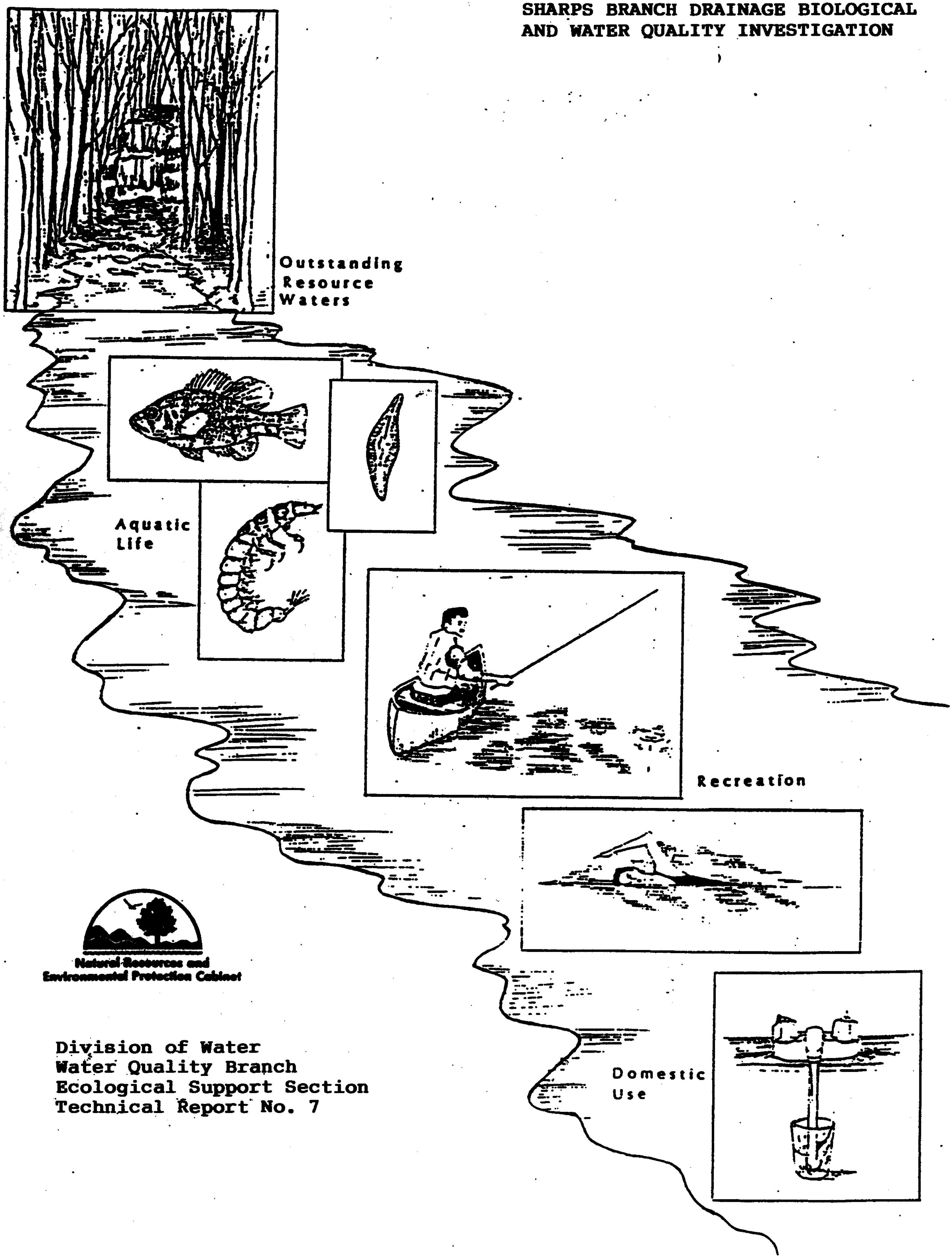
SHARPS BRANCH DRAINAGE BIOLOGICAL AND WATER QUALITY INVESTIGATION



Natural Resources and Environmental Protection Cabinet

Division of Water
Water Quality Branch
Ecological Support Section
Technical Report No. 7

Sharps Branch Drainage
Biological and Water Quality Investigation

By

Biological Branch
Division of Environmental Services

Technical Report No. 7

June 6, 1983

List of Contributors

Robert W. Logan
Project Leader

Gary V. Beck
Microbiologist

Samuel M. Call
Aquatic Invertebrate Zoologist

Ronald E. Houp
Aquatic Invertebrate Zoologist

Michael R. Mills
Ichthyologist

Stephen D. Porter
Phycologist

Clifford C. Schneider
Aquatic Invertebrate Zoologist

Donald K. Walker
Botanist

Abstract

A biological and physicochemical investigation of Arrow Spring and Sharps Branch (in Simpson County) was conducted on March 18, 1983. The purpose was to determine the existing water quality and the impact to the receiving stream caused by the Kentucky Agricultural Energy Corporation (KAEC), an ethanol refinery. Arrow Spring, a first order stream, flows in a northerly direction approximately 0.8 km (0.5 mi), where it joins Sharps Branch. Sharps Branch flows in an easterly direction approximately 2.6 km (1.6 mi), where it joins West Fork Drakes Creek. The West Fork of Drakes Creek is used by Franklin, Kentucky (population 7,738) as a source for its public water supply (RMI 46.8).

Except for elevated nitrates, the physicochemical data was typical of small streams of the region. However, data prior to this survey shows substances high in organic nutrients had been discharged into the Arrow Spring drainage. Biological data, from this study, indicated Sphaerotilus natans, a filamentous bacteria, had covered the stream substrate from Arrow Spring to the confluence with the West Fork of Drakes Creek, and was having an adverse effect on all aquatic life. Growth of Sphaerotilus was of nuisance proportion and represents a threat to water quality, particularly for domestic water supply use. The extent of Sphaerotilus growth had impaired the aesthetics and recreational uses of the Arrow Spring and Sharps Branch drainage. The presence of Sphaerotilus and the extent of growth in the drainage is an indication that improperly treated wastes have been discharged to Arrow Spring for an extended period of time.

The Sphaerotilus impact to the Arrow Spring drainage produced by the discharge of KAEC is a violation of 401 KAR 5:031, Section 3, (3), (4) and (5).

Table of Contents

List of Figures and Tables

Recommendations

1. Maintain maximum waste water treatment plant (WWTP) operational efficiency to minimize the entry of growth promoting substances from KAEC into Arrow Spring.
2. Remove any carbon sources, which will lower the carbon to nitrogen and carbon to phosphorus ratios, reducing favorable conditions for growth.
3. The KAEC WWTP should consider using one of the discharge strategies discussed in this report (page 18-20).
4. An immediate growth inhibition of Sphaerotilus should be prevented as it may adversely impact downstream water supplies (Franklin), cause odor problems and have an adverse effect on the warmwater aquatic habitat of the West Fork Drakes Creek.
5. Instream chlorination to remove the Sphaerotilus growth should only be used as a last resort and monitored closely to keep the impact to the receiving stream and downstream water supplies to a minimum.
6. For instream treatment, refer to Table 6 on the recommended chemical treatments for the control of iron bacteria in groundwaters.
7. The following parameters should be monitored until the nuisance growths of Sphaerotilus are controlled:
   - ethanol
   - BOD
   - COD
   - TOC
   - DO
   - pH
   - total phosphorus
   - $NO_2 + NO_3 - N$
   - NH_3-N
   - total dissolved solids
   - conductivity
8. Monitor the presence of Sphaerotilus and the extent of growth until nuisance growth conditions are eliminated.

