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Biochemical Characterization of Estuarine Benthic Microbial Communities for Use in Assessing Pollution Impacts

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ABSTRACT: Monitoring the benthic microbial community offers a means of assessing biological changes in response to pollutants at the base of the estuarine food web. Traditional methods of microbial community analysis are inadequate because they require removal of the microorganisms from their habitat for culture on laboratory media, resulting in bias. Biochemical techniques, however, allow the microbial community structure to be analyzed without removing the microorganisms from their habitat. We have used analyses of phospholipid fatty acids (PLFA) to characterize benthic microbial community structure in Biscayne and Pensacola Bays, FL, and to relate changes in microbial community structure to sources of metal pollution. Sediment samples were obtained from clean and contaminated areas of each bay system. PLFA were analyzed by capillary gas chromatography after modified Bligh-Dyer extraction and silicic acid column chromatography. Principal components analysis was used to distinguish geographic areas, and stations within these areas, from one another based on either geochemical or microbial PLFA data. Canonical correlation was used to construct a linear relationship between metal concentrations and microbial PLFA characteristics, but was confounded by sediment grain size. Polluted stations were generally characterized by high metal concentrations, fine-grain sediments, high lipid phosphate, high trans/cis fatty acid ratios, high bacterial PLFA, and low eucaryotic PLFA.

KEY WORDS: fatty acids, microbial lipids, metals, principal components analysis, canonical correlation, estuaries, sedimentary community structure

With increasing urbanization of Florida's coastline, estuarine pollution has become a serious problem in several areas of the state. As the state's population continues to increase, the potential for severe perturbations to estuarine biological communities also increases. Studies by the Florida Department of Environmental Regulation have shown that contamination of estuarine sediments by metals is already a problem in some areas [*1*]. Regulatory agencies need reliable methods for assessing the impacts of pollutants and for developing regulatory criteria to deal with pollution problems. We have investigated the use of recently developed techniques in biochemical ecology to assess the effects of estuarine pollution on benthic microbial communities.

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Benthic microbiota are an important component of the estuarine food web whose roles include recycling organic and inorganic nutrients and serving as a food source for higher organisms either directly or via the detrital food chain. On average, 50% of total ecosystem production enters the detrital food chain and 50% of this detritus is converted to bacterial biomass [*2*]. Since sediments are a repository for pollutants [*1*], pollution-induced changes in the sediment microbiota might offer an "early warning" of pollutant damage before any effects on macrobiota are noticeable.

Results of laboratory studies on the effects of metals on microorganisms are ambiguous, as illustrated in a recent review by Duxbury [*3*]. Depending on the experimental conditions, metals appear to have deleterious effects on microorganisms in some cases, and in other cases, little or no effect. Field studies indicate that microbial diversity decreases in response to metal contamination [*4*]. With increasing metal concentrations, the percentage of metal-resistant bacteria in some aquatic microbial communities increases [*5*]. In assessing the toxicity of metals using microcosms of benthic microbiota, Barnhart and Vestal [*6*] found the relative order of toxicity to be the same as determined by bioassays with higher organisms, but greater concentrations of metals were required to produce a toxic effect on microorganisms. They believe that the latter result occurred because in the microcosms, which were intended to mimic natural conditions, environmental factors such as chelation and adsorption to particles mitigated the effects of metals.

The studies cited in the previous paragraph were performed using classical microbiological methods, that is, isolation and identification of microorganisms or measurement of microbial activity in field samples or in laboratory microcosms. A problem with the classical methods, however, is that only a small portion of the microbial community is viable in any given culture medium [*7*]. Similarly, the creation of a microcosm creates a perturbation of the natural community structure [*8*]. Therefore, one can never be certain that the tests are truly representative of the in situ microbial community.

Analysis of lipids from benthic microbial communities can provide information about community structure and biomass without the biases intrinsic in the classical methods. Lipids can be extracted directly from sediment samples without removing the microorganisms from their substrate; components of the lipid extract are, therefore, representative of the entire microbial community. In particular, analysis of the ester-linked fatty acids of phospholipids (PLFA) has proven itself as a sensitive and reproducible means of studying microbial community structure [*8,9*]. The relative proportions of different PLFAs can be used to determine qualitative differences among microbial communities. Reproducible shifts in PLFA composition occur with experimental manipulations of microbial communities [*8–11*]. Analyses of PLFA have been used to study aquifer microbiota [*12*], microbial colonization of sand grains [*13*], and biofouling communities [*14*]. Inferences about microbial community structure and detrital inputs to marine sediments have been made based on the composition of extractable fatty acids [*15,16*].

This study tests the feasibility of using biochemical techniques to assess changes in benthic microbial community structure in response to sediment characteristics and metal concentrations. It is the first large-scale application in the field of biochemical techniques since the previously mentioned works were performed in the laboratory or under carefully controlled field conditions. The large amount of data generated by the biochemical analysis mandated the use of multivariate statistical procedures to reveal relationships between PLFA composition and sediment characteristics.

