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Oxolinic acid in aquaculture waters: can natural attenuation through photodegradation decrease its concentration?

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Abstract

Quinolones, such as oxolinic acid (OXA), are antimicrobials commonly used in aquaculture. Thus, its presence in the aquatic environment surrounding aquaculture facilities is quite easy to understand. When present in aquatic environment, pharmaceuticals may be subjected to several attenuation processes that can influence their persistence. Photodegradation, particularly for antibiotics, can have significant importance since these compounds may be resistant to microbial degradation. OXA photodegradation studies reported in literature are very scarce, especially using aquaculture waters, but are markedly important for an appropriate risk assessment. Results hereby presented showed a decrease on photodegradation rate constant from 0.70 ± 0.02 h^{-1} in ultrapure water to 0.42 ± 0.01 h^{-1} in freshwater. The decrease on photodegradation rate constant was even more pronounced when brackish water was used (0.172 ± 0.003 h^{-1}). In order to understand which factors contributed to the observed behaviour, environmental factors, such as natural organic matter and salinity, were studied. Results demonstrated that dissolved organic matter (DOM) may explain the decrease of OXA photodegradation observed in freshwater. However, a very sharp decrease of OXA photodegradation was observed in solutions containing NaCl and in synthetic sea salts, which explained the higher decrease observed in brackish water. Moreover, under solar radiation, the use of an $^1O_2$ scavenger allowed us to verify a pronounced retardation of OXA decay, suggesting that $^1O_2$ plays an important role in OXA photodegradation process.

Keywords: Photolysis; water treatment; humic substances (HS); reactive oxygen species (ROS); matrix effects.

1. INTRODUCTION

Aquaculture industry has been facing a huge development in the last few years, more than all other sectors of animal food-production, now accounting for 50% of the world's fish used for food (FAO, 2020; Romero et al., 2012). However, this development has been accompanied by some practices potentially harmful for both animal and human health (Zeghioud et al., 2019). In order to prevent or treat infections due to bacterial diseases, aquaculture farmers frequently make an intensive use of antibiotics (Romero et al., 2012; Zeghioud et al., 2019). Such abusive use is underneath the consumption of undetected antibiotics in food, which can cause allergies and toxicity and also may lead to the development of antibiotic-resistant bacteria (Rasul and Majumdar, 2017; Zeghioud et al., 2019). Bacterial resistance phenomenon is recognized as one of the most serious threats to public health currently, since both animals and animal waste are potential reservoirs of multi-resistant genes that can be transmitted directly or indirectly to humans through contact and food consumption (Caruso, 2016; WHO, 2020).

Quinolones, such as oxolinic acid (OXA), which constitute an antimicrobial family characterized by broad spectrum, high potency and theoretically low occurrence of side effects, are commonly used in aquaculture (Lin et al., 2010; Sturini et al., 2014; Zeghioud et al., 2019). OXA may be administered through both medicated feed and bathing treatments and good clinical effects at low dosage rates are observed in both fresh and saltwater aquaculture (Lai and Lin, 2009). After administration, the pharmaceutically active form of OXA is commonly present in recirculating aquaculture water and/or released into surface waters because it is metabolized to a small extent (Lai and Lin, 2009). It was already estimated that more than 50 kg of OXA may be released

per year via faecal excretion into the environment by Greek fish farms (Rigos et al., 2004).

Since the presence of antibiotics in the aquatic environment can cause toxic effects on aquatic organisms and promote the development of antibiotic resistance, discharges from aquafarms are of great concern (Zhong et al., 2018). After release, environmental persistence is a key issue determining the exposure concentrations and effects of pharmaceuticals (Caracciolo et al., 2015). On the other hand, pharmaceuticals' environmental persistence usually depends, to a large extent, on the activity of microorganisms with the capability to degrade them (Caracciolo et al., 2015). Still, since antibiotics may resist microbial degradation, alternative processes, such as photolysis, may be more influential on their persistence and effective in their degradation than biological processes (Pouliquen et al., 2007). Photolysis has, in fact, been considered as an effective way to decrease antibiotics' concentration in the environment (e.g. Oliveira et al., 2019; Silva et al., 2020). It can happen by one of two ways: directly, the pollutant itself absorbing the radiation that causes its transformation, or indirectly, photosensitizer absorbing the light and then generating photoreactants, such as hydroxyl radicals ($^{\bullet}OH$) or singlet oxygen ($^{1}O_2$), that will then interact with the pollutant resulting in its chemical transformation (Oliveira et al., 2019). On the other hand, self-sensitization processes can also occur. In this case, after the photon is absorbed directly by the pollutant, it reaches the triplet excited state and can transfer the energy to dissolved organic matter (DOM) or H_2O to form reactive oxygen species (ROS).

Even though photodegradation has been studied for a wide range of antibiotics, literature on OXA photodegradation is very scarce. One of the few exceptions is the work by Turiel et al. (2005), who reported that 80% of OXA remained unaltered in river

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water samples, after five months stored at ambient temperature under direct sunlight, pointing to a recalcitrant behaviour of OXA in the aquatic environment. Pouliquen et al. (2007) also observed a low contribution (10%) of photolysis on OXA degradation in water, being more stable to photolysis than other quinolones, which was considered to presage a higher environmental impact of OXA comparatively with other antibiotics from the same class. On the other hand, TiO_2-catalysed photodegradation was proven to be 100% efficient in OXA degradation (Pereira et al., 2013).

Considering that information on the fate and persistence behaviour of OXA in aquatic environments, and specifically in aquaculture waters, is very limited and that this is a type of knowledge needed for appropriate risk assessment and management regarding the discharge of antibiotics, this work aimed at: (i) understand about the OXA photodegradation behaviour when present in natural water samples, representative of fresh and brackish aquaculture; (ii) gather knowledge about the influence of water characteristics, such as the presence of organic matter or salinity, and the role they play on OXA photodegradation rate; and (iii) conclude about which reactive species may be involved on the photodegradation of OXA.

