EFFECTS OF AN OIL SPILL ON THE COMPOSITION AND STRUCTURE OF THE PERIPHYTON COMMUNITY IN CASINO CREEK AND BIG SPRING CREEK NEAR LEWISTOWN, MONTANA

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SUMMARY

Four composite periphyton samples were collected in March 1999 from Casino Creek and Big Spring Creek in central Montana after several hundred gallons of used motor oil were released into Casino Creek. Samples were collected following DEQ standard operating procedures, processed and analyzed using standard methods for periphyton, and evaluated following modified USEPA rapid bioassessment protocols for wadeable streams.

Aside from coating substrates and macroalgae, the oil did not appear to have an immediate effect on the species composition and structure of the periphyton community. The lack of a toxic or chemical effect may be due to cold water temperatures at the time of the spill, dilution and flushing of toxic chemicals, low concentrations of volatile and soluble compounds in the oil, and the relatively short time that had elapsed between the spill and periphyton sampling. The spilled oil may not have had its full biological effect until much later when water temperatures were higher.

Diatom association metrics appeared to indicate problematic levels of siltation at all of the sampling sites. Siltation levels were highest in Casino Creek and in Big Spring Creek below Casino Creek. Siltation index values in Big Spring Creek were higher than those recorded in August 1998 for the same reach of stream. It's possible that the spilled oil served as a substrate for motile diatoms and had the same effect on the siltation index as do fine particles of inorganic sediment. It's also possible that the elevated siltation index levels were due entirely or in part to the recorded abundance of motile, cold season diatoms in the genera *Navicula* and *Nitzschia*.

INTRODUCTION

Near the first of March 1999, several hundred gallons of used motor oil were released into Casino Creek, a tributary of Big Spring Creek south of Lewistown, Montana. Several days later, Anne Tews, an employee of the Montana Department of Fish, Wildlife and Parks, conducted a field assessment of the spill.

This report is based on 4 composite periphyton samples that were collected by Tews during her field assessment. This report evaluates the effects of the oil spill on the species composition and community structure of periphyton (benthic algae) communities in Casino Creek and Big Spring Creek. Using biocriteria for wadeable mountain streams in Montana, the report also assesses the impacts of the spill on aquatic life uses in the two streams.

The periphyton or phytobenthos community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity of streams in western Montana (Bahls et al. 1992).

Plafkin et al. (1989) and Stevenson and Bahls (1999) list several advantages of using periphyton in biological assessments of streams:

- Algae are universally present in large numbers in all streams and unimpaired periphyton assemblages typically support a large number (>30) of species;
- Algae have rapid reproduction rates and short life cycles, making them useful indicators of short-term impacts;
- As primary producers, algae are most directly affected by physical and chemical factors, such as temperature, nutrients, and toxins;
- Sampling is quick, easy and inexpensive, and causes minimal damage to resident biota and their habitat;
- Standard methods and criteria exist for evaluating the

composition, structure, and biomass of algal associations;

- Identification to species is straightforward for the diatoms, for which there is a large body of taxonomic and ecological literature;
- Excessive algae growth in streams is often correctly perceived as a problem by the public;
- Periphyton and other biological communities reflect the *biological integrity*[1] of waterbodies; restoring and maintaining the biological integrity of waterbodies is a goal of the federal Clean Water Act;
- Periphyton and other biological communities integrate the effects of different stressors and provide a measure of their aggregate impact; and
- Periphyton and other biological communities may be the only practical means of evaluating impacts from non-point sources of pollution where specific ambient criteria do not exist (e.g., impacts that degrade habitat or increase nutrients).

Periphyton is a diverse assortment of simple photosynthetic organisms called algae, and other microorganisms that live attached to or in close proximity of the stream bottom. Most algae, such as the diatoms, are microscopic. Diatoms are distinguished by having a cell wall composed of opaline glass--hydrated amorphous silica. Diatoms often carpet a stream bottom with a slippery brown film.

Some algae, such as the filamentous greens, are conspicuous and their excessive growth may be aesthetically displeasing, deplete dissolved oxygen, interfere with fishing and fish spawning, clog irrigation intakes, create tastes and odors in drinking water supplies, and cause other problems.

