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The use of peracetic acid for estrogen removal from urban wastewaters: E2 as a case study

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Abstract 17β-Estradiol (E2) is a natural estrogen produced by the feminine endocrine system. It is excreted mainly through urine and feces. Exposure to E2 may affect the reproductive system of both animals and humans, especially since the removal of E2 in conventional processes and technologies present in the wastewater treatment plants is not sufficient. Chlorine is one of the most studied and used oxidant worldwide. Although there are studies that demonstrate the endocrine disrupting compounds removal like E2, its reaction with organic matter can originate by-products, namely, trihalomethanes, which are known to have high toxic potential. The main aim of the present study was to evaluate the removal of E2 (50 μg E2 L^{-1}—maximum concentration) using peracetic acid (PAA), a seeming cleaner and innocuous alternative to chlorine. To this end, a series of jar tests were performed, using different peracetic acid concentrations (1, 5, 10, and 15 mg L^{-1}) and contact times (10, 15, and 20 min). The results obtained showed that a peracetic acid concentration of 15 mg L^{-1} with a contact time of 20 min had a removal efficacy of approximately 100%. The second main goal of this study was to evaluate the ecotoxicological potential of the tested treatments on the zebrafish *Danio rerio*. Several oxidative stress biomarkers were evaluated, namely glutathione *S*-transferase, lipid peroxidation, and catalase, besides vitellogenin. Both peracetic acid and E2 caused significant increases in the oxidative stress biomarkers, although this did not lead to increased lipid peroxidation levels. In addition, peracetic acid significantly decreased the estrogenic activity of E2, as indicated by decreased vitellogenin levels. Peracetic acid demonstrated to have great potential as an alternative disinfectant for chlorine treatments, and indications for future research are discussed.

Keywords 17β-Estradiol (E2) removal · Endocrine disrupting compounds · Wastewater peracetic acid · Bioassays

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Introduction

According to the IPCS (2002) and UNEP/WHO (2012), an endocrine disrupting compound (EDC) may be defined as "chemicals, or chemical mixtures, that interfere

with normal hormone action." These substances can disperse rapidly into the environment and main retention basins are underground waters, rivers, and lakes (Barreiros et al. 2016; Auriol et al. 2006). There has been increasing concerns related with the exposure to EDC since they may exert toxicity effects at very low concentrations although many research gaps remain including determining concentrations considered safe in relation to these types of compounds (Bila and Dezotti 2007, Chen et al. 2017; Vilela et al. 2018).

The introduction of EDC into the aquatic environment can occur through two distinct ways, namely, through diffuse sources and through a point source (single location). The discharge of domestic or industrial influents and leaching of landfills are examples of the latter source. As for the diffuse sources, the main ones are agricultural run-off, aquaculture, and wash off from roadways (Bolong et al. 2009; Ting and Praveena 2017; Maurício et al. 2018).

In the EU, EDC is included in the *Watch List* of emerging substances due to their ubiquity in the environment, as well as their number of uses in the industry, agriculture, and domestic use (EC 2015). In particular, 17β-estradiol (E2) has been given special attention due to its active estrogenicity and potential risk to ecosystems and human life (Cong et al. 2017; Schiliró et al. 2009). In this context, E2 is considered as a priority pollutant through the Water Framework Directive (WFD) of the European Union (Dai and Liu 2017). E2 is a hormone of the feminine reproductive system, responsible for the maintenance of sexual characteristics, such as breast growth and proliferation of epithelium cells (Hassani et al. 2016; Barreiros et al. 2016). The excretion of E2 is mainly through urine and feces, in conjugated form (Guedes et al. 2014; Auriol et al. 2006; Hu et al. 2003; Xu et al. 2012). The quantity of E2 released can vary, primarily due to population structural differences, their age and individual metabolism, as well as ethnic and gender differences, amongst other factors. The concentration range of E2 release is from several hundred to a few units, expressed in nanograms per liter (Xu et al. 2012; Zhang et al. 2011; Barreiros et al. 2016).

