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**PM chemical composition and oxidative potential of the soluble fraction of particles at two sites in the urban area of Milan, Northern Italy**

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(Article begins on next page)



## UNIVERSITÀ DEGLI STUDI DI TORINO

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## Highlights

- Concentrations of traffic-related PM chemical species at LEZ site were lower than at TR site
- The oxidative potential ( $OP^{DDT}$  and  $OP^{DCFH}$ ) of PM was comparable at TR and LEZ
- OP methods (DDT and DCFH) differed in respect to correlation with PM composition
- $OP^{DDT}$  and  $OP^{DCFH}$  can provide complementary information about the oxidative capacity of particles

# 1 **PM chemical composition and oxidative potential at a traffic site and in** 2 **a low emission zone in Milan (Northern Italy)**

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## 14 **Abstract**

15 Recent epidemiological evidence support the hypothesis that health effects from inhalation of  
16 ambient particulate matter (PM) are governed by more than just PM mass, since specific chemical  
17 components have been identified as important contributors to mortality and hospitality  
18 admissions, even when these end points are unrelated to PM mass.

19 We studied the chemical composition and the oxidative potential (OP) of PM samples from Milan  
20 at two sites which were differently impacted by traffic source: a traffic site (TR) and a limited  
21 emission zone site (LEZ), which located outside and inside the restricted traffic area of Milan,  
22 separately. Two a-cellular assays; dithiothreitol (OP<sup>DTT</sup>) and 2,7 dichlorofluorescein (OP<sup>DCFH</sup>) were  
23 used to characterize the OP of PM.

24 PM samples from LEZ site showed significantly lower concentrations of traffic-related chemical  
25 components compared to TR site. The decrease in the concentrations from TR to LEZ was  
26 maximum for elemental carbon (EC), with a LEZ/TR ratio of 0.64 ( $\pm 0.18$ ), and a significant  
27 reduction ( $p < 0.01$ ) was also observed for polycyclic aromatic hydrocarbons (LEZ/TR =  $0.73 \pm 0.16$ ),  
28 elements (Mn, Cu, Zn, Cd, Pb: LEZ/TR ranged between 0.64-0.82), OC (LEZ/TR =  $0.85 \pm 0.12$ ) and  
29  $\text{NH}_4^+$  ( $0.92 \pm 0.07$ ).

30 OP values, expressed as OP/m<sup>3</sup>, were comparable between sites both for OP<sup>DTT</sup> (TR =  $0.15 \pm 0.06$ ;  
31 LEZ =  $0.15 \pm 0.07$ ) and OP<sup>DCFH</sup> (TR =  $0.24 \pm 0.13$ ; LEZ =  $0.20 \pm 0.11$ ), thus not showing any significant  
32 difference in the oxidative potential of particles from TR and LEZ. OP<sup>DTT</sup> and OP<sup>DCFH</sup> showed  
33 contrasting seasonal and daily trends, and the two OP assays resulted to be sensitive to different  
34 chemical properties of Milan PM samples. We found that OP<sup>DTT</sup> correlated positively only with  
35 Global Radiation (Spearman's  $r_s = 0.38$ ,  $p < 0.05$ ), which could be considered as a proxy for high  
36 concentrations of secondary oxidizing organics, while OP<sup>DCFH</sup> was related to various PM chemical

37 species, mainly correlating with PM total mass ( $r_s = 0.65$ ;  $p < 0.01$ ) and elements (e.g. Zn,  $r_s = 0.67$ ; As,  
38  $r_s = 0.65$ ;  $p < 0.01$ ).

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40 **Keywords:** Particulate matter, chemical speciation, urban area, redox activity, DTT, DCFH

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## 1. Introduction

Air quality is a serious problem for the effects on population health, mainly in urban areas where anthropic activity and sources are concentrated. The Northern Italy is a known hotspot for atmospheric pollution levels, and Milan, the biggest city of Northern Italy, is characterized by high PM levels which are often above limit and quality values indicated by the European Union (EU) air quality Directive 2008/50/EC for PM<sub>10</sub> and PM<sub>2.5</sub> mass concentrations (Perrone et al., 2012).

Traffic is the major PM primary source in Milan, and previous studies have evaluated the effectiveness of various policies already undertaken or to be undertaken in the Province of Milan to limit traffic emissions of PM and NO<sub>x</sub> (Chiesa et al., 2014). The establishment of limited emission zones (LEZ) is one of the measures introduced in an attempt to reduce traffic emissions in urban areas, driving toward lower concentrations of PM. Since 2008 the Municipality of Milan has established a LEZ which permanently restricts the most polluting vehicles entering the historic city centre. Despite LEZ have become regarded as an important way to improve urban air quality, and there are thought to be approximately 200 LEZ currently in existence in Europe (Sadler and Consultants Ltd, 2015), there are very few experimental studies quantifying their impact on air quality, as recently well reviewed by Holman et al. (2015). In Milan, only one study had been done so far by using monitored data to assess if PM mass concentration and black carbon (BC), a component of PM and marker of primary exhaust source, reduce inside the LEZ area compared to outside due to a reduction in the local traffic source (Invernizzi et al., 2011). According to Invernizzi et al. (2011) there isn't any difference in PM mass concentration, but a significant reduction of BC concentrations was found from outside to inside the LEZ. When speaking about PM pollution, an important matter besides the PM mass concentration, is the chemical composition of particles, since it has been demonstrated the relation between PM physico-chemical properties and the biological effects induced by particles (Lippman and Chen, 2009). PM chemical composition, influenced both by sources and atmospheric processes, can vary significantly in time and space, and various studies have investigated as also the observed adverse health effects induced by exposure to PMs from different sampling periods and sites are different (Nawrot et al., 2009; Perrone et al., 2013).

The induction of oxidative stress by ambient PM is considered to play a central role in the adverse effects of particles on human health (Araujo et al., 2009). The ability of particle components to participate in a redox cycle has been proposed to be a key mechanism for the generation of ROS (reactive oxygen species) which cause the oxidative stress in cellular systems (Nel et al., 2006). The hazard posed by particles cannot be explained by a single parameter, however the redox properties, referred to as the oxidative potential (OP) of particles, are considered to be a promising and integrative metric for assessing the health effects induced by particles, also providing hazard information for risk assessment purposes (Borm et al., 2007). In this context, different a-cellular tests have been

82 developed to measure the OP of PM, and various studies tested a-cellular assays to  
83 investigate the oxidative potential of ambient particles (Charrier et al. 2012; Sauvain et  
84 al., 2013; Yang et al. 2014).

85 In this work we investigated the variations in the chemical composition and OP of PM  
86 samples from the urban area of Milan (2013). In particular we studied the effect of local  
87 traffic source on both the chemical composition and the OP values of particles by  
88 sampling total suspended particles (TSP) at two different sites in Milan: a site affected by  
89 local traffic source (TR site), and a site with a reduced impact of traffic within LEZ (LEZ  
90 site). The chemical composition of PM was analyzed in detail including: the carbonaceous  
91 fraction, inorganic ions, elements, and trace organic compounds, for a total of 57  
92 chemical species measured in the PM samples of Milan. The OP of particles was assessed  
93 by two different chemical assays (a-cellular methods): the dithiothreitol (DTT) assay and  
94 the 2,7 dichlorofluorescein (DCFH) assay.

95 The main aim of our study was to analyze i) if there are significant differences in the  
96 concentration of PM mass and chemical components, and in the OP values at the TR and  
97 the LEZ site (PTS samples were collected simultaneously at sites, and daily values  
98 compared); ii) which differences there are in the OP measures from two independent  
99 chemical methods, that is OP measured by DTT ( $OP^{DTT}$ ) and by DCFH ( $OP^{DCFH}$ ) assay; iii) the  
100 relation between the chemical composition of PM samples and their OP, both  $OP^{DTT}$  and  
101  $OP^{DCFH}$  (PTS samples were collected during different seasons at TR, presenting an high  
102 chemical compositional and OP variability).

103 So far we didn't find experimental studies which provided such detailed chemical  
104 characterization of PM samples with the objective to evaluate the effect of the reduction  
105 of traffic source within LEZ on PM pollution and composition. Also, there are few studies  
106 which analyzed so many PM chemical components which related to the oxidative  
107 potential of particles (Janssen et al., 2014; Charrier et al., 2015; Yang et al., 2015,).

## 109 **2. Material and Methods**

### 111 **2.1 The sampling sites**

112 PM was sampled at 2 locations in Milan, with different traffic source characteristics.

113 The two sites are: a traffic site (TR), located within the Campus of the University of Milano  
114 Bicocca, about 10 meters away a crossroad (Viale Sarca-Viale Chiese); a limited traffic site  
115 in the city centre within the low emission zone (LEZ) (Figure 1). The LEZ site was located in  
116 the courtyard of National Museum of Science and Technology "Leonardo da Vinci" of  
117 Milan, at a distance of about 15 meters away from the traffic road (Via San Vittore).

118 The Municipality of Milan established for the first time in 2008 a LEZ, restricting certain  
119 vehicles entering a 8.2 km<sup>2</sup> area in the historic city centre, known as the Ecopass zone. At  
120 the end of 2011 the scheme was replaced by a combined LEZ and urban road charging  
121 scheme (congestion charge) known as "Area C" (Comune di Milano, 2015). The activation

122 time is during all weekdays from 7.30 a.m. to 19.30 p.m. (Thursday from 7.30 a.m. to  
123 18.00 p.m.). During the activation time, the access to LEZ is forbidden to pre Euro 1  
124 gasoline and pre Euro 3/III diesel vehicles, and it is regulated by the payment of the  
125 congestion charge for other vehicles.

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127 *(approximate location of Figure 1)*

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## 2.2 Particle sampling

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The sampling campaigns were conducted during 2013 in 3 seasonal periods at the TR site:  
16th-24th January, 8th-18th July, 02th-15th October. During January and July the PM was  
collected by daily samples (24 h). In October, the sampling was simultaneously performed  
at both TR and LEZ, during working days, from 8.00 a.m. to 18.00 p.m. (daytime 10-hour  
sampling), when traffic limitation inside the LEZ was implemented.

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PM samples were taken using two identical high-volume samplers ( $200 \text{ l min}^{-1}$ , ECHO-PUF,  
TCR Tecora, Milan, Italy), collecting the total suspended particles (TSP) on a quartz fiber  
filter (QFF; diameter 102 mm; Whatman, USA; baked at  $600^\circ\text{C}$  for 2 h to reduce the  
impurities). Before and after sampling, filters were equilibrated (48 h at 35% RH, ambient  
T) and weighted with a microbalance ( $10 \mu\text{g}$  precision) in order to measure TSP  
concentration in ambient (unit:  $\mu\text{g m}^{-3}$ ). All sampled filters were kept in the dark at  $-20^\circ\text{C}$   
(to avoid photo-degradation and evaporation) until the chemical and oxidative potential  
analysis. In order to guarantee the quality of the PM measurements and analysis, totally  
six blank field filters were used during these campaigns (2 In January, 2 in July and 2 in  
October).

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## 2.3 Sample preparation

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Composition of PM was measured in detail, including the carbonaceous fraction (organic  
carbon, OC, and elemental carbon, EC), inorganic ions, elements, and trace organic  
compounds: carboxylic acids, alkylamines, polycyclic aromatic hydrocarbons (PAHs) and  
n-alkanes. The oxidative potential of PM was assessed by two different chemical assays  
(a-cellular methods): the DTT assay and the DCFH assay.

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Quartz filters were cut in punches to perform all the analysis. Four punches were used to  
perform the following chemical analysis: ion chromatography (IC, spot of  $2.5 \text{ cm } \emptyset$ ), gas  
chromatography coupled to mass spectrometry (GC-MS, spot of  $4.5 \text{ cm } \emptyset$ ), thermal  
optical transmission (TOT; rectangular spot of  $1 \times 1.5 \text{ cm}^2$ ) and inductively coupled plasma  
mass spectrometry (ICP-MS; spot of  $2.5 \text{ cm } \emptyset$ ). Two punches (spot of  $2.5$  and  $1.1 \text{ cm } \emptyset$   
each) of each filter were cut for OP measurements: one was measured by DTT assay, and  
the other was measured by DCFH assay.

160

## 2.4 Chemical analysis



161 PM samples were analysed for EC/OC, inorganic ions, elements and trace organic  
162 compounds: carboxylic acids, alkylamines, PAHs and *n*-alkanes. The approach used for  
163 chemical characterization of PM samples is described below.

