THE TOXICITY OF SOFTWOOD LEACHATE IN AQUATIC AND TERRESTRIAL ENVIRONMENTS

BY

KRISTINA L. A. FARMER

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

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ABSTRACT

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Concerns over the potential toxicity of leachate derived from softwood logs in remote storage areas lead Manitoba's Clean Environment Commission to request an investigation into the toxicity of softwood leachate in aquatic and terrestrial environments. This study had two main objectives: 1. To determine if leachates derived from the softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) are toxic to aquatic organisms; and 2. To determine the impact of softwood leachate on carbon and nitrogen mineralization and microbial functional diversity in an Eluviated Dystric Brunisol (Ahe horizon) and an Orthic Gray Luvisol (Ae horizon and LFH layer).

Softwood leachate was generated by soaking Jack Pine and Black Spruce logs in dechlorinated tap water for up to 60 days. Aquatic toxicity was assessed with rainbow trout (*Onchorhynchus mykiss*), *Daphnia magna*, and Microtox (*Photobacterium phosphoreum*) toxicity tests. Toxicity, observed at each trophic level, generally increased with log soaking duration. Toxic leachate was also generated after significant precipitation events at an outdoor simulated log storage site.

Leachate generated by soaking logs for 30 days was used to evaluate the effects on soil microbial processes. Soil samples were amended with single 25%, 5% or 1% by volume leachate treatments or with control water. Respiration rates and amount of mineralized nitrogen in each incubated sample were determined regularly over 20 weeks. Leachate additions had little effect on weekly microbial respiration in each soil horizon. Compared to the controls, increased, reduced and equal N mineralization was observed in the Ahe Brunisol, LFH Luvisol and Ae Luvisol leachate treated samples, respectively.

Soil functional diversity was assessed via examination of the rate and pattern of substrate usage on Biolog™ microtitre plates inoculated with extracts of leachate amended soils. Colour production in each well was recorded over 72 hours. Leachate treatment of the Ahe samples resulted in the rapid metabolization of a greater number of substrates compared to the control. Rates of substrate metabolization in the leachate treated LFH samples was greater than in the control. Treatment with Jack Pine leachate resulted in slower substrate use than the Black Spruce treatment. Leachate treatment of the Ae Luvisol samples had no effect on the number and rates of substrates utilized. In general, the functional diversities of the forest soils assayed were either not affected, or were stimulated by the single addition of softwood leachate.

Softwood leachate had negative impacts on aquatic organisms, while the effects of softwood leachate on soil microbial processes varied. From the results of this study, it can be concluded that the uncontrolled runoff of softwood leachate from log storage yards into water bodies must be prevented.

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FOREWARD

This thesis has been prepared in the manuscript format in accordance with the Department of Soil Science guidelines. The referencing style used throughout the thesis follows that of the Canadian Journal of Soil Science. Three manuscripts will be submitted for publication to the Journal of Environmental Quality. These papers are entitled: 1. The toxicity of softwood leachate to aquatic organisms; 2. The effect of softwood leachate on functional diversity of forest soils; and 3. The effects of softwood leachate on C and N mineralization in forest soils.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Remote storage of logs has recently been shown to be a source of toxic compounds in hardwood storage yards in British Columbia (Goudey and Taylor 1992). In some cases, storage yards are located near sensitive aquatic ecosystems such that drainage reaches the water body. Some softwood log yards may be located in similarly susceptible drainage areas. It is therefore important to investigate whether leachates from softwood logs are toxic in the aquatic environment and to determine their impact in terrestrial environments. If toxic effects are observed, ecosystem management plans for the placement of forest log yards and log piles may be required to alleviate environmental risk.

Research on the aquatic toxicology of pulp and paper effluents has revealed that many of the toxic compounds are wood-derived (Easty et al. 1978; Thakore et al. 1989; O'Connor et al. 1992). The composition of wood is usually differentiated into major cell wall components and extraneous components. The cell wall is composed of cellulose, hemicelluloses and lignin. These are structural wood constituents and, with the exception of part of the lignin, are insoluble in solvents and water (Karau 1975; Sjostrom 1993). These components should not contribute a great deal to the toxicity of effluents. In contrast, the extraneous components, or extractives, are non-structural wood constituents, many of which are soluble in neutral organic solvents and water. Wood extractives include

resin acids, fatty acids and phenols. The actual amount and composition of extractives present differs among wood species, different parts of the tree and tree age. The aquatic of mechanical pulping effluent is due mainly to naturally occurring wood extractives which leach from wood during the pulping process (O'Connor et al. 1992).

Natural processes can also lead to the generation of wood leachates. Leachate can be produced when wood extractives, expelled from wounds in logs post-harvest, are washed off with rain water. Wood extractives may also be released from water-saturated wood fibres via the process of diffusion. Wood constituents will continue to leach provided water contact and wood fibre saturation are maintained (Liu et al. 1995). Taylor (1994) demonstrated the potential for log storage piles to produce leachate when it was determined that an aspen log pile, exposed to natural weather conditions, generated significant quantities of toxic leachate over a 23 month period. During this study, only 5% of the supply of leachable material was removed from the wood. Leachate production could therefore continue indefinitely.

Although the toxicity of wood-derived compounds to aquatic organisms has been documented, little research has been conducted on the effects of these compounds in terrestrial environments. Wood leachate produced from log storage piles will enter surface water bodies directly via overland flow or will percolate down through the soil to groundwater with subsequent migration to the water body. Soils are often regarded as "environmental filters or buffers" (Gregorich et al. 1994) because of their ability to retain and degrade chemical and biological materials. Different soils will have distinct

attenuation capacities. Processes such as sorption and volatilization are significant, but soil microorganisms will play an integral role in the degradation of these compounds. The effects of the wood leachate on soil microbial processes are not known. The production of wood leachate is a natural forest process. Unfortunately, log storage piles may lead to the production of large volumes of highly concentrated leachate solutions. Although it is probable that soil microorganisms capable of metabolizing this type of substrate exist in forest soils, it may be possible to overload the system. Toxic effects at the microbial level may indicate negative impacts in the entire soil system. The evaluation of changes in various soil microbial processes is therefore important for assessing the effect of leachate in the total environment.

Hardwood leachate was found to be toxic to aquatic organisms (Goudey and Taylor 1992). As softwood trees are of major importance to Manitoba's forest industry (Abitibi-Price Inc. 1990), Manitoba's Clean Environment Commission requested an investigation into the environmental effects of softwood leachate. With the support of the Manitoba Model Forest and Manitoba Environment, the objective of this study was to determine whether leachates derived from the softwood species Jack Pine and Black Spruce have toxic effects on aquatic organisms and soil microorganisms. The results of this investigation may lead to the future development of management plans for the placement of log storage areas.

2. Literature Review

2.1 Introduction

Wood is an extremely important natural resource in Canada. One of the reasons the Europeans first settled the "New World" was to take advantage of this valuable commodity. In Canada's early years natural resources were extracted from the land without thought of future consequences. For example, river log drives were deemed an economic necessity of the times and therefore a public right. This practice was regarded as acceptable without knowledge of the possible adverse effects. The story is similar for pulp and paper mills discharging whole effluents into natural water systems. A more recent concern is the production of toxic leachates from wood and wood waste storage sites. Storage practices such as these are viewed as necessary and justifiable but evidence has emerged relating them to possible adverse effects in aquatic environments (Taylor 1994). These examples display the importance of investigating all potential sources of pollution and developing mitigating technologies *before* problems are detected.

The protection of aquatic resources is a major concern in Canada. There now exists both Federal and Provincial legislation pertaining to activities which may cause damage to aquatic ecosystems. The Canadian Fisheries Act is often used to initiate prosecutions against parties who intentionally or unintentionally discharge "deleterious substances" into waters "frequented by fish" (Fisheries Act 1991, Section 36(3)). This legislation

guards against the *possibility* of fish populations being harmed by industry and does not require actual proof of deleterious effects on fish populations (Walden and Howard 1977). Pulp and paper mills are an obvious target for scrutiny under this legislation. As a result, the effects of this industry on the aquatic environment, which range from tainting to acute toxicity in fish, have been the subject of much research. What causes these effects? Is it the chemicals added in the pulping process, or constituents from the wood itself? Studies indicate that some of the toxic constituents in effluents have natural origins.

Research studies into the effects of wood-derived compounds in aquatic environments have been conducted and are ongoing. Unfortunately, the effects of these compounds in terrestrial environments are largely unknown. Application of pulp and paper mill effluents and wood residues to agricultural lands has recently become an accepted disposal method (Kannan et al. 1990; Liu et al. 1995). It is believed that any "toxic" compounds added to the soil will be sorbed or degraded by microorganisms. The soil's capacity for natural attenuation is not limitless. The effects of these types of compounds on the soil system as a whole must be investigated before this disposal method becomes a more prevalent practice.

2.2 Wood Structure

Trees are generally classified into two different groups: 1. Coniferous or softwood trees; and 2. Deciduous or hardwood trees. The main differences between the groups are the length of time the leaves remain on the tree, and the structure and make-up of the wood

(Walker 1989). Coniferous trees retain their needle-like leaves for at least two seasons and contain soft, resinous and non-porous wood. Deciduous trees, however, shed their broad leaves annually and contain porous wood (Hosie 1979; Farrar 1995).

Although major differences exist between softwood and hardwood trees, all trees contain similar structural components. The two primary types of wood tissue are phloem and xylem. Phloem is the bark of the tree and comprises 10 to 15 percent of tree dry weight. Sap travels from the roots to the tree top through the phloem, which is essentially dead tissue (Wangaard 1981; Walker 1989). The major woody material of trees is called xylem. Xylem tissue is organized into concentric growth rings. These rings are formed via the interruption of rising sap. This usually occurs in late fall, but may also occur during periods of drought, therefore these rings are not necessarily formed annually (Walker 1989). Xylem tissue is differentiated into sapwood or heartwood. Sapwood is the physiologically active part of the xylem. It provides support to the stem, conducts water upward and stores food. Sapwood tends to be a lighter colour than heartwood and is located in the cambial growth zone of the tree between the bark and inner wood. As new sapwood forms due to cell division in the cambium tissue, the interior sapwood converts to heartwood (Harada and Cote 1985; Wangaard 1981; Sjostrom 1993). Heartwood, located in the central part of the wood, is the physiologically dead part of xylem. Its only role in tree structure is support. The transition of sapwood to heartwood is accompanied by the formation of various organic substances called extractives and extraneous materials (Harada and Cote 1985). The dark colour of heartwood is thought to be attributed to the production and secretion of these compounds (Wangaard 1981).

2.3 Wood Chemistry

Wood (xylem) is a complex material whose composition can generally be classified as either cell wall or extraneous components. Cell wall components, which include lignin and polysaccharides, provide the primary structure of wood. Lignin is a natural cementing and encrusting material. It is deposited between wood cells binding them together in a rigid structure. It is an aromatic (phenolic), amorphous substance, generally insoluble in common solvents (Schubert 1965; Browning 1967; Wangaard 1981) and resistant to biodegradation (Swift et al. 1979). In softwoods, lignin constitutes approximately 26 to 29 percent of dry weight, while it constitutes 19 to 26 percent of dry weight in hardwoods (Forestry Branch 1951). The greater part of wood is composed of cellulose. Cellulose is the most abundant carbohydrate in nature and is the most abundant compound in plant cell walls (Hori and Elbein 1985), constituting approximately one half of wood substance (Browning 1967). It is a polymer of several thousand glucose units. Cellulose is always accompanied by other types of polysaccharides called hemicelluloses. The hemicelluloses differ from cellulose in that they consist of glucose as well as other sugars (galactose, mannose, xylose and arabinose) (Liu et al. 1995). Together, these substances provide the framework and matrix system for the wood cell (Harada and Cote 1985).

Extractives or extraneous components are the other major constituent of wood. They are secondary, non-structural components of wood and bark. Most are produced by the tree as a line of defense against predatory insects. Extractives can generally be extracted by neutral solvents such as water, alcohol, benzene, ether or acetone, and typically constitute

2 to 5 percent of the dry wood weight (O'Connor et al. 1992). This is an extraordinarily diverse and numerous group of compounds which may be either lipophilic or hydrophilic in nature. The major classes of extractives include resins and fatty materials, as well as their acids, alcohols, terpenoids and phenolic substances. The quantity and types of extractives found in wood tissues vary widely among tree families, species, tissues types, as well as with the age of the tree, position within the tree and possibly rate of tree growth (Hillis 1985; Browning 1967; Sjostrom 1993; Forestry Branch 1951; Leach and Thakore 1973). Extractive compounds will generally be found in highest concentration in tree bark and heartwood.

Resin acids, fatty acids and phenols are the extractives most often linked to toxicity in aquatic systems. Resin acids are characteristic and important constituents of conifers which serve to protect wood tissue from insects. They are located in the ray and resin canals of conifer woody tissue, as well as in exudates produced following injury to the inner bark or outer sapwood layer. The most common resin acids include abietic acid, palustric acid, neoabietic acid, dehydroabietic acid, pimaric acid and isopimaric acid (Browning 1967). Although resin acids generally degrade readily, dehydroabietic acid tends to be very persistent in aquatic environments, rendering it one of the most studied resin acids (Brownlee et al. 1977). Dehydroabietic acid has been identified as the major pulp mill effluent component observed in receiving waters greater than 2 kilometers downstream from a source (Fox 1977), and in sediments 1 kilometer from the same source (Brownlee and Strachan 1977). Fatty acids, which are commonly found in both deciduous and coniferous trees, are located in ray parenchyma cells and function as

energy reserve sources (Brouzes 1976). Common types of fatty acids include palmitic acid, oleic acid, linoleic acid and linolenic acid (Sjostrom 1993; Browning 1967). Aquatic toxicity of C_{18} fatty acids increases with increasing degree of saturation (Leach and Thakore 1973). Phenolic compounds are found in softwood trees but are more prevalent in hardwoods. While they occur throughout the tree, phenols are primarily located in bark and cambial tissues. Up to 10 percent of the mass of aspen tree bark is made up of these compounds (Palo 1984). Phenolics act primarily as a line of defense against microbial infection and grazing by wood-boring insects and animals (Lindroth et al. 1988; Palo 1984).

2.4 Pulp and Paper Mill Effluent

The discharge of pulp and paper mill effluent into aquatic ecosystems is a major environmental concern. This concern is amplified by the enormous volumes continually released into the environment. In many cases, research has revealed that effluent from various mill processes is acutely toxic to aquatic life (Leach and Thakore 1976; Rogers et al. 1975; Johnsen et al. 1995; McDonald 1978), although this toxicity varies with both the process type and wood mix used. While individual compounds present in effluent may be toxic to aquatic organisms, this toxicity is greatly modified by such factors as dissolved oxygen content, pH, and suspended solids concentration. In fact, extreme changes in any of these factors could, in themselves prove to be "toxic". While some toxic constituents are added during pulp processing, it seems that most of the toxicity is wood-derived.

2.4.1 Processing Methods

There are a number of different processing methods employed in the pulp and paper industry. Three of the major methods utilized in North America include the groundwood / mechanical, sulfite, and Kraft processes. In each process log pieces are first subjected to debarking, which is typically achieved hydraulically.

2.4.1.1 Groundwood / Mechanical. The groundwood / mechanical pulping method utilizes a simple grinding of wood pieces in order to separate and remove individual fibres. This is a non-chemical process which produces a pulp with high lignin-content and relatively short fibres (Marier 1973). The pulp is then subject to screening, refining, cleaning and filtering. Pulp made this way is most often used for newsprint (Abitibi Price Inc. 1990). As this process yields a pulp lower in strength than from other processes, groundwood / mechanical pulp is often supplemented with sulfite or Kraft pulp for improved strength (Marier 1973).

2.4.1.2 Sulfite. In the sulfite pulping process, wood chips are subjected to chemical digestion in acidic bisulfite (Marier 1973) to produce easily-bleached, high-quality fibre. During digestion, approximately 54% of wood constituents are solubilized. The dissolved lignin and remaining acid are washed away, leaving the cellulose fibres, which are then cleaned and refined. The digestion liquid has high biological oxygen demands (BOD) associated with it, therefore recovery is practiced as much as possible (Marier 1973). A combination of mechanical and high-yield sulfite processing, in which digestion occurs in sulfuric acid (Abitibi Price Inc. 1990), is now becoming one of the

most common pulping methods world-wide as it has reduced BOD loads associated with it (O'Connor et al. 1992).

2.4.1.3 Kraft. Kraft pulp mills utilize wood digestion in alkaline mixtures of sodium salts. Approximately 52% of the wood constituents are solubilized in this process producing a very strong fibre. Recovery and recirculation of the digestion mixture is usually performed. This pulping process results in smaller water and air pollution hazards than the bisulfite sulfite process described above (Marier 1973).

2.4.2 Methods of Assessing Aquatic Toxicity

Aquatic toxicity can be assessed using a number of standard acute toxicity tests. Three of the more common tests are performed on Rainbow Trout (*Onchorhynchus mykiss*) (McLeay and Sergy 1990), water fleas (*Daphnia magna*) (Sprague and McLeay 1990; Buikema et al. 1980), and luminescent bacteria (*Photobacterium phosphoreum*) (Alberta Environmental Centre 1990; Bulich 1984, 1986). The objective of acute toxicity tests is to determine the concentration of a test material that produces a deleterious effect, usually death, in the test organisms during a short-term exposure under controlled conditions (Parrish 1985). A 50% response is the most reproducible measure of toxicity for a test material. The median lethal concentration (LC50) is the concentration which will cause death to 50% of the test population. The median effective concentration (EC50) is the concentration that will cause some other species-selected effect, usually immobilization, or reduction in light output for luminescent bacteria.

2.4.3 Factors Modifying Aquatic Toxicity

2.4.3.1 Dissolved Oxygen. Pulp and paper mill effluents typically have high biological and chemical oxygen demands resulting in low dissolved oxygen water contents. Fish respiration rates increase in low oxygen waters to ensure adequate oxygen uptake. This increased rate of breathing causes more toxicant to pass over the gills and be absorbed into the body per unit time. As a result, high BOD discharges effectively make fish more susceptible to natural environmental pressures (Sprague 1985; Brouzes 1976). Fish may become stressed to the point where they succumb to ordinarily sublethal toxicant concentrations (Kruzynski 1979). High BOD levels complicate the measurement of acute toxicity of pulp mill effluents to fish (Walden and Howard 1977).

2.4.3.2 pH. The pH of an effluent is also a factor influencing toxicity. The ionic form of many chemicals is affected by solution pH. For example, at the pH of many receiving waters (pH 7.5 and lower), chlorine is primarily found in the more toxic, free form. Leach and Thakore (1976) reported that resin acid toxicity increases substantially with decreasing pH. At pH 6.4, a 5 mg L^{-1} solution of dehydroabietic acid is more toxic than a 10 mg L^{-1} solution at pH 7.5. This change in toxicity directly corresponds to the ionic form of the organic molecule. At low pH, acidic compounds will be unionized and therefore more lipid soluble and toxic. As the pH increases towards neutrality, these compounds become ionized. Water soluble ionized compounds are excreted readily and are therefore less toxic than unionized compounds (Haygreen and Bowyer 1989).

2.4.3.3 Suspended Solids. High levels of suspended solids may also pose a considerable risk to aquatic life. Suspended solids can cause serious damage to fish gills. As well, they can result in decreased light penetration into waters resulting in changes in primary production and the distribution of some aquatic organisms (Sprague 1985). Solids that settle to the bottom form benthic deposits causing modification of physical aquatic habitats. These deposits smother eggs on spawning grounds and other invertebrate bottom fauna, impeding oxygen diffusion (Karau 1975; Sprague 1985; Servizi et al. 1971). Biodegradation of these solids can also lead to anoxic conditions. The European Inland Fisheries Advisory Commission (EIFAC) (1969) recommends $\leq$25 mg L^{-1} suspended solids for optimal protection of fisheries. Although high concentrations of suspended solids can have negative impacts in aquatic environments, it is interesting to note that some suspended organic materials may actually have a detoxifying action against certain pollutants by removing them from the aquatic system via sorption or chelation (Sprague 1985).

2.4.4 Toxic Compounds

2.4.4.1 Chlorine. Many pulp and paper mills employ chlorine as a bleaching agent. Chlorine is very toxic to aquatic life. Its primary mode of action is irritation and damage of the external and, particularly, the respiratory epithelia (Dandy 1972; Servizi and Martens 1974). It can also irreversibly denature (inactivate) enzymes by reacting with enzyme sulphydryl groups. Recovery does not occur when fish are placed in clean water (Green and Stumpf 1946; EIFAC 1973). Chlorine in pulp mill effluent may be found in

the active "free" form as either the hypochlorite ion (OCl^-) or hypochlorous acid (HOCl), found as chloramines (N-Cl), or bound to lignin compounds, resin acids or phenolics (Brouzes 1976; Walden and Howard 1977). Merkens (1958), found that free chlorine is more toxic to Rainbow Trout than the chloramine, although the toxicities are in the same order of magnitude. Dandy (1972) reported that brook trout survived for 9, 8, 48 hours and greater than 7 days in test solutions containing 0.35, 0.08, 0.04 and 0.005 mg L^{-1} HOCl respectively. Chlorine, as well as the by-products of its use, in pulp and paper mill effluents had previously been linked to the induction of mixed function oxidase (MFO) enzymes in fish (Munkittrick et al. 1991). These enzymes belong to a family of iron-containing hemoproteins which oxidize organic substances, increasing their rate of excretion and decreasing their toxicity. Recent research showing induction after exposure to non-bleaching effluents suggests that effluent components other than chlorine cause the induction of MFOs in fish (Pesonen and Andersson 1992; Lindstrom-Seppa et al. 1992; Munkittrick et al. 1994; Friesen et al. 1994; Payne et al. 1987), therefore, naturally occurring compounds can not be ruled out as MFO inducers.

2.4.4.2 Natural Constituents. The majority of the toxicity in pulp and paper mill effluent can be attributed to natural constituents washed from wood (Thakore et al. 1989; O'Connor et al. 1992; Wong et al. 1978; Easty et al. 1978; Leach and Thakore 1976; Walden and Howard 1977). Resin acids account for the greatest toxic load in effluents derived from mechanical, sulfite and Kraft pulping processes. They contribute 90% of the toxicity of hydraulic debarker effluent generated from spruce, pine and fir (BC Research 1977) and 60 to 90% of acute toxicity of mechanical pulping effluents

generated from spruce and Lodgepole Pine (BC Research 1975). Leach and Thakore (1976) determined the acute toxicity of different resin acids to Rainbow Trout in 96 hour static bioassays. It was found that toxicity decreased from: isopimaric acid > palustric acid > abietic acid > pimaric acid > dehydropimaric acid. Pre-lethal exposures of fish to resin acids cause symptoms of respiratory distress including higher cough frequency and increased gill ventilation (Taylor et al. 1988).

The toxicity of effluents generated from various types of wood will depend on the chemical composition of the mix. Softwood effluents are generally more toxic than hardwood effluents as a direct result of wood chemistry; softwoods have more resin acid-derived toxicity (O'Connor et al. 1992; Wong et al. 1978). In comparison, toxicity in hardwood tree species effluents is derived mainly from phenols (Goudey and Taylor 1992), the growth hormone juvabione (Thakore et al. 1989), and fatty acids (O'Connor et al. 1992), with only minor contributions from resin acids. O'Connor et al. (1992) demonstrated the differences in toxicity of simulated mechanical pulping effluents derived from both softwood and hardwood tree species. Black Spruce effluents (LC50 40%) were at least two times more toxic to fathead minnows than aspen effluents (LC50 80%).

In natural situations, the impact of pulp and paper effluent discharge on water environments is highly complex and variable. Effects depend on the physical and chemical characteristics of the effluent, the composition of the receiving water, and the relative water flows affecting dilution and dispersal of the wastes (Walden and Howard

1977). Effects can be decreased substantially if treatment occurs prior to discharge, as the majority of toxic wood-derived compounds are biodegradable to varying degrees (Easty et al. 1978; Leach and Thakore 1976; Owens 1991). Various biological treatment processes can remove 66 to 100% of resin acids, 59 to 100% of bleach toxicants and 79 to 100% of BOD from Kraft effluents (Easty et al. 1978). Treatment of this type could theoretically render pulp and paper mill effluents non-toxic.

2.5 Toxicity Derived From Wood

Wood itself can be toxic to aquatic life. As was described in the previous section, most of the aquatic toxicity of pulp and paper mill effluents is derived from wood constituents. The question now is whether or not these compounds are made available exclusively by industrial processing, or if they may also be released by natural processes. Investigations prove that compounds toxic to aquatic organisms leach naturally out of wood stored in water and on land.

2.5.1 Seasoning

It is common practice for pulp mills to allow wood to season for a period of time prior to processing. Seasoning, also referred to as deresination (Nugent et al. 1977), is required to reduce deposition of resinous substances, called "pitch", on pulp machinery. Pitch can cause difficulties in machine operations, as well as contribute to the dirt level in the finished pulp (Douek and Allen 1978). Wood can be stored either in chip or roundwood (log) form. Seasoning, which occurs more rapidly from chips, alters the chemical composition of wood (Springer 1978; Nugent et al. 1977). The resin acid content of

wood actually increases post-harvest as surviving parenchyma cells produce a surge of acid immediately prior to death (Nugent et al. 1977). Other than the initial increase in resin acids, extractive compounds begin to degrade with seasoning. Fatty acids are particularly sensitive. They undergo enzymatic hydrolysis to form free fatty acids and are then consumed by oxidation to eventually form carbon dioxide and water (O'Connor et al. 1992; Nugent et al. 1977). As well, all extractive compounds are subject to microbial attack. These changes are almost completely stopped at sub-zero temperatures (Nugent et al. 1977). Although pitch reduction is beneficial to the pulping process, long seasoning periods are avoided as losses of wood fibre become problematic (Springer 1978).

Many of the compounds which are "lost" via the seasoning process are toxic to aquatic organisms. Although biodegradation does occur, it is possible that some of the compounds that are problematic in pulping processes may be removed intact from the wood and allowed to enter the environment.

2.5.2 Water and Wood

The use of water in log storage is a common practice. High wood moisture contents prevent wood from drying out, cracking and forming chinks, and hinder the spread of fungal infections (Sorge and Shulten 1994; Schaumburg 1973), ensuring wood quality and reducing wood wastage. Logs are either stored in water or on land. On land, logs are sprayed with water periodically, a process called land-decking. In either situation, there is a pollution potential caused by the leaching of organic substances from the wood.

2.5.2.1 Water Storage. The storage of wood in water poses a threat to aquatic environments. Large amounts of bark are usually deposited into water bodies during log storage. The problems associated with suspended solids generated during such storage practices have been investigated thoroughly (Servizi et al. 1971; Schaumburg 1973; Brouzes 1976; Thurlow and Associates 1977). Log storage may cause modifications to river banks and lake shorelines, possibly increasing sedimentation. Wood floatage causes the release of organic leachates into surrounding waters. The quantity of material released depends on the wood species, amount of bark, surface area of exposed logs and the circulation of water (Karau 1975). Schaumburg (1973) reported that the open ends of floating logs are responsible for a substantial portion of the soluble organics released. The actual quantity lost will be influenced by the degree of water movement around the logs. High water circulation rates will tend to promote leaching, while low circulation rates will cause concentration gradients to form, slowing the diffusion of substances from wood to water (Thurlow and Associates 1977). However, this lack of water movement may also affect leachate dilution, causing higher leachate concentrations to form in low flow areas than in high flow areas.

2.5.2.2 Land storage. The leaching of organic substances is just as likely to occur from land-decked wood as from wood floatage. Sorge and Shulten (1994) indicate that the groundwater below a land-decked spruce wood storage site in Germany was contaminated by inorganic and organic compounds leached from the wood. As well, leaching can occur in storage areas and wood waste piles where the only moisture added is from natural sources, either as precipitation or surface water flow. A study conducted

by Taylor (1994) shows that the pollution potential from remote wood storage piles can be significant. In a 23 month study, an aspen log pile generated significant quantities of toxic leachate when exposed to natural conditions of temperature and precipitation. In addition, only 5% of the supply of leachable material was removed from the wood. Taylor hypothesizes that leachate would be produced indefinitely as long as the logs are exposed to the weather.

2.5.2.3 Woodwaste. Woodwaste, or wood residue, is generated via wood processing, land clearing, timber harvesting and dry-land sorting. Wood residues are used for pulp and paper production and power generation in pulping (Appleby 1988), as well as in the production of "value-added" products such as building materials and fire logs (Liu et al. 1995). They are also used as soil amendments, landfill cover materials and as fill in construction. Leachate may be produced from woodwaste in the same manner that it is produced from land-decked wood. Water is added either via natural precipitation, or during fire fighting or fire prevention. Leachate production from woodwaste piles has recently become an issue in British Columbia. This has lead to the production of the manual entitled: Guidelines on storage, use and disposal of wood residues for the protection of fish and fish habitat in British Columbia (Liu et al. 1995).

2.5.3 Wood Leachate Characteristics

The chemical characteristics of wood leachate, formed in water or on land, are similar to those of pulp and paper mill effluents. In general, leachate constituents are the dissolved or suspended cold-water extractives of wood. Depending on the tree species, these may

include tannins, lignins, simple sugars, organic acids, short-chain alcohols, phenols, inorganic minerals, polysaccharides, resin and fatty acids, as well as the by-products of microbial decomposition (Liu et al. 1995; Taylor 1994). The parameters of concern for the protection of aquatic organisms include: pH, dissolved oxygen, biological and chemical oxygen demands (BOD and COD), total organic carbon (TOC), and toxicity. Wood leachates are generally acidic, with pH ranges of 3 to 6.5 (Peters et al. 1976; Haygreen and Bowyer 1989) and have low dissolved oxygen contents. The consequences of these factors on aquatic life have been discussed previously. The dark colour of most leachates can influence the distribution of some aquatic organisms. For example, it can decrease the ability to detect prey or, conversely, give the illusion of shelter (Sprague 1985). Most wood leachates have high dissolved organic material concentrations (Taylor 1994) which may be estimated by BOD, COD, or TOC. The BOD test estimates the oxygen requirement of microorganisms to oxidize the water-borne organic material in the leachate. COD estimates the chemical oxidation potential of the organic material. TOC is an actual measurement of the organic carbon content of the leachate. Due to their high organic content, wood leachates generally have high biological oxygen demands, and even higher chemical oxygen demands which cause low dissolved oxygen concentrations (Greenberg et al 1992; Liu et al. 1995; Taylor 1994).

2.5.4 Wood Leachate Toxicity

Wood leachate has been shown to be toxic to aquatic life. The toxicity of wood leachate is similar to that of mechanical pulping mill effluent, although a direct comparison cannot be made as the production of leachate involves cold-water rather than hot-water

extraction (Evans 1973). In general, the toxicity of leachate produced from softwood species is derived from resin acids, tannins and lignins, while the majority of hardwood toxicity can be attributed to phenolic substances (Goudey and Taylor 1992).

2.5.4.1 Softwood Leachate. Wood leachate generated from softwood species can be acutely toxic to aquatic organisms. 96 hour LC50s of 25 to 40 mg L^{-1}, 35 to 45 mg L^{-1}, 75 to 91 mg L^{-1} and greater than 50 mg L^{-1} were estimated by Pease (1974) for freeze-dried extracts of Sitka Spruce, Western Red Cedar, Western Hemlock and Yellow Cypress, respectively. In another experiment, Peters et al. (1976) produced acutely toxic leachate by submerging Western Red Cedar heartwood blocks into a continuous flow of dechlorinated tap water. The median survival time of Coho Salmon fry exposed to this leachate ranged from 18 to 25 hours. The exposure of carp to sublethal concentrations of Norway Spruce bark extract resulted in behavioural changes including delayed feeding, surface swimming and contact with the sides of the aquarium (Temmink et al. 1989). Levy et al. (1989) reported that juvenile Sockeye Salmon avoided a log dump site in a British Columbia lake. This behavioural change was attributed to the hypoxic conditions caused by leachate produced from the floating logs. The majority of the toxicity of softwood leachate can be attributed to the resin acid component. In addition, tannins in the bark of Western Hemlock and tropolones in Western Red Cedar heartwood are compounds of concern.

2.5.4.2 Hardwood Leachate. Wood leachate generated from hardwood species has also been found to contribute to aquatic toxicity. A study conducted by Goudey and Taylor

(1992) found that leachate generated from aspen wood was extremely toxic to aquatic life. Leachate used for toxicity testing was generated in the laboratory by immersing aspen wood chips into dechlorinated water. The LC50s for Rainbow Trout and *Daphnia* were determined to be 1 - 1.8% and 1.7 - 3.4% of undiluted leachate, respectively, while the EC50 for the Microtox test (luminescent bacteria) was found to be 0.2 - 0.3%. After determining the acute toxicity of lab-generated leachate, Taylor (1994) went on to study natural aspen leachate generation in remote field piles. This study confirmed the results of the laboratory study: aspen leachate is toxic to aquatic organisms. The production and toxicity of naturally generated leachate depends on such factors as the rate and timing of rainfall and snowmelt, penetration of the wood by precipitation, and possibly the effect of freezing and thawing. Although heavy rains remove more soluble material from wood in total, Taylor found that the greatest threat of acute toxicity from aspen woodpiles occurs in the spring and/or after moderate precipitation events. This is because heavier rains tend to produce a more dilute leachate. The major toxic component of aspen wood leachate is phenols, augmented by low pH and dissolved oxygen concentration.

