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Photoinduced toxicity of selected PAHs to the marine microalga *Phaeodactylum tricornutum*

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In this study, the effects of different concentrations of pyrene, phenanthrene, fluoranthene, and chrysene within the solubility limits of the chemicals on marine microalgal species of *Phaeodactylum tricornutum* were examined under UV-A and UV-B lights by application of batch toxicity tests. The algal species were also exposed to the individual PAHs under cool-white fluorescent bulbs in the same laboratory conditions in order to compare the results with the responses of UV-exposed organisms. EC_{50} values calculated with the trimmed Spearman Karber method demonstrate that the UV light dramatically enhances the toxicity of the selected model PAHs. In most cases, the magnitude of increase in PAH toxicity was directly related to the concentration of individual PAHs and the duration of UV-lighting.

Keywords: UV, PAH, toxicity, phototoxicity, microalga, bioassay.

Introduction

Aquatic ecosystems are affected by gradual changes in environmental factors. Ultraviolet-B radiation (UV-B, 280–315 nm) reaching the earth's surface has increased due to stratospheric ozone depletion as a result of anthropogenic activities.[1–3] Enhanced UV-B radiation may be a threat to many organisms, including those in aquatic ecosystems.[4–6] The toxicity of some chemicals including PAHs may increase in the presence of UV-light, probably due to the production of highly destructive singlet oxygen, peroxides, and hydroxyl radicals, in the membranes of the organism following uptake into the tissue which may damage the cell constituents.[7–10]

Polycyclic aromatic hydrocarbons (PAHs) are relatively wide-spread contaminants in the marine environment and 16 of those were screened as priority pollutants.[11] The sources of the PAHs in the aquatic environments may be natural and/or anthropogenic. Elevated PAHs in aquatic systems are typically found close to the urban areas as the result of surface run-off and atmospheric deposition. Due to photomodification, PAHs are structurally changed into many different compounds that shows, in most cases, more toxic effects on the organisms compared to the parent compounds.[4,10,12,13]

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Phytoplankton, the microscopic plants in the water column, are the first link of the food chain. Because of their critical position in aquatic ecosystems, any effect on phytoplankton may result in many ramifications in the food web. Organic pollutants such as PCBs and PAHs are hydrophobic, they are generally associated with suspended particles,[14] and may concentrate in the membranes of phytoplankton and transported to the different sites of the aquatic ecosystems. Also, the evidence that PAHs are absorbed to a large degree by living phytoplankton cells probably due to the high surface area of phytoplankton was correlated with chlorophyll a measurements performed by Kowalewska.[15] Especially during blooms, phytoplankton appears to clean the water and transport PAHs to the sediment.[15]

Photomodifed PAHs (generally oxyPAHs) are also often more reactive and acutely toxic than parent PAHs,[10,16] and are toxic to plants,[17] bacteria,[10] and invertebrates.[18] Phytoplankton as a living organism in the photic zone of the aquatic environments may be exposed to the elevated levels of oxyPAHs compared to the organisms living in the deeper zones. A mode of action of these compounds is inhibition of photosynthesis.[17] A large number of recent studies indicate a considerable sensitivity to solar ambient UV of phytoplankton communities distributed from polar to tropical habitats.[19,20] Satellite studies over the last decade indicate a significant global decrease in phytoplankton.[21] Solar UV inhibits photosynthesis, bleaches photosynthetic pigments, affects nitrogen metabolism and induce DNA damage.[22,23]

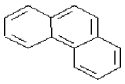
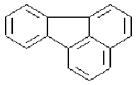
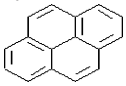
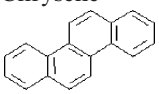
In recent years, the photo-induced toxicity of chemicals have been investigated intensively.[24–26] Direct toxic

effects of PAHs on several aquatic species have been demonstrated,[27–30] but there are few studies on the changes in toxic effects on phytoplankton in the presence of UV light.[20,22,31] Previous studies of UV-induced PAH toxicity have focused mainly on zooplankton and higher organisms,[29,32] while studies on microalgae are predominantly based on freshwater species.[33–36]

During the screening procedure of the individual PAHs used for this study, two criteria were considered i.e. the priority pollutants list of EPA[11] and the previous analytical studies on individual PAHs carried out in the Turkish coastal sites.[37,38] Studies of the coastal zone of northwestern Turkey have shown that total PAH concentrations are significantly higher in the sediments, mussels and water column in industrial and populated locations than in undeveloped areas and those selected PAHs consistently comprises the highest or second highest fraction of total PAHs in these locations.[37,39] The selected PAHs was also found highly widespread in the study area. Additionally, in some studies, concentrations of 3- and 4-ring PAHs were found highest in plankton, although the process of sorption of PAHs is complicated and is dependent on many parameters.[15]

Thus, in this study, the effects of individual PAHs of pyrene, phenanthrene, fluoranthene, and chrysene screened as priority pollutants[11] were investigated by application of microalgal (*Phaeodactylum tricornutum*) toxicity testing in the absence and presence of UV radiation at several exposure durations. Some properties of model PAHs used in toxicity testing studies were shown in Table 1.

Table 1. Selected model PAHs used in toxicity test systems

Model PAH	*Solubility (25°C) μg L^{-1}*	*Log Kow**
Phenanthrene 	435	4.46
Fluoranthene 	260	4.90
Pyrene 	133	4.88
Chrysene 	1.9	5.63

*Octanol-water partition coofficient

Materials and methods

Chemicals

PAHs were HPLC grade and purchased from Fluka-Riedel-de Haen Company. All other chemicals were AnalaR grade from Merck.

