CHAPTER 3

Using a battery of bioassays, benthic phytoplankton and the AUSRIVAS method to monitor long-term coal tar contaminated sediment in the Cache la Poudre River, Colorado.

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Abstract

This survey provides information on sediment toxicity and structural characteristics of the macroinvertebrates and benthic phytoplankton at 10 locations in the Cache la Poudre River after long-term exposure to coal tar residue. The application of the Australian river bioassessment system (AUSRIVAS) as well as a biotest battery was conducted to evaluate the river 'health' condition. Coal tar is a dense nonaqueous phase liquid of significant environmental concern due to its toxicity and persistence in the subsurface. Organisms like *Selenastrum capricornutum, Daphnia magna* and *Chironomus tentans*, representing different complexities in the biosphere, were selected as test systems for ecotoxicological studies. The results obtained in this study indicate that a biotest battery, macroinvertebrate and benthic phytoplankton communities are in principle, suitable biological tools for evaluation of toxic oil and coal-derived substances in long-term contaminated river sediment.

***Keywords**:* Coal tar, sediment toxicity, biotest battery, river bioassessment

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3.1 Introduction

High levels of contaminants in fresh water sediment may irreversible adverse effects to organisms that inhabit contaminated regions of ecosystems. The exposure to contaminated sediment particles is not only important for benthic organisms (Harkey *et al*. 1994) but also for algae, daphnids, or fish in case of resuspension of the particles (Knezovich *et al*. 1987). Sediment is an integral component of aquatic ecosystems, providing habitat, feeding and spawning for many aquatic organisms. Because sediment serves as a reservoir for contaminants, it is a source of contaminants to the water column and organisms (U.S. EPA 1997).

Various substances from conventional coal processing, e.g. polycyclic aromatic hydrocarbons (PAH), phenols and cyanides cause strong environmental concern because of their toxic, mutagenic or cancerogenic properties. Coal and oil gasification was a large industry in the United States that operated from the 1800s to the early 1950s. Manufactured gas plants in the U.S. used coal and oil to derive light-end hydrocarbons that were utilized for lighting and heating (Environmental Research & Technology 1984). A major byproduct of gas manufacturing processes was coal tar, a dark-colored, dense nonaqueous phase liquid. Coal tar typically was disposed onsite until abundant natural gas resources have eliminated the need of manufactured gas plants.

Freshwater pollutants have been monitored mainly by physical and chemical techniques (Hattingh 1979). However, these approaches are often impractical and have been deemed inadequate to provide information on unknown hazardous compounds and their potential harmful effects on man and aquatic ecosystems (Cairns & Gruber 1979). Since living organisms will show some response to hazardous levels of any chemicals, the use of biological sensors has become an alternative and increasingly important approach in the prediction and control of water pollution (Cairns *et al*. 1977). In the present study a battery of bioassays was used as a screening tool due to the fact that no single species is sensitive to all chemical effluents in an ecotoxicological risk assessment (Toussaint *et al*. 1995). Multispecies tests that incorporate functional relationships between trophic groups, such as grazers and producers, can warn of environmental damage resulting from secondary effects that are unpredictable from single species tests alone (Crossland 1984). A desirable property for a test battery member is that its sensitivity pattern should be different from other tests; i.e., to complement each other (Wangberg *et al.* 1995) and therefore both grazers and producers are included in most microcosm toxicity test methods (Cairns *et al.* 1986; Taub *et al.* 1985). Algae and aquatic invertebrates are attractive organisms because their generation spans are shorter than those of higher organisms such as fish.

In this article three different species representing different trophic levels were use to analyze the toxicity of remnants of coal tar in contaminated sediment of the Cache la Poudre River. The selected species were the fresh water algae *Selenastrum capricomutum,* and the invertebrates *Daphnia magna* and *Chironomus tentans*. The aim of the investigation carried out was firstly to determine the ecotoxicological effects of coal-relevant substances and bioconversion products on benthic invertebrates and phytoplankton community in a oil sheen area of the Cache la Poudre River, Colorado, and secondly to use a validated battery of sensitive bioassay to identify highly sediment polluted areas.

3.2 Material and methods

3.2.1. Study area

The Cache la Poudre River originates in the mountains of Colorado near the Continental Divide about 68 km west of Fort Collins. It has a drainage area of about 738 km^2, and flows northeastward about 194 km from its origin to its confluence with

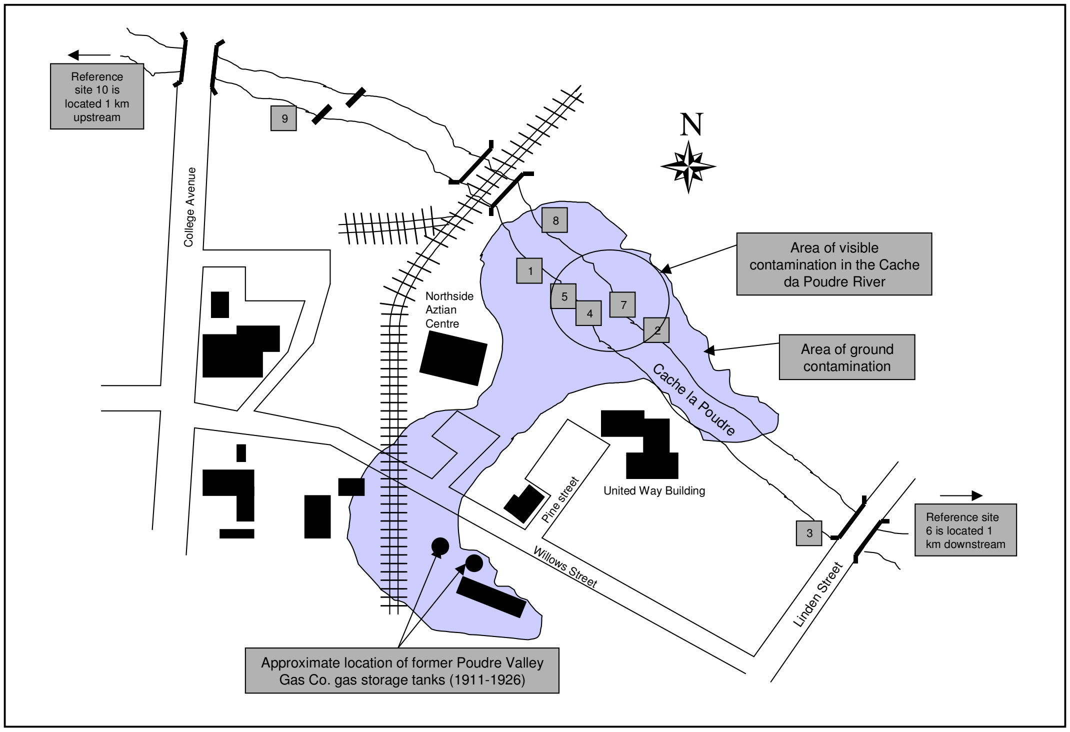

Figure 3.1 Map of the study area in Fort Collins showing the sampling sites on the banks of the Cache la Poudre River, Colorado.