Methodology

Study Areas

Two distinctly different estuarine areas, Biscayne Bay and Pensacola Bay, were chosen for this work. Biscayne Bay is a carbonate-rich, subtropical system in southeastern Florida. Pensa-

cola Bay is a temperate system in northwestern Florida containing terrigenous aluminosilicate sediments. These two bays are representative of two major sediment types found in Florida, and each has known metal contamination. Sediments and the associated microbiota were collected from two areas in each bay.

In Biscayne Bay (Fig. 1), collections were made in the vicinity of the Miami River (eleven stations; MR5 to MR15) and Snapper Creek (ten stations; SC1 to SC10). Miami River sediments contain relatively large metal enrichments, with metal concentrations decreasing with distance into the bay [*1*]. Snapper Creek drains a smaller, less urbanized area than the Miami River and has lower metal concentrations.[4] In Pensacola Bay (Fig. 2), samples were collected from Bayou Grande (ten stations; BG1 to BG10) and Bayou Chico (seven stations; BC1 to BC7). Because of the presence of industrial facilities along Bayou Chico, its sediments contain greater metal concentrations than those in Bayou Grande.[4] In both bay areas, stations were located roughly in a transect extending outward from the bayous and mouths of the rivers into the central portion of the bay. The station design was arranged to encounter a gradient of pollutant concentrations.

Limited information on organic contaminants[4] suggests that sediments in the Miami River, Bayou Chico and, to a lesser degree, Bayou Grande have elevated levels of polynuclear aromatic hydrocarbons (PAH) and polychlorinated biphenols (PCB) in addition to metals. However, PAH and PCB concentrations were low, generally below 1.0 mg/kg^{-1}, except at Bayou Chico Station 4, which had high PAH. Chlorinated hydrocarbon pesticides and phenolic compounds were very low at all sites in both bays. Because of the low concentrations of organics present, we examined only the relationships between sediment microbes and selected, potentially toxic metals.

Sampling Procedures

Sample Collection—The Biscayne Bay sites were sampled 2 to 3 July, 1985 and the Pensacola Bay sites sampled 21 to 23 July, 1985. Sediment samples were collected by SCUBA divers using 3-cm diameter cores to ensure the retrieval of undisturbed sediment. Care was taken to keep the layer of flocculent material at the sediment-water interface intact. Five replicate cores for microbial biochemistry were randomly taken at each station from within a .75 × 1.5 m rectangular grid to get an adequate representation of the microbial community [*10*]. Two additional cores were taken within the grid, one for sediment chemistry analysis and one for sediment grain size.

After retrieval, the top 2 cm of the microbial cores were extruded and washed through a 500-μm mesh sieve into a 250-ml centrifuge bottle with 2.5% saline. Large organisms and detrital material were retained by the sieve and thus, by definition, the sediment microbial community consisted of all the organisms passing through a 500-μm mesh. The samples were immediately preserved with 10% formalin [*17*]. Procedural blanks were prepared in the field by filling centrifuge bottles with saline and, during subsequent storage and analysis, treating them exactly as a sediment sample. Cores for sediment grain size and chemistry were extruded into glass bottles and stored on ice for transport to the laboratory.

Biochemical and Chemical Analyses

Lipid Analysis—After the removal of formalin [*17*], lipids were extracted from sediments by a modified Bligh-Dyer procedure according to White et al. [*18*]. An aliquot of the lipid extract was reserved for lipid phosphate analysis [*18*] and the remainder separated into neutral, glyco-, and phospholipid fractions by silicic acid column chromatography [*19*]. The phospholipid fatty

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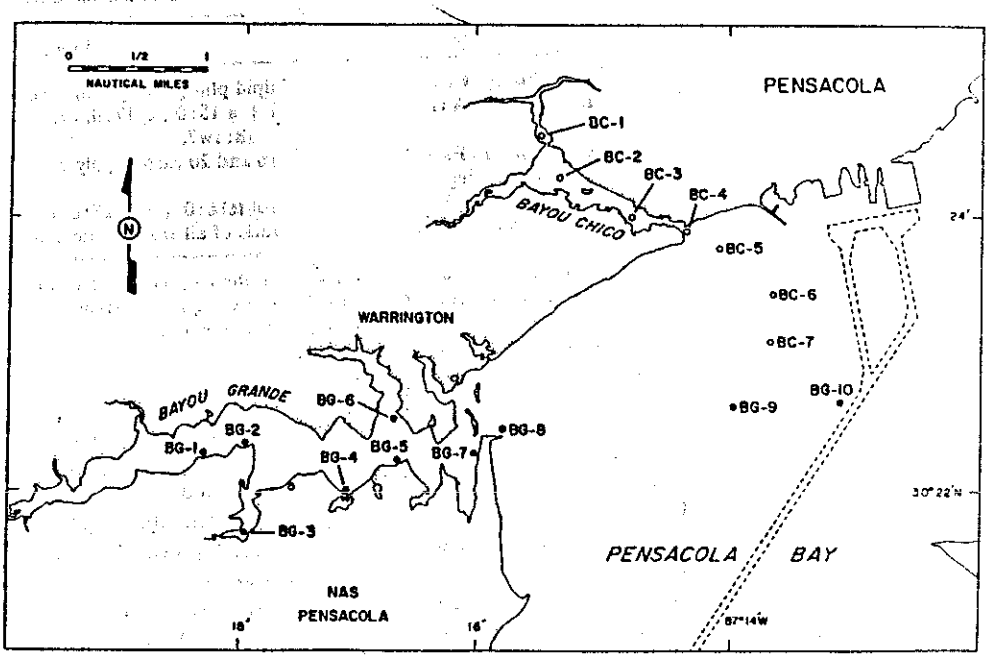