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[1] *Biological integrity* is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

PROJECT AREA AND SAMPLING SITES

The project area is located in Fergus County near the city of Lewistown in central Montana. Big Springs, a few miles southeast of Lewistown, is the source of Big Spring Creek and generates most of its streamflow. Major tributaries of Big Spring Creek, including Casino Creek, head on the north flanks of the Big Snowy Mountains, an outlier of the Middle Rockies Ecoregion. Study sites are located in the Montana Valley and Foothill Prairies Ecoregion (Omernik and Gallant 1987).

The surface geology of the Big Snowy Mountains consists of limestones and dolomites of Paleozoic age (Taylor and Ashley, undated). Lower in the watershed, where the study sites are located, Cretaceous shales of the Colorado Group are exposed (Taylor and Ashley, undated).

Natural vegegation in the study area is mixed grassland dominated by rough fescue, Idaho fescue, and bluebunch wheatgrass (USDA 1976). The main land uses in the Big Spring Creek watershed are agriculture, recreation, and urban development.

Periphyton samples were collected at one site on Casino Creek and 3 sites on Big Spring Creek (Table 1). One sample was collected from Big Spring Creek just above Casino Creek and two samples were collected at a distance of 1 mile and several miles below Casino Creek. Only one sample was collected from Casino Creek--below the spill--because of problems with access (Carol Endicott, MDEQ, personal communication).

Elevations at the sampling sites range from about 4,000 feet at the sites above Lewistown to about 3,900 feet at the lowest site on Big Spring Creek below Lewistown. Casino Creek and the upper 2 sites on Big Spring Creek are classified B-1 in the Montana Surface Water Quality Standards; Big Spring Creek below

Lewistown is classified B-2.

METHODS

Periphyton samples were collected following standard operating procedures of the MDEQ Planning, Prevention, and Assistance Division. Using appropriate tools, microalgae were scraped, brushed, or sucked from natural substrates in proportion to the rank of those substrates at the study site. Macroalgae were picked by hand in proportion to their abundance at the site. All collections of microalgae and macroalgae were pooled into a common container and preserved with Lugol's solution.

Samples were examined to estimate the relative abundance and rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described in Bahls (1993). Soft algae were identified using Prescott (1978), Smith (1950), and Whitford and Schumacher (1984). These books also served as the main references on the ecology of the soft algae.

After the identification of soft algae, raw periphyton samples were cleaned of organic matter using sulfuric acid, and permanent diatom slides were prepared in a high refractive index mounting medium following *Standard Methods for the Examination of Water and Wastewater* (APHA 1998). For each slide, between 429 and 476 diatom cells (858 to 952 valves) were counted at random and identified to species. The following were used as the main taxonomic and autecological references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Patrick and Reimer 1966, 1975. Lowe (1974) was also used extensively as an ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics (Table 2). A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999).

One additional metric was calculated for this study: percent of cells in the diatom family Epithemiaceae. This family is represented in rivers by two genera, *Epithemia* and *Rhopalodia*, which commonly harbor endosymbiotic nitrogen-fixing bluegreen algae (cyanobacteria) within their cells. A diatom association that contains a large percentage of cells in these genera may indicate nitrogen-limiting conditions, that is, low nitrogen to phosphorus ratios (Stevenson and Pan 1999).

Metric values from study sites were compared to numeric biocriteria developed for streams in the Rocky Mountain Ecoregions of Montana (Table 3). These criteria are based on metric values measured in least-impaired reference streams (Bahls et al. 1992) and on metric values measured in streams that are known to be impaired by various sources and causes of pollution (Bahls 1993).

The criteria in Table 3 distinguish among four levels of impairment and three levels of aquatic life use support: no impairment or only minor impairment (full support); moderate impairment (partial support); and severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor *biological integrity*, respectively.

Only metrics generated from periphyton samples collected in summer (June 21-September 21) may be compared with confidence to the biocriteria in Table 3. Metric values change seasonally and summer is the season in which reference streams and impaired streams were sampled for the purpose of biocriteria development. **Because the samples analyzed for this report were collected in winter, comparisons of metrics with the biocriteria in Table 3 must be made with caution.**

Quality Assurance. Several steps were taken to assure that the study results are accurate and reproducible. Upon receipt of the samples, station and sample information were recorded in a laboratory notebook and samples were assigned a unique number compatible with the Montana Diatom Database, e.g., 1819-01. The first part of this number (1819) designates the sampling site (Big Spring Creek above Casino Creek at Brewery Flats); the second part of the number (01) designates the number of periphyton samples that have been collected at this site to date for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with station and sample information provided by MDEQ. A portion of the raw sample was used to make duplicate diatom slides.