the South Platte River about 8 km east of Greeley. The river basin includes two physiographic provinces, the Southern Rocky Mountain Province and the Great Plains, which correspond to the two major ecoregions, Southern Rockies and Western High Plains (Dennehy *et al.* 1995). The Cache la Poudre River is an important water resource in Ft. Collins, since it supply drinking water to > 200 000 residents in Larimy County. It is further utilized for recreational and irrigation purposes. Water from the Cache la Poudre River also serves as a supplementary water source to the urban lakes (e.g., Sheldon Lake) in Ft. Collins (Sheldon Lake Drainage Improvement Project 2002, 2003a,b; Collins & Sprague 2005). Sampling sites were established on a 150-200 m oily sheen stretch of the Cache la Poudre River east of North College Avenue and west of Linden Street, Fort Collins (Fig. 3.1). The oily sheen was first discovered in September 2002 and is likely the remnants of coal tar, a byproduct from a gas company in business from 1904 to 1926. Over a long period of time, the oily substance has seeped through the sandy sediment about 4 m beneath the surface and eventually dipped down into the cracked layers of bedrock, spreading into the river (Lingle 2004).

Stream velocity, substratum type (i.e., percentage of cobbles, pebbles, gravel, sand and silt, and embeddedness of cobbles), substratum cover (i.e., filamentous algae, macrophytes and coarse particulate organic matter), and canopy cover were determined at each location where the phytobenthos was sampled. Substratum type and substratum cover were determined visually (Stevenson & Bahls 1999).

3.2.2 Toxicity testing

3.2.2.1 *Selenastrum capricornutum* biotest

In this study we used a 96-hour growth, standard freshwater algal toxicity test with the unicellular, crescent-shaped, green alga *Selenastrum capricornutum* (40-60 μm^3) that can be found in both eutrophic and oligotrophic freshwater environments. Methods used for culture and testing were as described by the Environmental Protection Agency (EPA)(U.S. EPA 1994). This method was originally developed for use with effluents, receiving waters, and pure compounds, but it is also suitable for testing sediment. The test was used to measure changes in cell density and chlorophyll content. Starter algae cultures were obtained from Aquatic Bio Systems Inc. Fort Collins, Colorado. Instructions for the preparation of glassware and nutrient stock solution were as detailed by the EPA (U.S. EPA 1994). Cultures were grown in a nutrient medium that include EDTA and then tested in a medium without EDTA. The cultures were grown in 250-mL glass Erlenmeyer flasks containing 100 mL culture medium. New cultures were started each week. The flasks were sealed with foam plugs to prevent contamination and placed in an incubation chamber at 25 ± 1 °C under continuous illumination (60-80 $\mu E/m^2/s$) supplied by cool light fluorescent lamps. The toxicity test was initiated after the stock cultures have reached a cell density of 1 x 10^2 cells/mL and were entering a log-phase growth stage. The same amount of sediment pore water obtained at the different sampling sites, while sampling macroinvertebrates with a core sampler, was filtered through a 0.45 µm filter to remove particle material. The test was conducted as a screen (100 % concentration). Nutrients without EDTA were added to ensure that reduction in growth was not due to nutrient limitation. The toxicity test was conducted in 250-mL Erlenmeyer flasks, with a test volume of 100 mL and three replicates per treatment. The negative control consisted of three replicates of the culture medium, prepared without EDTA. The inoculum density was adjusted to 1 x 10^6 cells/mL and each flask was inoculated with 1 mL stock culture. Each of the flasks were sealed with a foam plug and placed in an incubation chamber at 25 ± 1 °C with a continuous photoperiod. The flasks were swirled twice daily by hand and randomly repositioned in the incubation chamber. Cell density and chlorophyll*a* (Chl*a*) contents were measured of the triplicate samples at 24, 48, 72 and 96 hours and the mean count (cells/mL) was calculated for each treatment. Chl*a* was extracted from lyophilized GF filters using N,N-dimethylformamide for 2 h at room temperature. Chl*a* was measured photospectrometrically at 647 and 664 nm and calculated according to Porra *et al.* (1989). The following water quality measures: pH, alkalinity, hardness and temperature were taken at test initiation and termination.

3.2.2.2 *Daphnia magna* test

Standardized techniques involving whole sediment exposures, with acute or sublethal endpoints, have been used in the 48-hour toxicity test (American Society for testing and Materials, ASTM 2000). Test organisms *Daphnia magna* < 24 hour neonates were obtained from Aquatic Bio Systems Inc. Fort Collins, Colorado. They were cultured at 22 °C, under a photoperiod of 16:8 h light:darkness and a light intensity of 10 Ue/m^2/s. The culture medium was deionized water, with a pH 7.99, total hardness of 250 mg $CaCO_3$/L and total alkalinity of 96 mg $CaCO_3$/L. A static 48-hour acute test, with whole sediment samples, was conducted as a screen (100 % concentration). Each test container was a 250-mL glass beaker, and there were five replicates per treatment. Approximately 50 g sediment (wet weight) was placed in each beaker, and then 200 mL culture water was added, taking care not to disturb the sediments. After a settling period of 3 days as described (ASTM 2000), ten neonates were added to each container, and care was taken to release them below the water surface. For the negative control, the *Daphnia* were kept in pure water. Dissolved Oxygen (DO) and temperature was measured daily, while pH, ammonia, conductivity, alkalinity, and hardness were determined at test initiation and termination. After 24 h and 48 h the number of immobilized *Daphnia* was determined visually. *Daphnia* that sank to the bottom of the vessels within 15 s of having been slightly shaken were considered immobile. Data from the replicate beakers were pooled and treatments were compared to the negative control and reference sites.

3.2.2.3. *Chironomus tentans* test

The assessment of whole sediment toxicity involves a 10-day exposure of *Chironomus tentans* to the contaminated sediment. We conducted the toxicity test in 300-mL beakers with 8 replicate chambers. The sediment volume for the test was 100 mL with 175 mL of overlying water. Sediment of the different sampling sites were prepared the day before test initiation and allowed to equilibrate overnight following the instructions of the U.S. EPA (2000). The following day 10 second-to third-instars larvae were added per chamber under a 16:8 h light; dark photoperiod at 23 ± 1 °C. Overlying water in the test containers were renewed, with two volume replacements per day (U.S. EPA 2000). Hardness, alkalinity, conductivity, pH and ammonia were monitored at the beginning and end of the test while temperature and DO were monitored daily. After 10 days the mean survival of larvae exposed to test samples were compared to the mean survival of larvae exposed to controls and reference samples.

