Enhanced degradation of micropollutants by zero-valent aluminum activated persulfate: assessment of toxicity and genotoxic activity


ABSTRACT

Advanced oxidation of the aqueous Triton™ X-45 (TX-45), iopamidol (IOPA), ciprofloxacin (CIP) and bisphenol A (BPA) solutions via activation of persulfate (PS) with zero-valent aluminum (ZVA) was investigated. The study aimed at assessing the effectiveness of the PS/ZVA process in terms of target micropollutants (MPs) and toxicity abatements in raw surface water (RSW) and distilled water (DW). TX-45, CIP and BPA were completely degraded after 90-minute, 120-minute and 40-minute treatment, respectively, with PS/ZVA in DW, whereas 95% IOPA removal was achieved after 120-minute (MPs = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3). TX-45 (59%), IOPA (29%), CIP (73%) and BPA (46%) removal efficiencies decreased after 120-minute PS/ZVA treatment in RSW. In DW, Vibrio fischeri toxicities of original (untreated) MPs were found as: CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%), and as BPA (100%) > CIP (66%) > IOPA (62%) > TX-45 (35%) in RSW. Acute toxicities of MPs and their degradation products fluctuated during PS/ZVA treatment both in DW and RSW samples and resulted in different relative inhibition values after 120-minute. The original and PS/ZVA-treated TX-45, IOPA and BPA in DW exhibited neither cytotoxic nor genotoxic effects, whereas CIP oxidation ended up in degradation products with genotoxic effects.

Key words | acute toxicity and genotoxicity, bisphenol A, ciprofloxacin, iodinated X-ray contrast media, nanoscale zero-valent aluminum, non-ionic surfactants

INTRODUCTION

In the past decades, the presence of micropollutants (MPs) in water sources has raised considerable interest due to their potential harmful environmental and health impacts. The MPs group includes a wide variety of compounds such as pharmaceuticals, personal care products, hormones, industrial additives and household chemicals (Luo et al. 2014). The main sources of MPs are wastewater treatment plants for domestic sewage, wastewater from hospital effluents, chemical manufacturing plants, livestock and agriculture (Luo et al. 2014; Pasquini et al. 2014). Major consequences of MPs 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 (Fent et al. 2006). Recent studies demonstrated that conventional water/wastewater treatment plants are inadequate to effectively remove MPs, due to their complex molecular structure, chemical stability and low biodegradability (Gavrilescu et al. 2015). On the other hand, advanced oxidation processes (AOPs), characterized by the generation of highly reactive, non-selective free radicals such as sulfate (SO$_4^{2-}$, $E^0 = 2.6$ V) and hydroxyl (HO$, E^0 = 2.7$ V), offer a promising alternative to conventional treatment for the removal of MPs in water or wastewater. In the past years, persulfate (PS, S$_2$O$_8^{2-}$) has attracted increasing attention in AOP applications since it is more stable than hydrogen peroxide (HP) and has a relatively high redox potential ($E^0 = 2.01$ V). Upon thermal, chemical or photochemical activation, it is possible to generate SO$_4^{2-}$ from PS (Antoniou et al. 2010).
SO$_4^-$ generated in activated PS processes is more selective than the HO· for the oxidation of compounds with carbon-carbon double bonds and benzene rings (Anipsitakis & Dionysiou 2004; Huang et al. 2005). The activation of PS with nanoscale zero-valent iron and aluminum (ZVA) has exhibited a great potential in the oxidative treatment of refractory contaminants (Bokare & Choi 2009; Girit et al. 2015; Arslan-Alaton et al. 2017a, 2017b). Under acidic pH (pH < 4) and in the presence of dissolved oxygen (O$_2$), the ZVA/H$^+$/O$_2$ treatment system involves two major processes:

(i) corrosive dissolution of Al$^{3+}$ and simultaneous reduction of O$_2$ to hydroperoxyl radical(s), HO$_2^-$, that leads the formation of hydrogen peroxide (H$_2$O$_2$, HP); and

(ii) generation of HO· by an electron transfer mechanism from ZVA to HP (Bokare & Choi 2009).