On completion of the project, station information, sample information, and diatom proportional count data will be entered into the Montana Diatom Database. One set of diatom slides will be deposited in the University of Montana Herbarium in Missoula. The other set of slides will be retained by ***Hannaea*** in Helena.

RESULTS AND DISCUSSION

Results are presented in Tables 4, 5 and 6, located near the end of this report following the Literature Cited section. Spreadsheets containing completed diatom proportional counts, with species pollution tolerance classes (PTC) and calculated percent abundances, are attached as Appendix A.

SAMPLE NOTES

Big Spring Creek above Casino Creek (2). This sample was partly decomposed and smelled of hydrogen sulfide when opened on August 6. The bulk of this sample was sand and gravel. About 10% of the diatom cells were empty.

Casino Creek at Public Bridge (4). The bulk of this sample consisted of clumps of organic floc held together by a matrix of fungal hyphae. The sample was preserved with formalin. The *Cladophora* in this sample was also covered with organic floc. About 10% of the diatom cells were empty. The *Closterium* in this sample was smaller than the one in Big Spring Creek upstream and was probably a different species.

Big Spring Creek below Casino Creek (3). An oily residue covered everything in this sample. The soft algae were in poor condition. About 30% of the diatom cells were empty. The sample was preserved with formalin.

Big Spring Creek below Lewistown (1). This sample was partly decomposed and smelled of hydrogen sulfide when opened on August 6. The soft algae were in poor condition and were covered with a taupe (gray/brown/green) colored organic floc. An oily residue remained on the slide and coverslip after the sample was wiped off. About 10% of the diatom cells were empty.

NON-DIATOM ALGAE

The non-diatom algal flora was similar above and below the spill (Table 4). Diatoms and the filamentous green algae *Cladophora* and *Ulothrix* were present at all four of the sites. The desmid *Closterium* was common in Casino Creek and in Big Spring Creek both above and below Casino Creek.

Some differences did occur, however. The filamentous green algae *Spirogyra* and *Zygnema* were found in Big Spring Creek below Casino Creek but not above Casino Creek or in Casino Creek itself. The coenocytic chrysophyte *Vaucheria* was found only in Big Spring Creek below Casino Creek. *Oscillatoria*, a filamentous cyanophyte, was found only in Casino Creek.

Phormidium, another filamentous bluegreen, was found only in Casino Creek and in Big Spring Creek just below Casino Creek. However, in an earlier assessment of Big Spring Creek (Bahls 1999), *Phormidium* was found to be common throughout the upper reaches of the stream above Lewistown.

Not much can be made of these results because of the perennial and persistent nature of some non-diatom algae (especially *Cladophora* and *Vaucheria*) and the poor condition of these samples. Diatoms, because of their solitary growth habit and rapid division rates, are better suited for detecting the short-term effects of the oil spill.

DIATOMS

All of the major diatom species in Casino Creek and Big Spring Creek were Class 3 or pollution-sensitive taxa (Table 5). Class 3 diatoms typically *decrease* in abundance with increasing concentrations of organic waste (Lange-Bertalot 1979). However, all of these major species except *Achnanthes minutissima* exhibit eutrophic tendencies, meaning that they grow best in higher concentrations of inorganic nutrients such as nitrogen and phosphorus (Lowe 1974).

Three of the major diatom species--*Diatoma vulgare*, *Gomphonema olivaceum*, and *Nitzschia dissipata*--are cool season diatoms. These diatoms often dominate the diatom assemblages of mountain streams in the fall, winter, and spring (L. Bahls, unpublished data).

Species diversity values at all sites were normal for healthy mountain streams (Table 5). Diatom diversity in Big Spring Creek was slightly higher just below Casino Creek than it was above Casino Creek. The lowest diversity value was recorded in Big Spring Creek below Lewistown.

Casino Creek had the lowest pollution index value of 2.56. Nevertheless, this was still within the acceptable range for mountain streams in Montana. Pollution index values for Big Spring Creek above and below Casino Creek were virtually the same.

Siltation index values indicated problematic levels of fine sediments at all sites (Table 5). **If this assessment had been conducted during the summer index period**, Big Spring Creek above

Casino Creek would have been rated as suffering from minor silation, and both Casino Creek and Big Spring Creek below Casino Creek would have been moderately impaired by silation. Below Lewistown, the siltation index would have indicated severe impairment and nonsupport of aquatic life uses.

