Sediment Toxicity Testing:
Battery Test Evaluation of Shallow Urban Streams and the
Effect of Sampling Method on Toxicity

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ABSTRACT

Sediments have been recognized as a potential source of contamination in aquatic environments. Toxicity testing has been used as a tool for studying sediment related toxicity, although many aspects of the methodology are still evolving. However, it has been debated whether or not the results of laboratory assays can be extrapolated to ecosystems effects. As well, questions arise about changes that occur in sediments when they are manipulated in the laboratory during toxicity testing.

This study examined the effects of sampling method on sediment toxicity. Toxicity tests were performed on sediment which had been mixed (homogenized) and the toxicity compared to that of undisturbed sediment cores, where the integrity of the sediment had been maintained. Toxicity tests performed on the sediment included *Daphnia magna*, *Chironomus tentans*, and Microtox®. Chemical analysis of the sediment was also done, including metals, polynuclear aromatic hydrocarbons (PAH), ammonia, and metal/sulfide ratios.

It was found that the method of sampling affected toxicity of sediments to *Chironomus tentans* in about half the cases. However, the method of sampling did not affect toxicity to *Daphnia magna*, probably because the type of contaminants found in the sediments were not available to the *Daphnia*. *Daphnia magna* were unresponsive to all sediment tested. A bacterial luminescence bioassay (Microtox®) was also performed to compare the sensitivities of the different toxicity tests. It was
found that the Microtox® Solid Phase Test was the most sensitive of all the tests, and was able to distinguish between contaminated and uncontaminated sediment. The measurement of metal/AVS ratio in sediment was not found to be a useful tool in determining whether a sediment would be toxic or non-toxic to organisms.
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GLOSSARY

1S, 2S, 3S, 4S, and 5S - These are the sites sampled on Still Creek.

1M, 2M, 3M, 4M, 5M - These are the different times the reference site on Musqueam Creek was sampled.

AVS - Acid Volatile Sulfide (AVS) is a measure of the sulfide in a sediment sample. The amount of simultaneously extracted metals is also measured, and the molar ratio of metals to AVS is determined. If this ratio exceeds one, the sediment is said to potentially be toxic.

Mixed Sediment Samples - This refers to one of the sediment sampling methods used in this thesis. Details of sampling methodology can be found in Chapter 3.

Reference site - The site sampled on Musqueam Creek, Vancouver, B.C. is referred to as the reference site.

Sediment cores - This refers to one of the sediment sampling methods used in this thesis. Details of sampling methodology can be found in Chapter 3.

SEM - Simultaneously extracted metals (SEM) are the metals extracted simultaneously during the sulfide extraction process for SEM/AVS determination.

Test site - Sites sampled on Still Creek, Burnaby, B.C. are referred to as the test sites.
CHAPTER 1  INTRODUCTION

Sediment toxicity is determined by chemical analysis of the sediment, and by laboratory or in situ sediment toxicity tests. Chemical analysis verifies the presence or absence of contaminants. Toxicity tests are used to determine the biological significance of the contaminants, (i.e. if they are effecting the ecosystem).

The major limitation associated with sediment toxicity tests is the uncertainty associated with the extrapolation of these results back to the natural ecosystem. Uncertainty arises because sediment toxicity tests are typically performed on sediment which has been removed from the stream, and mixed (homogenized) so the integrity of the original sediment sample is lost. It is also probable that the sediment chemistry will be altered in this process and thus the toxicity of the sediment to organisms will also be altered. Since many management decisions are made using the results of the toxicity tests, it is important for decision makers to have confidence in their results.

This thesis examines how the process of mixing (homogenizing) alters the toxicity of stream sediments when compared to a relatively undisturbed sediment core sample.
CHAPTER 2  LITERATURE REVIEW AND TESTING RATIONALE

2.1  OBJECTIVES

This thesis set out to answer the following questions:

1. Is there a difference in toxicity between a mixed sediment sample and a relatively undisturbed sediment core taken from shallow urban streams?

2. How well does the measurement of SEM/AVS predict toxicity?

3. How sensitive is the Microtox® Solid Phase Test compared to the invertebrate tests done on the same samples?

This Chapter outlines the history of toxicity testing, describes each of the tests mentioned in the objectives, and discusses the rationale behind the toxicity tests chosen.

2.2  HISTORY OF SEDIMENT TOXICITY TESTING

Toxicity tests involve the exposure of organisms to contaminated water, effluent or sediment and documenting the effects. They serve a number of purposes: monitoring of water or sediment quality, evaluating sediment or water quality, and predicting toxicity of a specific compound, chemical or effluent before it is released into the environment.

In past years, pollution studies have focused on the water column. Canada recognized the need to develop biological test methods for monitoring and evaluating
water quality in the mid 1980s, and there now exists a set of standardized biological test methods to meet Environment Canada's needs regarding aquatic environmental monitoring and regulation (McLeay et al., 1991). These include acute lethality to rainbow trout \((Oncorhynchus mykiss)\), threespine stickleback \((Gasterosteus aculeatus)\), Ceriodaphnia, \(Daphnia\) spp., Microtox® \((Photobacterium phosphoreum)\), green algae \((Selenastrum capricomutum)\), and the fathead minnow \((Pimephales promelas)\).

In the past, little attention has been given to sediment quality, although it is a well known fact that many anthropogenic pollutants, both organic and inorganic, concentrate in sediment but are found only in low concentrations in the water column. It was often assumed that substances in the sediment were removed from circulation and were no longer a problem. However, these contaminants can be accumulated by and directly effect benthic organisms, or affect other aquatic organisms by becoming bioavailable through resuspension, leaching, or through the food chain. Thus, sediments can act as a source as well as a sink for pollutants.

The majority of literature regarding sediment toxicity began appearing in the 1980s. Early toxicity testing focused on acute toxicity of dredge material, and on work done by the US Army Corps of Engineers (Wright and Saunders, 1990). More recently, the importance of sediment toxicity testing has been realized by Governments in Canada, the United States and many European countries (Burton and Scott, 1992).
CHAPTER 2 LITERATURE REVIEW AND TESTING RATIONALE

Research on sediment toxicity has been limited by the complexity of sediment-water column and sediment-biota interactions (Giesy and Hoke, 1989). Water quality criteria have been based on total chemical concentrations (Di Toro et al., 1991), and this level is taken as a measure of bioavailable concentration. Water quality criteria protect inhabitants of the water column, and are not intended to protect organisms associated with sediments (Chapman, 1989). Recent literature has begun to address the question of sediment quality criteria (Di Toro et al., 1991; Chapman, 1991). Different methods have been proposed to determine sediment quality criteria, such as equilibrium partitioning as proposed by Di Toro et al., (1991) and the sediment quality triad as proposed by Chapman (1986). Sediment quality criteria are necessary in order to provide for long-term management of contaminated sediments (Chapman, 1989).

There are few standardized testing methods for sediment quality, and few sediment quality guidelines have been developed, unlike water quality testing and guidelines which have been extensively developed. Sediment quality objectives have been proposed for marine and freshwater sediments. The Wisconsin Department of Natural Resources developed guidelines for classification of dredged material for water disposal in 1985. These were updated in 1990 for cleanup at an EPA Superfund site (Bennett and Cubbage, 1991). The Ontario Ministry of the Environment developed guidelines for sediment evaluation which were based on the long-term effects on benthic organisms (Bennett and Cubbage 1991).
CHAPTER 2 LITERATURE REVIEW AND TESTING RATIONALE

Marine sediment quality chemical criteria were established for the Puget Sound area and are summarized by the Washington State Department of Ecology (1991). Similar sediment quality objectives have been proposed for Burrard Inlet, British Columbia (B.C. Environment, 1990). The general principal followed in the development of the Burrard Inlet objectives was that it would be below the lowest measured apparent effects threshold (AET) from Puget Sound criteria. The AET is the highest sediment concentration of a contaminant above which an adverse biological reaction is always observed (Alden and Rule, 1992). The main problem associated with criteria based on an AET is that it does not consider synergistic or antagonistic effects when more than one contaminant is present in situ. Regulatory sediment quality assessment should be based on site-specific toxicity testing rather than generic AET-based criteria or standards. AET could be used for identifying areas that have high sediment contamination (Alden and Rule, 1992).

Equilibrium partitioning has also been used to develop numerical sediment quality criteria (Meyer et al., 1993, Parkerton et al., 1993, Di Toro et al., 1991). This approach is based on the notion that contaminant concentration in the interstitial water is a good measure of bioavailable chemical concentration, and correlates with sediment toxicity (Meyer, 1993). This hypothesis needs further testing, however, before any conclusions on its usefulness can be made.
2.3 **MEASUREMENT OF SEDIMENT QUALITY**

Chapman and Long (1983) suggested that assessment of sediment quality should involve at least three measurements: concentrations of chemicals, toxicity of environmental samples, and assessment of resident biota populations. These three measurements are referred to as the sediment quality triad. Each of these alone is insufficient in assessing potential environmental damage. Chemical analysis can indicate quantities of contaminants. 'Hot spots' where there is a large concentration of a contaminant can be identified (Giesy and Hoke, 1989). However, some chemicals that are known to be toxic are not easily detected, or can be toxic at levels near detection limits. Other chemicals volatilize rapidly during sediment handling and are lost. Determination of contaminant concentrations provides no information about the bioavailability of compounds or their potential for causing adverse effects. A contaminant may be present in a sediment sample, but unless it elicits a toxic response to organisms, it may not be of concern. For example, metals can be present in high concentrations, but if irreversibly bound to sediment particles they will be unavailable to organisms.

Inventories of resident biota populations can detect changes in species diversity, or detect the presence or absence of a particular species from an area. Species diversity has been used as an indicator of stress on an ecosystem. Biodiversity usually declines as pollution/toxicity increases (Burton, 1991; Hall K.J., personal
communication). The presence of many different pollutants will result in a non-specific decrease in species richness and population size, (Sloof, 1985). In heavily polluted areas only one or two species may be able to survive. The presence or absence of a single species may be related to other factors besides pollution, e.g. predation or competition. Ecosystems are dynamic and constantly changing. However, many species can adapt to a changing environment, especially a slow change such as the slow build up of a pollutant over time.

Data from chemical analyses and biota surveys can be correlated to determine if the absence of a particular species occurs in an area of high contaminant concentration. Interpretation of these data is not always straightforward. Factors such as synergistic effects, antagonistic effects and changes in bioavailability are not assessed. Toxicity tests are the third component of the triad, and provide a direct measure of a sediment’s adverse effects. They are generally done using species that have been raised in the laboratory for many generations, and have never been exposed to pollutants. Since the tests are done in the laboratory, they may not accurately represent the conditions organisms are exposed to in the natural environment. However, they do provide a useful tool in a preliminary screening process or, together with chemical and ecological analyses, as part of a full assessment. Laboratory tests provide a link between the chemical analysis and the ecological survey, and allow for a more complete interpretation of results. This approach has been used in the Puget Sound area in Washington State, (Chapman, 1986). From
the data collected, a set of sediment quality criteria were derived. By using the sediment quality triad, site-specific sediment quality criteria were established. From this concept, sediment quality guidelines can be developed rather than rigid criteria (Chapman, 1991). That is, a ‘flexible framework’ should be developed that provides a workable range rather than a strict number or criterion, recognizing the natural variability of sediments. These may also be non-numerical, relying on actual sediment/benthos biological effects testing (Chapman, 1989).

Many different types of organisms have been used for toxicity testing. It is appropriate to perform toxicity tests on a sample with more than one species. Each sample will contain different contaminants, and each contaminant will vary in bioavailability, depending on a number of factors. Thus, employing a ‘battery of tests’ will ensure that a contaminated sediment will not be overlooked as ‘non-toxic’ when tested with a single species, and also provides more confidence a truly non-toxic sediment will be appropriately characterized. A battery of toxicity tests gives a measure of toxicity that accounts for bioavailability and interactions among toxicants (Giesy and Hoke, 1989). This approach has been used widely (Burton and Stemmer, 1988; Dutka et al., 1988, 1989; Dutka and Kwan, 1989; Santiago et al., 1993).

Dutka and Kwan (1989) applied the battery of screening tests approach to sediment extracts. Use of a battery of tests was able to show the whole area sampled was impacted by toxicants. It was noted that the make-up of the battery of tests is
important. In this case, *Daphnia magna* were sensitive to toxicants which the other tests were not, specifically for water extracts of sediment. Burton and Stemmer (1988) also utilized a battery of tests applied to waters, leachates and sediments. No one assay was able to consistently detect the presence of toxicants, emphasizing the need for a battery of tests in reliable toxicity assessments.

Dutka et al., (1989) used the battery of tests approach to study the Fraser River in British Columbia. Their results further confirmed the need for a battery of tests, and emphasized the importance in choosing the best tests for that site. It was found in this case that the *Daphnia magna* test was the most sensitive for indicating contaminant presence.

### 2.4 STANDARDIZED TESTS

The United States has a more developed set of standardized sediment toxicity tests than Canada. In 1990, the American Society for Testing and Materials, (ASTM), approved guidelines for whole-sediment toxicity tests with the marine amphipods *Rhepoxynius* and *Ampelisca*, the freshwater amphipod *Hyalella azteca*, and the freshwater midges *Chironomus tentans* and *Chironomus riparius* (Burton and Scott, 1992). Methods have also been approved for whole-sediment testing with *Daphnia* spp. and *Ceriodaphnia* spp.
A number of different organisms have also been suggested in the literature for assessing sediment toxicity (Giesy and Hoke, 1989, 1991; Burton, 1991), and will be discussed later, along with the rationale for choosing the tests done in this study.

Sediment toxicity has been evaluated by relating concentrations of toxic substances in sediments to known dose-response relationships. However, the efficiency of extraction methods and analyses are unknown, the bioavailability of compounds will differ in different sediment samples, and compound interactions (synergistic, additive, etc.) are not considered. Dose-responses can be calculated for a contaminated sediment, and the dose-response relationship can characterize the volume of sediment which exceeds a threshold value. It can also be used to determine how much a contaminated sediment would need to be diluted before it was deemed no longer a threat to benthic organisms, or what the possible toxicity of resuspended or dredged subsurface sediments might be (Giesy and Hoke, 1989).

The problems associated with dose-response sediment assessments include the choice of organism for the test, the positive or negative controls to use, the end points to be monitored, and choice of suitable test conditions.

To determine dose-response relationships with sediment, dilutions must be made. The sediment is diluted with a reference sediment which is not contaminated. More questions arise, such as: which sediment should be used as a reference sediment, how should the samples be mixed, and how long should they equilibrate? Dilutions
will also change the physical and chemical characteristics of the sediment, and any result will not be easily extrapolated to the ecosystem being studied.

The complications surrounding sediment dilutions have led to the use of pore water (Giesy et al., 1988) and sediment elutriates in sediment toxicity testing (Kwan and Dutka, 1990). In the generation of sediment elutriates, different extraction procedures have been used, such as Milli-Q water, DMSO, and ethanol, (Kwan and Dutka, 1990; Dutka et al., 1986; Schiewe et al., 1985). This method will extract contaminants in a liquid phase which can then be used with standardized tests such as *Daphnia magna*. However, the integrity of the sample is not maintained, and no information about actual bioavailability of the contaminants in the intact sediment is given. Questions arise such as: what solvent to use, how is the extraction done, and how long is the elutriate/sediment mixed before separation?

Pore water is usually extracted by centrifugation. Elutriate tests often give different results than pore water tests, and are often more toxic (Giesy and Hoke, 1989). Pore water, but not elutriate has been shown to be effective in predicting the presence of bulk sediment toxicity (Ankley et al., 1991a). Since burrowing benthic organisms are in contact with the pore water, and are exposed to contaminants via the porewater, toxicity tests using pore water are generally more relevant than toxicity tests done on an elutriate. Pore water testing allows the use of standardized test methods not designed for sediments, for example the *Daphnia magna* 48 hour acute test.
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Both the elutriate method and the pore water methods are based on the assumption that organisms receive most of their exposure to contaminants through contact with the pore water (Giesy and Hoke, 1991). This is true in many instances, but ignores the fact that some organisms feed directly on sediment detritus and are thus exposed to whole sediment. Toxicity depends not only on the phase tested, and the partitioning-fate characteristics of the sample, but also on the test organism's morphology, physiology, and feeding mechanisms. That is, if ingestion of contaminated sediment is the route of exposure, pore water and elutriate exposures will not adequately measure the toxicity of that sediment.

Whole sediment tests are often performed. Here, the test organism is exposed to the whole sediment, so the organism is exposed to any pollutant that is potentially bioavailable. There is no need to decide on methods of elutriate extraction or sediment dilution. These tests are more representative of the exposure to contaminants organisms receive in the natural stream environment.

There are a variety of methods used to collect and store sediment samples, and variable sediment storage times before samples are used for testing. Malueg et al., (1983) collected grab samples using a Ponar dredge. Samples reached the laboratory within 24 hours. Upon arrival, they were thoroughly mixed, stored at 5 °C and used in tests from 7 to 44 days later. Ingersoll and Nelson (1990) collected samples using a Ponar sampler. The upper 2-4 cm of the grab sample were placed
in a plastic container and stored at 4 °C. Sediment toxicity tests were performed within 15 days of sediment collection. Giesy et al (1990) also collected samples using a Ponar dredge. Samples were stored in plastic containers at 4 °C, and were used for testing within 30 days of collection. Sediment was sieved through a #18 sieve (1mm opening) before they were used for toxicity testing. Sasson-Brickson and Burton (1991) used an Ekman dredge or a Wildco hand coring device to collect sediment samples. Samples were placed in buckets, and transported to the laboratory where testing was begun immediately. Sediment was homogenized by mixing with a 'hand paddle'.

These few examples show the necessity for standardized procedures for sediment sampling and show how difficult it would be to compare results or organism sensitivities between these tests. Each used their own sampling method with or without sieving and storage.

Othoudt et al. (1991) examined the effects of storage on sediment toxicity. Samples were homogenized under an argon atmosphere and stored in plastic buckets at 4 °C. When sub-samples were taken for bioassays, the sediment was first re-homogenized. Using *Daphnia magna* and *Chironomus tentans* bioassays, it was determined that storage at 4 °C had no effect on toxicity over a 112 day period. However, in a review by Burton (1991), storage of sediments at 4 °C was found to alter metal toxicity to cladocerans, and change microbial activity. Organic
contaminant availability was also reported to change with storage. Dave (1992) also found storage increased sediment toxicity. Interstitial water (pore water) was also found to change chemically with storage. It is recommended by Burton (1991) that sediments to be used for toxicity testing should not be frozen, and should be kept at 4 °C under zero air space or N₂ for less than two weeks before testing.

Acid-volatile sulphide (AVS) concentrations are also altered with different storage and handling methods. When anoxic sediments are exposed to air, AVSs are oxidized, thus releasing metals that were bound to them. Copper/AVS ratio was shown to change from 5 to 130 within 10 days of storage (Bennett and Cubbage 1992b). AVS is discussed in detail later.

It is evident from the literature that there are no common methods used. Each laboratory has its own methods of sediment collection, homogenization, and storage time before tests are begun. Due to this lack of use of standardized procedures, it becomes difficult to compare studies, toxicity, and test sensitivities because sediments are altered in different ways by each methodology used. It has been recommended that further research be done regarding the effects of sediment collection and storage methods on toxicity (Schubauer-Berigan and Ankley, 1991).

In summary, sample collection, transport, storage and test manipulation can alter toxicological effects by increasing or decreasing toxicity of the sample in the laboratory as compared to the toxicity under field conditions. The degree to which the
toxicity has been altered is difficult, if not impossible, to measure. A complete understanding of the mechanisms of a pollutant's (or combination of pollutants) toxicity is required. For example, properties such as chemical speciation, volatility, solubility, partitioning and thermodynamics are necessary. These characteristics are for the most part unknown for complex environmental samples. Thus, the absolute toxicity of a sediment sample is rarely defined (Burton and Scott, 1992). A more useful measure is the relative toxicity of a sediment sample as compared to another. For example, upstream, "uncontaminated" sediments (when available) serve as a comparison to downstream "suspect" sediments. Parameters such as organic matter content, and particle size fractionation are important in choosing a reference sediment, and should be as similar to the test sediment as possible. This may be difficult due to the dynamic and variable nature of streams and rivers.

2.5 CONTAMINANTS, TOXICITY AND BIOAVAILABILITY

The bioavailability of a contaminant is the extent to which that contaminant can be toxic to or accumulated by biological organisms. Bioavailability is not an 'all or none' occurrence (Anderson, 1984). In one group of environmental conditions, a sediment may not show any toxicity. However, if the dissolved oxygen or pH conditions change in the overlying water, the sediment may become toxic. Many things can affect the bioavailability of contaminants associated with sediment, as outlined below.
2.5.1 Food Source and Feeding Habits

Bioavailability is a function of feeding, including the food source, nutritional preference and feeding rate. There exist a vast array of benthic dwelling organisms that are associated with sediments. Some examples are given in Table 2.1.

Table 2.1 Feeding and Life Habits of Some Freshwater Benthic Macroinvertebrates Associated with Sediments (Adapted from Adams, 1984).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Approx. # species</th>
<th>Respiratory Method</th>
<th>Mode of Existence</th>
<th>Feeding Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligochaeta</td>
<td>100's</td>
<td>Diffusion through integument</td>
<td>burrowers</td>
<td>sediment ingestion</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>50</td>
<td>Abdominal respiratory appendages</td>
<td>swimmers</td>
<td>scavengers, omnivores, chewers</td>
</tr>
<tr>
<td>Ephemeroptera</td>
<td>5</td>
<td>Tracheal gills</td>
<td>burrowers</td>
<td>collectors *</td>
</tr>
<tr>
<td>Trichoptera (Caddisflies)</td>
<td>5</td>
<td>Tracheal gills</td>
<td>burrowers</td>
<td>filterers **</td>
</tr>
<tr>
<td>Ptychopteridae</td>
<td>16</td>
<td>Atmospheric breathing tube</td>
<td>burrowers</td>
<td>collectors, sediment ingestion</td>
</tr>
<tr>
<td>Chironomidae (Midges)</td>
<td>500+</td>
<td>blood gills, integument</td>
<td>burrowers, tube builders</td>
<td>filterers ***, collectors, sediment ingestion</td>
</tr>
</tbody>
</table>

* Detritivores that gather deposited fine particulate organic matter from the surface of the sediment
** Detritivores that gather suspended fine particulate organic matter
***Chironomid larvae are known to exhibit more than one feeding mechanism within a species depending on the habitat of an individual.
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This table shows only a small portion of taxa, but demonstrates the wide variety of species, the diversity of life habits they have evolved, and the different feeding mechanisms employed by these organisms. The toxicity of chemicals also depends on life stage of the organism, stress factors (such as competition), and the presence or absence of other compounds.

2.5.2 Organic Carbon

Organic carbon is the principal controlling element in the bioavailability of hydrophobic organic chemicals that are sorbed to sediment. The clay content of sediment is also important in sorption of toxicants. For example, Eaton et al. (1983) have demonstrated a 35% reduction in the bioavailability of Kelthane© to fathead minnows in the presence of 65 mg/L suspended clay particles. Bioavailability is also affected by both total organic carbon (TOC) and dissolved organic carbon (DOC). For the solid phase, TOC is the most important. For the interstitial water, the DOC is most important (Adams, 1984). This is particularly true for lipophilic organic compounds. Soil/water partition coefficients are affected by organic carbon content, and the bound vs. non-bound concentration of a compound is a function of the presence of suspended particles. Uptake and accumulation of benzo(a)pyrene by Daphnia magna is reduced by 97% due to sorption of polynuclear aromatic hydrocarbons to organic matter in the water column. Thus, accumulation of hydrophobic chemicals such as benzo(a)pyrene depends on the amount of that chemical dissolved in the water column, as well as on the amount of chemical sorbed.
to particles that are taken up as food. Chemicals sorbed to organic matter have reduced bioavailability (McCarthy, 1983), but enter the food chain when organisms that feed on particles (filter feeders) ingest the hydrophobic compound.

2.5.3 Metals

2.5.3.1 Metals in Sediments

Metals are a natural component of the biosphere (Luoma, 1983). Some metals are essential for life, but all metals are toxic at high enough concentrations. When metals are discharged into the environment, they partition between the solid and liquid phases. A portion will complex with dissolved inorganic and organic ligands in solution. Some will associate with particulates through adsorption, coprecipitation, or be taken up by living organisms. Figure 2.1 is a schematic representation of metal partitioning between solid and liquid phases.

Sediments contain many sites for trace metals associations (Campbell et al., 1988). This includes adsorption onto particle surfaces; adsorption onto organic matter by complexation, chelation, or uptake by living organisms; and in the lattices of minerals. Other important sinks for metals include precipitation as carbonates, phosphates, and sulfides. The most important of these are outlined below:

1. Adsorbed metals includes metals associated with clays, organic matter, and iron/manganese oxides. Adsorption and desorption are highly pH dependent. Adsorption of cations decreases as pH decreases, and anions show the
Figure 2.1  Schematic representation of metal partitioning in the aquatic environment. Metals will partition amongst inorganic and organic ligands, adsorb to particulate matter, or be taken up by organisms. (adapted from Tessier and Campbell, 1987)

opposite correlation. Acidification results in increases concentrations of dissolved metals, although desorption is dependent on the organic matter content of the sediment.

2. Trace metals can be bound to carbonates. Carbonates in sediments are found as precipitates, shell debris, and limestone fragments, and can be an important sink for copper, zinc, and lead.
3. Oxides of iron and manganese are important in the toxicity of metals in sediments (Di Toro et al., 1991). Sediment iron and manganese oxides come from minerals such as magnetite, from precipitates, or as suspensions settling from the water column. These oxides are a major sink for trace metals, particularly for lead and zinc, which have a higher affinity for this fraction of the sediment (Campbell et al., 1988).

4. Sediment organic matter is a composite of plant and animal detritus in several stages of decomposition from colloidal to large fragments. The importance of organic matter as a sink is related to its abundance compared to the other phases. For example, binding of lead, zinc, and copper by the humic fraction is of greater importance in a sediment with low iron-oxides (Campbell et al., 1988).

5. Sulfides occur in sediments as detrital grains and as products of diagenesis (Campbell et al., 1988). The most abundant sediment sulfide is pyrite (FeS₂). Others include galena (PbS), sphalerite (ZnS) molybdenite (MoS₂) and cinnabar (HgS). These are found in very small quantities, if at all.

6. Approximately 25-50% of sediment trace metals are associated with the silicates. Primary minerals occur mainly in sand and silt fractions, and secondary silicate such as clays predominate in finer fractions.
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Exchangeable and adsorbed metals make up only a small percent of the total metals in sediment. Iron and manganese oxides and organic matter are the main sinks for the nonresidual component. Sediments are very dynamic, and the main sink can change spatially and temporally within a sediment.

2.5.3.2 Biological Processes
Many of the changes in metal partitioning are brought about by biological modes. Microorganisms can carry out chemical transformations that are otherwise thermodynamically not possible. These reactions are important as they may:
1) change the oxidation state to alter bioavailability;
2) produce volatile species; and
3) alter the toxicity of the metal.

Methylation of metals increases the toxicity and bioavailability of metals, and is the biological process which has received most attention. Mercury and tin are the important metals in this group, as well as the metalloids arsenic and selenium. Sediments are the major site for mercury methylation (Campbell et al., 1988).

Demethylation of metals in sediments has received less attention (Campbell et al., 1988), but is an important process because of the enhanced toxicity and bioavailability of the methylated species.

Oxidation and reduction of metals occurs as the chemistry in the environment changes, which is indirectly a result of microbial activities such as oxygen
consumption and production, denitrification, and hydrogen sulfide production. Many microorganisms may directly reduce or oxidize metals.

2.5.3.3 Metal Bioavailability

A metal is considered bioavailable to an organism when it can be taken up by the organism and can react with the 'metabolic machinery' of the organism (Campbell et al., 1988).

Sediments have been shown to act as a source of metals as well as a sink. Knowlton et al. (1983) showed that lead can be taken up by crayfish and macrophytes in the water column when the only source of lead is the underlying sediments.

Studies have also shown that the trace metal level in benthic organisms is not related to the total metal concentration in the sediment, but more so to easily extractable fractions (Tessier and Campbell, 1987). Different sediments will show different toxicity levels for the same total metal concentration. A severe acid digestion using sulfuric/hydrofluoric/nitric acids will give an estimate of the total metal concentration in a sediment. However, more useful information is provided by determining the exchangeable or reducible metals. These metals are more bioavailable (Calmano and Forstner, 1983) and are a better predictor of potential release of metals from sediments.

