Elimination of drugs of abuse and their toxicity from natural waters by photo-Fenton treatment

10 AUTHORS, INCLUDING:

Myriam Catalá
Instituto de Salud Carlos III
46 PUBLICATIONS **576** CITATIONS

SEE PROFILE

Raul Molina
King Juan Carlos University
39 PUBLICATIONS **933** CITATIONS

SEE PROFILE

Fernando Martinez Castillejo
King Juan Carlos University
56 PUBLICATIONS **1,261** CITATIONS

SEE PROFILE

Yolanda Valcarcel
King Juan Carlos University
58 PUBLICATIONS **500** CITATIONS

SEE PROFILE

Elimination of drugs of abuse and their toxicity from natural waters by photo-Fenton treatment

CrossMark

M. Catalá [a], N. Domínguez-Morueco [a], A. Migens [a], R. Molina [b], F. Martínez [b], Y. Valcárcel [c], N. Mastroianni [d], M. López de Alda [d], D. Barceló [d,e], Y. Segura [b,*]

[a] Department of Biology and Geology, Physics and Inorganic Chemistry, School of Experimental Sciences and Technology, Rey Juan Carlos University, C/ Tulipán s/n, E-28933 Móstoles, Madrid, Spain
[b] Department of Chemical and Environmental Technology, School of Experimental Sciences and Technology, Rey Juan Carlos University, C/ Tulipán s/n, E-28933 Móstoles, Madrid, Spain
[c] Research Group in Environmental Health and Ecotoxicology (ToxAmb), Department of Medicine and Surgery, Psychology, Preventive Medicine, Public Health, Immunology and Medical Microbiology, Faculty of Health Sciences, Rey Juan Carlos University, Avda. Atenas, s/n, E-28922 Alcorcón, Madrid, Spain
[d] Department of Environmental Chemistry, Institute for Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona 18–26, E-08034 Barcelona, Spain
[e] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

HIGHLIGHTS

- Photo-Fenton reactions eliminate drugs of abuse in natural fluvial water.
- High chemical degradation does not ensure toxicity elimination.
- Catalyst loading is critical for an efficient toxicity elimination.
- Toxicology testing is mandatory in decontamination assessment.

ARTICLE INFO

ABSTRACT

This paper investigates the elimination of drugs of abuse from six different chemical classes and their metabolites in natural fluvial waters (nearby the output of a sewage system). Mineralization of these substances and toxicological characterization before and after treatment by a heterogeneous photo-Fenton system has been evaluated. This advanced oxidation technology was able to significantly reduce the concentration of the drugs of abuse in all the tested conditions (different hydrogen peroxide and catalyst loadings). However, toxicological analyses measured as inhibition of fern spore mitochondrial activity, showed only a complete elimination of acute and chronic toxicity when a higher solid catalyst loading was used (0.6 g/L). A lower catalyst loading of 0.2 g/L was not enough for toxicity elimination. These results evidence the need for combining toxicological tests and chemical analyses in order to establish the effectiveness of the water treatment technologies based on advanced oxidation processes.

© 2015 Elsevier B.V. All rights reserved.

---

1. Introduction

The presence of emerging microcontaminants, in particular drugs of abuse (DAs) (and/or their metabolites) in the water cycle as a consequence of their widespread consumption and poor elimination in the water treatment plants is an issue of important concern. As a result of their continuous introduction into the aquatic environment, these substances are behaving in a pseudo-persistent manner and currently represent a new class of environmental emerging pollutants that require emergency attention. In many cases, these substances are persistent, bioaccumulative and potentially toxic to aquatic organisms

(Boles and Wells, 2010). According to the last Spanish survey on alcohol and drugs (EDADES, 2012), a general decrease or stabilization of drug consumption has been observed with one exception, namely the family of sedatives, including benzodiazepines, consumed last year by 11.4% of the population. Among illicit drugs, cannabis continues to be the most consumed substance, followed by cocaine.

The presence of these substances and/or their metabolites in the sewage system following consumption, and thereafter in the receiving aquatic environment, is not surprising. To this end, their illegal condition and/or abused use comes to aggravate the problem by preventing their adequate disposal and the control of residues. This fact, as well as the recent development of advanced analytical methods for their determination in water, has led to their detection in surface water and even in tap waters, which is a significant concern for public health authorities. The occurrence of DAs and benzodiazepines has been reported in different European surface waters (Zuccato et al., 2008; van Nuijs et al., 2009, Kasprzyk-Hordern et al., 2008; Terzic et al., 2010). In Spain, the occurrence of DAs and benzodiazepines in surface waters has been evaluated in several zones (Postigo et al., 2010; Gros et al., 2010; Valcárcel et al., 2012; Mendoza et al., 2014a, 2014b). As for pharmaceutical residues, the main origin of these substances is the sewage treatment plant (STP) effluents due to their inefficient elimination.

Although there are very few studies on the occurrence of DAs in drinking waters (see Mendoza et al., 2014a, 2014b and references therein), some substances such as cocaine, benzoylecgonine, epinephrine, the group of benzodiazepines, methadone and 2-ethylene 1,5-dimethyl 3,3-diphenylpyrrolidine (EDDP) have been repeatedly found in low quantities such as 13–76 ng/L for cocaine or 50–150 ng/L for the group of benzodiazepines. Both the contamination of the sources of drinking water and the inefficacy of water treatment systems are pointed out as the main cause of this contamination. Albeit a human health risk is not expected from the concentrations detected, the lack of toxicological data regarding chronic exposure and mixture toxicity recommend the total elimination of any kind of micropollutant from drinking water (Mendoza et al., 2014a, 2014b). Currently, the potential effects of these components on the environment, at medium and long terms, are also unknown and their strong biological effects must not be overlooked. These biologically active substances are designed to exert specific effects on cells, and therefore can have numerous negative effects on ecosystems. Due to the potential ecotoxicological risk of the drugs of abuse for ecosystems, they need to be removed from water, especially if it is intended for human consumption, and a solution to this problem might be the application of non-biological procedures, such as advanced oxidation processes (AOPs). The effectiveness of these techniques is based on the generation of hydroxyl radicals, which act as a powerful oxidizing agent with a high reactivity and low selectivity for the removal of organic compounds. Among AOPs, ozonation (Rodayan et al., 2014) and photo-oxidation processes such as photocatalysis with TiO_2 (Postigo et al., 2011a) and homogenous photo-Fenton with iron salts and hydrogen peroxide (Postigo et al., 2011a, 2011b) have been proposed as treatments for a variety of drugs (methadone, cocaine, benzoylecgonine, ketamine and oxycodone, among others). These processes, specially ozonation and homogeneous photo-Fenton showed a remarkable effect on drug degradation in simulated wastewaters or ultrapure water solutions at relatively high concentrations (100 μg/L–10 mg/L). On the other hand, the application of heterogeneous photo-Fenton treatment has been recently proposed as a remarkable effective technology for the degradation of drugs of abuse detected at low concentration (Valcárcel et al., 2012).