The spilled oil may have served as a substrate for motile diatoms and may have had the same effect on the siltation index as do fine particles of inorganic sediment. An assessment of Big Spring Creek conducted in August 1998 indicated no siltation problems in Big Spring Creek above Lewistown and only minor siltation below Lewistown (Bahls 1999). Certain cold season species of *Navicula* and *Nitzschia* were also more abundant in the winter samples. This may explain all or part of the elevated siltation index values in March as compared to August.

The disturbance index (% abundance of *A. minutissima*) indicated no significant physical, chemical, or biological disturbance had occurred in the days and weeks prior to sampling. The percent dominant species values were marginally large at two sites because of an abundance of cool season diatoms. Abnormal cells, which may result from toxins in the water, were not found at any of the sites.

Casino Creek had slightly more than a third of its diatom flora in common with sites in Big Spring Creek above and below Casino Creek (Table 5). It is not unusual for a tributary stream to have a diatom flora that is this different from that of the mainstem, especially if the streams have sources that are different geologically or hydrologically.

The two sites in Big Spring Creek above and below Casino Creek had very similar diatom floras, sharing nearly 80% of their diatoms. This would indicate that Casino Creek and its load of waste oil did not have a significant effect on Big Spring Creek.

The site below Casino Creek and the site below Lewistown had somewhat less than 60% of their diatom floras in common, indicating minor perturbations in this reach.

The immediate impact of the oil spill appeared to be limited to the physical coating of objects in the stream, including substrates and macroalgae. The oil did not appear to exert a toxic effect or to alter the composition and structure of the periphyton community.

There are several possible reasons why the oil spill did not have a measurable impact on the composition and structure of the periphyton community in Casino Creek and Big Spring Creek:

- The used motor oil may have contained only small amounts of volatile or soluble organic compounds;

- Dilution and flushing may have been sufficient to negate the effects of any soluble or volatile organics in the oil;

- Cold temperatures at the time of the spill may have kept the oil viscous and prevented it from mixing readily with stream water; and

- The short time elapsed between the spill and periphyton sampling may not have been long enough for the oil to have worked its full effect on the algae.

The full biological impact of the oil may have occurred much later when water temperatures were higher. The persistence of unweathered oil within sediments can have a long-term effect on the structure of benthic communities and cause the demise of sensitive aquatic species (USEPA 1986).

The diatom associations at the three Big Spring Creek sites were also assessed using Protocol II (Table 12) in Bahls (1993). This method compares selected metrics at study sites to the corresponding metrics at an upstream control site, in this case Big Spring Creek above Casino Creek at Brewery Flats (site #2). This approach can be used at any time of the year.

Metric scores and impairment ratings using Protocol II are presented in Table 6. In Protocol II, the upstream control site is assumed to be relatively unimpaired and automatically scores a perfect 100 percent for all metrics. The bioassessment using Protocol II (Table 6) indicates that the site below Casino Creek had excellent biointegrity and no impairment, and the site below Lewistown had good biointegrity and minor impairment from a lower diversity value, an elevated siltation index, and a somewhat lower than expected similarity index.

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Table 1. Location of periphyton sampling stations on Casino Creek and Big Spring Creek, MDFWP/MDEQ station code, sample number in the Montana Diatom Database, legal description, latitude/longitude, and sample date. Sites are listed in order from upstream to downstream. The latitude/longitude of the spill site is 47 02 40/109 25 15.

Location	Station Code	Sample Number	Legal Description	Latitude/ Longitude	Sample Date
Big Spring Creek above Casino Creek at Brewery Flats	#2	1819-01	T15BR18E23CAB	47 02 30 109 23 40	03/08/99
Casino Creek at Public Bridge (below oil spill)	#4	1820-01	T15NR18E22ADA	47 02 50 109 24 30	03/09/99
Big Spring Creek 1 mile below Casino Creek at Dunningtons	#3	1821-01	T15NR18E14CCC	47 03 10 109 24 00	03/09/99
Big Spring Creek below Lewistown at Tresch (Carroll Trail)	#1	1822-01	T15NR18E04	47 06 15 109 25 15	03/08/99

Table 6. Metric scores and impairment ratings for Big Spring Creek sites based on a comparison of diatom metrics at the study sites to metrics at the upstream control site above Casino Creek at Brewery Flats (Protocol II and Table 12 in Bahls 1993). Underlined values indicate full support of aquatic life uses with minor impairment; all other values indicate full support with no impairment.

