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COMPTE RENDU

Sixteenth Technical Seminar
on Chemical Spills

Seizième colloque technique
sur les déversements de produits chimiques

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Phytoremediation: An Industry Partner's Perspective

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Abstract

Chevron, an international petroleum company, is an active participant in the "growing" area of phytoremediation. For industry to adopt this new technology, it must offer opportunities for reliable site clean up and closure in conjunction with product stewardship, cost-effectiveness, and public and regulatory acceptance. Petroleum industry research has focused on the areas of metal and hydrocarbon clean up in soil, water, and groundwater. Our approach since the early 1990's is to work collaboratively with other industry members, universities, and government agencies to further the science of phytoremediation. Transfer of phytoremediation technology is an important aspect of its continued industry application and we have developed a communication tool described here. The future use of phytoremediation by industry customers depends on regulatory acceptance, scientific data proving it works and is predictable, and a clear approach on how and when to apply it.

1.0 Needs of the Petroleum Industry

The petroleum industry consists of oil, gas, and chemical operations located in diverse geographical and environmental conditions around the world. Conditions vary from tropical to arid climates, from urban to remote, and from developed to developing countries. Sites with potential for remediation can range from a former large refinery, chemical plant, or small urban gas station in such countries as the United States or Canada to large drilling sites or pipeline corridors in remote areas of Africa, Russia, or China.

The position of industry with regards to phytoremediation is driven by two main business factors: 1) risk and 2) cost. Risk embraces both efficacy (does it work?) and product stewardship (will it cause problems to humans or the environment?). In terms of efficacy, it must be clear that phytoremediation is scientifically sound and can in fact clean up to levels satisfactory to industry, regulators, and the public. There is a need to move phytoremediation to a greater level of predictability in order for industry customers to adopt it. Industry wants full site closure in an acceptable and correct manner; we do not want to do a sub-standard job of clean up and have a site haunt all of us in the future. An understanding of what endpoint levels are achievable and what overall environmental benefit is offered by phytoremediation must be better understood.

Product stewardship, the concept of understanding effects of and taking responsibility for all phases of a business operation from product design to final use and disposal, is part of Chevron's corporate policy of protection of our environment. Phytoremediation offers in-situ "ultimate disposal" treatment, not just transfer of waste to another site or entombment in place, which fits the concepts of good product stewardship.

The second business driver for industry to help develop phytoremediation and other biological treatment technologies is cost. In Chevron, there are in excess

of $1 billion in environmental reserves, funds that must be kept aside to cover future remediation of contaminated sites. Further, we spend over $100 million per year on remediation. There is a strong business need to reduce these costs and close these old sites, while concentrating on preventing and minimizing impact in the future.

Currently, several biological treatment technologies are used in the petroleum industry (Table 1). A main interest is the integration of phytoremediation with these approaches and the proper selection of the right tool for the right job, depending on cost and objectives. Phytoremediation is viewed as another tool in the toolbox of remediation technologies. Various disadvantages, advantages, and costs are associated with each technology and the challenge is to meld the best of each to obtain the most effective and economical remediation for a site.

2.0 Research Focus and Approach

Due to the nature of the petroleum business, business drivers, and geographical diversity, research has focused on basic questions related to the clean up of metals and hydrocarbons in very diverse types of soil, water, and groundwater conditions. The approach to this research has been one of collaboration with government, university, and other industry partners. Tables 2 through 6 summarize the questions, collaborators, and results of some of our phytoremediation research over the last 6 years. One question has centered around the effectiveness of phytoremediation to remediate petroleum contaminants such as total petroleum hydrocarbon (TPH), polynuclear aromatic hydrocarbons (PAHs), and benzene in soil and the mechanism that occurs in this process (Tables 2 and 3). A second question focuses on the ability of phytoremediation to inhibit groundwater flow and enhance degradation of TPH and volatile organic compounds in groundwater (Table 4). A third question has been the capability of plants to remove metals from water and soil and the associated mass balance (Tables 5 and 6). Results summarized in Tables 2 through 6 indicate effectiveness of phytoremediation in many cases.

2.1 Government Partnership

The Remediation Technologies Development Forum (RTDF) was established in 1992 by the US Environmental Protection Agency (USEPA) to foster public-private partnerships to develop innovative solutions to mutual hazardous waste problems (USEPA, 1997a). Seven action teams have been formed, with the Phytoremediation Action Team as one of those (USEPA, 1997b). Chevron is the co-leader of the Phytoremediation Action Team along with a representative from USEPA. There are 3 sub-groups:

1. Alternative Covers (vegetative caps)
2. Tricholorethylene in Groundwater
3. Total Petroleum Hydrocarbons in Soils

The most active sub-groups are Alternative Covers and TPH in Soils. Both groups have a combination of USEPA, university, military, and industry team members to develop phytoremediation applications. The Alternative Covers group has concentrated on appropriate models and is embarking on a field study. The TPH in Soils group developed a field protocol to study the efficacy of agricultural and native plants for degrading oil in soils. Ten field sites around the US are in progress, all conducted according to the standardized protocol for test design, sampling and sample analysis, test design, data collection, and statistical analysis.

2.2 Industry Partnerships

The general philosophy in the petroleum industry is that phytoremediation is good for all of us and our environment, so we put aside competitive interest and proprietary inclinations to collaboratively move this technology forward. The Petroleum Environmental Research Forum (PERF) was established in 1986 by petroleum companies under the National Cooperative Research Act with the purpose of developing technology, pollution control, and waste treatment for the petroleum industry. Projects are cooperatively proposed and funded. Two phytoremediation projects under this arrangement include a greenhouse comparison of phytoremediation and bioremediation (Remediation Technologies, 1997) and a follow-up field study with two field sites included as part of the RTDF project discussed above.

The American Petroleum Institute (API), a group of petroleum operators, studied effectiveness of halophytes to remediate salt-impacted soils (API, 1997) and is also considering a study on the role and mechanism of plants in plume control of groundwater containing MTBE.

2.3 University Partnerships

Cooperative work with universities has been accomplished in several ways through direct funding, joint funding, and donation of field sites and soil for greenhouse and laboratory work. Projects with the University of California at Berkeley, Kansas State University, and University of Cincinnati have greatly supplemented industry studies by focusing on basic research to understand the mechanisms of phytoremediation, how to optimize it, and to screen potential plant species.

3.0 Technology Transfer

A key factor for industry to continue interest in phytoremediation is transfer of technical knowledge into practical knowledge. One way Chevron is attempting to deal with this is through a new communication brochure (to be available hard copy and electronically) entitled "Phytoremediation for the Petroleum Industry" written collaboratively with the USEPA Hazardous Substance Research Center at Kansas State University (Kulakow *et al.*, 1999). The guidance includes sections on:

- How does phytoremediation work?"
- What types of contaminants can be treated by phytoremediation?
- When can phytoremediation be used?
- Is phytoremediation an option for your site?
- How to manage a phytoremediation project

These are the practical questions asked by industry today. A main concern is a clear understanding of the steps involved in using phytoremediation, such as illustrated in Figure 1.

4.0 Factors Affecting the Future Use of Phytoremediation by Industry

Phytoremediation is a complex technology. It has tremendous scientific appeal due to this complexity, but this also serves to make it complicated as a remediation approach. Much of the approach thus far has been very site-specific

with little reliability on what levels of clean up might be achieved and when they might be achieved. As a complex technology, it also offers many benefits not achieved by other remediation approaches, such as aesthetics, habitat renewal, in situ treatment, and so on. There needs to be a melding of the somewhat lack of exactness of the science with the great benefits it can offer in order to fully evaluate the place phytoremediation has in our remediation toolbox.

There are two main factors affecting the use of phytoremediation by industry in the future. One is the availability of efficacy and field test data that determines achievable endpoint levels, length of time, and depth of effectiveness of phytoremediation. The RTDF study is designed to provide some of this type of information, but available data is also needed for a number of other issues, e.g. groundwater. The second main factor for use in the future is guidance from regulators on what is acceptable for phytoremediation and where it can be used. Industry needs a clear message and clear steps on how to conduct and monitor phytoremediation in order to continue use of this important technology to accomplish the goals we all have of protecting the environment.

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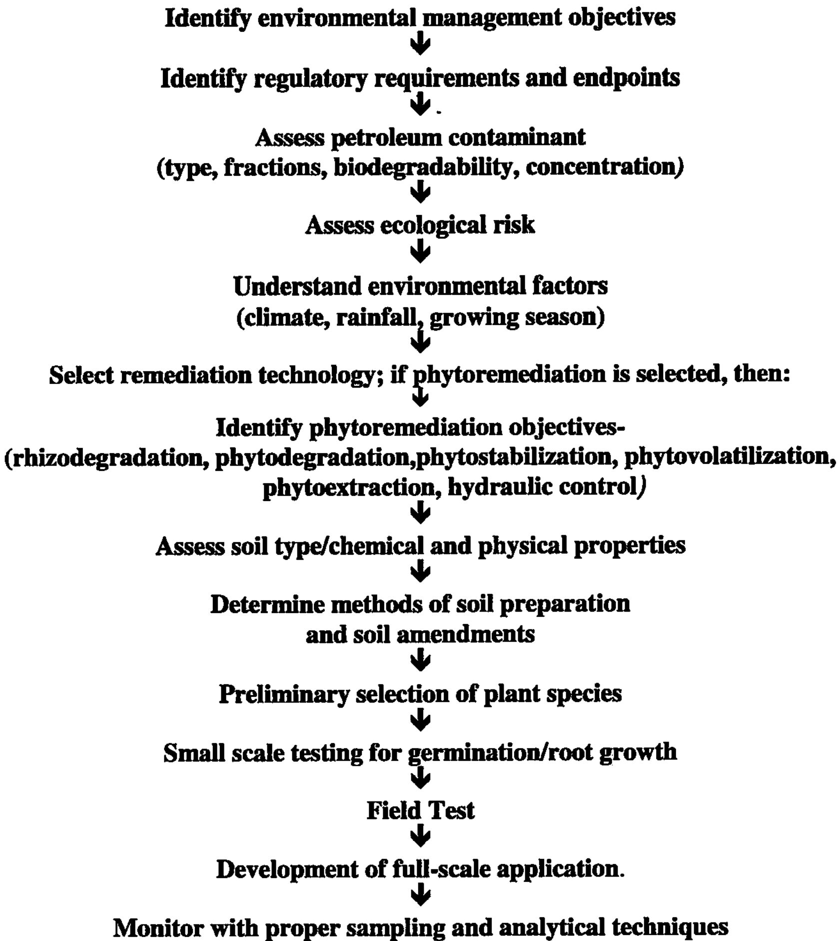

Figure 1 Steps of Phytoremediation

Table 1 Summary of Biological Treatment Technologies

Technology	Description	Advantages	Disadvantages	Cost
Composting (Windrow)	An aerobic biodegradation process in which waste is formed into piles, bulking agents are added, and aeration is provided by mechanical mixing. Heat is generated and may be conserved.	-Requires less land area and treatment period is generally shorter as compared to landfarming. -Run-on/off and leaching control are possible. -Higher potential of achieving uniform degradation of hydrocarbons as compared to other composting technologies. -Can be used to extend the "growing season" in cold climates. -No risk of accumulation of non-biodegradable constituents.	-Greater capital costs than for land treatment alternatives. -Air emissions control is difficult to obtain. -Residual compost material must be recycled or disposed. -Mechanical mixing is required for aeration.	Low to Moderate
Composting (Forced Aeration or Static Pile)	An aerobic biodegradation process in which aeration is provided to piles of waste and bulking agent by means of slotted pipes through which air can be forced with a blower or vacuum. Heat is generated and may be conserved.	-Air emissions control is possible, in addition to leaching and run-on/off control. -Requires less land area and treatment period is generally shorter as compared to landfarming. -Turning is not required to achieve aeration. -No risk of accumulation of non-biodegradable constituents. -Can be used to extend the "growing season" in cold climates.	-May have less uniform degradation of hydrocarbons compared to windrow composting if the compost is not thoroughly homogenized at start-up or if the piping system is not adequately designed to achieve uniform air flow. -Greater capital costs than for land treatment alternatives. -Residual compost material must be recycled or disposed.	Low to Moderate
Landspreading	Refers to a one-time application of waste to a soil surface. Fertilizer is usually added and the site is tilled.	-No risk of salts or metals accumulating. -Less long-term impact (not a permanent facility), so can generally be done adjacent to the site where the waste is generated.	-Higher risk that follow-up on maintenance (i.e., watering, monitoring) will be poor compared to permanent facility.	Low
In-Situ Biotreatment	Aeration, and nutrient and water addition are performed directly on a spill site.	-Excavation of oily soil not required.	-Effective only for superficial spills (treats top 6 - 12 inches). Specialized tilling equipment is required to treat soil depths of 12 to 24 inches.	Low

Table 1 Summary of Biological Treatment Technologies (continued)

Technology	Description	Advantages	Disadvantages	Cost
Landfarming	Managed chemical, physical and biological process involving controlled application of oily wastes to soil surface at specified loading rates and frequencies, using the assimilative capacity of the soil to decompose and contain the applied waste in the surface soil layer (top 6-12 inches).	-Easy to implement.	-Large area required to reduce loading impacts. -May require long treatment times or leave high residuals. -Application and mixing may be difficult for some wastes. -Accumulation of metals, salts, or hydrocarbons may occur after multiple applications of waste. -Treatment period is generally longer as compared to composting.	Low to Moderate
Composting (In-Vessel)	A specially-designed enclosed vessel is used to perform forced aeration composting.	-Greater oxygen transfer capabilities than static pile composting due to controlled environment. -"Closed loop" system provides better control of air emissions and leachate.	-Higher cost and more complex to operate than other composting systems. -May be difficult to implement at remote E&P sites.	Moderate to High
Bioslurry	A suspended growth biological process where the waste is suspended by the action of aeration and mixing in an aqueous slurry. The ex-situ bioslurry process uses a lined pit or tank.	-Promotes high biodegradation rates. -Dewatering of waste not required.	-High energy consumption. -Must be able to slurry the waste. -Water/solids separation after treatment may be difficult. -Water disposal may be required.	High

Table 1 Summary of Biological Treatment Technologies (continued)*

Technology	Description	Advantages	Disadvantages	Cost
Soil Venting	Vapor extraction wells are used to remove volatilized hydrocarbons from the unsaturated zone, and increase natural biodegradation rates by increasing oxygen to the subsurface.	-Addresses subsurface contamination of >12 inches without excavation of oily soil. -Air emissions can be controlled.	-Limited applicability (most effec-tive for light, volatile hydrocarbons such as condensates).	
Saturated Zone Biotreatment (Air sparging, etc.)	Bioremediation of dissolved hydrocarbon in the saturated zone is enhanced by delivery of oxygen to the subsurface using injection wells within the dissolved hydrocarbon plume to inject H_2O_2 or perform air sparging.	-Addresses contamination in both subsurface water and soil without requiring excavation. -Air emissions can be controlled.		Moderate to High
Phyto-remediation	The use of green plants to remove, degrade, or stabilize metals and organic compounds in soil and water	-Effective for a wide variety of contaminants: petroleum, PAHs, metals, explosives, pesticides, solvents, etc. -Treats soil, water, wetlands, groundwater -Can use in combination with other remediation technologies -Provides clean up in place (vs. traditional methods that entomb in place or transport contaminant) -Restores habitat -Favorable public appeal -Aesthetically pleasing -Regulator support	-Sites need to be maintained -Suitability of application depends on type/ level of contaminant and agronomic conditions -Slow (3+ years) -"Attractive nuisance" and food chain issues must be considered -Clean-up limited to soil depths within reach of plants' roots	Low

* Taken from McMillen and Lambertz, 1998.

Table 2 Chevron Phytoremediation Studies: Petroleum Contaminants in Soil (Laboratory/Greenhouse)

Contaminants	Site/Source	Collaborators	Plant(s)	Goal(s)	Results
TPH	• Wastewater treatment pond soil from refinery • CA	Kansas State University S.L. Lewis P. Kulakow A.P. Schwab M.K Banks	Study 1: • Bermuda grass • Fescue ------------------ Study 2: 30 grass species	Study 1: Optimal fertilizer rate ------------------------- Study 2: Plant species screen	• No difference in TPH degradation with fertilizer levels • 8 grass species identified as field candidates
TPH PAH	Artificial soil mix • Crude oil from CA • Gas pit sludge from TX	• Petroleum Environmental Research Forum • ReTec	• 7 grass species • Yellow clover	Phyto vs. Bio --Efficacy --Cost	• Phyto & bio effective • Initial costs similar; operating costs less with phyto if not irrigated
Benzene	[14 C] benzene	Phytokinetics A. Ferro	Alfalfa	• Benzene fate • Planted and unplanted systems	• Little uptake/ translocation • No volatilization
Phenanthrene Hexadecane	• Holding pond soil from refinery • CA	University of Calif. at Berkeley R. Miya M. Firestone	Wild Oats	Mechanism for rhizosphere biodegradation of hydrocarbons	In soil with plants vs. no plants: • Larger and less diverse community of PAH degraders in planted soils • Enhanced rates of PAH degradation
TPH PAH	• Land treatment unit soil at refinery • OH	University of Cincinnati J. Shann	Various species	• Evaluate bioavailability of PAHs in soil • Plant species screen	In progress

Table 3 Chevron Phytoremediation Studies: Petroleum Contaminants in Soil (Field)

Contaminants	Site/Source	Collaborators	Plant(s)	Goal(s)	Results
Low pH hydrocarbons --TPH --PAH	• Holding pond at refinery • CA	Kansas State University P. Kulakow A.P. Schwab M.K Banks	• Tall fescue • Grass & legume mix • Native CA grasses • Bulrush	Enhancement of microbial degradation in rhizosphere	• Some reduction in TPH, similar to tilled, watered, non-planted control • More rapid reduction TPH in planted soil • Wetland plants less effective than upland plants
Hydrocarbons	• Land farm at refinery • CA	Regional Water Quality Control Board	• Poplar • Grass and broadleaf species	•Stabilize contaminants •Vegetative cap	Water Board acceptance
Hydrocarbons	• Tank facility • Nigeria		Grass	Improve appearance of site; clean-up site	Visual improvement; no phytotoxicity to planted grass
TPH PAH	• Land treatment unit at refinery • OH	• US EPA/ RTDF S. Rock • Ohio EPA • University of Cincinnati	• Rye, legume, Fescue mix • Trees	Enhance degradation	In progress
TPH PAH	• Drained wastewater treatment pond • CA	• US EPA/ RTDF S. Rock • Region 9 EPA • Dept of Toxic Substances Control • Regional Water Quality Control Board	• Rye, legume, Fescue mix • Native grasses	Enhance degradation	In progress

Table 4 Chevron Phytoremediation Studies: Petroleum Contaminants in Groundwater (Field)

Contaminants	Site/Source	Collaborators	Plant(s)	Goal(s)	Results
TPH Volatile organics	• Marketing transfer terminal • UT	• US EPA S. Rock • Phytokinetics A. Ferro	• Hybrid Poplar (standard & pole-planted) • Juniper	• Inhibit migration of contaminants • Compare planting methods	• Lower concentration of volatile organics downgradient of trees • Pole plantings preferred installation method for low maintenance
Volatile Organics	• Former gas station • CO	Phytokinetics A. Ferro	• Hybrid Poplar	• Inhibit GW flow	In progress
Nitrates	• Chemical storage site • CA	Other industry	• Hybrid Poplar	• Nitrate uptake • Inhibit migration of contaminants	In progress
Fuel, MTBE, solvents, nitrates	• Superfund site • NV	Other industry	• Cottonwood trees	• Inhibit migration of contaminants	In progress

Table 5 Chevron Phytoremediation Studies: Metal Contaminants in Water (Greenhouse/ Field)

Contaminants	Site/Source	Collaborators	Plant(s)	Goal(s)	Results
Trace metals	• Refinery wastewater • Field • CA	CH2MHill H. Ohlendorf	• Bulrush • Brass buttons • Grasses • Cattails	• Wastewater treatment • Balance habitat & treatment (water levels, vegetative density, harvest frequency)	5 year management plan
Se	• Refinery wastewater • Field • CA	University California at Berkeley N. Terry D. Hansen	• Bulrush • Brass buttons • Grasses • Cattails	• Se mass balance • Se volatilization	• Effective Se removal (80% drop) • High rates of volatilization (10-70%)
Trace metals	• Refinery wastewater • Field • MS		• Bulrush • Cattails	• Reclaim lagoon • Wastewater treatment	In progress
8 metals • Hg • Cd • Cu • Pb • As • Se • Ni • Cr	• Artificially treated water • Greenhouse	University California at Berkeley A. Zayed N. Terry	6 species • Duck weed • Brass buttons • Cattails • Water hyacinth • Bulrush • Rabbitfoot Grass	• Identify wetland plants that take up metals • Analysis: ICP-AES, AA	• Duckweed—Hg, Cd, Se uptake • Brass buttons—Pb, Cu uptake

Table 6 Chevron Phytoremediation Studies: Metal Contaminants in Soil (Greenhouse/ Field)

Contaminants	Site/Source	Collaborators	Plant(s)	Goal(s)	Results
• Cd • Cr • Cu • Pb • Hg	• Landfarm • Field • OH	Bowser-Morner Associates J. Hewlett	10 grass & broadleaf species	Plant uptake of metals	• No Hg uptake • Alyssum-Pb uptake • Alyssum, cabbage, turnips-Cd, Cr, Cu uptake • No regulatory requirements for these metal levels, so no follow-up
• Cd • Cr • Pb	• Land treatment unit soil • Greenhouse • OH	University of Cincinnati J. Shann	Various species	• Evaluate bioavailability of metals in soil • Plant species screen	In progress

A Phytoremediation Technology Verification Protocol For Metal-Contaminated Matrices

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Abstract

The Environmental Technology Verification (ETV) Program is a joint Environment Canada – Industry Canada initiative designed to support the development and acceptance of new and innovative Canadian environmental technologies. The program provides standard protocols and decision-making procedures for credible and independent verifications of technology performance claims submitted by interested technology vendors; the program applicants. Successful verification entitles the company to use an ETV verification mark - licensed to ETV Canada Inc. by Environment Canada - along with an accompanying certificate, to enhance their marketing activities. Suppliers of equipment-based environmental services are also eligible to apply for verification within the program. Verifications are performed by ETV-approved "Verification Entities" - independent, impartial companies or individuals who have no stake in the particular, or a competing, technology.

To-date, fifteen technology-specific[1] protocols have been created to support the program, in addition to general verification report elements such as background information on the ETV Program, statistical analysis procedures for verifying claims, general and technology-specific references, and general and technology-specific glossaries.

A phytoremediation technology-specific protocol - recently created for the ETV Program - applies to *phytoremediation technologies or processes* that may be used to remediate *metal[2]-contaminated matrices* (i.e., soils, sediments, sludges, soil-like materials, water / groundwater, and wastewaters). The protocol addresses bench-scale treatability testing and technology development methods, field implementation considerations (e.g., sampling and process monitoring), and statistical aspects of process verification. This publication describes the basis for, and contents of, the phytoremediation protocol.

1.0 Introduction

Phytoremediation is essentially a generic term for any applied process that uses green plants and occasionally their associated microorganisms for remediating contaminated matrices. To-date, research and development efforts have created several distinct types of phytoremediation technologies suitable for organic

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[1] Technology-specific protocols have now been completed for: 1) water treatment, 2) wastewater treatment, 3) air emission control, 4) bioremediation, 5) stabilization / solidification, 6) physical / chemical remediation, 7) sewage biosolids stabilization, 8) resource recovery, 9) process control, 10) landfill liners, 11) oil spill control, 12) energy recovery, 13) analytical tools, 14) environmental instrumentation, and 15) phytoremediation.

[2] Ten specific metal or metalloid elements are covered in the protocol; no radionuclides are specifically included.

contaminants and inorganic contaminants (but rarely both together). Similarly to what has historically occurred with other environmental clean-up technologies (e.g., bioremediation), commercial use of phytoremediation has possibly preceded a full understanding of process controls and biological mechanisms affecting contaminant remediation. As the number of phytoremediation technology implementations increases, so will the number of project *evaluations*[3]. At present, there are relatively few instances of unbiased, third-party evaluations of phytoremediation technology implementations.

In order to provide some form of advice and guidance to technology developers, practitioners, scientists, regulators, and/or evaluators, ETV Canada commissioned the development of a phytoremediation technology verification protocol. The protocol aims to provide guidance on recommended requirements for successful implementation of specific phytoremediation technologies, which have been divided into two major classes as follows:

- Contaminant removal or transformation technologies including phytoextraction (or phytoconcentration) – including rhizofiltration phytotransformation / phytovolatilization; and,
- Contaminant stabilization technologies (i.e., phytostabilization), including sequestration / containment - including lignification and humification processes.

These technologies are based on the use of green plants and in some cases, their associated or symbiotic microorganisms. A more detailed description of the contents of the phytoremediation technology-specific protocol is provided later in this document but first we will discuss the Environmental Technology Verification process.

2.0 The Verification Process

An environmental technology verification involves the confirmation of a quantifiable performance claim supported by reliable data. Following a detailed, rigid protocol, a Verification Entity assesses the integrity of supplied or collected data and the validity of associated performance claim(s) based on the data provided.

The verification process is represented in appended flowchart schematics (Figures 1) that may be summarized as follows.

The technology vendor or applicant first submits a performance claim for the technology to ETV Canada. If the claim is verifiable and the application accepted, relevant information and data are forwarded to a selected Verification Entity to initiate the verification process. Following strict protocols and using specialists possessing the required skills, each vendor claim is thoroughly scrutinized as to its validity. If required, treatability testing is conducted to provide the Verification Entity with supplementary data or additional information supporting verification of the performance claim. All costs for supplementary testing are borne by the technology vendor / applicant.

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[3] The California Environmental Protection Agency, which established the framework for environmental technology verification protocols that we are following here, uses the terms *evaluations*, *validations*, *verifications*, and *authentications* synonymously, and we shall do so also.

The verification process consists of four defined stages, described in detail as follows.

2.1 Stage One - Pre-screening

To be eligible for application to the ETV program, the performance claim must relate to an environmental technology or an equipment-based environmental service, where the equipment performance is verifiable. The technology must offer an environmental benefit or address an environmental problem. It must meet minimum Canadian standards and/or national guidelines for the specific technology or claim as specified by ETV Canada and be currently available or commercially ready for full-scale application. Vendors with technologies that do not meet this criteria, but are ready for demonstration at pilot-scale or as a prototype commercial unit, should contact ETV Canada for advice regarding the planning of test programs to generate relevant data for subsequent claim verification.

If the technology meets the above criteria, the applicant submits a Pre-screening Application to ETV Canada. The Pre-screening Application is subsequently reviewed for eligibility and feasibility, and to ensure that no conflict of interest exists between the applicant and ETV Canada.

2.2 Stage Two - Application Review

If the technology and performance claim(s)[4] are eligible for application to the ETV program, the applicant submits a Formal Application. The Formal Application requests additional information about the technology, the claim to be verified, and data and information presently available to support the claim. ETV Canada reviews the Formal Application for completeness and determines acceptability for verification. If the application is not acceptable, the applicant may choose to modify and resubmit it. If the application is accepted, sufficient information from the applicant to initiate verification of the claim is submitted to ETV Canada. A confidentiality agreement is signed before any confidential information or data is passed to ETV Canada. ETV Canada then reviews the information and proposes a Verification Entity. ETV Canada discusses the scope of the proposed program with the applicant, and reaches agreement on an acceptable Verification Entity, including resolution of any conflict of interest between the applicant and the Verification Entity. If the proposed program and costs are accepted, the applicant enters into a contract with ETV Canada specifying the verification process and identifying the mutually agreeable Verification Entity.

2.2.1 Criteria for Specifying Claims

A performance claim typically has the following format: "The technology [name], as applied to _____, under operating conditions such as _____, will give results of _____." Applicants to the Environmental Technology Verification Program use the following three criteria for specifying claims about their technology:

i. *A claim must be specific and unambiguous.* A claim must clearly specify the minimum performance that is achievable with the technology, and not simply the maximum performance. For example, an unacceptable claim would state

[4] See "Criteria for Specifying Claims" in following section.

that a technology reduces emissions of a contaminant by *up to* x%, whereas an acceptable claim would state that a technology reduces emissions of a contaminant by *at least* y%.

A claim must clearly specify the operating conditions under which the claim is applicable. A claim must not be subject to more than one reasonable interpretation. Explicit and accurate information must be provided in order to clarify what the claim applies to.

Caution must be exercised when using relative or comparative terms in a claim. Expressions such as "better than" or "superior to", imply a comparison of technologies between vendors. To verify claims having these expressions requires the applicant to provide reliable, high quality data for the technology as well as for each of the competitive technologies. Using terms such as the "best" or "the only one in the world" requires the applicant to provide reliable, high quality data for all available technologies (in the world) to validate a performance claim.

A comparative term such as "improves" is an acceptable expression if the term is used to describe an advancement of the applicant's own technology and if the applicant has suitable data for both the baseline conditions (prior to the improvement) and the improved version.

Other comparative terms may be used in a performance claim, however, where necessary, a definition may be required to be included to ensure no misinterpretation of the performance claim wording.

ii. *A claim must be meaningful and nontrivial.* A claim must meet minimum standards and guidelines[5] for the technology. Where a federal standard is not available, the least stringent provincial standard shall apply.
iii. *A claim must be measurable and verifiable.* A claim must be measurable using acceptable test procedures and analytical techniques.

Examples of typical claims that could be verified - and may be relevant to phytoremediation technologies - are:

- contaminant removal/recovery: a claim specifies that a technology removes/recovers at least 95% of specified contaminant(s) in specified matrices (air, liquid, solid) with specified initial concentrations. This claim could be verified by providing data on the quantity of contaminant(s) removed/recovered by the technology as compared to the total amount of contaminant(s) to which the technology was subjected. The contamination could be measured on a mass or concentration basis, and could be expressed per process unit or per unit of time.
- contaminant degradation: a claim specifies that a technology degrades or destroys a contaminant in a specific matrix to a maximum concentration of "x" ppm, under specified operating conditions. This claim could be verified by providing data on contaminant concentrations in the matrix and any related matrices or environmental compartments.

[5] Refers to national or provincial standards and/or guidelines that are currently applicable. A preliminary list of some available standards or guidelines is presented in Appendix B of the General Verification Protocol.

- *mass conversion*: a claim specifies that a technology converts a specific quantity (on a mass basis) of a material from a contaminated matrix (such as soil, air, water) to another matrix. The conversion process must not yield extraneous contaminants or byproducts that may be deemed contaminants.

2.3 Stage three - Verification

The Verification Entity enters into a contract with ETV Canada to conduct the performance claim validation using a verification protocol designed for the specific technology sector. Before Verification Entities receive any confidential information or data, a confidentiality agreement between the applicant and ETV Canada is signed. The Verification Entity subsequently analyses the data from the applicant to validate the claim. If additional data is required, independent testing of the technology is conducted by an approved testing agency. This testing and its associated costs are borne by the applicant; however, ETV Canada may provide advice upon request to facilitate the testing.

The Verification Entity must prepare a report summarizing the verification, and this is then submitted to both ETV Canada and the applicant for review and approval. If the applicant's claim is substantiated, ETV Canada will arrange for the awarding of a Verification Certificate. If the claim cannot be substantiated, the applicant may choose to modify the performance claim, such that it may be substantiated using the existing data. Alternatively, the applicant may choose to conduct further testing to support the original claim. If additional verification work not covered by the original verification contract is required, this work will be subject to a further application fee.

2.3.1 Criteria for Claim Verification

For a claim to be verified, ETV Canada must be satisfied that the following four criteria are fulfilled:

- The technology provides an environmental benefit or addresses an environmental problem.
- The technology is based on sound scientific and engineering principles.
- The claim is fully supported by peer-review quality data generated through an independent testing process.
- The conditions of performance for the claim are clearly defined.

2.4 Stage Four - Award

If the applicant's claim is substantiated, ETV Canada prepares a final Verification Report and a Fact Sheet defining conditions of performance, and a Verification Certificate to be awarded to the successful applicant. The applicant is then entitled to use the Certificate, Fact Sheet and final Report in marketing activities.

3.0 Overview of ETV Technology-Specific Protocols

Each of the technology-specific verification protocols developed for ETV Canada contain a core set of common, general elements, including:

- a background section describing the scope of the protocol (e.g., contaminants included
- an introduction to the protocol contents (see below)

- a description of the technology

The protocols also include specific guidance and procedures that a Verification Entity (i.e., the verifier) follows to complete a verification of a specific performance claim for the technology in question. The guidance pertains to:

- a review of the application (i.e., to the ETV Program)
- a review of the specific technology
- a review of the data submitted by the applicant to support the performance claim
- a review of the performance claim itself
- preparation of the verification report

Additional information provided to the Verification Entity (and the applicant) includes:

- performance claim verification form and procedures
- background information on the ETV Program
- explanations for the verification criteria
- references for both general and technology-specific publications
- glossaries for both general and technology-specific terminologies
- additional relevant information

4.0 Overview of the Phytoremediation Verification Protocol

The following discussion relates to the specific contents of the phytoremediation specific verification protocol.

4.1 Metal Contaminants Covered in the Protocol

Any metal (or metalloid) species may be considered a "contaminant" if it occurs in a place where it is unwanted, or in a form or at a concentration that causes a detrimental environmental effect. However, only the following metallic elements have been considered in the protocol:

- Arsenic (As)
- Cadmium (Cd)
- Chromium (Cr)
- Copper (Cu)
- Lead (Pb)
- Mercury (Hg)
- Nickel (Ni)
- Selenium (Se)
- Silver (Ag)
- Zinc (Zn)

This list is based upon the metals/metalloids most commonly found at U.S. Superfund sites (i.e., contaminated sites identified by the U.S. EPA)(McLean and Bledsoe, 1992), cross-referenced with metals that are listed in Canadian federal or provincial clean-up criteria (e.g., CCME, 1997;Ontario MOEE, 1997)[6].

"Metals" and "metalloids" are variously defined in the literature. Hawley (1977) defines a metal as "An element that forms positive ions when its compounds are in solution and whose oxides form hydroxides rather than acids with water. Most

[6] A brief description of the physico-chemical properties and the CCME soil quality guidelines for the target metals / metalloids are appended to the Protocol report.

are crystalline solids with metallic luster, conductors of electricity, and have rather high chemical reactivity; many are quite hard and have high physical strength. They also form readily solutions (alloys) with other metals."

Of the above-listed elements, arsenic and selenium have been described as metalloids – elements that more closely resemble metals than they do the other traditionally-recognized non-metal elements. Hawley (1977) stated that the term metalloids "is no longer used by chemists", however one readily finds the terminology still being used in recent publications (e.g., CCME, 1997).

For the purposes of the protocol, the generally important physico-chemical characteristics of the above-mentioned metals (excepting the metalloids arsenic and selenium) are their:

- solid nature;
- silvery lustre;
- good conduction of heat and electricity; and,
- atomic densities greater than 6 g cm^{-3}.

Note that radionuclides are not included in the list, however some of the specific types of phytoremediation technologies listed in the protocol may in fact apply equally well to radionuclides (e.g., see Cornish *et al.*, 1995, for a discussion of ^{137}Cs, ^{239}Pu, ^{90}Sr, and $^{238,234}U$ phytoremediation feasibility).

Detailed discussions of metal chemistry and the fate of metals in the environment are not presented in the protocol report, as numerous excellent review publications have appeared in the literature in recent years[7]. However, it is important to note the significant, commonly-recognized, physico-chemical reactions that affect free metal concentrations in soils solutions as these reactions will in many cases directly affect the success (or lack of success) for phytoremediation. These reactions are:

- ion exchange and adsorption;
- oxidation / reduction reactions;
- acid / base reactions;
- precipitation / dissolution reactions;
- complexation / ligand formation;
- leaching; and,
- plant uptake.

4.2 Phytoremediation Technologies Covered in the Protocol

As mentioned earlier, the specific phytoremediation technologies covered by the protocol have been divided into two major classes as follows:

- Contaminant removal or transformation technologies including phytoextraction (or phytoconcentration) – including rhizofiltration phytotransformation / phytovolatilization; and,
- Contaminant stabilization technologies (i.e., phytostabilization), including sequestration / containment - including lignification and humification processes.

[7] Technology-specific references are appended to the protocol report.

These technologies are based on the use of green plants and in some cases, their associated or symbiotic microorganisms. The technologies or processes facilitate the:

- removal or displacement of metals from specific environmental compartments (e.g., soils, sediments) into other compartments (e.g., air);
- concentration of metals into a smaller volume of material;
- transformation of metals to less toxic forms; and/or,
- stabilization / containment of metals as less bio-available substances.

Apart from being either *contaminant removal / transformation* or *contaminant stabilization / containment* technologies, the specific technologies can further be classified according to the environmental scenarios in which they're applied; i.e., as either *terrestrial*, or *aquatic / wetlands* systems. Terrestrial systems usually involve surficially-contaminated soils that have uniform and sub-lethal concentrations of contaminant, and are physically, chemically, and biologically suitable for plant growth, culture, and cropping. Aquatic systems often involve floating plants (e.g., duckweed, water hyacinth) and/or rooted plants (e.g., reed beds, wetlands), and are also only suitable for specific contamination scenarios. Where required, distinctions between terrestrial and aquatic systems have been made if deemed relevant. Phytoremediation can also take place *in situ* (i.e., in place), or *ex situ*, with *ex situ* remediation being either on-site or off-site. *Ex situ* remediation processes involve engineered systems developed to support plant growth for decontaminating solids (e.g., soils, as in vegetated biocells), liquids (e.g., wastewaters, as in flow-through wetlands-type systems), or gas-phases (e.g., air, as in vegetated biofilters). *In situ* methods are the most common, and typically involve crop plantings at sites where specific physico-chemical characteristics and contaminant concentrations exist. The distinction between an *ex situ* or *in situ* technology is not always apparent, nor is it usually important, except perhaps in regards to regulatory requirements pertaining to site-specific clean-up operations.

Since phytoremediation is essentially a form of *bioremediation*, and since a wide variety of bioremediation technologies have generally been accepted as viable clean-up technologies worldwide, it follows that there should be few impediments to obtaining regulatory support for its use if scientifically-defensible development, implementation, and evaluation methods are used. Like bioremediation, phytoremediation is generally viewed as a cost-effective and environmentally-friendly method for reducing and/or eliminating toxic[8] xenobiotic contaminants from, or fixating, sorbing, and/or stabilizing contaminants within, specific contaminated environmental matrices. To be considered a true remediation technology, phytoremediation must successfully reduce or eliminate the actual or potential environmental impacts (or risks), of contaminants. Thus phytoremediation is readily distinguishable from *landscaping* or *ecological restoration* techniques that also use green plants in an environmental context, but are deemed to have a predominantly aesthetic value and are not specifically intended to treat xenobiotic contamination.

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[8] Reductions in toxicity normally result from reduced concentrations, transformation, or complete removal or destruction of a toxic material. In some cases, biological processes may result in increased toxicity due to the formation of intermediaries or metabolites more toxic than the parent compound and precautions must be taken to ensure that this is avoided.

As with any environmental clean-up technology (e.g., slurry bioremediation, thermal desorption, soil washing, etc.), phytoremediation has been developed and applied to specific types of contamination under specific environmental conditions. Since many phytoremediation processes have only recently been researched and/or developed, the limits for successful technology implementation have yet to be firmly established. However, some limitations have been documented for specific processes (e.g., the depth of contamination in particular soil types that are amenable to phytoextraction using non-woody plant species; or the maximum amount or concentration of metal contaminants that may be hyper-accumulated by specific plant species). As occurs with other ETV Program applications, the applicant (technology vendor) will be asked to provide specific operating ranges or limits for their specific technology.

4.2.1 Contaminant Removal Technologies

Removal technologies are those which result in phase transfer and/or transformation of the contaminant(s)-of-concern. Phase transfer of environmental pollutants should not normally be viewed as a clean-up method. However, the dilution effect and subsequent reduced risk that results when certain contaminants are removed from groundwater and released into the atmosphere via evapotranspiration processes in plants, has become an accepted method of site remediation in some jurisdictions (e.g., U.S.A.). The transformation of toxic contaminants to less toxic forms is seen as a favourable remediation option. Numerous and varied definitions of phytotransformation exist, and some authors choose to consider degradation as well as stabilization as "transformation" processes (e.g., Schnoor, 1997).

4.2.1.1 Phytotransformation / Phytovolatilization

For this protocol, phytotransformation will mean the applied use of plants that have shown an ability to transform certain toxic forms of inorganic contaminants to less toxic forms, either within the plant itself, or within the rhizosphere. The reduction or complete elimination of toxicity or environmental risk associated with the parent compound (i.e., contaminant) must be proven for the remediation to be considered successful.

Most definitions of phytotransformation pertain to organic contamination, for instance Newman *et al.* (1997) discussed the transformation of trichloroethylene (TCE) within hybrid poplar trees. However, transformation of metals / metalloids has been demonstrated (e.g., methylation of mercury; Steinnes, 1990), although a decrease in toxicity may not occur, and the role that plants may play in this process is not well documented at present.

Phytovolatilization applies to the use of specific plant species known to translocate and transpire volatile-contaminant-laden water (e.g., poplar trees and TCE). These plants are grown in strategic locations within, or in proximity to, areas of soil and/or groundwater contamination and the plants uptake and transpire the soluble contaminants. There are fewer instances of the phytovolatilization of inorganic versus organic contaminants, however phytovolatilization has been documented for both selenium and mercury (U.S. EPA, 1998).

4.2.1.2 Phytoextraction (or Phytoconcentration)

(Including Rhizofiltration)Phytoextraction refers to the applied use of certain plants that possess unique capacities for uptaking or hyperaccumulating specific inorganic elements (i.e., metals). The earliest definition of what constituted a hyperaccumulator plant depended upon the metal species in question. Baker and Brooks (1989) and Baker *et al.* (1991) defined hyperaccumulators as those whose leaves contained >100 mg Cd kg^{-1}, 1000 mg Ni and Cu kg^{-1}, or >10,000 mg Zn or Mn kg^{-1}, on a dry weight basis, when grown in metal-rich soils. This definition might be modified to apply to plants that contain greater than an order of magnitude of a particular metal species in their dried tissues, as compared to the levels that other plants would normally have.

Rhizofiltration involves the application of certain hyperaccumulator plants for extracting contaminants from aqueous wastestreams (e.g., contaminated groundwater). This technology has developed from wetlands engineering research, especially the use of artificial or constructed wetlands for wastewater treatment where many commercial successes have been documented and for which a large body of literature exists (refer to References). Dushenkov *et al.* (1995) describe their concept of rhizofiltration as a clean-up technology for metal and radionuclide-contaminated water, using sunflower (*Helianthus annuus*), Indian mustard (*Brassica juncea*), and other plant species.

4.2.2 Contaminant Stabilization Technologies

Contaminant stabilization technologies (defined below) are designed to reduce or completely eliminate environmental risk, however they differ from transformation technologies in that the contaminant does not change its molecular structure *per se* but is instead sequestered or compartmentalized in a bound or unavailable condition. Phytostabilization may involve using plants for remediating certain soil contamination scenarios by reducing or eliminating soil erosion, or leaching of contaminants from the soil into groundwater (Salt *et al.*, 1995). Additionally, certain soil amendments can be used to "bind" or stabilize the metals, preventing their movement away from the rhizosphere, thus minimizing their environmental impact. Berti and Cunningham (1997) discuss the processes of chemical stabilization combined with "biomining" or phytoextraction of metals by plants, and also growing plant biomass to restrict metal movement (leaching).

4.2.2.1 Sequestration / Containment (Including Lignification, Humification)

Sequestration is a term that may be used for any instance in which an ion is prevented from exhibiting its usual chemical properties due to close combination or association with an added material. Two groups of organic sequestering agents of economic importance are the aminopolycarboxylic acids (e.g., ethylene-diamine-tetraacetic acid or EDTA) and the hydroxycarboxylic acids (e.g., gluconic, citric, and tartaric acids)(Hawley, 1977). Commercial applications of phytoextraction have relied on sequestration processes, whereby chelates were used to bind inorganic contaminants that were then sequestered by plants, making the contaminants environmentally unavailable.

In lignification, metal contaminants may be incorporated into plant lignin, making the contaminant biologically unavailable, thus reducing the associated environmental risk. Lignin is a complex high molecular weight molecule that makes up 5-30% of the dry mass of plants. The structure of lignin is based on the phenyl

propanoid unit, which consists of an aromatic ring and a three-carbon side chain. Lignin is formed as an encrusted material associated with the cellulose and hemicellulose matrix of plant matter. A portion of lignin entering the soil does not undergo complete decomposition but reacts with microbially produced products in the formation of soil organic matter (SOM)(Paul and Clark, 1989). Lignification is a natural process that may be exploited for contaminant remediation via phytostabilization, however it might not be a permanent stabilization process as various species of fungi are known to degrade lignin, including the white-rot fungi (e.g., *Phanerochaete chrysosporium*, *Pleurotus ostreatus*), and brown-rot fungi (e.g., *Poria* spp. And *Gloeophyllum* spp.)(Paul and Clark, 1989).

Humification is the process of creating humus, which is "the dark-coloured major fraction of soil organic matter that is formed during the decomposition of organic residues and containing humic and fulvic acids and other poorly defined or unknown substances relatively resistant to decomposition" (Paul and Clark, 1989, p. 263). Humification may result in the incorporation of soil contaminants into the humus fraction of soil organic matter, which is a relatively stable environmental respository. Thus, humification is another natural process that may be exploited for contaminant remediation via phytostabilization.

4.3 Technology-Specific Factors and Processes

The stimulation of plant growth and plant-associated microbial communities capable of facilitating contaminant removal or stabilization is the ultimate goal of most if not all phytoremediation technologies. The use of amendments or methods designed to change the physico-chemical aspects of the contaminant(s), or the soil/water environment (e.g., chelates / ligands, surfactants, stabilizers, etc.), are also important technology elements. For *phytoextraction* and *phytostabilization*, there are many individual physical, chemical, and/or biological factors affecting processes involved in each technology application. The major or significant factors and processes for each of the individual technologies listed in this verification protocol are presented here. Associated with these factors and processes are characteristics or properties that somehow impact or affect the technology processes, and these are presented subsequently. This information is provided so that both the Applicant and the Verification Entity can better understand the elements of phytoremediation that are considered crucial to its technical success.

For *phytoextraction*, the major factors affecting the contaminant uptake or hyperaccumulation process are those factors that control:

i. The amount (concentrations) and speciation of the metals in the soil solution;
ii. Movement of the metal from the bulk soil to the soil solution and to the root surface;
iii. Transport of the metal from the root surface into the root; and,
iv. Translocation of the metal from the root to the shoot and/or leaves – and its storage therein.

For *phytovolatilization*, the factors affecting the uptake and volatilization processes are the same four factors mentioned above for phytoextraction, plus the factors that control:

v. Transpiration of metal from the shoot and/or leaves into the atmosphere.

For *phytostabilization / containment*, the factors affecting the contaminant stabilization (i.e., binding, sequestration, containment) processes – by the plant and/or its associated microorganisms – are the same four factors mentioned above for phytoextraction, plus the factors that control:

vi. Water infiltration and movement to the contaminated zone;
vii. Sorption (adsorption / desorption) of the metal from the soil solution;
viii. Sequestration of the contaminant within the plant, microbial biomass, or soil organic matter (e.g., as in lignification or humification).

4.4 Properties Affecting Technology-Specific Processes

The significant characteristics or properties that affect or impact terrestrial phytoremediation processes – as mentioned above – include, but are not limited to: Soil physical, chemical and biological properties such as:

- Soil textural analysis (i.e., % sand / silt / clay);
- Cation exchange capacity (CEC) - amount;
- Soil organic matter (SOM) – content and type;
- Soil pH;
- Soil redox potential (P_E);
- Microbial species and population numbers;
- Soil moisture content and matric potential;
- Soil mineralogy.

The site climate, including:

- Precipitation (type and amount);
- Temperature;
- Sunshine or photoperiod (amount);
- Wind (amount and patterns).

Plant species characteristics such as:

- Maximum rooting depth;
- Root growth habit (tap versus fibrous versus combination);
- Culture requirements (e.g., nutrients, photoperiod, water, temperature, etc.).

Special characteristics for any amendments (e.g., surfactants, chelates, etc.), such as:

- Mode and frequency of application;
- Mode of action;
- Required amount / concentration;
- Ranges / limits to operation and/or effectiveness.

For aquatic / wetlands phytoremediation, the following additional characteristics are also considered significant in relation to the remediation control processes in the aqueous environment:

- Nutrient (e.g., N, NO_2, NO_3, NH_3, P, K) concentrations;
- pH;
- Biochemical oxygen demand (BOD_5).

Applicants are advised to ensure that sufficient data has been collected regarding these factors / processes / characteristics / properties to support the specific technology verification application.

5.0 Summary

A phytoremediation technology verification protocol has been created for technology developers, practitioners, scientists, regulators, and/or evaluators, providing them with guidance on recommended requirements for successful implementation and/or evaluation of phytoremediation technologies applicable to metal-contaminated matrices. The protocol addresses bench-scale treatability testing and technology development methods, field implementation considerations (e.g., sampling and process monitoring), and statistical aspects of process verification.

This publication represents a synopsis of the Environmental Technology Verification (ETV) Phytoremediation Technology-Specific Verification Protocol. As such, the author has attempted to introduce the reader to the Environmental Technology Verification Program and its components, as well as the Verification Process requirements that a Phytoremediation Technology vendor or program applicant would encounter.

The complete protocol report may be obtained by contacting Mr. John McMullen at ETV Canada, c/o 2197 Riverside Drive, Suite 300, Ottawa, ON, K1H 7X3; Tel. (613) 247-1900 ext. 228; Fax. (613) 247-2228; email: jmcmullen@etvcanada.on.ca.

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Numerous other phytoremediation publications are referenced in the technology-specific protocol report available from ETV Canada.

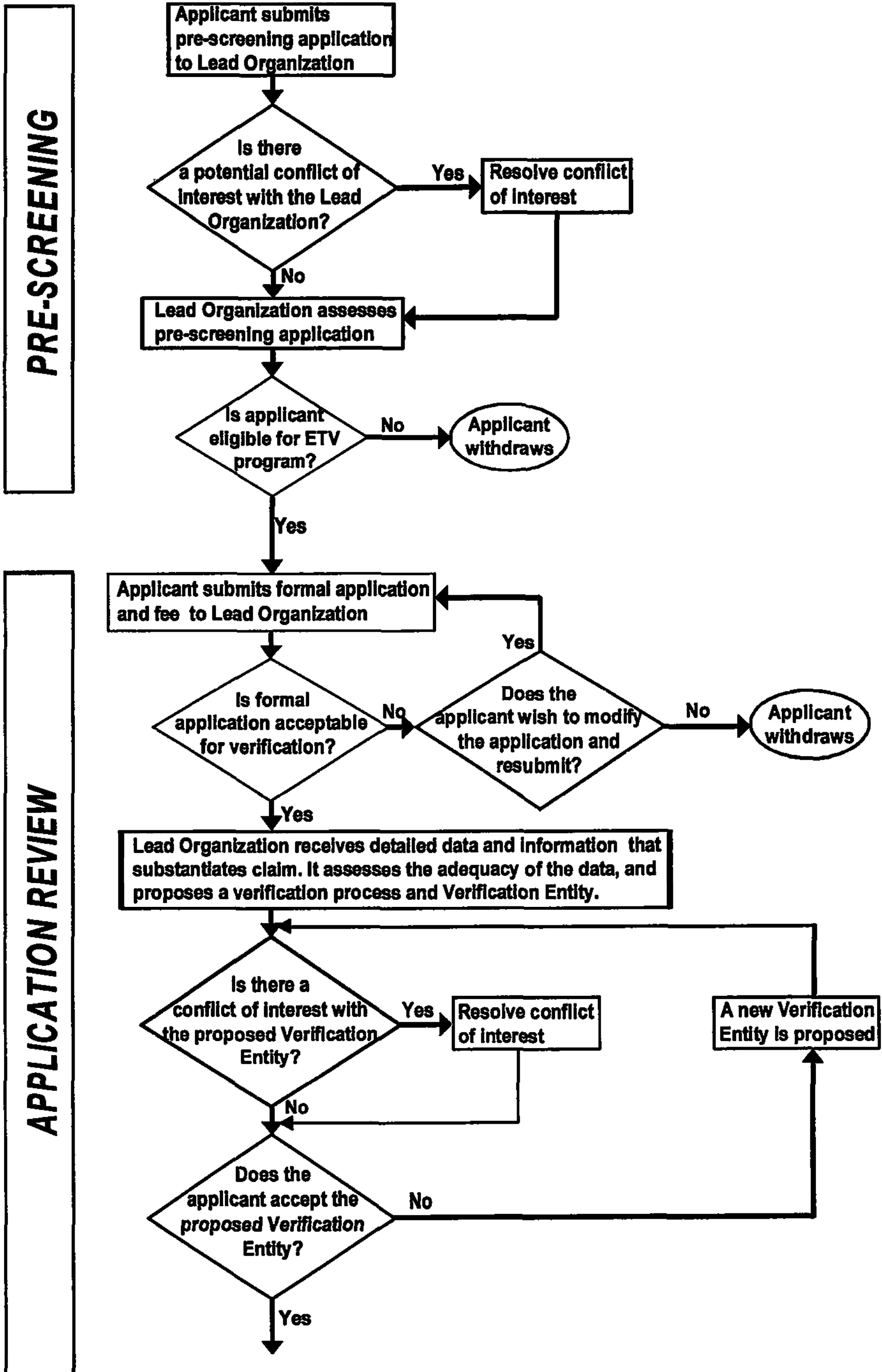
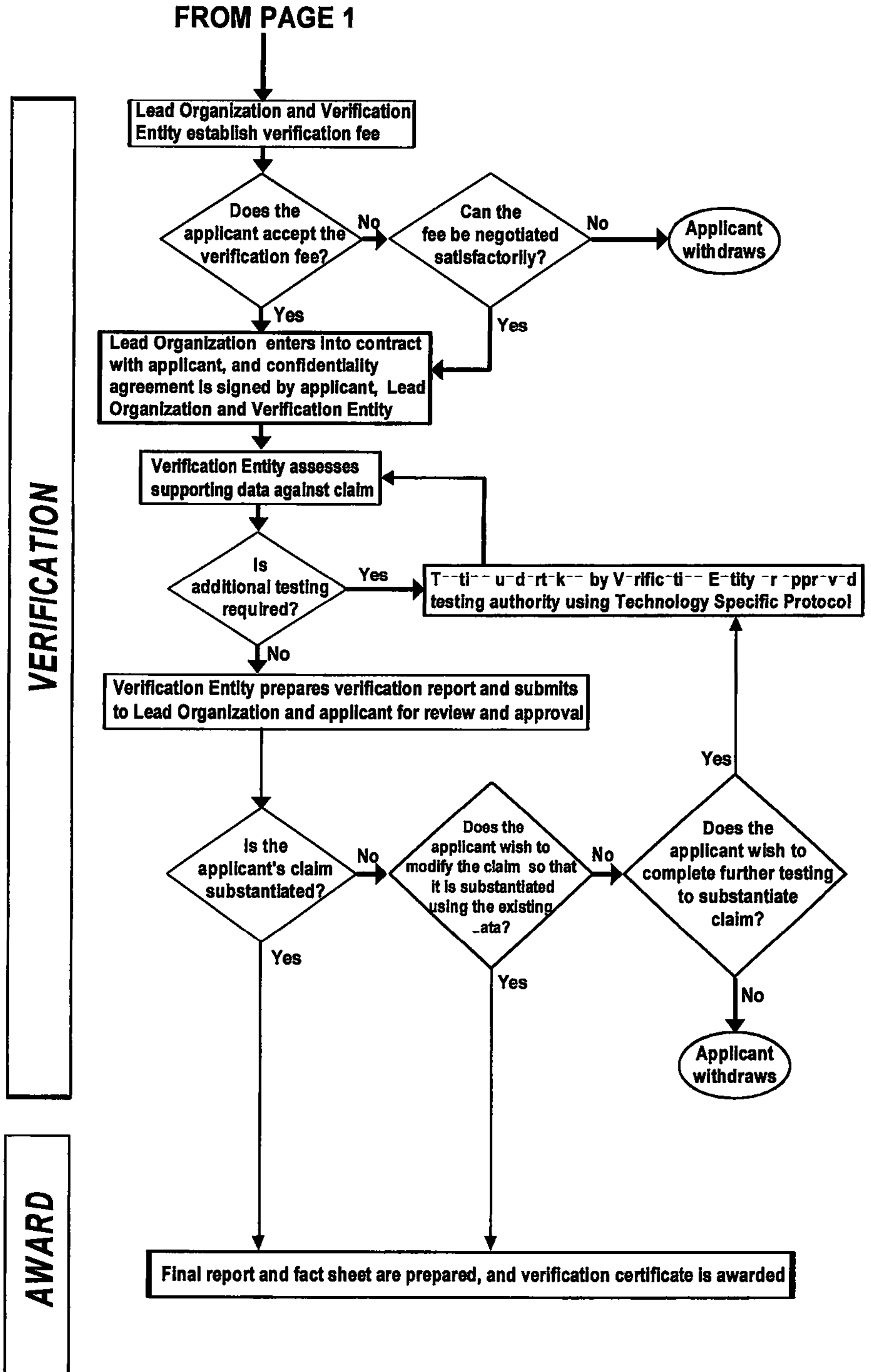


FIGURE 1: Proposed ETV Program Verification Sequence


FROM PAGE 1
VERIFICATION
Lead Organization and Verification Entity establish verification fee
Does the applicant accept the verification fee?
No
Can the fee be negotiated satisfactorily?
No
Applicant withdraws
Yes
Yes
Lead Organization enters into contract with applicant, and confidentiality agreement is signed by applicant, Lead Organization and Verification Entity
Verification Entity assesses supporting data against claim
Is additional testing required?
Yes
testing authority using Technology Specific Protocol
No
Verification Entity prepares verification report and submits to Lead Organization and applicant for review and approval
Yes
Is the applicant's claim substantiated?
No
Does the applicant wish to modify the claim so that it is substantiated using the existing
No
Does the applicant wish to complete further testing to substantiate claim?
Yes
Yes
No
Applicant withdraws
AWARD
Final report and fact sheet are prepared, and verification certificate is awarded

Development of a Worldwide Data Base on Metal Tolerant and Metal Accumulating Plants for Contaminated Site Remediation

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Abstract

Since the 1960s, the feasibility for employing a broad range of organisms, such as bacteria, fungi and algae for remediation purposes has come under increased scrutiny (Pelczar *et al.* 1993). This use of various organisms to extract toxic pollutants from the environment is known as bioremediation. More recently though, research focus has shifted towards the use of plant based systems for remediation, recovery, and rehabilitation of sites containing a variety of metals. Known as phytoremediation, this technology uses green plants to remove, contain, or render harmless environmental contaminants. The concept of using plants to treat waste water was known and applied over 300 years ago (Hartman, 1975). The use of plants to treat soil contaminants and waste products such as sludges and mine wastes is a more recent application of this kind of technology. Within the last 10 years, in particular, flowering plants, including wild species, crop plants and genetically altered plants, as well as aquatic ferns, have been the subject of intensive study for use as phytoremediation agents. Reviews and specific accounts describing phytoremediation have been widely published (Azadpour and Matthews, 1996; Black, 1995; Brown, 1995; Coghlan, 1997; Comis, 1995; Cunningham and Berti, 1993; Cunningham and Lee, 1995; Kim, 1996; Moffat, 1995; Parry, 1995; Rouhi, 1997; Salt *et al.*, 1995; Watanabe, 1997). This paper provides an overview of recent efforts by Environment Canada in the establishment of a worldwide database of naturally occurring aquatic and terrestrial plants having potential for remediation, recovery, and removal of metals at contaminated sites.

1.0 Introduction - Defining Phytoremediation and its Various Applications

The underlying success of phytoremediation is based on the capacities of certain plants to extract toxic substances or to tolerate their presence in the substrate or aquatic environments in which they grow. Some cultivated plants such as the common sunflower (Helianthus annuus) accumulate a wide range of metallic elements in substantial quantities as shown in hydroponic culture studies. Elements accumulated by sunflower plants include cadmium, cesium, chromium, copper, lead, manganese, nickel, strontium, uranium and zinc (Salt *et al.*, 1995). Another cultivated plant, Indian mustard (Brassica juncea), also has the ability to accumulate a wide range of metallic elements. In fact, it has been shown in field trials to accumulate lead to as much as 1.5% of its shoot dry weight (Watanabe, 1997). Alpine pennycress (Thlaspi caerulescens), a native plant of central Europe and the UK, has also been shown in field trials (Comis, 1995) and under natural conditions on mine

wastes in the UK (Baker *et al.*, 1994), to accumulate as much as 3% zinc (30,000 mg/g or parts per million) in its leaves without exhibiting toxic effects.

The highest rates of accumulation of metallic elements are by such plants as the New Caledonia tree Sebertia acuminata, a serpentine endemic. This plant has been shown to accumulate as much as 20% of its dry weight in nickel (Brown 1995). High levels of nickel accumulation, up to 22% total ash content, have been found in the Australian shrub Hybanthus floribundus (cited in Streit and Stumm, 1993 as first reported by Harborne, 1988).

The technology of phytoremediation relies on three main strategies for the use of plants or plant tissues outlined below and reviewed in greater detail by Salt *et al.* (1995).

Phytoextraction relies on growing plants that sequester large quantities of toxic elements within the aerial portion of the plant. Plants, whether wild species or cultivated varieties, are grown as crops that are harvested to remove the toxic elements accumulated within the plant. The harvested plant parts containing toxic elements from the contaminated site can be disposed relatively inexpensively as small quantities of toxic waste present in the plant ashes. If of commercial value, the toxic elements can be recovered through extraction procedures. Plants that have a sizable biomass above ground and that can be harvested several times a season to remove the toxic elements are the best candidates for phytoextraction. As well, to be most useful for phytoremediation, they should also have the ability to accumulate toxic elements in the order of 2-5% within the plant tissues (Brown *et al.*, 1995).

Biosorption is another form of phytoextraction applied to the removal of contaminants mainly from effluents using biological materials but not necessarily by entire living organisms. The most common biosorbent studies have been with algae. Species from several major algal groups have shown cell walls with extremely high adsorptive capacities for heavy metals (Kim *et al.*, 1995; Wang *et al.*, 1995; Wehrheim and Wettern, 1994; Wilson and Edyvean, 1995). Flowering plants, the main subject of the present undertaking, have also yielded examples of species that can serve as very efficient biosorbents. Creosote bush (Larrea tridentata), for example, ground up and used as a filtering material has a high capacity for removing Cu (Gardea-Torresdey *et al.*, 1996a).

Rhizofiltration is the use of plants whose roots absorb and sometimes precipitate contaminants from polluted waters. The most effective plants, such as grasses, are those whose roots regenerate rapidly and have large surface areas. Rhizofiltration works best where contaminants are present in low concentrations and in large volumes of effluent. Some plants are able to concentrate toxic metals, on a dry weight basis, to values ranging from 131 to 563 times the concentrations in the contaminant source (Dushenkov *et al.*, 1995).

Phytostabilization is an approach to remediation of sites contaminated with heavy metals that depends on the tolerance of plants to such elements. Plants can be useful phytostabilization agents even if they physically remove little or no contaminants as long as they have the ability to tolerate growth under such conditions. Contaminated sites generally support little vegetation. As a result, the barren soils are prone to erosion and leaching, processes that spread pollutants into

the environment. Plants that accumulate heavy metals and those that are merely metal-tolerant can be used to stabilize sites and often soil amendments are added to promote luxuriant plant growth.

2.0 Scope of Data Base

The main purpose of the present work was the compilation of a database representing a worldwide inventory of both terrestrial and aquatic plants of potential value for phytoremediation. Plants are defined here more specifically as vascular plants, including flowering plants, conifers and ferns and their allies. Species included in the database have demonstrated an ability or potential to tolerate, accumulate or hyperaccumulate specific metals or be useful as sorbents for metals. The following 19 elements served as the basis for plant selection and inclusion in the database:

Aluminum (Al)	Cobalt (Co)	Mercury (Hg)	Radium (Ra)
Arsenic (As)	Copper (Cu)	Molybdenum (Mo)	Strontium (Sr)
Beryllium (Be)	Chromium (Cr)	Nickel (Ni)	Uranium (U)
Cadmium (Cd)	Lead (Pb)	Palladium (Pd)	Zinc (Zn)
Cesium (Cs)	Manganese (Mn)	Platinum (Pt)	

The database includes both wild and cultivated species and varieties and also biological and ecophysiological information-where possible. Information on the following topics is also included: origin of the plants referenced in the studies; taxonomy, distribution, biological notes, environmental/health effects and uses of the species; cultivation practices; sources of material/species studied; weedy or other significant relatives; mode of action (re: tolerance and accumulation).

In the computerized searches for titles, papers were identified dealing with heavy metals and bacteria, algae, lichens, fungi and bryophytes (mosses and liverworts). Separate tables of species records have been compiled for these organisms. It was felt that records of these organisms such as algae, fungi and certain mosses may be useful since these organisms act as highly efficient biosorbents. Their inclusion in the database complements that of species of vascular plants that also serve as efficient biosorbents. The abilities of mosses and lichens to absorb relatively high concentrations of toxic metals make these organisms particularly useful as indicators of polluted environments. A particularly notable exception is sphagnum moss which serves as an excellent biosorbent (Gardea-Torresdey *et al.,* 1996b).

The Environment Canada library in Hull, Quebec, undertook an extensive computerized search of public access databases and commercial abstracting services based on key search words and the 19 elements for which data was required. Key search words and phrases included: phytoremediation, hyperaccumulate, heavy metals, heavy metal tolerance, heavy metal accumulation, sorption of heavy metals, serpentine, bioremediation, rhizofiltration, toxic metals, phytoextraction, microbial bioremediation, rhizosphere biodegradation, phytostabilization, constructed wetlands, biotransformation, decontamination, transgenic plants and phytoremediation, biomarkers of toxic exposures, vegetative remediation, trees and phytoremediation, phreatophytes, and treemediation.

The following major databases and resources were searched for relevant data:

AMICUS	ENV ABSTRACTS
BADADUQ	ENV LIBRARY
CAB	ENVIRO ET FAUNE
CANMET LIBRARY	LAURENTIAN
CBCA	POLTOX
CDN RESEARCH INDEX	UNCOVER
CHEMICAL ABSTRACTS ONLINE	U.S. EPA
CISTI CAT	U.S. PATENTS
CURRENT CONTENTS	WASTE INFO
ELIAS	WATER RESOURCES ABSTRACTS

Due to time restrictions and the existence of a major work summarizing terrestrial higher plants that hyperaccumulate metallic elements (Baker and Brooks, 1989), the search was primarily conducted for suitable references during the last 10 years. Although searches were initiated December 1, 1997 and conducted into March 1999, only references to the end of 1998 were included within the database.

As a result of these library searches, over 4200 titles of articles, books, abstracts and conference proceedings were retrieved. Based on title and abstract information, selections were made for works that appeared to contain specific data on species that related to their abilities to tolerate or accumulate one or more of the metallic elements in question.

Although the database was developed to be worldwide in scope, special attention was paid to ensuring that species native to Canada and the United States were included and that species from higher elevations in warmer regions were included since these are potentially useful candidates for phytoremediation in countries at higher latitudes.

Supplementary, but limited, data were obtained on the taxonomy, biology, ranges and uses of plants listed in the database from the author's personal library of botanical reference works as well as from the substantial holdings of the libraries at the Canadian Museum of Nature and at the Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa.

3.0 Basis for Inclusion of Species in Database

Beyond the general requirements that a species tolerate, accumulate or have the ability to adsorb any of the 19 metallic elements in question, certain specific levels of tolerance or accumulation had to be indicated in a publication for the species to be listed.

Initial emphasis was placed on entering species that were hyperaccumulators. The term hyperaccumulator was coined originally for plants accumulating >1000 mg/g (0.1%) nickel in their dried tissues (Brooks *et al.*, 1977). Reeves (1992) emphasized the point that hyperaccumulators, as first defined, were plants that accumulated Ni specifically in the above-ground tissues. Within the context of the present compilation, the definition of hyperaccumulation has been broadened to include species that take up the minimal levels of the elements (1000 mg/g) in any

part of the plant, including just cell walls, when biological materials are used as adsorbents.

As reviewed by Baker and Walker (1990), hyperaccumulators of manganese were later defined by Jaffré (1980) as plants containing >10,000 mg/g manganese in dried plant tissues. In their summary paper of terrestrial plants that hyperaccumulate metallic elements, Baker and Brooks (1989, Table 1) provided an overview of seven elements (Co, Cr, Cu, Pb, Mn, Ni, Zn) and their occurrence in various substrates, in vegetation in general, and in plants on ultramafic (serpentine) substrates. In this publication they also recognized hyperaccumulators of cobalt, copper and lead on the basis of the accumulation of >1000 mg/g of these elements. Since manganese and zinc are elements normally found at levels about 10 times higher in vegetation on both ultramafic and non-ultramafic soils than are Co, Cu, Pb and Ni, the minimum metal concentration for a species to be a hyperaccumulator for both Mn and Zn was set at 10,000 mg/g dry weight of plant material (Baker and Brooks, 1989).

No specific hyperaccumulation level was set for chromium by Baker and Brooks (1989), although they did report some questionable results indicating >1000 mg/g of chromium in two Zimbabwe plants (Dicoma niccolifera and Sutera fodina) published by Wild (1974). An evaluation of these species by Brooks and Yang (1984) yielded results at least 20 times lower. More recent work with alfalfa, Medicago sativa, (Tiemann *et al*, 1997) and aquatic species including Bacopa monnieri (Gupta *et al.* 1994), Azolla sp. (Priel, 1995), Spirodela polyrrhiza and Salvinia molesta (Srivastav *et al.*, 1994) and Pistia stratiotes (Sen *et al.*, 1987) indicate that these readily exceed the 1000 mg/g level in Cr accumulation. Normal levels in plant tissues are generally in the order of <1 mg/g (Baker and Brooks, 1989). For convenience, the 1000 mg/g level was adopted as the minimum level for recording a species as a hyperaccumulator of Cr. Species were recorded as accumulators if they contained at least 100 mg/g of Cr.

Cadmium hyperaccumulation was based on >100 mg/g, as indicated in Brown *et al.* (1995). Although minimum levels for hyperaccumulation of aluminum have not been proposed in the literature, a value of >1000 mg/g has been used for purposes of recording species in the database. This was inferred as being a reasonable value in light of results in Grauer and Horst (1990) that indicate Al-sensitive barley (Hordeum vulgare) and horse bean (Vicia faba) show toxicity at 1.85 mM (50 mg/g) and 9.3 mM (250 mg/g) respectively. Values of Al accumulation by a number of species recorded in the database are substantially higher than the 1000 mg/g level. Levels below this minimum value were recorded as representing cases of accumulation. Species that were indicated in published works as tolerating significant amounts of Al were also included in the database even when no concentrations were cited.

Arsenic levels in plants are generally <12 mg/g (Porter and Peterson, 1975). This is in the same range as elements such as copper and lead for which the hyperaccumulation levels are >1000 mg/g. The same concentration for hyperaccumulators of As has therefore been used in compiling the database. Only a single record of accumulation of molybdenum (>1500 mg/g) was noted in the literature. This was for Thlaspi caerulescens and was considered to represent hyperaccumulation.

Vegetation on substrates containing mercuric sulphide can contain mercury in the range of 0.2-10 mg/g of plant dry weight (Atri, 1983). This is roughly the same level of concentration found for various other elements such as copper, chromium or lead (Baker and Brooks 1989). For the present compilation of records, hyperaccumulators of Hg are based on an accumulation >1000 mg/g on a dry weight basis.

No reports of accumulation were found for beryllium, palladium, platinum and radium. A summary of the various concentration levels used for recording accumulation of the major elements surveyed is given in Table 1.

Lower concentrations for certain elements were recorded for a species if they were moderately elevated above normal background levels and they were given in connection with an analysis that indicated that the plant was an accumulator or hyperaccumulator of some other metals. Certain minimal levels were used, commonly 100-200 mg/g (dry weight) of accumulated metal in a plant, as the cut-off point for entering a record into the database. Such levels were also used to select accumulator species that had not been recorded as hyperaccumulators. The basic premise applied in recording species was that it is better to include more species at lower, but yet seemingly elevated levels of metals, than fewer.

Table 1: Concentration Level Guidelines for Accumulation and Hyperaccumulation of Metals by Plants Used for Recording Species in the Phytoremediation Database (mg/g dry wt. or ppm).

	Al	As	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn
Acc*	100	100	10?	100	100	100	100	200	100	200	500
Hyp*	1000	1000	100	1000	1000	1000	1000	10000	1000	1000	10000

- * - Accumulation minimum level; ** - Hyperaccumulation minimal level

Although a large number of species are known to grow over serpentine substrates, only those specifically shown to be hyperaccumulators or to accumulate substantial levels of heavy metals are included in the database. Plants simply listed in floras or other treatments as serpentine species are, therefore, not included.

A number of major works and summary documents contributed greatly to the compilation of this database. Of particular significance, in terms of numbers of species, is the review by Baker and Brooks (1989) on plants that hyperaccumulate metals and that of Reeves *et al.* (1996) on nickel-accumulating plants of ancient serpentine soils of Cuba. Two compilations on serpentine vegetation and serpentine ecology and one on heavy metal tolerance in plants were also very helpful in providing information on a global basis (Baker *et al.*, 1991; Roberts and Proctor, 1992; Shaw, 1990). The compilation of heavy metals in aquatic plants by Atri (1983) is a major source of information on this topic. Element concentrations for most of the species listed in this work, however, are quite low. The publication came to light late in the preparation of the initial phase of the phytoremediation database and was not included as a source for the present compilation of species. Any suitable records in

this publication will be added in a future update to the database planned for later in 1998. Similarly, the book on Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment (Markert, 1993) is a valuable general reference work and a source of specific data on metal concentrations for some species. This source will also be used for updating the database. The paper by Frankenberger and Losi in Skipper and Turco (1995) includes plants but, in particular, provides an excellent overview of microorganisms involved in bioremediation of chromium, mercury, arsenic and selenium. It does not provide specific data on levels of accumulation by the organisms that would permit the addition of species records into the database.

4.0 Database Organization

The PHYTOREM was compiled by Dr. Haber - National Botanical Systems under contract to Environment Canada. The database itself was compiled using Microsoft Access software. This particular database management software was chosen because of its widespread use throughout government departments and at many other agencies and for its interchange capability between several database and spreadsheet formats. The use of an older version of Access (i.e., version 2) was rationalized as being a suitable format in that the database would be accessible to a broader user group. Those with the recent Access 97 should have no problem converting and using the phytoremediation database directly.

The database consists of three species tables. The main species table includes only vascular plants. The two subsidiary tables includes species that are useful for bioremediation, in general; one includes algae, lichens, fungi and bryophytes, the other, bacteria. Two tables of references are provided. The main table includes all citations referred to in the database that relate to concentration levels of elements accumulated or tolerated by all species recorded in the database and the second is a table of general botanical and reference works consulted. A series of sample queries, three data entry forms and two report formats for printing out vascular plant records and the references for all species entries are also provided (Table 2).

Data for all organisms was originally entered into a single database table with the fields established for entry of information most pertinent to vascular plants. This table was subsequently divided to separate the species of primary concern, the vascular plants, from other organisms. The table that now includes the algae, lichens, fungi and mosses and that for bacteria were much simplified to reduce fields to a basic set to provide nominal information on taxonomy and primary information on the elements of concern in relation to bioremediation.

Table 2. PHYTOREM components.

Tables	Species tables: Plants Algae, lichens, fungi, mosses (including liverworts) Bacteria References tables: References - botany References - metals (all organisms)
Example Queries (plants)	Canadian plants United States plants Ni hyperaccumulators, worldwide Lead accumulators Mustard family (Brassicaceae) Mustards hyperaccumulating Pb Monocots (grasses and other groups) Aquatic plants (submersed and emergent species) Ferns and allies
Forms	Botany references data entry Metal references data entry Plant data entry
Report	Vascular plant species report References for all species entries

4.1 Data Field Descriptions

4.1.1 Plants Table

The primary table of data on vascular plants consists of 38 fields for recording data entry sequence and information on the taxonomy, distribution, habitat, biology, uses and data on metal accumulation or tolerance referenced to published works. Table 3 provides a description of the fields and their contents. To accommodate the substantial information and numerous references to data available on Indian mustard (Brassica juncea), the original database structure needed to be adapted from one containing primarily text fields to a combination of both text and memo fields. The latter store information in a more efficient manner that do not limit the maximum amount of data that can be entered per record. Memo fields can be searched and queries and filters can be applied, however, searches on short character strings such as an element symbol results in superfluous records being retrieved. Memo fields, also, cannot be sorted or indexed. To provide a more accurate search capability for critical data in some memo fields and to allow these fields to be sorted, a complimentary short text field was created. For example, the elem_action field (a text field) allows species to be sorted on the basis of the relative concentrations of elements recorded in the elem_conc_ref field (a memo field). A similar approach was taken to recording literature references. Many references exceed the 255 character field limit of Access. Separate fields allow references to be sorted by author, year and journal.

For fields lacking data or that were not completed because of time constraints in developing the initial phase of the database, a "Null" character string was inserted. This indicates a variable of no value or of unknown value.

It should be pointed out that several major works containing multiple species entries that summarize metal accumulation were used to enter many records in the database. Such references are recorded in the summary_ref field. The original publication cited in such summary publications is given in the primary_ref field. Because of time constraints, only a few of the original publications cited in such summary works were consulted. For other records listing references only in the primary_ref field, the original publications were used to extract data. In a few instances, only abstracts of papers were available for review.

Table 3: Field Descriptions for the Plants Table

Name	Type	Size	Description [and explanatory comments]
Entry_seq	Number	4	Automatic counter field to record sequence of data entry [allows multiple records taken from a single summary document to be grouped together for purposes of review and data management]
Type_org	Text	2	Vascular plant (VP); [field allows tables of different organisms to be combined and provides the ability to distinguish records by type of organism]
Growrh_form	Text	4	FErn, GRaminoid, HErb, SHrub, SUcculent, VIne, Tree
Sci_name	Text	150	Complete scientific name with authorities
Synonym	Text	255	Common synonym
Com_name	Text	100	Common English name(s)
Cv_strain	Text	255	Cultivar or strain name or code; also transgenic variants
Family	Text	20	Taxonomic category: family
Order	Text	20	Taxonomic category: order
Subclass	Text	20	Taxonomic category: subclass
Class	Text	15	Taxonomic category: class
Tax_notes	Memo	-	Taxonomic notes
Duration	Text	4	A(nnual), B(iennial), A/B, P(erennial), A/P, B/P

Table 3: continued

Name	Type	Size	Description [and explanatory comments]
Origin	Text	200	Country or region of origin of plants on which report(s) is based [Canadian and USA species or those of other continents or countries can be sorted]
World_range	Memo	-	World range of the species
Primary_habitat	Text	3	Terrestrial, Aquatic, Terrestrial/Aquatic (T, A, T/A); [provides the ability to distinguish between terrestrial and aquatic plants and marsh/wetland species (T/A)]
Hab_descr	Text	200	Habitat description [allows for sorts to distinguish plants from serpentine soils and to distinguish cultivated species (crop plants)]
Namer_occ	Text	1	North American occurrence: N(ative); E(xotic); X (not present); ? (status unknown); [allows North American species to be categorized]
NAmer_spp	Memo	-	Indication of whether other species in the genus are present in North America
Sig_relatives	Memo	-	Other species of significance in the genus
Cult_wild	Text	1	Crop plant ©, horticultural species (H), wild (W)
Cult_info	Memo	-	Propagation and/or test studies info and reference
Cult_source	Memo	-	Source of cultivated material as indicated in publication
Impact_attributes	Text	15	UN (unknown), WD (weed), NX (noxious properties), HY (hybridizes), PH (disease and insect pest host)
Impact_description	Memo	-	Documentation of potential impact [e.g., information on invasive potential of an exotic weed]
Uses	Memo	-	General uses of plant and references [medicinal, edible]
Bio_notes	Memo	-	Notes on such topics as pollination, dispersal mechanism & references
Gen_notes	Memo	-	Toxicity of metal to the plant and other pertinent info. & references

Table 3: continued

Name	Type	Size	Description [and explanatory comments]
Elem_conc_ref	Memo	-	Author reference and concentration of elements with organ of storage
Elem_form_ref	Text	200	Element form & reference if specifically indicated [e.g., Cu(II)]
Elem_chel_ref	Text	255	Element, chelate used & reference
Elem_action	Text	50	Hyperaccumulate (H); Accumulate (A); Tolerate (T) ; Rhizosphere concentration ®; Precipitate (P); [e.g., PbH]
Storage_sites	Text	50	Sites where element concentrations were measured [e.g., root, shoot, leaf]
Biofiltr_use	Text	2	Use for biofiltration (Y/N); [used to distinguish species useful for such a function as indicated in the publication]
Biofiltr_ref	Text	255	References to the biofiltration publications [generally the same as the primary reference]
Tolerance_info	Memo	-	Information on conditions under which tolerance occurs
Summary_ref	Text	50	Reference to summary papers where several to many species are listed
Primary_ref	Text	200	Primary literature reference (obtained from summary reference if summary document was used)

4.1.2 Tables for Bacteria, Algae, Lichens, Fungi and Bryophytes

Table 4 provides a listing of data fields for the table of bacteria records. Because bacteria were not a primary focus for the purposes of the phytoremediation database, only nominal information on the organisms themselves is provided. Similarly, a much reduced set of data fields are provided for algae, lichens, fungi and bryophytes (Table 5).