2.6 Effects in Terrestrial Environments

2.6.1 Natural Attenuation

Discharge on land is a recognized disposal method for pulp mill effluents and wood leachate (Kannan et al. 1990; Liu et al. 1995). In addition, effluents and leachates are regarded as resources, providing water and nutrients to the soil. Through this disposal method, the toxicant load is naturally reduced as the solution permeates through the soil. Natural attenuation methods include sorption-desorption, abiotic and biotic degradation,

and volatilization (Johnson and Ryder 1988; Liu et al. 1995; Kookana and Rogers 1995). The effectiveness of natural attenuation will greatly depend on the properties of the soil, the soil microbial population and the effluent / leachate solution. Although this may be a cost-effective method of disposal, the potential environmental impacts have yet to be identified. It may be possible to overload the system, surpassing the natural attenuation capacity of the soil.

2.6.2 Chlorinated Constituents

The effects of wood constituents in terrestrial environments has not received adequate attention, although studies on the effect of chlorinated pulp mill effluents in soils have been conducted. The environmental concerns associated with land application of chlorinated compounds include: 1. contamination of water sources; 2. phytotoxicity; 3. introduction into the human food chain; and 4. toxicity to soil biota (Kookana and Rogers 1995). The negative impacts of chlorinated compounds in aquatic systems have been discussed previously. It has been observed that the yields of beets and potatoes irrigated with untreated pulp mill effluents are negatively affected (Sev and Papazov 1971), while irrigation with treated effluents results in no negative effect (Abasheyeve et al. 1993) or better than average yields (Narum et al. 1979). The long term effects of this type of irrigation are not known. Compounds such as pentachlorophenol (PCP) can bioaccumulate in earthworms (Haimi et al. 1992), and have caused inhibition of microbial growth (Ruckdeschel et al. 1987). Brezny et al. (1993) observed a 28% increase in the total number of bacteria, as well as a 40% decrease in Gram-positive bacteria, in a soil treated with chlorinated lignin compounds. Chlorolignins are not

considered a major toxic concern due to their high molecular weight (Kringstad and Lindstrom 1984), although studies have not been conducted on their smaller, abiotic and biological transformation products (Kookana and Rogers 1995).

2.6.3 Non-Chlorinated Constituents

The effects of non-chlorinated wood constituents in terrestrial environments have not been studied as thoroughly as the chlorinated compounds. In fact, data on the sorption and mobility of resin acids in soils, as well as their toxicity to soil fauna and flora, is scarcely available in the literature (Kookana and Rogers 1995). One study showed the effect of aspen leachate on lettuce seed germination and root elongation. The leachate had no effect on seed germination, while root elongation was reduced by 50% by a leachate concentration of 25% by volume (Goudey and Taylor 1992). The authors concluded that the toxicity of aspen leachate to plant life is weak compared to the toxicity observed to aquatic life.

2.6.4 Soil Microbial Processes

Evaluation of changes in soil microbial processes may provide the most useful information on the effects of pulp mill effluents and wood leachates on soils. In essence, soils act as "environmental filters or buffers" (Gregorich et al. 1994) by retaining and degrading chemical and biological materials. Soil microorganisms play an integral role in breaking down these materials, maintaining the quality of the soil.

2.6.4.1 Soil Quality. Soil quality is a difficult term to define as it encompasses a wide array of physical, chemical and biological properties. A number of definitions have been proposed in the literature, all dealing with the degree of fitness or health of a soil, and its ability to support life (Gregorich et al. 1994; Parr et al. 1992; Granatstein and Bezdicek 1992). One of the more comprehensive definitions of soil quality is "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental productivity, and promote plant and animal health" (Doran and Parkin 1994). The term "sustain" implies that a high quality soil will maintain its structure and function over time even while encountering external stress (Costanza 1992). One of the key attributes of a stable, high quality soil is an active, diverse soil microorganism community. The resiliency of the microbial community in the soil will affect the quality of a soil, as soil microorganisms are primarily responsible for soil biological functioning.

2.6.4.2 Soil Microorganisms as Indicators of Soil Quality. The use of indicators in biological systems is a common practice (Hellawell 1986). In general, an indicator is a species or property which reflects the existence of certain environmental conditions in a system. Changes in some component of the system, occurring as a result of physical, chemical and/or biological soil processes, should theoretically be observed in the indicator before being observed in the system itself. As microbial processes are an integral part of soil function within the ecosystem, soil microorganisms may be effective bio-indicators of soil quality. Soil microbes basically serve as sensitive "early warning indicators" for changes in soil systems; prompt and accurate responses to

perturbations in the soil system may be detected in microbial populations and processes long before they are noticed in physical and chemical soil properties (Turco et al. 1994). In general, soil microbial processes provide much of the resiliency or capacity to ameliorate stress in the soil system (Karlen et al. 1992). Unfortunately, many of the processes performed by soil microorganisms are only detected in their absence. This means that a disturbance will have occurred prior to its detection.

A number of soil microbiological criteria may be used as indicators of changes in soil quality. According to Visser and Parkinson (1992) these criteria may be evaluated at three different levels: population, community and soil ecosystem.

2.6.4.3 Population Level Studies. Population level studies, which usually require isolation and culturing of soil microorganisms, generally do not provide effective information for the assessment of soil quality (Visser and Parkinson 1992). Although relatively simple to perform, there are a number of problems associated with these studies. As only organisms in the soil solution are extracted and subsequently isolated and up to 95 to 99 percent of bacteria found in soils do not culture well (Killham 1994), the potentially "important" microorganisms may not be identified. Conversely, as the culture environment is less variable and more selective than the soil environment, culturable soil microorganisms may show more activity in culture than they would in the soil environment. It is important to understand that abundance does not necessarily equate with importance in soil system functioning.

2.6.4.4 Community Level Studies. Community level studies will be more useful in assessing soil quality than population-based studies, as soil functioning processes are more evident. Changes in composition and/or activity of microbial communities might have immediate and lasting effects, either positive or negative, on ecosystem functioning. Traditional methods for evaluating soil microbial communities generally focus on taxonomic diversity. There are a number of limitations associated with these methods, the greatest of which is that it is difficult, if not impossible, to assess the total microbial species complement of a soil (Garland and Mills 1994; Parkinson and Coleman 1991). As well, there is a general lack of information relating taxonomic diversity to soil function; some rare species may actually have a greater role in soil function than more prevalent ones (Zak et al. 1994). An assessment of the functional, rather than taxonomic, diversity of a soil may provide more useful information because changes in functional diversity of a soil microbial community may have lasting effects on ecosystem function (Perry et al. 1989). Garland and Mills (1991) proposed the use of microtitre plates containing a wide range of substrates to assess functional differences between soil microbial communities. The assessment is based upon the sole-source carbon utilization patterns of the bacterial community and does not attempt to characterize the numbers or identities of the species present. Functional changes observed at the microbial level could effectively forecast changes in the general quality of soils at an ecosystem level (Zak et al. 1994).

2.6.4.5 Ecosystem Level Studies. The study of soil microorganisms at the ecosystem level is linked closely to community level studies in that the processes in soil functioning are studied. As in community level studies, the "interactions (of the) team, rather than the performances of individuals" are measured (Tate 1995). The focus of ecosystem level studies is to select and evaluate sensitive processes which have maximum functional relevance and can be useful in rapid screening procedures (Klein et al. 1986).

In the soil environment, the effects of physical, chemical and/or biological perturbation may be assessed through monitoring changes in various soil processes; altered processes within the system may lead to cycle imbalances, indicating changes in soil quality. Two fundamental processes are the mineralization of soil carbon and nitrogen (Visser and Parkinson 1992). The ability of the soil to support biological life can be assessed through the measurement of C mineralization as CO_2 production, or respiration (Killham 1994; Tate 1994; Nadelhoffer 1990). Nitrogen mineralization, the conversion of organic N to a plant available inorganic form (NH_4 and NO_3), is of primary importance to the regulation of forest productivity (Zak et al. 1993; Nadelhoffer 1990). This process is sensitive to variations in the soil environment (Visser and Parkinson 1992; Klein et al. 1986). The addition of pulp mill effluent or wood leachate to soils could potentially cause changes in the soil C and N pools. Analysis of these changes may provide information on the balance between soil nutrient turnover and energy input, which are indicative of soil quality.

2.7 Conclusion

Wood contains compounds that are toxic to aquatic life. These compounds, including resin acids, fatty acids and phenols, may be released from wood into the environment via pulp and paper practices and/or the natural processes of leaching. The protection of aquatic organisms from deleterious compounds released via either method is federally legislated. Guidelines for land disposal of effluents and leachates have been recently published in British Columbia. Unfortunately, the environmental effects emphasized in these guidelines are related to the aquatic ecosystem; the effects of wood-derived compounds in terrestrial environments have yet to be investigated thoroughly.

A number of forestry policy objectives have been adopted in the province of Manitoba. The main objective is "...to ensure and promote forest activities that are environmentally sound and to maintain the environmental integrity of the forest ecosystem" (Canada-Manitoba Partnership Agreement in Forestry 1993). The effects of pulp and paper mill effluents and wood leachates to both aquatic and terrestrial ecosystems must receive attention to ensure environmental integrity. Toxicity of these solutions to aquatic organisms has been documented. Toxicity to vegetation, soil fauna and soil microorganisms must now be assessed. If required, management plans for the possible containment and disposal of wood leachates should be prepared and implemented.

3. THE TOXICITY OF SOFTWOOD LEACHATE IN AQUATIC ENVIRONMENTS

3.1 Abstract

Concerns over the potential toxicity of leachate derived from softwood logs in remote storage areas lead Manitoba's Clean Environment Commission to request an investigation into the toxicity of softwood leachate in aquatic environments. The objective of this study was to determine if leachates from the softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) are toxic to aquatic organisms. Leachate was generated by soaking either Jack Pine or Black Spruce logs in dechlorinated City of Winnipeg tap water at a ratio of 2.5:1 water : wood w/w for periods of 2 to 60 days. Aquatic toxicity was assessed using 96-hour, semi-static tests with Rainbow Trout (*Onchorhynchus mykiss*), 48-hour multi-concentration and 24-hour single-concentration tests with *Daphnia magna*, and 15-minute Microtox tests. Toxicity was observed at all three trophic levels and generally increased with increased log soaking duration. Jack Pine and Black Spruce leachates were also generated in a field study. Logs were held in weather-exposed crib structures. After significant precipitation events, the leachates were sampled and tested for toxicity using *Daphnia* and Microtox tests. Toxicity was observed only in leachates generated from relatively small rain events. Larger events tended to produce more dilute solutions.

3.2 Introduction

Remote land storage of logs has recently been shown to be a source of toxic compounds in hardwood storage yards in British Columbia (Goudey and Taylor 1992). Some storage yards are located near sensitive aquatic ecosystems such that drainage from these yards reaches the water. As softwood log yards may be located in similarly susceptible drainage areas, it is important to investigate whether leachate from softwood logs is toxic. If so, ecosystem management plans for the placement of forest log yards and log piles may be required to alleviate possible environmental risk.

Much research has been conducted on the effects of pulp and paper mill effluents in aquatic environments (Easty et al. 1978, Thakore et al. 1989, O'Connor et al. 1992; Robinson et al. 1994). Toxic compounds from different types of mills have been identified; some of these are added during processing, but many are derived from the wood itself. Wood composition is usually differentiated into major cell wall components and extraneous components. Cellulose, hemicelluloses and lignin, the main constituents of the cell wall, provide structural support. With the exception of a small part of the lignin, these compounds are insoluble in solvents and water (Karau 1975; Sjostrom 1993). In view of their low solubilities, these components should not contribute a great deal to the toxicity of effluents. In contrast, the extraneous components, or extractives, are non-structural wood constituents, many of which are soluble in neutral organic solvents and water. Wood extractives include resin and fatty acids, phenols and certain neutral compounds such as juvabione, and dehydrojuvabione. The suite of extractives present will vary with wood species, parts of the tree and tree age. The toxicity of mechanical pulping effluent is due

mainly to these naturally occurring extractives which leach from wood during the pulping process (O'Connor et al. 1992).

The potential for toxic materials to leach from wood storage piles into aquatic environments has, until recently, received little attention. Historically, the right to float wood in lakes and rivers, and to stockpile logs in forests and mill compounds has been looked upon as an economic necessity and, therefore, acceptable. Research indicates an association between these practices and possible environmental hazard. Schaumburg (1973) observed that the soluble organic matter leached from Douglas Fir, Ponderosa Pine and Hemlock logs into water, if in high concentration, exerted both biological and chemical oxygen demands and had deleterious effects, including acute toxicity, on fish and other aquatic life. Goudey and Taylor (1992) found that leachate from aspen logs was similarly toxic in aquatic environments. These results suggest that the storage of logs in the forestry industry may contribute a significant pollution load to the environment as a result of the leaching of organic compounds from wood and bark.

The first objective of this study was to determine whether or not leachates generated by soaking Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) logs are toxic to aquatic organisms. Toxicity was assessed using Rainbow Trout (*Onchorhynchus mykiss*), water fleas (*Daphnia magna*) and luminescent bacteria (*Photobacterium phosphoreum*). The second objective was to assess how the toxicity of the leachates, if present, changed with duration of log soaking. It was hypothesised that leachate derived from soaking softwood logs would exhibit toxicity to aquatic organisms as a result of the solubilization of extractive compounds and that this toxicity was dependent upon duration of soaking.

3.3 Materials and Methods

3.3.1 Leachate Generation

Softwood leachate was produced using Jack Pine and Black Spruce logs obtained from the Pine Falls Paper Company in Pine Falls, Manitoba, in June, 1995. Logs were cut into 0.30 m pieces and immersed in 60 L of dechlorinated City of Winnipeg tap water contained in fiberglass tanks (0.6 m x 0.6 m x 0.375 m). A total of two log soaking "trials" were conducted. each consisting of 5 soaking periods of 2, 7, 14, 30 and 60 days. Wood obtained on October 5, 1996 from the Pine Falls Paper company was used in Trial 2. On average, the Jack Pine logs used for each experiment had a diameter of 13.7 cm and a total weight of 24.4 kg. The water : wood ratio w/w for Jack Pine was 2.46. The Black Spruce logs had an average diameter of 11.8 cm. An average of 24.7 kg Black Spruce wood was soaked in each period resulting in a water : wood ratio of 2.43. Logs were mixed every second day to aid the diffusion of compounds from wood to water. A total of 7 L of water was added to the 60-day soak in Trial 2 to counter the effects of passive evaporation on leachate concentration. After the appropriate soaking period, log pieces were removed from the tanks and the remaining leachate solution was sampled.

3.3.2 Physical, Chemical and Biological Characteristics

Preliminary measurements of pH, dissolved oxygen and conductivity were taken for both leachates prior to dilution of samples for toxicity testing. Samples were sent to the Environmental Sciences Centre (ESC), Winnipeg, after each soaking period for physical and chemical characterization. The following analyses were performed. Total coliform concentration was estimated using the most probable number (MPN) multiple tube

fermentation procedure. The results of the examination of the replicate tubes and dilutions were reported as the MPN of organisms present per 100 mL (ESC Principle of Method A152.01). Biochemical oxygen demand (BOD) was determined by comparing the dissolved oxygen content of a sample at the beginning and the end of a 5-day incubation period (ESC Principle of Method A006.01). Chemical oxygen demand (COD) was determined by oxidizing all organic compounds in the sample with potassium dichromate and sulphuric acid followed by titration with ferrous ammonium sulphate (ESC Principle of Method A007.01). Total solids were determined by weighing a portion of the sample after drying to constant weight at 105°C. The residue retained after passing a measured volume of the sample through a Whatman 934-AH glass microfibre filter (1.5 μm) constituted the total suspended solids, while the dissolved solids were assessed by passing the sample through Whatman GF/C glass microfibre filter (1.2 μm) and drying to constant weight (ESC Principle of Method A009.02). Total carbon (TC) was assessed by injecting the sample into a combustion tube where the carbon in the sample was oxidized to carbon dioxide. Carbon dioxide was then detected by a non-dispersive infrared gas analyzer (NDIR). Total inorganic carbon (IC) was determined by injecting the sample into a reactor vessel containing 25% phosphoric acid (H_3PO_4), followed by CO_2 detection by NDIR. Total organic carbon was calculated as the total carbon content less the inorganic carbon content (ESC Principle of Method A609.03). Total Kjeldahl nitrogen was assessed via digestion of the sample with a sulphuric acid solution containing perchloric acid and selenium dioxide catalysts, followed by phenate colourimetry (ESC Principle of Method A217.02). Total phosphorous was determined by sample digestion with a sulphuric acid-persulfate mixture followed by reduction with

stannous chloride in an aqueous sulphuric acid medium and colourimetric analysis (ESC Principle of Method A208.02). True colour was measured by visual comparison of centrifuged samples against chloroplatinate standards (ESC Principle of Method A001.01). Duplicate samples of leachate were submitted for analysis for each soaking period. Detailed descriptions for the previous methods can be found in Appendix II.

3.3.3 Rainbow Trout Toxicity Tests

Rainbow trout (*Onchorhynchus mykiss*) of the D. Sundalsora strain were obtained as swim-up fry (4 months, average weight 0.17 g) from the Rockwood Aquaculture Research Centre, Gunton, Manitoba. The fish were held in large aerated tanks with a flow-through supply of City of Winnipeg dechlorinated tap water maintained at 10°C. The fish were fed Trout Chow #1 three times per week. The daily light cycle was 12 hours of light and 12 hours of dark. No disease was apparent throughout the holding period. Mortalities in the holding tanks never exceeded 2% prior to the commencement of an experiment.

Ten separate 96-hour Rainbow Trout toxicity tests were performed throughout the experiment, one for each log soaking period. Trial 1 consisted of the first set of log soaks (2 to 60 days). Trial 2 consisted of the second set of log soaks (2 to 60 days) (Table 3.1). Duplicate 6-L fish tanks were prepared for each of 6 concentrations of leachate by volume: 0 (control), 6.25, 12.5, 25, 37.5 and 50%. Each tank was placed into a controlled environment chamber maintained at 10°C with a 12-hour photoperiod. A total of ten fish were added to each tank. All fish were unfed, i.e. feeding had not occurred in

the 24 hours prior to testing. A semi-static experimental design was employed; 50% of the tank volume was replaced after 48 hours. Aeration was required. Fish were checked for abnormal behaviour and mortality after 1, 4, 12, 24, 48, 72 and 96 hours. Abnormal behaviour included increased respiratory or "coughing" rates, erratic swimming behaviour, surfacing, discolouration and loss of equilibrium. Fish were considered dead when they failed to show opercular or other activity and did not respond to gentle prodding (McLeay and Sergy 1990). Temperature, pH, dissolved oxygen and conductivity of each tank was monitored and recorded at the same times. After 96 hours, the remaining fish were anaesthetized in tricaine methanesulphonate (MS 222). The fork length (mm) and weight (g) of all fish was measured. Percent mortality and lethal concentration (LC50) values were calculated for each leachate type at each soaking time using probit analysis. The LC50 value is the concentration which is lethal to 50% of the population over a specified period of time. The lower the LC50 value, the greater the toxicity of the sample. One requirement for this calculation is that there must be at least two concentrations with partial mortalities, i.e. concentrations yielding 0 or 100% mortalities cannot be used. In cases where probit analysis could not be used, the LC50 was calculated by averaging the highest concentration with 0% mortality and the lowest concentration with 100% mortality (Gelber et al. 1985).

Table 3.1 Commencement dates for log soaking periods and Rainbow Trout toxicity tests.

	Soaking Period	Soak Period Start Date	Rainbow Trout Toxicity Test Start Date
Trial 1	2 d	August 14, 1995	August 16, 1995
	7 d	August 14, 1995	August 21, 1995
	14 d	August 14, 1995	August 28, 1995
	30 d	August 19, 1995	September 18, 1995
	60 d	August 24, 1995	October 23, 1995
Trial 2	2 d	October 28, 1995	October 30, 1995
	7 d	October 30, 1995	November 6, 1995
	14 d	November 6, 1995	November 20, 1995
	30 d	October 28, 1995	November 27, 1995
	60 d	October 13, 1995	December 11, 1995

3.3.4 *Daphnia* Toxicity Tests

All *Daphnia* toxicity tests were conducted by the ESC, Winnipeg using method D112.01. Two different test methods were employed: 1. A multi-concentration, 48-hour, static acute toxicity test for leachate samples; and 2. A single concentration, 24-hour, static acute toxicity test for control water. The shorter test was selected for the control because of cost factors and because toxicity was not expected at 100% concentration. *Daphnia* neonates less than 24 hours old were used for all tests. Duplicate leachate solutions with concentrations of 100, 50 and 1% leachate by volume were used for the 48-hour test. The 24-hour test did not require dilution. Ten organisms were placed into 200 mL of the appropriate solution using a disposable plastic pipette. All samples were kept in a controlled environment chamber maintained at 20°C ± 2°C with a controlled photoperiod of 16 hours light, 8 hours dark. Test organisms were observed for immobilization or mortality at 2, 24 and 48 hours (2 and 24 hours only for the 24-hour test). Death was

indicated by the lack of movement of the body, appendages and heart, as observed through a dissecting microscope. Results of the 48-hour test were reported as LC50s (probit calculation). Results of the 24-hour test were reported as pass or fail. A test resulting in 50% mortality or greater was designated a fail.

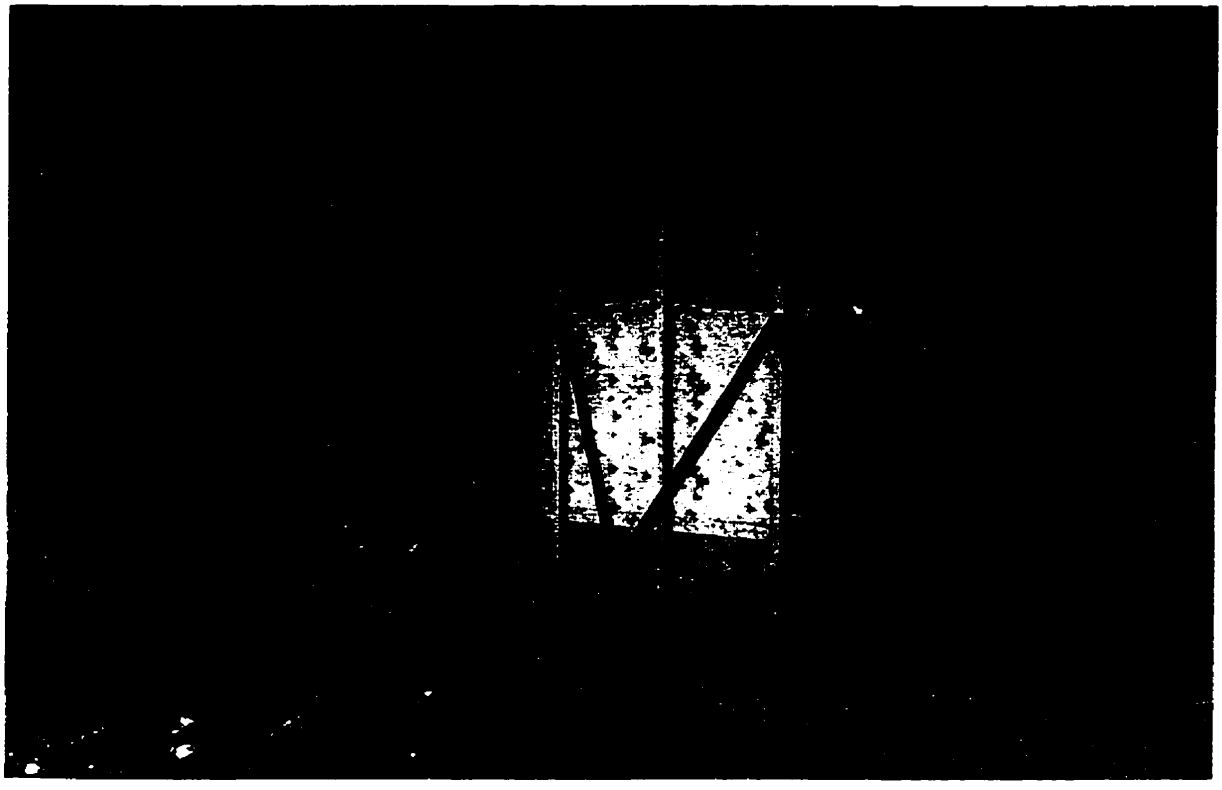
3.3.5 Microtox Toxicity Test

The Microtox toxicity tests were performed by Norwest Laboratories, Edmonton. The standard operating procedures used were adapted from Alberta Environmental Centre (1990) and Western Canada Microtox Users Committee (1991). This test is based upon the monitoring of changes in the light emission of a population of luminescent bacteria (*Photobacterium phosphoreum*) when exposed to a toxic substance or a sample containing toxic materials. The test is performed by rehydrating freeze dried Microtox reagent, which contains 10^8 bacteria per vial, and determining the initial light output of the stabilized bacterial suspension. Aliquots of the sample are then added to the bacterial suspensions and light measurements are made at 5 and 15 minute intervals. A dilution control blank is used to correct time-dependent changes in light output. The test end-point is measured as the effective concentration of a test sample which reduces light emission by 50% after 15 minutes at 15°C (EC50 15 MIN). The lower the EC50 value, the higher the toxicity of the sample.

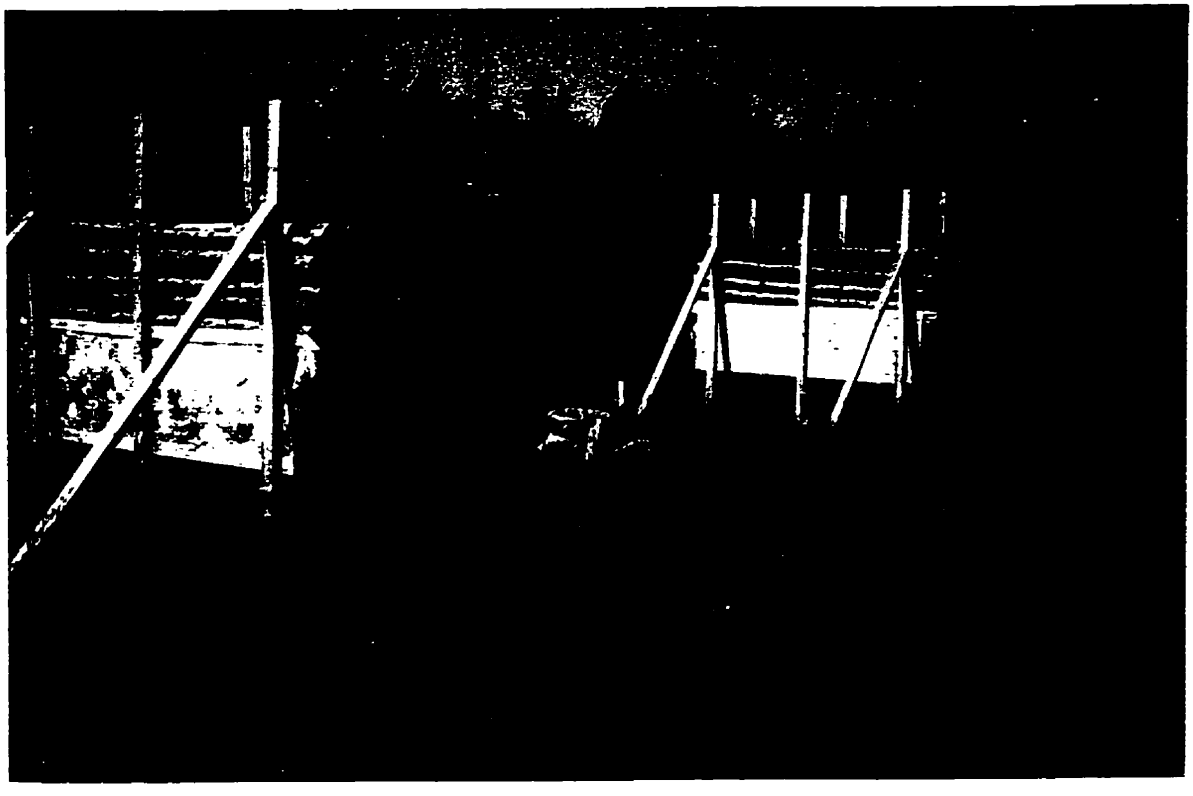
3.3.6 Field Study

Two log cribs were constructed and placed at the University of Manitoba Glenlea Research Station. One crib was filled with fresh, 2.40 m Jack Pine logs, while the other

was filled with fresh, 2.40 m Black Spruce logs (Appendices Ia and Ib). Figures 3.1 and 3.2 depict the design of the structures. The actual crib dimensions were 2.40 m long x 1.20 m high x 0.60 m wide. The sides of the cribs were constructed with plywood. The inside of the crib was lined with polyethylene plastic. Each crib was placed on a shop table 0.75m high. One end of each table was jacked up approximately 5 cm to facilitate drainage of leachate into a polyethylene-lined container on the ground at the opposite end. A polyethylene-lined container was also placed between the two cribs to catch rainwater to be used as a control. There were no obstructions to exposure to the weather as the nearest trees were 17 m away. The collection containers were checked for leachate after every rainfall. If sufficient leachate volumes were produced, samples were taken for *Daphnia* toxicity testing at a commercial laboratory. This experiment ran from July 1995 to October 1995. In June 1996, the logs were replaced (Appendices Ic and Id) and the sides of the cribs were lowered to allow greater exposure to precipitation. Leachate collection continued until the end of August 1996. A total of 6 samples in 1995 and 5 samples in 1996 were taken. Microtox assays were performed on three samples from the 1996 season for comparison to *Daphnia* results. Rainbow trout toxicity tests were not conducted due to volume limitations.



3.1 Structure of log crib located at Glenlea Research Station.



3.2 Black Spruce (left) and Jack Pine (right) log cribs, and control water collection container (centre) at Glenlea Research Station.

3.3.7 Statistical Analyses

Changes in each physical and chemical characteristic, as well as toxicity to trout, *Daphnia* and Microtox, over time and soaking trial were assessed using 2 factor ANOVAs for each treatment type. Mean comparisons between the two leachate treatments and the control for each of these parameters were performed using 3 factor ANOVAs with treatment, soaking time and trial number as factors. In all statistical analyses, duplicate leachate samples were treated as replicates; true replicate samples were not possible due to logistical limitations. Homogeneity of variance was assessed using Bartlett's test. Mean separation was performed by applying Fisher's Least Significant Difference (LSD) test ($p = 0.05$). Correlations of the results from the Rainbow Trout, *Daphnia magna* and Microtox toxicity tests were performed. All statistical tests were performed using CoStat 5.0 (CoHort Software, Minneapolis, MN).

3.4 Results and Discussion

3.4.1 Softwood Leachate Characteristics

Physical and chemical parameters of the leachate samples were measured prior to the commencement of toxicity testing. The pH of both wood leachates decreased with increased log soaking duration (Table 3.2). Black Spruce leachate generated in Trial 1 was generally more acidic than that generated in Trial 2. The opposite was true for Jack Pine. The increase in acidity over time in the leachates was probably related to continued release of organic acids from the logs (Goudey and Taylor 1992), although this was not confirmed. Black Spruce leachate was significantly more acidic than Jack Pine leachate,

while both leachate types were more acidic than the control water (Table 3.3). The pH ranges of the undilute leachate samples may themselves prove toxic to aquatic life. The lethality of water with pH in the range of 4 to 5 to fish has been documented in various laboratory studies (Baker and Schofield 1982; EPA 1971). Beggs and Gunn (1986) observed the failure of recruitment in lake trout populations in a lake acidified to pH <5.5. At pH <5.0, lake trout were lost from the lake altogether. It is important to note that fish will avoid acidic waters if possible (Wells 1915; Gunn and Noakes 1986) but have the ability to rebound from short exposures to acidic waters (EPA 1971). Acidic softwood leachate could therefore have varying effects on aquatic life depending on the characteristics of the receiving waters.

Table 3.2 Leachate physical, chemical and biological characteristics over five log soaking periods (2 to 60 days).

