REVIEW OF
SEDIMENT MONITORING
TECHNIQUES

MEND Project 4.7.4

This project was funded by Energy, Mines and Resources Canada and the British Columbia Ministry of Energy, Mines and Petroleum Resources under the Canada/British Columbia Mineral Development Agreement

November 1990

REVIEW OF SEDIMENT MONITORING TECHNIQUES

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November 1990

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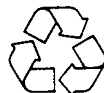

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EXECUTIVE SUMMARY

The primary objective of this literature review is to provide the British Columbia Acid Mine Drainage Task Force with information and guidance on sediment contamination issues. The review focuses on assessment of sediment quality through sediment chemistry, bioassay testing and benthic macroinvertebrate community structure. Analytical methods for sediment chemistry are reviewed and summarized. Sampling devices, design, handling and preparation are evaluated with respect to sediment toxicity testing. Freshwater sediment bioassays with bacteria, alga, invertebrates and fish are reviewed; acute, sublethal, and chronic (= life cycle) endpoints are discussed. Application of several bioassays in a multitrophic or battery approach is recommended. The utility of benthic macroinvertebrate community structure and function as measures of sediment quality is discussed. Sampling methods and design are reviewed;-natural substrate sampling is recommended over artificial substrates. Recommendations are made for sorting, taxonomic identification and statistical analysis of benthic infauna data sets. This review stresses the importance of quality assurance/quality control (QA/QC) in sampling, sample handling, chemical analysis, bioassay testing and taxonomic analyses. Finally, guidelines for sediment chemistry, bioassay testing (test battery) and benthic macroinvertebrate community assessment are provided; an interpretive framework (The Triad) is suggested which will effectively combine the information from these independent measures.

ACKNOWLEDGEMENTS

This study was funded by the Department of Energy, Mines and Resources of Canada and the British Columbia Ministry of Energy Mines and Petroleum Resources, under the Canada/British Columbia Mineral Development Agreement. This study was conducted by E.V.S. Consultants. The report was written by Laura Dear, Elizabeth Power and Wade Gibbons and reviewed by Dr. Peter Chapman and Mr. Robert Deverall (Analytical Services Laboratory); it was produced by Arlene Booth and Janice Linton. E.V.S. Consultants wishes to acknowledge the assistance of Mr. Benoit Godin (Environment Canada).

1.0 INTRODUCTION

Organic and inorganic contamination of aquatic sediments may be the result of past or present discharge/disposal practices; this problem is gaining prominent attention both nationally and internationally. Aquatic sediments, particularly those of high organic content, tend to have an affinity for many organic and inorganic contaminants. Depending on the ambient physical and chemical conditions at a particular site, sorption can affect contaminant persistence, toxicity, uptake and transport (Seelye et al., 1982; Breteler and Saska, 1985; Lewis and MacIntosh, 1986). Sediments are known to act as sinks and can contain many toxic substances at levels much higher than the concentrations in the overlying water.

Many persistent contaminants are immediately lethal or have long-term deleterious effects. Such contaminants are sorbed to sediments in the aquatic environment where they remain over long periods of time and can affect those organisms living in and coming into contact with the sediments. Organisms living in the sediment (the benthos) are ecologically important and/or a food source for commercially important organisms, such as trout, salmonids and invertebrates, such as crayfish. These sediment-bound toxicants can affect benthic organisms through direct contact, and by ingestion of contaminated particles. In addition, toxicants may be released into the overlying water column and affect aquatic organisms by acidification, dredging, bioturbation, storm events, or complexation.

In all cases, the contaminant is active (or bioavailable) only if released from the sediment and passed to the target organism across a biological barrier. Sediment toxicity, as measured by bioassays, is hence a consequence of its desorptive capacities, and the direct toxicity of the contaminant. Detection of sediment-bound contaminants is suggestive of toxic potential, but is insufficient without a meaningful biological toxicity appraisal.

In the case of acid mine drainage, sediment contamination may represent a significant problem with respect to residual contamination, even after source elimination. However, bioassay techniques have not been widely applied to acid mine drainage problem in fresh water systems; to date, approaches have concentrated on documenting the degree and distribution of contaminants, without attempting to address the question of bioavailability and ecological significance.

A sediment monitoring program really depends on the questions which a study sets out to address. The following approaches are used to characterize sediments:

- bulk sediment analysis: chemical characterization of the sediment which provides an assessment of the total concentrations of various contaminants.

- physical analysis: characterization of physical characteristics such as grain size, organic carbon.

- bioassay testing: toxicity tests to examine the bioavailability of contaminants.

- benthic macroinvertebrate community structure.

1.1 Objectives

The primary objective of this review is to provide the British Columbia Acid Mine Drainage Task Force with a review which will provide information and guidance on sediment contamination issues. The review, based on in-house and readily available literature, has the following objectives:

1. To provide an overview of the types of tests available for the chemical analysis of sediments (including bulk total analysis, elutriate tests, and fraction/extraction procedures). Sediment characterization based on physical parameters is also discussed.

2. To evaluate the freshwater sediment bioassays which are currently available. This evaluation will review these protocols with respect to specific criteria which are important to data quality and data utility, including the application of marine techniques to freshwater lakes and rivers.

3. To provide an overview of field and laboratory techniques (i.e., sampling techniques, preservation of samples, quality assurance/quality control (QA/QC) and benthos sampling) used in sediment quality assessment to provide a set of guidelines which would be useful in selecting the most effective approaches to sediment monitoring.

4. To develop an interpretative framework for sediment assessment and implementation, related to acid mine drainage. Areas which require further investigation will be identified, as well as possible "next steps" for development of this field.

1.2 **Approach**

Based on discussions with Acid Mine Drainage Task Force members, evaluation of freshwater sediment bioassays was targeted as a focus of this literature review. In addition, we were encouraged to draw upon our experience in the field of sediment assessment to provide background information and an interpretive framework for this review. This review is not intended to be exhaustive, but rather to summarize the state-of-the-art in the field of sediment assessment. A section on sediment chemistry is included to provide an overview currently available analytical techniques. The level of effort expended is described in the letter proposal (Appendix A). The report is divided into sections according to each of the above objectives; the primary focus of the review is on the first objective.

2.0 SEDIMENT CHEMISTRY

The types of tests available for the chemical analysis of sediments include:

- bulk total analysis;
- elutriate test;
- fractionation/extraction procedures.

The utility of any one test depends on the purpose of the study, as each particular test provides specific information. Specific chemical analyses should be selected well in advance so that an appropriate sampling regime can be determined. Once a list of parameters is agreed upon, the appropriate sample containers and preservation techniques can be specified.

2.1 Sample Collection and Handling

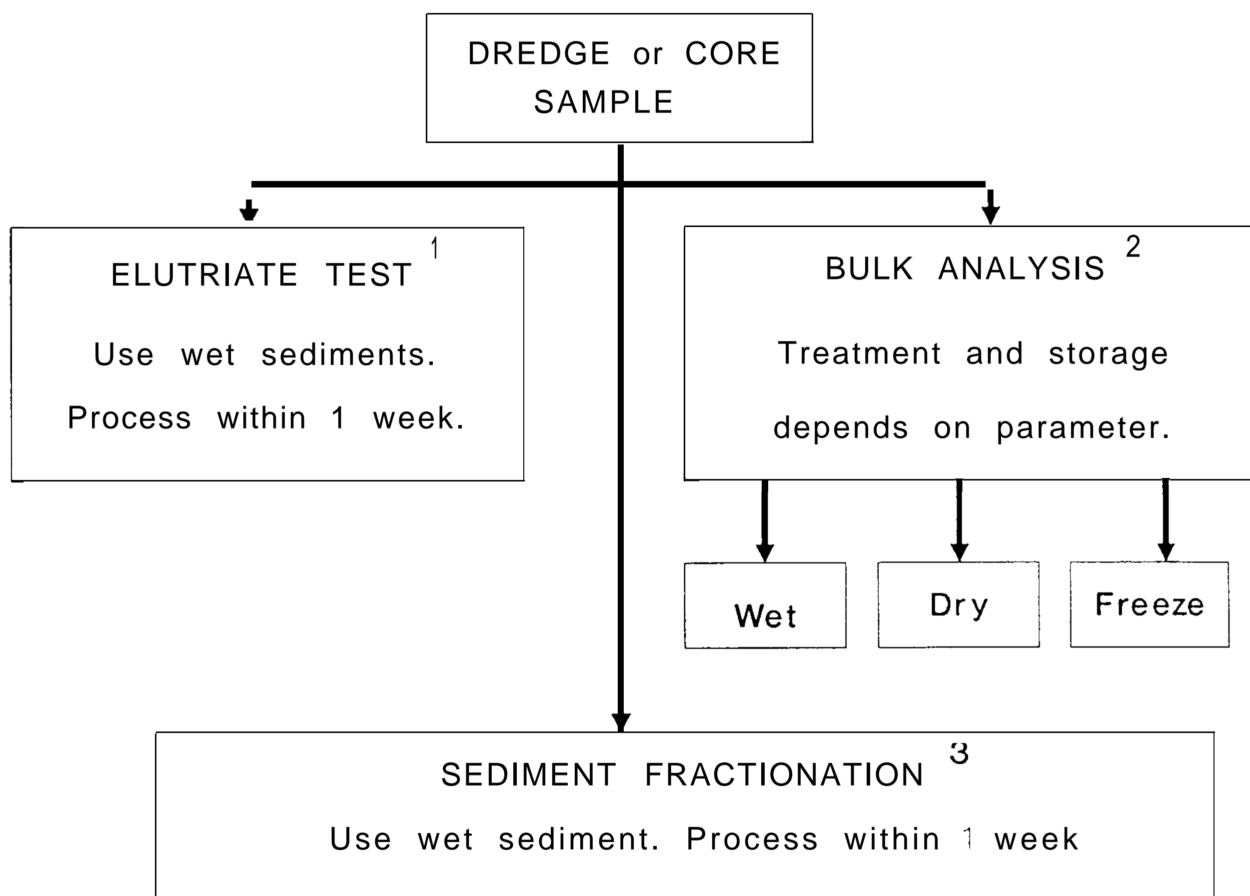
Appropriate field technique is critical to the collection of high quality data. Sampling errors will be manifested in the form of limited accuracy and/or the collection of a non-representative sample. The main objective of field collections is to ensure the site is adequately characterized. This can be addressed by:

- collecting representative samples;
- using appropriate sampling and handling techniques; and
- properly preserving the samples until they are analyzed.

Collection of representative samples and selection of appropriate sample locations is discussed in detail by Plumb (1981). Sample collection is discussed elsewhere in this document (c.f., section 3.3) and in detail by Plumb (1981). Sample handling methods will be discussed in this section as they are determined by the type of test, since all three of the test types discussed in this section (bulk analysis, elutriate test, and fractionation) have specific sample handling requirements (c.f. Figure 1).

The elutriate test indicates the ability of chemical constituent to migrate from the solid phase to the liquid phase. Because chemical constituents migrate differentially, sample alterations due to drying, freezing and air oxidation should be avoided. Fractionation tests provide information on the partitioning of chemical components among different chemical phases. As with samples for elutriate tests, samples should not be dried, frozen or air oxidized. Bulk analysis ascertains total concentrations of the chemical constituents; chemical speciation is less important, freezing and drying may not cause sample degradation. Where potential volatile compounds (e.g., methyl-mercury) may be present, precautions should be taken to avoid losses during sample handling. It is important to emphasize that a sample stored in a dried or frozen state can only be analyzed by bulk analysis, and cannot be used for elutriate tests or fractionation studies.

As it is not always possible to analyze samples immediately after collection, samples may require preservation to avoid chemical changes during storage. A sample may change physically, chemically, and biologically after it is collected. Preservation slows but may not arrest these modifications. Quality Assurance/Quality Control (QA/QC) for sediment chemistry sampling, handling, and analysis is discussed in detail in Section 5.3.



1 Mobility of Constituents

2 Total Sediment Concentration

3 Elemental Distribution in Sediment

source: Plumb, 1981

Figure 1. Methods of sediment sampling for the three sediment chemical analyses: Bulk, Elutriate, and Fractionation.

2.2 Chemical Analyses

2.2.1 Bulk Analysis

A bulk analysis provides a measure of the total concentration of a specific constituent in a sample. Total sediment concentrations can be used to compare different sites and to identify major point sources. However, the harshness of the "total" extraction procedure does not provide information on chemical distribution or speciation within the sample.

Various digestion procedures have been used to dissolve the metals contained in sediment samples, and can be classified based on their effectiveness at dissolving metals: total dissolution with hydrofluoric, fuming nitric or perchloric acids; strong acid digestion using hot strong acids such as nitric; and mild acid leach using dilute acid (IJC, 1988; PSEP, 1986). The silicate minerals are completely dissolved when total dissolution is used, and recoveries for most metals are high. Almost all metals (including, cadmium, copper, lead, mercury, silver, and zinc) are dissolved by strong acid digestion except for some minerals (PSEP, 1986). Hence, concentrations of metals that are in the minerals, for example, iron, aluminum, manganese, chromium, and nickel, are underestimated. The mild acid leach procedure provides low retrieval for most metals (PSEP, 1986).

Total Digestion

A hydrofluoric acid/aqua regia total acid digestion was recommended by the PSDDA/PSEP workshop members for total metal analysis of marine sediments (see Rantala and Loring, 1975 for detailed methodology). The recommended procedure is as follows:

- 200 mg of dry ground sample is weighed into a decomposition vessel;

- add 0.5 mL concentrated nitric acid (HNO,) and heat on low setting; add a few drops of hydrogen peroxide to aid digestion;

- cool and add 0.75 mL concentrated hydrochloric acid (HCl) and 3.0 mL concentrated hydrofluoric acid (HF). Seal vessel and heat for 2 hours in an oven;

- cool and add 2.5% boric acid; reseal and reheat for 1 hour in the oven;

- cool and bring contents to 25 mL with distilled/deionized water; and,

- analyze directly by acceptable spectroscopy techniques.

A second method recommended in PSEP (1986) is the procedure followed by the NOAA Status and Trends Program:

- 0.2 g of dry ground (100 mesh) sample is digested with nitric, perchloric, and hydrofluoric acids in a fluoropolymer bomb;

- add 1 mL of 4:1 HNO_3:$HClO_4$; seal bomb cap and place in a 130°C oven for 4 h;

- cool, add 3 mL of HF. Reseal and reheat overnight (8-12 h);

- cool and add 20 mL distilled/deionized water; shake vigorously and add contents to a 30 mL acid washed polyethylene bottle; and,

- analyze as per acceptable spectroscopy techniques.

The above procedures assume that 200 mg of sediment is representative of the whole sample, or the sample has been prepared (dried, pulverized, sieved, etc.,) to ensure it is representative.