Table 4: Field Descriptions for the Bacteria Table

Name	Type	Size	Description [and explanatory comments]
Entry_seq	Number	4	Automatic counter field to record sequence of data entry [allows multiple records taken from a single summary document to be grouped together for purposes of review and data management]
Type_org	Text	2	Bacteria (BA); [field allows tables of different organisms to be combined and provides the ability to distinguish records by type of organism]
Sci_name	Text	150	Complete scientific name with authorities
Cv_strain	Text	255	Strain name or code
Tax_notes	Memo	-	Taxonomic notes
Origin	Text	200	Country or region of origin of bacteria on which report(s) is based
Cult_source	Memo	-	Source of cultured material as indicated in publication
Bio_notes	Memo	-	General notes on important/ interesting aspects of the biology & references
Gen_notes	Memo	-	General notes on procedures of the study or results and references
Elem_conc_ref	Memo	-	Author reference and concentration of elements
Elem_form_ref	Text	200	Element form & reference if specifically indicated [e.g., Cu(II)]
Elem_chel_ref	Text	255	Element, chelate used & reference
Elem_action	Text	50	Hyperaccumulate (H); Accumulate (A); Tolerate (T) [e.g., PbH]
Storage_sites	Text	50	Sites where element concentrations were measured [e.g., cell wall]
Biofiltr_use	Text	2	Use for biofiltration (Y/N); [used to distinguish species useful for such a function as indicated in the publication]
Biofiltr_ref	Text	255	References to the biofiltration publications [generally the same as the primary reference]
Summary_ref	Text	50	Reference to summary papers where several to many species are listed
Primary_ref	Text	200	Primary literature reference (obtained from summary reference if summary document was used)

Table 5 Field Descriptions for the Algae, Lichens, Fungi and Bryophytes (mosses) Table

Name	Type	Size	Description [and explanatory comments]
Entry_seq	Number	4	Automatic counter field to record sequence of data entry [allows multiple records taken from a single summary document to be grouped together for purposes of review and data management]
Type_org	Text	2	Algae (AG); Lichens (LI); Fungi (FU); Bryophytes (BR) ; [field allows tables of different organisms to be combined and provides the ability to distinguish records by type of organism]
Sci_name	Text	150	Complete scientific name with authorities
Synonym	Text	255	Common synonym
Com_name	Text	100	Common English name(s); [or general name of the group if available]
Cv_strain	Text	255	Strain name or code
Family	Text	20	Taxonomic category: family
Order	Text	20	Taxonomic category: order
Subclass	Text	20	Taxonomic category: subclass
Class	Text	15	Taxonomic category: class
Tax_notes	Memo	-	Taxonomic notes
Origin	Text	200	Country or region of origin on which report(s) is based [Canadian and USA species or those of other continents or countries can be sorted]
Primary_habitat	Text	3	Terrestrial, Aquatic, Terrestrial/Aquatic (T, A, T/A); [provides the ability to distinguish between terrestrial and aquatic organisms]
Cult_wild	Text	1	Cultured © or wild (W)
Cult_info	Memo	-	Propagation and/or experimental conditions and reference
Cult_source	Memo	-	Source of cultivated material as indicated in publication
Gen_notes	Memo	-	Toxicity of metal to the organism, etc. and other pertinent info. & references

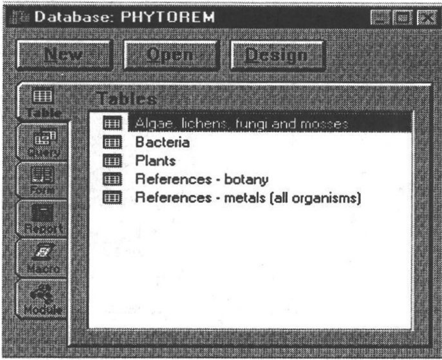
Table 5 continued

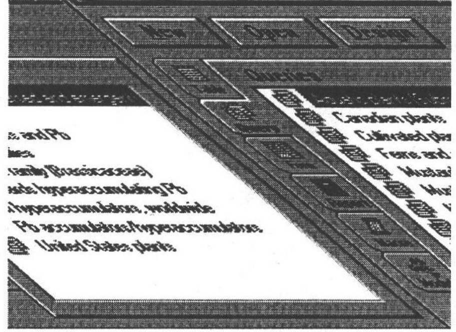
Name	Type	Size	Description [and explanatory comments]
Elem_conc_ref	Memo	-	Author reference and concentration of elements
Elem_form_ref	Text	200	Element, form & reference, if specifically indicated [e.g., Cu(II)]
Elem_chel_ref	Text	255	Element, chelate used & reference
Elem_action	Text	50	Hyperaccumulate (H); Accumulate (A); Tolerate (T) [e.g., PbH]
Biofiltr_use	Text	2	Use for biofiltration (Y/N); [used to distinguish species useful for such a function as indicated in the publication]
Biofiltr_ref	Text	255	References to the biofiltration publications [generally the same as the primary reference]
Tolerance_info	Memo	-	Information on conditions under which tolerance occurs
Summary_ref	Text	50	Reference to summary papers where several to many species are listed
Primary_ref	Text	200	Primary literature reference (obtained from summary reference if summary document was used)

5.0 Database Use and Search Capabilities

5.1 Using the Phytoremediation Database

Once the phytorem.mdb file is opened in Microsoft Access (either version 2 or later releases; see Fig. 1), a simple way to view some of the data is to open the sample queries provided. A series of sample queries that demonstrate a range of search possibilities have been included with the database and are accessible through the database window under query (Fig. 1b). Even the novice user of Microsoft Access should be able to simply change the criterion characteristic for the query by selecting design mode and entering beside the criterion heading another standard symbol for a metal or other query criterion.





a b

Figure 1: Microsoft Access (ver. 2.0) database window showing contents of the tables menu (a) and query menu (b) for the phytorem.mdb.

In the present state of database development, the following text fields are relatively complete providing access to all species records:

Taxonomy and species origin Sci_name (botanical name of species) Family Order Subclass Class	Cultivation Cult_wild Elements and concentrations Elem_conc_ref Elem_action
Geography and habitat Origin (of samples/species tested) Primary_habitat (terrestrial; aquatic)	References Summary_ref Primary_ref

Data sets can be quickly retrieved by applying an appropriate filter. Data sets of species fulfilling one or more filter criteria can be viewed in tabular format, however, due to the numerous fields in the database, only a fraction of the data for any one species can be seen. A more complete view of data on any one particular species in a data set retrieved through the filtering process is possible by using the data entry form (Figure 2).

Plants

Field	Value	Field	Value
Entry_seq:	289	Cult_wild:	C
Type_org:	VP	Cult_info:	See Dushenkov et al. 1995 for experimental meth
Growth_form:	HE	Cult_source:	Dushenkov et al. 1995 & 1997: cv 173874 & cv
Sci_name:	Brassica juncea (L.) Czern.	Impact_attributes:	WD, HY, PH
Synonym:		Impact_description:	Widely escaped from cultivation as a weed of wa
Com_name:	Indian mustard; leaf mustard; Chinese mustard; b	Uses:	This is one of the important oil seed crops in India
Cv_strain:	Dushenkov et al. 1995 (cv 173874); Kumar et a	Bio_notes:	Detailed fact sheet based on Duke 1983 is avail
Family:	Brassicaceae	Gen_notes:	Studies on impact of Selenium on Brassica races
Order:	Capparales	Elem_conc_ref:	Dushenkov et al. 1995 (root of cv. 173874): Pb [
Subclass:	Dilleniidae	Elem_form_ref:	Dushenkov et al. 1995: Cd(NO3)2.4H20; Pb(NO3)2
Class:	Magnoliopsida	Elem_chel_ref:	Blaylock et al. 1997: EDTA used for Pb
Tax_notes:	Highly variable species with a number of des	Elem_action:	PbH; PbP; ZnH; NiH; CuH; CrA; CdA; UrA
Duration:	A	Storage_sites:	Root; shoot

Record: 289 of 572

Form View

Figure 2: Portion of the Plants Data Entry Form for the phytorem.mdb.

5.1.2 Search Hints and Notes

The following comments provide some guidance in undertaking searches and compiling data subsets for review purposes.

- The present database does not contain records of plants accumulating radium. If the user were to add records to the database of species accumulating radium, the standard symbol for radium, "Ra", would need to be altered for the purpose of data retrieval. Inappropriate records would be retrieved if the standard symbol were used. A replacement symbol such as "Rd" should be used if one wished to sort or filter records on the basis of the presence of this element. The program cannot distinguish between records containing "Ra" and those that include entries for chromium hyperaccumulation (CrA) in the elem_action field. This is not a problem if searches were based solely on the three character codes based on the element and whether the species is an accumulator (RaA, CrA) or hyperaccumulator (RaH, CrH). Such a modified replacement symbol was required to distinguish between records of species containing uranium "U" and copper "Cu". The standard symbol for uranium has been replaced with "Ur" to allow for searches on this element. Three species are currently in the database that accumulate uranium.
- To retrieve all records of species accumulating, hyperaccumulating, tolerating or precipitating elements, a query or filter criterion using only the symbol for the element should be used in relation to the elem_action field, e.g., "*Pb*". To be more specific in retrieval of species accumulating or hyperaccumulating a particular element, the criterion should include additional letters as appropriate, e.g., "*PbH*". The asterisk (a replacement symbol for any other characters coming before or after) should be used as shown. If the asterisk is not used, only records

for which the two or three letter character string occurs at the beginning of the field will be retrieved in the data subset. When simply using the "find" feature of Microsoft Access, asterisks are not needed since one can set the search parameters to be case sensitive and to find the character string anywhere within a field.

- There is no separate field to distinguish coniferous species, however, the single species presently recorded in the database can be retrieved by using the family Pinaceae or by retrieving all records with a growth_form of TR (tree).
- Certain species were reported in publications in relation to their use as rhizofiltration agents for heavy metals and were so recorded in the database. In other instances, studies dealing with nutrient culture experiments were not directly aimed at identifying such agents but have been recorded in some instances in the database under biofiltr_use as "Y(es)" because of their potential for such use.

6.0 Summary of Database Contents

With the limited time and funding available, database development focused on maximizing the number of species records in the database. Of secondary importance, given these restrictions, was the entry of supplementary information in such data fields as worldwide range, habitats, biology and uses. With the restriction of including only species that have been shown to accumulate or tolerate levels of heavy metals above certain minimal levels, a total of 775 species records have been compiled.

The following summary derived from the plants table provides an overview of the contents of some of the main fields for which data entry is relatively complete.
Families - 76
Orders - 39
Sub class - 9
Class (monocots and dicots)- 2
Countries of origin of species listed (based on names used in the publications) - 39

The greatest proportion of species recorded in the plants table of the database (a total of 465) are presently listed as accumulating, hyperaccumulating or tolerating a single heavy metal of the 19 elements surveyed in the literature. Additionally, 66 species have been recorded as taking up 2 elements and a further 25 of accumulating 3 elements. The balance of 15 species are ones with the broadest capabilities for heavy metal accumulation. These are able to accumulate at least 4 or more elements. The species are summarized below with the number of elements and relative degree of accumulation (Accumulation, Hyperaccumulation, Precipitation).

6.1 Aquatic and Wetland Plants

Azolla filiculoides; water-fern (Africa; floating) - 4 (CuA; NiA; PbA; MnA)
Bacopa monnieri; water hyssop (India; emergent species) - 5 (HgA; CuH; CrH; PbA; CdH)
Eichhornia crassipes; water hyacinth (pantropical/subtropical; troublesome weed) - 6 (CdH; CrA; ZnA; HgH; PbH; CuA)

Hydrilla verticillata; hydrilla (southern Asia but introduced and spreading as a troublesome weed in the warmer states of the USA) - 4 (CdH; CrA; HgH; PbH)
Lemna minor; duckweed (native to North America and widespread) - 4 (PbH; CdH; CuH; ZnA)
Pistia stratiotes; water-lettuce (pantropical and native to southern USA but an a aquatic weed) - 4 (CuT; CdT; HgH; CrH)
Salvinia molesta; water-fern (India) - 4 (CrH; NiH; PbH; ZnA)
Spirodela polyrrhiza; giant duckweed (native to North America) - 5 (CdH; NiH; CrH; PbH; ZnA)
Vallisneria americana; tapegrass (native to Europe and North Africa but widely cultivated in the aquarium trade - 4 (CuH; CdH; CrA; PbH)

6.2 Crop Plants

Brassica juncea; Indian mustard - 7 (PbH; PbP; ZnH; NiH; CuH; CrA; CdA; UrA)
Helianthus annuus; sunflower - 4 (PbH; UrH; SrH; CsH) [Salt *et al.* (1995) also list Cr(VI), Cd, Cu, Mn, Ni and Zn as being readily absorbed by the roots of plants grown hydroponically.]

6.3 Terrestrial plants

Agrostis castellana; bentgrass (Portugal) - 5 (AsH; PbA; ZnA; MnA; AlA)
Thlaspi caerulescens; alpine pennycress (Europe) - 7 (ZnH; CdH; CoH; CuH; NiH; PbH; CrA)
Athyrium yokoscense; fern (Japan) - 4 (CuH; CdA; ZnH; PbH)

The frequency distribution of records based on the 19 heavy metals recorded within the plants table of the database is given in Table 6 and Figure 3. No records are present of species accumulating beryllium, palladium, platinum or radium. Species with the highest concentration of the elements recorded in the database are listed below in tabular form. Names used are as given in the original publications cited and concentration values are in mg/g dried plant matter of roots or shoots, except where noted differently.

These results point to the interesting observation that certain cultivated plants are potentially very important phytoremediation agents. Not only can species such as sunflower (Helianthus annuus), Indian mustard (Brassica juncea) and alfalfa (Medicago sativa) accumulate a range of toxic metallic elements, but they also appear to have some of the highest capabilities for taking up certain elements.

Table 6: Frequency Distribution of Records Based on 19 Heavy Metals

Al: Solidago hispida (Canada) - 6820	Mn: Macadamia neurophylla (New Caledonia) - 51,800
As: Agrostis tenuis (=A. capillaris; cult.) - 2000	Mo: Thlaspi caerulescens (Europe) - 1500-8000
Cd: Vallisneria spiralis (India) - 6242	Ni: Psychotria douarrei (New Caledonia) - 47,500 [Hybanthus floribundus (Australia) - 22% total ash content]
Co: Haumaniastrum robertii (Africa) - 10,200	Pb: Brassica juncea (cult.) - 26,200
Cr: Medicago sativa (cult.) - 7700	Sr: Helianthus annuus (cult.) - high absorb. rate
Cs: Helianthus annuus (cult.) - high absorb. Rate	Ur: Helianthus annuus (cult.) - >15,000
Cu: Larrea tridentata (USA) - 23,700 (biosorption)	Zn: Thlaspi caerulescens (Europe) - 52,000
Hg: Pistia stratiotes (pantropical) - 1100	

Table 7: Number of Records Within the Plants Table Containing Each of the 19 Elements

Aluminum	25	Cesium	1	Lead	79	Nickel	372	Strontium	1
Arsenic	4	Cobalt	27	Manganese	28	Palladium	0	Uranium	3
Beryllium	0	Copper	67	Mercury	8	Platinum	0	Zinc	48
Cadmium	37	Chromium	35	Molybdenum	1	Radium	0		

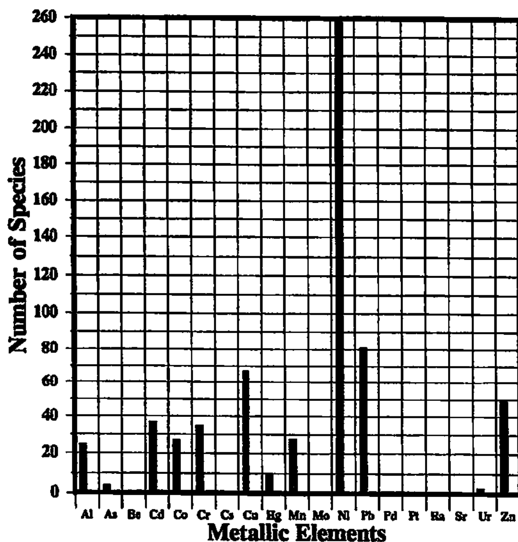


Figure 3. Histogram of the Number of Species Records in the Plants Database Table for Each of the 19 Elements.

The symbol for uranium has been changed from standard usage to reflect its modified form (Ur) adopted in the database to facilitate accurate sorting of records.

7.0 Data Update Requirements

7.1 Factors Limiting Record Inclusion

Although the database contains a considerable number of species records, many that should be included, based on the selection criteria used, were likely missed. Their exclusion results from several factors. The process of scanning only the titles or abstracts of potentially suitable publications during the selection process for obtaining interlibrary loans results in publications not being included. The minimal levels of heavy metal concentrations used to recognize species accumulating elements is another major contributing factor influencing selection of species. Although some flexibility was exercised in applying these lower concentration levels as selection criteria, species below these minimal levels could potentially be of value for phytoremediation. There are many crop species, in particular, that may prove to be useful in phytoremediation that are here excluded due to their low, but still elevated levels of heavy metal accumulation.

Time and cost restrictions have also limited the development of the database. Computerized library searches and the procurement of photocopies through interlibrary loans is a costly component of database development and has contributed toward limiting data entry.

7.2 Update Requirements

The database was compiled in a widely used database management format using Microsoft Access. This should allow recipients and potential users of this database to update information on existing species and to add new species based on suitable selection criteria.

The compilation of supplementary information dealing with the taxonomy, distribution, habitat preferences, reproduction, general biology, uses, culturing techniques and ecological impacts is extremely time-consuming. Only limited information of this type has been included and only for some of the better known species. At present, the plants database table also lacks the full characterization of plants based on the storage sites of metals accumulated. Once all records have this field attribute completed, a sort can be undertaken that separates plants that merely accumulate heavy metals in their roots from those translocating these to the shoots. Plants accumulating substantial quantities or hyperaccumulating metallic elements in the aboveground shoots are species most useful as agents for phytoextraction of elements through cropping of aboveground biomass.

Few references to studies attempting to clarify mechanisms of tolerance or accumulation of metals by plants are presently included in the database. The database would benefit considerably from the addition of such studies. Species useful in remediation of radionuclides are also poorly represented in the database. Greater emphasis needs to be placed on finding additional publications dealing with this subject.

Plants are also of considerable potential value in the remediation of organic compounds and as stabilizers of contaminated sites. Further literature searches emphasizing species that could be useful for these purposes would also add to the value of the database as a general resource of information on plants adaptable to the remediation of a broad range of toxic inorganic and organic waste products. The database was not meant, however, to serve as an encyclopedic repository of species information. Its main purpose is to provide easy access to published works dealing with species of highest potential for phytoremediation projects and basic research. It does not replace the need for in-depth bibliographic searches needed to undertake a comprehensive research program or remediation project.

The two ancillary database tables, one for bacteria and the second for algae, lichens, fungi and mosses, provide only initial listings of species and do not represent to any degree a comprehensive compilation. The exceedingly high adsorptive capacity for heavy metals of many of the algae, in particular, warrants that a more intensive literature search be conducted for this diverse group of organisms. They have a high potential for commercial applications for remediation of contaminated effluents.

For users of this database who have ample computer disk storage capacity, images can also be added to the database or a subset of the database for demonstration and teaching purposes. One or more OLE Object fields can be added to the database and graphic files can be viewed conveniently as part of a data entry form. A convenient graphics format is the PCX file format that is readily viewed using the

Windows Paintbrush software present on most computers using the Windows graphical interface.

8.0 Conclusions

With an anticipated and demonstrated increase in plant based remediation and restoration applications in Canada, this data base is expected to provide a valuable tool to assist in pre-selection of appropriate plant cultivar selection. Further, with increased interest being shown by the agricultural and mining community on molecular techniques for metal sequestration and recovery, this inventory of biological starter materials can provide valuable insight into possible genetic materials necessary to adapt / modify representative Canadian plant species that may be more appropriate to the circumstances. Finally, it is currently planned that this database, unique to metal accumulating and tolerant plants will be followed by future initiatives that identify analogous plant species that could be utilized for remediation and restoration of sites containing contaminated with organic materials (PHYTOPET) and radioactive materials (PHYTORAD).

9.0 Acknowledgments

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Using Terrestrial Plant Species to Extract Zinc and Manganese from Contaminated Sediment

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Abstract

Sediments are both carriers and potential sources of heavy metals in polluted aquatic systems. A greenhouse experiment was conducted to evaluate the effect of adding inorganic fertilizer (N, P_2O_5 and K_2O) and a chelating agent (EDTA) on growth of five terrestrial plant species and on Zn and Mn accumulation in the plant shoots. Surface sediment (0-10 cm) was collected from a site near Île aux Corbeaux on the St. Lawrence River (Quebec, Canada). Each treatment was replicated three times in a randomized complete block design.

A good growth of wild turnip (*Brassica rapa*), Indian mustard (*Brassica juncea*), black mustard (*Brassica nigra*), red fescue (*Festuca rubra*) and timothy (*Phleum pratense*) was established on these sediments. Inorganic fertilizers added to the substrates helped increase the dry matter yield of the terrestrial plant species. Application of fertilizers to sediment increased Zn and Mn accumulation in Indian mustard, black mustard and wild turnip. Also, shoot concentration and accumulation of Mn and Zn were appreciably higher in Indian mustard grown in sediment treated with chelate. Application of chelate to the sediment had little or no effect on shoot accumulation of Zn and Mn in other *Brassica* species and grasses.

These results indicate that Indian mustard would be a valuable plant species for extraction of Zn from fertilized sediment. Bioassay results indicate that after plant growth the fertilized sediment was not toxic to aquatic organisms. A field test must be conducted to confirm the greenhouse results before recommending use of this plant for phytoremediation of sediment contaminated with heavy metals.

1.0 Introduction

Île aux Corbeaux is located in the heart of the Lake St. Pierre archipelago in the freshwater portion of the St. Lawrence River. In 1994, during work to control shoreline erosion, old beacon batteries were discovered along a stretch of shoreline of about 450 meters. Riverside residents used these batteries together with stones to control shoreline erosion. Lapping, waves and ice action had, however, caused the batteries to disintegrate, contaminating adjacent sediment with heavy metals—zinc (Zn) and manganese (Mn) in particular.

Three zones, a total of 325 m^2, caused particular concern. The degree of sediment contamination was highly variable. In 1998, sediment where the remains of the batteries were found, showed high concentrations of Mn and Zn—as much as 5385 and 4208 mg/kg respectively. On the other hand, Mn and Zn concentrations as low as 6 mg/kg and 19 mg/kg respectively were measured close to these sites.

A number of site decontamination scenarios have been suggested, including phytoremediation on a terrestrial area near the contaminated zone. Phytoremediation,

which is becoming more and more popular (Giasson & Jaouich, 1998), is a technology that uses plants to extract metal from contaminated soil (Cunningham & Berti, 1993). Most phytoremediation has been performed on soil or in hydroponic systems (Brown *et al.*, 1995; Huang *et al.*, 1997; Ebbs & Kochian, 1997,1998), but there is little data on the potential of terrestrial plants for decontaminating metal-rich sediment. Data on phytoextraction of heavy metals could help in guiding phytoremediation of sediment from Île aux Corbeaux.

As is well-known, plants mobilize certain quantities of trace elements (Kabata-Pendias & Pendias, 1992) and heavy metals from mine tailings (Karam & Azzaria, 1989). This suggests that heavy metal loads can perhaps be reduced in this way. However, successful plant colonization of solid geologic materials containing heavy metals depends, among other things, on the fertility of the substrate and the plant species (Karam & Aajjane, 1997; Sauvesty & Karam, 1998).

The objective of this exploratory work was to find out if certain crucifers and gramineae grown in fertilized sediment treated with a chelate have potential for accumulating Zn and Mn in their aboveground biomass. We also wanted to find out if adding chelate to fertilized sediment affected the level of toxicity of the substrate to aquatic organisms.

2.0 Materials and Methods

Surface sediment (0-10 cm) was collected from a site near Île aux Corbeaux on the St. Lawrence River (Quebec, Canada). Samples were split in half, one half being assayed for chemical analysis and environmental sediment toxicity assessment (Bombardier & Turcotte, 1999), on the assumption that bioassays integrate the effects of all sediment toxicants (Miller *et al.*, 1985), and the remainder air-dried, crushed, mixed as a composite sample and retained for a greenhouse experiment.

Air-dried sediment samples (650 g) were placed in plastic pots and inorganic fertilizer applied at a rate of 0, 1x or 2x, where x was 54 mg/kg N (urea) + 39 mg/kg P_2O_5 (superphosphate) + 70 mg/kg K_2O (muriate). The pots were then placed on a greenhouse bench top in a randomized block design with three replicates. One week after the fertilizer application, five different species were planted: wild turnip (*Brassica rapa*), Indian mustard (*Brassica juncea*), black mustard (*Brassica nigra*), red fescue (*Festuca rubra*) and timothy (*Phleum pratense*). The wild turnip seeds were collected from an industrial site; the other species were market-provided. The amount of seeds added to the substrate was 3 cm^3 for wild turnip and 5 cm^3 for the other species. The photoperiod was 16 hours, and greenhouse temperature was maintained at 25°C. Fifteen and seven days before harvesting, chelate (EDTA 0.01M) was applied to the fertilized sediment and control at a rate of 0, 1x and 2x, where x was 165 mg/kg EDTA for each day of application.

After six weeks of growth, the aboveground portion of the plants was cut and oven-dried at 65°C for 72 hour and the dry weight yield was recorded. Subsamples were digested in a nitric-perchloric acid mixture (1:2) and then analysed for Mn and Zn using atomic absorption spectrophotometry (ASS). Following harvest, sediment used for the three replications of each treatment was mixed to form a composite, then air-dried and retained for chemical analysis. Total Zn and Mn in the substrate was extracted by digesting 0.5 g sediment in a mixture of concentrated acids (20 mL

HNO_3, 20 mL $HClO_4$ and 20 mL HF) in a Teflon beaker; the residue was taken up in 0.5 N HNO_3. Bioavailable Zn and Mn were extracted with DTPA-TEA-$CaCl_2$ solution (Liang & Karamanos, 1993). Metallic cations were determined by ASS. The same analytical procedure was applied to sediments prior to the greenhouse assays.

A total of 15 cultivated sediment samples were assayed with alga (*Selenastrum capricornutum*), hydra (*Hydra attenuata*) and bacteria (*Vibrio fischeri*) (Microtox™) as described by Environment Canada (1992) for the alga, Trottier (1996) for the hydra and Microbics (1992) for the bacteria.

For each plant species, an analysis of variance of the main effect of fertilizer rate and chelate rate and their interaction on shoot dry matter yield was carried out using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute Inc., 1990).

3.0 Results and Discussion

3.1 Sediment Properties

The general characteristics of the sediment collected were as follows: surface sediment was medium-textured (65.3% fine sand, 22.3% silt and 8.1% clay); pH measured in water was neutral to slightly alkaline (7.07 to 7.97); all sediment samples had carbonate, consistent with the alkaline pH of the sediment. Total organic carbon content was very low (0.5%). Surface sediment contained, on average, 761 mg/kg total N, 1123 mg/kg total P and 692 mg/kg total K. Total Zn content of the sediment ranged from 2 to 4208 mg/kg and total Mn from 5 to 5385 mg/kg. High proportions of the bioavailable Zn and Mn were in the oxide and carbonate fractions (data not shown) as designated by Tessier *et al.* (1979). Low Zn (2.9%) and Mn (0.4%) fractions were found in easily exchangeable form.

3.2 Effect of Fertilization and Chelation on Plant Growth

In general, the application of NPK fertilizer to sediment enhanced the growth and the shoot dry matter yield (SDMY) of most plant species (Figure 1). Without exception, the smallest yields were obtained from the control, due to the low availability of essential elements. Analysis of variance (Table 1) revealed a very highly significant effect of NPK fertilizer rate ($P < 0.001$) on SDMY of wild turnip, Indian mustard, red festuca and timothy, but only a significant effect ($P = 0.05$) on SDMY of black mustard. The response of the plant species to NPK fertilizer could be attributable to the improvement in sediment fertility. All treatments considered, the main effect of fertilizer rate on plant DMY was greater than the main effect of chelate rate, as indicated by the higher F value (Table 1).

Metal concentrations in shoot tissue were used to measure transfer of metal from the sediment. Without exception, average metal concentrations in the shoots of all plants increased depending on NPK fertilizer rate (Figure 2). Inorganic fertilization increased Mn and Zn concentrations in plant shoots. Amount of metal accumulated in the shoot tissue was calculated from aboveground plant tissue concentration and dry matter production. Indian mustard, black mustard and wild turnip accumulated more Mn than the two grass species. Compared to the control treatment, the highest rate of inorganic fertilization produced a 7.4-, 6.0- and 5.5-fold increase in Mn for wild turnip, black mustard and Indian mustard respectively.

The general trend in plant uptake of sediment-derived Zn was similar to that of Mn uptake (Figure 2). In general, Zn concentration in shoot tissue increased with fertilizer rate, with Zn concentration greatest in Indian mustard. Black mustard was the exception with its highest Zn concentration at NPK rate 1. However, differences in plant tissue Zn concentrations between the control and the highest NPK fertilizer rate were smaller than those observed for tissue Mn concentrations.

Chelate treatment effects on shoot Zn and Mn concentrations are presented in Figure 3. As the figure indicates, shoot Mn and Zn concentration and accumulation increased appreciably in Indian mustard grown in sediment treated with chelate. Application of chelator to the fertilized sediment had little or no effect on shoot accumulation of Zn and Mn in other *Brassica* species and grasses. It resulted in only a slight increase in metal concentration in shoot tissue. Indian mustard was the exception.

These results are consistent with those of Ebbs & Kochian (1998), who showed that shoot Zn concentration and accumulation increased significantly for Indian mustard following addition of EDTA to Zn-contaminated soil. As mentioned by these authors, grass species generally did not respond to chelating agent application. These results indicate that Indian mustard would be a valuable plant species for shoot Zn accumulation from fertilized sediment.

3.3 Effect of Chelate on Substrate Toxicity

One of the goals of the project was to determine if applying fertilizer and chelate to sediment might increase substrate toxicity. To this end, three ecotoxicological tests were performed on nine sediment subsamples taken before the start of the greenhouse experiment and fifteen subsamples of cultivated sediment (three substrates per plant species). The tests were as follows: the Microtox™ (solid phase) assay, the growth inhibition test using the alga *Selenastrum capricornutum* and the (sub)lethality test with the hydra *Hydra attenuata*. Not only did the series of tests consider a variety of species (three) and trophic levels (three), it also evaluated several types of effects: luminescence inhibition (Microtox™), growth inhibition (alga) and (sub)lethality (hydra). In addition, three levels of toxicity were investigated (acute lethality, acute sublethality and chronic sublethality).

The bioassays showed that the uncultivated subsamples were not toxic to the bacteria *V. fischeri* (Microtox™) or the coelenterate *Hydra attenuata*. Three of the subsamples, were, however, potentially toxic to the alga *Selenastrum capricornutum*, in one case the potential was high and in the other two it was moderate. In all three cases, sediment concentration of bioavailable Zn (Zn-DTPA) was high (Table 2).

However, sediment that had been fertilized and cultivated was not toxic to any of the three organisms tested. On the contrary, this sediment stimulated growth of *S. capricornutum*, sometimes more than 100%. This may have been attributable to the presence of fertilizing elements and the decrease in metal concentrations in the cultivated sediment.

4.0 Conclusion

The greenhouse experiment demonstrated the beneficial effect of combined NPK fertilization on growth of plant species in sediment. In addition, fertilization promoted accumulation of Mn and Zn in the aboveground portion of Indian mustard,

black mustard and wild turnip. This was further promoted by applying EDTA chelate to the growth medium, especially in the case of Indian mustard. Growth and development of terrestrial plants helps decontaminate the environment, as indicated by biotesting with the alga *S. capricornutum*. Last, the results obtained cannot be used to determine total plant species capacity for recovering metal from sediment since metal content of the root tissue was not measured.

5.0 Acknowledgements

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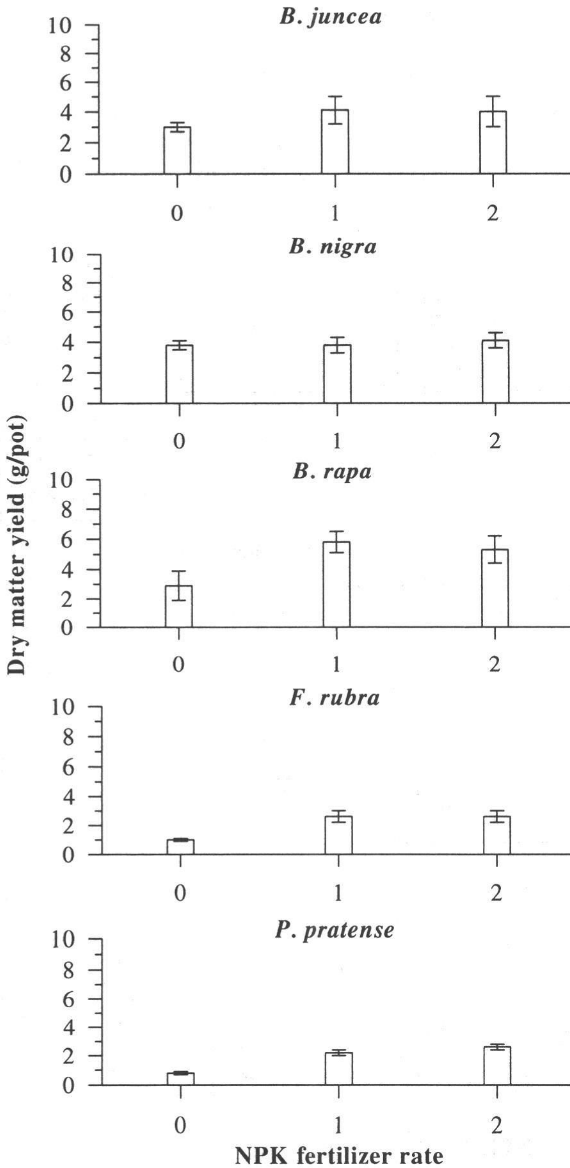

Figure 1 Effect of NPK Fertilizer Rate (all chelate treatments grouped) on Shoot Dry Matter Yield of Terrestrial Plant Species (averages and standard deviations)

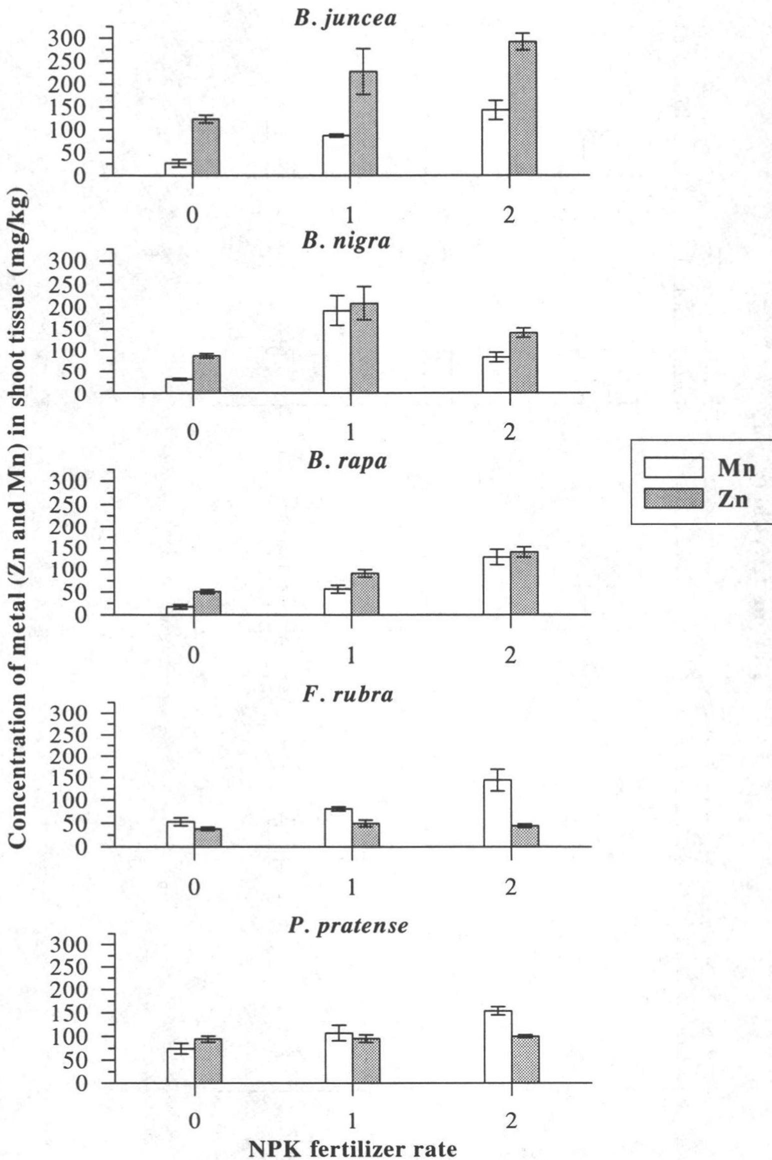

Figure 2 Effect of NPK Fertilizer Rate (all chelate treatments grouped) on Shoot Zn and Mn Concentrations (averages and standard deviations)

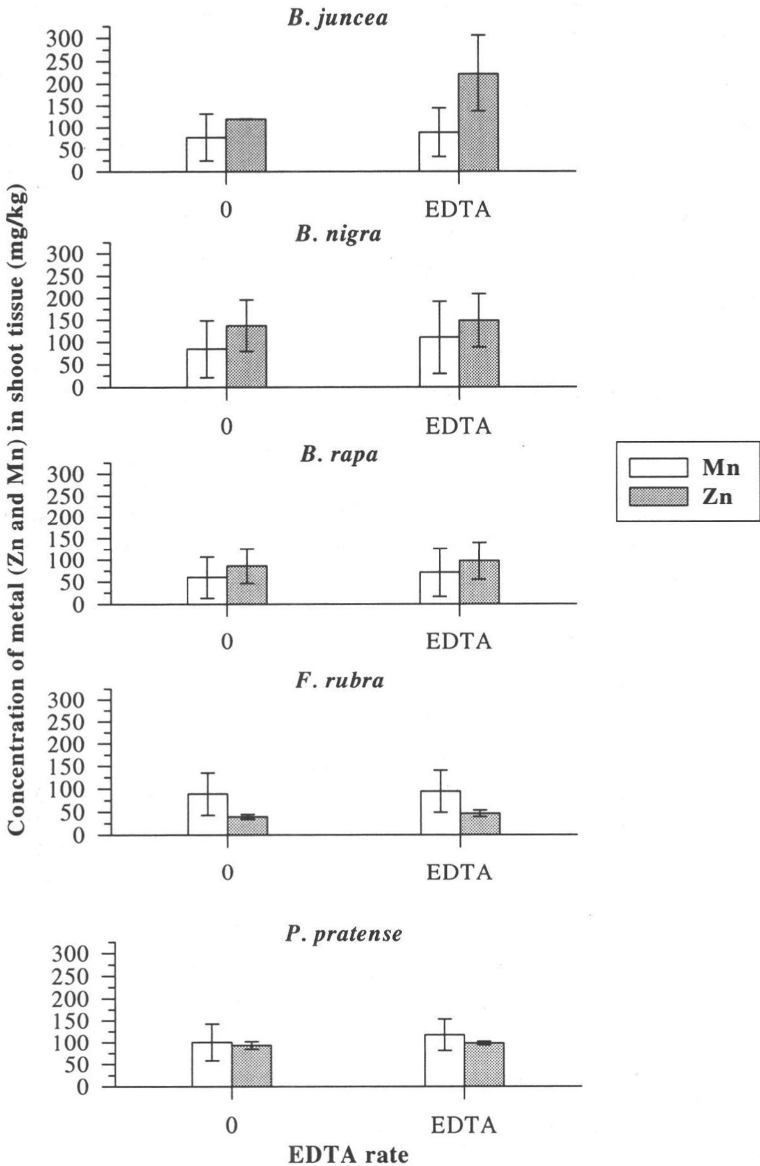

Figure 3 Effect on Shoot Zn and Mn Concentrations of Adding Chelate to Fertilized Sediments (all fertilizer treatments grouped) (averages and standard deviations)

Table 1 Summary of Analysis of Variance (Significance of *F* Values) to Determine the Effect of Fertilizer (NPK) Rate, Chelate (EDTA) Rate and their Interaction on Shoot Dry Matter Yield of Five Plant Species Grown on a Sediment Substrate under Greenhouse Conditions.

Source of variation	Degree of freedom	Wild turnip	Indian mustard	Black mustard	Red fescue	Timothy
Main effects						
Fertilizer rate (F)	2	129.3***	35.1***	4.0*	81.7***	244.5***
Chelate rate (C)	2	25.1***	9.1**	3.0*	6.9**	2.8 NS
Interaction (FxC)	4	7.3*	24.5***	9.5***	0.8 NS	1.9 NS

*,**,*** *F* values significant at $P = 0.05$, 0.01 and 0.001 respectively
NS: not significant.

Table 2 Bioassay Response to Nine Subsamples of Sediment Prior to the Greenhouse Experiment

	Bioassays				
Subsample	Microtox (*Vibrio fischeri*)	*Selenastrum capricornutum*	*Hydra attenuata*	Bioavailable Mn (mg/kg)	Bioavailable Zn (mg/kg)
1	Non-toxic	Non-toxic	Non-toxic	4.9	6.8
2	Non-toxic	Moderately toxic	Non-toxic	23.6	178.0
3	Non-toxic	Highly toxic	Non-toxic	15.2	646.5
4	Non-toxic	Moderately toxic	Non-toxic	37.9	354.0
5	Non-toxic	Non-toxic	Non-toxic	9.2	22.4
6	Non-toxic	Non-toxic	Non-toxic	39.3	30.8
7	Non-toxic	Non-toxic	Non-toxic	38.4	38.8
8	Non-toxic	Non-toxic	Non-toxic	22.5	25.8
9	Non-toxic	Non-toxic	Non-toxic	11.3	12.0

Constructed Wetlands for Treatment of Dissolved Phase Hydrocarbons

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Abstract

A pilot scale constructed wetland is being tested as an innovative remedial solution for surface treatment of condensate-contaminated groundwater. The wetland was implemented in 1997 to assess its potential as a lower cost alternative to mechanical-based treatment.

This paper presents results on the following: (a) hydrocarbon removal efficiency, (b) hydrocarbon removal mechanisms, (c) winter operation, and (d) effect of hydrocarbons on vegetation health.

Inflow water to the wetland contains between 15 to 20 mg/L of C_5-C_{10} hydrocarbons, including 50% BTEX compounds. Hydrocarbon removal efficiency in the wetland is 100% during the summer months. During winter, 100% of the hydrocarbons are also removed in the wetland, with the use of subsurface aeration. The wetland is operated without subsurface aeration during the spring, summer, and fall. Treatment efficiency in the wetland decreases to 60% and 30% hydrocarbon removal during spring and late fall, respectively. Hydrocarbons not removed in the wetland are subsequently removed along the outflow channel. Without aeration, temperature appears to be a significant factor in the variable removal rates. At present, the main hydrocarbon removal mechanism appears to be volatilization, and to a lesser extent, biodegradation and dilution. Plant uptake is not a factor at present. Winter operation has been successful for two winters, through the use of subsurface aeration coupled with surface straw insulation. Laboratory simulation of field conditions showed no effect on plant growth. Field observations indicate plants are healthy, and currently cover 1/3 of the wetland.

1.0 Introduction

A constructed wetland was implemented at the Gulf Strachan Gas Processing Plant in 1997 to evaluate its feasibility for treating extracted groundwater contaminated with natural gas condensate, as an alternative to conventional treatment. The use of constructed wetlands, also referred to as engineered or artificial wetlands, has been well documented for treatment of acid mine drainage and municipal wastewater (Hammer, 1989; Reed *et al.*, 1995). However, the use of constructed wetlands for treatment of dissolved phase hydrocarbons is relatively new. Wetlands have been evaluated for tertiary treatment (*i.e.,* polishing) of low levels of dissolved hydrocarbons (Litchfield, 1993; Nix and Bishay, 1996). However, this application is one of the first wetland applications for primary treatment, and may be the first of its kind for treatment of condensate-contaminated groundwater.

The site is located near Rocky Mountain House, approximately 200 km northwest of Calgary, Alberta. A pump and treat system has been operating since

1992 to prevent off-site migration of dissolved and free phase hydrocarbons. The extracted groundwater is being treated by a mechanical system with primary treatment provided by a shallow tray air stripper. Life-cycle remediation costs indicate that long-term pump and treat system operation costs can be substantial. In many cases O&M costs are much higher than the initial capital expenditure. A pilot scale constructed wetland was implemented in 1997 to assess its potential as a lower cost, reduced maintenance alternative to conventional treatment (CAPP, 1998; 1999).

This paper presents results on the following: (a) hydrocarbon removal efficiency; (b) hydrocarbon removal mechanisms; (c) feasibility of winter operation; and, (d) effect of hydrocarbons on vegetation health.

2.0 Design, Operation and Monitoring

2.1 Conceptual Design

During 1998, the constructed wetland system was comprised of four stages, described below:

- groundwater extraction;
- pre-wetland aeration tank;
- constructed wetland; and
- outflow channel

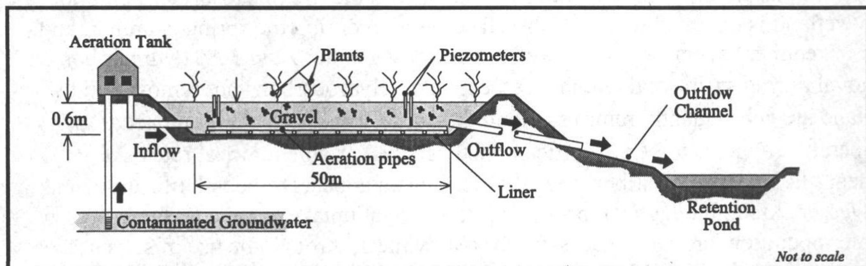

Figure 1 Constructed Wetland Cross Section Schematic

2.1.1 Groundwater Extraction

Groundwater containing dissolved phase hydrocarbons is pumped from recovery wells, located near the south boundary of the site (Figure 1). Groundwater extraction rates vary seasonally between 40 to 70 L/min. At present, 25% of the extracted groundwater is directed to the pilot scale wetland, with the remainder treated by the air stripper system. The water generally contains between 15 and 20 mg/L of total purgeable hydrocarbons (TPH, C_5 to C_{10}), containing approximately 50% BTEX compounds (benzene, toluene, ethylbenzene, and xylenes).

2.1.2 Pre-Wetland Aeration Tank

During 1997 and 1998, extracted groundwater was pumped into a 20,000 L pre-wetland aeration holding tank. Free phase hydrocarbons present in the extracted water are separated from the inflow water in the tank. The tank is aerated to promote precipitation of dissolved iron and manganese and stimulate aerobic biodegradation. Some hydrocarbon removal also occurs within the tank due to aeration.

2.1.3 Constructed Wetland

The wetland consists of a gravel-filled cell with surface dimensions of 50 m long x 17 m wide x 0.6 m deep. To protect groundwater quality beneath the wetland, the cell is bermed and double lined.

A network of 13 piezometers was installed for water monitoring (Figure 2). Four double piezometers were installed (CW-2A/B, 5A/B, 8A/B, and 11A/B) along the flow centreline to evaluate potential differences between near surface and subsurface water. The "A" series are screened at approximately 0.0 to 0.3 metres below ground surface (mbgs), and the "B" series piezometers are screened at approximately 0.3 to 0.6 mbgs. Nine piezometers with screen intervals between 0.0 to 0.6 mbgs were also installed (CW-1, 3, 4, 6, 7, 9, 10, 12, 13).

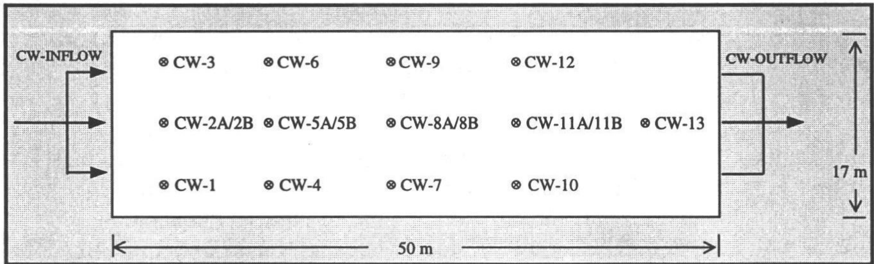

Figure 2 Piezometer Locations in Constructed Wetland

Planted vegetation consists primarily of *Phragmites australis* (phragmites) reed grass, an indigenous species chosen for its deep root penetration and robust growth. The phragmites were 0.3 m high, and in good health when planted. Approximately 400 mature cattails, *Typha latifolia,* were also planted during July 1998. The cattails were randomly distributed among the phragmites. All plants were installed at approximately 0.6 m spacing.

2.1.4 Outflow Channel

Wetland discharge flows into a heated sump at the NE corner of the wetland, and is discharged into a 15 m long surface outflow channel. This channel is gravel-filled, and drains to an adjacent pre-existing surface water retention pond. Should hydrocarbons reach the retention pond, infiltrating water flows downgradient for re-capture by recovery wells. Alternatively, the wetland discharge may be recirculated directly back to the wetland inflow.

2.2 Operation

During 1998, the average inflow rate to the wetland was approximately 12 L/min (17 m^3/d). The residence time of water in the wetland at this rate is approximately 14 days. The water surface was maintained at approximately 0.1 m below surface to reduce potential impact on wildlife, and allow a buffer against potential ice or frost buildup. The approximate volume of water in the wetland is 180,000 L, based on a gravel porosity of 36%.

To stimulate plant growth and enhance biodegradation potential, nutrients were added at regular intervals between May 21 to October 15, 1998, using a dual Dositron in-line feeder system. Three different nutrient regimes were used, depending on the season. During spring, a 10-52-10 mixture (% Nitrogen-Phosphorous-Potassium) was used to stimulate root growth. During summer a 50:50 mixture of calcium nitrate and 20-10-20 was used to stimulate plant growth, and monopotassium phosphate (0-51-34) was used in the fall for reproduction/budding. The plants were dormant during winter.

To reduce the potential for freeze-up during winter operation, air was injected into the base of the wetland from late November to early May. Air was injected into sixteen aeration lines at a constant pressure of 33.5 kPa with an air compressor. Outflow points on the north side of the wetland were periodically checked to confirm that air flow was occurring across the entire width of the wetland. As an additional precaution, the wetland surface was insulated at surface with 0.15 m of straw.

2.3 Methods

Depth to water, and field measurements of pH, electrical conductivity, and dissolved oxygen were monitored at regular intervals using handheld instruments. Water and air temperature were monitored continuously using on-site monitors equipped with data loggers. Flow rates were measured with an in-line flowmeter.

Water samples were collected on three occasions in 1997, and six occasions in 1998. Samples were submitted for laboratory analysis of main ions and dissolved iron and manganese (Table 3) and BTEX and total purgeable hydrocarbon (TPH, C_5-C_{10}) analyses. The iron and manganese samples were filtered in the field.

Vapours at surface were monitored using six vapour collection vessels (VCVs), inserted into the surface of the wetland along the flow centreline. The VCVs consist of 45 gallon steel drums, with the top end sealed and fitted with a swagelock valve for sampling. The bottom end is open, and inserted below the water surface of the wetland, leaving a 0.18 m headspace above surface for collection of vapours. Hydrocarbon vapour concentrations were measured using a GasTech Organic Vapour Analyzer (OVA) calibrated to hexane gas. The O_2 and CO_2 concentrations were measured with a GasTech 4 Gas Analyzer. The 16 hour period was chosen so that the VCV headspace would not constrain the volume of emitted vapours. Over a longer monitoring period, the headspace could reach vapour equilibrium resulting in a conservative estimate of mass flux to surface.

To evaluate the effect of hydrocarbons on plant health, laboratory and field evaluations were conducted. A laboratory study was conducted in 1997 to determine the effect of dissolved phase hydrocarbons on the survival and growth of the phragmites reed grass. A total of 29 plants were exposed to dissolved hydrocarbons collected from Gulf Strachan over seven weeks, simulating field conditions with respect to gravel, nutrients, light exposure, and hydrocarbon concentrations. Another 29 plants were exposed to the same conditions, except clean water was used. Water was changed twice weekly in both cases. Over a period of seven weeks, the plants were monitored weekly for mortality and stress. Mortality was defined as the proportion of plants exhibiting necrosis (dry tissue) exceeding approximately 50% of the leaves and/or shoot. Symptoms of stress were based on: number of tillers

(shoots), shoot length, leaf emergence, percent chlorosis (yellowing of above ground tissue), and panicle appearance.

To evaluate plant health in the wetland, 10 test plots, each 2 m^2, were selected at random. Plant measurements were made on during July and August, 1997, and in July, 1998. The number of shoots per plant, height of tallest tiller, and number of leaves on the tallest tiller were individually compared for the ten test plots. Root penetration was also determined for both phragmites and cattails.

3.0 Hydrocarbon Removal Effectiveness

3.1 Pre-Wetland Aeration Tank

Based on water sampling of the influent and effluent at the tank, aeration of extracted water in the pre-treatment tank resulted in a reduction of BTEX concentrations by 10% to 37%. A reduction in TPH concentrations of 20 to 45% was observed. Aeration also resulted in an increase in dissolved oxygen levels from 0.1 mg/L to 3.4 mg/L. Dissolved iron and manganese concentrations were not significantly affected by the aeration.

3.2 Wetland

Analytical results indicate that removal of BTEX and TPH were similar. Results showing TPH and BTEX concentrations at the wetland inflow (CW-IN) and wetland outflow (CW-OUT) are shown below in Table 1. Also shown are aeration status, average water flow rate, water temperatures near the wetland inflow and outflow, and air temperature measured on site.

Within the wetland, most of the BTEX removal occurs near the inflow (*i.e.*, prior to CW-8A) (See Figure 3, below). Steady state conditions are apparent during the latter portion of the wetland. Hydrocarbons remaining in the water at the wetland outflow are subsequently removed within the outflow channel.

Total hydrocarbon removal was observed on September 21, due to natural processes occurring within the wetland. Monitoring on May 20, June 4 and November 19 indicate respective BTEX removals of 42%, 52%, and 33% of the total input flux to the wetland.

Comparing May 6, 1998 data to May 20, 1998 data illustrates the importance of aeration for mass removal during colder temperatures. With subsurface aeration, BTEX removal efficiency is typically 100% (*i.e.*, May 6, 1998). The only exception was in early January 1998, when aeration efficiency was reduced due to iron precipitation buildup on the aeration lines (Table 1). After subsequent replacement of the aeration lines, this problem has not reoccurred.

3.3 Outflow Channel

As shown in Figure 3 below, approximately 40 to 60% of the hydrocarbon influx reached the wetland outflow on June 4 and November 19, 1998. Sampling at the outflow channel discharge indicates these remaining hydrocarbons were completely removed within the outflow channel, prior to discharge to the retention pond.

Table 1 Flow Rate, Temperature, TPH and BTEX Concentration vs. Time

Date Sampled	CW Aeration	Average Water Flow Rate (L/min)	Average Temperature: Water CW-2A (°C)	Average Temperature: Water CW-13 (°C)	Average Temperature: Air Outside (°C)	Total Purgeable Hydrocarbons (C_3-C_{12}) CW-IN (mg/L)	CW-OUT (mg/L)	TPH Removal	Total BTEX CW-IN (mg/L)	CW-OUT (mg/L)	BTEX Removal
Sept. 11/97	No	33	-	14.4	13.4	13.7	0.02	99.9%	9.63	<0.0045	100%
Oct. 9/97	No	14	-	9.4	7.1	22.6	6.6	71%	12.1	2.85	76%
Nov. 20/97	Yes	24	6.5	3.4	-5.1	19.8	<0.04	100%	9.54	<0.0045	100%
Jan. 14/98	Yes	24	5.7	0.5	-22.0	22.2	11.6	48%	8.60	3.42	60%
Feb. 4/98	Yes	19	7.1	0.6	-2.7	8.6	<0.04	100%	5.1	<0.0036	100%
Feb. 18/98	Yes	15	6.6	0.6	-3.6	27.7	0.9	97%	10.9	<0.0016	100%
May 6/98	Yes	7	8.4	6.6	13.0	8.4	0.2	98%	4.5	0.002	99.9%
May 20/98	No	11	9.1	8.6	10.4	9.6	6.1	36%	7.9	4.6	42%
Jun. 4/98	No	10	8.6	9.4	12.2	15.0	6.2	59%	11.0	5.3	52%
Jun. 17/98	No	7	9.8	9.5	11.0	15.9	6.5	59%	7.4	2.9	61%
Sept. 21/98	No	15	9.0	12.3	10.7	12.7	<0.1	100%	6.0	<0.00056	100%
Nov. 19/98	No	17	5.8	2.5	-6.2	13.8	8.8	36%	7.0	4.7	33%

The percentage reduction in total BTEX concentration across the wetland and at the outflow channel discharge during 1998 is shown below in Figure 3.

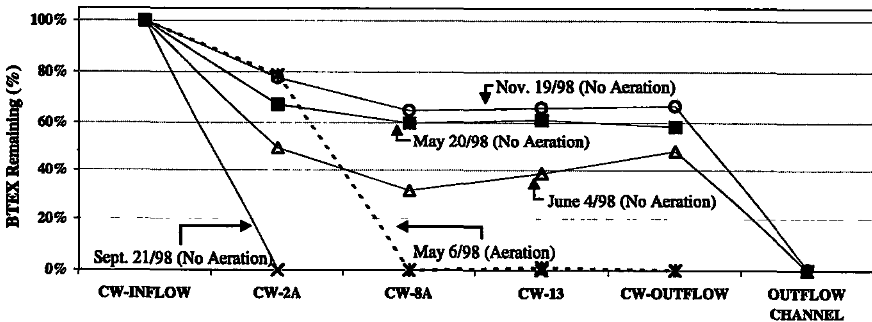

Figure 3. BTEX Concentrations in Wetland and Outflow Channel

4.0 Hydrocarbon Removal Mechanisms

4.1 General

As discussed above, hydrocarbon removal appeared to occur primarily near the inflow to the wetland. A lack of observed removal over the latter portion of the wetland relative to the inflow may be due to (a) reduced volatilization after CW-2A due to laminar flow within the wetland (*i.e.*, insufficient mixing between deep and shallow water) and (b) anaerobic conditions in the water after CW-2A resulting in reduced biodegradation rates.

Removal mechanisms in the outflow channel were not evaluated as part of the study. The rapid rate of hydrocarbon removal observed in the outflow channel could be due to turbulent flow along the outflow channel, resulting in enhanced exposure of the water to atmosphere.

As noted above, significant seasonal variability is observed with respect to hydrocarbon removal. The reason for the apparent improved removal efficiency on June 4 relative to May 20 may be due to the addition of nutrients commencing May 21, resulting in potentially enhanced microbial activity. Another potential factor, temperature, did not vary significantly during this period (see Table 1 above). The reason for the increased treatment in September relative to November does, however, appear to be temperature related. The water temperature in September was 3°C higher at the inflow and 10°C higher at the outflow relative to November. The higher water temperature would result in:

(a) increased hydrocarbon volatility, due to increased vapour pressure and Henry's Law Constant (Montgomery and Welkom, 1991); and
(b) increased biodegradation rates due to increased bioactivity at higher temperatures (Atlas, 1997).

A discussion of the expected important hydrocarbon removal mechanisms is presented below (volatilization, biodegradation, dilution, and plant uptake). Sorption is not considered to be a major factor, as there is no soil in the wetland, and a biofilm accumulation on the gravel surface is not apparent at present.

4.2 Volatilization

Volatilization was measured using six vapour collection vessels (VCVs) (see Section 2.3). The average hydrocarbon concentrations were measured to be 450 ppm over the first 6 m of the wetland, 60 ppm over the next 19 m of the wetland, and 45 ppm over the final 25 m of the wetland.

Hydrocarbon (HC) removed due to volatilization and biodegradation was calculated during the first 6 m of inflow, corresponding to the distance between the inflow and piezometer CW-2A, and the first VCV along the flow line. Calculations are made using data from November 19, 1998. As shown below, hydrocarbon mass removed due to volatilization accounts for approximately 53% of the total mass removal on this date.

<u>Total water volume in first 6 m of wetland</u>

Water volume = (volume wetland)(% porosity)(% distance along flow path)
= (180,000 L)(36% porosity)(6m/50m)
= 22,000 L

Total Hydrocarbon (HC) mass removed in first 6 m of wetland

- Assume the concentration measured at CW-2A is representative of conditions across the first 6 m of flow in the wetland

HC removed = (volume water) ([HC]water decrease between inflow and CW-2A)
= (22,000 L)(2.7 mg/L)
= 58.3 g of hydrocarbon

Mass removed due to volatilization

- Assume headspace measured at VCV near CW-2A is representative of conditions over the first 6 m of flow within the 17 m wide wetland
- Assume 1 mol gas = 22.4 L
- Assume hexane (C_6) is representative of hydrocarbon vapours

HC Volatilized= (Volume VCV)(6 m x 17 m)($[HC]_{vap}$)(1 mol air/22.4 L)(ave. HC mol. mass)
= (17,850L_{air})(450 ppm_v)(1E-06)(86.2g C_6/mole)
= 30.9 g

% Volatilized = (30.9 g HC volatilized) / (58.3 g HC removed)(100%)
= 53% of total mass

4.3 Aerobic Biodegradation

Aerobic biodegradation was assessed based on DO levels and microbial population. Aerobic conditions were present only near the inflow, as DO levels typically dropped from 3.4 mg/L at the inflow to 0.6 mg/L at CW-2A (located 6 m into the bed). The DO decrease of 2.8 mg/L is calculated to account for approximately 32% of the total hydrocarbon mass removed, assuming no inorganic oxygen uptake, based on consistent iron and manganese concentrations in the wetland. Uptake due to plants is not considered a factor due to the shallow root penetration into the water at present (maximum 0.1 m). This estimate does not account for additional oxygen influx from atmosphere. See calculations below:

Total Hydrocarbon (HC) mass removed in first 6 m of wetland

- Assume the concentration measured at CW-2A is representative of conditions across the first 6 m of flow in the wetland

HC removed = (volume water) ($[HC]_{water}$ decrease between inflow and CW-2A)
= (22,000 L)(2.7 mg/L)
= 58.3 g of hydrocarbon

Mass removed due to aerobic biodegradation

- Assume 3.5 mg/L DO consumed = 1 mg/L HC biodegraded (Hinchee *et al.*, 1992)
- Assume all DO consumption is due to aerobic biodegradation

HC Biodegraded = (DO consumption (mg/L))(1mg HC/3.5 mg DO)(22,000 L)
= 18.5 g

% Biodegraded = (18.5 g HC biodegraded) / (58.3 g HC removed)(100%)
= 32% of total HC removal

Microbial populations in the water were evaluated by collecting duplicate water samples from the wetland inflow and outflow for analysis of aerobic and anaerobic heterotrophs, and total hydrocarbon-degrading microbes. Samples were analyzed at the University of Alberta, Department of Microbiology using the most probable number (MPN) estimation method. Populations were enumerated under aerobic and anaerobic conditions for two week and four week periods, respectively.

The number of aerobic heterotrophs (3×10^5/mL) did not change between the inflow and outflow. The population of hydrocarbon degraders (3.4×10^3/mL in both influent and effluent) is considered to be relatively low. Overall, these results suggest the level of aerobic biodegradation occurring in the water may be limited. However, microbial populations in biofilm on the gravel matrix could be significantly higher than present in the water (Atlas, 1997).

4.4 Anaerobic biodegradation

Anaerobic conditions are present throughout the wetland, based on DO levels of less than 1.0 mg/L. Microbial enumeration indicate the anaerobic heterotroph population increased slightly between the inflow (2.3×10^2/mL) to the outflow (1.4×10^3/mL). These populations are considered relatively low. In general, anaerobic biodegradation of hydrocarbons is much slower than aerobic biodegradation (*i.e.* factor of 10) (Atlas, 1997; Morgan *et al.*, 1993). Based on the lack of hydrocarbon removal within the wetland after CW-8A, (see Figure 3, above), anaerobic biodegradation does not appear to be a major removal factor (*i.e.*, if anaerobic biodegradation were occurring, a steady decline in hydrocarbon concentrations across the wetland would be expected).

4.5 Dilution

Based on rainfall data, dilution does not appear to be a major factor at Strachan. Daily rainfall varied from 0 to 32 mm. A typical rainfall event was 10 mm. Based on a catchment area of approximately 1,000 m^2, a 10 mm rainfall equates to approximately 10,000 L of water in the wetland. Assuming a volume of water of 180,000 L in the wetland, the rainfall would result in a 6% dilution factor.

4.6 Plant uptake

Uptake by plants refers to the absorption of contaminants into the plant tissue (Cunningham *et al.*, 1996), resulting in a reduction in contaminant levels in the water. At this time, plant uptake does not appear to be a major factor, for the following reasons: (a) relatively shallow root penetration (maximum 0.1 m), resulting in minimal root/water contact over the 0.6 m water depth in the wetland; and, (b) essentially no change in hydrocarbon removal rates in 1998 compared to 1997, despite a 23% increase in plant density.

5.0 Effects of Hydrocarbons on Plant Growth

5.1 Laboratory Results

A total of 29 plants were exposed to dissolved hydrocarbons over seven weeks, simulating field conditions with respect to gravel, nutrients, light exposure, and hydrocarbon concentrations. Another 29 plants were exposed to the same conditions, except clean water was used. The laboratory results indicated the thirty

plants subjected to hydrocarbons at field concentrations (*i.e.*, 20 mg/L TPH; 10 mg/L BTEX) showed no adverse effects with respect to survivability and health (Komex International, 1997). One plant death was observed in the plant group subjected to hydrocarbons, and two plant deaths occurred in the control group. Statistical analysis indicate there was no difference in density, change in maximum shoot height, root and shoot fresh and dry weights, and root to total biomass ratio between the contaminated and control treatments. These results suggest that the addition of hydrocarbon contaminated water to the wetland would not adversely affect the health of the phragmites.

5.2 Field Observations

At the wetland, detailed vegetation assessment indicates the plants have remained healthy following commencement of hydrocarbon addition in August 1997, with negligible discoloration or signs of stress. Following winter dormancy, the plants have increased in size and density and currently cover approximately 33% of the wetland, from a starting point of 10%. In 1998, the root penetration of the phragmites and cattails was observed to be 6 cm and 10 cm, respectively, a 50% increase over 1997. No adverse effects of competition between the cattails and phragmites were observed.

6.0 Economic Evaluation

A comparative economic analysis of the constructed wetland versus a conventional air stripper system is not possible at this time. This is due to the fact that the wetland has not yet reached full treatment capacity, pending establishment of the plant community. Once the treatment capacity is established, the size of a full scale wetland can be calculated, enabling an evaluation of the economics.

7.0 Summary

Inflow water to the wetland contains between 15 to 20 mg/L of C_5-C_{10} hydrocarbons, including 50% BTEX compounds. Hydrocarbon removal efficiency in the wetland is 100% during the summer months. During winter, 100% of the hydrocarbons are also removed in the wetland, with the use of subsurface aeration. The wetland is operated without subsurface aeration during the spring, summer, and fall. Treatment efficiency in the wetland decreases to 60% and 30% hydrocarbon removal during spring and late fall, respectively. Hydrocarbons not removed in the wetland are subsequently removed along the outflow channel. Without aeration, temperature appears to be a significant factor in the variable removal rates. At present, the main hydrocarbon removal mechanism appears to be volatilization, and to a lesser extent, biodegradation and dilution. Plant uptake is not a factor at present. Winter operation has been successful for two winters, through the use of subsurface aeration coupled with surface straw insulation. Laboratory simulation of field conditions showed no effect on plant growth. Field observations indicate plants are healthy, and currently cover 1/3 of the wetland.

Overall, the wetland displayed promising results for hydrocarbon removal during summer operation. The treatment effectiveness of the outflow channel was shown to be similar to the wetland during lower temperatures in spring and fall.

8.0 Acknowledgements

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Validation of a Phytoremediation Computer Model

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Abstract

The use of plants to stimulate the remediation of soils contaminated with recalcitrant hydrocarbons represents an effective method. A recently developed phytoremediation model may be useful in predicting the fate of recalcitrant compounds in soil. The model was developed to simulate the fate of recalcitrant hydrocarbons interacting with plant roots in unsaturated soil. The objective is to provide data to validate and calibrate the model. Lysimeters were constructed and filled with soil contaminated with 10 [mg kg^{-1}] TNT, PBB, and chrysene. Vegetated and unvegetated treatments were conducted in triplicate. Data acquisition included contaminant concentrations in the soil, plant roots and herbage, root distribution, microbial activity, plant water use, and soil moisture. The simulation results for TNT demonstrate that the present model can predict contaminant concentration with time and depth under actual field conditions.

1.0 Introduction

Phytoremediation, the use of plants to stimulate the remediation of contaminated soil, represents an effective, low-cost, cleanup method (Jones, 1991) and can be applied to various sites. Plants have grown naturally in many hazardous waste sites or have been planted for aesthetic purposes or to provide a medium for stabilization (Chang and Corapcioglu, 1997; 1998). Phytoremediation also can be used to clean up many types of contamination, such as metals, pesticides, solvents, explosives, crude oil, PAHs, and landfill leachate (U.S. EPA, 1996). Plant roots are important factors that contribute the enhanced bioremediation. Root exudates, which can be used for microbial substrates, increase soil microbial populations and activity (Aprill and Sims, 1990; Mcfarlane *et al.*, 1990; Nair *et al.*, 1993). Roots may also reduce movement of contaminants in soil by extracting water (Schnoor, 1993; Gatliff, 1994). Plants may absorb, translocate, and sequester organic contaminants, which removes them from the soil environment (Cunningham *et al.*, 1995).

Mechanistic computer models assist in predicting the fate of contaminants in soils using various remediation processes. This mathematical modeling is an economical approach to collecting relevant data while minimizing the acquisition of irrelevant data and guides the user in identifying and quantifying the governing mechanisms. A recently developed phytoremediation computer model may be used as a predictive tool to assess the effectiveness of various plants on the remediation of soil contaminated by several classes of recalcitrant compounds. The model was developed to simulate the transport and fate of recalcitrant contaminants interacting with plant roots in partially saturated soils. A time-specific root distribution model is incorporated into a vertical unsaturated soil water flow equation. However, the model requires extensive testing with field data for validation and calibration to prove its

reliability. The objective of this study is to provide empirical data for such validation and calibration.

2.0 Materials and Methods

Field lysimeters constructed of 0.1 m diameter PVC tubing, 1.5 m in length, were filled with soil contaminated with 10 mg kg^{-1} 2,4,6-trinitrotoluene (TNT), 2,2',5,5'-tetrabromobiphenyl (PBB), and chrysene and placed in the ground at the Texas A&M University Research Farm near College Station, TX. Each lysimeter was sectioned longitudinally into two separable halves to simplify the destructive sampling of roots and soil. The bottoms of the lysimeters were fitted with porous caps to allow the drainage of excess moisture into a collection jar. This construction was found to be robust, permitting the lysimeters to be filled with moist soil, which was compacted to the required bulk density (1.4 g cm^{-3}). The lysimeters were filled with a Weswood loam soil. This soil has a pH of 7.9, organic carbon matter content of 1.0%, and a sand, silt and clay content of 23%, 47%, and 30%, respectively. Contaminants were mixed into soil not previously exposed to PAHs, PBBs, or TNT spills and packed in the lysimeters to a bulk density of 1.4 g cm^{-3}. Vegetated (Johnson grass) and unvegetated (fallow) treatments were conducted in triplicate. Moisture was controlled by covering the lysimeters with a transparent roof. Lysimeters were watered twice weekly to adjust the soil moisture to field capacity.

Columns were destructively sampled each time period. Samples of vegetation, roots, and soil were collected and measured for contaminant concentrations. To determine the influence of roots on bioremediation, the columns were divided into six sections (0-10, 10-30, 30-60, 60-90, 90-120, 120-150 cm).

Analysis of the TNT, PBB, and chrysene were conducted using an immunoassay procedure (Strategic Diagnostic Inc.) validated by the U.S. EPA Office of Solid Waste (U.S. EPA, 1995).

Data acquisition included contaminant concentration in the soil, plant roots and herbage, time-specific root distribution throughout the soil, microbial activity in the bulk soils and rhizosphere, plant water use, and soil moisture.

3.0 Model Development

The model system consists of two main parts. A time-specific root distribution model was incorporated into a vertical, unsaturated soil-water flow equation and contaminant transport equation to handle the effect of plants on water flow and the transport and fate of contaminants in the unsaturated soil.

A general macroscopic equation uses Darcy's law for one-dimensional water flow, which includes a root water uptake sink term for the soil-water flow model in the vadose zone:

$$\frac{\partial \theta_w}{\partial t} - \frac{\partial}{\partial z}\left[K_w\left(\frac{\partial h_w}{\partial z} - 1\right)\right] + L_d q_{av} = 0 \tag{1}$$

where h_w is the pressure head in bulk soil [L] related to θ_w [L^3 L^{-3}], the volumetric water content of bulk soil by the soil-moisture characteristic curve; K_w is the

unsaturated hydraulic conductivity [LT^{-1}]; L_d is the rooting density, defined as the length of roots per unit volume of soil [LL^{-3}]; and, q_{av} is the mean daily uptake rate [L^{3}L^{-1}T^{-1}].

The increase in rooting depth z_m with time may be written as Borg and Grimes (1986):

$$z_m = z_T Sin_f \tag{2}$$

where z_m is the current rooting depth [L]; z_T [L] is the maximum rooting depth to be achieved at time to maturity $t = t_T$; and, *Sinf* is a single sine function which delineates a sigmoidal distribution with time:

$$Sin_f = 0.5 + 0.5\sin[3.03 \cdot (t/t_T) - 1.47] \tag{3}$$

The root growth model is coupled with the root distribution model to evaluate the propagation of roots through the soil profile:

$$L_d = L_{Td} Sin_f \, e^{-\frac{f_T}{Sin_f} z} \tag{4}$$

where f_T and L_{Td} are f and the maximum value of L_d in topsoil when z_m reaches the maximum rooting depth z_T. After fully grown, a plant assumably attains constants f_T and L_{Td}. z is the depth positively oriented downward and f [L^{-1}] is constant over depth at a time period for plant growth.

The mean daily water uptake rate at specific time can be estimated using the transpiration rate:

$$q_{av} = \frac{Ef}{L_{md}(1 - e^{-fz_m})} \tag{5}$$

where E is the transpiration rate from a unit soil surface area [L^{3}L^{-2}T^{-1}] and L_{md} is the maximum rooting density at specific time.

All possible mechanisms that can occur in the rhizosphere are combined into a mass balance equation for the aqueous phase contaminant in vegetated, unsaturated soils: (1) a linear isotherm equilibrium sorption model is used to express contaminant loss in the aqueous phase due to adsorption onto the solid matrix; (2) sorption and uptake by roots are considered as a function of contaminant hydrophobicity and rooting density; (3) different Monod-type biodegradation models in the soild matrix and the rhizosphere were used. Because most bacteria in the natural subsurface have a tendency to attach to solid particles, a rapid increase of microorganisms due to abundant root releases such as exudates, lysates, and mucilages may cause the build-up of a biofilm typically occurring within 50 μm from the root surface, where the microbial population is 50-100 times greater than in bulk soil (Foster *et al.*, 1983).

Generally, the biofilm is fully penetrated by substrates (Rittmann, 1993). Therefore, the chemical concentrations in the biofilm the soils tested can be assumed to be the same as the average bulk aqueous phase concentrations (Corapcioglu, 1992); (4) the interphase mass transfer of organic contaminants by partitioning between the gas and water phase is considered using equilibrium and kinetic models.

4.0 Model Validation and Conclusions

Data of the root distribution throughout the soil and contaminant concentrations of 30 and 90 days after planting were used for model validation purposes. The proposed root growth and distribution models of equations (2)-(4) were tested against field data with Johnson grass samples. The results are consistent with studies indicating that rooting densities, while declining exponentially with depth, normally increase with time.

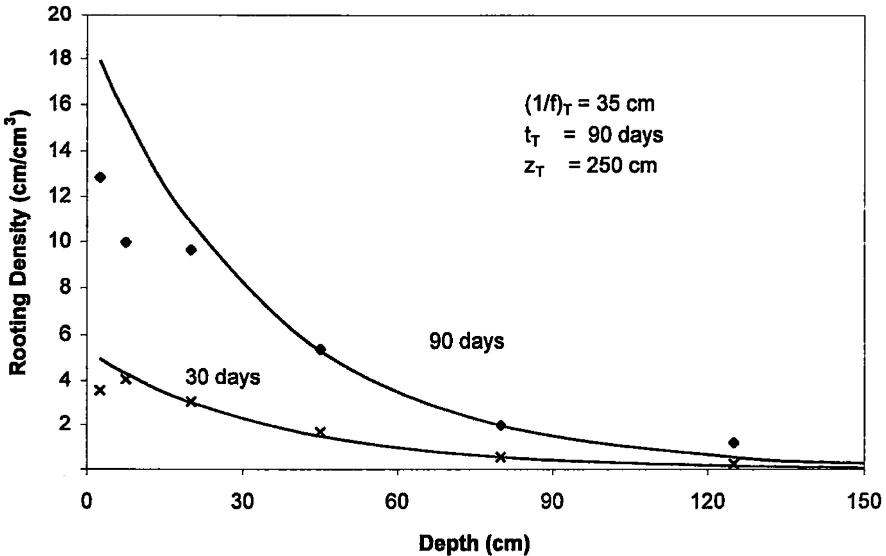

Figure1 Simulation of Rooting Density Profiles for Johnson Grass After 30 and 90 Days.

Figure 1 shows the simulated and experimental values are well matched. The rooting density of top soil was slightly over-estimated, possibly because the roots in the top 30 cm were stressed by high temperatures and low water availability during the hot summer period.

The warm season grass samples were collected 30 and 90 days after planting for chemical analysis using immunoassay-testing procedures. Contaminants decreased in all treatments. Of the three contaminants, the largest loss in soil-chemical concentration was from TNT, which decreased to approximately 3-30 [μg

kg^{-1}] after 90 days. The predicted values are well matched with field data reaching to a uniform concentration after the depth of 50 cm (Figure 2).

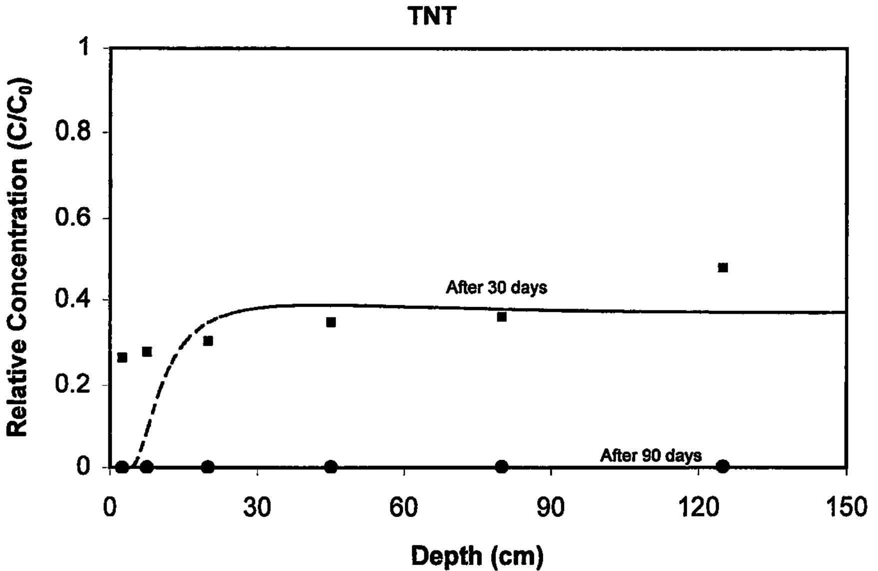

Figure 2 Model Results Compared with TNT Data Obtained in Vegetated Lysimeters.

The simulation results demonstrate that the present model can predict contaminant concentration with time and depth under actual field conditions. Other model parameters are being evaluated and samples will continue to be evaluated during a two-year period. The validated and calibrated computer model can provide insight into the selection and optimization of phytoremediation at contaminated sites.

5.0 Acknowledgments

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Assessment of the Role of Plants in the Bioremediation of Two Hydrocarbon-Contaminated Soils

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Abstract

Two contaminated soils, one with creosote and the other with oil, were used to examine the potential for plant-assisted bioremediation in the presence of each of the following crops: wheat (*Triticum aestivum*), canola (*Brassica rapa*), sunflower (*Helianthus annuus*), fababean (*Vicia faba*), and alsike clover (*Trifolium hybridum*). The crops were grown to maturity and the soils and plant tissues subsequently extracted with dichloromethane to measure dichloromethane-extractable organic materials (DEO). The soil DEO concentrations were compared with non-planted greenhouse control samples of each soil as well as with reserved time zero soil samples. At the end of three months, no significant differences in DEO concentrations were detected between all soils examined. At the end of 6 months, the DEO concentrations of the greenhouse soils had decreased compared with the reserved samples, however there was no significant change in concentration due to the presence of any of the plant species. Preliminary examination of tissues from fababeans and canola grown in the oil-contaminated soil suggest that the plants may have taken up oil-derived hydrocarbons.

1.0 Introduction

Bioremediation is a popular option for cleaning up soils contaminated with hydrocarbons. The least intrusive systems, such as land-spreading, or low-input systems, such as biopiling, often take advantage of the indigenous microbial communities which may have adapted to the local contamination. These systems may benefit from maximizing the potential of the ecosystem to metabolize the contaminant (Bossert and Compeau, 1995).

Phytoremediation, in the most direct sense of the word, refers to the removal of contaminants from soil through plant uptake. Additionally, plants may serve to enhance bioremediation by providing an enriched habitat for soil microorganisms in the rhizosphere, breaking and rebuilding soil aggregates, protecting the soil surface from erosion, and creating channels into the soil for aeration and microbial habitation (Cunningham and Ow, 1996; Shimp *et al.*, 1993).

Shimp *et al.* (1993) list six reasons for the inclusion of plants in remediation systems: 1) Plants utilize solar energy, thereby providing additional energy to the bioremediation system; 2) Plant development and tissue subsamples may be used as indicators of contaminant toxicity or remediation success; 3) Plants may reduce the spread of a contaminant in soil by removing water from the soil, maintaining a small water potential near the surface; 4) The rhizosphere is densely colonized by a variety of microorganisms which may be able to degrade a wide variety of organic contaminants; 5) Many plants have mechanisms for transporting oxygen to the rhizosphere; and, 6) Plants are aesthetically pleasing. A seventh reason could also be

included, 7) Plants physically protect the surface of disturbed soils from the erosive actions of wind and water (Schnoor *et al.*, 1995).

The rhizosphere is the zone of carbon and microbial enrichment that immediately surrounds the plant root (Wild, 1988). The root provides surfaces for microbial colonization additional to the soil solids, and the root exudates and exfoliated meristem cells provide rich carbon and energy sources for the microbial community. Up to 100-times more organisms may colonize the root surface than do the bulk soil (Paul and Clark, 1989). Different plant species support different communities, however the communities are typically much larger than in the surrounding soil (Shimp *et al.*, 1993). Even in contaminated soils, significantly more microorganisms (Lee and Banks, 1993) or larger microbial biomasses (Walton and Anderson, 1990) are found in vegetated soils than in non-vegetated soils.

Plant root exudates are generally simple organic compounds such as organic acids, amino acids, fatty acids, etc. (Hsu and Bartha, 1979; Shimp *et al.*, 1993) which are readily used as cosubstrates during the metabolism of more complex, less desirable organic contaminants (Hsu and Bartha, 1979). Plant root exudates may arise from "leaks" of cellular materials when sloughed root cap cells rupture, "secretions" of extracellular plant compounds, and "mucilages". The secretions and "leaks" may include enzymes, aliphatic and aromatic hydrocarbons, amino acids, sugars, and low molecular weight carbohydrates (Burken and Schnoor, 1996). Mucilages are gel-like materials composed of low molecular weight carbohydrates. The addition of dead root tissue, or of the entire root following plant senescence to soil is termed rhizodeposition. The extent of rhizodeposition and exudation depends on many plant characteristics. The entire rooting system of an annual plant decays in the soil following senescence whereas only a portion of the root system of a perennial is decomposed. To examine the role of plant exudates in facilitating bioremediation, Reilley *et al.* (1996) stimulated non-vegetated soils spiked with ^{14}C-pyrene with applications of organic acids to mimic root exudates. They measured greater quantities of $^{14}CO_2$ evolving from these soils than from spiked, non-vegetated, non-amended soils, providing evidence that the presence of root exudates as cosubstrates may be instrumental in plant-enhanced systems.