3.2.2.4 Data analyses of biotest endpoints

The test results were recorded on standard Excel spreadsheats for data processing, and statistical analysis was performed using SYSTAT® 7.0.1 (1997). The responses of bioassay samples were subjected to an analysis of variance ($p \leq 0.05$) using SYSTAT® 7.0.1 (1997) compared to examine the endpoint variability of each test. All toxic responses were modeled by probit function (LT50) where number of dead/total number is a probit function of time.

3.2.2.5 Sampling of macroinvertebrates and phytobentos

Macroinvertebrates and phytobentos were collected at weekly intervals with a corer at each of the ten sampling sites from August to October 2004. Four core samples were taken monthly at the ten sampling sites (Fig. 3.1). A random sampling procedure was used to reduce hydrobiological variability between sites (Voelz & Ward 1991). The corer was driven into the substrate to an approximate depth of 10 cm. Cobble-sized rock within the corer were transferred to a bucket and scrubbed. The other material within the corer was removed by hand. Macroinvertebrates, organic matter, and remaining water were passed through a sieve with 75 µm apertures. These materials were placed in labeled 0.5-L glass jars with plastic lids and preserved in 10% (v/v) formalin solution. The sediment water was fixed with buffered 5% (v/v) formaldehyde for determination of benthic phytoplankton composition, community structure and identification of taxa. Cells greater than 30 µm in diameter were counted first at x 125 magnification; smaller taxa were then enumerated at x 1250 using the strip-count method American Public Health Association (APHA 1989). Diatoms were identified after clearing in acid persulphate. Biovolumes of more abundant taxa were estimated by measuring cell dimensions of at least 20 individuals and using closest geometric formulae (Willen 1976). Identification of taxa was done according to described methods of Krammer and Lange-Bertalot (1986-1991) and Patrick and Reimer (1975). In the laboratory, the benthic samples were washed through a series of three sieves to separate the following size classes: > as 1 mm, 250 µm-1 mm, and 75-250 µm. Each sample was sorted with an illuminated dissecting microscope at 20x magnification. Organisms were counted and placed in labeled glass vials containing 70% (v/v) ethanol before taxonomic identification to the lowest possible taxonomic category, according to Merritt and Cummins (1996) and Thorp and Covich (1991). The macroinvertebrate and benthic phytoplankton collected at the reference sites were analyzed and used to build an Australian River Assessment System (AUSRIVAS)-type predictive model (Table 3.1) for the oil sheen area.

Table 3.1 O/E index and ecological health rating for the long-term contaminated sediment-sampling sites in the Cache la Poudre River.

Site	O/E*	Rating
1	0.43	Moderately impacted
2	0.00	Severely degraded
3	0.44	Moderately impacted
4	0.00	Severely degraded
5	0.00	Severely degraded
6	0.68	Mildly impacted
7	0.00	Severely degraded
8	0.45	Moderately impacted

*E number of taxa expected from those predicted with > 50 percent probability of occurrence; 0, number of taxa observed from the list of those with > 50 percent probability of being at the site; O/E, Observed/Expected ratio. This should be near 1.0 for sites similar to reference conditions in the Cache la Poudre River. Sites with O/E < as 0.8 are below reference in the Cache la Poudre River.

The habitat data of the eight sites judged to be damaged were compared with the reference sites to provide an assessment of the ecological health of the river in damaged areas (Simpson & Norris, 2000; Humphrey *et al*. 1995, 2000). Only those taxa which had a probability of 50% or greater of occurring at a damaged sampling site, were used to calculate the number of expected (E) taxa. The observed (O) number of taxa was obtained by summing the number of taxa actually recorded at the damaged sampling site with expected probabilities of > 50 percent. The O/E index was calculated to provide a measure of the difference between the observed and expected macroinvertebrate and benthic phytoplankton distributions. An O/E ratio near 1 is equivalent to reference while a ratio less than about 0.8 indicates that 20% of the expected taxa were missing in the benthic environment of the damaged site and it is below reference condition.

3.3 Results

3.3.1 Physicochemical characteristics

The substratum at each sampling site in the Cache la Poudre River consisted predominantly of pebbles, sand and silt-clay. These three size fractions collectively constituted over 85% of the mineral substratum, while cobble stones were only present at sites 1, 8, 9, 10 (Fig. 3.2).

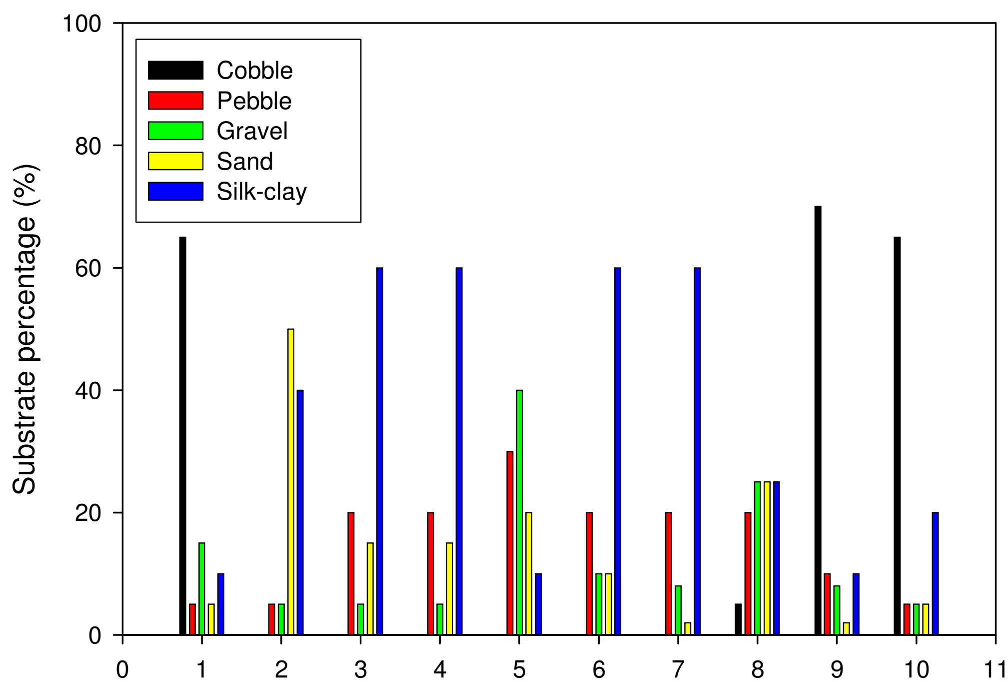

Figure 3.2 Substratum composition of the ten sampling sites in the Cache la Poudre River, Colorado**.**

The flow regime was measured on six occasions when the flow was high, medium and low, respectively (Fig. 3.3). During the whole period of our investigation the pH at all of the ten sampling sites was above seven (Fig. 3.3).

