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Use of solar advanced oxidation processes for wastewater treatment: Follow-up on degradation products, acute toxicity, genotoxicity and estrogenicity

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HIGHLIGHTS

- 53 micropollutants detected in wastewater effluent.
- The most used toxicity tests as well as other methods are tested and discussed.
- Genotoxicity and Estrogenic response are complementary to other bioassays.
- Successful follow-up of wastewater treatment processes by solar AOPs.

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ABSTRACT

Wastewater tertiary treatment by advanced oxidation processes is thought to produce a treated effluent with lower toxicity than the initial influent. Here we performed tertiary treatment of a secondary effluent collected from a Waste Water Treatment Plant via homogeneous (solar/HSO_5^-/Fe^{2+}) and heterogeneous (solar/TiO_2) solar advanced oxidation aiming at the assessment of their effectiveness in terms of contaminants' and toxicity abatement in a plain solar reactor. A total of 53 organic contaminants were qualitatively identified by liquid chromatography coupled to high-resolution mass spectrometry after solid phase extraction. Solar advanced oxidation totally or partially removed the major part of contaminants detected within 4.5 h. Standard toxicity tests were performed using *Vibrio fischeri*, *Daphnia magna*, *Pseudokirchneriella subcapitata* and *Brachionus calyciflorus* organisms to evaluate acute and chronic toxicity in the secondary or tertiary effluents, and the EC_{50}% was calculated. Estrogenic and genotoxic tests were carried out in an attempt to obtain an even sharper evaluation of potential hazardous effects due to micropollutants or their degradation by-products in wastewater. Genotoxic effects were not detected in effluent before or after treatment. However, we observed relevant estrogenic activity due to the high sensitivity of the HELN ERα cell line.

1. Introduction

Water scarcity is becoming an increasingly acute issue in many regions of the world. Taking cognizance of the need for this crucial resource, wastewater treatment plants (WWTPs) offer the most promising source of recycled water. Obviously, reusable wastewater should not contain any toxic or xenobiotic substances like pharmaceuticals, pesticides and, especially, endocrine-disrupting compounds (Köck-Schulmeyer et al., 2013). Most of these recalcitrant compounds count among the so-called 'emerging contaminants' (ECs). In view of their widespread presence and potential impact, ECs must be removed from wastewater before discharge or reuse.

Recent studies report that WWTPs fail to remove ECs and other pollutants normally present in the ng–μg L^{-1} range of

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concentration (Lacey et al., 2008; Göbel et al., 2005). Hansen (2007) and Pal et al. (2010) reported the ecological risk that ECs may cause via continuous penetration in the aquatic environment. Major consequences are: feminization of higher organisms, microbiological resistance and accumulation in soil, plants and animals, short-term and long-term toxicity, endocrine-disrupting effects, and antibiotic resistance of microorganisms (Bolong et al., 2009; Fent et al., 2006). The hazard of these micropollutants in the environment does not only depend on their concentrations but also their metabolites or degradation by-products, which can sometimes prove more harmful that the parent compounds (Vulliet et al., 2001; Scrano et al., 2002, 2004). Cocktails of compounds represent an issue that had to be addressed because the toxicity of a mixture cannot be easily determined by summing up individual toxicities of the mixture components. Thus, predicting the impact of a wastewater stream on the ecology of a receiving body hinges on determining the toxicity of the outlet effluent.

There is currently no scientifically recognized definition of toxicity, but a lay of definition would sum up the adverse effects posed by a substance to living organisms (European Union, 2012). Toxicity research focuses on organisms from bacteria to algae, invertebrates, and others. Wastewater contains a cocktail of organic compounds, some of which maybe toxicant. For this reason, biomonitors have been developed and standardized to detect toxicity in water samples, by analyzing usually response patterns in living organisms, such as inhibition of luminescence or inhibition of growth (Calleja et al., 1986). However, these methods are not always sensitive at low concentration of contaminants (Rizzo et al., 2005), making it necessary to accurately plan a selection of assays that may have to be used simultaneously to adequately assess toxicity (Rizzo, 2011).

The EU Water Framework Directive requires a “good chemical and biological status” of all water bodies by 2015 (Water Framework Directive, 2000/60/EC). To achieve this objective, hinges on developing efficient technologies to create effective wastewater treatment protocols is necessary. Advanced oxidation processes (AOPs) are emerging as lead candidates for removing ECs in wastewater as unselective radicals (hydroxyl or sulfate) can mineralize organic matter at high reaction rates. A disadvantage regarding the use of AOPs is the additional cost of producing radicals, i.e. the reagent and/or energy consumption needed to activate the mechanisms. Some AOPs, like photo-Fenton and heterogeneous photocatalysis with TiO_2 can be driven by solar irradiation (Malato et al., 2009; Plantard et al., 2012; Brienza et al., 2014; Quiñones et al., 2015). Several reports suggest that AOPs may produce an effluent with higher toxicity than the initial wastewater influent due to the formation of oxidation intermediate products, which highlights the need to carry out toxicity tests when applying AOPs (Li et al., 2013; Garcia-Käufer et al., 2012).

