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Original article

Efficiency of advanced oxidation processes in lowering bisphenol A toxicity and oestrogenic activity in aqueous samples

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Bisphenol A (BPA) is a well-known endocrine disruptor with adverse oestrogen-like effects eliciting adverse effects in humans and wildlife. For this reason it is necessary to set up an efficient removal of BPA from wastewaters, before they are discharged into surface waters. The aim of this study was to compare the efficiency of BPA removal from aqueous samples with photolytic, photocatalytic, and UV/H₂O₂ oxidation. BPA solutions were illuminated with different bulbs (halogen; 17 W UV, 254 nm; and 150 W UV, 365 nm) with or without the TiO₂ P-25 catalyst or H₂O₂ (to accelerate degradation). Acute toxicity and oestrogenic activity of treated samples were determined using luminescent bacteria (*Vibrio fischeri*), water fleas (*Daphnia magna*), zebrafish embryos (*Danio rerio*), and Yeast Estrogen Screen (YES) assay with genetically modified yeast *Saccharomyces cerevisiae*. The results confirmed that BPA is toxic and oestrogenically active. Chemical analysis showed a reduction of BPA levels after photolytic treatment and 100 % conversion of BPA by photocatalytic and UV/H₂O₂ oxidation. The toxicity and oestrogenic activity of BPA were largely reduced in photolytically treated samples. Photocatalytic oxidation, however, either did not reduce BPA toxic and oestrogenic effects or even increased them in comparison with the baseline, untreated BPA solution. Our findings suggest that chemical analysis is not sufficient to determine the efficiency of advanced oxidation processes in removing pollutants from water and needs to be complemented with biological tests.

KEY WORDS: *bioassay; endocrine disrupting compounds; photocatalysis; photolysis; toxicity*

The presence of endocrine disrupting compounds (EDCs) in surface waters has been noted since the early 1980s (1, 2). This is a broad class of compounds that includes natural and synthetic oestrogens (such as 17 β -oestradiol, 17 α -ethinyloestradiol), phytoestrogens (isoflavonoides), some pesticides (atrazine, dieldrin), surfactants (alkylphenol-ethoxalates), and industrial compounds like bisphenol A (BPA) (3-6). Endocrine disruptors interfere with the endocrine

system and disrupt the physiological functions of hormones. One of the adverse effects is the feminisation of fish and other aquatic organisms in large rivers that diminishes their reproduction potential (7). Other effects include mutations (1, 8) associated with higher incidence of cancer and lower reproductive ability in men (9).

As EDCs reach the aquatic environments via sewage systems and wastewater treatment plants (2, 10), efficient treatment technologies are

necessary to remove oestrogens and xenoestrogens from wastewaters. However, conventional biological treatment in wastewater treatment plants is not completely efficient in removing these compounds from wastewaters, so they are eventually discharged into receiving waters. Bisphenol A (BPA) is a commercially important chemical (11), used in the production of epoxy resins, polycarbonate products, and plasticisers and as a polymerisation inhibitor in polyvinyl chloride (PVC). It enters the aquatic environment through municipal wastewater discharges and leachate from landfills. The available data on BPA toxicity include studies on fish, aquatic invertebrates, and macrophytes (12-14). Reported levels in freshwater are low, less than $1 \mu\text{g L}^{-1}$ (11). However, BPA concentrations in landfill leachate may reach as high as 17.2 mg L^{-1} (15). Some studies (16, 17) suggest that continuous exposure to even low BPA levels could result in endocrine disruption.

Advanced oxidation processes (AOPs) are based on chemical reactions with hydroxyl radicals and include Fenton oxidation, ozonation, photolytic, and photocatalytic oxidation. Photocatalytic degradation of organic contaminants in water, typically involving TiO_2 as a catalyst, is an emerging technology whose key advantages include non-toxicity, photochemical stability, and high activity. The efficiency of direct photolysis with ultraviolet irradiation can be enhanced when combined with hydrogen peroxide ($\text{UV}/\text{H}_2\text{O}_2$) as a strong oxidant, whose photolytic dissociation yields hydroxyl radicals and accelerates degradation (18-24). Laboratory studies (16, 18-22) have demonstrated that various organic pollutants can readily be degraded by AOPs into less harmful products. However, this is not always the case. Some of the degradation by-products may have a stronger biological effect than the original compound (23). For this reason, treated samples should be biologically tested for residual toxicity and oestrogenicity to determine the potential risks of AOP treatment.

The aim of our study was to degrade BPA in aqueous samples with AOPs, compare the toxicity and oestrogenic activity of the baseline BPA sample with treated samples, and identify the most efficient AOP method.

