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Phototransformation of 2,4-Dichloroaniline in a Surface Freshwater Environment: Effects on Microbial Assemblages

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Most organic pesticides, with the exception of very persistent compounds, will undergo transformations either chemically or biologically shortly after application. Most toxicity studies, however, do not take into account the complicated interactions between or among a pesticide compound and its transformation products, and the possible transformation of a pesticide compound into intermediate products of equal or greater toxicity than their parent compound. Photochemical and microbial degradation have been recognized as two important removal forces of many organic pesticides in natural surface waters (Hwang et al. 1986). However, microbial activity is also subjected to inhibitory effect due to the toxicity of pesticide pollutants. Any pesticide that inhibits natural microbial consortia will interfere with microbially-mediated biogeochemical cycling of essential elements and toxicants in natural ecosystems and lead to adverse delayed environmental health impacts. The photoinduced toxicity to aquatic organisms at low concentrations have been reported for some organic pesticide and PAHs compounds (Khan et al. 1973; Landrum et al. 1987; McConkey et al. 1997). This may occur via photosensitization reactions (e.g., production of singlet oxygen) (Ankley et al. 1994) and photooxidation of the compounds to more toxic products such as diols and quinones (David and Boule 1993). Because these chemical species are more water soluble than the parent compounds, aquatic organisms may be exposed to higher concentrations of photoproducts than the parent compounds. Therefore, this presents a greater toxic risk to aquatic organisms and ultimately humans, because of direct exposure and/or bioaccumulation through food chains. The extent of this problem suggests organic contaminants and their photoproducts may be degrading aquatic ecosystems and jeopardizing environmental health. Therefore, it is of dire importance to determine the effects of pesticide transformation products on microbial biota while conducting an ecotoxicology study.

Chloroanilines are formed from microbial transformation of various phenylcarbamate, phenylurea and acylanilide herbicides. Chloroanilines' toxic action is mainly due to the inhibition of acetylcholine esterase (Schmitz 1996). Degradation of chloroanilines was found to be considerably enhanced by UV irradiation (Hwang et al. 1987). The reaction intermediates formed are usually more stable, and sometimes more toxic (Halmann 1996). Biological assay procedures have been routinely used to monitor the environmental impact of many pesticides. Among them, microbial bioassays are also applied for ecotoxicity measurements, based on the assumption that microorganisms can act as surrogates for higher organisms in the ecosystem and microbial tests are relatively simple, rapid and inexpensive. In this study a simultaneous incubation system was used to assess the influence of photolysis on microbial toxicity of 2,4-dichloroaniline. The effect of 2,4dichloroaniline and its known photoproducts on bacterial assemblages was assessed with measurements of total bacterial numbers and

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bacterial heterotrophic activity of D-glucose mineralization. Transformation rate of 2,4-dichloroaniline was measured with high performance liquid chromatography.

MATERIALS AND METHODS

Surface freshwater samples were collected in the summer of 1996 from the Mississippi River in Vicksburg, Mississippi. At the time of sampling, temperature and pH were measured. The pH of the Mississippi river sample ranged from 7.0 to 7.2, and the temperature ranged from 25°C to 28°C. 2,4-Dichloroaniline (analytical grade) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Ethyl acetate (absolute), acetonitrile (HPLC grade), and water (HPLC grade) were obtained from Fisher Scientific Company, Atlanta, Georgia. ^{14}C-UL-D-glucose (S.A.: 246 mCi/mmol) was obtained from Sigma Chemical Company, St. Louis, Missouri.