The purpose of this experiment was to compare the changes in dichloromethane extractable organic materials (DEO) in two contaminated soils with and without plants during the growing period. Five plant species were chosen to represent a range of commercially and agriculturally important crop species: wheat (*Triticum aestivum*), canola (*Brassica rapa*), sunflower (*Helianthus annuus*), fababean (*Vicia faba*), and alsike clover (*Trifolium hybridum*).

2.0 Materials and Methods

2.1 Soils

Samples from two weathered contaminated soils were used in this experiment: an oil-contaminated soil and a creosote-contaminated soil. The oil contaminated soil, collected near Devon, Alberta is loam textured, pH 7.7 and contained 5.6 mg DEO g^{-1} soil from an oil-well blowout in 1947. The creosote contaminated soil, collected from a site in Edmonton, Alberta is silty clay textured, pH 7.3, and is contaminated with 1.5 mg DEO g^{-1} soil, residual from a wood treatment plant on the site between 1924 and 1988. A third "soil", a pristine (non-contaminated) potting mix, that was an uncharacterized blend of peat, sand and vermiculite, was included as a soil control.

2.2 Plants

Five plant species, common in prairie cropping systems were selected: *Triticum aestivum*, cv. Neepawa (wheat), *Brassica rapa*, cv. El Dorado (canola), *Helianthus annuus*, cv. DO827 (sunflower), *Vicia faba*, cv. Orion (fababean), and *Trifolium hybridum*, cv. Dawn (alsike clover).

2.3 Experimental

The experimental design was factorial: 3 soils × 5 plant species. A subtreatment was applied to the legumes (clover and fababeans): fertilization with P and K, no N. Each species was seeded to a depth of ~1.5 times the seed diameter into each of the three soils in triplicate. All plants, except *T. hybridum* were thinned to 5 plants per pot. Wheat, canola, fababean, and alsike clover were planted in 15-cm (diameter) pots containing ~2 kg soil, and the sunflower was planted into 17.5-cm pots containing ~2.25 kg soil. These pots were placed on top of inverted 10-cm pots within 15-cm MacConkey pots (Figure 1). The environmental conditions were set at 21°C for 16 h with a light intensity of 275 µE (day period) and 18°C for 8 h (night period). The humidity was approximately 25% R.H. All pots were watered daily. Wheat, canola, fababean, and sunflower were harvested after 3 months of growth. Alsike clover, a two season crop, was harvested after 6 months. As incubation controls, unplanted pots of the experiméntal soils were incubated with the planted pots. Each pot received fertilization monthly with a solution of 20-20-20 (N-P_2O_5-K_2O) equivalent to 0.0125 g N pot^{-1}. The unplanted, fababean, and alsike clover pots were further replicated to include a 0-20-20 treatment, receiving the same quantity of P and K as did the +N pots. Plants were harvested using stainless steel scissors which were cleaned between treatments. The plant tissues were collected in pre-weighed brown paper bags. When harvested, the above-ground plant materials were collected in brown paper bags and dried at 35°C to a constant weight. The soils were collected in plastic bags and stored at 4°C for further analysis.

2.4 Leachate

At the end of each month, leachate subsamples (20 mL) were collected immediately after watering the pots. The pots were watered so that the water ran through the soil and filled, but did not overflow, the MacConkey pot (Figure 1). The amount of water used to achieve this was not added in measured quantities. These samples were analyzed for dissolved organic carbon (DOC) on an Astro 2001 Series 2 Soluble C Analyzer with an ultraviolet source. The analysis used 1 M sodium persulfate as the oxidizing agent, and 85% phosphoric acid as the sparging solution. The calibration standard was a 100 mg L^{-1} aqueous solution of potassium acid phthalate.

2.5 Soil and Plant Analysis

The DEO were Soxhlet-extracted from the soils with dichloromethane (Rutherford *et al.*, 1998). Subsamples of ~10 g (oven dry basis) soil were placed in cellulose thimbles and mixed with an equal amount of anhydrous $MgSO_4$ to ensure a dry extraction. Dichloromethane (200 mL) was cycled through the Soxhlet apparatus overnight at a rate of 4 cycles h^{-1}. The heat source was a sandbath set at 75°C. The collected extract was reduced under vacuum to < 5-mL. This extract was quantitatively transferred to a 25-mL volumetric flask and brought to volume with

fresh dichloromethane. A 5-mL aliquot was transferred to a pre-weighed aluminum dish and the dichloromethane allowed to volatilize. The tarry residue remaining was weighed on a precision balance (to 4 decimal places) and designated total DEO. Soils were only collected for extraction following the harvest of the mature plants, hence the soils planted with alsike clover were not extracted at 3 mo, in order to allow the plant growth to continue undisturbed.

The above-ground plant tissues were ground in a Wylie mill to pass through a 0.5 mm mesh screen. Ground plant tissue subsamples (1-3 g) were weighed into cellulose thimbles and Soxhlet extracted overnight with 200 mL dichloromethane. Dichloromethane was selected as an extractant in order to remove similar types of compounds as those removed from the soils. Plugs of glass wool held the ground plant material in the cellulose thimbles to prevent its floating during extraction. Following Soxhlet extraction, the plant extracts were reduced under vacuum to < 5 mL and quantitatively transferred to a 10 mL volumetric flask and brought to volume with fresh dichloromethane.

Both soil and plant extracts were stored in borosilicate glass scintillation vials and immediately weighed before storage at 4°C.

Selected plant extracts (fababean and canola from each of the three soils) were analyzed for evidence of contaminant-hydrocarbon uptake using a Carlo Erba 5160 HRGC (high resolution gas chromatograph) connected to a Finnigan 4500 MS (mass spectrometer). Financial constraints prevented the analysis of all plant species; the fababean was selected due to its relatively strong growth, the canola because of its importance as an oilseed. Prior to analysis the extract residues were re-dissolved in hexane and passed through a column of anhydrous aluminum oxide in order to collect specifically the aromatic and saturated fractions. The HRGC had a Restik XTI-5 fused silica column (30 m, 0.25 mm ID, 0.25 μm df.) and the carrier gas was helium (35 cm s^{-1}). The GC/MS (gas chromatograph/mass spectrometer) used multiple ion detection (0.053 s $mass^{-1}$). The ion source temperature was 150°C, the electron multiplier was 1350 ev, and the transfer line temperature was 300°C. The optimal run time for the fababean tissues was 40 min and for the canola tissues was 20 min.

2.6 Statistics

A one-way ANOVA, with an LSD means separation was conducted using SAS (Windows 6.10) PROC GLM to determine if there were differences among treatments, within soils. The same test was conducted on the above-ground dry matter measurements to determine if there were differences in plant yields among plants, within soils. Significant differences in the quantity of C measured in the collected leachates among months, within plant-soil treatments were analyzed as a "split plot in time" with means separation analysis.

3.0 Results

The yield of above-ground dry matter from plants grown in the contaminated soils was the same as, or less than the yield of dry matter from the same plant grown in the control soil, except for one treatment (Figure 2). The fababeans grown in the creosote-contaminated (-N) soil had a larger above-ground dry matter yield than did the fababeans grown in either the control soil or the oil-contaminated soil. Unfortunately, none of the replicates for the fababeans in creosote-contaminated (+N) soil succeeded in establishing themselves, likely due to poor soil physical conditions

rather than chemical inhibition. The soil was very prone to clodding and did not provide a homogenous medium for germination.

The monthly leachate data followed the same overall trend for the two contaminated soils (Figure 3). As time progressed, the concentration of C in the leachate generally decreased. The exceptions to this were the sunflower- and fababean- planted oil-contaminated soil in which the C concentration did not change over the 3-month growing period and the fababean- and sunflower-planted creosote-contaminated soil in which the concentration of C in the leachate increased over the 3-month period.

No significant loss of DEO was measured in either contaminated soil during the 3-month growing period (Figure 4). Over the 6-month growing period, a significant loss in DEO was measured in both clover and clover (-N) for both soils (Figure 5). These losses were not significantly different from each other, nor were they different from the losses measured in both of the non-planted pots for both soils.

Observations made following solvent extraction of both soils and all the plants was the wide range of colours of the extracts of the same soil (yellow to dark brown), and the wide variety of shades of green for the same plant species, grown in different soils. This prompted further analysis of selected extracts.

Gas chromatography/MS of fababean and canola tissues grown in all three soils, suggests that there may have been uptake of hydrocarbons into the tissues of the plants grown in the oil-contaminated soil (Figures 6; 7). The "control" in the diagrams refers to the pristine potting mix. The possible uptake was determined by comparing the scans of the plant extracts from the contaminated soils with those of the plants grown in the pristine control soil. The chromatogram of the plant tissue grown in the creosote-contaminated soil does not differ from the chromatogram of the same plant tissue grown in the control soil. However, the chromatogram of the fababean plant tissue grown in the oil-contaminated soil shows enrichment of C_{16}, C_{17}, and C_{18} hydrocarbons, as well as the hump between 16 and 21 min that is characteristic of naphthenic hydrocarbons. The chromatogram of the canola tissues grown in oil-contaminated soil (Figure 7) shows a hump of unresolved hydrocarbons, likely alkanes, between 10 and 15 min that is not seen in the canola grown in the other two soils. These possible enrichments of contaminant-hydrocarbons were not quantitatively measured, only qualitatively detected.

4.0 Discussion

The wheat and canola grew poorly on the contaminated soils relative to those in the pristine soil. The other species produced no less above-ground dry matter than their counterparts grown in the pristine soil. This is in contrast to studies where low levels of contaminated municipal sludge (Baghdady and Saad, 1992; Wild and Jones, 1992) and PAHs have stimulated plant growth (Sims and Overcash, 1983) in a range of plants (carrot-*Daucus carota*, fababean, rye-*Secale cerealis*). Decreased plant yields in fuel-contaminated soils have been measured before and are not surprising (Chaîneau *et al.*, 1997). These are likely due to poor chemical and physical conditions in the contaminated soils as well as toxicity of high levels of contaminant to the plants.

When the overlying soil was teased away, the fababeans in the creosote-contaminated soil had germinated, but failed to take root. This was likely due to the clodding of the creosote-contaminated soil whenever it was watered, reducing contact

between the soil, moisture, and the large seed. The watering system disrupted the soil surfaces, causing the floatation and reorganization of smaller surface clods into larger clods. It seems that the creosote-contaminated soil in the (-N) pots clumped around the seeds, maintaining the required contact for establishment. This could be a random event, or, more likely, is an artifact of where they were located in the growth chamber relative to the impact of watering, as the (-N) pots were further away from the hose than were the (+N) pots. The oil-contaminated and control soils showed no tendency to clump, rather they "settled" following watering. The seeds of all the other plants included were much smaller than those of the fababean, and likely established in niches around and under the clods in the creosote-contaminated soil.

The loss of soluble C from the contaminated soils was greatest early in the experiment. It may be that the simpler C substrates were metabolized to more water-soluble forms, such as the production of C_2 units by metabolic oxidation of straight-chain alkanes, following the first addition of nutrients to stimulate the soil microorganisms. Oddly, the sunflower-planted creosote-contaminated soil lost greater amounts of soluble C as the experiment progressed, while the sunflower-planted oil-contaminated soil decreased in month 2, but rose again in the last month. It may be that the organisms stimulated by sunflowers are better able to mineralize contaminants to soluble forms than the organisms favored by the other plants. It may also be that sunflowers produce larger quantities of root exudates and sloughed materials under the conditions of this experiment than do the other plants included and that these are the detected C forms increasing in the leachate as the plants mature. It is also possible that much of the C detected in all of the leachate samples was plant-derived, rather than due to the mineralization of the contaminant.

Based on the DEO data (Figure 3 and 4), the presence of plants did not remove more contaminants from soil. The only factor in this experiment that did relate to hydrocarbon removal from these soils was time. All of the soils, vegetated and non-vegetated, when incubated for 6 months lost the same amount of DEO. These findings are similar to those of Wiltse *et al.* (1998), who found the no difference in crude oil-derived DEO in soils planted with alfalfa compared with non-vegetated soils after 6 months, but did measure the same significant decrease in the soils compared to their original DEO contents. We can contrast these findings with a number of other experiments that did find enhanced removal of contaminants in the presence of plants. Papers that describe enhanced removal of contaminants in the presence of plants generally tend to look at one specific component of contamination or at "spikes". Wild and Jones (1992) measured significant decreases in the total concentration of 14 PAHs, ranging between naphthalene and coronene in soils after 82 d of carrot growth. Aprill and Sims (1990) measured significant losses of PAHs from contaminated soils with prairie grass vegetation, compared with non-vegetated controls. Reilley *et al.* (1996) measured significantly greater removal of added pyrene and anthracene in petroleum-contaminated soils planted with grasses or alfalfa compared with non-vegetated controls. Plant-microbe associations known to enhance 2-chlorobenzoic acid disappearance from soils are not as efficient when the contaminant is present in a mixture of contaminants (Siciliano and Germida, 1998).

It is possible that our experiment would have benefited from analyzing the DEO extracts for specific contaminants. The experiments cited in which specific PAHs were studied report no data on dichloromethane extractable organic material

changes. The sensitivity of analyses for individual components is greater than the sensitivity of the DEO analysis.

Another explanation for the lack of plant-enhanced disappearance of DEO is that both soils included in this studied are *weathered contaminated soils*. Soils that are contaminated with residual heavy materials that remain after the contaminant has aged in the soil, rather than with the individual compounds, light phase contaminants, or pesticides evaluated by others which have dominated this body of literature. The weathering of contaminants in soil may result in the uneven distribution of the contaminant through the soil matrix (Hatzinger and Alexander, 1995). Lowest concentrations of contaminant may be located at the surface of aggregates, where microorganisms, water, and air may interact to metabolize the substrate. Highest concentrations may be located within aggregates, where the contaminant is protected from metabolism. Not only may the proportion of the soil explored and modified by the root systems be too small, the part of the soil explored may have too little contamination for the rhizosphere to be of significance to the whole soil. The observed decrease in DEO after 6 mo was observed in both contaminated soils, regardless of the presence or absence of plants, therefore the causal agent of the enhanced DEO removal must be unrelated to treatment. The percolation of water through these soils over the longer incubation period (6 mo) may have slowly reorganized these aggregates or forced the redistribution of some of the DEO such that it was exposed and subsequently removed.

Several papers have been published which examine plant uptake of contaminants. In agreement with the findings presented here, Goodin and Webber (1995) observed no uptake of anthracene or benzo(a)pyrene by rye, soybean, or cabbage grown in spiked, sludge-treated soil, based on acetone-hexane Soxhlet extracts of the tissues. Methanol extracts of a fescue, sudangrass, and alfalfa, grown in pyrene- and anthracene- contaminated soil also all failed to show evidence of incorporation of these contaminants into plant tissues (Reilly *et al.*, 1996). However, plant roots, grown in contaminated soils have been shown to accumulate organic constituents from the contaminant (Schroll and Scheunert, 1992; Wild and Jones, 1992). Schnoor *et al.* (1995) categorize contaminant potential for plant uptake by the K_{ow} of the contaminant: direct uptake and accumulation is most likely for compounds with a log K_{ow} between 0.5 and 3; compounds with a log $K_{ow}>3$ are too strongly bound to root surfaces to be transported to above ground tissues and; those with a log $K_{ow}<0.5$ do not sorb sufficiently to be transported through plant membranes.

Our results did suggest the possibility that contaminant-derived aliphatic hydrocarbons were recoverable from selected plants (fababean, canola) grown in the oil-contaminated soil. Carbon chains of 16, 17, and 18 units were identified in the chromatogram of fababean that were neither seen in the plant tissues grown in the creosote-contaminated nor the pristine soils. As well, in both the fababean and canola unresolved hydrocarbons are observed uniquely in the oil-contaminated soil.

Much of the literature published about plant-enhanced bioremediation of soils has focused on the beneficial aspects. Our examination of DEO contents before and after incubation and in vegetated and non-vegetated controls would suggest that the five plants selected in the two soils studied do not enhance bioremediation. However, the hints of plant accumulation of hydrocarbons from the oil-contaminated soil suggest that plants may be a minor fate of xenobiotic C in soils.

The results of this paper indicate that the role of plants in bioremediation systems, both as enhancers of bioremediation systems and as possible sinks of contaminant C should be studied further. Gross measurements of DEO materials extracted from cropped soils compared to those from non-cropped controls show no evidence of plant facilitation of bioremediation. However, the GC chromatographs of dichloromethane extracts of fababean and canola tissues indicate that unique hydrocarbons are present in the plants grown in oil-contaminated soil which do not appear in the plants grown in pristine or creosote-contaminated soil. The potential for plant uptake of some contaminants should be examined further. An alternate explanation for the observed change in hydrocarbon distribution in the plant tissues could be that the presence of oil in the growth medium caused the plant to change the types and sizes of its own hydrocarbons as they are produced. The detection of larger compounds eluting after 17 min in the canola tissues grown in pristine and creosote-contaminated soils is not observed in those grown in oil-contaminated soils. This could lend strength to such an alternative hypothesis.

Careful attention must be given to the age of the contaminant in the soil and to a careful accounting of both individual pollutants and the total content of contaminant C. Due to the increasing complexity of hydrocarbon residues as they weather in soil, conclusions drawn from artificially contaminated systems should be regarded as only a starting point for further research, rather than as models for field situations. Furthermore, future experiments into the potential for bioremediation should use both gross analyses (*ie*, DEO, total PAHs) as well as compound-specific analyses. Our work, in comparison with published literature, suggests that one measurement without the other may yield misleading results.

5.0 Acknowledgements

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Table 1 Characteristics of Two Hydrocarbon-Contaminated Soils

Soil	pH	EC[1] (dS m^{-1})	Total C	CO_3^{-2}-C	DEO-C[2]	Texture[4]
			mg g^{-1} soil			
Oil-contaminated	7.7	0.34	27.7	0	18.1	Loam
Creosote-contaminated	7.3	1.25	37.9	8.3	10.7	silty clay

[1]Electrical Conductivity
[2]Dichloromethane Extractable Organic Carbon
[3]Water holding capacity
[4]Rutherford *et al.*, 1998

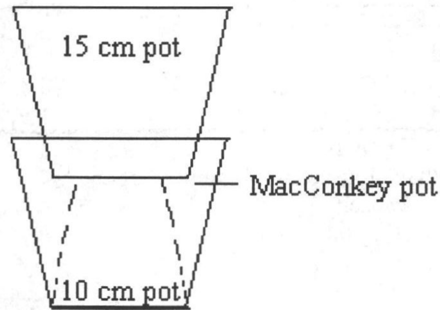


Figure 1 Leachate Collection Apparatus

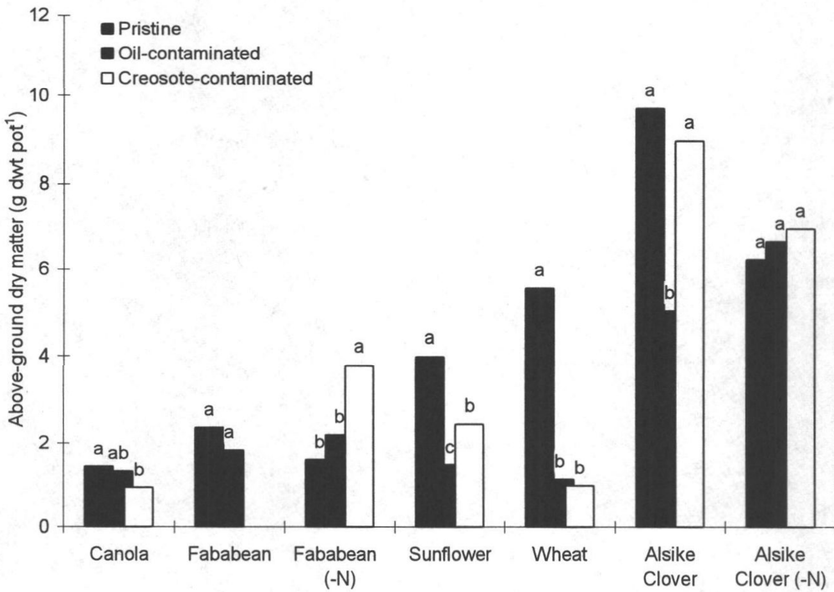


Figure 2 Above-Ground Dry Matter Yields (g dry weight pot^{-1}) from Five Plant Species

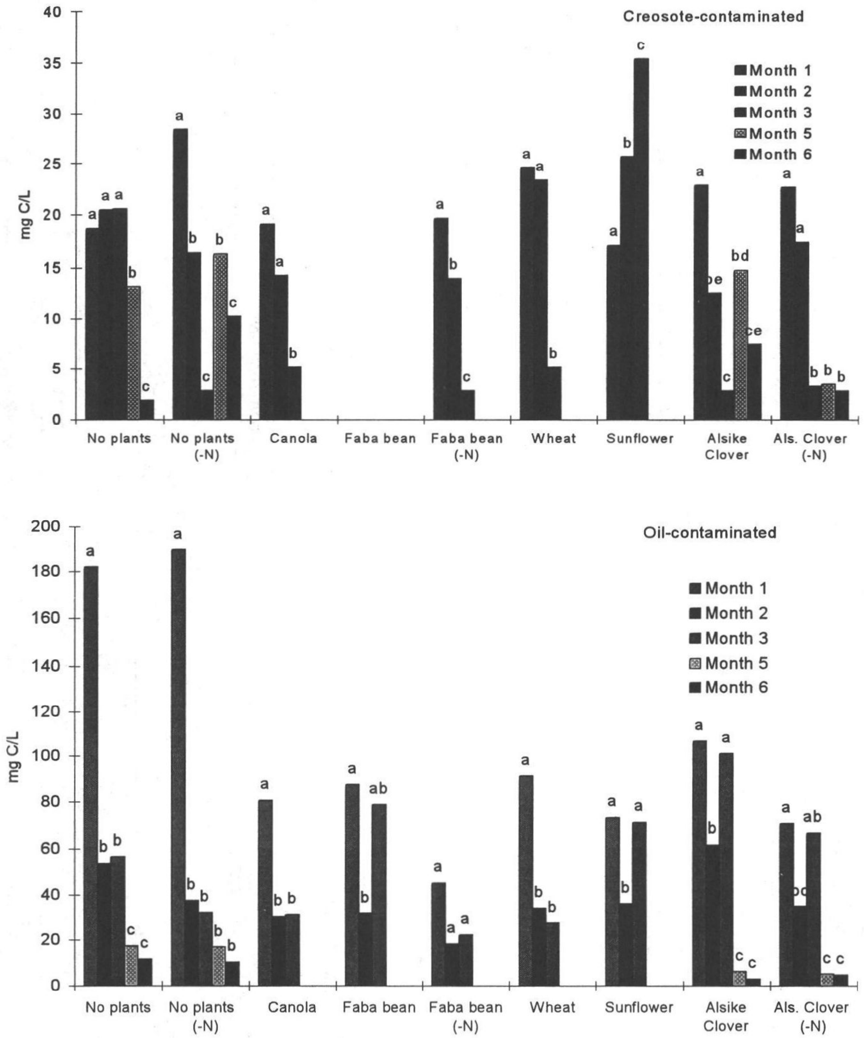
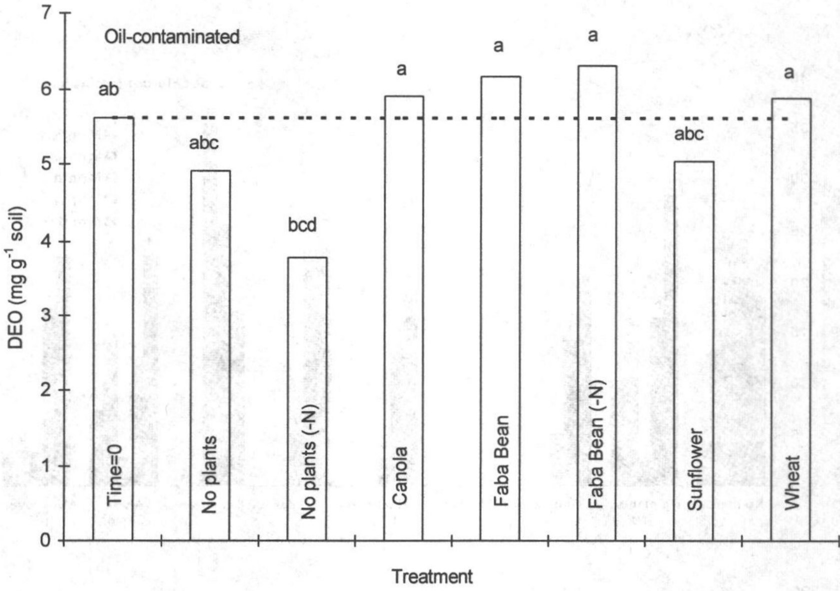


Figure 3 Soluble Organic C Contents of Leachate Collected from Two Contaminated Soils Over 6 (Comparisons are only valid within plant ($p<0.05$))



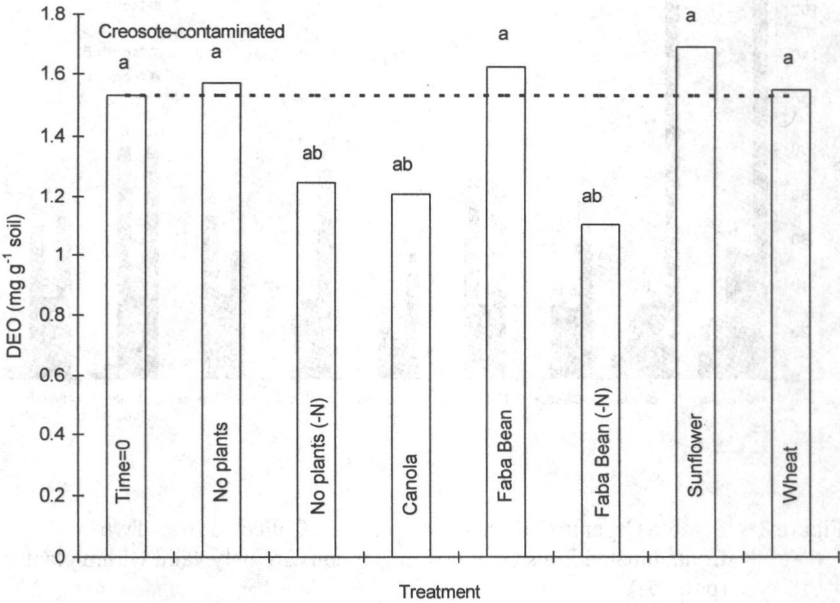


Figure 4 Soil DEO Concentrations at Time=0, and Time=3 mo

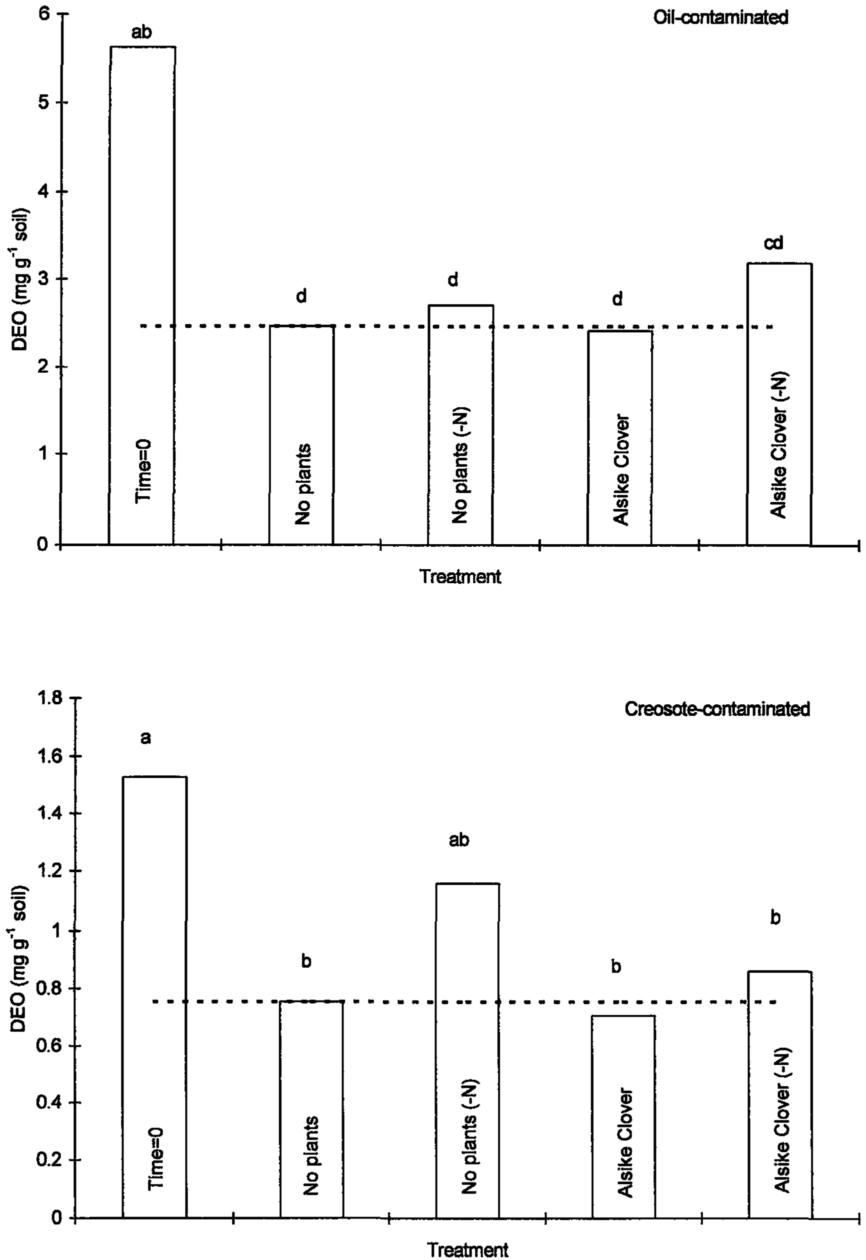


Figure 5 Soil DEO Levels at Time=0, and Time=6 mo

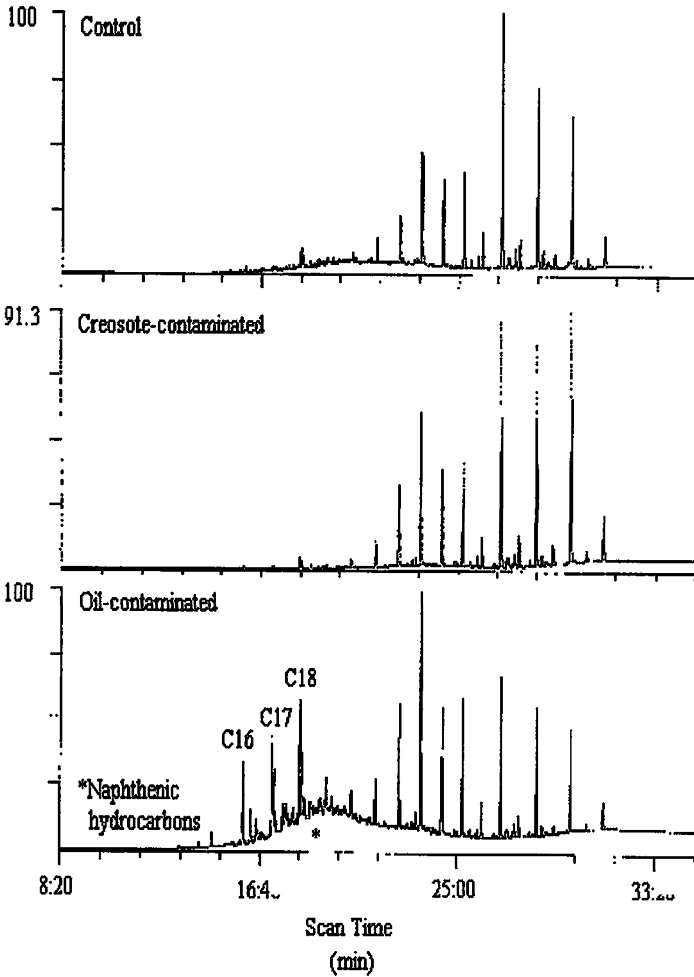


Figure 6 GC/MS Chromatograms of the Tissue Extract of Fababean (+N) Grown in All Three Soils

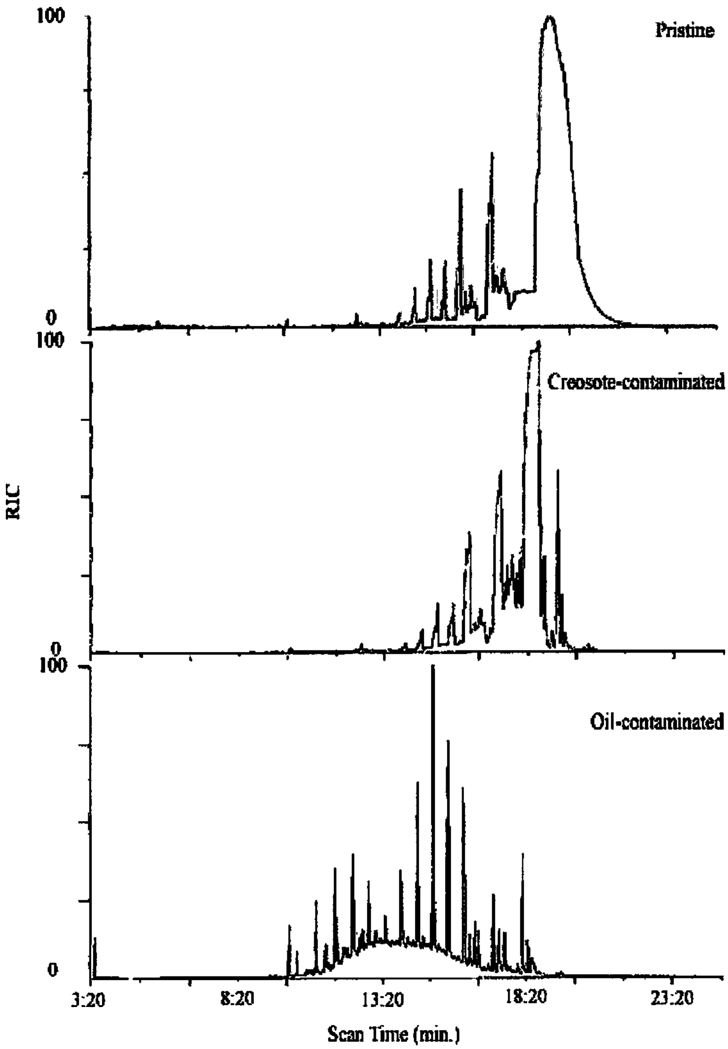


Figure 7 GC/MS Chromatograms of The Tissue Extract of Canola Grown in All Three Soils

Assessment of Phytoremediation as an *In-Situ* Technique for Cleaning Oil-Contaminated Sites

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Abstract

Phytoremediation, the use of plants and their associated microorganisms for the *in situ* treatment of contaminated soils, is a steadily emerging technology that holds the promise of effective and inexpensive cleanup of various hazardous wastes. It capitalizes on the natural, synergistic relationships among plants, microorganisms, and the environment. Based on a review of relevant literature, we discuss the key mechanisms and special considerations involved in the phytoremediation of petroleum hydrocarbons. Several studies indicate that phytoremediation is effective at degrading, containing and transferring petroleum hydrocarbons in soil. Little published information exists on the application of phytoremediation to oil-contaminated sites in Canada. As well, only a handful of studies look at the specific mechanisms of phytoremediation for petroleum hydrocarbons.

1.0 *Introduction*

Petroleum hydrocarbons are naturally-occurring chemicals exploited by humans (Committee on In Situ Bioremediation *et al.*, 1993). Representative types include alkanes (e.g., methane, ethane, propane), aromatics (e.g. benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX), polycyclic aromatic hydrocarbons (PAHs; e.g., naphthalene, phenanthrene, anthracene, benzo[*a*]pyrene), and creosote (Committee on In Situ Bioremediation *et al.*, 1993; Mackay, 1991). During the past century, our reliance on petrochemicals has resulted in the contamination of a significant number of sites (Bauman, 1991). In Saskatchewan alone, it is estimated that there are several hundred sites contaminated with petroleum hydrocarbons (Carlson, 1998). Today, environmental managers can choose from a variety of approaches to remediate petroleum-contaminated soil and groundwater. These approaches range from intensive engineering techniques to natural attenuation, a "hands-off" approach that relies entirely on natural processes to remediate sites with no human intervention.

Phytoremediation is a method that is intermediate between engineering and natural attenuation, as it employs human initiative to enhance natural attenuation. It involves the *in situ* use of plants and their associated microorganisms (Figure 1) to degrade, contain or render harmless contaminants in soil or groundwater (Cunningham *et al.*, 1996). It depends on natural, synergistic relationships among plants, microorganisms, and the environment. Consequently, phytoremediation does not require intensive engineering techniques or excavation. However, human intervention in phytoremediation may involve establishing appropriate plants and microorganisms or applying agronomic techniques (e.g., fertilizer application and tillage) to enhance natural degradation or containment processes.

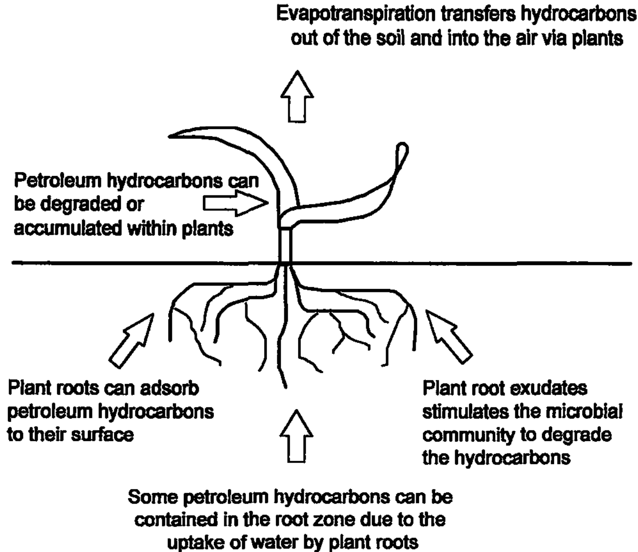

Figure 1 The Degradation, Containment, or Transfer of Petroleum Hydrocarbons in Soil via Interactions With Plants and Microorganisms.

Phytoremediation has been used to effectively remediate a variety of contaminants in soil and groundwater. Various plants – including canola (*Brassica napus* L.), oats (*Avena sativa*), and barley (*Hordeum vulgare*) – have been shown to tolerate and accumulate metals such as selenium, copper, cadmium and zinc (Banuelos *et al.*, 1997; Ebbs *et al.*, 1997; Brown *et al.*, 1994). Hybrid poplar trees (*Populus deltoides* x *nigra*) reduce the concentration of nitrate in surficial groundwater (Schnoor *et al.*, 1995) as well as take up and degrade the herbicide atrazine from contaminated soils (Burken and Schnoor, 1997). Forage grasses inoculated with bacteria tolerate and degrade individual chlorinated benzoic acids as well as mixtures of these compounds (Siciliano and Germida, 1998a). Alamo switchgrass (*Panicum virginatum*) accumulates the radionuclides Cesium-137 (^{137}Cs) and Strontium-90 (^{90}Sr), compounds present in nuclear fallout from weapons testing and reactor accidents (Entry and Watrud, 1998). Various grasses and leguminous plants, together with their associated microorganisms, increase the removal of petroleum hydrocarbons from contaminated soil (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996). These are just a few of the many examples of phytoremediation at work.

The objective of this paper is to evaluate the effectiveness of phytoremediation as a tool for cleaning up soil and groundwater contaminated with petroleum hydrocarbons. This objective was achieved by reviewing relevant literature on phytoremediation of petroleum hydrocarbons. Information was collected and summarized for any plant or microbe found in the literature relating to

phytoremediation of petroleum hydrocarbons. Grasses, herbs, shrubs, and deciduous trees were the general types of plants considered; no information was found for coniferous trees. Bacteria, protozoans, and fungi were the microorganisms considered. Literature was reviewed regarding organisms in terrestrial and wetland ecosystems together with those that influence groundwater (e.g., deciduous trees and microbes). Changes over time in the interactions among contaminants, plants and microorganisms also were considered.

A database is currently under construction in conjunction with this literature review. The purpose of the database is to act as an inventory of plant species that tolerate and/or phytoremediate petroleum hydrocarbons. Information included in the database is summarized in Table 1.

Table 1 Information in the Database on Plants That Tolerate or Phytoremediate Petroleum Hydrocarbons

General Information	Experimental Information	Plant Information
Common name of plant	Laboratory or field experiment	Family of plant
Scientific name of plant	Initial control and treatment concentrations of contaminant	Growth form (fern, grass, herb, shrub, tree)
Cultivar, strain, or code, (including transgenic variants)	Post-experiment control and treatment concentrations of contaminant	Morphology (type of root or shoot system, nitrogen fixation)
Contaminant of concern	Soil characteristics	Growth duration (annual, biennial, perennial)
Effect of organism on contamination	Age of plant at 1st exposure (seed, post-germination, mature)	Primary habitat (terrestrial, aquatic, wetland)
Mechanism involved in phytoremediation (degradation, rhizosphere effect, containment, transfer, tolerance)	Requirements for phytoremediation (specific nutrients, addition of oxygen)	Habitat description (including temperature preferences, salinity, soil texture, topography)
	Contaminant storage sites in the plant (root, shoot, leaf, no storage)	Western Canadian occurrence
		North American occurrence
		World range
		Cultural information
		Natural history notes
		Other species of significance in the genus

2.0 Mechanisms for the Phytoremediation of Petroleum Hydrocarbons

Plants and microorganisms remediate petroleum-contaminated soil and groundwater through three main mechanisms. These include degradation and containment as well as transfer of the contaminant from the soil to the atmosphere (Figure 1) (Cunningham *et al.*, 1996; Siciliano and Germida, 1998b; Sims and Overcash, 1983). The following information explores in greater detail these mechanisms of petroleum-hydrocarbon phytoremediation.

2.1 Degradation

Plants and microorganisms are involved both directly and indirectly in the degradation or transformation of petroleum hydrocarbons into products (e.g., alcohols, acids, carbon dioxide, and water) that are generally less toxic and less persistent in the environment than their parent compounds (Eweis *et al.*, 1998). Plants and microorganisms can degrade petroleum hydrocarbons independently of each other (refer to Sections 2.1.2 and 2.1.3). However, one of the most important factors involved in the degradation of petroleum hydrocarbons through phytoremediation is the interaction between plants and microorganisms in the rhizosphere effect.

2.1.1 The Rhizosphere Effect

The rhizosphere is the region of soil closest to the roots of plants and is, therefore, under the direct influence of the root system. Plants provide root exudates of carbon, energy, nutrients, enzymes and oxygen to microbial populations in the rhizosphere (Cunningham *et al.*, 1996; Campbell, 1985; Vance, 1996). In fact, exudates of sugars, alcohols, and acids can amount to 10 to 20% of annual plant photosynthate (Schnoor *et al.*, 1995) and can provide sufficient carbon and energy to support large numbers of microbes (e.g., approximately $10^8 - 10^9$ vegetative microbes per gram of soil in the rhizosphere; Erickson *et al.*, 1995). Due to the presence of these exudates, microbial populations and activities are 5 to 100 times greater in the rhizosphere than in soil not in contact with plant roots (i.e., bulk soil) (Figure 2) (Atlas and Bartha, 1998; Gunther *et al.*, 1996; Anderson *et al.*, 1993; Paul and Clark, 1989). This plant-induced enhancement of the microbial population is referred to as the rhizosphere effect (Atlas and Bartha, 1998). It is believed that the rhizosphere effect leads to increased degradation of organic contaminants in the rhizosphere.

Several studies serve as examples of the rhizosphere effect in the phytoremediation of petroleum hydrocarbons. Gunther *et al.* (1996) found higher microbial numbers and activity coupled with increased degradation in hydrocarbon-contaminated soil planted to ryegrass (*Lolium perenne* L.) compared to unvegetated soil. The authors suggested that plant roots stimulated microbes, which enhanced the degradation of the hydrocarbons. Jordahl *et al.* (1997) reported that populations of microorganisms capable of degrading benzene, toluene, and xylene were five times more abundant in the rhizosphere of poplar trees (*Populus deltoides* x *nigra* DN-34, Imperial Carolina) than in bulk soil; the rhizosphere and bulk soil had not been previously exposed to benzene, toluene or xylene. Nichols *et al.* (1997) found higher numbers of organic chemical degraders in rhizosphere and contaminated soils compared to bulk and uncontaminated soils, respectively. Plants creating the rhizosphere in this experiment included alfalfa (*Medicago sativa* L.) and alpine

bluegrass (*Poa alpina* L.), while the contaminants included hexadecane, (2,2-dimethylpropyl) benzene, *cis*-decahydronaphthalene, benzoic acid, phenanthrene, and pyrene.

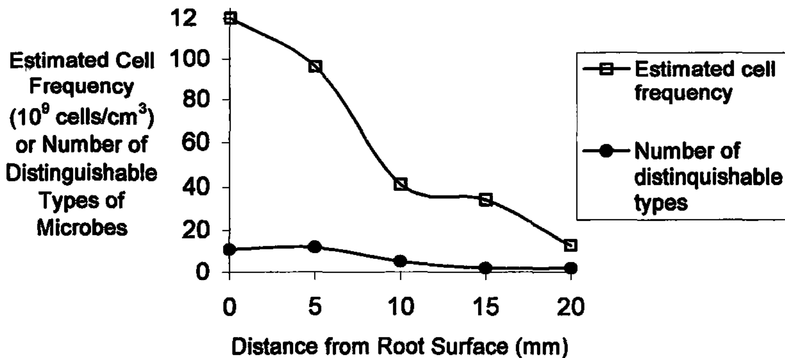

Figure 2 Number of Microorganisms at Increasing Distance from the Root Surface (Adapted from Paul and Clark, 1989)

Several experiments suggest, however, that the mineralization of petroleum hydrocarbons from soil may not be enhanced by the rhizosphere effect. Ferro *et al.* (1997) found that alfalfa (*Medicago sativa* Mesa, var. Cimarron VR) planted in artificial loamy sand soils did not increase the rate or extent of mineralization of [^{14}C]benzene compared to unplanted, artificial loamy sand soils. Similarly, crested wheatgrass [*Agropyron desertorum* (Fisher ex Link) Schultes] did not increase the rate or extent of mineralization of [^{14}C]phenanthrene when planted and unplanted systems were compared (Ferro *et al.*, 1994). In this experiment, however, the authors suggested that the lack of significant difference between mineralization in planted and unplanted systems may have been due to the rapid mineralization of [^{14}C]phenanthrene by microbes prior to establishment of the plant root systems and, therefore, prior to the presence of a rhizosphere effect in the soil (Ferro *et al.*, 1994).

2.1.2 A Closer Look at the Role of Plants in Degradation

Evidence regarding the direct degradation of petroleum hydrocarbons by plants is somewhat dated and limited in quantity. Durmishidze (1977) summarized various studies, primarily from the USSR, on degradation pathways for hydrocarbons in plants. Corn seedlings, tea, and poplar shoots were reported as metabolizing methane into various acids, including formic, malic, citric, succinic and fumaric acid as well as the amino acids leucine, glutamic acid, α-alanine, and glycine. The assimilation of [^{14}C]methane, [1,2-^{14}C]ethane, [1,3-^{14}C]propane, [1,4-^{14}C]butane, and [1,5-^{14}C]pentane was recorded for bean and corn seedlings, as well as tea, grape, walnut and quince. The ability to assimilate *n*-alkanes and liberate $^{14}CO_2$ was identified in leaves and roots of both whole and cut plants. As well, the pathway of conversion for alkanes in plants was generalized as:

n-alkane → primary alcohols → fatty acids → acetyl-CoA → various compounds

Durmishidze (1977) also reported that benzene, toluene, and xylene are metabolized in cereal grasses within 2 to 3 days, in corn within 4 to 5 days, and in root crops within 5 to 6 days. Phenol was reported as the primary conversion product of benzene in plant tissues, with the subsequent production of pyrocatechol, muconic acid, followed by fumaric acid and then succinic, malic, glycolic, malonic, and oxalic acid. The primary cleavage products of toluene were given as glycol, as well as glyoxalic, fumaric, succinic, and malic acid. Benzpyrene was reported to be metabolized by 14-day-old corn and bean plants, alfalfa, rye grass, chick pea, cucumbers, squash, orchard grass, and vetch, with the amount of degradation ranging from 2 to 18% of the benzpyrene taken up by the plant and varying with plant type.

Substantial information exists on the indirect roles played by plants in the degradation of petroleum hydrocarbons. These roles include: (i) supply of root exudates for the increase of microbial populations and cometabolic degradation processes, (ii) release of root-associated enzymes capable of transforming organic pollutants, and (iii) effects of plants and their root systems on physical and chemical soil conditions (Gunther *et al.*, 1996).

Perhaps the most important indirect role played by plants in the degradation of petroleum hydrocarbons is providing root exudates that induce the rhizosphere effect (see Section 2.1.1). Root exudates and the types of microbial communities they support are likely to be a site-time-plant specific phenomenon (Siciliano and Germida, 1998b). Site and time factors include variables such as soil type, nutrient levels, pH, water availability, temperature, oxygen status of soil, light intensity, and atmospheric carbon dioxide concentration, all of which significantly affect the type and quantity of root exudates (Siciliano and Germida, 1998b). Root exudate patterns are also known to be dependent upon plant species and stage of plant development. Hegde and Fletcher (1996) found the release of total phenolics by the roots of red mulberry (*Morus rubra* L.) increased continuously over the life of the plant with a massive release at the end of the season accompanying leaf senescence.

Cometabolism is the process by which a compound that cannot support the growth of microorganisms can be modified or degraded when another growth-supporting substrate is present (Cunningham and Berti, 1993). Both plant exudates and petroleum hydrocarbons can provide energy to support populations of microbes that co-metabolize specific petroleum hydrocarbons. Ferro *et al.* (1997) hypothesized that plant exudates may have served as co-metabolites, accelerating the biodegradation of pyrene in the rhizosphere. Kanaly *et al.* (1997) found that benzo[*a*]pyrene is almost completely degraded (95% degradation) by microbes in soil containing suitable co-substrates present in a crude oil mixture; the specific compounds acting as co-substrates in the crude oil mixture were not identified. Benzo[*a*]pyrene is a large, five-ring PAH that is typically persistent in the environment. This persistence is thought to be due to the inability of microorganisms to directly use benzo[*a*]pyrene for energy and growth, which emphasizes the importance of cometabolism in its degradation.

Another indirect role plants play in the degradation of petroleum hydrocarbons involves the release of enzymes from the roots. These enzymes are capable of transforming organic contaminants by inducing chemical reactions in soil. Schnoor *et*

al., (1995) identified plant enzymes as causative agents in the transformation of contaminants mixed with sediment and soil; isolated enzyme systems included dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase. These findings suggest that plant enzymes might have significant spatial effects beyond the plant itself as well as temporal effects after the death of the plant (Cunningham *et al.*, 1996).

Finally, plants and their roots can indirectly influence degradation by altering the physical and chemical condition of the soil. Living plants have extensive root systems that help to bring plants, microbes, nutrients, oxygen and contaminants into contact with each other. As well, plants provide organic matter to the soil, which can influence the bioavailability of contaminants (i.e., the extent to which a contaminant is available to interact with living organisms), especially since organic matter binds lipophilic organic contaminants, including some petroleum hydrocarbons. Organic matter is provided as root exudates, as well as through the loss of root cap cells and the excretion of mucigel – a gelatinous, lubricating substance that aids root penetration of the soil (Cunningham *et al.*, 1996).

2.1.3 A Closer Look at the Role of Microorganisms in Degradation

Currently, microorganisms are used to destroy or immobilize organic contaminants in the absence of plants in a process referred to as *bioremediation.* In this section, we focus on issues concerning the role of microorganisms in the degradation of petroleum hydrocarbons in the presence of plants – a mechanism of phytoremediation. Key issues include: reasons for microbial degradation of petroleum hydrocarbons, differences in degradation by various organisms, and characteristics of microbial communities involved in degradation. The ability of microorganisms to reduce the toxicity of contaminants to the point where plants grow under otherwise adverse conditions is also of interest.

Microbial degradation of organic contaminants normally occurs because the microorganisms can use the contaminant for their own growth and reproduction (Committee on In Situ Bioremediation *et al.*, 1993). Organic contaminants not only provide the microorganisms with a source of carbon, they also provide electrons that the organisms use to obtain energy. Basic microbial metabolism of contaminants involves aerobic respiration (respiration in the presence of oxygen). However, variations in metabolism include anaerobic respiration, secondary utilization and cometabolism, using inorganic compounds as electron donors, fermentation, and reductive dehalogenation.

In general, the metabolic processes of microorganisms act on a wider range of compounds, carry out more difficult degradation reactions, and transform a contaminant into more simple molecules than plants (Cunningham and Berti, 1993). However, not all microorganisms degrade organic contaminants in the same manner. The pathway of aerobic degradation of PAHs by prokaryotic microorganisms, such as bacteria, involves a dioxygenase enzyme, the incorporation of two atoms of molecular oxygen into the contaminant, and the production of less toxic compounds such as acids, alcohols, carbon dioxide and water (Gibson and Subramanian, 1984; Eweis *et al.*, 1998). In contrast, degradation by eukaryotic fungi initially involves the incorporation of only one atom of oxygen into the PAH, which is similar to the degradation mechanism found in mammals (Sutherland, 1992; Cerniglia, *et al.* 1986;

Cerniglia and Gibson, 1979). Moreover, whereas most fungal transformations result in compounds that are less toxic than the parent PAHs, some of the minor metabolites produced during fungal degradation of these compounds are more toxic than the parent compound itself (Sutherland, 1992).

Certain characteristics of microorganisms and microbial communities may influence their establishment and use in phytoremediation. For instance, the composition and size of the microbial community in the rhizosphere is dependent on the type of plant species, the age of the plant and soil type (Campbell, 1985; Atlas and Bartha, 1998; Bossert and Bartha, 1984). The microbial community in the rhizosphere may also depend on the exposure history of the plant roots to contaminants (Anderson *et al.*, 1993). Soil microbial communities may experience selective enrichment of contaminant-tolerant species when exposed to the contaminants for a prolonged period of time, which may result in enhanced degradation of the contaminant (Anderson *et al.*, 1993). On the other hand, some species of bacteria appear to be able to degrade a wide variety of rarely-occurring compounds without having to first adapt to contaminated conditions (Siciliano and Germida, 1998b). Catabolic pathways in pseudomonads, for example, allow these bacteria to degrade a variety of aromatic contaminants, such as toluene, *m*-xylene, and naphthalene, without having to synthesize a large number of different enzymes (Houghton and Shanley, 1994).

Evidence also suggests that degradation of certain contaminants occurs only when a specific consortium of microbes occur together at a contaminated site (Anderson *et al.*, 1993). Lappin *et al.* (1985) isolated five species of bacteria from the rhizosphere of wheat that could grow on and degrade the herbicide mecoprop [2-(2-methyl 4-chlorophenoxy)propionic acid] as long as two or more species occurred together. Individually, none of the species could degrade mecoprop. More recently, White and Alexander (1996) reported finding a consortium of microbes that could utilize phenanthrene sorbed to soil without first desorbing it. This has important implications for bioavailability, in that it appears bacteria may not require that certain contaminants be in the aqueous phase before degradation can occur.

Another role played by microbes involves their ability to reduce the phytotoxicity of contaminants to the point where plants can grow in adverse soil conditions and thereby stimulate degradation of other contaminants (Siciliano and Germida, 1998b). Radwan *et al.* (1995) found that the plant *Senecio glaucus* grew along the polluted border of an oil lake in the Kuwaiti desert. Interestingly, the plant roots and adhering sand particles were white and clean, while the surface of the transitional zone between the root and shoot was black and polluted. The authors suggested that microbes detoxified contaminants in the rhizosphere, thus allowing the plants to survive in the oil-contaminated soils. Rasolomanana and Balandreau (1987) found improved growth of rice in soil to which oil residues were applied. The authors hypothesized that the increased growth resulted from the removal of the oil residues by various bacterial species of the genus *Bacillus*, which used plant exudates to cometabolize the oil residues in the rhizosphere.

2.2 Containment and Transfer of Contaminants

Additional mechanisms of phytoremediation include containment of petroleum hydrocarbons as well as their transfer from the soil to the atmosphere by

way of evapotranspiration from the plant. Containment can involve the accumulation of contaminants within the plants as well as adsorption of the contaminants onto roots. Durmishidze (1977) reported that rice seedlings take up [^{14}C]methane through their roots and that bean and corn seedlings take up [^{14}C]methane, [1,2-^{14}C]ethane, [1,3-^{14}C]propane, [1,4-^{14}C]butane, and [1,5-^{14}C]pentane through their roots and leaves. Benzene, toluene, and xylene were also reported as entering plants with irrigation water and becoming incorporated into the metabolic processes of the plants (Durmishidze 1977). However, more recent studies indicate that the amount of petroleum hydrocarbons taken up by plant roots from contaminated soil is relatively small (e.g., 2 to 8% accumulation of soil-applied [^{14}C]benzene in alfalfa shoots and less than 2% in roots) (Ferro et al. 1997; Edwards 1983). Generally, adsorption onto the surface of roots occurs to a greater extent in roots with higher lipid contents and when the contamination includes lipophilic PAHs with four or more rings (Schwab et al. 1998; Reilley et al., 1996; Sims and Overcash, 1983).

Containment can also occur by several other means. For example, plants can act as organic pumps that transpire water, thus preventing contaminant migration by keeping the contaminants near the roots (Schnoor et al., 1995; Aprill and Sims 1990). Plants can act indirectly to contain contaminants by supplying enzymes that bind the contaminants into soil organic matter (or humus) in a process called humification and by increasing soil organic matter content, which allows for humification (Cunningham *et al.*, 1996).

As mentioned above, phytoremediation may also involve the transfer of petroleum hydrocarbons from the soil to the atmosphere. Wiltse *et al.* (1998) observed leaf burn in alfalfa plants growing in soil contaminated with crude oil. The authors suggested that an unidentified compound from the contaminated soil was translocated and volatilized through the plant. The leaf burn gradually disappeared as the experiment progressed, indicating the dissipation of the contaminants responsible for the effect. Watkins *et al.* (1994) found that the volatilization of [^{14}C]naphthalene was enhanced in sandy loam soil planted to bell rhodesgrass (*Cloris gayana*) compared to unplanted soil. The results of the study suggested that naphthalene was taken up by the roots of the grass, translocated within the plant and volatilized through the stems and leaves. The authors noted that this mechanism of removal would reduce the amount of naphthalene available for biodegradation, but may have implications for air quality monitoring and regulatory compliance.

3.0 Special Considerations with Phytoremediation

There are several special considerations that must be taken into account when applying phytoremediation to contaminated sites. Three of the most important considerations include the influence of environmental conditions on phytoremediation, the establishment of appropriate plants and microorganisms, and the concentrations of petroleum hydrocarbons at the contaminated site.

3.1 The Influence of Environmental Conditions on Phytoremediation

A variety of environmental conditions affect or alter the mechanisms of phytoremediation. Soil type, structure and organic matter content can limit the bioavailability of petroleum contaminants (Otten *et al.*, 1997; Boyle and Shann, 1998; Alexander *et al.*, 1997; Cunningham *et al.*, 1996). Plants that accumulate PAHs can

experience toxic effects, such as reduced growth, resulting from photomodifications and photosensitizations of PAHs exposed to solar ultraviolet radiation, which penetrates into plant tissues (Ren *et al.*, 1994; Huang *et al.*, 1993; Duxbury *et al.*, 1997). Water content in soil and wetlands affects plant/microbial growth and the availability of oxygen required for aerobic respiration (Eweis *et al.*, 1998). Fertilizers increase degradation by increasing availability of and reducing competition for limited nutrients in oil-contaminated soil (Steffensen and Alexander, 1995; Schwendinger, 1968). Finally, temperature changes affect the rates at which various processes take place, with the rate of microbial degradation generally doubling for every 10 °C increase in temperature (Eweis *et al.*, 1998; Wright *et al.*, 1997; Dibble and Bartha, 1979). Together with biodegradation, the various environmental factors act to bring about the weathering – or the loss of certain fractions – of contaminant mixtures, with the end result being that the more resistant compounds remain in the soil (Bossert and Bartha, 1984).

3.2 Establishment of Appropriate Plant Species and Microorganisms

Successful phytoremediation of petroleum hydrocarbons may require the establishment of appropriate plants and microorganisms at the contaminated site. Factors to consider include the types of plants to be established, the influence of contamination on germination of plants, the types of microbes to be established, and the effectiveness of inoculating contaminated soils with microorganisms.

Legume and grass species have been singled out for their potential use in phytoremediation of sites contaminated with petroleum hydrocarbons (Table 2) (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996). Legumes are nitrogen-fixing plants, which gives them an advantage over non-legume plants in that they do not have to compete with other plants and microorganisms for limited supplies of available soil nitrogen at oil-contaminated sites (Gudin and Syratt, 1975). Aprill and Sims (1990) suggested that the following characteristics of prairie grasses make them superior vehicles for phytoremediation: (i) they are characterized by fibrous root systems with the maximum root surface area (per m^3 of soil) of any plant type, (ii) they develop extensive root systems that may penetrate the soil to a depth of up to 3 m, and (iii) they exhibit an inherent genetic diversity which may yield a competitive advantage. Whatever the plant chosen for phytoremediation, the species must be well adapted to the climate of the region, making length of growing season, average temperature, and rainfall important considerations in phytoremediation planning (Cunningham *et al.*, 1996).

Another important factor in establishing plants at oil-contaminated sites involves getting the plants to germinate (Cunningham *et al.*, 1996). Germination of the seeds is enhanced when soil is moist (but not too wet), the temperature is appropriate, and the soil is not toxic to the seeds. A good way of knowing if the plants to be used for phytoremediation will germinate successfully is to carry out germination tests in the contaminated soil prior to planting.

Table 2 Plants That Tolerate or Phytoremediate Petroleum Hydrocarbons*

Tolerant plants	Plants that phytoremediate
alfalfa (*Medicago sativa*)	alfalfa (*Medicago sativa* L.)
birdsfoot trefoil (*Lotus corniculatus*)	big bluestem (*Andropogon gerardi*)†
black medick (*Medicago lupulina*)	blue grama (*Bouteloua gracilis*)†
Melilotus altissima	Canada wild-rye (*Elymus canadensis*)†
Psoralea bituminosa	common buffalograss (*Buchloe dactyloides*)
Robinia pseudacacia	fescue (*Festuca arundinacea* Schreb.)
Vicia tetrasperma	Indiangrass (*Sorghastrum nutans*)†
white clover (*Trifolium repens*)	little bluestem (*Schizachyrium scoparius*)†
	Meyer zoysiagrass (*Zoysia japonica* var. Meyer)
	prairie buffalograss (*Buchloe dactyloides* var Prairie)
	ryegrass (*Lolium perenne* L.)
	side oats grama (*Bouteloua curtipendula*)†
	sudangrass (*Sorghum vulgare* L.)
	switchgrass (*Panicum virgatum*)†
	Verde kleingrass (*Panicum coloratum* var. Verde)
	western wheatgrass (*Agropyron smithii*)†

* Source: Gudin and Syratt (1975); Aprill and Sims (1990); Qiu *et al.* (1997); Gunther *et al.* (1996); Reilley *et al.* (1996).

† Aprill and Sims (1990) evaluated the phytoremediation potential of these plants as a group, not as individual species. Switchgrass was, however, also investigated as an independent species by Reilley *et al.* (1996).

A variety of microorganisms, some of which are associated with the roots of plants, are reportedly involved in the degradation of petroleum hydrocarbons (Table 3). In general, the bacteria *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Corynebacterium*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Nocardia*, and *Mycobacterium*, are reported as the most active bacterial species in the degradation of hydrocarbons in soil (Bossert and Bartha, 1984). A diversity of fungi also degrade hydrocarbons in soil, including *Aspergillus ochraceus*, *Cunninghamella elegans*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, and *Syncephalastrum racemosum*, which oxidize various PAHs, such as anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, fluoranthene, fluorene, naphthalene, phenanthrene, pyrene, as well as methyl-, nitro-, and fluoro-substituted PAHs (Sutherland, 1992). Many others are listed in Table 3.

Table 3 Microorganisms in Soil That Degrade Hydrocarbons*

Bacteria		Fungi	
Achromobacter	*Micrococcus*	*Acremonium*	*Monilia*
Acinetobacter	*Mycobacterium*	*Aspergillus*	*Mortierella*
Alcaligenes	*Norcardia*	*Aureobasidium*	*Paecilomyces*
Arthrobacter	*Proteus*	*Beauveria*	*Penicillium*
Bacillus	*Pseudomonas*	*Botrytis*	*Phoma*
Brevibacterium	*Rhodococcus*	*Candida*	*Phanerochaete*
Chromobacterium	*Sarcina*	*Chrysosporium*	*Rhodotorula*
Corynebacterium	*Serratia*	*Cladosporium*	*Saccharamyces*
Cytophaga	*Spirillum*	*Cochliobolus*	*Scolecobasidium*
Erwinia	*Streptomyces*	*Cunninghamella*	*Sporobolomyces*
Flavobacterium	*Vibrio*	*Cylindrocarpon*	*Sprotrichum*
	Xanthomonas	*Debaryomyces*	*Spicaria*
		Fusarium	*Syncephalastrum*
		Geotrichum	*Tolypocladium*
		Gliocladium	*Torulopsis*
		Graphium	*Trichoderma*
		Humicola	*Verticillum*

* Source: Bossert and Bartha (1984); Barnsley (1975); Heitkamp and Cerniglia (1989); Grosser *et al.* (1995); Sutherland (1992); Cerniglia and Gibson (1979); Bumpus *et al.* (1985); Radwan *et al.* (1995).

It is not an uncommon experience for soil and plant inoculants to be out-competed by native microflora (Cunningham *et al.*, 1996). Indeed, this is true even with some symbiotic relationships (e.g. soybean and their *Bradyrhizobia* symbiont). Nevertheless, there are reports that inoculating contaminated sites with microorganisms can be an effective means of producing enhanced degradation of soil contaminants. For example, Grosser *et al.* (1995) identified that the isolation and reintroduction of *Mycobacterium* species capable of degrading phenanthrene, anthracene, and pyrene, resulted in mineralization above that found with just indigenous microbes. Likewise, Siciliano and Germida (1997) found that inoculating two pseudomonad bacterial species onto meadow brome (*Bromus biebersteinii*) increased degradation of 2-chlorobenzoic acid (a PCB-degradation compound) in soil. Schwendinger (1968) identified a reduced adaptation time and greater total CO_2 production over a 7-week period after seeding *Cellulomonas* (a cellulose-decomposing bacteria) into sandy loam soil with relatively high levels of oil (100 ml/kg soil).

3.3 Concentration of Petroleum Hydrocarbons

Within certain concentration ranges, plants and microbes can tolerate petroleum hydrocarbons, thus laying the foundation for the phytoremediation of contaminated sites. Phytoremediation of petroleum hydrocarbons may be ineffective, however, if concentrations of the contaminants are either too high (causing toxicity) or too low (resulting in poor bioavailability). Phytoremediation may also be

ineffective if concentrations of other compounds, such as salts and metals, occur in high concentrations with petroleum hydrocarbons at contaminated sites.

Low concentrations of contaminants may limit the extent to which phytoremediation can further reduce contaminant levels. If microbial uptake and metabolism of organic compounds stops at low concentrations, microorganisms will not be physiologically capable of reducing contaminant concentrations to levels lower than those already present (The Committee on In Situ Bioremediation et al. 1993). Low concentrations may also cause microbes capable of degrading the contaminant to switch to alternative substrates or may result in the death of the microbes due to lack of sustenance (Committee on In Situ Bioremediation *et al.*, 1993; Hrudey and Pollard, 1993). Similarly, readily biodegradable contaminants in groundwater may remain undegraded or degrade very slowly if their bioavailability is low due to low concentrations (Committee on In Situ Bioremediation *et al.*, 1993).

Conversely, concentrations of contaminants that are too high can result in a toxic response and/or death of microorganisms and plants – again limiting the effectiveness of phytoremediation. For example, a microbial population may exhibit a treatable concentration range above which the contaminant inhibits or prevents metabolic activity. In turn, this would then prevent the growth of new microbial biomass needed to stimulate rapid removal of the contaminant (Hrudey and Pollard, 1993; Committee on In Situ Bioremediation *et al.*, 1993). Rogers *et al.* (1996) reported that various plants, including red clover, alfalfa, birdsfoot trefoil, white clover, alpine bluegrass, tilesy sage, bering hairgrass, reed canarygrass, and quackgrass were killed shortly after exposure to greater than 2000 mg of a mixture of organic chemicals per kilogram of soil (or 0.2%). The organic mixture contained equal molar amounts of benzoic acid, hexadecane, 2,2-dimethyl 4,npropyl-benzene, phenanthrene, pyrene, and either cis-decahydronaphthalene (cis-decalin) (at 25 °C only) or cycloheptane (at 10 °C only).

Certain levels of petroleum hydrocarbons can be tolerated by a wide variety of plants. Schwendinger (1968) found that oat plants continued to grow – without exhibiting symptoms of severe damage – when exposed to approximately 3% (w/w) oil in soil. As well, fairly sensitive crops, such as tomato, kale and leaf lettuce, could tolerate a considerable quantity of crude oil in the soil, though the amount of crude oil tolerated was species dependent (Schwendinger 1968). Annual Kuwaiti plants, such as *Senecio glaucus*, grow in moderately to weakly contaminated areas containing less than 10% (w/w) oil sediments (Radwan *et al.*, 1995). Carr (1919) identified that soybean could tolerate rather large amounts of crude oil (4%) mixed with the soil and that the growth of soybeans in sandy peat soil was even improved through the addition of small amounts of crude oil (0.75%). Rogers *et al.* (1996) found that of the nine plant species tested (i.e., alfalfa, red clover, white clover, birdsfoot trefoil, alpine bluegrass, Bering hairgrass, reed canary grass, quackgrass, and tilesy sage), most grew well in soil contaminated with 2000 mg kg^{-1} (0.2%) or less of the same mixture of organic chemicals described above.

Even if concentrations of petroleum hydrocarbons are within the range tolerated by plants, other contaminants, such as salts and metals, may be present at levels that prevent effective phytoremediation. High salinity levels can disrupt protein structures, denature enzymes and dehydrate cells (Atlas and Bartha 1998). As

well, high metal concentrations may inhibit microbial metabolism (Hrudey and Pollard 1993).

4.0 Summary

Phytoremediation is steadily emerging as a tool for the clean-up of soils contaminated with petroleum hydrocarbons. Plants used in successful trials include various grass and legume species (Aprill and Sims, 1990; Qiu *et al.*, 1997, Gunther *et al.*, 1996, and Reilley *et al.*, 1996). Plants and their associated microorganisms can directly and indirectly remediate petroleum-contaminated soil and groundwater through three main mechanisms: (i) degradation, (ii) containment and (iii) the transfer of the contaminant from the soil to the atmosphere (Cunningham *et al.*, 1996; Siciliano and Germida, 1998b; Sims and Overcash, 1983).

Degradation is accomplished by both plants and microorganisms, either independently or through joint interactions. Whereas there is ample evidence to demonstrate that microorganisms play a direct and important role in the degradation of various petroleum hydrocarbons, there is only limited evidence that plants degrade petroleum hydrocarbons directly (Durmishidze, 1977). Plants do, however, play a myriad of significant – though indirect – roles in the degradation of petroleum hydrocarbons. These roles include supplying root exudates for microbial use, releasing root-associated enzymes that degrade contaminants in the soil, and altering the physical/chemical nature of the soil to promote phytoremediation (Gunther *et al.*, 1996; Erickson *et al.*, 1995; Siciliano and Germida, 1998b; Schnoor *et al.*, 1995; Cunningham *et al.*, 1996).

Plants can play a direct role in containing petroleum hydrocarbons within a given area as well as in facilitating the transfer of petroleum hydrocarbons from the soil to the atmosphere. Plants act to prevent petroleum hydrocarbons from spreading through adsorption and uptake, as well as by acting as organic pumps that isolate contaminants in the root zone (Ferro *et al.*, 1997; Schnoor *et al.*, 1995; Aprill and Sims, 1990). Plants also can transfer volatile petroleum hydrocarbons (e.g., naphthalene) from the soil to the atmosphere via evapotranspiration (Watkins *et al.*, 1994). Although this mechanism removes contaminants from the soil, health risks may still arise since the contaminant has simply been moved to the atmosphere, which can serve as an alternative source of exposure.

Certain petroleum hydrocarbons are easier to phytoremediate than others. In general, BTEX compounds are relatively easy to remediate because they are (i) rapidly degraded in the presence of oxygen, (ii) relatively soluble making them bioavailable, and (iii) can serve as the primary electron donor for many bacteria widely distributed in nature (Committee on In Situ Bioremediation *et al.*, 1993). Large and lipophilic compounds, such as the four and five-ring PAHs, are more difficult, but not impossible, to remediate. The difficulty arises from their limited bioavailability, which is a result of their adsorption to soil organic matter and clay as well as their limited ability to pass through the cellular membranes of plants and microbes (Cookson, 1995). However, cometabolism by microorganisms has been shown to lead to the degradation of some large PAHs, such as benzo[*a*]pyrene (Kanaly *et al.* 1997). In general, weathering processes, involving volatilization, evapotranspiration, photomodification, hydrolysis, and biotransformation, selectively reduce the concentration of easily-degradable contaminants, making older sites more

difficult to phytoremediate than newer sites (Cunningham *et al.*, 1996; Bossert and Bartha, 1984).

The effectiveness of phytoremediation is somewhat site-specific in that it can be affected by environmental conditions. Phytoremediation potential may be restricted in soils with abundant organic matter or clay – soil components that tend to reduce the bioavailability of petroleum hydrocarbons (Otten *et al.*, 1997; Cunningham *et al.*, 1996). Phytoremediation is facilitated by adequate quantities of nutrients, water and oxygen and is enhanced at warmer temperatures (Wright *et al.*, 1997). However, phytoremediation may be inhibited if contaminant concentrations are too high or too low (Committee on In Situ Bioremediation *et al.* 1993; Hrudey and Pollard, 1993; Rogers *et al.*, 1996; Atlas and Bartha, 1998).

There are several gaps in the literature that are worth noting. First, there appears to be little or no information available regarding efforts to phytoremediate sites in Canada that are contaminated with petroleum hydrocarbons. Perhaps the most applicable paper in this regard is the study conducted by Rogers et al. (1996) which dealt with the selection of cold-tolerant plants for growth in soils contaminated with organics. Second, there is little detailed evidence on the mechanisms involved in the phytoremediation of petroleum hydrocarbons. For example, studies often state that concentrations of petroleum hydrocarbons are reduced in soil to a greater extent when plants are present than in their absence (e.g., Aprill and Sims, 1990 and Qiu *et al.,* 1997). Although these results indicate that phytoremediation is effective in reducing the contaminant load in soil, they, unfortunately, do not identify the specific mechanisms that cause the reduction.

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Microbial Based Strategies for Assessing Rhizosphere-Enhanced Phytoremediation

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Abstract

Contaminated soils at installations built by the U.S. Department of Defense in cold regions may present human and environmental health risks. Many installations are remote and relatively inaccessible. The expenses to mobilize and demobilize cleanup efforts, short treatment seasons, and limited infrastructure result in high costs and restrict treatment options. Rhizosphere-enhanced biotreatment—a low-cost, easily implemented treatment technology that relies on stimulating indigenous microorganisms—overcomes many of the limitations, but wider application is hampered by the scarcity of information for predicting success across a range of soil-contaminant-climate settings and lack of suitable means to monitor progress. Coupling chemical and microbial data from field research-demonstration sites may provide insight to the important processes and suggest more robust monitoring techniques. Our results measured changes in culturable bacterial diversity and suggested a relationship with decreases in contaminant concentrations. This approach may provide a biological method of monitoring phytoremediation progress, completion, or both.

1.0 Introduction

In cold regions, treatment rates are reduced by low temperatures, brevity of treatment season, or both. Site monitoring often is difficult because of the remote locations of many sites, the inherent heterogeneity of contaminant distribution, and the accumulation of numerous small contaminant releases that have occurred in the general area. Phytoremediation may be an attractive treatment option for these sites, yet our knowledge and experience with using phytoremediation to treat contaminated soils, especially in cold regions, is imperfect. These specifics combine to limit application of phytoremediation.

Although phytoremediation is inexpensive to implement and maintain, an inherent trade-off is longer treatment times than for many "traditional" technologies. The magnitude of savings is site specific, but in general, implementing phytoremediation can be a relatively low-cost option (Figure 1).

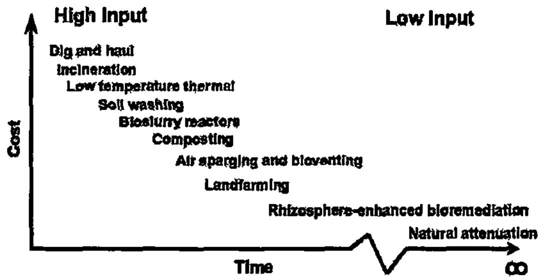

Figure 1 Relative Costs and Treatment Times for Selected Remediation Options

For treatment technologies that are aggressive, sampling and analysis can be done before and during the treatment process. Contaminant concentrations can be readily monitored and the treatment process continued until remediation goals are met. The other extreme, natural attenuation, is increasingly viewed as an acceptable treatment for benzene, toluene, ethyl benzene and xylene (BTEX)-contaminated groundwater. For contaminated groundwater, treatment times and rates often can be realistically predicted because there is a database of groundwater chemistry from past monitoring of plumes. Because groundwater systems are mixed and subsurface conditions are relatively constant, sample heterogeneity is reduced and monitoring is facilitated.

By contrast, phytoremediation would be applied to relatively shallow contamination of less mobile, and also less biodegradable, contaminants. Moreover, much of the system, defined by the rooting depth, is subject to changes in temperature and moisture and is generally not a well-mixed system. Understandably, acceptance and use of phytoremediation may be delayed because of longer treatment times and the uncertainty of achievable rates and endpoints. To overcome these uncertainties, requirements to increase spatial sampling density, temporal sampling frequency, or both, may be imposed. Additional monitoring requirements increase overall treatment costs and can counteract many of the benefits of using phytoremediation. Although we are gaining experience in using phytoremediation at a number of sites, we are somewhat limited in our ability to effectively predict success.

There is convincing evidence from both laboratory and field studies showing that phytoremediation can be effective for treating contaminated soils. Although the majority of these studies were conducted in temperate climates (Anderson *et al.*, 1993; Aprill and Sims, 1990; Cunningham and Ow, 1996; Cunningham *et al.*, 1996; Reilley *et al.*, 1996, Schwab *et al.*; 1995; and Wiltse *et al.*, 1998), some were conducted in a subarctic climate (e.g. Reynolds *et al.*, 1999). From these studies the operative mechanisms for phytoremediation appear to be largely contaminant dependent. For many organic contaminants, especially petroleum compounds, the generally accepted phytoremediation mechanism is enhanced microbial activity in the rhizosphere, which in turn accelerates the rate of degradation of contaminants. Plant-

produced compounds may serve as cometabolites for more recalcitrant compounds, and this may result in lower contaminant concentration endpoints than can be obtained without plants (Fletcher *et al.*, 1995). We propose that a potential tool for evaluating phytoremediation, monitoring endpoints, or perhaps predicting long-term success of phytoremediation may be based on changes in the soil microbial ecology. We hypothesized that changes in the microbial ecology may be more apparent than subtle changes in contaminant concentrations, and therefore provide a practical monitoring or confirmation tool (Figure 2).

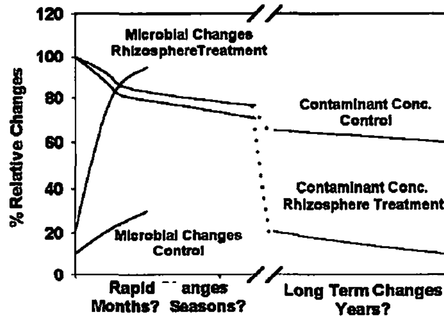

Figure 2 Theoretical Changes in Soil Microbial Characteristics and Contaminant Concentrations during Phytoremediation

2.0 Materials and Methods

In late July,1995 we initiated a comparison of rhizosphere-enhanced biotreatment to natural attenuation at a petroleum-contaminated site in Fairbanks, Alaska. We compared the effects of vegetation and nutrient additions (rhizosphere treatment) on remediating both diesel- and crude-oil-contaminated soils with treatments that consisted of no vegetation and no nutrient amendments (control treatments). For both diesel- and crude-oil-contaminated soils, contamination resulted from a combination of old and more recent releases. We sampled twice a year, in late fall and early spring for a approximately two years and measured changes in petroleum concentrations. Concomitantly, we characterized the microbial populations by different indices, including species richness (d) and the Shannon-Weaver diversity index ($\overline{H}$).

Arctared red fescue (*Festuca rubra*) and annual ryegrass (*Lolium multiflorum*) were chosen for their cold hardiness and rapid growth, respectively. Both grasses have extensive root distribution and tolerance to low fertility soils. Seeds were planted each spring as a mix consisting of equal seed masses of each species. We fertilized only at the beginning of the experiment by hand-broadcasting commercially available agricultural fertilizer, granular 20-20-10. The fertilizer was surface-applied at 620 g/m^2, which approached the maximum fertilizer rate that we could use without inhibiting microbial activity by inducing osmotic stress in the soils at this site (Walworth *et al.*, 1997). We hand spread the fertilizer on the surface at a near-maximum rate, because this technique could readily be used at remote field sites with

minimal equipment and frequency. After the initial fertilizer application, no further fertilizer was added.

For each soil, approximately 170 subsamples were taken prior to seeding or fertilization and composited by rotary mixer. These large composite samples, one for each soil, were then apportioned into fine mesh, cylindrical, open topped bags that were buried vertically in the plots. Sufficient bags were buried so that triplicate soil samples could be obtained from each plot at each sampling time. Plots were approximately 3 m^2.

For microbial characterization, soil samples were serially diluted and plated on 0.1 strength tryptic-soy agar to determine viable numbers of bacteria (Zuberer, 1994). Numbers of aerobic, heterotrophic bacteria were determined following incubation of the plates for three days at 20 ± 1°C. For each soil sample characterized, we evaluated between 50 and 100 randomly chosen isolates from dilution plates having between 30 and 300 colonies. Bacterial isolates were identified to the species level by characterizing their fatty acid methyl ester (FAME) profiles following the procedures outlined by Sasser (1990) and Sasser and Wichman (1991) in which fatty acid (FA) profiles are identified by comparison to a bacterial reference library (MIDI, 1995). Isolates that we could not identify using the library were given an internal laboratory identifier and added to the library. Unknown isolates having fatty acid profiles distinctively different from other unknowns were treated as individual species. Unknowns having similar fatty acid profiles were characterized as individuals of the same, although unidentified, species.

We used two indices, species richness (d) and Shannon-Weaver index ($\overline{H}$).

