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Influence of Spatial and Temporal Variations on Organic Pollutant Biodegradation Rates in an Estuarine Environment

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The influence of spatial and temporal environmental variations on rates of organic pollutant biodegradation were assessed by using heterotrophic uptake kinetics. These studies were conducted at three sites, representing the gradient from freshwater to estuarine to marine systems. Of the compounds tested, total uptake V_{max} rates decreased in the order of nitrilotriacetic acid, *m*-cresol, chlorobenzene, and 1,2,4-trichlorobenzene. In general, the freshwater site exhibited the highest uptake rates, with somewhat lower rates at the estuarine site. Rates at the marine site were much lower than at the other sites, except during the winter. Metabolic rates at both the freshwater and estuarine areas were significantly decreased during periods of low water temperature. Rates at the marine site were relatively uniform throughout the year. Linear regression analysis was used to compare *m*-cresol biodegradation rates to characteristics of the microbial community, which included direct microscopic counts, CFU counts, and cellular incorporation of amino acids. The observed rates did not consistently correlate well with any of the measured characteristics of the microbial community.

Since aquatic environments constitute a major sink for anthropogenic pollutants, knowledge of the fate of organic contaminants in these systems is essential. The fate of a chemical in any environment will be influenced by the interaction of complex physical, chemical, and biological factors. The persistence of the compound will, in most cases, be most significantly affected by its biodegradability. Biodegradation of any specific substance is dependent on both its structure and the environment in which it is placed. Regardless of whether the chemical is inherently metabolizable or not, its ultimate breakdown will be dictated more by environmental factors than by the nature of the chemical or the degradative agents that might be present (1, 7).

Many studies have been directed toward elucidating the biochemical mechanisms by which single pure chemicals are broken down by pure cultures. Relatively few researchers have addressed the degradation of contaminants by using natural microbial communities (4, 9, 11). Natural environmental variability precludes making extrapolations from laboratory studies of biodegradation until the influence of these variables on the degradative activity of the microbial community is known.

Estuarine ecosystems are characterized by spatially and temporally discontinuous inputs of terrestrially derived materials, by areas of rapid sedimentation, and by salinity gradients, all of which contribute to their highly variable nature. Also, since many estuarine environments receive pollutants directly from coastal industries and indirectly from upstream sources, they constitute relevant areas for biodegradation investigations. In this study, we used the heterotrophic uptake method of biodegradation assessment (10) to evaluate the influence of spatial and temporal variations on pollutant metabolism by natural microbial communities in a North Carolina estuary.

MATERIALS AND METHODS

Site description and sampling procedure. Samples were collected from three sites within the Newport River Estuary system near Morehead City, N.C. (Fig. 1). The Newport River system drains a largely forested watershed of approximately 340 km^3. The upstream site is a narrow creek located in the freshwater portion of the river near the village of Newport. The major organic inputs are from surface runoff, resulting in highly colored water due to humic content. Particulate material tends to be low in concentration and small in size. The estuarine area (21 km^2) is broad, shallow (average depth of 1 m at mean low tide), and subjected to considerable wind-driven mixing. There is a minimal but continuous input of water from the Newport River. Nutrient inputs are primarily from large salt marshes that line the estuary. The water has higher and more variable concentrations of particles than the upstream site. The estuarine sampling site is about 10 km downstream from the freshwater site. The marine sampling area is located approximately 5 km offshore in Onslow Bay, due west of Cape Lookout, N.C. This

is a typical continental shelf region with some nutrient input from coastal areas.

Samples were collected just below the surface in acid-cleaned, 10-liter polyethylene containers. The samples were returned to the laboratory within 1 h of collection. At the time of sampling, the temperature and salinity were measured.