Laboratory conditions

Toxicity tests were carried out at a constant temperature (22 ± 2°C) either under fluorescent bulbs (3500–4000 lux) or under the lighting system consisting of UV-B, UV-A and standard fluorescent bulbs (Philipps). UV-A and UV-B intensity were measured using a Macam Model 103 radiometer (Livingstone, Scotland, UK). UV-A intensity ranged from 172–180 μW cm^{-2} and UV-B intensity ranged from 4.2–6.3 μW cm^{-2}. No-UV treatments were conducted under fluorescent lighting only. Levels of UV in the fluorescent (No-UV) treatments were negligible.

Microalgae Bioassays

Stock and working solutions were prepared by dissolving individual PAHs in HPLC grade acetone and by pipetting necessary amounts of stock solutions into 250 mL of culture media to achieve the desired concentrations respectively. During the preparation of working solutions, the solubility limits of the selected PAHs were considered for the fact that soluble compounds in water are more bioavailable to aquatic organisms.

The cultures were exposed to several concentrations of PAHs under fluorescent bulbs while, only three different concentrations of PAHs were chosen during the exposure under UV system (Table 2). Culture media was made of filtered clean seawater (045 μm-membrane Millipore) collected from the surface waters (22 ppt) of Marmara Sea (Istanbul Strait, known as relatively cleaner site) and modified f/2 nutrient medium.[40] Carrier controls were prepared by adding the same amount of acetone (0.1%) as in the PAHs' culture flasks. Stock cultures of *Phaeodactylum* cells were added to the flasks with a starting concentrations of 10000 cells mL^{-1}. Batch tests were performed as previosly described by Okay et al.[41]

Table 2. The working concentrations of PAHs

Model PAH	*Working concentrations under fluorescent bulbs (μg L^{-1})*	*Working concentrations under UV system (μg L^{-1})*
Pyrene	20; 40; 80; 100;120	40; 80;120
Phenanthrene	40; 100; 200; 300; 400	100; 200; 400
Fluoranthene	25; 50; 100; 125; 250	50; 100; 250
Chrysene	0.2; 0.4; 1.0; 1.2; 1.4; 1.8	0.2; 1.0; 1.8

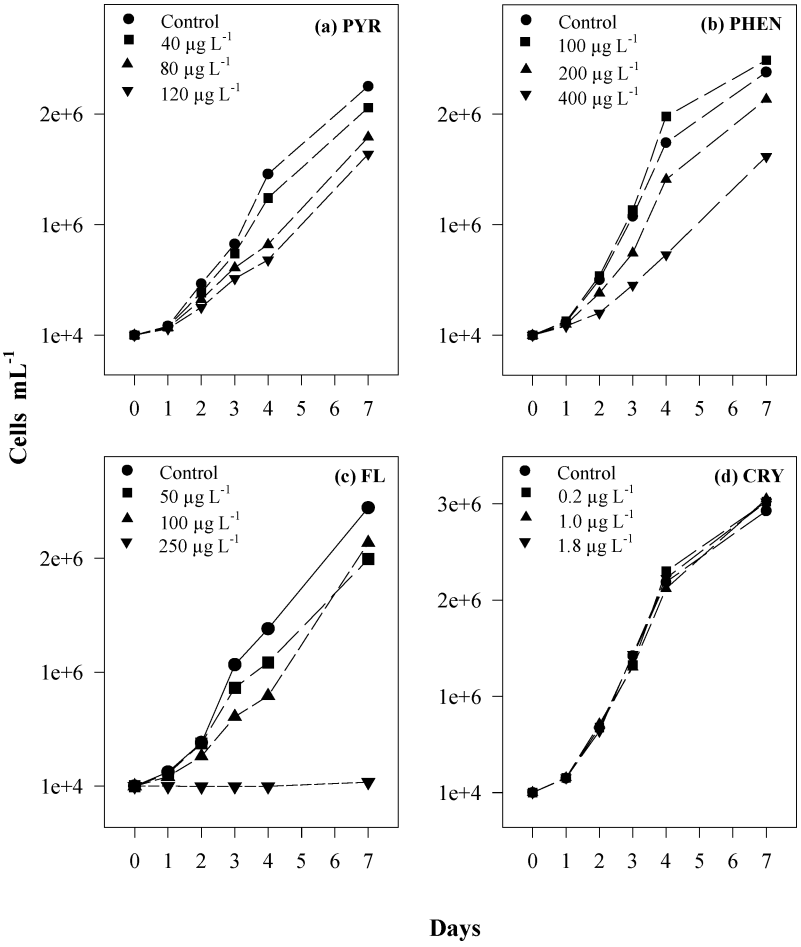


Fig. 1. Effect of individual PAHs on the growth of *Phaeodactylum tricornutum* under fluorescent lamps. (a) PYR: Pyrene; (b) PHEN: Phenanthrene; (c) FL: Fluoranthene; (d) CRY: Chrysene.