Strong Acid Digestion

While many authors agree that total digestion procedures for metal characterization provide for total metal solubilization, certain factors such as the quantity of organic matter or the presence of various anions may effect the degree of metal recovery (IJC, 1988). In addition, perchloric, hydrofluoric and nitric acids generally require special equipment or great care to ensure laboratory safety (IJC, 1988). It has been shown that sufficiently long digestion by a 1:1 mixture of concentrated HCl and HNO, at a high temperature is comparable to digestion methods using perchloric and hydrofluoric acids (IJC, 1988). The method is as outlined below (IJC, 1988):

- To 2.0 g of dry ground (100 mesh) add 10 mL each of concentrated HCl and HNO, (teflon beakers with polyethylene reflux caps are recommended);

- Transfer to a hotplate and heat slowly to 95°C. Digestion is carried out for 5 hours and samples should be rotated every 30 to 90 minutes to ensure *even* heating;

- After 5 hours, cool and decant the digestate to a 100 mL volumetric flask;

- Transfer the remaining sediment to centrifuge tubes and mix with 25 mL of 1% 12M HCl in deionized/distilled water, cap and shake for 3 minutes; decant supernatant into the volumetric flask; and,

- Metals in this solution can be analyzed by acceptable spectroscopy techniques.

2.2.2 Sediment Elutriate Tests

The elutriate test is a short-term, sediment-leaching procedure. It consists of agitating a known volume of sediment with a known volume of site water. The supernate is filtered, and the filtrate is analyzed. The test is an indicator of the chemical constituents likely to be released to the water column during a dredge/disposal/filling operation (Keeley and Engler, 1974; Lee et al., 1975; Plumb, 1981). The 4:1 water to sediment ratio used in the test is based on hydraulic dredging ratios, results from this test are likely to overestimate the release from less powerful dredging procedures (Plumb, 1981).

The elutriate test provides information on the potential effects of a disposal operation on water quality. Results can be compared to appropriate water quality guidelines. It should be noted that comparing elutriate test concentrations with criteria would be "conservative", as site dilution is not incorporated (Plumb, 1976). Therefore, elutriate test results exceeding water quality guidelines do not necessarily imply water quality impacts.

The elutriate test is a simulation of the dredging and disposal process and the supernatant that results from the vigorous agitation of a 4:1 site water/sediment mixture is intended to approximate a dredging slurry. The following methodology is modified from Plumb (1981):

- Subsample 1000 mL of both dredging and disposal site water;

- filter disposal site water through a 0.45 μ pore-size membrane filter that has been pre-rinsed with 100 mL disposal site water (discard this filtrate);

- subsample sediment and mix with unfiltered site water in an approximate 4:1 volumetric ratio (usually 1000 g dry sediment: 4000 g water);
- cap flask and shake for 30 min and then let settle for 1 h;
- decant the supernatant and centrifuge;
- vacuum filter sample through a 0.45 μ pore membrane filter;
- elutriate can be analyzed (in triplicate) by atomic absorption.

2.2.3 Sediment Fractionation

Trace metals may be associated with various phases of the sediments, they may be (1) adsorbed (e.g., to clays, humic acids, etc.); (2) carbonate-bound; (3) bound to iron and/or manganese oxyhydroxides; (4) bound to organic matter; (5) bound to sulphide; (6) matrix bound (bound in lattice positions) (Tessier et al., 1977; Tessier and Campbell, 1987; Pardo et al., 1990). Owing to the fact that these fractions behave differently under different environmental conditions, total heavy metal content (bulk analysis) does not completely describe the sediment chemistry; speciation studies are therefore recommended to estimate the toxicity potential (Pardo et al., 1990).

The two basic speciation studies recommended for sediments are theoretical, based on thermodynamic calculations (Pardo et al., 1990), and experimental (sequential extraction). Several fractionation methodologies (see Förstner, 1982; Tessier and Campbell, 1987) involve leaching the sample with progressively stronger reagents so that the metal concentrations can be determined in each extract. The assumption behind fractionation studies is that a specific reagent will define a specific phase of a sediment constituent.

To selectively extract sediment-bound metals from a specific fraction a number of procedures may be followed (Tessier and Campbell, 1987). Once the reagents have been selected, it must be determined in which order to apply them. For a given set of reagents, the sequence used will influence the partitioning obtained. There is a potential problem with readsorption, i.e., the extraction procedure may cause a shift in the metal distribution (Tessier and Campbell, 1987).

Sample procedures will be briefly outlined, following Plumb (1981); Table 1 presents alternative extraction strategies. A sediment sample is split in half; one half used for the interstitial water testing and the second half used for all other analyses.

- Sample collection and handling must be carried out without introducing chemical changes (e.g., oxidation).
- The initial separation removes the interstitial water and is carried in a glove bag maintained with positive pressure nitrogen.
- The exchangeable phase is determined on the unused sample half. A 20 g subsample is mixed with 100 mL of 1 N ammonium acetate, shaken for 1 hour and centrifuged. Filter the sample through 0.45 μ pore membrane filters and analyze immediately.
- The easily reducible phase is performed on the solid residue from the exchangeable phase extraction. The first extraction is carried out with 0.1 M hydroxylamine hydrochloride-O.01 M nitric acid. The solid residue is used in the organic and sulphide phase and is extracted with 1 N ammonium acetate.
- The solid residue remaining from the sulphide phase extraction is extracted with citrate-dithionate in the moderately reducible phase.
- The remaining solid residue is extracted with hydrofluoric acid and nitric acid in the residual phase extraction.

2.2.4 Conventional Parameters

Although this section is restricted to the chemical analysis of metals in sediments, a brief discussion on the analysis of physical parameters (conventionals) will follow. Sediment characterization will depend on the study objectives and the contaminants of concern. However, a set of characterization parameters should be included which are known to influence toxicity and or sorptive properties, to aid in data interpretation. The following procedures are summarized from an unpublished ASTM document (Burton and Landrum, 1989), refer to that publication (cited in Burton and Landrum, 1989) for complete methodologies.

Table 1. Examples of sample extraction procedures (from Tessier and Campbell, 1987).

Step	Method			
	1	2	3	4
I	$MgCl_2$	NH_4OAc	NH_4OAc	$NH_2OH \bullet HCl/HNO_3$
II	NaOAc/HOAc	$NH_2OH \bullet HCl/HNO_3$	NH_2O/HNO_3	$NH_2OH \bullet HCl/HOAc$
III	$NH_2OH \bullet HCl/HOAc$	$(NH_4)_2C_2O_4/H_2C_2O_4$	H_2O_2/HNO_3	$Na_2S_2O_4$
IV	H_2O_2/HNO_3	H_2O_2/HNO_3	$Na_2S_2O_4$	$KClO_4/HCl/HNO_3$
V	$HF/HClO_4$	HNO,	HF/HNO3	HF/HNO_3

1 Tessier et al., 1979.
2 Forstner, 1982.
3 Engler et al., 1977.
4 Chao and Theobald, 1976.

2.2.4.1 Carbon Fraction

Carbon fractions which may be of importance in determining toxicant fate and bioavailability include: total organic carbon, dissolved organic carbon, dissolved inorganic carbon, particulate organic carbon, and particulate reactive carbon. The organic carbon content of sediments has been measured by wet oxidation methods after Menzel and Vaccaro (1964). This method is also useful for the determination of the organic carbon content of water. A titration method (after Walkley and Black, 1934), a modification of the titration method (Yeomans and Bremmer, 1988) or combustion after removal of carbonate by the addition of HCl and subsequent drying (Wood et al., 1987) have also been employed for organic carbon analysis.

2.2.4.2 Particle Size

Particle sizing of sediments can be measured by numerous methods (Allen, 1975) and the most effective method is dependent on the particle properties of the sample (Singer et al., 1988). Particle size is often conducted by wet sieving (Duncan and Lattaie, 1979; Plumb (1981). Particle size classes may be determined by the hydrometer method (Day, 1965; Patrick, 1958), the pipet method (Rukavina and Duncan, 1970), settling techniques (Sanford and Swift, 1971), X-ray absorption (Rukavina and Duncan, 1970) and laser light scattering (Cooper et al., 1984). To obtain definite particle sizes for the fine material, that which may be ingested by benthos, a Coulter (particle size) counter method may be employed (McCave and Jarvis, 1973; Vanderpleog, 1981). This method gives the fraction of particles with an apparent spherical diameter and is superior to the pipette method if adequate quality assurance steps are taken (Shideler, 1976). Another potential method for determining the particle size distribution of a very fine fraction is through the use of electron microscopy (Leppard et al., 1988). The collection technique for the very fine materials can result in aggregation to longer colloidal structures (Leppard et al., 1988; Leppard, 1986; Burnison and Leppard, 1983). Comparisons of particle sizing methods have shown some to produce similar results and other not, which may be attributed to differences in the particle property being measured, i.e., the Malvern Laser Sizer and Electrozone Particle Counter are sizing techniques, and the hydrophotometer and SediGraph determine sedimentation diameter through particle settling (Singer et al., 1988; Welch et al., 1979; Kaddah, 1974). Size analysis techniques for fine-grained sediments are generally based on settling velocity, although some, such as sieving, coulter counter and microscopy, measure actual particle dimensions on cross-sectional area (IJC, 1988).

2.2.4.3 Cation Exchange Capacity

Cation exchange capacity provides information relevant to metal bioavailability studies (Black, 1965) and sorption of anionic surfactants. Amorphic oxides of iron and manganese and reactive particulate carbon have been implicated as the primary characteristics influencing metal sorption potential in sediments (Crecelius et al., 1987; Jenne and Zachara, 1987). "Reactive" particulate carbon is that portion which equilibrates with the aqueous phase. Various selective extraction methods have been recommended to determine bioavailable fractions of metals in sediments (Chao and Zhou, 1983). At this time no *one* method is clearly superior to the others. This may be due, in part, to site specific characteristics which influence bioavailability (desorption/equilibration processes). Easily extractable fractions are usually removed with cation displacing solutions, e.g., neutral ammonium acetate, chloride, sodium acetate, or nitrate salts (Lake et al., 1984). Sequential extraction procedures are discussed in more detail in section 2.2.3.

2.2.4.4 Eh

Eh measurements are particularly important for metal speciation and for determining the extent of sediment oxidation. These measurements are potentiometric and measured with a platinum electrode relative to a standard hydrogen electrode (Plumb, 1981). Care must be taken in probe insertion and to allow equilibration to occur when measuring Eh. Redox gradients in sediments often change rapidly over a small depth and are easily disturbed. Because of the rapid changes in Eh during storage, it is preferable to take these measurements as soon as is practical in the field.

3.0 SEDIMENT BIOASSAY TESTING

3.1 Introduction

Sediment bioassays provide information on toxicity and bioavailability which cannot be provided by chemical analyses alone. They are generally subject to less variability than are benthic surveys, but they are not specific to any particular group of chemicals. Acute lethal testing in the freshwater environment can use a number of species, as described in this section.

In terms of problems with acid mine drainage, sediment contamination represents a significant problem with respect to residual contamination, even after source elimination. However, sediment bioassay techniques have not been widely applied to acid mine drainage problems in freshwater systems; to date, approaches have concentrated on documenting the degree and distribution of contaminants, without attempting to address the significance or bioavailability of the various components.

3.2 Metals **in Sediments and Bioavailability**

Metals are rapidly lost from the aqueous phase by precipitation but the precise prediction of metal partitioning in sediments is difficult (Jones, 1986). Aquatic sediment can become contaminated with inorganic chemicals which can precipitate as insoluble, inorganic compounds, adsorb to inorganic or organic particulate matter or dissolve in sediment pore water. Precipitated hydrous ferric oxides, which are frequently associated with aqueous mine waste, appear to control soluble metal concentrations in effluent leaving the mine. Studies of the distribution and partitioning of metals at a mine in Wales suggest similarities in the behaviour of zinc and cadmium and of silver, copper and lead (Jones, **1986).** These contaminants can directly affect benthic organisms by becoming bioavailable through resuspension, leaching or sediment ingestion.

Sediment analyses are used for selection of critical sites for routine water sampling for non water-soluble contaminants that are rapidly adsorbed by particulate matter, and thus escape detection by water analyses. Sediment data are important in environmental investigations, where short term or past pollution events are not detected by water analyses **(Förstner** and Whittman, 1979). Sample preparation poses many problems with respect to the assessment of metal species in sediments and pore water. Metal partitioning of anoxic sediments must be performed with great care under oxygen-free conditions and sediment extractions should follow appropriate procedures (reviewed in **Förstner,** 1987).

Most pollutants can generally be assumed to be concentrated in the tine particle fraction of sediment (Sakai et al., **1986),** which is related to both grain size and organic carbon (Karickhoff et al., 1979; Mudroch, 1984). Sakai et al., **1986)** clearly demonstrated that by sieving sediments, heavy metal concentrations generally increased with decreasing particle size. Schneider and Weiler (1984) developed a grain size correction procedure to reduce grain size effects by normalization of the total trace metal concentration to a conservative metal; data for sediments of differing grain size were then compared. This approach has theoretical applications, but is not generally useful for evaluating sediment quality, since it does not address the question of bioavailability any more than total metal concentrations do.

Dissolved organic matter in aquatic systems is widely known to reduce the bioavailability of heavy metals (Karickhoff et al., 1979; Landrum et al., 1985, 1987). Metals in lake sediments were found to be distributed in a bi-modal fashion with higher concentrations in the particle size from 250-63 μm and less than 4 μm. Metal content seems to be associated with organic content in the first fraction, while in the small fraction size, sorption with clay particles and co-precipitation with Fe/Mn oxides and hydroxides are most likely responsible (Mudroch, 1984). In the Rhine River in Europe, analyses showed a bi-modal distribution of metal in relation to particle size (> 63 μm) due to organic content and < 0.2 μm due to the clay particles effect (Forstner and Whittmann, 1979). Determination of metals in riverine sediments seems to be correlated with particle size. Hiromutsu et al. (1986) suggest particle size cut-offs of <71 μm; Ricket et al. (1977) used <20 μm, and DOE/DFO (1979) used particle size less than 150 μm, because these particles are small enough to be ingested by benthic invertebrates.

3.3 Sampling Methods for Bioassay Samples

A critical aspect of any environmental evaluation is the field sampling program. Any errors incurred during sampling will manifest themselves by limiting the utility of the data. The objective of sediment sampling for biological and chemical analysis is to obtain samples which will characterize the area sampled. Sample size should be small enough to be conveniently handled, yet sufficient to provide appropriate quantities for all the analyses. Also, consideration must be given to site selection and replicate sampling. This section focuses on sediment sampling techniques for bioassay testing; sample handling and quality assurance/quality control for sediment samples are discussed in Section 5.2.

In lakes, basic limnological information should be recorded; this might include the following: area, mean depth, maximum depth, volume of the lake, discharge of inlet/outlet, retention time of the water, alkalinity, pH, nutrient, dissolved oxygen (D.O.), redox potential, chlorophyll a, and transmittance. In flowing waters, the following information should be recorded: water flow rates, water depth, turbidity, alkalinity, pH. and D.O.

3.3.1 Sampling Devices

There are three broad classifications of sediment collecting devices: corers, grabs and dredges. Corers generally produce the least disturbed sediments; grabs and dredges collect larger surface samples which

in some cases may be considered to be qualitative. These methods are widely used for sampling in freshwater and marine sediments, but are not always appropriate for sampling in moving waters.

For monitoring purposes, the surface layer of sediment usually provides the most valuable information because it has the most recent accumulation of heavy metals and the greatest interaction with surface waters, and therefore the greatest possibility of remobilisation and direct contact with benthic invertebrates. Superficial sediments in accumulation areas are usually very loose with water content values over 90%. It is generally difficult to collect good samples if thin layers (< 1 cm) are sampled (Hakanson, 1980).

