Available online at www.sciencedirect.com
ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 187 (2007) 186–195
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Photolysis, oxidation and subsequent toxicity of a mixture of polycyclic aromatic hydrocarbons in natural waters

Hilla Shemer [1], Karl G. Linden *

Department of Civil and Environmental Engineering, Duke University, 121 Hudson Hall Engineering Building, Box 90287, Durham, NC 27708-0287, USA

Received 8 June 2006; received in revised form 7 October 2006; accepted 7 October 2006
Available online 10 November 2006

Abstract

Photodegradation of a mixture of three polycyclic aromatic hydrocarbons fluorene (FLU), dibenzofuran (DBF), and dibenzothiophene (DBT) using UV and UV/H_2O_2 processes was studied. Treating a mixture of the PAHs stimulated a more realistic contamination composition present in polluted water. Effects of pH, PAH concentration, and water matrix composition on removal rates and efficiencies of these compounds are discussed. Batch experiments were conducted using both monochromatic low pressure (LP, 253.7 nm) and polychromatic medium pressure (MP, 200–400 nm) UV sources, in a quasi-collimated beam setup. A synergistic effect was observed during direct photolysis and LP-UV/H_2O_2 of the mixture as compared to photodegradation as a single component in an aqueous solution. Similar results were obtained for FLU using MP-UV/H_2O_2 whereas, degradation of DBF and DBT was inhibited in a mixture. Natural water enhanced the direct photolysis compared to laboratory buffered water, whereas, degradation of the PAHs in the natural water was inhibited using the UV/H_2O_2 process. Toxicity testing using a luminescent inhibition bioassay was correlated to intermediates generated during UV-based oxidation reactions.

Keywords: Photodegradation; UV irradiation; Quantum yield; Hydroxyl radicals; Toxicity

1. Introduction

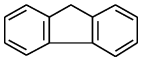
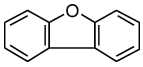
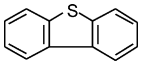
Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different compounds introduced to the environment by incomplete combustion of coal, oil, and wood, improper storage or disposal of fuels and oils, and wood treatment processes. PAHs and their oxidation products are usually found as a mixture containing two or more of these compounds. PAHs were identified in industrial and municipal wastewater, effluents, rain, surface and drinking water, sediment, soils, and plants [1–3]. PAHs are of concern because many of them are toxic, carcinogenic, and they tend to bioaccumulate in aquatic organisms. Exposure to wildlife and humans can occur through advective and/or diffusive transport of contaminants and microbially produced intermediates to the overlying water column, where these chemicals can be subject to photolytic attack. Microbial transformations of photoproducts can also occur through the activity of suspended or sediment bacteria.

PAHs are removed from the environment by volatilization, photo- and chemical oxidation, adsorption to soil particles, leaching, bioaccumulation and biodegradation [4]. Among the engineered processes bioremediation is generally considered having cost and technical advantages [5]. Advanced oxidation processes (AOPs), by which hydroxyl radicals are generated in order to destroy the organic contaminant, were also studied as an alternative to or in combination with biodegradation for removal of PAHs from aqueous solutions [6–10]. The highly reactive hydroxyl radicals, generated during AOPs, can lead to complete mineralization of the pollutant but most typically lead to formation of products of higher polarity and solubility in water such as phenols, quinones, and acids [11–12]. These metabolites may be far more toxic as compared to their parent compounds [13–16]. For accurate assessment of the hazards of PAHs (as a sole component or in a mixture), it is necessary to know the potential of engineered or naturally occurring environmental processes to cause toxicological effects due to the formation of these oxidation by-products. Little information

* Corresponding author. Tel.: +1 919 6605196; fax: +1 919 6605219.
E-mail addresses: shilla@duke.edu (H. Shemer), kglinden@duke.edu (K.G. Linden).
[1] Tel.: +1 919 6605518; fax: +1 919 6605219.

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.10.018

Table 1
Physical and chemical properties of FLU, DBF, and DBT [23]

Compound	Structure	MW (g mol^{-1})	Water solubility (μM)	log K_{OW}
FLU		166.2	12.0	4.22
DBF		168.2	18.4	4.12
DBT		184.3	7.9	4.44

exists concerning the potency of multiple PAHs in well-defined bioassay systems. Toxicity of PAHs in aquatic environments was assessed using various whole organism assays such as fish, macrophytes, protozoa, algae, and bacteria [16]. Luminescent bacteria have been found to be particularly useful in evaluating toxicant impacts [16] and provide a measure of sub-lethal response for a single or a mixture of pollutants. Chemical analysis may be complemented by the application of bioassays that give an integrated measure of toxicity. Non-target pollutants as well as reduced availability of toxicants and combined effect such as antagonistic or synergistic interactions are considered when using a biological test systems [17].

Fluorene (FLU), dibenzofuran (DBF) and dibenzothiophene (DBT) are three ring PAHs largely derived from anthropogenic sources (e.g., petroleum products), although they can also form through natural processes (e.g., forest fires). These compounds share fluorene's basic molecular structure, only differing by one atom in the bridge of the furan ring: C in FLU, O in DBF, and S in DBT (Table 1). Like many PAHs, these compounds are sparingly soluble with low volatility and high K_{OW} (Table 1) and in soils and sediments they strongly associate with dissolved or particulate organic matter [18–19] that may significantly increase apparent solubility [20] yet often reduces bioavailability and toxicity [21]. Interaction with precipitated organic matter may also reduce toxicity of PAHs [22].

In this research the kinetics and quantum yields for treating DBF ($C_{13}H_{10}O$), FLU ($C_{13}H_{10}$), and DBT ($C_{13}H_{10}S$) in a mixture, using UV and UV/H_2O_2 were determined. Treating a mixture of the PAHs stimulated a more realistic contamination composition present in polluted water. Effects of pH, organics concentration, and matrix composition on the removal rates and efficiencies of these compounds were studied. Photolysis of each compound in a mixture was compared to their photodegradation as a sole component in buffered laboratory water. Luminescent inhibition toxicity bioassay was used to evaluate the efficiency of the UV-based oxidation reactions.

2. Methods and materials

2.1. Materials

DBF and FLU (98% purity) were purchased from Alfa Aesar (MA, USA), DBT (98% purity) from Aldrich (MO, USA), hydrogen peroxide (30% in water) and HPLC grade acetonitrile from Fisher Chemicals (NJ, USA), HPLC grade water from Acros Organics (Geel, Belgium). All chemicals were used as received and all solutions were prepared with de-ionized (DI) water.

2.2. Experimental setup

Two UV sources were studied for the degradation of DBF, FLU, and DBT, low pressure mercury (LP) vapor germicidal lamps (ozone-free, General Electric #G15T8) and medium pressure (MP) Hg arc lamps (Hanovia Co., Union, NJ). The former emits essentially monochromatic light at 253.7 nm, while the latter has various outputs ranging from about 200 nm to above 400 nm. Photolysis was carried out in a quasi-collimated beam apparatus with and without the addition of up to 25 mg L^{-1} hydrogen peroxide. A mixture of PAHs, at initial concentration of 2 μM each, were exposed in batches to average UV fluences of up to 1000 mJ cm^{-2}, at ambient temperature. The UV fluence was defined as the energy over the 200–300 nm range and at 254 nm for the MP and LP lamps, respectively.

