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Distribution of Autotrophic Nitrifying Bacteria in a Polluted River (the Passaic)†

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The abundance of nitrifying bacteria, determined by most-probable-number procedures, within habitats of the Passaic River was as follows: rooted aquatic plants > algae ≅ rocks > sediments ≫ water. On the average, NH_4^+ oxidizers were 540-fold more abundant in the topmost 1 cm of sediment than in the water, and NO_2^- oxidizers were 250-fold more abundant. The population densities in this surface sediment at two nearby stations, one with a predominantly mineral stream bed and the other an organic ooze, did not differ significantly. Large numbers of nitrifiers were present to a depth of about 5 cm in a mineral sediment core.

If ammonium in wastewater treatment plant effluent is nitrified after discharge, it consumes dissolved oxygen. Whether this potential demand on the oxygen supply is exerted in the receiving stream, and if so in what stretch, is often an important and controversial aspect of water quality management. A case in point is the Passaic River. The nitrification component of a proposed waste load allocation scheme for this river was challenged by a regional planning group, apparently on the basis that the sequence of nitrogen transformations characteristic of nitrification is not observed with downstream progression (2). That is, despite elevated levels of ammonium over most of the Passaic main stem, and summertime water temperatures favorable for nitrification, a clearly defined buildup of nitrate is not observed. An alternative position is that nitrification occurs but is masked by other nitrogen transformations, particularly ammonification and denitrification within the stream bed. This controversy is treated in detail elsewhere (7).

Basic data in this problem area were sought in the present investigation, which concerns the distribution of nitrifying bacteria in Passaic River habitats.

MATERIALS AND METHODS

Sample collection and preparation. Sampling was within a few meters of the river bank, where the water depth was about 20 cm. Water samples were taken at the surface. To obtain surface sediment (defined as the topmost sediment approximately 1 cm thick), a glass tube (40 by 4.8 cm ID) was gently pushed about 8 cm into the stream bed. A small shovel was then forced under the buried end of the tube to lift it out. The overlying water was discarded by siphon, and the surface sediment was transferred to a sterile jar. Three cores were taken from each station, and the surface sediment was composited.

For the examination of deeper sediment levels, the glass tube was pushed deeper into the sediment. The overlying water was discarded, and the tube and contents were taken to the laboratory. Samples were obtained from different depths as the material was allowed to descend from the tube, deepest level first.

Rocks (5 to 10 cm in diameter) were removed from the stream bed and allowed to drain. The rock surface that had faced the stream water was scraped with a spatula. The scrapings were placed into sterile phosphate buffer dilution water (1).

Algae and plants were handled gently to avoid disturbing attached sediment and other material. Rooted plants were severed at the mud-water interface. The specimens were drained and placed into dilution water. Plants were identified by means of keys (5, 9).

The samples were chilled for transport to the laboratory. Inoculations and measurements of pH, dissolved oxygen, particulate matter, and NO_2^- were completed on the day of collection. Samples were stored at 4°C for NH_4^+ and NO_3^- determinations. (For NO_3^-, 1% [vol/vol] concentrated H_2SO_4 was added.) These determinations were usually performed within 36 h.

Pebbles, twigs, leaf fragments, etc., were removed from the sediment samples, which were then homogenized with a sterile glass rod. A 10-ml portion was combined with 90 ml of dilution water, shaken vigorously by mechanical means, and diluted serially. A comparable portion was oven dried at 104°C and weighed.

Rock, scrapings and specimens of algae and plants suspended in dilution water were blended in the cold for 1 min at approximately 15,000 rpm and diluted

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serially. A portion of the lowest dilution was used for the dry weight determination.

Where results are expressed gravimetrically, it is on the basis of oven dry weight.

Physical, chemical, and bacteriological determinations. Water samples were analyzed as follows: temperature, mercury thermometer; pH, glass electrode; dissolved oxygen, azide modification of the Winkler method (1); total organic carbon, Beckman infrared analyzer; NH_4^+, phenate method (1); NO_2^-, sulfanilamide-naphylethylenediamine (12); NO_3^-, brucine-sulfanilic acid method (10); particulate matter in suspension, membrane filtration (0.45 μm) and oven drying. Inorganic nitrogen species in the sediment were determined by the procedures of Bremner (3).