Compounds used. Radiolabeled compounds used as substrates for biodegradation assessments included the following: *m*-[*ring-U*-^{14}C]cresol (specific activity, 36 mCi/mmol; Amersham Corp., Arlington Heights, Ill.); chloro[*U*-^{14}C]benzene (specific activity, 19.7 mCi/mmol; Amersham); nitrilotri[1-^{14}C]acetic acid (specific activity, 53 mCi/mmol; Amersham); and 1,2,4-trichloro[*U*-^{14}C]benzene (specific activity, 13.1 mCi/mmol; Pathfinder Laboratories, St. Louis, Mo.). Solutions were prepared in 95% ethanol at a concentration of 1 μCi/ml, with the exception of nitrilotriacetic acid (NTA), which was prepared in a 50% ethanol-water solution at 2 μCi/ml. All chemicals were shown by gas chromatographic analysis to be more than 99% pure. A mixture of ^{3}H-labeled amino acids (New England Nuclear Corp., Boston, Mass.) was used to measure community heterotrophic activity, and $NaH^{14}CO_3$ (New England Nuclear) was used to assess CO_2 recovery.

Community measurements. Metabolism of the organic pollutants was measured by the technique reported by Pfaender and Bartholomew (10). This protocol measures both the uptake of radiolabeled substrate into microbial cells and subsequent respiration to $^{14}CO_2$. The use of multiple substrate concentrations permits the calculation of the kinetic parameters V_{max} and K_m (15). Samples were distributed into vessels, amended with labeled substrate, and incubated for short periods (4 to 10 h) at the temperature of the water at the time of sampling. Controls consisted of similarly treated samples amended with $HgCl_2$ to inhibit biological activity. After incubation, the $^{14}CO_2$ produced from respiration was trapped from the headspace above acidified samples, and substrate uptake into cells was determined by filtering the water sample through membrane filters (0.2-μm pore size). Uptake and respiration values were corrected for abiotic activity by means of the $HgCl_2$-treated samples. Further details are provided in a previous report (10).

Microbial community size was measured for all samples by both acridine orange direct counting (AODC) (5) and CFU by the spread plate technique (3). The CFU medium was composed of Casamino acids, 1.0 g; cellobiose, 0.4 g; sodium acetate, 0.4 g; yeast extract, 0.2 g; $(NH_4)_2SO_4$, 0.3 g; and purified agar, 20.0 g, dissolved in 1 liter of water from the sample site. Community metabolism was assessed for all samples as the cellular incorporation of a mixture of radiolabeled amino acids according to the technique of Azam and Holm-Hansen (2). These measurements were done concurrently with biodegradation assessments.

RESULTS

One of the principal advantages of using the kinetic approach to assess pollutant biodegradability is that it provides the option to calculate the kinetic parameters V_{max} and K_m. Since a large percentage of the uptake experiments in this study appeared to yield saturation-type kinetics, a Hanes-Woolf linear transformation of the uptake and respiration data has been used to give V_{max} and K_m values for each sampling site and date. A linear correlation coefficient of 0.9 or better was used as a criterion for linearity in calculating V_{max} values. *m*-Cresol degradation was assessed extensively at the three sites during approximately a 1-year period, with water temperatures ranging from 7°C in January to 32°C in August. Figure 2 shows V_{max} values for *m*-cresol total uptake (incorporation into cells plus $^{14}CO_2$ production) at the three sites