Several studies have indicated that the main part of estrogen pollution comes from point sources (Auriol et al. 2006; Schiliró et al. 2009; Bolong et al. 2009; Caliman and Gavrilescu (2009); Falconer et al. 2006; Pereira et al. 2011; Prasse et al. 2015). In addition, these case studies showed behavioral and morphological difference in aquatic fauna downstream from estrogen-containing effluent discharges. These differences include (i) alteration in the sexual development and mating process of some species (Hamid and Eskicioglu 2012), (ii) a ratio decrease between males and females (Dias et al. 2015) due to the process of feminization through the synthesis of VTG, (iii) an increase of cases of hermaphroditism, premature death of offspring, inhibition of testicular growth (Prasse et al. 2015; Bila and Dezotti 2007), and (iv) a decrease in functionality of the immune system in aquatic mammals, associated with the exposure to these compounds (Bila and Dezotti 2007; Ahmed et al. 2017). Similarly, there are also a number of risks for humans, mainly not only due to the consumption of animals that contain estrogens in their systems but also due to the consumption of water containing these compounds (Nollet and Lambropoulo 2017; Caliman and Gavrilescu 2009). It is known that the endocrine disruption in human beings can lead to infertility, reproductive system malfunctions, changes in the thyroid function (Bolong et al. 2009; Caliman and Gavrilescu 2009), increase in the occurrence of cases of cancer (breast, ovaries and prostate) (Bolong et al. 2009; Adeel et al. 2017), and an increase in the sperm count and testicular enlargement (Bolong et al. 2009; Pereira et al. 2011).

Currently, the wastewater treatment plants (WWTP) are designed to eliminate phosphorus, nitrogen, and carbon and only a small fraction, associated with current conventional wastewater treatments, of emerging compounds is removed simultaneously, in which about 10% of estrogens are removed through, for example, biological treatment (Auriol et al. 2006). The remaining fraction flows through the treatment plant (Auriol et al. 2006), making WWTP one of the main sources of estrogen pollution (Adeel et al. 2017; Auriol et al. 2006). However, there are a few studies that indicate that EDC and namely E2 can be removed from wastewaters in very high percentages. These treatments consist in multi barrier treatments, advance oxidations, modified biological systems, like MBR, or even conventional disinfection processes (Maurício et al. 2018). Wastewater disinfection is of extreme importance when it comes to the removal of pathogenic organisms to avoid contact with humans and animals (ECDC 2011). This process is also particularly important for EDC, because they can be removed through this process (Bolong et al. 2009). Chlorine (Cl) is one of the most used oxidants in wastewater disinfection, which is effective in the removal of EDC, namely estrogens, and

ensures high efficiency. Previous studies have proven that for an initial concentration of 100 ng L^{-1} of E2, a chlorine dose of 2 mg L^{-1} and a contact time of 30 min are sufficient to decrease the concentration below its detection limits of 30 ng L^{-1} (Pereira et al. 2011; Dias et al. 2015; Freese and Nozaic 2004; Ahmed et al. 2017). Despite the high removal efficiencies of chlorine, its biggest disadvantage is the formation of toxic and carcinogenic by-products, such as trihalomethanes (THM) (Du et al. 2017; Freese and Nozaic 2004).

As an alternative to chlorine, peracetic acid (PAA) has been considered as a potential good alternative option (Luukkonen et al. 2014). Its disinfection potency is similar to chlorine or ozone, being as effective in the removal and inactivation of pathological organisms (Antonelli et al. 2013; Azzellino et al. 2011; Luukkonen and Pehkonen 2017). The disinfection process of PAA is through a chemical pathway, unlike e.g. UV, which makes it simple to operate, with a quick start and low maintenance costs (Wagner et al. 2002; Rizzo et al. 2019). The disinfecting action of PAA is through the release of active oxygen or the production of reactive hydroxyl radicals that attack the bacterial cell causing the destruction of the cell wall and membrane as well as certain enzymes and DNA (Collivignarelli et al. 2017; Karpova et al. 2013; Luukkonen et al. 2014). As compared to chlorine treatment, one of the biggest advantages of this disinfectant is that it only produces innocuous by-products, like acetic acid, oxygen, and water, with little toxic potential for the aquatic environment (Kitis 2004; Henao et al. 2018; Rizzo et al. 2019). However, its mode of action could dictate that PAA may potentially provoke oxidative stress to beneficial organisms in waterbodies receiving PAA-treated WW (Chhetri et al. 2014). Most studies evaluating the efficacy of PAA have so far only focused on the removal of microorganisms, indicating that its potential to remove EDC like E2 remains poorly known (Bonetta et al. 2017; Rizzo et al. 2019). There is no evidence, however, of any endocrine disruption potential of PAA itself in human health and ecotoxicological studies (Henao et al. 2018).

The aim of the present study was to assess the efficacy of PAA in the removal of E2 from an urban wastewater. To this end, jar tests were conducted to determine the E2 concentration reduction. In addition, the estrogenic activity (vitellogenin—VTG), antioxidant enzyme activities (glutathione *S*-transferase—GST, and catalase—CAT), and oxidative stress (lipid peroxidation—LPO) were determined in zebrafish (*Danio rerio*) following exposure to the highest E2 and PAA concentrations evaluated in the jar test. Effects of PAA on wastewater quality (pH, chemical oxygen demand—COD, total suspended solids—TSS) were also determined as to evaluate whether PAA-treated wastewater remained within the limits set for these parameters in the EU.

