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17-β-Estradiol: Significant reduction of its toxicity in water treated by photocatalysis

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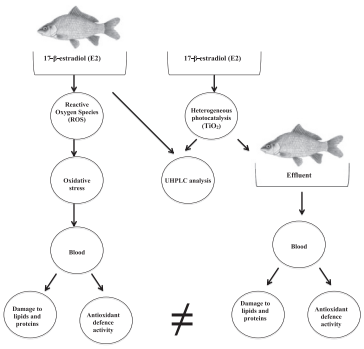
HIGHLIGHTS

- Water with E2 induces oxidative stress in *C. carpio*.
- This study confirms the efficiency of the heterogeneous photocatalysis to remove E2 from water.
- Treated water significantly reduces the oxidative stress in *C. carpio*.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to assess the efficiency of photocatalysis by TiO_2 on the removal of 17-β-estradiol (E2) (at environmentally relevant concentrations) and the toxicity caused by this emerging pollutant. After 60 min of TiO_2/UV treatment at pilot scale (14 L), E2 was removed from water approximately 85%. The toxicity was established by using *Cyprinus carpio* as bioindicator organism and oxidative stress biomarkers (OSB): [lipid peroxidation level (LPX), hydroperoxide content (HPC) and protein carbonyl content (PCC)] and enzymes [superoxide dismutase (SOD) and catalase (CAT)]. It was found that the photocatalytic treatment led to significantly reduce OSB in approximately 85–95%. Thus, it can be concluded that heterogeneous photocatalysis by TiO_2 is an efficient process to eliminate the toxicity caused by E2 and thus to remediate water polluted with this molecule.

Abbreviations: AOPs, advanced oxidation processes; CAT, catalase; CHP, cumene hydroperoxide; E2, 17-β-estradiol; HPC, hydroperoxide content; LPX, lipid peroxidation; MDA, malondialdehyde; PCC, protein carbonyl content; OSB, oxidative stress biomarkers; ROS, Reactive oxygen species; SOD, superoxide dismutase.

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1. Introduction

Photocatalysis has been proven to be a highly efficient Advanced Oxidation Process (AOP) (Avilés-García et al., 2018; Rosales et al., 2019; Yap et al., 2019; Hurtado et al., 2018; Mirzaei et al., 2017). The most widely used photocatalyst has been TiO_2, because of its capability to oxidize drugs dissolved in water, non-toxicity, low-cost and its long-term photostability, also this photocatalyst can carry out the degradation of up to 75% of some organic compounds (Tran et al., 2019; Durán et al., 2018). Although this method is effective for the removal of contaminants it can also generate metabolites, sometimes even more toxic than the original compound (Murgolo et al., 2018; Šojić Merkulov et al., 2018), hence it is necessary to evaluate this type of methodology to secure its safety use, mainly on the removal of emerging contaminants like endocrine disruptors. These substances are known to produce negative impacts to the human reproductive system, increase the risk of cancer and cause disturbances in the immune and nervous system, (Sumpter and Johnson, 2005), even at very low concentrations. In this context, 17-β-estradiol (E2) is an estrogen considered as a priority pollutant by the Water Framework Directive (WFD) of the European Union (Dai and Liu, 2017). E2 reaches the environmental waters from different sources but mainly from the animal and human urine, either by natural excretion or increased by the consumption of synthetic hormonal products for birth control or for the massive production of milk in the dairy industry. Estrogenic compounds are not completely removed by the wastewater facilities, and have been released in the effluents (Ferguson et al., 2001), reaching environmental waters between the ranges of ng/L to μg/L (Valdés et al., 2015; Díaz-Torres et al., 2013; Oğuz and Kankaya, 2013; Singh et al., 2010; Nasu et al., 2001). Furthermore, some negative outcomes have been reported in aquatic organisms at the same range of concentrations; reduced fertility, induce male feminization, reduced sperm count, induce the production of vitellogenin and vitelline envelope proteins, induce oxidative stress and so on (Lei et al., 2013; Moura Costa et al., 2010; Thilagam et al., 2010; Ahmad et al., 2009; Robinson et al., 2007; Jobling et al., 2004; Thomas-Jones et al., 2003; Kang et al., 2002; Rose et al., 2002). Thus, it is critical the development of new and effective methodologies for the efficient removal of this type of compounds from the water, but at the same time these new methodologies must also be friendly with the environment and the organisms that inhabit them.

The removal of E2 by photocatalysis with TiO_2 has already been reported (Alvarez-Corena et al., 2016; Arlos et al., 2016; Zhang et al., 2007), however, such works have focused mainly on the E2 degradation at either non-relevant environmental concentration or without an assessment of the produced metabolites. Thus, although E2 removal is efficient by photocatalysis, from a toxicological point of view it is of paramount importance to establish whether or not the treated water by such a technology can be discharged or not.

Therefore, the aim of this study was to assess the reduction of toxicity exerted by photocatalysis applied for the removal of 17-β-estradiol using *Cyprinus carpio* as bioindicator. This was achieved by evaluating the prooxidant effect and the antioxidant effect induced in the blood of the common carp after its exposure to water contaminated with E2; and comparing it against the exposure to the same water after its photocatalytic treatment with TiO_2. The prooxidant effect was evaluated by the determining the following biomarkers: lipid peroxidation level (LPX), hydroperoxide content (HPC) and protein carbonyl content (PCC); and the antioxidant effect will be evaluated by the determination of the activities of the SOD and catalase.

