

Response to the Referees.

In addition to the replies published on the ACPD Discussion, we highlight here all specific changes to the revised text of the manuscript (in red) in response to the Referees' comments.

Reply to REFEREE 1:

We thank the Reviewer for the constructive criticisms. Below we provide a point-by-point rebuttal to his/her specific comments. However, his/her remarks about the lack of recognition of past studies do not hold, as many of the papers suggested by the Referee have already been included in the references list of the manuscript, and clearly the Reviewer simply missed them.

These authors have reported associations between their ROS measurement and WSOC in a number of past studies and asserted that the WSOC was secondary (for example, [Daher et al., 2012; Saffari et al., 2013; 2014]). The authors should note this and clearly state what is new about this work, ie, that the processing may be heterogeneous?

REPLY: The statistical association between ROS activity (cellular assay) and WSOC concentrations has already been investigated at a relatively small number of sites (see the review by Saffari et al. [Environ. Sci. Technol. 2014, 48, 7576–7583]). The originality of the present study relies on two aspects:

1. As the Referee pointed out, we investigate here the effect of heterogeneous processes (fog formation) on ROS activity in the particles.
2. Past experiments were based on aerosol samples in which WSOC and other redox-active agents (including metals) were actually physically mixed together, and their contributions to ROS activity could be disentangled only with statistical tools. Here instead, we provide observations of ROS activity of bulk aerosol particles and for interstitial aerosols, with the latter characterized by being naturally depleted of soluble compounds as a consequence of fog scavenging. Conversely, fog water samples largely recover WSOC of the original particle population while the insoluble species are mostly left out in the interstitial aerosol phase. We believe that the exploitation of the natural partitioning effect of fog on aerosol species exhibiting different water solubilities is an original contribution of our study to the research on the nature of the chemical species governing redox activity in the aerosol.

There is a substantial body of published literature on oxidative potential, albeit with different assays, that discusses the effect of oxidation on increased toxicity. Examples include chamber studies and analysis of ambient data (discussed more below). None of these, which are very pertinent to this paper, are cited in this work.

REPLY: The references suggested by the reviewer have already been included in the manuscript. See the detailed comment below.

No evidence or reference is provided establishing that this assay (that is, this specific ROS measurement) is linked to adverse human health effects, although a health connection is implicitly assumed throughout. It would appear the implicit assumption is that because this is a cellular assay it can be directly connected to adverse human health responses, but there are many components to a cellular assay that can lead to various responses, so the connection is not established until empirically proven. This could be done by citing comparisons of their assay responses to other assays that have established links to health outcomes or oxidative stress markers or cite specific associations between this assay and health effects. As the paper stands, there really is no basis for asserting that these results specifically apply to human health, instead the author need to qualify this assertion throughout the paper.

REPLY: The association between ROS activity and adverse health outcomes is certainly a subject of ongoing investigation. Nevertheless, it is not true that cellular ROS assays have not been connected to adverse human health responses, and it is unfair to state that “*As the paper stands, there really is no basis for asserting that these results specifically apply to human health*”, because the link between ROS assays and human health effects has already been established in few epidemiological studies, which have already been explicitly cited in the paper (Page 4, Line 28).

Finally, there is the question of importance on a broader scale and associated assertions by the authors of wide ranging impacts. The authors suggest that populations are commonly exposed to aerosol that has been fog-processed, but is this true, what is the evidence for this? Quantitative support for this assumption should be provided to demonstrate that this mechanism is truly of broad importance, as stated. Overly expansive statements of the importance of this work should also be avoided throughout.

REPLY: Fog and low-level clouds are transient phenomena in the atmosphere but their occurrence can in fact be very high in certain areas of the globe. This is especially true for highly-populated regions in orographic basins in wintertime. Cermak et al. (2009) showed that several pollution hotspots in Europe, including Benelux, the Ruhr district, the basins of Paris and London and the Po Valley, experience low-level clouds and fogs for 35% to 60% of the days in winter months. The fraction of fog days in fall/winter in the Californian Central Valley (6.5 millions inhabitants) is ca. 20% according to Baldocchi and Waller (2014). Fog frequencies of ca. 10% in winter are also characteristic of the Yangtze River corridor (Niu et al., 2010), and even greater values (20% to more than 35%) are typical of the Indo-Gangetic plain (Saraf et al., 2011). All these regions of the globe commonly experience PM pollution peaks in winter months, during the fog season. In this season of the year, the same stable weather conditions favor the accumulation of air pollutants and fog formation. Therefore, fog-processing is potentially a major driver for secondary aerosol formation in wintertime at all these sites. In Gilardoni et al. (2016), we provided a first estimate of SOA produced by aqueous-phase processing of smoke particles in Europe: 0.1 to 0.5 Tg of organic carbon per year, corresponding to 4 – 20% of total primary OA emissions in the region.

The above text has been included in the Introduction section of the revised version of the manuscript.

Could not find any data on the various sample sizes (N).

REPLY: The information can be found on page 2 Lines 33-35, and Page 3 Lines 1-5:

"Fog samples were collected from 30 November to 30 December 2015 at the meteorological station Giorgio Fea in San Pietro Capofiume (44°39'15" latitude, 11°37'29" longitude), a rural site located 30 km northeast of Bologna (Italy) in the eastern part of the Po Valley (northern Italy). From 30 November to 4 December, an

intensive observation period was scheduled, with the concurrent sampling of fog and aerosol samples and the deployment of a HR-ToF-AMS (Aerodyne Research) for online aerosol measurements. During the sampling campaign, a total of 6 aerosol samples and 16 fog samples were collected. Additionally, fog samples collected after the intensive observation period (after 4 December) were pooled in groups of two or three for the analysis of metals and Oxidative Potential. "

We further specify that the full chemical analyses (WSOC, ion chromatography, metals) was performed on 6 aerosol samples and 7 fog samples. ROS activity analysis was carried out on 20 samples (= 6 aerosol + 7 unfiltered fog + 7 filtered fog samples). Finally, sample size information is specified in every table in the present version of the manuscript.

We have therefore included the following lines of text in the revised version of the manuscript: "The full chemical analyses (WSOC, ion chromatography, metals) were performed on 6 aerosol samples and 7 fog samples." and "ROS activity analysis was carried out on 20 samples (= 6 aerosol + 7 unfiltered fog + 7 filtered fog samples)." in Sections 2.4 and 2.5, respectively.

The authors measured and report ROS of collected fog water and claim this is potentially linked to adverse health. How does this happen? Is the exposure route through inhaling fog drops? Likely not. Instead the argument is that the fog serves mainly as a chemical reactor that produces the toxic species. The drops evaporate and the fine PM is now more toxic. This assumes that all species in the fog contributing to ROS remain during evaporation, but it is stated that much of it is small molecular weight organic acids, which are likely very volatile and lost. If the fog ROS is equivalent to the ambient PM ROS, than these volatile species play no role. There seems to be some inconsistency in the author's arguments. Maybe this can be clarified.

REPLY: The Referee is right in pointing out that the volatility characteristics of redox-active WSOC in fog water eventually affect exposure. If we hypothesize that these are truly absorbed VOCs, such as formic acid, than their partitioning is completely reversible. However, the Referee assumes that fog droplets dry out once inhaled. On the contrary, fog droplets are expected to travel along the respiratory tract, just alike the droplets of nebulized solutions produced by aerosol generators for clinical use. Airways are much warmer with respect to ambient air but also humid close to saturation, and water vapor diffuses quicker than temperature (a physical process which is also at the basis of cloud condensation nuclei counters), therefore evaporation of fog droplets along their travel in the respiratory tract can be much reduced. The size range of Po Valley fog droplets, spanning between 3 and 30 μm (Heintzenberg et al., 1998) indicates that the inhaled fraction can deposit all over the respiratory tract down to the lungs. VOC evaporating from drying droplets can be exhaled but also become adsorbed to the wet tissues of the airways. The issue of the deposited fraction of the volatile fraction of fog solutes is complex and cannot be fully assessed in this paper. It is worthwhile to note, however, that non-volatile SOA are also expected to form from aqueous oxidation of water-soluble VOC and SVOC. For instance, evidence for dimerization of phenolic compounds in Po Valley fog was presented by Gilardoni et al. (2016). Such SOA compounds exhibit a quinoid structure and are potentially redox-active. The correlation found in this study between ROS activity and oxalic acid concentration (a tracer for highly oxidized, low-volatility aqueous SOA) supports the hypothesis of redox-active WSOC components of reduced volatility.

Finally, in respect to the Referee's comment "If the fog ROS is equivalent to the ambient PM ROS, than these volatile species play no role", we would like to specify that, as discussed in the paper, on a per-volume basis, daytime aerosols and fog exhibit comparable ROS, but this is only due to the large mass concentration of daytime aerosols. However, on a per-mass basis, which is the relevant metric here in discussing toxicity, ROS activity of fog is 2.5 times larger than that of both daytime and nighttime aerosols. This is clearly shown in

the most important figure of the paper (Figure 1). So there is actually no inconsistency in the results and the statements made in the manuscript.

