Reply (in green) 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 NO_2 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).

Page 3, Line 15-16: The conversion of µg/mL to µg/m3 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 µg mL^{-1}, were converted into µg 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 (µg 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 (PM1 ~ 35 μg/m^3 in daytime, Fig. S1).

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.

Page 7, Line 14-16: Although, it seems possible that the high correlation of inorganic species such as SO4-2, 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 SO4-2 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 basis (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 SO_2 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 SO_2 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 SO4-2 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.