2. MATERIALS AND METHODS

2.1. Chemicals

OXA (Fisher Scientific, 98%) stock solution (10 mg L^{-1}) was prepared by dissolving the compound in 0.03 mol L^{-1} sodium hydroxide solution sonicating for 60 min. Then, the working solution was prepared in 0.001 mol L^{-1} phosphate buffer and pH was adjusted to 7.3 using hydrochloric acid (2 mol L^{-1}) (NormaPur, 37%). Phosphate buffer stock solution (1 L) was prepared using 0.05 mol of sodium dihydrogen phosphate dihydrate (Fluka, Biochemika, $\geq$ 99.5%) and 0.05 mol di-sodium hydrogen

phosphate dihydrate (Fluka, Biochemika, $\geq$ 99%), which was diluted to 0.001 mol L^{-1} and pH adjusted to 7.3 using hydrochloric acid (NormaPur, 37%). Synthetic sea salt solution (21‰) was prepared dissolving Red Sea Salt (Red Sea Europe) in phosphate buffer solution (0.001 mol L^{-1}, pH 7.3). Sodium chloride solution 21‰ (Fluka, 99.5%) was also prepared in phosphate buffer solution (0.001 mol L^{-1}, pH 7.3). A stock solution of sodium azide (NaN_3, Riedel-de Haën, 99%) was prepared at a concentration of 0.023 mol L^{-1} and isopropanol (C_3H_8O, Sigma-Aldrich, 99.8%) stock solution was prepared at a concentration of 1 mol L^{-1}, both in ultrapure water (Milli-Q plus 185, Millipore).

For high-performance liquid chromatography with a UV-vis detector (HPLC-UV) analysis, methanol (Fisher Scientific, HPLC grade) and formic acid (Sigma-Aldrich, > 98%) were used.

2.2. Instrumentation

OXA quantification was performed using HPLC-UV. The device consisted of a Waters Alliance 2695 Separations Module equipped with a Waters 2487 Dual Absorbance detector. Separation was carried out at 25 °C using a 150 mm × 4.6 mm i.d. ACE® C18 column-PFP (5 μm particle size) connected to a 4.6 mm i.d. ACE® 5 C18 guard column. The mobile phase consisted of methanol: 0.1% formic acid, 45:55 (*v/v*), at a flow rate of 0.9 mL min^{-1}. An injection volume of 20 μL was used and detection was performed at 270 nm wavelength. Methanol and 0.1% formic acid aqueous solutions were filtered through a 0.2 μm polyamide membrane filter (Whatman) before use as mobile phase.

For the irradiation experiments a solar radiation simulator Solarbox 1500 (Co.fo.me.gra, Italy) was used. This instrument is equipped with a xenon arc lamp (1500 W) and UV filters that limit the transmission of light below 290 nm. All

experiments were performed with a constant irradiation of 55 W m^{-2} (290–400 nm), which corresponds to 550 W m^{-2} in the spectral range, according to the manufacturer. The level of irradiance and temperature was monitored by a multimeter (Co.fo.me.gra, Italy) equipped with a UV 290–400 nm band sensor and a black standard temperature sensor. A parabolic reflection system was used to ensure irradiation uniformity in the chamber, which was kept refrigerated by an air-cooling system.

2.3. Photodegradation experiments

OXA aqueous solutions (20 mL) in ultrapure water and natural samples were irradiated, in triplicate, in quartz tubes (internal diameter × height = 1.8 × 20 cm) covered with Parafilm M$^{®}$. Each set of experiments was accompanied by dark controls prepared in the corresponding matrix, which were maintained inside the solar simulator during the same time as the irradiated solutions and under identical conditions apart from the irradiation (quartz tubes covered by aluminium foil), in order to prove the absence of any thermal and/or microbiological degradation. Aliquots (500 μL) of irradiated OXA solutions and dark controls were withdrawn from quartz tubes throughout time, stored in the dark at 4 ºC and analysed within 24 h.

Initially, the effect of OXA concentration on its photodegradation was studied using two concentrations, 100 and 250 μg L^{-1} in solutions prepared in ultrapure water, containing 0.001 mol L^{-1} phosphate buffer and pH adjusted to 7.3. Furthermore, in order to understand the photodegradation behaviour of OXA in natural water samples, OXA solutions were prepared in two water samples with different characteristics. Both samples (freshwater and brackish water) were collected in Aveiro (Portugal). After collection, samples were filtered through 0.45 μm nitrocellulose membrane filters (Millipore), stored at 4 ℃ and used within 7 days. The salinity, conductivity and pH of

these natural water samples were measured using a Multi 3320 meter from WTW. Also, dissolved organic carbon (DOC) was measured using a Total Organic Carbon analyser, TOC-VCPH, from Shimadzu. For the DOC analysis, samples were acidified with 2% (*v/v*) HCl 2 mol L^{-1} to remove the inorganic carbon.

To evaluate the influence of DOM in OXA photodegradation, humic substances (HS) were used as model compounds, namely humic acids (HA), fulvic acids (FA) and the XAD-4 fraction (the most hydrophilic fraction of organic matter), which were extracted and isolated from an estuarine water from Ria de Aveiro (Aveiro, Portugal) as described by Santos et al. (1994) and Esteves et al. (1995). OXA solutions with each of the HS fractions (20 mg L^{-1}) were prepared in 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3. The concentration of HS fractions (20 mg L^{-1}) was selected considering that freshwater DOC concentration is usually between 2 and 10 mg L^{-1} (Leech et al., 2009) and that DOC of HS is about 50% of their concentration (Santos et al., 1994; Esteves et al., 1995).

Finally, the effect of salinity was evaluated by preparing OXA standard solutions in presence of 21‰ NaCl or 21‰ synthetic sea salts and 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3.

Isopropanol (C$_3$H$_8$O) and sodium azide (NaN$_3$) were used to evaluate the influence of $\cdot$OH and ^{1}O$_2$, respectively, on OXA photodegradation. For this purpose, OXA standard solutions were prepared in 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3, containing 5 mmol L^{-1} of NaN$_3$ or 20 mmol L^{-1} of C$_3$H$_8$O.