The quality of treated water requires the application of not only chemical but also biological methods to detect contaminants as well as to assess the possible effects on the environment and public health. Biological assays are presented as innovative and rapid tests and indicators of toxic effects of water contamination (Farré et al., 2007). The small number of taxa used in bioassays is currently one of the main concerns of environmental toxicology, since the use of an appropriate range of taxons allows ensuring ecologically relevant results. Some years ago, a novel microbioassay of phytotoxicity with fern spores has been published (Catalá et al., 2009). This method combines biological and ecological relevance with high sensitivity and simplicity, making it a low cost tool for ecotoxicological monitoring (Catalá et al., 2010). It has been successfully used in the assessment of the toxicity of polycyclic aromatic compounds, industrial pollutants (Marugán et al., 2012), environmental concentrations of pharmaceutical compounds (Feito et al., 2012), environmental water samples (Rodríguez-Gil et al., 2010; Esteban et al., 2012) as well as in the follow-up of water treatment technologies (Rodríguez-Gil et al., 2010; Marugán et al., 2012). Due to the use of selected robust biomarkers, this method has been demonstrated to improve sensitivity of validated methods to environmental micropollution (Esteban et al., 2012).

In this context, the main objective of this study was to evaluate the influence of a mesoporous Fe-based catalyst and hydrogen peroxide concentrations on a heterogeneous photo-Fenton system for the elimination of illicit drugs in complex natural fluvial waters. The assessment of the efficacy was performed by chemical analysis and also by acute and sub-chronic toxicity bioassays, as combined techniques to evaluate the removal of the DAs and by-products after the treatments.

2. Materials and methods

2.1. Environmental water sampling

The Madrid region, with 809 inhabitants per km^2, is the most densely populated region of Spain and one of the most densely populated in Europe. Its area, 8 028 km^2 (1.6% of the Spanish territory), is occupied by an estimated population of 6,495,551 inhabitants. A total of 86.94% of the population is concentrated in a conurbation composed of the city of Madrid (3,207,247 inhabitants) (49.9%) and the municipalities in the metropolitan area (2,370,678 inhabitants) (36.9%) (INE base, 2012). A surface water sample was collected in March 2012 from the Manzanares River, approximately 100 m downstream from the point of emission of Butarque STP due to its characteristics for drug presence observed in previous studies (Mendoza et al., 2014a, 2014b. This STP serves a population equivalent to 1,440,000, and treats 432,000 m^3/day with biological activated sludge. The samples were collected in 1 L of amber polyethylene terephthalate (PET) bottles, and the pH, conductivity and temperature were measured immediately after collection. Once collected and during shipment samples were kept frozen at −20 °C.

2.2. Photo-Fenton treatment

The experimental set-up for the photo-Fenton reactor was a batch-type reactor consisting of a vessel of 12 cm of diameter and 1 L in volume with the irradiation lamp axially immersed. The UV–Visible irradiation was provided by a 150 W medium pressure mercury lamp (Heraeus TQ-150). The lamp was surrounded by a quartz jacket in which a copper sulphate aqueous solution circulates to block radiation at wavelengths shorter than 313 nm. In a typical run, the vessel was filled with 1 L of the water sample. A powder silica-supported iron oxide (Fe_2O_3/SBA-15) was used as the heterogeneous photo-Fenton-like catalyst with an easy separation after the treatment. This catalyst was prepared following a method described elsewhere (Martínez et al., 2005). The Fe_2O_3/SBA-15 catalyst was characterized by containing crystalline iron oxides, in the form of hematite, supported on a meso-structured SBA-15 silica support. The iron catalyst was suspended into the aqueous solution (0.1 or 0.6 g/L) and the pH was initially adjusted to ca. 3 with H_2SO_4 (0.1 M) and left uncontrolled during the treatment. The initial hydrogen peroxide concentration used for the treatments was calculated according to the theoretical stoichiometric amount for the complete mineralization of the total organic carbon (TOC) of the water towards CO_2 and H_2O following reaction (1), one half and one quarter of that concentration (coded as 100%, 50% and 25%, respectively,

equivalent to ca. 1, 0.5 and 0.25 mL of H_2O_2 per litre of wastewater sample treated)

$$C(TOC) + 2H_2O_2 \rightarrow CO_2 + 2H_2O. \qquad (1)$$

The temperature of the water samples in the cylindrical vessel was set at ca. 22 °C. The solution was stirred at 500 rpm to ensure the complete dispersion of the catalyst within the reaction medium. The duration of the treatments was long-stand up to 6 h until the hydrogen peroxide was totally consumed.

After 6 h, the samples were filtered through 0.22 μm nylon membrane filters to remove the heterogeneous catalyst prior to be analysed using macroscopic parameters such as TOC (total organic carbon) and pH. The total hydrogen peroxide consumption was confirmed using an iodometric titration method. The iron leached from the solid catalyst was measured by ICP-AES analysis in the filtered solution using a Varian Vista AX spectrometer. One aliquot of the treated samples was submitted to quantify the concentration of drugs of abuse (DAs). Another aliquot was frozen for further toxicity testing after pH neutralization with KOH (pH 7.0 ± 0.5).

The average efficiency of elimination of the drugs was calculated as a percentage referring the remaining concentration of a given compound after the treatment to its concentration in raw river water. When the substance was not detected in the treated water, half of the limit of determination (LODet) was used as putative remaining concentration.

2.3. Drug analysis

Upon reception in the laboratory for analysis, samples were fortified with a solution containing deuterium labelled analogues of the studied compounds at the following concentration: 50 ng/L for the compounds CO-d3, BE-d8, MOR-d3, the benzodiazepines DIAZ-d5, LOR-d4 and ALP-d5, METH-d3, THC-d3, OH-THC-d3 and THC-COOH-d3, and 20 ng/L for the remaining compounds-EPH-HCl-d3, AM-d5, MA-d14, MDMA-d5, CE-d3, LSD-d3, HER-d9, 6ACM-d6, and EDDP-d3. For the LSD metabolite 2-oxo-LSD deuterium labelled analogue compounds were not commercially available and for this reason LSD-d3 was used as surrogate standard for its quantification. The surface water sample was then vacuum filtered through 1 μm glass fibre filters, followed by 0.45 μm nylon membrane filters (Whatman International Ltd, Maidstone, England), and all samples were subsequently stored in amber PET bottles in the dark at −20 °C until analysis.

