Low to negligible photoactivity of lake-water matter in the size range from 0.1 to 5 um

Low to negligible photoactivity of lake-water matter in the size range from 0.1 to 5 µm

Marco Minella,[a] Fabio Romeo,[a] Davide Vione,[a,b,]* Valter Maurino,[a] Claudio Minero [a]

[a] *Dipartimento di Chimica Analitica, Università di Torino, Via P. Giuria 5, 10125 Torino, Italy.*

[b] *Centro Interdipartimentale NatRisk, Università di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy.*

* Corresponding author. Phone +39-011-6707838; Fax +39-011-6707615; E-mail: *davide.vione@unito.it*; URL: *http://naturali.campusnet.unito.it/cgi.bin/docenti.pl/Show?_id=vione*

Abstract

We found that the photochemical generation of reactive transients such as singlet oxygen and triplet states upon irradiation of different lake water samples is accounted for by components smaller than 0.10 µm. Compared with radiation absorption by the smaller compounds, particulate matter was often unable to absorb and/or scatter radiation to a significant extent, which is consistent with the lack of a measurable photochemical activity. In one case, significant radiation absorption/scattering was also connected with negligible photochemical activity. It is also shown that filtration of the lake water samples at 0.10 µm was able to effectively remove the suspended particles, differently from the usually suggested filtration at 0.45 µm.

Keywords: Surface-water photochemistry; particulate matter; dynamic light scattering; trimethylphenol; furfuryl alcohol.

Introduction

Sunlight-driven reactions play an important role in the functioning of the aquatic ecosystems. Photochemical reactions are able to influence radiation absorption by modifying the concentration and the nature of the absorbing components. This phenomenon is known as photobleaching and can favour the penetration of harmful UVB and UVA radiation in water bodies (Brinkmann et al., 2003; Bracchini et al., 2004; Sulzberger and Durisch-Kaiser, 2009). Radiation absorption by photoactive species, also called photosensitisers, induces the production of reactive transients such as $^{\bullet}OH$

radicals, singlet oxygen ($^1O_2$) and reactive triplet states (T*). These transients, as well as others derived from them such as the carbonate radical, are involved into the transformation of dissolved components including xenobiotics. The most important photosensitisers in surface waters are dissolved organic compounds, nitrate and nitrite. All of them yield $^{\bullet}OH$ upon irradiation, while T* and $^1O_2$ are only produced by organic compounds (Hoigné, 1990). T* and $^1O_2$ are involved into the transformation of many dissolved molecules of environmental importance such as amino acids, phenylurea herbicides, sulphonamide antibiotics and the histamine antagonist cimetidine (Gerecke et al., 2001; Latch et al., 2003; Boreen et al., 2005; Boreen et al., 2008).

Chromophoric Dissolved Organic Matter (CDOM), which is operationally defined as the organic matter that passes through a filter of 0.45 µm pore size and is able to absorb sunlight, is a known major photosensitiser in surface waters (O'Sullivan et al., 2005). Much less is known about the photosensitising properties of larger components of organic matter, but it has been found for instance that algae and algal residues are able to produce reactive oxygen species upon irradiation, including $^{\bullet}OH$ and $^1O_2$ (Zepp and Schlotzhauer, 1983; Wang et al., 2007). Particulate or colloidal organic/inorganic matter might thus play a significant role in the photochemical processes that take place in surface waters (*e.g.* irradiated semiconductor colloids can generate reactive oxygen species) (Borer et al., 2009). The purpose of the present paper was to get further insight into the photochemical activity of larger organic/inorganic components in surface waters. To this purpose, water samples taken from the surface layer of different lakes were subjected to different filtration procedures (filters with pore sizes of 5, 0.45 and 0.10 µm). After filtration, samples were irradiated to quantify the photochemical generation of T* and $^1O_2$. Furfuryl alcohol and trimethylphenol were used as probe molecules for $^1O_2$ and T*, respectively (Hoigné, 1990; Halladja et al., 2007; Al Housari et al., 2010). The results of sample filtration were checked by means of the dynamic laser light scattering technique, by which it is possible to obtain the hydrodynamic radius distribution of suspended particles.

Experimental section

Additional information including instrumental details is reported in the Supplementary Material, hereafter SM.

Sampling and sample treatment. Water samples were taken from the surface layer of the lakes under study and transported to the laboratory under refrigeration. Sampling dates are reported in Table 1. Preliminary filtration to remove the coarse material was carried out by means of Sartorius

Minisart filters (cellulose acetate, pore diameter 5 µm). When relevant, further filtration was carried out with Millipore MF membranes (cellulose acetate, pore diameter 0.45 µm) and Whatman Anotop plus filters (pore diameter 0.10 µm). Irradiation of the sample aliquots filtered at 5 µm was started as soon as possible to avoid transformation due to microbiological processes, the other aliquots were stored under refrigeration until irradiation.

Sample characterisation. The total suspended solids (TSS) in the 5 µm-filtered samples were measured by the APAT CNR IRSA method n. 2090 (Belli et al., 2004), which implies vacuum filtration on 0.45 µm pore-diameter filter membranes. The weighting of the dried membranes before and after filtration was carried out with a Shimadzu AX120 balance, with a sensitivity of 0.1 mg.

The size distribution of suspended particles in the samples, filtered at 5, 0.45 and 0.1 µm, was evaluated by Dynamic Light Scattering (DLS) and the absorbance $A_1(\lambda)$ by UV-vis spectrophotometry. Nitrate was determined by ion chromatography. Nitrite was quantified following the procedure of Kieber and Seaton (1996), which involved pre-column derivatisation with 2,4-dinitrophenylhydrazine and analysis in liquid chromatography (HPLC-UV). Non-Purgeable Organic Carbon (NPOC) and inorganic carbon were determined with a Shimadzu TOC analyzer. Nitrate, nitrite, NPOC and inorganic carbon (IC) were determined as the average of runs carried out at least in triplicate. The error bounds to the analytical data represent $\pm\sigma$ (see Table 1).