In order to overcome the low solubility of the PAHs in water, methanolic stock solutions of the PAHs mixture were placed in a glass vessel, the methanol was evaporated with a gentle stream of N_2, after which buffer was added at a desired pH and the solution was covered and stirred overnight to dissolve the chemicals. Experiments were carried out in a 70 mm × 50 mm crystallization dish with surface area of 34.2 cm^2 open to the atmosphere. A schematic description of the experimental setup is described in detail elsewhere [24]. A 100 mL aqueous solution was gently stirred to maintain homogeneity. Exposure times necessary to achieve UV fluences from 0 to 1000 mJ cm^{-2} were determined from the average irradiance as calculated with a spreadsheet program using the lamp spectrum, solution absorbance, incident irradiance read from a calibrated radiometer (1700IL, SED 240/W, International Light, Peabody, MA), and the measured Petri-factor for the dish (0.96 for the MP lamp and 0.97 for the LP lamp). Incident photon irradiance (E cm^{-2} s^{-1}) was measured by taking the lamps emission spectrum with a diode array spectrometer (Ocean Optics, Dunedin, FL) whose response was calibrated using a NIST traceable standard D2 lamp (Thermo Oriel, Stratford, CT). All experiments were performed in duplicate. No loss of PAHs due to volatilization or/and hydrolysis was observed in unexposed stirred samples.

Photodegradation of the three PAH compounds in a mixture was compared to their photodegradation as a sole component, in 2 mM phosphate buffer solution (PBS), at pH 7. Furthermore, the

effect of the following parameters on the degradation rate of each compound was studied in a mixture of the three PAHs: (i) pH at 4 (2 mM acetate buffer), 7 (2 mM phosphate buffer), and 10 (2 mM borate buffer); (ii) initial concentration of the PAHs at (0.8, 2 and 4 μM of each compound) at pH 7; (iii) degradation in natural water obtained from Eno River NC, USA and from Atlantic Woods Superfund Site located on an estuary of the Elizabeth River VA, USA; (iv) anions present in natural water including chloride (0, 10, 100, and 10,000 mg L^{-1}) and sulfate (0, 10, 100, and 1500 mg L^{-1}) were added to 2 mM phosphate buffer originated from NaCl and Na_2SO_4 salts, respectively. The effect of phosphate ions was measured using 0–20 mM PBS solution at pH 7.

Experiments to identify singlet oxygen and hydroxyl radicals, formed during direct photolysis in natural water, were performed by adding 0.001 M sodium azide or 0.085 M 2-propanol to a mixture of PAHs. Azide ions scavenge singlet oxygen and hydroxyl radicals while 2-propanol scavenges only hydroxyl radicals. It is assumed that oxidation resulting from these oxidizing species will be inhibited in the presence of azide or 2-propanol.

2.3. Sampling

Samples from the rivers were taken near the shore, 10–20 cm below the water surface, and collected in 4 L amber glass bottles. Samples were filtered under vacuum the same day of sampling using washed Millipore glass fiber filters of 0.45 μm pore size. The filtrates were refrigerated in amber glass bottles in the dark at 4 °C. UV exposure experiments were conducted within 3 days of sampling.

2.4. Analysis

Samples (1 mL) were extracted for analysis using C-18 (7.5 mm × 150 mm) reversed phase Varian Prostar HPLC (Varian, Inc., Palo Alto, CA) equipped with a photodiode array detector. Isocratic elution was used with a mobile phase of 70:30 HPLC grade acetonitrile and water, at flow rate of 1.5 mL min^{-1}, 100 μL injection, absorbance detection at 242–252 nm. The retention times were 5.7, 6.2 and 7.1 min for DBF, FLU, and DBT, respectively.

Water quality was determined using the following methods: total organic carbon (TOC) was measured by a Tekmar Dohrmann Apollo 9000 Total Carbon analyzer in accordance with Standard Method 5310A [25]. Turbidity was determined using Hach 2100N turbidimeter. pH of the solution was determined using a Thermo Orion 920A pH meter. Alkalinity and total hardness were measured according to the Standard Methods 2320 and 2340 [25], respectively. Anion concentrations were measured with DX-120 Dionex ion chromatograph equipped with an AS 14A column, elluent 8 mM Na_2CO_3 and 1 mM $NaHCO_3$.

2.5. Toxicity assay

Bioluminescence inhibition assay, based on a marine gram negative bacterium, *Vibrio fischeri* (NRRL B-11177 (ATCC)), was used for acute toxicity estimation of a mixture of DBF, FLU, and DBF. Light production is directly proportional to the metabolic activity of the bacterial population and any inhibition of enzymatic activity causes a corresponding decrease in bioluminescence [26]. Prior to toxicity testing, cultures of *V. fischeri* were grown in photobacterium broth (Fluka) at 15 °C in darkness with continuous mixing. Cultures were harvested after 3 days by centrifuging 17.5 mL at 5000 × *g* for 5 min at 15 °C. The pellet was re-suspended in 100 mL 2% NaCl to obtain OD at 600 nm from 0.82 to 0.86 (measured by a UV spectrophotometer-Cary Bio100, Varian, Inc., Palo Alto, CA). A 0.5 mL aliquots of culture suspension were added to a 48-well tissue culture plate (Corning Inc., costar 3548). After 10 min incubation at 15 °C in the 48-well culture plate, the luminescence was measured using FLUOstar OPTIMA fluorescence measurement system (BMG Labtech, Inc., Germany) with spectral range of 240–740 nm. Wells were then dosed with 0.5 mL of the tested solution and incubated in darkness for 30 min at 15 °C, after which the bacterial luminescence was measured again. Tested solutions were added to the wells in triplicates, and luminescence was measured twice for each plate. Toxicity assays were conducted for mixtures of PAH solution after exposure to direct photolysis at UV fluences from 0 to 2500 mJ cm^{-2} and for UV/H_2O_2 (25 mg L^{-1}) treatment at UV fluences from 0 to 1000 mJ cm^{-2}. The assay was carried out in duplicate in aqueous solution without solubility enhancing agents in order to exclude unwanted interactions with the PAHs. Control samples consisting of bacterial suspension in 2% NaCl with 2 mM PBS, the media of the UV exposure experiments, were included along with the test sample. For the UV/H_2O_2 additional controls were conducted for the effect of the enzyme catalase, which was added to the PBS solution in order to destroy residual H_2O_2 prior to analysis. The decrease in bacterial luminescence (% inhibition) due to addition of the PAHs was determined as follows [15]:

$$\%\ \text{inhibition} = 100 \times \left(1 - \frac{L_t C_0}{L_0 C_t}\right) \quad (1)$$

where L_0 and C_0 are the luminescence of test samples and control at $t = 0$. L_t and C_t are luminescence values for test samples and control measured after 30 min incubation.