Treatment	Soaking Time	pH	Dissolved Oxygen	Conductivity	Solids Total	Solids Dissolved	Solids Suspended	Kjeldahl N	Total Coliform
	days		mg L^{-1}	µmhos cm^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg N L^{-1}	MPN 100 mL^{-1}
(A) Black Spruce	2	6.23 a	3.63 b	167 d	295 d	280 d	14.0 b	0.83 d	87000 b
	7	5.70 b	2.53 d	179 d	400 c	375 c	27.5 b	1.26 d	92500 b
	14	4.83 c	3.23 c	218 c	670 b	655 b	16.5 b	1.89 c	140000 a
	30	4.75 d	3.93 a	290 b	615 b	595b	23.5 b	2.38 b	77000 b
	60	3.65 e	2.45 d	425 a	865 a	820 a	43.5 a	3.81 a	9757 c
	Trial 1	3.19 b	4.79 a	276 a	n/a	n/a	n/a	n/a	116000 a
	Trial 2	4.98 a	1.51b	236 b	n/a	n/a	n/a	n/a	46500 b
(B) Jack Pine	2	6.33 a	2.63 ab	182 e	300 d	265 d	33.0 b	1.26 d	136667 a
	7	5.40 b	2.30 b	219 d	475 b	450 b	22.0 c	2.08 c	150000 a
	14	4.93 d	3.18 ab	254 c	475 b	455 b	24.0 bc	2.60 b	150000 a
	30	5.03 d	2.50 b	288 b	380 c	370 c	15.0 c	2.46 b	81575 b
	60	5.15 c	0.78 c	369 a	810 a	765 a	47.0 a	5.42 a	77000 b
	Trial 1	5.39 a	3.02 a	283 a	n/a	n/a	n/a	n/a	146000 a
	Trial 2	4.92 b	1.53 b	241 b	n/a	n/a	n/a	n/a	93400 b
(C) Control	2	7.50 b	8.38 b	169 b	120 a	120 a	<5.00 ns	0.34 b	74.0 ns
	7	7.93 a	8.83 b	175 ab	130 a	125 a	<5.00 ns	0.48 a	111 ns
	14	7.93 a	8.50 b	165 b	110 a	110 a	<5.00 ns	0.36 b	1.75 ns
	30	7.85 a	9.50 a	183 a	76 b	76 b	<5.00 ns	0.34 b	0.00 ns
	60	7.85 a	9.78a	183 a	115 a	115 a	<5.00 ns	0.36 b	0.00 ns
	Trial 1	7.78 ns	8.84 ns	175 ns	n/a	n/a	n/a	n/a	71.7 ns
	Trial 2	7.74 ns	9.15 ns	175 ns	n/a	n/a	n/a	n/a	3.44 ns

A, B and C show results from individual 2 factor ANOVAs for each treatment with soaking duration and trial number as factors. Data represent the average of 2 replicates from two soaking trials, except for the solids and Kjeldahl nitrogen data, which are averages of the 2 replicates from the second soaking trial only. Means in the same column followed by the same letter are not significantly different (Least significant difference test, P=0.05).

Table 3.2 (cont.) Leachate physical, chemical and biological characteristics over five log soaking periods (2 to 60 days).

Treatment	Soaking Time	Total P	Biological O_2 Demand	Chemical O_2 Demand	Carbon		
					Total	Organic	Inorganic
	days	mg P L^{-1}	mg L^{-1}	mg L^{-1}	mg C L^{-1}	mg C L^{-1}	mg C L^{-1}
(A) Black Spruce	2	0.74 d	115 e	378 d	134 e	108 e	26.6 a
	7	1.00 c	238 d	573 c	236 d	218 d	18.3 b
	14	2.47 b	383 c	1010 b	333 c	315 c	17.8 b
	30	2.64 b	453 b	945 b	444 b	435 b	9.2 c
	60	4.61 a	643 a	2000 a	705 a	693 a	11.4 c
	Trial 1	2.68 a	336 b	1152 a	386 a	366 a	19.3 a
	Trial 2	1.90 b	473 a	809 b	355 b	341 b	14.0 b
(B) Jack Pine	2	0.76 d	118 e	365 e	106 d	84.3 d	17.5 ns
	7	2.59 c	300 d	680 d	252 c	232 c	19.8 ns
	14	3.93 b	345 c	985 b	289 bc	265 c	23.8 ns
	30	3.65 b	410 b	878 c	328 bc	310 b	17.9 ns
	60	6.26 a	553 a	1550 a	525 a	506 a	19.3 ns
	Trial 1	4.00 a	289 b	955 a	275 b	248 b	27.4 a
	Trial 2	2.87 b	401 a	828 b	325 a	311 a	11.9 b
(C) Control	2	0.01 ns	<5.00 ns	15.0 bc	47.3 ns	27.4 ns	19.9 ns
	7	0.01 ns	<5.00 ns	10.0 c	24.5 ns	8.03 ns	16.5 ns
	14	0.02 ns	<5.00 ns	21.8 ab	21.5 ns	5.43 ns	16.7 ns
	30	0.02 ns	<5.00 ns	16.0 bc	25.1 ns	6.63 ns	18.5 ns
	60	0.22 ns	<5.00 ns	25.8 a	25.6 ns	7.60 ns	19.0 ns
	Trial 1	0.02 ns	<5.00 ns	22.5 a	33.9 ns	16.0 ns	17.9 ns
	Trial 2	0.02 ns	<5.00 ns	12.9 b	25.3 ns	6.90 ns	18.2 ns

Table 3.3a Comparison of physical, chemical and biological characteristics between log soaking trials, log soaking duration and treatment type.

		pH	Dissolved Oxygen	Conductivity	Total Coliform	Total P	Biological O_2 Demand	Chemical O_2 Demand	Total Carbon	Organic Carbon	Inorganic Carbon
			mg L^{-1}	µmhos cm^{-1}	MPN 100 mL^{-1}	mg P L^{-1}	mg L^{-1}	mg L^{-1}	mg C L^{-1}	mg C L^{-1}	mg C L^{-1}
Trial Number	1	3.67 b	5.55 a	245 a	88200 a	2.31 a	210 b	710 a	238 ns	217 ns	21.6 a
	2	5.20 a	4.06 b	217 b	53800 b	1.60 b	280 a	550 b	242 ns	220 ns	14.7 b
Log Soaking Time	2 days	5.88 a	4.88 b	173 e	75800 a	0.55 d	77.0 e	253 e	96.0 e	73.0 e	21.3 a
	7 days	5.12 c	4.55 c	191 d	80900 a	1.20 c	181 d	421 d	171 d	152 d	18.2 bc
	14 days	3.28 e	4.97 b	212 c	96700 a	2.14 b	244 c	671 b	232 c	195 c	19.4 ab
	30 days	5.23 b	5.31 a	254 b	70900 a	2.10 b	289 b	613 c	466 b	250 b	15.2 d
	60 days	4.90 d	4.33 c	325 a	28900 b	3.63 a	400 a	1190 a	454 a	438 a	16.3 cd
Treatment	Black Spruce	3.49 c	3.15 b	256 b	92100 b	2.29 b	397 a	981 a	370 a	354 a	16.6 b
	Jack Pine	5.09 b	2.28 c	262 a	118000 a	3.44 a	345 b	892 b	300 b	279 b	19.6 a
	Control	7.76 a	9.00 a	175 c	39.4 c	0.02 c	5.00 c	17.7 c	29.6 c	11.2 c	18.1 ab
Main Effects	**df**										
Trial	1	***	***	***	***	***	***	***	ns	ns	***
Soaking Time	4	***	***	***	***	***	***	***	***	***	**
Treatment (Trt.)	2	***	***	***	***	***	***	***	***	***	*
Interaction											
Trial x Time	4	***	**	**	ns	***	***	***	***	***	**
Trial x Trt.	2	***	***	***	*	***	***	***	***	***	***
Time x Trt.	8	***	***	***	**	***	***	***	***	***	***
Trial x Time x Trt.	8	***	***	***	**	***	***	***	***	***	ns

* Results from 3 factor ANOVAs for each parameter with trial number, soaking duration and treatment as factors. Means in the same column followed by the same letter are not significantly different (Least significant difference test, P = 0.05).

Table 3.3b Comparison of solids and Kjeldahl nitrogen over time and between the treatments in Trial 2 only.

		Solids			Kjeldahl
		Total	Dissolved	Suspended	N
		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg N L^{-1}
Log Soaking Time	2 days	238 c	222 d	17.3 b	0.808 e
	7 days	433 b	317 c	18.2 b	1.43 d
	14 days	418 b	407 b	15.2 b	1.62 c
	30 days	357 bc	347 c	14.5 b	2.00 b
	60 days	597 a	567 a	31.8 a	3.19 a
Treatment	Black Spruce	569 a	545 a	25.0 a	2.03 b
	Jack Pine	488 a	461 b	28.2 a	2.76 a
	Control	169 b	109 c	5.00 b	0.364 c
Main Effects	df				
Time	4	***	***	***	***
Trt.	2	***	***	***	***
Interaction					
Time x Trt.	8	***	***	***	***

* Results from 2 factor ANOVAs for each parameter with soaking duration and treatment as factors. Means in the same column followed by the same letter are not significantly different (Least significant difference test, P = 0.05).

Total and organic carbon increased significantly with time in both leachate treatments (Table 3.2). Differences in the inorganic carbon content over time were observed in the Black Spruce leachate only. The percentage of the total carbon which was inorganic was extremely small. On average, Black Spruce leachate had 7.3% and Jack Pine leachate had 8.3% inorganic carbon. Each soaking trial yielded similar results. The total and organic carbon contents of the Black Spruce leachate were significantly higher than in the Jack Pine leachate. The carbon contents of each leachate were significantly greater than the control (Table 3.3), indicating that the carbon was derived from the logs. The

general effects of increased carbon content in the leachates over time may be observed in other leachate parameters. For example, the biological oxygen demand (BOD) of each leachate increased significantly with increased log soaking duration (Table 3.2). Greenberg et al. (1992) suggest that BOD values increase with concentration of organic compounds; oxygen is required for the degradation of these compounds. In this study, it was determined that total and organic carbon content were significantly correlated to BOD in both leachates (Table 3.4). As with the carbon values, BOD values for Black Spruce were significantly greater than for Jack Pine. The biological oxygen demand of the control samples was insignificant compared to those of the leachates (Table 3.3).

Table 3.4. Correlations between carbon content and biological oxygen demand.

	Parameter	**Correlation Coefficient (r)**	**P<0.05**
Black Spruce	BOD vs. Total C	0.77	1.81e-4 ***
	BOD vs. Organic C	0.78	1.54e-4 ***
Jack Pine	BOD vs. Total C	0.88	2.98e-7 ***
	BOD vs. Organic C	0.88	2.20e-7 ***

Trends in the chemical oxygen demand of the leachates and the control water were similar to those of the BOD (Table 3.2). The only exception was observed in the Jack Pine 30 day soak sample, where there was a slight decrease in COD. COD values are usually higher than BOD. This is because both the biodegradable and non-biodegradable organic matter fractions are oxidized in the assessment of COD while only the biodegradable fraction is analyzed in the BOD analysis. The COD values were expected to be approximately 2 times the value of BOD, as this is most often the case found with pulp and paper mill effluents (Wong et al. 1979). In most instances, the COD of each

leachate was at least 2 times greater than BOD. The oxygen demands in each of the leachates, as described by the BOD and COD, could cause the formation of anoxic conditions in stagnant, slow-moving, or ice-covered waters which can be detrimental to aquatic life.

As a result of the high oxygen demands of the leachates, dissolved oxygen (DO) levels were significantly reduced in each leachate compared to the control water. However, statistical analysis revealed that the Jack Pine leachate had a lower oxygen concentration than the Black Spruce leachate even though Jack Pine had both lower carbon contents and oxygen demands than Black Spruce (Table 3.3). No pattern of decrease in the dissolved oxygen concentration over time was evident in either leachate; the levels were low throughout each soaking period and each trial (Table 3.2). Although fish mortalities due to deficient oxygen occur at levels below approximately 2 mg L^{-1} for most fish in laboratory settings (Sprague 1985), aquatic tests usually require a minimum oxygen concentration of 60% of saturation, corresponding to approximately 6 mg L^{-1} at 10°C (McLeay and Sergy 1990). Jones (1964) reported that the critical dissolved oxygen level for Rainbow Trout in natural conditions is 6 to 7 mg L^{-1} at 5 to 10°C. Below this level, fish activity is restricted. Therefore, waters containing high concentrations of either type of wood leachate would probably not support aquatic life for extended periods unless the dissolved oxygen content was increased by sufficient dilution.

Increases in the electrical conductivity of each leachate with increased log soaking time indicate that ionic compounds were continuously released from the logs into the water

(Mackereth et al. 1978). The leachate conductivities were significantly greater than that of the control water. The identities of the ionic compounds present in the leachate samples were not determined. It is therefore not known whether these compounds could pose a threat to aquatic organisms themselves. It is important to note that the mobility and potential toxicity of ionic compounds can be altered with changes in pH (Sprague 1985).

The amount of solids (total, dissolved and suspended) also increased over time in each leachate treatment (Table 3.2). (The data for the solids content of the leachates are based upon the second soaking trial only because a full data set was not obtained from ESC for Trial 1.) Total solids increased significantly with increased log soaking duration for both types of leachate. Statistically significant differences were not detected between the two leachates, but both were greater than the control (Table 3.3), indicating that material was leaching from the wood. The majority of the solids were in the dissolved phase (95% of the total). Rainbow trout can be acclimated to waters with up to 20 000 mg L^{-1} dissolved solids (Brouzes 1976). As the highest concentrations produced in the Black Spruce and Jack Pine leachates were 820 and 765 mg L^{-1}, respectively, the total concentrations of dissolved solids in the leachates would probably not cause an increase in the toxicity to aquatic organisms unless one or more of the dissolved compounds was itself toxic. The suspended solids, which are low relative to the dissolved fraction, may actually pose more of a risk to aquatic life. The problems associated with the suspended solids generated during log storage in water have been investigated thoroughly (Servizi et al. 1971; Schaumburg 1973; Brouzes 1976; Thurlow and Associates 1977). Suspended

solids can cause serious damage to fish gills. As well, they cause prevent the penetration of light into waters resulting in changes in the distribution of some aquatic organisms (Sprague 1985). Biodegradation of these solids can also lead to anoxic conditions. European Inland Fisheries Advisory Commission (EIFAC) (1969) recommends $\leq$25 mg L^{-1} suspended solids for optimal protection. As the suspended solids were 43.5 mg L^{-1} in the Black Spruce leachate and 47 mg L^{-1} in the Jack Pine leachate after 60 days of log soaking, a potential problem could exist in low flow waters.

Nutrients were released from the logs to the water with time; Kjeldahl nitrogen and total phosphorous levels increased with increased soaking time for both leachates (Table 3.2). Jack Pine leachate had significantly higher levels of both nutrients than the Black Spruce leachate. The control had negligible levels of each nutrient in comparison (Table 3.3).

The concentration of coliform bacteria in each leachate decreased with increased log soaking duration. The coliform concentration in the control water samples, which did not vary over time, was insignificant compared to the leachate samples. The reduction in total coliform bacteria with increased log soaking time could be a result of increased toxicity of the leachates to bacteria. This could be due to either a greater number, or an increased concentration of toxic compound(s) being released from the wood over time. Alternatively, as total coliform counts are typically used as indicators of water pollution, the results may actually indicate an increase in water quality. The results do not permit discrimination between these two opposing conclusions. One other possible explanation for the reduced coliform concentrations could be nutrient limitation. As both N and P

levels in the leachates increased with time it is unlikely that these were limiting factors. True colour in a water sample is caused by colloidal organic compounds (Greenberg et al. 1992). The colour of each leachate exceeded the detection limits of the analysis (>50 Colour Units), indicating a relatively high concentration of organic compounds in solution. In comparison, the control samples were all <5 Colour Units. Both leachates appeared to be the colour of strong, yellowish tea. A typical tea has a true colour of 100 Colour Units (Greenberg et al. 1992). A concentrated softwood leachate, if released into small waterways with slow mixing rates, could possibly affect aquatic life by decreasing the amount of light penetration through the water (Sprague 1985).

3.4.2 Rainbow Trout Toxicity

Concentration effects within each soaking experiment were evident in Rainbow Trout exposed to both Black Spruce and Jack Pine leachate (Figures 3.3 - 3.12). The test tanks containing the 50% Black Spruce and Jack Pine leachate treatments were initially more acidic and had lower dissolved oxygen contents than the remaining, less concentrated tanks. Table 3.5 shows the initial pH and dissolved oxygen levels of the 50% leachate test tanks (the greatest concentration used in the study) and the control tanks immediately prior to fish exposure. Both the dissolved oxygen concentration and the pH increased after the commencement of aeration of the test tanks. The initial ranges of dissolved oxygen and pH in these samples probably did not cause direct toxic effects to Rainbow Trout in of themselves. A possible exception may have occurred in the 60-day log soaks, in which the pH values of the leachates were significantly less than 6.0. However, low dissolved oxygen and pH levels could influence leachate toxicity to fish in

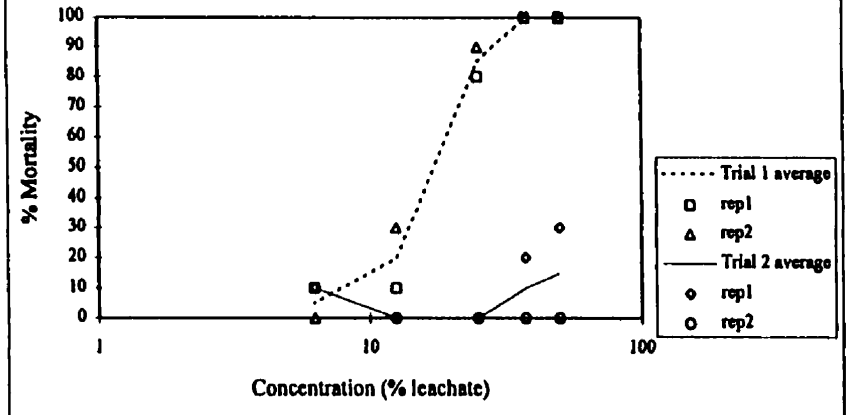


Figure 3.3 Percent mortality of rainbow trout exposed to varying concentrations of Black Spruce leachate generated by soaking logs for 2 days.

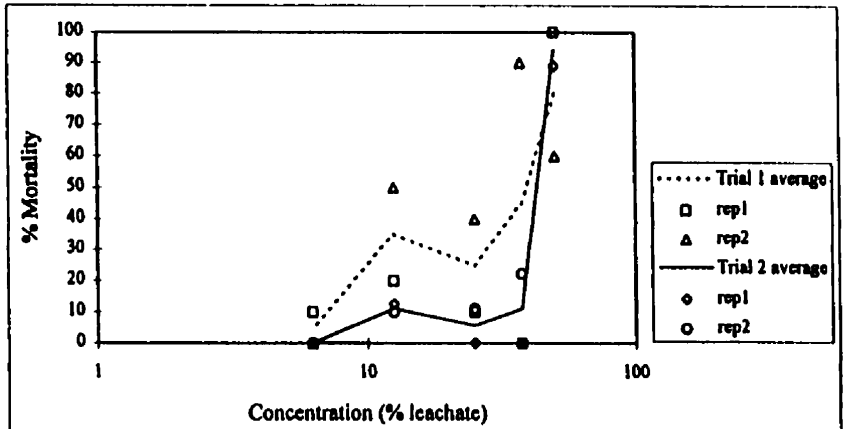


Figure 3.4 Percent mortality of rainbow trout exposed to varying concentrations of Jack Pine leachate generated by soaking logs for 2 days.

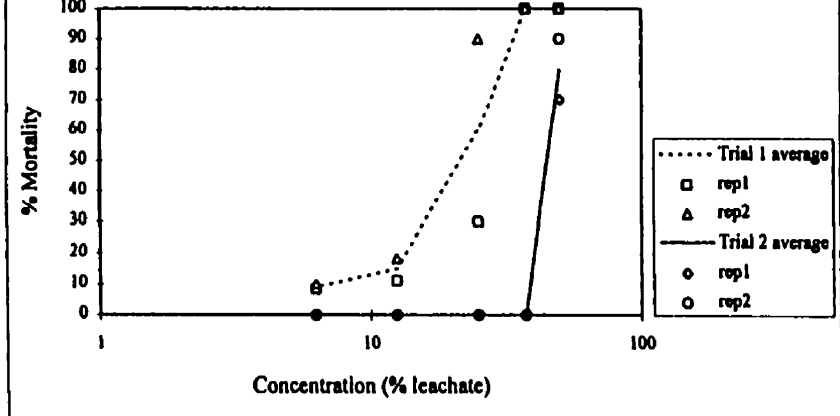


Figure 3.5 Percent mortality of rainbow trout exposed to varying concentrations of Black Spruce leachate generated by soaking logs for 7 days.

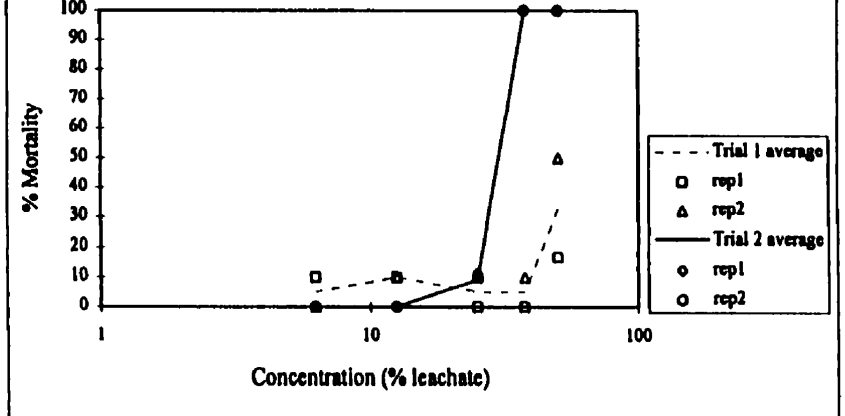


Figure 3.6 Percent mortality of rainbow trout exposed to varying concentrations of Jack Pine leachate generated by soaking logs for 7 days.

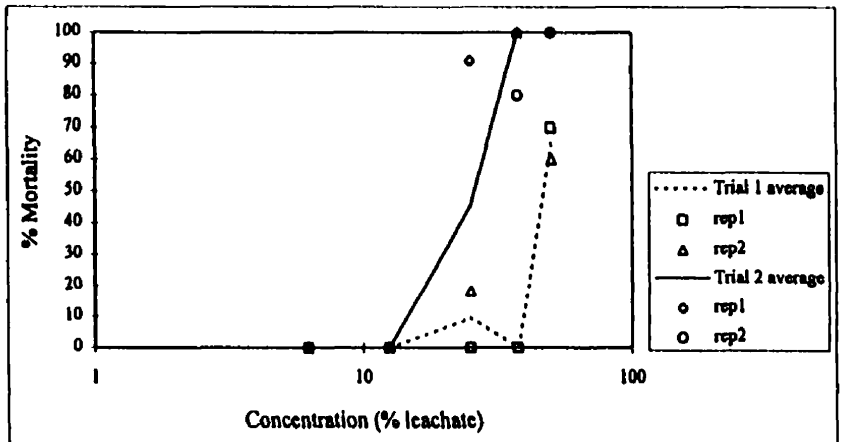


Figure 3.7 Percent mortality of rainbow trout exposed to varying concentrations of Black Spruce leachate generated by soaking logs for 14 days.

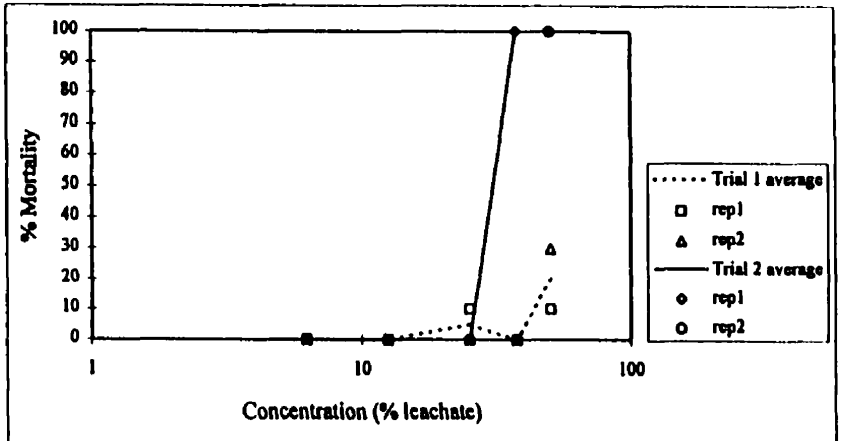


Figure 3.8 Percent mortality of rainbow trout exposed to varying concentrations of Jack Pine leachate generated by soaking logs for 14 days.

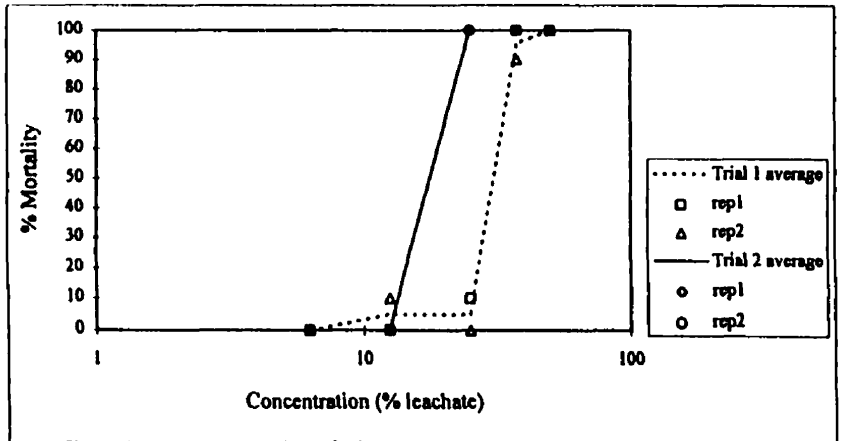


Figure 3.9 Percent mortality of rainbow trout exposed to varying concentrations of Black Spruce leachate generated by soaking logs for 30 days.

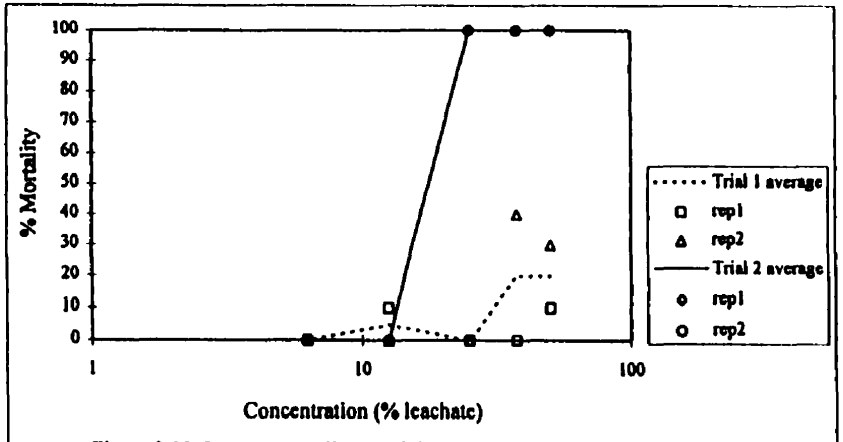


Figure 3.10 Percent mortality of rainbow trout exposed to varying concentrations of Jack Pine leachate generated by soaking logs for 30 days.

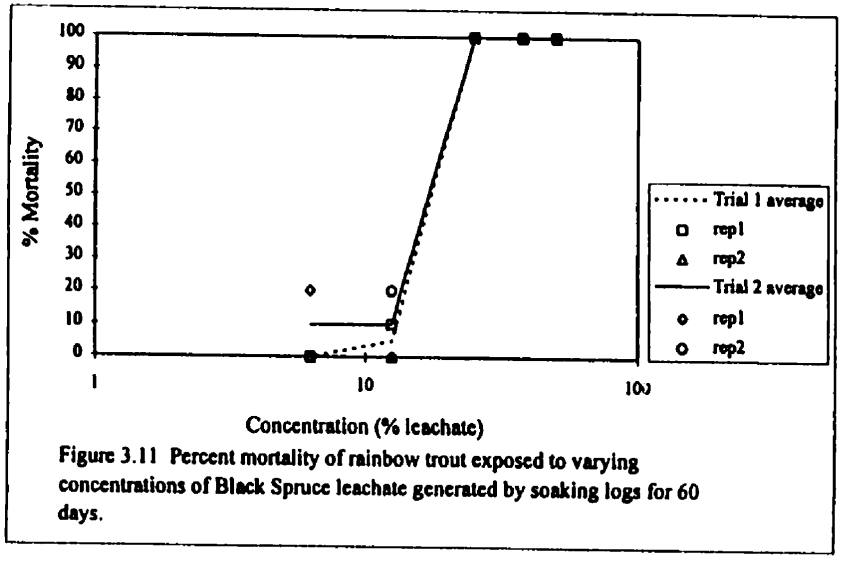


Figure 3.11 Percent mortality of rainbow trout exposed to varying concentrations of Black Spruce leachate generated by soaking logs for 60 days.

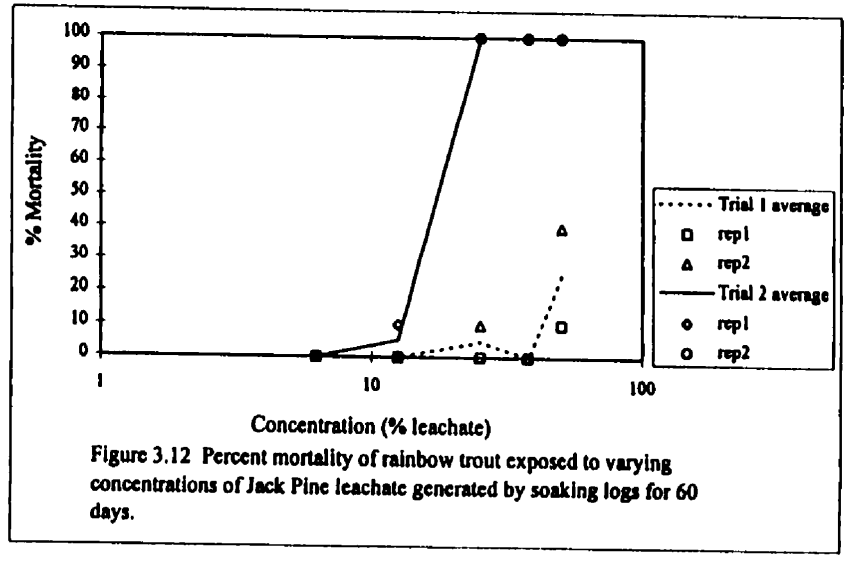


Figure 3.12 Percent mortality of rainbow trout exposed to varying concentrations of Jack Pine leachate generated by soaking logs for 60 days.

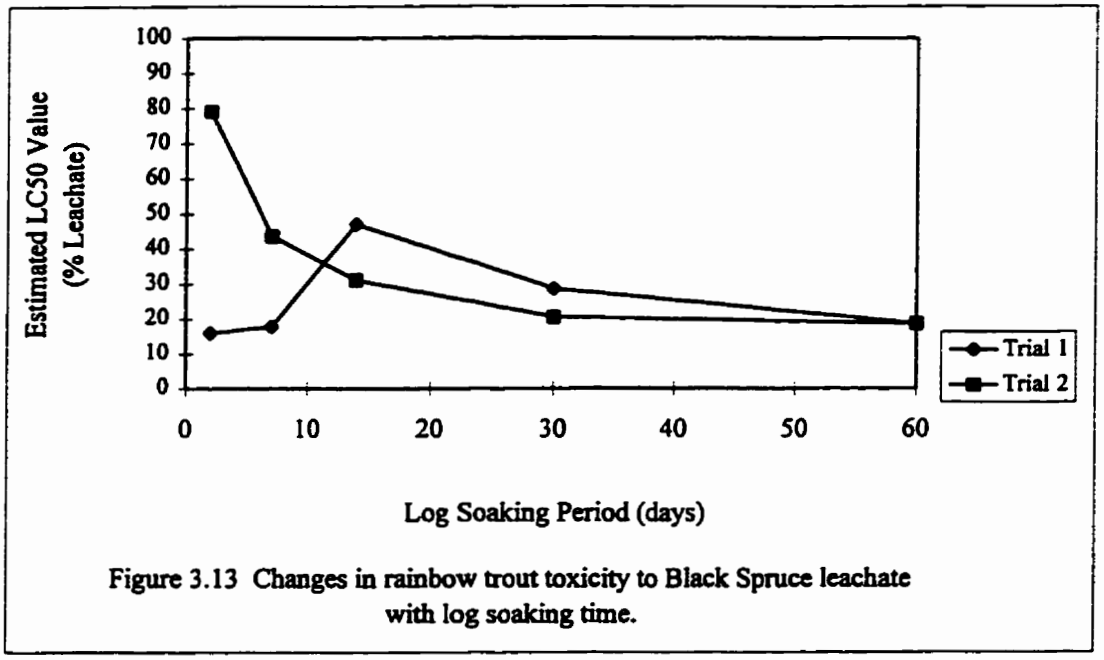


Figure 3.13 Changes in rainbow trout toxicity to Black Spruce leachate with log soaking time.