Irradiation experiments. The lake water samples (20 mL) were spiked with furfuryl alcohol (FFA, 2.0 mM) or 2,4,6-trimethylphenol (TMP, 2.0 mM) and placed into cylindrical Pyrex glass cells (diameter 4.0 cm, height 2.5 cm), tightly closed with a lateral screw cap. The cells were irradiated from the top under a set of five UVA lamps (TL K 40W/05, Philips, emission maximum 365 nm) and magnetically stirred during irradiation. Two replicates were made of each irradiation experiment and the results were averaged. Blank runs were carried out, in the absence of irradiation, by wrapping the cells in aluminium foil and placing them under the lamp with magnetic stirring. The time evolution of TMP and FFA was also monitored upon irradiation in Milli-Q water, to assess the direct photolysis of the two compounds under the adopted experimental set-up.

The UV irradiance (295-400 nm) reaching the cells was 20.5±0.5 W m^{-2}, measured with a CO.FO.ME.GRA. (Milan, Italy) power meter. The photon flux in solution was 3.88×10^{-6} Einstein L^{-1} s^{-1}, actinometrically determined with the ferrioxalate method (Kuhn et al., 2004) by taking into account the trends with wavelength of the fraction of radiation absorbed by ferrioxalate and of the quantum yield of Fe^{2+} photoproduction. Figure A-SM shows the emission spectrum of the lamps,

taken with an Ocean Optics SD2000 CCD spectrophotometer and normalised to the actinometry data, taking into account the transmittance of the Pyrex glass of the cells (Albinet et al., 2010).

After the scheduled irradiation times, the aqueous solutions were analysed by HPLC-UV (see SM for the conditions).

Assessment of the formation rates of triplet states and $^1O_2$. Radiation absorption by organic compounds in surface water yields the excited singlet states, which by inter-system crossing can be transformed into the longer-lived and more reactive triplet states, T* (Hoigné, 1990). Singlet oxygen is mainly produced by reaction between T* and O_2, and a suitable probe molecule to measure its formation rate (R_{1O2}) is FFA (Hoigné, 1990). In the presence of FFA, there is competition between the thermal deactivation of $^1O_2$ upon collision with the solvent ($k_d = 2.5 \cdot 10^5$ s^{-1}; Wilkinson and Brummer, 1981) and reaction between $^1O_2$ and FFA (rate constant $k_{FFA,1O2} = 1.2 \cdot 10^8$ M^{-1} s^{-1}; Wilkinson and Brummer, 1981). From the competition kinetics and under the steady state approximation for $^1O_2$, one obtains R_{1O2} as follows:

$$R_{^1O_2} = R_{FFA} \frac{k_{FFA,^1O_2} [FFA]_o + k_d}{k_{FFA,^1O_2} [FFA]_o} \qquad (1)$$

where $[FFA]_o$ is the initial concentration of FFA and R_{FFA} is the experimentally measured initial rate of FFA transformation. The time trends of FFA concentration ($[FFA]_t$ vs. t), after fitting with pseudo-first order kinetic equations ($[FFA]_t = [FFA]_o\, e^{-k\,t}$), yield $R_{FFA} = k\, [FFA]_o$.

The triplet states T* would mainly react with O_2 to give $^1O_2$ or undergo various deactivation reactions. All these processes have a lumped rate constant $k' \approx 5 \cdot 10^5$ s^{-1} (Canonica and Freiburghaus, 2001). However, T* can also react with dissolved organic molecules. TMP is a suitable probe to measure the formation rate of T* (Halladja et al., 2007). If TMP is added in sufficient amount to the system, the T* that are photogenerated with rate R_{T*} would either react with TMP (second-order rate constant $k_{TMP,T*}$) or undergo other processes with decay constant k'. In a previous field study it has been found that $k_{TMP,T*} = 3.0 \cdot 10^9$ M^{-1} s^{-1} (Al Housari et al., 2010), which compares well with the value of $2 \cdot 10^9$ M^{-1} s^{-1} reported by Halladja et al. (2007) in the presence of humic acids under irradiation. An additional issue is that TMP could also react with $^1O_2$, with second-order rate constant $k_{TMP,1O2} = 6.3 \cdot 10^7$ M^{-1} s^{-1} (Tratnyek and Hoigné, 1994). The reaction between TMP and T* would reduce the formation rate of $^1O_2$, which would be proportional to the fraction of T* that does not react with TMP. The contribution to TMP transformation of the reaction with singlet oxygen can be expressed as follows:

$$R_{TMP}^{^1O_2} = R_{^1O_2} \cdot \frac{k'}{k' + k_{TMP,T*}\left[TMP\right]_o} \cdot \frac{k_{TMP,^1O_2}\left[TMP\right]_o}{k_d + k_{TMP,^1O_2}\left[TMP\right]_o} \qquad (2)$$

where $[TMP]_o$ is the initial concentration of TMP, and R_{1O2} is given by equation (1). By excluding the reaction of TMP with $^1O_2$, from the competition kinetics and under the steady state approximation for T* one gets the following expression for R_{T*}:

$$R_{T*} = (R_{TMP} - R_{TMP}^{^1O_2}) \frac{k_{TMP,T*}\left[TMP\right]_o + k'}{k_{TMP,T*}\left[TMP\right]_o} \qquad (3)$$

where R_{TMP} is the experimentally measured initial rate of TMP transformation. The time trends of TMP concentration showed straight profiles. By fitting the $[TMP]_t$ vs. t trends with pseudo-zero order kinetic equations ($[TMP]_t = [TMP]_o\,(1 - k\,t)$), one gets $R_{TMP} = k\,[TMP]_o$.