Metric	Big Spring Cr. above Casino Cr. (2)	Big Spring Cr. below Casino Cr. (3)	Big Spring Cr. below Lewistown (1)
Shannon Diversity Index	100	101	<u>80</u>
Pollution Index	100	101	105
Siltation Index	100	81	<u>57</u>
Similarity Index	100	80	<u>52</u>

Table 5. Percent abundance of major diatom species[1] and values of selected diatom association metrics for periphyton samples collected from Casino Creek and Big Spring Creek in March 1999. <u>Underlined values</u> indicate full support of aquatic life uses with minor impairment; **bold values** indicate partial support of aquatic life uses with moderate impairment; <u>**underlined and bold values**</u> indiate nonsupport of aquatic life uses with severe impairment based on criteria for wadeable mountain streams in Table 3.

Species/Metric (Pollution Tolerance Class)	Big Spring Cr. above Casino Cr. (2)	Casino Cr. at Bridge (4)	Big Spring Cr. below Casino Cr. (3)	Big Spring Cr. below Lewistown (1)
Achnanthes minutissima (3)	18.91	2.38	18.48	6.64
Diatoma vulgare (3)	2.63	2.27	4.27	14.22
Gomphonema olivaceum (3)	3.36	25.97	2.56	3.15
Navicula tripunctata (3)	6.20	5.19	4.49	13.87
Nitzschia dissipata (3)	1.58	12.66	3.42	7.81
Nitzschia fonticola (3)	12.71	0.87	16.35	27.16
Number of Cells Counted	476	462	468	429
Shannon Species Diversity	4.51	3.98	4.57	3.60
Pollution Index	2.69	2.56	2.72	2.82
Siltation Index	<u>36.79</u>	**43.73**	**45.49**	<u>**64.91**</u>
Disturbance Index	18.91	2.38	18.48	6.64
Number of Species Counted	52	41	59	37
Percent Dominant Species	18.91	<u>25.97</u>	18.48	<u>27.16</u>
Percent Abnormal Cells	0.00	0.00	0.00	0.00
Similarity Index	37.61		38.34	58.91
(Sites 2 and 3)		79.95		

[1] A major diatom species is here defined as one that accounts for 10.0 percent or more of the diatom cells that were counted at one or more stations in a sample set.

Table 4. Estimated relative abundance of algal cells and rank by volume of diatoms and genera of non-diatom algae in periphyton samples collected from Casino Creek and Big Spring Creek in March 1999. R = rare, C = common, VC = very common, A = abundant, VA = very abundant.

Taxa	Big Spring Cr. above Casino Cr. (2)	Casino Cr. at Bridge (4)	Big Spring Cr. below Casino Cr. (3)	Big Spring Cr. below Lewistown (1)
Chlorophyta				
Cladophora	C(2)	A(2)	VC(4)	A(2)
Closterium	C(4)	C(6)	C(6)	
Spirogyra			A(3)	C(4)
Ulothrix	C(3)	VC(3)	C(5)	C(6)
Zygnema				C(5)
Chrysophyta				
Diatoms	VA(1)	VA(1)	VA(2)	VA(1)
Vaucheria			VA(1)	A(3)
Cyanophyta				
Oscillatoria		VC(4)		
Phormidium		C(5)	C(7)	

Table 3. Criteria for rating levels of biological integrity, environmental impairment or natural stress, and aquatic life use support in wadeable **mountain** streams of Montana using selected metrics for benthic diatom associations. The lowest rating for any one metric is the overall rating for the study site.

Biological Integrity/ Impairment or Natural Stress/Use Support	Diversity Index (Shannon)	Pollution Index	Siltation Index	Disturbance Index	Number of Species Counted	Percent Dominant Species	Percent Abnormal Cells	Similarity Index[1]
Excellent None/Full Support	>2.99	>2.50	<20.0	<25.0	>29	<25.0	0.0	>59.9
Good/Minor Full Support	2.00-2.99	2.01-2.50	20.0-39.9	25.0-49.9	20-29	25.0-49.9	>0.0-<1.0	40.0-59.9
Fair/Moderate Partial Support	1.00-1.99	1.50-2.00	40.0-59.9	50.0-74.9	10-19	50.0-74.9	1.0-9.9	20.0-39.9
Poor/Severe Nonsupport	<1.00	<1.50	>59.9	>74.9	<10	>74.9	>9.9	<20.0