3. RESULTS AND DISCUSSION

3.1. Photodegradation in ultrapure water and in natural aquatic environments

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Kinetic experimental results on the photodegradation of OXA throughout time at different antibiotic initial concentrations (100 and 250 μg L^{-1}) in ultrapure water are presented in Figure 1a). Results are shown together with the corresponding fittings to the pseudo-first order equation $C/C_0 = e^{-kt}$, in which k is the rate constant (h^{-1}), t is time, and C and C_0 are the concentration of OXA at a given irradiation time and the initial concentration, respectively. Also, OXA half-life times ($t_{1/2}$) were calculated as ln2/k.

As may be seen in Figure 1a), OXA photodegradation occurs faster at 100 than at 250 μg L^{-1}. Indeed, concentration has already been shown to influence degradation since photolysis can be decreased due to photon limitation occurring at higher initial concentrations (Chowdhury et al., 2011). This effect has been confirmed in this work, since photodegradation rate constants of 1.24 $\pm$ 0.02 and 0.70 $\pm$ 0.02 h^{-1} and $t_{1/2}$ of 0.56 $\pm$ 0.01 and 0.99 $\pm$ 0.04 h (Table 1) were determined at OXA concentrations of 100 and 250 μg L^{-1}, respectively.

Figure 1

Table 1

As previously highlighted, there are few published results on OXA photodegradation, which were obtained at initial OXA concentrations between 1 and 20 mg L^{-1} (Lai and Lin, 2009; Turiel et al., 2005; Zeghioud et al., 2019). Such concentrations are higher than those used in this work, so, in agreement with the concentration effect here determined, resulted in $t_{1/2}$ values much higher than those observed in this study. For instance, Lai and Lin (2009) used 20 mg L^{-1} of OXA and obtained $t_{1/2}$ that varied between 55.2 and 115.2 h, depending on the matrix (Table 2). Although OXA photodegradation studies in literature are scarce, for other quinolones there are some published results (Table 2). Pseudo-first order rate constants can vary a lot depending on the quinolone under study. For example, Sturini et al. (2012), reported values between 3.66 and 39.6 h^{-1}.

Table 2

Since water matrix can influence the photodegradation behaviour of organic compounds, OXA was spiked into different water matrices in order to obtain a final concentration of 250 μg L^{-1}. As already mentioned, OXA is used in medicated feed and bathing treatments in both fresh and saltwater aquaculture (Lai and Lin, 2009). Thus, in order to represent both types of aquaculture, fresh and brackish water were used as matrices. Properties of collected samples are presented in Table 3. The obtained results on OXA photodegradation in these matrices are depicted in Figure 1a). It must be highlighted that photodegradation experiments in these matrices were conducted at the natural pH of each water sample (in the range 7.3–8.5). It is known that pH influences the speciation of OXA (pKa = 5.94 ± 0.20), and thus its photodegradation behaviour. However, for the pH of the aqueous matrices used in this work, OXA is in its negative form and, therefore, pH effects on speciation may be neglected.

Table 3

Results in Figure 1a) make evident that OXA photodegradation in freshwater and, especially, in brackish water is slower than in ultrapure water. This is confirmed by the corresponding k and $t_{1/2}$ presented in Table 1. As for the latter, the value determined in ultrapure water was 0.99 ± 0.04 h, while in freshwater and in brackish water $t_{1/2}$ was 1.65 ± 0.03 h and 4.03 ± 0.04 h, respectively. These results demonstrate that the OXA photodegradation rate is slower in the natural matrices, particularly in brackish water, with a $t_{1/2}$ four times higher than the observed in ultrapure water. Consequently, as compared with ultrapure water, it may be expected a larger OXA persistence in the aquatic environment, along with potential risks, such as enhancing microbial resistance through prolonged exposure of microorganisms to OXA. Thus, it is important to understand how environmental factors, such as natural organic matter and salinity, influence the OXA photodegradation behaviour.

3.2. Influence of HS in OXA photodegradation

Organic matter may result in opposite effects on the photodegradation rate (Silva et al., 2016a; Silva et al., 2016b; Oliveira et al., 2019). If on one side, organic matter can act as a photosensitizer (enhancing the photodegradation by being promoted to a transient excited state, which may react directly with the pollutant or react with oxygen present in solution, forming ROS); on the other side, it can also have an inhibitory effect (generally, by acting as a filter (inner filter effect), decreasing the radiation available for the pollutant). Thus, the global effect of organic matter will be a balance between these two opposite contributions.

Results obtained for OXA photodegradation under the presence of HS, namely HA, FA and XAD-4 fraction, are shown in Figure 1b), which evidences a decrease in photodegradation rate in the presence of HS. This is confirmed by the corresponding k and $t_{1/2}$ values in Table 1. As may be observed, OXA $t_{1/2}$ varied between 1.70 ± 0.05 and 2.38 ± 0.07 h in the presence of the different fractions of HS, which are higher than the value obtained in ultrapure water (0.99 ± 0.04 h). Thus, in this case, the inhibitory effect of HS prevailed in relation to their photosensitizing effect. DOM inhibition has already been observed for the photodegradation of antibiotics, such as sulfonamides (Wenk and Canonica, 2012; Oliveira et al., 2019). Also, Prabhakaram et al. (2009) reported an inhibition of quinolone antibiotics photodegradation rate in the presence of HA and FA (Table 2). Apart from the inner filter effect, inhibition can also result from other processes as the scavenging/quenching of reactive species and back-reductions to the parent compound by excited triplet states of dissolved organic matter ($^{3}CDOM^{*}$). Results obtained in this work (Figure 1b)) using different HS fractions demonstrated a

higher inhibition in the presence of HA, than in the presence of FA and XAD-4 (Table 1).

