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FIRST EVALUATION OF THE EFFECT OF MICROORGANISMS ON STEADY STATE HYDROXYL RADICAL CONCENTRATIONS IN ATMOSPHERIC WATERS

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Abstract

Clouds are complex multiphasic media where efficient chemical reactions take place and where microorganisms have been found to be metabolically active. Hydroxyl radical is the main oxidant in cloud water, and more generally in the atmosphere, during the day and drives the cloud oxidative capacity. However, only one measurement of the steady state hydroxyl radical concentrations in cloud water has been reported so far. Cloud chemistry models are used to estimate the hydroxyl radical concentrations with values ranging from 10^{-12} to 10^{-15} M that are surely overestimated due to a lack of knowledge about the speciation of the organic matter acting as a sink for hydroxyl radicals. The aim of this work is to quantify the concentration of hydroxyl radicals at steady state in rain and cloud waters and to measure the impact of native microflora on this concentration. First, the non-toxicity of terephthalic acid as probe is controlled before the analysis in real atmospheric water samples. Higher

concentrations of hydroxyl radicals are found in cloud waters than in rain waters, with a mean value "1.6 ± 1.5" × 10^{-16} M and "7.2 ± 5.0" × 10^{-16} M for rain and cloud waters respectively and no real impact of microorganisms was observed. This method allows the measurement of steady state hydroxyl radical levels at very low concentrations (down to 10^{-17} M) and it is biocompatible, fast and easy to handle. It is a useful tool, complementary to other methods, to give a better overview of atmospheric water oxidant capacity.

Keywords

Cloud water, photochemistry, radical chemistry, oxidative capacity, microorganisms interaction.

1. Introduction

Clouds are multiphasic systems where many chemical reactions occur, particularly in droplets (Faust, 1994; Herrmann et al., 2015; Lelieveld and Crutzen, 1991). Very efficient photochemical processes take place inside droplets and chemical reactions within clouds can be faster than the equivalent reactions in the gas phase. The aqueous phase chemistry leads to oxidation processes that can be driven by reactions with radicals. Among the radicals, hydroxyl radical is the most efficient oxidant in the atmosphere (Lelieveld et al., 2004). Several works were carried out on hydroxyl radical chemistry in cloud to define $^{\bullet}$OH sources and reactivity (Blough and Zepp, 1995; Chameides and Davis, 1982; Jacob, 1986; Mauldin III et al., 1997). Main sources of hydroxyl radical are i) hydrogen peroxide, nitrite and nitrate photolysis (Yu and Barker, 2003; Zafiriou and Bonneau, 1987; Zellner et al., 1990; Zepp et al., 1987), ii) iron *via* ligand-to-metal charge-transfer reactions or photo-Fenton chemistry (Deguillaume et al., 2004, 2005; Faust and Hoigné, 1990; Faust and Zepp, 1993; Nakatani et al., 2007; Weschler et al., 1986), iii) the reaction of ozone towards the superoxide radicals and

iv) the mass transfer of •OH from the gas phase to the aqueous phase (Arakaki and Faust, 1998; Faust and Allen, 1993). In terms of •OH sinks, the main way is the reactivity with dissolved organic matter (Arakaki et al., 2013). However, due to the complexity of this environmental matrix, the organic composition is poorly characterized (Herckes et al., 2013); for example, only 21% of the organic matter in cloud waters sampled at the puy de Dôme station is characterized (Bianco et al., 2016). Furthermore, microorganisms are also present and metabolically active in cloud water. They can survive in spite of numerous stress factors like osmotic shocks, temperature changes and freeze-thaw cycles (Amato et al., 2007a; Joly et al., 2015). In order to survive, they use organic matter as a source of carbon such as short chain aldehydes and carboxylic acids (Amato et al., 2007b; Ariya et al., 2002; Vaïtilingom et al., 2011) but they also have an effect on the oxygenated compounds. As aerobic cells, they are able to consume or either neutralize Reactive Oxygen Species (ROS) like hydroxyl radical, singlet oxygen and hydrogen peroxide. Cloud microorganisms, present in cloud water, have the capacity to degrade hydrogen peroxide and can contribute to the degradation of organic compounds during the day and even more during the night (Vaïtilingom et al., 2010, 2013).

Up to now, the hydroxyl radical concentrations have been estimated using cloud chemistry models. These models consider multiphase chemistry and the mass transfer from the gas to the aqueous phase. Simulated concentrations range from 10^{-12} to 10^{-15} M (Deguillaume et al., 2004, 2005, Herrmann et al., 2000, 2010; Mouchel-Vallon et al., 2017; Tilgner and Herrmann, 2010; Tilgner et al., 2013) depending on the chemical scenarios and on the complexity of the chemical mechanisms. For example, the amounts of organic matter and iron are key parameters that drive the level of •OH. Moreover, formation rates of hydroxyl radical in irradiated rain or cloud waters are measured in the laboratory (Faust and Allen, 1993; Nakatani et al., 2007) and are used to evaluate cloud chemistry models (Bianco et al., 2015);

they express the maximum of •OH concentration formed without taking into account the different scavengers present in cloud water. The steady state hydroxyl radical concentrations have been estimated in different surface waters (Arakaki et al., 1999; Brezonik and Fulkerson-Brekken, 1998; Haag and Hoigné, 1985; al Housari et al., 2010; Mill et al., 1980; Qian et al., 2001; Russi et al., 1982; Zepp et al., 1987; Zhou and Mopper, 1990) but, to our knowledge, the study of Anastasio and McGregor (2001) is the only one on cloud water. In their experiments, they used benzene and benzoic acid as chemical probes at concentrations ranging from 50 to 1500 µM and analyzed reaction products by HPLC. Because the most concentrated species in cloud water are in the µM range, addition of a probe in high concentration can impact the biological activity which is controlled by the environment (Metallo and Vander Heiden, 2013; Shingler, 2003; Tropel and Meer, 2004). Toxicity issues could also arise. Existing methods must then be adapted to study the impact of microorganisms. Moreover to take biological variability into account several experiments must be performed in parallel with strictly identical conditions.

The main goal of our study is to evaluate the stationary concentration of OH radicals in atmospheric waters and the potential impact of microorganisms on this concentration. For that purpose we had to develop a specific method which main characteristics are i) to take into account the native microorganisms, and their viability, ii) the use of small volume of sample (300 µL/well) for one measurement so 6 mL for a complete analysis, and iii) the direct and fast measurement by fluorescence, without chromatography technics.

2. Experimental section

2.1. Chemicals

Terephthalic acid (TA) (Sigma Aldrich) and 2-hydroxyterephthalic acid (TAOH) (Atlantic Research Chemical), with a purity of 98 and 97% respectively, are used without additional purification. Acetic acid and H_2SO_4 are from Acros Organic. Formic acid, oxalic acid, succinic acid, K_2SO_4, $MgCl_2$-$6H_2O$, NO_3NH_4, NaH_2PO_4-$2H_2O$, Na_2HPO_4-$2H_2O$, ferrozine and hydrogen peroxide (30% in water, not stabilized) are from Fluka. $CaCl_2$-$2H_2O$, NaCl, horse radish peroxydase, Tris-EDTA buffer solution, dimethylsulfoxide (DMSO), $FeCl_3$-$6H_2O$ and ethylenediamine-N,N'-disuccinic acid (EDDS) are from Sigma Aldrich. NaOH is from Merck and 4-hydroxyphenylacetic acid is from Alfa Aesar®.