164 EC and OC were quantified by means of an EC/OC carbon analyzer (Sunset Laboratory  
165 Inc., USA) by thermal optical transmission, TOT, using the NIOSH 5040 protocol, and detail  
166 on methods employed are given by Birch and Cary (1996).

167 Inorganic ions, carboxylic acids and alkylamines were determined by extracting filters in  
168 ultrapure water (18.2 M $\Omega$  cm<sup>-1</sup> Milli-Q water system, Millipore, Billerica, MA, USA). Filter  
169 (spot 2.5 cm  $\varnothing$ ) was extracted in water by ultrasonic bath (three cycles of 10 min, 1.5 ml  
170 H<sub>2</sub>O MilliQ each time); the extract was then filtered (0.45  $\mu$ m pore size PTFE filter, Alltech  
171 USA) and analysed (within 24h of extraction) by IC (ICS-2000, Dionex) with a conductivity  
172 detector. Cation determination, inorganic cations (Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup> and Ca<sup>++</sup>) as well as  
173 alkylamines (methyl- MA, dimethyl- DMA, trimethyl- TMA, ethyl- EA, diethyl- DEA,  
174 triethylamine TEA, as ammonium salts), was performed by means of an IonPac CS17  
175 separation column (4x250 mm, Dionex) and guard column CG17 (4x40 mm, Dionex), with  
176 gradient MSA (methansulfonic acid) elution at 1.0 ml min<sup>-1</sup>: 0-5 min = 3 mM MSA; 5-15  
177 min = linear gradient 0.3 mM min<sup>-1</sup> MSA; 15-25 min = linear gradient 1 mM min<sup>-1</sup> MSA;  
178 25-29 min = 40 mM MSA; 29-36 min= 3 mM. The anions, including inorganic anions (F<sup>-</sup>, Cl<sup>-</sup>,  
179 NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>) as well as mono and dicarboxylic acids (formiate, acetate, propionate,  
180 oxalate, succinate, glutarate), were analyzed by means of an IonPac AS11A-5  $\mu$ m  
181 separation column (4x250 mm, Dionex) and guard column AS11A- 5  $\mu$ m (4 x 40 mm,  
182 Dionex), using KOH as eluent at 1.2 mL min<sup>-1</sup> with a gradient concentration program (0-12  
183 min: 1.0 mM; 12-30 min: linear gradient up to 28 mM; 30-33 min: 28 mM).

184 For PAHs and *n*-alkanes, filters were extracted using dichloromethane: the extract was  
185 then filtered, evaporated (under N<sub>2</sub>) and dissolved in isooctane for GC-MS analysis (GC  
186 6850; MS 5973, Agilent), as described in detail in our previous papers (Sangiorgi et al.,  
187 2014; Perrone et al., 2012). 12 PAHs were determined: benzo[a]anthracene (BaA),  
188 cyclopenta[cd]pyrene (CPcdP), chrysene (CHR), benzo[b+j]fluoranthene (BbF+BjF),  
189 benzo[k]fluoranthene (BkF), benzo[e]pyrene (BeP), benzo[a]pyrene (BaP),  
190 dibenzo[a,h]anthracene (DBahA), indeno[1,2,3-cd]pyrene (IcdP), benzo[ghi]perylene  
191 (BghiP), dibenzo[a,e]pyrene (DBaep). Concentrations of the homologues series from C20  
192 to C32 were quantified for *n*-alkanes (C20-C32).

193 For elemental characterization, the aerosol-loaded filters (spot 2.5 cm  $\varnothing$ ) were digested  
194 by a microwave oven (Milestone, Ethos ONE) according to the current legislation of the  
195 European Community in the field of air quality monitoring (UNI 14902:2005). The  
196 digestion mixture was composed of sub-boiling HNO<sub>3</sub> and ultra-pure H<sub>2</sub>O<sub>2</sub> (Sigma-Aldrich,  
197 St. Louis, MO, USA). The resulting solutions were filtered and diluted to 15 ml with H<sub>2</sub>O  
198 MilliQ. All the elements were determined by a magnetic sector inductively coupled  
199 plasma mass spectrometer (SF-ICP-MS) Element 2 by Thermo Scientific (Bremen,  
200 Germany), and further information on the analysis is provided under Supplemental

201 Material . The relative standard deviations for all elements in each sample were always  
202 lower than 5 %. NIST SRM 1648a (Urban Particulate Matter) was used to verify that  
203 analyte concentrations were within 15% of the certified values before proceeding with  
204 sample analysis.

205 For all chemical species measured, the detection limit (DL) was calculated by analyzing  
206 the blank field filters, that is filters which were not used in the sampling but handled,  
207 extracted and analysed in the same way as the samples. DL values were computed as the  
208 mean signal of all analysed blank field filters plus three times the standard deviation.  
209 Concentrations of different chemical compounds in the ambient PM samples were  
210 considered if they were over the obtained DL, otherwise they were indicated as: <DL.  
211 Chemical compounds with more than 50% of ambient data <DL were not reported.  
212

## 213 **2.5 Oxidative potential assays**

214 Oxidative potential (OP) is defined as a measure of the capacity of PM to oxidize target  
215 molecules, i.e. by generating ROS in environments without cells (a-cellular test). Various  
216 a-cellular tests exist to assess the oxidative capacity of PM, each with a different  
217 sensitivity to the oxidizing compounds. In this study two independent assays, the DTT  
218 assay and the DCFH assay, were both used to assess the OP of PM samples.

219 The two assays we used to assess OP of particles, both  $OP^{DTT}$  and  $OP^{DCFH}$ , are a  
220 measurement of the oxidative potential of the soluble fraction of particles: particles  
221 collected on filters were extracted as described in paragraph 2.5.1 and 2.5.2, and the  
222 insoluble fraction was always removed by filtration before the DTT and the DCFH assay.  
223

### 224 **2.5.1 DTT method standard procedure**

225 The DTT assay provides an estimate of the redox activity of a sample based on the ability  
226 of redox active compounds in the PM samples to catalyze electron transfers between DTT  
227 and oxygen, thereby generating ROS. DTT, a dithiol compound, is the reducing reactive,  
228 which shares similarities with cellular reducing species in the biological systems, e.g.  
229 glutathione (Held et al., 1996). Briefly, the rate at which DTT is consumed under a  
230 standardized set of conditions is monitored, and the rate of DTT consumption is  
231 proportional to the concentration of the catalytically active redox-active species in the  
232 PM samples. Redox-active compounds catalyze the reduction of oxygen species to  
233 superoxide by DTT, which is oxidized to DTT-Disulfide. The remaining DTT is allowed to  
234 react with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), generating 2-nitro-5-thiobenzoic acid  
235 (TNB), which is the "colored" species produced in this reaction and has a high molar  
236 extinction coefficient ( $14150 \text{ M}^{-1} \text{ cm}$  at 412 nm) in the visible range.

237 Previous works have successfully applied the DTT assay to estimate the oxidative  
238 potential of PM ambient samples, and various DTT method optimizations have been  
239 proposed (Charrier et al., 2012; Li et al., 2009; Rattanavaraha et al., 2011).

240 The standard DTT procedure we followed is: first, 750  $\mu\text{l}$  of 0.1M phosphate buffer (pH=  
241 7.2) and 250  $\mu\text{l}$  of 0.1 mM DTT in phosphate buffer solution were added to a 4ml amber-  
242 glass vial that was shaken continuously on a shake table and the temperature was  
243 maintained at 37° C using an heated bath; then, at time zero, 300  $\mu\text{l}$  of PM extraction  
244 solution (or external standard: 0.021 mg ml<sup>-1</sup> 1,4-naphtoquinone, 1,4 NQ) were added to  
245 the mixture, and 10  $\mu\text{l}$  of 10.0 mM DTNB in phosphate buffer solution were added at  
246 known times. For each sample (PM or external standard) we prepared 5 vials, and DTNB  
247 was added in each of them at five different times: 0, 5, 10, 15 or 20 min. The remaining  
248 DTT reacts fast with DTNB forming TNB, which is stable in the solution for at least 2h at  
249 room temperature, and TNB was quantified using a 1 cm path length quartz cell in a  
250 Thermo UV/VIS spectrophotometer. The rate of DTT loss ( $\mu\text{M min}^{-1}$ ) was calculated from  
251 the slope of the linear regression of the five points of DTT concentration versus time (0-20  
252 min) (Figure S1 in Supplementary Material). As suggested by Charrier et al. (2012) we  
253 avoid the use of EDTA, a chelator that is sometimes used in the assay to reduce the rate  
254 of DTT loss in the blank, but significantly suppress the OP response induced by metals  
255 present in the PM samples. To reduce the background oxidation, which can seriously  
256 affect the reproducibility and stability of the DTT assay, we pretreated the phosphate  
257 buffer with Chelex 100 resin (sodium form, Biorad), a cation exchange resin that removes  
258 trace metals (high affinity for iron and copper) from phosphate buffer solutions.  
259 To measure OP of particles by DTT assay, filters (spot of 2.5 cm  $\varnothing$ ; 0.2-3.8 mg of PM  
260 collected on the spot) were extracted by ultrasonic bath (10 min) in 4 ml methanol (High  
261 purity grade >99.9 %, Riedel-de-Haën). The extract was then filtered (0.45  $\mu\text{m}$  pore size  
262 PTFE filter, Alltech USA), evaporated (under N<sub>2</sub>) and dissolved in 1.6 ml water solution 0.1  
263 M phosphate buffer to be treated with the DTT assay. The extraction of filters in  
264 methanol was chosen in agreement with what reported by Yang et al. (2014). Yang et al.  
265 (2014), and others (Verma et al., 2012; Rattanavaraha et al., 2011), found that for PM  
266 ambient samples the methanol extracts produce significantly higher DTT reactivity than  
267 the water extracts, suggesting that the use of methanol to retrieve DTT reactive  
268 components might be more efficient than using water because of methanol extraction  
269 properties for both hydrophilic compounds and hydrophobic organic compounds.

270

### 271 **2.5.2 DCFH method standard procedure**

272 The DCFH assay is commonly used for visualizing ROS generation at cellular level but has  
273 also been used for determining the OP of PM as a-cellular assay . In the DCFH assay, the  
274 presence of oxidizing species is assessed from the rapid oxidation of DCFH to a  
275 fluorescent compound (DCF) and, in the presence of the horseradish peroxidase (HRP),  
276 the test allows a high sensitivity of  $\sim 1.1 \text{ nmol/m}^3$  and calibrated with known  
277 concentration of H<sub>2</sub>O<sub>2</sub>.

278 Our protocol used an off-line ROS analyzer according to the method of King et al. (2013)  
279 to measure the filters and determine their ROS content. We modified it a bit, as we used

280 the on-line incubate compared to the King's method: instead of extracting the filter  
281 directly in 2'-7'-dichlorofluorescein- horseradish peroxidase solution (King's method), we  
282 extracted the filter in MilliQ water, and then the extract solution was filtered and injected  
283 in the continuous running 2'-7'-dichlorofluorescein, horseradish peroxidase solution  
284 system. The final chemical concentration, including 2'-7'-dichlorofluorescein, horseradish  
285 peroxidase going through the instrument, is the same as King's method. The process of  
286 our off-line DCFH assay is shown in Supplementary Material FigureS2.  
287 In DCFH assay, for every filter, we used two methods to extract, one with ultrasound and  
288 the other one without ultrasound. In this paper we considered only the  $OP^{DCFH}$  results  
289 without ultrasounds in the extraction process, as we found that ultrasound can  
290 significantly affect the ROS measurement results (Figure S3 in Supplementary Material).  
291 Many other evidences also showed that ultrasound have an influence on ROS  
292 measurements (Fuller et al., 2014, Mark et al., 1998, Milijevic et al., 2014). One possible  
293 explanation is the sonication of water with dissolved air present is known to create  
294 hydroxyl radicals due to the high temperature and pressure created by the collapse of  
295 bubbles formed by cavitation, and the sonication-derived free radicals has been shown to  
296 impact on OP results by DCFH (Milijevic et al., 2014).  
297 The process of filter extraction without ultrasound in DCFH assay was the following: filters  
298 (spot of 1.1 cm  $\varnothing$ ) were dissolved in 3 ml ultra-pure water, incubated 15 min at 30 °C  
299 water bath (Cole-Parmer, Switzerland), then vortex on Vortex Genie 2 (Bender& Hobein  
300 AG, Switzerland) for 3 min, and filtered through a 0.45  $\mu$ m Nylon Membrane filter  
301 (Infochroma, Switzerland). The filtered water extract was diluted to 15mL and then  
302 processed in the DCFH assay measurement.