These HS purified fractions were fully characterized (Oliveira et al., 2019). In what respects elemental analysis (results depicted in Table 4) it may be seen that the XAD-4 fraction has a lower carbon content than HA and FA; contrarily, the oxygen content of XAD-4 is higher. This indicates greater content of oxygen functional groups on XAD-4 fraction than in the other two fractions used. Thermogravimetric analysis (data not shown), indicated that HA are the most thermo-resistant and are decomposed at temperatures above 300 ºC, thus it may be concluded that HA constitute a fraction with higher aromaticity and lower functional and aliphatic groups content when compared with FA and XAD-4 fractions. The UV spectra of 10 mg L^{-1} in presence of the three HS fractions were performed in the range 200-600 nm and showed the decrease of absorbance values from 200 to 600 nm for all HS isolates (Figure 2). The differences in absorbance intensities are most likely caused by the difference in chromophore concentrations. The greater content of chromophores allows for more light absorption, which can be confirmed by the UV spectra.

Table 4

Figure 2

Considering the characterization of the HS fractions, HA is the most hydrophobic fraction, being more enriched in aromatic and/or chromophoric groups, followed by FA and XAD-4. Also, XAD-4 fraction is the richest fraction in oxygen functional groups with lower carbon but higher oxygen content than HA and FA. Results suggest an inverse correlation between the HS aromaticity and their photosensitizing effect, which is coincident with the observations of Oliveira et al. (2019) and Silva et al. (2016a). HA fraction, presenting higher aromaticity, has higher ability to absorb light and thus, a higher inner filter effect, which would explain the lower OXA photodegradation rate in

the presence of this fraction. Wenk and Canonica (2012) also found a correlation between the DOM aromatic degree and the antioxidant activity. DOM of terrestrial origin, with high aromaticity, was shown to have an inhibition effect higher than DOM of aquatic origin, with low aromaticity, and thus more likely to act as antioxidant (Wenk and Canonica, 2012).

The inhibitory effect of DOM may explain, at least partially, the slower OXA photodegradation observed in fresh and brackish water as compared with ultrapure water (Figure 1a)). In fact, apart from other influencing factors in these complex matrices, the larger inhibitory effect observed in brackish water (9.8 mg L^{-1} DOM, $k = 0.172 \pm 0.003$ h^{-1}) than in freshwater (8.3 mg L^{-1} DOM, $k = 0.42 \pm 0.01$ h^{-1}), may be related to some extent with DOM concentration.

3.3. Influence of salinity in OXA photodegradation

Although the DOC of fresh and brackish water samples was very similar, salinity was a main difference between them. Salinity has been shown to have different influence in the photodegradation behaviour of organic pollutants. Amongst sulfonamide antibiotics, salinity was considered to inhibit photodegradation in the case of sulfamethoxazole (Oliveira et al., 2019), but an opposite effect was observed for sulfadiazine, with reactive halogen species (RHS) being considered responsible for the enhancement of sulfadiazine photodegradation observed in saline waters (Zhao et al., 2019).

Results represented in Figure 1c) demonstrate small differences between photodegradation of OXA in ultrapure water and in presence of 21‰ NaCl solution. However, using 21‰ synthetic sea salts solution, the decrease in OXA photodegradation was very sharp, with $t_{1/2}$ increasing from 0.99 ± 0.04 h, in ultrapure

water, to 4.25 ± 0.04 h, in synthetic sea salts. Indeed, the $t_{1/2}$ of OXA in synthetic sea salts ($t_{1/2} = 4.25 \pm 0.04$ h) was just slightly higher to that observed in brackish water ($t_{1/2} = 4.03 \pm 0.04$ h). Synthetic sea salts from *Red Sea Salt* contain ions like calcium, magnesium, and carbonates, being free of synthetic additives, nitrates, phosphates, or heavy metals (Red Sea, 2019). The increase in $t_{1/2}$ observed in synthetic sea salts, as compared with NaCl, may be attributed to OXA stabilization due to chelate formation between the quinolone and divalent cations in the synthetic sea salts solution, since one of the main properties of quinolones is the chelate binding of metal cations to the carbonyl ring and one of the carboxylic oxygens (Rigos et al. 2004; Turel, 2002). Moreover, carbonates present in the synthetic sea salt solution, such as bicarbonate (HCO_3^-), could inhibit OXA photodegradation since they can act as $^{\cdot}OH$ scavengers (Acero et al., 2019; Bian and Zhang, 2016), via a main mechanism, as follows (Bian and Zhang, 2016):

$$HCO_3^- + {}^{\cdot}OH \rightarrow CO_3^{-\cdot} + H_2O \tag{1}$$

In fact, indirect photolysis mediated by $^{\cdot}OH$ has already been pointed out to have an important role in the photodegradation of some fluoroquinolones (Zhang et al., 2019). $^{\cdot}OH$ may come from the reduction of superoxide ($O_2^{\cdot-}$), which is produced from the one-electron reduction of O_2, which is the first step in the formation of ROS (Xiao et al., 2020).

3.4. Influence of the presence of scavengers

The influence of $^{\cdot}OH$ and $^1O_2$ on OXA photodegradation was assessed by using C_3H_8O and NaN_3, respectively. The obtained results for OXA C/C_0 throughout irradiation time are represented in Figure 1d) and the corresponding kinetic parameters are depicted in Table 1.

C_3H_8O is a relatively selective and routinely used $^{\bullet}OH$ scavenger. The mechanism of reaction between these two species is shown in Eq. (2).

$$^{\bullet}OH + (CH_3)_2CHOH \rightarrow H_2O + (CH_3)_2C^{\bullet}OH \qquad (2)$$

$^{\bullet}OH$ has been pointed out to have an important role in the photodegradation of some fluoroquinolones. Surprisingly, and as may be seen in Figure 1d), OXA photodegradation was faster in the presence of C_3H_8O, than in ultrapure water. In agreement, k was higher in the presence of this $^{\bullet}OH$ scavenger (k increased from 0.70 ± 0.02 h^{-1} (in ultrapure water) to 1.32 ± 0.06 h^{-1} (in presence of the scavenger)) (Table 1). The increase in the photodegradation rate indicates that isopropanol increases OXA photodegradation. The presence of isopropanol (20 mmol L^{-1}) alters the properties of the aqueous solvent and it is known that changes in the solvent may induce changes in triplet state (mainly when involving carbonyl groups) affecting the rate of photodegradation (Cowan and Drisko, 1976), in this case, increasing OXA photodegradation rate. Notwithstanding, these results do not allow to make definite conclusions on the role of $^{\bullet}OH$ in OXA photodegradation.