FIG. 2—*Station locations in Pensacola Bay, FL.*

was subtracted from the raw data. Each FAME was then expressed as its percentage of the total identified FAME. FAME nomenclature is as described by Guckert et al. [*8*].

Sediment Chemistry—Sediment metal concentrations were determined according to Ryan et al. [*22*]. The seven metals analyzed were cadmium, chromium, copper, lead, zinc, mercury, and arsenic. Sediment grain size was determined by sieving and pipette analysis according to Folk [*23*].

Statistical Analysis

Data Reduction—The biochemical data were arranged into five groups, which were then used in the statistical analyses. The five groups, described in Table 1, were: lipid phosphate (LPO), bacterial marker PLFA (BAC), eucaryotic marker PLFA (EUC), sulfate-reducing bacteria marker PLFA (SRB), and ratio of *trans-* to *cis*-monoenoic PLFA (T/C). Lipid phosphate is a measure of microbial biomass which correlates well with other measures of biomass such as extractable ATP [*18*]. The marker PLFA groups were selected to represent different subsets of the microbial community according to lipid compositions reported in the literature [*9,10, 16,24–28*]. The ratio of *trans-* to *cis*-monoenoic PLFA has been suggested as an indicator of microbial "stress" by Guckert et al. [*27*] because the ratio increased in starving bacterial cells and in experimental microcosms of estuarine microbiota that were manipulated to produce anaerobic conditions.

Statistical Analyses—Statistical analyses were performed using the Minitab and BMDP statistical programs with the Florida State University CYBER 760 computer. Plots of normal scores versus residuals for the metals and biochemical variables indicated that the data were not normally distributed. After a log transformation, similar plots showed the data to approximate a normal distribution. All data were log transformed before any statistical analysis. Mean values from the replicate cores were used for all statistical procedures.

TABLE 1—*Grouped biochemical data.*

Group	Components
Lipid Phosphate (LPO)	lipid phosphate g^{-1} dry sediment
Bacterial PLFA (BAC)	i + a 15:0[a], cy17:0, cy19:0, i + a 17:0, 18:1w7c
Eucaryotic PLFA (EUC)	18 and 20 carbon polyenoic PLFA
Sulfate-reducing bacteria PLFA (SRB)	10Me16:0, i + a 17:0, cy17:0
Trans/cis ratio (T/C)	ratio of all *trans*-monoenoic to *cis*-monoenoic PLFA

[a]Fatty acid nomenclature as described by Guckert et al. Ref *8*. Number of carbons : number of double bonds. Prefixes designate iso (i) or anteiso (a) branching or cyclopropyl (cy) structure. Distance (X) of double bond from methyl end (w) and cis or trans bonding is given by wXc or wXt.

The two multivariate statistical techniques used to analyze the data were principal components analysis and canonical correlation. Principal components analysis is a data reduction technique used to construct weighted linear combinations of variables (principal components) that account for as much of the original total variability as possible. Each principal component (PC) is uncorrelated with all of the other PCs [*29*]. Principal components analysis can be used as an exploratory tool that allows one to summarize a data set by reducing a large number of variables into a smaller set of PCs, to interpret the PC according to their component weighted variables, and to look at correlations among variables by clustering the variables into PC [*30*]. All variables are scaled to a mean of zero and unit standard deviation. Factor loadings, coefficients expressing the relative importance of each variable to each PC, are then calculated. Finally, a factor score is computed for each PC at each station, where the factor score is equal to the summation of the standardized value of each variable multiplied by its factor loading. Factor scores thus express the degree to which each station possesses the quality described by that factor [*30*]. Factor scores can be plotted to graphically illustrate similarities and differences among stations.

