Effects of Liming on Soil Respiration, Fungi Diversity and Abundance in a Metal-Contaminated Region in Northern Ontario

By

Kassandre Goupil

Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science (MSc) in Biology

School of Graduate Studies
Laurentian University
Sudbury, Ontario

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE

Laurentian Université/Université Laurentienne
School of Graduate Studies/École des études supérieures

Title of Thesis Titre de la thèse	EFFECTS OF LIMING ON SOIL RESPIRATION, FUNGI DIVERSITY AND ABUNDANCE IN A METAL-CONTAMINATED REGION IN NORTHERN ONTARIO
Name of Candidate Nom du candidat	Goupil, Kassandre
Degree Diplôme	Master of Science

Department/Program Département/Programme	Biology	Date of Defence Date de la soutenance	April 28, 2014

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Kabwe Nkongolo
(Supervisor/Directeur de thèse)

Dr. Peter Beckett
(Committee member/Membre du comité)

Dr. Graeme Spiers
(Committee member/Membre du comité)

Dr. Nandakumar Kanavillil
(External Examiner/Examinateur externe)

Approved for the School of Graduate Studies
Approuvé pour l'École des études supérieures
Dr. David Lesbarrères
M. David Lesbarrères
Director, School of Graduate Studies
Directeur, École des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

I, **Kassandre Goupil**, hereby grant to Laurentian University and/or its agents the non-exclusive license to archive and make accessible my thesis, dissertation, or project report in whole or in part in all forms of media, now or for the duration of my copyright ownership. I retain all other ownership rights to the copyright of the thesis, dissertation or project report. I also reserve the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or project report. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that this copy is being made available in this form by the authority of the copyright owner solely for the purpose of private study and research and may not be copied or reproduced except as permitted by the copyright laws without written authority from the copyright owner.

Abstract

At present, little is known concerning the fungi communities inhabiting the Greater Sudbury Region. This study aimed at identifying the fungal species and abundance in limed and unlimed areas contaminated with metals. Samples were collected from the LFH soil layer from Wahnapitae Hydro-Dam, Daisy Lake, Kingsway, Kelly Lake, Hagar, Onaping Falls and Capreol. Limed and unlimed areas were compared for soil metals, pH, fungi diversity, abundance and seasonal soil respiration. Fungi from soil samples were cultured using Sabouraud Dextrose Agar and Malt Extract Agar. A total of 52 fungi species from 34 genera were identified. There was a significantly higher fungal diversity in the limed areas compared to the samples from unlimed sites based on SDA medium data. Fungi abundance followed the same trend. Significantly higher soil respiration rates were recorded for limed sites compared to unlimed sites. Summer soil respiration rates correlated ($r = 0.50$) with total fungal abundance.

Acknowledgements

I would first like to thank my supervisor, Dr. Nkongolo, for his constant devotion, guidance and expertise towards fulfilling this research project. I am also greatly appreciative towards Dr. Nasserulla's help in identifying many of the fungi species. I also must sincerely thank my committee members, Dr. Spiers and Dr. Beckett, for their assistance and knowledge in completing this research project. Furthermore, I would like to express gratitude towards Irene McAuley for her added support concerning this research project. Her knowledge regarding the safe operation of the laboratory equipment, safety regulations in the laboratory and media preparation proved greatly helpful to me. Equally, I am grateful to all my esteemed labmates for their continued assistance throughout this research project. Their support is greatly appreciated.

Table of Contents

List of Figures

List of Tables

Chapter 1: Literature Review

1.1 Metal Contamination in the Greater Sudbury Region (Ontario, Canada)

Sudbury, Ontario, Canada, at the southern edge of the Canadian Shield, is located in a forest zone transitional to the northern boreal (coniferous) forest and the southern deciduous (hardwood) forest (Winterhalder, 1984). As such, Sudbury is significantly diversified in terms of flora and fauna. For instance, this forest zone, categorized by Rowe (1959), as the Great Lakes – St. Lawrence Forest Region, was once heavily characterized by Red and White Pine whereas the southern Algonquin section was characterized with White Pine having formed an admixture with tolerant hardwoods, namely Sugar Maple and Yellow Birch. Perhaps the most striking feature about the topographical region of Sudbury is that it is located at the heart of a mineral-rich geological basin (Winterhalder, 1984). This elliptical depression of the landscape, being thirty-eight miles long by seventeen miles wide and stretching out to both the northeast and southwest regions of the area, significantly impacts the vegetation of the area (Winterhalder, 1984).

The construction of the Canadian Pacific Railway (CPR) mainline in the 1880s quickly led to Sudbury's economic growth and development by means of mining and forestry and as such, the city became a major mining centre (Howey, 1938). Logging practices were quickly established in Sudbury which included the deletion of the larger Red and White Pine (Winterhalder, 1984). While it was originally believed that the removal of such species would only have a minimal environmental effect in terms of land degradation, continuous logging and further removal of timber started to significantly barren the land (Winterhalder, 1984). Furthermore, forest fires were an often occurrence due to the slash that was being left behind (Winterhalder, 1984). As a result, land was quickly denuded, especially in the vicinity of the roast yards (Winterhalder, 1984). The first roast yard and production-oriented smelter was

established in 1888 in Copper Cliff (Winterhalder, 1984). The process of roasting copper-nickel ore, involved removing sulphur from the pyrrhotite (Fe_7S_8); this being associated with chalcopyrite ($CuFeS_2$) as well as pentlandite $(Ni, Fe)_9S_8$) (Boldt, 1967). This process was accomplished by aerobic heating to a satisfactory temperature in order to begin a process of exothermic, self-maintaining combustion (Boldt, 1967). The beds of ore utilized were subsequently ignited and left to burn for months prior to being loaded into cars with steam shovels to be smelted in furnaces (Winterhalder, 1984). Benedickson (1973) later recorded that throughout the early stages of heap-roasting, the Canadian Copper Co. utilized wood at a rate of 40,000 cords per annum.

The increased demand for nickel in the late 1890's led to the development of 80 roast heaps and 9 furnaces in the area by 1901, which was significant considering the size of the region (Winterhalder, 1984). Furthermore, between 1913 and 1916, the Mond Nickel Company subsequently removed all forms of woody vegetation, including tree stumps, from the Coniston area in order to supply the fuel demands of the roast beds (Watson et al., 1972). However, they later opted to follow the example of the Norwegians and abandon open-bed roasting in 1929 due to land desolation and damage (Peck, 1978). As such, the Mond Nickel Company located in Coniston, opted to operate their roast beds only in the winter and not in the summer (Peck, 1978). At this time, the Coniston roast yard was barren and dominated by metal-contaminated soils because of close proximity to the Coniston smelters which clearly demonstrated the relevance of a study by Turcotte in 1981. He examined the hypothesis that the widespread devastation of vegetation and the toxicity of soils in the Sudbury area were resulting factors of early roast bed processes. These processes coupled with the original logging activities caused irreversible damage to valuable organic material, leaf litter and soil nutrients (Watson et al.,

2012). Other influences that led to the barren land were the deposition of wastes, including slag and mill tailings (Winterhalder, 1984). While the abolishment of these open roast yards was later established, their long term effects have extensively impacted Sudbury's landscape (Amiro et al., 1981). Specifically, open roast yards were replaced with centralized smelters, and while a safer alternative, they have equally significantly contributed to the detrimental effects on the Greater Sudbury Region landscape and vegetation.

Smelting processes released sulfur dioxide and metals (Hutchinson & Whitby, 1974). These SO_2 emissions combined with water vapour in the atmosphere to produce acid rain which impacted the Sudbury landscape. During the century of mining and smelting, more than 100 million tonnes of sulfur dioxide and tens of thousands of tonnes of cobalt, copper, nickel as well as iron ores were released into the Earth's atmosphere (Freedman et al., 1980). As a result, over 40 square miles of land in the Sudbury region were subjected to extreme pollution and were rendered desolate (DeLestard, 1967). An additional 140 square miles were heavily impacted by the early 1970s as only herbaceous, shrub and small tree growth could be supported within this area (DeLestard, 1967). Accordingly, 'barren lands' surrounding the smelters were created and characterized by bare and sparsely vegerated land, severely eroded hilltops, acidic ($pH < 4$) and metal contaminated soils (Freedman and Hutchinson 1980b; Amiro and Courtin 1981). Surrounding the barrens, the 'semi-barren lands' had better soil conditions ($pH > 5$) but nevertheless, had many bare hilltops (Gorham and Gordon 1960) and many sensitive plant species such as epiphytic lichens (LeBlanc et al., 1972). Leblanc and his co-workers (1972) were able to correlate atmospheric pollution with the distribution of lichens inhabiting bark. Nieboer et al. (1972) demonstrated a similar relationship in terms of a decrease in rock lichen diversity adjacent to smelters, while additionally demonstrating an increase in their metal

content. These effects were felt as far as St. Charles, roughfly 50 to 60 km from Sudbury (DeLestard, 1967). DeLestard (1967) stated that this extreme land desolation and damage was due to airborne pollution from the Copper Cliff, Coniston and Falconbridge smelters. A loss in soil nutrients quickly occurred because of increased loss of vegetation and soil erosion in the area (Winterhalder, 1984). Soil chemistry within the area was majorly affected by increased soil acidity which led to decreases in available calcium and magnesium and increases in available aluminium and contaminant heavy metals (Huettl 1993; Winterhalder 1996). In fact, in 1971, Antonovics et al. discovered a considerable difference concerning particular soil characteristics in soils having an elevated metal content compared to normal soils which possessed blooming vegetation. These differences were observed in nutrient content, organic matter content and texture, through field observations (Antonovics et al, 1971). The increased acidity of these soils affected the solubility of certain nutrients that reached toxic levels, leading to a decline in plant growth and soil erosion in the area. Because a loss in microflora and fauna was encountered, the topsoil equally suffered and became deficient in available phosphorous, nitrogen, calcium, magnesium and manganese. Additionally, Dudka et al. (1995) reported that elemental soil contamination in the Sudbury mining and smelting area was quite evident in terms of cadmium, cobalt, copper, chromium, iron, manganese, nickel, sulphur and zinc with copper and nickel being the primary contaminants of the region. The bioavailability of these elements in the soil crucially impacts soil fungal metabolism and development (Kendrick, 2000).

1.2 Fungi and Soil Elements

Fungi play a crucial role in soil formation, organic matter decomposition as well as in the transformations of toxic metabolites found in the soil (Ritz et al., 2004). They are heterotrophic eukaryotes that have the ability to produce enzymes which allow them to absorb these toxic

compounds as food which they typically absorb at the various growing points of their indefinite "body", which is comprised of many finely branched tubes called hyphae (Kendrick, 2000). In order to thrive in their environment, fungi need a wide range of elements that are categorized into two groups (macronutrients and micronutrients) according to their respective required amounts for normal and optimal growth (Kendrick, 2000). The macronutrients include potassium (K), utilized in carbohydrate metabolism, enzyme activity, and maintaining ionic balance; phosphorus (P), which is an essential component of nucleic acids and for energy transfer mechanisms; magnesium (Mg), used as an enzyme activator necessary in ATP metabolism; sulphur (S), is a component of some amino acids, vitamins and other sulfhydryl compounds; and calcium (Ca), used as an enzyme activator that is regularly found in membranes (Kendrick, 2000). Micronutrients, often referred to as trace elements, include iron (Fe), that is found in cytochromes, heam apoenzymes, and pigments; copper (Cu), used as an enzyme activator and involved in pigments; and manganese (Mn), zinc (Zn), and molybdenum (Mo), which are all enzyme activators (Kendrick, 2000).

Different species of fungi require different levels of intake of these macro and micro nutrients, which are made available to them based on the substrate they inhabit (Kendrick, 2000). Metal ions are directly or indirectly involved in all the many aspects of fungal growth, metabolism as well as differentiation. While some metals are essential for fungi growth such as K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Co and Ni, many other metals do not seem to have an apparent function (Gadd, 1992). They include Rb, Cs, Al, Cd, Ag, Au, Hg and Pb (Gadd, 1992). Nevertheless, all the elements listed above have the ability to interact with fungal cells because they can be accumulated by physico-chemical mechanisms and transport systems of altering specificity (Gadd, 1992). Metal effects in fungi can vary a great deal between organisms, strains,

the stage of growth and different vegetative and reproductive forms of the same organism (Gadd & Mowll 1985; Sabie & Gadd 1990). However, extreme high or low concentrations of some metal ions can perturb or eventually inhibit fungi growth (Al-Janabi, 2010).

1.3 Fungi and Soil Liming

While some fungi prefer more acidic areas of a specific pH range, others are more accustomed to alkaline environments (Kendrick, 2000). Generally speaking however, fungi usually prefer more acidic conditions and grow well when germinating on acidic soil with a pH range of 2 to 7 where overall optimum fungal growth is observed at a pH of 5 (Smith & Doran, 1996). At highly acidic or alkaline pH levels, organic matter mineralization is slowed down or stopped which is attributed to poor microbial activity linked to bacteria (Smith & Doran, 1996). At low pH levels, nitrification and nitrogen fixation will also be inhibited (Smith & Doran, 1996). Furthermore, the solubility of heavy metals as well as the mobility and degradation of herbicides and insecticides are pH dependant (Smith & Doran, 1996). All these factors play a key role in fungi diversity and abundance in a specific site or region which in turn, greatly influence soil respiration and overall soil quality for this same region. Consequently, the quality of the soil for a general region must be well maintained within certain elemental levels and pH ranges in order to allow for a wide range of fungal diversity and abundance.

In addition to participating in soil decomposition, fungi are exceedingly diverse, essential in many food webs and participate heavily in nutrient cycling (Newbound, 2008). Symbiotic mycorrhizal relationships are also critical in many ecosystems as they provide necessary plant nutrition, aid in seedling establishment and in plant community structures (Newbound, 2008). Accordingly, fungi are functionally important in ecosystems but their relevance in these

ecosystems is often overlooked. However, some previous studies have analyzed the development of fungi species as affected by pH and soil type (Weyman-Kaczmarkowa & Pedziwilk, 2000). For instance, Weyman-Kaczmarkowa & Pedziwilk (2000) have documented the effects of liming ($Ca(OH)_2$) on the numbers of colony-forming units (CFU) and the fungi biomass present in two soil types, namely loamy sand and a loose sandy soil, during a 90 day laboratory experiment. Their analysis revealed that raising the pH of the loose sandy soil from an original value of 4.5 to 7.0 and, subsequently, 9.0, decreased the mean fungal CFU numbers by 50 % as well as their biomass by 42 % and 68 % respectively in comparison to the unlimed controls used in the study (Weyman-Kaczmarkowa & Pedziwilk, 2000). Their analyses equally revealed that the liming of the loamy sand was more strongly inhibited in comparison to the loose sandy soil (Weyman-Kaczmarkowa & Pedziwilk, 2000). This resonates with Smith and Doran's findings in 1996, conducted in Wisconsin, USA, which stipulated that overall optimum fungi growth is typically observed at a pH of 5. Hence, soil with lower of higher pH values would thus have less impressive fungal yields.

Other studies have analyzed fungal populations and their diversity in organically amended agricultural soils, which is to say, soils which have been treated with organic fertilisers, namely: farm yard manure, vermicompost, plant compost, and integrated compost (Swer, Dkhar & Kayang, 2011). Using the serial dilution plate method, scientists in India were able to quantify fungal population counts and conclude that a wide range of fungal populations were comparatively higher in organically amended plots as compared to the controls utilized in the study (Swer, Dkhar & Kayang, 2011). This lends support towards the positive effects of soil amendment on fungi diversity and abundance for a specific region.

Lastly, studies have also examined the effects of heavy metals on various fungi species, including saprotrophic soil fungi. Such studies have revealed that fungi have evolved various response mechanisms which limit the toxic effect of metals to their cells (Baldrian, 2010). These previously mentioned studies are novel because they allow the examination of pH and metal fungal dynamics in ecologically disturbed and newly remediated regions. Little is known concerning the fungal populations inhabiting the Greater Sudbury Region which has been ecologically contaminated by SO_2 fumigation and aerial fallouts provoqued by decades of mining, roasting and smelting operations. This study will allow the documentation and classification of fungal communities within this region.

1.4 Soil Amendment in the Sudbury Region

Sudbury, desolate and denuded due to smelting and mining activities by the 1970's, needed to embark on land reclamation. The primary restoration efforts began in 1969 when Laurentian University and the Ontario Department of Lands and Forests started investigating revegetation strategies for the entire area (Lautenbach, 1987). Laurentian University's Keith Winterhalder's earlier work demonstrated that low pH and low nutrient levels coupled with elevated copper and nickel levels greatly limited plant growth within the area (Winterhalder, 1983). Thus, in 1973, a multi-disciplinary technical advisory committee was established in order to organize reclamation efforts in Sudbury (Lautenbach, 1987). This committee consisted of members of Laurentian University, the International Nickel Company Limited, Falconbridge Nickel Company Limited, the Ontario Ministries of Environment, Natural Resources, Northern Development and Mines, the local conservation authority as well as local and regional governments with the addition of many volunteers (Lautenbach, 1987). Inco Incorporated and

Falconbridge Limited, now known as Vale and Glenecore, also contributed significantly to land reclamation efforts (Lautenbach, 1987). These reclamation efforts included adding dolomitic and calcitic limestone in order to neutralise, detoxify and facilitate the revegatation of the acidic and metal contaminated soils in the Sudbury region (Winterhalder 1996). This soil amendment strategy proves useful concerning the recolonization of species because it facilitates the germination of existing and incoming seed banks due to the increase in soil pH (Winterhalder, 1996). The pH of the metal contaminated soils is increased because ions in the liming agents combine with hydrogen ions already in the soil in order to produce water and carbon dioxide which reduces the acidity of the soil (Winterhalder, 1996). Toxic metals found in the soil may also be eliminated by precipitation as a carbonate or a hydroxide (Winterhalder, 1996). Furthermore, when the soil is neutralized by the lime, the hydroxylation of the trivalent aluminium ion will occur thus contributing to soil amendment although theoretically, the copper ions could still negatively influence root growth (Winterhalder, 1996). Specifically, dolomitic limestone contains calcium magnesium carbonate while calcitic limestone contains calcium carbonate. Each liming agent was chosen for their specific contents and respective soil enhancing properties for the Sudbury region. While calcium has been reported to improve membrane integrity amongst other things, the combined action of both, dolomitic and calcitic limestone, are known to generate better results (Winterhalder, 1996). They actively participate in the competitive exclusion of metal ions (Winterhalder, 1996). Further reasoning for their combined usage is attributed to the fact that the sole use of calcitic limestone induces a magnesium deficiency in addition to an antagonistic effect between magnesium and nickel (Winterhalder, 1996). The ground limestone was manually placed over the targeted soil by emptying bags over a grid. This results in a unique distribution pattern where specific areas of a targeted region possess

varying degrees of lime, from zero to optimal to excessive. Consequently, this offers a favourable and random distribution in vegetation which allows for an ecological variation of plants.

1.5 Soil Respiration

Soil respiration is known as an ecosystem process by which carbon dioxide is released from the soil by means of root respiration, microbial decomposition of litter and soil organic matter as well as faunal respiration (Yiqi & Zhou, 2010). Soil respiration is intricately linked to the general processes occurring in ecosystems because it is strongly related to ecosystem productivity, soil fertility, as well as regional and global carbon cycles (Yiqi & Zhou, 2010). Accordingly, due to the fact that the global carbon cycle regulates climate change, it is evident that soil respiration is also linked to climate change as well as carbon trading and environmental policy (Yiqi & Zhou, 2010). The study of soil respiration has now become a multidisciplinary subject. The role of fungi in soil respiration is of prime importance.

Temperature along with moisture content, are the most important environmental factors affecting microbial growth and activity in soils (Pietikåinen, Pettersson & Bååth, 2005). Due to the fact that the global warming issue has received a lot more attention in recent years, the importance of the temperature dependence of soil microorganisms is crucial since these microorganisms are the main group producing CO_2 through the decomposition of organic material in the soil (Pietikåinen et al., 2005). By measuring the respiration rate (total activity) for a specific soil, the temperature dependence of soil microorganisms can be determined (Pietikåinen et al., 2005). Little is known concerning the temperature dependence of different groups of soil microorganisms like bacteria and fungi (Pietikåinen et al., 2005). However, with

the use of the acetate-in-ergosterol incorporation technique, one can estimate the fungal activity in the soil (Pietikåinen et al., 2005). It has been documented that the instantaneous soil respiration rate will often increase when temperature reaches up to around 40 °C or more; this phenomenon occurring even in soils from cold climates (Pietikåinen et al., 2005) such as the Northern Ontario Region. Interestingly, this is not due to the growth of thermophilic organisms which would occur at higher temperatures because the same effect is observed even in short incubation times (a few hours) which would not allow for substantial thermophilic development (Pietikåinen et al., 2005). Contrarily, soil bacterial and fungal growth rates in cold climates typically have optimum temperatures below 30 °C (Pietikåinen et al., 2005). In the present study, soil retrieval containing fungal colonies was achieved in the summer of 2012 and 2013 when optimum values of soil respiration theoretically should have been achieved due to environmental temperature. Persson et al. (1999) also discovered a difference in the temperature dependence of respiration rate in a forest soil and agricultural soil. They revealed that a lower minimum temperature for respiration was required in the agricultural compared to the forest soil (Persson et al. 1999). A possible explanation for this difference lies within a shift in the relative importance of fungi and bacteria as decomposers; fungi are more essential in the forest soils and are considered more active at low temperatures in comparison to bacteria (Persson et al., 1999; Ågren, 2000; Lipson, Schadt & Schmidt, 2002; Schadt et al., 2003).

While the evaluation of soil respiration to determine soil health is widely adopted, the determination of the individual contributions of the different microorganisms to the total metabolism involved in the soil is rather difficult to undertake in terms of methodology (Pietikåinen et al., 2005). However, according to a model developed by Anderson and Domsch (1973), bacterial and fungal respiration can indeed be estimated using inhibitors. In the soil used

to develop the method, they obtained a ratio of bacterial to fungal respiration of about 20/80 which supports the hypothesis that the fungal biomass dominates the metabolism of many forest soils as previously mentioned (Anderson & Domsch, 1974). Biomass measurements by Satchell (1971), Shields et al. (1973), Parkinson (1973), and Parkinson as cited by Clark and Paul (1970) also support Anderson and Domsch's findings. They indicated that soil biological biomass was dominated by the fungal component. Consequently, fungi are considered to be the major decomposers in these soils and as such, their diversity and abundance will directly influence the soil respiration outputs for a specific region. Therefore, fungi diversity and abundance need to be properly assessed for the Greater Sudbury Region.

Domsch and Anderson (1973) reported that soil containing the highest percentage of bacterial activity had a pH of 2.9 while the soil containing the least bacterial activity had a pH of 7.5. However, optimal and minimal fungal activity does not follow this trend as bacterial and fungal soil requirements are different. In fact, fungi have previously been stated to be more tolerant of heavy metals as a group in comparison to bacteria (Doelman, 1985; Hiroki, 1992). Furthermore, different biomass measurements or plate counting techniques have revealed that heavy metals will affect bacteria and fungi in a different manner in the soil (Hiroki, 1992; Khan & Scullion, 2002; Maliszewska et al., 1985; Muller et al., 2001). Moreover, metal speciation affects fungi growth because the chemical, physical and biological behaviour of various trace and heavy metals found in the soil will direct the movement and outcome of these metals in the soil as well as subsequent uptake by fungi (Roberts et al., 2005). Thus, because metal speciation can cause a potential threat to organisms, such as fungi, soil respiration will equally be affected. In fact, by analyzing soil in a spruce forest in Southern Sweden, Rajapaksha, Tobor-Kaplon & Bååth (2004) reported that soil respiration rate was slightly affected by metal contamination and

they found that a clear dose-response effect was encountered at added metal concentrations above 4 mmol kg^{-1}. In fact, they discovered that the highest levels of Cu and Zn contamination resulted in a 30% reduction of soil respiration rate compared to the control soil (Rajapaksha et al. 2004). Furthermore, the metal contamination of Zn and Cu was not differentiated in terms of metal effect on soil respiration (Rajapaksha et al., 2004). It should also be noted that soil respiration rates in the metal-contaminated soils gradually decreased in comparison to the non-contaminated control samples throughout the first 3 days post metal addition, after which soil respiration became stable (Rajapaksha et al., 2004). However, respiration rates following 10 and 60 days of incubation were not significantly different (Rajapaksha et al., 2004). Lastly, following the addition of lime, a short increase in respiration rates in the contaminated soils was apparent (Rajapaksha et al., 2004).

1.6 Government Regulations and Revegetation

The establishment of government regulations, the perfection of new methods for removing sulfur from the ore, and the reduction of smelter fumes, were measures developed in order to allow for significant reductions in emissions (Gunn, 1996). Sudbury was able to receive federal, provincial and municipal funding to commence various reclamation projects such as fertilizing and seeding which subsequently allowed for the colonization of many plants within the vicinity. For instance, in 1974, in the Coniston Creek valley, many grass species, legumes as well as wildflower species had colonized the land within a year following the remediation treatment. Other remediation efforts included adding herbaceous covers and trees to the area such as jack pine (*Pinus banksiana*), red pine (*Pinus resinosa*), black spruce (*Picea mariana*), hemlock (*Conium macularum*), and balsam fir (*Abies balsamea*) to name a few. These efforts

were germane to Sudbury's re-greening prossess. With them, numerous areas, including the O'Donnell roast yard area, have been completely re-colonized by native plants (Watson et al., 2012). This particular roast yard was naturally re-colonized due to low levels of metals in its' surrounding area (Watson et al., 2012). In fact, only the roast yard itself remains toxic and this, a resulting factor of metal leaching by rainwater into the soil (Watson et al., 2012). Lastly, an indirect but effective effort to improve revegetation in the area was by the construction of a 381 meter "superstack" at Copper Cliff and the Coniston smelter closure in 1972 as well as emission reductions at the Copper Cliff and Falconbridge smelters (McCall et al., 1995). Subsequently, an environmental study by Potvin and Negusanti (1995) demonstrated that emissions of sulfur dioxide and metal particulates had declined by around 90 percent in the area. The resulting effect from a decrease in emissions was a healthier environment. It is by examining specific areas of restoration having been subjected to a decrease in emissions from existing smelters and having undergone restoration in terms of tailings basins, uplands and surrounding lakes, that we can better understand their resulting impacts on the fungi populations inhabiting the Greater Sudbury Region.

1.7 Objectives

The objectives of this present study are to assess soil fungi diversity and abundance in selected unlimed and limed areas contaminated with metals in the Greater Sudbury Region. The effects of liming on soil respiration outputs were also determined. We hypothesize that land reclamation, in particular liming, has significantly increased soil fungi diversity, abundance and soil respiration outputs within the Greater Sudbury Region (GSR).

Chapter 2: Soil Metal Analysis in Limed and Unlimed Areas in the Greater Sudbury Region (Ontario, Canada)

2.1 Introduction

For decades, the Greater Sudbury Region has thrived economically because of its' geological basin, namely because of its' nickel, copper and other metal and mineral deposits. Subsequent roasting and smelting of these elements have caused sulphur dioxide fumigations and metal particulate depositions which have led to various detrimental effects on the overall environmental quality of the Sudbury region (Winterhalder, 1996). Airborne pollution from the Copper Cliff, Coniston and Falconbridge smelters quickly caused the extreme desolation of the Sudbury landscape (DeLestard, 1967). Semi-barren to entirely barren areas in proximity of the smelters were created as a result of this airborne pollution (Winterhalder, 1996) which gave Sudbury its' well known and characteristic ecologically disturbed appearance. Furthermore, concentrations of certain metals such as nickel and copper have been detected in higher concentrations in areas around the aforementioned smelters in comparison to areas further away (Amiro et al., 1981). Accordingly, Hutchinson and Whitby (1977) have detected over 3000 ppm and 2000 ppm of nickel and copper respectively having spread widely in surface soils. Metal contaminated and acidified soils have led to phytotoxic effects in plants (Winterhalder, 1996) and possible mycotoxic effects in fungi according to Kendrick (2000) and Al-Janabi (2010). This has dictated the species diversity of plants and fungi within the region.

Soil amendment and revegetation within the Greater Sudbury Region included the addition of ground limestone within the area. Specifically, dolomitic and calcitic limestone were added to barren lands in the hopes of neutralizing the soil in order to later on add fertilizer, various grasses, herbs and trees to further the re-greening process. Dolomitic limestone was added in 1978 by the city of Greater Sudbury. Between 1978 and 2013, 3400 ha of land in

Sudbury were limed (Lautenbach et al., 1995; VETAC, 2013). The calcium and magnesium carbonate components are both essential to neutralize soil metal toxicity as they synergistically generate a competitive exclusion of metal ions from the root-hair's exchange complex, which alone, they are unable to do (Winterhalder, 1996). Since the addition of the limestone in the 1970's, once barren lands have now become increasingly populated by vegetation due to an increase in soil pH which prompted the decreased bioavailability of certain metals past toxic levels. Further reclamation efforts at the hands of devoted groups like the Vegetation Enhancement Technical Advisory Committee (VETAC), Vale Canada Limited, Glencore and many volunteers were also undertaken in order to maximize the re-greening of this area. While a lot is known in regards to the successful growth of various plants and trees having been re-introduced in the once barren lands, little is known in regards to the fungi species inhabiting these areas and how their diversity and abundance has been affected by liming.

The objective of this component of the present study was to examine the effects of dolomitic and calcitic limestone addition to barren Sudbury lands on soil toxicity and soil chemistry.

2.2 Materials and Methods

2.2.1 Site Description and Substrate Sampling / Collection

The liming of the targeted sites was previously performed up to 30-40 years ago through the Sudbury's Regional Land Reclamation Program using dolomitic limestone (Winterhalder, 1996). Soil samples of limed and adjacent unlimed areas from seven sites were collected within the Greater Sudbury Region in Ontario, Canada. The selected sites include Wahnapitae Hydro-Dam, Daisy Lake, Kingsway, Kelly Lake, Hagar (St. Charles), Onaping Falls and Capreol; the

last three sites were used as reference sites (Figure 1). For each limed, unlimed and reference site, three samples were collected. Soil samples were collected from the top organic horizon (LFH) and were air dried and stored in sealed plastic bags prior to preparation for chemical and fungi analysis.

2.2.2 Soil pH and Metal Analysis

Soil pH was measured in water and a neutral salt solution pH (0.1 M $CaCl_2$) (Carter et al. 1993). Methodology for total metal analysis was executed as described by Abedin et al. (2012). For the estimation of total metal concentrations, a 0.5 g soil sample was treated with 10 mL of 10:1 ratio HF:HCl, heated to 110 ^{0}C for 3.5 hours in open 50 mL Teflon™ tube in a programmable digestion block to dry down samples, followed by the addition of 7.5 mL of HCl and 7.5 mL of HNO_3 and heating to 110 ^{0}C for another 4 hours to dry gently. The samples were then heated to 110 ^{0}C for 1 hour following addition of 0.5 mL of HF, 2 mL of HCl and 10mL of HNO_3 to reduce sample volume to 8-10 mL. On cooling, the samples are made to 50mL with ultrapure water for subsequent analysis by plasma spectrometry.

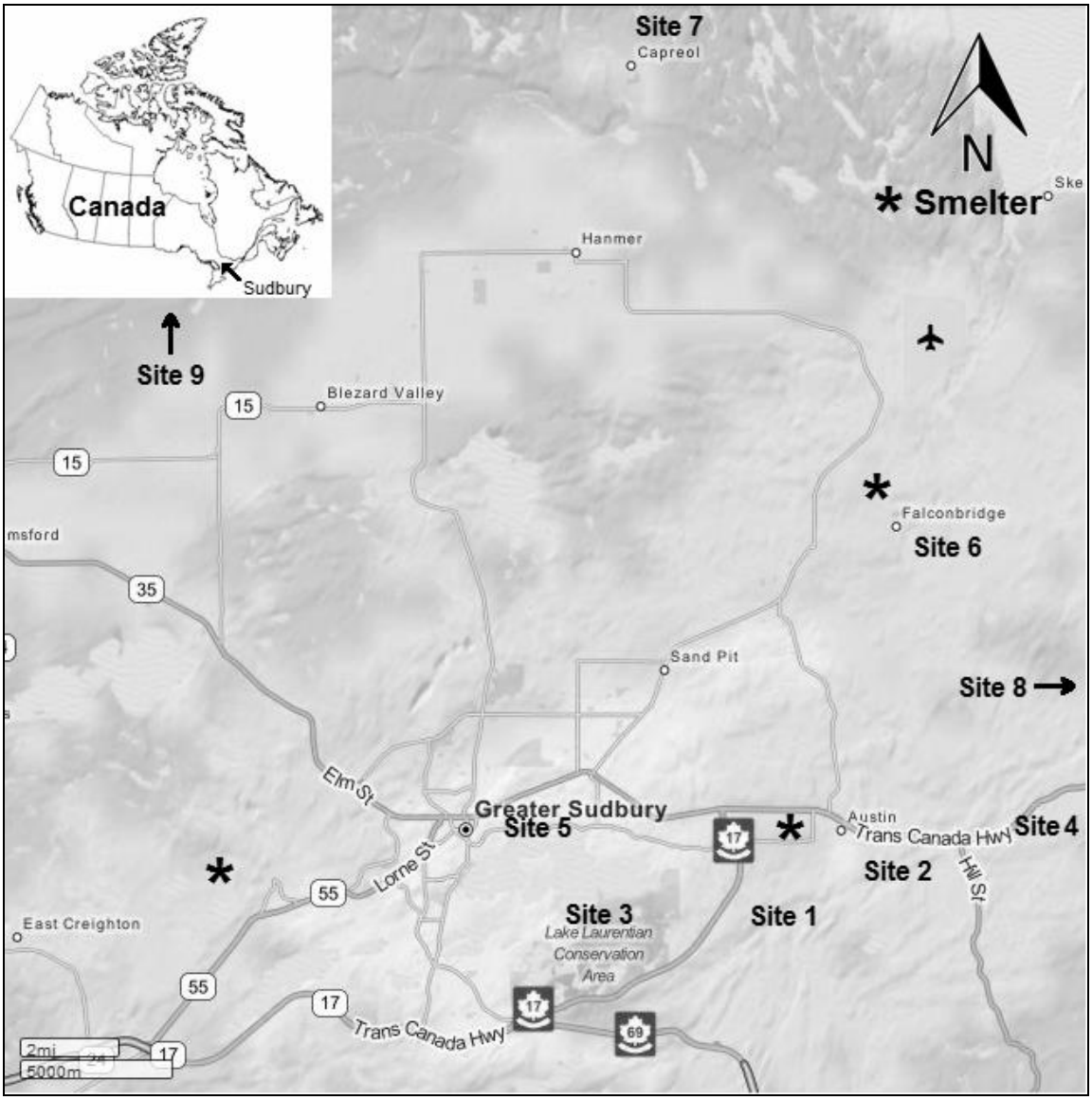


Figure 1: Location of sampling area from the Greater Sudbury Region. Site 1: Daisy Lake; Site 2: Wahnapitae Hydro-Dam; Site 3: Laurentian; Site 4: Kukagami; Site 5: Kingsway; Site 6: Falconbridge; Site 7: Capreol; Site 8: Hagar, Site 9: Onaping Falls and ≈ 91km from Sudbury towards Timmins; and Site 10: Kelly Lake. Sites 7, 8 and 9 were used as reference sites. Hagar (St Charles to be exact) is outside the map as a reference site and is about 50-60 km from Sudbury. All reference sites were chosen based on their distance (~50 km) from smelters. Sites 3, 4 and 6 were uniquely used for metal analysis and not for fungal analysis.