\[
\begin{align*}
\text{Al}^0 & \rightarrow \text{Al}^{3+} + 3e^- \quad (1) \\
\text{O}_2 + \text{H}^+ + e^- & \rightarrow \text{HO}_2^- \quad (2) \\
2\text{HO}_2^- & \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \quad (3) \\
\text{Al}^0 + 3\text{H}_2\text{O}_2 & \rightarrow \text{Al}^{3+} + 3\text{HO}^- + 3\text{OH}^- \quad (4)
\end{align*}
\]

The oxidation capacity of the ZVA/H$^+$/O$_2$ system relies on the formation of HO·, which possesses strong oxidizing capability towards a variety of organic compounds. For example, the removal of aqueous acetaminophen [N-(4-hydroxyphenyl) ethanamide, ACTM] using the ZVA/H$^+$/air-O$_2$ system was investigated by Zhang et al. (2012). More than 99% of 2 mg/L aqueous ACTM was removed in 16 h, at pH 1.5 and 25 ± 1°C by the ZVA/H$^+$/air-O$_2$ treatment system and higher ZVA concentrations enhanced the removal. Bokare & Choi (2009) examined 4-chlorophenol (4-CP) as the model pollutant to explore the potential of ZVA/H$^+$/air-O$_2$ treatment. Nearly complete (>95%) 4-CP degradation was achieved in 10 h under air-O$_2$ equilibrated conditions (ZVA = 1 g/L; 4-CP = 100 μM; initial pH = 2.5). The addition of oxidants (e.g. HP or PS) is expected to substantially improve the degradation rates of organic pollutants via ZVA-mediated treatment processes (Girit et al. 2015; Arslan-Alaton et al. 2017a, 2017b). It has been reported that in the presence of ZVA, PS could be activated under acidic conditions through direct electron transfer from the ZVA surface to PS to generate SO$_4^-$ (Arslan-Alaton et al. 2017a, 2017b):

\[
2\text{Al}^0 + \text{S}_2\text{O}_7^{2-} + 6\text{H}^+ + 1.5\text{O}_2 \rightarrow 2\text{Al}^{3+} + 2\text{SO}_4^- + 3\text{H}_2\text{O}
\]

In a recent study, Cheng et al. (2015) examined the performance of acid-washed ZVA (AW-ZVA) in the presence of HP to remove phenol. The effect of HP addition (0–8 mM) on phenol removals by AW-ZVA was also investigated in this study. Increasing the initially added HP concentration from 1 mM to 2 mM increased the phenol removal efficiency; however, further increase did not improve phenol degradation.

During the application of AOPs, the main concern is associated with the formation of various degradation products that can potentially be more toxic, estrogenic, teratogenic, mutagenic, carcinogenic, genotoxic and/or persistent than the original compound/pollutant (Rizzo 2011; Garcia-Käufer et al. 2012). Thus, information provided by the target pollutant measurements alone is not sufficient to assess the effectiveness of the AOPs and their risks associated with the degradation products. In addition, the natural water matrices may change the degradation products formed during the application of AOPs, which in turn may lead to different levels of biological activity depending on the variability in the water quality characteristics. Thus bioanalytical tools are necessary to examine the impact of individual MPs and their degradation products but also the effects exerted by water/wastewater as a whole (Papa et al. 2016). Among the variety of bioanalytical tools currently studied, a set of bioassays must be conducted for ecotoxicological assessment. As recommended by Escher et al. (2014), the ‘ideal’ battery test should consist of indicator bioassays that are able to cover a wide range of cellular toxicity pathways and take into account each mode of action: the non-specific (baseline), the specific (e.g. estrogenicity) and the reactive (e.g. genotoxicity) toxicity.

In the present work, a PS/ZVA treatment process for the removal of MPs in distilled water (DW) and raw surface water (RSW) spiked with MPs was investigated. Four extensively used chemicals that enter the aquatic environment through different discharge routes and ultimately form MPs were selected:

(i) a diphenyl methane plasticizer, bisphenol A (BPA)

(ii) an octylphenol ethoxylate-based non-ionic surfactant, Triton™ X-45 (TX-45)
(iii) an iodinated aromatic X-ray contrast medium, iopamidol (IOPA).
(iv) a fluoroquinolone-type antibiotic, ciprofloxacin (CIP).

