	0.26	0.39*	0.11	0.39	0.38
	nss-SO ₄ ²⁻	-0.01	0.03	-0.04	-0.27	0.30	0.51***
	MSA ⁻	-0.14	-0.06	0.02	0.00	-0.04	0.23
	Al	-0.14	-0.03	-0.01	0.13	-0.03	0.25
	Ba	0.56***	0.47***	0.62***	0.57***	0.26	0.24
	Cd	0.44***	0.14	0.56***	0.14	-0.07	-0.18
	Ce	0.16	0.18	0.37	0.77***	0.10	0.18
	Co	-0.28	-0.23	-0.30	-0.24	-0.19	-0.22
	Cr	0.21	0.10	0.50***	0.49***	0.28	0.14
	Cu	0.63***	0.62***	0.64***	0.74***	0.49**	0.42*
	Fe	0.42***	0.44***	0.63***	0.71***	0.20	0.34
	La	-0.15	0.04	-0.16	0.12	0.13	0.20
	Mn	0.12	0.23	0.59***	0.53***	0.08	0.44**
	Mo	0.00	0.12	0.01	0.14	-0.12	0.00
	Ni	-0.05	0.02	0.07	0.06	-0.02	0.17
	P	0.00	0.12	0.37	0.47**	0.10	0.45**
	Pb	0.35	0.12	0.38	-0.02	0.13	0.63***
	Sr	-0.13	0.05	-0.13	0.01	-0.07	0.21
	Ti	-0.10	0.02	0.09	0.20	0.02	0.34
	V	-0.04	-0.01	0.17	-0.09	0.10	0.27
	Zn	0.15	0.21	0.20	0.13	-0.11	0.23
	OC	0.79***	0.42***	0.86***	0.54***	0.53***	0.12
	EC	0.79***	0.52***	0.87***	0.61***	0.63***	0.38
	SOC	0.32*	-0.04	0.38	0.08	0.37	-0.07
	POC	0.79***	0.52***	0.88***	0.61***	0.63***	0.38
	Water Soluble OC	Oxalate	0.26	0.31*	0.51***	0.20	0.37
Acetate		0.60***	0.44***	0.76***	0.52***	-0.02	0.19
Glycolate		0.45***	0.37***	0.79***	0.68***	0.10	0.24
Propionate		0.49***	0.22	0.51***	0.43		
Formate		0.56***	0.50***	0.73***	0.55***	-0.02	0.35
Pyruvate		0.26	0.16	0.31	0.41	-0.54	-0.92

