Mercury levels in fungal fruiting bodies from interior and coastal forests of the Bay of Fundy region, New Brunswick, Canada

by

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B.Sc. Esfahan University / Iran, 1999

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

Master of Science in Forestry and Environmental Management

In the Graduate Academic Unit of Forestry

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Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

July, 2007

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ABSTRACT

Ectomycorrhizal (ECM) fungal fruiting bodies have been proposed as suitable accumulators of heavy metals such as mercury (Hg). This study was to examine ECM fungal fruiting bodies as suitable indicators of environmental Hg pollution, and to estimate an annual Hg sequestration by the fruiting bodies from the underlying soil substrate. Sampling of common fungal fruiting bodies, underlying soil, and surrounding mosses were conducted from selected forests on Grand Manan Island, the south-west shore region at Lepreau and New River Beach, and the interior at Fredericton, of the province of New Brunswick, Canada. Across this gradient, atmospheric fog-borne Hg input decreases from island-to-coast-to-mainland.

This study determined total mercury concentration (THg) variations of the fungal fruiting bodies by taxonomical groups (family, genus, and species), type of body part (cap and stalk), developmental stages, and elemental sulphur (S), nitrogen (N), and carbon (C) contents. THg concentrations of the fungal tissue were positively correlated with fungal S content, decreased from cap to stalk, and decreased with increasing developmental stages (emerging > mature > senescent).

THg concentrations of the fungal fruiting bodies increased with increasing THg concentrations of F-layer, and decreasing total S levels and soil depth. Also, the fruiting bodies surrounded by *Polytrichum jumiperium* and *Pleuzorium shreberi* (with the highest mean THg concentrations) had higher mean THg concentrations than bodies near *Sphagnum* sp. and *Ptilium crista-castrensis* (with the lowest mean THg concentrations) and non-moss sites.

Similar to previous studies, Hg in the soil bonded to S groups of organic soil and in the mineral soil its concentration decreased with decreasing soil C content. THg concentrations of the forest floor were associated with greater THg in moss tissue and affected by moss species type. THg concentrations in the moss increased with increasing S content of the moss tissue and THg concentrations of the soil, and with decreasing tissue height and S content of the soil.

This study revealed that high variations in the THg concentrations of the fungal fruiting bodies, moss, and soil were found to obscure the use of these three matrices as suitable Hg pollution in the study locations. Particularly, THg concentrations in these matrices were slightly influenced by location (island > coast > mainland). Annual extent by THg sequestrated by fungal fruiting bodies from the underlying soil layers were determined to be insignificant in terms of overall Hg quantities within the soil, and estimated inputs of annual atmospheric Hg deposition rates.

Particularly, variation of THg concentrations by developmental stages is a new work that will assist in developing a sampling design procedure that would minimize this variation. This new investigation also, is a preliminary step for further studies on ECM fungal species.

Key words: developmental stage, ectomycorrhizal, fungal fruiting bodies, forest, mercury, moss, soil layer, soil organic matter.

ACKNOWLEDGEMENTS

A study of this size could not be completed without the help of many people whose encouragement and support are appreciated.

I have been honored to work with Dr. Paul. A. Arp since May 2003 when I started working as a research assistant with the Mercury project. His comprehensive view on research and his commitment to provide only high-quality work have made a deep impression on me. I owe him a great deal of gratitude for showing me correct approach to research. I would like to thank him for his patience, effort, and financial support. He also encouraged me to attend and present papers at national and international conferences.

I also would like to acknowledge my advisory committee: Dr. Michael D. B. Burt, Dr. David W. Malloch, and Dr. Graham Forbes. At the early stage of this study, Dr. Malloch helped me in performing efficient mushroom sampling and mushroom identification. I also thank his wife, Louise, for her hospitality. Dr Burt put much effort into reading, discussing, and providing me with valuable comments on this study. Dr. Forbes' advice on conducting an improved sampling procedure helped me to achieve the goal of this study more effectively.

There are some very special individuals whose contributions have facilitated the progress of my study. Charlie D. Ritchie, a Ph.D. candidate, was one of the very first people who guided me through Mercury research. His early work on Mercury

research in the Bay of Fundy region was much appreciated. Mark Castonguay provided geographical and GIS data for the study locations, and helpful information regarding this issue. His time and effort were very much appreciated. Mercury laboratory analyses would not be done without the timely, accurate, and efficient performances of Janet Otorowski, Dragana Perkman, and Tri Ningsih. I also would like to thank Duc Banh who performed part of the laboratory analyses and undoubtedly his assistance in almost all computer technical issues was a great help. During this study, I had the pleasure to work with Aaron Townsend, Traver Connors and Diana Banh whose assistance have been highly beneficial. My office-mates provided a very friendly environment especially Laura Sweeney whose friendship and helpfulness are greatly appreciated.

This study has been supported and funded by the Collaborative Mercury Research Network (COMERN). As a student, my involvement in such a strong and sociable organization gave me the chance to access up-to-date Mercury research and discussion on Mercury issues.

I am deeply grateful to my husband, Ali, for his patience and support with field photography and sampling. Also, the birth of our son, Abtin brought us much joy and happiness in this period. I would like to express my gratitude to my parents for their dedication to encouraging me and helpful guidance throughout my life.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

This thesis deals with analyzing the total mercury (THg) concentrations in forest-based ectomycorhizal (ECM) fungi, as related to fungal species, soil substrate [litter (L), fermentation (F) and humification (H) horizons of the forest floor; top mineral soil horizon (A) below the forest floor], and geographic gradient of atmospheric Hg deposition (off-shore island, coast, interior) in south-western New Brunswick. This research was done to elucidate whether fungi play, at least in principle, a role in the terrestrial Hg bio-accumulation pathways, and could serve as terrestrial indicators of local and regional Hg pollution. In general, little is known about physical, chemical and metabolic specifics and extent of Hg accumulation and related transformations within their tissues and soil-based substrates, although it has been suggested that some of these fungi could be Hg hyper-accumulators, especially if substrate-based Hg concentrations are enhanced by way of atmospheric deposition, or through local Hg pollution (Kalac & Svoboda 2000). The objectives of this research are to:

1) Generate information about THg concentrations in the fruiting bodies (caps, stalks) of a number of fungal species common to the south-western region of New Brunswick, along a geographic gradient starting from Grand Manan Island, to the main coast at Lepreau and New River Beach, and the mainland interior at Fredericton (specifically the forest of the University of New Brunswick).

2) Determine the extent to which these species tend to bio-accumulate Hg, based on field-sampled THg concentrations in their soil substrates (L-, F-, H-, and A-layers), in the absence or presence of mosses.

3) Discern whether THg concentrations in the fungi and in their substrates (forest floor, mineral soil, moss carpets surrounding the fungal fruiting bodies) can be predicted based on multi-linear regression analysis, where this analysis encompasses a variety of predictor variables, such as geographic location (off-shore island, mainland coast, mainland interior), forest edge versus forest interior, S, N, and C elemental content of fungi, and soil substrates, soil layer type (L, F, H, A), absence or presence of mosses (by moss species). For example, high S content in fungal tissues could signal a high rate of fungal Hg retention (Minagawa et al. 1980); in contrast, high S contents in soils could signal the opposite situation. Also, increased levels of atmospheric Hg deposition along the geographic gradient from Fredericton to Grand Manan Island could result in a proportional increase in the Hg concentrations in the cap and stalk tissues of fungi that are commonly occurring along this gradient. In addition, higher catch of Hg-containing fog water along forest issues (Ritchie et al. 2006) could potentially lead to increased Hg concentrations in fungi that grow underneath the forest edge than within the forest interior a few tens of meters away.

As such, this thesis mostly focuses on examining trends in Hg concentrations in fungi, specifically in tissues of the fungal fruiting bodies, and their mycelial substrates as accessed through field sampling. Not included in this research are any particular biochemical assays and examinations that would shed light on specific

metabolic processes by which Hg accumulates in certain species and soils and not in others. However, the results of this thesis may provide a general framework for launching such studies by way of hypothesis generation. This thesis also does not include an analysis on the transference dynamics of Hg into soils from atmosphere, from soils into fungi, or from fungi into higher trophic levels. As such, the thesis only provides information on Hg concentrations in fungi and their substrates at the field sampling time. It will take another effort to learn how these concentrations might change over time when, e.g., atmospheric deposition rates for Hg would increase or decrease significantly, how and when Hg specifically enters into fungal tissues, and whether the Hg is being retained or further transformed or metabolized in these tissues.

THESIS OUTLINE

This thesis has the following structure:

Chapter 1: Introduction: aim and scope of research objectives, and thesis outline.

Chapter 2: Literature review: general background on Hg in terrestrial ecosystems, with emphasis on Hg accumulations in fungal fruiting bodies, moss and soil.

Chapter 3: Material and method: Study area.

Chapter 4: Material and method: Methods and sampling procedure.

Chapter 5: Results and discussion: THg concentrations in fungal fruiting bodies, by taxonomical order, and location and relating to elemental sulphur (S), nitrogen (N), and carbon (C) contents of the fungal fruiting bodies.

Chapter 6: Results and discussion: Elemental Hg and S, N, and C contents in mosses common to the sampling areas of this study, by species and location, and relating THg and S, N, and C contents in mosses to the underlying soil substrate and other variables.

Chapter 7: Results and discussion: THg concentrations in soil, by soil layer, location, soil thickness, and elemental S, N, and C contents of the soil.

Chapter 8: Results and discussion: Ecological considerations regarding Hg accumulations in fungal fruiting bodies, especially an evaluation of species-specific bioconcentration factors (BCFs), where this factor is quantified as the ratio of Hg concentration in the fungal fruiting body divided by the THg concentration of the F-layer of the forest floor.

Chapter 9: Summary and suggestions for further work.

Appendix I: Amino acid composition of some fungal fruiting bodies.

Appendix II: Fungal taxonomical groups, fungal attributes, and moss habit and characteristics.

Appendix III: Data and metadata.

CHAPTER 2: LITERATURE REVIEW

Mercury, derived from the Latin name Hydrargyrum (Hg, liquid silver), is a heavy metal that occurs in liquid form at room temperature, and evaporates easily. Industrially, mercury is derived from mining and processing cinnabar ore (vermilion; HgS): Hg vapor is generated from cinnabar by heating to a temperature of about 500 ºC (Cotton & Cotton 1999). Hg vapor is then captured through cooling.

Hg is distributed naturally and industrially throughout the world through a continuing sequence of emissions and deposition pathways (Gustin et al. 2003) Primary natural Hg emission sources are volcanic eruptions (Stracquadanio et al. 2003). Locally, Hg often occurs in sulfide ore deposits in the form of HgS, or cinnabar (Kim et al. 2004). Mercury is also a minor but environmentally significant associate of fossil fuel deposits (coal, tar and oil sands) (Sunderland & Chmura 2000b; Sunderland & Chmura 2000a). Wind erosion of soils (dust), sea spray, natural forest and brush fires, and photo-chemically induced evasion of Hg from surface waters and plant surfaces lead to the re-emission of surface-deposited Hg back into the atmosphere (Richardson et al. 2003). Industrial emission sources are fossil-fuel burning power plants (Pacyna et al. 2006), and municipal and medical waste incinerators (Keating et al. 1997).

Hg occurs in three oxidation states Hg^0, Hg^+ and Hg^{2+}in aqueous solutions and the terrestrial environment (Morita et al. 1998). [Univalent Hg ions (Hg_2^{2+}) are not stable because ligands such as NH_3 (ammonia), $-NH_2$ (amines), OH^- (hydroxide), CN^- (cyanide), SCN^- (thiocyanide), S^{2-} (sulfide) and $CH3COO^-$ (acetate) lead to the dis-

proportionation (simultaneous oxidation and reduction transformation) of Hg_2^{2+} to Hg^0 and Hg^{2+}, e.g.

$Hg_2^{2+} + 2\ OH^- \rightarrow Hg\ (l) + HgO\ (s) + H_2O$;

$Hg_2^{2+} + S^{2-} \rightarrow HgS\ (s)$; and

$Hg_2^{2+} + 2\ CN^- \rightarrow Hg\ (l)$ (Cotton & Cotton 1999)].

Hg in the atmosphere undergoes various reactions, from the gas phase to the aqueous phase, and back, by way of a cycle of photochemical oxidation and reduction reactions. Gas phase oxidation of Hg vapor that results in subsequent absorption of Hg^+ and Hg^{2+} by air particles is very slow (Brosset & Lord 1991). However, in the atmosphere, aqueous Hg reactions in cloud-water and on rain-drop surfaces increases the amount of oxidized Hg which can then be easily deposited to other parts of the ecosystem through wet and dry deposition. This oxidation is facilitated by the presence of air-borne ozone (Brosset & Lord 1991; Iverfeldt & Lindqvist 1986; Munthe 1992). Mercuric sulfide (HgS), mercuric oxide (HgO) and mercuric chloride ($HgCl_2$) are dominant examples of divalent Hg species (Garrels & Christ 1965).

Methyl mercury (MeHg) is the most common organic form of Hg. Methylation of Hg^{2+} occurs in soil and sediments based on the action of sulfate-reducing bacteria and possibly other pathways as well (Batten & Scow 2003). Other organic Hg forms refer to bimethyl (Me_2Hg), and ethyl (Et_2Hg) mercury (Pacyna et al. 2006). Both are highly neurotoxic because of their rapid absorption by and transference across cellular membranes throughout faunal tissues, including skin and other blood barriers to various organs (Marn-Pernat et al. 2005; Pazderov et al. 1974). In Canada, MeHg is of

concern in fish tissue, especially in northern Canada where regional and international Hg emissions have caused elevated Hg input in to the fresh water over decades (New England Governors Eastern Canadian Premiers Conference & Committee on the Environment 2001). In humans, the frequent fish consumption, specifically fish from exposed aquatic ecosystems to Hg species, accelerates the MeHg toxicity depending on the fish type, type of accumulated MeHg (hydrophobic MeHg chloride and MeHg – thiol complexes) in the fish tissue and the transformation of Hg species in the gastrointestinal tract (Harris et al. 2004).

A number of useful properties of Hg have led to its application in numerous household, medical, and industrial products. Metallic mercury conducts electricity, combines easily with other metals (except platinum and iron), is used to measure temperature and pressure, and works as a catalyst in chemical reactions. Also, antibacterial and antifungal properties of both methyl and ethyl Hg resulted in ubiquitous and continued uses as preservatives in medical preparations, grain products, and chemical products in general (Risher et al. 2002). However, for human health reasons, excessive industrial Hg uses have been limited by banning Hg as an additive in agricultural products, paints, pesticides and immunizing vaccines, and there is a progressive reduction of Hg in industrial and household emissions, batteries and dental use (Keating et al. 1997).

The thermo-dynamic stability ranges of Hg compounds in aqueous solution and soils with changing pH and aeration conditions can be gleaned from the Eh-pH diagram in Figure 2.1 (Silva et al. 1991, Atlas of Eh-pH diagrams 2005): in an

oxidizing condition (Eh>0.4), $HgCl_2$ and Hg $(OH)_2$ are the dominant inorganic species in solution. Under reducing conditions (Eh<0.4), Hg precipitates as HgS. $Hg(OH)_2$ is

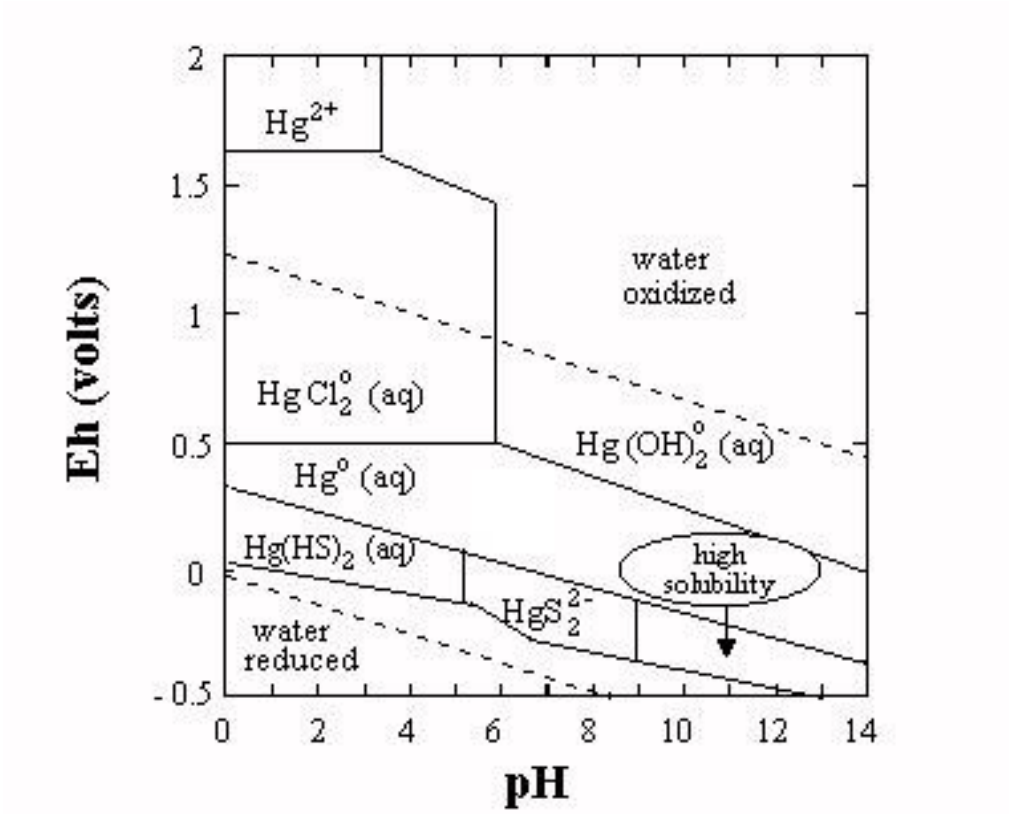


Figure 2.1. Eh (Redox Potential) versus pH for the main inorganic Hg species (Adapted from Silva et al. 1991).

stable at high pH, while $HgCl_2$ dominates in low pH solution and can form volatile $HgCl_2$ when Cl^- ions are present in high concentrations. Under moderate redox conditions, as can be found inside cellular tissues, Hg pre-existing in various oxidized states such as Hg^{2+}, at least in principle, can be converted to Hg^0. As such, Hg would then evaporate from these tissues. In mineral and organic soils, Hg is also complexed by soil organic matter (SOM) (Loux 1998), with sulfide groups being the dominant ligand (Ravichandran 2004), and by clay fractions, with absorption of Hg ions to the surface clay (Farrah & Pickering 1978).

HG EMISSIONS

In Canada, various natural and anthropogenic emissions of Hg have come under scrutiny (Richardson et al. 2003), supported in part by political action such as the Mercury Action Plan of the New England and Eastern premiers Round-table on the Environment (NEG/ECP). For example, Atlantic Canada receives approximately 12 % of anthropogenic Hg emission from US and Canadian sources (Pilgrim et al. 2000). Particularly, the Bay of Fundy in Atlantic Canada receives long-range Hg emissions into the coastal waters and ecosystems (Percy et al. 2004). In this case, frequent mid-summer and mid-autumn fog events are expected to contribute to local Hg deposition patterns into the forests (Pleijel & Munthe 1995). Forest fires are likely to contribute to emissions and atmospheric re-deposition mostly by precipitation, across the country, each summer (Sigler et al. 2003; Turetsky et al. 2006).

ATMOSPHERIC HG DEPOSITION

Spatial patterns of atmospheric Hg deposition are influenced positively by increased surface roughness as influenced by terrain type and vegetation cover, with forest canopies being particularly rough (Weathers et al. 2000). In comparison, forest ecosystems capture more of the atmospherically transported Hg than nearby fields and other open areas (St Louis et al. 2001). Deposition rates of Hg also increase substantially at high-elevation sites by way of cloud interception (Malcolm et al. 2003).

In general, coniferous vegetation and forest edges are more effective than deciduous vegetation in capturing air-borne droplets and aerosol particles (Kolka et

al. 1999; Weathers et al. 2000): deciduous leaves have large and flat surfaces and so capture, absorb and retain less air-borne materials than the diffuse needle-structure of coniferous canopies. Over time, Hg gradually accumulates in leaves from year to year, and with increasing surface roughness (Ericksen et al. 2003). Among deciduous trees, birch trees have a particularly high capacity for absorbing and storing Hg in the foliage (St Louis et al. 2001).

Sampling canopy throughfall (wash-off intercepted precipitation from plant leaves and needles, stems and branches to the forest soil surface) has become a means to determine a significant part of canopy-to-soil transference of water, nutrients, and pollutants in forest ecosystems: water dripping from the foliage during and after precipitation contains some of the deposited and surface-absorbed Hg, and the overall canopy-to-soil transference rate is given by the amount of throughfall per day times the Hg concentration of the sampled water at that time (Kolka et al. 1999; Rea et al. 2000; Rea et al. 2001). Determining the amount of Hg transference in litterfall captures the other dominant part of overall canopy-to-soil Hg transference (Ericksen et al. 2003; Gabriel & Williamson 2004; St Louis et al. 2001).