2.2. Irradiation setup

Lamps used for the irradiation setup are tropical terrarium bulb call Reptile UVB (13 W) from Exo Terra®. The polychromatic emission spectrum of the irradiation system reaching the solution is recorded using an optical fiber with a charge coupled device (CCD) spectrophotometer (Ocean Optics USD 2000 + UV–vis) which is calibrated using a DH-2000-CAL Deuterium Tungsten Halogen reference lamp. The total irradiance in the UV region of solar spectrum (290 and 400 nm) reaching the solution is then estimated to be 915 μW cm−2. In Fig. 1, the lamp emission is compared with the solar spectrum measured at the puy de Dôme station under cloudy or clear sky conditions.

2.3. Sampling and analysis of natural atmospheric waters (cloud and rain)

Rain samples were collected in September 17th, October 28th and December 2nd, 2015 and on February 17th and September 15th, 2016 on OPGC (Observatoire de Physique du Globe de Clermont-Ferrand) building roof (435 m above sea level, 45°76' North, 3°11' East, France) with a homemade pluviometer, composed of sterilized Erlenmeyer flask (2 000 mL) and sterilized funnel. Cloud waters were collected on February 16th, April 1st, June 1st and 5th and

July 2nd, 2016 at the puy de Dôme station (1 465 m above the sea level, 45°46' North, 2°57' East, France) that belongs to the atmospheric survey networks EMEP (the European Monitoring and Evaluation Program), GAW (Global Atmosphere Watch) and ACTRIS (Aerosols, Clouds, and Trace gases Research Infrastructure). For this, a sterilized single-stage cloud collector was used as described in Deguillaume et al. (2014).

Cloud and rain samples are kept in the dark and stored at 4°C before the experimentation. Chemical composition analyses are performed on cloud and rain samples through pH, total organic carbon (TOC) concentration, iron concentration and ionic species concentrations. The pH is measured before and after experimentation with a pH meter MP 225 (Mettler Toledo). TOC analyses are performed with TOC 5050A analyzer (Shimatzu). Concentrations of Fe(II) and Fe(III) are quantified by spectrophotometric assays after complexation with ferrozine (colorimetric complexing agent) as described by Parazols et al. (2007). Ionic species are quantified by ion chromatography with a Dionex DX320 column for anions and a Dionex ICS1500 column for cations. For microbiological characterization, cells are counted by flow cytometry. Cultured microorganisms are grown on R2A medium at 5 and 17°C. ATP is measured with BioThema© ATP Biomass kit HS.

Measurements of hydroxyl radical concentrations in atmospheric waters are performed under irradiation and with filtered or non-filtered samples. Waters are filtered just before filling the plate, thanks to a Minisart® PES filter (pore size of 0.22 µm and filter diameter of 28 mm from Sartorius, Germany) under sterilized conditions.

All the vessels are washed with hydrochloric acid solution (2 M) and rinsed with ultrapure water and then sterilized.

2.4. Determination of steady state hydroxyl radical concentrations

The detection of $^{\bullet}OH$ radical is based on the reaction with TA (Fig. 2):

Among the products formed 2-Hydroxyterephthalic acid (TAOH) is detected and quantified due to its strong fluorescence. However, TAOH is not the unique byproduct and could be instable under irradiation (Page et al., 2010). For these reasons, TAOH formation yield (γ_{TAOH}) was previously described and evaluated as a function of irradiation conditions, temperature and pH by Charbouillot *et al.* (2011). In the same work the second order rate constant between TA and $^{\bullet}$OH was determined, k_{TA} = "4.1 ± 0.1" × 10^9 M^{-1} s^{-1}.

In order to obtain the real steady state concentration of $^{\bullet}$OH ($[^{\bullet}OH]_{ss}$), with natural organic matter present in cloud sample, we used the formation rate of TAOH (Rf_{TAOH}) to calculate the steady state hydroxyl radical concentrations as follows:

$$\frac{\frac{d[TAOH]}{dt}}{\gamma_{TAOH}} = k_{TA}.[TA].\left[^{\bullet}OH\right]_{SS} \quad (1)$$

So the steady state hydroxyl radical concentration can be calculated as follows:

$$\left[^{\bullet}OH\right]_{SS} = \frac{\frac{d[TAOH]}{dt}}{\gamma_{TAOH}.k_{TA}.[TA]} \quad (2)$$

To determine the steady state concentration, the linear part of the curve between Rf_{TAOH} and [TA] is used (Fig. S3). In fact, these conditions represent a low concentration of the probe in order to take into account the impact of all the natural scavengers present in cloud aqueous phase. The slope of the linear part of the equation is then divided by the second-order rate constant between TA and $^{\bullet}$OH (k_{TA}) and by the TAOH formation yield (γ_{TAOH}) as described in equation 2. These experimental conditions enable the determination of the real steady state concentration by taking into account the impact of all the chemical compounds present in the environment. Moreover, the use of TA as probe and the formation of TAOH which is detected by fluorescence spectroscopy, allows for reaching concentrations of $^{\bullet}$OH in the order of tenth of femtomolar.

For blank determination 8 measures have been performed in the dark. Limit of detection was calculated as mean $_{blank}$+ 3SD $_{blank}$ whereas LOQ was determined as mean $_{blank}$+ 10SD $_{blank}$.

All the experimental and calculation details are presented in the supplementary data file (Diagram S1, Fig. S1, S2, S3). Note that DMSO is used in order to stop the generation of TAOH during the experiments through light irradiation and/or different chemical processes occurring, thanks to its high reactivity with $^{\bullet}OH$ (k_{DMSO} = 6.6 10^9 M^{-1} s^{-1}) (Buxton et al., 1988). The addition of 5 µL of DMSO (1 mM) immediately after the sampling does not change significantly the quantification of $^{\bullet}OH$ (Fig. S4).

Finally we tested this protocol to measure OH radical concentrations at the steady state in controlled conditions using a microcosm mimicking cloud water conditions (artificial marine cloud medium, artificial light, H_2O_2 and Fe-EDDS (ethylenediamine-N,N'-disuccinic acid) complex as a source of radicals). The results are reported in the Supplementary Data (Figures S5 and S6).

Wilcoxon test for paired data statistical analysis are performed on hydroxyl radical concentration values to see significant difference (R software 3.5.0 used with α = 5%).

2.5. Measurement of hydrogen peroxide concentration

H_2O_2 concentrations are measured using an accurate enzymatic fluorimetric assay with a 4-hydroxy-phenylacetic acid that produces a fluorescent dimeric compound with hydrogen peroxide. This method is described in detail in the paper of Vaïtilingom et al. (2013).

2.6. Assessment of the probe non-toxicity

The non-toxicity of TA to microorganisms was evaluated on *Pseudomonas graminis* 13b-3, DQ512786 isolated from cloud water sampled at the puy de Dôme station. *Pseudomonas graminis* 13b-3 is incubated in R2A medium at 17°C and 130 rpm. After one day of