Materials and methods

Wastewater characterization

The wastewater used in this study was collected from the WWTP "Quinta do Conde" that is located in the Sesimbra region near Lisbon (Portugal) and discharges its treated effluent into the River Tagus basin (Fig. 1). This WWTP was designed to collect and treat a flow of 19,300 m^3 day^{-1} of urban wastewater corresponding to approximately 94,000 equivalent inhabitants. It provides secondary and tertiary treatments, with oxidation ditches and a final ultra-violet disinfection system before the effluent is discharged. This WWTP also includes an internal water reuse system.

The wastewater used in this work was a secondary effluent, i.e., collected after the secondary decantation. The main physical-chemical characteristics of the wastewater were determined using the methods described in APHA (2005) and are shown in Table 1, in "Results and discussion."

The wastewater was filtered was vacuum filtered with an operating pressure of 4 bar (KNF Neuberger N035AN) with 1.2 μm followed by a 0.4-μm glassfiber filter from Filter Lab (MFV3) and paper filter from Macherey-Nagel (MN GF5).

Peracetic acid assay

Three jar tests were conducted to evaluate the efficiency of PAA (Merck KGaA; concentration 38 to 40%) in the removal of 50 μg E2 L^{-1} (Alfa Aesar (L03801) with 99% purity), in order to study a worst-case scenario. These E2 and PAA concentrations were derived by diluting stock solutions prepared in methanol and distilled water, respectively. Each treatment was conducted with three replicates, each consisting of a glass jar containing 1 L treatment solution. After the required contact time had elapsed, the PAA reaction was stopped

Fig. 1 Location of the wastewater treatment plant station used as source of the wastewater evaluated

through the addition of 100 mg sodium thiosulfate L^{-1} (Gehr et al. 2003).

To determine the E2 removal efficiency with different PAA dosages, E2 concentrations were measured after the treatments. This was done through stir bar sorptive extraction (SBSE) followed by high performance liquid chromatography with diode-array detection (HPLC-DAD), as detailed in Maurício et al. (2018). Under these analytical conditions, the limits of detection (LOD) and quantification (LOQ) were calculated according to Shrivastava and Gupta (2011)—LOD = 7.82 μg L^{-1} and LOQ = 19.80 μg L^{-1}.

Different contact times were equally evaluated (10, 15, and 20 min) in a total of 3 assays. For each assay, the glass jars were placed in a *Jar test* device at 200 rpm (rotation speed).

The extraction was performed by SBSE method, using a *Gerstel Twister™ PDMS* (GC 011555–001-00) bar. The 1-L samples were divided into four Erlenmeyers with 250 mL capacity and salted with NaCl (100 g L^{-1}), covered with a black plastic bag and agitated during a 3-h period at 900 rpm. Afterwards, for the desorption process to take place, the bars were placed in 5-mL vials, with 3 mL of acetonitrile (*Carlo Erba Reagents*) and agitated during 3 additionally hours at 300 rpm. After the agitation, the vials where warmed in a water bath, at a 60 °C temperature during approximately 10 min, followed by 5 min of ultrasounds (*Julabo* USR 3/2). To avoid any solids in this process, the samples were submitted to an acrodisc filtration (*Whatman*, Anatop 10, 0.2 μm, ∅ = 10 mm) and evaporated up to the conic part and sonicated a second time to prevent the E2 loss. Finally, the remaining solution was transferred to 2-mL vials with inserts and evaporated until dryness and then recovered with 100 μL of methanol (*Honeywell/Riedel-de Haën™*).

Table 1 Wastewater characterization

Parameter	Value
pH	7.9
TSS (mg L^{-1})	< 10
Chemical oxygen demand (COD) (mg O_2 L^{-1})	67
Nitrogen (N—mg N L^{-1})	35
Phosphorus (P—mg P L^{-1})	2
E2 (ng L^{-1})	n.d.

n.d. non-detectable

The analysis was made using HPLC-DAD injection (Waters® 2690 separation module (Milford, MA, USA) coupled to a WaersTM 996 photodiode array detector). The E2 was quantified at 281 nm, and the determination was performed using a Luna C18 column (Phenomenex, 5-μm particle size, LC Column size 150 × 3 mm) and a precolumn C18, 3 mm Milli-Q ultra-pure water with 1% of formic acid (HCOOH, Panreac, 98% purity, MW 46.03 g mol^{-1}) and acetonitrile used as the mobile phase, with an isocratic 50:50 gradient composition. The analysis time was 10 min, in which E2 was detected at analysis time of 4.4 min. The flow rate was 0.55 mL min^{-1}, and the sample injection volume was 20 μL. The chromatograms were acquired with a MassLynxTM software data acquisition system.