2. Materials and methods

2.1. Test substances

17β- estradiol (CAS number 50-28-2, >98.0% purity) $C_{18}H_{24}O_2$, 272.38 MW, was purchased from Sigma-Aldrich. All solvents used were HPLC grade. 17β- estradiol was dissolved first in acetonitrile to prepare stock solution from which all working concentrations were obtained. Unless otherwise stated, reagents in this section were obtained from Sigma-Aldrich (St. Louis MO).

2.2. Specimen procurement and conservation

Bioindicator, *Cyprinus carpio* of 14.83 ± 1.42 cm in length and weight 53.09 ± 6.4 g were used. The specimens came from the Tiacaque carp center, in the Mexico State. Fish were brought to the aquarium in plastic containers with oxygenated water and subsequently stocked in 15 L tanks with dechlorinated tap water added with salts and adapted to test conditions for 40 days before to the experiment. Carps were fed with Pedegral Silver™ fish food once daily and water was changed every 24 h so that the tanks were in healthy conditions. The characteristics of the tap water were determined daily and their values were maintained in the following intervals: temperature 21 ± 1.5 °C, O_2 concentrations >90%, pH 7.4–8.0, total alkalinity 16.3 ± 4.1 mg/L, total hardness 19.1 ± 0.3 mg/L. Natural photoperiod was used during the whole experiment.

2.3. Water treatment

This process was carried out in a relative new technology, a photocatalytic downflow bubble column reactor that allowed to process 14 L of water per batch. Two concentrations of the 17β- estradiol [1 ng/L (C1) and 1 μg/L (C2)] were assessed. These concentrations were selected because they were environmentally relevant, found in different parts of the world (Valdés et al., 2015; Díaz-Torres et al., 2013; Oğuz and Kankaya, 2013; Singh et al., 2010; Nasu et al., 2001). The initial pH was 6.0. The employed catalyst was the commercially available TiO_2 Degussa P25 and the loading was 100 mg/L. It is well known that this catalyst exhibits a specific surface area of 50 m^2/g and the main crystalline phase is Anatase.

The main feature of the employed bubble column reactor is that the gas is concomitantly injected with the liquid at the top of the column. A Venturi effect at the inlet is exploited to break up the gas and homogeneously disperse it as 3.4 mm bubbles into the liquid. The radiation lamp was placed in the lower part of the column radially centred. The liquid was recirculated to the photocatalytic reactor by means of a 1 HP pump. Nevertheless, a more detailed description of the reactor can be found in Martín del Campo et al. (2011).

During the reaction time (60 min), temperature (25 ± 3 °C) and oxygen flowrate (50 mL/min) were monitored and kept constant. The power of the UV radiation source was 400 W and emitted radiation with a wavelength of 254 nm. Samples were taken at different times (0, 5, 10, 15, 20, 25, 30, 45 and 60 min) during the reaction to control the degradation of the molecule within the treatment process. At the end of reaction, water was recovered and filtered to separate the catalyst (TiO_2) and it was placed in plastic bottles protected from light for its conservation and later use in the exposure systems.

2.4. HPLC methodology

Specific determination of 17β-estradiol in the samples of water withdrawn from the reactor was made using high performance liquid chromatography equipped with a multiple UV wavelength detector (Vanquish Thermoscientific UHPLC 1100). UV detector was set at 200 nm for all analysis. Separations was performed with a C18 column 5 μm, 120 Å (4.6 × 150 mm) which was maintained at 40 °C with a flow rate of 1 mL/min; run time was 8 min; injection volume was 50 μL; elution was achieved under isocratic conditions, 50:50 (*v*/v) mixture of water [pH adjusted to 2.6 with 99% phosphoric acid (spectrophotometric grade)]:acetonitrile (HPLC grade).

The determination of the 17β-estradiol concentration in carp blood was also carried out at 12, 24, 48, 72 and 96 h before and after the

treatment at the two environmentally relevant concentrations referred to in Section 2.3.

Plasma samples underwent the following extraction procedure for separation of 17 β-estradiol from binding proteins prior to analysis. The plasma samples were extracted with diethyl-ether and quick frozen. The resulting supernatant (ether phase) contains the steroid and was placed in a 30 °C water bath for evaporation under nitrogen. The steroid residues were then reconstituted in PBSG (phosphate buffered saline pH 7.0 with 1% gelatin) and stored at −20 °C until assayed. 17 β-estradiol concentration was followed by using high performance liquid chromatography equipped with a multiple UV wavelength detector (Vanquish Thermoscientific UHPLC 1100), using the afore mentioned conditions.

2.5. Toxicity studies

Adult organisms (*Cyprinus carpio*) of 16.42 ± 1.61 cm in length and weight 61.82 ± 7.6 g were exposed at two different environmental relevant concentrations of E2 (1ng/L (C1) and 1 μg/L (C2)) and at different exposure times (12, 24, 48, 72 and 96 h). One group was exposed to the untreated water and other group was exposed to the treated water. For the evaluation of toxicity tests, 6 systems were used for each exposure time: 1) Control before treatment, 2) C1 before treatment, 3) C2 before treatment, 4) Control after treatment, 5) C1 after treatment and 6) C2 after the treatment. Each system (20 L capacity tanks) contained 10 L of treated or untreated water and in them 5 fish of the above-mentioned characteristics were placed. Water composition and physicochemical characteristics similar to the acclimatization period were used in this study. The systems were made in quintuplicate. A total of 150 carp specimens were used in the experiment.

