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2.0 METHODS

2.1 Field Collection Methods

2.1.1 Benthos

Benthos samples were collected from the bottom of Lake Michigan at all sampling stations for benthic community analyses and substrate characterization (particle size category and distribution). Samples were collected with a standard Eckman Dredge. Five replicate samples were collected for benthic community analysis and three replicate samples were collected for sediment characterization. When removed from the water, each Eckman Dredge sample was washed into a bucket, then transferred to a 1.0 L wide-mouth plastic sample container. Samples for benthic community analysis were flooded with clean water then preserved with 3% formalin solution prior to shipment for laboratory preparation and analysis. Samples reserved for sediment characterization were not preserved prior to laboratory analysis.

2.1.2 Phytoplankton

Phytoplankton samples were collected at each sample location for taxonomic identification and community structure analysis. Four replicate 1.0 L grab samples were collected from composite lake water samples also used for water chemistry samples and chlorophyll-a samples. Lake Michigan water was collected from the surface to approximately one foot above the bottom by a 12 volt battery-powered submersible pump, and composited into a five gallon bucket. Phytoplankton grab samples, chlorophyll-a samples, water chemistry samples, and field determined water quality parameters were obtained from the composite water sample. Phytoplankton samples were preserved with 2.0 to 3.0 mL Lugol's solution prior to shipment and preparation for analysis. Net phytoplankton samples were also obtained concurrent with zooplankton sample collection utilizing an 80μm mesh plankton net. Methods are further discussed under the zooplankton section.

2.1.3 Zooplankton

Zooplankton samples were obtained at sites S120, S340, S650 and S1000 by passing a large volume of water through an 80μm mesh net of 0.5 meters diameter fitted with a revolving/count flowmeter calibrated for the net size. Prior to deployment of each tow, the beginning number of the flowmeter was recorded. The volume of water sampled was determined by the revolutions and counts attained by the flowmeter during the sample period. The sample net was towed by a motorboat driven at slow speed in circles around each site marker. The net was positioned to the inside of the circle with sufficient distance behind the wash and wake of the boat to intercept undisturbed waters. All zooplankton samples were a composite of material collected from the water column for a timed towing period of three minutes (approximately 6 circles around each site marker). After retrieval of the net, the final flowmeter number was recorded, the net contents were rinsed down to the plankton bucket attached to the bottom of the net, the bucket was completely rinsed into a 500 mL labelled plastic sample container, and the sample was preserved with 3 to 4 mL of Lugol's solution prior to shipment and preparation for analysis.

2.1.4 Ichthyoplankton

Ichthyoplankton (larval fish) samples were collected from S120, S340, S650, and S1000 by using a 300 μm mesh net towed behind the boat. The ichthyoplankton net was fitted with a revolving/count flowmeter calibrated for the diameter of the net (0.5 meters), and procedures for towing were identical to zooplankton collection methods. All ichthyoplankton samples were collected for a tow period of ten minutes. Upon retrieval of the sampling net, the final number from the flowmeter was recorded, all net contents were rinsed down into the plankton bucket attached to the bottom of the net, and the plankton bucket was completely rinsed into a 500 mL labelled plastic sample container preserved with 3% (vol:vol) formalin solution prior to shipment.

2.1.5 Periphyton

Periphyton growth on site buoy markers (float periphyton) from S120, S340, S650, and S1000 was quantitatively sampled from a known surface area of the buoys. The marker buoys at each

sampling location also served as an artificial substrate for periphyton colonization. SCUBA divers removed the floats from the water to assure minimal disturbance of colonized algae. On board the field boat, several small pieces of buoy approximately 2 to 3 square inches were cut from each marker with a razor blade and placed in Whirl-Pak bags. Deionized water and 1.0 mL of Lugol's solution was added to each sample bag to keep the periphyton wet and preserved. until preparation for analysis.