Annual wet Hg deposition in New Brunswick Atlantic regions is ≤ 10 ppt and its variation from year to year is not significantly different (Table 2.1; Figure 2.2 - 2.3). However some regions in North America have wet Hg deposition as high as 16-18 ppt (Figure 2.3). Coastal catchments in the Atlantic region receive higher wet Hg deposition than mainland sites (Table 2.1).

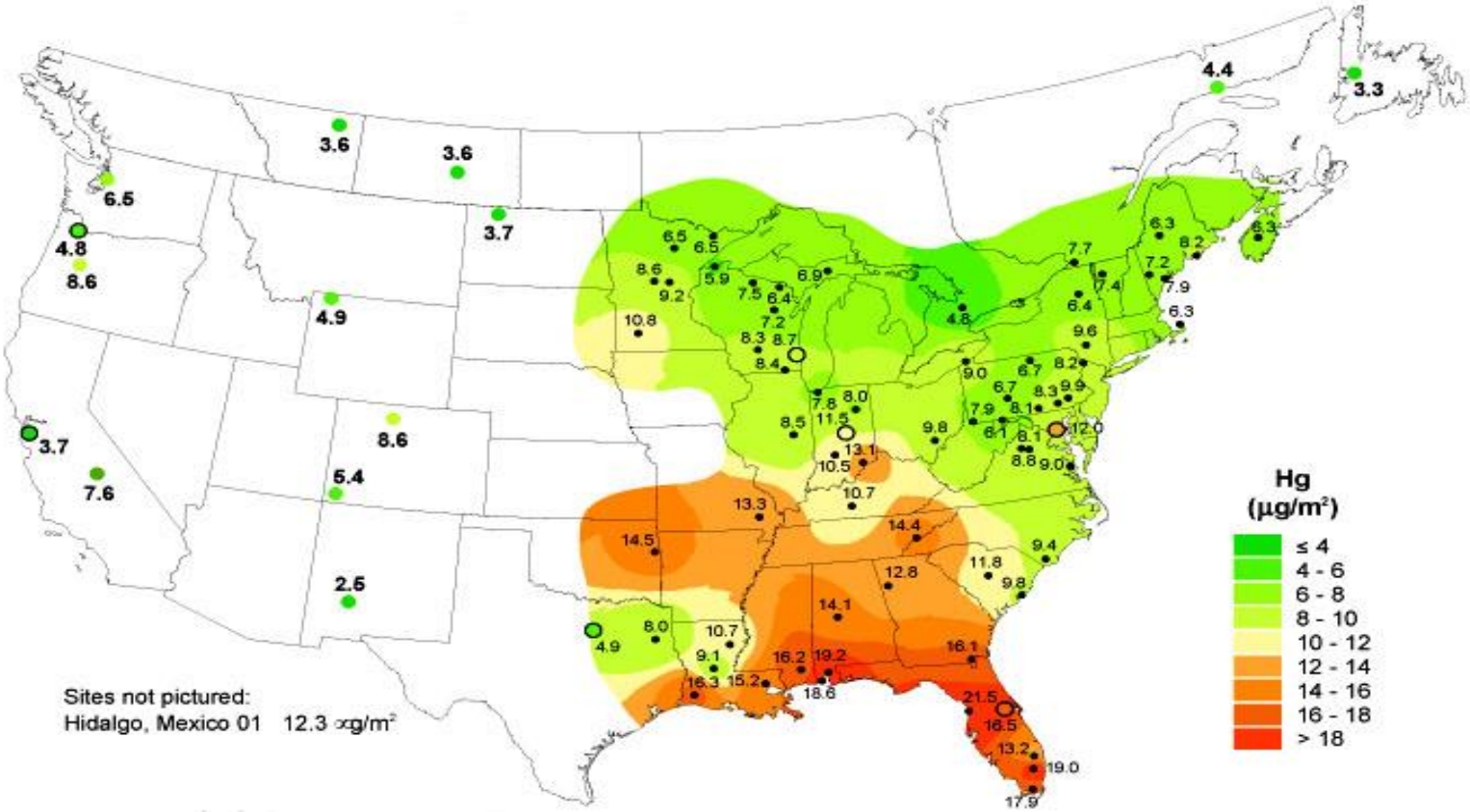


Figure 2.2. Map of mass of THg wet deposition per area (µg/m^2) in 2005 (Adapted from National Atmospheric Deposition Program/ Mercury deposition Network).

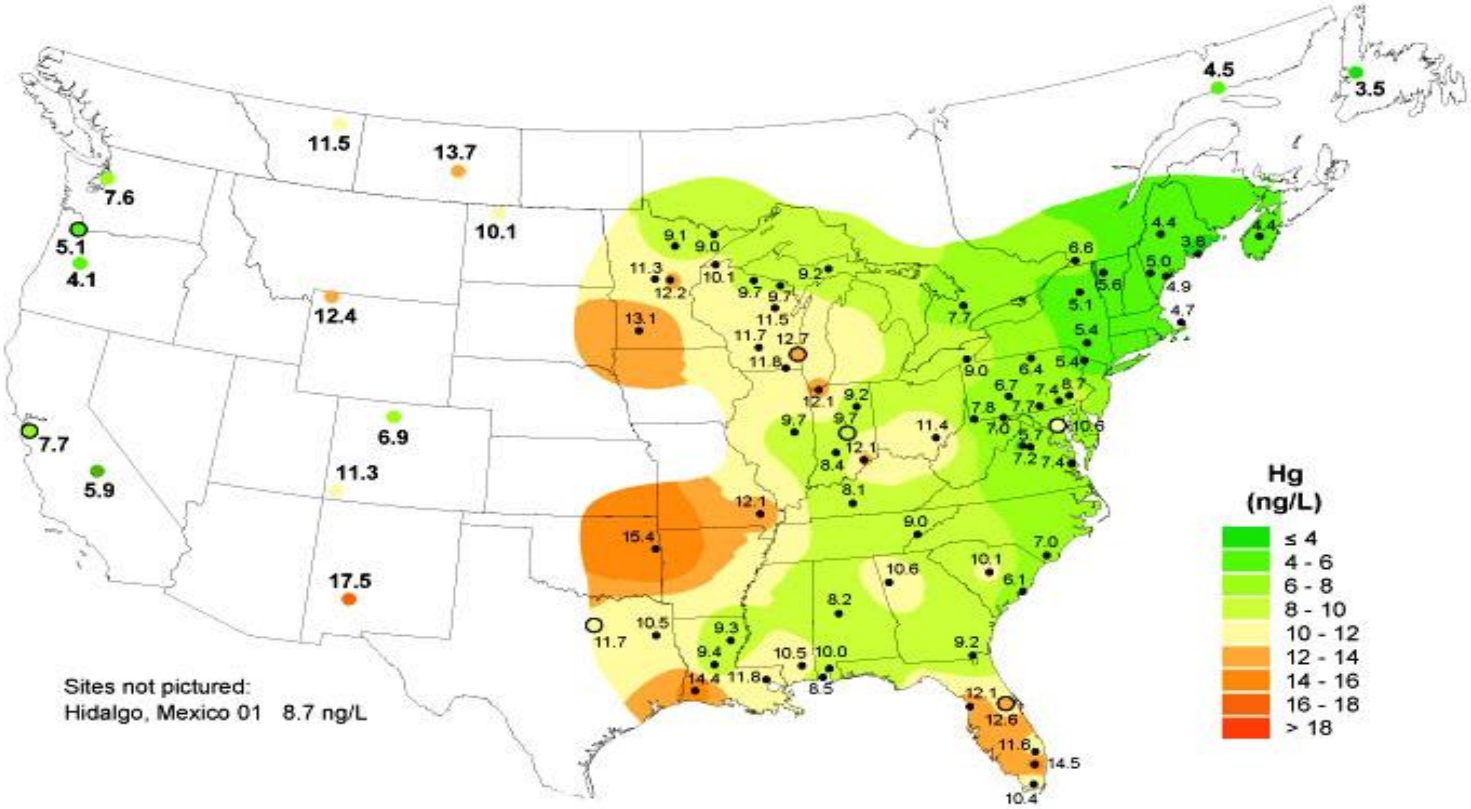


Figure 2.3. Map of THg concentrations (ng/L) from wet deposition in 2005 (Adapted from National Atmospheric Deposition Program/Mercury deposition Network).

Table 2.1. Reported annual THg wet deposition in St. Andrews, New Brunswick operating station and Maine, US stations close to central New Brunswick and the Bay of Fundy coastal catchments.

Year*	Coast	Inland	Coast	Inland
	ppt (ng/L)		$\mu g/m^2$	
2005	4.4	4.4	8.2	6.3
2004	10.2	7.2	10.2	6.5
2003	5.6	6.0	7.2	6.4
2002	5.1	4.8	8	4.1
2001	8	6.2	5.5	4
2000	7	5.1	7	5.1
1999	6.1	5.5	8	6.9
1998	6.1	6	9	6.8

* reported data are from collected weekly precipitation samples in a year-term period in operating stations.

HG IN SOIL AND WATERSHEDS

Despite low Hg content in crustal minerals, elevated Hg concentration in soils generally accrue from continued atmospheric deposition and from the accumulating litter of plants and animals (Davis et al. 1997). Soil may contain elemental mercury (Hg^0), inorganic mercury (Hg^{2+}) and organic mercury ($MeHg^+$). Elemental mercury (Hg^0) is relatively volatile and has low solubility in an aqueous solution, hence, upon its production, Hg^0 is readily released from soil to the atmosphere.

It has been determined that approximately 95 % of THg in soil is bivalent (Revis et al. 1990). The majority of this Hg is bound to soil humus by way of reduced S^{2-} (sulfide) groups (Xia et al. 1999). Under anaerobic soil and sediment conditions, sulfate-reducing bacteria are able to convert Hg^{2+} ions to HgS and $MeHg^+$ (Compeau & Bartha 1985). HgS has a very low solubility in aqueous solution (11×10^{-17} ppb,

dw at 25 ^{o}C), hence HgS easily accumulates in soils and sediments, and comprises approximately 88 % of THg in soil (Revis et al. 1990).

In the soil, Hg speciation depends on the redox potential of the Hg-surrounding matrix (soil, vegetation type, water, air), on pH, on the nature of available Hg-binding ligands such as chloride and sulphide, on organic matter, and on landscape and climate conditions of the area (Gabriel & Williamson 2004). In watersheds, MeHg is produced in wet, anaerobic areas and is transported from there into adjacent water bodies such as ponds, streams, lakes and rivers (Lee & Iverfeldt 1991). Once within the water, Hg and MeHg is easily bio-accumulated through binding on organic-water interfaces as provided by water-filtering tissues (an integrated procedure for removing and filtering adsorbed substances to tissue surface) of aquatic plants, vertebrates (e.g., fish gills), and invertebrates, ranging from single-cell organisms (phyto- and zoo-plankton) to multi-cellular tissues, or biofilms (mainly algae) growing on rocks, to plant surfaces (Chen & Folt 2005; Kainz et al. 2002). High retention rates, or low turn-over rates, of MeHg within organisms lead to a trophic build-up of MeHg in muscle and brain tissues, with highest MeHg levels registered in top predators such as fish-eating otter, fish, seals, and birds such as loons (Wong et al. 1997).

HG IN FUNGI

On entry into the soil, Hg is available for uptake by ground vegetation including fungi, which are known to be effective in sequestering heavy metals from soil substrates (Demirbas 2001; Falandysz & Danisiewicz 1995; Kalac et al. 1991;

Kalac et al. 1996). Hyphal filaments provide the conduit for translocating Hg from the substrate into the fungal fruiting bodies. for example, adding radioactive Hg ions into sterile wheat straw columns, , resulted in the accumulation of radioactive Hg in the fruiting body *of Pleurotus cornucopiae* (Brunnert & Zadrazil 1980).

There are some studies that established different Hg accumulation pattern in saprotrophic fungi (wood decomposers and lawn decomposers) versus mycorrhizal species. Saprotrophic species accumulate more Hg, and this is related to higher Hg availability, and higher enzyme activities, especially in lawn decomposers (Alonso et al. 2000; Laaksovirta & Lodenius 1979).

Ectomycorrhizal (ECM) fungal species are known to protect their host tree against excessive heavy metals such as Hg (Taylor 2000) by traslocating Hg from soil and storing Hg in their tissue. ECM species interact with the host tree by intercellular mycelium developments and connections. The mycelium also grow out from the mycelia mantle into the surrounding soil matrix, thereby increasing the volume of soil accessed for water, nutrient and heavy metal extraction (Figure 2.4). Not all the Hg content accumulated in the mycelium, however, may be transferred into the fruiting body. A long-lived mycelium can be expected to have much higher Hg accumulations than short-lived and fast growing mycelia.

Several studies have been done on measuring Hg concentrations in the fruiting bodies of wild ECM fungal species (Isildak et al. 2004; Kalac et al. 1996; Malinowska et al. 2004). These studies established that Hg concentrations in the

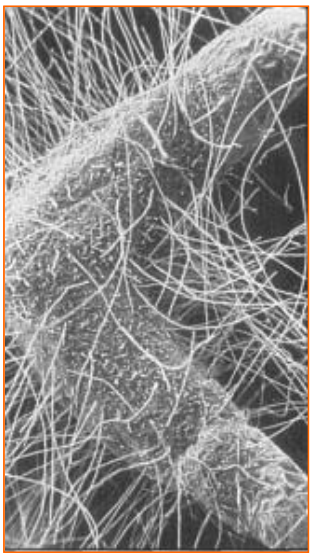


Figure 2.4. Scanning electron micrograph of pine branch ectomycorrhizal, mantle covering, and abundant external hyphae. (Photo courtesy of Hugues Massicote). (Adapted from Amaranthus & Pacific Northwest Research Station Portland 1998).

fruiting bodies of some species are higher in Hg-contaminated soils than in unaffected soils (Kalac et al. 1996; Svoboda et al. 2002).

Accumulation of Hg in fruiting bodies of ECM fungal species is species-dependent and is relative to the amount of available Hg in underlying soil substrate (Demirbas 2001; Falandysz et al. 2003). According to the Environmental Protection Agency (EPA) guideline, bioconcentration of Hg in living plants occurs through uptake, translocation, transformation, and retention of Hg from their surrounding environment. Hence, bioconcentration factor (BCF = Hg concentration in dried fungal tissue / Hg concentration in the soil substrate) is calculated for mushrooms and plants. BCF value of about 250 has been observed with *Boletus edulis* while the BCF values less than 200 have been reported for the other studied ECM fungal species; fungal caps had higher BCF values than the stalks (Falandysz et al. 2002; Falandysz et al. 2003; Kalac et al. 1996; Kalac & Svoboda 2000). Demirbas (2001) reported that

Amanita muscaria has a lower Hg concentration (up to 1,900 ppb, dw) than *Amanita vaginata* (up to 3,200 ppb, dw) when grown on the same substrate under the same conditions. However, Cu, Mn and Zn accumulations were much higher in *Amanita muscaria* than Hg. *Hydnum repandum* also has a higher tendency to uptake Pb and Cd than Hg from natural forest soils (Pb: 2,500 ppb, dw, Cd: 3,400 ppb, dw, Hg: 600 ppb, dw, dry weights) (Tuzen et al. 1998b).

Wild fungal species tend to accumulate more Hg than cultivated species (Tuzen et al. 1998b; Vetter & Berta 2005). For instance, *Agaricus bisporus*, the most popular cultivated fungal species, takes up about half as much Hg than wild *Agaricus* species (Falandysz et al. 1994). It is possible that the difference between Hg accumulation in wild and cultivated fungal fruiting bodies is due to a fast-growing mycelium with a high fructification rate within the cultivated species (Demirbas 2001).

Limited data are available on the proportion of accumulated MeHg in fungal tissues. Approximately 16% of THg is reported as MeHg in some species (Minagawa et al. 1980; Fischer et al. 1995). Some ECM species take up MeHg directly from humus or their symbiotic plants while some saprotrophic fungal species transform inorganic Hg ions to MeHg (Fischer et al. 1995).

S, N, and C are essential non-metallic elements for fungal growth. Average S, N, and C contents of 0.5, 5, and 49 % were reported in the fungi fruiting bodies, respectively (Bowen 1966). The C content in fungi differs because different species utilize variable amounts and forms of C from the substrate (Ainsworth et al. 1965; Harley & Smith 1983). C content is not necessarily correlated with THg

concentrations in fungal fruiting bodies and it is more likely that C just provides the structure of amino acids, carbohydrates, and fat. (Harley & Smith 1983; Weete 1974). Hence, amount of C in the fungal fruiting bodies is not a good predictor of THg concentrations. The N and S contents in fungal fruiting bodies are species-related, and are also related to fungal age and N and S availability in the substrates (Ainsworth et al. 1965). Specifically, ECM fungi utilize inorganic sources of N and simple amino acids including S-amino acids (methionine and cystine) derived from the fungal decay of organic matter (Harley & Smith 1983). Within the fungal fruiting bodies, S-amino acids (either singly or part of the protein complex) generally provide the location of S-Hg binding sites (Fischer et al. 1995; Kojo & Lodenius 1989). The S-amino acid and protein composition of the fungal fruiting bodies also depends on the species and the amount and type of N and S sources in the substrate (Fujihara et al. 1995) (Table I A: Appendix I). In total, fungal protein contains about 70 % of the total nitrogen (Fujihara et al. 1995). Other N-based components such as chitin, fat and carbohydrate comprise less than 10 % of the fungal fruiting bodies and these amounts increase as the fruiting body matures (Dikeman et al. 2005). The amount of total protein of some of the fungal fruiting bodies is summarized in Table 2.2.

Table 2.2. Total protein (TP) contents (%) of the fungal fruiting bodies.

Wild ECM fungi	TP*	Cultivated ECM fungi	TP*	Saprotrophic fungi	TP*
Amanita vaginata[1]	27.8	*Agaricus bisporus*[1]	38.6	*Marasimus oreades*[1]	40.2
Boletus edulis[1]	33.1	*Coprinus comatus*[1]	33.7	*Pleurotus ostreatus*[1]	24.7
Cantharellus cibarius[1]	16.2	*Lentinus edodes*[1]	29.4	*Tricholoma albobruneum*[1]	21.0
Cantharellus cibarius[3]	9.9	*Lentinus edodes*[2]	18.6		
Lactarius deliciosus[1]	25.9				
Russula xerampelina[1]	21.3				
Suillus granulatus[1]	24.7				
Suillus grevillei[2]	17.8				

* Percentage (%) of the total protein (% N x 6.25) on a dry weight basis.
[1] (Petrovska 2001) ; [2] (Fujihara et al. 1995) ; [3] (Danell & Eaker 1992).

Differences in THg concentrations in fungal species are likely due to differences in the molecular make-up of specific Hg binding sites (S-Hg). In general, Hg accumulates in the fungal fruiting bodies by bonding to sulphydryl (thiol or –SH) groups that are associated with the S amino-acid components of high-molecular-weight (HMW) proteins. Caps have almost two times higher amounts of such proteins than stalks (Kojo & Lodenius 1989). Hg-HMW protein contents were found in *Boletus edulis* and *Agaricus bisporus* (Wuilloud et al. 2004). In contrast, Hg- low-molecular-weight (LMW) protein bonds involving metallothionines were observed for *Lentinum edodes* (Wuilloud et al. 2004). *Cantharellus cibarius*, compared to some other wild growing fungi, was found to have a lower numbers of thiol-Hg binding sites which explained its lower Hg content (Danell & Eaker 1992). In contrast, cultivated *Agaricus bisporus* having high methionine content, was found to have a low Hg content (Tuzen et al. 1998a). In general, methionine and cystine represent a very low fraction of the total amino acid composition of the fungal fruiting bodies (about 2 %) (Table II A: Appendix I) , and these amino acids are thought to be

involved in the transference of Hg and other heavy metals such as Cd from the mycelium to the fungal fruiting bodies (Surinrut et al. 1987).

With mycorrhizal fungi and with Hg hyper-accumulators in general THg retention is generally associated with non-protein-bond thiol groups (Hg–S-H) (Kojo & Lodenius 1989). This is most notably so for *Boletus edulis* (with high THg concentrations up to 10,000 ppb, dw) and *Amanita muscaria* (with THg concentrations up to 600 ppb, dw), with a 55% non-protein thiol content (Kojo & Lodenius 1989). To illustrate, *Hydnum repandum*, compared to *Amanita muscaria*, has higher total methionine and cystine concentrations (approx. 3 times higher) but lower non-protein SH and Hg concentrations. *Russula* sp. is also found to have low non-protein SH and THg concentrations. In *Cantharellus cibarius* and *Cantharellus tubaeformis* non-protein SH groups were not detected. In contrast, *Suillus* sp. has a high amount of non-protein SH groups (approx. 90 %) but still low THg concentration in its fruiting bodies. From the above studies, species-related Hg accumulation in the fungal tissue is not only related to non-protein SH groups and this suggests that factors other than non-protein SH groups, such as overall Hg availability, mycelial substrate conditions and cellular Hg^{2+} reduction processes could also be important in fungal Hg retention. For example, the presence of the Hg^{2+} and MeHg reductase enzyme (Silver & Phung 2005; Wiatrowski et al. 2006) could be crucial in keeping overall Hg concentrations in cellular tissues low. Whether this enzyme occurs in fungal tissues is not known, but Hg^{2+} reductase activities have been reported in bacterial cultures, and play an important role in the Hg-detoxification process (Nascimento and Chartone-souza 2003). Heavy-metal tolerance of

underground ECM fungal structures has been attributed to metal-binding proteins and peptides (Bellion et al. 2006). Specifically, Cu and Cd tolerance of wild-growing hypae of *Russula emetica*, *Russula delica* and *Boletus edulis* has been related to the binding of these metals by way of metallothionine complexes (Morselt et al. 1986). Heavy-metal tolerance and binding with ECM species likely contributes to the overall health of pine, fir and oak forests (Molina et al. 1993).

