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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1. Introduction

High levels of contaminants in freshwater sediment may have irreversible adverse effects on 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 (US 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 US used coal and oil to derive light-end hydrocarbons that were utilized for lighting and heating (Environmental Research and 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 eliminated the need for 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 and Gruber, 1979). Since living organisms will show some response to hazardous levels of any chemical, 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 used to analyze the toxicity of remnants of coal tar in contaminated sediment of the Cache la Poudre River. The selected species were the freshwater algae *Selenastrum capricornutum*, and the invertebrates *Daphnia magna* and *Chironomus tentans*. The aim of the investigation carried out here was firstly to determine the ecotoxicological effects of coal-relevant substances and bioconversion products on benthic invertebrates and phytoplankton community in an 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.

2. Material and methods

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 194km from its origin to its confluence with 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). 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. 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 and Bahls, 1999).

2.2. Toxicity testing

2.2.1. *Selenastrum capricornutum* biotest

In this study, we used a 96-h growth, standard freshwater algal toxicity test with the unicellular, crescent-shaped, green alga *S. capricornutum* (40-60 μm^3) that can be found in both eutrophic and oligotrophic freshwater environments. The methods used for culture and testing were as described by the Environmental Protection Agency (EPA) (US 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 (US EPA, 1994). Cultures were grown in a nutrient medium that included EDTA and were 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 reaches a cell density of 1×10^2 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 macroinverte-brates with a core sampler, was filtered through a 0.45 mm filter to remove particulate material. The test was conducted as a screen (100% concentration). Nutrients without EDTA were added to ensure that the 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 1×10 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 2571 °C with a continuous photoperiod. The flasks were swirled twice daily by hand and randomly repositioned in the incubation chamber. Cell density and chlorophylls (Chl*a*) contents of the triplicate samples were measured at 24, 48, 72 and 96 h and the mean count (cells/ml) was calculated for each treatment. Chl*a* was extracted from lyophilized GF filters using iV,iV-dimethylformamide for 2h at room temperature. Chl*a* was measured photo-spectrometrically 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.

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* <24h 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 of 7.99, total hardness of 250mg $CaCO_3$/l and total alkalinity of 96mg $CaCO_3$/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 50g sediment (wet weight) was placed in each beaker, and then 200ml 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 were measured daily, while pH, ammonia, conductivity, alkalinity and hardness were determined at test initiation and termination. After 24 and 48h, the number of immobilized *Daphnia* was determined visually. *Daphnia* that sank to the bottom of the vessels within 15s of having been slightly shaken were considered immobile. Data from the replicate beakers were pooled

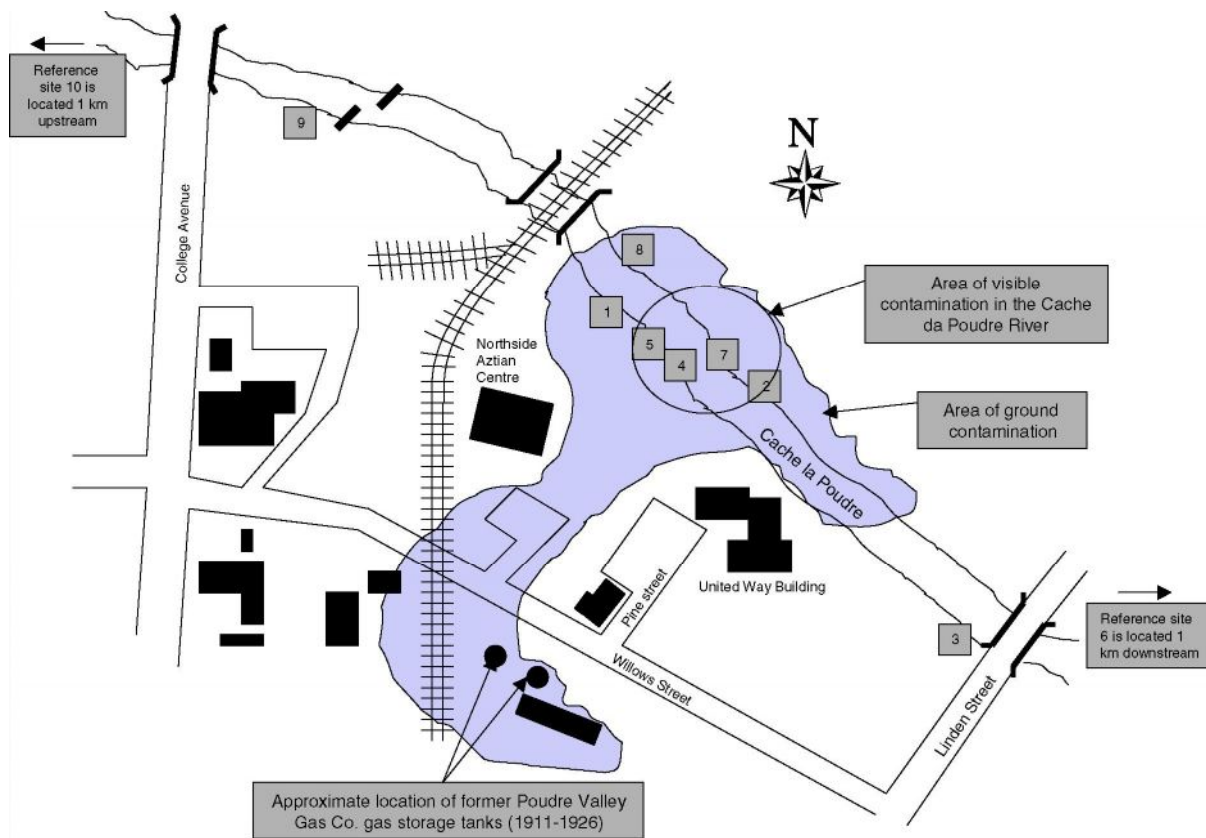and treatments were compared to the negative control and reference sites.