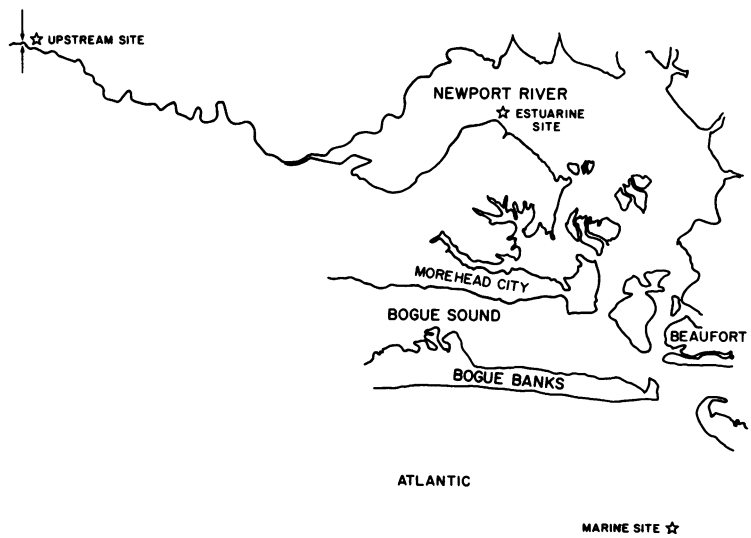

FIG. 1. Sample sites in the Newport River Estuary of North Carolina.

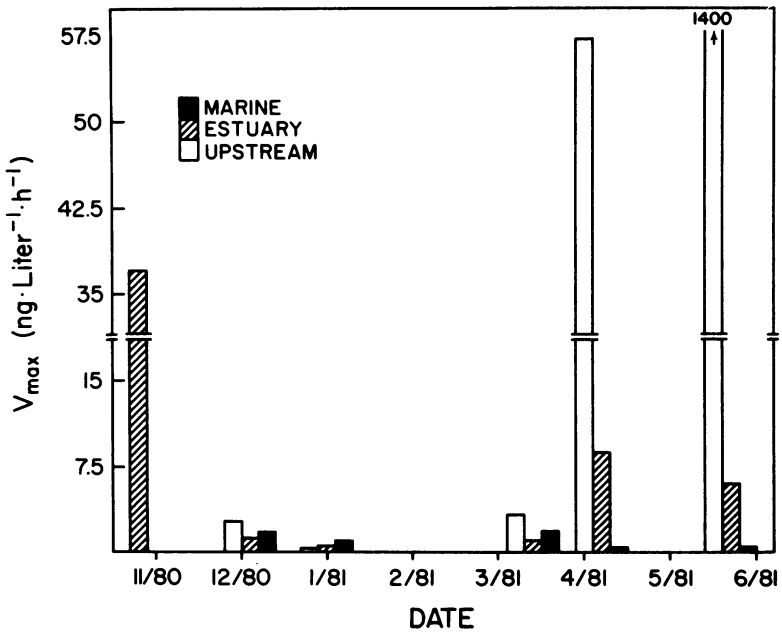

FIG. 2. Variation in *m*-cresol V_{max} with sample site and date.

throughout the year. The K_m values for the samples were remarkably similar, having a range of 0.6 to 1.8 μg · liter^{-1} for all sample times and sites. This figure demonstrates dramatic changes in *m*-cresol V_{max} over this period, and these changes appear to be related to changes in water temperature at the sites. In the warmer months, there were also dramatic site differences in *m*-cresol V_{max} rates, with the upstream site having higher values than the estuarine site, which in turn had higher values than the marine site. The *m*-cresol V_{max} rates for the marine site fluctuated much less during the year than those of the upstream or estuarine sites, the values being similar to those of the other sites during the colder months.

In addition to *m*-cresol, microbial uptake of chlorobenzene, NTA, and trichlorobenzene was assessed at the three sites at different times throughout the year. A comparison of uptake V_{max} values for these compounds (Table 1) indicates decreasing uptake rates in the order of

TABLE 1. Summary of kinetic parameters for chlorobenzene, NTA, and 1,2,4-trichlorobenzene[a]

Date	Site	Temp (°C)	Chlorobenzene		NTA		Trichlorobenzene	
			V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
9-25-80	Upstream	—[b]	13	1.0				
	Estuarine	27	10	1.1				
	Marine	—	<1	—				
11-7-80	Upstream	12			500	55	7.5	6.5
	Estuarine	14			200	110	<1	—
	Marine	17			—	—	<1	—
2-13-81	Upstream	7	<1	—	56	60		
	Estuarine	6	<1	—	175	60		
	Marine	8	<1	—	85	65		
5-26-81	Upstream	24	14	5.1			<1	—
	Estuarine	28	4.9	3.0			7.9	7.0
	Marine	24	1.7	4.1			2.3	4.5

[a] V_{max}, Nanograms per liter per hour; K_m, micrograms per liter. A V_{max} value less than 1 ng · liter^{-1} · h^{-1} represents a metabolic rate less than that detectable by this technique.

