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Multivariate Statistical Analyses of 96-Hour Sediment Bioassay and Chemistry Data

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Freshwater sediments serve an important role in the quality of freshwater communities but have often been neglected in community assessments. Several authors have reported freshwater systems with "healthy" water quality which support depauperate benthic faunal assemblages as a result of unobtrusive perturbations within the sediments (Prater and Anderson 1977a, Lehmkuhl 1979). As a result of increased concern over toxic substances in freshwater sediments, renewed interest has been focused on the importance of sediment quality and sediment-organism, sediment-water column interactions.

Beginning in 1977, an investigation of the sediment quality in five harbors of Lake Michigan was conducted using the principal procedures then utilized to evaluate dredged sediments: bulk sediment chemistry (USEPA 1977), the elutriate test (USEPA/COE 1977) and 96-hour sediment bioassays (Prater and Anderson 1977 a,b). The study was an attempt to resolve some of the concerns expressed with regard to these procedures. The principal objective was to complete thorough statistical analyses of the data using univariate and multivariate techniques to determine if relationships existed between chemical and bioassay results. The results of the univariate analyses have been presented elsewhere (Hoke and Prater 1980, Laskowski-Hoke and Prater 1981 a,b).

MATERIALS AND METHODS

Five harbors on Lake Michigan were selected for the study: Indiana Harbor, IN; Grand Haven Harbor, MI; New Buffalo Harbor, MI; Green Bay Harbor, WI and Marinette-Menominee Harbor, WI-MI. A Ponar dredge was used to collect five L of sediment from all stations in each harbor. After collection, samples were maintained at 4 °C and transported to the laboratory for bioassay and chemical analyses. Four L of sediment were utilized for bioassay testing and one L was utilized for bulk and elutriate chemical analyses.

The 96-hour sediment bioassays were conducted using the procedure of Prater and Anderson (1977 a,b) and conformed to U.S. EPA guidelines (1978). Hexagenia limbata Walsh, Daphnia magna Straus, Lirceus fontinalis Rafinesque and Pimephales promelas Rafinesque were the test species utilized. Hexagenia limbata and L. fontinalis were collected from unperturbed natural populations while P. promelas and D. magna were obtained from in-house laboratory cultures.

Standard methodologies (USEPA 1974, 1977) were used to analyze the bulk and elutriate chemical samples for total solids, total volatile solids, total phosphorus, oil & grease, TKN, NH_3, NO_3 + NO_2, Cl, SO_4, Cu, Cr, Pb, Ni, Fe, Cd, Mn, Zn, Ba, As, Hg, CN and particle size. When applicable, the pre-test and post-test waters from the bioassays were analyzed for the same parameters. Chemical leaching from the test sediments during the bioassays was calculated by subtracting the pre-test water concentration of each parameter from the post-test water concentration. These values were collectively termed the "difference" chemistry.

Two statistical packages, Statistical Package for the Social Sciences (SPSS) and Biomedical Computer Programs P-Series (BMDP) were used for data analyses. Since the total number of bioassay analyses performed was 40 (N = 40), the canonical correlation (Hotelling 1935, Cooley and Lohnes 1971) of bioassay data and the three groups of chemical data was performed separately. Canonical correlation analysis is restricted to data sets in which the total number of variables is less than the sample size. The bulk chemistry, elutriate chemistry, difference chemistry and bioassay data contained 33, 19, 15 and 3 variables, respectively. The fourth bioassay variable, mortality of L. fontinalis, was eliminated before analysis as a result of small sample size (N = 20).

The purposes of the canonical correlation analyses were to examine the data for correlations between groups of variables (e.g. between bioassay and elutriate chemistry data sets) and to reify (Marriot 1974) or reduce the number of variables in each chemical data set to the minimum number accounting for the greatest amount of variance in the bioassay data set. Variables were eliminated based on their standardized coefficients (weights) for the canonical variables as determined during preliminary analyses using the SPSS subprogram CANCORR.

Final analyses were performed using the BMDP-6M program. This program generated the canonical component loadings representing the correlation between the raw data for a given variable and the canonical variable constructed during the canonical correlation procedure. These loadings measured the relative importance of a single variable to the canonical correlation

and were necessary for redundancy analyses (Stewart and Love 1968).

RESULTS AND DISCUSSION

Reification of each chemistry data set was performed by eliminating parameters with coefficients (weights) of ≤ 0.30 in the initial analyses. Initial analysis of the bioassay and bulk chemistry data sets eliminated 12 bulk chemistry parameters Final analysis of these data sets (Table 1) generated one pair of canonical variates ($N = 40$, $R_c = 0.943$, $p \leq 0.05$) with an eigenvalue or shared variance between the bioassay and bulk chemistry data sets of 0.889.

Table 1. Final canonical correlation analysis of mortality (Set I) and bulk chemistry data (Set II).