At the end of the exposure period, fish were removed from the systems and placed in a tank with 50 mg/L of clove oil as an anesthetic. Anesthetized specimens were placed in a lateral position and blood was collected with a heparinized 1-mL hypodermic syringe by puncture of the caudal vessel performed laterally near the base of the caudal peduncle, at mid-height of the anal fin and ventral to the lateral line. Six hundred microliters of blood samples were collected in heparinized tubes, placed in 1 mL of Tris buffer solution pH 7. The supernatant was centrifuged at 12, 500 ×*g* for 15 min at −4 °C. The serum obtained was used to determine the oxidative stress determinations. The biomarkers evaluated were: lipid peroxidation level (LPX), hydroperoxide content (HPC) and protein carbonyl content (PCC), in addition to activities of antioxidant enzymes, SOD and CAT. All evaluated biomarkers were analyzed using the supernatant.

2.6. Oxidative stress evaluation

2.6.1. Determination of LPX (Buege and Aust, method 1979)

50 μL of supernatant liquid were added with 450 μL of Tris HCl solution (150 mM) and 1 mL of TCA-TBA solution (0.375% thiobarbituric in 15% trichloroacetic acid). Subsequently, the mixture was incubated at 37 °C for 35 min and then vortexed, immediately samples were placed in boiling water for 45 min and suddenly cooled down in a fridge at −20 °C to cause a thermal shock. Following this, samples were centrifuged at 3500 rpm for 10 min and finally the absorbance was measured at 535 nm. The obtained data were reported as millimole of malondialdehyde per milligram of protein. This calculation was made using the molar extinction coefficient (MEC) of 1.56 × 105/M/cm.

2.6.2. Determination of HPC (Jiang et al., method, 1992)

100 μL of supernatant (deprotonated with 10% trichloroacetic acid) was mixed with 900 μL of reaction mixture (0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange and 4 mM 90% butylhydroxytoluene in methanol). The mixture was incubated for 60 min at 25 °C and preserved from the light. Then, the absorbance was read at 565 nm. Results were interpolated in a pattern curve and results were expressed in nanomole of cumene hydroperoxide per milligram of protein.

2.6.3. Determination of PCC (Levine et al., 1994, Parvez and Raisuddin, 2005, Burchman, method 2007)

Precipitate was mixed with 150 μL of 2,4 -dinitrophenyl hydrazine (10 mM in 2 M HCl). Then, the solution was incubated at 25 °C for 1 h in the dark. After incubation, 500 μL of trichloroacetic acid (20%) were placed and the sample was allowed to stand for 15 min at 4 °C. Subsequently, the solution was centrifuged at 11,000 rpm for 5 min.; supernatant was discarded and the button was washed three times with 1 mL of ethanol:ethyl acetate (1:1). Immediately, precipitate was dissolved in 1 mL of guanidine (6 M), pH 2.3, and incubated for 30 min at 37 °C. Finally, absorbance was read at 366 nm, and expressed as micromolar reactive carbonyls formed (C=O) per milligram of protein, using the MEC of 21,000 M /cm.

2.7. Determination of the antioxidant enzymes

2.7.1. Determination of SOD activity (Misra and Fridovich, 1972)

40 μL of supernatant was added with 260 μL of carbonate buffer solution (50 mM sodium carbonate and 0.1 mM EDTA) at pH 10.2, and with 200 μL of adrenaline (30 mM). Absorbance was read at 480 nm, at 30 s and 5 min. The enzyme activity was measured by interpolating the data in a pattern curve and results were expressed as SOD UI per milligram of protein.

2.7.2. Determination of CAT activity (Radi et al., method 1991)

30 μL of supernatant was added with 420 μL of buffer solution (0.3 M sucrose, 1 mM EDTA, 5 mM HEPES, and 5 mM KH2PO4) and 300 μL of H_2O_2 solution (20 mM). Subsequently, the absorbance was measured at 240 nm, at 0 and 60 s. Results were expressed as millimole of H_2O_2 per milligram of protein.

2.7.3. Determination of total proteins (Bradford method 1976)

50 μL of supernatant was added with 150 μL of H_2O distilled and 2.5 mL of Bradford's reagent, then vortexed and allowed to stand protected from light for 5 min at 25 °C. Finally, the absorbance of the solution was measured at 595 nm.

2.8. Considerations used for animal welfare in this study

This protocol was reviewed and approved by the Bioethics Committee of the Universidad Autónoma del Estado de México (UAEM) to ensure that it was carried out in accordance with institutional standards for the care of animal test subjects. Provisions set out in the official Mexican norm on the production, care and use of laboratory animals (NOM-062-ZOO, 1999) were also taken into account.

2.9. Statistical analysis

The data obtained from the oxidative stress determinations were analyzed by the one-way analysis of variance (ANOVA), followed by a Bonferroni multiple comparison ($p < 0.05$).

3. Results

3.1. Photocatalytic degradation of E2

Fig. 1 shows the E2 degradation profile under UV radiation and using TiO_2 Degussa P25 as photo-catalyst. It should be noticed that the maximum removal of E2 observed in Fig. 1 is about 85%. It is worth pointing out that by any means this value is the maximum achieved during the process but the concentration of E2 after 60 min of treatment was lower and out of the equipment detection limit. Therefore, it can be assured that the removal efficiency of the assessed technology is higher

than 85%. Also, as consequence of the detection limit of the used HPLC, a degradation profile when using C1 could not be produced and therefore is not included in fig. 1. Nevertheless, such solution was also treated at the same reaction conditions than C2 solution and used to conduct toxicology tests.