2,4-Dichloroaniline was dissolved in acetonitrile and added to 50 mL of water sample into 150-mL quartz flasks (GM Associates Incorporation, Oakland, California) and incubated in triplicate. The final concentration was 10 mg/L. The flasks allowed > 85% transmission of light at wavelength of > 260 nm (Hwang and Maloney 1996). The flasks were suspended in an outdoor tub which contained continuous running water in order to maintain the water temperature at 28±l°C. The water level in the flask was about 3 cm below the surface of the cooling water. A research radiometer (model IL 1700, International Light Incorporation, Newburyport, Massachusetts) was placed beside the tub to measure UV irradiance. The dark control consisted of flasks being wrapped with aluminum foil. All bottles were capped with silicon stoppers. At the termination of incubation, dichloroaniline and the photoproducts were recovered from 10-mL subsamples by three successive extractions with 1 mL of ethyl acetate after the water pH had been adjusted to 10 with addition of 0.1N NaOH. Extraction recovery of the parent compound was greater than 95%. The extracts were then blown down to 1 mL under nitrogen gas. The extract was then analyzed for the parent compound using HPLC. Total bacterial numbers were determined with Acridine Orange Direct Counting (AODC) of epifluorescence microscopy technique (Hobbie et al. 1977; Hwang and Maloney 1996).

In a separate experiment, the effect of dichloroaniline and its photoproducts on bacterial assemblages was measured with heterotrophic mineralization of ^{14}C-D-glucose and total bacterial numbers. Dichloroaniline was added to 25 mL of autoclaved and filter-sterilized river water in quartz flasks to make the final concentration 20 mg/L. The water containing dichloroaniline was exposed to midday sunlight outdoors for up to 3 hr. Then equal amount of fresh unamended river water was added to the photo-exposed water and incubated in darkness for 1 day at 25°C in the laboratory. Treatments included: darkness control (i.e., no dichloroaniline was added), darkness (i.e., exposure to dichloroaniline in darkness) and exposure to sunlight for 1 hr, 2 hr, and 3 hr respectively. Radiolabeled glucose (final concentration 1 µg/L) dissolved in ethanol was added to the water samples after the preexposure and incubated at 25°C in darkness for 1 hr. At the termination of the incubation, 0.5 mL of 2 N H_2SO_4 was added to the sample, and the $^{14}CO_2$ produced was trapped by 2-phenylethylamine-soaked filter papers (Hwang and Manoley 1996). The radioactivity of the filter paper was measured with liquid scintillation spectrometry (Packard model TR 1700).

Samples for AODC measurement were preserved right after the incubation in darkness was completed. Dichloroaniline concentration was measured with high performance liquid chromatography (HPLC). The column used was the reverse phase Nova pack C-18 HPLC column (Waters Chromatography Division, Milford, Massachusetts). A

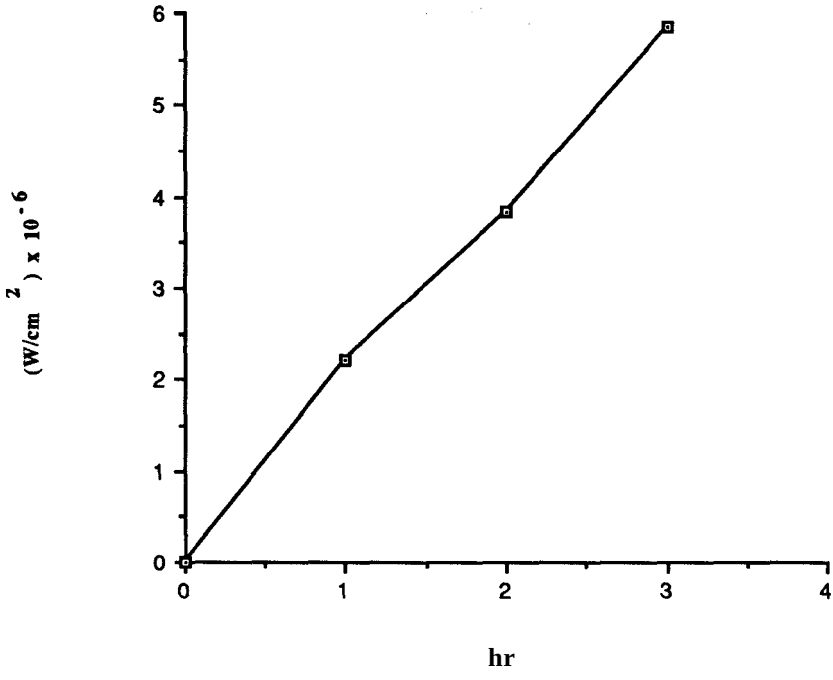

Figure 1. UV irradiation received during the experiment.

