RELATIVE CONTRIBUTION OF PHOTOLYSIS AND MICROBIAL DEGRADATION TO CHLOROANILINES REMOVAL IN ESTUARINE WATER

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INTRODUCTION

Aromatic amides and carbamate derivatives include several important pesticides and plant growth regulators. Their toxicity is mainly due to the inhibition of acetylcholine esterase. Many of these compounds undergo slow hydrolysis reaction. However, their decomposition is significantly enhanced by ultraviolet (UV) irradiation (Halmann 1996). Chloroanilines are formed from many of these pesticides as the initial microbial transformation products and may be substantially more resistant to further degradation (Neilson 1994). Aniline and chlorinated anilines enter the estuarine environments by a variety of routes (Bartha 1971; Kaufmann and Blake 1973). They can also enter as waste effluents from dye manufacturing plants (Lyons et al. 1984). The fate of these xenobiotics is of interest because of their toxicity to aquatic organisms and their threat to human health (Messner et al. 1979).

Photolysis and microbial degradation both have been found to be important degradative processes affecting many organic contaminants in aquatic environments (Matsumura 1982; Pignatello et al. 1983). An important advantage of photolysis is that it may be performed at low or ambient temperatures and complete mineralization of the organic contaminants may be achieved. At pH 7, the half-lives of aniline, p-chloroaniline, p-nitroaniline, and 4-bromo- 3-chloroaniline by direct photolysis were 180, 4.6, 880, and 61 minutes, respectively (Halmann 1996). Photo- degradation occurs primarily in the presence of UV light, and biodegradation is primarily conducted by microorganisms (Matsumura 1982; Hwang et al. 1986a). Microbial degradation and photolysis rates in natural waters vary with the season. Direct photolysis rates of benzopyrene were lower in winter than in summer, while surface UV irradiation is reduced in the winter relative to summer (Zepp and Baughman 1977). Microbial degradation rates of hydrocarbons were lower in winter due to lower temperatures (Cooney et al. 1985). In aquatic environments, one degradative process may predominate over another, or microbial degradation and photolysis may be equally important. For example, in a freshwater lake the degradation of 2,4,5-trichloroaniline, which did not occur in the dark, was significantly higher in sunlit untreated lakewater than in sunlit poisoned lakewater (Hwang et al. 1986a). Similar trends were also reported for degradations of polychlorinated phenols in an estuarine environment (Hwang et al. 1986b).

p-Chloroaniline was photolyzed to produce photoproducts such as 4-chloronitrosobenzene and 4-chloronitrobenze (Miille and Crosby 1983). Photoproducts of aniline in natural water included azobenzenes (Zepp et al. 1981). The photoproducts are subject to further photolysis and biodegradation, via dechlorination and oxidative processes with ultimate mineralization to CO_2 (Matsumura 1982). The attenuation of UV irradiation by dissolved organic compounds and suspended particulates are expected to result in lower photolysis rates for chloroanilines in natural waters relative to distilled water. However, photosensitizers present in natural water can accelerate photolysis rates of certain compounds (Larson et al. 1989). The photodegradation of anilines was strongly enhanced in the presence of riboflavin at 5 μM, assumably due to the sensitized photolysis reaction (Halmann 1996). Thus for assessment of persistence of selected organic contaminants, it is essential that we understand the kinetics and mechanisms of photolysis and biodegradation of the contaminants in a given ecosystem. The objectives of this study are to review the previous data and determine the relative contribution of photolysis and microbial degradation to removal of chloroanilines, as the two processes significantly affect their fate in estuarine water.

MATERIALS AND METHODS

Sampling and Chemicals. Surface estuarine water (top 10 cm) samples were collected from Skidaway River, an estuarine river located near Savannah, Georgia. At the time of sampling, temperature, pH, and salinity were

measured. The biological, chemical, and physical properties of the water samples are listed in Table 1. Ring-U-^{14}C-2,4,5-trichloroaniline (14.8 mCi/mmol), aniline phosphate (17.6 mCi/mmol), p-chloroaniline (10.2 mCi/mmol), and 2,4-dichloroaniline (12.5 mCi/mmol) were obtained from Sigma Chemical Company. ^{14}C- sodium bicarbonate (50 mCi/mmol) and ^{14}C-U-labeled D-glucose (200 mCi/mmol) were obtained from DuPont NEN Research Products.