A number of studies, such as chamber studies, have shown that if you take primary emissions, (say from a combustion source, like an automobile) and oxidize them, the oxidative potential substantially increases [Li et al., 2009] [McWhinney et al., 2011]. Likewise, chamber experiments in which SOA is produced from various VOCs show that some compounds, such as those found in biomass burning emissions, when oxidized have high intrinsic oxidative potentials [McWhinney et al., 2013]. It has even been shown that fresh soot that is subsequently oxidized has substantially increased oxidative potential (eg, [Antinolo et al., 2015; Shiraiwa et al., 2012]). All of these results are extremely pertinent to this work, but never cited nor discussed.

REPLY: On the contrary, several of these studies have already been cited in the manuscript (Page 2, Lines 9-13).

A variety of elemental concentrations of transition metals were measured, which are claimed to be redox active. Take Fe, for example. In the soluble form is redox active, but the insoluble form is not. Most measured elemental Fe is not soluble (many references show this) so no association to the water-soluble form, and hence redox activity, can be assumed a priori. The point here is that the use of elemental metal concentrations to infer toxicity through an oxidative stress response is not correct. This must be rectified in the manuscript.

REPLY: Our observations indicate a specific contribution of metals to ROS activity of PM distinct from that of SOA (Table 3). At the same time, filtration experiments showed that ROS activity is contributed by both soluble and insoluble components of the aerosol (Figure 2). Since several metals showed an enrichment in interstitial particles (like Cr, Mn, Fe, Cd and Pb, Figure S3b) which are depleted of soluble compounds, we explained the ROS activity of the unfiltered extracts of interstitial aerosol samples (Figure 2) with the presence of water-insoluble transition metals. This does not exclude a contribution of metals also to the soluble fraction of redox-active particulate matter (which, for daytime aerosols, can be large). Contrary to the Referee's indications, there is evidence that the soluble fraction of metals such as V, Zn, As and Cd can be > 50% in submicron aerosol samples (PM_{2.5}) (Heal et al.; 2005). Past experiments dealing with fog chemistry in the Po Valley (Mancinelli et al., 2005) highlighted large soluble fractions for several transition metals (e.g., 77% and 81% for Zn and Cu, respectively). The same study showed that, even if Fe is preferentially distributed to the insoluble core of fog droplets, the soluble fraction still accounts for more than 1/3 of total elemental iron. Therefore, a contribution to ROS activity from partially-soluble transition metals such as Fe cannot be ruled out and, on the contrary, is expected.

Past studies on metal solubility in Po Valley fog (Mancinelli et al., 2005) are now cited in Section 3.1 of the revised manuscript.

The authors assert there are policy implications, but is it really a novel finding that aged biomass burning smoke is toxic? There are many publications on the toxicity of smoke to humans (some should have been cited). The main finding here is that cloud process increases the ROS produced by rat macrophage. This specific finding should be stated in the context of overall known toxicity of smoke. (Ie, the authors could state something along the lines of, smoke is known to be toxic, here we show that fog processing of the smoke, increases the toxicity...).

REPLY: Although we agree with the Reviewer that there have been many studies indicating the toxicity of biomass burning particles, the main point of the paper and its novelty is the resulting increased toxicity from fog processing, and not the toxicity of biomass burning aerosol itself. This is clearly a novel finding which has not been reported previously. Nonetheless, we accept the Reviewer's comment and we will cite relevant studies on the toxicity of smoke particles to humans.

One important review study on smoke particles toxicity (Naeher et al., "Woodsmoke health effects: A review", *Inhalation Toxicology*, 19, 67-106, 2007) is now included in the Introduction section of the revised manuscript: "In the rural areas of the Po Valley, wood smoke can be responsible for adverse health effects on humans (Naeher et al., 2007)...".

Finally, the last line of the main text states: The enhanced toxicity of fog droplets observed in this study suggests that the historical reduction of fog frequency may result in an unintended improvement of air quality in many continental areas, overlapping also with the deliberate reduction of PM emissions put into practice since the early 90's in many developed countries. This assumes that fogs are more effective than other atmospheric processes (eg, aqueous reactions in haze or gas phase oxidation followed by partitioning) in converting wood smoke emissions to species toxic to humans. Is there any evidence for this? The point is what proof do the authors have that if the fogs were not present the aerosol would not still chemically evolve over time to a similar toxicity as fog-processed smoke?

REPLY: This Referee's comment is highly speculative. Our conclusion statement in the paper is a direct implication of our own findings. We do not claim that the in-fog processes are the most efficient processes governing ROS activity in the Po Valley aerosol, but, since fog frequency has decreased with time – as it is documented by visibility and liquid water content data –, the resulting specific impact is a parallel decrease in redox-active species concentrations in the particles. We are not aware if in the meantime something has changed also in respect to all other possible processes involving redox-active compound formation in SOA. We simply have no data for this hypothesis, nor Referee has provided data in support of it.

References:

Baldocchi, D., and Waller, R. (2014), Winter fog is decreasing in the fruit growing region of the Central Valley of California, *Geophys. Res. Lett.*, 10.1002/2014GL060018.

Cermak, J., R. M. Eastman, J. Bendix, S. G. Warren (2009), European climatology of fog and low stratus based on geostationary satellite observations, *Q. J. R. Meteorol. Soc.*, 135, 2125–2130.

Heal, M. R., Hibbs, L. R., Agius, R. M., & Beverland, I. J. (2005). Total and water-soluble trace metal content of urban background PM 10, PM 2.5 and black smoke in Edinburgh, UK. *Atmos. Environ.*, 39(8), 1417-1430.

Heintzenberg, J., M. Wendisch, B. Yuskiewicz, D. Orsini, A. Wiedensohler, F. Stratmann, G. Frank, B. G. Martinsson, D. Schell, S. Fuzzi, G. Orsi (1998), Characteristics of haze, mist and fog, *Contr. Atmos. Phys.*, 71, 21 -31.

Gilardoni, S., Massoli, P., Paglione, M., Giulianelli, L., Carbone, C., Rinaldi, M., Decesari, S., Sandrini, S., Costabile, F., Gobbi, G. P., Pietrogrande, M. C., Visentin, M., Scotto, F., Fuzzi, S., and Facchini, M. C. (2016): Direct observation of aqueous secondary organic aerosol from biomass-burning emissions, *PNAS*, 113, 10013-10018, 10.1073/pnas.1602212113.

Niu, F., Z. Li, C. Li, K.-H. Lee, M. Wang (2010), Increase of wintertime fog in China: Potential impacts of weakening of the Eastern Asian monsoon circulation and increasing aerosol loading, *J. Geophys. Res.* 115, doi:10.1029/2009JD013484.

Mancinelli, V., S. Decesari, M. C. Facchini, S. Fuzzi, F. Mangani, Partitioning of metals between the aqueous phase and suspended insoluble material in fog droplets (2005), *Ann Chim.*, 95, 275-290.

Saraf, A. K., A. K. Bora, J. Das, V. Rawat, K. Sharma, S. K. Jain (2011), Winter fog over the Indo-Gangetic Plains: mapping and modelling using remote sensing and GIS, *Nat. Hazards*, 58, 199–220.

Reply to REFEREE 2

Decesari et al. present a very interesting and novel analysis of Reactive Oxygen Species (ROS), including examinations of ROS in aerosol and fog and the effects of fog scavenging and chemistry on aerosol ROS. The design of the experiment, which takes advantage of differential fog scavenging of different aerosol ROS components (e.g., metals vs. WOC), is particularly clever. The manuscript is well written, concise and even spare in its style, effectively conveying a lot of information in a compact form.

Reply: we thank the Referee for her/his comments, which turn helpful in clarifying specific points of the manuscript.

I have a few, mostly minor, comments that should be addressed:

- 1. In performing mass balances of ROS and other components across aerosol and fog over time, the authors are assuming that there are no significant changes due to factors such as changes in boundary layer depth and fog drop deposition. Nocturnal cooling of the top of a fog layer typically leads to entrainment of air from above the boundary layer and associated growth in boundary layer depth. The entrained air is likely to have very different composition than the air originally in the boundary layer. This entrainment of air of differing composition can alter the mass balance. Likewise, significant deposition of fog water over the course of an episode can substantially cleanse the boundary layer of scavenged particles, again altering the mass balance. These factors need to be clearly outlined as sources of possible error in the mass balance analysis that is central to the paper.*

REPLY: The Referee is right. Nocturnal cooling at top of the fog layer makes this unstable, as documented by previous observations at the site (Wobrock et al., 1998). Occult depositions of fog were also investigated in past campaigns (Jaeschke et al., 1998). In general, the real atmosphere is not a closed system in any dimensions, even horizontally. Therefore, our interpretation of the results, assuming a “box model” where sinks and external sources of materials are ignored, can be valid only in a first approximation. Our assumption is primarily supported by the mass balance achieved between daytime PM concentrations and the lumped nighttime PM and fog components concentrations (Fig. S1): the mass balance shows that PM behaved approximately conservatively in the PBL in the days considered in this study. We acknowledge, however, that the PM mass balance can be affected by compensating fluxes of particles in and out the PBL (entrainments and depositions).

