INTRODUCTION:
Occurrence of pharmaceuticals in the aquatic reservoirs represents an important environmental problem due to their continuous discharge and persistence in the aquatic environment for months and years even after their elimination at the sewage treatment plants [1]. Several studies have evidenced that these compounds are difficult to remove from waters in the sewage treatment plant (STP) with conventional purification treatments [2]. Therefore, advanced oxidation processes (AOPs) have been developed as an alternative for the removal of pharmaceuticals and their metabolites from wastewater. Application of these treatments generate reactive oxygen species (ROS) that may reach superficial waters through discharges of effluents from STP. The main aim of this study was to clarify if increased levels of ROS produced after application of pharmaceutical treatments could represent an oxidative challenge to the aquatic organisms.

MATERIAL AND METHODS:

A primary effluent obtained from a STP (Badajoz, Spain) was spiked with nine selected pharmaceuticals (acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol and sulfamethoxazole) so as the resulting wastewater have 200 ng µl⁻¹ of each compound.

For this treatment ozone was produced from dry air in an ozone generator (Ozonil OZva). The duration of chemical treatment was 5 h.

Analytical methods
Pharmaceutical concentration in samples was determined by high performance liquid chromatography using a LaChrom Elite equipment (VWR International, Hitachi, Barcelona, Spain). Detection limit for accurate measurements of concentrations was ~ 2 ng µl⁻¹.

Total organic carbon (TOC) of effluent was determined by a TOC-VCSH Shimadzu Analyzer (VWRInternational).

Chemical oxygen demand (COD) of effluent was measured following the standard dichromate reflux method in a Dr. Lange spectrophotometer [3].

Biological oxygen demand (BOD) of effluent was measured following the respirometric method.

Results and discussion

Effluents characterization before and after pharmaceuticals spiking and after biological and single ozonation treatments is shown in Table 1. The levels of all pharmaceuticals among the effluent without treatment and the effluents with biological, single ozonation and with biological+O3 CPC (dark) were below the detection limits of the HPLC method applied that was ~ 2 ng µl⁻¹.

Table 1. Effluents characterization before and after pharmaceuticals spiking and after biological and single ozonation treatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Total organic carbon, TOC (mg C TV)</th>
<th>Chemical Oxygen Demand, COD (mg O2 TV)</th>
<th>Biological Oxygen Demand, BOD5 (mg O2 TV)</th>
<th>BOD5/COD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>7.4</td>
<td>56</td>
<td>228</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Spiked effluent</td>
<td>7.8</td>
<td>61</td>
<td>237</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Biological treatment</td>
<td>7.4</td>
<td>26</td>
<td>52</td>
<td>30</td>
<td>0.57</td>
</tr>
<tr>
<td>Biological + O3 CPC (dark)</td>
<td>7.9</td>
<td>12.16</td>
<td>14.87</td>
<td>11</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Note: Pharmaceuticals used: acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol and sulfamethoxazole (200 ng µl⁻¹ each one).

Fig. 1. Activity of superoxide dismutase (SOD) in D. magna juveniles exposed to non treated (spiked effluent) and treated effluents from sewage treatment plant. Values are expressed as mean±S.E.M. (number of pools n=4).

Fig. 2. Activity of catalase (CAT) in D. magna juveniles exposed to non treated (spiked effluent) and treated effluents exposed to sewage treatment plant. Values are expressed as mean±S.E.M. (number of pools n=4).

Fig. 3. Levels of lipid peroxidation (LPO) measured as TBARS in D. magna juveniles exposed to non treated (spiked effluent) and treated effluents from sewage treatment plant. Values are expressed as mean±S.E.M. (number of pools n=4).

REFERENCES: