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Bacterial Community Structure and Function along a Heavy Metal Gradient

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The response of the planktonic, sediment, and epilithic bacterial communities to increasing concentrations of heavy metals was determined in a polluted river. None of the communities demonstrated a pollution-related effect on bacterial numbers (viable and total), heterotrophic activity, resistance to Pb or Cu, or species diversity as determined by either the Shannon-Wiener diversity index or rarefaction. The lack of correlation between concentrations of heavy metals and resistance in the sediment bacterial community was investigated and found to be due at least in part to the high pH of the river water and the resultant reduction in heavy metal toxicity. The three different communities demonstrated characteristic profiles based on the relative abundances of bacterial strains grouped according to functional similarities.

Heavy metals represent a significant source of pollution for the aquatic environment. While many heavy metals are essential elements at low levels, they can exert toxic effects at concentrations encountered in polluted environments. In response to toxic concentrations of heavy metals, many aquatic organisms, including microorganisms, can develop tolerance (15). Since microorganisms mediate many important processes in the aquatic environment, including self purification and nutrient recycling, the development of heavy metal tolerance by the microbial community would allow these important functions to be maintained despite heavy metal inputs to the environment. If, however, certain members of the microbial community cannot develop resistance to the same extent as the rest of the community, their contribution to the community will be lost, and the ability of the community to perform the above-mentioned processes will be impaired.

Community structure analysis is used by ecologists to study the effects of pollutants. Microbial ecologists have been more reluctant to use this approach because of the selectivity of microbial culture techniques and because of the extensive testing which must be done to characterize the microbial isolates that represent the members of the community (2). However, it has been shown that grouping of isolates at the species level is unnecessary; even a limited number of tests can serve to characterize microbial cultures sufficiently to permit grouping of bacterial strains into closely related clusters that have ecological relevance (20). If tests are selected because they represent important community processes, information can be obtained about community function as well as about structure.

In the present study, the response of three natural bacterial communities to heavy metal pollution was studied. These three communities, planktonic, sediment, and epilithic, were expected to react differently to heavy metal stress. The planktonic community, exposed to continually fluctuating concentrations of heavy metals, would be in contact with ambient concentrations of heavy metals but for periods of time insufficient for selection of tolerant organisms. The sediment bacteria, remaining in situ, would adapt to prevailing concentrations of heavy metals, but because sediments are sinks for heavy metals, these concentrations could represent historical as well as current inputs to the aquatic environment. Epilithic bacteria, developing seasonally in situ, could be expected to adapt to prevailing concentrations of heavy metals (19). By comparing the response of these three communities along a naturally occurring gradient of heavy metals in an urban river, the capacity of aquatic microorganisms to adjust to heavy metal inputs can be determined.

(A preliminary report of this research has been presented [D. Dean-Ross, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, Q80, p. 295].)

MATERIALS AND METHODS

Site description. The Maumee River is formed by the confluence of the St. Joseph River and the St. Mary's River in Fort Wayne, Ind. Before leaving Fort Wayne, it receives effluent from many local industries and the municipal sewage treatment plant and runoff from an abandoned hazardous waste site. Because of the historical importance of electrical wire industries in Fort Wayne, copper and lead were selected for study. Six sampling stations were selected based on known or suspected sources of heavy metals for the river. Station 1 was on the Maumee River approximately 1 mi (ca. 1.6 km) from the confluence of the St. Mary's River and the St. Joseph River. Station 2, also on the Maumee River, was 500 m below the municipal sewage treatment plant outfall. Station 3 was located where a small ditch receiving effluent from several industries joins the river. Station 4 was at the downstream boundary of the hazardous waste site. Station 5 was located on the St. Mary's River in a residential area. Station 6 was located on the St. Joseph River at the Indiana University-Purdue University campus. The locations of the sampling stations are indicated in Fig. 1.

Sampling. Water samples for bacteriological analysis were collected aseptically and transported to the laboratory, where they were analyzed within 8 h of collection. Water samples for heavy metal analysis were acidified with 5 ml of concentrated HNO_3 per liter and stored at 4°C until extraction and analysis. Sediment samples were collected in shallow water with a sterile spatula. Samples were placed in sterile bottles and stored at 4°C until bacteriological analysis, which was always completed within 24 h of sampling. A

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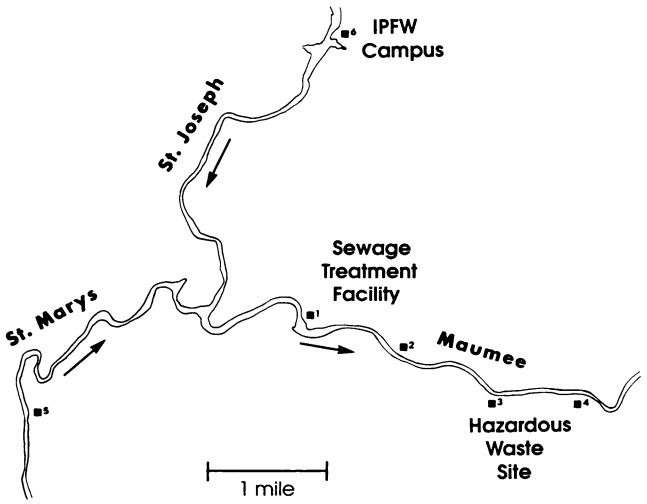

FIG. 1. Locations of sampling stations. Arrows denote current direction. IPFW, Indiana University-Purdue University at Fort Wayne.

portion of the sample was weighed, acidified, and stored at 4°C until extraction and analysis for heavy metal content. For representative samples of the epilithic community, Teflon strips were placed in the river at the sampling stations (16). They were held in place by attaching a line to a weight. A polystyrene float on the line insured that the Teflon strips would not sink to the bottom. Strips were left in place for 5 to 6 weeks, after which time they were removed, placed in sterile bottles containing river water collected at the station, and transported to the laboratory for immediate bacteriological analysis. The Teflon strip from station 5 was lost owing to vandalism.