The analysis of the selected compounds in the collected samples was performed following a previously described fully automated method based on on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE-LC–MS/MS) (Valcárcel et al., 2012). The isotope dilution method based on the peak area obtained for each analyte and its corresponding deuterated analogue was used for quantification. Calibration curves obtained for the analytes were linear between 0.1 ng/L (or the limit of quantification if higher) and 1000 ng/L, with correlation coefficients (r^2) higher than 0.99 in all cases. The method limits of detection (LODs) and determination (LODet) were experimentally estimated from the on-line SPE-LC–MS/MS analysis of water samples as the concentration of analyte giving a signal-to-noise ratio of 3 and 8, respectively. All samples were analysed in triplicate and a blank was analysed every 2 real samples in order to check for possible system carryover. Any target illicit drug or metabolite in any analysed sample was positively identified if the retention time of the analyte in the sample was within ± 2% of the retention time of the analyte in the aqueous standard mixture, and the relative abundance of the two selected SRM transitions for each analyte in the sample was within ± 20–50% of the ratio produced by the commercial standard.

2.4. Biotoxicity assays

2.4.1. Plant material for toxicity assays
Polystichum setiferum spores, a typical fern of riparian habitats, were sampled in NW Spain, A Coruña province, in the riverbank of the Sar River (Santiago de Compostela). Fragments of leaf were collected with mature but closed sporangia. Spore release was promoted by drying the fragments on smooth paper for a week in the laboratory. Spores were stored dry at 4 °C in darkness until use. Spores were sieved and suspended in gametophyte culture medium as detailed in Feito et al. (2013).

2.4.2. Bioassays
The acute (48 h) and subchronic (1 week) phytotoxicities of autoclaved water samples (pH corrected to 7.0 ± 0.5) were evaluated measuring the mitochondrial activity of wood fern spores (*P. setiferum*). Aliquots containing 8×10^4 spores were prepared and 1.5 mL of each water sample was added (n = 6). Counting was done by means of a Z2 Coulter® particle count and size analyser (Beckman Coulter). Control samples contained 1.5 mL of culture medium (n = 6). Spores were incubated in controlled conditions (20 °C, PAR 35 μmol m^{-2} s^{-1} 16 h photoperiod) for 48 h and 168 h (1 week). After exposure, the spore suspensions were sterilised with commercial bleach (47–65 g L^{-1} NaClO, 1:100, 5 min) and washed twice with fresh medium. The measurement of the different endpoints was performed as described in Feito et al. (2013).

2.4.3. Toxicity data statistical analysis
The absorbance data of the samples were treated by the R- and R-Commander software. Bioassays for each treatment and exposure time were prepared in replicate (n = 6). Data are expressed as mean ± standard error. To check the assumptions of normality and homoscedasticity, the Shapiro–Wilk and the Bartlett tests were applied respectively. Since the assumption of independence was met, the analysis of variance two-way ANOVA was used in order to determine significant differences between treatments at each time. A p-value < 0.05 was considered significant in all cases.

3. Results and discussion

3.1. Drugs of abuse and chemical characterization

As previously mentioned, this paper investigates the optimization of a heterogeneous photo-Fenton process for the elimination of drugs of abuse in natural freshwater. In addition to chemical efficacy, the ability to reduce ecotoxicity by the photo-Fenton treatment was also assessed under different hydrogen peroxide and catalyst loadings.

Table 1 shows the results of drug chemical characterization in the initial natural fluvial water sample. TOC and pH showed values of 60 mg/L and 6.6, respectively. All drugs of abuse studied, with the exception of those belonging to the groups of cannabinoids and lysergic compounds, amphetamine (amphetamine-like compound, ALC), cocaethylene (cocainic), and heroin (opioid) and its metabolite 6-acetylmorphine (6ACM), were detected.

The average results obtained in terms of drug concentrations in this sample were higher than those found in other European (Zuccato et al., 2008; Kasprzyk-Hordern et al., 2008) and Spanish (Boleda et al., 2009; Postigo et al., 2010; Valcárcel et al., 2012) studies, probably due to the high density of population in Madrid. Only two substances were found with lower concentrations than in the rest of the studies, namely, ephedrine (ALC) and benzoylecgonine (cocaine metabolite). In the latter case, concentrations were similar to those of cocaine, while the reported concentrations of this metabolite are usually higher than those of the parent compound (Postigo et al., 2010; Valcárcel et al., 2012). On the other hand, this sample was characterized by a relatively high

Table 1
Drugs of abuse detected in freshwater (Manzanares River) downstream a STP.

Chemical group	Drug	CAS number	LODet (ng/L)	Concentration (ng/L)
ALCs	Amphetamine	300-62-9	4.28	n.d.
	Ephedrine	24221-86-1	0.45	156.33
	MDMA	42542-10-9	1.26	27.03
	Methamphetamine	4846-07-5	1.28	1.90
Benzodiazepines	Alprazolam	28981-97-7	3.60	6.79
	Diazepam	439-14-5	1.40	138.33
	Lorazepam	846-49-5	14.60	123.00
Cannabinoids	OH-THC	36557-05-8	1.26	n.d.
	THC	1972-08-3	6.81	n.d.
	THC-COOH	56354-06-4	4.19	n.d.
Cocainics	Benzoylecgonine	519-09-5	0.60	7.22
	Cocaethylene	529-38-4	1.47	n.d.
	Cocaine	50-36-2	0.13	7.27
LSD	LSD	50-37-3	1.80	n.d.
	OXO-LSD	111295-09-1	0.16	n.d.
Opioids	6ACM	2784-73-8	0.64	n.d.
	EDDP	66729-78-0	0.57	97.53
	Heroin	561-27-3	1.66	n.d.
	Methadone	76-99-3	0.62	26.97
	Morphine	57-27-2	1.45	3.57

n.d.: not detected.

concentration of alprazolam, diazepam and lorazepam (anxiolytic benzodiazepines) and methadone and EDDP as opioids.