3. Results and discussion

3.1. Mixture versus sole component

Degradation of the mixture of DBF, FLU, and DBT using UV irradiation and UV/H_2O_2, were compared to the photodegradation of each compound as a sole component, in 2 mM phosphate buffer solution at pH 7 (Fig. 1). Plotting ln([PAH]/[PAH]$_0$) versus UV fluence resulted in linear relationship indicating pseudo first order degradation kinetics for both direct photolysis (Fig. 1A) and UV/H_2O_2 degradation (Fig. 1B) in a mixture and as a sole component. The term [PAH]/[PAH]$_0$ represents the ratio between the residual to initial concentration of each compound. The slopes of Fig. 1 gives the UV fluence based pseudo first order rate constant, k'_d. Results for both lamp types are summarized in Fig. 2A. The decay performance of the respective

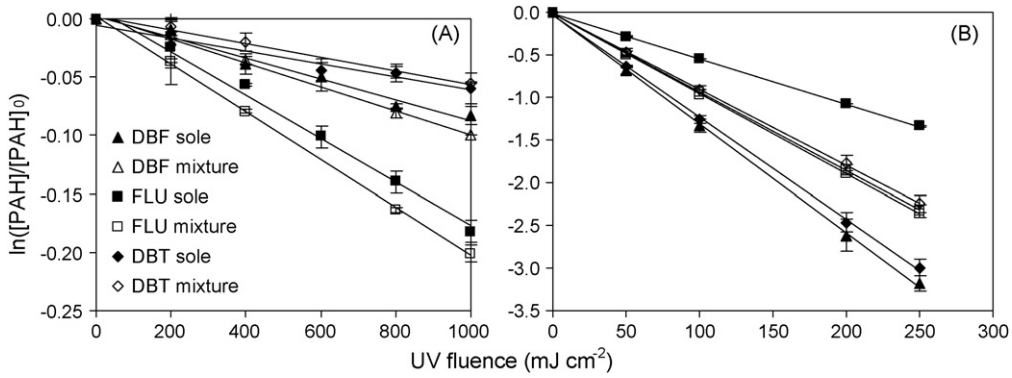


Fig. 1. Direct photolysis using LP lamp (A) and 25 mg L^{-1} hydrogen peroxide assisted photodegradation using MP lamp (B) of DBF, FLU and DBT as a sole component and in a mixture in 2 mM phosphate buffer at pH 7.

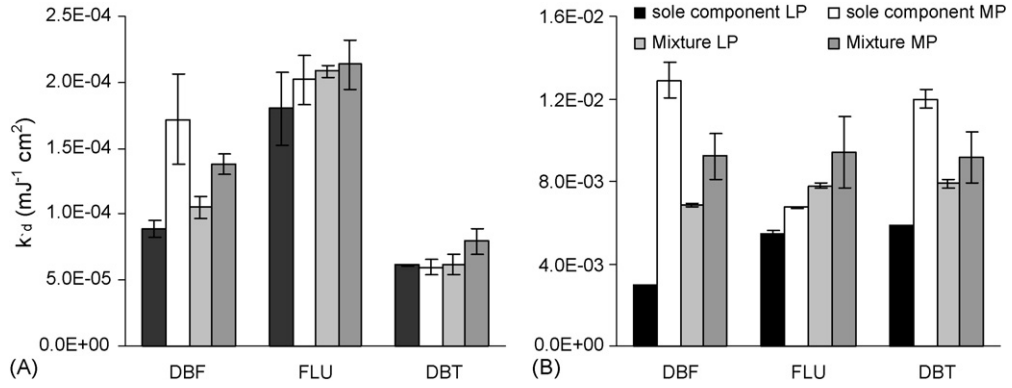


Fig. 2. Direct (A) and hydrogen peroxide (B) assisted photodegradation rate constants of DBF, FLU and DBT as a sole component and in a mixture, using both LP and MP lamps in 2 mM phosphate buffer at pH 7.

UV sources were directly compared via the UV fluence-based rate constants, which is considered a more accurate basis for comparing photochemical reactions than time based kinetics [24].

Direct photolysis in a mixture of the three compounds DBF, FLU and DBT, using the LP lamp, was slightly accelerated as compared to their photolysis as a sole component in phosphate buffer solution, as shown in Fig. 2A. The same results were obtained for FLU and DBT, using the MP lamp, whereas, degradation of DBF was found to be faster when irradiated as a sole component in the solution. The synergic effect observed during photolysis of the mixture may be caused by reaction of the molecules of PAHs with the primary oxidizing agents formed by their excited state such as PAH radical cations [7]. In a mixture of organic compounds attenuation and shading of the UV light can occur since there is an overlap in the absorbance spectrum of the various compounds hence, the ratios between the fractions of light absorbed by each of the compounds changes throughout the wavelength range (Fig. 3). The effect of FLU and DBT on the fraction of light absorbed by the DBF can be seen clearly at the maxima range of its absorbance between 250 and 300 nm which coincides well with the main emission bands of the MP lamp, a logical explanation for the antagonistic effect on decay of DBF in the mixture. Whereas, less significant overlap exists between the maxima absorbance of FLU and DBT and the emission of the MP lamp.

Next the quantum yields (Φ) for each of the PAHs, in a mixture and as a sole component, was calculated using Eqs. (2) and (3). Quantum yield is defined as the number of moles of product formed or reactant removed per Einstein of photons absorbed by the reactant

$$\frac{\mathrm{d}[\mathrm{PAH}_i]}{\mathrm{d}t} = \Phi_i \sum_{\lambda} E_{\mathrm{p}}^{0}(\lambda) f_{\mathrm{PAH}_i(\lambda)} (1 - \exp^{-(2.303 a(\lambda) z)}) \quad (2)$$

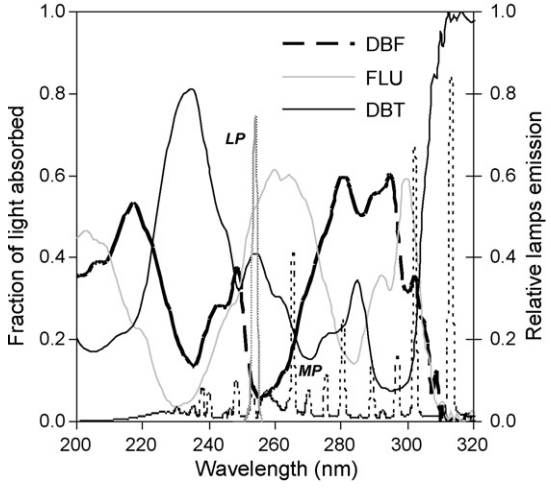


Fig. 3. Fraction of light absorbed by DBF, FLU, and DBT in a mixture of 2 μM each and relative LP and MP UV lamps emission spectra.