Results and Discussion

Table 1 reports the Total Suspended Solids (TSS) content of the samples pre-filtered at 5 µm, which varies from negligible to around 1.2 mg L^{-1}. In the case of Lake Candia, TSS is also consistent with the known data concerning the seasonal trend of algal growth (Giussani et al., 1990). Algae could thus make up a significant fraction of the TSS found in the Candia sample of 22 September. The pre-filtration at 5 µm was carried out to enable analysis of the samples by laser light scattering, which would be hampered by the presence of few large particles.

Dynamic light scattering (DLS) analysis of filtered samples. First of all, preliminary DLS measurement of filtered Milli-Q water was carried out to check for the possible release of particles by the filters, which could be excluded. The DLS size distributions of suspended particles in the filtered (5, 0.45 and 0.1 µm) samples of Lake Candia (sampling date 5 March 2010) and Lake Viverone (24 May 2010) are reported in Figure 1, while the other samples are shown in Figure H-SM. As is usual in DLS, the distributions are reported by considering equal to 1 the intensity of the most populated size mode in each sample. This explains the shift to the left of peak maxima with filtration, because previously minor peaks can become the main ones when larger particles are eliminated. Note that the DLS analysis does not allow evaluation of the absolute amount of particles in each population. In almost all cases the 0.1 µm filtration left samples without detectable particles

(autocorrelation function equal to that of Milli-Q water). A single exception is the Viverone sample filtered at 0.1 μm, which showed a significant scattering caused by particles with a hydrodynamic radius roughly equal to 100 nm.

A common, operational definition of dissolved compounds is that they are able to pass through 0.45 μm pore-size filters. By showing a size distribution of suspended particles in the samples filtered at 0.45 μm, with the single exception of the sample from Lake Avigliana (Figure H-SM), the DLS experimental evidence does not support the common assumption.

Irradiation experiments. First of all, no transformation of TMP or FFA was detected upon irradiation in Milli-Q water, thereby excluding any direct photolysis of the two compounds under the adopted experimental conditions. Figure 2 shows the time trends of TMP (2a) and FFA (2b) in the filtered samples (at 5, 0.45 and 0.10 μm) taken from Lake Candia in March. The lack of TMP or FFA transformation in the dark allows the exclusion of significant biological processes. If direct photolysis and biological activity play a negligible role, the transformation of TMP and FFA under irradiation can be ascribed to the formation and reactivity of transients such as triplet states T* (mainly for TMP transformation), $^1O_2$ (for FFA) and $^\bullet OH$ (for both TMP and FFA). The radicals $^\bullet OH$ are produced by irradiation of nitrate, nitrite and CDOM (Takeda et al., 2004). From the concentration data of NO_3^- and NO_2^- reported in Table 1 and the contribution of the two specie to $^\bullet OH$ photoproduction under the adopted irradiation device (Vione et al., 2009), one derives a formation rate of $^\bullet OH$ around 10^{-14} - 10^{-13} M s^{-1} for nitrate and 10^{-12} - 10^{-11} M s^{-1} for nitrite. One can thus expect transformation of up to 1 μM TMP or FFA after 30 h irradiation, that is, no more than 0.05% of their initial concentration values. As far as irradiated CDOM is concerned, it is often the main $^\bullet OH$ source in surface waters. However, although its contribution to $^\bullet OH$ may be higher than that of nitrite, it is of the same order of magnitude (Takeda et al., 2004; Vione et al., 2006). Therefore, $^\bullet OH$ photoproduction by CDOM would not modify the conclusions that can be derived by considering the role of nitrite and nitrate.

Figure 2 shows that very similar time trends can be observed for TMP in the irradiated samples, irrespective of the filtration procedure (5, 0.45 or 0.10 μm). Practically the same observation holds for FFA. The presence of suspended solids in the Candia sample pre-filtered at 5 μm is supported by both DLS measurements (Figure 1a) and Total Suspended Solids (TSS) determination (Table 1). The gradual elimination of the suspended material upon filtration at 0.45 and 0.10 μm, which can be monitored by DLS, was not able to substantially modify the transformation of either TMP or FFA. This finding suggests that suspended solids, as well as dissolved molecules larger than 0.10 μm give a low to negligible contribution to the fomation of either T* or $^1O_2$. Figure 3a reports the

absorption spectra of water from Lake Candia, subjected to different filtration procedures. The overlap of the spectra suggests that most of the absorbance is accounted for by components smaller than 0.10 µm. Larger components as well as suspended solids give a negligible contribution to either radiation absorption or scattering, which would be counted as absorption in the reported UV-vis spectra. This finding is compatible with the observed lack of photochemical activity of components larger than 0.10 µm (Figure 1).

In contrast, Figure 3b provides evidence of some effect on the absorption spectra that is connected with filtration of water from Lake Viverone. Note that spectral data were taken from 200 to 900 nm (here only the interval between 300 and 500 nm is reported, which is relevant to the adopted irradiation conditions) and that all the spectra overlapped at the highest wavelength values, which excludes baseline-related issues. According to Figure 3b, the suspended particles and, possibly to a lesser extent, dissolved material between 0.10 and 0.45 µm could absorb or scatter radiation. However, Figure 4a shows that the time trend of TMP was practically the same upon irradiation of the Viverone samples filtered at 5, 0.45 or 0.10 µm. In the same figure it is also confirmed the lack of TMP transformation in the dark, already observed for Candia. In the case of FFA (Figure 4b), there are similar trends for the irradiated samples filtered at 5 and 0.10 µm. The FFA trend in the irradiated 0.45 µm sample was very similar to the dark runs, and degradation was slower compared to the 5 and 0.10 µm samples. This might imply production of some $^1O_2$ by suspended particles in the 5 µm sample, with such particles being largely removed by 0.45 µm filtration. However, strangely enough, the generation of $^1O_2$ would be restored upon filtration at 0.10 µm. Overall, the very limited transformation of FFA upon irradiation of the Viverone samples makes it difficult to draw a sound conclusion.