Heavy metal analyses. Total metals were extracted by nitric acid digestion, and acid-extractable metals were extracted by heating with hydrochloric acid (1). For each method, an appropriate portion of the sample was digested, filtered through a 0.45-μm-pore-diameter membrane filter, and adjusted to the original volume. Extracts were analyzed for Zn, Pb, Ni, Cd, and Cu on an Instrumentation Laboratories atomic absorption spectrophotometer (model 551). Metal concentrations in sediment were calculated on a dry weight basis.

Bacteriological analyses. Total viable heterotrophic bacterial counts were determined by the spread plate technique with half-strength nutrient agar (Difco Laboratories). Enumeration of heterotrophs tolerant of Cu and Pb was performed by supplementing the half-strength nutrient agar with $Cu(SO_4)_2$ or $Pb(NO_3)_2$ to yield final concentrations ranging from 0.001 to 1.0 mM in order-of-magnitude increments. Plates were incubated at 22°C and counted after 1 week. The fraction of heterotrophs tolerant of various concentrations of Cu or Pb was calculated by comparison to the number of heterotrophs on unsupplemented half-strength nutrient agar. Half-strength nutrient agar was selected to minimize the interactions between heavy metals and components of bacterial growth media observed by other workers (4, 5). Because of these interactions, the concentration of free metal in the medium is lower than the concentration of metal added to the medium by an unknown amount. Consequently, the concentration of metal added to the medium will be referred to as the nominal concentration to emphasize that it differs from the actual concentration of free metal in the medium. Total direct bacterial counts were performed by acridine orange epifluorescence microscopy by the method of Scheraga et al. (22). Heterotrophic activity was determined by measuring the utilization of a ^{14}C-labeled amino acid mixture by the single-concentration method of Gocke (9). Absorption (amount of radiolabel retained on a filter with a pore size of 0.22 μm) and mineralization (amount of $^4CO_2$ evolved) were determined separately and added together to calculate turnover time for river water and attached communities. For sediments, only mineralization was used. All data are the result of averaging four replicates and subtracting a killed-cell control.

TABLE 1. Total heavy metal concentrations in Maumee River sediments

Station	ppm[a] of:				
	Pb	Cu	Cd	Ni	Zn
5	40	36	1.4	34	130
6	48	32	3.7	41	120
1	255	124	8.9	101	450
2	204	83	10.0	79	240
3	428	669	24.6	118	560
4	180	407	42.7	103	330

[a] Dry weight basis.

Characterization of bacterial strains. Isolates for characterization were obtained by selecting colonies at random from the unsupplemented half-strength nutrient agar plates. Colonies were transferred to nutrient broth and allowed to grow for 2 to 4 days at 22°C. Nutrient broth cultures were used to perform the following physiological tests. Resistance to Cu, Zn, Pb, and Cd was determined with half-strength nutrient agar supplemented with 1.0 mM $Cu(SO_4)_2$, $ZnSO_4$, or $Pb(NO_3)_2$ or 0.1 M $CdCl_2$. Growth on glucose, acetate, citrate, serine, glutamate, and glycine was determined by adding the carbon sources at a concentration of 0.2% (wt/vol) to a mineral salts medium of the following composition per liter: KH_2PO_4, 0.11 g; K_2HPO_4, 0.65 g; NH_4NO_3, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.025 g; $FeCl_3 \cdot 6H_2O$, 2.5 mg; $MnCl_2 \cdot 4H_2O$, 1 mg; and $ZnSO_4 \cdot 7H_2O$, 3.5 mg. Assays for nitrate reduction and denitrification were performed by the method of Focht and Joseph (7). Starch hydrolysis was determined by streaking cultures on starch agar (Difco) and observing yellow zones surrounding the colonies after flooding plates with Gram iodine (24). Gelatin hydrolysis was determined by streaking cultures on nutrient agar (quarter strength) supplemented with 4 g of gelatin per liter and observing cleared zones around the colonies. Utilization of cellulose was determined by supplementing nutrient agar (quarter strength) with powdered carboxymethyl cellulose (0.1%) and observing cleared zones around the colonies. Lipolysis was determined by the development of turbidity in liquid medium prepared by the addition of 0.1% olive oil to mineral salts medium. Growth on aromatic compounds was determined by supplementing mineral salts medium with 0.1% phenol or benzoic acid. Growth on biphenyl was determined by placing biphenyl crystals in the lids of the inverted petri dishes containing mineral salts medium.