303

## 304 **2.6 Data analysis**

305 We compared PM chemical species concentrations and OP values measured in the same  
306 sampling days (1-15 October) at the TR and LEZ site in Milan to evaluate whether there  
307 were significant spatial variations between the two urban sites (paragraph 4.2). We  
308 calculated average concentrations at TR and LEZ, and significant differences ( $p < 0.05$ ) were  
309 assessed by Student's t test. Also we calculated daily ratios between the two sites  
310 (LEZ/TR) for OP values and PM chemical components, and the mean ratios were tested to  
311 see if they were statistically significantly ( $p < 0.01$  or  $p < 0.05$ ) different from 1. The day-to-  
312 day correlation of OP values and chemical species concentrations for the two sampling  
313 sites was assessed by using the Pearson correlation coefficient ( $R^2$ ).

314 We attempted to identify the association between OP measured in PM samples of Milan  
315 and other variables, including PM chemical composition, as discussed in paragraph 4.4.  
316 Univariate correlation between OP values ( $OP/m^3$  both  $OP^{DTT}$  and  $OP^{DCFH}$ ) and meteo  
317 variables, gas pollutant concentrations, PM mass concentration and PM species  
318 concentrations was calculated using Spearman rank correlation coefficient ( $r_s$ ), and  
319  $p < 0.01$  or  $p < 0.05$  were considered statistically significant.

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### 3. Results

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#### 3.1 PM chemical composition

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Table 1 presents average concentrations for single chemical compounds in PM samples of different seasons (winter (W), summer (SU) and late summer (late-SU) at TR site) and from different sites (TR and LEZ) in Milan. Atmospheric concentrations (per  $\text{m}^3$  air) for the carbonaceous fraction (OC, EC and total carbon, TC), elements, inorganic ions, carboxylic acids, alkylamines, PAHs and n-alkanes in PM samples, together with meteo data, gas pollutants ( $\text{O}_3$  and  $\text{NO}_x$ ) and TSP mass concentration are reported as mean and standard deviation. PAH and n-alkane concentrations were measured only for late-SU PM samples, both from TR and LEZ. Meteo data (Global radiation  $\text{W m}^{-2}$ , temperature  $^\circ\text{C}$ , relative humidity RH) and gas pollutant ( $\text{O}_3$  and  $\text{NO}_x$ ) concentrations were obtained from the Lombardy Regional Agency for Environmental Protection (ARPA, 2015), which monitors the air quality of Milan at various stations set inside and outside LEZ.

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PM concentrations at TR site were 2 times higher in W ( $\text{TSP} = 129 \pm 60 \mu\text{g m}^{-3}$ ) compared to SU and late-SU campaigns, when similar TSP concentrations of  $50 \pm 7 \mu\text{g m}^{-3}$  (SU) and  $57 \pm 19 \mu\text{g m}^{-3}$  (late-SU) were measured. SU and late SU campaigns also generally showed similar atmospheric concentrations of chemical compounds, while concentrations were higher in W for most of primary pollutants (EC and elements) and nitrates.

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For the TR site and the LEZ site, PM samples collected simultaneously during late-SU campaign showed very similar mass concentrations ( $\text{TSP}$  was  $57 \pm 19 \mu\text{g m}^{-3}$  at the TR site and  $52 \pm 19 \mu\text{g m}^{-3}$  at the LEZ site), but concentrations at the TR site were higher for some chemical species compared to the LEZ site. For example, EC was  $2.6 (\pm 1.1) \mu\text{g m}^{-3}$  at the TR site and  $1.6 (\pm 0.6) \mu\text{g m}^{-3}$  at the LEZ site, and similar differences were observed also for elements (e.g. Cu, Zn, Ba, Pb) and for PAHs ( $\text{TR} = 2.43 \pm 1.74 \text{ ng m}^{-3}$ ;  $\text{LEZ} = 1.68 \pm 0.95 \text{ ng m}^{-3}$ ) from traffic.

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*(approximate location of Table 1)*

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#### 3.2 OP values

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Average values for OP of PM samples from different seasons (W, SU, late-SU at TR site) and sites (TR and LEZ) in Milan are reported in Table 2.  $\text{OP}^{\text{DTT}}$  and  $\text{OP}^{\text{DCFH}}$  results are presented per  $\text{m}^3$  air ( $\text{OP}/\text{m}^3$ ), as well as expressed per mg PM ( $\text{OP}/\text{mg}$ ).

$\text{OP}^{\text{DTT}}/\text{m}^3$  was  $0.04 (\pm 0.03)$  in W, and it was  $0.10 (\pm 0.05)$  and  $0.15 (\pm 0.06)$  respectively in SU and late-SU at the TR site. Thus SU and late-SU PM samples showed  $\text{OP}^{\text{DTT}}/\text{m}^3$  values 2-3 times higher compared to PM samples from W. Such a seasonal trend of  $\text{OP}^{\text{DTT}}$  was even more evident when expressed per mg PM, being  $\text{OP}^{\text{DTT}}/\text{mg}$  significantly lower in W ( $0.33 \pm 0.18$ ) compared to SU ( $1.92 \pm 1.07$ ) and late-SU ( $3.43 \pm 2.94$ ).

358  $OP^{DCFH}/m^3$  had an opposite seasonal trend compared to  $OP^{DTT}/m^3$ , and W PM samples  
359 from TR site showed higher  $OP^{DCFH}/m^3$  values ( $0.36\pm 0.07$ ) than SU ones ( $0.14\pm 0.07$ ). In W,  
360  $OP^{DCFH}/m^3$  was more than 2 times higher than in SU; such a difference was similar to  
361 differences in PM mass concentrations (Table 1), so that when expressed per mg PM,  
362  $OP^{DCFH}/mg$  didn't show any significant contrast between seasons, ranging from 2.73  
363 ( $\pm 1.29$ ) in SU and 4.02 ( $\pm 1.77$ ) in late-SU.

364 For the TR site and the LEZ site, PM samples collected during late-SU campaign showed  
365 very similar OP values, and neither  $OP^{DTT}$  nor  $OP^{DCFH}$  evidenced significant differences  
366 between the two sites (Table 2, and Table S1).

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368 *(approximate location of Table 2)*

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## 370 **4. Discussion**

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### 372 **4.1 PM chemical composition and OP in Milan**

373 The major chemical components of PM in Milan were OC (also converted to OM, organic  
374 matter, as  $1.6*OC$ ; Turpin and Lim, 2001), EC,  $SO_4^{2-}$ ,  $NO_3^-$ ,  $NH_4^+$  and mineral dust  
375 (estimated based on measurements of elements including Al, Ca, Mg, Fe, K; Putaud et al.,  
376 2010). These single chemical components accounted on average 26% (OM), 4.1% (EC),  
377 6.8% ( $SO_4^{2-}$ ), 16.6% ( $NO_3^-$ ), 6% ( $NH_4^+$ ) and 15% (mineral dust) of particle (TSP) mass in  
378 Milan, so the sum of these components occupied most ( $\cong 75\%$ ) mass of our PM samples.  
379 The chemical composition we analyzed for PM in Milan is consistent with that reported by  
380 Putaud et al. (2010) for urban sites across Southern Europe (contributions to PM10 mass:  
381  $OM=26\%$ ;  $EC=6\%$ ;  $SO_4^{2-}=12\%$ ;  $NO_3^-=9\%$ ; mineral dust=21%).

382 The concentrations (Table 1) of trace primary organic compounds, PAHs and n-alkanes,  
383 we measured in PM samples are very similar to that we measured previously in Milan in  
384 the years 2006-2009 (Perrone et al., 2012), while in the present study we found higher  
385 concentrations for carboxylic acids, which are typically attributed to secondary source.  
386 The average concentration of carboxylic acids we measured at TR site was maximum in  
387 SU ( $\Sigma CAs= 0.96\pm 0.29 \mu g m^{-3}$ ), and it was quite high if compared with other summer  
388 campaigns in Milan in the years 2006-2009, when the average concentration of  $\Sigma CAs$  was  
389  $0.47\pm 0.07 \mu g m^{-3}$  for PM10 samples (Perrone et al., 2012). This could also suggest an  
390 important contribution of secondary organics in the PM samples of Milan during this  
391 summer 2013 campaign.

392 In the present study we measured for the first time in Milan the concentrations of other  
393 trace organic compounds, alkylamines, which was founded low, but detectable,  
394 concentrations of  $DMA^+$  ( $0.04 \mu g m^{-3}$  in SU;  $0.09-0.10 \mu g m^{-3}$  in W and late-SU) and  $TEA^+$   
395 ( $0.36-0.42 \mu g m^{-3}$  in late-SU) in the ambient PM. Despite the very important role that  
396 these alkylamines are supposed to play in aerosol chemistry and secondary formation,

397 there are still very few data about their concentrations in PM ambient samples (Ge et al.,  
398 2011).

399 The OP of daily PM samples of Milan has been assessed as both  $OP^{DTT}$  and  $OP^{DCFH}$ ,  
400 highlighting some interesting features and also differences (as discussed in paragraph 4.3)  
401 between the two independent a-cellular tests we used to measure the oxidative potential  
402 of particles. The OP of PM in Milan had been investigated once by only DCFH assay (Daher  
403 N., et al., 2012). Daher et al. (2012) reported that  $OP^{DCFH}$  was greatest during January-  
404 February, and that  $OP^{DCFH}/m^3$  in winter PM was 2-3 fold higher than in summer PM in  
405 Milan. We also found a similar seasonal trend for  $OP^{DCFH}$  (Table 2), while in contrast the  
406 oxidative potential of Milan PM was maximum in summer if measured as  $OP^{DTT}$ . We point  
407 out that, although OP of Milan PM showed a significant intra-day variability (Figure 2), still  
408  $OP^{DTT}$  and  $OP^{DCFH}$  clearly showed a different seasonal behavior, being daily  $OP^{DCFH}$  always  
409 high, while daily  $OP^{DTT}$  always low, in winter.

410 In the following paragraphs we will discuss differences in the PM chemical composition  
411 and OP at the two sites, TR and LEZ, in Milan (paragraph 4.2). Also we analyze in detail  
412 which information can be derived on the oxidative potential of PM in Milan by: looking at  
413 the contrasting seasonal and site trends of  $OP^{DTT}$  and  $OP^{DCFH}$  (paragraph 4.3); correlating  
414 daily  $OP^{DTT}$  and  $OP^{DCFH}$  with daily PM chemical composition (paragraph 4.4).

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416 *(approximate location of Figure 2)*

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#### 418 **4.2 Differences in the PM chemical composition and OP at TR and LEZ sites**

419 We compared average concentrations measured in the same sampling days (late-SU  
420 campaign, 1-15 October) at the TR and LEZ site in Milan. Significant differences in PM  
421 mass concentration, chemical species concentrations and OP values between the two  
422 sites were assessed by Student's t-test (Table S1). The average concentrations at the two  
423 sites were statistically comparable for PM mass, OP values (both  $OP^{DTT}$  and  $OP^{DCFH}$ ) and  
424 concentration of most of chemical compounds. The only exception was for EC, two  
425 elements (Cu and Zn) and one PAH (CHR), which average concentrations at sites resulted  
426 significantly different ( $p < 0.05$ ). The average concentrations of EC, as well as that of Cu, Zn  
427 and CHR, were significantly lower at LEZ site than TR site, and this can be explained by the  
428 reduced impact of the traffic source at LEZ site. EC, as well as PAHs and some elements,  
429 derives from exhaust and not-exhaust (elements) emissions of circulating vehicles, and in  
430 Milan they are mainly emitted by traffic source (Perrone et al., 2012).