HG IN MOSS

Mosses have been used extensively as heavy metal pollution indicators in different terrestrial environments (Gramatica et al. 2006). The mechanisms by which mosses take up and accumulate metals in their tissue tends to be related to the extent of heavy-metal deposition from the atmosphere, either directly under open condition, or indirectly under closed forest canopy conditions via throughfall (Gjengedal & Steinnes 1990; Ruhling & Tyler 2004). Mosses soak up moisture, nutrients and pollutants from the atmosphere during rain and fog events. Mosses tend to produce a thick carpet, and trap canopy debris (needles, leaves, and small twigs) and filter throughfall, rain, and snowmelt. Brown & Brumelis (1996) found that metal concentrations in *Hylocomium splendens*, a feather moss, increased with age of the green segments of moss tissue, and decreased with distance from the pollution source. As mosses grow, longer exposure periods result in higher metal concentrations that involve metal translocation from inter-cellular to intra-cellular locations (Brown & Brumelis 1996). The absence of cuticles and the presence of large surface areas facilitate the general absorption and translocation of water, nutrients and pollutants to

intra-cellular spaces. Roots are generally absent, but water below the moss carpet is still available for uptake by upward capillary flow, which is further encouraged by surface-based evapo-transpiration (Foster 1984). Laboratory experiments with Zn and *Hylocomium splendens*, and with Hg and *Pleurozium schreberi* and *Sphagnum* sp. showed that these mosses strongly retain metals, and these metals are not easily leached, especially not from young moss tissues (Brown & Brumelis 1996; Lodenius & Tulisalo 1995).

CHAPTER 3: STUDY AREA

The data collected for this study are from three study areas: Fredericton (UNB Research forests), the mainland coast (near Point Lepreau to New River Beach), and an off-shore island (Grand Manan Island) (Figures 3.1 - 3.2). These locations were chosen because atmospheric Hg input through fog droplets has been determined to be the highest on Grand Manan Island, lower on the New Brunswick coastline, near Lepreau, and lowest in Fredericton (Ritchie et al. 2006). Captured Hg-fog droplets and precipitation potentially could lead to the highest Hg deposition to soil, ectomycorrhizal (ECM) fruiting bodies, and mosses on the island. However, Hg absorption to the same matrices could be lower along the coast and the lowest on the mainland. Preliminary sampling at these locations showed that THg concentrations varied by matrix type: water << mosses < soil < lichens < soil-based fungal fruiting bodies (Nasr et al. 2005) (Table 3.1). This study showed that higher retention of air-borne Hg by forests tends to be associated with higher THg concentrations in mosses, soil, lichens, and soil-based fungi in Grand Manan Island than coast and mainland (island > coast > mainland) (Nasr et al. 2005).

Fungi and associated substrates (soils and mosses) were sampled in these areas at specific locations, as shown in Figures 3.1 and 3.2, and summarized in Table 3.2.

Table 3.1. THg concentrations in water (ppt) and lichens, mosses, fungal fruiting bodies, and soil samples (ppb, dw) from Grand Manan Island, Lepreau and Fredericton (Nasr et al. 2005).

Sample type	Grand Manan		Lepreau		Fredericton	
	Range	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Water (fog & rain) [a]	42-453	235	4-33	13	4	4
Lichens (*Usnea* sp.) [b]	5-560	260	190-290	210	70-180	140
Mosses [b]	6-150	100	40-150	80	50-80	62
Fungal fruiting bodies [b]	24-6000	1050	3-9000	808	8-2400	561
Soil [b]	40-800	260	6-470	203	12-390	150

[a] THg concentration (ppt), [b] THg concentration (ppb, dw)

Table 3.2. Geographical locations, GPS coordinates, and area (ha) of the study locations from Grand Manna Island, New Brunswick coastal forests and Fredericton.

Point ID	Forest Location	GPS location		Sampling area
		West	**North**	**ha**
	Fredericton (mainland):			
1	UNB-Woodlot 1A	66.6419	45.9199	1.56
2	UNB-Woodlot 1B	66.6416	45.9193	
3	UNB-Woodlot 2	66.6369	45.9168	3.31
4	UNB-Woodlot 3	66.6406	45.9161	3.84
5	UNB-Woodlot 4	66.6433	45.9097	2.22
6	UNB-Woodlot 5	66.67454	45.9133	5.23
	Bay of Fundy coast:			
7	Cranberry Head 1	66.338	45.1349	97.59
8	Cranberry Head 2	66.3355	45.1273	
9	New River Beach	66.5235	45.1226	18.03
10	Little Lepreau	66.4876	45.1386	11.87
11	Chance Harbour	66.3659	45.1395	10.36
	Grand Manan Island:			
12	Seal Cove	66.8507	44.6433	24.11
13	Deep Cove	66.8762	44.6161	9.76
14	Southern Head	66.8833	44.6061	23.39

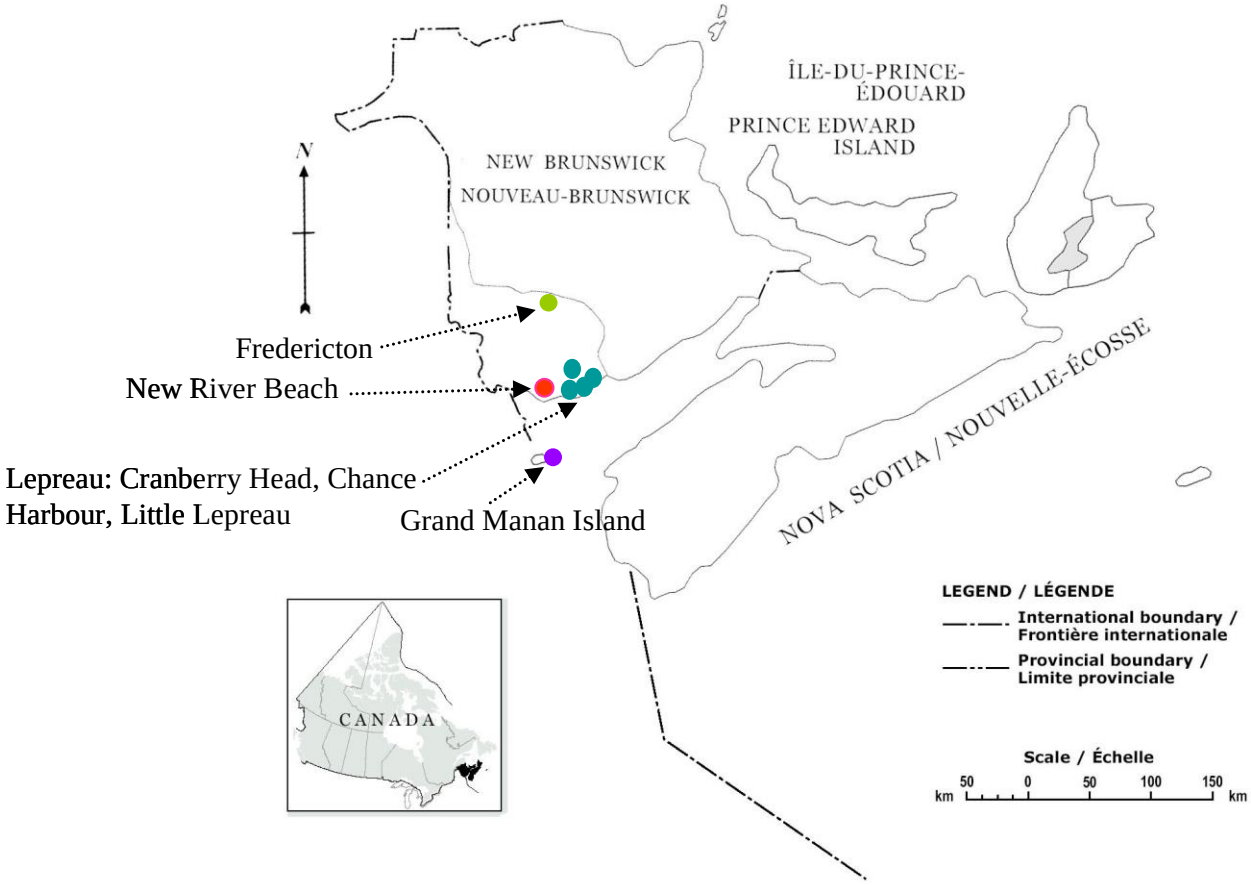


Figure 3.1. Location of the study areas within the province of New Brunswick, Canada (Adapted from Natural Resources Canada, 2002).

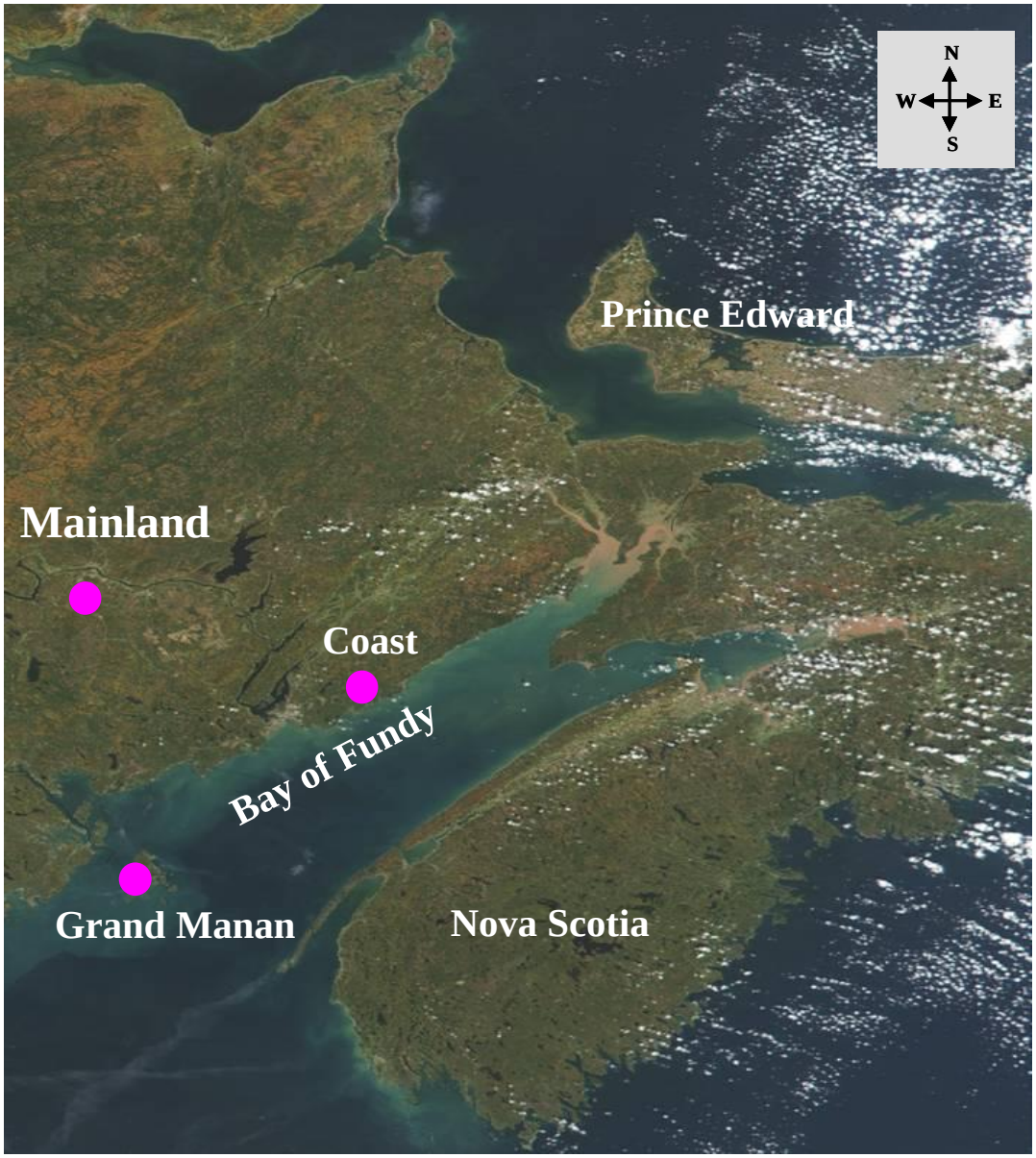


Figure 3.2. Satellite map of the study locations (Adapted from NASA, 2001). Island: Grand Manan Island. Coast: Lepreau and New River Beach. Mainland: Fredericton, UNB Research forests.

GRAND MANAN ISLAND

Grand Manan Island is located in New Brunswick south-western part of the Bay of Fundy. The upland forests along the southern and western coast of Grand Manan Island are exposed to high fog drifts, especially during mid-summer, on and near cliffs rising about 100 m above sea level. The presence of softwood forests at these locations likely contributes to the overall capture and retention of the atmospherically carried Hg. The tree species are balsam fir (*Abies balsamea*), spruce (*Picea rubens* and *Picea mariana*), birch (*Betula* sp.), beech (*Fagus* sp.), and a small component of maple (*Acer* sp.). Selected locations were Deep Cove, Seal Cove, and Southern Head covering areas of about 10, 24, and 23 ha, respectively. Soils, mosses and mushrooms were collected from these locations (Figures 3.3 - 3.4.; Table 3.2).

NEW BRUNSWICK COASTAL FORESTS

Along the New Brunswick coastline, 5 forested locations near Lepreau and New River Beach were selected for soil and mushroom sampling (Figures 3.5 – 3.6; Table 3.2). The locations are Cranberry Head 1 and 2, Chance Harbour, Little Lepreau, and New River Beach covering areas of about 98, 10, 11, and 18 ha. The forests in Cranberry Head and New River Beach rise about 20- 50 m above sea level. Selected locations from Chance Harbour and Little Lepreau are interior forest locations in coastal New Brunswick. The tree species are almost entirely balsam fir (*Abies balsamea*), spruce (*Picea* sp.), birch (*Betula* sp.), beech (*Fagus* sp.), and a small component of larch (*Larix laricina),* maple (*Acer* sp.), and pine (*Pinus* sp.). Specifically, the dominant tree species in Chance Harbour were balsam fir and birch.

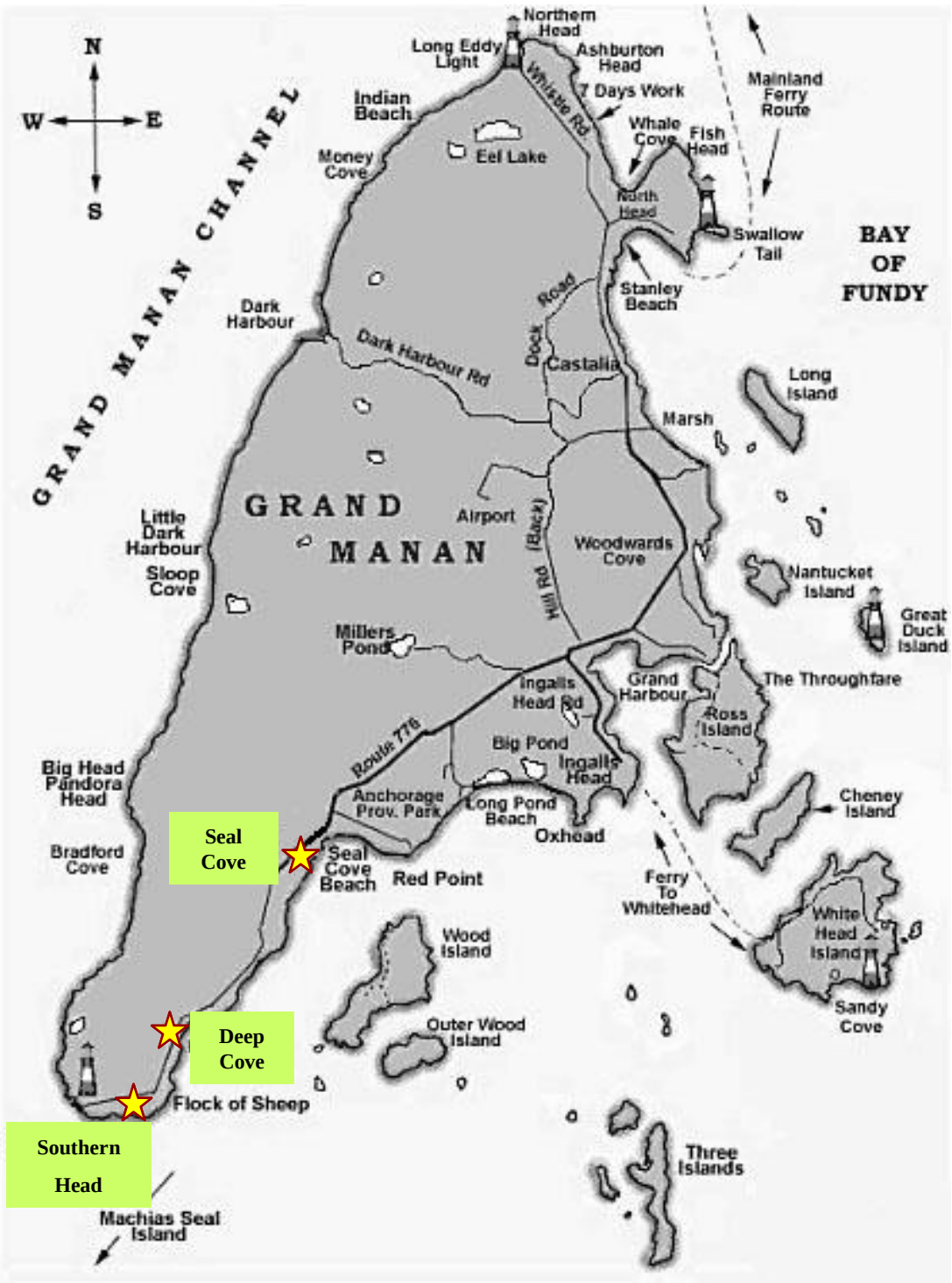


Figure 3.3. Study locations from Grand Manan Island (Adapted from The Whale Camp, 1998-2007).

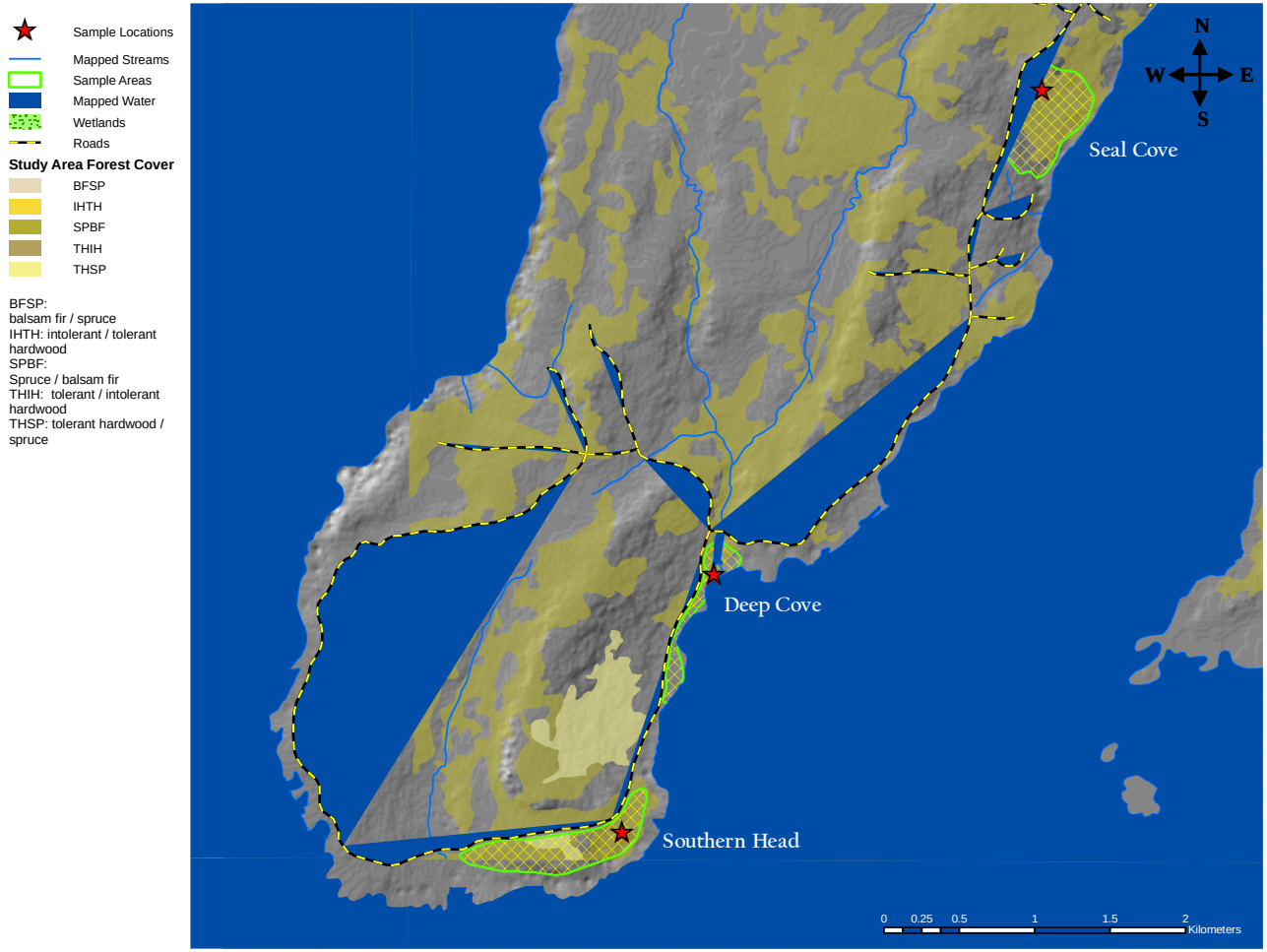


Figure 3.4. Area and forest stand condition of selected forests from Grand Manan Island. The Spruce-balsam fir forest stands were located on the southern east side of the island on the 100 – 200 m high cliffs.