Treatment performance was assessed in terms of target pollutant removals. The ecotoxicological effects were measured on two target organisms: the photobacterium Vibrio fischeri (V. fischeri) and the microalga Pseudokirchneriella subcapitata (P. subcapitata) in order to focus on taxa playing different roles in the trophic web (primary decomposer and producer, respectively). The genotoxicity of untreated and PS/ZVA-treated effluents was evaluated with the UMU-Chromo test, which has been developed and standardized to detect the genotoxicity of native, non-concentrated aqueous samples (ISO 13829 2000), and has been proposed as a screening test for monitoring the genotoxicity of individual pollutants as well as surface and wastewater samples (Hamer et al. 2000; Zegura et al. 2009).

MATERIALS AND METHODS

Materials

Commercial-grade ZVA nanoparticles (Brunauer–Emmett–Teller surface area: 10–20 m²/g; particle size: 100 nm; purity: >99.9%) were purchased from US Research Nanomaterials, Inc. (Houston, USA). TX-45, with an average ethoxylate chain length of approximately five, ((C₆H₄O)ₙC₄H₉₂O; CAS Nr. 9002-93-1; purity: 98%) was purchased from Merck (Germany). IOPA (777 g/mol; C₁₁H₂₂N₂O₆; CAS Nr. 60166-93-0) is a non-ionic radiographic contrast agent commercially available as ‘Pamiray 300/370’ injectable solution. IOPA was purchased from BIEM Pharmaceutical Co. A.S. (Ankara, Turkey). BPA (228 g/mol; C₁₃H₁₆O₂; CAS Nr. 80-05-7; purity: 99.9%) and CIP (331 g/mol; C₁₇H₁₈FN₃O₃; CAS Nr. 85721-33-1; purity ≥98%) as well as potassium persulfate (PS; 270 g/mol; K₂S₂O₈; CAS Nr. 7727-21-1; >99.9%) were purchased from Sigma-Aldrich (USA). Chromatographic-grade acetonitrile and methanol (MeOH) were obtained from Merck (Germany). Aqueous MP solutions were prepared in DW (Arium 61316RO, Sartorius AG, Germany). The RSW sample was taken from the influent of a local water treatment plant located in Istanbul, Turkey, treating lake water (total organic carbon = 7.6 mg/L; dissolved organic carbon (DOC) = 7.2 mg/L; suspended solids = 12 mg/L; color = 48 Pt-Co units; alkalinity = 115 mg CaCO₃/L; hardness = 110 mg CaCO₃/L; PO₄³⁻ = 0.17 mg/L; SO₄²⁻ = 15.5 mg/L; NO₃⁻ = 2.2 mg/L; Cl⁻ = 21 mg/L; pH = 7.9).

Experimental procedures

Aqueous MP solutions were treated in a 500 mL-capacity borosilicate glass reactor at room temperature (23 ± 2 °C) at fixed initial MP concentrations of 2 mg/L (BPA = 8.8 μM; TX-45 = 4.7 μM; IOPA = 2.6 μM; CIP = 6.0 μM) based on the calculated detection limits for both high performance liquid chromatography and bioassays and enabled direct analytical assessment of individual samples without further concentration. PS oxidation (in the absence of ZVA) of MPs was examined at pH 3 for 120 min. The more conventional ZVA/H⁺/O₂ (in the absence of PS) treatment was explored at pH 5 with 1 g/L ZVA as a control experiment. PS/ZVA treatment experiments were conducted with 2 mg/L MPs aqueous solutions in the presence of 1 g/L ZVA at pH 3 for 120 minutes. The selection of the studied working conditions was based on experiments that have been reported recently (Arslan-Alaton et al. 2017a, 2017b). The experimental procedure of a typical run was described elsewhere (Girit et al. 2015). Samples were taken at regular time intervals from the reactor and filtered through 0.22 μm Millipore membranes (Millipore Corp., USA) to separate ZVA from the reaction solution. All experiments were conducted in duplicate and average values were taken when presenting the results.