196 incubation, 6 mL of the culture are centrifuged for 3 min at 12 500 rpm. Cells pellets are
197 washed with NaCl 0.8% and cloud artificial medium. Optical density (OD) is taken with a
198 spectrophotometer (at $\lambda = 575$ nm) to obtain cell concentrations that are equal to 10^6 cells mL^{-1}
199 corresponding to concentration 10 times higher than in natural cloud water. Incubations are
200 done in cloud artificial marine media mimic the typical cloud chemical composition deriving
201 from marine influence. The used concentrations are also ten times higher than those reported
202 in natural cloud water, in order to keep the same ratio between cell and organic compound
203 concentrations. In fact, it has been showed that, at constant ratio "cell concentration/degraded
204 chemical compound concentration" and in the range of concentrations investigated,
205 biodegradation rates are independent of the absolute cell and chemical concentrations
206 (Vaïtilingom et al., 2011): acetic acid 2.0×10^{-4} M, formic acid 1.45×10^{-4} M, oxalic acid
207 3.0×10^{-5} M, succinic acid 1.5×10^{-5} M, $MgCl_2$-$6H_2O$ 1.0×10^{-4} M, $CaCl_2$-$2H_2O$ 4.0×10^{-4} M, K_2SO_4 5.0×10^{-5} M, NaCl 2.0×10^{-3} M, NO_3NH_4 8.0×10^{-4} M, NaOH 1.1×10^{-3} M and
208
209 H_2SO_4 3.15×10^{-4} M. pH solution is adjusted to 6.1 close to the average pH of natural clouds
210 representative of air mass from marine origin (Deguillaume et al., 2014). The solution is
211 sterilized with 0.22 µm PES filter (Polyethersulfone, diameter 28 mm, membrane of type
212 16532-k, Sartorius Stedium biotech, Germany) and kept in the dark at 17°C.
213 After incubation of cells, the media is tested with different concentrations of TA. At 0, 47 and
214 72 min of incubation, culture is done in R2A plate incubated at 17°C and ATP concentration
215 is measured with an ATP Biomass kit HS, BioThema©.
216
217 **3. Results**
218
219 *3.1. Validation of the non-toxicity of terephthalic acid*

In order to see the impact of microorganisms on hydroxyl radical concentration, it is crucial to check the non-toxicity of our probe. *Pseudomonas graminis* 13b-3 isolated in cloud water at the puy de Dôme station is chosen as a model strain because it is representative of the major actors of the cloud microbiota and on which we had already a lot of information available. First it belongs to the *Pseudomonas* genus which is widely present in puy-de-Dôme cloud samples. From cultural experiments we found this genus in 60% of our samples (Vaïtilingom et al., 2012) and metatranscriptomics data showed that *Pseudomonas* strains are among the most active in cloud waters (Amato et al., 2017). In addition we choose the strain *Pseudomonas graminis* 13-b3 as a model strain because we already studied its metabolism in Vaïtilingom et al (2010, 2011), Husarova et al (2011) and Wirgot et al (2017).

This strain is incubated at a concentration of "1.5 ± 0.3" × 10^6 cells.mL^{-1} with 3 different probe concentrations (0, 4 and 10 µM) in "artificial marine" cloud medium. The evolutions of cell counts and of ATP concentrations as a function of time are presented in Fig. 3.

Whatever the TA concentration, the number of bacteria remains within the same order of magnitude and so no impact of probe concentration is detectable. Similarly, no major change in ATP concentrations is observed with time under all the tested conditions, ranging from 1.6 to 2.6 × 10^{-6} pmol.$cell^{-1}$ with a mean value of 2.0 × 10^{-6} pmol.$cell^{-1}$.

Wirgot et al (2017) showed that *Pseudomonas graminis* 13-b3 metabolism and particularly its ATP content are very sensitive to environmental conditions. For instance the ATP concentration was highly impacted by the presence of H_2O_2, therefore *Pseudomonas graminis* 13-b3 is a good model to evaluate the impact of our probe on ATP concentration. In the same paper we showed that the results issued from the study of this model strain (a strong correlation between H_2O_2 and ATP concentrations) was valid when 32 cloud samples were considered, containing a very diverse biodiversity. In this sense *P. graminis* 13-b3 was a pertinent model for all the cloud microbiota.

Therefore we can conclude from the results of the present study that terephthalic acid does not impact *Pseudomonas graminis* 13-b3 viability and energy. In addition we expect that all the microorganisms in cloud water behave in a similar way than our model strain concerning TA toxicity.

Consequently, this probe can be used for measuring microorganism's impact on hydroxyl radical concentration.

3.2. Hydroxyl radical steady state concentration in atmospheric waters

With this method, $[^{\bullet}OH]_{ss}$ was evaluated under irradiation (2 hours) in various natural atmospheric waters, 5 rain waters and 5 cloud waters sampled in 2015 and 2016. Characterization of these atmospheric waters (including meteorological, physicochemical and biological parameters) and their retro-trajectories over 120 h are presented in supplementary data (Tables S1 and S2, Fig.S5 and S6, respectively).

During the two hours of irradiation the concentrations of $^{\bullet}OH$ are evaluated at different sampling times. At the same time, hydrogen peroxide concentrations are followed and no decrease of the concentration is observed (data not shown). The steady state hydroxyl radical concentrations obtained in rain and cloud waters are shown in Fig. 4. The standard deviations for steady state concentration are calculated from plots of Rf_{TAOH} versus TA concentrations.

Measured hydroxyl radical concentrations for atmospheric waters range from 2.8×10^{-17} M (in rain water) to 2.0×10^{-15} M (in cloud water). Steady state hydroxyl radical concentrations in cloud waters are higher than in rain waters, with values in cloud water ranging from 2.6×10^{-16} to 2.0×10^{-15} M with a mean value of $"7.2 \pm 5.0" \times 10^{-16}$ M and values in rain water ranging from 2.8×10^{-17} to 4.3×10^{-16} M with a mean value of $"1.6 \pm 1.5" \times 10^{-16}$ M. Thus the value of $[^{\bullet}OH]_{ss}$ is more than four times higher in cloud waters than in rain waters. On the contrary, no significant differences are observed between our 5 cloud samples or our 5

rain samples. Moreover, no real correlation is found between the main parameters (Fe, H_2O_2, NO_3^- and TOC) and OH radical concentration. The only tendencies observed are for the rain samples with the highest TOC concentrations where the calculated OH radical concentrations are the lowest and for the cloud samples where the OH radical concentrations increase with the increase of nitrate concentration. These tendencies support the fact that TOC is a major sink for OH radicals in natural water (Arakaki et al., 2013) and nitrate/nitrite could be an important photochemical source of OH radical in such medium (Kaur and Anastasio, 2017). However, from our results, no major influence of the air mass origins of the studied clouds and rains could be detected on $[^{\bullet}OH]_{ss}$, 5 samples are not enough and more samples should be analyzed to really conclude on this point.

After assessing the concentration of hydroxyl radicals in real atmospheric waters, the same experiments are performed with filtered waters in order to see if microorganisms can impact $[^{\bullet}OH]_{ss}$ (Fig. 4). Concentration of hydroxyl radicals without (filtered) or with (non-filtered) microorganisms in rain waters show similar results except for two samples where concentrations are higher with the presence of microorganisms (September 17th, 2015 and September 15th, 2016). This observation is even more obvious with cloud waters. Systematically, the steady state concentrations of $^{\bullet}OH$ are higher with microorganisms than without microflora. However the difference is not statistically significant (p-value = 0.81 for rain waters and 0.06 for cloud waters, paired Wilcoxon test used), microorganism impact seems to be low, but more data are needed to strengthen this observation.

4. Atmospheric implications and Conclusion

A convenient method has been developed for the determination of steady state hydroxyl radical concentration in real atmospheric waters. Indeed, terephthalic acid (TA) used as a probe is non-toxic for microorganisms and therefore allows working with natural samples and

to study the impact of the microflora. Moreover, TA is soluble in water and not volatile so the experiments can be performed in open-air conditions that are essential to work on natural samples with microorganisms. Finally another positive aspect is that because of the formation of a fluorescent product (TAOH) on which basis to evaluate the concentration of $^{\bullet}OH$, the technique is very sensitive with a limit of quantification near 10^{-17} M and the measurement is easy to perform and fast. So, the use of TA is a very sensitive probe allowing direct assessment of the steady state concentration of hydroxyl radicals considering the sinks naturally present in the sample; it is complementary to the evaluation of the hydroxyl radical formation rates obtained in many previous studies (Faust and Allen (1993) in cloud; Nakatani *et al.* (2007) in rain and river, and Bianco *et al.* (2015) in cloud water). This result is thus important for atmospheric scientists as the steady state hydroxyl radical concentration is really a crucial parameter corresponding to the oxidant capacity of cloud water and more generally of the atmosphere.