Bioavailable metals were estimated by extracting 5 g of soil with 20 ml of 0.01M $LiNO_3$ in a 50-ml centrifuge tubes in a shaker under ambient lighting conditions for 24 hours at 20 ^{0}C (Abedin et al. 2006; Abedin et al. 2012). The pH (LiNO3) of the suspension was measured prior to centrifugation at 3000 rpm for 20 minutes, with filtration of the supernatant through a 0.45 um filter into a 20 ml polyethylene tube and made to volume with deionized water. The filtrate was preserved at approximately 3 ^{0}C for analysis by ICP-MS. The quality control program completed in an ISO 17025 accredited facility (Elliot Lake Research Field Station of Laurentian University) included analysis of duplicates, Certified Reference Materials (CRM's), Internal Reference Materials (IRM's), procedural and calibration blanks, with continuous calibration verification and use of internal standards (Sc, Y, Bi) to correct for any mass bias. All concentrations were calculated in mass/mass dry soil basis. The data obtained for all elements of interest in analyzed CRM soil samples were within ± 12% of the certified level.

2.2.3 Statistical Analysis

The data for the metal levels in soil samples were analyzed using SPSS 7.5 for Windows™, with all data being transformed using a $\log_{10}$ transformation to achieve a normal distribution. Variance-ratio test was done with an assumption of data normality in the underlying population distributions of the data. ANOVA, followed by Tukey's HSD multiple comparison analysis, were performed to determine significant differences ($p < 0.05$) among the sites. Data from analysis of samples from limed and unlimed areas were compared using Student t-test.

2.3 Results

Results for total and bioavailable metal and nutrient content as well as pH measurements are described in Tables 1 to 7.

2.3.1 Total Metals

The estimated levels of total metal concentrations in the soil samples for the different sites from the Sudbury Region in Canada are listed in Table 1 with highest concentrations consistently measured in the top organic horizon (LFH). All the total metal concentrations obtained from the dominantly mineral horizon layers (Ae, Bm, BC, and C) were within the OMOE guidelines with the exception of Copper (Table 1). There were significant differences between soil horizons among all the sites for the total amount of nutrients and metal elements (Table 1). Table 3 showcases the mean concentration of total elements in the limed and unlimed organic surface horizons (LFH) of soils from the Sudbury region sites with highest concentrations majorily measured in the unlimed sites. However, no significant differences were observed between limed and unlimed sites for either total metal or nutrient contents with the exception of total arsenic (Table 3).

2.3.2 Bioavailable Metals

The concentration of bioavailable elements in Sudbury samples from the different soil horizons varied from < analytical detection limits to 0.32 mg kg^{-1} for arsenic, 0.22 mg kg^{-1} to 0.35 mgkg^{-1} for cobalt, 0.80 mg kg^{-1} to 8.20 mg kg^{-1} for copper, 4.57 mg kg^{-1} to 92.32 mg kg^{-1} for magnesium, 4.07 mg kg^{-1} to 18.93 mg kg^{-1} for manganese, 1.13 mg kg^{-1} to 5.17 mg kg^{-1} for nickel, 0.03 mg kg^{-1} to 0.38 mg kg^{-1} for strontium, and 0.30 mg kg^{-1} to 1.24 mg kg^{-1} for zinc (Table 2). Overall, the portion of total metals that was available to biota was very small. In fact, only 1.1% and 0.8% of total copper and nickel respectively were bioavailable (Table 5).

2.3.3 Effect of Liming

The pH in unlimed sites was consistent with that documented for soils on coarser textured soils with coniferous vegetation on the Canadian Shield of at < 4, classified as extreme acid (McKeague et al. 1979; Spiers et al. 2012). The pH in limed sites was significantly higher ranging from 4.12 to 6.75 in the top organic layer (Table 7). No differences were observed among soil profiles analyzed for pH (H_2O) and pH ($CaCl_2$). The liming did maintain an increase in soil pH from extremely acid to slightly acid, even 30 to 40 years after dolomitic limestone applications. Significantly, no major differences were observed between limed and unlimed areas with regards to total element concentrations with the exception of arsenic (Table 3). As expected, the limed samples contained higher levels of total calcium and magnesium compared to unlimed sites (Table 3). On the other hand, there were significantly higher contents of bioavailable aluminum, iron, manganese, and strontium in unlimed sites compared to limed areas (Table 4). The levels of bioavailable cobalt, copper, nickel, and zinc were also lower in limed compared to unlimed areas (Table 4).

Table 1: Mean total concentrations of elements in selected soil horizons from sites in the Greater Sudbury region (concentrations are in mg kg^{-1}, dry weight)

Horizons	Elements																
	Al	As	Ca	C	Co	Cu	Fe	K	P	Pb	Mg	Mn	S	Ni	N	Sr	Zn
OMOE	-	12	-	-	50	91	-	-	-	250	-	1100	-	75	-	-	360
LFH	16445a	38.8a	5174a	142263a	42a	882a	25227a	7795b	654a	118.6a	1823a	279ab	1588a	1003a	6030a	57a	76a
Ae	15368a	8.29b	2900a	16836b	11b	158b	13785b	10040a	273b	11.34b	798b	329ab	922b	68b	349b	43a	36b
Bm	16482a	5.96b	3128a	17760b	15b	104bc	20491a	9992a	378b	4.31c	968b	351a	1022b	44b	416b	35a	63a
BC	15118a	1.84b	2977a	14465b	12b	65c	19300a	9978a	323b	5.16c	929b	249ab	1023b	39b	304b	30a	55a
C	17466a	3.77b	3391a	17236b	12b	79c	22267a	9998a	292b	7.39c	990b	195b	1179b	41b	409b	34a	42b

Means in columns with a common subscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$).
7 sites: Daisy Lake, Dam, Laurentian, Kukagami, Kingsway, Falconbridge, Capreol.

Table 2: Bio-available nutrients and metals in selected soil horizons from sites in the Greater Sudbury region (concentrations are in mg kg^{-1}, dry weight)

Horizons	Elements															
	Al	As	Ca	Cd	Co	Cu	Fe	K	P	Pb	Mg	Mn	Na	Ni	Sr	Zn
LFH	71.45a	0.32	162.86a	<DL	0.26a	8.20a	77.19a	149.54a	14.68a	0.27	92.32a	18.93a	15.75a	5.17a	0.38a	1.24a
Ae	28.61b	0.07	35.09b	<DL	0.22a	2.67a	29.04b	16.11a	0.91b	<DL	24.28b	13.34a	5.79b	1.98b	0.07b	0.31b
Bm	18.70b	<DL	23.79b	<DL	0.35a	1.37a	14.98bc	13.51a	0.18b	<DL	16.62b	15.66a	5.21b	1.78b	0.06b	0.36b
BC	15.87b	<DL	15.14b	<DL	0.23a	0.80a	7.52c	8.73a	<DL	<DL	10.34b	6.35a	4.43b	1.13b	0.03b	0.32b
C	16.9b	<DL	12.44b	<DL	0.23a	1.09a	11.41c	9.96a	<DL	<DL	4.57c	4.07a	4.32b	1.52b	0.08b	0.30b

Means in columns with a common subscript are not significantly different based on Tukey multiple comparison test ($p \geq 0.05$). 7 sites: Daisy Lake, Dam, Laurentian, Kukagami, Kingsway, Falconbridge, Capreol. <DL indicates concentrations below detectable level.

Table 3: Total nutrient and metal content in the organic surface horizon (LFH) of limed and unlimed sites from the Greater Sudbury region sites (concentrations are in mg kg^{-1}, dry weight)

Sites	Elements[a]																
	Al	As*	Ca	C	Co	Cu	Fe	K	P	Pb	Mg	Mn	S	Ni	N	Sr	Zn
Limed	13666 ±1439	1.46 ±1.28	6920 ±2044	124267 ± 26533	41 ±14	952 ±343	20800 ±3634	7066 ±316	571 ±93	91.23 ±45	2309 ±672	198 ±41	1290 ±147	991 ±485	5343 ±1170	58 ±12	68 ±21
Unlimed	17783 ±2573	18.98 ±5.82	4106 ±617	136583 ±39404	42 ±11	1021 ±356	29000 ±3364	8540 ±187	600 ±37	120.3 ±43	1548 ±194	242 ±15	2001 ±205	1061 ±398	6345 ±1860	62 ±10	73 ±16

[a] Results are expressed as mean values ± standard error based on three replicates (n = 3)
*Represents significant difference between treatments based on t-test ($p \geq 0.05$)
Limed and Unlimed sites: Daisy Lake, Dam and Kingsway.

Table 4: Bio-available nutrients and metals in the organic surface horizon (LFH) of limed and unlimed sites from the Greater Sudbury region sites (concentrations are in mg kg^{-1}, dry weight)

Sites	**Elements**															
	Al*	As	Ca*	Cd	Co	Cu	Fe*	K	P	Pb	Mg*	Mn*	Na	Ni	Sr*	Zn
Limed	43.3	0.14	252.8	<DL	0.12	7.21	42.07	98.13	6.14	0.21	169.1	3.71	23.43	4.15	0.07	0.48
Unlimed	77.8	0.10	82.3	<DL	0.31	12.04	108.6	129.22	6.05	0.16	39.78	13.95	13.66	6.85	0.33	1.48

*Represents significant difference between treatments based on t-test ($p \geq 0.05$). Limed and No limed sites: Daisy Lake, Wahnapitae Hydro Dam and Kingsway. <DL indicates concentrations below detectable level.

Table 5: Proportion of bioavailable relative to total elements for the LFH horizon of soils from the Greater Sudbury region sites

Sampling sites	Elements*													
	Al	As	Ca	Cd	Co	Cu	Fe	K	P	Pb	Mg	Mn	Ni	Zn
Daisy Lake	0.65	2.28	2.19	<DL	0.61	0.87	0.33	1.33	1.00	0.15	3.11	4.35	0.73	1.53
Dam	0.36	<DL	3.18	<DL	0.43	1.19	0.34	0.99	1.06	<DL	5.39	2.14	0.51	0.98
Laurentian	0.78	<DL	1.22	<DL	0.56	0.70	0.19	3.71	0.47	0.18	1.40	5.38	0.35	0.99
Kukagami	0.60	<DL	3.77	<DL	2.61	1.96	0.84	2.75	4.68	0.78	3.06	12.59	1.39	2.97
Kingsway	0.23	<DL	2.38	<DL	0.48	0.95	0.18	1.99	0.94	0.14	4.56	4.19	0.49	1.40
Falconbridge	0.10	0.64	3.43	<DL	0.29	0.75	0.15	0.53	0.66	0.21	9.90	0.79	0.59	0.39
Capreol (Reference Site)	0.28	<DL	3.99	<DL	1.25	1.11	0.22	4.58	0.53	0.41	4.13	0.47	1.37	0.26

*Values as percent (%).

<DL indicates concentrations below detectable level.

Table 6: Correlation between total and bio-available metal and nutrient content in soil profiles from the Greater Sudbury region sites

Element	LFH	Ae	Bm	BC	C
Aluminum	0.06	-0.03	-0.23	-0.31	-0.79*
Barium	0.29	-0.35	-0.19	0.01	0.09
Calcium	0.93*	0.49	0.54	-0.02	-0.10
Cobalt	0.65	0.90*	0.49	0.79*	0.51
Copper	0.90*	0.45	0.45	0.34	0.24
Iron	0.02	0.13	0.27	0.69	0.98*
Potassium	-0.002	0.65	0.06	0.34	-0.26
Magnesium	0.83*	0.42	0.77*	0.46	-0.21
Manganese	0.88*	0.99*	0.82*	0.95*	0.74
Sodium	-0.17	-0.18	-0.31	-0.49	-0.15
Strontium	0.49	0.28	0.05	0.02	0.13
Nickel	0.89*	-0.23	0.19	0.16	0.87*
Phosphorus	0.21	0.01	-0.15	0	0
Zinc	0.49	-0.05	0.27	0.43	0.60

*Represents strong correlation between two variables. 7 sites: Daisy Lake, Wahnapitae Hydro Dam, Laurentian, Kukagami, Kingsway, Falconbridge and Capreol.

Table 7: The pH of the LFH layer from the Greater Sudbury region sites

Sampling sites		**Layer 1 (LFH)**	
	Type	pH H_2O	pH $CaCl_2$
Daisy Lake	Unlimed	4.04	3.87
	Limed	4.12	4.05
Dam	Unlimed	3.82	3.56
	Limed	6.75	6.34
Kingsway	Unlimed	3.87	2.35
	Limed	4.67	4.35
Kelly Lake	Unlimed	3.75	3.37
	Limed	6.41	6.13
Hagar (reference site)	Unlimed	3.50	3.23
Onaping Falls (reference site)	Unlimed	4.84	3.92
Capreol (reference site)	Unlimed	3.92	3.43

2.4 Discussion

Soil metal contamination proves to be very detrimental to overall soil quality because it interferes with the soil's natural capability to function (Karlen et al., 1997). Additionally, metal contamination is unfavourable towards the vegetation and microorganisms inhabiting the soil from which they derive their nutrients (Winterhalder, 1996). Phytotoxic effects caused by an accumulation of metals in the soil may influence the optimum growth and development of plants (Beckett & Davis, 1988). Likewise, mycotoxic effects in fungi caused by high bioavailable levels of toxic metals may also unfavour metabolism, development, and differentiation (Gadd, 1986; Gadd & White, 1989). The chemical toxicology of metals released in the environment by natural and anthropogenic sources is of increasing concern in many regions worldwide, including the Greater Sudbury Region. For this particular region, soil acidity coupled with elevated total aluminium, copper and nickel levels were the major factors restricting vegetative growth within the area thus causing the blackened and barren land of Sudbury (Winterhalder, 1996).

Data from the present study shows that the highest total metal concentrations occurred in the upper soil horizon (LFH). The total amounts of arsenic, carbon, cobalt, copper, phosphorus, lead, magnesium, sulfur, nickel and nitrogen were found to be significantly higher in the first soil horizon compared to the other soil depths (Table 1). Thus, it seems as though metal particulate accumulation in this soil layer is caused by an aerial cause rather than an internal cause. Overall, the level of bioavailable metals was significantly lower than total metal values. Higher bioavailable concentrations of aluminium, calcium, iron, potassium, magnesium, sodium, nickel, strontium and zinc were also found in the top soil layer compared to lower horizons (Table 2). A significant association was revealed concerning the levels of total and bioavailable calcium, copper, magnesium, manganese and nickel found in the soil (Table 6).

The Greater Sudbury Region has undergone many modifications over the years in terms of soil chemistry due to decades of SO_2 fumigations as well as metal particulate accumulations in the soil (Winterhalder, 1996). These aformentionned events caused the soil to become very acidic in many areas and consequently, caused several metals to be more readily biologically available to plants and soil microbes (Winterhalder, 1996). As a result, dolomitic and calcitic limestone were added manually as well as aerially in some sites in order to modify its' existing soil chemistry (Winterhalder, 1996). Results of the present study showed higher pH values in limed areas compared to unlimed sites. This indicates the lasting effects of liming performed 30-40 years ago. Metal analysis revealed significantly lower concentrations of total arsenic and bioavailable aluminium, iron and strontium in limed sites compared to unlimed sites. These findings are consistent with Winterhalder's (1983) reports concerning the occurence of toxic levels of aluminium in the soil when soil pH decreases. Furthermore, the high levels of bioavailable calcium and magnesium in the limed sites reflects the addition of the dolomitic and calcitic limestone having been applied 30-40 years ago.

Soil pH can influence metal and nutrient uptake in plants. Nutrient uptake is a complex concept in which different mechanisms are proposed for a variety of elements found in the soil. Plants require nutrients and metals for a variety of purposes. For instance, zinc is essential for plant growth, structure and function (Ahmad & Erum, 2010). While zinc does not cause phytotoxic effects in plants at low levels (Paschke et al., 2000), increased concentrations of zinc in the soil can cause toxic effects in plants (Chaney & Oliver, 1996). Zinc deficiency in plants can also be detrimental. When this occurs, intervenial chlorosis (molting) will be present in which light green to pale yellow coloration will apear between the midrib and secondary veins (Storey, 2006). The role of nickel in the metabolic processes of higher plants is also critical

(Ahmad & Erum, 2010) but excessive levels can be toxic. These excessive levels of nickel in the soil are generally caused by anthropogenic sources. Copper is also essential to plant health because it acts as a structural element in regulatory proteins, participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism, and hormone signaling (Marschner, 1995; Raven et al. 1999). When present in high levels in the soil, Cu can become highly toxic which can lead to symptoms such as chlorosis, necrosis, stunting, leaf discoloration, and inhibition of root growth (Van Assche & Clijsters, 1990; Marschner, 1995). Consequently, the chemical toxicology of the soil is directly related to the survival or death of plants.

Soil pH also greatly regulates fungal metal and nutrient uptake. At low pH levels, the accessible levels of nutrients to plants and fungi will be affected. Additionaly, at high or low pH levels, decreased or ceased organic matter mineralization rates will be encountered (Smith & Doran, 1996). As such, soil acidity can directly or indirectly affect many of the complex vital prossesses of fungi. Metal effects in fungi can vary a great deal between organisms, strains, the stage of growth and different vegetative and reproductive forms of the same organism (Gadd & Mowll 1985; Sabie & Gadd 1990). A specific organism can undergo several mechanisms, both dependant and independant of metabolism and affected by environmental factors, in order for potentially toxic metal species to be detoxified (Gadd, 1992). In addition, many other elements that are not considered to be true metals according to their chemical definitions can also cause detrimental effects to fungi species because they exhibit varying degrees of toxicity and accumulation by fungi (Gadd, 1992). These include metalloids, actinides and lanthanides such as uranium and thorium (Gadd, 1992). For example, Caesium is of current concern as it relates to environmental contamination as well as accumulation by the biota, including fungi, and

transferring to other organisms such as humans (Gadd, 1992). Being either toxic metals or metalloids, they nevertheless can exert harmful effects on fungi due to their strong coordinating abilities (Ochiai, 1987). The toxic effects that fungi species may experience include the blocking of functional groups of biologically important molecules such as enzymes and transport systems for essential nutrients and ions, the displacement or substitution of essential metal ions from biomolecules and functional cellular units, conformation modification, denaturation, and inactivation of enzymes and disruption of cellular and organellar membrane integrity (Ochiai, 1987). Almost every aspect of fungi metabolism, growth and differentiation can be affected by toxic metals. The effects of metals will vary with specific fungi species and the level of metal concentration as well as with other physico-chemical factors (Gadd, 1986; Gadd & White, 1989).

While smelters are still in use within the region, the pH values documented through this present study show that the liming performed 30-40 years ago continues to provide long term advantages. In fact, soil pH values within the vicinity were once recorded to be as low as 2.2 to 2.4 before liming (Winterhalder, 1996). Additionaly, lower bioavailable concentrations of aluminium, iron and strontium in limed sites, compared to unlimed sites, may be attributed to a decrease in soil acidity in the treated sites.

Chapter 3: Analysis of Soil Fungi Diversity and Abundance in Limed and Unlimed Areas Contaminated with Metals in the Greater Sudbury Region (Ontario, Canada)

3.1 Introduction

Mining and smelting processes as well as activities associated with them have caused a variety of negative effects to the Greater Sudbury Region environment. For instance, SO_2 fumigation and the accumulation of numerous metal particulates, have damaged soil quality and ultimately, the vegetation and fungi populations inhabiting the region (Winterhalder, 1996). In fact, Balsilie et al. (1978) have documented a decrease in the numbers of soil fungi and bacteria near the location of the historic smelter sites. Additionally, they have been able to show a restriction in the degree of colonization of roots of oxeye daisy (*Chrysanthemum leucanthemum*) and devil's paintbrush (*Hieracium aurantiacum*) by vesicular arbuscular mycorrhizal fungi in the Sudbury barrens (Balsilie et al., 1978).

A decrease in industrial emissions was put into effect as it was earlier hyphothesised that reductions in emissions would lead to the rapid colonization of vegetation in the barrens mainly because it was believed that SO_2 fumigation was the main factor directly limiting vegetative improvement (Winterhalder, 1996). Nevertheless, remediation projects were also commenced in 1978 (Winterhalder, 1996). Remediation projects consisted of neutralizing soil pH with the addition of lime as well as adding fertilizer, seed distribution and tree planting (Winterhalder, 1996). In fact, tree planting has been germane to Sudbury's regreening process. It allowed for the introduction of new tree species while at the same time, expanding native species. Consequently, because many tree species as well as grasses and herbs were introduced into the environment, more organic matter was readily made accessible to fungi for decomposition purposes. The intracacies of soil-fungal interactions are crucial in determining fungi diversity and abundance

for a particular region. Thus, soil chemistry, metal binding capacity and metal speciation within the soil are key concepts that need to be adequately assessed and properly understood in order to sufficiently identify metal-fungal dynamics for a specific region. The examination of fungal diversity for a particular area is critical as it is an essential factor in the determination of their sustainability (Nielsen & Winding, 2002). Additionally, fungal abundance is another prime indicator of their population health (Nielsen & Winding, 2002). Since fungi play such a crucial role in soil formation, organic matter decomposition as well as in the transformations of toxic metabolites found in the soil according to Ritz et al. (2004), soil amendment strategies adopted in damaged ecosystems, such as liming, require extreme diligence to ensure that all fungal nutrient, metal, substrate requirements are addressed and achieved.

The objective of this component of the present study is to determine fungi diversity and abundance in selected limed and unlimed areas contaminated with metals in the Greater Sudbury Region.

3.2 Materials and Methods

3.2.1 Soil Sampling and Processing

Soil sampling was done as described in section 2.2.1 in regards to the Wahnapitae-Hydro Dam, Daisy Lake, Kingsway, Kelly Lake, Hagar, Onaping Falls and Capreol sites. Every soil sample from every site was dried at room temperature (25 °C) for 48 hours by opening and exposing the plastic bags to air. The soil was then sifted with a 2 mm sieve in order to remove all forms of debris. Three soil samples from each of the limed, unlimed or reference sites were mixed together respectively to produce a ‘master’ limed, unlimed or reference soil sample to be used later for fungal analysis. Samples from the “master mix” were used for serial dilutions. It

should be noted that for all samples, only the top 5 cm (LFH layer) of organic soil was used because it was hypothesized that the majority of fungi colonies would be present within this soil horizon.

3.2.2 Fungal Isolation Method

All materials, except for the soil, were autoclaved before use. Serial dilutions were subsequently prepared. For that purpose, 10.0 g of soil was mixed with 90.0 mL of sterile H_2O resulting in a dilution of 1/10. A sterile stopper was then firmly placed on the Erlenmeyer flask containing the 1/10 dilution. The solution was shaked vigorously for 1 hour. Four successive dilutions by a factor of 10 were then conducted. Using a sterile 1-mL pipet, 1 mL of the mixture was mixed with 9.0 mL of sterile water to make a 1/100 dilution. The mixture was vortexed for approximately 30 seconds. The 1/1000 dilution was prepared by mixing 1 mL of the 1/100 solution with 9.0 mL of sterile water. The same procedure was followed to prepare the 1/10 000 and 1/100 000 dilutions.

For the purporse of this study, two medium types were utilized, namely Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA). SDA is a multipurpose growing medium for numerous fungal species whereas, MEA is an ideal medium for the cultivation of yeasts and molds. For each medium (SDA and MEA), six plates including a control and different diluted solutions were prepared and duplicated. Dilution factors on the plates included 1/100, 1/1000, 1/10 000, 1/100 000 and 1/1 000 000. These five serial dilutions were prepared in order to achieve the highest sensitivity of detection of fungi on the agar plates. The labelled plates were inoculated with 0.1 mL of each solution respectively. The inocula were evenly spread over the surface of the agar plate using a sterile glass rod. The plates were then incubated at 26°C until

satisfactory growth appeared which is to say, until colony masses were easily observed. Following this, the plates were examined every day and different colonies started being recorded roughly 3-4 days after incubation.

3.2.3 Slide preparation

All standard microscope slides were cleaned with 95 % ethanol prior to use. A few drops of Lactophenol cotton blue were then placed on the slides in question. With the aid of a pin probe, parts of a fungal colony were placed onto the slide. A cover slip was then placed onto the mount. Slides were then properly labeled and air dried before storage. Fungi identification was achieved via classification guides based on microscopic morphology and colony appearance on the media. Classification guides included: Detection and isolation of soil fungi (Davet & Rouxel, 2000); Compendium of soil fungi (Domsch, Grams & Anderson, 2007); Idenfication of fungi: an illustrated introduction with keys, glossary, and guide to literature (Dugan, 2006); A laboratory guide to common *Penicillium* species (Pitt, 1985); Pictorial atlas of soil and seed fungi: morphologies of cutured fungi and key to species (Watanabe, 1937); and Mucorales (Zycha, 1970).

3.2.4 Statistical Analysis

Fungal diversity and fungal abundance between unlimed and limed soil samples were analyzed using Student t- tests. One-Way Analysis of Variance (ANOVA) tests were also conducted between limed, unlimed and reference soil samples concerning fungal diversity and abundance. Individual differences were determined using Tukey's multiple range test.

3.3 Results

3.3.1 Fungi Diversity

All fungal species identified in limed and unlimed sites as well as in the reference sites are described in Table 8. Figures 2 - 4 are venn diagrams showcasing different fungi species and their shared or specific site location. The corresponding fungi phyla are listed in Table 9. Figures 5 to 7 are pie charts illustrating fungi phyla appearing in limed, unlimed or reference sites. Data for fungi appearance in different dilution factors is described in Tables 18 to 39. Fungi abundance for all the targeted sites is described in Tables 40 to 59. Tables 60 to 81 summarize the time of appearance as well as the colony description of the fungi species in the agar plates and over the microscope.

A total of 52 fungi species belonging to 34 genera were identified (Table 8). Some species were specific to one or two sites, while others were present in the majority of the sites including the reference sites. A total of 23 fungi species were identified in samples from limed areas based on fungi growth on SDA medium plates, while 17 species were observed in samples from unlimed sites (Table 8). A total of 18 fungi species were found in the reference sites (Table 8). The reference sites of Hagar, Onaping Falls and Capreol contain both unique and shared fungi species amongst each other as well as amongst the targeted limed and unlimed sites (Table 8). These results indicate that fungal diversity is higher in limed soils compared to unlimed soils based on SDA medium growth.

Fungi growth on the MEA medium revealed the presence of 21 species in samples from limed areas and 19 species from unlimed areas (Table 8). The reference sites of Hagar, Onaping Falls and Capreol had various distinctive and common fungi species amongst each other as well as amongst the targeted limed and unlimed sites (Table 8). A total of 20 species were found

growing in the reference sites (Table 8). These results suggest that fungal diversity is slightly higher in limed soils compared to unlimed when grown on MEA medium. This trend is however less significant when fungi species growing on SDA medium was considered.

The most frequently occurring fungi species on the SDA medium include *Cryptococcus neoformans, Mucor indicus, Penicillium fellutanum, Rhizopus stolonifer, Sprorotrichum schenckii, Trichoderma harzianum* and *Trichophyton mentagrophytes* (Table 8). For the MEA medium, *Cryptococcus neoformans, Penicillium citrinum*, *Rhizopus stolonifer*, *Streptomyces scabies, Trichophyton mentagrophytes* and *Trichophyton tonsurans* were the most prevalent species (Table 8). In general, 41 species were identified on SDA medium and 36 species on MEA medium (Table 8).

Optimal fungal growth was observed on SDA medium at the dilution factors of 10^{-1} to 10^{-4} and at 10^{-1} to 10^{-3} dilution factors for the MEA medium. In both media cases, the majority of the fungal growth was observed within the first 7 to 10 days post inoculation.

3.3.2 Fungi Phylum Identification

Limed areas: Limed areas were primarily composed of Ascomycota, followed by fungi from Basidiomycota, Zygomycota, Deuteromycota and Oomycota phyla respectively (Figure 5).

Unlimed areas: Unlimed areas were primarily composed of Ascomycota, followed by fungi from Zygomycota, Basidiomycota, Deuteromycota and Oomycota phyla respectively (Figure 6).

Reference sites: The reference sites were primarily composed of Ascomycota, followed by fungi from Zygomycota, Basidiomycota, Oomycota and Deuteromycota phyla respectively (Figure 7).

3.3.3 Fungi Species Identification

Figures 8 and 9 illustrate the number of different fungi species observed in limed, unlimed and reference sites for SDA and MEA media respectively.

Wahnapitae Hydro-Dam site: The most common species identified on SDA medium in the limed areas include *Penicillium fellutanum*, *Rhodotorula mucilaginosa*, *Trichoderma harzianum* and *Trichophyton mentagrophytes* (Table 18). For the unlimed areas, the most prevalent fungi species include *Eupenicillium shearii*, *Penicillium fellutanum*, *Sporotrichum schenckii* and *Trichophyton mentgrophytes* (Table 19).

For the MEA medium, *Mycoderma aceti* and *Trichophyton mentagrophytes* were the most frequent species in the limed areas (Table 20). For the unlimed areas, the most prevalent fungi species include *Cryptococcus neoformans*, *Mycoderma aceti* and *Trichophyton mentagrophytes* (Table 21). Greater fungal species diversity is apparent in limed soil samples compared to samples from unlimed areas when grown on the SDA medium. Fungal diversity is slightly greater in unlimed soil samples compared to limed soil samples when grown on the MEA medium.

Daisy Lake site: The most common species growing on the SDA medium in the limed soil include *Mortierella isabellina*, *Penicillium chrysogenum* and *Sporotrichum schenckii* (Table

22). For unlimed soil, the most prevalent fungi species include *Acremonium strictum*, *Penicillium lividum* and *Rhizopus stolonifer* (Table 23).

For the MEA medium, the most common species in the limed soil samples include *Penicillium chrysogenum*, *Sporotrichum schenckii* and *Trichophyton mentagrophytes* (Table 24). For the unlimed soil samples, *Acremonium strictum*, *Saprolegnia parasitica* and *Verticillium dahlia* were the most prevalent (Table 25). Greater species diversity is apparent in limed soil samples as opposed to unlimed soil samples for the SDA medium. Fungal diversity is also greater in samples from limed areas compared to unlimed soil samples when grown on the MEA medium.

Kingsway site: For SDA medium, *Candida albicans, Penicillium fellutanum, Rhizopus stolonifer*, and *Trichophyton mentagrophytes* were the most common species in the limed areas (Table 26). Whereas, *Cunninghamella bertholletiae* and *Saprolegnia parasitica* were the most prevalent fungi species in the unlimed areas (Table 27).

For the MEA medium, the most common species in the limed soil were *Mycoderma aceti* and *Trichophyton mentagrophytes* (Table 28). *Cryptococcus neoformans*, *Saprolegnia parasitica*, *Sporotrichum schenckii* and *Trichophyton mentagrophytes* were the most prevalent in the unlimed soil (Table 29). Greater fungal diversity was observed in the limed soil samples compared to the samples from the unlimed areas for the SDA medium. Fungal diversity is greater in the unlimed soil samples compared to the limed soil samples for the MEA medium.

Kelly Lake site: The most common species identified on SDA medium in the limed areas include *Cryptococcus neoformans, Penicillium crustaceum* and *Sporotrichum schenckii* (Table

31). For the unlimed areas, the most prevalent fungi species include *Cryptococcus neoformans* and *Rhizopus stolonifer* (Table 30).

For the MEA medium, the most common species identified in the limed soil were *Penicillium lividum* and *Streptomyces scabies* (Table 33). For the unlimed soil, the most prevalent species include *Acremonium strictum*, *Actinomyces bovis*, and *Alternaria alternata* (Table 32). Greater fungal diversity was observed in the limed soil samples compared to the samples from unlimed areas for both the SDA and MEA media.

Hagar (reference site): The species identified on SDA medium include *Cunninghamella bertholletiae, Eupenicillium shearii, Mucor indicus, Penicillium crustaceum* and *Trichophyton mentagrophytes* (Table 34).

For the MEA medium, the species identified include *Penicillium crustaceum, Rhizopus stolonifer, Streptomyces scabies, Trichophyton concentricum, Trichophyton mentagrophytes* and *Trichophyton tonsurans* (Table 35).

Onaping Falls (reference site): The species identified on SDA medium include *Allescheria boydii, Candida glabrata, Candida lusitaniae, Cryptococcus neoformans, Mucor indicus, Penicillium citrinum,* and *Penicillium decumbens* (Table 36).

For the MEA medium, the species identified include *Blastomyces dematitidis, Coccidioides immitis, Mucor indicus, Penicillium citreonigrum, Penicillium citrinum, Penicillium montanense, Pythium debaryanum, Rhodotorula mucilaginosa, Trichophyton faviforme, Trichophyton tonsurans,* and *Trichosporon mucoides* (Table 37).

Capreol (reference site): The species identified on SDA medium include *Absidia corymbifera*, *Blastomyces brasiliensis*, *Blastomyces dermatitidis*, *Cladosporium sphaerospermum*, *Mucor indicus*, *Penicillium montanense*, *Trichoderma harzianum*, *Trichophyton mentagrophytes*, and *Trichophyton tonsurans* (Table 38).

For the MEA medium, the species identified include *Blastomyces dermatitidis*, *Candida lusitaniae*, *Cryptococcus neoformans*, *Epicoccum purpurascens*, *Penicillium citrinum*, *Pythium debaryanum*, *Rhizopus stolonifer*, *Sporotrichum schenckii*, *Streptomyces scabies*, and *Trichophyton faviforme* (Table 39).

3.3.4 Fungi Diversity

No significant differences were observed between limed and unlimed areas for the SDA medium analysis (Table 10). However, the trend revealed a higher fungal diversity in the limed soil samples compared to the samples from unlimed soil. There was a significant difference between limed and unlimed areas for the MEA medium data (Table 10). Slightly higher fungal diversity was recorded for the unlimed soil samples compared to the samples from limed soil.

There was a significantly higher fungal diversity in the limed areas compared to the samples from unlimed sites based on SDA medium data (Table 11). There was no significant difference for fungal diversity between limed, unlimed or reference sites based on the MEA medium analysis (Table 11).

3.3.5 Fungi Abundance

Wahnapitae Hydro-Dam site: The most abundant species identified on SDA medium in samples from the limed area at the Wahnapitae Hydro-Dam site is *Penicillium fellutanum* with a total abundance of 7.77 x 10^7 c.f.u./g, followed by *Mycoderma aceti* with 3. 65x 10^7 c.f.u./g, and *Trichoderma harzianum* with 2.52 x 10^6 c.f.u./g (Table 40). The most abundant species on unlimed soil on SDA medium is *Tricophyton mentagrophytes* with a total abundance of 1.22 x 10^7 c.f.u./g followed by *Sporotrichum schenckii* with a total value of 6.88 x 10^6 c.f.u./g and *Eupenicillium shearii* with 3.89 x 10^6 c.f.u./g (Table 41). In general, higher fungal abundance was observed in samples from limed sites compared to unlimed soil samples for SDA medium (Figure 10). Fungal abundance values on MEA medium could not be estimated with accuracy because of the extremely fast growing nature of the fungi.

Daisy Lake site: Analysis of samples from the limed areas in Daisy Lake revealed that the most abundant species was *Sporotrichum schenckii* with a total abundance of 3.38 x 10^6 c.f.u./g, followed by *Penicillium chrysogenum* with 2.65 x 10^6 c.f.u./g, and *Mortierella isabellina* with an abundance of 2.42 x 10^6 c.f.u./g on the SDA medium (Table 42). For samples from the unlimed areas, *Acremonium strictum* with an abundance of 5.26 x 10^7 c.f.u./g was the most abundant species detected on SDA medium (Table 43). This was followed by *Penicillium lividum* with a total abundance of 5.16 x 10^7 c.f.u./g, and *Rhizopus stolonifer* with 6.0 x 10^6 c.f.u./g (Table 43). In general, higher fungal abundance was observed in unlimed soil samples compared to limed soil samples for the SDA medium but this result being attributed to topographic reasons of the Daisy Lake site (Figure 10).

Sporotrichum schenckii with a total abundance of 6.18 x 10^6 c.f.u./g was the most abundant species identified on samples from the limed areas growing on MEA medium (Table

44). This was followed by *Penicillium chrysogenum* with a total abundance of 2.46 x 10^6 c.f.u./g, and *Trichophyton mentagrophytes* with a total abundance of 1.03 x 10^6 c.f.u./g (Table 44). For samples from the unlimed areas, *Acremonium strictum* with a total abundance of 1.37 x 10^8 c.f.u./g was the most abundant species observed on the MEA medium (Table 45). The abundance of *Penicillium citrinum* and *Saprolegnia parasitica* were 1.32 x 10^7 c.f.u./g and 9.25 x 10^6 c.f.u./g respectively (Table 45). In general, the trend based on all the serial dilutions shows higher fungal abundance in unlimed soil samples compared to limed soil samples but this being once again attributed to topographic reasons (Figure 11).