Table 2
Quantum yields of DBF, FLU, and DBT in a mixture and as a sole component at 254 nm (LP lamp) and 200–350 nm (MP lamp)

Compound	LP		MP	
	Sole component	Mixture	Sole component	Mixture
DBF	$(1.29 \pm 0.08) \times 10^{-2}$	$(9.60 \pm 0.16) \times 10^{-3}$	$(4.30 \pm 1.11) \times 10^{-3}$	$(2.19 \pm 0.04) \times 10^{-3}$
FLU	$(2.53 \pm 0.82) \times 10^{-3}$	$(2.57 \pm 0.41) \times 10^{-3}$	$(5.20 \pm 0.69) \times 10^{-3}$	$(2.64 \pm 0.19) \times 10^{-3}$
DBT	$(1.72 \pm 0.45) \times 10^{-3}$	$(9.97 \pm 0.73) \times 10^{-4}$	$(1.71 \pm 0.17) \times 10^{-3}$	$(8.69 \pm 0.46) \times 10^{-4}$

$$f_{\mathrm{PAH}_i(\lambda)} = \sum_{\lambda} \frac{\varepsilon_{\mathrm{PAH}_i} C_{\mathrm{PAH}_i}}{\varepsilon_{\mathrm{PAH}_i} C_{\mathrm{PAH}_i} + \varepsilon_{\mathrm{PAH}_{ii}} C_{\mathrm{PAH}_{ii}} + \varepsilon_{\mathrm{PAH}_{iii}} C_{\mathrm{PAH}_{iii}}} \quad (3)$$

where Φ_i is the quantum yield for removal of compound PAH_i (mol E^{-1}); $E_{\mathrm{p}}^{0}(\lambda)$ the volume averaged UV photon flux (E L^{-1} s^{-1}) at wavelength λ calculated by dividing the incident photon irradiance (E cm^{-2} s^{-1}), measured using a spectrometer, by the photon energy (mJ E^{-1}) and the depth of the solution (cm) in wavelength range of 200–350 nm for the MP lamp and at λ = 254 nm for the LP lamp; f_i the fraction of light absorbed by compound PAH_i calculated using Eq. (3); $a(\lambda)$ the solution absorbance (cm^{-1}); z the depth of solution (cm); $\varepsilon_{\mathrm{PAH}_i}$ the molar absorption coefficient (M^{-1} cm^{-1}) and C_{PAH_i} is the molar concentration. Summation in Eqs. (2) and (3) was taken over the wavelength range 200–350 nm for the MP lamp, and at λ = 254 nm for the LP lamp.

Quantum yield of each compound in a mixture was found to be slightly lower (by a factor of 1.3–2.0) than the quantum yield calculated for each PAH irradiated as a sole component using both UV lamps (Table 2), although slightly higher degradation rates were observed in the mixture. Miller and Olejnik [7] reported an increase in quantum yields of benzo[*a*]pyrene (BAP) and FLU by a factor of 2–3 in BAP-chrysene (CHR) and BAP–FLU mixtures, respectively, in relation to photolysis of individual PAHs. Whereas, a decrease of quantum yield was observed for BAP in a BAP–FLU mixture.

Hydrogen peroxide (25 mg L^{-1}) assisted rates of degradation of DBF, FLU, and DBT in a mixture were higher as compared to their degradation as a sole component, using the LP lamp (Fig. 2B), although the steady state concentration of hydroxyl radicals, calculated using Eq. (4), in the mixture (8.5×10^{-13} M) was lower than in all three independent systems ($(1.1–5.1) \times 10^{-12}$ M). These results suggest that the oxidation reaction in a mixture includes other oxidizing agents such as organic radicals ($\mathrm{ROO}^{\bullet}$) formed by reaction of the PAHs with the hydroxyl radicals. Another indication of the occurrence of additional reactions in a mixture (along with hydroxyl radical) was the similar degradation rates of the three compounds, while as individual components they followed the decreasing order of DBT ≥ FLU > DBF (LP) and DBF > DBT > FLU (MP).

$$[^{\bullet}\mathrm{OH}]_{\mathrm{SS}} = \frac{\sum_{\lambda} R_{\mathrm{OH}}^{\mathrm{form}}}{\sum_{i} R_{\mathrm{S,OH}}[\mathrm{s}]_i} = \frac{\sum_{\lambda} E_{\mathrm{p}}^{0}(\lambda)(1 - \exp^{-(2.303a(\lambda)z)}) f_{\mathrm{H_2O_2}}}{\sum_{i} R_{\mathrm{S,OH}}[\mathrm{s}]_i} \quad (4)$$

$$f_{\mathrm{H_2O_2}(\lambda)} = \sum_{\lambda} \frac{\varepsilon_{\mathrm{H_2O_2}} C_{\mathrm{H_2O_2}}}{\sum_{i} \varepsilon_{\mathrm{PAH}_i} C_{\mathrm{PAH}_i} + \varepsilon_{\mathrm{H_2O_2}} C_{\mathrm{H_2O_2}}} \quad (5)$$

where $[^{\bullet}\mathrm{OH}]_{\mathrm{ss}}$ is the steady-state concentration of hydroxyl radicals radicals; $R_{\mathrm{OH}}^{\mathrm{form}}$ the formation rate of hydroxyl radicals; $f_{\mathrm{H_2O_2}}$ the fraction of light absorbed by hydrogen peroxide calculated using Eq. (5) and $R_{\mathrm{S,OH}}^{\mathrm{form}}[\mathrm{s}]_i$ is the sum of the scavenging rate of hydroxyl radicals. In buffered laboratory water the scavengers include the PAH compounds ($k_{\mathrm{OH,DBT}} = 1.8 \times 10^{10}$ M^{-1} s^{-1}; $k_{\mathrm{OH,DBF}} = 1.6 \times 10^{10}$ M^{-1} s^{-1}; $k_{\mathrm{OH,FLU}} = 1.4 \times 10^{10}$ M^{-1} s^{-1}, [27]) and hydrogen peroxide ($k_{\mathrm{OH,H_2O_2}} = 2.7 \times 10^{7}$ M^{-1} s^{-1}, [28]).

Lower rates of degradation of DBF and DBT in a mixture were observed using the MP lamp as compared to their decay as a sole component (Fig. 1B). These results can be explained by the higher fraction of light absorbed by the hydrogen peroxide in the presence of each compound separately rather than in a mixture of all three PAHs. The higher fraction of light absorbed by the hydrogen peroxide correlates to a higher concentration of hydroxyl radicals: 2.1×10^{-15} M in the solution containing the mixture of PAHs as compared to 0.9×10^{-14} and 2.0×10^{-14} M for DBT and DBF, respectively, irradiated as sole components. It is not clear why the degradation of FLU in a mixture was higher as compared to its degradation as a sole component given that similar observations regarding the fraction of light absorbed by the hydrogen peroxide and the concentration of hydroxyl radicals were found when comparing its degradation alone and in a mixture.

3.2. pH effect

Variation of pH from 4 to 10 did not exert any influence on the direct photolysis rate of each of the PAHs in a mixture under the conditions applied, as indicated statistically by analysis of covariance (ACNOVA) with the pH as the covariant. These results were expected since the PAHs studied showed no change of extinction coefficient with pH.