These results confirmed that the drugs of abuse are not entirely eliminated by the wastewater treatment plants, as previously reported (Mendoza et al., 2014a, 2014b; Zuccato et al., 2008;). Zuccato and Castiglioni (2009) showed that while amphetamine and methamphetamine as cocaine and its metabolites, were reduced by 70–90%, reaching sewage effluents in the range of ng/L, the reduction of ecstasy was less than 50%, leading to the presence of tens of ng/L. Furthermore, substances such as methadone and its metabolites were shown to be highly resistant, with a reduction of only 9%.

Additional to chemical characterization, a bioassay using fern spores was performed to assess the actual toxicity of the river natural water. Fig. 1 shows fern spore mitochondrial activity after acute (Fig. 1A) and sub-chronic (Fig. 1B) exposure to raw fluvial water. Regardless of the length of the exposure, acute or sub-chronic, raw fluvial water induced a statistically significant reduction in mitochondrial activity over 70% (referred to controls with culture medium), indicating high toxicity. Albeit contamination by priority pollutants has been greatly reduced and classical and validated bioassays usually show no toxicity, the ecological quality and the biodiversity of the surface waters of industrialized countries is continually decreasing (Esteban et al., 2012). The use of alternative bioassays performed on critical phases of biota lifecycle, such as the fern spore bioassay, helps to identify the risks derived from micro and emerging pollution in a cost-effective manner (Esteban et al. 2012; Rodriguez-Gil et al., 2013).

3.2. Evaluation of heterogeneous photo-Fenton for DA removal and toxicity reduction

In the first approach, several blank experiments were carried out in order to assess the influence of UV–vis irradiation and the catalyst addition (Table 2) as compared to photo-Fenton experiments which include the iron-catalyst and the hydrogen peroxide. The effect of the UV–Vis irradiation was evaluated in the absence of catalyst and oxidant, whereas the catalyst addition blanks were conducted with two different solid catalyst loadings (0.1 and 0.6 g/L) in the absence of UV–vis light and oxidant. Other blank experiments such as dark Fenton or photo-oxidation with hydrogen peroxide in the absence of catalyst were not assessed due to the low oxidant consumption at these operation conditions. The activity of the solid catalyst decreased significantly at room temperature under
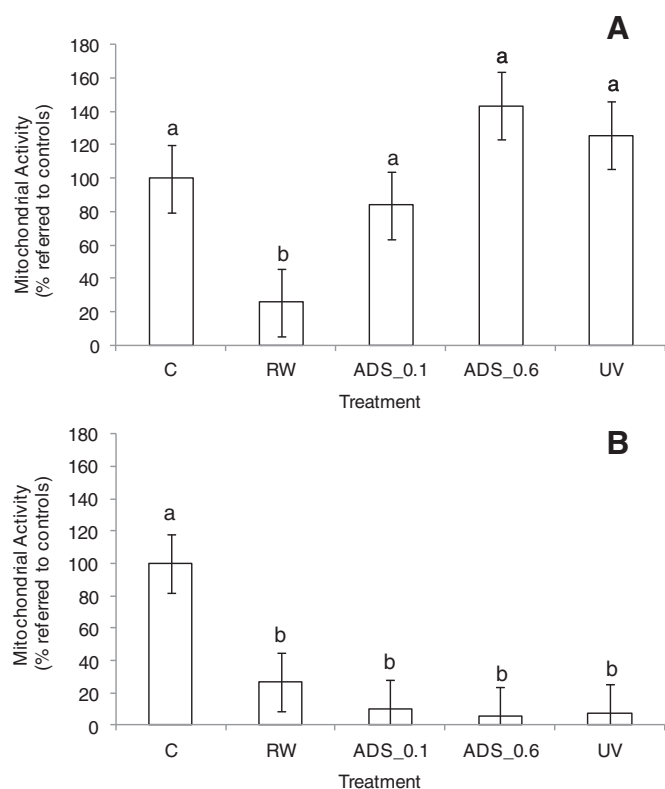

Fig. 1. Acute (A) and subchronic (B) toxicity of raw water (RW) and blank experiment samples expressed as percentage of variation in mitochondrial activity from controls (culture medium, C). Columns represent mean values (n = 6), bars represent standard error. Letters a and b represent statistically significant groups defined by the Tukey-b test.

dark conditions, and the photolysis of hydrogen peroxide is dramatically reduced using UV–Vis irradiation higher than 313 nm.

In the blank experiment performed with only UV–VIS irradiation, only two of the compounds studied, namely, diazepam and EDDP (methadone metabolite), were detected (although at concentrations below their limit of determination). In the blank experiment carried out to assess the effect of the catalyst addition (0.1 and 0.6 g/L), 8 out of the 20 compounds tested were found, namely, the ALCs ephedrine, ecstasy (MDMA) and amphetamine, the benzodiazepines diazepam and lorazepam, the metabolite of cocaine benzoylecgonine, and the opioids EDDP and methadone. An increase in the concentrations after these blank experiments was observed for ephedrine, ecstasy, metamphetamine and benzoylecgonine, mainly using the lower catalyst loading (0.1 g/L). Table 2 also summarizes the mean chemical efficiency of removal of the blank experiments for each group of drugs of abuse. The concentration of all groups present in the river water was reduced by more than 70% using only UV–vis light. The presence of the solid iron catalyst led to a strong reduction of the opioid concentration (75–83%) and a moderate reduction of benzodiazepines (32–56%), however a strong increase in the concentration of cocainics (225–159%) and a moderate increase of ALCs (51–11%) were observed.

At first sight, the attainment of negative efficiencies may seem striking. However, we must keep in mind that the sample processed is a natural river water. The only pre-treatment performed was freezing and the gravitational decantation of the heaviest solids in suspension before preliminary blank experiments. Fluvial natural waters are extremely complex and contain dissolved inorganic and organic substances, particles of different sizes in suspension as well as a rich microbiota including microscopical animals, plants, fungi, protists and prokaryotes (bacteria). UV–vis treatment can kill most of the microbiota but many organisms may survive freezing and be biologically active during the catalyst addition blank experiment without irradiation. The release of cell contents,

Table 2
Drugs of abuse concentration (ng/L) after blank experiments of micro-contaminated fluvial water.