Clustering of bacterial strains. Characters were coded as positive (present), negative (absent), or uncomparable (denitrification in the absence of nitrate reduction). Similarities were calculated with the simple matching coefficient S_{SM} (25). The isolates were clustered by the unweighted pair-

TABLE 2. Extractable heavy metal concentrations in Maumee River sediments

Station	ppm[a] (% of total metal) of:				
	Pb	Cu	Cd	Ni	Zn
5	35.2 (87.1)	28.5 (78.9)	1.4 (100.0)	2.2 (6.4)	96 (74.4)
6	42.0 (87.7)	21.9 (69.5)	3.2 (86.5)	25.1 (61.1)	64 (52.0)
1	217.8 (85.3)	93.1 (75.1)	8.9 (100.0)	65.3 (64.7)	257 (56.5)
2	166.3 (81.6)	52.5 (63.6)	8.8 (88.0)	47.5 (60.3)	125 (52.7)
3	377.0 (88.1)	531.5 (79.4)	21.3 (86.6)	63.9 (54.2)	279 (50.1)
4	158.6 (88.3)	367.5 (90.3)	42.0 (98.4)	73.2 (70.9)	210 (64.6)

[a] Dry weight basis.

group average method (25). Groups were defined at the 85% similarity level (20).

Calculation of diversity indices. Diversity indices were calculated by two methods. The first is the Shannon-Wiener diversity index, $H' = -\Sigma p_i \log p_i$, where p represents the number of isolates in a cluster divided by the total number of isolates in a sample. Logarithmic base 2 was used for the calculation. The second method utilized rarefaction (20, 23). In this approach, results from samples containing a different number of isolates are expressed as the number of expected groups in a sample of an investigator-determined size.

RESULTS

Heavy metal analyses. Analysis of water samples revealed no detectable levels of any of the heavy metals at any station. Analysis of total heavy metal concentrations in sediments (Table 1) revealed a gradient in heavy metal concentration with lowest values at stations 5 and 6, representing sediment quality upstream of major heavy metal inputs, and highest values at stations 3 and 4, just downstream of significant heavy metal inputs to the Maumee River. To emphasize this gradient, we arranged stations in order of increasing pollution severity in Tables 1 and 2.

Extractable metal concentrations (Table 2), which may be considered to represent the quantity of total metal which is biologically available (12), showed a trend similar to that of total metal concentrations. The percentage of total metal that was extractable, however, varied with the metal and the sampling location. Each metal showed a characteristic range of percentages, indicating that a characteristic proportion of total metal was extractable for each metal.

Bacterial abundance and activity. The abundance of bacteria in water, sediment, and epilithon was assessed with direct counts and viable counts. Heterotrophic activity was determined for each community (Table 3). In the water column, no particular trends in bacterial abundance were noted, but amino acid turnover times were slightly higher at stations 5 and 6 than at the Maumee River stations (1 and 4). In sediment samples, the number of viable bacteria was approximately three to five times higher at the Maumee River stations than at stations 5 and 6. For the epilithic bacteria, all three parameters measured showed increased levels at the Maumee River stations as compared with station 6.

Bacterial resistance to heavy metals. Pb and Cu were selected at representative heavy metals for comparisons of resistance. These two metals were selected prior to initiating the study because they were suspected to occur in Maumee River sediments in high concentrations and because comparatively few data were available on the development of resistance to these metals by natural bacterial communities. The three bacterial communities showed different patterns of reactions to increasing concentrations of the heavy metals. With the exception of bacteria collected at station 3, the planktonic bacteria showed poor adaptation to both Pb and Cu (Table 4). A significant portion of the planktonic bacteria demonstrated resistance to nominal concentrations of Cu and Pb up to 0.1 mM. A 1 mM Cu concentration reduced the number of viable bacteria below the detection limit (<300 CFU/g [dry weight] of sediment). However, a significant portion of the bacterial community tolerated the same nominal concentration of Pb.

In contrast, the sediment bacteria were much more resistant to both metals. The sediment bacteria (Table 5) grew in Pb concentrations of up to 0.1 mM, while the highest concentration (1.0 mM) inhibited viable bacteria with from 27 to 55% of the bacteria demonstrating resistance. The sediment bacteria showed a similar pattern of resistance to Cu, except that 1.0 mM Cu inhibited the bacteria to a much

TABLE 3. Activity of Maumee River bacteria in water column, sediment, and epilithon

Station	Water column			Sediment			Epilithon		
	Direct counts[a] (10^6)	CFU/ml[a] (10^4)	TT[b] (h)	Direct counts[a] (10^8)	CFU/g[c] (10^7)	TT (h)	Direct counts[a] (10^8)	CFU/ml[c] (10^7)	TT (h)
5	8.3	6.4	7.34	9.76	3.8	3.73			
6	8.9	1.41	5.10	16.2	3.7	1.59	1.6	1.5	0.282
1	8.11	6.1	2.14	10.9	12.9	1.62	6.2	14.2	0.033
2	12.3	14.6	3.61	10.6	17.4	0.71	5.8	14.4	0.050
3	9.7	8.9	5.43	7.07	20.5	0.80	7.4	10.8	0.006
4	11.1	10.4	3.46	7.83	10.8	1.11	4.6	17.9	0.063

[a] At least 25 fields or 200 cells were counted.
[b] TT, Turnover time.
[c] Average of three replicates.

TABLE 4. Fraction of planktonic bacteria resistant to lead and copper

Station	Fraction of planktonic bacteria resistant to the following nominal concn (mM) in half-strength nutrient agar of:							
	Pb				Cu			
	0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
1	0.92	0.70	0.62	0.09	0.97	0.80	0.61	<0.01
2	0.57	0.56	0.50	0.39	0.67	0.67	0.49	<0.01
3	1.1	1.0	1.1	0.38	1.1	1.1	0.80	<0.01
4	0.65	0.53	0.36	0.09	0.79	0.93	0.36	<0.01
5	0.73	0.34	0.51	0.15	1.02	0.66	0.47	<0.01
6	0.79	0.48	0.41	0.36	0.94	0.65	0.35	<0.01

greater extent than did 1.0 mM Pb. No trend of increasing heavy metal resistance was noted along the heavy metal gradient; bacterial communities from stations 5 and 6 showed the same percentage of resistance as did those from stations 1 to 4.