431 The day-to-day correlation of PM mass concentrations, chemical species concentrations  
432 and OP values for the two sampling sites, TR and LEZ, was assessed by using the Pearson  
433 correlation coefficient ( $R^2$ ) (Table S1). Daily PM mass concentrations measured at TR and  
434 LEZ were well correlated ( $R^2 = 0.75$ ), as well as correlation between the two sites was high  
435 for daily concentrations of most chemical compounds. In particular, daily concentrations  
436 of all major chemical components of PM ( $OC$ ,  $NH_4^+$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ) were highly correlated

437 ( $R^2=0.83-0.99$ ) between sites, indicating that the daily variations of concentrations  
438 observed at sites for major chemical species and PM mass were strongly influenced by  
439 day-to-day variations over the wider urban area of Milan, due to daily variations of both  
440 atmospheric conditions and widespread sources. A some lower correlation between the  
441 two sites was found for daily concentrations of other chemical compounds, and this could  
442 be explained by the influence of time-varying local source characteristics, as variations in  
443 the local traffic source (e.g. EC,  $R^2=0.64$ ) and local resuspension (e.g. Fe, Ba, Al, Ca,  
444  $R^2=0.54-0.24$ ) at each site. For specific chemical compounds, the very low correlation  
445 between sites for daily concentrations was mainly caused by very low measured values  
446 and/or many values below the DL (e.g. Gd, Ni, Co). Daily OP values measured at TR and  
447 LEZ site were highly correlated for  $OP^{DTT}$  ( $R^2$  was 0.69 and 0.91 for  $OP^{DTT}/m^3$  and  $OP^{DTT}/mg$   
448 PM, respectively). Also  $OP^{DCFH}/m^3$  values were correlate ( $R^2=0.63$ ) at sites, while  
449 correlation was low for  $OP^{DCFH}/mg$  ( $R^2=0.13$ ).

450 Figure 3 pictures ratios between sites (LEZ/TR), calculated from daily values for OP (per  
451  $m^3$ ), PM mass and chemical species concentrations (results are also reported in  
452 Supplemental Material, Table S1). A ratio LEZ/TR < 1 indicates that daily concentrations  
453 measured at LEZ were lower than daily concentrations measured at TR site. The lowest  
454 average ratios, LEZ/TR < 0.8, were for BaA (0.59), Cu (0.64), EC (0.64), followed by most of  
455 the PAHs (CPcdP, CHR, BaP,  $\Sigma 11$ PAHs, BeP, BbJF, DBaeP, BkF, I123cdP) and most of the  
456 elements (Zn, Pb, Mo, Ba, Fe, Mn), together with two n-alkanes (C25 and C26) and TC.  
457 Daily concentrations of all these chemical components (with average ratio LEZ/TR < 0.8)  
458 resulted significantly lower at the LEZ site compared to the TR site ( $p < 0.01$  or  $p < 0.05$ ; the  
459 only exception was Ba because of a high standard deviation). Also we found significant  
460 differences ( $p < 0.01$ ) between the two sites for daily concentrations of BghiP  
461 (LEZ/TR = 0.81), Cd (LEZ/TR = 0.82), OC (LEZ/TR = 0.85) and  $NH_4^+$  (LEZ/TR = 0.92). The lowest  
462 LEZ/TR values (< 0.8) were encountered for chemical species which are associated to the  
463 local traffic source, and for these species daily concentrations measured at the LEZ site  
464 were on average 20-41% lower compared to the TR site because of the reduced  
465 contribution of traffic at LEZ.

466 For example, EC daily concentration at LEZ site was 36% lower compared to TR site. A  
467 previous study was performed in Milan in 2009 to assess differences between sites placed  
468 in and out the LEZ area, and even higher contrasts were reported for the soot carbon  
469 fraction (measured as BC), being on average the BC concentration 47-61% at sites within  
470 the LEZ of that measured outside the LEZ (Invernizzi et al., 2011). Other studies run in  
471 German showed a 14-16% reduction of EC concentrations by comparing sites within and  
472 outside LEZ in Berlin (Lutz et al., 2009). Although our measurement campaign refers to a  
473 restricted period of the year (1-15 October 2015), the observed EC reduction in the LEZ  
474 area of Milan very well confirms the differences of BC concentrations we measured at  
475 two sites inside and outside Milan LEZ (BC inside LEZ is  $43 \pm 8\%$  lower compared to  
476 outside LEZ) during a one-year long campaign in 2014-2015 (our data, not yet published).



477 The lowest LEZ/TR values we found were for low molecular weight PAHs (LEZ/TR was  
478 0.59-0.68 for BaA, CPcdP and CHR), while LEZ/TR values were some higher for high  
479 molecular weight PAHs (IcdP, BghiP and DBahA: LEZ/TR=0.80-0.85). Therefore PAHs daily  
480 concentrations at LEZ site were lower (15-41%) compared to TR site, and this reduction in  
481 the concentrations was greater for low molecular weight PAHs (32-41%) than for high  
482 molecular weight PAHs (15-20%). This could be attributed to the reduced contribution at  
483 LEZ site primarily of traffic diesel, as diesel vehicles mainly emit low 4-ring PAHs, while 5-6  
484 ring PAHs are prevalent in gasoline vehicles (Perrone et al., 2014; Ravindra et al., 2008). In  
485 the Milan LEZ the access is forbidden to pre Euro3/III diesel vehicles, which are estimated  
486 to be the main contributors to PAHs emissions from traffic in Milan (Perrone et al., 2014).  
487 Traffic source contribution to PAHs concentrations at sites can be assessed by using  
488 specific PAH diagnostic ratios (relative molecular concentration ratios between PAHs)  
489 which are characteristic of a given emission source. Traffic source can be identified by  
490 BaA/(BaA+CHR) and BaP/BghiP ratios, >0.35 and >0.6 respectively (Tobiszewski and  
491 Namiesnik, 2012), and both sites in Milan indicated the relevant contribution of traffic,  
492 particularly at TR (BaA/(BaA+CHR)=0.40±0.02; BaP/BghiP=0.83±0.07), where values were  
493 higher compared to LEZ site (BaA/(BaA+CHR)=0.37±0.02; BaP/BghiP=0.73±0.06).  
494 PM chemical components not emitted from traffic showed comparable concentrations at  
495 TR and LEZ site (LEZ/TR≅1). This is the case of PM chemical components from secondary  
496 sources, like carboxylic acids (LEZ/TR=0.97-1.10), alkylamines (LEZ/TR=1.09-1.17),  
497 sulphates (LEZ/TR=1.13), nitrates (LEZ/TR=0.92), as well as species from other primary no  
498 traffic sources, such as Al, Ca and Ca<sup>++</sup> (LEZ/TR=0.98-1.06) mainly from dust resuspension  
499 and K<sup>+</sup> (LEZ/TR=0.91), also from biomass burning (Perrone et al., 2012). For all these  
500 species which are not purely emitted from traffic, daily concentrations at the two sites in  
501 Milan didn't evidence any significant difference, indicating how concentrations at the two  
502 sites, TR and LEZ, actually differed for the contribution of the only traffic source and not  
503 of other sources (secondary sources and no-traffic primary sources).  
504 OP daily values at TR and LEZ sites were comparable for both OP<sup>DTT</sup> (LEZ/TR=1.07) and  
505 OP<sup>DCFH</sup> (LEZ/TR=0.91), thus not showing any significant difference in the oxidative  
506 potential value of particles between sites. Janssen et al. (2014) found that OP at a  
507 continuous traffic site can be higher compared to the urban background, though the  
508 difference was not large for OP<sup>DTT</sup> contrary to other OP measures, e.g. OP<sup>ESR</sup> and OP<sup>AA</sup>.  
509 The fact that OP/m<sup>3</sup> daily values measured at TR and LEZ sites in Milan were comparable  
510 and correlated would indicate that day-to-day variations of OP<sup>DTT</sup> and OP<sup>DCFH</sup> observed at  
511 sites was more influenced by, and thus representative of, values of the oxidative potential  
512 of PM over the wider Milan urban area. OP<sup>DTT</sup> and OP<sup>DCFH</sup> results are discussed in more  
513 detail in the next paragraphs 4.3 and 4.4.

514  
515 *(approximate location of Figure 3)*  
516

### 4.3 OP<sup>DTT</sup> and OP<sup>DCFH</sup>

The oxidative potential of PM samples was assessed by two different chemical methods, OP<sup>DTT</sup> and OP<sup>DCFH</sup>, which use different measurement units and metrics (OP<sup>DTT</sup>= the rate of DTT consumption in time,  $\Delta[\text{DTT}]\mu\text{M min}^{-1}$ ; OP<sup>DCFH</sup>= the total H<sub>2</sub>O<sub>2</sub> equivalent consumption of DCFH), and the results are not directly comparable as absolute values. Both OP<sup>DTT</sup> and OP<sup>DCFH</sup> are an estimate of the oxidative potential of PM samples which vary because of different physico-chemical properties of particles, as it is discussed in paragraph 4.4. OP<sup>DTT</sup> and OP<sup>DCFH</sup> make use of a different reducing agent, DTT and DCFH respectively, to assess the redox activity of a PM sample, so the OP<sup>DTT</sup> and the OP<sup>DCFH</sup> assay can respond in a different way between them because they react not in the same way to redox active compounds in the sample (Sauvain et al., 2013).

Actually, the trends observed for the OP values of Milan PM samples showed some significant differences if we compare OP<sup>DTT</sup> and OP<sup>DCFH</sup> results, and such differences are evident for seasonal variations.

OP<sup>DTT</sup> clearly showed the maximum OP/m<sup>3</sup> values in summer and late-summer, while the minimum was in winter. For OP<sup>DCFH</sup> it was the opposite, with the highest OP/m<sup>3</sup> values obtained for winter PM samples at TR site (Table 2). A higher OP<sup>DTT</sup> of summer PM compared to winter PM was also found by Charrier et al. (2012) when measuring at an urban site in Fresno, CA.

Average OP<sup>DCFH</sup> content in Milan ambient air was 0.22 nmol/m<sup>3</sup>, which is comparable to many other publications using DCFH assay measured ROS concentrations in ambient PM. For example, OP<sup>DCFH</sup> is 0.16 nmol/m<sup>3</sup> in spring 2012, in Georgia, southeastern United States, measured by King et al.(2013) when using off-line DCFH assay; Hung and Wang measured the OP<sup>DCFH</sup> is 0.058 nmol/m<sup>3</sup> in aerosol particles collected at a sidewalk in Taipei (Hung and Wang, 2001); Average OP<sup>DCFH</sup> is ~1.2 nmol/m<sup>3</sup> in January and February 2004 in Flushing, New York (Venkatachari, et al., 2007); OP<sup>DCFH</sup> is up to 0.38 nmol/m<sup>3</sup> in aerosol phase in West Los Angeles during summer in 2001 (Hasson, et al., 2003); OP<sup>DCFH</sup> is 0.1 nmol/m<sup>3</sup> in Summer 2003, in Rubidoux, Riverside county California (Venkatachari et al., 2005).

### 4.4 Correlation between OP and PM chemical constituents

We assessed the correlation between OP values (OP/m<sup>3</sup>, both OP<sup>DTT</sup> and OP<sup>DCFH</sup>) and meteorological variables (Global Radiation, T, RH), gas pollutant concentrations (O<sub>3</sub> and NO<sub>x</sub>), PM mass concentration (TSP) and PM composition using the Spearman correlation coefficient (r<sub>s</sub>) (Table 3).

OP<sup>DTT</sup> showed the highest correlation with Glob Rad (r<sub>s</sub>=0.38), and it was the only significant (p<0.05) positive correlation we found between OP<sup>DTT</sup> and all the meteorological variables we considered. OP<sup>DTT</sup> showed a very low positive correlation also with T, O<sub>3</sub> and few PM chemical constituents, including Ni, SO<sub>4</sub><sup>2-</sup>, acetic and oxalic acid.

Contrary, OP<sup>DTT</sup> was negatively correlated with most of PM chemical constituents, and the

557 highest anti-correlation was observed between  $OP^{DTT}$  and Gd, Nd, Mo, Pb (Spearman's  $r$   
558 ranging between -0.69 and -0.52;  $p < 0.01$ ) and other metals (V, Cd, Fe, Zn, Ce: Spearman's  
559  $r$  ranging between -0.46 and -0.40;  $p < 0.05$ ).