Drugs		UV–vis		Catalyst load_0.1		Catalyst load_0.6	
		[ng/L]	Efficiency	[ng/L]	Efficiency	[ng/L]	Efficiency
ALCs	Amphetamine	n.d.		n.d.		n.d.	
	Ephredrine	n.c.	>99%	333.67	−113%	302.30	−93%
	MDMA	n.d.	>95%	27.37	−1%	14.00	48%
	Methamphetamine	n.d.	>33%	2.60	−37%	1.66	13%
	Average efficiency		76%		−50%		51%
Benzodiazepines	Alprazolam	n.c.	>47%	n.c.	>47%	n.c.	>47%
	Diazepam	0.89	>99%	52.40	62%	8.27	94%
	Lorazepam	n.d.	>88%	109.33	11%	73.30	40%
	Average efficiency		74%		32%		56%
Cannabinoids	OH-THC	n.d.		n.d.		n.d.	
	THC	n.d.		n.d.		n.d.	
	THC-COOH	n.d.		n.c.		n.c.	
	Average efficiency						
Cocainics	Benzoylecgonine	n.c.	>92%	46.87	−549%	37.33	−417%
	Cocaethylene	n.d.		n.c.		n.c.	
	Cocaine	n.c.	>98%	n.c.	>98%	n.c.	>98%
	Average efficiency		95%		−225%		−159%
LSD	LSD	n.d.		n.d.		n.d.	
	OXO-LSD	n.d.		n.d.		n.d.	
	Average efficiency						
Opioids	6ACM	n.d.		n.d.		n.d.	
	EDDP	0.22	99%	8.81	91%	1.06	99%
	Heroin	n.d.		n.d.		n.d.	
	Methadone	n.c.	>98%	5.69	79%	0.72	97%
	Morphine	n.d.	>59%	n.d.	>59%	n.c.	>59%
	Average efficiency		83%		75%		83%

n.d.: not detected.
n.c.: not confirmed.

including active enzymes, of the microorganisms killed can also contribute to strong chemical changes in the sample including the enzymatic transformation of drugs and the deconjugation of metabolites. The increase in the concentration of certain psychoactive pharmaceuticals with comparable chemical structure to illicit drugs has been repeatedly observed in the wastewater treatment plants (Calisto and Esteves, 2009; Esteban et al., 2012). Vieno et al. (2007) explained this effect by the reversion of glucuronides and other conjugated metabolites to the parent compounds by chemical and enzymatic deconjugation. Our results seem to evidence a similar mechanism for the observed increase in some compounds.

Toxicological characterization is based on the toxic effect of the different treated waters on mitochondrial activity of spores. Reduced mitochondrial activity corresponds to high toxicity since the mitochondrion is the main source of ATP, the energetic currency of cells. Mitochondrial activity reduction can be due to both the decrease of the number of spores caused by death, and a general decrease in the activity of the spore population. In both cases, this reduced activity may be attributed to a severe toxicity caused by the drugs of abuse analysed and/or other substances not analysed in the water. Depending on the exposure time, the cells are dealing with acute (24–48 h) or subchronic toxicity (1 week). Before spore exposure, the pH of all the samples was neutralized to discard pH effect on toxicity.

Blank experiment products (filtered, autoclaved and neutralized) induced no apparent acute toxicity but an extreme sub-chronic toxicity since all treatments induced a dramatic significant reduction of mitochondrial activity after one week of exposure (Fig. 1). It is noteworthy that although blank experiments yielded TOC reductions above 79% (Table 3) and good efficiencies in drug elimination (Table 2), they did not succeed in the elimination of fluvial pollution toxicity. Incomplete mineralization and formation of more toxic chemical species could explain this effect (Bolong et al., 2009; Marugán et al., 2012).

The photo-Fenton treatment of the raw water sample was studied using the two different solid catalyst loadings of 0.1 g/L and 0.6 g/L and three concentrations of hydrogen peroxide (100%, 50% and 25% of stoichiometric calculated amount) in order to assess the influence of

these crucial variables in the photo-Fenton process. Table 4 shows concentrations of drugs of abuse after the photo-Fenton treatments using Fe catalyst loading 0.1 g/L. None of the target compounds were detected (or its presence confirmed) using 25%, 50% or 100% of stoichiometric hydrogen peroxide. The mean chemical removal efficiency of the photo-Fenton treatment using iron catalyst 0.1 g/L for each group of drugs is also shown, reaching values higher than 75% in all cases, independently of the hydrogen peroxide concentration initially added. Cocainics and opioids were eliminated by more than 95% and 85%, respectively. Since the presence of many substances was not detected or confirmed, the removal efficiency is referred to the limit of determination, and therefore when the initial concentration is very close to this value, the percentage of reduction is relatively low and the efficiency should be understood as the minimum reached by this treatment. In the samples analysed after the photo-Fenton treatments using the highest loading of Fe-based catalyst (0.6 g/L), three compounds were determined, MDMA, diazepam and EDDP, at concentrations below 0.8 ng/L (Table 5). The mean removal efficiency after photo-Fenton experiment using 0.6 g/L was also greater than 75% for all of the different groups of drugs. Despite the differences in certain DAs, the general results evidence that the initial hydrogen peroxide concentration does not affect the chemical removal of the drugs under study. It is important to mention that the hydrogen

Table 3
Macroscopic characterization of the river water, blank and photo-Fenton treatments.

Samples	pH	TOC	% TOC reduction
River water	6.6	60.6	–
Photolysis	3.1	12.8	79
Catalyst_0.1	3.1	9.7	84
Catalyst_0.6	3.2	8.3	86
Fe[0.1 g/L] 100%	3.1	8.9	85
Fe[0.1 g/L] 50%	3.0	8.8	85
Fe[0.1 g/L] 25%	3.1	8.7	85
Fe[0.6 g/L] 100%	2.9	10.3	83
Fe[0.6 g/L] 50%	3.2	10.3	83
Fe[0.6 g/L] 25%	3.0	12.5	79

Table 4
Drugs of abuse concentration (ng/L) after photo-Fenton treatments using 0.1 g/L of Fe-based catalyst loading and different concentrations of H_2O_2.