Epilithic bacterial communities demonstrated a resistance pattern that was intermediate between that of the planktonic and the sediment communities. The epilithic bacteria were adapted to the lowest nominal concentration (0.001 mM) of both Pb and Cu (Table 6), while concentrations of 0.01 and 0.1 mM resulted in a decline in the tolerance of both metals. As with the other communities, a 1.0 mM concentration of both heavy metals significantly inhibited the bacteria, although a slight increase in resistance to both Cu and Pb was noted in communities collected from the stations exhibiting the highest concentrations of heavy metals in the sediment (stations 3 and 4).

Effect of the pH of the medium on resistance. Since the sediments were alkaline (pH, 8.4 to 9.2) and the pH of the laboratory media was 6.8, we decided to determine whether pH might have an effect on the toxicity of the heavy metals in situ. Accordingly, heavy metal-supplemented nutrient media were prepared as described above with filtered river water (collected at station 6) with a pH of 8.4. At this higher pH, Pb at a concentration of 1.0 mM no longer had any demonstrable inhibitory effects (Table 7): bacteria showed the same level of growth at that concentration as on unsupplemented medium. In the case of Cu, a slight decrease in inhibitory effects was noted: bacteria showed some tolerance of 1.0 mM Cu at pH 8.4. However, a discrepancy still existed between the apparent tolerance of the bacteria of the in situ concentration of Cu and the 90% inhibition of the same bacteria by 1 mM Cu (65 ppm) in laboratory media.

Distribution of bacterial groups. A total of 65 groups of isolates were identified by the abbreviated numerical taxonomy procedure used. The distribution of the most abundant groups at each station is illustrated in Fig. 2, 3, and 4 for planktonic, sediment, and epilithic bacterial communities, respectively. Groups having only one representative per community type were not plotted to save space, but they were included in the calculation of species diversity indices.

Some groups were common to all community types (groups 1 and 2), while others were found associated predominately with the water column (group 4), sediment (groups 5, 6, 8, 9, 10, 14, and 25), or the epilithon (groups 7 and 11). Group 1 consisted of isolates which could utilize individual carbon sources with the exception of the aromatic compounds, did not possess hydrolytic enzymes, and were resistant to Pb and Cd. These characteristics appeared to be evenly distributed and not associated with any particular community. On the other hand, groups associated with the water column showed a reduced ability to utilize individual carbon sources, no hydrolytic activity, and limited resistance to heavy metals (groups 4, 6, and 34). A large group not illustrated in Fig. 1 but associated with the water column consisted of isolates which grew in nutrient broth but failed to grow on any of the characterization media. Sediment bacteria in general demonstrated an ability to utilize glucose, several amino acids, and citric and acetic acids, hydrolytic activity, and resistance to lead as well as the ability to utilize one or more aromatic compounds (groups 5, 6, 8, 9, and 14) or resistance to several heavy metals (groups 10 and 35). Groups associated with the epilithon showed many characteristics in common with the sediment bacteria but lacked the ability to utilize aromatic compounds (groups 7 and 11).

In comparing distributions of groups along the gradient, it was apparent that the planktonic community, as expected, demonstrated little or no gradient effect. On the other hand, the sediment community showed some effect in that some groups were more abundant in the Maumee River stations than in the relatively nonpolluted stations (groups 1, 3, and 4), while one group showed the opposite trend (group 6).

TABLE 5. Fraction of sediment bacteria resistant to lead and copper

Station	Fraction of sediment bacteria resistant to the following nominal concn (mM) in half-strength nutrient agar of:							
	Pb				Cu			
	0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
1	1.11	0.88	0.87	0.27	1.14	0.98	0.69	0.004
2	0.89	0.96	1.04	0.28	1.08	0.82	0.86	0.004
3	0.94	0.95	0.88	0.30	0.90	1.05	0.71	0.011
4	1.17	1.18	1.15	0.46	1.25	1.14	0.97	0.017
5	1.12	1.06	1.09	0.55			1.02	<0.001
6	1.18	1.18	0.90	0.47	1.14	1.09	0.71	<0.001

TABLE 6. Fraction of epilithic bacteria resistant to lead and copper

Station	Fraction of epilithic bacteria resistant to the following nominal concn (mM) in half-strength nutrient agar of:							
	Pb				Cu			
	0.001	0.01	0.1	1.0	0.001	0.01	0.1	1.0
1	1.0	1.13	0.21	0.06	1.00	0.81	0.62	<0.01
2	0.80	0.95	0.74	0.13	1.01	0.96	0.47	<0.01
3	0.77	0.72	0.62	0.12	0.96	0.85	0.60	0.03
4	0.85	0.72	0.12	0.12	0.87	0.82	0.54	0.01
6	0.61	0.61	0.48	0.03	0.85	0.71	0.52	<0.01

TABLE 7. Effect of the pH of river water on the fraction of sediment bacteria resistant to lead and copper

Station	Fraction of sediment bacteria resistant to the following nominal concn (mM) in half-strength nutrient agar[a] of:							
	Pb				Cu			
	0.01	0.1	0.5	1.0	0.01	0.10	0.50	1.0
1	0.90	0.94	1.01	1.00	0.86	0.81	0.20	0.04
2	1.16	1.15	1.14	1.07	1.04	1.12	0.33	0.11
3	1.00	0.98	0.96	1.02	1.01	1.01	0.29	0.05
4	1.09	1.03	1.02	1.03	0.98	0.96	0.40	0.14
6	1.00	1.01	1.04	1.03	0.90	1.10	0.26	0.03

[a] Prepared with filtered river water, pH 8.4.