Here we set out to take a snapshot of the issues currently questionable. No doubt exists that it is necessary to determine what kind of contaminant residues are persisting in wastewater secondary effluents before their discharge, and assess the toxicity of an effluent also in the presence of great dilutions. But, what kind of bioassay might be more effective to do that? By adopting a specific tertiary treatment is it possible to foresee a real abatement of toxicity? Are AOPs the most relevant and efficient treatment technologies for micropollutants’ degradation and toxicity abatement?

To try to answer these questions, we proceeded through several steps: (i) identification of the micropollutants present in the wastewater; (ii) assessment of the toxic potential of wastewater based on different standard assays; (iii) evaluation of the estrogenic and genotoxicity potential of wastewater; (iv) comparison of efficiency of two solar AOPs according to two major criteria: destruction of contaminants and evolution of toxicity.

2. Materials and methods

2.1. Reagents and wastewater

Wastewater (WW) was taken from a WWTP in southern France designed to treat 35,227 $m^3\ day^{-1}$ of inlet flow. Real WW effluent collected downstream of the WWTP secondary biological treatment stage had the following mean characteristics: pH = 7.2 ± 0.2; conductivity = 669 ± 21 μS cm^{-1}; [TOC] = 26.3 ± 0.6 mg L^{-1}; [Cl^-] = 77.9 ± 0.3 mg L^{-1}; [NO_3^-] = 9.9 ± 0.2 mg L^{-1}; [HCO_3^-] = 108.8 ± 7.2 mg L^{-1}; [Ca^{2+}] = 52 ± 4 mg L^{-1}; [Na^+] = 67 ± 3 mg L^{-1}; [K^+] = 15 ± 2 mg L^{-1}. Sampled effluent was used on the same day as it was collected.

All reagents used for chromatographic analyses were LC/MS grade.

Solar heterogeneous photocatalytic experiments were carried out using a slurry suspension (0.7 g L^{-1}) of Evonik P-25 titanium dioxide (surface area 54 $m^2\ g^{-1}$). Solar photo-Fenton experiments were performed using iron sulfate (100 μM of $FeSO_4 \times 7H_2O$), Oxone®(PMS) monopersulfate (200 μM of $HKSO_5 \times 0.5\ HKSO_4 \times 0.5\ K_2SO_4$) and sulfuric acid, obtained from Sigma Aldrich. To avoid the precipitation of iron during solar photo-Fenton processes, pH was adjusted to 2.6 with sulfuric acid.

The free concentration of Fe (II) was determined at the beginning and the end of process by potentiometric micro-titration (Abulkibasha et al., 2013). The loss of Fe (II) free concentration was less than 10%.

2.2. Analytical equipment and methods

Contaminant concentrations were measured by liquid chromatography–electrospray–orbitrap mass spectrometry (Exactive Plus Orbitrap, ThermoScientific) using an ion spray source in positive and in negative mode. Separation was done on a HPLC system (Accelera 1250 pump, ThermoScientific) equipped with a Betabasic C-18 analytical column (150 mm × 2.1 i.d., 3.5 μm particle size) at 0.2 mL min^{-1} flow rate. The mobile phase consisted of a binary mixture of solvent A (0.1% formic acid/water) and B (acetonitrile). The gradient was operated from 10 to 30% A for 10 min, 30–90% A for 5 min, held at 100% for 5 min, then back to initial conditions in 5 min.

A solid phase extraction procedure was applied to the wastewater sample using Oasis HLB LP cartridges (500 mg, 6 mL, Waters). Pre-concentration was performed according to Bueno et al. (2009), and a final concentration factor of 100:1 was obtained.

2.3. Toxicity analyses

Ecotoxicological evaluation was performed on sampled effluent using *Vibrio fischeri*, *Daphnia magna*, *Pseudokirchneriella subcapitata* and *Brachionus calyciflorus* as test organisms to evaluate acute and chronic aquatic toxicity according to standard procedures: (i) bioluminescence inhibition of marine bacterium *V. fischeri* after 30-min exposure (ISO 1134-3:2007); (ii) 48-h immobilization of *D. magna* (ISO 6341:1996); (iii) 72-h growth inhibition of *Pseudokirchneriella subcapitata* green algae (ISO 8692:2012); (iv) 48-h growth inhibition of *B. calyciflorus* rotifers (ISO, 20666:2008). Assays with *V. fischeri* and *D. magna* are considered acute toxicity tests while assays with *P. subcapitata and B. calyciflorus* as are chronic toxicity tests. Samples were classified according to their toxicity using EC_{50} values as established by Calleja et al. (1986). This classification system is based on wider ranges of outcome percentages of effect, considering the concentration where 50% of maximal

effect is observed the effect concentration at 50% (EC50), and consisted of four toxicity classes (1–4): Class 1, when EC50≤<25% (very toxic); as Class 2, when <25% < EC50 < 75% (toxic); as Class 3, when EC50 = 75% (slightly toxic); and Class 4, when EC50 > 75% (non toxic) (Table 1).