Species richness (d) was defined as (Odum, 1971; Pielou, 1975):

$$d = (S-1)/\log N \qquad (1)$$

where S = number of species
N = number of individuals

The Shannon-Weaver index ($\overline{H}$) was defined as (Shannon and Weaver, 1963):

$$\overline{H} = (C/N)(N \log N - \Sigma n_i \log n_i) \qquad (2)$$

where C = 2.3
N = number of individuals
n_i = number of individuals in the ith species

The diversity index $\overline{H}$ incorporates terms for the total number of individuals and the number of members of each species within the community.

Soil total petroleum hydrocarbon (TPH) was extracted by sonication with CH_2Cl_2. Anhydrous Na_2SO_4 was added to the soil during extraction as a drying agent. Extracts were analyzed by GC-FID.

3.0 Results

Soil TPH concentrations in both the natural attenuation and rhizosphere treatments decreased relative to the initial TPH concentrations, which were approximately 8350 mg/kg and 6200 mg/kg for diesel- and crude-oil-contaminated-soils, respectively (Figure 3). For figures 3-7, error bars represent the treatment mean +/- 1 S.E. The rhizosphere treatment had significantly lower TPH concentrations after approximately 640 days of treatment for both the diesel- and crude-oil-contaminated soils. For each treatment in the crude-oil-contaminated soil TPH concentrations decreased, but they remained greater than TPH values in the corresponding treatments in the diesel-contaminated soil. At 640 days, TPH values were approximately 2500 and 1400 mg/kg for the control and rhizosphere treatments, respectively, in the crude-oil-contaminated soil (Figure 3). Corresponding TPH values for the diesel-contaminated soil were approximately 2200 and 700 mg/kg.

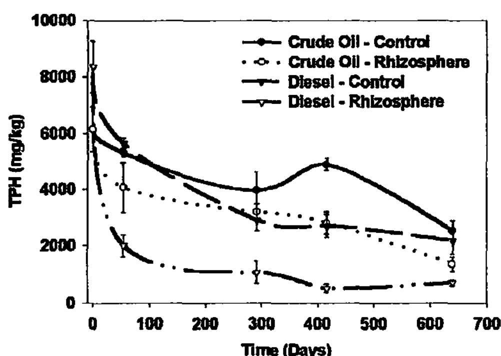

Figure 3 TPH Concentration Changes in Diesel- and Crude-Oil-Contaminated Soils during Remediation Treatments.

In the diesel-contaminated soil, diversity, expressed as both species richness (d) and the Shannon-Weaver index ($\overline{H}$), initially increased after approximately 300 days for both the control and rhizosphere treatments (Figures 4 and 5). For the control treatment, $\overline{H}$ was stable at 420 and 640 days (Figure 5).

Using d and $\overline{H}$ as indicators, we showed that bacterial diversity increased after contaminant concentrations in the soil had reached relatively low levels. This effect, as well as the decrease in contaminant concentration, was greater in the rhizosphere treatment compared to the control treatment. From approximately 300 to 640 days, TPH concentrations remained above 2000 mg/kg in the control treatment,but had dropped to approximately 700 mg/kg in the rhizosphere treatment after 420 days. During this time increases in diversity, expressed as d, were relatively constant for the control treatment but accelerated for the rhizosphere treatment. Continued increases in $\overline{H}$ were seen only for the rhizosphere treatment.

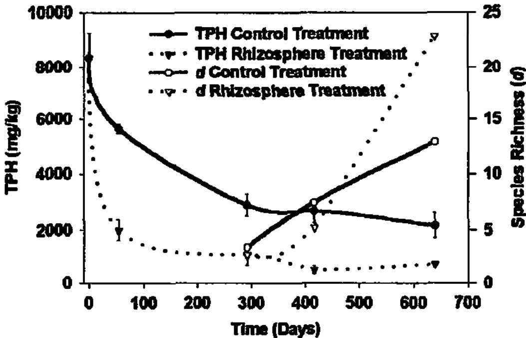

Figure 4 TPH and Bacterial Species Richness in the Diesel-Contaminated Soil during Remediation

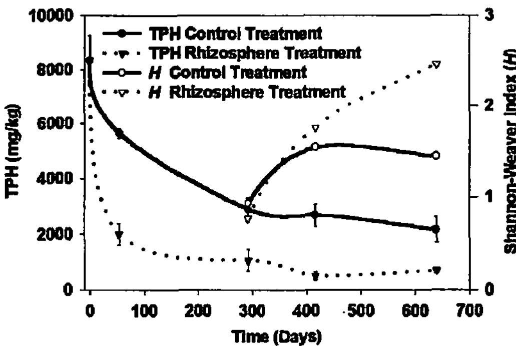

Figure 5 TPH and Shannon-Weaver Index for Bacteria in the Diesel-Contaminated Soil during Remediation

In the crude-oil-contaminated soil, species richness values were lower for both the control and rhizosphere treatments than for the diesel-contaminated soil (Figures 4 and 6). Moreover, there was little change in species richness from 300 to 640 days, compared to the increases observed in the diesel-contaminated soils. Diversity expressed using the Shannon-Weaver index showed trends similar to the *d* values (Figure 7).

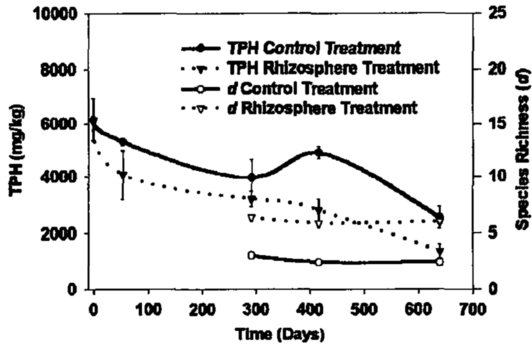

Figure 6 TPH and Bacterial Species Richness in Crude-Oil-Contaminated Soil

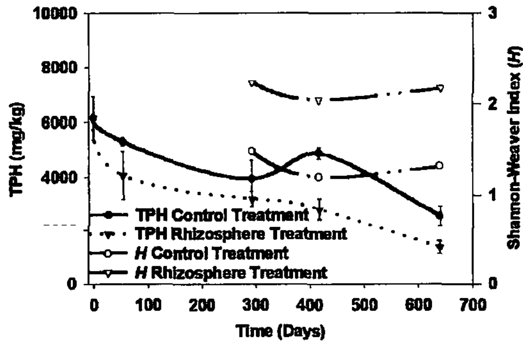

Figure 7 TPH and Shannon-Weaver Index for Bacteria in Crude-Oil-Contaminated Soil

4.0 Discussion and Conclusions

Microbial ecologists use the term diversity to indicate the heterogeneity of the microbial populations within a community occupying a given habitat (Hauxhurst *et al.*, 1981). Communities with low diversities tend to be relatively specialized which can be an indication of severe environmental stress (Hauxhurst *et al.*, 1981). When the microbial community is altered by stress, community structure changes as does the diversity of the community (Atlas, 1984). Generally, introduction of moderate to high levels of pollutants into the habitat results in decreased microbial diversity due to toxicity of the pollutant that eliminates sensitive species. This, in turn, reduces competition and results in enrichment of tolerant populations (Atlas, 1984; Mills and Wassel, 1980; Peele *et al.*, 1981).

Crude oil and gasoline contamination have been shown to reduce species diversity (Atlas, 1984). The greatest diversity reduction was noted in an Arctic tundra pond for the more toxic hydrocarbons found in gasoline where only one

species survived and proliferated. The crude oil amendment resulted in a gradual reduction of $\overline{H}$ = 4 to $\overline{H}$ = 2 over several weeks. The results indicated that petroleum hydrocarbons reduced microbial diversity and reflected fewer species, but increased numbers of metabolically specialized microorganisms.

In addition to diversity, microbial communities can be characterized by productivity (Atlas and Bartha, 1993). Greater diversity generally coincides with decreased productivity, reflecting the increased interactions and complexities of a mature community that has reduced productivity. Conversely, high productivity systems are more likely to be dominated by a few species and these selected species are likely to have more individuals that are highly productive. For contaminated soils undergoing phytoremediation, or for bioremediation in general, we may be able to use changes and stability of the microbial community structure to make inferences about the bioavailability of the remaining contaminants.

A challenge with using bioremediation is the residual contaminant that is slow to degrade. This results in contaminant concentrations that are increasingly recalcitrant, less bioavailable, or both and, when plotted against treatment time, become asymptotic to the time axis (Figure 8).

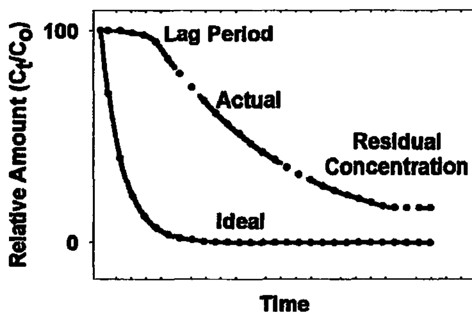

Figure 8 Idealized and Typical Concentration Changes during Biotreatment

Data for the diesel contaminated soil (Figures 4-5) suggest that, as remediation nears "completion," and biodegradable and bioavailable contaminant carbon is used—as indicated by the low TPH concentrations—bacterial diversity increases. This occurs in both the control and the rhizosphere treatments. However, in the control treatment, TPH concentration does not decrease to the extent that it does in the rhizosphere treatment, and bacterial diversity in the control treatment, expressed as either $\overline{H}$ or d, does not increase to the extent that it does in the rhizosphere treatment.

In the crude-oil-contaminated soil (Figures 6-7), contaminant concentrations remain higher and bacterial diversity is more stable than in the diesel-contaminated soil. These data suggest that the bacterial community in the crude-oil-contaminated soil is, in the terminology of Atlas and Bartha (1993), still both relatively productive and less diverse compared to the diesel-contaminated soil. The combination of higher TPH values and stable but relatively low bacterial diversity in the crude-oil-

contaminated soil suggests that some of the contaminant carbon in the control treatment is more bioavailable than in the rhizosphere treatment.

These data are encouraging in suggesting a means to evaluate "completeness" of bioremediation, but we caution that they represent only two soils and we have characterized only the bacterial component of these systems. The fungal component of most soils is generally believed to have significant contaminant degradation potential, but isolation and characterization are more difficult than for bacteria.

Measurement of microbial diversity, community structure, contaminant degrader activity, and frequency of degradative genes could be combined to enhance our understanding of remediation processes (Langworthy et al., 1998; Mills and Wassel, 1980; and Song and Bartha, 1990). An improved understanding of the time-dependent relationships between contaminant concentration changes and microbial community changes, coupled with improved techniques to readily characterize microbial communities, may provide a useful tool for monitoring the functioning of phytoremediation, evaluating desirable endpoints when bioavailable contaminants are diminished, or both.

5.0 Acknowledgments

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The Response of *Scirpus pungens* to Crude Oil Contaminated Sediments

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Abstract

The wetland plant *Scirpus pungens* is commonly found along the shores of the St. Lawrence River. This plant is of ecological importance as it provides a unique habitat for a diverse number of biota and is essential for the control of coastal erosion. The potential impact of an accidental oil spill on wetlands dominated by this species is unknown. To partially resolve this question, *Scirpus pungens* and sediments were recovered from a site near Ste. Croix, Quebec for an exposure study using medium-light crude oil contaminated sediments.

Transplants in oiled (range: 1.14 - 72.9 g/kg) and unoiled (control) sediments were maintained in a greenhouse facility to monitor time-series changes in plant height, new growth, and mortality over a sixty-three day period. While some growth was evident under all treatment conditions, significant differences in productivity were observed. Visual results showed that plants exposed to high concentrations of oiled sediment (36.5 - 72.9 g/kg) were considerably smaller than those exposed to control and/or lightly-contaminated sediments (<4.56 g/kg). Statistical analyses of weight and stem density data showed that elevated oil concentrations significantly decreased plant biomass. Mortality was significantly correlated to oil concentration and reached 87% in the highest concentration. Study results indicate that transplants of *Scirpus pungens* were able to survive, grow, and produce new shoots in sediments contaminated with crude oil in a range of concentrations comparable to those associated with oil spill incidents.

1.0 Introduction

Each year, millions of tonnes of petroleum products are transported on the St. Lawrence River, a sinuous and often shallow waterway that at times can be difficult to navigate at some points because of strong currents, rapids, tidal influences and ice. Hundreds of oil spills occur within this waterway each year, but fortunately the

majority are minor. However, given the volume of refined and crude products transported (18.7 million tonnes in 1988) and the capacity of tankers (160,000 tonnes) within the system, concerns over the environmental impact of a major spill cannot be discounted (Brander-Smith, 1990).

The St. Lawrence River is a fragile wetlands habitat, home to 140 rare plant species, 260 bird species, 240 fish species, 5 seal species and 18 species of whale (Brander-Smith, 1990). Vast areas of wetlands comprised of nearly pure stands of *Scirpus pungens* (formerly *Scirpus americanus*) are found along the shores of the river. These plants provide a unique habitat and food source for aquatic biota and migratory birds such as the Greater Snow Goose (*Chencaerulescens atlantica*). In addition, they mitigate coastal erosion by attenuating waves and tidal action, and by promoting the sedimentation and stabilisation of fine particles (Yang, 1998; Brouillet and Coursol, 1996; Giroux and Bédard, 1995 ; Bélanger and Bédard, 1994 ; Hill, 1973; Lemieux, 1973).

This study examined the sensitivity of *S. pungens* to oiled sediments to provide data on potential recovery rates of the species in the event of an accidental oil spill within the St. Lawrence River.

2.0 Materials and Methods

2.1 Plants

S. pungens regenerates rapidly by producing rhizomal clones; sexual reproduction accounts for less than 5% of its seedlings (Giroux & Bédard, 1995). Thus, for this experiment, individual culms of *S. pungens* were recovered in the summer of 1998 from a site near Ste. Croix de Lotibinière (46°37' N, 71°45' W) on the south shore of the St. Lawrence River. The plants were transplanted into Pro-mix BX® (Premier-Canada Limited), a commercial potting mixture formulated with peat moss and inorganic nutrients. After two weeks, healthy plants of 220 to 400 mm in length were selected for exposure to test sediments.

2.2 Sediment

Sediment from a wetland site adjacent to Ste. Croix de Lotbinière was also recovered for the experimental study. Analysis showed that this test sediment is composed of silt (60%), fine sand (35%) and clay (5%). A cement mixer was used to homogenise the sediment with nutrient amendments prior to use. Visible rocks and organic matter were removed manually and 7 L of homogenised test sediment were placed into each of 30 two-gallon high-density PVC buckets. Nutrient amendments in each test bucket were 0.913 g triple super phosphate (0-46-0, IMC-Agrico Inc., Canada) and 3.059 g prilled ammonium nitrate (34-0-0, J.R. Simplot Co., Canada).

2.3 Oil

A medium, sulphurous crude oil (MESA) was obtained from the Petro Canada refinery in East Montreal, Quebec, Canada. To simulate residual oil that reaches the shore following a spill incident, the oil was artificially weathered by aeration for 12 hours in a large Nalgene® container. Samples of the weathered oil characterised by GC/MS showed that weathering removed approximately 12% of the lighter, more volatile fractions.

2.4 Experimental Design

Nine treatment concentrations of the weathered crude in sediment were prepared (1.14, 2.28, 4.56, 9.12, 18.24, 27.35, 36.47, 54.71, and 72.94 g oil/kg) to cover an experimental range up to that of saturation. Each concentration was replicated three times (27 oil-treated buckets) along with three additional buckets containing sediment and nutrients only which served as controls. An Exomixer power drill attachment (A. Richard Ltd., Canada) was used to homogenise all treatment and control buckets after the application of the fertilisers and oil.

Six culms of *S. pungens* from which all root and rhizome material was trimmed to within 1.5cm of the base of the stalk, were weighed individually and hand transplanted into test sediments within buckets. The buckets were placed in a random block design within a cold-frame shelter that served as a greenhouse during the experiment. Water levels were kept at ≅1cm above the sediment surface throughout the 63-day experimental period (August 21 to October 21, 1998).

2.5 Data Collection

Changes in height of the six original *S. pungens* transplants were measured weekly throughout the first 57 days of the experiment. Four new shoots from each bucket were also chosen for measurement to represent growth rates of new emergent plants in each of the buckets. These plants were randomly selected based on pre-defined height (between 50 and 250 mm in order to allow for good growth potential) and health criteria (lack of parasitism and early mortality). The variability in growth and plant density between the different buckets reflects natural variability in the height of the new stalks selected for repeated measures. Measurements on new growth were recorded on Days 54, 57 and 60 of the experiment. Evidence of plant mortality was recorded once per week over 57 days for the original plants, and twice a week between Days 54 and 60 for new growth. Plants were considered dead when no green tissue was visible. For this reason, mortality might not have been recorded for several days or weeks following the stress period that caused mortality.

Other variables, measured regularly throughout the experiment were bucket weight (to estimate evapotranspiration), relative humidity, temperature and irradiance. Meteorological data was provided by Environment Canada's Environmental and Weather Services Offices because on-site measurement equipment failed.

At the end of the experiment, biomass was collected manually from all of the buckets. All samples were washed to remove residual sediment and the number shoots per bucket was recorded. Biomass was quantified by weight at the end of the experiment since abundant growth in each bucket made individual plant measurements impossible. Total biomass, above ground biomass, below ground biomass, root biomass and stem biomass were determined separately. After determining wet weight, the plants were dried at 105°C for 60 h to constant weight then recorded. Twenty seven representative *S. pungens* culms were chosen at the beginning of the experiment to calculate initial dry weight using the same procedure.

Data was analysed using SYSTAT® 8.0 (SPSS Inc.) for GLM repeated measures, regression analysis, ANOVA and t-tests. SAS® 6.12 was used to perform contrast analysis.

3.0 Results

3.1 Biomass

Using the one-way ANOVA model, no significant biomass ($F(9, 170) = 0.924$; $p = 0.506$) or height differences $F(8, 135) = 0.993$; $p = 0.448$) were found amongst the 30 buckets at the beginning of the experiment. Absolute variation in total plant biomass was from 49.69g/bucket dry weight in one control bucket and 7.33g/bucket for one of the most highly contaminated buckets. According to a paired t-test, biomass was significantly higher at the end of the experiment than at the beginning ($t = 21.996$; $p = 0.001$). Data from the end of the experiment was log transformed and fitted by linear regression, which indicated that increasing treatment levels had significant inhibitory effects on total dry biomass (Table 1, Figure 1).

Table 1 Descriptive Statistics and Regression Analysis for *Scirpus pungens* Biomass.

Parameter	N	Mean (g)	Standard deviation	r^2	t	p
Total dry weight (g)	30	24.11	11.26	0.799	-10.54	0.001
Above ground dry weight(g)	30	3.23	1.94	0.795	-10.42	0.001
Below ground dry weight (g)	30	10.44	4.74	0.787	-10.18	0.001
Root dry weight (g)	30	8.57	3.78	0.768	-9.63	0.001

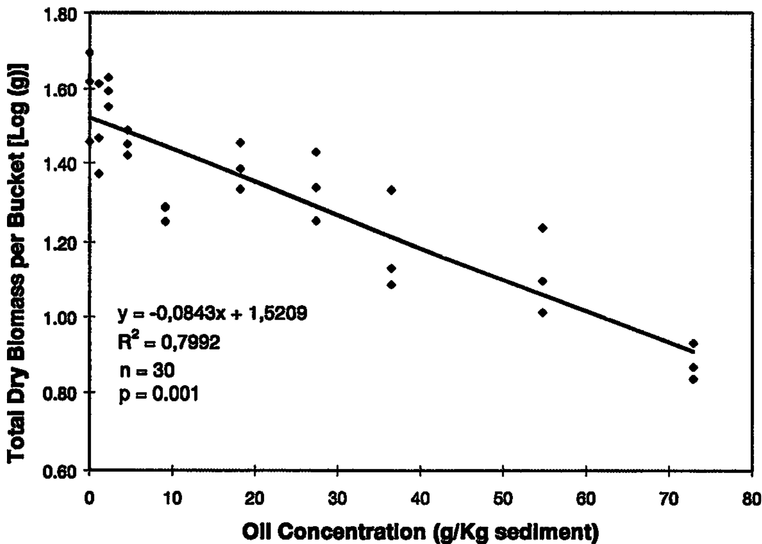

Figure 1 Total Dry Biomass for Each Treatment by Day-57.

One-way ANOVA with Tukey pairwise comparison test on log-transformed data determined where significant treatment differences lie and whether there was a trend in the biomass data (entire plant, above ground growth, etc.). Significant differences in plant biomass were observed between the unoiled control sediments

and those with low (<4.56 g/kg), and high oil concentrations (36.5 – 72.9 g/kg). Significant differences were also found between moderately oiled sediments (9.12 – 27.3 g/kg) and 72.9 g/kg (Table 2). These results were consistent for all of the partial and total biomass calculations.

Table 2 Tukey Pairwise Analysis of Differences of Total Dry Biomass at Treatment Oil Concentrations (g/kg).[1]

[Oil]	0	1.1	2.3	4.6	9.1	18.2	27.4	36.5	54.7	72.9
0	1.000									
1.1	0.791	1.000								
2.3	0.974	1.000	1.000							
4.6	0.480	1.000	0.982	1.000						
9.1	*0.014	0.342	0.139	0.647	1.000					
18.2	0.061	0.754	0.429	0.959	0.999	1.000				
27.4	0.005	0.169	0.060	0.389	1.000	0.973	1.000			
36.5	*0.001	*0.006	*0.002	*0.019	0.573	0.212	0.823	1.000		
54.7	*0.001	*0.001	*0.001	*0.004	0.197	0.052	0.388	0.999	1.000	
72.9	*0.001	*0.001	*0.001	*0.001	*0.004	*0.001	*0.011	0.262	0.678	1.000

[1] Significant Differences are marked with an asterisk.

3.2 Mortality

Based on the criteria for mortality, death due to transplantation shock would not be recorded for several days to weeks. For statistical analysis of oil-induced mortality, data was corrected for death due to initial transplantation shock. This was achieved by discounting from the data set all plants from the first four weeks of the experiment that were considered dead and randomly removing plants from other treatments so that all treatments would have an equal number of observations. Percent mortality increased with increasing oil concentration (Figure 2).

Mortality data from the last day of the experiment was also used in probit regression analysis. Data from one treatment (2.28 g oil/kg sediment) was removed because of 0% mortality which this statistical analysis cannot utilize. (Predicted mortality for this treatment was close to 7%). Results showed that increasing oil concentration correlated significantly with mortality (t-value = 5.188 ; p = 0.001). Treatment 9 (72.9 g oil/kg sediment) had the greatest mortality (87%), which was significantly different from all other treatments whose mortality rates varied from 0 - 33% with an average of 12%.

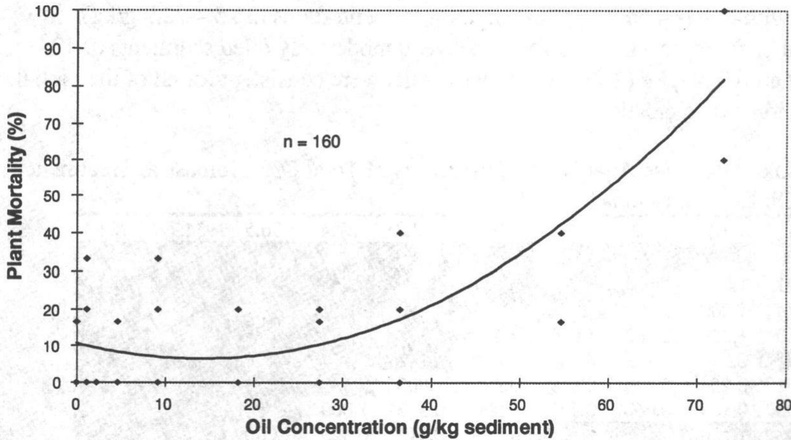

Figure 2 Mortality of Original Plants.

3.3 Stem Density

The number of stems per bucket varied from 25 to 62 with an average of 35 stalks and a standard deviation of 9.408 (Figure 3). Regression analysis performed on log transformed data showed that 47% of the variability in stem density was due to treatment effects ($r^2 = 0.470$; $p = 0.001$). Regression and one-way ANOVA analysis showed that the differences are significant (Table 3), while Tukey pairwise comparison showed there were significant differences between the control and treatments with oil concentrations of 54.7 and 72.9 g/kg sediment (Table 4).

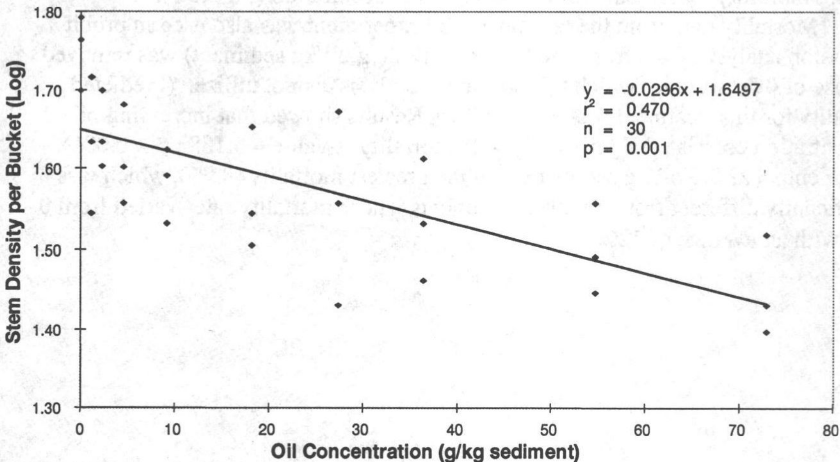

Figure 3 Stem Density per Treatment (Day-57).

Table 3 ANOVA Results for Comparison of *S. pungens* Stem Density.

Factor	Error df	df	MS	F	P
Stem density	20	9	0.114	3.814	0.006

Table 4 Tukey Pairwise Comparison of Significant Differences of *S. pungens* Stem Density.

	54.71 g/kg (P)	72.94 g/kg (P)
Control buckets	0.014	0.003

3.4 Plant Growth

The effects of increasing oil concentration on plant growth were readily apparent by visual observation (Figure 4). Growth appeared to be stimulated in the presence of oil at concentrations less than <4.56 g/kg sediment when compared to the growth of the control plants. At higher oil concentrations, up to 27.4 g/kg sediment, concentration dependent inhibitory effects were observed. Due to the shelter provided by the walls of the cold-frame structure, plants in the experimental buckets thrived past the natural senescence period for this species in mid September observed at the field site. At sediment oil concentrations above 36.4 g/kg sediment, growth and new shoot generation were significantly reduced. Major differences in plant density and vigour were visible in the most highly contaminated treatments.

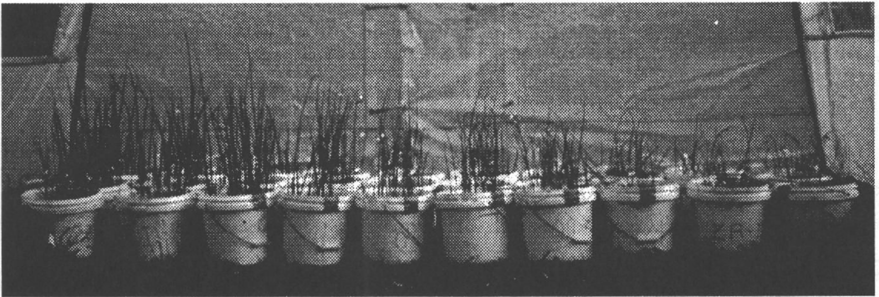

Figure 4 *S. pungens* at the End of the Experiment. Oil Concentration Increases from Left to Right.

The growth of the original 6 culms of *Scirpus pungens* and that of the 4 representative new shoots in each experimental bucket were analysed using the GLM repeated measures and polynomial contrasts. All treatments followed the same growth pattern throughout the experiment. Plant growth was evident over the first 4 weeks (Figure 5) at which point growth levelled off and increasing mortality was observed.

Statistical analysis of growth data for the original plants does not convey the same results as visual observations of the plants would imply. Although there were significant differences between the height and growth of plants due to treatment and block effects, no clear pattern emerged from the analysis. Growth of the transplants occurred over the first 4 weeks only, however significant height differences were observed throughout the experiment (probably due to mortality of plants). Contrast analysis of data from the first 4 weeks delineated significant differences between the control and treatments and between blocks. However no apparent trends could be distinguished (Table 5).

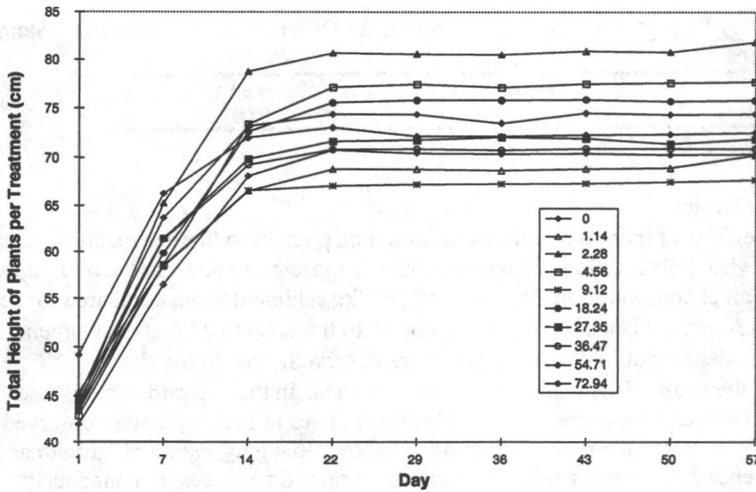

Figure 5 Growth Trends for the Original Plants in Each Treatment.

Table 5 Contrast Analysis on Original Plant Growth for First 22 Days.

Days of Contrast	Source	Treatments Compared (Corresponding P values for significant differences only)								
		0-1	**0-2**	**0-3**	**0-4**	**0-5**	**0-6**	**0-7**	**0-8**	**0-9**
7 - 1	Block 1	(0.008)								
7 - 1	Block 2		(0.002)						(0.002)	
7 - 1	Block 3									(0.051)
14 - 7	Block 1		(0.015)	(0.026)		(0.001)				
14 - 7	Block 2		(0.033)	(0.007)						
14 - 7	Block 3			(0.016)						
22 - 7	Block 1					(0.002)				
22 - 7	Block 2									
22 - 7	Block 3	(0.001)		(0.016)						

Representative data on the growth of new emergent plants was collected between Days 54 and 60 inclusive. Significant differences between the control and oiled treatments 5 and 7- 9 (18.24, and 36.5 – 72.9 g/kg sediment) were linked to the increasing oil concentrations of the treatments. Between Day-54 and Day-57 there was also a significant growth difference for these plants when grouped by treatment levels (Table 6). However no significant differences were evident between Day-57 and Day-60. In terms of differences in environmental test conditions, there is no evidence of a block effect or an interaction between treatments and blocks.

Height data for these specific dates showed significant treatment and block effects; however, the interaction of these 2 factors was not significant. For each of the three sample dates, control data is significantly different from experimental buckets containing sediments treated with 4.56, 27.4, 36.5 and 72.9 g oil/kg sediment respectively.

Table 6 Polynomial Contrasts of New Plant Growth by Experimental Date.

Source	Days of Contrast	df	MS	F	Pr > F
A vs B	60 - 54	1	394.8	8.60	0.0045
A vs C	60 - 54	1	1121.6	24.42	0.0001
B vs C	60 - 54	1	216.6	4.72	0.0331
0 vs 5	60 - 54	1	330.0	7.18	0.0091
0 vs 7	60 - 54	1	302.3	6.58	0.0123
0 vs 8	60 - 54	1	784.2	17.07	0.0001
0 vs 9	60 - 54	1	255.0	5.55	0.0211
A vs B	57 - 54	1	677.6	6.34	0.0140
A vs C	57 - 54	1	748.0	7.00	0.0100

A = treatments 1, 2 and 3 (1.14, 2.28, 4.56 g/kg sediment)
B = treatments 4, 5, and 6 (9.12, 18.24, 27.35 g/kg sediment)
C = treatments 7, 8, and 9 (36.47, 54.71, 72.94 g/kg sediment)
0 = control (0 g/kg sediment)

4.0 Discussion

As biomass was not significantly different between buckets at the beginning of the experiment it was assumed that all buckets initially contained similar biomass and that intertreatment differences at the end of the experiment were due to differences in independent variables. Analysis of dry biomass showed that there was significant growth over the course of the experiment for all of the oil treatments which was consistent with visual records. In summary, healthy culms of *Scirpus pungens* recovered from the shores of the St. Lawrence River were able to survive transplant shock and grow in sediments contaminated with a medium sulphurous crude oil at concentrations as high as 72.9 g/kg.

Analysis of dry weight at the end of the experimental period (Day-63) showed that there were significant differences in the extent of plant growth in response to oil-induced stress. These results were consistent with visual data and analyses of biomass. Plants were not significantly affected by low concentrations of oil in the sediment. No data were found in the literature for the response of *Scirpus pungens* to different degrees of oiling, but our data are consistent with results for *Spartina alterniflora*, which was not affected by oil applications of <5 g/kg of medium and light crude oils (Alexander and Webb, 1987). Heavily-contaminated sediment produced significantly less biomass than did lightly- and moderately-contaminated sediment, and all parts of the plant were affected similarly. Oiling reduced plant growth and impacted the photosynthetic capacity of the plants. This was apparent in the form of a brownish mottling effect on the stem of plants subjected to the 3 highest concentrations (36.5 – 72.9 g oil/kg sediment). Pezeshki *et al.* (1998) have noted significantly decreased stomatal conductances in *Scirpus olneyi* subjected to two crude oils. Important photosynthetic decreases were also noted by Lin and Mendelssohn (1996) in *Spartina patens* exposed to moderate (4 L/m^2) contamination with a Louisiana crude oil in a bucket experiment.

It is clearly evident, both visually and statistically, that the oil within sediments was responsible for reducing stem density. Our observations of stem density did not take into consideration the size of the stalks, their state of emergence or their viability. Had all or only new emergent stems been considered for analysis, pairwise analysis may have revealed more significant differences. Previous studies

on plant response to oiling (Lin and Mendelssohn, 1996) have shown highly significant treatment effects on the stem density when only above ground biomass was analysed. Due to logistical constraints, all new shoots were not analysed in this experiment. Only four shoots could be chosen for this analysis because there were not enough healthy individuals of similar size in the heavily-oiled treatments to allow a larger sampling effort.

Plants in lightly oiled sediments appeared in good health. Indeed plants in treatments of 1.14 and 2.28 g oil/kg sediment seem to have benefited from mild oil applications, having increased plant height compared with controls. Plants in moderately contaminated sediment (9.12 – 27.35 g oil/kg) were shorter and appeared to be less vigorous than control and lightly oiled (<4.56 g/kg) specimens. Heavily contaminated sediments appeared to allow only limited shoot generation and contained less vigorous plants in comparison with all other treatments. Inhibitory effects on metabolic processes were evident in the form of a brown mottling of their stems after two weeks of exposure.

Statistical analysis may be unable to resolve fine differences in treatment effects perhaps because all plants were not measured for growth throughout the experiment. Considering the high individual variability among the six culms per experimental bucket, it was not surprising that statistical analysis did not identify clear changes in plant height and growth trends. Furthermore, the database limitation was magnified in the probit analysis that discounted all data from one treatment that exhibited 0% mortality and in contrast analysis which discounted all dead plants. This limited the predictive power of the statistical results, as the plants experiencing the greatest response were not considered in the analyses.

Better representation of population effects might have been achieved by measuring all plants in each treatment. Increasing concentrations of crude oil in sediments resulted in significantly reduced biomass.

In support of visual findings, data suggested a negative effect of oiled sediments on the growth of new shoots. However, due to the limited collection period (Day-54 to Day-60), the statistical results cannot accurately represent long-term trends. Had data been collected over a longer period and on all emergent shoots, significant trends might have been evident.

Over the experimental period of 57 days, *Scirpus pungens* was capable of surviving and producing some new growth in sediments contaminated with crude oil at concentrations up to 72.9 g/kg sediment. This was above the 1% level of contamination usually found after an oil spill (Sergy *et al.*, 1998).

In the event of an actual oil spill, vegetation within a wetland can also be severely damaged if aerial parts come in contact with the oil. This scenario was not evaluated here; the reported tolerance of *Scirpus pungens* to oil only applies to oiled-sediment exposure. Nevertheless, the results obtained provide evidence that transplants may be used to accelerate natural recovery in wetlands impacted by an accidental oil spill. This activity would limit erosion and may accelerate the natural biodegradation rate of oil. *Scirpus pungens* can aerate marsh sediments by way of its lacunae (aerenchyma) that allow it to transport oxygen from the atmosphere and its photosynthetic parts to its rhizomes and roots within the sediment. In nature, optimal oil degradation rates are often limited by nutrient and oxygen availability (Lee and Levy, 1989; 1991). Furthermore, the rhizosphere may provide stimulatory growth

substances (plant exudates) to the indigenous microbiota as well as an improved physical habitat. Vegetative transplantation has been used in terrestrial environments for the cleanup of hazardous wastes (Schnoor *et al.*, 1995) including polycyclic aromatic hydrocarbons (Banks and Schwab, 1993). While this process, described as phytoremediation, has not been used as a marine oil spill countermeasure, recent greenhouse studies with wetland plants (*Spartina* sp.) showed that the oil degradation rate in sediments was significantly enhanced by the application of fertiliser in conjunction with the presence of transplants (Lin and Mendelssohn, 1998). Phytoremediation of oil spills with *Scirpus pungens* will be evaluated in a future study.

5.0 Conclusions

Visual findings, biomass, stem density and mortality indicate that oil contaminated sediments will impact the growth of transplanted culms of *Scirpus pungens*. However, within the concentration range reported for coastal oil spills, *Scirpus pungens* will likely survive. In the event of an accidental oil spill along the St. Lawrence River, restoration of wetland habitats may be accelerated by the transplantation of *Scirpus pungens*. This countermeasure will help prevent erosion of the wetland sediments and may accelerate the degradation rates of stranded residual oil.

6.0 Acknowledgements

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Field Scale Prototype Anaerobic/Wetlands Cells For Removing Heavy Metals from Water

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Abstract

A passive treatment system utilizing an anaerobic digester followed by three plant based treatment cells has been built in Trail, British Columbia, site of the world's largest non-ferrous smelter. Water becomes contaminated with heavy metals as it passes through discarded materials from the smelter and through a former Arsenic storage area. This water, containing large amounts of dissolved Zn, Cd, and As is collected and pumped to the pilot scale treatment facility.

The facility, capable of treating 12-15,000 L of water each day includes a large anaerobic digester that utilizes waste by-product from the pulp and paper industry as a matrix for Sulphur Reducing Bacteria. The partially treated water then flows through a series of hydroponics cells containing a mixture of metal-resistant fast growing plants. The system uses gravity based hydroponics flow-through and solar powered aeration cells between garden cells.

During the summer months transpiration will yield expected (or greater) results. All plants are perennials and after two months of operation there were no signs of impaired plant functioning.

1.0 Site Problem

A historical vegetation-capped landfill, near the Cominco smelter in Trail BC, produces a leachate that contains toxic metals. Prior to 1997, rainwater percolating through the landfill dissolved metals and traveled downwards through an extensive layer of sand until bedrock was reached. At this point it moved laterally, eventually making its way to Stoney Creek and into the Columbia River. In 1996, Cominco Ltd. hired Klohn-Crippen, an engineering consulting firm to characterize the seepage flows and geohydrology of the Stoney Creek basin. They used the characterization study to design and build a seepage collection system in 1997. This collection system funneled the seepage water into a system of collection sumps. From the final sump it is pumped to a lime-based Effluent Treatment Plant.

Table 1 Approximate Total Volume of Water and Metal Contaminants Entering Stoney Creek Daily From Landfill site and Arsenic Storage Area.

Total Volume/Day	Containing Following Heavy Metals (ppm)			
	Arsenic	Cadmium	Zinc	Lead
77,000 L	45	3.64	205	0.056

Table 1 shows the average amount of water that enters Stoney Creek daily after flowing downwards through the now-capped land fill site. While passing through discarded material from the landfill it dissolves metals including Zinc, Cadmium and trace amounts of other metals. Arsenic entered the creek from a storage area on the

other side of the valley. To arrive at the figures in the table volumes from separate seeps have been combined and amounts calculated to reflect metal contaminant based on percentage of total volume.

To evaluate an alternative treatment technology that could be built near the seeps, Northern Water, Environment & Training Services was contracted by Cominco Ltd. to design and build a prototype phytoremediation treatment system.

2.0 System Design and Construction

During the summer of 1997 a series of self-contained sub-surface flow wetland cells were constructed on a Cominco property. The property was ideal topographically and, in addition, had a stream of clean water that ran year round. A local contractor, Jim Hall, was hired to build three treatment cells and two aeration cells. The cells were designed and built using typical constructed wetland techniques (Kadlec and Knight, 1996).

Cells were planted with fast growing plants and kept watered using the clean water from the local stream for the first year while root growth was established. The first cell is 50 m^2 and includes grasses, selected shrubs and perennials and several species of hybrid poplar and willow. The second cell, also 50 m^2, contains several species of grasses some of which are indigenous to the immediate area. The third treatment cell, 300 m^2 is planted with *Typha latifolia*. A final large lined holding pond was built as the final stage in the system. Water is held there for final testing before being delivered back to the environment. Figure 1 shows a plan of the completed system. Construction of the plant cells was completed during the summer of 1997 and the anaerobic cell in the spring of 1998.

Oxygen depletion during treatment in each cell was an important system design consideration. Accordingly, a 6 m^2 aeration cell was built between cells one and two and two and three. These cells serve as the location for a control system to set water level in the preceding cell and they are convenient sample points for testing purposes.

3.0 Bench-scale Testing

With the co-operation of Dr. W. Rauser and Dr. B Husband at the University of Guelph, space was secured in the greenhouse for bench scale testing. Models of the system as built in Trail were constructed and planted with the species being investigated. Large 77 L containers, each containing one or more of the metals found as contaminants in the leachate were used to provide water at a rate that corresponded to that planned for the field-scale system. Three systems were exposed to solutions containing 5, 10 and 40% of the metals present in the leachate as well as a control system using deionized water.

This greenhouse research showed that the metal levels in full-strength leachate were too high for the chosen plants. Metal levels would need to be reduced by 50 - 90% if complete treatment of the leachate by wetlands were to be successful. For experimental purposes both dilution and pre-treatment using an anaerobic digester were considered. The second option was chosen as it would provide complete treatment and allow the pilot plant to be scaled up to treat the seepages in situ.

During the same period Cominco Research personnel examined the efficacy of various biomass materials to remove metals in an anaerobic environment. This work showed that as much as 90% of the metals present in the leachate could be removed using a locally available residual biomass product from a nearby pulp mill. Therefore,

during the early summer of 1998 a large, 500 m3 anaerobic digester was built to provide a complete treatment system.

4.0 Design of Anaerobic Cell

Three main factors are crucial to the design and sizing of an anaerobic cell. These include the volume of water to be treated, the concentration of metals present and the treatment area required to do so, and the composition of the organic biomass.

The potential to treat 20,000 L per day (13.9 L/min) was used to estimate cell area. The volume sized 'rule of thumb' used in calculation based on metal concentrations is the removal of 0.3 mol/(m^3d) of metal where the volume component is the total volume neglecting the pore space and moisture content (Dvorak et al, 1991; Hedin et al, 1989; Gusek and Wildeman, 1997). Zinc content can range up to 500 mg/L and Cd. up to 10 mg/L and As up to 45 mg/L. Therefore:

$$\text{Cell Volume} = (155 \text{ mol/d})/((0.3 \text{ mol/(m}^3\text{d})) = 517 \text{ m}^3 \quad (1)$$

While the area based 'rule of thumb' is estimated to be between 10 m^2min/L and 20 m^2min/L (Gusek and Wildeman, 1997). This factor is pH dependent in the range of 5-7 with higher pH values requiring lower loading factors. Therefore:

$$\text{Cell Area} = (20\text{m}^2\text{min/L}) \text{ X } (13.9 \text{ L/min}) = 278 \text{ m}^2 \quad (2)$$

The composition of biomass used was 60% pulp mill residuals, 35% sand and 5% cow manure.

Based on these parameters and allowing for adjustments due to site characteristics a cell was constructed that was 24 m x 18 m at the top with sides that sloped to a bottom area that was 18 m x 10 m. The total depth was 3.5 meters. The retention time was increased by the addition of two 400 m^2 baffles that divided the biological treatment medium into three equal layers with a flow through gap at opposite ends of each layer. Water to be treated enters at bottom of cell and is discharged at the top.

5.0 Results

Late in the 1998 growing season the construction work had been completed and all necessary pipelines laid. Metal contaminated water was then pumped into the anaerobic and following cells and allowed to remain for several days to allow for a period of stabilization and a first flush of the dissolved organic material. After a few days grab samples were then taken from (Figure 1):

- A valve stem installed at the top of the vertical header that delivers water to the bottom of the anaerobic cell. This provided the raw sample of contaminated water.
- From the outflow of this cell as it enters a specially constructed small aeration cell,
- At the outlet of each of the three subsequent plant based treatment cells.

An initial set of samples was taken August 9th, followed by further samples in late August, late September and early October. In total in 1998, four sets of samples (09/08/98, 24/08/98, 22/09/98, 02/10/98) each including five points were sampled and assayed using ICP/AES for the three metals of interest, Zn, Cd, and As. Table 2

shows the results of assays completed on the water samples indicating the levels found after passage through each cell and an indication of the percentage removed from each cell. This last figure is based on the metal content of the water that enters the cell and shows the effectiveness of each cell in removing that element.

Table 2 Zinc, Cadmium & Arsenic (mg/l) in Each Stage of a Four-Stage Biologically Based Treatment System. Percent Removed Represents the Percentage of Dissolved Metal Entering the Cell that was Removed During Passage Through that Cell.

Date Stage	Zinc	% removed	Cadmium	% removed	Arsenic	% removed
09/08/98 1st input	*130*		*3.6*		*45*	
output to trees	78	40.00	0.43	88.00	8.1	82.00
output to grass	7.9	89.90	0.3	30.23	1.5	81.48
output to *Typha*	4.3	45.57	0.18	40.00	0.62	58.66
output to pond	0.12	97.21	0.01	94.40	0.05	91.90
% removed from all stages	*99.90*		*99.70*		*99.89*	
24/08/98 1st input	*205*		*3.4*		*42.0*	
output to trees	78	61.95	0.45	86.76	9.5	77.38
output to grass	31	60.26	0.36	20.00	5.0	47.37
output to *Typha*	24	22.58	0.29	19.44	3.4	32.00
output to pond	3.9	59.11	0.1	65.52	0.79	76.76
% removed from all stages	*98.09*		*97.06*		*98.12*	
22/09/98 1st input	*140*		*2.3*		*23*	
output to trees	88	37.14	0.16	93.04	8.2	64.35
output to grass	74	15.90	0.14	12.5	8.4	(+2.3)
output to *Typha*	1.9	97.43	0.04	71.42	1.5	82.14
output to pond	0.38	80.00	0.02	50.00	0.48	68.00
% removed from all stages	*99.72*		*99.1*		*97.91*	
02/10/98 1st input	*75*		*1.0*		*17*	
output to trees	72	4.00	0.21	79.00	8.0	52.94
output to grass	50	30.55	0.11	47.62	5.8	27.50
output to *Typha*	0.56	98.88	0.02	81.82	0.82	85.86
output to pond	0.54	3.57	0.01	50.00	0.56	31.70
% removed from all stages	*99.28*		*99.00*		*96.71*	

Figures 2, 3 and 4 graphically illustrate the nature of the reduction in metal presence that takes place. For each metal of concern it shows that the largest proportion of the removal process is completed in the first cell but that removal does continue as the water passes through each of the following cells. The initial value is the concentration of a metal in the untreated water as it flows into the anaerobic cell. The 2nd value is the output of the anaerobic cell/input to the tree cell; 3rd value is the output of the tree cell/input to the grass cell; 4th value is the output of the grass cell/input to the *Typha cell.* The final value is output of *Typha* cell, which is the input into the holding cell.

The system was designed to treat 10-15,000 liters a day during the summer months when plants are growing most rapidly and using water and nutrients. Unfortunately contaminated water was not introduced until relatively late in the growing season giving less than a full season's results. As a consequence the results shown in Figures 2,3 & 4 and Table 2 are considered as preliminary. In 1999, a full growing season trial is planned.

5.1 Metal Speciation

The system design included a layer of non-woven geotextile laid down over the biosolids mixture and a layer of sand on top of that, however, a certain amount of metal sulphides were expected to move through this filtering mechanism into the plant containing cells. A filtering mechanism had been included at the outlet of each of the cells before water exited to the aeration units. The system had been designed with an understanding that anaerobic activity would take place in all of the cells. As a result of this activity we anticipated that additional metal sulphides would be formed. This did indeed occur, and the results are evident when examining the continuous reduction of metals in the water as it flows through each stage of the system. However, since samples were taken from aeration cells and from the exit of the anaerobic cell some metal sulphides were included in each sample. Therefore, some of the dissolved metals measured present in the cells could be metal sulphides as assays did not differentiate between dissolved and insoluble colloidal metals.

5.2 Volume Treated

Setting flow rates was a trial and error procedure. Since the metal-containing water was being introduced for the first time the system was initially kept at less than 50% potential treatment volume. This ensured that a steady state anaerobic condition was attained in all cells and that plants would not be flooded with concentrations of metals that were not sustainable to life.

Table 3 Volume Treated (L/d) Measured as Output of the Anaerobic Cell

Date	Volume leaving anaerobic cell
12/09/98	10,944
13/09/98	12,470
14/09/98	13,252
15/09/98	17,855
16/09/98	13,828
17/09/98	13,603
18/09/98	13,358
19/09/98	14,842
20/09/98	13,714
21/09/98	6,768
22/09/98 *	13,896
23/09/98	14,592
24/09/98	14,784
25/09/98	8,304
26/09/98	8,640
27/09/98	8,640
28/09/98	12,144
29/09/98	12,638
30/09/98	13,171
Average (19 days)	12,497

* Note that there were samples taken for assay during the time frame described in this chart as well as one immediately following this time period on October 2nd.

The average flow through during this period is what is expected during operations throughout the summer months. Flow through was markedly lower on four

days when levels in the anaerobic cell had dropped below the threshold required to maintain adequate flow. The anaerobic cell is charged over a 1-2 day period that supplies about 1 week for the other downstream cells.

Following this period the system was once again slowed down as plants were well advanced into senescence and transpiration had stopped or severely slowed down.

6.0 Future Considerations

We are encouraged by the results obtained to date and are looking forward to a full growing season of operations. We expect to begin operations this spring as soon as snow cover has gone, plants have begun to emerge and leaf buds have broken. Additional tree material will be planted and the system checked against over-wintering damage. Solar powered aeration units will be re-installed in the aeration cells and augmented as required.

A complete season's operations will provide greater understanding of the systems potential and, at the same time, ensure a detailed record of operation values throughout spring, summer and fall.

Now that the system is fully planted and plants have reached a mature level of root growth, additional procedures will be implemented that will ensure a more detailed testing of many facets of the system's operating parameters.

This season we will:

- Plant a tree cover on landfill and install measuring equipment to monitor changes in water flow through
- Investigate metal speciation in the anaerobic cell with particular reference to arsenic;
- Research passive filters to remove metal sulphides from suspension;
- Assess each plant species ability to sequester metals;
- Install flow meters at inlets and outlets of the three tiered wetlands system to monitor treatment rates more thoroughly;
- Determine the most efficient residence time in the anaerobic digester while still ensuring adequate flow rates.

An important aspect of the work this summer will be to begin to understand the life expectancy of the system. Core samples of the biological residue in the anaerobic cell will be taken and analyzed to determine levels of metal sulphide build-up and amounts of carbon and sulphur remaining.

Future work to determine winter treatment rates is required to develop a year-round treatment system. This is desirable as the seeps flow year-round. However, this winter work is dependent on the success of the summer trials and will require additional capital expenditure.

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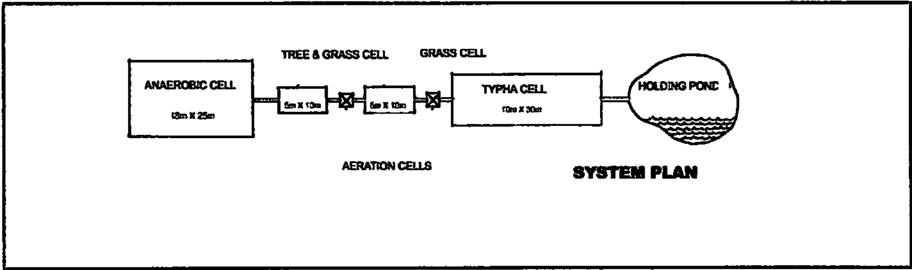

Figure 1 Plan of Prototype System as Installed in Trail British Columbia for Removal of Heavy Metals from Landfill Leachate. Water Enters System at Left of Anaerobic Cell and Flows Downhill through each Cell to Holding Pond. Collection Points are at the Exit from each Cell.

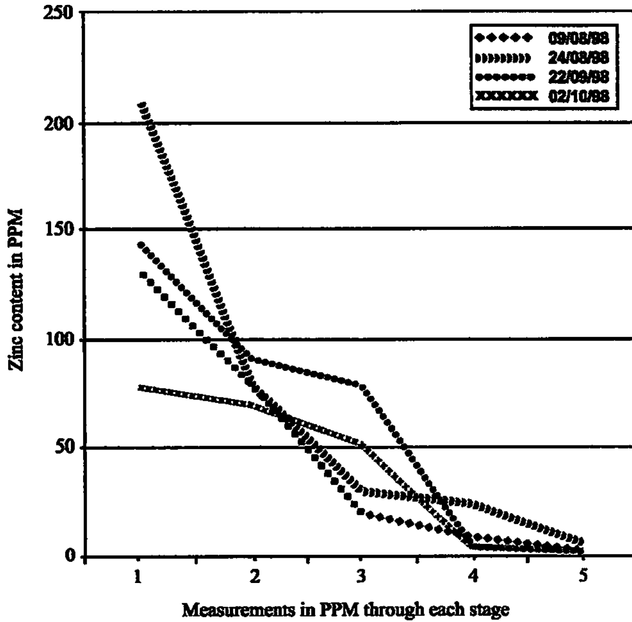

Figure 2 Zinc Reduction in Four-Stage Biological Remediation System

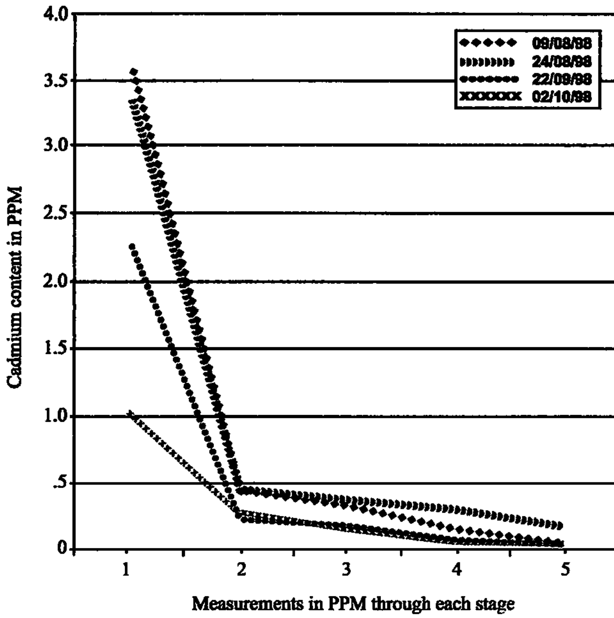

Figure 3 Cadmium Reduction in Four-Stage Biological Remediation System

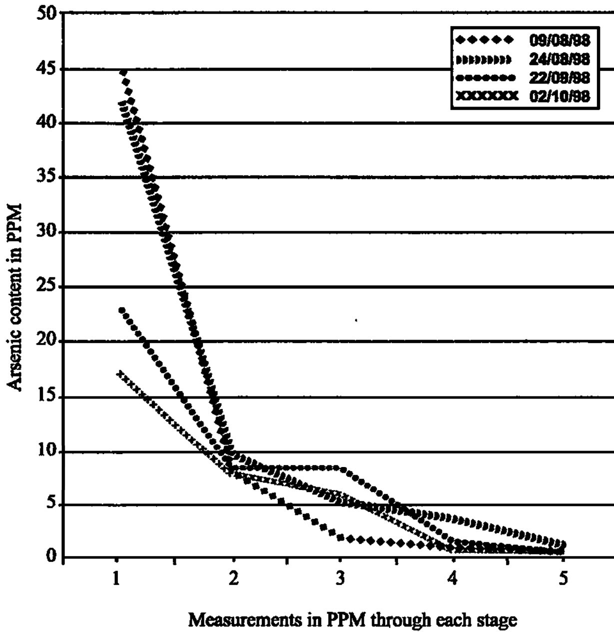

Figure 4 Arsenic Reduction in Four-Stage Biological Remediation System

Preliminary Report and Design of Using Jack Pines for the Phytoremediation of Diesel-contaminated Soils in Northern Saskatchewan

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Abstract

The purpose of this study is to evaluate the viability of Jack Pines (*Pinus banksiana*) for the phytoremediation of diesel-contaminated soils in Northern Saskatchewan. Monitoring of these sites of concern showed that the diesel concentrations ranged from 0 ppm to 30,000 ppm. Jack Pine is an ideal species to use for the revegetation and final clean up of these sites because it is native to the region and is closely related to pines that have the ability to stimulate degradation of hydrocarbons. The first phase of this study is to establish and evaluate Jack Pine seedlings in a greenhouse setting, thereby providing a controlled environment and continuous growth for accelerated results. If preliminary results in Phase 1 indicate seedling establishment is likely to succeed, plantings will be done on site to evaluate the species ability to grow in diesel-contaminated soils and the effectiveness with which these seedlings can enhance microbial degradation of diesel under natural, dynamic conditions. This report will give design criteria for the study and present preliminary results.

1.0 Introduction

One of the prices of an industrialized society is the production of potentially hazardous waste products, and the difficulties faced in the safe and economical disposal of these products. There is an enormous need for efficient, adaptable technology to clean up soil or water that has been contaminated by hazardous substances (Reisinger, 1995).

Phytoremediation, the use of plants to reclaim contaminated soils or water, is a relatively new technology within the remediation industry (Adler, 1996). Plant growth contributes to remediation of hydrocarbons primarily by stimulating the activity of soil microbes. Transpiration or direct metabolic breakdown of the hydrocarbon by the plants may also occur. In addition, plants encourage soil water to flow towards the root zone, thus drawing the contaminants towards the area of greatest microbial activity. Figure 1 is an illustration of the processes involved in the phytoremediation of hydrocarbons (Cunningham and Berti, 1993). At the same time plants also prevent wind and water erosion, and decrease the risk of groundwater contamination by trapping surface water (Reisinger, 1995). Phytoremediation is easier and cheaper than conventional methods of cleaning-up contaminated sites, and can potentially be applied to almost any situation.

SaskTel, Saskatchewan's telecommunications corporation, has several radio tower sites in northern Saskatchewan that have some level of diesel contamination from spills associated with their generator systems. To comply with provincial environmental regulations, SaskTel must ensure that the soil hydrocarbon concentrations of these contaminated sites meet the accepted risk guidelines. SaskTel has begun to decommission these sites by enhancing the microbial degradation of the

diesel in the topsoil through the addition of fertilizers. In order to degrade subsoil hydrocarbons, bioventing wells have been installed. Following the decommissioning phase, long-term reclamation will continue, relying heavily on the natural microbial degradation of the hydrocarbons. Phytoremediation, which is believed to aid in the speed and effectiveness of soil recovery, will be used in conjunction with this natural microbial degradation.

Several species of pine (*Pinus* spp.) have been used in phytoremediation, and at least one species has been found to stimulate degradation of hydrocarbons (Looney, 1998). It is reasonable to expect that Jack Pine (*Pinus banksiana*), which is native to northern Saskatchewan, would be effective for phytoremediation of diesel. If so, the vegetation established for phytoremediation could also serve as part of the final revegetation plan for the decommissioned sites.

2.0 Study Area

The first phase of this study is being conducted under controlled conditions, in the Shand Greenhouse located in Estevan, SK.

Phase 2, the field trials, will be conducted on various remote radio tower sites in Northern Saskatchewan. Monitoring of these sites has shown diesel concentrations ranging from 0 ppm to 30,000 ppm.

3.0 Methods

Seedling establishment is being evaluated in the Shand Greenhouse, to provide a controlled environment for the experiment, and continuous growth for accelerated results. The experiment consists of:

- plantings of seedlings in sand with a range of diesel concentrations;
- a control planting of seedlings in sand with no diesel, for comparison of seedling response; and
- control pots of sand with diesel and no seedlings, for comparison of diesel degradation.

3.1 Procedures for Soil Spiking

Fifty litres of playground sand was obtained from a local landscaper. It was sterilized to prevent unwanted microbial breakdown of the introduced diesel. Diesel (8.65 litres) purchased from a local gas station was mixed into the sand. The contaminated sand was stored in plastic containers with a porous base, which fed into a collecting pan. The containers were stored in a well-ventilated area to allow for volatilization and drainage of the surplus diesel. The sand was watered and mixed at regular intervals. Soil and effluent were then tested for BTEX (benzene, toluene, ethylbenzene, and xylenes) and TPH (total petroleum hydrocarbons) levels to ensure their levels did not exceed environmental regulatory guidelines. Dilution ratios were calculated to obtain the desired volumes and concentrations for the growing medium. After the soil spiking procedure was completed, the contaminated soil was transported to the Shand Greenhouse where the planting of the Jack Pines took place.

3.2 Planting

Seedlings were planted at approximately the same stage of growth as those that would be planted on the sites. They were planted in 2 litre pots large enough to accommodate two years of growth. Planting medium was playground quality sand,

neither sterilized nor inoculated. Diesel-treated medium was combined with varying amounts of clean medium to create 5 different concentration levels, plus a control with no diesel contamination. As well, pots of soil with the control and the 5 varying concentrations were created, but no seedlings were planted in them in order to see how the natural degradation of the diesel compares to the seedling assisted breakdown of the hydrocarbons. Soil samples were taken from each of the five concentrations and tested for TPH and BTEX.

Shand Greenhouse associates created the box to house the seedlings, which is shown in a schematic drawing (Figure 2). The box is seated on an angle iron frame with adjustable legs. The back, top, front, and ends of the box itself are made of a plexiglass. The bottom of the box overlaps with the rest of the box and is bolted at the plexiglass frame. The front plexiglass slides up and is held by a pin, enabling staff members to easily care for and observe plant growth and health. A fan is mounted at the top end of the last piece on the right of the entire structure and acts as the ventilation system within the structure. As well, a drainage canal is featured along the front end of the structure, which collects the runoff from the Jack Pines after each watering. This effluent has been collected into a large container and will be tested for BTEX and TPH at the end of the first year's growth, to ensure that it can be released safely into the greenhouse's sewer system.

Pots were placed in three blocks within the box, according to a randomized block design (Figure 3). Each block contains three replicates of all diesel concentration classes, as well as the controls.

3.3 Monitoring and Care of Seedlings

Green house staff monitor the survival, growth, and vigour of plants daily in the various treatments. Observations such as aberrations from the norm, poor seedling health, seedling deaths, and notable differences between the sets are recorded. A representative soil moisture reading is taken with the use of a water meter. Watering is conducted as needed according to these results. If the water meter has determined that the seedlings do require watering, a fertilizer mixture of nitrogen (20-10-20 at 100 g/L of nitrogen), Epson salts, and calcium nitrate (15-01-15 at 100g/L of nitrogen and 76 g/L of calcium) is added to the irrigation system that is used to water the Jack Pine seedlings.

3.4 Monitoring of the Effluent

Irrigation of the plants as per the Greenhouse's typical watering regime for tree seedlings is expected to result in low concentrations of hydrocarbons being leached with the irrigation water. Effluent tests of the diesel sand mixture were conducted after the initiation of the program. During watering of the soil and plants, two 250 mL sample jars were used to collect water from the midstream of the water flow. The samples were then sent to Enviro-Test Labs for BTEX and TPH analysis. Another testing of the effluent will be done at the end of the first year's growth to ensure that the BTEX and TPH levels are well below the acceptable risk guidelines.

3.5 Soil and Plant Sampling

At the end of the first year's growth, plant biomass calculations and soil hydrocarbon concentrations will be measured. Two composite soil samples from each of the concentrations, including the controls, will be gathered in 250 mL glass

jars and sent for analysis. The plant biomass calculations will be done by collecting a sample of the Jack Pines growing in each concentration. The plants will be separated into roots, stems, and leaves, then dried and weighed. Soil and plant biomass sampling will be performed a second time, at the end of the second year of growth.

4.0 Results

4.1 Soil and Effluent Chemistry

The results of the effluent testing following the planting are illustrated in Table 1. Results of these tests were compared to the CCME (Canadian Council Ministers of the Environment) guidelines for the protection of aquatic life and the Canadian Water Quality Guidelines for recreation. To date, the levels of hydrocarbon in the effluent have been well below the acceptable risk guidelines and standards set by the CCME for the protection of aquatic life and the Canadian Water Quality Guidelines for recreation (no visible sheen, discoloration or noticeable odour). It is expected that the levels of BTEX and TPH in the effluent will continue to decrease over time. However, testing of the effluent will be completed at the end of the first year's growth in August of 1999 as well as at the end of the experiment in August of 2000.

Table 2 illustrates the BTEX and TPH levels of the soil, as well as the concentration levels. These results will be compared with the second test results to see if the use of the seedlings has increased the breakdown of the hydrocarbons. Results for soil and effluent testing are very preliminary at point.

4.2 Seedling Growth

As phase 1 is only partially completed, results of this study are still very preliminary. The seedlings have been monitored daily by greenhouse staff. The plants were placed in a dormancy period from Dec.05, 1998 to Jan.07, 1999. The first seedling death was recorded Sept.17/98, one month after the seedlings were planted. By March 10th, 32 of the original 54 seedlings (59.3%) were still alive. Most of the seedling deaths that did occur happened in the month of January. There is no apparent pattern to the deaths regarding hydrocarbon concentrations as seedlings are surviving in all concentration classes. Seedling survivorship by location and concentration is indicated in Figure 4.

There is one clear pattern of seedling growth. All but one of the seedlings grown in uncontaminated soil are dead. It is speculated that the pure sand soil the Jack Pine are growing in could not hold the nutrients added by the fertilizer, and the plants could not obtain the compounds they needed for survival.

Location within the house may also be having a slight influence on seedling growth and development. The average height of seedlings in block one is 16.3 cm ($\pm$ 2.46), 21.9 cm ($\pm$ 4.91) in block two and 23.0 cm ($\pm$ 4.15) in block three. This indicates perhaps that the closer the seedlings are to the natural sunlight and ventilation system, the better they are growing. Figure 5 illustrates seedling height by location and concentration.

5.0 Conclusions

Phase one of this study is to establish and evaluate Jack Pine seedlings in a greenhouse setting. From the results of the first phase of the experiment, we will be able to determine the feasibility of performing more extensive testing of this method

of phytoremediation on site in Northern Saskatchewan. The preliminary results thus far have proven that Jack Pines are able to survive in soil highly contaminated with hydrocarbons. Based on these results, the use of Jack Pines in the remediation of surface soils contaminated with hydrocarbons looks promising. Jack Pines, growing in their natural ecosystem should have the potential to aid the process of natural microbial degradation of hydrocarbons. Therefore, in addition to reclaiming sites in Northern Saskatchewan with a native plant species, Jack Pines should also be involved in the continual breakdown of hydrocarbons beneath the topsoil, that are presently contaminating the soil. The potential of other members of the Pinaceae family native to northern Saskatchewan should also be explored.

6.0 Acknowledgements

The authors wish to thank the staff at Shand Greenhouse for their assistance with this project. In addition, Laurie Hammond, Ron Quinn and Sherry Hohn of ERIN Consulting Ltd. have also provided advice and assistance during this project. Dr. Hanna would also like to acknowledge the support of John MacDonald, Manager of SaskTel's Environmental Health and Safety Department.

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Table 1 Effluent Test Results (ug/L)

	Benzene	Toluene	Ethylbenzene	Xylenes
Sample*	<0.5	<0.5	<0.5	<0.5
CCME (aquatic)	300	300	700	5000

*Sample is a composite, taken from the runoff water from all pots in the study

Table 2 Soil Test Results (ug/g) at Time of Planting

Soil Concentration	Benzene	Toluene	Ethylbenzene	Xylenes	Total Volatiles
2100	<0.01	<0.01	<0.01	0.06	0.5
2400	<0.01	<0.01	0.02	0.15	0.9
6900	<0.01	<0.01	0.06	0.14	4
10000	<0.01	<0.01	0.19	0.45	11
22000	<0.01	0.12	0.26	0.47	14
23000	<0.01	0.33	1.1	3	34
CCME (agricultural)	0.05	0.1	0.1	0.1	

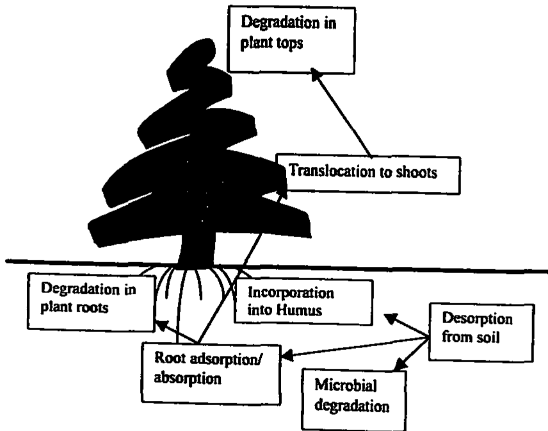


Figure 1 Phytoremediation of Organic Soils (Cunningham, 1993)

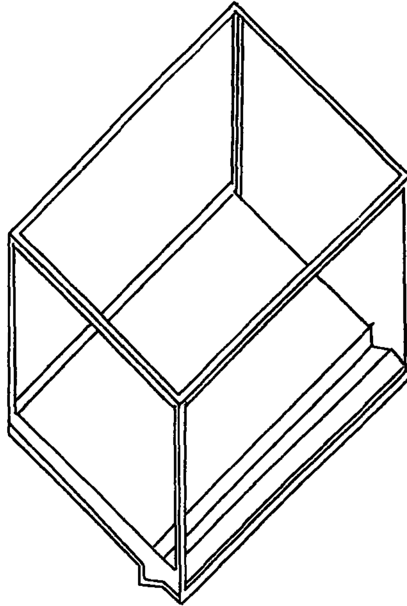
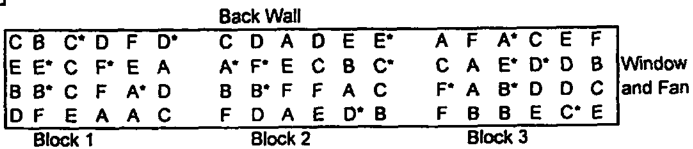


Figure 2 Schematic Drawing of Plexiglass Box Constructed to House Jack Pines

Door



Legend
A = 0 ppm B = 2100 ppm C = 2400 ppm D = 6900 ppm
E = 10000 ppm F = 22000 ppm * = control plots (no seedlings)

Figure 3 Placement of Jack Pine Pots Within Plexiglass Box

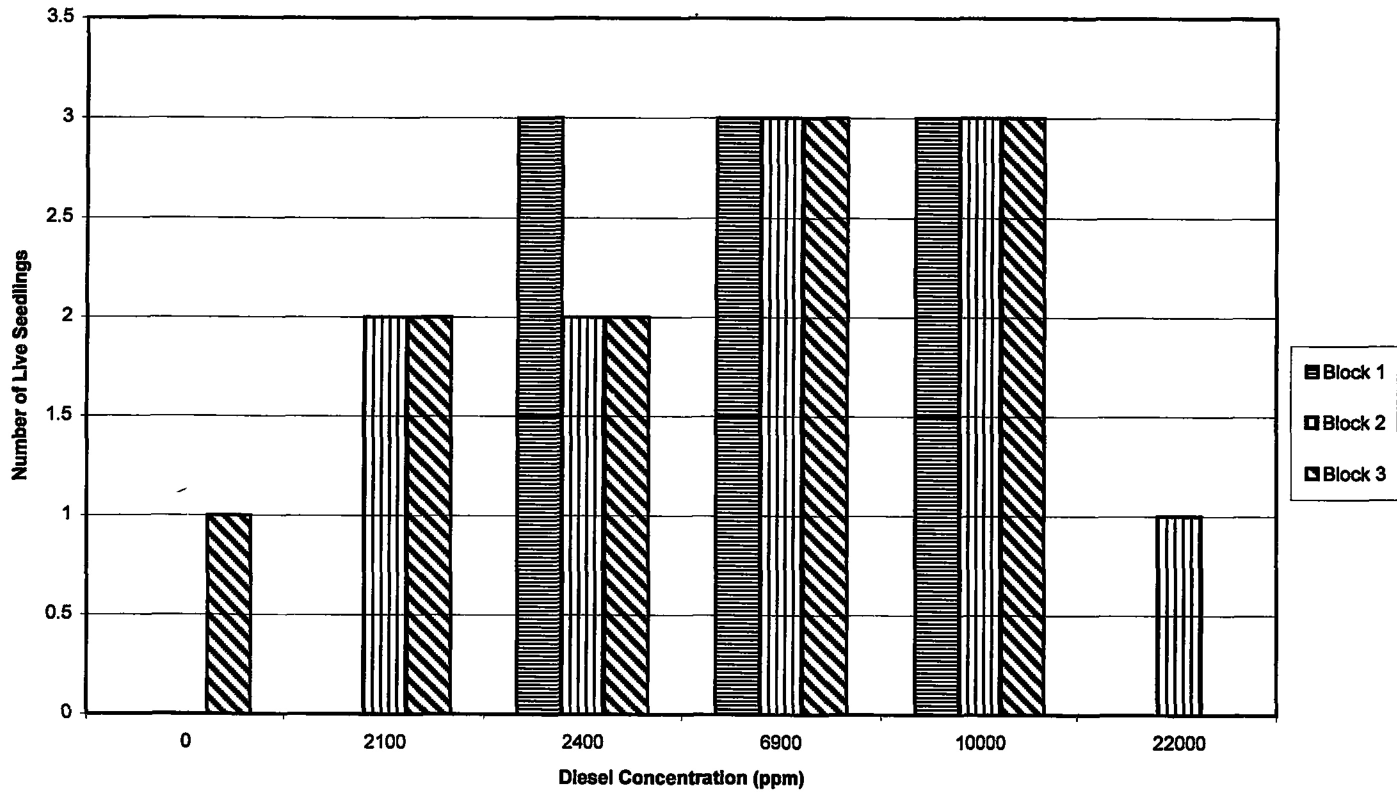


Figure 4 Jack Pine Seedling Survivorship (March 10,1999)

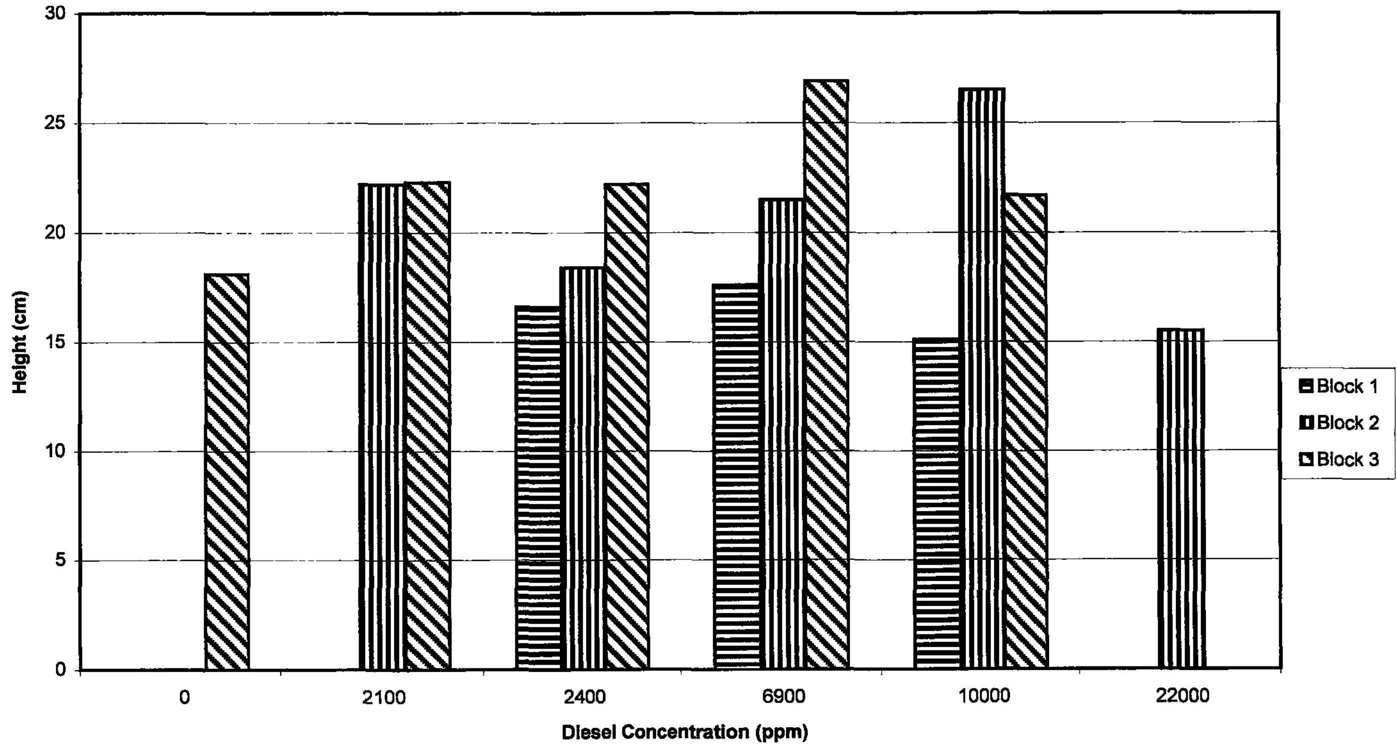


Figure 5 Average Heights of Jack Pine Seedlings per Concentration Class (March10,1999)

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Cover Photograph
Effect of oil contamination on the growth of *Scirpus punges.*

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Photographie de la couverture
Effet de la contamination d'hydrocarbures sur la croissance de *Scirpus punges.*

Courtoise de: D. Longpré

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Solvent Vapour Monitoring in Work Space by Solid Phase Micro Extraction

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Abstract

Solid phase micro extraction (SPME) is a fast, solventless alternative to conventional charcoal sampling/carbon disulfide extraction for volatile organic compounds (VOC). In this work SPME was compared to the active sampling technique in a typical lab atmosphere. Two different types of fibre liquid coatings were evaluated for suitability for concentrating low molecular weight VOC at ambient levels of vapour concentration. The polydimethylsiloxane (PDMS) fibre, a good general purpose fibre used for semi-volatile work was found to be unsuitable despite the thick coating. The new Carboxen/PDMS fibre in a field sampling kit was found to be excellent for VOC work. The field kit was convenient to use but variation in individual fibre, up to a factor 4, was noted. Calibration studies showed an optimal exposure time of 15 min, with a repeatability of about 25 % for a broad spectrum of organic vapour, with a minimum detectable amount in the range of 0.1 ppm. This works reports an application of SPME in monitoring solvent vapour at a bench where dichloromethane (DCM) was used. Comparisons were made with the charcoal sampling. Adsorption characteristics, calibration procedures and the advantages and limitations of SPME will be discussed.

1.0 Introduction

Compared to the charcoal sampling/carbon disulphide extraction used in NIOSH method #1005, SPME is a rapid and simpler sample preparation technique. By combining sample extraction and collection in one step, and a simple way of introducing the analytes onto an analytical instrument, it offers the benefits of minimal sample loss, maximum sample utilisation since there is no dilution involved, reduction of sample turnaround time and solvent usage required in traditional sample extraction. In a more recent survey of their application in VOC analysis, it has been applied to detection of gasoline in fire debris (Almirall *et al.*, 1996), odors in drinking water (Mindrup *et al.*, 1998), and flavour and aroma volatiles in whole fruits (Mindrup *et al.*, 1998).

In this work, the use of SPME for solvent vapour in the lab space is described. By extension of this type of work, we can anticipate similar application to ambient air survey work, which constitutes an important role for routine environment monitoring as well as in emergencies spill situations The heart of SPME sampling technology is the liquid coating on the fibre, of which a variety of different chemistries are available, depending on the type of analytes to be measured. We have also employed the general purpose 100 μm non-bonded PDMS fibre which have been successfully applied to headspace/immersion SPME to concentrate BTEX compounds from water accommodated fraction (WAF) samples as well as semi-volatiles such as PCB in water. With their universality of adsorption characteristics for most organic compounds, they should be good candidates for general analytical work without having to employ many different fibres with different chemistries.

For evaluation, we have also employed the Carboxen/PDMS SPME fibres with a film thickness of 75 µm. The Carboxen is a porous carbon layer which traps low molecular weight VOC and is said to be suitable for trace level monitoring work.

The fibre is contained in a portable field sampler which has a built-in septum system that allows the exposed fibre to be retracted and safely transported back to the lab for analysis without any loss or contamination. The sampler body is made from a durable lightweight polymer with an aluminum nosepiece that acts as a temperature shield during thermal desorption in the GC. There are five slots in the needle guide/depth gauge control the depth of needle insertion into a sample container, or into the injection port during fibre desorption.

In the case of air analysis, the fibre is exposed for a pre-determined amount of time and then thermally desorbed (Llompart *et al.*, 1998). Used as a passive sampler but with very good sensitivity because of the favourable partition coefficient of most organics on Carboxen/PDMS, this technique is far simpler and faster to implement than active collection using sorbent tubes, especially in view of the toxic carbon disulphide extraction of the charcoal tube samples. Consistent with what other workers have reported (Shirey,1997), very good sensitivity was found for volatiles in the two types of SPME. In that regards the SPME technique is far superior to the charcoal tube sampling by a sensitivity factor of at least 100. Despite the high sensitivity and ease of employment of SPME in air analysis, the Carboxen coated fibre showed an unacceptable level of variation in adsorption characteristics from fibre to fibre. We are reporting here results of lab space vapour concentration using charcoal sampling and SPME. We have investigated the performance characteristics of SPME and compared to charcoal tube sampling. Optimal exposure time was evaluated. Figures of merit such as detection limit, linearity range and repeatability studies were collected.