[b] —, Data not available.

NTA, chlorobenzene, and trichlorobenzene. Uptake V_{max} values for these compounds varied throughout the year in a manner similar to *m*-cresol, with the uptake of chlorobenzene and trichlorobenzene being essentially unmeasurable during the coldest months. In general, between-site differences were also similar to those observed with *m*-cresol, with the highest uptake rates at the upstream site, followed by the estuarine and marine environments.

Since the data presented in Fig. 2 and Table 1 indicated an apparent relationship between temperature and rates of pollutant biodegradation, a linear regression plot of *m*-cresol uptake V_{max} versus site temperature was constructed (Fig. 3) by using data from the estuarine site. The *m*-cresol V_{max} data used include those shown in Fig. 2 and additional data from previous dates on which only estuarine samples were collected. Although the correlation coefficient (*r*) for temperature versus V_{max} is 0.95 for temperatures below 20°C, the overall correlation coefficient covering the full range of in situ temperatures is 0.57. These results suggest that low ambient temperatures are a dominant influence on biodegradation rates for *m*-cresol, but at higher temperatures, other influences become important.

To examine relationships between uptake rates and other characteristics of the environment, we measured certain parameters that may influence the microbial community, as well as community size and general metabolic activity. These data are shown in Table 2 for those dates on which *m*-cresol metabolism measurements were made. These values were used in conjunction with *m*-cresol V_{max} data to do correlations by means of linear regression analysis. The correlation matrix is shown in Table 3. All correlation coefficients other than with *m*-cresol V_{max} were generated by using parameters from Table 2 combined with data from other sample dates. The *m*-cresol V_{max} correlations used only the data in Table 2. Table 3 shows that the results obtained with the two counting methods do not correlate strongly with one another or with amino acid turnover rates. Although not shown in Table 3, correlations of amino acid

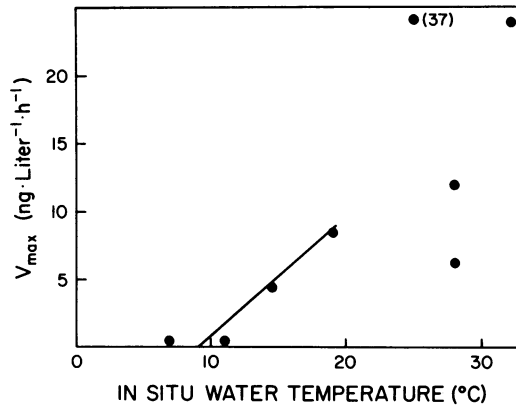

FIG. 3. Linear regression analysis of *m*-cresol V_{max} at the estuarine site versus temperature. The correlation coefficient (*r*) below 20°C is 0.95, whereas the overall coefficient is 0.57.

TABLE 2. Measured sample site characteristics

Date	Site	Temp (°C)	Salinity (‰)	CFU/ml (×10⁴)	AODC/ml (×10⁶)	Amino acid turnover rate (h⁻¹)
12-5-80	Upstream	8	0	—[a]	1.3	0.018
	Estuarine	11	26	13	3.0	0.026
	Marine	11	34	—	2.5	0.071
1-2-81	Upstream	7	0	6	3.0	0.003
	Estuarine	7	4	14	2.0	0.002
	Marine	10	26	12	1.0	0.036
3-12-81	Upstream	12	0	0.8	1.6	0.031
	Estuarine	14	24	10.0	1.7	0.010
	Marine	14	33	0.8	2.8	0.042
4-3-81	Upstream	17	0	5.4	3.9	0.083
	Estuarine	19	26	5.4	6.6	0.250
	Marine	14	34	0.32	3.3	0.038
5-26-81	Upstream	24	0	4.8	8.6	1.0
	Estuarine	28	24	2.1	9.5	1.0
	Marine	24	35	1.0	2.2	0.30

[a] —, Data not available.