Analytical procedures and instrumental analyses

An Agilent 1100 system (Agilent Technologies, USA) with a diode array detector was employed for the measurement of MPs. The chromatographic conditions used for BPA, TX-45 and IOPA have been described elsewhere (Girit et al. 2015; Arslan-Alaton et al. 2017a, 2017b). For CIP measurement, a C18 Novapack column (3.9 mm × 150 mm; 5 μm particle size; Waters, USA) was employed as a stationary phase, while the mobile phase was a mixture of 0.1% CH₂O₂/MeOH-H₂O (60:40, v/v). The flow rate and temperature of the column were set as 0.8 mL/min and 25 °C, respectively. The instrument detection limits of TX-45, IOPA, CIP and BPA for an injection volume of 100 μL were calculated as 28, 78, 3.3 and 3.0 μg/L, respectively. Anion analysis was conducted using a Dionex ICS-1500 ion chromatography unit equipped with a conductivity detector, a Dionex IonPac AG14A (4 × 50 mm) guard column and a Dionex IonPac AS14A (4 × 250 mm) analytical column. All other analyses for conventional parameters were performed as defined in Standard Methods (APHA 2005).
Acute toxicity and genotoxic activity measurements

Before conducting the toxicity tests, residual PS in the test samples was removed with sodium thiosulfate (Olmez-Hanci et al. 2014). In order to eliminate their interference with the toxicity and genotoxicity test results, Al^{3+} residuals in the reaction solutions were removed in the form of Al(OH)_3 flocs by pH adjustment to 6–7 using 1 N NaOH, precipitation and membrane filtration steps prior to acute toxicity/genotoxicity analysis.

Acute toxicity

The toxicity towards the photobacterium V. fischeri was measured with a BioTox™ test kit (Aboatox Oy, Finland) based on the percent relative luminescence inhibition after an incubation period of 15 minutes in accordance with the ISO 11348-3 (2008) protocol. The acute toxicity towards the freshwater microalga P. subcapitata was determined using Algaltokit F™ (MicroBioTests, Inc., Ghent, Belgium) microbiotests according to the procedure described in ISO 8692 (2002). Percent relative growth inhibition rates were calculated after an incubation period of 72 h on the basis of a toxicant-free control. The application of these standard methods for the assessment of toxicity on V. fischeri and P. subcapitata has been described in more detail elsewhere (Olmez-Hanci et al. 2014). Acute toxicity results were reported as percent relative inhibition with respect to a control during MPs treatment in DW and RSW samples. A positive control sample with potassium dichromate was also included for each test and all bioassays were run in triplicate.

Genotoxicity

Test systems to determine the genotoxicity or mutagenicity can be grouped by the biological system employed and their genetic endpoint detected. Bioassays with prokaryotes (i.e., Salmonella) enable the detection of agents that induce gene mutation and primary DNA damages. Bioassays using Salmonella typhimurium strain TA1535/pSK1002 have been developed and standardized (ISO 15829 2000) for the determination of genotoxicity. Additionally, the inhibition of bacterial growth could be used as a parameter to measure cytotoxic potential of the samples (Masood & Malik 2013). In the present study, the umuC test (in triplicate) was carried out according to the procedure described by ISO 15829 (2000). The UMU-Chromo Test™ test kit was supplied by EBPI Environmental Bio-Detection Products Inc. (Canada). According to the ISO 15829 (2000) protocol, genotoxicity is measured by quantifying the induction ratio as the ratio of β-galactosidase activity of samples and controls in relation to the growth inhibition of the bacteria Salmonella typhimurium TA1535 (pSK1002). In the present study, several dilutions (namely 1:1.5, 1:3.0, 1:6 and 1:12) of the original and treated MP samples together with positive, negative and solvent controls were tested in accordance with the test protocol. For each sample dilution, the growth factor G, the β-galactosidase activity U_S and induction ratio I_R were calculated as given below:

\[ G = \frac{A_{600,S} - A_{600,B}}{A_{600,NC} - A_{600,B}} \]

\[ U_S = \frac{A_{420,S} - A_{420,B}}{A_{420,NC} - A_{420,B}} \]

\[ I_R = \left( \frac{1}{G} \right) \times \frac{A_{420,S} - A_{420,B}}{A_{420,NC} - A_{420,B}} \]

where \( A_{600,S} \) and \( A_{420,S} \) are the absorbance of sample S at 600 nm and 420 nm; \( A_{600,B} \) and \( A_{420,B} \) are the absorbance of the blank B at 600 nm and 420 nm; \( A_{600,NC} \) and \( A_{420,NC} \) are the absorbance of the negative control (NC) at 600 nm and 420 nm. Wavelengths of 600 nm and 420 nm were used to measure bacterial growth and quantify β-galactosidase activity, respectively. The optical density was measured using a Bio Rad Benchmark Plus microplate spectrophotometer. The whole test was considered valid if \( G > 0.5 \) and the positive controls reach an \( I_R \) value >2. Calculation of G allowed identification of toxic growth inhibitory effects, and \( G < 0.5 \), representing >50% inhibition of the biomass growth, was considered to be indicative of samples being cytotoxic. An \( I_R > 1.5 \) was taken as the threshold at which the sample was considered as genotoxic (ISO 15829 2000).