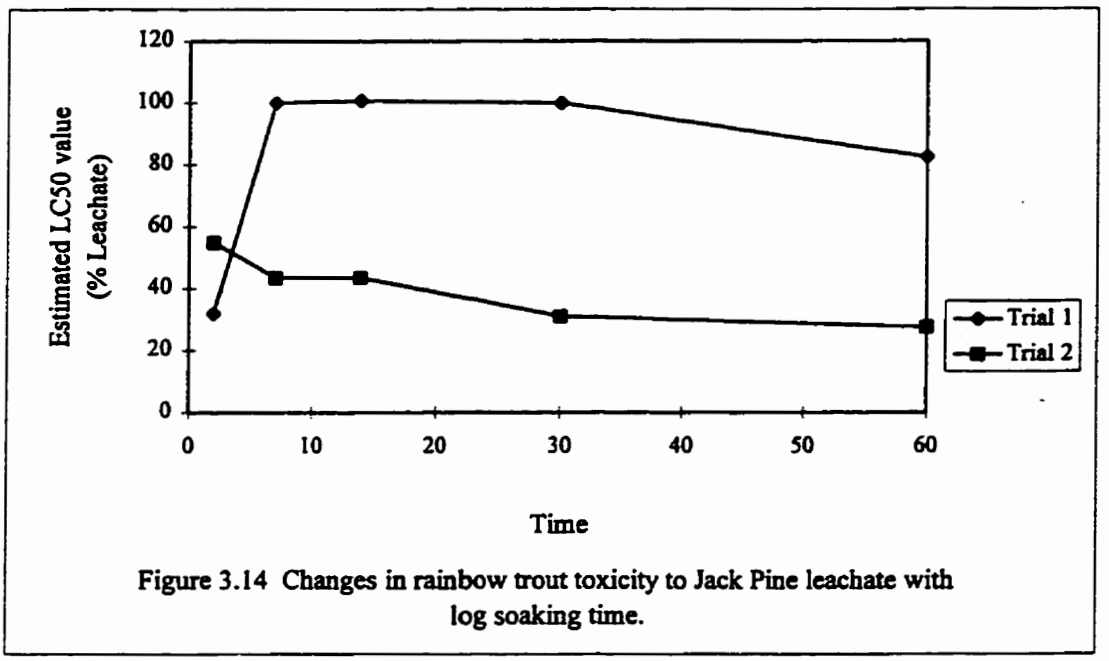


Figure 3.14 Changes in rainbow trout toxicity to Jack Pine leachate with log soaking time.

an indirect manner. Low dissolved oxygen levels may induce increased fish respiration resulting in greater exposure of fish to dissolved toxicants as water passes over the gills more frequently. As mentioned previously, pH can change the ionic form of many chemicals and this can affect toxicity. Leach and Thakore (1976) reported that resin acid toxicity increased substantially with decreasing pH: at pH 6.4, a 5 mg L^{-1} solution of dehydroabietic acid was more toxic than a 10 mg L^{-1} solution at pH 7.5. In most cases, toxicity decreases as the pH increases towards neutrality (Haygreen and Bowyer 1989).

Table 3.5 Initial dissolved oxygen and pH values of test tanks containing a 50% leachate concentration.

	Black Spruce				**Jack Pine**				**Control**			
	Trial 1		**Trial 2**		**Trial 1**		**Trial 2**		**Trial 1**		**Trial 2**	
Soak Period	DO (mg L^{-1})	pH	DO (mg L^{-1})	pH	DO (mg L^{-1})	pH	DO (mg L^{-1})	pH	DO (mg L^{-1})	pH	DO (mg L^{-1})	pH
2d	8.84	nm	8.00	7.20	7.10	nm	6.40	7.10	11.18	nm	11.7	7.95
7d	9.75	nm	7.38	6.55	8.65	nm	5.70	6.10	11.55	nm	11.7	7.95
14d	7.28	nm	7.60	6.30	6.80	nm	8.35	7.10	11.55	nm	12.0	8.20
30d	7.75	5.40	8.3	5.8	6.90	6.10	8.20	5.90	11.8	8.1	12.0	8.15
60d	7.10	4.70	7.30	4.70	7.25	6.40	6.90	4.50	12.2	7.75	11.6	7.85

†All values are the means of duplicate test tanks. No pH measurements were taken for the 2, 7 and 14 day soaks in trial 1.

Toxicity of wood leachate to Rainbow Trout was assessed by the percentage of mortalities in each tank. In general, the toxicity of Black Spruce leachate increased with increased log soaking time. This was expected as it allows time for a greater number and/or a greater concentration of natural compounds to diffuse from the wood into the water. While no significant differences were detected between the first three soaking periods, the percentage of mortalities increased significantly after the 30 day soak and again after the 60 day soak (Table 3.6). In contrast, significant differences in percent

mortalities were not detected between the log soaking times in the Jack Pine leachate. Black Spruce leachate generated in the first soaking trial was significantly more toxic than that of the second trial. The opposite occurred in the Jack Pine leachate. Diffusion of compounds should occur more readily from wood with a higher moisture content as it would take less time for the saturation point to be reached. The logs used in the second soaking trial were more fresh than those from the first trial. It was therefore expected that the second trial would yield more concentrated leachate solutions. This seems to have occurred in the Jack Pine only. Although analyses were not performed on the logs themselves, it is probable that variations in the chemical composition of the logs existed and acted as confounding factors. In general, Black Spruce leachate was found to be more toxic than the Jack Pine leachate (Table 3.7). A graphical representation of the changes in the LC50 values at each soaking time reemphasizes this information (Figures 3.13 and 3.14). A decrease in the LC50 value corresponds to a greater toxic effect.

Table 3.6 Rainbow trout mortality when exposed to softwood leachate of varying concentration.

Factor	Black Spruce Mean Mortality (%)	Jack Pine Mean Mortality (%)
Concentration		
50%	87 a	63 a
37.5%	75 b	50 b
25%	48 c	26 c
12.5%	6.5 d	7.0 d
6.25%	3.5 d	6.0 d
Soaking Time		
2 day	36 c	30 ns
7 day	37 c	28 ns
14 day	36 c	24 ns
30 day	49 b	31 ns
60 day	63 a	36 ns
Soaking Trial		
1	49 a	14 b
2	38 b	45 a

Values represent the mean of two replicates from two soaking trials. Means in the same column followed by the same letter are not significantly different. (Least significant difference test, P=0.05).

Table 3.7 Rainbow trout mortality mean comparisons.

Factor	Mean Mortality (%)
Soaking Time	
2 day	29.5 c
7 day	26.0 c
14 day	29.8 c
30 day	38.8 b
60 day	49.3 a
Soaking Trial	
1	32.2 a
2	37.2 a
Treatment	
Black Spruce	44.2 a
Jack Pine	25.2 b
Main Effects	**P <0.05**
Time	***
Trial	ns
Treatment	***
Interaction	
Time x Trial	***
Time x Treatment	ns
Trial x Treatment	***
Time x Trial x Treatment	ns

Values represent the mean of two replicates from two soaking trials. Means in the same column followed by the same letter are not significantly different. (Least significant difference test, P=0.05).

3.4.3 *Daphnia* Toxicity

Both types of softwood leachate were toxic to *Daphnia magna*. Toxicity was described using LC50 values; the lower the LC50, the greater the toxic effect. Toxicity increased with increased log soaking duration in each leachate (Table 3.8). Black Spruce leachate produced during Trial 1 was more toxic than the leachate produced during Trial 2, while no significant differences in toxicity to *Daphnia* were detected between the two soaking trials for the Jack Pine leachate. Black Spruce leachate was significantly more toxic to *Daphnia* than Jack Pine leachate (Table 3.9).

Table 3.8 Toxicity of softwood leachate to *Daphnia magna*.

Factor	Black Spruce LC50 (%)	Jack Pine LC50 (%)
Soaking Time		
2 day	76 a	89 a
7 day	73 a	95 a
14 day	56 b	54 b
30 day	34 c	49 b
60 day	27 c	45 b
Soaking Trial		
1	48 b	72 ns
2	58 a	62 ns

Values represent the mean of two replicates from two soaking trials. Means in the same column followed by the same letter are not significantly different. (Least significant difference test, P=0.05).

Table 3.9 *Daphnia magna* mortality when exposed to Black Spruce and Jack Pine leachate generated by soaking logs for various amounts of time.

Factor	Mean LC50 (%)
Soaking Time	
2 day	82.5 a
7 day	83.6 a
14 day	55.0 b
30 day	41.6 c
60 day	36.2 c
Soaking Trial	
1	59.8 a
2	59.7 a
Treatment	
Black Spruce	53.2 b
Jack Pine	66.4 a
Main Effects	
Time	***
Trial	ns
Treatment	***
Interaction	
Time x Trial	***
Time x Treatment	ns
Trial x Treatment	**
Time x Trial x Treatment	**

Values represent the mean of two replicates from two soaking trials. Means in the same column followed by the same letter are not significantly different. (Least significant difference test, P=0.05).

3.4.4 Microtox Toxicity

The Microtox assay was performed on the leachates generated in the second trial only. The pattern of change was similar in both leachates: a major increase in toxicity after the 2 day soak followed by a relatively stable toxicity level (Table 3.10). In both leachates, a slight decrease in toxicity was noted after 60 days of log soaking. These results suggest that it takes at least two days for the more toxic compounds to leach, or for the concentrations of these compounds to become great enough to produce toxic effects at the bacterial trophic level. It may also be possible that some of the toxic compounds become degraded somewhat after 60 days, although further testing is needed to determine this. As with the previous two assays, the Black Spruce leachate produced significantly greater toxic effects than the Jack Pine leachate ($p = 0.05$) (Table 3.11).

Table 3.10 Toxicity of softwood leachate to luminescent bacteria as assessed by the Microtox assay.

Factor	Black Spruce EC50 (%)	Jack Pine EC50 (%)
Soaking Time		
2 day	26 a	32 a
7 day	6.2 b	5.6 c
14 day	2.5 c	6.0 bc
30 day	2.8 c	5.1 c
60 day	5.4 b	7.2 b

Values represent the mean of two replicates from one soaking trial. Means in the same column followed by the same letter are not significantly different. (Least significant difference test, P=0.05).

Table 3.11 Toxicity of softwood leachate to luminescent bacteria (Microtox assay).

Factor	Mean EC50 (%)
Soaking Time	
2 day	28.6 a
7 day	5.88 b
14 day	4.23 c
30 day	3.93 c
60 day	6.25 b
Treatment	
Black Spruce	8.47 a
Jack Pine	11.1 b
Main Effects	
Time	***
Treatment	***
Interaction	
Time x Treatment	***

Values represent the mean of two replicates from the second soaking trial. Means in the same column followed by the same letter are not significantly different (Least significant difference test, P=0.05).

3.4.5 Toxicity Correlations

The results from the *Daphnia* and Rainbow Trout toxicity tests were highly correlated with each other for the Jack Pine and Black Spruce leachates generated in trial 2 only (Table 3.12). The correlation coefficients were -0.95 and -0.96 for Jack Pine and Black Spruce, respectively (CoStat 5.0). The correlations were negative due to the different methods of describing toxicity in Rainbow Trout and *Daphnia*: toxicity increased with increased percent mortality values in the trout, while toxicity increased with decreased LC50 value in *Daphnia*.

The results from the Microtox tests (Trial 2 only) were correlated with the results from the trout tests for both leachates. The correlation coefficients were -0.64 and -0.71 for

the Jack Pine and Black Spruce leachates, respectively. In contrast, the results from the *Daphnia* and Microtox tests were correlated for the Black Spruce leachate only (r = 0.82). High correlations between the results from fish toxicity tests and the Microtox test have been reported previously. Lebsack et al. (1981) calculated correlation coefficients of 0.82 and 0.97 for tests of fossil fuel process waters on Rainbow Trout and flathead minnows, respectively. These tests employed flow-through experimental designs. High correlations between the Microtox and *Daphnia* toxicity tests have also been reported. Vasseur et al. (1984) quantified the toxicity of 39 different industrial effluents using these two tests. The corresponding correlation coefficient obtained was 0.96. The lower correlations observed in trial 1 of this study may be related to the experimental design employed. As a semi-static toxicity test with aeration was used, the potential toxicity of the leachates could have been affected if toxic, volatile compounds were present in the leachates.

Table 3.12 Correlation of toxicity results from Rainbow Trout, *Daphnia magna* and Microtox toxicity tests for Jack Pine and Black Spruce leachate.

	Toxicity Tests	**Correlation Coefficient (r)**	**P<0.05**
Jack Pine			
Trial 1	Rainbow trout vs. *Daphnia*	-0.41	ns
Trial 2	Rainbow trout vs. *Daphnia*	-0.95	***
Trial 2	Rainbow trout vs. Microtox	-0.64	*
Trial 2	*Daphnia* vs. Microtox	0.62	ns
Black Spruce			
Trial 1	Rainbow trout vs. *Daphnia*	-0.37	*
Trial 2	Rainbow trout vs. *Daphnia*	-0.96	***
Trial 2	Rainbow trout vs. Microtox	-0.71	*
Trial 2	*Daphnia* vs. Microtox	0.82	**

†Correlations performed using two replicates from each soaking period. Trials were dealt with separately.

3.4.6 Field Leachate Experiment

The field generated leachates were not subject to physical and chemical characterization. The results of the toxicity tests performed at the Environmental Sciences Centre on the field-generated leachate produced during the 1995 season were inconclusive as the *Daphnia* did not survive in any treatment including the control rain water. Although the *Daphnia* cultures used in toxicity testing undergo monthly life cycle studies and regular interference toxicant bioassays to ensure health, the actual dates of these tests were not provided. It is therefore possible that the mortalities were caused by factors other than the field treatments. In view of this, the results from this season were not accepted. The 5 leachate samples generated during the 1996 season were sent to Norwest Labs for *Daphnia* bioassays. The health of the organisms was verified on June 25, 1996 through a bioassay with zinc sulphate as a reference toxicant. On three occasions, the leachate volume generated at the field site was sufficient to have both Microtox and *Daphnia* bioassays performed. *Daphnia* toxicity to Black Spruce and Jack Pine leachates was observed in the June 24 and July 10, 1996 leachate samples. Moderate toxicity was also observed in the August 20, 1996 Jack Pine leachate sample (Table 3.13). Toxicity was observed at varying levels in each sample analyzed by the Microtox assay. Black Spruce leachate ranged from extremely toxic on June 24, 1996 to moderately toxic on August 6, 1996, to slightly toxic on August 20, 1996. The Jack Pine leachate was very toxic on June 24, 1996 and slightly toxic on August 6, 1996. No effect was observed in the August 20, 1996 assay. The decrease in toxicity with time may be due to either temporal factors or to the volume of precipitation with which each leachate sample was generated. Taylor (1994) observed that heavier precipitation events tended to produce a more dilute,

and correspondingly less toxic, aspen leachate. The precipitation events which produced the toxic Jack Pine and Black Spruce leachate samples were the smallest leachate-producing events during the summer. If similar amounts of material were extracted from the wood during each precipitation event, the reduced water volume could be responsible for the more concentrated leachate. Analysis of the conductivities of the toxic leachates support this argument. The toxic leachates produced in June and July had significantly higher conductivities and lower pH values than the non-toxic samples, suggesting that more concentrated leachates were produced.

Table 3.13 Field generated leachate toxicity and chemical characteristics.

Treatment	Sampling Date 1996	Rain mm	Test	*Daphnia magna* % mortality or LC50	Microtox EC 50 (15min)	pH	DO mg L^{-1}	Conductivity mS cm^{-1}
Black Spruce	June 24	6.8	P/F	100% mortality Fail	12.5% Fail (extremely toxic)	4.6	5.02	0.355
	July 10	10.8	LC50	51% Toxic	n/a	5.1	2.44	0.310
	August 6	64	LC50	> 100% Non-lethal	63.9% Fail (mod. toxic)	7.2	2.33	0.079
	August 20	24.6	LC50	> 100% Non-lethal	85.4% Pass (slightly toxic)	6.4	2.13	0.103
	August 22	16.5	LC50	> 100% Non-lethal	n/a	6.7	2.50	0.107
Jack Pine	June 24	6.8	P/F	100% mortality Fail	30.7% Fail (very toxic)	4.3	7.37	0.250
	July 10	10.8	LC50	69% Toxic	n/a	4.6	4.77	0.207
	August 6	64	LC50	> 100% Non-lethal	83.4% Pass (slightly toxic)	6.0	3.75	0.056
	August 20	24.6	LC50	89.4% Mod. Toxic	>100% Pass (non-toxic)	6.1	2.30	0.084
	August 22	16.5	LC50	> 100% Non-lethal	n/a	6.6	1.83	0.082
Control	June 24	6.8	P/F	20 % Pass (slightly toxic)	>100% Pass (non-toxic)	6.5	8.93	0.031
	July 10	10.8	LC50	> 100% Non-lethal		7.5	7.2	0.04
	August 6	64	LC50	> 100% Non-lethal	>100% Pass (non-toxic)	7.8	8.15	0.017
	August 20	24.6	LC50	> 100% Non-lethal	>100% Pass (non-toxic)	8.4	9.03	0.021
	August 22	16.5	LC50	> 100% Non-lethal		8.1	7.1	0.031

3.5 Summary and Conclusions

Leachates derived from the softwood species Black Spruce and Jack Pine, when generated in the laboratory at a 2.5:1 w/w ratio, are toxic to Rainbow Trout, *Daphnia* and luminescent bacteria. Toxicity generally increased in all three trophic levels as the log soaking period was increased. Black Spruce leachate was more toxic than Jack Pine leachate in all toxicity tests. The results of the physical and chemical analyses of the water after the various log soaking periods verify that compounds were released from the logs to the water and that the concentration of these compounds increased with increased log soaking duration. The identities of these compounds were not determined in this study, but previous investigations have determined that water soluble compounds such as carbohydrates, phenols, organic acids, phosphate, potassium and metal ions, and lipophilic compounds such as resin and fatty acids, are released by softwood during water storage of timber (Borga et al. 1996). Wood extractives such as phenols and resin and fatty acids have toxic properties in aquatic environments (Leach and Thakore 1976; Borga et al. 1996). The acidity of the generated leachates may also produce toxic effects in aquatic organisms. As dilute leachate samples were used in this study, toxicity due to acidity was probably an issue for the 50% concentration of the 60 day leachates only. The extremely low dissolved oxygen content of the leachates was not a factor in this study because aeration was performed throughout the assays. In natural systems, the low oxygen concentrations could become a major issue depending on the characteristics of the receiving waters.

Results from the field study indicate that leachate generated from rain falling on logs is also toxic to *Daphnia* and luminescent bacteria. The LC50 and EC50 values obtained for the *Daphnia* and Microtox assays, respectively, although within similar concentration ranges, were slightly greater than those obtained with laboratory generated leachate. This was expected because of the longer exposure of the logs to water in the lab. As the field setup did not allow for any logs to be immersed in water for any period of time, a natural log pile could produce a more concentrated, and therefore more toxic leachate, particularly if log piles are located in water-collecting areas. It also follows that larger log piles could potentially generate greater volumes of toxic leachate. Concentrations can vary according to the type and amount of precipitation.

It is important to note that the toxicity of softwood leachate will depend on a number of factors. One such factor is the wood species from which the leachate is derived; tree species differ in the amounts and types of compounds in their composition. Another important factor is the age of harvested logs prior to exposure to precipitation; the composition of wood, including the extractives content, can change with time after harvest (O'Connor et al. 1992). It is therefore important to realize that leachates derived from different wood storage piles will vary in composition and toxicity. Knowing that leachate can have toxic effects to aquatic organisms and that the degree of toxicity can vary, all remote log storage piles should be placed such that drainage of leachate will not reach susceptible water bodies.

4. EFFECTS OF SOFTWOOD LEACHATE ON CARBON AND NITROGEN MINERALIZATION IN FOREST SOILS.

4.1 Abstract

Concerns over the potential toxicity of leachate derived from softwood logs in remote log storage areas lead Manitoba's Clean Environment Commission to request an investigation into the toxicity of softwood leachate in both aquatic and terrestrial environments. The objective of this study was to determine if leachate from the softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) had an effect on microbial respiration and nitrogen mineralization in two forest soils: 1. an Eluviated Dystric Brunisol (Ahe horizon); and 2. an Orthic Gray Luvisol (Ae horizon and LFH layer). Leachate was generated by soaking pine and spruce logs in water for 30 days. Soil samples were brought to field capacity with a single 25%, 5% or 1% by volume leachate treatment or with control water. Samples were incubated for up to 20 weeks. Weekly respiration rates were determined by quantifying the amount of CO_2 evolved. Nitrogen mineralization was determined regularly via soil extraction with KCl and analysis for NH_4 and NO_3 by spectrophotometric methods. The single addition of softwood leachate had little effect on microbial respiration in each soil horizon and sampling period. N mineralization was also unaffected in the leachate-treated samples of the Ahe Brunisol and Ae Luvisol. However, compared to the control, N mineralization was reduced in the LFH Luvisol samples treated with leachate. These results suggest that the single addition of relatively dilute softwood leachate on forest soils will have minimal impact on soil microbial processes.

4.2 Introduction

The production of wood leachates from log storage piles has become a recent concern. Trees are often harvested and stockpiled in remote locations for up to one year prior to being transported to the mill. During storage, there is a potential for wood leachate to be produced after precipitation events. This can occur when natural wood extractives, released from wounds in the logs immediately after harvesting, are washed off by rain water. Leachate may also be produced when wood extractives diffuse from water saturated wood fibres (Liu et al. 1995). Wood constituents will continue to leach provided water contact and wood fibre saturation are maintained. Taylor (1994) demonstrated the potential for remote hardwood storage piles to produce leachate when it was determined that an aspen log pile, exposed to natural weather conditions, generated significant quantities of leachate over a 23 month period. During this study, only 5% of the supply of leachable material was removed from the wood, resulting in indefinite leachate production. In a previous study, it was determined that aspen leachate is toxic to aquatic organisms (Goudey and Taylor 1992). As softwood trees are of importance to Manitoba's forest industry, these findings lead Manitoba's Clean Environment Commission to request an investigation into the effects of softwood leachate in the environment. The softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) are two important trees used in pulp and paper production in Manitoba (Abitibi-Price Inc. 1990). It was previously determined that leachates derived from these tree species exhibit toxicity to aquatic organisms (Chapter 3). The objective of this work was to determine whether or not leachate derived from Jack Pine and Black Spruce logs

have similar negative effects in the terrestrial environment. In particular, are naturally occurring soil processes impacted by the addition of softwood leachate?

In the soil environment, the effects of physical, chemical and/or biological perturbation may be assessed by monitoring changes in various soil processes. Two fundamental processes are the mineralization of soil carbon and nitrogen. The ability of the soil to support biological life can be assessed through the measurement of C mineralization as CO_2 production, or respiration (Nadelhoffer 1990). Nitrogen mineralization, the conversion of organic N to a plant available inorganic form (NH_4 and NO_3), is of primary importance to the regulation of forest productivity (Zak et al. 1993; Nadelhoffer 1990). This process is sensitive to variations in the soil environment (Visser and Parkinson 1992). Although the conversion of NH_4 to NO_3 is not prevalent in acidic forest soil environments, NO_3 production has been detected in incubated forest soils (Weber and Gainey 1962; Heilman 1974). The addition of softwood leachate to forest soils may cause changes in the soil C and N pools. Analysis of these changes may provide information on the balance between soil nutrient turnover and energy input, which are indicative of soil quality. The objective of this study was to determine if the processes of C and N mineralization in forest soils are affected by the addition of Jack Pine and Black Spruce leachates.

4.3 Materials and Methods

4.3.1 Leachate Production

Softwood leachate was produced using Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) logs obtained from the Pine Falls Paper Company in Pine Falls,

Manitoba on October 5, 1995. Logs were cut into 30 cm pieces on October 10 and October 12, 1995, covered with polyethylene plastic and stored in an unheated shed. Leachate production began on June 12, 1996 by immersing log pieces in dechlorinated City of Winnipeg tap water. Totals of 24.8 kg Jack Pine and 24.0 kg Black Spruce wood were soaked separately for 30 days in 60 L water contained in fiberglass tanks (0.6 m x 0.6 m x 0.375 m). The water : wood ratios w/w were 2.42 and 2.50 for Jack Pine and Black Spruce, respectively. The average moisture content of each log type at the time of soaking was 18.3% ± 1.6% for Jack Pine and 14.7% ± 2.1% for Black Spruce. Logs were mixed every second day to aid the diffusion of compounds from wood to water. After the 30 day soaking period, log pieces were removed from the tanks and the remaining leachate solution was transferred into amber-coloured glass bottles and stored at 4°C.

4.3.2 Leachate Characterization

Portions of the leachates were sent to Norwest Labs, Winnipeg (NWL) for physical and chemical characterization. The physical and chemical parameters measured included the following analyses: 1. Biochemical oxygen demand (BOD) by a standard 5-day incubation at 20°C. Dissolved oxygen was measured with an oxygen meter (NWL method BOD 08202); 2. Total solids by gravimetric analysis of the evaporated residue from a portion of the sample at 105°C (NWL method TS 10471L); 3. Suspended solids by gravimetric analysis of the residue retained by a 934-AH 1.5 μm glass microfibre (NWL method TSS 10401L); 4. Dissolved solids by filtration and gravimetric analysis of the evaporated residue at 105°C (NWL method TDS 1045L); 5. Total carbon by an

automated UV digest with colourimetric CO_2 dialysis (NWL method TC 06015); 6. Total organic carbon via an auto persulfate UV digest followed by CO_2 dialysis (NWL method TOC 06005L); 7. Total Kjeldahl nitrogen by a total block digest of the sample with K_2SO_4 / HgO and H_2SO_4 followed by auto phenate colourimetry (NWL method TKN 07021P); and 8. True colour by visual comparison of a filtered sample with chloroplatinate standards (NWL method COLO 02021L). The results of these analyses were compared to results from previously generated leachates.

4.3.3 Study Soils

Two types of soils were sampled on July 4, 1996 in the Manitoba Model Forest area: 1. An Eluviated Dystric Brunisol (Sec. 14 - Tp. 17 - Rg. 8-EPM; 50° 27'; 96° 23'); and 2. An Orthic Gray Luvisol (Sec. 16 - Tp. 24 - Rg. 9-EPM; 51° 03'; 96° 17'). The soil horizons / layers used in the study included the Ahe horizon of the Brunisol (herein denoted as Ahe), and the Ae horizon and LFH layer of the Luvisol (herein denoted as Ae and LFH, respectively). The characteristics and properties of these soils are outlined in Tables 4.1 and 4.2.

Table 4.1 Characteristics of the study soils.

(a) Horizon	Depth (cm)	Eluviated Dystric Brunisol Description
F	2 - 0	Poorly decomposed organic matter; fibrous, containing pine needles, lichen and grasses; diffuse boundary.
Ahe	0 - 7	Dark yellowish brown (10YR 5/2 m, 10YR 4/4 d); sand; single grain structureless; abundant roots; diffuse boundaries.
Ae	7 - 14	Brownish yellow (7.5YR 5/8 m, 10YR 6/8 d); sand; single grain structureless; diffuse boundaries.
Bm	14 - 50	Reddish yellow (7.5YR 6/4 m, 7.5YR 6/6 d); sand; single grain structureless; few roots; diffuse boundaries.
C	50 +	Yellow (10YR 5/4 m, 10YR 7/6 d); sand; single grain structureless; few roots; diffuse boundaries.

(b) Horizon	Depth (cm)	Orthic Gray Luvisol Description
LFH	10 - 0	Easily recognizable at surface to more fibric and humified at the base; abundant roots; abrupt boundary.
Ae	0 - 8	Light brownish gray (10YR 4/3 m, 10YR 6/2 d); silty clay loam; weak subangular blocky; moderate roots; abrupt boundaries.
AB	8 - 14	Light brownish gray(10YR 3/2 m, 10YR 6/2 d); silty clay loam to silty clay; moderate to medium blocky; moderate roots; abrupt boundary.
Bt	14 - 40	Dark gray (10YR 3/3 m, 10YR 4/1 d); silty clay; moderate coarse prismatic breaking to strong medium blocky; few roots; abrupt boundary.
Ck	40 +	Light gray (2.5Y 5/2 m, 10YR 7/2 d); silty clay loam to silty clay; weak subangular blocky; few to no roots; abrupt boundary.

Table 4.2 Properties of the study soils.

Property	Luvisol		Brunisol
	LFH	Ae	Ahe
% sand	n/a	12.0	92.0
% silt	n/a	38.0	4.0
% clay	n/a	50.0	4.0
Particle size analysis	n/a	clay	sand
Total organic carbon (%)	37.7	2.41	0.17
Organic matter (%)	67.1	4.29	0.3
Nitrogen (%)	1.14	0.1	<0.10
C:N ratio	33.1	24.1	17 - 29 ‡
pH (1:2 ratio in 0.01M $CaCl_2$)	5.1 †	5.2 †	5.0 †
CEC (cmol(+) kg^{-1})	67.6	24.2	1.1
Base saturation (%)	82	77	n/a
Exchangeable Ca (meq $100g^{-1}$)	40.5	9.14	0.95
Exchangeable Mg (meq $100g^{-1}$)	12.8	8.66	0.33
Exchangeable Na (meq $100g^{-1}$)	0.24	0.16	0.08
Exchangeable K (meq $100g^{-1}$)	1.69	0.68	0.06
Electrical conductivity (1:1 in water; mS cm^{-1})	0.17 †	0.13 †	0.06 †

All analyses conducted at Norwest Labs, Winnipeg, excluding those indicated by "†", which were performed in the Department of Soil Science. "‡" indicates published C:N ratios for a similar soil (Smith and Ehrlich 1964, 1967).

The vegetation growing on the Brunisolic soil was primarily Jack Pine with an understory of lichen (*Cladonia* spp.), feather mosses (*Pleurozium schreberi*, *Hylocomium splendens*), bearberry (*Arctostaphylos uva-ursi*), blueberry (*Vaccinium angustifolium* Ait.), and twinflower (*Linnaea borealis L.*). The Luvisolic soil was predominantly vegetated with mixed wood: Black Spruce, White Spruce (*Picea glauca*), Balsam Fir (*Abies balsamea*) and Trembling Aspen (*Populus tremuloides*). The understory vegetation consisted of strawberry (*Fragaria virginiana* Dcne.), bunchberry (*Cornus canadensis* L.) and snowberry (*Symphoricarpos albus* (L.) Blake). Samples of Ahe, Ae, and LFH were placed into labeled plastic bags and stored at 4°C. Immediately prior to use, Ahe and Ae were sieved and LFH was ground and sieved using a 2 mm mesh screen.

Large root fragments were removed manually. All samples were maintained at field moisture levels.

Soil samples were weighed and placed into 30 mL borosilicate glass vials. The Ahe and Ae samples contained 10 g equivalent dry soil. The LFH samples contained 0.6 g equivalent dry soil. A 25%, 5%, 1% Jack Pine or Black Spruce leachate treatment or control water was added to 28 samples of each soil horizon / layer. These concentrations were selected based upon previous toxicity test results of the leachates to luminescent bacteria (Microtox) (Chapter 3). The volumes added were adjusted to bring each soil sample to field capacity. Each group of 28 samples was divided into 4 replicate 1 L Mason jars containing 50 mL acidified water (pH 4). A 20 mL borosilicate glass vial containing 14 mL of 2M KOH was added to each jar prior to sealing. A total of 84 jars contained treated soil samples. Four control jars containing only the KOH vial and acidified water were included. All jars were incubated at 26°C for 20 weeks. Jars were opened 3 times per week and aerated with oxygen throughout the incubation period.

4.3.4 Carbon Mineralization

The rate of carbon mineralization (microbial respiration) was determined by trapping CO_2 generated within the jar in 2.0M KOH. Traps were replaced every 7 days and tightly capped until analysis. The CO_2, trapped as K_2CO_3, was precipitated using excess 0.8M $BaCl_2$ solution. The residual alkali was then titrated with 1M HCl (Zibilski 1994). The total amount of soil in each jar decreased as the assay progressed. All calculations were adjusted for the change in soil mass. Values for C mineralization were expressed as mg C (g oven dry soil)$^{-1}$. Analytical accuracy was assessed via titration of standard samples consisting of 14 mL KOH and 5mL 1M $KHCO_3$.

4.3.5 Nitrogen Mineralization

Nitrogen mineralization was determined via analysis of soil samples for NH_4^+-N and NO_3^--N. Samples of non-amended soil, leachate, and soil treated with leachate were analyzed at time 0. Further analyses were performed at weeks 2, 4, 6, 8, 12, 16 and 20 on the contents of one vial from each jar. Nitrogen extractions were conducted on all samples by adding 50 mL 2M KCl solution, shaking for 30 minutes and filtering through Whatman #2 filter paper. The filtrate was frozen immediately following extraction. Standard solutions of NH_4^+-N and NO_3^--N were prepared according to Maynard and Kalra (1993). Colourimetric analysis of all samples was conducted using a flow injection analyzer. Net mineral N was calculated as the sum of the NH_4^+-N and NO_3^--N and expressed as μg N (g oven dry soil)$^{-1}$. Quality control measures included 10% analytical replication, and the inclusion of standard samples of KNO_3 and $(NH_4)_2SO_4$ (Maynard and Kalra 1993) for assessment of analytical accuracy.