Biological assays

The toxicity tests were carried out with young zebrafish (*Danio rerio*) obtained from a commercial source (Aquaplante, Portugal) and acclimatized to laboratory conditions for 2 weeks prior to testing. Animals were

housed in a closed-circuit system consisting of a 100-L volume aquarium filled with filtered dechlorinated tap water with pH 7.1 ± 0.1; temperature 20 ± 1 °C; photoperiod 12:12 (light:dark), and continuous aeration (> 6 mg O_2 L^{-1}).

At the beginning of the tests, adult fish of both sexes (weight 0.3 ± 0.1 g; length 2.4 ± 0.2 cm; mean ± SD) were randomly distributed over five polystyrene test tanks containing 10-L test medium to assess the following treatments (all $n = 7$): control, solvent control (0.05% methanol V/V), 50 μg E2 L^{-1}, 15 mg PAA L^{-1}, and 50 μg E2 L^{-1} + 15 mg PAA L^{-1}. E2 stock solutions were prepared in methanol and distilled water. Then adequate aliquots of E2 and/or PAA stock solutions in distilled water were added to aquaria for exposure tests. The E2 and PAA concentrations were selected since they corresponded to the (highest) concentrations evaluated in the jar tests (c.f. "Peracetic acid assay"). The methanol concentration used in the solvent control was the same as in E2 and E2 + PAA treatments. During the 7 days test, fish were fed daily adding libitum with commercial fish food (Tetramin®). Test solutions were renewed every 48 h.

After the 7 days exposure period, zebrafish were collected and euthanized on ice by cervical sectioning Subsequently, each specimen was homogenized individually by trituration (Tissue Master 125 homogenizer) in 3.0 mL PBS buffer solution (Na_2HPO_4 with KH_2PO_4, KCl and NaCl, all Sigma-Aldrich, in 1 L Milli-Q water, pH = 7.4) and equally divided into two microtubes (1.5 ml) as replicas. Then, samples were centrifuged for 15 min (10,000×*g* at 4 °C), transferred to new microtubes (1.5 mL) and stored at − 80 °C until further analysis.

Glutathione *S*-transferase (GST) activity, lipid-peroxidation catalase (CAT) activity, and VTG were determined. The GST activity was determined at 340 nm following a procedure first described by Habig et al. (1974) and adapted for 96-well microplates (Diniz et al. 2013). The lipid peroxidation was assessed by adapting the TBARS method, which is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) resulting in a compound absorbing at 532 nm (Ohkawa et al. 1979). The catalase (CAT) activity was determined spectrophotometrically at 540 nm as described in Johansson and Borg (1988) and adapted for 96-well microplates. Finally, the determination of VTG was carried out by the ELISA method adapted from Denslow et al. (1999) and following the same procedure as described in Diniz et al. (2010).

The total amount of proteins in samples was carried out by the Bradford method (1976). A microplate reader (Bio-Rad, Benchmark, USA) was used for all the spectrophotometric measures. Enzyme activities and VTG concentrations were expressed according to samples total protein.

Complementary analysis

To further understand the PAA effect, it was performed two additional determinations—COD and pH. It was important to evaluate these two specific parameters because they are always present in international standards and legislation. Also, it is described that there still is a lack of knowledge concerning the PAA possible interference in COD value and determination (analytical standard method) and in the final wastewater pH (after PAA addition).

Data analysis

Results were analyzed using the non-parametric Mann-Whitney *U* test since statistics assumptions were not fulfilled. Statistical analyses were performed with the Statistica software (Statistica version 8.0; Statsoft Inc., Tulsa, OK, USA, 2007) at a significance level of 5%.

Results and discussion

Wastewater characterization

In Table 1, the wastewater main characteristics are shown.

The information obtained for the wastewater main quality parameters (Table 1) corresponded to a common secondary effluent, from an urban wastewater treatment plant. It was also verified that total all parameters, namely chemical oxygen demand, suspended solids, nitrogen, and phosphorus values, were according to the discharge EU legislation limits.

Efficacy of PAA in the removal of E2

The E2 removal efficiency obtained for the different PAA treatments (concentrations and contact times) are visualized in Fig. 2.