Summary

1. The data indicate that KAEC was responsible for the Sphaerotilus growth in the Arrow Spring and Sharps Branch drainage. Discussion with plant personnel indicated that no growths of Sphaerotilus were observed in Arrow Spring prior to KAEC's initial operation.
2. The impact of nutrients and carbonaceous materials, including ethanol, was apparently continuous and manifested in the heavy growth of Sphaerotilus natans.
3. The discharge and/or leak to Arrow Spring by KAEC has resulted in a fish kill.
4. The extent of Sphaerotilus growths over the entire substrate, from the surface origin of Arrow Spring to the confluence of Sharps Branch with West Fork Drakes Creek (approximately two miles), had an adverse effect on aquatic life.
5. The aesthetics provided by Arrow Spring and Sharps Branch drainage have been impaired, due to the presence of Sphaerotilus.
6. Sphaerotilus represents a potential threat to proper water treatment at the Franklin Water Treatment Plant (WTP).
7. The available habitat for algae was largely restricted by dense growths of Sphaerotilus, which helps to explain the relative lack of filamentous algae. The diatom community at all three collection sites consisted of rheophilic and aerophilic forms, which are typically found in well oxygenated streams with pH values greater than 7.0. The Arrow Spring station was dominated by Navicula cryptocephala and Navicula lanceolata, both of which are characteristic of nutrient enriched streams and considered to be tolerant to high levels of organic pollution.
8. At Arrow Spring 19-1, only four species of macroinvertebrates were collected. Twenty four species of macroinvertebrates were collected in Sharps Branch upstream of Arrow Spring (19-2), while fourteen species were collected at the

downstream station (19-3). At the two collection sites (19-1 and 19-3), virtually all the available habitats for invertebrates had been eliminated by the smothering effects of Sphaerotilus. Sharps Branch (19-2) above the confluence with Arrow Spring possessed a diverse benthic community in proportion to stream size and available habitats.

9. The contamination of the Arrow Spring drainage has resulted in the apparent elimination of fish communities. There were eight species of fish collected at station 19-2. In contrast, no species were collected at station 19-3. The four sculpin fry, collected at Arrow Spring (19-1), indicates this area was used for fish spawning.

10. The impact to the Arrow Spring drainage by KAEC shows them to be in violation of 401 KAR 5:031, Section 3. This section states, in part, that the following minimum water quality criteria are applicable to all surface waters. Surface waters shall not be aesthetically or otherwise degraded by substances that: (3) produce objectionable color, odor, taste or turbidity; (4) injure, be toxic to or produce adverse physiological or behaviorable responses in humans, fish, shellfish and aquatic life; (5) produce undesirable aquatic life or result in the dominance of nuisance species.

INTRODUCTION

On March 18, 1983, the Biological Branch of the Division of Environmental Services conducted a biological evaluation and water quality survey of Arrow Spring and Sharps Branch, which are tributaries of the West Fork Drakes Creek, in southern Simpson County near Franklin, Kentucky. The purpose of this survey was to determine the impact of byproducts from an ethanol refinery, operated by Kentucky Agricultural Energy Corporation (KAEC), to water quality and aquatic life in the drainage. Samples were collected at three sites during a normal flow period (Table 2).

The Sharps Branch drainage occupies a small portion of the Drakes Creek segment in Simpson County. The segment is designated as 03 (Green River) 019 (Drakes Creek).

Basin Impacts and Stream Uses

There are two known discharges to the drainage above Arrow Spring. One is the I-65 rest area, a 20,000 gpd capacity wastewater treatment plant (WWTP) that discharges to a sink hole and the other is the Cracker Barrel Restaurant WWTP, with a design capacity of 10,000 gpd that discharges to an interrupted stream.

Methods

Water samples were analyzed in accordance with the latest edition of Standard Methods for the Examination of Water and Wastewater (APHA 1981) and United States Environmental Protection Agency's (U.S. EPA) Methods for Chemical Analysis of Water and Waste (U.S. EPA 1979). Field turbidity measurements were taken with an HF Instruments Model DRT-15 trubidimeter. Field conductivity was determined with a Yellow Springs Instrument Company (YSI) Model 33 S-C-T meter. Field measurements for dissolved oxygen (DO) and water temperature were conducted with a YSI Model 54A oxygen meter. An Analytical Measurements Model 707B pH meter was used for field pH.

Table 1
KAEC Physicochemical Data Collected by DOW

Date	Source	Ethanol (mg/l)	BOD mg/l	COD mg/l	TSS mg/l	pH SU	NH_3-N mg/l
Feb. 23, 1983	Franklin WTP	36.3	-	-	-	-	-
Feb. 23, 1983	Arrow Springs	89.1	-	-	-	-	-
Feb. 23, 1983	Franklin WTP	36.9	30.0	67.0	2.0	-	-
Feb. 23, 1983	Arrow Springs	70.0	146.0	190.0	24.0	-	-
Feb. 23, 1983	Arrow Springs at Sharps Branch	54.9	61.0	94.0	15.0	-	-
Feb. 23, 1983	Clean Side Sharps Branch	<10.0	2.1	3.8	7.0	-	-
Feb. 23, 1983	W.F. Drakes Creek above Sharps Branch	<10.0	1.9	2.7	10.0	-	-
Feb. 23, 1983	W.F. Drakes Creek below Sharps Branch	<10.0	12.0	23.0	8.0	-	-
March 2, 1983	KAEC Neutralization Pond	-	17.1	31.6	13.0	3.3	17.4
March 2, 1983	Final Clarifier	-	333.0	806.0	154.0	7.3	1.43
March 2, 1983	Final Discharge at Arrow Springs	-	106.0	263.0	159.0	8.8	3.56
March 11, 1983	City of Franklin Clear/well	<0.5	-	-	-	-	-
March 11, 1983	Franklin City Hall	<0.5	-	-	-	-	-
March 11, 1983	Sharps Branch below Arrow Springs	<0.5	-	-	-	-	-
March 11, 1983	W.F. Drakes Creek above Sharps Branch	<0.5	-	-	-	-	-
March 11, 1983	W.F. Drakes Creek below Sharps Branch	<0.5	-	-	-	-	-
March 11, 1983	W.F. Drakes Creek below water plant	<0.5	-	-	-	-	-
March 11, 1983	W.F. Drakes Creek B.G. #1	<0.5	-	-	-	-	-
March 11, 1983	W.F. Drakes Cr. B.G. #2	<0.5	-	-	-	-	-
March 11, 1983	Simpson Co. W.D. Key Stop	<0.5	-	-	-	-	-
March 11, 1983	KAEC 24 hr. Composite #001	<0.5	-	-	-	-	-
March 16, 1983	KAEC 24 hr. Composite #002	69.1	-	-	-	-	-
March 16, 1983	Franklin Distribution Line Walker's Equipment Co.	<0.5	-	-	-	-	-
March 16, 1983	Simpson Co. W.D. Key Stop	<0.5	-	-	-	-	-
March 16, 1983	Franklin D.L. Minute Mart	<0.5	-	-	-	-	-
March 16, 1983	Franklin Clear Well	<0.5	-	-	-	-	-
March 16, 1983	W.F. Drakes Creek below Water Plant Intake	<0.5	-	-	-	-	-
March 16, 1983	W.F. Drakes Creek above Sharps Branch	<0.5	-	-	-	-	-
March 16, 1983	W.F. Drakes Creek below Sharps Branch	<0.5	-	-	-	-	-