Estrogenic activity was assayed using a cell line expressing an estrogen receptor. The stably-transfected cell line was obtained previously as per Escande et al. (2006). Generation of HELN ERα cell line was developed in two steps. First, Hela cells were transfected with GAL4RE-ERE-bGlob-Luc-SVNeo plasmids to generate the HELN cell line. Second, HELN cells were transfected with estrogen receptor alpha (ERα) plasmid to obtain the HELN ERα cell line. Selection was performed using neomycin (or G418) at 1 mg mL^{-1} and puromycin at 0.5 μg mL^{-1}. The HELN ERα cell line was cultured in phenol red-free GIBCO® Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12) supplemented with 5% dextran-coated charcoal fetal calf serum (DCC-FCS) for the strain culture. In the experimental procedure, HELN-ERα cell lines were seeded at a density of 4×10^4 cells/well in 96-well white opaque tissue culture plates for 24 h (Greiner CellStar, D. Dutscher, France) and maintained in 5% DCC-FCS. The cells were exposed to serial dilution of the sampled water to obtain a dose–response curve, and incubated for 16 h. At the end of incubation, luciferase activity was assessed by addition of d-luciferin (0.3 M) and measured on a microtiter plateluminometer (Top Count, Perkin Elmer). Results are expressed as percentage of luciferase activity. For each dilution, estrogenic potency, corresponding to the concentration yielding half-maximum luciferase activity (EC_{50} value), was determined.

The *in vitro* genotoxic assay gamma-H2AX measurement was performed with the LS 174T cell line as described by Khoury et al. (2013). Briefly, LS cell line was dispensed in 96-well cell culture plates (40×10^3 cells, 200 μL/well), and after 24-h incubation with dilutions, the cells were washed with DPBS, fixed with 4% formaldehyde (Electron Microscopy Science), permeabilized with 0.2% Triton X-100, blocked with MAXblock Blocking medium (Active Motif, Belgium), and incubated with an anti-gammaH2AX antibody (Cell Signaling). This antibody was then detected by an infrared fluorescent secondary antibody (CF770, Biotium). In parallel, the DNA was colored by a fluorescent molecular probe (TO-PRO-3 iodide, Molecular Probes). The plate was then read in a scanner (Odyssey Infrared Imaging Scanner, Li-CorScienceTec, LesUlis, France) for infrared fluorescence. The simultaneous detection of both fluorescences made it possible to quantify the molecules of gamma-H2AX as ratio of H2AX gamma to quantity of DNA.

2.4. Solar photoreactor

Solar photocatalytic experiments were carried out in laboratory setup previously described (Brienza et al., 2014). The photoreactor consisted in a module of three independent 30-cm wide, 100-cm high, 2-cm thick flat "panels". Solar panels were oriented southwards and inclined at an angle of about 42° (in order to maximize average solar irradiation). The irradiated volume was 6 L. A 14 L stirred-reservoir was filled with the WW. The solution was continuously re-circulated by a pump (volumetric pump New-Jet 3000) from the bottom to the top of the photoreactor panels and from there to the reservoir with 55 cycles h^{-1} for 248 cycles/experiment. Reactor panels were covered by a special polymethyl methacrylate (PMMA) sheet, which transmits 85% of the incident UV radiation (Janin et al., 2013). Global solar radiation was measured with a pyranometer (Kipp & Zonen CMP3) effective in the wavelength range 310–2800 nm. UV measurements were obtained on Kipp & Zonen UV-A 3C probe set for the range 300 and 400 nm. Temperature was not controlled and varied from 20 to 30 °C.

All experiments were ended after 4 h 30 min of solar irradiation with an average of solar UV power of 70 W/m^2, and samples were collected for analyses.

Table 1
Degrees of effluent toxicity according to Calleja et al. (1986).

EC_{50} (% v/v)	Effluent characterization
<25%[a]	Very toxic
25–75%	Toxic
75%	Slightly toxic
>75%	Non-toxic

[a] % of diluted effluent.

2.4.1. Photolysis

Simple solar photolysis experiments were performed as above using WW without the addition of titanium dioxide.

Solar heterogeneous photocatalysis at initial pH of 7.2: after filling the tank with WW and adding catalyst (TiO_2), the system was homogenized for 15 min in the darkness. TiO_2 concentration was defined as a function of optical pathway: according to reactor design, a TiO_2 concentration of 0.7 g L^{-1} leads to more than 90% passing light absorption by the catalyst and, as a result, to a near-optimal degradation rate (Plantard et al., 2012). At conclusion of the treatment cycles, in order to remove the TiO_2 powder before analysis, the samples were filtered through 0.45 μm PTFE filters.