RESULTS AND DISCUSSION

Treatability of MPs with ZVA and ZVA-activated PS

In order to assess the oxidation capacity of the PS/ZVA treatment system for TX-45, IOPA, CIP and BPA degradation, some preliminary experiments were conducted in DW (MPs = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM or 0.50 mM; pH = 3; t = 120 min). Additionally, the degradation of MPs with mere PS oxidation (in the absence of ZVA) and ZVA/
H⁺/O₂ treatment (in the absence of PS) was also examined. MPs abatement profiles as a function of treatment time for mere PS oxidation and the ZVA/O₂/H⁺ system are presented in Supporting Information Figures S1 and S2, respectively (available with the online version of this paper). Table 1 also summarizes percent overall MPs removal efficiencies obtained from these preliminary experiments. As can be seen from Figures S1 and S2 as well as Table 1, TX-45, IOPA and BPA abatements were poor for mere PS oxidation and for ZVA/H⁺/O₂ treatment as well. These results indicated that the ZVA/H⁺/O₂ treatment system as well as mere PS oxidation were not effective for the removal of TX-45, IOPA and BPA. On the other hand, high removal efficiency was achieved for CIP (58%) at the end of 120-minute ZVA/O₂/H⁺ treatment. As aforementioned in situ generated HP in the aqueous suspension of ZVA serves as a precursor of the HO¹, which is primarily responsible for the effective CIP oxidation in the ZVA/H⁺/O₂ system (Bokare & Choi 2009).

As expected, PS activation with ZVA greatly enhanced the degradation rates of MPs as compared to mere PS oxidation and ZVA/H⁺/O₂ treatment. External addition of 0.25 mM PS to the reaction medium increased the TX-45 and IOPA removal efficiencies to 86% and 75%, respectively, at the end of 120-minute PS/ZVA treatment. Nonetheless CIP and BPA were completely degraded after 120-minute and 40-minute treatment with the PS/ZVA (0.25 mM) process. As can be seen from Table 1 increasing the initial PS concentration improved the TX-45 (from 86% to 100%) and IOPA (from 75% to 95%) removals. In contrast, CIP removal efficiencies decreased with increasing the initial PS concentration from 0.25 mM (100%) to 0.50 mM (69%). Correspondingly, the time required for complete removal of BPA increased with increasing the initial PS concentration. These observations could be attributed to the well-known SO⁴⁻ scavenging effect of excessive PS (Arslan-Alaton et al. 2017a).

### Effect of the water matrix

In order to assess the process performance of the PS/ZVA treatment process for MPs removal in RSW samples, a set of degradation experiments were conducted under the following optimized reaction conditions: MPs = 2 mg/L; ZVA = 1 g/L; pH = 3; PS = 0.25 mM (for CIP and BPA) and 0.50 mM (for TX-45 and IOPA). Figure 1(a) and 1(b) delineates time-dependent changes in TX-45, IOPA, CIP and BPA concentrations during PS/ZVA treatment in DW and RSW, respectively. In the natural water sample, a dramatic decrease in TX-45, IOPA, CIP and BPA removal rates was observed owing to the presence of organic and inorganic pollutants. The removal efficiencies of TX-45, IOPA, CIP and BPA were lower in RSW than in DW, which could be attributed to the presence of natural organic materials that act as electron donors, thus limiting the availability of H₂O₂ for the reaction with HO¹.