In this study, the measured values of the steady state hydroxyl radical concentrations are within the range of few 10^{-16} M with mean values found in cloud water of "7.2 ± 5.0" × 10^{-16} M and in rain water of "1.6 ± 1.5" × 10^{-16} M.

As we can see in the Figure 5, our values are close to those published in different studies from cloud, rain and fog waters even though our average of steady state OH radical concentration in rain waters is around 10 times lower than those evaluated by Arakaki et al. (1999) and Albinet et al. (2010). This difference can be explained by the typology of the sampling sites. Indeed, Clermont-Ferrand area is much smaller than Torino or Higashi-Hiroshima and so less polluted area with less photochemical sources of OH radical. This is confirmed by the average concentration of nitrate in rain water measured in each site; Torino: 2.05 mg L^{-1}, Higashi-Hiroshima 1.86 mg L^{-1} and Clermont-Ferrand 0.68 mg L^{-1}. On the other hand, our $[^{\bullet}OH]_{ss}$

values found in cloud waters are within the same range than those measured by Anastasio and McGregor (2001) in cloud waters and by Kaur and Anastasio (2017) in fog waters.

If we compare our mean values, the steady state hydroxyl radical concentration is four times higher in cloud water than in rain water. This higher concentration in cloud water can be also explained considering a higher concentration of photochemical sources of hydroxyl radicals in cloud than in rain waters (Lin and Peng, 1999). For example, our average concentrations of nitrate are 11.06 mg L^{-1} in cloud water and 0.68 mg L^{-1} in rain water.

Our results and comparison with the other studies show that nitrate (nitrite) could be the major precursors for photoformed $^{\bullet}OH$ in rain and cloud waters. This observation is in agreement with the results published in the paper of Kaur and Anastasio (2017) which found that nitrate/nitrite ions are responsible for 70% of photoformed $^{\bullet}OH$ in fog waters. In contrast, the study of Bianco et al. (2015) concluded that hydrogen peroxide is the most significant source of this radical (between 70 and 90%) in cloud waters sampled at the the puy de Dôme station. This discrepancy could be due to the origin of the air masses (continental, marine, polluted), but also to the different kind of water fog or cloud and so the physicochemical properties of the collected water which can drive the photochemical reactivity. In the study of Bianco et al. (2015) no polluted samples was analysed and the puy de Dôme station in a remote site from sources of pollution.

On the contrary, our values are much lower than most of the values estimated by cloud chemistry models. Depending on the chemical scenarios (emission/deposition rates and initial chemical conditions) and on the chemical mechanisms, the estimated $^{\bullet}OH$ concentrations vary from 10^{-12} M to 10^{-15} M (Deguillaume *et al.*, 2010; Herrmann *et al.*, 2010; Mouchel-Vallon *et al.*, 2017; Tilgner and Herrmann, 2010; Tilgner *et al.*, 2013). The highest concentration measured in the present study (~10^{-15} M) is close to the minimal value simulated by the cloud chemistry models. This demonstrates that the models tend to

overestimate the hydroxyl radical concentration as previously mentioned by Arakaki *et al.* (2013). This can be explained by two reasons. First, the models are expected to underestimate the radical sinks because all the organic scavengers cannot be exhaustively considered in the aqueous chemical mechanism. Secondly, models are simulating the mass transfer of $^{\bullet}OH$ from the gas to the aqueous phase and consequently take into account an additional source not considered in the present study. This additional source is estimated to represent 69% of the total OH radical concentration present in aqueous phase (Kaur and Anastasio, 2017).

Moreover, thanks to this useful method, we have shown that the endogenous microflora has no significant impact on the steady state hydroxyl radical concentrations. The microorganisms could be a sink of $^{\bullet}OH$ radicals as any other organic matter present in clouds; however this quenching effect is suspected to be negligible as microorganisms represent a low fraction of the total organic carbon (evaluated to 1.7% by Bauer *et al.*, 2002). On the contrary they could produce $^{\bullet}OH$ radicals by a passive mechanism. Indeed Samake *et al.* (2017) have recently reported that dead microorganisms used as bioaerosol models (bacteria and fungi) can produce ROS, although the method used does not allow to identify the type of radicals formed. Finally our results can be interpreted in two ways: i) the production or quenching of $^{\bullet}OH$ is too low due to the low concentration of microorganisms in clouds thus no change in $[^{\bullet}OH]_{ss}$ can be measured; ii) the rates of production and consumption of $^{\bullet}OH$ by microorganisms are not negligible but within the same range of order and thus the resulting $[^{\bullet}OH]_{ss}$ remains constant.

This convenient tool will allow the evaluation of atmospheric water oxidant capacity; it should be applied now to measure numerous samples at different sites (various altitudes, latitudes, seasons, *etc.*). The $^{\bullet}OH$ concentrations at the steady state will be used to evaluate and constrain cloud chemistry models that simulate the organic matter transformations in clouds and thus their composition, their evolution and lifetime. This will help to better assess

the effect of cloud of the transformation of atmospheric organic matter that plays a crucial role on air quality and climate.

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REFERENCES

Albinet, A., Minero, C., and Vione, D., 2010. Photochemical generation of reactive species upon irradiation of rainwater: Negligible photoactivity of dissolved organic matter. Sci. Total Environ. 408, 3367–3373.

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A.-M., 2007a. Microorganisms isolated from the water phase of tropospheric clouds at the puy de Dôme: major groups and growth abilities at low temperatures. FEMS Microbiol. Ecol. 59, 242–254.

Amato, P., Demeer, F., Melaouhi, A., Fontanella, S., Martin-Biesse, A.-S., Sancelme, M., Laj, P., and Delort, A.-M., 2007b. A fate for organic acids, formaldehyde and methanol in cloud water: Their biotransformation by micro-organisms. Atmos. Chem. Phys. 7, 4159–4169.

Anastasio, C., and McGregor, K.G.,2001. Chemistry of fog waters in California's Central Valley: 1. In situ photoformation of hydroxyl radical and singlet molecular oxygen. Atmos. Environ. 35, 1079–1089.

Arakaki, T., and Faust, B.C., 1998. Sources, sinks, and mechanisms of hydroxyl radical ($^{\bullet}$OH) photoproduction and consumption in authentic acidic continental cloud waters from Whiteface Mountain, New York: The role of the Fe(r) (r = II, III) photochemical cycle. J. Geophys. Res. 103, 3487–3504.

Arakaki, T., Miyake, T., Shibata, M., and Sakugawa, H., 1999. Photochemical formation and scavenging of hydroxyl radical in rain and dew waters. Nippon Kag. Kaish. 5, 335–340.

Arakaki, T., Anastasio, C., Kuroki, Y., Nakajima, H., Okada, K., Kotani, Y., Handa, D., Azechi, S., Kimura, T., Tsuhako, A., et al., 2013. A general scavenging rate constant for reaction of hydroxyl radical with organic carbon in atmospheric waters. Environ. Sci. Technol. 47, 8196–8203.

Ariya, P.A., Nepotchatykh, O., Ignatova, O., and Amyot, M., 2002. Microbiological degradation of atmospheric organic compounds. Geophys. Res. Lett. 29, 34-1-34–4.

Bauer, H., Kasper-Giebl, A., Löflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., and Puxbaum, H., 2002. The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. Atmos. Res. 64, 109–119.