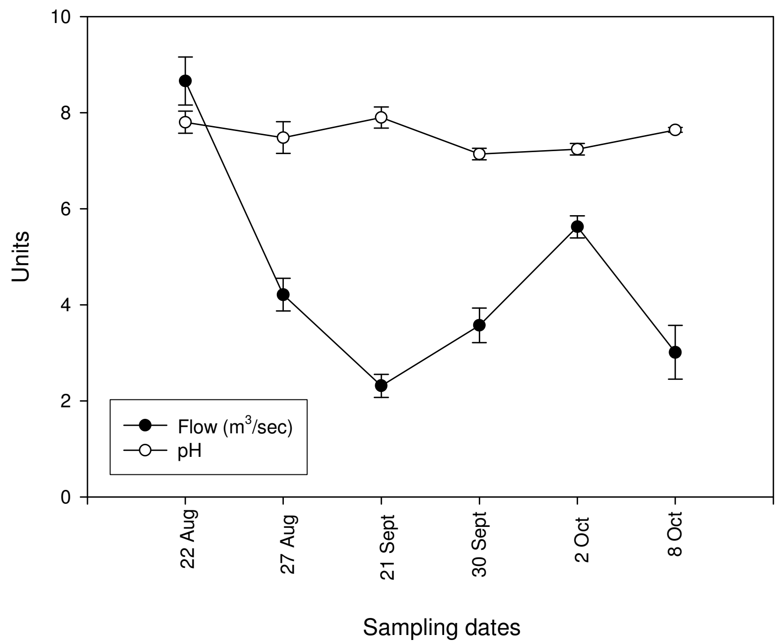

Figure 3.3 Variations in flow and pH from August 22 to October 8 in the Cache la Poudre River, Colorado. Error bars indicate standard deviations from the mean value.

3.3.2 *Selenastrum capricornutum* biotest

Results of the present study indicate that the values of Chl*a* content were subjected to considerable variation according to the different sampling sites and the exposure period (Fig. 3.4b). The control and reference sites 9 and 10 revealed no significant differences in Chl*a* content upstream. In the case of sampling sites 2, 4, 5 and 7, a

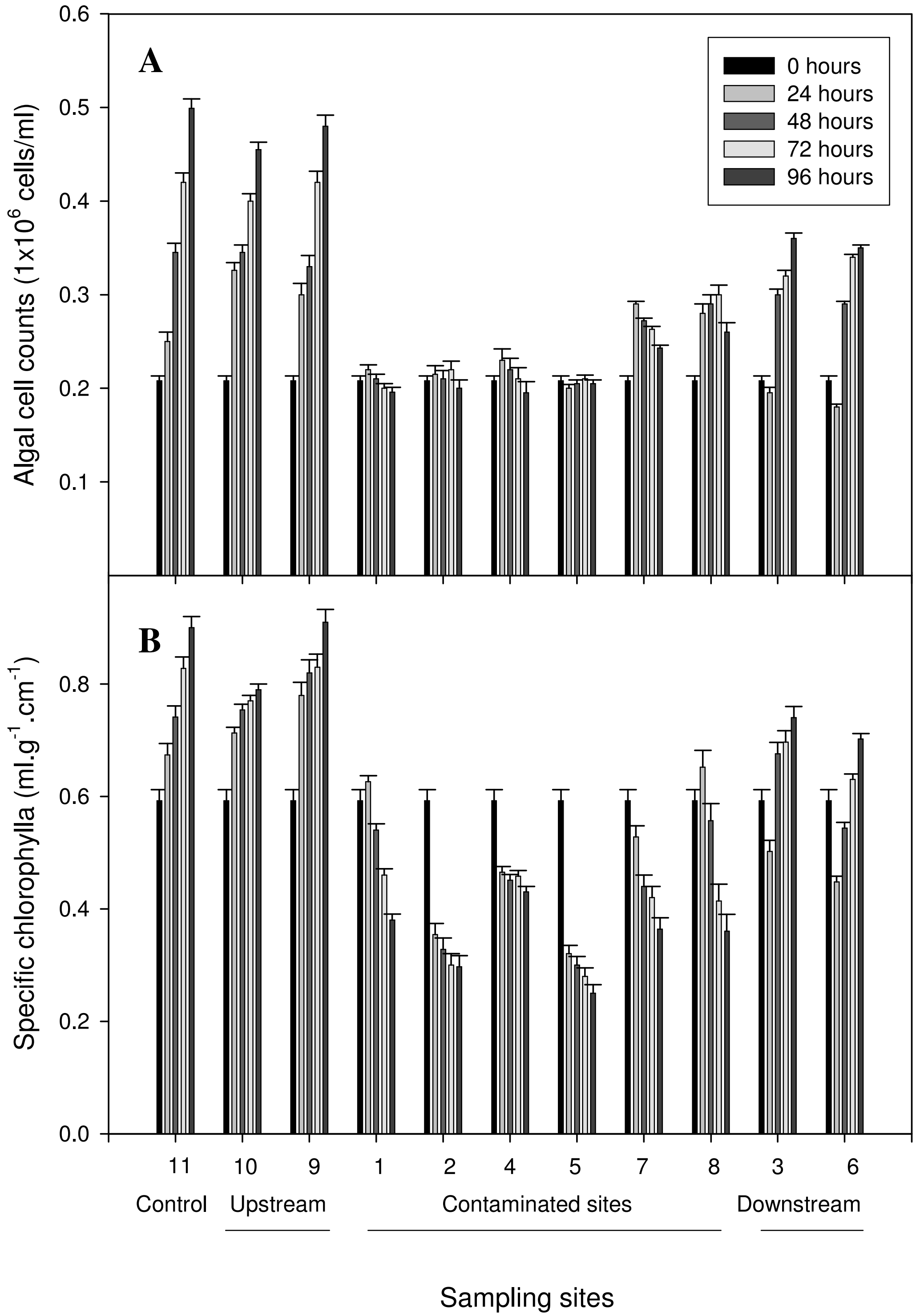

Figure 3.4 Changes in *Selenastrum capricornutum* cell counts (A) and specific Chl*a* content (B) at the different sampling sites in the Cache la Poudre River, Colorado. Error bars indicate standard deviations from the mean value.