- 2. Lines 9-12 of abstract: this sentence should be rewritten to more clearly distinguish primary and secondary particle source contributions that the authors are referring to.*

REPLY: Estimates of the relative contributions of biomass burning and secondary sources to PM mass concentrations during the November 2015 fog campaign are currently not available. Nevertheless, the AMS

proxies for biomass burning POA and for oxidized organic aerosols (m/z 60 and m/z 44, in Table 2) indicates that the organic composition during the November 2015 field campaign is compatible with that observed during the previous experiments at the site where quantitative source apportionment of submicron PM was carried out (Gilardoni et al., 2016). This is why we stated that “Po Valley PM, which is mainly composed of biomass burning and secondary aerosols” in the abstract, without reporting the actual shares of PM mass. The point here is that the PM composition in a rural area like the Po Valley is *qualitatively* very different from that of urban environments, much more impacted by traffic emissions and more extensively studied for ROS.

3. *Section 2.2: more information should be presented concerning cleaning of the fog sampler and any contamination contained in collector blanks.*

REPLY: The collector and its strings were extensively cleaned at the beginning of the fog season (approximately two weeks before the samples object of this study were collected). In particular, the strings (stainless steel) were carried to a chemical laboratory where they were gently brushed to remove any stuck residues from fog sampling in the previous winter and washed with milliQ water in an ultrasonic bath. Finally, they were brought back to the field and mounted on the fog collector before first the automatic sampling system was switched on around mid-November. This information will be added to Section 2.2.

The above text is now available in Section 2.2 providing the full specifics of the fog sampling protocol.

4. *P.3, line 12: This should be the PVM-100 not PVM-10.*

REPLY: correct. It is PVM-100.

5. *P.3, lines 15-16: I suggest you explain to the reader that multiplying by LWC yields “air equivalent concentrations”*

REPLY: we will add the following sentence to the text: “Concentrations of analytes in fog samples, expressed in $\mu\text{g mL}^{-1}$, were converted into $\mu\text{g m}^{-3}$ by multiplying with the fog liquid volume (mL m^{-3}). The latter was not estimated by the mass of sampled fog water multiplied by the flux of the fog collector, because the collection efficiency is typically much smaller than 1 (about 40%, according to Fuzzi et al. 1997). We used instead the liquid water content (LWC) measured by the PVM-100 and averaged over the sampling time of the fog collector to multiply by the concentrations of chemicals in fog water to get air equivalent concentrations ($\mu\text{g m}^{-3}$) of the fog components”.

6. *Section 2.3: Please explain to the reader why you chose to filter the fog samples. Suspended particulate matter inside fog drops is also scavenged material that should be considered as part of the overall system ROS mass balance.*

REPLY: true, and in fact we performed ROS analyses on both filtered and unfiltered extracts. Since fog chemical components partition selectively between the soluble and insoluble phases, performing the analysis of ROS activity with and without filtration provides useful information on the nature of the redox-active compounds (Fig. 2).

7. P.3, line 25: change “chromatographers” to “chromatographs”

REPLY: will be corrected.

8. P. 4, lines 9-10: I am confused why the authors would extract fog water with Milli-Q water. Is this statement in error?

REPLY: This was mentioned in error. Please see below the corrected sentence: “In this method, samples (filters) were first extracted using an initial sonication period for 15 min with high-purity 10 Milli-Q (18 mΩ) water.”

The actual sentence included in the revised manuscript is: “In this method, samples (only filters in this step) were first extracted using an initial sonication period for 15 min with high-purity 10 Milli-Q (18 mΩ) water.”

9. P. 5, line 29: change “what observed” to “what is observed”

REPLY: will be corrected.

10. P. 5, line 30: change “ammonia” to “ammonium” since you are discussing ionic species here

REPLY: will be corrected.

11. P. 6, line 15: change “adsorbed” to “absorbed”

REPLY: accepted.

12. P. 6, lines 35-36: The statement “fog scavenging denudes particles of WS components” is misleading. It sounds like material is being stripped off particles while what is really happening is that the fog is selectively scavenging some particles and leaving others intact in the aerosol. Please rephrase.

REPLY: Accepted. The sentence will be changed into “as fog scavenges the WS components of the particles”.

To account for another comment from Referee 3 on the same section, the full paragraph has been rephrased as follows: “The most critical difference between daytime and interstitial (i.e., unscavenged) aerosols is that the latter are depleted of WS components, including WSOC (Figure 1) and inorganic aerosols (Figures S2 and S3) which are efficiently scavenged by fog. Therefore, as discussed above, the higher ROS activity of daytime aerosols compared to that of nighttime/interstitial aerosols must be attributed to WS components, while, at nighttime, the aerosol ROS activity is mainly driven by WI components.”

13. P. 9, lines 3-5: This sentence needs to be rewritten to improve the grammar.

REPLY: the text will be rephrased as follows: “The uptake of reactive gases could explain the excess of WSOC in fog water compared to the scavenged fraction of daytime PM1 (Figure 1 c,d) as well as the higher intrinsic toxicity of fog components, as shown in Figure 1 b”.

REFERENCES:

Fuzzi, S., Orsi, G., Bonforte, G., Zardini, B., and Franchini, P. L.: An automated fog water collector suitable for deposition networks: Design, operation and field tests, *Water Air and Soil Pollution*, 93, 383-394, 10.1023/a:1022108801083, 1997.

Gilardoni, S., Massoli, P., Paglione, M., Giulianelli, L., Carbone, C., Rinaldi, M., Decesari, S., Sandrini, S., 10 Costabile, F., Gobbi, G. P., Pietrogrande, M. C., Visentin, M., Scotto, F., Fuzzi, S., and Facchini, M. C.: Direct observation of aqueous secondary organic aerosol from biomass-burning emissions, *Proceedings of the National Academy of Sciences of the United States of America*, 113, 10013-10018, 10.1073/pnas.1602212113, 2016.

Jaeschke, W., Dierssen, J. P., Guenther, A., Schickedanz, U., Wolf, A., Ricci, L., Arends, B. G., Mass fluxes and chemical pathways during a fog event, *Contr. Atmos. Phys.*, 71, 1, 145 – 157, 1998.

Wobrock, W., Jaeschke, W., Schell, D., Teichmann, U., Wendisch, M., Mertes, S., Laubach, J., Fuzzi, S., Orsi, G., Observations of the turbulence structure of wind, temperature and liquid water content in a foggy surface layer, *Contr. Atmos. Phys.*, 71, 1, 171 – 187, 1998.

Reply to REFEREE 3

The paper from Decesari et al., measures the capability of aerosols to generate reactive oxygen species during the fog conditions in Po, Valley, Italy. The authors use a suitable assay, which is biologically relevant to indirectly interpret the toxicity of ambient aerosols. The study is well conducted although the sample size is small (n ~ 6). However, the reviewer can understand the intricacies involved in collecting the samples with enough mass for conducting all the toxicity and chemical analysis, particularly in the ambient conditions as in the study. The measurements are novel and this is probably the first time, ROS activity of the fog has been measured. The manuscript is very well written and the results are interesting and highly useful from the perspective of policy intervention, particularly in controlling the trans boundary movement of the pollutants. I support the publication of this manuscript. However, I have few comments below, which can help the authors to further improve upon their work.

REPLY: we thank the Referee for the useful comments. We provide a point-by-point reply below.

*Page 1, Line 34: There have been many epidemiological evidences showing the links between traffic pollution and adverse health effects (e.g. Brunekreef et al., *Journal of Exposure Science and Environmental Epidemiology* (2007) 17, S61–S65; doi:10.1038/sj.jes.7500628). Janssen et al., 2011 is not the first evidence.*

REPLY: correct, however, most epidemiological studies used NO₂ concentrations to assess the effect of differential exposures to traffic pollution within cities on human health, while Janssen et al. (2011) was among the first studies employing black carbon as a tracer for primary PM.

Page 2, Line 1: Bates and Fang et al., 2015 were not the toxicological studies. Bates et al., 2015 should be considered as epidemiological study and Fang et al., 2015 didn't report any direct linkages with the health impacts.

REPLY: The Referee is right about Bates et al. (2015). Its quotation in the text will be transferred to Page 1, line 34 among the citations of other epidemiological studies. The study of Fang et al., (2015) can instead be considered a toxicological study, as it makes use of a chemical assay for ROS activity (DTT assay).

The text in the revised version of the manuscript reads: "Research on traffic-related PM has provided a first epidemiological evidence of the links between adverse health effects and PM chemical composition (Janssen et al., 2011; Bates et al., 2015), in line with the results of several toxicological studies (Nel, 2005; Cassee et al., 2013; Fang et al., 2015), while outside urban areas etc."