MATERIALS AND METHODS

Photolytic, photocatalytic, and UV/H₂O₂ oxidation

Nine 250 mL samples containing BPA (Sigma-Aldrich, Steinheim, Germany, CAS No. 80-05-7) in the concentration of 20 mg L^{-1} in either tap or ultrapure water ($18.52 \text{ M}\Omega \text{ cm}^{-1}$, Milli Q; ELGA LabWater, VWS, Buckinghamshire, UK) were treated in a two- or three-phase, batch slurry open glass photoreactor at room temperature ($20 \text{ }^\circ\text{C}$), magnetically stirred at 600 rpm (10 g), and continuously purged with purified air at a flow rate of 300 mL min^{-1} (see Figure 1). This concentration of BPA was selected since a study of environmental BPA measured a maximum of 17.2 mg L^{-1} BPA in untreated leachate (15). After a 30 min dark period (to allow for sorption to reach equilibrium) the samples were illuminated for six hours with either of three bulbs: a halogen bulb (GE Hungary Kft., Budapest, Hungary; 150 W; visible light), a UV high-pressure mercury lamp (Dr. Hönle AG, Gräfelfing, Germany; 150 W, with the wavelength range between 300 and 400 nm and peak emission at 365 nm), and a UV low-pressure mercury lamp (Dr. Hönle AG; 17 W; with peak emission at 254 nm). The lamps were laid in a tap water cooling jacket and immersed vertically in the suspension. In photocatalytic and $\text{UV}/\text{H}_2\text{O}_2$ oxidation, we added to the suspension 0.5 g L^{-1} of TiO_2 P-25 (Degussa-Hüls AG, Frankfurt, Germany) and 2.5 mL of 37% H_2O_2 , respectively to accelerate

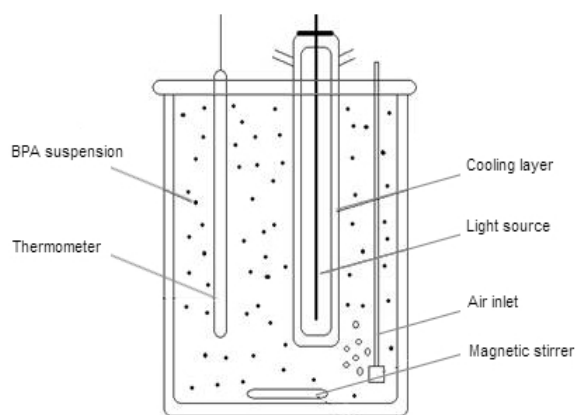


Figure 1 A schematic diagram of batch slurry reactor

BPA degradation. One untreated sample (sample 0) with the baseline BPA concentration of 20 mg L⁻¹ was used to compare the removal efficiency of the oxidation methods tested.

Chemical analysis

BPA degradation was determined by analysing residual BPA in liquid-phase samples that were collected from the batch slurry reactor every 15 min in the first hour and then on every full hour over the remaining five hours. For the analysis we used an Agilent series 1100 high-performance liquid chromatograph (HPLC; Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary solvent pump, autosampler, and UV detector ($\lambda=210$ nm) working at a constant temperature of 30 °C. Its limit of detection (LOD) was 0.2 mg L⁻¹. Separations were performed on a Phenomenex Luna C18 5- μ m column (250x4.6 mm; Phenomenex, Torrance, CA, USA) with the mobile phase consisting of methanol (MeOH) and ultrapure water (Milli Q) in the ratio of 75:25, at a flow rate of 1 mL min⁻¹ (26).

Toxicity tests

The toxicity of the baseline and treated BPA samples was tested using luminescent bacteria *Vibrio fischeri*, water fleas (*Daphnia magna* Straus, 1820), and zebrafish (*Danio rerio* Hamilton-Buchanan, 1822) embryos. We used all three toxicity tests due to different sensitivity of the test systems to toxic chemicals. The inhibition of bioluminescence activity (luciferin-luciferase system) in *Vibrio fischeri* is a rapid, reproducible, and low-cost bacterial assay that correlates well with other acute toxicity tests (27, 28). Water fleas are widely used in short- and long-term aquatic toxicity studies (29, 30). Zebrafish (*Danio rerio*) has been a model organism in ecotoxicology testing since 2005, when fish embryo toxicity testing became mandatory for routine sewage surveillance (31).

Bioluminescence was measured on a LUMISTox 300 luminometer (Dr. Lange GmbH, Düsseldorf, Germany) according to the ISO 11348-2:1998 test protocol (32) using freeze-dried bacteria *Vibrio fischeri* NRRL-B-11177 (Dr. Lange GmbH). The luminescent bacteria were exposed to the baseline and treated samples at 15 \pm 0.2 °C for 30 min. The percentage of inhibition was calculated for each tested sample relative to baseline.