4.3.6 Statistical Analysis

One and two-factor analyses of variance (Microsoft Excel Version 7.0, Microsoft Corporation, Redmond, Washington; CoStat Version 5.01, CoHort Software, Minneapolis, Minnesota) were performed to determine the existence of significant differences in both C and N mineralization between treatments in each soil horizon / layer. Mean separation was accomplished with Fishers Least Significant Difference Test.

4.4 Results and Discussion

4.4.1 Leachate Characteristics

The leachates generated in the lab were chemically similar to previously generated leachates (Chapter 3). Both leachates were characterized by high organic carbon contents, with C:N ratios of 77.6 and 133 for Jack Pine and Black Spruce, respectively (Table 4.3).

Table 4.3 Chemical and biological characteristics of Jack Pine and Black Spruce leachate generated by soaking logs in water for 30 days.

Analysis	Jack Pine Leachate	Black Spruce Leachate
Total Coliform (CFU 100 mL^{-1})	20	30
BOD (mg L^{-1})	500.5	601
True Colour (ColourUnit)	90	275
Dissolved solids (mg L^{-1})	472	656
Suspended solids (mg L^{-1})	40.5	31
Total solids (mg L^{-1})	471	649.5
Total carbon (mg C L^{-1})	333.5	447
Inorganic carbon (mg C L^{-1})	8.65	7.9
Organic carbon (mg C L^{-1})	324.5	439.5
Total Kjeldahl nitrogen (mg N L^{-1})	4.87	3.85

4.4.2 Carbon Mineralization

The weekly rate of C mineralization generally decreased over time (Figures 4.1 to 4.6), probably due to a reduction in the amount of substrate in the soil available for microbial oxidation. This trend was most evident in the Ahe horizon and LFH layer. The mineralization rates in each treatment decreased until week 17, when a surge of respiration was detected. The rate remained high through week 18 and then decreased again. In contrast, no distinct pattern of change in the rate of C mineralization was

evident in any treatment in the Ae horizon, although the same rate increase occurred at week 17.

Jack Pine and Black Spruce leachate addition generally had little effect on the rate of C mineralization in each soil horizon and each sampling period over the 20 week incubation. A small number of statistically significant differences in mean values of mineralized C between treatments in each sampling period were detected. These differences were small relative to the total amount of C respired and did not follow a discernible pattern. The apparent lack of treatment effects may be due to a number of reasons. Firstly, although the chemical constituents of the leachate produced for this study were not identified, softwood leachates are generally composed of aromatic and lignin-like carbon compounds (Liu et al. 1995; Taylor 1994). These compounds may not be metabolized as readily as other C substrates occurring in the soils (Swift et al. 1979). Secondly, although the undilute softwood leachates had high C:N ratios, the actual amount of C added to each soil sample was small relative to the total C respired. The amount added may have been insufficient to induce changes in microbial respiration. Lastly, the absence of significant differences in each week may also be due to poor statistical sensitivity of the analyses resulting from the low error degrees of freedom from too few replications, combined with the natural variation in the data on a weekly basis. This would mean that leachate may actually have more of an effect on this microbially mediated process than observed.

Although treatment effects were generally not observed in each sampling period, one interesting observation occurred in the LFH over the first three weeks of incubation. In these samples, the 25% Jack Pine treatment displayed reduced C mineralization rates

compared to all other treatments. This may have been caused by a lack of adaptation of the indigenous microorganism population in the LFH to Jack Pine leachate as the Luvisolic soils were sampled in a mixed wood forest devoid of Jack Pine tree species. This time may have been required to stimulate the soil microorganism community capable of degrading Jack Pine leachate. Other than the apparent retardation in the LFH, the overall results do not indicate that the addition of softwood leachate had an effect on C mineralization at each sampling period.

The addition of a carbon-rich substrate to a soil sample should have caused an increase in C mineralization. This generally did not occur. An examination of the cumulative C mineralized over the 20 week incubation period in each treatment suggests that the addition of leachate caused no change in the Ahe samples while caused a slight decrease in the C mineralized in the Ae and LFH samples compared to the control (Table 4.4). This could be due to a slight toxic quality of the leachate. Analysis of the percent change in respiration in the Luvisolic soils from the control suggests that the effect of Jack Pine leachate was greater than that of Black Spruce. A general trend of decreasing effect with decreasing concentration is also evident. Although this data suggests a treatment effect exists, this cannot be tested statistically due to the cumulative nature of the data (Note: The cumulative values have large standard errors associated with them, therefore Table 4.4 only provides a general summary). If these effects are real, then the addition of softwood leachate to the Luvisolic soil horizons could negatively affect the microbial activity in Luvisolic soils, effectively impacting the soil's ability to support biological life (Nadelhoffer 1990).

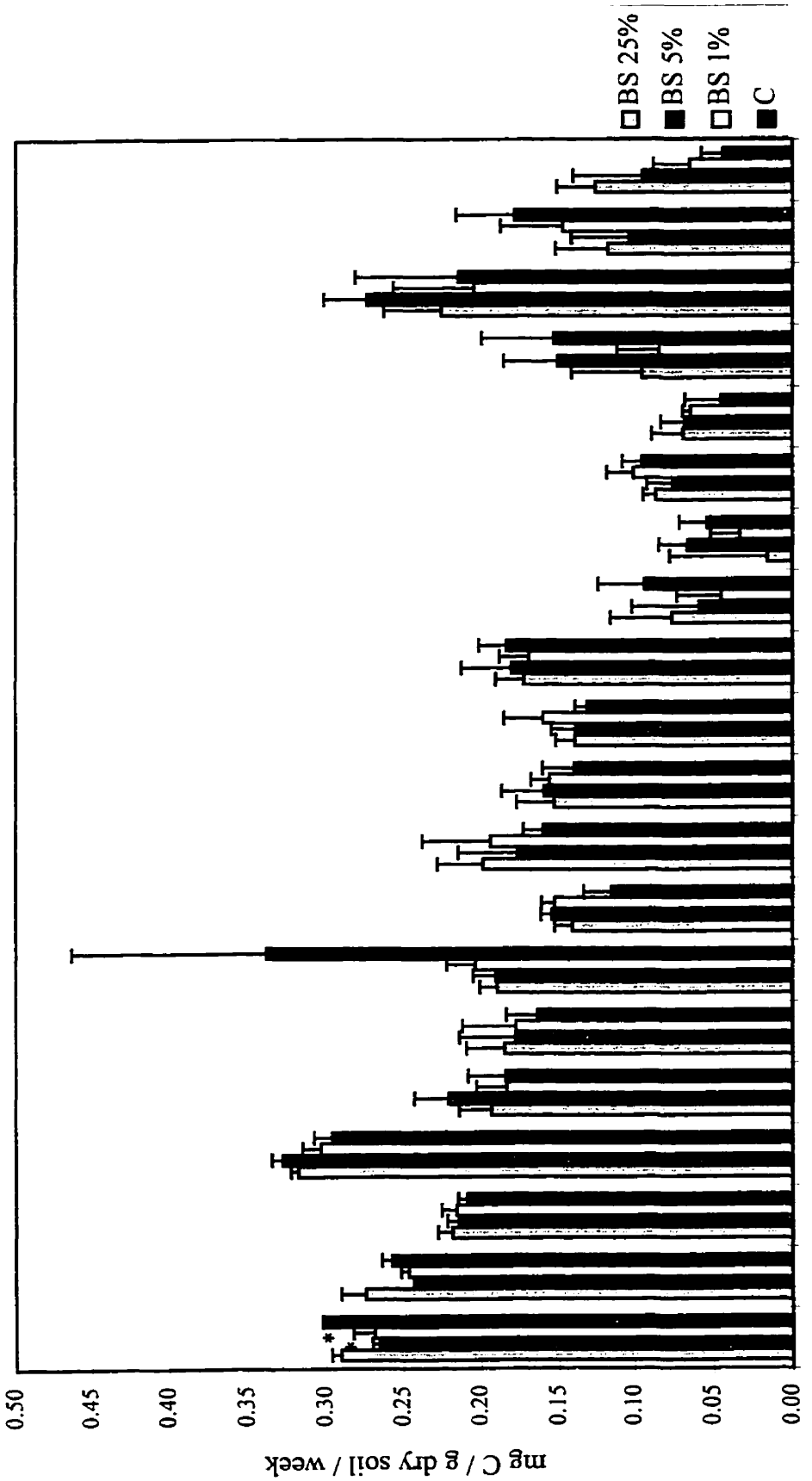


Figure 4.1 Changes in the rate of C mineralization over time in the Ahe horizon of a Brunisol treated with Black Spruce leachate. Values are the mean of 4 replicates.

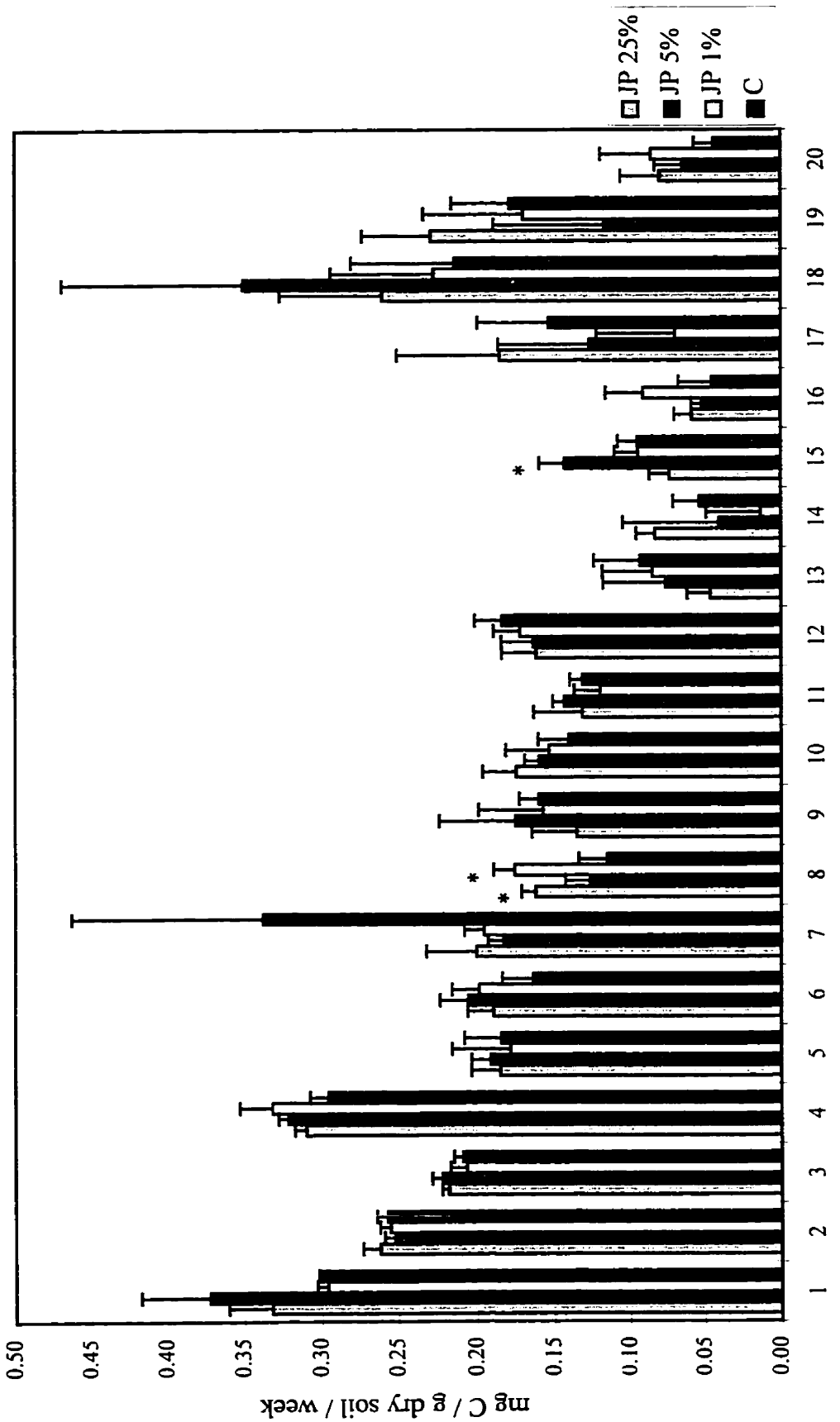


Figure 4.2 Changes in the rate of C mineralization over time in the Ahe horizon of a Brunisol treated with Jack Pine leachate. Values are the mean of 4 replicates.

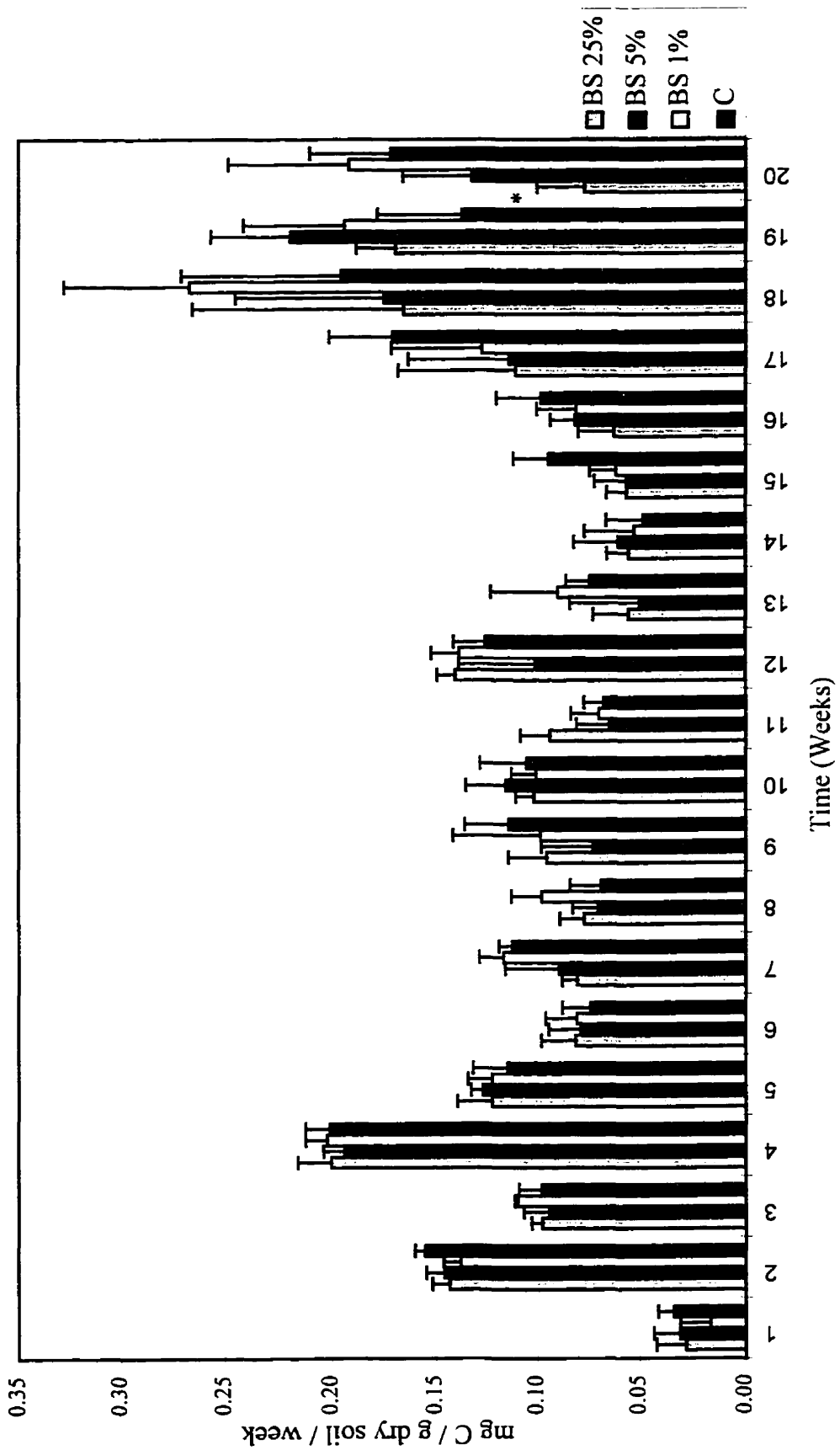


Figure 4.3 Changes in the rate of C mineralization over time in the Ae horizon of an Orthic Gray Luvisol treated with Black Spruce leachate. Values are the mean of 4 replicates.

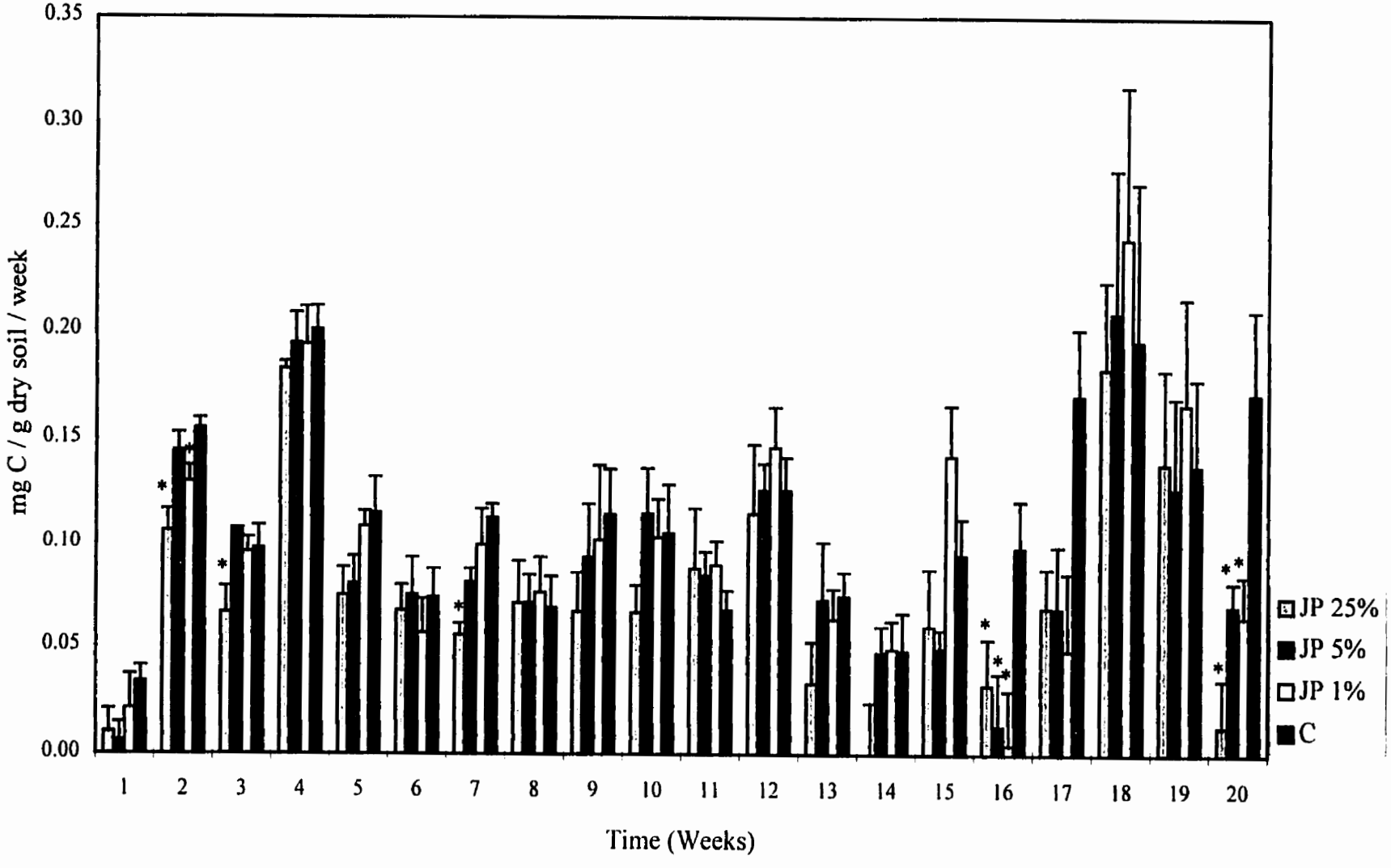


Figure 4.4 Changes in the rate of C mineralization in the Ae horizon of an Orthic Gray Luvisol treated with Jack Pine Leachate. Values are the mean of 4 replicates.

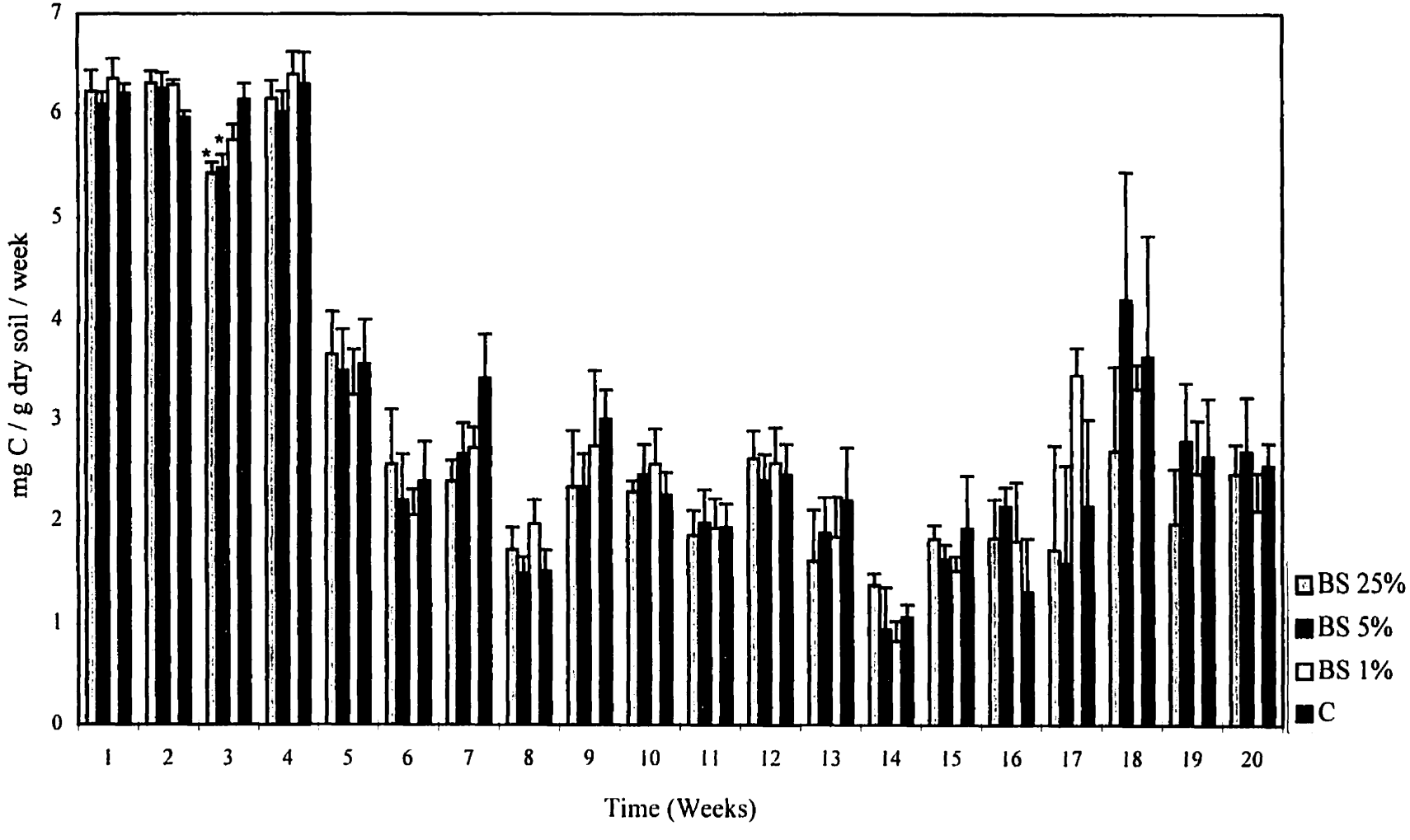


Figure 4.5 Changes in the rate of C mineralization over time in the LFH layer of an Orthic Gray Luvisol treated with Black Spruce leachate. Values are the mean of 4 replicates.

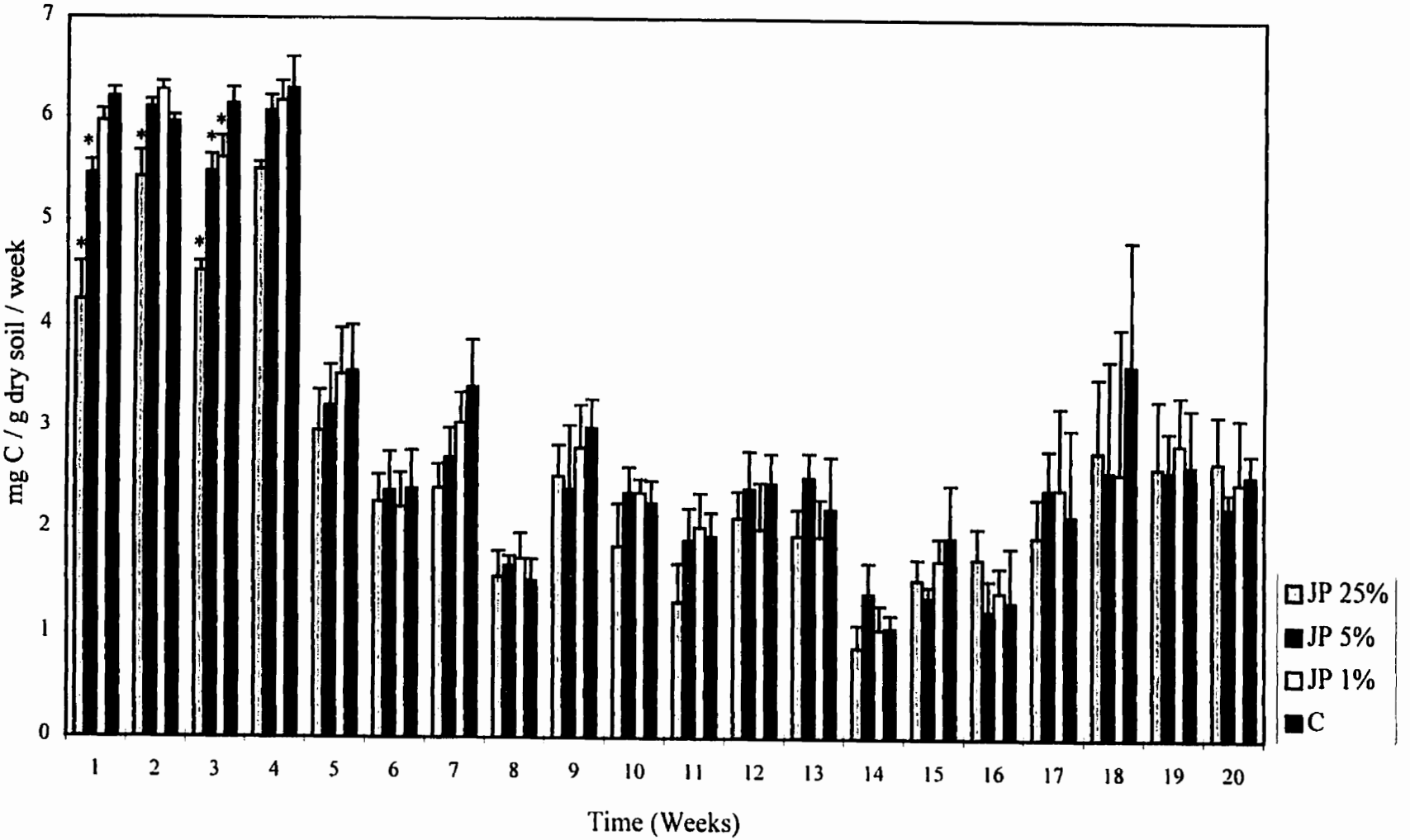


Figure 4.6 Changes in the rate of C mineralization over time in the LFH layer of an Orthic Gray Luvisol treated with Jack Pine leachate. Values are the mean of 4 replicates.

Table 4.4 Cumulative carbon mineralization over 20 weeks in soil samples treated with softwood leachate.

	JP			BS		
	25%	5%	1%	25%	5%	1%
	---------mg C (g oven dry soil)$^{-1}$---------					
Ahe Brunisol						
(A) Soil + Leachate Treatment	3.48	3.49	3.28	3.28	3.34	3.17
(B) Control Soil	3.36	3.36	3.36	3.36	3.36	3.36
(C) Total C in Leachate Treatment	0.02	0.00	0.00	0.03	0.01	0.00
A - B - C	0.10	0.13	-0.08	-0.11	-0.03	-0.19
% Change From Control	2.9%	3.7%	-2.4%	-3.2%	-0.8%	-5.7%
Ae Luvisol						
(A) Soil + Leachate Treatment	1.50	1.83	2.00	2.01	2.07	2.36
(B) Control Soil	2.26	2.26	2.26	2.26	2.26	2.26
(C) Total C in Leachate Treatment	0.02	0.00	0.00	0.03	0.01	0.00
A - B - C	-0.78	-0.43	-0.26	-0.28	-0.20	0.10
% Change From Control	-34.5%	-19.1%	-11.4%	-12.2%	-8.8%	4.2%
LFH Luvisol						
(A) Soil + Leachate Treatment	52.7	58.4	60.1	59.0	60.7	61.9
(B) Control Soil	62.6	62.6	62.6	62.6	62.6	62.6
(C) Total C in Leachate Treatment	0.02	0.00	0.00	0.03	0.01	0.00
A - B - C	-9.92	-4.20	-2.50	-3.63	-1.91	-0.70
% Change From Control	-15.8%	-6.7%	-4.0%	-5.8%	-3.0%	-1.1%

Values represent the addition of the means of 4 replicates per week for 20 weeks.

4.4.3 Nitrogen Mineralization

Mineral N levels generally increased over time in each soil horizon and leachate treatment (Figures 4.7 to 4.12). Statistically significant differences in the mean values of mineral N were detected between the various leachate treatments in weeks 4 in the Ahe, weeks 2, 4, 6, 12 and 16 in the Ae, and weeks 2, 6, 12 and 20 in the LFH. It should be noted that the amounts of NO_3-N were negligible compared to NH_4-N, presumably due to the acidic nature of the forest soils sampled.

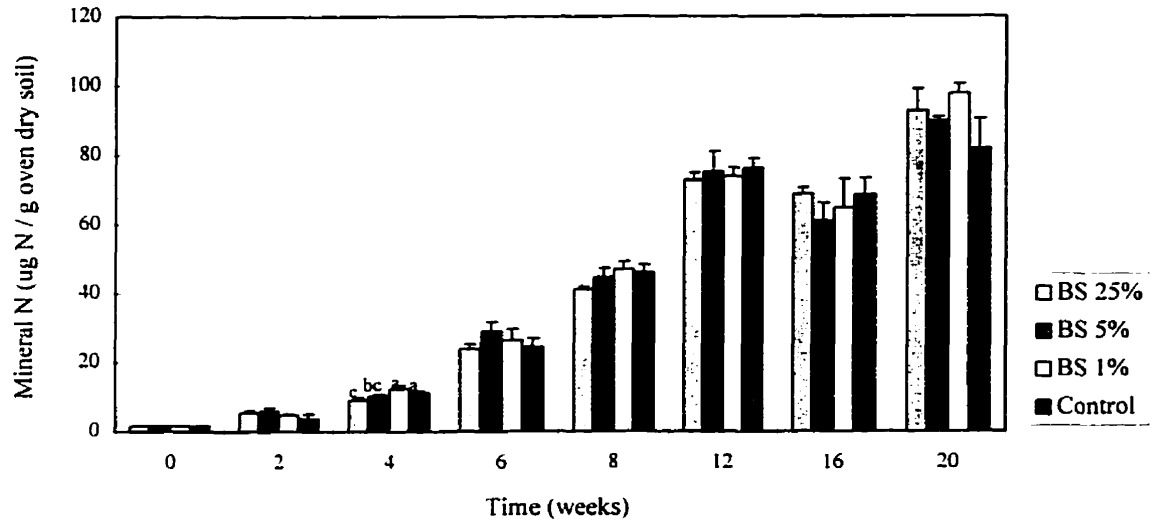


Figure 4.7 Changes in mineral N over time in the Ahe horizon of a Brunisolic soil treated with Black Spruce leachate. Values are the mean of 4 replicates.