Drugs		25%		50%		100%	
		[ng/L]	Efficiency	[ng/L]	Efficiency	[ng/L]	Efficiency
ALCs	Amphetamine	n.d.		n.d.		n.d.	
	Ephredrine	n.d.	>99%	n.d.	>99%	n.d.	>99%
	MDMA	n.d.	>95%	n.d.	>95%	n.c.	>95%
	Methamphetamine	n.d.	>32%	n.d.	>33%	n.d.	>32%
	Average efficiency		76%		76%		76%
Benzodiazepines	Alprazolam	n.c.	>47%	n.c.	>47%	n.c.	>47%
	Diazepam	n.d.	>99%	n.d.	>99%	n.d.	>99%
	Lorazepam	n.d.	>88%	n.d.	>88%	n.d.	>88%
	Average efficiency		78%		78%		78%
Cannabinoids	OH-THC	n.d.		n.d.		n.d.	
	THC	n.d.		n.d.		n.d.	
	THC-COOH	n.d.		n.d.		n.d.	
	Average efficiency						
Cocainics	Benzoylecgonine	n.c.	>92%	n.c.	>92%	n.c.	>92%
	Cocaethylene	n.d.		n.d.		n.d.	
	Cocaine	n.c.	>98%	n.c.	>98%	n.c.	>98%
	Average efficiency		95%		95%		95%
LSD	LSD	n.d.		n.d.		n.d.	
	OXO-LSD	n.d.		n.d.		n.d.	
	Average efficiency						
Opioids	6ACM	n.d.		n.d.		n.d.	
	EDDP	n.c.	>99%	n.c.	>99%	n.d.	>99%
	Heroin	n.d.		n.d.		n.d.	
	Methadone	n.d.	>98%	n.c.	>98%	n.d.	>98%
	Morphine	n.d.	>59%	n.d.	>59%	n.d.	>59%
	Average efficiency		85%		85%		85%

n.d.: not detected.
n.c.: not confirmed.

peroxide added in these experiments is completely depleted after reactions except in the case of 100%–0.6 g/L of catalyst, where the concentration of remained oxidant is lower than 10 mg/L. It should also be pointed out that the iron leached out to the solution from the solid catalyst in all the photo-Fenton experiments was lower than 0.1 mg/L. Therefore, the homogeneous contribution to the overall activity in terms of TOC and DA reduction should be negligible.

Regarding the TOC removal of blanks and photo-Fenton treatments (Table 3), no differences were observed among them, regardless of the different photo-Fenton operation conditions (oxidant and iron catalyst

Table 5
Drugs of abuse concentration (ng/L) after the photo-Fenton treatments using 0.6 g/L of Fe-based catalyst loading and different concentrations of H_2O_2.

Drugs		25%		50%		100%	
		[ng/L]	Efficiency	[ng/L]	Efficiency	[ng/L]	Efficiency
ALCs	Amphetamine	n.d.		n.d.		n.d.	
	Ephredrine	n.d.	>99%	n.d.	>99%	n.d.	>99%
	MDMA	0.17	95%	n.c.	>95%	n.d.	>95%
	Methamphetamine	n.d.	>33%	n.d.	>33%	n.d.	>33%
	Average efficiency		77%		76%		76%
Benzodiazepines	Alprazolam	n.c.	>47%	n.c.	>47%	n.c.	>47%
	Diazepam	0.79	99%	n.d.	>99%	n.d.	>99%
	Lorazepam	n.d.	>88%	n.d.	>88%	n.d.	>88%
	Average efficiency		78%		78%		
Cannabinoids	OH-THC	n.d.		n.d.		n.d.	
	THC	n.d.		n.d.		n.d.	
	THC-COOH	n.d.		n.d.		n.d.	
	Average efficiency						
Cocainics	Benzoylecgonine	n.c.	>92%	n.c.	>92%	n.c.	>92%
	Cocaethylene	n.d.		n.d.		n.d.	
	Cocaine	n.c.	>99%	0.32	96%	n.c.	>98%
	Average efficiency		95%		96%		95%
LSD	LSD	n.d.		n.d.		n.d.	
	OXO-LSD	n.d.		n.d.		n.d.	
	Average efficiency						
Opioids	6ACM	n.d.		n.d.		n.d.	
	EDDP	0.15	>99%	n.d.	>99%	n.d.	>99%
	Heroin	n.d.		n.d.		n.d.	
	Methadone	n.c.	>98%	n.d.	>98%	n.d.	>98%
	Morphine	n.d.	>59%	n.d.	>59%	n.d.	>59%
	Average efficiency		85%		85%		85%

n.d.: not detected.
n.c.: not confirmed.

concentrations) or the absence of UV–vis irradiation or catalyst and oxidation for the photolysis and catalyst addition blanks.

The effect of photo-Fenton with a solid catalyst loading of 0.1 g/L is shown in Fig. 2. Only the highest concentration of H_2O_2 eliminated acute toxicity from fluvial water (Fig. 2A). None of the photo-Fenton treatments with 0.1 g/L eliminated sub-chronic toxicity. However, photo-Fenton processes using an Fe-based catalyst loading of 0.6 g/L (Fig. 3) eliminated both acute and sub-chronic toxicities. This is a relevant feature since both 0.1 and 0.6 g/L led to virtually the same chemical results in terms of TOC. It must be highlighted that the process involving the addition of 0% H_2O_2 increased by more than 40% the acute mitochondrial activity of spores (Fig. 3A). Although fern spores have a higher respiration rate than controls, this event can be considered as low to moderate toxicity. It has been frequently reported that the presence of low levels of toxic substances resulted in a stimulation of cell activity due to compensatory mechanisms, an effect known as hormesis (Calabrese, 2008; Calabrese and Blain, 2009). This effect has been frequently observed in spore development bioassays using natural waters (Rodríguez-Gil et al., 2010; Esteban et al., 2012) or low toxicant doses (Marugán et al., 2012; Feito et al., 2012, 2013).

Most environmental regulations establish requirements for discharges of urban wastewater treatment plants in terms of chemical parameters. As this work demonstrates, the TOC removal or even the elimination of DAs are not representative parameters for the efficient degradation of these micropollutants, because their concentration represents a very low percentage of the total organic carbon of the water and they can be degraded towards other by-products with remarkable ecotoxicity. Although in terms of macroscopic characterization all treatments led to very similar results (Table 3), the effectiveness of the photo-Fenton process in terms of toxicity depends on the loading of
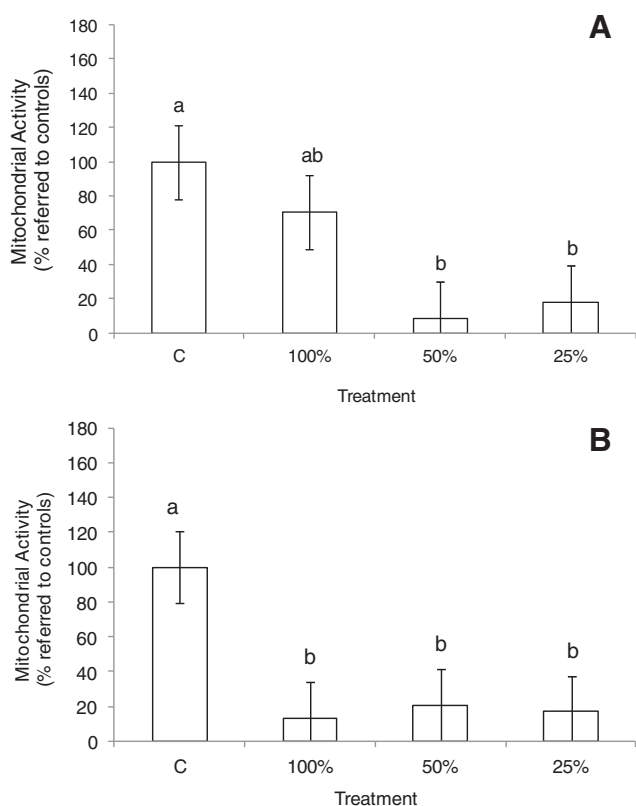