Dredge

An Ekman grab can be used for collection of undisturbed sediment samples (Forstner, 1977). Cornwell (1986) used the Ekman to retrieve samples from the bottom while a plastic corer liner was used to retrieve the sample for analysis. Mudroch (1984) used a Shipek grab sampler for particle size analysis. Grabs are most useful in lakes or in very slow moving rivers. In rivers, another approach may be to look for small pockets of fine grained sediment and use an Eckman grab (Ricket et al., 1977). Surficial sediment can be removed by a clean plastic scoop for analysis (Plumb, 1981).

Corer

For lakes, Forstner (1979) did not recommend the gravity corer for surface sediment analysis since the shock disturbs the fragile surface layer. On the other hand, undisturbed core samples for trace metal profile were obtained with a KB corer with plastic liner by slowly lowering, not dropping, the corer into the sediment. Plumb (1981) suggested use of a piston corer to collect undisturbed sediment. The same type of corer was used for sedimentary records by Davison et al. (1985).

Multiple cores provide a spatial dimension to the chemical stratigraphy of lake sediments that can help elucidate the various processes that control sediment deposition and thereby contribute to a more accurate reconstruction of environmental history (Engstrom and Swain, 1986). Morris and Kwain (1988) found that temporal (vertical) and spatial trends in sediment accumulations of metals could be investigated using core samples for a lake in Ontario, Canada.

A freeze-core has been used for particle size analysis of river sediments, although the possibility of contamination of sediments by the copper brass sampler should be considered (Derksen, 1985). Hand

cores with plastic liners can be easily driven into soft sediments and are very practical for sampling along river banks and shoals.

Sediment Traps

Sediment traps are used to provide insight into such processes as nutrient cycling, production, sediment accumulation, atmospheric input and water column transformations of both dissolved and particulate matter (Asper, 1988; Wesche et al., 1989). There are many different designs and applications, but the general principle is that these samplers trap suspended sediments by collecting particles which fall in from the overlying water. Well positioned, the sediment trap can collect a large amount of new material (Hakanson, 1976; Carlton, 1975). Shape and form of sediment traps are variable and may change the rate of sedimentation; additionally, retrieval procedures also affect the amount of sediment collected (Bloesch and Burns, 1980). Differences between deposition and bed load transport cannot be determined from sediment trap samples. Rather, the samples give an estimate of the amount of material potentially available to settle in a particular area (Welton and Ladle, 1979).

Sediments which are collected, particularly by “above sediment” surface traps, do not accurately represent the in *situ* sediments for a particular site, primarily because the particle size range of trapped sediments does not represent the particle size range of bedded sediments. Since particle size is known to affect adsorption of contaminants, samples collected by sediment traps will not accurately reflect bedded sediments. Sediment traps are really designed for measuring ***in situ flux*** of particulate matter, and are still useful for this purpose. However, sediment traps designed for use in small rivers and streams (Welton and Ladle, 1979) are the more applicable for sediment sampling purposes because they are inserted in the stream bed and are more likely to accumulate sediments in a manner similar to bedded sediments. These samplers collect the material which settles between stones in the interstitial spaces and may be useful at sites where large stones preclude sampling by dredge or core.

Interstitial water

Extraction of interstitial water from sediments is performed by leaching, centrifugation or squeezing. Recent ***in situ*** methods include pore water sampling using sediment “peepers” (Hesslein, 1976). The syringe method (Derksen, 1985) allows interstitial water samples to be taken from gravel as deep as 20-25 cm. This method should be used in clastic stream sediments.

3.3.2 Sample Design

Sampling design theory is a large field of study, but some specific points will be made here. There are several aspects to sample design which should be addressed before a study team initiates sample collection including: overall site locations, determination of background levels or reference sites (if appropriate), sediment volumes required and replication.

The bottom dynamics should be established in order to avoid interference with resuspended and hydrodynamically complex situations difficult to interpret (Hakanson, 1980). Therefore, in lakes, it is suggested that sampling close to river mouths be avoided, as river action and bottom conditions often show considerable variability in time and space. Also to be avoided are subaquatic slopes with inclinations >4-5%, and topographical bottle necks (i.e., between islands). Ideally, a preliminary survey would be conducted to identify appropriate sample sites. Particle size analysis will provide useful information because coarse materials are found in erosional areas and finer materials are found in accumulation areas. Also, the percentage of water in the sediment, expressed as wet weight, may describe the relationship between accumulation vs. transportation vs. erosion (Hakanson, 1980), and can be represented graphically. For Swedish lakes, Hakanson (1980) suggested that samples should provide an even area cover of the lake or sub-basin with at least 5 samples to determine a proper mean estimate. In rivers, visual assessment of "accumulation" and "erosion" sites can usually be done for grab samples.

Establishing background levels of contamination is a critical task which can be accomplished by the following:

- using background levels measured if sampling occurred during the pre-industrial period, as it is done in mineral exploration (Forstner, 1977),

- from core fractioning and ^{210}Pb, ^{137}Cs and ^{14}C measurement or other references such as pollen analysis and sedimentation rate determination (Davison et al., 1985; Forstner and Wittman, 1979),

- by comparison to standard reference levels of geological features (Turekiana and Wedephol, 1961, quoted by Hakanson, 1980, Forstner, 1977),

- by using heavy metal concentrations in the large fraction size (> 10 mesh size or 2 mm) as background levels (Hiromutsu et al., 1986), and

- by selection of several upstream reference sites, outside of the zone of acid mine drainage influence.

Determination of the sample size for hypothesis testing depends on estimation of the variability of the parameters (Sokal, 1969), and on the amount of sediment required to conduct all the testing. In general, replication should be determined on a case-by-case basis with regard to the variability of the sample, ideally determined in a preliminary survey.

3.3.3 Sample Handling

Sample handling methods, with respect to quality assurance/quality control are provided in Section 5.2, but methods specific to bioassay sample handling are discussed here. It is generally agreed that sediments to be used for toxicity testing should not be frozen (Chapman and Becker, 1986, Malueg et al., 1986, Dillon, 1983; Swartz et al., 1985), although freezing may be appropriate for chemical measurements on samples to be stored or for testing of chemical extracts (e.g., Microtox). Samples for bioassay testing should not be held longer than 4 weeks, with 6 weeks as a maximum holding time; these times are based on convention and experience more than published data, and may change as more information becomes available. Sediment samples should be stored in tightly sealed bags, in the dark at 4°C. Plumb (1981) recommended refrigeration of the samples in an air tight container to preserve the anaerobic integrity of the sample for metal analysis.

3.3.4 Sample Preparation

The biological effects of toxic sediments have been investigated using bulk sediment chemical analysis (Malueg et al., 1984a,b), elutriate tests (Samoiloff et al., 1980; Nebeker et al., 1984a, 1988; Dawson et al., 1988, Sloterdijk et al., 1989), solid-phase sediment tests (Prater and Anderson, 1977a,b; Cairns et als., 1984; Nebeker et al., 1984a; Schuytema et al., 1988), recirculating apparatus (Prater and Anderson, 1977; Malueg et al., 1984a,b), and by making differential extractions with various extractants to selectively remove particular classes of toxic substances (Samoiloff et al., 1983; Dutka and Kwan, 1988).

The exposure route for contaminants is an important consideration; the test is directed towards a phase of the "sediment system" (e.g., direct contact with bulk sediment, exposure to pore water, exposure to overlying water, ingestion of sediments). Direct exposure to sediments is ideal because it most closely approximates the **field** situation for benthic invertebrates. Testing of overlying water examines the bioavailability of sediment contaminants to water column organisms and pore water testing examines the component of field sediments that many researchers believe is the route of exposure to benthic invertebrates. Testing of pore water and sediment extracts also allows examination of dose response relationships, to determine the relative toxicity and assess probability of effect (Giesy et al., 1990). Historically, sediment dilutions (with clean sediment) have not been as widely used as dilutions of sediment-extracts and pore water, perhaps because of a lack of understanding on how to mix sediments.

All assessment strategies are limited by several factors. It is difficult to determine if chemicals present in the sediment are available to exert a toxic effect to animals in the field situation because the chemicals may be bound tightly to the sediment particles rendering them biologically inactive. The proportion of toxic substances extracted that are available to cause toxicity are unknown; elutriates test only the soluble components of the sediment, and do not address the toxicity due to particle ingestion or contaminant contact by benthic organisms. The rigorous shaking and extraction processes may not simulate real-world conditions and may change bioavailability by altering oxidation states and sorption characteristics of the sediments (Jenne and **Zachara,** 1988). Additionally, some contaminants may be toxic to one group of organisms, but not another, so selection of test species is important (Burton et al., 1989; Malueg et al., 1983).

Giesy et al. (1990) compared the relative sensitivities of bioassay tests, using different exposure regimes for each (pore water from the sample diluted by pore water from a reference area, diluting whole --sediment with sediment from the reference area and then testing the resulting pore water, and diluting whole sediment with sediment from the reference area and then using the whole sediment). The four bioassay tests used were: Microtox (15 min.), ***Daphnia magna* (48** h lethality), ***Hexagenia limbata*** (168 h, acute lethality) and ***Chironomus tentans*** (10 d, lethality and weight gain). While the three dilution techniques gave similar results with some assays, they gave very different results in other assays. The dose response relationships determined by the three dilution techniques would be expected to vary with sediment, **toxicant** and bioassay type, and Giesy et al. (1990) concluded that the dose response relationship derived from each technique needs to be interpreted accordingly.

The use of standard, non-benthic bioassay organisms allows comparison of results of tests using sediment extracts or elutriates with previously established surface water criteria. The relationship between the results of pore water and elutriate tests and those conducted with whole sediments is unclear (Nebeker et al., 1984a), in part, because the partitioning of toxicants from the solid phase into the pore water, and thus bioavailability, is dependent on many chemical and physical processes, most of which are not well understood and seldom characterized with respect to sediment toxicity bioassays (Giesy et al., 1990).

Dose response relationships for toxicants can be determined by spiking sediments of a defined character with known concentrations of a single toxicant or mixtures. Sediment spiking experiments are used to determine if sediment bound contaminants are available to organisms. Nebeker et al. (1986a) spiked sediments with Cd to examine their toxicity to *Duphniu magna* and *Hyalella azteca* in sediment bioassays, relative to water column bioassays. The toxicity of dissolved Cd in the sediment tests was similar to the toxicity of Cd in the water only tests, indicating that the test organisms were not obtaining appreciable amounts of Cd from the sediment or from suspended or dissolved organic materials (Nebeker et al., 1986a). If toxicity is due only to the free metal ion, then the presence of ligands may reduce the free Cd concentration and hence the toxicity. Although the spiking technique provides useful information about toxicity of specific contaminants, it does not provide sufficient information to determine *in situ* effects.

Ideally, bioassay testing and chemical analyses would be conducted on bedded sediments, collected by methods which obtain whole sediments. However, where this is not possible, testing of sediment collected by sediment traps could be used to characterize the chemistry and toxicity of those sediments. However, the results would have to be interpreted with caution, recognizing that only one component of the system's sediment was being tested.

3.4 Review of Sediment Bioassay Techniques

Many environmental processes affect metal availability to aquatic organisms (Luoma, 1983); although chemical analyses of sediments provide valuable information, they should not replace direct biological measurements such as bioassays. The choice of test organisms is important, since there are many choices of species, taxa, routes of exposure and protocols, each of which has advantages and disadvantages.

A large number of sediment bioassays have been developed and used in recent years for fresh waters (Cairns et al., 1984; Malueg et al., 1984a,b; Nebeker et al., 1984a; Prater and Anderson, 1977a,b;

Table 2. Summary of lethal bioassay tests for freshwater sediments.

Organism	Group	Species	Endpoint	Author(s)
Invertebrates				
crustacean	cladocem	*Daphnia magna*	mortality	Prater & Anderson, 1977a,b Cairns et al., 1984 Maleug et al., 1984a,b Nebeker et al., 1984a, 1986 Schuytema et al., 1984 Giesy et al., 1988 Athey et al., 1989 Burton et al., 1989 Larson, 1989
	amphipod	*Hyalella azteca*	mortality	Nebeker et al., 1984a
		Gammarus lacustris	mortality	Nebeker et al., 1984a
Insects	midge	*Chironomus tentans*	mortality	Nebeker et al., 1984a,b
	mayfly	*Hexagenia limbata*	mortality	Prater & Anderson, 1977a,b Malueg et al., 1984a,b Nebeker et al., 1984a Schuytema et al., 1984
Annelids	oligochaete	*Stylodrilus heringianus*	mortality	Keilty et al., 1988
			sediment avoidance	Keilty et al., 1988 White & Kielty, 1988
		Limnodrilus hoffmeisteri	mortality	Keilty et al., 1988
			sediment avoidance	Keilty et al., 1988 White & Kielty, 1988

Table 3. Summary of sublethal bioassay tests for freshwater sediments.

Organism	Group	Species	Endpoint	Author(s)
Fish				
		Pimephales promelas	development	Dawson et al., 1988
			growth	Leblanc & Surprenant, 1985 Norberg & Mount, 1985 Dawson et al., 1988
Invertebrates				
Crustaceans	cladocern	***Daphnia magna***	young survival	Cairns et al., 1984
	amphipod	***Hyalella azteca***	growth	Nebeker et al., 1984a, 1986 Nebeker & Miller, 1988 Burton et al., 1989
			bioaccumulation	Nebeker et al., 1984a
		Gammarus lacustris	growth	Nebeker et al., 1984a
			bioaccumulation	Nebeker et al., 1984a
Insects	midge	***Chironomus tentans***	bioaccumulation	Nebeker et al., 1984a
			larval growth	Cairns et al., 1984 Malueg et al., 1984a,b Nebeker et al., 1984a, 1988 Giesy et al., 1988
	mayfly	***Hexagenia limbata***	bioaccumulation	Prater & Anderson, 1977a,b Malueg et al., 1984a,b Nebeker et al., 1984a
Oligochaetes		***Tubifex tubifex***	growth and reproductive success	Milbrink, 1987 Wiederholm et al., 1987
		Limnodrilus hoffmeisteri		Wiederholm et al., 1987
		Limnodrilus cfaparedeanus		Wiederholm et al., 1987
		Limnodrilus udekamianus		Wiederholm et al., 1987
		Potamothrox hammonienis		Wiederholm et al., 1987

Schuytema et al., 1984). Bioassay tests vary with respect to bioassay type (lethal, sublethal, genotoxic, and bacterial) and to bioassay organism (ranging from bacteria through to fish). Tables 2 and 3 summarize lethal and sublethal freshwater sediment bioassays, respectively; chronic (= full life cycle) tests are indicated. It has been widely noted that different bioassay tests can produce different bioassay results (Samoiloff et al., 1983; Cairns et al., 1984, Chapman et al., 1984), hence it is recommended that toxicity testing incorporate more than one endpoint. Ideally, as outlined in Chapman and Long (1983), sediment bioassay testing should incorporate acute lethal, sublethal, chronic and genotoxic or bacterial responses. At least three tests are recommended for a "test battery", in order to adequately describe sediment toxicity.

Generally, both lake and river samples are tested using the same bioassay protocols. Bioassay organisms are considered to be "test systems" and, since tests are usually conducted under static conditions, lacustrine bioassay organisms are favoured.