Fig. 4 presents the effect of pH on the UV/H_2O_2 oxidation rates of DBF, FLU, and DBT in a mixture. It can be observed that the increase of pH from 4 to 7 led to an increase in the rate of degradation of all three PAHs. However, further increase of pH up to 10 reduced the oxidation rates back to the level obtained at pH 4. When the pH of an aqueous solution increases, the fraction of radiation that hydrogen peroxide absorbs becomes stronger [12] hence, more hydroxyl radicals are formed. Nevertheless, hydroxyl radical scavenging effect by hydrogen peroxide (Eq. (6)) also increases at higher pH values [12] and hydrogen per-

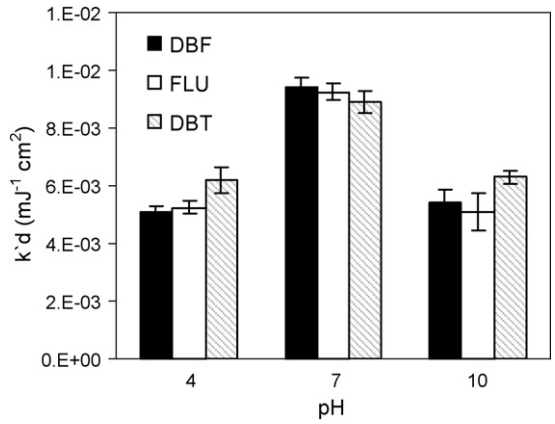


Fig. 4. UV/H_2O_2 rates constants of photodegradation of DBF, FLU and DBT in a mixture, using MP lamps in 2 mM acetic (pH 4), phosphate (pH 7) and borate (pH 10) buffers.

oxide undergoes self-decomposition (Eq. (7)) at neutral to high pH with rate constants of 2.3×10^{-2} and 7.4×10^{-2} min^{-1} at pH 7.0 and 10.5, respectively [29].

$$^{\bullet}OH + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \quad (6)$$

$$H_2O_2 \rightarrow \frac{1}{2}O_2 + H_2O \quad (7)$$

Combinations of these effects, self-decomposition, scavenging, and photodegradation into hydroxyl radicals could explain the effect of the pH on the degradation of the PAHs during UV/H_2O_2 reactions.

3.3. Initial concentration of PAHs

The influence of the initial concentration of the PAHs on the direct photolysis rate using the MP UV lamp was studied in a mixture of the three compounds at concentrations ranging from 0.3 to 4 μM for each compound. Total organic load of the solution ranged from 0.9 to 12 μM, which is the sum of the individual concentrations of these compounds. It was found that the direct photolysis of all three compounds was not affected by the initial concentration of the organic compounds studied, as indicated statistically by analysis (ACNOVA) performed on log([PAH]/[PAH]$_0$) with the initial concentration (PAH$_0$) of each of the compounds as the covariant (data not shown). Similar to the direct photolysis results, no effect of the concentration, from 0.3 to 4 μM, was found for the degradation of the PAHs in the presence of 25 mg L^{-1} hydrogen peroxide. Gregory et al. [30] reported no significant concentration effect on the quantum yield of dibenzothiophene sulfoxide over a range of 0.5–30 mM. In contrast Miller and Olejik [7] and Beltran et al. [6] reported higher rates of photodegradation at higher initial concentration of FLU, chrysene, benzo[*a*]pyrne, phenanthrene, and acenaphthene. These results were explained by higher absorption by the organic compound as their concentration was increased and consequently the observed rate of the reaction increased. Yet in both these studies each compound was irradiated as a sole component using a monochromatic 254 nm UV lamp.

3.4. Natural water

Photodegradation of DBF, FLU and DBT in a mixture, was conducted in natural water obtained from the Eno River near Durham, NC, and from Atlantic Woods Superfund Site located on an estuary of the Elizabeth River in VA. The water quality is presented in Table 3. The apparent pseudo-first order rate constants were compared to those obtained in phosphate buffered water (Fig. 5).

Direct photolysis was enhanced in the natural water as compared to purified buffered water (Fig. 5A), probably through reactions with photo-oxidants (i.e. agents inducing oxidative transformations of natural water components and pollutants). Reactive transients in natural surface water may include hydroxyl radicals, singlet molecular oxygen, excited triplet states of chromophoric dissolved organic matter (DOM) and a variety of radical species ($^{\bullet}$OOR), e.g. radical cations of aromatic structures, phenoxyl and peroxyl radicals [31], carbonate radicals ($^{\bullet}CO_3^-$), generated from the reaction of hydroxyl radicals with either carbonate or bicarbonate ion, and aqueous electron ($e_{(aq)}^-$), a highly reactive and strong reducing species formed upon photolysis of DOM by sunlight and scavenged by nitrate [32]. The great variety of aquatic photo-oxidants, which are formed upon photosensitization by DOM, are still largely unidentified due to the complex and variable chemical composition of DOM. It was further shown that DOM-derived photo-oxidants other than the hydroxyl radical or singlet oxygen dominate the photosensitized transformation of various substituted phenols in fulvic and humic acid solutions, lake waters and seawater [31]. Experiments to identify singlet oxygen and hydroxyl radicals were performed by adding 0.001 M sodium azide or 0.085 M 2-propanol to a mixture of PAHs solution. At neutral pH, azide ions scavenge both singlet oxygen and hydroxyl radicals at rate constants of 7.9×10^8 and 1.2×10^{10} M^{-1} s^{-1}, respectively, and 2-propanol scavenges hydroxyl radicals at rate constant of 1.9×10^{10} M^{-1} s^{-1} [33]. It was found that both singlet oxygen and hydroxyl radicals were not important for the observed decay of the PAHs in both natural waters studied, using both UV lamp types. Similar results were obtained by Canonica and Freiburghaus [31] in regard to phenol photosensitization from DOM, and Vialaton and Richard

Table 3
Water quality of natural water obtained from Eno River North Carolina and from Elizabeth River Virginia, USA

Water	pH	TOC (mg L^{-1} C)	Turbidity (NTU)	Hardness (mg L^{-1} $CaCO_3$)	Alk (mg L^{-1} $CaCO_3$)	Cl^- (mg L^{-1})	NO_3^- (mg L^{-1})	SO_4^{-2} (mg L^{-1})
Eno River	7.5	9.1	7.9	24	15	12.3	1.4	9.4
Elizabeth River	8.1	24.8	0.5	3000	66	9986.3	0.6	1243.6

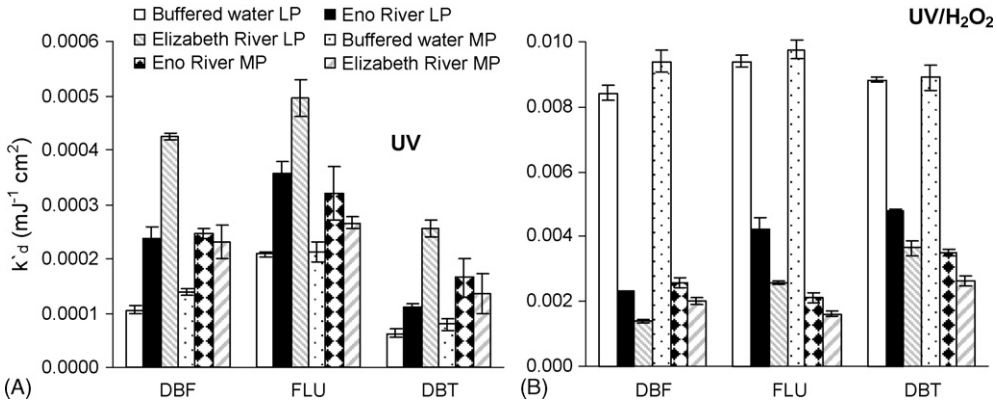


Fig. 5. Rate constants for direct photolysis (A) and 25 mg L^{-1} hydrogen peroxide assisted degradation (B) in buffered and natural water obtained from Eno River North Carolina and from Elizabeth River Virginia.