Bianco, A., Passananti, M., Perroux, H., Voyard, G., Mouchel-Vallon, C., Chaumerliac, N., Mailhot, G., Deguillaume, L., and Brigante, M., 2015. A better understanding of hydroxyl radical photochemical sources in cloud waters collected at the puy de Dôme station – experimental versus modelled formation rates. Atmos. Chem. Phys. 15, 9191–9202.

Bianco, A., Voyard, G., Deguillaume, L., Mailhot, G., and Brigante, M., 2016. Improving the characterization of dissolved organic carbon in cloud water: Amino acids and their impact on the oxidant capacity. Sci. Rep. 6.

Blough, N.V., and Zepp, R.G., 1995. Reactive oxygen species in natural waters. In Active Oxygen in Chemistry, C. S. Foote, J. Valentine, A. Greenberg and J. F. Liebman (Springer), pp. 280–330.

Brezonik, P.L., and Fulkerson-Brekken, J., 1998. Nitrate-induced photolysis in natural waters: Controls on concentrations of hydroxyl radical photo-intermediates by natural scavenging agents. Environ. Sci. Technol. 32, 3004–3010.

Buxton, G.V., Greenstock, C.L., Helman, W.P. and Ross, A.B., 1988. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($^{\bullet}OH/^{\bullet}O^{-}$) in aqueous solution. J. Phys. Chem. Ref. Data 17, 513–886.

Chameides, W.K., and Davis, D.D., 1982. The free radical chemistry of cloud droplets and its impact upon the composition of rain. J. Geophys. Res. 87, 4863–4877.

Charbouillot, T., Brigante, M., Mailhot, G., Maddigapu, P.R., Minero, C., and Vione, D., 2011. Performance and selectivity of the terephthalic acid probe for OH as a function of temperature, pH and composition of atmospherically relevant aqueous media. J. Photochem. Photobiol. A 222, 70–76.

Deguillaume, L., Leriche, M., Monod, A., and Chaumerliac, N., 2004. The role of transition metal ions on HOx radicals in clouds: A numerical evaluation of its impact on multiphase chemistry. Atmos. Chem. Phys. 4, 95–110.

Deguillaume, L., Leriche, M., and Chaumerliac, N., 2005. Impact of radical versus non-radical pathway in the Fenton chemistry on the iron redox cycle in clouds. Chemosphere 60, 718–724.

Deguillaume, L., Desboeufs, K.V., Leriche, M., Long, Y., and Chaumerliac, N., 2010. Effect of iron dissolution on cloud chemistry: From laboratory measurements to model results. Atmos. Poll. Res. 1, 220–228.

Deguillaume, L., Charbouillot, T., Joly, M., Vaïtilingom, M., Parazols, M., Marinoni, A., Amato, P., Delort, A.-M., Vinatier, V., Flossmann, A., et al., 2014. Classification of clouds sampled at the puy de Dôme (France) based on 10 yr of monitoring of their physicochemical properties. Atmos. Chem. Phys. 14, 1485–1506.

Faust, B.C., 1994. Photochemistry of clouds, fogs, and aerosols. Environ. Sci. Technol. 28, 217A–222A.

Faust, B.C., and Allen, J.M., 1993. Aqueous-phase photochemical formation of hydroxyl radical in authentic cloudwaters and fog waters. Environ. Sci. Technol. 27, 1221–1224.

Faust, B.C., and Hoigné, J., 1990. Photolysis of Fe (III)-hydroxy complexes as sources of OH radicals in clouds, fog and rain. Atmos. Environ. 24, 79–89.

Faust, B.C., and Zepp, R.G., 1993. Photochemistry of aqueous iron (III)-polycarboxylate complexes: Roles in the chemistry of atmospheric and surface waters. Environ. Sci. Technol. 27, 2517–2522.

Haag, W.R., and Hoigné, J., 1985. Photo-sensitized oxidation in natural water via OH radicals. Chemosphere 14, 1659–1671.

453 Herckes, P., Valsaraj, K.T., and Collett, J.L., 2013. A review of observations of organic
454 matter in fogs and clouds: Origin, processing and fate. Atmos. Res. 132, 434–449.
455 Herrmann, H., Ervens, B., Jacobi, H.-W., Wolke, R., Nowacki, P., and Zellner, R., 2000.
456 CAPRAM2.3: A chemical aqueous phase radical mechanism for tropospheric chemistry.
457 Journal of Atmospheric Chemistry 36, 231–284.
458 Herrmann, H., Hoffmann, D., Schaefer, T., Bräuer, P., and Tilgner, A., 2010. Tropospheric
459 aqueous-phase free-radical chemistry: Radical sources, spectra, reaction kinetics and
460 prediction tools. ChemPhysChem 11, 3796–3822.
461 Herrmann, H., Schaefer, T., Tilgner, A., Styler, S. A., Weller, C., Teich, M., Otto, T., 2015.
462 Tropospheric aqueous-phase chemistry: kinetics, mechanisms, and its coupling to a changing
463 gas phase. Chem. Rev. 115 (10), 4259–4334.
464 al Housari, F., Vione, D., Chiron, S., and Barbati, S., 2010. Reactive photo-induced species in
465 estuarine waters. Characterization of hydroxyl radical, singlet oxygen and dissolved organic
466 matter triplet state in natural oxidation processes. Photochem. Photobiol. Sci. 9, 78–86.
467 Husarova, S., Vaïtilingom, M., Deguillaume, L., Traïkia, M., Vinatier, V., Sancelme, M.,
468 Amato, P., Matulova, M., Delort, A.-M, 2011. Biotransformation of methanol and
469 formaldehyde by bacteria isolated from clouds. Comparison with radical chemistry. Atmos.
470 Environ. 45, 6093-6102.

471 Jacob, D.J., 1986. Chemistry of OH in remote clouds and its role in the production of formic
472 acid and peroxymonosulfate. J. Geophys. Res. 91, 9807–9826.
473 Joly, M., Amato, P., Sancelme, M., Vinatier, V., Abrantes, M., Deguillaume, L., and Delort,
474 A.-M., 2015. Survival of microbial isolates from clouds toward simulated atmospheric stress
475 factors. Atmos. Environ. 117, 92–98.
476 Kaur, R. and Anastasio, C., 2017. Light absorption and the photoformation of hydroxyl
477 radical and singlet oxygen in fog waters. Atmos. Environ., 164, 387-397.

Lelieveld, J., and Crutzen, P.J., 1991. The role of clouds in tropospheric photochemistry. J. Atmos. Chem. 12, 229–267.

Lelieveld, J., Dentener, F.J., Peters, W., and Krol, M.C., 2004. On the role of hydroxyl radicals in the self-cleansing capacity of the troposphere. Atmos. Chem. Phys. 4, 2337–2344.

Lin, N.-H., and Peng, C.-M., 1999. Estimates of the contribution of below-cloud scavenging to the pollutant loadings of rain in Taipei, Taiwan. Terrestrial, Atmos. Ocean. Sci. 10, 693–704.

Mauldin III, R.L., Madronich, S., Flocke, S.J., and Eisele, F.L., 1997. New insights on OH: measurements around and in clouds. Geophys. Res. Lett. 24, 3033–3036.

Metallo, C. M.; Vander Heiden, M. G., 2013. Understanding Metabolic Regulation and Its Influence on Cell Physiology. Mol. Cell. 49 (3), 388–398.

Mill, T., Hendry, D.G., and Richardson, H., 1980. Free-radical oxidants in natural waters. Science 207, 886–887.