It is also worth noticing that, in the context of E2 removal by photocatalysis with TiO_2, the catalyst loading employed in this work is about 10 times less than that reported by other authors and the reaction volume is approximately 20–30 times higher than previous works (Álvarez-Corena et al. 2016; Arlos et al., 2016; Zhang et al., 2007). In order to establish the effect of light an experiment without catalyst was conducted and after 60 min of reaction the removed E2 was only 30%. This highlights the effect of the photo-catalyst on the E2 removal rate. In addition, an adsorption experiment, i.e. catalyst without light, was also performed. The maximum attained E2 removal by adsorption was determined to be 5% after 60 min of treatment.

3.2. 17β- estradiol concentration in blood of common carp

Table 1 shows the results of the water added with two environmentally relevant concentrations of 17-β-estradiol and its change with time in water and in blood of common carp. As can be seen for both, C1 and C2, the concentration of 17-β-estradiol decreases in water and gradually increases in common carp blood. In the photocatalytically treated waters, for both C1 and C2 solutions, 17-β-estradiol was not identified in any of the exposure times, likewise, this contaminant was not identified in blood.

3.3. Oxidative stress

3.3.1. LPX results

Fig. 2 shows the results regarding the lipoperoxidation level in blood of *Cyprinus carpio* exposed to E2 at two concentrations C1 and C2; before (AC1 and AC2) and after (DC1 and DC2) of the photocatalysis treatment with TiO_2. Graph in Fig. 2, shows a significant increase ($P < 0.05$) of lipoperoxidation with respect to the control group in the water without treatment at 12 h in AC1 (116%), at 24 h in AC1 (110%) and in AC2 (112%), at 48 h in AC1 (112%) and AC2 (117%), at 72 h in AC1 (116%) and finally at 96 h in AC1 (114%) and AC2 (122%). Noteworthy, is the difference in the grade of lipoperoxidation observed at all times, except at 12 h, in both concentrations if we compare the results obtained from the exposure to water before and after treatment.

3.3.2. HPX results

Fig. 3 shows the results referring to the content of hydroperoxides in blood of *Cyprinus carpio* exposed to E2 at two concentrations C1 and C2; before (AC1 and AC2) and after (DC1 and DC2) of the photocatalysis treatment with TiO_2. Graphic shows a significant increase of hydroperoxide content ($P < 0.05$) with respect to the control in both concentrations tested in the water without treatment, at 12 h AC1 (324%) AC2 (715%), at 24 h AC1 (209%) AC2 (826%) and at 48 h in AC1 (186%) AC2 (397%). However, 72 h and 96 h only show a significant increase of hydroperoxide content ($P < 0.05$) with respect to the control at the highest concentration; AC2 (244%) and AC2 (221%) respectively. On the other hand, a significant decrease ($P < 0.05$) of the hydroperoxide content with respect to the control was observed at the lowest concentrations tested in the treated water at 72 h and 96 h, in DC1 (24%) and DC1 (21%) respectively. Important to remark that at all times of exposure there are differences between the two concentrations tested before and after the treatment; AC1 vs DC1 and AC2 vs DC2.

3.3.3. PCC results

Fig. 4 shows the results concerning the protein carbonylated content in blood of *Cyprinus carpio* exposed to E2 at two concentrations C1 and C2; before (AC1 and AC2) and after (DC1 and DC2) of the photocatalysis treatment with TiO_2. Graphic shows a significant increase ($P < 0.05$) on the protein carbonylated content with respect to the control group, in both concentrations tested and in both groups, with treatment and without treatment, at 12 h in both concentrations without treatment AC1 (122%) and in AC2 (157%), and also in the highest concentration after the treatment, DC2 (109%), at 24 h in both concentrations without treatment AC1 (124%) and in AC2 (186%), and also in the lowest concentration after the treatment, DC1 (110%). After 48 h, a significance increase only were observed in the groups without the water treatment, at 48 h in AC2 (125%), at 72 h in AC1 (123%) and AC2 (154%) and finally at 96 h in AC1 (160%) and in AC2 (189%). On the other hand, a