### Table 1

Comparison of overall percent MP removal efficiencies obtained after 120-minute PS oxidation as well as ZVA/O₂/H⁺ and PS/ZVA treatments in DW

<table>
<thead>
<tr>
<th>Treatment system</th>
<th>TX-45</th>
<th>IOPA</th>
<th>CIP</th>
<th>BPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS (0.50 mM PS)</td>
<td>20</td>
<td>10</td>
<td>10a</td>
<td>10</td>
</tr>
<tr>
<td>ZVA/O₂/H⁺</td>
<td>22</td>
<td>8</td>
<td>58</td>
<td>18</td>
</tr>
<tr>
<td>PS/ZVA (0.25 mM PS)</td>
<td>86</td>
<td>75</td>
<td>100</td>
<td>100b</td>
</tr>
<tr>
<td>PS/ZVA (0.50 mM PS)</td>
<td>100c</td>
<td>95</td>
<td>69</td>
<td>100d</td>
</tr>
</tbody>
</table>

Treatment conditions: MP = 2 mg/L; ZVA = 1 g/L; pH = 3; T = 25 °C.

<table>
<thead>
<tr>
<th>PS</th>
<th>0.25 mM</th>
</tr>
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<tbody>
<tr>
<td>0.50 mM</td>
<td></td>
</tr>
<tr>
<td>40 min</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
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</table>

Figure 1 Time-dependent changes in TX-45, IOPA, CIP and BPA concentrations during PS/ZVA treatment in DW (a) and RSW (b). Treatment conditions: MP = 2 mg/L; DOC_{org} = 7.2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3; T = 25 °C. Error bars are standard deviations of triplicate measurements.
inorganic competitors for SO$_4^{2-}$. The corresponding TX-45, IOPA, CIP and BPA removal efficiencies in RSW were 59%, 29%, 73% and 46%, respectively. These attained results are consistent with the presence of organic (i.e., natural organic matter) and inorganic (i.e., nitrate, chloride, phosphate) compounds present in the RSW sample capable of scavenging SO$_4^{2-}$. It is expected that the presence of water components inhibits oxidation of MPs. Similar results were also obtained by Baeza & Knappe (2011) who suggested that the presence of radical scavengers in lake water and wastewater treatment plant effluent matrices was the dominant factor that decreased biochemically active compound (sulfamethoxazole, sulfamethazine, sulfadiazine, trimethoprim, BPA and diclofenac) transformation rates. Dimitroutla et al. (2012) also observed a decrease in the oxidation rate of several MPs of one order of magnitude when comparing the reaction in pure water with a wastewater matrix. Moreover, advanced oxidation of organic pollutants in real water matrices might lead to the formation of different intermediates types than in pure water that could lower overall process efficiency, since these species could be less reactive with the SO$_4^{2-}$ or HO$^-$ (Kiwi et al. 2000).

Changes in acute toxicity

Considering that the degradation and toxicity pattern of MPs will be affected by the characteristics of the natural water sample, changes in acute toxicity and genotoxicity were examined during PS/ZVA treatment of MPs in DW and RSW. Percent relative inhibition values before (t = 0) and during PS/ZVA treatment of MPs in DW and RSW samples are presented for V. fischeri and P. subcapitata in Figures 2 and 3, respectively. Toxicity analysis of the original and PS/ZVA-treated MP samples demonstrated different patterns for the studied test organisms (V. fischeri and P. subcapitata) as well as the water matrix. The percent relative inhibitions of the original (untreated) TX-45, IOPA, CIP and BPA samples in DW towards V. fischeri were in decreasing order of CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%). On the other hand, in the natural water sample bearing MPs, the decreasing order of relative inhibitory effects were obtained as CIP (42%) > IOPA (27%) > BPA (12%) > TX-45 (6%). From the above findings it might be inferred that the V. fischeri toxicities in the DW samples were suppressed as compared with the RSW, except for IOPA. The original IOPA in the DW sample was practically non-toxic towards V. fischeri, whereas an inhibitory effect of 27% was observed for RSW sample. CIP appeared to be the most toxic MP towards V. fischeri for both water matrices.

The obtained findings indicate that the toxicity results depend on complex interaction between the pollutant type (its physicochemical properties), water matrix and test organism.