Figure 1_rev

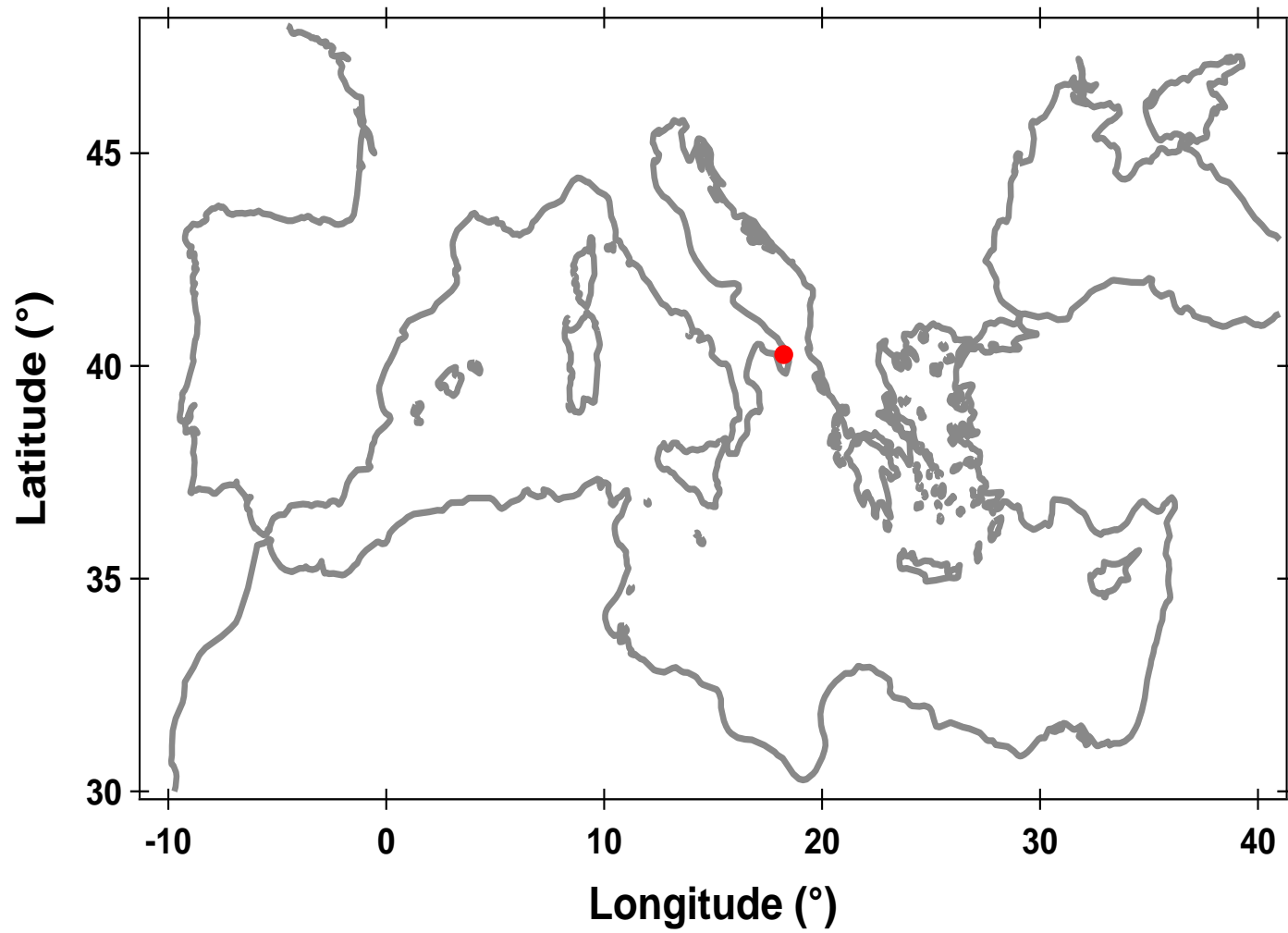