Mouchel-Vallon, C., Deguillaume, L., Monod, A., Perroux, H., Rose, C., Ghigo, G., Long, Y., Leriche, M., Aumont, B., and Patryl, L., 2017. CLEPS 1.0: A new protocol for cloud aqueous phase oxidation of VOC mechanisms. Geosci. Model Dev. 10, 1339–1362.

Nakatani, N., Ueda, M., Shindo, H., Takeda, K., and Sakugawa, H., 2007. Contribution of the photo-Fenton reaction to hydroxyl radical formation rates in river and rain water samples. Anal. Sci. 23, 1137–1142.

Page S.E., Arnold, W.A., McNeill K., 2010. Terephthalate as a probe for photchemically generated hydroxyl radical. J. Environ. Monit. , 12, 1658-1665.

Parazols, M., Marinoni, A., Amato, P., Abida, O., Laj, P., Mailhot, G., Delort, A.-M., and Sergio, Z., 2007. Speciation and role of iron in cloud droplets at the puy de Dôme station. J. Atmos. Chem. 57, 299–300.

Qian, J., Mopper, K., and Kieber, D.J., 2001. Photochemical production of the hydroxyl radical in Antarctic waters. Deep Sea Res. Part I Oceanogr. Res. Pap. 48, 741–759.

Russi, H., Kotzias, D., and Korte, F., 1982. Photoinduzierte hydroxylierungsreaktionen organischer chemikalien in natürlichen Gewässern-Nitrate als potentielle OH-radikalquellen. Chemosphere 11, 1041–1048.

Samake, A., Uzu, G., Martins, J.M.F., Calas, A., Vince, E., Parat, S., and Jaffrezo, J.L., 2017. The unexpected role of bioaerosols in the oxidative potential of PM. Sci. Rep. 7.

Shingler, V., 2003. Integrated Regulation in Response to Aromatic Compounds: From Signal Sensing to Attractive Behaviour. Environ. Microbiol. *5* (12), 1226–1241.

Tilgner, A., and Herrmann, H., 2010. Radical-driven carbonyl-to-acid conversion and acid degradation in tropospheric aqueous systems studied by CAPRAM. Atmos. Environ. 44, 5415–5422.

Tilgner, A., Bräuer, P., Wolke, R., and Herrmann, H., 2013. Modelling multiphase chemistry in deliquescent aerosols and clouds using CAPRAM3.0i. J. Atmos. Chem. 70, 221–256.

Tropel, D., van der Meer, J. R., 2004. Bacterial Transcriptional Regulators for Degradation Pathways of Aromatic Compounds. Microbiol. Mol. Biol. Rev. 68 (3), 474–500.

Vaïtilingom, M., Amato, P., Sancelme, M., Laj, P., Leriche, M., and Delort, A.-M., 2010. Contribution of microbial activity to carbon chemistry in clouds. Appl. Environ. Microbiol. 76, 23–29.

Vaïtilingom, M., Charbouillot, T., Deguillaume, L., Maisonobe, R., Parazols, M., Amato, P., Sancelme, M., and Delort, A.-M., 2011. Atmospheric chemistry of carboxylic acids: Microbial implication versus photochemistry. Atmos. Chem. Phys. 11, 8721–8733.

Vaïtilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A.I., Amato, P., and Delort, A.-M., 2012. Long-term features of cloud microbiology at the puy de Dôme (France). Atmos. Environ. 56, 88–100.

Vaïtilingom, M., Deguillaume, L., Vinatier, V., Sancelme, M., Amato, P., Chaumerliac, N., and Delort, A.-M., 2013. Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds. Proc. Natl. Acad. Sci. 110, 559–564.

Weschler, C.J., Mandich, M.L., and Graedel, T.E., 1986. Speciation, photosensitivity and reactions of transition metal ions in atmospheric droplets. J. Geophys. Res. 91, 5189–5204.

Wirgot, N., Vinatier, V., Deguillaume, L., Sancelme, M., Delort, A.-M., 2017. H_2O_2 modulates the energetic metabolism of the cloud microbiome. Atmos. Chem. Phys. 17, 14841-14851.

Yu, X.-Y., and Barker, J.R., 2003. Hydrogen peroxide photolysis in acidic aqueous solutions containing chloride ions. I. Chemical mechanism. J. Phys. Chem. A 107, 1313–1324.

Zafiriou, O.C., and Bonneau, R., 1987. Wavelength-dependent quantum yield of OH radical formation from photolysis of nitrite ion in water. Photochem. Photobiol. 45, 723–727.

Zellner, R., Exner, M., and Herrmann, H., 1990. Absolute OH quantum yields in the laser photolysis of nitrate, nitrite and dissolved H_20_2 at 308 and 351 nm in the temperature. Atmos. Chem. 10, 411–425.

Zepp, R.G., Hoigne, J., and Bader, H., 1987. Nitrate-induced photooxidation of trace organic chemicals in water. Environ. Sci. Technol. 21, 443–450.

Zhou, X., and Mopper, K., 1990. Determination of photochemically produced hydroxyl radicals in seawater and freshwater. Mar. Chem. 30, 71–88.

Figure captions:

Fig. 1: Comparison of actinic fluxes of the lamp used in the present work together with the ones measured under natural conditions during cloudy and sunny situations at the puy de Dôme station (Solar emission in cloudy condition was measured in October 16th, 2013 and in sunny condition in September 23th, 2013). Black line represents the lamp actinic flux, blue line is the actinic flux measured under cloudy condition and red line is the actinic flux measured under sunny condition. Dotted lines represent molar absorption coefficient (M^{-1} cm^{-1}) for Fe-EDDS complex (pink) and for H_2O_2 (green).

Fig. 2: Reaction between TA and $^{\bullet}OH$.

Fig. 3: Evolutions of the number of cells (A) and of the concentration of ATP (B) as a function of time (0, 47 and 72 min) in the presence of 3 probe concentrations (0, 4 and 10 µM).

Fig. 4: Concentrations of hydroxyl radicals at steady state in rain and cloud waters without (white histogram) and with microorganisms (dash histogram). Error bars represent standard variation from plots of Rf_{TAOH} versus TA concentrations.

Fig 5: Measured of steady state hydroxyl radical concentrations by different research groups in different atmospheric waters (cloud, fog and rain).