decrease in Chl*a* content of *Selenastrum capricornutum* was significant during the whole exposure time of the experimental run. On the other hand, at sampling site 3 and 6 downstream, the *Selenastrum capricornutum* growth was inhibited up to the third day of incubation and thereafter, the *Selenastrum capricornutum* started to recover. Variation in counts of *Selenastrum capricornutum* cells of the different sampling sites over a period of 96 hours is represented in Fig. 3.4a. At sampling sites 3 and 6, *Selenastrum capricornutum* started to recover by the end of the third day of exposure and attained a higher count at the end of the 96 hours while sampling sites 2, 4, 5 and 7 resulted in a decreased of *Selenastrum capricornutum* cell count by the end of the exposure time. The total *Selenastrum capricornutum* count decreased in response to the coal tar residue compared with the control and reference site 9 and 10.

3.3.3 Fresh water *Daphnia magna* and *Chironomus tentans* biotests

The percentage of survival of daphnids in 100% concentration for 48 hr of exposure is indicated in Fig. 3.5. At sampling sites 2 and 5, the survival of *Daphnia magna* to the whole-sediment toxicity test was 0% after 48 h of exposure, while at sampling site 7 only 20% survived. Coal tar residue had little effect on the survival rate of *Daphnia magna* at sampling sites 3 and 6 downstream.

The survival endpoint results for *Chironomus tentans* following a 10-day exposure to whole-sediment toxicity test are illustrated in Fig. 3.5. The sediment of sites 9 and 10 were used as a reference. Sampling site 5 was highly toxic and contained 13.3% survivors, while sampling sites 2, 4 and 7 had a survivor percentages of 40, 40, 26.66%, respectively. The trend observed in this data set is comparable to the data obtained from the *Selenastrum capricornutum* and *Daphnia* bioassay, and is highly significant ($p \leq 0.001$).

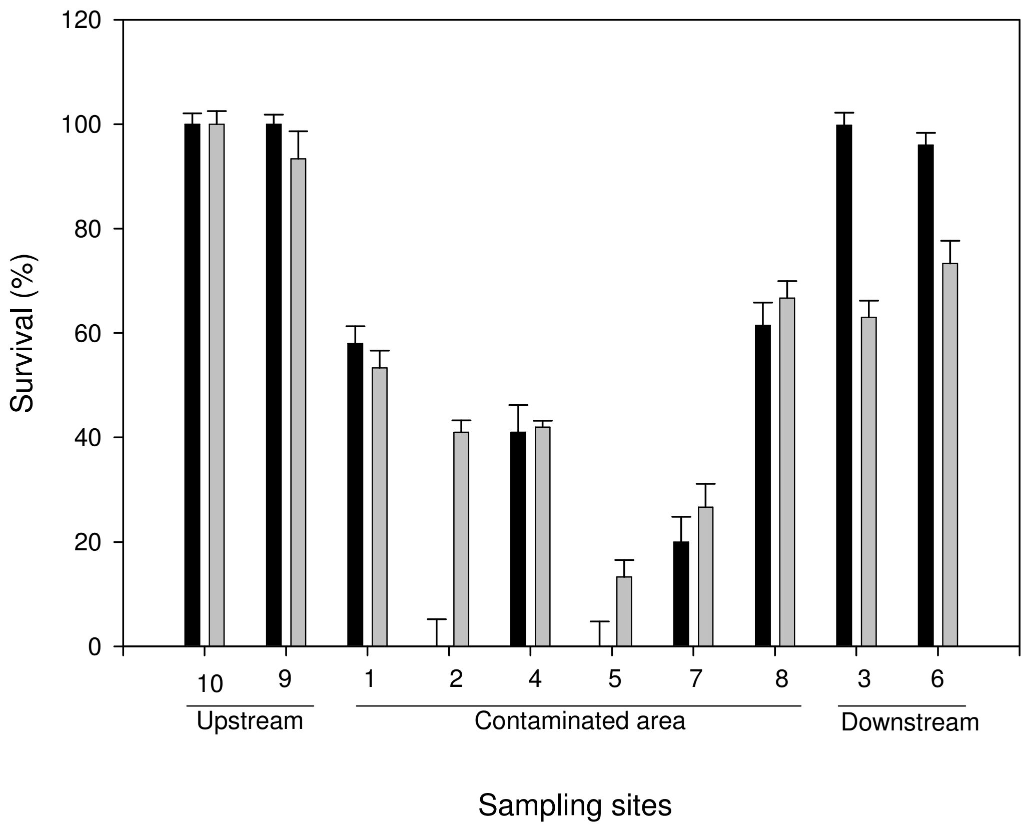

Figure 3.5 Results of the survival rates of *Daphnia magna* (48 h) and *Chironomus tentans* (240 h) after biotesting of sampling sites. Error bars indicate standard deviations from the mean value.

3.3.4 Macroinvertebrate Response

Eighty percent of the organisms collected at the sampling sites belong to the following taxonomic groups: Annelida (Oligochaeta), Diptera (Chironomidae), Ephemeroptera

(*Tricorythodes minutus*), Tricoptera (*Hydropsyche* spp.) and Nematoda, while the relative abundance of other taxonomic groups was low (not shown). The highest diversity and evenness were both recorded at site 10, while the habitat at the reference and most other sampling sites did not support any aquatic vascular plant biomass. Analysis of data upstream and downstream of the oily sheen revealed that there were a significantly higher number of organisms with a low number of taxa downstream at sites 3 and 6, than upstream ($p \leq 0.0029$). Only a few taxa occurred at these sites, and diversity values at locations 3 and 6 were consistently lower than the reference locations 9 and 10 upstream during the sample period. The major taxa, comprising of collector-gatherers (Chironomidae; Oligochaeta) was much higher at sites 9 and 10, upstream from the oily sheen, than at the other sampling sites. Sites 3 and 6 downstream had the highest relative abundance of collector-gatherers (Nematoda, Oligochaeta), followed by lower numbers of collector-filterers (Trichoptera) during our survey (Fig. 3.6). Very few chironomids, scrapers and shredders were either eliminated or reduced in numbers at the sampling sites.

Using the AUSRIVAS-method, it was found that sampling sites 2, 4, 5 and 7 were severely degraded with none of the expected macroinvertebrate families found. The sampling sites 9 and 10, which were ± 200 m and 1 km upstream, respectively from the oily sheen were selected as unmodified reference sites and used as a baseline for judging change.