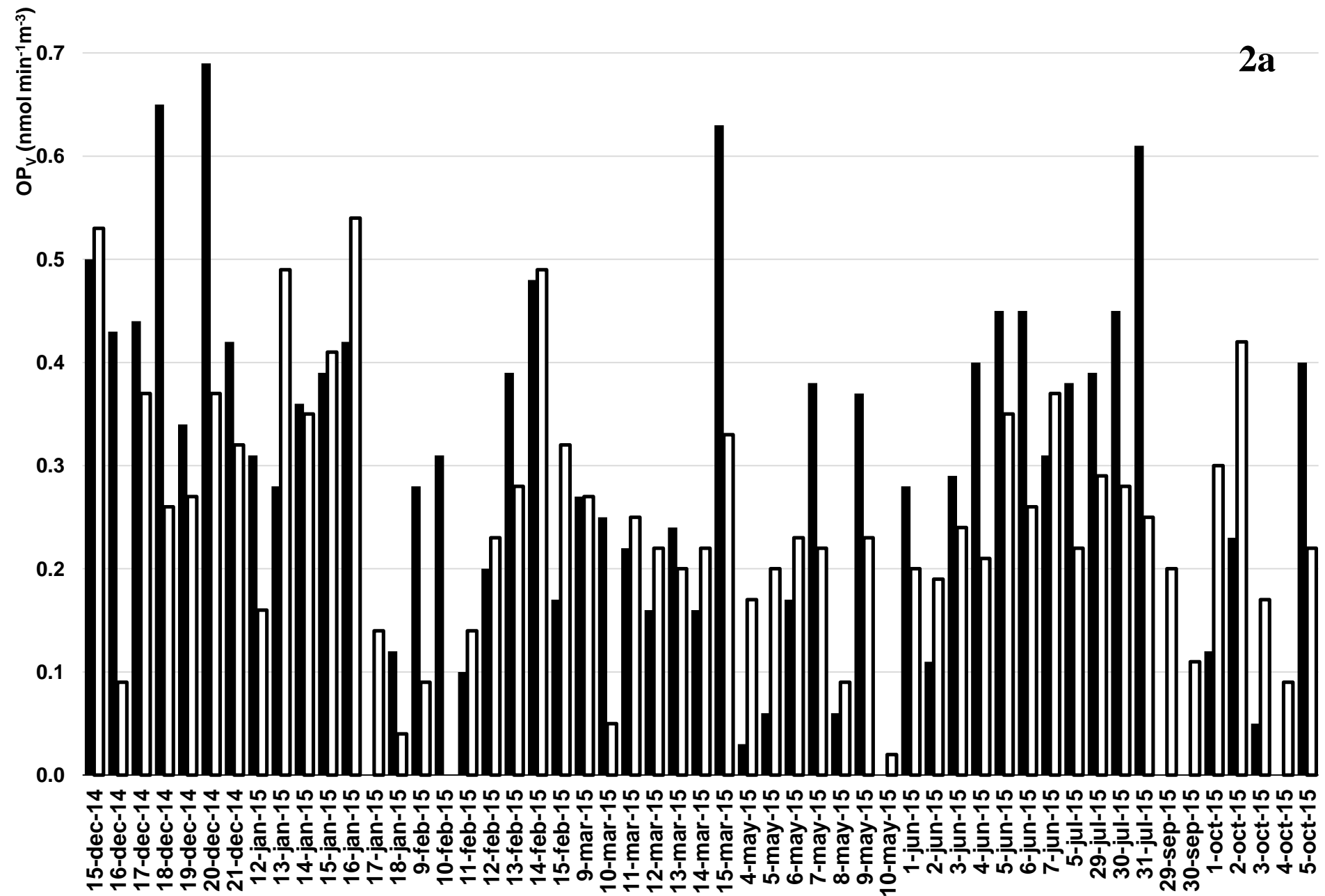
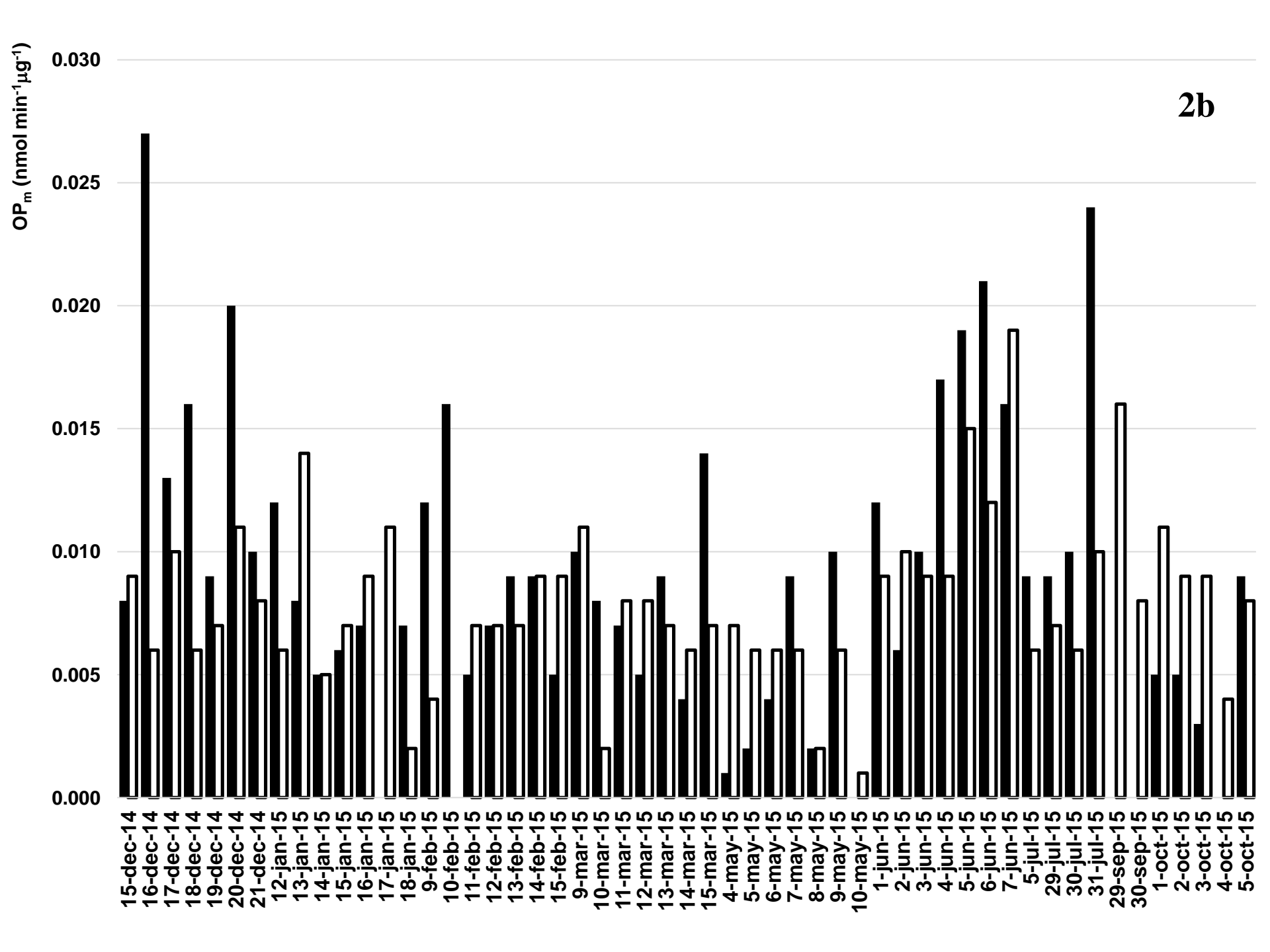