Median inhibition concentration (30-min IC₅₀) for BPA was calculated using the LumiSoft data acquisition software (Dr. Lange LUMISsoft, Düsseldorf, Germany) based on the reduced light emission obtained in diluted samples containing the following BPA concentrations: 0.3 mg L⁻¹, 0.6 mg L⁻¹, 1.3 mg L⁻¹, 2.5 mg L⁻¹, 5.0 mg L⁻¹, 10.0 mg L⁻¹, and 16.0 mg L⁻¹.

For the second acute toxicity test we obtained our water flea population from the Institut für Wasser, Boden und Lufthygiene des Umweltbundesamtes, Berlin, Germany. Water fleas were cultured in 3-litre aquaria with reconstituted water (M4) at 20 \pm 1 °C with a 16:8 h light:dark cycle and fed daily with the alga *Desmodesmus subspicatus*. Acute toxicity was tested according to the ISO 6341:1996 guidelines (33) with a few modifications. Two test groups of ten 24-hour-old neonates were used for each treated sample and control. The animals were exposed to 30 mL of test samples in glass Petri dishes for 24 h. Toxic effects were quantified by determining the inhibition of mobility of the tested animals (27). Median effective concentration (24-h EC₅₀) for BPA was determined using the probit analysis of results obtained in diluted samples containing the following BPA concentrations: 5 mg L⁻¹, 7.5 mg L⁻¹, 10 mg L⁻¹, 12.5 mg L⁻¹, 15 mg L⁻¹, 17.5 mg L⁻¹, and 20 mg L⁻¹.

The third toxicity test was performed with zebrafish according to the ISO 15088:2007 guidelines (34). Adult zebrafish were cultured in 45-litre glass aquaria with tap water, kept at 26 \pm 2 °C under a 12:12-hour light and dark cycle. Fish were fed three times a day with commercially available dried fish food (Tetramin) and *Daphnia magna*. Following the procedure described by Kammann et al. (35) we placed a plastic spawning box in the aquaria to obtain eggs. Fertilised eggs were placed in 24-well plates containing 1 mL of a sample, one egg per well. Lethal and sublethal toxic effects of zebrafish embryos exposed to different dilutions of the initial BPA sample and treated samples were evaluated after 48 h of exposure. Toxic effects were evaluated following the procedure described by Nagel (31) and Schulte and Nagel (36) (see Tables 2 and 3 in Results and Discussion). Median lethal concentration (48-h LC₅₀) and median effective concentration (48-h EC₅₀) were determined by probit analysis based on responses to BPA concentrations obtained by dilutions of the baseline sample: 5 mg L⁻¹, 10 mg L⁻¹, 15 mg L⁻¹, and 20 mg L⁻¹.

Oestrogenic activity test

Oestrogenic activity of the baseline and treated samples was determined with the Yeast Estrogen Screen (YES) assay using a recombinant yeast strain *Saccharomyces cerevisiae*, as described by Routledge and Sumpter (37). First we concentrated 250 mL of the baseline and treated samples to 1 mL using the solid phase extraction (SPE) method described by Bistan et al. (26). Concentrated samples and serial dilutions of the baseline BPA solution were transferred to 96-well microtitre plates and dried in sterile conditions. Then we added 200 μ L of yeast-containing growth medium (optical density at 620 nm was 1.0) to each well and after 48 to 52 h at 34 °C measured the absorbance at 575 and 620 nm on a microtitre plate reader PowerWave XS (BioTek, Winooski, Vermont, USA).

The validity of the assay was confirmed with a positive control (tested concentrations were in the range of 2.6 ng L⁻¹ to 1.36 μ g L⁻¹ of 17 β -oestradiol), negative control (0.025-1.57 μ g L⁻¹ of progesterone), and a blank (yeast in the growth medium and chlorophenol red- β -D-galactopyranoside) (26). Oestrogenic activity of the treated samples was expressed as relative estrogenic activity (REA), where the estrogenic activity of treated samples was compared to the oestrogenic activity of baseline solution of BPA.

RESULTS AND DISCUSSION

BPA removal

Table 1 compares BPA removal efficiency after six hours between photolytic, photocatalytic, and UV/H₂O₂ oxidation. The latter two processes showed the highest efficiency, with 100 % BPA degradation within the first 90 minutes (Figure 2). The least efficient was photolysis with visible light (3 % conversion).