Page 3, Line 15-16: The conversion of $\mu\text{g}/\text{mL}$ to $\mu\text{g}/\text{m}^3$ needs more description. What was the sampling flow rate, etc?

REPLY: we will add the following text: "Concentrations of analytes in fog samples, expressed in $\mu\text{g mL}^{-1}$, were converted into $\mu\text{g m}^{-3}$ by multiplying with the fog liquid volume (mL m^{-3}). The latter was not estimated by the mass of sampled fog water multiplied by the flux of the fog collector, because the collection efficiency is typically much smaller than 1 (about 40%, according to Fuzzi et al. 1997). We used instead the liquid water content (LWC) measured by the PVM-100 and averaged over the sampling time of the fog collector to multiply by the concentrations of chemicals in fog water to get air equivalent concentrations ($\mu\text{g m}^{-3}$) of the fog components".

Page 4, Line 14: The unfiltered fraction was directly assayed for the ROS activity but this fraction would be containing a lot of quartz fibers as well. These fibers are also shown to be toxic to the cells. How did the authors make sure that their results are well controlled in this environment?

REPLY: As explained in Section 2.5, the effect of quartz fibers from the filters is accounted for by the analysis of the blanks. Unfiltered extracts of blank filters ($n = 2$) exhibit a ROS activity of 280 ± 13 Fluorescence Units (FU, measured by the microplate reader), which, upon filtration, decreases to 27 ± 23 FU. These analyses confirm that the contribution of filter fibers to ROS activity is substantial, in agreement with the Referee's comments. Nevertheless, the ROS activity measured for the samples is much greater than the blank levels (levels as high as 3896 ± 350 FU for the not-filtered and 1518 ± 118 FU for the filtered sample fractions were measured, respectively). The filters were in fact highly loaded, as they originated from sampling for 12h with a HiVol system in a polluted atmosphere ($\text{PM}_{10} \sim 35 \mu\text{g}/\text{m}^3$ in daytime, Fig. S1).

We added the following text to the new Section 2.5: "The ROS activity of filter blanks ($N = 2$) was on the order of 280 ± 13 fluorescence units (FU) for unfiltered extracts and of 27 ± 23 FU for filtered extracts. For comparison, the ROS activity of PM_{10} filter samples was as high as 3896 ± 350 FU and 1518 ± 118 FU for unfiltered and filtered extracts, respectively."

Page 4, Line 22-23: What was the level of blank? And how much was the typical response from the sample in comparison to blanks?

REPLY: see reply to previous comment.

Page 6, Line 34: The secondary ionic species like what? Are these secondary ionic species shown to be toxic or ROS- active?

REPLY: the reasoning in this part of the text was based solely on similarities in the scavenging behavior of redox-active compounds (measured by ROS activity) with that of all the chemical species analyzed in daytime and night-time PM samples. The actual association of redox-active compounds to chemical agents was discussed in depth in the following section when the toxicological potential of organic and inorganic compounds is taken into account. However, we agree that the reference to secondary ionic species can be misleading here, and will modify the text by just stating “Therefore, as discussed above, the higher ROS activity of daytime aerosols compared to that of nighttime/interstitial aerosols must be attributed to WS components, while WI compounds dominate ROS activity at night”, and discuss the identification of potential redox-active chemicals in Section 3.2.

The actual text included in the revised version of the manuscript reads: “Therefore, as discussed above, the higher ROS activity of daytime aerosols compared to that of nighttime/interstitial aerosols must be attributed to WS components, while, at nighttime, the aerosol ROS activity is mainly driven by WI components.”

Page 7, Line 14-16: Although, it seems possible that the high correlation of inorganic species such as SO₄²⁻, NO₃⁻, etc. could be due to their co-linearity with the WSOC, however, recent studies (Environmental Science & Technology 51 (5), 2611-2620; Environ Sci Technol. 2012, 46(12):6637-44) have shown that SO₄²⁻ enhanced acidity of the aerosol can solubilize the metals (such as Fe and Cu), which are known to generate ROS. Do the authors think to include such possibility in their work?

REPLY: this is an interesting possibility. However, the acidity of fog is highly variable geographically, depending on the availability of atmospheric acidic (e.g., sulfuric, nitric and hydrochloric acids and acidic sulfates) and basic (ammonia) species. The historical trends of fog pH in the Po Valley (Giulianelli et al., 2014) show that the overall acidity has drastically decreased in the last 20 years, as a consequence of the reduction in SO₂ emissions, which was not accompanied by similar reductions in ammonia emissions that remained instead relatively constant with time. In recent years, fog pH in San Pietro Capofiume was around 6 or higher. Therefore, a possible effect on ROS from metal solubilization remains questionable in this specific environment. Nevertheless, we will add the following text to the paper to make clear to the reader that the attribution of ROS activity of fog to WSOC cannot be generalized, as multiple mechanisms can become important in different environments: “It should be noted, however, that inorganic acids can indirectly affect the ROS activity of fogs and wet aerosols by solubilizing redox-active metals (Oakes et al., 2012; Fang et al., 2017). Such mechanisms can be important for areas where SO₂ levels are high, while they are less plausible for environments such as the Po Valley where aerosols and fogs are largely neutralized by ammonia, as demonstrated by the typical fog pH of about ~ 6 , according to Giulianelli et al., 2014).”

The paragraph in Section 3.2 in the revised manuscript now reads: “It should be noted, however, that inorganic acids could indirectly affect the ROS activity of fogs and wet aerosols by solubilizing redox-active metals (Giulianelli et al., 2014; Oakes et al., 2012; Fang et al., 2017). Such mechanisms can be important for areas where SO₂ levels are high, while they are less plausible for environments such as the Po Valley where aerosols and fogs are largely neutralized by ammonia, as demonstrated by the typical fog pH of about ~6 , according to Giulianelli et al. (2014).”

Page 8, Line 14: See my earlier comment, could the toxicity of Fe, Ni and Cu be due SO₄²⁻ enhanced acidity and the solubility of these metals?

REPLY: see the reply to previous comment.

Page 9, Line 4: There should be "with" between "water" and "respect"

REPLY: will be corrected.

Page 10, Line 2: What do you mean by scavenging rate of ROS? I think this sentence needs to be either further cleared or modified.

REPLY: we meant the scavenging rate of total redox-active compounds, as measured by ROS activity. We will rephrase the text.

Now reads: "the scavenging rate of total redox-active compounds (71% of ROS activity) which is higher than etc.".

Enhanced toxicity of aerosol in fog conditions in the Po Valley, Italy

Stefano Decesari¹, Mohammad H. Sowlat², Sina Hasheminassab², Silvia Sandrini¹, Stefania Gilardoni¹, Maria Cristina Facchini¹, Sandro Fuzzi¹, Constantinos Sioutas²

¹Institute of Atmospheric Sciences and Climate of the National Research Council of Italy, Bologna, Italy

²Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA, USA

Correspondence to: Stefano Decesari (s.decesari@isac.cnr.it)

Abstract. While numerous studies have demonstrated the association between outdoor exposure to atmospheric particulate matter (PM) and adverse health effects, the actual chemical species responsible for PM toxicological properties remain a subject of investigation. We provide here reactive oxygen species (ROS) activity data for PM samples collected at a rural site in the Po Valley, Italy, during the fog season (i.e., November-March). We show that the intrinsic ROS activity of Po Valley PM, which is mainly composed of biomass burning and secondary aerosols, is comparable to that of traffic-related particles in urban areas. The airborne concentration of PM components responsible for the ROS activity decreases in fog conditions, when water-soluble species are scavenged within the droplets. Thanks to this partitioning effect of fog, the measured ROS activity of fog water was contributed mainly by water-soluble organic carbon (WSOC) and secondary inorganic ions rather than by transition metals. We found that the intrinsic ROS activity of fog droplets is even greater (> 2.5 times) than that of the PM on which droplets are formed, indicating that redox-active compounds are not only scavenged from the particulate phase, but are also produced within the droplets. Therefore, even if fog formation exerts a scavenging effect on PM mass and redox-active compounds, the aqueous-phase formation of reactive secondary organic compounds can eventually enhance ROS activity of PM when fog evaporates. These findings, based on a case study during a field campaign in November 2015, indicate that a significant portion of airborne toxicity in the Po Valley is largely produced by environmental conditions (fog formation and fog processing) and not simply by the emission and transport of pollutants.