The N_3^- ions are relatively selective quenchers of $^1O_2$ (Eq. 3), thus, if these species are involved in the process inhibition of OXA photodegradation should be observed under the presence of sodium azide.

$$N_3^- + {}^1O_2 \rightarrow N_3^{\bullet} + O_2^{\bullet} \qquad (3)$$

In presence of NaN$_3$, k presented an evident decline from 0.70 ± 0.02 h^{-1} (in ultrapure water) to 0.438 ± 0.007 h^{-1} (Table 1). The pronounced retardation of OXA decay under solar radiation in the presence of this scavenger allows to infer that the self-sensitized photo-oxidation processes of $^1O_2$ generation was involved in the OXA photodegradation. To the best of our knowledge, the participation of $^1O_2$ in the photodegradation of OXA was here assessed for the very first time. Nonetheless,

several authors have already highlighted that $^1O_2$ plays an important role in the photodegradation of other fluoroquinolones (Ge et al., 2010; Geng et al., 2020; Niu et al., 2016).

4. CONCLUSIONS

The main purpose of this work was to understand the OXA photodegradation behaviour in aquaculture water samples and gather information about the influence of water characteristics, such as organic matter and salinity. Results allowed us to conclude that OXA persistence is much higher in environmental water samples than in ultrapure water, especially when present in matrices with high salinity values, such as brackish water. Organic matter also has a negative effect on OXA photodegradation rate, which might partially explain the decrease of the photodegradation rate observed in the freshwater sample. In what concerns the species that may be involved on the photodegradation of OXA in ultrapure water, results suggest that self-sensitized photo-oxidation processes of $^1O_2$ generation were involved in this process.

REFERENCES

Acero, J.L., Real, F.J., Benitez, F.J., Matamoros, E., 2019. Degradation of neonicotinoids by UV irradiation: Kinetics and effect of real water constituents. Sep. Purif. Technol. 211, 218–226.

Bian, X., Zhang, J., 2016. Photodegradation of sulfadiazine in aqueous solution and the affecting factors. J. Chem. 2016, 1–5.

Caracciolo, A.B., Topp, E., Grenni, P., 2015. Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. J. Pharm. Biomed. Anal. 106, 25–36.

Caruso, G., 2016. Antibiotic Resistance in Fish Farming Environments: A Global Concern. J. FisheriesSciences.com 10(4), 9–13.

Chowdhury, R.R., Charpentier, P.A., Ray, M.B., 2011. Photodegradation of 17b-estradiol in aquatic solution under solar irradiation: Kinetics and influencing water parameters. J. Photochem. Photobiol. Chem. 219, 67–75.

Cowan, D.O, Drisko, R.L., 1976. Photochemical Techniques and the Photochemistry of Ketones. In: Elements of Organic Photochemistry, pp. 75-130 (Cowan, D.O., Drisko, R.L., Ed.). New York and London Plenum Press.

Esteves, V.I., Cordeiro, N.M.A., Duarte, A.C., 1995. Variation on the adsorption efficiency of humic substances from estuarine waters using XAD resins. Mar. Chem. 51:61–66.

Esteves, V.I., Otero, M , Duarte A.C., 2009. Comparative characterization of humic substances from the open ocean, estuarine water and fresh water. Org. Geochem. 40, 942–950.

Food and Agriculture Organization of the United Nations (FAO), 2020, http://www.fao.org/home/en/, accessed January 2020.

Ge, L., Chen, J., Wei, X., Zhang, S., Qiao, X., Cai, X., Xie, Q., 2010. Aquatic Photochemistry of Fluoroquinolone Antibiotics: Kinetics, Pathways, and Multivariate Effects of Main Water Constituents. Environ. Sci. Technol. 44, 2400–2405.

Geng, C., Liang, Z., Cui, F., Zhao, Z., Yuan, C., Du, J., Wang, C., 2020. Energy-saving photo-degradation of three fluoroquinolone antibiotics under VUV/UV irradiation: Kinetics, mechanism, and antibacterial activity reduction. Chem. Eng. J. 383, 123145.

Lai, H.-T., Lin, J.-J., 2009. Degradation of oxolinic acid and flumequine in aquaculture pond waters and sediments. Chemosphere 75, 462-468.

Leech, D.M., Snyder, M.T., Wetzel, R.G., 2009. Natural organic matter and sunlight accelerate the degradation of 17b-estradiol in water. Sci. Total Environ. 407, 2087-2092.

Lin, J.-S., Pan, H.-Y., Liu, S.-M., Lai, H.T., 2010. Effects of light and microbial activity on the degradation of two fluoroquinolone antibiotics in pond water and sediment. J. Environ. Sci. Health B 45:5, 456-465.

Niu, X.-Z., Busetti, F., Langsa, M., Croué, J.-P., 2016. Roles of singlet oxygen and dissolved organic matter in self-sensitized photo-oxidation of antibiotic norfloxacin under sunlight irradiation. Water Res. 106, 214–222.

Oliveira, C., Lima, D.L.D., Silva, C.P., Otero, M., Esteves, V.I., 2016. Photodegradation behaviour of estriol: An insight on natural aquatic organic matter influence. Chemosphere 159, 545-551.

Oliveira, C., Lima, D.L.D., Silva, C.P., Calisto, V., Otero, M., Esteves, V.I., 2019. Photodegradation of sulfamethoxazole in environmental samples: The role of pH, organic matter and salinity. Sci. Total Environ. 648, 1403–1410.

Pereira, J.H.O.S, Reis, A.C., Queirós, D., Nunes, O.C., Borges, M.T., Vilar, V.J.P., R.A.R. Boaventura, 2013. Insights into solar TiO2-assisted photocatalytic oxidation of two antibiotics employed in aquatic animal production, oxolinic acid and oxytetracycline. Sci. Total Environ. 463 464, 274-283.

Pouliquen, H., Delépée, R., Larhantec-Verdier, M., Morvan, M.-L., Bris, H.L., 2007. Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionised water, freshwater and seawater under abiotic conditions. Aquaculture 262, 23-28.