system consisting of Waters 510 HPLC Pump and a Waters Model U6K Universal Liquid Chromatography Injection fitted with a 2 mL injection loop was used. Detection was achieved at 254 nm using a Waters 996 photodiode array detector. Mobile phase consisted of 0.75 mL/min of HPLC grade acetonitrile and 0.50 mL/min of HPLC grade water. Difference in experimental data between different treatment groups was determined with student-t-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Photochemical degradation process of chloroanilines in aquatic environments was linear according to the first order rate decay (Hwang et al. 1987). In August, freshwater samples from the Mississippi River at Vicksburg, Mississippi, was incubated with 2,4-dichloroaniline (10 mg/L) at 28°C for 3 hr in midday sunlight. The UV irradiance integrated between 325 and 375 nm by the radiometer) increased linearly from 0 to 5.85 x 10^{-6} watts/cm^2 after 3 hr (Figure 1). After incubation, the effects of dichloroaniline and its photoproducts mixtures on bacterial assemblages were measured with glucose mineralization and AODC technique. Glucose mineralization reflects the bacterial heterotrophic activities. Acridine orange direct counting technique, AODC, is a method which measures the total bacterial numbers (including the dormant individuals) within a water sample.

Bacterial mineralization rate of D-glucose in darkness control group was 0.048±0.005 µg/L/hr (Table 1). The glucose mineralization rate of 1-hr, 2-hr and 3-hr exposure

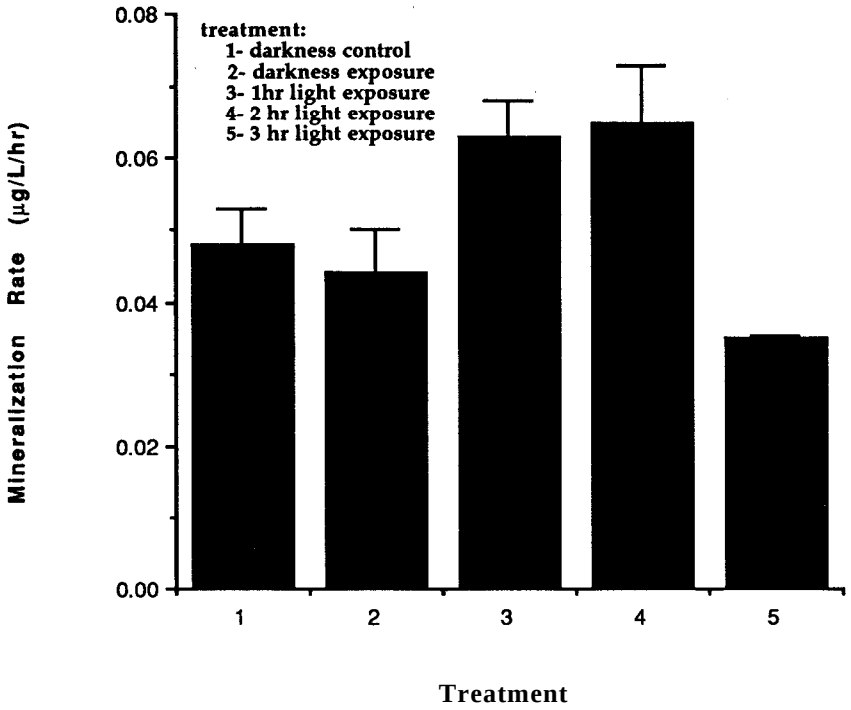

Figure 2. Effect of 2,4-dichloroaniline and/or its photoproducts on microbial mineralization of glucose. Numbers are expressed as mean ± 1 standard deviation (n=3). Darkness control refers to the group that was incubated in darkness without adding 2,4-dichloroaniline.