Table 1 continued

Date	Source	Parameter(s)					
		Ethanol (mg/l)	BOD mg/l	COD mg/l	TSS mg/l	pH SU	NH_3-N mg/l
March 16, 1983	Sharps Branch above Arrow Springs	< 0.5	-	-	-	-	-
March 16, 1983	Sharps Branch below Arrow Springs	< 0.5	-	-	-	-	-
March 16, 1983	Arrow Springs above discharge pipe	< 0.5	-	-	-	-	-
March 16, 1983	Arrow Springs below discharge pipe	< 0.5	-	-	-	-	-
March 16, 1983	KAEC Equilization Pond	3.5	6.9	23.0	6.6	0.45	-
March 16, 1983	KAEC final discharge 24 hr. composite	211.0	724.0	148.0	7.0	0.09	-
March 16, 1983	KAEC final discharge grab sample	221.0	532.0	133.0	7.3	0.16	-
March 18, 1983	Arrow Springs	< 0.5	-	-	-	-	-
March 18, 1983	Sharps Branch above Arrow Springs	< 0.5	-	-	-	-	-
March 18, 1983	Sharps Branch below Arrow Springs	< 0.5	-	-	-	-	-
March 19, 1983	Franklin WPI clear well 24 hr. composite	< 0.5	-	-	-	-	-
March 20, 1983	Franklin WPI clear well 24 hr. composite	< 0.5	-	-	-	-	-
March 21, 1983	Franklin WPI clear well 24 hr. composite	< 0.5	-	-	-	-	-
March 25, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 26, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 27, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 28, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 29, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 30, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
March 30, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
April 1, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
April 2, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-
April 3, 1983	Franklin WTP clear well	< 0.5	-	-	-	-	-

Biological samples were collected utilizing a variety of techniques. Qualitative algal samples were procurred by selectively scraping or siphoning material from all available habitats. Samples were preserved in the field with 5% buffered formalin and transported to the Division of Environmental Services (DES) biological laboratory for analysis. Diatoms were treated with 30% hydrogen peroxide and potassium dichromate to remove organic material (van der Werff 1955), and several slides randomly scanned for the presence of rare taxa.

Macroinvertebrate qualitative samples were taken by selectively picking various substrate types and by collecting in different habitats with a triangular kick net. All invertebrate samples were preserved in the field in 70% alcohol solution and transported to the DES biological laboratory for enumeration and identification. The trophic relationships follow those outlined by Merritt and Cummins (1978) and Hawkins and Sedall (1982). Aquatic macroinvertebrates were placed into one of three categories (i.e. tolerant, facultative and intolerant), generally based on information presented by Weber (1973) and Hart and Fuller (1974). These categories are defined by Beck (1955) and Weber (1973) as follows: tolerant organisms are associated with gross organic contamination and are generally capable of thriving under anaerobic circumstances; facultative organisms are capable of tolerating a wide range of environmental conditions, including moderate levels of organic enrichment, but cannot exist under anaerobic conditions; intolerant organisms are sensitive to even moderate levels of organic enrichment and are generally unable to withstand even moderate reductions of dissolved oxygen.

Fish were collected using a 3.4 m by 1.2 m, 0.3 cm mesh, common sense minnow seine and kick nets. Both pool and riffle areas and all recognizable habitat types were sampled. The fish samples were preserved in 10% formalin solution and transported to the DES biological laboratory for enumeration and identification.

Bacteriological samples were collected from directly below the water's surface in sterile 250 ml, wide mouth, sterile, nalgene jars, placed on wet ice and returned for analysis to the DES biological laboratory within six hours. Analyses for total coliform, fecal coliform and fecal streptococcus bacteria were performed using the membrane filter techniques outlined by Bordner et al. (1978). Confirmation of Sphaerotilus natans was accomplished by phase contrast microscopy and comparison with physical properties of the Sphaerotilus-Leptothrix group (Dondero 1975).

PHYSICAL EVALUATION

Arrow Spring, a first order perennial stream, originates underground at latitude 36° 40' 57", longitude 86° 33' 53" and flows in a northerly direction approximately 0.8 km (0.5 mi). It joins Sharps Branch, a second order perennial stream, at mile point 1.6 (Figure 1). Sharps Branch, which is approximately 6.4 km (4.0 mi) long, flows in an easterly direction, joining the West Fork of Drakes Creek at mile point 50.0 and 50.2 (a small island splits the stream at the mouth). The average stream gradient for Sharps Branch is 8.4 m/km (27.5 ft/mi).

Physiographically, the area lies in the Pennyroyal Plain Subsection of the Highland Rim, a section of the Interior Low Plateaus Province (Quarterman and Powell 1978). The area is characterized by karst topography and is sometimes called the "Cavernous Limestone Plateau". Numerous sinking streams occur on gently rolling topography. The Pennyroyal Plain is underlain primarily by Ste. Genevieve, St. Louis and Warsaw limestone of Mississippian age (McDowell et al. 1981). The major soil groups and characteristics for the subbasin are given in Table 1. Elevations range from 700 ft above mean sea level (msl) in the headwaters of Sharps Branch to 590 ft above msl at its confluence with West Fork Drakes Creek.

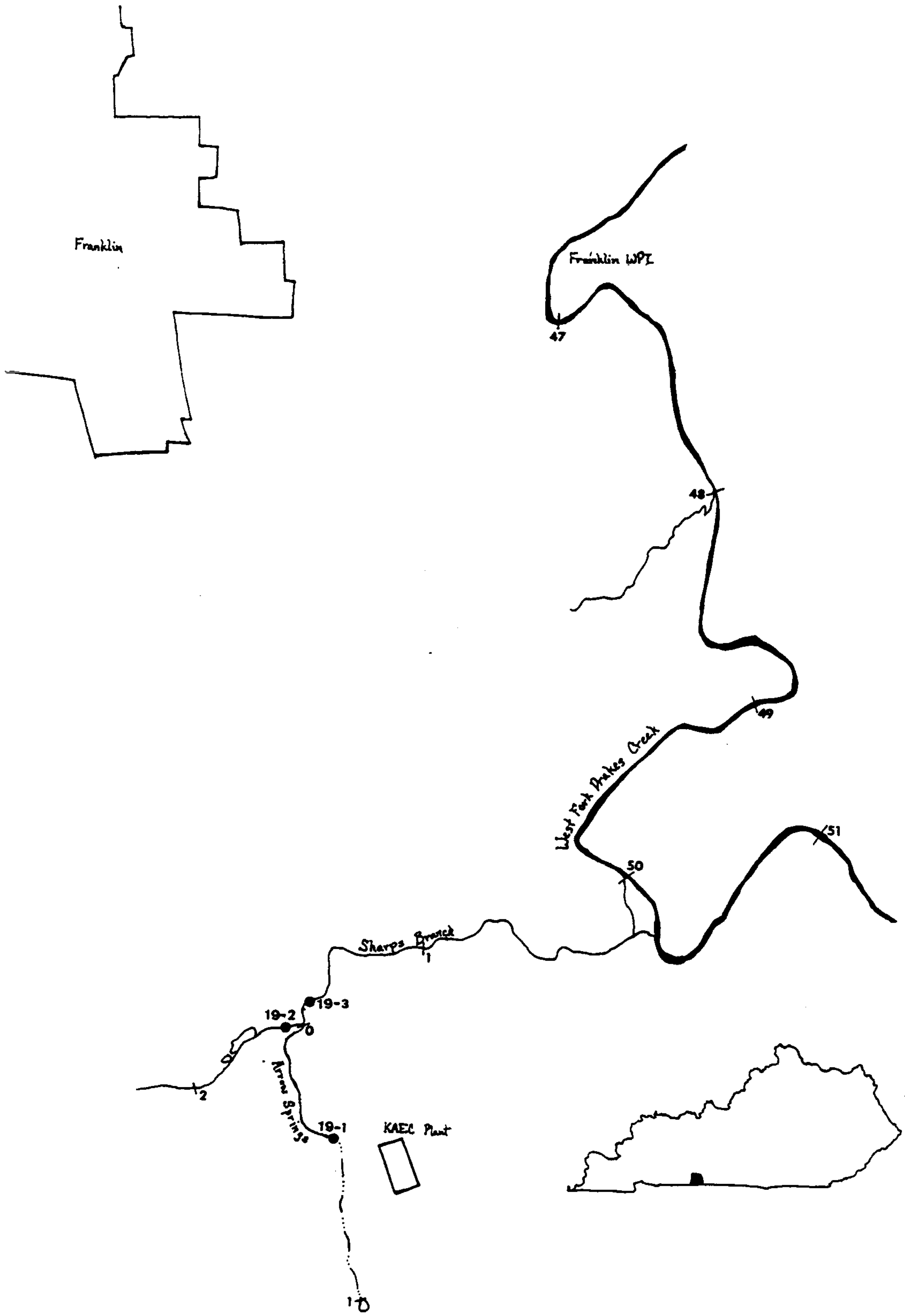
Table 2
Soils of the
Sharps Branch Drainage