Shore periphyton recognized as the matted filamentous green growth attached to rocks and surfaces along the shoreline. This shore algae is a complex of macro-algae such as the filamentous *Cladophora glomerata*, *Spyrogyra* sp. *Oedogonium* sp. common to most waters. Of recent interest in the Laurentian Great Lakes is the presence and distribution of the red algae (Rhodophyta) *Bangia atropurpurea* in the shore periphyton. Most filamentous algae found along the shoreline environment support an attached complex community of micro-algae including other smaller filamentous forms, diatoms, and other cellular and colonial algae. *Bangia atropurpurea* is not an algae that supports an associated attached (epiphyte) assemblage. As a result, when *Bangia atropurpurea* becomes a significant component of the macro-algae, there is typically a concurrent decrease in the epiphyte community and biomass. According to Lowe *et al* (1982) the lack of epiphyte growth on *Bangia atropurpurea* is a result of the mucilaginous nature of the readily soluble outer cell wall (sulphated galactans) that prevents attachment. This reduction in epiphytic growth would typically reduce biomass and total community diversity. This bioassay samples the shore algae to determine the extent of growth of *Bangia atropurpurea* within and outside the Amoco Cove area.

Shore periphyton attached to rocks situated along the Amoco Cove and Whihala Beach shoreline was qualitatively sampled. Filamentous algae from within the exposed splash zone above the water line, to a depth of approximately 0.5 meters were sampled and composited. Algae were removed from the rock substrates by scraping with a brush, collection with an aspirator, or a combination of both methods. Algae samples were stored in a 250 mL plastic sample container and immediately preserved with Lugol's solution prior to preparation for analysis.

Periphyton associated with the shifting sand bottom substrates was collected to determine if a substantial epipsammon (attached to sand) community existed. Replicate samples of the sandy bottom substrates were collected in 28.2 cm^2, 118 mL plastic containers with snap-on tops by SCUBA divers. Collection protocols were designed to sample epipsammon as well as refugee algae dislodged from other sites and sources. Collection procedures involved the following steps:

1) carefully place the inverted plastic container into the sediments trapping the sand (bottom) and water interface,

2) slide a large metal spatula through the surrounding sediments and onto the inverted container to trap the sample,

3) remove the container and spatula from the bottom, turn upright slowly without removing spatula seal and let settle for several seconds,

4) slowly slide off spatula and snap on sample container top, return to surface.

Four replicate samples of epipsammon algae that is associated with the sandy bottom substrates were collected at each study site. Upon retrieval at the surface, all samples were preserved with 2-3 mL of Lugol's algal preservative and prepared for shipment.

2.1.6 Algal Bioassay

Ambient Lake Michigan waters and undiluted effluent samples were collected on July 25 for use as test conditions for an algal bioassay. Depth composited Lake Michigan water samples were collected in the field at each of S120, S340, and S3500 as described for phytoplankton sample collections. Samples of undiluted effluent were obtained from the Amoco Whiting site prior to the discharge pipe as a composite sample using a permanent automated sampler operated by Amoco personnel. All water and effluent samples were immediately stored on ice and shipped overnight to the ADVENT laboratory.

2.1.7 Water Chemistry

Water chemistry samples for laboratory analysis were collected in the field as a composite of waters from the surface to approximately 1.0 foot above the bottom with a submersible battery-powered pump as described for phytoplankton composite sample collection. Samples for laboratory analysis were transferred to pre-labelled sample bottles by a dipping container to prevent contamination of composited site water. All water chemistry samples were immediately treated according to EPA preservation guidelines and stored on ice until received by the analytical laboratory.

2.1.8 In-situ Water Quality

Water quality parameters that typically change immediately following collection were determined in the field for the composite sample and *in situ* at depth in Lake Michigan as appropriate. The field water quality parameters were determined with the aid of a calibrated Hydrolab Surveyor 3 multi-parameter transmitter and probe. Water quality parameters and physio-chemical parameters recorded *in situ* included the following:

- **Total Alkalinity** as mg/L $CaCO_3$. Determined by titration with weak sulfuric acid to Bromocresol green-methyl red pH 4.5 endpoint.
- **pH** as standard pH units. Measured by Hydrolab Surveyor 3.
- **Dissolved Oxygen** in mg/L. Measured by Hydrolab Surveyor 3.
- **Specific Conductance (Conductivity)** in μmhos/cm. Measured by Hydrolab Surveyor 3.
- **Temperature** in °C. Measured by Hydrolab Surveyor 3.
- **Depth** measured in meters by Hydrolab Surveyor 3.
- **Secchi disk depth** measured with an 8" standard pattern Secchi disk.