3.4.1 Microorganisms

The use of microorganisms as test organisms is based on the assumption that at least some biochemical and physiological systems are conserved, and that toxicants elicit their effects due to similar interactions with biomolecules present in many organisms. Owing to differing modes of actions of toxicants and to the differences in physiologies and biochemistries of organisms, not all organisms can be expected to respond with equal sensitivities. Bacteria are generally equally sensitive or less sensitive to metals than are plant or animal cells (Babich and Stotzky, 1985). The variation in microbial bioassays' sensitivities to extracts, and deviations from the responses of higher organisms have made it less widely accepted as a means for assessing sediment toxicity.

3.4.1.1 Bacterial Luminescence

3.4.1.1.1 **Microtox^R**

The Microtox^R assay is a bacterial luminescence bioassay that was developed by Beckman, Inc., in 1977, as a rapid screening alternative to standard acute toxicity tests using fish or invertebrates (Bulich, 1984). The test is based on the reduction in bioluminescence of the marine bacterium (*Photobacterium phosphoreum*) (NRRL

B-l 1177) by toxicants. The metabolism of the luminescent bacteria is influenced by low levels of toxicants and, occasionally, stimulants. Any alteration of metabolism affects the intensity of the organism's light output. By sensing these changes in light output, the presence and relative concentration of toxicants can be obtained by establishing the EC50 levels from graphed data: EC50 being that concentration of toxicant causing a 50% reduction in light from the baseline level (Bulich, 1986, Dutka, 1988). It is simple (sediment extracts are prepared) and can be completed in a short period of time with replication. The bacteria are available in a freeze-dried state, and can be reconstituted in the field. In general, advantages of this test are that it is among the most rapid available, it is fully applicable to the field, and all reagents are available commercially. Technical disadvantages are that start-up requires some initial expense, the reaction requires some stabilization time and there may still be some problems with pH adjustment, timing of the reaction, salinity, coloured or turbid samples, and extraction or concentration of the toxicant in field samples.

The impacts of metals on Microtox fluorescence have been investigated by Bulich et al., (1981), Dutka and Kwan (1982, 1984), Qureshi et al., (1984), Vasseur et al., (1984a,b,1986), Greene et al. (1985), Reteuna et al. (1986), Tarkpea et al. (1986), Thomas et al. (1986), Surowitz et al. (1987), Elnabarawy et al. (1988), Morel et al. (1988) and Parrott (1988). Intercomparisons are complicated due to the use of different exposure times, pH and assay temperatures. For some metals, the toxicity (as assessed by the Microtox assay) increases with temperature (Vasseur et al., 1984a) and a slow, progressive response is evident for most metals regardless of temperature (Dutka and Kwan, 1982; Vasseur et al., 1984a,b; Elnabarawy, 1986).

The Microtox[R] assay has been extensively studied and the results for a large number of pure compounds and complex mixtures have been compared with those of acute bioassay using both fish and invertebrates (Lebsack et al., 1981; Qureshi et al., 1982; Schiewe et al., 1985). In a general review (Munkittrick et al., in press), Microtox is not as sensitive to inorganic chemicals as *Daphnia* or the rainbow trout, but is comparable to the fathead minnow. *Duphnia* were more sensitive than Microtox to copper (60 to 370x), chromium (100), cadmium (>60), arsenate (6.5), zinc (2.0 to 9.6), mercury (1.0 to 2.7) and cobalt (1.2). Rainbow trout were more sensitive to cadmium (400), copper (30) and zinc (22), but less sensitive to mercury (0.38) and arsenate (0.81). Fathead minnow were more sensitive to cadmium (<100), and of approximately the same sensitivity as Microtox to chromium (1.0 to 1.9), copper (0.006 to >40) and zinc (0.1 to 1.2), but less sensitive to cobalt (0.32) and mercury (0.44). In comparison with other bacterial assays, Microtox also showed a lower sensitivity to lead (Dorward and Barisas, 1984), cadmium (De Zwart and Sloof, 1983) and nickel (Dutka and Kwan, 1984).

Sediment Extracts and Leachates

Microtox has been used to monitor sediment toxicity by Dutka et al. (1986, 1988a,b) and Kaiser et al. (1987, 1988), as well as Hamdy and Post (1985), Hermens et al. (1985), Kwiatkowski and Nadeau (1985), Schiewe et al. (1985), Calleja et al. (1986), Williams et al. (1986), Symons and Sims (1988) and True and Hayward (1988). These studies seldom quantified contaminants present in the leachates, and compared toxicity on the basis of positive response versus negative, or toxic/non-toxic designations.

Although numerous attempts have been made to examine sediment-associated toxicity, the issue is controversial and can be complicated. Microtox is much more sensitive to organic extracts using 1% DMSO than water extracts (Dutka and Kwan, 1988), but interpretation must be made carefully. Problems exist with organic extraction techniques and the toxicity of the solvent carrier. In laboratory studies, DMSO at the 1% level was not toxic to microassays used by Dutka and Kwan (1988a,b) in their screening battery and no synergistic effects were detected between the toxicant and 1% DMSO solution. Dutka (1988) cautions that a) more rigorous extraction procedures may measure toxicants which are not biologically available, b) the solvent may potentiate the response and c) increasing the complexity of the test also increases the expense and time commitment to procedures which are only meant for monitoring and prioritization. Several authors only use pore water extract as an estimate of biologically-available toxicants (e.g., Giesy et al., 1988).

Ribo et al. (1985) recommended the use of rotary evaporation and fractionation to examine samples. The procedure is not effective for metals, organo-metals, electrolytes, pH and surfactants and can only be used to identify relative differences. Results were comparable to levels of volatile halocarbons which have been considered a'general indicator of contaminant levels (Ribo et al., 1985).

In interassay comparisons involving sediment extracts, Microtox was 875x less sensitive (based on differences in EC/LC50s; range 42-3000) than *Duphnia* to leachates involving electroplating sludge (Fe, Cd, Cr, Ni, Cu,; pH 4.4-9.1) and pesticide waste (bendiocarb, propham, azinphos methyl, chloropropham, dimethoate, dichlorvos) (Calleja et al., 1986). Microtox was also reported to be less sensitive to DDT (Calleja et al., 1986). In a large scale comparison of Superfund site toxicity, Microtox did not show a positive response 65% of the time to toxicity which was detected by *Duphniu* or algal tests (Greene, pers. comm.). In a comparison of 326 samples, 48% were toxic to algae, 41% to *Duphniu* and 21% to Microtox. The Microtox test did not show any toxicity response to samples of electroplating waste (Greene, pers. comm.). Of the 326 samples, Microtox did identify 4 samples as being toxic which were missed by *Daphnia* and the algal tests.

In a study of water extracts of sediments, Microtox failed to document any positive responses, although *Daphnia* was positive 4/19 times (Dutka and Kwan, 1988). Microtox did not show any positive responses to water-extracts of lake sediments, although *Daphnia* showed a response to 63 of 94 samples (Dutka and Gorrie, 1989). The ECHA dipstick was positive in 27 samples, *Spirillum* in 1, and Microtox was negative in all. Only 59% of ECHA positive responses were also positive in *Daphnia* bioassays and Dutka and Gorrie (1989) again emphasize that it is unwise to use a single toxicant testing procedure.

3.4.1.1.2 Mutatox

This is a relatively new test, developed by the Microbics Corporation and is based on the use of a dark mutant strain of ***Photobacterium phosphoreum,*** M169, to screen for the presence of genotoxic agents. This test will pick up chemicals which are (a) DNA damaging agents, (b) DNA intercalating agents, (c) direct mutagens which either cause base substitution or are frame shift agents and (d) DNA synthesis inhibitors. The test procedures are similar to those followed in the Microtox test with incubation of Ml69 cells, cell media and sample being carried out at 22 ± 2°C. Light level is read after 18 ± 1 hour contact and compared to negative controls (dilution water, solvent concentration used and sodium azide).

3.4.1.2 Beta-galactosidase

SOS-Chromotest

The SOS-Chromotest is a genotoxicity test described by Dutka et al. (1986) and Xu et al. (1987). This assay also uses colorimetric indicators for activity of the beta-galactosidase (BGSD) enzyme system as an indicator of toxicant impact. The strain of *E. coli used* (K12-PQ37) has been genetically altered so that the galactosidase enzyme acts as an indicator of activity of cellular DNA repair mechanisms. The SOS-repair system is induced after DNA damage, and in this strain of bacteria, one of the control genes for the SOS system has been fused to an operon which acts to turn-on the BGSD enzyme when the SOS-system is activated (Quillardet et al., 1982). The test therefore provides an indication of chemical pollution which may have genotoxic or mutagenic effects (Dutka, 1988), and the BGSD activity acts as a marker for genotoxic impacts. To distinguish between toxicants which kill the bacteria and those causing DNA damage, the assay also incorporates simultaneous

determinations of alkaline phosphatase (AP) activity. Positive recording of AP activity ensures that the bacteria are still viable during the test.

Although the SOS-Chromotest assay only requires a 2 h incubation period, the test requires that bacteria be grown in culture overnight prior to the assay, and results are usually read in a photometer, although identification of toxic samples can be subjectively estimated by visual comparisons. Although the assay is available commercially from the same company as the Toxichrome test (Orgenics Ltd., Israel), the increased complexity limits its usefulness as a field microassay at present. Results are comparable to those obtained with the Ames mutagenicity test, and 90% of 180 pure chemicals tested so far have shown positive results in both the Ames and SOS-Chromotest (Vigerstad et al., 1987). Advantages of the SOS-Chromotest include the short test time span and its ideal application to situations where cell division is not possible. The results are not obscured by nutrient or microbial contamination (Quillardet et al., 1985). The SOS-Chromotest responds at lower levels of contaminants than those affecting Microtox luminescence (Dutka et al., 1986) or algal growth (Hat-wood et al., 1988).

The SOS-Chromotest, as it is now designed, cannot test an effluent or environmental sample at more than 4.76% of the original sample strength. Organics must be dissolved in DMSO or ethanol, and the regression analysis associated with the test commonly defines an EC50 >25% of sample strength, but the relationship cannot be further investigated (Greene, pets. comm.). Work on adapting the technique for relative toxicity analysis is currently under way. Other disadvantages include the fact that the test is subject to batch variability (Dutka et al., 1988a,b) and that the test requires overnight pre-incubation and sample preparation.

Toxi-Chromotest

This is a fairly rapid (2-3 h) bacterial colourmetric assay in kit form which may eventually replace the Microtox[R] test (Dutka, pers. comm.). The Toxi-chromotest *can* be used to test for toxicant activity in water and sediment extracts. The assay is based on the ability of substances (toxicants) to inhibit the *de nova* synthesis of an inducible enzyme, beta galactosidase, in a highly permeable mutant of *E. Coli. The* sensitivity of the test is enhanced by exposing the bacteria to stressing conditions (provided by kit materials) after which they are rehydrated in a cocktail containing a specific inducer of beta galactosidase and essential factors required for the recovery of the bacteria from their stressed condition. The activity of the induced enzyme is detected by the hydrolysis of a chromogenic substrate. Toxic materials interfere with the recovery process and thus with the synthesis of the enzyme and the colour reaction.

This test has been in the evaluation process for the past three years at several major laboratories, including Environment Canada in Quebec (Dr. C. Blaise's laboratory), U.S. EPA at Corvallis (Dr. J. Greene's laboratory) and the Canadian National Water Research Institute in Burlington (Dr. B. Dutka's laboratory). Unpublished data show that in a comparison of 120 samples taken from Superfund sites, 65% of samples were designated as toxic by the *Daphnia* or algal bioassays, but only 2 samples were positive in the Toxichrome test. These samples were those with the highest toxicity as designated by the algal bioassay and the sample which the daphnid bioassay designated as second most toxic (Greene, pets. comm.). This suggests that the Toxichrome test has some of the same insensitivities as the Microtox test, sometimes requiring either high contaminant levels or pre-concentration of the sample to obtain positive results.

3.4.1.3 ATP-TOX

The concentration of ATP per bacterial cell remains relatively constant and stable throughout all phases of growth, thus bacterial densities can be easily estimated by measuring the ATP content of the test system. When rapidly growing bacterial cells are exposed to toxicants, growth inhibition usually occurs. After several life cycles the toxic effect can be estimated by comparing sample cell growth to the control via ATP content. The ATP-TOX test usually uses *E. coli* because of its sensitivity, but any bacterial species with a short duplication period may be utilized, including *P. fluoresces* and *Salmonella typhimurium TA98 (Ames* test bacterium). Each species has its own sensitivity spectrum, and is usually the most sensitive microassay when batteries of tests are used. This sensitivity is attributed to the fact that a positive result is recorded at 1% inhibition, whereas other tests usually rely on a change in the EC50 (50% inhibition).

The assay has been described in detail by Xu and Dutka (1987), and the test reagents are commercially available. However, testing requires excessive sample manipulations and technicians require practice to obtain reproducible results (Greene, pets. comm.). Furthermore, in addition to interfering with bacterial growth and/or survival, toxicants also interfere with luciferase activity during the ATP determinations, and therefore the observed light output reduction associated with a positive result is a sum of the toxicant impacts on growth, survival and luciferase inhibition of the assay. Combining the results of ATP-TOX assays using *E. coli* and *P. fluorescens* brings the sensitivity of the assay up to the level of Microtox, but the Microtox assay is still faster (Xu and Dutka, 1987). Results of ATP-TOX tests showing greater than 20% inhibition have correlated well with *Duphnia* mortality (Dutka et al., 1988b).

3.4.1.4 Dehydrogenase Enzymes

This test is a measure of heterotrophic potential. Dehydrogenases are an enzyme class which is a major representative of the enzymes which catalyze oxidation reactions by transferring electrons down the electron transport system. During incubations (generally less than 90 min), a redox-indicator dye is added to produce a colour changes (Elnabarawy, 1986). Various dyes have been used (reviewed in Bitton, 1983; Bitton et al., 1986), including methylene blue, triphenyl tetrazolium chloride (TIC), tetrazolium blue, resazurin and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride (INT).

In general,-heavy metals (Ag, Hg, Cr) are more toxic to dehydrogenase enzymes than organics (pulp mill effluent, phenol, formaldehyde) (Bitton, 1983). Measurement of dehydrogenase activity can be affected by several variables including oxygen conditions, sediment age, extraction procedure, incubation time and diluent type as well as sediment volume (Burton and Lanza, 1985). Dehydrogenase techniques do not correlate well with macrobioassays and are generally less sensitive than Microtox.

3.4.2 Algae

Algae are suitable as test organisms due to their morphological simplicity, ease of culture and wide distribution. *Selenastrum capricornutum* has been used to test sediment elutriates and pore water, by measuring ^{14}C uptake by the algae, after exposure to the sample (Ross et al., 1988). A series of various concentrations of elutriates is used for each test. The algal fraction bioassay (AFB) has been used by Department of Fisheries and Oceans in the Great Lakes (IJC, 1988) as a short term screening test (Munawar and Munawar, 1982, 1987; cited in IJC, 1988). The AFB uses indigenous communities to examine differential response of a productivity measure, such as ^{14}C uptake, of various size fractions. This test can be used to evaluate elutriates or solid phase (overlying water). However, elutriates may require filtration to remove particles which would inhibit light; it is expected that this practice would also results in removal of metals sorbed to particles. Overall, the development of algal bioassays is very promising as a tool for sediment assessment, but the tests are not yet widely enough used or validated for incorporation into a test battery.