VARIABLE	COCANVAR 1	LOCANVAR 1
Set I		
% Mortality *P. promelas*	0.271	-0.084
% Mortality *H. limbata*	-1.030	0.966
% Mortality *D. magna*	0.026	0.672
Rd_1		0.413
Set II		
Total Kjeldahl nitrogen	0.780	0.223
*Retained 10	-0.612	0.046
*Retained 30	-1.011	0.191
*Retained 60	-1.421	-0.075
*Retained 140	-1.070	0.258
*Passed 140	-1.060	-0.212
Chromium	0.640	0.734
Copper	-1.208	0.767
Iron	0.897	0.771
Lead	-1.509	0.823
Rd_2		0.232
R_c^2 (Eigenvalue)		0.889
R_c ($a \leq .05$)		0.943
X^2, D.F.		96.58, 30
Significance		0.000

*Seive size analysis

The elimination of the 12 chemical parameters resulted in a small loss of information but rendered the chemistry data set more manageable. The final loadings illustrated a positive correlation between the mortalities of H. limbata and D. magna and bulk chemistry Pb, Fe, Cu and Cr. The redundancy of the bioassay data given the bulk chemistry data was 0.413 while redundancy of the bulk chemistry data given the bioassay data was 0.232.

Reification of the elutriate chemistry data set eliminated 10 of the initial 15 variables. Final analysis (Table 2) generated two pairs of canonical variates ($N = 40$, $R_c = 0.863$ and 0.578, $p \leq 0.05$) with eigenvalues of 0.746 and 0.334, respectively. All pairs of canonical variates generated during an analysis were orthogonal or uncorrelated and all pairs of variates except the initial pair accounted for residual shared variance.

Final loadings on the first pair of canonical variate illustrated a positive correlation between the mortalities of H. limbata and D. magna and elutriate chemistry Ni, NH_3, TKN and SO_4. Loadings on the second pair of canonical variates illustrated a positive correlation between the mortality of P. promelas and elutriate chemistry TKN and SO_4. Total redundancy of the bioassay data given the elutriate chemistry data was 0.379 while redundancy of the elutriate chemistry data given the bioassay data was 0.241.

Initial analysis of the bioassay and difference chemistry data sets eliminated 13 chemical variables. The final analysis (Table 3) produced two pairs of canonical variates ($N = 40$, $R_c = 0.891$ and 0.611, $p \leq 0.05$) with eigenvalues of 0.794 and 0.373, respectively. Final loadings on the first pair of canonical variates illustrated that the mortalities of H. limbata and D. magna were positively correlated with difference chemistry CN and Zn and negatively correlated with suspended solids and Hg. Loadings on the second pair of canonical variates illustrated a positive correlation between D. magna mortality and difference chemistry Pb, Hg and Zn while P. promelas mortality was negatively correlated with these same parameters. Total redundancy of the bioassay data given the chemistry data was 0.427 while redundancy of the chemistry data given the bioassay data was 0.292.

The canonical correlation analyses generated results similar to those generated in the bivariate correlation analyses (Hoke and Prater 1980, Laskowski-Hoke and Prater 1981 a,b). The bulk chemistry concentrations of Cr, Cu, Fe and Pb were im-

portant in relationship to the mortalities of H. limbata and D magna. These heavy metals were positively correlated with the linear composites representing the bioassay and bulk chemistry data and contributed the greatest amount of information to the shared variance between these linear composites.

Numerous investigators have reported on the acute toxicity of heavy metals in water to different species of aquatic insects. Documentation of the effects of heavy metals in sediments on the acute mortality of aquatic invertebrates is virtually non-existent. However, the incorporation of heavy metals into sediments cannot totally negate their toxic effects on aquatic invertebrates, particularly those which burrow into and ingest sediments.

Burrowing mayflies of the genus Hexagenia ingest large amounts of sediment (Zimmerman et al. 1975) and digest detritus with varying amounts of inorganic matter as their primary food source (Cummins 1973). Mean values of assimilation efficiency indicated materials ingested by nymphs were high in digestibility (Zimmerman et al. 1975) while Hunt (1953) concluded that some nutritional value must be derived from ingested inorganic mud. Pavlyutin (1970) demonstrated that some organisms are capable of assimilating a percentage of the ash fraction in their food.

Heavy metals in water also have exhibited toxicity to the cladoceran, D. magna. It seems unlikely that bulk chemistry of the sediments would be related to the mortality of a planktonic organism, however, the mortality of D. magna was positively correlated with sieve size < 0.250 mm but > 0.105 mm. The incorporation of heavy metals, in association with petroleum residues or organics, into this sediment fraction as a result of colloid formation and/or absorption may have influenced mortality. Unprecipitated particles or particles resuspended by H. limbata burrowing activity may have become accesible to D. magna during feeding. The heavy metals associated with the particulates could then have been released as a result of chemical reactions occurring in the intestinal tract of the organism (Lee and Plumb 1974).