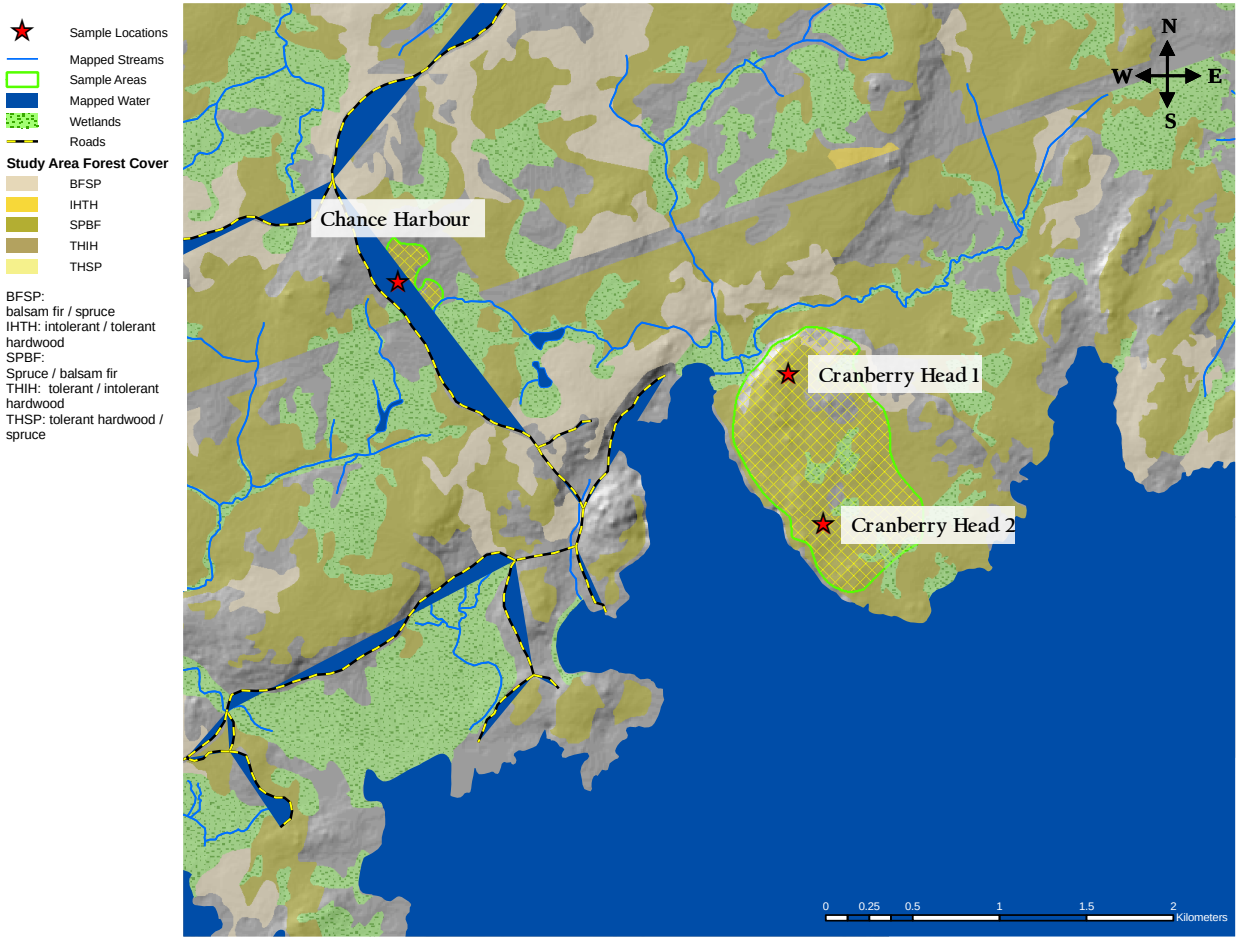


Figure 3.5. Area and locations of New Brunswick coastal study areas (a). (1) Cranberry Head 1 and 2: The Spruce-balsam fir forest stands located on a cliff, approximately 20 m above the ocean. (2) Chance Harbour: Interior spruce-balsam fir forest stand.

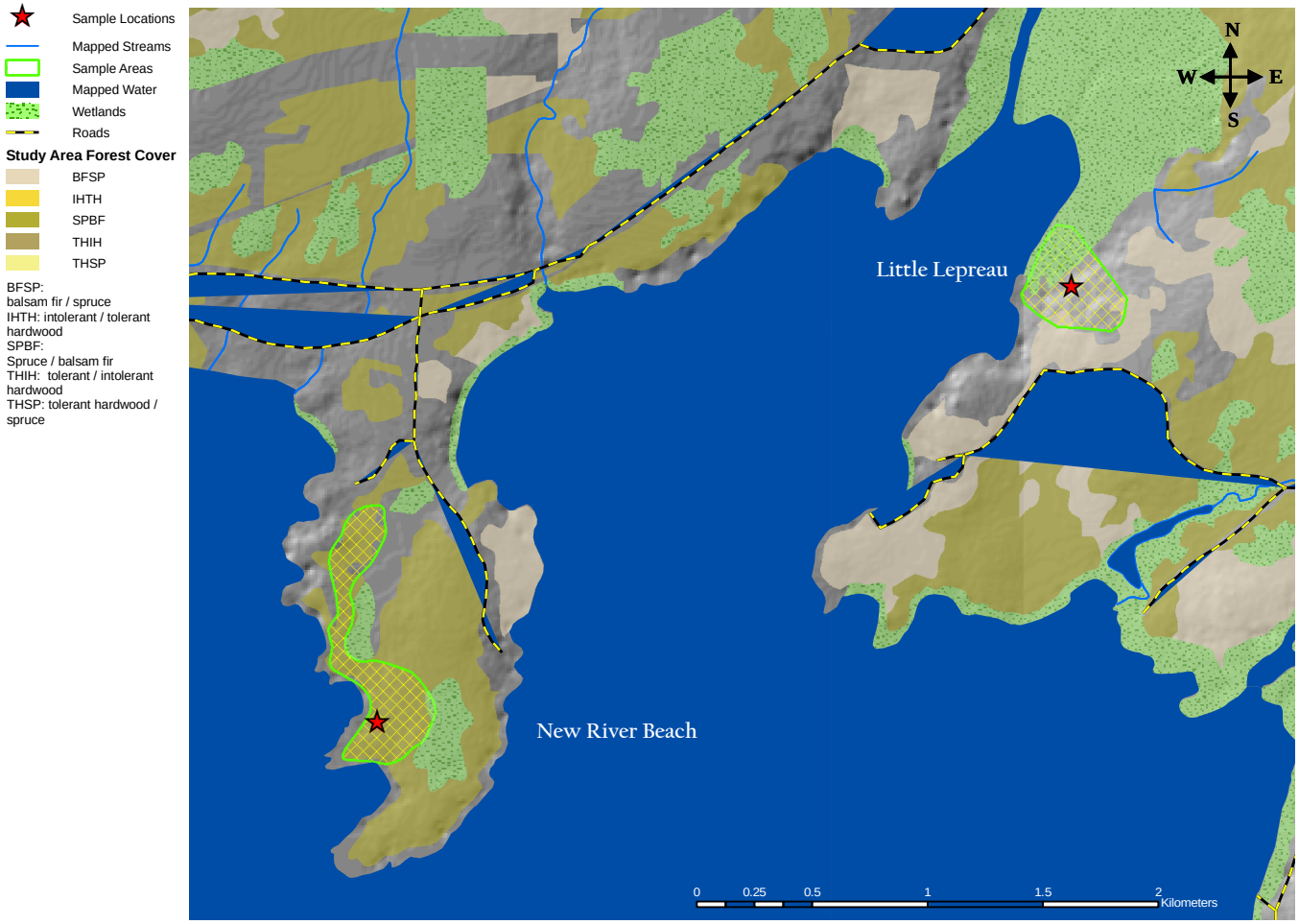


Figure 3.6. Area and locations of New Brunswick coastal study areas (b). (3) New River Beach: the Spruce-balsam fir forest stands located on a cliff, approximately 20 – 50 m above the ocean directing to flat uncovered Sphagnum sp. peat; (4) Little Lepreau: Interior balsam fir forest.

UNB FOREST

The 1200 ha UNB forest marks part of the southern border of Fredericton. Six locations were selected within this forest (Figure 3.7; Table 3.2). In the spruce-balsam fir stands (UNB 1A, UNB 1B, UNB 3, UNB 4, and UNB 5), the majority of tree species were balsam fir (*Abies balsamea*), spruce (*Picea* sp.), birch (*Betula* sp.), beech (*Fagus* sp.), maple (*Acer* sp.) and a small component of larch (*Larix laricina),* and pine (*Pinus* sp.). In the tolerant hardwood stands (UNB 2), birch, beech, and maple were dominant, with a smaller component of larch at forest edges. By selecting a hardwood stand, host-specific *Fuscoboletinus viscidus* were mostly found close to larch at the edge of the hardwood stand. The area of sampled forests ranged from 2-5 ha. The reasons for sampling a smaller area of mainland forests, compared to the coastal and the island sites, were limited similar forest type and limited availability of fungal fruiting bodies during the sampling period.

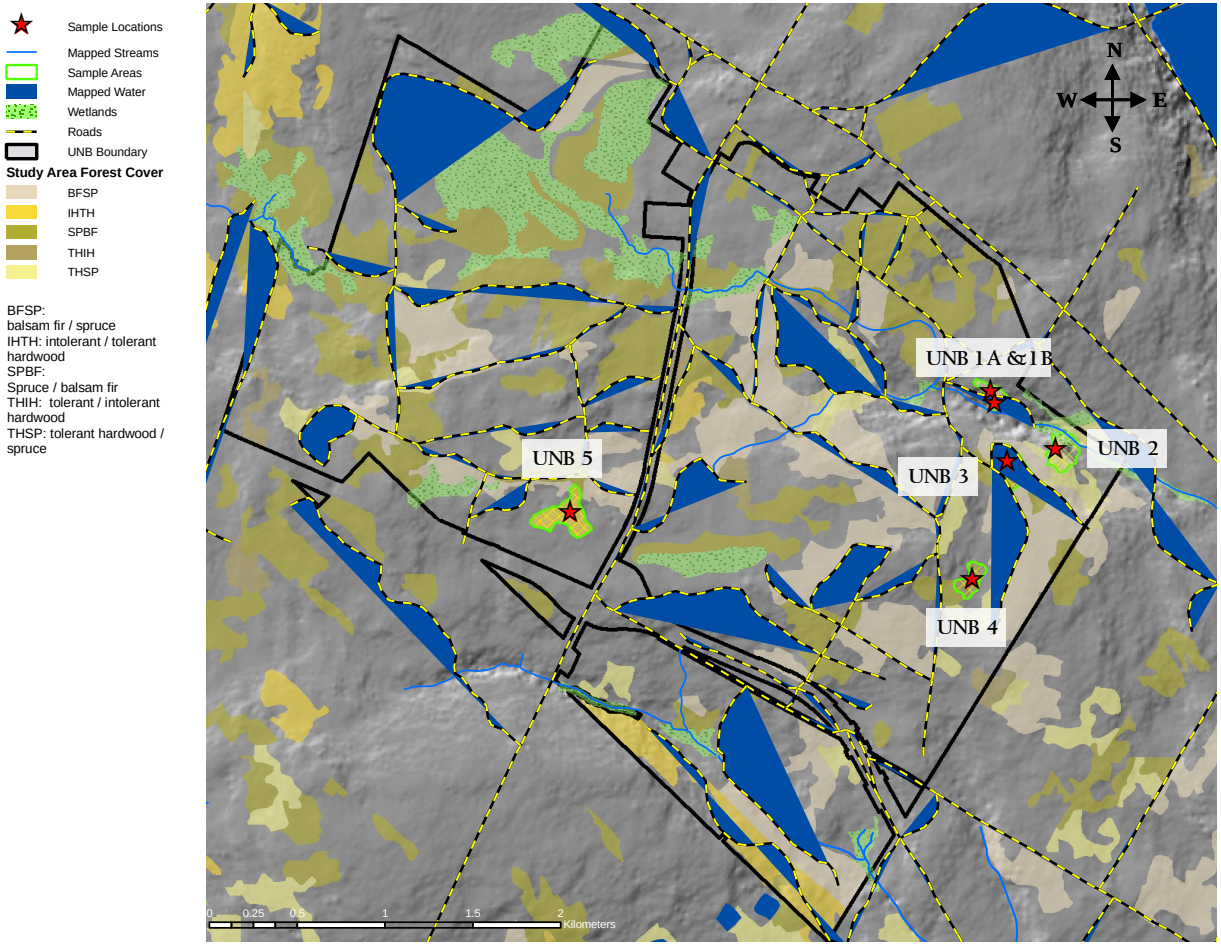


Figure 3.7. Area and locations of the mainland forests near Fredericton. The Spruce-balsam fir forests: UNB 1A, UNB 1B, UNB 3, UNB 4, and UNB 5, Tolerant hardwood-spruce forests: UNB 2.

CHAPTER 4: METHODODOLOGY

FIELD SAMPLING PROCEDURE

Sampling of fungal fruiting bodies, mosses and soil at each study location occurred in summer and fall of 2004 and 2005, from late July to late October. GPS location and photographs of the collected fruiting bodies were obtained at the time of sampling, and mushroom size, colour, odour, texture, and other properties were noted and recorded. The Ainsworth Dictionary of Fungi (Ainsworth et al. 2001) and the Field Guide to North American Mushrooms (Lincoff & National Audubon Society 1981) were used for species identification. The taxonomical order of the collected fungal fruiting bodies is summarized in Figure II A (Appendix II). Soil horizon and moss species identification was done with a field guide, mostly at the sampling place (Arp 2002).

The sampling was conducted over a range of areas up to 100 ha from 200 x 200 m plots from the forest edge to the forest interior from the selected sites within each geographical location (island, coast, and mainland) (Chapter 3). The sampling design is portrayed in Figure 4.1. Fruiting bodies of the same species were collected at least 5-10 m apart to avoid pseudo-replication. In some cases, two or more samples were collected from the same spot to allow for proper species identification. Collected fruiting bodies were separated into caps and stalks, and categorized by size (diameter) and developmental stage. The diameter of caps varied depending on the fungal species and developmental stage; these diameters were measured as follows: I

– 1 to 4 cm, II - 1 to 19 cm, and III – 2 to 21 cm. The developmental stages of the fruiting bodies (Figures 4.2- 4.3) were categorized as follows:

- Stage I: just emerging, not fully-developed, with spore-bearing part still covered by partial veil ;
- Stage II: fresh fully-developed (mature); and
- Stage III: fully-developed to old fully-developed (senescent).

Sampling involved retrieving the following materials: caps and stalks of commonly found ECMs, litter (L: 0.5-3 cm), fermented (F-:1.5-8 cm), humus (H: 0.5-2 cm), and eluviated/ rich organic matter (A-: 4-15 cm) layers of soil at each location that yielded a cap and stalk sample, and a moss sample when present. The soil surface at each sampling spot was categorized as follows (Figure 4.3):

- Litter: where the surface soil is covered by leaves, needles and tree debris;
- Moss: the surface soil is covered just by mosses, and litter is absent; and
- Litter and moss: the surface soil is partially covered by litter and moss.

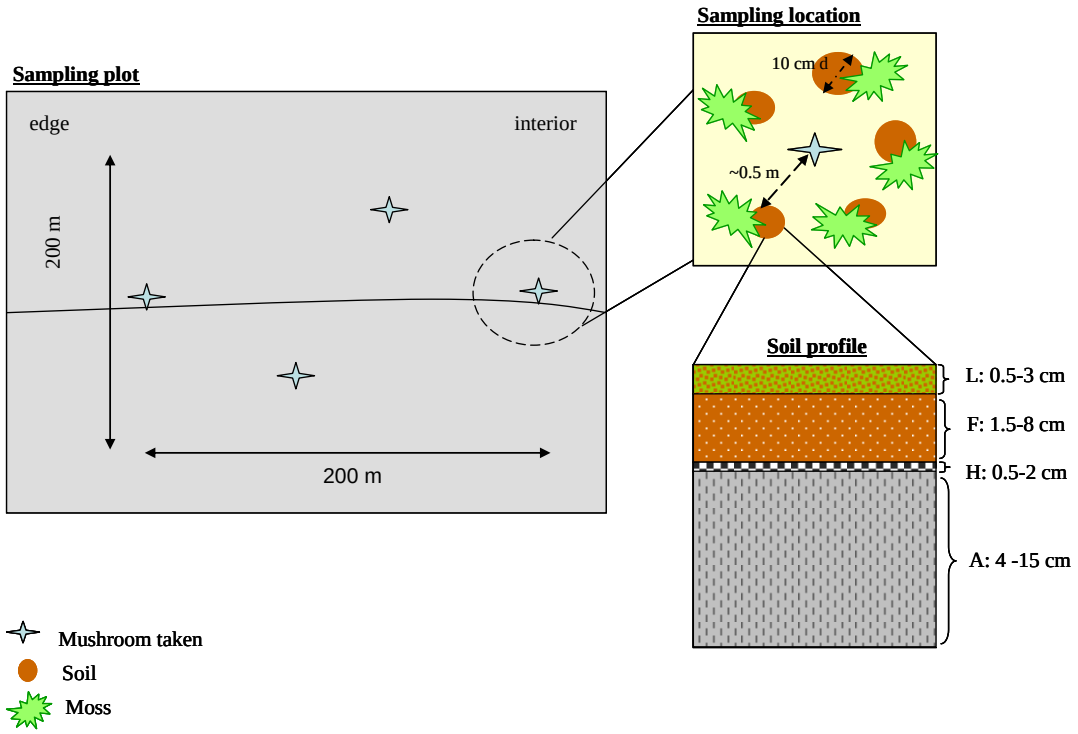


Figure 4.1. Diagram of the field sampling design.

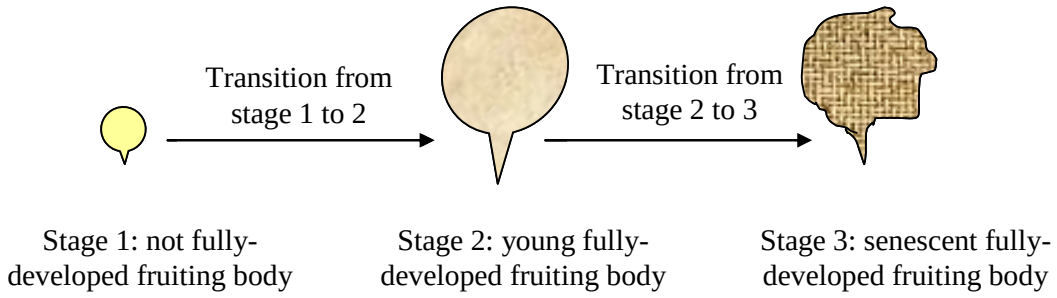


Figure 4.2. Developmental stages of fungal fruiting bodies.

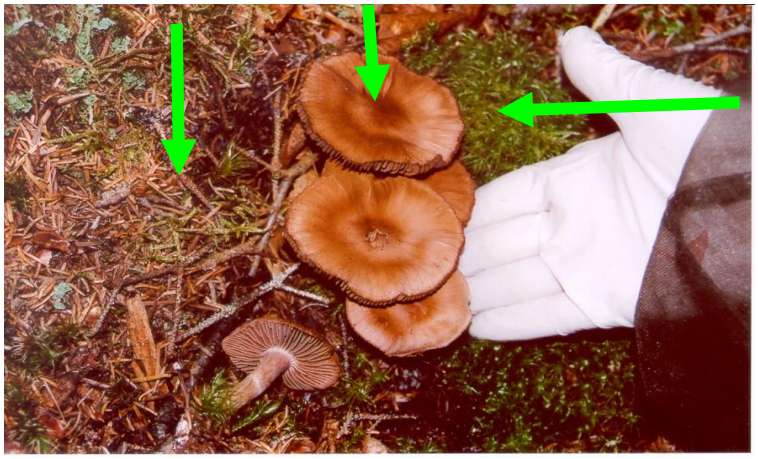
Litter

Not fully-developed
Amanita muscaria

Bazzania trilobata

Not fully-developed
Boletus sp.