Solar homogenous photocatalysis by photo-Fenton process at pH 2.6 using PMS as oxidant agent. The pilot reservoir was filled with 14L of WW secondary effluent and pH was adjusted to 2.6 by adding sulfuric acid in order to optimize the photo-Fenton reaction. After 15 min of homogenization, iron salt was added (100 μM as $FeSO_4 \times 7H_2O$) and homogenized for another 15 min in the darkness. Peroxymonosulphate (PMS) was then added in single 200 μM dose as Oxone®, and the process was started. PMS-to-Fe^{2+} ratio was defined in agreement with Mahdi Ahmed and Chiron (2014).

3. Results and discussion

3.1. Contaminants determined in the wastewater

The secondary WWTP effluent (before the solar AOP treatment) was screened for a broad spectrum of pollutants using known retention times, accurate masses, and fragment ions adopting the method developed by Bueno et al. (2009). A total of 53 micropollutants were detected and the main organic contaminants were pharmaceuticals and pesticides (Table 2).

It was not surprising to find non-steroidal anti-inflammatory drugs (NSAIDs) in our sample as they rank among the most commonly used pharmaceuticals worldwide. Several studies have reported the presence of this category of pharmaceuticals in WWTP effluents (Zorita et al., 2009; Jelic et al., 2011). The NSAIDs detected here were *acetanominophen*, *antipyrine*, *indomethacin*, *ketoprofen*, *ketorolac*, *mefenamic acid*, *methylprednisolone*, *propyphenazone*. We also identified the beta-blockers *nadolol*, *metoprolol*, *propranolol* and *sotalol*, which form another major class of prescription drugs. Maurer et al. (2007) stated that the elimination of these pharmaceuticals depends on the hydraulic retention time in WWTPs. It is our opinion that both inadequacy of bacteria load and excess of dilution together with reduced contact time in the biological-treatment basin could be a rationale behind of the unsuccessful removing of recalcitrant substances in WWTPs. One of the most typical pharmaceuticals studied and detected in the environment is *carbamazepine*, an anticonvulsant lipid, which was also present in our sample. The micropollutants identified included also antibiotics, which are the most frequently prescribed drugs to humans

Table 2
List of emerging contaminants detected in wastewater effluent samples.

Pharmaceuticals	
➢ **Nonsteroidalanti-inflammatory drugs** Acetaminophen, Indomethacin, Ketoprofen, Ketorolac, Mefenamic acid, Methylprednisolone, Propyphenazone	➢ **Antihistamines** Loratadine
➢ **Antidepressants** Amitriptylline, Citalopram, Floxetine, Paroxetine, Venlafaxine	➢ **Anesthetics** Mepivacaine
➢ **Antibiotics** Amoxicilina, Enrofloxacin, Oxolinic acid	➢ **Antihypertensives** Metoprolol, Nadolol, Propanolol, Sotalol
➢ **Central nervous system stimulants** Cafeine, Paraxanthine	➢ **Antiprotozoals** Metronidazole
➢ **Anticonvulsant lipid regulators** Carbamazepine, Primidone, Fenofibric acid	➢ **Ant infective agents** Norfloxacin, Ofloxacin, Sulfamethazine, Sulfamethoxazole, Sulfapyridine,Trimetropim
➢ **Antimicrobials** Clotrimazole	➢ **Anti-ulcer drugs** Ranitidine
➢ **Chemotherapy drugs (alkylating agents)** Cyclophosphamide, Ifosfamide	➢ **Bronchodilators** Salbutamol, Terbutaline
➢ **Estrogen antagonists** Tamoxifen	
Pesticides	
➢ **Herbicides** Atrazine, Isoproturon, Simazine, Terbutryn	➢ **Algaecides** Irgarol
➢ **Antimicrobials** 2-(Thiocyanomethylthio) benzothiazole (TCMTB)	
Others	
Cotinine Leucomalachite green 4-AA 4-AAA	Malachite green Nicotine Theobromine

and animals in modern medicine. A high percentage of antibiotics (up to 90%) get excreted without undergoing metabolism in the hosting body. This occurrence and the high consumption of antibiotics in the world may explain their widespread presence in the environment (Huang et al., 2001). In this pharmaceutical category, we detected *sulfamethoxazole* which is often used in combination with *trimethoprim* that was also identified in our sample.

WW effluent was collected from southern France in a major farming area (predominantly olive and grape crops), so we expected to find pesticides in our sample. The pesticides detected were: *isoproturon*, *atrazine* (now forbidden), *simazine*, *terbutryn*, *2-(thiocyanomethylthio) benzothiazole*, and *irgarol*. Excluding *2-(thiocyanomethylthio) benzothiazole* and *irgarol*, these are the most common pesticides used in olive farming in Mediterranean countries and their residues sometimes find their way into olive oil (Hernando et al., 2007). It is therefore important to remove them and evaluate their toxicity and the consequences of their uptake.