Kingsway site: The most abundant species identified on SDA medium in samples from the limed area include *Candida albicans* with a total abundance of 1.12 x 10^8 c.f.u./g, followed by *Penicillium fellutanum* with 4.34 x 10^7 c.f.u./g, and *Trichophyton mentagrophytes* with 3.06 x 10^7 c.f.u./g (Table 46). The most abundant species in the unlimed soil include *Cunninghamella bertholletiae* with a total abundance of 3.42 x 10^7 c.f.u./g, followed by *Saprolegnia parasitica* with 1.10 x 10^6 c.f.u./g and *Penicillium crustaceum* with a value of 7.85 x 10^5 c.f.u./g (Table 47). It is evident that fungal abundance is higher in limed soil samples compared to unlimed soil samples based on the trend in the serial dilutions for the SDA medium (Figure 10).

For the MEA medium, the most abundant species identified in the limed soil were *Mycoderma aceti* with a total abundance of 6.60 x 10^7 c.f.u./g, followed by *Trichophyton mentagrophytes* with 2.88 x 10^7 c.f.u./g and *Rhizopus stolonifer* with 1.50 x 10^6 c.f.u/g (Table 48). For the unlimed samples, the most abundant species was *Cryptococcus neoformans* with a total abundance of 1.66 x 10^8 c.f.u./g (Table 49). This was followed by *Sporotrichum schenckii* with 1.47 x 10^7 c.f.u./g, and *Trichophyton mentagrophytes* with 3.42 x 10^6 c.f.u./g (Table 49). In

general, the trend based on all the serial dilutions shows higher fungal abundance in unlimed soil samples compared to limed soil samples for the MEA medium (Figure 11).

Kelly Lake site: The most abundant species identified on SDA medium in samples from the limed area at the Kelly Lake site include *Cryptococcus neoformans* with a total value of abundance of 2.66 x 10^8 c.f.u./g, followed by *Sporotrichum schenckii* with 1.36 x 10^7 c.f.u./g and *Penicillium crustaceum* with 3.69 x 10^6 c.f.u./g (Table 51). The most abundant species in the unlimed area include *Cryptococcus neoformans* with a total abundance of 1.06 x 10^8 c.f.u/g, *Rhizopus stolonifer* with a total value of 1.39 x 10^7 c.f.u/g and *Trichoderma harzianum* with a total abundance value of 1.10 x 10^6 c.f.u/g (Table 50). In general, the trend based on all the serial dilutions demonstrates higher fungal abundance in the limed soil samples compared to the unlimed soil samples (Figure 10).

Streptomyces scabies with a total abundance of 4.89 x 10^8 c.f.u/g was the most abundant species identified on samples from the limed area growing on MEA medium (Table 53). This was followed by *Allescheria boydii*, *Blastomyces dermatittidis*, and *Sporoblomyces roseus* with a total abundance of 5.0 x 10^6 c.f.u/g, followed by *Penicillium* lividum with 8.60 x 10^5 c.f.u/g (Table 53). The most abundant species in unlimed areas was *Penicillium montanense* with a total abundance of 3.86 x 10^8 c.f.u/g, followed by *Alternaria alternata* with 2.68 x 10^8 c.f.u/g, and *Acremonium strictum* with 2.51 x 10^7 c.f.u/g (Table 52). In general, the trend based on all the serial dilutions shows higher fungal abundance in unlimed soil samples compared to limed soil samples (Figure 11).

Hagar (reference site): The most abundant species on SDA medium for the reference site of Hagar was *Trichophyton mentagrophytes* with 1.01 x 10^8 c.f.u./g followed by *Eupenicillium*

shearii with a total abundance of 6.04 x 10^7 c.f.u./g, and *Penicillium crustaceum* with 3.79 x 10^7 c.f.u./g (Table 54).

For the MEA medium, the most abundant species was *Trichophyton tonsurans* with 1.59 x 10^8 c.f.u./g followed by *Trichophyton concentricum* with a total abundance of 1.45 x 10^8 c.f.u./g, and *Trichophyton mentagrophytes* with 6.43 x 10^7 c.f.u./g (Table 55).

Onaping Falls (reference site): The most abundant species on the SDA medium for Onaping Falls was *Penicillium citrinum* with a total abundance of 2.79 x 10^8 c.f.u./g (Table 56). This was followed by the species of *Candida glabrata* with a total abundance value of 2.57 x 10^8 c.f.u./g and *Penicillium decumbens* with a total abundance of 2.10 x 10^8 c.f.u./g (Table 56).

For the samples growing on the MEA medium, the most abundant species was *Trichosporon mucoides* with a total abundance of 1.23 x 10^8 c.f.u./g, followed by *Penicillium citreonigrum* with 1.54 x 10^7 c.f.u./g, and *Penicillium citrinum* with 1.27 x 10^7 c.f.u/g (Table 57).

Capreol (reference site): The most abundant species growing on the SDA medium for Capreol was *Trichophyton mentagrophytes* with a total abundance of 1.05 x 10^8 c.f.u./g (Table 58). Then, *Blastomyces dermatitidis* and *Penicillium montanense* with a total abundance of 4.89 x 10^7 c.f.u./g and 1.98 x 10^7 c.f.u./g, respectively (Table 58).

For the MEA medium, the most abundant species was *Sporotrichum schenckii* with a total abundance of 1.61 x 10^9 c.f.u./g (Table 59). This was followed by *Epicoccum purpurascens* with 9.83 x 10^8 c.f.u./g and *Streptomyces scabies* with 6.92 x 10^8 c.f.u./g (Table 59). While our CFU/g counts are elevated, they represent the top range of possible fungi colony forming units and reflect the usage of 5 dilution factors in which numerous fungi species were documented and highly abundant.

Table 8: Fungi species isolated from the different sites within the Greater Sudbury Region

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
SDA Medium							
Absidia corymbifera							X
Acremonium strictum		*					
Actinomyces bovis			+				
Allescheria boydii						X	
Blastomyces brasiliensis		+					X
Blastomyces dermatitidis							X
Candida albicans			+				
Candida glabrata						X	
Candida lusitaniae						X	
Cladosporium sphaerospermum							X

Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Cryptococcus neoformans	+		+ *	+ *		X	
Cunninghamella bertholletiae			*		X		
Eupenicillium shearii	+ *				X		
Histoplasma capsulatum				+			
Microsporum gallinae	+						
Mortierella isabellina		+					
Mucor indicus		+		+	X	X	X
Mucor mucedo		*					
Mycoderma aceti	+		+				
Penicillium chrysogenum		+		+			
Peniclilium citrinum						X	

Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar		
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Penicillium crustaceum			*	+	X		
Penicillium decumbens						X	
Penicillium fellutanum	+ *		+ *				
Penicillium lividum		*					
Penicillium montanense				*			X
Pythium debaryanum				*			
Rhizopus stolonifer	+	+ *	+ *	+ *			
Rhodotorula mucilaginosa	+						
Saccharomyces cerevisiae				+			
Saprolegnia parasitica			*				
Sporotrichum schenckii	*	+ *	+ *	+			

Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Stachybotrys chartarum			+				
Trichoderma harzianum	+ *			*			X
Trichophyton concentricum		+					
Trichophyton mentagrophytes	+ *		+ *		X		X
Trichophyton rubrum		*	*				
Trichophyton tonsurans			*				X
Trichosporon mucoides			+				
Trichothecium roseum	+						
Verticillium dahliae	+		+				

Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
MEA medium							
Acremonium strictum		*		*			
Actinomyces bovis		+		*			
Allescheria boydii				+			
Alternaria alternata				*			
Blastomyces dermatitidis				+		X	X
Candida lusitaniae							X
Coccidioides immitis						X	
Cryptococcus neoformans	*	+	*	+ *			X
Epicoccum purpurascens							X
Gliocladium viride				*			

Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Mucor indicus						X	
Mycoderma aceti	+ *		+				
Penicillium chrysogenum		+					
Penicillium cinnamopurpureum		+					
Penicillium citreonigrum						X	
Peniclilium citrinum		*		+		X	X
Penicillium crustaceum		*		+	X		
Penicillium fellutanum		+					
Penicillium lividum			*	+			
Penicillium montanense				*		X	
Pythium debaryanum				+		X	X
Rhizopus stolonifer			+	+	X		X

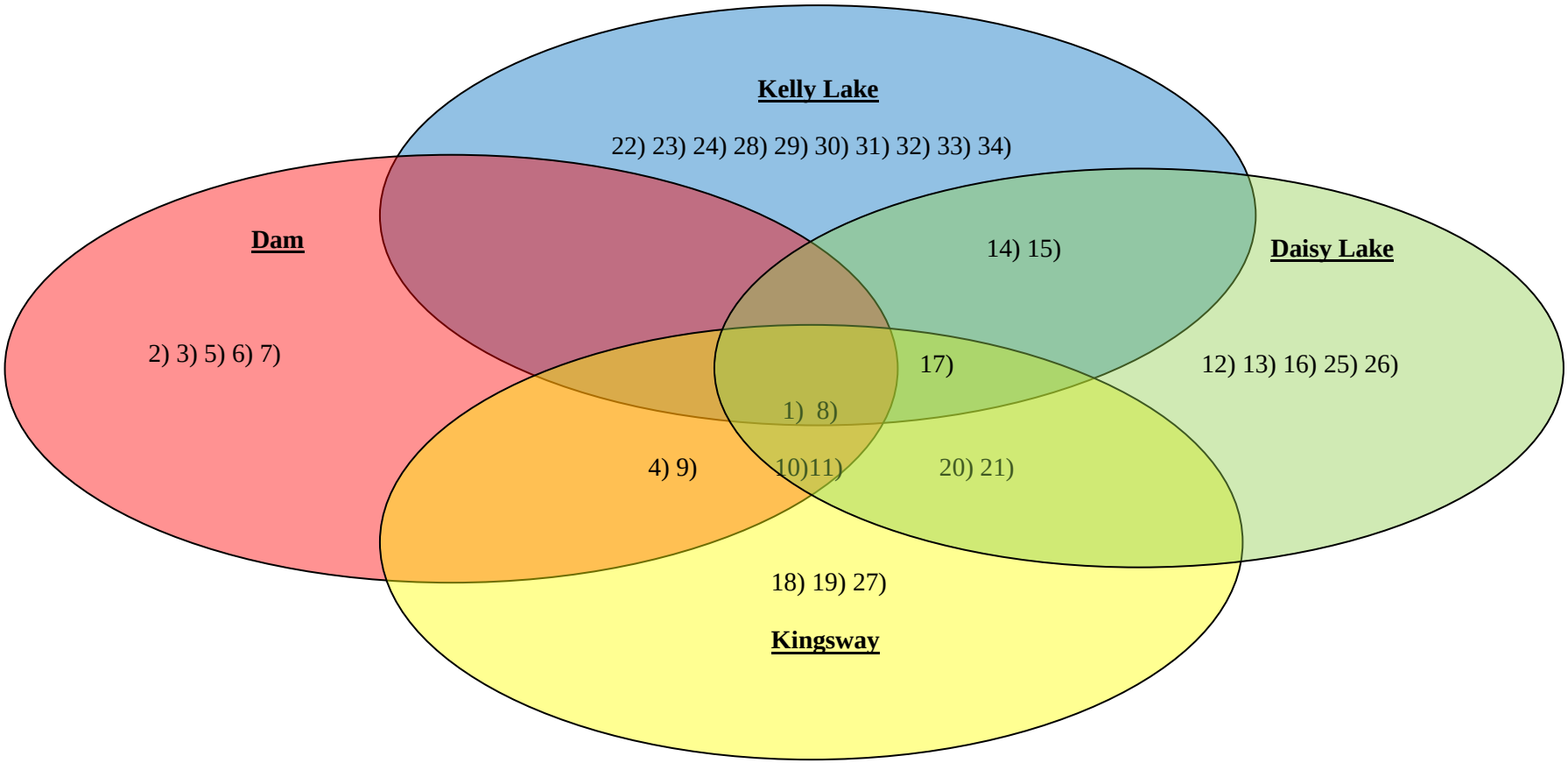
Table 8: (continued)

Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Rhodotorula mucilaginosa	+ *					X	
Saprolegnia parasitica		*	*				
Sporoblomyces roseus				+			
Sporotrichum schenckii		+	*				X
Streptomyces scabies			*	+	X		X
Trichophyton concentricum					X		
Trichophyton faviforme						X	X
Trichophyton mentagrophytes	+ *	+ *	+ *	*	X		
Trichophyton rubrum		*					
Trichophyton schoenleini		*					
Trichophyton tonsurans		*	+ *		X	X	

Table 8: (continued)

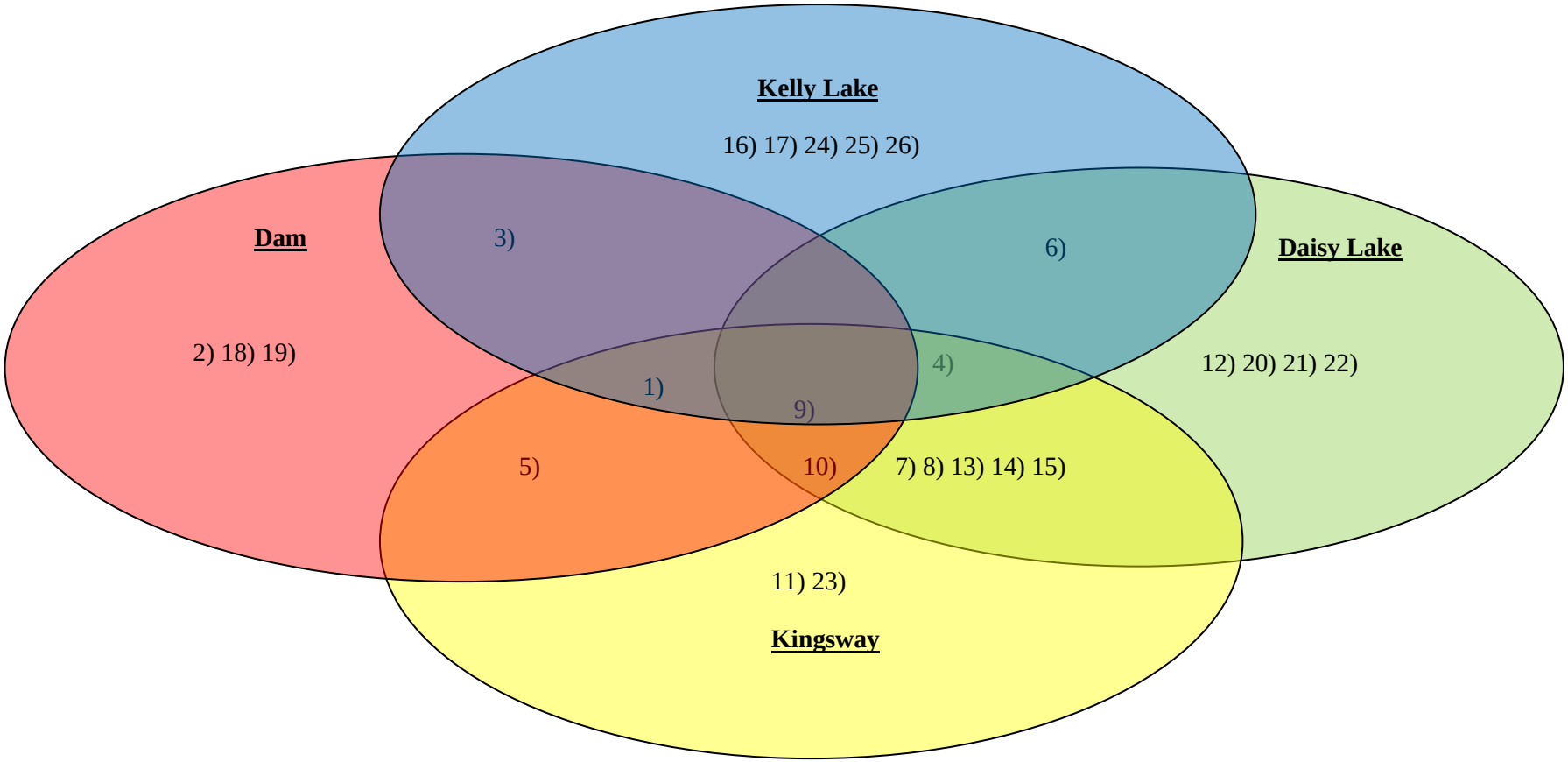
Species	Sites						
	Limed (+): WHD	Daisy Lake	Kingsway	Kelly Lake			
	Unlimed (*): WHD	Daisy Lake	Kingsway	Kelly Lake	Hagar	Onaping Falls	Capreol
Trichophyton violaceum	+						
Trichosporon mucoides	+					X	
Verticillium dahlia	*						

WHD = Wahnapitae Hydro-Dam
"+" represents fungi species presence in limed area
"*" represents fungi species presence in unlimed area
"X" represents fungi species presence in reference site



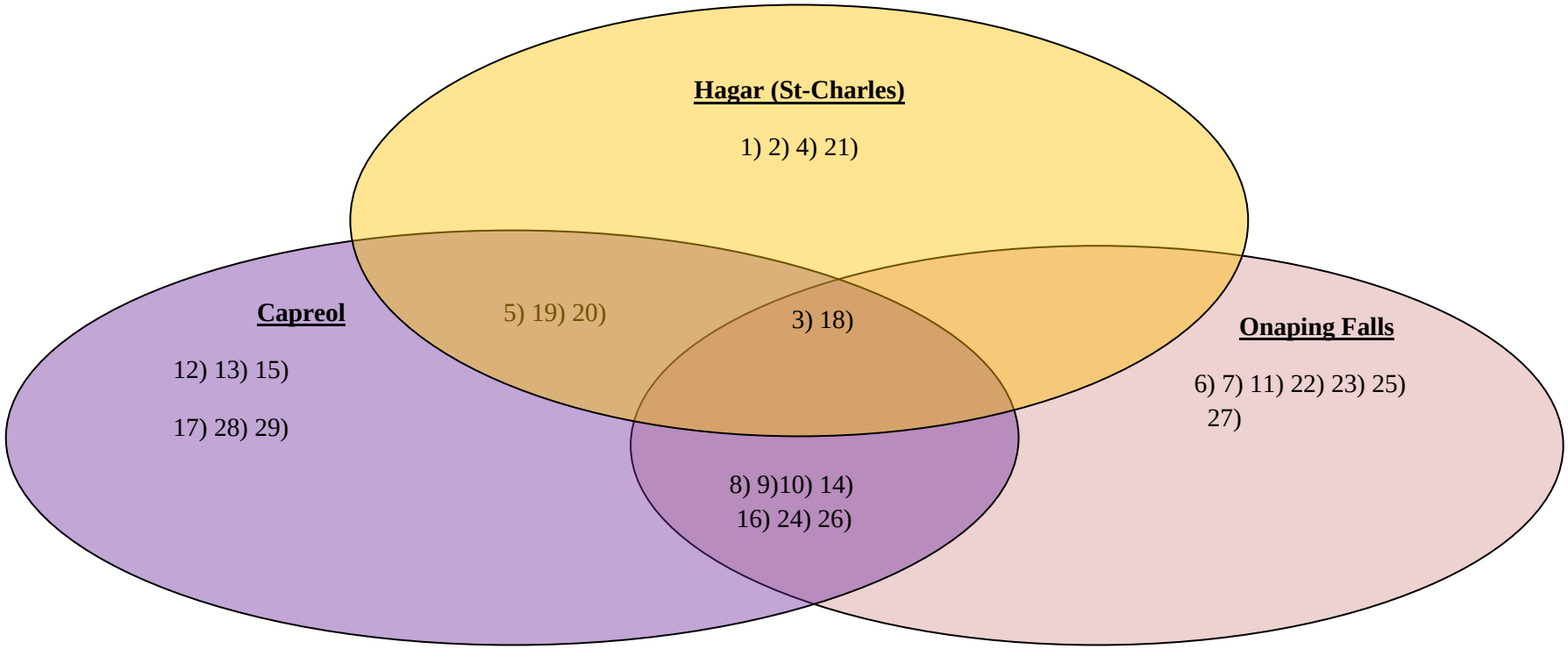
1) *Cryptococcus neoformans*
2) *Eupenicillium shearii*
3) *Microsporium gallinae*
4) *Mycorderma aceti*
5) *Rhodotorula mucilaginosa*
6) *Trichoderma harzianum*
7) *Trichothecium roseum*
8) *Rhizopus stolonifer*
9) *Verticillium dahliae*
10) *Penicillium fellutanum*
11) *Trichophyton mentagrophytes*
12) *Blastomyces brasiliensis*
13) *Mortierella isabellina*
14) *Mucor indicus*
15) *Penicillium chrysogenum*
16) *Trichophyton concentricum*
17) *Sporotrichum schenckii*
18) *Stachybotrys chartarum*
19) *Candida albicans*
20) *Actinomyces bovis*
21) *Trichosporon mucoides*
22) *Histoplasma capsulatum*
23) *Penicillium crustaceum*
24) *Saccharomyces cerevisiae*
25) *Penicillium cinnamopurpureum*
26) *Trichophyton violaceum*
27) *Trichophyton tonsurans*
28) *Allescheria boydii*
29) *Blastomyces dermatitidis*
30) *Penicillium citrinum*
31) *Penicillium lividum*
32) *Pythium debaryanum*
33) *Sporoblomyces roseus*
34) *Streptomyces scabies*

Figure 2: Venn diagram illustrating common fungi species among limed areas based on SDA and MEA medium analysis



1) *Cryptococcus neoformans*
2) *Eupenicillium shearii*
3) *Trichoderma harzianum*
4) *Rhizopus stolonifer*
5) *Penicillium fellutanum*
6) *Acremonium strictum*
7) *Penicillium crustaceum*
8) *Trichophyton rubrum*
9) *Trichophyton mentagrophytes*
10) *Sporotrichum schenckii*
11) *Cunninghamella bertholletiae*
12) *Mucor mecedo*
13) *Penicillium lividum*
14) *Trichophyton tonsurans*
15) *Saprolegnia parasitica*
16) *Penicillium montanense*
17) *Pythium debaryanum*
18) *Mycoderma aceti*
19) *Rhodotorula mucilaginosa*
20) *Penicillium citrinum*
21) *Trichophyton schoenleini*
22) *Verticillium dahliae*
23) *Streptomyces scabies*
24) *Actinomyces bovis*
25) *Alternaria alternata*
26) *Gliocladium viride*

Figure 3: Venn diagram illustrating common fungi species among unlimed areas based on SDA and MEA medium analysis



1) *Cunninghamella bertholletiae*
2) *Eupenicillium shearii*
3) *Mucor indicus*
4) *Penicillium crustaceum*
5) *Trichophyton mentagrophytes*
6) *Allescheria boydii*
7) *Candida glabrata*
8) *Candida lusitaniae*
9) *Cryptococcus neoformans*
10) *Penicillium citrinum*
11) *Penicillium decumbens*
12) *Absidia corymbifera*
13) *Blastomyces brasiliensis*
14) *Blastomyces dermititidis*
15) *Cladosporium sphaerospermum*
16) *Penicillium montanense*
17) *Trichoderma harzianum*
18) *Trichophyton tonsurans*
19) *Rhizopus stolonifer*
20) *Streptomyces scabies*
21) *Trichophyton concentricum*
22) *Coccidioides immitis*
23) *Penicillium citreonigrum*
24) *Pythium debaryanum*
25) *Rhodotorula mucilaginosa*
26) *Trichophyton faviforme*
27) *Trichosporon mucoides*
28) *Epicoccum purpurascens*
29) *Sporotrichum schenckii*

Figure 4: Venn diagram illustrating common fungi species among reference sites based on SDA and MEA medium analysis

Table 9: Fungi species and phyla identified in samples from sites within the GSR

Species	Phylum
Absidia corymbifera	Zygomycota
Acremonium strictum	Ascomycota
Actinomyces bovis	Deuteromycota
Allescheria boydii	Ascomycota
Alternaria alternata	Ascomycota
Blastomyces brasiliensis	Ascomycota
Blastomyces dermatitidis	Ascomycota
Candida albicans	Ascomycota
Candida glabrata	Ascomycota
Candida lusitaniae	Ascomycota
Cladosporium sphaerospermum	Ascomycota
Coccidioides immitis	Ascomycota
Cryptococcus neoformans	Basidiomycota
Cunninghamella bertholletiae	Zygomycota
Epicoccum purpurascens	Ascomycota
Eupenicillium shearii	Ascomycota
Gliocladium viride	Ascomycota
Histoplasma capsulatum	Ascomycota
Microsporum gallinae	Ascomycota
Mortierella isabellina	Zygomycota

Table 9: (continued)

Species	Phylum
Mucor indicus	Zygomycota
Mucor mucedo	Zygomycota
Mycoderma aceti	Ascomycota
Penicillium chrysogenum	Ascomycota
Penicillium cinnamopurpureum	Ascomycota
Penicillium citreonigrum	Ascomycota
Peniclilium citrinum	Ascomycota
Penicillium crustaceum	Ascomycota
Penicillium decumbens	Ascomycota
Penicillium fellutanum	Ascomycota
Penicillium lividum	Ascomycota
Penicillium montanense	Ascomycota
Pythium debaryanum	Oomycota
Rhizopus stolonifer	Zygomycota
Rhodotorula mucilaginosa	Basidiomycota
Saccharomyces cerevisiae	Ascomycota
Saprolegnia parasitica	Oomycota
Sporoblomyces roseus	Basidiomycota
Sporotrichum schenckii	Ascomycota
Stachybotrys chartarum	Ascomycota

Table 9: (continued)

Species	Phylum
Streptomyces scabies	Deuteromycota
Trichoderma harzianum	Ascomycota
Trichophyton concentricum	Ascomycota
Trichophyton faviforme	Ascomycota
Trichophyton mentagrophytes	Ascomycota
Trichophyton rubrum	Ascomycota
Trichophyton schoenleini	Ascomycota
Trichophyton tonsurans	Ascomycota
Trichophyton violaceum	Ascomycota
Trichosporon mucoides	Basidiomycota
Trichothecium roseum	Ascomycota
Verticillium dahliae	Ascomycota

GSR = Greater Sudbury Region

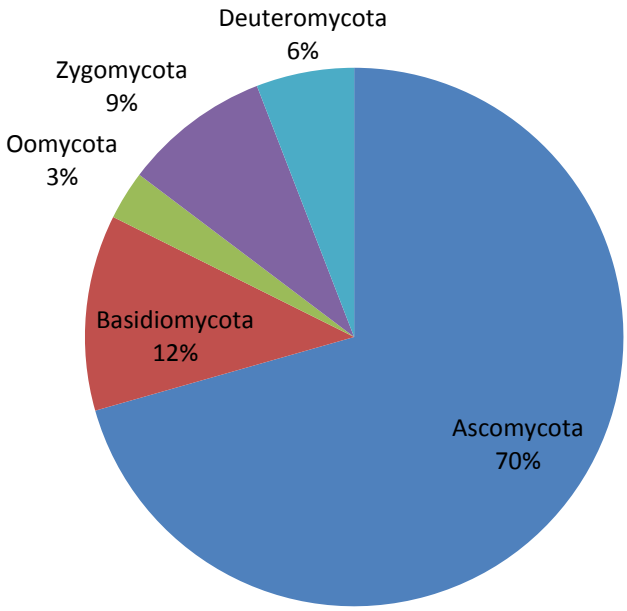


Figure 5: Fungi phyla present in limed areas

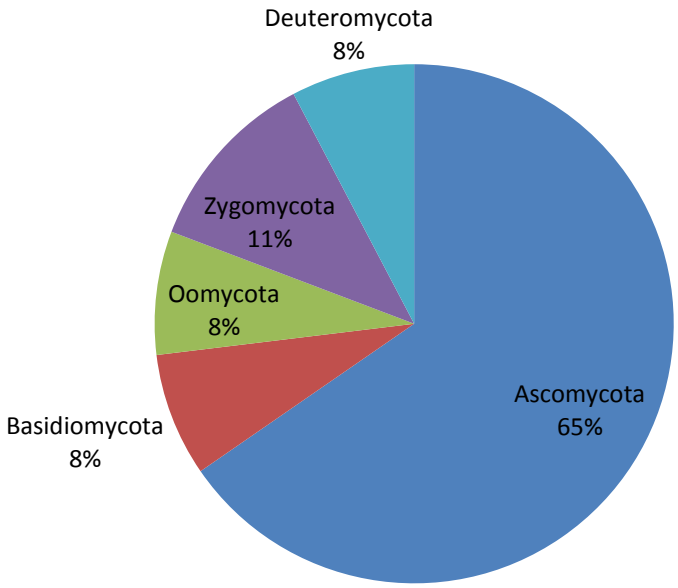


Figure 6: Fungi phyla present in unlimed areas

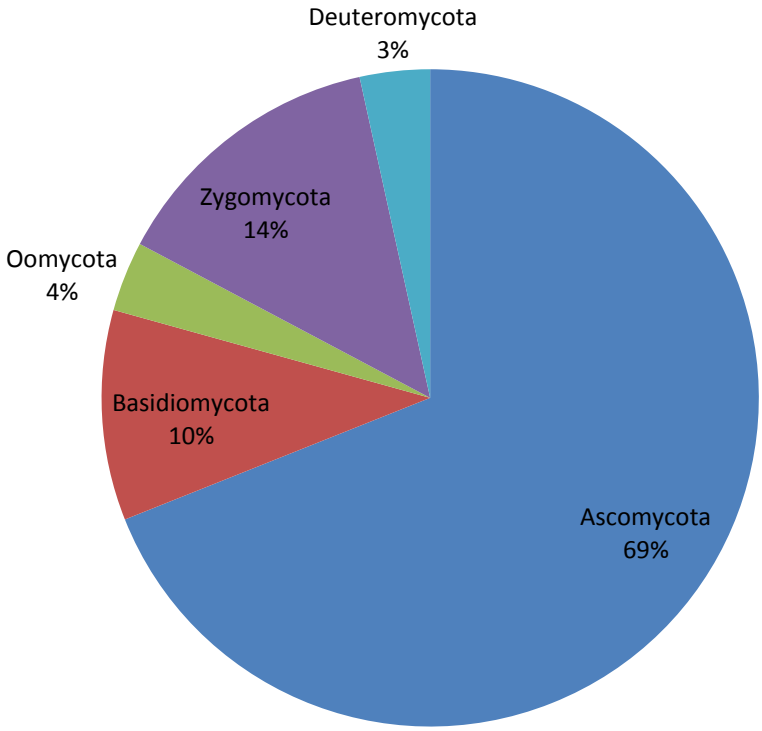


Figure 7: Fungi phyla present in reference sites

Table 10: Number of different fungi species within medium for limed and unlimed soil samples

Treatment	**Number of species (Mean)**	
	SDA	**MEA**
Limed	7.25 a	5.125 a
Unlimed	5.125a	5.625 b

Limed and unlimed sites include: Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake. Means in a column with different subscript are significantly different based on t-test ($P \leq 0.05$).

Table 11: Number of different fungi species within medium for limed, unlimed and reference sites

Treatment	Number of species (Mean ± SE)	
	SDA	**MEA**
Limed	7.25 a ±0.53	5.125 a ±0.95
Unlimed	5.125 b ±0.44	5.625 a ±0.42
Reference sites	5.833333 ab ±0.31	7.5 a ±0.85

Limed and unlimed sites include: Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake. Reference sites include: Hagar, Onaping Falls and Capreol. Means in a column with different subscript are significantly different based on Tukey's multiple comparison test ($P \leq 0.05$). SE = Standard error.