[33] who reported no effect of these additives on 4-chloro-2-methylphenol and nitrobenzene photo-transformation.

By applying the LP lamp the efficiency of the direct photolysis of all three compounds was faster in the Elizabeth River than in the Eno River water, suggesting that the much higher DOC in the Elizabeth River water contributed to higher photosensitized transformation of the PAHs in these waters. Whereas, using the MP lamp the direct photolysis was faster in the Eno River water probably due to a higher fraction of light absorbed by each target pollutant in the Eno River water. Background absorbance of the Elizabeth River water was higher as compared to the Eno River water (Fig. 6). Light filtering effects due to competition between the UV absorbing species, such as humic material, and the target pollutants is known to cause a reduction of the degradation efficiency.

Hydrogen peroxide assisted degradation of the three PAHs studied using both UV lamps showed much lower rates as compared to laboratory buffered water, as well as lower decay in Elizabeth River water as compared to Eno River water (Fig. 5B). These results were expected due to the scavenging characteristics of DOC and alkalinity. Both these parameters are higher in the Elizabeth River than in Eno River water. Alkalinity, originating from bicarbonate ions (pH < 8.3), scavenge the hydroxyl radicals as shown in Eq. (8).

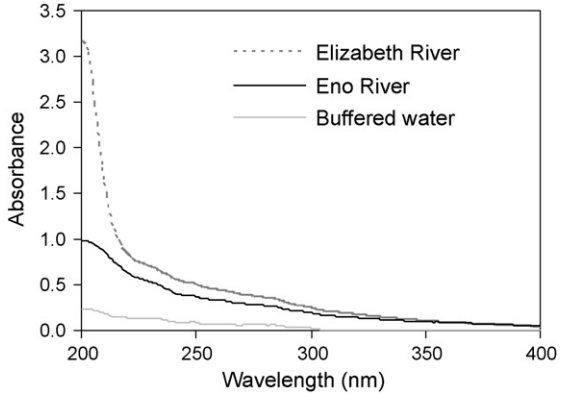


Fig. 6. Background absorbance of phosphate buffer and natural waters tested at UV wavelengths of 200–400 nm.

$$^{\bullet}OH + HCO_3^- \rightarrow H_2O + {}^{\bullet}CO_3^- \quad k = 8.5 \times 10^6\ M^{-1}\ s^{-1} \tag{8}$$

The formed carbonate radicals are highly selective and react relatively slowly with organic compounds [34]. They may also react with hydrogen peroxide to originate peroxy radical ($HO_2^{\bullet}$) [35] which is a much less reactive species than the hydroxyl radical. Natural organic matter not only acts as a hydroxyl radical scavenger but as explained previously, it can filter the UV light reducing the fraction of the incident light intensity available for hydrogen peroxide [35], which is the major source of hydroxyl radicals in the UV/H_2O_2 system. The fraction of light absorbed by the hydrogen peroxide was found to be 1.2- to 7.3-fold higher in the Eno River water than in Elizabeth River water at an equivalent UV fluence. This observation was also reflected by the steady state concentration of hydroxyl radicals that was 2.6-fold higher in the Eno River water than in the Elizabeth River water, 8.3×10^{-17} and 2.1×10^{-16} M, respectively. The steady state concentration of hydroxyl radicals was calculated using Eq. (4), where the denominator also included the scavenging rates of bicarbonate ions ($k_{OH,HCO_3^-} = 8.5 \times 10^6\ M^{-1}\ s^{-1}$) and NOM ($k_{OH,NOM} = 2.5 \times 10^4\ M^{-1}\ s^{-1}$) [36].

Inorganic anions, such as Cl^-, SO_4^{2-}, and $H_2PO_4^-$/HPO_4^{2-}, may effect overall reaction rates by scavenging the hydroxyl radicals to form less reactive inorganic radicals ($^{\bullet}Cl^-$, $^{\bullet}Cl_2^-$, $^{\bullet}HOCl^-$, $^{\bullet}SO_4^{2-}$) that further take part in oxidation reactions [37]. Differences in the anionic content of the natural water did not appear to affect the degradation rate of the PAHs. This conclusion is based on experiments conducted in 2 mM phosphate buffer, which showed that chloride (0–100 mg L^{-1}) and sulfate (0–1500 mg L^{-1}) each separately and as a mixture did not affect the rate of degradation of the mixture of PAHs using a MP lamp and 25 mg L^{-1} hydrogen peroxide. Although, slight inhibition (7–8%) was observed in the presence of 10,000 mg L^{-1} Cl^-. Further experiments showed no effect of phosphate ions (0–1900 mg L^{-1}) on the decay of the PAHs. Gultekin and Ince [34] reported only a slight inhibition of color removal using LP lamp and 4.1 mM hydrogen peroxide at chloride concentration

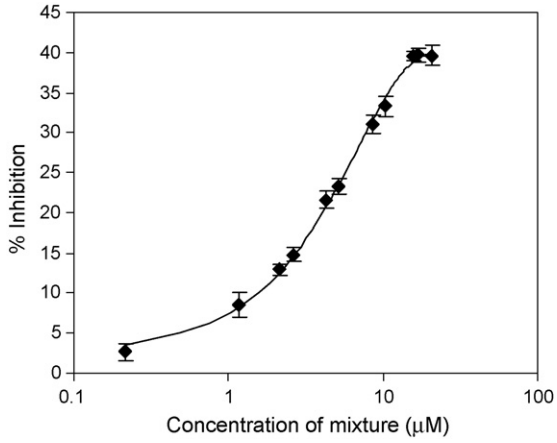


Fig. 7. Dose–response of *V. fischeri* to a mixture of DBF, FLU and DBT.

below 100 mM (3.5 g L^{-1}). At higher concentrations >100 mM of chloride a significant decrease in degradation of the color was observed. The presence of chloride and sulfate led to a decrease in the rate of depletion of atrazine and hydrogen peroxide during H_2O_2/Fe^{3+} reaction [37]. The low concentrations of nitrate ions in the natural water and the fact that during UV/H_2O_2 process the production of hydroxyl radicals is much more effective than from the photolysis of nitrate [38], it can be concluded that nitrate did not affect the degradation rate of the PAHs.

3.5. Toxicity

A combined toxicity effect was evaluated by applying the mixture of DBF, FLU, and DBT at equal concentrations to *V. fischeri* bacteria. A dose–response curve was obtained by exposure of *V. fischeri* to a concentration series of the mixture of the PAHs. Bacterial response was measured as percentage inhibition of luminescence and plotted versus the total concentration (μM) of the mixture in 2 mM phosphate buffer (Fig. 7). Error bars represent the standard deviation of $n = 6$. An increase in the concentration of the mixture resulted in increased inhibition of luminescence, with NOEL (no observed effect level) of 1.2 μM as the sum of the three compounds.