The free metal ion is usually the most toxic and the most easily bioavailable (Burton, 1991). The partitioning of a metal between sediment and aqueous phases is
important in the bioavailability of that metal. Some metals, such as those bound in
the matrices of inorganic compounds (e.g. clays), are probably unimportant
biologically. Metals associated with organic matter such as humic materials are more
likely to be biologically available if they are weakly bound.

Many factors affect the ability of a metal to be taken up by an organism, including the
metal form. Other factors which can affect uptake of metals by organisms includes
temperature, which can affect the rate of metal transformation and the amount of
metal accumulated by the organism (Campbell et al., 1988). The rate of biological
processes usually doubles with each 10 °C increase in temperature. Metal efflux as
well as influx is affected by temperature (Campbell et al., 1988). Increased
temperature increases efflux of arsenic, cobalt, iron, zinc, and inorganic mercury, but
has no net effect on bioaccumulation. Salinity affects metal speciation as well as
osmotic conditions which will effect permeability of the organism’s membranes. The
pH affects metal speciation and solubility. It can also affect biological uptake, as it is
thought hydrogen ions compete with metal ions at the surface of cells (Campbell et
al., 1988). Cadmium, copper, and zinc uptake and toxicity decreases with increasing
hydrogen ion concentration. Lead, on the other hand, shows an increase in toxicity
with increasing hydrogen ion concentration. Metal-metal interactions affect metal
speciation, uptake, and the fate of the metal inside the organism (Campbell et al.,
1988).
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2.5.3.4 Biological Effects of Metals

Most metals associate with proteins in organisms, and thus can have toxic effects on endocrinology, cell biology, haematology, and physiological functions such as respiration and osmoregulation (Campbell et al, 1988). Metal toxicity differs between organisms, and also depends on factors such as pH and water hardness. Salinity also effects toxicity of metals.

Metal toxicity also depends on whether individual metals or mixtures of metal are present (Campbell et al, 1988). Mixtures of metals can have synergistic, antagonistic, or additive effects. For example, cadmium and selenium act antagonistically, and copper and nickel act synergistically. Cadmium can inhibit the toxicity of copper, and iron can inhibit the toxicity of cadmium. The toxicity of eight metals (arsenic, cadmium, chromium, copper, mercury, lead, nickel and zinc) to Daphnia magna was found to be additive (Enserink et al., 1991).

2.5.3.5 Measurement of Metal Bioavailability

It is possible to measure metal accumulation by an organism in vitro, but without a full comprehension of the physical, biological, and chemical mechanisms involved in metal uptake and metabolism in benthic organisms in the natural conditions encountered in situ, the relevance of such a measurement is questioned. Such measurements can give us information, but cannot tell us how bioavailable that metal is in a particular sediment.
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The free ion of a metal is the most bioavailable, but it is often difficult to measure the concentration of the free ion and relate this concentration to biological effects. There is also variability in biological responses to metals depending on the mode of feeding, digestive processes and excretion. Digestive processes can affect metal partitioning in sediment ingesting organisms.

Since sulfide levels are important in metal partitioning, a measure of the sulfide levels and the metals extractable from the sediment by a cold hydrochloric acid extraction has been used to determine the bioavailability of metals in sediments, and hence sediment toxicity (Di Toro et al., 1991, 1992; Carlson et al., 1991). The sulfide fraction is referred to as the acid-volatile sulfide (AVS) fraction. The metal concentration that is concurrently extracted is called the Simultaneously Extracted Metals (SEM). If the molar ratio of metals to sulfide exceeds one, the sediment is deemed toxic as some metals are not bound to sulfide and thus potentially available. For a ratio of less than one, the sediment is said to be non-toxic with respect to metals.

SEM/AVS ratios in sediments have been evaluated and compared to toxicity in oligochaetes and snails for the same sediments (Carlson et al., 1991). The results showed that for cadmium and nickel, a SEM/AVS ratio of greater than 1 did predict sediment toxicity to the organisms.
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Di Toro et al. (1992) used the SEM/AVS ratio to discriminate toxic from nontoxic sediments. Of the 117 experiments performed, only 7% were misclassified as toxic using the theory that when the ratio was less than 1 the sediment was nontoxic and when the ratio was greater than 1 the sediment was considered toxic. When a more conservative interpretation was adopted (when the ratio is less than 1 the sediment is nontoxic), all experiments were classified correctly. Toxicity were verified with toxicity tests using two species of amphipods, a snail, and an oligochaete. All experiments were done using spiked sediments.

2.5.4 Ammonia

Ammonia is important in porewater toxicity, (Burton, 1991). It is produced by bacteria as an end product of the decomposition of organic matter, and is more typical in organic, anoxic sediments receiving nutrient input. NH$_4^+$ predominates in most waters, however, the ratio of NH$_4^+$:NH$_3$ is dependent on the pH and the temperature which may increase the levels of non-ionized ammonia (NH$_3$). Mean 48 hour and 96 hour LC$_{50}$ values for freshwater invertebrate range from 1.10 to 22.8 mg NH$_3$/L and 0.56 to 2.48 mg NH$_3$/L for fish (WHO, 1986).

Ammonia is a widely used industrial chemical, and is found in cleaning fluids and fertilizers. Urban runoff is also a source of ammonia, and varies seasonally from 0.18 mg N/L in fall to 1.4 mg N/L in spring (WHO, 1986). It is also formed during
breakdown or decomposition of organic matter, and is a component of human sewage.

2.5.5 Polynuclear Aromatic Hydrocarbons (PAH)

Polynuclear aromatic hydrocarbons (PAH) are often found in polluted sediments. They are important because of their toxicity, persistence, and potential link to carcinogenicity. PAH are formed by both natural and anthropogenic sources. The majority of PAH in the environment originate during incomplete combustion of organic matter at relatively high temperatures (e.g. 700 °C). Pyrolysis of organic material at temperatures as low as 100 - 150 °C can also lead to the production of PAH (Neff, 1985). PAH are also directly biosynthesized by bacteria, fungi and plants. Many of the PAH that are biosynthesized are not true PAH as they contain oxygen, nitrogen or sulfur substituents. Human sources include industrial processes such as pyrolysis of kerosene to form benzene and other solvents, coke production, gas production from petroleum, coal gasification, waste incineration, power and heat generation, open fires, rubber tire wear, and gasoline and diesel vehicles (Neff, 1979).

PAH can enter the aquatic environment by a number of routes, including spillage and seepage of fossil fuels, discharge of domestic and industrial waste, fallout or rainout from air, and runoff from land. Biosynthesis of PAH may occur in freshwater aquatic environments, but is likely to be restricted to anoxic sediments, and the PAH
probably would be immobile (Neff, 1985). Surface runoff and fallout from the air are the main sources of high molecular weight PAH, while petroleum spillage is the main source of total PAH.

Sediments are the ultimate sink for PAH. In the aquatic environment PAH tend to adsorb to the surface of organic and inorganic particulates. These particulates settle out and carry the PAH with them. PAH are quite persistent in sediment, and can accumulate to concentrations of 1000 times that of the overlying water, (Neff, 1985). Once deposited on the bottom, PAH are much less susceptible to photochemical, chemical, or biological degradation than they were in the water column. This hydrophobic behavior also results in the potential for PAH to accumulate in the lipids of aquatic organisms, particularly if the PAH are not readily metabolized (Kauss and Hamdy, 1991).

It is generally accepted that the low molecular weight PAH (e.g. naphthalene) are more toxic than high molecular weight PAH, probably because the low molecular weight compounds are more water soluble and more bioavailable than the high molecular weight compounds (Roberts et al, 1989).

PAH in street dust samples in Tokyo showed the predominant PAH were 3 and 4 ring structures, indicating automobile exhaust as the major source. Street dust is a source of PAH in urban runoff, which ultimately ends up in the sediments of streams, lakes or coastal waters (Takada et al., 1990).
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There appears to be an association between PAH peaks in sediment and storm events (Evans et al, 1990). There is a time lag of four to thirty days between storm events and PAH incorporation into sediments. This time-lag is governed by many factors, but most importantly the sites with the shortest time lag are adjacent to point sources of PAH input. Other factors include the site history, river hydrodynamics, and relation of storm peak to coincident river flow. During heavy storm events, PAH are quickly washed into rivers associated with particulate matter, and rapidly become associated with river sediments (Evans et al., 1990).

Metabolism of PAH is required as a prerequisite for PAH-induced carcinogenesis and mutagenesis (Neff, 1985). This is done via the mixed-function oxygenase (MFO) enzyme system, which is common in crustaceans, fish and molluscs. Among invertebrates, MFO is restricted to some species of Arthropoda and Annelida.

PAH have been found in the tissues of a wide variety of organisms (Neff, 1985). This indicates an ability to accumulate PAH from the water or sediment. The extent of accumulation of PAH in different species depends on the structure of the hydrocarbon, the feeding strategy of the organism, and their capacity to metabolize PAH. Bivalve molluscs have low ability to metabolize PAH and tend to accumulate PAH from the water column and retain them in their tissues. *Daphnia pulex* accumulate and release PAH metabolites rapidly, due to their greater PAH-metabolizing ability (Neff, 1985). In Daphnids, the bioaccumulation potential of
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PAH is increased by a factor of 10 with each additional ring on the PAH structure (Neff, 1985). Elimination of all PAH by Daphnids is rapid, however, with half-lives ranging from 0.4 to 5 hours. Most aquatic species degrade and excrete PAH rapidly. Even species which lack the ability to metabolize PAH can release PAH rapidly. Thus, food chain biomagnification of PAH occurs to a limited extent (Neff, 1985).

Amphipods accumulate higher concentrations of PAH than clams, probably due to different feeding mechanisms (Varanasl et al., 1985). Other bottom feeders have also been shown to concentrate PAH. Tubifex worms can concentrate levels six times that of the sediment; carp can concentrate PAH twenty times sediment levels (Morton, 1983). Bioavailability of PAH is significantly reduced when DOC levels are increased, as seen with freshwater amphipods, which show substantial reduction in accumulation of benzo(a)pyrene (BaP) when DOC is present. BaP is highly hydrophobic. For less hydrophobic compounds such as phenanthrene, the uptake is not significantly reduced in the presence of DOC (Di Toro et al., 1991). Varanasl et al. (1985) showed that only a fraction of BaP in sediment is bioavailable, the rest being too tightly bound to sediment particles for significant uptake in organisms.

Only PAH with a molecular weight range from naphthalene (MW 128) to fluoranthene and pyrene (MW 202) are acutely toxic to aquatic organisms, as the solubility falls below the concentration required to elicit a response (Neff, 1985).
Acute toxicity does not reflect the potential impact of chronic low level PAH contamination. Chronic effects include abnormal sea urchin egg cleavage, decreased copepod egg production, altered fish feeding behavior and interference of cell membrane function, and PAH induced cancer (Neff, 1985).

2.5.6 Bioturbation

Bioturbation is described as ‘the stirring or movement of sediments by the activity of benthic organisms’ and is a major factor in contaminant availability. Bioturbation by benthic organisms can occur in different ways (Reynoldson, 1987):

- pumping of pore water into overlying water
- introducing water into the sediment
- depositing faecal pellets
- disruption of horizontal and vertical stratum.

Bioturbation can increase aeration and can affect Eh and pH gradients, which can in turn affect partitioning of contaminants in the sediment and perhaps result in release of a contaminant to the water column or to the pore water.

Bioturbation may or may not cause sediments to be resuspended. Sediment ‘working’ has been reported to loosen the upper layers of estuarine sediment which were then readily resuspended by currents (Campbell et al., 1988). Bivalves and polychaetes pellet sediments, which results in decreased stability of the sediments
which are more easily resuspended. However, activities such as tube-building tends to stabilize the sediments, decreasing resuspension.

Chironomids have been found to reduce ammonia levels in the top 5-10 cm. Tubificids and chironomids increase sediment transport of electron acceptors such as O\textsubscript{2}, NO\textsubscript{3}-, and SO\textsubscript{4}-2 (Burton, 1991).

Experiments using microcosms have shown that sediments reworked by tubificid worms cause the upward movement of pollutants through the sediments at a rate of 0.3 - 1.0 mm per day (Campbell et al., 1988). Although organic compounds were moved to the surface sediments from depths of up to 10 cm, only a small proportion of these compounds was shown to be released into the water column. Chironomid larvae have been shown to increase the movement of iron and manganese from lake sediments to the water column.

2.6 EXAMPLES OF SEDIMENT TOXICITY TESTS

Toxicity is expressed and measured at the organism level (Harris et al., 1990), but will affect the ecosystem at a community or population level. The validity of any test will depend on its relevance to the \textit{in situ} conditions and how well data can be extrapolated to represent the \textit{in situ} conditions. Most toxicity tests are performed using a single species in a test container. Since ecosystems are complex and interactions exist between species and between individuals of a species, it is perhaps advantageous to use more than one species together in one test container.
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In the late 1970s, Anderson and Prater (1980) reported a 96 hr sediment bioassay system that used three organisms - *Hexagenia limbata*, *Pimephales promelas* and *Daphnia*. The equipment took up substantial space, although it was relatively inexpensive. It was also a flow through system which required large volumes of water. They had 'erratic' results with *Daphnia*, and *Pimephales* was not sensitive at all to the sediments tested.

There appears to be a lack of correlation between laboratory test results and field responses. Results obtained from *in situ* testing may not be accurately predicted by the testing of sediments in the laboratory (Sasson-Brickson and Burton, 1991). An attempt was made to develop an *in situ* sediment exposure unit that would allow for testing in the field, thus eliminating the need to remove sediment from the ecosystem and do testing in an artificial laboratory situation (Sasson-Brickson and Burton, 1991). *Ceriodaphnia dubia* were used in an acute test, and the results of the *in situ* test were compared to laboratory tests. The sediment samples used for the laboratory experiments were grab samples combined and mixed. Higher mortality was found in the *in situ* tests as compared to the laboratory tests showing that traditional sample collection and laboratory toxicity test methods may alter the water and sediment toxicity that occurs *in situ*. While it is true that removal of sediment from the site interferes with the sample integrity, *in situ* studies are often impractical, especially in remote sites if personnel must be sent to perform the test. They are also subject to vandalism, and there appears to be no means of standardizing for
environmental factors such as storm events which can radically alter the chemistry of a stream for short periods. What is needed is a sediment toxicity test that minimizes alterations to the sample integrity, but allows the testing to be done in the laboratory so conditions can be standardized, and chronic endpoints such as total number of young can be examined.

*Daphnia magna*, a common water flea, inhabit the water column, yet they spend some time feeding on bottom sediments. They are widely used in laboratory tests, and a standard protocol exists for culturing and testing these organisms (EPS, 1990). They are easy to culture, and are a relatively sensitive species to contaminants. *Ceriodaphnia dubia* is closely related to *Daphnia*, and has also been used extensively. However, they seem to be more difficult to culture, and intermittently show high mortality or low reproduction in culture, making evaluation of test results difficult (DeGraeve and Cooney, 1987; Cooney et al., 1992; Patterson et al., 1992). Eleven laboratories in the United States were involved in an inter laboratory study using *C. dubia* (DeGraeve et al., 1992). Only 56% of the tests were valid because laboratories were unable to initiate tests successfully, or because control survival rate was <80%. *Ceriodaphnia dubia* are also very small, and the young are difficult to see and count. These cladocerans are not recommended for sediment bioassays (Bennett and Cubbage, 1992c). They are applicable where contaminants are redispersed into the water column. Initial attempts to use *C. dubia* in bulk sediment
assays by Ankley et al. (1991a) proved unsuccessful, as organism recovery from test and control sediments was variable.

Bacteria have been used as an assay organism. The Microtox® assay is the most common assay used. It is generally equal or more sensitive to metals than plant or animal cells. *Photobacterium phosphoreum*, the bacteria used in the Microtox® assay, have been found to be particularly sensitive to copper, but less sensitive than *Daphnia* to mercury and cadmium (Giesy and Hoke, 1989).

Amphipods are also used frequently as assay organisms. *Rhepoxynius abronius* and *Hyalella azteca* are both used routinely. Amphipods have proved difficult to culture in the laboratory. Borgmann et al. (1989) have successfully cultured *Hyalella azteca*, but they noted a constant mortality in the cultures that was thought not due to cannibalism. If the culture conditions produce a high mortality rate, the reliability of test results must be questioned. Amphipods also have a relatively long life cycle, and are not recommended for inclusion in a battery of screening tests for these reasons (Giesy and Hoke, 1989). For testing chronic toxicity in amphipods, four to six weeks is required, compared to less than three weeks for *Daphnia magna*, (Borgmann et al., 1989). Toxicity to *Hyalella* does not correlate with metal, PAH or ammonia concentrations (Borgmann and Norwood, 1993).

Mayfly nymphs, *Hexagenia limbata*, are burrowing insects. They bury themselves in sediment and thus would be appropriate as a sediment bioassay organism.
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*Hexagenia* cannot be raised well in the laboratory (Bennett and Cubbage, 1992c), and reproduce only in May and June. They are very sensitive to toxic chemicals in sediments (Giesy and Hoke, 1989), but because of culturing and reproduction limitations, they are not recommended for inclusion in a battery of tests.

Bennett and Cubbage (1992c) evaluated a number of toxicity tests on the basis of reliability, ecological relevance, cost per analysis, ease, testing time and problems encountered. *Hyalella azteca*, Microtox® and *Hexagenia* (although only a seasonal assay) were recommended as sediment toxicity tests. These three species showed the greatest reliability in demonstrating the toxic effects of sediments where metals and PAH were the main contaminants. Most assays tested by Bennett and Cubbage (1992c) were based on acute endpoints.

Dutka et al. (1989) evaluated the toxicity of Fraser River sediments with a battery of screening tests, including Microtox®, SOS genotoxicity test, ATP-TOX test, and a 48h *Daphnia magna* test. These tests were performed on sediment elutriates, and *Daphnia magna* proved to be the most sensitive for indicating the presence of contaminants with toxicant activity.

Giesy and Hoke (1989, 1991) ranked a number of available sediment tests on the basis of rapidity, simplicity, replicability, cost, sensitivity, and ecological relevance. The assays recommended for inclusion in a battery of tests were Microtox®, an algal assay, a *Chironomus tentans* 10-day test, and a *Daphnia magna* 48 hr test.
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The Washington State Department of Ecology have set standards for sediment quality (Washington State, 1991). Sediments should be tested using two acute tests and a chronic test. The Ontario Ministry of the Environment is currently drafting sediment toxicity testing methods. Test organisms include *Pimephales, Hexagenia* and *Chironomus*.

2.7 RATIONALE FOR SAMPLING TECHNIQUES CHOSEN

The ecological significance of toxicity tests has often been questioned. As soon as sediment is removed from the site, it is altered, and any results obtained in the laboratory may not represent what actually occurs in the environment, (Sasson-Brickson and Burton 1991). However, it is usually impractical or impossible to do any tests *in situ*, making laboratory testing more practical and economic.

Numerous studies fail to recognize that there are many chemical, physical and biological processes involved in sediment toxicity, and that sediments are dynamic, changing spatially on scales of microns to millimeters and temporally on scales of minutes to months. With this in mind, Burton (1991) has questioned whether the eradication of sediment integrity, when grab sampling, allows for data that can be extrapolated to *in situ* conditions. It is impossible to sample sediment without some degree of disruption and alteration of the integrity of the sample. Extrapolation of results to *in situ* conditions becomes more difficult as the degree of sample disruption increases. Dredge sampling can result in the loss of fine particles, water soluble
compounds and volatiles. They are limited to fine-grained sediments, as are core samplers (Burton 1991). Core samplers can allow for the stratification of sediments to remain intact.

The upper few centimeters of sediment is the active part of the ecosystem. Deeper sediments are more unchanging and more inert, but may be re-exposed to the active ecosystem through dredging, intense storms, or hydrogeological events, (Giesy and Hoke, 1989). Sampling method can therefore have drastic effects on sediment integrity, disrupting physiochemical and biological gradients. Sampling may affect contaminant partitioning, complexation, speciation, and bioavailability, and thus affect sediment toxicity. It is thus necessary to collect and process sediments in a manner that is least disruptive to sediment integrity (Burton, 1991).

Burgess and Scott (1992) raised the following questions regarding the design of toxicity tests:

* Do standard sediment sampling and holding procedures significantly alter critical sediment parameters, i.e. stratigraphy, porosity and chemical reactivity?

* Should only intact sediment cores be used in tests without homogenization?
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- Should sediments be manipulated in terms of sampling and storage if those actions result in the mortality of bioturbating organisms and chemically active bacteria?

In this study, two methods of sediment sampling were compared. The commonly used method of grab samples which are pooled and homogenized was compared to a small hand cored sample with which the sample integrity has been maintained as much as possible. Sediments used in the Daphnia and chironomid toxicity tests were not stored, and the Microtox® tests were performed within one week of sample collection.

2.8 RATIONALE FOR TOXICITY TESTS CHOSEN

As discussed, there is no one optimal assay to determine all facets of sediment toxicity. A battery of tests is more suitable. For example, acute tests may be appropriate for some situations where contaminants are found at high levels. Chronic tests will be more appropriate in situations where contaminants are found in lower amounts, and may not cause acute responses. Every test site is unique, and the choice of toxicity tests must reflect this. After a thorough search of available literature it was decided to use the following tests (justification for the selection follows):

- *Daphnia magna* 48 hr acute toxicity test on pore water
- Microtox® basic test on pore water
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- Microtox® solid phase test on whole sediment
- *Daphnia magna* 3-brood (14 day) chronic toxicity test
- *Chironomus tentans* 14-day chronic toxicity test

To maintain the integrity of the sample as much as possible, undisturbed sediment cores were used in testing sediment toxicity, and the toxicity results compared to homogenized or mixed grab samples. Samples were not sieved to remove indigenous organisms as this would alter sediment integrity too much. Also, the presence of indigenous organisms would allow for a more natural situation where bioturbation and species interactions could freely occur.

It was also decided that more than one organism should be tested in the same container. This not only saved space, but allowed for any effects of bioturbation to be augmented. Malueg et al. (1983) tested *Daphnia* and *Hexagenia* together and separately with sediments. It was concluded that the presence of the *Hexagenia* in the sediment intensified the response of *Daphnia magna* since the *Daphnia* are sensitive to dissolved materials and to particulates released from the sediments by *Hexagenia* activity. Nebeker et al. (1984b) exposed *Daphnia* and chironomids to sediment in the same test vessel. The *Daphnia* test was a 48 hr test, and the *Chironomus* test was 10 days in duration. For the present investigation, the chironomid and *Daphnia* chronic tests were conducted in the same vessel, and were...
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used to compare the toxicity of the two sampling techniques. The Microtox® solid phase test and regular test were done to compare toxicity of whole sediment to pore water.

*Daphnia* were chosen because they are a commonly used test species, and standard methods for their use in such tests has been developed (EPS, 1990). Toxicants leaching from the sediment into the water column would be available for the *Daphnia* living in the water column. Rather than looking at survival alone as an indicator, number of young per brood and total number of young were used as more subtle detectors of toxicity. *Daphnia* are easier to culture than *Ceriodaphnia*, which are reported as at least as sensitive as *Daphnia*. *Daphnia* are also larger, and the young are easier to count than *Ceriodaphnia* (DeGraeve and Cooney, 1987).

*Chironomus tentans* were chosen as a sediment dwelling organism as they are easy to culture (Bedard et al., 1991), are relatively sensitive to sediment bound toxicants, and are in direct contact with the sediment. The larval stages graze on detritus and also filter food particles from the overlying water. Survival and growth were the end points used. Giesy and Hoke (1991) suggest the use of a chironomid growth reduction assay rather than a simple test of survival as it 'increases the discriminatory power of an assay'. *Chironomids* have been observed to show deformities in mouth parts due to contaminants in the sediment (Reynoldson, 1987). They have been shown to be sensitive to metal contaminated sediments (Wentsel et
CHAPTER 2 LITERATURE REVIEW AND TESTING RATIONALE

They are also important in the cycling of residues into and from sediments due to bioturbation (Giesy and Hoke, 1989). The second instar larvae were selected as they are the most sensitive life stage (Nebeker et al., 1984a) and are easier to find in the sediment than younger stages due to their bright red colour. They are also very easy to find when surviving animals are screened from sediments at the end of a test. A 14-day test has been used by other researchers (Mosher et al, 1982; Wentsel et al 1977; Othoudt et al, 1991). A ten day test was used by Rosiu et al (1989) and a 15 day test Nebeker et al (1984b).

Microtox® was chosen as it is a simple and fast test. The test is based on the luminescent bacterium *Photobacterium phosphoreum*. The bacteria produce luminescence via luciferase, which reduces flavin (a long chain aldehyde) and oxygen to produce light (Chang et al., 1981). The bacterial luminescent path is a branch of the electron transport chain, and the flow of electrons in this chain can be used as an indicator of the metabolic ‘state’ of the cell. Thus, in the presence of a contaminant, luminescence is expected to decline in direct proportion to the toxicant concentration. A commercially available test system has been developed by Microbics Corporation and is called Microtox®. The bacteria are available in lyophilized form from Beckman Inc., and stored at -20 °C. The Microtox® assay has been shown to be as sensitive or more sensitive than acute lethality tests with rainbow trout, fathead minnows and *Daphnia* when exposed to pure organic compounds, but less sensitive to inorganic compounds (Munkittrick et al., 1991). In
CHAPTER 2 LITERATURE REVIEW AND TESTING RATIONALE

Effluent testing, Microtox® seems to be less sensitive as the toxicity of the effluent increases. As mentioned, it has been included in batteries of tests (Dutka and Kwan, 1989; Dutka et al, 1989; Giesy and Hoke, 1989).

Microtox® was developed as an effluent or water phase test. It has been used in testing sediment pore water, and sediment elutriates. Because pore water contains only water-soluble contaminants their effects on the Microtox® bacteria give estimates of toxicity of water-soluble contaminants only. The basic test cannot be used on the sediment, because the sediment particles will absorb the light given off by the bacteria. However, a method has been recently developed where bacteria are placed directly in contact with the sediment, and after 15 minutes incubation, the mixture is centrifuged, and the activity of the bacteria in the supernatant is determined (Brouwer et al., 1990). The advantages of this test are that the bacteria are in direct contact with the sediment, which would expose them to sediment bound toxicants. Disadvantages include the choice of a reference sediment, which should have similar sediment properties such as sediment grain size and organic content, and also there is probably some element of stress put on the bacteria during the centrifugation step which may effect results.

Microbics Corporation have developed a modification of the Microtox® assay that also exposes the bacteria directly to the sediment, but uses a filter system that is not toxic to the bacteria themselves. This eliminates the centrifugation step, and
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eliminates the need for radioactively labeled bacteria. The new Solid Phase Test (SPT) shows good correlation with a benthic bioassay (organism not given) when tested on marine sediments (Tung et al., 1991). Microtox® is a convenient way to compare the toxicity of the pore water and the solid phase through the two types of tests available. Using the solid phase sediment assay is more ecologically relevant than a test of a sediment extract or elutriate because there is less manipulation and chemical/physical alteration of the sediment. Microtox® SPT detects soluble/insoluble, organic/inorganic contaminants. The bacteria are exposed to particle-bound contaminants. The solid phase test and the pore water basic test complement each other in their prediction of sediment toxicity. Tay (1992) were able to show the Microtox® SPT was more sensitive than the basic test on pore water. Giesy and Hoke (1991) have recommended *C. tentans, D. magna*, and *P. phosphoreum* for use in sediment toxicity bioassays.

2.9 SUMMARY

Toxicity is expressed and measured at the organism level (Harris et al., 1990), but affects the ecosystem at a community or population level. The validity of any battery of tests will depend on the relevance of the results to the *in situ* conditions and how well the data can be extrapolated to represent the *in situ* conditions. The choice of organisms used in toxicity testing is important, as not all organisms are sensitive to all contaminants.
Sediments act as the ultimate sinks for contaminants, and can act as a source for contamination. Tests to determine toxicity of sediments should incorporate at least one sediment dwelling organism. In this case, *Chironomus tentans* was selected. Chironomids are easily cultured (Bedard et al., 1991), and have been used in sediment toxicity testing by others. (A 14-day test was used by Mosher et al., 1982; Wentzel et al., 1977, and Othoudt et al., 1991. A ten day test was used by Rosiu et al. (1989) and a 15 day test Nebeker et al. (1984b)). Chironomids are also important in the cycling of residues into and from sediments due to bioturbation (Giesy and Hoke, 1989), which make them ideal for use in a test system where more than one species is utilized.

*Daphnia magna* were also selected for use. They are a commonly used test species, and standard methods for their use have been developed (EPS, 1990). Contamination released from sediments by chironomid activity would be available for the Daphnids living in the water column. Standard methods also exist for Daphnia magna 48 hr toxicity tests, (EPS, 1990). This test was used to determine the toxicity of the sediment pore water.