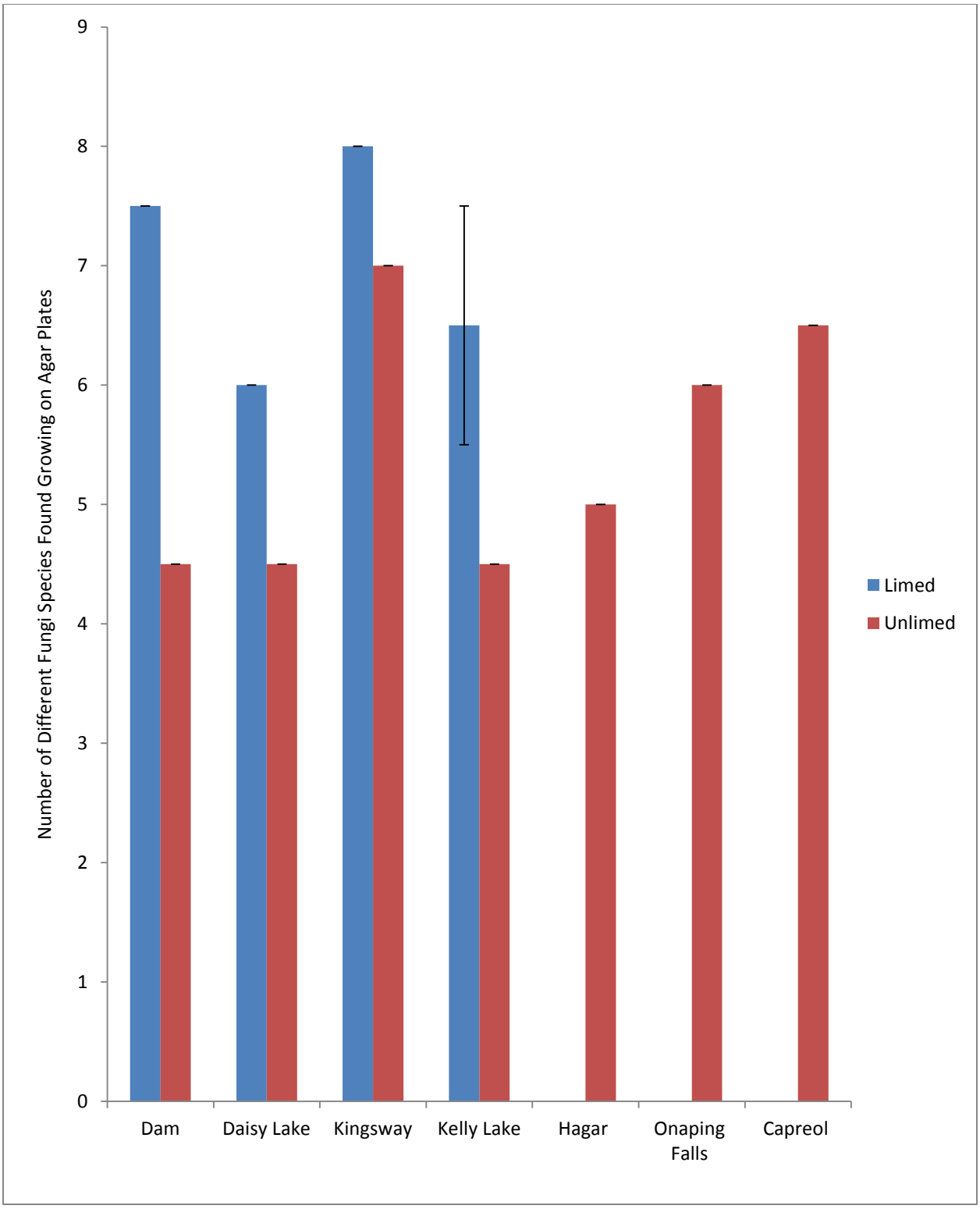


Figure 8: Fungi diversity identified in limed and unlimed samples on SDA medium for all targeted sites with standard error bars

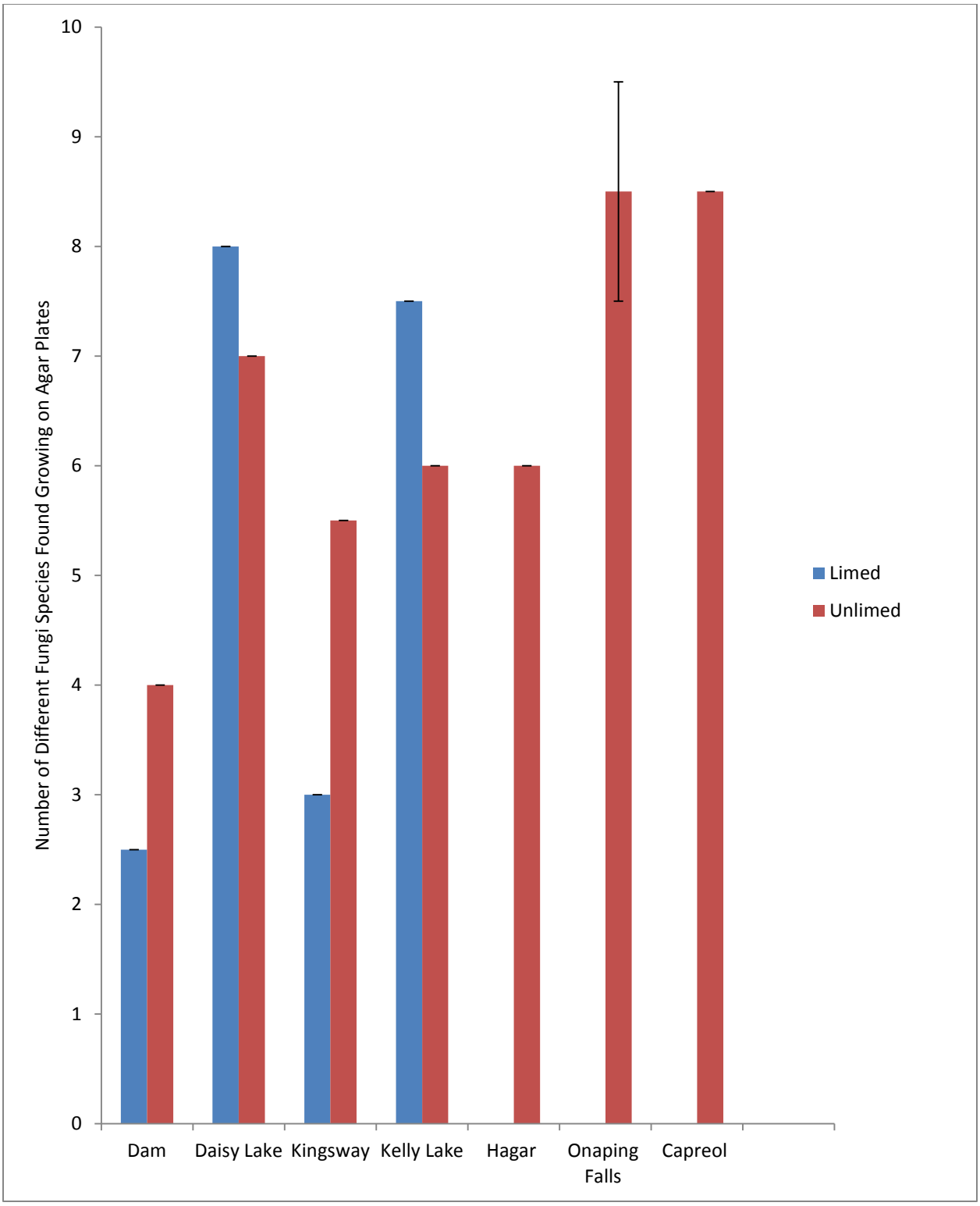


Figure 9: Fungi diversity identified in limed and unlimed samples on MEA medium for all targeted sites with standard error bars

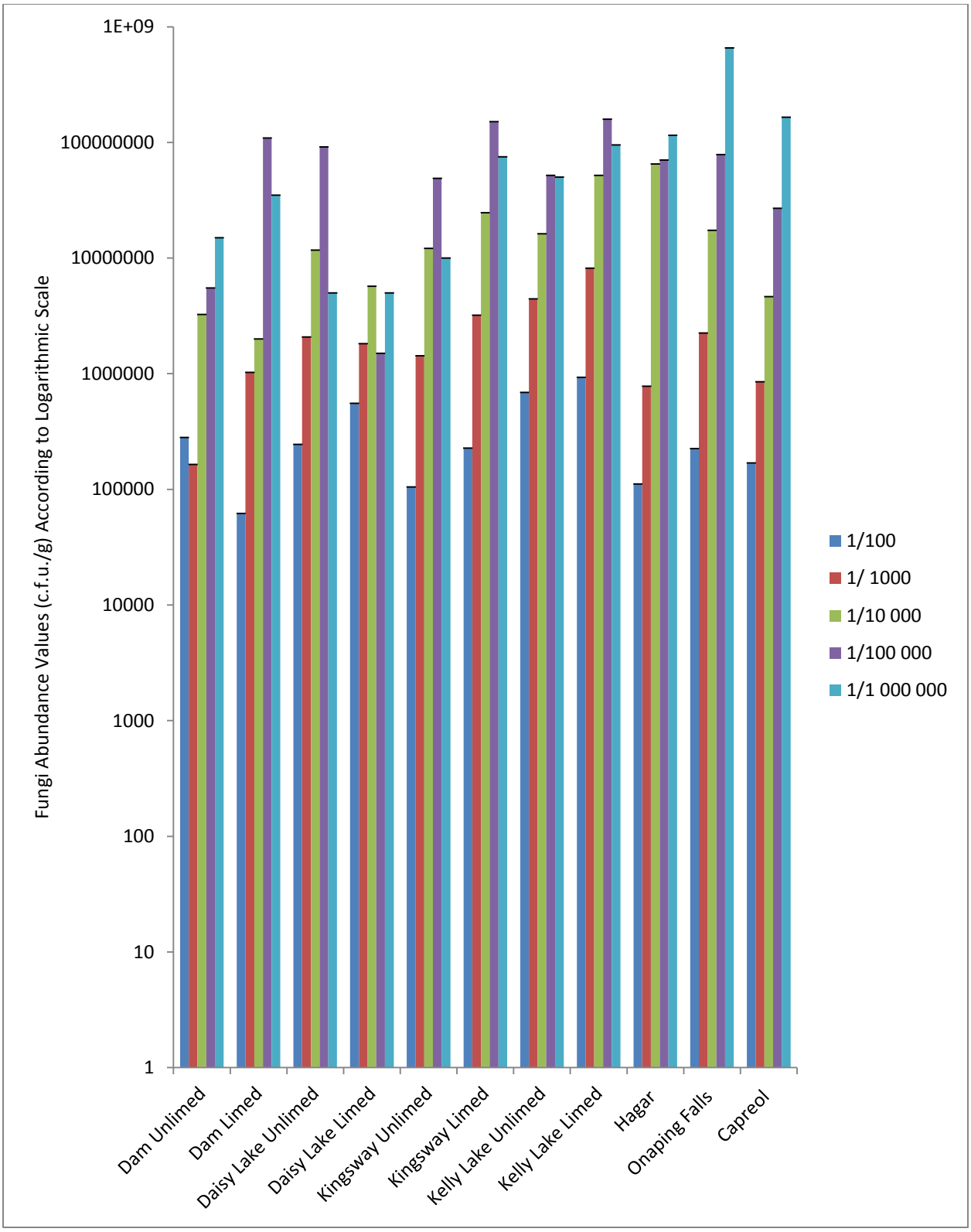


Figure 10: Fungal abundance for different dilution factors for unlimed and limed areas of all targeted sites based on SDA medium analysis with standard error bars on logarithmic scale

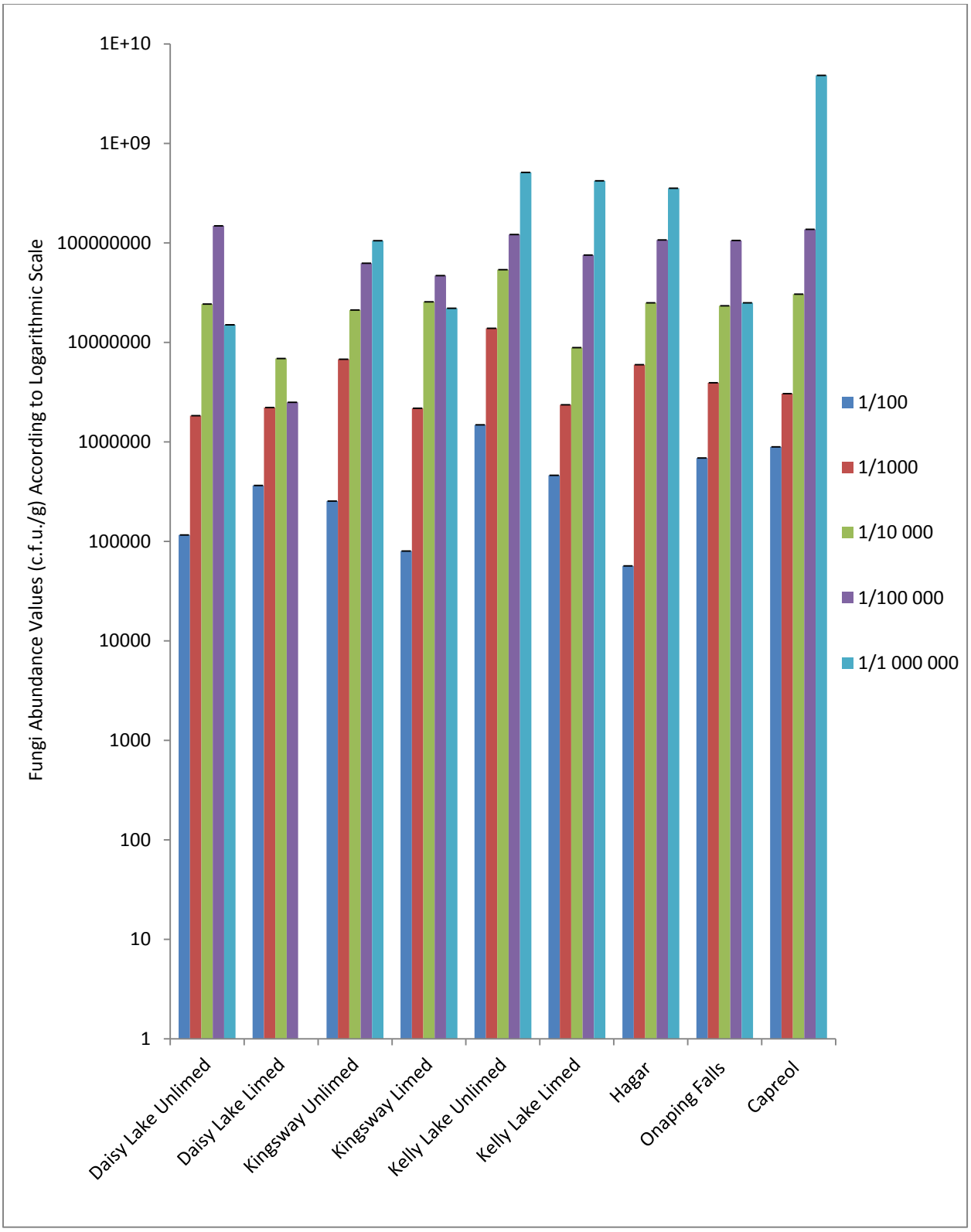


Figure 11: Fungal abundance for different dilution factors for unlimed and limed areas of all targeted sites based on MEA medium analysis with standard error bars on logarithmic scale

Table 12: Mean fungi abundance within media for limed and unlimed soil samples

	Dilutions				
	1/100	1/1000	1/10 000	1/100 000	1/1 000 000
SDA					
Limed	3.74×10^5 a	3.55×10^6 a	2.10×10^7 a	1.05×10^8 a	5.25×10^7 a
Unlimed	3.29×10^5 a	2.39×10^6 a	1.08×10^7 a	4.76×10^7 a	2.0×10^7 a
MEA					
Limed	2.26×10^5 a	1.68×10^6 a	1.03×10^7 a	3.12×10^7 a	1.10×10^8 a
Unlimed	4.63×10^5 a	5.60×10^6 a	2.47×10^7 a	8.30×10^7 a	1.57×10^8 a

Limed and unlimed sites include: Wahnapitae Hydro-Dam, Daisy Lake, Kingsway, and Kelly Lake. Means in a column with common subscript is not significantly different based on T-test ($P \leq 0.05$). Abundance units are colony forming units/ gram of soil (c.f.u/g).

Table 13: Mean fungi abundance within media for limed, unlimed and reference soil samples

Treatment	**Abundance (Mean c.f.u./g. ± SE)**	
	SDA	**MEA**
Limed	1.82×10^8 a $\pm 5.17 \times 10^7$	1.54×10^8 a $\pm 7.84 \times 10^7$
Unlimed	8.11×10^7 b $\pm 1.73 \times 10^7$	1.61×10^8 a $\pm 4.44 \times 10^7$
Reference sites	4.0×10^8 a $\pm 1.22 \times 10^8$	1.36×10^9 b $\pm 8.43 \times 10^8$

Limed and unlimed sites include: Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake. Reference sites include: Hagar, Onaping Falls and Capreol. Means in a column with different subscript are significantly different based on Tukey's multiple comparison test ($P \leq 0.05$). SE = Standard error.

3.4 Discussion

Smith and Doran (1996) described that fungi growth is generally observed in the soil with a pH between 2 and 7 with an optimum of 5. Consequently, most fungal species prefer acidic soils. However, some fungi have a better growth rate in alkaline soils compared to acidic soils (Kendrick, 2000). In fact, soil pH affects many processes. For example, organic matter mineralization is slowed down or stopped at highly acidic or alkaline pH levels, which is attributed to poor microbial activity linked to bacteria (Smith & Doran, 1996). At low pH levels, nitrification and nitrogen fixation will also be inhibited (Smith & Doran, 1996). Furthermore, the solubility of heavy metals as well as the mobility and degradation of herbicides and insecticides are pH dependant (Smith & Doran, 1996). Soil pH affects cation availability which in turn influences aggregate stability because multivalent cations, like calcium ions, act as bridges between organic colloids and clays (Smith & Doran, 1996). All these factors play a key role in fungi diversity and abundance in a specific site or region.

The effects of soil liming include an intricate change in the total nutrient availability in the soil as well as an increase in soil pH (Mengel & Kirby, 1987). Some nutrients prove more useful for some species of fungi than others and the levels of such macro and micro nutrients in the substrate dictate the survival of fungi species (Kendrick, 2000). Fungi utilize a variety of metals to accomplish their vital prossesses. In the present study, the total and bio-available concentration of nutrients and metal elements in the limed and unlimed organic surface horizons (LFH) of soils were determined for Al, As, Ca, Cd, Co, Cu, Fe, K, P, Pb, Mg, Mn, N, Na, Ni, S, Sr and Zn in order to better understand their role on fungi sustainability. The total amounts of arsenic, carbon, cobalt, copper, phosphorus, lead, magnesium, sulfur, nickel and nitrogen were found to be significantly higher in the first soil horizon compared to other soil depths (Table 1).

Higher bioavailable concentrations of aluminium, calcium, iron, potassium, magnesium, sodium, nickel, strontium and zinc were also found in the top soil layer compared to lower horizons (Table 2). Elemental analysis also revealed significantly lower concentrations of total arsenic and bioavailable aluminium, iron and strontium in limed sites compared to unlimed sites. Furthermore, high levels of bioavailable calcium and magnesium were found in limed sites reflecting the addition of the dolomitic and calcitic limestone that was applied 30-40 years ago.

Fungi have the ability to use many metals or elements as food sources while other organisms prove to be intolerant to many metals in their soil environment even at low concentrations. In the present study, we found that the levels of bioavailable Al, As, Ca, Co, Cu, Fe, K, P, Pb, Mg, Mn, Na, Ni, Sr and Zn were 71.45 $mgkg^{-1}$, 0.32 $mgkg^{-1}$, 162.86 $mgkg^{-1}$, 0.26 $mgkg^{-1}$, 8.20 $mgkg^{-1}$, 77.19 $mgkg^{-1}$, 149.54 $mgkg^{-1}$, 14.68 $mgkg^{-1}$, 0.27 $mgkg^{-1}$, 92.32 $mgkg^{-1}$, 18.93 $mgkg^{-1}$, 15.75 $mgkg^{-1}$, 5.17 $mgkg^{-1}$, 0.38 $mgkg^{-1}$, and 1.24 $mgkg^{-1}$ respectively in the LFH layer. Macronutrients are of prime importance for fungi (Kendrick, 2000). Potassium (K), is utilized in carbohydrate metabolism, enzyme activity, and maintaining ionic balance (Kendrick, 2000). Phosphorus (P), is an essential component of nucleic acids and for energy transfer mechanisms (Kendrick, 2000). Magnesium (Mg), is utilized as an enzyme activator necessary in ATP metabolism (Kendrick, 2000). Sulphur (S), is a component of some amino acids, vitamins and other sulfhydryl compounds (Kendrick, 2000). Lastly, calcium (Ca), is utilized as an enzyme activator and is regularly found in membranes (Kendrick, 2000). Fungi also utilize many micronutrients, often referred to as trace elements, which include: iron (Fe), which is found in cytochromes, heam apoenzymes, and pigments; copper (Cu), utilized as an enzyme activator also involved in pigments; and manganese (Mn), zinc (Zn), and molybdenum (Mo), which are all enzyme activators (Kendrick, 2000). Metals are directly or indirectly involved in all the many

aspects of fungal growth, metabolism as well as differentiation. While some elements are essential for fungi growth such as K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Co and Ni, many other elements do not seem to have an obvious function (Gadd, 1992). They include Rb, Cs, Al, Cd, Ag, Au, Hg and Pb (Gadd, 1992). Nevertheless, all the elements stated above interact with fungal cells and can be accumulated by physico-chemical mechanisms and transport systems of altering specificity (Gadd, 1992). These metal ions may compete with H^+ for sites on ligands and as a result, H^+ may be regarded as a borderline ion (Nieboer & Richardson, 1980). Thus, when an increase in acidity occurs, protonation in the ligand anion will transpire and an increase in the concentration of the free metal ion will also be encountered (Hughes & Poole, 1991). Metal effects in fungi can also vary a great deal between organisms, strains, the stage of growth and different vegetative and reproductive forms of the same organism (Gadd & Mowll 1985; Sabie & Gadd 1990). Our results clearly demonstrate the relevance of this last statement as some fungi species were observed growing in less metal contaminated sites but were not present in highly metal contaminated sites. For example, *Mucor indicus* was present at the Kelly Lake and Daisy Lake limed sites but was not found growing in the unlimed areas of these sites. In comparison, *Mucor mecedo* was only present at the Daisy Lake unlimed site. It is possible that the bioavailability of certains elements affects the growth of these two strains of fungi species in a different manner in the soil. Fungal species that are present in both limed and unlimed soil might have evolved various response mechanisms which limit the toxic effect of metals to their cells as stated by Baldrian (2010).

Other nutritional requirements for fungi involve carbon nutrition (Kendrick, 2000). In fact, most fungal species are primarily distinguished from other organisms by their inability to fix organic carbon (Kendrick, 2000). As such, fungal species need to rely on the simplest

compound available to them as a source of energy which is that of the monosaccharide glucose (Kendrick, 2000). Unlike other carbon sources, glucose does not require to be enzymically broken down to simpler molecules before it can be absorbed by fungi (Kendrick, 2000). Fungi species can thus metabolize glucose at any given time since they already possess all the necessary or constitutive enzymes in order to do so (Kendrick, 2000). Fungi species may also rely on other carbon sources such as fructose, manose and galactose which are readily available to them as well but often enough there is a delay before assimilation can commence (Kendrick, 2000). This being attributed to the fact that such specific sugar processing fungi enzymes are not necessarily awaiting usage and as such, fungi species may take quite some time to properly recognise the nature of the substrate and to synthesize the proper enzymes; this process is called induction as it produces adaptive enzymes (Kendrick, 2000). Furthermore, if a significant concentration of glucose is present, this may in fact suppress the production of the enzymes that are intricately linked to other substrates (Kendrick, 2000). However, if the reverse scenario proves to be the case and only a little glucose is present, the induction process may be fuelled and shorten the lag phase on many substrates consequently (Kendrick, 2000).

In nature, fungi need to deal with mixed substrates and perhaps one of the most important cases of mixed substrate involves lignin (Kendrick, 2000). In fact, Basidiomycetes, such as *Cryptococcus neoformans* (present in all 4 limed sites), have the ability to degrade lignin into carbon dioxide but cannot use lignin as a sole source of carbon and as such, will only degrade it in the presence of another accessible carbon source, such as cellulose, cellobiose or glucose (Kendrick, 2000). Fungi may actually only use lignin because it brings them better access to the cellulose or to release available nitrogen (Kendrick, 2000). Consequently, the varying degree of

these nutritional requirements among the sites might explain why some species are site specific such as *Gliocladium viride* found only in the unlimed site of Kelly Lake.

All these factors heavily influence fungi diversity and abundance growing in limed or unlimed soil and within one site or within a general region such as the Greater Sudbury Region. In fact, the Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake sites have common physical and chemical features because they are within the same geographical region however, soil composition, vitamin and nutrient levels as well as moisture content are also site specific. This explains why some fungi species are unique to some sites while others are commonly found growing in all four targeted limed or unlimed sites. For example, for the limed soil, the fungi species of *Cryptococcus neoformans* and *Rhizopus stolonifer* were found commonly growing in all four targeted sites, namely Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake, while many other fungi species were site(s) specific (Figure 2). Therefore, some sites are better suited for some phyla of fungi to grow and thrive based on their respective soil properties. This also applies to the reference sites utilized (Figure 4).

Climatic factors also influence fungi diversity as well as abundance within a region in general. Physical parameters such as temperature, light and even gravity have been found to have significant effects on many different species of fungi (Kendrick, 2000). Some psychrophilic fungi have been documented to grow at temperatures below 0 °C while at the other extreme, some thermophilic fungi have the ability to grow and thrive at temperatures above 50°C (Kendrick, 2000). Some fungi require light for fruiting purposes while other fungi species seem to be completely indifferent to illumination (Kendrick, 2000). Several macrofungi are extremely sensitive to gravity while numerous microfungi are totally oblivious to gravity (Kendrick, 2000). Consequently, because the sites of Wahnapitae Hydro-Dam, Daisy Lake, Kingsway, Kelly Lake,

Hagar, Onaping Falls and Capreol are geographically spread, they each have different topographic settings which allow for slightly different temperatures and illumination rates (at times) which could have a small effect on their respective fungi populations.

Culture media also requires a specific source of nitrogen due to the fact that no fungi species have the ability to fix atmospheric nitrogen (Kendrick, 2000). Often, numerous fungi may use nitrate, though ammonium nitrogen is well known to be more universally metabolized (Kendrick, 2000). Also, urea, amino acids and many polypeptides as well as proteins are available to fungi (Kendrick, 2000). In culture media, the nitrogen source for many fungi is hydrolysed casein, which is a mixture of amino-acids (Kendrick, 2000). Vitamins or coenzymes as they are often referred to, are necessary for fungi development in minute amounts (Kendrick, 2000). While numerous fungi species have the ability to create their own vitamins, many are deficient in thiamin which is involved in carboxylation, biotin which is also involved in carboxylation, riboflavin which is necessary in dehydrogenation, pyridoxine, involved in transamination, and nicotinic acid, which is involved in dehydrogenation as well as others (Kendrick, 2000). Sometimes, the absence of some vitamins may inhibit fungi growth while in other cases, additions of vitamins may simply increase growth rates (Kendrick, 2000). Consequently, the presence or absence of vitamins in the environment or under laboratory conditions may intricately alter fungi development and their corresponding diversity and abundance for a specific region. As a result, the chemical differences between SDA and MEA media in terms of nitrogen content and vitamin levels can possibly explain the documented variancy in fungi species between the sites. While culture media must also contain water, several conidial fungi and yeast are known to be the most xerotolerant organisms known in the world and as such, are able to grow at water activities (a_w) as low as 0.70 (Kendrick, 2000).

In nature, fungi will try obtaining their many requirements by any means possible but when grown on media, nutrients must be added to ensure that fungal colonies will grow (Kendrick, 2000). Two fungal media, namely SDA and MEA, were utilized in this study. Both media are chemically different from one another and allow for variations in terms of fungal diversity and abundance. SDA, a multipurpose medium, is among one of the most widely used fungal media as it allows for numerous fungi species to grow. SDA medium contains sources of carbon, nitrogen as well as vitamins just like all fungal media utilized for commercial purposes. Among its' ingredients, SDA medium contains glucose (to be utilized as a carbon source), and neopeptone and polypeptone agar (utilized as a solidifying agent for nitrogen). SDA pH is around 5.6 which is slightly acidic and appropriate for the growth of a multitude of fungi species that have an optimal growth at a pH of 5 according to Smith and Doran (1996). Commercially available SDA can include antibiotics such as streptomycin, which does not harm fungi. Such formulations are known as "restrictive" or "selective" while SDA formulations that have no inhibitor are known as "permissive". In order to view and document other possible fungi species growing in sites within the Greater Sudbury Region, MEA medium was also used. This medium is suited for the isolation, cultivation and enumeration of yeasts and molds. MEA medium also provides sources of carbon, protein and nutrients that are necessary for the growth of microorganisms. Because MEA medium contains high concentrations of maltose and other saccharides as energy sources, it primarily allows for yeasts and molds to grow while also allowing other fungi species to grow and restricting bacterial growth on Petri dishes. MEA medium uses Dextrin and Glycerin as carbon sources and Peptone is utilized as a nitrogen source. Also, bacteriological agar is the solidifying agent for this particular strand of medium. MEA medium has a pH of 4.7. As it was previously stated, more fungal diversity was observed

on the SDA medium in comparison to the MEA medium. Specifically, 41 fungi species were identified on SDA medium and 36 species on MEA medium (Table 8).

The present study revealed a level of fungal diversity that supports Smith and Dorans' findings in 1996. Significantly more fungal diversity was found in limed soil samples compared to unlimed soil samples for all sites when grown on SDA medium (Table 11). For the MEA medium, an opposite trend was observed where more fungal diversity was recorded in the unlimed soil samples compared to the limed soil samples except in the case of the Daisy Lake and Kelly Lake sites where slightly more fungi species were found growing in the limed areas. These results can possibly be explained by the chemical and pH differences between SDA and MEA media utilized for the purpose of this experiment. In general, MEA medium allows for the rapid cultivation of more acidophilic fungi, which is to say, yeasts, which grow profusely reaching highly abundant counts on petri dishes while simultaneously allowing other types of fungi to grow as well.

The fungal abundance variability follows the same trend as the species diversity. Significantly higher fungal abundance was observed in the limed areas compared to the unlimed areas concerning SDA medium, with the exception of the Daisy Lake site (Table 13). A closer look of the Daisy Lake site shows that the topography plays a key role in fungi distribution since the limed area was located uphill and the unlimed area was downhill. This may have resulted in soil erosion causing depletion of metals, nutrients and fungi from the top horizon of the limed areas and accumulation of these elements in the unlimed areas. These results support Weyman-Kaczmarkowa & Pedziwilk's findings in 2000 documenting the effects of liming ($Ca(OH)_2$) on the numbers of fungal colony-forming units (CFU). Although a different type of limestone was utilized in their study, they nevertheless recorded elevated mean fungal CFU numbers when soil

pH reached 4.5 (Weyman-Kaczmarkowa & Pedziwilk, 2000) as was the case in our study. While our CFU/g counts are elevated, they represent the top range of possible fungi colony forming units and reflect the usage of 5 dilution factors in which numerous fungi species were documented and highly abundant. A closer look at our CFU/g counts for individual fungi species is consistent with the literature. For instance, Anastasi et al. (2005) have determined the CFU count for many fungi species in their study including *Pseudallescheria boydii*. Their study revealed an abundance of 7.3×10^5 CFU/g dwt for this particular fungi species. By comparison, our study revealed the presence of *Allescheria boydii*, commonly referred to as *Pseudallescheria boydii*, in the Onapping Falls site with an abundance of 2.35×10^4 c.f.u./g (Table 56). For the MEA medium, an opposite trend was observed where more fungal abundance was recorded in the unlimed sites compared to the limed sites (Table 13). This again can possibly be explained by the chemical ingredients and lower pH of the MEA medium which is better suited for yeast growth. Higher yeast counts were in fact documented for all sites for MEA medium.

Chapter 4: Analysis of Soil Respiration as an Indicator of Soil Microbial Activity in Limed and Unlimed Areas in the Greater Sudbury Region (Ontario, Canada)

4.1 Introduction

Soil fertility is crucial for overall soil quality within an ecosystem (Doran et al., 1997). Both terms are linked as they define the ability of a soil to function by examining the degree of biological activity occurring in the soil which is in turn, linked to soil quality parameters such as soil aggregation, plant nutrient and energy cycling and general soil tilth (Doran et al., 1997). Typically, soil quality may be determined by analyzing a variety of soil properties. According to the Soil Quality Institute of the United States Department of Agriculture (1999), the physical, chemical and biological properties of the soil must be well evaluated in order to determine soil fertility and soil quality for a specific region. They have deemed the physical properties of a soil to include bulk density, water content, infiltration rate, aggregate stability, slacking, and morphological estimations. Conversely, soil chemical properties consist of pH, electrical conductivity (EC), and soil nitrate levels (Soil Quality Institute, 1999). Lastly, they have stipulated the biological properties measured in the soil to include soil respiration and the presence of earthworms. Soil respiration rates are particularly critical in the assessment of soil health, primarily in cases where soil amendment strategies have been executed, because the levels indicate the complete extent of biological activity of living microorganisms available in the soil (Doran et al., 1997). Consequently, bacterial and fungal components of the soil are functionally important and must be properly assessed.

Currently, laboratory and field methods for measuring soil respiration prove to be somewhat impractical for a variety of reasons. For instance, many techniques are known to be time consuming, labor intensive, require specialized knowledge and equipment, and involve

rather extensive calculations (Doran et al., 1997). However, a simple, inexpensive and a relatively quick method of measuring soil biological activity and/or respiration can be achieved by using the Solvita Soil Test (Doran et al., 1997). The Solvita system has been deemed to offer great promise as a substitute for more refined methods of quantitatively measuring soil respiration in the laboratory and the field (Doran et al., 1997). Consequently, this technology has the ability to accurately estimate soil CO_2 respiration to provide an adequate estimation of microbial biomass found in the soil.

The objective of this component is to measure soil respiration as an indicator of microbial activities and soil health in selected limed and unlimed areas in the Greater Sudbury Region.

4.2 Materials and Methods

4.2.1 Assessment of Soil Respiration

Soil samples collected in the summer of 2012-13 were sampled as described in section 2.2.1. Based on the same methodology, additional soil samples were collected from the LFH layer (top organic layer) of each targeted site in the fall of 2013 in order to analyze seasonal influences on soil respiration. Soil respiration was assessed using the CO_2 – burst application of the Solvita Soil Test. Soil samples were dried in an incubator at 45 °C for 24 hours prior to the test in order to remove all traces of soil moisture. Dried soil samples were weighed into a capillary cup after placing a fiber filter in the bottom of the cup. Each beaker, containing 40 grams of dried soil, was placed into a glass jar with the use of forceps. Following this, 20 mL of distilled water was placed into the glass jar with special attention to not spill on the soil sample. It is important to note that wetting the soils will stimulate a flush of carbon dioxide. A CO_2 probe was then inserted into the glass jar using forceps. The lid of the glass jar was then screwed on

tightly. Jars were opened 24 hours later to remove the probes. Probe color was determined by inserting the probe face-up into the Digital Color Reader (DCR) and by selecting the CO_2-Low mode. The DCR reads the color number on the first line as well as the CO_2 – C in mg/kg (ppm) on the second line. Interpretations of the DCR provided data were based on Solvita's overall guidelines (Solvita, 2013). Temperature and rainfall were recorded daily for one month prior to sampling. Climatic data was obtained via the Government of Canada daily data report, measured at the Sudbury Airport.

4.2.2 Statistical Analysis

Summer and fall soil respiration data for limed, unlimed and reference sites were compared using One-Way ANOVA. Association between respiration data and fungal abundance was determined using Spearman Correlation analysis. CO_2-C data from the analysis of summer and fall data were also compared using Spearman Correlation and Student t-test.

4.3 Results

4.3.1 Analysis of Temperature and Rainfall

Mean temperature for the 30 days prior to sampling in June was significantly higher (14°C) compared to October-November that was only 4.6°C. Likewise, mean rainfall for May-June was 85.8 mm and was higher than the 49.3 mm rainfall in the 30 days prior to fall sampling on November 9th 2013.

4.3.2 Seasonal Soil Respiration and Relationship to Fungal Abundance

Interpretations of the soil respiration data were based on Solvita's professional guidelines which can be visualized by appendix E. According to their guidelines, a soil having an ideal state of biological activity contains a microbial biomass above 1,200 ppm and below 2,500 ppm.

Whereas, a soil that requires new applications of stable organic matter contains a microbial biomass of less than 1,200 ppm.

Summer 2012-13: Based on the Solvita Soil Test for soil respiration, the DCR indicated that the Kingsway unlimed site, the Kelly Lake unlimed site and the reference site of Hagar (St-Charles) require new applications of stable organic matter and have a microbial biomass of < 1,2000 ppm. Whereas, the Kingsway limed site, the Kelly Lake limed site, the Wahnapitae Hydro-Dam limed and unlimed sites, and the reference sites of Onaping Falls and Capreol have an ideal state of biological activity and an adequate organic matter level.

Summer soil respiration rates were correlated ($r = 0.50$) with total fungal abundance within the targeted sites. Respiration rates for the reference sites, with the exception of the Hagar site, were higher or similar to those documented for the limed sites. Soil respiration data for limed and reference sites were not significantly different. Sensible differences were observed between limed and unlimed sites.

Fall 2013: Based on the Solvita Soil Test for soil respiration, the DCR indicated that the Wahnapitae Hydro-Dam unlimed site and the Kelly Lake unlimed site require new applications of stable organic matter and have a microbial biomass of < 1,2000 ppm. Whereas, the Wahnapitae Hydro-Dam limed site, the Kelly Lake limed site, the Kingsway limed and unlimed sites, and all three reference sites (Hagar, Onaping Falls and Capreol) have an ideal state of biological activity and an adequate organic matter level.

Significantly higher soil respiration rates were recorded for limed sites compared to unlimed sites. Respiration rates for the reference sites were very similar to those documented for the limed sites. Soil respiration data for limed and reference sites were not significantly different.

No significant differences were found between summer and fall soil respiration rates. There was no correlation between summer and fall data ($r = 0.21$).

Table 14: Weekly mean temperature and total rainfall for May 11th - June 11th 2012-13 in Sudbury

Week	Mean Temperature (°C)	Total Rainfall (mm)
1	10.15	4.1
2	13.85	31.9
3	14.75	2.4
4	13.4	44.2
5	18.65	3.2

Table modified from 'Government of Canada Daily Data Report for May 11th – June 11th 2012-13'. Data was obtained at the Sudbury Airport.

Table 15: Weekly mean temperature and total rainfall for Oct. 9th - Nov. 9th 2013 in Sudbury

Week	Mean Temperature (°C)	Total Rainfall (mm)
1	12	1.0
2	6.5	1.2
3	1.5	1.5
4	1.9	20.6
5	1.2	25

Table modified from 'Government of Canada Daily Data Report for Oct.9th –Nov.9th 2013'.
Data was obtained at the Sudbury Airport.

Table 16: Total fungal abundance and soil respiration for limed, unlimed and reference sites in the GSR

Site	Total Fungal Abundance (c.f.u./g)	CO_2-C (mg/kg (ppm))	Microbial Biomass
Summer 2012			
Hagar (St-Charles)	7.44×10^8	38	Requires new applications of stable organic matter. Biomass <1,2000 ppm.
Kingsway limed	3.50×10^8	65	Ideal state of biological activity and adequate organic matter level.
Kingsway unlimed	2.68×10^8	56	Requires new applications of stable organic matter. Biomass <1,2000 ppm.
Wahnapitae Hydro-Dam limed	1.50×10^8	72	Ideal state of biological activity and adequate organic matter level.
Wahnapitae Hydro-Dam unlimed	2.41×10^7	65	Ideal state of biological activity and adequate organic matter level.
Summer 2013			
Capreol	$3{,}65 \times 10^9$	81	Ideal state of biological activity and adequate organic matter level.
Kelly Lake limed	$8{,}22 \times 10^8$	71	Ideal state of biological activity and adequate organic matter level.
Kelly Lake unlimed	3.83×10^8	50	Requires new applications of stable organic matter. Biomass <1,2000 ppm.
Onaping Falls	9.12×10^8	68	Ideal state of biological activity and adequate organic matter level.