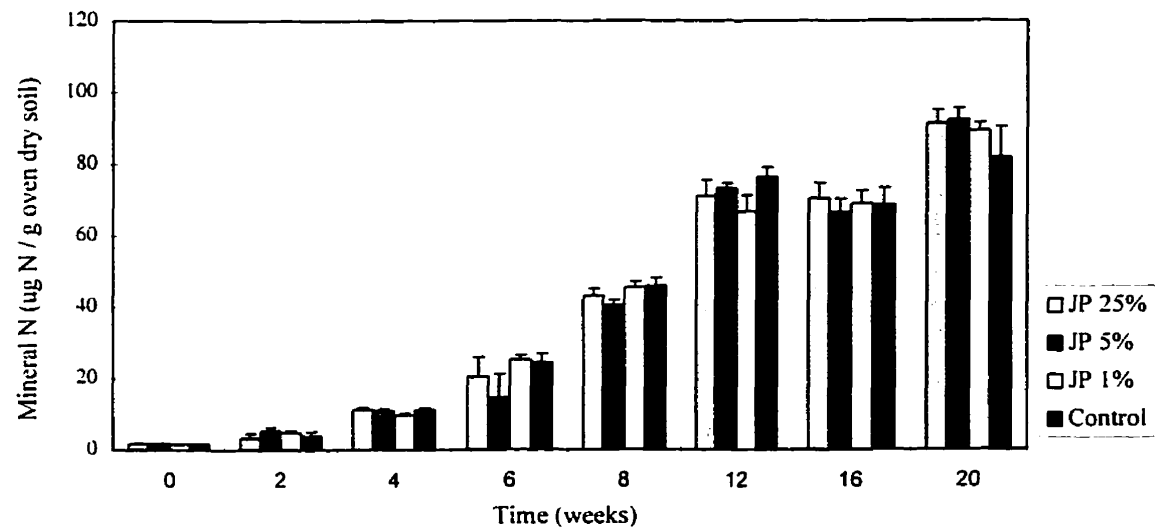


Figure 4.8 Changes in mineral N over time in the Ahe horizon of a Brunisolic soil treated with Jack Pine leachate. Values are the mean of 4 replicates.

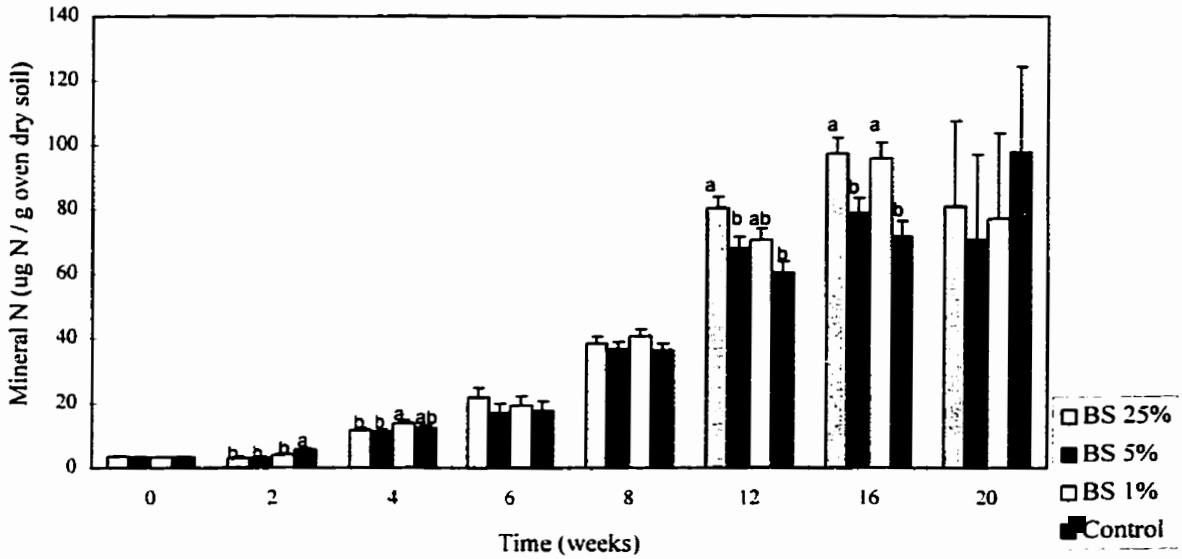


Figure 4.9 Changes in mineral N over time in the Ae horizon of the Luvisolic soil treated with Black Spruce leachate. Values are the mean of 4 replicates.

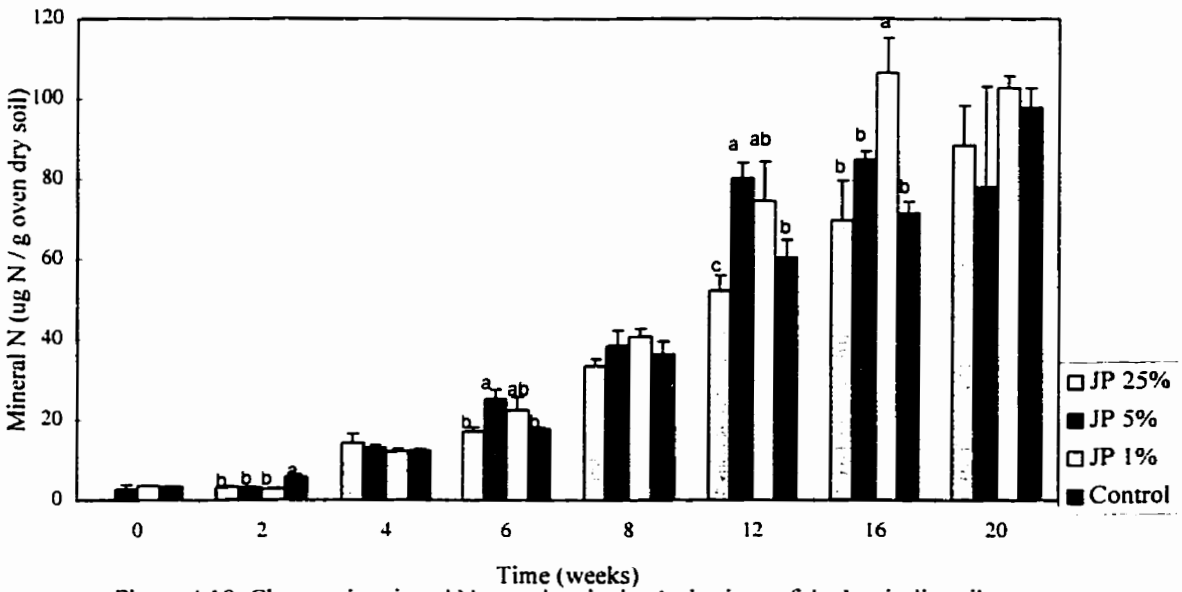


Figure 4.10 Changes in mineral N over time in the Ae horizon of the Luvisolic soil treated with Jack Pine leachate. Values are the mean of 4 replicates.

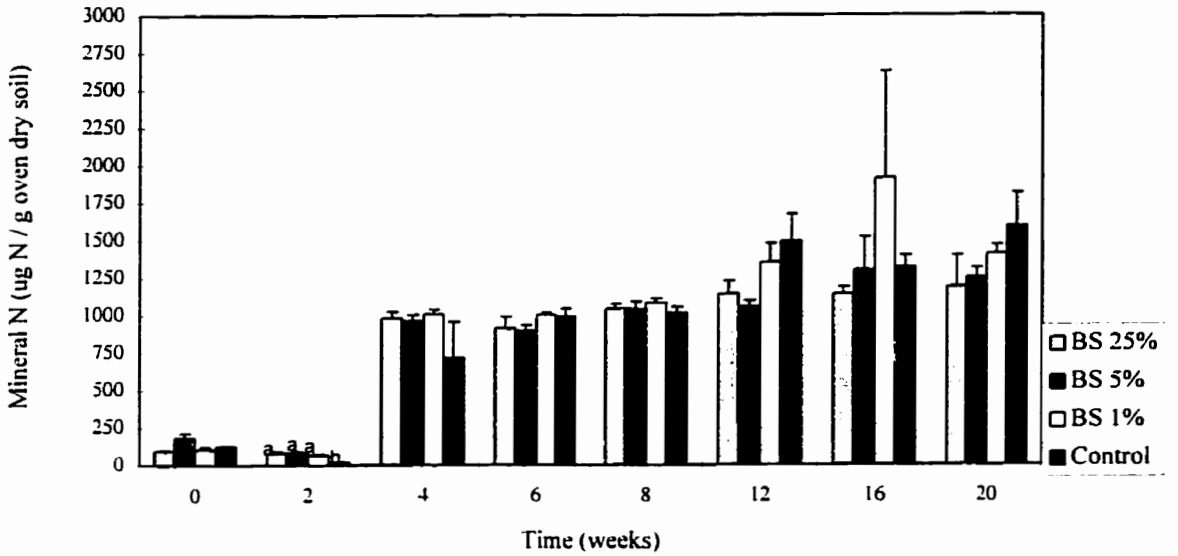


Figure 4.11 Changes in mineral N over time in the LFH layer of a Luvisolic soil treated with Black Spruce leachate. Values are the mean of 4 replicates.

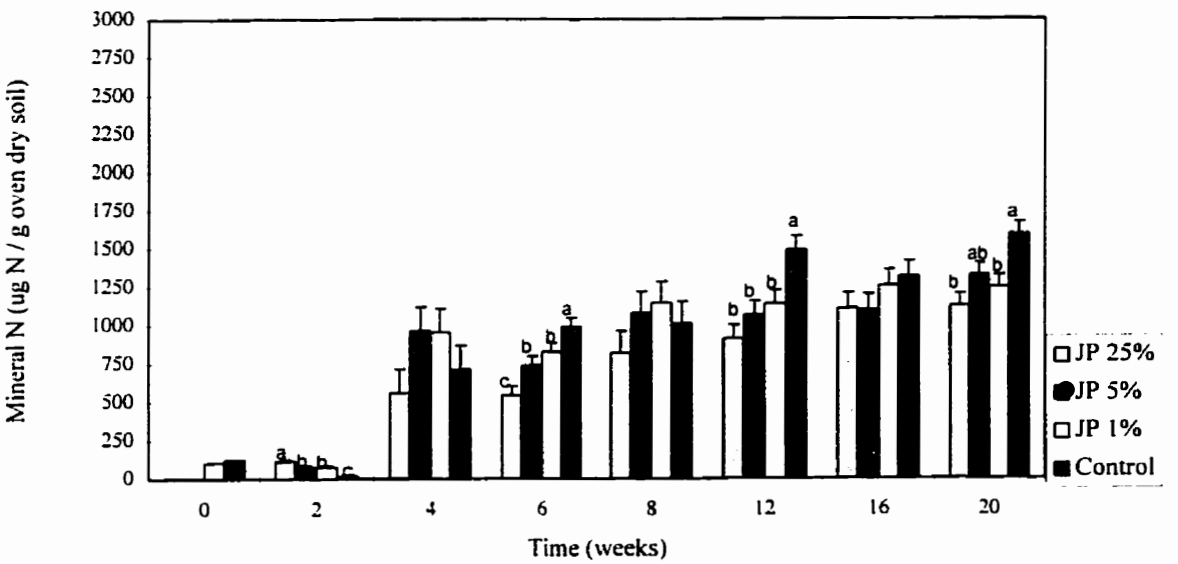


Figure 4.12 Changes in mineral N over time in the LFH layer of a Luvisolic soil treated with Jack Pine leachate. Values are the mean of 4 replicates.

The most interesting observation in this study was that the process of N mineralization was not suppressed as expected following leachate addition. The C:N ratios of the leachates were 77.6 and 133 for Jack Pine and Black Spruce, respectively, while the C:N ratios of the soils were 17 to 29, 24.1 and 33.1 for the Ahe, Ae and LFH, respectively. The addition of a substrate with a C:N ratio greater than that of the soil C:N ratio should have caused net immobilization of N (White et al. 1988). This generally did not occur.

N mineralization increased with time in all treatments of the N-deficient Ahe horizon of the Brunisolic soil. The single addition of softwood leachate seems to have had no effect on N mineralization as few statistically significant differences between the treatments and the control were observed. As previously discussed, the lack of statistical differences may be due to a number of factors, one of which being that the volume of leachate added may have been insufficient to cause changes in the measured soil microbial processes.

Table 4.5 Net nitrogen mineralization after 20 weeks in soil samples treated with softwood leachate compared to control samples.

	JP			BS		
	25%	5%	1%	25%	5%	1%
	——————μg N (g oven dry soil)$^{-1}$——————					
Ahe Brunisol						
(A) Soil + Leachate Treatment	91.4	92.3	89.3	92.8	89.8	97.7
(B) Control Soil	81.9	81.9	81.9	81.9	81.9	81.9
(C) Mineral N in Leachate Treatment	9.78	0.43	0.94	1.32	1.21	1.38
A - B - C	-0.31	10.0	6.43	9.60	6.72	14.4
% Change from Control	-0.38%	12.2%	7.86%	11.7%	8.20%	17.6%
Ae Luvisol						
(A) Soil + Leachate Treatment	88.5	78.0	103	81.0	70.5	77.1
(B) Control Soil	97.8	97.8	97.8	97.8	97.8	97.8
(C) Mineral N in Leachate Treatment	7.68	0.33	0.74	1.04	0.95	1.09
A - B - C	-17.0	-20.1	4.46	-17.8	-28.3	-21.8
% Change from Control	-17.4%	-20.6%	4.56%	-18.2%	-28.9%	-22.3%
LFH Luvisol						
(A) Soil + Leachate Treatment	1130†	1330	1250†	1190	1250	1410
(B) Control Soil	1600	1600	1600	1600	1600	1600
(C) Mineral N in Leachate Treatment	106	4.62	10.2	14.4	13.1	15.0
A - B - C	-576	-275	-360	-424	-363	-205
% Change from Control	-36.0%	-17.2%	-22.5%	-26.5%	-22.7%	-12.8%

Values for A, B and C represent the means of four replicates. Values of A followed by "†" are statistically different from the control mean (B). Mean separation performed using Fisher's Least Significant Difference test.

The process of mineralization also occurred in both Luvisolic horizons throughout the incubation period, although reduced levels of mineralization compared to the control were observed. In the Ae samples, these reduced mineral N levels occurred in the leachate-treated soils after 2 weeks of incubation. In subsequent sampling periods in which statistically significant differences between treatments were observed, N mineralization was greater in leachate-treated samples than the control. These

differences were generally small relative to the total amount of mineral N in each sample and did not follow a pattern. However, after 20 weeks of incubation, slightly reduced levels of N mineralization were observed (Table 4.5). As these differences were not statistically significant, it must be concluded that the single addition of softwood leachate had minimal impact on N processes in the Ae horizon of the Luvisolic soil within a 20 week period.

The opposite was observed in the LFH samples. In these samples, statistically significant net N mineralization in leachate-treated samples compared to the control was only observed in week 2 (Figure 4.11 and 4.12). N immobilization, compared to the control, was observed in all other sampling periods in which statistically significant differences between treatment means were found (Figure 4.12), including after 20 weeks of incubation (Table 4.5). As N mineralization was reduced in the leachate-treated LFH soils, it is possible that leachate additions could reduce the available N for plant uptake.

The hypothesis of the study was that the addition of a carbon-rich substrate would cause increased microbial respiration and immobilization of N. This generally did not occur as expected. In fact, little change in respiration and/or N mineralization was observed in the Ahe of the Brunisol. In contrast, reduced respiration occurred in both the Ae and the LFH of the Luvisol, with no real effect to N mineralization in the Ae and reduced N mineralization in the LFH. Evaluation of the C:N ratios of each soil and of the leachate additions does not appear to explain the results of the study. It is possible that the addition of softwood leachate to the Luvisolic soils produced toxic effects in the soil microorganism population, resulting in a reduction in the overall soil biological activity.

4.5 Summary and Conclusions

Single additions of Jack Pine and Black Spruce leachates, prepared in the laboratory at a water : wood soaking ratio of 2.5:1, had varying effects on the processes of C and N mineralization in two Manitoba forest soils. The hypothesis of this study was that net mineralization of C and immobilization of N would occur as a result of the addition of a substrate with a wide C:N ratio. It is difficult to conclude that C mineralization was affected by the addition of leachate due to the small number of statistically significant differences between the treatments at each sampling period. However, examination of the cumulative data shows possible reductions in soil microbial respiration in the Luvisolic soils due to the addition of softwood leachate, although these results cannot be tested statistically. Analysis of the cumulative mineral N data indicates that immobilization, compared to the control, occurred only in the LFH of the Luvisol, while no treatment effect was observed in either the Ahe of the Brunisol or the Ae of the Luvisol.

Leachate solutions generated in log storage yards could be more or less concentrated than the solutions generated for this study depending on the size of the log pile and the amount and type of precipitation. Small precipitation events could produce small volumes of highly concentrated leachate. Conversely, large events could produce large volumes of more dilute leachate (Taylor 1994). Forest soils adjacent to log storage areas may therefore be subject to a wide spectrum of leachate concentrations. The leachate concentrations used in this study were toxic to aquatic organisms (Chapter 3). While it is

not known whether the toxicity of softwood leachate to aquatic organisms would be affected by the percolation of leachate through soil prior to entering the water, it is known that relatively dilute softwood leachate demonstrated little or no effect on C and N mineralization in forest soil horizons. Further studies involving more concentrated leachate solutions, as well as repeated leachate applications, should be conducted before proper recommendations for locating log storage sites can be made.

5. THE EFFECT OF SOFTWOOD LEACHATE ON THE FUNCTIONAL DIVERSITY OF FOREST SOIL MICROBIAL COMMUNITITES

5.1 Abstract

Evaluation of the functional diversity of a soil microbial community provides insight into ecosystem functioning. Functional, or metabolic diversity may be assessed via examination of the rate and pattern of substrate usage on Biolog™ microtitre plates. The objective of this study was to determine the effects of leachate from the softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*) on the functional diversity of the microbial community of two forest soils: 1. An Eluviated Dystric Brunisol (Ahe horizon); and 2. An Orthic Gray Luvisol (LFH layer and Ae horizon). Leachate was generated by soaking pine and spruce logs in water for 30 days. Biolog™ plates, inoculated with extracts of soils treated with 25%, 5% or 1% by volume leachate or control water, were incubated and read manually over 72 hours. Colour production in each well was recorded. Compared to the control, a greater number, or diversity, of substrates were metabolized at a more rapid rate in the plates inoculated with leachate-treated Brunisolic soil. The leachate treatments did not have a significant effect on the functional diversity of the Ae horizon of the Luvisol. In contrast, leachate treatment of the LFH samples caused more rapid substrate metabolization than the control. Extracts of soil treated with Jack Pine leachate had slightly slower metabolism than those treated with Black Spruce leachate. In general, the functional diversities of the forest soils assayed were either not affected, or were stimulated by the single addition of softwood leachate.

5.2 Introduction

Soil microorganisms can be used as sensitive biological indicators of soil quality (Turco et al. 1994). The decomposition of organic compounds and cycling of nutrients, processes which are central to the function of soils, are mediated by microorganisms. Traditional methods for evaluating soil microbial populations generally focus on taxonomic diversity. There are a number of limitations associated with these methods, the greatest of which is that it is difficult, if not impossible, to assess the total microbial species complement of a soil (Garland and Mills 1994; Parkinson and Coleman 1991). As well, there is a general lack of information relating taxonomic diversity to soil function; certain rare species may actually have a greater role in soil function than more prevalent ones (Zak et al. 1994). An assessment of the functional, rather than taxonomic, diversity of a soil may provide more useful information because changes in functional diversity of a soil microbial community may have lasting effects on ecosystem function (Perry et al. 1989). Garland and Mills (1991) proposed the use of microtitre plates containing a wide range of substrates to assess functional differences between soil microbial communities. The assessment is based upon the sole-source carbon utilization patterns of the bacterial community. This approach does not attempt to characterize the numbers or identities of the species present. Functional changes observed at the microbial level could effectively forecast changes in the general quality of soils at an ecosystem level (Zak et al. 1994).

The microbial community inhabiting the soil responds to physical, chemical and/or biological perturbations. One such perturbation could be the natural flush of organic

compounds from downed trees after a precipitation event. It is probable that the microbial community in forest soils would be adapted to the natural range of substrates released from the logs in varying concentrations. However, a large log pile could produce a more concentrated leachate solution depending on the size of the pile and the volume of water in contact with the wood. This leachate may have toxic effects on the surrounding biota. The objective of this study was to determine if the functional diversity of the microbial community in forest soils is impacted by the addition of a laboratory generated leachate derived from the softwood species Jack Pine (*Pinus banksiana*) and Black Spruce (*Picea mariana*). The results could have implications on the placement of large, remote log storage areas in the forest industry.

5.3 Materials and Methods

5.3.1 Leachate Production

Softwood leachate was produced using Jack Pine and Black Spruce logs obtained from the Pine Falls Paper Company in Pine Falls, Manitoba on October 5, 1995. Logs were cut into 30 cm pieces on October 10 and October 12, 1995, covered with polyethylene plastic and stored in an unheated shed. Leachate production began on June 12, 1996 by immersing log pieces in dechlorinated City of Winnipeg tap water. Totals of 24.8 kg Jack Pine and 24.0 kg Black Spruce wood were soaked separately for 30 days in 60 L water contained in fiberglass tanks (0.6 m x 0.6 m x 0.375 m). The water : wood ratios w/w were 2.42 and 2.50 for Jack Pine and Black Spruce, respectively. The average moisture content of each log type at the time of soaking was 18.3% ± 1.6% for Jack Pine and 14.7% ± 2.1% for Black Spruce. Logs were mixed every second day to aid the

diffusion of compounds from wood to water. After the 30 day soaking period, log pieces were removed from the tanks and the remaining leachate solution was transferred into amber-coloured glass bottles and stored at 4°C.

5.3.2 Leachate Characterization

Portions of the leachates were sent to Norwest Labs, Winnipeg (NWL) for physical and chemical characterization. The physical and chemical parameters measured included the following analyses: 1. Biochemical oxygen demand (BOD) by a standard 5-day incubation at 20°C. Dissolved oxygen was measured with an oxygen meter (NWL method BOD 08202); 2. Total solids by gravimetric analysis of the evaporated residue from a portion of the sample at 105°C (NWL method TS 10471L); 3. Suspended solids by gravimetric analysis of the residue retained by a 934-AH 1.5 μm glass microfibre (NWL method TSS 10401L); 4. Dissolved solids by filtration and gravimetric analysis of the evaporated residue at 105°C (NWL method TDS 1045L); 5. Total carbon by an automated UV digest with colourimetric CO_2 dialysis (NWL method TC 06015); 6. Total organic carbon via an auto persulfate UV digest followed by CO_2 dialysis (NWL method TOC 06005L); 7. Total Kjeldahl nitrogen by a total block digest of the sample with K_2SO_4 / HgO and H_2SO_4 followed by auto phenate colourimetry (NWL method TKN 07021P); and 8. True colour by visual comparison of a filtered sample with chloroplatinate standards (NWL method COLO 02021L). The results of these analyses were compared to results from previously generated leachates.

5.3.3 Study Soils

Two types of soil were sampled on July 4, 1996 in the Manitoba Model Forest area: 1. An Eluviated Dystric Brunisol (Sec. 14 - Tp. 17 - Rg. 8-EPM; 50° 27'; 96° 23'); and 2. An Orthic Gray Luvisol (Sec. 16 - Tp. 24 - Rg. 9-EPM; 51° 03'; 96° 17'). The soil horizons / layers used in the study included the Ahe horizon of the Brunisol (herein denoted as Ahe), and the Ae horizon and LFH layer of the Luvisol (herein denoted as Ae and LFH, respectively). The characteristics and properties of these soils are outlined in Tables 5.1 and 5.2.

The vegetation growing on the Brunisolic soil was primarily Jack Pine with an understory of lichen (*Cladonia* spp.), feather mosses (*Pleurozium schreberi*, *Hylocomium splendens*), bearberry (*Arctostaphylos uva-ursi*), blueberry (*Vaccinium angustifolium* Ait.), and twinflower (*Linnaea borealis L.*). The Luvisolic soil was predominantly vegetated with mixed wood: Black Spruce, White Spruce (*Picea glauca*), Balsam Fir (*Abies balsamea*) and Trembling Aspen (*Populus tremuloides*). The understory vegetation consisted of strawberry (*Fragaria virginiana* Dcne.), bunchberry (*Cornus canadensis* L.) and snowberry (*Symphoricarpos albus* (L.) Blake). Samples of Ahe, Ae, and LFH were placed into labeled plastic bags and stored at 4°C. Immediately prior to use, Ahe and Ae were sieved and LFH was ground and sieved using a 2 mm mesh screen. Large root fragments were removed manually. All soil samples were maintained at field moisture levels.

Table 5.1 Characteristics of the study soils.

(a)		Eluviated Dystric Brunisol
Horizon	**Depth (cm)**	**Description**
F	2 - 0	Poorly decomposed organic matter; fibrous, containing pine needles, lichen and grasses; diffuse boundary.
Ahe	0 - 7	Dark yellowish brown (10YR 5/2 m, 10YR 4/4 d); sand; single grain structureless; abundant roots; diffuse boundaries.
Ae	7 - 14	Brownish yellow (7.5YR 5/8 m, 10YR 6/8 d); sand; single grain structureless; diffuse boundaries.
Bm	14 - 50	Reddish yellow (7.5YR 6/4 m, 7.5YR 6/6 d); sand; single grain structureless; few roots; diffuse boundaries.
C	50 +	Yellow (10YR 5/4 m, 10YR 7/6 d); sand; single grain structureless; few roots; diffuse boundaries.

(b)		Orthic Gray Luvisol
Horizon	**Depth (cm)**	**Description**
LFH	10 - 0	Easily recognizable at surface to more fibric and humified at the base; abundant roots; abrupt boundary.
Ae	0 - 8	Light brownish gray (10YR 4/3 m, 10YR 6/2 d); silty clay loam; weak subangular blocky; moderate roots; abrupt boundaries.
AB	8 - 14	Light brownish gray(10YR 3/2 m, 10YR 6/2 d); silty clay loam to silty clay; moderate to medium blocky; moderate roots; abrupt boundary.
Bt	14 - 40	Dark gray (10YR 3/3 m, 10YR 4/1 d); silty clay; moderate coarse prismatic breaking to strong medium blocky; few roots; abrupt boundary.
Ck	40 +	Light gray (2.5Y 5/2 m, 10YR 7/2 d); silty clay loam to silty clay; weak subangular blocky; few to no roots; abrupt boundary.

Table 5.2 Properties of the study soils.

Property	Luvisol		Brunisol
	LFH	Ae	Ahe
% sand	n/a	12.0	92.0
% silt	n/a	38.0	4.0
% clay	n/a	50.0	4.0
Particle size analysis	n/a	clay	sand
Total organic carbon (%)	37.7	2.41	0.17
Organic matter (%)	67.1	4.29	0.3
Nitrogen (%)	1.14	0.1	<0.10
C:N ratio	33.1	24.1	17 - 29 ‡
pH (1:2 ratio in 0.01M $CaCl_2$)	5.1 †	5.2 †	5.0 †
CEC	67.6	24.2	1.1
Base saturation (%)	82	77	n/a
Exchangeable Ca (meg $100g^{-1}$)	40.5	9.14	0.95
Exchangeable Mg (meg $100g^{-1}$)	12.8	8.66	0.33
Exchangeable Na (meg $100g^{-1}$)	0.24	0.16	0.08
Exchangeable K (meg $100g^{-1}$)	1.69	0.68	0.06
Electrical conductivity (1:1 in water; mS cm^{-1})	0.17 †	0.13 †	0.06 †

All analyses conducted at Norwest Labs, Winnipeg, excluding those indicated by "†", which were performed in the Department of Soil Science. "‡" indicates published C:N ratios for a similar soil (Smith and Ehrlich 1964, 1967).

Samples of field moist Ahe, Ae and LFH equivalent to 10 g oven dry soil were measured into 50 mL glass beakers. Triplicate samples were amended with 25%, 5% or 1% by volume Jack Pine or Black Spruce leachate extracts or with control water. The concentrations selected were based upon previous toxicity results using the Microtox assay (Chapter 3). The final moisture contents of the soil horizon / layer samples were 118%, 149% and 120% of field capacity for Ahe, Ae and LFH, respectively. Beakers were covered with parafilm and incubated for 7 days at 20°C.

The microbial community was extracted from the soil by blending each soil sample with 90 mL sterile 0.85% physiological saline for 2 minutes at high speed (10^{-1}). Following this, 1 mL of the slurry was added to 99 mL sterile 0.2% water agar (10^{-3}). A portion of the 10^{-3} solution was transferred to a sterile plastic reagent reservoir. Aliquots of 100 µL were added to each of 96 wells on both Gram Positive and Gram Negative Biolog™ (Biolog Inc., Hayward, CA) microtitre plates (Garland and Mills 1991; Zak et al. 1994). Each plate contained 95 separate carbon substrates plus 1 control well; the two plates combined for a set of 128 different carbon substrates (Appendices IIIa and IIIb). In addition to the substrate, each well also contained nutrients, salts and a redox-sensitive tetrazolium violet dye. During carbon source utilization (respiration), the dye is reduced resulting in the formation of insoluble formazan which appears as a purple residue at the bottom of the wells (Bochner and Savageau 1977; Winding 1994; Garland 1996a; Zak et al. 1994). All inoculated plates were incubated at 25°C for 72 hours. The plates were shaken during incubation to provide more even distribution of oxygen (Garland 1996b) and to prevent cells from settling to the bottom of the wells (Winding 1994). Plates were read manually at 12, 24, 36, 48, 60 and 72 hours and scored for the appearance of colour. Intensity values were assigned to each cell based on the time at which colour was first evident in each well, e.g. value of 6 for colour development at 12 hours, 5 for colour development at 24 hours, etc. Wells which remained colourless throughout the incubation period were given a value of 0, indicating no reaction. Functional diversity was calculated as the percentage of substrates utilized during the 72 hour incubation period. Two factor analysis of variance (CoStat 5.0, CoHort Software, Minneapolis, Minnesota) was conducted to determine significant differences in colour development intensity and functional diversity between leachate treatments.

5.4 Results and Discussion

The leachates generated in the lab were chemically similar to previous laboratory generated leachates (Chapter 3). Both leachates were characterized by high organic carbon contents, with C:N ratios of 77.6 and 133 for Jack Pine and Black Spruce, respectively, high oxygen demands, and relatively high amounts of dissolved solids. Both leachates were amber coloured (Table 5.3).

Table 5.3 Physical and chemical characteristics of Jack Pine and Black Spruce leachate generated by soaking logs in water for 30 days.

Analysis	Jack Pine Leachate	Black Spruce Leachate
BOD (mg L^{-1})	501	601
True Colour (ColourUnit)	90	275
Dissolved solids (mg L^{-1})	472	656
Suspended solids (mg L^{-1})	41	31
Total solids (mg L^{-1})	471	650
Total carbon (mg C L^{-1})	334	447
Inorganic carbon (mg C L^{-1})	8.7	7.9
Organic carbon (mg C L^{-1})	325	440
Total Kjeldahl nitrogen (mg N L^{-1})	4.87	3.85

†Values are the average of 2 duplicate samples. All analyses performed by Norwest Labs, Winnipeg.

The addition of Jack Pine and Black Spruce leachate to samples of the Ahe horizon of the Brunisol promoted a greater diversity and rate of substrate utilization in the Biolog™ plates compared to the control treatment in all cases (Table 5.4). Statistically significant differences were not detected between the two types of leachate treatments or between the various concentrations. One explanation for the higher number of substrates metabolized in the plates inoculated with leachate-treated soils is that microorganisms

capable of degrading these compounds may have been added with the leachate treatment. Another explanation for the increased activity is that the Brunisolic soil is naturally substrate limited. The total organic carbon content of the Ahe horizon was 0.17% (Table 5.2). The addition of a carbon rich leachate treatment may have caused the stimulation of the indigenous soil microbial community. It is important to re-emphasize that functional diversity, quantified by the number of substrates metabolized on the Biolog™ plate, does not necessarily equate to a greater number of organisms present in the microbial community. However, it does mean that there are organisms present which can metabolize a greater range of substrates, i.e. perform a greater range of metabolic functions within the soil. A greater rate of substrate utilization suggests that the microorganisms are more capable of readily metabolizing the specific compounds.