The test flasks were incubated for 4 days under either fluorescent or UV system and then the cultures incubated under UV radiation were transferred to the fluorescent lightining system, and the bioassay continued for 3 more days to observe whether the cells would recover in the absence of UV radiation. The changes in algal concentration were determined by direct cell count using a Coulter Counter (BECKMAN Z2). Since PAH toxicity is known to be dependent on the intensity and duration of UV exposure, UV radiation was applied 1, 2, 3 and 4 hours per day. The flasks were repositioned within the experimental space to minimize possible spatial differences in illumination and temperature on growth.

Results and discussion

Acetone used throughout the toxicity tests as carrier solvent (0.1%) did not show any negative effect on the growth of *Phaeodactylum* cells compared to the results obtained from the control cultures containing no acetone. The first series of experiments were accomplished under fluorescent light in the absence of UV lighting. Figure 1 shows the results of toxicity tests carried out under fluorescent bulbs (no-UV). Although the tests were performed in a wider range consisting of five or six (for Chrysene) concentrations, only the results of three concentrations were included in the graphs for a clear representation. The toxic effect of pyrene on *Phaeodactylum tricornutum* is presented in Figure 1a. The toxic effect estimated from the cell counts started from the second day and at fourth day of the test, 80 μg L^{-1} and 120 μg L^{-1} of pyrene inhibited the algal growth by 43% and 53% respectively.

The growth curves of algal cells incubated in 100 μg L^{-1}, 200 μg L^{-1} and 400 μg L^{-1} phenanthrene solutions are presented in Figure 1b. Similar to the results obtained for pyrene, a clear dose-response relationship can be seen from the figure after the application of 200 μg L^{-1} phenanthrene. The concentration of 100 μg/L phenanthrene showed a stimulative effect on the growth of algae. The growth of algae was inhibited by 19% and 58% in the 200 μg L^{-1} and 400 μg L^{-1} phenanthrene solutions respectively.

Table 3. EC_{50} (96 hours) values of PAHs under fluorescent and UV lamps for *Phaeodactylum tricornutum*

Type of lighting	*PYR** ($\mu g\ L^{-1}$)	*PHEN** ($\mu g\ L^{-1}$)	*FL** ($\mu g\ L^{-1}$)	*CRY** ($\mu g\ L^{-1}$)
Fluorescent	106.26	346.98	106.86	—
1 h UV day^{-1}	40.00	292.49	106.86	—
2 h UV day^{-1}	52.78	297.20	81.68	—
3 h UV day^{-1}	44.90	121.23	75.85	1.00
4 h UV day^{-1}	49.56	124.96	64.43	0.63

*PYR: Pyrene; PHEN: Phenanthrene; FL: Fluoranthene; CRY: Chrysene.

In Figure 1c representing the counts of algal cells in 50 $\mu g\ L^{-1}$, 100 $\mu g\ L^{-1}$ and 250 $\mu g\ L^{-1}$ fluoranthene shows that the dose-response relationship discontinued after 4th days of exposure. The difference between 50 $\mu g\ L^{-1}$ and 100 $\mu g\ L^{-1}$ was dissapeared on day 7, although complete inhibition of algal growth was observed in 250 $\mu g\ L^{-1}$ fluoranthene solution throughout the test period. The growth of *Phaeodactylum* up to 1.8 $\mu g\ L^{-1}$ chrysene, which is the highest concentration applied under the solubility limit of chrysene did not show any toxic effect compared to the control during the incubation of cells under fluorescent bulbs (Fig. 1d).

The results of the second series of experiments performed under UV lighting were represented in Figures 2, 3, 4 and 5 for pyrene, phenanthrene, fluoranthene and chrysene, respectively. The figures (a and b within the series) generated for only two concentrations of each PAH show the changes in cell concentrations relative to controls incubated under UV with several time courses. It should be noted that the incubation under UV lighting system was stopped after Day 4, so that the cell counts obtained on Day 7 illustrate the possible recovery of *Phaeodactylum* cells after 3 days incubation under fluorescent lights following UV application.

Figure 2 created for pyrene shows that cultures incubated in 40 $\mu g\ L^{-1}$ (Fig. 2a) did not show a significant difference compared to the cultures incubated under fluorecent lighting. In the case of 120 $\mu g\ L^{-1}$, inhibition was more pronounced and began from the second day. Four hours per day UV application to the cultures in 120 $\mu g\ L^{-1}$ pyrene solution almost stopped the growth completely. EC_{50} values were calculated with the trimmed Spearman–Karber method using US EPA statistical computer program (Version 1.5) are demonstrated in Table 3. Without UV radiation, *Phaeodactylum* cells' EC_{50} for pyrene was 106.26 $\mu g\ L^{-1}$ According to the data, after application of UV radiation, the EC_{50} values dropped to levels between 40.00 and 52.78 $\mu g\ L^{-1}$ (for 2 and 3 hours application respectively) which indicates a 2-3 fold change in sensitivity compared to the cultures incubated in the absence of UV radiation. A linear relationship was not found between the inhibition of algal growth and duration of UV exposure.

The responses of *Phaeodactylum* cultures incubated in 100 $\mu g\ L^{-1}$ and 400 $\mu g\ L^{-1}$ phenanthrene concentrations were shown in Figure 3. UV applications of 1 and 2 hours showed no effect on the growth according to the cell numbers determined on the first day for both concentrations compared to the responses of cultures incubated under fluorescent lights. In both concentrations, 3 and 4 hours/day applications of UV radiation had a pronounced effect. Since 400 $\mu g\ L^{-1}$ phenanthrene results in a clear inhibition (Fig. 1b) on the cell growth compared to the cultures in 100 $\mu g\ L^{-1}$ phenanthrene, the effect under UV lighting system was more significant and after 3 days, recovery of cells under fluorescent light following 4 hours/day of UV application was not possible.