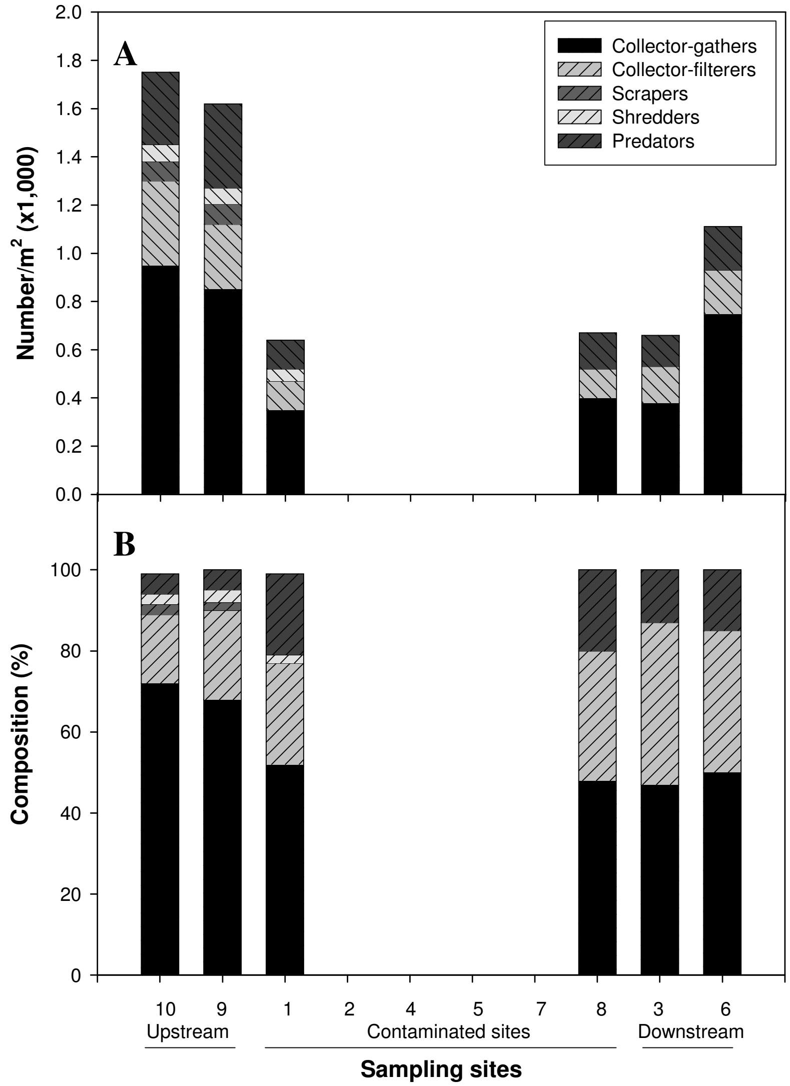

Figure 3.6 (A) Abundance (individuals/m^2) and (B) percentage composition of macroinvertebrate functional feeding groups recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004.

3.3.5 Benthic phytoplankton response

High diversity of species and abundance were recorded in case of diatoms with respect to the two reference sites 9 and 10. Green algal species were relatively uncommon at all the sampling sites. The only genera that dominate the contaminated areas was *Didymosphenia geminata* (Fig. 3.7).

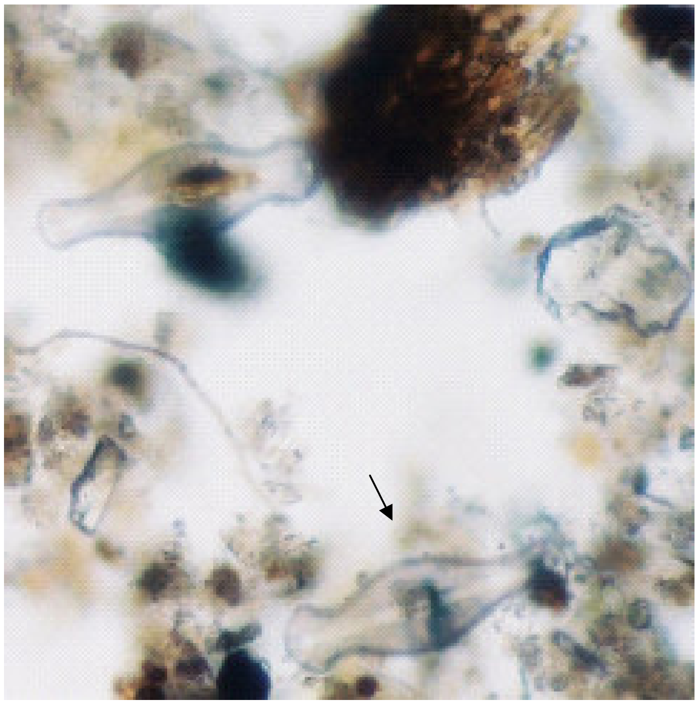

Figure 3.7 Bacillariophyceae, *Didymosphenia geminata* (indicated by a arrow), the only taxon in large abundance in the contaminated sediment. Unstained, bright-field microscopy, 200 x.

This diatom genera was much more abundant in the highly contaminated sampling sites 2, 4, 5, and 7 than at any other site upstream or downstream, including the reference sites 9 and 10 (Fig. 3.8).