2.0 Procedure

2.1 Charcoal tube Sampling and Analysis

NIOSH method #1005 was used as a basis of comparison. The survey was carried out in Nov 1966 in response to a concern about the work space solvent vapour concentration, especially DCM which was used in testing interfacial tension. Air was drawn through a 2-stage (100/50mg) SKC charcoal tube by a SKC Airchek sampler (model 224-PCXR7) at a flow rate of 0.1 L/min, the flow rate was calibrated and controlled by a variable orifice. Sample description and location are described as follows,

Sample 1 (collected on Nov16)	Interfacial bench background, no other activities in the lab
Sample 2 (collected on Nov24 morning)	Interfacial test bench (not active but with activities going on in the lab)
Sample 3 (collected Nov24 afternoon)	lab 345 near GC/MSD; background
Sample 4 (collected on Nov24 afternoon)	Interfacial test bench (active)

As soon as the sampling was completed, the charcoal tubes were capped and stored in a fridge. To ascertain that there was no DCM breakthrough, the front and back portions (100/50 mg) of the tube were extracted separately in a 4-mL capped vial. Prior to extraction the charcoal was spiked with 100 µg of 1,2-Dichlorobenzene

as a recovery standard. A 1-mL aliquot carbon disulphide was added slowly and the contents were allowed to sit for 30 min with an occasional shaking. In parallel with the sample extraction, a matrix spike was also performed by spiking a blank charcoal tube with 132 µg DCM. Prior to analysis, 100-µg of d8-toluene was added to each vial as an internal standard to account for the difference in sample volume (nominally 1-mL) and variation in instrument response throughout the analysis.

Initially a GC with flame ionisation detector (FID) was used for the analysis. The advantage of FID was that the carbon disulphide would not produce a solvent peak to interfere with the analytes of interest. It was found however the solvent produced a high background in general to make identification of analytes difficult. Subsequently all analyses was carried out on on a bench top GC/MS system (HP 5890 Series II GC/GCD) equipped with a 30 M SPB-1 megabore column (0.5mm id, 1.5 µm film). The oven was kept at 30°C for 5 min and heated to a final temperature of 150°C at the rate of 15°C/min. The GCD was operated in selected ion monitoring mode set up automatically with the injection of a nominally 100-ppm solvent mixture standard in scan mode. A 1-µL aliquot was injected manually in splitless mode. Calibration of the system was by a 132 ppm native DCM solution with 100 ppm each of recovery and internal standard. The DCM peak eluted at 2.9 m just before the CS_2 peak. Ion mass at 85 was used to quantify the DCM in the extract, corrected for the response of internal standard.

2.2 SPME

The 100-µm polydimethylsiloxane used in a manual holder and 75-µm Carboxen/polydimethylsiloxane fibre housed in a field sampling kit was conditioned at 270°C in the heated injection port of another GC for 30 min prior to use. The field sampling kit has a built-in septum sealing system that the fibre can be retracted into, thereby protecting the fibre from further exposure. The fibres were deployed at the same sampling points as the charcoal tubes. Corresponding with sample 2, a duplicate SPME sample was also taken.

The fibre was inserted into the injection port of the same GC/GCD system and analysed with the same instrumental condition as described above. By the nature of SPME method in which the fibre is exposed in the media under study, there is no further extraction or concentration so no recovery or internal standard was used. Calibration of the SPME was carried out by injecting 1 µL DCM (in a mixture of 9 common solvents used in our lab) in a 5-L Tedlar bag. The mass of solvent which must be added to a given volume of air at 25° and 1 atm is given by the equation (Photovac 10S plus user's manual)

$$m = 4.1 \times 10^{-8} \times M \times V \times c$$

Where

c=conc. in ppm
m=mass of DCM
M=molecular wt in AMU in g and V=volume of air

The concentration of DCM vapour in the Tedlar bag was calculated to be 265 ppm by the equation.

3.0 Results and Discussions

3.1 Preliminary Study

Before the actual sampling, a preliminary study was carried out in which a 5-L Tedlar bag was prepared with 265 ppm DCM. Two separate charcoal tube samples were taken each with 2.5L of air sampled. Analysis was carried out as described. The values found were 319 and 145 ppm. While the first tube agreed reasonably well with the 'true' value of 265 ppm, the second one did not. The variation may be due to non-uniform vapour distribution. There was no breakthrough of DCM from the first stage of 100 mg charcoal tube as evident from non-detectable amount of DCM in the back half. A blank charcoal tube was exposed for 30 min in lab air without connecting to the pump. It was analysed in the same manner and found to be below detection limit. Recovery standards showed a range of recoveries between 53 to 68% in the extraction method. Instrument detection limit was 10 μg/mL, for a sample volume of 3 L method detection limit is 3μg/L or 1ppm of DCM vapour in air.

Problems exist, however, with proper calibration of the Carboxen fibre. Each fibre was calibrated by static exposure in an atmosphere with a known vapour concentration. This was carried out by inserting into a Tedlar bag having a known DCM concentration for the same amount of time the fibre was exposed in the lab atmosphere. The static atmosphere in the bag was not the same as in an open atmosphere, the condition of which was far more dynamic. In a typical lab environment there might be appreciable air current such that vapour concentration at a fixed spot might neither be constant nor static, making it hard to approximate the actual flux the fibre is subjected to. An attempt was made to create a more dynamic situation: the SPME fibre was inserted into the Tedlar bag through a septum valve, the holder body was then attached to a mechanical shaker and agitated gently for the duration of exposure. It was thought by doing so, the boundary layer between the fibre and the air would not become static and saturated. This is akin to stirring the water in immersion SPME to minimise localised saturation. Results summarised in Figure 1 show however there was no difference between the two methods (shaking or dynamic vs. no shaking or static) of calibration within the precision of SPME measurement. This fact underlies the difficulty involved with most passive sampling device such as the 3-M solvent exposure badges.

3.2 Adsorption Study, PDMS vs. Carboxen

Low molecular weight VOC vapour such as DCM presents a special problem with the conventional 100 μm PDMS fibre: the volatility is such that adsorbed DCM can diffuse readily back out from the surface , as a result of which the response of the PDMS fibre was found to be very poor. By comparison, the mixed phase Carboxen/PDMS phase retains DCM well. The pores of Carboxen, a chemically modified charcoal, are described as tapered (private communication, Supelco) so the smaller gaseous molecules diffuse deeper into the pores relative to the heavier molecules. They become adsorbed until desorption by heat. Comparison study of the two types of fibres were carried out by exposing one pair of each type to an ambient air with low vapour source of DCM, benzene and toluene. After 30 min, the fibres were analysed by GC/GCD. While the Carboxen fibres both showed measurable amounts of all three solvent vapour, the PDMS had only non-detectable and trace level of vapour on the fibre (Figure 2).

As a basis of comparison, a properly conditioned Carboxen SPME was exposed for 30-min on a lab bench. This bench was an actively in use, with about 10 capped vials (40-mL) of various solvent nearby used to make dilutions of working standards. The solvents, commonly used throughout our lab, included pentane, hexane, iso-octane, benzene, toluene, xylenes, DCM, methanol, ethanol and carbon disulphide. That location would provide a typical lab atmosphere with measurable solvent vapour to test the validity of SPME. Five replicate measurements were made consecutively. GC/GCD analysis of the exposed fibre showed most of the solvent had indeed detectable amounts of vapour adsorbed on the fibre, except for the polar solvents. For clarity only results of DCM, benzene and toluene data were used to illustrate this point (Figure 3). Repeatability of the same fibre for five runs was about 20% RSD (relative standard deviation). The repeatability reflected the variation of the atmosphere as well as the process of adsorption and analysis. It should be pointed out the experiment was carried out in an unprotected and open area in a typical lab. The data showed the vapour concentration over that bench was reasonably constant and measurable by SPME. The actual concentration of solvent vapour however was not known because the fibre was not calibrated for each solvent.

Having established the repeatability of a single fibre, four fibres of the same type were then exposed at the same location. After exposure each fibre was stored in the field sampler body sealed by a septum before analysis. Results showed response of the fibres varied by as much as a factor of 4 (Figure 3). The sealing system was previously established to be effective in maintaining the adsorbed VOC for at least 8-hr so the differences were not due to sample re-evaporation. This variability was a serious drawback of employing different fibres in air monitoring work, since it would make comparison of data from different sampling sites difficult, unless each fibre was calibrated individually.

3.3 Time Exposure Study

A time exposure experiment was then carried out in the dynamic environment as outlined above at exposures ranging from 2 to 30 min. Results are shown in Figure 4, which illustrates most solvent vapours showed a more or less increasing adsorption profile, peaking at 15 min. It is interesting to know with most volatile solvents, exposure time as short as 2 min showed a measurable response.

3.4 DCM measurement in lab atmosphere

Prior to actual use, the fibres were calibrated using a 5-L Tedlar bag with a DCM vapour concentration of 265 ppm. Results of lab air samples are summarised in table 1, showing the charcoal tube and the corresponding SPME values. Only sample 1 and 4 had measurable amount of DCM on the charcoal tubes. In most cases there was non-detectable amount of DCM in the back portion of the tube, so no breakthrough occurred at a sampling rate of 0.1 L/min for a total volume of 3 L. The only exception is in sample 4 collected during active duties at the interfacial lab. The back half had 3 μg/L (1 ppm) and amounts to less than 4%. This is at the detection limit of the method and can be considered insignificant.

The corresponding SPME results are also shown. Duplicate SPME at the site where sample 2 was collected showed the DCM concentration to be at 4 and 9 μg/L (1.1 and 3 ppm). Agreement was in general good, considering the rather poor sensitivity of liquid injection of DCM in CS2 by GC/GCD. There were detectable

amounts of DCM by all 4 SPME fibres. Examination of SPME sample chromatograms of the GC/GCD analyses typically showed the DCM peak at several hundred thousand counts. The same from wet chemical extraction had only several thousand counts (at least 100 times less) and poorly formed peak shapes such that quantitation became difficult. Because no solvent was used, the baseline was also much cleaner in the SPME runs without the solvent peak swamping out all signals between 3 to 6 min. Also the SPB-1 methylsiloxane GC column used for headspace (HS) analysis of water samples for BTEX compounds might not be suitable for solvent analysis.

The recovery of the 1,2-dichlorobenzene (recovery standard) added to the charcoal samples was generally good at 75% with a variation of 11%. Recovery of native DCM from the matrix spike was 46 to 26 % respectively for front/back half, showing the fairly high degree of variation in the analysis of DCM due to the low instrument sensitivity.

The 'dirtiest' sample, corresponding to when the interfacial tests were performed had a DCM concentration of 78 μg/L (26ppm) by the NIOSH method and 50μg/L (16ppm) by SPME. Background concentration ranged from 17μg/L (6ppm) to non-detectable (<1ppm). They are all below the regulatory limit.

3.5 Limitation of SPME in Multi-sampling Program

For any meaningful air monitoring work a number of samplers need to be employed simultaneously. The individual variability of the SPME is thus a serious drawback. According to Supelco, the Carboxen phase fibre is hand coated to a specific thickness on the SPME substrate so it is conceivable a quality control problem might cause such variability. The uniformity of the Carboxen particles dispersed in the PDMS phase might also play a part in the adsorption process. Moreover, in actual usage, as a fibre 'ages' due to physical damage such as chaffing, chipping and heat stress the durability of the coating would also come into question. In view of this shortcoming, the use of SPME as a routine quantitative monitoring tool is at the moment marginal, however, as a qualitative sampling tool it was excellent because of the simplicity and high sensitivity, exposure as short as 2 min would be sufficient to obtain a 'snap shot' profile of the vapour present in the atmosphere under investigation.

4.0 Conclusion

Using charcoal tube sampling and wet extraction, the present GC/GCD set-up was not very sensitive for DCM analysis due to the high background introduced by the solvent peak. While the Carboxen SPME is much more sensitive (by a factor of at least 100) and much simpler to use. Only very short exposure time is needed to provide a general characterisation of the quality of air at the sampling site. At present, there is too much fibre-to fibre variation to be a useful routine air-sampling tool.

5.0 References

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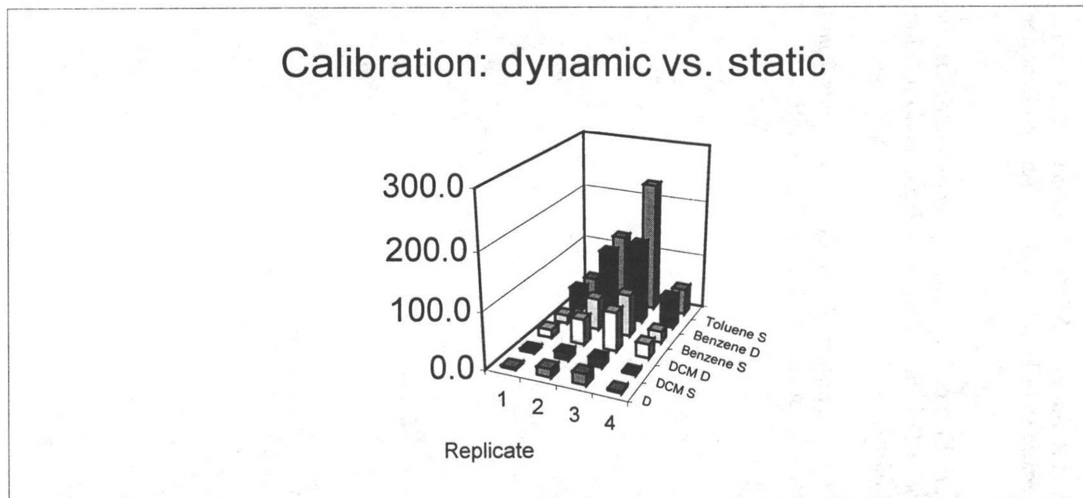
Llompart, M., K. Li, and M. Fingas, *"Headspace SPME for the Determination of Volatile and Semi-Volatile pollutants in Water and Air"*, Journal of Chromatography, Vol. 824, pp. 53-61, 1998.

Shirey, R., *"SPME/Capillary GC Analysis of Solvents from Water at Low ppb Levels"*, Supelco Reporter, Vol. 16, No. 2, p. 6, 1997

Replicate	DCM D	DCM S	Benzene D	Benzene S	Toluene D	Toluene S
1	3.0	4.2	14.6	14.5	39.2	35
2	14.4	12.6	45.0	56.0	120.0	126
3	19.2	15.0	69.0	74.0	144.0	231
4	3.4	2.7	24.8	20.0	55.5	46



D=Dynamic; S=Static

Figure 1 Calibrating SPME by Tedlar Bag, Static vs Dynamic Mode

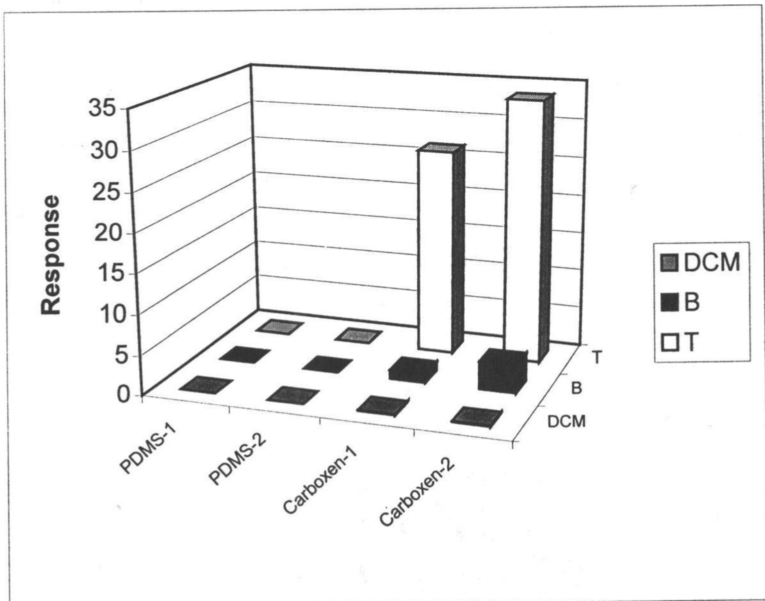
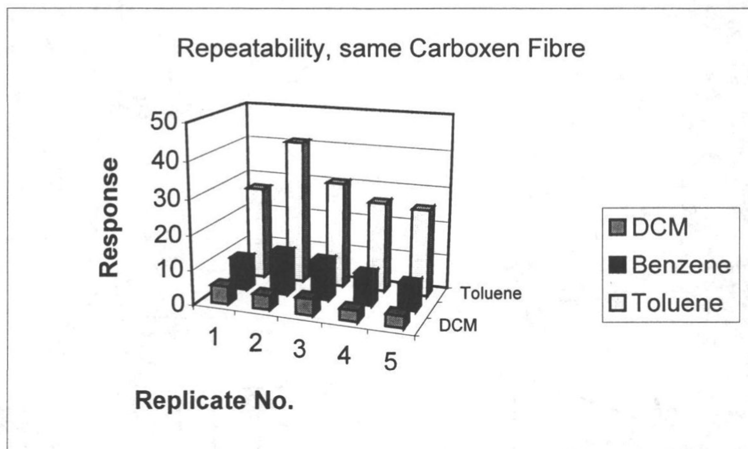


Figure 2 VOC response, PDMS vs. Carboxen SPME
Sampling Location: open working bench, 30 min exposure

Same Fibre



Different Fibres

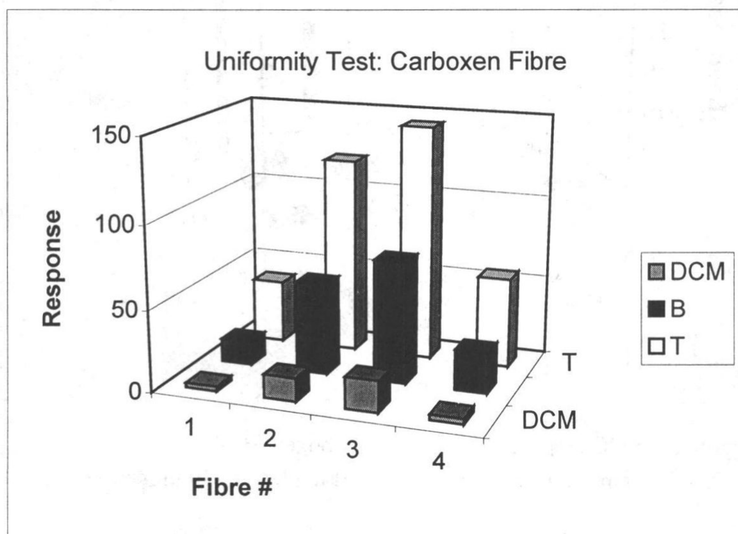


Figure 3: Repeatability of 75-um Carboxen/polymethylsiloxane Fibre
Condition: 45 min exposure, near solvent vials on work bench

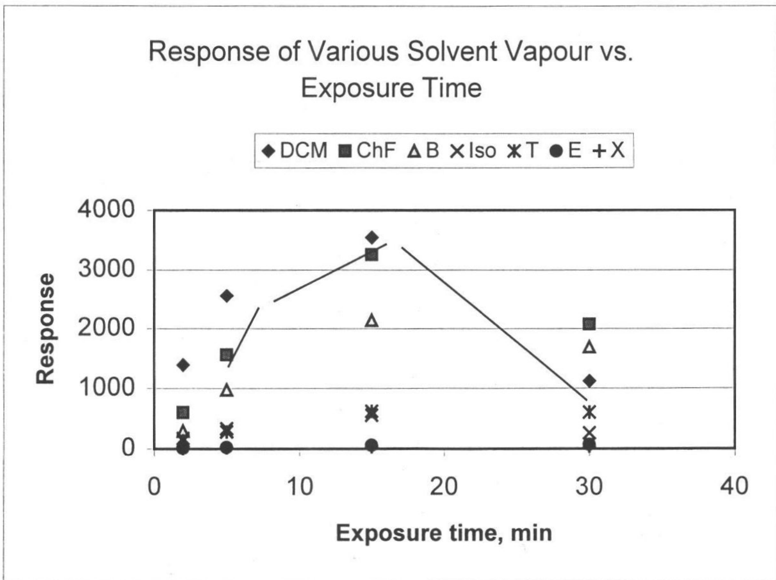


Figure 4 Time Exposure Study

Table: DCM in Lab Air, pump/charcoal vs. SPME method

	Location		Charcoal tubes	SPME	duplicate
Sample 1(Nov16)	Interfacial bench background	frt	17	34	
		bk	0		
Sample 2(Nov24 morning)	Interfacial test bench ('clean': non active)	frt	0	4	9
		bk	0		
Sample 3(Nov24 afternnon)	lab 345 near GC/MSD; background	frt	0	6	
		bk	0		
Sample 4(Nov24 afternnon)	Interfacial test bench ('dirty': active)	frt	78	50	
		bk	3		

Results in µg DCM/L; charcoal tube sampling flow rate 0.1L/m for 30m, SPME (Carboxen) exposed for 30m

Duplicating Conditions for Field Testing of Carbon Dioxide: A Modeller's Dream Becomes A Technician's Nightmare

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Abstract

This paper summarizes attempts made to explain anomalous carbon dioxide (CO_2) readings observed during a sampling exercise in 1994. The original data was collected downwind from a diesel fire using portable CO_2 meters. The research team postulated that temperature, humidity, radio frequency interference, and/or diesel fumes could have contributed to the anomalous readings found by the front-line instruments. A laboratory study, incorporating these factors singly and in combination, and using multiple instruments, was undertaken to attempt to determine which, if any, of these conditions were responsible for the uncharacteristic trends found in the field sampling. Though trends were observed in this laboratory study, they failed to explain the field results. In 1997, the opportunity to return to the 1994 site and set up a sampling array similar to the 1994 instrument array was presented. With a specific goal in mind, the research team was intent on capturing and defining the anomalous results. The number of CO_2 monitors was quadrupled. At one sampling station four monitors from each of two manufacturers, for a total of eight monitors, were positioned. The number of burns was increased from three to twelve thereby delivering more data. With the additional instruments/testing came the need for additional technical support, calibration gases, and data analysis. What began as a routine sampling task had become a carefully pointed attack on the CO_2 produced by the diesel fire. What the results were able to show tells us more about the unpredictable nature of field testing and the inability to control environmental factors than they did about our original problem - explaining results that didn't fit with our prediction of how the instruments should have responded. For future testing, we have learned about the practical limitations and expectations of these portable CO_2 instruments in field monitoring tasks.

1.0 Introduction

1.1 Past Experiments

The task of accurately measuring for air contamination in open systems is a challenging one, especially when one hopes to use this data to form predictions regarding total emissions from the source. In this study, portable carbon dioxide (CO_2) meters were used to measure emissions from diesel burn experiments in Mobile, Alabama. Understandably, there are limitations associated with portable instruments. In an application which includes moderately difficult conditions, where

the temperature may fluctuate from 20 to 50 degrees Celsius, where the relative humidity varies greatly in a short period of time, and where other parameters exist which may affect the signal generated by the CO_2 monitor, these instruments are being asked to perform at their limits. The application discussed in this study was the measurement of CO_2 produced by an in-situ fuel burn, but it would be applicable to any testing where CO_2 monitors are being used to measure levels close to ambient levels and where conditions are somewhat different from typical indoor locales. The measurement of CO_2 during in-situ fuel burn testing has been performed in the past. These authors are aware of at least four such occurrences, including three mesoscale burn tests (Walton *et al*, 1993, Fingas *et al*, 1993, Fingas *et al*, 1996) and the Newfoundland Offshore Burn Experiment (NOBE)(Fingas *et al*, 1995). In both the NOBE and two of the mesoscale burns, the fuel burned was crude oil. It was the in-situ burn from the 1994 test (Fingas *et al*, 1996) at Mobile, Alabama, where diesel fuel was burned, that some interesting trends appeared in the CO_2 data. Shortly after ignition of the fuel, the CO_2 level was observed to drop significantly below initial readings, followed some minutes later by a "surge" to a level higher than initial levels and finally a tailing off back to ambient levels after the burn had been extinguished for some time. This trend was most obvious with monitoring stations situated closest to the burn pan (30m downwind), but was noticeable up to 75m away (Figure 1). That CO_2 would be produced by combustion was not a surprise. The curiosity was the drop in observed CO_2 levels shortly after the ignition of the fuel.

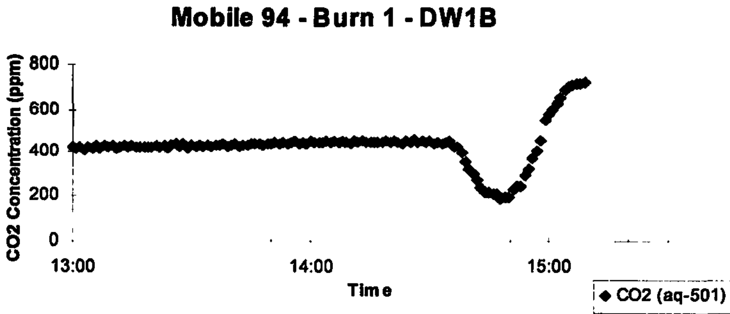

Figure 1. The CO_2 pattern during this burn had a dip that was most apparent at stn DW1B (30m downwind). Note that burn ignition occurred at approx. 14:32 and was extinguished at approx. 15:02.

It was noted that during the first few minutes of the burn, when the CO_2 level was seen to drop, other measured parameters were seen to be changing rapidly. For example, in that same span, the local temperature was seen to rise from an initial value of 27C to as high as 55C at the nearest monitoring stations (also those which showed the most exaggerated CO_2 dip). The possible correlation with effects from the burning diesel fuel invited further investigation.

In a laboratory study which was undertaken to attempt to discover the cause of these unusual readings (Goldthorp *et al*, 1997), the effects of temperature, humidity, particulate, radio-frequency interference (RFI), and diesel fumes were measured by the instrument used in the 1994 Mobile tests, as well as other portable CO_2 meters. Changes in these parameters could certainly influence the signal produced by the meters, but not in a way that would offer any clear answers to the anomalous results in 1994.

Another possibility to consider is that the instruments were reading true in the 1994 burn experiment. When we look at the readings from the meter, we see that the level of CO_2 dropped well below *ambient* levels for approximately 20 minutes. It is unlikely that the test conditions would have produced a real decrease in levels of CO_2. For this to be true, the burn dynamics would have to predict that CO_2 was consumed by combustion, which we know is not the case. In fact, if anything, there will be CO_2 generated by the burn through all phases of the test, including the initial phase where the fire has the fastest growth. Even when the plume from the fire is directed away from the monitoring station, fresh air moving to feed the fire would tend to contain ambient CO_2 concentrations. The measured CO_2 would not be expected to drop below the ambient level at any point during the short duration of the in-situ burn.

Had this been a reading from a single instrument, it would have been rejected as bad data - something we tend to do when results are not following a pattern we expect. In this case, the effect was measured by several instruments simultaneously, such that the results cannot be discarded casually. This leads back to the initial conclusion that another parameter was affecting the CO_2 signal measured by the portable monitors used during the burn experiment. Failure to repeat this effect in the laboratory tests may have been due to either a) we have yet to identify the cause of "the dip" or b) that the closed laboratory model was unable to reproduce the field conditions.

1.2 Instrumentation

The portable CO_2 detectors used employ a non-dispersive infra-red (NDIR) detector. The instruments used in the testing were the CD-1 (Armstrong Instruments, Nepean, ON) and the aq-501 (Metrosonics, Rochester, NY). The CD-1 has an analog meter and an analog output. The aq-501 has a digital meter and internal datalogging, as well as sensors for temperature and humidity. Both of the instruments had an upper detection limit of 5000 ppm CO_2.

For the CD-1, an external datalogger was used. The datalogger used was the CR-10 (Campbell Scientific, Logan, UT).

The calibrations were made using ultra-zero nitrogen and 4686 ppm certified standard gas mixture of CO_2 in a balance of zero air (Matheson Gas Products, Whitby, ON).

1.3 The 1997 Mobile Burns

The opportunity to perform more sampling arose in 1997, when the USCG, in cooperation with NIST, was testing fireproof oil boom at the USCG Fire and Safety Test Detachment facility in Mobile Bay, Alabama. The fuel used in the testing was diesel, the same as in the 1994 burns. As well, the general location was the same as

the 1994 burns. The testing was to take place over several days and, during that time, several burns were to be conducted, most of which were at least one hour in duration. While it is impossible to reproduce identical field conditions, this was as close as we could expect to achieve. When the invitation to sample on-site was offered, we were more than happy to attend.

The planning was underway. From the standpoint of CO_2 monitoring only (there were several other parameters to be measured), 13 sampling locations were chosen relative to the burn pan: one upwind station, 6 stations in the direction that the plume was most likely to travel, and six stations along the sides. Since a 3-D profile of the CO_2 was desired, 31 instruments were required at these 13 stations, such that the six main stations all had 4 instruments, each monitoring a different height above ground level. Also, since two different models of portable CO_2 monitoring instruments were being used, it was decided that at one of the stations, four of each instrument would be used, for comparison of responses under the same conditions, bringing the field instrument total to 35. As unpredictable as field projects often are, the decision to bring a few spare instruments, a wise decision in retrospect, brought the number of CO_2 monitors to be taken to the 1997 Mobile burns to 40 instruments! This was four times the number of instruments used in the 1994 tests.

The difficulties began to become apparent well before the sampling was to take place. Instrument inventories do not often include more than one or two like instruments. Between the sampling partners of Environment Canada's Emergencies Science Division and US Environmental Protection Agency's Environmental Response Team, along with the Weston/REAC Air Monitoring Group, 9 instruments were owned outright. The rest consisted of the entire available inventory of three major rental companies. Orders for full-size calibration gas cylinders were placed, in anticipation of daily calibration of each of these instruments, each consuming as much as 2L of gas per calibration. Extra hands would be required to make those calibrations in the few minutes between arriving on-site and the beginning of the burn testing for the day. With gear in tow, we were as ready as we were going to be when we arrived at the USCG facility in September 1997.

While the CO_2 monitors had internal batteries which would be sufficient for at least 8 hours of use, the task of bringing them in from their stations at the end of each day to be charged, only to return them to their stations in the morning, was too daunting. Since there was power provided on-site by large generators, extension cords were run to all of the stations to power the instruments. More than once, data was rejected due to extension cords being uncoupled over the three-week project. Because the instruments had their internal battery, the readings would appear normal in the morning, only to discover much later in the day that things had gone awry as the battery had run down. Thankfully, this was not a common occurrence.

In order to protect the instruments from the elements overnight (not to mention the island's resident ant colonies, who found rubber seals particularly appetizing), the instruments were wrapped in plastic each night. It was found that this sped up the morning ritual of preparing for sampling, since instruments could be left connected to power bars and datalogging cables. The day usually began with a mad scramble as the sampling team raced from station to station to unbag all of the instruments, then turned them on to get the maximum warm-up time prior to

calibration, which required at least six people with two or more Tedlar bags of calibration gas each. With precision teamwork, this operation usually concluded mere minutes before the ignition of the first burn of the day, often no more than an hour from the time the boat docked on Little Sand Island, the USCG burn test facility.

2.0 Results and Discussion

As an experiment, the 1997 Mobile burns went extremely well. Unlike the 1994 testing where three burns, each of approximately 20 minute duration, took place, the 1997 tests featured 12 burns, most of which were at least an hour in duration. Most of the time, the wind was favourable, that is to say that the plume was directed over our "downwind" sampling stations most of the time. The instruments, CO_2 monitors and others, performed as well as we could have expected. Tight scheduling by the USCG and NIST allowed for a great deal of testing in a relatively short period. Sample handling was done with due care, even when the clock was ticking. The data collected, by the CO_2 monitors alone, consisted of more than 50,000 data points recorded for later interpretation. Yet, through the course of this experiment, we did not find any explanation for the anomalous results seen during the 1994 tests.

In fact, the CO_2 plots looked remarkably ordinary (Figure 2). We searched through the plots for any notable valleys. In two instances, there was some interesting curves produced, during the burns on September 29th and 30th. On these two burns, we focused our interest.

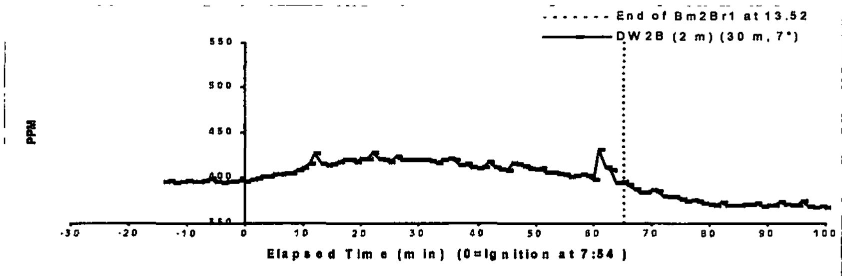

Figure 2: "Normal" CO_2 readings measured during a burn on September 26th, 1997. Data has been slope corrected.

The first notable event occurred during a short burn on September 29th. The CO_2 level at several of the downwind station showed a very slight dip, followed by an upwards trend which tailed off towards the end of the burn (Figure 3). This may have been more notable had not the upwind station (72 metres upwind from the burn) shown the same trend (Figure 4). In the 1994 burn, when the downwind stations measured the anomalous results, the upwind station had recorded no appreciable change. This instance appeared to be the result of some changes in the ambient levels and not from the burning diesel fuel.

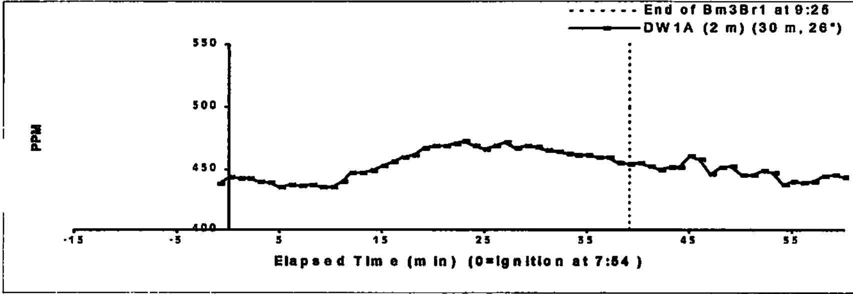

Figure 3: Carbon dioxide measured by Metrosonics aq-501 at station DW1A, September 29th, 1997. Data has been slope corrected.

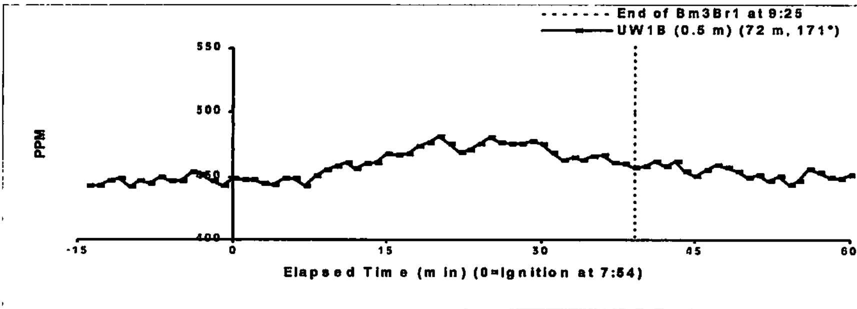

Figure 4: Carbon dioxide measured by Metrosonics aq-501 at station UW1B, September 29th, 1997. Data has been slope corrected.

The second interesting plot occurred the following day during a prolonged burn of over two hours. Midway through the burn, several of the downwind stations showed a persistent drop in the CO_2 levels, followed by a recovery towards the end of the burn (Figure 5). A check with the upwind stations showed steady background readings (Figure 6). A possible solution presented itself when the results from the

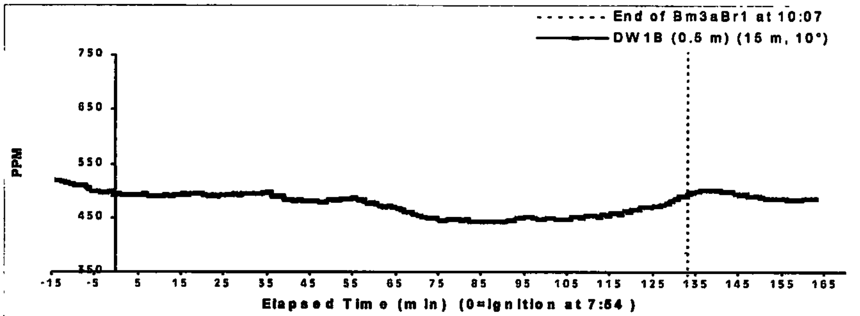

Figure 5: Carbon dioxide measured by Metrosonics aq-501 at station DW1B, September 30th, 1997. Data has been slope corrected.

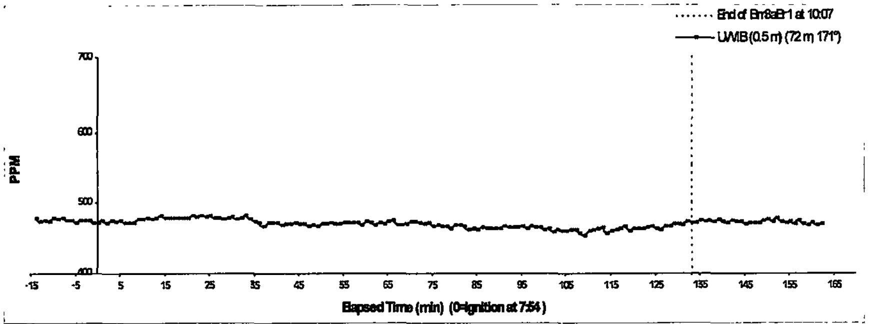

Figure 6: Carbon dioxide measured by Metrosonics aq-501 at station UW1A, an upwind location, September 30th, 1997. Data has been slope corrected.

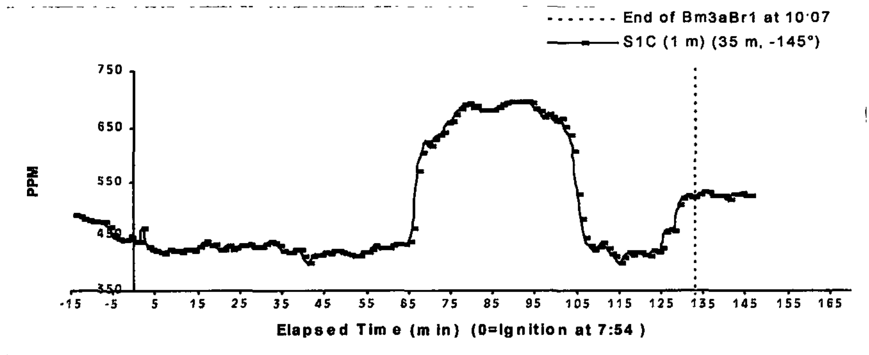

Figure 7: Carbon dioxide measured by Metrosonics aq-501 at station S1C, September 30th, 1997. Data has been slope corrected.

side stations were observed. One of the stations to the West of the burn pan showed a large extended rise in the CO_2 level over the same time as the dip in the "downwind" stations (Figure 7). A look at the weather pattern data on that day showed that the wind was changing considerably, but that correlation of the CO_2 changes with a wind direction change was not apparent (Table 1). Since wind conditions did not appear to play a role, other factors must be considered. Also of note is that, during this burn, there was an attempt made to quench the fire, but that the fire continued to burn slowly until, near the end, there was a breach in the fire boom and the remainder of the fuel burned uncontrolled until it was consumed. Using this information, we can postulate that in order to quench the fire, the generator/pumps for the firehose were activated. As it turns out, the station closest to the firehose pump/generator was station S1C, the station where the high readings ocurred. It is obvious, also, that the depression in the CO_2 readings at the downwind stations did not ever go below normal ambient levels, as happened in 1994. Also, unlike the 1994 data, there is a valid hypothesis as to why the CO_2 readings dropped in this data set - the quenching of the fire.

Table 1: CO_2 Results at three stations and Wind Direction on September 30th, 1997

Time	CO_2 at UW1B (ppm)	CO_2 at DW1B (ppm)	CO_2 at S1C (ppm)	Wind Direction (degrees from North)
8:00	472	496	419	246
8:05	479	492	418	243
8:11	478	495	438	238
8:16	482	493	431	230
8:21	478	494	433	280
8:26	479	494	437	295
8:31	467	490	420	294
8:36	472	483	412	295
8:41	466	479	421	319
8:46	472	485	412	315
8:51	472	483	430	313
8:56	468	471	433	270
9:01	469	**462**	**572**	245
9:07	469	**452**	**641**	230
9:12	466	**448**	**691**	252
9:17	462	**445**	**680**	276
9:22	464	**444**	**692**	281
9:27	464	**450**	**696**	248
9:32	464	**449**	**673**	123
9:37	461	**448**	**637**	149
9:42	455	**452**	432	170
9:47	464	**457**	416	185
9:52	466	464	415	90
9:57	465	472	422	63
10:03	470	481	507	72
10:08	472	498	527	81
10:13	476	502	523	95
10:18	474	496	529	83
10:23	474	491	ND	125
10:28	471	487	ND	127
10:33	469	484	ND	128

Though massive quantities of data were collected, none matched the pattern that was seen in one instance in 1994. Considering the unpredictable nature of field testing, this is not surprising. However, though efforts were made to reproduce the sampling conditions in 1997, there were some notable differences. The main difference between the burn experiments was the nature of the burn. The 1994 burns were full-pan, uncontrolled burns, while the 1997 burns were contained by a fire boom ring and the fuel delivery rate was metered. As a result, the 1997 burns were much smaller (though longer duration) and consequently did not occur as close to the instruments and the rapid temperature change was not observed.

3.0 Conclusions

For the purposes of predicting the CO_2 produced by burning diesel fuel in an open system, this experiment was a success. Those who use this data to develop emission models should be pleased. Unfortunately, the puzzle of 1994 has yet to be solved, as no light has been shed on the cause of the anomalous readings. Because of this, we do not know when or if this effect may repeat nor how to correct for it if it does. The effort and teamwork demonstrated in this project was commendable and the reward is quality data - lots of it. A good emission model comes at a high price. The logistics of the sampling exercise, the significant capital and staffing cost, as well as the hours of data interpretation, all contribute to the investment in quality data. The portable CO_2 monitors used throughout this testing were used in conditions that would not be considered normal operating conditions, but generally performed well and we would not hesitate to use them for a similar task. As for the unexplained results found in 1994, we can only hope that we do not encounter the same phenomenon again until fortunes dictate that we are able to discover the cause.

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A Closer Look at the Use of a Portable Infra-red Analyzer for Low-Level Hydrocarbon Emissions

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Abstract

Previously, it was reported that, during some on-site tests, a portable infra-red (IR) analyzer was used successfully to monitor for hydrocarbon vapours. The detection limit of the IR detector is much lower than that of most other hydrocarbon vapour monitors and can be used in situations where, as in most ambient air, the levels are often less than a milligram per cubic metre (mg/m^3). The advantage of providing continuous sampling data is that it may indicate trends in the hydrocarbon vapour emissions that may not be apparent using a grab-type sample. The initial tests were designed to determine if the IR detector was capable of monitoring the low level hydrocarbons in a field situation. The findings from that initial work has led to more recent work which expanded the testing to include an upwind IR monitor, shortened sampling cycles to produce more data, and added canister samples collected outside the burn period. The metered grab samples, using the Summa canisters, were collected over a one-hour period and any results would therefore reflect an average value over the hour. The IR analyzer, with a sampling cycle of approximately one minute, was able to produce a near real-time distribution of the hydrocarbon vapours in the test site emissions. Because the testing parameters and methods are quite different, it is difficult to compare these two methods, but indications suggest strongly that the use of this portable IR instrument could help to describe the hydrocarbon emissions downwind from a source, as well as to monitor for these hydrocarbons continuously, including situations where the levels are below detection limits of most portable detectors.

1.0 Introduction

1.1 The Test Site

The location for the test was the United States Coast Guard (USCG) Fire and Safety Test Detachment test facility on Little Sand Island in Mobile Bay, Mobile, Alabama. This test site has been used in the past for similar test procedures (Walton *et al*, 1995, Fingas *et al*, 1993). The source of the hydrocarbon vapour emissions was a test tank which contained seawater onto which was pumped fresh diesel fuel which was ignited and allowed to burn in a controlled fashion for one hour. The quantity of fuel was replenished over the duration of the test to sustain the burning for the full hour. When possible, as many as three burns, each of one hour duration, were performed in a day. When multiple burns were performed, a minimum of 1 hour

between each burn was observed. The first set of burn events for this study took place on September 26th, 1997 and October 1st, 1997 (Goldthorp *et al.*, 1998). The second and most recent set took place between August 25th and September 9th,1998. Results from the latter set will be discussed. Both the Summa canisters and the infra-red detectors were among several instruments used to monitor the burn emissions. For this set of data, the Summa canisters were placed side-by-side with the IR detector. As a background, for each Summa and IR sample collected, there was an ambient sample collected at an upwind location. Power was supplied to the upwind sampling instruments by a large on-site diesel-powered generators that operated continuously throughout the day. The generator was situated away from the sample stream. Downwind instruments had shore power provided.

At the beginning of each day, test instruments were turned on and allowed to warm up for the maximum allowable time. The Summa canisters that were used during the burns were opened and closed manually in coordination with the burn event. Thus those canisters were used to collect sample solely while the burn was taking place. Also, during the burns associated with boom 4 in Mobile 1998, a second pair of canisters were used to collect air samples between the burn events, thus, they were turned on when a burn was complete and turned off again when the next burn began. The IR analyzer monitored continuously throughout the day, collecting samples both during and between burns.

1.2 Summa Canister Sampling and Analysis

The canisters used for collecting the air samples were 6L stainless steel pre-cleaned and evacuated Summa canisters. The canisters are widely used for ambient air sampling. An adjustable restricter orifice was used at the inlet of the canister to meter the flow to approximately 25 mL/minute. For a series of three one-hour tests, this would yield a sample volume of approximately 4.5L. This sample volume was deemed to be the maximum while maintaining a constant flow through the valve since, as the canister fills up, it becomes impossible for the weaker vacuum to keep the same flow rate through the orifice. The canister valve was operated manually. Each restricter orifice was purged thoroughly before the field tests and baked out overnight between uses.

The analysis was performed by the Analysis and Air Quality Division (AAQD) of Environment Canada using a cryogenic preconcentration technique with a high resolution gas chromatograph and quadrupole mass-selective detector (GC-MSD) as described in EPA Methods T)-14 (Winberry *et al.*, 1988) and TO-15 (Winberry, 1997). An Entech Model 7000 preconcentrator with auto-sampler (Entech Instruments, Inc., Simi Valley, CA) was used for sample preconcentration. The instruments used for species identification and quantification were a Hewlett-Packard 5890 series II chromatograph with a Hewlett-Packard 5970 MSD. Volatile Organic Compounds (VOC) were separated on a 60 m, 0.32 mm I.D. fused silica capillary column with a 1.0 μm film thickness of J&W (J&W Scientific Inc., Folsom, CA) DB-1 bonded liquid phase.

All samples were diluted with clean, humidified air in order to provide sufficient positive canister pressure for proper operation of AAQD analytical systems. Air from each canister was drawn through the preconcentrator's multi-stage trapping system and sample volumes were measured with a mass flow controller. A gaseous

mixture of internal standard was added in combination with 500 mL of the sample into a glass bead trap maintained at -170C. A three-stage concentration technique called Microscale Purge and Trap was used to separate water from the organic sample components. The sample with the internal standard was concentrated to approximately 0.5 mL in the cryogenic glass bead trap. The trap was then heated to 25C while slowly flushing with 50 mL of helium to transfer the organics to a secondary Tenax trap maintained at -50C. This process results in the transfer all of the VOCs with less than 1 μL of water. Then, while heating to 180C, the VOCs were back-flushed to be further focused on an open-tubular focusing trap at -160C. This cryofocusing trap was then ballistically heated to 100C, resulting in rapid injection of VOCs onto the analytical column.

Temperature programming of the GC column was used to obtain optimum results. Column temperature was held initially at -60C for 3 minutes, then raised to 250C at a rate of 8 degrees per minute. The GC-MSD was operated in the selected ion monitoring mode (SIM). Identification of target analytes by SIM analysis is based on a combination of chromatographic retention time and relative abundance of selected monitored ions.

An instrument calibration standard was prepared using stock gas standards prepared in the laboratory of the AAQD from three multi-component liquid mixtures and Scott certified gas mixture cylinders (Scott Environmental Technology Inc., Plumsteadville, PA). Quantification was based on daily 3-point linear regression calibration curves obtained from analysis of this external standard mixture.

1.3 Portable Infra-red Analyzer

The two infra-red analyzers used in the field tests were both Bruel & Kjaer (B&K) Multi-gas Monitor Type 1302 (Brüel & Kjaer, Nærum, Denmark). The B&K 1302 has an optical filter carousel which holds six discreet wavelength filters. The gas selectivity is dependant on the optical filters installed in the unit. The detection is performed on a closed cell using a paired photo-acoustic detector system. This method can be used in a field situation effectively, due to the ability to measure the differential values of the two detectors and eliminating effects of external vibration. Although portable, the instrument was connected to an AC source while in use.

The 1302 instruments were set up to monitor continuously throughout the sampling period. As soon as a sample had been analyzed, the cell was flushed and a new sample was drawn into the detection cell to begin the next analysis. Each cycle took approximately 2 minutes. For each sample cycle, data was generated for each of the wavelength filters. Data was stored internally by each 1302 instrument. One of the filters in each instrument is used to measure water vapour concentration. The results from this one filter were used to compensate for water vapour interference of the other filters. Results were calculated in mg/m^3. The data is stored with three significant figures. The resolution for this instrument is dependant on the calibration factor used and the range of the values during the test period. For this field test, the resolution was 0.01 mg/m^3, unless the value exceeded 10 mg/m^3, in which case the resolution was 0.1 mg/m^3.

Of the optical filters installed in the 1302 instruments, the filter that was used as a means to detect total hydrocarbons for this study allows light with wavelength between 3.3-3.5 μm to enter the detection cell. This waveband is known to be in the

range of IR energy absorbed by a typical carbon-hydrogen (C-H) bond, such as in a -CH_3 or -CH_2 group. This is a common organic molecular bond found on all aliphatic hydrocarbon vapours, as well as aromatic and other functional group compounds with an aliphatic portion or ligand. For example, benzene does not show an IR peak in this range, but ethylbenzene, toluene, and xylenes do, due to their aliphatic portions. The detection limit and the response factor varies depending on the compound. Because of the varied response to a wide range of compounds and a bias towards more responsive compounds, this is a general screening method and the value generated may not reflect the true balanced VOC concentration when the composition of the sample is unknown. The calibrant for this test was a certified Scott gas mixture of propane in air. Results will be presented in propane equivalents.

2.0 Results

2.1 Summa Canister Samples

Full laboratory analysis of the Summa canister samples includes speciated concentrations of 143 VOC/air toxic compounds (listed in Appendix A). Not all of the compounds are visible in the selected IR waveband. In order to present a comparison to results found with the IR instrument, a number of the compounds not visible with the selected IR filter were removed from the list. A total was then found for the remaining compounds. This total was used as an approximation of the VOCs in the sample which could be compared with the IR results. For each sample, there was a matching upwind sample collected. The values for each downwind sample was adjusted (reduced) by the total found at the upwind sample location (full results presented in Appendix B). A summary of the results is shown in Table 1 below.

Table 1: Totals for selected VOCs in relevant Summa samples

Burn Number †	1.1, 1A.1,2,3	2.1, 2.2	3.1, 3.2, 3.3	4.1, 4.2, 4.3 during burns	4.1, 4.2, 4.3 post-burn
Adjusted Total (μg/m^3)	5.69	4.87	1.98	1.53	1.76

†cumulative samples collected over consecutive burn periods

2.2 Infra-red Analyzer Data

Results generated for Figures 1 through 4 were done so by taking the differential values between the upwind and downwind stations. A positive result indicates that the downwind station had relatively higher readings than the upwind station. A negative reading is indicative of higher readings at the upwind station, not that unusual since the wind shifted considerably throughout the experiment. The data was recorded by the internal data logger of the IR detector. Results for the IR detector were recorded in parts per million (ppm). For the purposes of comparison with the cumulative Summa samples, they have been converted to μg/m^3. An average VOC concentration over the period of each burn was calculated as shown in Table 2.

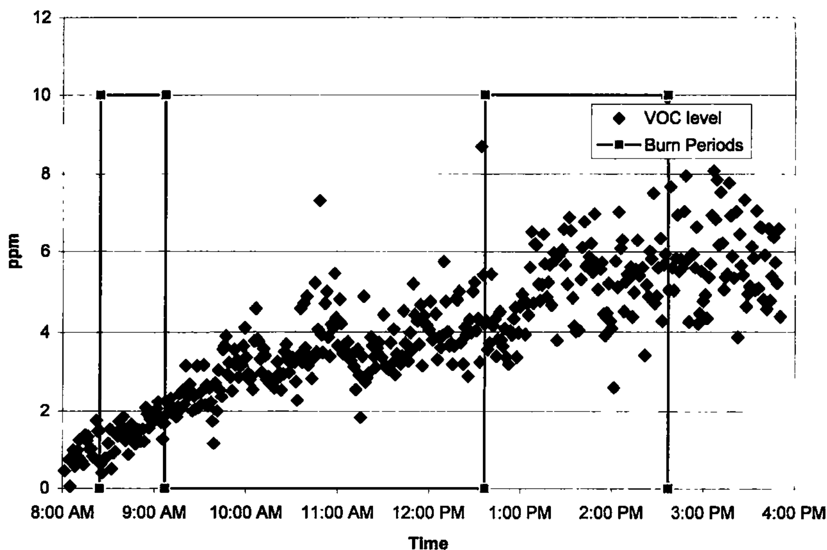

Figure 1 - Differential "VOC" levels from IR detectors
Boom 1 - Burn 1, Boom 1A - Burn 1, August 25, 1998

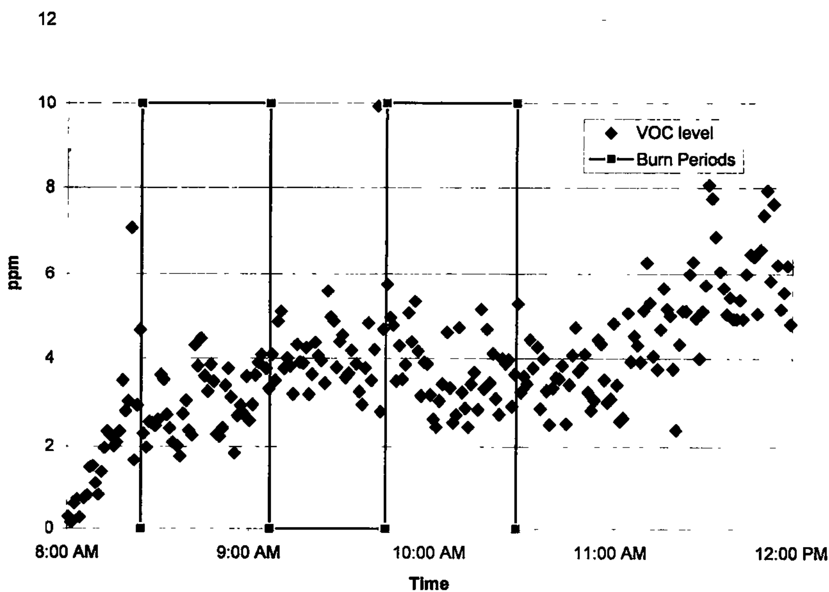

Figure 2 - Differential "VOC" levels from IR detectors
Boom 1A, Burns 2,3, August 26/98

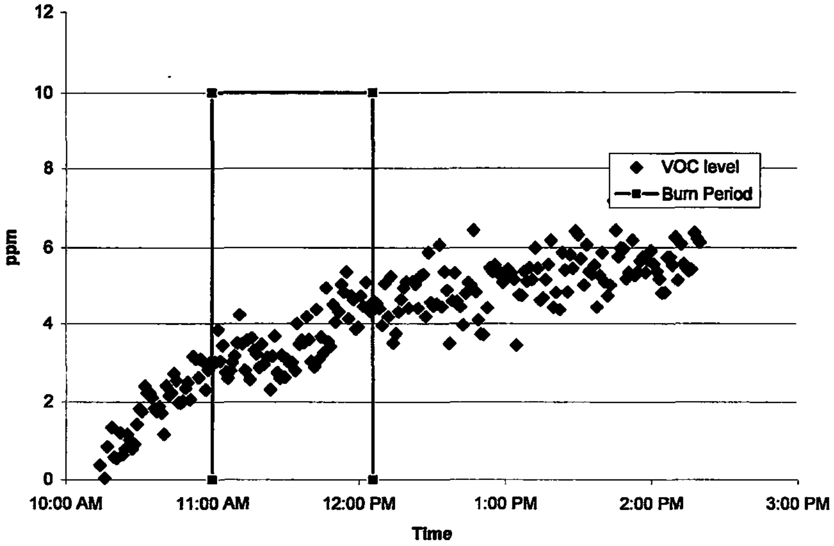

Figure 3 - Differential "VOC" levels from IR detectors
Boom 4, Burn 1, September 8, 1998

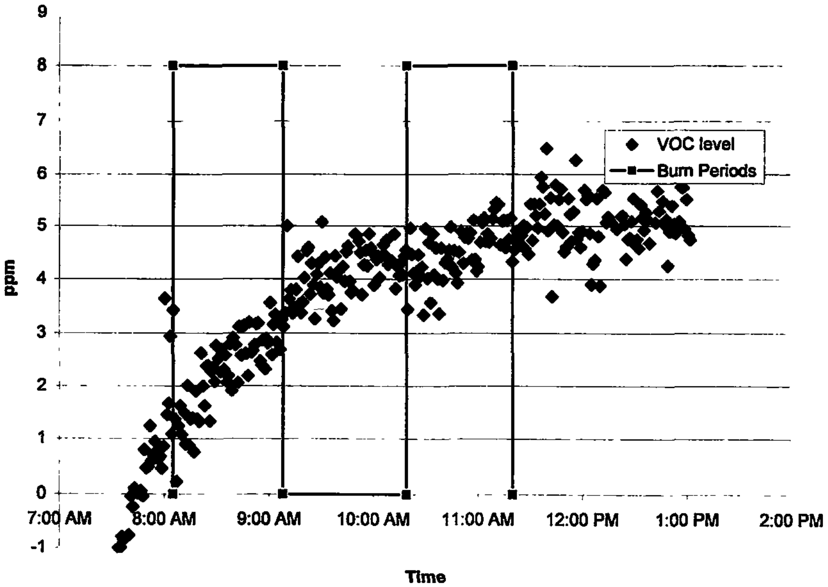

Figure 4 - Differential "VOC" levels from IR detectors
Boom 4, Burns 2,3, September 9, 1998

Table 2: Average VOC Concentration Using IR Detector

Burn Number	Average VOC Conc.* (μg/m^3)	Burn Status
1.1	9.27	ON
1A.1	34.2	ON
1A.2	20.3	ON
1A.3	24.9	ON
2.1	27.1	ON
4.1	23.8	ON
Post 4.1	31.7	OFF
4.2	15.5	ON
Post 4.2	27.5	OFF
4.3	30.2	ON
Post 4.3	34.1	OFF

*propane equivalent

3.0 Discussion

3.1 Experimental Conditions

While this experiment improved on the preliminary tests done the previous year (Goldthorp et al, 1998) in that the experimental validity of the data was much better, there were other factors which restricted the ability to make conclusions based on the gathered data. Although in the preliminary tests we were not able to make a direct comparison between canister sample results and IR data, due to instruments not being side by side, the field conditions were favourable. That is to say that, in the previous test series, the smoke plume being sampled was directed towards the instruments downwind of the burn tank. The stations at which the instruments were placed were deemed to be the prime locations to collect airborne vapours from the test tank, based on predicted wind patterns. These predictions held true in 1997, when the preliminary tests took place. These patterns were not consistent while the 1998 samples were being collected. Unfortunately, logistics does not allow the field operators to ideally place all of the equipment prior to each burn, but rather to outline a grid and maintain the instruments at those locations throughout the test period. A number of the tests conducted in this experiment proceeded although the smoke plume did not pass directly over the sampling instruments (placed in the location with the highest *likelihood* of being beneath the smoke plume). This has a severe effect on the experimental data. For one, in conditions where there was no upwind/downwind bias of the paired IR instruments relative to the smoke plume (in other words, the plume was as likely to swing over the "upwind" station as the "downwind " station), there was no experimental value in sampling. This was the case during the second

burn for boom 2 and all of the burns for boom 3. The conditions were so unfavourable towards the experiment that the IR instruments were not used during these burns, even though canister samples were collected.

Secondly, inconsistent sampling conditions during the collection of cumulative samples (with the Summa canisters) leads to potentially lower values as the VOC concentration is averaged to include both favourable and unfavourable sampling situations. For example, a burn for which the conditions are favourable (smoke plume directed over sampler) will produce samples with the highest concentration of airborne vapours due to the burn. A burn for which the conditions are not favourable (smoke directed away from the sampler) will produce samples that are less affected by the burn. When these samples are combined, as with the cumulative samples in this experiment, the quantities are diluted and an average concentration determined by the analysis will be lower than when the conditions are consistently favourable. In comparison with results collected in 1997, the VOC results presented here are consistently lower, due largely to the change in field conditions while the sampling was conducted.

3.2 Direct Comparison

When these two detection methods are compared directly, there are a number of considerations and allowances which must be made. As these methods are both capable of detecting volatile organic compounds, they are using different definitions of the "total VOC", which we have tried to rectify by eliminating some of the speciated compounds detected by the GC/MSD to more closely approximate what is being detected by the IR detector. However, the IR analyzer is strongly biased towards some compounds and the calibration is made to a single compound (propane) from which the "total" number is derived. Also, while the integration software in the IR instrument is designed to compensate for effects of temperature and humidity changes, large variances from calibration parameters can cause deviation in results (Williams, 1998).

Table 3: Direct Comparison of IR average with cumulative Summa Samples ($\mu g/m^3$)

Burn Number	1.1, 1A.1, 1A.2, 1A.3	2.1, 2.2	4.1, 4.2, 4.3	Post-burn 4.1, 4.2, 4.3
"VOC" from IR method (average)	22	27*	23	31
"VOC" from Summa Samples	5.7	4.9	1.5	1.8

* IR result from burn 2.1 only

With results as low as they are, it is difficult to determine if the results from the IR detectors are reliable, since the values found in the canister samples are below the detection limit for most of the VOC compounds detectable by the IR detectors. In order to better correlate the instrument response with the laboratory analysis, the concentration of hydrocarbons needs to be higher by probably two orders of magnitude, so that the values found would be well above the detection limit. At these

levels, it is difficult and ill-advised to derive any direct correlation between these two methods. Rather, we will concentrate on observed trends and expand on ideas presented in 1997.

3.3 Observed Trends

In preliminary testing, using only one IR detector, it was suggested that levels were, on average, lower during the burn than in the periods preceding and following the burn period. When using a background instrument for simultaneous data collection, this trend is clarified and validated. Understandably, the highest spikes were observed while fuelling was taking place prior to the burns. But also, the early part of the burn, in most cases, showed a persistent drop in the downwind VOC level. This level seemed to recover to pre-burn levels approximately 15-20 minutes into the burn period, but the net effect is that the average concentration would be lower than what was measured, on average, in the time previous to and following the burn. These trends were found consistently with the IR data throughout the experiment. It was suggested by trends in the preliminary test that in the period of time following the burn, there could be some residual emissions due to the inefficient combustion which occurs in the final stage of the burn, when the hot fuel residue which does not get consumed by the open fire is vaporized. Interestingly, the cumulative Summa canister sample collected in the period following the boom 4 burns did show agreement with the trend observed with the IR instruments. Both the uncorrected and background-corrected totals found higher VOC levels in the post-burn cumulative sample than the cumulative sample collected while the burn was taking place, as shown in Appendix 2.

Short-term elevations in VOC levels which occurred immediately prior to burn periods are likely due to the fuelling which lasted approximately 8 minutes prior to each burn. Preliminary testing using one instrument suggested that, since operators were in close proximity, that human activity might be the cause of the elevated levels. In this experiment, the comparison to a background level shows clearly that the spike is consistently downwind and, since operators were present at both stations, the elevated level can be more clearly linked with the fuelling. Although VOC levels in the post-burn period often fluctuated as much or more than at other times, markedly elevated levels were not consistently observed. Throughout the experiment, other small spikes were observed, but no gross fluctuations from background levels were found.

The advantage of real-time instrumentation is the ability to show short-term changes in the monitoring conditions, should they arise. Overall, the level of hydrocarbons, although low, was seen to be relatively consistent over the burn period, with slightly lower levels in the first 15-20 minutes. This would indicate that emissions from the burn do not vary widely over burn period. This experiment strengthens the confidence in the continued use of Summa canister sampling as a reasonable means to predict the hydrocarbon emissions from a burn of diesel fuel.

All of the observed trends are limited by the low VOC results found during the experiment. What cannot be disputed, however, is that without the IR detector in continuous sampling mode over the full day, including the monitoring of burn emissions as well as fuelling and pre- and post-burn periods, these trends could not have been observed at all.

4.0 Conclusions

While the aim of this experiment was to determine if IR instrumentation could be used to determine the level of hydrocarbons/VOC in low-level emissions, the levels proved to be too low to adequately postulate a correlation with traditional sampling methods. This lack of experimental control is, unfortunately, a reality when practising field experiments under variable conditions. The experiment was improved greatly by the introduction of a second upwind IR instrument and the differential levels of VOC helped to clarify the trends of VOC emissions, if not to correlate with the laboratory analysis. The indication that the early part of the burn may generate the lowest amount of VOC emissions is interesting, and may suggest that the open fire is hot and efficient enough to consume the majority of the VOCs from the fuel, as well as some of the ambient vapours. This trend would certainly be clarified with more favourable field conditions and more testing.

With the use of the two methods, we can get a more complete picture of the emission patterns. While with the IR instrument, we can offer near-real-time trends not available with canister sampling, the GC/MSD offers us speciation of compounds which the portable IR instrument cannot do. It may still be possible to formulate a relationship between these two sampling methods and to eventually consider IR detectors for on-site measurement of low-level hydrocarbon emissions. In the interim, these instruments have shown to be reliable in the field and can be used with confidence to show emission patterns and possible anomalies which cannot be determined using traditional sampling methods. When used in support of traditional sampling methods in emission monitoring, the IR detectors can be valuable in providing near-real-time information not previously available.

5.0 References

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Appendix A - Compounds Detected by Laboratory Analysis
(shaded compounds selected for comparison with IR results)

Results in µg/m3	25-Aug-98	25-Aug-98	25-Aug-98
Compound Name	DW2B	DW1B	DW3B
1-Propene	1.193	1.889	1.045
1-Propene	11.131	9.891	12.288
Freon 22 (Chlorodifluoromethane)	0.591	0.760	0.594
Freon 12 (Dichlorodifluoromethan)	2.456	2.553	2.465
Propyne			
Chloromethane	4.708	1.740	1.458
Isobutane (2-Methylpropane)	2.301	2.575	2.266
Freon 114 (1,2-Dichlorotetrafluor)	0.162	0.162	0.152
Vinylchloride (Chloroethylene)	0.177	ND	ND
1-Butene/2-Methylpropene	2.010	1.789	1.049
1,3-Butadiene	ND	0.358	0.127
Butane	5.145	5.436	5.313
t-2-Butene	0.125	0.202	0.112
2,2-Dimethylpropane	0.073	0.069	0.084
Bromomethane	0.627	0.115	0.386
1-Butyne	ND	ND	ND
c-2-Butene	0.140	0.184	0.097
Chloroethane	0.799	0.129	0.096
2-Methylbutane	6.180	6.386	6.141
Freon 11 (Trichlorofluoromethane)	2.529	15.479	2.040
1-Pentene	0.281	0.453	0.223
2-Methyl-1-Butene	ND	0.257	ND
Pentane	4.162	4.683	4.394
Isoprene (2-Methyl-1,3-Butadiene)	1.873	2.046	2.070
Ethylbromide	0.086	ND	ND
t-2-Pentene	0.099	0.178	0.061
1,1-Dichloroethylene	0.074	0.080	ND
c-2-Pentene	0.091	0.188	0.097
Dichloromethane	0.929	0.579	0.339
2-Methyl-2-Butene	0.324	0.487	0.228
Freon 113 (1,1,2-Trichlorotrifluor)	0.770	1.080	0.748
2,2-Dimethylbutane	0.260	0.433	0.266
Cyclopentene	0.055	0.083	0.031
t-1,2-Dichloroethylene	0.120	ND	0.067
4-Methyl-1-Pentene	ND	0.153	ND
3-Methyl-1-Pentene	ND	ND	ND
Cyclopentane	0.267	0.381	0.267
1,1-Dichloroethane	ND	ND	ND
2,3-Dimethylbutane	0.363	0.719	0.355
t-4-Methyl-2-Pentene	ND	ND	ND
2-Methylpentane	NDR	NDR	NDR
c-4-Methyl-2-Pentene	ND	ND	ND
3-Methylpentane	0.828	1.815	0.933
1-Hexene/2-Methyl-1-Pentene	ND	0.690	ND
c-1,2-Dichloroethylene	ND	0.074	ND
n-Hexane	1.879	2.293	1.771

* samples shown for illustration only - full results presented in Appendix B

Appendix A - Compounds Detected by Laboratory Analysis
(shaded compounds selected for comparison with IR results)

Compound			
Chloroform	0.417	0.259	0.203
t-2-Hexene	ND	ND	ND
2-Ethyl-1-Butene	ND	0.152	ND
t-3-Methyl-2-Pentene	ND	ND	ND
c-2-Hexene	ND	ND	ND
c-3-Methyl-2-Pentene	ND	ND	ND
2,2-Dimethylpentane	ND	ND	ND
1,2-Dichloroethane	0.053	0.089	0.048
Methylcyclopentane	0.428	1.190	0.418
2,4-Dimethylpentane	ND	0.331	0.120
1,1,1-Trichloroethane	0.322	0.330	0.324
2,2,3-Trimethylbutane	ND	ND	ND
1-Methylcyclopentene	0.062	0.136	0.028
Benzene	1.188	1.553	0.894
Carbontetrachloride	0.532	0.463	0.490
Cyclohexane	0.292	0.620	0.262
2-Methylhexane	0.574	1.524	0.571
2,3-Dimethylpentane	0.270	0.766	0.259
Cyclohexene	ND	ND	ND
3-Methylhexane	0.619	1.644	NDR
Dibromomethane	0.110	0.072	0.091
1,2-Dichloropropane	ND	ND	ND
Bromodichloromethane	NDR	NDR	NDR
Trichloroethylene	0.134	0.125	0.173
1-Heptene	ND	1.000	ND
2,2,4-Trimethylpentane	0.398	1.624	0.496
t-3-Heptene	ND	ND	ND
c-3-Heptene			
Heptane	1.051	1.518	1.217
t-2-Heptene	ND	ND	ND
c-2-Heptene	ND	ND	ND
c-1,3-Dichloropropene	ND	ND	ND
2,2-Dimethylhexane	ND	NDR	ND
Methylcyclohexane	0.633	1.277	0.775
2,5-Dimethylhexane	0.156	0.361	0.184
2,4-Dimethylhexane	0.111	0.427	0.154
t-1,3-Dichloropropene	ND	ND	ND
1,1,2-Trichloroethane	ND	ND	ND
Bromotrichloromethane	ND	ND	ND
2,3,4-Trimethylpentane	ND	0.593	0.175
Toluene	3.196	6.040	3.760
2-Methylheptane	0.375	0.837	0.462
1-Methylcyclohexene	ND	0.117	ND
4-Methylheptane	ND	0.440	0.200
Dibromochloromethane	0.066	0.037	ND
3-Methylheptane	0.289	0.793	0.375
c-1,3-Dimethylcyclohexane	0.141	0.286	0.134
t-1,4-Dimethylcyclohexane	0.080	0.152	0.088
EDB (1,2-Dibromoethane)	0.279	0.620	0.236

* samples shown for illustration only - full results presented in Appendix B

Appendix A - Compounds Detected by Laboratory Analysis
(shaded compounds selected for comparison with IR results)

2,2,5-Trimethylhexane	0.038	0.193	0.047
1-Octene	ND	0.619	ND
Octane	0.609	1.002	0.577
t-1,2-Dimethylcyclohexane	0.106	0.264	0.131
t-2-Octene	ND	ND	ND
Tetrachloroethene	0.288	0.190	0.397
c-1,4/t-1,3-Dimethylcyclohexane	0.037	0.113	0.043
c-2-Octene	ND	ND	ND
Chlorobenzene	0.419	0.181	0.106
Ethylbenzene	1.332	2.588	1.436
m/p-Xylene	4.887	8.509	5.960
Bromoform	0.084	0.049	0.038
1,4-Dichlorobutane	ND	ND	ND
Styrene	0.096	1.650	0.233
1,1,2,2-Tetrachloroethane	0.068	0.125	0.046
o-Xylene	1.300	2.925	1.624
1-Nonene			
n-Nonane	0.771	1.439	0.485
iso-Propylbenzene	0.092	0.147	0.067
3,6-Dimethyloctane	ND	0.147	ND
n-Propylbenzene	0.239	0.353	0.213
3-Ethyltoluene	0.493	1.101	0.452
4-Ethyltoluene	0.302	0.550	0.246
1,3,5-Trimethylbenzene	0.312	0.953	0.450
2-Ethyltoluene	0.227	0.521	0.183
tert-Butylbenzene	ND	ND	ND
1,2,4-Trimethylbenzene	0.923	2.215	0.793
1-Decene			
Benzylchloride	0.171	ND	ND
1,3-Dichlorobenzene	0.124	0.049	0.041
Decane	0.803	1.708	0.453
1,4-Dichlorobenzene	0.168	0.091	0.070
iso-Butylbenzene	0.052	0.069	0.031
sec-Butylbenzene	0.040	0.070	0.027
1,2,3-Trimethylbenzene	0.279	0.770	0.211
p-Cymene	0.211	0.254	0.165
1,2-Dichlorobenzene	0.190	0.067	0.057
Indan	0.081	0.186	0.069
1,3-Diethylbenzene	0.082	0.139	0.043
1,4-Diethylbenzene	0.257	0.457	0.147
n-Butylbenzene	0.076	0.119	0.048
1,2-Diethylbenzene	0.069	0.053	ND
Undecane	1.112	3.487	0.514
1,2,4-Trichlorobenzene	0.986	0.159	0.118
Naphthalene	1.184	0.791	0.310
Dodecane	1.745	6.501	0.674
Hexachlorobutadiene	0.529	0.048	0.032
Hexylbenzene	0.284	0.217	0.095

* samples shown for illustration only - full results presented in Appendix B

Appendix B - Selected Speciated Components and VOC Totals for all Samples

Sample Date	25-Aug-98	25-Aug-98	27-Aug-98	27-Aug-98	3-Sep-98	3-Sep-98	9-Sep-98	9-Sep-98	9-Sep-98	9-Sep-98
Sample Location	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B
Misc. Information	Boom 1, 1A	Boom 1, 1A	Boom 2	Boom 2	Boom 3	Boom 3	Boom 4	Boom 4	Post-Burn	Post-Burn
Initial Volume (mL)	2755	4356	1013	1296	3964	4314	3680	4547	5815	4272
Dilution Factor	3	2	8	7	2	2	2	2	2	2
Results in µg/m3										
Compound Name										
1-Propene	1.193	1.739	1.828	0.797	1.281	1.882	0.593	0.918	0.288	0.529
1-Propene	11.131	8.719	4.708	4.480	5.209	4.733	2.983	2.799	3.497	3.262
Isobutane (2-Methylpropane)	2.301	1.877	0.749	0.703	2.230	2.248	1.343	1.202	1.557	1.356
1-Butene/2-Methylpropene	2.010	1.788	3.660	1.097	1.046	1.632	0.383	0.638	0.522	0.442
1,3-Butadiene	ND	ND	ND	ND	0.107	0.319	ND	ND	ND	ND
Butane	5.145	4.359	1.453	1.446	5.080	4.496	2.625	2.061	2.792	2.599
t-2-Butene	0.125	0.228	ND	ND	0.144	0.184	0.063	0.167	0.101	0.101
2,2-Dimethylpropane	0.073	0.059	ND	ND	0.069	0.070	0.051	0.044	0.053	0.035
c-2-Butene	0.140	0.267	ND	ND	0.153	0.158	ND	0.118	0.066	0.084
Chloroethane	0.799	0.168	ND	ND	0.106	0.123	0.064	0.086	0.093	0.096
2-Methylbutane	6.180	5.236	1.744	1.872	7.118	6.666	2.331	1.844	2.421	2.264
1-Pentene	0.281	0.445	0.875	ND	0.324	0.527	ND	0.131	0.097	0.094
2-Methyl-1-Butene	ND	ND	ND	ND	0.225	0.226	ND	ND	ND	ND
Pentane	4.162	3.699	1.201	1.199	4.025	3.741	1.627	1.307	1.914	1.780
Isoprene (2-Methyl-1,3-Butadier	1.873	2.861	0.984	1.616	0.468	0.600	0.388	0.695	0.878	0.770
Ethylbromide	0.086	0.258	ND	ND	ND	ND	ND	ND	ND	ND
t-2-Pentene	0.099	0.104	ND	ND	0.177	0.185	ND	0.027	ND	0.023
c-2-Pentene	0.091	0.152	ND	ND	0.186	0.188	ND	ND	ND	0.057
2-Methyl-2-Butene	0.324	0.392	ND	ND	0.477	0.464	0.083	0.102	0.111	0.108
2,2-Dimethylbutane	0.260	0.207	ND	0.161	0.396	0.372	0.137	0.118	0.183	0.132
Cyclopentene	0.055	0.051	0.108	ND	0.108	0.069	ND	0.023	0.030	0.028
4-Methyl-1-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methyl-1-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyclopentane	0.267	0.240	ND	ND	0.330	0.284	0.125	0.079	0.113	0.108
2,3-Dimethylbutane	0.363	0.302	ND	ND	0.451	0.421	0.136	0.092	0.127	0.102

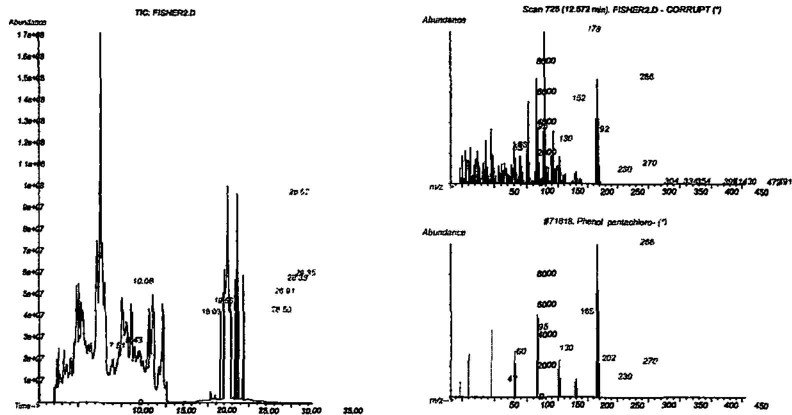

Figure 3: SFE-GC/MSD analysis of Fisher Soil, showing PCP

Appendix B - Selected Speciated Components and VOC Totals for all Samples

t-4-Methyl-2-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2-Methylpentane	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR	NDR
c-4-Methyl-2-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methylpentane	0.828	0.961	ND	0.384	0.974	1.018	0.352	0.326	0.493	0.407
1-Hexene/2-Methyl-1-Pentene	ND	0.444	1.139	ND	0.304	0.640	ND	0.253	ND	ND
n-Hexane	1.879	2.092	0.527	0.610	2.096	1.993	0.703	0.564	0.888	0.790
t-2-Hexene	ND	ND	ND	ND	ND	0.052	ND	ND	ND	ND
t-3-Methyl-2-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-2-Hexene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-3-Methyl-2-Pentene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-Dimethylpentane	ND	ND	ND	ND	0.070	ND	ND	ND	ND	ND
Methylcyclopentane	0.428	0.611	ND	ND	0.675	0.510	0.220	0.114	0.267	0.149
2,4-Dimethylpentane	ND	0.134	ND	ND	0.194	0.200	ND	ND	ND	ND
2,2,3-Trimethylbutane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1-Methylcyclopentene	0.062	0.037	ND	ND	0.095	0.067	0.028	0.056	0.030	0.027
Cyclohexane	0.292	0.324	ND	ND	0.245	0.199	0.109	0.068	0.109	0.086
2-Methylhexane	0.574	0.421	ND	ND	0.498	0.613	0.170	0.146	0.224	0.159
2,3-Dimethylpentane	0.270	0.194	ND	ND	0.371	0.413	0.122	ND	0.122	0.118
Cyclohexene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-Methylhexane	0.619	NDR	ND	ND	0.646	0.677	NDR	0.179	NDR	0.218
1-Heptene	ND	0.320	0.640	ND	ND	0.480	ND	ND	ND	ND
2,2,4-Trimethylpentane	0.398	0.334	0.254	0.359	0.682	0.912	0.155	0.145	0.160	0.146
t-3-Heptene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-3-Heptene										
Heptane	1.051	0.704	0.370	0.484	0.821	0.806	0.252	0.241	0.363	0.322
t-2-Heptene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-2-Heptene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,2-Dimethylhexane	ND	ND	ND	ND	ND	NDR	ND	ND	ND	ND
Methylcyclohexane	0.633	0.338	ND	0.130	0.345	0.330	0.122	0.098	0.158	0.141
2,5-Dimethylhexane	0.156	0.096	ND	ND	0.123	0.169	0.040	0.059	ND	0.039
2,4-Dimethylhexane	0.111	0.095	ND	ND	0.122	0.186	0.061	0.030	ND	0.051
2,3,4-Trimethylpentane	ND	0.112	ND	ND	0.252	0.340	ND	0.043	ND	ND
Toluene	3.196	2.561	1.211	1.366	3.700	3.780	1.104	0.997	1.289	1.189

Appendix B - Selected Speciated Components and VOC Totals for all Samples

2-Methylheptane	0.375	0.172	ND	ND	0.275	0.300	ND	0.106	0.134	0.093
1-Methylcyclohexene	ND	ND	ND	ND	ND	ND	ND	0.056	0.054	ND
4-Methylheptane	ND	0.094	ND	ND	0.128	0.117	ND	ND	ND	ND
3-Methylheptane	0.289	0.176	ND	ND	0.293	0.311	ND	0.090	0.121	0.081
c-1,3-Dimethylcyclohexane	0.141	0.052	ND	ND	0.070	0.069	0.025	0.029	0.057	0.036
t-1,4-Dimethylcyclohexane	0.080	0.034	ND	ND	0.050	0.054	ND	ND	0.027	ND
2,2,5-Trimethylhexane	0.038	0.039	ND	ND	0.080	0.083	ND	0.016	ND	ND
1-Octene	ND	0.581	1.272	ND	0.674	0.469	ND	ND	ND	ND
Octane	0.609	0.314	0.265	0.294	0.510	0.483	0.133	0.138	0.174	0.136
t-1,2-Dimethylcyclohexane	0.106	0.047	ND	ND	0.074	0.066	ND	ND	ND	ND
t-2-Octene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
c-1,4/t-1,3-Dimethylcyclohexan	0.037	ND	ND	ND	0.033	0.036	ND	ND	ND	ND
c-2-Octene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethylbenzene	1.332	1.996	0.253	0.258	0.802	0.785	0.235	0.220	0.200	0.208
m/p-Xylene	4.887	7.135	0.703	0.768	2.454	2.490	0.563	0.477	0.504	0.488
Styrene	0.096	0.550	ND	ND	0.128	0.095	ND	0.045	0.049	0.047
o-Xylene	1.300	1.537	0.266	0.286	0.872	0.831	0.235	0.199	0.242	0.223
1-Nonene										
n-Nonane	0.771	0.397	ND	0.545	0.610	0.435	0.233	0.112	0.186	0.150
iso-Propylbenzene	0.092	0.068	0.079	0.057	0.083	0.075	0.029	0.033	0.037	0.030
3,6-Dimethyloctane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Propylbenzene	0.239	0.181	0.108	0.117	0.246	0.200	0.076	0.084	0.068	0.061
3-Ethyltoluene	0.493	0.422	0.118	0.196	0.640	0.545	0.129	0.102	0.116	0.097
4-Ethyltoluene	0.302	0.234	0.140	0.161	0.349	0.301	0.090	0.104	0.084	0.074
1,3,5-Trimethylbenzene	0.312	0.322	0.076	0.120	0.344	0.288	0.067	0.047	0.049	0.047
2-Ethyltoluene	0.227	0.200	0.091	0.107	0.297	0.218	0.075	0.063	0.065	0.049
tert-Butylbenzene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,2,4-Trimethylbenzene	0.923	0.716	0.294	0.368	1.069	0.893	0.241	0.193	0.185	0.170
1-Decene										
Decane	0.803	0.453	0.282	0.298	0.767	0.419	0.267	0.127	0.215	0.120
iso-Butylbenzene	0.052	0.033	ND	ND	0.031	0.030	0.017	0.024	0.022	0.019
sec-Butylbenzene	0.040	0.029	ND	0.036	0.037	0.033	0.022	0.018	0.027	0.018
1,2,3-Trimethylbenzene	0.279	0.174	0.118	0.109	0.258	0.200	0.075	0.063	0.059	0.061

Appendix B - Selected Speciated Components and VOC Totals for all Samples

p-Cymene	0.211	0.191	ND	ND	0.071	0.102	0.076	0.070	0.084	0.071
1,3-Diethylbenzene	0.082	0.037	0.084	0.063	0.121	0.094	0.034	0.036	0.038	0.023
1,4-Diethylbenzene	0.257	0.142	0.123	0.166	0.306	0.208	0.109	0.103	0.072	0.069
n-Butylbenzene	0.076	0.040	ND	0.077	0.076	0.057	0.039	0.042	0.042	0.026
1,2-Diethylbenzene	0.069	ND	ND	ND	0.045	0.043	0.027	0.038	0.028	ND
Undecane	1.112	0.386	0.200	0.318	1.045	0.401	0.469	0.153	0.205	0.134
Naphthalene	1.184	0.320	0.397	0.298	0.430	0.290	0.191	0.243	0.119	0.123
Dodecane	1.745	0.326	0.302	0.406	1.139	0.360	0.751	0.253	0.222	0.204
"VOC" Total	**66.02**	**60.33**	**26.62**	**21.76**	**56.33**	**54.36**	**20.58**	**19.06**	**22.43**	**20.67**

ND = Not Detected
NDR = Not Detected due to incorrect ion ratio
XS = Concentration exceeded detector range

Sample Date	25-Aug-98	25-Aug-98	27-Aug-98	27-Aug-98	3-Sep-98	3-Sep-98	9-Sep-98	9-Sep-98	9-Sep-98	9-Sep-98
Sample Location	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B	DW2B	UW1B
Misc. Information	Boom 1, 1A	Boom 1, 1A	Boom 2	Boom 2	Boom 3	Boom 3	Boom 4	Boom 4	Post-Burn	Post-Burn
Initial Volume (mL)	2755	4356	1013	1296	3964	4314	3680	4547	5815	4272
Dilution Factor	3	2	8	7	2	2	2	2	2	2

Sampling and Analysis of Shredded Automobile Residue

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Abstract

Shredded automotive residue usually contains contaminants of concern such as Polychlorinated Biphenyls (PCBs) and heavy metals. Automotive shredder waste is very heterogeneous and contains many other shredded materials. Because of this, focus must be placed on acquiring and processing large samples and designing a system to achieve a highly-representative sample of the material. Various methods of designing and implementing a sampling program are described.

