PERIPHYTON MONITORING IN THE BAYOU SYSTEM, 1996-1997

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INTRODUCTION

This report presents results of the periphyton sampling conducted in September, 1996 and 1997 on the Bayou system for the Federal Facilities Oversight Unit (FFOU). Results of the first UK periphyton study are summarized below. The endpoints for all studies were biomass, chlorophyll *a*, and the Autotrophic Index (AI). The Autotrophic Index (AI), defined as the ratio of biomass to chlorophyll *a*, was calculated for each sampling station. Values between 50 - 200 are considered acceptable, *i.e*. indicate no impact.

March to September, 1989 Collections

A previous study was conducted in 1989 from March through November on the Bayou system to investigate the effects of pollutants from effluent outfalls on green algae (Birge *et al*., 1989). Collections were made using floating rack samplers with artificial substrates (APHA, 1995). Five sampling periods of two weeks each were performed in Big Bayou Creek from March through November, and three sampling periods of two weeks each were conducted in Little Bayou Creek from July through September. Stream stations sampled included BB1 and BB3-BB9 on Big Bayou Creek and LB2-LB4 on Little Bayou Creek. To the extent possible, sampling locations were selected to normalize for tree canopy, sunlight penetration, *etc*. The AI mean values obtained for Big Bayou Creek in the 1989 study ranged from 97 (BB3, BB8) to a high of 200 at BB5. The limit of 200 was exceeded in one sample from BB5. This station also was shown to be impacted by ecological studies and stream chemistry. AI values >200 for the most part correlate with inhibition of chlorophyll *a* production, and AI values <50 may be attributed to various degenerative changes within the stream system, including the breakdown of chlorophyll *a* and alteration of biomass by siltation, *etc*., leading to a higher proportion of non-chorophyll containing organic and mineral materials. In Little Bayou Creek, AI values were in the mid-range for LB2 and LB3, indicating no impact on the chlorophyll *a*-containing perihyton. However, at LB4 the AI was >624, indicating very heavy impact. The principal sources of pollution for this site would be the North-South Ditch and runoff from the active landfill. This is the stream area in which the USGS found substantial variation in stream discharge, conductivity, temperature and other parameters (Evaldi and McClain, 1989).

MATERIALS AND METHODS

Sample Collection

Generally, periphyton is collected on artificial substrate samplers suspended in the water column, as was done in the 1989 collections. However, during the more recent sampling events of 1996 and 1997, the UK group used the USGS method (Porter *et al*., 1993) for direct collection of periphyton from natural substrates by scraping the surfaces.

Sample Analysis

Procedures were followed as indicated in *Standard Methods* (APHA, 1995). Chlorophyll *a* was extracted in acetone and optical density (OD) measurements were made at 664 nm (chlorophyll *a*), 647 nm (chlorophyll *b*), 630 nm (chlorophyll *c*), and 750 nm (turbidity corrections) using a Spectronic GeneSys 5 spectrophotometer. After initial measurements, the acetone extract was acidified and the absorbance was again measured, at 665 nm and other wavelengths, and corrected for turbidity. Acidification converts chlorophyll *a* to pheophytin, and the difference before and after acidification relates to the amount of chlorophyll *a* originally present in the sample. Lorenzen (1967) determined that if the ratio of absorbance before acidification ($OD664_b$) to absorbance after acidification ($OD665_a$) is 1.7 or greater, it indicates a high proportion of chloropyll *a* and a healthy system; whereas a ratio of 1.0 indicates little or no chlorophyll and equates with pure pheophytin.

Analyses were performed on chlorophylls *a*, *b*, and *c* which were quantified using Formula 1 given below (Wetzel and Likens, 1991; APHA, 1995), where:

$C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$
$C_b = 21.03(OD647) - 5.43(OD664) - 2.66(OD630)$
$C_c = 24.52(OD630) - 7.60(OD647) - 1.67(OD664)$

where:

C_a, C_b, and C_c = concentration (mg/L) of chlorophyll *a, b, c*, respectively.
OD664, OD647, and OD663 = corrected optical densities (with a 1 cm light path) at the respective wavelengths (*i.e.* OD664 = Absorbance at 664 - Absorbance at 750).

Formula 1

$$\text{Chlorophyll } a \text{ (mg/m}^2\text{)} = \frac{C_a \text{ X extract volume (L)}}{\text{surface area (m}^2\text{)}}$$

Biomass was determined by dividing ash-free weight (mg) by surface area (cm^2). The Autotrophic Index was calculated as the ratio of biomass to chlorophyll *a*. The acceptable range of AI values is 50 - 200; impact is indicated by values >200 or <50.

RESULTS

September 23, 1996 Collections

The FFOU periphyton study was undertaken to compare the new data with the previous periphyton results, water chemistry and ecological data in order to assess changes in stream conditions and the impact of stream discharges on periphyton. In 1996, samples were collected from Big Bayou Creek at stations BB1, BB2, and BB7-BB9, and from Little Bayou Creek at stations LB2-LB5 (Table 1). Results of this sampling event generally agreed with those from the 1989 study cited above. In Big Bayou Creek, biomass ranged from 606 (BB8) to 2022 (BB1) and chlorophyll *a* ranged from 1.1 (BB8) to 13.7 (BB1). Chlorophyll *a* concentrations were suppressed within the effluent receiving zone, ranging from 1.1 to 5.4 µg/L as compared with 13.7 µg/L at the upstream reference (*i.e*. BB1). The AI values at stations BB8 and BB9 ranged from about 250 to 551 (Table 1). In Little Bayou Creek, LB4 was heavily impacted, the chlorophyll *a* and biomass were markedly lower, and the AI value was 1141. As indicated above, AI values >200 are considered indicative of ecological impact and poor stream quality. Based upon the 1996 AI values, the quality of Little Bayou Creek and sectors of Big Bayou Creek may have deteriorated somewhat compared with the 1989 survey, and this is in agreement with findings for metal pollutants (Birge and Price, 1997).