The Hydrolab Surveyor 3 was used to measure temperature, conductivity, dissolved oxygen, pH, and depth at relevant intervals of 1.0 ft from the surface to just above the bottom to determine depth-related water quality profiles.

2.1.9 Effluent Water Quality

A Hydrolab Datasonde 3 datalogger was deployed directly in the Outfall 001 header box. The stand-alone unit was programmed to collect hourly readings of temperature, conductivity, and pH for the two month period of May 12, 1994 to July 12, 1994.

2.2 Laboratory Methods

2.2.1 Benthos

Benthos samples were analyzed by Mr. Henry Zimmermann, a benthic invertebrate entomologist with several years experience in the Great Lakes including southern Lake Michigan. Mr. Zimmermann processed the benthic samples using standard EPA techniques (EPA 1989). All samples were sieved with a 250 μm screen, then organisms were sorted and identified from the remaining debris from a white enamel pan with the aid of Bausch and Lomb zoom dissecting scope (1-30 X total magnification). A general scan of the sieved sediments was conducted to check for unaccounted specimens. Head capsules of Chironomid specimens were mounted on microscope slides and viewed with a Leitz compound microscope at 100-400 X total magnification for proper identification.

2.2.2 Phytoplankton

The 1.0 L phytoplankton grab samples were checked for sufficient preservation and allowed to completely settle for a minimum of 24 hours following receipt at the AATA laboratory. Approximately 900 mL of water was removed without disturbing the settled material by using a siphon constructed of aquarium hose in order to concentrate the settled phytoplankton into a sample volume of 100 mL. Based on an evaluation of the amount of material settled, each sample was then completely transferred to a 150 mL beaker, covered with a watch glass and allowed to resettle for a period of 24 hours to further concentrate the samples. Final

concentration of the phytoplankton sample to a volume of 20 mL was performed by removal of remaining excess water by siphon.

Concentrated phytoplankton samples were enumerated and identified to the lowest practical taxonomic level at 600 X total magnification using a Palmer-Maloney plankton counting chamber (volume 0.1 mL). Diatoms typically cannot be successfully identified at 600 X total magnification, and were only enumerated for density estimation purposes. Following evaluation of the concentrated phytoplankton sample in three separate preparations of the Palmer-Maloney counting chamber, the total volume of the concentrated sample was measured in a 25 mL graduated cylinder and allowed to settle for a minimum of 24 hours. Following the final settling period, settled material from all site replicates was combined and used to prepare a permanent diatom slide for diatom taxonomic identification and relative percent abundance determination for the sample site. A minimum of 600 diatom frustules were identified from each site to characterize the diatom portion of the phytoplankton community.

2.2.3 Zooplankton

Zooplankton net samples were evaluated by Dr. Edward Reed, formerly of Colorado State University (Professor Emeritus, retired). Zooplankton net samples were completely mixed and split for storage as backup phytoplankton and zooplankton samples for species confirmation. Zooplankton samples used for evaluation were subsampled for observation because of the high number of organisms in the split sample. All organisms from subsamples were sorted and separated from debris then identified and enumerated with the aid of a dissecting microscope ranging from 10 to 130 X total magnification. Identification was augmented by a compound microscope when higher total magnification was required.

2.2.4 Ichthyoplankton

Ichthyoplankton samples were delivered to the Colorado State University Larval Fish Laboratory for analysis. Identification and enumeration of ichthyoplankton samples were conducted using accepted methods and protocols consistent with the standard operational procedures of the Larval Fish Laboratory.