At the lower PAA concentrations, a removal efficiency of 9–90% (1 mg PAA L^{-1}), 28–70% (5 mg PAA L^{-1}),

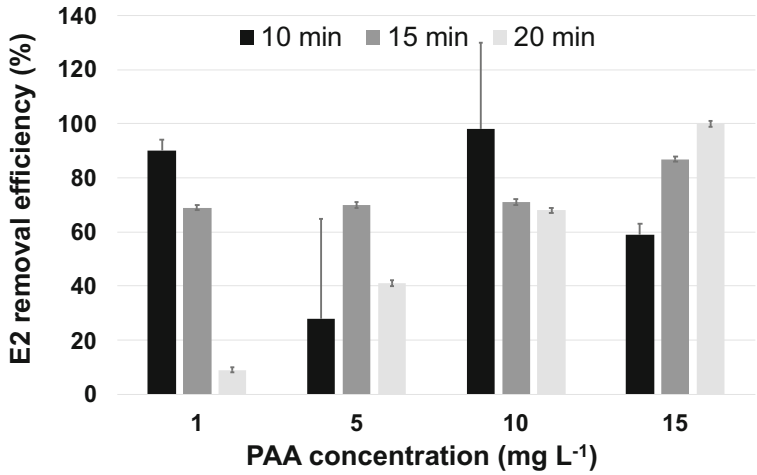

Fig. 2 Jar test results—E2 removal efficiency with different PAA treatments (concentrations and contact times)

and 68–98% (10 mg PAA L^{-1}) could be attained (Fig. 2). Interestingly, increasing the contact time at these PAA concentrations did not consistently result in greater removal efficiencies. Only at the highest PAA concentration tested (15 mg PAA L^{-1}), a clear increase in efficiency with increasing contact time could be denoted: Efficiencies increased from 59 ± 4% (10 min) to 87 ± 1% (15 min) up to 100 ± 3% (20 min; Fig. 2).

The relative importance of PAA concentration and its contact time with WW has been a matter of debate in the past decade. Several authors indicated that PAA disinfection efficacy depends more on dosage than on its contact time (e.g., Azzellino et al. 2011; Luukkonen et al. 2014), whereas other authors concluded the contrary (e.g., Dell'Erba et al. 2007; Chhetri et al. 2014). According to Coyle et al. (2014), both contact time and the applied disinfectant dosage are significant factors in achieving a satisfactory disinfection level. Microbial inactivation models also usually rely on both PAA concentration and contact time (e.g., Antonelli et al. 2013; Santoro et al. 2007). In our study, it appears that a sufficient PAA concentration (15 mg L^{-1}) is a prerequisite, after which the PAA contact time is crucial for an effective E2 removal (Fig. 2). This was further evaluated by plotting the E2 removal efficiency as a function of the PAA concentration multiplied with its contact time (Fig. 3).

No significant correlation could be demonstrated when using all data ($r = 0.48$; DF = 10; $p > 0.05$; Fig. 3a). As may be deducted from Fig. 3a, this is especially due to the scatter in data at "PAA concentration X contact time" values < 150. Indeed, when excluding the latter data, a positive correlation was obtained ($r = 0.92$; DF = 3; $p < 0.05$; Fig. 3b). Although it should be noted that this is based on few data points ($n = 5$), this indicates that a "PAA concentration × contact time" value of 150 (e.g., 15 mg PAA L^{-1} for 10 min or 10 mg PAA L^{-1} for 15 min) would assure an E2 removal efficiency of about 64%. The proposed (annual average)

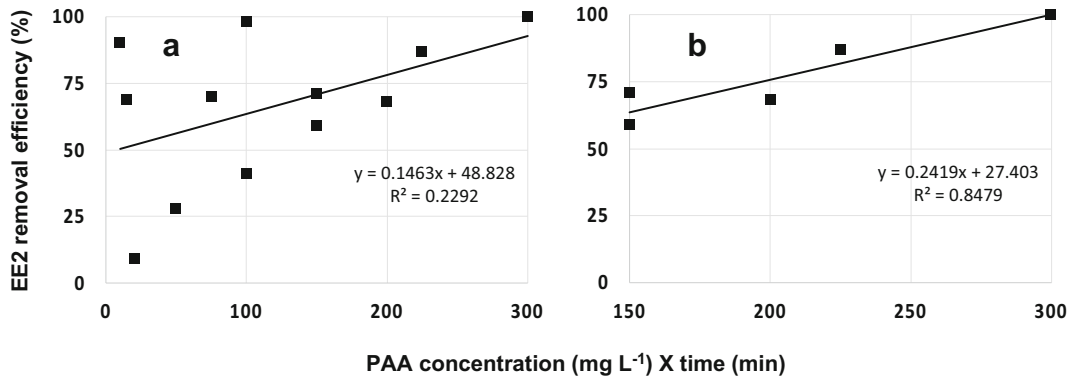

Fig. 3 E2 removal efficiency as a function of the PAA concentration multiplied with its contact time. **a** All data included. **b** With values < 150 excluded

environmental quality standards for E2 in the EU are below 1 ng L^{-1} (0.4 ng L^{-1} for inland surface waters and 0.08 ng L^{-1} for other surface waters; EC 2015). To achieve these standards, a removal efficiency of 100% is likely to be needed for WW effluents containing detectable levels of E2. This thus indicates that a PAA concentration × contact time value of 300 (e.g., 15 mg PAA L^{-1} for 20 min) would be needed (Fig. 3b).