Fig. 3. Acute (A) and subchronic (B) toxicity of photo-Fenton treatments using 0.6 g/L of Fe-based catalyst loading expressed as percentage of variation in mitochondrial activity from controls (culture medium). Columns represent mean values (n = 6), bars represent standard error. Letters a and b represent statistically significant groups defined by the Tukey-b test.
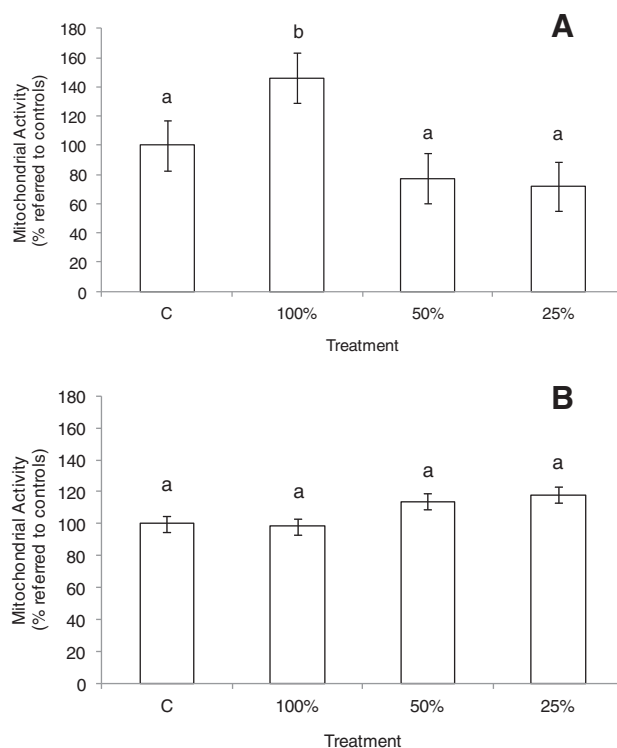
the iron catalyst. The higher loading used, 0.6 g/L, abolished both acute and sub-chronic inhibition of mitochondrial activity.

The photo-Fenton treatment led to high removals of the DAs using very low concentrations of the oxidant (25%). However, taking into account the toxicological assessment, this low concentration of hydrogen peroxide was only effective when the photo-Fenton treatment employed 0.6 g/L of Fe catalyst. The addition of the adequate concentration of H_2O_2 is especially important, not only to design cost-effective procedures, but also from the ecotoxicological point of view. A special concern, from the biological approach, is the environmental release of free radicals and peroxides overproduced during water treatment since they can attack membranes, proteins and even the genetic material (Cadenas, 1989). These pro-oxidants are extremely toxic irrespective of the type of organism or ecosystem, and their discharge in the environment must be absolutely prevented.

It is important to note that this study is limited to a particular fluvial sample. Although its characteristics are similar to other freshwaters from similar basins, future studies should extend the range of water samples after different sewage outfalls. It must be noted that the influence of the water matrix does not seem to have a crucial role on the efficiency of the heterogeneous photo-Fenton treatment, as it has been reported in other works for the application of advanced oxidation processes in the degradation of other emerging pollutants with different type of water matrices (Rodriguez-Gil et al., 2013). Finally, the use of rapid cost-efficient microbioassays such as the fern spore has demonstrated to be not only useful, but even necessary for the follow-up and optimization of decontamination procedures. Nonetheless, in order to achieve a relevant ecotoxicological characterization of the success in the decontamination, a complete battery of bioassays using ecologically relevant organisms and biologically relevant endpoints should be systematically implemented.

Fig. 2. Acute (A) and subchronic (B) toxicity of photo-Fenton treatments using 0.1 g/L of Fe-based catalyst loading expressed as percentage of variation in mitochondrial activity from controls (culture medium). Columns represent mean values (n = 6), bars represent standard error. Letters a and b represent statistically significant groups defined by the Tukey-b test.

4. Conclusions

This work deals with the potential health and environment risks due to the presence of drugs of abuse in natural fluvial waters, especially those intended for human consumption. It has been demonstrated that the heterogeneous photo-Fenton processes eliminated the presence of these compounds. The concentration of most drugs of abuse studied was successfully reduced below the analytical detection limit, while only a few of those compounds, such as diazepam, EDDP or MDMA, remained at very low concentrations. In terms of TOC reduction all treatments exhibited very similar results. However, toxicological data showed that although photo-Fenton treatments led to high efficiencies in drug elimination, they did not succeed in the elimination of fluvial pollution toxicity. Moreover, toxicity might increase due to reaction products, especially peroxides and oxidant by-products. It was observed that the efficacy of the chemical system depended on the loading of the iron catalyst used in the treatment. Only when using the highest Fe-based catalyst loading (0.6 g/L) both acute and sub-chronic inhibition of mitochondrial activity were observed. These results indicate the need of performing a combined toxicological and chemical analysis for the thorough and safe evaluation of the water treatment efficacy and confirm photo-Fenton as a good alternative for the treatment of river water intended for human consumption.

Authors contributions

YV, FM and MC designed the study. YS and RM designed and performed the photo-Fenton treatments. AM, under the direction of MC, performed the bioassays, prepared figures and tables and did data analysis. ND assisted with manuscript preparation. NM, under the direction of MLA and DB, performed the analysis of the drugs of abuse. All the authors have participated in the manuscript writing and/or revision under the coordination of YS as the corresponding author.

Acknowledgements

References

Boleda, R., Galcerán, T., Ventura, F., 2009. Monitoring of opioids, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. Water Res. 43 (4), 1126–1136.

Boles, T.M., Wells, M.J.M., 2010. Analysis of amphetamine and methamphetamine as emerging pollutants in wastewater and wastewater-impacted streams. J. Chromatogr. A 1217, 2561–2568.