Litter *Cortinarius sp.*



Dicranum polysetum

Figure 4.3. Forest surface soil type. Above: litter (leaves, needles, and tree debris). Middle: moss (*Bazzania trilobata*). Below: litter and moss (*Dicranum polysetum*).

In total, soil samples (minimum 10 g) were collected from 10 cm wide soil divots within 0.5 m of each mushroom. Also, to have a sufficient sample size, samples of moss common across the study areas were collected. Altogether there were *Sphagnum* sp. samples (5 - 10 cm height), *Ptilium crista-castrensis* samples (5 - 10 cm height), *Polytrichum juniperinum* samples (5 - 10 cm height), and *Pleurozium schreberi* samples (5 - 10 cm height). No moss, soil or fungal samples were taken from areas with mosses that were not common across the three study locations, such as *Dicranum polysetum* and *Bazzania trilobata* (Figure 4.3). Across the three main study sites, THg concentrations were determined for 400 caps, 338 stalks, and 50 whole fruiting bodies. All samples were handled with clean Powder Free Latex gloves. Mosses and soil samples were stored in ziplock bags and kept cool until processed. The fungal tissues were wrapped in parchment paper and kept in ambient air prior further identification and measurements.

SAMPLE PREPARATION AND LABORATORY ANALYSIS

In the laboratory, plants, substrate debris, coarse fragments and stones, slugs and worms were removed from mushrooms, soil and moss samples without introducing Hg contaminants, using plastic knives and gloves. The size of each cap (diameter, cm) and the size of each stalk (height, cm) were measured, then stored in clean polypropylene vials, and sealed with Teflon caps for measuring moisture content and elemental (Hg, S, N, and C) analyses.

All samples were weighed wet and dry, to determine moisture content. Drying was done by way of freeze-drying samples, with a moisture trap maintained at –50 °C, under vacuum, over the course of 48 hours (Figure 4.4). To avoid evaporative Hg losses from the samples, all dried samples were kept in capped polypropylene vials in a dark, sealed desiccator. Prior to analysis, dried samples were ground into a fine powder using a ceramic mortar and pestle. Before and after each grinding, the mortar and pestle were washed with deionized water and dried. The homogenized samples were stored in clean polyethylene vials in the desiccator until analysis.

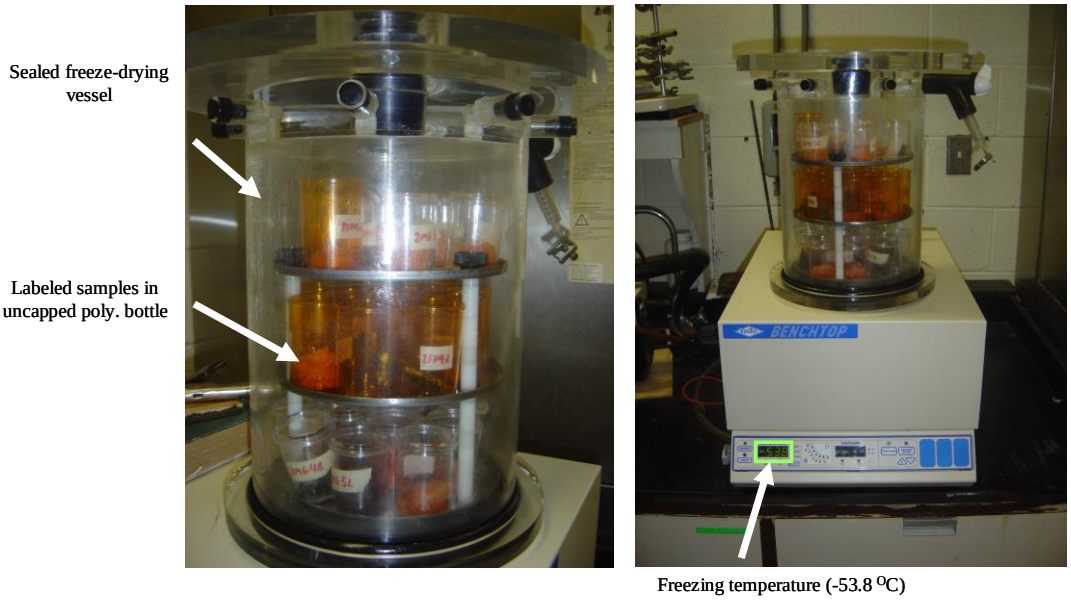


Figure 4.4. Freeze dryer.

THg analysis was carried out with a DMA-80 Direct Mercury analyzer, following EPA method 7473 (Figures 4.5 - 4.6; DMA-80 analyzer manual). A maximum 500 mg of homogenized sample was weighted in a small nickel sample boat. The boat then entered a sealed pyrolysis chamber. Each analysis cycle starts

with preheating the sample at 300 °C, followed by pyrolysis at 850 °C. Elemental Hg was released from the thermally decomposing samples under a pure oxygen flow. Then the released Hg vapour is then temporarily trapped by way of a gold amalgamator at the end of the pyrolysis tube, from where the Hg is then released again towards the detection cell through heating. Hg detection was enabled by way of Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS), with the fluorescence induced by ultraviolet light with a 253.7 nm wavelength. Total analysis time for each sample amounts to 5 min, with a 0.02 ng of Hg / sample instrumental detection limit (IDL).

Data quality control measures involved including two blanks and two standard samples on the Hg analysis tray, which provided for the automated processing of 40 samples over the course of about 4 hours. PACS-2 and the LICHEN standards for marine sediment and lichen, respectively, were used checking the accuracy of the DMA-THg results for the soil and vegetation samples, respectively. The differences between quoted and measured THg concentrations remained consistently within ± 5 % of the certified value. A calibration curve (the response of the method to known quantities of Hg in an analyte) was drawn for quantities of Hg in DORM (dogfish flesh: certified Hg reference material) (R^2 = 0.9996) (Figure 4.7). The precision of measurements were within ± 6 % when performing repeated sample analyses. The DMA-80 Direct Mercury analyzer also provided a data monitoring interface by which the results of each sample determination were automatically and electronically recorded, for current or later review of the THg concentration per sample, and of the details about each photometrically captured THg fluorescence signal, as generated for

each sample. Deviations from the normal appearance of the fluorescence signal would require a repeat of the analysis of that sample. Such deviations usually signaled that the pyrolysis reactor tube was in need of replacement. In general, this tube enables the un-interrupted analysis of about 1000 samples. To avoid any interference from high-to-low THg concentrations from sample to sample, samples with approximately the same THg concentrations were analyzed in batches, and batches were separated by blanks. In average, the mean 0.2 ng Hg / sample (1.8 ng Hg/g) were observed in blanks during sample analyses, generally with the higher values for blanks at the very beginning of each batch.

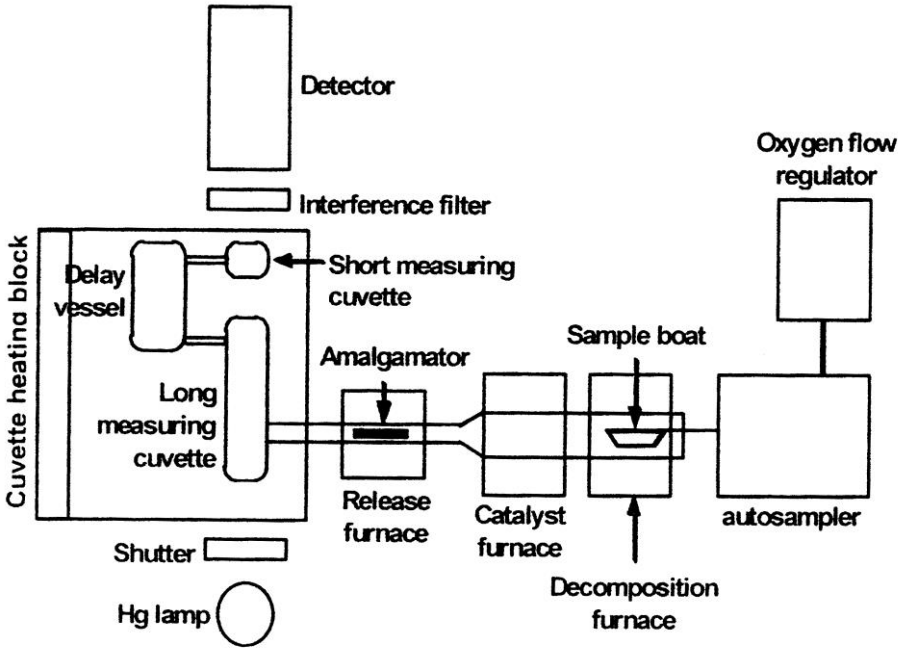


Figure 4.5. Schematic of DMA-80 THg analyzer (Adapted from DMA-80 THg analyzer manual).

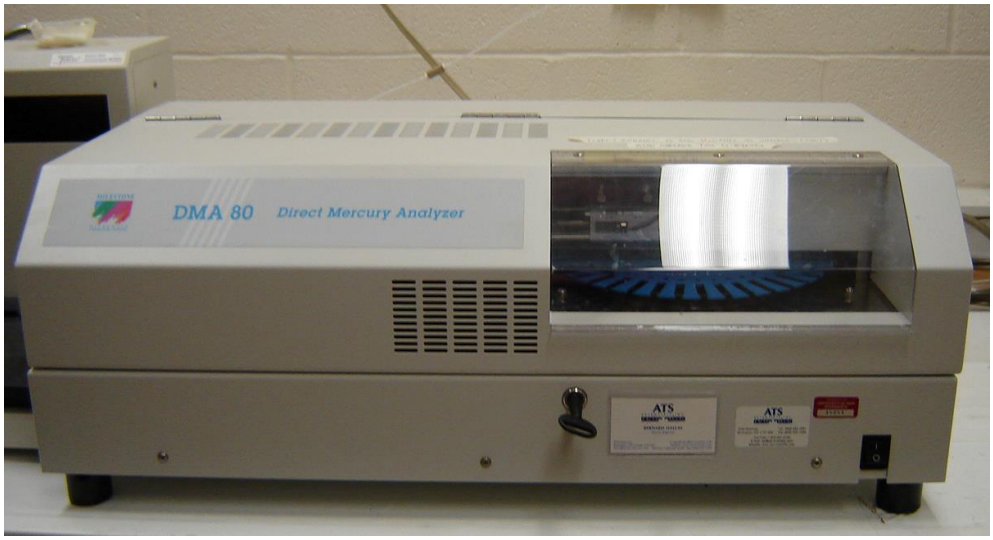


Figure 4.6. DMA-80 THg analyzer.

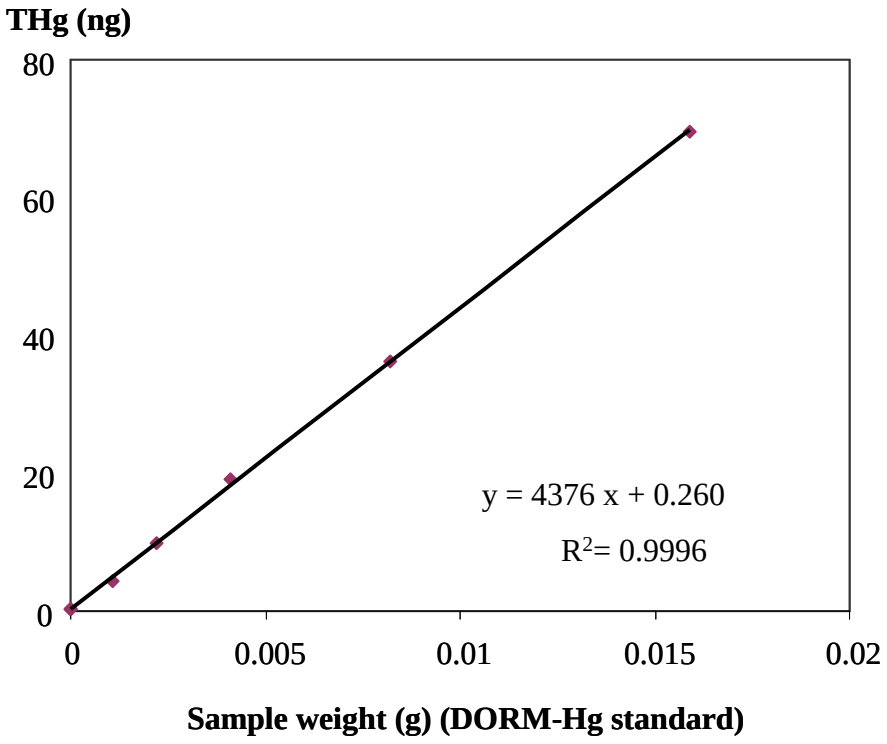


Figure 4.7. Calibration curve of THg concentrations (ppb, dw) for certified DORM standard.

The same samples were analyzed for total elemental S, N, and C contents with the LECO® CNS-2000 analyzer by combusting small portions (500 mg) of sieved, oven-dried samples at 1500 °C in a flow of pure O2 (Figure 4.7). All elemental concentrations were expressed on the freeze-dried weight basis (dw).

Figure 4.8. LECO CNS-2000.

DATA ANALYSIS

THg concentrations, elemental S, N, and C contents and other measurable elements such as substrate thickness (soil, moss), cap and stalk sizes along with categorical variables described in Figure 4.8 were all entered on one Microsoft Excel (2003) spreadsheet. Hg, S, N, and C in fungi, mosses, and soil were subject to statistical analysis; descriptive summaries (averages, standard deviations, ranges, maximum, minimum), were done by group (species, location, substrate, etc; also see

table 4.8). This included generating box plots, to explore the variability of data as a whole and by group. In each box plot, the data was shown by displaying the 25, 50, and 75 percentiles of the data within each group. These plots also displayed individual points lower and higher than the 25 and 75 percentiles (outliers), respectively by colored circles outside each individual box. The significant effects of categorical variables were examined by ANOVA. The effect was significant if calculated F-value was higher than F-critical ($F_{0.05, df1, df2}$) at P-value < 0.0001 or if F-value was higher than F-critical but at higher P-value than 0.0001. Relationships between variables were examined using simple (dependent variable is related to only one independent variable) and multiple (dependent variable is related to two or more independent variables) regression analyses. The goal of performing regression analyses was to establish a set of independent variable(s) that could explain the variance of the dependent variable at a significant level. In general, in analyses of this study, dependent variable was THg concentration and independent variables were continuous and categorical variables described in Fig 4.8. The regression analyses were performed based on the following major assumptions:

(1) the data is normally distributed,

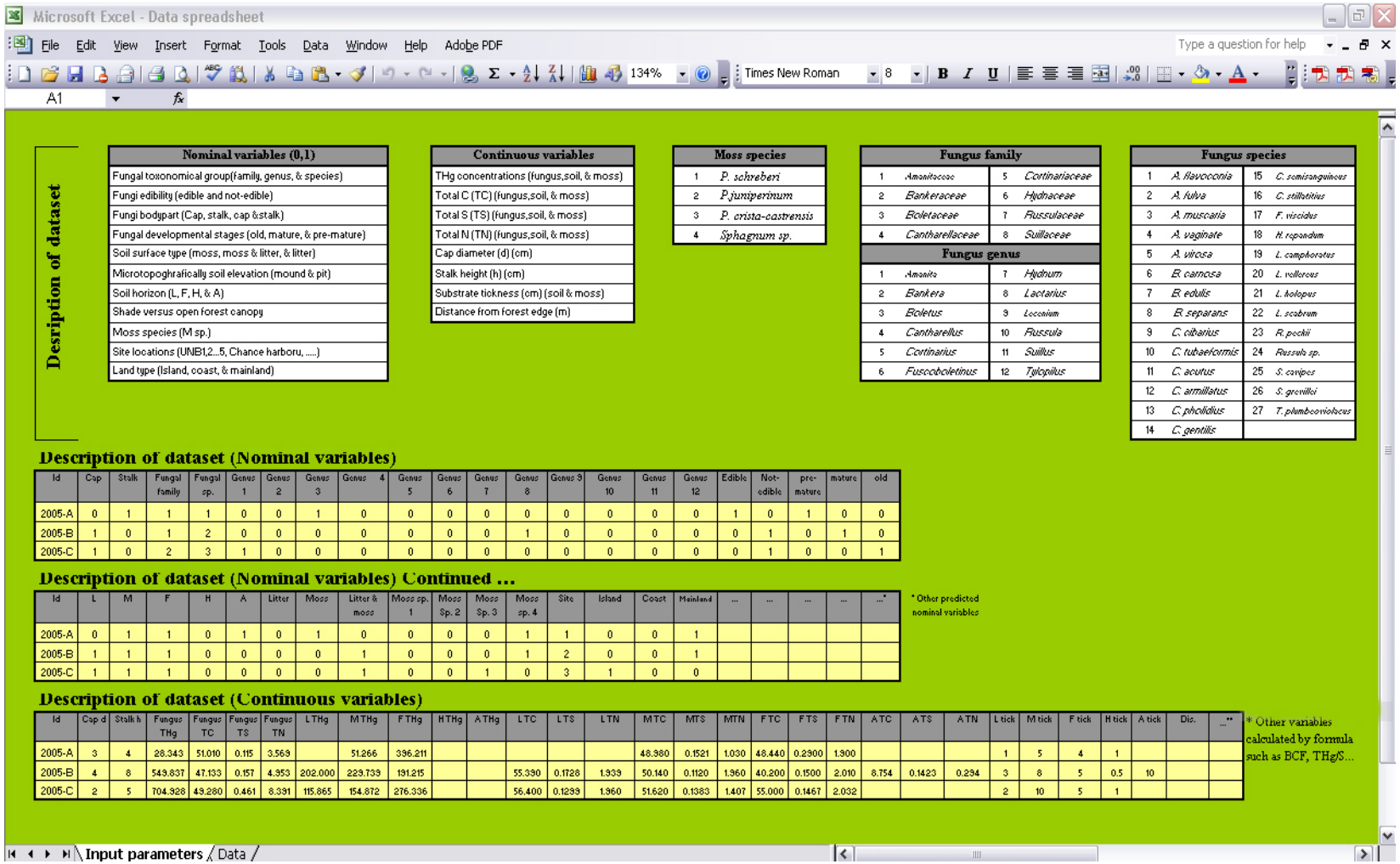
(2) there is a linear relationship between dependent variable and independent variables,

(3) independent variables are not linearly related, and

(4) the model is unbiased because the average error is zero (the sum of squares of estimated error is minimal).

The regression results were summarized by way of number of analysed sample (N), standard error of estimates for the regression coefficients as well as t- and P-values, coefficient of variation (R^2) for the goodness of fit of the best-fitted regression models, the root mean square error (RMSE) for the residuals of the actual versus best-fitted model estimates. The sign (minus or plus) of the regression coefficients was used to ascertain whether the predictor variables that were associated with the best-fitted model were positively or negatively related to the dependent variable. For example, THg concentrations in fungal tissues should generally be positively related (+) to increasing fungal S content, and fungal S content should increase with increasing fungal N content. In contrast, fungal THg content would be expected to decrease (-) with increasing S content of the soil. All statistical analyses were done with StatView software (Version 5, 1992-1998).

The quality of the data was ascertained by checking the raw data with box plots and regression plots. For the case of outliers, all chemical analyses were repeated to determine whether the outliers were real or the result of analytical errors. In general, all elemental analyses were essentially free of analytical errors; however, typographical errors occurred on occasions. Most errors were made in soil layer classifications: a soil layer with a C % above 20 % would not constitute an A-layer. In contrast, an H-layer with a low C % would not be an L-, F-, or A-layer. Most erros were corrected with reassigning the field determined A and H specifications for individual soil samples, as needed.