As far as the other lake water samples are concerned, the results of the irradiation experiments are reported in Figures B-D (SM), and the absorption spectra following the different filtration procedures are shown in Figures E-G (SM). In most cases, practically no difference in the trends of TMP and FFA can be observed upon irradiation of the samples filtered at 5, 0.45 or 0.10 µm. The single exception is represented by the time trend of TMP under irradiation in the 0.45-µm filtered sample from Lake Candia (sampling date 22 September, Figure D(a)-SM). In that case, transformation of TMP was faster than in the irradiated 5 and 0.10 µm-filtered samples. It may be possible that a fraction of TMP photo-transformation in the sample filtered at 0.45 µm is accounted for by components with dimensions between 0.10 and 0.45 µm, which would be removed by filtration at 0.10 µm. However, under this hypothesis it is not easy to explain why the sample filtered at 5 µm, which should contain the 0.10-0.45 µm components as well, behaves in more or

less the same way as that filtered at 0.10 µm. Other explanations (*e.g.* accidental contamination of the 0.45 µm sample) cannot, therefore, be excluded.

Formation rates of T* and $^1O_2$. From the time evolution data of TMP and FFA it has been possible to determine the formation rates of the triplet states (R_{T*}) and of $^1O_2$ (R_{1O2}) in the samples, which would largely depend on the contribution of components smaller than 0.10 µm. Table 1 reports the values of R_{T*} and R_{1O2}, calculated with equations (1-3), for the lake water samples filtered at 0.10 µm. It is $R_{T*} > R_{1O2}$, which means that not all of T* react with O_2 to produce $^1O_2$. The ratio R_{1O2} $(R_{T*})^{-1}$ varies from 0.2 to 0.6, indicating that in the studied samples the percentage of T* that could produce $^1O_2$ varied from 20 to 60%. Interestingly, 2 mM TMP would be able to scavenge 93% of photogenerated T*. By halving $[TMP]_o$, the scavenged fraction would decrease little (85%). It is thus possible to account for the zero-order time evolution of TMP in the irradiated samples.

Implications for photochemical reactivity. The irradiation experiments suggest that most of T* and $^1O_2$ are produced by compounds smaller than 0.10 µm. Larger dissolved components (0.10 – 0.45 µm) and suspended particles up to 5 µm would play a minor to negligible role. In many cases the larger species would also absorb and/or scatter radiation to a limited or negligible extent compared to the smaller compounds. In one case (Lake Viverone) there was evidence of a significant absorption and/or scattering by larger components, which was nevertheless connected to a negligible photochemical activity toward the transformation of TMP or FFA. The lack of reactivity combined with lack of radiation absorption, which was the most common scenario, implies that components larger than 0.10 µm were not present in sufficient amount to influence the transformation of the adopted substrates by their photochemical reactivity. In contrast, lack of reactivity in the presence of significant radiation absorption would point to a negligible intrinsic photoactivity of the larger components. Reasons could be either that the reactive species are not produced, or that they are produced but also consumed before they leave the suspended particles or the large dissolved molecules. Interestingly, evidence is available that organic matter contains moieties that are able to scavenge photoproduced $^1O_2$ (Latch and McNeill, 2006) or T* (Canonica and Laubscher, 2008).

Conclusions

The combination of filtration and DLS measurement of the size distribution of suspended particles suggests that filtration at 0.45 µm is not sufficient to remove all the suspended solids. To the latter purpose, filtration at 0.10 µm would be required. The common definition of the analytical parameters labelled as "dissolved" could be re-defined on the light of the experimental evidence here reported, which supports a filtration step with pore sizes smaller than 0.45 µm (*e.g.* 0.1 µm).

As far as the generation of T* and $^1O_2$ is concerned, most of the photochemical reactivity of the studied lake water samples was accounted for by dissolved components smaller than 0.10 µm. In most cases, larger species would absorb and/or scatter radiation negligibly when compared with smaller components. In one case the absorption and/or scattering of radiation was detectable, but it was connected with negligible photoactivity.

Acknowledgements

Financial support by PNRA – Progetto Antartide is gratefully acknowledged.

References

Al Housari, F., Vione, D., Chiron, S., Barbati, S., 2010. Reactive photoinduced species in estuarine waters. Characterization of hydroxyl radical, singlet oxygen and dissolved organic matter triplet state in natural oxidation processes. Photochem. Photobiol. Sci. 9, 78-86.

Albinet, A., Minero, C., Vione, D., 2010. Phototransformation processes of 2,4-dinitrophenol, relevant to atmospheric water droplets. Chemosphere 80, 753-758.

Belli, M., Centioli, D., de Zorzi, P., Sansone, U., Capri, S., Pagnotta, R., Pettine, M., 2004. Analytical methods for water (in Italian), APAT, Rome, method n. 2090.

Boreen, A. L., Arnold, W. A., McNeill, K., 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an SO_2 extrusion photoproduct. Environ. Sci. Technol. 39, 3630-3638.

Boreen, A. L., Edhlund, B. L., Cotner, J. B., McNeill, K., 2008. Indirect photodegradation of dissolved free amino acids: The contribution of singlet oxygen and the differential reactivity of DOM from various sources. Environ. Sci. Technol. 42, 5492-5498.

Borer, P., Sulzberger, B., Hug, S. J., Kraemer, S. M., Kretzschmar, R., 2009. Photoreductive dissolution of iron(III) (hydr)oxides in the absence and presence of organic ligands: experimental studies and kinetic modelling. Environ. Sci. Technol. 43, 1864-1870.

Bracchini, L., Cozar, A., Dattilo, A. M., Falcucci, M., Gonzales, R., Loiselle, S., Hull, V., 2004. Analysis of extinction in ultraviolet and visible spectra of water bodies of the Paraguay and Brazil wetlands. Chemosphere 57, 1245-1255.

Brinkmann, T., Sartorius, D., Frimmel, F. H., 2003. Photobleaching of humic rich dissolved organic matter. Aquat. Sci. 65, 415-424.

Buxton, G. V., Greenstock, C. L., Helman, W. P., 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, 1027-1284.