[1] The Similarity Index or Percent Community Similarity (Whittaker 1952) may be used to compare a study site to an unimpaired upstream control site on the same stream. This metric measures the degree of floristic similarity between diatom associations at the two sites and is the sum of the smaller of the two percent abundance values for each species that is common to both sites. Adjacent riffles on the same stream, without intervening tributaries or environmental perturbations, will generally have at least 60% of their diatom floras in common (Bahls 1993). PCS may also be used to guage the relative amount of impairment or recovery that occurs between adjacent study sites: >59.9% = very similar floras, no change; 40.0-59.9% = somewhat similar floras, minor change; 20.0-39.9% = somewhat dissimilar floras, moderate change; <20.0% = very dissimilar floras, major change.

Table 2. Diatom association metrics used to evaluate biological integrity in Montana streams: reference, range of values in Montana streams, and expected direction of metric response to increasing anthropogenic perturbation or natural stress.

Metric	Reference	Range of Values	Expected Response
Shannon Species Diversity	Bahls 1979	0.00-5.00+	Decrease[1]
Pollution Index[2]	Bahls 1993	1.00-3.00	Decrease
Siltation Index[3]	Bahls 1993	0.00-90.0+	Increase
Disturbance Index[4]	Barbour *et al.* 1999	0.00-100.0	Increase
No. Species Counted	Bahls 1979, 1993	0-100+	Decrease[1]
Percent Dominant Species	Barbour *et al.* 1999	5.0-100.0	Increase
Percent Abnormal Cells	McFarland *et al.* 1997	0.0-20.0+	Increase
Similarity Index	Whittaker 1952	0.0-80.0+	Decrease

[1] Shannon diversity and species richness may increase somewhat in naturally nutrient-poor mountain streams in response to slight to moderate increases in nutrients or sediment.

[2] This is a composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species.

[3] Computed as the sum of the percent abundances of all species in the genera *Navicula*, *Nitzschia*, and *Surirella*. These are common genera of predominantly motile taxa that are able to maintain their positions on the substrate surface in depositional environments.

[4] Computed as the percent abundance of *Achnanthes minutissima*. This attached taxon typically dominates early successional stages of benthic diatom associations and resists chemical, physical and biological disturbances in the form of metals toxicity, substrate scour by high flows and fast currents, and grazing by macroinvertebrates.

APPENDIX A: DIATOM PROPORTIONAL COUNTS

Sample	Genus/Species/Variety	PTC	Count	Percent
182201	Achnanthes biasolettiana	3	4	0.47
182201	Achnanthes lanceolata	2	4	0.47
182201	Achnanthes minutissima	3	57	6.64
182201	Amphora pediculus	3	27	3.15
182201	Cocconeis pediculus	3	2	0.23
182201	Cocconeis placentula	3	8	0.93
182201	Cymbella affinis	3	2	0.23
182201	Cymbella microcephala	2	3	0.35
182201	Cymbella silesiaca	2	3	0.35
182201	Cymbella sinuata	3	8	0.93
182201	Diatoma vulgare	3	122	14.22
182201	Fragilaria construens	3	2	0.23
182201	Fragilaria leptostauron	3	2	0.23
182201	Fragilaria vaucheriae	2	2	0.23
182201	Gomphoneis herculeana	3	4	0.47
182201	Gomphonema minutum	3	2	0.23
182201	Gomphonema olivaceum	3	27	3.15
182201	Gomphonema parvulum	1	2	0.23
182201	Gomphonema pumilum	3	3	0.35
182201	Gomphonema truncatum	3	1	0.12
182201	Navicula capitatoradiata	2	3	0.35
182201	Navicula cryptotenella	2	64	7.46
182201	Navicula gregaria	2	4	0.47
182201	Navicula menisculus	2	4	0.47
182201	Navicula minuscula	1	2	0.23
182201	Navicula reichardtiana	2	6	0.70
182201	Navicula tripunctata	3	119	13.87
182201	Nitzschia capitellata	2	2	0.23
182201	Nitzschia dissipata	3	67	7.81
182201	Nitzschia fonticola	3	233	27.16
182201	Nitzschia heufleriana	3	16	1.86
182201	Nitzschia linearis	2	2	0.23
182201	Nitzschia palea	1	8	0.93
182201	Nitzschia sociabilis	2	25	2.91
182201	Rhoicosphenia curvata	3	8	0.93
182201	Surirella minuta	2	2	0.23
182201	Synedra ulna	2	8	0.93