Contaminants formed from dipyrone metabolites such as *4-aminoantipyrine* (4-AA) and *4-acetylaminoantipyrine* (4-AAA) were detected.

3.2. Wastewater "standard" toxicity results

The WW sample was assessed for acute and chronic toxicity by direct-contact tests using four species: *V. fischeri*, *D. magna*, *P. subcapitata* and *B. calyciflorus*. Based on the toxicity evaluation criteria reported in Table 1, the WW effluent was categorized as non-toxic. A partial toxic effect from all the tested organisms was observed for *V. fischeri*, with an EC_{50} value of 80% (Table 3) *versus* 90% for *D. magna* and *B. calyciflorus* and 98% for *P. subcapitata*. These findings seem to reassuring that the selected WWTP can release a non-toxic effluent despite of the large number of micropollutants we determined in the ng–μg L^{-1} concentration range.

To better ascertain the validity of the ecotoxicological tests used and their response, we repeated the tests on an artificially-contaminated WW. Secondary biological treatment effluent sampled from the WWTP was spiked with a known concentration (1 mg L^{-1} each) of 5 micropollutants such as mepanipyrim, sulcotrione, 2-methyl-4-chlorophenoxyacetic acid (MCPA), ibuprofen and diclofenac. They were not present in raw wastewater effluent but they were selected as pesticides and pharmaceuticals representative of the most common organic products widely found in WW. With the exception of *V. fischeri*, for which EC_{50} did not change with the additional pollutants, all the other toxicity responses increased considerably (Table 3). Given that the standard *V. fischeri* test (only 30 min of contact time) is ill-adapted for assessing low/mid-range concentrations of contaminants, the highest toxic effect observed in artificially-contaminated WW sample was for *P. subcapitata*, with an estimated EC_{50} value of 8.6% *versus* 12.1% for *B. calyciflorus* and 21.9% for *D. magna*. These results do not pose a question about the suitability of standard toxicity measurements, but indicate that the limit of concentration able to be explored using these standard bioassays should be fairly established and, consequently, different specific tests should be adopted to refine the identification of hazardous effects linked to the presence of micropollutants in WW.

Table 3
Wastewater toxicity according to different tests.

Organism	EC_{50} (% v/v) raw WW[a]	EC_{50} (% v/v) spiked WW[a]
V. fischeri	80%	80%
D. magna	90%	21.9%
B. calyciflorus	90%	12.1%
P. subcapitata	98%	8.6%

[a] WW: wastewater.

3.3. Wastewater composition and toxicity after treatment

As already detailed in Section 3.1, the emerging contaminants

are listed in Table 2.

The aims of this work were to observe the efficiency of solar photocatalysis processes as tertiary WW treatment for removing emerging contaminants and evaluate toxicity before and after the treatment. For this purpose, it was not necessary to quantify all emerging contaminants identified, and the efficiency of used treatment was reported as percent reduction of averaged area peaks for each class of micropollutants respect to the raw effluent (Fig. 1); moreover, the efficiency of the solar AOPs was reported as percentage reduction of each individual micropollutant (Figs. 2 and 3) in an attempt to have an accurate and detailed (even if complex) analysis; finally, toxicity was evaluated before and after the treatment based on information observed using specific genotoxicity and estrogenic tests.

Simple solar photolysis was unable to effectively remove micropollutants. The variation of peak areas of all the pollutants detected in the raw secondary effluent after the photolysis process was negligible (data not shown).

Heterogeneous photocatalysis with TiO_2 led to complete (or undetectable presence) or partial degradation of a large number of pharmaceuticals and pesticides belonging to different classes. As shown in Fig. 1, antibiotics and antimicrobial pesticides were no longer detectable with a percentage of reduction close to 100. Degradation of antidepressants, anticonvulsant lipid regulators, estrogen antagonists, antihypertensive, antiprotozoals and herbicides was more than 70%, while abatement of chemotherapy antagonists and anti-infective agents was between 50 and 70% (on the basis of average peak areas). This process was inefficient in removing recalcitrant compounds like nonsteroidal anti-inflammatory drugs, nervous stimulant, bronchodilator agents, algaecide and others such as malachite green and theobromine as shown in Fig. 2.

In the case of solar photo-Fenton, after 4.5 h of illumination time, the presence of antimicrobials drugs was no longer detected. The process leading also to the disappearance of other micropollutants (>70% decrease in average of peak areas) such as antidepressants, chemotherapy drugs, estrogen antagonists, antiprotozoals and other (Fig. 3). Degradation of nonsteroidal anti-infiammatory drugs, antibiotics, anticonvulsant lipid regulators, antihypertensives, anti-ulcer agents was in the range 50–70%.

As for TiO_2, solar photo-Fenton was unable to effectively mineralize ECs such as: nervous stimulants, anesthetics, bronchodilator agents, algaecides and others like malachite green and theobromine as shown in Fig. 3.