3.4.3 Invertebrate Bioassays

Benthic invertebrates are intimately associated with sediments, either burrowing in or living and feeding on the sediment. They are relatively stationary and are therefore representative of the ambient conditions. Some invertebrates are long-lived and can accumulate contaminant body burdens over time.

Early studies assessing the toxicity of contaminated sediment have mainly been restricted to single-species testing of freshwater invertebrates, including ***Duphniu magna*** (Cairns et al., 1984; Larson, 1989; Malueg et al., 1984a,b; Nebeker et al., 1984a, 1986, 1988, Schuytema et al., 1984; Athey et al., 1989; Burton et al., 1989), *Hyalella azteca* (Nebeker et al., 1984a; Nebeker and Miller, 1988), *Gammarus pulex* (Cairns et al., 1984; Nebeker et al., 1984a), *Chironomus* sp. (Nebeker et al., 1984b, 1988; Giesy et al., 1988) and oligochaetes (Klump et al., 1987; Milbrink, 1987; Wiederholm et al., 1987; Kielty et al., 1988; White and Kielty, 1988).

3.4.3.1 *Daphnia Magna*

Daphnia magna is the most commonly tested freshwater organism (Cairns et al., 1984; Malueg et al., 1984a,b; Nebeker et al., 1984a, 1986, 1988; Schuytema et al., 1984; Athey et al., 1989; Burton et al., 1989; Larson, 1989). It has generally been shown to be the most sensitive animal used for sediment tests, especially to metals (Malueg et al., 1984a,b; Nebeker et al., 1984a; Dutka and Kwan, 1988; Athey et al., 1989). Sediment bioassays using ***D. magna*** are relatively inexpensive and require little special equipment (Nebeker et al., 1984a). *Daphnia* bioassays are usually acute (48-h in duration), using mortality as an endpoint (ASTM, 1984), although chronic (7- and 10-d) studies are not uncommon (Nebeker et al., 1984a). Full life cycle and acute tests have also been developed for ***Ceriodaphniu dubia***, another cladoceran species, but this smaller bioassay -- organism has not been widely used for testing sediments.

Duphniu filter feed on algae, bacteria, and small particles of debris from the water. Although they are planktonic, they feed at the surface of the sediments and come in contact with particulate-bound toxicants both at the sediment water interface, and in the interstitial waters. Sediment bioassay testing using ***Duphniu*** are generally of three types, which are representative of major exposure routes: sediment-elutriate tests (Nebeker et al., 1984a; Athey et al., 1989; Burton et al., 1989), bulk sediment and water tests (Cairns et al., 1984; Nebeker et al., 1984a, 1986; Schuytema et al., 1984; Athey et al., 1989; Burton et al., 1989), and solvent-extracted component tests (Larson, 1989).

Nebeker et al. (1984a) conducted 48-h solid-phase (sediment and water) beaker tests and sediment elutriate tests with ***Duphnia. These*** **tests,** run in conjunction with one another, are recommended as a fast, simple and inexpensive approach to initial screening for acute toxicity (Nebeker et al., 1984a). Cairns et al. (1984) found that the order of sensitivity to copper in sediments was ***Daphnia >Chironomus>Hyallela>Gammarus. Daphnia*** have been shown to be more sensitive than ***Hexagenia limbata*** (Malueg et al., 1983). Elutriate and solid phase tests conducted together are valuable because the combination shows when the sediment is toxic but is not releasing dissolved toxicants into the overlying water column.

Chronic (= life cycle) tests have a valuable function in determining if impairment of growth and reproduction can occur in sediments that do not exhibit acute toxicity. ***Daphnia*** have been used successfully in chronic tests in water-only bioassays (Nebeker et al., 1984a; 1986) solid-phase bioassays (Nebeker et al., **1984a),** and sediment elutriate samples (Nebeker et al., **1984a,** 1988). The life cycle ***D. magna*** bioassay is extensively used and produces repeatable results. Cultures are readily available and subject to low levels of population variations, which improves inter-laboratory calibration. Studies are run for **7-** or 10-d periods with number of surviving young and adults as endpoints. Seven day chronic tests are preferable to 10 d tests, as the tests take less time, and generate the same type of data (Nebeker et al., 1988).

3.4.3.2 Amphipods

Hyalella azteca and ***Gammarus lacustris*** are the two freshwater amphipod species discussed most frequently in the literature with respect to sediment toxicity (Cairns et al., 1984; Nebeker et al., **1984a, 1986;** Nebeker and Miller, 1988; Schuytema et al., 1988; Burton et al., 1989). ***H. azteca*** is the preferred species as they reproduce continually, grow rapidly, and are relatively easy to rear under laboratory conditions (Nebeker et al., 1984a). They maintain intimate contact with and burrow in the sediment and exhibit consistently high control survival (Nebeker et al., 1984a).

Amphipods, while very sensitive to toxic organic chemicals (Nebeker et al., **1984a),** appear to be less sensitive to metal-contaminated sediments (Cairns et al., 1984; Nebeker et al, 1986). The toxicity of Cd in sediment to ***H. azteca was*** similar to the toxicity of cadmium in water-only tests, indicating that the test animals were not obtaining appreciable amounts of cadmium from the sediment or from suspended or dissolved organic materials (Nebeker et al., 1986).

Lethal bioassay (48- and 96-h) procedures generate reliable results and are relatively simple and inexpensive (Nebeker et al., 1984a). However, acute sediment bioassays using *Hyalella* appear to be less sensitive than the longer chronic studies. Burton et al. (1989) evaluated multitrophic level sediment toxicity studies and found *H. azteca* to be the least sensitive organism, with no response to elutriates and marginal toxicity to whole sediments. However, lethality and growth effects at 10-, 20-, and 30-d periods recorded acute and chronic toxicity in the test sediments, indicating that 48-h may be inadequate to detect toxicity (Burton et al., 1989).

Chronic studies with *H. azteca* range in duration from 10- to 30-d (Cairns et al., 1984; Nebeker et al., 1984a, 1986; Schuytema et al., 1988; Nebeker and Miller, 1988; Burton et al., 1989), but life cycle tests with *Hyalella* are not presently practical because recovery of the young is very difficult and this introduces experimental error (Chapman, 1986).

3.4.3.3 Insects

The two freshwater insects discussed most commonly in the literature with respect to sediment toxicity are the mayfly, *Hexagenia limbata,* and the midge, *Chironomus tentans*. Tests reported are generally chronic, using 25-d adult emergence (Nebeker et al., 1984a; 1988) and 10 or 15-d larval growth and survival (Cairns et al., 1984; Malueg et al., 1984 a,b; Nebeker et al., 1984a, 1988; Giesy et al., 1988) as endpoints. Various sediment toxicity test procedures are reported: bulk phase sediment and water (Nebeker et al., 1984a), sediment elutriate (Nebeker et al., 1984a), and a sediment and water multi-species test chamber (Prater and Anderson, 1977 a,b; Cairns et al., 1984; Malueg et al., 1984 a,b; Nebeker et al., 1984a).

The rationale and some general methods for testing of *H. limbata* are described in Nebeker et al. (1984a), Fremling and Mauck (1980) and Henry et al. (1986). *H. limbata* require a finely textured, high organic content sediment in which to burrow (Henry et al., 1986), and therefore physical sediment characteristics (i.e., grain size and organic carbon) may complicate the interpretation of bioassay data for this test. Since *H. limbata* can be difficult to obtain and culture, and since the life cycle is relatively long, Giesy et al. (1990) examined *D. magna* and C. *tetans* as possible alternatives, and found that either assay can predict the response of this amphipod species.

Burrowing mayflies of the genus *Hexagenia* ingest large quantities of sediment (Zimmerman, 1975) and detritus (Cummins, 1973); therefore, sediment-bound contaminants could be released during digestion in the gut of

these animals, hence resulting in greater exposure than would be observed from exposure to pore water alone. However, Giesy et al. (1990) did not find that whole sediments were more toxic to *H. limbata* than pore water, and they discuss possible reasons for this.

Bioconcentration studies using *H. limbata*, and the amphipods, *H. azteca* and G. *lacustris*, are designed to run for 28 d (Nebeker et al., 1984a). The *Chironomus* 10- or 15- d larval growth test has three advantages over each of these: the duration of study is up to two weeks shorter, two sets of data can be generated from one test set-up, and after weighing, the larvae are readily retained for bioconcentration studies. The sediment can be screened to collect the larvae, which can be frozen and stored for later tissue analysis (Nebeker et al., 1984a). *H. limbata* are considered to be more convenient for bioconcentration studies because of the greater biomass available for tissue analysis; however, *H. limbata* has demonstrated high control mortality due to competition for space and cannibalism (Cairns et al., 1984; Nebeker et al., 1984a).

Ten day acute sediment toxicity studies using C. *tentuns use* larval survival as an endpoint (Cairns et al., 1984; Malueg et al., 1984 a,b; Nebeker et al., 1984a, 1988; Giesy et al., 1988). Based on lethality, the C. *tentans* assay was less sensitive than *D.* magna, but growth inhibition was sensitive and the most discriminatory of the three assays tested by Giesy et al. (1988): Microtox^R, *D. magna* (48-h LC50), and C. *tentuns* (10-d larval survival and growth). The sediments that were classified as toxic to *D. magna* were also those that caused the greatest growth reduction of C. *tentuns* (Giesy et al., 1988).

Second instar C. *tentuns* larvae (12-d postoviposition) were used by Nebeker et al. (1984b) because they are large enough to work with and are the most sensitive life stage of this species to a number of toxicants, including metals. This species has been recommend for use in solid phase tests because it is easy to rear and test under laboratory conditions (Nebeker, 1984a); the growth endpoint is very sensitive to sediment contaminants and allows better discrimination among sediment toxicities than does lethality for either *D. magna* or *H. limbata* (Giesy et al., 1990).

Mixed-species tests are of two types: a %-h Prater-Anderson (1977a,b) *D. magna* and I-I. *limbata* bioassay, and a 10-d *H. limbata* larval growth and survival study with *D. magna* added during the first and last two days of the study (Malueg et al., 1984a,b). The %-h test developed by Prater and Anderson (1977a) is commonly used to assess the toxicity associated with freshwater dredged sediments. While the 96-h test duration is sufficient for testing the acute toxicity of sediments, it does not sufficiently describe longer-term exposure responses. The mixed-species exposure procedures described by Malueg et al. (1984a,b) and Leblanc and Surprenant

(1985) may more adequately describe long-term exposure situations, but are impractical due to the use of elaborate recirculating apparatus.

3.4.3.4 Oligochaetes

Tubificid oligochaetes may be found in a wide variety of aquatic. environments covering pure oligotrophic to hypereutrophic waters (Milbrink, 1987). As a group, the oligochaetes are one of the predominant components of the benthos of most lake ecosystems. The worms live in and feed from the sediments and are thus exposed to pollutants both through feeding and bodily contact. They are the last organisms to disappear when conditions deteriorate, and among the first to reappear when conditions improve (Milbrink, 1987).

Heavy metal studies with oligochaetes have concentrated almost exclusively on tubificids, in particular ***Limnodrilus hoffmeisteri*** and ***Tubifex tubifex*.** Field surveys of heavy metal levels in sediments and tubificids and laboratory studies with metals in solution indicate that tubificids are tolerant of certain metals (Chapman et al., 1980). The significance of this tolerance is that tubificids can be used in field surveys of heavy metals as monitoring tools (Chapman et al., 1982), and possibly as indicators of heavy metal pollution (Wiederholm et al., 1987).

Annelid worms are considered to be of excellent use as bio-indicators in LC50 tests for the assessment of heavy metal contamination (Chapman et al., 1980, 1982; Chapman and Brinkhurst, 1984). For many contaminants, particularly heavy metals, the uptake process by benthic invertebrates is clearly related to the ambient concentration of the contaminant in the sediment. Uptake of the contaminant may result in toxic body burdens but is mediated by bioavailability and species excretory and regulatory processes. The range of variability of bioaccumulation rates for the Oligocheata is summarized by Reynoldson (1987).

Short-term studies using lethality (Kielty et al., 1988) and sediment avoidance (Kielty et al., 1988, White and Kielty, 1988) as endpoints have been reported. Keilty et al. (1988) determined the %-h LC50 values for oligochaetes, ***Stylodrilus heringianus*** and ***L. hoffmeisteri*, *exposed*** to endrin-contaminated sediments. Very high LC50 values for both oligochaete species were reported (in comparison to LC50 values for other aquatic species tested in aqueous endrin solutions). The high sorptive properties and concurrent reduction in availability are primarily responsible for the low toxicity. These data suggest that oligochaetes may be able to withstand substantial amounts of highly toxic, sorbed pollutants. However, the EC50 burrowing avoidance values for both species were found to be approximately 4.6 and 150 times lower than their %-h LC50 values,

for *S. heringianus* and *L. hoffmeisteri*, respectively. Both species burrowed into contaminated sediment and then returned to the surface in numbers somewhat proportional to the sediment concentration and the length of exposure.

Chronic sediment toxicity studies using freshwater oligochaetes commonly use growth and reproductive success as endpoints (Milbrink, 1987; Wiederholm et al., 1987). Wiederholm et al. (1987) cultured five species of worms (*Tubifex tubifex, L. hoffmeisteri, L. claparedeanus, L. udekemianus*, and *Potamothrox hammonienis*) for 0.5 to 1.5 years in lake sediments with substantial amounts of heavy metal contamination. Growth and reproduction were generally affected earlier than survival in metal-contaminated sediments. *T. tubifex* was chosen as the best bioassay organism as it permitted easy access to larval material, and information regarding growth and birth rates was generated quickly.

Milbrink (1987) provides an example of the particular sensitivity of standardized tubificid bioassays in the detection of heavy metal contamination of lake sediments. In heavily contaminated sediments a considerable time-lag both in growth rate and in production of young was observed. After 60 days, weights were reduced and no cohort animals were left by day 200. Growth rates, reproductive success and the timing of consecutive reproductive events of cohort individuals was found to be indicative of toxic events (Milbrink, 1987).

Sediment toxicity to oligochaetes appears to be modified by several factors. For example, feeding masked the toxicity of metal-contaminated sediments to *T. tubifex* (Milbrink, 1987) and mixed species testing revealed that toxicity of contaminated sediment to *L. hoffmeisteri* was reduced in the presence of *S. heringianus* (Keilty and White, 1988). *T. tubifex* was found to be inappropriate as a test organism for the testing of oligotrophic sediments, as it will not grow and reproduce, even in the absence of pollutants (Wiederholm et al., 1987).

3.4.4 Fish Bioassays

The use of early life stage (ELS) tests and life cycle chronic tests has been emphasized increasingly in the evaluation of sublethal effects of toxicants to fish (Brungs, 1969; Leblanc and Surprenant, 1985; Norberg and Mount, 1985; Pickering and Gast, 1972; Schuytema et al., 1988). Fathead minnows (*Pimephales promelas*) are the most commonly used species for ELS owing to their widespread distribution, relative ease of laboratory culture, and the existence of a large toxicity database (Norberg and Mount, 1985).