TABLE 3. Linear correlation coefficient matrix for temperature, microbial community size, and metabolism measurements[a]

Determination and site	AODC			CFU			Amino acid turnover rate			Temp		
	Upstream	Estuarine	Marine	Upstream	Estuarine	Marine	Upstream	Estuarine	Marine	Upstream	Estuarine	Marine
CFU												
Upstream							0.03			0.48		
Estuarine								−0.63			−0.69	
Marine									0.11			−0.37
AODC												
Upstream				0.76			0.35			0.86		
Estuarine					−0.79			0.66			0.74	
Marine						−0.51			0.68			−0.13
m-Cresol V_{max}												
Upstream	0.94			0.17			0.99			0.85		
Estuarine		0.86			−0.88			0.02			0.39	
Marine			−0.28			0.08			0.59			−0.39

[a] Numbers represent the correlation coefficients (*r*) from linear regression analysis.

turnover and temperature were not strong (0.57, 0.44, and 0.45 for upstream, estuarine, and marine sites, respectively). There are no general correlations of *m*-cresol V_{max} with any of the other parameters, although at specific sites (with AODC in upstream and estuarine environments or with amino acid turnover at the upstream site), significant relationships are apparent.

DISCUSSION

The procedure used in this study for measurement of biodegradation rates (10) provides a rapid means of estimating both the rates and kinetics resulting from the microbial metabolism of the pollutant substrates. Both incorporation of label into cell biomass and production of $^{14}CO_2$ are measured. By using short incubation periods (<12 h) and low concentrations of substrate, uptake rates similar to those that would occur under natural conditions are generated, and problems encountered by long incubation and high concentrations are minimized (4). The K_m and V_{max} calculations are used to define the rate of metabolism. These parameters incorporate both the activity of the degrading population and the effects of substrate concentration (8). The only assumption made in the calculation of kinetic parameters is that the naturally occurring substrate concentrations are low relative to the amounts added. This assumption seems realistic, since the environments sampled are subject to minimal inputs of organic contaminants from anthropogenic sources.

It is significant to note that, although the three environments sampled are associated with the Newport River Estuary area, they represent three distinctly different ecosystems. Since the three sites are subject to different environmental factors, it is likely that different microbial communities are present. These differences are reflected in the observed rates of biodegradation of the compounds examined. With the exception of the winter months, the general pattern shows the highest rates at the upstream site, with decreasing rates through the estuarine and marine areas. The marine microbial community does not appear to be influenced by changes in temperature, as evidenced by the relatively constant rates of both pollutant and amino acid metabolism throughout the year. This could be the result of either the community being adapted to low temperatures or the rates of metabolism being dictated by nutrient limitation. Unlike the marine site, the upstream and estuarine V_{max} rates, in addition to amino acid metabolism values, are consistently much less in the winter months than at other times of the year. Figure 3 demonstrates that at temperatures below 20°C, there is a strong correlation between *m*-cresol V_{max} and temperature. Above 20°C, other influences on metabolic activity are apparently more significant than in situ temperature. Several workers have reported decreased pollutant biodegradation rates with samples obtained during cold-water periods (11, 16); however, the reported metabolic rates were not measured at in situ temperatures.

