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MICROFLORA OF THE YELLOWSTONE RIVER
II. PERTURBATIONS THROUGH BILLINGS

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INTRODUCTION

The last floristic survey of the Yellowstone River through Billings was accomplished 20 years ago when the river was receiving a pollution burden much greater than it receives today. In 1955, no bottom organisms occurred in the first 11 miles below waste outfalls at Billings; sewage "fungus" was commonplace; and taste and odor problems were chronic (7).

Today, due largely to the application of pollution control technology, the situation is much improved and the river is getting cleaner (2). Nevertheless, the Yellowstone River from Laurel to Billings remains water quality limited because of discharges from the Laurel and Billings sewage treatment plants and wastewater discharges from three oil refineries, a beet sugar factory and a coal-fired steam-electric plant. There are also a number of nonpoint source sediment and oil problems in this reach of the river (5).

This paper describes the response of Yellowstone River algae to a variety of waste discharges originating in the Laurel-Billings municipal-industrial complex. Emphasis is placed on the relationship between the composition of benthic diatom associations and ambient concentrations of selected algal nutrients. This investigation was conducted as a contribution to the biological portion of a waste load allocation study prepared for this section of the Yellowstone by the Montana Department of Health and Environmental Sciences.

METHODS

Sampling Stations and Schedule

Nine stations were sampled from Laurel downstream to Huntley, including the Clarks Fork River and Yegen Drain:

I. Yellowstone River at Laurel (above Laurel wastewater discharge and confluence with the Clarks Fork River).

II. Clarks Fork River at mouth.

III. Yellowstone River at Duck Creek Bridge.

IV. Yellowstone River at South Bridge (Billings).

V. Yellowstone River below Corette plant.

VI. Yellowstone River at East Bridge (Billings).

VII. Yegen Drain at mouth.

VIII. Yellowstone River below Yegen Drain (above Billings wastewater discharge).

IX. Yellowstone River near Huntley.

Periphyton samples and water samples for algal nutrients were collected at these stations on the dates listed in Table I.

Table I. Sampling Schedule*

	Station and Date								
	I	II	III	IV	V	VI	VII	VIII	IX
Nutrient Samples	9/9	9/9	9/9	9/9	7/22	9/9	10/23	10/7	9/9
Periphyton Samples	9/9	9/9	9/9	9/9	9/16	9/9	11/1	11/1	9/9

*All samples taken in 1975 except the nutrient sample at Station V, which was collected in 1974.

Field and Laboratory Procedures

At each station, periphyton samples were obtained by scraping natural substrates in proportion to the surface area of each type that was exposed for colonization. (Rocks predominated at most stations). Substrates from both sluggish and rapidly flowing water were sampled in order to minimize possible bias caused by current effects. This procedure allows for collection of a composite sample that is representative of the range of physical conditions prevailing at each site at the time of collection.

From each sample, a subsample was taken and scanned microscopically to determine the presence and relative importance of non-diatom algae. Then, in a manner prescribed by the Environmental Protection Agency (4), each sample was acidified and oxidized, a permanent mount was prepared, and a diatom species proportional count was performed.

Nutrient analyses were performed at the Department of Health and Environmental Sciences' water laboratory in Helena following methods outlined by the American Public Health Association (1).

RESULTS

Algal Nutrients

The results of algal nutrient analyses are presented in Table II. The Yegen Drain (VII) was a major contributor of all measured forms of nitrogen and phosphorus. The Clarks Fork

Table II. Algal Nutrients in the Yellowstone River through Billings (All values in mg/l)

Nutrient	Station								
	I	II	III	IV	V	VI	VII	VIII	IX
$NO_3 + NO_2$ (Total as N)	0.05	0.38	0.05	0.02	0.06	0.05	0.34	0.12	0.06
Ammonia (Total as N)	<0.01	<0.01	<0.01	<0.01	--	<0.01	3.2	0.46	<0.01
Nitrogen (Kjeldahl, Total as N)	0.18	0.45	0.24	0.26	--	0.15	4.0	1.0	0.24
Phosphate (PO_4 as P)	0.006	0.010	0.009	0.004	0.023	0.005	0.100	0.022	0.020
Phosphorus (Total as P)	0.013	0.016	0.013	0.016	--	0.014	0.124	0.032	0.027

River (II) introduced appreciably higher levels of nitrate while phosphate was elevated below the Corette plant (V). Overall, comparing nutrient concentrations at Laurel (I) and Huntley (IX), nitrogen components were not appreciably increased by discharges through Billings, but phosphate and total phosphorus were.