Periphyton was a less sensitive parameter for monitoring the impact of effluent chemicals than were ecological metrics for macroinvertebrates or tissue residues in sentinel species (Birge *et al*., 1992). Although the AI is responsive to metal pollution and is a useful endpoint for localizing surface water contamination (Geyer *et al*., 1984; Smetts and Rittman, 1990), this procedure does not appear sensitive enough to monitor low concentrations of metals or TCE.

September 29, 1997 Collections

Samples were collected at stations BB1 and BB6-BB8 in Big Bayou Creek, and at station LB4 in Little Bayou Creek. Table 2 presents the results of this sampling event. Values were adjusted for turbidity, sampling errors, *etc*. Biomass and chlorophyll *a* values generally were low. There appeared to be some overall suppression of chlorophyll *a* and periphyton production. AI values fell outside the acceptable range at all stations except BB1, which was the reference (Table 2). Station BB6, which had the highest AI value (852), was the site on Big Bayou Creek where water column metals generally have been found at higher concentrations (Birge *et al*., 1992). In Little Bayou Creek, the optical density measurements for LB4 at 750 nm were approximately five to ten times greater (*i.e*. 1.256) than for non-chlorophyll substances (*e.g*. 664 nm) at other stations, *e.g*. 0.148 at BB6. This introduced appreciable error, and a reliable AI could not be determined for LB4.

Due to the atypical results at LB4, it is likely that this station remains impacted.

The $OD664_b/OD665_a$ ratios ranged from 1.35 (BB6) to 1.53 (BB7) for Big Bayou Creek, and 1.20 for LB4 on Little Bayou Creek. Except for LB4, these ratios were mid-range as compared with 1.7 (pure chlorophyll *a*) and 1.0 (pure pheophytin). It should be noted that the lowest ratio, 1.2 for LB4, approached 1.0 which is given as the value for pheophytin,the breakdown product of chlorophyll *a* (Lorenzen, 1967; APHA, 1995)

As indicated by Birge and Price (1997), recent water column and sediment metal concentrations in the Bayou system were equal to or greater than those reported in 1989. Also, pH, conductivity, and metal pollution, as previously indicated by Birge *et al*. (1998), may have contributed to stream impact. The findings of the periphyton studies and the earlier reports generally are in agreement. As previously noted, station LB4 was the most impacted with respect to chlorophyll-containing periphyton. However, periphyton may not be the "best" parameter for monitoring chemical pollution or ecological impact.

In view of the above observations, there are several conclusions which may be drawn -

- Periphyton studies in 1989, 1996, and 1997 all indicate some degree of impact in the effluent receiving zone of Big Bayou Creek, and occasionally the more downstream stations.

- The most impacted station monitored was LB4 on Little Bayou Creek. This did not appear to be related to the discharge of upstream effluents (*i.e*. 002, 012, 011, 010).

- The impact at LB4 was observed over a period of ten years, suggesting the need for a focused study upon this area of the Bayou drainage, as well as the North-South Ditch which enters Little Bayou Creek upstream of the LB4 monitoring station.

- The periphyton assays, while useful in assessing and localizing magnitude of impact of PGDP effluents, were less sensitive than species richness and other parameters as measured in macroinvertebrate communities or tissue residues analyzed in fish sentinel monitors (*e.g*. stoneroller minnow, longear sunfish, green sunfish; Birge *et al*. 1992).

- If further monitoring is conducted with periphyton, it is recommended that artificial substrates be used.

Table 1. Photosynthetic Pigment Concentrations and Autotrophic Indices (AI) for Periphyton Collected September 23, 1996 from Big and Little Bayou Creeks, Paducah, KY.

Sampling Station	Chl *a* (mg/m^2)	Chl *b* (mg/m^2)	Chl *c* (mg/m^2)	Biomass (mg/m^2)	AI
BB1	13.73	2.68	4.94	2022.22	147.3
BB2	10.38	3.04	3.21	1652.78	159.3
BB7	5.45	1.14	1.59	947.37	174.0
BB8	1.10	0.42	0.90	605.56	551.5
BB9	6.44	3.50	5.38	1608.33	249.9
LB2	7.24	0.00	1.11	2761.11	381.5
LB3	4.87	2.26	4.47	2050.00	421.0
LB4	1.02	0.47	0.75	1163.89	1141.5
LB5	2.43	5.01	6.54	733.33	302.2

Table 2. Photosynthetic Pigment Concentrations and Autotrophic Indices (AI) for Periphyton Collected September 29, 1997 from Big and Little Bayou Creeks, Paducah, KY.

Sampling Station	$664_a/665_b$	Chl *a* (mg/m^2)	Chl *b* (mg/m^2)	Chl *c* (mg/m^2)	Biomass (mg/m^2)	AI
BB1	1.44	7.6	2.15	2.47	784	103
BB6	1.35	9.2	1.92	1.80	7385	852
BB7	1.53	10.2	0.65	1.80	312	31
BB8	1.37	7.2	0.92	1.57	1946	270
LB4	1.20	10.9	1.53	2.86	874	---

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