1 Introduction

There is a rapidly growing body of epidemiological evidence identifying major health impacts associated with population exposure to airborne particulate matter (PM), including, but not limited to, respiratory and cardiovascular diseases, as well as neurodegenerative effects (Pope et al., 2002; Pope et al., 2004; Dockery and Stone, 2007; Davis et al., 2013; Gauderman et al., 2015). Even if the international air quality standards for atmospheric PM are based on mass concentrations (PM₁₀ and PM_{2.5} for particles of diameter below 10 or 2.5 μm , respectively), the WHO acknowledges that it is likely that not every PM component is equally important in causing these health effects (WHO, 2007, 2013). Research on traffic-related PM has provided a first epidemiological evidence of the links between adverse health effects and PM chemical composition (Janssen et al., 2011; Bates et al., 2015), in line with the results of several toxicological studies (Nel, 2005; Cassee et al., 2013; Fang et al., 2015), while outside urban areas, the characterization of PM and associated

health effects is sparse. During transport in the atmosphere, particles emitted by traffic are progressively diluted and eventually transformed by chemical processes that enrich them in secondary components (Zhang et al., 2007; Crippa et al., 2014). Moreover, the oxidation of reactive volatile organic compounds (VOCs) supplies a range of water-soluble compounds (e.g., formaldehyde, glyoxal), partitioning into the particulate phase at high relative humidities (deliquescent aerosols and cloud/fog droplets), where they can be further oxidized into new secondary organic compounds (so called “aqueous SOA”) (Ervens et al., 2011). The current understanding of the health impacts of PM secondary components is certainly much more limited than for traffic-related aerosols. Although recent chamber studies have indicated increased oxidative potential of PM emitted from a variety of sources (e.g., combustion or biomass burning) after undergoing oxidation and/or secondary formation (Antinolo et al., 2015; McWhinney et al., 2011; McWhinney et al., 2013), there is a lack of direct field toxicological observations, which is partly caused by the difficulty of disentangling the secondary PM fraction from other oxidized organic material of primary nature in field conditions. In this study, we investigate the toxicological properties of PM at a polluted rural site, where the presence of fog is responsible for aerosol scavenging and deposition but also for secondary aerosol formation through aqueous-phase processes. Fog and low-level clouds are transient phenomena in the atmosphere but their occurrence can be high in wintertime in certain areas of the globe, including many highly-populated sites enclosed in orographic basins. (Cermak et al., 2009) showed that several pollution hotspots in Europe, including Benelux, the Ruhr district, the basins of Paris and London and the Po Valley, experience low-level clouds and fogs for 35% to 60% of the days in winter months. The fraction of fog days in fall/winter in the Californian Central Valley (6.5 millions inhabitants) is ca. 20% according to (Balducchi and Waller, 2014). Fog frequencies of ca. 10% in winter are also characteristic of the Yangtze River corridor (Niu et al., 2010), and even greater values (20% to more than 35%) are typical of the Indo-Gangetic plain (Saraf et al., 2011). All these regions of the globe commonly experience PM pollution peaks in winter months, during the fog season. In this season of the year, the same stable weather conditions favor the accumulation of air pollutants and fog formation. Therefore, fog-processing is potentially a major driver for secondary aerosol formation in wintertime at all these sites. In (Gilardoni et al., 2016), we provided a first estimate of secondary organic aerosols produced by aqueous-phase processing of wood smoke particles in Europe: 0.1 to 0.5 Tg of organic carbon per year, corresponding to 4 – 20% of total primary organic aerosol emissions in the region. In the present study, aerosol chemical and toxicological measurements were carried out in the Po Valley, Italy, where radiation fogs frequently occur during the cold season (up to 25% of the time in fall-winter months in rural areas, according to recent studies (Giulianelli et al., 2014). With approximately 20 million inhabitants (~30% of the Italian population), the Po Valley area has the highest population density across the country, and ranks among the top European “pollution hotspots” in terms of mortality attributable to PM exposure (Kiesewetter and Amann, 2014). In the rural areas of the Po Valley, wood smoke can be responsible for adverse health effects on humans (Naeher et al., 2007), while little information is available on the toxicological properties of secondary aerosols, including SOA species formed upon smoke particles by chemical processing in fog water and deliquesced aerosols. We provide here the first measurements of the

redox activity (as a proxy for toxicological potential) of fog water and interstitial (i.e., unscavenged) aerosols. The toxicological assay used in this study is capable of quantifying the oxidative potential associated with ambient PM, initiated via generation of reactive oxygen species (ROS) due to the interaction of target cells with redox-active components of ambient PM (Landreman et al., 2008; Daher et al., 2012; Verma et al., 2012b). We show that ROS, in the rural Po Valley PM, occur in concentrations comparable to that of a PM in a megacity, and that the ROS levels are even amplified in fog water with respect to the PM fraction scavenged within fog droplets. Results from the present study provide a basis for prospective epidemiological programs to evaluate how fog scavenging/processing of PM impacts on human health.

2 Methods

2.1 Measurement site

Fog samples were collected from 30 November to 30 December 2015 at the meteorological station Giorgio Fea in San Pietro Capofiume (44°39'15" latitude, 11°37'29" longitude), a rural site located 30 km northeast of Bologna (Italy) in the eastern part of the Po Valley (northern Italy). From 30 November to 4 December, an intensive observation period was scheduled, with the concurrent sampling of fog and aerosol samples and the deployment of a HR-ToF-AMS (Aerodyne Research) for online aerosol measurements. During the sampling campaign, a total of 6 aerosol samples and 16 fog samples were collected. Additionally, fog samples collected after the intensive observation period (i.e., after 4 December) were pooled in groups of two or three for the analysis of metals and oxidative potential.

2.2 Aerosol and fog-water sampling

Aerosol samples were collected by a PM1 Tecora Echo High Volume sampler equipped with a PM1 Digital sampling head, operating at 500 L min⁻¹ flow. One daytime and one nighttime sample were collected every day from 9:00 to 18:00 LT and from 18:00 to 9:00 LT respectively, on prewashed and prebaked Pall quartz fiber filters. Samples were wrapped with aluminum foil, zipped in plastic bags, and stored in freezer at -20°C until analysis. Fog samples were collected using an automated, computer driven active string collector described in (Fuzzi et al., 1997). A Particulate Volume Monitor PVM-100 (Gerber, 1991), used to determine fog liquid water content (LWC) at 1 min time resolution, was used to activate the string collector. **The collector and its strings were extensively cleaned at the beginning of the fog season, two weeks before the samples of this study were collected. In particular, the stainless-steel strings were carried to the CNR-ISAC chemical laboratory in Bologna where they were gently brushed to remove any stuck residues from fog sampling in the previous winter and washed with ultrapure (milliQ) water in an ultrasonic bath. Finally, they were brought back to the field and mounted on the fog collector before first the automatic sampling system was switched on around mid-November.** A LWC threshold of 0.08 g m⁻³ was chosen for the activation as an indicator of fog presence, which roughly corresponds to a 200 m visibility. **Concentrations of analytes in fog**

samples, expressed in $\mu\text{g mL}^{-1}$, were converted into $\mu\text{g m}^{-3}$ by multiplying with the fog liquid volume (mL m^{-3}). The latter was not estimated by the mass of sampled fog water multiplied by the flux of the fog collector, because the collection efficiency is typically much smaller than 100% (about 40%, according to Fuzzi et al. 1997). We used instead the liquid water content (LWC) measured by the PVM-100 and averaged over the sampling time of the fog collector to multiply by the concentrations of chemicals in fog water to obtain air equivalent concentrations ($\mu\text{g m}^{-3}$) of the fog components.

2.3 Chemical analysis of water-soluble components

Prior to chemical analysis a quarter of each aerosol quartz-fibre filter was extracted with 10 mL of 18-M Ω Milli-Q water by sonication for 30 minutes. Liquid extracts were filtered to remove quartz residues, then analyzed for Water Soluble Organic Carbon (WSOC) with a total organic carbon analyzer (Shimadzu TOC-5000A). Fog samples were filtered on 47 mm quartz-fibre filters (Whatman, QMA grade) within few hours after collection to remove suspended particulate. Conductivity and pH measurements were carried out immediately, then samples were stored frozen until further analysis. WSOC was determined in the same way as for aerosol filter extracts, in addition the ionic composition of fog was determined by two Thermofisher ICS_2000 ion chromatographs equipped with an IonPac AS11 2x250 mm separation column for anions and IonPac CS16 3x250 mm Dionex separation column, self-regenerating suppressors and KOH and MSA as eluents for anions and cations respectively. In both cases a gradient elution allowed the separation and detection of both inorganic and organic anions and cations.

2.4 Total elemental analysis of aerosol and fog samples

The magnetic sector inductively coupled plasma mass spectrometry (SF-ICP-MS; Thermo-Ginnigan Element 2) unit was used for the total elemental analysis of the aerosol and fog samples. Detailed information about the procedure can be found elsewhere (Zhang et al., 2008) (Okuda et al., 2014). Briefly, the samples are first digested in an automated microwave-aided (oven-aided in case of fog samples) digestion system (Milestone ETHOS+) using a mixture of ultra-high-purity acids (1.0 mL of 16 M nitric acid and 0.25 mL of 12 M hydrochloric acid). Then, the digestates are analyzed by SF-ICPMS after being diluted to 15 mL with high-purity water (18 m Ω). A combination of the SF-ICPMS instrumental analysis, method blanks, and digestion recoveries were used to estimate the analytical uncertainties. A total of 49 elements were determined using this method. Further details of the analytical method can be found in (Zhang et al., 2008) and (Okuda et al., 2014). The full chemical analyses (WSOC, ion chromatography, metals) were performed on 6 aerosol samples and 7 fog samples.