Canonical correlation is a multivariate procedure that can be used to analyze linear relationships between two groups of variables. The two groups of variables are called the predictor set and the criterion set. The canonical correlation procedure constructs maximally correlated canonical variate pairs, each consisting of a weighted linear combination of the predictor variables (CVI) and a weighted linear combination of the criterion variables (CVII). Each succeeding canonical variate pair is uncorrelated with its predecessor and with succeeding pairs, and each pair has a successively smaller correlation coefficient. Canonical variable loadings and canonical variate scores are analogous to PC factor loadings and factor scores. Canonical correlation can be used to predict information about one set of variables from information contained in a second set [*29,30*].

Results and Discussion

Mean values and ranges of the seven metals and sediment grain sizes at each of the four sites are presented in Table 2. In Biscayne Bay, metal concentrations were greater at the Miami River stations than at the Snapper Creek stations. The highest metal concentrations occurred at Miami River Stations 5, 6, and 7, upstream in and at the mouth of the Miami River; metal concentrations were generally an order of magnitude lower seaward of Station 7. At Snapper Creek stations, cadmium, copper, lead, and zinc decreased with distance from shore. In contrast, chromium values were highest at the outermost Stations 9 and 10. Stations 1, 2, and 3 at the Bayou Chico site in Pensacola Bay contained the highest concentrations of chromium, lead and zinc of any stations in this study. With the exception of these three stations, metal concen-

TABLE 2—*Mean values (and ranges) of sediment metal concentrations and grain size in Pensacola and Biscayne Bays, FL.*

Site	N	Grain Size (mm)	Metal Concentrations (mg kg^{-1})						
			Cadmium	Chromium	Copper	Lead	Zinc	Mercury	Arsenic
Biscayne Bay									
Miami River	11	0.07 (0.02 – 0.14)	0.90 (0.09 – 3.9)	40.3 (8.8 – 110)	65.0 (2.3 – 380)	80.5 (1.8 – 260)	116 (10.0 – 410)	1.14 (0.09 – 3.7)	3.0 (0.80 – 6.9)
Snapper Creek	10	0.15 (0.05 – 0.20)	0.10 (0.04 – 0.19)	7.7 (2.6 – 12.0)	2.2 (0.6 – 5.7)	3.3 (1.3 – 9.4)	7.7 (3.5 – 15.0)	0.03 (0.02 – 0.05)	2.2 (0.08 – 3.2)
Pensacola Bay									
Bayou Chico	7	0.06 (0.02 – 0.19)	1.1 (0.09 – 3.3)	374 (4.6 – 2000)	45.5 (4.6 – 110)	85.9 (5.0 – 340)	629 (18.0 – 1300)	0.43 (0.04 – 0.90)	4.9 (0.80 – 15.0)
Bayou Grande	10	0.14 (0.02 – 0.27)	1.3 (0.02 – 6.7)	80.8 (1.7 – 250)	12.0 (0.5 – 42.0)	32.0 (0.6 – 120)	78.4 (1.0 – 220)	0.16 (0.01 – 0.38)	8.5 (0.2 – 26.0)

trations were similar in Bayou Chico and Bayou Grande. Metal concentrations tended to decrease with distance from the upper end of Bayou Chico; no such trend was evident at Bayou Grande.

Although the range of sediment grain sizes overlapped at the Miami River and Snapper Creek sites, mean grain size was larger at Snapper Creek. Similar results were obtained at Bayou Chico and Bayou Grande, with Bayou Grande having a larger mean grain size.

Mean values and ranges of the biochemical parameters at each of the four sites are presented in Table 3. Lipid phosphate, indicative of microbial biomass, was greatest at the Bayou Chico stations, as was the ratio of *trans*- to *cis*-monoenoic fatty acids. The Snapper Creek stations had the lowest mean lipid phosphate, trans/cis ratio, and percentage of sulfate-reducer fatty acids, and the largest percentage of eucaryotic fatty acids. The highest mean percentage of sulfate-reducer fatty acids was found at the Miami River stations.

Principal Components Analysis of Metals—The metals data set consisted of one value per station for each of seven metals and sediment grain sizes. All of the metals were highly correlated with each other and all were inversely correlated with sediment grain size (Table 4).

Eight PCs were extracted from this data set, with PC1 and PC2 accounting for 82 and 7%, respectively, of the total variance. Factor loadings for each of the eight variables are given in Table 5. All of the metals, with the exception of arsenic, had relatively large loadings on PC1; arsenic had the largest loading on PC2. Sediment grain size had a negative loading on both PC1 and PC2. These results indicate that the metals, with the exception of arsenic, tended to increase or decrease in concert and that all were more concentrated in fine-grained sediments.