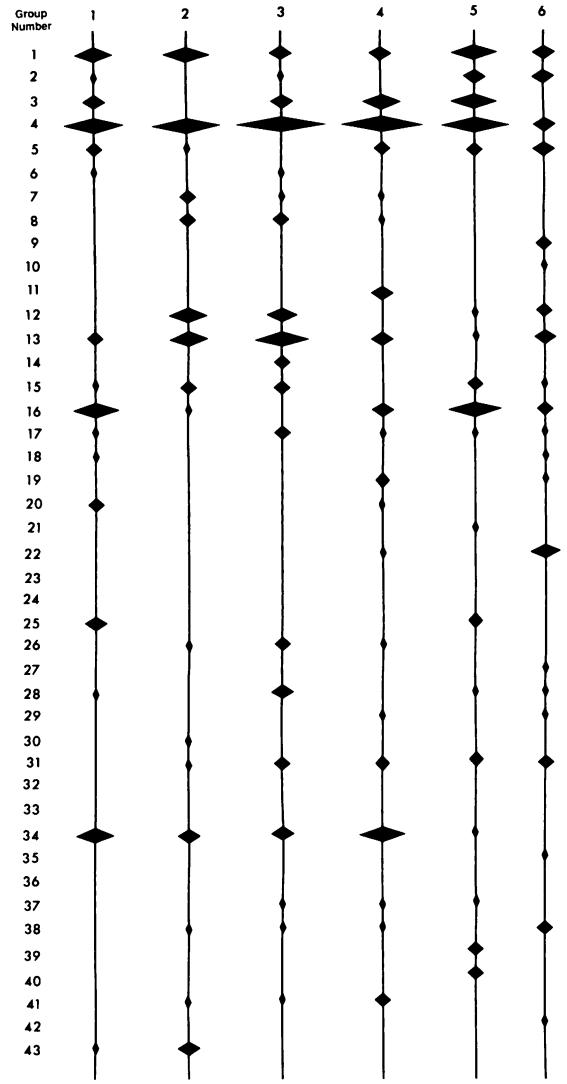

FIG. 2. Distribution of planktonic bacterial groups. Numbers in the first column on the left refer to groups as determined by cluster analysis. Numbers above vertical lines refer to station numbers. The horizontal axis of the diamonds is proportional to the number of isolates clustered in the designated group. No diamond for a group signifies that no isolates at that station clustered in the group.

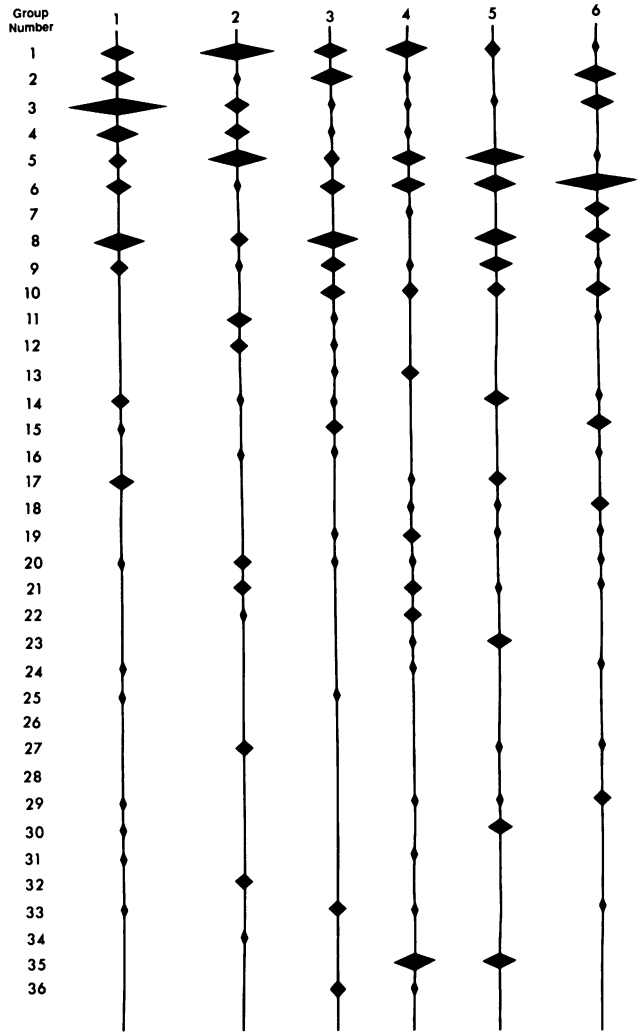

FIG. 3. Distribution of sediment bacterial groups. See the legend to Fig. 2 for details.

As with the sediment bacteria, some gradient-associated trends in group size could be seen in the epilithic bacteria. Again, groups 1 and 4 appeared to be associated with the more heavily polluted stations, while groups 2 and 7 were associated with the St. Joseph River station. The significance of these distributions was more difficult to judge in the case of the epilithon, owing to the loss of samples from station 5. Another factor which made analysis difficult was the low number of isolates in the epilithon datum set. Although equivalent numbers of isolates were selected, many isolates failed to grow after inoculation into nutrient broth. Thus, fewer isolates were available for clustering from the epilithon than from the sediment or planktonic communities.