A different pattern of activity was detected in the Luvisolic soil samples. In contrast to the Brunisolic soil, the Luvisolic soils were not substrate limited. The total organic carbon contents of these soils were 2.41% and 37.7% for the Ae and LFH, respectively (Table 5.2). The addition of a substrate-rich leachate would probably not greatly affect the rate or numbers of substrates used. This was precisely what occurred in the Ae horizon; no significant differences were detected between the treatments for both diversity and rate of utilization (Table 5.4). Similarly, leachate and concentration did not have a significant effect on the diversity of substrates used in the LFH layer. However, treatment effects were observed for the rate of substrate utilization in the LFH samples (Table 5.4). The plates inoculated with the Black Spruce amended soil extract showed a greater rate of substrate utilization than the Jack Pine amended soil extract. As Black

Spruce trees are commonly found on this soil, the indigenous microbes might be better adapted to Black Spruce leachate and thus capable of metabolizing it more readily than the Jack Pine leachate. This lack of adaptation was also noted in a previous study, in which reduced microbial respiration was observed in samples of the LFH from a Luvisolic soil after treatment with Jack Pine leachate (Chapter 4). Increased microbial numbers may have also occurred as a result of the addition of substrate. The differences between rate of substrate utilization for the LFH layer and the Ae horizon of the Luvisol are probably owing to differences in number and/or activity of the soil microorganisms. It is reasonable to expect to find higher microbial numbers in the LFH layer of a soil than in the other horizons as this is where primary decomposition occurs (Swift et al. 1979). As well, leachate would naturally filter through the LFH before reaching the A horizon resulting in a greater exposure of the microbial population in the LFH to Black Spruce leachate.

Colour production in the control wells occurred in 4 out of the 42 plates inoculated with each of the Ahe and Ae soil extracts. In comparison, 7 of the 42 plates inoculated with a LFH soil extract had positive control wells (Table 5.5). In all cases, the control wells developed colour at a slower rate than for the plate in total. Colour production in the control wells occurred between 60 and 72 hours for each soil horizon, while average plate colour production occurred within 48 to 60 hours for the Ahe and Ae samples and within 24 to 36 hours for the LFH. The control wells did not contain carbon substrate initially, but did have the tetrazolium indicator dye. The positive responses in the control wells suggest that substrate may have been added with the soil extract supporting respiration

and subsequent colour production. The higher number of positive control wells in the LFH samples compared to the Ae and Ahe samples may be due to the relatively high percentage of substrate in this soil layer.

Table 5.4 Mean diversity and rate index of substrate utilization in Gram negative and Gram positive Biolog™ microtitre plates for: (a) the Ahe horizon of an Eluviated Dystric Brunisol; (b) the Ae horizon of an Orthic Gray Luvisol; and (c) the LFH layer of an Orthic Gray Luvisol, all treated with Jack Pine and Black Spruce leachates of varying concentrations.

			Diversity				Substrate Utilization Rate Index			
Soil Horizon		**Trt.**	**Gram Negative**		**Gram Positive**		**Gram Negative**		**Gram Positive**	
(a) Ahe Brunisol	Leachate	BS	83%	ns	78%	ns	2.82	ns	2.58	ns
		JP	81%	ns	74%	ns	2.74	ns	2.43	ns
	Conc.	25%	89%	a	83%	a	3.03	a	2.86	a
		5%	87%	a	80%	a	2.98	a	2.64	a
		1%	86%	a	84%	a	3.01	a	2.81	a
		0%	67%	b	57%	b	2.10	b	1.72	b
(b) Ae Luvisol	Leachate	BS	90%	ns	86%	ns	3.08	ns	2.90	ns
		JP	89%	ns	85%	ns	2.97	ns	2.83	ns
	Conc.	25%	89%	ns	82%	ns	3.08	ns	1.75	ns
		5%	90%	ns	87%	ns	3.04	ns	2.86	ns
		1%	89%	ns	88%	ns	3.04	ns	2.93	ns
		0%	89%	ns	85%	ns	2.97	ns	2.92	ns
(c) LFH Luvisol	Leachate	BS	98%	ns	95%	ns	4.42	a	4.36	a
		JP	97%	ns	95%	ns	4.22	b	4.24	b
	Conc.	25%	98%	ns	95%	ns	4.41	a	4.34	a
		5%	98%	ns	95%	ns	4.38	a	4.36	a
		1%	98%	ns	95%	ns	4.42	a	4.31	a
		0%	96%	ns	95%	ns	4.07	b	4.18	b

Means followed by different letters are significantly different at $p = 0.05$. Means are the average of 3 replicates.

Table 5.5 Control wells with colour development.

Soil	Treatment	Hours to Colour Production	Intensity Value	Plate Type
Ahe Brunisol	JP 1%	72	1	GP
	BS 25%	48	3	GN
	BS 5%	72	1	GP
	BS 1%	72	1	GP
Ae Luvisol	BS 25%	72	1	GN
	BS 25%	72	1	GP
	BS 1%	60	2	GP
	BS 1%	60	2	GP
LFH Luvisol	JP 5%	72	1	GP
	JP 1%	72	1	GN
	BS 25%	72	1	GN
	BS 25%	72	1	GN
	BS 25%	60	2	GN
	BS 25%	72	1	GP
	BS 5%	72	1	GP

The coefficients of variation associated with studies of soil biological populations are typically high. Often, they are greater than 50% of the mean (Bonmati et al. 1991). This high degree of variation may be due to the natural variation of biological systems and/or the methodological limitations of assessing them. The coefficients of variation associated with these analyses of functional diversity using the Biolog™ plates were relatively low compared to characteristic soil biological studies (Table 5.6). This type of assay by-passes typical methodological problems because it assesses the metabolic function of the soil as a whole. As well, the microbial population may demonstrate significant metabolic redundancy which would lower the variation. It should also be noted that results are replicable. Thirty-one substrates are duplicated on the Gram negative and Gram positive plates. A comparison of the duplicates shows that a positive match, i.e. a substrate used on one plate was also used on the other plate, occurred on average 95% of the time. Exact intensity matches for the duplicate substrates occurred 65% of the time.

Table 5.6 Coefficients of variation between replicate samples for three soil horizons treated with softwood leachate.

	Diversity		**Intensity**	
	GN	**GP**	**GN**	**GP**
Ahe Brunisol	9.0%	9.4%	12.0%	15.2%
Ae Luvisol	3.7%	4.6%	5.3%	4.7%
LFH Luvisol	1.6%	1.1%	2.6%	1.9%

5.5 Summary and Conclusions

Microorganisms play an integral role in nutrient cycling in soil. In a forest ecosystem, wood leachate naturally enters the soil environment, therefore the microorganisms present should be capable of metabolizing it. Yet it may be possible to overload the system resulting in toxic effects to the microbial population. This was not observed using leachate produced in the lab at a soaking ratio of 2.5:1 w/w by weight, although the concentrations used in this study were found to be toxic to aquatic life and luminescent bacteria (Microtox). Instead, the leachate added to the soil either had no effect or, in a few cases, stimulated the microbial population somewhat. Differences between the effects of the three leachate concentrations on functional diversity were not observed. Perhaps more concentrated treatments would produce different results. The results from this study indicate that in general, the metabolic functions of the soil microbial population, as assayed using the Biolog™ microtitre plate, were not negatively impacted by the single addition of softwood leachate.

6. GENERAL DISCUSSION

Wood-derived compounds have been identified as the major toxic constituents in pulp and paper mill effluents (Thakore et al. 1989; O'Connor et al. 1992; Wong et al. 1978; Easty et al. 1978; Leach and Thakore 1976; Walden and Howard 1977) and log water-storage areas (Karau 1975; Schaumburg 1973). Recently, it was determined that leachate derived from aspen logs is acutely toxic to aquatic life. One objective of this study was to determine whether softwood leachate, derived from Jack Pine and Black Spruce logs, was also toxic to aquatic organisms. The other objective was to determine the effects of softwood leachate on soil microbial processes. The effects of wood compounds in terrestrial environments have received little attention although the disposal of pulp and paper mill effluents and woodwaste leachates on soil has been recommended in British Columbia (Liu et al. 1995). It was determined in this study that concentrated wood leachate solutions are toxic to aquatic organisms and may cause negative effects in some soil environments.

Softwood leachates were generated by soaking Jack Pine and Black Spruce logs in water for periods of 2 to 60 days. In general, longer soaking periods produced more concentrated solutions due to extended leaching of wood-derived compounds from the wood into the water. The elevated electrical conductivities of the leachate solutions illustrated this point (Mackereth et al. 1978). Both softwood leachates were

characterized by acidic pH, high organic carbon contents and low dissolved oxygen concentrations. The increase in acidity over time in the leachates was attributed to the continued release of organic acids from the logs, although this was not confirmed. Leachate pH ranged from 6.3 to 3.6. As water with pH in the range of 4 to 5 can be acutely toxic to fish (Baker and Schofield 1982; EPA 1971), the undiluted leachate samples may themselves prove toxic to aquatic life. Depending on the characteristics of the receiving waters, the ability of the aquatic organisms to avoid acidic areas (Wells 1915; Gunn and Noakes 1986), and their ability to rebound from short exposures to acidic waters (EPA 1971), acidic softwood leachate could have varying impacts.

Organic carbon increased significantly with soaking time in each leachate treatment. The general effects of increased carbon content may be observed in other leachate parameters. For example, the biological oxygen demand (BOD) of each leachate increased with organic carbon concentration. As oxygen is required for the degradation of these compounds (Greenberg et al. 1992), a corresponding reduction in dissolved oxygen levels were observed. Waters containing high concentrations of either type of wood leachate would therefore not support aquatic life for extended periods of time.

Softwood leachates derived from Jack Pine and Black Spruce logs were acutely toxic to Rainbow Trout, *Daphnia*, and luminescent bacteria. Although the identities of the chemicals contained in each solution were not identified, it is likely that resin and fatty acids were toxic major constituents, as these compounds have been associated with the aquatic toxicity of softwood mechanical pulping effluents (O'Connor et al. 1992).

Previous studies have indicated that effluents derived from softwood wood-mixes are more toxic than those derived from hardwood (O'Connor et al. 1992). Leachate derived from hardwood (aspen) logs was observed to be extremely toxic to aquatic organisms. The LC50 values for Rainbow Trout were 1 to 5% (Taylor and Goudey 1992). In contrast, the LC50 values obtained for Rainbow Trout exposed to softwood leachate ranged from 21 to 29% and 31 to 100% for Black Spruce and Jack Pine, respectively. The hardwood leachate was generated by soaking aspen wood chips for 35 days in dechlorinated water at a soaking ration of 9:1 w/w, while the softwood leachates were generated by soaking intact log pieces for 30 days in dechlorinated water at a soaking ration of 2.5:1 w/w. The aspen leachate would presumably be more concentrated than the softwood leachates due to increased surface area and soaking time. Due to these differences in leachate generation method, direct comparisons between softwood and hardwood leachate toxicity cannot be made.

The effects of softwood leachate addition in soil were not as pronounced as in the water. The leachate concentrations used in both soil studies were selected based upon the results of the Microtox luminescent bacteria toxicity tests. The EC50 values for the 30 day leachate were 2.8% and 5.1% for Black Spruce and Jack Pine, respectively. Leachate solutions ranging from 1% to 25% were then selected for the soil studies. Organisms in water environments are almost immediately exposed to chemical and/or biological additions. Although dissolved organic matter and/or suspended solids may sorb some toxic compounds (Sprague 1985), it is likely that a large proportion will be taken in by aquatic organisms via respiration, ingestion or diffusion. Due to the nature of the soil environment, soil microorganisms should not be exposed as substantially. Soil colloids

provide numerous surfaces for sorption. As well, many pore spaces may not come into contact with leachate additions. Although the Microtox assay assesses toxic effects to the bacterial trophic level, the results from aquatic tests do not necessarily translate to effects in the terrestrial environment. It may be possible that more highly concentrated solutions than those used would cause greater effects than observed.

The microbially mediated processes of carbon and nitrogen mineralization in forest soils were not greatly affected by the addition of softwood leachate. The hypothesis of this study was that the addition of a carbon-rich substrate would cause increased microbial respiration and immobilization of N (White et al. 1988). This generally did not occur. Carbon mineralization, assessed via microbial respiration, was generally not affected on a weekly basis. The only exception to this occurred during the first three weeks of incubation of the LFH samples of the Luvisol treated with Jack Pine leachate; reduced respiration, compared to the control, was observed. Assessment of the cumulative respiration in each of the three soil horizons tested suggests that microbial respiration may have been affected by the addition of leachate, although statistical analysis was not possible. If these values are real, then the addition of softwood leachate promoted biological activity in the substrate-limited Ahe horizon of the Brunisolic soil, but caused a reduction in biological activity in the Ae horizon and the LFH layer of the Luvisolic soil. Jack Pine leachate solutions caused greater reductions in cumulative respiration in the Luvisolic soils. The indigenous microbial community in the Luvisolic soil would not have been adapted to this type of leachate, as Jack Pine trees usually do not grow on fine-textured Luvisolic soils.

The effects of leachate addition on soil N mineralization were similar to those on C mineralization. The single addition of softwood leachate had no effect on the mineralization of N in the N-deficient Ahe Brunisol. Although the process of mineralization occurred in both Luvisolic horizons throughout the incubation period, reduced levels of mineralization compared to the control were observed. In the Ae horizon, these reduced levels were not statistically significant. It can therefore be concluded that the single addition of softwood leachate had minimal impact on this process. However, the reductions in N mineralization in the Jack Pine leachate-treated LFH soils were significant. Leachate addition to the Luvisolic soils may result in a reduction in the overall soil biological activity.

The microbial functional diversity (metabolic diversity), as illustrated using Biolog™ microtitre plates, was either not affected or was slightly enhanced due to the addition of softwood leachate. In the Ahe Brunisol samples, a greater number of substrates were utilized at a faster rate in the plates inoculated with treated soil. This has been attributed to the fact that the Brunisol was substrate-limited; the addition of leachate may have stimulated the indigenous microbial population. In the Luvisolic soils, the number of substrates metabolized did not change with leachate addition. However, the rate at which they were metabolized increased in the LFH samples. This seems to contradict the results from the previous study, in which negative and/or toxic effects were seen at the soil microbial level. It must be re-emphasized that in the method used (Biolog™ plates), the numbers and identities of the microorganisms present were not determined. It is therefore possible that the leachates may have been toxic to certain species, allowing new species the opportunity to perform the same metabolic functions; soil microbial systems have the capacity for metabolic redundancy.

7. SUMMARY AND CONCLUSIONS

The findings of this study are summarized below:

1. Softwood leachates derived from Jack Pine and Black Spruce logs are characterized by acid pH, low dissolved oxygen, and high organic carbon contents.

2. Softwood leachates, generated by soaking Jack Pine and Black Spruce log pieces at a 2.5:1 water : wood ratio w/w for 2 to 60 days, were toxic to aquatic life. The mean mortalities of Rainbow Trout exposed to 50% leachate by volume, the highest concentration tested, were 87% and 63% for Black Spruce and Jack Pine, respectively. The average LC50 values obtained in the *Daphnia* toxicity tests were 53% and 66% for Black Spruce and Jack Pine, respectively, while the average EC50 values in the Microtox assay were 8.58% and 11.2% for Black Spruce and Jack Pine, respectively. Leachate toxicity generally increased with log soaking duration at each trophic level.

3. Softwood leachate generated at an outdoor log pile after relatively small rain events was toxic to *Daphnia* and luminescent bacteria. Larger rain events produced leachates which tended to be less toxic or non-toxic due to increased dilution.

4. The single addition of Black Spruce and Jack Pine leachates to samples of the Ahe horizon of a Brunisolic soil produced no significant changes in C mineralization on

either a weekly or cumulative basis. N mineralization was also not affected by leachate treatment. The apparent lack of treatment effects may have been caused by: 1. An insufficient amount of C added to the soil; 2. Possible recalcitrance of the C compounds present in the leachate compared to native soil C compounds; and 3. Poor statistical sensitivity of the analyses combined with the natural variation of the data.

5. The single addition of Black Spruce and Jack Pine leachates to samples of the Ae horizon of a Luvisolic soil produced no significant changes in C mineralization on a weekly basis. In the LFH layer, reduced respiration over the first three weeks of incubation occurred in the Jack Pine leachate-treated samples. This reduction was probably due to a lack of adaptation of the indigenous soil microbial population in the Luvisol to Jack Pine leachate as this tree species does not grow on this type of soil. The cumulative respiration data illustrates that Jack Pine leachate may have caused toxic effects in both Luvisolic soil horizons as an apparent reduction in the cumulative C mineralization occurred. The effects of the Black Spruce leachate were not as pronounced. The single addition of softwood leachate had minimal impact on N processes in the Ae horizon of the Luvisolic soil, while N mineralization was reduced in the leachate-treated LFH samples.

6. The functional diversity of the Ahe horizon of the Brunisol was enhanced by the addition of softwood leachate. No effect of the leachate was observed in the Ae Luvisol, but substrates were utilized at a faster rate in the plates inoculated with leachate amended LFH Luvisol samples.

Softwood leachate was acutely toxic to all aquatic life tested. Log storage yards should be located such that leachate drainage does not reach adjacent water bodies to ensure the protection of aquatic life. The effects of softwood leachate addition to soil were more varied. This study determined that leachate derived from Jack Pine logs causes reductions in biological activity in fine-textured Luvisolic soils. Jack Pine log storage areas should therefore not be located on this type of soil. As Jack Pine trees do not normally grow on this type of soil, this recommendation should not be difficult to implement.

The leachate concentrations used in both soil studies, which ranged from 1% to 25%, were selected based upon the results of Microtox aquatic toxicity tests, in which EC50 values were 2.8% and 5.1% for Black Spruce and Jack Pine, respectively. The impacts of softwood leachate on soil microorganisms should be less than those on aquatic organisms due to the nature of the soil habitat; wood-derived compounds may become sorbed to soil particles. Therefore, soil microorganisms would not be exposed to toxic compounds to the same extent as would organisms in aquatic environments. Future studies on the effects of softwood leachate in soil should therefore include more concentrated leachates than those used in this study. As well, a study on the effects of leachate percolation through soil on aquatic toxicity would be very informative. This would verify whether buffer zones between log piles and water bodies would be sufficient for the protection of aquatic life, or if other measures, such as leachate containment and treatment, are required.

8. CONTRIBUTION TO KNOWLEDGE

The negative effects of pulp and paper mill effluents in aquatic environments have been the focus of much research. Many of the toxic compounds found in these effluents are derived from wood. It was recently determined that significant quantities of these toxic compounds can be released from aspen log storage piles. This study, requested by Manitoba's Clean Environment Commission, determined that leachates derived from Jack Pine and Black Spruce logs are also toxic to aquatic organisms.

Very little work has been performed on the effects of wood compounds in soil. This is despite the fact that guidelines have been drafted for the disposal of pulp and paper mill effluents and wood waste leachates on soil in British Columbia. It was assumed that these materials would be naturally attenuated in the soil environment. We have determined that softwood leachates may also exert toxic effects in some soil environments. Changes observed in soil microbial processes may indicate impacts in the soil environment as a whole.

The results of this study provides useful information for forest managers. Guidelines and/or plans for the placement of log storage piles should be implemented as large volumes of concentrated leachate solutions may be generated from log storage areas after precipitation events.

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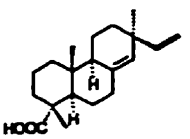
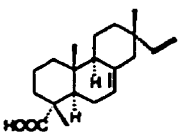
10. APPENDICES

Appendix I. Structures of common resin and fatty acids.

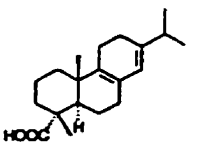
Resin Acids



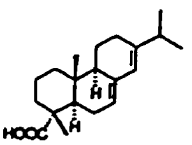
Pimaric Acid



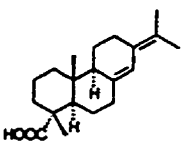
Isopimaric Acid



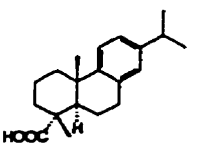
Palustric Acid



Abietic Acid



Neoabietic Acid



Dehydroabietic Acid

Fatty Acids

Linoleic Acid	$CH_3(CH_2)_4CH{=}CH\text{-}CH_2\text{-}CH{=}CH(CH_2)_7COOH$
Linolenic Acid	$CH_3\text{-}(CH_2\text{-}CH{=}CH)_3(CH_2)_7COOH$
Oleic Acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Palmitic Acid	$CH_3(CH_2)_{14}COOH$

Appendix IIa. Jack Pine logs used in field leachate study at Glenlea, Manitoba July 1995.

Jack Pine Logs (approximately 2.4 m long)					
Number	Diameter (m)	Approximate Surface Area (m^2) Ends	Cylindrical	Total	Volume (m^3)
1	0.065	0.007	0.490	0.497	0.730
2	0.135	0.029	1.02	1.05	3.15
3	0.090	0.013	0.679	0.691	1.40
4	0.085	0.011	0.641	0.652	1.25
5	0.120	0.023	0.905	0.927	2.49
6	0.100	0.016	0.754	0.770	1.73
7	0.140	0.031	1.06	1.09	3.39
8	0.145	0.033	1.09	1.13	3.63
9	0.090	0.013	0.679	0.691	1.40
10	0.145	0.033	1.09	1.13	3.63
11	0.120	0.023	0.905	0.927	2.49
12	0.105	0.017	0.792	0.809	1.90
13	0.125	0.025	0.942	0.967	2.70
14	0.175	0.048	1.32	1.37	5.29
15	0.070	0.008	0.528	0.535	0.847
16	0.150	0.035	1.13	1.17	3.89
17	0.115	0.021	0.867	0.888	2.29
18	0.070	0.008	0.528	0.535	0.847
19	0.150	0.035	1.13	1.17	3.89
20	0.190	0.057	1.43	1.49	6.24
21	0.110	0.019	0.829	0.848	2.09
22	0.145	0.033	1.09	1.13	3.63
23	0.135	0.029	1.02	1.05	3.15
24	0.110	0.019	0.829	0.848	2.09
25	0.135	0.029	1.02	1.05	3.15
26	0.195	0.060	1.47	1.53	6.57
27	0.160	0.040	1.21	1.25	4.42
28	0.165	0.043	1.24	1.29	4.70
29	0.070	0.008	0.528	0.535	0.847
30	0.115	0.021	0.867	0.888	2.29
Total	**Average**	**Total**	**Total**	**Total**	**Total**
30	0.1	0.783	28.1	28.9	86.1

Appendix IIb. Jack Pine logs used in field leachate study at Glenlea, Manitoba June 1996.

Jack Pine Logs (approximately 2.4 m long)					
Number	Diameter (m)	Approximate Surface Area (m^2) Ends	Cylindrical	Total	Volume (m^3)
1	0.092	0.013	0.694	0.707	0.016
2	0.096	0.014	0.724	0.738	0.017
3	0.131	0.027	0.988	1.01	0.032
4	0.110	0.019	0.829	0.848	0.023
5	0.149	0.035	1.12	1.16	0.042
6	0.158	0.039	1.19	1.23	0.047
7	0.085	0.011	0.641	0.652	0.014
8	0.175	0.048	1.32	1.37	0.058
9	0.100	0.016	0.754	0.770	0.019
10	0.147	0.034	1.11	1.14	0.041
11	0.183	0.053	1.38	1.43	0.063
12	0.124	0.024	0.935	0.959	0.029
13	0.163	0.042	1.23	1.27	0.050
14	0.115	0.021	0.867	0.888	0.025
15	0.148	0.034	1.12	1.15	0.041
16	0.182	0.052	1.37	1.42	0.062
17	0.153	0.037	1.15	1.19	0.044
18	0.153	0.037	1.15	1.19	0.044
19	0.110	0.019	0.829	0.848	0.023
20	0.150	0.035	1.13	1.17	0.042
21	0.077	0.009	0.581	0.590	0.011
22	0.145	0.033	1.09	1.13	0.040
23	0.133	0.028	1.00	1.03	0.033
24	0.095	0.014	0.716	0.730	0.017
25	0.095	0.014	0.716	0.730	0.017
26	0.133	0.028	1.00	1.03	0.033
27	0.140	0.031	1.06	1.09	0.037
28	0.100	0.016	0.754	0.770	0.019
29	0.163	0.042	1.23	1.27	0.050
30	0.087	0.012	0.656	0.67	0.014
31	0.131	0.027	0.988	1.01	0.032
32	0.070	0.008	0.528	0.535	0.009
33	0.130	0.027	0.980	1.01	0.032
34	0.057	0.005	0.430	0.435	0.006
35	0.142	0.032	1.07	1.10	0.038
36	0.065	0.007	0.490	0.497	0.008
37	0.110	0.019	0.829	0.848	0.023
Total	**Average**	**Total**	**Total**	**Total**	**Total**
30	0.1	0.837	29.3	30.2	0.032

Appendix IIc.Black Spruce logs used in field leachate study at Glenlea, Manitoba July 1995.

Black Spruce Logs (approximately 2.4 m long)					
Number	Diameter (m)	Approximate Surface Area (m^2) Ends	Cylindrical	Total	Volume (m^3)
1	0.125	0.025	0.942	0.967	1.47
2	0.120	0.023	0.905	0.927	1.36
3	0.105	0.017	0.792	0.809	1.04
4	0.120	0.023	0.905	0.927	1.36
5	0.120	0.023	0.905	0.927	1.36
6	0.155	0.038	1.17	1.21	2.26
7	0.125	0.025	0.942	0.967	1.47
8	0.180	0.051	1.36	1.41	3.05
9	0.060	0.006	0.452	0.458	0.339
10	0.095	0.014	0.716	0.730	0.851
11	0.120	0.023	0.905	0.927	1.36
12	0.075	0.009	0.565	0.574	0.530
13	0.145	0.033	1.09	1.13	1.98
14	0.120	0.023	0.905	0.927	1.36
15	0.090	0.013	0.679	0.691	0.763
16	0.120	0.023	0.905	0.927	1.36
17	0.105	0.017	0.792	0.809	1.04
18	0.110	0.019	0.829	0.848	1.14
19	0.145	0.033	1.09	1.13	1.98
20	0.095	0.014	0.716	0.730	0.851
21	0.135	0.029	1.02	1.05	1.72
22	0.125	0.025	0.942	0.967	1.47
23	0.100	0.016	0.754	0.770	0.942
24	0.085	0.011	0.641	0.652	0.681
25	0.105	0.017	0.792	0.809	1.04
26	0.080	0.010	0.603	0.613	0.603
27	0.115	0.021	0.867	0.888	1.25
28	0.150	0.035	1.13	1.17	2.12
29	0.120	0.023	0.905	0.927	1.36
30	0.160	0.040	1.21	1.25	2.41
31	0.100	0.016	0.754	0.770	0.942
32	0.130	0.027	0.980	1.01	1.59
33	0.130	0.027	0.980	1.01	1.59
34	0.070	0.008	0.528	0.535	0.462
35	0.070	0.008	0.528	0.535	0.462
36	0.100	0.016	0.754	0.770	0.942
37	0.125	0.025	0.942	0.967	1.47
38	0.150	0.035	1.13	1.17	2.12
39	0.060	0.006	0.452	0.458	0.339
40	0.095	0.014	0.716	0.730	0.851
Total	**Average**	**Total**	**Total**	**Total**	**Total**
40	0.113	0.855	34.2	35.0	51.3

Appendix IId. Black Spruce logs used in field leachate study at Glenlea, Manitoba June 1996.

Black Spruce Logs (approximately 2.4 m long)					
Number	Diameter	Approximate Surface Area (m^2)			Volume
	m	Ends	Cylindrical	Total	(m^3)
1	0.140	0.031	1.06	1.09	0.037
2	0.101	0.016	0.762	0.778	0.019
3	0.180	0.051	1.36	1.41	0.061
4	0.123	0.024	0.927	0.951	0.029
5	0.119	0.022	0.897	0.919	0.027
6	0.128	0.026	0.97	0.991	0.031
7	0.142	0.032	1.07	1.10	0.038
8	0.122	0.023	0.920	0.943	0.028
9	0.096	0.014	0.724	0.738	0.017
10	0.072	0.008	0.543	0.551	0.010
11	0.160	0.040	1.21	1.25	0.048
12	0.125	0.025	0.942	0.967	0.029
13	0.109	0.019	0.822	0.841	0.022
14	0.128	0.026	0.965	0.991	0.031
15	0.086	0.012	0.648	0.660	0.014
16	0.098	0.015	0.739	0.754	0.018
17	0.132	0.027	1.00	1.02	0.033
18	0.160	0.040	1.21	1.25	0.048
19	0.084	0.011	0.633	0.644	0.013
20	0.124	0.024	0.935	0.959	0.029
21	0.095	0.014	0.716	0.730	0.017
22	0.087	0.012	0.656	0.668	0.014
23	0.130	0.027	0.980	1.01	0.032
24	0.137	0.029	1.03	1.06	0.035
25	0.155	0.038	1.17	1.21	0.045
26	0.144	0.033	1.09	1.12	0.039
27	0.164	0.042	1.24	1.28	0.051
28	0.192	0.058	1.45	1.51	0.069
29	0.092	0.013	0.694	0.707	0.016
30	0.125	0.025	0.942	0.967	0.029
31	0.088	0.012	0.664	0.676	0.015
32	0.083	0.011	0.626	0.637	0.013
33	0.145	0.033	1.09	1.13	0.040
34	0.133	0.028	1.00	1.03	0.033
35	0.054	0.005	0.407	0.412	0.005
36	0.145	0.033	1.09	1.13	0.040
37	0.110	0.019	0.829	0.848	0.023
38	0.125	0.025	0.942	0.967	0.029
39	0.116	0.021	0.875	0.896	0.025
Total	**Average**	**Total**	**Total**	**Total**	**Total**
39	0.122	0.962	35.8	36.8	1.15

Appendix III. Detailed description of physical and chemical analyses performed on leachate samples by the Environmental Sciences Centre (ESC), Winnipeg.

1. The total coliform concentration was estimated using the most probable number (MPN) multiple tube fermentation procedure. The results of the examination of the replicate tubes and dilutions were reported as the MPN of organisms present per 100 mL. This number is based upon a Poisson distribution (random dispersion) and is therefore an estimate of the mean density of coliform bacteria present in a sample. The coliform group is defined as all aerobic, and facultative anaerobic, Gram-negative, non-spore forming, rod shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C. The presence of the coliform group was confirmed by the formation of gas in Brilliant Green Bile Broth (ESC Principle of Method A152.01; Greenberg et al 1992).

2. Biochemical oxygen demand (BOD) is the quantity of oxygen required for biological and chemical oxidation of water-borne organic substances under specific test conditions. BOD was determined by comparing the dissolved oxygen content of a sample at the beginning and the end of a 5-day incubation period. Oxygen concentration was measured using an oxygen probe (ESC Principle of Method A006.01; Greenberg et al 1992).

3. The chemical oxygen demand was determined by oxidizing all organic compounds in the sample with potassium dichromate and sulphuric acid. The excess dichromate was titrated with ferrous ammonium sulphate. The amount of organic material susceptible to oxidation is proportional to the potassium dichromate consumed (ESC Principle of Method A007.01; Greenberg et al 1992).

4. Total solids were determined by weighing a portion of the sample after drying to constant weight at 105°C. The residue retained after passing a measured volume of the sample through a Whatman 934-AH glass microfibre filter (1.5μm) constituted the total suspended solids, while the dissolved solids were assessed by passing the sample through Whatman GF/C glass microfibre filter (1.2μm) and drying to constant weight (ESC Principle of Method A009.02; Greenberg et al 1992).

5. Total carbon (TC) was assessed by injecting the sample into a combustion tube, thermostatically controlled at 680°C, containing an oxidative catalyst. The water in the sample was vaporized and the organic carbon was oxidized to carbon dioxide. A high purity carrier gas carried the combustion product from the TC combustion tube, through an inorganic carbon reactor vessel, where the products were cooled and dried by a dehumidifier, to a non-dispersive infrared gas analyzer (NDIR) for carbon dioxide detection. Analog output from the NDIR generated a peak whose area was calculated by a calibrated data processor. Total inorganic carbon (IC) was determined by injecting the sample into an IC reactor vessel containing 25% phosphoric acid (H_3PO_4). Under acidic conditions, only the IC component in the sample is decomposed to carbon dioxide. A carrier gas transports the CO_2 to the NDIR for detection and quantification. Total organic

carbon in the sample was calculated as the total carbon content less the inorganic carbon content (ESC Principle of Method A609.03; Greenberg et al 1992).

6. Total Kjeldahl nitrogen was assessed via digestion of the sample with a sulphuric acid solution containing perchloric acid and selenium dioxide catalysts. This converted organic nitrogen to ammonium sulphate. The digest was neutralized with sodium hydroxide solution and treated with alkaline phenol and sodium hypochlorite reagent to form a blue colour, designated as indophenol. Sodium nitroprusside was then added as a catalyst. The colour intensity at 630 nm is proportional to the total Kjeldahl nitrogen concentration (ESC Principle of Method A217.02; Greenberg et al 1992).

7. Total phosphorous was determined by digestion of the sample with a sulphuric acid-persulfate mixture to release the organically bound P as phosphate. Digestion with acid hydrolyzed polyphosphate to orthophosphate which reacted with ammonium molybdate to form heteropoly molybdophosphoric acid. This was reduced with stannous chloride in an aqueous sulphuric acid medium to form molybdenum blue, which was then measured colourimetrically at 660 nm (ESC Principle of Method A208.02; Greenberg et al 1992).