groups were 0.063±0.008 µg/L/hr, 0.065±0.008 µg/L/hr, 0.035±0.0002 µg/L/hr, respectively (Table 1). The mineralization rate of the 3-hr exposure group was significantly lower than the 1-hr and 2- hr exposure groups (t-test, $p \leq 0.05$; Table 1, Figure 2). The data of concurrent measurements of the total bacterial numbers are shown in Table 1 and Figure 3. The results indicated that there was no significant difference in the amount of bacteria present between the darkness control and the darkness group. However, total bacterial numbers increased by 79% over the darkness control in the 3-hr exposure group, while exposure groups 1 and 2 increased by 24% and 65% respectively. This result indicates that bacterial abundance increased and no toxicity occurred after exposure to the mixture of dichloroaniline and its photoproducts. The lower glucose mineralization activity of the 3-hr exposure group was puzzling. We hypothesized that these results came about due to competitive utilization/uptake among the substrates, assuming that a similar uptake system exists for all members of the microbial population to respond in the same way to the substrates (i.e. photoproducts and glucose) (Atlas and Bartha 1993). Total bacterial numbers in the photo-exposure groups increased dramatically, assumably as a result of bacterial utilization of the degradation products that were produced during photochemical and microbial degradation. Our experiment also indicated that there was no dichloroaniline degradation occurring in the darkness group. This finding is consistent

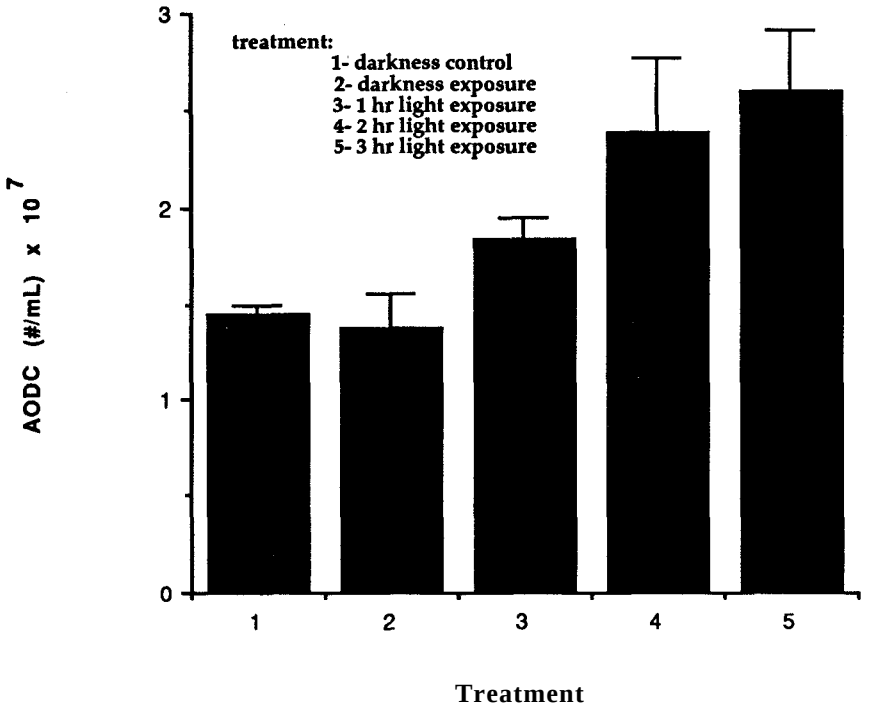

Figure 3. The effect of 2,4dichloroaniline and/or its photoproducts on total bacterial numbers. Numbers are expressed as mean ± 1 standard deviation (n=3).