Sample	Genus/Species/Variety	PTC	Count	Percent
182101	Achnanthes biasolettiana	3	34	3.63
182101	Achnanthes lanceolata	2	14	1.50
182101	Achnanthes minutissima	3	173	18.48
182101	Amphipleura pellucida	2	1	0.11
182101	Amphora inariensis	3	9	0.96
182101	Amphora pediculus	3	33	3.53
182101	Aulacoseira granulata	3	1	0.11
182101	Caloneis bacillum	2	2	0.21
182101	Campylodiscus hibernicus	2	1	0.11
182101	Cocconeis pediculus	3	4	0.43
182101	Cocconeis placentula	3	33	3.53
182101	Cymbella affinis	3	9	0.96
182101	Cymbella hebridica	3	8	0.85
182101	Cymbella mexicana	3	1	0.11
182101	Cymbella microcephala	2	15	1.60
182101	Cymbella minuta	2	14	1.50
182101	Cymbella silesiaca	2	2	0.21
182101	Cymbella sinuata	3	5	0.53
182101	Diatoma hiemale	3	11	1.18
182101	Diatoma vulgare	3	40	4.27
182101	Fragilaria construens	3	7	0.75
182101	Fragilaria leptostauron	3	15	1.60
182101	Fragilaria pinnata	3	10	1.07
182101	Fragilaria vaucheriae	2	2	0.21
182101	Frustulia vulgaris	2	2	0.21
182101	Gomphonema angustatum	2	2	0.21
182101	Gomphonema minutum	3	7	0.75
182101	Gomphonema olivaceum	3	24	2.56
182101	Gomphonema parvulum	1	3	0.32
182101	Meridion circulare	3	3	0.32
182101	Navicula acceptata	2	2	0.21
182101	Navicula capitata	2	2	0.21
182101	Navicula capitatoradiata	2	6	0.64
182101	Navicula cryptotenella	2	54	5.77
182101	Navicula lanceolata (Ag.) Ehr.	2	1	0.11
182101	Navicula libonensis	2	1	0.11
182101	Navicula minima	1	2	0.21
182101	Navicula minuscula	1	2	0.21
182101	Navicula reichardtiana	2	8	0.85
182101	Navicula tripunctata	3	42	4.49
182101	Navicula viridula	2	3	0.32
182101	Nitzschia acicularis	2	2	0.21
182101	Nitzschia amphibia	2	6	0.64
182101	Nitzschia angustatula	2	2	0.21
182101	Nitzschia dissipata	3	32	3.42
182101	Nitzschia fonticola	3	153	16.35
182101	Nitzschia gracilis	2	15	1.60
182101	Nitzschia heufleriana	3	26	2.78
182101	Nitzschia liebetruthii	3	3	0.32
182101	Nitzschia linearis	2	11	1.18
182101	Nitzschia palea	1	8	0.85
182101	Nitzschia paleacea	2	2	0.21
182101	Nitzschia recta	3	3	0.32
182101	Nitzschia sigmoidea	3	2	0.21
182101	Nitzschia sociabilis	2	22	2.35
182101	Nitzschia vermicularis	2	3	0.32
182101	Surirella angusta	1	2	0.21
182101	Surirella minuta	2	11	1.18
182101	Synedra ulna	2	25	2.67