As can be seen from Figure 2(a) and 2(b), the inhibitory effect of aqueous MP solutions towards V. fischeri fluctuated in the case of PS/ZVA treatment both in DW and RSW, most probably due to the formation and subsequent oxidation of MP degradation products. The V. fischeri toxicities of CIP and BPA remained nearly constant throughout the PS/ZVA treatment in DW, determined as 52% and 36%, respectively, after 120-minute treatment. PS/ZVA-treated TX-45 in DW resulted in a slightly higher toxicity towards V. fischeri (26%) than in RSW. As is reported in the related literature, the step-wise loss of the side chain ethoxylate units of alkylphenol ethoxylates typically results in more hydrophobic and hence more toxic intermediates (Hill & Jans 2005). In the case of IOPA, 120-minute
PS/ZVA treatment in DW did not result in toxic degradation products towards V. fischeri. The original V. fischeri toxicities of CIP, IOPA, BPA and TX-45 were 42%, 27%, 12% and 6%, respectively. It is apparent from Figure 2(b) that, after 120-minute PS/ZVA treatment, TX-45 (8%) and BPA (1%) were found to be practically non-toxic, whereas 19% and 23% relative inhibitions were still evidenced for IOPA and CIP, respectively.

According to the P. subcapitata growth inhibition test results given in Figure 3(a) and 3(b), the untreated BPA (100%), CIP (66%) and IOPA (62%) in DW samples were highly toxic. On the other hand, untreated TX-45 (35%) was not as toxic as BPA, CIP and IOPA. Similar to V. fischeri toxicities, the inhibitory effect of the original BPA and CIP samples was suppressed in RSW, measured as 56% for BPA and 19% for CIP. It is interesting to point out that the P. subcapitata toxicity of IOPA in RSW sample increased to 88%, whereas TX-45 toxicity in RSW (39%) was close to the value being observed in DW (35%).

The increasing and decreasing trend of toxicity towards P. subcapitata was also evidenced during PS/ZVA treatment of the investigated MPs in DW and RSW samples. As can be seen from Figure 3(a) the acute inhibitory effect showed no significant change within 120-minute PS/ZVA treatment of TX-45 (from 35% to 40%) and CIP (from 66% to 64%) in DW. On the other hand, the untreated IOPA in DW showed an increase in its inhibitory effect from 62% to 95% after 120-minute treatment, most possibly due to the presence of some degradation products being more toxic than the original IOPA solution (Arslan-Alaton et al. 2017). In the case of BPA in DW, the relative inhibition of 100% decreased to 80% after 120-minute treatment time, which was still very high.

In general, the bioassay performed with P. subcapitata in DW appeared to be more sensitive to the investigated MPs degradation products among the selected bioassays. P. subcapitata toxicity patterns differed appreciably for RSW samples; during PS/ZVA treatment of BPA, percent relative inhibition increased from 56% to 64% after 120 minutes, which was not practically different from that of the untreated BPA. In the case of IOPA, the relative inhibition fluctuated during the treatment and reached a value (85%) which was at the same level of the untreated IOPA (88%). On the other hand, TX-45 degradation products exhibited a low toxic effect towards P. subcapitata after 120 minutes (around 25%). For PS/ZVA treatment of CIP in RSW, P. subcapitata toxicity of CIP increased from 19% to 57% after 120 minutes, attesting to the fact that degradation products were more toxic towards P. subcapitata than the original CIP.

It should be pointed out here that the removal efficiencies of the investigated MPs and aquatic toxicity patterns being observed before, during and after treatment with PS/ZVA process in DW and RSW matrices were quite different from each other. Furthermore, the sensitivity of microorganism used in toxicity tests towards MPs and their degradation products was found to be different. Consequently, based on the data obtained in this study, it is advisable to adopt different specific toxicity tests to refine the identification of toxic/inhibitory effects linked to the presence of MPs in different water matrices.