Limed and unlimed sites include: Kingsway, Kelly Lake and Wahnapitae Hydro-Dam. Reference sites include: Hagar (St-Charles), Onaping falls, and Capreol. GSR = Greater Sudbury Region. A correlation was found between total fungal abundance and soil respiration ($r = 0.50$).

Table 17: Fall soil respiration rates for limed, unlimed and reference sites in the GSR

Site	CO_2-C (mg/kg (ppm))	Microbial Biomass
Fall 2013		
Capreol	70	Ideal state of biological activity and adequate organic matter level.
Hagar (St-Charles)	68	Ideal state of biological activity and adequate organic matter level.
Onaping Falls	69	Ideal state of biological activity and adequate organic matter level.
Kingsway limed	71	Ideal state of biological activity and adequate organic matter level.
Kingsway unlimed	69	Ideal state of biological activity and adequate organic matter level.
Kelly Lake limed	71	Ideal state of biological activity and adequate organic matter level.
Kelly Lake unlimed	50	Requires new applications of stable organic matter. Biomass <1,2000 ppm.
Wahnapitae Hydro-Dam limed	71	Ideal state of biological activity and adequate organic matter level.
Wahnapitae Hydro-Dam unlimed	38	Requires new applications of stable organic matter. Biomass <1,2000 ppm.

Limed and unlimed sites include: Kingsway, Kelly Lake and Wahnapitae Hydro-Dam. Reference sites include: Hagar (St-Charles), Onaping falls, and Capreol. GSR = Greater Sudbury Region.

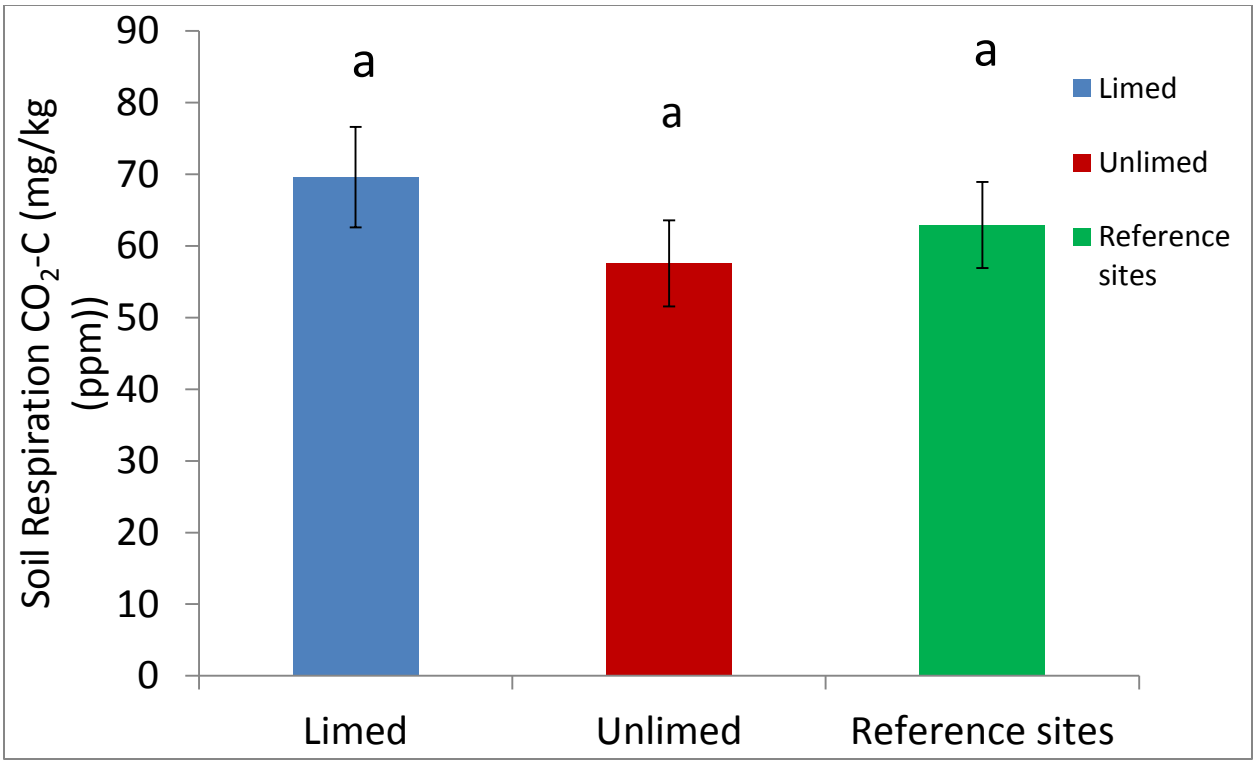


Figure 12: Mean summer soil respiration rates for unlimed, limed and reference sites in the Greater Sudbury Region with standard error bars.

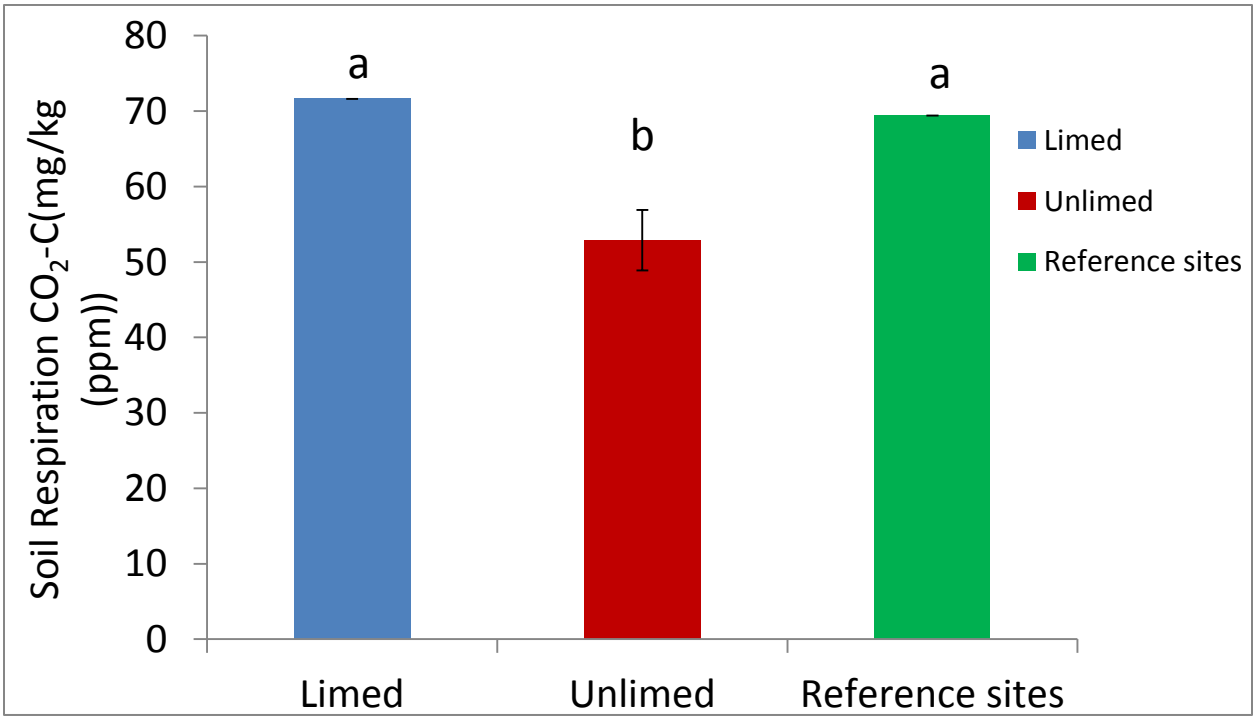


Figure 13: Mean fall soil respiration rates for unlimed, limed and reference sites in the Greater Sudbury Region with standard error bars.

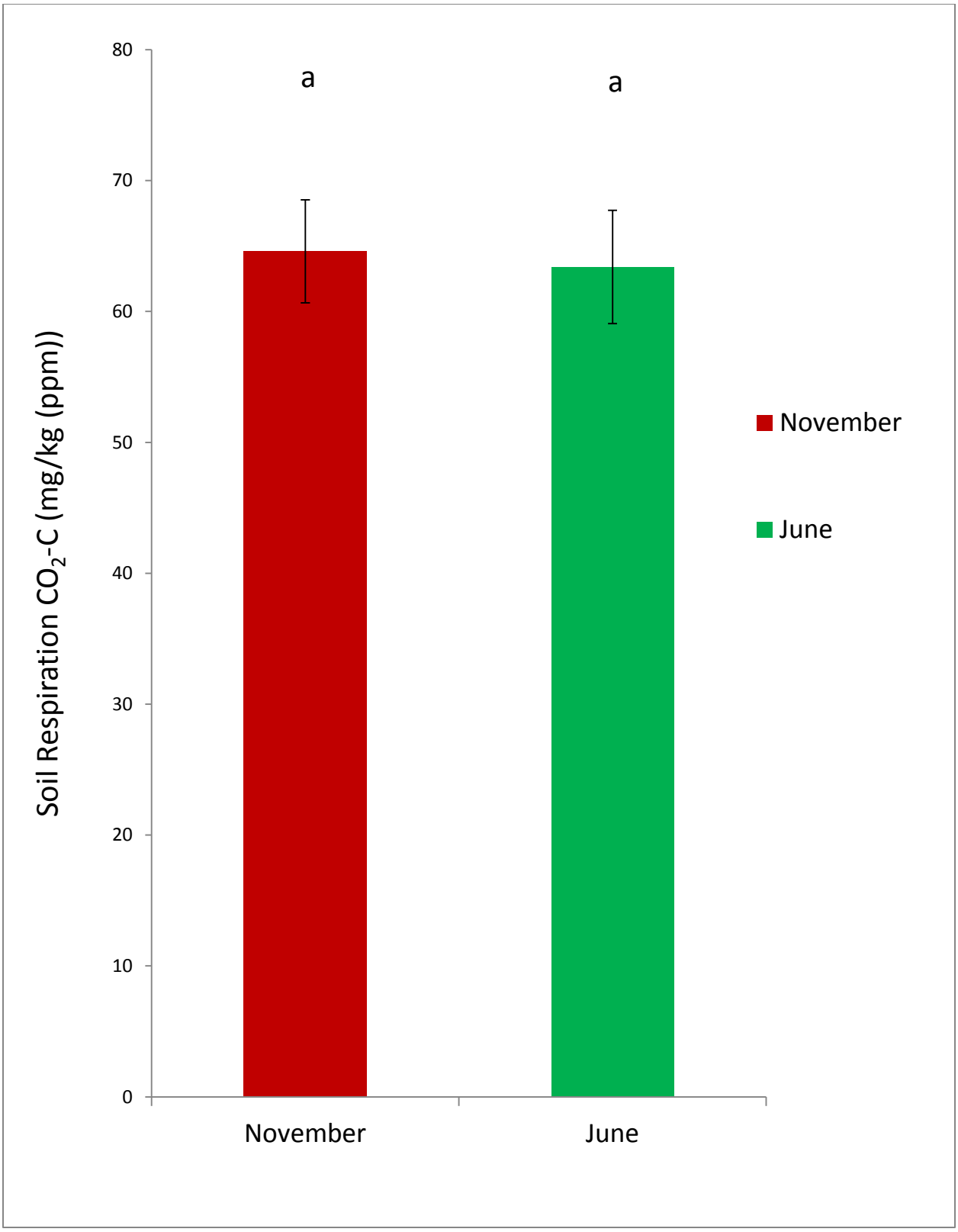


Figure 14: Mean June and November soil respiration rates for the Greater Sudbury Region with standard error bars. No correlation was found between summer and fall data (r = 0.21).

4.4 Discussion

Soil Respiration and Climatic Conditions

The major factors influencing soil respiration include soil moisture affecting microbial activity (Greaves & Carter, 1920), bacterial decomposition as a source of CO_2 efflux (Turpin 1920), soil diffusion kinetics (Lundergardh, 1927), and the existing correlation of CO_2 production with the rate of diffusion through the soil (Smith & Brown 1933). The mean rates of soil respiration (g C m^{-2} yr^{-1}) vary among global biomes. However, climate change has caused a substantial increase in terrestrial CO_2 flux to the atmosphere from 1960 to present, particularly in temperate and tropical biomes compared to high latitude biomes (Anderson, 2011). Laboratory studies examining the effects of global warming on soil respiration have also indicated that the microbial respiration responses to warming as assessed by Q_{10} measurements can fluctuate significantly from soils from different latitudes (Bekku et al., 2003). Variations in temperature and precipitation patterns brought on by climate change will ultimately produce major boundary shifts in the biomes, particularly in transitions between grasslands and forests (Anderson, 2011). Consequently, these transitions will affect the fungi communities in many regions and ultimately, soil respiration rates occurring in these environments.

Precipitation patterns and soil moisture significantly affect soil microbial communities and their respiratory behaviours (Anderson, 2011). Some studies have examined the relevance of this association in order to better understand the possible effects of climate change, among other natural phenomenons, on soil respiration and soil health as well as to fully comprehend the extent of the role of microbial communities in soil respiration. For instance, Cook and Orchard (2008) have examined the relationships between soil respiration and soil moisture in recent decades.

We have now come to realize that soil microbial populations have adapted to many of the worlds unfavourable environmental conditions such as stresses from repeated cycles of precipitation and drying (Anderson, 2011). This results in the creation of strong selection pressures to adapt to these aformentioned irregular and volatile weather conditions (Anderson, 2011). Microbial activity will be reduced or will cease below critical levels of soil moisture which in turn, will stimulate the formation of desiccation-resistant dormant stages such as spores or cysts in some microbial species (Anderson, 2011). Soil fungi have the ability to produce hyphal strands in order to bridge across air-filled pores and are active at a water potential as low as -15 MegaPascals (MPa) (Swift et al., 1979). In comparison, bacteria are inactive below -1.0 to -1.5 MPa (Swift et al., 1979). This clearly demonstrates some of the key differences between fungal and bacterial communities inhabiting the soil. Other microorganisms such as protists are also known to have developed this adaptability to unfavourable climatic conditions. For example, one of the most common protists in the soil, the naked amoebae, is known to encyst at low levels of soil moisture but will excyst when satisfactory levels of soil moisture are present (Anderson, 2011).

Soil respiration on a global scale (g C m^{-2} yr^{-1}) is also linearly related to mean annual precipitation (mm), with a slope of ~0.5 (Raich & Schlesinger, 1992). Additionally, Risch and Frank (2006) have been able to identify a positive correlation between soil respiration and seasonal moisture ($r = 0.65$, $p < 0.05$) when studying a temperate grassland in North America. Overall seasonal moisture levels may thus dictate average soil respiration rates for particular regions. Consequently, variations in summer and fall moisture levels cause variations in microbial structure and soil respiration outputs for these same seasons. In fact, the correlation between soil respiration in the summer and fall in the present study was only $r = 0.21$. Key

factors to this seasonal variation in soil respiration include seasonal variations in mean temperature (°C), total rain (mm), total snow (cm) and total precipitation (mm). Mean monthly temperatures from May 11th to June 11th 2012-13 were higher than the mean monthly temperature from October 9th to November 9th 2013 during the study. Total rainfall (mm) followed the same trend. Consequently, bacteria and fungi dynamics in the soil will be greatly modified during seasonal changes. Additionally, soil respiration outputs will be partially dictated on soil porosity, soil aeriation levels in relation to soil water content as well as the differential physiological responses of the microbial community (Lou & Zou, 2006). Field observations have also revealed a curvilinear relation between soil CO_2 efflux and soil moisture with CO_2 efflux being mainly limited at the lowest and highest moisture levels and optimal in the optimum soil moisture ranges (Bowden et al., 1998; Xu et al., 2004). This is also consistent with previous experimental reports (Ino & Monsi, 1969).

With increasing attention towards the global warming issue in recent years, the significance of the temperature dependence of soil microorganisms has become crucially important since microorganisms are the main group producing CO_2 through the decomposition of organic material in the soil (Pietikåinen et al., 2005). At present, little is known concerning the temperature dependence of different groups of soil microorganisms such as bacteria and fungi (Pietikåinen et al., 2005). However, we do know that soil respiration is strongly linked to temperature. The instantaneous soil respiration rate will often increase when temperature reaches up to around 40 °C or more; this phenomenon occurring even in soils from cold climates (Pietikåinen et al., 2005). Interestingly, this is not due to the growth of thermophilic organisms which would occur at higher temperatures since the same effect is observed even in short incubation times (a few hours) which would not allow for substantial thermophilic growth

(Pietikåinen et al., 2005). Contrarily, soil bacterial and fungal growth rates in cold climates typically have optimum temperatures below 30 °C; this is coupled by the fact that activity values decrease at higher temperatures (Pietikåinen et al., 2005). In this study, soil retrieval containing fungi colonies was achieved in the summer when optimum values of soil respiration theoretically should have been achieved due to environmental temperature. Soil retrieval was equally undertaken in the fall of 2013. Mean monthly temperatures from May 11th to June 11th 2012-13, and October 9th to November 9th 2013 ranged from 14°C to 4.6°C respectively. Because these temperatures, while still being warm, were below 30 °C, bacterial and fungal growth rates are considered to have been optimal in Sudbury at this time. Optimum fungal growth rates have allowed for optimum soil respiration rates within the targeted areas. In fact, total fungal abundance was strongly correlated with summer soil respiration rates ($r = 0.50$).

Soil Respiration and Liming

Summer soil respiration rates in the reference sites were similar or higher than those documented for the limed sites with the exception of the Hagar site. For this latter site, it would appear as though other biotic and abiotic factors are influencing soil chemistry and bacterial or fungal driven respiration rates. It is also possible that within this site, there exists a high degree of variability from sampling. Although our soil respiration measurements do not differentiate between bacterial and fungal metabolic activity, we can conclude that through the addition of lime, fungi, and possibly other microorganisms, have returned to the ecosystem as prime decomposers, based on our diversity and abundance results, and are actively participating in soil respiration in the area thus aiding overall soil quality and recovery within the region. The documentation of 52 fungal species belonging to numerous fungi phyla have allowed us to conclude that many fungi populations have been re-established in the once exceptionally

ecologically disturbed environment. In fact, soil pH has been linked to microbial respiration responses in previous studies. Domsch and Anderson (1973), through their study, have concluded that the soil containing the highest percentage of bacterial activity had a pH of 2.9 while the soil containing the least bacterial activity had a pH of 7.5. Conversely, fungal activity does not follow this trend as bacterial and fungal soil requirement are very different in many aspects.

Soil Respiration and Soil Metal Contamination

Fungi are considered to be more tolerant of heavy metals as a group in comparison to bacteria (Doelman, 1985; Hiroki, 1992). Furthermore, it has been previously determined that heavy metals will affect bacteria and fungi differently in the soil (Hiroki, 1992; Khan & Scullion, 2002; Maliszewska et al., 1985; Muller et al., 2001). Metal contamination of aluminium, cobalt, copper, and iron, and possible excessive amounts of elements, namely potassium, manganese and strontium were higher in unlimed areas compared to limed areas which could have inhibited fungal diversity and abundance within the region and ultimately, have affected fungal soil respiration outputs. This is consistent with a study conducted by Rajapaksha et al. (2004), where soil respiration rate was slightly negatively affected by metal contamination and a clear dose-response effect was encountered at added metal concentrations above 4 mmol kg^{-1}. Furthermore, their study revealed that following the addition of lime, a short increase in respiration rate in the contaminated soils was apparent (Rajapaksha et al., 2004). Therefore, this soil amendment strategy was successful for promoting soil respiration.

Chapter 5: General Conclusions

The Greater Sudbury Region is known for its' rich geological basin, and has thrived economically because of its' nickel, copper and other metal and mineral deposits. Subsequent mining, roasting as well as smelting of these elements have caused sulphur dioxide fumigations and metal particulate depositions which have led to various detrimental effects on the overall environmental quality of the Greater Sudbury Region. Metal contaminated and acidified soils have caused phytotoxic effects in plants and mycotoxic effects in fungi. Physiologically, the excess of some metal ions or nutrients can have adverse effects on fungi growth, metabolism and differentiation. The objectives of this study were to assess fungal diversity and abundance along with soil respiration in limed and unlimed areas contaminated with metals in the Greater Sudbury Region.

Overall, the portion of total metals that was available to biota was very small. Higher concentrations of total aluminium, arsenic, copper, iron, potassium, manganese, nickel, strontium, and zinc and bioavailable concentrations of aluminium, copper, iron, potassium, manganese, nickel, strontium and zinc were observed in unlimed sites compared to limed sites. The pH ranged from 4.12 to 6.75 in the limed areas compared to 3.75 to 4.04 in the unlimed areas. A total of 52 fungi species belonging to 34 genera were identified based on agar plate morphology and microscopic description using classification guides. A total of 41 species were identified on SDA medium and 36 species on MEA medium. The most frequent fungi species on the SDA medium include *Cryptococcus neoformans*, *Mucor indicus*, *Penicillium fellutanum*, *Rhizopus stolonifer*, *Sporotrichum schenckii*, *Trichoderma harzianum* and *Trichoderma mentagrophytes*. The most prevalent fungi species on the MEA medium include *Cryptococcus neoformans*, *Penicillium citrinum*, *Rhizopus stolonifer*, *Streptomyces scabies*, *Trichophyton*

mentagrophytes and *Trichphyton tonsurans*. These species belong to an assortment of fungal phyla, each with their own specific and necessary metal and nutrient intake requirements. Fungi from limed areas were primarily composed of Ascomycota, followed by fungi from Basidiomycota, Zygomycota, Deuteromycota and Oomycota phyla respectively. Whereas, for the unlimed sites, Ascomycota was the most abundant phylum, followed by fungi from Zygomycota, Basidiomycota, Deuteromycota and Oomycota phyla respectively. The reference sites were primarily composed of Ascomycota, followed by fungi from Zygomycota, Basidiomycota, Oomycota and Deuteromycota phyla respectively. While the frequencies (%) of fungi phyla between unlimed, limed and reference sites were quite similar, the difference lies within the fungal species composition for every type of site.

Several fungi species were found in all the sites while some species were site-specific. For instance, *Cryptococcus neoformans* and *Rhizopus stolonifer* were common in all the limed sites, namely Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake. *Trichophyton mentagrophytes* was common in all the unlimed sites of Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake.

The study equally revealed that through remediation and soil amendment activities, in particular, soil liming, the Wahnapitae Hydro-Dam, Daisy Lake, Kingsway and Kelly Lake sites have increased their mean fungal diversity and abundance yields. A total of 23 different fungi species were identified in samples from limed areas compared 17 species for unlimed sites. This difference was found to be significant for the SDA medium. Significantly higher fungal abundance values were recorded for limed sites compared to unlimed soil for SDA medium. Fungal diversity based on growth on MEA medium revealed no significant difference between limed and unlimed sites. A total of 21 species were found in samples from the limed sites

compared to 19 species documented on unlimed soil. No significant differences were recorded for fungal abundance for limed and unlimed sites for the MEA medium. The most abundant fungi species found in the limed sites was *Steptomyces scabies* with a total abundance of 4.89 x 10^8 c.f.u./g. By comparison, the most abundant fungi species found in the unlimed sites was *Penicillium montanense* with a total abundance of 3.86 x 10^8 c.f.u./g.

Temperature and rainfall were recorded daily for one month prior to soil sampling. Climatic data was obtained through the Government of Canada daily data report, measured at the Sudbury Airport. Mean temperature for the 30 days prior to sampling in June was significantly higher (14°C) compared to October-November that was only 4.6°C. Likewise, mean rainfall for May-June was 85.8 mm and was higher than the 49.3 mm rainfall in the 30 days prior to fall sampling on November 9th 2013. Soil respiration measurements support the fungal diversity and abundance data. Higher soil respiration rates were recorded for all the limed sites compared to the unlimed sites. In fact, sensible differences were observed between limed and unlimed sites. The means for the summer CO_2-C outputs in the limed and unlimed sites were 69.6 ppm and 57.6 ppm repectively. Summer soil respiration rates were correlated (r = 0.50) with total fungal abundance within the targeted sites. In general, respiration rates for the reference sites, with the exception of the Hagar site, were higher or similar to those documented for the limed sites. As in the summer, fall soil respiration data was higher in samples from limed sites compared to unlimed sites. The means for the fall CO_2-C outputs in the limed and unlimed sites were 71.6 ppm and 52.85 ppm respectively. No significant differences were found between summer and fall soil respiration rates.

In conclusion, the present study revealed that dolomitic and calcitic limestone applied 30 to 40 years ago still has an effect on soil toxicity, fungi growth, microbial biomass, and ecosystem sustainability in the Greater Sudbury Region. As such, our hypotheses were validated.

Literature Cited

Abedin, J., Spiers, G. 2006. Metal bioavailability in smelter-impacted land systems. In Proceedings, 31st Annual Meeting and Conference of the Canadian Land Reclamation Association, Ottawa, Ontario, 1-17.

Abedin, J., Beckett, P., & Spiers, G. 2012. An evaluation of extratants for assessment of metal phytoavailability to guide practices in acidic soils capes in northern regions. Canadian Journal of Soil Science, 92, 253-268.

Ågren, G.I. 2000. Temperature dependence of soil organic matter. Ambio. 29, 55–55.

Ahmad, S. S., Erum, S. 2010. Soil and Environment. 29 (2), 110.

Al-Janabi, S. A. A. H. 2010. Toxic effect of heavy metals on dermatophytes. Mycoses 54 (4): 345-349.

Amiro, B.D., Courtin, G.M. 1981. Patterns of vegetation in the vicinity of an industrially disturbed ecosystem, Sudbury, Ontario. In C. D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada. Maralte Publishing, the Netherlands.

Anastasi, A., Varese, G., Marchisio, V. 2005. Isolation and Identification of fungal communities in compost and vermicompost. Mycologia. 97:33-44.

Anderson, J. P. E., Domsch, K. H. 1974. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology, 21(3), 314-322.

Anderson, O.R. 2000. Abundance of terrestrial gymnamoebae at a northeastern U.S. site: A four-year study, including the El Nino winter of 1997-1998. J Eukaryot Microbiol 47:148-155.

Anderson, O.R. 2011. Soil Respiration, Climate Change and the Role of Microbial Communities. Protist. 162: 679-690.

Antonovics, J., Bradshaw, A. D. and Turner, R. G. 1971. Heavy metal tolerance in plants. Advances in ecological research pp. 1-85 Academic Press.

Baldrian, P. 2010. Effect of heavy metals on saprotrophic soil fungi. Soil Biology Volume 19, pp.263-279.

Balsillie, D., McIlveen, W.D., and Winterhalder, K. 1978. Problems of regeneration of stressed ecosystems. In Proceedings of the 7 1 st Annual meeting of the Air Pollution Control Association, 25-30 June 1978, Houston, Tex. Pap. 78-44.6.

Beckett, P.H.T., Davis, RD.1988. Upper critical levels of toxic elements in plants. New Phytologist 79: 95-106.

Bekku, Y.S., Nakatsubo T., Kume, A., Adachi, M., Koizumi, H. 2003. Effects of warming on the temperature dependence of soil respiration rate in Artic, temperate and tropical soils. Appl Soil Ecol 22: 205-210.

Benedickson, J. 1973. Sudbury, nickel & INCO: early history. Alternatives 3(3): 6-9.

Boldt, J.R., Jr. 1967. The Winning of Nickel. Longmans Canada Ltd.: Toronto. 487 pp.

Bowden, R.D., Newkirk, K.M., Rullo, G.M. 1998. Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. Soil Biol. Biochem. 30: 1591-1597.

Carter, M. R. 1993. Soil sampling and methods of analysis. Lewis Publishers (eds.), Florida.

Chaney, R. L.; Oliver, D. P. In Contaminants and the Soil Environment in the Australia-Pacific Region; R. Naidu et al., Eds.; Kluwer Academic Publishers: Dordrecht, 1996; p 259.

Clarbone, J., Keller, W., and Griffiths, R. W. 1998. Effects of changes in acidity on aquatic insects in rocky littoral habitats of lakes near Sudbury, Ontario. Restoration Ecology (6):376-389.

Clark, F. E., E. A. Paul.1970. The microflora of grassland. Adv. Agron. 22: 375-435.

Cook, F.J., Orchard, V.A. 2008. Relationships between soil respiration and soil moisture. Soil Biol Biochem 40: 1013-1018.

Davet, P., Rouxel, F. 2000. Detection and Isolation of Soil Fungi. Science Publishers, Inc., Post Office Box 699, Enfield, New Hampshire 03748,USA. 188 pp.

DeLestard, L.P.G. 1967. A history of the Sudbury Forest District. Sist. His. Series No.21. Ont. Dept. Lands and Forests, Toronto. 90 pp.

Doelman, P. 1985. Resistance of soil microbial communities to heavy metals, p. 369–384. In V. Jensen, A. Kjøller, and L. H. Sørensen (ed.), Microbial communities in soil. Elsevier, London, United Kingdom.

Domsch, K. H., Gams, W, and Anderson, T-H. 2007. Compendium of Soil Fungi. IHW-Verlag, Soil fungi - 672 pages.

Doran, J., Kettler, T. and Tsivou, M. 1997. Field and Laboratory Solvita Soil Test Evaluation. USDA-ARS, University of Nebraska, Lincoln. Pp 1-6.

Dudka, S., Ponce-Hernandez, R., and Hutchinson, T. C. 1995. Current level of total element concentrations in the surface layer of Sudbury's soils. Science of the Total Environment, 162(2), 161-171.

Dugan, F. M. 2006. Identification of fungi: an illustrated introduction with keys, glossary, and guide to literature. American Phytopathological Society, Science - 176 pages.

Freedman, B., Hutchinson, T.C. 1980a. Pollutants imputs from the atmosphere and accumulations in soils and vegetation near a nickel-copper smelter in Sudbury, Ontario, Canada. Canadian Journal of Botany, 58:108-132.

Freedman, B., Hutchinson, T.C. 1980b. Long term effects of smelter pollution at Sudbury, Ontario, on forest community composition. In C. D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada. Maralte Publishing, the Netherlands.

Gadd, G.M., Mowll, J.L. 1985. Copper uptake by yeast-like cells, hyphae and chlamydospores of Aureobasidium pullulans. Experimental Mycology **(**9)***:*** 230–240.

Gadd, G.M., White, C.1989a. Heavy metal and radionuclide accumulation and toxicity in fungi and yeasts. In: PooleRK, GaddGM, eds. Metal-microbe interactions. Oxford : IRL Press, 19-38.

Gadd, G. M. 1992. Interactions of fungi with toxic metals. Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, Scotland, UK.

Gorham, E, Gordon, A.G. 1960. Some effects of smelter pollution north-east of Falconbridge, Ontario. In C. D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada. Maralte Publishing, the Netherlands.

Greaves, J.R., Carter, E.G. 1920. Influence of moisture on bacterial activities of the soil. Soil Sci 10:361-387.

Gunn, J., M. 1996. Restoring the smelter-damaged landscape near Sudbury Canada. Restoration and Management Notes 14: 129-136.

Hiroki, M. 1992. Effects of heavy metal contamination on soil microbial populations. Soil Sci. Plant Nutr. 38:141–147.

Howey, F.R. 1938. Pioneering on the C.P.R Mutual Press Ltd., Ottawa. Ottawa. 160 pp.

Huettl, R. F. 1993. Forest soil acidification. Angewandte Botanik, (67): 66-75.

Hughes, M.N, Poole, R.K. 1991. Metal speciation and microbial growth – the hard (and soft) facts. Journal of General Microbiology ***(***137)***:*** 725–734.

Hutchinson, T. C., L. M. Whitby. 1974. Heavy metal pollution in the Sudbury mining and smelting region of Canada. In Soil and vegetation contamination by nickel, copper and other metals. Environmental Conservation (1):123-132.

Hutchinson, T. C. , L. M. Whitby. 1977. The effects of acid rainfull and heavy metal particulates on a boreal Forest ecosystem near the Sudbury smelting region of Canada. Water, Air, Soil Pollution, Volume 7, Issue 4, pp 421-438.

Ino, Y., Monsi, M. 1969. An experimental approach to the calculation of CO_2 amount evolved from several soils. Jpn J Bot 20:153-188.

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline, R. G., Harris, R. F., Schuman, G. E. 1997. Soil quality: a concept, a definition, and framework evaluation (a guest editorial). Soil Sci. Soc. Am. J. 61: 4-10.

Keller, W., Gunn J. W. 1995. Lake water quality improvements and recovering aquatic communities. pp 67-80. In: Gunn, J.M. (ed) Restoration and Recovery of an Industrial Region, Springer Verlag, New York.

Kendrick, B. 2000. The Fifth Kingdom. Third Edition. Pp. 150-154.

Khan, M., Scullion, J. 2002. Effects of metal (Cd, Cu, Ni, Pb or Zn) enrichment of sewage sludge on soil micro-organisms and their acitivities. Appl. Soil Ecol. 20:145–155.

Lautenbach, W.E., Miller, J., Beckett, P. J., Negusanti, J.J. and Winterhalder, K. 1995. Municipal land restoration program: The regreening process. In J. Gunn Ed, Restoration and recovery of an industrial region: Progress in restoring the smelter-damaged landscape near Sudbury, Canad, pp.109-122. New York: Springer-Verlag.

Lautenbach, William, E. 1996. The greening of Sudbury. J. Soil Water Conservation. 228-231.

LeBlanc, F., Rao, D.N. and Comeau, G. 1972. The epiphytic vegetation of Populus balsamifera and its significance as an air pollution indicator in Sudbury, Ontario. In C. D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada.Maralte Publishing, the Netherlands.

Lipson, D.A., Schadt, C.W and Schmidt, S.K. 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. Microb. Ecol. 43, 307–314.

Lundegardh, H. 1927. Carbon dioxide evolution of soil and crop growth. Soil Sci 23:417-453.

Luo, Y., Zhou, X. 2006. Soil Respiration and the Environment. Academic Press, Burlington, MA.

Maliszewska, W., S. Dec, H. Wierzbicka, and A. Woz´niakowska. 1985. The influence of various heavy metal compounds on the development and activity of soil micro-organisms. Environ. Pollut. 37:195–215.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London.

McCall, J., Gunn J. M., and Struik, H. 1995. Photo interpretive study of recovery of damaged lands near the metal smelters of Sudbury, Canada. Water Air Soil Pollution. 85: 847-852.

McKeague, J.A., J.G. Desjardins, and M.S. Wolynetz. 1979. Minor elements in Canadian soils. LRRI-27. Agriculture Canada, ResearchBranch, Ottawa.

Mengel, K., Kirby, E. A. 1987. Principles of Plant Nutrition, 4th edn. International Potash Institute, Bern, Switzerland.

Michelutti, B., Weiseman M., 1995. Engineered wetlands as a tailings rehabilitation strategy. Pp 135-141. In: Gunn, J.M. (ed) Restoration and Recovery of an Industrial Region, Springer-Verlag, New York.

Muller, A. K., K. Westergaard, S. Christensen, and S. J. Sørensen. 2001. The effect of longterm mercury pollution on the soil microbial community. FEMS Microbiol. Ecol. 36:11–19.

Newbound, M. 2008. Fungal diversity in remnant vegetation patches along an urban to rural gradient. Australasian Mycologist, 28: 74-77.

Nieboer, E., Ahmed, H. M., Puckett, K. J. and Richardson, D.H.S. 1972. Heavy metal content of lichens in relation to distance from a nickel smelter in Sudbury, Ontario. Lichenologist (5):292-304.

Nieboer, E., Richardson, D.H.S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environmental Pollution **(**1)***:*** 3–26.

Nielsen, M. N., Winding, A. 2002. Microorganisms as indicators of soil health. Denmark: National Environmental Research Institute. Pp.399.

Norman, D. Yan, Keller, W. and Gunn, J. M. 1995. Liming of Sudbury lakes: lessons for recovery of aquatic biota from acidification. pp 195-204. In: Gunn, J.M. (ed) Restoration and Recovery of an Industrial Region, Springer Verlag, New York.