Degradation Measurements. Radiolabeled or unlabeled compounds were dissolved in acetone and added to 60 ml of water in 150-ml quartz flasks for study of photochemical and microbiological degradation. Quartz flasks (Quartz Scientific, Inc.) allowed more than 85% transmission of light of wavelength > 260 nm. Acetone was added at a concentration less than 1 x 10^{-6} M, and no photosensitization by acetone in distilled water of the test compounds were observed at this concentration. Approximately 0.1 μCi of the candidate radioactive compound was added to the flasks and incubated in triplicates. Final concentration of each compound in the flasks was 25 μg/L. Flasks were suspended in an outdoor tank through which estuarine water was continuously circulated and water level in the flask was about 3 cm below the surface. Flasks were covered with aluminum foil for dark experiments. Formaldehyde (final concentration 0.4%) was added to poison distilled water (buffered at the pH 7.7 ± 0.2 with 0.016M phosphate) and a group of the sunlit estuarine water sample as poisoned control. Ultraviolet absorption by formaldehyde solution at this concentration was found to be negligible. Parent compound disappearance (transformation) and $^{14}CO_2$ appearance (mineralization) were determined for the degradation study. The flasks were exposed to midday sunlight (10 a.m.-2 p.m.) for transformation study or incubating for up to 3 days for mineralization study. The $^{14}CO_2$ produced was collected and radioassayed as described in Hwang et al. (1989). To determine disappearance rates of the test chemicals, the samples containing parent compound were extracted three times with ethyl acetate after the pH was adjusted to 10 with 2N NaOH. Extracts were concentrated by evaporation under nitrogen. Analyses of the extracts were conducted by a HPLC Waters (UV-VIS photodiode array detector) system equipped with a 4.6 x 250 mm reverse-phase octyl column of 5 μm ultrasphere (mobile phase: 50:50 acetonitrile:water; flow rate, 1.0 ml/min; detector wavelength, 254 nm).

Transformation rate constants and mineralization rate constants were calculated assuming the reactions were first-order. K_p (first-order degradation rate constant) was expressed in units of h^{-1} or d^{-1} . The half-life was calculated by the equation: $t_{1/2} = 0.693/k_p$. The first-order rate constants were corrected for abiotic loss in darkness (< 5%) and light screened by dissolved organic matter. Experiments were conducted on sunny days and solar irradiation was integrated hourly with a radiometer (LI-COR Inc., Model LI-550B; active range, 400-700 nm). The light screening factor (S) was the ratio of the valerophenone (0.01 mM) photolysis rate constants in estuarine water to the rate constant in distilled water (Skurlatov et al. 1983). The valerophenone solution was exposed to midday sunlight for up to 1 h. Analyses for valerophenone were conducted with HPLC using a reverse-phase octyl column and mobile phase of methanol:water (70:30; flow rate 2.0 ml/min; wavelength 260 nm).

Microbial Biomass and Activity Measurements. Relative rates of bacterial heterotrophic activity were determined by measuring uptake of 3H-glucose (0.45 nM of D-[6-^{3}H.(N)]-glucose; 30 Ci/mmol). A correction factor of 0.5 for ^{3}H-D-glucose respiration to ^3H_2O was applied for final calculation of turnover rate (Hwang et al. 1986b). Similarly, the uptake of $^{14}CO_2$ by algae under sunlight was determined by adding 1 μCi of ^{14}C-sodium bicarbonate to the estuarine water and incubated in sunlight for 4 h (Hwang et al. 1986b). Total bacterial numbers were determined by direct microscopic counting with epifluorescence microscopy of acridine orange-stained specimens (Hobbie et al. 1977). Total microbial biomass was determined with measurements of particulate adenosine triphosphate (ATP) (Hodson et al. 1981). Chlorophyll a, used as a measure of phytoplankton biomass, was determined fluorometrically using the method described in Strickland and Parsons (1972).

RESULTS AND DISCUSSION

Degradation rates, including transformation and mineralization of the test compounds in darkness, sunlight poisoned, and sunlight untreated are listed in Table 2. Note that no degradation (detection limit: 0.1 ng for the test compounds) was observed in poisoned dark samples. The photo-transformation of the test compounds followed a first-order equation (Figure 1). The relative rates of photolysis in both buffered distilled water and estuarine water decreased in the order: p-chloroaniline, 2,4,5-trichloroaniline, 2,4-dichloroaniline, and aniline (Table 2). The trend of the direct photolysis can be predicted by comparing the absorbance and quantum yields of the compounds (Hwang et al. 1987). The photo-transformation (disappearance of parent compound by photolysis) rate constants for p-chloroaniline,