Species diversity along the gradient. Shannon-Wiener diversity indices varied from 2.54 to 3.04 for planktonic bacteria, 2.66 to 5.40 for sediment bacteria, and 2.08 to 2.58 for epilithic bacteria (Table 8). The indices showed no correlation with the heavy metal gradient for any of the communities. Rarefaction values (all stations from all communities being compared at the same sample size) varied from 9.36 to 11.65 for planktonic bacteria, 10.07 to 14.57 for sediment bacteria, and 8.05 to 10.18 for epilithic bacteria. As with the Shannon-Wiener index, no effect of the heavy metal gradient could on the distribution of the rarefaction values could be noted. Both indices revealed a trend in diversity as related to community type, with the sediment community showing the highest diversity and the epilithic community showing the lowest.

DISCUSSION

It was expected that gradient-related effects on bacterial community structure and function would be present in view of the observed heavy metal concentration gradient along the Maumee River. However, the only observed gradient-related effect was an increase in bacterial activity in all three communities in stations along the Maumee River as compared with stations in the St. Joseph River and St. Mary's River. It is likely that increased nutrient loading from

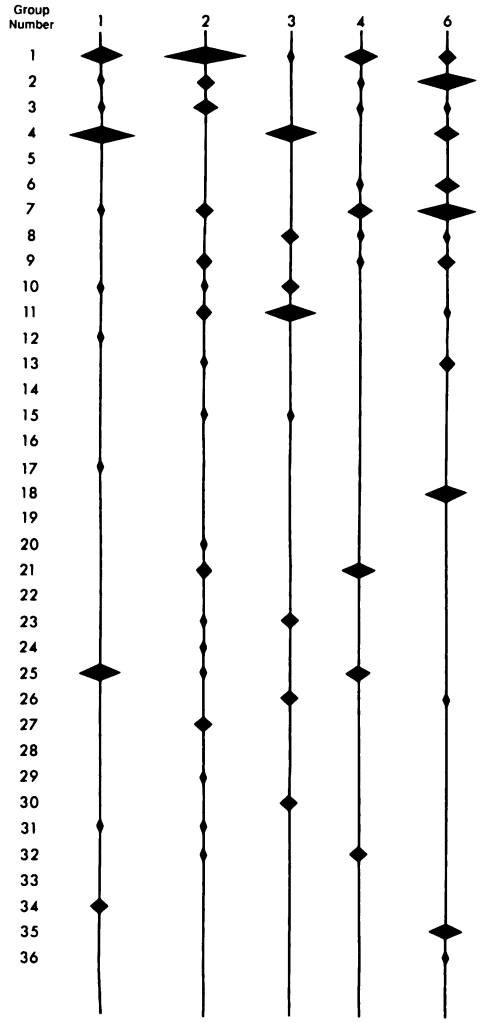

FIG. 4. Distribution of epilithic bacterial groups. See the legend to Fig. 2 for details.

various sources of effluent for the Maumee River contribute to the higher bacterial activity in this river.

Of the three communities studied the planktonic bacterial community demonstrated the lowest adaptation to heavy metals. This result was expected because of the negligible levels of heavy metals detected in the water and because of the transient and fluctuating nature of the contact between bacteria and dissolved heavy metals. With one exception, the epilithic bacterial community showed a similar response. This exception was a slight increase in the proportion of bacteria resistant to Cu at stations 3 and 4, the stations showing the heaviest concentrations of heavy metals in the sediment. Without a more extensive sampling program, it is difficult to determine whether this was a real adaptation to variations in the concentrations of heavy metals in the water or simply a random fluctuation in the bacterial analysis.

Despite increases in extractable heavy metals from 2- to 25-fold, no corresponding increases in resistance to heavy metals were noted in the sediment bacterial community. This result is in marked contrast to the results of other studies of soils and sediments contaminated with heavy metals. Houba and Remacle (13) showed a positive correlation between heavy metal concentrations and the percentage of bacteria resistant to heavy metals in samples collected from three

TABLE 8. Species diversity indices

Community and station	Species diversity index	
	Shannon-Wiener	Rarefaction[a]
Water		
1	2.67	9.97
2	2.54	9.36
3	2.71	10.10
4	2.81	10.46
5	2.61	10.02
6	3.04	11.55
Sediment		
1	2.71	10.07
2	2.66	10.11
3	2.84	10.80
4	5.40	14.57
5	4.66	12.48
6	2.90	10.93
Epilithon		
1	2.22	8.59
2	2.57	10.18
3	2.08	8.05
4	2.31	9.38
6	2.58	9.73

[a] Calculated based on a sample size of 15.

aquatic environments, including the sedimentation pond of a zinc-copper factory. Hornor and Hilt (12) showed a similar positive correlation between the development of zinc resistance and zinc concentrations in polluted stream sediments. Similar correlations have been noted in heavy metal-contaminated soils (6) and in estuarine sediments (18, 26).

In view of the widespread occurrence of correlations between heavy metal concentrations and resistant bacteria, the lack of such a correlation in the present study is noteworthy. A possible explanation is that bacteria are not exposed to the same concentrations of heavy metal in situ as in laboratory-prepared media. As has already been noted above, components of bacterial growth media can complex heavy metals and remove them from solution, thus reducing their apparent concentrations in the media (4, 5). If this were the only factor operating in the present situation, bacteria tested on laboratory media should have been adapted to concentrations of heavy metals in laboratory media that were as high as, if not higher than, concentrations observed in the field. This was not the case in the present situation; bacteria were inhibited in laboratory media by the same concentrations of heavy metals that they encountered with no apparent inhibitory effects in the field.