Microtox® is a bacterial toxicity test. It was selected because it is simple and fast. The solid phase test determines the toxicity of the solid phase directly, and the basic test determines toxicity of liquid samples,(pore water in this case). The Microtox® solid phase test was compared to the other solid phase sediment tests to determine
its relative sensitivity. The Microtox® basic test was compared with the Daphnia 48 hr pore water toxicity test.

SEM/AVS determination has been reported as an indicator of sediment toxicity (Di Toro et al., 1980). This test is used to predict if metal contaminated sediments are toxic to organisms based on the molar ratio of simultaneously extracted metals (SEM) and acid volatile sulfide (AVS). This analytical method was selected in order to compare the results obtained from the toxicity tests to the SEM/AVS ratios.

The tests selected for this research allowed for comparisons to be made between tests and between sampling method. They allowed for identification of toxic and non-toxic sediments, and any differences in sediment toxicity between sampling methods to be determined.
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3.1 TEST SITE AND REFERENCE SITE.

Two urban creeks were used in this study to determine if the method of sediment sampling influences sediment toxicity. Still Creek and Musqueam Creek were chosen and are shown in Figure 3.1.

![Map of Greater Vancouver](image)

Figure 3.1  Map of Greater Vancouver, showing the study site (Still Creek) and the reference site (Musqueam Creek)

Still Creek in Burnaby, B.C. was chosen as the study site (Figure 3.2). Still Creek is the largest tributary of the Brunette River watershed. It flows into Burnaby Lake, which drains to the Brunette River and ultimately to the Fraser River at New Westminster. Its upper reaches are in predominantly residential areas and, as it flows towards Burnaby Lake, it enters industrial areas and flows beside major
vehicular routes such as Lougheed Highway, Trans-Canada Highway, and Boundary Road.

![Map of the Brunnette watershed showing sites sampled on Still Creek.](image)

Figure 3.2 Map of the Brunnette watershed showing sites sampled on Still Creek. For a description of the sampling sites, see Table 3.1.

Flow characteristics are important in the transportation and deposition of particulates. The upper reaches of the Brunnette watershed have slopes of up to 10%, while the lower reaches are generally flat, with slopes of <5%. As a result, the upper reaches have a higher flow velocity than the slow flowing lower reaches. In particular, the
region of the creek around Willingdon and Douglas Avenues has very low velocities, especially during low rainfall periods.

This area of Still Creek is influenced by backwater effects from Burnaby Lake which also controls flows. The creek has been classified as a storm sewer by the Greater Vancouver Regional District (Bindra and Hall, 1977), and parts of the creek are completely culverted. The Creek is still culverted in many areas, and the water levels in the Creek are greatly affected by storm events.

The area of the Brunette watershed is 6060 ha, which is divided into four predominant landuses (Hall and Anderson, 1988), namely 42% residential, 31% open space and forested, 15% commercial and institutional, and 5.5% industrial. Industrial areas are concentrated along the middle reach of Still Creek and the north side of Burnaby Lake.

The Brunette River basin has been studied since the early 1970's (Hall et al. 1976; Bindra and Hall, 1977). In 1976, sediment trace metal concentrations were evaluated in the Brunette basin on sediments collected in 1973. It was found that toxic materials (metals and chlorinated hydrocarbons, such as DDT) had accumulated to levels found in other river basins with a much longer history of industrialization/urbanization. Hall et al. (1976) found that street surface materials were a major source for trace metal contamination, especially lead and zinc. Land use was also a major factor in determining contaminant levels. Highest levels of
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PCBs occurred in commercial/industrial areas and the highest average concentration of DDT occurred in residential areas. It was also found that where chlorinated hydrocarbons were present, concentrations were higher in stream sediments than in surface material, indicating that sediments acted as a sink for these contaminants.

A more recent study (Duynstee, 1990) looked at changes in sediment metal levels. Sediments collected in 1989, were compared to sediments analyzed in 1973 (Hall et al. 1976). Sediments were analyzed for cadmium, chromium, copper, iron, nickel, manganese, lead and zinc. Land use and traffic volume information was also gathered for both the sampling periods, and analyzed for correlations. There was an overall decrease in lead and chromium levels in the watershed sediments, related in part to the use of unleaded gasolines. Levels of copper, manganese and zinc in sediments were found to have increased beyond world averages.

Musqueam Creek was chosen as the reference creek (Figure 3.3). This creek runs through Pacific Spirit Park, under SW Marine Drive and through the Musqueam Indian Reservation. It then passes through a golf course before reaching the Fraser River estuary. The site chosen to sample was upstream of the golf course, but downstream of Marine Drive. This is because upper reaches often dry up in summer months, and would be unsuitable for sampling. Lower reaches are affected by runoff from the golf course which may contain pollutants such as fertilizers and pesticides. It is one of the only streams in Vancouver that contains a viable fish population.
Research by Bindra and Hall (1977) showed elevated levels of metals in the upper reaches of Still Creek, and thus there was no suitable upstream reference site.

Figure 3.3 Map of Musqueam Creek, the reference site.

3.2 SAMPLING METHOD

Five sites were sampled on Still Creek (Figure 3.2). Musqueam Creek was sampled at the same general location (Figure 3.3) each time, but sometimes upstream and sometimes downstream of Crown Street, depending on the flow in the Creek. Site descriptions are summarized in Table 3.1.
Table 3.1 Description of sampling sites where sediments were collected for toxicity testing.

<table>
<thead>
<tr>
<th>SITE NUMBER</th>
<th>SITE LOCATION</th>
<th>DESCRIPTION</th>
<th>SAMPLE DATE</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>Still Creek at Willingdon</td>
<td>Creek runs under Willingdon Street, in an industrial area. Trans Canada highway to South</td>
<td>July 4, 1992</td>
<td>49 15' 28&quot; N, 123 00' 11&quot; W</td>
</tr>
<tr>
<td>2S</td>
<td>Still Creek at Douglas</td>
<td>Most downstream location sampled. Industrial area.</td>
<td>August 2, 1992</td>
<td>49 15' 35&quot; N, 122 58' 59&quot; W</td>
</tr>
<tr>
<td>3S</td>
<td>Still Creek at Atlin</td>
<td>Most upstream location sampled. Highly residential area, some sewage leakage into creek.</td>
<td>August 24, 1992</td>
<td>49 14' 31 N, 123 02' 36&quot; W</td>
</tr>
<tr>
<td>4S</td>
<td>Still Creek at Lougheed</td>
<td>Creek runs under Lougheed Highway, through peat bog, industrial area.</td>
<td>October 14, 1992</td>
<td>49 15' 58&quot; N, 123 01' 05&quot; W</td>
</tr>
<tr>
<td>5S</td>
<td>Still Creek at Westburne</td>
<td>Small tributary of Still Creek, sampled near where it joins main branch. Runs under main railway tracks, industrial area.</td>
<td>December 4, 1992</td>
<td>49 15' 53&quot; N, 122 58' 60&quot; W</td>
</tr>
<tr>
<td>1-5M</td>
<td>Musqueam Creek (Reference Site)</td>
<td>Reference site. Small creek running through Pacific Spirit Park. Sampled just upstream of the Musqueam Indian Reserve and golf course, and downstream of Marine Drive.</td>
<td>Sampled at all dates above.</td>
<td>49 14' 45&quot; N, 123 11' 30&quot; W</td>
</tr>
</tbody>
</table>
CHAPTER 3 METHODOLOGY

Two types of sampling methods were used to determine if the sampling method affected toxicity. The first will be referred to as ‘cores’. A small glass corer of dimensions 50 mm ID X 130 mm was used to take a shallow sediment core. At each site, 10 cores were taken, starting at the most downstream location and working upstream in order to minimize disturbance of the sediments. The second method used was a ‘mixed’ sample. At each site, enough sediment for 10 mixed samples was taken at the same location as the core. The farthest downstream sample was taken first. Four cores were taken, and randomly one was saved as a core, and the others were put in a bucket. This was done at all 10 locations.

The 10 individual cores to be kept as cores were put aside. The bucket of sediment was thoroughly mixed, and enough sediment removed for the mixed samples. This portion was put into plastic bags for transportation to the laboratory. The sampling was done by the same person each time to keep the methodology consistent. The rest was also aliquoted into bags and labeled appropriately for the chemical and physical analyses. Bagged samples were stored in the dark in a cooler. The cores were kept as undisturbed as possible in holders which minimized any motion. On the same day as a site on Still Creek was sampled, a site on Musqueam Creek was sampled as the reference sediment. The same procedure was followed. This procedure resulted in 10 core samples and enough sediment for 10 mixed samples at each site.
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The sediment core sampling method can be done with very little disturbance of the sediment. Distinct layers can be seen in samples, and algae growth on the surface is even maintained if present. This method eliminates the need for homogenizing the sample, and eliminates the uncertainty that each sample is equally homogenized (Ditsworth et al. 1990). However, there are limitations to the hand core sampling technique. The sediments should not be rocky or pebbly, as the glass corer can break. Also, the water cannot be too deep, as samples are taken by hand. Musqueam Creek is quite shallow, and the depth was never greater than one foot. Each site at Still Creek varied in depth. The water levels were deepest at site 2S (two to three feet deep), which proved the most difficult site to sample. The rest varied from 6 inches to 2 feet, and were relatively easy to sample.

Samples collected for SEM/AVS analysis were collected in an acid washed glass jar. As the sediment sample was being removed (discrete sample), a stream of nitrogen was blown into the jar. As little air space as possible was left at the top of the jar, and nitrogen was blown in when this was not achieved.

Since Still Creek is in an urban watershed, its water levels and flows are greatly affected by storm events. For this reason, sampling was done at least three days after any precipitation was measured. Evans et al. (1990) studied the effects of storm events on sediment PAH levels. They showed the time lag between the storm event and the incorporation of PAH into the sediments varied between 4 and 30 days.
Coincident flows are reached quickly in Still Creek. Preliminary observations indicated flow returned to 'normal' within two days after a storm event. Thus, it was decided to sample after at least three dry days (after a storm event) in order for flows to subside. Flows in Musqueam Creek were less affected by storm events because runoff is reduced in treed areas as opposed to paved, urban areas.

All sediment to be used in the *Daphnia* and *Chironomid* 10 day tests was placed in the 22 °C incubator upon arrival at the laboratory. This was done to bring the sediment to the temperature used for the toxicity tests so that the *Daphnia* and *Chironomids* were not temperature shocked. All sediment for chemical analyses was held at 4 °C until analyzed. SEM/AVS samples were analyzed immediately. While the SEM/AVS is running, sediments for the acute toxicity test with *Daphnia* were centrifuged to extract pore water in order to avoid effects of storage on the pore water chemistry. Samples for metal analyses and PAH analysis had additional preparation steps before they could be analyzed (Section 3.3.1 and 3.3.2). Samples for Microtox® solid phase and basic test were prepared prior to running the test, and were stored at 4 °C until this time.

Core samples were placed in the 22 °C incubator and aerated. Mixed samples were prepared by re-homogenizing the sediment in the plastic bags, and aliquoting it into ten 300 ml beakers. Culture water (as described in Section 3.5) was placed over the
sediment and the sediment was allowed to equilibrate overnight in the 22 °C incubator. All samples were aerated carefully to avoid any mixing of the sediments.

Sediment sampling was done in the morning, usually between 9 am and 1 pm. Bioassays were set up, SEM/AVS analyzed in all samples, and pore water extracted by 9 pm, within 12 hours of sample collection.

3.3 ANALYTICAL METHODS

Analytical methods are summarized in Table 3.2, and described in detail below.

3.3.1 PAH Analysis

PAH analysis was done based on the methods used by Hall et al. (unpublished) and Ingersoll and Nelson (1990), with some modifications. All glassware was rinsed in 1:1 acetone:hexane solvent prior to use.

A portion of sediment was collected in a clean glass jar at each study site on the same day as sampling was done. This portion of sediment was air dried in the laboratory for one week. Replicate 10 g samples of sediment from each study site were mixed with 5 g of anhydrous sodium sulfate (Na₂SO₄). The samples were transferred to clean 125 ml glass bottles, and 75 ml of solvent (1:1 acetone : hexane) was added. The samples were then extracted for one hour on a wrist action shaker.

The solvent was subsequently decanted into an Erlenmeyer flask. At this point, the solvent extracts from the same sample were combined. Earlier experiments had
Table 3.2 Summary of analytical methods used in sediment toxicity testing of Still Creek and the reference site, Musqueam Creek.

<table>
<thead>
<tr>
<th>ANALYSIS</th>
<th>METHOD</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYSICAL ANALYSES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent Dry Weight</td>
<td>Dry at 103 C</td>
<td>APHA, 1989</td>
</tr>
<tr>
<td>Percent Organic Matter</td>
<td>Fire at 550 C</td>
<td>APHA, 1989</td>
</tr>
<tr>
<td>Particle Size Analysis</td>
<td>Dry Sieving</td>
<td>Ken Hall, personal communication</td>
</tr>
<tr>
<td>CHEMICAL ANALYSES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVS &amp; SEM</td>
<td>Atomic absorption, (metals)</td>
<td>Allen et al., 1991</td>
</tr>
<tr>
<td></td>
<td>Colorimetric, (sulfides).</td>
<td>APHA, 1989</td>
</tr>
<tr>
<td>Ammonia</td>
<td>Colorimetric</td>
<td>APHA, 1989</td>
</tr>
<tr>
<td>Total Metals</td>
<td>ICP, Aqua regia digestion</td>
<td>Chemex Labs</td>
</tr>
<tr>
<td>&lt;63 um Metals</td>
<td>Atomic absorption, aqua regia digestion</td>
<td>Murphy et al., 1990.</td>
</tr>
<tr>
<td>TOXICITY ASSAYS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>Pore water, 48 hour acute</td>
<td>EPS, 1990</td>
</tr>
<tr>
<td><em>D. magna</em></td>
<td>Solid phase, 14 day chronic</td>
<td>Gersich and Milazzo, 1990</td>
</tr>
<tr>
<td><em>C. tentans</em></td>
<td>Solid phase, 14 day chronic</td>
<td>Mosher et al., 1982.</td>
</tr>
<tr>
<td>Microtox Basic Test</td>
<td>Pore water, standard assay</td>
<td>Microbics Inc., 1989</td>
</tr>
<tr>
<td>Microtox SPT</td>
<td>Solid Phase test</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 3 METHODOLOGY

shown that this was required to increase the concentration of the PAH in the samples. Another 75 ml was added to each bottle, and shaken for another hour. The solvent fraction was added to the appropriate Erlenmeyer flasks. The sample was then filtered through a #2 Whatman filter paper containing about 5 g Na$_2$SO$_4$. There was 300 ml solvent extract from the sediment samples.

The solvent was evaporated down to about 5 ml on a rotary evaporator (Model #?), transferred to a glass conical centrifuge tube and blown down with nitrogen to 1 ml. Samples were then transferred to 10 ml in cyclohexane. Sample extracts were subsequently run through a silica gel column to separate PAHs from other contaminants before samples were analyzed on a gas chromatograph (GC).

The silica gel column was prepared as follows:

Glass wool was placed at the bottom of a glass column and washed twice with methylene chloride. A slurry of 10 g silica gel + 40 ml methylene chloride was prepared and pored into a glass column of ID 1 cm with capacity to hold this volume. Once settled, a layer of 1-2 cm anhydrous sodium sulfate was added to the column. The valve was then opened, and the methylene chloride allowed to elute from the column until it just covered the Na$_2$SO$_4$ layer. Forty ml of pentane was added, and allowed to elute to the Na$_2$SO$_4$ layer. The sample extract was then pipetted carefully onto the column, and the sample container rinsed with 2 ml cyclohexane and transferred to the column. The column was eluted with 25 ml pentane followed by 25
CHAPTER 3 METHODOLOGY

ml 4:6 methylene chloride: pentane. This second eluent contained the PAH, and was saved for GC analysis.

The GC used was a 5880A Hewlett Packard series gas chromatograph. The column was a DB5 fused silica capillary column. Injection volume was 1 uL. The initial column temperature was 40 °C with the temperature program increasing linearly at 5 °C/minute to a final temperature of 300 °C for 25 minutes. Even with cleanup, samples from Still Creek were very dirty, and the baseline was very noisy. No spike/recovery experiments were done. Standards of known concentrations were run to determine retention times. Detection limits were 0.05 ug PAH /g dry weight sediment.

3.3.2 Metal Analysis, < 63 um fraction

The whole sediment was initially dried at 103 °C and disaggregated with a mortar. The < 63 um fraction was separated by sieving. This fraction was then fired at 550 °C to remove organics. In a small Erlenmeyer flask, 0.5 g of sediment was weighed. Twenty five ml aqua regia (3:1 HCl:HNO3) and some glass boiling beads were added, and the sample was boiled to near dryness. Another 25 ml of aqua regia was added, and again boiled to near dryness. At this point, 10 ml of 1% HNO3 is added, and the samples were left to cool for 30 minutes. Samples were then filtered through #40 Whatman filters, and analyzed by atomic absorption (Murphy et al, 1990).
A Thermo-Jarrell Ash Video 22 Atomic absorbance spectrophotometer was used. This model uses a double beam system, and dual channels. In this type of AA system, the light source is divided into a reference beam and a sample beam, and the read out given represents a ratio of sample and reference beams. This means that any fluctuations in the intensity of the source do not result in fluctuations in the read out, resulting in a more stable baseline. Standard curves were prepared for each metal, and were in the correct range for the metal levels in the samples. The detection limits for the AA are given in Table 3.3.

### Table 3.3 Detection limits for atomic absorption.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Detection Limit (ug/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.2</td>
</tr>
<tr>
<td>Zn</td>
<td>0.1</td>
</tr>
<tr>
<td>Mn</td>
<td>0.06</td>
</tr>
<tr>
<td>Ni</td>
<td>0.28</td>
</tr>
<tr>
<td>Cd</td>
<td>0.1</td>
</tr>
<tr>
<td>Fe</td>
<td>0.05</td>
</tr>
<tr>
<td>Cr</td>
<td>0.2</td>
</tr>
<tr>
<td>Pb</td>
<td>0.2</td>
</tr>
</tbody>
</table>

3.3.3 **AVS and SEM analysis.**

The AVS and SEM analysis used methods described by Allen et al. (1991) and Di Toro et al. (1990).
CHAPTER 3 METHODOLOGY

The sample was collected by immersing an acid washed glass jar under water and scooping the sediment. The sample was purged with nitrogen while under water and not exposed to the atmosphere. Immediately upon arrival in the laboratory, the sample was extracted for AVS and SEM. A 10 g sample was rapidly weighed and added to the AVS apparatus. The apparatus used is shown in Figure 3.4. The sediment was purged with nitrogen for ten minutes. Using a syringe and a screw cap with a septum, 20 ml of 6 M HCl was added to the flask containing the sediment. This step converted the AVS in the sample to hydrogen sulfide (H$_2$S) by acidification at room temperature. The H$_2$S was purged from the sample under a stream of nitrogen and was trapped in 30 ml 0.5N NaOH. The amount of sulfide was then determined using the methylene blue colorimetric method as described in APHA (1989). The SEM constitutes the metals that were extracted from the sediment during the acidification step. These were filtered through a 0.2 um acid resistant filter (Sartorius cellulose nitrate filters, 47 mm diameter). The concentration of metals was determined by atomic absorption. Metals analyzed were lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr), and iron (Fe). For more details, please refer to Allen et al, (1991).

Each time the SEM/AVS protocol was followed, a standard solution of sulfide (S$^{-2}$) was added, and the percent recovery calculated. This was accounted for in all further calculations of AVS concentrations. The acid solution (the SEM portion) was used as
Figure 3.4 Graphic representation of the equipment used in AVS/SEM determination. (a) nitrogen cylinder; (b) gas washing bottles; (c) flow controller; (d) reaction flask; (e) & (f) sulfide traps with NaOH.

3.3.4 Total Metals

*Aqua regia* digestion and inductively coupled plasma (ICP) atomic emission spectrometry were used to determine the total metals in the sediment samples. Chemex Labs Inc. conducted the ICP analysis for 32 metals. Sediment samples were sieved to 35 mesh, and ground to approximately 150 mesh. Concentrated nitric acid was added to 1 g of sediment, and refluxed. This sample was cooled, and HCl added. The sample was refluxed again. The digestion process takes a total of 2 - 2.5
hours. The sample was then diluted with distilled water to 25 ml and analyzed. One set of standards, one blank, and one duplicate were run with the samples. The detection limits are given in Table 3.4.

Table 3.4  Detection limits for ICP analysis.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Detection Limit (ug/g dry wt.)*</th>
<th>Metal</th>
<th>Detection Limit (ug/g dry wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>0.2</td>
<td>Mg(%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Al(%)</td>
<td>0.01</td>
<td>Mn</td>
<td>5</td>
</tr>
<tr>
<td>As</td>
<td>2</td>
<td>Mo</td>
<td>1</td>
</tr>
<tr>
<td>Ba</td>
<td>10</td>
<td>Na(%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Be</td>
<td>0.5</td>
<td>Ni</td>
<td>1</td>
</tr>
<tr>
<td>Bi</td>
<td>2</td>
<td>P</td>
<td>10</td>
</tr>
<tr>
<td>Ca(%)</td>
<td>0.01</td>
<td>Pb</td>
<td>2</td>
</tr>
<tr>
<td>Cd</td>
<td>0.5</td>
<td>Sb</td>
<td>2</td>
</tr>
<tr>
<td>Co</td>
<td>1</td>
<td>Sc</td>
<td>1</td>
</tr>
<tr>
<td>Cr</td>
<td>1</td>
<td>Sr</td>
<td>1</td>
</tr>
<tr>
<td>Cu</td>
<td>1</td>
<td>Ti(%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fe(%)</td>
<td>0.01</td>
<td>U</td>
<td>10</td>
</tr>
<tr>
<td>Ga</td>
<td>10</td>
<td>V</td>
<td>1</td>
</tr>
<tr>
<td>Hg</td>
<td>1</td>
<td>W</td>
<td>10</td>
</tr>
<tr>
<td>K(%)</td>
<td>0.01</td>
<td>Zn</td>
<td>2</td>
</tr>
</tbody>
</table>

* All values in ug/g dry weight (ppm), except those with %.

3.3.5 Ammonia

Ammonia in the pore water was analyzed using an automated colourimetric analyzer (Lachat Instruments, 1990). Pore water was extracted by centrifugation of sediment samples. Since ammonia is volatile and will leave the sample slowly, samples were adjusted to pH 3-5 with sulfuric acid and stored at 4 °C until analyzed. Ammonia reacts with alkaline phenol and hypochlorite forming indophenol blue which is proportional to the ammonia concentration. Sodium nitroprusside was added to
CHAPTER 3  METHODOLOGY

intensify the colour, which was measured photometrically at 630 nm. Samples were never stored more than three days before being analyzed. The system was checked with a standard every ten samples, and recalibrated if necessary. The detection limit was 0.05 mg/L. The method used was that of Lachat Instruments, (1990).

3.4  PHYSICAL PROPERTIES

3.4.1  Percentage Dry Weight
Crucibles were dried in the 103 °C oven, cooled, and preweighed. Wet sediment was added, and the combined weight of the dish and sediment was recorded. The sediment was dried overnight at 103 °C. The crucibles were cooled and weighed. Each sample was weighed in triplicate.

3.4.2  Percentage Organic Matter
Crucibles were prefired at 550 °C, cooled and weighed. The samples that had been dried as in Section 3.4.1 were then added and the crucibles reweighed. Samples were fired for 2-3 hr at 550 °C, cooled and weighed. Each sample was weighed in triplicate.

3.4.3  Particle Size Analysis
A large portion of disaggregated sediment (usually 700 g or more) was dried in the 103 °C oven. Once dry, the sample was placed in a series of Tyler brass sieves and shaken for 30 minutes. The proportion of sediment in each sieve was weighed. The finest fraction, < 63 um, was analyzed for metals.
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Sieves fractions determined were: >2 mm, 0.5 mm (coarse sand), 0.25 mm (medium sand), 0.125 mm (fine sand), 0.063 mm (very fine sand), <0.063 mm (silt and clay).

3.5 CULTURING TEST ORGANISMS

3.5.1 *Daphnia magna*

The organisms used to start the culture was obtained from EVS Consultants, North Vancouver, B.C. The method used to maintain the culture and to obtain test organisms was that of Environment Canada, (EPS, 1990). Organisms were cultured in moderately hard culture water, hardness 80-100 mg/l as CaCO$_3$, and pH 7.4-7.8.

Organisms were fed YCT (Yeast, trout chow, and Cerophyll mixture) which was provided by EVS Consultants, and algae (*Ankistrodesmus*, *Selenastrum* and *Chlamydomonas*). The ratio was 1 ml YCT plus 9 ml algae (mixture of the above algae species) per litre of culture water. They were fed on Monday, Wednesday and Friday.

Organisms were cultured at 25 ± 1 °C, on a light cycle of 16 h light : 8 h dark. Dissolved oxygen, pH and conductivity were monitored on feeding days, prior to changing the culture water. The brood number of each culture, and the date of birth were also recorded.

3.5.2 *Chironomus tentans*

The culture was started from egg masses obtained from EVS Consultants, North Vancouver, B.C.. The culture method was a modification of methods from EVS
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Consultants, the OME draft protocol (Bedard et al. 1991), and Nebeker et al. (1984a, 1984b)

Culture water was moderately hard synthetic water, the same water used to culture *Daphnia*. Chironomids were cultured at the same temperature and light cycle as the *Daphnia* cultures.

Sediments used for the cultures were obtained from the headwaters of Musqueam Creek, and were fired at 550 °C before using.

The culture was started by preparing a 20 L aquarium tank as follows. A 1-2 cm layer of sand was placed in the tank, and culture water was added to about 10 cm depth. The tank was aerated overnight, and egg masses were added the next day. Food was added after two days, when the eggs had hatched. The chironomids were fed with Tetra Min flake fish food, which was prepared as follows: 20 g of fish food and 200 ml culture water were blended in a blender for 3 mins, aliquoted into 100 ml containers, and stored at 4 °C until used. To use, the mixture was shaken well, then 3 ml of the slurry was added to each tank.

The tanks also received *Daphnia magna* YCT food (Section 3.5.1), and algae. Approximately 10 ml was added, 1 ml YCTF to 9 ml algae.

The feeding schedule was as follows:

- Monday: 3 ml Tetra Min
• Wednesday: 3 ml Tetra Min

• Friday: 3 ml Tetra Min + 10 ml Daphnia diet

If the tanks looked too turbid and cloudy, the amount fed was reduced. If the Chironomids were on the surface of the sand between feedings, the amount fed was increased. (Chironomids do not normally come out of their burrows unless they are hungry). Dissolved oxygen, pH, and conductivity were measured on feeding days prior to the addition of food. Adults start emerging after approximately 30 days.

To obtain egg masses, adult chironomids must be collected. Up to 5 tanks were maintained as continuous cultures in order to have a constant supply of adults. The adults were collected in a large Erlenmeyer flask containing 300 ml culture water with a dissolved sugar cube (Figure 3.5). A long strip of nitex screen (mesh size # 102) was placed in the flask, and the adults collected. At least 10-15 adults were collected. The ratio of females to males varied from 2:3 to 3:4.

Egg masses appeared in 1-2 days. As soon as one appeared, it was removed and placed in a prepared culture tank. At least two egg masses were used in each test tank. The egg masses hatched in approximately 2-3 days. Feeding started at this time. Hatching was counted as day 0, and the chironomids were used for testing on day 10, at the second instar larval stage.
3.6 TOXICITY TESTS METHODOLOGY

3.6.1 Reference Toxicity Tests

A reference toxicity test is a test done with a standard chemical in order to assess the sensitivity of organisms.
For *Daphnia magna*, the reference toxicant used was ZnSO$_4$ (EPS, 1990). A ZnSO$_4$ stock solution of 100 mg/l was made by diluting ZnSO$_4$ in culture water, hardness 80-100 mg/l. The stock solution was stored at 4 °C. Concentrations used for reference toxicant testing were 0, 0.8, 1.1, 1.5, 2.0, and 2.7 mg/l. Ten Daphnids were added to 200 ml of each concentration of ZnSO$_4$, and survival recorded after 24 and 48 hr. A reference toxicant test was done every time a *Daphnia magna* 48 hr test was done on the pore water. LC$_{50}$'s were calculated each time. (See Table 3.6).