The environmental presence of endocrine-disrupting substances has fast become a burning environmental issue, making it vital to evaluate the presence of estrogenic activity in water bodies.

In this work, the estrogenic activity of endocrine-disrupting substances was measured with cell lines expressing estrogen receptor alpha (ERα). In *vitro* assays using reporter gene activation in stably-transfected cell lines provide robust, sensitive and specific bioassays for screening endocrine activity, like estrogenic activity. Estrogenic power, i.e. the concentration yielding half-maximum luciferase activity (EC_{50}), was determined, and our estrogenic assay showed relevant levels of estrogenic activity present in our sample.

Among the micropollutants detected, there was *tamoxifen*. Berry et al. (1990) and Parker et al. (1993) found that *tamoxifen* is able to inhibit the action of estradiol and at the same time exhibits a slight estrogenic activity. We assayed *tamoxifen* using the method of Escande et al. (2006) and demonstrated beyond any doubt that this pharmaceutical compound is a highly effective inhibitor of estradiol, as the cell line developed through this method featured specific estrogen receptors not affected by the potential estrogenic activity of *tamoxifen.*

The high sensitivity of the method adopted here made it

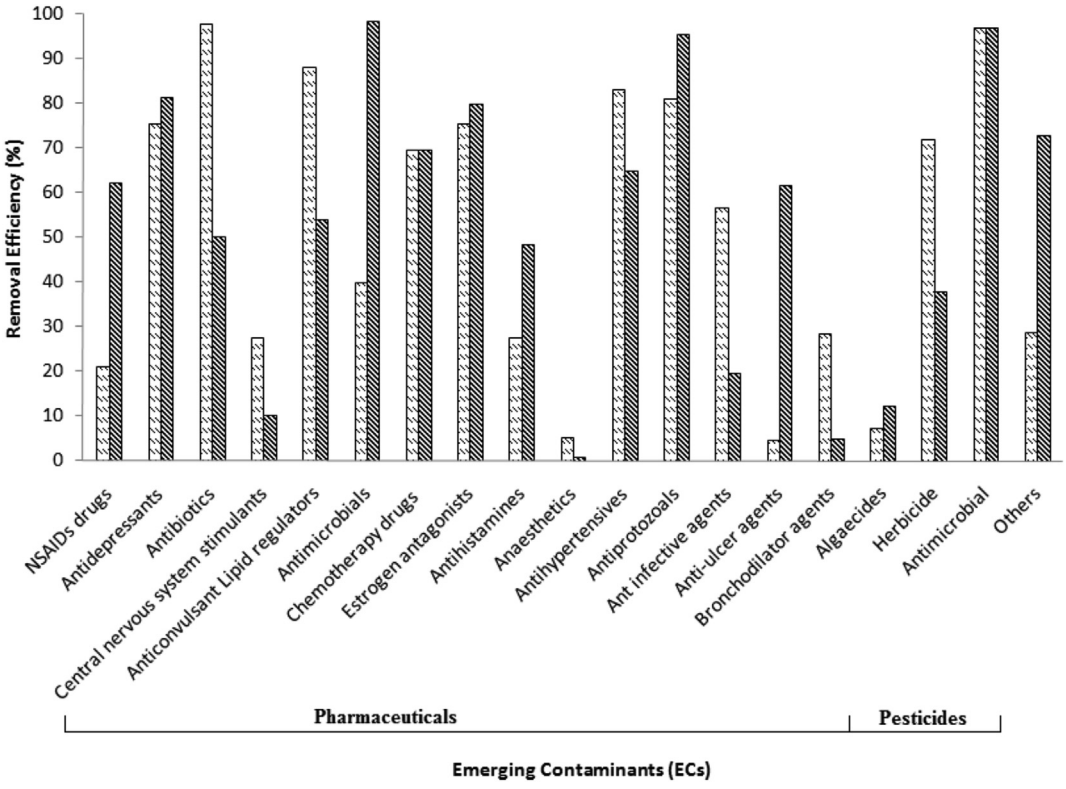

Fig. 1. Emerging contaminants removed by solar advanced oxidation processes: (▨) TiO_2/solar light and (▧) PMS/Fe(II)/solar light.