The processes used to shred automotive and other similar scrap are reviewed and the characteristics of the various streams given. Typical contents of the various streams are described and densities given.

Calculations for the minimum number of samples for a given pile are reviewed. Procedures used to acquire samples are reviewed and suggestions made as to optimal procedures to acquire representative samples. Procedures and sampling equipment are summarized.

1.0 Introduction

Shredded automotive waste can contain contaminants of concern such as PCBs and heavy metals. Automotive shredder residue (ASR) is very heterogeneous and contains many other shredded materials such as industrial machinery, farm machinery, domestic waste, industrial waste, drums, electrical appliances, industrial electrical equipment, soil, and general garbage. In addition it may contain larger portions of the same material that have bypassed the shredding process. It may also contain completely extraneous materials that have been dumped or spilled onto the piles. Because of this, focus must be placed on acquiring and processing large samples and designing a system to achieve a highly representative sample of the pile. Some sampling and analytical schemes which collect and extract only a few grams of sample may result in data that are not representative of the entire mass of the residue piles.

Shredded automotive residue derives from the extraction of the ferrous material from a shredder. Some processes are shown in Figure 1. It is important to emphasize that each recycling operation is somewhat different. Some operations only have a shredder and a magnetic conveyor or roller unit. Other operations separate the shredded material into as many as 7 separate components, several of which are recycled. Approximately 25% of a car's mass is not recycled by land-filling (CARE, 1999). The same source estimates that 5% of a car's weight consists of rubber, mostly tires, and 50% of the landfill is rubber. It is felt by the present authors, however, that given current tire recycling that this figure is probably much less, probably as little as 5%.

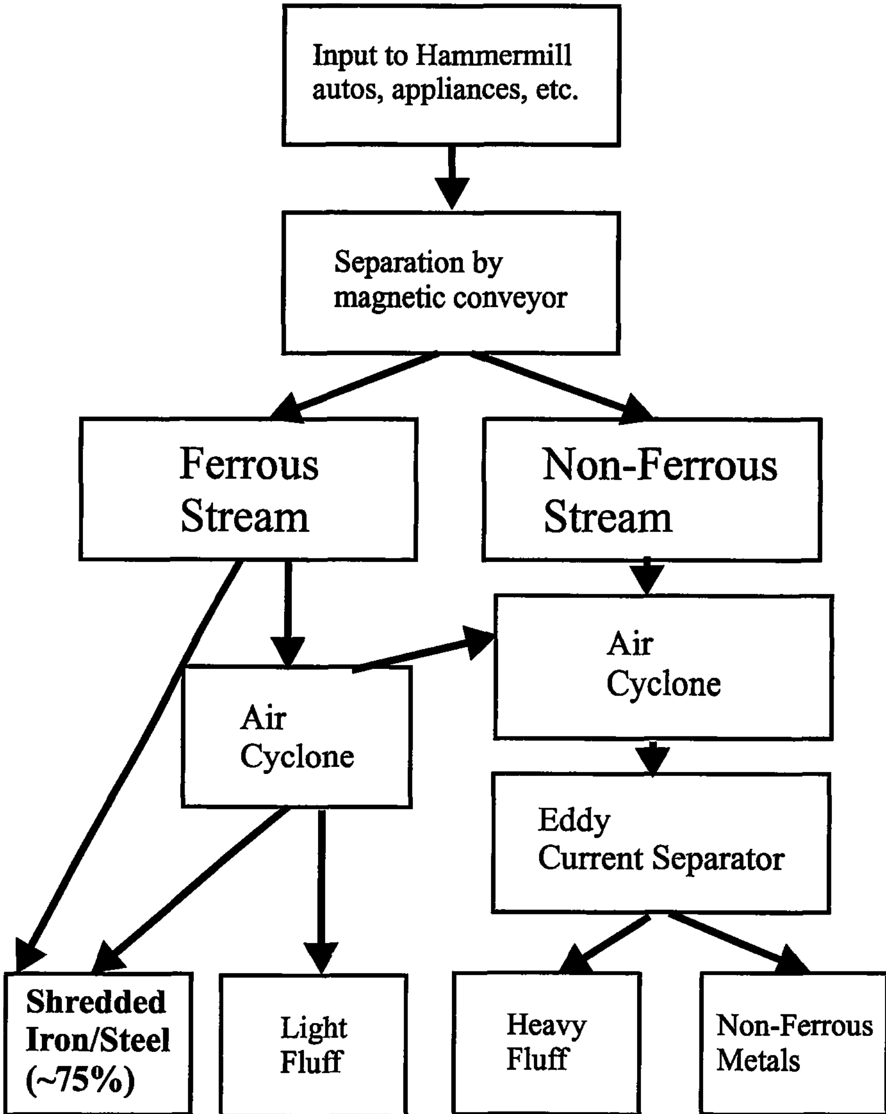

Figure 1 Processes in Automotive Shredding

Alternatives to the land-filling of ASR have been proposed, including use of the material as a day-cover at landfills, recycling of selected plastics, use as a source of fuel, and recycling of bulk as consumer products such as fence posts, picnic tables, flower pots, etc. Day (1996) evaluated ASR as a landfill day cover and measured the leachability of metals. Day noted that ASR retained metals very well and with the other good characteristics of the material, was an excellent day cover. Consideration of the PCB content and its leaching into the environment was not made.

2.0 Characteristics of ASR

Day (1996) summarized the characteristics of several ASR samples, as shown in Table 1.

Table 1 **Characteristics of Selected ASR Samples**
(Day, 1996)

Parameter	Units	Study of Several Sites (1990)	1991 Sample	1995 Sample	European Study 1996
Bulk density	kg/m^3	404	495	409	358
Moisture	%	6	16	4.3	6.6
Particle size	%				
	<2 mm	37	33	47	44
	2 to 26.5 mm	45	51	36	38
	>26.5	18	16	17	18
Calorific value	MJ/kg	15	12	13	11
Ash residues	%	58	61	60	59
Elemental Analysis	%				
	C		28	30	33
	H		4	4	4
	N		0.9	1.3	0.6
	Cl		0.5	5.7	1.6
	S		0.3	0.3	0.2
Metals	%				
	Fe		18	13	16
	Cu	1.1	0.6	0.8	2.4
	Zn	0.9	0.9	0.7	0.8
	Al		1.4	1.1	2.9
Heavy Metals	Pb	0.28	0.24	0.4	0.65
	Cd	0.007	0.005		0.006
	Cr	0.04	0.02	0.04	0.07
	Ni			0.07	0.09

Day *et al.* (1993) also studied ASR from 5 shredder operations in Ontario and Quebec and took 22 samples and analyzed them. The results of this are shown in Table 2.

Table 2 **Characteristics of ASR from Canadian Sources**
(Day *et al*. 1993)

Parameter	Units	Minimum Value	Maximum Value	Average
Bulk density	kg/m^3	218	728	404
Moisture	%	0.94	26	6
Particle size	%			
	<2 mm	17	69	37
	2 to 26.5 mm	25	54	45
	>26.5	6.6	39	18
Calorific value	MJ/kg	7.1	16	12
Ash residues	%	41	73	58
Metals	%			
	Zn	0.37	1.5	0.92
	Pb	0.12	0.61	0.28
	Cd	0.0017	0.043	0.0065
	Cr	0.009	0.1	0.042
	Cu	0.26	2.4	1.1
Leachable Metals	ppm			
	Zn	160	400	250
	Pb	0.9	60	7
	Cd	0.3	2.6	0.66
	Cr	<.3	0.39	0.32
	Cu	<.3	5.2	0.69

Table 2 and 3 show that a large percent of the ASR (between 40 to 75 %) can consist of ash or unburnable material. This is the primary reason that the use of ASR as a supplementary fuel is not efficient. Furthermore, the calorific value is low. As can be seen by the high metal content, disposal of ash from ASR would result in a potential threat to the environment from leachable metals, whereas the leaching of metals from ASR itself is lower. Day and Awadalla (1995) studied the leachability of metals from ASR. Table 3 shows the average leachability of heavy metals using different protocols. It is important to note the protocols can yield very different results.

Table 3 **Leachability of Metals from ASR**
(Day and Awadalla, 1995)

Metal	Average Concentrations Leached in mg/L Protocol			
	Quebec	Ontario	EPTOX	TCLP
Zinc	215	81	89	118
Lead	4.4	2	1.5	5.7
Cadmium	0.5	0.3	0.3	0.4
Chromium	<.3	<.3	<.3	<.3
Copper	1.1	1.7	1.5	1.9

Westat (1991) reported on a study of 7 sites and as many as 28 samples to characterize the PCB, lead and zinc content of ASR. This is summarized in Table 4.

Table 4 **Analysis of ASR samples for PCB, Lead and Cadmium**
(Westat, 1991)

Sample Type	Input Type	Average Concentration (ppm) PCBs	Lead	Cadmium	Number of Samples	Number of Sites
fresh fluff	auto	32	2700	47	28	7
fresh fluff	white goods	80	3100	48	15	5
fresh fluff	mixed input	180	4600	46	9	3
stored fluff		68	3900	35	10	5
spillover*		28	6100	32	5	5
ferrous		0.2			8	6
non-ferrous		1			5	3
soil		44	2200	22	8	4

** spillover is light fluff that collects on the side of conveyors*

Westat (1991) also reported on a study to characterize the concentration of various substances in some ASR samples. This data is presented in Table 5.

Table 5 **Components of Selected Fluff Samples**
(Westat, 1991)

Component(s)	Percent of Total Weight Sample 1	Sample 2	White Goods	Mixed Inputs	Average
Metals, wire and glass	11	2	3	2	5
Soft plastics, foams, soft rubber, vinyl	17	14	8	17	14
Fabrics, paper and wood	17	28	9	26	20
Hard materials, hard plastics, hard rubber	9	2	10	5	7
Fines, dirt, dust	40	38	65	45	47
Other	6	16	5	5	8

The present authors took two random samples of ASR and sorted these into several different categories. Each category was then weighed and the volume estimated. The resulting data are shown in Table 6.

Tables 5 and 6 show that about half of the weight of ASR samples is composed of fine materials and that about half of the volume is constituted of foam and fabric material. This sets the stage for the difficulty in getting and analyzing a representative sample of the material for contaminants such as PCBs.

3.0. PCBs in ASR

Westat (1991) studied the PCBs and other hazardous materials in major components of the fluff. These data are summarized in Table 7. This shows that the PCBs reside mostly in the fine and absorbent materials, however there is a large amount of variability in data because of the broad categories of components used.

Table 6 **Analysis of Two ASR Samples**

By Weight

Item	Weight	%	Volume	%
misc. fines	428.2	40.7	3	14.5
mixed foam/fluff	167.3	15.9	4	19.3
carpet/insulation	100.7	9.6	4	19.3
nonferous scrap	66.6	6.3	0.5	2.4
polyurethane foam	64.7	6.2	3.8	18.3
wood	57.2	5.4	1	4.8
plastic parts	36.8	3.5	1	4.8
domestic waste	28.0	2.7	0.7	3.2
rubber parts	24.8	2.4	0.5	2.4
cardboard	17.7	1.7	0.7	3.2
rubber tubing	17.2	1.6	0.1	0.6
electrical wire	14.1	1.3	0.3	1.6
glass	12.5	1.2	0.1	0.6
fabric	10.3	1	0.5	2.4
styrofoam	4.8	0.5	0.5	2.4

By Volume

Item	Weight	%	Volume	%
mixed foam/fluff	167.3	15.9	4	19.3
carpet/insulation	100.7	9.6	4	19.3
polyurethane foam	64.7	6.2	3.8	18.3
misc. fines	428.2	40.7	3	14.5
wood	57.2	5.4	1	4.8
plastic parts	36.8	3.5	1	4.8
domestic waste	28.0	2.7	0.7	3.2
cardboard	17.7	1.7	0.7	3.2
nonferous scrap	66.6	6.3	0.5	2.4
rubber parts	24.8	2.4	0.5	2.4
fabric	10.3	1	0.5	2.4
styrofoam	4.8	0.5	0.5	2.4
electrical wire	14.1	1.3	0.3	1.6
rubber tubing	17.2	1.6	0.1	0.6
glass	12.5	1.2	0.1	0.6

Weight is in grams, Volume is relative

This table shows that the content of each component is variable, however

Table 7 **PCBs in Components of Selected Fluff Samples**
(Westat, 1991)

	Sample 1		Sample 2		White Goods		Mixed Inputs	
Component(s)	% weight	PCBs (ppm)	% weight	PCBs (ppm)	% weight	PCBs (ppm)	% weight	PCBs (ppm)
Metals, wire and glass	11	13	2	9.9	3	0.06	2	39
Soft plastics, foams, soft rubber, vinyl	17	66	14	7	8	35	17	260
Fabrics, paper and wood	17	37	28	12	9	24	26	63
Hard materials, hard plastics, hard rubber	9	11	2	24	10	5.5	5	46
Fines, dirt, dust	40	43	38	29	65	62	45	140
Other	6		16		5		5	

there is little PCB on impermeable material, and a large portion of the PCBs are in the fines and in the permeable foams. Analysis by the present authors shows similar results, however, each component has not been analyzed.

4.0 Effect of Heterogeneous Sampling

The effect of heterogeneous sampling can be illustrated by using a numerical example such as given in Table 8. The PCB content of each sample was determined using Table 7 or estimated on the basis of past analysis by the authors. Column 1 lists the names of the material; column 2, the weight of the material and 3, the percentage by weight. Column 4 gives the relative volume, and 5 the relative volume percent of the sample. Column 6 gives the approximate number of items in a lot and obviously, the number of fines is a default number. The next column, gives the PCB concentration in the particular component and column 8 gives the contribution of that particular component to the overall PCB concentration. The last row in the column shows that this particular sample had an overall PCB concentration of 89 ppm. The next three sets of columns show the effect on the overall PCB concentration, of various sub-sampling schemes. The first sub-sampling scheme, known as sampling by equal weight of components, shows that this method results in an overall concentration value of 28 ppm. Example 2 shows the effect of taking one piece of each component which results in a PCB concentration of only 8 ppm for the entire sub-sample. Example 3 shows the effect of taking representative pieces of the sub-sample, a procedure which is advocated in the literature. The net result of this method is to show a PCB concentration of 21 ppm for the entire sub-sample.

Sub-sampling inevitably results in lower PCB values because of the fact that the heavier pieces have very little PCB content and because the highest PCB content is in the fines portion, which is often not proportionately taken in sub-sampling procedures. This illustrates the importance of correct sampling procedures and analyzing as large a portion of the material as is possible, to avoid sub-sampling.

5.0 Calculation of Minimum Samples

The residue from automotive and scrap metal shredding is not homogeneous, therefore appropriate measures must be taken to ensure that representative and sufficient samples are taken from piles (EPA, 1993; EPA 1992). The accepted statistical method for calculating the minimum number of samples is given by:

Table 8 **Example of the Effects of Sub-sampling on PCB Content**

Item	weight	%	Volume	%	Approximate number	PCB conc. in fraction(ppm)	PCB contribution	Example 1 Component weight (g)	Equal Weight Sampling	Example 2 Component weight (g)	one piece of each	Example 3 Component weight (g)	representative pieces
misc. fines	428.2	40.7	3	14.5	1000	200	81.4	10	20	9	0	5	10
mixed foam/fluff	167.3	15.9	4	19.3	10	20	3.18	10	2	17	3.4	15	3
carpet/insulation	100.7	9.6	4	19.3	7	20	1.92	10	2	14	2.8	15	3
nonferrous scrap	66.6	6.3	0.5	2.4	5	0.1	0.01	10	0.01	13	0.01	15	0.02
polyurethane foam	64.7	6.2	3.8	18.3	12	30	1.86	10	3	5	1.5	15	4.5
wood	57.2	5.4	1	4.8	20	0.2	0.01	10	0.02	3	0.01	15	0.03
plastic parts	36.8	3.5	1	4.8	8	0.3	0.01	10	0.03	5	0.02	5	0.02
domestic waste	28.0	2.7	0.7	3.2	10	0.4	0.01	10	0.04	3	0.01	5	0.02
rubber parts	24.8	2.4	0.5	2.4	3	1	0.02	10	0.1	8	0.08	5	0.05
cardboard	17.7	1.7	0.7	3.2	8	3	0.05	10	0.3	2	0.06	15	0.45
rubber tubing	17.2	1.6	0.1	0.6	2	0.2	0	10	0.02	9	0.02	15	0.03
electrical wire	14.1	1.3	0.3	1.6	5	0.02	0	10	0	3	0	0	0
glass	12.5	1.2	0.1	0.6	15	0.02	0	10	0	1	0	0	0
fabric	10.3	1	0.5	2.4	12	2	0.02	10	0.2	1	0.02	0	0
styrofoam	4.8	0.5	0.5	2.4	12	2	0.01						
	(grams)				**overall PCB content** (ppm)		**89** True amount		**28**		**8**		**21**

$n = (t_{0.2})^2 \, s^2/\Delta$ (1)

Where: n is the minimum number of samples
$t_{0.2}$ is the students t number given the degrees of freedom
(This is derived from a look-up table, but only ranges from 1.6 to 1.3 for 3 to 100 samples, therefore the square of this value can be taken conservatively as 2.5)
s is the sample standard deviation
Δ is RT-x' Where RT is the regulatory threshold or 50 ppm and x' is the average parameter value, here for PCBs

Equation (1) then reduces to:

$n = 2.5 \, s^2/(50\text{-}x')^2$ (2)

Minimum Value a:
Use nominal values, s= 25, half of the regulatory value
x = 25, ie. half of the values are at regulatory and half not

$n = 2.5(25)^2/(25)^2$

$n = 3$

Minimum Value b:
Based on the values used by EPA in 17 study sites (Westat, 1991), the sample numbers were: 4, 3, 3, and 2. Thus this would suggest a minimum of 4 samples per pile.

Minimum Value c:
based on the values of standard deviation and averages obtained by EPA in the Westat study for a similar site (mixed input):
s=170 x' = 180

$n = 2.5(170)^2/(50\text{-}180)^2$

$n = 4$

Setting the minimum value:

The minimum samples per pile should be 3 or 4 based on the three approaches noted above.

6.0 Sampling Procedures

Some sampling procedures are given in the literature, however most are manual or semi-manual techniques. Since most ASR is stored in large piles or pits, often covering several hectares, manual sampling techniques are ineffective and could result in the taking of unrepresentative samples. All sampling techniques in the literature will be briefly reviewed with their best application noted. It is imperative that representative samples of the ASR piles be taken and analyzed. As was demonstrated above, sub-sampling in the laboratory can result in errors because the PCBs are not homogeneously distributed throughout the ASR material. Thus taking a greater proportion of the more contaminated material results in high values and vice versa. Because the highest amounts of PCBs are found in the fine and porous material, the usual result of poor sampling practice is values less than the actual average. To avoid errors, extreme care must be taken to sample the piles randomly, take as much representative sample from a given hole as is possible and then to analyze the largest sample as possible.

6.1 Sampling Small Piles by Cone and Quartering

Day *et al.* (1993) describe a procedure used for reducing small piles to samples. The procedure was used to sample small amounts of sample directly from the shredder and would be best used for such operations. A large sample of ASR is placed on a concrete pad using a front end loader. The pile is mixed and formed into a pile approximating a cone. The operator of the loader then divides and separates the pile into four quadrants. Two opposite quadrants are separated and removed. The two remaining quadrants are recombined into a cone. This procedure is repeated until the pile is reduced to the sample size needed for analysis.

6.2 Sampling Tall Piles

Piles that are higher than a cell dimension might be sampled by dividing the pile into vertical cells as well as horizontal cells (EPA, 1993). This procedure is similar to the one described below, except that it is conducted on the basis of cells at different levels. The particular difficulty with this method is obtaining material in cells near the center. There are also concerns about the homogeneity of the material in cells because material may settle through the entire pile resulting in differences between the content of materials in the bottom and top. Unless the pile is very high it might be best to dig through the entire height of a pile and then sample as best along the entire height.

6.3 Dimensioning piles and selecting those cells for samples

Procedures were analogous to those recommended by EPA (1992, 1993). Each pile is divided into multiple cells of 3m by 3m by 3m (or total depth - if less than 3 m) and random numbers used to pick the cells to sample. Random numbers between 0 and 1, are generated using a random function generator, such as the RAN function in Excel and are used to select the sample cells. Piles are divided into cells directly on site to achieve a greater degree of randomness. Piles are measured using a tape measure (both physical and optical) with an accuracy within about 3 m. Then the cells are assigned a number, starting at the northwest corner and proceeding in an easterly direction. The figure below illustrates the numbering of cells.

The total number of cells is established from the measurements and on the basis that each one is 3 m in each dimension.

The cells to be sampled are chosen by using the random numbers in successive order. When the total number of cells in a pile is established, the cells to be sampled are calculated as follows:

1.) Determine a multiplier by dividing the number of cells by 100 or 1000 if there are more than 100 cells.

2.) Take the random numbers in order by either 2 digits if 100 was used or by 3 digits if 1000 was used and multiply by the multiplier to yield the cell to be sampled.

3.) Order the cells to be sampled (5 minimum in each case) so that the backhoe can obtain samples conveniently. If a cell could not be reached because of obstacles, the next randomly-selected cell is sampled.

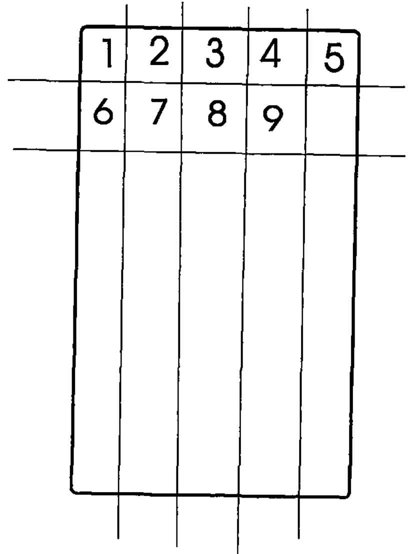

Figure 2 Example of Cell Numbering

Example. If a the pile has 34 cells, divide this by 100 to yield a multiplier of 0.34. Then take the first 5 random numbers, first 2 digits (they are for illustration purposes: 0.63, 0.90, 0.60, 0.17, and 0.34, taken then as 63, 90, 60, 17 and 34) and multiply these by 0.34. This yields (rounded off to 2 digits) 21, 31, 20, 6 and 12. These cells to be sampled were then mapped in distances in metres.

6.4 Reducing Samples Using Griding

One method of reducing large samples has been suggested (EPA, 1993). A string grid is superimposed over the pile. Then a few cells are chosen at random and all material in the grid is removed and placed in another area. Care is taken to ensure that all fine material is removed so that a representative sample is chosen. This is repeated until the sample is reduced to what can be analyzed in one sample. The griding method is not used extensively since it requires extensive manual work and it is difficult to remove the material in a grid quantitatively and exactly.

6.5 Sampling Using a Backhoe

The following describes a procedure developed by Environment Canada for sampling piles using a backhoe. The area is mapped and marked for sampling as described in section 6.3 above. Cells, chosen by the random procedure, are marked and the backhoe moves to each successive position. Before collecting the first sample and each subsequent sample, the bucket of the backhoe is decontaminated (See decontamination procedures in Section 7).

The shovels and scoops for sampling are also decontaminated using the same procedure noted in Section 7. The backhoe then exposes the cell to be sampled and mixes a scraping of the material down the hole. The operator takes one bucket of this and puts it in a position where the sampling crews can reach it.

The sampling crew inspects the load and if there are pieces too big to fit into

the sample bottle, these are cut with snips or other tools. It is suggested that a sample bottle of at least 2 L in capacity is used. This is the largest pre-washed bottle normally available. Pieces are usually smaller than 7 x 7 x 14 cm or less than 9.5 cm in diameter; standard ASR is within this size range. The sampling crew then mixes the backhoe bucket sample thoroughly with a fork or shovel and then takes random samples with a scoop and places them into the sample bottle. Acetone washings, pressure wash water and an unused sample bottle are sometimes preserved for analysis of any possible background PCBs.

7.0 Decontamination of Sampling Equipment

The backhoe bucket and all tools which come into contact with the ASR, should be decontaminated before the first use each day, and before sampling at each new location. A pressure wash is usually used first, followed by a light acetone rinse. All decontamination acetone should be contained in a pan and transferred to appropriate waste containers pending sampling and analysis to determine ultimate disposal.

The backhoe bucket can be steam-cleaned before leaving the construction company yard. At the ASR sampling site, any remaining bulk material is removed by scraping and brushing using a steel shovel, steel scrapers, and stiff bristled brooms or brushes (ASTM, 1994; Environment Canada, 1995). Then the bucket is washed using a pressure wash (ASTM, 1994; Environment Canada, 1995) applied with a mobile sprayer or a large back-pack sprayer. The bucket is finally rinsed with pesticide grade acetone (Environment Canada, 1995) using a Teflon wash bottle. The bucket is air-dried prior to its next use.

All shovels, forks, cutting tools and scoops are decontaminated before the first use each day, and before sampling at each new location. Acetone is contained in a tank or pan and transferred to appropriate waste containers pending sampling and testing to determine the ultimate disposal. The remainder of the decontamination procedure is the same as for the bucket.

To evaluate these decontamination procedures the following samples should be collected: Acetone rinse samples of the backhoe bucket, shovels, forks, and scoops. These samples were collected after removal of all bulk material, but before further decontamination as described above (ASTM, 1994); and final acetone rinse samples of the backhoe bucket, shovels, forks, and scoops after equipment decontamination. These samples might be collected once after every ten decontamination washings (ASTM, 1994).

9.0 Analysis

Analysis is performed using a standard method (eg. Environment Canada, 1997). Samples are only air-dried before extraction, thus the amounts of PCBs would be under-determined because of the remaining water content (ie. The concentration of PCBs is actually slightly higher on a dry-weight basis.). If samples were to be dried at an elevated temperature, such as in an oven, some of the PCBs could be evaporated. Extraction is usually performed by Soxhlet and often multiple extractions are needed because of the large sample size. Extracts are combined prior to the application of the standard method. Samples are often screened by GC-ECD and results calibrated with Aroclor mixtures.

Table 9 shows typical results from two successive sampling and analysis

Table 9 Example of PCB Content in ASR

Description	% Moisture	Aroclor 1242, µg/g	Aroclor 1254, µg/g	Total Aroclor µg/g
Pile 1 - Older Material				
Year 2	24.7	19.6	5.6	25.2
Year 2	22.2	19.5	6.8	26.3
Year 2	27.8	89.5	21.4	111
Year 2	21.9	57.6	11.3	68.9
Year 2	20.2	19.2	16.5	35.6
Year 2	16.7	38.1	46.6	84.7
Year 1	19.3	39.1	0	39.1
Year 1	17.1	29.1	0	29.1
Year 1	21.8	29.7	0	29.7
Year 1	19.3	23.5	0	23.5
Year 1	21.4	34.5	0	34.5
Year 1	28.4	44.3	0	44.3
Year 1	24.3	48	0	48
Year 1	14	37.8	0	37.8
Year 1	28.1	18.1	5.7	23.8
Year 1	32.9	18.2	12.1	30.3
Average	*22.5*	*35.4*	*7.9*	*43.2*
Standard Deviation	*5*	*18.7*	*12.4*	*24.6*
Pile 2 - Older Material				
Year 2	24.1	48.8	11.1	60.0
Year 2	20.4	27.7	11.5	39.2
Year 2	21.3	35.6	13.6	49.3
Year 2	22.4	47.1	14.5	61.6
Year 2	22.3	27.0	8.3	35.3
Year 2	29.9	35.9	11.1	46.9
Year 1	26.7	24	33.3	57.3
Year 1	23.2	17.4	0	17.4
Year 1	23.1	19.2	14.7	33.9
Year 1	31	24.2	0	24.2
Year 1	27.5	34.5	12	46.5
Average	*24.7*	*31*	*11.8*	*42.9*
Standard Deviation	*3.5*	*10.4*	*8.8*	*14.4*
Pile 3 - Older Material				
Year 2	15.2	33.4	9.8	43.2
Year 2	13.4	34.5	16.1	50.6
Year 2	17.5	74.3	14.7	89.0
Year 2	18.6	50.9	19.6	70.5
Year 2	13.1	18.0	9.1	27.1
Year 2	20.1	35.6	15.0	50.6
Year 2	13.3	66.2	14.8	81.0
Year 1	29	170	0	170
Year 1	30.8	45.3	0	45.3
Year 1	39.3	134.5	0	134.5
Year 1	36.9	67.7	0	67.7
Year 1	32.6	50.1	0	50.1
Year 1	32.6	44.7	0	44.7
Year 1	23	18.3	18.8	37.1
Year 1	30.8	45.1	0	45.1
Year 1	28.8	49.5	0	49.5
Average	*24.7*	*58.6*	*7.4*	*66*
Standard Deviation	*8.9*	*40.3*	*8*	*37.8*

Description	% Moisture	Aroclor 1242, µg/g	Aroclor 1254, µg/g	Total Aroclor µg/g
Pile 4 - Newer Material				
Year 2	56.5	48.9	21.9	70.8
Year 2	47.2	58.8	23.9	82.6
Year 2	13.7	36.1	10.2	46.3
Year 2	42.6	30.1	46.3	76.4
Year 2	55.4	99.1	25.7	125
Year 2	21.3	48.2	15.2	63.4
Average	*39.5*	*53.5*	*23.9*	*77.4*
Standard Deviation	*17.9*	*24.6*	*12.4*	*28.4*
Pile 5 - Newer Material				
Year 2	26.3	67.1	19.5	86.6
Year 2	34.5	28.2	6.4	34.6
Year 2	28.4	42.4	9.3	51.7
Year 2	26.6	81.5	19.3	101
Year 2	23.3	69.8	14.8	84.6
Year 2	30.3	48.9	10.4	59.3
Year 2	11.2	40.1	0	40.1
Year 2	18.5	61.3	0	61.3
Average	*25.5*	*54*	*11.4*	*65.4*
Standard Deviation	*7.3*	*19.1*	*7.1*	*26.4*
Pile 6 - Newer Material				
Year 2	38.5	115.7	10.2	126
Year 2	32.3	36.5	9.9	46.4
Year 2	43.2	34.3	8.2	42.4
Year 2	24.8	35.0	7.7	42.8
Year 2	31.8	38.1	15.2	53.3
Year 2	25.2	37.4	9.8	47.2
Year 2	30.4	31.1	7.8	38.9
Year 2	35.4	22.0	6.8	28.9
Year 1	37	30.2	0	30.2
Year 1	31.6	130.4	0	130.4
Year 1	40.2	45.4	11	56.4
Year 1	31.7	47.6	47.2	94.8
Year 1	28.5	41.8	10.7	52.5
Year 1	26.1	65	0	65
Average	*32.6*	*50.7*	*10.3*	*61.1*
Standard Deviation	*8.6*	*32.3*	*11.6*	*32.7*
Pile 7 - Newer Material				
Year 2	21.3	101.4	19.3	121
Year 2	30.9	50.7	12.9	63.6
Year 2	45.4	51.9	16.0	67.9
Year 2	29.2	45.4	14.4	59.7
Year 2	49.4	35.6	7.6	43.1
Year 2	44.7	50.2	9.8	60.1
Average	*36.8*	*55.8*	*13.3*	*69.2*
Standard Deviation	*11.2*	*23.1*	*4.2*	*28.6*
Overall samples				
Average	*28.9*	*48*	*12.2*	*60.2*
Standard Deviation	*11*	*27.7*	*9.9*	*31.8*
Difference Between Years Samples (positive indicates first year is more)				
Average	*18.8*	*19.6*	*-26*	*-6.4*
Standard Deviation	*-10.3*	*-9.2*	*8.1*	*-27.5*

campaigns at the same site. Statistics on the results are also given. It is important to recognize that piles are not homogeneous and there may be as much variance between samples from within a pile as between piles. This is an important point because some of the statistics that are often applied to these situations are invalid. Examples of this include the use of t-tests to evaluate the compliance of a sample. These statistical tests presume that the material is more or less homogeneous.

Table 9 illustrates several important issues regarding the distribution of PCBs in ASR:

1) The variance is highest for Aroclor 1242 at this site. This Aroclor was used in capacitors and indicates that the greatest variance is due to variance in capacitor input. These could be derived from capacitors on small electrical equipment such as appliances, but usually from the shredding of industrial capacitors.

2) Aroclor 1254, which shows a lesser variance, but varies nonetheless, derives almost exclusively from electrical transformers. Although it can be found in small transformers and even motors, it is usually found in larger electrical transformers.

3) The variance in a pile is often as large as between piles.

4) The difference between the results from two years of analysis is not large, but could simply be a function of the normal variance between holes.

5) This site is typical and has an overall average of over 60 ppm PCB content which would classify the material in the entire site as contaminated. The PCB concentration limit in Canada (and many other countries) is 50 ppm, above which waste is considered to be contaminated and requires special treatment.

6) Table 9 does not show sample depth, but depth in these samples varied between 1 and 9 metres. The average depth was about 3 metres. On the older piles at this site, it was found that samples of ASR below the high water line showed low PCB concentrations. This is probably due to the association of the PCBs with oil that floats up and down with the spring high water, thus effectively leaving the portions below the water line with less PCB (and oil), than the upper levels.

7) In many piles, certain samples can have markedly higher or lower PCB content. This is simply as a result of the material being shredded at the time. There is little mixing of ASR once it leaves the shredding facility and is transported to the piles.

10. Concluding Remarks

ASR is a very heterogeneous material that often contains significant levels of PCBs. It has been shown that sampling and analysis of the material requires a careful sampling plan and implementation of such. Inappropriate sampling and sub-sampling can result in inaccurate results because the PCB content of ASR resides primarily in the fine material and secondarily in the porous material. Impermeable material such as wire or glass, contains very little PCBs. Site mapping and sampling procedures are suggested in this paper to ensure that representative samples are taken and analyzed.

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Coupled Supercritical Fluid Extractor-Gas Chromatography / Mass Spectrometry for Quick Extraction of Organic Analytes from Solids

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Abstract

An attempt has been to couple supercritical fluid extraction (SFE) to a capillary gas chromatography/mass spectrometry (GC/MS) system equipped with a standard on-column injector (OCI). The coupled system offers the convenience of an online extraction system with a sophisticated analytical instrumental to enable fast characterisation of emergency response samples, with a turnaround time of well under one hour with minimal sample handling. There is also the benefit of maximising the sensitivity of the instrumentation because the entire sample extract is utilised without any dilution. Collection of the extracted analytes occurs inside the OCI and the pre-column but without the benefit of cryogenic cooling only high molecular weight compounds can be trapped. Peak shape for these high-boiling compounds are comparable to on column injection of the same analytes in liquid. This article reports the hardware adaptation of the Suprex PrepMaster SFE to the Hewlett Packard HP 5890 GC/5971 Mass Selective Detector (MSD). Optimisation of SFE was carried out with respect to pressure, temperature, time and the use of modifiers. Comparison to conventional solid/liquid extraction was made for a few selected materials and problems encountered with this system are also discussed.

1.0 Introduction

The principal goal of direct analysis of samples by coupling SFE, a solventless extraction technique to a lab grade analytical instrument is to provide rapid screening or quantitation of target analytes in a complex matrix. There are several advantages of SFE from the standpoint of emergencies spill analysis: it requires no solvent and the associated disposal problem which means the technique is amenable to be carried out in the field (Li *et al.*, 1995a). The solvating power of the fluid can be easily fine-tuned by varying pressure/temperature on the instrument. Because supercritical fluid carbon dioxide (used in this study) expands to a gas, the time consuming task of concentrating the raw extract in the case of a conventional solid/liquid extraction is eliminated. SFE can be carried out off-line in which the fluid is allowed to expand into a solvent or on a sorbent trap has been reported (Li *et al.*, 1995b).

An on-line extraction is one in which the fluid enters the analytical instrument via a suitable interface; the target analytes are trapped or accumulated on the capillary column, using the liquid phase coating as the collection medium. Upon completion of extraction, the GC/MSD program is started and the trapped analytes partitions throughout the length of the column in the usual manner. Since the entire amount of

extractable material goes into the analytical system, sensitivity is maximised without the extra dilution as in the case of offline collection or conventional extraction. Other than weighing or loading the sample into the extraction cell, there is minimal sampling handling, which could also result in little or no analyte loss from sample workup.

We have investigated the feasibility of linking the SFE to a GC/MSD with the following goals in mind: firstly, using the existing configuration the GC/MSD with little or no modification so that normal operation of the GC/MSD system is not impaired. This point is important since in a mobile laboratory with vehicle portable instrumentation, there is usually a limited number of analytical instruments; maximum flexibility is desirable in handling different sample introduction to these instruments. Secondly, little or no intervention once the sample is loaded into the extraction cell of the SFE. Thirdly, the system should be able to extract target analytes quantitatively or at least semi-quantitatively, with comparable chromatographic performance to conventional extraction methods. Finally to be useful in emergencies response work the entire analysis should take place within a one-hour period.

2.0 Experimental

All SFE-GC/MSD analyses were performed on a Hewlett Packard HP 5890 Series II GC, interfaced directly to the 5971 MSD with electron impact ionisation (70eV) and helium carrier gas with a nominal flow rate of 0.9 mL/min. The GC was equipped with an OCI using the duckbill valve, which can be parted manually to allow the fused silica restrictor or an OC injection needle to be introduced. A blank wide bore (3M x 0.53 mm id.) pre-column was coupled to a narrow-bore capillary column (HP-1, 30M x 0.25mm id, 0.25 µm film thickness) using a press-fit glass union.

Extractions were performed entirely using supercritical fluid extraction grade carbon dioxide (Air Research Speciality Gases) on the Suprex PrepMaster SFE. The instrument is capable of holding two extraction cells and a dual set of flow paths to perform two sequential extractions. The fluid enters the SFE and the pressure is stepped up by a dual reciprocating piston pump, capable of a maximum pressure of 400 atmosphere. The oven maximum temperature is 150 °C mainly due to the limitation of the valve seals. In this work only one cell was used. The weighed sample was loaded into the extraction cell (1.7 mL with a glass insert, effective cell volume 0.5-mL). An outlet restrictor (15- to 30-µm fused silica tubing) controlled the flow rate of the supercritical fluid through the cell. The temperature and pressure of extraction was set and maintained by the controller of the SFE. A typical extraction program was an initial static extraction of 5 min during which the fluid permeated the sample, followed by a dynamic extraction of 20-30 min. During that time the 2-position static/dynamic outlet valve rotated to the dynamic position, allowing the pressurised contents to flow out from the cell. Pressure of 150-300 atmospheres and oven temperature of 60 °C were used. In an offline collection the restrictor was dipped into 1.5 mL of toluene in a 2-mL volumetric flask. In an online mode, the restrictor was inserted into the OCI of the GC/MSD system. After the dynamic

extraction, the restrictor was pulled out from the OCI. The GC was then started, the OCI temperature was ramped from 60 °C to 260 °C and the temperature program proceeded normally. The solvent delay of 5 min was enough for the carbon dioxide to be flushed from the instrument by the helium carrier gas. No modification of the OCI was made to maintain maximum flexibility of the instrument.

3.0 Results and Discussion

3.1 Coupled SFE-GC/MSD: requirements and problems

In an online collection, the supercritical carbon dioxide carries all extractable materials via the restrictor and deposits it into the head of the GC pre column and capillary column through the OCI. This results in maximum sensitivity because there is no sample dilution involved as in conventional extraction. At the same time impurities of the SF are concentrated in the same manner and present as a background, superimposing on the sample GC profile. It is important to purchase the highest grade of SF possible and maintain proper care of in-line filters (in between supply source cylinder and instrument) to minimise the background. In spiked filter studies in which analytes were spiked into small filter strips, the background was not excessive due to the relatively clean matrix. In complex matrices, the chromatogram is usually quite complicated, making identification of analyte peaks almost impossible. The use of a MS as a GC detector is essential for online collection to work for it provides spectral information of the target analytes for positive identification.

Because the SF carbon dioxide expands to nearly 3 orders of magnitude in volume upon depressurisation once it enters the GC (Hawthorne *et al.*, 1989); the excess gas flow creates a problem for the GC. The actual amount of gas is dependent on the pressure of the extraction cell and the diameter of restrictor. Sometimes, even for a 15-μm restrictor the pressure can exceed the vacuum capacity of the MSD, causing it to shut down. The sealing system of the OCI is a duck bill valve which opens and closes by a spring loaded mechanism. Before extraction starts, the restrictor is inserted into the OCI, which then closes around it. If the valve seals completely around the silica tubing without any leaks, sample is transfer is 100% and the entire flow enters the GC. The OCI is chosen to be the inlet for interfacing because of the convenient sealing system and the ability to heat up rapidly at the rate of 100 °C/min, releasing the concentrated extract in a relatively narrow band. However, since the existing system has no cryogenic cooling, trapping of the extracts occurs by physical deposition on the 1-metre length of the pre-column and adsorption by the liquid phase coating on the capillary column, at the starting temperature of the GC at about 10 degree above ambient temperature.

Several types of restrictor interface configurations were also tried. They include a Suprex heated transfer line accessory with a 1/32 in id. stainless steel tubing and a temperature controller. The tubing was crimped to give a flow of about 100 mL/min. During extraction it was pushed into the OCI with a septum seal system rather than the duckbill. This configuration of interface had a high transfer rate of extractable which shortened the extraction time, plus an advantage that the heated transfer line eliminated ice blockage (occurs frequently with unheated restrictors).

This however failed because of the excess flow. To reduce the flow, fused silica tubing of 30-µm was tried. The smaller diameter permitted operation of the GC but the flow rate was still too high, causing the GC head pressure gauge to go to maximum ('pegged'), and at times shut down the MS. A smaller diameter 15-µm restrictor was tried but recovery was generally less than 10 %. Also the smaller diameter was more susceptible to ice blockage. A final configuration of the 20-µm was deemed a reasonable compromise between adequate sample transfer rate and the MS vacuum requirement.

Because of the capacity of the narrow bore GC capillary column (suited for semi-volatile analysis), large amounts of extractable in the sample creates overloading. Excessive water also could potentially cause ice blockage, although the reference soils with the low moisture level did not cause any problem.

3.2 Optimisation

The following optimisation experiments were performed by spiking a small piece of filter paper (0.5 g) with 0.4µg Aroclor 1254 and subjecting to online extraction for 10 min. The area sum of 5 dominant pentachloro-biphenyl peaks in the Aroclor was used to compare yields from various extraction conditions. For comparison, repeatability of the system was investigated using 300 atm (atmosphere) at an oven temperature of 60 °C. Results were varied, indicating the persistent poor performance of the transfer line and the overpressure condition (Table 1).

3.2.1 Pressure

Extraction pressure was investigated to improve the recoveries. Pressures of 100, 200 and 300 atm were attempted. As expected, there was some evidence that higher pressure performed better, due to the increasing solvating strength of the fluid (Table 1). In general, each 100-atm incremental increase in pressure increased the recovery approximately by 2-fold. There was however too much variation in the data especially at higher pressure due to frequent MS shut down from the high flow into the GC. The high source pressure triggered a vacuum shutdown by turning off power to the diffusion pump. Subsequent extractions had to be performed at 200 atm.

3.2.2 Temperature

Extractor oven temperatures were studied at 60, 90 and 120 °C. The effect of higher temperature was to decrease fluid density but improved the analyte solubility in the fluid. In the case of PCB-spiked filters much better recovery was obtained at 60° C (Table 1).

3.2.3 Modifier

The effect of modifier was summarised in Table 1, showing the use of 50 µL toluene, acetone, DCM and a control (no modifier). Within the variation of the data, the control (without modifier) worked as well as the ones with toluene and acetone. DCM appeared to be not as effective. The benefits of modifier are well documented in normal SFE and apparently they had the same beneficial effect in the extraction of semi-volatiles by promoting better analyte solubility in the fluid. In the case of online collection, the modifier entrained in the SF carbon dioxide contributes an additional total organic loading, trapped and concentrated on the GC column so the amount used should be kept within the pumping capacity of the vacuum system. The type of

modifier needs consideration as well: toluene remains a liquid at the 50 °C GC initial oven temperature and thus recondenses on the column, whereas DCM is gaseous under identical condition.

3.2.4 Extraction Times

Generally after 20 min of dynamic extraction, no significant amount of analytes could be recovered. Overall recovery however was very low; at best 50-75% for the strongest retained high molecular weight PAH.

3.3 Performance of the SFE-GC/MSD: comparing to conventional extraction and analysis

A requirement of quantitative analysis is that the peak shape of the analyte has to be reasonably sharp for identification. Since the analytes are concentrated at the head of the column and only migrated down the column once temperature commenced (at least for the high molecular weight compounds), it is anticipated the sample band is comparable to liquid injection. Lighter compounds would not be trapped adequately so they tend to spread out, giving broader peak shapes. In some extreme cases they would be swept through the entire length of the column and lost. The present system does not have the cryogenic cooling capability, implying the trapping/concentration performance is severely compromised. To assess the applicability of this coupled extraction technique, a filter was spiked with a EPA Method 525 mixture, consisting of 13 PAHs, 8 PCBs, 12 pesticides, 9 base-neutrals and pentachlorophenol. Results showed very poor recovery for the early eluting compounds such as the 2- and 3-ring PAH, and only moderate recoveries for the late eluting compounds including the 4-to 6-ring PAH. Figure 1 shows chromatograms of the extracted spiked filter, compared to an OCI liquid injection of 1/10 the same Method 525 mixture. Recoveries across a broad spectrum of contaminants were about 20-25 %.

To provide a basis for comparison, filters spiked with a PAH mixture were extracted using the standard conditions outlined above and were collected offline in 1-mL toluene. The collection experiment was repeated three times. Online collection of the same matrix showed uniform recovery across all PAH in the mixture: the early eluting PAH was collected equally well as the late eluting PAH. Recoveries were in the range of 65-90%.

The expanded gas created an over pressure situation even with the small diameter restrictor (20-μm), putting an tremendous stress on the capillary column. An examination of the mid molecular weight 4- ring PAH and high molecular weight 6-ring PAH shows that although the recovery is not satisfactory, peak shapes are comparable to that of liquid injection of a standard mixture (Figure 2). Periodic injections of liquid standards showed the liquid coating was gradually deteriorating from the high loading of organic onto the column. This was especially evident after extractions of real soil samples.

Even though the recovery was not quantitative, reference materials (RM) were extracted to assess the performance of the coupled system. Matrices extracted included airborne particulate (National Institute of Standards and Technology, NIST SRM 1649) and a naturally PAH contaminated soil (SRS103-100 certified reference material, from Fisher Scientific)

In both cases, as expected, the recoveries were only on the average of 20% for the strongest retained PAH and negligible for the lighter PAH (Table 2). For the detectable target analytes, a library search nonetheless yielded the correct identification for some target compounds, in the sample size as little as a few mg. This was demonstrated in the detection and identification of Pentachlorophenol in a 1-mg aliquot of the SRS103-100 soil (Figure 3). Recovery for this compound is also acceptable at 79 % compared to the less than 50 % of most of the PAH. Operating the MS in selected ion monitoring mode can further enhance the high sensitivity potential for the coupled technique. From a sample size of 1-2 mg, method detection limit could be in the ppb range (pg/mg). Such sensitivity is clearly an advantage when only limited amount of sample is available. High analyte concentration would easily exceed the column capacity of 50 ng, causing distortion of peak shape and poor separation.

4.0 Conclusion

Without cryogenic cooling, the potential of the present coupled SFE-GC/MSD system was not realised. Interfacing the two instruments though easily carried out by a restrictor tubing inserted into the existing OCI of the GC, was not quantitative and needs further optimisation to meet the operational pressure requirement of the GC/MSD. Despite these shortcomings, the potential advantages were demonstrated by the correct identification through a spectral library search of target analytes in real soil samples in as little as 1 mg, all in well less than 1 hr.

5.0 Acknowledgements

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Table 1: Optimisation Experiments of SFE-GC/MSD

(A) Repeatability: 60° C oven; 300 atm,10 min, 50 μL Toluene, 0.4 μg PCB spike

	Number of Extractions		
Peaks	1	2	3
1	1622	814	1215
2	1087	625	835
3	1566	944	1200
4	2632	760	1531
5	2170	945	909
Total	9077	4088	5690

(B) Effect of Pressure: 60° C oven; 10 min, 50 μL Toluene, 0.4 μg PCB spike

Peaks	100 atm	200 atm	300 atm
1	750	327	1100
2	525	256	950
3	750	540	1200
4	520	288	1020
5	468	418	950
Total	3013	1829	5220

(C) Effect of Temperature: 300 atm; 10 min, 50 μL Toluene, 0.4 μg PCB spike

Peaks	60 °C	90 °C	120 °C
1	1100	610	502
2	950	270	250
3	1200	380	290
4	1020	360	180
5	950	250	150
Total	5220	1870	1372

(D) Effect of Modifier: 300 atm; 10 min, 50 μL each modifier, 0.4 μg PCB spike

Peaks	Control	DCM	Acetone	Toluene
1	2280	1075	2670	1100
2	920	384	37	950
3	990	520	550	1200
4	910	258	333	1020
5	520	220	354	950
Total	5620	2457	3944	5220

Table 2: PAH Results of SRS 103-100 Certified Reference Material, µg/g

	Reference Value	Coupled SFE-GC/MSD (n=2)	% Recovery
ANTHRACENE	431	120	28
PHENANTHRENE	1925	670	35
PYRENE	1075	450	42
BENZO(a)ANTHRACENE	264	257	97
CHRYSENE	316	138	44
BENZO(a)PYRENE	97	45	46
INDENO(1,2,3-cd)PYRENE	32	27	84
PENTACHLOROPHENOL	1425	1127	79

PAH Concentrations from Urban Dust SRM 1649, in µg/g

	Certified value	Coupled SFE-GC/MSD (n=2)	
PHENANTHRENE	4.1+/-0.4	0.4	10
ANTHRACENE	0.4+/-0.1	0.1	25
FLUORANTHRENE	6.5+/-0.2	3.1	48
PYRENE	5.3+/-0.3	2.2	42
BENZ(a)ANTHRACENE	2.2+/-0.1	1.2	55
BENZO(a)PYRENE	2.5+/-0.1	1.6	64
PERYLENE	0.7+/-0.1	0.4	57
INDENO(1,2,3-cd)PYRENE	3.1+/-0.6	2.5	81
BENZO(g,h,i)PERYLENE	4.0+/-1.1	2.7	68

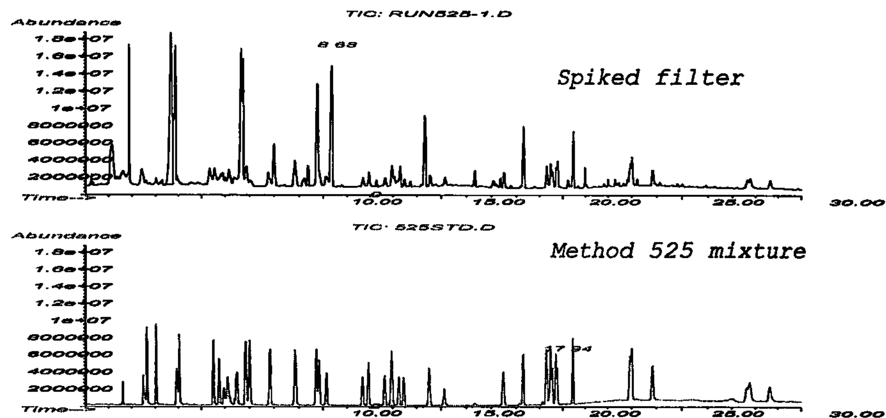

Figure 1: Spiked filter SFE-GC/MSD analysis

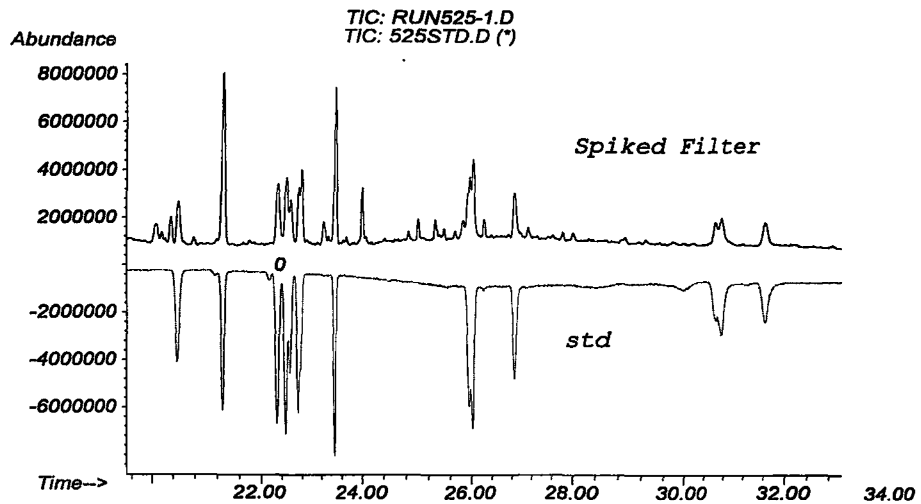

Figure 2: Peak Shape Comparison

Summary of Spill Events in Canada 1984-1995

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Abstract

This paper presents a synopsis of the *Summary of Spill Events in Canada; 1984-1995* report published last November by Environment Canada. This analysis of spill information provides a statistical summary of reported spill incidents in Canada during the time period studied and identifies key findings with respect to spills that impact on the environment. These findings can be used to provide focus for prevention and preparedness activities in the various sectors examined.

1.0 Introduction

Environment Canada receives and responds to spill reports involving hazardous substances, twenty-four hours a day, seven days a week. Spill-report information may be provided directly to Environment Canada by an individual caller reporting a spill, or via any one of Environment Canada's many partners, including other federal departments and provincial and territorial governments. All information received is entered into a national computer database called the National Analysis of Trends in Emergencies System (NATES) where it can be analyzed.

This paper provides a summarized view of spill information and the resulting trends for the period 1984-1995. Findings are presented on the following: the number and quantity of spills in Canada, seven major industrial and public sectors which incur spills, the causes of and reasons for the spills, the sources of spills for the top five reasons, broad material categories, and the effects of spills on the receiving environment.

2.0 Purpose of the Report

The purpose of the report is to provide a summary review of reported spill incidents in Canada, including the identification of spill trends, covering the years 1984 to 1995. The data used in producing the report were gathered by Environment Canada, or provided by various regional or provincial agencies and other government departments.

Using simple statistical techniques, results of the analyses are summarized and presented in the report as tables, graphs and pie charts. The report provides information on causes and reasons for reported spills, and the number and sources of those spills. Users of this information will be able to look at trends and focus on specific problem areas. The results presented are meant to help Canadians in their efforts to reduce the frequency of spills and the severity of their environmental impact.

3.0 Data Gathering and Standardization

The Environmental Emergencies Branch of Environment Canada administers the national spill database NATES (National Analysis of Trends in Emergencies System). This database was established in 1973 as a part of the overall Environmental Emergencies Program. Its role is to record information received through voluntary reporting of pollution incidents involving hazardous substances.

NATES captures most significant spill events reported each year. However, NATES is only one of the data sets used. Provincial and territorial data are obtained by Environment Canada through informal information exchange agreements and some data is obtained through agreements with other federal government departments. For the purposes of the report and this paper, all of the data sets are referred to as NATES data.

Data used in the report were collected from various sources, and therefore some standardization was required to compile the data for analysis. Some aspects of the data were standardized, such as units of measurement (e.g. volume to mass), substance names and re-categorization (e.g. grouping categories or chemicals and sectors or breaking a large category into sub-categories).

In reviewing the results presented in this paper, it should be kept in mind that there are some limitations to the completeness and accuracy of the data, which may have some bearing on the results.

Although every effort was made to capture all available spill data for the 1984-1995 time period, there are some data missing. For example, there are limited spill data from the Province of Quebec, and a very complete data set for the Province of Ontario. Although this may not significantly affect the overall trend analysis results on a national basis, it does show biased trend results for some provinces when compared with others.

An inherent limitation of most spill information collected in Canada, including NATES data, is that the end result generally does not look back to the point of origin. For example, spill hotlines capture the initial spill report information and disseminate it as appropriate. However, once the initial information has been circulated, follow-up information and activities are generally not filtered back to the initial contact point.

Spill volume is an example of first report information that can change over the course of an incident, but is not usually updated on the initial spill report. The volume of a spill is usually underestimated at the beginning of an incident. Also, if a mixture of substances are spilled, it is not usually known what concentration of each substance is present in the actual spill. For these reasons, the volumes recorded may be approximations. In spite of some of the inaccuracies, this information is still quite useful in providing an overview of relative increases and decreases over the years.

Since reporting spill incidents to NATES is not mandatory, the data do not represent a comprehensive picture of all spills reported in Canada. The data do, however, provide a good sampling of information with which to perform analyses and obtain trends. While the actual numbers presented may not be definitive, the resulting trends are useful in identifying areas where Canadians can be more proactive in reducing the number of spills of substances harmful to our environment.

4.0 National Spill Trends and Statistics

The number of spills reported annually increased steadily between 1984 and 1988 and has remained relatively constant since that time for the period studied. Sections 3.1 to 3.6 summarize national spill statistics in Canada and their distribution. The awareness and reporting of spills has increased through the mid-1980s and early 1990s as spill-reporting requirements were implemented through legislation in various territories and provinces all across Canada. Public awareness of the need for reporting spills has also increased.

4.1 Number of Reported Spills by Year

The NATES spill data set used for the purpose of this study contains over 94 000 spill reports for the period 1984-1995. The number of reported spills has more than doubled during the period examined (see Table 1). A noticeable increase is evident in 1988; this increase can be largely attributed to the implementation of Ontario's provincial spill-reporting requirements in that year. From 1988 to 1995, the number of spills remains relatively constant. Spill reporting has become a standard function for organizations handling hazardous substances. It appears that the annual spill numbers are leveling off, with minor fluctuations.

4.2 Total Quantity of Reported Spills by Year

Significant increases in the total reported quantity spilled annually appear to have occurred in the years 1987, 1992, 1993 and 1995 (see Table 1). Further analysis reveals that several large sewage spills were reported during these years. If these sewage spills are excluded and an average is taken for all years analyzed, the average amount spilled by year is 413 000 tonnes.

It is important to note that the spill quantity is the mass of the total discharged product and not the mass of the contaminant. In Canada, the majority of large spills consist of effluent, sewage and mine tailings. A single major spill can have a significant impact on the total quantity spilled during a given year.

The impact of a spill on the environment depends on the toxicity and the concentration of the substance, on the volume spilled and on the receiving environment. Therefore the size of the spill does not necessarily determine the environmental impact.

4.3 Number of Reported Spills by Province and Territory

When examining the number of reported spills by province and territory, there appear to be large differences, making it seem as though some provinces experience more spills than others. However, these numbers are a reflection of the number of spills *reported* rather than the actual number of incidents. For example, there appears to be a large difference in incident numbers between Ontario and Quebec. However, with similar rates of reporting, the expected result is that Quebec would have only marginally lower numbers of incidents than Ontario. This assumption is based on the size of industry and transportation sectors in the Province of Quebec.

The provinces of Ontario and Alberta have the largest number of reported spills. Both of these provinces produce and handle large volumes of hazardous materials. Alberta has a large petroleum industry, handling and transporting large volumes of product, resulting in more frequent spills. Ontario has a large number of

spill incidents due to a large and diversified industrial base and a high volume of transportation of hazardous materials. Good data capture by multiple organizations in these two provinces is also a factor in the higher number of reported spills.

4.4 Reported Spills by Month and Season

According to the NATES data, the summer months of June through August have the greatest number of reported incidents, at 29% of total events (see Figure 1). This could be attributed to an increase in transportation activities during these months; however, there are many possible variables in each industry within the different sectors. The reasons for an increase of incidents reported during these months could be better explained by individually examining each industry in the public and private sectors. Some of these variables might include increases or decreases in production, plant shut downs and startups, etc.

4.5 Reported Spills by Broad Sectors

The broad business sector, including manufacturing, handling and transportation of hazardous materials, as well as their on-site use and storage, represents the greatest number of incidents at 75% of all reported spills. It should be noted that the business sector is generally very diligent in reporting spills. Public relations and profile in the community are key concerns for industry. Private citizens tend to spill frequently in smaller amounts but may be unaware of reporting requirements or concerned about possible enforcement action. This category represents 7% of all spills. Government (including all federal, provincial, territorial and municipal governments as well as government-owned facilities) accounts for 17% of all reported spills.

The 'other/unknown' category accounts for one percent of spills. In some cases, the details surrounding the incident may not be known at the time of the initial spill report. If little or no follow-up is carried out to amend the original report, or the information is not known the spill is accounted for under 'other/unknown'.

4.6 Distribution of Spills by Quantity and by Year

The majority of reported spills are in quantities of less than one tonne. Comparison of two six-year periods, 1984-1989 and 1990-1995, indicates an increase of 69% in the number of reported spills of less than one tonne (see Table 2). This is likely a reflection of increased reporting, as a larger number of small spills are being reported. Nevertheless, these small spills can have cumulative effects on the environment and on humans.

The quantity spilled is often unknown when an incident is first reported. The 'unknown' category for spill size represents a significant number of spills (32%) and increases substantially over the study period. More accurate data reporting would allow for greater focus in prevention efforts.

There are 11% fewer reported spills in the 1-10 tonne category for the 1990-1995 period than for the six preceding years. The number of spills of this size appears to decrease over the last four years. The 10-100 tonne category decreases 20% for the years 1990-1995, compared with the 1984-1989 period. This is good news as spills of this magnitude can have a significant impact on the environment.

In the >100 tonne category, there has been a gradual increase in the number of spills exceeding 100 tonnes since 1984. Examination of the two six-year periods indicates an increase of 59% in the number of reported spills >100 tonnes.

5.0 Summary Findings of Reported Spills in Seven Major Sectors

A subset from the main spill data listed above in section 3.0 to 3.6 has been further examined to focus on seven major sectors: chemical, government, metallurgy, mining, petroleum, pulp and paper, and the service industries. These seven sectors represent 63% of the total spills reported and 93% or the total reported quantity spilled from 1984 to 1995 (see Tables 3 and 4). The seven sectors vary in size, therefore reasonable comparisons among sectors are not possible.

5.1 Spills in the Chemical Sector

The number of reported incidents in the chemical sector from a low of 70 in 1984 to a high of 784 in 1994. In 1995, there was a 33% decrease from the previous year in the number of spills reported. The quantity spilled generally declines after 1989.

The Canadian Chemical Producers Association, an industry association which includes most of the major chemical producers in Canada, has implemented widespread programs to improve prevention, preparedness and response to incidents involving product handled by their members. These programs may have contributed to the reduction in total quantity spilled over the period examined.

5.2 Spills in the Government Sector

This sector includes federal, provincial and municipal levels of government and their operations and holdings. Spills by the government sector also include municipal sewage releases which result from flooding or overflow. The number of spills reported in the government sector increases steadily after 1987. The quantity of material spilled remained relatively constant over the years 1984 to 1991. For the 1992-1995 time period, over 96% of the total spill quantities are composed of sewage spills greater than 1 000 tonnes.

5.3 Metallurgy Sector

The number of spills reported in this sector gradually increases from 31 spills reported in 1984 to a high of 703 spills in 1992, followed by a decrease to 431 spills in 1995. A large number of spills in this sector are large-quantity 'mill water' or 'dirty water' spills that are ten to a hundred times larger than other spills. Roughly 70% of the total quantity spilled in 1993 can be attributed to one 'dirty water' spill.

5.4 Mining Sector

From 1988, the annual number of spills in the mining sector remains in the range of 172 to 199 spills per year. From 1992 to 1995, the spill quantity declines. The peak in 1984 and 1987 are caused by a single large spill in each year, 87 000 tonnes of mining mill effluent and 100 000 tonnes of mine tailings respectively.

5.5 Petroleum Sector

The petroleum sector shows two periods of slow increase in the number of spills: the first from 1984 to 1990, and the second from 1992 to 1995. Data for the latter part of the study period was unavailable. Taking into account the drop in 1992, the quantity of spills in the petroleum sector reported over the 12-year period varies with no apparent trend.

5.6 Pulp and Paper Sector

The number of spills reported for this sector rises steadily throughout the period, from an average of 98 spills per year during 1984-1989 to an average of 354 spills per year during the 1990-1995 time frame. With the exception of 1987, the quantity spilled remains relatively consistent. The peak in 1987 is caused by one spill of 65 000 tonnes of white water.

5.7 Service Industry Sector

In this report, the service industry sector includes all types of services, including maintenance contractors, specialized industrial services and dry cleaning services. The number of spills in the service industry sector increases almost fivefold from 1984 to 1995. There is no discernible trend in the quantity spilled, as the quantity fluctuates greatly from year to year. The anomalies in quantity spilled in 1986 and 1987 are caused by single, large spills in each of those two years.

6.0 Summary Findings for Causes and Reasons for Spills in Seven Major Sectors

The 'cause' of a spill is 'what went wrong' while the 'reason' for a spill is 'why it went wrong'. Pipe leaks account for the majority of causes of spills in the seven sectors examined. Other significant known causes include discharge, process upset and overflow.

The top five reasons (or root causes) for spills include equipment failure, human error, corrosion, storm/flood and material failure, accounting for 63% of all reasons (see Table 5). In each of the seven sectors, equipment failure and human error are included among the top three reasons for spills. Further analysis of the source of spills by reason provides additional focus for prevention activities.

In this section, the cause, reason and source of spills are shown as a percentage of the number of reported spills in the seven sectors.

6.1 Causes of Spills in Seven Major Sectors

The cause of a spill relates to how a spill happened. Examination of these causes by persons working in the various sectors can assist in preventing similar events from happening in the future.

The leading causes of spills vary greatly from sector to sector (see Figure 2). Process upset is the leading cause of spills in the chemical and metallurgical sectors, while discharge is the most frequent cause of spills in the government and pulp and paper sectors. Pipe leaks are the primary cause of spills in the mining sector, while the most important cause of spills in the service industry is container leaks.

6.2 Reasons for Spills in Seven Major Sectors

This section presents the main reasons (sometimes referred to as 'root causes') for spills in the seven selected sectors. In some cases the 'unknown' category is quite large, totaling almost 17% of all spills in the seven selected sectors.

As stated previously, the selected sectors are of different sizes. Each sector is therefore examined independently. There are, however, some apparent trends visible when reasons for spills in the various sectors are examined as a group. Equipment failure and human error are among the top three reasons reported for every one of the seven sectors. Focused prevention and preparedness efforts in these two areas may contribute significantly to a reduction in the number of spills in these sectors.

Thirty percent of all spills in the chemical sector are due to equipment failure, while 15% are attributed to human error and 13% to intentional discharge. Equipment failure refers to the failure of systems and machinery, not to failure of the actual containment material or from corrosion of containment materials in piping and tanks.

In the government sector, the main reasons for spills are: storm, flood (25%), equipment failure (22%), and human error (10%). From a search of the database (not shown), the most frequent source of government-sector spills are waste water treatment plants, leading to the conclusion that 'storm, flood' is an important reason for sewage spills. This type of spill is often the result of overflow that occurs when rainfall exceeds the capacity of the treatment plant or sewer system.

Equipment failure accounts for 32% of the reasons for spills in the metallurgy sector, and human error for 11%. Similar percentages were determined for the mining sector, with 31% of the reasons for spills attributed to equipment failure and 14% to human error. Material failure and corrosion accounted for an additional 9% of reasons for spills in the mining sector.

Equipment failure (24%), corrosion (24%), and human error (18%) collectively account for two thirds of the reasons for spills in the petroleum sector.

Equipment failure is the reason for over one-third (37%) of reported spills in the pulp and paper sector. Human error accounted for 15% and power failure for 6%.

The service-industry sector, including businesses such as dry cleaning, construction and janitorial services, reported human error as the reason for 23% of all spills, with equipment failure accounting for another 15%.

In summary, the most commonly reported reason for spills in the seven sectors is equipment failure (25%), followed by human error (16%) and corrosion (12%). In each of the seven sectors, equipment failure and human error are included among the top three reasons for spills.

6.3 Sources of Spills

The "source" of a spill is the specific type of installation or vehicle that failed. By examining what sources were involved for each of the top five reasons, prevention and preparedness efforts can be more precisely targeted.

As noted earlier, the top five reasons (or root causes) for spills include equipment failure, human error, corrosion, storm/flood and material failure.

Table 6 details the sources of the top five reasons for spills for the seven chosen sectors, providing a view of the relative importance of each source.

Overall, the most frequent source of spills for the top five reasons is the production field (25%), followed by other industrial plants (16%), sewage treatment and sewers (11%), pipelines (11%), tank trucks and other motor vehicles (10%), storage (all types) (8%), service stations (4%) and other sources (15%).

6.3.1 Source of Spills Where Reason is Equipment Failure

Equipment failure refers to failure of system components including anti-overflow devices or electronic controllers. Spills in the production field (29%) and in other industrial plants (26%) are the top two sources of spills resulting from equipment failure. Tank trucks and other motor vehicles account for 9%, and storage for 7%.

These two sources both contain extensive piping, handling, containment, and storage systems that are all subject to equipment failure. Regular equipment inspection and maintenance are of great value in reducing the frequency of spills due to equipment failure.

Overall for all reasons examined, equipment failure is the highest reason reported representing 40% of all reported incidents in the seven sectors examined.

6.3.2 Source of Spills Where Reason is Corrosion

Spills in the production field (which includes mines and oil wells) and from pipelines account for 42% and 45% of the spills attributed to corrosion respectively. Pipeline spills account for 11% of the total reported spills in the seven sectors.

Equipment and piping used in these environments are exposed to extreme temperatures, weather conditions and moisture. The equipment is not normally housed in buildings and structures that would protect them. Pipelines are exposed to acidic soils and moisture. All of these factors contribute to corrosion.

6.3.3 Source of Spills Where Reason is Material Failure

Material failure is defined as the failure of the containment material for any given system. Failure is usually the result of poor design, substandard containment materials, or incompatibility between the containment system and the product being contained. The two most common sources of spills attributable to material failure are the production field and other industrial plants. Each accounts for 23% of the total spills caused by material failure. Tank trucks and other motor vehicles are the source of 14% of spills caused by material failure, while spills in storage facilities are the source of 10%.

6.3.4 Source of Spills Where Reason is Human Error

The most common source for spills where the reason is human error is 'tank trucks and other motor vehicles', at 22% of the total spills. Spills in the production field and in other industrial plants each represent 16% of the total spills due to human error.

All of the major sources of spills where the reason is human error have fairly equal values. Personnel in all areas of work make errors. Human error may be reduced by altering workplace design, and also by determining and then eliminating

the factors which contribute to human error. Training is often the recommended approach for reducing incidents related to human error.

When examining the total number of reported incidents in the seven sectors, human error was the second highest reason reported representing 25% of the total number.

6.3.5 Source of Spills Where Reason is Storm or Flood

Seventy-one percent of spills attributable to a storm or flood are sewage spills from either a sewage treatment plant or a sewer. Run-off causing overflows during periods of major precipitation, particularly from storm and sanitary sewers, constitutes one of the major reasons for sewage spills. Sewage treatment plants and sewers account for 11% of the total number of spills.

7.0 Summary Findings for Spills and Environment Affected

The extent of the impact of any given spill depends on many factors: the nature and concentration of the product, the environment affected, weather conditions and the quantity spilled. Most spills occur in small quantities, thus limiting the area of environmental impact. The environmental medium impacted for almost half of the reported spills is land. Environmental impacts to waterways occur in roughly one fifth of the incidents. While land-based spills can cause significant environmental impact and may migrate to groundwater, spills to waterways are generally more serious as they can impact entire habitats and disrupt food chains. The impact of a single marine spill can affect algae and plankton, fish, birds and marine life. In almost all land-based spills, there is damage to vegetation and property.

Some spills, due to the properties of product spilled, may persist in the environment for an extended period of time.

7.1 Environment Affected

The environmental impact of a spill is often more dependent upon the receiving environment than on the amount of material spilled. Depending on the substance, it may be possible for a land spill to be cleaned up immediately.

An examination of all the reported incidents with regards to the medium into which the spill was discharged reveals that spills discharged to land occur more frequently than spills to any other environmental medium (48% of reported spills), followed by freshwater (15%), air (6%) and saltwater (5%). More than one environmental medium may be affected by a single spill - the 'other' category in this analysis refers to multi-media spills as well as spills that were held in some sort of containment area (26%).

Releases to air account for only 6% of reported incidents. This low rate may be partly explained by the fact that releases to air are not always visible and may therefore be reported less often. Many land-based spills may also involve a release to air, but at the time of the initial report are described as land-based spills.

Waterways are second to land as the environmental medium most often affected. Groundwater may be impacted more frequently than statistics reveal (less than 1%); the existence or extent of groundwater contamination is often unknown at the time of the initial spill report and is rarely captured.

7.2 Percentage Distribution of Environment Affected by Province and Territory

By examining the environmental media affected, by province and territory, the largest number of spills to land are found to occur in Saskatchewan, Alberta, Manitoba, the Northwest Territories and the Yukon Territory. There are a large proportion of facilities in the petroleum and mining industries located in these provinces, which in part accounts for the large number of spills affecting land. The Atlantic provinces (including Newfoundland) and British Columbia show high numbers of spills occurring in the saltwater environment, which is related to the marine activity on the east and west coasts. Quebec and Ontario have a higher number of spills affecting freshwater environments, which can be explained by the density of human population and activities adjacent to the St. Lawrence River, the Great Lakes and other fresh water bodies.

7.3 Percentage Distribution of Environment Affected by Spill Category

Every spill has the potential to affect one or more environmental media. The chart shows how different types of spilled material - divided into three spill categories: wastes, effluents and others; oils and hydrocarbons; and chemicals. There are over 1 000 substances listed in the database.

Nearly half of the incidents impacting air are from chemicals, with about 35% from oils and hydrocarbons. Land is most affected by spills of oils and hydrocarbons (nearly 70%), and saltwater and groundwater environments are, in a majority of cases, also impacted by oils and hydrocarbons. The freshwater medium is primarily impacted by wastes and effluents.

7.4 Percentage Distribution of Reported Consequences of Spills

Although data of reported consequences of spills are limited, the data available indicated that property damage is the most frequently listed consequence (41%), followed by vegetation damage (38%). The fact that leaking pipes result in a large number of spills helps to explain the high percentage of reported consequences in these two categories.

Data for specific consequences such as oiled birds (<1%), contaminated drinking water (<1%) and income loss (<2%) are very limited and are therefore shown grouped in the 'other consequences' category. This category also includes the consequences not already indicated (approximately 14%) for a combined total of 16% of all reported consequences.

The number of reported fish kills is also quite low. There are several reasons that may contribute to an explanation. The evidence of a consequence of this type may not always be visible at the time of the initial report. Evidence of an impact may only become obvious days or weeks later.

As large number of spills affect both freshwater and saltwater, more complete reporting is required in this area in order to ensure that incident reports are updated if additional follow-up data become available. This would result in a more accurate representation of the impact of spills on fish, bird and other wildlife populations.

7.5 Spills of MIACC List 1 Substances

The Major Industrial Accidents Council of Canada (MIACC) and its partners have developed lists of hazardous substances that have a potential for causing harm to

people and the environment if released in an industrial accident. 'List 1' is the short list of high priority substances which are commonly found in Canada, in facilities and transport. The five List 1 substances involved in the highest number of spills are ammonia, chlorine, hydrochloric acid, propane and gasoline.

Gasoline is the most frequently spilled of the MIACC List 1 substances. The number of gasoline spills decreases substantially in 1992 and remains close to this value for the following three years. Gasoline is also the List 1 product spilled in the largest quantity with a total of 19 730 tonnes reported spilled between 1984 and 1995. One single incident, a pipeline leak in 1992, accounts for 6 200 tonnes (96% of the total for 1992). The second largest spill occurred in a storage depot in 1984 and accounts for 1 575 tonnes (28% of the total for 1984). Apart from these two peaks, the quantity reported per year decreases gradually throughout the period.

Anhydrous ammonia (including solutions >35%) and hydrochloric acid follow gasoline as the substances most frequently spilled. Both reached a high of nearly 60 reported spills in the late 1980s. The frequency of ammonia spills has leveled off to approximately 40 per year, while hydrochloric acid spills have generally declined to levels close to 30 per year. The quantity of both ammonia and hydrochloric acid releases varies over the years studied, with no discernible trend.

Chlorine spills number in the order of 20 to 30 spills per year from 1988 to 1995. The number of spills gradually increases between 1984 and 1993, and declines slightly from 1993 to 1995. Apart from one large incident in 1986 (which involved a release of 408 tonnes due to a pipe leak in a storage area), the quantity of chlorine reported spilled per year does not change significantly, except for an increase in the last two years.

The spill frequency of propane since 1988 increases from about 20 spills per year to about 30 spills. There is no discernible trend in spill quantities for propane.

8.0 Breakdown of Reported Number and Quantity of Spills by Material Categories

The breakdown of reported number and quantity of spills by material categories examines the distribution of spill events by the broad types of materials spilled - oils, non-oils (including chemical spills) and wastes and effluents. Oil spills total 58% of reported spills, while non-oils account for 24% and wastes and effluents account for the remaining 18%. The prevalent use of fossil fuels for powering vehicles, heating buildings, generating energy, and a myriad of industrial uses underlines the importance of oils as an independent category. The frequency of spills is directly proportional to material usage, therefore it is not surprising to find that the highest spill rate is in the oils category.

In examining the broad material categories for total reported quantity spilled, the tonnage spilled is dominated by the wastes and effluents category at 89% of the total. Most of these spills are municipal sewage releases, often due to storm or floods that result in the overflow or bypass or storm and sanitary sewage systems.

8.1 Percent of Spills by Material Category

A more detailed view of material categories examines the distribution of the number of reported spills by percentage (see Figure 3).

Fuel oils and gasoline, the most common fossil fuels currently used in Canada, account for 30% of the spill events in Canada. Crude oil, the source of these fuels, accounts for only 9% of the spills; with other oils and other hydrocarbons accounting for another 19%. Saltwater, a component of the mixture that comes out of the oil well mixed with the crude, has always been reported as a separate substance (e.g. a two-tonne spill might be registered as one tonne of crude oil and one tonne of saltwater); it accounts for 7% of the spills. This indicates that the majority of crude oil spills occur at the stage where the crude is still in a mixture with saltwater. Acids and bases (among the most common in use in Canada are sulphuric acid and sodium hydroxide) comprise 3% of spills in the country. Corrosive gases (the most common being chlorine and ammonia) account for 1% of the total. The category called 'other chemicals and substances', which includes pesticides, plastic precursors, paints, salts, and a myriad of industrial chemicals, comprises 13% of spills.

9.0 Key Findings of the *Summary of Spill Events In Canada: 1984-1995*

Key findings are identified to help focus the efforts of Environment Canada and other agencies involved in the prevention, preparedness and response to spills.

The following are key findings:

- Spill reporting in Canada has improved steadily since 1984. More stringent legislation and increased awareness of spill-reporting requirements have contributed to better reporting and more complete data for analysis.
- Forty-four percent of reported spills are smaller spills of less than one tonne.
- Seven major sectors selected for analysis are implicated in 65% of all reported spills. These are the chemical, government (including all levels of government and their operational facilities and holdings), metallurgy, mining, petroleum, pulp and paper, and service industry sectors.
- The top five reasons for spills are equipment failure, human error, corrosion, material failure, and storm or flood.
- The largest spills are consistently sewage or effluent spills, often the result of a storm or flood.
- The environmental medium most frequently affected by spills is land.
- The main reported consequences of spills are vegetation and property damage.
- Fifty-eight percent of the total number of reported spills involve oil and petroleum products.
- Wastes and effluents account for 89% of the total quantity of reported spills.
- Generally speaking, the number and quantity of spills increased steadily from 1984 into the early 1990s, followed by an overall decline to the end of the study period in 1995.
- There is a trend toward a high number of small spills being reported possibly indicating a greater awareness and sensitivity to all types of environmental emergencies.