Neamtu and Frimmel (20) reported low BPA conversion when exposed to UV-C light in deionised water and increased conversion in the presence of hydrogen peroxide, which is in agreement with our results. Also similar to our findings, Nomiya et al. (38) reported very high BPA degradation in water with TiO₂.

Toxicity findings

All toxicity tests showed concentration-dependent inhibitory effects on *Vibrio fischeri* luminescence, water flea mobility, and zebrafish embryos development (Figure 3, Table 2). The 30-min IC₅₀ for *Vibrio fischeri* was 2.4 mg L⁻¹ (95 % CI: 2.4-2.5 mg L⁻¹) and 24-h EC₅₀ for water fleas was 12.5 mg L⁻¹ (95 % CI: 11.3-14.1 mg L⁻¹). The most pronounced sublethal effect of BPA was lack of body pigmentation; the 48-h EC₅₀ was 7.5 mg L⁻¹ (95 % CI: 5.0-11.5 mg L⁻¹), and the 48-h LC₅₀ for

Table 1 Experimental conditions of the photolytic/photocatalytic/H₂O₂ oxidation of Bisphenol A (BPA), the BPA conversion and removed BPA.

Sample	Water	Light	Catalyst	Conversion (%)	Residual BPA (mg L ⁻¹)
0 (baseline sample)	Ultra pure	without	without	-	20.0
1	Ultra pure	UV 254 nm, 17 W	without	84	3.2
2	Tap water	UV 254 nm, 17 W	without	73	5.4
3	Ultra pure	UV 365 nm, 150 W	without	16	16.8
4	Ultra pure	UV 365 nm, 150 W	TiO ₂ P-25	100	-
5	Tap water	UV 365 nm, 150 W	TiO ₂ P-25	100	-
6	Ultra pure	UV 254 nm, 17 W	without*	100	-
7	Ultra pure	UV 365 nm, 150 W	without*	100	-
8	Ultra pure	halogen	without	3	19.4
9	Ultra pure	halogen	TiO ₂ P-25	82	3.5

*H₂O₂ was used to accelerate the degradation rate of BPA
- zero value was obtained

Table 2 Percentage of observed lethal and sublethal effects on zebrafish embryos (*Danio rerio*) after 48 h exposure to different concentrations of BPA.

Toxic effects (%)	Treated samples (mg L ⁻¹)				
	0	5	10	15	20
Lethal effects					
Egg coagulation	-	20	10	20	30
Missing tail detachment	-	-	-	-	20
Lack of somite formation	-	-	-	-	-
Missing heartbeat	-	-	-	-	10
Embryo deformation	-	-	-	10	30
Sublethal effects					
Lack of eye development	-	-	-	-	-
Lack of movement	-	-	-	-	-
Lack of eye pigmentation	-	-	-	57	100
Missing blood circulation	-	-	-	-	-
Lack of body pigmentation	-	25	56	100	100
Lack of otoliths	-	-	-	-	-
Yolk sac edema	-	-	-	29	75

- zero value was obtained

Danio rerio was 18.2 mg L⁻¹ (95 % CI: 16.9-20.2 mg L⁻¹).

A correlation between BPA concentration and luminescence inhibition was not established for the treated samples. In fact, methods that most efficiently removed BPA (samples 6 and 7) showed similar luminescence inhibition as the baseline sample, whereas photocatalysis with TiO₂ (sample 4) showed about 80 % inhibition (Figure 4). In other words, the treated samples were still toxic to *Vibrio fischeri*. We are not inclined to attribute higher toxicity of samples 6 and 7 to H₂O₂ because peroxide

concentration in these samples was 5 mg L⁻¹ and aquatic toxicity data report only slight toxicity to microorganisms (30-min EC₅₀ = 30 mg L⁻¹) (39). Instead, the toxicity of these and other samples with high BPA removal efficiency is more likely associated with oxidation by-products, as suggested by Neamtu and Frimmel (20). In contrast to our findings, Chiang et al. (16) reported a decrease in toxicity to *Vibrio fischeri* during TiO₂ photocatalytic oxidation of BPA, despite the confirmed formation of oxidation by-products. Olmez-Hanci et al. (23) reported similar toxicity to ours due to the formation of oxidation