Bolong, N., Ismail, A.F., Salim, M.R., Matsuura, T., 2009. A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination 239, 229–246.

Cadenas, E., 1989. Biochemistry of oxygen toxicity. Annu. Rev. Biochem. 58 (1), 79–110.

Calabrese, E.J., 2008. Hormesis: why is important to toxicology and toxicologist. Environ. Toxicol. Chem. 27, 1451–1474.

Calabrese, E.J., Blain, R.B., 2009. Hormesis and plant biology. Environ. Pollut. 157, 42–48.

Calisto, V., Esteves, V.I., 2009. Psychiatric pharmaceuticals in the environment. Chemosphere 77 (10), 1257–1274.

Catalá, M., Esteban, M., Rodriguez-Gil, J.L., Quintanilla, L.G., 2009. Development of a naturally miniaturised testing method based on the mitochondrial activity of fern spores: a new higher plant bioassay. Chemosphere 77 (7), 983–988.

Catalá, M., Esteban, M., Quintanilla, L.G., 2010. Working with Ferns: Mitochondrial Activity of Fern Spores for the Evaluation of Acute Toxicity in Higher Plant Development. Springer, New York, pp. 237–247.

EDADES, 2012. Encuesta domiciliaria sobre Alcohol y Drogas en España 2011/2012. Delegación del Gobierno para el Plan Nacional sobre Drogas. Available at:, www.mspsi.es (Latest access on January 2013).

Esteban, S., Valcárcel, Y., Catalá, M., González Castromil, M., 2012. Psychoactive pharmaceutical residues in the watersheds of Galicia (Spain). Gac. Sanit. 26 (5), 457–459.

Farré, M., Martínez, E., Barceló, D., 2007. Validation of interlaboratory studies on toxicity in water samples. TrAC Trends Anal. Chem. 26 (4), 283–292.

Feito, R., Valcárcel, Y., Catalá, M., 2012. Biomarker assessment of toxicity with miniaturized bioassays: diclofenac as a case study. Ecotoxicology 21, 289–296.

Feito, R., Valcárcel, Y., Catalá, M., 2013. Preliminary data suggest that venlafaxine environmental concentration could be toxic to plants. Chemosphere 90, 2065–2069.

Gros, M., Petrovic, M., Ginebreda, A., Barcelo, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36 (1), 15–26.

INE base, 2012. National Statistics Institute on-line database. Available at, http://www.ine.es/inebmenu/mnu_padron.htm (Latest access on March 2013).

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42 (13), 3498–3518.

Martínez, F., Calleja, G., Melero, J.A., Molina, R., 2005. Heterogeneous photo-Fenton degradation of phenolic aqueous solutions over iron-containing SBA-15 catalyst. Appl. Catal. B Environ. 60, 185–194.

Marugán, J., Bru, D., Pablos, C., Catalá, M., 2012. Comparative evaluation of acute toxicity by Vibrio fischeri and fern spore based bioassays in the follow-up of toxic chemicals degradation by photocatalysis. J. Hazard. Mater. 213, 117–122.

Mendoza, A., Rodríguez-Gil, J.L., González-Alonso, S., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2014a. Drugs of abuse and benzodiazepines in the Madrid Region (Central Spain): Seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. Environ. Int. 70, 76–87.

Mendoza, A., López de Alda, M., González-Alonso, S., Mastroianni, N., Barceló, D., Valcárcel, Y., 2014b. Occurrence of drugs of abuse and benzodiazepines in river waters from the Madrid Region (Central Spain). Chemosphere 95, 247–255.

Postigo, C., López de Alda, M.J., Barceló, D., 2010. Drugs of abuse and their metabolites in the Ebro River basin: occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environ. Int. 36 (1), 75–84.

Postigo, C., Sirtori, C., Oller, I., Malato, S., Maldonado, M.I., López de Alda, M., Barceló, D., 2011a. Photolytic and photocatalytic transformation of methadone in aqueous solutions under solar irradiation: kinetics, characterization of major intermediate products and toxicity evaluation. Water Res. 45 (16), 4815–4826 (15).

Postigo, C., Sirtori, C., Oller, I., Malato, S., Maldonado, M.I., López de Alda, M., Barceló, D., 2011b. Photolytic and photocatalytic transformation of cocaine in aqueous solutions under solar irradiation: kinetics, characterization of major intermediate products and toxicity evaluation. Appl. Catal. B Environ. 104 (1–2), 37–48.

Rodayan, A., Segura, P.A., Yargeau, V., 2014. Ozonation of wastewater: removal and transformation products of drugs of abuse. Sci. Total Environ. 487, 763–770.

Rodríguez-Gil, J.L., Catalá, M., González Alonso S., Romo, Maroto R., Valcárcel, Y., Segura, Y., Molina, R., Melero, J.A., Martínez, F., 2010. Heterogeneous photo-Fenton treatment for the reduction of pharmaceutical contamination in Madrid Rivers and ecotoxicological evaluation by miniaturized fern spores bioassay. Chemosphere 80, 381–388.

Rodriguez-Gil, J.L., San Sebastián Sauto, J., González-Alonso, S., Sánchez Sánchez, P., Valcarcel, Y., Catalá, M., 2013. Development of cost-effective strategies for environmental monitoring of irrigated areas in Mediterranean regions: traditional and new approaches in a changing world. Agric. Ecosyst. Environ. 181, 41–49.

Terzic, S., Senta, I., Ahel, M., 2010. Illicit drugs in wastewater of the city of Zagreb (Croatia) — estimation of drug abuse in a transition country. Environ. Pollut. 158 (8), 2686–2693.

Valcárcel, Y., Martínez, F., González, Alonso S., Segura, Y., Catalá, M., Molina, R., Montero-Rubio, J.C., Mastroianni, N., López de Alda, M., Postigo, C., Barceló, D., 2012. Drugs of abuse in surface and tap waters of the Tagus River basin: heterogeneous photo-Fenton process is effective in their degradation. Environ. Int. 41, 35–43.

van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H., Covaci, A., 2009. Cocaine and metabolites in waste and surface water across Belgium. Environ. Pollut. 157 (1), 123–129.

Vieno, N.M., Harkki, H., Tuhkanen, T., Kronberg, L., 2007. Occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. Environ. Sci. Technol. 41 (14), 5077–5084.

Zuccato, E., Castiglioni, S., 2009. Illicit drugs in the environment. Philos. Trans. R. Soc. A 367, 3965–3978.

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027–1032.