Non-Diatom Algae

Diatoms dominated the flora at all stations except in and below Yegen Drain where *Oscillatoria* and *Stigeoclonium* were the dominant algae, respectively. *Euglena* and a filamentous bacterium resembling *Sphaerotilus* were also evident at these two sites. *Cladophora glomerata* was abundant at Laurel and below the Corette discharge. The remaining 11 genera of non-diatom algae--all greens and blue-greens--were relatively uncommon.

Diatoms

The principal features of benthic diatom associations at the nine sites are given in Table III.

The seven major taxa are those that contributed 10 percent or more relative abundance in one or more collections. Pollution tolerances for these taxa were obtained from Cholnoky (3) and Lowe (6). Generally, *Achnanthes minutissima* and *Cymbella affinis* are intolerant of organic pollution; *Diatoma vulgare* and *Nitzschia dissipata* will tolerate only weak organic pollution but thrive where oxidation is complete; and *Navicula cryptocephala* var. *veneta* and *Nitzschia palea* are tolerant of organic pollution. The characteristically aerophilous *Navicula mutica* was an anomaly in the Yellowstone River. The total abundance of *Nitzschia* species is generally regarded as a suitable indicator of nitrogenous pollution. With one exception, to be discussed later, these indicator taxa behaved as expected considering their pollution tolerances and the nature and amount of enrichment. Relative abundance values for all major taxa were reasonably close at the stations bracketing the study section (stations I and IX).

The most striking feature about the diatom community measures in Table III is the position held by the Yegen Drain collection (VII). Here, the numbers of taxa observed and counted were both conspicuously and unexpectedly maximum. On the other hand, diatom associations in the Clarks Fork River (II) and below the Corette plant (V) had the fewest taxa, indicating they may have been subject to some perturbation.

DISCUSSION AND CONCLUSIONS

The diatom association below the Corette plant (station V) had the fewest taxa and the highest relative abundance for a single taxon of any association at all of the other stations

Table III. Structure of Benthic Diatom Associations in the Yellowstone River through Billings

Parameter	Station I	II	III	IV	V	VI	VII	VIII	IX
Major Taxa (recorded as percent of total population)									
Achnanthes minutissima	2.0	35.8	25.4	25.0	18.6	21.0	7.4	1.7	9.9
Cymbella affinis	11.6	18.7	23.7	21.2	42.3	15.8	14.4	1.7	16.5
Diatoma vulgare	15.6	0.3	6.7	1.9	3.0	5.7	3.8	2.8	4.9
Navicula cryptocephala v. *veneta*	4.6	8.7	3.8	5.2	3.0	4.1	6.3	29.7	5.5
Navicula mutica								11.7	
Nitzschia dissipata	25.8	8.7	7.3	11.1	16.9	18.0	15.5	5.6	19.7
Nitzschia palea	3.7	2.0	4.7	3.0	1.2	2.7	9.0	27.8	4.1
Total *Nitzschia* species	30.7	15.8	16.8	21.0	20.5	27.1	34.6	41.7	35.8
Taxa Observed	46	42	51	52	39	47	58	55	46
Taxa Counted	32	28	33	37	27	36	49	37	31
Cells Counted	352	358	342	368	338	366	367	360	345

sampled (Table III). These findings might be interpreted as indicators of stress on the aquatic biota. Although nutrient data at this site are incomplete and out-dated, phosphate did appear to be significantly more concentrated here than upstream (Table II). However, the abundance of the saprophobic diatoms *Cymbella affinis* and *Achnanthes minutissima* and the relatively minor importance of *Nitzschia* species indicate chemical water quality below the Corette plant to be rather good. Because *C. affinis* is a summer diatom, i.e., it prefers warmer waters (6), the stress causing dominance of this taxon and depressed floristic variety at this location might be brought on by elevated temperature in the plant's thermal plume rather than by some chemical constituent introduced into the river. Unfortunately, temperature data were not collected and this possibility cannot be confirmed.