2,4-dichloroaniline, and 2,4,5-trichloroaniline in estuarine water ranged from 0.01 to 0.45 h^{-1} with half-lives ranging from 2 to 68 h. Aniline was photolyzed rapidly in summer but slowly in winter, with half-lives of 36 and 125 h in summer and in winter, respectively. Photo-mineralization ($^{14}CO_2$ production by photolysis in poisoned water) half-lives ranged from 30 to 189 d for p-chloroaniline, 2,4-dichloroaniline, and 2,4,5-trichloroaniline and 103 to 355 d for aniline. Photolysis rates for all compounds were higher in summer than in winter, assumably due to higher surface irradiance in summer. For example, the photo-transformation rate constants of p-chloroaniline decreased from 0.45 to 0.18 h^{-1} from summer to winter (Table 2). Although particulates concentration was much higher in summer (data not shown), the increase in irradiance appeared to be a more important factor than the increase in light attenuation by particulates in the summer.

The photolysis rates were higher in buffered distilled water than in estuarine water when the attenuation of light by estuarine water (i.e., screening factor), was taken into account. The primary process responsible for the transformation and mineralization of certain compounds (e.g., 2,4-dichloroaniline) was photolysis (Table 2). During the incubation periods (up to 3 d) there was no significant microbial (i.e., dark) degradation of p-chloroaniline, 2,4-dichloroaniline and 2,4,5-trichloroaniline (data not shown). The dark mineralization rate constants of aniline in summer and winter were 0.005 and 0.0009 d^{-1}, respectively. Since bacterial total numbers were comparable in two seasons and bacterial turnover of glucose was faster in summer than in the winter, we speculate that the changes in temperature in the two seasons were significantly affecting microbial degradation rates.

In sunlit estuarine water both photolysis and microbial degradation can act simultaneously on the compounds. As indicated above, there was no detectable microbial (dark) degradation of p-chloroaniline, dichloroaniline, and trichloroaniline in estuarine water. However, after these compounds were transformed by photolysis, microbial consortia appeared to mineralize the photoproducts (Hwang et al. 1996). The summer mineralization rate constants of trichloroaniline under sunlight conditions were 0.017 and 0.06 d^{-1} in poisoned and untreated estuarine water, respectively (Table 2). In the winter microbial mineralization of chloroanilines' photoproducts markedly decreased. For aniline, microbial degradation rates were lower than photolysis rates throughout the year.

Our studies indicated rapid photolysis of p-chloroaniline and trichloroaniline in both distilled and estuarine water. Aniline photolyzed rapidly in the summer but at much lower rates in the winter, presumably due to the lower intensity of light in the UV-blue region in the winter. At latitude 30°N the irradiance at 304 nm and 460 nm decreases 3.5-fold and 1.9-fold, respectively, from summer to winter (Mill et al. 1982). Aniline was reported to have a sensitized photolysis rate constant of 0.12 h^{-1} in humic acid solutions at pH 6 under May sunlight (Zepp et al. 1981), which was higher than the rate constant determined in our study. Presumably, this was due to the higher level of dissolved organic matter in their solutions. Under fluorescent lamps, p-chloroaniline was reported to have a photo-transformation rate constant of 0.79 h^{-1}, while 3,4-dichloroaniline had a summer photolysis rate constant of 0.04 h^{-1} in both distilled water and filtered seawater (Miller and Crosby 1983). The photolysis rate constant of p-chloroaniline and 2,4-dichloroaniline in estuarine water was computed to be 0.45 and 0.03 h^{-1}, respectively (Table 2). Thus, our values were similar to those determined by others, even though the water and irradiance source were different.

When the screening factor was taken into account, the photolysis rates of all candidate compounds were higher in distilled water than in estuarine water. Thus, sensitization was not observed in all cases of our study. Although the photolysis rates of chloroaniline and trichloroaniline are high in surface waters, if the entire water column is considered influence of photolysis will be much smaller. However, aniline compounds and their photoproducts below photic depth could be brought back to the surface by vertical mixing forces in estuarine water. Based on the average depth of the river (5 m) and the velocities of wind and tides, it takes about 1 h for a complete vertical mixing for the river. Thus, as a result of such mixing, the chloroanilines should be subject to photolysis through the entire water column. In spite of such mixing, microbial degradation should be a major degradative process for chloroanilines at greater depths. Since microbial adaptation often requires long exposure periods before degradation occurs (Pignatello et al. 1983), our incubation periods (3 d or less) may not have been long enough to observe microbial degradation of chloroanilines. Microbial degradation rates of aniline in surface estuarine water were lower than photolysis throughout the year. However, if the whole estuary is considered, microbial degradation may be more important than photolysis for aniline, due to the high light attenuation effect of the estuary.