Evaluations of the chronic toxicity of sediments to fathead minnows are less common in the literature (Dawson et al., 1988, Leblanc and Surprenant, 1985). Dawson et al. (1988) examined the effects of metal-contaminated sediment extracts and a reference toxicant (zinc sulphate) on developmental morphology, growth and mortality of exposed fathead minnow embryos. The potential toxicity of sediments related primarily to the effective Zn concentration in the extracts.

Norberg and Mount (1985) present a rapid method to estimate the chronic toxicity of effluents to newly hatched minnow larvae (< 24h old). Tests are run for 7 d under static-renewal conditions with survival and growth as endpoints. Bioassays have been run on seventy effluents including refinery, metal-plating, and pulp and paper effluents; this paper represents results using zinc, copper and Dursban. Results were obtained quickly, cost-effectively, with good sensitivity, based on comparisons with other chronic data. However, the methods discussed are for effluent testing; this procedure would be modified for testing sediment extracts.

The rainbow trout alevin yolk conversion efficiency bioassay measures the lethal and sublethal effects of toxicants on rainbow trout alevins. The procedures are currently being revised, by E.V.S. Consultants, and the test is being adapted for use *in situ. The* test is based on studies conducted by Hodson and Blunt (1981, 1986) which examined the efficiency of the conversion of yolk to body tissue. Response variables measured include embryo and alevin mortality, time to hatch, developmental rate and yolk conversion efficiency. Although this test (described below) has not been utilized to evaluate acid mine drainage sediments, it has the potential to be adapted for sediments either by elutriate or overlying water testing.

The test currently runs for 3 weeks with rainbow trout eyed embryos placed in beakers containing test solutions. The test is a static-renewal bioassay, with test solutions changed three times per week. The number of dead embryos and alevins, and the number of newly hatched alevins, is recorded daily. The test is terminated when most of the embryos have reached the swim-up stage, and have used most of their yolk. Surviving alevins are preserved in formalin and, once the preserved specimens have hardened, the remaining yolk is removed from the body and both yolk and body are weighed.

Embryo and alevin mortality, as well as mean age at hatch, can be calculated; accelerated hatch is a common response to low levels of toxicants. Developmental rate can be assessed from yolk and body weights. If a toxicant retards development, exposed individuals should have larger yolks and smaller bodies than do controls. Toxicants may also affect the efficiency of the conversion of yolk to body tissue. Efficiency is defined as the weight of body tissue produced per unit weight of yolk used. Differences in efficiency are assessed by comparing regressions of body weight on yolk weight. If exposed individuals have smaller bodies than do

controls at any given yolk weight, they are clearly not converting their yolk as efficiently. The difference in efficiency between exposed individuals and controls represents the energy cost of exposure. Measuring yolk conversion efficiency is equivalent to measuring growth at a fixed ration, except that in this case the ration (yolk) is provided by the test organisms themselves.

In future test development, this bioassay will be conducted on alevins only. Beginning exposure at the eyed embryo stage does provide data on embryo mortality and time to hatch, but these effects on embryos should be examined in tests restricted to embryos only. Differences in time to hatch can confound the assessment of yolk conversion efficiency. The duration of the test will be restricted to 10-14 days because, as alevins approach starvation, their yolk conversion efficiency declines. Thus, individuals developing more rapidly may appear to be less efficient.

Leblanc and Surprenant (1985) assessed toxicity by monitoring effects on three species, tested concurrently: fathead minnow (*Pimephales promelas*), the midge (*Paratanytarsus parthenogenica*), and the cladoceran (*Daphnia magna).* While this test is more complete with respect to intraspecific toxicity assessment, it was more time-consuming (21 d) and less cost-effective (water circulation system) than either Dawson et al. (1988) or Norberg and Mount (1985).

McLeay et al. (1987) exposed underyearling Arctic grayling (*Thymallus arcticus*) for 4 d to suspensions of fine organic and inorganic sediment and for 6 weeks to inorganic sediment under lab conditions. The test sediments were collected from an active placer mine. The exposures evoked sublethal responses but did not cause gill damage. After six weeks of exposure to sediment concentrations greater than 100 mg/L, fish had impaired feeding activity, reduced growth rates, experienced downstream displacement, colour changes and decreased resistance to reference toxicant. Stress responses (elevated or more varied blood sugar levels, depressed leucocrit values) were recorded after short exposure to organic sediments and the inorganic sediments caused the fish to surface. This study and others have demonstrated that a number of sediment characteristics besides concentration in suspension (e.g., grain size, shape, hardness and organic content) can modify their effect on fish. This approach is not considered practical for application as a sediment bioassay because it is time consuming, complex, and may not address effects other than those caused by turbidity.

In general, fish bioassays are not as appropriate for sediment evaluation as invertebrate bioassays, primarily because fish are not as intimately associated with the sediment. However, fish bioassays are useful if the study is focusing on overlying water effects.

3.4.5 Multitrophic analysis

Numerous investigators have emphasized the importance of using multiple toxicity tests in evaluations of pollutants in aquatic ecosystems (LeBlanc, 1984; Burton and Stemmer, 1988; Burton et al., 1989). A battery of tests is preferred because species sensitivity to toxicants varies due to differing modes of action and metabolic processes (Burton et al., 1989). In addition, ecosystem sensitivity is influenced by a myriad of factors, such as indigenous species sensitivity, alteration of toxicity, seasonal effects and food web interactions. There has also been concern over the validity and effectiveness of using single-species surrogates, e.g. *D. magna, P. promelas*, rather than resident species or multispecies tests in the evaluation of ecosystem impacts (Miller et al., 1984). Relative sensitivity frequently varies with the site and/or toxicants under investigation. It is appropriate that a test battery be used which is comprised of multiple, sensitive, species that are representative of different trophic levels.

It is apparent that no one single species toxicant assay can be used to detect ecosystem impacts due to the varying target sites and factors which influence sensitivity and differing temporal response times of ecosystem components. Very few comprehensive multitrophic level studies have been conducted (Burton et al., 1989; Dutka and Kwan, 1988, Fairchild et al., 1987). The battery test approach appears to be the most diagnostic with respect to sediment toxicity (Dutka and Kwan, 1988). In general, bioassays are selected for use in a battery to offer a range of taxa, exposure routes, and timespans.

4.0 BENTHIC MACROINVERTEBRATES

4.1 Introduction

Benthic macroinvertebrates are relatively sedentary organisms that inhabit or depend upon the sedimentary environment for their various life functions. Consequently, they are sensitive to both long-term and short-term changes in sediment and water quality. Benthic macroinvertebrates play a key role in lotic (running waters) and lentic (standing waters) ecosystems due to their intermediate position linking primary production (e.g., periphyton) and higher trophic levels (i.e., fish). Macroinvertebrates are particularly suitable as ecological indicators in aquatic systems as they meet many of the requirements inherent in the criteria established by Ryder and Edwards (1985) for biological indicators of ecosystem quality. Benthic communities show cumulative effects of present/past conditions, they have low mobility and relatively long life cycles (Wilhm,

1975), their ecological relationships are relatively well understood (Herricks and Cairns, 1982), sampling procedures are relatively well developed, the group is heterogeneous in that a single sampling technique collects a considerable number of species from a wide range of phyla, and macroinvertebrates are generally abundant (Mason, 1981).

4.2 Utility of Benthic Macroinvertebrates in Assessing Contaminated Sediments

Benthic macroinvertebrate community structure and function assessments have many different applications. Changes in-community structure and function have been used extensively to monitor impacts associated with a variety of anthropogenic pollutants. Documented alterations in benthic community structure have been related to crude oil exposures in ponds and streams (Rosenburg and Wiens, 1976; Cushman and Goyert, 1984), acidification of lakes of the Canadian shield (Raddum and Fjelleim, 1984; Okland and Okland, 1980), heavy metal contamination of lake sediments and streams (Wiederholm, 1984a,b; Waterhouse and Farrell, 1985), organic contamination in lentic systems (Krieger, 1984; Rosas et al., 1985) and acid/mine-stressed conditions in lakes and ponds (Simpson, 1983; Armitage and Blackburn, 1985). Monitoring of riffle zoobenthos in streams receiving acid mine drainage (Koryak et al., 1972) found high numbers of individuals comprised of few species. The number of insect species present steadily increased with progressive neutralization until amphipods and oligochaetes appeared.

Site-specific knowledge of surface water quality, habitat quality, sediment chemistry and sediment toxicity provide the best context in which to interpret benthic community assessment data (Davis and Lathrop, 1989). Alone, benthic macroinvertebrates can be used to screen for potential sediment contamination based on spatial gradients in community structure, but they should not be used alone to definitively determine sediment quality. Benthic macroinvertebrate data must be integrated with other available data (e.g., surface water quality, habitat quality, sediment chemistry and toxicity results as in the integrated Sediment Quality Triad approach; Chapman et al., 1987) to evaluate sediment quality.

4.3 Sampling Methods for Benthic Infauna Samples

Although benthos community analysis has been used extensively to monitor aquatic stress (e.g., characterize contamination of bottom sediments), there has been little standardization of methodologies. This has been, in part, due to the great number of investigations using benthos community responses, but has also resulted

from investigators attempting to account for the major sources of variation of the aquatic habitat (e.g., temporal and spatial variation) which may be influential in restructuring the benthic invertebrate community.

The primary objective of benthic macroinvertebrate community and function analysis, in relation to contaminated sediments, is to provide sufficient data and evidence to assist in characterizing the quality of contaminated sediments. In general, assessment of benthic communities has been approached from a structural or functional perspective. Community structure is defined as a measurement of biotic characteristics at a specific point in time, e.g., abundance, diversity, and species composition. Community function is the measurement of any process (rate) of the ecosystem, e.g., species colonization rate. The biomonitoring of benthic communities is normally done from a structural perspective because structural studies normally take less time and are more conventional, thus permitting comparisons to be easily made with benthic data from other studies (Mattews et al., 1982).

Benthic macroinvertebrates can be collected from natural or artificial substrates depending on the specific conditions. Natural substrates should be sampled when possible; however, there will be circumstances in which artificial substrates must be used because natural substrates cannot be sampled effectively. In addition, there may also be situations where it would be advantageous to use artificial substrates in conjunction with natural substrates. The choice of sampling method is, in many cases, dictated by sediment characteristics, water depth, water flow, inter-site variability, the specific question to be addressed and methods utilized in previous studies.

4.3.1 Natural Substrates

Sampling benthos from the natural substratum facilitates direct comparisons between the structure of the impacted benthos community and the contamination of the surrounding sediments. There are a variety of samplers described in the literature with which to collect benthos from every imaginable sediment type. The selection of a sampling device for the collection of benthic macroinvertebrates is dependent on the habitat of interest. There have been different samplers created for lotic and lentic habitats, riffle and pools, deep and shallow sites and the list continues. The type of habitat (lentic or lotic) and the type of sediment are by far the largest considerations for choice of sampling device.

Lakes and Large Rivers

There are variety of grabs and corers for sampling the bottoms of lakes and rivers. The most popular grabs are the Ekman grab for sampling silt, muck, and sludge in water with little current; Peterson and Ponar grabs for sampling sand, gravel, and mud and clay especially in swift currents; and the Shipek grab for a variety of substrates in either lakes or rivers. The Ekman is not as effective in areas of heavy macrophytic growth and debris but is much lighter and easier to use than the Ponar in softer substrates (Davis and Lathrop, 1989). In habitats of abundant benthos populations, core samplers have been found to be the best devices, especially for studying depth distribution of organisms in the sediments. In muddy substrates, cores have also been shown to be the preferred sampling device to collect a quantitative sample (G.L.W.Q.B., 1988). The surface area sampled by a particular sampling device should also be considered. Downing (1979) demonstrated that sampling variance of lake benthos was reduced when the sampler size was increased. Similarly, when estimating the abundance of stream benthos, Morin (1985) concluded that larger samplers yielded estimates that were less variable than those obtained by small samplers. Comprehensive articles by Elliott and Drake (1981a,b; 1982) and Elliott and Tullett (1978) have reviewed and tested many references to marine and freshwater sampling devices.

Shallow Streams

Devices such as the Surber sampler or square foot sampler (Surber, 1937), the T-sampler (Mackie and Bailey, 1981), Hess bottom sampler (Hess, 1941), Neill sampler (Neill, 1938) are common devices for sampling macroinvertebrate populations in shallow (< 0.5 m deep) and fast flowing streams. All samplers utilize the current velocity of the stream to funnel disturbed animals into a downstream collecting container. Drift nets have also been used to collect drifting organisms over a definite interval of time.

4.3.2 Artificial Substrates

An artificial substrate is defined as any device used to mimic specific features of the aquatic environment into which it is placed. Artificial substrates are typically used to sample aquatic habitats that can not be sampled effectively using conventional devices (e.g., grabs). In the case of assessing acid mine drainage effects, natural community studies would be more useful. However, in cases where it may not be possible to sample field sediments, artificial substrates could be used, recognizing that any measurable effects would be related to water column contamination originating from acid mine drainage, as well as from the surficial sediments in the intermediate area.

While there are numerous types of artificial substrates, a rock filled wire basket is commonly used to assess benthic macroinvertebrate communities. This method has been well documented as an acceptable artificial substrate sampler for the monitoring of macroinvertebrate communities (Rabeni et al., 1985; Mason et al., 1973; Hellawell, 1978; Mason, 1981; Merritt et al., 1984). Artificial substrates provide more control of substrate conditions and permit sites to be located close to the contaminant source where natural substrates may not be sampled appropriately. Artificial substrates present an unoccupied habitat for benthic organism colonization that is consistent from site to site. The number and types of colonizing organisms may be influenced by the quality of water and sediment at a particular site. It should be noted that the purpose of the artificial substrate sample is to monitor changes in macroinvertebrate communities over time and space, and that they do not necessarily reflect the true macroinvertebrate community. There are numerous advantages and disadvantages to artificial substrates:

Advantages

- allow collection of data from locations which cannot be sampled effectively by other means
- permit standardized sampling
- reduced variability compared with other types of sampling
- require less operator skill than other methods
- convenient to use
- permit nondestructive sampling of an environment
- permit greater flexibility in sampling programs

Disadvantages

- colonization dynamics incompletely known; comment: this is considered the most serious disadvantage. The only remedy to this is more comparison studies.

- nonrepresentative sampling under either natural or polluted conditions; comment: artificial substrates are used only as a relative measure, and are not to be equated with natural substrate. Substrates should be positioned as close to natural substrate as is possible.

- artificial substrates require long exposure time to obtain a sample; comment: artificial substrates are left in for 6-8 weeks.

- loss of fauna on retrieval of samplers; comment: a fine net is used to retrieve substrates to prevent loss of invertebrates.

- unforeseen losses of artificial substrates; comment: place more artificial substrates in habitats than are required by the sample design.

- inconvenient to use, logistically awkward; comment: this is site specific and must be addressed on a site by site basis.