In the canonical correlation analysis of the bioassay and elutriate chemistry data, the loading of SO_4 on the linear composite of the elutriate chemistry data slightly exceeded the rule-of-thumb cut-off point for interpretation (Cooley and Lohnes 1971). It could, however, indicate the importance of the redox potential of the sediments in the release of NH_3 and organic N during the elutriate test. The importance of elutriate TKN in the results was undoubtedly an artifact related to the importance of NH_3 and organic N in relationship

to test organism mortality and the measurement of both NH_3 and organic N in the TKN analyses.

The first pair of canonical variates from the analysis of the difference chemistry and bioassay data illustrated the association of Hg with organic material as suspended solids (Leuthart and Spencer 1977, Schindler and Alberts 1977) and the inverse relationship of Hg and suspended solids with mortality of H. limbata and D. magna. This may indicate that fallout of organic particulates, with incorporation into the sediments, adversely affects burrowing organisms. Cyanide and Zn were positively related to the mortality of these two species, possibly through disruption of respiratory functions. The second pair of canonical variates illustrated a positive relationship between D. magna mortality and difference chemistry Pb, Hg and Zn while P. promelas mortality was negatively related to these same parameters. These heavy metals may have been associated with particulates which P. promelas do not ingest or they may have been associated with petroleum residues which P. promelas could avoid.

The canonical correlation analyses illustrated significant relationships between the bioassay and various chemistry data sets. As an analytical tool, it facilitated the identification of the most important variables in each of the chemistry data sets relative to the bioassay data and confirmed the results of the univariate analyses. The multivariate analyses also illustrated that the redundancy of the bioassay data set was greater than that of each of the chemistry data sets.

Although direct cause and effect relationships between mortality of test organisms and concentrations of chemical parameters are not postulated, these results add credence to the potential for such relationships. The current state-of-the-art does not permit establishment of direct cause and effect relationships between sediment contaminants and mortality of test organisms. However, the importance of physical exposure to contaminated sediments and the opportunity for ingestion of these sediments would seem to be excellent areas of concentration for future research into the mechanisms of toxicological cause and effect.

Table 2. Final canonical correlation analysis of mortality (Set I) and elutriate chemistry data (Set II). The significant correlations (R_c), $\alpha \leq .05$, are presented; as well as the coefficients for the canonical variables of each set (COCANVAR), correlation of the canonical variable with the original variables (LOCANVAR), and the redundancy (Rd) of each set given the other set.

VARIABLE	COCANVAR 1	LOCANVAR 1	COCANVAR 2	LOCANVAR 2
Set I				
% Mortality *P. promelas*	0.132	0.160	1.092	0.959
% Mortality *H. limbata*	-1.229	0.960	-0.342	0.089
% Mortality *P. magna*	0.393	0.403	0.381	-0.044
Rd_1 (.379)		0.276		0.103
Set II				
Ammonia	-1.439	0.562	-3.695	0.267
Total Kjeldahl Nitrogen	1.090	0.483	4.039	0.440
$NO_3 + NO_2$	0.358	-0.167	-0.303	0.151
Sulphate	-0.489	0.301	0.531	0.458
Nickel	-0.602	0.848	-0.098	0.123
Rd_2 (.241)		0.207		0.034
R_c^2 (Eigenvalue)		0.746		0.334
R_c		0.863		0.578
X^2,D.F.		63.16, 15		15.95,8
Significance		0.000		0.043

Table 3. Final canonical correlation analysis of mortality (Set I) and difference chemistry data Set (II). The significant correlations (R_c), $\alpha \leq .05$, are presented; as well as the coefficients for the canonical variables of each set (COCANVAR), correlation of the canonical variables with the original variables (LOCANVAR), and the redundancy (Rd) of each set given the other set.

VARIABLE	COCANVAR 1	LOCANVAR 1	COCANVAR 2	LOCANVAR 2
Set I				
% Mortality *P. promelas*	0.477	-0.285	0.052	-0.365
% Mortality *H. limbata*	-1.017	0.892	-0.949	-0.126
% Mortality *D. magna*	0.066	0.664	1.298	0.693
Rd_1 (.427)		0.349		0.078
Set II				
Suspended Solids (105°C)	0.171	-0.614	-0.360	0.217
Cyanide	-0.393	0.793	-0.090	0.282
Lead	0.678	-0.132	0.558	0.365
Mercury	0.215	-0.568	1.084	0.406
Nickel	-0.302	-0.255	-0.871	-0.253
Zinc	-0.672	0.667	0.476	0.502
Rd_2 (.292)		0.246		0.046
R_c^2 (Eigenvalue)		0.794		0.373
R_c		0.891		0.611
X^2, D.F.		78.71, 18		25.02, 10
Significance		0.000		0.005

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