In summary, photolysis was an important degradative process in removing aniline and chloroanilines from surface estuarine water. The decrease in winter photolysis rates correlated to a decrease in surface irradiance while microbial degradation decrease correlated to a decrease in temperature. The data reported in this study can be used to predict the persistence of the aniline compounds in the estuary. In addition, our findings suggest the efficacy of shallow pretreatment in enclosures exposed to solar UV to photochemically degrade the compounds before release to the estuary. Alternatively, releasing the compounds at the surface of the estuarine water to maximize the photolysis before the effluents can mix with the more saline water below.

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REFERENCES

Bartha, R. 1971. Fate of herbicide-derived chloroanilines In soil. J. Agric. Fed. Chem. 19: 385-387.

Cooney, J.J., S.A. Silver, and E.A. Beck. 1985. Factors influencing hydrocarbon degradation in three freshwater lakes. Microb. Ecol. 11: 127-137.

Halmann, M.M. 1996. Photodegradation of water pollutants. Boca Raton, Fla: CRC Press.

Hobbie, J.E., R.J. Daley, and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228.

Hodson, R.E., A.E. Maccubbin, and L. R. Pomeroy. 1981. Dissolved adenosine triphosphate utilization by free-living and attached bacterioplankton. Mar. Biol. 64: 43-51.

Hwang, H.-M., R.E. Hodson, and R.F. Lee. 1986a. Photochemical and microbial degradation of 2,4,5-trichloroaniline in a freshwater lake. Appl. Environ. Microbiol. 50: 1177-1180.

Hwang, H.-M., R.E. Hodson, and R.F. Lee. 1986b. Degradation of phenol and chlorophenols by sunlight and microbes in estuarine water. Environ. Sci.Technol. 20: 1002-1007.

Hwang, H.-M., R.E. Hodson, and R.F. Lee.1987. Degradation of aniline and chloroanilines by sunlight and microbes in estuarine water. Water Res. 21: 309-316.

Hwang, H.-M., R.E., Hodson, and D.L. Lewis. 1989. Microbial degradation kinetics of toxic organic chemicals over a wide range of concentrations in natural systems. Environ. Toxicol. Chem. 8: 65-74.

Hwang, H.-M., and S.W. Maloney. 1996. A study of microbial transformations of trichloroaniline and p-cresol using size-fractionation technique. Bull. Environ. Contam. Toxicol. 56: 343-350.

Kaufmann, D.D, and J. Blake. 1973. Microbial degradation of several acetamide, acylanilide, carbamate, toluidine and urea pesticides. Soil. Biol. Biochem. 5: 297-308.

Larson, R.A., D.D. Ellis, H.-L. Ju, and K.A. Marley. 1989. Flavin-sensitized photodecomposition of anilines and phenols. Environ. Toxicol. Chem. 8: 1165-1170.

Lyons, C.D., S. Katz, and R. Bartha. 1984. Mechanisms and pathways of aniline elimination from aquatic environments. Appl. Environ. Microbiol. 48: 491-496.

Matsumura, F. 1982. Degradation of pesticides in the environment by microorganisms and sunlight. In: Matsumura F, Murti CRK (eds) Biodegradation of Pesticides. New York: Plenum Press.

Messner, B., J. Berndt, and I. Baum. 1979. Changes in fatty acid and cholesterol synthesis in rat liver slices due to aromatic amines formed during the degradation of some herbicides. Bull. Environ. Contam.Toxicol. 21: 831-836.

Miille, M.J., and D.G. Crosby. 1983. Pentachlorophenol and 3,4-dichloroaniline as models for photo-chemical reactions in seawater. Mar. Chem.14: 111-120.

Mill, T., W.R. Mabey, D.C. Bomberger, T.-W. Chou, D.G. Hendry, and J.H. Smith. 1982. Laboratory protocols for evaluating the fate of organic chemicals in air and water. EPA-600/3-82-022. Washington, D. C.: U.S. EPA.

Miller, G.C. and D.G. Crosby. 1983. Photooxidation of 4-chloroaniline and N-(4-chlorophenyl)-benzenesulfonamide to nitroso- and nitro-products. Chemosphere 12: 1217-1227.