8. True colour was measured by visual comparison against a colour disc. The disc was routinely calibrated against chloroplatinate standards where 1 unit of colour was produced by 1 mgL^{-1} platinum in the form of the platinate ion. A range of standards from 5 to 50 colour units was used. Samples were subject to centrifugation prior to analysis to remove turbidity (ESC Principle of Method A001.01; Greenberg et al 1992).

Appendix IVa. Rainbow trout toxicity test using 2 day leachate (Trial 1). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		1.5		7.5		12		24		29		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10	3	7	1	6	6	0		0		0	10	100
50%	2		10		10		10		10	4	6	1	5	4	1	1	0		0	10	100
37.5%	1		10		10		10		10		10		10	5	5	5	0		0	10	100
37.5%	2		10		10		10		10	1	9		9	4	5	3	2	2	0	10	100
25%	1		10		10		10		10		10		10	3	7	5	1			8	80
25%	2		10		10		10		10		10		10	2	8	7	1			9	90
12.5%	1		10		10		10		10		10		10	1	9		8			2	20
12.5%	2		10		10		10		10		10		10		11	3	7			3	30
6.25%	1		10		10		10		10		10		10		10	1	9			2	20
6.25%	2		10		10		10		10		10		10		10		10			0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		1.5		7.5		12		24		29		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1	10		10		10		10		5	5	0	5	4	1	1	0		0	10	100
50%	2	10		10		10		10			10	2	8	1	7	2	5	1	4	6	60
37.5%	1	10		10		10		10			10		10		10		10			0	0
37.5%	2	10		10		10		10			10		10	2	8	2	6	5	1	9	90
25%	1	10		10		10		10			10		10		10	1	9		9	1	10
25%	2	10		10		10		10			10		10	2	8	1	7	1	6	4	40
12.5%	1	10		10		10		10			10		10	1	9		9	1	8	2	20
12.5%	2	10		10		10		10			10		10	1	9	2	7	2	5	5	50
6.25%	1	10		10		10		10			10		10		10		10	1	9	1	10
6.25%	2	10		10		10		10			10		10		10		10			0	0

Control Water

Conc.	Tank #	Time (Hours) 0		1.5		7.5		12		24		29		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1	10		10		10		10		10		10		10		10		10		0	0
0%	2	10		10		10		10		10		10		10		10		10		0	0

Appendix IVb. Rainbow trout toxicity test using 7 day leachate (Trial 1). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		1		4		12		24		48		52		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10	2	8	8	0		0		0		0	10	100
50%	2		10		10		10		10	4	6	6	0		0		0		0	10	100
37.5%	1		10		10		10		10	3	7	6	1	1	0		0		0	10	100
37.5%	2		10		10		10		10		10	8	2	1	1		1	1	0	10	100
25%	1		10		10		10		10		10		10		10	2	8	1	7	3	30
25%	2		10		10		10		10		10	6	4	1	3	2	1		1	9	90
12.5%	1		9		9		9		9		9		9		9		9	1	8	1	11
12.5%	2		11		11		11		11		11		11		11	2	9		9	2	18
6.25%	1		10		10		10		10		10		10		10		10	1	9	1	10
6.25%	2		10		10		10		10		10	1	9		9		9		9	1	10

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		1		4		12		24		48		52		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10		10		10		10		10	2	8	2	20
50%	2		10		10		10		10		10	3	7		7	2	5	1	4	6	60
37.5%	1		10		10		10		10		10		10		10		10		10	0	0
37.5%	2		10		10		10		10		10		10		10		10	1	9	1	10
25%	1		10		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10		10	1	9	1	10
12.5%	1		10		10		10		10		10		10		10		10	1	9	1	10
12.5%	2		10		10		10		10		10		10	1	9		9		9	1	10
6.25%	1		10		10		10		10		10		10		10		10	1	9	1	10
6.25%	2		10		10		10		10		10		10		10		10		10	0	0

Control Water

Conc.	Tank #	Time (Hours) 0		1		4		12		24		48		52		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1	10			10		10		10		10		10				10	7	3	7	70
0%	2	10			10		10		10		10		10				10	1	9	1	10

Appendix IVc. Rainbow trout toxicity test using 14 day leachate (Trial 1). (D = dead; A = alive).

Black Spruce Leachate

		Time (Hours)																		Mortality	
Conc.	Tank #	0		1		5		12		24		47		51		72		96			
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10		10	1	9	1	8	4	4	1	3	7	70
50%	2		10		10		10		10		10	3	7	1	6	2	4		4	6	60
37.5%	1		10		10		10		10		10		10		10		10		10	0	0
37.5%	2		10		10		10		10	1	9		9		9	9	0		0	10	100
25%	1		10		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10	1	9		9		9		9	1	10
12.5%	1		10		10		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10		10		10	0	0
6.25%	1		10		10		10		10		10		10		10		10		10	0	0
6.25%	2		10		10		10		10		10		10		10		10		10	0	0

Jack Pine Leachate

		Time (Hours)																		Mortality	
Conc.	Tank #	0		1		5		12		24		47		51		72		96			
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10		10		10		10		10	1	9	1	10
50%	2		10		10		10		10	1	9		9	1	8		8	1	7	3	30
37.5%	1		10		10		10		10		10		10		10		10		10	0	0
37.5%	2		10		10		10		10		10		10		10		10		10	0	0
25%	1		10		10		10		10		10		10		10		10	1	9	1	10
25%	2		10		10		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10		10		10	0	0
6.25%	1		10		10		10		10		10		10		10		10		10	0	0
6.25%	2		10		10		10		10		10		10		10		10		10	0	0

Control Water

		Time (Hours)																		Mortality	
Conc.	Tank #	0		1		5		12		24		47		51		72		96			
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10		10		10		10		10		10		10	0	0
0%	2		10		10		10		10		10		10		10		10		10	0	0

Appendix IVd. Rainbow trout toxicity test using 30 day leachate (Trial 1). (D = dead; A = alive).

Black Spruce Leachate

		Time (Hours)																	
		0		1		12		25		48		53		73.5		96		Mortality	
Conc.	Tank #	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10	10	0		0		0		0		0	10	100
50%	2		10		10		10	10	0		0		0		0		0	10	100
37.5%	1		10		10	1	9	5	6	6	0		0		0		0	10	100
37.5%	2		10		10		10		10	4	6	1	5	3	2	1	1	9	90
25%	1		10		10		10		10		10	1	9		9		9	1	10
25%	2		10		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10	1	9		9	1	10
6.25%	1		10		10		10		10		10		10		10		10	0	0
6.25%	2		10		10		10		10		10		10		10		10	0	0

Jack Pine Leachate

		Time (Hours)																	
Conc.	Tank #	0		1		12		25		48		53		73.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10		10		10		10	1	9	1	10
50%	2		10		10		10		10		10		10	1	10	2	7	3	30
37.5%	1		10		10		10		10		10		10		10	0	10	0	0
37.5%	2		10		10		10		10		10		10	1	9	0	6	4	40
25%	1		10		10		10		10		10		10			0	10	0	0
25%	2		10		10		10		10		10		10			0	10	0	0
12.5%	1		10		10		10		10	1	9		9			0	9	1	10
12.5%	2		10		10		10		10		10		10			0	10	0	0
6.25%	1		10		10		10		10		10		10			0	10	0	0
6.25%	2		10		10		10		10		10		10			0	10	0	0

Control Water

		Time (Hours)																	
Conc.	Tank #	0		1		12		25		48		53		73.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		9		9		9		9		9		9		9	0	9	0	0
0%	2		9		9		9		9		9		9		9	0	9	0	0

Appendix IVe. Rainbow trout toxicity test using 30 day leachate (Trial 1). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		0.5		1		4		9		49		73.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10	7	4	4	0		0		0		0		0		0	10	100
50%	2		10	5	5	5	0		0		0		0		0		0	10	100
37.5%	1		10	1	10	9	0		0		0		0		0		0	10	100
37.5%	2		9		9	8	1	1	0		0		0		0		0	10	100
25%	1		9		9		9		9	9	0		0		0		0	10	100
25%	2		10		10		10		10	10	0		0		0		0	10	100
12.5%	1		10		10		10		10		10		10	1	9		9	1	10
12.5%	2		10		10		10		10		10		10		10		10	0	0
6.25%	1		10		10		10		10		10		10		10		10	0	0
6.25%	2		10		10		10		10		10		10		10		10	0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		0.5		1		4		9		49		73.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10		10		10	1	9		9	1	10
50%	2		10		10		10		10		10	2	8	1	7	1	6	4	40
37.5%	1		10		10		10		10		10		10		10		10	0	0
25%	1		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10	1	9		9	1	10
12.5%	1		10		10		10		10		10		10		10		10	0	0
12.5%	2		9		9		9		9		9		9		9		9	0	0
6.25%	1		10		10		10		10		10		10		10		10	0	0
6.25%	2		10		10		10		10		10		10		10		10	0	0

Control Water

Conc.	Tank #	Time (Hours) 0		0.5		1		4		9		49		73.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10		10		10		10		10		10	0	0
0%	2		10		10		10		10		10		10		10		10	0	0

Appendix IVf. Rainbow trout toxicity test using 2 day leachate (Trial 2). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		0.5		7.25		12.75		25.75		52		52.5		53.25		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10	1	9		9	2	7		7		7	1	6	4	40
50%	2		10		10		10		10		10		10		10		10		10		10	0	0
37.5%	1		10		10		10		10		10		10		10		10	1	9		9	1	10
37.5%	2		9		9		9		9		9		9		9		9		9	1	8	1	10
25%	1		10		10		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10		10		10		10	0	0
16.6%	1		10		10		10		10		10		10		10		10		10		10	0	0
16.6%	2		10		10		10		10		10	1	9		9		9		9		9	1	10
12.5%	1		10		10		10		10		10		10		10		10		10	1	9	1	10
12.5%	2		9		9		9		9		9		9		9		9		9		9	0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		0.5		7.25		12.75		25.75		52.0		52.5		53.3		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
66.6%	1		9		9		9		9		9		9		9		9	4	5	4	1	8	80
66.6%	2		9		9		9		9		9		9	2		5	5	2	0			9	90
50%	1		9		9		9		9		9		9		9		9		9			0	0
50%	2		9		9		9		9		9		9		9		9	2	7			2	20
37.5%	1		9		9		9		9		9		9		9		9		9			0	0
37.5%	2		9		9		9		9		9		9		9		9	1	8			1	10
25%	1		9		9		9		9		9		9		9		9		9			0	0
25%	2		9		9		9		9		9	1	8		8		8	1	7			2	20
12.5%	1		9		9		9		9		9		9		9		9		9			0	0
12.5%	2		9		9		9		9		9		9		9		9		9			0	0

Control Water

Conc.	Tank #	Time (Hours) 0		0.5		7.25		12.75		25.75		52.0		52.5		53.3		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10				10		10		10		10		10	1	9	1	10
0%	2		10		10		10				10		10		10		10		10			0	0

Appendix IVg. Rainbow trout toxicity test using 7 day leachate (Trial 2). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		7		13.25		25		49.5		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10	4	6	2	4	1	3	7	70
50%	2		10		10		10		10	4	6	4	2	2	0	10	100
37.5%	1		10		10		10		10		10		10		10	0	0
37.5%	2		10		10		10		10		10		10		10	0	0
25%	1		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10	0	0
16.6%	1		10		10		10		10		10		10		10	0	0
16.6%	2		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10	0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		7		13.25		25		49.5		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
66.6%	1		10		10		10	4	6	6	0		0		0	10	100
66.6%	2		10		10		10	6	4	4	0		0		0	10	100
50%	1		10		10		10		10	9	1		1		1	9	90
50%	2		10		10		10	1	9	7	2	2	0		0	10	100
37.5%	1		10		10		10		10	1	9	1	8		8	2	20
37.5%	2		10		10		10		10		10	1	9		9	1	10
25%	1		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10	0	0

Control Water

Conc.	Tank #	Time (Hours) 0		7		13.25		25		49.5		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10				10		10		10	0	0
0%	2		10		10		10				10		10		10	0	0

Appendix IVh. Rainbow trout toxicity test using 14 day leachate (Trial 2). (D = dead; A = alive).

Black Spruce Leachate

		Time (Hours)																			
Conc.	Tank #	0		0.5		4.25		11		24		29.25		48		60		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10		10		10	10	0		0		0		0		0	0	0	10	100
50%	2		10		10		10		10	10	0		0		0		0		0	0	0	10	100
37.5%	1		10		10		10		10	1	9	1	8	4	4	1	3		3	1	2	8	80
37.5%	2		10		10		10		10	1	9	4	5	4	1		1		1		1	9	90
25%	1		11		11		11		11		11		11		11		11		11		11	0	0
25%	2		10		10		10		10		10		10		10		10		10		10	0	0
16.6%	1		9		9		9		9		9		9		9		9		9		9	0	0
16.6%	2		12		12		12		12		12		12		12		12		12		12	0	0
12.5%	1		10		10		10		10		10		10		10		10		10		10	0	0
12.5%	2		11		11		11		11		11		11		11		11		11		11	0	0

Jack Pine Leachate

		Time (Hours)																			
Conc.	Tank #	0		0.5		4.25		11		24		29.25		48		60		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
66.6%	1		10		10		10	10	0		0		0		0		0		0		0	10	100
50%	1		13		13		13		13		13		13	5	7	4	3	2	1	1	0	13	100
50%	2		10		10		10		10		10		10	9	1	1	0		0		0	10	100
37.5%	1		10		10		10		10		10		10		10		10		10		10	0	0
37.5%	2		10		10		10		10		10		10		10		10		10		10	0	0
25%	1		10		10		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10	10	0		0			10	100
12.5%	2		10		10		10		10		10		10		10		10		10			0	0

Control Water

		Time (Hours)																			
Conc.	Tank #	0		0.5		4.25		11		24		29.25		48		60		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10		10		10		10		10		10		10		10	0	0
0%	2		10		10		10		10		10		10		10		10		10		10	0	0

Appendix IVi. Rainbow trout toxicity test using 30 day leachate (Trial 2). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		0.5		4.5		12		24.5		48		72.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10		10	10	0		0		0		0		0		0	10	100
50%	2		12		12	12	0		0		0		0		0		0	12	100
37.5%	1		10		10		10	5	5	4	1	1	0		0		0	10	100
37.5%	2		10		10		10	4	6	6	0		0		0		0	10	100
25%	1		10		10		10		10		10	5	5	2	3		3	7	70
25%	2		10		10		10		10		10	7	3	2	1		1	9	90
16.6%	1		10		10		10		10		10		10		10		10	0	0
16.6%	2		10		10		10		10		10		10		10		10	0	0
12.5%	1		11		11		11		11		11		11		11		11	0	0
12.5%	2		11		11		11		11		11		11		11		11	0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		0.5		4.5		12		24.5		48		72.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
66.6%	1		10		10	10	0		0		0		0		0		0	10	100
50%	1		10		10	2	8	8	0		0		0		0		0	10	100
50%	2		10		10	1	9	9	0		0		0		0		0	10	100
37.5%	1		10		10		10		10	2	8	2	6	6	0		0	10	100
37.5%	2		10		10		10		10	4	6	2	4	3	1		1	9	90
25%	1		10		10		10		10		10		10		10		10	0	0
25%	2		10		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10		10	0	0

Control Water

Conc.	Tank #	Time (Hours) 0		0.5		4.5		12		24.5		48		72.5		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10				10		10		10		10		10		10	0	0
0%	2		10				10		10		10		10		10		10	0	0

Appendix IVj. Rainbow trout toxicity test using 30 day leachate (Trial 2). (D = dead; A = alive).

Black Spruce Leachate

Conc.	Tank #	Time (Hours) 0		1		1.5		1.75		4		6.25		12.5		24		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10	6	4		4	2	2	2	0		0		0		0		0		0		0	10	100
50%	2		10	1	9		9	3	6	6	0		0		0		0		0		0		0	10	100
37.5%	1		10		10		10		10		10		10	10	0		0		0		0		0	10	100
37.5%	2		10		10		10		10		10		10	10	0		0		0		0		0	10	100
25%	1		11		11		11		11		11		11		11	2	9	9	0		0		0	11	100
25%	2		10		10		10		10		10		10		10	2	8	8	0		0		0	10	100
16.6%	1		10		10		10		10		10		10		10		10		10		10		10	0	0
16.6%	2		10		10		10		10		10		10		10	1	9	1	8		8			2	20
12.5%	1		10		10		10		10		10		10		10		10	1	9	1	8			2	20
12.5%	2		10		10		10		10		10		10		10		10		10		10			0	0

Jack Pine Leachate

Conc.	Tank #	Time (Hours) 0		1		1.5		1.75		4		6.25		12.5		24		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
50%	1		10	1	9	8	1		1	1	0		0		0		0		0		0		0	10	100
50%	2		10	3	7	5	2		2	2	0		0		0		0		0		0		0	10	100
37.5%	1		10		10		10		10		10	5	5	5	0		0		0		0		0	10	100
37.5%	2		10		10	2	8		8		8	4	4	4	0		0		0		0		0	10	100
30%	1		10		10		10		10		10		10	2	8	8	0		0		0		0	10	100
30%	2		10		10		10		10		10		10	5	5	5	0		0		0		0	10	100
25%	1		10		10		10		10		10	1	9		9		9		9	1	8		8	2	20
25%	2		10		10		10		10		10		10		10		10		10		10		10	0	0
12.5%	1		10		10		10		10		10		10		10		10		10		10		10	0	0
12.5%	2		10		10		10		10		10		10		10		10		10		10		10	0	0

Control Water

Conc.	Tank #	Time (Hours) 0		1		1.5		1.75		4		6.25		12.5		24		48		72		96		Mortality	
		D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	Number	Percent
0%	1		10		10		10		10		10		10		10		10		10		10		10	0	0
0%	2		10		10		10		10		10		10		10		10		10		10		10	0	0

Appendix Va. Characteristics of leachates generated in a preliminary soak of Black Spruce logs, wood chips and bark chips in dechlorinated City of Winnipeg tap water for 24 hours.

Time	0 hours			2.5 hours			7.5 hours			19 hours			24 hours		
	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH
Logs	14.0	11.6	-	16.0	10.6	7.4	17.8	10.7	7.1	20.1	6.00	6.9	20.0	4.20	6.7
Wood Chips	12.5	11.9	-	15.8	11.3	7.3	19.0	10.2	7.0	21.0	8.85	7.1	21.0	8.40	6.9
Bark Chips	12.5	11.8	-	16.0	10.5	7.1	19.0	7.65	5.4	21.0	2.00	4.3	21.0	1.40	4.2

Appendix Vb. Characteristics of leachates generated in a preliminary soak of Jack Pine logs, wood chips and bark chips in dechlorinated City of Winnipeg tap water for 24 hours.

Time	0 hours			2.5 hours			7.5 hours			19 hours			24 hours		
	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH	Temp. °C	Dissolved Oxygen (mg L^{-1})	pH
Logs	14.0	11.6	-	16.0	11.1	7.3	18.0	9.79	6.3	20.5	4.85	6.5	21.0	0.90	6.5
Wood Chips	12.2	11.6	-	16.0	11.1	7.3	19.0	9.40	6.1	21.0	4.40	6.4	21.0	1.30	6.3
Bark Chips	12.5	11.6	-	16.5	11.3	7.1	19.5	7.60	6.1	21.0	1.20	6.3	21.5	0.60	6.2

Appendix VI. Canadian System of Soil Classification.

The classification of soils in Canada is based upon the following hierarchy:

Order. Taxa at the order level are based upon properties of the pedon (the smallest three-dimensional unit at the surface of the earth that is considered a soil) that reflect the nature of the environment and the effects of the dominant soil-forming processes.

Great Group. Soil taxa formed by the subdivision of each order. Each great group carries with it the differentiating criteria of the order to which it belongs. In addition, taxa at the great group level are based upon properties that reflect differences in strengths of dominant processes or a major contribution of a process in addition to the dominant one.

Subgroup. Subgroups are formed by the subdivision of the great group. They carry the differentiating criteria of the order and the great group to which they belong. Also, subgroups are differentiated on the basis of the kind and arrangement of horizons.

Level	Eluviated Dystric Brunisol	Orthic Gray Luvisol
Order	• under forest vegetation • brownish-coloured Bm horizons • well to imperfectly drained	• under forest vegetation • eluvial Ae and illuvial Bt horizons • well to imperfectly drained
Great Group	• no Ah horizon, or < 10 cm thick • pH < 5.5 • low base-saturated parent materials	• mean annual soil temperature < 8°C • high base saturation
Subgroup	• eluvial horizon (Ae) at least 2 cm thick	• orthic - conformity to the central concept of the great group

The major mineral horizons are A, B and C. These horizons contain 17% or less organic C by weight.

A - This is the mineral horizon formed at or near the surface in the zone of leaching or eluviation of materials in solution or suspension, or of maximum in-situ accumulation of organic matter, or both. The accumulation of organic matter is usually expressed morphologically by a darkening of the surface soil (Ah). Conversely, the removal of organic matter is usually expressed by a lightening of the soil colour usually in the upper part of the solum (Ae).

B - This is the mineral horizon characterized by enrichment in organic matter, sesquioxides, or clay; or by the development of soil structure; or by a change in colour denoting hydrolysis, reduction, or oxidation. Clay accumulation is indicated by finer soil textures and by clay cutans coating peds and lining pores (Bt).Colour changes include relatively uniform browning (brawnification) due to oxidation of iron (Bm).

C - This is a mineral horizon comparatively unaffected by the pedogenic processes operative in A and B. C horizons containing carbonate minerals are denoted Ck.

Three major organic horizons are L, F, and H, which are developed primarily from the accumulation of leaves, twigs and woody materials. They are usually not saturated with water for prolonged periods.

L - This is an organic horizon that is characterized by an accumulation of organic matter derived mainly from leaves, twigs, and woody materials in which the original structures are easily discernible.

F - This is an organic horizon that is characterized by an accumulation of partly decomposed organic matter derived mainly from leaves, twigs, and woody materials. Some of the original structures are difficult to recognize. The material may be partly comminuted by soil fauna as in moder, or it may be a partly decomposed mat permeated by fungal hyphae as in mor.

H - This is an organic horizon that is characterized by an accumulation of decomposed organic matter in which the original structures are indiscernible. This horizon differs from F by having greater humification due chiefly to the action of organisms. It is frequently mixed with mineral grains, especially near the junction with a mineral horizon.

(Agriculture Canada Expert Committee on Soil Survey 1987).

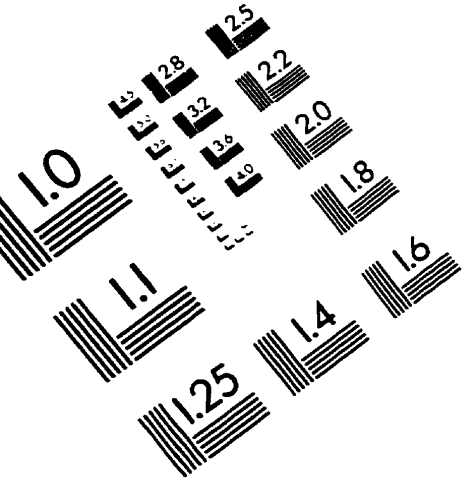
Appendix VIIa. Substrates on the Gram negative Biolog™ Microtitre plates.

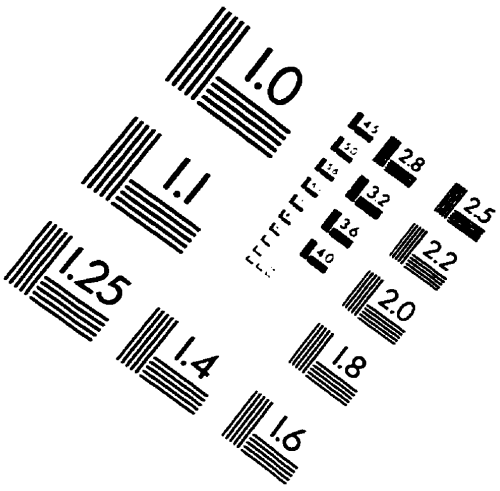
Gram Negative Biolog™ Plate					
Cell	**Substrate**	**Cell**	**Substrate**	**Cell**	**Substrate**
A1	water	**D1**	acetic acid	**G1**	L-histidine
A2	a-cyclodextrin	**D2**	cis-aconitic acid	**G2**	hydroxy L-proline
A3	dextrin	**D3**	citric acid	**G3**	L-leucine
A4	glycogen	**D4**	formic acid	**G4**	L-ornithine
A5	tween 40	**D5**	D-galactonic acid lactone	**G5**	L-phenylalanine
A6	tween 80	**D6**	D-galacturonic acid	**G6**	L-proline
A7	N-acetyl-D-galactosamine	**D7**	D-gluconic acid	**G7**	L-pyroglutamic acid
A8	N-acetyl-D- glucosamine	**D8**	D-glucosaminic acid	**G8**	D-serine
A9	adonitol	**D9**	D-glucuronic acid	**G9**	L-serine
A10	L-arabinose	**D10**	a-hydroxybutyric acid	**G10**	L-threonine
A11	D-arabitol	**D11**	B-hydroxybutyric acid	**G11**	D,L-carnitine
A12	cellobiose	**D12**	*f*-hydroxybutyric acid	**G12**	*f*-amino butyric acid
B1	i- erythritol	**E1**	p-hydroxy phenylacetic acid	**H1**	urocanic acid
B2	D-fructose	**E2**	itaconic acid	**H2**	inosine
B3	L-fucose	**E3**	a-keto butyric acid	**H3**	uridine
B4	D-galactose	**E4**	a-keto glutaric acid	**H4**	thymidine
B5	gentiobiose	**E5**	a-keto valeric acid	**H5**	phenyl ethylamine
B6	a-D-glucose	**E6**	D,L-lactic acid	**H6**	putrescine
B7	m-inositol	**E7**	malonic acid	**H7**	2-amino ethanol
B8	a-D-lactose	**E8**	propionic acid	**H8**	2,3-butanediol
B9	lactulose	**E9**	quinic acid	**H9**	glycerol
B10	maltose	**E10**	D-saccharic acid	**H10**	D,L-a-glycerol phosphate
B11	D-mannitol	**E11**	sebacic acid	**H11**	glucose-1- phosphate
B12	D-mannose	**E12**	succinic acid	**H12**	glucose-6- phosphate
C1	D-melibiose	**F1**	bromo succinic acid		
C2	B-methyl D-glucoside	**F2**	succinamic acid		
C3	D-psicose	**F3**	glucuronamide		
C4	D-raffinose	**F4**	alaninamide		
C5	L-rhamnose	**F5**	D-alanine		
C6	D-sorbitol	**F6**	L-alanine		
C7	sucrose	**F7**	L-alanyl-glycine		
C8	D-trehalose	**F8**	L-asparagine		
C9	turanose	**F9**	L-aspartic acid		
C10	xylitol	**F10**	L-glutamic acid		
C11	methyl pyruvate	**F11**	glycyl-L-aspartic acid		
C12	mono-methyl succinate	**F12**	glycyl-L-glutamic acid		

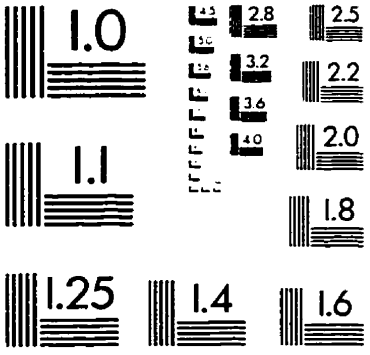
Appendix VIIb. Substrates on the Gram positive Biolog™ Microtitre plates.

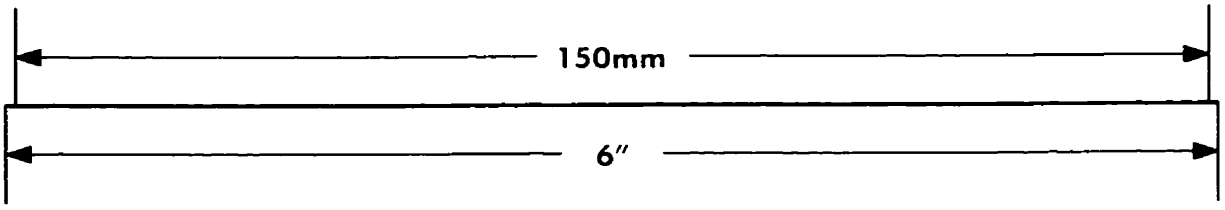
Gram Positive Biolog™ Plate					
Cell		**Cell**		**Cell**	
A1	water	**D1**	B-methyl-D- glucoside	**G1**	alaninamide
A2	a-cyclodextrin	**D2**	a-methyl-D- mannoside	**G2**	D-alanine
A3	B-cyclodextrin	**D3**	palatinose	**G3**	L-alanine
A4	dextrin	**D4**	D-psicose	**G4**	L-alanyl-glycine
A5	glycogen	**D5**	D-raffinose	**G5**	L-asparagine
A6	inulin	**D6**	L-rhamnose	**G6**	L-glutamic acid
A7	mannan	**D7**	D-ribose	**G7**	glycyl-L-glutamic acid
A8	tween 40	**D8**	salicin	**G8**	L-pyroglutamic acid
A9	tween 80	**D9**	sedoheptulosan	**G9**	L-serine
A10	N-acetyl-D- glucosamine	**D10**	D-sorbitol	**G10**	putrescine
A11	N-acetyl-D-mannosamine	**D11**	stachyose	**G11**	2,3-butanediol
A12	amygdalin	**D12**	sucrose	**G12**	glycerol
B1	L-arabinose	**E1**	D-tagatose	**H1**	adenosine
B2	D-arabitol	**E2**	D-trehalose	**H2**	2'-deoxy adenosine
B3	arbutin	**E3**	turanose	**H3**	inosine
B4	cellobiose	**E4**	xylitol	**H4**	thymidine
B5	D-fructose	**E5**	D-xylose	**H5**	uridine
B6	L-fucose	**E6**	acetic acid	**H6**	adenosine-5'-monophosphate
B7	D-galactose	**E7**	a-hydroxybutyric acid	**H7**	thymidine-5'-monophosphate
B8	D-galacturonic acid	**E8**	B-hydroxybutyric acid	**H8**	uridine-5'-monophosphate
B9	gentiobiose	**E9**	*f*-hydroxtbutyric acid	**H9**	fructose-6- phosphate
B10	D-gluconic acid	**E10**	p-hydroxyphenyl acetic acid	**H10**	glucose-1- phosphate
B11	a-D-glucose	**E11**	a-keto glutaric acid	**H11**	glucose-6- phosphate
B12	m-inositol	**E12**	a-keto valeric acid	**H12**	D-L-a-glycerol phosphate
C1	a-D-lactose	**F1**	lactamide		
C2	lactulose	**F2**	D-lactic acid methyl ester		
C3	maltose	**F3**	L-lactic acid		
C4	maltotriose	**F4**	D-malic acid		
C5	D-mannitol	**F5**	L-malic acid		
C6	D-mannose	**F6**	methyl pyruvate		
C7	D-melezitose	**F7**	mono-methyl succinate		
C8	D-melibiose	**F8**	propionic acid		
C9	a-methyl-D- galactoside	**F9**	pyruvic acid		
C10	B-methyl- galactoside	**F10**	succinamic acid		
C11	3-methyl- glucose	**F11**	succinic acid		
C12	a-methyl-D- glucoside	**F12**	N-acetyl-L- glutamic acid		

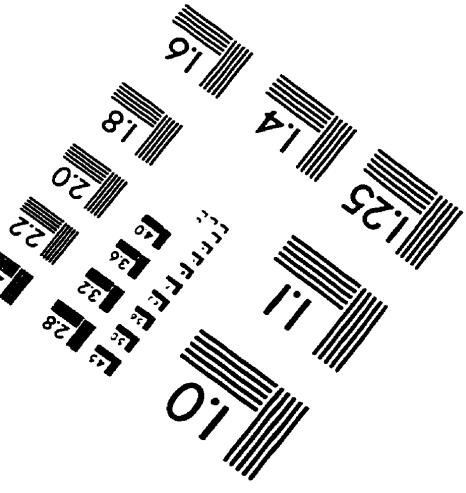
IMAGE EVALUATION
TEST TARGET (QA-3)

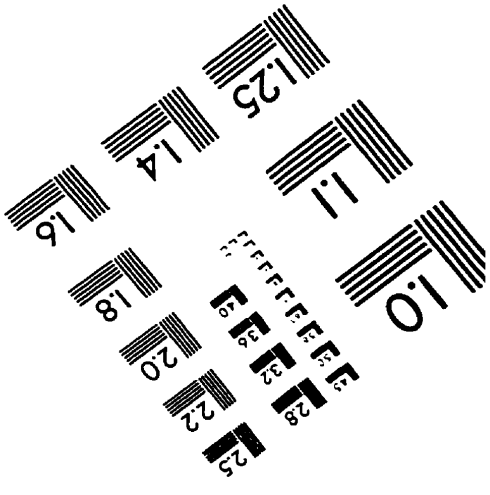












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