TABLE 3—*Mean values (and ranges) of the grouped biochemical variables[a] in Pensacola and Biscayne Bays, FL.*

Site	N	LPO (μmole g^{-1})	BAC (%)	EUC (%)	SRB (%)	T/C
Biscayne Bay						
Miami River	11	0.16 (0.02 − 0.36)	30.5 (26.1 − 35.5)	9.0 (6.2 − 15.4)	9.9 (5.7 − 14.1)	0.039 (0.014 − 0.082)
Snapper Creek	10	0.05 (0.01 − 0.21)	28.3 (23.1 − 39.2)	12.1 (6.4 − 22.4)	6.9 (2.9 − 9.7)	0.033 (0.024 − 0.048)
Pensacola Bay						
Bayou Chico	7	0.55 (0.10 − 1.2)	30.5 (23.9 − 41.4)	9.1 (3.1 − 20.5)	7.7 (5.0 − 10.8)	0.064 (0.032 − 0.086)
Bayou Grande	10	0.24 (0.02 − 0.90)	27.5 (19.1 − 38.0)	8.1 (3.1 − 18.6)	9.7 (5.0 − 15.1)	0.052 (0.022 − 0.118)

[a]Descriptions of the biochemical variables are in Table 1.

TABLE 4—*Pearson correlation coefficients[a] for metals and sediment grain size (n = 38).*

	Cadmium	Chromium	Copper	Lead	Zinc	Mercury	Arsenic	Grain size
Cadmium	1.00	...	...	...	...	...	...	...
Chromium	0.84	1.00	...	...	...	...	...	...
Copper	0.81	0.72	1.00	...	...	...	...	...
Lead	0.90	0.84	0.82	1.00	...	...	...	...
Zinc	0.90	0.90	0.83	0.91	1.00	...	...	...
Mercury	0.82	0.68	0.77	0.85	0.79	1.00	...	...
Arsenic	0.73	0.75	0.62	0.65	0.71	0.54	1.00	...
Grain size	−0.78	−0.78	−0.79	−0.82	−0.83	−0.74	−0.77	1.00

[a]All are significant at $P < 0.05$.

TABLE 5—*Factor loadings for metals and sediment grain size on PC1 and PC2.*

Variable	Factor Loading PC1	PC2
Cadmium	0.77	0.55
Chromium	0.60	0.70
Copper	0.80	0.41
Lead	0.84	0.46
Zinc	0.76	0.59
Mercury	0.91	0.25
Arsenic	0.28	0.93
Grain size	−0.62	−0.67

Factor scores for each station are plotted in Fig. 3. Groupings of the data according to site are readily apparent. The Snapper Creek stations are located in a cluster to the left of center. Within the cluster, Stations 1, 2, 3, and 4, with relatively larger metal concentrations, have the least negative values on PC1. Station 1 stands out from the rest by its larger negative value on PC2, which was due to its relatively low concentration of arsenic. Miami River stations are near and to the right of center and are distinguished from each other primarily by their position along the PC1 axis. The Miami River stations with the lowest metal concentrations and largest sediment grain sizes, Stations 9, 10, 14, and 15, are located closest to the Snapper Creek cluster. Station 5, located upstream in the Miami River, lies farthest to the right along the PC1 axis

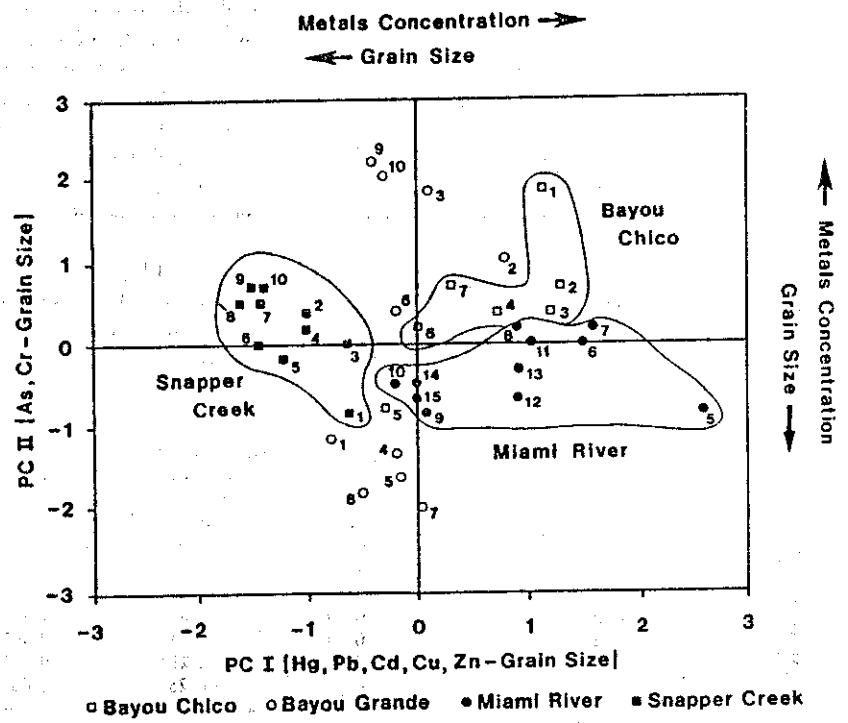

FIG. 3—*Factor scores from principal components analysis of metals and sediment grain size.*

because it had the greatest metal concentrations. The rest of the Miami River stations, with intermediate metal concentrations, are located between the two extremes. Bayou Chico stations are generally located in the upper right quadrant and are separated along both PC axes. Station 1 had the highest value on PC2 because of its relatively large arsenic and chromium concentrations. Station 5 was separated by its larger grain size and lower metal concentrations. The Bayou Grande stations are located in two clusters along the PC2 axis, indicating that arsenic, chromium, or sediment grain size accounted for most of the differences among stations at this site.