To the best of our knowledge, Block et al. (2015) is the only other study that evaluated the efficacy of PAA in the removal of E2. These authors evaluated contact times of 10 and 20 min with PAA concentrations of 1, 5, and 10 mg PAA L^{-1} to disinfect 5 μg E2 L^{-1}. Efficacies above 79% were obtained for all treatments, but not always with a clear dose-response relationship like in our study. At PAA concentration × contact time values of both 100 (5 mg PAA L^{-1} for 20 min and 10 mg PAA L^{-1} for 10 min) and 200 (10 mg PAA L^{-1} for 20 min), E2 removal efficacies of about 90% were obtained, respectively (values calculated by the authors based on the data in Block et al. 2015). In our study, an E2 removal efficiency of only approximately 70% was attained at these conditions (Fig. 3). This lower removal efficiency as compared to the study by Block et al. (2015) may have several reasons. Firstly, different PAA sources were tested in the two studies (Merck KGaA and PeroxyChems's VigorOx® in this and the Block et al. (2015) study, respectively). Different PAA formulations contain different components hampering the comparison of results from studies using different PAA sources (Luukkonen and Pehkonen 2017). Secondly, Block et al. (2015) evaluated PAA efficacy in distilled water, whereas (filtered) wastewater was used in the present study. Since PAA efficacy is known to be greater in neutral to acidic solutions with lower COD levels (Eramo et al. 2017; Luukkonen et al. 2014), this may also at least partly explain the differences in PAA efficiencies attained. Removal efficiencies in preliminary tests that we conducted with distilled water indeed revealed E2 removal efficiencies of 100% even at the lowest PAA concentration (1 mg L^{-1}) and contact time (10 min) evaluated (data not shown). Thirdly, the E2 concentration used in the present study (50 μg L^{-1}) was approximately five times higher than that in Block et al. (2015; 9 μg L^{-1}). E2 concentrations measured in surface waters are typically in the nanograms per liter range (Adeel et al. 2017; Vilela et al. 2018), although waterbodies to which livestock has direct access may contain higher E2 levels (Pal et al. 2010). There is thus a need to continue monitoring E2 in waterbodies receiving WWTP effluents and those influenced by livestock. Subsequently, E2 removal efficiencies by PAA at environmental-realistic E2 concentrations determined from such studies should be further evaluated.

Effects of PAA on wastewater quality

Acetic acid, hydrogen peroxide, and water are the degradation products of PAA (Chhetri et al. 2014). The formation of acetic acid during PAA degradation may thus lead to increased levels of total organic carbon (TOC) and chemical oxygen demand (COD) (Collivignarelli et al. 2017; Luukkonen and Pehkonen 2017). Reported typical (theoretical or measured) increases in COD levels range from 2 to 4 mg L^{-1} for each 1 mg PAA L^{-1} dosed (Cavallini et al. 2013; Kitis 2004; Luukkonen and Pehkonen 2017). Subsequently, the expected increase in COD levels at the highest PAA concentration evaluated in the present study (15 mg L^{-1}) would be 30 to 60 mg L^{-1}. The increases in COD as measured in distilled water and WW, however, were 9.0 mg L^{-1} and 18 mg L^{-1}, respectively. The actual increase in COD is known to depend on the method applied and the chemical composition of the PAA form (Luukkonen and Pehkonen 2017; Luukkonen et al. 2014). In the present study, for example, we used the open reflux boiling method, which is known to potentially lead to volatilization of organic material (Baldry et al. 1995). In any case, both the measured (60 mg L^{-1}) and theoretical (max. 101 mg L^{-1}) final COD levels after the maximum PAA concentration evaluated in this study are below 125 mg L^{-1}, which is the trigger value for WW discharges to waterbodies in the EU (EC 1991).