Fig. 1. Map of the study area in Fort Collins showing the sampling sites on the banks of the Cache la Poudre River, Colorado.

2.2.3. *Chironomus tentans* test

The assessment of whole sediment toxicity involves a 10-day exposure of C. *tentans* to the contaminated sediment. We conducted the toxicity test in 300-ml beakers with eight replicate chambers. The sediment volume for the test was 100ml with 175 ml of overlying water. Sediments of the different sampling sites were prepared the day before test initiation and allowed to equilibrate overnight following the instructions of the US EPA (2000). The following day, 10-second-to-third-instar larvae were added per chamber under a 16:8 h light:dark photoperiod at 23±1 C. Overlying water in the test containers was renewed, with two volume replacements per day (US 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 as compared to the mean survival of larvae exposed to controls and reference samples.

2.2.4. Data analyses of biotest endpoints

The test results were recorded on standard Excel spreadsheets 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 the probit function (LT50) where the number of dead/total number is a probit function of time.

2.2.5. Sampling of macroinvertebrates and phytobentos

Macroinvertebrates and phytobentos were collected at weekly intervals with a corer at each of the 10

sampling sites from August to October 2004. Four core samples were taken monthly at the 10 sampling sites (Fig. 1). A random sampling procedure was used to reduce hydro-biological variability between sites (Voelz and Ward, 1991). The corer was driven into the substrate to an approximate depth of 10cm. Cobble-sized rocks within the corer were transferred to a bucket and scrubbed. The other material within the corer was removed by hand. Macroinvertebrates, organic matter and the 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 125 **x** magnification; smaller taxa were then enumerated at 1250 **x** using the strip-count method (American Public Health Association (APHA), 1989). Diatoms were identified after clearing in acid persulfate. Biovolumes of more abundant taxa were estimated by measuring cell dimensions of at least 20 individuals and using the closest geometric formulae (Willen, 1976). Identification of taxa was done according to the 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: > 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 for the oil sheen area. 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 and 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%. 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 close to 1 is equivalent to the 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 the reference condition (Table 1).

Table 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% probability of occurrence; O, number of taxa observed from the list of those with > 50% probability of being at the site; *O/E,* observed/expected ratio. This should be close to 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.