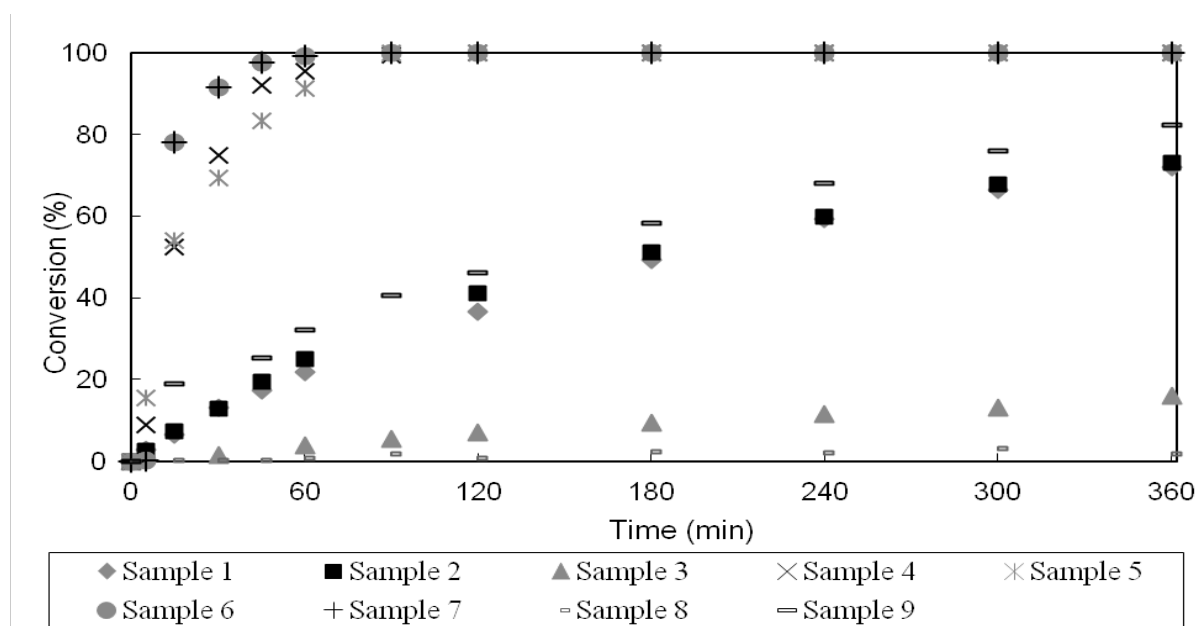


Figure 2 BPA conversion as a function of time obtained over various experimental conditions and catalysts.

Table 3 Percentage of observed lethal and sublethal effects on zebrafish embryos (*Danio rerio*) after 48 h exposure to samples treated with photolytic/photocatalytic/H₂O₂ oxidation

Toxic effects (%)	Treated samples									
	0	1	2	3	4	5	6	7	8	9
Lethal effects										
Egg coagulation	30	10	10	10	10	10	10	100	-	-
Missing tail detachment	20	-	-	-	20	40	50	-	20	-
Lack of somite formation	-	-	-	-	-	-	-	-	-	-
Missing heartbeat	10	-	-	-	20	40	40	-	-	-
Embryo deformation	30	-	-	-	30	70	70	-	-	-
Sublethal effects										
Lack of eye development	-	-	-	-	-	-	-	-	-	-
Lack of movement	-	-	-	-	11	45	56	-	-	-
Lack of eye pigmentation	100	-	11	45	22	33	-	-	70	-
Missing blood circulation	-	-	-	-	-	-	-	-	-	-
Lack of body pigmentation	100	55	78	89	89	89	78	-	100	78
Lack of otholits	-	-	-	-	-	-	-	-	-	-
Yolk sac edema	75	-	-	45	78	67	78	-	50	11

- zero value was obtained

products, but they also reported a gradual drop as these products degraded after 90 minutes.

The results of the mobility inhibition test with water fleas after 24 h of exposure only reinforce our bioluminescence findings (Figure 4). High toxicity (100 % immobility of water fleas) remained in all samples treated with photocatalytic oxidation or UV/H₂O₂ oxidation, regardless of the type of illumination or reaction medium (samples 4-7 and 9) and despite nearly complete BPA conversion. In

comparison, the samples treated with photolysis showed lower toxicity, even though BPA was still present. Sample 2 showed 10 % immobility, which is within the range of allowed immobility for controls and therefore not considered toxic (33). The water flea toxicity test has confirmed what the luminescent bacteria toxicity test suggested, that the major cause of toxicity were toxic intermediates produced by photocatalytic and UV/H₂O₂ oxidation. As with peroxide, we are not inclined to attribute the