The chironomid reference toxicity test was conducted with a solution of CdCl$_2$. A stock solution of 100 mg/L was prepared with culture water and stored at 4 °C. The reference toxicity test was done simultaneous to the sediment toxicity testing. Both reference and toxicity tests were performed using chironomids cultured in the same tank and thus of the same age (i.e. ~ 10 d old). Different dilutions of CdCl$_2$ were made (0.5 mg/L, 1 mg/L, 2.5 mg/L, 5.0 mg/L, 10 mg/L), and placed into ice cube trays. One chironomid was added per compartment, and 8-10 chironomids were used for each dilution. Mortality was recorded at 24, 48, 72 and 96 hr.

3.6.2 *Daphnia magna* 48 hour Acute Toxicity Test

The pore water was extracted by centrifuging sediment at 3700 rpm (Beckman CS-6 centrifuge) for 30 minutes. The supernatant was removed and used in the 48 hr *Daphnia magna* acute test. Dilutions were made using culture water, hardness 80-100 mg/L. The following pore water concentrations were used: 100%, 50%, 25%,
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12.5%, 0%. One Daphnid was added per 10 ml volume. Beakers containing 20 ml and two Daphnids were used. There were five replicates to give 10 *Daphnia* per dilution. (N.B. If there was a shortage of *Daphnia*, less than ten *Daphnia* were used per dilution of pore water). None of the pore water samples were aerated. The 48 hr LC<sub>50</sub> was calculated as described in Appendix I.

3.6.3 *Daphnia magna* Three Brood Chronic Toxicity Test
The *Daphnia* used for this 14-day test were the same brood as that used in the 48 hr test, but because the sediment samples were left overnight to allow the mixed sample to settle, the *Daphnia* were 24 - 36 h old at the start of the 14-day test. This protocol worked well, as the tests were done at 22 °C, slowing the growth rate of the *Daphnia*. The 14-day test has been used by other researchers (Gersich and Milazzo, 1990; Adams and Heidolph, 1985). Twenty four hour old *Daphnia* have been used by Devillers et al. (1990). As mentioned the tests were done at 22 °C, with a light cycle of 16 hr light : 8 hr dark. The cores had dimensions of 50 mm ID X 130 mm. The sediment was 60 - 70 mm deep, allowing the overlying water to make up the volume. The mixed samples were in 300 ml beakers as described in section 3.2. These were left at 22 °C overnight and the tests were started the next day. Core samples and mixed samples were treated in the same way as follows. About 2/3 of the overlying water was removed without disturbing the sediment, and replaced with fresh dilution water (pH 7.4-7.8, hardness 80-100 mg/l). If fine sediments were disturbed during water removal, the removal procedure was stopped. In each test vessel, a plastic 50
CHAPTER 3 METHODOLOGY

ml Falcon brand conical centrifuge tube was placed. This tube was specially prepared. The conical end of the Falcon tube had been cut off, and was the top end of the Daphnid chamber. The screw cap had a hole bored to within a few millimeters of the edge, without destroying the thread on the screw cap. The cap was removed, a small square of nitex screen (mesh # 102) placed on the tube and the screw cap replaced over the mesh. When in the test vessels, the volume of liquid was about 30 ml. To each of these, a single Daphnid (24-36 hr old) was placed. (See Figure 3.6).

Figure 3.6 Sediment core sample with Daphnid chamber in place. A single Daphnid was placed in the chamber for the duration of the 14-day test. When the overlying water is replaced, the adult was removed, and the young left on the Nitex screen insert, which was replaced.
CHAPTER 3 METHODOLOGY

Test vessels were fed with 2 ml of YCT/algae mix, and .25 ml Tetra min chironomid food. Every second day thereafter, Daphnids were removed using a wide bore pipette and placed in a holding beaker with culture water. The modified Falcon tube was removed from the test vessel, and the nitex netting replaced. Any young produced by each daphnid were easily counted and removed from the test vessel with minimal disruption of the sediment. About 2/3 of the overlying water was replaced with fresh culture water, and the Falcon tube replaced. The same Daphnid was returned to the same test sediment, and the test container was replaced in the incubator. End points were survival, total number of young, and number of young per brood. Daphnids and chironomids were tested in the same test vessel so that any natural interactions between the species would occur, such as bioturbation by the chironomids causing a release on contaminants into the water column. This procedure also saved space and time in the toxicity testing process.

3.6.4 *Chironomus tentans* 14-day Chronic Toxicity Test

There have been attempts to standardize the sediment test methods with *C. tentans* (Ankley et al., 1993). However, these efforts have not resulted in a consensus as to the best test methods. The method used in this research was that of EVS Consultants, North Vancouver, B.C.

Test organisms were obtained by placing 2-3 egg masses in a 20 L tank with 1-2 cm of culture sediment and 10 cm culture water. The tank was aerated overnight before
CHAPTER 3  METHODOLOGY

the egg masses were placed in the tanks, then continuously aerated. The egg masses were allowed to hatch, and larvae were fed according to the same schedule as the culture tanks. They were used for testing when they were 10 ± 2 days old (second instar larvae). It was preferable to use 8 - 10 day old larvae rather than 10 -12 day old larvae to prevent the chances of pupation during later stages of the toxicity test. Larvae were retrieved from the culture tank by sieving and sifting through the sediments using small paint brushes. Five second instar larvae were added to each test vessel shortly before the Daphnia were added. As mentioned the test vessels were fed every second day with YCT/algae and Tetra min food. The endpoints measured were survival and growth (dry weight of survivors) after 14 days of exposure to the sediments.

3.6.5 Microtox®

Microtox® is a toxicity test system that uses the bioluminescent bacteria Photobacterium phosphoreum to measure toxicity. The bacteria emit light which is diminished in response to the presence of toxicants. There are two types of Microtox® tests, the basic Microtox® test for aqueous samples and the solid-phase test (SPT) for sediment or soil samples.

The basic Microtox® test exposes the luminescent bacteria to four dilutions of an aqueous sample (in this case, pore water). When exposed to a toxicant, the light emitted by the bacteria decreases in direct proportion to the amount of toxicant
present. The luminescence is measured after 5 and 15 minutes of exposure, and the EC\textsubscript{50} is calculated using Microtox\textsuperscript{®} software. The EC\textsubscript{50} is the effective concentration at which there is a 50\% reduction in the luminescence.

The Microtox\textsuperscript{®} Solid-Phase Test (SPT) permits the measurement of the effect of toxicants bound to sediment particles (Microbics Corp., 1991). The test allows the bacteria to come in direct contact with the solid particle-bound toxicants in an aqueous suspension containing both soluble and insoluble organic and inorganic compounds.

For the SPT, the sediment was centrifuged for 30 minutes at 3700 rpm in a Beckman CS-6 model centrifuge. The resulting supernatant (pore water) was removed and used as the pore water tested using the basic Microtox\textsuperscript{®} test. The sediment (solid phase) was then mixed thoroughly, and 0.4 g was weighed out. The Microtox\textsuperscript{®} reagent was reconstituted, and diluted with the Solid-Phase Diluent. Serial dilutions were made, and after 20 min, the solid phase was filtered out, and the Microtox\textsuperscript{®} bacteria were incubated for 5 and 15 minutes. At these time intervals, the amount of luminescence was measured. The data was then reduced using the Microtox\textsuperscript{®} SPT software, and the EC\textsubscript{50} was calculated.

3.7 **STATISTICAL METHODS**

Survival data for both the chironomid and *Daphnia* 14-day tests were analyzed using a 2 X 2 (fourfold) contingency table, where the null hypothesis (H\textsubscript{0}) tested was that
survival of the organisms was independent of the sampling method used. The alternate hypothesis was that survival was associated with the sampling method (Zar, 1984).

The number of young produced by *Daphnia* (total, and per brood) and the dry weight of the chironomids were analyzed using a t-test, (Zar, 1984). Sample calculations can be found in Appendix I.

LC50 values were calculated using the method outlined by EPS (1990). The method can be found in Appendix II. EC 50 values in the Microtox® tests were calculated by the Microtox® data analyzer program, supplied with the Microtox® instrument (Microbics Corp., 1989). Sample calculations of the method used by Microtox® can be found in Appendix III.

Correlation of toxicity with the results of the chemical analyses was done using regression analysis to create 'best fit' linear correlations. The goodness of fit of the data to the regression line is given by $r^2$. If the regression line was a perfect fit to the data, (i.e. there is a linear relationship) $r^2 = 1$ (Zar, 1984). Multiple regression analysis was also done in order to determine the relationships between the dependent variables and more than one independent variable, to give an $R^2$ value to measure goodness of fit.

The linear relationship between variables was also calculated using Spearman's Rank Correlation (Zar, 1984). The rank correlation ranges between -1 and +1 where
-1 and +1 indicate a perfect linear relationship between the ranks of the two variables. All statistical analyses were done by computer using SPSS®, Release 6.0.
CHAPTER 4 RESULTS AND DISCUSSION

4.1 INTRODUCTION

Still Creek sediments varied greatly in their physical and chemical characteristics at each site sampled. The reference site (Musqueam Creek) was sampled at one location, but sediment characteristics varied over the course of the sampling period. The physical and chemical data showing spatial and temporal variability at each site are summarized in Section 4.2.

This thesis set out to answer three questions, as outlined in Section 2.1. The first of these questions was whether the sediment sampling method affected the toxicity of sediments to *Daphnia magna* and *Chironomus tentans* in 14 day tests. The results are presented in Section 4.3. Also presented below is a comparison of the toxicities of Still Creek sediment and Musqueam Creek sediment, (Section 4.4).

The second question examined the relative sensitivity of the Microtox® solid phase test (SPT) and basic test, as compared to the other toxicity tests. The results of these tests are given in Section 4.5, and the results are compared to the results of the other toxicity tests performed on sediment and pore water samples.

The third question explored the usefulness of the SEM/AVS ratio in predicting sediment toxicity. Results are provided in Section 4.6. Results from the SEM/AVS ratios are compared to the results of other toxicity tests performed using the same sediments.
CHAPTER 4 RESULTS AND DISCUSSION

In addition, the metal and PAH concentrations were examined for each site and compared to the toxicity data (Sections 4.7 and 4.8). Toxicity results were examined to see if toxic sediments were found where sediment quality criteria suggested they should. Sulfide, ammonia and organic matter are also examined with respect to toxicity in Sections 4.9 and 4.10. Finally, Section 4.11 looks at the surrounding landuse at each site sampled.

4.2 PHYSICAL AND CHEMICAL SEDIMENT ANALYSES

Each site sampled on Still Creek differed in physical and chemical properties. The reference site was sampled at the same location each time, and showed variability between sampling dates. Results of the chemical and physical analyses performed on the sediments are given below.

4.2.1 Physical Analyses

Sediments were analyzed for percentage dry weight, percentage organic matter, and particle size fractionation (Table 4.1).

Still Creek particle size fractionation samples ranged from 1.14% silt and clay (< 63 µm) at site 3S to 19.6% at site 5S. Site 5S also had a high proportion of organic matter (23.66%) and a very low percent dry weight (14.38%).

Musqueam Creek, the reference site, was sampled at one general location. The particle size fractionation ranged from 1.5% to 3.81% silt and clay (< 63 µm fraction).
Table 4.1  Particle size fractionation and sediment description for Still Creek (S) and Reference (M) sediments.

<table>
<thead>
<tr>
<th>SITE NUMBER</th>
<th>Sediment Fraction (as percent (%) of total)</th>
<th>Sediment Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;2mm *</td>
<td>0.5-2mm Coarse sand</td>
</tr>
<tr>
<td>1S</td>
<td>28.21</td>
<td>16.27</td>
</tr>
<tr>
<td></td>
<td>Sandy at centre of creek, oily, black goo at sides</td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>62.49</td>
<td>21.33</td>
</tr>
<tr>
<td></td>
<td>Sandy, black sediment, oily sheen</td>
<td></td>
</tr>
<tr>
<td>3S</td>
<td>41.64</td>
<td>13.52</td>
</tr>
<tr>
<td></td>
<td>Sandy, sewage odour, iron precipitate at stream edges</td>
<td></td>
</tr>
<tr>
<td>4S</td>
<td>11.77</td>
<td>30.77</td>
</tr>
<tr>
<td></td>
<td>Peaty, dark brown to black organic sediment, oily sheen</td>
<td></td>
</tr>
<tr>
<td>5S</td>
<td>6.21</td>
<td>37.45</td>
</tr>
<tr>
<td></td>
<td>Dark brown sediment, wood-fibre material</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>40.05</td>
<td>20.44</td>
</tr>
<tr>
<td></td>
<td>Sandy, clean.</td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>26.61</td>
<td>9.88</td>
</tr>
<tr>
<td></td>
<td>Sandy, clean.</td>
<td></td>
</tr>
<tr>
<td>3M</td>
<td>3.53</td>
<td>9.52</td>
</tr>
<tr>
<td></td>
<td>Sandy, clean.</td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>25.59</td>
<td>24.36</td>
</tr>
<tr>
<td></td>
<td>Sandy, clean.</td>
<td></td>
</tr>
<tr>
<td>5M</td>
<td>1.56</td>
<td>47.43</td>
</tr>
<tr>
<td></td>
<td>Sandy, clean.</td>
<td></td>
</tr>
</tbody>
</table>

*ALL VALUES ARE GIVEN AS PERCENT (%)
CHAPTER 4 RESULTS AND DISCUSSION

4.2.2 Ammonia and Sulfide

Pore water ammonia levels and sediment sulfide levels in Musqueam Creek increased during the dry summer months (2M and 3M) and fell as autumn approached (4M and 5M) when higher flows flushed these contaminants from the sediment. The highest concentration of ammonia in the reference sediments was 0.882 mg N/L (site 3M). The highest sulfide levels were recorded in the same sample (0.0997 umoles/g dry weight), (Table 4.2).

Table 4.2 Sediment Chemical analyses for Still Creek (S) and Reference (M) sediments.

<table>
<thead>
<tr>
<th>Site Number</th>
<th>Dry Weight (%)</th>
<th>Organic Matter (%)</th>
<th>Sulfide (umoles/g dry wt)</th>
<th>Ammonia (mg N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>66.03</td>
<td>2.28</td>
<td>0.653</td>
<td>5.349</td>
</tr>
<tr>
<td>2S</td>
<td>81.47</td>
<td>1.34</td>
<td>0.189</td>
<td>1.496</td>
</tr>
<tr>
<td>3S</td>
<td>79.66</td>
<td>1.76</td>
<td>0.076</td>
<td>20.934</td>
</tr>
<tr>
<td>4S</td>
<td>35.43</td>
<td>10.66</td>
<td>0.644</td>
<td>7.149</td>
</tr>
<tr>
<td>5S</td>
<td>14.38</td>
<td>23.66</td>
<td>0.222</td>
<td>0.575</td>
</tr>
<tr>
<td>1M</td>
<td>79.25</td>
<td>1.85</td>
<td>0.0232</td>
<td>NS</td>
</tr>
<tr>
<td>2M</td>
<td>80.11</td>
<td>1.01</td>
<td>0.05</td>
<td>0.256</td>
</tr>
<tr>
<td>3M</td>
<td>67.5</td>
<td>2.24</td>
<td>0.0997</td>
<td>0.882</td>
</tr>
<tr>
<td>4M</td>
<td>80.09</td>
<td>0.97</td>
<td>0.0462</td>
<td>0.569</td>
</tr>
<tr>
<td>5M</td>
<td>74.66</td>
<td>0.83</td>
<td>0.0094</td>
<td>0.251</td>
</tr>
</tbody>
</table>

NS = NOT SAMPLED

Ammonia fluctuations were also reported by Schubauer-Berigan and Ankley (1991).

Seasonal changes in redox conditions were thought to affect the cycling of nitrogen,
which would result in an increase or decrease in ammonia. Ammonia alone was not responsible for the toxicity they observed. Changes in oxidative conditions which affected ammonia also influenced the toxicity or availability of other contaminants, such as metals.

The highest level of ammonia recorded in Still Creek was at site 3S (20.93 mg N/L). This site, however, had the lowest sulfide levels of all Still Creek samples (0.076 umoles/g dry wt.). The highest sulfide levels were at sites 1S (0.653 umoles/g dry weight) and site 4S (0.644 umoles/g dry weight). High ammonia levels at site 3S can probably be attributed in part to the presence of a strong sewage odour in the creek at this site. This might be caused by cross connections between the storm and domestic sewers in this area.

Figure 4.1 shows the fluctuations in both ammonia and sulfide concentrations in the creeks. Still Creek sulfide and ammonia varies site to site, whereas the reference site levels seem to peak in August (site 3M). The toxicity of ammonia and sulfide is discussed in Section 4.9.

4.2.3 Organic Matter

Organic matter content in sediments from the reference site (M) varied over the course of the summer and fall (Table 4.2). Levels were found to peak in August, (3M) at 2.24%, during a long dry period when the creek was not flushed out by rainfall.
Organic matter in Still Creek sediments varied from site to site. Highest levels were found at site 5S (22.66%), where the sediment had substantial quantities of wood fibre material. (Table 4.2). Lowest organic matter levels were found at site 2S (1.34%).

4.2.4 Polynuclear Aromatic Hydrocarbons (PAH)
PAH were not found at the reference site (Musqueam Creek) in levels detectable by GC analysis. PAH levels found in Still Creek are summarized in Table 4.3. PAH
levels found in Still Creek sediments are compared to sediment quality guideline levels in Table 4.4.

Table 4.3 Summary of PAH levels found in Still Creek sediments by GC analysis.

<table>
<thead>
<tr>
<th>PAH</th>
<th>1S (ug/g dry wt)</th>
<th>2S (ug/g dry wt)</th>
<th>3S (ug/g dry wt)</th>
<th>4S (ug/g dry wt)</th>
<th>5S (ug/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAPTHALENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.04</td>
<td>&lt;</td>
</tr>
<tr>
<td>ACENAPHTYLENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.47</td>
<td>&lt;</td>
</tr>
<tr>
<td>ACENAPHTHENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.40</td>
<td>&lt;</td>
</tr>
<tr>
<td>FLUORENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.13</td>
<td>&lt;</td>
</tr>
<tr>
<td>PHENANTHRENE</td>
<td>0.31</td>
<td>0.13</td>
<td>0.17</td>
<td>0.65</td>
<td>&lt;</td>
</tr>
<tr>
<td>ANTHRACENE</td>
<td>0.04</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.53</td>
<td>&lt;</td>
</tr>
<tr>
<td>FLUORANTHRENE</td>
<td>0.30</td>
<td>0.11</td>
<td>0.22</td>
<td>1.30</td>
<td>&lt;</td>
</tr>
<tr>
<td>PYRENE</td>
<td>0.26</td>
<td>0.12</td>
<td>0.20</td>
<td>1.18</td>
<td>&lt;</td>
</tr>
<tr>
<td>BENZO(a)ANTHRACENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>9.40</td>
<td>&lt;</td>
</tr>
<tr>
<td>CHRYSENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>0.41</td>
<td>&lt;</td>
</tr>
<tr>
<td>BENZO(a)PYRENE</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>TOTAL PAH</td>
<td>0.91</td>
<td>0.36</td>
<td>0.59</td>
<td>14.51</td>
<td>&lt;</td>
</tr>
<tr>
<td>Normalized PAH (mg PAH/Kg OC)</td>
<td>40</td>
<td>27</td>
<td>33</td>
<td>136</td>
<td>&lt;</td>
</tr>
</tbody>
</table>

Grey shading indicates PAH in exceedance of one or more of the criteria.

Using the established criteria for Burrard Inlet (BCE 1990), Still Creek sediments exceed the criteria at three of the five sites sampled. Site 4S has the greatest levels of PAH, with a total PAH concentration of 14.5 ug/g dry wt. Three and four ring PAH were the most abundant (phenanthrene, fluoranthene, benzo(a)anthracene and pyrene). This site was adjacent to Lougheed Highway, which was a likely source of PAH. Street dust from a metropolitan area in Tokyo (Takada et al., 1990) have been found to contain predominantly three and four ring PAH. Suedel et al. (1993) found organic carbon normalized PAH to be better predictors of toxicity than bulk sediment concentrations. These are also given in Table 4.3.

PAH toxicity is discussed in detail in Section 4.8.
CHAPTER 4 RESULTS AND DISCUSSION

Table 4.4 PAH Sediment Quality Criteria.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Burrard Inlet Objectives* (ug/g)</th>
<th>Wisconsin Dept. of Natural Resources** (mg/Kg OC)</th>
<th>Washington State Dept. Ecology Marine *** (mg/Kg OC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PAH</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.2</td>
<td>1240</td>
<td>99</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>0.06</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.05</td>
<td>92</td>
<td>16</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.05</td>
<td>-</td>
<td>23</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.15</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.1</td>
<td>-</td>
<td>220</td>
</tr>
<tr>
<td>Flouranthene</td>
<td>0.17</td>
<td>1216</td>
<td>160</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.26</td>
<td>-</td>
<td>1000</td>
</tr>
<tr>
<td>Benzo(a)Anthracene</td>
<td>0.13</td>
<td>-</td>
<td>110</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.14</td>
<td>-</td>
<td>110</td>
</tr>
<tr>
<td>Benzo(a)Pyrene</td>
<td>0.16</td>
<td>89</td>
<td>99</td>
</tr>
</tbody>
</table>

* = (Coquitlam - Pitt River Area Burrard Inlet Water Quality Assessment and Objectives, BCE 1990)
** = (Schuettelpelz, 1990)

All Values as ug/g dry weight, except iron as % and those indicated as mg/Kg Organic Carbon (OC)

4.2.5 Metal Concentrations

Metals selected for discussion here are nickel, copper, zinc, lead, and chromium. These metals were selected because they are thought to be the most toxic of the trace metals (Khangarot and Ray, 1987, 1989). They are also the metals most commonly associated with anthropogenic inputs (Jennett et al., 1980). Although also considered toxic, cadmium and mercury are not discussed, as mercury was not analyzed and cadmium was not found in levels above detection. SEM and < 63 um sediment fraction metals were analyzed using atomic absorption, as discussed in the methodology section. Sediments were also analyzed for cadmium, but none of the sediment samples had detectable levels of this metal using AA. The full 32 metal ICP scan is shown in Appendix VIII.
QA/QC for samples analyzed by AA (Chapter 3) included a sample of sediment being extracted on two separate occasions. The values are presented in Table 4.5.

Table 4.5 QA/QC for simultaneously extracted metals (SEM) and AA analysis.

<table>
<thead>
<tr>
<th>METAL</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>REPEAT #1 Dec. 4th (ug/g dry wt)</td>
</tr>
<tr>
<td>Cu</td>
<td>1.5</td>
</tr>
<tr>
<td>Zn</td>
<td>4.1</td>
</tr>
<tr>
<td>Mn</td>
<td>12.3</td>
</tr>
<tr>
<td>Ni</td>
<td>0.2</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Fe</td>
<td>247</td>
</tr>
<tr>
<td>Cr</td>
<td>2.4</td>
</tr>
<tr>
<td>Pb</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Overall, metal levels were much higher in Still Creek than in Musqueam Creek, as expected by the surrounding land use for each creek (Section 4.11). Table 4.6 shows the metal concentration in Still Creek (1S, 2S, 3S, 4S, and 5S) and Musqueam Creek (1M, 2M, 3M, 4M, and 5M) sediments.

From Table 4.6, it can be seen that the metals associated with the < 63 um fraction were generally lower than the SEM metals, and much less than the ICP total metal levels, as would be expected. This was true for all but copper, which was higher in the < 63 um fraction than in the SEM three times out of five in Still Creek sediment. When the < 63 um fraction metals were higher than the SEM metals, the sediment
Table 4.6 Metal concentrations in sediments from Still Creek and the reference site, Musqueam Creek. Includes SEM, < 63 µm and total (ICP) metals. Each is calculated as µg/g dry weight whole sediment.

<table>
<thead>
<tr>
<th>Metal Conc.</th>
<th>Site 1S</th>
<th>Site 2S</th>
<th>Site 3S</th>
<th>Site 4S</th>
<th>Site 5S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP</td>
<td>&lt;63um</td>
<td>ICP</td>
<td>&lt;63um</td>
<td>ICP</td>
</tr>
<tr>
<td>Zn</td>
<td>122</td>
<td>4.92</td>
<td>67.86</td>
<td>164</td>
<td>9.86</td>
</tr>
<tr>
<td>Ni</td>
<td>17</td>
<td>0.28</td>
<td>2.407</td>
<td>16</td>
<td>0.72</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>Cu</td>
<td>51</td>
<td>8.75</td>
<td>15.9</td>
<td>50</td>
<td>23.24</td>
</tr>
<tr>
<td>Cr</td>
<td>178</td>
<td>0.52</td>
<td>3.328</td>
<td>154</td>
<td>0.27</td>
</tr>
<tr>
<td>Fe</td>
<td>17500</td>
<td>330</td>
<td>27.52</td>
<td>19400</td>
<td>310</td>
</tr>
<tr>
<td>Pb</td>
<td>58</td>
<td>3.39</td>
<td>53.3</td>
<td>66</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metal Conc.</th>
<th>Site 1M</th>
<th>Site 2M</th>
<th>Site 3M</th>
<th>Site 4M</th>
<th>Site 5M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICP</td>
<td>&lt;63um</td>
<td>ICP</td>
<td>&lt;63um</td>
<td>ICP</td>
</tr>
<tr>
<td>Zn</td>
<td>22</td>
<td>1.96</td>
<td>6.407</td>
<td>26</td>
<td>3.03</td>
</tr>
<tr>
<td>Ni</td>
<td>9</td>
<td>0.31</td>
<td>0.998</td>
<td>10</td>
<td>0.71</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>Cu</td>
<td>6</td>
<td>6.08</td>
<td>0.254</td>
<td>7</td>
<td>6.76</td>
</tr>
<tr>
<td>Cr</td>
<td>162</td>
<td>0.4</td>
<td>0.052</td>
<td>163</td>
<td>0.18</td>
</tr>
<tr>
<td>Fe</td>
<td>13400</td>
<td>240</td>
<td>636</td>
<td>14000</td>
<td>930</td>
</tr>
<tr>
<td>Pb</td>
<td>6</td>
<td>0.79</td>
<td>3.108</td>
<td>8</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Shading indicates values that meet or exceed at least one of the criteria levels in Table 4.7
< indicates less than detection level
CHAPTER 4  RESULTS AND DISCUSSION

tended to be more toxic. Metal toxicity to *Chironomus tentans* and *Daphnia magna* is discussed in Section 4.7.

Copper levels in Musqueam Creek (< 63 um fraction only) increased during the dry summer, from 1M through 3M. By the 4M sampling period, the < 63 um copper levels had started to decline again as rain flushed out the system. This trend, however, doesn't follow in the total or SEM metal levels. Overall, copper levels (<63 um) are quite close to ICP copper levels for whole sediment, indicating that most of the copper is in the <63 um fraction. Note that < 63 um copper was greater than the total copper at site 5S.

As discussed in Chapter 2, there are a number of numerical sediment quality guidelines that have been recommended to evaluate sediment quality. Table 4.6 shows (highlighted) those sites at which the sediment metal levels exceed the sediment quality guidelines, which are given in Table 4.7.

From Table 4.6, it is obvious there are more exceedances of the criteria in Still Creek sediments than the reference sediments. Reference sediments exceed the criteria only in total (ICP) metals, not SEM or < 63 um metals. Still Creek sediments exceed the criteria at all sites. There were more exceedances using SEM than < 63 um methods, particularly at site 4S.

Reference sediment did not exceed any of the lead criteria, which is to be expected as there is little vehicular traffic in the area. Lead levels at site 5S were also low.
CHAPTER 4 RESULTS AND DISCUSSION

Table 4.7 Sediment quality guidelines for metals in freshwater, marine and terrestrial environments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Burrard Inlet Objectives* (ug/g dry wt)</th>
<th>Wisconsin Dept. of Natural Resources ** (ug/g dry wt)</th>
<th>Ontario Provincial Sediment Quality Guidelines*** (ug/g dry wt)</th>
<th>Washington State Dept. Ecology Marine **** (ug/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>20</td>
<td>10</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1</td>
<td>1</td>
<td>0.6</td>
<td>51</td>
</tr>
<tr>
<td>Chromium</td>
<td>60</td>
<td>100</td>
<td>26</td>
<td>260</td>
</tr>
<tr>
<td>Copper</td>
<td>100</td>
<td>100</td>
<td>16</td>
<td>390</td>
</tr>
<tr>
<td>Iron (%)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Lead</td>
<td>30</td>
<td>50</td>
<td>31</td>
<td>450</td>
</tr>
<tr>
<td>Nickel</td>
<td>45</td>
<td>100</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Zinc</td>
<td>150</td>
<td>100</td>
<td>120</td>
<td>410</td>
</tr>
</tbody>
</table>

* = (Coquitlam - Pitt River Area Burrard Inlet Water Quality Assessment and Objectives, BCE 1990)
** = (Schuettbelz, 1990)
*** = (Persaud et al, 1991)

All Values as ug/g dry weight, except iron as % and those indicated as mg/kg Organic Carbon (OC)

This site is also not directly affected by vehicular traffic, but is adjacent to railway tracks. Lead was found to exceed criteria levels at all the other sites sampled in Still Creek. Highest lead levels in Still Creek were found in the SEM extraction of sediments from site 4S, which is adjacent to Lougheed Highway. Levels reached 166 ug/g dry weight, which exceeds all three criteria in Table 4.7. The highest lead in total metal extraction of sediment was site 3S (Atlin Street), which is unusual as this is a residential neighbourhood.
CHAPTER 4  RESULTS AND DISCUSSION

Three of the sites (1S, 2S, and 4S) were sampled in 1976 and analyzed for metals (Hall et al., 1976). Changes in metal levels between 1976 and 1992 are shown in Table 4.8.