2.2.5 Periphyton

Marker Buoy (Float) Periphyton. Periphyton collected from the marker buoy material was removed by brushing and washing the sample into a 60 mL beaker. Each replicate sample was kept separate and surface areas of all cut pieces of buoy material were measured to determine the total area scraped per replicate sample. The contents of the beaker were measured with a graduated cylinder to determine dilution and concentration factors. Three types of counts were performed to document the taxonomic makeup and abundance data for each sample. First, a 5.0 mL aliquot of thoroughly mixed sample was used to prepare permanent diatom microscope slides for species identification and enumeration at 1,000 to 12,000 X total magnification.

A second evaluation was conducted to determine the density of the diatom portion of the float periphyton. Enumeration of diatom algae cells was conducted with the aid of a hemacytometer (Improved Neubauer ruling) counting chamber at 400 X total magnification. Ten suspensions of completely mixed sample were individually introduced to the hemacytometer (0.0001 mL volume) and all diatoms enumerated within the two grids of the chamber for a total of 20 counts. Diatom density estimates were determined from the volume of the sample, the surface area of the marker buoys samples, and the volume and mean number of diatoms enumerated in the hemacytometer.

A third evaluation was conducted to account for the non-diatom portion of the periphyton and utilized a Palmer-Maloney counting chamber (volume 0.10 mL). A suspension of thoroughly mixed sample was introduced into the Palmer-Maloney counting chamber and non-diatom algae were analyzed at 400 X total magnification for identification and enumeration data. A minimum of 1.5 Palmer Maloney counting chamber values were analyzed per sample. When one half of a Palmer-Maloney counting chamber was analyzed and no new species were encountered, a sufficient sample was believed to be analyzed. Density estimates for the non-diatom algae were determined from the volume of the sample, the surface area of the marker buoy samples, and the volume and enumeration data from the Palmer-Malony counting chamber analysis. Float periphyton density estimates included diatom periphyton density plus non-diatom periphyton density values.

Shore Periphyton. Matted filamentous periphyton sample replicates collected from rock substrates near Outfall 001 and rock substrates at Whihala Beach ("reference site" located west of the Amoco Facility) were used to determine if gross differences existed in this growth form between the two sites. Prior to viewing with the aid of a microscopic, each sample of scraped algae was individually spread out in a large tray to untangle the algal mat and visually detect the presence of different algal forms by color, size, etc. Microscopic analysis of the shore periphyton included a minimum of 5 large (0.5 area of microscope slide) wet mount preparations of algae, including evaluation of all algal material visually different or unique. Taxa were identified at 400 X total magnification and categorized by one of the three groups for abundance (5=dominant, 3=common, 1=rare). Categories were used in lieu of other quantitative measures because of the inaccuracies and difficulties of sampling and counting a thick filamentous algal mat. The epiphytic algal community attached and associated with the tangled filamentous mat was heavily dominated by a few characteristic diatoms. Separate sample processing for diatom analysis and taxonomy was considered unnecessary and therefore not conducted.

Epipsammon Periphyton. Samples of epipsammon (sand associated) periphyton collected by SCUBA divers from the shifting fine sand substrates required a series of sample treatment steps to separate the algae from the substrates. The procedure used to isolate epipsammon algae from the sandy substrates is listed below:

1) Completely irrigate and suspend the sediment with deionized water within the confines of the sample container used in the field.

2) Mix the sediment by hand for a period of 10-15 seconds using a teflon laboratory spatula or similar tool to dislodge algae from the sand grains.

3) Allow the suspended sediments to settle for a period of approximately 1 - 2 minutes depending upon silt and clay composition of sediments.

4) Remove, and retain supernatant water with large bore pipette and store in a 60 mL glass vial or similar suitable container. Minimize collection of sediment material.

5) Concentrate the collected algae by allowing the suspension in the glass vial to settle for a period of 12 hours followed by removal of excess water. This step also allows for more supernatant to be added to the sample vial in subsequent steps.

6) Repeat steps 1 - 5 above until microscopic evaluation of the last supernatant contains ten or fewer algal cells per field of view (100 X total magnification).

The above procedure removed, isolated and concentrated algae associated with the epipsammon substrate collections.