Neilson, A.H. 1994. Organic chemicals in the aquatic environment: distribution, persistence, and toxicity. Boca Raton, Fl: Lewis Publishers.

Pignatello, J.J., M.M. Martinson, J.G. Steiert, R.E. Carlson, and R.L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. Appl. Environ. Microbiol. 46: 1024-1031.

Skurlatov, Y.I., R.G. Zepp, and G.L. Baughman. 1983. Photolysis rates of (2,4,5-trichlorophenoxy) acetic acid and 4-amino-3,5-trichloropicolinic acid in natural waters. J. Agri. Fed. Chem. 31: 1065-1071.

Strickland, J.D., and T.R. Parsons. A Practical Handbook of Seawater Analysis. 1972. Fisheries Research Board of Canada, Ottawa.

Zepp, R.G., and G.L. Baughman. 1977. Prediction of photochemical transformation of pollutants in the aquatic environment. In: Aquatic pollutants: transforomation and biological effects. (ed) O. Hutzinger, V. Lelyveld and B.C.J. Zoeteman. New York: Pergamon Press.

Zepp, R.G., G.L. Baughman, and P.F. Schlotzhauer. 1981. Comparison of photochemical behavior of various humic substances in water-I. Sunlight induced reactions of aquatic pollutants photosensitized by humic substances. Chemosphere 10: 109-117.

Table 1. Physical, chemical, and biological properties of Skidaway River water*

Season and temperature (°C)	Acridine orange direct count (number/mL) x 10^6	Particulate ATP (µg/L)	Chlorophyll-a (µg/L)	^{14}C-bicarbonate uptake		^{3}H-glucose uptake	
				Turnover time (h)	Specific turnover rate**	Turnover time (h)	Specific turnover rate†
Biological properties							
Summer (27-29°C)	7.0 ± 1.4 (5.5-8.3)	1.6 ± 0.6 (1.0-2.3)	7.9 ± 2.2 (5.5-9.8)	289 ± 220 (133-444)	4.4	2.0 ± 0.4 (1.6-2.4)	7.1 n = 9
Winter (7-23°C)	4.5 ± 2.9 (1.1-9.8)	3.0 ± 1.1 (1.8-4.6)	8.3 ± 4.2 (4.2-12.8)	1370 ± 923 (573-2312)	0.9	3.7 ± 0.7 (3.0-4.6)	6.0 n = 14
Physical and chemical properties							
Temperature (°C)	21.7 ± 6.3 (7-29)				pH	7.7 ± 0.2 (7.4-8.0)	
S††	0.85 ± 0.04 (0.82-0.88)						

*Expressed as the mean ± 1 SD (range).

**Defined as the turnover rate divided by chlorophyll-a concentration ($h^{-1} \cdot L \cdot / \mu g \cdot 10^{-4}$).

†Defined as the turnover rate divided by acridine orange direct numbers ($h^{-1} \cdot$ mL./ cell$\cdot 10^{-8}$).

††Screening factor is the ratio of valerophenone photolysis rate in estuarine water to that in distilled water.