Table 4.8 Comparison of Still Creek metal concentrations at three sites from 1976 and present (1992) data.

<table>
<thead>
<tr>
<th>METAL</th>
<th>SITE 1S</th>
<th>SITE 2S</th>
<th>SITE 4S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hall et al. 1976</td>
<td>This Study 1992</td>
<td>% Change</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.5</td>
<td>&lt;0.5</td>
<td>-</td>
</tr>
<tr>
<td>Chromium</td>
<td>100</td>
<td>178</td>
<td>78</td>
</tr>
<tr>
<td>Copper</td>
<td>684</td>
<td>51</td>
<td>-93</td>
</tr>
<tr>
<td>Iron</td>
<td>23800</td>
<td>17500</td>
<td>-26</td>
</tr>
<tr>
<td>Nickel</td>
<td>33.6</td>
<td>17</td>
<td>-49</td>
</tr>
<tr>
<td>Lead</td>
<td>440</td>
<td>58</td>
<td>-87</td>
</tr>
<tr>
<td>Zinc</td>
<td>206</td>
<td>122</td>
<td>-41</td>
</tr>
</tbody>
</table>

NT = Not tested
1992 data are for ICP, while 1976 data are for AA.

Since 1976, all metal levels at sites 1S and 2S appear to have decreased, except chromium which has increased at site 1S and was not analyzed at 2S in 1976. At site 4S, however, only nickel and lead appear to have decreased, the others seem to have increased. The decrease in lead could be attributed to lead free gasoline. This was countered by an overall increase in traffic in Greater Vancouver which may contribute to the apparent increase in other metal levels.

Metals and toxicity is discussed in detail in Section 4.7.
4.3 SAMPLING METHOD AND TOXICITY

To address the question of how sampling method affects the toxicity of a sediment sample, toxicity tests were done using samples taken in the same sample set using the two sampling methods discussed in the methodology section. *Daphnia magna* and *Chironomus tentans* were then exposed to the sediments for 14 days, and various endpoints examined. The raw toxicity data and statistics are presented in Appendix IV.

4.3.1 Reference and Pore Water 48 hr Toxicity Tests

Reference toxicity tests were done each time a toxicity test was performed. The reference toxicity test results are presented in Table 4.9.

Table 4.9 48 hr LC$_{50}$ reference toxicity test data for *Daphnia magna* and *Chironomus tentans*.

<table>
<thead>
<tr>
<th></th>
<th>Daphnia magna REFERENCE TOXICITY TEST</th>
<th>Chironomus tentans REFERENCE TOXICITY TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLE</td>
<td>48 Hr LC50 ZnSO4 (mg/L)</td>
<td>SAMPLE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 Hr LC50 CdCl2 (mg/L)</td>
</tr>
<tr>
<td>1</td>
<td>1.95</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1.18</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1.52</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1.16</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>1.75</td>
<td>5</td>
</tr>
<tr>
<td>AVG</td>
<td>1.512</td>
<td>AVG</td>
</tr>
<tr>
<td>STD</td>
<td>0.311</td>
<td>STD</td>
</tr>
<tr>
<td>VAR</td>
<td>0.097</td>
<td>VAR</td>
</tr>
<tr>
<td>MAX</td>
<td>1.95</td>
<td>MAX</td>
</tr>
<tr>
<td>MIN</td>
<td>1.16</td>
<td>MIN</td>
</tr>
</tbody>
</table>

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CHAPTER 4 RESULTS AND DISCUSSION

Reference toxicity tests were done to evaluate the health of the organisms used in the toxicity tests. The *Daphnia magna* had an average LC₅₀ of 1.5 mg/L ZnSO₄. The culture water was moderately hard (80-100 mg CaCO₃/L). This is within the range found by Berglind and Dave (1984), who had an LC₅₀ of 1.1 mg/L in hard water (300 mg CaCO₃/L) and 1.7 mg/L in soft water (50 mg CaCO₃/L).

Elder (1989) summarized literature where *Daphnia* 48 hour LC₅₀’s had been done. The 48 hr LC₅₀ for zinc ranged from 0.068 to 5.1 mg/L.

Pore water from Still Creek and the reference site was extracted by centrifugation, as discussed in Chapter 3. *Daphnia magna* 48 hr LC₅₀ toxicity tests were done. None of the sediment pore water samples were toxic to the Daphnia. LC₅₀ concentrations are calculated as a percent of the pore water (Table 4.10). Pore water with LC₅₀’s greater than 100% are considered nontoxic.

Table 4.10  Pore water 48 hr LC₅₀ data for *Daphnia magna*.

<table>
<thead>
<tr>
<th>Site</th>
<th><em>Daphnia magna</em> Pore water LC₅₀ (actual value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (all)</td>
<td>non-toxic</td>
</tr>
<tr>
<td>1S</td>
<td>non-toxic</td>
</tr>
<tr>
<td>2S</td>
<td>non-toxic</td>
</tr>
<tr>
<td>3S</td>
<td>525%</td>
</tr>
<tr>
<td>4S</td>
<td>101.6%</td>
</tr>
<tr>
<td>5S</td>
<td>136%</td>
</tr>
</tbody>
</table>
Reference toxicity tests using CdCl₂ were also done on *Chironomus tentans* at the start of each set of tests. The results are presented in Table 4.9. The 48 hr LC₅₀ ranged from 5.04 mg/L to 0.5 mg/L (average 2.3 mg/L). Timmermans et al. (1992) and Pascoe (1987) both found that the 24 hr LC₅₀ of *Chironomus riparius* was 2.1 mg/L. Timmermans et al. (1992) noted impaired growth and development of *C. riparius* in Cd concentrations of 0.007 and 0.002 mg/L. Pascoe et al. (1989) found male *C. riparius* emergence was delayed when exposed to 0.15 mg/L cadmium.

The lowest chironomid 48 hr LC₅₀ was at sample time 5 (which corresponds to samples 5S and 5M). Although chironomid survival was poor in Still Creek sediments at this time (13% in mixed and 52% in cores) the survival in the reference sediments was good (73% mixed, 67% cores). However, the dry weight of chironomids at the end of the 14-day tests was low in both the Still Creek and reference sediments, perhaps indicating that the low 48 hr LC₅₀ did in fact mean the culture was in poorer health than at other times. The lowest survival rate of chironomids in the reference sediments was seen at 3M, which was a reasonably healthy culture (48 hr LC₅₀ of 1.55 mg/L). Thus, toxicity in the reference sediments at this time is likely due to sediment contaminants.
4.3.2 *Daphnia magna* 14 Day Test

4.3.2.1 *Daphnia magna* Survival

Figure 4.2 shows the percentage survival of *Daphnia magna* over the 14 day tests. There was no significant difference in daphnid survival between the mixed and core samples at P<0.05. Only one site had a significant difference in daphnid survival between the mixed and core sediments at P<0.10, (site 3S). This site had very high ammonia levels in the pore water. This site also exceeded the sediment quality criteria for phenanthrene and fluoranthene, and for < 63 um fraction copper and SEM lead levels.

It was thought that due to the process of mixing and homogenization of sediments, or through bioturbation, contaminants might be released into the water column. Schmidt-Dallmier et al. (1992) showed that the exposure of aquatic organisms to suspended sediments can impair growth and survival, and increase bioaccumulation of sediment-associated contaminants. It is evident that if a release of contaminants from the sediment occurred, it was not in high enough quantities to effect *Daphnia* survival. In most cases there was no significant difference in survival between mixed and core sediments. This was possibly due to the fact 2/3 of the overlying water was replaced every second day, and contaminants would not have had an opportunity to accumulate. Also, many of the contaminants present in Still Creek, such as the PAH, are hydrophobic and would remain in the sediment fraction.
Figure 4.2  Percent Survival of *Daphnia magna* in the 14 day test, comparison of sampling methods. * P<0.10 (See Table 3.1 for site and sampling data identification.)

Bennett and Cubbage (1992a) found *Daphnia* unresponsive to samples containing high levels of PAH. They attributed this to the low water solubility of these compounds. Suedel et al. (1993) found daphnids were less responsive to fluoranthene than chironomids were, probably because chironomids are in direct contact with the sediment. Enserink et al., (1991) found *Daphnia* responded to water soluble metals. Eight metals were tested singly, and in combination. It was found that the effect of the metals on *Daphnia* was additive. Water soluble metals are more
bioavailable than sediment bound metals, and any metal exchange from sediment to water was not large enough to affect daphnids in this study.

*Daphnia* survival was examined over the 14 days of the experiment. Although there was no significant difference between any of the sediments, *Daphnia* mortality did occur. That is, it took longer than the first 48 hours for any mortality to occur. (Figure 4.3.)

Site 1S and 1M best illustrate this phenomenon. The Reference (M) site mixed samples and core samples were the least toxic to the *Daphnia*. It is interesting to note that survival was 100% up to day 4. Any mortality that occurred, happened after this time. If only acute 48 hour testing had been done, the chronic effect (mortality after 4 days) would have been missed.

At sites 2S and 2M, a similar pattern developed after 2 days for the mixed sediments at each site, and after 8 days for the 2S cores (Figure 4.3(b)). At sites 3S and 3M, there was a drop in *Daphnia* survival in the 3S cores after day 2, which leveled off after day 6 (Figure 4.3 (c)). Site 3S had very high pore water ammonia levels, and a strong raw sewage smell. Sites 4S and 4M exhibited mortality after day 4, and the *Daphnia* were most sensitive to the 4S cores (Figure 4.3 (d)).

The first mortalities at sites 5S and 5M were observed after day 6. Daphnia in cores from both sites showed poor survival. Again, note that there is no statistical
Figure 4.3 *Daphnia magna* mortality examined over the 14 days of the test. (a), (b), (c), (d), and (e) show sites 1, 2, 3, 4, and 5 respectively. Both Still Creek and Musqueam Creek results are shown on each graph.
difference in survival at any of the sites, but that a chronic trend appeared to develop with respect to survival.

Overall, mortality seemed to occur 4 to 6 days after the *Daphnia* were exposed to the sediments, and continued to occur to the end of the exposure time (14 days). Only rarely did *Daphnia* mortality occur in the first 48 hours.

4.3.2.2 Total Number of Young

The average total number of young produced by *Daphnia* over the 14 days in each sediment sample type is shown in Figure 4.4.

There was a significant difference (P<0.05) between the mixed sediment samples and the cores with respect total number at only one site, namely 3S. The number of young produced by *Daphnia* in the mixed sediments was greater than that produced by *Daphnia* in the core samples at this site. There was a layer of scum on top of the core samples that was not present when samples were mixed which may have affected the *Daphnia*. This sample had a strong sewage odour, and the water was quite cloudy throughout the experiment.

4.3.2.3 Number of Young Per Brood

The number of young produced per brood is shown in Table 4.11. This proved to be a more sensitive endpoint than survival. There was a significant difference between sampling methods in the number of young produced in the first brood in sediment from sites 1S and 5S. In 1S sediments, the *Daphnia* in the core samples had a
Figure 4.4 *Daphnia magna* total number of young, comparing mixed and core sampling method over 14 days for both Still Creek and reference sediments.

greater number of young than those in the mixed, while *Daphnia* in the 5S mixed samples had a greater number of young than in the cores.

In the second brood, there was a significant difference between the sampling methods at sites 1S and 2S. In 1S sediments, the *Daphnia* in the mixed samples had a greater number of young, while *Daphnia* in 2S sediment core samples had a greater number of young.
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Table 4.11 Average total number of young per brood, comparing core and mixed sampling methods at $P<0.05$ confidence level.

<table>
<thead>
<tr>
<th>Site</th>
<th>Brood 1</th>
<th>Brood 2</th>
<th>Brood 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>YES*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>no</td>
<td>YES*</td>
<td>no</td>
</tr>
<tr>
<td>3S</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>4S</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>5S</td>
<td>YES**</td>
<td>no</td>
<td>YES**</td>
</tr>
<tr>
<td>1M</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>2M</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>3M</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>4M</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>5M</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

* indicates daphnids in cores had greatest number of young per brood
** indicates daphnids in mixed samples had greatest number of young per brood

In the third brood, there was only a difference in number of young between sampling methods at site 5S. *Daphnia* in the mixed sediments had a greater number of young. Site 5S contained a high level of organic matter, unidentified complex hydrocarbons (as determined by GC analysis), and high copper concentrations exceeding the sediment quality criteria (in Table 4.7) that could be responsible for chronic effects such as the difference in number of young between *Daphnia* exposed to core and mixed sediment samples.

4.3.2.4 Summary of *Daphnia* Data

It appears that the *Daphnia* were not sensitive to the difference in sampling methods. *Daphnia* survival was not a sensitive endpoint for the types of samples used here.
CHAPTER 4 RESULTS AND DISCUSSION

This could be partly due to the static-renewal nature of the tests, which did not allow for any build-up of contaminants in the water column. About 2/3 of the overlying water was removed every second day, and replaced with new culture water. Contaminants could leach from the sediments to the overlying water in amounts that might elicit chronic effects, but were not allowed to accumulate. In an actual creek, water is constantly flowing so although this experiment was not flow-through, it is somewhat more representative of the stream conditions than a static test.

The total number of young was sensitive to the sampling method only at site 3S. This could have been due to the ammonia levels in this particular sample, which were very high.

The number of young per brood was the most sensitive endpoint for the *Daphnia* 14-day tests. There was no consistency as to differences between sampling methods. *Daphnia magna* appeared to be affected more by the contaminants present than the method of sampling.

4.3.3 *Chironomus tentans* 14 Day Test

4.3.3.1 Dry Weight

Chironomids were more sensitive to the method of sampling than the *Daphnia*, possibly due to their direct contact with the sediment through their life history and feeding habits. Figure 4.5 shows the chironomid dry weights in survivors from the core and mixed samples.
Figure 4.5 Dry weight of surviving chironomids at the end of the 14 day test, comparison of core and mixed sampling methods. Bars indicate the standard deviation, and * indicates a significant difference at P<0.05.

Only two out of ten cases, sites 3S and 5S, showed a significant difference in dry weight of chironomids between the cores and mixed samples at P>0.05. Site 3S had high ammonia levels and exceeds the sediment quality criteria levels for copper and lead, as was shown in Table 4.6. Ammonia is potentially volatilized by the mixing process, which could account for the reduced toxicity to chironomids in the mixed samples. Lead is readily accumulated by chironomid larvae, and slows growth (Timmermans et al., 1992). Copper accumulation in *Chironomus riparius* is accompanied by significant growth impairment at concentrations as low as 0.009...
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mg/L (Timmermans et al., 1992). Cadmium, chromium and zinc have been found to reduce chironomid emergence (Wentsel et al., 1978). Thus, mixing the sediments at sight 3S may have altered sediment toxicity, and could account for the observed difference in toxicity to chironomids between the mixed and core sediments. Site 3S also exceed the sediment quality criteria for PAH. However, Bennett and Cubbage (1992a) found chironomids insensitive to PAH contaminated sediments.

Site 5S exceeded the sediment quality criteria levels for copper. At this testing time, however, the chironomid culture may not have been as healthy as previous times (Section 4.3.1).

4.3.3.2 Survival
There was a significant difference in chironomid survival between sample methods in 4 out of 10 sets of toxicity tests. In all cases, survival was greater in the cores, (Figure 4.6). Mixed sediment appear to give a representation of a "worst-case scenario" in this test. This is possibly due to the mixing process rendering contaminants such as metals more bioavailable and thus the sediment becomes more toxic.

4.3.4 Summary
Sediment sampling is generally done by grab sampling (Stemmer et al., 1990). This method of sampling results in a loss of sample integrity, thereby altering the biological, physical (stratification) and chemical properties of the sample.
Figure 4.6  *Chironomus tentans* survival in reference (M) and Still Creek (S) sediments, comparing the sampling methods. * indicates a significant difference at $P<0.05$.

environment. In this section, the toxicity of two methods of sediment sampling was compared.

Chironomids were more sensitive than *Daphnia magna* to the sediments tested, possibly because they are in direct contact with the sediments. The chironomids were sensitive to the different sampling methods. Survival of chironomids was
always greater in the core samples when there was a significant difference between the sampling methods.

The core samples had a distinct top layer of fine particles of sediment. This would be mixed up and effectively diluted in the mixed samples. Depending on the sediment sample, this could provide a readily available food source in the portion of the sediment inhabited by the chironomids (i.e., the top few centimeters). In a mixed sample, this fine material is distributed throughout the test vessel, and therefore less readily available. On the other hand, the mixed samples would make some of the deeper sediments available to the chironomids which would otherwise have been buried. These deeper sediments could contain higher levels of contaminants that may affect chironomid growth and/or survival.

With respect to chironomid survival, it appears that a mixed sample gives a 'worst case scenario'. Chironomid dry weight as an endpoint was not able to distinguished between sampling methods. Whether the mixed samples are more or less toxic than the core samples would depend on the types of contaminants present and their location within the sediments (e.g. vertical stratification), as well as their bioavailability compared to undisturbed core sediments.

4.4 SAMPLING SITE AND TOXICITY

The toxicity of sediments from Still Creek was compared to the toxicity of the reference sediments. The raw data tables are presented in Appendix V.
4.4.1 *Daphnia magna* 14 Day Test

The *Daphnia* showed no difference in survival between sediments from the test sites in Still Creek and the reference site in Musqueam Creek, (data in Table 1, Appendix V).

Total number of young was a more sensitive endpoint than survival when comparing Still Creek and reference sediments. In three out of ten experiments, there was a significant difference in total number of young between Still Creek and the reference site (P<0.05). The data are presented in Table 2, Appendix V. There was a significant difference in total number of young between 2S and 2M mixed sediments, but not between 2S and 2M cores. There was also a significant difference in total number of young between 3S and 3M cores, and between 3S and 3M mixed sediments. 3S is the Still Creek site which had very high ammonia in the sediment pore water, and was likely contaminated with sewage. This site exceeded the sediment quality criteria for fluoranthene, phenanthrene, copper and lead. The *Daphnia* were more sensitive to this site than the other sites. Qureshi, et al. (1982) found daphnids more sensitive than Microtox® basic test to ammonia, zinc and copper.

The number of young per brood was not a sensitive endpoint when comparing the reference site toxicity to the test sites. The data are presented in Tables 3, 4, 5, Appendix V. There was a significant difference in number of young per brood
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between 5S and 5M in mixed sediments in the first brood. *Daphnia* in 5S samples produced more young in the first brood than those in the reference sediments. There were no differences in the number of young in the second brood.

There was a difference in young per brood in the third brood between 5S and 5M core samples. *Daphnia* in the reference sediments produced more young in the third brood than the *Daphnia* in the 5S cores. Thus, at sites 5S and 5M it appears there was a chronic effect on the *Daphnia* in 5S cores as there is no significant difference between 5S and 5M cores until the third brood. Site 5S exceeded the sediment quality criteria for copper.

4.4.2 *Chironomus tentans* 14 day test

Chironomid survival was sensitive to the differences between Still Creek sediments and the reference sediments in 5 out of 10 cases. (Figure 4.7).

There was no difference in the survival of chironomids between sites 3S and 3M in either the mixed or the core sediments. Survival was poor overall at 3S and 3M sample date, (August 24, 1992), although the 96 hr LC50 test performed at this time was approximately average (Table 4.9). There had been no rainfall in the Greater Vancouver area for over a month, and the reference site appeared to degrade in quality at this time. Survival was greatly improved by the next sampling date (October), as there had been a number of rainfall events to "flush out" the system. When there was a significant difference in survival between Still Creek and the
Figure 4.7 *Chironomus tentans* survival over the 14 day test, comparing Still Creek to the reference site. This figure shows the difference between (a) reference (M) and Still Creek (S) mixed sediments and (b) reference and Still Creek core sediments.
reference site, chironomids in reference sediments always survived better than chironomids in Still Creek sediments.

Site 4S had the highest sediment PAH (total PAH of 14.5 ug / g dry weight). The naphthalene concentration was 0.036 ug/g dry weight. This site was very toxic to chironomids with respect to survival. Survival was only 12% in the mixed sediments and 8% in the cores. Darville and Wilhm (1984) found that *Chironomus attenuatus* had an LC_{50} value of 13 mg/L to naphthalene, and life cycle exposures at naphthalene concentrations of less than 0.5 mg/L resulted in minimal effect. Naphthalene levels in Still Creek were unlikely to have had an effect on chironomids, as the sediment PAH levels were low, and PAH solubility is low. Other PAH, however, were elevated, and may have contributed to mortality and growth reduction.

The dry weights of chironomids from Still Creek and reference sediments are compared in Figure 4.8. Five out of ten cases showed a significant difference in dry weight between Still Creek and reference sediments at P<0.05 confidence interval. In each case, the chironomids grown in the reference sediments had greater weights than those grown in Still Creek sediments for both sampling methods.

*Chironomus tentans* were also used by Bennett and Cubbage (1992a) for toxicity testing of PAH contaminated sediments. PAH concentrations reached up to 33,000 mg/Kg dry weight, yet chironomids were unaffected by the elevated PAH
concentrations. Only one out of the four sites they tested was statistically different from the control with respect to chironomid dry weight.

4.4.3 Summary

*Daphnia* were more sensitive to the source of the sediment and the contaminants present than they were to the different sampling methods. Based on survival, chironomids were equally sensitive to sampling method used and which site the sediment was from (S or M). This is due to the chemical nature of the contaminants found in Still Creek, such as PAH or metals which are bound to the sediments. Contaminants would tend to stay bound to particulate matter, and remain in the solid phase, where they would be more available to chironomids than to *Daphnia*. Any contaminants that might have been released into the water column did not elicit a toxic response in *Daphnia*. Suedel et al. (1993) found chironomids to be more sensitive than *Daphnia magna* to fluoranthene, one of the PAH found in Still Creek sediment. Bennett and Cubbage (1992a) found *Daphnia magna* insensitive to PAH despite high levels of PAH in sediment samples.

As would be expected, at the sites at which there was a significant difference in chironomid survival between Still Creek sediments and the reference sediments, there was also a significantly higher dry weight of those chironomids which survived in Still Creek sediments as compared to those from reference sediments.
Figure 4.8 *Chironomus tentans* dry weight, 14 day test. Comparison of Still Creek and reference site sediments, cores (a) and mixed (b). Bars indicate the standard deviation. * indicates a significant difference at P<0.05.
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Malueg et al. (1983) found that the presence of *Hexagenia* intensified the *Daphnia* response due to the physical activity of *Hexagenia*. Chironomids and daphnids were placed in the same test vessel. It was hoped bioturbation by the chironomids would release contaminants into the water column. This was not the case in the research presented here. *Daphnia* were not sensitive to the sediments tested. It is possible that the static-renewal test did not allow sufficient time for accumulation of contaminants in concentrations high enough to elicit a toxic response.

4.5 Microtox® Results

Table 4.12 shows the results of both the solid phase and basic Microtox® tests. The pore water from Musqueam Creek sediment (the reference site) was not toxic to the Microtox® bacteria during all five sampling periods. Still Creek sediment pore water was toxic only at site 3S. Toxicity at this site was most likely due to ammonia, as concentrations at this site reached 20.9 mg N/L. The 15 minute EC\textsubscript{50} at this site was 38.2\% (approximately 8 mg N/L). Site 1S pore water gave a 15 minute EC\textsubscript{50} of >100\%, which is essentially non-toxic.

Qureshi et al. (1982), however, found Microtox® basic test was not sensitive to ammonia (5 minute EC\textsubscript{50} was 3,607 mg/L total N). Hoke et al. (1992), however, found that ammonia concentration caused toxicity to Microtox® bacteria. Toxicity was also due, in part, to copper, zinc and aluminum when concentrations were elevated. Ammonia (NH\textsubscript{3}) can also form complexes with zinc and copper, which
CHAPTER 4  RESULTS AND DISCUSSION

could make these metals more soluble and perhaps more available to the microorganisms.

Table 4.12 Results of Microtox® pore water and solid phase tests for Still Creek and Musqueam Creek sediments.

<table>
<thead>
<tr>
<th>SITE</th>
<th>MICROTOX BASIC TEST (PORE WATER)</th>
<th>MICROTOX SOLID PHASE TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 MINUTE EC50</td>
<td>15 MINUTE EC50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1S</td>
<td>non-toxic*</td>
<td>&gt; 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3S</td>
<td>non-toxic</td>
<td>38.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(22.3 - 65.4%)</td>
</tr>
<tr>
<td>4S</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5S</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td>3M</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
<tr>
<td>5M</td>
<td>non-toxic</td>
<td>non-toxic</td>
</tr>
</tbody>
</table>

* Non-toxic indicates increased luminescence over the blank
# indicates confidence range greater than 100%

In sediments of greater than 9.2 mg PAH /Kg dry weight, Tay et al. (1992) found a significant correlation between Microtox® basic test and sediment PAH concentration. Only one site in Still Creek exceeded 9.2 mg PAH/Kg dry weight (Site 4S). Total PAH concentration was 14.5 mg PAH /Kg dry weight at site 4S, and the pore water from this site was not toxic to the Microtox® bacteria in the basic test. Bennett and Cubbage (1992a) found that the Microtox® was sensitive to PAH contamination in pore water in three out of four cases. They found much higher PAH
concentrations (up to 33,000 mg PAH/Kg dry weight), and also used a sediment extraction procedure before testing the toxicity with Microtox® basic test.

The solid phase Microtox® test was more sensitive than the basic Microtox® test which is done using sediment pore water. Still Creek sediments were all toxic to the Microtox® bacteria to some extent, the most toxic sites being 3S and 4S. Microtox® bacteria have been found to be sensitive to copper in the basic Microtox® test (Giesy and Hoke, 1989). Sites 3S and 4S both had elevated copper concentrations in the sediment (< 63 μm fraction) which exceeded the sediment quality criteria. The copper associated with solid phase of the sediment must also be toxic to the Microtox® bacteria.

The reference sediments were overall not very toxic to the Microtox® bacteria. The most toxic of the reference sediments was 3M, in August 1992 when there had been no rain for some time, and the flow through the creek was low. However, metal levels were not elevated, and no PAH were detectable at this site. Microtox® SPT confidence ranges were very high in those reference sediments which did elicit a response.

Since the Daphnia were generally unresponsive to any of the sediments, correlation of daphnid endpoints with Microtox® results was not performed.

In order to determine correlations between contaminants present in the sediment and Microtox® SPT results, linear regression and Spearman's correlation were
performed on the data. In some cases, toxicity is not observed until threshold
centrations are met (Jacobs et al., 1993). Thus, Spearman’s rank correlation is
better suited in assessing the relationship between toxicity and potentially toxic
centrations, as Spearman’s coefficients measure non-linear relationships better
than linear coefficients.

Table 4.13 shows the $r^2$ values for the linear correlations and the Spearman’s
correlation coefficients. Since Musqueam sediments were not toxic to the Microtox®
bacteria, the correlation values were not calculated. The results presented are for
Still Creek sediments only.