3. Results

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. 2). The flow regime was measured on six occasions when the flow was high, medium and low (Fig. 3). During the whole period of our investigation, the pH at all of the 10 sampling sites was above 7 (Fig. 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. 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 decrease in Chl*a* content of *S. capricornutum* was significant during the whole exposure time of the experimental run. On the other hand, at sampling site 3 and 6 downstream, the *S. capricornutum* growth was inhibited up to the third day of incubation and thereafter the S. capricornutum started to recover. The variation in counts of *S. capricornutum* cells of the different sampling sites over a period of 96 h is represented in Fig. 4A. At sampling sites 3 and 6, *S. capricornutum* started to recover by the end of the third day of exposure and attained a higher count at the end of the 96h while sampling sites 2, 4, 5 and 7 resulted in a decrease of *S. capricornutum* cell count by the end of the exposure time. The total *S. capricornutum* count decreased in response to the coal tar residue compared with the control and reference site 9 and 10.
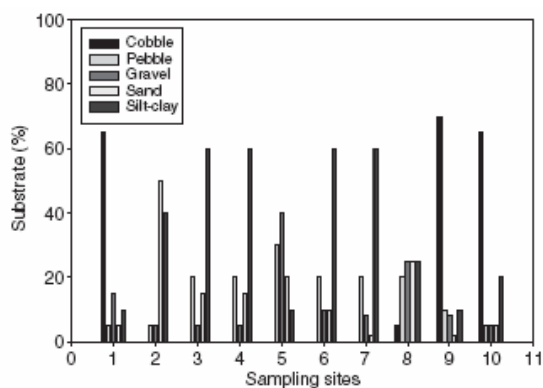

Fig. 2. Substratum composition of the 10 sampling sites in the Cache la Poudre River, Colorado.
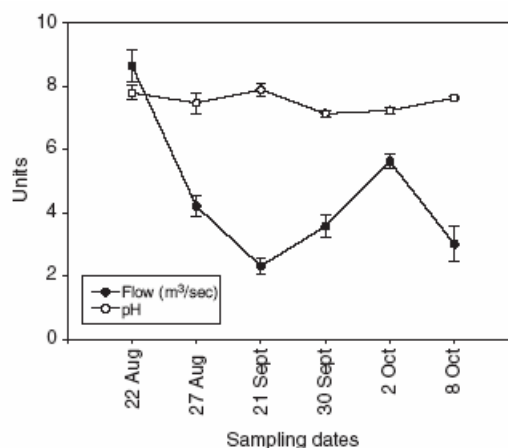

Fig. 3. Variations in flow and pH from August 22 to October 8 in the Cache la Poudre River, Colorado. Error bars indicate standard deviations from the mean value.

3.3. Freshwater *Daphnia magna* and Chironomus tentans *biotests*

The percentage of survival of daphnids in 100% concentration for 48h of exposure is indicated in Fig. 5. At sampling sites 2 and 5, the survival of *D. magna* to the whole-sediment toxicity test was 0% after 48h of exposure, while at sampling site 7 only 20% survived. Coal tar residue had little effect on the survival rate of *D. magna* at sampling sites 3 and 6 downstream. The survival endpoint results for *C. tentans* following a 10-day exposure to whole-sediment toxicity test are illustrated in Fig. 5. The sediments 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 survivor percentages of 40, 40 and 26.66%, respectively. The trends observed in this data set are comparable with the data obtained from the *S. capricornutum* and *Daphnia* bioassay, and are highly significant ($p \leq 0.001$).