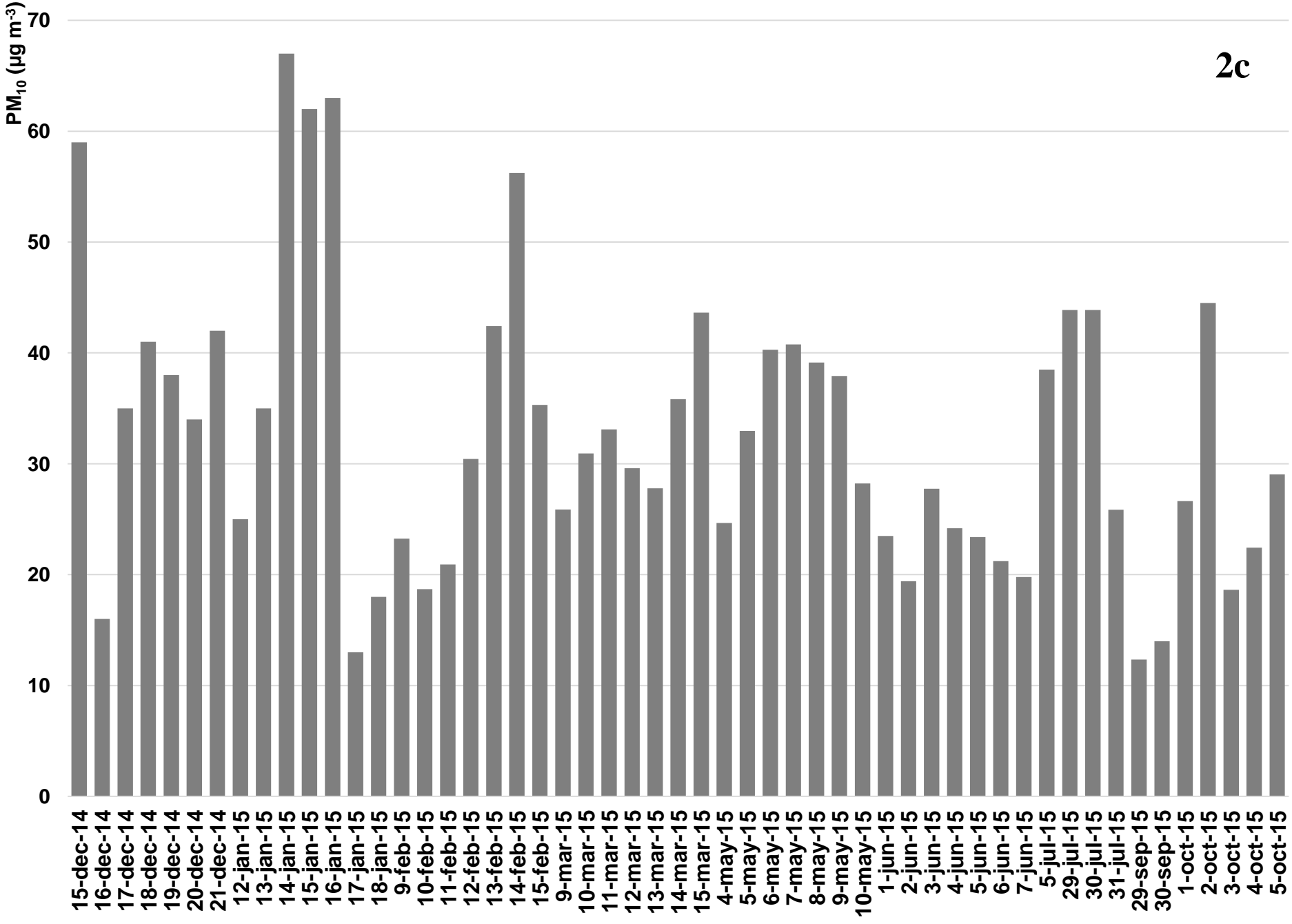