Sample	Genus/Species/Variety	PTC	Count	Percent
182001	Achnanthes lanceolata	2	4	0.43
182001	Achnanthes minutissima	3	22	2.38
182001	Amphora inariensis	3	1	0.11
182001	Amphora pediculus	3	18	1.95
182001	Aulacoseira granulata	3	8	0.87
182001	Caloneis bacillum	2	5	0.54
182001	Cocconeis pediculus	3	2	0.22
182001	Cocconeis placentula	3	19	2.06
182001	Cymbella affinis	3	9	0.97
182001	Cymbella minuta	2	37	4.00
182001	Diatoma tenue	2	1	0.11
182001	Diatoma vulgare	3	21	2.27
182001	Fragilaria capucina	2	5	0.54
182001	Fragilaria vaucheriae	2	84	9.09
182001	Frustulia vulgaris	2	4	0.43
182001	Gomphonema angustatum	2	5	0.54
182001	Gomphonema minutum	3	5	0.54
182001	Gomphonema olivaceum	3	240	25.97
182001	Gomphonema parvulum	1	10	1.08
182001	Melosira varians	2	1	0.11
182001	Meridion circulare	3	1	0.11
182001	Navicula capitatoradiata	2	15	1.62
182001	Navicula cryptotenella	2	61	6.60
182001	Navicula menisculus	2	8	0.87
182001	Navicula minima	1	6	0.65
182001	Navicula pelliculosa	1	2	0.22
182001	Navicula reichardtiana	2	28	3.03
182001	Navicula tripunctata	3	48	5.19
182001	Navicula trivialis	2	4	0.43
182001	Nitzschia apiculata	2	2	0.22
182001	Nitzschia dissipata	3	117	12.66
182001	Nitzschia fonticola	3	8	0.87
182001	Nitzschia gracilis	2	4	0.43
182001	Nitzschia heufleriana	3	10	1.08
182001	Nitzschia liebetruthii	3	2	0.22
182001	Nitzschia palea	1	2	0.22
182001	Nitzschia sigmoidea	3	1	0.11
182001	Nitzschia sociabilis	2	13	1.41
182001	Rhoicosphenia curvata	3	8	0.87
182001	Surirella minuta	2	73	7.90
182001	Synedra ulna	2	10	1.08

Sample	Genus/Species/Variety	PTC	Count	Percent
181901	Achnanthes biasolettiana	3	37	3.89
181901	Achnanthes clevei	3	3	0.32
181901	Achnanthes lanceolata	2	13	1.37
181901	Achnanthes minutissima	3	180	18.91
181901	Amphipleura pellucida	2	2	0.21
181901	Amphora inariensis	3	3	0.32
181901	Amphora libyca	3	4	0.42
181901	Amphora pediculus	3	30	3.15
181901	Caloneis bacillum	2	2	0.21
181901	Campylodiscus hibernicus	2	0	0.00
181901	Cocconeis pediculus	3	3	0.32
181901	Cocconeis placentula	3	28	2.94
181901	Cymatopleura elliptica	2	2	0.21
181901	Cymatopleura solea	2	2	0.21
181901	Cymbella affinis	3	41	4.31
181901	Cymbella cuspidata	3	0	0.00
181901	Cymbella hebridica	3	3	0.32
181901	Cymbella microcephala	2	50	5.25
181901	Cymbella minuta	2	2	0.21
181901	Cymbella silesiaca	2	12	1.26
181901	Cymbella sinuata	3	2	0.21
181901	Diatoma hiemale	3	7	0.74
181901	Diatoma tenue	2	2	0.21
181901	Diatoma vulgare	3	25	2.63
181901	Diploneis oblongella	3	3	0.32
181901	Fragilaria capucina	2	2	0.21
181901	Fragilaria construens	3	3	0.32
181901	Fragilaria leptostauron	3	29	3.05
181901	Fragilaria pinnata	3	17	1.79
181901	Fragilaria vaucheriae	2	3	0.32
181901	Frustulia vulgaris	2	2	0.21
181901	Gomphonema angustatum	2	2	0.21
181901	Gomphonema minutum	3	8	0.84
181901	Gomphonema olivaceum	3	32	3.36
181901	Gomphonema parvulum	1	15	1.58
181901	Melosira varians	2	4	0.42
181901	Navicula capitata	2	2	0.21
181901	Navicula capitatoradiata	2	6	0.63
181901	Navicula cryptotenella	2	59	6.20
181901	Navicula gregaria	2	2	0.21
181901	Navicula menisculus	2	5	0.53
181901	Navicula reichardtiana	2	10	1.05
181901	Navicula tripunctata	3	59	6.20
181901	Navicula ventralis	2	0	0.00
181901	Nitzschia amphibia	2	2	0.21
181901	Nitzschia dissipata	3	15	1.58
181901	Nitzschia fonticola	3	121	12.71
181901	Nitzschia gracilis	2	6	0.63
181901	Nitzschia heufleriana	3	27	2.84
181901	Nitzschia linearis	2	4	0.42
181901	Nitzschia palea	1	4	0.42
181901	Nitzschia sociabilis	2	4	0.42
181901	Nitzschia vermicularis	2	11	1.16
181901	Surirella minuta	2	13	1.37
181901	Synedra ulna	2	29	3.05