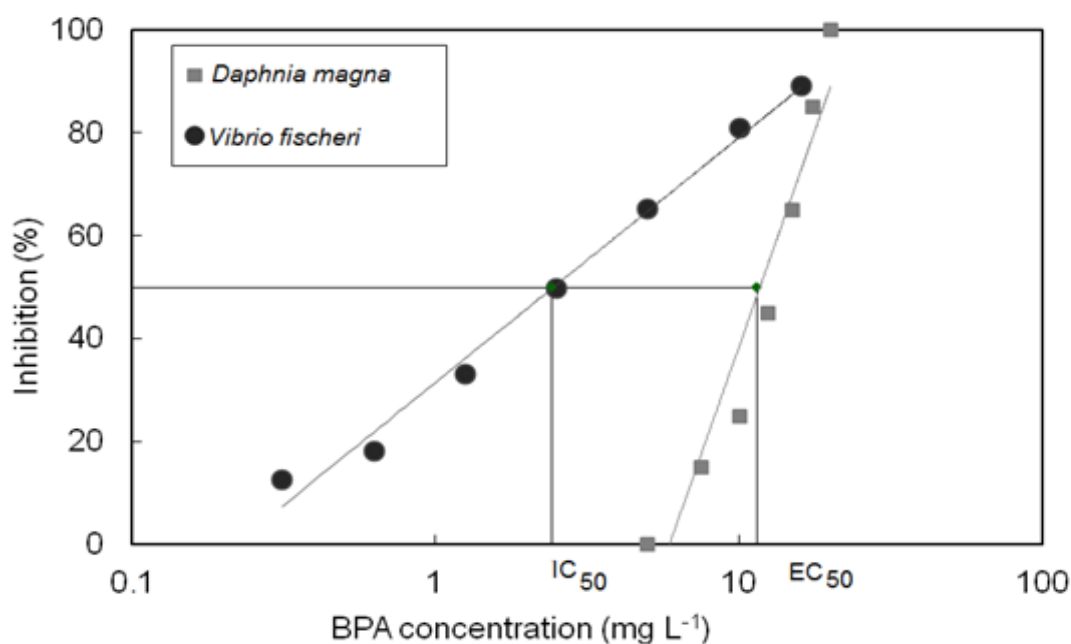


Figure 3 Inhibition of bioluminescence in luminescent bacteria (*Vibrio fischeri*) and inhibition of mobility of water fleas (*Daphnia magna*), dependent on different BPA concentrations. IC₅₀ represents the 30 min IC₅₀ for *Vibrio fischeri*. EC₅₀ represents the 24-h EC₅₀ for *Daphnia magna*

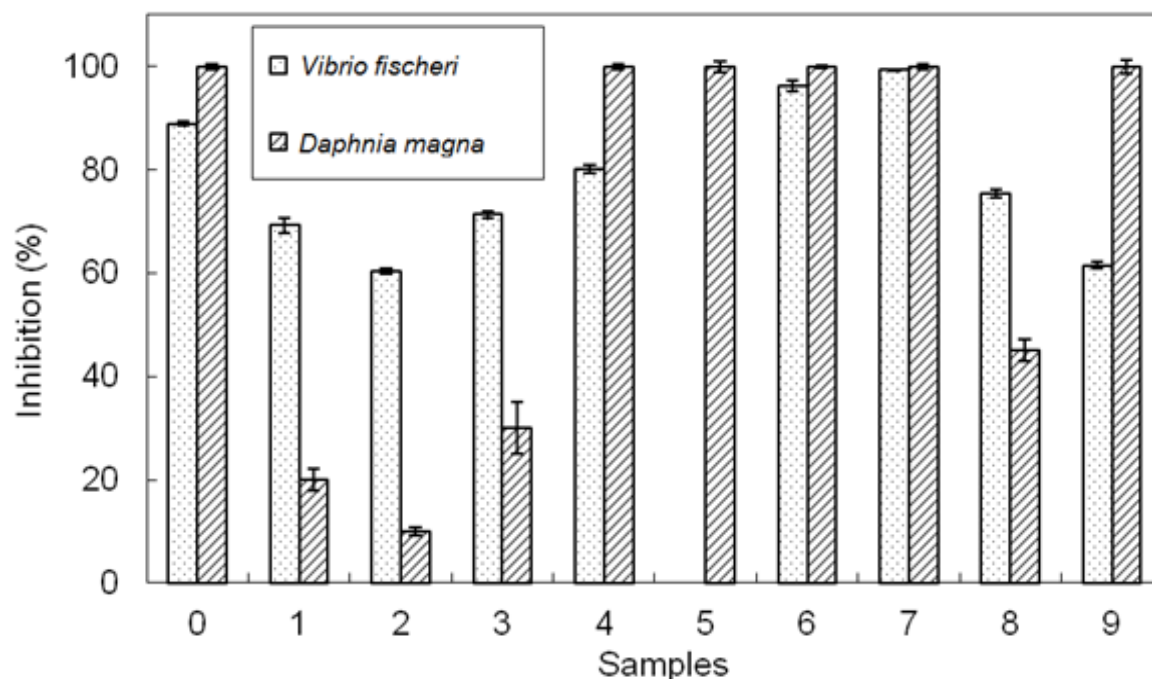


Figure 4 Inhibition of bioluminescence in luminescent bacteria (*Vibrio fischeri*) and inhibition of mobility of water fleas (*Daphnia magna*) in samples treated with photolytic/photocatalytic/ H_2O_2 oxidation. Sample 0 was the baseline sample containing BPA in the concentration of 20 mg L^{-1} . Luminescence inhibition was not measured in sample 5 due to technical problems

observed toxic effects to TiO_2 dissolution, as Chiang et al. (16) did not detect titanium ions in the end-product solutions.