Analysis of the algae isolated from the epipsammon included initial observation at 360 X total magnification to identify clumps and tangles of macro-algae (primarily filamentous) in a Sedwick-Rafter algal counting chamber (volume 1.0 mL). The initial observations indicated that the epipsammon community consisted of diatoms which were numerically dominated by a few taxa. Subsequently, permanent diatom microscope slides were prepared for identification and enumeration. Due to the dominance of a few taxa, combined with on overall low richness for all sites, a shortened count of 100 frustules for each station was performed at 1000 X total magnification.

2.2.6 Algal Bioassay

Algal bioassay procedures were patterned after the *Selenastrum capricornutum* Printz Algal Assay Bottle Test (USEPA 1978). Two algal species were testes: *Selenastrum capricornutum* and *Scenedesmus quadricauda*. These algae are commonly used in laboratory test procedures. Species of *Scenedesmus* have been found among the phytoplankton in southern Lake Michigan. The algal cultures were obtained from commercial suppliers.

The algal bioassay was designed to test two questions: first, was there any difference in samples taken from outside the effluent dispersion zone from samples taken inside the effluent dispersion zone; and second, would there be any differences expected at dilutions of 20:1 or 40:1 (roughly the dispersion at two sample sites within the effluent dispersion zone). To assure that the test

met quality expectations, two laboratory controls were tested, one using filtered algal growth media and one using unfiltered algal growth media: healthy growth in these controls would indicate that the test was conducted properly and the algae were capable of normal growth. Both algal species were tested in triplicate under all conditions, except that only *Selenastrum* was used in the unfiltered laboratory control tests.

The bioassay design permitted several statistical comparisons, shown in Table 2-1.

Table 2-1. Algal Bioassay Design

Algal Bioassay Design Statistical Comparisons						
Treatment Group		Treatment Comparison				
		A	B	C	D	E
A	S3500 Sample: Lake Michigan reference water outside effluent dispersion zone		1	1	2	3
B	S340 Sample: Estimated 40:1 dilution inside effluent dispersion zone	1				3
C	S120 Sample: Estimated 20:1 dilution inside effluent dispersion zone	1			2	
D	95% S3500 water and 5% Effluent (20:1 dilution)	2		2		
E	97.5% S3500 water and 2.5% Effluent (40:1 dilution)	3	3			
F	Lab Controls: Filtered and unfiltered algal growth medium					

1 This comparison evaluates "outside" vs. "inside" effluent dispersion zone.
2 This comparison evaluates impact of a 20:1 treated effluent dilution.
3 This comparison evaluates impact of a 40:1 treated effluent dilution.

The tests were initiated by inoculating algal cells from rapidly growing (log-phase) cultures into each tests flask at an initial density of 2,000 cells/mL. The test lasted 8 days. The general experimental conditions were:

- Tests were conducted in temperature-controlled room at 25°C under continuous light.
- Lighting was by cool white fluorescent light and reached an intensity of 360 to 440 foot candles at the top and bottom of each flask. Light intensity was measured with a General Electric Model 214 hand-held light meter.
- Each 2000 mL Erlenmeyer flask contained 400 mL of test solution. Flasks were covered with aluminum foil. A small hole in each foil cap permitted gas exchange.
- Flasks were randomly placed on shelves to minimize potential effects of subtle differences due to shelf position. Positions were changes daily, again by random assignment, to further minimize any position effects.

Algal growth was measured by cell counts during the test and by algal dry weight and chlorophyll-a measurement at the conclusion of the test. Chemical parameters were measured on excess media from each treatment group, including analyses for nutrients, major ions and general parameters.

Cell counts were done using a hemacytometer counting slide. A few mL of shaken test solution was withdrawn by pipette and loaded onto one side of the counting slide. A second sample was taken from the same flask and loaded onto the other side. Microscopic cell counts were made and the average of the two sides was recorded as the value for that replicate.