While burdened with a much heavier nutrient load (Table II), Yegen Drain (VII) had more diatom taxa than other study sites (Table III). One explanation might be that Yegen Drain offers a greater diversity of habitats and a physical environment, in terms of substrate, depth, temperature and flow regime, favorable to a larger variety of benthic diatoms. This situation deserves more attention and illustrates the fact that factors other than pollution load are responsible for biological variety in streams, making it difficult, if not impossible, to assess water quality on this basis alone.

Nitzschia palea is one of several diatom species that thrives on organic nitrogen compounds (8). Its abundance at Station VIII was apparently in response to the heavy nitrogen load introduced by the Yegen Drain (Tables II and III). The capacity of *N. palea* to deaminate amino acids and liberate free ammonia to the atmosphere may have been responsible in part for the rapid reduction in all nitrogen species below Yegen Drain and the eventual recovery to near baseline levels at Huntley (IX). With four times the nitrogen load, it is not clear why the Yegen Drain (VII) had only one-third the *N. palea* population of the Yellowstone station downstream (VIII). This may have been due to more intense competition and/or physical factors less favorable to *N. palea* in Yegen Drain.

Except for nitrate (Table II), water quality at the mouth of the Clarks Fork River (Station II) appeared to be quite good despite the relatively low number of diatom taxa (Table III). However, from the standpoint of water quality the Clarks Fork River carries a high silt load, which probably has a greater effect on the biota than any chemical parameter (9). Nevertheless, the Clarks Fork and discharges through Laurel had no discernible negative effect on Yellowstone River periphyton at Duck Creek Bridge (Station III).

The phytoplankton data from the 1955 Yellowstone survey (7) are not strictly comparable to the data reported here.

Nonetheless, it is evident that water quality has improved considerably in the intervening 20 years. On the whole, comparing samples from Laurel and Huntley, Yellowstone River periphyton was not indelibly affected by perturbations through Billings in 1975. Self-purification and recovery of the microflora from pollution may be considered complete at Huntley.

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EMBRYOLOGY OF *EHRETIA ACUMINATA* R. BR.

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INTRODUCTION

Ehretioideae is the second sub-family of Boraginaceae consisting of ten genera and about 98 species (Engler and Prantl, 1897). Hooker (1885) considers Ehretioideae as a tribe and includes only three genera viz., *Ehretia*, *Coldenia* and *Rhabdia* (=*Rotula*). However, Hutchinson (7) has elevated Ehretioideae to the status of a family Ehretiaceae and includes all the genera that are contained in the sub-family Ehretioideae of Engler as well as those of Cordioideae, in addition to *Duckeodendron* which he considers to be of uncertain position. He has separated these genera depending upon the arborescent habit of most of the members and has included the Ehretiaceae under the order Verbenales.

In this sub-family, plants exhibit herbaceous to tree-like habit. The ovary is four-lobed and the style is terminal. Occasionally the style is bipartite. The stigma is capitate and the fruit is fleshy and drupaceous. In South India, this sub-family is represented by one species of *Coldenia*, eight species of *Ehretia* and one species of *Rotula* (5).

Ehretioideae has not received much attention by the embryologists. Svensson (19) who has done the most extensive work on the family Boraginaceae has also omitted this sub-family due to the inavailability of properly fixed material. However, recently there have been a few reports on the embryology of this sub-family which include the investigations of Venkateswarlu and Atchutaramamurti (20) on *Coldenia procumbens*, Johri and Vasil (9) on *Ehretia laevis* and Nagaraj and Fathima (15, 17) on *Rotula aquatica*. The present investigation deals with microsporogenesis, development of anther, megasporogenesis, development and organization of megagametophyte, fertilization, development of endosperm and the embryo, structure and development of pericarp and seed coat in *Ehretia acuminata* R. Br. which is a tree with alternate and glabrous leaves flowering during March - June. The flowers are arranged in densely compound terminal and axillary panicles.

MATERIALS AND METHODS

Young flower buds, flowers and fruits of *Ehretia acuminata* were collected from Lal Baugh, Bangalore, Karnataka, India, during March - July and fixed in formalin-acetic acid-alcohol for 24 hours. They were stored in 70 percent alcohol. Customary methods of dehydration, infiltration and paraffin embedding were followed. Sections were cut at 5-20 microns thickness and stained in Heidenhein's iron alum haematoxylin. Fast green or eosin was used as counter stain.