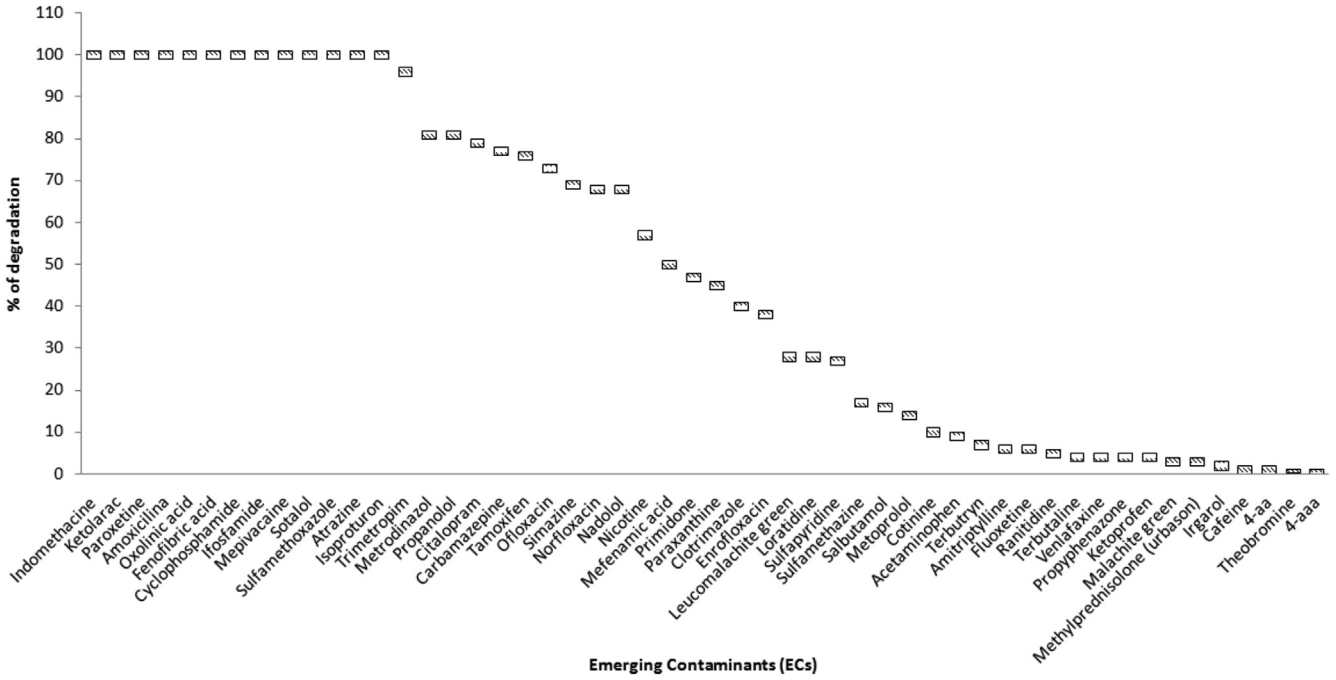

Fig. 2. Percentage degradation of emerging contaminants (ECs) via solar heterogonous photocatalysis using suspended TiO_2.

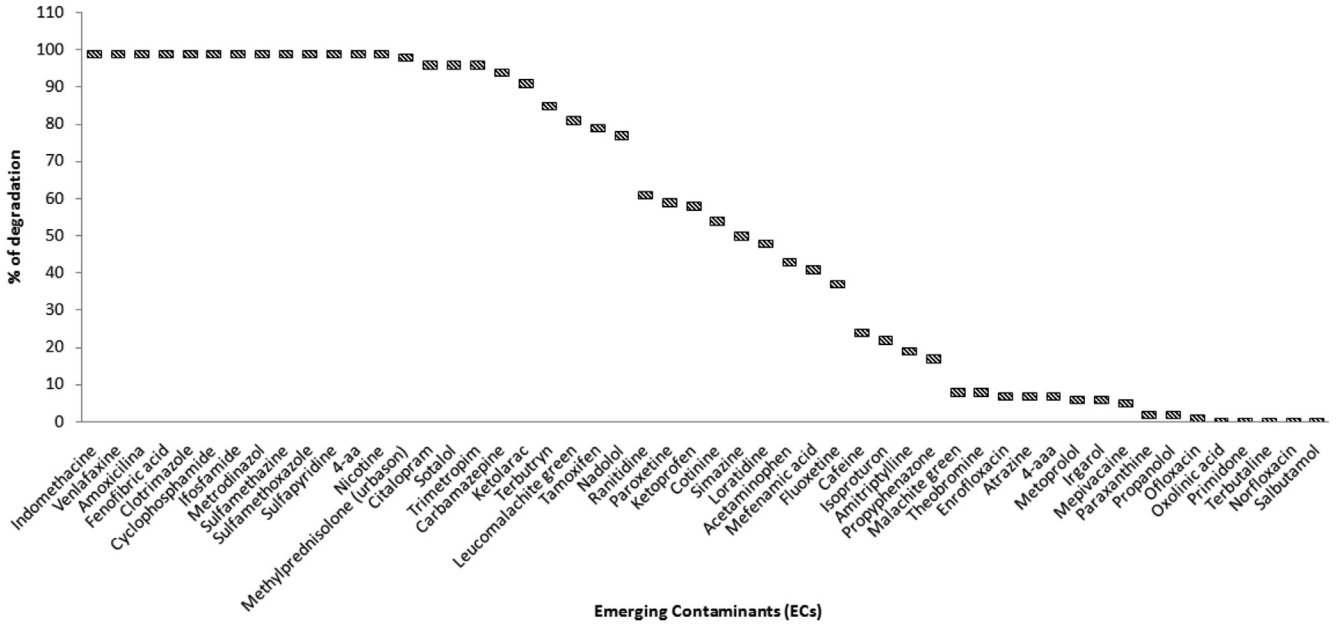

Fig. 3. Percentage degradation of emerging contaminants (ECs) via solar homogeneous photocatalysis using PMS as oxidant agent.