Table 4.13 Linear Correlation, Spearman’s correlation coefficients and Microtox®
toxicity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear Correlation Coefficient</th>
<th>Spearman’s Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter</td>
<td>0.084</td>
<td>-0.300</td>
</tr>
<tr>
<td>PAH</td>
<td>0.322</td>
<td>-0.800</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.180</td>
<td>-0.800</td>
</tr>
<tr>
<td>Sulfide</td>
<td>0.278</td>
<td>-0.200</td>
</tr>
<tr>
<td>&lt;63 umm metals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>0.566</td>
<td>-0.900</td>
</tr>
<tr>
<td>Copper</td>
<td>0.204</td>
<td>0.300</td>
</tr>
<tr>
<td>Lead</td>
<td>0.357</td>
<td>-0.667</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.315</td>
<td>0.500</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.012</td>
<td>-0.200</td>
</tr>
<tr>
<td>Sum all</td>
<td>0.181</td>
<td>-0.800</td>
</tr>
<tr>
<td>ICP-Total Metals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>0.046</td>
<td>-0.300</td>
</tr>
<tr>
<td>Copper</td>
<td>0.266</td>
<td>-0.900</td>
</tr>
<tr>
<td>Lead</td>
<td>0.210</td>
<td>-0.600</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.049</td>
<td>-0.154</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.006</td>
<td>-0.400</td>
</tr>
<tr>
<td>Sum all</td>
<td>0.156</td>
<td>-0.600</td>
</tr>
</tbody>
</table>

Shading highlights those variables where the absolute
value of the correlation is greater than 0.800
CHAPTER 4 RESULTS AND DISCUSSION

Contaminant concentrations in Still Creek sediments correlated with Microtox® SPT to a greater extent using Spearman's coefficient than with linear regression (Table 4.13). Microtox® toxicity correlated with PAH, ammonia, < 63 um fraction chromium, sum of the < 63 um fraction metals, and ICP copper concentrations in Still Creek sediments. Jacobs et al. (1993) also found that contaminants (PAH) correlated significantly with toxicity using Spearman's correlation procedure, but did not conduct a linear correlation. Total sediment copper (using ICP analysis) correlated with Microtox® toxicity, but < 63 um fraction copper did not. The opposite was true for chromium concentrations and Microtox® toxicity. Giesy et al. (1988) and Tay et al. (1992) found low sensitivities of the Microtox® bacteria (P. phosphoreum) to sediment metals in the Microtox® basic pore water test. Giesy et al. (1989) found Microtox® sensitive to copper. Codina et al. (1993) found the Microtox® basic test sensitive to zinc and copper, but not as sensitive to cadmium, chromium and nickel. Santiago et al. (1993) found a correlation of 0.69 between Microtox® elutriate basic test and lead. No correlation was found between the Microtox® elutriate test and PAH concentrations.

The Microtox® SPT was sensitive to contaminated sediments. The confidence ranges were small and there were more sediment samples that were toxic to the bacteria than was found using pore water and the basic Microtox® test. This was also found by Tay et al. (1992), where ten out of twelve samples examined were toxic using the Microtox® SPT, and only five out of twelve samples were toxic using
the basic Microtox® test on the pore water. However, the SPT did not perform as well in less contaminated sediments, (i.e. the reference sediments). The confidence ranges become much larger, (See Table 4.12), and the EC50 is not as clear as when samples are more contaminated, (See appendix VI for examples).

It appears that there is not one factor alone that is causing toxicity in the sediments tested with the Microtox® bacteria. Any toxicity observed is obviously due to a combination of all the contaminants present in a given sample.

4.6 SEM/AVS RATIO AND TOXICITY

SEM/AVS ratio is believed to be an indicator of sediment toxicity (Di Toro et al., 1990). Ratios for the tests in this study are found in Table 4.14. All ratios for the SEM/AVS were greater than 1, even at the reference site (M). The highest ratio was 14.69 at site 3S. This site was toxic to both Daphnia magna (survival) and Chironomus tentans (dry weight and survival), Microtox® Basic Test and Solid Phase Test.

AVS concentration in sediments varies seasonally (Di Toro et al., 1990, 1991), peaking in late summer and early spring. This correlates with microbial and biological activity in the sediments. Musqueam Creek AVS levels peaked in late summer, (August). However, in Still Creek, AVS dropped off during the summer, and by October, had increased again. Because each site was so different in the physical and chemical make-up of the sediment, a seasonal trend cannot be examined for
CHAPTER 4 RESULTS AND DISCUSSION

Still Creek sediment. Each site would need to be tested over a long period, as was done for Musqueam Creek. An investigation of seasonal changes was beyond the scope of this study.

Table 4.14 Total SEM/AVS ratios in Still Creek and Musqueam Creek (reference) sediments. Sum of metals includes lead, copper, zinc, nickel and cadmium.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TOTAL SEM (umoles/g dry wt)</th>
<th>AVS CONC. (umoles/g dry wt)</th>
<th>RATIO SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>1.586</td>
<td>0.653</td>
<td>2.43</td>
</tr>
<tr>
<td>2S</td>
<td>1.525</td>
<td>0.189</td>
<td>8.08</td>
</tr>
<tr>
<td>3S</td>
<td>1.120</td>
<td>0.076</td>
<td>14.69</td>
</tr>
<tr>
<td>4S</td>
<td>5.556</td>
<td>0.644</td>
<td>8.63</td>
</tr>
<tr>
<td>5S</td>
<td>0.990</td>
<td>0.223</td>
<td>4.45</td>
</tr>
<tr>
<td>1M</td>
<td>0.134</td>
<td>0.023</td>
<td>5.79</td>
</tr>
<tr>
<td>2M</td>
<td>0.134</td>
<td>0.050</td>
<td>2.68</td>
</tr>
<tr>
<td>3M</td>
<td>0.129</td>
<td>0.100</td>
<td>1.29</td>
</tr>
<tr>
<td>4M</td>
<td>0.064</td>
<td>0.046</td>
<td>1.38</td>
</tr>
<tr>
<td>5M</td>
<td>0.026</td>
<td>0.009</td>
<td>2.77</td>
</tr>
</tbody>
</table>

A study of the seasonal and spatial concentrations of AVS in lake sediments (Howard and Evans, 1993) showed that AVS concentrations change corresponding to anoxic conditions. Stream environments are very dynamic, and may be more or less anoxic due to changes in flow, for example. Thus, changes in the AVS concentrations mean changes in metal bioavailability, and changes in the AVS concentration due to storage of sediment would also mean a change in toxicity.
CHAPTER 4  RESULTS AND DISCUSSION

It was found that SEM/AVS ratios (meaning the sum of the SEM metals) did not correlate well (using regression analysis) with the chironomid 14-day test. The highest ratio was between chironomid mortality in Musqueam Creek cores and SEM/AVS ratio \(r^2 = .619\) (Table 4.15)

Table 4.15 Multiple linear regression analysis between chironomid toxicity test endpoints and SEM/AVS ratio.

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>Cu/AVS</th>
<th>Cu + Ni/AVS</th>
<th>Cu + Ni + Zn/AVS</th>
<th>SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ &amp; STILL</td>
<td>0.123</td>
<td>0.127</td>
<td>0.361</td>
<td>0.34</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.167</td>
<td>0.589</td>
<td>0.712</td>
<td>0.619</td>
</tr>
<tr>
<td>STILL</td>
<td>0.001</td>
<td>0.151</td>
<td>0.054</td>
<td>0.068</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>Cu/AVS</th>
<th>Cu + Ni/AVS</th>
<th>Cu + Ni + Zn/AVS</th>
<th>SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ &amp; STILL</td>
<td>0.245</td>
<td>0.356</td>
<td>0.379</td>
<td>0.076</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.049</td>
<td>0.667</td>
<td>0.667</td>
<td>0.07</td>
</tr>
<tr>
<td>STILL</td>
<td>0.306</td>
<td>0.496</td>
<td>0.999</td>
<td>0.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>Cu/AVS</th>
<th>Cu + Ni/AVS</th>
<th>Cu + Ni + Zn/AVS</th>
<th>SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ &amp; STILL</td>
<td>0.261</td>
<td>0.309</td>
<td>0.314</td>
<td>0.317</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.356</td>
<td>0.499</td>
<td>0.632</td>
<td>0.77</td>
</tr>
<tr>
<td>STILL</td>
<td>0.003</td>
<td>0.135</td>
<td>0.136</td>
<td>0.068</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>Cu/AVS</th>
<th>Cu + Ni/AVS</th>
<th>Cu + Ni + Zn/AVS</th>
<th>SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ &amp; STILL</td>
<td>0.066</td>
<td>0.134</td>
<td>0.23</td>
<td>0.201</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.122</td>
<td>0.862</td>
<td>0.956</td>
<td>0.097</td>
</tr>
<tr>
<td>STILL</td>
<td>0.003</td>
<td>0.387</td>
<td>0.527</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Grey shading highlights those variables where the R2 value is greater than .8

When multiple regression analysis is done, it appears the combination of Cu, Ni, and Zn/AVS ratio is the best indicator of toxic sediment (Table 4.15).

The previous statistical analysis assumes a linear relation. However, the relationship between SEM/AVS and toxicity is not linear, and we therefore would not expect a linear correlation between the results of this analysis and any other test. When the metal/AVS ratio is less than one, there is theoretically little or no toxicity due to
CHAPTER 4 RESULTS AND DISCUSSION

metals. When this ratio exceeds 1, there are more bioavailable metals in the sediment since they are not bound as insoluble sulfides and toxicity is expected to increase.

When Spearman's correlation coefficients are calculated, there is a correlation between lead/AVS ratio and chironomid mortality, and between nickel/AVS and chironomid dry weight in Musqueam (reference) sediments (Table 4.16). There was also a correlation of 0.975 between the total SEM/AVS ratio and chironomid mortality in reference mixed sediments.

Table 4.16 Spearman's correlation coefficients and metal/AVS ratios.

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>Nickel AVS</th>
<th>Lead AVS</th>
<th>Zinc AVS</th>
<th>Copper AVS</th>
<th>SEM/AVS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES MUSQ&amp;STILL</td>
<td>0.049</td>
<td>0.328</td>
<td>0.529</td>
<td>0.524</td>
<td>0.657</td>
<td>0.610</td>
</tr>
<tr>
<td>MUSQ</td>
<td>-0.500</td>
<td>-0.300</td>
<td>-0.600</td>
<td>-0.369</td>
<td>0.300</td>
<td>-0.564</td>
</tr>
<tr>
<td>STILL</td>
<td>0.500</td>
<td>0.500</td>
<td>0.700</td>
<td>0.500</td>
<td>-0.300</td>
<td>0.400</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>Nickel AVS</th>
<th>Lead AVS</th>
<th>Zinc AVS</th>
<th>Copper AVS</th>
<th>SEM/AVS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES MUSQ&amp;STILL</td>
<td>-0.251</td>
<td>-0.771</td>
<td>-0.398</td>
<td>-0.500</td>
<td>-0.355</td>
<td>-0.402</td>
</tr>
<tr>
<td>MUSQ</td>
<td>-0.821</td>
<td>-0.462</td>
<td>0.462</td>
<td>0.158</td>
<td>0.462</td>
<td>0.290</td>
</tr>
<tr>
<td>STILL</td>
<td>0.410</td>
<td>-0.410</td>
<td>-0.154</td>
<td>-0.410</td>
<td>0.410</td>
<td>0.716</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>Nickel AVS</th>
<th>Lead AVS</th>
<th>Zinc AVS</th>
<th>Copper AVS</th>
<th>SEM/AVS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED MUSQ&amp;STILL</td>
<td>-0.266</td>
<td>0.079</td>
<td>0.371</td>
<td>0.753</td>
<td>0.776</td>
<td>0.576</td>
</tr>
<tr>
<td>MUSQ</td>
<td>-0.667</td>
<td>-0.975</td>
<td>-0.103</td>
<td>0.658</td>
<td>0.975</td>
<td>0.237</td>
</tr>
<tr>
<td>STILL</td>
<td>-0.100</td>
<td>0.600</td>
<td>0.300</td>
<td>0.600</td>
<td>0.200</td>
<td>0.100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>Nickel AVS</th>
<th>Lead AVS</th>
<th>Zinc AVS</th>
<th>Copper AVS</th>
<th>SEM/AVS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED MUSQ&amp;STILL</td>
<td>-0.384</td>
<td>-0.427</td>
<td>-0.079</td>
<td>-0.410</td>
<td>-0.518</td>
<td>-0.407</td>
</tr>
<tr>
<td>MUSQ</td>
<td>-0.700</td>
<td>-0.200</td>
<td>0.500</td>
<td>-0.205</td>
<td>0.200</td>
<td>0.205</td>
</tr>
<tr>
<td>STILL</td>
<td>-0.200</td>
<td>0.200</td>
<td>0.500</td>
<td>0.200</td>
<td>-0.600</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Shading highlights those variables where the Spearman's correlation coefficient is greater than 0.8
CHAPTER 4  RESULTS AND DISCUSSION

Other researchers (e.g. Carlson et al., 1991; Di Toro et al., 1992) were able to show low or no toxicity in sediments with a metal/AVS ratio of less than one, and an increase in mortality once the ratio exceeded 1 in a single step curve. They measured cadmium and/ or nickel levels, and demonstrated that it was possible to predict when a sediment will NOT be acutely toxic (Di Toro et al., 1992).

Only one site in Still Creek (2S) had a nickel/AVS ratio of >1 (1.55) (See Appendix XI). Chironomids had a low survival rate (36% in mixed sediments, 29% in cores), and had low dry weights when grown in sediments from this site. *Daphnia magna* were not sensitive to this site. All other sites had Ni/AVS ratios <1, and some were more toxic to the chironomids than 2S.

Ankley et al. (1991b) found that samples with a SEM (cadmium + nickel)/AVS ratio greater than one were consistently toxic to *Hyalella*. However, they did not purge their samples with nitrogen before transporting them to the laboratory for analysis. Samples were also stored for an unspecified length of time at 4 °C. Bennett and Cubbage (1992b) found that the Cu/AVS ratios changed from 5 to 130 within 10 days (unspecified storage conditions). Ankley et al. (1991b) stored sediments for toxicity testing for 3 to 4 months before chemical analysis and toxicity tests were performed. Storage of sediments for extended periods can also alter the toxicity (Burton, 1991), and it has been recommended that sediments be stored for less than two weeks if they are to be used for toxicity testing.
Ankley et al. (1993) showed that Cu/AVS ratios of less than 1 accurately predicted nontoxic sediments. However, when Cu/AVS ratio exceeded 1, there was no consistency in toxicity. Some samples were toxic while some were not, even though ratios exceeded 1.

Three out of five sites in Still Creek had copper/AVS ratios <1, and toxicity varied. However, the site with the lowest chironomid survival (4S) was one of the sites with a ratio >1. Site 4S had a Cu/AVS ratio of 1.13. This site was chronically and acutely toxic to chironomids. Site 4S was also the most toxic to the Microtox® SPT (15 min EC50 of 0.4%). The site with the highest copper/AVS ratio was 3S, which also was toxic to chironomids and daphnids. Bennett and Cubbage (1992b) found that there was no significant decrease in Chironomus tentans survival or dry weight with any of the sediments tested, indicating C. tentans is not sensitive to copper toxicity. Any toxicity at site 3S was not exclusively linked to copper, as the site was also high in ammonia, lead, and PAH concentrations. Site 3S was also toxic to the Microtox® bacteria in the SPT test (15 minute EC50 of 3%).

A study of lake sediments and Cu/AVS ratios also showed that a high ratio did not necessarily indicate toxic conditions (Bennett and Cubbage, 1992b). Cu/AVS ratios ranged from 0.3 to 59. Sediments with a ratio of 0.6 showed the greatest number of significant toxic effects, although these effects were not conclusively linked to copper. A high ratio did not necessarily indicate toxic sediments. A possible
CHAPTER 4 RESULTS AND DISCUSSION

explanation is that the copper is not necessarily free copper (Cu\(^{2+}\)), and that binding phases other than AVS may control copper availability in sediments. These phases are not yet defined. For example, the sediments in the Bennett and Cubbage (1992b) study were lake sediments which had been contaminated due to copper additions to kill algae. Still Creek sediments are from a flowing shallow stream bed, and the source of the copper is from less specific sources such as copper pipes or street sediments, indicating different sediment chemistry and thus different controlling factors in metal availability. The Bennett and Cubbage (1992b) study found sediment pore water copper to be a better indicator of toxicity. Pore water metal analysis was not done on Still Creek sediments in this investigation.

As mentioned, all ratios of total SEM to AVS in this study exceeded 1, even in the reference sediments. Thus, it was impossible to predict sediment toxicity based on this ratio. Thus SEM/AVS ratios alone is not a sufficient means of predicting metal bioavailability or toxicity in freshwater sediments. Individual metal/AVS ratios are better at predicting toxic sediments. Cu/AVS seems to be the best indicator of potentially toxic sediments. When the ratio was > 1, sediments were toxic, but toxicity was not conclusively due to the copper.

4.7 METAL CONCENTRATIONS AND TOXICITY

Three different methods were used to extract metals from sediments used in the toxicity tests, as discussed previously.
CHAPTER 4 RESULTS AND DISCUSSION

*Daphnia magna* are affected by metal ions in the following order of decreasing toxicity: Hg>Ag>Cu>Zn>Cr>Cd>Pb>Ni (Khangarot and Ray, 1987). *Chironomus tentans* are influenced by metal ions in the following order of decreasing toxicity: Ag>Hg>Cu>As>Cd>Zn>Cr>Pb>Co>Ni (Khangarot and Ray, 1989). The toxicity of silver (Ag) and mercury (Hg) were reported as 6683 and 2397 times (respectively) more toxic to chironomids (48 hr EC50) than Ni. It would be expected that the sediments containing the highest levels of mercury or silver would be more toxic to daphnids and chironomids. Kszos et al. (1992) also showed *Daphnia magna* to be insensitive to nickel at concentrations of up to 160 ug/L in 21 day exposures. Timmermans et al. (1992) found Cd, Zn, Pb and Cu were readily accumulated by *Chironomus riparius*, and growth was impaired.

Trace metal uptake is thought to be associated with nutrient uptake in the digestive tract or with body diffusion across the body-covering of chironomid larvae. Wentzel et al. (1977) also found a relationship between sediment metal concentration and mean length and weight of chironomid larvae.

The ICP analysis of total sediments included 32 metals, (See Appendix VI, Table 1). There was no mercury (Hg) (detection limit 1 ug/g dry weight) detected in any of the sediments analyzed. The highest levels of silver (Ag) were found in sediment from site 4S. This site was very toxic to *chironomids* and to Microtox® SPT, but not to *Daphnia*. This may be due to the low solubility of the metal, and also because the
overlying water in the toxicity tests was changed every second day, which would not allow for any metals to accumulate in the water column. Sediments from site 4S also had the highest levels of copper and zinc as measured by ICP analysis. Site 5S had the highest chromium and nickel levels. This site was not toxic to *Daphnia*, but was toxic to chironomids, especially in the mixed sediments, which were more toxic to chironomids than the cores.

Enserink et al. (1991) showed that the combined toxicity of eight metals to *D. magna* in water was additive. In the research presented here, *Daphnia* were found to be unresponsive to sediment metal concentrations. Metals were not allowed to accumulate in the test vessels, as the overlying water was replaced every second day.

The metal concentrations in the sediments from the <63 um sediment fraction were shown in Table 4.6. Copper, zinc and nickel were highest in site 5S sediments, which were toxic to chironomids but not *Daphnia*. Chromium and lead concentrations were highest at site 4S. These metal toxicities are quite similar to those found by ICP analysis, although overall metal concentrations are less when only the <63 um fraction is analyzed. This site was acutely toxic to chironomids (i.e. there was low survival).

The < 63 um metals and SEM were slightly elevated in sample 3M, which was sampled in August after an extended period of no rain. It appears the overall metal
concentrations did not change, but that there was an increase in the easily extracted metals (SEM) and metals in the fine particles (<63 um).

Linear regression was calculated for < 63 um and ICP metal concentrations (Table 4.17). Overall, chironomid mortality and dry weight did not correlate well with either ICP metals or < 63 um metal concentrations. This was also found to be the case by Tay et al. (1992) and Giesy et al. (1988). The highest single metal correlation was between chironomid dry weight (mixed sediment) and < 63 um fraction zinc. \( r^2 = .944 \). Nickel concentration correlated more often than the other metals with chironomid toxicity test end points.

No correlation existed between total ICP metal concentration or the sum of the <63 um fraction metals, and any of the endpoints tested using both linear regression analysis and Spearman's correlation coefficients.

Using Spearman's correlation, there were more correlations between single metal concentrations and chironomid toxicity test end points than with linear correlation, particularly ICP total sediment chromium and chironomid dry weight in the mixed sediments (Table 4.18). As expected, there was a greater number of correlations when more than one metal is considered in the multiple regression analysis.
Table 4.17 Linear regression analysis ($r^2$ values) for *chironomid* mortality and dry weight, and metal concentrations.

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>&lt;63um Chromium</th>
<th>&lt;63um Copper</th>
<th>&lt;63um Lead</th>
<th>&lt;63um Nickel</th>
<th>&lt;63 um SUM ALL</th>
<th>CP - Total Chromium</th>
<th>CP - Total Copper</th>
<th>CP - Total Lead</th>
<th>CP - Total Nickel</th>
<th>ICP - Total Zinc</th>
<th>ICP - Total SUM ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td>0.466</td>
<td>0.058</td>
<td>0.727</td>
<td>0.306</td>
<td>0.372</td>
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<td>0.637</td>
<td>0.47</td>
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<tr>
<td>MUSQ&amp;STILL</td>
<td>0.816</td>
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<td>0.263</td>
<td>0.062</td>
<td>0.059</td>
<td>0.24</td>
<td>0.329</td>
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<td>0.058</td>
<td>0.023</td>
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<td>0.153</td>
<td>0.744</td>
<td>0.256</td>
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<td>0.811</td>
</tr>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chironomid Dry Weight</td>
<td>&lt;63um Chromium</td>
<td>&lt;63um Copper</td>
<td>&lt;63um Lead</td>
<td>&lt;63um Nickel</td>
<td>&lt;63 um SUM ALL</td>
<td>CP - Total Chromium</td>
<td>CP - Total Copper</td>
<td>CP - Total Lead</td>
<td>CP - Total Nickel</td>
<td>ICP - Total Zinc</td>
<td>ICP - Total SUM ALL</td>
</tr>
<tr>
<td>CORES</td>
<td>0.245</td>
<td>0.237</td>
<td>0.088</td>
<td>0.378</td>
<td>0.467</td>
<td>0</td>
<td>0.413</td>
<td>0.273</td>
<td>0.216</td>
<td>0.234</td>
<td>0.155</td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.079</td>
<td>0.245</td>
<td>0.078</td>
<td>0.055</td>
<td>0.072</td>
<td>0.131</td>
<td>0.607</td>
<td>0.26</td>
<td>0.038</td>
<td>0.201</td>
<td>0.025</td>
</tr>
<tr>
<td>STILL</td>
<td>0.001</td>
<td>0.059</td>
<td>0.229</td>
<td>0.001</td>
<td>0.011</td>
<td>0.025</td>
<td>0.198</td>
<td>0.312</td>
<td>0.002</td>
<td>0.096</td>
<td>0.612</td>
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<td></td>
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</tr>
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<td>Chironomid Mortality</td>
<td>&lt;63um Chromium</td>
<td>&lt;63um Copper</td>
<td>&lt;63um Lead</td>
<td>&lt;63um Nickel</td>
<td>&lt;63 um SUM ALL</td>
<td>CP - Total Chromium</td>
<td>CP - Total Copper</td>
<td>CP - Total Lead</td>
<td>CP - Total Nickel</td>
<td>ICP - Total Zinc</td>
<td>ICP - Total SUM ALL</td>
</tr>
<tr>
<td>MIXED</td>
<td>0.343</td>
<td>0.004</td>
<td>0.364</td>
<td>0.361</td>
<td>0.12</td>
<td>0.034</td>
<td>0.147</td>
<td>0.364</td>
<td>0.256</td>
<td>0.243</td>
<td>0.311</td>
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<tr>
<td>MUSQ&amp;STILL</td>
<td>0.253</td>
<td>0.068</td>
<td>0.474</td>
<td>0.861</td>
<td>0.719</td>
<td>0.275</td>
<td>0.607</td>
<td>0.251</td>
<td>0.186</td>
<td>0.268</td>
<td>0.124</td>
</tr>
<tr>
<td>STILL</td>
<td>0.062</td>
<td>0.497</td>
<td>0.341</td>
<td>0.892</td>
<td>0.274</td>
<td>0.004</td>
<td>0.454</td>
<td>0.108</td>
<td>0.008</td>
<td>0.577</td>
<td>0.008</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chironomid Dry Weight</td>
<td>&lt;63um Chromium</td>
<td>&lt;63um Copper</td>
<td>&lt;63um Lead</td>
<td>&lt;63um Nickel</td>
<td>&lt;63 um SUM ALL</td>
<td>CP - Total Chromium</td>
<td>CP - Total Copper</td>
<td>CP - Total Lead</td>
<td>CP - Total Nickel</td>
<td>ICP - Total Zinc</td>
<td>ICP - Total SUM ALL</td>
</tr>
<tr>
<td>MIXED</td>
<td>0.053</td>
<td>0.011</td>
<td>0.123</td>
<td>0.184</td>
<td>0</td>
<td>0.069</td>
<td>0.778</td>
<td>0.2</td>
<td>0.006</td>
<td>0.61</td>
<td>0.1</td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.441</td>
<td>0.094</td>
<td>0.202</td>
<td>0</td>
<td>0.016</td>
<td>0.122</td>
<td>0.847</td>
<td>0.31</td>
<td>0.037</td>
<td>0.264</td>
<td>0.163</td>
</tr>
<tr>
<td>STILL</td>
<td>0.229</td>
<td>0.731</td>
<td>0</td>
<td>0.286</td>
<td>0.944</td>
<td>0.697</td>
<td>0.775</td>
<td>0.731</td>
<td>0.488</td>
<td>0.811</td>
<td>0.185</td>
</tr>
</tbody>
</table>

Grey shading highlights those variables where the R2 value is greater than .8.
Table 4.18 Spearman's correlation coefficients for metal concentrations and *Chironomus tentans* end points.

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>&lt;63um Chromium</th>
<th>&lt;63um Copper</th>
<th>&lt;63um Nickel</th>
<th>&lt;63um Zinc</th>
<th>&lt;63 um SUM ALL</th>
<th>ICP - Total Chromium</th>
<th>ICP - Total Copper</th>
<th>ICP - Total Nickel</th>
<th>ICP - Total Zinc</th>
<th>ICP - Total SUM ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td>MUSQ&amp;STILL</td>
<td>0.833</td>
<td>0.225</td>
<td>0.829</td>
<td>0.517</td>
<td>0.511</td>
<td>0.304</td>
<td>0.043</td>
<td>0.637</td>
<td>0.775</td>
</tr>
<tr>
<td></td>
<td>MUSQ</td>
<td>0.790</td>
<td>0.300</td>
<td>0.200</td>
<td>-0.300</td>
<td>-0.600</td>
<td>-0.600</td>
<td>-0.600</td>
<td>0.500</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>STILL</td>
<td>0.500</td>
<td>-0.200</td>
<td>0.072</td>
<td>-0.600</td>
<td>0.500</td>
<td>0.300</td>
<td>-0.200</td>
<td>0.500</td>
<td>0.600</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>&lt;63um Chromium</th>
<th>&lt;63um Copper</th>
<th>&lt;63um Nickel</th>
<th>&lt;63um Zinc</th>
<th>&lt;63 um SUM ALL</th>
<th>ICP - Total Chromium</th>
<th>ICP - Total Copper</th>
<th>ICP - Total Nickel</th>
<th>ICP - Total Zinc</th>
<th>ICP - Total SUM ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td>MUSQ&amp;STILL</td>
<td>-0.471</td>
<td>-0.544</td>
<td>-0.500</td>
<td>-0.614</td>
<td>-0.614</td>
<td>-0.026</td>
<td>-0.734</td>
<td>-0.650</td>
<td>-0.631</td>
</tr>
<tr>
<td></td>
<td>MUSQ</td>
<td>0.410</td>
<td>-0.359</td>
<td>0.051</td>
<td>0.359</td>
<td>-0.205</td>
<td>0.564</td>
<td>-0.667</td>
<td>-0.053</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>STILL</td>
<td>0.154</td>
<td>-0.462</td>
<td>0.263</td>
<td>-0.051</td>
<td>-0.051</td>
<td>-0.205</td>
<td>0.154</td>
<td>0.154</td>
<td>-0.051</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>&lt;63um Chromium</th>
<th>&lt;63um Copper</th>
<th>&lt;63um Nickel</th>
<th>&lt;63um Zinc</th>
<th>&lt;63 um SUM ALL</th>
<th>ICP - Total Chromium</th>
<th>ICP - Total Copper</th>
<th>ICP - Total Nickel</th>
<th>ICP - Total Zinc</th>
<th>ICP - Total SUM ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED</td>
<td>MUSQ&amp;STILL</td>
<td>0.711</td>
<td>-0.310</td>
<td>0.713</td>
<td>0.505</td>
<td>0.006</td>
<td>0.195</td>
<td>0.328</td>
<td>0.479</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td>MUSQ</td>
<td>0.664</td>
<td>-0.718</td>
<td>0.664</td>
<td>0.716</td>
<td>0.308</td>
<td>0.309</td>
<td>-0.401</td>
<td>-0.395</td>
<td>0.544</td>
</tr>
<tr>
<td></td>
<td>STILL</td>
<td>0.400</td>
<td>-0.700</td>
<td>0.410</td>
<td>-0.900</td>
<td>-0.300</td>
<td>0.200</td>
<td>-0.900</td>
<td>-0.051</td>
<td>0.400</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>&lt;63um Chromium</th>
<th>&lt;63um Copper</th>
<th>&lt;63um Nickel</th>
<th>&lt;63um Zinc</th>
<th>&lt;63 um SUM ALL</th>
<th>ICP - Total Chromium</th>
<th>ICP - Total Copper</th>
<th>ICP - Total Nickel</th>
<th>ICP - Total Zinc</th>
<th>ICP - Total SUM ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td>MUSQ&amp;STILL</td>
<td>-0.244</td>
<td>-0.128</td>
<td>-0.312</td>
<td>-0.323</td>
<td>-0.079</td>
<td>-0.072</td>
<td>-0.517</td>
<td>-0.396</td>
<td>-0.856</td>
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<tr>
<td></td>
<td>MUSQ</td>
<td>0.700</td>
<td>0.030</td>
<td>0.300</td>
<td>0.000</td>
<td>-0.100</td>
<td>0.200</td>
<td>-0.390</td>
<td>-0.054</td>
<td>-0.354</td>
</tr>
<tr>
<td></td>
<td>STILL</td>
<td>0.300</td>
<td>0.900</td>
<td>0.308</td>
<td>0.500</td>
<td>0.900</td>
<td>0.600</td>
<td>-0.300</td>
<td>0.300</td>
<td>0.700</td>
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</tbody>
</table>

Grey shading highlights those variables where the absolute value of $R^2$ is greater than 0.8
Table 4.19 Multiple linear regression analysis of chironomid toxicity, <63 um fraction metals, and total metal concentration (ICP analysis).