Sites selected to use with artificial substrates should be as similar as possible to reduce variability due to natural differences. Baskets should be anchored to the bottom if conditions permit, allowing a more natural community to colonize the substrates. If this is not physically possible, then substrates can be suspended in the water column as close to the natural substrate as possible. Whether the baskets are on the bottom or suspended, substrates should be positioned at all stations in the same manner. Basket samples should be used when flow conditions are relatively stable. The artificial substrates should remain in the stream for a six to eight week colonization period. Artificial substrates should be removed from the stream using a dip net (of decided mesh aperture, (c.f. Section 4.5) to be held behind and below the basket during retrieval to prevent loss of organisms. Current flow will wash organisms into the mesh net. The debris and organisms on the baskets, rocks, and dip net should be cleaned into a pail, separated from the mud by washing on a screen and preserved.

4.4 Sampling Design

Benthic macroinvertebrate communities are monitored from both a spatial and temporal perspective in order to assess possible impacts. The number of sites established within a study area depends on the nature of the problem and the particular habitat. Sites selected should be as similar as possible in substrate, current velocity, and depth to minimize the natural variability found in benthic communities. The sampling design

should include assessing benthos communities from the contaminated sites, or close to the source of contamination, and from sites used as a control or reference. In lotic systems, samples should be collected progressively downstream to monitor the extent of the zone of impact. Similarly, in lakes, samples should be collected progressively outward from the source of contamination. The natural conditions of the reference sites should be as similar as possible to the natural conditions found at the contaminated sites, differing only in characteristics which are directly, or indirectly, related to the source of contamination.

When sampling benthos at a particular site, it is imperative to collect enough samples to minimize the intra-site variability. If the variability of estimates within a site is large, then differences in these estimates among sites will be difficult to detect. A preliminary study should be performed to estimate the number of samples required to effectively assess potential differences among sites. The preliminary study will involve the use of a quantitative method to determine the mean and variance of invertebrate abundance values at a series of sites of interest. This information permits the determination of the degree of precision (D), which is the percentage error expressed as the standard error of the mean (Elliott, 1977); a degree of precision of 20% (0.20) is considered adequate for many benthic studies. The equation used is based on the assumption that the benthic macroinvertebrate community has a negative binomial distribution, which is the distribution most commonly found in benthic organisms.

$$D=(1/nx + 1/nk)^{0.5}$$

where:

D = degree of precision
n = sample number
x = sample mean
k = index of dispersion $((x' - s^2/n)/s^2 - x)$

4.5 Sorting and Taxonomic Identification

Benthos samples collected in the field must be sieved to separate the macroinvertebrates from the sediments. Selecting the appropriate mesh size is an ongoing problem faced by scientists when sieving macrobenthos. A small mesh size ($<200\ \mu m$) captures most of the benthic organisms; however, sorting time is increased dramatically and the net is more susceptible to damage. Conversely, a mesh size which is large ($>500\ \mu m$) will reduce sorting time, but may not capture all early instar organisms. Obviously a compromise has to be made. For most studies investigating benthos community structure, a mesh aperture of 500 μm is probably sufficient; however, the objectives of the particular study need to be taken into consideration.

Benthos samples collected from natural, or artificial substrates should be preserved with 70% ethanol. Phloxine-B, a histological stain, may also be added to facilitate sorting. Formalin (5-10%), which is an excellent preservative, can be used but is not recommended because of potential human health effects. Each sample should be washed in a sieve (of the decided mesh size) to remove excess preservative, stain, etc. There are many reports in the literature discussing techniques for benthos sorting. U.S. EPA (1973), Pennack (1978), Merritt et al. (1984), and A.P.H.A. (1989) offer excellent guidance for sampling sorting. Each sample should be sorted into major taxonomic groups before detailed identifications are made.

The minimum taxonomic level of identification for organisms collected should be as follows (Alberta Environment, 1990):

Phylum for Aschelminthes

Class for Coelenterata

Family for Annelida and Diptera

Subfamily for Chironomidae

Genus for Mollusca, Ephemeroptera, Trichoptera, and Plecoptera

Order for remaining Arthropoda

Common species should be identified to the lowest practical level. The level of identification is dictated by the objectives of the particular study. Species level identification for all organisms are not necessary for a successful program and commonly depends on the availability of local taxonomic keys (Davis and Lathrop, 1989). Relevant information which may be extracted from the taxonomic data includes: total number of organisms per sample; total number of invertebrate taxa per sample; total number of major groups; and, the characterization of dominant groups in the benthic community.

4.6 Statistical Analysis

There are many methods which are used to condense taxonomic data into a more compact, usable form, despite the inevitable loss of information. One of the most popular methods of condensing data is to describe

the benthic community data at each site by a numerical index. Some of the most commonly used methods of this type have been described by Hellawell (1978); each have their advantages and disadvantages, and all have been used in assessment of industrial related impacts. The numerical index methods range from simple estimates (e.g., abundance per taxon, total abundance, taxonomic richness) to pollution indices (e.g., Saprobic index - Zelinka and Mat-van, 1966; Chandlers index - Chandler, 1970); diversity indices (e.g., Shannon Diversity index, Shannon, 1948), and, comparative indices (e.g., Bray-Curtis index - Bray and Curtis, 1957).

The most relevant basic data are obtained using descriptive statistics, including mean abundance (number/m*), taxonomic richness, variance (SD), range and coefficient of variation (CV) to obtain a general comparison among sites. The differences of total abundance and abundances of major taxonomic groups among sites can be tested using analysis of variance (ANOVA). Prior to running an ANOVA, benthic data should be examined to determine whether the data are normally distributed. Non-normal data should be transformed using an appropriate transformation (typically a logarithmic transformation) in order to satisfy the assumption of normality in parametric statistics.

The analysis of the benthos data can also include, but not necessarily be limited to, the following statistical techniques:

- Tabulation of benthic invertebrate data in accord with sensitive, facultative and tolerant groups based on their sensitivity to environmental contaminants;

- Application of community diversity indices such as the Shannon-Weaver Diversity Index (Shannon, 1948), Keefe-Bergensen Diversity Index, Creckanowski's Coefficient;

- ANOVA or a non-parametric alternative to compare total numbers, total number of taxa and number of dominant taxa among sites;

- Reciprocal averaging ordination;

- Cluster analysis and principal components analysis;

- Multivariate analysis of variance;

- Multiple discrimination analysis.

Multivariate methods can be used to assess the relationship among sites. Cluster analysis, a multivariate classification method, can be used to group sites with similar species composition and abundance. Species abundance data can be transformed to reduce problems associated with unusually high values. Coefficients from similarity or distance indices can then be used to cluster sites based on a cluster linkage method. Similarity indices (e.g., Bray-Curtis index - Bray and Curtis, 1957; proportional similarity index) and distance measures (e.g., Euclideon distance) can be used to determine similarity between sites. Ordination techniques (e.g., Principal Component Analysis, Factor Analysis) can be used if results from the cluster analysis demonstrate distinct associations of sites. Ordination assumes that a relationship exists between species abundance and environmental factors. In ordination analysis, sites are separated or associated on one or more axes that are interpreted with knowledge of known environmental conditions.

A correlation matrix can be constructed in order to determine associations of biological variables (e.g., abundance, diversity) with environmental variables (e.g., water depth, current velocity, chemical variables) that may be influencing macroinvertebrate communities. This statistical method can provide an assessment of variables which cannot be controlled but that can greatly influence benthic communities.

5.0 QUALITY ASSURANCE/QUALITY CONTROL

5.1 Introduction

These quality assurance guidelines have been developed for personnel collecting samples and laboratories providing analytical data (chemical and bioassay) for sediments. Contracts for analyses by laboratories should be accompanied by a statement of work detailing these analytical and quality control requirements. The objectives of these Quality Assurance guidelines are to:

- Maximize generation of quality analytical data,
- Ensure complete documentation and defensibility of methods and data,
- Ensure compatibility of methods and results from different proponents and contract laboratories,

- Clearly define the various QA/QC requirements that must be followed.

Testing facilities are responsible for enforcing QA/QC measures by continuous supervisory review and random "spot-checks" by designated QA/QC officers.

5.2 Sampling and Handling

The generation of quality data begins with the collection of the sample; therefore the integrity of the sample collection process is of importance to analytical laboratories and the success of the investigation. Samples must be collected in such a way that no foreign material is introduced to the sample and no material of interest escapes from the sample prior to analysis (details of sampling procedures are found in Section 3.3). To ensure sample integrity, the following must be considered:

- Handling of samples or contact with contaminating materials/surfaces must be minimized,
- Samples must be collected in appropriate clean, inert containers and preserved in a fashion which ensures that no material of interest is lost due to adsorption, chemical or biological degradation, or volatilization,
- Appropriate volumes of sample must be collected to ensure that the required detection limits can be met and quality control samples can be analyzed,
- Samples must be properly shipped to the laboratory, in the appropriate time frame, to ensure that holding times for the analyses are met.

5.3 Chemistry

Standard laboratory practices for cleanliness as applied to glassware, reagents, solvents, gases and instruments must be followed. Work surfaces should be kept clean and high level samples should not be processed simultaneously. Reagents should be of the highest quality and checked for purity, strength, deterioration with time, and contamination. Class A volumetric glassware should be thoroughly cleaned and calibrated when necessary. Balances should be checked frequently, with certified weights and records kept. All instruments

must be calibrated on a routine basis (at least daily/each time instruments are "set-up"), with the maintenance of appropriate standards and operation logs of performance. All analytical laboratories should provide details of sample extraction, workup and analysis.

The chemistry QA/QC program includes the analysis of quality assurance samples to define the precision and accuracy of the method for the type of sample under investigation. All documentation of QA/QC procedures must be checked and signed by a senior chemist, and retained for audit purposes. The following quality assurance samples should be employed:

- Method Blank - usually distilled water with added reagents, which is carried through the entire analysis as a check on laboratory contamination (also called a reagent blank). At least one full method blank should be run for each batch.

- Duplicate - a homogenous sample is split either in the field (field duplicate) or in the laboratory prior to digestion (analytical duplicate) with the duplicate presented to the analyst as an additional sample to check for precision. Duplicates should not be tested consecutively. At least 15-20% or one sample per-batch (whichever is greater) should be duplicated to provide an indication of reproducibility. If more than two splits are analyzed the term replicate is normally used.

- Check Standard - a procedure that is standardized with calibration standards prior to analyzing the samples. The analytical response to the standards is checked by frequently analyzing one or more standards along with the samples. The check standards are prepared independently of the calibration standards.

- Spike - a known amount of analyte added to a sample to provide information on matrix effects (on compounds of interest) and apparent accuracy. Surrogate spike compounds can be used to evaluate analytical recovery from each sample.

- Standard Reference ("Control") Material - a material that contains a known concentration of the analyte in question. Based upon a reliable documentation of the analyte concentration, a reference material is certified by agencies such as the National Bureau of Standards and the National Research Council (NRC); standard reference materials are available from NRC and EP. At least one standard reference material should be analyzed per batch. For those

parameters where standard reference materials are available, these can be analyzed in lieu of parameter spikes.

The following field-initiated QA/QC procedures can also be used:

- Transfer (Preservation) Blanks - a sample container is filled with distilled water to the same volume as that for samples and preserved as if it were a normal water or sediment sample. This blank is then sent to the laboratory for analysis.

- Cross-Contamination Blanks - decontaminated sample-handling equipment (spatulas, augers, core barrels) is wiped with a clean lab tissue, which is then placed in a sealable container. Alternatively, equipment is rinsed with distilled/deionized water, and the water is collected and preserved as if it were a normal sample.

- Blind Replicate Samples - collected sample is homogenized and split in the field into at least three identical aliquots, and each aliquot is treated and identified as a separate sample. The replicates are sent blind to the laboratory. The mean, standard deviation, and relative percent standard deviation are calculated by the project QA/QC coordinator. Alternatively/in addition, a collected sample may be split in the field into two aliquots, and one aliquot sent for analysis to a different or "reference" laboratory. The relative percent difference is calculated by the project QA/QC coordinator. If project constraints require the use of more than one laboratory, their comparability must be established using certified reference materials.

- Blind Standard Reference Materials - standard reference material is placed in a sample container at the time of sample collection and sent blind to the laboratory. The percent recovery is calculated by the project QA/QC coordinator

5.4 **Bioassays**

The following QA/QC procedures apply to sediment bioassays in general and are additional to general good laboratory practices of proper documentation, cleaning, avoidance of contamination and maintenance of appropriate test conditions. All unusual observations and deviations from established procedures must be recorded and reported.

Test organisms

Only healthy organisms of similar size and life history stage should be used in bioassays. Taxonomic identifications of bioassay organisms must be confirmed by a qualified taxonomist. Health of test organisms is evaluated using negative and positive controls.

Negative controls

All bioassays must be conducted using well-established negative (clean) controls. For every test series with a particular organism or biological system, one bioassay test chamber or series of chambers must contain clean, inert sediment plus diluent seawater. The complete bioassay series must be repeated if more than 10% of the control animals die or show evidence of sublethal effects.

Positive controls

All bioassay should be conducted using well-established positive controls (reference toxicants). Reference toxicants are used to provide insight into mortalities or increased sensitivity that may occur as a result of disease, changes to tolerance/sensitivity, and loading density; results of all positive controls should be tiled for continuous comparison. Reference toxicants can also provide insight into nonlethal effects that occur due to acclimation, insensitivity, or stress tolerance developed in handling and bioassay. Accordingly, concurrent bioassays using a reference **toxicant** should be implemented for each test series. The **toxicant** should be at least reagent or analytical grade and should be added to dilution water without the use of solvents and other carriers (except water) if possible.

Reference test samples

Control sediments are generally those from which test animals were collected. Physical and chemical sediment characteristics (e.g., grain size, organic content) of reference sediments may be very different from those of the test sediments. Where this is the case, one or more reference sediments should be added to the test series. Reference sediments should be collected from an area documented to be free from chemical contamination and should represent the range of important physical and chemical variables found in the test sediments. Data derived from such a reference tests can be used to partition **toxicant** effects from unrelated effects, such as those of sediment grain size.

Blind testing

All treatment and bioassay containers should be randomized and tested without laboratory personnel knowing sample identities. Replicates of each treatment should be assigned a code number during testing and randomized in the test sequence.

Maintenance/monitoring of water quality

Bioassays involving exposure of organisms in aqueous media require that the media be uncontaminated and that proper water quality conditions be maintained to ensure the survival of the organisms and to ensure that undue stress is not exerted on the organisms unrelated to the test sediments. At a minimum, the following variables must be measured at the beginning and termination of testing: conductivity, dissolved oxygen, pH, and temperature.

5.5 Taxonomic Quality Assurance/Quality Control

A thorough and effective Quality Assurance/Quality Control (QA/QC) Program is the principle means of maintaining the accuracy and precision of field and laboratory analyses to assure scientific credibility. The QA/QC program should also standardize and minimize possible errors in computation and reporting of results.

Sources of error in taxonomic analyses include the experience and training of the taxonomist, the degree of subsampling, sieve sizes, sorting skills, level of analysis (e.g., to the family, genus or species level), the correctness of identifications and the type of statistical or numerical treatment applied to the data. Laboratory data sheets should contain entries for all pertinent biological and physical data. They may then be easily reproduced and appended to the final report, where they will be available should any questions arise. Laboratory data sheets should include entry spaces for:

- station number
- replicate number
- collection gear
- water depth

- date and time of collection
- names of laboratory personnel
- sediment grain size composition (percent gravel, percent sand, percent silt and percent clay)
- columns for numbers of specimens, species names,. species codes, initials of the taxonomists performing the identifications and comments on the specimens (size, reproductive condition, etc.).