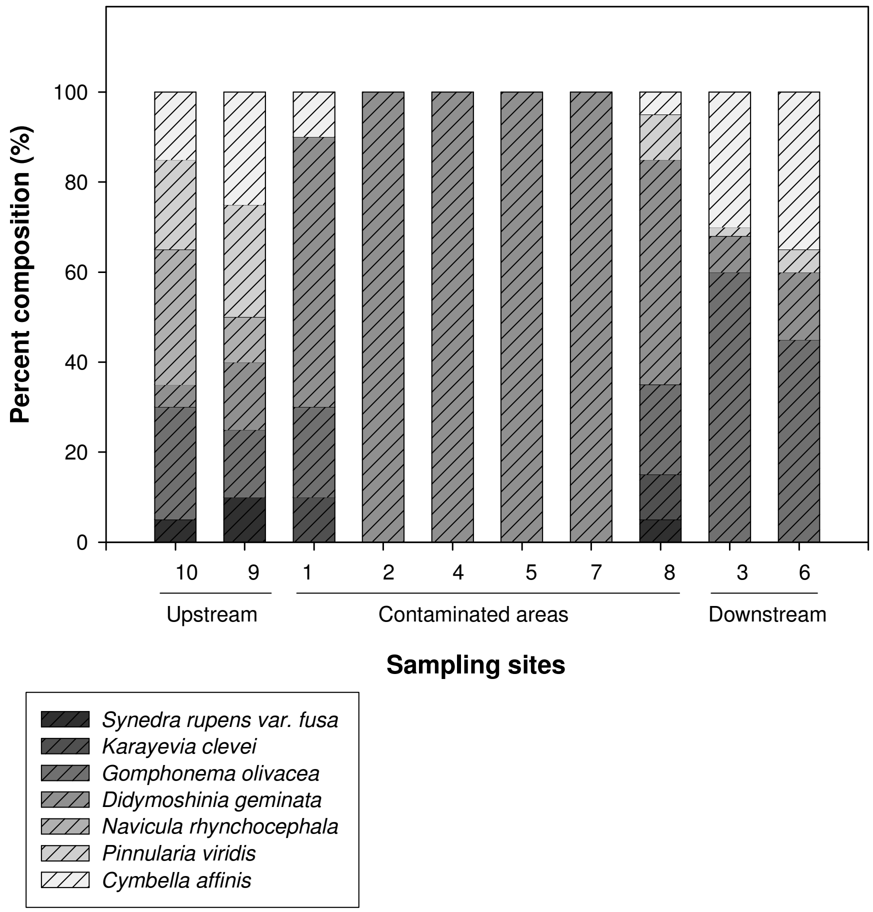

Figure 3.8 Percentage composition of different Bentic phytoplankton species recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004.

Comparisons with the reference sites 9 and 10, revealed that sampling sites 3 and 6 downstream from the oily sheen, have a lower species diversity than the upstream sites, and that only a few taxa occurred at these sites of which the genera *Gomphonema olivacea* and *Cymbella affinis* were the major taxa with *Didymosphenia geminata* in much lower abundance.

3.4 Discussion

3.4.1 Physicochemical characteristics

Douglas (1958) and Cattaneo *et al*. (1997) recognized that different sizes of stone supported different densities and species of epilithic algae, which was likely the result of differences in their susceptibility to flood disturbance. Distribution of stream macroinvertebrates is also closely related to substrate characteristics (Minshall 1984). The substratum in the Cache la Poudre River consisted predominantly of pebbles, sand and silt-clay (Fig. 3.2). The flow regime was measured (Fig. 3.3), since this is an important factor shaping structure of benthic-algal assemblages in the Cache la Poudre River due to the fact that diatom immigration onto bare substrata may increase with reduced current speed (Stevenson 1983). The influence of velocity, like discharge, may interact with other variables (e.g., substrate, food supply, DO) to determine habitat conditions for macroinvertebrates (Ward 1992). Winter *et al*. (2003) reported that a higher proportion of diatom species were indicative of a $pH > 7$ and a lower proportion of species indicative of a $pH < 7$ which showed that the structure of benthic-algal assemblages correlated strongly with pH.

3.4.2 *Selenastrum capricornutum* biotest

Phytoplankton, benthic and epiphytic microalgae, and macroalgae are energy sources critical to most aquatic ecosystems. Changes in their density and composition can affect the chemical and biological quality of the habitat (Round 1981). The impacts of petroleum pollutants on algae are subject to variation due to the great variability in both the chemical composition of the pollutants, as well as algal population (Morales-Loo & Goutz 1990; Herman *et al*. 1991). Furthermore, algae have been shown to be more sensitive to complex wastes than fish or invertebrates (Miller *et al*. 1978). Results of the present study indicate that the values of Chl*a* content were subjected to considerable variation according to the different sampling sites and the exposure period (Fig. 3.4b). The total *Selenastrum capricornutum* count decreased in response to the coal tar residue compared with the control and reference sites 9 and 10. This findings support previous studies by Amman and Terry (1985) and Tukaj (1978) with respect to the effect of several organic pollutants on algae.

3.4.3 Fresh water *Daphnia magna* and *Chironomus tentans* biotests

The water flea *Daphnia magna*, is the recommended and perhaps, the most widely used test organism for toxicity. Tremendous numbers of papers are available regarding the use of daphnids in such evaluation, through the estimation of the median lethal concentration (LC50) or Time (LT50) values (ISO 1982). Fig. 3.5 shows the percentage of survival of daphnids in 100% concentration for 48 hours of exposure. The response of *Daphnia magna* to the whole-sediment toxicity test was similar to the *Chironomus tentans* and *Selenastrum capricornutum* bioassay test, excepted for sampling sites 3 and 6 downstream which correlated strongly with the reference sites 9 and 10. This indicates that *Daphnia* was not as sensitive to exposure of coal tar residue downstream as in the case of the *Selenastrum capricornutum* and *Chironomus tentans* bioassays.

The accumulation of pollutants is maximum in the upper few centimeters of sediment which are important for a number of biological processes (Levin & Kimball 1985). *Chironomus tentans* is therefore an ideal species for toxic sediment assessment because it comes into contact with the compounds through both physical contact and ingestion of sediment material. Furthermore researchers also have demonstrated that natural factors do not significantly affect the survival, growth, and reproduction of *Chironomus* ssp. (Ankley *et al*. 1994; Day *et al*. 1995b).

3.4.4 Macroinvertebrate Response

Macroinvertebrate communities have been commonly used to assess stream water quality, and most studies on environmental monitoring and assessment of stream ecosystems focused on the change of community structure or functional organization of macroinvertebrate as responses to environmental stresses (Rosenberg & Resh 1993).

The organisms in the major taxonomic groups: Annelida (Oligochaeta), Diptera (Chironomidae), Ephemeroptera (*Tricorythodes minutus*), Tricoptera (*Hydropsyche* spp.) and Nematoda accounted for more than 80% of the organisms collected at the 10 sampling sites. However, the relative abundance of other taxonomic groups was low, which may be partly due to the absence of any acquatic plant biomass. Gregg and Rose (1985) indicated that aquatic plants increased physical heterogeneity and created more diverse habitats for the attachment of benthic macoinvertebrates. Cummins (1974, 1975) suggested that food resources are a major determinant of stream macroinvertebrate distribution and proposed a classification of stream macroinvertebrates into functional feeding groups (Cummins 1974). Distributional patterns of functional feeding groups reflect resource distribution and use, and facilitate the understanding of organic matter processing in river ecosystems (Vannote *et al*. 1980). Therefore, it has been suggested that the bioassessment of river water quality based on functional feeding groups of macroinvertebrates may be superior to that based on community structure alone because it reflects more ecologically significant attributes of rivers (Rabeni *et al.* 1985). In our study the mean number of taxa in each functional feeding group significantly differed among the 10 sampling sites. The major taxa, comprising of collector-gatherers (Chironomidae; Oligochaeta) was much higher at the reference sites, upstream from the oily sheen (Fig. 3.6). Few scrapers and shredders were observed downstream that probably indicates the effect of oily substances in the water. Other studies have observed higher abundances of some chironomid and oligochaete taxa related to petroleum contamination (Rosenberg & Wiens 1976; Woodward & Riley 1983), however no apparent oil-induced increases in these groups were observed downstream of the oily sheen during our study. The most severely affected taxa in the study were Chironomidae, Trichoptera and Ephemeroptera; the sensitivity of these groups to petroleum exposure has been documented elsewhere (Barton & Wallace 1979).