Soil[1] Association	Slope[2] %	Drainage[2] Class	Potential[1] Sediment Runoff	Infiltration[1]	Septic Tank[1] Absorbtion Rating
Cumberland	2-10	Well Drained	Medium	Moderate	Slight
Pembroke	2-12	Well Drained	Medium	Moderate	Slight
Crider	3-12	Well Drained	Low	Moderate	Slight

(1) Weston (no date)

(2) Bailey and Winsor (1964)

Figure 1
Map of Arrow Spring and Sharps Branch Drainage
Depicting Sampling Locations



The pool and riffle habitats were well developed in Arrow Spring and Sharps Branch. Other stream habitats included undercut banks, root mats, submerged logs and a variety of substrates. Streamside vegetation was generally well developed and provided a good buffer from surface runoff. Land use in the subbasin is predominately agriculture, with some forested areas.

On March 18, 1983, stream flow, measured approximately 100 yards below the origin of Arrow Springs, was 0.15 m^3/s (5.31 ft^3/s). The flow in Sharps Branch, measured upstream of Arrow Spring at RMI 1.6, was 0.04 m^3/s (1.47 ft^3/s); at RMI 1.5, downstream of Arrow Spring, the flow was 19 m^3/s (6.78 ft^3/s). The historic minimum flow recorded at Arrow Spring was 0.02 m^3/s (0.62 ft^3/s) (DOW wasteload allocation file).

Arrow Spring

Station 03019001 (19-1)

This station was located at Arrow Spring, on the KAEC property. The stream is first order with moderate gradient and was characterized by a long riffle, as it emerged from the base of a cliff, and a long pool downstream. The substrate consisted of boulders, cobble and small pebbles, all covered by four to five inches of *Sphaerotilus natans*.

Sharps Branch

Station 03019002 (19-2)

This station was located on Sharps Branch, approximately 500 ft above the confluence of Arrow Spring, at RMI 1.7. The stream is second order with moderate gradient. Riffles and runs were common with occassional pools. The substrate was cobble, pebble and gravel. No sedimentation was apparent and the substrate was unimbedded. No *Sphaerotilus* growths were observed.

Sharps Branch

Station 03019003 (19-3)

This station was located at RMI 1.5, just below the confluence of Arrow Spring. The stream is second order with moderate gradient. The stream channel was characterized by riffles and runs alternating with pools. Substrates consisted of boulders, cobble and pebbles, most of which was covered by a four to five inch growth of *Sphaerotilus*.

PHYSICOCHEMICAL EVALUATION

In general, physicochemical parameters measured at all three stations on March 18, 1983, were indicative of satisfactory water quality (Tables 3 and 4). The physicochemical data revealed values typical of small hardwater, alkaline streams in the region, when compared with STORET ambient data, with the exception of elevated nitrates. With the exception of manganese, there were no violations of Kentucky Surface Water Standards. The water quality of Arrow Spring and the Sharps Branch drainage is sufficient to support a diverse aquatic community and provide a suitable raw water source for drinking water for the Franklin water treatment plant (WTP) with the absence of impacts from KAEC.

Water quality data collected on February 23, 1983, indicated KAEC impacted Arrow Spring, Sharps Branch and West Fork Drakes Creek, at least as far as the Franklin WTP (Table 2). Subsequent sampling for ethanol on and after March 11, 1983 found levels at those sites to be below laboratory detection limits. Chemical and biochemical oxygen demand levels (COD, BOD, respectively) in samples collected from Arrow Spring at Sharps Branch and the Franklin WTP on the same date indicated the waste was high in organic materials.

TABLE 3
KAEC Metal Data Collected By DOW

March 2, 1983

Parameter	KAEC Neutralization Pond	Final Discharge to Arrow Springs
Cadmium, total, mg/l	0.026	0.005
Chromium, total, mg/l	0.021	0.016
Copper, total, mg/l	0.019	0.014
Iron, total, mg/l	4.68	1.79
Lead, total, mg/l	0.090	0.020
Manganese, total, mg/l	0.660	0.290
Nickel, total, mg/l	0.085	0.059
Zinc, total, mg/l	0.360	0.141

Table 4

KAEC Data Collected By DES

March 18, 1983

Parameter	19-1	19-2	19-3
pH	7.3	7.7	7.6
Alkalinity, mg/l	181.0	148.0	167.0
Acidity, mg/l	12.2	4.2	8.1
Hardness, mg/l	199.0	152.0	188.0
BOD, mg/l	0.8	0.5	0.8
COD, mg/l	1.3	< 1.0	2.8
TOC, mg/l	5.0	3.0	< 3.0
SS, mg/l	< 1.0	4.0	3.0
TDS, mg/l	191.0	150.0	180.0
Sulfate, mg/l	4.5	3.0	5.0
Sulfide, mg/l	< 0.1	< 0.1	< 0.1
Chloride, mg/l	8.0	8.0	8.3
TKN, mg/l	0.08	0.05	0.07
Nitrite, mg/l	0.03	< 0.01	0.03
Nitrate, mg/l	4.6	4.4	4.4
NH_3-N, mg/l	0.08	0.04	0.07
Calcium, mg/l	64.2	46.4	56.6
Sodium, mg/l	3.84	3.12	3.84
Potassium, mg/l	12.6	11.8	12.2
Magnesium, mg/l	9.06	6.38	8.22
Iron, mg/l	0.076	0.118	0.142
Manganese, mg/l	0.069	0.044	0.078
Cadmium, mg/l	0.002	0.001	0.001
Lead, mg/l	< 0.010	< 0.010	< 0.010
Copper, mg/l	0.002	0.002	0.002
Zinc, mg/l	0.004	0.004	0.004

BACTERIOLOGICAL EVALUATION

A total of three samples were collected from Arrow Spring (19-1) and Sharps Branch (19-2, 19-3). These samples were analyzed for total coliform, fecal coliform and fecal streptococcus bacteria. The results (Table 5) indicated acceptable total coliform, fecal coliform and fecal streptococcal levels. Fecal coliform/fecal streptococcus ratios (FC/FS) were not definitive. The low values found in sample analyses were in the gray zone of interpretation (between 1.0 and 3.5) (U. S. EPA 1973), but it is probable that the fecal pollution was animal in origin.

Table 5
Bacteriological Data

Date	Station No.	Source	TC Per 100 ml	FC Per 100 ml	FS Per 100 ml	FC/FS Ratio
18 Mar 83	19-1	Arrow Spring	520	240	140	1.7
18 Mar 83	19-2	Sharps Branch	400	250	88	2.8
18 Mar 83	19-3	Sharps Branch	2,000	200	170	1.2

TC - Total Coliform

FC - Fecal Coliform

FS - Fecal Streptococcus

Microscopic examination of unidentified growth on the substrate indicated the presence of <u>Sphaerotilus</u> <u>natans</u>, a filamentous bacteria. The filamentous bacteria possessed distinct cylindrical sheaths, lacked chlorophyll, were gram-negative, and possessed internal ellipsoidal or rod-shaped cells within the sheath and swarm cells outside the sheath. Only false branching was not detected by staining. Prior to KAEC's first production, no Sphaerotilus was observed in Arrow Spring, according to personal communication with Gregg McCarty, chemist, KAEC.