Hypothetically, the addition of an acidic substance like PAA to a WW could lead to a drop in pH levels. Previous studies, however, have indicated that this decrease is not significant at realistic PAA treatment concentrations (Cavallini et al. 2013; Luukkonen and Pehkonen 2017). Luukkonen et al. (2014), for example, determined that the decrease in pH levels after administration of PAA may be determined by multiplying the PAA dose applied (in mg L^{-1}) with 0.033. The pH values in the WW treated with PAA indeed remained the same or dropped only slightly from 7.9 (value prior to PAA treatment) to 7.9 (0 mg PAA L^{-1}), 7.9 (1 mg PAA L^{-1}), 7.8 (5 mg PAA L^{-1}), 7.6 (10 mg PAA L^{-1}), and 7.5 (15 mg PAA L^{-1}). As for COD, the pH values thus also remained within the limits (pH = 6.0 to 9.0) as set in EC

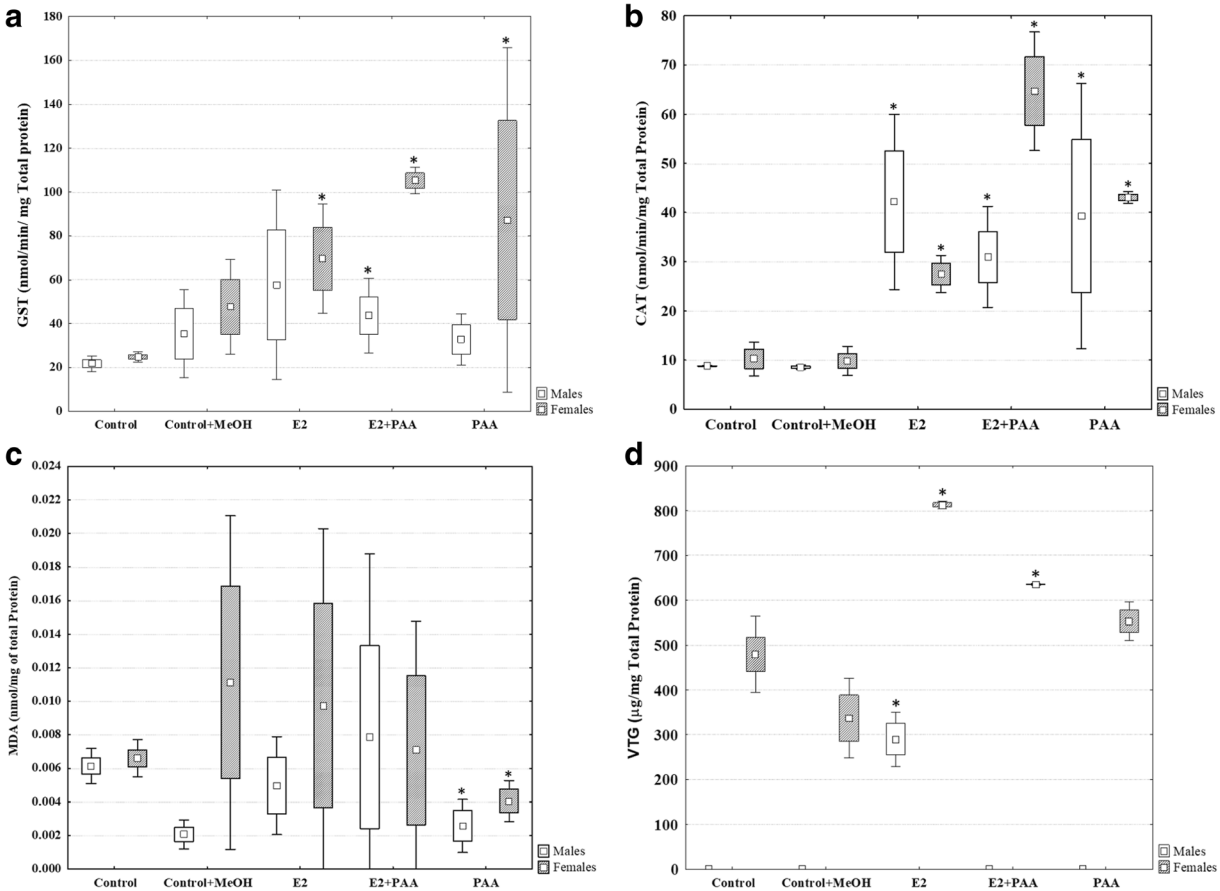

Fig. 4 **a** Ecotoxicological responses of E2 and PAA on zebrafish. GST activity: Middle point: mean; whisker value: standard deviation; box value: standard error. Asterisk means significant differences ($p < 0.05$) from the respective controls. **b** Ecotoxicological responses of E2 and PAA on zebrafish. CAT activity: Middle point: mean; whisker value: standard deviation; box value: standard error. Asterisk means significant differences ($p < 0.05$) from the respective controls. **c** Ecotoxicological responses of E2 and PAA on zebrafish. MDA concentrations: Middle point: mean; whisker value: standard deviation; box value: standard error. Asterisk means significant differences ($p < 0.05$) from the respective controls. **d** Ecotoxicological responses of E2 and PAA on zebrafish. VTG concentrations: Middle point: mean; whisker value: standard deviation; box value: standard error. Asterisk means significant differences ($p < 0.05$) from the respective controls

(1991). TSS levels measured after any of the PAA treatments made also adhered to this Directive (TSS < 10 mg L^{-1}; data not shown; EC 1991).