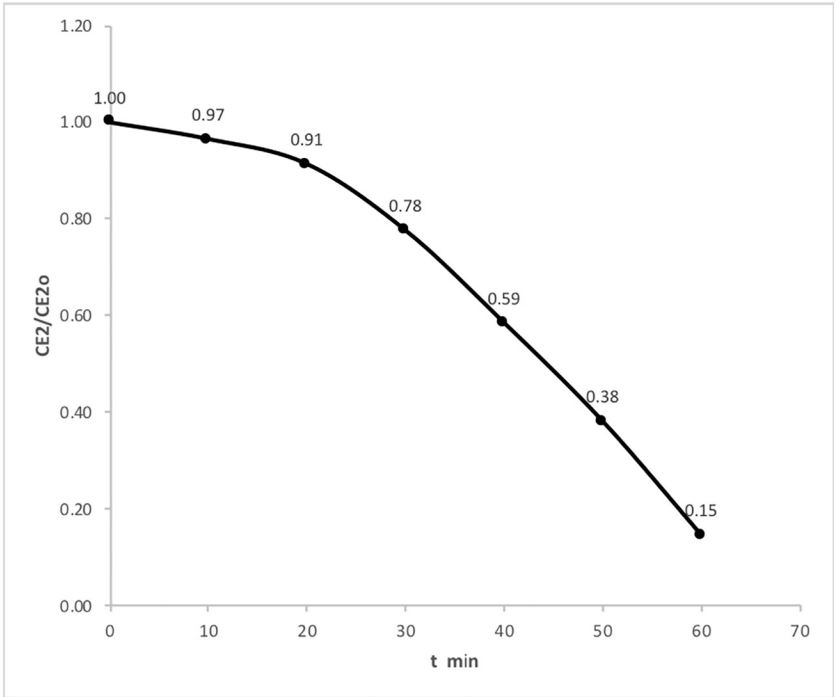

Fig. 1. 17-β-Estradiol concentration evolution profile with time. Reaction volume = 14 L, $T = 298$ K, $Q_{O2} = 50$ mL/min.

Table 1
Concentration of 17-β-estradiol in water and blood of Cyprinus carpio before and after photocatalytic treatment.

Time	Water without photocatalytic treatment C1	17-β-Estradiol in blood of common carp in water without photocatalytic treatment C1	Water with photocatalytic treatment C1	17-β-Estradiol in blood of common carp in water with photocatalytic treatment C1	Water without photocatalytic treatment C2	17-β-Estradiol in blood of common carp in water without photocatalytic treatment C2	Water with photocatalytic treatment C2	17-β-Estradiol in blood of common carp in water with photocatalytic treatment C2
0 h	1 ± 0.0 ng/L	0 ± 0.0 ng/L	NI	NI	1 ± 0.0 μg/L	0 ± 0.0 μg/L	NI	NI
12 h	0.8 ± 0.02 ng/L	0.12 ± 0.03 ng/L	NI	NI	0.87 ± 0.03 μg/L	0.10 ± 0.01 μg/L	NI	NI
24 h	0.7 ± 0.01 ng/L	0.23 ± 0.01 ng/L	NI	NI	0.81 ± 0.05 μg/L	0.16 ± 0.03 μg/L	NI	NI
48 h	0.5 ± 0.03 ng/L	0.42 ± 0.02 ng/L	NI	NI	0.62 ± 0.04 μg/L	0.33 ± 0.02 μg/L	NI	NI
72 h	0.4 ± 0.02 ng/L	0.54 ± 0.04 ng/L	NI	NI	0.53 ± 0.03 μg/L	0.41 ± 0.01 μg/L	NI	NI
96 h	0.2 ± 0.01 ng/L	0.76 ± 0.03 ng/L	NI	NI	0.35 ± 0.04 μg/L	0.58 ± 0.04 μg/L	NI	NI

NI = Unidentified.

significant decrease ($P < 0.05$) with respect to the control group was observed, at 12 h DC1 (68%), 48 h AC1 (84%) and at DC2 (72%) and finally at 72 h in DC2 (87%). It's important to remark a difference between the two concentrations tested before and after the treatment at 12, 72 and 96 h, and only in the highest concentration at 24 and 48 h.

3.4. Antioxidant enzymes activity

3.4.1. SOD activity

Fig. 5 shows SOD activity in blood of *Cyprinus carpio* exposed to E2 at two concentrations C1 and C2; before (AC1 and AC2) and after (DC1 and DC2) of the photocatalysis treatment with TiO_2. Graphic shows a significant increase of SOD activity ($P < 0.05$) with respect to the control group in both concentrations tested at all exposure times for the group exposed to the water without treatment, at 12 h AC1 (140%), AC2 (136%), at 24 h AC1 (119%), AC2 (125%), at 48 h AC1 (156%), AC2 (130%), at 72 h AC1 (134%) AC2 (120%), and finally at 96 h AC1 (126%), AC2 (128%). On the other hand, there was a significant decrease ($P < 0.05$) of SOD activity with respect to the control group respectively at 12 h in DC2 (86%). A difference in the SOD activity between the two concentrations tested before and after the water treatment was observed at all times.

3.4.2. CAT activity

Fig. 6 shows CAT activity in blood of *Cyprinus carpio* exposed to E2 at two concentrations C1 and C2; before (AC1 and AC2) and after (DC1 and DC2) of the photocatalysis treatment with TiO_2. A significant increase was observed ($P < 0.05$) with respect to the control group, at 12 h AC1 (114%), AC2 (120%), at 24 h AC1 (129%), AC2 (122%), at 48 h AC1 (120%), AC2 (130%), at 72 h AC1 (139%), AC2 (134%) and DC2 (119%) and finally at 96 h AC1 (140%) and AC2 (173%). On the other hand, there was a significant decrease ($P < 0.05$) of the CAT activity with respect to the control at 24 h DC1 (55%), DC2 (85%), at 48 h DC1 (88%) and at 96 h DC1 (47%) and DC2 (85%). Finally differences were found in all concentrations tested before and after the water treatment at all times.

4. Discussion

The toxicological response of *Cyprinus carpio* was assessed using oxidative stress biomarkers, after its exposure to water contaminated with E2 at two different environmental relevant concentrations. The estrogenic compound E2 it is known for being metabolized in the liver by the microsomal cytochrome *p*450 monooxygenase system (Guengerich, 1990) forming hydroxylated compounds. Likewise, the biotransformation of E2 generates semiquinones, which with the help of molecular O_2 produce quinones, forming also the radical anion superoxide that is capable of producing cellular oxidation (Cavalieri et al., 2000).