560  $OP^{DCFH}$  (nmol/m<sup>3</sup>) showed a positive correlation PM mass concentration ( $r_s = 0.65$ ;  
561  $p < 0.01$ ), which accorded with many other previous studies on this topic (Araujo et al.,  
562 2009; Saffari et al., 2013; Daher et al., 2014).  $OP^{DCFH}$  also showed a positive correlation  
563 with the major chemical components of particles including OC, TC,  $SO_4^{2-}$ ,  $NO_3^-$  and  $NH_4^+$  ( $r_s$   
564 = 0.49-0.57;  $p < 0.01$ ),  $OP^{DCFH}$  was positively correlated with Ca and metals like Mn, Co, Zn  
565 and As ( $r_s = 0.50-0.67$ ;  $p < 0.01$ ), and to a lesser extent with Al, Cu, Cd, Cr, F<sup>-</sup> and  $Ca^{++}$  ( $r_s$   
566 = 0.36-0.44;  $p < 0.05$ ).

567  $OP^{DTT}$  and  $OP^{DCFH}$  were related, positively and/or negatively ( $OP^{DTT}$ ), with different  
568 variables, indicating how DTT and DCFH assays seem to be sensitive to different chemical  
569 properties of PM samples.

570  $OP^{DTT}$  positively correlated mainly with Global Radiation and, to a lesser and low extent,  
571 also with T, O<sub>3</sub> and PM chemical components deriving from secondary photochemical  
572 source ( $SO_4^{2-}$  and oxalic acid). Global Rad and all the meteorological-chemical variables which  
573 were positively related with  $OP^{DTT}$  were high in SU and late-SU, when the highest  $OP^{DTT}$   
574 values were measured. Global Radiation is typically associated to an high secondary  
575 organic aerosol (SOA) formation, so it could be speculated that the observed positive  
576 correlation between  $OP^{DTT}$  and Global Radiation could indicate that the high oxidative  
577 potentials measured by the DTT assay are associated to an high SOA content (not  
578 measured) of particles in SU and late-SU days, as also discussed in paragraph 4.1 for  
579 carboxylic acid concentrations. SOA is rich in oxidized organic compounds, mainly  
580 carbonyl compounds and organic acids (Zhang et al., 2004); studies have shown how the  
581 DTT reactivity results from the thiol oxidation by carbonyl/acidic groups, while on the  
582 contrary they are not reactive with the DCFH assay (Sauvain et al., 2013). Various authors  
583 report that the DTT assay positively responds to the presence of SOA in PM samples  
584 (McWhinney et al., 2011), as well as to the presence of specific carbonyl compounds such  
585 as quinones (Kumagai et al., 2002).

586 Some studies report that the DTT assay, besides to be sensitive to oxidizing organic  
587 compounds, is also sensitive to metal concentrations in ambient PM samples, in  
588 particular, as we did, if the use of EDTA is avoided in the assay (Charrier et al., 2012).  
589 Contrary, we found that  $OP^{DTT}$  was negatively correlated with metal (except Ni)  
590 concentrations in the samples. The OP we measured is only from the soluble fraction  
591 of particles (PM samples extracted in methanol for DTT assay), and our OP measures  
592 don't regard to the insoluble PM fraction (extracted PM samples were filtered before OP  
593 tests, both for the DTT and the DCFH assay). A recent study showed that dissolved metals  
594 such as  $Zn^{2+}$  (but not  $Ni^{2+}$ ) may undergo complexation with DTT, with a consequent  
595 stabilization of DTT toward oxidation (Sauvain et al., 2013). Such a stabilization of DTT  
596 toward oxidation in the presence of soluble metal ions is reported also by Uzu (et al.,

597 2011) in the case of lead-rich particles. The negative correlation we observed between  
598 metal concentrations and  $OP^{DTT}$  of the Milan PM samples could be due to this process of  
599 complexation of DTT with metals in solution. Actually, DTT mimic antioxidant molecules  
600 (glutathione for example) in biological systems, so it is argued that the occurring of the  
601 complexation with metal ions would indicate a removal of antioxidants, thereby depriving  
602 the cells of a protection mechanism toward oxidation: this is in agreement with the  
603 observed cellular toxicity for example of ZnO particles, which is attributed to soluble  $Zn^{2+}$   
604 (Xia et al. 2008).

605  $OP^{DCFH}$  seemed to be positively influenced by many chemical components of PM,  
606 including metals and various compounds, which are relevant to their mass contribution to  
607 particles (OC, TC,  $SO_4^{2-}$ ,  $NO_3^-$ ,  $NH_4^+$ ) and PM mass. Their individual effect on  $OP^{DCFH}$  values  
608 is difficult to assess, and it could be due to the correlation between various chemical  
609 variables. What we can say is that, while  $OP^{DTT}$  was clearly not influenced by TSP  
610 concentration, high  $OP^{DCFH}$  values were associated with the total mass concentration of  
611 particles (and, likewise, with the concentrations of all major PM chemical components).

612

613 *(approximate location of Table 3)*

614

## 615 **5 Conclusions**

616 We analyzed the chemical composition and the OP of PM from Milan at two sites which  
617 were differently impacted by traffic source: a traffic site (TR) and a limited emission zone  
618 site (LEZ), which located outside and inside the restricted traffic area of Milan, separately.  
619 The sampling campaign was performed simultaneously at TR and LEZ (October 2013),  
620 analysis results showed that daily concentrations of some chemical components from  
621 primary traffic source were significantly lower at the LEZ than at the TR site. The decrease  
622 in the concentrations from TR to LEZ was maximum for EC (-36%), PAHs (between -32%  
623 and -0.41% for low molecular weight PAHs: BaA, CPcdP and CHR) and some elements  
624 (between -27% and -0.37% for Cu, Zn, Pb, Mo, Fe, Mn). We confirm the results of a  
625 previous study (Invernizzi et al., 2012) which demonstrated that traffic restriction causes  
626 a reduction in the BC (or EC) concentrations within LEZ in Milan, and moreover we add  
627 that a significant reduction is also observed for other compounds (PAHs and elements)  
628 which are known for their relevance on health effects they may cause on population  
629 exposed. For this reason, data as ours can be particularly helpful to policy and decision  
630 makers in evaluating the effectiveness of traffic regulations on the impact on air quality  
631 within LEZ.

632 The measurement of OP is relevant to get information on the oxidative stress caused by  
633 exposure to PM, an important mechanism of action by which PM can lead to adverse  
634 health effects. We used two different a-cellular assays, DTT and DCFH, which are between  
635 the most frequently used to assess the OP of ambient particles. Average and daily OP  
636 values measured at TR and LEZ were comparable both for  $OP^{DTT}$  and  $OP^{DCFH}$ , thus not

637 showing any significant difference in the oxidative potential value of particles between  
638 sites and indicating that OP observed at sites was more influenced by, and thus  
639 representative of, values of the oxidative potential of PM over the wider Milan urban  
640 area.

641 By sampling at different seasons at TR site, a seasonal trend was observed for OP of Milan  
642 ambient air PM. In particular both  $OP^{DTT}$  and  $OP^{DCFH}$  showed a clear seasonal trend, but it  
643 was the opposite, with maximum  $OP^{DTT}$  values ( $OP^{DTT} \text{ m}^{-3}$  and  $OP^{DTT} \text{ mg}^{-1} \text{ PM}$ ) measured in  
644 summer, while  $OP^{DCFH}$  was highest in winter. The different results we observed between  
645  $OP^{DTT}$  assay and  $OP^{DCFH}$  assay Milan ambient air PM measurements seem to be due to the  
646 sensitivity of DTT and DCFH assays to different chemical properties of PM samples. We  
647 found that  $OP^{DTT}$  and  $OP^{DCFH}$  were related to meteo and PM chemical variables, but the  
648 effects were not the same.  $OP^{DTT}$  correlated positively with Global Radiation (probably a  
649 proxy for high concentrations of secondary organic aerosol, rich in carbonyl compounds  
650 which are reactive with DTT) and correlated negatively with metals (because DTT can  
651 complex with ion metals inhibiting their reactivity).  $OP^{DCFH}$  correlated positively with TSP  
652 mass, many of the major PM chemical components and metals. It is still difficult to say  
653 which assay can be indicated as the best to evaluate the real oxidative stress caused by  
654 PM exposure, and it should be considered at least a combination of the DTT and DCFH  
655 assays to provide complementary information relative to the quantification of the  
656 oxidative capacity of particles.

657

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663

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**Table 1** Meteorological data, gas pollutants (NO<sub>x</sub> and O<sub>3</sub>; μg m<sup>-3</sup>), particulate matter (TSP μg m<sup>-3</sup>) and chemical species (μg m<sup>-3</sup> or ng m<sup>-3</sup>) concentrations in TSP samples from the TR site (W, SU and late-SU campaigns) and the LEZ site (late-SU campaign). Mean ± standard deviation (SD). PAH and ALK concentrations were measured only for late-SU PM samples