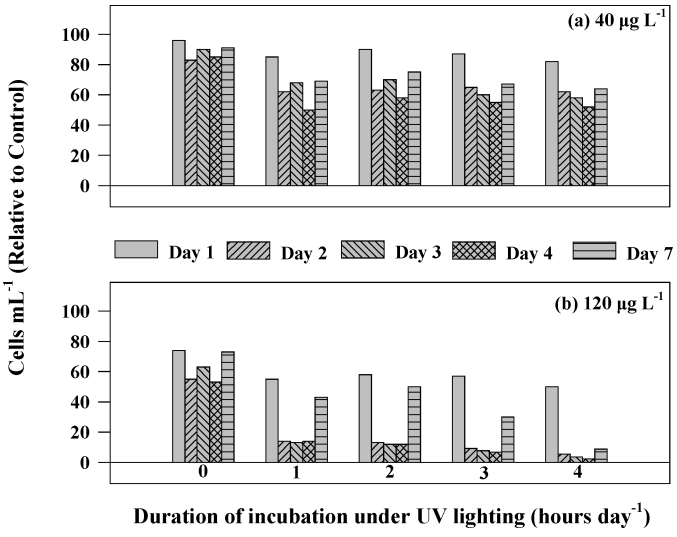


Fig. 2. Effect of pyrene on *Phaeodactylum tricornutum* under UV radiation at several incubation times.

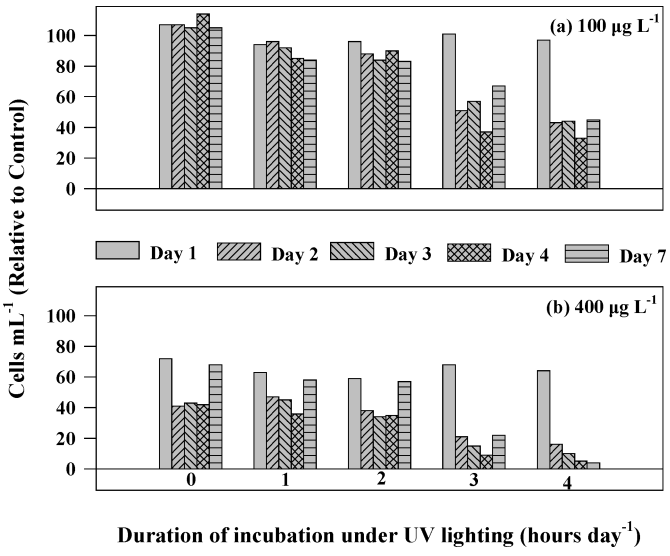


Fig. 3. Effect of phenanthrene on *Phaeodactylum tricornutum* under UV radiation at several incubation times.

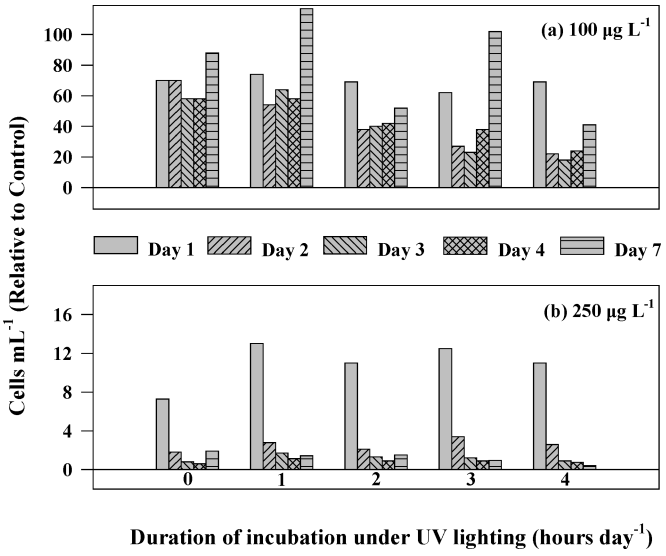


Fig. 4. Effect of fluoranthene on *Phaeodactylum tricornutum* under UV radiation at several incubation times.

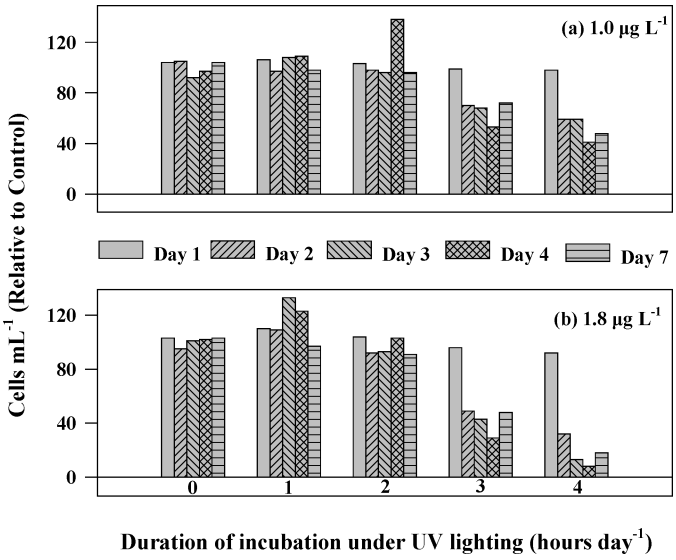


Fig. 5. Effect of chrysene on *Phaeodactylum tricornutum* under UV radiation at several incubation times.