Our results (Fig. 2) show a significant increase in the lipidperoxidation level in common carp blood, evident for both concentrations at 24, 48 and 96 h. ROS are responsible for initiating damage in the lipids of the cell membrane; the resulting free radicals lipids propagate lipidperoxidation process (LPX), generating a chain reaction where the final products are aldehydes, ketones, esters, and alcohols. Allowing the accumulation of HPX that eventually decompose into a wide variety of products such as malondialdehyde, hexanal and 4-hydroxynonenal (Konigsberg, 2008). This repetitive process leads the membrane to lose its physicochemical properties and culminates with the cell death (Velázquez and Vega, 2004). In accordance with the above, or results

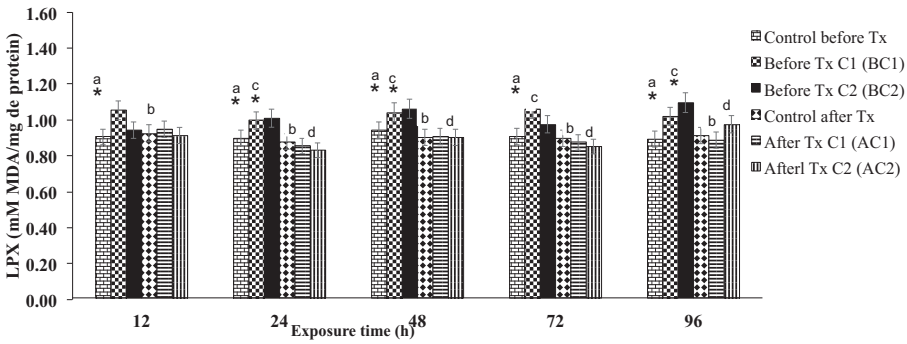

Fig. 2. Lipid peroxidation (LPX) in blood of *Cyprinus carpio* exposed to water contaminated with 17-β-estradiol (E2) [1 ng (C1) and 1 μg (C2)] before its treatment, for 12, 24, 48, 72 and 96 h; and after its treatment (TiO_2/UV) at the same exposure times. The bars represent the average of three repetitions ± standard deviation. MDA is malondialdehyde. Asterisk represent significantly different compared with the control (*$p < 0.05$, ANOVA and Bonferroni multiple comparison).

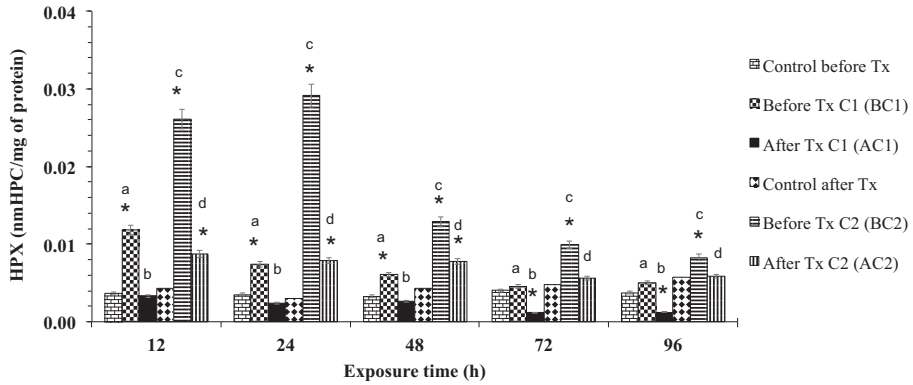

Fig. 3. Hydroperoxide content (HPC) in blood of *Cyprinus carpio* exposed to water contaminated with 17-β-estradiol (E2) [1 ng (C1) and 1 μg (C2)] before its treatment, for 12, 24, 48, 72 and 96 h; and after its treatment (TiO2/UV) at the same exposure times. The bars represent the average of three repetitions ± standard deviation. CHP is cumene hydroperoxide. Asterisk represent significantly different compared with the control (*$p < 0.05$, ANOVA and Bonferroni multiple comparison).

(Fig. 3) also demonstrates a significant increase of hydroperoxide content in the blood of the common carp for both concentrations tested and at all exposure times. Hydroperoxides are formed at the stage of propagation of the LPX, when polyunsaturated fatty acids (linoleic, linolenic and arachidonic), are attacked due to its low energy of activation and high reactivity, forming the peroxidation of the lipids (Céspedes and Castillo, 2008). Other important biomolecules susceptible for oxidative damage are proteins, particularly side chains with cysteine and methionine residues are major targets for the oxidative action of reactive oxygen and nitrogen species (Stadtman, 2004). 4-Hydroxy-2-nonenal, a lipid peroxidation product, can also react with side chains of proteins resulting in the carbonylation of proteins (Nadkarni and Sayre, 1995). Our results (Fig. 4) show a significant increase in the protein carbonylation content in the blood of the common carp, evident for both concentrations.

Oxidative stress has been defined as the exposure of living matter to various sources that produce a rupture of the balance that must exist between pro-oxidant factors and antioxidant mechanisms responsible for eliminating these chemical species, either by a deficit of these defences or for an exaggerated increase in the production of ROS (Venero, 2002). For the toxicity assessment we also evaluate the activity of two antioxidant enzymes. SOD and CAT enzymes are two major biomarkers of oxidative damage that can be induced by various environmental pollutants under pro-oxidant conditions, initially increased to counterattack oxidative stress but prolonged exposure causes their depletion, leading to oxidative damage to macromolecules, such as lipid peroxidation, protein and DNA damage (Bebianno et al., 2005). Our results (Figs. 5 and 6) show a significant increase in the activity of SOD and CAT activity in the blood of the common carp, evident for both concentrations and at all exposure times.