Autotrophic NH_4^+-oxidizing and NO_2^--oxidizing bacteria population densities were estimated by most-probable-number (MPN) procedures, using 10-fold dilutions with 10 replicate tubes per dilution (11). The incubation was at 28°C for 35 days.

RESULTS

Passaic water phase. The Passaic drainage basin includes swamp, woodland, farmland, suburbs, and, for the terminal one-third of its length, a densely populated and industrialized urban area. The river system receives 328 industrial, municipal, and institutional point source discharges registered under the National Pollution Discharge Elimination Permit Program (N.J. Department of Environmental Protection, personal communication). Consequently, the main stem of the river is seriously polluted over most of its length, as demonstrated in the water quality of the 41 samples tested as part of this investigation. Samples were taken at 11 stations on 17 different days over the period July 1970 to June 1972, along a 64-km (40-mile) stretch from just above the town of Chatham to just above the river's mouth in Newark Bay. Two stations in the midsection of the river were most intensively sampled (at the crossings of U.S. Route 46 and Interstate 80). The means of all the data are (milligrams per liter): dissolved oxygen, 4.8; total organic carbon, 14; NH_4^+-N, 4.2; NO_2^--N, 0.24; NO_3^--N, 1.3; particulate matter (n = 28), 32. The medians are identical or slightly lower. The median pH was 7.1.

The samples were also examined for nitrifying bacteria, as summarized in Table 1 (top). Plotting the MPNs against each of the physical and chemical variables for visual inspection, and statistical treatment of the data by means of stepwise linear regression analysis, did not reveal any strong correlation between population density and the environmental variables examined (V. A. Matulewich, M.S. thesis, Rutgers University, New Brunswick, N.J., 1974; 8). This was true when the analysis included only those sets of data representing the warmer seasons (n = 36; sample temperatures, 19 to 26°C) and when the relatively few wintertime sets were included (n = 5; 1 to 5°C).

Passaic solid phase. Eight samples of surface sediment from the two intensively sampled stations were analyzed for inorganic nitrogen species. The means of the data are (milligrams per liter): NH_4^+-N, 76.8; NO_2^--N, 0.32; NO_3^--N, 21.5.

Bacterial enumerations were performed with surface sediment from eight stations, rock scrapings from three stations, algae from two stations, and rooted aquatic plants from six stations. The dominant stream bed material in the study reach was light-brown, sandy or silty sediment, except at I-80 where it was a black organic ooze. Rocks were generally sparce on the stream bed. The rock scrapings were light brown, and the only microscopically recognizable forms were diatoms. Clumps of floating algae were seen only occasionally. The algal samples were predominantly filamentous green forms. Aquatic plants were abundant only in localized areas. The plants are listed later.

On the average, nitrifying bacteria were most numerous in association with the rooted aquatic

TABLE 1. *Nitrifying bacteria in Passaic River water and solid-phase environments*

Environment	NH_4^+ oxidizers			NO_2^- oxidizers			No. of observations
	Range	Mean	Median	Range	Mean	Median	
	MPN/ml	MPN/ml	MPN/ml	MPN/ml	MPN/ml	MPN/ml	
Water	5.6-2,780	395	150	0[a]-702	30	4	41
Surface sediment[b]	1,280-1,620,000	214,000	62,000	116-42,800	7,422	3,990	23
	MPN/g	MPN/g	MPN/g	MPN/g	MPN/g	MPN/g	
Surface sediment[b]	1,190-3,860,000	462,000	147,000	121-94,500	15,900	5,300	23
Rock associated	832,000-3,920,000	2,090,000	1,520,000	80,500-274,000	153,000	106,000	3
Algae associated	31,000-4,680,000	2,360,000		399-136,000	68,200		2
Plant associated	1,910-61,200,000	11,400,000	3,420,000	847-12,300,000	1,350,000	125,000	13

[a] <0.1/ml (one sample).