			Traffic (TR) site						LEZ site	
			winter - W		summer - SU		late summer - lateSU		late summer - lateSU	
			Jan-Feb 13 (n=5)		July 13 (n=7)		1-15 Oct 13 (n=10)		1-15 Oct 13 (n=10)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Meteo Data	Global Rad	W m <sup>2</sup>	54 ± 25		247 ± 41		158 ± 82		158 ± 82	
	T	°C	4.2 ± 1.3		25.7 ± 0.8		15.9 ± 1.3		15.9 ± 1.3	
	RH	%	78.4 ± 8		54 ± 3.6		69.9 ± 16.0		69.9 ± 16.0	
Gas pollutants	NO <sub>x</sub>	μg m <sup>-3</sup>	160.8 ± 44		53 ± 5.3		112.5 ± 35.1		108.4 ± 29.7	
	O <sub>3</sub>	μg m <sup>-3</sup>	8.4 ± 6		85 ± 10.4		25.2 ± 15.1		20.3 ± 10.0	
Particulate matter	TSP	μg m <sup>-3</sup>	129 ± 60		50 ± 7		57 ± 19		52 ± 19	
TSP chemical composition	Carbonaceous fraction (μg m <sup>-3</sup> )	OC	26.5 ± 17.5		7.5 ± 0.8		9.0 ± 3.2		7.4 ± 1.9	
		EC	6.2 ± 2.9		1.5 ± 0.4		2.6 ± 1.1		1.6 ± 0.6	
Elements (μg m <sup>-3</sup> )		TC	32.8 ± 20.3		9.0 ± 0.9		11.6 ± 4.2		9.0 ± 2.4	
		Al	0.7 ± 0.4		0.3 ± 0.1		0.4 ± 0.2		0.4 ± 0.3	
Inorganic ions (μg m <sup>-3</sup> )		Ca	3.4 ± 1.8		1.2 ± 0.3		1.9 ± 0.4		1.9 ± 0.5	
		V	0.0037 ± 0.0016		0.0017 ± 0.0004		0.0017 ± 0.0008		0.0014 ± 0.0007	
		Mn	0.053 ± 0.023		0.016 ± 0.002		0.022 ± 0.007		0.016 ± 0.006	
		Fe	4.2 ± 2.0		0.9 ± 0.1		1.1 ± 0.6		0.7 ± 0.3	
		Co	0.0017 ± 0.0008		0.0004 ± 0.0001		0.0007 ± 0.0008		0.0006 ± 0.0003	
		Ni	0.0080 ± 0.0033		0.0018 ± 0.0004		0.0105 ± 0.0076		0.0119 ± 0.0084	
		Cu	0.161 ± 0.075		0.032 ± 0.009		0.047 ± 0.027		0.026 ± 0.010	
		Zn	0.229 ± 0.129		0.049 ± 0.013		0.064 ± 0.026		0.039 ± 0.009	
		As	0.0021 ± 0.0012		0.0009 ± 0.0002		0.0012 ± 0.0005		0.0010 ± 0.0004	
		Cd	0.0037 ± 0.0023		0.0006 ± 0.0003		0.0007 ± 0.0004		0.0006 ± 0.0003	
		Ba	0.078 ± 0.041		0.016 ± 0.005		0.025 ± 0.014		0.016 ± 0.007	
		Ce	0.0021 ± 0.0009		0.0008 ± 0.0001		0.0007 ± 0.0003		0.0006 ± 0.0003	
		Nd	0.0006 ± 0.0004		0.0002 ± 0.0000		0.0002 ± 0.0001		0.0002 ± 0.0002	
		Gd	0.00047 ± 0.00022		0.00014 ± 0.00004		0.00008 ± 0.00005		0.00007 ± 0.00002	
		Pb	0.143 ± 0.081		0.022 ± 0.023		0.016 ± 0.011		0.010 ± 0.005	
	C2-C5 mono and dicarboxylic acids (CAs) (μg m <sup>-3</sup> )	acetic	CH3COO-	0.18 ± 0.08		0.09 ± 0.02		0.12 ± 0.03		0.12 ± 0.03
formic		HCOO-	0.11 ± 0.03		0.07 ± 0.02		0.12 ± 0.01		0.13 ± 0.01	
glutaric		O2C(CH2)3CO2	0.04 ± 0.01		0.08 ± 0.01		0.04 ± 0.02		0.04 ± 0.01	
succinic		O2C(CH2)2CO2	0.13 ± 0.02		0.22 ± 0.04		0.12 ± 0.04		0.13 ± 0.04	
malonic		O2CCH2CO2	0.52 ± 0.38		<0.07		0.11 ± 0.02		0.13 ± 0.05	
oxalic		O2CCO2	0.36 ± 0.05		0.49 ± 0.21		0.33 ± 0.12		0.36 ± 0.16	
		SCAs	<b>1.32 ± 0.53</b>		<b>0.96 ± 0.29</b>		<b>0.75 ± 0.18</b>		<b>0.73 ± 0.21</b>	
Alkylamines (μg m <sup>-3</sup> )	dimethylamine	DMA+	0.10 ± 0.03		0.04 ± 0.00		0.09 ± 0.02		0.10 ± 0.01	
	triethylamine	TEA+	<0.32		<0.32		0.36 ± 0.02		0.42 ± 0.10	
* Polycyclic aromatic hydrocarbons (PAHs) (ng m <sup>-3</sup> )	benzo[a]anthracene	BaA			0.21 ± 0.16		0.11 ± 0.07		0.11 ± 0.07	
	cyclopenta[cd]pyrene	CPcdP			0.19 ± 0.19		0.19 ± 0.19		0.11 ± 0.08	
	chrysene	CHR			0.29 ± 0.13		0.29 ± 0.13		0.19 ± 0.07	
	benzo[b]fluoranthene	BbF			0.54 ± 0.33		0.54 ± 0.33		0.39 ± 0.18	
	benzo[k]fluoranthene	BkF			0.15 ± 0.12		0.15 ± 0.12		0.11 ± 0.06	
	benzo[e]pyrene	BeP			0.27 ± 0.20		0.27 ± 0.20		0.19 ± 0.12	
	benzo[a]pyrene	BaP			0.24 ± 0.21		0.24 ± 0.21		0.15 ± 0.10	
	dibenzo[a,h]anthracene	DBaA			0.03 ± 0.03		0.03 ± 0.03		0.02 ± 0.02	
	indeno[1,2,3-cd]pyrene	I123cdP			0.22 ± 0.17		0.22 ± 0.17		0.17 ± 0.11	
	benzo[ghi]perylene	BghiP			0.27 ± 0.20		0.27 ± 0.20		0.22 ± 0.18	
	dibenzo[a,e]pyrene	DBaP			0.03 ± 0.01		0.03 ± 0.01		0.02 ± 0.01	
		Σ11PAHs			<b>2.43 ± 1.74</b>		<b>2.43 ± 1.74</b>		<b>1.68 ± 0.95</b>	
* n-Alkanes (ALKs) (ng m <sup>-3</sup> )	n-eicosane	C20			2.06 ± 1.10		2.06 ± 1.10		2.12 ± 1.20	
	n-heneicosane	C21			1.14 ± 0.42		1.14 ± 0.42		0.92 ± 0.47	
	n-docosane	C22			2.38 ± 0.90		2.38 ± 0.90		2.46 ± 1.31	
	n-tricosane	C23			2.63 ± 0.96		2.63 ± 0.96		2.15 ± 1.00	
	n-tetracosane	C24			3.65 ± 1.32		3.65 ± 1.32		3.11 ± 1.31	
	n-pentacosane	C25			4.91 ± 1.52		4.91 ± 1.52		3.72 ± 1.46	
	n-hexacosane	C26			4.18 ± 1.52		4.18 ± 1.52		2.93 ± 1.19	
	n-heptacosane	C27			6.04 ± 2.21		6.04 ± 2.21		5.04 ± 3.11	
	n-octacosane	C28			4.27 ± 1.30		4.27 ± 1.30		3.33 ± 1.67	
	n-nonacosane	C29			7.45 ± 2.76		7.45 ± 2.76		6.56 ± 3.85	
	n-triacontane	C30			3.45 ± 1.06		3.45 ± 1.06		2.92 ± 1.77	
	n-hetriacontane	C31			5.90 ± 1.85		5.90 ± 1.85		5.12 ± 2.62	
	n-dotriacontane	C32			2.42 ± 0.74		2.42 ± 0.74		2.01 ± 0.92	
		ΣC20-C32			<b>50.48 ± 14.88</b>		<b>50.48 ± 14.88</b>		<b>41.71 ± 19.50</b>	

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805**Table 2** Oxidative potential (OP) measures (per volume air, m<sup>-3</sup>; per PM mass, mg<sup>-1</sup>) of TSP samples from the TR site (W, SU and late-SU campaigns) and the LEZ site (late-SU campaign). Mean ± standard deviation (SD)

OP measure	metric	unit	Traffic (TR) site						LEZ site	
			winter - W		summer - SU		late summer - lateSU		late summer - lateSU	
			Jan-Feb 13 (n=5)		July 13 (n=6)		1-15 Oct 13 (n=10)		1-15 Oct 13 (n=10)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
OP <sub>DTT</sub>	per unit air	Δ [DTT] μM min <sup>-1</sup> m <sup>-3</sup>		0.04 ± 0.03		0.10 ± 0.05		0.15 ± 0.06		0.15 ± 0.07
OP <sub>DCFH</sub>	volume (m <sup>3</sup> )	nmol H <sub>2</sub> O <sub>2</sub> equiv m <sup>-3</sup>		0.36 ± 0.08		0.14 ± 0.07		0.24 ± 0.13		0.20 ± 0.11
OP <sub>DTT</sub>	per unit PM	Δ [DTT] μM min <sup>-1</sup> mg <sup>-1</sup>		0.33 ± 0.18		1.92 ± 1.07		3.34 ± 2.94		3.80 ± 2.92
OP <sub>DCFH</sub>	mass (mg <sup>-1</sup> )	nmol H <sub>2</sub> O <sub>2</sub> equiv mg <sup>-1</sup>		2.99 ± 1.52		2.73 ± 1.29		4.02 ± 1.77		3.74 ± 1.41

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**Table 3** Spearman correlation ( $r_s$ ;  $n > 20$ ) between meteo-PM chemical data and OP measures ( $OP_{DTT}$  and  $OP_{DCFH}$ ; expressed per  $m^3$ ).

		$OP_{DTT}$ (n=29)	$OP_{DCFH}$ (n=31)	
<i>Meteo data</i>	Global Rad	<b>0.38 *</b>	<b>-0.38 *</b>	
	T	0.28	-0.17	
	RH	-0.19	<b>0.41 *</b>	
<i>Gas pollutants</i>	$NO_x$	-0.15	0.25	
	$O_3$	0.22	-0.22	
<i>PM total mass</i>	TSP	-0.15	<b>0.65 **</b>	
<i>PM chemical</i>	OC	-0.30	<b>0.51 **</b>	
	EC	-0.27	0.30	
	TC	-0.33	<b>0.49 **</b>	
	Al	-0.21	<b>0.41 *</b>	
	Ca	-0.19	<b>0.55 **</b>	
	V	<b>-0.46 *</b>	0.30	
	Mn	-0.35	<b>0.50 **</b>	
	Fe	<b>-0.43 *</b>	0.31	
	Co	-0.22	<b>0.55 **</b>	
	Ni	0.18	0.40	
	Cu	-0.24	<b>0.40 *</b>	
	Zn	<b>-0.40 *</b>	<b>0.67 **</b>	
	As	-0.13	<b>0.65 **</b>	
	Cd	<b>-0.46 *</b>	<b>0.39 *</b>	
	Ba	-0.21	0.34	
	Ce	<b>-0.43 *</b>	0.18	
	Nd	<b>-0.58 **</b>	0.30	
	Gd	<b>-0.69 **</b>	<b>0.44 *</b>	
	Pb	<b>-0.52 **</b>	0.31	
	Mo	<b>-0.55 **</b>	0.12	
	$F^-$	<b>-0.47 *</b>	<b>0.37 *</b>	
	$Cl^-$	-0.35	0.35	
	$NO_3^-$	-0.11	<b>0.57 **</b>	
	$SO_4^{2-}$	0.16	<b>0.53 **</b>	
	$NH_4^+$	0.06	<b>0.57 **</b>	
	$K^+$	<b>-0.39 *</b>	0.33	
	$Ca^{++}$	-0.27	<b>0.36 *</b>	
	acetic	CH3COO-	0.07	0.24
	glutaric	O2C(CH2)3CO2	-0.19	-0.14
	succinic	O2C(CH2)2CO2	-0.03	0.16
	oxalic	O2CCO2	0.14	0.31
	sum carboxylic acids	$\Sigma$ CAs	-0.08	0.25
sum PAHs	$\Sigma$ 11PAH	-0.05	0.18	
sum <i>n</i> -alkanes	$\Sigma$ C20-C32	-0.31	-0.22	

\*  $p < 0.05$

\*\*  $p < 0.01$

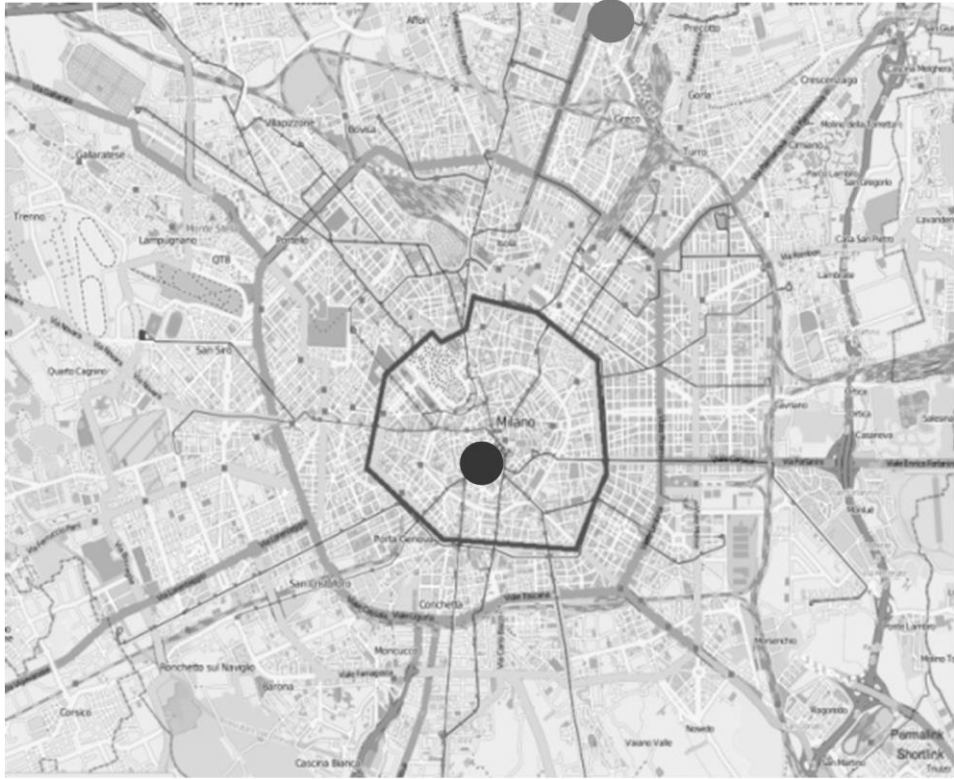
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813 **Figure 1** Map of the city of Milan, with the location of the two sampling sites: the traffic site (TR site) and  
814 the site within the low emission zone (LEZ site). LEZ area is delimited by the dark line in the map.

815 **Figure 2**  $OP^{DTT}$  and  $OP^{DCFH}$  of daily PM (total suspended particle, TSP) samples in Milan. Values are  
816 presented per  $m^3$  air ( $OP/m^3$ ) and expressed per mg TSP ( $OP/mg$  PM). \* = not measured. Error bars indicate  
817 coefficient of variations (CVs) for duplicate measurements.