possible to successfully detect estrogenic activity even when chemical analysis was unable to ascertain the presence of any known estrogenic compound, i.e. when contaminants were under the limit of detection of the analytical method implemented (Fig. 4). The estrogen screen detects chemical compounds that bind to, and activate, the estrogen receptor in raw wastewater effluent. For this reason, it was used as a relevant test for the evaluation of the estrogenic activity in our WW before and after applying AOP treatments, i.e. solar heterogeneous and solar homogeneous photocatalysis.

Results reported (Fig. 4) the inefficiency of photolysis. In fact, it was even confirmed by the estrogenic test, as estrogenic activity remained constant after the solar photolysis. Profile curve of estrogen activity is the same detected in raw wastewater. Solar heterogeneous and homogeneous photocatalysis applied had also a positive effect on effluent toxicity by reducing estrogenic activity levels. As shown in Fig. 4, the percentage of luciferase activity decreased and the effluent could be considered as environmentally friendly.

The genotoxicity test, based on the quantification of the phosphotylation of the histon H2AX that reflects global genotoxicity insult, was carried out in our raw wastewater, but no genotoxic

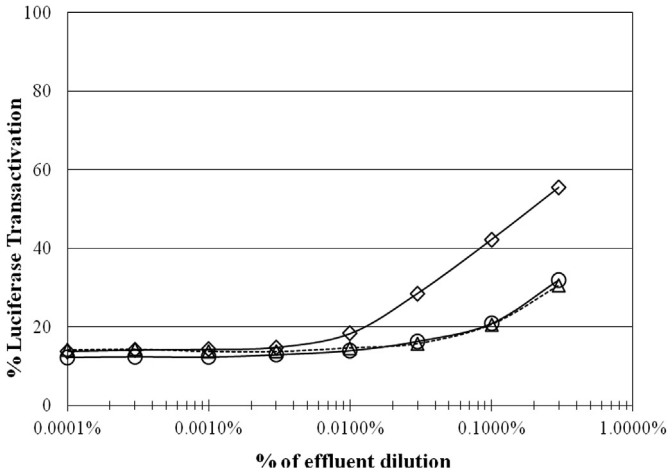

Fig. 4. Estrogenic activity profile determined in effluent after 4.5 h application of different solar advanced oxidation treatments: (-◊-) raw water same as photolysis; (-o-) PMS/Fe(II)/solar light; (···Δ···) TiO_2/solar light.

effect was observed same as after the applied treatment (data not shown).

4. Conclusion

Much of the research led available in literature has been carried out with contaminated wastewater at known concentrations to study photodegradation behavior (Klamert et al., 2009; Miranda-García et al., 2011), and little attention has been paid to advanced oxidation processes applied directly on WW samples. In fact, in several works the wastewater effluents were pre-treated before applying solar advanced oxidation processes as a tertiary treatment. Pre-treatment step was done using concentrated H_2SO_4 to remove carbonates and the effluents were considered ready for AOPs treatment when the total inorganic carbon (TIC) was below 2 mg L^{-1}(Pietro-Rodríguez et al., 2012a, 2012b; Klamert et al., 2013). The originality of this work is that the secondary wastewater effluent was treated using solar advanced oxidation process without any pre-treatment, expect for solar photo-Fenton that was developed at pH 3 using adequate quantity of sulfuric acid. We would like to consider this work as a simulation of a really feasible tertiary treatment of WW.

Based on the results obtained, both the solar advanced oxidation processes experimented exhibited capacity as technologies for decreasing effluent toxicity. This success was also confirmed by the high number of micropollutants degraded as well as the decrease in estrogenic activity.

The main findings of our research are:

- ❖ Advanced analytical methods such as LC-Qq LIT-MS detected the presence of 53 micropollutants, including pharmaceuticals, pesticides and other organics.
- ❖ Ecotoxicity bioassay results showed that wastewater effluents are not toxic or slightly toxic according to the applied organisms.
- ❖ *Pseudokirchneriella subcapitata* was the most sensitive model organism for detecting toxicity at relatively high concentrations of contaminants.
- ❖ The estrogenic assay detected environmentally relevant levels of estrogenic activity due to the presence of estrogenic compounds, even if at very low concentrations, and even when chemical analysis was unable to detect the estrogen.
- ❖ Solar advanced oxidation processes showed that emerging contaminants at low concentrations can be successfully degraded and estrogenic activity decreased
- ❖ No genotoxicity was observed in any of the samples

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