10.0 Conclusion and the Path Forward

Spill reporting has improved considerably over the 1984-1995 period. Collecting spill information and examining the findings enables both public and private sectors to examine the source of spills. More detailed analysis of spill data

can assist in determining what efforts to undertake to prevent these spills from occurring and to minimize their impact to the environment through preparedness and timely response to spill events. By understanding how and why spills occur, government and partners can develop pollution prevention plans that focus their efforts in protecting the environment by identifying and managing the associated risks.

Specific prevention activities are being developed as follow up to the *Summary of Spill Events in Canada; 1984-1995* report by Environment Canada. The Environmental Emergencies Branch continues to encourage the Major Industrial Accidents Council of Canada (MIACC) to engage the petroleum, pulp and paper and mining sectors to participate more actively in prevention activities dealing with the manufacture, storage, transportation, distribution, handling and use and disposal of hazardous substances. Workshops are developed for the federal sector to increase their awareness in areas or prevention activities and technologies, contingency planning, and proper reporting of spills. The Department maintains a leadership role in areas such as risk assessment, process safety, life-cycle management of hazardous substances, and spills on federal lands.

11.0 Acknowledgments

The Environmental Emergencies Branch elected to undertake the spills summary report project through the approach of a self-directed work team. The team members who produced the report are: Dora Boersma, Patricia Charlebois, Gilles Cloutier, Asit Hazra, Carol Lau and Raj Thakur.

12.0 References

Environment Canada, 1998, *Summary of Spill Events in Canada; 1984-1995*, Report EPS 5/SP/3, Hull, Quebec, Canada, 81 p., 1998.

Table 1 - Total Reported Spills by Year, 1984-1995

Total Reported Spills by Year, 1984-1995		
Year	**Total Number of Spills**	**Total Quantity Spilled (tonnes)**
1984	3,361	367,421
1985	4,308	274,017
1986	4,997	633,965
1987	5,114	1,403,892
1988	8,260	200,472
1989	9,246	462,376
1990	9,764	320,983
1991	9,938	442,672
1992	9,020	1,793,201
1993	9,711	2,284,921
1994	10,578	788,217
1995	9,913	1,711,869
Total	**94,210**	**10,684,006**

Table 2 - Spill Size Distribution

Spill Size Distribution					
Number of Reported Spills					
Year	**< 1 tonne**	**1-10 tonnes**	**10-100 tonnes**	**>100 tonnes**	**unknown spill size**
1984	1,224	912	513	116	596
1985	1,721	1,056	619	122	790
1986	2,256	1,290	602	147	702
1987	2,339	1,238	650	171	716
1988	3,704	1,507	656	134	2,259
1989	4,034	1,358	677	136	3,041
1990	4,608	1,631	730	200	2,595
1991	4,547	1,571	781	216	2,823
1992	3,995	709	301	269	3,746
1993	4,145	881	383	217	4,085
1994	4,106	918	400	229	4,925
1995	4,368	826	377	180	4,160
Total	**41,047**	**13,897**	**6,689**	**2,137**	**30,438**

Table 3 - Number of Reported Spills in Seven Sectors

Number of Reported Spills in Seven Sectors							
Year	Chemical	Government	Metallurgy	Mining	Petroleum	Pulp & Paper	Service Industry
1984	70	223	31	153	1,831	38	94
1985	130	200	58	83	2,053	44	104
1986	206	206	181	118	2,398	73	157
1987	179	228	139	124	2,512	63	208
1988	405	981	360	172	3,021	148	281
1989	582	1,080	392	172	2,971	224	346
1990	588	1,320	361	191	3,157	312	408
1991	552	1,487	508	195	3,139	291	434
1992	667	1,991	703	194	1,144	340	427
1993	754	1,957	618	186	1,531	371	456
1994	784	2,165	599	199	1,577	458	464
1995	534	2,204	431	184	1,642	353	484
Total	**5,451**	**14,042**	**4,381**	**1,971**	**26,976**	**2,715**	**3,863**

Table 4 - Total Quantity of Reported Spills in Seven Sectors (tonnes)

Total Quantity of Reported Spills in Seven Sectors (tonnes)							
Year	Chemical	Government	Metallurgy	Mining	Petroleum	Pulp & Paper	Service Industry
1984	1,783	142,556	4,860	113,078	72,121	2,948	433
1985	12,399	140,820	314	16,105	46,029	35,447	211
1986	16,160	11,267	23,923	29,972	62,232	28,138	431,886
1987	17,128	133,863	87,665	126,939	89,773	90,608	616,308
1988	5,498	58,480	23,497	6,752	29,444	26,933	1,115
1989	7,194	189,169	51,266	42,899	120,765	16,322	228
1990	6,629	84,194	79,178	35,247	50,284	35,845	310
1991	1,619	185,449	32,449	26,172	43,963	46,491	5,106
1992	827	1,386,991	193,435	58,667	11,164	25,494	5,625
1993	1,519	677,529	1,425,753	12,094	62,725	35,612	190
1994	178	678,622	27,489	7,262	18,174	19,751	197
1995	325	1,576,576	11,791	4,783	18,176	49,224	763
Total	**71,259**	**5,265,518**	**1,961,620**	**479,969**	**624,852**	**412,814**	**1,062,374**

Table 5 - Top Five Reasons for Spills in Seven Sectors

Top Five Reasons for Spills in Seven Sectors		
Top Five Reasons	Total Number of Spills	% of Top Five Reasons
Equipment Failure	14,941	40%
Human error	9,346	25%
Corrosion	7,048	19%
Storm, flood	4,004	11%
Material failure	2,024	5%
Total	**37,363**	**100%**

Table 6 - Number of Spills by Source of the Top Five Reasons in Seven Sectors

Source	Total Number of Spills	% of Number of Spills
Production field	9,360	25%
Other industrial plants	6,149	16%
Sewage treatment and sewers	4,089	11%
Tank trucks and other motor vehicles	3,762	10%
Pipeline	3,945	11%
Storage (all types)	2,900	8%
Service station	1,316	4%
Other	5,842	15%
Total	**37,363**	**100%**

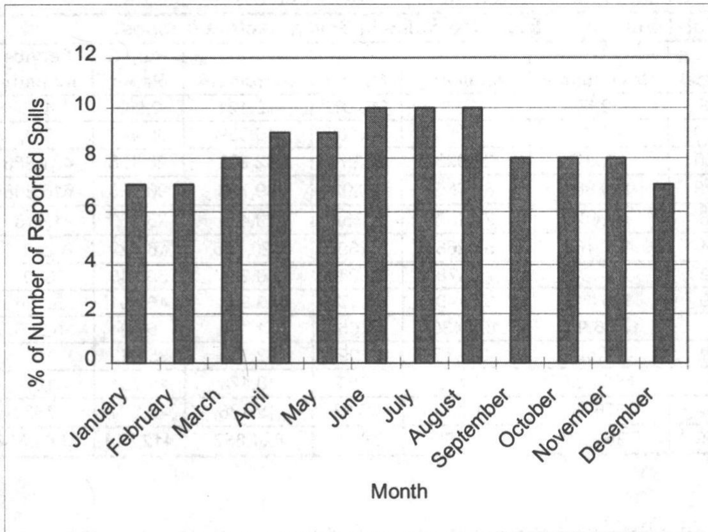

Figure 1 - Breakdown of Reported Spills by Month, 1984-1995

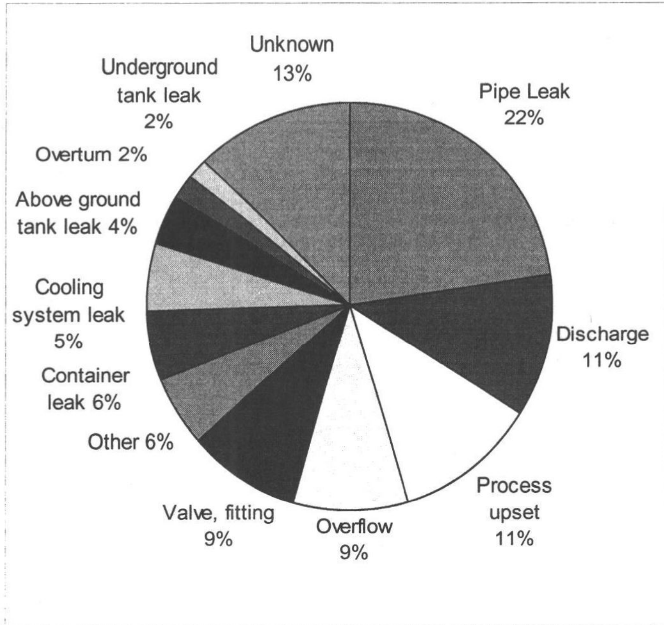

Figure 2 - Causes of Spills in Seven Major Sectors

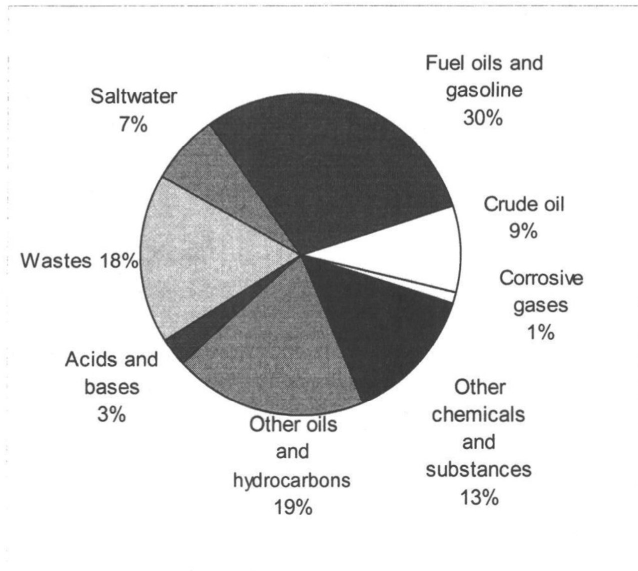

Figure 3 - Percentage Distribution of Spills by Material Category

Decontaminating Residences Sprayed With Methyl Parathion

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Abstract

The United States Environmental Protection Agency/Environmental Response Team Center (U.S. EPA/ ERTC) conducted a study to determine if there were a method to reduce indoor surface concentrations of methyl parathion to less than 15 micrograms per 100 square centimeters ($\mu g/100cm^2$). Methyl parathion is a limited-use insecticide that is designed for outdoor use on certain agricultural crops. However, during the 1980s and 1990s, methyl parathion was sprayed in numerous homes and businesses throughout the country.

1.0 Introduction

In December 1994, the U.S. EPA first became involved in Lorain County where methyl parathion was used within homes and businesses. During this incident, U.S. EPA Region V, along with other governmental agencies, developed protocols for relocation, decontamination, reconstruction, and re-habitation. As part of this response, a priority system for characterization was developed. Each affected residence was assigned a level number from 1 to 4, with Level 1 homes requiring the highest priority, and Level 4 homes requiring no action. These level designations were based on contaminant concentrations within each residence, as well as, blood and urine concentrations of each occupant. Over 230 Level 1 and 2 homes were remediated with the majority of these located in an apartment complex.

The Lorain County incident revealed that, based on time and cost, demolition and removal of the contaminated materials were the most efficient means of remediation. This was determined due to the fact that a series of re-decontaminations and re-sampling events would expend more time and money, in addition to potentially destroying items that were being cleaned.

In November 1996, it was discovered by U.S. EPA Region IV that methyl parathion had been sprayed in several hundred homes and businesses throughout the Mississippi Gulf Coast region. The primary area of contamination was in Jackson County, centered around the cities of Pascagoula and Moss Point. The estimated number of Level 1 and 2 homes exceeded 600. There were also significant logistical differences between the incidents in Lorain County and Jackson County. In Lorain County most of the affected homes were near each other, whereas in Jackson County some of the affected homes were over 60 miles apart.

The U.S. EPA Region IV based its response on the protocols established by U.S. EPA Region V in Lorain County. In addition to the aforementioned incidents, there have been other affected regions. Methyl parathion misapplication has been discovered in Hattiesburg, MS, Memphis, TN, and Chicago, IL, with rumors that the illegal pesticide use has spread to parts of Florida, Arizona, and California.

As the Jackson County project progressed, three phases of remedial options evolved. Phase I involved the use of six test houses that were contaminated with methyl parathion. These houses were sampled prior to decontamination, after decontamination, and after application of a sealant. Phase II was initiated after review of the data from Phase I. Phase II was a fixed lab treatability study of contaminated building materials obtained from Jackson County. Phase III was determined based on results from the lab treatability study. This phase involved elevating the internal temperature of contaminated homes to between 60°C and 75°C.

The objective of this study was to determine if there is a potential remedial method, other than demolition and removal, to reliably reduce the concentrations of methyl parathion to less than 15 micrograms per 100 square centimeters ($\mu g/100cm^2$) within homes and businesses.

2.0 Methodolgy

2.1 Phase I - Test House Study

With several hundred homes in need of decontamination, the U.S. EPA selected test houses to determine if there were potential sealing methods that would reduce concentrations of methyl parathion to below acceptable limits. All of the test houses were classified as Level 1, based on initial wipe sampling conducted by the U.S. EPA Region IV Pesticides Branch and the Mississippi State Department of Agriculture.

As part of the Jackson County project, the ERTC selected a total of 6 test houses to evaluate other remedial options and verify the decontamination effectiveness. The test house selection and procedure was an ongoing process. The results from one house led to the methodology to be utilized at the next house. Test House 1 was selected to evaluate various surfaces and procedures. Test House 2 was used to examine the ability of two different primer/sealers to seal the contaminants and the discoloration associated with the decontamination procedure. At Test House 3, clear sealers were tested on soft woods. At Test House 4 one sealer was used on all walls within multiple rooms. The initial sampling results from Test House 5 were below action levels, therefore further tests were not conducted. Test House 6 was used to verify the results of Test House 4, and to test a new sealer.

The six residences were evaluated using different sealing techniques on a variety of materials (i.e., dry wall, paneling, tongue and groove paneling, etc.). Differing decontamination and sealing procedures were attempted to determine the effectiveness of each.

Various commercially and locally available sealants were evaluated. Polyurethane, acrylic, latex-based paint sealants, and oil-based paint sealants were tested to determine if methyl parathion and its breakdown products could be encapsulated using sealants.

In accordance with U.S. EPA/ERTC/REAC Standard Operating Procedure (SOP) #2011: Chip, Wipe, and Sweep Sampling, wipe samples were collected from a 10 centimeter (cm) by 10 cm area using a disposable template and a cotton gauze pad moistened with isopropyl alcohol. Wipe samples were analyzed according to the U.S. EPA/ERTC/REAC SOP #1826: Analysis of Methyl Parathion in Wipe Samples by Gas Chromatograph/Mass Spectroscopy (GC/MS).

Air samples were collected following a modified NIOSH Method 5600, Pesticides, Organophosphorus. The air sampling train consisted of a 410-mg washed XAD-2 sorbent with a quartz fiber filter connected to a personal sampling pump. All samples were collected from 12 inches above the floor at a flow rate of 1 liter per minute (L/min.) for 240 minutes (4 hours). Air samples were analyzed according to the U.S. EPA/ERTC/REAC SOP #1825: Analysis of Methyl Parathion in Air Samples by GC/MS).

Initial wipe samples were collected from a number of locations within each home prior to decontamination. These locations included: bedrooms, kitchens, utility rooms, living rooms, family rooms, and bathrooms. The types of material sampled included: wallboard, baseboard, ceilings, counter tops, and cabinets. Notes and measurements were taken along with each sample in order to prevent re-sampling of the same area.

A second set of wipe samples was collected at a minimum of 24-hours after decontamination. A third set of wipe samples was collected at a minimum of 24-hours after the sealing process. In one case, a second sealing was performed followed by a fourth set of wipe samples.

Air samples were only collected in Test House 1 and 6. Samples were collected in the kitchen and in a bedroom at Test House 1, and in all 3 bedrooms at Test House 6.

2.2 Phase II - Treatability Study

Kiber Environmental Services (Kiber) of Atlanta, GA was subcontracted to perform a laboratory treatability study. Kiber collected building materials from homes contaminated with methyl parathion at the Jackson County Pesticide site. The materials were collected from houses under remediation. The materials included plastic baseboards, wood baseboards, wood paneling, dry wall, kitchen cabinets, bathroom cabinets, and Formica. Kiber subjected these materials to chemical, encapsulation, and physical tests.

Chemical treatment of methyl parathion was used as a method to remediate building materials. Kiber performed treatment by saturating test materials with a chemical reagent for 24 hours. Wipe samples were collected prior to and after treatment. Reagents evaluated were water, sodium hydroxide (50%), hydrogen peroxide (50%), sodium perborate (0.5%), sodium persulfate (4%), sodium percarbonate (1%), Windex (100%), potassium permanganate (20%), Dawn detergent (50%), sulfuric acid (10%), UltraKleen (17%), and ammonia (100%).

Encapsulation testing was performed by using commercially available sealants, including polyurethane, lacquer, enamel, paint, and primer. The encapsulants tested were a water based polyurethane, Krylon White Laquer Spray, Colorall Spray Enamel, Varathane Liquid Plastic, Rustoleum Fast Dry Primer, BEHR

Hi-Gloss Polyurethane, Krylon Interior/Exterior Spray, Kilz Sealer Primer, Rustoleum Appliance Enamel, and Krylon Epoxy Enamel. Typical application was through the use of an aerosol spray can. Each encapsulant was applied and allowed to dry for three days. Samples were collected from untreated building materials, and from sealed building materials. The building materials tested were drywall, Formica, press board, wood baseboard, and wood paneling.

Kiber determined that there were three types of physical treatment that could aid in the breakdown of methyl parathion. The physical treatments were heat, humidity, and ultraviolet radiation. These treatments were tested alone and in combination in order to determine which was the most effective. The physical treatments tested were heat at 53°C, ultraviolet light at 365 nanometers (nm), heat at 33°C with ultraviolet light at 365nm, heat at 60°C, heat at 75 °C, and heat at 100°C.

For the physical treatment study, the building materials tested by Kiber included dry wall, wood paneling, Formica, wood baseboard, and wood cabinets. Each building material was analyzed in triplicate. These triplicate samples were analyzed after various reaction times to evaluate the breakdown over time. The heat at 53°C, and both UV tests samples were analyzed prior to physical treatment, as well as, after 3, 7, and 14 days of treatment. The heat at 60°C, 75°C and 100°C tests samples were analyzed prior to physical treatment, as well as, after 1, 2, 3, 9, and 15 days of treatment.

2.3 Phase III - Heat Treatment Study

Based on the results of the treatability study, ERTC conducted a heat treatment study on 4 homes. Two trailer homes were set aside for the study in September 1997. A third trailer was studied in November 1997, and in January 1998 a heat treatment study occurred at a single-family home. Using an external heating and power supply, these homes were heated to between 60 °C and 75 °C for 4 to 10 days. Air and wipe samples were collected before, during, and after the heating events.

In September 1997, two trailers which were contaminated with methyl parathion at the Jackson County Pesticide site were moved to a central staging location. In order to best assess the heat treatment method, a new application of methyl parathion was sprayed within the trailers. Heat was supplied to the interior of the trailers utilizing two external heaters. Trailer 1 was heated with a gasoline powered heater, and trailer 2 was heated with an electrical heater. Each trailer had two bedrooms, one bathroom, and a common living room/kitchen area.

Temperature and relative humidity readings within the trailers were recorded prior to introduction of heat. Each trailer reached the target temperature of 60°C within 3-hours. Temperature and relative humidity readings were monitored throughout testing.

Wipe and air sampling was performed prior to the introduction of heat into the trailers to establish baseline conditions. Wipe samples were collected from areas sprayed with methyl parathion as well as from clean areas. Once the temperature within the trailer reached 60°C, wipe and air sampling was performed twice per day for the first 3 days and once on the fourth day. After sample collection on the fourth day, the heat was turned off, and air sampling ended. On the fifth day, 24 hours after

heating ended, a last series of wipe samples were collected. After the wipes were collected, bulk samples (drywall, paneling, and carpet) were collected from 10 areas.

In November 1997, a third contaminated trailer located at 1629 Wynedote Drive in Gautier, MS was heated for seven days. The trailer had three bedrooms, two bathrooms, and a common living room/kitchen area. The residents of the trailer were relocated during treatment and restoration.

Heat was supplied to the interior of the trailer by an external electric heater powered by a diesel generator. The heated air was channeled into the trailer through a network of ducts. The main duct entered through the living room window and branched to both ends of the trailer. Smaller ducts branched off the main duct into the main bathroom, middle and east bedrooms.

Temperature readings within the trailer were recorded prior to heating. The trailer reached the target temperature of 75°C within 5 hours. Trailer temperature readings were monitored throughout the 7-day test.

Initial sampling was performed prior to the introduction of heat into the trailer to identify baseline conditions. Initial wipe samples were collected at 40 locations throughout the trailer. Samples were collected from baseboards, cabinets, and discolored areas. One vacuum dust composite sample was collected from the trailer floor. The north side of each carpeted room (master bedroom, living room, middle bedroom and east bedroom) was vacuumed.

Each day air samples were collected in duplicate from the living room. On the second day, ambient air samples were collected from four locations surrounding the trailer.

Due to generator failure, the heating supply was interrupted twice. The first outage occurred on the second day of the test and lasted 4 hours. The lowest temperature recorded during this outage was 63°C. On the sixth day of the test, the generator failed again. Power was not restored for 12 hours. The lowest temperature recorded during this outage was 20°C.

After seven full days the heat was turned off. Sixteen hours after heating ended, post samples were collected. Ten post wipe samples were collected. One set of post heating duplicate air samples were collected from the living room. The post heating vacuum sampling was also collected. The south side of each carpeted room (master bedroom, living room, middle bedroom and east bedroom) was vacuumed.

In January 1998, a fourth contaminated home located at 9075 Hall Road in Grand Bay, AL was heated for ten days. The home had three bedrooms, two bathrooms, living room, and kitchen. Heat was supplied to the interior of the home by an external electric heater powered by a diesel generator. The heated air was channeled into the house through a network of ducts. The main duct entered through the living room window. Smaller ducts branched off the main duct into each room.

Temperature readings within the house were recorded prior to heating. The home reached the target temperature of 75°C within 5 hours. Internal temperature readings were monitored throughout the 10-day test.

Initial wipe samples were collected at 25 locations prior to the introduction of heat. Duplicate air samples were collected each day from the living room.

After one week, the heating process was interrupted and the house was vented for 7 hours. The venting was assisted by the blower on the heater at 9,000 cubic feet

per minute. At the conclusion of the venting process, the house was closed and heated again. The house reached 75°C within two hours.

After ten days the heat was turned off. At that time, 40 post heating wipe samples were collected. The next day, one set of post heating duplicate air samples were collected from the living room.

3.0 Results and Discussion

3.1 Phase I - Test House Study

A variety of procedures were conducted on Test House 1 (2519 Ridgeway) to evaluate sealants on walls and wood cabinets. Extremely variable results were encountered after the sealing, indicating that the spray application of methyl parathion was not uniform. This led to inconsistent sampling results due to the fact that only small areas of the house were used for testing. It was determined that entire rooms would have to be tested in order to evaluate sealant effectiveness. It was also found that spraying of the sealant was more effective than brushing; additionally, the attempts to seal wood cabinets were unsuccessful. This failure could have resulted from the lack of complete decontamination, or from the solvent in the sealer (acrylic & polyurethane) acting as a mobilizer for the residual methyl parathion in the wood material. Table 1 presents the Test House 1 methyl parathion wipe sample results, and methyl parathion air sample results.

The goal for Test House 2 (4207 2nd Street) was to evaluate the KILZ sealant's ability to cover the yellow staining produced by the decontamination procedure and its ability to seal in contaminants, in order to meet the cleanup criteria of 15 $\mu g/100cm^2$. The work performed in Test House 2 consisted of decontamination with a 10 percent Ultra-Kleen (UK) solution followed by a 2 percent UK wash, then repeating the process. Separate rooms were sprayed with latex and oil-based KILZ Sealant and the walls were left to dry. The post sealing results showed that the walls would meet the decontamination cleanup criteria. Visual observations indicated that the latex-based paint did not mask the yellow staining associated with the decontamination procedure. Table 2 presents the Test House 2 methyl parathion wipe sample results.

Test House 3 (5301 MLK Blvd) was tested to determine the effectiveness of clear sealers on stained tongue and groove wood paneling. The results from Test House 3 verified the previous results (from Test House 2) that it was difficult to decontaminate and seal soft woods (i.e., heart pine and manufactured paneling). None of the sealed material met the cleanup criteria. Based on the information derived from the wood testing, the only two successful decontamination options for wood were to: 1) remove, or 2) seal with KILZ. However, sealing with KILZ would permanently change the appearance by masking the natural wood.

On Test House 4 (3837 Jefferey Road) the U.S. EPA conducted a variety of decontamination and sealing methods to finalize a wall-based method. The living room and 2 bedrooms were decontaminated using various concentrations of UK solutions and were then sealed with oil-based KILZ. A third bedroom was not decontaminated but was sealed with two coats of oil-based KILZ. The post sealing wipe results for all rooms were below the decontamination criteria of 15 $\mu g/100cm^2$.

No sealing work was conducted on Test House 5 as the initial wipe sample

concentrations were below the action level.

Phase I concluded with Test House 6 (6742 Anna Ave.). The testing procedures in this house were the same as Test House 4, however, a new sealer was used on the baseboards. All of the post sealing sample results met the cleanup criteria except for one baseboard sealed with the new sealer, Enterprise Stain Block, Oil Primer/Sealer.

The results from the five test houses indicate that the wipe sample cleanup criteria can be met if materials are decontaminated and sealed. The cleanup criteria can also be met by covering the material with two coats of oil-based KILZ without decontamination. However, only painted surfaces (i.e. drywall and baseboards) can be treated without altering the external appearance of the material. Additionally, long-term leaching effects were not studied.

3.2 Phase II - Treatability Study

Kiber observed significant variability in the analytical results due to the non-heterogeneous nature of the method of application of the methyl parathion. However, certain trends were observed during the chemical treatment process.

Sodium hydroxide at a 50% concentration was effective at reducing methyl parathion concentrations to below 15 $\mu g/100cm^2$. However, treatments with weaker sodium hydroxide concentrations were not able to reduce methyl parathion below 15 $\mu g/100cm^2$.

Sodium percarbonate and potassium permanganate were able to achieve the treatment criteria for some but not all of the material tested. However, due to the variability of the testing data, no strong conclusions were able to be made regarding these chemical additives.

Review of the data from the encapsulation testing reveals that most products tested are capable of reducing the concentrations of methyl parathion on a sealed surface. However, the data does indicate that several products were more effective. The products include Krylon White Lacquer, Colorall Spray Enamel, and BEHR Hi-Gloss Polyurethane. Note that the variability in the treatment process was noted between different materials, making accurate comparison of different products difficult.

Treatment with heat alone at 53°C was effective at reducing methyl parathion concentrations. Treatment was effective on most material, but it was not always able to reduce concentrations below 15 $\mu g/100cm^2$. Treatment of drywall using the 53°C heat alone was generally effective, resulting in significant reduction within 3 to 7 days. The results of the treatment on other material types showed slightly reduced treatment effectiveness.

Treatment with the UV light alone did not produce a concentration reduction trend. Kiber theorized that while UV light should have been successful, methyl parathion soaked into the building materials, and the UV light was only able to treat the surface of the material.

Treatment with heat at 33°C and the UV light provided the poorest treatment. Very little breakdown occurred at temperatures less than 50°C.

Treatment with heat at 60°C, 75°C, 100°C provided the most positive results. Heat at 60°C showed that wipe samples on all of the building materials tested were at

acceptable limits (under 15 μg/100cm^2) within 15 days after the beginning of heating. Heat at 75°C showed that wipe samples on all of the building materials tested were at acceptable limits by the third day of heating. Heat at 100°C showed that wipe samples on all of the building materials tested were at acceptable limits after the first day of heating.

The results from the treatability study indicate that the most viable remedial option would be to heat a residence between 60°C and 75°C. This conclusion led to the evolution of Phase III, the heat treatment study.

Tables 3 and 4 present the trailer 1 methyl parathion wipe sample results. wipe sample results.Table 5 presents the methyl parathion air sampling results from trailers 1 and 2.

Table 6 presents the wipe sample results from 9075 Hall Road. Table 7 presents daily air sample results from 9075 Hall Road.

The results from the heat treatment study indicate that heating to 75°C provides an effective means for remediating a residence contaminated with methyl parathion. While this method does offer a method to reduce methyl parathion concentrations to acceptable limits (less than 15 μg/100cm^2), the trade off occurs in the form of heat damage. Most plastic moldings, plastic light switches, plastic outlet covers, and plastic heating registers sustained damage during the heating process. Also as a consequence of the heat some cabinets and counters tops began to separate. Additionally, wall paper peeled and drywall cracked and separated. protocol.

4.0 Conclusion

The results from the heat treatment study indicate that heating a home to 75°C provides an effective means for remediating a residence contaminated with methyl parathion. While this method does offer a method to reduce methyl parathion concentrations to acceptable limits (less than 15 μg/100cm^2), the trade off occurs in the form of heat damage.

Table 1: Test House 1 Methyl Parathion Wipe Sample Results in µg/100cm²

Location	Pre-Decon Results	Post-Decon Results	Post-Sealing Results	Sealant Used
Living Room Baseboard 1	176	766	11	KILZ Spray
Living Room Baseboard 2	33.7	243	6.22	KILZ Spray
Hallway Wall	116	82	69.5	KILZ Total One
Hallway Ceiling	89.4	38.2	36.9	Not Sealed
Bathroom Baseboard	34.4	51.9	4.9	KILZ Total One
Kitchen Wall - Low	205	225	3.87	KILZ Spray
Kitchen Baseboard	474	310	5.75	KILZ Spray
Kitchen Floor Tile	82.2	98.6	58.7	Not Sealed
Kitchen Wall - High	60.3	256	30.8	KILZ Total One
Kitchen Ceiling	12.1	8.11	0.92	Not Sealed
Kitchen Counter	115	75	27.7	Not Sealed
Kitchen Wall near Counter	477	328	16.9	Spray Polyurethane
Kitchen Cabinet Door	3.65	75.1	Not Sampled - Discarded	Acrylic Spray
Kitchen Cabinet Bottom	1790	35	53.1	Spray Polyurethane
Kitchen Cabinet Side	35.4	14.4	25.6	Acrylic Spray

Table 1a Test House 1 Methyl Parathion Air Sample Results in µg/m³

Location	Pre-Decon Results	Post Decon Results
Kitchen	3.20	1.00
Bedroom	1.37	1.03

µg/100cm² - Micrograms per 100 square centimeters.
µg/m³ - Micrograms per cubic meter.

Table 2: Test House 2 Methyl Parathion Wipe Sample Results in µg/100cm²

Location	Post-Decon Results	Post Sealing Results	Sealant Used
Location 1 - Blue bedroom wall next to door (200cm²)	6.46	Not Sampled	Latex-based KILZ
Location 2 - Blue bedroom wall under West window (200cm²)	0.39 J	5.86	Latex-based KILZ
Location 3 - Blue bedroom wall under South window (200cm²)	1.85	Not Sampled	Latex-based KILZ
Location 4 - Blue bedroom wall near closet (200cm²)	23.9	7.44	Latex-based KILZ
Location 5 - Master bedroom wall near closet door (200cm²)	2.44	Not Sampled	Oil-based KILZ
Location 6 - Master bedroom wall under South window (200cm²)	4.06	Not Sampled	Oil-based KILZ
Location 7 - Master bedroom wall under bay window (200cm²)	0.72 J	1.67	Oil-based KILZ
Location 8 - Master bedroom heater wall (200cm²)	77.1	3.78	Oil-based KILZ
Location 9 - Small bedroom wall composite (400cm²)	4.06	12.1	Latex-based KILZ
Location 10 - Living room panel wall composite (400cm²)	0.26 J	Not Sampled	Not Sealed
Location 11 - Bathroom wall composite (200cm²)	3.87	Not Sampled	Not Sealed
Location 12 - Family room wall composite (300cm²)	1.41 J	Not Sampled	Not Sealed
Location 13 - Laundry room wall composite (200cm²)	U	Not Sampled	Not Sealed
Location 14 - Mud room panel wall composite (200cm²)	42.9	Not Sampled	Not Sealed
Living room ceiling above front window (100cm²)	Not Sampled	8.64	Not Sealed
Master bedroom ceiling above center of bay window (100cm²)	Not Sampled	0.31 J	Not Sealed

µg/100cm² - Micrograms per 100 square centimeters.
J - Compound present below Method Detection Limit (MDL) of 1.5 µg/100cm².
U - Not detected above MDL.

Table 3: Trailer 1 Methyl Parathion Wipe Sample Results in μg/100cm^2

Location	9/15 Pre Heat	9/16 AM	9/16 PM	9/17 AM	9/17 PM	9/18 AM	9/18 PM	9/19 AM	9/20 PostHeat
East Bedroom Drywall Top	5.5	4.9	3.7	1.9	1.1J	1.8	3.5	1.5	1.5
East Bedroom Drywall Middle	31	9.4	6.0	4.3	3.1	4.3	3.6	2.9	1.7
East Bedroom Drywall Bottom	13	8.9	4.6	4.8	3.6	4.0	4.0	3.3	2.2
Master Bedroom Drywall Top	3.3	14	1.9	2.3	1.4J	1.9	1.6	1.6	0.8J
Master Bedroom Drywall Middle	3.5	9.1	1.7	2.6	1.2J	1.5	3.0	1.2J	1.0J
Master Bedroom Drywall Bottom	3.5	12	1.9	1.7	1.4J	1.6	1.6	1.4J	1.0J
Dining Area Paneled Wall Top	740	1800	740	45	110	73	320	750	500
Dining Area Paneled Wall Middle	2500	2400	1400	67	1800	220	110	1000	840
Dining Area Paneled Wall Bottom	3300	3500	190	1400	2000	3400	2500	1600	53
Kitchen Outside Drywall Top	33	2.8	5.6	4.9	23	6.8	11	7.7	1.9
Kitchen Outside Drywall Bottom	21	4.9	5.2	7.0	16	7.8	9.7	9.8	1.5
Kitchen Inner Drywall Top	27	3.5	4.5	3.4	7.5	3.6	6.1	7.1	1.7
Kitchen Inner Drywall Middle	22	4.3	3.5	4.6	8.8	5.6	6.5	3.6	1.9
Kitchen Inner Drywall Bottom	34	6.1	7.2	5.2	18	4.9	8.7	4.2	2.3
West Bedroom Paneled Wall Top	18	6.6	4.1	4.4	7.5	5.0	5.7	0.7J	0.5J
West Bedroom Paneled Wall Middle	30	3.6	3.0	3.2	6.2	4.1	4.1	0.5J	U
West Bedroom Paneled Wall Bottom	25	5.3	2.9	2.3	4.4	3.3	5.1	0.8J	0.5J
West Bedroom Drywall Top	14	2.8	3.6	2.0	4.9	4.3	5.3	0.8J	1.4J
West Bedroom Drywall Middle	7.8	3.9	2.0	1.9	5.8	1.1J	3.9	1.7	0.9J
West Bedroom Drywall Bottom	13.0	4.5	3.4	3.0	5.1	2.8	2.8	2.4	1.4J

μg/100cm^2 - Micrograms per 100 square centimeters.
J - Compound present below Method Detection Limit (MDL) of 1.5 μg/100cm^2.
U - Not detected above MDL.

Table 4: Trailer 1 Methyl Parathion Wipe Sample Results in μg/100cm^2

Location	9/15/97 Pre Heat	9/20/97 Post Heat
Master Bathroom Counter Top	4900	74
Refrigerator Door	100	1.9
Bath Tub	220	1.1J
Master Bedroom East - Clean Drywall	NS	U
Master Bedroom East - Clean 2" x 4"	NS	0.4J
Kitchen - Clean Drywall	NS	U
Kitchen - Clean 2" x 4"	NS	1.1J

μg/100cm^2 - Micrograms per 100 square centimeters.
NS - Not Sampled.
J - Compound detected below method detection limit (MDL)of 1.5 μg/100cm^2.
U - Not detected above MDL.

Table 5: Trailer 1 and 2 Methyl Parathion Air Sampling Results in μg/m³

Location	9/15/97 1000 to 1200	9/15/97 1630 to 0030	9/16/97 0800 to 1600	9/17/97 0000 to 0800	9/17/97 0800 to 1600	9/18/97 0000 to 0800	9/18/97 0800 to 1600	9/19/97 0000 to 0800
Trailer 1 - Living Room	32	155	189	59	94.5	72	99	91
Trailer 1 - West Bedroom	29	90	72	15	34	6.6	31	19
Trailer 2 - Living Room	14	144	21	0.13J(1)	21	14.2	11	2.9
Trailer 2 - West Bedroom	22	85	39	11	0.31J(2)	18.7	4.6	7.2

μg/m³ - Micrograms per cubic meter.
J - Compound present below Method Detection Limit (MDL).
U - compound not detected above the MDL. The MDL was volume dependent and ranged from 0.48 to 1.73 μg/m³.
(1) - Total pump failure occurred. Timer showed completion of program, however pump had zero flow capability.
(2) - Tubing became disconnected due to heat.

Table 6: Methyl Parathion Wipe Sample Results in $\mu g/100cm^2$ from 9075 Hall

Location	Initial Sample 1/19/98	Post Sample 1/29/98
Kitchen - Northeast corner - Top of cabinet	5200	1.2 J
Kitchen - Northeast corner - Back splash	320	0.5 J
Kitchen - Northeast corner - Counter top below Back splash	120	U
Kitchen - West wall - Wall behind refrigerator location	19	2.0
Kitchen - Under refrigerator location - Floor	6.9	1.8
Kitchen - Cabinet under sink - Next to J-pipe	30	11
Kitchen - Top cabinet right of refrigerator - Right corner	1.8	U
Kitchen - Lower cabinet right of refrigerator - Top left	2.4	0.6 J
Kitchen - Cabinet over stove - Next to vent pipe	340	1.7
Kitchen - Cabinet next to living room - Lower right	550	5.3
Kitchen - Top of cabinet above stove - Next to vent pipe	NS	1.5 J
Kitchen - West wall - Wall above wallpaper in middle	NS	1.4 J
Kitchen - Counter top - Left of sink	NS	U
Kitchen - Back splash - Between stove and sink	NS	1.1 J
Kitchen - Top drawer - In cabinet right of refrigerator	NS	0.8 J
Kitchen - Top drawer - In cabinet right of stove	NS	1.0 J
Kitchen - Cabinet left of sink - Bottom shelf	NS	28
Kitchen - Cabinet between sink and stove - Bottom shelf	NS	14
Kitchen - Upper cabinet left of sink - Bottom shelf	NS	0.7 J
Kitchen - In front of sink - Floor	NS	6.4

$\mu g/100cm^2$ - Micrograms per 100 square centimeters.
U - Not detected above the Method Detection Limit (MDL).
J - Compound present below MDL of 1.5 $\mu g/100cm^2$.
NS - Not sampled.

Table 6 (continued): Methyl Parathion Wipe Sample Results in µg/100cm^2 from 9075 Hall Road

Location	Initial Sample 1/19/98	Post Sample 1/29/98
Dinning area - China cabinet on east wall - Bottom shelf	860	4.7
Refrigerator - Right side	26	0.4 J
Living room - South wall - On baseboard near outlet	5.9	1.4 J
Dryer - Front top in center	5.4	U
Washer - Right side top in center	2.5	U
Freezer - Back left side by vents	2.2	U
Washer/dryer area - By water pipes - Floor	11	0.9 J
Washer/dryer area - By water pipes - Baseboard	28	0.8 J
Water heater area - Right wall - Baseboard	9.5	0.7 J
Water heater area - Right side - Floor	6.0	2.2
Bathroom - Vanity base - By wall and drain pipe	50	4.1
Bathroom - Baseboard between toilet and bathtub	10	U
Bathroom - Floor between toilet and bathtub	6.1	4.1
Bathroom - Vanity top right drawer in back	NS	2.1
Bathroom - Counter top by Back splash under light switch	NS	U
Bathroom - Back splash under light switch	NS	U
Living room - China cabinet - Bottom shelf in corner	NS	1.0 J
Northwest corner bedroom - East wall - Amber stain	NS	U
Northwest corner bedroom - North wall - Drywall by outlet	4.1	0.5 J
Northwest corner bedroom - North side - Floor by outlet	0.7 J	U

µg/100cm^2 - Micrograms per 100 square centimeters.
U - Not detected above the Method Detection Limit (MDL).
J - Compound present below MDL of 1.5 µg/100cm^2.
NS - Not sampled.

Table 7: Methyl Parathion Air Sample Results in μg/m^3 from 9075 Hall Road

Date & Time	Living Room	Living Room Duplicate
08 January 1998 - 1350 to 1750	0.78$^{(1)}$	NS
20 January 1998 - 1020 to 1420	140	130
21 January 1998 - 1000 to 1400	96	85
22 January 1998 - 0933 to 1333	61	NS
23 January 1998 - 1010 to 1410	43	43
24 January 1998 - 1020 to 1420	34	34
25 January 1998 - 1015 to 1415	46	33
26 January 1998 - 0945 to 1345	31	30
27 January 1998 - 0937 to 1337	25	23
28 January 1998 - 0945 to 1345	12	11
29 January 1998 - 1000 to 1400	17	13
30 January 1998 - 0811 to 1211	U$^{(2)}$	U$^{(3)}$

μg/m^3 - Micrograms per cubic meter.
U - Not detected above the method detection limit of 0.52 μg/m^3.
NS - Not sampled.
(1) - Sample collected in kitchen.
(2) - Sample and duplicate collected in kitchen.
(3) - Sample collected in bedroom.

The Evaluation of the Steam and Iron Assisted Leaching (SIAL) Process.

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Abstract

While performing experiments for the clean up of soils contaminated with Soviet Samin missile fuel, it was observed that the presence of water vapour enhanced the thermal desorption of the Samin. Removal of these compounds was noted at lower than expected temperatures. The literature also indicates that this is possible for PolyChlorinated Biphenyls (PCB)'s and PentaChloroPhenols (PCP) (Krabbenhoft, *et al.*, 1996). While performing unrelated experiments to determine the effect of different acids on the leaching of metals from incinerator wastes, a significant reduction in the amount of dioxins and furans was observed. From these initial observations the following process was developed.

This project will investigate the effect of pH, Pressure, Temperature, and Iron on the removal of PCB's and Doixins/Furans from soils using nitric acid. The process investigated is an ex-stitu, batch system. If successful, then a full scale unit will be designed and built. This paper will discuss the effect of different operational parameters, but will not investigate the actual mechanisms of the process.

1.0 Objectives

Various experiments will be undertaken to determine the effectiveness of different parameters on the process. These parameters will include temperature and pH, pressure, and the presence of iron. It is anticipated that once the optimum conditions have been determined, a full scale reactor be built.

2.0 The Process

The process involves acidifying the soil with nitric acid, applying heat, under low pressure and condensing the vapours. It is expected that the organic contaminants will be transferred to the condensate or destroyed. The inorganic contaminants should remain in the soil. It is suspected that these metals will be more readily leached from the acidified soil. The temperatures that will be used are below the decomposition temperature of the organic contaminants. A maximum temperature of 300 C will be utilised.

The condensate should then be treated by running it through an Advanced Oxidation Process (AOP) reactor. This reactor uses UV light and oxidants to destroy organic contaminants in water. Previous testing has shown that enhanced UV oxidation results in the destruction of the PCB's and Dioxins (Argue, 1992).

2.1 The Soil

The soil used to test the process was to be from Environment Canada. Environment Canada had no soils available at the time for this project. It was decided to use the soils available. The soil used was from a landfill in Finland. This site is contaminated with PCB's, Dioxins/Furans, and heavy metals. The contamination

arose from the deposition of incinerator slag at the site. Only one soil was tested, due to budgetary and time constraints.

2.2 The Reactor

A bench scale reactor was used to test the process. This unit was designed to process 2 Kilograms of soil in a batch mode. Below is a photo of the reactor. This reactor was a modified pressure cooker. The pressure inside the reactor was regulated with a check valve set to release at 20 PSI. A second valve was set to release at 50 PSI. This acted as a safety. The reactor was also equipped with a manual valve so that the system could be pressurised or vented. A pressure gauge was installed to record the internal pressure, as well as a thermal couple to monitor temperature. This thermal couple was connected to a controller, which in turn regulated a heating element. The controller was set to either 200c or 300c depending on the experiment. The reactor was heated by various methods, the most successful being a 60,000 BTU propane burner. The exhaust from both the check valve and the release valve were connected to a condenser. The off gasses were condensed and collected at this point.

The first reactor was decommissioned after the seventh run due to metal fatigue. A new reactor was constructed. This second reactor was built with thicker walls as well as incorporating a stirrer.

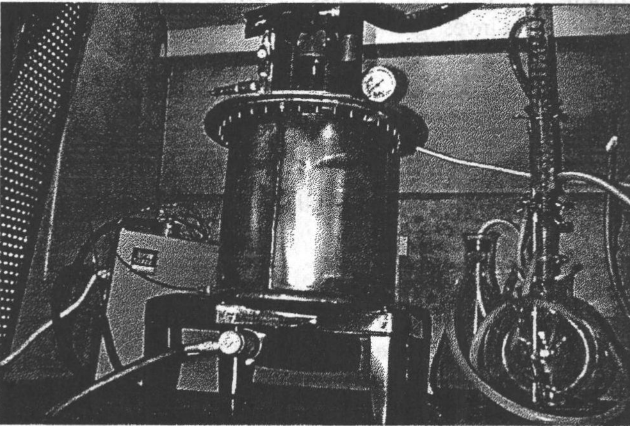

Figure 1 The Reactor

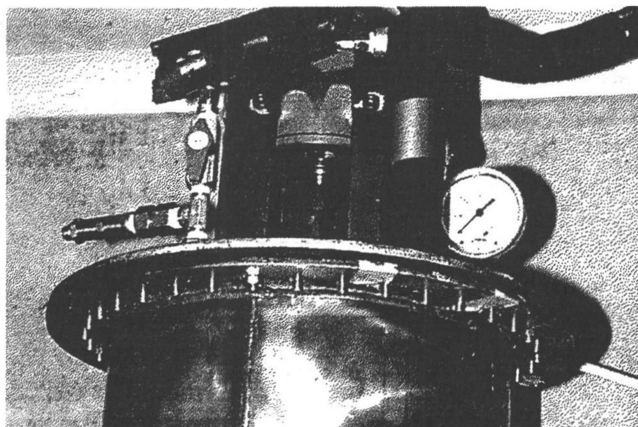
Figure 2 Close up of Valves and Gauge

3.0 Results

The following runs were performed:

Table 1 The Test Matrix

Run #	pH	Temp	Pressure
1	2	300	20
2	2	300	20
3	2	300	0
4	6	300	20
5	2	300	0
6	2	200	20
7	6	300	20
8	2	300	20
9	6	300	20
10	6	300	0
11	2	300	20

The effect of iron could not be determined, due to the lack of time and resources.

The samples were analysed for pH, moisture, PCB's and, Dioxins/Furans. It was noted in the first set of results (runs 1 through 7) that the PCB's followed the same trend as the Dioxins and Furans. In order to reduce the cost of analysis, only PCB's were tracked for the next set of runs (runs 8 through 11).

3.1 Removal Efficiency

Table 2 Removal Efficiency

Run #	pH	Max Temp Attained	Pressure	% Removal of PCB's	% Removal TEQ's
1	2	130	20	41	23
2	2	329	20	82	34
3	2	297	0	28	14
4	6	328	20	95	80
5	2	296	0	67	N.D.
6	2	225	20	60	35
7	6	332	20	89	80
9	6	168	20	84	N.D.
10	6	177	0	83	N.D.

N.D = Not determined.

From the results it can be seen that the percentage removal of PCB's is better than for Dioxins/Furans. This may be due to the lower initial concentration of Dioxin/Furan as compared to the PCB concentration. A neutral medium is better for the removal of these compounds from soil than an acidic medium. This operating condition results in condensate comprising of more than one phase. Running at elevated pressures increases the efficiency of removal under all conditions. The higher temperature runs also showed better performance. Due to analytical problems, the results for runs 11 and 8 could not be determined.

4.0 Discussion

The best conditions for running the reactor are neutral, 300c, and under pressure. The worst conditions were acidic, high temperature, and no pressure. The removal efficiency is not the only concern of this project at this time. When determining the mass balance for the system, it was observed that the contaminants were lost. The mass of the system does not balance since the initial mass does not equal the final mass plus the mass in the condensate. There can be two explanations for this. The first is that the compounds of interest are being lost (atmosphere, poor analytical recovery, irreversible binding with soil and/or particulates). The second is that the compounds are being destroyed.

Investigation of the chromatographs for evidence of destruction indicates that the latter is the case. One would expect that the post run soils would have proportionately more light components (due to the break down of the heavier components) than in the pre run soil. This was not observed. The proportion of light compounds to heavy compounds remains the same. Conversely there is evidence in the chromatographs of the condensate phase that destruction does take place. It can be seen that there is a greater amount of lighter compounds in the condensate, with less of the heavier components. This may indicate that the compounds that are carried over are being broken down in the vapour phase. Further investigations are necessary. Radio labelled compounds could be used to track the migration of individual compounds. When the system was run using acid, the off gas was reddish in colour.

This may indicate the production of NOx. This must be addressed in the design of a larger scale reactor.

The number of test runs was limited by the sample analysis. Sample analysis for PCB's and Dioxin/Furans is both time consuming and costly. Even with assistance from Environment Canada, the turn around time for samples sometimes exceeded six weeks. The cost per sample was in the $700.00 range. The operation of the reactor was quick, with a capacity of one run per day. The most time consuming part of the process was cleaning the reactor from the previous run.

5.0 Conclusions and Recommendations

The process as tested shows a significant reduction in the amount of PCB's and Dioxin/Furans in the soil. Preliminary evidence indicates that the compounds are being destroyed in the vapour phase, but further investigations are required for confirmation. The efficiency varies with the run conditions. Up to 95% of PCB's and 80% of the Dioxins/Furans were removed from the soil. It is anticipated that soils with greater contamination will yield better removal efficiencies. The use of a slightly higher temperature may also lead to improvement.

The following are recommendations for future study. To better determine the exact mechanisms of the process, a less complex soil matrix should be tested. This could be done by spiking a clean soil. The spike could also be a single compound, such as a single congener, as opposed to an Aroclor. The mass balance should be investigated in greater detail. If indeed the compounds are being destroyed then an experiment should be set up to investigate this aspect. The effect of metals in the soils should be investigated to see if they have an effect on the system. Different types of soils should also be investigated. Once an optimised system is set up, an AOP study should be carried out.

In conclusion, this process shows great promise, but further investigations are warranted.

6.0 Acknowledgements

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Sources, Pathways and Environmental Fate of Organochlorine Compounds in the Arctic

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Abstract

Over the past six decades, the arctic has been exposed to many organochlorines and heavy metal contaminants originating from very distant regions of the world. These organochlorines have both industrial and agricultural (pesticides) uses and include such substances as toxaphene, chlorobenzenes, trichloroethane (DDT), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCHs). It has been shown that the Arctic ecosystems are particularly susceptible to these organochlorines. These extremely persistent, lipophilic and bioaccumulative contaminants have been shown to reach elevated concentrations in both plants and animals. Many researchers have shown that high levels of the contaminants caused toxicological disorders such as: immunal dysfunction, reproductive disorders, reduced learning abilities, and developmental abnormalities.

1.0 Introduction

The Arctic, often referred to as 'north of 60', is a vast and cold region comprising of 40 percent of Canada's land area. It is sparsely populated and always considered pristine because of its remoteness and lack of industrial activities.

It is no surprise to anyone that the highest concentrations of organic contaminants are found in areas with highly industrialized or agricultural inputs. However, it is mind-boggling how the once-pristine, far-remote regions of the world such as the Arctic have been infiltrated with persistent, hydrophobic organic and inorganic toxic substances (see Figure 1). Most of these contaminants are anthropogenic in origin and have either industrial or agricultural uses (Wania and Mackay, 1995). Rappe in 1974 predicted that persistent organic contaminants migrate thousands of miles through the atmosphere from their point of release as gases and aerosols to these remote regions of the globe. Temperatures in tropical and temperate regions at lower latitudes where they are heavily used tend to favour volatilization from the Earth's crust, while cold and Arctic climate at higher latitudes tend to favour condensation and deposition from the atmosphere onto snow, ice, soil, water and vegetation (Wania and Mackay, 1993a).

The migration of contaminants of different volatilities can be likened to gas chromatography elution. Contaminants of different volatilities migrate through the atmosphere at varying speeds. For example, smaller molecules and highly volatile compounds often remain airborne for very long periods of time and therefore migrate much faster, while less volatile or heavier contaminants migrate very slowly and do not stay in the air as long. This phenomenon of differential contaminant mixtures

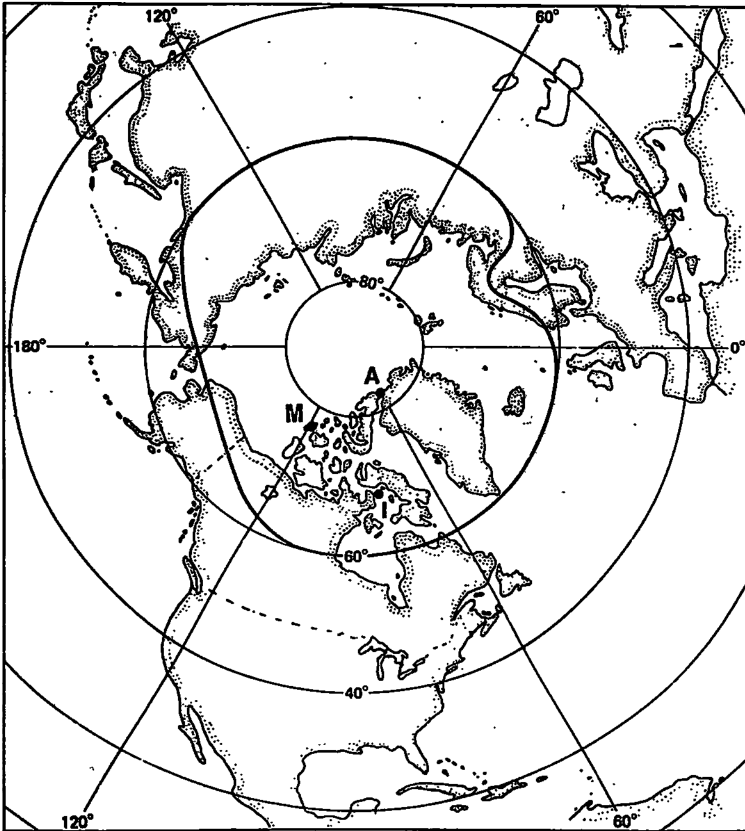

Figure 1 The Arctic above 60° Latitude

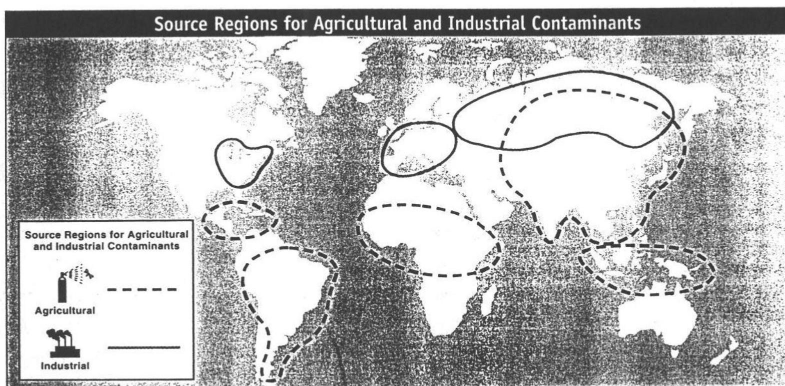

Figure 2 Global Main Source Regions of Agricultural and Industrial Contaminants

deposition by long-range transport along increasing latitudinal or varying temperature gradients has been coined several terms e.g. global distillation, global fractionation, cold condensation etc. Many investigations dealing with contaminant concentrations in the atmosphere at different latitudes or temperatures have been published (Stern *et al.*, 1997; Muir *et al.*, 1996; 1994; Oehme *et al.*,1996; Iwata *et al.*, 1995). Even though some significant local inputs have been documented in the Canadian Arctic, such as the Distant Early Warning (DEW) military radar sites (Bright *et al.*, 1995; Reimer *et al.*, 1993b), the predominant pathway to the northern latitudes is generally believed to be atmospheric transport in the gas phase and aerosol, and to a lesser extent by rivers from southern latitudes.

These contaminants are of some concern because they are very stable to oxidation, hydrolysis, photolysis, and biodegradation is very slow in northern climates. In addition to their persistence, some also bioaccumulate and biomagnify at all trophic levels.

The three main divisions of contaminants of some concern for human health and the environment found in the Arctic are: 1) persistent organic pollutants such as organic pesticides, chlorinated industrial organic compounds, and polyaromatic hydrocarbons; 2) heavy metals; 3) radionuclides. This paper will deal with the persistent organic pollutants. In this paper, the words pollutant and contaminant will be used interchangeably, but strictly speaking, a contaminant is any substance that is foreign to a place. It may or may not be harmful depending on the prevailing concentrations. On the other hand, a pollutant is an anthropogenic substance that is capable of doing some damage to human health or the environment (in air, water, or land). In other words, a pollutant is a contaminant in effect, but a contaminant may or may not necessarily be a pollutant.

Arctic Environmental Contaminants of Concern (Barrie *et al.*, 1992) include:

1.) Organic Pesticides
- Chlorobornanes (CHBs)
- Chlorobenzenes (CBZs)
- Hexachlorocyclohexanes (HCHs)
- Chlordanes
- DDT/DDE
- Aldrin/dieldrin
- Endosulphan
- Others (trifluralin,etc.)

2.) Chlorinated Industrial Organic Compounds
- Chlorobenzenes (CBZs)
- Polychlorinated biphenyls (PCBs)
- Dioxins and Furans (TCDDs and TCDFs)

3.) Polyaromatic hydrocarbons (PAHs)
- Benzo[a]pyrene (B[a]P)

4.) Metals

Cadmium (Cd)
Lead (Pb)
Mercury (Hg)

5.) Radionuclides

Cesium (^{137}Cs)
Plutonium ($^{239,240}Pu$)
Iodine (^{129}I)
Strontium (^{90}Sr)

6.) Acids

Sulphur oxides
Nitrogen oxiides

2.0 Sources and Occurrence

As the name implies, persistent organic pollutants are organic, hydrophobic anthropogenic substances and their metabolites (e.g., PCBs, HCB, dioxins, furans), chlorobenzenes, chlorinated pesticides (e.g., DDT, DDE, toxaphene, chlordane, HCH, aldrin and dieldrin and polyaromatic hydrocarbons (PAHs). The organo-chlorines are human-made just like most pollurtants, but unlike the PAHs and metals, they form a unique group in that they do not have any natural sources. The first hint of the presence of these pollutants in the Arctic came from pilots in early 1950s having to fly frequently through Arctic haze which has since been confirmed as clear evidence of contamination as a result of long-range transport over time (Hargrave *et al.*, 1988; Gregor, 1989). Another evidence of Arctic contamination by foreign chemicals came from toxicological studies which showed that marine mammals and fish in the Arctic do have relatively high levels of these organic pollutants. Given the levels and the identity of these pollutants, they have to come from far distant and foreign places, outside the Arctic Circle (Stern *et al.*, 1997; Barrie *et al.*, 1992)

2.1 Pesticides

Most of the pesticides of concern found in the far North are chlorinated substances, and are produced in the southern latitudes and places far removed from the Arctic such as the United States, United Kingdom, France, Russia, Denmark, Germany, South Africa etc. Although the production and uses of some of these pesticides (e.g. dichlorodiphenyltrichloroethane or DDT) have been stopped in North America and many European countries because of their toxicities, their application is still continuing in many developing countries especially in the tropics, Africa, Central America, South America, China, India and South East Asia (see Figure 2). DDT is of great concern because of its high biomagnification and toxicity in terrestrial and aquatic food chains. Ever since its introduction in 1945, the global use of DDT has been enormous with an estimated production of 1.5×10^6 t in 1974. One estimate of the annual production in India alone in the mid-80s was 1.8×10^4 t.

Chlorobenzenes have a variety of uses depending on the degree of chlorination (see Table 1). Some are used as organic solvents, reagents in many industrial processes, farm applications (see Table 1) and chemical reactions. For example,

hexachlorobenzene is used as a fungicide, as an additive for pyrotechnic compounds for military uses, as an intermediate in the production of sundry dyestuffs, porosity controller in the manufacture of electrodes. It is sometimes used in organic synthesis, as a wood preservative, and pharmaceuticals. Chlorobenzenes are very cheap to produce, and their low cost has resulted in their widespread and intense use all over the world especially in the developing countries. Their use in tropical and warm climate tends to enhance their global transport. Hexachlorobenzene is also formed as a by-product of hexachloro-hexane production and old electrode reactions. Some hexachlorobenzene release often occurs during manufacture either as mixtures in effluents, spills or vapour being ventilated. Hexachlorobenzene is also a by-product of chlorination of benzene to produce other less chlorinated pesticides and other sources of release include the use of other chlorinated pesticides, incomplete combustion, old dump sites and inappropriate discharges. However, while the use of some of these organochlorine pesticides still continues in countries such as in Africa, Asia, Latin America, Eastern Europe, and Russia, their production and use are generally being phased out or discontinued; they are gradually being replaced by third generation pesticides that are phosphorus- or nitrogen-based. The third generation pesticides have much shorter half-lives than the organochlorines and their toxic modes of action are far more specific and well understood. Hexachlorobenzene has a high bioaccumulation potential in aquatic food chains because of its high lipophilicity and long half-life in biota (Niimi, 1987).

Ordinarily, technical grade hexachlorocyclohexane (HCH) consists of many isomers such as α-HCH (55-70%), β-HCH (5-14%), δ-HCH (6-10%) and γ-HCH (10-18%) and about 5% other impurities (Baumann *et al.*, 1980); the composition can also be varied to suit different specifications e.g. 99% γ-HCH. It is widely used as a broad spectrum pesticide for plants, animals and soil insecticide. The γ-HCH (lindane) is currently in use in its pure form for a wide variety of agricultural and forestry applications in North America and Europe. The production and use of other isomers have been stopped in most countries. Technical grade was banned in Canada in 1971. During applications, significant amounts are lost to the atmosphere from spray drift and from evaporation of spray droplets. Release to the atmosphere also occurs from slow volatilization from treated soils and plant surfaces. Besides, wind erosion can also carry pesticides-coated soils for long distances (Welch *et al.*, 1991). HCH is still widely used in Asia and China where annual uses have been estimated to around 6×10^4 t in the mid to late 1970s.

Endosulphan (hexachlorohexahydromethano-2,3,4-benzodioxathiepin-3-oxide) is a widely used insecticide on cash crops and in orchards. When ingested, it is readily eliminated or decomposes in organisms and therefore does not bioaccumulate. In Canada and the United States, the annual usage is 100 t in the early 1970s and 900 t in the early 1980s, respectively.

Also detected in the Arctic are the aldrin, dieldrin, and endrin groups. They are extensively used as insecticides in the United States until 1973. Aldrin is specially used as a termiticide, and can be oxidized by soil bacteria, insects and animals to dieldrin, one of the strongest carcinogens of the major organochlorine pesticides. It has a potency 10-15 fold greater than that of heptachlor, chlordane or HCH. Dieldrin is a *stereoisomer of endrin*. Dieldrin has not been used in Canada since 1981, even though its usage is still permitted for ground injection of termites. Aldrin has been banned in

the United States, Germany, and former USSR. Estimates of usage for Central and South America were 1377 t for endrin and 925 t for aldrin. The annual usage in the United States between 1981-1985 was 450-680t.

Toxaphene is another pesticide that has been found in the Arctic. It was commonly used in the 1960s and '70s mostly on cotton in the Southern and South Central United States with smaller amounts for weeds in soyabeans farms. It now has limited use in Canada, mostly to control ectoparasites in livestock.

Chlordane has also been detected to significant extent in the Arctic air, fauna, and snow. It consists of a mixture of many components e.g. hepta-, octa-, and nona-chloro-substituted dicyclopentadienes. Its uses were similar to those of DDT, mostly as an insecticide for seed dressings and coatings. A current use is as termiticide. Of the components, heptachlor is the most active, the most predominant (70%), the most stable in light and air, highly hydrophobic and is often stored in fat. One of its oxidation products has been shown to be highly carcinogenic, having a half-life of several years and very resistant to many chemical and biological transformations.

Table 1 Production and Major Uses of Organochlorines (Barrie, 1992).

Organochlorine	Use	Period of Manufacture	Production Estimate (Mt)
PCBs	Dielectric, hydraulic fluid	1930-present; new product prohibited 1980	Global 1.2 US 0.6
DDT	Insecticide	1940-present; deregistered 1986; most uses phased out 1970-78	Global 1.5 US 1
Toxaphene	Insecticide	1947-present; deregistered 1982; phased out in the 1970s	US 0.5
Chlordane	Insecticide	1945-1988; use restricted in 1970; agric. use eliminated 1985	US 0.25
Hexachloro-benzene (HCB)	Fungicide	1915-present;	US 0.1
Hexachlorocyclo-hexane (HCH)	Insecticide	1945-present; mixed isomers banned 1971; use of lindane restricted	US 0.4
Aldrin/dieldrin	Insecticide	1950-present	US 0.11

2.2 Chlorinated Industrial Organic Compounds

PCBs on the other hand, are industrial chemicals used as transformer fluids and

as a flame retardant. Even though, still in use, their production has been stopped in the late seventies. The major producer was Mosanto in the United States, PCBs are still in use all over the globe. In both temperate and tropical regions, release of PCBs into the environment occurs during spills, leaks, fires, and deliberate discharges. Other contaminants like dioxins and furans are byproducts of production of phenoxyacetic acid pesticides or combustion products of PCBs while DDE is a metabolite of DDT. Both PCBs, DDT and DDE have low volatility to be transported in the gas phase and as particulates. Estimates of the residence times in the atmosphere for PCBs and DDT are close to 45-100 days.

Some domestic sources of PCBs have also been identified such as Distant Early Warning (DEW) line sites and dumps. However, the resulting contamination has been shown to be extremely localised (Bright *et al.*, 1995; Reimer *et al.*, 1993b).

Chlorobenzenes are also industrial chemicals, and have been discussed above.

2.3 Polyaromatic Hydrocarbons (PAHs)

Polyaromatic hydrocarbons are also organic contaminants found in large quantities in the far north and widely distributed in the environment. They do not have chlorines atoms attached but all the same are very stable, with some being potentially carcinogenic and mutagenic. Because they are very stable substances, they can be carried by air currents for long distances. However, some can react with hydroxy radicals in the air to produce reactive diols and epoxides that can bind to deoxyribonucleic acid (DNA). PAHs have both natural and anthropogenic origins. PAHs are significant components of crude oil. Natural sources in the environment include: natural fires, all forms of spills and seeps of petroleum, wood, oil, peat, coal deposits, and plant terpenes on aromatization, (e.g. pimanthrene, cadalene, acenaphthylene, and retene). Processes such as thermal and bacterial degradation of plants and animal remains in lakes, crude oil, and marine sediments are also important sources of the highly alkylated PAHs such as simonellite and retene. Anthropogenic sources include all forms of incomplete combustion of fossil fuels by man e.g. automobiles exhausts, coal-combustion emissions, man-made fires from coal gas, wood, gasoline etc. Other sources are forest and house fires, aluminum smelting, foundries etc. Some small quantities are produced by diagenesis and microorganisms. PAHs produced in combustion at high temperatures usually consist of non-alkylated compounds while diagenesis forms highly alkylated PAHs which are very common in crude oils and ancient sediments. The anthropogenic sources of PAHs have increased dramatically since the last century as a result of increased combustion of fossil fuels. For example, a survey of PAHs in agricultural soils from the United Kingdom showed about a 4-fold increase in the last century, with levels in the 1980s higher than those in the 1950s and 1960s (Jones *et al.* 1989). Levels of PAHs in forest lakes in Finland in the 1970s and 1980s were also higher than levels in the 1950s and 1960s (Wickstrom and Tolonen, 1987). While the use of coal has been declining since the second World war, the use of liquid fuels, especially in automobiles, jet engines and airplanes has sky-rocketed since and this change can be seen in the PAHs patterns deposited from emissions. PAHs samples obtained from many locations in the north often showed mixtures of both natural and anthropogenic sources. Annual emissions in the United States during the late 1970s have been estimated at around 11,000 t. Estimates of global annual anthropogenic emissions of benzo-a-pyrene (B[a]P) was 5,044 t of

which the United States (1976) contribution was about one quarter. For the mid-1980s, estimates of the B[a]P annual emissions are 655 t. According to Suess (1976), benzo-a-pyrene was about 1-20% of the total PAHs emitted. PAH emissions can be both in the gas and particulate phases. McVeety and Hites (1988) have shown that PAHs in non-urban lake sediments in the eastern United States indicated that atmospheric emissions of PAHs peaked in the 1950s which corresponded to the dramatic decline in coal use as a heating fuel.

Metals and radionuclides are both natural and anthropogenic; the former could be mobilized by volcanoes eruptions, winds, mining, smelting, incineration while the later could be released from development and testing of thermonuclear devices, processing of nuclear fuels and improper disposal of nuclear reactor and spent fuels. Metals and radionuclides will not be discussed in this paper.

3.0 Transport

3.1 Factors Affecting Long-range Transport

As discussed above, large quantities of organochlorine and PAHs are produced or in use in the southern latitudes from where they are released in part and/or dispersed. These substances possess several characteristics (see Tables 2 and 3) which make them good candidates for long-range transport to the Arctic (Barrie *et al.*, 1992). The transport of a substance through different compartments in the environment depends on many properties such as vapour pressure (VP), Henry's Law constant (H), and many partition coefficients such as octanol/air, organic matter/water, octanol/water etc. These are reflected in their:

1.) low water solubility
2.) medium to high volatility (vapour pressure)
3.) high adsorption or octanol/water partition coefficient
4.) long atmospheric residence times when adsorbed on particulates or as vapour
5.) high biological and chemical stability

Some examples of substances that have these properties are PCBs, chlorinated pesticides, some PAHs and several other substances that have not surfaced yet in the Arctic e.g. aromatic ethers, chlorinated paraffins, chlorinated solvents, chlorinated fire retardants, lubricants, refrigerants, and plasticizers. The residence times for many of these substances in the atmosphere are of the order of several weeks and more which are ample enough for them to reach the far North.

3.1.1 Water Solubility

Low solubility in water is important because extremely soluble substances leach quickly through the soil during precipitation, or after dissolution in large water bodies end up in sediments or readily entrapped in ice or snow thus making these contaminants unavailable for long-range transport by air. Chemicals with high solubility tend to stay in aquatic environment where they can be adsorbed or undergo biodegradation. At low concentrations, the amount of a substance that can be partitioned into the air from water bodies and therefore available for long range transport can be determined from Henry's law constant or under equilibrium conditions approximated by the partition coefficient K_{aw}. Henry's Law constant (H) is

Table 2 Properties of Some Organochlorines and PAHs found in the North

Substance	Henry's Law K Freshwater 20°C (Pa/m³/mol)	Henry's Law K Seawater -2°C (Pa/m³/mol)	Soil half-life (days)	Vap. pressure (Pa 20°C)
PCBs 33	22.6	3.3	-	1.2×10^{-2}.
PCBs 52	24.1	3.6	-	9.0×10^{-3}
PCBs 101	18.1	2.7	-	1.5×10^{-3}
PCBs 153	10.0	1.5	-	2.5×10^{-4}
γ-HCH	0.20	0.029	49	3.9×10^{-2}
Dieldrin	1.1	0.16	130	5.6×10^{-3}
p,p'-DDT	2.4	0.36	497	2.6×10^{-4}
Toxaphene	0.61	0.090	-	6.1×10^{-3}
NAP	28.0	4.1	54	17.5
PHEN	2.6	0.38	171	6.0×10^{-2}

the ratio of the concentration or partial pressure of the substance in air (P) to its concentration in water at equilibrium (C_{liq}) and it is temperature and compound dependent:

$$H = P/C_{liq}. \quad (1)$$

Where

P is the partial vapour pressure (Pa)

C_{liq} is the concentration in water (mol/m³)

Hence as H increases, partitioning to water becomes less.

Also

$$K_{aw} = \frac{P \times M}{1000\, S \times RT} \quad (2)$$

where P = Vapour pressure (N/m²)

R = Gas constant (J/mol x K)

T = Temperature (K)

S = Water solubility (kg/m³)

M = Molecular mass (kg/mol).

The vapour pressure of a contaminant and the surface area of atmospheric aerosol determine the gas/particle partitioning. At air temperatures of -90 to 50°C which prevails in the Arctic, the vapour pressure of the supercooled liquid is referred

to here, since most of these contaminants are solids in pure form at such temperatures (Bidleman, 1988; 1995).

Substances that are transported by water currents must have some degree of solubility e.g. chlorobenzenes (see Table 3) and hexachlorohexane even though long-range transport by this route is very slow. Aqueous convection refers the movement of such substances when dissolved in water. For suspended particles gravity flow often predominates but evapotranspiration moves dissolved species upwards through concentration gradient created. Sediments entrapment is often the result of gravity flow.

Hence, movement of these contaminants in the soil or water results from diffusion and advection. In the main, diffusion is driven by concentration gradient, while advection is driven by pressure, gravity, density, and thermal gradients. Sleep and Sykes (1989), and Wania and Mackay (1993a) have investigated the diffusion coefficients in gases and liquids and concluded that gaseous molecular diffusion coefficients exceed those of liquids by 4 or 5 orders of magnitude and therefore gaseous diffusion will always prevail over liquid diffusion.

The exchange of contaminants between the atmosphere and earth's surface can also occur by rain and snow removal of gaseous and particulates, and transfer of gaseous compounds through air-sea, air-lake and air-land surfaces. Atmospheric phase distributions of many contaminants have been estimated with the Junge-Pankow (J-P) adsorption model (Pankow, 1987) using the equation:

$$\phi = c\theta / (VP + c\theta) \tag{3}$$

where

ϕ is the fraction of the total airborne compound
VP is the liquid-phase vapour pressure (Pa)
θ is the atmospheric aerosol surface area (cm^2/cm^3)
c is a function of themodynamics of adsorption and surface area occupied by the sorbate molecule (17.2 Pa-cm)

Contaminants can be incorporated into snow as gases or particles. The deposition of contaminants by precipitation is a one-way process from the atmosphere to the surface. The deposition flux (F) is the product of the concentration of the contaminant in snow (C_s) or rain (C_r) and the rate of precipitation (R_p):

$$F = C_s C_r R_p. \tag{4}$$

Where

F is the deposition flux
C_s is the concentration of contaminant in snow (ng/Kg)
C_r is the concentration of the contaminant in rain (ng/L^{-1})
R_p is the rate of precipitation (Kg/min)

Hoff *et al.*, (1995) has estimated gas exchange between snowpack and the atmosphere. The cumulative flux (ΣF_i) from atmosphere to surface via snow has been calculated as follows:

$$\Sigma F_i = C_{vwm,i} \cdot \Sigma P_i \quad (5)$$

Where

$C_{vwm,i}$ is the precipitation amount weighted mean concentration of the contaminant in the snowmelt (ng/L)

ΣP_i is the total precipitation amount for the period (mm)

and ΣF_i is the cumulative flux (ng/m^2)

Cumulative flux represents an estimate of the cumulative input of a contaminant to the snowpack by snow.

Table 3 Chemical Properties of Selected PCBs, Chlorobenzenes and DDT at 25°C. From Barrie *et al.*, 1992

Chemical	Mol. Wt. (g/mol.)	Vap. Press (Pa)	Aq. Sol. (g/m^3)	Log K_{ow}	Log K_{oa}
Chlorobenzenes					
1,2-	147.0	196	118	3.4	4.36
1,2,3-	181.5	28	21	4.1	5.19
1,2,3,4-	215.9	5.2	7.8	4.5	5.64
1,2,4,5-	215.9	0.72	1.27	4.5	5.63
penta	250.3	0.22	0.65	5.0	6.27
hexa-	284.8	0.0023	0.005	5.5	6.90
p,p'-DDT	354.5	0.00002	0.003	6.2	10.09
PCBs					
4-	188.7	0.271	1.2	4.5	6.78
4,4'-	223.1	0.0048	0.06	5.3	7.67
2,4,5-	257.5	0.132	0.14	5.6	7.96
2,3,4,5-	292	0.005	0.02	5.9	8.74
2,2',4,4',6,6'-	360.9	0.00048	0.002	7.0	8.99

3.1.2 Medium to High Volatility

Volatility or vapourization is the gaseous loss of substances to the atmosphere either on exposure to the air or when dissolved or suspended in other matrices. Even though most organochlorine pesticides are solid at room temperature, they can readily volatilize into the air from farm fields after application, or from surface waters after deposition in rain snow or from aerosol particles (Barrie *et al.*, 1992). The volatility of a substance depends on its vapour pressure which in turn depends on temperature. The relationship between vapour pressure and temperature (absolute) can generally be expressed by the Classius-Clapeyron equation:

$$\log_{10} VP = m/T + b \quad (6)$$

Where

VP is the vapour pressure in Pa
m is the slope of the graph-plot
T is the absolute temperature in K
and b is a constant. The constants m and b have been determined for many chlorine pesticides.

The rate at which a chemical dissipates from the surface of a matrix or itself is controlled primarily by diffusion. Since there is relatively little air movement around the matrix, a vapourized substance is transported first through a stagnant air boundary layer only by molecular diffusion. The rate of movement from such a surface is determined by the diffusion coefficient and the vapour density of the chemical. Many factors can change the rate of volatilization only through their effect on the thickness of the stagnant boundary layer e.g. wind, temperature, vegetation cover etc. Reversible exchange of gases also occur on soil, vegetation organic matter and snow. The mechanism of exchange on water is pretty well understood, often described as the "two-film" model (Liss and Slater, 1974). The exchange rate is controlled by molecular diffusion across thin air and water films at the interface. The net gas flux can be estimated by the equation:

$$F = K_l[C_w - C_a RT/H] \qquad (7)$$

and

$$1/K_l = 1/k_l + RT/Hk_g \qquad (8)$$

Where
C_w is the concentration of chemicals in water (dissolved) (g/cc)
C_a is the concentration in air (gaseous) (g/m^3)
H is the Henry's Law constant
R is the gas constant
T is the temperature (K)
K_l is the overall mass transfer coefficient
k_l is the mass transfer coefficient for the water film
k_g is the mass transfer coefficient for the air film

Substances with medium volatility can be transported both in the gaseous phase and as particulates while those with high volatility will be as vapour only. Major toxaphene sources for Canada have been shown to be emissions from previously treated farm fields in the southern United States such as Alabama, Mississipi etc. based on 5-day back trajectories of air mass movement (Hoff *et al.*, 1992).

3.1.3 High Adsorption or Octanol/Water Partition Coefficient

Generally, substances with high adsorption or octanol/water partition coefficient are lipophilic, hydrophobic and readily adhere to organic particulates on which they can be carried for very long distances in the atmosphere. High lipid solubility is also an important property that protects these contaminants from the elements that can result in degradation or metabolism for some time. Release of these contaminants back to the atmosphere from fat deposits will depend on their octanol/air

partition coefficients (see Table 3). It can be seen from Table 3 that release of these contaminants from fat to water is more facile than from fat to air.

3.1.4 Long Atmospheric Residence Times

Long residence times require ability to float in the air for long periods, or to vapourize and condense repeatly (see mechanism of transport below). Very small particles can travel long distances while big ones do not travel as far. According to Biddleman *et al.*, 1981, the atmospheric residence times for PCBs and chlorinated pesticides in the atmosphere is of the order of weeks (45-100 days) which is ample time for them to disperse around the northern hemisphere (20-30 days).

3.1.5 High Biological and Chemical Stability

In order to survive the long journey to the north, a high degree of chemical and biological stability are required. These include chemical attacks and reactions that can result in degradation of the chemicals such as hydrolysis, radical attacks, photolysis and biodegradation. However, it is known that some of these contaminants do partially undergo some of these transformations.

4.0 Pathways and Environmental Fate

The main routes for the entry of contaminants into the Arctic are by air, ocean current, ice and rivers. Determinations of organochlorines in air, snow and sea water conclusively demonstrated that atmospheric supply was the major route for the transport to the Arctic Oceans surface layers (Hargrave *et al.*, 1988; Bidleman *et al.*, 1989; Patton *et al.*, 1989; Barrie *et al.*, 1992). It has also been suggested that similar transport pathways exist for the volatile and semi volatile PAHs.

4.1 Atmospheric Transport

Most organochlorine compounds do volatilize as soon as they are applied to the soil or spilled especially in the tropics (Takeoka *et al.*, 1991) and to lesser extent in the temperate regions. This implies that under normal environmental conditions, large fractions of their masses can exist in the vapour phase or on atmospheric particles or adsorbed or dissolved on the Earth's surface. They are subsequently transported to the cold remote regions such as the Arctic and the Antarctic. The way they move, depends on how their mass is partitioned between different compartments and how they interact with the surfaces e.g. snow, water or soil. How the substance returns to the atmosphere depends on whether they are in the gas or particle phase. Partitioning between gas and particle phases depends on many factors such as temperature, molecular weight and particle surface area available for adsorption. Gas and particle partition has been investigated by Junge (1977); Pankow (1987, 1988); and Ligocki and Pankow (1989). Particle deposition and resuspension between the atmosphere and a surface can occur at different times than those of a gas. While particle deposition depends on physical factors such as particle size distribution, surface roughness and wind speed, gaseous exchange depends on chemical reactions with the surface. As already discussed, volatilization from soils also depends on Henry's Law constant, water evaporation rate, soil sorption coefficient, temperature and wind speed. Ottar (1989) has indicated that organochlorines are subject to systematic long-term transfer from warmer to colder climate because they are continuously deposited and re-emitted

via volatilization. This phenomenon is often referred to as global distillation, global fractionation, or cold condensation. The theory is rather simplistic because it ignores losses through other processes such as advection by ocean currents, hydrolysis, sedimentation on particulates, and seasonal changes on volatilization rates. It has been postulated that transport of organochlorine to the Arctic occurs in multiples of cycles that allow contaminants to reach very long distances in a very short time and are as follows:
1.) evaporation in warm temperature from the earth's surface
2.) transport by air (wind current) (see Figure 4)
3.) condensation in cold temperature on the earth's surface
This cycle is repeated again and again (see Figure 3). The series of cycles of evaporation, air transport, and condensation have been referred to as grasshopper effect or multi-hop transport. Most organochlorine and PAHs are multi-hop contaminants. The less volatile organochlorines such as DDT, some PAHs (e.g.Ba[a]P), heavy metals, and acids are one-hop compounds. The pathways of these compounds are characterized by a) longer residence times in winter (~20-30 days) than in summer (~3-7 days) and b) stronger south to north transport into the Arctic from Eurasia. Multi-hop compounds can also reenter the atmosphere from the Earth's surface under warmer conditions than when they were deposited on sudden exposure of ocean surface to the atmosphere after being covered by ice, or resuspension of dust or snow by winds.