Prabhakaran, D., Sukul, P., Lamshöft, M., Maheswari, M.A., Zühlke, S., Spiteller, M., 2009. Photolysis of difloxacin and sarafloxacin in aqueous systems. Chemosphere 77, 739-746.

Rasul, R.G., Majumdar, B.C., 2017. Abuse of Antibiotics in Aquaculture and it's Effects on Human, Aquatic Animal and Environment. Haya: Saudi J. Life Sci. 2, 81-88.

Red Sea, 2019. Retrieved December, 2019, from www.redseafish.com/red-sea-salts/red-sea-salt/.

Rigos, G., Nengas, I., Alexis, M., Troisi, G.M., 2004. Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. Aquat. Toxicol. 69, 281-288.

Romero, J., Feijoó, C.G., Navarrete, P., 2012. Antibiotics in Aquaculture – Use, Abuse and Alternatives, in Health and Environment in Aquaculture, ed. Carvalho, E.D., IntechOpen, 159-198.

Santos, M.E.; Esteves, V.I.; Amado, F.L.; Duarte, A.C.; Humic Substances in the Global Environment and Implications on Human Health, in Senesi N., Miano T. M. (Eds.) Elsevier, Amsterdam, 1994, pp. 877–882.

Silva, C.P., Lima, D.L.D., Otero, M., Esteves, V.I., 2016a. Photosensitized Degradation of 17 β-estradiol and 17 α-ethinylestradiol: Role of Humic Substances Fractions. J. Environ. Qual. 45, 693–700.

Silva, C.P., Lima, D.L.D., Groth, M.B., Otero, M., Esteves, V.I., 2016b. Effect of natural aquatic humic substances on the photodegradation of estrone. Chemosphere 145, 249-255.

Silva, C.P., Oliveira, C., Ribeiro, A., Osório, N., Otero, M., Esteves, V.I., Lima, D.L.D., 2020. Sulfamethoxazole exposure to simulated solar radiation under continuous flow mode: Degradation and antibacterial activity. Chemosphere 238, 124613.

Sturini, M., Speltini, A., Maraschi, F., Pretali, L., Profumo, A., Fasani, E., Albini, A., 2014. Environmental photochemistry of fluoroquinolones in soil and in aqueous soil suspensions under solar light. Environ. Sci. Pollut. Res. 21, 13215–13221.

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Fasani, E., Albini, A., 2010. Photochemical Degradation of Marbofloxacin and Enrofloxacin in Natural Waters. Environ. Sci. Technol. 44, 4564–4569.

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Irastorza, E.A., Fasani, E., Albini, A., 2012. Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight. Appl. Catal. B 119-120, 32–39.

Turel, I.; The interactions of metal ions with quinolone antibacterial agents, 2002. Coord. Chem. Rev. 232, 27–47.

Turiel, E., Bordin, G., Rodríguez, A.R, 2005. Study of the evolution and degradation products of ciprofloxacin and oxolinic acid in river water samples by HPLC-UV/MS/MS-MS. J. Environ. Monit. 7, 189–195.

Vasconcelos, T.G.; Henriques, D.M., König, A., Martins, A.F., Kümmerer, K., 2009. Photo-degradation of the antimicrobial ciprofloxacin at high pH: Identification and biodegradability assessment of the primary by-products. Chemosphere. 76, 487-493.

Wenk, J., Canonica, S., 2012. Phenolic Antioxidants Inhibit the Triplet-Induced Transformation of Anilines and Sulfonamide Antibiotics in Aqueous Solution. Environ. Sci. Technol. 46, 5455-5462.

World Health Organization, 2020, https://www.who.int/, accessed January 2020.

Xiao, Y, Carena, L., Näsi, M.-T., Vähätalo, A.V., 2020. Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron. Water Res. 177, 115782.

Zhao, Q., Fang, Q., Liu, H., Li, Y., Cui, H., Zhang, B., Tian, S., 2019. Halide-specific enhancement of photodegradation for sulfadiazine in estuarine waters: Roles of halogen radicals and main water constituents. Water Res. 160, 209–216.

Zhang, Z., Xie, X., Yu, Z., Cheng, H., 2019. Influence of chemical speciation on photochemical transformation of three fluoroquinolones (FQs) in water: Kinetics, mechanism, and toxicity of photolysis products. Water Res. 148, 19–29.

Zeghioud, H., Kamagate, M., Coulibaly, L.S., Rtimi, S., Assadi, A.A., 2019. Photocatalytic degradation of binary and ternary mixtures of antibiotics: reactive species investigation in pilot scale. Chem. Eng. Res. Des. 144, 300–2309.

CRediT author statement

Vitória L. Louros: Methodology, Formal analysis, Investigation, Data Curation, Visualization

Carla P. Silva: Term, Conceptualization, Methodology, Writing - Review & Editing

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Term	Definition
Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs, implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing - Review &	Preparation, creation and/or presentation of the published

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Project administration	Management and coordination responsibility for the research activity planning and execution
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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of interests

Table 1. Data on pseudo first-order rate constants (k (h^{-1})), determination coefficient (R^2), half-lives ($t_{1/2}$ (h)) obtained for different matrices under simulated solar radiation. (SD is the standard deviation, n=3)

Sample	$C_{0\ OXA}$ ($\mu g\ L^{-1}$)	$k \pm$ SD (h^{-1})	R^2	$t_{1/2} \pm$ SD (h)
Ultrapure water	100	1.24 ± 0.02	0.9995	0.56 ± 0.01
Ultrapure water	250	0.70 ± 0.02	0.9955	0.99 ± 0.04
Freshwater	250	0.42 ± 0.01	0.9976	1.65 ± 0.03
Brackish water	250	0.172 ± 0.003	0.9984	4.03 ± 0.04
HA	250	0.29 ± 0.01	0.9905	2.38 ± 0.07
FA	250	0.32 ± 0.01	0.9957	2.15 ± 0.05
XAD-4	250	0.41 ± 0.0.	0.9947	1.70 ± 0.05
Sea salts	250	0.163 ± 0.005	0.9982	4.25 ± 0.04
NaCl	250	0.93 ± 0.02	0.9978	0.74 ± 0.02
NaN$_3$	250	0.4.8 ± 0.007	0.9991	1.58 ± 0.02
C$_3$H$_8$O	250	1.22 ± 0.06	0.9930	0.53 ± 0.04

Table 2. Pseudo-first order rate constants (k (h^{-1})) determined in the literature for the photodegradation of quinolone antibiotics under natural or simulated solar irradiation.