The results obtained and mentioned until now, as a result of the exposure of the teleost fish to contaminated water with E2 are in accordance with previous published studies. Gutiérrez-Gómez et al. (2016) said that E2 can produce the phenomenon of oxidative stress in *C. carpio*, the organs of the fish affected with this process were, blood, brain, gills, kidney and liver; Thilagam et al., 2010 reported that estrogenic drug E2 induce an increment in the ROS, which consequently induce DNA alteration, lipid peroxidation and an increase of the glutathione enzyme in the hepatic tissue of the Japanese sea bass *Lateolabrax japonicas*; Moura Costa et al., 2010, reported an increase in SOD and CAT activities in the liver of the silver catfish *Rhamdia quelen*; and Maria et al., 2008 reported an increase in lipid peroxidation and a decrease in DNA integrity in gills, blood and liver of the sea bass *Dicentrarchus labrax L* after its exposure to E2.

On a second part of this study, contaminated water with E2 was treated during 60 min with a heterogeneous photocatalytic process, using TiO_2 as a catalyst in ultraviolet light presence for the oxidation and remediation of the water from this estrogenic compound. After and during the treatment, samples were taken and analyzed by HPLC. Our results (Fig. 1) demonstrate that after 60 min, E2 was removed in a high percentage from the water. These results are in accordance with the previous studies which also report a high efficiency for the removal of E2 using advanced oxidation process as chlorination, ozonation and heterogeneous photocatalytic process (Alvarez-Corena et al., 2016; Kralchevska et al., 2012; Snyder et al., 2006; Moriyama et al., 2004; Hu et al., 2003; Ohko et al., 2002; Coleman et al., 2000). For the

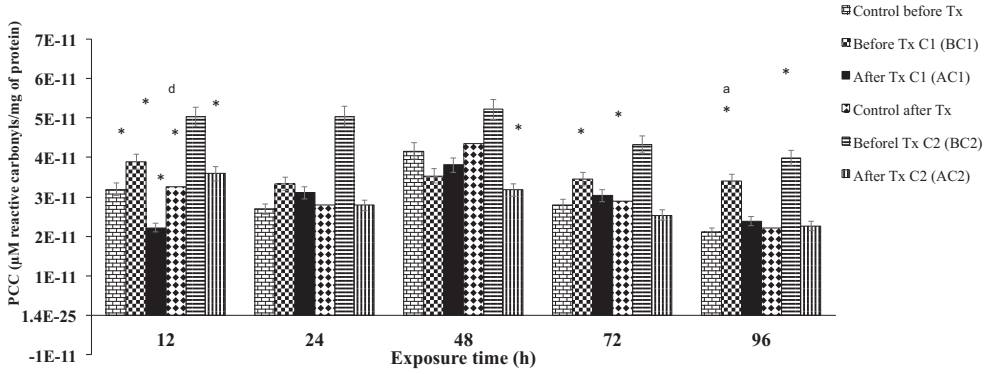

Figure 4. Protein carbonylated content (PCC) in blood of *Cyprinus carpio* exposed to water contaminated with 17-β-estradiol (E2) [1 ng (C1) and 1 μg (C2)] before its treatment, for 12, 24, 48, 72 and 96 h; and after its treatment (TiO2/UV) at the same exposure times. The bars represent the average of three repetitions ± standard deviation. Asterisk represent significantly different compared with the control (*$p < 0.05$, ANOVA and Bonferroni multiple comparison).

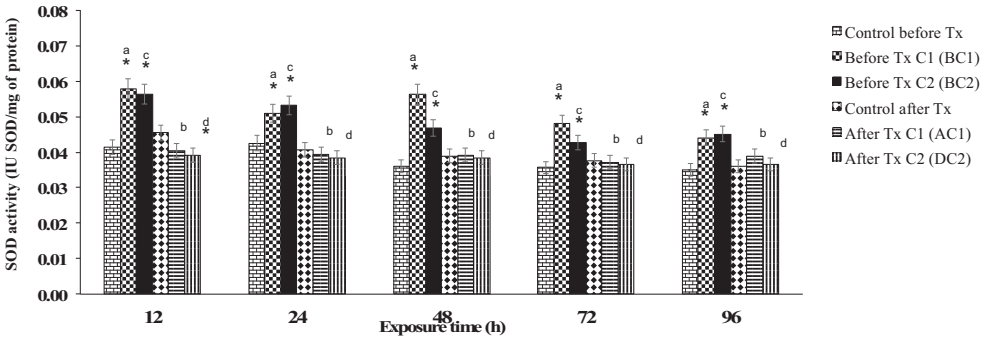

Figure 5. Superoxide dismutase (SOD) activity in blood of *Cyprinus carpio* exposed to water contaminated with 17-β-estradiol (E2) [1 ng (C1) and 1 μg (C2)] before its treatment, for 12, 24, 48, 72 and 96 h; and after its treatment (TiO2/UV) at the same exposure times. The bars represent the average of three repetitions ± standard deviation. Asterisk represent significantly different compared with the control (*$p < 0.05$, ANOVA and Bonferroni multiple comparison).

purposes of our investigation we just focused on the remediation of E2 in the water and no metabolites were assessed on the HPLC technique.