3.4. Macroinvertebrate response

Eighty percent of the organisms collected at the sampling sites belong to the following taxonomic groups: Annelida (Oligochaeta), Diptera (Chironomi-dae), Ephemeroptera (*Tricorythodes minutus*), Tricoptera (*Hydropsyche* spp.) and Nematoda, while the relative abundance of other taxonomic groups was low (Fig. 6). 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 was 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, Oligo-chaeta), followed by lower numbers of collector-filterers (Trichoptera) during our survey (Fig. 6). Very few chironomids, scrapers and shredders were either eliminated or reduced in numbers at the sampling sites.
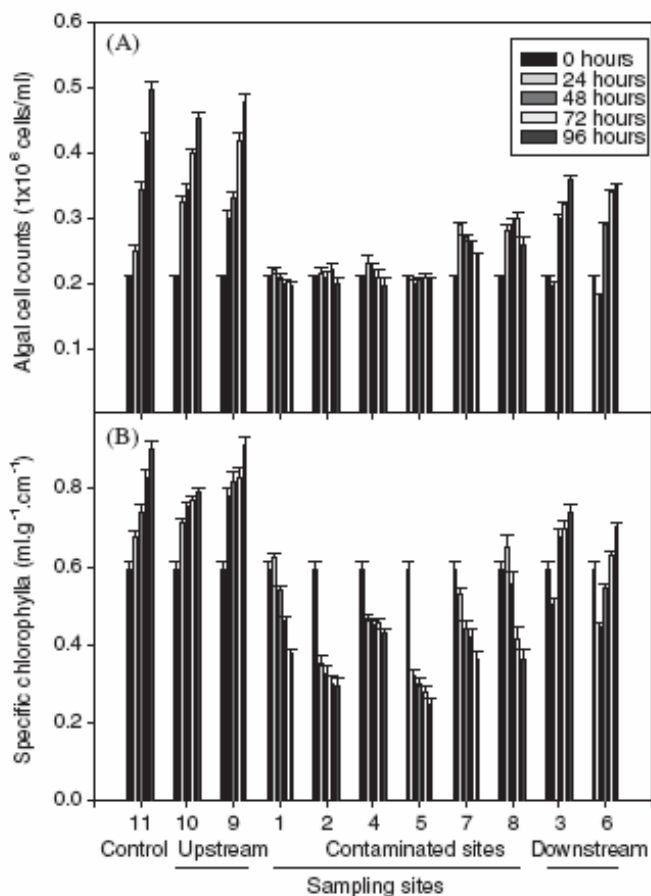

Fig. 4. Changes in *Selenastrum capricornum* cell counts (A) and specific Chl*a* content (B) at the different sampling sites in the Cache la Poudre River, Colorado. Error bars indicate standard deviations from the mean value.
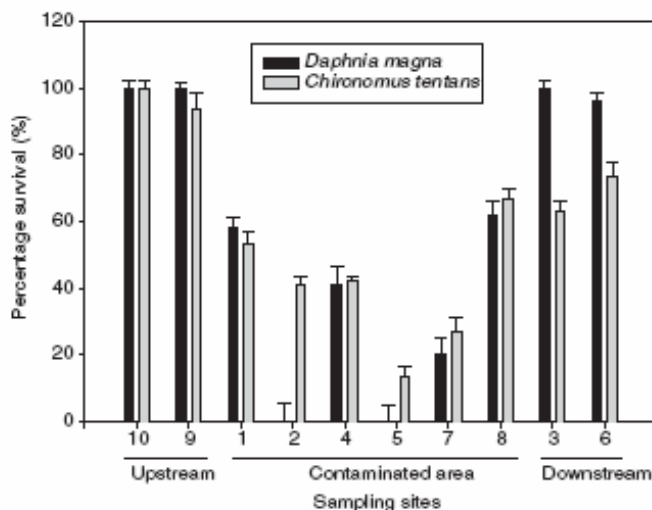

Fig. 5. Results of the survival rates of *Daphnia magna* (48 h) and *Chironomus tentans* (240 h) after biotesting of sampling sites. Error bars indicate standard deviations from the mean value.

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. Sampling sites 9 and 10, which were 7 200m and 1km upstream, respectively, from the oily sheen were selected as unmodified reference sites and used as a baseline for judging change.

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 dominated the contaminated areas was Didymosphenia geminata. 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. 7). Comparisons with the reference sites 9 and 10 revealed that sampling sites 3 and 6 downstream from the oily sheen had 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.
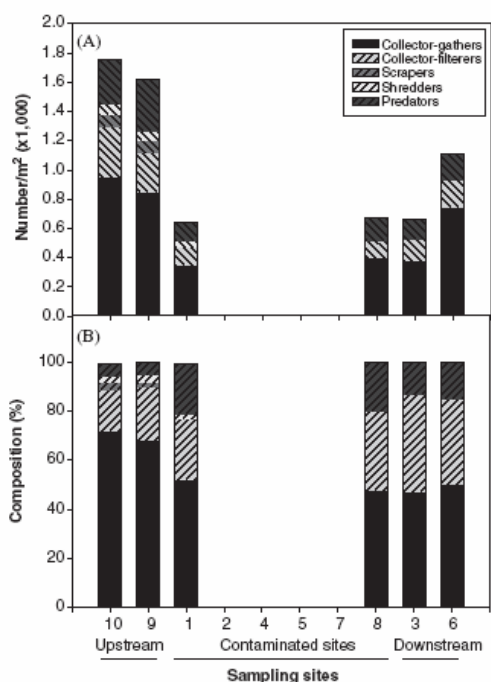