Ochiai, E.I. 1987. General principles of biochemistry of the elements. New York : Plenum Press.

Parkinson, D. 1973. Techniques for the study of soil fungi. Bull. Ecol. Res. Commun. (Stockholm), 17:29-36.

Paschke, M. W. Redente, E. F. and Levy, D. B. Environmental Toxicity and Chemistry 2000, 19, 2751.

Peck, G.R. 1978. The not-so-distant past. Sudbury Star. 30 September, 1978.

Persson, T., Breland, T.A., Seyferth, U., Lomander, A., Kåtterer, T., Henriksen, T.M., and Andrén, O. 1999. Carbon and nitrogen turnover in forest and arable soil in relation to substrate quality, temperature and moisture. Tema Nord 560, 131–152.

Peters, T.H. 1984. Rehabilitation of mine tailings: a case of complete reconstruction and revegetation of industrially stressed lands in the Sudbury area, Ontario, Canada. pp 403-421 In: P.J. Gunn (ed.) Restoration and Recovery of an Industrial Region, Springer-Verlag, New York.

Pietikåinen, J., Pettersson, M. and Bååth, E. 2005. Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiology Ecology, 52: 49–58.

Pitt, J. I. 1985. A Laboratory Guide to Common Penicilium Species. Commonwealth Scientific and Industrial Research Organization, 184 pages.

Potvin, R., J. Negusanti. 1995. Declining industrial emissions, improving air quality and reduced damage to vegetation. pp. 51-65. In: J.M. Gunn (ed.) Restoration and Recovery of an Industrial Region, Springer-Verlag, New York.

Raich, J.W., Schlesinger, W.H. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44: 81-99.

Rajapaksha, R. M. C. P., Tobor-Kaplon, M. A and Bååth, E. 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Appl. Environ. Microbiol.70(5):2966-2973.

Raven, J.A, Evans, M.C.W. and Korb, R.E. 1999. The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. Photosynth. Res. 60:111-149.

Risch, A.C., Frank, D. A. 2006. Carbon dioxide fluxes in a spatially and temporally heterogeneous temperate grassland. Oecologia 147: 291-302.

Ritz, K. ,Young, I. M. 2004. Interactions between soil structure and fungi. Mycologist 18 (2): 52-59.

Roberts, D., Nachtegaal, M. and Sparks, D. 2005. Speciation of metals in soils. Soil Sci. Sco. Am. Book series number 8. Pp. 619-654. USA.

Rowe, J.S. 1959. Forest Regions of Canada. Bulletin 123, Canada Department of Northern Affairs and National Resources, Forestry Branch. 71 pp.

Sabie, F.T., Gadd, G.M. 1990. The effect of zinc on the yeast-mycelium transition of *Candida albicans* and examination of zinc uptake at different stages of growth. Mvcological Research 94*:* 952–958.

Satchell, J. E. 1971. Feasibility study of an energy budget for Meathop Wood. In Productivity of forest ecosystems, ecology and conservation. No. 4. Edited by P. Duvigneaud. Paris: UNESCO.

Schadt, C.W., Martin, A.P., Lipson, D.A. and Schmidt, S.K.. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301, 1359–1361.

Shields, J. A., E. A. Paul, W . E. Lowe, and D.Parkinson. 1973. Turnover of microbial tissue in soil under field conditions. Soil Biol. Biochem. 5: 753-764.

Smith F.B., Brown, P. E. 1933. The diffusion of carbon dioxide through soils. Soil Sci 35:413-423.

Smith, J.L, J.W. Doran. 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In Methods for assessing soil quality. Soil Science Society of America Special Publication (49): 169-182.

Soil Classification Working Group. 1998. The Canadian System of Soil Classification. 3rd ed. Agriculture and Agri-Food Canada Publication, 1646, 187.

Soil Quality Institute. 1999. Soil Quality Test Kit Guide. United States Department of Agriculture.

Solvita. 2013. Official Solvita Guideline Soil CO_2 Respiration Test. Woods End Laboratories Inc. Mt Vernon, USA.

Spiers, G. A., Wren, C. D. and McLaughlin, D. 2012. Distribution of chemicals of concern. In C.D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada. Maralte Publishing, the Netherlands.

Storey, J. B. 2006. Zinc. Handbook of plant nutrition. Pp.411-435 CRC Press.

Swer, H., Dkhar, M. S. and Kayang, H. 2011. Fungal population and diversity in organically amended agricultural soils of Meghalaya, India. Journal of Organic Systems, 6 (2). 3-12.

Swift, M., Heal, O.W. and Anderson, J.M. 1979. Decomposition in Terrestrail Ecosystems. University of California Press, Berkeley, CA.

Turcotte, C. K. 1981. A comparative study of soils and vegetation in the vicinity of two roast yards in Sudbury, Ontario. Hons. B.Sc. Thesis, Laurentian University. 116 pp.

Turpin, H. W. 1920. The carbon dioxide of the soil air. Cornell Univ Agr Expt Sta Mem 32: 319-362.

Van Assche F, Clijsters H. 1990. Effects of metals on enzyme activity in plants. Plant Cell Environ. 13:195-206.

VETAC. 2013. Annual Report. Regreening Program.

Watanabe, T. 1937. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. Lewis Publishers. Boca Raton. F.L. U.S.A.

Watson, W. Y., D.H.S. Richardson. 1972. Appreciating the potential of a devastated land. The Forestry Chronicle (48):312-315.

Watson, G., Greenfield, M., Butler, M. and Wren, C. 2012. Historical smelter emission and environmental impacts. In C. D. Wren, ed. Risk assessment and environmental management: A case study in Sudbury,Ontario, Canada. Maralte Publishing, the Netherlands.

Weyman-Kaczmarkowa, W. and Pedziwilk, Z. 2000. The development of fungi as affected by pH and type of soil, in relation to the occurrence of bacteria and soil fungastatic activity. Microbiol Res. 155(2):107-12.

Wilderman, T. 1991. Handbook for constructed wetlands receiving acid mine drainage. Risk Reduction Engineering Library, U.S. Environmental Protection Agency, Cincinnati, OH.

Winterhalder, K. 1983. The use of manual surface seeding, liming and fertilization in the reclamation of acid metal-contaminated land in the Sudbury, Ontario mining and smelting region of Canada. Environmental Technology Letters (4): 209-216.

Winterhalder, K. 1984. Environmental Degradation and Rehabilitation in the Sudbury Area. Laurentian University Review. Vol. XVI (2). Pp. 15-47.

Winterhalder K. 1995. Early history of human activities. In: Gunn J, editor. Environmental restoration and recovery of an industrial region. NewYork: Springer-Verlag; p. 17–31.

Winterhalder, K. 1996. Environmental degradation and rehabilitation of the landscape around Sudbury, a major mining and smelting area. Environmental Reviews, 4, 185-224.

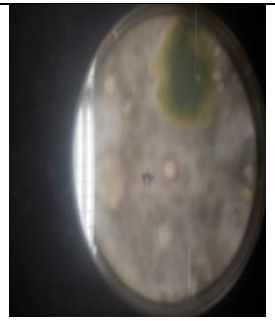
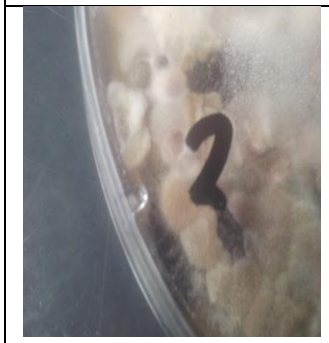
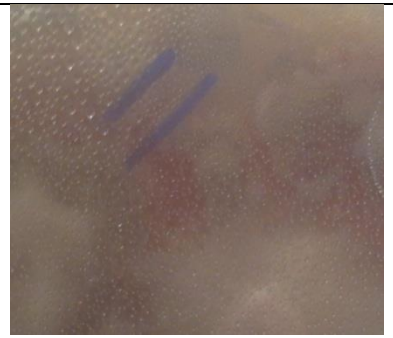
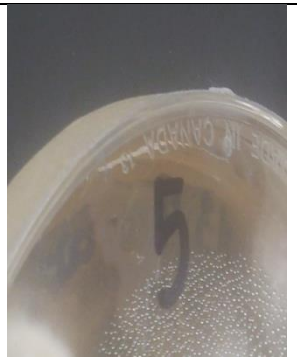
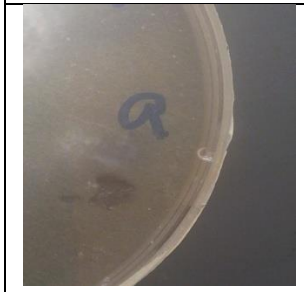
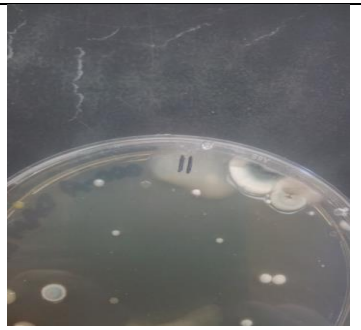
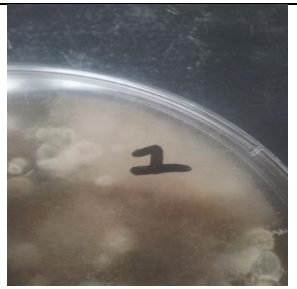
Xu, L., Baldocchi, D.D. and Tang, J. 2004. How soil moisture, rain pulses and growth alter the response of ecosystem respiration to temperature. Global Biogeochem Cy 18:GB4002.

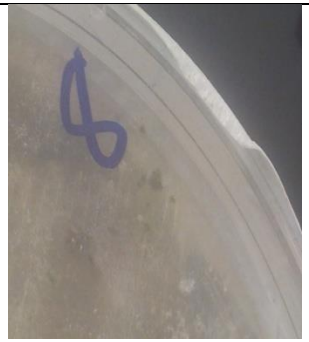
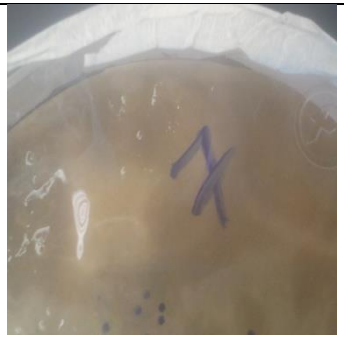
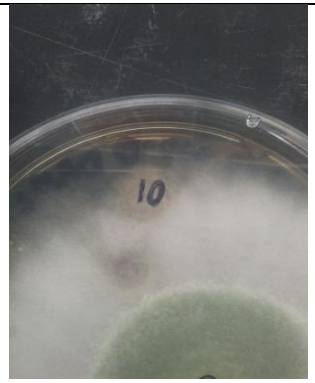
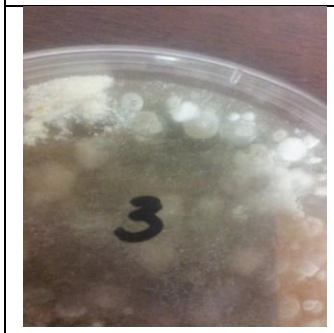
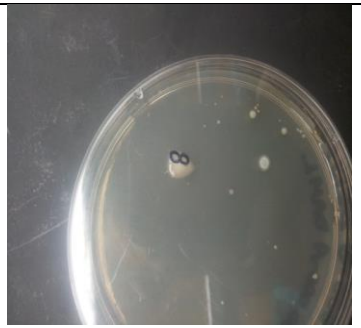
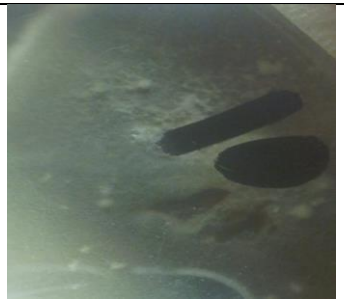
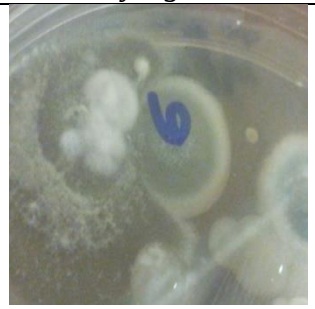
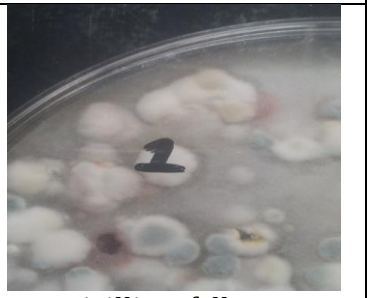
Yiqi, L., Zhou, X. 2010. Soil respiration and the environment. Academic press.

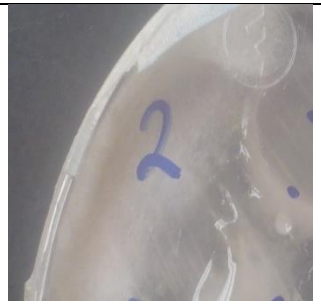
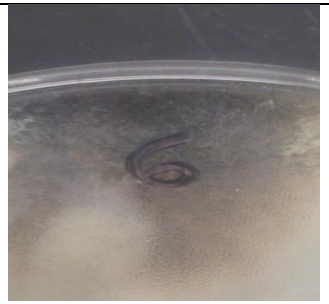
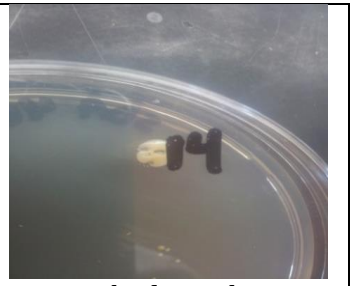
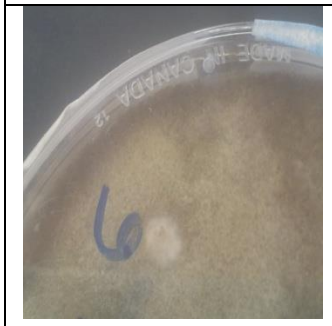
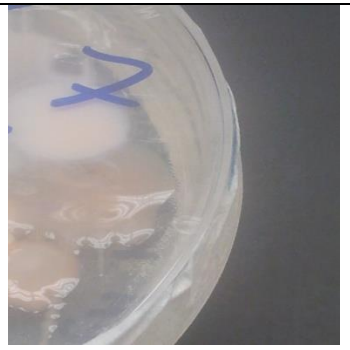
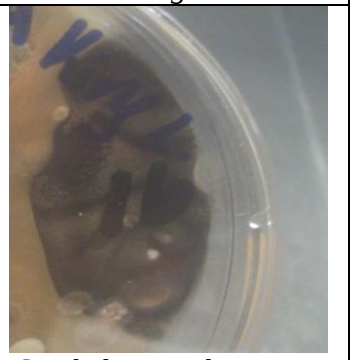
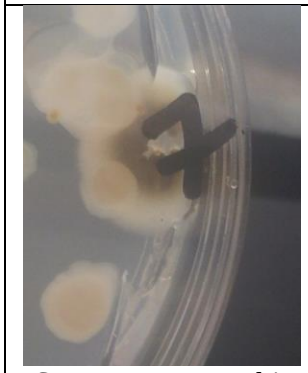
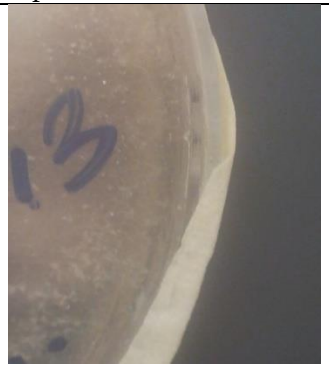
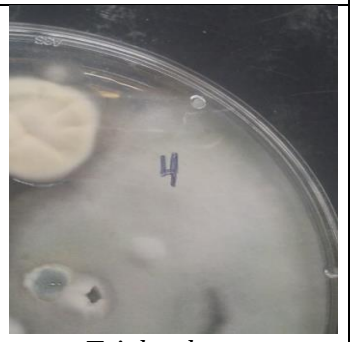
Zycha, H., Siepmann, R. 1970. Mucorales. Lubrecht & Cramer Limited, Science - 355 pages.

Appendices

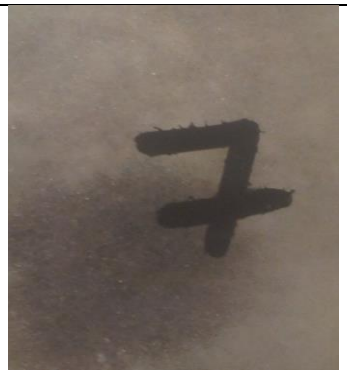
Appendix A - Illustrations of fungi species on agar media

 *Absidia corymbifera*	 *Acremonium strictum*	 *Actinomyces bovis*	 *Allescheria boydii*	 *Alternaria alternata*
 *Blastomyces brasiliensis*	 *Blastomyces dermatitidis*	 *Candida albicans*	 *Candida glabrata*	 *Candida lusitaniae*
 *Cladosporium sphaerospermum*	 *Coccidioides immitis*	 *Cryptococcus neoformans*	 *Cunninghamella bertholletiae*	 *Epicoccum purpurascens*

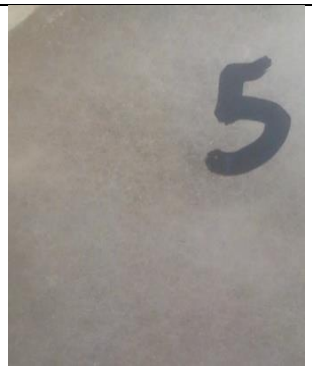
 *Eupenicillium shearii*	 *Gliocladium viride*	 *Histoplasma capsulatum*	 *Microsporum gallinae*	 *Mortierella isabellina*
 *Mucor indicus*	 *Mucor mucedo*	 *Mycoderma aceti*	 *Penicillium chrysogenum*	 *Penicillium cinnamopurpureum*
 *Penicillium citreonigrum*	 *Peniclilium citrinum*	 *Penicillium crustaceum*	 *Penicillium decumbens*	 *Penicillium fellutanum*

 *Penicillium lividum*	 *Penicillium montanense*	 *Pythium debaryanum*	 *Rhizopus stolonifer*	 *Rhodotorula mucilaginosa*
 *Saccharomyces cerevisiae*	 *Saprolegnia parasitica*	 *Sporoblomyces roseus*	 *Sporotrichum schenckii*	 *Stachybotrys chartarum*
 *Streptomyces scabies*	 *Trichoderma harzianum*	 *Trichophyton concentricum*	 *Trichophyton faviforme*	 *Trichophyton mentagrophytes*

Trichophyton rubrum



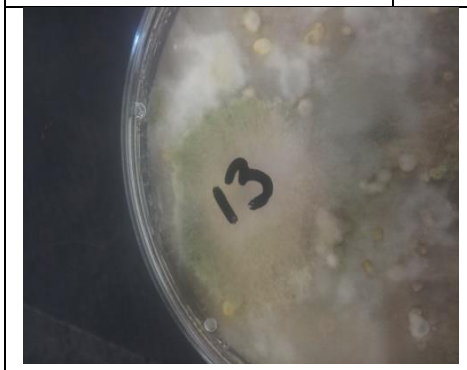
Trichophyton schoenleini



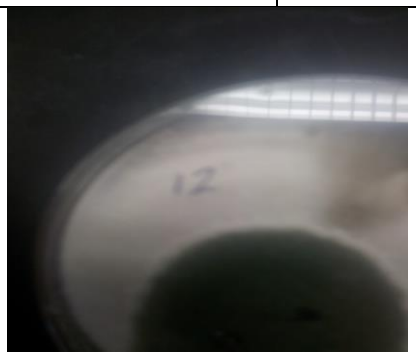
Trichophyton tonsurans

Trichophyton violaceum

Trichosporon mucoides

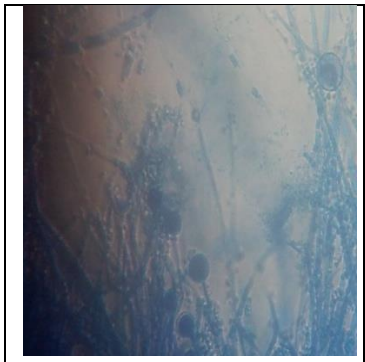
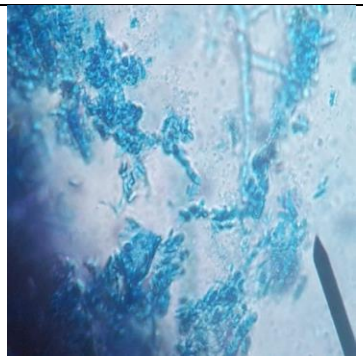
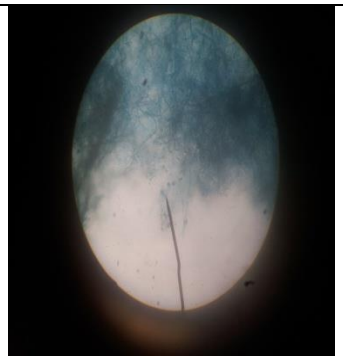
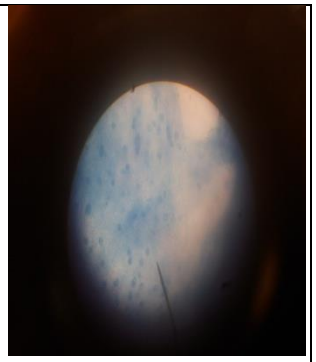
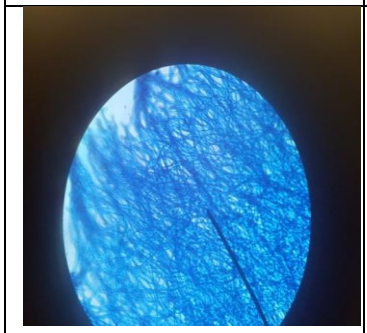
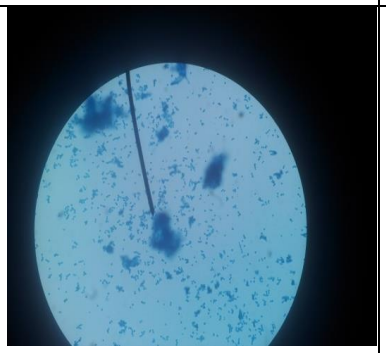
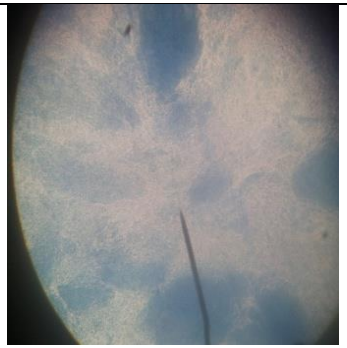
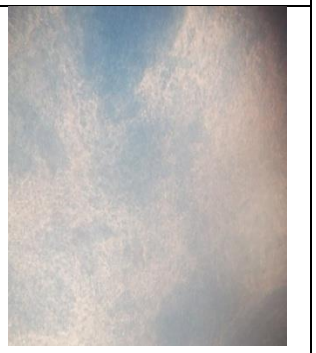
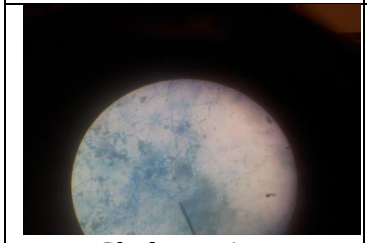
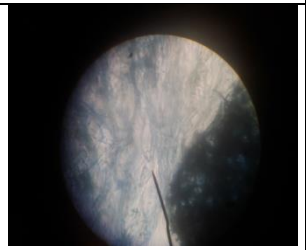
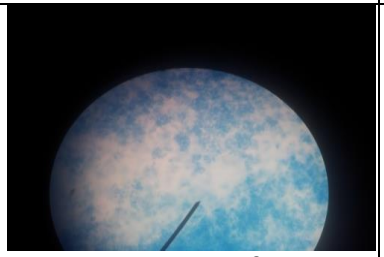
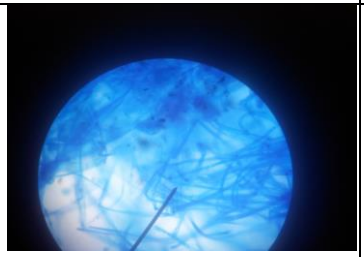
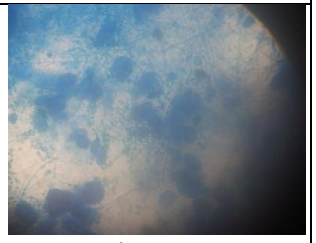


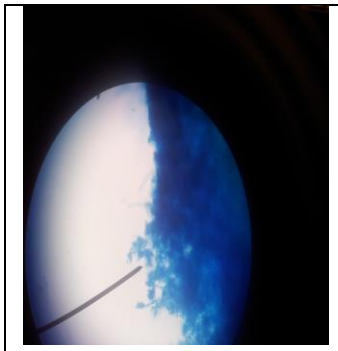
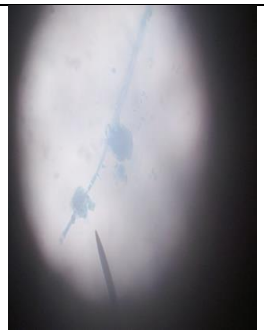
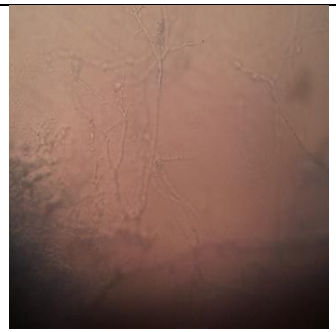
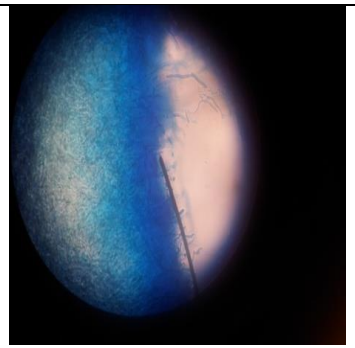
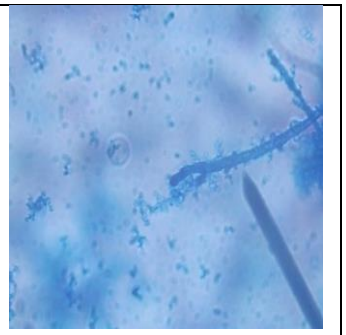
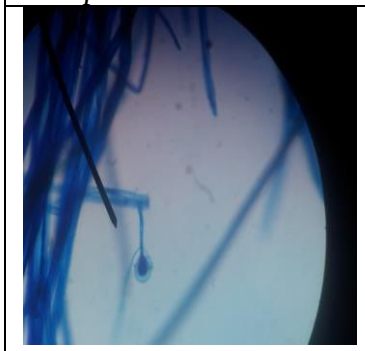
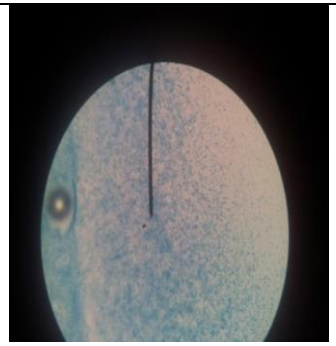
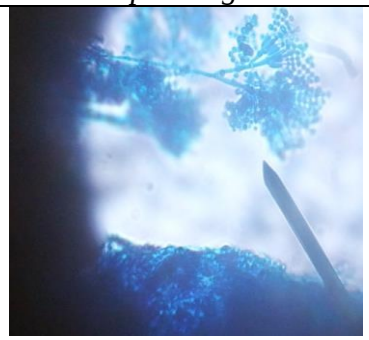
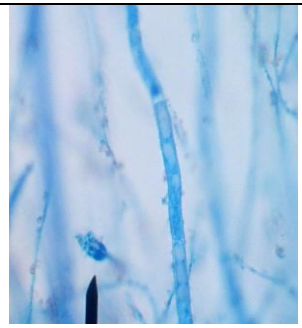
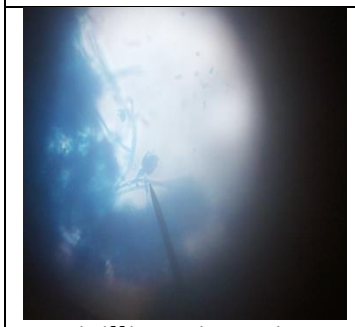
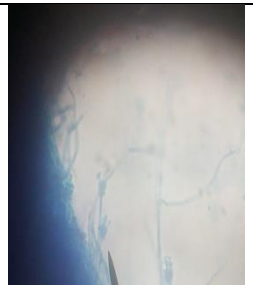
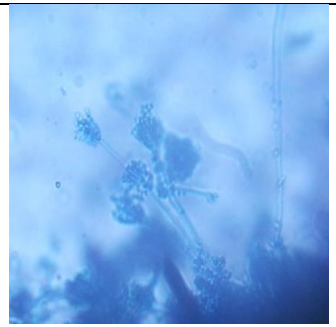
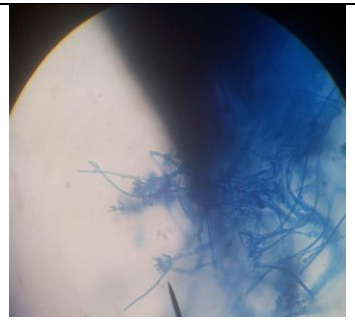
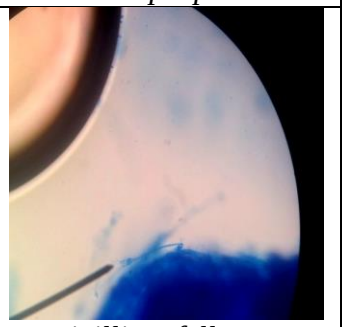
Trichothecium roseum

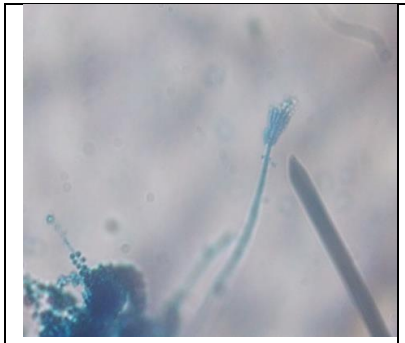
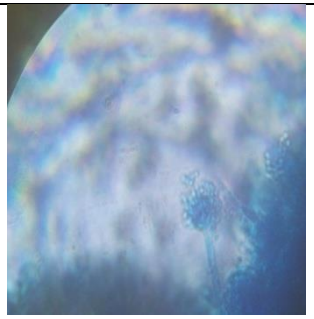
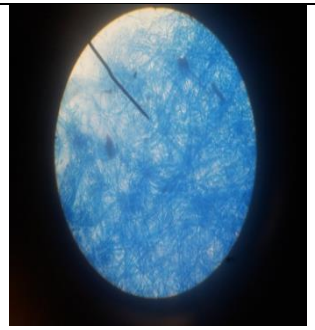
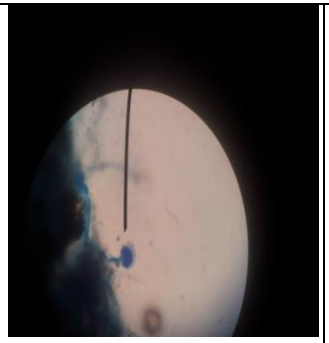
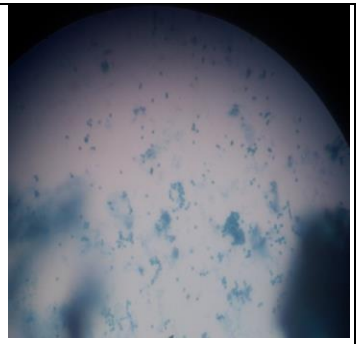
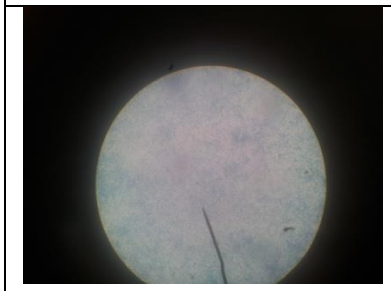
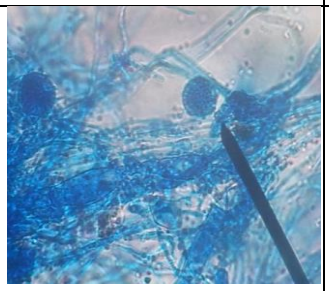
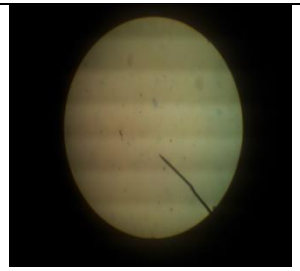
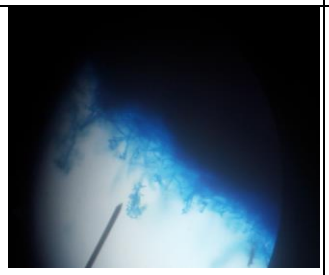
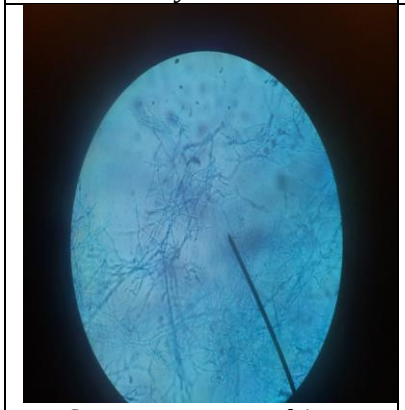
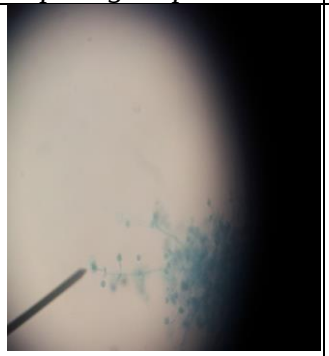
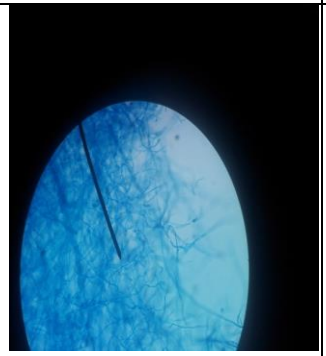
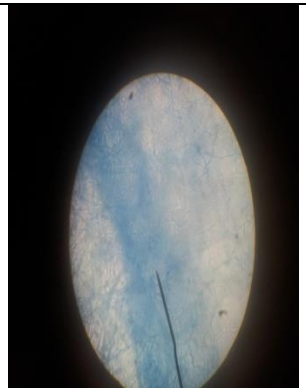
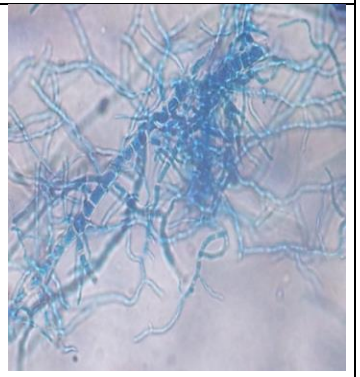


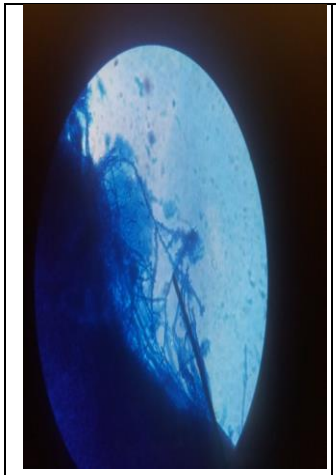
Verticillium dahlia

Appendix B - Illustrations of fungi species via microscope

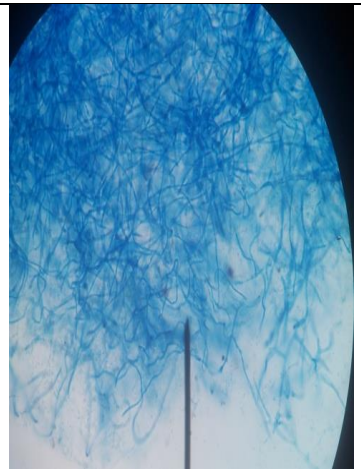
 *Absidia corymbifera*	 *Acremonium strictum*	 *Actinomyces bovis*	 *Allescheria boydii*	 *Alternaria alternata*
 *Blastomyces brasiliensis*	 *Blastomyces dermatitidis*	 *Candida albicans*	 *Candida glabrata*	 *Candida lusitaniae*
 *Cladosporium sphaerospermum*	 *Coccidioides immitis*	 *Cryptococcus neoformans*	 *Cunninghamella bertholletiae*	 *Epicoccum purpurascens*

 *Eupenicillium shearii*	 *Gliocladium viride*	 *Histoplasma capsulatum*	 *Microsporum gallinae*	 *Mortierella isabellina*
 *Mucor indicus*	 *Mucor mucedo*	 *Mycoderma aceti*	 *Penicillium chrysogenum*	 *Penicillium cinnamopurpureum*
 *Penicillium citreonigrum*	 *Peniclilium citrinum*	 *Penicillium crustaceum*	 *Penicillium decumbens*	 *Penicillium fellutanum*

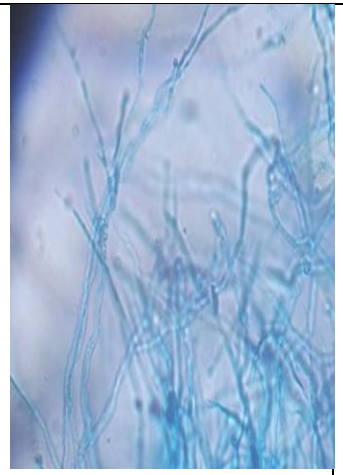
 *Penicillium lividum*	 *Penicillium montanense*	 *Pythium debaryanum*	 *Rhizopus stolonifer*	 *Rhodotorula mucilaginosa*
 *Saccharomyces cerevisiae*	 *Saprolegnia parasitica*	 *Sporoblomyces roseus*	 *Sporotrichum schenckii*	 *Stachybotrys chartarum*
 *Streptomyces scabies*	 *Trichoderma harzianum*	 *Trichophyton concentricum*	 *Trichophyton faviforme*	 *Trichophyton mentagrophytes*



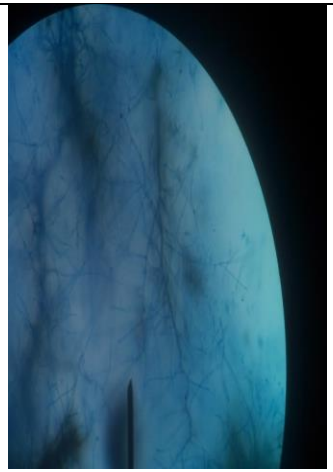
Trichophyton rubrum



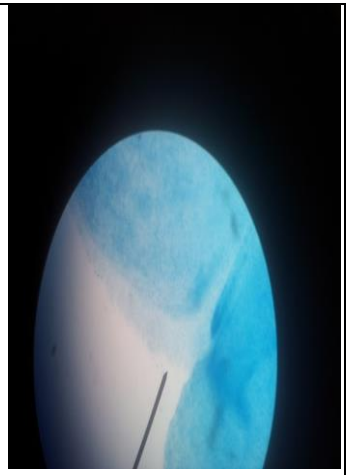
Trichophyton schoenleini



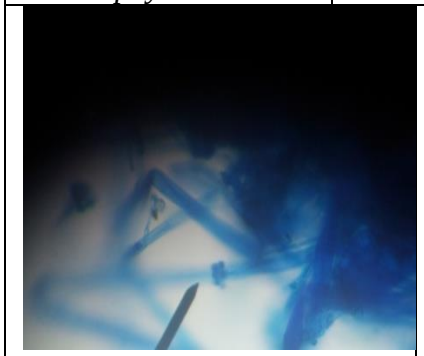
Trichophyton tonsurans



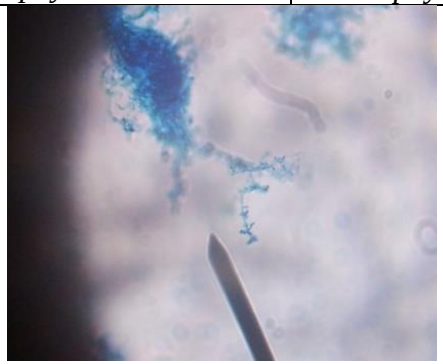
Trichophyton violaceum



Trichosporon mucoides



Trichothecium roseum



Verticillium dahlia

Appendix C - Photographs of sites used in the study

a)

b)

c)

d)

Figure 15: Photographs of the Wahnapitae Hydro-Dam site, demonstrating limed and unlimed areas utilized for soil samples. Photographs a) and c) are limed areas and b) and d) are unlimed.

a)

b)

c)

d)

Figure 16: Photographs of the Kingsway site, demonstrating limed and unlimed areas utilized for soil samples. Photographs a) and c) are limed areas and b) and d) are unlimed.