One factor known to affect the solubility of heavy metals is pH. Hahne and Kroontje (10) investigated the effect of pH on the behavior of heavy metals, including lead, and found that under alkaline conditions lead, like other heavy metal salts, has a lower solubility than under acid conditions. The decrease in the heavy metal availability under alkaline conditions will produce a corresponding decrease in toxicity for microorganisms (8). In the present study, the interaction between pH and heavy metal toxicity was investigated, and it was found that the use of river water rather than distilled water to prepare media altered the resistance pattern of the sediment bacteria. When river water was used in the medium, Pb no longer inhibited the sediment communities along the gradient. Cu was still significantly inhibitory, although the number of resistant bacteria increased an average of 10-fold at the highest concentration of Cu tested.

In addition to the above-noted effect of pH on solubility and hence availability of heavy metals, other factors, such as hardness, alkalinity, ion exchange, complexation to organic and inorganic ligands, and sorption onto hydrous oxides and organic colloids, have also been shown to affect the availability of heavy metals in sediments (21) and the effects of heavy metals on microorganisms (3). All of these factors interact to alter the bioavailability of heavy metals to biota and to obscure the relationship between the apparent toxicity of heavy metals and the total metal concentration. An example of this is given in the work of Hornor and Hilt (12), who found that a concentration of 512 ppm of Zn completely inhibited the development of sediment bacteria on laboratory medium while a concentration of 3435.7 ppm was tolerated in the sediment from which the bacteria were sampled.

We propose that a similar phenomenon occurred in the present study, producing a situation in which the concentration of bioavailable metal in the laboratory medium greatly exceeded the concentration of bioavailable metal in the sediment. Thus, because the concentrations of metals in the sediment did not reach levels that were toxic for bacteria, no resistance to the metals developed in the bacterial community. This result underscores the importance of environmental factors on the toxicity of heavy metals. The concentration of a heavy metal as determined by nitric acid digestion or exhaustive hydrochloric acid extraction is not sufficient to categorize toxicity for biota. The physiochemical properties of the environment must also be characterized with respect to their interactions with heavy metals.

While bacterial abundance and activity were unaffected along the heavy metal gradient, qualitative effects were noted in the distribution of particular groups, particularly in the sediment samples. Groups which were more abundant in the Maumee River sediments (groups 1, 3, and 4) were characterized by a lack of ability to utilize aromatic carbon sources, a lack of ability to utilize glucose and/or glutamate, a lack of hydrolytic activity, and resistance to Pb. The ability to utilize other amino acids, citrate, and acetate was not a determining factor. Group 6, which was more abundant at stations 5 and 6, had a more extensive metabolic capacity, including the ability to utilize benzoate and biphenyl and gel hydrolysis. However, metabolic capacities lacking in groups 1, 3, and 4 were present in other groups which were evenly distributed along the gradient. Therefore, it cannot be concluded that the communities developing in the more heavily polluted stations were suffering any restriction in functional diversity.

Comparisons among the three communities were more productive. Groups that were more abundant in the water column were characterized by a limited capacity to utilize individual carbon sources. For example, members of group 4 could utilize glucose or glutamate but none of the other individual carbon sources tested or any complex carbon sources. Groups 16 and 34 were characterized by the ability to utilize both glucose and glutamate but by a limited ability to utilize other carbon sources. Members of group 4 showed scattered resistance to Pb, while groups 16 and 34 were characterized by a complete lack of resistance to any of the heavy metals tested. In addition, there was a large group of organisms isolated from the water column which grew in nutrient broth but which did not grow on any of the test media. Thus, a picture emerges of a community with complex nutritional requirements.

The sediment community, on the other hand, was composed of bacterial strains with diverse metabolic capabilities. Groups that were relatively abundant in the sediment were characterized by the ability to utilize most if not all of the carbon sources tested, including one or more aromatic compounds (groups 5, 6, 8, 9, and 14), and resistance to one or more heavy metals (groups 10 and 35).

The most striking characteristic of the epilithic community was the lack of ability of strains to be subcultured in laboratory media. More bacterial strains were selected from epilithic plates than from the other plates, yet far fewer of the former strains grew in nutrient broth. Of the strains which could be subcultured and tested, those that were relatively abundant in the epilithic community were able to utilize at least glucose and glutamate (groups 1, 3, and 7) if not several individual carbon sources (group 4 and 11). In addition, groups 7 and 11 were resistant to more than one heavy metal.

The qualitative variations in the distributions of groups along the gradient were not sufficient to alter either of the species diversity indices calculated from the data. While ecologists have noted that a decrease in diversity often accompanies environmental stress, this is by no means a universal observation, particularly where microbial communities are concerned. In a study of the effects of acid mine drainage on bacterial community diversity in water and sediment, Wassel and Mills (27) found a significantly lower diversity at sites affected by acid mine drainage than at control sites. On the other hand, Hood et al. (11) found an increase in bacterial diversity in communities affected by petroleum contamination, apparently because of a nutrient enrichment effect. Martin and Bianchi (17) found that bacterial diversity within a phytoplankton community fluctuated depending on the status of the algal component of the community. Diversity was moderately high under oligotrophic conditions, increasing during periods of exponential growth (blooms) of the phytoplankton and decreasing during periods of phytoplankton mortality. Diversity also differed during the growing season, being higher in the autumn than in the spring. Marine bacterial communities developing in near-shore and offshore locations in the Gulf of Alaska showed uniformly high diversities in winter and summer (14). In the present study, the lack of gradient-related effects on either of the diversity indices is in agreement with bacterial activity measurements which suggest that heavy metals are not exerting deleterious environmental effects under the conditions prevailing in the Fort Wayne river system.