The results of the toxicity test on zebrafish embryos show fewer lethal and sublethal effects in samples treated with photolytic oxidation (samples 1-3 and 8) than in the baseline sample or samples treated with photocatalytic or UV/H_2O_2 methods (Table 3). The only detectable lethal effect was egg coagulation. In samples 1-3 it was observed in 10 % of the exposed embryos, which is not considered a toxic effect (35).

In the photolytically treated samples toxic effects correlated with residual BPA, but this was not the case with photocatalytically treated samples, as they turned out to be more toxic to zebrafish embryos (with the exception of sample 9) even though they did not contain any residual BPA. Again, this test confirms the implications of the luminescence and mobility tests that most of the toxic effects can be attributed to oxidation by-products of photocatalysis and UV/H_2O_2 treatment.

YES assay

Our YES test only confirms the trends established by the three toxicity tests. We found that BPA is

oestrogenically active and approximately 10,000 times less potent than oestradiol, which is similar to what Li et al. (40) have reported. Our testing has also showed that the oestrogenic activity of BPA is concentration dependent since the beta-galactosidase activity with the highest tested BPA concentration of 20 mg L^{-1} was 1.98 and with the lowest tested concentration of 0.3 mg L^{-1} it was 1.53 (which corresponds to the activity established in the progesterone control sample).

In the main experiment however, oestrogenic activity was BPA concentration-dependent only in samples treated with photolytic methods (UV or visible light), whereas in samples treated with the photocatalytic and UV/H_2O_2 oxidation no such correlation was observed (Figure 5). In samples treated with UV/H_2O_2 oxidation (samples 7 and 8) and photocatalytic oxidation with visible light (sample 9) oestrogenic activity equalled baseline BPA sample (sample 0) even though these samples contained no BPA.

An interesting deviation from the trends observed in the three toxicity tests was the difference in oestrogenic activity between samples 4 and 5, even though both were treated photocatalytically and only