[b] The topmost sediment, approximately 1 cm thick. Both sets of entries are based on the same MPNs.

plants, followed by rock scrapings, algae, and surface sediment (Table 1). Population densities in the sediment and water phases can be compared easily, since the MPNs of both materials are expressed volumetrically. Based on the means, NH_4^+ oxidizers in the surface sediment outnumbered those in the water by a factor of 540×, and NO_2^- oxidizers did so by 250×.

Nitrifying bacteria in different levels of a sediment core were enumerated (Table 2). Ammonium oxidizer MPNs were highest from approximately 2 to 5 cm, decreasing thereafter. Nitrite oxidizers decreased sharply in number at depths greater than 10 cm. Although there could have been some downward displacement of cells as a result of the sampling procedure, any such effect is believed to have been minor.

As shown in summary form in Table 1, nitrifiers were generally most abundant in association with rooted aquatic plants. More details are provided in Table 3. The highest MPNs were derived from a specimen of *Potomogeton pusillus*. A second specimen of *P. pusillus*, and also *P. gramineus* and *P. zesteriformis*, were less densely colonized. Three observations of *Nuphar advena* yielded an extremely wide range of NH_4^+ oxidizer MPNs.

Comparison between Passaic stations. The stations at U.S. 46 and I-80 are about 0.8 km (0.5 mile) apart, and there are no intervening outfalls. Nevertheless, the nature of the stream bed differed sharply, as noted previously, because the river widens just upstream of station I-80, prompting deposition of suspended matter. At the I-80 station there was a faint H_2S odor and gas bubbles were seen. The U.S. 46 station was free from H_2S odor and visible gasification.

Eight pairs of water-phase MPN values and six of surface sediment were available for the comparison. (These are included in Table 1.) A pair represents one sample from each station taken on a common day. Each pair of water samples was taken on a different day, over a span of 13 months (sample temperature range, 3 to 26°C). Each pair of sediment samples was also taken on a different day, over a span of 12 months (sample temperature range, 18 to 26°C). To make the comparisons, it was necessary to eliminate the variability in the MPNs resulting from seasonal or daily variations in the environmental conditions. This was done statistically by the procedure of Fertig and Heller (6). The following comparisons were made: NH_4^+ oxidizers in the water at U.S. 46 versus those at I-80, and similarly for NO_2^- oxidizers; NH_4^+ oxidizers in the surface sediment (topmost 1 cm) at U.S. 46 versus those at I-80, and similarly for NO_2^- oxidizers. In all of the comparisons there were no significant differences between the MPNs at the two stations on any given day ($P < 0.05$).

Comparison to a headwater reach. For comparative purposes a limited number of samples from a brook that joins the Rockaway River, a tributary of the Passaic, were examined. This brook is free of effluent point source inputs. The mean nitrogen concentrations of three samples were (milligrams per liter): NH_4^+-N, 0.07; NO_2^--N, 0.016; NO_3^--N, 0.51.

Most of the resultant MPNs (Table 4) are within the range of comparable observations on the Passaic, and at the lower end of that range. The exception outside of the range is the sample of rock scrapings, in which both nitrifier groups were more abundant. These scrapings were

TABLE 2. *Nitrifying bacteria in a sediment profile of the Passaic River*[a]

Depth (cm)	NH_4^+ oxidizers		NO_2^- oxidizers	
	MPN/ml	MPN/g	MPN/ml	MPN/g
0–1	275,000	720,000	27,500	72,000
2–3	622,000	1,010,000	27,500	44,800
4–5	792,000	1,240,000	10,100	15,900
7–8	133,000	193,000	4,930	7,170
9–10	62,000	74,700	15,000	18,000
14–15	2,400	2,220	47	43
20–21	622	551	16	15

[a] The core, removed on 20 January 1972 from a station near Chatham, consisted of alternating bands of light-brown, silty and sandy sediment. In the overlying water the NH_4^+ oxidizer MPN was 6.2/ml, and NO_2^- oxidizers were <0.1/ml (not detected).