From the EC_{50} values (Table 3), the effect of UV lighting duration can be observed clearly. EC_{50}values for fluorescent and 1–2 hours/day UV applications were found 346.98 μg L^{-1}, 293 μg L^{-1} and 297μg L^{-1} respectively, while the values dropped to 121.23 μg L^{-1} for 3 hours, and 124.96 μg L^{-1} for 4 hours UV applications.

Figure 4 summarizes the results of fluoranthene exposures under fluorescent and UV lighting. From the growth curves (Fig. 1c), it is clear that 250 μg/L fluoranthene almost stops the growth ; that behavior can also be seen from Figure 4a and Figure 4b. An additional effect of UV application up to 4 hours day^{-1} to the cultures incubated in 250 μg L^{-1} fluoranthene seems negligible. Although there was no difference between the EC_{50}values incubated under fluorescent and under UV application of 1 hour, the EC_{50}values calculated for the cultures of 2,3 and 4 hours UV application (Table 3) indicate that duration of UV radiation was linearly related to inhibition of cell growth. EC_{50} values was dropped to 64.43 μg L^{-1} after 4 hours of UV exposure which is nearly the half of the value found for the EC_{50} values incubated under fluorescent and after 1 hour per day UV application.

Chrysene concentrations of 1.0 and 1.8 applied in this study showed no pronounced effect on *Phaeodactylum* for 1–2 hours/day of UV exposure, although 3 and 4 hours day^{-1} applications, particularly for the higher concentration caused a clear inhibition on the growth (Fig. 5). EC_{50} values could not be calculated for the cultures incubated under fluorescent and under 1 and 2 hours of UV application since the growth of the cells was not inhibited. The application of 3 and 4 hours of UV affected the growth significantly i.e., the EC_{50} values for those exposures were found 1.00 μg L^{-1} and 0.63 μg L^{-1} respectively (Table 3).

When the previous studies on the phototoxic effects of individual PAHs were examined, a very complicated situation was met and this causes a great adversity to compare the results because of the variations in the parameters such as organisms, selected end points, UV light intensities and/or radiation type (UVA or UVB), duration of exposure, concentrations of PAHs (especially the differences between the concentrations above and below the solubility limits) etc. Even with the same organisms, very different results, were obtained.

In this study, the toxic effect of pyrene on *Phaeodactylum tricornutum* presented in Figure 1a was significant. In a previous study with the same algae and pyrene tested between 20 μg L^{-1}–100 μg L^{-1} in a similar test design, a clear dose-response relationship was not found throughout the test duration.[41] One of the reason for pyrene to be found toxic in 96 hours'test in this study may be the difference in the temperature which was higher (2°C) in this study compared to the previous one. As is well known the temperature affects both the metabolism of the organisms and the fate of the chemicals.

In the same study, the acute-short term toxicity test (6 hours) results depending on the ability of ^{14}C uptake with *Phaeodactylum* showed that pyrene is acutely toxic to the algae (EC_{50} = 68–70 μg L^{-1}).[41] From Table 3, without UV radiation, *Phaeodactylum* cells' $EC_{50}$for pyrene was found as 106.26 μg L^{-1} which was higher (less toxic) compared to the toxicity tests performed by measuring the ^{14}C uptake rate.[41] That shows that the test design and endpoint selection for toxicity tests are very important and the results may differ significantly.

In a fluoranthene exposure study, Southerland and Lewitus[31] tested the physiological response of *Ankistrodesmus* (an estuarine benthic green microalga) to fluoranthene addition at 19 μg/L, which was a very low concentration compared to the concentrations applied in this study (50 μg L^{-1}–250 μg L^{-1}), no significant

effect on growth rate was observed. On the other hand, in another fluoranthene +UV exposure study carried out with *Daphnia magna*, fluoranthene toxicity was higher after a 2 hour exposure to solar-simulating UV light, if organisms were allowed to accumulate the substance for 24 hours prior to irradiation.[42] No enhanced toxicity was observed if the solutions were irradiated before the daphnids were added, thus it was concluded that the acute phototoxicity of fluoranthene was predominantly due to photoactivation of accumulated or adsorbed molecules through the production of either singlet oxygen or free radicals by UV light. In this study although the phytoplankton cultures had enough time (4 days) to bioaccumulate the compound, no additional effect of UV lighting could be observed until the exposure was extended to 4 hours day^{-1}. It should also be remarked that no recovery of the cultures exposed to 4 hours day^{-1} was observed. In fact, in most cases the inhibition on day 7 was lower compared to the results obtained on day 4. There may be several reasons for that mainly the batchwise design of the algal toxicity testing systems, which has several disadvantages. One of the disadvantage of those systems is imposibility of reaching a stable steady state in the test chamber i.e. the parameters in the vessels such as the concentration/the structure of the original chemical, the number of cells and the amount of nutrients change permanently. As a result of that, for example, the amount of chemical per cell has been decreasing during the exposure period and inhibition may decrease towards the end of the test.