Figure 2_rev



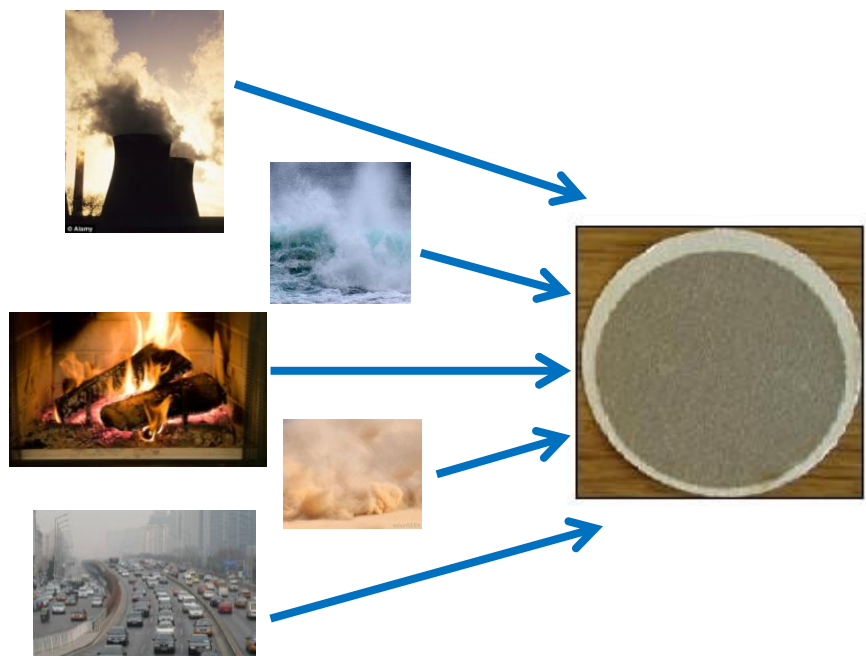


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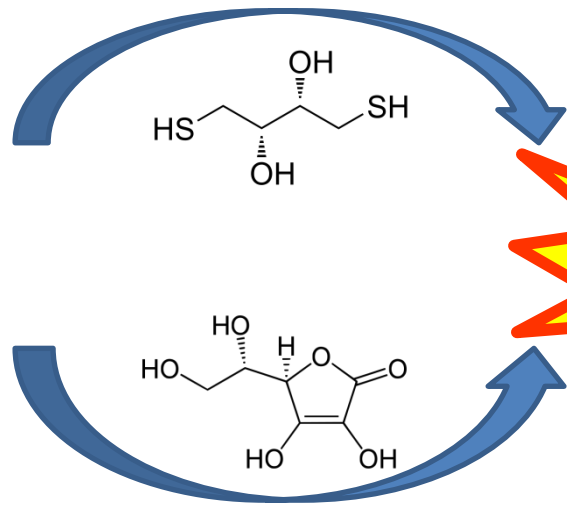


Highlights

- Oxidative potential is assessed for PM₁₀ collected at a Central Mediterranean Site.
- Two cell-free assays yield similar OP_{DTT} and OP_{AA} responses.
- Association of OP_V^{AA} and OP_V^{DTT} with PM chemical components varies with seasons.
- Metals and primary organic carbon are the main responsible for PM-induced OP.



Dithiothreitol assay



Ascorbic Acid assay



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