General Information

Sphaerotilus natans, a filamentous bacteria, is usually associated with polluted water and is also known as "sewage fungus", "slime growth", or "iron bacteria". The appearance of sewage fungus in quantity is always connected with other obvious chemical and biological indications of organic pollution, such as foul odors, deficiency of oxygen, abundance of ammonia nitrogen and a paucity of other aquatic fauna (Butcher 1932). It can be found in streams receiving paper mill wastes, sugar refining wastes, brewery wastes, municipal wastes (Phaup 1968) and, as in this case, ethanol refinery wastes.

Sphaerotilus has a high efficiency for removing nutrients from dilute solution. It can rapidly produce a gross infestation, covering all submerged surfaces, which can extend for several miles below the entry of a polluting discharge (Curtis 1969). Growth is very unsightly and can cause unpleasant odors when decomposing (Curtis 1969). Due to the reduction of oxygen, when growing or decomposing, it has an adverse affect on all stream life. Sphaerotilus is a nuisance organism and has long been recognized as a biological indicator of organic pollution (Dondero 1975).

Growth Requirements

The basic requirements for growth are a carbon source and a nitrogen source, plus essential vitamins which the organism is incapable of synthesizing. The conditions that elicit heavy growths of Sphaerotilus are the following:

1. Carbon Source

A source of organic carbon compounds, such as glucose, galactose, sucrose, maltose, mannitol, sorbitol, succinate, fumarate, butyrate, butanol, glycerol, sodium lactate, sodium pyruvate, sodium acetate and ethanol can be used by *Sphaerotilus* for growth (Stokes 1954). With alcohols and sugars supplying the carbon source, *Sphaerotilus* is able to outcompete other organisms for the nutrients. *Sphaerotilus* will not grow well if the carbon source is removed.

2. Nitrogen

Sphaerotilus natans is able to use ammonia, nitrates and nitrites as the sole nitrogen source (Phaup 1968).

3. Continuous Flow of Nutrients

The extent of *Sphaerotilus* growth is in direct proportion to the concentration of nutrients present (Wuhrmann 1964). Nutrients must be continuously supplied for an extended period of time to cause heavy growths of *Sphaerotilus* (Amberg and Elder 1957; Amberg and Cormack 1960; Amberg et al. 1962). *Sphaerotilus* can bloom from quite low levels of organic pollution. Low concentrations of organic nutrients will sustain *Sphaerotilus* growth, if they are continuous (Dondero 1975). Phaup and Gannon (1967) found excellent growths of *Sphaerotilus* in experimental river channels with a flow of 0.58 ft/sec to 1.49 ft/sec, with only sucrose (1-40 mg/l) added to the influent river water.

4. Trace Elements

Calcium and magnesium are necessary for growth. Iron and phosphates may be required in trace quantities, but are toxic in low concentrations (Phaup 1968).

Other Ecological Requirements

1. Oxygen

   Sphaerotilus natans is an aerobe, but the minimum requirement for oxygen is not high (greater than 2.5 ppm).

2. Temperatures

   According to Curtis (1969), most laboratory data indicate an optimum growth temperature of 25-30°C. Stokes (1954) found a growth range of 15-40°C for Sphaerotilus, with an optimum temperature for growth of 30°C. Most field studies indicate growth is more extensive at lower winter temperatures (Curtis 1969). However, Cawley (1958) reported *Sphaerotilus* growth below a pollution source on the Altamaha River to be limited to 200 yards, except in winter, when the growth extended for 15 miles.

3. Alkaline conditions are most favorable for growth (pH 6.8-8.0), with inhibition occurring at pH 6.2 (Bahr 1953).

Effects of Growths

The byproducts of the death and decomposition of *Sphaerotilus* have an adverse effect on water quality for the following reasons:

1. Effects on Aquatic Life

   The higher biota of streams suffer more than they benefit from the presence of *Sphaerotilus*. Organisms in the immediate vicinity may suffer from the physical alterations of their habitat. There is evidence that the slime growth enveloping the stream bed is damaging to the benthic animals that serve as food for fish (Avery 1970, Gaufin and Tarzwell 1955) and to the eggs of walleyed pike (Smith and Kramer 1963) and salmonid fish (Rassmussen 1955). Decomposition of *Sphaerotilus* severely reduces oxygen levels and adversely affects all river life (Curtis 1969). Oxygen consumption of decomposing Sphaerotilus is 10-20 times greater per unit of dry weight than that of normally occurring aquatic macrophytes (Ministry of Technology 1966).

2. Effects on Domestic Water Supply

The complete and immediate growth inhibition of Sphaerotilus may be detrimental to the stream and downstream water supplies. The decomposition of Sphaerotilus and the resulting by-products (e.g. H_2S, color, D.O. depletion) may impart taste and odor to the water, thus adversely impacting downstream water supplies. Since it has previously been demonstrated that past impacts from KAEC have been observed in the Franklin WTP, it may be presumed that effects of a quick die-off of Sphaerotilus would also be felt at that WTP. Hydrogen Sulfide (H_2S) produced during decomposition is very toxic to all forms of aquatic life, as well as causing taste and odor problems. However, because of the unpleasant taste and odor which result when sulfides occur in water, it is unlikely that any person or animal will consume a harmful dose (McKee and Wolf 1963). The color produced by decomposition is aesthetically objectionable.

Control

Sphaerotilus is difficult to remove once it has become established; therefore, it is recommended that one or more of the following be implimented to control Sphaerotilus growths.

1. Control of Sphaerotilus is preferably achieved by preventing entry of those substances promoting growth of this organism into Arrow Spring.
2. Hattingh (1963) found that bulking in biological treatment plants was due to the combined effects of high carbon to nitrogen and carbon to phosphorus ratios, favoring Sphaerotilus growth. Therefore, removing

any carbon sources (e.g. ethanol), thus lowering these ratios, should have a positive effect in removing the growth from the stream.

3. To develop a significant growth of Sphaerotilus, the supply of nutrients must be continuous. Using laboratory and actual stream monitoring, Amberg and Elder (1957), Amberg and Cormack (1960) and Amberg, Cormack and Rivers (1962) showed that impounding the carbon source (spent sulfite liquors) and intermittently discharging was effective in reducing slimes. Amberg and Elder (1957) indicated a daily 2, 4 or 6 hour discharge period resulted in no growth. Amberg and Cormack (1960) reported that 2 hours of discharge, with 22 hours of storage, was best for controlling slime growth. They further note that a discharge period of 24 hours in combination with 2 to 5 days storage was effective in reducing 80% of the slime growth. It would, therefore, seem logical that the application of a daily discharge period of no more than six hours in duration would aid in reducing Sphaerotilus growth in Arrow Spring.

4. Until Sphaerotilus growth subsides, the KAEC WWTP effluent and Arrow Spring 19-1 should be monitored for the following parameters:

o ethanol	o total phosphorus
o BOD	o $NO_2 + NO_3 - N$
o COD	o NH_3-N
o TOC	o total dissolved solids
o DO	o conductivity
o pH	

These parameters reflect the characteristics of wastewater associated with grain based ethanol refineries.

5. It has been observed that chlorine will prevent the instream growth of Sphaerotilus (Table 6). However, the use of this chemical and others for control would have considerable impact on the stream and the downstream domestic water supply. Cullimore and McCann (1975) summarized chemical treatments (Table 6) recommended for the control of iron bacteria in ground waters. They also reported iron bacteria are very resistent to chemical methods of control, perhaps due to protective slime layers and other cell coatings and the clumping together of cells to form thick layers. The cell coatings contain ferric and manganic oxides and hydroxide deposits, which could restrict the diffusion of the chemical agents and/or directly react with them.