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LITERATURE CITED

1. **American Public Health Association.** 1981. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
2. **Atlas, R. M.** 1984. Use of microbial diversity measurements to assess environmental stress, p. 540–545. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
3. **Babich, H., and G. Stotzky.** 1980. Environmental factors that influence the toxicity of heavy metal and gaseous pollutants to microorganisms. Crit. Rev. Microbiol. **8**:99–145.
4. **Cooney, J. J., and G. W. Pettibone.** 1986. Metals and microbes in toxicity testing. Toxicity Assessment **1**:487–499.

5. **Duxbury, T.** 1986. Microbes and heavy metals: an ecological overview. Microbiol. Sci. **3**:330–333.
6. **Duxbury, T., and B. Bicknell.** 1983. Metal-tolerant bacterial populations from natural and metal-polluted soils. Soil Biol. Biochem. **15**:243–250.
7. **Focht, D. D., and H. Joseph.** 1973. An improved method for the enumeration of denitrifying bacteria. Soil Sci. Soc. Am. Proc. **37**:698–699.
8. **Gadd, G. M., and A. J. Griffiths.** 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. **4**:303–317.
9. **Gocke, K.** 1977. Comparison of methods for determining the turnover times of dissolved organic compounds. Mar. Biol. **42**:131–141.
10. **Hahne, H. C. H., and W. Kroontje.** 1973. Significance of pH and chloride concentration on behavior of heavy metal pollutants: mercury (II), cadmium (II), zinc (II), and lead (II). J. Environ. Qual. **2**:444–450.
11. **Hood, M. A., W. S. Bishop, Jr., F. W. Bishop, S. P. Meyers, and T. Whelan III.** 1975. Microbial indicators of oil-rich salt marsh sediments. Appl. Microbiol. **30**:982–987.
12. **Hornor, S. G., and B. A. Hilt.** 1985. Distribution of zinc-tolerant bacteria in stream sediments. Hydrobiologia **128**:155–160.
13. **Houba, C., and J. Remacle.** 1980. Composition of the saprophytic bacterial communities in freshwater systems contaminated by heavy metals. Microb. Ecol. **6**:55–69.
14. **Kaneko, T., R. M. Atlas, and M. Krichevsky.** 1977. Diversity of bacterial populations in the Beaufort Sea. Nature (London) **270**:596–599.
15. **Klerks, P. L., and J. S. Weiss.** 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut. **45**: 173–205.
16. **Lewis, D. A., H. P. Kollig, and T. L. Hall.** 1983. Predicting 2,4-dichlorophenoxyacetic acid ester transformation rates in periphyton-dominated ecosystems. Appl. Environ. Microbiol. **46**:146–151.
17. **Martin, Y. P., and M. A. Bianchi.** 1980. Structure, diversity, and catabolic potentialities of aerobic heterotrophic bacterial populations associated with continuous cultures of natural marine phytoplankton. Microb. Ecol. **5**:265–279.
18. **Mills, A. L., and R. R. Colwell.** 1977. Microbiological effects of metal ions in Chesapeake Bay water and sediment. Bull. Environ. Contam. Toxicol. **18**:99–103.
19. **Mills, A. L., and L. M. Mallory.** 1987. The community structure of sessile bacteria stressed by acid mine drainage. Microb. Ecol. **14**:219–232.
20. **Mills, A. L., and R. A. Wassel.** 1980. Aspects of diversity measurement for microbial communities. Appl. Environ. Microbiol. **40**:578–586.
21. **O'Donnel, J. R., B. M. Kaplan, and H. A. Allen.** 1985. Bioavailability of trace metals in natural waters, p. 485–501. *In* R. D. Caldwell, R. Purdy, and R. C. Bahner (ed.), Aquatic toxicology and hazard assessment: seventh symposium, ASTM STP 854. American Society for Testing and Materials, Philadelphia, Pa.
22. **Scheraga, M., M. Meskill, and C. D Litchfield.** 1979. Analysis of methods for the quantitative recovery of bacteria sorbed onto marine sediments, p. 21–39. *In* C. D. Litchfield and P. L. Seyfried (ed.), Methodology for biomass determinations and microbial activities in sediments. American Society for Testing and Materials, Philadelphia, Pa.
23. **Simberloff, D.** 1978. Use of rarefaction and related methods in ecology, p. 150–165. *In* K. L. Dickson, J. Cairns, Jr., and R. J. Livingston (ed.), Biological data in water pollution assessment: quantitative and statistical analyses, ASTM STP 652. American Society for Testing and Materials, Philadelphia, Pa.
24. **Skerman, V. B. D.** 1967. A guide to the identification of the genera of bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.
25. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco.
26. **Timoney, J. F., J. Port, J. Giles, and J. Spanier.** 1978. Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York Bight. Appl. Environ. Microbiol. **36**:465–472.
27. **Wassel, R. A., and A. L. Mills.** 1983. Changes in water and sediment bacterial community structure in a lake receiving acid mine drainage. Microb. Ecol. **9**:155–169.