818 **Figure 3** Average ratios (error bar indicates SD of average) calculated from the daily values (n=10) at the LEZ  
819 and TR site for  $OP/m^3$  ( $OP^{DTT}$  and  $OP^{DCFH}$ ), PM mass (TSP) and chemical concentrations. Values are in  
820 ascending order, and dark bars highlight [LEZ]/[TR] values < 0.8. LEZ/TR significantly different from 1: \*p  
821 <0.05, \*\*p< 0.01

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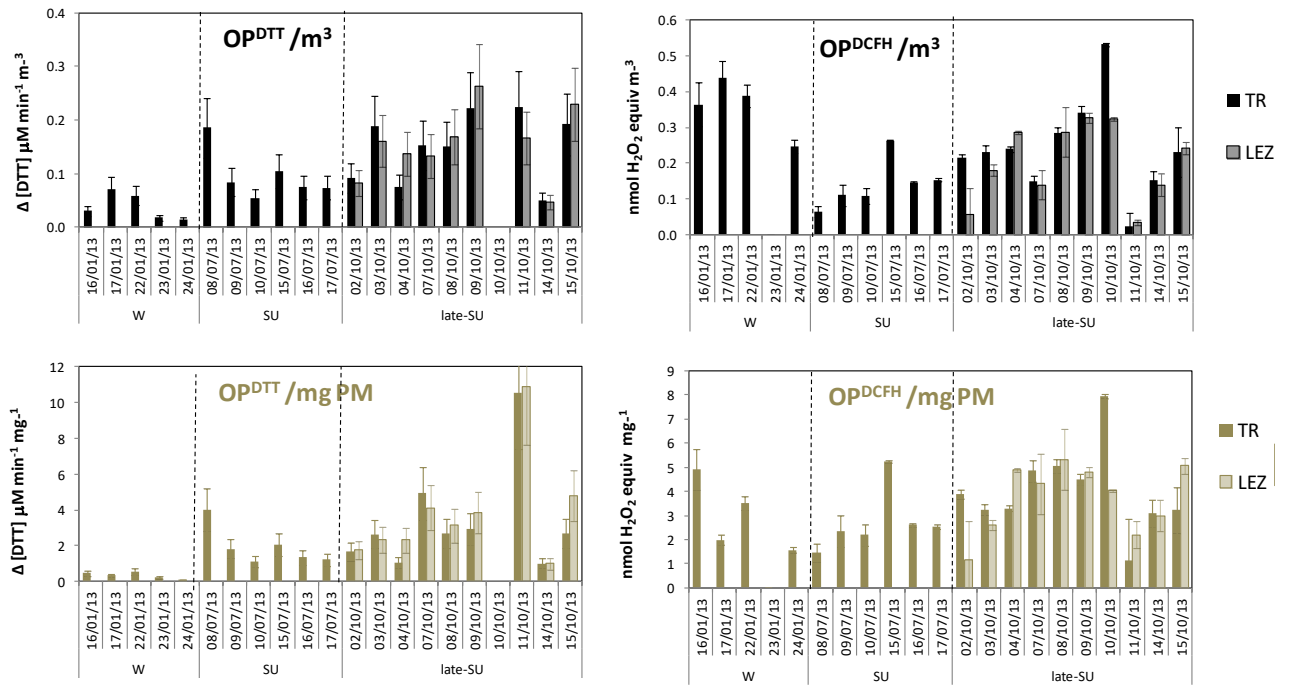


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**Figure 1**

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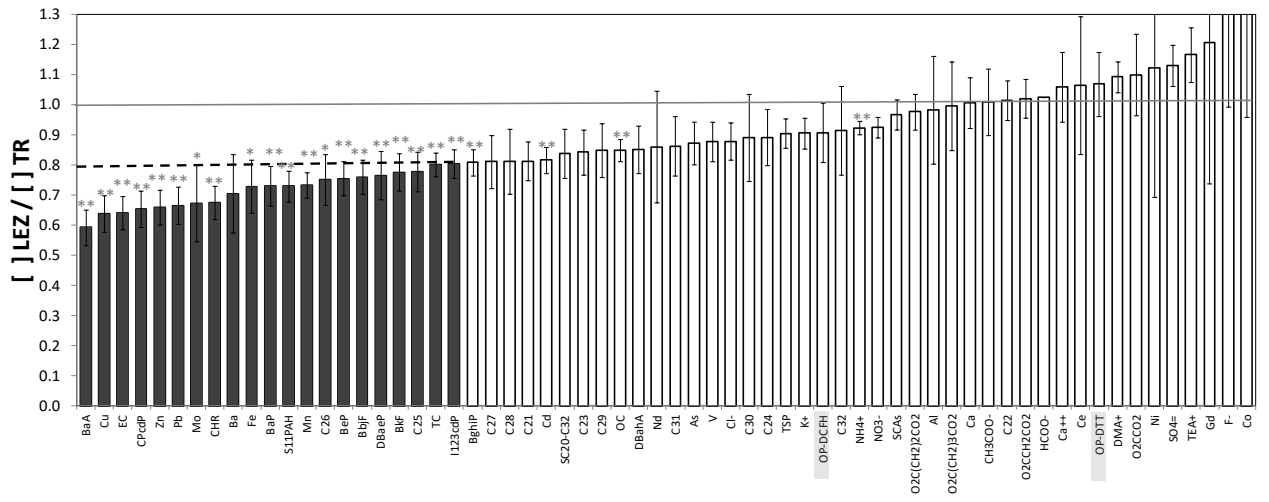
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Figure 2



833

834

Figure 3

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836 **Supplementary Material**

837

838 **PM Chemical Analysis**

839 **Elemental analysis**

840 A magnetic sector inductively coupled plasma mass spectrometer (SF-ICP-MS) Element 2 by  
841 Thermo Scientific (Bremen, Germany) was used to quantify in TSP samples following elements: Al,  
842 As, Ba, Ca, Cd, Ce, Co, Cu, Fe, Gd, Mn, Mo, Nd, Ni, Pb, V and Zn. Mass resolution (low or medium)  
843 and isotope selection were optimized for each element to ensure resolution of spectral  
844 interferences and maximize sensitivity. A minimum of triplicate 180 s analyses when working at  
845 low resolution, and quadruplicate when working at medium resolution, was conducted on each  
846 sample following a 60 s uptake and stabilization period.

847 After each sample the nebulizer system was rinsed for 2 min with 2% sub-boiling HNO<sub>3</sub>, to  
848 eliminate carry-over. Sets of instrumental blanks and calibration verification checks were run at  
849 frequent intervals during the sequence. The calibrations were performed with standard solutions  
850 prepared in aliquots of sample blanks. Procedural blanks were also prepared to assess metal  
851 contribution from the filters, bombs, ultrapure water and purified acids used in this procedure. All  
852 signals for samples were obtained after subtraction of their appropriate procedural blank values.

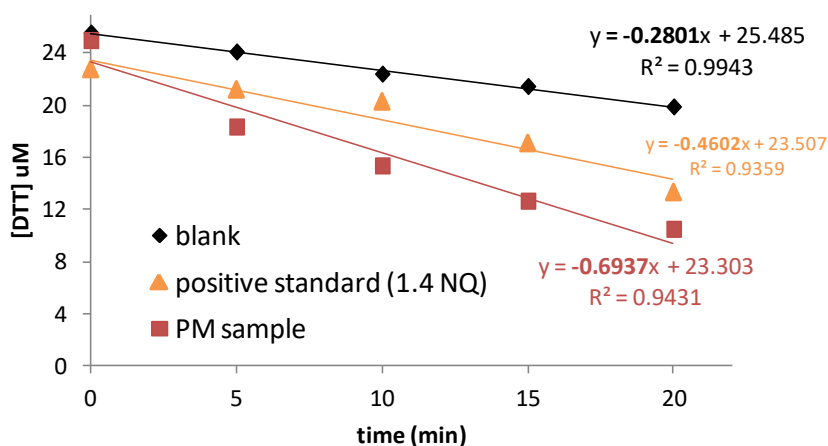
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855 **Oxidative potential (OP) measures**

856 **DTT method**

857 Rates of DTT loss ( $\mu\text{M min}^{-1}$ ) were determined from a linear regression of five points of DTT  
858 concentration versus time (0-20 min), as illustrated in Figure S1. The blank consists of 300  $\mu\text{l}$  of 0.1  
859 M phosphate buffer solution (no added redox-active species), the positive external standard  
860 consists of 300  $\mu\text{l}$  of 1,4-napthoquinone (1,4 NQ) ( $0.021 \text{ mg ml}^{-1}$ ). We ran at least one blank and  
861 one positive control with every experiment. The rate of DTT loss for each PM sample was  
862 calculated from the slope of the linear regression and then blank corrected by subtracting the  
863 average blank rate ( $n=26$ ) we processed. The positive control was quite well reproducible between  
864 different experiments, with a coefficient of variation (CV%) of 30%.

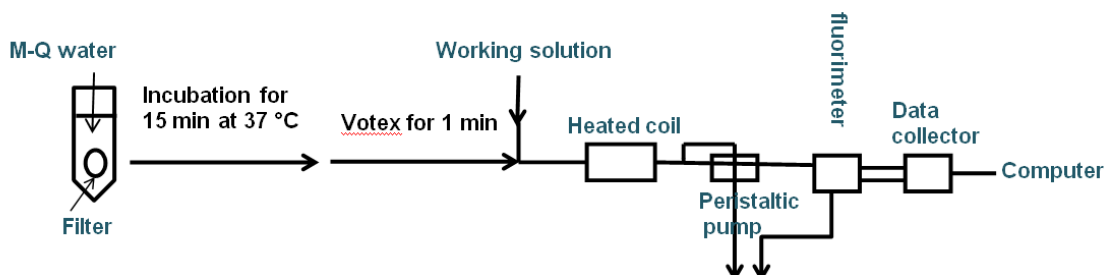


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866 **Figure S1.** The rate of DDT loss for each sample (blank, positive standard and PM extract) is  
867 determined as the slope of its sample regression line

868

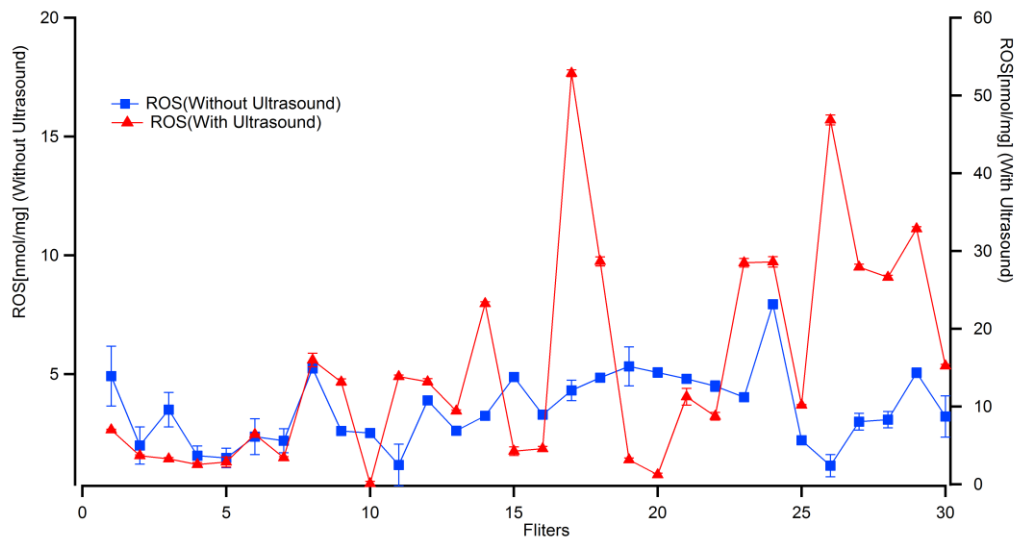
869 **DCFH method**



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871

**Figure S2** ROS off-line measurement process



872

873 **Figure S3** ROS contents in ambient PM (nmol/mg) when using extract process with and without ultrasound