Table 2. Photolysis and microbial degradation of aniline and chloroanilines*

Compound and water	Season (temp °C)	Midday surface irradiance (Einsteins $m^{-2}h^{-1}$)	Transformation		Mineralization	
			Rate constant (h^{-1})	Half-life (h)	Rate constant (d^{-1})	Half-life (d)
Aniline						
Distilled**	Summer (29)	5.2 ± 0.7	0.021 ± 0.008	33	0.0079 ± 0.0009	88
	Winter (14)	2.9 ± 0.8	0.013 ± 0.007	53	0.0023 ± 0.0003	301
Estuary (poisoned)	Summer (29)	5.2 ± 0.7	0.019 ± 0.002	36	0.007 ± 0.001	103
	Winter (14)	2.9 ± 0.8	0.006 ± 0.004	125	0.0020 ± 0.0001	355
Estuary	Summer (29)	5.2 ± 0.7	0.025 ± 0.003	27	0.021 ± 0.003	33
	Winter (14)	2.9 ± 0.8	0.010 ± 0.006	71	0.0037 ± 0.0001	189
Estuary (dark)	Summer (29)		0.004 ± 0.001	173	0.005 ± 0.001	139
	Winter (14)		< DL		0.0009 ± 0.0003	770
p-Chloroaniline						
Distilled	Summer (25)	5.9 ± 0.6	0.8 ± 0.1	1	0.029 ± 0.003	24
	Winter (15)	2.6 ± 0.9	0.26 ± 0.02	3	0.0126 ± 0.0009	55
Estuary (poisoned)	Summer (25)	5.9 ± 0.6	0.45 ± 0.04	2	0.023 ± 0.002	30
	Winter (15)	2.6 ± 0.9	0.18 ± 0.01	4	0.010 ± 0.002	68
Estuary	Summer (25)	5.9 ± 0.6	0.49 ± 0.05	1	0.084 ± 0.007	8
	Winter (15)	2.6 ± 0.9	0.26 ± 0.02	3	0.030 ± 0.007	23
Estuary (dark)	Summer (25)		< DL		0.0012 ± 0.0002	578
	Winter (15)		< DL		< DL	
2,4-Dichloroaniline***						
Distilled	Summer (25)	5.8 ± 0.7	0.071 ± 0.009	10	0.009 ± 0.001	77
	Winter (13)	4.6 ± 0.4	0.033 ± 0.005	21	0.007 ± 0.001	98
Estuary (poisoned)	Summer (25)	5.8 ± 0.7	0.027 ± 0.005	26	0.008 ± 0.004	84
	Winter (13)	4.6 ± 0.4	0.010 ± 0.001	68	0.004 ± 0.001	189
Estuary	Summer (25)	5.8 ± 0.7	0.027 ± 0.009	26	0.014 ± 0.005	49
	Winter (13)	4.6 ± 0.4	0.008 ± 0.003	82	0.008 ± 0.003	92
2,4,5-Trichloroaniline						
Distilled	Summer (29)	4.5 ± 0.1	0.37 ± 0.07	2	0.021 ± 0.003	32
	Winter (16)	3.0 ± 1.0	0.11 ± 0.01	6	0.012 ± 0.006	58
Estuary (poisoned)	Summer (29)	4.5 ± 0.1	0.10 ± 0.03	6	0.017 ± 0.003	42
	Winter (16)	3.0 ± 1.0	0.09 ± 0.01	8	0.008 ± 0.001	87
Estuary	Summer (29)	4.5 ± 0.1	0.140 ± 0.008	5	0.06 ± 0.01	12
	Winter (16)	3.0 ± 1.0	0.09 ± 0.02	8	0.016 ± 0.001	44

*^{14}C-labeled compounds were incubated in quartz flasks at a concentration of 25 µg/L. In transformation measurement, all compounds except aniline were exposed to midday sunlight for 4 h, and half-lives were reported as "light hours". Aniline was exposed to sunlight and darkness for up to 3 days because of its slower photolysis rate. < DL: < Detection Limit.

**Distilled water was buffered at pH 7.7 ± 0.2 (0.016M phosphate).

***Degradation of 2,4-dichloroaniline and 2,4,5-trichloroaniline in darkness were negligible.

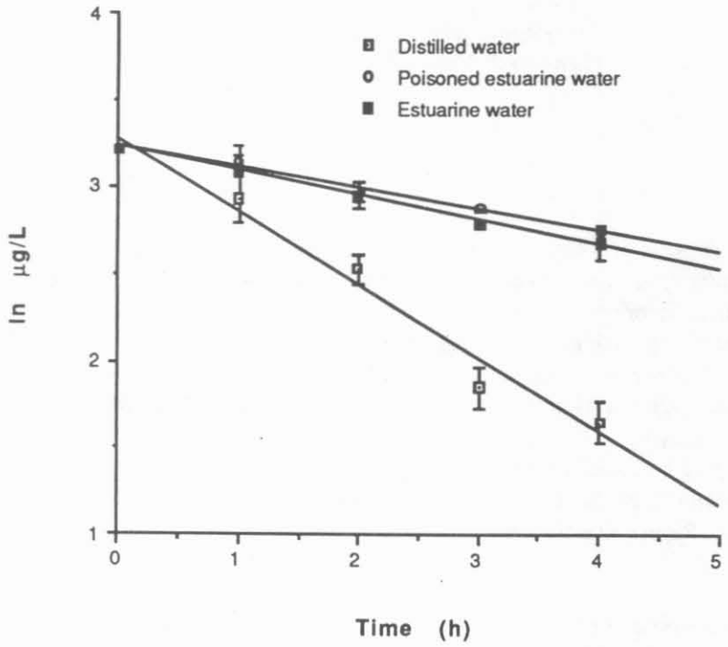

Figure 1. Transformation of 2,4-5-trichloroaniline (25 µg/L) in August. Temperature was 29^{0}C and pH was 7.6. Vertical bars represent 1 s.d. with n = 3, r = 0.97-0.99.