The oligochaete *Lumbriculus variegatus* was exposed to multiple concentrations of pyrene and fluoranthene for 96 hours in the absence and in the presence of UV radiation and pyrene was 4-fold more potent than fluoranthene in a UV exposure study.[43] When comparing the EC_{50} values found for pyrene and fluoranthene in this study, pyrene was 1.30–2.65 fold more toxic than fluoranthene for all UV exposures.

Kowalewska[15] reported that individual PAHs interact differently with phytoplankton cells depending on their physicochemical properties and molecular structure. PAHs of higher symmetry, such as anthracene, chrysene, and dibenzoanthracene absorbed more easily in cells and form more stable complexes than compounds of lower symmetry, which may be adsorbed only on the outer surface or on detritus particles. This may be a reason for chrysene to show a higher toxic effect (lower EC_{50}-Table 3) compared to the phenanthrene and fluoranthene after 3–4 hours/day UV exposure.

In a study with *Daphnia magna* as the test species, EC_{50} values for pyrene, phenantrene, fluoranthene and chrysene were all estimated to be greater than 1024 $\mu g\ L^{-1}$ before UV exposure.[44] Those values are very much higher than those found in this study. EC_{50} values after 24 hours UV exposure in the same study differed significantly and was 1.38, 378, 5.01 and >1024 for pyrene, phenanthrene, fluoranthene and chrysene respectively, indicating that the phototoxicity ranking of those chemicals is as PYR > FL > PHEN > CRY, which was the same in this study when comparing the values obtained after 24 and 48 hours. The same ranking was also reported by Schirmer et al.[45] who carried out a similar study with fish cells. Although the EC_{50} values differ greatly for some PAHs, depending on many factors especially on the sensitivity of organisms at different trophic levels, the phototoxicity ranking of parent PAHs did not changed in these studies despite the fact that the methodology and test organisms are different. In a study carried out with the same organism (*Daphnia magna*), EC_{50} values for pyrene were between 29.2 $\mu g\ L^{-1}$ and 54.8 $\mu g\ L^{-1}$[46] which is very low compared to the result (>1024 $\mu g\ L^{-1}$)obtained by Wernersson,[44] before UV exposure. In the same studies after UV exposure, EC_{50} values were given ranging from 3.0 $\mu g\ L^{-1}$ to 30.0 $\mu g\ L^{-1}$ and 1.38 $\mu g\ L^{-1}$ pyrene by Nikkila et al.[46] and by Wernersson[44] respectively.

EC_{50} values were not detected before and after the applications of SSR in the presence of pyrene for both short-term and long-term applications with using different endpoints (inhibition of luminescence and inhibition of growth) in a marine bacterial bioassay system utilizing *Vibrio fischeri* and various PAHs at concentrations above the solubility limits under Simulated Solar Radiation (SSR; UVA+UVB+Fluorescent bulbs).[47] Phenanthrene exposures of *Vibrio fisheri* in the same test system resulted in EC_{50} values between 6.72 ± 0.35 mg L^{-1} to 8.09 ± 0.33 mg L^{-1} . Additional to that in the same study, EC_{50} values for both PAHs were very high compared to the exposure studies in the literature remarking the differences in the sensitivities of the organisms used in toxicity test systems.[47]

Conclusions

As with other environmental stress factors, UV-B causes species-specific responses with a high degree of intraspecies variation. In this study, *Phaeodactylum tricornutum* showed enhanced sensitivity to the selected model PAHs in the presence of UV irradiation in accordance with the responses of most aquatic species in the previous studies. The degree of toxicity was dependent on the concentration of the PAHs and duration of UV radiation. In most cases, the sensitivity of *Phaeodactylum* to the PAHs was 2–3 times greater under UV exposure than when exposed to PAHs alone. These results confirm that PAHs used in this study possess a phototoxic mode of action. In the field, the consequences of UV exposure may be different. For example, UV doses may be higher than those simulated in the laboratory, or individual PAH phototoxicity may be compounded by the co-occurrence in tissues of other phototoxic PAHs.

Although there is significant evidence that increased UV-B exposure is harmful to all aquatic organisms—as was indicated in several studies—including phytoplankton by inhibiting growth and photosynthesis, damage at the whole ecosystem level is still uncertain and responses may not be

limited to simple decreases in primary production that may cause detectable differences in ecosystem biomass in time. Thus, further studies should aim to determine the effect of individual and combined effects of PAHs + UV exposures at typical intensities and durations in the field on common organisms at several trophic levels by considering the site-specific ranges of PAH concentrations in different matrices of aquatic environments.

Acknowledgments

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References

[1] Stolarski, R.; Bojkov, R.; Bishop, L.; Zerefos, C.; Staehelin, J.; Zawodny, J. Measured trends in stratospheric ozone. Science. **1992**, *256*, 342–345.

[2] Kerr, J.B.; McElroy, C.T. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science. **1993**, *262*, 1032–1034.

[3] Taalas, P.; Kaurola, J.; Kylling, A.; Shindell, D.; Sausen, R.; Dameris, M.; Grewe, V.; Herman, J.; Damski, J.; Steil B. The impact of greenhouse gases and halogenated species on future solar UV radiation doses. Geophys. Res. Lett. **2000**, *27*, 1127–1130.

[4] Mallakin, A.; McConkey, B.J.; Miao, G.; McKibben, B.; Snieckus, V.; Dixon, G.D.; Greenberg, B.M. Impacts of structural photomodification on the toxicity of environmental contaminants: anthracene photooxidation products. Ecotox. Environ. Safe. **1999**, *43*, 204–212.