As soon as the Arctic region is reached, the contaminants become trapped since the climate is very cold, and ice cover very large that there is little opportunity for further evaporation except in summer but is usually limited within the Arctic. Gaseous exchange between snowpack/ice and atmosphere has been well documented (Semkin *et al.*, 1996). The snowpack burden estimate (B) has been defined as:

$$B = Cp_{snow}\, d_{snow} = CSWE \qquad (9)$$

where
C is the contaminant concentration
p_{snow} is the snowpack density
d_{snow} is the snowpack depth
and the product of p_{snow}, and d_{snow} is SWE or snow water equivalent

Some evidence of HCB volatilization in the Arctic has been reported for the Canadian Ice Island snowpack (Hargrave *et al.*, 1988). However, Arctic is the final destination, and accumulation in all ecosystems has begun. Contaminants on reaching the Arctic can land on snow, rivers, vegetation, and lakes, directly from the air or precipitation by rain or snow. In the spring, as the snow melts contaminants can then be carried into lakes, rivers, oceans or get trapped in ice or stay on vegetation surface and from there enter the food chain.

4.2 Oceans

The major ocean waters flowing into the Arctic Ocean pass from the North Atlantic through Fram Strait and the Barents Sea and from the North Pacific through Bering Strait. In terms of contaminant transport, most contaminants will reside in the

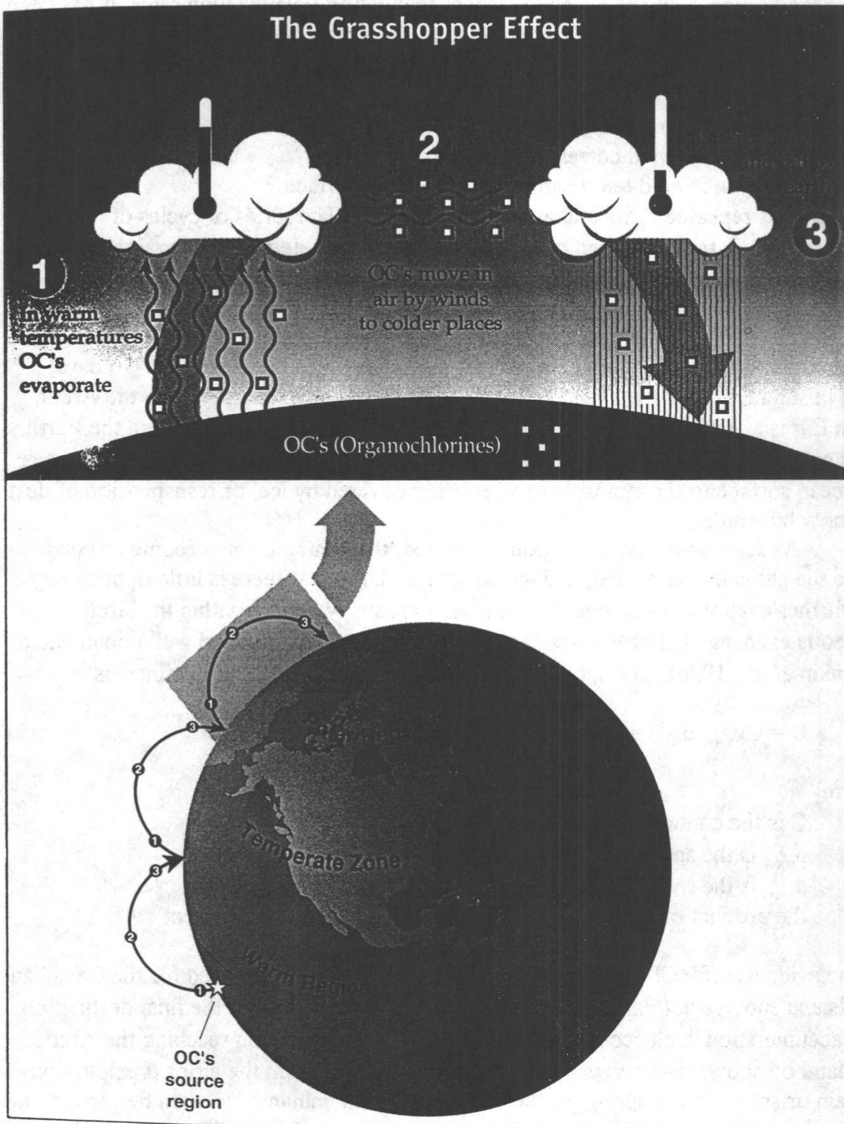

Figure 3 The HOP and HOP-HOP/Grasshopper Effect

Figure 4 Arrows Showing Dominant Air Currents

top layer of approximately 0-200 m. The determining force of the North Atlantic currents originates at the Gulf Stream, in the Gulf of Mexico (see Figure 5). These currents transport substantial quantities of the persistent, soluble, organochlorine contaminants from the eastern seaboard of North America and heat from the Gulf to northern and western coasts of Europe. The system continues to move northward, then through the Barents sea and Fram Strait until the Arctic is reached (Dahlgaard, 1995). During transport, contaminants are diluted, dispersed, volatilized or scavenged by other particulates. Concentration changes have been employed to determine the extent of dilution during transport. The surface currents take years or decades to transport water and contaminants from the temperate and tropical regions to the Arctic Ocean. On the other hand, very little contaminants are expected from the Pacific ocean gyre, since the net flow of water through the Indonesian Archipelago is from the Pacific Ocean to the Indian Ocean (Verschell *et al.*, 1995)

4.3 Rivers

Rivers are also important for the transport of organochlorine contaminants into the Arctic. Rivers contribute less than 3% of the total water entering the Arctic Ocean, with the rest coming from the Atlantic Ocean and Barents Sea (80%) and the Pacific (17%) (see Figure 6). There are other smaller rivers that discharge into the Arctic Archipelago. Rivers in general contribute only small amounts of contaminants into the Arctic. The major inflowing river to the Arctic Ocean is the Mackenzie river with nine others in Russia. These rivers bring in rich sediments, nutrients as well as large quantities of suspended particles, dissolved organic matter and contaminants. Arctic lakes can hence act as sinks for suspended organic contaminants. The mean residence time for the Arctic Ocean surface layer is 12.5 years. Arctic ocean currents also move 96% of the water flowing out of the Arctic annually by the Canadian Archipelago (34%), western Fram Strait (62%) and the rest as ice.

For the Mackenzie River, the total PCBs on the suspended fraction was 13-24% during the 1993 summer, the remaining rivers have very low suspended burden load. The quantities of contaminants entering the Arctic Ocean by rivers can be estimated from the following equation:

$$F_{r/o} = \int(C_w + C_{ss}S)Vdt \qquad (10)$$

where

Cw is the concentration of the substance dissolved in river water

$F_{r/o}$ is the amount of contaminant transferred

C_{ss} is the concentration of the substance per unit mass of suspended sediment

S is the mass concentration in the river of suspended sediment

V is the volume flow rate of river water into the ocean

Estimates of many contaminants have been made. Concentrations of organo-chlorines in some rivers were found to be variable and often undetectable. Water columns to sediment exchange is also an important pathway for contaminants in shelves, deltas, and oceans. Many contaminants are transported to the sediments as associated with particulates. These particle-associated contaminants can be released to pore-waters and diffuse to overlying water layers, or may be resuspended during storms, or be incorporated into the bottom frazil ice or be ingested by foraging biota and enter the

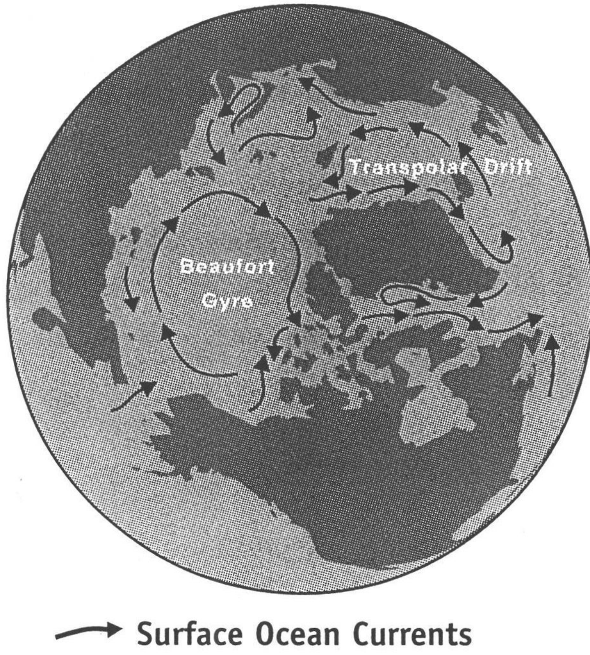

Figure 5 The Arctic Surface Ocean Currents

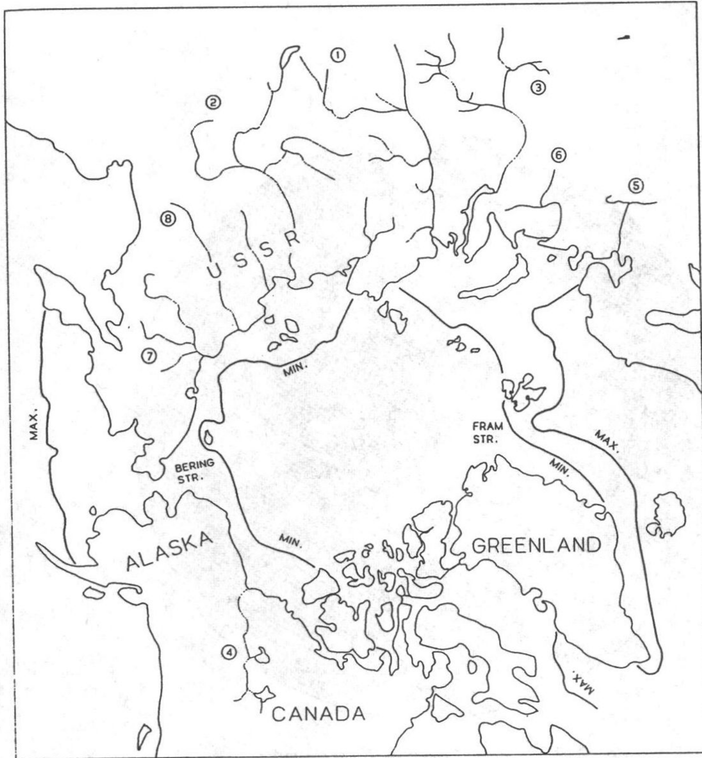

Figure 6 The Major Rivers with Minimum and Maximum Ice Build in the Arctic

food chain.

5.0 Arctic Environment Levels

Organochlorine contaminants are of special concern in the North because traditional foods which are the primary route of human exposure in the Arctic have been shown to contain some amounts of these contaminants (see Tables 4-10). For example, levels of total DDT in human tissue or PCBs and DDE in mothers' milk in the Arctic are considerably higher than those of their counterparts in the south. This reflects consumption of high, trophic-level species of food containing these contaminants (Dewailly *et al.*, 1992). The relative abundance of organochlorines in arctic air is ΣHCH > ΣPCBs > toxaphene ~ Σchlordane ~endosulfan>pentachloroanisole >chloroveratoles > ΣDDT ~ dieldrin >octachlorostyrene > methoxychlor ~ endrin > trifluralin ~mirex; in sea water the order is α-HCH > HCBs >γ-HCH ~ CHBs(toxaphene) >Σchlordane > PCBs > DDTs; in the Mackenzie river it is ΣPCBs > ΣHCH > ΣDDT > Σchlordane. The PCBs levels in the vicinity of the DEW Line and local dump sites have been shown to be much higher (see Table 11 and Figure 7). The environmental levels of different contaminants in various compartments are as shown below in the following tables:

Table 4 Atmospheric Organochlorine Concentrations at Alert, Dunay and Tagish ($pg.m^{-3}$) (Barrie *et al.*, 1992)

Location	ΣCBz	ΣHCH	ΣCHLOR	ΣDDT	ENDRIN	ΣTOX
Alert 1993	97.1	71.7	5.19	0.82	0.18	4.43
1994	94.3	76.9	6.65	1.40	0.20	5.33
Dunay 1993	85.7	50.9	4.55	0.93	0.28	4.70
Tagish 1993	52.7	91.3	5.63	1.39	0.22	5.12
1994	44.0	80.4	5.19	1.51	0.2	5.35

Table 5 HCH Burden in the Top 200 m Water Column of the Arctic Ocean ($\mu g/m^2$) (Hargrave, 1988; MacDonald *et al.*, 1996)

Location	Date	α-HCH	γ-HCH
Ice Island	1986	800	97
Beaufort Sea	1992	680	87
Chukchi Sea	1993	460	120
Lincoln Sea	1993	510	90

Table 6 **Organochlorine Compounds Detected in River Water (Northern Quebec, 1987) (ng/L). From Langlois (1987)**

Contaminant	Hudson Bay	Ungava Bay
γ-HCH	1.1	0.8
Total PCB	<9	<9
p,p'-DDT	<0.4	<0.4
p,p'-TDE	<0.4	<0.4
0,p-DDT	<0.4	<0.4
p,p'-DDE	<0.4	<0.4
Dieldrin	<0.4	<0.4

Table 7 **Organochlorine Concentrations on Snowpack in the Arctic, 1987 (pg/L) (Gregor, 1989)**

Contaminant	Median	Range
PCB	910	257-1,770
α-HCH	910	143-42,700
γ-HCH	700	83-10,500
α-Endosulfan	170	ND-4,880
Dieldrin	290	ND-4,420
HCB	20	ND-104
sum-DDT	93	ND-1,380
sum-CBZ	240	ND-915
sum-PAH	19,000	190-149,000

Table 8 Concentrations of Organochlorines in Lower Trophic Level Biota (ng/g lipid wt.) (Hargrave *et al.*,; 1992; Muir *et al.*, 1996)

Species	Region	ΣDDT	ΣPCBs	ΣHCH
Epontic Particles	Ice Island Barrow St.	20-70 150-360	40-360 20-360	10-280 160-230
Zooplankton				
25-125 µm	Ice Island	<8-150	10-490	2-200
>500 µm	Barrow St	2-20	4-20	90-180
Amphipods				
Pelagic	Barrow St.	3-60	1-230	70-390
Benthic	Arctic Ocean	2200-25900	5700-34000	6-420
Fish				
Turbot	Cumberland Sound	626-1044	914-1619	59-117
Four-horn Sculpin	Wellington Bay	92.9	171-521	100
Bivalves				
Clams	Sanikiluaq	33.7	61.8	<5.6

Table 9 Total PAH levels in Arctic Marine Biota (µg/kg wet et.) (Muir *et al.*, 1992)

Location	Year	Species	Range
Beaufort Sea Tuktoyatuk Resolute Bay	1984 1984	Inconnu (*Stenodus leucichthys* Arctic cod (*Boreogadus saida*)	1.4-1.5 4.5-14.8
Beaufort Sea	1983	Least cisco (*C. Sardinella)* Flounder (Platichthys stellatus) Flounder (*Liopesetta glacialis)* Eel pout *(Lycodes reticulatus*) Whitefish (*C.nasus)*	58 63 10 6 49
Beaufort Sea (Liverpool Bay)	1983	Herring *(Clupea harengus P.*)	24
Cape Hatt, Baffin Island	1980	Bivalve *(Mya truncata)*	1-11

Table 10 Comparative Concentrations of Organochlorines in human milk of Aboriginal and non-Aboriginal (ng/g lipid). (Dewailly, 1995)

Location	Year	Chlordane	Cis Nonachlor	Trans-nonachlor	Oxychlordane
Southern Canada	1992	0.37	2.89	17.5	13.4
Greenland (Inuit)	1993	11.6	3.12	1463	862

Table 11 Range of PCB Concentrations (ppb) at 22 Abandoned DEW line Stations (Holtz *et al.*, 1986)

Range	Number of Sites
>100,000	2
10,000-100,000	1
1,000-10,000	3
100-1,000	4
10-100	6
1-10	3
<1 (not contaminated)	3

6.0 Biological Effects

The toxicological effects of organochlorine on mammals and vertebrates reproduction tend to vary from species to species. For instance, DDT has a clear toxic effect on the reproduction of rabbits and birds but not on beagle dogs. The mechanism involves abnormal rates of steroid hormone metabolism as a result of liver enzymes induction. Birds were known to suffer increased susceptibility to duck hepatitis virus when fed PCBs. Several aquatic mammals such as the ringed seal, killer whales, river otters, bottlenose dolphins etc. have been reported to show pathological and population changes for which exposure to organochlorine contaminants has been implicated as a cause (Gilbertson, 1989). Likewise minks fed coho salmon found to be contaminated with 10-15 µg/g of PCBs suffered reproductive impairment. Since the traditional Inuit foods are thus contaminated with organochlorine compounds, their PCBs and pesticides intake exceeds the recommended daily intake (TDI). The associated risks in this population are not clearly known. The main concern with PCB exposure has been for the fetus and for breast-fed infants and the sensitivity of the developing fetus and the increasing burden for the developing infant. PCBs, HCBs and DDT have high bioaccumulation potential in terrestrial and aquatic food chains because of long half-lives in biota and high lipophilicity. One unfortunate result of the stress of these contaminants is the continuation of decreasing biological diversity and the dominance by long-lived species.

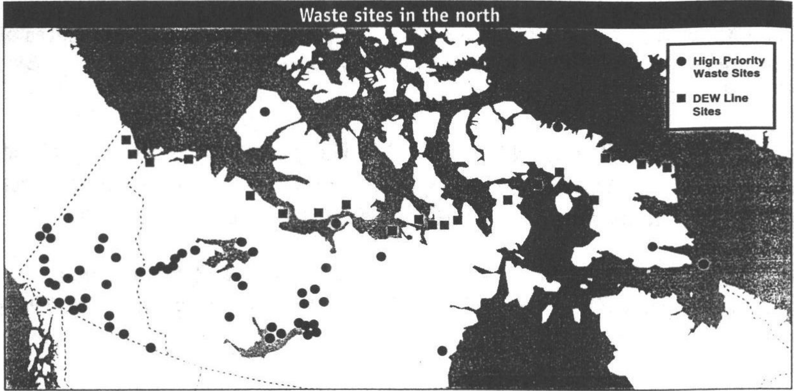

Figure 7 The DEW Line and Waste Dump (High Priority) Sites in the Canadian Arctic

7.0 Conclusion

Monitoring of environmental contaminants in the Arctic should be intensified, but reduction in further long-range transport of environmental contaminants will require a joint international effort.

8.0 References

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Use of Microtox® and a lux-modified *Pseudomonas fluorescens* to Assess the Toxicity of Salinity Contaminated Sites

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Abstract

Many industrial sites associated with the petroleum industry are contaminated with hydrocarbons, metals and salinity. The Microtox® acute toxicity bioassay is a popular, widely used test that has been used frequently to determine the relative toxicity of these and other types of contaminated soils, as well as individual chemicals and contaminated groundwaters and freshwaters. This study compares the toxicological response of the marine Microtox® bacteria, *Vibrio fisheri*, and a lux-modified soil and freshwater bacteria, *Pseudomonas fluorescens*, to saline contamination. *P. fluorescens* is shown to be more sensitive to saline contamination, and a more appropriate bioassay organism for the assessment of contaminated terrestrial, freshwater and groundwater samples than the Microtox® bacteria, *V. fisheri*.

1.0 Introduction

Bacterial toxicological assays have several advantages over traditional bioassays with higher-order plants and animals. Their high surface to volume ratio and simple morphology provides target sites at or near the cytoplasmic membrane, where many important metabolic pathways are located (Franson, 1995). These cellular characteristics, combined with respiration rates 10 to 1000 times greater than mammalian cells, the large number of organisms (10^6) that can be used in a single test, and the ease of maintaining cultures, make bacteria ideal for use in toxicological analyses (Franson, 1995).

The light emitting reaction of the bacterial luciferase system involves the oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain fatty aldehyde in the presence of molecular oxygen:

$$FMNH_2 + RCHO + O_2 \rightarrow FMN + RCOOH + H_2O + h\nu \quad (490 \text{ nm}) \qquad (1)$$

where R represents a long-chain alkyl group and h and ν represent Planck's constant and frequency respectively (Steinberg *et al.*, 1995; Iizumi *et al.*, 1998). Because toxic compounds destroy cellular metabolism and consequently eliminate light production, the response of bioluminescent marine bacteria to toxic compounds can be quantified easily and accurately by measuring the rate of light production (Environment Canada, 1992; Franson, 1995; Iizumi *et al.*, 1998). Over the last two decades, bioluminescent bacteria toxicity assays have consequently become increasingly popular as fast, relatively inexpensive and sensitive toxicity assays.

The most popular bioluminescent bacterial toxicity bioassay is the Microtox® acute toxicity bioassay, which uses the marine bacteria *Vibrio fisheri*

NRRL B-11177*. In this liquid phase toxicity bioassay, bioluminescent bacteria are exposed to a small volume of aqueous test sample, and the reduction in bacterial luminescence after exposure to the test sample is measured after a specified period of time (e.g. 15 minutes). The effective concentration of chemical or test sample causing a 50% inhibition of light (EC_{50}) is reported (Environment Canada, 1992). The Microtox® test is currently marketed by Azur Environmental (Carlsbad, CA).

The liquid phase Microtox® acute toxicity bioassay has been used to evaluate the toxicity of many chemicals in aqueous solution (Ribo and Kaier, 1983; Greene *et al.*, 1985; Miller *et al.*, 1985; Reteuna *et al.*, 1989; Ribo *et al.*, 1989; Carlson-Ekvall and Morrison, 1995; Ruiz *et al.*, 1997; Gustavson *et al.*, 1998), as well as contaminated groundwaters and wastewaters (Ankley *et al.*, 1990; Svenson *et al.*, 1996; Boyd *et al.*, 1997a; Aruldoss and Viraraghavan, 1998). This Microtox® bioassay has also been adapted for assessment of the toxicity of soils and sediments. Distilled water extracts of soils and sediments have been used to evaluate the toxicity of hydrocarbon, pesticide, metals and organics contamination (Calleja *et al.*, 1986; Dasappa and Loehr, 1991; Galli *et al.*, 1994; Ronnpagel *et al.*, 1995; Dombroski *et al.*, 1996; Ramanathan and Burks, 1996; Hauser *et al.*, 1997; Goicolea *et al.*, 1998). Saline extracts (Dombroski *et al.*, 1996; Guzzella *et al.*, 1996; Cheung *et al.*, 1997; Matthiessen *et al.*, 1998) and organic solvent extracts (O'Connell *et al.*, 1997; Zemanek *et al.*, 1997; Pavoni *et al.*, 1998; Sunahara *et al.*, 1998) have also been used to analyze contaminated soils and sediments using the liquid phase Microtox® bioassay.

A solid phase Microtox® bioassay was developed in the early 1990s to allow for a more direct assessment of soil and sediment toxicity (Environment Canada, 1992). In this test, the Microtox® bacteria, *V. fisheri*, are acclimatized in a soil/water slurry, allowing direct contact of the bacteria with the solid sample Reduction in bacterial luminescence is measured after separation of the water and soil phases (Environment Canada, 1992). This solid phase Microtox® bioassay has been used to analyze metals, hydrocarbon and phenol contaminated soils (Ronnpagel *et al.*, 1995; O'Connell *et al.*, 1997; Goicolea *et al.*, 1998; Marwood *et al.*, 1998; Knoke *et al.*, 1999), as well as contaminated freshwater and marine sediments (Demuth *et al.*, 1993; Kwan and Dutka, 1995; Ronnpagel *et al.*, 1995).

As shown above, the Microtox® test has been very popular and widely used, and most assays of environmental contamination have been performed on terrestrial and freshwater samples, including petroleum industry sites. However, the Microtox® bacteria, *V. fisheri*, is an inappropriate choice of bioassay organism for analysis of these types of samples, since bioassay organisms should be selected from naturally occurring organisms from the environment where the sample is taken, and therefore represent the actual impact of the contamination on the ecosystem (Ankley *et al.*, 1989; Keddy *et al.*, 1995; Paton *et al.*, 1995a; Paton *et al.*, 1995b; Boyd *et al.*, 1997b). Due to differences in physiology, marine organisms may have a profoundly different toxicological response than terrestrial or freshwater organisms (Hall Jr and Anderson, 1995).

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* *Vibrio fisheri* NRRL B-11177 is also known as *V. fisheri* (Beijerinck 1889), *V. fisheri* (Lehmann and Neumann 1896), *V. fisheri* strain 7151, *Photobacterium phosphoreum* (Cohn 1878), and *P. phosphoreum* NRRL B-11177

At petroleum industry sites, the inapplicability of the Microtox® bioassay is compounded. Due to activities such as crude oil dewatering and desalting, and disposal of process wastes into on-site pits, petroleum industry sites may be heavily contaminated with salinity, in addition to metals and hydrocarbons (Pollard and Hrudey, 1992; Zemanek *et al.*, 1997). Since *V. fisheri* is a marine bacteria which has an osmotic requirement for a highly salinity environment, it is not expected to be sensitive to saline contamination.

Studies have shown the Microtox® assays to exhibit variable sensitivity and reproducibility, especially between freshwater and seawater samples (Miller *et al.*, 1985; Ankley *et al.*, 1989; Environment Canada, 1992; Keddy *et al.*, 1995; Dombroski *et al.*, 1996; Guzzella *et al.*, 1996; Marwood *et al.*, 1998). Keddy *et al.* (1995) reviewed toxicity assays for assessment of high priority contaminated sites in Canada under the National Contaminated Sites Remediation Program, and recommended the development and use of a soil or freshwater bacteria for use in toxicity testing of terrestrial, freshwater and freshwater sediment samples, rather than use of the Microtox® bioassay.

The development of a fast and easy bacterial toxicity bioassay using a terrestrial or freshwater bacteria is limited by the fact that bioluminescence is largely a marine trait (Paton *et al.*, 1995a). However, the isolation of lux genes from *V. fisheri*, and insertion of these genes into a variety of freshwater and soil bacteria, has allowed the development of luminescent terrestrial and freshwater bacterial assays for a variety of industrial applications, including acute toxicity testing (Meikle *et al.*, 1992; Paton *et al.*, 1995a; Paton *et al.*, 1997; Ben-Israel *et al.*, 1998; Iizumi *et al.*, 1998; Palmer *et al.*, 1998).

The ubiquitous gram-negative bacterium, *Pseudomonas fluorescens* 10586, has been transformed into a luminescent bacteria by the addition of a multi-copy plasmid containing the *V. fisheri* lux genes (Kleeberger *et al.*, 1983; Paton *et al.*, 1995a; Boyd *et al.*, 1997b). Because of its natural occurrence in both soil and freshwater, *P. fluorescens* is a more ecologically relevant toxicity bioassay than *V. fisheri* for these types of environmental samples, and can reflect the actual impact of environmental toxicants on bacteria in freshwater and terrestrial environments. (Paton *et al.*, 1995a; Paton *et al.*, 1995b). This lux-modified bacteria has been successfully used to assess the toxicity of individual metals and organics (Paton *et al.*, 1995a; Boyd *et al.*, 1997b; Boyd *et al.*, 1998), and contaminated groundwaters, wastewaters and soils (Paton *et al.*, 1995b; Boyd *et al.*, 1997a; Palmer *et al.*, 1998; Sousa *et al.*, 1998). Since *P. fluorescens* has minimal osmotic requirements and is not a marine organism, it is expected to be more sensitive to saline contamination than *V. fisheri*.

This paper investigates the response of the Microtox® bacteria, *Vibrio fisheri*, and a lux-modified *Pseudomonas fluorescens* to saline contamination, and mixed saline-chromium and saline-phenol contamination.

2.0 Materials and Methods

2.1 Bioassay Cultures

Pseudomonas fluorescens construct 10586r pUCD607 was kindly provided by Grahame Paton of the University of Aberdeen. *P. fluorescens* was grown at 25°C to mid-exponential phase in Luria-Bertani broth containing 50 mg/mL kanamycin. A 10mL aliquot was centrifuged at 5000rpm for 5 minutes, suspended in Minimal Salts

Media (MSM), and recentrifuged. The washed bacterial pellet was reconstituted in 0.5 mL MSM (*P. fluorescens* inoculum). Freeze dried Microtox® bacteria, *Vibrio fisheri* NRRL 11177, were obtained from Azur Environmental (Carlsbad, CA) and stored at –20°C until use. The bacteria were reconstituted in 1mL Microtox® Reconstitution Solution (*V. fisheri* inoculum).

2.2 Toxicity Bioassays

Bioassay methods were modified from standard Microtox® acute bioassay methods as described in Environment Canada (1992). The *P. fluorescens* and *V. fisheri* bioassays were performed at 25°C and 15°C respectively. 10µL of inoculum was added to disposable borosilicate glass vials containing 0.5mL diluent (MSM for *P. fluorescens* and 2%NaCl in DDW for *V. fisheri*). The inoculated vials were vortexed, and allowed to reach maximum luminescence over 5 to 15 minutes. Initial luminescence of each vial was read (time=0) and 0.5mL of test solution was added to each vial. The vials were vortexed, and luminescence was read on a Microbics 2055 Toxicity Analyzer (Carlsbad, CA) at 15 minutes. Vials were inoculated and read sequentially at 15 second intervals to ensure precise incubation times. All bioassays were performed at least in triplicate.

2.3 Test Solutions

Test solutions were made with analytical grade phenol, potassium dichromate (Cr^{6+}) and sodium chloride (NaCl) provided by Sigma Chemical Company (St. Louis, MO) and distilled, deionized water (DDW). Serial dilutions of Cr^{6+} and phenol were made at salinities of 0 0.25, 0.5, 1, 1.5, 3 and 4% (w/v) NaCl for *P. fluorescens*, and 2, 2.25, 2.5, 3, 3.5, 4 and 6% NaCl for *V. fisheri*. The *V. fisheri* bioassay samples were prepared to represent saline contaminated samples which were osmotically adjusted with 10% Microtox® Osmotic Adjustment Solution (22%NaCl). Although the purpose of osmotic adjustment is to standardize the salinity of the samples for the test, osmotic adjustment is routinely performed without consideration of the salinity of the sample (Environment Canada, 1992).

Although osmotic adjustment with sodium chloride is the standard Microtox® test procedure, the use of sucrose as a osmotic adjuster is as an acceptable method for increasing sensitivity to metals (Environment Canada, 1992). However, researchers have shown that Microtox® results of sucrose bioassays correlated poorly with results of sodium chloride bioassays, showed greater toxicity to only select metals, and did not improve bioassay sensitivity overall (Ankley *et al.*, 1990). Sodium chloride is therefore the best osmotic adjustment method available for the Microtox® test.

2.4 Data Analysis

Because of the osmotic adjustment and dilution steps *V. fisheri* and *P. fluorescens* bioassays, the salinity of a sample will differ from the final test salinity in the bioassay. In the *P. fluorescens* bioassay, the 1:1 dilution step will cause a 50% decrease in salinity between the sample and the test solution. Due to the osmotic adjustment and 1:1 dilution steps in the *V. fisheri* bioassay, the sample salinity will be adjusted by 2%+(Sample %Salinity/2). Since it is the response of the bacteria to the salinity in the original sample which is of interest in toxicity bioassays, comparisons between *V. fisheri* and *P. fluorescens* are made based on the original sample salinity.

Percent change in luminescence was calculated compared to controls (MSM for *P. fluorescens* and 2%NaCl for *V. fisheri*) as described in Environment Canada (1992). $EC_{50(15min)}$ values were calculated by linear regression; log transformations of the data were made in order to maximize fit (as determined by apparent line fit and r^2 valves) as required. Mean $EC_{50(15min)}$ values and 95% confidence intervals (α=0.05) were calculated according to Freund and Simon (1992).

3.0 Results

3.1 Influence of Salinity on Luminescence of *Pseudomonas fluorescens* and *Vibrio fisheri*

Pseudomonas fluorescens. The bioassay results indicate that NaCl causes a inhibitory effect on luminescence at all sample concentrations (see Figure 1). As sample salinity increases from 0.25% to 1.5% sample salinity, inhibition decreases from about 45% to almost zero . However, further increases in salinity cause dramatic decreases in luminescence to almost 100% inhibition at 3% sample salinity, and complete inhibition at greater salinities.

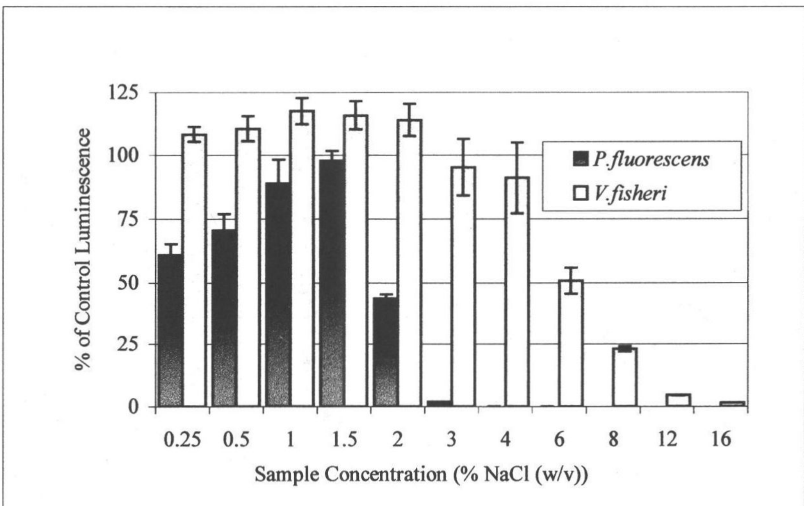

Figure 1. Response of *P. fluorescens* and *V. fisheri* to Salinity

Vibrio fisheri . Luminescence occurs over a much larger range of sample salinities for *V. fisheri* than *P. fluorescens* (see Figure 1). Stimulation of luminescence is fairly constant at about 10% from 0.25 to 1% NaCl, and increases slightly to about 15% in the 1-2% range. Greater NaCl sample concentrations cause a gradual decrease in luminescence, but *V. fisheri* is still slightly luminescence at 16% sample salinity.

3.2 Influence of Salinity on *Pseudomonas fluorescens* Cr^{6+} and Phenol $EC_{50(15min)}$

At 0% sample salinity, the Cr^{6+} $EC_{50(15min)}$ value is 657(±51)ppm (see Table 1, Figure 2). As sample salinity increases from 0 to 0.5%, the $EC_{50(15min)}$ does not change significantly (as determined by overlap of 95% confidence intervals of the mean $EC_{50(15min)}$ values). Increases in salinity to 0.75 and 1.5% causes a significant increase in the $EC_{50(15min)}$ to about 1000 ppm, but further increases severely increases toxicity. At 2% salinity, the chromium is ten times more toxic than at 0% salinity ($EC_{50(15min)}$ decreases ten-fold), and luminescence is totally inhibited at 4% salinity.

Table 1 . Response of *P. fluorescens* to Chromium and Phenol in the Presence of Salinity

Sample	Test	Chromium				Phenol			
NaCl(%)	NaCl(%)	EC_{50}(ppm)	95%CI	n	r^2	EC_{50}(ppm)	95%CI	n	r^2
0	0	657	51	120	0.842	558	64	16	0.917
0.25	0.125	702	61	12	0.929	344	157	12	0.709
0.5	0.25	662	59	24	0.831	270	46	12	0.998
1	0.5	966	61	37	0.883	116	51	12	0.983
1.5	0.75	1042	97	12	0.952	48	29	12	0.750
2	1	65	64	50	0.806	79	13	40	0.788
4	2	NA	NA	NA	NA	NA	NA	NA	NA

NA=Too toxic to allow EC50 calculation, CI=Confidence Interval

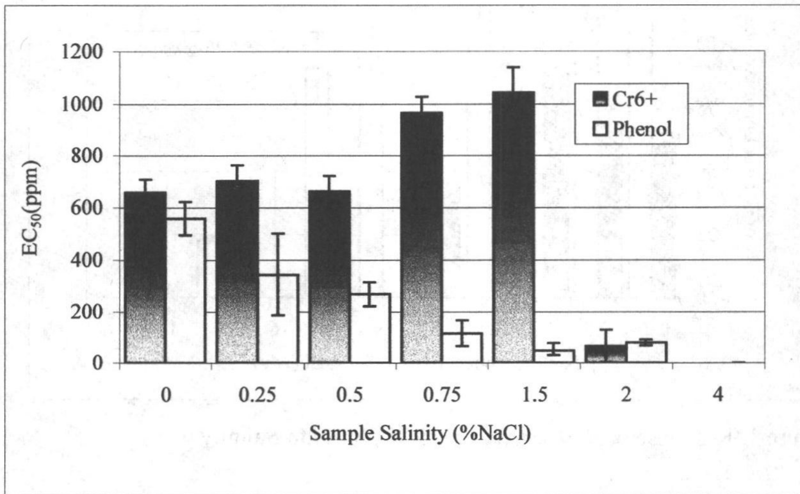

Figure 2. Response of *P. fluorescens* to Chromium and Phenol in the Presence of Salinity

At 0% sample salinity, the phenol $EC_{50(15min)}$ value is 558(±64)ppm (see Table 1, Figure 2). As the sample salinity increases, the toxicity of phenol increases dramatically. Phenol is twice as toxic at 0.25-0.5% salinity, five times as toxic at

0.75% salinity, and eight times as toxic at 1.5-2% salinity, than at 0% salinity. At 4% salinity, all luminescence is inhibited.

3.3 Influence of Salinity on *Vibrio fisheri* Cr^{6+} and Phenol $EC_{50(15min)}$

The Cr^{6+} $EC_{50(15min)}$ is about ten times lower for *V. fisheri* than for *P. fluorescens*; 63.53(±3.81)ppm at 0% sample salinity (see Table 2, Figure 3). At 0.25% sample salinity, the $EC_{50(15min)}$ value decreases substantially to 40ppm. Further increases in salinity to 2% cause decreasing toxicity compared to 0% sample salinity, but chromium is about three times more toxic at 4% sample salinity than at 0% sample salinity.

Table 2. Response of *V. fisheri* to Chromium and Phenol in the Presence of Salinity

Sample	Test	Chromium				Phenol			
NaCl(%)	NaCl(%)	EC_{50}(ppm)	95%CI	n	r^2	EC_{50}(ppm)	95%CI	n	r^2
0	2	63.53	3.81	30	0.986	27.61	2.10	24	0.958
0.25	2.125	40.83	9.24	16	0.957	33.27	4.05	15	0.941
0.5	2.25	66.44	8.46	24	0.945	31.50	4.03	24	0.879
1	2.5	71.62	11.60	24	0.899	28.05	2.11	24	0.959
1.5	2.75	75.97	9.73	15	0.960	36.86	3.37	16	0.961
2	3	78.75	5.65	24	0.976	24.43	1.84	24	0.963
4	4	16.87	5.14	15	0.556	8.62	1.59	39	0.835

CI=Confidence Interval

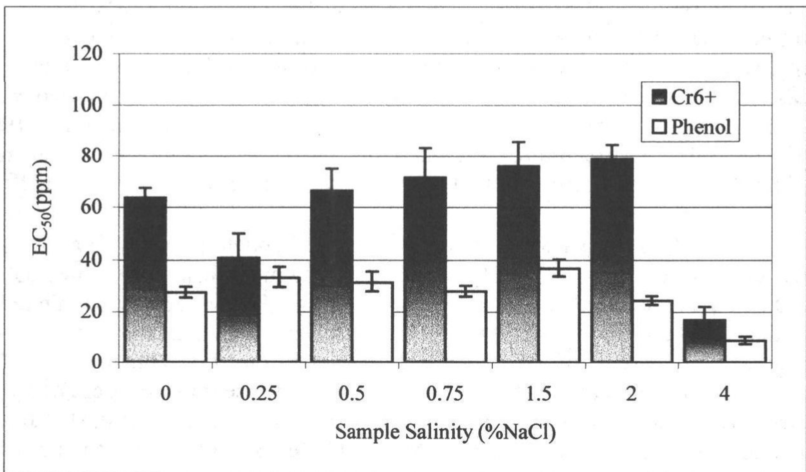

Figure 3. Response of V. fisheri to Chromium and Phenol in the Presence of Salinity.

Unlike *P. fluorescens*, the *V. fisheri* $EC_{50(15min)}$ shows no dramatic trends with increases in salinity (see Table 2, Figure 3). As in the Cr^{6+} bioassays, the $EC_{50(15min)}$ of *V. fisheri* for phenol is about 10 times lower than that of *P. fluorescens*, at 27.61(±2.10) ppm. The $EC_{50(15min)}$ values are fairly constant from 0 to 2% salinity,

showing slight increases at 0.25% and 1.5% sample salinity. At 4% sample salinity the $EC_{50(15min)}$ decreases to about half of that at 0% sample salinity.

4.0 Discussion

The results of this study indicate that *P. fluorescens* is much more sensitive to salinity contamination than *V. fisheri*. *P. fluorescens* exhibits some inhibition of luminescence at all salinities tested, while *V. fisheri* luminescence is stimulated at low to moderate sample salinities, and luminesces over a much larger range of salinities. The sample salinity required to inhibit luminescence in *V. fisheri* is over four times higher that that required for *P. fluorescens* (>16% compared to >3%).

Both *V. fisheri* and *P. fluorescens* exhibit decreased sensitivity to chromium in the presence of salinity. Previous studies have shown salinity to have a protective effect on bioassay organisms; chloride can form relatively non-toxic complexes with metal contamination and mitigate the toxic effect of metals (Ankley *et al.*, 1989; Ribo *et al.*, 1989; Ankley *et al.*, 1990; Brecken-Folse *et al.*, 1994; Carlson-Ekvall and Morrison, 1995; Hall Jr and Anderson, 1995; Hall Jr. *et al.*, 1995; Hauser *et al.*, 1997; Ferguson and Hostrand, 1998; Shaw *et al.*, 1998). This effect is exhibited over low ranges of salinity, where metals are ionic (most toxic) in the absence of salinity, and increasingly complexed with chloride with increased salinity. Since chromium and salinity alone are inhibitory to *P. fluorescens*, and chromium is most toxic in the absence of salinity, it is likely that any decrease in toxicity is due to the interaction of the metal and the chloride.

Although *V. fisheri* exhibits the same decreased toxicity response to chromium in the presence of salinity, complexation of chromium cannot explain the decrease in sensitivity for *V. fisheri*. Osmotic adjustment of samples for the Microtox® bioassay requires the test salinity to be at least 2%. At this level of salinity, substantial complexation of metals with chloride is expected, so further increases of salinity should have minimal consequences on complexation (Ribo *et al.*, 1989; Hall Jr and Anderson, 1995; Hall Jr. *et al.*, 1995; Ferguson and Hostrand, 1998; Shaw *et al.*, 1998). It is therefore more likely that the decreased sensitivity is due to the stimulation of luminescence at higher salinities, masking some of the toxic effects of the chromium.

Increased salinity causes substantial increases in phenol toxicity to *P. fluorescens;* phenol being twice as toxic at 0.25%, and more than twenty times as toxic at 1.5% sample salinity, than at 0% salinity. Since *P. fluorescens* is inhibited by both salinity and phenol, the increased toxicity of phenol with increased salinity indicates additive toxicity of these chemicals. Although specific mechanisms of salinity interaction with non-metals are largely unknown, the toxic response of an organism can be exasperated by physiological stress (Ankley *et al.*, 1990; Hall Jr and Anderson, 1995; Hall Jr. *et al.*, 1995; El-Alfy and Schlenk, 1998). The osmotic stress that a terrestrial and freshwater bacteria will experience in saline conditions, may therefore contribute to the increased phenol toxicity exhibited by *P. fluorescens* in the presence of salinity. Although osmotic stress would also occur in the chromium bioassay, the decreased toxicity of the chromium in the presence of salinity would counteract the deleterious effects of the salinity contamination.

V. fisheri phenol toxicity exhibits little response to increased salinity, as the phenol $EC_{50(15min)}$ changes minimally in the range of 0.25-2% sample salinity, and is only twice as toxic at 4% sample salinity. Phenol is not expected to react with

chloride, and osmotic stress due to salinity should be minimal, since *V. fisheri* is a marine bacteria, so results again indicate that stimulation of luminescence at higher salinities masks some of the toxic effects of the phenol.

The results of this study indicate that *P. fluorescens* is more sensitive to salinity alone and in mixed samples than *V. fisheri*, but also that *V. fisheri* is about ten times more sensitive to chromium and phenol than *P. fluorescens*. Although sensitivity is an important consideration of a toxicity bioassay, it is essential to consider the ultimate purpose of the toxicity testing when selected a bioassay organism. A very sensitive, but not environmentally applicable, bioassay can be used to detect low concentrations of environmental contamination, but would provide no more information on the environmental impact of the contamination than would traditional analytical chemical analysis. Where the purpose of toxicity testing is to demonstrate the response of applicable bioassay organisms and to mimic the effects of contamination in terrestrial, freshwater or groundwater environments, *P. fluorescens* is a more appropriate bioassay than the Microtox® bacteria *V. fisheri*.

5.0 Conclusions

The marine nature of the Microtox® bacteria, *V. fisheri*, limits the bioassay's applicability to analysis of terrestrial, freshwater and groundwater contamination. The required osmotic adjustment of environmental samples with sodium chloride influences the bacterial luminescence, and can substantially change the toxic profile of the sample. Use of Microtox® to analyze saline contaminated sites, such as are often found in the petroleum industry, is especially limited, since *V. fisheri* does not exhibit a toxic response to salinity, and the stimulation of luminescence by salinity may mask toxic effects of other types of toxicants in mixed samples. Environmental applicability, sensitivity to saline contaminated samples, and lack of required osmotic adjustment of samples makes the lux modified *P. fluorescens* bacterial bioassay a far more appropriate toxicity test for the assessment of terrestrial, freshwater and groundwater samples than the Microtox® test.

6.0 Acknowledgements

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The Dilemma of Agrochemicals

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Abstract

Agrochemicals is a general term to describe a chemical compound or mixture used to enhance the productivity of farm crops. The term agrochemicals refers to fungicides, pesticides, herbicides, nematocides as well as fertilizers, soil conditioners and plant hormones. This paper will stress the influence of the pesticides, herbicides and fungicides when discussing the dilemma of agrochemicals. There is no doubt that the use of some types of agrochemicals causes much emotional response in the general public. Agrochemicals not only have a history of environmental problems, but they are a current concern and will continue to be so in the future. The evidence of severe detrimental effects on society is undeniable. The ecology of a region, for example, can be influenced by the side-effects of the agrochemicals where they are applied. Although these examples of the hazards of using agrochemicals is an undesirable feature that science is struggling with to solve, these conditions have to weighed against the benefits that will result from their use. It has been estimated that the use of agrochemicals have decreased crop losses by some 17 to 78 percent. The benefits of additional crops to feed a growing population cannot be understated. As well, the ability to control vector-borne diseases by pesticides has saved hundreds of thousands of lives and hundreds of millions of illnesses from malaria alone since 1945. This paper will examine the problems associated with the use of agrochemicals and the on going trials of science to minimize the impact of these toxicants on our environment and health.

1.0 Introduction

The approximate percentage of the American population that lived on farms and supplied food to the nation was about 90% in the 19th century, 5% in the 1960's and 3-4% today (Chambers, 1994a). A reason for the dramatic shift in the farm populations had to do with the use and manufacture of synthetic organic pesticides. Prior to the development of synthetic pesticides the farmer's only solution to protect one's harvest were with tillage implements, mowing, use of crop seeds free of weed seeds, trap crops, crop rotations and site selection (Barnard *et al.*, 1997a). This was labour intensive and not very effective. The advent of pesticides has been estimated to have increased crop yields by 1.3 to 12 fold by decreasing crop losses by 17 to 78% (Chambers, 1994a). Crop losses even with the application of pesticides are still approximately 13% loss due to insects, 12% loss to pathogens and 12% loss to weeds (Chambers, 1994a). Today in the developing countries, over 60% of the economically active population depends on agriculture (Wesseling *et al.*, 1997a).

The word pesticide refers to a chemical compound capable of being used to control the effect of pests on agricultural crops, humans, households and commercial operations. There are several chemical classifications of pesticides such as: organophosphates; organic acids; triazines; carbamates; chlorinated hydrocarbons. The pesticides can also be classified by their functional groups: metal inorganic/organic (i.e. copper oxychloride, barium polysulphide, elements such as Arsenic, Copper, Mercury bound to carbon); phosphorus (i.e. parathion, malathion, diazon); nitrogen (i.e. aldicarb, carbaryl, carbofuran, cycloate, diallate); halogenated (i.e. dieldrin, DDT, DDD, HCH-Lindane); sulphur (i.e. classes include: sulphide, sulphate, sulphite, sulphonic acid) (Chmielewska-Horvathova and Robinson, 1996). This paper will concentrate on herbicides (weed control), fungicides (disease control) and insecticides (insect control).

The production of pesticides has dramatically increased, in 1980 approximately 0.5 million tonnes of pesticides were used worldwide. In 1985 there were 3 million tonnes of formulated pesticides used (Wesseling *et al.*, 1997b). In 1988, there were 7.2 million kg of pesticides applied in the province of Ontario alone of which 70% were herbicides (Zhu *et al.*, 1998a). Use of insecticides and herbicides, for example in the U.S. on pastures and crop land, was 99 million kg of active ingredients (a.i.) in 1964 to 277 million kg a.i. in 1992 (Majewski, 1998a). The figure below gives an overview of the amount of pesticides used in one country, the US from the years 1964 to 1995.

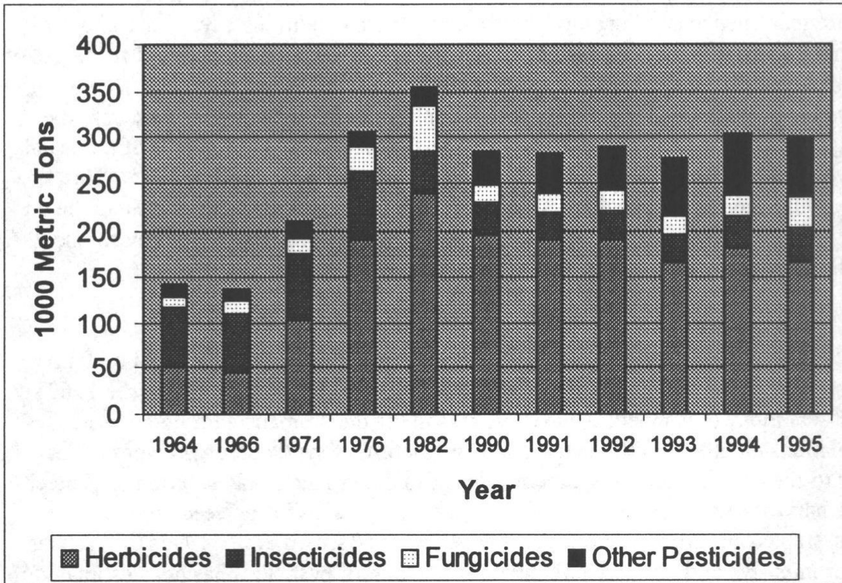

Figure 1 Active Ingredients Applied to Selected US crops 1964-95 (Barnard *et al.*, 1997f)

One observation to note is that there is a general trend to use herbicides more often than any other type of pesticide. Figure 1 also demonstrates a slight drop with the amount of insecticides applied to crops, this is primarily because organochlorine insecticides (i.e. chlordane, toxaphene, DDT) were replaced with synthetic pyrethroids and other insecticides that are applied at much lower rates (Barnard *et al.*, 1997b). Fungicides, shown on Figure 1, are the smallest class of pesticides, representing only 6% of the quantity of all pesticides types, but they are necessary for the appearance and quality of the fruit and vegetable crops (Barnard *et al.*, 1997c).

There are about 600 basic pesticides in approximately 50,000 formulated products on the market (Chambers, 1994b). The various pesticide formulations consist of 1-20% active ingredients and 80-99% carrier diluent (Kirk-Othmer, 1980). The active ingredient is the intended toxicant. It is the component which repels, kills, attracts or controls the pest. The formulation, or carrier diluent, can be in the form of dusts, water dispersions, emulsions, granules, aerosols and solutions.

The increase in food due to pesticides has allowed major cultural changes since less people had to work on the farms and could donate more of their time to other endeavors. The evolution of pesticides has increased the amount of food available, reduced the price of food, increased productivity and reduced many labour intensive cultural practices.

2.0 The Problems of Pesticide Use

Although pesticides have reduced losses to agriculture due to disease, insects and weeds, it is not a solution without problems. There is a growing concern about the safety of pesticide residue on food, contamination of the media and occupational exposures. Also, the effects of the pesticides and their respective transformation products on human health and the environment were not fully understood. There were unexpected detrimental effects and unwanted residues located in many places. Some of the problems of pesticides will now be addressed.

Some pesticides bioaccumulate, for example, DDT an insecticide, synthesized in 1874, was found to partition into cell membranes and remains stored in the adipose tissues or fat cells of the organism (Chambers, 1994c). Higher trophic levels of the food chain can then acquire and accumulate high concentrations of the chemical via ingestion. The concentrations listed in Figure 2 are actual values of DDT.

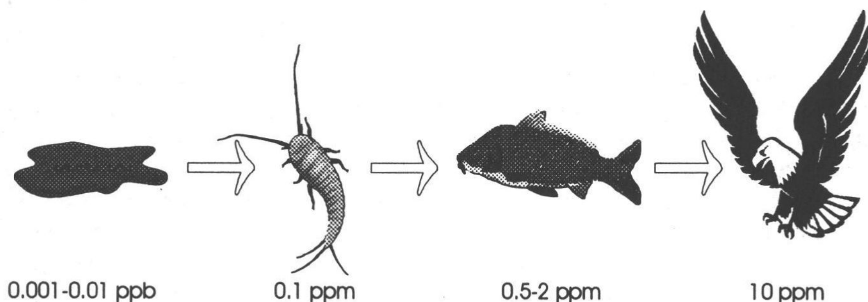

Figure 2 Bioaccumulation of DDT (Chambers, 1994d).

Toxicologists showed that levels of pesticides that were once believed to be safe for humans and the environment were not (Ryan, 1997a). Later it was discovered that exposure to some pesticides posed risks of cancer, genetic mutations, sterility and birth defects (Laughlin, 1993). Terminology such as the 'Pesticide Reference Dose' was implemented. A chronic dose means: "the level of exposure to a specific pesticide that a person could receive every day over a seventy-year period without experiencing appreciable risk". An Acute dose means: "the level of exposure to a specific pesticide that a person could receive with no appreciable immediate risk" (Ryan, 1997b). Table 1 demonstrates some examples of pesticides which are suspected to have caused an increase in the risk of these conditions.

Table 1 Suspected Toxicity of Some Pesticides

Toxicity	Some Suspected Pesticides
Carcinogenicity (induces cancer)	2,4-D, Endrin, Linuron, Rotenone, Captan, Maleic hydrazide
Teratogenicity (malformations, deviations from normal development)	Cyanazine, Carbaryl, Endrin, Rotenone, Benomyl
Mutagenicity (genetic changes, can be passed to offspring)	Diallate, Trifluralin, Dimethoate, Carbaryl, Rotenone, Benomyl, Maleic hydrazide
Neurotoxicity (damage to the nervous system)	EPN, Maneb, Paraquat
Reproductive Effects (causes damage or adverse effects to reproduction)	Dimethoate, Rotenone, Benomyl, DBCP (dibromochloropropane)

(Wesseling *et al.*, 1997c)(Barnard *et al.*, 1997d)

3.0 Classifications of Toxicity

Pesticides have been detected in every media. For example, since 1971 in the US, more than 130 pesticides and their respective derivatives have been detected in groundwater. Thirty-five of these samples had concentrations greater than health advisory levels (Wade *et al.*, 1998). Severe rainstorms just after pesticide application to crops increases herbicide runoff (Fawcett *et al.*, 1994a). Over-spraying, spray drift, leaching, volatility and solubility of the pesticide will also cause contamination into surface waters.

Since chemicals have been detected in water a classification system has been designed to determine the level of toxicity to fish. One organization called GESAMP (Group of Experts on the Scientific Aspects of Marine Pollution) has assessed damage according to the following categories shown in Table 2. The fish are exposed to a chemical, in this case a pesticide, for a duration of 96 hours in which the concentration at which half the fish perish has been determined, referred to as the Threshold Limit Medium (TLm). This value is then used to indicate how detrimental the pesticide is to

fish. For comparisons sake, the table will only list Rainbow trout as the type of fish, but in fact there are all varieties of marine and freshwater life that are tested for their sensitivity to a chemical exposure. As well the time for the test may vary from 24, 48 or 96 hours duration. A few examples of pesticide toxicity relative to Rainbow trout at 96 hours have been shown in Table 2.

Table 2 GESAMP Classification for Toxicity to Fish

Rating	96 hr TLm (mg/L)	Pesticide	Rainbow Trout 96 hr TLm (mg/L)
Highly Toxic	<1	DDT	0.007
Moderately Toxic	1-10	2,4-D	1.1
Slightly Toxic	10-100	Dimethoate	20
Practically non-toxic	100-1000	Bentazone	190
Non-hazardous	>1000	Maleic Hydrazide	1,435

(Montgomery, 1993a)

Soil contamination is also a major concern at many agrochemical facilities. There are classifications systems to assist the farmer in determining to what degree the pesticide is mobile and the persistence of the chemical. Koc or "soil organic carbon sorption coefficient" indicates which chemicals are likely to be more mobile. Table 3 shows the experimental values indicative of mobility and some examples. Some pesticides adsorb very tightly to the soil (Koc>100) such as trifluralin, paraquat and glyphosate. These chemicals are lost mainly with the sediment. Pesticides with extremely low adsorption (Koc<0.1) are lost with subsurface drainage (Fawcett *et al.*, 1994b).

Table 3 Pesticide Mobility

Mobility Class	Koc (mg/L)	Pesticide	Koc (sand loam soil) (mg/L)
Very Mobile	<15	2,4-D	14
Mobile	74-15	Dimethoate	33
Moderately Mobile	499-75	Prometon	150
Slightly Mobile	4000-500	Diazinon	1,543
Non-Mobile	>4000	DDT	130,000

(Roberts, 1996)(Montgomery, 1993b)

The half-life of the chemical is used to determine the persistence of the pesticide in the soil. Half-life of the pesticide is defined as the time required to degrade to half its initial concentration (Hornsby *et al.*, 1996). Table 4 is a

classification system to determine persistence of the pesticide, some examples are shown.

Table 4 Persistence Classification

Persistence	Half-life (days)	Name of Pesticide	Half-life (days)
Very Persistent	>60	DDT	3,800
Moderately Persistent	22-60	Diazinon	32
Slightly Persistent	5-21	2,4-D	15
Nonpersistent	<5	Malathion	1 (organic-rich)

(Roberts, 1996)(Montgomery, 1993c)

Table 3 and 4 demonstrate that DDT is both classified as very mobile and very persistent. The longer a pesticide remains in the environment, the more potential there is for it to come in contact with unintended species. All agricultural uses of DDT have been prohibited in the US in 1972, but it can be used for public health emergencies such as malaria (Arbuckle and Sever, 1998a). Given the low cost of DDT and the re-emergence of vector-borne diseases (malaria, dengue fever) these factors are likely to increase the pressure to use this pesticide in developing countries (Wesseling *et al.*, 1997c).

Agrochemicals have also been detected in the air. The global circulation of pesticides is accomplished by the transport, deposition and recycling of these chemicals in the atmospheric compartment. For example, air sampling devices detected evidence of long-term transport of pesticides across Ontario and Lake Ontario during the months of June and July, 1993-4 (Zhu *et al.*, 1998b). They were also measured in the remote location of Chukchi sea, which is several thousand miles away from pesticide usage (Rice and Chernyak, 1997).

The Midwest along the Mississippi River is known to be the highest density of agricultural activity and harvested crop land in the U.S. In this area a total of 140 different herbicides and insecticides are used by 10 different states which border the Mississippi River. In a random air sampling experiment about the region, the results showed: 25 out of 45 pesticides and transformation products were detected; 15 out of 25 herbicides and 2 herbicide transformation products were detected; 8 out of 18 insecticides and 1 transformation product were detected (Majewski *et al.*, 1998b). The results were detected in units of ng/m^3. The data illustrates that agrochemicals can become airborne in the ambient atmosphere and as a result humans, forests and other ecosystems will be exposed to low concentrations of these chemicals.

The exposure to chemicals in air has also prompted a classification system to determine at what level the chemicals are toxic. One such system is used by the American Conference of Governmental Industrial Hygienists (ACGIH). A threshold limit value-time weighted average (TLV-TWA) is introduced. The TLV-TWA is defined by "the time-weighted average concentration of a conventional 8 hour workday and a 40 hour workweek, to which it is believed that nearly all workers may

be repeatedly exposed, day after day, without adverse effects" (ACGIH, 1998a). A worker may be defined as a healthy individual weighing 150 pounds from age 25 to 44 (Genium, 1998a). In order to arrive at this value a few species of animals are tested with the same chemical, for example, monkey, rat, rabbit, Guinea pig. The animal that is the most sensitive is used to determine the lowest concentration at which there is found to be adverse effects due to inhalation of the chemical. This value is then decreased by extrapolated the value to humans by a factor of 100. The value of 100 is protective measure to extrapolate the value from animals to humans (10) and for the sensitivity range for humans (10). Table 5 shows some examples of TLV's.

Table 5 TLV-TWA of some pesticides

Pesticides	TLV-TWA (mg/m^3)
Diazinon	0.1
DDT	1
2,4-D	10

(ACGIH, 1998b)

The TLV-TWA value does not however give an indication as to what exposure would be necessary to cause adverse effects in humans. Another value called the Immediately Dangerous to Life and Health (IDLH) is by definition, "the maximum concentration form which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects" (Genium, 1998b). Table 6 demonstrates a few IDLH's.

Table 6 Some Examples of Pesticides and Their Respective IDLH's

Pesticide	IDLH (mg/m^3)
4,6-dinitro-o-cresol	5
Endrin	200
2,4-D	500
Lindane	1,000
Malathion	5,000
Dimethyl phthalate	9,300

(Montgomery, 1993d)

There are other methods that have been created to demonstrate the toxicity of chemicals. There is the World Health Organization (WHO) classification shown below for chemicals that are consumed and have contacted skin. This classification like the Environmental Protection Agency (EPA) are based on the LD50 and LC50 values. LD50 is defined as, "the single dose of a substance that causes the death of

50% of an animal population from exposure to the substance by any route other than inhalation" (Genium, 1998c). LC50 refers to, "the concentration of a material in air that on the basis of laboratory tests (respiratory route) is expected to kill 50% of a group of test animals when administered as a single exposure in a specific time period , usually 1 hour" (Genium, 1998c). Below are Tables 7 and 8 showing the WHO and EPA classifications, respectively.

Table 7 WHO Toxicity Classification

Class	Oral		Dermal	
	Solids	Liquids	Solids	Liquids
Extremely Hazardous	≤5	≤20	≤10	≤40
Highly Hazardous	6-50	21-200	11-100	41-400
Moderately Hazardous	51-500	201-2000	101-1000	401-4000
Slightly Hazardous	≥501	≥2001	≥1001	≥4001

Table 8 EPA Toxicity Classification

Class	LD50 for Rats		LC50 for Rats	Eye Effects	Skin Effects
	Oral (mg/kg)	Dermal (mg/kg)	Inhalation (mg/L)		
I	≤50	≤200	≤0.2	Corrosive; corneal opacity not reversible within 7 days	Corrosive
II	50-500	200-2,000	0.2-2.0	Corneal opacity reversible within 7 days; irritation persisting for 7 days	Severe irritation at 72 hours
III	500-5,000	2,000-20,000	2.0-20	No corneal opacity; irritation reversible within 7 days	Moderate irritation at 72 hours
IV	≥5,000	≥20,000	≥20	No irritation	Mild or slight irritation at 72 hours

(Royal Society of Chemistry, 1994)

4.0 Disposal

The toxic nature of pesticides places demands on toxicologists to determine how to safely handle and dispose of the chemicals. Some of the pesticides over time were banned from use or highly restricted. For example, DDT, BHC, DBCP were all banned in 1972, 1978 and 1985 respectively (Arbuckle and Sever, 1998c). As well, pesticides such as Dieldrin and Heptachlor can be used under restrictive conditions (Fawcett *et al.*, 1994b). The disposal of unwanted pesticides presents a formidable problem. Across rural America millions of tons of unwanted agricultural pesticides have accumulated in thousands of barns. Farmers are storing the pesticides because of changes in production or crops or the pesticides have been banned due to their toxicity. For example, Minnesota and Wisconsin both are storing waste pesticides estimated to be in the order of 3 million pounds (1994 statistics) and 4 million (1991 statistics) respectively (Centner, 1998a). As well, in the state of Georgia a survey was taken and it was discovered that of the approximately 1400 farmers, 23% of the unwanted pesticides had been in storage for just slightly less than 5 years (Centner, 1998b). At present in the United States, 30 states lack laws or guidelines that would establish the ground rules for collecting unwanted agricultural pesticides (Centner, 1998c). This is an undesirable situation that poses risks to humans and potential contamination of the environment. At present there is no permanent legislation, funding or program for disposal of pesticides in the US (Centner, 1998c).

The usual methods of disposal of pesticides are excavation, landfilling or incineration (Dzantor *et al.*, 1993). However, waste translocation from one site to another or the conversion of wastes into other forms without fully addressing the problem of contaminant detoxification must be addressed.

Bioremediation is another option for disposal of pesticides. This technique applies the controlled use of microbiological systems to detoxify the waste. The results suggest that combinations of land farming and biostimulation with organic amendments may be used to decontaminate certain herbicide wastes in soil effectively (Dzantor *et al.*, 1993a).

5.0 Export

Part of a solution with so much unwanted pesticides is to export the chemicals to other countries. Through the years 1995 to 1996, the US exported 338 million pounds of pesticides of which 6%, or 21 million pounds, were pesticides banned for use in the United States (Christen, 1998). Developing countries are exposed to large amounts of highly toxic pesticides and to pesticides banned or severely restricted in industrialized countries (Wesseling *et al.*, 1997d). Table 9 shows exports from only one country, the US. Note that Table 9 only shows pesticides that were banned, severely restricted never registered or were for restricted use only.

Table 9 Pesticide Exports in Pounds from US Ports, 1992-1995

Category	1992	1993	1994	1995	1996
Banned, discontinued	5,926,583	4,901,465	8,535,417	6,496,218	5,139,284
Severely restricted	6,152,495	8,321,638	4,597,626	5,869,905	5,823,220
Never registered	4,541,905	2,474,569	2,974,326	5,832,291	5,522,533
Restricted use	57,762,642	70,683,137	72,184,305	73,886,934	79,100,341
Total	74,383,625	86,380,809	88,291,674	92,085,348	95,585,378

(FASE, 1998)

Exports to other countries prompted new laws to protect developing countries from the inherent hazards of pesticide use. In September 1998 an international treaty was signed by 57 countries, including the United States, to defend against unwanted imports of certain hazardous chemicals and pesticides. Basically the treaty, referred to as Prior Informed Consent (PIC), states that any chemical that is banned or severely restricted in at least two participating countries cannot be exported without prior permission from the importing country (Christen, 1998a). Importing countries will have to decide which pesticides they can manage safely and those they wish to exclude. The problem has always been that developing countries do not have strong customs and enforcement infrastructures to implement training and informed use of the various formulations of pesticides.

Clif Curtis from the World Wildlife Fund states in the absence of effective regulatory regimes, "it's a nice concept that won't get very far". He points to problem centers such as India and China where pesticides are produced domestically. In these countries the PIC treaty will have no effect because of lack of trade-related and border crossing controls (Christen, 1998).

6.0 Developing Countries

To protect populations of developing countries from adverse pesticide health effects, policy debates have concentrated on safe-use strategies. Demonstration and training of adequate agricultural practices, improvement of registration process, control of exportation of hazardous substances and chemical risk assessment have been implemented by regulatory agencies (Wesseling *et al.*, 1997e).

In developing countries enforcement is required to ensure that there is no indiscriminate use and inappropriate handling of the chemicals. However, the issue of safe handling of pesticides is hindered by illiteracy, short term profit motives, poor legislation, eating recently sprayed food, early re-entry to sprayed fields and lack of enforcement of policies and procedures (Wesseling *et al.*, 1997f).

Farmers in Malaysia, Thailand, the Philippines, Cameroon, Kenya, Zimbabwe, the Jordan Valley, China, Bolivia, South Africa, Central America all demonstrate

inadequate pesticide management (inaccessible technical assistance; use of increased dosages; overly frequent spraying; faulty equipment; no protective equipment; inadequate practices of storage and disposal of pesticide containers) (Wesseling *et al.*, 1997g).

Surveys taken in developing countries lead to the following results. In Ecuador exposure comes from mixing pesticides with hands and a stick (36 out of 40 farms); no protective clothing other than rubber boots (38 out of 40 farms); disposing of chemicals in an unsafe manner (35 out of 40 farms) (Cole *et al.*, 1997). These types of practices lead to chronic hand dermatitis in over 80% of the cases (Cole *et al.*, 1997a). In the US, the rate for atopic and contact dermatitis is 32 per 1000 people, 12% for hand eczema in southern Sweden and 2% contact dermatitis for Hispanic grape, tomato and citrus workers in California (Cole *et al.*, 1997b).

In Ghana pesticide containers were used for storing drinking water (5 out of 10 farms). In Eastern Africa at an abandoned pesticide store, local people found obsolete and highly toxic chemicals in drums. They poured out the contents and used the drums for building material and water vessels (Lambert, 1997). Current guidelines by Alberta Environment, Canada require the residual concentration left from a used pesticide container before disposal to be 500 ppm or less, after a triple rinse (Guidotti *et al.*, 1994).

A survey from 123 farmers in Ghana determined that 30% of the farmers store the pesticide in the bedroom (for security reasons-expensive pesticides), 17% in the storeroom or another room in the house. When asked about re-entry times the survey showed: 39% returned after a few hours; 28% after 1 day; 20% after 2-3 days (Clarke *et al.*, 1997). Current guidelines state that treated areas must be declared out of bounds for a period of 3-7 days. If re-entry must be done before this time then personal protective equipment must be worn (Parmeggiani, 1991). As well, 28% of the farmers never used any form of personal protective clothing (Clark *et al.*, 1997). The longer the re-entry interval, the less risk for intoxication and exposure from the chemicals.

Nicaraguan law prohibits anyone under the age of 16 to be working with pesticides. However, in a survey of 458 people, 43% of the respondents that were in this age group admitted working with pesticides (Keifer *et al.*, 1996).

A survey was done on Mexican farmers who were spraying crops with DDT. In 1992 the data showed the most important route of exposure was the consumption of highly contaminated foods. The results indicated that in 1994 the predominant source of contamination was inhalation of the vapors (Waliszewski *et al.*, 1996).

Despite the fact that million dollar training programs on safe pesticide use are being carried out worldwide, there are still major problems with safety (Wesseling *et al.*, 1997j). The World Health Organization (WHO) estimates from hospital admission data, that 3 million people (2 million suicide poisonings) suffer severe pesticide poisonings worldwide annually (Keifer *et al.*, 1996a). It should be noted that in California, where medical professionals are legally obligated to report pesticide related illness, estimated that only 1-2% of pesticide illness cases were officially reported (Keifer *et al.*, 1996a). This survey sheds light on the probability that developing countries report even less of the pesticide poisonings. Table 10 demonstrates an example of reported health issues due to pesticides from countries around the world.

Table 10 Reported Health Problems Due to Pesticides

Country	Health Issue
Ukraine	Rates of spontaneous abortions and premature deliveries were twice as high in female sugar-beet growers compared with women of other rural occupations (Arbuckle and Sever, 1998b)
Finland	1973-1975, women working in agriculture had the highest spontaneous abortion rate. Compared with all women: farmers, and gardeners had a significantly higher abortion rate of 11.6%, farmhands had a rate of 7.9% (Arbuckle and Sever, 1998h)
China	Survey of 2872 women who had used pesticides during their pregnancies were at higher risk of spontaneous abortions and birth defects (Arbuckle and Sever, 1998d)
Costa Rica	High rates of male sterility found among Dibromochloropropane exposed banana workers (Wesseling *et al.*, 1997k)
Andhra Pradesh, India	High frequency of abortions and stillbirths among grape vine workers exposed to pesticides (Wesseling *et al.*, 1997k)
Egypt	Elevated risk for chromatid breaks and gaps and sister chromatid exchanges among workers formulating organophosphate compounds (Wesseling *et al.*, 1997k)
United States	Amounts of DDT and DDE residues in serum and mammary adipose tissue encountered in general population (Wesseling *et al.*, 1997k)
Malaysia	Whole-blood cholinesterase levels depressed by 25%, for 45% of the pesticide users exposed to organophosphates (Wesseling *et al.*, 1997l)
Latin American Countries	Found on locally consumed food levels of: lead arsenate, EBDCs, chlorothalonil, endosulfan, copper oxychlorate exceeding national tolerances (Wesseling *et al.*, 1997j)
Sweden	Frequency of spontaneous abortions near a herbicide plant was reported to be significantly higher, 15%, than in the comparison areas, 9% (Arbuckle and Sever, 1998f)
Nicaragua	458 interviewed, 48% reported having been "poisoned or made ill". Many incidents (38%) involved a mixture of pesticides (Keifer *et al.*, 1996)
Bolivia	2-10% of agricultural workers reported previous occupational poisonings (Wesseling *et al.*, 1997h)

Table 10 Continued Reported Health Problems Due to Pesticides

Carchi, Ecuador	Highest provincial rates of pesticide poisonings 22 cases per 100,000 population (Cole *et al.*, 1997)
Guatemala	70% of melon and broccoli growers interviewed reported poisoning symptoms over a period of two years, which they associated with applying pesticides (Wesseling *et al.*, 1997i)

It should be noted that there are significant methodological problems when compiling statistics from developing countries in regards to pesticide poisonings. Some examples of the factors that hinder the accuracy of the statistics are: imprecision in the characterization of exposure; limited sample size; quantification of the dose exposure and failure to account for potential confounding factors (Arbuckle and Sever, 1998g).

7.0 Theories

Previously, each pesticide was analyzed in the laboratory to determine its acceptable tolerance level. This level was determined independently from the other pesticides. However, toxicologists have shown that many chemicals can act synergistically. In other words, the toxic effect of one pesticide can be enhanced when mixed with other pesticides that act through the same metabolic pathway. These cumulative effects are acknowledged by setting an upper limit through an estimated lifetime toxic exposure rather than simply limiting the amount of individual pesticide that is know to have been sprayed on an agricultural product (Ryan, 1997c).

In 1996 the Food Quality Protection Act (FQPA) of the United States moved away from the Delany Clause to what is referred to as the "Risk Cup". The legislatively dictated but scientifically untenable "zero tolerance" of pesticide residues will be abandoned. New scientific analytical instruments are detecting more and more residues of pesticides on food. In 1994, the Food and Drug Administration, which tested raw or processed foods, found that 1% had residues which exceeded tolerances and less than 40% had any detectable residues. This implies that the remaining ~ 60% had detectable residues (Barnard *et al.*, 1997e). This implies that infants, the elderly, and those with compromised immune systems will have guidelines to limit their consumption of pesticides. This is an attempt by the Environmental Protection Agency (EPA) to set a lifetime upper limit to the amount of risk from aggregate chemical exposure for US citizens (Ryan, 1997a).

The hypothesis presumes that each of us has a level of pesticide tolerance exposure that we can withstand without harm to our health. The cup is filled by the risk arising from each pesticide exposure (through air, water or diet). If the cup is full, exposure to that pesticide must be reduced in order to reduce the human population risk (Ryan, 1997b). The current perspective on pesticide consumption implies that you will digest pesticides or derivatives of pesticides to some degree throughout a lifetime. The risk cup is an attempt to minimize the inevitable so that there will be no ill health effects.

A new theory of disease called Toxicant-Induced Loss of Tolerance (TILT) pertains to chemical sensitivity. The process follows a two-step procedure: exposure to high levels, or repeated exposure to lower levels of a chemical, followed by

exposure levels that have no observable or experimental observations that affect people. The theory of TILT implies that the body appears to lose the ability to adapt to low-level chemical exposures. "TILT manifests itself as a loss of tolerance to everyday chemical, food, and drug exposures in affected persons, possibly leaving these individuals more susceptible to other diseases." (Ashford and Miller, 1998). This remains a theory since it has yet to be scientifically verified.

8.0 Resistance is Futile

Pest resistance is a growing issue. If a species of insect contains the necessary biotypes that are resistant to the active ingredient of the pesticide, then that individual will survive to pass the resistant trait on to the next generation. As the generations continue to multiply, the resistant trait now becomes part of the dominant species of the population. The pesticide and its intended use then becomes uneffective. These effects are being seen since close to 200 insect and arachnid pests have developed resistance to one or more of the pesticides that are in use (Uri, 1997). Resistance to the herbicides atrazine, cyanazine and simazine has been shown for corn and sorghum. In an attempt to suppress the weeds, insects and fungicides, farmers have been using new chemicals that exhibit less toxic properties than the earlier pesticides (Király, 1996). As well different agricultural practices are being used to make it more difficult for the pests to adapt. The farmer may, for example, rotate pesticides with various modes of action, use different active ingredients in combination, alterations in planting, lower the application rates and utilize a mixture of mechanical (physical barriers) or non-chemical control procedures (Uri, 1997).

In 1995, problems such as pest resistence and pesticide toxicity prompted the National Research Council to seek alternative approaches to complement current pest management procedures (Uri, 1997a). One of the alternative methods is a biologically based pest control technology or biopesticides. Biopesticides are genetically engineered horticultural crops with the idea of building in resistence to insects, diseases, and herbicide tolerance. Since 1993, biopesticides have accounted for 6% of the world market at the manufacturing level (Menn, 1996). As indicated in Table 11 there are many advantages to researching gene technology.

Table 11 Comparison of Synthetic Chemical Pesticides and Biotechnology

Agrochemical	Registration Costs	Time (years)	Safety Studies
Pesticides	$10-12 million	5-8	120
Biopesticides	$1-1.2 million	1-2	12

(Froyd, 1997)

At a glance one can notice that the biotechnology looks promising for saving time and money. The faster the product is on the market, the quicker the company can collect a profit.

In 1995, about 13% of the US cotton acreage was bioengineered with a protein from the bacteria *Bacillus thuringiensis* (Bt). (Uri, 1997b). The toxin binds to the midgut cell receptors causing swelling and eruption of the stomach epithelial cells (Menn, 1996a). The bioengineered seeds were to produce a bacterial pesticide to

control cotton bollworms, European corn borer and other insects (Uri, 1997b). There are currently over 50 biopesticides in the US alone (Menn, 1996b).

Research is also being conducted on baculovirus or more specifically a strain called *Autographa californica* (ACAL). It is an insect-specific toxic protein that will not harm mammals but attacks insects that destroy cotton and vegetables. Once the insects have been infected with the ACAL baculovirus the protein produces the toxin inside the insect which first paralyzes it causing the insect to stop feeding (Menn, 1996c).

There are some problems with the biopesticides. Currently, the technology has not translated to match field performances of the manufactured chemical synthetic pesticides. The biopesticides have yet to function adequately under variations of temperature and moisture content, as well as providing consistent disease control (Froyd, 1997a). They also have a narrow range of insects that can be inhibited from eating the plants. Problems of self life and mass production pose additional scientific research. Also, resistance to the transgenic crops has already been detected by the diamond back moth (*Plutella xylostella*) located in the Phillippines and Hawaii (Menn, 1996d). It will be difficult to provide herbicide tolerance and disease resistance via genetic engineering from transfer of a single gene. Research no doubt will have to comprehend how to transfer perhaps more than one gene in order to provide the plant with the necessary defenses.

Another alternative method is to use organically grown products. This system promotes and enhances biodiversity of the ecosystem, soil biological activity and respects biological cycles. At present, organic food products account for 1% of the total retail food sales in the US (Uri, 1997a). The consumer has to accept the possibility that the produce may not be blemish-free.

9.0 A Case Study

In Kuttanad, South India there is a rice farmland yielding a high variety of paddy cultivation of which there is always the problem of recurring floods. The environment sustained a natural ecological balance with the flood waters providing a cleaning effect and also transporting nutrient rich sediments back to the fields. The ecosystem provided for earthworms, snails, frogs, water snakes, birds etc. and so insecticides were not necessary.

However, one day the market realized that the frogs could be sold for meat. This demand depleted the region of frogs. Since the frogs were greatly reduced, the number of snakes also quickly diminished. With the depletion of snakes from the region to other areas came mice and rats. With the frogs gone, other insects came such as stem-borers, swarming caterpillars etc. All together, this influx lead to a diminished return of the rice paddy fields.

In order to combat the ravaging insects, insecticides (i.e. folidol, endrin) were introduced. With the annihilation of the pests it was noticed that the toxic sprays either poisoned some of the wild life or the swans, rats, mice, turkeys, fowl etc. migrated to other areas to find additional food.

When the larger animals left the area, aphids, which were immune to the sprays, ravished the rice paddy fields. It was then that highly toxic chemicals (i.e. dimecron, hinoden, metaciden) were used. Through the misuse of the application of these more toxic pesticides, the chemicals obliterated soil bacteria, tiny soil worms and

other creatures. The flushing action of the recurring floods partially reduced the toxic action of the pesticides but the overall chemical exposure left the ecosystem unable to sustain animal or aquatic life (Balchand, 1997).

This case study is a good example that pesticides do work. It is also an example of how scientists need to view the "big picture" as opposed to the details. In this example a simple reintroduction of frogs to the area would have eventually balanced the system once again. Sometimes it is not always obvious how to solve dilemmas.

10.0 Conclusion

Given that prospect that the population will increase, this will drive the need for more sustainable agricultural productivity. It is projected that by the year 2005 an additional 10 million acres each will be required for corn and wheat, as well 8 major crops, theoretically, will have to increase acreage by some 5-10%. Research into alternative solutions then becomes a necessity (Uri, 1997c).

Indonesia, The Netherlands and Sweden are all countries that are attempting to move away from agrochemicals and are finding more reliance for plant protection on biological and cultural control (Kopisch-Obuch, 1996). These changes no doubt were the result of a growing awareness of excessive use and the negative effects of pesticides in the past.

The problem of agrochemicals is that they are needed to achieve adequate food production and protection from vector-borne diseases, but evidence suggests that they have also been detrimental to living organisms and ecosystems. The dilemma is not just about feeding a population. It is about understanding the complex interactions of nature so that one can utilize the limited resources, while at the same time, sustaining and preserving the vitality of the planet for the years to come. A complex interaction that was unknown until recently, for example, is the poisonous frog *Dendrobates pumilio*. It was found that after the frog eats its fill of an ant called *Solenopsis azteca*, the frogs make a defensive coat from the toxins that the ants produce (Rouhi, 1999). When the pesticides made the ants disappear, the frog lost the capacity to protect itself. These are the types of interactions that are 'hidden' within ecosystems. Using pesticides will always be a balance between risk and benefit.

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