Canonica, S., Freiburghaus, M., 2001. Electron-rich phenols for probing the photochemical reactivity of freshwaters. Environ. Sci. Technol. 35, 690-695.

Canonica, S., Laubscher, H. U., 2008. Inhibitory effect of dissolved organic matter on triplet-induced oxidation of aquatic contaminants. Photochem. Photobiol. Sci. 7, 547-551.

Cory, R. M., McKnight, D. M., 2005. Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ. Sci. Techol. 39, 8142-8149.

Gerecke, A. C., Canonica, S., Müller, S. R., Scharer, M., Schwarzenbach, R. P., 2001. Quantification of dissolved organic matter (DOM) mediated phototransformation of phenylurea herbicides in lakes. Environ. Sci. Technol. 35, 3915-3923.

Giussani, G., Debernardi, R., Ruffoni, T., 1990. Three years of experience in biomanipulating a small euthropic lake – Lago di Candia (Northern Italy). Hydrobiologia 200, 357-366.

Halladja, S., Ter Halle, A., Aguer, J. P., Boulkamh, A., Richard, C., 2007. Inhibition of humic substances mediates photooxigenation of furfuryl alcohol by 2,4,6-trimethylphenol. Evidence for reactivity of the phenol with humic triplet excited states. Environ. Sci. Technol. 2007, 41, 6066-6073.

Hoigné, J., 1990. Formulation and calibration of environmental reaction kinetics: Oxidations by aqueous photooxidants as an example. In: Aquatic Chemical Kinetics, Stumm, W. (Ed.), Wiley, NY, pp. 43-70.

Kieber, R. J., Seaton, P. J., 1996. Determination of subnanomolar concentrations of nitrite in natural waters. Anal. Chem. 67, 3261-3264.

Latch, D. E., Stender, B. L., Packer, J. L., Arnold, W. A., McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: Cimetidine and ranitidine. Environ. Sci. Technol. 37, 3342-3350.

Latch, D. E., McNeill, K., 2006. Microheterogeneity of singlet oxygen distributions in irradiated humic acid solutions. Science 311, 1743-1747.

Maddigapu, P.R., Bedini, A., Minero, C., Maurino, V., Vione, D., Brigante, M., Mailhot, G., Sarakha, M., 2010. The pH-dependent photochemistry of anthraquinone-2-sulphonate. Photochem. Photobiol. Sci. 9, 323-330.

O'Sullivan, D. W., Neale, P. J., Coffin, R. B., Boyd, T. J., Osburn, S. L., 2005. Photochemical production of hydrogen peroxide and methylhydroperoxide in coastal waters. Mar. Chem. 97, 14-33.

Sulzberger, B., Durisch-Kaiser, E., 2009. Chemical characterization of dissolved organic matter (DOM): A prerequisite for understanding UV-induced changes of DOM absorption properties and bioavailability. Aquat. Sci. 71, 104-126.

Takeda, K., Takedoi, H., Yamaji, S., Ohta K., Sakugawa, H., 2004. Determination of hydroxyl radical photoproduction rates in natural waters. Anal. Sci. 20, 153-158.

Tratnyek, P. G., Hoigné, J., 1994. Photo-oxidation of 2,4,6-trimethylphenol in aqueous laboratory solutions and natural waters: kinetics of reaction with singlet oxygen. J. Photochem. Photobiol. A: Chem. 84, 153-160.

Vione, D., Falletti, G., Maurino, V., Minero, C., Pelizzetti, E., Malandrino, M., Ajassa, R., Olariu R.-I., Arsene, C., 2006. Sources and sinks of hydroxyl radicals upon irradiation of natural water samples. Environ. Sci. Technol. 40, 3775-3781.

Vione, D., Rinaldi, E., ;Minero, C., Maurino, V., Olariu, R. I., Arsene, C., 2009. Studies regarding groundwater quality at rural sites. 2. Photochemical generation of $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals upon UV-A irradiation of nitrate-rich groundwater. Rev. Chim. 60, 551-554.

Vione, D., Bagnus, D., Maurino, V., Minero, C., 2010. Quantification of singlet oxygen and hydroxyl radicals upon UV irradiation of surface water. Environ. Chem. Lett. 8, 193-198.

Wang, L., Zhang, C., Wu, F., Deng, N., 2007. Photodegradation of aniline in aqueous suspensions of microalgae. J. Photochem. Photobiol. B: Biol. 87, 49-57.

Wilkinson, F., Brummer, J., 1981. Rate constants for the decay and reactions of the lowest electronically excited singlet-state of molecular oxygen in solution. J. Phys. Chem. Ref. Data 10, 809-1000.

Zepp, R. G., Schlotzhauer, P., 1983. Influence of algae on photolysis rates of chemicals in water. Environ. Sci. Technol. 17, 462-468.

Table 1. Analytical data relevant to the studied lake water samples. Nitrate, nitrite, NPOC, IC (inorganic carbon) and pH have been measured on the samples filtered at 0.45 µm. TSS = Total Suspended Solids content, determined by 0.45 µm filtration of the samples pre-filtered at 5 µm. The accuracy of pH measurements is within 0.1 units. R_{T*} and R_{1O2} are referred to the samples filtered at 0.10 µm. Lake Candia has an average depth of 5.9 m and a surface of 1.35 Km^2. For Lake Viverone the corresponding data are 22.5 m and 5.72 Km^2, for Lake Grande, Avigliana, 19.5 m and 0.89 Km^2. The lakes are all located in Piedmont (NW Italy).