The AUSRIVAS-method, widely used in the USA and Canada (Reynoldson & Metcalfe-Smith 1993), was used in the survey study of the Cache la Poudre River (Table 3.1). Sampling sites 2, 4, 5 and 7 were severely degraded with none of the expected macro-invertebrate families found, which show a good parallelism with the results of the battery of bioassays and also indicated the areas of highly contaminated sediment, because due to the complexities associated with coal tar migration in subsurface media many coal tar accumulations are difficult to locate (Cohen & Mercer 1993).

3.4.5 Benthic phytoplankton response

Algae are ubiquitous, ecologically important, and sensitive to a broad range of stressors and respond rapidly to changes in water chemistry (McCormick & Cairns 1997). Variation in community structure and function within benthic macrohabitats results from local variation in abiotic forces that are not always easily measured or readily apparent to the unaided eyes. For example, small-scale changes in current velocity (e.g., 5 cm/s) can alter patterns of algal biomass accumulation on the substrate (Stevenson 1983) and therefore sampling was taken at different current velocities (Fig. 3.3). The epipsammic community in the Cache la Poudre River, dominated by diatoms, was very firmly attached to sand particles, and it seems to be well adapted for existence in a highly variable environment (Miller *et al.* 1987). The best use of diversity-related indices in river assessments is probably as an indicator of changes in species composition when comparing impacted and reference assemblages (Jüttner *et al.* 1996). Some investigators have found that diversity decreases with pollution (Rott & Pfister 1988), that diversity can increase with pollution (van Dam 1982), and that diversity changes differently depending upon the type of pollution (Jüttner *et al.* 1996). Patrick (1973) furthermore predicted that some pollutants would differentially stimulate growth of some species and thereby decrease evenness of species abundances, while toxic pollution could increase evenness and that severe pollution could decrease species numbers. In the case of our study high diversity of species and abundance were recorded in case of diatoms with respect to the two reference sites 9 and 10 (Fig. 3.8). Morales-Loo and Goutz (1990) and Herman *et al*. (1991) found that petroleum hydrocarbons in the aquatic environments could have subtle effects on the relative abundance of algal species, inhibiting the growth of some sensitive species and promoting the growth of tolerant ones.

We found in our study that the use of benthic diatoms as indicators of environmental conditions in the Cache la Poudre River was important for three basic reasons (1) their importance in ecosystems; (2) their utility as indicators of environmental conditions, and (3) their ease of use. Diatom importance in river ecosystems is based on their fundamental role in the food webs (Lamberti 1996), oxygenation of surface waters and linkage in the biogeochemical cycles (Kim *et al*. 1990; Mulholland 1996). As one of the most species-rich components of river communities, diatoms are important elements of biodiversity and genetic resources in rivers (Patrick 1961).

3.5 Conclusion

In conclusion, based on data we suggest that *Selenastrum capricornutum*, *Daphnia magna* and *Chironomus tentans* should be included as a battery of tests for assessing acute toxicity from coal tar, although time, conditions of exposure, and species specificity were different and may have influenced the results. The toxicological properties of coal tar contaminated sediment are currently unknown. However, since neat coal tar has been demonstrated to be highly carcinogenic to laboratory animals, it is reasonable to presume that coal tar contaminated soil represents a concern to human health (Goldstein *et al*. 1998; Gaylor *et al*. 2000). In addition, the presence of coal tar residue contaminated sediment in the environment brings about significant implications in the community structure of aquatic organism as seen in our study. In the present study we have focused on the exposure of macroinvertebrates and phytoplankton to residue of coal tar and the evaluation of lethal responses of species under laboratory conditions and on the benthic community's response under natural conditions when challenged with a long-term exposure. Because of the numerous routes of exposure we found that, almost any species may be affected by coal tar. Also, because coal tars differ in their chemical composition from site to site based on production methods and temperatures, fuel stock, length and method of storage, leaching of chemicals by nearby soils and water resources, and weathering conditions, the use of coal tar collected from a single manufactured gas plant site as in the case of our study, do not adequately represent the environmental effects of all coal tars. The dominance by a single phytoplankton taxon that was significantly higher at the contaminated sampling sites, than in the case of the reference areas were most probably due to environmental stress of coal tar residue. Nutrient or organic enrichment, or toxic conditions, in streams can cause such shifts in algal communities from dominance by one species to dominance by other taxa (Gausch *et al.* 1998; McCormick & Stevenson, 1998). Such changes in algal taxonomic composition can profoundly affect food web interactions and ecosystem dynamics (McCormick & Cairns 1997).

The results presented in this study show that the importance of the biotest battery in the determination of toxicity of contaminated sediment lies in the fact that these are measures of the organism's response to simultaneous influences of various environmental parameters affecting its toxicity. The main point of the test battery was to include living organisms at two or more trophic levels to determine the potential effects based on diverse endpoints. Although we did not attempt to measure residue concentrations of the coal tar, we found that the biotest battery is a very useful, sensitive and inexpensive tool to detect toxicants in the environment. The results of our study indicate that changes in the macrovertebrates and benthic algae composition can also be used as bioassessment to identify potential environmental hazards at polluted coal tar sites.

Figure 3.9 Clean-up of the Cache la Poudre, 2004-2005. Before (A) and during (B) restoration.

In October 2004, after reaching an agreement that divided the cost of the cleanup project among Xcel Energy, which operated the gas plant, Schrader Oil Co., which now owns much of the former gas plant property, and the city of Fort Collins, which owns property south of the river contaminated by coal tar and other chemicals, EPA contractors began excavating the contaminated property behind Northside Aztlan Community Center, 200 Willow St. The EPA's plan is to divert and drain a portion of the river, remove as much contaminated soil and groundwater as it could from the riverbed and the contaminated site behind Aztlan, then install a 680 foot long plastic barrier between the contaminated site and the south bank of the Poudre River (Darst 2005) (Fig. 3.9).

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