Microsoft Excel - Data spreadsheet

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A1

Desription of dataset

Nominal variables (0,1)
Fungal taxonomical group(family, genus, & species)
Fungi edibility (edible and not-edible)
Fungi bodypart (Cap, stalk, cap &stalk)
Fungal developmental stages (old, mature, & pre-mature)
Soil surface type (moss, moss & litter, & litter)
Microtopoghrafically soil elevation (mound & pit)
Soil horizon (L, F, H, & A)
Shade versus open forest canopy
Moss species (M sp.)
Site locations (UNB1,2...5, Chance harboru, ...)
Land type (Island, coast, & mainland)

Continuous variables
THg concentrations (fungus,soil, & moss)
Total C (TC) (fungus,soil, & moss)
Total S (TS) (fungus,soil, & moss)
Total N (TN) (fungus,soil, & moss)
Cap diameter (d) (cm)
Stalk height (h) (cm)
Substrate tickness (cm) (soil & moss)
Distance from forest edge (m)

Moss species	
1	*P. schreberi*
2	*P.juniperinum*
3	*P. crista-castrensis*
4	*Sphagnum sp.*

Fungus family			
1	*Amanitaceae*	5	*Cortinariaceae*
2	*Bankeraceae*	6	*Hydnaceae*
3	*Boletaceae*	7	*Russulaceae*
4	*Cantharellaceae*	8	*Suillaceae*

Fungus genus			
1	*Amanita*	7	*Hydnum*
2	*Bankera*	8	*Lactarius*
3	*Boletus*	9	*Leccinum*
4	*Cantharellus*	10	*Russula*
5	*Cortinarius*	11	*Suillus*
6	*Fuscoboletinus*	12	*Tylopilus*

Fungus species			
1	*A. flavoconia*	15	*C. semisanguineus*
2	*A. fulva*	16	*C. stillatitius*
3	*A. muscaria*	17	*F. viscidus*
4	*A. vaginata*	18	*H. repandum*
5	*A. virosa*	19	*L. camphoratus*
6	*B. carnosa*	20	*L. volleneus*
7	*B. edulis*	21	*L. holopus*
8	*B. separans*	22	*L. scabrum*
9	*C. cibarius*	23	*R. pechii*
10	*C. tubaeformis*	24	*Russula sp.*
11	*C. acutus*	25	*S. canipes*
12	*C. armillatus*	26	*S. grevillei*
13	*C. phoilidius*	27	*T. plumbeoviolaceus*
14	*C. gentilis*		

Description of dataset (Nominal variables)

Id	Cap	Stalk	Fungal family	Fungal sp.	Genus 1	Genus 2	Genus 3	Genus 4	Genus 5	Genus 6	Genus 7	Genus 8	Genus 9	Genus 10	Genus 11	Genus 12	Edible	Not-edible	pre-mature	mature	old
2005-A	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
2005-B	1	0	1	2	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0
2005-C	1	0	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1

Description of dataset (Nominal variables) Continued ...

Id	L	M	F	H	A	Litter	Moss	Litter & moss	Moss sp. 1	Moss Sp. 2	Moss Sp. 3	Moss sp. 4	Site	Island	Coast	Mainland	...	...	...	...	...*
2005-A	0	1	1	0	1	0	1	0	0	0	0	1	1	0	0	1					
2005-B	1	1	1	0	0	0	0	1	0	0	0	1	2	0	0	1					
2005-C	1	1	1	0	0	0	0	1	0	0	1	0	3	1	0	0					

* Other predicted nominal variables

Description of dataset (Continuous variables)

Id	Cap d	Stalk h	Fungus THg	Fungus TC	Fungus TS	Fungus TN	LTHg	MTHg	FTHg	HTHg	ATHg	LTC	LTS	LTN	MTC	MTS	MTN	FTC	FTS	FTN	ATC	ATS	ATN	L tick	M tick	F tick	H tick	A tick	Dis.	...**
2005-A	3	4	28.343	51.010	0.115	3.569		51.266	336.211						48.380	0.1521	1.030	48.440	0.2300	1.300				1	5	4	1			
2005-B	4	8	549.837	47.133	0.157	4.953	202.000	229.739	191.215			55.390	0.1728	1.339	50.140	0.1120	1.360	40.200	0.1500	2.010	8.754	0.1423	0.294	3	8	5	0.5	10		
2005-C	2	5	704.928	49.280	0.461	8.391	115.865	154.872	276.336			56.400	0.1239	1.360	51.620	0.1383	1.407	55.000	0.1467	2.032				2	10	5	1			

* Other variables calculated by formula such as BCF, THg/S...

Input parameters / Data

Figure 4.9. Model-user in Excel (Microsoft Corporation 2003) used for StatView Input.

CHAPTER 5: THg IN FUNGAL FRUITING BODIES

INTRODUCTION

THg concentrations in fungal fruiting bodies are known to vary widely, by several orders of magnitude (Chapter 2). The sources of these variations, however, are not known, nor is there sufficient information by which these variations can be predicted in general. The available information in the literature is highly fragmented, and essentially no information is available regarding the role of fungi as ecological pathways for Hg bio-accumulation in terrestrial ecosystems (Chapter 2). The main goal of this chapter is to summarize THg, S, N, and C elemental contents in the fruiting bodies of fungi for the sampling areas in this study, to explore the means by which these variations can be summarized, and by which these variables relate to each other, by substrate type and location. The specific objectives were:

- to compare THg concentrations in the fruiting bodies by taxonomical groups (family, genus, and species), developmental stage of the fruiting body (early, mature, senescent), body part (caps and stalks), and location (island, coast, and mainland) locations;
- to determine whether there is an association between elemental Hg and elemental S, N and C contents in the fungal fruiting bodies;
- to provide additional information of THg accumulation or retention in fungal fruiting bodies such as ratio of THg in caps versus stalks, by fungal taxonomical group; and

- to derive a predictor model for THg concentrations in fungal fruiting bodies using species type, elemental content, growth stage, and type of body part.

METHODS

Fruiting bodies were collected as described in Chapter 4 from the three study locations (island, coast, and mainland). THg concentrations of the fungal fruiting bodies were determined by taxonomical group (family, genus, species), type of body part (cap, stalk, the whole fruiting body), stage of development (early, mature, senescent), and location (island, coast, mainland) were compiled in an Excel spreadsheet (Microsoft Corporation 2003) (Figure 4.9; Chapter 4). ANOVA was performed to test the significance effect of categorical variables such as type of body part, developmental stages, and location on THg concentrations of the fungal fruiting bodies. The results showed that each variable had significant effect on the variation of THg concentrations in the fungal fruiting bodies, while their interactions (described in Table 5.1) did not significantly influenced the THg variations (Table 5.1). For example, F-values for type of body parts, location, and developmental stage were larger than F-critical and I concluded that these factors had significant influence on the THg concentrations at P-value < 0.0001. On the other hand, although the F-values for (type of body part x developmental stages) and (type of body part x location) interactions were smaller than F-critical I concluded that these interactions were not significant at P-values of 0.3098 and 0.1103. The analyses were facilitated by grouping the THg, S, N, and C data by the same categories and by generating the corresponding box plots displaying the 25, 50, and 75 percentiles of the THg, S, N,

and C data within each group. In each box plot the lowest, highest, and median values were shown by the lowest and highest vertical angles, and middle diameter of a box, respectively. Also higher and lower outliers were depicted by colored circles outside the box. The variations of the THg concentration ratio in cap versus stalk and stage of development were analyzed similarly by category using box plots. The significant effect of each categorical variable on THg, S, N, and C contents of the fungal fruiting bodies were also tested by performing ANOVA for each individual box plot, also see Chapter 4.

Table 5.1. ANOVA table for THg concentrations (ppb) in the fungal fruiting bodies from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Type of body part	1	19,521,126.1	19,521,126.1	20.7	<0.0001
Developmental stage	2	24,357,435.9	12,178,717.9	12.9	<0.0001
Location	2	29,203,701.5	14,601,850.7	15.5	<0.0001
Type of body part x developmental stages *	2	2,214,388.0	1,107,194.0	1.2	0.3098
Type of body part x location *	2	4,172,018.1	2,086,009.1	2.2	0.1103
Developmental stages x location	4	6,672,821.5	1,668,205.4	1.8	0.1334
Type of body part x developmental stages x location	4	1,772,539.6	443,134.9	0.5	0.7579
Residual	657	619,621,455.0	943,107.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,1,657}$ = 3.84; F-value > F critical
F (critical) $_{0.05,2,657}$ = 2.99; F-value > F critical
* F (critical) $_{0.05,2,657}$ = 3.84; F-value < F critical
F (critical) $_{0.05,4,657}$ = 2.37; F-value < F critical
Type of body part (cap, stalk); developmental stage: (early, mature, senescent); location (island, coast, mainland)

These data were analyzed by multiple regression analysis to determine whether independent variables such as taxonomical group, stage of development, location, type of body part, and by elemental content (S, N, and C) affected THg concentrations as dependent variable. The elemental variations across the groups were subjected to bi-variate and multi-variate linear regression analysis. Bi-variate regressions were used to determine relationships between THg concentrations and elemental S, N, and C contents, and between S and N. The multi-variate analysis was used to generate a simple predictor equation by which THg concentrations in the fungal fruiting bodies can be estimated knowing fungal family, genus, species, and fungal tissue type (cap and stalk), and location. The multiple Regression analysis was performed based on the following main assumptions: (1) the sum of the squares of the estimated error (SSE) is minimal, (2) keeping other independent variables constant, each independent has linear relationship with dependent variable, and (3) independent variables are not related to each other, and (4) data is normally disturbed. Since THg concentrations of the fungal fruiting bodies were in a wide range, logarithms transformation was performed in multiple regression analysis to improve the linearity of the relationships. Regression results were summarized in terms of the regression coefficient, R^2, the root mean square error of the residuals (actual values minus best-fitted values), by the standard error of estimates for each regression coefficient, and the associated t- and p-values. All statistical analyses were performed in StatView statistical package (version 5, 1992-1998).

RESULTS AND DISCUSSION

Altogether, the samples involved 27 fungal species from 12 genera were analyzed and these species were common across the study locations. All species, however, were not equally abundant at each site. Characteristics and attributes of these species are summarized in Table II A (Appendix II). The overall mean THg concentration in the fungal fruiting bodies was 822 ppb, dw and it varied by four orders of magnitude. Among taxonomical families (Figure 5.1), *Bankeraceae* (1761 ppb, dw), *Cortinariaceae* (1336 ppb, dw), *Boletaceae* (895 ppb, dw), and *Amanitaceae* (712 ppb, dw) had the highest mean THg concentrations. *Suillaceae* (431 ppb, dw), *Russulaceae* (346 ppb, dw), and *Hydnaceae* (249 ppb, dw) had intermediate mean THg concentrations, and *Cantharellaceae* (74 ppb, dw) had the lowest mean THg concentration. ANOVA for fungal family showed that the calculated F-value (27.7) is much larger than the critical value ($F_{0.05,7,719} = 2.00$) and so it was concluded that there was a significant difference among fungal families at P-value < 0.0001 (Table 5.2).

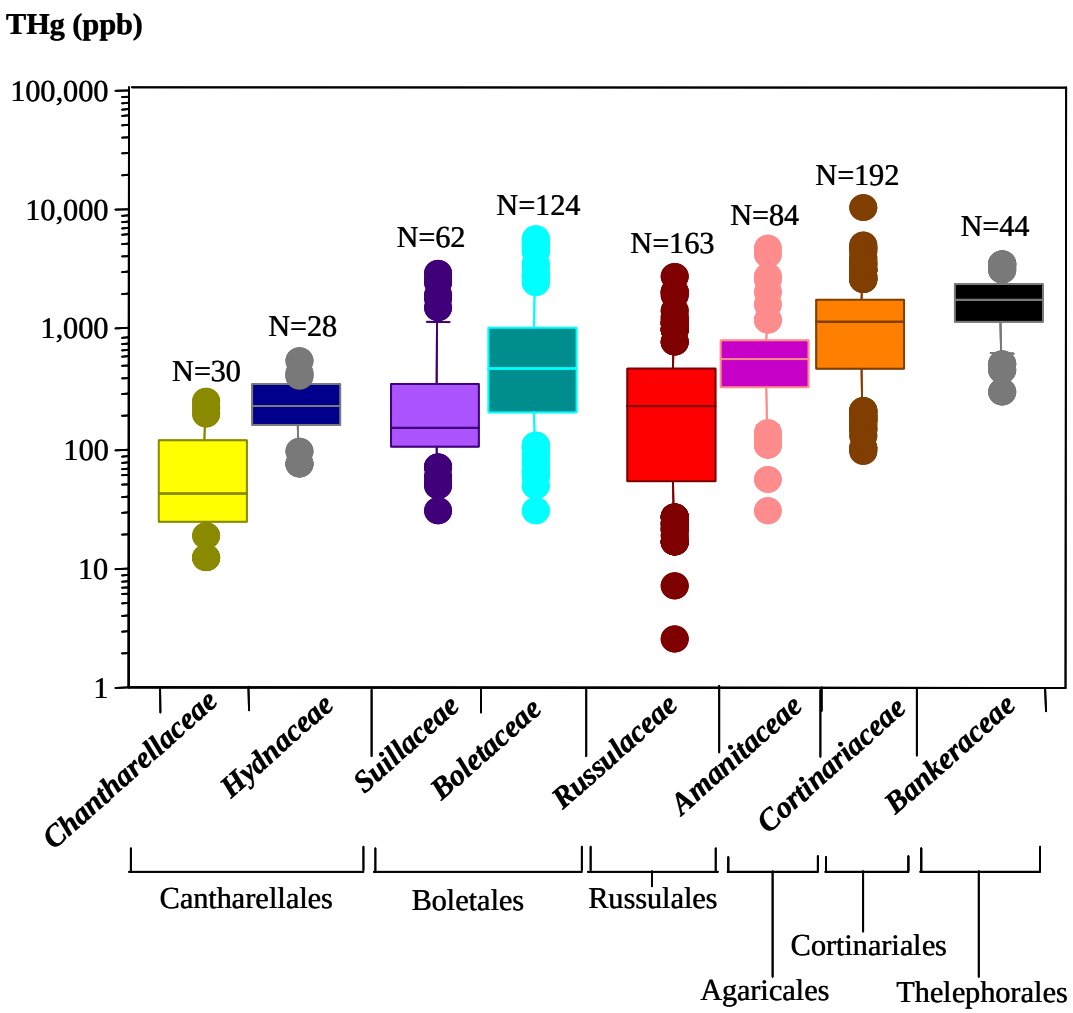


Figure 5.1. Box plot for THg concentrations (ppb, dw) in fruiting bodies, by order and family.

Table 5.2. ANOVA table for THg concentrations (ppb) of the fungal family from the three study locations (island, coast, and mainland) (Figure 5.1).

Source of variance	DF	SS	MS	F-value	P-value
Fungal family	7	163,581,787.3	23,368,826.8	27.7	<0.0001
Residual	719	605,707,706.1	842,430.8		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,7,719}$ = 2.00; F-value > F critical

Within each family, THg varied as follows (Figure 5.2):

- *Boletaceae*: mean THg concentrations decreased in order from *Boletus* (2161 ppb, dw) to *Leccinum* (399 ppb, dw), *Fuscoboletinus* (326 ppb, dw), and *Tylopilus* (297 ppb, dw).
- *Russulaceae*: *Lactarius* had the highest mean THg concentration (482 ppb, dw) and *Russula* had the lowest mean THg concentration (242 ppb, dw).

By performing ANOVA analysis for fungal genus, the test statistics was F-value of 30.8 (Table 5.3). Since the F-value is larger than F-critical ($F_{0.05,11,715} = 1.83$) I concluded that THg concentrations of the fungal genus were significantly different at P-value < 0.0001.

Among the species, *Boletus edulis*, *Bankera carnosa* and *Cortinarious armillatus* had the highest mean THg concentrations of 2,838, 1,761 and 1,696 ppb, dw, respectively (Figure 5.3). *Cantharellus cibarius* had the lowest mean THg concentration of 46 ppb, dw. The significant effect of fungal species on variation of THg concentrations were tested by ANOVA (Table 5.4). The results showed that F (critical) ($F_{0.05,26,700} = 1.51$) was much smaller than the test statistic (F-value = 26.3) and so there was a significant difference in THg concentrations of the fungal species at P-value < 0.0001.

We compared these results with other studies that are mostly done in Europe on THg concentrations in fungal fruiting bodies (Table 5.5). In these studies, depending on the county (city or region), THg concentrations in *Boletus edulis* varied from about 1000 to 10,000 ppb, dw in a wide range. The same as other studies, this study showed that fruiting bodies of *Boletus edulis* had high mean THg

concentrations regardless a local and/or long-range Hg deposition in the sampling sites. Literature values for *Amanita muscaria* (400 ppb, dw) and *Cantharellus tubaeformis* (120 ppb, dw) were similar to the results of this study (i.e., 601 and 128 ppb, dw, respectively). Of *Amanita* genus sampled in the current study, *Amanita vaginata* had the lowest mean THg concentrations of 266 ppb, dw. Falandysz & Bielawski (2001), Demirbas (2001), and Zurera et al. (1986) reported mean THg concentrations of about 500 ppb, dw in *Amanita vaginata* from not Hg-contaminated sites. Also Demirbas (2001) reported a sharply increase of THg concentration in the fruiting bodies of this species in Hg-enriched soil substrate, showing that in some extent this species effectively uptake Hg from its substrate. Higher mean THg concentrations than found in this study were reported for *Hydnum repandum* (600 ppb, dw) and *Leccinum scabrum* (up to 2000 ppb, dw) (Table 5.5). In the present study, all species from *Cortinarius* (the second highest Hg containing genus), excluding *Cortinarius semisanguineus* (381 ppb, dw), had mean THg concentrations higher than 800 ppb, dw, with the highest mean value of 2,804 ppb, dw in *Cortinarius stillatitius*. Laaksovirta & Lodenius (1979) reported average THg concentrations of 80 ppb, dw in *Cortinarius* which is much lower than was found in this study. In the literature, *Suillus grevillei* was reported to be an Hg hyper-accumulator, amounting to mean THg concentrations of 2,500 ppb, dw (Falandysz et al. 2004). In the present study, the mean THg concentration for this species was 557 ppb, dw. Mean THg concentrations for *Russula* of 242 ppb, dw were similar to those of other studies (Table 5.5).

In this study, decomposers (another fungi group that decompose litter materials in forest floor) had THg concentrations of about 120 to 2,420 ppb, dw which was a smaller range than mycorrhizal fungi (Table IIIB: Appendix III). Laaksovirta & Lodenius (1979) reported that lawn and wood fungi had higher THg concentrations than mycorrhizal fungi. For example, *Marasmium oreades* (a lawn fungus) had THg concentrations that varied from 3,000 to 16,000 ppb, dw depending on collection site. In our study, THg concentrations in the same species, *Marasmium oreades*, varied from 146 to 2,420 ppb, dw (Table IIIB: Appendix III).

Across all sites, mean THg concentrations in edible and non-edible fungi were 950 and 740 ppb, dw, respectively, (ANOVA: F-value > F-critical, P-value = 0.0015) (Table 5.6). These averages include the highest and lowest Hg in the two most common edible fungi: *Boletus edulis* and *Cantharellus cibarius*, respectively.

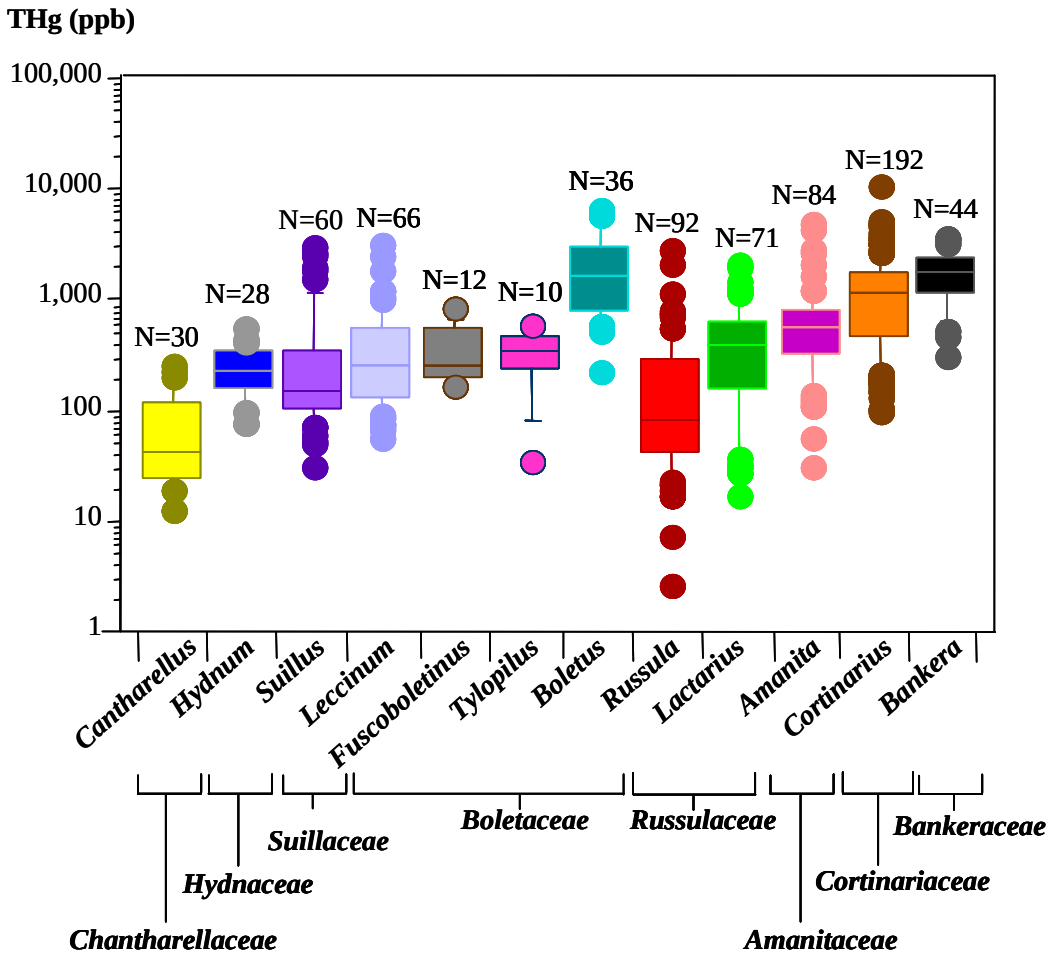


Figure 5.2. THg concentrations (ppb, dw) in fruiting bodies, by genus.