Principal Components Analysis of Biochemistry Data—Variables used in the analysis were the five grouped biochemical variables and sediment grain size. Sediment grain size was included in the analysis because a preliminary examination of the data indicated that both biochemical and metal data might be considerably influenced by sediment particle size. Pearson product-moment correlation coefficients for the biochemical variables and grain size are shown in Table 6. The greatest correlation was an inverse relationship between lipid phosphate and sediment grain size ($r = -0.72$). In turn, lipid phosphate was directly correlated with bacterial PLFA, suggesting that the overall increase in biomass in the fine-grained sediments was due to an increased bacterial contribution. An inverse relationship between bacterial biomass and sediment grain size has been reported for other marine systems [*30–32*]. The trans/cis ratio correlated directly with lipid phosphate, bacterial PLFA, and sulfate-reducer PLFA, and inversely with eucaryotic PLFA. The positive correlation between the trans/cis ratio and bacterial PLFA in our samples may indicate that although bacterial biomass increases in the fine-grained sediments, many of the bacteria are stressed or dormant [*27*], perhaps as a result of competition or localized nutrient limitations at some of the stations. Further work is needed to elucidate the meaning of the trans/cis ratio in estuarine sediments.

Although six principal components were extracted from the data set, PC1 and PC2 accounted for 43 and 20%, respectively, of the total variability. Factor loadings for PC1 and PC2 are given in Table 7. PC1 was most heavily influenced by lipid phosphate, with an equally large inverse weighting by sediment grain size, indicative of the overall relationship between microbial biomass and sediment grain size. Eucaryotic PLFAs had a large positive loading on PC2. Sulfate-reducing bacteria and trans/cis ratio had the largest negative loadings. The loading of eucaryotes and sulfate-reducing bacteria at the opposite ends of the PC2 axis may occur because the sulfate reducers are anaerobic bacteria. If one assumes that many of the microeucaryotes are aerobic organisms, then the proportion of eucaryotes should decrease in anaerobic mud favored by sulfate-reducing bacteria.

Factor scores for each station are plotted in Fig. 4. Some grouping of the stations by site is evident. Most of the Snapper Creek stations were located in the upper left quadrant in a region characterized by a large proportion of eucaryotic microorganisms and large grain sizes. Stations 1 and 10 were separated from the main group of Snapper Creek stations by their position along

TABLE 6—*Pearson product-moment correlation coefficients for biochemical variables and sediment grain size (n = 38).*

Variables	LPO	BAC	EUC	SRB	T/C
LPO	1.00				
BAC	0.37[b]	1.00			
EUC	−0.20	0.01	1.00		
SRB	0.26	0.43[b]	−0.38[b]	1.00	
T/C	0.50[b]	0.29[b]	−0.36[b]	0.33[b]	1.00
Grain size	−0.72[b]	−0.25	0.03	−0.21	−0.31[b]

[a]Biochemical variables as defined in Table 1.
[b]Significant at $P < 0.05$.

TABLE 7—*Factor loadings for biochemical variables[a] on PC1 and PC2.*

Variable	Factor Loading PC1	PC2
LPO	0.88	−0.18
BAC	0.56	−0.24
EUC	0.08	0.85
SRB	0.27	−0.72
T/C	0.48	−0.57
Grain Size	−0.87	−0.05

[a]Biochemical variables as defined in Table 1.