Quality Assurance/Quality Control procedures for taxonomic analyses should include:

- Complete sorting (including resorting), processing and/or laboratory records for each sample.
- 10% of all samples should be randomly selected and resorted by individuals different from those who conducted the original sorting.
- All sieved sediments should be held for one year and be available thereafter if desired.
- A voucher collection consisting of specimens representative of each species (or lowest taxonomic unit of classification).
- Taxonomic references used for the identification of all organisms cited in the appendix to the final report.

Any unusual/and/or ambiguous identifications could be submitted to appropriate experts in that area. For example, expert verification might include:

Oligochaetes	R. Brinkhurst (Institute of Ocean Sciences, Victoria)
Amphipods	E. Bousfield (National Museum, Ottawa)
Chironomids	D.R. Oliver (Biosystematics Research Institute, Ottawa)
Water mites	D. Cook (Wayne State University, Michigan)

Stoneflies K. Cummins (Oregon State University)

6.0 GUIDELINES

6.1 **Introduction**

We have not yet developed an ecological science capable either of detecting the stresses imposed upon natural ecosystems by anthropogenic activities, or of predicting the long term effects of such stresses (Schindler, 1987). However, we must apply the tools at hand and develop and utilize new methods. This review has summarized and evaluated biological methods for sediment assessment, specifically with respect to acid mine drainage. This section of the report will briefly address the last objective of the study, which is to develop an interpretative framework and identify future directions of sediment assessment issues at acid mine drainage sites.

6.2 **Sediment Chemistry**

Chemical analysis of sediments may include bulk total analysis, elutriate tests and fractionation/extraction procedures. The selection of the method depends on the purpose of the study. Bulk analysis measures the total concentration of a chemical in a sample. Elutriate tests measure the chemicals likely to be released to the water column during disruption (e.g., dredging, erosion) of the sediment. Sediment fractionation selectively extracts sediment bound chemicals (i.e., metals) to estimate toxicity potential. Section 2.0 discusses specific methodologies for each of these analytical approaches. Since the physical characteristics of sediments are related to chemical bioavailability, there are a number of parameters which should be measured including: carbon fraction, particle size, cation exchange capacity and Eh.

6.3 **Bioassay Testing**

It has been clearly demonstrated that bioassay testing of freshwater sediments has considerable merit as a method of sediment quality assessment (Cairns et al., 1984; Malueg et al., 1984a,b; Nebeker et al., 1984a,b). However, there is a critical need for direct application of these methods to acid mine drainage sites; very little work has been published on the toxicity of sediments from acid mine drainage sites. From the literature, it is evident that metal contaminated sediments can be toxic and it is expected that there will be cause for

concern related to metal toxicity at acid mine drainage sites, particularly under low pH conditions when metals are more likely to become dissolved. Section 3.0 provides details and evaluation of the various aspects of sediment assessment using bioassay techniques. The basic recommendations are summarized below:

Sampling

- Sampling devices should be selected on a case-by-case basis. The preferred methods of collection are a grab or corer.
- Sample locations should be chosen based on prior information about sediment transport, and the sites need to adequately represent the area being influenced by acid mine drainage.
- Sediment samples for bioassay testing should be stored in tightly sealed bags, in the dark at 4°C for periods of no longer than six weeks before test initiation.

Bioassav protocols

Selection of sediment bioassays should take into account the following (Nebeker et al., 1984a): ease of culture, cost, sensitivity, large data base, no special equipment requirements, intimate contact with sediment and control survival. For acid mine drainage sediments, we recommend that the following bioassay tests be included in a test battery for sediment quality assessment:

- ***Daphnia magna***: Although ***D. magna is*** not a benthic organism, it has been selected as an appropriate test species to investigate contaminated sediment toxicity because it is a standard bioassay organism used to determine the toxicity of water, effluents, sediments, and sediment elutriates. The toxicities of many contaminants (including metals) to ***D. magna*** have been measured, and there is a large data base available to compare results. The ***D. magna*** assay is sensitive, reproducible, inexpensive, and practical. Both acute (48-h) and chronic (7-d) assays are recommended.
- ***Hyallela azteca***: This amphipod species is easy to culture and widely used as a bioassay test species. As an acute bioassay with a lethality endpoint, it provides a clear measure of toxicity and is known to be sensitive to metals.

- ***Chironomus tentans*: *C. tentans*** is a representative of the group of insects known as midges, which have a wide distribution during their larval stages of development in freshwater sediments. C. ***tentans*** spend the bulk of its life cycle in the top few centimetres of sediment, and are hence important in the cycling of sediment residues. Nebeker et al. **(1984a)** recommend C. ***tentans*** as the benthic insect of use for solid phase tests, as it is easy to rear and test in the laboratory. Ten day old **second-instar** larvae are most suitable for testing as they are large enough to work with, are the most sensitive of the three larger larval **instars,** and allow two larval molts during a 10-d exposure (Nebeker et al., **1984a).** We recommend that the chronic (= partial life cycle) test be used.

6.4 Benthic Macroinvertebrates

Benthic macroinvertebrate community structure and function assessments have been used extensively to monitor impacts associated with a variety of anthropogenic inputs, including acid mine drainage. Section 4.0 provides details of methodologies utilized and the basic recommendations are summarized below:

- Consider site-specific characteristics when developing a sampling program (e.g., sediment conditions, surface water conditions and habitat quality).

- Sample natural substrates using the appropriate equipment and try to standardize the substrate which is sampled. Collect enough samples to minimize intra-site variability; if possible conduct a preliminary study to examine population variance for the site.

- Sort, enumerate and identify the samples using full quality assurance/quality control to guarantee the data quality.

- In statistical analyses, start out with descriptive statistics and then **ANOVA.** From there it can determined whether multivariate data analyses are appropriate.

6.5 Interpretative Framework

The approach suggested is a multi-faceted, integrative assessment of the environment. Integrative assessments of environmental health are preferred because there is not adequate knowledge to determine and document environmental quality using single measures. This is particularly true for systems that are contaminated by complex mixtures of chemicals. To effectively monitor or assess environmental quality, three basic questions should be addressed:

1. What is (are) the contaminant(s);

2. What is the extent of contamination; and,

3. What are the potential effects on biota.

These questions may be answered by incorporating the following parallel components or disciplines:

- Contamination: chemical analyses of water and/or sediments.

- Toxicity: water and/or sediment **bioassays.**

- Biological alteration ***(in situ):*** changes in the numbers and types of plants and animals in the pond.

The Sediment Quality Triad approach (Chapman et al., 1987) consists of three components: sediment chemistry which measures contamination, sediment **bioassays** which measure toxicity, and biological community structure ***(in situ*/field** parameters) which measure alteration. Information provided by each component is unique and complementary. For instance, sediment chemistry provides no information on biological effects; sediment bioassays are conducted under laboratory conditions and may not be directly applicable to ***in situ*** conditions; ***in situ*** alteration may be. due to biological and/or physical effects that are unrelated to contamination and toxicity.

Together, all three components provide the strongest evidence presently available for determining pollution-induced degradation. For example, if there are high levels of contamination, toxicity and alteration of aquatic environments, the burden of evidence indicates degradation. In the reverse case, low levels of contamination,

toxicity and alteration indicate non-degraded conditions. Intermediate responses provide differential levels of information shown as indicated in Table 4.

Sediments are a major repository for persistent toxic substances discharged into the aquatic environment, including metals. Assessment of sediment conditions through the Sediment Quality Triad can provide valuable information about water quality and contaminant loading to the aquatic environment. The Triad has utility as a both a reactive or proactive tool. When degradation has already occurred, our responses (e.g., mitigation, remediation) are reactive. However, when contamination and toxicity are high but alteration has not been determined, the Triad would serve a proactive function by indicating that the receiving environment is stressed by toxic chemicals and that additional stresses may precipitate community alteration. The Triad approach provides a means to:

- determine problem areas of sediment contamination where pollution-induced degradation is occurring;

- prioritize degraded areas, and their environmental significance; and,

- predict where such degradation will occur based on levels of contamination and toxicity.

Table 4. Information provided by differential Triad responses.

Triad Components			Possible Conclusions
Contamination	**Toxicity**	**Alteration**	
+	+	+	strong evidence for pollution-induced degradation
			strong evidence that there is no pollution-induced degradation
+			contaminants are not bioavailable
	+		unmeasured chemicals or conditions exist with the potential to cause degradation
		+	alteration is not due to toxic chemicals
+	+		toxic chemicals are stressing the system
	+	+	unmeasured toxic chemicals are causing degradation
+		+	chemicals are not bioavailable or alteration is not due to toxic chemicals

Responses are shown as either positive (+) or negative **(-)**, indicating whether or not measurable differences from control, reference conditions or measures are determined.

The Triad concept is important since it is designed to determine, and ultimately monitor, trends in sediment quality. Sediment deposition zones accumulate and integrate toxic chemical inputs from multiple sources over time, thus assessment of sediment quality is essential to determine trends in environmental quality.

6.51 Approach

Chemistry

The first component of the Sediment Quality Triad is sediment chemistry. Specifically, identification and quantification of contaminants present in the sediments must be undertaken. Analysis of the sediment enables characterization of the degree of contamination by specific compounds. Auxiliary analyses of grain size will provide a basis for normalizing data for different types of sediments. Bulk chemical analyses are detailed in Section 2.2.1.

Sediment Toxicity Bioassays

Field-collected sediments typically contain a complex mixture of chemicals, depending on local sources. There is rarely any single chemical that can be identified as causing toxic responses observed in the laboratory or the field, although having a single mine discharging to the aquatic environment may make toxicant identification easier. However, different organisms will respond differently to chemical contaminants in sediments (Swartz et al., 1982). To provide a realistic assessment of sediment toxicity, more than one bioassay test is required. Ideally, a range of tests would be used including acute lethal, acute sublethal and chronic tests (Chapman and Long, 1983). As outlined in Section 3.0, the following tests and test organisms are recommended:

- *Daphnia magna:* both acute (48-h) and chronic (7 d);
- *Hyallela azteca*: sublethal acute (10 d) lethality; and
- *Chironomus tentans*: chronic (10 d) partial life-cycle.

Benthic Infaunal Analysis

Sediment chemistry analyses will provide data on types and quantities of chemicals that may be affecting the biota. Sediment bioassays will indicate whether the contaminated sediments are toxic to the biota, but this will be done in laboratory tests which do not mimic the real environment. Consequently, there is a need to determine whether the benthic infauna, which live in the sediments, are affected by the measured chemical contamination. Comparison of bioassay and infauna data will allow separation of non-pollution related effects on the infauna in the natural environment may be affected by chemical contamination. Section 4.0 provides detailed methodologies for benthic macroinvertebrate community structure and function assessments, and recommendations are outlined in Section 6.4.

6.5.2 Data Analysis

Background

Appropriate statistical analysis of biological and chemical data are essential to the success of any monitoring or ecosystem assessment program. Chemical, biological, and toxicological data collected using methods summarized elsewhere in this document should be analyzed to maximize an understanding of the data. A framework that utilizes (analyzes) these data is the Triad, described by Chapman (1986).

There are two "basic" methodologies that have been used to analyze Triad data. The first method was developed by Chapman (1986) and is based on ratios of individual parameters between test sites and reference sites. The second method was developed by Chapman et al. (1989) who utilized non-parametric ranking to describe the relative contamination of test sites to reference sites. These two methodologies are outlined below.

Ratio-to-Reference

The best example of an analysis of data utilizing the Triad approach is described in Chapman et al. (1987). Chapman et al. (1987) suggest that the Triad can be used to determine pollution-induced degradation both spatially and temporally by generating indices that represent individual aggregate characterizations of the respective chemistry, toxicology and biology. Chemical, toxicological and biological data can be used to obtain

Ratio-to-Reference (RTR) values. Generally, the reference data set come from an "unpolluted" reference site (e.g., upstream of discharge point).

To determine RTR values, individually measured parameters are divided by the reference values for those same parameters. This process "normalizes" the data into dimensionless numbers that can be compared even when large differences in measurement units exist. An index of chemical contamination can be calculated for each site by separately determining RTR values for groups of similar chemicals (i.e., metals). In some cases, these values can be combined into a single mean chemistry RTR value. An index of toxicity can be generated by calculating the mean of the bioassay RTR values. An index of biological "health" alteration can be determined in the same manner as for toxicity, using biological community structure data.

The composite RTR values for each Triad measure can provide useful visual indices. For example, these values can be plotted on scales with a common origin and placed at 120 degrees from each other such that each of the three values becomes the vertex of a triangle. A relative measure of the degree of degradation can be derived by calculating and comparing the areas of the triangles for each station and/or site. These plots also serve to visually define the characteristics of the reference time period. The Triad approach is appealing because data are presented in a simple, easy to interpret manner.

Non-Parametric Ranking

As well as RTRs, rankings can also be assigned to chemical, toxicological and biological data for statistical comparisons of the data (Chapman et al., 1989). For all chemistry data, the sample with the lowest level of a chemical is scored as 1 and the highest is scored with a number that is equal to the number of time periods or samples that are to be ranked. Tied data should be scored with by summing the tied ranks and dividing by the number of ties. Overall ranks based on each of chemical, toxicological and biological data can be determined by comparing rankings for chemicals, toxicity tests and biological indices using Kendall's coefficient of concordance (Zar, 1984). If measurements are made over time (e.g., monitoring to document reclamation), high concordance will indicate that chemical, toxicological, and biological parameters are changing in the same direction (i.e., improving or degrading). Low concordance would indicate that chemical, toxicological and biological data are changing independently of each other.

7.0 FUTURE RESEARCH

During this review, data gaps and research needs were identified to provide a focus for future investigations regarding the sediment quality of water bodies affected by acid mine drainage. The following research topics are recommended for future investigations of sediment quality issues:

1. Development of sediment quality guidelines to facilitate evaluation of field sites.

2. Application of a battery of bioassay tests to sediments collected from acid mine drainage sites.

3. Simultaneous measurement of sediment chemistry, toxicity and infaunal community structure in the Triad approach.

4. Comparative bioassay testing to compare the sensitivity of various freshwater bioassays.

5. Further investigations into the effects of grain size on sediment toxicity from two perspectives: 1) how it changes metal bioavailability and 2) how it physically affects the sensitivity of bioassay species.

6. Develop an interpretive framework for evaluation of freshwater sediments at acid mine drainage sites; this would included investigation into possible numerical sediment chemistry guidelines as well as development of biological testing procedures.

7. Examine the physical characteristics of sediment which are related to a propensity to sorb metals, then develop a predictive model for acid mine drainage sites where chemical contamination and toxicity could be problems.

8. Investigate the conditions of mine operation and acid mine drainage which are most likely to contribute to sediment contamination and toxicity.

9. Examine natural variability of benthic macroinvertebrate communities at sites with a range of grain sizes; this will assist in addressing the issue of assigning sources of sample variability. It would be useful to compare sample variability at a range of acid mine drainage vs. reference sites.

10. Develop a standard approach to benthos sampling which will **maximize** the sensitivity of the community measures, yet require as little sampling and analysis effort as possible. This would include preparation of sampling and sample analysis protocols so that investigations of acid mine drainage sites yield comparable data over time, and between sites.

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