Table 6

Recommended chemical treatments for the control of iron bacteria in ground waters*

Treatment**	Site	Reference
110 lb oxalic acid, 50 lb sulphamic acid, 50 lb wetting agent, 0.25 lb inhibitor	Wells	Grainge and Lund (1969)
Elimination of dissolved CO_2 by elevation of pH to above 8.3	Wells	Ellis (1932)
Residual chlorine, 0.2 $mg.l^{-1}$	Lab tests	Grainge and Lund (1969)
Hydrogen perioxide, 100 $mg.l^{-1}$ and phosphate inhibitor	Lab tests	Grainge and Lund (1969)
Hypochlorite, 0.438%	Wells	Machmeier (1971)
Residual chlorine, 50–100 $mg.l^{-1}$ for 2 h	Wells	Machmeier (1971)
Shock chlorination with 5.25% hypochlorite	Wells	Machmeier (1971)
Hydrochloric acid (muriatic acid), 14-21%	Screened wells	Schafer (1974)
Sulphamic acid, 7.5–10% (several hours contact time)	Screened wells	Schafer (1974)
Hydroxyacetic acid, 4.7–7% (contact time related to pH of water)	Screened wells	Schafer (1974)
Chlorine gas to give 500 $mg.l^{-1}$	Wells	Schafer (1974)
LBA (Liquid Antibacterial Acid, USA Patent 3085929), 5% (treat for 36 h)	Wells	Luthy (1964)
Recycling of hypochlorite solutions	Water supplies	Rao (1970)
Hydrochloric acid treatment followed by 300 $mg.l^{-1}$ chlorine, 18 h contact	Wells	Mogg (1972)
Calcium hypochlorite, 715 $mg.l^{-1}$	Wells	Schafer (1974)
Lithium hypochlorite, 0.14%	Wells	Schafer (1974)
Sodium hypochlorite, 0.14%	Wells	Schafer (1974)
Chlorine dioxide gas (limited use)	Wells	Schafer (1974)
Potassium permanganate, 0.1–0.2%	Wells	Schafer (1974)
Continuous chlorination	Wells	Woods (1973)
Acrolein, 0.1–30 $mg.l^{-1}$ (restricted use)	Water systems	Woods (1973)

* Take from Cullimore and McCann (1975)

**All concentrations mentioned refer to final concentrations in ground water.

BIOLOGICAL EVALUATION

Based on recent data and Kentucky Water Quality Standards, the biological data of Arrow Spring indicated waste from KAEC had adversely impacted the aquatic habitat and water quality for an extended period of time. The impact of nutrients and carbonaceous materials, including ethanol, was apparently continuous and manifested in the heavy growth of *Sphaerotilus natans* at stations 19-1 and 19-3. This resulted in the reduction and/or elimination of aquatic flora and fauna from these two stations. The *Sphaerotilus* impact to the Arrow Spring drainage produced by KAEC shows them to be in violation of 401 KAR 5:031, Section 3, (3), (4) and (5).

Algae

Attached algae were collected at the three sampling sites from all available substrates. A total of 107 taxa were identified from these sites (Appendix A). Station 19-2 supported the greatest number of species (87), while station 19-1 contained nearly 50% fewer taxa (45). Station 19-3 had somewhat intermediate species richness (65), most likely due to dilution effects from the higher quality water of Sharps Branch, as well as immigration of typical stream diatoms which exhibit a broad range of environmental tolerance. The primary limiting factors at the impacted sites (19-1, 19-3) appear to be reduced habitat for algal colonization and water quality limitations.

The available habitats at stations 19-1 and 19-3 were largely restricted to the dense growths of *Sphaerotilus*, a phenomenon which has been discussed previously. The occurrence of these extensive bacterial growths explains the relative lack of filamentous algae at these sites, since *Sphaerotilus* is able to colonize stream substrates at much faster rates than filamentous algae. The dominant periphytic taxa, exclusive of diatoms, were *Vaucheria*, a tolerant, aerophilic taxon (Prescott 1962), and *Euglena*, an alga which is tolerant of "high organic pollution" (Palmer 1969). In contrast, station 19-2 was characterized by a diversity of filamentous red, green and blue-green algae, as well as placcoderm desmids. Vaucheria and Euglena were not present. The difference in the algal community structure (as well as species richness) is due to greater habitat diversity at 19-2 (Sphaerotilus was not observed there).

The diatom community at all three sites consisted of rheophilic and aerophilic forms, which are typically found in well oxygenated streams with pH values greater than 7.0 (Lowe 1974). Station 19-1 was dominated by Navicula cryptocephala and Navicula lanceolata, both of which are characteristic of nutrient enriched streams (Lowe 1974). The former taxon is considered to be tolerant to high levels of organic pollution (Palmer 1969). Species richness was the lowest observed in the study and was generally limited to tolerant forms including soil diatoms. In contrast, station 19-2 contained a greater diversity of diatoms, with much speciation noted in typical stream genera (Achnanthes, Cymbella, Gomphonema and Surirella). Many species in the community are typically found in streams with moderate to high quality water and low turbidity (Harker et al. 1979, 1980). Diatom species richness at station 19-3 was intermediate between the above mentioned sites, most likely due to immigration (drift) from station 19-2. These additional species were present in relatively low numbers. The dominant species in the community were similar to those observed at station 19-1.

Aquatic Macrophytes

Submerged aquatic macrophytes were collected at stations 19-1 and 19-3. Growths of Potamogeton foliosus (leafy pondweed) were sparse to moderate in riffle and run areas at both sites, while moderate growths of Egeria densa (water weed) were noted in the riffle area at station 19-3. No submerged aquatic macrophytes were observed at station 19-2. The occurrence of these aquatic plants may be due to increased nutrient availability, as well as a more organic substrate at those sites.

Macroinvertebrates

Invertebrate collections were taken at three selected sites (Appendix B). At the Arrow Spring (19-1) site, virtually all of the available habitats for invertebrates had been eliminated by the smothering effects of *Sphaerotilus natans*. A total of four taxa were collected. One of those, *Tubifex* sp., an aquatic worm that is considered to be very tolerant to organic waste, lives in the substrate. Another species, *Aphanolaimus* sp., an aquatic nemotode, has temporarily exploited *Sphaerotilus* as habitat and a food source. These organisms were considered to be a nuisance because of their prolific numbers. Two species of aquatic snails, *Elimia* sp. and *Physa* sp., were also able to tolerate the conditions there.

Sharps Branch (19-2) above the confluence with Arrow Spring possessed a diverse benthic community in proportion to stream size and available habitats. A total of 24 species were collected. Of those, nine species were Ephemeropterans (mayflies), which made up most of the species diversity, and contained members in several functional feeding groups. Those organisms have successfully partitioned most of the available food and habitat resources. Other orders were not as speciose, but were an integral part of the community structure.

The benthic substrate at 19-3 was covered with *Sphaerotilus*, again eliminating the invertebrate habitats and organisms, except for several small areas of concrete that served as pads for a low water bridge. Water velocity through those areas was fast enough to keep them free of *Sphaerotilus*, which in turn allowed a group of tolerant Dipterans (midges) and Trichopterans (caddisflies) to occur there. The other taxa collected are attributed to downstream drift from Station 19-2.

Fishes

Fish collections were attempted at all three sampling locations. Four sculpin fry (*Cottus carolinae*), collected with kicknets, were the only fish taken in the area of Arrow Spring (19-1). This is an indication that the area was used for spawning by that species and possibly others. However, the survival of any fry spawned there is probably minimal, due to lack of food and suitable habitat. Avery (1970) and Gaufin and Tarzwell (1955) found that slime growths which envelope stream beds damaged benthic animals that serve as food for fish.

The collections in Sharps Branch above the confluence with Arrow Spring (19-2) revealed a fish community typical of small, second-order streams (eight species) (Appendix C). In addition, several large specimens of longear sunfish (Lepomis megalotis) were captured and released. In contrast, no fish were captured in Sharps Branch below the mouth of Arrow Spring (19-3), despite the presence of diverse habitat. This is probably attributable to a degradation of the water quality, the Sphaerotilus growths, the lack of food organisms or most likely a combination of these factors.