TABLE 3. *Nitrifying bacteria associated with aquatic plants in the Passaic River*[a]

Plant[b]	NH_4^+ oxidizers (MPN/g)	NO_2^- oxidizers (MPN/g)
Potomogeton pusillus	61,200,000	12,300,000
P. pusillus	2,380,000	56,600
P. gramineus	12,000,000	125,000
P. zesteriformis	3,580,000	638,000
Nuphar advena	1,910	1,120
N. advena	221,000	847
N. advena	10,400,000	5,840
Ceratophyllum demersum	1,780,000	1,780,000
C. demersum	10,100,000	412,000
Sagittaria cuneata	39,200,000	2,080,000
Elodea cancedensis	1,110,000	1,950
Polygonum natans	3,070,000	165,000
Glyceria striata	3,420,000	43,700

[a] Sampled during the summer of 1971.

[b] *P. pusillus*, small pondweed; *P. gramineus*, variable pondweed; *P. zesteriformis*, flatstem pondweed; *N. advena*, yellow pond lily; *C. demersum*, coontail; *S. cuneata*, arrowhead (Northern); *E. cancedensis*, elodea; *P. natans*, waterweed (water smartweed); *G. striata*, foulmeadow grass.

TABLE 4. *Nitrifying bacteria in water and solid-phase environments of a brook that joins the Rockaway River*

Environment	NH_4^+ oxidizers	NO_2^- oxidizers	No. of observations
	MPN/ml[a]	MPN/ml[a]	
Water	139	2.4	3
Surface sediment[b]	23,000	7,020	2
	MPN/g[a]	MPN/g[a]	
Surface sediment[b]	16,800	5,140	2
Rock associated	10,10,000	1,660,000	1
Algae associated	997,000	23,000	1
Plant associated	3,070,000	135,000	1

[a] Means where there were multiple observations.

[b] The topmost sediment, approximately 1 cm thick. Both sets of entries are based on the same MPNs.

green as the result of the presence of green algae.

DISCUSSION

Compared to the water phase, the solid-phase habitats were densely populated with nitrifying bacteria. Of the solid materials examined, sediment was least densely populated. Nevertheless, sediment is of special interest since it constitutes essentially all of the stream bed of the Passaic main stem over the study reach.

The relative number of nitrifiers in the surface sediment and in the overlying water is estimated as follows. At times of low stream flow, when the removal of dissolved oxygen through nitrification is potentially most troublesome, a water depth of 1 m is representative of many parts of the upper and middle Passaic. Assuming a column height of 101 cm (100 cm of water and 1 cm of sediment), and using the mean MPNs derived from all of the Passaic water and surface sediment samples, 16% of the NH_4^+ oxidizers are calculated to be in the water and 84% in the surface sediment. For NO_2^- oxidizers the values are 29 and 71%, respectively. These estimates are similar to those obtained for the Trent River (4). Judging from this exercise, the Passaic water column would rarely contain as many nitrifiers as the underlying surface sediment.

The relationship between the estimated nitrifier population density and nitrification is not known. The presence of higher nitrate levels in surface sediment than in water, as shown here, suggests that the sediment is a relatively active site of nitrification. More direct evidence for this process in Passaic sediment is available in the form of the suppression of oxygen uptake by allylthiourea, a specific inhibitor of autotrophic NH_4^+ oxidation (J. Cirello, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1975). Similarly, 2-chloro-6-(trichloromethyl)-pyridine, another specific inhibitor of NH_4^+ oxidation, suppressed CO_2 fixation in an estuarine sediment to a depth of a few centimeters (14).

Unambiguous evidence of nitrification in an effluent receiving stream is the demonstration of the NH_4^+-to-NO_3^- sequence, as has been observed in a few streams all with clearly defined recovery zones below the point sources of pollution (13). However, the absence of this sequence does not prove the absence of nitrification, since this could be masked by other nitrogen transformations, particularly ammonification and denitrification. This may describe the Passaic River, which does not display the characteristic nitrification sequence even though NH_4^+ levels and, during summertime low stream flow periods, the residence times and temperatures are favorable for the process. Whether or not the wastewater NH_4^+ received by the Passaic is nitrified within its bounds remains unknown (7). The present report emphasizes that in any assessment of this problem, not only the water column but also the stream bed must be taken into account.

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