At the end of the 8 day test, 300 mL of each test solution was filtered through a pre-weighed glass fiber filter with 0.7 micron pore size. The algae retained on the filter were dried at 104°C and weighed, giving an algal dry weight. Samples of rapidly-growing algal culture were similarly filtered and weighed to estimate the initial dry weight of the original algal inoculum. The net growth of algae was determined as the difference between day 8 biomass and the initial biomass, and expressed as dry weight of algae per liter.

The filtrate from the 300 mL sample was mixed with other replicates for each treatment and used for chemical analysis. The remaining test sample (approximately 100 mL) was used to measure chlorophyll-a. A measured volume was filtered onto 0.7 micron glass fiber filters, then

the filter and retained algae were sonicated to facilitate extraction on chlorophyll-a with 90% acetone. Chlorophyll-a was measured flourometrically and expressed as mg chlorophyll-a per liter.

2.3 Analytical and Statistical Methods

Numerical count data associated with species identifications were used to determine the following community structural parameters and characteristics as presented by Ludwig and Reynolds (1988).

- Richness: the total number of different taxa,
  Hill's N1 value as the number of dominant taxa.
- Diversity: Shannon-Weiner Diversity Index,
  Simpson's Diversity Index,
  Hill's N1 value as a percent of richness.
- Evenness: Hill's E5 value.

Richness is a measure of the number of different taxa in a sample collection. Richness values typically represent species level identification, and the values play an important role in determination of other community metrics such as density. Hill's N1 value is the number of abundant species in the sample (e.g. the number of species that are not rare), where each species is weighted by its abundance. Hill's N1 is determined using the Shannon-Weiner Diversity (see discussion below) value as:

$$N1 = e^{H'};$$

where: H' is the Shannon-Weiner Diversity value for the community, and
$e = 2.7183$

Hill's N1 is an appealing representation of the dominant species in the community because of the easy interpretation with respect to structure and diversity for persons unfamiliar with

diversity indices. Hill's N1 value when compared to total richness gives an intuitive evaluation of how many species in the community may be considered ecologically functional. For convenience, Hill's N1 may be presented as a percent of total richness.

Diversity is a measure of how the abundance is distributed among the different number of taxa (richness). A community with a richness of 20 different taxa, each of which are represented by 15 specimens (total of 300 organisms) will have a much different diversity than 20 different taxa represented by five specimens for each of 18 taxa and 105 specimens for two taxa (total of 300 organisms). Measures of diversity were represented by the Shannon-Weiner Diversity Index (H'); Simpson's Diversity Index (λ); and Hill's N1 value as discussed above. Both the Shannon-Weiner Diversity Index and Simpson's Diversity Index have been used extensively as a descriptive community parameters in ecological studies. The Shannon-Weiner Index considers the abundance of each species in the community and index values increase in magnitude as community diversity increases. A community with one species, regardless of abundance has a Shannon-Weiner Diversity of zero. Maximum values of H' typically range from 3.8 to 4.5 for well developed benthic communities in running waters, or from complex substrates within lakes, and well developed complex planktonic communities.

The Shannon-Weiner Diversity Index values are determined by:

$$\text{Shannon-Weiner Diversity Index: } H' = -\sum_{n=1}^{s} (p_i \ln p_i)$$

where: p_i = is the proportional abundance of the *i*th species, and
s = the total number of species in the community.

The Shannon-Weiner Diversity Index value is used to determine Hill's N1 number of abundant taxa as discussed above.

Simpson's Diversity Index (λ) is a widely used measure of diversity which ranges from zero (for maximum diversity) to 1.0 (for no diversity). Maximum diversity (zero index value) is attained when all species present are of equal abundance. Simpson's Diversity Index is determined by:

$$\textbf{Simpson's Diversity Index:} \quad (\lambda) = \sum_{i=1}^{s} \frac{n_i(n_i - 1)}{n(n - 1)} \quad ;$$

where: n_i = the number of individuals of the *i*th species,
n = the total number of individuals in the sample, and
s = the total number of species in the sample.