2.5 ROS analysis

The ROS assay, an in vitro exposure assay of PM extracts to rat alveolar macrophage cells (cell line NR8383) (Landreman et al., 2008), was used as a measure of PM toxicity of the samples. In this method, samples (only filters in this step) were first extracted using an initial sonication period for 15 min with high-purity 10 Milli-

Q (18 mΩ) water. The samples are then continuously agitated in the dark at room temperature for 16 h, followed by another 15-min sonication and 1-min agitation by a vortex mixer. A portion of the sample suspension was passed through 0.22 μm polypropylene syringe filters (referred to as filtered samples, representing WS components), while the other portion of the sample suspension underwent the ROS assay without filtration (referred to as the unfiltered samples)(Shafer et al., 2016). ROS activity analysis was carried out on 20 samples (= 6 aerosol + 7 unfiltered fog + 7 filtered fog samples).

In the in vitro exposure and ROS detection step, the membrane-permeable 2',7'-dichlorodihydro- fluorescein diacetate (DCFH-DA) probe gets deacetylated by cytoplasmic esterases to 2',7'-dichlorodihydro- fluorescein (DCFH) upon entering the macrophage cell. In the presence of oxidizing species (for example, the ROS species generated due to exposure to toxic PM components), DCFH is converted to its fluorescent form, which is DCF. In the next step, an M5e microplate reader (Molecular Devices, CA, USA) is used to determine the fluorescence intensity of each cell after the exposure at 488 nm excitation and 530 nm emission. The raw fluorescence data were corrected using blanks and normalized using Zymosan (ZYM) positive controls (i.e., β-1,3 polysaccharide of D-glucose from Sigma Aldrich, MO, USA) (Shafer et al., 2016). Filter blanks were used to account for the potential impact of the biologically active quartz filters on the ROS results. The ROS activity of filter blanks (N = 2) was on the order of 280 ± 13 fluorescence units (FU) for unfiltered extracts and of 27 ± 23 FU for filtered extracts. For comparison, the ROS activity of PM1 filter samples was as high as 3896 ± 350 FU and 1518 ± 118 FU for unfiltered and filtered extracts, respectively. Following subtraction of the contribution of blanks, the overall per-mass oxidative potential (representing the inherent toxicity) of samples was reported in units of Zymosan equivalents. The per-volume ROS activity (i.e., normalized by the volume of air samples) of samples was also calculated via multiplying the mass-based ROS by the PM mass concentration of a given sample. This provides a measure of the actual toxicity of the air inhaled by individuals, which is a very important parameter in exposure assessment studies (Wittkopp et al., 2013; Zhang et al., 2007; Delfino et al., 2010).

2.6 Statistical analyses

Spearman rank correlation was used to explore the association of individual components of the aerosol and fog with the ROS activity. We also applied the principal component analysis (PCA) to the ambient concentrations of the chemical components of the aerosols in order to identify the source factors that contribute to PM levels and ROS activity. In this analysis, the VARIMAX-normalized rotation approach was employed to identify the uncorrelated source factors (Henry, 1987). Additionally, we also applied the multi-linear regression (MLR) analysis to identify the source factors (as represented by the relevant species) that mostly contribute to ROS activity of aerosols and fog water. In this analysis, several combinations of species with high loadings in the PCA were regressed against the ROS levels, and the ones leading to the highest R2 value were kept in the final solution. The statistical significance was evaluated at both P values of <0.1 and <0.05.

3 Results

3.1 ROS activity of fog water and aerosol samples

When fog forms, aerosol particles are selectively scavenged into fog droplets, with the smaller and more hydrophobic particles left unscavenged as interstitial aerosol. This process tends to be selective with respect to specific chemical components (“partitioning”) and represents a useful way to study the toxicological properties of externally-mixed PM components under field conditions. Past studies in the Po Valley (Facchini et al., 1999; Gilardoni et al., 2014) highlighted that fog scavenging efficiency for different chemical components of atmospheric particles is related to their water solubility, with higher scavenging efficiencies for water-soluble (WS) species (e.g., inorganic ions and water-soluble organic carbon, WSOC) and lower scavenging efficiencies for hydrophobic compounds (e.g., elemental carbon and several metals) (Gilardoni et al., 2014). Therefore, during fog episodes, fog droplets are enriched in WS components, while water-insoluble (WI) species dominantly partition into interstitial aerosols (Hallberg et al., 1992; Fuzzi et al., 1988). Results from the present study are in good agreement with the published literature on the effect of fog scavenging on the aerosol properties: Figures S2 and S3 in Supporting Information indicate that organic as well as inorganic WS components (e.g. MSA, oxalate, nitrate, and sulfate) and metals with high WS fractions (including Ca, Na, and Mg) have the highest scavenging rate, whereas other combustion-related species with lower solubility (i.e. Fe, Cr, and Zn) are scavenged much less efficiently. Metals can be also scavenged by fog (Mancinelli et al., 2005) but not to the same extent as secondary organic and inorganic species, resulting in higher metal enrichment of the interstitial aerosol. The results on partitioning of WSOC between interstitial aerosols and fog water are presented in Figure 1c,d. Contrary to the chemical species discussed above, WSOC is a complex mixture of chemical species originating from a variety of sources. According to previous studies in the region (Gilardoni et al., 2014; 2016), oxidized particulate organic compounds include biomass burning products as well as secondary organic species, which also account for the products of transformation of biomass burning compounds upon fog processing. As can be seen in Figure 1c, the mass fraction of WSOC in PM₁ was comparable between daytime and interstitial aerosols, analogously to what is observed for some of the WS ionic species, like sulfate and oxalate, and contrary to others (e.g., nitrate and ammonium) which are considerably depleted in interstitial particles with respect to daytime aerosol. However, the airborne concentration of WSOC in daytime aerosol was approximately two times higher than that of interstitial aerosols (Figure 1c). Additionally, the concentration of WSOC was much higher in the fog water than in daytime and nighttime aerosols, both on a per-mass and per-volume basis.

We present here for the first time results on fog scavenging effects on ROS activity of the aerosol. The average ROS activity of daytime (i.e, without fog) and nighttime (in-fog as well as interstitial) aerosol bulk extracts and for fog water is reported in Figure 1a,b. On a per m³ of air volume basis, the ROS activity of fog water and daytime aerosol are quite comparable and about four times greater than that of interstitial aerosol. However, per PM mass ROS activities of the daytime and interstitial aerosols are very similar (1877±849 and 1870±1229 microgram Zymosan/mg of PM, respectively), while the ROS activity of fog water was

considerably higher (5194 ± 610 MicrogramZyosan/mg of PM) than that of both types of aerosols. The variability of ROS activity of PM in the Po Valley in the fog season is therefore complex. The extrinsic (on a per-volume basis) ROS activity in PM decreases upon fog scavenging similarly to total PM₁ mass concentrations (-70% and -66%, respectively) causing the toxicity of interstitial particles being comparable to that of the original aerosol population. In contrast, the ROS activity of fog water is clearly higher than the scavenged fraction of ROS activity of PM (calculated as the difference between daytime and interstitial PM activity). This trend reflects very closely that of WSOC levels (Figure 1c,d). The excess of WSOC mass in fog water with respect to daytime aerosol indicates that organic solutes in fog droplets do not originate simply from aerosol scavenging, and that additional sources from aqueous-phase processing of **absorbed** volatile organic compounds must be taken into account. Secondary organic aerosol (SOA) sources in fog water could therefore explain the “amplification” of ROS activity with respect to the parent aerosol population.

To further explore the difference in the toxicity of daytime and interstitial aerosols, the ROS activity of daytime and nighttime aerosols was also evaluated based on the filtered ROS analysis protocol, representing the toxicity of the WS components (Figure 2a,b). As already mentioned, volume-based ROS activity of daytime aerosols was 3-5 times higher than that of interstitial aerosols, but this difference becomes even greater in the filtered extracts (a factor of 7, see Figure 2a). It should be noted that during the daytime, the per PM-mass filtered ROS activity of aerosols (which relates to the redox activity of only the WS fraction) is almost half of the unfiltered ROS levels (representing the redox activity of both WS and WI fractions). However, during nighttime, the per PM mass ROS activity of the WS fraction contributes approximately only 25% to the overall per-mass ROS aerosol activity (Figure 2b). These results imply that during daytime, WS and WI PM fractions contribute roughly equally to the ROS activity of the aerosol, whereas during nighttime, the WI components (comprising mostly elements, metals and insoluble carbonaceous material) contribute to as much as 75% of the aerosols ROS activity. Therefore, even if daytime and interstitial aerosols exhibit the same intrinsic toxicity (Figure 1b), this is driven by different chemical composition in the two aerosol populations. The most critical difference between daytime and interstitial (i.e., unscavenged) aerosols is that the latter are depleted of WS components, including WSOC (Figure 1) and inorganic aerosols (Figures S2 and S3) **which are efficiently scavenged by fog. Therefore, as discussed above, the higher ROS activity of daytime aerosols compared to that of nighttime/interstitial aerosols must be attributed to WS components, while, at nighttime, the aerosol ROS activity is mainly driven by WI components.** It is also noteworthy that the highest ROS activity was observed in the fog water, which is enriched in WS components compared to the daytime and interstitial aerosols.