Even though the advanced oxidation process we tested demonstrates to be efficient for the removal of E2, it is well known that during the process many secondary byproducts could be formed (Alvarez-Corena et al., 2016), even more, in some cases metabolites still have estrogenic activity and could be more toxic than parent compound or the oxidative conditions of the process per se could be harmful for aquatic organisms (Zhang et al., 2008; Irmak et al., 2005; Alum et al., 2004; Huber et al., 2004).

In this treatment, the maximum removal of E2 observed is about 85%. E2 removal kinetics it is typically reported to be of pseudo first order. In this case, however, two main kinetic stages can be distinguished in Fig. 1. A slow one that dominates during the first 20 min of reaction. After this time, reaction rate increases and a new slow down would be expected once E2 concentration is much lower. This third stage is not observed due to the analytical equipment limitation on detecting the E2 concentration beyond 60 min of treatment. The first stage can be related to an induction period that might depend on the reaction volume (14 L in this case) and therefore has not been reported previously. It is plausible that because of the large treated volume, at the beginning of treatment is the light acting while it takes some time for the oxidant species to be produced at the photocatalytic surface and transferred to the water where the E2 oxidation might me occurring. Hence, it is important to test its toxicity to be sure that the process is not only efficient but also friendly with the environment.

Likewise, we measured the concentration of E2 in water and blood of *C. carpio* at the two concentrations used and the different times of exposure and we observed that the concentration of E2 in water is decreasing with respect to time and in blood the drug is increasing. Also, it is observed in Table 1, that in the waters treated with the photocatalytic method no amount of E2 was identified neither in water and much less in blood of the carp. It should be mentioned that in the chromatogram no additional peak was observed that made us suspect the presence of any of the metabolites of E2.

For the third and final part of this study, we exposed the common carp to the water treated with the photocatalytic process, and we measure the same biomarkers as in the first part for its subsequent contrast and comparison. Our results (Fig. 2) show a significant decrease in the degree of lipid peroxidation in the blood of the common carp compared with the previous results, evident for both concentrations at all times. In accordance with the above, or results (Fig. 3) also demonstrates a significant decrease of hydroperoxide content in the blood of the common carp for both concentrations tested, at all exposure times compared with the previous results. Furthermore, for C1 all HPX values calculated were below to the obtained for the controls and for C2, it reach the same level of the controls only until 96 h. Regarding with the protein carbonylation (Fig. 4), it is also evident a significant decrease of PCC in the blood of the common carp for both concentrations tested, at all exposure times compared with the previous results, except for C1 at 48 h. In like manner, the activity of the antioxidant enzymes SOD and CAT decreased in the blood of the common carp for both concentrations tested, at all exposure times compared with the previous results.

Results described above are in accordance with previous reports in literature using other biomarkers and under different advanced oxidation processes. Hu et al., 2003, report a decrease in estrogenic activity of a contaminated water after its aqueous chlorination using NaClO, likewise Ermawati et al., 2007; Alum et al., 2004 and Huber et al., 2004, reported a decrease in the estrogenic activity of a contaminated water after an ozonation process, finally Chen et al., 2007, reported a decrease in estrogenic activity of a contaminated water after its treatment using an UV/H_2O_2 process. Even though estrogenic activity and oxidative stress are two different biomarkers, both of them are interconnected, White et al., 2010, Mobley and Brueggemeier, 2004 report that

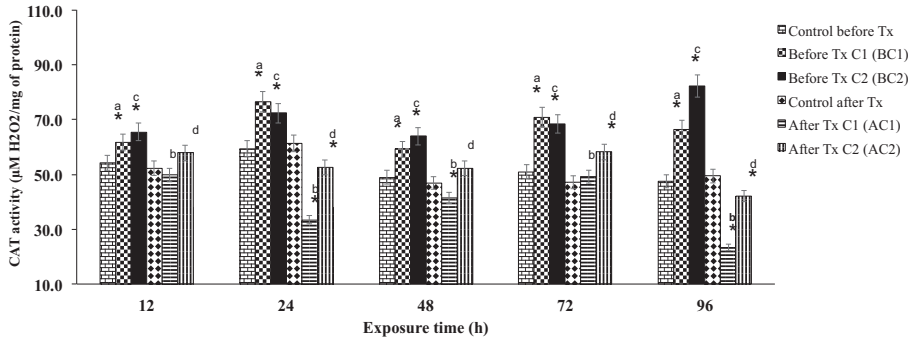

Fig. 6. Catalase (CAT) activity in blood of *Cyprinus carpio* exposed to water contaminated with 17-β-estradiol (E2) [1 ng (C1) and 1 μg (C2)] before its treatment, for 12, 24, 48, 72 and 96 h; and after its treatment (TiO_2/UV) at the same exposure times. The bars represent the average of three repetitions ± standard deviation. Asterisk represent significantly different compared with the control (*$p < 0.05$, ANOVA and Bonferroni multiple comparison).

E2 induce changes in antioxidant status by reducing the ability of cells to metabolize reactive oxygen species via a endocrine disruptor mediated mechanism.

5. Conclusions

The removal of E2 under the conditions used in the treatment was good, approximately 85%. The toxicity of the water added with E2 was reduced between 85 and 95% once it was treated by heterogeneous photocatalysis using TiO_2. This was demonstrated by the reduction of the values of the biomarkers of cellular oxidation and antioxidant activities, which practically reached basal levels.

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