A fish kill was reported on Sharps Branch but no counts of numbers were made by the district fishery biologist due to low numbers of dead fish seen (Wayne Davis, pers. comm.).

Appendix A

Algal Synoptic List for the
Sharps Branch Drainage

Taxa	Station 19-1	19-2	19-3
Chlorophycophyta (Green Algae)			
Chlamydomonas sp.	-	X	-
Cladophora glomerata	-	X	-
Closterium acerosum	X	X	X
Cl. ehrenbergii	-	X	-
Cl. moniliferum	X	X	-
Cosmarium sp.	-	X	-
Oedogonium spp.	-	X	-
Scenedesmus sp.	-	X	-
Stigeoclonium sp.	-	X	X
Ulothrix tenerrima	-	X	-
Chrysophycophyta			
Chrysophysae (Golden Algae)			
Vaucheria sp.	X	-	X
Bacillariophyseae (Diatoms)			
Achnanthes sp.	-	X	-
A. affinis	-	X	X
A. clevei	-	X	-
A. deflexa	-	X	-
A. lanceolata var. *dubia*	X	X	X
A. minutissima	-	X	X
Amphora ovalis var. *pediculus*	X	-	-
Am. perpusilla	-	X	X
Am. submontana	-	X	X
Caloneis bacillum	X	X	-
Cocconeis cf. *fluviatilis*	-	X	-
C. pediculus	-	X	X
C. placentula var. *euglypta*	-	X	X
Cyclotella meneghiniana	-	-	X
Cyc. stelligera	-	X	-
Cymatopleura solea	X	X	X
Cymbella spp.	-	X	X
Cym. sp. K	-	X	X
Cym. affinis	-	X	X
Cym. minuta	X	X	X
Cym. prostrata	-	X	-
Cym. prostrata var. *auerswaldii*	X	X	X
Cym. sinuata	-	X	X
Cym. triangulum	X	-	-

Algal Synoptic List for the
Sharps Branch Drainage

Taxa	Station 19-1	19-2	19-3
Cym. tumida	-	X	X
Cym. turgidula	X	-	-
Diploneis sp.	-	X	X
Eunotia sp.	-	X	-
Frustulia rhomboides var. amphipleuroides	-	X	-
F. rhomboides var. viridula	-	X	-
F. vulgaris	X	X	X
Gomphonema spp.	-	X	-
G. acuminatum	-	-	X
G. angustatum	X	X	X
G. angustatum var. sarcophagus	-	X	X
G. clevei	X	X	X
G. parvulum	X	X	X
G. truncatum	-	X	-
Gyrosigma scalproides	X	X	X
Gy. spencerii	-	-	X
Hantzschia amphioxys	X	-	-
Melosira varians	X	X	X
Meridion circulare	X	X	X
M. circulare var. constrictum	X	-	-
Navicula spp.	X	X	X
Nav. cf. cincta	-	-	X
Nav. cryptocephala	X	X	X
Nav. cuspidata	-	-	X
Nav. gottlandica	-	X	X
Nav. hustedtii	-	X	X
Nav. lanceolata	X	X	X
Nav. mutica	X	-	-
Nav. paucivisitata	X	X	X
Nav. pupula	-	X	X
Nav. radiosa var. parva	-	X	
Nav. radiosa var. tenella	X	X	X
Nav. rhynchocephala	X	X	X
Nav. salinarum var. intermedia	X	X	X
Nav. symmetrica	X	-	X
Nav. tripunctata	-	X	X
Neidium affine var. amphirhynchus	X	-	-
Nitzschia spp.	-	X	-
Nit. acula	X	-	-
Nit. apiculata	-	X	-
Nit. denticula	-	X	-
Nit. dissipata	X	X	X
Nit. fonticola	-	X	X
Nit. frustulum	-	X	-
Nit. gandersheimiensis	-	X	-
Nit. intermedia	X	-	X

Algal Synoptic List for the
Sharps Branch Drainage

Taxa	Station 19-1	19-2	19-3
Nit. linearis	X	X	X
Nit. palea	X	-	X
Nit. cf. rautenbachiae	-	X	X
Nit. recta	-	X	-
Nit. sinuata var. tabellaria	-	X	X
Pinnularia spp.	X	-	X
Rhoicosphenia cruvata	X	X	X
Surirella angusta	X	X	X
Sur. linearis var. helvetica	-	X	-
Sur. ovalis	-	X	X
Sur. ovata	X	X	X
Sur. ovata var. salina	-	X	-
Sur. stalagma	-	X	X
Sur. tenera var. nervosa	-	X	-
Sur. sp.	-	X	-
Synedra sp.	-	X	-
Syn. acus	X	X	X
Syn. rumpens var. familiaris	X	X	X
Syn. rumpens var. fragillarioides	X	X	X
Syn. ulna	X	X	X
Euglenophycophyta (Euglenoid Algae)			
Euglena spp.	X	-	X
Rhodophycophyta (Red Algae)			
Audouinella violacea	-	X	X
Cyanochloronta (Blue-green Algae)			
Dactylococcopsis falcatus	-	X	-
Microcoleus lyngbyaceous	-	X	-
Schizothrix calcicola	X	X	X
Sch. mexicana	-	X	X
TOTAL TAXA	45	87	65

Total Taxa Observed In Study - 107

Appendix B

Macroinvertebrate Synoptic Species List for the

Sharps Branch Drainage

Taxa	Stations 19-1	19-2	19-3
Oligochaeta			
Tubifex tubifex	50	-	-
Lumbriculus sp.	-	2	-
Nemotoda	TNTC	-	TNTC
Gastropoda			
Elimia sp.	2	-	1
Elimia laqueata	-	2	-
Physa sp.	15	-	-
Ephemeroptera			
Stenonema femoratum	-	48	-
Stenonema mediopunctatum	-	2	-
Eurylophella minimella	-	2	-
Eurylophella bicolor	-	2	-
Heptagenia sp.	-	1	-
Isonychia sp.	-	8	-
Baetis sp.	-	10	-
Paraleptophebia sp.	-	1	-
Plecoptera			
Isoperla sp.	-	3	-
Coleoptera			
Psephenus herricki	-	2	-
Dubiraphia sp.	-	1	-

Macroinvertebrate Synoptic Species List for the

Sharps Branch Drainage

Taxa	Stations		
	19-1	19-2	19-3
Trichoptera			
Cheumatopsyche sp.	-	1	-
Hydropsyche sp.	-	1	5
Isopoda			
Lirceus fontinalis	-	6	-
Odonata			
Calopteryx sp.	-	3	-
Decopoda			
Cambarus sp.	-	1	-
Orconectes sp.	-	1	-
Hirudinea			
Helobdella stagnalis	-	1	-
Diptera			
Pedicia sp.	-	2	-
Hydrobaenus pallipes	-	1	1
Cricotopus trimulus	-	1	-
Tribelos sp.	-	1	-
Diamesa sp.	-	-	15
Dicrotendipes modestus	-	-	1
Stictochironomus sp.	-	-	1
Phaenopsectra sp.	-	-	1
Cricotopus tremulus gp. sp.	-	-	7
Orthocladius obumbratus	-	-	9

Macroinvertebrate Synoptic Species List for the

Sharps Branch Drainage

Taxa	Stations		
	19-1	19-2	19-3
Orthocladius mallochi	-	-	11
Polypedilum convictum	-	-	1
Phaenopsectra flavipes	-	-	1
Eukiefferiella bavarica gp.	-	-	2
Amphipoda			
Gammarus sp.	-	-	3
Total Number of Taxa	4	24	14
Total Individuals	67	103	52

Appendix C

Fish Synoptic Species List for the

Sharps Branch Drainage

Taxa	Stations		
	19-1	19-2	19-3
Lampetra aepyptra	-	1	-
Semotilus atromaculatus	-	14	-
Phoxinus erythrogaster	-	3	-
Lepomis cyanellus	-	7	-
Lepomis macrochirus	-	11	-
Lepomis megalotis	-	2	-
Cottus carolinae	4*	2	-
Etheostoma spectabile	-	1	-
Total Individuals	4	41	0
Total Species	1	8	0

* = Fry

Appendix D

Literature Cited

(APHA) American Public Health Association. 1981. Standard methods for the examination of water and wastewater, 15th edition. Am. Publ. Heal. Assoc., Am. Water Works Assoc., Water Poll. Contr. Fed.