3.2 Association of ROS activity to PM and fog chemical components

To investigate the main species driving the ROS activity in fog water, we performed Spearman rank correlation as well as Multilinear regression (MLR) analysis between per-volume concentrations of all species and per-volume ROS activity of fog water, the results of which are presented in Tables 1, 2, and in Table 3. As can be seen in Table 1, most of the WS species were strongly correlated with the ROS activity of fog.

However, this does not necessarily mean that all of these species, including inorganic ions, are responsible for the aerosol redox activity. Previous studies have indicated that some of these associations with toxicologically innocuous species (e.g., inorganic ions) are observed because of the co-linearity of inorganic ions with important redox active species, including WSOC, and not because of the toxicity of these components per se (Ntziachristos et al., 2007) (Cho et al., 2005) (Verma et al., 2012a). For instance, (Verma et al., 2012a) found strong positive correlations between the redox activity of quasi-ultrafine PM (PM_{0.18}) and NO₃⁻, SO₄²⁻, NH₄⁺, and WSOC; however, the authors concluded that the strong correlations observed between these inorganic ions and redox activity is because of their co-linearity with WSOC (as also shown in Table S1) rather than their own toxicity, as these components are not mechanistically active in these assays (Cho et al., 2005). **It should be noted, however, that inorganic acids could indirectly affect the ROS activity of fogs and wet aerosols by solubilizing redox-active metals (Giulianelli et al., 2014; Oakes et al., 2012; Fang et al., 2017). Such mechanisms can be important for areas where SO₂ levels are high, while they are less plausible for environments such as the Po Valley where aerosols and fogs are largely neutralized by ammonia, as demonstrated by the typical fog pH of about ~6, according to Giulianelli et al. (2014).**

The attribution of ROS activity of fog to dissolved organic substances was also confirmed by the results of the MLR analysis (Table 3), demonstrating that WSOC alone explains 98% of the variability in the fog water ROS levels. We observed much smaller correlation coefficients between the ROS activity of fog water samples and elemental/metallic components, which have been shown to correlate with particle toxicity in earlier studies (Ntziachristos et al., 2007) (Cho et al., 2005) (Hu et al., 2010) (Verma et al., 2012a). This is due to the lower concentration of metals/elements in the fog water compared to daytime and interstitial aerosols, as shown in Figure S3.

To investigate the major PM chemical species that contribute to the ROS activity of daytime and interstitial aerosols, we performed a principal component analysis (PCA) followed by a linear correlation and a multilinear regression (MLR) analysis on the data pertaining to aerosols samples. It should be noted that, due to the limited number of aerosol samples analyzed for ROS activity (a total of 6), the data for both daytime and nighttime (i.e., interstitial) aerosols were combined together, and the following analyses were performed on the pooled data. Table S2 presents the results of the PCA conducted on the chemical components of daytime and interstitial aerosols. As can be seen in the table, two source factors were resolved, together explaining 95% of the variance in the data. The first source factor comprises species that most likely are of combustion origin (e.g., traffic, power plants), even though some of them (e.g., Fe, Mn, and Cu) may also come from non-exhaust traffic sources in other areas (Sanderson et al., 2014). The metals in this group, including V, Fe, Cu, Mn, and Ni, are considered toxic and known as redox-active metals (Argyropoulos et al., 2016; Sowlat et al., 2016; Wang et al., 2016). The second source factor consists of water-soluble components, including WSOC as well as the ionic components (methane-sulfonate, oxalate, sulfate, and nitrate) which are tracers of secondary aerosols (Hu et al., 2010; Hasheminassab et al., 2013). We should point out that the PCA grouping of the chemical species reflects both the possible day-to-day variations in the contributions of the specific sources to PM mass as well as the diurnal cycles in concentrations governed by the specific fog

scavenging rates (Figures S2, S3). Table 2 shows the Spearman rank correlation coefficients between the WS components and elemental/metallic species of the aerosols and the corresponding ROS levels. Very strong positive correlations were observed between the ROS activity of aerosol samples and many of the WS components, which are also known tracers of secondary organic aerosols (SOA); for example, the Spearman rank correlation coefficients between the ROS activity and the concentrations of MSA and oxalate were 0.83 and 0.83, respectively. Similar as in the case of fog water, the observed association of inorganic ions and low-molecular weight organic acids with the aerosol ROS activity is probably because of the co-linearity of the ionic species and (unspeciated) redox-active organic compounds. Interestingly, correlation coefficients are greater for the SOA tracers (oxalate, MSA) than for the bulk WSOC mixture ($R = 0.66$) and for the whole organic mass measured by the AMS ($R = 0.43$), suggesting that the ROS activity is prevalently contributed by the secondary fraction of particulate organic matter. However, Table 2 also shows strong positive and statistically significant correlations for several metal/elemental species, such as Fe, Ni, and Cu, which are primary components of the aerosol. The correlation results were also corroborated by the outcome of the MLR analysis (Table 3), indicating that MSA (representative of the secondary organic aerosols source factor) and Ni (representative of the primary combustion-related source factor) were highly correlated with the ROS activity in the aerosol samples. Judging by the standardized coefficients (0.79 and 0.30 MicrogramZymosan/m³ air for MSA and Fe, respectively) and partial correlation values (0.94 and 0.73 for MSA and Fe respectively), the secondary organic aerosol fraction (represented by MSA in the MLR analysis) overall has a higher contribution to the aerosol ROS activity compared to combustion-related metals (represented by Fe in the MLR analysis). The results discussed above imply that during daytime, the aerosol ROS activity is driven by a combination of WS organics and redox active metals. However, during nighttime, when fog scavenging partitions the WS components into the water phase and enriches the nighttime (or interstitial) aerosols with metals, the aerosol ROS activity is mainly driven by elemental/metallic components, while the ROS activity of fog is mostly driven by WS organic compounds.

WSOC is the main driver of toxicity in fog water and also a likely contributor of toxicity of PM₁ during daytime when the contribution of WS components to toxicity is significant (Fig. 2). Submicron organic aerosols in rural Po Valley originate mainly from combustion processes (biomass burning) but a significant fraction of mass is the product of chemical transformations occurring in wet aerosols and fog droplets (“aqueous SOA”) (Gilardoni et al., 2016). Biomass burning organic tracers and their products of oligomerization formed in the aqueous phase contain hydroquinone and catechol moieties that can participate to redox reactions leading to ROS formation. The formation of such redox active organic compounds occurs in the aqueous phase, which explains a correlation with ionic tracers such as oxalate. Another source of reactive WSOC dissolved in deliquesced aerosols and especially in fog water is the uptake of water-soluble reactive gases. It is well known that organic vapours, like low-molecular weight organic acids and carbonyls, can account for a significant fraction of WSOC in fog (up almost 50% according to (Collett et al., 2008)). Some, like formic acid, exhibit ROS activity (Du et al., 2008). In this study, the contribution of low-molecular weight organic acids to WSOC was on average $8\% \pm 4\%$, which represents a lower limit for the contribution of WS VOC to fog solutes (as

formaldehyde and other carbonyls were not measured). The uptake of reactive gases could explain the excess of WSOC in fog water compared to the scavenged fraction of daytime PM1 (Figure 1 c,d) as well as the higher intrinsic toxicity of fog components, as shown in Figure 1 b.

4 Discussion and Conclusions

5 This study reports ROS activity data for PM1 and fog water samples at a continental rural area virtually free of local traffic emissions and where aerosol mass is prevalently contributed by biomass burning and secondary organic and inorganic components (Gilardoni et al., 2014). The aerosol chemical composition differed substantially from that of urban PM for which the induced oxidative potential has been documented by several studies (Saffari et al., 2014) and can be put in relation to the redox-active activity of traffic-related aerosols (Ning and Sioutas, 2010). Despite the very limited in situ traffic emissions at the Po Valley site, the ROS levels recorded for PM (1879 ± 851 and 1873 ± 1199 MicroZymosan/mg during day and night respectively) and fog samples (4142 ± 1347 MicroZymosan/mg) in our study are comparable with those reported by (Saffari et al., 2014), who reported ROS values of approximately 800 and 6000 MicrogramZymosan/mg PM in Los Angeles (LA) for PM2.5 and quasi-ultrafine PM (PM0.25), respectively. Additionally, in a more recent on-road study conducted in 2015 inside two major freeways of Los Angeles, the average (\pm SD) per-mass ROS activity of PM2.5 particles was found to be 3660 (± 1743) and 3439 (± 3058) MicrogramZymosan/mg PM for I-110 and I-710, respectively (Shirmohammadi et al., 2017), indicating that the intrinsic toxicity of the Po Valley fog water is higher than that of PM2.5 particles collected on the freeways of Los Angeles.