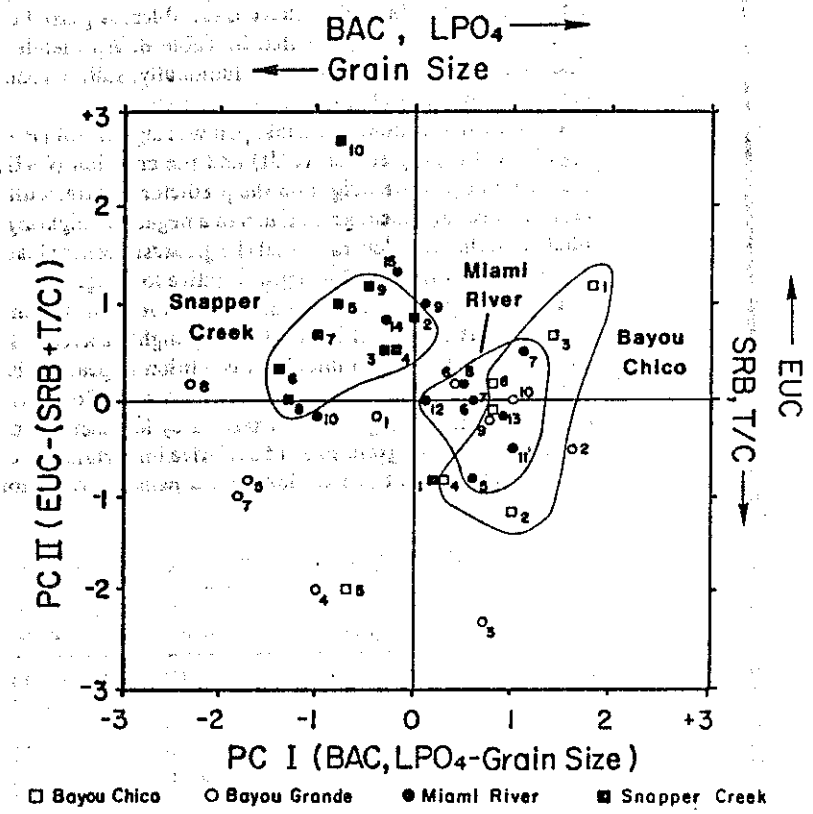

FIG. 4—*Factor scores from principal components analysis of biochemical variables and sediment grain size.*

the PC2 axis. Differences at these two stations were related primarily to their biochemical characterisitics, with sediment grain size having little influence on PC2 (Table 7). Station 1 was distinguished by its high lipid phosphate content and large proportion of bacterial and sulfate-reducer fatty acids. Station 10 was separated from the other stations by its high eucaryotic PLFA and low sulfate-reducer PLFA concentrations. Miami River stations were located in two distinct clusters. Stations in and near the mouth of the Miami River occupied a cluster in the right center of the plot, indicating slightly higher lipid phosphate values with moderate levels of eucaryotic and sulfate-reducer PLFAs. The remainder of the Miami River stations (Stations 9, 10, 14, and 15) had lower lipid phosphate and higher eucaryotic PLFA values than the other Miami River stations, and were more closely aligned with Snapper Creek stations. These were the four distinctly different Miami River stations indicated by the principal components analysis of the metals data (Fig. 3) as geochemically similar to the Snapper Creek stations. Bayou Chico stations were located primarily in the right half of the figure. These stations, containing some of the highest metal concentrations measured during this study, were similar to the inner, metal-contaminated group of Miami River stations with respect to factor scores on PC1, but exhibited a wider spread along the PC2 axis because of their range of values for eucaryotic and sulfate-reducer PLFAs. The Bayou Grande stations were spread throughout the lower half of the plot, separated along both PC1 and PC2.

Canonical Correlation of Biochemistry and Metal Data—Canonical correlation was performed using the metal and sediment grain-size data as the predictor variables and the grouped microbial lipid data as the criterion variables. A partial correlation matrix from the canonical correlation analysis is presented in Table 8. All metals were positively correlated with lipid phosphate and bacterial PLFA. Additionally, sulfate-reducing bacterial PLFAs and the trans/cis ratio were correlated with several metals.

Only the first canonical variate pair was significant ($P < 0.05$). The canonical variable loadings for both the predictor (CVI) and the criterion (CVII) variates are shown in Table 9. All metals had a positive weight on the predictor variate, with cadmium and zinc having the greatest influence. Sediment grain size had a negative weighting on the predictor variate. Lipid phosphate and the trans/cis ratio had the greatest positive loading on the criterion variate, whereas eucaryotic PLFA exerted a slight negative loading.

The value of the canonical variate pair for each station is plotted in Fig. 5. If the canonical correlation is successful in constructing highly correlated variate pairs, then the variate pairs will lie along a diagonal line. This condition appears to be true for this data set.

Some clustering of stations is evident. Snapper Creek stations are generally found in the lower left quadrant, in a region characterized by low metal concentrations, high eucaryotic PLFAs, and large sediment grain size. The relative importance of eucaryotic PLFAs is evident when you consider that all but one station have a negative value for the criterion variate. Miami River

TABLE 8—*Pearson correlation coefficients for biochemical variables,[a] metals, and sediment grain size ($n = 38$).*

	LPO	BAC	EUC	SRB	T/C
Cadmium	0.87[b]	0.46[b]	−0.14	0.35[b]	0.56[b]
Chromium	0.81[b]	0.35[b]	−0.04	0.23	0.55[b]
Copper	0.79[b]	0.48[b]	−0.17	0.37[b]	0.46[b]
Lead	0.85[b]	0.49[b]	−0.22	0.39[b]	0.55[b]
Zinc	0.90[b]	0.42[b]	−0.13	0.31[b]	0.58[b]
Mercury	0.71[b]	0.33[b]	−0.14	0.28	0.26
Arsenic	0.63[b]	0.33[b]	0.13	0.15	0.26
Grain size	−0.77[b]	−0.32[b]	0.08	−0.28	−0.34

[a]Biochemical variables as defined in Table 1.
[b]Significant at $P < 0.05$.