Evenness has recently become a popular measure of the distribution of species abundance among the total number of taxa much like diversity. However, evenness is typically described as a measure of how relative abundances diverge away from equal abundance among all members of the community. Evenness measured by Hill's modified ratio (E5) is unaffected by species richness, and therefore preferable over other evenness metrics in cases where species richness is expected to be limited. Hill's modified ratio is intuitively easy to understand as the value approaches zero as a single species becomes more and more dominant in the community. Determination of Hill's modified ratio as an evenness metric utilizes Simpson's Diversity Index value, and the Shannon-Weiner Diversity Index value (in the form of Hill's N1 number of abundant taxa). Evenness a measured by Hill's modified ratio is determined by:

$$\textbf{Hill's modified ratio Evenness:} \quad E5 = \frac{(1/\lambda) - 1}{e^{H'} - 1}$$

where: eH' = Hills N1 number of abundant taxa,
λ = Simpson's Diversity Index value.

Complete discussions on the calculations and use of Hill's N1, Simpson's Diversity Index, Shannon-Weiner Diversity Index, and Hill's modified ratio evenness (E5), are presented in Ludwig and Reynolds (1988). Richness, diversity, and evenness community structure parameters were determined with statistical software programs for the IBM provided by Ludwig and Reynolds (1988).

Community structure parameters were used to evaluate biological collections within the dispersion zone and outside the dispersion zone. Sites situated inside the dispersion zone included S120 and S340 as determined by *in-situ* water quality screening and previous dye dispersion studies. Sites situated outside the dispersion zone included S650, S1000, S2000 and S3500. Community structure parameters for all replicate samples inside and outside the dispersion zone were subjected to Analysis of Variance (ANOVA) procedures to indicate if statistical differences existed in mean parameter values inside and outside of the dispersion zone.

Comparisons among communities using structural characteristics is typically sufficient to identify differences between biological samples. However, community structure characteristics are based upon the distribution of organisms among different taxa. Although not common, two communities with nearly identical density and richness would show comparable diversity values but may contain no species in common. An evaluation of the taxonomic makeup of any community is valuable with respect to insight into the ecology and function of the community.

Taxonomic comparisons between the biological collections were performed using the Bray-Curtis Percent Dissimilarity Index (PD). The Bray-Curtis Percent Dissimilarity Index is but one of many similarity indices available, but is favored over presence-or-absence based indices because abundance of each common species is considered in the comparison. The PD is calculated by first:

Calculating Percent Similarity (PS):

$$PS_{jk} = \frac{2W}{A + B}(100)$$

where,

$$W = \sum_{i=1}^{s} [\min(X_{ij}, X_{ik})] ; \qquad A = \sum_{i=1}^{s} X_{ij} ;$$

and,

$$B = \sum_{i=1}^{s} X_{ik} ;$$

and,

X_{ij} represents the abundance of the *i*th species in the *j*th community,
X_{ik} represents the abundance of the *i*th species in the *k*th community.

A comparison of any two communities with identical species and abundance distributions would result in a percent similarity (PS) of 100%. Bray-Curtis Percent Dissimilarity (PD) is computed by:

$$PD = 100 - PS$$

The Bray-Curtis expression as PD is a useful expression as a distance complement of the PS which may be used in ecological distance based metrics and analyses. The Bray-Curtis PD was used to determine the magnitude of differences in taxonomy and the distribution of taxa among the sample sites. Determination of the PD value was done using the specific statistical software programs for the IBM computer as discussed in Ludwig and Reynolds (1988). Standard descriptive data summary statistics, necessary data transformations and ANOVA procedures were performed using the IBM computer and Microsoft Excel software with added statistical applications.

Statistical procedures and evaluation of algal assay data using *Selenastrum capricornutum* and *Scenedesmus quadricauda* (cell counts, chlorophyll, and dry weight biomass) consisted of analysis of variance (ANOVA) procedures performed to compare all treatments for a given

species. If significant differences were found, the appropriate multiple range tests (Dunnett's procedure for parametric data and Wilcoxon's procedure for nonparametric data) were performed to identity significantly different exposures. To compare a field sample to analogous laboratory-prepared samples, the Student's t-test was performed. Statistical significance was determined at the 95 percent confidence level.