Toxicity of a mixture of PAHs as measured by bioluminescence inhibitions after exposure to UV fluences ranged from 0 to 2000 mJ cm^{-2} was correlated with the decay in concentration of PAHs as analyzed analytically using HPLC (Fig. 8A and B). For direct photolysis an increase of the toxicity was observed for UV fluences ranging from 500 to 1500 mJ cm^{-2}, while the parent compounds were degraded. Hence, the increased toxicity can be related to the intermediates generated during photo-oxidation reaction. The toxicity decreased back to approximately the initial value at a UV fluence of 2000 mJ cm^{-2}. As the irradiation continued the by-products along with the parent compound were further photolyzed and hence the overall toxicity decreased. Photoinduced toxicity is derived from two photochemical processes: photosensitization and photomodification [15,39,40]. During a photosensitization reaction intracellular singlet-state oxygen and other reactive oxygen species (ROS) are generated. Theses species can cause oxidative damage to bacteria via membrane, protein, and DNA damage [41]. In the case of photomodification, organic compounds are structurally altered to a variety of compounds. Photomodification of PAHs is most likely to result in oxygenated products, such as dibenzothiophene 5-oxide and dibenzothiophene [42], flourene 5-oxide [43], 9-fluorenone and 9-fluorenol [44], many of which are regarded as more toxic

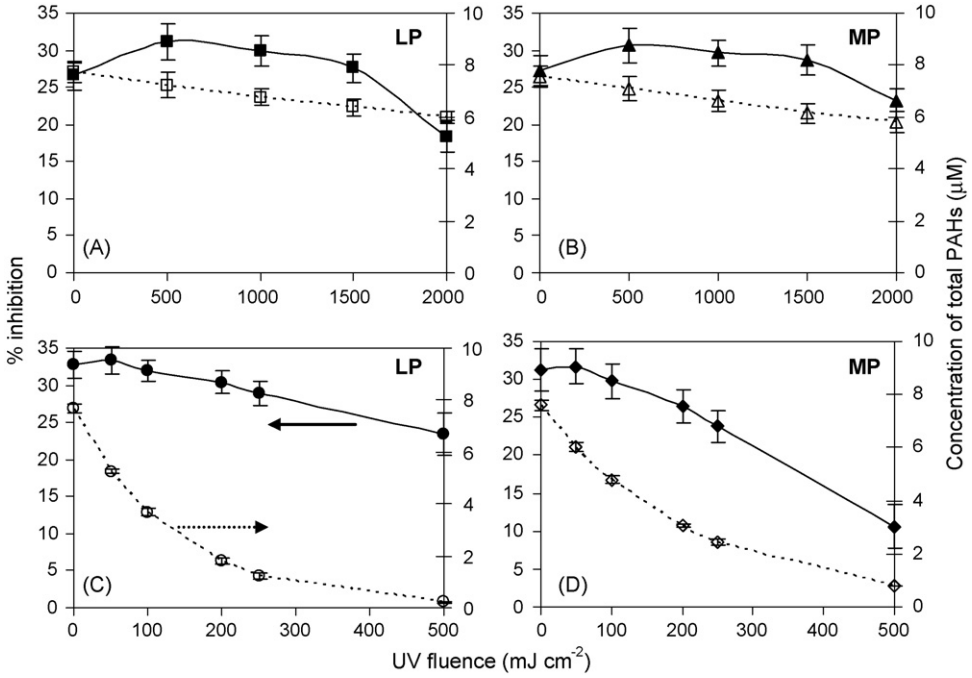


Fig. 8. Toxicity (solid line) and concentration change as measured using HPLC (dash line) during direct photolysis (A and B) and UV/H_2O_2 (C and D). Error bars represents the standard deviation of $n = 12$.

than their parent compound [13–15]. Unlike most experimental setups described in the literature, at which the bacteria along with the PAHs were exposed to light, in this research phosphate buffer solution of a mixture of PAHs were irradiated either by UV alone or in the presence of hydrogen peroxide and then mixed with the bacteria. Thus the observed toxicity could be attributed solely to the remaining parent compound and its degradation by-products (photomodification toxicity) rather than the short-lived singlet oxygen and ROS (photosensitized toxicity). The half-life of these species is only microseconds therefore they cannot affect the bacteria given the experimental setup in this research (the duration between the end of the UV irradiation and the beginning of the bioassay was approximately an hour).

During the UV/H_2O_2 reaction an increase in toxicity was observed after exposure to 50 mJ cm^{-2} after which a linear decrease of the toxicity was found (Fig. 8C and D). Again, it is expected that formation of oxidation products increased the toxicity of the solution to the *V. fischeri* bacteria and as the UV irradiation continued, these intermediate compounds, along with the parent compound were degraded. Possible toxicity effect of hydrogen peroxide was eliminated by destruction of its residual concentration using the enzyme catalase prior to the bioassay. Chemical analysis using HPLC indicated 97% and 90% removal of the sum of the three compounds using LP and MP lamps, respectively, at a UV fluence of 500 mJ cm^{-2}. Whereas, only 29% and 66% decrease in toxicity was measured in the LP and MP irradiation systems, respectively. These results support the conclusion that toxic oxidation by-products are formed as a result of the application of the advanced oxidation process UV/H_2O_2. Furthermore, it can be concluded that nearly complete decay of the parent compounds did not indicate that the hazardous impact of the target pollutants were also removed. Hence, chemical analysis should be complemented by the application of bioassays that provide an integrated measure of toxicity. Non-target pollutants as well as reduced availability of toxicants and combined effects such as antagonistic or synergistic interactions are considered when using biological test system [17].

The same trends of increased toxicity under direct photolysis and linear reduction at UV fluences above 50 mJ cm^{-2} in the UV/H_2O_2 process, were also observed for each of the compounds degrading as a sole component in phosphate buffer solution. Although compared to the mixtures, much lower toxicity values were measured for DBF and FLU as sole components while, higher toxicity was found for DBT as a sole component [27]. Loibner et al. [17] found additive bioluminescence inhibition effects to *V. fischeri* for low molecular weight PAHs acenaphthene (ACE), FLU, phenantherene (PHE), naphthalene, and acenaphthylene. For a mixture of ACE, FLU, PHE, and pyrene, Swartz et al. [45] observed antagonistic interactions for the amphipod *Rhepoxynius abronius*. Verhiest et al. [46] found synergistic PAH interactions of PHE, FLU, and benzo[*k*]fluoranthene measured in whole-sediment tests using *Daphnia magna*, *Hyallela azteca*, and *Chironomus riparius* as test organisms. These results emphasize the complexity of predicting and evaluating the behavior of a compound in a mixture solely by chemical analysis and the need for caution in over evaluating toxicological data from a single testing approach.