	Nitrate, M	**Nitrite, M**	**NPOC, mg C L^{-1}**	**IC, mg C L^{-1}**	**TSS, mg L^{-1}**	**pH**	**R_{T*}, 10^{-9} M s^{-1}**	**R_{1O2}, 10^{-9}M s^{-1}**
Candia, 5 Mar 2010	$(5.5\pm1.3)\cdot10^{-6}$	$(5.3\pm2.1)\cdot10^{-7}$	4.19±0.18	12.77±0.18	0.8±0.2	6.2	9.29±0.62	3.57±0.55
Candia, 27 Apr 2010	$(9.6\pm1.4)\cdot10^{-6}$	$(1.7\pm0.2)\cdot10^{-6}$	5.60±0.06	5.16±1.06	<0.1	6.6	14.0±1.3	2.78±0.37
Viverone, 24 May 2010	$<0.5\cdot10^{-6}$	$(1.1\pm0.7)\cdot10^{-7}$	3.29±0.06	22.21±0.46	0.2±0.1	7.4	6.19±1.36	3.70±0.39
Avigliana, 13 Jun 2010	$(2.2\pm0.1)\cdot10^{-5}$	$(3.6\pm0.4)\cdot10^{-7}$	3.75±0.29	38.03±0.02	0.4±0.2	8.3	11.0±0.22	3.06±0.16
Candia, 22 Sep 2010	$(2.7\pm1.0)\cdot10^{-6}$	$(3.6\pm0.2)\cdot10^{-7}$	6.57±2.67	12.23±0.15	1.2±0.2	7.3	12.2±1.5	5.19±0.10

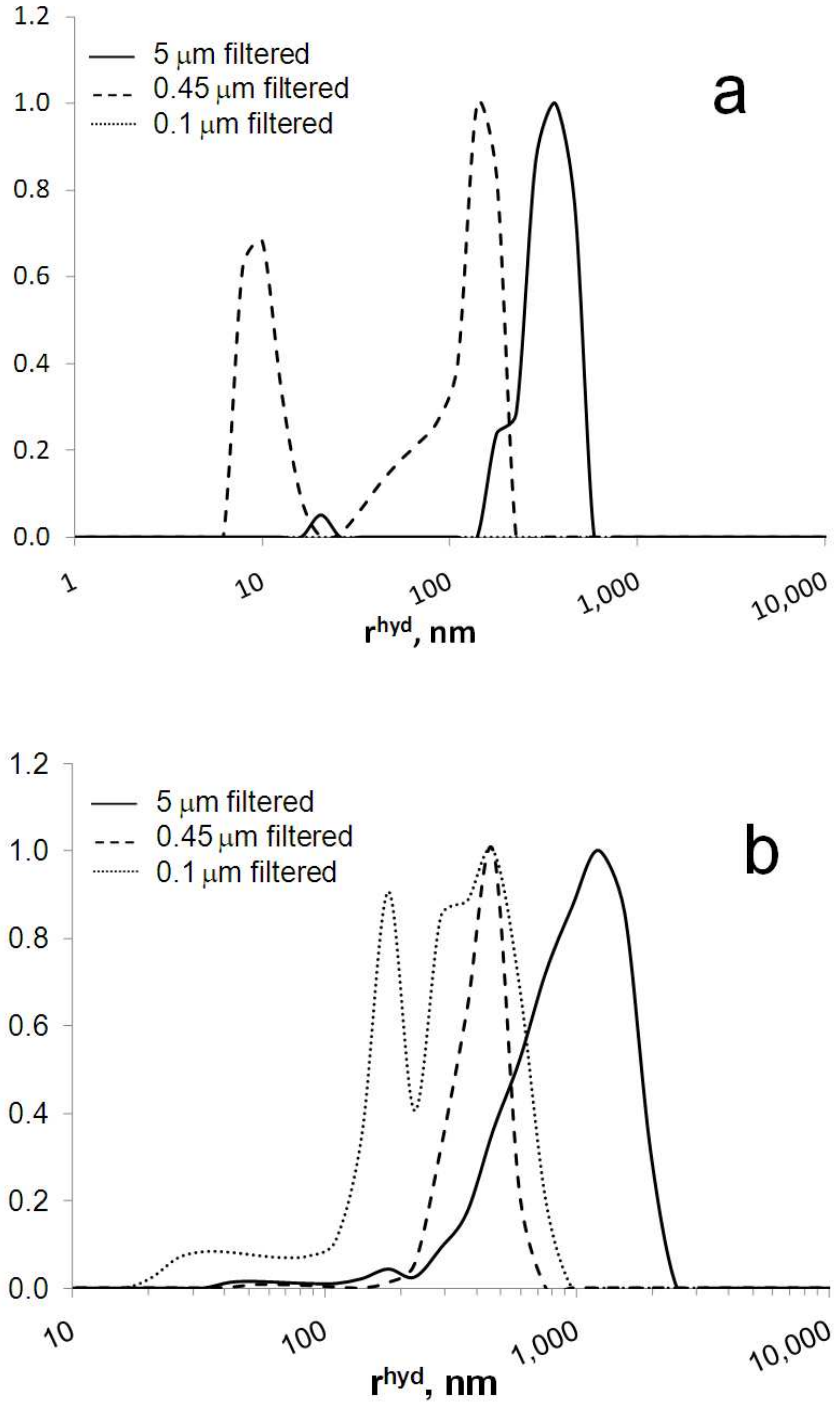

Figure 1. Hydrodynamic radii distribution of suspended particles in the samples filtered at 5, 0.45 and 0.10 µm.

(a) Lake Candia (5 March 2010). Note the absence of suspended particles upon filtration at 0.10 µm.

(b) Lake Viverone (24 May 2010).