Antibiotic	Matrix	pH	Conductivity (mS cm^{-1})	k (h^{-1})	$t_{1/2}$ (h)	Reference
CIP				13.2		
DAN				39.6		
ENR	Freshwater	7.7	0.23	14.4		Sturini et al., 2012
LEV				11.4		
MAR				3.66		
MOX				20.4		
DIF	Ultrapure	5.6		0.8		
	HA (10 mg L^{-1})	4.3		0.4		
	FA (10 mg L^{-1})	4.3		0.5		Prabhakaram et al., 2009
SARA	Ultrapure	5.6		0.3		
	HA (10 mg L^{-1})	4.6		0.2		
	FA (10 mg L^{-1})	4.6		0.2		
CIP	Ultrapure	9			0.4	Vasconcelos et al., 2009
		5			0.8	
MAR	Freshwater	8	0.38	5.6		Sturini et al., 2010
ENR				9		
OXA	Eel pond water	8.4	0.91		55.2	
	Shrimp pond water	8.1	18		115.2	Lai et al., 2009
FLU	Eel pond water	8.4	0.91		55.2	
	Shrimp pond water	8.1	18		45.6	

Note: CIP: ciprofloxacin; DAN: danofloxacin; DIF: difloxacin; ENR: enrofloxacin; FLU: flumequine; LEV: levofloxacin; MAR: marbofloxacin; MOX: moxifloxacin; OXO: oxolinic acid; SARA: sarafloxacin.

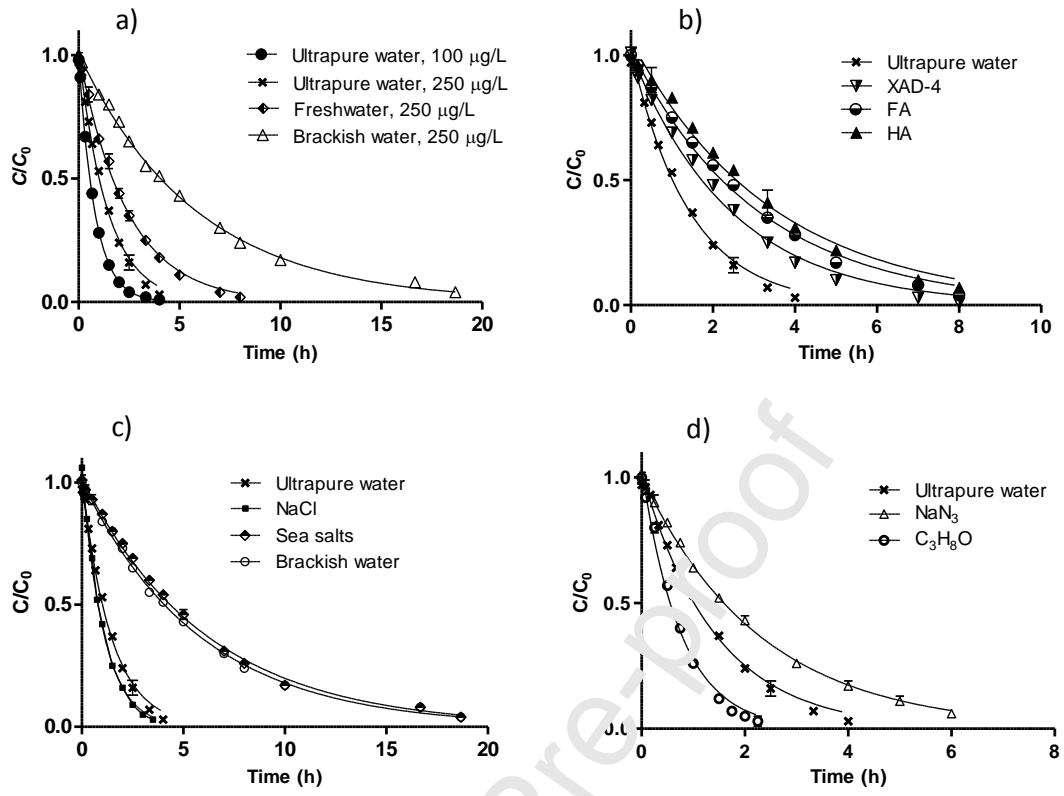**Table 3.** Physico-chemical characteristics of environmental water samples.

Sample	pH	Conductivity (mS cm^{-1})	Salinity (‰)	DOC (mg C L^{-1})
Freshwater	7.3 ± 0.3	0.26	0.00	8.3 ± 0.4
Brackish water	8.6 ± 0.3	50.70	33.20	9.8 ± 0.3

Table 4 - Elemental analysis of HS fractions used.

HS Fraction	Elemental analysis (%)				
	C	H	N	S	O
HA	54.8	4.0	3.6	1.8	32.4
FA	54.5	4.7	1.9	1.0	34.9
XAD-4	48.8	4.2	3.1	1.2	40.5

Table 4 - Elemental analysis of HS fractions used.