4. Conclusions

A synergic effect on degradation was observed during direct photolysis of a mixture containing DBF, FLU, and DBT, as compared to their photodegradation as a single component in aqueous solutions. Variation of pH from 4 to 10 did not exert any influence on the direct photolysis rate of the three PAHs, whereas, an increase of pH from 4 to 7 led to an increase in the rate of degradation during UV/H_2O_2 reaction. Further increase of pH up to 10 reduced the oxidation rates. Concentration ranges from 0.3 to 4 μM for each compound did not affect the removal rates and efficiencies of these compounds by UV and UV/H_2O_2 reactions. Direct photolysis was enhanced in the natural water as compared to purified buffered water probably through reactions with photo-oxidants, although the contribution of singlet oxygen and hydroxyl radicals to the decay of the PAHs was ruled out. Degradation in the natural waters compared to laboratory water was inhibited using UV/H_2O_2 due to the scavenging characteristics of DOC and alkalinity. Anionic content of the natural water (Cl^-, $SO_4{}^{2-}$ and $NO_3{}^-$) did not affect the degradation rate of the PAHs. An inhibition of luminescence toxicity assay indicated formation of toxic intermediates generated during UV-based photolysis and oxidation reactions after which oxidative degradation of these by-products along with the parent compounds resulted in reduced toxicity.

Acknowledgement

This research was made possible by funding to the Duke University Basic Research Center through the Superfund Basic Research Program, grant number P42 ES-010356.

References

[1] D.P. Arfsten, D.J. Schaeffer, D.C. Mulveny, Ecotoxicol. Environ. Saf. 33 (1996) 1–24.
[2] M. Maier, D. Maier, B.J. Lloyd, Water Res. 34 (2000) 773–786.
[3] T. Jamroz, S. Ledakowicz, J.S. Miller, B. Sencio, Environ. Toxicol. 18 (2003) 187–191.
[4] N. Nadarajah, J. Van Hamme, J. Pannu, A. Singh, O. Ward, Appl. Microbiol. Biotechnol. 59 (2002) 540–544.
[5] Y. Zeng, P.K.A. Hong, D.A. Wavrek, Environ. Sci. Technol. 34 (2000) 854–862.
[6] F. Beltran, G. Ovejero, J. Garcia-Araya, J. Rivas, Ind. Eng. Chem. Res. 34 (1995) 1607–1611.
[7] J.S. Miller, D. Olejnik, Water Res. 35 (2001) 233–243.
[8] J.S. Miller, D. Olejnik, Ozone Sci. Eng. 26 (2004) 453–464.
[9] K.M. Lehto, J.A. Puhakka, H. Lemmetyinen, Biodegradation 14 (2003) 249–263.
[10] M.P. Fasnacht, N.V. Blough, Environ. Sci. Technol. 37 (2003) 5767–5772.
[11] G. Stucki, M. Alexander, Appl. Environ. Microbiol. 53 (1987) 292–297.
[12] F.J. Beltran, G. Ovejero, J. Rivas, Ind. Eng. Chem. Res. 35 (1996) 891–898.
[13] X.D. Huang, D.G. Dixon, B.M. Greenberg, Environ. Toxicol. Chem. 12 (1993) 1067–1077.
[14] X.D. Huang, D.G. Dixon, B.M. Greenberg, Ecotoxicol. Environ. Saf. 32 (1995) 194–200.
[15] B.J. McConkey, C.L. Duxbury, D.G. Dixon, B.M. Greenberg, Environ. Toxicol. Chem. 16 (1997) 892–899.

[16] Y.S. El-Alawi, B.J. McConkey, D.G. Dixon, B.M. Greenberg, Ecotoxicol. Environ. Saf. 51 (2002) 12–21.
[17] A.P. Loibner, O.H.J. Szolar, R. Braun, D. Hirmann, Environ. Toxicol. Chem. 23 (2004) 557–564.
[18] J.F. McCarthy, Arch. Environ. Contam. Toxicol. 12 (1983) 559–568.
[19] H.M. Liu, G. Amy, Environ. Sci. Technol. 27 (1993) 1553–1562.
[20] P. Lassen, L. Carlsen, Chemosphere 38 (1999) 2959–2968.
[21] C.E.W. Steinberg, M. Haitzer, R. Bruggemann, I.V. Perminova, N.Y. Yashchenko, V.S. Petrosyan, Int. Rev. Hydrobiol. 85 (2000) 253–266.
[22] J.E. Weinstein, J.T. Oris, Environ. Toxicol. Chem. 18 (1999) 2087–2094.
[23] J.P. Incardona, T.K. Collier, N.L. Scholz, Toxicol. Appl. Pharmacol. 196 (2004) 191–205.
[24] C.M. Sharpless, K.G. Linden, Environ. Sci. Technol. 37 (2003) 1933–1940.
[25] AWWA Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, American Water Works Association and Water Environment Federation, 1998.
[26] S. Parvez, C. Venkataraman, S. Mukherji, Environ. Int. 32 (2006) 265–268.
[27] H. Shemer, K.G. Linden, Water Res., 2007 (In Press).
[28] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 17 (1998) 513–883.
[29] W. Chu, Chemosphere 44 (2001) 935–941.
[30] D.D. Gregory, Z.H. Wan, W.S. Jenks, J. Am. Chem. Soc. 119 (1997) 94–102.
[31] S. Canonica, M. Freiburghaus, Environ. Sci. Technol. 35 (2001) 690–695.
[32] M.W. Lam, K. Mantuco, S.A. Mabury, Environ. Sci. Technol. 37 (2003) 899–907.
[33] D. Vialaton, C. Richard, Aquat. Sci. 64 (2002) 207–215.
[34] I. Gultekin, N.H. Ince, J. Environ. Sci. Health, Part A: Environ. Sci. Eng. 39 (2004) 1069–1081.
[35] C.H. Liao, M.D. Gurol, Environ. Sci. Technol. 29 (1995) 3007–3014.
[36] J. Hoigne, H. Bader, Ozone Sci. Eng. 1 (1979) 73–85.
[37] J. De Laat, G.T. Le, B. Legube, Chemosphere 55 (2004) 715–723.
[38] M. Sorensen, F.H. Frimmel, Water Res. 31 (1997) 2885–2891.
[39] J.L. Newsted, J.P. Giesy, Environ. Toxicol. Chem. 6 (1987) 445–461.
[40] S.N. Krylov, X.-D. Huang, L.F. Zeiler, D.G. Dixon, B.M. Greenberg, Environ. Toxicol. Chem. 16 (1997) 2285–2295.
[41] A.W. Girotti, Photochem. Photobiol. 38 (1983) 745–751.
[42] F. Traulsen, J.T. Andersson, M.G. Ehrhardt, Anal. Chim. 392 (1999) 19–28.
[43] J. Sabaté, J.M. Bayona, A.M. Solanas, Chemosphere 44 (2001) 119–124.
[44] J. Rivas, F.J. Beltran, O. Gimeno, M. Carbajo, Ind. Eng. Chem. Res. 45 (2006) 166–174.
[45] R.C. Swartz, S.P. Ferraro, J.O. Lamberson, F.A. Cole, R.J. Ozretich, B.L. Boese, D.W. Schults, M. Behrenfeld, G.T. Ankley, Environ. Toxicol. Chem. 16 (1997) 2151–2157.
[46] G. Verhiest, B. Clément, G. Blake, Ecotoxicology 10 (2001) 363–372.