874

## Results for chemical composition and OP of PM samples at TR and LEZ

Particulate matter	TSP	$\mu\text{g m}^{-3}$	TR site (n=10)		LEZ site (n=10)		LEZ/TR (n=10)		$R^2 (n=10) (c)$		
			mean	SD	mean	SD	(a)	(b)			
			57 ± 19		52 ± 19		0.91 ± 0.16		0.75		
Oxidative Potential (OP)	per unit air volume ( $\text{m}^3$ )	OP <sup>DTT</sup>	$\Delta [\text{DTT}] \mu\text{M min}^{-1} \text{m}^{-3}$	0.15 ± 0.06	0.15 ± 0.07		1.07 ± 0.32		0.69		
		OP <sup>DCFH</sup>	nmol H <sub>2</sub> O <sub>2</sub> equiv $\text{m}^{-3}$	0.24 ± 0.13	0.20 ± 0.11		0.91 ± 0.31		0.63		
	per unit PM mass (mg PM)	OP <sup>DTT</sup>	$\Delta [\text{DTT}] \mu\text{M min}^{-1} \text{mg}^{-1}$	3.34 ± 2.94	3.80 ± 2.92		1.26 ± 0.47		0.91		
		OP <sup>DCFH</sup>	nmol H <sub>2</sub> O <sub>2</sub> equiv $\text{mg}^{-1}$	4.02 ± 1.77	3.74 ± 1.41		1.06 ± 0.49		0.13		
TSP chemical composition	Carbonaceous fraction	OC	$\mu\text{g m}^{-3}$	9.0 ± 3.2	7.4 ± 1.9		0.85 ± 0.12	**	0.83		
		EC	$\mu\text{g m}^{-3}$	2.6 ± 1.1	1.6 ± 0.6	*	0.64 ± 0.18	**	0.64		
	Elements	TC	$\mu\text{g m}^{-3}$	11.6 ± 4.2	9.0 ± 2.4		0.80 ± 0.13	**	0.78		
		Al	$\mu\text{g m}^{-3}$	0.4 ± 0.2	0.4 ± 0.3		0.98 ± 0.50		0.43		
		Ca	$\mu\text{g m}^{-3}$	1.9 ± 0.4	1.9 ± 0.5		1.01 ± 0.19		0.54		
		V	$\mu\text{g m}^{-3}$	0.0017 ± 0.0008	0.0014 ± 0.0007		0.88 ± 0.21		0.85		
		Mn	$\mu\text{g m}^{-3}$	0.022 ± 0.007	0.016 ± 0.006		0.73 ± 0.13	**	0.79		
		Fe	$\mu\text{g m}^{-3}$	1.1 ± 0.6	0.7 ± 0.3		0.73 ± 0.28	*	0.51		
		Co	$\mu\text{g m}^{-3}$	0.0007 ± 0.0008	0.0006 ± 0.0003		1.36 ± 1.07		0.03		
		Ni	$\mu\text{g m}^{-3}$	0.0105 ± 0.0076	0.0119 ± 0.0084		1.12 ± 0.86		0.04		
		Cu	$\mu\text{g m}^{-3}$	0.047 ± 0.027	0.026 ± 0.010	*	0.64 ± 0.19	**	0.81		
		Zn	$\mu\text{g m}^{-3}$	0.064 ± 0.026	0.039 ± 0.009	*	0.66 ± 0.18	**	0.39		
		As	$\mu\text{g m}^{-3}$	0.0012 ± 0.0005	0.0010 ± 0.0004		0.87 ± 0.21		0.75		
		Cd	$\mu\text{g m}^{-3}$	0.0007 ± 0.0004	0.0006 ± 0.0003		0.82 ± 0.14	**	0.91		
		Ba	$\mu\text{g m}^{-3}$	0.025 ± 0.014	0.016 ± 0.007		0.71 ± 0.39		0.45		
		Ce	$\mu\text{g m}^{-3}$	0.0007 ± 0.0003	0.0006 ± 0.0003		1.06 ± 0.72		0.38		
		Nd	$\mu\text{g m}^{-3}$	0.0002 ± 0.0001	0.0002 ± 0.0002		0.86 ± 0.45		0.52		
	Gd	$\mu\text{g m}^{-3}$	0.00008 ± 0.00005	0.00007 ± 0.00002		1.21 ± 1.05		0.05			
	Pb	$\mu\text{g m}^{-3}$	0.016 ± 0.011	0.010 ± 0.005		0.67 ± 0.20	**	0.75			
	Mo	$\mu\text{g m}^{-3}$	0.001 ± 0.001	0.001 ± 0.001		0.67 ± 0.38	*	0.33			
	Inorganic ions	F-	$\mu\text{g m}^{-3}$	0.02 ± 0.02	0.02 ± 0.02		1.32 ± 0.99		0.01		
		Cl-	$\mu\text{g m}^{-3}$	0.7 ± 0.8	0.6 ± 0.8		0.88 ± 0.19		0.99		
		NO <sub>3</sub> -	$\mu\text{g m}^{-3}$	10.9 ± 6.6	10.2 ± 6.5		0.92 ± 0.11		0.98		
		SO <sub>4</sub> <sup>2-</sup>	$\mu\text{g m}^{-3}$	4.2 ± 2.6	4.5 ± 2.6		1.13 ± 0.21		0.95		
		NH <sub>4</sub> <sup>+</sup>	$\mu\text{g m}^{-3}$	4.2 ± 2.9	4.0 ± 3.0		0.92 ± 0.07	**	1.00		
		K <sup>+</sup>	$\mu\text{g m}^{-3}$	0.71 ± 0.74	0.62 ± 0.63		0.91 ± 0.16		0.96		
		Ca <sup>++</sup>	$\mu\text{g m}^{-3}$	1.6 ± 0.7	1.5 ± 0.5		1.06 ± 0.36		0.25		
		C2-C5 mono and dicarboxylic acids (CAs)	acetic	CH <sub>3</sub> COO-	$\mu\text{g m}^{-3}$	0.12 ± 0.03	0.12 ± 0.03		1.01 ± 0.31		0.01
			formic	HCOO-	$\mu\text{g m}^{-3}$	0.12 ± 0.01	0.13 ± 0.01		1.02 ± 0.30		0.00
			glutaric	O <sub>2</sub> C(CH <sub>2</sub> ) <sub>3</sub> CO <sub>2</sub>	$\mu\text{g m}^{-3}$	0.04 ± 0.02	0.04 ± 0.01		1.00 ± 0.39		0.01
	succinic		O <sub>2</sub> C(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub>	$\mu\text{g m}^{-3}$	0.12 ± 0.04	0.13 ± 0.04		0.98 ± 0.18		0.52	
	malonic		O <sub>2</sub> CCH <sub>2</sub> CO <sub>2</sub>	$\mu\text{g m}^{-3}$	0.11 ± 0.02	0.13 ± 0.05		1.02 ± 0.14		0.58	
	oxalic		O <sub>2</sub> CCO <sub>2</sub>	$\mu\text{g m}^{-3}$	0.33 ± 0.12	0.36 ± 0.16		1.10 ± 0.41		0.45	
	$\Sigma$ CAs			$\mu\text{g m}^{-3}$	<b>0.75 ± 0.18</b>	<b>0.73 ± 0.21</b>		0.97 ± 0.15		0.72	
	Alkylamines	dimethylamine	DMA <sup>+</sup>	$\text{ng m}^{-3}$	0.09 ± 0.02	0.10 ± 0.01		1.09 ± 0.09		0.94	
		triethylamine	TEA <sup>+</sup>	$\text{ng m}^{-3}$	0.36 ± 0.02	0.42 ± 0.10		1.17 ± 0.27		0.10	
		Polycyclic aromatic hydrocarbons (PAHs)	benzo[a]anthracene	BaA	$\text{ng m}^{-3}$	0.21 ± 0.16	0.11 ± 0.07		0.59 ± 0.19	**	0.82
	cyclopenta[cd]pyrene		CPcdP	$\text{ng m}^{-3}$	0.19 ± 0.19	0.11 ± 0.08		0.65 ± 0.19	**	0.91	
	chrysene		CHR	$\text{ng m}^{-3}$	0.29 ± 0.13	0.19 ± 0.07	*	0.68 ± 0.17	**	0.58	
	benzo[b+ij]fluoranthene		BbJf	$\text{ng m}^{-3}$	0.54 ± 0.33	0.39 ± 0.18		0.76 ± 0.18	**	0.75	
	benzo[k]fluoranthene		BkF	$\text{ng m}^{-3}$	0.15 ± 0.12	0.11 ± 0.06		0.78 ± 0.20	**	0.80	
	benzo[e]pyrene		BeP	$\text{ng m}^{-3}$	0.27 ± 0.20	0.19 ± 0.12		0.75 ± 0.18	**	0.90	
	benzo[a]pyrene		BaP	$\text{ng m}^{-3}$	0.24 ± 0.21	0.15 ± 0.10		0.73 ± 0.21	**	0.85	
	dibenzo[a,h]anthracene		DBaA	$\text{ng m}^{-3}$	0.03 ± 0.03	0.02 ± 0.02		0.85 ± 0.25		0.90	
	indeno[1,2,3-cd]pyrene		I123cdP	$\text{ng m}^{-3}$	0.22 ± 0.17	0.17 ± 0.11		0.80 ± 0.15	**	0.94	
	benzo[ghi]perylene		BghiP	$\text{ng m}^{-3}$	0.27 ± 0.20	0.22 ± 0.18		0.81 ± 0.14	**	0.97	
	dibenzo[a,e]pyrene		DBaEP	$\text{ng m}^{-3}$	0.03 ± 0.01	0.02 ± 0.01		0.77 ± 0.24	*	0.58	
$\Sigma$ 11PAHs			$\text{ng m}^{-3}$	<b>2.43 ± 1.74</b>	<b>1.68 ± 0.95</b>		0.73 ± 0.16	**	0.88		
n-Alkanes (ALKs)	n-eicosane		C20	$\text{ng m}^{-3}$	2.06 ± 1.10	2.12 ± 1.20		1.05 ± 0.28		0.88	
	n-heneicosane		C21	$\text{ng m}^{-3}$	1.14 ± 0.42	0.92 ± 0.47		0.81 ± 0.21	*	0.59	
	n-docosane	C22	$\text{ng m}^{-3}$	2.38 ± 0.90	2.46 ± 1.31		1.01 ± 0.21		0.84		
	n-tricosane	C23	$\text{ng m}^{-3}$	2.63 ± 0.96	2.15 ± 1.00		0.84 ± 0.23		0.51		
	n-tetracosane	C24	$\text{ng m}^{-3}$	3.65 ± 1.32	3.11 ± 1.31		0.89 ± 0.28		0.28		
	n-pentacosane	C25	$\text{ng m}^{-3}$	4.91 ± 1.52	3.72 ± 1.46		0.78 ± 0.20	**	0.53		
	n-hexacosane	C26	$\text{ng m}^{-3}$	4.18 ± 1.52	2.93 ± 1.19		0.75 ± 0.25	*	0.32		
	n-heptacosane	C27	$\text{ng m}^{-3}$	6.04 ± 2.21	5.04 ± 3.11		0.81 ± 0.26		0.82		
	n-octacosane	C28	$\text{ng m}^{-3}$	4.27 ± 1.30	3.33 ± 1.67		0.81 ± 0.32		0.17		
	n-nonacosane	C29	$\text{ng m}^{-3}$	7.45 ± 2.76	6.56 ± 3.85		0.85 ± 0.27		0.85		
	n-triacontane	C30	$\text{ng m}^{-3}$	3.45 ± 1.06	2.92 ± 1.77		0.89 ± 0.43		0.13		
	n-hetriacontane	C31	$\text{ng m}^{-3}$	5.90 ± 1.85	5.12 ± 2.62		0.86 ± 0.30		0.63		
	n-dotriacontane	C32	$\text{ng m}^{-3}$	2.42 ± 0.74	2.01 ± 0.92		0.91 ± 0.44		0.01		
	$\Sigma$ C20-C32		$\text{ng m}^{-3}$	<b>50.48 ± 14.88</b>	<b>41.71 ± 19.50</b>		0.84 ± 0.24		0.57		

(a) Mean concentrations at LEZ and TR are significantly different \* p&lt;0.05

(b) Ratio (LEZ/TR) of the daily concentrations at the TR and LEZ site is significantly different from 1 \* p&lt;0.05, \*\* p&lt;0.01

(c) Correlation between daily concentrations measured simultaneously at the two sites. Bold characters indicate values which are highly correlated ( $R^2 > 0.9$ )

877 **Table S1** PM (TSP) mass concentration, oxidative potential and chemical composition of PM measured at  
878 the TR site and at the LEZ site in Milan (1-15 October 2013)  
879