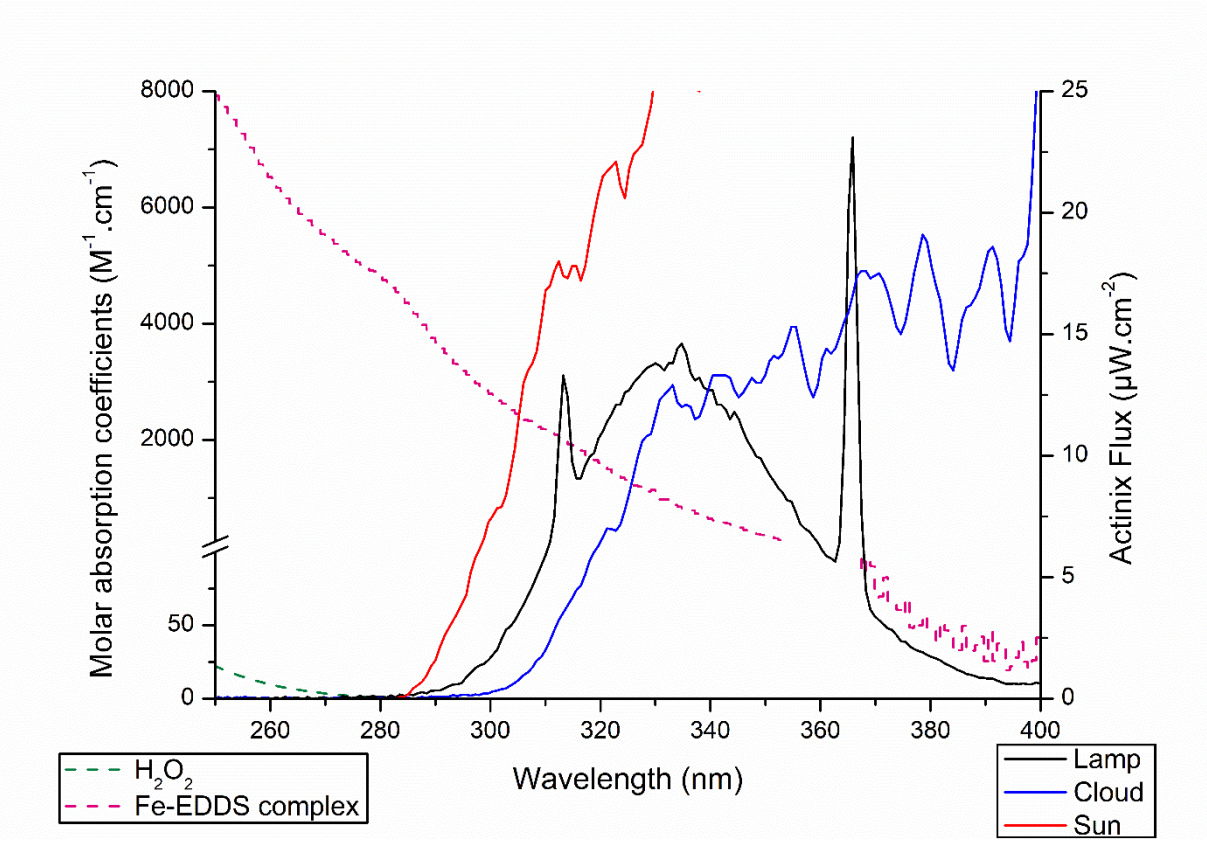
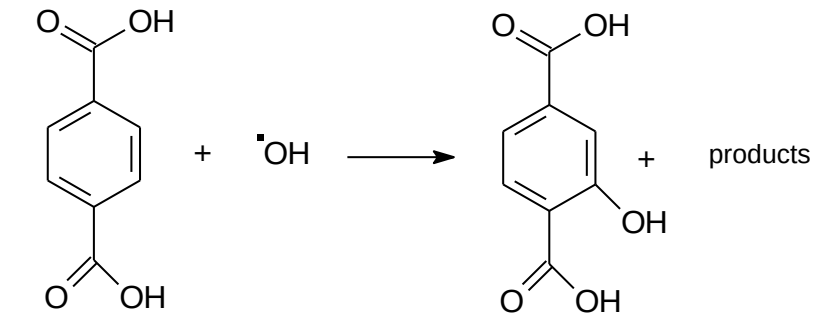


Fig. 1



Terephthalic acid 2-hydroxyterephthalic acid (TAOH)

Fig. 2

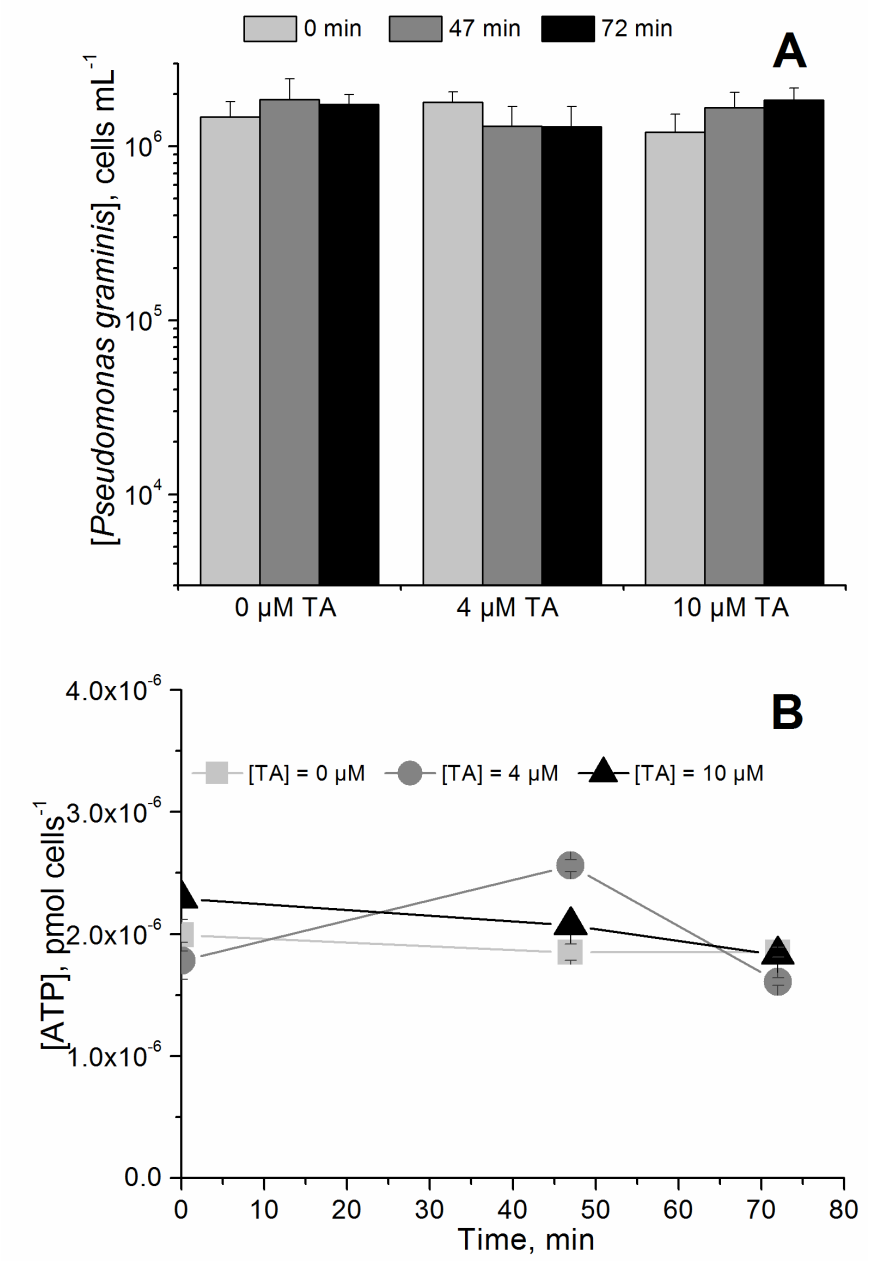
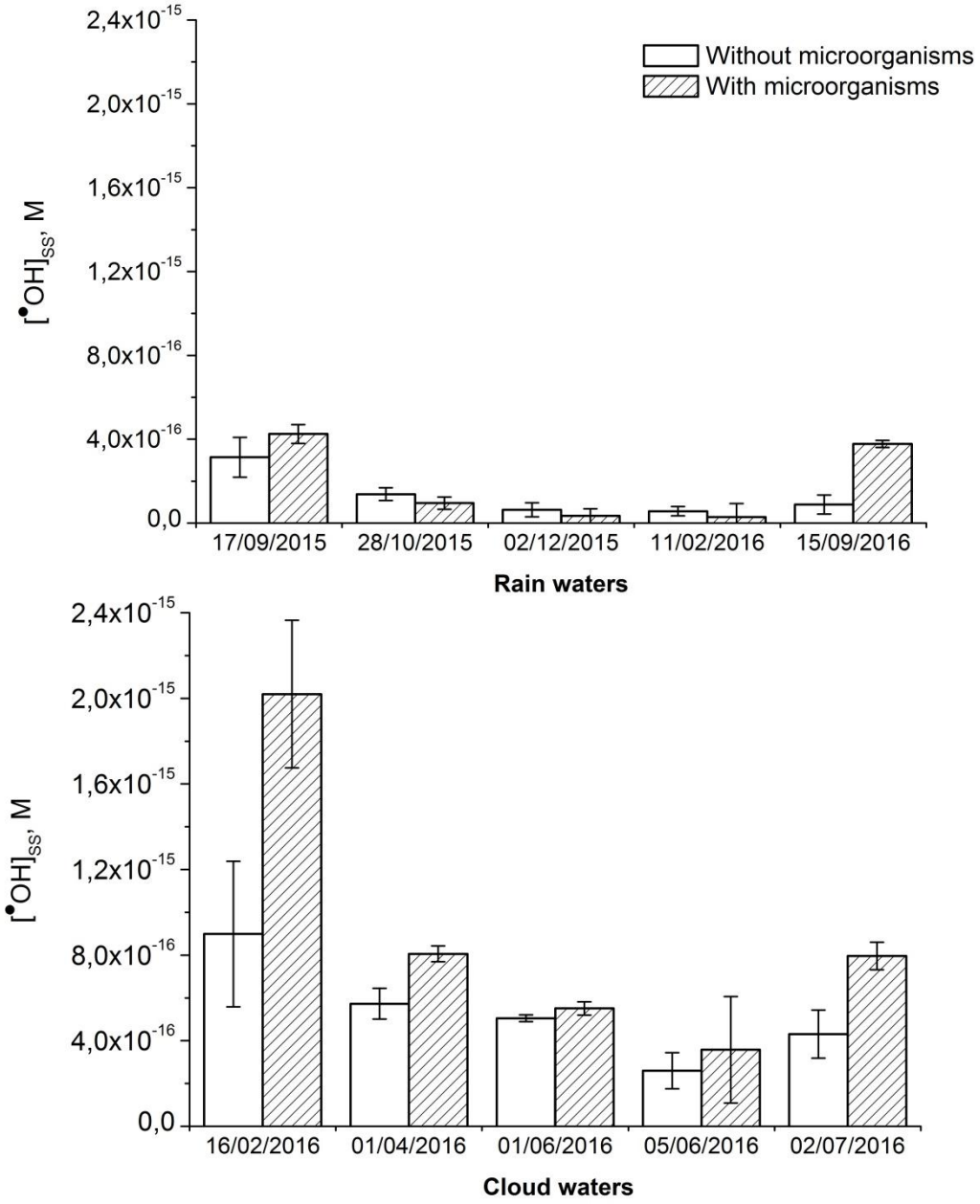