[5] Tuveson, R.W.; Wang, G.R.; Wang, T.P.; Kagan, J. Light-dependent cytotoxic reactions of anthracene. Photochem. Photobiol. **1990**, *52*, 993–1002.

[6] Holm-Hansen, O.; Lubin, D.; Helbling W. Ultraviolet radiation and its effects on organisms in aquatic environments. In: Environmental UV Photobiology, Young R.A.; Moan J.; Björn L.O.; Nultsch W., eds. Plenium Press, New York/London, 1993.

[7] Kagan, J.; Wang, T.P.; Benight AS. The phototoxicity of nitro polycyclic aromatic hydrocarbons of environmental importance. Chemosphere. **1990**, *20*, 453–466.

[8] Foote, C.S. Definition of type I and type II photosensitized oxidation: guest editorial. Photochem. Photobiol. **1991**, *54*, 659.

[9] McCloskey, J.T.; Oris, J.T. Effect of anthracene and solar ultraviolet radiation exposure on gill ATPase and selected hematologic measurements in the bluegill sunfish (*Lepomis macrochirus*). Aquat. Toxicol. **1993**, *24*, 207–218.

[10] McConkey, B.J.; Duxbury, C.L.; Dixon, D.G.; Greenberg, B.M. Toxicity of a PAH photooxidation product to the bacteria *Photobacterium phosphoreum* and the duckweed *Lemna gibba*: effects of phenanthrene and its primary photoproduct, phenanthrenequinone. Environ. Toxicol. Chem. **1997**, *16*, 892–899.

[11] Sun, F.; Littlejohn, D.; Gibson, M.D. Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection. Anal. Chim.Acta. **1998**, *364*, 1–11.

[12] Huang, X.,D.; Dixon, D.G.; Greenberg, B.M. Increased polycyclic aromatic hydrocarbon toxicity following their photomodification in natural sunlight: impacts on the duckweed *Lemna gibba* L. G-3. Ecotox. Environ. Safe. **1995**, *32*, 194-200.

[13] Miller, K.P.; Ramos, K.S. Impact of cellular metabolism on the biological effects of benzo[*a*]pyrene and related hydrocarbons. Drug. Metab. Rev. **2001**, *33*, 1–35.

[14] Baker, J.E.; Eisenreich, S.J. PCBs and PAHs as tracers of particulate dynamics in large Lakes. J. Great Lakes Res. **1989**, *15*, 84–103.

[15] Kowalewska, G. Phytoplankton—the main factor responsible for transport of polynuclear aromatic hydrocarbons from water to sediments in the Southern Baltic ecosystem. ICES J. Mar.Sci. **1999**, *56*, 219–222.

[16] Nikolaou, K.; Masclet, P.; Mouvier, G.; Sources and chemical reactivity of polynuclear aromatic hydrocarbons in the atmosphere—A Critical-Review. Sci.Total Environ. **1984**, *32*, 103–132.

[17] Huang, X.D.; McConkey, B.J.; Babu, T.S.; Greenberg, B.M. Mechanisms of photoinduced toxicity of photomodified anthracene to plants: Inhibition of photosynthesis in the aquatic higher plant *Lemna gibba* (duckweed). Environ.Toxicol. Chem. **1997**, *16*, 1707–1715.

[18] Steevens, J.A.; Slattery, M.; Schlenk, D.; Aryl, A.; Benson, W.H. Effects of ultraviolet-B light and polycyclic hydrocarbon exposure on sea urchin development and bacterial bioluminescence. Mar. Environ. Res. **1999**, *48*, 439–457.

[19] Wangberg, S.A.; Selmer, J.S.; Gustavson, K. Effects of UV-B radiation on carbon and nutrient dynamics in marine plankton communities. J. Photochem. Photobiol. B. **1998**, *45*, 19–24.

[20] Estevez, M.S.; Malanga, G.; Puntarulo, S. UV-B effects on Antarctic *Chlorella* sp. cells. J. Photochem. Photobiol. B. **2001**, *62*, 19–25.

[21] Gregg, W.W.; Conkwright, M.E. Decadal changes in global ocean chlorophyll. Geophys. Res. Lett. **2002**, *29*.

[22] Ghetti, F.; Hermann, H.; Häder, D.P.; Seidlitz, H. K. Spectral dependence of the inhibition of photosynthesis under simulated global radiation in the unicellular green alga *Dunaliella salina*. J. Photochem. Photobiol.B. **1999**, *48*, 166–173.

[23] Klisch, M..; Häder, D.P. Effects of solar radiation on phytoplankton. Recent Res. Devel. Photochem. Photobiol. **1999**, *3*, 113–121.

[24] Larson, R.A.; Berenbaum, M.R. Environmental phototoxicity. Environ. Sci. Technol. **1988**, *22*, 354–360.

[25] Arfsten, D.P.; Schaeffer, D.J.; Mulveny, D.C. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: a review. Ecotoxicol. Environ. Safe. **1996**, *33*, 1–24.

[26] Huovinen, P.S.; Soimasuo, M.R.; Oikari, A.O.J. Photoinduced toxicity of retene to *Daphnia magna* under enhanced UV-B radiation. Chemosphere. 2001, *45*, 683–691.

[27] Ren, L.; Zeiler, L.F.; Dixon, G.D.; Greenberg, B.M. Photoinduced effects of polyaromatic hydrocarbons on *Brassica napus* (Canola) during germination and early seed development. Ecotoxicol. Environ. Safe. **1996**, *33*, 73–78.