The data in Fig. 2 and Table 1 show that the uptake V_{max} rates for NTA were the highest of all compounds tested. Whereas the V_{max} ranges from a high of 500 ng · liter^{-1} · h^{-1} at the upstream site in November to a low of 50 ng · liter^{-1} · h^{-1} at the same site in February, the K_m values were much less variable. Several workers

have reported higher V_{max} rates for NTA, 1.9×10^6 ng · liter^{-1} · h^{-1} for Ohio River water (13), and 3.38×10^6 ng · liter^{-1} · h^{-1} in soil (14). However, when first-order rate constants (V_{max}/K_m) are compared, the values of 0.018 h^{-1} calculated from Thompson and Duthie (13) for Ohio River water and 0.009 h^{-1} for soil (14) are closer to the 0.001 to 0.009 h^{-1} values found in our study. The lower rates we measured may reflect a smaller or less active microbial community within these coastal environments.

The metabolic rates of *m*-cresol are intermediate between those of NTA and the more slowly degraded chlorinated aromatics. Chlorobenzene and 1,2,4-trichlorobenzene V_{max} rates were approximately equal. It should be noted that little $^{14}CO_2$ production was detected with trichlorobenzene, whereas chlorobenzene showed approximately 30 to 40% of the metabolized label appearing as $^{14}CO_2$. It is possible that the uptake of trichlorobenzene does not result in extensive alterations. The absence of $^{14}CO_2$ production may indicate uptake into cells and either no subsequent degradation or cometabolic alteration. A review of the environmental biodegradation literature revealed no relevant degradation rate information for *m*-cresol, chlorobenzene, or 1,2,4-trichlorobenzene.

There are several kinetic approaches to the measurement and prediction of aquatic biodegradation rates. Paris et al. (9) suggest that second-order rate constants based on substrate concentration and initial community size can be used to provide a site-independent estimate of the microbial breakdown of pollutants. Larson (8) suggests that estimates should be based on the kinetics of metabolism, since degradative activity is the phenomenon of interest. Since most pollutants are present in the environment at very low concentrations and the aquatic microbial community may be largely inactive at any given time (6, 12), it is questionable whether general descriptors of the total number of microorganisms would necessarily relate to metabolic processes carried out by a relatively small part of the community. One of the objectives of our effort was to assess whether directly measured rates of biodegradation related to measured characteristics of the microbial community.

In our study, two measures of community size were made (AODC and CFU). Table 3 shows that these two parameters do not consistently correlate well with measured V_{max} values for *m*-cresol, although there are good correlations with AODC at the upstream and estuarine site. These two measures of community size do not correlate well with one another or with a general measurement of microbial activity, amino acid turnover rate. The V_{max} rates for *m*-cresol and amino acid turnover correlate well only at the upstream site, for which the best relationship with V_{max} and AODC is also found. The absence of consistent, strong correlations is most likely the result of the changes in general community parameters (AODC, CFU, and amino acid turnover) not adequately reflecting changes in the populations or activity of *m*-cresol utilizers. Although consistent linear correlations between measured parameters were not apparent, this does not prove a lack of association. It is possible that the characteristics are related in a nonlinear manner. The data seem to indicate that at the upstream site, *m*-cresol degraders are either a very consistent proportion of the total community or they respond to changes in their environment in a manner similar to the total community. Since the correlation data seem to indicate that some relationships do exist between community characteristics and biodegradation rates, but that the relationships are neither simple or consistent, there is an obvious need for additional research on the influence of environmental variation and rates of pollutant breakdown.

These studies have shown that saturation-type kinetics can be applied to the measurement of aquatic biodegradation rates. These rates are strongly influenced by spatial and temporal variations in the environment and do not correlate consistently with general characteristics of the microbial community. It is possible that some compounds may be utilized by a large portion of the microbes present in certain environments. For these compounds and environments, general measures of community characteristics may relate to degradation processes. Our data would seem to indicate that for the compounds used, direct measurements of biodegradation rates are necessary if the inherent variability of the environment is to be reflected in assessments of pollutant breakdown.

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