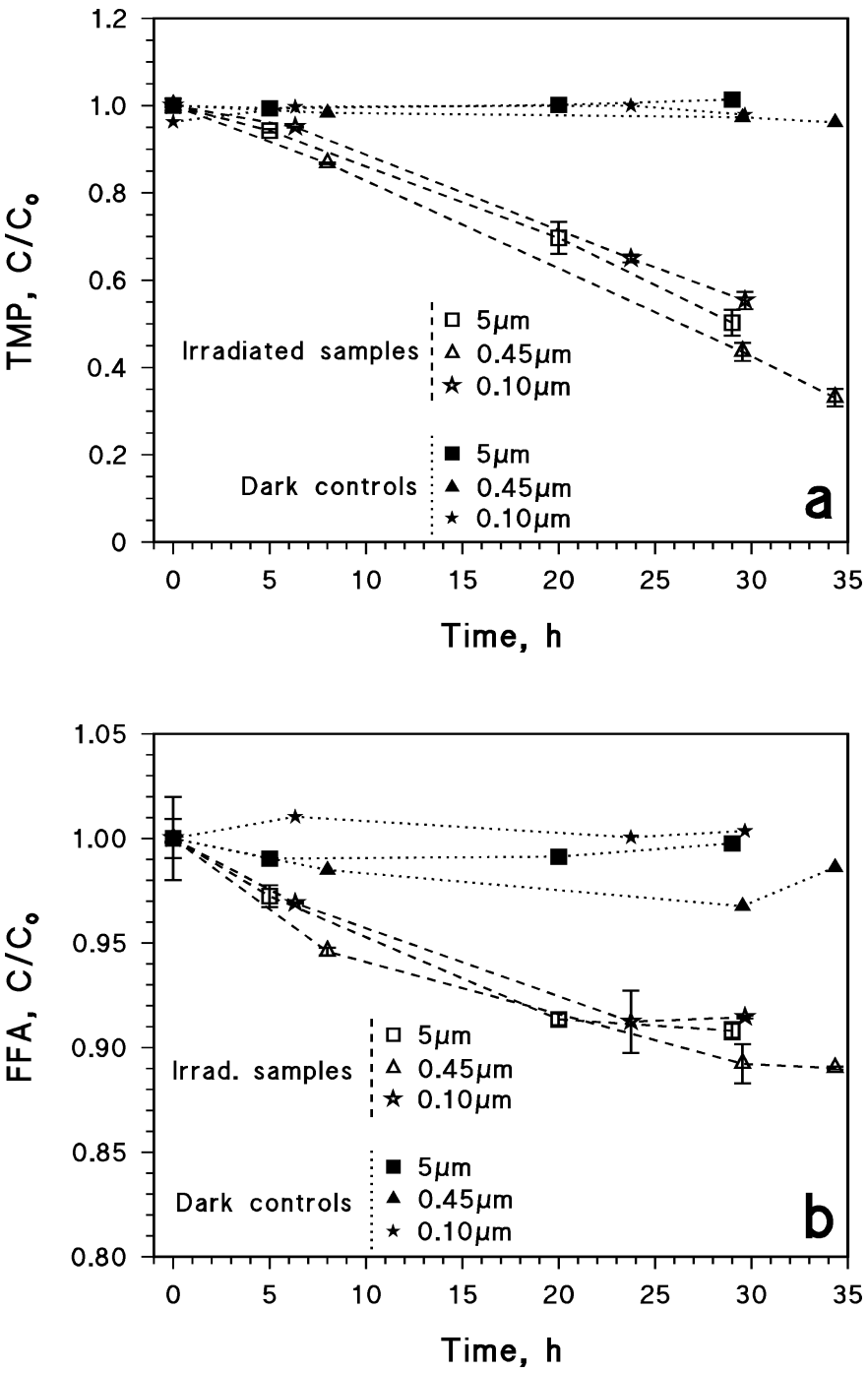

Figure 2. Time trends of **(a)** 2.0 mM TMP and **(b)** 2.0 mM FFA in water from Lake Candia (5 March 2010) filtered at 5, 0.45 and 0.10 µm, both in the dark and under irradiation.