Appendix D – Sudbury Soil Horizons

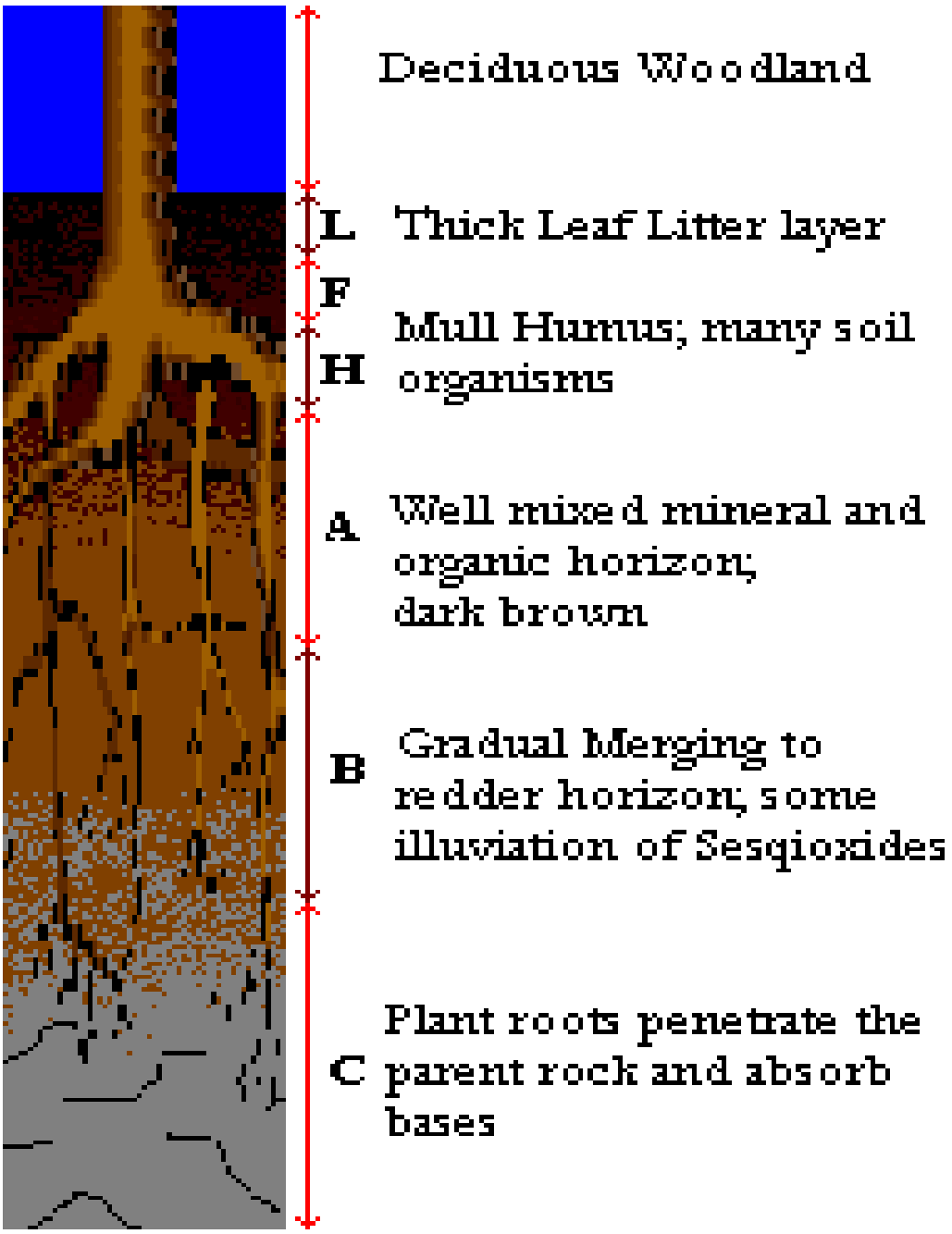


Figure 17: Sudbury soil horizons demonstrating LFH layer to be used for fungal analysis

Appendix E- Professional Soil Test Interpretation by Solvita

PROFESSIONAL SOIL TEST INTERPRETATION

Test Result ppm CO_2-C	N-Mineralization	Potential Biomass
> 100	High N-Potential soil. Likely sufficient N for most crops.	Soil very well supplied with organic matter. Biomass >2,500 ppm.
61 - 100	Moderately-high. This soil has limited need for supplemental N.	Ideal state of biological activity and adequate organic matter level.
31 - 60	Moderate Level. Supplemental N is most likely indicated.	Requires new applications of stable organic matter. Biomass <1,200ppm.
6 - 30	Moderate-Low - will not provide sufficient N for most crops.	Low in organic structure and microbial activity. Biomass < 500ppm.
0 - 5	Little biological activity; Requires significant fertilization.	Very inactive soil. Biomass < 100 ppm. Consider long-term care.

Appendix F – Sample Calculation for Abundance of Fungi Species

Fungi Abundance Formula:

Abundance (c.f.u./g) = Number of colonies / (volume pipetted (mL) x dilution)

Sample calculation:

Eupenicillium shearii colony found in Wahnapitae Hydro-Dam unlimed soil on SDA medium

Abundance = 57 colonies/ (0.1 mL x 10^{-3}) = 5.7 x 10^{6} c.f.u./g

Units utilized are: colony forming units/gram. All calculations for abundance values were determined via this formula for all species.

Appendix G

Table 18: Fungi species isolated from samples from limed area at Dam using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans					A	
Eupenicillium shearii					A	
Microsporium gallinae					B	
Mycoderma aceti					A	A
Penicillium fellutanum		AB	AB		A	
Rhizopus stolonifer		B				
Rhodotorula mucilaginosa		AB	AB		AB	
Trichoderma harzianum		A	A	AB	B	
Trichophyton mentagrophytes		AB	AB			
Trichothecium roseum		B				
Verticillium dahliae					B	

Table 19: Fungi species isolated from samples from unlimed area of Dam using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Eupenicillium shearii		AB	AB	AB	B	
Penicillium fellutanum		AB	AB	AB		
Sporotrichum schenckii			AB	AB	AB	A
Trichoderma harzianum			A			
Trichophyton mentagrophytes		AB	AB	AB	AB	B

Table 20: Fungi species isolated from samples from limed area of Dam using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Mycoderma aceti		AB	AB	AB		
Rhodotorula mucilaginosa						B
Trichophyton mentagrophytes		AB	AB	AB	AB	AB

Table 21: Fungi species isolated from samples from unlimed area at Dam using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococus neoformans		AB	AB	B		
Mycoderma aceti		AB	AB	B		
Rhodotorula mucilaginosa				B		A
Trichophyton mentagrophytes		AB	AB	AB	AB	AB

Table 22: Fungi species isolated from samples from limed area at Daisy Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces brasiliensis		AB				
Mortierella isabellina		AB	AB	AB		
Mucor indicus		A				
Penicillium chrysogenum		AB	AB	AB		
Rhizopus stolonifer		AB	AB			
Sporotrichum schenckii		AB	AB	AB	AB	
Trichophyton concentricum					B	B

Table 23: Fungi species isolated from samples from unlimed area at Daisy Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum		AB	AB	AB	A	
Mucor mucedo				B		
Penicillium lividum				B	AB	
Rhizopus stolonifer					AB	B
Sporotrichum schenckii			A			
Trichophyton rubrum				B		

Table 24: Fungi species isolated from samples from limed area at Daisy Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Actinomyces bovis		AB				
Cryptococcus neoformans					A	
Penicillium chrysogenum		AB	AB	AB		
Penicillium cinnamopurpureum		AB				
Penicillium fellutanum				B		
Sporotrichum schenckii		AB	AB	AB	AB	
Trichophyton mentagrophytes			AB	AB		
Trichophyton violaceum		AB				
Trichosporon mucoides			B	A		

Table 25: Fungi species isolated from samples from unlimed area at Daisy Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum			AB	AB	B	
Penicillium citrinum				AB	B	
Penicillium crustaceum						A
Saprolegnia parasitica				AB	AB	B
Trichophyton mentagrophytes					B	B
Trichophyton rubrum				A	A	
Trichophyton schoenleini		B				
Trichophyton tonsurans				B	A	
Verticillium dahliae		AB	AB			

Table 26: Fungi species isolated from samples from limed area at Kingsway using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Actinomyces bovis					A	A
Candida albicans			AB	AB	AB	
Cryptococcus neoformans						B
Mycoderma aceti						B
Penicillium fellutanum			AB	AB	AB	
Rhizopus stolonifer		AB	AB	AB		
Sporotrichum schenckii			AB	A		
Stachybotrys chartarum					A	
Trichophyton mentagrophytes		AB	A	AB	AB	AB

Table 26: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichosporon mucoides					B	
Verticillium dahliae						B

Table 27: Fungi species isolated from samples from unlimed area at Kingsway using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans					B	
Cunninghamella bertholletiae		AB	AB	AB	AB	A
Penicillium crustaceum			AB	A		
Penicillium fellutanum				A	A	
Rhizopus stolonifer		B	B			
Saprolegnia parasatica		AB	AB	AB		
Sporotrichum schenckii						A
Trichophyton mentagrophytes				B	A	
Trichophyton rubrum					B	
Trichophyton tonsurans				A		

Table 28: Fungi species isolated from samples from limed area at Kingsway using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Mycoderma aceti		AB	AB	AB	AB	AB
Rhizopus stolonifer					B	
Trichophyton mentagrophytes			AB	AB	AB	
Trichophyton tonsurans					B	

Table 29: Fungi species isolated from samples from unlimed area at Kingsway using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans		AB	AB	AB	AB	AB
Penicillium lividum					A	
Saprolegnia parasitica		AB	AB	AB		
Sporotrichum schenckii		AB	AB	AB	AB	
Streptomyces scabies					B	
Trichophyton mentagrophytes		AB	AB	AB		
Trichophyton tonsurans				A		

Table 30: Fungi species isolated from samples from unlimed area at Kelly Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans		AB	AB	AB	AB	AB
Penicillium montanense				A	AB	
Pythium debaryanum				A		
Rhizopus stolonifer		AB	AB	AB	AB	
Trichoderma harzianum				AB	B	

Table 31: Fungi species isolated from samples from limed area at Kelly Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans		AB	AB	AB	AB	AB
Histoplasma capsulatum				B	B	
Mucor indicus		B		B		
Penicillium chrysogenum		B				
Penicillium crustaceum		B	AB	AB	AB	
Rhizopus stolonifer		AB				
Saccharomyces cerevisiae				AB	A	
Sporotrichum schenckii		AB	AB	AB	AB	

Table 32: Fungi species isolated from samples from unlimed area at Kelly Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum		B	AB	AB	AB	
Actinomyces bovis		AB	AB	AB	B	
Alternaria alternata		AB	AB	AB	AB	AB
Cryptococcus neoformans						A
Gliocladium viride		AB		B		
Penicillium montanense				AB	AB	B
Trichophyton mentagrophytes					B	B

Table 33: Fungi species isolated from samples from limed area at Kelly Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Allescheria boydii						B
Blastomyces dermatittidis						B
Cryptococcus neoformans			A	B		
Penicillium citrinum					A	
Penicillium crustaceum		A				
Penicillium lividum		AB	AB			
Pythium debaryanum					AB	
Rhizopus stolonifer		AB				
Sporoblomyces roseus						B
Streptomyces scabies		AB	AB	AB	AB	AB

Table 34: Fungi species isolated from samples at Hagar using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cunninghamella bertholletiae		B	B	AB		
Eupenicillium shearii		AB	AB	AB	AB	AB
Mucor indicus		B	B	AB		
Penicillium crustaceum			B	AB	AB	AB
Trichophyton mentagrophytes				AB	AB	AB

Table 35: Fungi species isolated from samples at Hagar using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Penicillium crustaceum				AB	AB	AB
Rhizopus stolonifer		AB	AB	A	AB	
Streptomyces scabies					AB	
Trichophyton concentricum				A	AB	AB
Trichophyton mentagrophytes				AB	AB	AB
Trychophyton tonsurans				AB	AB	AB

Table 36: Fungi species isolated from samples at Onaping Falls using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Allescheria boydii		AB	A			
Candida glabrata		AB	AB	AB	AB	AB
Candida lusitaniae						A
Cryptococcus neoformans					B	
Mucor indicus		AB	AB	AB		
Penicillium citrinum			AB	AB	AB	AB
Penicillium decumbens			AB	B	AB	AB

Table 37: Fungi species isolated from samples at Onaping Falls using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces dermatitidis					B	
Coccidioides immitis		B				
Mucor indicus			A	B		
Penicillium citreonigrum		AB	AB			
Penicillium citrinum		AB	AB	AB	AB	A
Penicillium montanense			AB	AB	AB	A
Pythium debaryanum		B				
Rhodotorula mucilaginosa			A	AB		
Trichophyton faviforme					B	
Trichophyton tonsurans						B
Trichosporon mucoides		AB	AB	AB	AB	B

Table 38: Fungi species isolated from samples at Capreol using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Absidia corymbifera					B	B
Blastomyces brasiliensis						A
Blastomyces dermatitidis		AB	AB	AB	AB	AB
Cladosporium sphaerospermum						A
Mucor indicus				AB	AB	
Penicillium montanense			AB	AB	AB	AB
Trichoderma harzianum					B	
Trichophyton mentagrophytes					AB	AB
Trichophyton tonsurans						A

Table 39: Fungi species isolated from samples at Capreol using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces dermatitidis		AB				
Candida lusitaniae				A		
Cryptococcus neoformans					AB	AB
Epicoccum purpurascens		AB	AB	AB	AB	AB
Penicillium citrinum					B	
Pythium debaryanum					A	
Rhizopus stolonifer		AB	AB	AB	B	
Sporotrichum schenckii				AB	AB	B
Streptomyces scabies		AB	AB	AB	A	AB
Trichophyton faviforme					A	B

Table 40: Fungi abundance in soil samples from limed area at Dam using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans	A				1.0×10^6	
Eupenicillium shearii	A				5.3×10^7	
Microsporium gallinae	B				1.0×10^6	
Mycoderma aceti	A				3.0×10^6	7.0×10^7
Penicillium fellutanum	A	3.3×10^4	3.1×10^5		1.55×10^8	
	B	2.5×10^4	1.5×10^5			
Rhizopus stolonifer	B	1.0×10^3				
Rhodotorula mucilaginosa	A	2.1×10^4	6.7×10^5		1.0×10^6	
	B	2.7×10^4	7.3×10^5		1.0×10^6	

Table 40: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichoderma harzianum	A	3.0×10^3	5.0×10^4	2.2×10^6		
	B			1.8×10^6	1.0×10^6	
Trichophyton mentagrophytes	A	6.0×10^3	7.0×10^4			
	B	7.0×10^3	7.0×10^4			
Trichothecium roseum	B	1.0×10^3				
Verticillium dahliae	B				2.0×10^6	

Table 41: Fungi abundance in soil samples from unlimed area at Dam using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Eupenicillium shearii	A	3.4×10^4	5.7×10^5	1.0×10^6		
	B	3.2×10^4	5.5×10^5	6.0×10^5	5.0×10^6	
Penicillium fellutanum	A	1.85×10^5	9.1×10^5	1.6×10^6		
	B	2.04×10^5	9.7×10^5	1.4×10^6		
Sporotrichum schenckii	A		3.0×10^4	5.0×10^5	1.0×10^6	1.0×10^7
	B		3.0×10^4	2.0×10^5	2.0×10^6	
Trichoderma harzianum	A		3.0×10^4			
Tricophyton mentagrophytes	A	5.4×10^4	9.0×10^4	7.0×10^5	2.0×10^6	
	B	5.2×10^4	1.0×10^5	5.0×10^5	1.0×10^6	2.0×10^7

Table 42: Fungi abundance in soil samples from limed area at Daisy Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces brasiliensis	A	1.46×10^5				
	B	1.23×10^5				
Mortierella isabellina	A	5.2×10^4	5.3×10^5	1.7×10^6		
	B	4.7×10^4	4.3×10^5	2.1×10^6		
Mucor indicus	A	1.0×10^3				
Penicillium chrysogenum	A	4.0×10^4	8.0×10^5	1.5×10^6		
	B	5.4×10^4	9.2×10^5	2.0×10^6		
Rhizopus stolonifer	A	2.0×10^4	1.9×10^5			
	B	2.7×10^4	1.5×10^5			
Sporotrichum schenckii	A	1.7×10^4	2.9×10^5	1.9×10^6	1.0×10^6	
	B	2.9×10^4	3.3×10^5	2.2×10^6	1.0×10^6	
Trichophyton concentricum	B				1.0×10^6	1.0×10^7

Table 43: Fungi abundance in soil samples from unlimed area at Daisy Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum	A	2.5×10^5	2.14×10^6	1.08×10^7	8.0×10^7	
	B	2.39×10^5	2.0×10^6	9.8×10^6		
Mucor mucedo	B			1.0×10^5		
Penicillium lividum	A				4.9×10^7	
	B			2.3×10^6	5.2×10^7	
Rhizopus stolonifer	A				1.0×10^6	
	B				1.0×10^6	1.0×10^7
Sporotrichum schenckii	A		2.0×10^4			
Trichophyton rubrum	B			4.0×10^5		

Table 44: Fungi abundance in soil samples from limed area at Daisy Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Actinomyces bovis	A	6.8×10^4				
	B	5.8×10^4				
Cryptococcus neoformans	A				2.0×10^6	
Penicillium chrysogenum	A	8.2×10^4	7.0×10^5	2.9×10^6		
	B	9.7×10^4	6.5×10^5	5.0×10^5		
Penicillium cinnamopurpureum	A	4.8×10^4				
	B	4.3×10^4				
Penicillium fellutanum	B			1.2×10^6		
Sporotrichum schenckii	A	1.53×10^5	1.29×10^6	3.9×10^6	1.0×10^6	
	B	1.59×10^5	1.07×10^6	2.8×10^6	2.0×10^6	

Table 44: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton mentagrophytes	A		2.6×10^5	4.0×10^5		
	B		3.0×10^5	1.1×10^6		
Trichophyton violaceum	A	1.0×10^4				
	B	9.0×10^3				
Trichosporon mucoides	A			1.0×10^6		
	B		1.7×10^5			

Table 45: Fungi abundance in soil samples from unlimed area at Daisy Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum	A		1.04×10^6	1.57×10^7		
	B		1.28×10^6	2.05×10^7	2.36×10^8	
Penicillium citrinum	A			2.4×10^6		
	B			3.0×10^6	2.1×10^7	
Penicillium crustaceum	A					1.0×10^7
Saprolegnia parasitica	A			1.9×10^6	5.0×10^6	
	B			6.0×10^5	1.0×10^6	1.0×10^7
Trichophyton mentagrophytes	B				1.0×10^6	1.0×10^7
Trichophyton rubrum	A			1.5×10^6	1.0×10^7	
Trichophyton schoenleini	B	1.0×10^3				

Table 45: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton tonsurans	A				2.3×10^7	
	B			2.9×10^6		
Verticillium dahliae	A	1.13×10^5	6.5×10^5			
	B	1.18×10^5	6.9×10^5			

Table 46: Fungi abundance in soil samples from limed area at Kingsway using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Actinomyces bovis	A				1.0×10^7	6.0×10^7
Candida albicans	A		9.6×10^5	1.0×10^7	2.3×10^7	
	B		9.0×10^5	1.23×10^7	1.77×10^8	
Cryptococcus neoformans	B					1.0×10^7
Mycoderma aceti	B					1.0×10^7
Penicillium fellutanum	A		2.8×10^5	7.7×10^6	4.2×10^7	
	B		3.0×10^5	6.7×10^6	3.0×10^7	
Rhizopus stolonifer	A	1.89×10^5	1.40×10^6	1.04×10^7		
	B	2.19×10^5	1.49×10^6	1.15×10^7		
Sporotrichum schenckii	A		7.9×10^5	1.0×10^5		
	B		7.7×10^5			

Table 46: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Stachybotrys chartarum	A				1.0×10^6	
Trichophyton mentagrophytes	A	2.0×10^4	2.2×10^5	7.2×10^6	6.0×10^6	2.0×10^7
	B	2.7×10^4		1.08×10^7	7.0×10^6	1.0×10^7
Trichosporon mucoides	B				6.0×10^6	
Verticillium dahliae	B					4.0×10^7

Table 47: Fungi abundance in soil samples from unlimed area at Kingsway using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans	B				2.0×10^7	
Cunninghamella bertholletiae	A	8.8×10^4	7.3×10^5	9.5×10^6	2.5×10^7	1.0×10^7
	B	1.04×10^5	8.1×10^5	5.2×10^6	1.7×10^7	
Penicillium crustaceum	A		6.0×10^5	5.0×10^5		
	B		4.7×10^5			
Penicillium fellutanum	A			5.8×10^6	1.9×10^7	
Rhizopus stolonifer	B	3.0×10^3	6.0×10^4			
Saprolegnia parasatica	A	8.0×10^3	9.0×10^4	1.8×10^6		
	B	7.0×10^3	1.0×10^5	2.0×10^5		
Sporotrichum schenckii	A					1.0×10^7

Table 47: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton mentagrophytes	A				1.0×10^6	
	B			1.0×10^5		
Trichophyton rubrum	B				1.0×10^6	
Trichophyton tonsurans	A			1.2×10^6		

Table 48: Fungi abundance in soil samples from limed area at Kingsway using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Mycoderma aceti	A	8.9×10^4	1.22×10^6	1.5×10^6	2.0×10^7	3.0×10^7
	B	7.1×10^4	1.12×10^6	4.3×10^7	2.1×10^7	1.4×10^7
Rhizopus stolonifer	B				3.0×10^6	
Trichophyton mentagrophytes	A		1.06×10^6	4.6×10^6	4.1×10^7	
	B		9.5×10^5	2.1×10^6	8.0×10^6	
Trichophyton tonsurans	B				1.0×10^6	

Table 49: Fungi abundance in soil samples from unlimed area at Kingsway using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans	A	6.1×10^4	1.58×10^6	6.3×10^6	4.5×10^7	1.5×10^8
	B	5.1×10^4	6.0×10^5	8.8×10^6	6.1×10^7	6.0×10^7
Penicillium lividum	A				1.0×10^6	
Saprolegnia parasitica	A	1.2×10^4	1.4×10^5	9.0×10^5		
	B	6.0×10^3	7.0×10^4	5.0×10^5		
Sporotrichum schenckii	A	1.49×10^5	8.1×10^5	3.5×10^6	6.0×10^6	
	B	1.13×10^5	7.9×10^6	4.0×10^6	7.0×10^6	
Streptomyces scabies	B				5.0×10^6	
Trichophyton mentagrophytes	A	5.1×10^4	1.03×10^6	1.6×10^6		
	B	6.4×10^4	1.41×10^6	2.7×10^6		

Table 49: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton tonsurans	A			1.4×10^7		

Table 50: Fungi abundance in soil samples from unlimed area at Kelly Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans	A	5.22×10^5	4.4624×10^6	1.28×10^7	3.6×10^7	2.0×10^7
	B	7.98×10^5	4.05×10^6	1.63×10^7	3.9×10^7	8.0×10^7
Penicillium montanense	A			5.0×10^5	1.0×10^6	
	B				1.0×10^6	
Pythium debaryanum	A			2.0×10^5		
Rhizopus stolonifer	A	4.0×10^4	2.8×10^5	8.0×10^5	1.6×10^7	
	B	1.8×10^4	9.0×10^4	7.0×10^5	1.0×10^7	
Trichoderma harzianum	A			5.0×10^5		
	B			7.0×10^5	1.0×10^6	

Table 51: Fungi abundance in soil samples from limed area at Kelly Lake using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cryptococcus neoformans	A	8.2×10^5	7.54×10^6	3.93×10^7	1.13×10^8	1.1×10^8
	B	8.44×10^5	6.19×10^6	3.58×10^7	1.39×10^8	8.0×10^7
Histoplasma capsulatum	B			1.0×10^5	1.0×10^6	
Mucor indicus	B	2.0×10^3		2.0×10^5		
Penicillium chrysogenum	B	2.3×10^4				
Penicillium crustaceum	A		3.4×10^5	1.5×10^6	4.0×10^6	
	B	2.9×10^4	2.2×10^5	3.0×10^5	1.0×10^6	
Rhizopus stolonifer	A	1.8×10^4				
	B	2.0×10^3				
Saccharomyces cerevisiae	A			1.14×10^7	4.2×10^7	
	B			8.2×10^6		
Sporotrichum schenckii	A	7.5×10^4	1.02×10^6	2.8×10^6	4.0×10^6	

Table 51: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
*Sporotrichum schenckii**	B	5.1×10^4	1.04×10^6	4.4×10^6	1.4×10^7	

Table 52: Fungi abundance in soil samples from unlimed area at Kelly Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Acremonium strictum	A		1.1×10^5	3.7×10^6	2.0×10^7	
	B	1.0×10^4	4.0×10^4	5.4×10^6	2.1×10^7	
Actinomyces bovis	A	2.0×10^3	1.2×10^5	1.5×10^6		

Table 52: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
*Actinomyces bovis**	B	1.0×10^3	7.0×10^4	3.0×10^5	3.0×10^6	
Alternaria alternata	A	1.334×10^6	1.327×10^7	4.3×10^7	8.2×10^7	1.3×10^8
	B	1.507×10^6	1.401×10^7	4.99×10^7	1.11×10^8	9.0×10^7
Cryptococcus neoformans	A					1.0×10^7
Gliocladium viride	A	2.6×10^4				
	B	9.2×10^4		2.0×10^6		
Penicillium montanense	A			7.0×10^5	1.0×10^6	
	B			9.0×10^5	1.0×10^6	7.7×10^8
Trichophyton mentagrophytes	B				3.0×10^6	2.0×10^7

Table 53: Fungi abundance in soil samples from limed area at Kelly Lake using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Allescheria boydii	B					1.0×10^7
Blastomyces dermatittidis	B					1.0×10^7
Cryptococcus neoformans	A		1.0×10^4			
	B			1.0×10^5		
Penicillium citrinum	A				2.0×10^6	
Penicillium crustaceum	A	1.0×10^3				
Penicillium lividum	A	8.9×10^4	6.4×10^5			
	B	6.1×10^4	9.3×10^5			
Pythium debaryanum	A				1.0×10^6	
	B				1.0×10^6	
Rhizopus stolonifer	A	2.0×10^3				
	B	4.0×10^3				
Sporoblomyces roseus	B					1.0×10^7

Table 53: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Streptomyces scabies	A	3.98×10^5	1.79×10^6	9.4×10^6	7.5×10^7	4.4×10^8
	B	3.69×10^5	1.34×10^6	8.2×10^6	7.2×10^7	3.7×10^8

Table 54: Fungi abundance in soil samples at Hagar using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Cunninghamella bertholletiae	A			8.0×10^5		
	B	7.3×10^4	2.9×10^5	1.0×10^5		
Eupenicillium shearii	A	6.0×10^4	5.4×10^5	4.5×10^6	1.5×10^7	2.0×10^7
	B	8.0×10^4	3.4×10^5	4.4×10^6	2.6×10^7	5.0×10^7
Mucor indicus	A			1.7×10^6		
	B	1.0×10^4	3.0×10^5	1.0×10^8		
Penicillium crustaceum	A			5.7×10^6	1.6×10^7	2.0×10^7
	B		9.0×10^4	6.1×10^6	1.8×10^7	1.0×10^7
Trichophyton mentagrophytes	A			2.7×10^6	3.7×10^7	7.0×10^7
	B			4.0×10^6	2.9×10^7	6.0×10^7

Table 55: Fungi abundance in soil samples at Hagar using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Penicillium crustaceum	A			3.0×10^6	6.0×10^6	7.0×10^7
	B			6.3×10^6	1.0×10^7	1.0×10^8
Rhizopus stolonifer	A	5.8×10^4	4.1×10^6	8.0×10^5	4.0×10^6	
	B	5.5×10^4	7.8×10^6		5.0×10^6	
Streptomyces scabies	A				1.8×10^7	
	B				1.3×10^7	
Trichophyton concentricum	A			7.3×10^6	3.5×10^7	9.0×10^7
	B				2.8×10^7	1.3×10^8

Table 55: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton mentagrophytes	A			8.2×10^6	5.0×10^6	3.0×10^7
	B			8.4×10^6	1.7×10^7	6.0×10^7
Trichophyton tonsurans	A			6.9×10^6	3.7×10^7	1.0×10^8
	B			8.9×10^6	3.6×10^7	1.3×10^8

Table 56: Fungi abundance in soil samples at Onaping Falls using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Allescheria boydii	A	2.0×10^3	4.0×10^4			
	B	5.0×10^3				
Candida glabrata	A	1.96×10^5	1.65×10^6	2.14×10^7	3.3×10^7	2.5×10^8
	B	2.30×10^5	1.94×10^6	6.60×10^6	9.9×10^7	1.0×10^8
Candida lusitaniae	A					1.0×10^7
Cryptococcus neoformans	B				1.0×10^6	
Mucor indicus	A	1.0×10^4	5.0×10^4	2.0×10^5		
	B	8.0×10^3	6.0×10^4	1.0×10^5		
Penicillium citrinum	A		2.0×10^5	1.6×10^6	7.0×10^6	4.4×10^8
	B		4.3×10^5	3.5×10^6	7.0×10^6	1.0×10^8
Penicillium decumbens	A		5.0×10^4		5.0×10^6	1.7×10^8
	B		8.0×10^4	1.3×10^6	5.0×10^6	2.4×10^8

Table 57: Fungi abundance in soil samples at Onaping Falls using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces dematittidis	B				1.0×10^6	
Coccidioides immitis	B	1.0×10^3				
Mucor indicus	A		3.0×10^4			
	B			3.0×10^5		
Penicillium citreonigrum	A	1.37×10^5	1.5×10^5			
	B	1.30×10^5	2.1×10^5			
Penicillium citrinum	A	1.8×10^4	2.4×10^5	2.1×10^6	8.0×10^6	1.0×10^7
	B	3.6×10^4	1.8×10^5	9.0×10^5	4.0×10^6	
Penicillium citreonigrum	A		1.12×10^6	4.0×10^6	8.0×10^6	1.0×10^7
	B		1.42×10^6	4.3×10^6	2.0×10^6	
Pythium debaryanum	B	1.0×10^3				
Rhodotorula mucilaginosa	A		1.0×10^4	9.0×10^5		
	B			4.0×10^5		
Trichophyton faviforme	B				1.0×10^6	

Table 57: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Trichophyton tonsurans	B					1.0×10^7
Trichosporon mucoides	A	5.56×10^5	1.92×10^6	1.51×10^7	7.6×10^7	
	B	5.01×10^5	2.58×10^6	1.86×10^7	1.117×10^8	2.0×10^7

Table 58: Fungi abundance in soil samples at Capreol using SDA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Absidia corymbifera	B				3.0×10^6	1.0×10^7

Table 58: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces brasiliensis	A					1.0×10^7
Blastomyces dermatitidis	A	1.89×10^5	7.1×10^5	2.6×10^6	7.0×10^6	3.0×10^7
	B	1.50×10^5	7.8×10^5	3.5×10^6	3.0×10^6	5.0×10^7
Cladosporium sphaerospermum	A					1.0×10^7
Mucor indicus	A			4.0×10^5	1.0×10^6	
	B			4.0×10^5	1.0×10^6	
Penicillium montanense	A		8.0×10^4	1.7×10^6	7.0×10^6	1.0×10^7
	B		1.3×10^5	7.0×10^5	1.0×10^7	1.0×10^7
Trichoderma harzianum	B				1.0×10^6	
Trichophyton mentagrophytes	A				1.5×10^7	9.0×10^7
	B				6.0×10^6	1.0×10^8
Trichophyton tonsurans	A					1.0×10^7