<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>METALS (&lt;63um fraction)</th>
<th>METALS (ICP, TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
<td>Cr+Cu</td>
</tr>
<tr>
<td>Chironomid Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.466</td>
<td>0.469</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.816</td>
<td>0.899</td>
</tr>
<tr>
<td>STILL</td>
<td>0.193</td>
<td>0.216</td>
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<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>METALS (&lt;63um fraction)</th>
<th>METALS (ICP, TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
<td>Cr+Cu</td>
</tr>
<tr>
<td>Chironomid Dry Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CORES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.245</td>
<td>0.338</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.079</td>
<td>0.246</td>
</tr>
<tr>
<td>STILL</td>
<td>0.001</td>
<td>0.06</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>METALS (&lt;63um fraction)</th>
<th>METALS (ICP, TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
<td>Cr+Cu</td>
</tr>
<tr>
<td>Chironomid Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.343</td>
<td>0.387</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.253</td>
<td>0.616</td>
</tr>
<tr>
<td>STILL</td>
<td>0.062</td>
<td>0.572</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>METALS (&lt;63um fraction)</th>
<th>METALS (ICP, TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cr</td>
<td>Cr+Cu</td>
</tr>
<tr>
<td>Chironomid Dry Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.053</td>
<td>0.105</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.441</td>
<td>0.445</td>
</tr>
<tr>
<td>STILL</td>
<td>0.229</td>
<td>0.932</td>
</tr>
</tbody>
</table>

Grey shading highlights those variables where the R2 value is greater than .8
CHAPTER 4 RESULTS AND DISCUSSION

The ICP individual metal concentrations seem to be a better indicator of sediment toxicity, especially for the mixed sediment samples (Table 4.19).

SEM concentration did not correlate with the chironomid end points. (Table 4.20 ).

Table 4.20 Multiple linear regression analysis of SEM, OM and PAH, and *Chironomus tentans* end points.

<table>
<thead>
<tr>
<th></th>
<th>Chironomid Mortality</th>
<th>Chironomid Dry Weight</th>
<th>Chironomid Mortality</th>
<th>Chironomid Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEM</td>
<td>SEM+OM</td>
<td>SEM</td>
<td>SEM+OM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+PAH</td>
<td></td>
<td>+PAH</td>
</tr>
<tr>
<td>CORES</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.698</td>
<td>0.699</td>
<td>0.725</td>
<td>0.357</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.001</td>
<td>0.001</td>
<td>N/A</td>
<td>0.872</td>
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<tr>
<td>STILL</td>
<td>0.745</td>
<td>0.774</td>
<td>0.775</td>
<td>0.872</td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.051</td>
<td>0.166</td>
<td>0.384</td>
<td>0.149</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.033</td>
<td>0.252</td>
<td>N/A</td>
<td>0.027</td>
</tr>
<tr>
<td>STILL</td>
<td>0.418</td>
<td>0.511</td>
<td>0.936</td>
<td>0.257</td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.314</td>
<td>0.458</td>
<td>0.547</td>
<td>0.149</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.357</td>
<td>0.364</td>
<td>N/A</td>
<td>0.027</td>
</tr>
<tr>
<td>STILL</td>
<td>0.012</td>
<td>0.872</td>
<td>0.972</td>
<td>0.257</td>
</tr>
</tbody>
</table>

Grey shading highlights those variables where $R^2$ is greater than 0.8

When PAH and organic matter (OM) are also brought into the calculations, there is a
correlation with Still Creek sediment PAH, SEM and OM and chironomid bioassay end points.

4.8 SEDIMENT PAH

The predominant PAH present in Still Creek sediment were phenanthrene, anthracene, fluoranthene, and pyrene (Table 4.3). PAH levels in Musqueam Creek (reference) sediment were below the detection levels.

PAH levels were analyzed by linear regression to see if any of the chronic or acute endpoints investigated in the toxicity tests correlated with the PAH concentration. PAH concentration did not correlate with the chironomid data. (The highest correlation was $r^2 = .719$, between chironomid mortality in Still Creek core sediments and PAH concentration.)

Using Spearman's correlation analysis (Table 4.22), there was again no correlation between sediment PAH concentration and the chironomid data.

However, when multiple regression was performed on the PAH, organic matter, and ammonia concentration were included, the correlation values increase (Table 4.21). This is also true for SEM, PAH and OM.

Landrum et al (1991) found that sediment concentrations of total PAH in the range of 100 ug/g dry weight were required to produce mortality in amphipods. The highest PAH concentration in the sediments from Still Creek was 14.5 ug/g dry weight (site
4S). Thus, PAH were probably not solely responsible for chironomid mortality, but may have played a role in the chronic toxicity to chironomids (decreased dry weight). Bennett and Cubbage (1992a) found PAH concentrations of up to 33000 mg/Kg had no effect on chironomid survival.

Table 4.21 Multiple linear correlation analysis, $r^2$ values for PAH, ammonia and sulfide.

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>SULFIDE</th>
<th>PAH</th>
<th>ammonia</th>
<th>organic matter (OM)</th>
<th>PAH + OM</th>
<th>PAH + OM + ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.34</td>
<td>N/A</td>
<td>0.11</td>
<td>0.065</td>
<td>0.557</td>
<td>0.604**</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.619</td>
<td>N/A</td>
<td>0.964</td>
<td>0.317</td>
<td>N/A</td>
<td>0.970**</td>
</tr>
<tr>
<td>STILL</td>
<td>0.067</td>
<td>0.719</td>
<td>0</td>
<td>0.012</td>
<td>0.764</td>
<td>0.776</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>SULFIDE</th>
<th>PAH</th>
<th>ammonia</th>
<th>organic matter (OM)</th>
<th>PAH + OM</th>
<th>PAH + OM + ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.076</td>
<td>N/A</td>
<td>0.232</td>
<td>0.158</td>
<td>0.162</td>
<td>0.468**</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.069</td>
<td>N/A</td>
<td>0.02</td>
<td>0.05</td>
<td>N/A</td>
<td>0.381*</td>
</tr>
<tr>
<td>STILL</td>
<td>0.239</td>
<td>0.323</td>
<td>0.22</td>
<td>0.069</td>
<td>0.432</td>
<td>0.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Mortality</th>
<th>SULFIDE</th>
<th>PAH</th>
<th>ammonia</th>
<th>organic matter (OM)</th>
<th>PAH + OM</th>
<th>PAH + OM + ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.317</td>
<td>N/A</td>
<td>0.1</td>
<td>0.304</td>
<td>0.38</td>
<td>0.494**</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.769</td>
<td>N/A</td>
<td>0.255</td>
<td>0.217</td>
<td>N/A</td>
<td>0.788*</td>
</tr>
<tr>
<td>STILL</td>
<td>0.058</td>
<td>0.014</td>
<td>0.565</td>
<td>0.818</td>
<td>0.819</td>
<td>0.968</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chironomid Dry Weight</th>
<th>SULFIDE</th>
<th>PAH</th>
<th>ammonia</th>
<th>organic matter (OM)</th>
<th>PAH + OM</th>
<th>PAH + OM + ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIXED</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSQ&amp;STILL</td>
<td>0.202</td>
<td>N/A</td>
<td>0.03</td>
<td>0.52</td>
<td>0.524</td>
<td>0.533**</td>
</tr>
<tr>
<td>MUSQ</td>
<td>0.097</td>
<td>N/A</td>
<td>0.376</td>
<td>0.07</td>
<td>N/A</td>
<td>0.877*</td>
</tr>
<tr>
<td>STILL</td>
<td>0.069</td>
<td>0.332</td>
<td>0.004</td>
<td>0.666</td>
<td>0.9</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Grey shading highlights those variables where the R2 value is greater than .8

* PAH levels were below detection in Musqueam Creek, and thus are not in this calculation
** This R2 does not include a PAH concentration for Musqueam, as PAH were below detection
CHAPTER 4 RESULTS AND DISCUSSION

However, Ingersoll and Nelson (1990) used *Chironomus riparius* to test the toxicity of PAH and PCB contaminated sediments. Survival in control sediments was 70%, and survival in contaminated sediments was as low as 6%. The total PAH at one of their sampling locations was 8.5 ug/g dry weight, which is similar to PAH levels at site 4S (14.5 ug/g dry weight), the site most toxic to chironomids.

Jacobs et al. (1993) found that correlation between PAH and toxicity to Microtox® depended on the statistical method used. They showed that the total PAH concentration significantly correlated with toxicity using Spearman’s correlation, but
CHAPTER 4 RESULTS AND DISCUSSION

not with linear regression. This was also found to be true in this thesis. PAH concentration (total) did not correlate with the Microtox® SPT results using linear regression analysis, but did correlate using Spearman's correlation coefficients.

4.9 SULFIDE, AMMONIA AND TOXICITY

Ammonia concentration did not appear to be correlated with toxicity (Table 4.22) except using Spearman's correlation analysis, which showed a correlation coefficient of 0.8 between both chironomid mortality and dry weight in mixed sediments, and ammonia concentration. Schubauer-Berigan and Ankley (1991) were also unable to correlate ammonia concentrations to toxicity, and concluded that ammonia was only one of several toxicants in the sediments tested contributing to toxicity. They also noted that the fate of other toxicants such as metals could also be influenced by the same changes in oxidative conditions that affect ammonia concentration. Ammonia may also affect the bioavailability of metals. Copper and Zinc complexes such as Cu(NH₃)₄²⁺ and Zn(NH₃)₄²⁺, are quite soluble, and are thus potentially more bioavailable.

In the research presented here, toxicity due to ammonia appeared to occur in sediments from site 3S, which was toxic to Microtox®, chironomids, and daphnids.

Bennett and Cubbage, (1992a) reported toxicity to *Chironomus tentans* in sediments that were high in sulfide. They attributed some of this toxicity to the sulfides, as increased sulfide levels also corresponded to Microtox® toxicity. Sulfide was thought
CHAPTER 4 RESULTS AND DISCUSSION

not to be solely responsible for the Microtox® toxicity, as sulfide would be removed due to sediment oxidation, but high sulfide was thought to play a significant role in chironomid toxicity.

When linear correlation analysis was done on sulfide levels, it was found there was a correlation only between the reference site sulfide levels and chironomid mortality, not between Still Creek sulfide levels and chironomid mortality. Chironomid mortality in the reference mixed samples showed a greater correlation with sediment sulfide ($r^2 = 0.769$) than the cores ($r^2 = 0.619$). Note that sulfide levels in Musqueam Creek were 10-fold lower than the sulfide concentrations in Still Creek.

Sulfide concentrations did not correlate with the chironomid data using Spearman's correlation.

Bennett and Cubbage (1992a) reported a correlation between sulfide concentration and the Microtox® basic test. There was no such correlation in the research presented here, nor was there a correlation between Microtox® SPT and sulfide.

4.10 ORGANIC MATTER AND TOXICITY

Organic matter is an important parameter to measure because metals, PAH and other hydrophobic contaminants can be associated with this fraction, (Cooper and Harris, 1974). A positive linear relationship has been shown to exist between PAH concentration and organic matter content (Evans et al., 1990). This was not found for Still Creek PAH and percent organic matter, ($r^2 = 0.013$). When site 5S is
CHAPTER 4 RESULTS AND DISCUSSION

removed from the calculation, the correlation becomes 0.997. PAH at site 5S were below detection limits. The organic matter at this site was large wood fibre pieces, which resulted in a very high percent organic matter. Thus, the site was dissimilar to the others, and when removed from the correlation, the relationship between percent organic matter and PAH becomes linear, as found by Evans et al. (1990).

Bioavailability is often assumed to be inversely proportional to the sediment organic carbon content (Suedel et al., 1993). Uptake and accumulation of benzo(a)pyrene by *Daphnia magna* was shown to be reduced by 97% due to adsorption of PAH to organic matter (McCarthy, 1983). Humic acid concentration was shown to have an effect on toxicity to *Chironomus riparius* (Bruner and Fisher, 1993).

The percentage organic matter in the sediments used in toxicity testing was analyzed, and linear correlation analyses are presented in Table 4.21. The SPT Microtox® test did not correlate well with organic matter content using linear regression or Spearman's correlation. (Table 4.22)

The mortality of chironomids from Still Creek mixed sediments correlated well with organic matter content ($r^2 = .818$), but there was no correlation between chironomid mortality and Still Creek core sediments ($r^2 = .012$). There were no other significant correlations with organic matter content and the chironomid end points.

There was no correlation between reference (Musqueam Creek) sediment toxicity and the percent organic matter in either core or mixed sediments.
CHAPTER 4 RESULTS AND DISCUSSION

When multiple linear regression is examined, there is a correlation of $r^2 = .819$ between PAH + organic matter and chironomid mortality and $r^2 = .90$ between PAH + organic matter and chironomid dry weight in Still Creek mixed sediments.

4.11 LAND USE AND TOXICITY

Urban development results in an increase in runoff due to the increase in impermeable surfaces as compared to rural or forested areas. The contribution of contamination to the environment by stormwater runoff can be significant, and in an urban environment is the largest contributor of non-point source pollution. Stormwater has been shown to contain metals (Hall and Anderson, 1988) and PAH (Evans et al. 1990). The majority of contaminants associated with stormwater runoff are deposited 5 - 25 m from the road (Lygren et al., 1984), or within 15 m of the road (Johnson and Harrison, 1984). The highest concentration of particulate-associated lead was found within 10 m of the highway.

Hall and Anderson (1988) sampled stormwater runoff in the Brunette watershed, and found the highest levels of cadmium, chromium, copper, nickel, lead, and zinc occurred in runoff from industrial and commercial areas. Open and/or green space showed lower metal levels in stormwater runoff than the commercial/industrial areas.

Duynstee (1990) used Geographic Information Systems (GIS) to study land use and metal contamination in the Brunette watershed. She was able to show that lead and chromium levels in sediments have decreased between 1973 and 1989. The
CHAPTER 4 RESULTS AND DISCUSSION

decrease in lead was due to the use of unleaded fuel. Copper and zinc levels increased in this time, and was attributed to an increase in traffic volumes. Copper was shown to have a strong correlation to commercial land use and traffic. Lead levels in the 1973 data also correlated to traffic patterns. In the sediments analyzed here, lead and chromium were found to have decreased since 1976, but there had been an increase in copper and zinc.

PAH were shown to be a major component of street dust, which is washed into Still Creek during rain events. The highest total PAH and the greatest number of different PAH were found at site 4S, which was the site adjacent to Lougheed Highway. Lower levels of PAH were found at sites 1S, 2S and 3S. Sites 1S and 2S are close to major traffic routes, but are protected by narrow buffer zones of green space. Site 3S is in a residential area, and PAH may be from sources other than vehicular traffic, such as waste oil or leakage entering the system. There were no PAH above detection levels at site 5S. This site is adjacent to railway tracks, but not directly impacted by vehicular traffic.

There were no detectable PAH and only low concentrations of metals in sediments from the reference site. The reference creek was sampled upstream of the Indian reserve and golf course, but downstream of Marine Drive. The traffic volume along Marine Drive is much less than along Lougheed Highway, and overall loading of pollutants is reduced. Also, the sediment sampling location on Musqueam Creek
CHAPTER 4 RESULTS AND DISCUSSION

was more than 25 metres from the highway, and would not be affected by stormwater runoff, as reported by Lygren et al. (1984). Musqueam Creek is also surrounded by green space, which would aid in contaminant removal before they can enter the waterway.

True and Hayward (1990) showed that although marine sediments near urban environments contained high concentrations of toxic compounds, the sediments were not necessarily toxic to Microtox® using pore water and sediment extraction methods, and the Microtox® Basic test method. Toxicity depends on the bioavailability of contaminants.

In the research presented in this document, the Microtox® basic test was slightly more sensitive to Still Creek than to the reference sediment. The Microtox® SPT was, however, a better indicator of toxicity. When pore water was analyzed, toxicity as measured by the Microtox® basic test is due to water-soluble contaminants. When solvent extracts are used, information regarding the actual bioavailability to the bacteria is lost. However, the solid phase test is a sediment contact assay, and is therefore the most suitable for determining sediment toxicity.

It appears that the contaminants and the toxicity in sediments sampled and tested were impacted by the surrounding land use, as indicated by metal, PAH and overall toxicity data.
4.12 SUMMARY OF TOXICITY

The toxicity data for Still Creek and the reference site, Musqueam Creek, have been summarized in Table 4.23.

*Daphnia magna* survival was most affected by 3S cores sediments, but not the 3S mixed sediments. This is most likely due to the high ammonia levels in those sediments, and perhaps the mixing process resulted in chemical changes in the sediment, rendering it non-toxic with respect to survival. *Microtox*® basic test was also sensitive to sediment from this site. The highest SEM/AVS ratio was found in sediments from site 3S. This site also had the highest copper/AVS ratio.

Chironomid dry weight was most sensitive to site 5S. However, the culture may not have been as healthy at this time, as indicated by the reference toxicity test with CdCl₂. Chironomid survival was lowest in site 4S, and the dry weight of the surviving chironomids was also low. *Microtox*® SPT was also most sensitive to sediments from site 4S. PAH and metal levels were the highest in the sediment from site 4S.

Chironomid survival was affected by the sampling method, and in 4 out of 10 cases, survival was greater in the core samples, indicating that the mixing process increased toxicity to chironomids.

Overall, metals seem the best indicator of toxicity, especially < 63 um fraction copper. Different statistical methods (linear and multiple regression or Spearman’s
correlation) gave different results with respect to correlation of data. From the multiple correlation results, it is obvious that there was more than one factor contributing to toxicity, which is to be expected.

The results of the toxicity tests and chemical analyses indicate that no one test or parameter can be used to determine the toxicity of a sediment sample. In Still Creek sediments, it was found that the nature of the contaminants affected *Daphnia magna* toxicity more than the method of sampling. For the chironomids, the method of sampling did alter sediment toxicity at some of the sites tested. Contaminants present also affected chironomid survival and growth.

Giesy et al. (1988) found little correlation between toxicity tests. They found that none of the assays accurately predicted the results of the other two toxicity tests. Some sites were deemed toxic by one or two assays, but not toxic to the other assays.

Giesy et al. (1990) found *D. magna* LC$_{50}$ (lethality) and *C. tentans* EC$_{50}$ (growth) about equally sensitive. In the research presented here, *C. tentans* growth was found to be more sensitive than both *D. magna* survival and reproduction.

The Microtox® SPT was found to be more sensitive than the pore water test. Brouwer et al. (1990) also found this to be true. The Microtox® SPT was the most sensitive test to the sediments tested here. This test would be a useful test in an initial screening process for determining sediment toxicity. If a sediment was not toxic
Table 4.23 Summary of toxicity data for Still Creek and the reference site, Musqueam Creek.

<table>
<thead>
<tr>
<th>Musqueam Cr.</th>
<th>Daphnid 14 Survival % mixed</th>
<th>Daphnid 14d Total # young mixed</th>
<th>Chironomid Survival % mixed</th>
<th>Chironomid Dry wt (mg) mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cores</td>
<td></td>
<td>cores</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>90</td>
<td>38.4</td>
<td>73</td>
<td>2.1</td>
</tr>
<tr>
<td>2M</td>
<td>100</td>
<td>36.2</td>
<td>35</td>
<td>1.5</td>
</tr>
<tr>
<td>3M</td>
<td>100</td>
<td>19.8</td>
<td>22</td>
<td>2.0</td>
</tr>
<tr>
<td>4M</td>
<td>90</td>
<td>32.0</td>
<td>69</td>
<td>2.7</td>
</tr>
<tr>
<td>5M</td>
<td>100</td>
<td>34.1</td>
<td>73</td>
<td>1.2</td>
</tr>
<tr>
<td>Avg.</td>
<td>96.0</td>
<td>32.1</td>
<td>54.4</td>
<td>1.9</td>
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</table>

<table>
<thead>
<tr>
<th>Still Creek</th>
<th>Daphnid 14 Survival % mixed</th>
<th>Daphnid 14d Total # young mixed</th>
<th>Chironomid Survival % mixed</th>
<th>Chironomid Dry wt (mg) mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cores</td>
<td></td>
<td>cores</td>
<td></td>
</tr>
<tr>
<td>1S</td>
<td>60</td>
<td>40.3</td>
<td>30</td>
<td>1.3</td>
</tr>
<tr>
<td>2S</td>
<td>90</td>
<td>49.3</td>
<td>36</td>
<td>1.5</td>
</tr>
<tr>
<td>3S</td>
<td>100</td>
<td>50.0</td>
<td>26</td>
<td>2.0</td>
</tr>
<tr>
<td>4S</td>
<td>100</td>
<td>37.9</td>
<td>12</td>
<td>1.1</td>
</tr>
<tr>
<td>5S</td>
<td>90</td>
<td>28.3</td>
<td>13</td>
<td>0.4</td>
</tr>
<tr>
<td>Avg.</td>
<td>88.0</td>
<td>41.2</td>
<td>23.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Shading indicates significant difference between sampling methods.
CHAPTER 4 RESULTS AND DISCUSSION

to the Microtox® SPT, it was probably not toxic to the other bioassays, as seen with the reference sediments. All Still Creek sediments were toxic to Microtox® SPT, and further testing using chironomids or Daphnia was required.

The organisms selected for a battery of toxicity tests should be chosen based on the type of contaminants present. Here, the contaminants were associated with the sediment, and thus Daphnia were not affected. However, chironomids and the Microtox® SPT were suitable for the type of contaminants present.
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1 OBJECTIVES

This study set out to answer three questions, outlined in Section 2.1. These objectives were met as follows:

1. Does the sampling method affect toxicity?

   It was found that *Daphnia magna* acute (survival) and chronic (number of young) bioassays were generally insensitive to the types of contaminants found in Still Creek, and thus were not sensitive enough to indicate changes in toxicity between the two sampling methods.

   *Chironomus tentans* was more sensitive to the types of contaminants found in Still Creek, and it was found that the sampling method did affect sediment toxicity. Generally, the process of mixing (homogenizing) the sediment increased the toxicity of sediment. One exception was when ammonia levels were very high, and mixing decreased sediment toxicity which was most likely due to oxidation of the ammonia.

2. How well does SEM/AVS predict sediment toxicity?

   The ratio of the sum of the SEM metals and AVS concentration was not a good indicator of toxicity. Individual metal/AVS ratios were a better indicators, but no one metal was able to predict toxicity in all cases. Also, when metal/AVS ratios were less than 1, the sediment was not always non-toxic. Thus SEM/AVS ratio
was not a reliable predictor of sediment toxicity. Rather than measure relative ratios, it may be more informative to look at the absolute concentration of SEM above AVS which may then indicate toxicity.

3. **How sensitive is the Microtox® SPT compared to other toxicity tests?**

The Microtox® Solid Phase Test (SPT) was the most sensitive of all the toxicity tests evaluated for stream sediments. Since the reference sediments were not toxic to the Microtox® SPT but Still Creek sediments were, the test was able to distinguish between a stream with little or no contaminant loading and one with high contaminant loading. Thus, this test would be useful as an initial screening tool, or in a sediment quality monitoring program.

5.2 **CONCLUSION**

Sediment toxicity tests are used to determine if a contaminated sediment is capable of eliciting a biological effect on the aquatic ecosystem. These toxicity tests are carried out in the laboratory, which raises uncertainty as to whether the results of these tests correspond to *in situ* effects on indigenous biota. The reason for the uncertainty is that the sediment toxicity tests are carried out in an artificial environment on a narrow range of species and life stages, and thus do not mimic the natural environment. Also, as soon as the sediment is removed from the stream, its integrity has been altered. Any processes (such as mixing or sieving) done to the sediment will further alter chemical and physical properties of the sediment, casting
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

greater doubt on the validity of extrapolating results from the laboratory to the natural environment.

A common method of sampling has been to take grab samples at a study site and combine into a composite sample by mixing (or homogenizing) the sediment. The toxicity of sediment sampled by this mechanism was compared to the toxicity of relatively undisturbed sediment, collected using a small glass corer.

It was found that in about half the sites sampled there were statistical differences in the chironomid toxicity tests measured between the two sampling methods. In some cases, the end points were more sensitive to the sample method than to the site sampled (i.e. endpoints did not discriminate between the test site and the reference site).

*Daphnia magna* and *Chironomus tentans* were the two species used to test for differences in toxicity of a sediment sample using two methods. Endpoints measured for the *Daphnia magna* test were survival, number of young per brood, and total number of young. Survival was not a sensitive endpoint for the type of contaminants present in Still Creek. Total number of young and number of young per brood were more sensitive endpoints, and showed a greater number of significant differences between Still Creek and reference sediments. Overall, *Daphnia magna* were not affected as much by the sampling method as they were by the contaminants present.
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

Chironomus tentans were more sensitive to the sediments tested than Daphnia magna, probably because they were in direct contact with the sediment not only as a habitat, but as a food source. The endpoints measured in the chironomid test were survival (acute) and growth (chronic). Both showed measurable responses. Chironomids were most sensitive to site 5S (growth) and site 4S (acute). Metal levels were elevated at both site 4S and 5S. PAH were elevated at site 4S, but were below detection levels at site 5S.

Malueg et al. (1983) found that the presence of Hexagenia intensified Daphnia magna response due to physical activity of Hexagenia in the sediments. This was not found in this research between chironomids and Daphnia, probably due to the nature of the contaminants in the sediment. Bennett and Cubbage (1992c) found that migration of sediment bound toxicants to the water column must occur before Daphnia magna would demonstrate sediment toxicity. No such migration occurred in their tests, rendering these organisms ineffective as toxicity indicators under these circumstances.

Giesy et al. (1990) found that a sediment dilution causing a 50% reduction in C. tentans weight and the LC50 concentration for the D. magna 48 hr lethality test were not statistically different, indicating similar sensitivity to a particular sediment sample. It was found in this study that chironomids were more sensitive than the Daphnia, and that Microtox ® SPT was the most sensitive. Again, this might be due to the
nature of the contaminants found in Still Creek. Santiago et al. (1993) also found \textit{Daphnia} the least sensitive of the assays they performed, which included Microtox®, and Burton (1989) found no toxicity in water and sediment tests to \textit{Daphnia magna}. Burton and Scott (1992) ranked sediment toxicity tests according to sensitivity, range and discriminatory ability. They found \textit{D. magna} 7 day reproduction and \textit{Chironomus tentans} 10 day survival ranked highest.

Other toxicity tests done on sediment samples were a pore water acute toxicity test using \textit{Daphnia magna}, and a Microtox® basic test on the pore water. None of these toxicity tests were sensitive to the sediment pore water, except at site 3S, the Still Creek site which had very high ammonia concentrations. The solid phase Microtox® test (SPT) was also performed and was considered a more sensitive test than the Microtox® basic test, as the bacteria responded to all five Still Creek sediments, and was sensitive to one of five Musqueam Creek sediment samples.