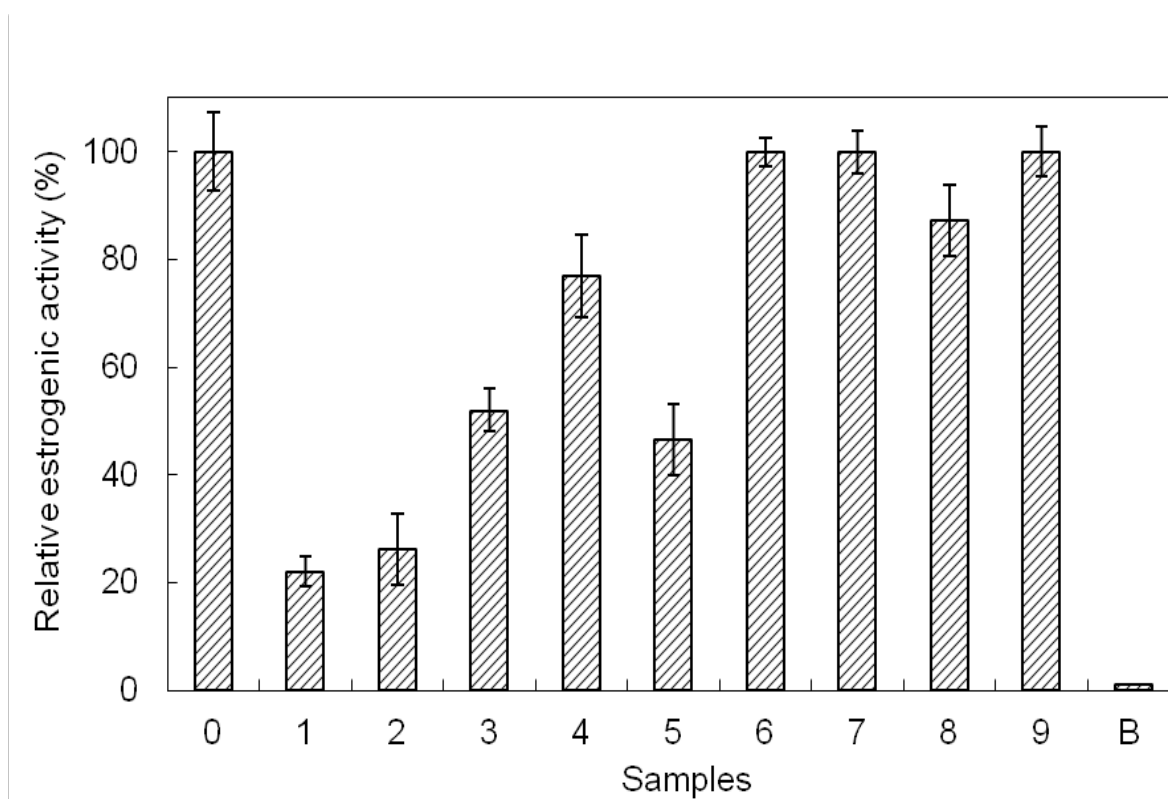


Figure 5 Relative oestrogenic activity of samples treated with photolytic/photocatalytic/ H_2O_2 oxidation, calculated according to the estrogenic activity of 20 mg L^{-1} BPA. Sample 0 was the baseline sample containing BPA in the concentration of 20 mg L^{-1} , while sample B served as a blank (ultrapure water without BPA).

differed in water medium. We do not have a reasonable explanation for the observed difference, and it should be further investigated in future studies.

The results of the oestrogenicity test point to oxidation intermediates as the culprit for high findings, whose presence has been confirmed by several other reports (20-25).

The results of the bioassays used in our study show inter-species differences in the responses to the baseline BPA and treated samples, which were lower in the zebrafish embryos than in water fleas and luminescent bacteria. These findings suggest that reliable biomonitoring of waters should always rely on several test species.

Our findings clearly show that current pollutant removal techniques can result in the formation of harmful by-products and that chemical analysis of water pollutants is not reliable enough to determine the most efficient wastewater treatment method. Chemical analysis should therefore be combined with toxicity and oestrogenic activity testing to help in pollution prevention.

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Izvleček

Učinkovitost odstranjevanja strupenosti in estrogenske aktivnosti bisfenola A v vodnih vzorcih, tretiranih z naprednimi oksidacijskimi procesi

Bisfenol A je dobro poznan motilec endokrinega sistema z estrogensko aktivnostjo in škodljivimi učinki na ljudi in živali. Zaradi tega se pojavlja potreba po učinkovitem odstranjevanju BPA iz odpadnih voda. V raziskavi smo proučevali učinkovitost odstranjevanja BPA iz vodnih vzorcev s procesi fotolitske, fotokatalitske in UV/H₂O₂ oksidacije. Med poskusom smo vodne vzorce osvetljevali s tremi različnimi žarnicami (halogensko, 17 W UV, 254 nm in 150 W UV, 365 nm). V procesih foto(kata)litske oksidacije smo uporabili H₂O₂ in katalizator TiO₂ P-25 (Degussa). Akutno strupenost in estrogensko aktivnost BPA ter tretiranih vzorcev smo določili s testi strupenosti na luminiscentne bakterije (*Vibrio fischeri*), vodne bolhe (*Daphnia magna*), zarodke cebric (*Danio rerio*) in YES testom z gensko spremenjenimi kvasovkami *Saccharomyces cerevisiae*. Rezultati so pokazali, da je BPA za vodne organizme strupen in estrogensko aktiven. Kemijske analize so pokazale zmanjšanje vsebnosti BPA po fotolitski oksidaciji in popolno odstranitev BPA iz vodnih vzorcev po fotokatalitski oksidaciji in UV/H₂O oksidaciji. Strupenost in estrogenska aktivnost sta se zmanjšali pri vzorcih, tretiranih s fotolitsko oksidacijo, sorazmerno z zmanjšanjem vsebnosti BPA. Pri fotokatalitsko tretiranih vzorcih se kljub popolni pretvorbi BPA strupenost in estrogenska aktivnost nista zmanjšali ali pa sta se celo zvišali v primerjavi z izhodnim vzorcem. Ugotovili smo, da ugotavljanje učinkovitosti odstranjevanja BPA iz vodnih vzorcev z naprednimi oksidacijskimi procesi z uporabo kemijskih analiz ne zadostuje. Potrebna so biološka testiranja, ki dokažejo morebitno preostalo strupenost in estrogensko aktivnost obdelanih vzorcev.

KLJUČNE BESEDE: *biološki test; motilci endokrinega sistema; fotokataliza; fotoliza*

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