Table 59: Fungi abundance in soil samples at Capreol using MEA medium

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Blastomyces dermatitidis	A	1.49×10^5				
	B	1.37×10^5				
Candida lusitaniae	A			7.0×10^6		
Cryptococcus neoformans	A				1.0×10^7	1.7×10^8
	B				2.0×10^6	1.2×10^8
Epicoccum purpurascens	A	7.4×10^4	4.9×10^5	2.0×10^5	3.4×10^7	2.0×10^7
	B	1.88×10^5	3.4×10^5	1.61×10^7	1.6×10^7	1.88×10^9
Penicillium citrinum	B				1.0×10^6	
Pythium debaryanum	A				2.0×10^6	
Rhizopus stolonifer	A	3.0×10^3	1.0×10^5	6.0×10^5		
	B	3.0×10^3	1.1×10^5	4.0×10^5	1.0×10^6	
Sporotrichum schenckii	A			6.1×10^6	1.07×10^8	
	B			6.3×10^6	4.0×10^6	3.1×10^9

Table 59: (continued)

Species		Dilution				
	Replicate 1	1/100 A	1/1000 A	1/10 000 A	1/100 000 A	1/1000 000 A
	Replicate 2	1/100 B	1/1000 B	1/10 000 B	1/100 000 B	1/1000 000 B
Streptomyces scabies	A	6.03×10^5	2.43×10^6	1.10×10^7	9.5×10^7	9.7×10^8
	B	6.28×10^5	2.62×10^6	1.34×10^7		2.9×10^8
Trichophyton faviforme	A				1.0×10^6	
	B					2.0×10^7

Table 60: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Dam using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 10	Waxy and creamy beige, irregular shape, on side of plate	Globose to ovoid budding yeast-like cells
Eupenicillium shearii	Day 4	Dark green, very small, circular	Appearance of lenticular ascospores with two closely appressed equatorial flanges and biverticillate penicilli
Microsporium gallinae	Day 5	Waxy dark beige, circular, small	Septated hyphae producing microconidia and macroconidia; macroconidia are clevate-shaped and microconidia are unicellular and ovoid-shaped
Mycoderma aceti	Day 4	Waxy beige, very small, dot/circular	Elongated ovoid-shaped spores that form chains

Table 60: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium fellutanum	Day 4	Cottony white, very small circular in shape	Presence of conidiophores with irregularly located metulae of variable length, terminating in definite vesicles; conidia are dark green in color
Rhizopus stolonifer	Day 4	Dark brown/black/charcoal, wool texture, around the edge of plate, half-circle shape	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores

Table 60: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Rhodotorula mucilaginosa	Day 12	Small, yellow, circular colony	Many spores in a rounded to ovoid shape
Rhodotorula mucilaginosa	Day 12	Very small, dark charcoal/black, circular	Many spores in a rounded to ovoid shape
Trichoderma harzianum	Day 5	Furry/fuzzy forest green, colony is quiet large, irregular in shape but often circular	Conidia are globose and pale green in color; present at tips of septate hyphae in agglomerations or free (not on hyphae)

Table 60: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 4	Waxy white, spread over entire plate, ever so slightly wooly	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichothecium roseum	Day 12	Hair-like colony, strands of white/grey/green present	Conidiospores ellipsoidal to pyriform on hyphae
Verticillium dahliae	Day 10	Cottony white, widely spread, irregular in shape	Globose to elongate microsclerotia, without dark hyphae or dark mycelium

Table 61: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Dam using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Eupenicillium shearii	Day 4	Dark green, very small, circular	Appearance of lenticular ascospores with two closely appressed equatorial flanges and biverticillate penicilli
Penicillium fellutanum	Day 4	Cottony white, very small dot-like in shape - circular in shape	Presence of conidiophores with irregularly located metulae of variable length, terminating in definite vesicles; conidia are dark green in color

Table 61: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Sporotrichum schenckii	Day 4	Yellow in color, colony is in the middle of a circular white colony, yellow colony is circular and cottony	Septate hyaline hyphae, conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Trichoderma harzianum	Day 5	Furry/fuzzy forest green, colony is quiet large, irregular in shape but often circular	Conidia are globose and pale green in color; present at tips of septate hyphae in agglomerations or free (not on hyphae)

Table 61: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 4	Small dot-like rust colored colony	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton mentagrophytes	Day 4	Wooly white, colony is usually around the edges of the plate, colony goes from wooly white to transparent around its sides, fairly large	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 62: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Dam using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Mycoderma aceti	Day 9	Dark forest green in color, very minuscule (dot-like), spread over entire plate but particularly on the sides of the plate, circular	Elongated ovoid-shaped spores that form chains
Rhodotorula mucilaginosa	Day 13	Yellow, somewhat circular form, small, yeast like	Many spores in a rounded to ovoid shape
Trichophyton mentagrophytes	Day 2	Yeast like, light beige, spread over entire surface of plate, globulose-irregular forms	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 63: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Dam using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococus neoformans	Day 6	Dark pink in color, somewhat circular, small in size, yeast-like	Globose to ovoid budding yeast-like cells
Cryptococus neoformans	Day 2	Yeast like, light lime green with brown dots/spots, irregular forms over plate, entire surface of plate is covered	Globose to ovoid budding yeast-like cells
Mycoderma aceti	Day 13	Dark forest green in color, very minuscule (dot-like), spread over entire plate but particularly on the sides of the plate, circular	Elongated ovoid-shaped spores that form chains
Mycoderma aceti	Day 6	Cottony white, very small, circular, growing on the dark brown colonies, the white colony is almost moss like	Elongated ovoid-shaped spores that form chains

Table 63: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Rhodotorula mucilaginosa	Day 9	Orange, small, somewhat circular but its form is not completely circular	Many spores in a rounded to ovoid shape
Rhodotorula mucilaginosa	Day 13	White to transparent in color, edges of the colony appear fuzzy and jagged, colony is circular, medium size	Many spores in a rounded to ovoid shape
Rhodotorula mucilaginosa	Day 9	Pale yellow, very small, circular colony	Many spores in a rounded to ovoid shape
Trichophyton mentagrophytes	Day 2	Yeast like, light beige, spread over entire surface of plate, globulose-irregular forms	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 64: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Daisy Lake using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Blastomyces brasiliensis	Day 5	Waxy, very light beige, circular colony, medium size, edges appear slightly jagged	A variety of conidia can be be observed including pyriform microconidia; round narrow base budding yeast cells are present
Mortierella isabellina	Day 5	Circular shape, thin edge is transparent, the middle of the colony is fuzzy, medium in size	Coenocytic fungi, mitosporangia visible, only a few spores are visible
Mucor indicus	Day 9	Long, thin, grey hair like projections on colony	Sporangiophores are hyaline, erect and repeatedly sympodially branched and have long branches;

Table 64: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Mucor indicus**	*	*	sporangia is brown and columella are pyriform; sporangiospores are ellipsoidal
Penicillium chrysogenum	Day 7	Dark forest green, very small, circular shape	Presence of brush-shaped conidiophores which possess blue-green conidia at their tips
Rhizopus stolonifer	Day 5	Rust colored, irregular shape but somewhat circular, small colony	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the

Table 64: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Rhizopus stolonifer**	*	*	sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores
Sporotrichum schenckii	Day 13	Cottony white with traces of yellow, has many lines emanating from the center of the colony and outwards, very large colony, shape is that of a half circle	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval

Table 64: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Sporotrichum schenckii**	*	*	and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Sporotrichum schenckii	Day 16	Beige/white, texture appears rough like that of a rock, small	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae

Table 64: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Sporotrichum schenckii	Day 12	Cottony white, circular, small-medium colony	Septate hyaline hyphae, conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Trichophyton concentricum	Day 13	Cottony white but has traces of yellow, especially around the edges, very large colony on the edge of the plate, shape is that of a half circle	Presence of balloon shaped chlamydoconidia

Table 65: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Daisy Lake using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Acremonium strictum	Day 2	Cottony white colony, irregular shape but sometimes circular, edges are very ragged, colony is medium in size	Presence of septate hyphae which give rise to thin, tapered phialides; conidia are unicellular and hyaline and are present in mucoid heads or unconnected chains, hyphae is also hyaline
Mucor mucedo	Day 4	Grey- like hairy colony, irregular shape, growing on edge of plate, hair like projections are very thin and short	Spores are simple or branched and form apical, globular sporangia which are supported by a column-shaped columella

Table 65: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium lividum	Day 4	Dark forest green, very small and circular colony, cottony texture	Conidia are blue, ellipsoidal and are present on distinctly vesiculate penicilli
Rhizopus stolonifer	Day 4	Rust colored, very small and circular colony, cottony texture	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores
Rhizopus stolonifer	Day 9	Black-charcoal, very fuzzy, circular shape, medium size colony	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the

Table 65: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Rhizopus stolonifer**	*	*	sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores
Sporotrichum schenckii	Day 3	Yellow, irregular shape, on the edge of the plate, colony appears fuzzy, has very little grey dots on it	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae

Table 65: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton rubrum	Day 2	Very light beige/transparent, circular, slight deviation between middle of the colony and edge of the colony (variation in color), radiant lines emanating from the center of the colony outwards	Many clavate microconidia and cigar-shaped macroconidia are present

Table 66: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Daisy Lake using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Actinomyces bovis	Day 6	Waxy beige, very small, circular	Central mass of granular substance where chains of cocci/spores are visible; mycelia threads are found with extremities being club-shaped
Cryptococcus neoformans	Day 10	Yeast like (waxy beige/yellow), the colony also has little black/ brown dots on it, colony is circular, small	Globose to ovoid budding yeast-like cells
Penicillium chrysogenum	Day 5	Dark brown, rust like color, irregular shape, very small, spread to entire plate, fuzzy texture	Presence of brush-shaped conidiophores which possess blue-green conidia at their tips

Table 66: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium cinnamopurpureum	Day 6	Dark green, circular, small	Stipes are monoverticillate, vesiculate and bear long gradually tapering phialides
Penicillium fellutanum	Day 9	Cottony dark beige, circular, medium size	Presence of conidiophores with irregularly located metulae of variable length, terminating in definite vesicles; conidia are dark green in color
Sporotrichum schenckii	Day 9	Cottony white, circular, medium sized colony	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are

Table 66: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Sporotrichum schenckii**	*	*	unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Trichophyton mentagrophytes	Day 3	Transparent/white circular-like colony, edges are fuzzy and more transparent than the middle of the colony	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 66: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 9	Cottony lime, circular-irregular shape, medium size	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton violaceum	Day 4	Black/dark brown colored, irregular shape (spider-web-like), small, dispersed around the whole pate, texture is unclear	Hyphae are branched and segmented and there is presence of chlamydospores
Trichosporon mucoides	Day 8	Very vibrant red, irregular shape, some areas of it appear more pink, yeast-like	Hyphae is septate and presence of arthroconidia

Table 67: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Daisy Lake using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Acremonium strictum	Day 10	Grassy green, minuscule dots form the colony into irregular shapes, texture is rough looking	Presence of septate hyphae which give rise to thin, tapered phialides; conidia are unicellular and hyaline and are present in mucoid heads or unconnected chains, hyphae is also hyaline
Penicillium citrinum	Day 6	Cottony white, small, circular shape	Penicilli consist of 3-5 divergent and vesiculate metulae which bear long columns of conidia
Penicillium crustaceum	Day 9	Dark forest green and white, rough texture, shape is that of a half circle	Conidiophores are long and bear predominantly terverticillate penicilli
Saprolegnia parasitica	Day 14	Waxy light beige with hints of pink in the middle, yeast like, irregular form	Oogonium are present containing oospores and are aseptate

Table 67: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Saprolegnia parasitica	Day 6	Orange/pink, circular, small, texture is slightly fuzzy	Oogonium are present containing oospores and are aseptate
Trichophyton mentagrophytes	Day 7	Olive green, small, circular, cotton texture	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Tricophyton mentagrophytes	Day 5	Dark brown, irregular shape (spider web-like colony), growing on the edge of the plate, fuzzy-like texture	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton rubrum	Day 14	Waxy light beige with hints of yellow in the middle, yeast like, irregular form	Many clavate microconidia and cigar-shaped macroconidia are present

Table 67: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton schoenleini	Day 8	Bright orange, circular shape, texture is slightly fuzzy	Characteristic antler "nail head" hyphae also known as "favic chandeliers" are present
Trichophyton tonsurans	Day 3	Light white – transparent colony, white hair like projections on the colony, the projections are long and thin, very large colony, oval shape	Hyphae are irregular, branched and septate; microconidia are clavate form
Trichophyton tonsurans	Day 3	Waxy white/transparent, irregular shape, some center lines going outwards on the colony	Hyphae are irregular, branched and septate; microconidia are clavate form
Verticillium dahliae	Day 4	Cottony white, irregular shape, small in size, dispersed over entire plate	Globose to elongate microsclerotia, without dark hyphae or dark mycelium

Table 68: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Kingsway using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Actinomyces bovis	Day 15	Black, circular, rough and slimy texture	Central mass of granular substance where chains of cocci/spores are visible; mycelia threads are found with extremities being club-shaped
Actinomyces bovis	Day 15	Small, irregular shape, very light beige-transparent, texture appears slightly rough	Central mass of granular substance where chains of cocci/spores are visible; mycelia threads are found with extremities being club-shaped
Candida albicans	Day 3	Dark forest green, very small, circular	Spherical to subspherical budding yeast-like cells present

Table 68: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 14	Very milky, yeast like, beige-brown-light pink, growing on the edge of the plate, irregular shape	Globose to ovoid budding yeast-like cells
Mycoderma aceti	Day 8	Milky beige/pale yellow, small, circular, yeast like	Elongate ovoid shaped spores that form chains
Penicillium fellutanum	Day 5	Cottony white, small, circular	Presence of conidiophores with irregularly located metulae of variable length, terminating in definite vesicles; conidia are dark green in color
Rhizopus stolonifer	Day 2	Very cottony, white, spread over entire plate	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and

Table 68: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Rhizopus stolonifer**	*	*	many brown or colorless spores
Sporotrichum schenckii	Day 3	Small, circular, rust colored	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae

Table 68: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Sporotrichum schenckii	Day 4	Black-charcoal, circular, small	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Stachybotrys chartarum	Day 15	Dark olive green, colony growing on the edge of the plate, half oval, some lines are present on the colony, colony appears slightly fuzzy	Phiales bearing conidia arose from dark conidiophores; conidia are ellipsoidal and hyaline

Table 68: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 4	Yeast like, very small, circular to irregular form, beige	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton mentagrophytes	Day 6	Yellow, very small, dot-like	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichosporon mucoides	Day 5	White, texture is rough, circular, medium size	Hyphae is septate and presence of arthroconidia

Table 68: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Verticillium dahliae	Day 10	Beige/light brown, small, circular, texture is somewhat yeast like, outward lines on the culture	Globose to elongate microsclerotia, without dark hyphae or dark mycelium
Verticillium dahliae	Day 10	Brown/black/charcoal, small, circular, texture is somewhat yeast like, outwards lines on the culture	Globose to elongate microsclerotia, without dark hyphae or dark mycelium
Verticillium dahliae	Day 15	Very small, circular, colony is bright orange with an outer edge of light beige, colony appears slightly fuzzy	Globose to elongate microsclerotia, without dark hyphae or dark mycelium

Table 69: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Kingsway using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 9	Milky beige, small, circular shape	Globose to ovoid budding yeast-like cells
Cunninghamella bertholletiae	Day 8	Brown/yellow/a bit of pale green, colony is more or less round shaped, rough texture like that of a rock, colony growing upwards	Erect, branched sporangiophores which end in globose shaped vesicles from which several one-celled, globose echinulate develop on swollen denticles
Penicillium crustaceum	Day 5	Very small, dark forest green, cicular	Conidiophores are long and bear predominantly terverticillate penicilli

Table 69: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium fellutanum	Day 8	Dark forest green, colony growing on the edge of plate, irregular shape, somewhat fuzzy	Presence of conidiophores with irregularly located metulae of variable length, terminating in definite vesicles; conidia are dark green in color
Rhizopus stolonifer	Day 3	Grey/purple filaments (hair-like), filaments are very thin and long, colony is medium size	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores

Table 69: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Saprolegnia parasatica	Day 3	Very small, rust colored, cottony appearance	Oogonium are present containing oospores and are aseptate
Sporotrichum schenckii	Day 9	Beige/light brown, small, circular, texture is somewhat fuzzy, outward lines on the culture	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae

Table 69: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 6	White, entire surface of plate, flat like colony	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton mentagrophytes	Day 2	Cottony/fuzzy white, irregular shape, over entire plate	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton rubrum	Day 5	Yeast like, circular, transparent beige	Many clavate microconidia and cigar-shaped macroconidia are present

Table 69: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton tonsurans	Day 9	Light brown, small, circular, texture is somewhat fuzzy, outward lines on the culture	Hyphae are irregular, branched and septate; microconidia are clavate form

Table 70: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed area at Kingsway using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Mycoderma aceti	Day 2	Yeast like with fuzzy white texture growing on it (like that of a cloud), entire plate or selected areas (circular shape in selected areas)	Elongated ovoid spores that form chains
Rhizopus stolonifer	Day 5	Yeast like transparent culture with purple hair like projections growing on it, medium size, filaments are very thin and short	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores

Table 70: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 3	Cottony white, circular, small to medium size	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton mentagrophytes	Day 3	Rust colored, small, irregular to circular shape, yeast like	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton mentagrophytes	Day 5	Yeast like transparent culture with white hair like projections growing on it, medium size, filaments are very thin and short	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 70: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 2	Yeast like, lime green, irregular to circular in shape	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton tonsurans	Day 6	Dark olive green, circular, cottony texture, slight line of transparencey on the edges of the colony	Hyphae are irregular, branched and septate; microconidia are clavate form

Table 71: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed area at Kingsway using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 2	Yeast like, irregular form, slightly transparent in color	Globose to ovoid budding yeast-like cells
Penicillium lividum	Day 7	Fuzzy brown/charcoal/grey, irregular shape, small	Conidia are blue, ellipsoidal and are present on distinctly vesiculate penicilli
Saprolegnia parasitica	Day 3	Rust colored, circular, very small, texture is slightly cottony	Oogonium are present containing oospores and are aseptate
Sporotrichum schenckii	Day 3	Cottony white, circular, small	Septate hyaline hyphae; conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia: type 1 are

Table 71: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Sporotrichum schenckii**	*	*	unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attached to the sides of the hyphae
Streptomyces scabies	Day 9	Yeast like, light beige-transparent with pale green in the center, irregular shape	Hyphae are branched with swirls at the end
Trichophyton mentagrophytes	Day 3	Yeast like, lime colored, irregular or circular shapes	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 71: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton tonsurans	Day 5	Fuzzy white colony, its base being yeast like, the white projections are thin and short	Hyphae are irregular, branched and septate; microconidia are clavate form

Table 72: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed areas at Kelly Lake using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 2	Waxy and creamy beige, irregular shape, on side of plate	Globose to ovoid budding yeast-like cells
Penicillium montanense	Day 7	Cottony white, very small colony	Typical monoverticillate penicilli; long stipe, phiales very evident and round conidia present
Pythium debaryanum	Day 7	Yeast-like, milky, irregular or round shape, small to medium sized colony, light rust colored	Coenocytic hyphae present, the oogonia usually contains one single oospore, the antheridia contains an elongated and club-shaped antheridium
Rhizopus stolonifer	Day 3	Cottony white, irregular shape, mostly growing on edges of plates, fairly large colony	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate

Table 72: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
*Rhi zopus stolonifer**	*	*	with a sporangium which contains a columella and many brown spores
Trichoderma harzianum	Day 7	Fuzzy forest green, colony is medium to large in size, irregular in shape or often circular	Conidia are globose and pale green in color; they are present at tips of septate hyphae in agglomerations or free (not on hyphae)

Table 73: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed areas at Kelly Lake using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cryptococcus neoformans	Day 3	Yeast-like, milky beige, very small, mostly circular, spread over entire plate	Globose to ovoid budding yeast-like cells
Histoplasma capsulatum	Day 3	Thin colony, transparent and beige color around the edges, medium in size, circular	Filamentous mould, macroconidia are thick walled with tubercles and microconidia are smooth walled
Mucor indicus	Day 4	Fuzzy/cottony light beige with charcoal dots at extremities of colony projections	Sporangiophores are hyaline, erect and repeatedly sympodially branched and have long branches, sporangia is brown and columella are pyriform, sporangiospores are ellipsoidal

Table 73: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium chrysogenum	Day 4	Dark yellow/orange, very small, somewhat circular, texture seems somewhat fuzzy at certain areas and more rugged at others	Typical terverticillate penicilli; presence of brush shaped conidiophores which possess blue-green conidia at their tips
Penicillium crustaceum	Day 8	Cottony white and forest green in coloration, medium in size, circular	Typical terverticillate penicilli; conidiophores are long and bear predominantly terverticillate penicilli
Rhizopus stolonifer	Day 4	Cottony grey/charcoal, very fuzzy, slight black dots present on colony, colony forming on edges of plate, irregular shape	Broad hyphae with few or no septa, presence of many stolons among mycelia connecting the sporangiophores, the sporangiophores terminate with a sporangium which contains a columella and

Table 73: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
**Rhizopus stolonifer*	*	*	many brown or colorless spores
Saccharomyces cerevisiae	Day 4	Small, milky light yellow, circular	Blastoconidia are present; they are unicellular, globose, and ellipsoid to elongate in shape; budding is observable; no hyphae present
Sporotrichum schenckii	Day 3	Cottony white, fuzzy, small, somewhat circular	Septate hyaline hyphae, conidiophores and conidia are present, conidiophores are sympodial and not easily differentiated from vegetative hyphae, two types of conidia present: type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2 are

Table 73: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
* *Sporotrichum schenckii*	*	*	brown in coloration and oval which are attached to the sides of the hyphae

Table 74: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from unlimed areas at Kelly Lake using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Acremonium strictum	Day 3	Irregular shape, transparent, colony appears very thin, slight striations on it, medium in size	Presence of septate hyphae which give rise to thin, tapered phialides; conidia are unicellular and hyaline and are present in mucoid heads or unconnected chains, hyphae is also hyaline
Actinomyces bovis	Day 9	Dark pink color, circular in shape, fuzzy texture, small	Central mass of granular substance where chains of cocci/spores are visible; mycelia threads are found with extremities being club-shaped
Alternaria alternata	Day 2	Milky beige/transparent, very small, circular, yeast-like, quickly overpopulated entire plate, extremely fast growing	Conidiophores are pale brown and straight; secondary conidiophores are usually short and one-

Table 74: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
**Alternaria alternata*	*	*	celled; conidia are pale brown or slight yellow coloration and are obclavate; septa are vertical and transverse; chains of conidia are present in groups
Cryptococcus neoformans	Day 3	Milky beige, yeast-like, small to medium in size, circular form is often found, more irregular shapes are also found	Globose to ovoid budding yeast-like cells
Gliocladium viride	Day 15	Very small, forest green color, circular or irregular shapes observable, colonies forming in clusters	Presence of specifically erect and penicillate conidiophores accompanied by phialides which possess one-celled and hyaline to green conidia in groups or heads; conidia are ovate

Table 74: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium montanense	Day 10	Dark forest green, irregular shape or circular shape, cottony in texture	Typical monoverticillate penicilli; long stipe, phiales very evident and round conidia present
Trichophyton mentagrophytes	Day 7	Very small, cottony white, circular, striations quite evident forming radiant pattern	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 75: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples from limed areas at Kelly Lake using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Allescheria boydii	Day 13	Fuzzy texture, light grassy green color which turned to charcoal with time, circular shape, medium in size	The presence of spherical ascomata (cleistothecia) and fusiform or ellipsoidal ascospores present
Blastomyces dermatitidis	Day 15	Dark orange/yellow coloration, very small, circular, appears yeast-like	Present in filamentous form and produces spores directly on the wall of the hyphae; no fruiting bodies present
Cryptococcus neoformans	Day 10	Small, yeast-like, pink coloration	Globose to ovoid budding yeast-like cells
Penicillium citrinum	Day 8	Cottony forest green, very small, circular shape	Penicilli consist of 3-5 divergent and vesiculate metulae which bear long colums of conidia

Table 75: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium crustaceum	Day 10	Dark green coloration, very small, colony forms in clusters, circular shape	Conidiophores are long and bear predominantly terverticillate penicilli
Penicillium lividum	Day 10	Small, grassy green, circular shape, texture is fuzzy	Conidia are blue, ellipsoidal and are present on distinctly vesiculate penicili
Pythium debaryanum	Day 3	Transparent, circular, growing on the sides of the plate, possesses slight striations, colony appears smooth, medium in size, slightly different texture than that of yeast but similar	Coenocytic hyphae present, the oogonia usually contains one single oospore, the antheridia contains an elongated and club-shaped antheridium
Rhizopus stolonifer	Day 8	Fuzzy projections with charcoal tips, irregular shape, medium in size	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the

Table 75: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
**Rhizopus stolonifer*	*	*	sporangiophores terminate with a sporangium which contains a columella and many brown to colorless spores
Sporobolomyces roseus	Day 13	Circular colony, medium size, salmon to light rose color, yeast-like	The blastoconidia are very common; they are oval to elongate; kidney-shaped ballistoconidia are also observable
Streptomyces scabies	Day 2	Milky texture, light beige, yeast-like, very fast growing, colonies are growing connectively, irregular in shape	Hyphae are branched with swirls at the end

Table 76: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Hagar using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cunninghamella bertholletiae	Day 3	Wooly white, circular shape, quite large, looks somewhat like a white daisy	Erect, branched sporangiophores which end in globose shaped vesicles from which several one-celled, globose echinulate develop on swollen denticles
Eupenicillium shearii	Day 5	Dark green, small, in proximity to cottony white cultures, circular	Appearance of lenticular ascospores with two closely appressed equatorial flanges and biverticillate penicilli

Table 76: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Mucor indicus	Day 3	Wooly/hairy, grey/charcoal, irregular shape, long filaments present	Sporangiophores are hyaline, erect and repeatedly sympodially branched and have long branches; sporangia is brown and columella are pyriform; sporangiospores are ellipsoidal
Penicillium crustaceum	Day 5	Cottony white, very small, cicular	Conidiophores are long and bear predominantly terverticillate penicilli
Trichophyton mentagrophytes	Day 3	Waxy beige, circular, inward lines on it, small in size	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 76: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 5	Fuzzy beige/transparent, irregular but large shape, many small lines on it	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae

Table 77: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Hagar using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium crustaceum	Day 5	Dark green, very small, circular	Conidiophores are long and bear predominantly terverticillate penicilli
Rhizopus stolonifer	Day 3	Fuzzy/wooly white with grey projections, irregular shape, fairly large	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores

Table 77: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Rhizopus stolonifer	Day 3	Wooly white with little black dots, on entire surface of plate, irregular shape	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores
Streptomyces scabies	Day 8	Glossy beige, yeast like, circular, small	Hyphae are branched with swirls at the end
Trichophyton concentricum	Day 3	Very flat surface, transparent, little line like projections on the colony, jagged edges on the colony	Presence of balloon shaped chlamydoconidia

Table 77: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichophyton mentagrophytes	Day 5	Cottony white, circular, very small, everywhere on the plate	Round microconidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton tonsurans	Day 5	Waxy beige, circular, small size, many on plate	Hyphae are irregular, branched and septate; microconidia are clavate form

Table 78: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Onaping Falls using SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Allescheria boydii	Day 2	Fuzzy light grey, irregular shape, large colony, fast growing	The presence of spherical ascomata (cleistothecia) and fusiform or ellipsoidal ascospores present
Candida glabrata	Day 5	Small, yeast-like, beige in coloration, circular in shape	Many ovoid, budding yeast-like cells or blastoconidia; no pseudohyphae was observed
Candida lusitaniae	Day 4	Cottony white, medium in size, circular shape, colony is growing in an upwards fashion	Many subglobose, ovoid, or elliptical budding yeast-like cells or blastoconidia are present
Cryptococcus neoformans	Day 4	Circular, beige and brown, small to medium size, yeast-like colony	Globose to ovoid budding yeast-like cells
Mucor indicus	Day 2	Fuzzy charcoal/grey, large colony, fast growing	Sporangiophores are hyaline, erect and repeatedly

Table 78: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
**Mucor indicus*	*	*	sympodially branched and have long branches; sporangia is brown and columella are pyriform; sporangiospores are ellipsoidal
Penicillium citrinum	Day 3	Cottony white, medium in size, circular	Penicilli consist of 3-5 divergent and vesiculate metulae which bear long columns of conidia
Penicillium decumbens	Day 4	Forest green coloration, cottony, mostly circular colony	Conidiophores are fairly long; stipes are slim, monoverticillate; only a few phiales; conidia are ellipsoidal

Table 79: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Onaping Falls using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Blastomyces dematitidis	Day 4	Orange/pink coloration, mostly circular, medium in size, yeast-like colony	Present in filamentous form and produces spores directly on the wall of the hyphae; no fruiting bodies were observed
Coccidioides immitis	Day 3	Small in size, circular, very dark brown-black coloration, fuzzy texture	Arthroconidia are barrel-shaped; hyphae are present and are thinly septate and hyaline
Mucor indicus	Day 3	Colony is composed of white fuzz with grey/brown dots on the tips of hair-like projections, irregular shape, medium to large sized colonies	Sporangiophores are hyaline, erect and repeatedly sympodially branhed and have long branches; sporangia is brown and columella are pyriform; sporangiospores are ellipsoidal

Table 79: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Penicillium citreonigrum	Day 4	Cottony white, small to medium in size, mostly circular in shape	Slender stipes; conidia are small and smooth walled
Penicillium citrinum	Day 3	Fuzzy white, has somewhat hair-like projections that are very short, irregular shape	Penicilli consist of 3-5 divergent and vesiculate metulae which bear long columns of conidia
Penicillium montanense	Day 4	Forest green, cottony, small to medium, circular mostly	Typical monoverticillate penicilli; long stipe, phiales very evident and round conidia present
Pythium debaryanum	Day 5	Very small colony, red dots are present on what seems like a white fuzzy colony	Coenocytic hyphae present; the oogonia usually contains one single oospore; the antheridia contains an elongate and club-shaped antheridium

Table 79: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Rhodotorula mucilaginosa	Day 3	Bright yellow coloration, yeast-like, mostly circular or irregular in shape, small or very small in size	Many spores in a rounded to ovoid shape
Trichophyton faviforme	Day 3	White projections on colony, fuzzy, irregular shape	Hyphae are irregular and broadly shaped at specific regions and septate; conidia are more or less clavate form
Trichophyton tonsurans	Day 3	Middle is light forest green, contour is white and beige/pink, fuzzy texture, circular	Hyphae are irregular, branched and septate; microconidia are clavate form
Trichosporon mucoides	Day 2	Yeast-like, light beige, very small, circular to irregular shape	Hyphae is septate and presence of arthroconidia

Table 80: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Capreol SDA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Absidia corymbifera	Day 4	Light beige and brown coloration, beige coloration is in the middle of colony and brown coloration on the edges, striations are present on colony, medium in size	Aseptate hyphae is observed and is fairly wide; sporangiophores arise from locations on the stolon which lie between the rhizoids; sporangiophores are rather long and they apically give rise to a conical apophysis below the columella
Blastomyces brasiliensis	Day 3	Circular, medium size, striations on colony, beige, yellow, white and transparent coloration	Filamentous form is observed with many spores on the wall of the hyphae; no fruiting bodies were present
Blastomyces dermatitidis	Day 2	Cottony white or transparent, irregular shape or circular shape, medium to large size	Present in filamentous form and produces spores directly on the wall of the hyphae; no fruiting bodies present

Table 80: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Cladosporium sphaerospermum	Day 5	Forest/grassy green coloration, colonial colonies, circular shape, fuzzy colonies, very small	Conidiophores are quite erect, robust, and septate; conidia are spherical and are in branched chains
Mucor indicus	Day 2	Fuzzy grey/brown, medium in size, irregular shape	Sporangiophores are hyaline, errect and repeatedly sympodially branched and have long branches; sporangia is brown and columnela are pyriform; sporangiospores are elipsoidal
Penicillium montanense	Day 3	Forest green coloration, cottony texture, small size, circular shape	Typical monoverticillate penicilli; long stipe, phiales very evident and round conidia are present

Table 80: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Trichoderma harzianum	Day 4	Grassy green, fuzzy-cottony texture, irregular shape	Conidia are globose and pale green in color; present at tips of septate hyphae in agglomerations or free (not on hyphae)
Trichophyton mentagrophytes	Day 5	Yeast-like, beige coloration, irregular shape, small to medium in size	Round conidia in grape like clusters; presence of spiral hyphae and cigar shaped macroconidia which are attached to hyphae
Trichophyton tonsurans	Day 5	Fuzzy white, circular, very small, colonial colonies	Hyphae are irregular, branched and septate; microconidia are clavate form

Table 81: Time of fungi colony appearance and characterization of colony based on visual observation on agar plate and microscope from soil samples at Capreol using MEA medium

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Blastomyces dermatitidis	Day 5	Dark brown, very small, colony ressembles small specks, circular or oval shape, growing on entire plate	Present in filamentous form and produces spores directly on the wall of the hyphae; no fruiting bodies were oberved
Candida lusitaniae	Day 5	Light pink, circulr shape, very small, yeast like	Many subglobose, ovoid, or elliptical budding yeast-like cells or blastoconidia are present
Cryptococcus neoformans	Day 5	Small, grey coloration, circular shape, yeast-like	Globose to ovoid budding yeast-like cells
Cryptococcus neoformans	Day 9	Yeast-like, light milky beige color, irregular shape	Globose to ovoid budding yeast-like cells
Epicoccum purpurascens	Day 3	Forest green at the beginning of development which turned black with time, small, circular, cottony texture	Hyphae are septate; short conidiophores originating from the hyphae have been observed to form clusters;

Table 81: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
**Epicoccum purpurascens*	*	*	conidiophores give rise to conidia and the young conidia are round, non-septate and pale while the mature conidia are rough, verrucose and contain vertical to transverse septa as well as have a funnel shaped base
Penicillium citrinum	Day 3	Orange in middle of colony with forest green contours, circular, medium size, cottony texture	Penicilli consist of 3-5 divergent and vesiculate metulae which bear long columns of conidia
Pythium debaryanum	Day 3	Transparent with hints of white, large in size, irregular shape, lobular edges	Coenocytic hyphae present, the oogonia usually contains one single oospore, the antheridia contains an elongated and club-shaped antheridium

Table 81: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Rhizopus stolonifer	Day 3	Fuzzy white/grey projections, colony grows on edges of the plate, irregular shape, medium to large shape	Broad hyphae with few or no septa, presence of many stolons among the mycelia connecting the sporangiophores; the sporangiophores terminate with a sporangium which contains a columella and many brown or colorless spores
Sporotrichum schenckii	Day 3	Cottony white, small to medium in size, circular in shape	Septate hyaline hyphae, conidiophores and conidia are present; conidiophores are sympodial and not easily differentiated from vegetative hyphae; two types of conidia:type 1 are unicellular, hyaline, oval and at the tips of the conidiophores and type 2: brown in coloration and oval which are attched to the sides of the hyphae

Table 81: (continued)

Species	Time of appearance of incubation	Agar plate description of colony	Microscopic description of colony
Streptomyces scabies	Day 2	Yeast-like, milky beige, small, circular or irregular shape, light rust coloration also observable	Hyphae are branched with swirls at the end
Trichophyton faviforme	Day 4	Beige, white and transparent colonies, smooth edges, half circles in terms of formation, fuzzy texture, medium size	Hyphae are irregular and broadly shaped at specific regions and septate; conidia are more or less clavate form