0 min
47 min
72 min
A
[Pseudomonas graminis], cells mL-1
10^6
10^5
10^4
0 µM TA
4 µM TA
10 µM TA
B
4.0x10-6
3.0x10-6
2.0x10-6
1.0x10-6
0.0
[TA] = 0 µM
[TA] = 4 µM
[TA] = 10 µM
[ATP], pmol cells-1
0
10
20
30
40
50
60
70
80
Time, min

Fig. 3


Without microorganisms
With microorganisms
[•OH]ss, M
2,4x10-15
2,0x10-15
1,6x10-15
1,2x10-15
8,0x10-16
4,0x10-16
0,0
17/09/2015
28/10/2015
02/12/2015
11/02/2016
15/09/2016
Rain waters
16/02/2016
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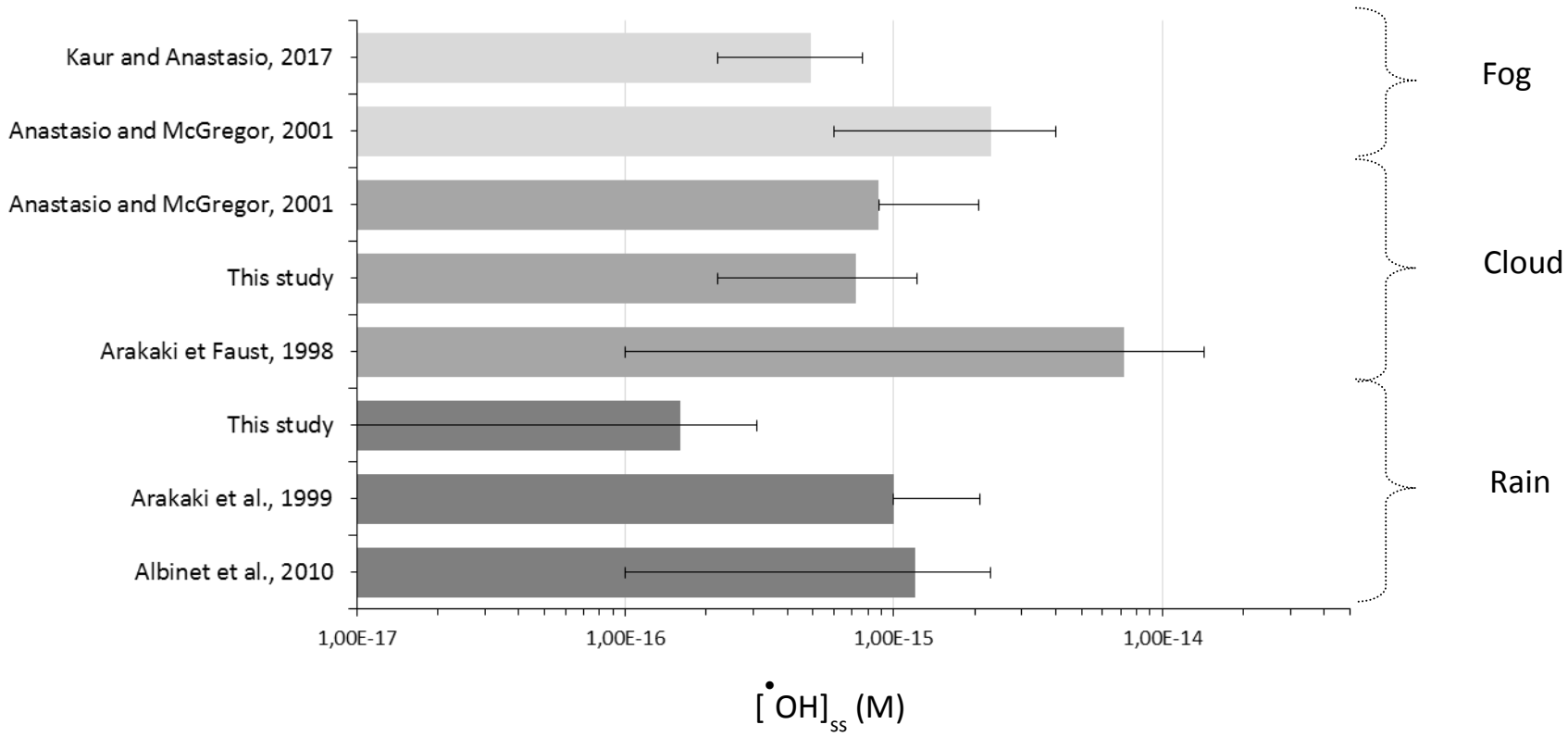
05/06/2016
02/07/2016
Cloud waters

Fig. 4


Kaur and Anastasio, 2017
Anastasio and McGregor, 2001
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Arakaki et al., 1999
Albinet et al., 2010
1,00E-17
1,00E-16
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[•OH]ss (M)
Fog
Cloud
Rain

Fig 5