[28] Chandler, G.T.; Shipp, M.R.; Donelan, T.L. Bioaccumulation, growth and larval settlement effects of sediment associated polynuclear aromatic hydrocarbons on the estuarine polycheate, *Streblospio benedicti*. J. Exp. Mar. Biol. Ecol. **1997**, *213*, 95–110.

[29] Boese, B.L.; Lamberson, J.O.; Swartz, R.C.; Ozretich, R.;Cole, F. Photoinduced toxicity of PAHs and alkylated PAHs to a marine infaunal amphipod (*Rhepoxynius abronius*). Arch. Environ. Contam. Toxicol. **1998**, *34*, 235–240.

[30] Lotufo, G.R. Lethal and sublethal toxicity of sediment associated fluoranthene to benthic copepods: application of the critical body residue. Aquat. Toxicol. **1998**, *44*, 17–30.

[31] Southerland, H.A.; Lewitus, A.J. Physiological responses of estuarine phytoplankton to ultraviolet light-induced fluoranthene toxicity. J. Exp.Mar. Biol. Ecol. **2004**, *298*, 303–322.

[32] Weinstein, J.E.; Oris, J.T.; Taylor, D.H. An ultrastructural examination of the mode of UV-induced toxic action of fluoranthene in the fathead minnow, *Pimphales promelas.* Aquat. Toxicol. **1997**, *39*, 1–22.

[33] Bastian, M.V.; Toetz, D.W. Effect of eight polynuclear aromatic hydrocarbons on growth of *Anaebena flos-aquae*. Bull. Environ. Contam. Toxicol. **1982**, *29*, 531–538.
[34] Gala, W.R.; Giesy, J.P. Photoinduced toxicity of anthracene to the green algae *Selenastrum capricornutum*. Arch. Environ. Contam. Toxicol. **1992**, *23*, 316–323.
[35] Gala, W.R.; Giesy, J.P. Using the carotenoid biosynthesis inhibiting herbicide, Fluoridone, to investigate the ability of carotenoid pigments to protect algae from the photo induced toxicity of anthracene. Aquat.Toxicol. **1993**, *27*, 61–70.
[36] Gala, W.R.; Giesy, J.P. Flow cytometric determination of the photoinduced toxicity of anthracene to the green algae *Selenastrum capricornutum*. Environ. Toxicol. Chem. **1994**, *13*, 831–840.
[37] Telli-Karakoç, F.; Tolun, L.; Henkelmann, B.; Klimm, C.; Okay, O.S.; Schramm, K.W. Polycyclic aromatic hydrocrbons (PAHs) and polychlorinated biphenyls (PCBs) distributions in the Bay of Marmara Sea: Izmit Bay. Environ. Pollut. **2002**, *119*, 383–397.
[38] Tolun, L.T.; Martens, D.; Okay, O.S.; Schramm, K. W. Polycyclic Aromatic Hydrocarbon contamination in coastal sediments of the Izmit Bay (Marmara Sea): Case studies before and after the Izmit Earthquake. Environ. Int. **2006**, *32*, 758–765.
[39] Okay, O.S.; Tolun, L.; Telli- Karakoç, F.; Tüfekçi,V.; Tüfekçi, H.; Morkoç, E. Izmit Bay ecosystem after Marmara earthquake and subsequent fire: The long term data, Mar. Pollut. Bull. **2001**, *42*,361–369.
[40] Guillard, R.R.L.; Ryther, J.H. Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve). Grand Canad. J. Microbiol. **1962**, *8*, 229–239.
[41] Okay, O.S.; Tüfekçi, V.; Donkin, P. Acute and chronic toxicity of pyrene to the unicellular marine alga *Phaeodactylum tricornutum.* Bull. Environ. Contam.Toxicol. **2002**, *68*, 600–605.
[42] Wernersson, A.S.; Dave, G. Effects of different protective agents on the phototoxicity of fluoranthene to *Daphnia magna.* Comp. Biochem.Phys.C. **1998**, *120*, 373–381.
[43] Ankley, G.T.; Erickson, R.J.; Sheedy, B.R.; Kosian, P.A.; Mattson, V.R.; Cox, J.S. Evaluation of models for predicting the phototoxic potency of polycyclic aromatic hydrocarbons. Aquatic Toxicol. **1997**, *37*, 37–50.
[44] Wernersson, A.S. Predicting petroleum phototoxicity. Ecotoxicol. Environ. Safe. **2003**, *54*, 355–365.
[45] Schirmer, K.; Chan, A.G.; Greenberg, B.M.; Dixon, D.G.,; Bols, N.C. Ability of 16 priority PAHs to be phototoxic to a cell line from the rainbow trout gill. Toxicology. **1998**, *127*, 143–155.
[46] Nikkila, A.K.; Penttinen, S.; Kukkonen, J.V.K. UV-B-induced acute toxicity of pyrene to the waterflea *Daphnia magna* in natural freshwaters. Ecotox. Environ. Safe. **1999**, *44*, 271–279.
[47] El-Alawi, Y.S.; Dixon, D.G.; Greenberg, B.M. Effects of a preincubation period on the photoinduced toxicity of polycyclic aromatic hydrocarbons to the luminescent bacterium *Vibrio fisheri.* Environ.Toxicol. **2001**, *16*, 277–286.