Images source: Flaticon

$t_{1/2}$ (ultrapure water) = 0.99 ± 0.04 h
$t_{1/2}$ (brackish water) = 4.03 ± 0.04 h
$t_{1/2}$ (freshwater water) = 1.65 ± 0.03 h
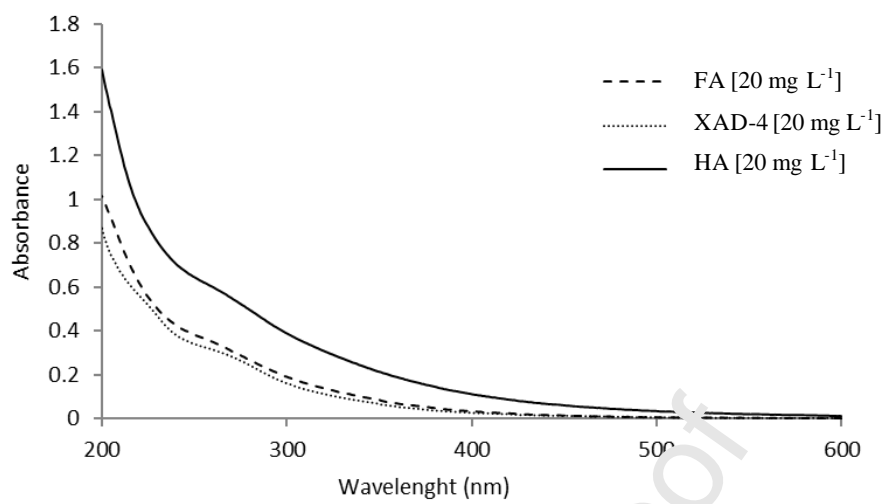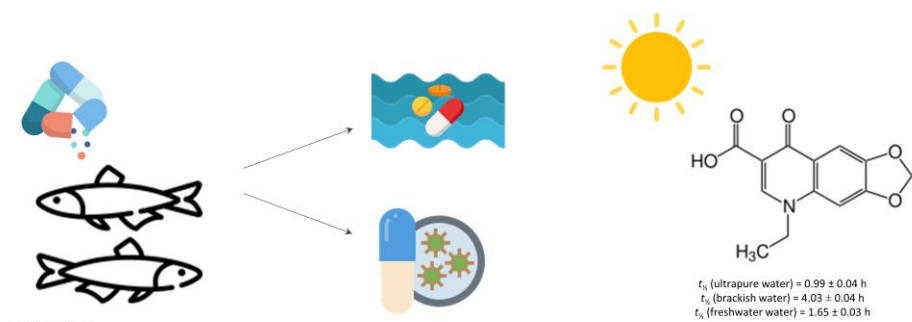
Highlights

- Photodegradation influences antibiotics' persistence in different types of matrices

- $t_{1/2}$ increased from 0.99 h in ultrapure water to 1.65-4.03 h in natural waters

- Dissolved organic matter may explain the photodegradation decrease in freshwater

- Salinity may explain the decrease in photodegradation in brackish water

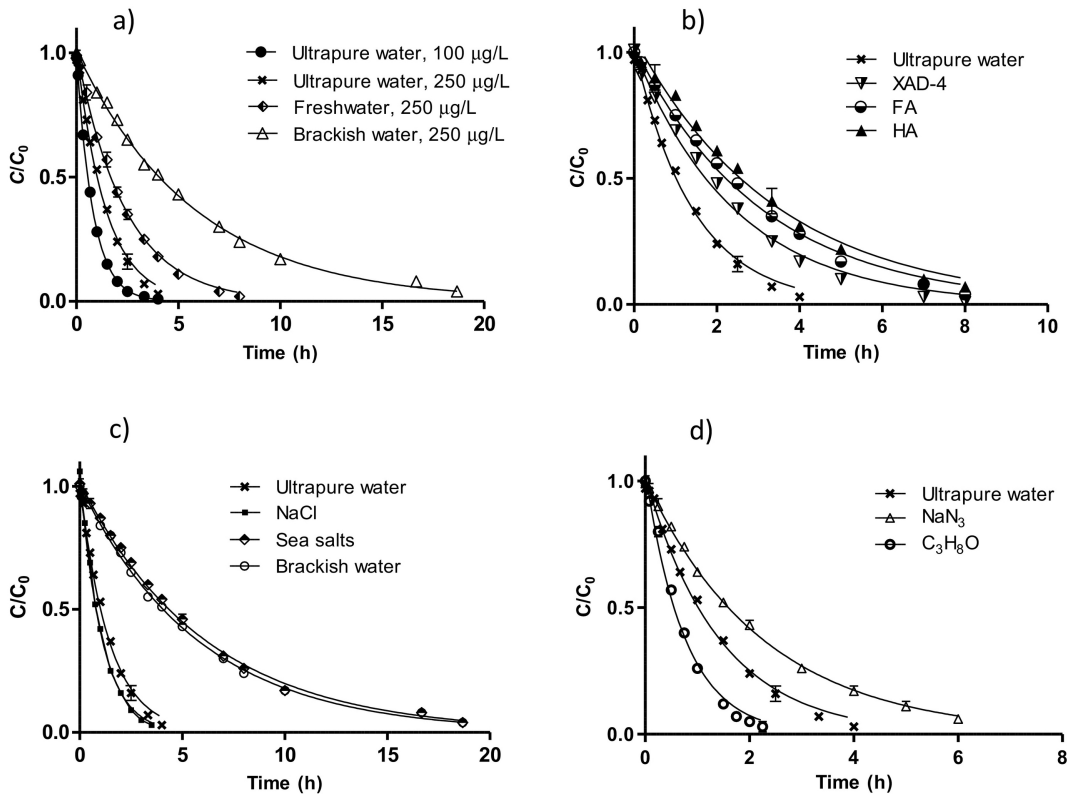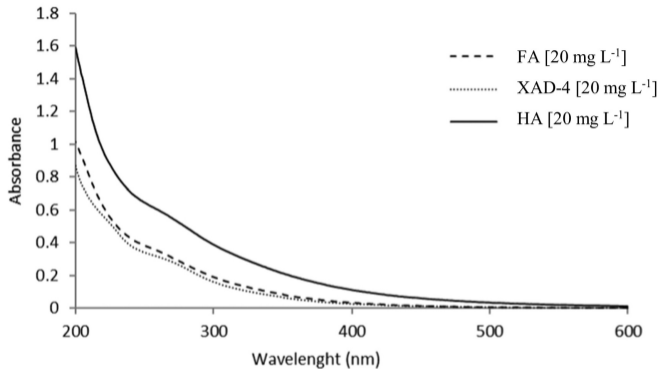- Results suggest that $^1O_2$ plays an important role in OXA photodegradation process

Figure 1

Figure 2