Ecotoxicological responses of E2 and PAA on zebrafish

Fish mortality remained below 10% in all treatments. Published 3-4d LC50 values of E2 for adult fish range from 3.5 mg L^{-1} (*Oryzias latipes*; Kashiwada et al. 2002) to 4.3 mg L^{-1} (*Kryptolebias marmoratus*; Rhee et al. 2011), whereas Saili et al. (2012) reported a 5d LC50 of > 6.8 mg L^{-1} for zebrafish embryos. At the E2 concentration of 50 µg L^{-1} as evaluated in the present study, lethal effects were thus indeed not anticipated. Regarding PAA, however, the test concentrations used in the present study (1 to 15 mg L^{-1}) could be expected to have lethal effects given the reported 4d-LC50 values of 0.35 and 1 mg L^{-1} for adult zebrafish (Henao et al. 2018). The absence of such effects may be related to the fast degradation of PAA since a follow-up study conducted in our laboratory demonstrated that a PAA concentration of 15 mg L^{-1} dropped to about half after 10 min (7.6–7.9 mg PAA L^{-1}) and to about one-third after 15 min (4.8 mg L^{-1}) (unpublished data). In addition, PAA degradation products exhibit neglectable toxicity to aquatic life (Chhetri et al. 2014).

Although PAA did not exert effects on fish survival, it did cause significant effects at the biochemical level

(Fig. 4). For example, increased CAT levels ($p < 0.05$) were denoted in the PAA treatment (Fig. 4b). The stimulation of this antioxidant defense mechanism may be attributed to the formation of hydrogen peroxide during PAA degradation (Chen et al. 2017; Chupani et al. 2014).

Since the LPO (MDA content) showed a trend to decrease compared to controls (Fig. 4c), this increase in CAT activity, which is considered to be reversible (Chupani et al. 2014), appeared capable of preventing oxidative stress in organisms' cells. Subsequently, future (pilot) experiments evaluating the use of PAA in WWTP should consider an optimized residence time to avoid or minimize (sublethal) toxic effects on aquatic organisms in WW receiving waters.

On the other hand, chlorine compounds (sodium hypochlorite, chlorine dioxide) that are currently mostly used as disinfectants are known to have a much greater toxic potential to aquatic organisms than PAA (Elia et al. 2006).

The E2 and E2 + PAA treatments showed similar responses on CAT and LPO as those discussed above for PAA (Fig. 4b, c).

In addition, these treatments caused significant increased levels of GST (only for females in the E2 treatment; Fig. 4a) besides CAT. The induction of these antioxidant mechanisms in the E2 treatments may be explained with the fact that E2 biotransformation in the liver leads to the formation of radical anion superoxide that is capable of producing cellular oxidation (Cavalieri et al. 2000; Orozco-Hernández et al. 2019).

Increased VTG levels in both male and female zebrafish after E2 exposure have previously been demonstrated in several studies (Rose et al. 2002; Van den Belt et al. 2014; Holbech et al. 2006). In line with this, VTG levels in E2-exposed males were comparable with those in untreated females, whereas VTG was not detected in males receiving any of the other treatments (Fig. 4d). In addition, VTG levels of females in the E2 treatment were approximately three times higher than those found in the controls (Fig. 4d). PAA addition to E2 exposed females led to a significant decrease in E2 levels, supporting the high E2 removal efficiency of PAA discussed in point—efficacy of PAA in the removal of E2.

Conclusions

From this study, it may be concluded that PAA has a high efficiency in the removal of E2, both in terms of its concentration as in decreasing its toxic potential. This demonstrates the great potential of PAA as an alternative to chlorine compounds in the disinfection WW, the more since no substantial effects on wastewater quality parameters were observed. Future studies should evaluate whether PAA concentrations and/or contact times may be reduced at environmental-realistic E2 concentrations. In addition, effluents of different physical-chemical composition should be evaluated to test this efficacy for a wider range of WW. Chronic toxicity studies may confirm that organisms in waterbodies receiving a continuous flow of PAA-treated WW do not suffer from unacceptable risks, especially when compared to chlorine treated WW. In the longer run, the practical application of PAA as a substitute for chlorine products as a disinfectant should be accessed by implementing pilot plants in WWTP and by monitoring communities of receiving water bodies, since PAA treatment is easily incorporable in a WWTP, especially when compared to other alternatives for EDC removal such as membrane systems.

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