Amberg, H. R. and J. F. Cormack. 1960. Factors affecting slime growth in the lower Columbia River and evaluation of some possible control measures. Pulp Pap. Canada, 61:T70-T80.

__________ and __________, and M. R. Rivers. 1962. Slime growth control by intermittent discharge of spent sulfite liquor. TAPPI, 45:770-779.

__________ and R. Elder. 1957. Intermittent discharge of spent sulfite liquor. Trans. Am. Soc. Civ. Engrs., 122:1179-1186.

Avery, E. L. 1970. Effects of domestic sewage on aquatic insects and salmonids of the east Gallatin River, Montana. Water Res., 4:165-177.

Bahr, H. 1953. Zur biologic von Sphaerotilus natans Kützing. Schweiz. Z. Hydrol., 15:285-301.

Bailey, H. H. and J. H. Winsor. 1964. Kentucky Soils. University of Kentucky Agricultural Experiment Station. Misc. Publ. 308. Lexington, KY.

Beck, W. M., Jr. 1955. Suggested method for reporting biotic data. Sew. Ind. Wastes, 27:1193-1197.

Bordner, R., J. Winter and P. Scarpino, editors. 1978. Microbiological methods for monitoring the environment, water and wastes. Environ. Monit. Supp. Lab., U. S. EPA., EPA-600/8-78-017.

Butcher, R. W. 1932. Contribution to our knowledge of the ecology of sewage fungus. Trans. Br. Mycol. Sol., 17:112-123.

Cawley, W. A. 1958. An effect of biological imbalance in streams. Sew. Ind. Wastes, 30:1174-1182.

Cullimore, D. R. and A. E. McCann. 1977. The identification, cultivation and control of iron bacteria in ground water. Aquat. Microbiol. 6:219-261.

Curtis, E. J. C. 1969. Sewage fungus: its nature and effects. Water Res., 3:298-311.

Dias, F. F., H. Okrend and N. C. Dondero. 1968. Growth promoting activity of spent sulfite liquor for Sphaerotilus natans growing in a continuous flow apparatus. Appl. Microbiol., 68:276-278.

Dondero, N. C. 1975. The Sphaerotilus-Leptothrix Group. Ann. Rev. of Microbiol., 29:407-428.

Ellis, D. 1932. Iron bacteria. Fredrick A. Stokes and Co., New York.

Literature Cited

Gaufin, A. R. and C. M. Tarzwell. 1955. Environmental changes in a polluted stream during winter. Am. Midl. Nat., 54:77-88.

Grainge, J. W. and E. Lund. 1969. Quick culturing and control of iron bacteria. J. Am. Water Works Assoc., 61:242-245.

Harker, D. F., Jr., S. M. Call, M. L. Warren, Jr., K. E. Camburn, P. Wigley. 1979. Aquatic biota and water quality survey of the Appalachian Province, eastern Kentucky. Ky. Nat. Pres. Comm. Tech. Rept.

________________, R. R. Hannan, M. L. Warren, Jr., L. R. Phillipe, K. E. Camburn, R. S. Caldwell, S. M. Call, G. J. Fallo and D. VanNorman. 1980. Western Kentucky coal field: preliminary investigations of natural features and cultural resources. Introduction and ecology and ecological features of the western Kentucky coal field, Ky. Nat. Pres. Comm. Tech. Rept.

Hart, C. W., Jr. and S. L. H. Fuller, editors. 1974. Pollution ecology of freshwater invertebrates. Academic Press, New York, NY.

Hattingh, W. J. H. 1963. Activated sludge studies - 3. Influence of nutrition on bulking. Water Treat. J., 9:476-480.

Hawkins, C. P. and J. R. Sedall. 1981. Longitudinal and seasonal changes in functional organization of macroinvertebrate communities in four Oregon streams. Ecology, 62:387-397.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries, 6:21-27.

Lowe, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. Nat. Environ. Res. Cent., Off. Res. Devel. U. S. EPA., Cincinnati, OH. EPA-670/4-74-005.

Luthy, R. G. 1964. New concept for iron bacteria control in water wells. Water Well, 24:29-30.

Machmeier, R. E. 1971. Chlorination of private water supplies. Univ. of MN., Ag. Exten. Serv. Bull., M-156.

Merritt, R. W. and K. W. Cummins. Editors. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co. Dubuque, IA.

McDowell, R. C., G. J. Grabowski, Jr. and S. L. Moore. 1981. Geologic map of Kentucky. U. S. Geol. Surv. and the Eleventh Ky. Geol. Survey, Lexington, KY.

McKee, J. E. and H. W. Wolf. 1974. Water quality criteria. State Water Resour. Contr. Bd., Sacramento, CA.

Literature Cited

Ministry of Technology. 1966. Water pollution research. H.M.S.O., London, England.

Mogg, J. L. 1972. Practical corrosion and incrustation guidelines for water wells. Ground Water, 10:6-11.

Oregon State University. 1971. Slime growth evaluation of treated pulp mill waste. Water Poll. Contr. Res. Series, 1204 ODLQ. U.S. EPA., 08/71:1-54.

Palmer, C. M. 1969. A composite rating of algae tolerating organic pollution. J. Phycol., 5:78-82.

Phaup, J. D. 1968. The biology of Sphaerotilus species. Water Res., 597-614.

__________ and J. Gannon. 1967. Ecology of Sphaerotilus in an experimental outdoor channel. Water Res., 1:523-541.

Prescott, G. W. 1962. Algae of the western Great Lakes area. Wm. C. Brown Co., Dubuque, IA.

Quarterman, E. and R. L. Powell. 1978. Potential ecological/geological natural landmarks of the interior low plateaus. U.S. Dept. Int., Washington, D.C.

Rao, C. S. G. 1970. Occurrence of iron bacteria in the tube well water supply of Howrah. Environ. Heal., 12:273-280.

Rassmussen, C. 1955. Verh. Int. Verein Limnol., 12:819-822.

Schafer, D. C. 1974. The right chemicals are able to restore or increase well yield. The Johnson Drillers J., 1-4:13.

Smith, L. L. and R. H. Kramer. 1963. Survival of walleye eggs in relationship to wood fibres and Sphaerotilus natans in the Rainy River, Minnesota. Trans. Am. Fish. Soc., 92:220-234.

Stokes, J. L. 1954. Studies on the filamentous sheathed iron bacterium Sphaerotilus natans. J. Bacteriol., 67:2780-2791.

(U.S. EPA) United States Environmental Protection Agency. 1973. Current practices in water microbiology. Off. Water Prog., United States Environ. Prot. Agency.

__________ 1979. Methods for chemical analysis of water and wastes. Environ. Monit. Supp. Lab., Off. Res. Devel. U. S. EPA., Cincinnati, OH. EPA-600/4-79-020.

van der Werff, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. Int. Ver. Theor. Angew. Limnol. Verh., 12:276-277.

Literature Cited

Weber, C. I., ed. 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. U.S. EPA., Cincinnati, OH. EPA-670/4-73-001.

Weston, R., Inc. no date. The river basin water quality management plan for Kentucky, Green River. Ky. Dept. Nat. Res. Environ. Prot., Frankfort, KY.

Woods, G. A. 1973. Bacteria: friends or foes? Che. Engin., 80:81-84.

Wuhrmann, K. 1964. River bacteriology and the role of bacteria in self-purification of rivers. Princ. and Appl. in Aquat. Microbiol., John Wiley, New York, NY.