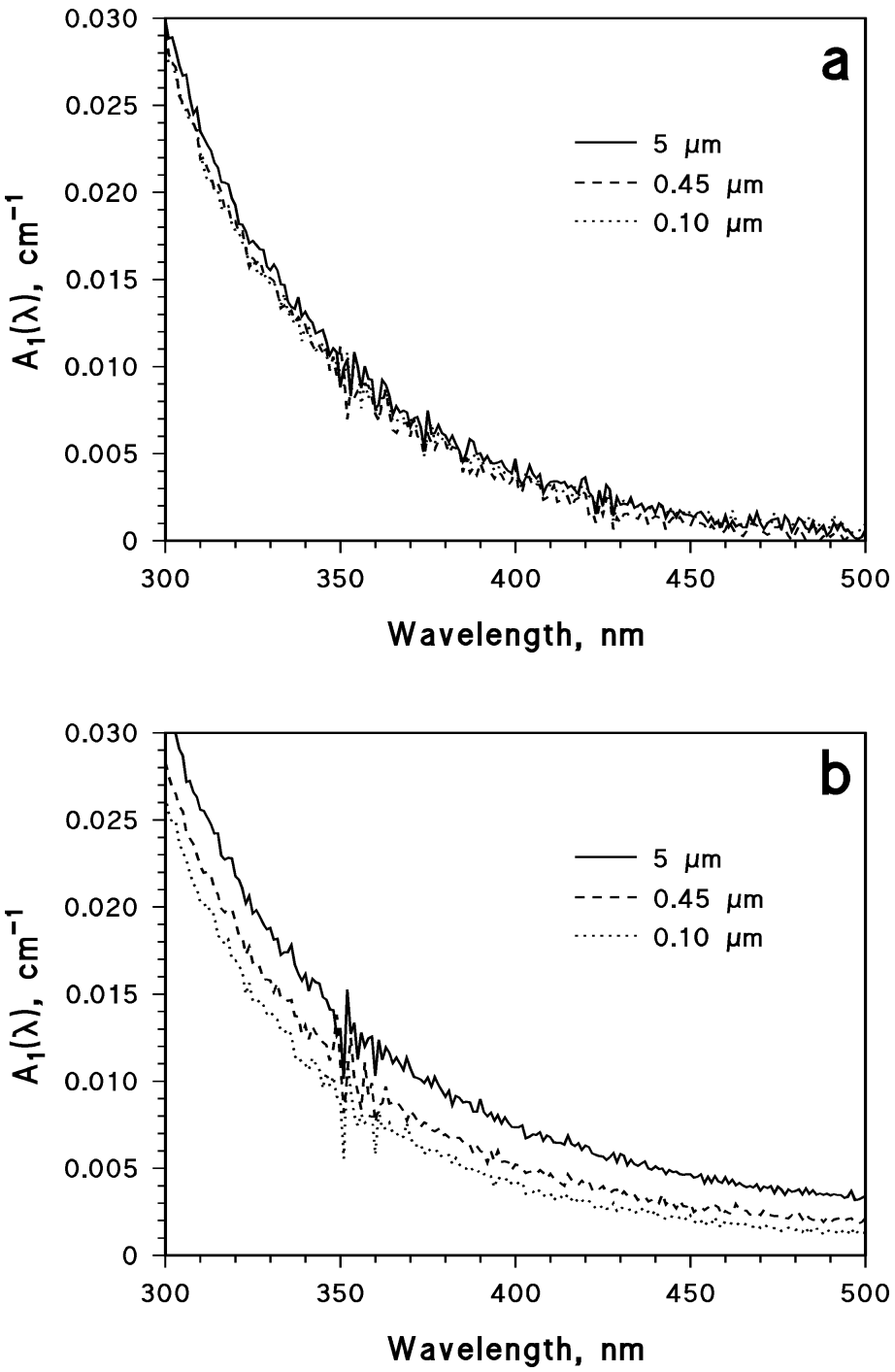

Figure 3. Absorption spectra $A_1(\lambda)$ (optical path length b = 1 cm) of water from **(a)** Lake Candia (5 March 2010) and **(b)** Lake Viverone (24 May 2010), filtered at 5, 0.45 and 0.10 µm.

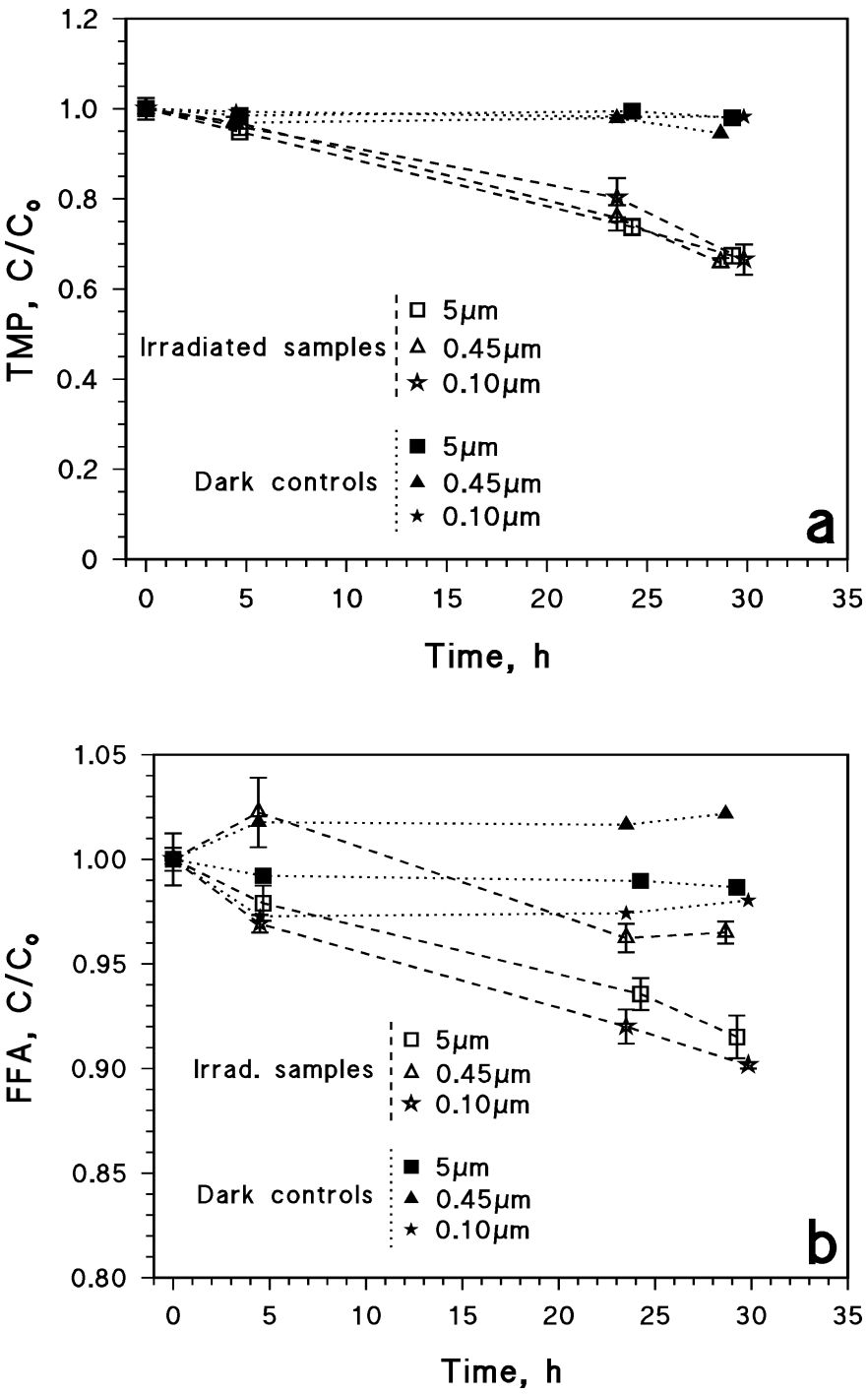

Figure 4. Time trends of **(a)** 2.0 mM TMP and **(b)** 2.0 mM FFA in water from Lake Viverone (24 May 2010) filtered at 5, 0.45 and 0.10 μm, both in the dark and under irradiation.