Table 1. Effect of 2,4dichloroaniline and/or its photoproducts on microbial mineralization of glucose and total bacterial numbers.

darkness control	darkness	light (1 hr)	light (2 hr)	light (3 hr)
A. Glucose mineralization* (µg/L/hr)				
0.048±0.005	0.044±0.006	0.063±0.005	0.065±0.008	0.035±0.0002
B. AODC #: (10 /mL)				
1.45 ±0.05	1.4±0.2	1.8±0.1	2.4±0.4	2.6±0.3

*Glucose was added at a final concentration of 1 µg/L after the water samples had been exposed to the chemicals for 1 day. Glucose mineralization was measured after 1 hr incubation at 25°C. Numbers are expressed as mean ± 1 standard deviation (n=3). Darkness control: incubation in darkness without dichloroaniline; darkness: incubation in darkness with dichloroaniline; light-exposure groups: incubation in light with dichloroaniline for up to 3 hr. See Materials and Methods for details.

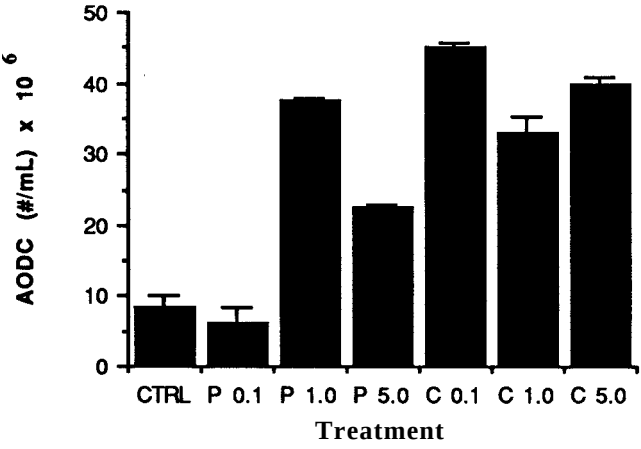

Figure 4. Effect of phenol and catechol on total bacterial numbers. CTRL: control; P (phenol) added at 0.1, 1.0, and 5.0 mg/L levels; C (catechol) added at 0.1,1.0, and 5.0 mg/L levels. Numbers are expressed as mean ± 1 standard deviation (n=3).

with our previous studies with estuarine system (Hwang et al. 1987).

After transformation of 2,4-dichloroaniline by photolysis, microbes were able to further mineralize the photoproducts. In our previous study with an estuarine system, microbial mineralization rate of trichloroaniline was proportional to the duration of photochemical exposure (Hwang et al. 1987). However, the effect of the photoproducts on microbial consortia was not conducted in that study. In this particular experiment photoproducts of 2,4 dichloroaniline were not identified. Nonetheless, chloroanilines have been shown to photodegrade via dechlorination and hydroxylation to phenols and catechols (Matsumura 1982; Miller et al. 1980). The effect of phenol and catechol on total bacterial numbers was also determined in this study and the results are indicated in Figure 4. The amount of phenol and catechol was added up to 5.0 mg/L ($\leq$ 50% of the concentration of 2,4-dichloroaniline added). Except for the treatment with 0.1 mg/L of phenol, exposures to phenol (at final concentrations of 1.0 and 5.0 mg/L, respectively) and catechol (at final concentrations of 0.1, 1.0, and 5.0 mg/L, respectively) significantly stimulated bacterial numbers up by 5 folds. Therefore, this finding indicates that indeed bacterial consortia could utilize stable photoproducts and increase their population sizes.

Photo-induced toxicity of dichloroaniline was not observed in this study. On the contrary, the photoproducts of 2,4-dichloroaniline was found to increase total bacterial number and bacterial heterotrophic activity in most of the light-exposure groups. Selected photoproducts could serve as growth substrates for bacterial consortia and affect biogeochemical fates of other dissolved organic substrates.

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