Table 5.3. ANOVA table for THg concentrations (ppb, dw) of the fungal genus from the three study locations (island, coast, and mainland) (Figure 5.2).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	247,259,713.6	22,478,155.8	30.8	<0.0001
Residual	715	522,029,779.9	730,111.6		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square

F (critical) $_{0.05,11,715}$ = 1.83; F-value > F-critical

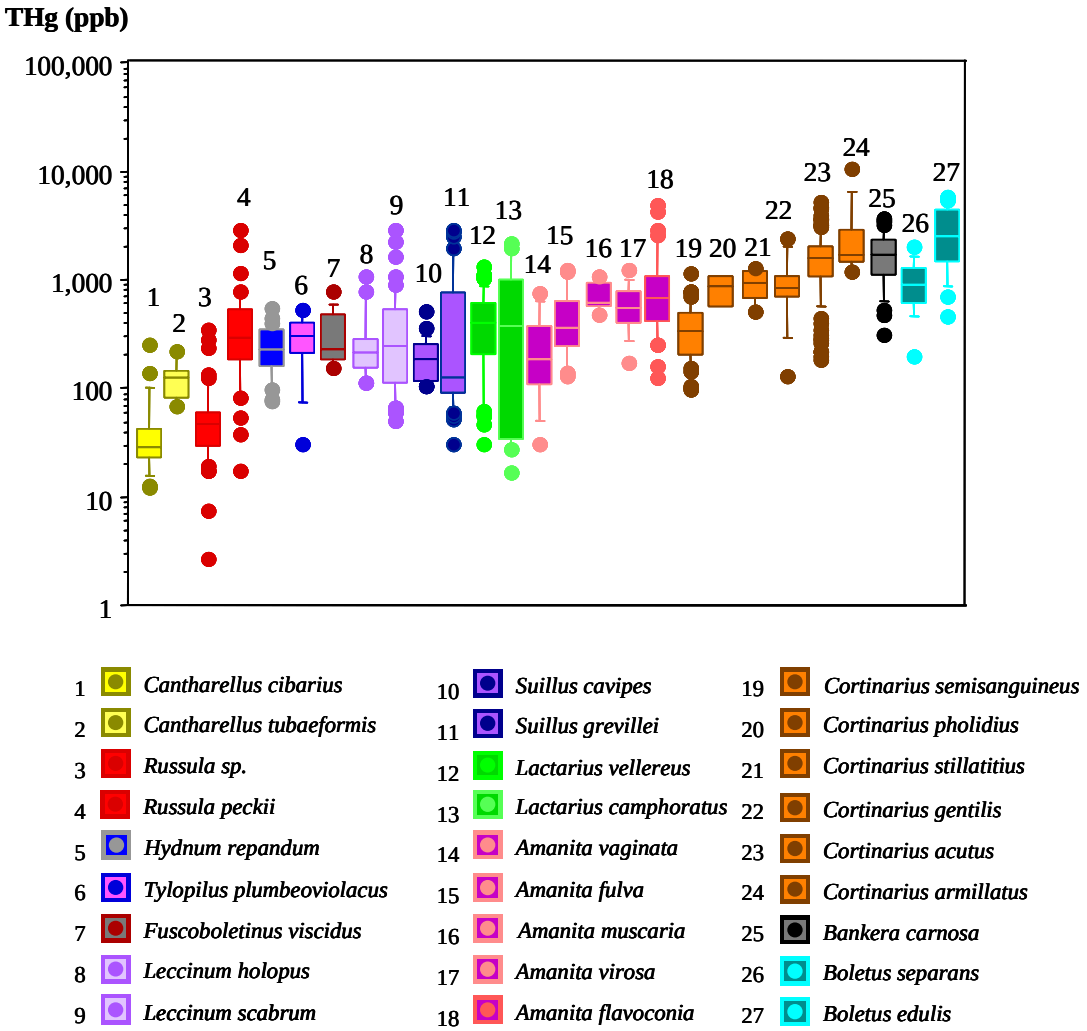


Figure 5.3. THg concentrations (ppb, dw) in fruiting bodies, by species (each number before the species name corresponds to the specified box plot in the Figure).

Table 5.4. ANOVA table for THg concentrations (ppb, dw) of the fungal species from the three study locations (island, coast, and mainland) (Figure 5.3).

Source of variance	DF	SS	MS	F-value	P-value
Fungal species	26	379,792,247.7	14,607,394.1	26.3	<0.0001
Residual	700	389,497,245.7	556,424.6		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,26,700}$ = 1.51; F-value > F-critical

Table 5.5. Comparison of THg concentrations (ppb, dw) in the fruiting bodies from different studies. Data from other studies, data from this and other studies; and data from this study.

Species	<500 ppb	500-1000 ppb	1000-2000 ppb	2000-5000 ppb	5000-10000 ppb	References
Amanita Fulva						(Falandysz et al. 2004)
Amanita muscaria						(Demirbas 2001; Falandysz et al. 2001; Falandysz & Bielawski 2001; Falandysz et al. 2002; Falandysz et al. 2003; Falandysz et al. 2004; Laaksovirta & Lodenius 1979)
Amanita vaginata						(Demirbas 2001; Falandysz & Bielawski 2001; Zurera et al.1986)
Boletus edulis						(Demirbas 2001; Falandysz & Bielawski 2001; Falandysz et al. 2002; Falandysz et al. 2003; Laaksovirta & Lodenius 1979)
Cantharellus cibarius						(Falandysz et al. 2002; Falandysz et al. 2003; Laaksovirta & Lodenius 1979; Svoboda et al. 2002)
Cantharellus tubaeformis						(Laaksovirta & Lodenius 1979)
Cortinarius sp.						(Laaksovirta & Lodenius 1979)
Hydnum repandum						(Tuzen et al. 1998)
Leccinum scabrum						(Falandysz et al. 2001; Falandysz et al. 2002; Svoboda et al. 2002)
Russula sp.						(Demirbas 2001; Falandysz et al. 2002; Falandysz et al. 2004; Laaksovirta & Lodenius 1979; Tuzen et al. 1998)
Suillus grevillei						(Falandysz et al. 2002; Falandysz et al. 2004; Laaksovirta & Lodenius 1979; Vetter & Berta 1997)

Table 5.6. ANOVA table for THg concentrations (ppb, dw) of the fungal fruiting bodies based on the edibility of the tissue from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Edibility	1	10,578,989.1	10,578,989.1	10.1	0.0015
Residual	725	758,710,504.4	1,046,497.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,\ 1,725}$ = 2.60 ; F-value > F-critical
Edibility: edible and non-edible fungal fruiting bodies

The genera common to the three study sites were *Amanita*, *Boletus*, *Cortinarius*, *Lactarius* and *Russula* (Figure 5.4; Table 5.7): mean THg concentrations in *Boletus* decreased from island to mainland (island > coast > mainland). *Amanita* and *Cortinarius* had higher mean THg concentrations on the island and along the coast than at the mainland locations. *Lactarius* had higher mean THg concentrations on the island than the coast and mainland locations. For *Russula*, mean THg concentrations decreased from coast to mainland to island (coast > mainland > island). The island-to-coast-to-mainland decrease of mean THg in *Amanita*, *Boletus*, and *Lactarius* suggests that Hg exposure and retention is higher on the island and coast compared to the mainland sampling location. Due to not-similar *Cortinarius* representatives from the three study locations (island: N = 5, coast: N = 108, mainland: N = 75), this genus can not be compared among the three locations. By performing ANOVA for *Amanita*, *Boletus*, *Lactarius* and *Russula* from the three study locations the calculated F-values for genus (F-value = 58.9) and location (F-value = 10.1) were much higher than the critical values of 2.60 and 2.99, respectively at P < 0.0001 level (Table 5.8). The F-value for genus and location interaction was also higher than the critical value at P = 0.0035 level (Table 5.8). From the above test statistics I concluded that mean THg concentrations of the fungal fruiting bodies were

significantly different among fungal genus. Also geographical gradient (island, coast, and mainland) of fungal tissue has significantly influenced the THg concentrations of the fruiting bodies. However, the probability of genus x location were not significant, compared to genus and location effects, individually.

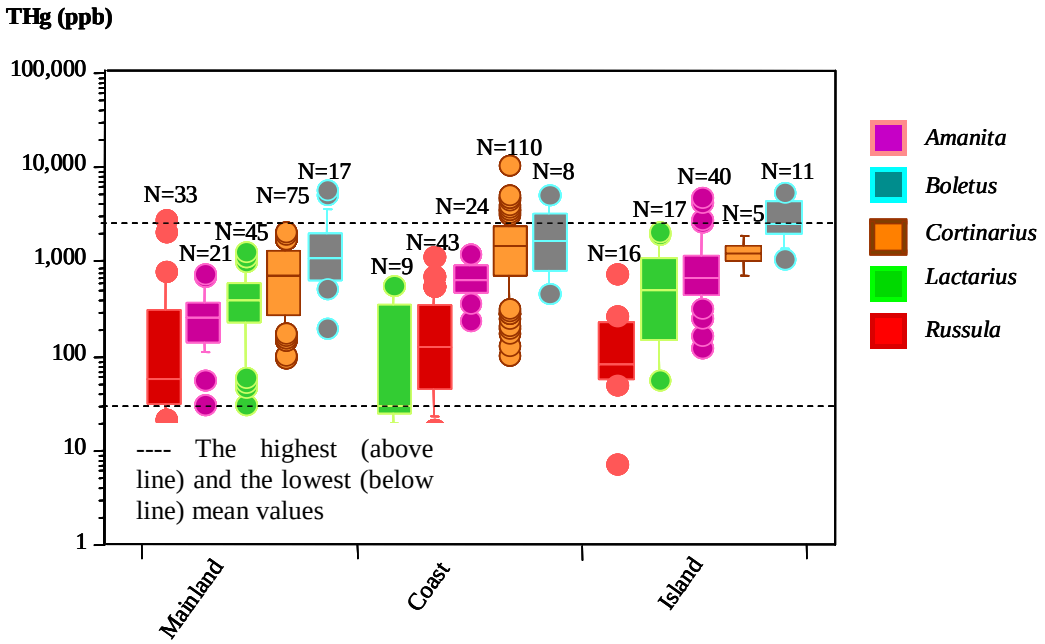


Figure 5.4. THg concentrations (ppb, dw) in fruiting bodies, by genus and location (island, coast, and mainland).

Table 5.7. THg concentrations (ppb, dw) in the fruiting bodies, by genus and location (island, coast, and mainland).

Location and genus	N	Mean	Std. Dev.	Std. Error	Min	Max
		ppb			ppb	ppb
Island	85	1068	1213	132	7	5520
Amanita	36	1039	1068	178	124	4855
Boletus	11	2948	1445	436	1065	5520
Cortinarius	5	1241	428	191	715	1898
Lactarius	17	721	664	161	57	2145
Russula	16	153	178	45	7	740
Coast	193	1176	1309	94	3	10475
Amanita	24	689	268	55	243	1220
Boletus	8	2136	1642	580	459	5206
Cortinarius	108	1683	1421	137	103	10475
Lactarius	9	188	249	83	17	621
Russula	44	224	243	37	3	1158
Mainland	194	660	766	55	19	5805
Amanita	25	278	189	38	30	757
Boletus	17	1662	1608	390	200	5805
Cortinarius	75	834	594	69	98	2106
Lactarius	45	451	316	47	31	1323
Russula	32	311	614	109	19	2860

Table 5.8. ANOVA table for THg concentrations (ppb, dw) of common fungal genus in the three study locations (island, coast, and mainland) (Figure 5.4).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	3	98,940,107.3	32,980,035.8	58.9	<0.0001
Location	2	11,290,302.2	5,645,151.1	10.1	<0.0001
Fungal genus x location	6	11,180,817.6	1,863,469.6	3.3	0.0035
Residual	271	151,793,871.6	560,125.0		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square

F (critical) $_{0.05,2,271}$ = 2.99; F-value > F-critical

F (critical) $_{0.05,3,271}$ = 58.9; F-value > F-critical

F (critical) $_{0.05,6,271}$ = 3.3; F-value > F-critical

Location: island, coast, mainland

* *Cortinarius* has been excluded from the analysis of this table.

Specifically, THg concentrations in Amanita were significantly different in the three study locations (island, coast, and mainland) (F-value > F-critical, P-value < 0.0001) (Table 5.9). For this species, there was a consistently significant decrease in THg content from coast to mainland (Table 5.10).

Table 5.9. ANOVA table for Log_{10}(THg) of *Amanita* in the three study locations (island, coast, and mainland).

Source	DF	SS	MS	F-value	P-value
Location	2	4.0	2.0	20.449	< 0.0001
Residual	81	8.0	0.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
Location: island, coast, mainland
F (critical) $_{0.05,2,81}$ = 2.99; F-value > F-critical

Table 5.10. Comparison of THg concentrations (ppb, dw) in *Amanita* from the three locations (island, coast, and mainland).

Location	Mean Diff.	DF	t-value	P-value
Island vs coast	277.192	61	1.267	0.2098
Coast vs mainland	424.743	43	6.027	< 0.0001
Island vs mainland	701.935	58	3.032	0.0036

Across all sites, the average elemental composition (S, N, and C) of the fungal fruiting bodies was 0.2 % sulphur (S), 4.3 % nitrogen (N), and 46% carbon (C), see Figures 5.5 - 5.7. The average S, N, and C contents of the fungal fruiting bodies were significantly different among fungal genus (ANOVA: F-value > F-critical, P-value < 0.0001), see Tables 5.11 – 5.13. Among fungal species, *Boletus edulis* had the highest mean S and N contents of 1 and 10 %, respectively. However, Bernas et al. (2006) reported a lower mean S content for *Boletus edulis* at 0.45 %, and that was similar to the amount reported for cultivated *Agaricus bisporus*, and higher than values reported

for cultivated *Pleurotus ostreatus* (Total S = 0.21 %). For the other species in this study, N and S contents varied from 3 – 7 % and 0.10-0.4 %, respectively. *Boletus* and *Leccinum* had the lowest mean C content of about 42 %. The average total C content of other genera was about 45-49 % (Figure 5.7).

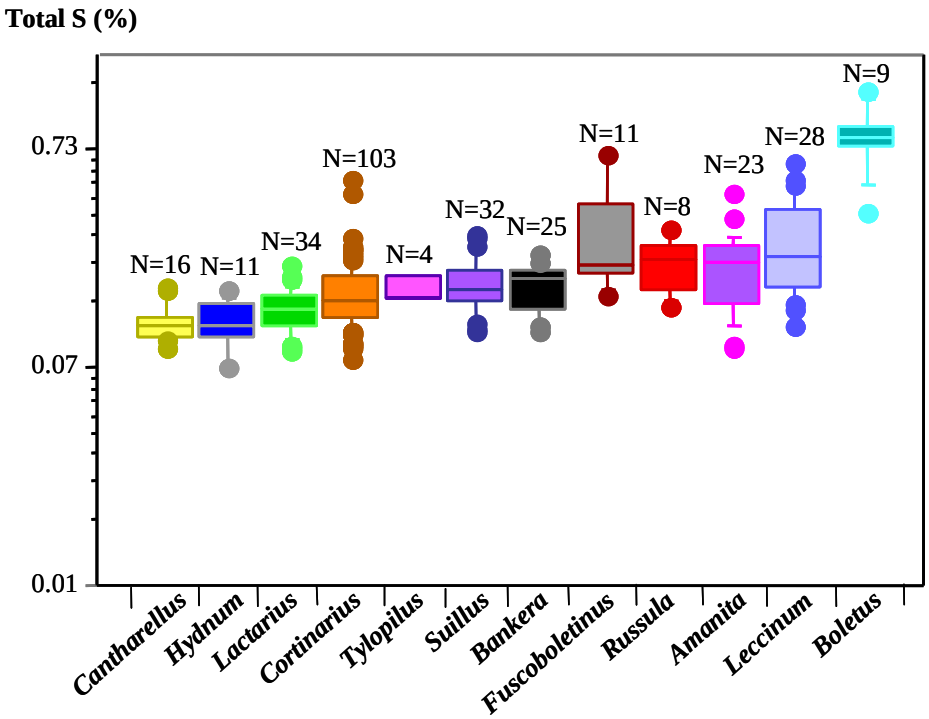


Figure 5.5. Total S contents (%) in the fruiting bodies, by genus.

Table 5.11. ANOVA table for elemental S contents (%) of the fungal genus from the three study locations (island, coast, and mainland) (Figure 5.5).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	4.7	0.4	53.9	<0.0001
Residual	292	2.3	0.01		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,11,292}$ = 1.83; F-value > F-critical

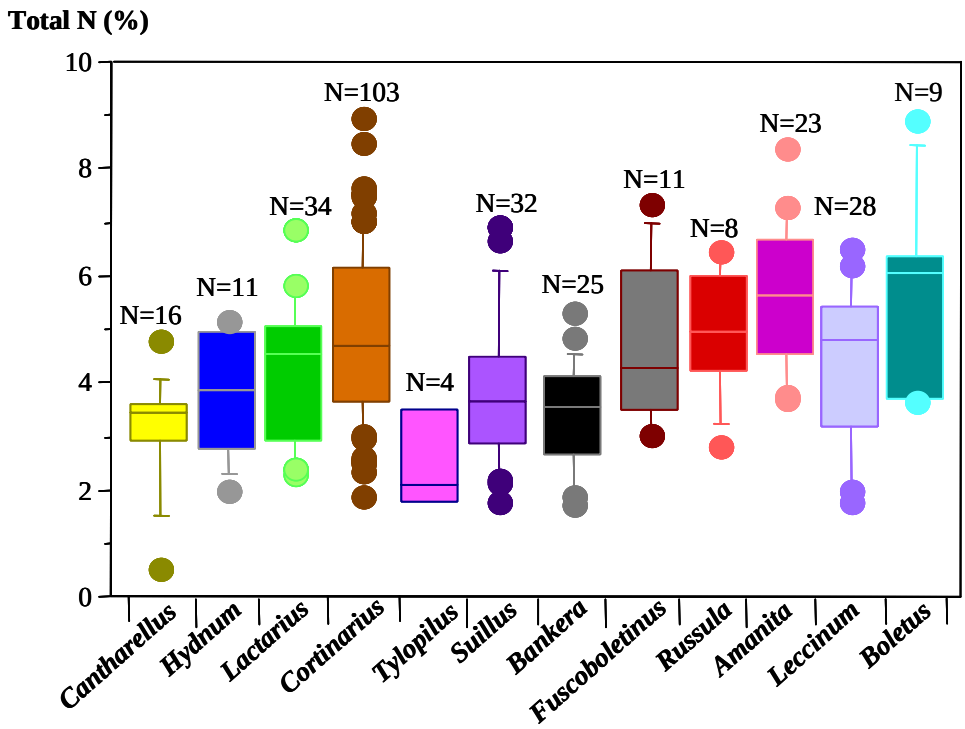


Figure 5.6. Total N contents (%) in the fruiting bodies, by genus.

Table 5.12. ANOVA table for elemental N contents (%) of the fungal genus from the three study locations (island, coast, and mainland) (Figure 5.6).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	142.9	13.0	5.4	<0.0001
Residual	292	708.3	2.4		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,11,292}$ = 1.83; F-value > F-critical

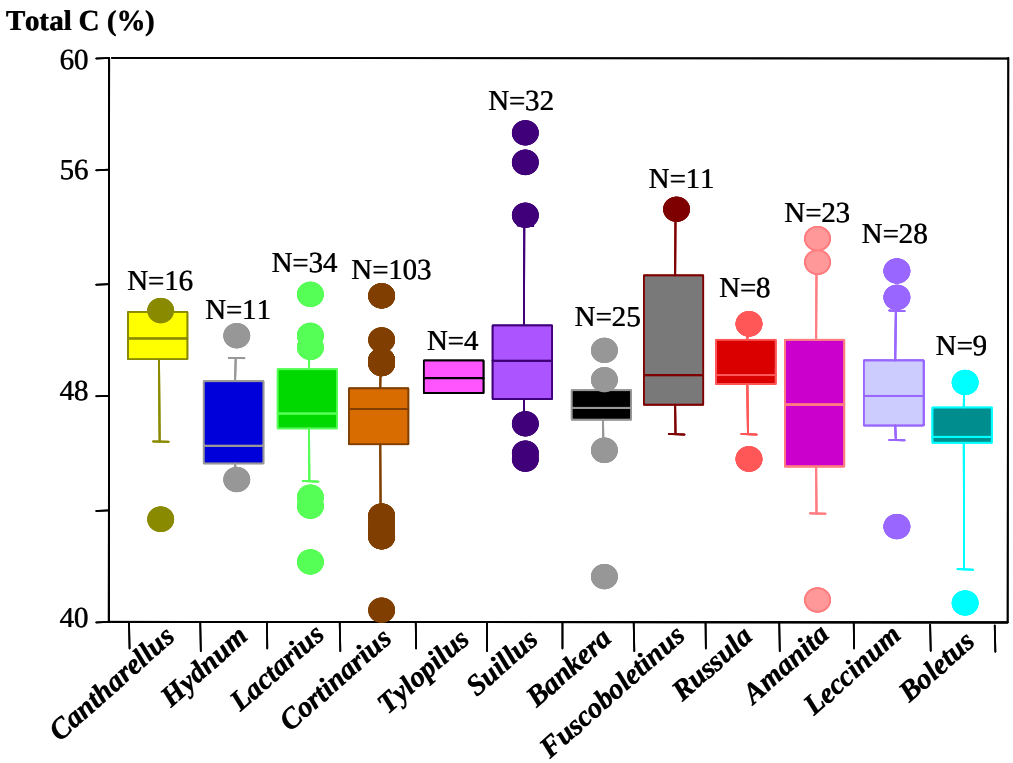


Figure 5.7. Total C contents (%) in the fruiting bodies, by genus.