10 Although direct comparison of the ROS activity of the fog and aerosol samples collected in this study with those of ambient aerosol previously reported by other studies is associated with some uncertainties (due mainly to the different size range of the particles collected and the fact that the ROS activity appears to be strongly particle size-dependent (Sioutas et al., 2005; Saffari et al., 2014), nonetheless these results point to possible health effects associated with PM exposure during fog episodes in the Po Valley, the toxicity of which are comparable or, in many cases, higher than that of the highly toxic traffic-related PM. Our results show that the toxicity of aerosol particles accumulating in orographic depressions at the mid-latitudes during the cold season, which is normally peak concentration season for PM in many continental areas, can be further amplified by the formation of fog, whose intrinsic toxicity is even greater than that of the original aerosol particles scavenged into the droplets. Moreover, the redox potential of fog solutes is mainly driven by oxidized organic compounds, which also explains the excess of ROS activity in fog water with respect to the scavenged fraction of the aerosol. The effect of secondary organic species on ROS activity in fog and aerosols in the Po Valley is clearly demonstrated by the fact that fog water exhibits a higher intrinsic toxicity with respect to PM1, despite its depletion of redox active metals as a consequence of the systematically different scavenging rates between WS and WI aerosol species. The contribution of WS secondary species to ROS activity is supported also by: a) the results of MLR analysis, b) the scavenging rate of total redox-active compounds (71% of ROS activity) which is higher than that for most combustion-related metals (typically <

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60%), corroborating the contribution of hygroscopic particles to ROS activity; and c) the results obtained from the filtered extracts indicating that ca. 50% of ROS activity is attributable to WS species in daytime (i.e., out-of-fog) conditions. The origin of secondary organic compounds responsible for the ROS activity of aerosol WSOC in the Po Valley cannot fully be elucidated based on this set of data. However, previous observations at the same site in the fog season showed that SOA are produced in large amounts by aqueous reactions in fog droplets and deliquesced aerosols starting from organic compounds emitted from biomass burning (Gilardoni et al., 2016). Production of biomass burning SOA is accompanied by the formation of redox active organic compounds, including hydroquinones. We can therefore speculate that the enhancement of ROS activity in fog water is at least partly irreversible, as evaporating fog droplets become enriched on newly-formed redox-active secondary species. The relevance of secondary sources of toxicity in fog and fog-processed aerosols calls for more stringent controls on possible precursor emissions, which should be pursued by policy makers including international authorities (as secondary PM components concentrations are often triggered by transboundary pollution) (Kiesewetter and Amann, 2014). Finally, the health effects of the exposure to fog water toxics depends on fog frequency, and in turn on climate conditions and climate change. Substantial reductions (-50%) of fog occurrence in the last 30 years have been documented for the Po Valley and many other European locations, and these dramatic changes have been attributed to global warming and changes in cloud condensation nuclei concentrations (Vautard et al., 2009; Giulianelli et al., 2014). The enhanced toxicity of fog droplets observed in this study suggests that the historical reduction of fog frequency may result in an unintended improvement of air quality in many continental areas, overlapping also with the deliberate reduction of PM emissions put into practice since the early 90's in many developed countries.

Acknowledgements

This research was funded by Regione Emilia Romagna as part of the "Supersito" project (Decreto Regionale 428/10) and co-funded by the European Union's Seventh Framework Programme (FP7/2007-797 2013) under grant agreement no 603445 (BACCHUS). The authors would also like to acknowledge the financial support from the United States National Institute of Allergy and Infectious Diseases (award number: 5R01AI065617-15) and the National Institutes of Health (grant number: 5R01ES024936-02).

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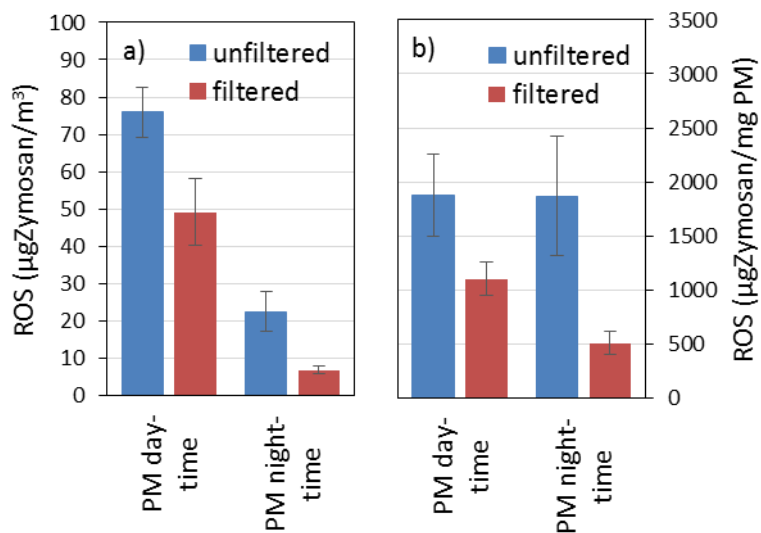
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5 Figure 2: Per-volume (MicrogramZyosan/m³ air) (a) and per-mass (MicrogramZyosan/mg PM) (b) ROS activity of the aerosol samples collected in the Po Valley in fall 2015. The values for aerosol samples are based on both filtered (representing the redox activity of WS components) and unfiltered (representing the redox activity of both WS and WI components) ROS analysis protocols. Bars represent geometric means and error bars correspond to one standard error (SD).

Table 1 - Spearman rank correlation coefficients between per-volume concentrations of water-soluble species as well as metals/elements in the fog water samples and the corresponding ROS levels. Correlation coefficients which were statistically significant (at P<0.05) are highlighted in bold.

Species	Corr Coeff	Species	Corr Coeff
Water-soluble components			
Acetate	0.72*	Cl ⁻	0.86
Dimethylamine	0.58	K ⁺	0.93
Ethylamine	0.90	Mg²⁺	0.90
Formate	0.75*	Na ⁺	0.89
Methansulfonate	0.89	NH₄⁺	0.82
Methylamine	0.68*	NO ₂ ⁻	-0.75
Oxalate	0.52	NO₃²⁻	0.97
Trimethylamine	0.89	SO ₄ ²⁻	0.61
Ca²⁺	0.86	WSOC	0.93
Metals/Elements			
Na	0.26	Ni	0.26
Mg	0.60	Cu	0.49
Al	0.49	Zn	0.60
Ca	0.49	As	0.26
Ti	0.49	Cd	0.49
V	0.26	Ba	0.49
Cr	0.49	Pb	0.60
Mn	0.60	Th	0.49
Fe	0.49		

*Denotes statistical significance at P<0.1.

Table 2 - Spearman rank correlation coefficients between concentrations of the metals/elements, markers of organic aerosol, and water-soluble (WS) components in the aerosol samples and the corresponding ROS levels. Statistically significant ($P < 0.05$) correlation coefficients are highlighted in bold.

Species	Corr Coeff	Species	Corr Coeff
Markers of organic aerosol			
m/z 44 (marker of OOA ¹)	0.43	m/z 60 (marker of BBOA ²)	0.31
Water-soluble components			
Organic matter	0.43	K ⁺	0.60
WSOC	0.66	Mg ²⁺	0.62
Acetate	0.66	Na ⁺	0.60
Formate	0.66	NH ₄ ⁺	0.31
Methansulfonate	0.83	NO ₃ ⁻	0.66
Oxalate	0.83	SO ₄ ²⁻	0.66
Cl ⁻	0.66		
Elements/metals			
Na	0.54	Ni	0.77*
Mg	0.03	Cu	0.77*
Al	-0.25	Zn	0.60
Ca	0.33	As	0.66
Ti	-0.52	Cd	0.31
V	0.74	Ba	-0.15
Cr	0.26	Pb	0.66
Mn	0.66	Th	0.14
Fe	0.77*		

*Denotes statistical significance at $P < 0.1$.

5 1 OOA: Oxygenated organic aerosol

2 BBOA: Biomass burning organic aerosol

Table 3 - Output of multiple linear regression (MLR) analysis using ROS activity as the dependent variable and ambient concentrations of the measured chemical species as independent variables.

Category	Species	Standardized coefficients	Units	Partial R	R	P-value	R ²
Fog Samples	WSOC	0.97	µg Zymosan/ m ³ air	0.98	0.98	<0.0001	0.96
Aerosols	MSA	0.79	µg Zymosan/ m ³ air	0.94	0.97	0.016	0.94
	Fe	0.30	µg Zymosan/ m ³ air	0.73		0.161	