Overall, metal levels were the best indicator of toxicity. The <63 um fraction metals correlated with chironomid end points more often in the core samples, while total metals (ICP metals) correlated with chironomid endpoints more often in mixed sediments. Site 4S exceeded the sediment quality criteria in 6 out of 7 of the metals shown (Table 4.6). The only metal which did not exceed the criteria was cadmium. The most toxic site to the chironomids (chronic) was 5S, which had very high copper (< 63 um fraction). PAH levels at this site were below detection. Toxicity, however is
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

not due to copper alone. There were also some unidentified peaks (GC analysis) which may or may not have contributed to toxicity. The site most toxic to chironomids (acute) was site 4S. This site exceeded the PAH sediment quality criteria for 9 individual PAH, and for total PAH. Site 1S was acutely and chronically toxic to chironomids, and exceeded the PAH sediment quality criteria for 3 individual PAH and had elevated metals.

The SEM/AVS ratio was not a good indicator of toxicity. The ratio of the sum of the SEM (Zn, Pb, Cr, Cu, and Ni) to AVS was greater than 1 at all sites, and was therefore not a good indicator of toxicity. The individual metals/AVS ratios were not particularly useful. Cu/AVS was greater than 1 at two sites, both of which were toxic to chironomids. Toxicity was not exclusively linked to copper toxicity. When the ratio was less than 1, sites were both toxic and non-toxic. Multiple regression analysis indicated that Cu+Ni+Zn/AVS was the most useful indicator of chironomid toxicity for metal/AVS analysis. The opposite was true in Di Toro et al. (1992). They found in all experiments that a ratio of <1 was always a non-toxic sediment. However, they used spiked sediments, where no other contaminants were likely to be present.

The statistical methods used in analyzing data is also important. It was demonstrated here, and by Jacobs et al. (1993) that toxicity end points and contaminant concentrations correlated in more cases when Spearman’s correlation coefficients
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

were used rather than linear regression because of the non-linear nature of the Spearman’s correlation.

The research done in this study indicates the difficulty facing regulators in determining whether or not a stream is contaminated and if it is polluted. (Contaminated simply indicates the presence of contaminants while polluted indicates the presence of contaminants and the ecological impact made by those contaminants). Sampling methods changed the toxicity of sediment to chironomids, and caution must be used when extrapolating laboratory toxicity tests results to the natural environment. When there was a significant difference between mixed and core sediment sampling methods, the mixed were more toxic to chironomids. Thus, mixed samples represent a worst case scenario, and cores are perhaps more representative of in situ conditions.

However, it is not always practical to take undisturbed cores, as was done in this study, because the method requires a relatively shallow stream and sandy or fine sediments. It may also be impractical to transport undisturbed cores over longer distances compared to the ease in transporting mixed samples in plastic or glass containers.

A full understanding of all factors involved is necessary before laboratory tests can be extrapolated to real world decisions. All three parts of the sediment triad should be carried out when possible, as observed toxicity does not always correlate with
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

contaminant concentrations. Two of the three triad components were completed in this study. However, due to limited funding, it was not possible to complete a benthic inventory.

Seasonal changes in toxicity must also be considered. The reference site, Musqueam Creek, was sampled at the same location each time, but the toxicity and the chemical analyses results changed each sample date (See Tables 4.1, 4.2 and 4.6). Of note were the ammonia and metal concentrations, which show it is not adequate to sample a site once and make decisions based on that one sample. Schubauer-Berigan and Ankley (1991) noted that ammonia concentrations fluctuated during the course of their study, and also noted that pore water toxicity did not always correlate to ammonia concentration, indicating more than one contaminant was responsible for toxicity. Only one pore water sample (3S) was toxic to Microtox®, and was the site which had high ammonia levels. There was no correlation of ammonia and chironomid end points, except at Musqueam Creek. However, ammonia was ten fold lower in Musqueam Creek than in Still Creek.

The fate of metals could be affected by the same changes in oxidative conditions that affect ammonia toxicity. Thus, toxicity can change independent of contaminant loading, and can add to the difficulty in interpreting sediment toxicity assessments based solely on toxicity tests.
CHAPTER 5  CONCLUSION AND RECOMMENDATIONS

The most sensitive species is not always the best species to use in toxicity testing. *Daphnia* are generally thought to be a sensitive species (Dillon et al., 1990), but they were found to be unresponsive to the sediments tested in this study, possibly due to the hydrophobic nature of contaminants. Thus, regulatory and management decisions should not be based on the 'most sensitive species' approach. Although it may be the least expensive approach in the short term, it may be a very expensive approach in the long term if contamination is overlooked. A battery of tests may cost more, and chronic tests may take a little longer, but the end result and the confidence we can place in management and regulatory decisions will outweigh the initial costs. The complexity of the ecosystem should not be ignored. The Microtox® SPT should be used as an initial screening test as it was sensitive to all Still Creek sediments, but not to Musqueam Creek sediments. Other tests should then be chosen based on the type of contaminants present.

5.3  RECOMMENDATIONS

1. Chemical analyses should be as extensive as possible, but within reason. In this study, metal levels in pore water samples should have been measured, ammonia should have been monitored in the overlying water during the 14 day tests, and sediment PCB levels should have been determined. PCB levels have been
shown to be toxic to aquatic organisms (Ingersoll and Nelson, 1990), and previous research on Still Creek showed some elevated levels in sediments.

2. Sample method can affect sediment toxicity, and management decisions and regulations should take this into account when extrapolating laboratory results to the ecosystem. Many questions remain unanswered regarding the changes that occur in sediments when they are manipulated in the laboratory during toxicity testing. Although it appeared in most cases that if toxicity was altered, the process of mixing increased toxicity, yet this was not true in all cases. Thus, future studies which reduce the uncertainty, and increase our knowledge of how toxicity is manifested in ecosystems is required. That is, more emphasis should perhaps be placed on understanding how contaminants influence complex systems rather than developing methods to detect adverse effects of contaminants.

3. Seasonal/temporal changes in a stream's chemistry may affect contaminant bioavailability or toxicity. Thus, it is important to consider the temporal aspect of stream toxicity, as well as contaminant loading when discussing contamination and toxicity.
CHAPTER 6 REFERENCES


CHAPTER 6 REFERENCES


CHAPTER 6 REFERENCES


CHAPTER 6 REFERENCES


Student's t-test

An example of a two sample t-test for a two tailed hypothesis where $H_0: \mu_1 = \mu_2$ and $H_A: \mu_1 \neq \mu_2$ is shown below. Here, the null hypothesis ($H_0$) states that there is no difference between the mean dry weight of chironomids grown in mixed sediments and in cores. The alternative hypothesis ($H_A$) is that there is a significant difference between the mean dry weights of chironomids grown in mixed sediments and in core samples. The following example is the data for 4S cores and 4S mixed. The calculation below shows that there is no significant difference in dry weight between chironomids from mixed sediments and those from sediment core samples. $H_0$ was therefore accepted because $t (-1.487)$ was found to be within the 95% confidence limit of 2.306.

If the calculated value of $t$ were to be greater than the 95% confidence limit, (which is found in a table at the back of almost any statistics text) then the $H_0$ would have been rejected and the $H_A$ is accepted, which means that there would have been a significant difference between the dry weights of the samples.
4S MIXED
.001
.0011
.0003
.0018
.0019
.0005

4S CORES
.0017
.0008
.0017
.0025

\( n_1 = 6 \)
\( n_2 = 4 \)
\( v_1 = 5 \)
\( v_2 = 3 \)
\( \bar{X}_1 = 0.0011 \)
\( \bar{X}_2 = 0.0017 \)
\( SS_1 = \text{variance} \times v_1 \)
\( = 1.78 \times 10^{-6} \)
\( SS_2 = \text{variance} \times v_2 \)
\( = 1.09 \times 10^{-6} \)

\( S^2 p = \frac{SS_1 + SS_2}{v_1 + v_2} = 3.59 \times 10^{-7} \)

\( S = \frac{S^2 p}{n_1 + \frac{S^2 p}{n_2}} = 0.000387 \)

\( t = \frac{X_1 - X_2}{S_{X_1, X_2}} = -1.487 \)

\( t_{0.05, (2), v} = 2.306 \)

Therefore, do not reject Ho.

Figure 1  Example Calculation of a two sample t-test.
2X2 (fourfold) Contingency Table

This calculation shown below was used to determine if sample method affected survival of Daphnia or chironomids. The example used below is the survival of chironomids in 4S mixed and 4S core sediments. The null hypothesis states that survival of the organism is independent of sampling method. The alternate hypothesis is that survival is associated with sampling method.

<table>
<thead>
<tr>
<th></th>
<th>DEAD</th>
<th>ALIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4S MIXED</td>
<td>44</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>4S CORES</td>
<td>44</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>TOTAL</td>
<td>88</td>
<td>10</td>
<td>98</td>
</tr>
</tbody>
</table>

\[ X_c^2 = \frac{n( f_{11}f_{22} - f_{12}f_{21} - n/2)^2}{(C_1)(C_2)(R_1)(R_2)} \]

\[ X_c^2 = \frac{49( (44)(4) - (6)(44) - 98/2)^2}{(88)(10)(50)(48)} = 0.0706 \]

\[ v = 1 \]
\[ X_{0.05,1}^2 = 3.841 \]

Therefore do not reject Ho.

Figure 2  Example of a fourfold contingency table.
Example Calculation for an LC50 Test

The figure below shows an example calculation of an LC50. LC50 is the median lethal concentration, which is the concentration at which the toxicant is estimated to be lethal in 50% of the test organisms. The numbers used are from EPS, (1990). In the example, ten Daphnids were tested at each of five concentrations, as was done in this thesis for ZnSO4. The percentage mortality is then plotted against the concentration of the reference toxicant using logarithmic-probability paper ("log-probit") and a line drawn by eye to best fit the results. The LC50 concentration was found to be 5.6 mg/L in this example.

Figure 1  Plot of mortality on log-probit paper to determine the LC50.
Example Calculation for a Microtox® EC\textsubscript{50} Test

The EC\textsubscript{50} is effective sample concentration which causes a 50\% reduction in bacterial luminescence. The Microtox® software automatically performs the following calculations and statistics. Microtox® uses the reduction in bioluminescence as an indicator of toxicity, and assumes a linear relationship between the concentration of a toxicant and the response of the organism. The relationship is as follows:

\[ G_t = \frac{I_{tc}}{I_t} - 1 \]

Where \( G \) is the observed gamma; \( I_{tc} \) is the light level (I) at incubation time (t) of the control (c) or blank; and \( I_t \) is the light level (I) at incubation time (t), for the various sample concentrations used. (The linear regression analysis described below calculates an estimated gamma, and calculates the confidence interval for the observed data around the generated linear line).

The gammas can then be plotted against concentration on log-log paper, and a line drawn through the points. The concentration at the point where the line crosses gamma = 1 is the EC\textsubscript{50} concentration.

The computer not only determines the EC\textsubscript{50}, but also uses conventional linear regression statistics for greater confidence in the result. Thus, the equation is converted to linear form:

\[ \log (C) = b \log (G) + \log (a) \]
This equation describes a line with slope $b$ and intercept of $\log(a)$. With this linear function, least squares statistics can be used to derive the EC$_{50}$ with confidence intervals. The 95% confidence factor is derived from the following formula:

$$\exp \left( K_{oc} \left( (1-r^2) \left( 1+\left( (\ln G - \ln \bar{G}) / \sigma G \right)^2 \right)^{5} \right) \right)$$

Where $\sigma$ is the standard deviation.

The confidence limits are then determined by multiplying and dividing the EC$_{50}$ value by the confidence factor.
The tables below show summarized data for the testing of significant differences between endpoints of mixed sediment toxicity tests and core sediment toxicity tests.

Table 1  Comparison of *Daphnia magna* survival in sediment cores and mixed sediment samples, comparing toxicity of sampling methods.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLE TYPE</th>
<th>SURVIVAL (%)</th>
<th>SIGNIFICANT DIFFERENCE AT P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>MIXED</td>
<td>60</td>
<td>NS*</td>
</tr>
<tr>
<td>1S</td>
<td>CORE</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>MIXED</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>1M</td>
<td>CORE</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>MIXED</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>2S</td>
<td>CORE</td>
<td>80</td>
<td></td>
</tr>
<tr>
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<td>MIXED</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>2M</td>
<td>CORE</td>
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<td>57</td>
<td></td>
</tr>
<tr>
<td>3M</td>
<td>MIXED</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>3M</td>
<td>CORE</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>4S</td>
<td>MIXED</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>4S</td>
<td>CORE</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>MIXED</td>
<td>90</td>
<td>NS</td>
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<tr>
<td>4M</td>
<td>CORE</td>
<td>100</td>
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<tr>
<td>5S</td>
<td>MIXED</td>
<td>90</td>
<td>NS</td>
</tr>
<tr>
<td>5S</td>
<td>CORE</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>5M</td>
<td>MIXED</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>5M</td>
<td>CORE</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

*NS = NO SIGNIFICANT DIFFERENCE BETWEEN SAMPLING METHODS*
Table 2  Comparison of total number of young produced by *Daphnia* grown in mixed sediment and sediment core sampling methods.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLE TYPE</th>
<th>AVERAGE # YOUNG</th>
<th>STANDARD DEVIATION</th>
<th>VARIANCE</th>
<th>SIGNIFICANT DIFFERENCE IN MEANS AT P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>MIXED</td>
<td>40.3</td>
<td>19.7</td>
<td>386.9</td>
<td>NS</td>
</tr>
<tr>
<td>1S</td>
<td>CORE</td>
<td>56.2</td>
<td>14.3</td>
<td>204.6</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>MIXED</td>
<td>38.4</td>
<td>17.2</td>
<td>296.2</td>
<td>NS</td>
</tr>
<tr>
<td>1M</td>
<td>CORE</td>
<td>54.9</td>
<td>25.6</td>
<td>654.8</td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>MIXED</td>
<td>49.3</td>
<td>5.6</td>
<td>30.9</td>
<td>NS</td>
</tr>
<tr>
<td>2S</td>
<td>CORE</td>
<td>47.6</td>
<td>4.2</td>
<td>17.7</td>
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</tr>
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<td>2M</td>
<td>MIXED</td>
<td>36.2</td>
<td>9.8</td>
<td>95.7</td>
<td>NS</td>
</tr>
<tr>
<td>2M</td>
<td>CORE</td>
<td>45.5</td>
<td>13.1</td>
<td>172.2</td>
<td></td>
</tr>
<tr>
<td>3S</td>
<td>MIXED</td>
<td>50</td>
<td>10.6</td>
<td>112.3</td>
<td>SIG</td>
</tr>
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<td>CORE</td>
<td>29</td>
<td>8.5</td>
<td>72</td>
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<td>MIXED</td>
<td>19.8</td>
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<td>CORE</td>
<td>16.1</td>
<td>6.2</td>
<td>39</td>
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</tr>
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<td>4S</td>
<td>MIXED</td>
<td>37.9</td>
<td>18.1</td>
<td>328.1</td>
<td>NS</td>
</tr>
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<td>4S</td>
<td>CORE</td>
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<td>13.4</td>
<td>180.1</td>
<td></td>
</tr>
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<td>10</td>
<td>101.3</td>
<td>NS</td>
</tr>
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<td>4M</td>
<td>CORE</td>
<td>30.4</td>
<td>11.4</td>
<td>130.7</td>
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<td>5S</td>
<td>CORE</td>
<td>26.3</td>
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<td>236</td>
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<td>MIXED</td>
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<td>12.3</td>
<td>150.9</td>
<td>NS</td>
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<tr>
<td>5M</td>
<td>CORE</td>
<td>32.8</td>
<td>27.4</td>
<td>749.3</td>
<td></td>
</tr>
</tbody>
</table>

N.B. NS = NOT SIGNIFICANT; SIG = SIGNIFICANT
Table 3  Comparison of *Daphnia magna* number of young per brood between the sampling methods. Brood number 1.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLING METHOD</th>
<th>MEAN # YOUNG FIRST BROOD</th>
<th>SIGNIFICANT DIFF. IN MEAN AT P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>MIXED</td>
<td>7.9</td>
<td>SIG</td>
</tr>
<tr>
<td>1S</td>
<td>CORE</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>MIXED</td>
<td>9.6</td>
<td>NS</td>
</tr>
<tr>
<td>1M</td>
<td>CORE</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>MIXED</td>
<td>12.3</td>
<td>NS</td>
</tr>
<tr>
<td>2S</td>
<td>CORE</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>MIXED</td>
<td>15.9</td>
<td>NS</td>
</tr>
<tr>
<td>2M</td>
<td>CORE</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>3S</td>
<td>MIXED</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>3S</td>
<td>CORE</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>3M</td>
<td>MIXED</td>
<td>6.8</td>
<td>NS</td>
</tr>
<tr>
<td>3M</td>
<td>CORE</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>4S</td>
<td>MIXED</td>
<td>5.7</td>
<td>NS</td>
</tr>
<tr>
<td>4S</td>
<td>CORE</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>MIXED</td>
<td>6.7</td>
<td>NS</td>
</tr>
<tr>
<td>4M</td>
<td>CORE</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>5S</td>
<td>MIXED</td>
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<td>SIG</td>
</tr>
<tr>
<td>5S</td>
<td>CORE</td>
<td>6.9</td>
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</tr>
<tr>
<td>5M</td>
<td>MIXED</td>
<td>7.5</td>
<td>NS</td>
</tr>
<tr>
<td>5M</td>
<td>CORE</td>
<td>9.6</td>
<td></td>
</tr>
</tbody>
</table>

NS = NOT SIGNIFICANT  
SIG = SIGNIFICANT
Table 4  Comparison of *Daphnia magna* number of young per brood between the two sampling methods used in this thesis. Brood number 2.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLING METHOD</th>
<th>MEAN # YOUNG SECOND BROOD</th>
<th>SIGNIFICANT DIFF. IN MEAN AT P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>MIXED</td>
<td>13.8</td>
<td>SIG</td>
</tr>
<tr>
<td>1S</td>
<td>CORE</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>MIXED</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>1M</td>
<td>CORE</td>
<td>23.2</td>
<td></td>
</tr>
<tr>
<td>2S</td>
<td>MIXED</td>
<td>26.8</td>
<td>SIG</td>
</tr>
<tr>
<td>2S</td>
<td>CORE</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>MIXED</td>
<td>22.5</td>
<td>NS</td>
</tr>
<tr>
<td>2M</td>
<td>CORE</td>
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<td></td>
</tr>
<tr>
<td>3S</td>
<td>MIXED</td>
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<td>NS</td>
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<td>14.5</td>
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<tr>
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<td>MIXED</td>
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<td>NS</td>
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<td>4S</td>
<td>CORE</td>
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<tr>
<td>4M</td>
<td>MIXED</td>
<td>16.8</td>
<td>NS</td>
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<td>4M</td>
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<td>20.8</td>
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</tr>
<tr>
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<td>MIXED</td>
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<td>NS</td>
</tr>
<tr>
<td>5S</td>
<td>CORE</td>
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</tr>
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<td>5M</td>
<td>MIXED</td>
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<td>NS</td>
</tr>
<tr>
<td>5M</td>
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</tr>
</tbody>
</table>

NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 5  Comparison of number of *Daphnia magna* young per brood between the sampling methods used in this thesis. Brood number 3.

<table>
<thead>
<tr>
<th>SITE</th>
<th>SAMPLING METHOD</th>
<th>MEAN # YOUNG THIRD BROOD</th>
<th>SIGNIFICANT DIFF. IN MEAN AT P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>MIXED</td>
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<td>NS</td>
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</tr>
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<td>1M</td>
<td>MIXED</td>
<td>24</td>
<td>NS</td>
</tr>
<tr>
<td>1M</td>
<td>CORE</td>
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</tr>
<tr>
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<td>MIXED</td>
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<td>NA</td>
</tr>
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</tr>
<tr>
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<td>NA</td>
</tr>
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<td>CORE</td>
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<tr>
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<td>MIXED</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>3M</td>
<td>CORE</td>
<td>-</td>
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<tr>
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<td>NS</td>
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<tr>
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</tr>
<tr>
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<td>20</td>
<td></td>
</tr>
<tr>
<td>5S</td>
<td>MIXED</td>
<td>29</td>
<td>SIG</td>
</tr>
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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 6  Comparison of *Chironomus tentans* survival between sediment cores and mixed sediment samples.

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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 7  Comparison of *Chironomus tentans* dry weight between organisms grown in sediment cores and organisms grown in mixed sediment samples.

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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
APPENDIX V  DATA CALCULATIONS

Data Tables for Statistical Calculations (Sampling Sites)

The tables below show summarized data for the testing of significant differences between endpoints of Still Creek sediments and reference site endpoints.

Table 1  Comparison of *Daphnia magna* survival in Still Creek sediments and Reference sediments, comparing toxicity of sampling methods.

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NS = NOT SIGNIFICANT
Table 2  Comparison of total number of young produced by *Daphnia* grown in reference sediments and Still Creek sediments.

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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 3  Comparison of *Daphnia magna* number of young per brood between reference sediments and Still Creek sediments. Brood number 1.

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NS = NOT SIGNIFICANT  
SIG = SIGNIFICANT
Table 4 Comparison of *Daphnia magna* number of young per brood between reference sediments and Still Creek sediments. Brood number 2.

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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 5  Comparison of *Daphnia magna* number of young per brood between reference sediments and Still Creek sediments. Brood number 3.

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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
Table 6  Comparison of *Chironomus tentans* survival between sediment from the reference site and sediment from Still Creek.

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<th>SURVIVAL (%)</th>
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NS = NOT SIGNIFICANT  
SIG = SIGNIFICANT
Table 7: Comparison of *Chironomus tentans* dry weight between organisms grown in sediment from Still Creek and sediment from the reference site.

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<th>SITE</th>
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NS = NOT SIGNIFICANT
SIG = SIGNIFICANT
APPENDIX VI MICROTOX® SPT EXAMPLES

Microtox® Confidence Range Examples

Below are two examples of the Microtox® SPT results. Figure 1 shows a SPT from Still Creek. The sediment is quite toxic, and the confidence rage is small. However, when a sediment with low toxicity is tested, (Figure 2) the confidence range is very large.

FILE NAME: DOUG15.SPT
Sample Description:
STILL CREEK AT DOUGLAS, 15 MIN EXPOSURE
Procedure: SOLID-PHASE
Initial Concentration : 10 %
Test Time: 5 minutes

<table>
<thead>
<tr>
<th>NUMBER</th>
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<th>CONC.</th>
<th>GAMMA</th>
</tr>
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<tbody>
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<td>-0.08140*</td>
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<td>81</td>
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<td>-0.02469*</td>
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</table>

CONTROL It's : 79
*SLOPE = 1.6374

EC 50 = 11.5158 (95% CONFIDENCE RANGE: 11.0075 TO 12.0475)

Figure 1 Results of a Microtox® SPT on sediments from Still Creek.
MICROTOX DATA REPORT

FILE NAME: MJULY4I.SPT
TEST DATE: TEST TIME:

Sample Description:
Musqueam Creek, July 04 sample day, 15 min test

Procedure: SOLID-PHASE

Osmotic Adjustment: NO
Initial Concentration: 10 %
Test Time: 5 minutes

Dilution Factor: 2
Concentration Units: %

<table>
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CONTROL It's: 100

SLOPE = 0.0411

EC 50 = 10.9735 (95% CONFIDENCE RANGE: 2.3493 TO 153.3168)

I Used for calculations
* Invalid gammas

Figure 2 Results of a Microtox® SPT done on sediment with low toxicity (Musqueam Creek).
APPENDIX VII GC ANALYSIS AND RESULTS FOR PAH

GC Analysis of Sediment for PAH

Sediment from Still Creek and the reference site (Musqueam Creek) were analyzed for PAH using gas chromatography (GC). PAH levels were below detection for all Musqueam Creek sediments. Levels found in Still Creek at each sampling location are given in Table 4.3.

A sample GC scan for sample spiked with standards is given in Figure 1, and an example scan for Still Creek is given in Figure 2.
Figure 1   Example of GC analysis for PAH, standards.
Figure 2  Example of a sediment sample from Still Creek analyzed for PAH by GC analysis, (Sample 1S).
APPENDIX VIIIICP METAL ANALYSIS OF SEDIMENT

Below is the full list of 32 metals analyzed by ICP. The values are given in ug/g dry weight.

Table 1 Results of ICP analysis of sediments from Still Creek and Musqueam Creek.

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APPENDIX IX SINGLE METAL/AVS RATIOS

Below are the summary tables for each of the single metal/AVS ratios. Each is the ratio of metal (umoles/g dry wt) and sulfide (ummoles/g dry wt).

Table 1 Zinc/AVS ratios

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<th>SAMPLE</th>
<th>ZINC CONC. (umoles/g dry wt)</th>
<th>AVS CONC. (umoles/g dry wt)</th>
<th>RATIO SEM/AVS</th>
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<td>1.038</td>
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Table 2 Copper/AVS ratios.

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<thead>
<tr>
<th>SAMPLE</th>
<th>COPPER CONC. (umoles/g dry wt)</th>
<th>AVS CONC. (umoles/g dry wt)</th>
<th>RATIO SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>0.2502</td>
<td>0.6526</td>
<td>0.38</td>
</tr>
<tr>
<td>2S</td>
<td>0.1429</td>
<td>0.1887</td>
<td>0.76</td>
</tr>
<tr>
<td>3S</td>
<td>0.2351</td>
<td>0.0762</td>
<td>3.08</td>
</tr>
<tr>
<td>4S</td>
<td>0.7284</td>
<td>0.6436</td>
<td>1.13</td>
</tr>
<tr>
<td>5S</td>
<td>0.2079</td>
<td>0.2225</td>
<td>0.93</td>
</tr>
<tr>
<td>1M</td>
<td>0.0040</td>
<td>0.0232</td>
<td>0.17</td>
</tr>
<tr>
<td>2M</td>
<td>0.0413</td>
<td>0.0500</td>
<td>0.83</td>
</tr>
<tr>
<td>3M</td>
<td>0.0213</td>
<td>0.0997</td>
<td>0.21</td>
</tr>
<tr>
<td>4M</td>
<td>0.0000</td>
<td>0.0462</td>
<td>0.00</td>
</tr>
<tr>
<td>5M</td>
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<td>0.0094</td>
<td>0.00</td>
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</table>
Table 3 Nickel/AVS ratios.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>NICKEL CONC. (umoles/g dry wt)</th>
<th>AVS CONC. (umoles/g dry wt)</th>
<th>RATIO SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
<td>0.041</td>
<td>0.653</td>
<td>0.06</td>
</tr>
<tr>
<td>2S</td>
<td>0.293</td>
<td>0.189</td>
<td>1.55</td>
</tr>
<tr>
<td>3S</td>
<td>0.026</td>
<td>0.076</td>
<td>0.34</td>
</tr>
<tr>
<td>4S</td>
<td>0.337</td>
<td>0.644</td>
<td>0.52</td>
</tr>
<tr>
<td>5S</td>
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<td>0.223</td>
<td>0.59</td>
</tr>
<tr>
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<td>0.017</td>
<td>0.023</td>
<td>0.74</td>
</tr>
<tr>
<td>2M</td>
<td>0.023</td>
<td>0.050</td>
<td>0.47</td>
</tr>
<tr>
<td>3M</td>
<td>0.017</td>
<td>0.100</td>
<td>0.17</td>
</tr>
<tr>
<td>4M</td>
<td>0.000</td>
<td>0.046</td>
<td>0.00</td>
</tr>
<tr>
<td>5M</td>
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<td>0.009</td>
<td>1.46</td>
</tr>
</tbody>
</table>

Table 4 Lead/AVS ratios.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>LEAD CONC. (umoles/g dry wt)</th>
<th>AVS CONC. (umoles/g dry wt)</th>
<th>RATIO SEM/AVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1S</td>
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<td>0.653</td>
<td>0.39</td>
</tr>
<tr>
<td>2S</td>
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<td>0.189</td>
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<tr>
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<td>0.076</td>
<td>3.51</td>
</tr>
<tr>
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<td>0.644</td>
<td>1.24</td>
</tr>
<tr>
<td>5S</td>
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<td>0.223</td>
<td>0.87</td>
</tr>
<tr>
<td>1M</td>
<td>0.0146</td>
<td>0.023</td>
<td>0.63</td>
</tr>
<tr>
<td>2M</td>
<td>0.0102</td>
<td>0.050</td>
<td>0.20</td>
</tr>
<tr>
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<td>0.0174</td>
<td>0.100</td>
<td>0.17</td>
</tr>
<tr>
<td>4M</td>
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<td>0.046</td>
<td>0.26</td>
</tr>
<tr>
<td>5M</td>
<td>0.0123</td>
<td>0.009</td>
<td>1.31</td>
</tr>
</tbody>
</table>