Changes in genotoxic activity

Figure 4 presents the $I_G$ values obtained for the untreated and PS/ZVA-treated TX-45, IOPA, CIP and BPA in DW. The calculated G values demonstrated that neither the original nor the treated products of TX-45, IOPA and BPA were cytotoxic. As aforementioned, in the ISO 13829 test.
protocol, a growth factor (G) of less than 0.5 (representing 50% inhibition of biomass growth) is considered to be indicative of cytotoxic samples, and as indicated in the validity criteria of the above test protocol, the results cannot be evaluated if $G < 0.5$. In the present study, only CIP was found to be cytotoxic ($G = 0.23$) at the studied dilution ratios and due to $G < 0.5$ the $I_R$ could not be calculated. Both untreated (Figure 4(a)) and PS/ZVA-treated (Figure 4(b)) TX-45, IOPA and BPA samples had no genotoxic activity according to the umuC test results. However, PS/ZVA treatment of CIP in DW caused the formation of genotoxic degradation products after 120 minutes. The induction ratios of 1:0.1:5 and 1:0:3:0 dilutions were calculated as 3.42 and 1.54, respectively, for the PS/ZVA-treated CIP samples. As indicated in former related studies (Mersch-Sundermann et al. 1994; Kümmerer et al. 2000), topoisomerases-II inhibitors like CIP and ofloxacin are very strong genotoxicants for *Escherichia coli* PQ37 (SOS Chromo test) and mutagens for *Salmonella typhimurium* TA102 (Ames test) in the absence of an exogenous metabolizing system. In a recent study, CIP and samples of photolyzed aqueous CIP exerted heritable genotoxic effects in the HepG2 cell line (Garcia-Käufer et al. 2012). In this study, an aqueous CIP solution at a concentration of 0.4 mg/L (1.2 μmol/L) was found to meet the lowest observed effect level, as was assessed previously by dose-response experiments. It was also shown that the photolytic decomposition products of CIP and other fluoroquinolones are more genotoxic than the drug itself (Sanchez et al. 2005; Pereira et al. 2007). As has been emphasized in Garcia-Käufer et al. (2012), for the induction of heritable mutations or cancer in humans involved with exposure to CIP or its transformation products, more detailed studies, especially those applying *in vivo* toxicity tests, will be necessary.

CONCLUSIONS AND RECOMMENDATIONS

In the present study, activation of PS with nanoscale ZVA (1 g/L) particles was investigated in DW and RSW samples for the treatment of 2 mg/L TX-45, IOPA, CIP and BPA at pH 3 for 120 minutes. Changes in acute toxicity and genotoxicity patterns during PS/ZVA treatment of selected MPs were particularly studied. The major findings of this work are summarized below:

- Mere PS oxidation resulted in MP removals in the range of 10–22%.
- ZVA/O2/H+ treatment led to inefficient degradation of TX-45 (22%), IOPA (8%), CIP (58%) and BPA (18%) at the studied treatment conditions: MP = 2 mg/L; ZVA = 1 g/L; pH = 3; $T = 25^\circ$C; $t = 120$ min.
- On the other hand, PS activation with nanoscale ZVA greatly enhanced the MP removals, resulting in complete CIP (in 120 minutes) and BPA (in 40 minutes) degradation, whereas 86% and 75% removal efficiencies were realized for TX-45 and IOPA, respectively, with PS/ZVA treatment (MP = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM; pH = 3; $T = 25^\circ$C; $t = 120$ min).
- The decreasing order of MP removal efficiencies in DW at the optimized treatment conditions (MP = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3; $T = 25^\circ$C) were: BPA (100% in 40 minutes) > TX-45 (100% in 90 minutes) > CIP (100% in 120 minutes) > IOPA (95% in 120 minutes).
- The environmental characteristics of the water matrix (its inorganic and organic matter content) represented a
drawback for MPs removal. The efficiency of the PS/ZVA treatment system in RSW after 120 minutes followed the decreasing order of CIP (73%) > TX-45 (59%) > BPA (46%) > IOPA (29%).

- The percent relative inhibitions of the original TX-45, IOPA, CIP and BPA samples in DW were in order of CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%) towards V. fischeri, whereas the decreasing order was found as BPA (100%) > CIP (66%) > IOPA (62%) > TX-45 (35%) towards P. subcapitata.

- Acute toxicities of the original BPA and CIP samples were suppressed in the natural water samples. In the case of original IOPA, V. fischeri and P. subcapitata toxicities in RSW increased to 27% and 88%, respectively, compared to DW. P. subcapitata toxicity of TX-45 in DW and RSW samples were found to be at the same level (35–39%) whereas a lower V. fischeri toxicity was evidenced in RSW (6%) sample than in DW (15%).

- The V. fischeri and P. subcapitata toxicities fluctuated during PS/ZVA treatment both in DW and RSW samples and resulted in different relative inhibition values at the end of 120-minute treatment.

- According to the UMU-Chromo test results, the original and PS/ZVA-treated TX-45, IOPA and BPA samples exhibit neither cytotoxic nor genotoxic effects. Only CIP was found to be cytotoxic at an initial concentration of 2 mg/L and PS/ZVA-treated CIP resulted in the formation of genotoxic degradation products.

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