TABLE 9—*Canonical variable loadings.*

Predictor Variate		Criterion Variate	
Variable	Loading	Variable	Loading
Cadmium	0.93	LPO	0.97
Chromium	0.89	BAC	0.49
Copper	0.82	EUC	−0.18
Lead	0.89	SRB	0.37
Zinc	0.97	T/C	0.72
Mercury	0.70		
Arsenic	0.67		
Grain size	−0.78		

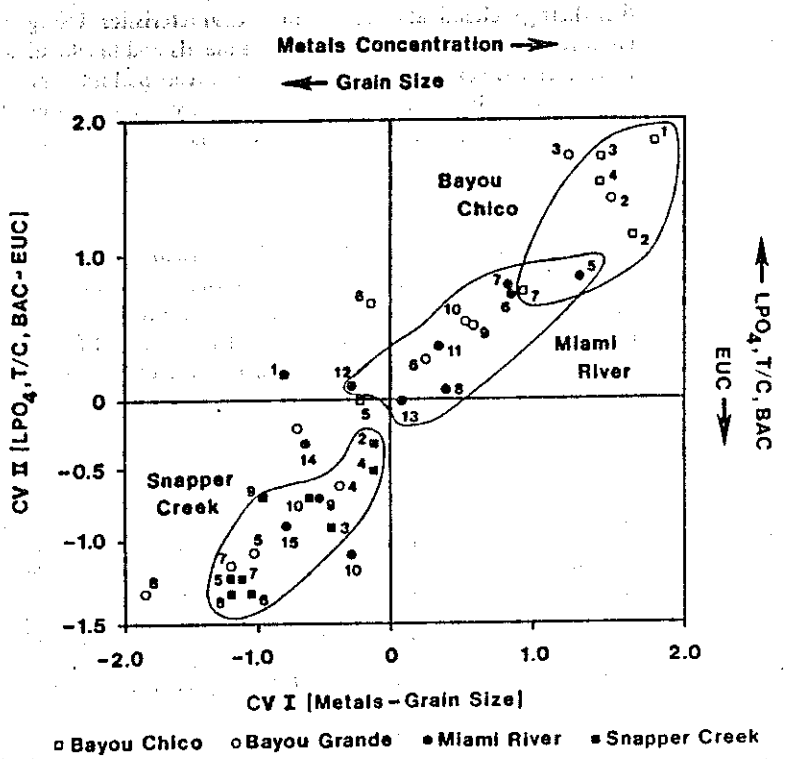

FIG. 5—*Canonical variate scores using metals and sediment grain size as the predictor variables and biochemical variables as the criterion variables.*

stations are near the center of the plot, with those having greater metal concentrations, finer-grained sediments, greater lipid phosphate values, and higher trans/cis ratios the farthest from the group of Snapper Creek stations. The Miami River stations with the lowest metal concentrations (Stations 9, 10, 14, and 15) closely resembled the group of Snapper Creek stations. Bayou Chico stations were primarily located in the far upper right quadrant because of their high metal content and high values for lipid phosphate and trans/cis ratio. Bayou Grande stations were located along the entire range of the criterion and predictor variate axes because of their large variation in both sediment and biochemical characteristics.

Conclusions

Refinements of these procedures are, of course, necessary before practical applications are possible. Both microbial community structure and metal concentrations are significantly affected by sediment grain size, as indicated by the large weighting factors for grain size in the principal components analysis. This relationship makes it difficult to attribute changes in the microbial community solely to the effects of metals. However, there are steps that can be taken to improve the interpretation of the data in subsequent work. Principal components analysis of individual PLFAs can be used to suggest grouping patterns that will provide better discrimination among stations, rather than using the groups chosen a priori in this work. Metal data can be converted to metal/aluminum ratios, which are not only better indicators of polluted sediments than are absolute metal concentrations [*32*], but also may be useful in factoring out the bias introduced by sediment particle size. Finally, inclusion of the low but measurable levels of a variety of organic contaminants in the data set may explain the additional variability of microbial biochemistry.

Principal components analysis has shown that stations can be distinguished based upon either their geochemical or biochemical characteristics. Using canonical correlation, a linear relationship was constructed between the metals and biochemical variables. This ability to determine a statistically valid relationship between pollutant metals and biochemical parameters representing microbial community structure suggests that biochemical monitoring of microbial communities may be a useful method for assessing the effects of estuarine pollution.

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