Fig. 6. (A) Percentage composition and (B) abundance (individuals/m²) of macroinvertebrate functional feeding groups recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004.
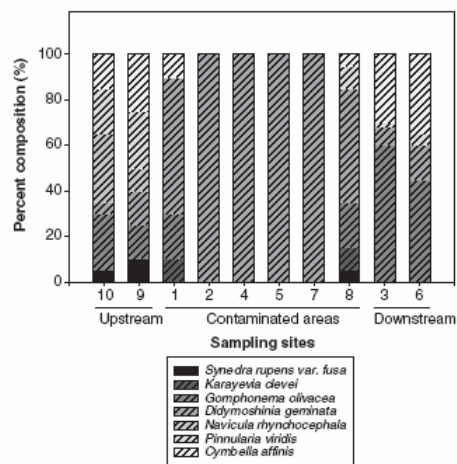

Fig. 7. Percentage composition of different bentic phytoplankton species recorded at the 10 sampling sites on the Cache la Poudre River, Colorado. Mean values from sampling during August to October, 2004.

4. Discussion

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 a result of differences in their susceptibility to flood disturbance. The distribution of stream macroinvertebrates is also closely related to substrate characteristics (Minshall, 1984; Watling, 1991). The substratum in the Cache la Poudre River consisted predominantly of pebbles, sand and silt-

clay. The flow regime is an important factor shaping the 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 was 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.

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 (O'Brien and Dixon, 1976; Morales-Loo and 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). The 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. The total *S. capricornutum* count decreased in response to the coal tar residue compared with the control and reference sites 9 and 10. These findings support previous studies by Amman and Terry (1985) and Tukaj (1978) with respect to the effect of several organic pollutants on algae.

4.3. Freshwater *Daphnia magna* and *Chironomus tentans* biotests

The water flea *D. 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). The response of *D. magna* to the whole-sediment toxicity test was similar to the *C. tentans* and *S. capricornutum* bioassay test, except 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 *S. capricornutum* and *C. tentans* bioassays.

The accumulation of pollutants is maximum in the upper few centimeters of the sediment, which is important for a number of biological processes (Levin and Kimball, 1985). *C. 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 have also demonstrated that natural factors do not significantly affect the survival, growth, and reproduction of *Chironomus* ssp. (Ankley et al., 1994; Day et al., 1995).

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 and 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 macro-invertebrates 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. Few scrapers and shredders were observed downstream which 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 and Wiens, 1976; Woodward and 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 and Wallace, 1979). The AUSRIVAS method, widely used in the USA and Canada (Reynoldson and Metcalfe-Smith, 1993), was used in the survey study of the Cache la Poudre River. Sampling sites 2, 4, 5 and 7 were severely degraded with none of the expected macroinvertebrate families found, which showed a good parallelism with the results of the battery of bioassays and also indicated the areas of highly contaminated sediment (Cohen and Mercer, 1993).

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 and Cairns, 1997; Rott et al. 1998). 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 eye. 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 samples were taken at different current velocities. The epipsammic community in the Cache la Poudre River, dominated by diatoms, was very firmly attached to sand particles, and it seemed 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 (Juttner et al., 1996). Some investigators have found that diversity decreases with pollution (Rott and Pfister, 1988), diversity can increase with pollution (Van Dam, 1982), and diversity changes differently depending upon the type of pollution (Juttner et al., 1996). Patrick (1973) furthermore predicted that some pollutants would differentially stimulate the growth of some species and thereby decrease the evenness of species abundances, while toxic pollution could increase evenness and severe pollution could decrease species numbers. In the case of our study, a high diversity of species and abundance were recorded in case of diatoms with respect to the two reference sites 9 and 10. 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.

In our study, we found 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).

5. Conclusion

In conclusion, based on data from the present study, 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 has significant implications in the community structure of aquatic organisms as seen in our study. 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 does 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 was most probably due to the 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 and Stevenson, 1998). Such changes in algal taxonomic composition can profoundly affect food web interactions and ecosystem dynamics (McCormick and 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 the potential environmental hazards at polluted coal tar sites.

Acknowledgments

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