Table 5.13. ANOVA table for elemental carbon contents (%) of the fungal genus from the three study locations (island, coast, and mainland) (Figure 5.7).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	1,653.4	150.3	4.1	<0.0001
Residual	292	10,588.1	36.3		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,11,292}$ = 1.83; F-value > F-critical

Plotting fungal THg concentrations and S content generated a scatter plot with 1) two slope groupings, with one group of species along the steep slope (group 1) and another group along the lower slope (group 2), and 2) group 3 with fungal species scattered at the based of the two slope groups (Figure 5.8 and Table 5.14).

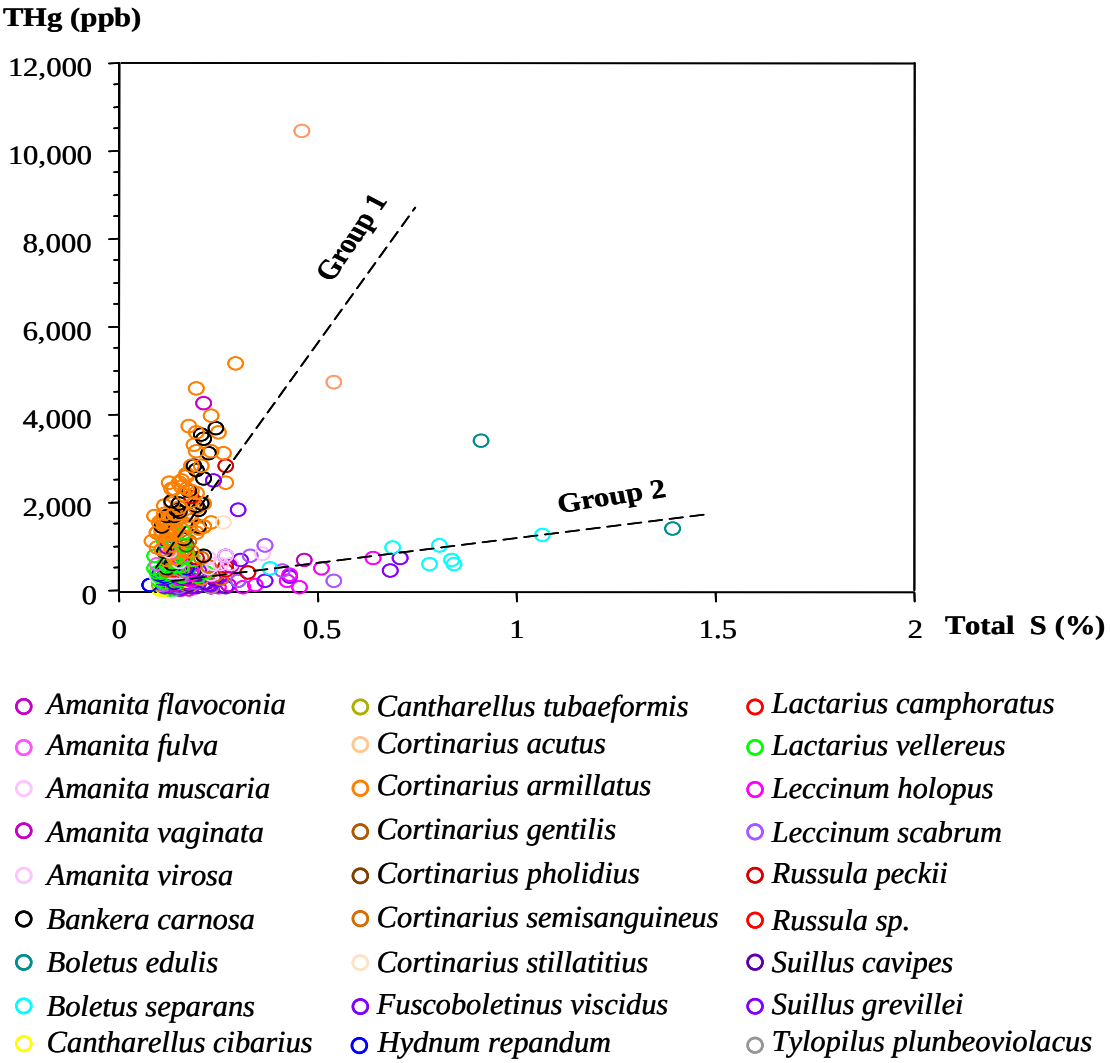


Figure 5.8. Bi-variate scattergram of THg concentrations (ppb, dw) versus total S (%) in the fruiting bodies.

Species with mean THg concentrations ≤ 500 ppb, dw are at the base of the two slopes (group 3). These species are *Hydnum repandum, Fuscoboletinus viscidus, Suillus cavipes, Russula peckii, Cortinarius semisanguineus, Cantharellus tubaeformis, Cantharellus cibarius, Leccinum holopus,* and *Suillus grevillei*. *Amanita virosa* (THg > 500 ppb, dw) fell between the two slopes. By splitting the THg- S scatter graph based on location, coast and island species were assigned to Group 1 and mainland species were assigned to Group 2, with these exceptions:

- mainland species *Amanita muscaria, Cortinarius armillatus, Cortinarius semisanguineus, Lactarius vellereus,* and *Russula peckii* were assigned to Group 1;
- the coastal *Russula peckii* species was assigned to Group *2*; *Suillus grevillei* was assigned to Group 1 and 2;
- the island *Suillus grevillei* and *Amanita flavoconia* were assigned to Group 1 and 2, depending in their THg content relative to S.

Generating bi-variate scattergrams showed that THg concentrations in fungal fruiting bodies were correlated to elemental S (Figure 5-8), and possibly N as well (Figure 5.9), but not to elemental C (Figure 5-10). As such, elemental S was correlated with elemental N for *Amanita vaginata, Bankera carnosa, Cantharellus tubaeformis, Cortinarius semisanguineus, Fuscoboletinus viscidus, Hydnum repandum, Russula peckii*, and *Suillus cavipes* (R^2= 0.596) (Figure 5.11).

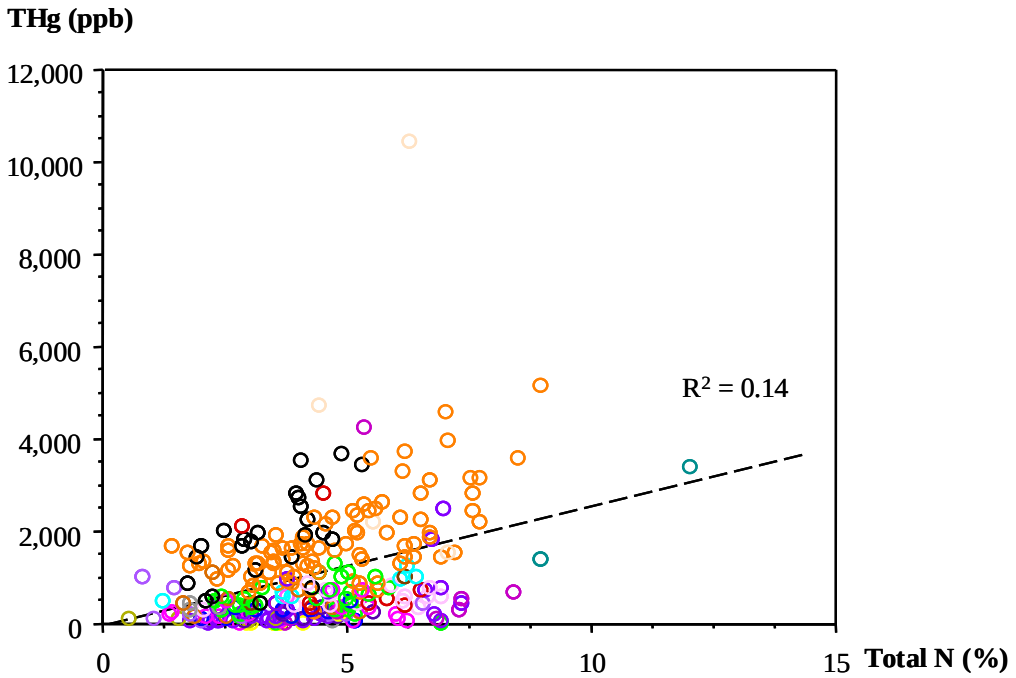
Positive correlations between S and N would be due to the joint occurrence of S and N in two important amino acids, i.e., cystine and methionine. For example, methionine and cystine content (of total protein) vary from 1 to 2.2 and 0.4 to 1.3 %

in *Amanita muscaria*, respectively (Table I A: Appendix I). In *Russula* genus, *Lactarius* has a high S-amino acid content of 2 % while its total protein is about 26 (Table 2.2: Chapter 2; Table I A: Appendix I). A content of less than 0.3 % S-amino acids has been reported for *Russula* (Table I A: Appendix I). In general, a high total protein content correlates with a high S and N content. As such, proteins amount to 15-46 % of dry fungal mass. Low versus high protein contents are species related (Table 2.2: Chapter 2). According to Petrovoska (2001), *Cantharellus cibarius*, compared to other ECM fungal species, has a low protein content of about 13 %. In contrast, the protein content of *Boletus edulis* is about 33 % (Table 2.2: Chapter 2). A total protein content of about 28 % has been reported for *Amanita vaginata* (Table 2.2: Chapter 2), but THg concentrations in this species are quite low compared to *Boletus edulis*.

Table 5.14. Number of available fruiting bodies on the Hg-S bi-variate scattergram (Figure 5.8), by species and location (island, coast, and mainland).

Fungi species	Group 1 *			Group2 *		
	Island	Coast	Mainland	Island	Coast	Mainland
	N	N	N	N	N	N
Amanita flavoconia	1			1		
Amanita fulva		1				
Amanita muscaria	4		4			
Amanita vaginata				2		4
Amanita virosa					6	
Bankera carnosa		23	2			
Boletus edulis			1			1
Boletus separans						7
Cantharellus cibarius						12
Cantharellus tubaeformis		4				
Cortinarius armillatus	2	52	32			
Cortinarius pholidius		2				
Cortinarius semisanguineus		2	6			
Cortinarius stillatitius		4				
Fuscoboletinus viscidus		1			5	5
Hydnum repandum		11				
Lactarius camphoratus			1			
Lactarius vellereus		2	31			
Leccinum holopus					11	8
Leccinum scabrum			2			7
Russula peckii			2		2	4
Suillus cavipes		9				
Suillus grevillei		4			3	16
Tylopilus plumbeoviolacus			4			

* Group 1: species from steep slope of Figure 5.7.; Group 2: species from lower slope of Figure 5.8.



- *Amanita flavoconia*
- *Amanita fulva*
- *Amanita muscaria*
- *Amanita vaginata*
- *Amanita virosa*
- *Bankera carnosa*
- *Boletus edulis*
- *Boletus separans*
- *Cantharellus cibarius*
- *Cantharellus tubaeformis*
- *Cortinarius acutus*
- *Cortinarius armillatus*
- *Cortinarius gentilis*
- *Cortinarius pholidius*
- *Cortinarius semisanguineus*
- *Cortinarius stillatitius*
- *Fuscoboletinus viscidus*
- *Hydnum repandum*
- *Lactarius camphoratus*
- *Lactarius vellereus*
- *Leccinum holopus*
- *Leccinum scabrum*
- *Russula peckii*
- *Russula sp.*
- *Suillus cavipes*
- *Suillus grevillei*
- *Tylopilus plunbeoviolacus*

Figure 5.9. Bi-variate scattergram of THg concentrations (ppb, dw) versus total N (%) in the fruiting bodies.

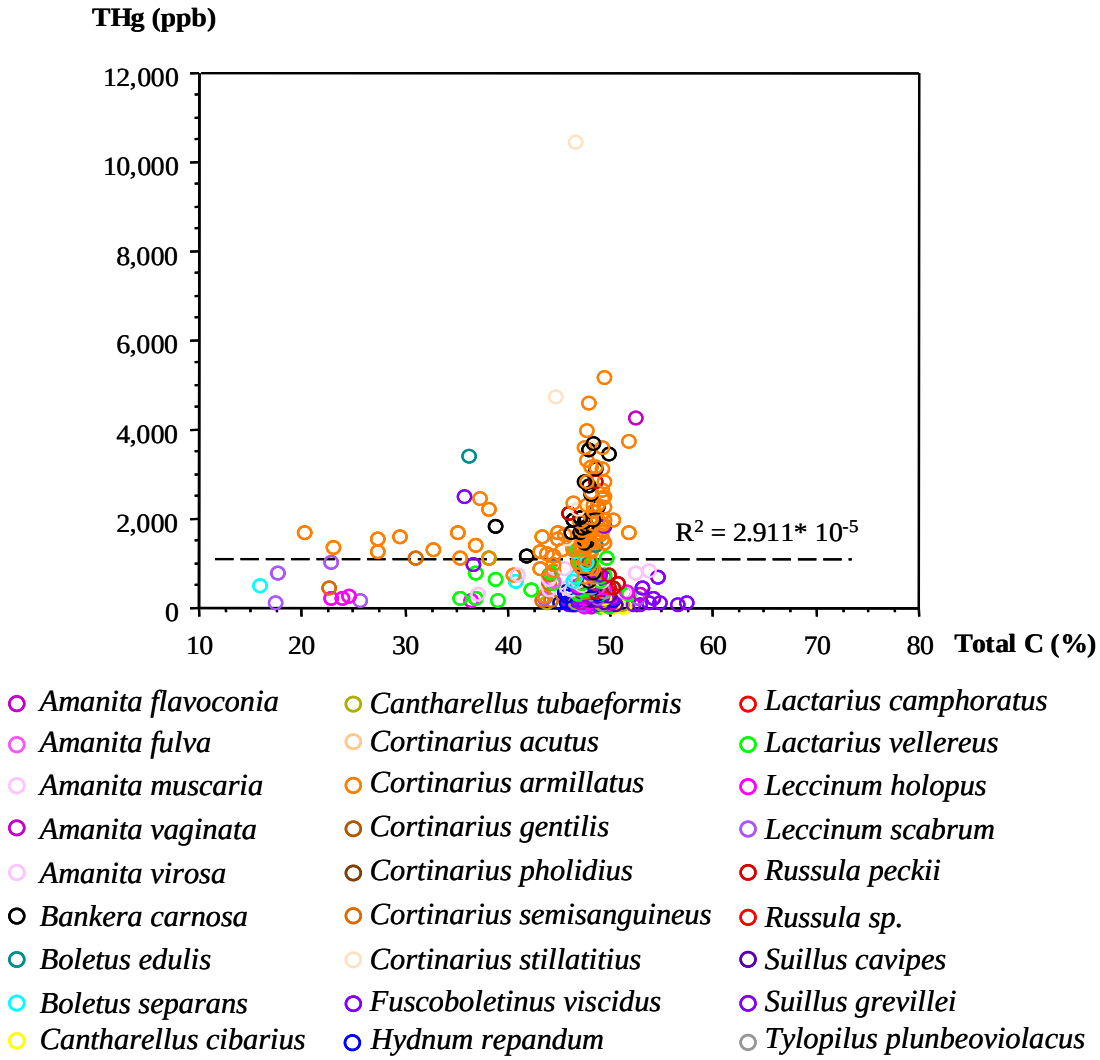


Figure 5.10. Bi-variate scattergram of THg concentrations (ppb, dw) versus total C (%) in the fruiting bodies.

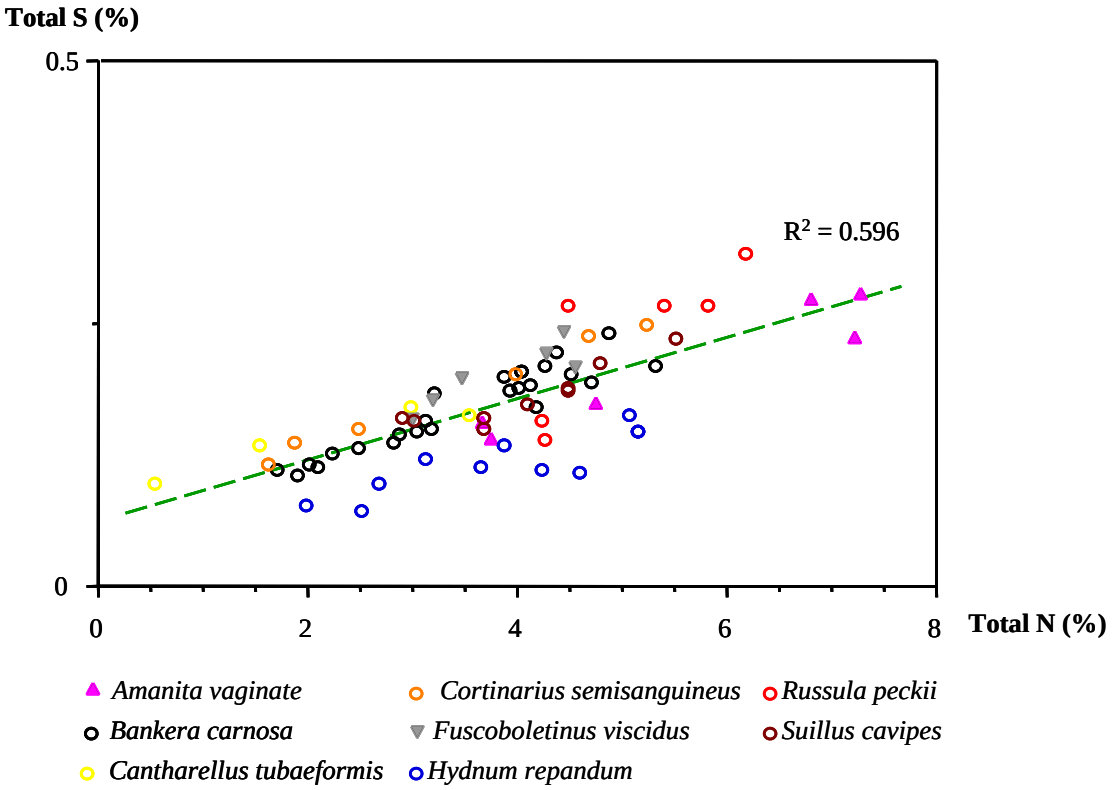


Figure 5.11. Bi-variate scattergram of total S (%) versus total N (%) in the fruiting bodies, by species.

A high S-amino acid content is likely an important factor affecting the Hg absorption by certain species. This is corroborated by the data that reveal a tighter correlation of Hg with elemental S than with elemental N content, where the latter is likely more tightly correlated with total protein or total amino-acid content. In general, some fungal species have a higher S-amino-acid content than other species. However, S content alone does not account for the Group 1 versus Group 2 differentiation in the THg versus S plot of Figure. The reason for this grouping remains unknown. However, it is possible that some species may have a specific Hg-binding capacity even though elemental S and S-amino acid content may be similar. If so, then such species (Group-1 species) would be Hg hyper-accumulators. Alternatively, Group-2 species may have the ability to eliminate some of the THg

accumulations by way of an enzymatically induced Hg^{2+} to $Hg^{(0)}$ reduction similar to bacteria (Silver & Phung 2005; Wiatrowski et al. 2006).

Multiple regression analysis was used to determine to what extent the observed variations in the THg concentrations of the fungal fruiting bodies could be attributed to variations regarding elemental S, N and C contents of the fungal tissues, type of fungal body part, developmental stages (not fully-developed, young fully-developed, and old fully-developed), and fungal genus. The best regression model ($P < 0.0001$) is summarized in Figure 5.12 and Table 5.15. This model contains the following Hg-retention predictor variables:

- total elemental S (%) in the fungal fruiting bodies,
- the generic identifiers for *Bankera* (+), *Cantharellus* (-), *Cortinarius* (+), *Lactarius* (+) and *Russula* (+),
- a cap (1) versus stalk (0) identifier, and
- a developmental stage identifier referring to fruiting bodies that were not fully-developed (0), were fully-developed (1), and started to decay (2).

Specifically, *Bankera*, *Cortinarius*, *Cantharellus*. *Amanita*, *Lactarius* and *Russula* factored strongly in this analysis because of the previously mentioned species-specific Hg retention differences. Species with average Hg retention are also important in this analysis, but do not enter as significant regression variables into the best-fitted modeling results. The elemental S content in the fungal fruiting bodies was one of the significant numerical variables to account for some of the observed THg variations. This is to be expected because as S increases, the more Hg is bound to the fungal tissues, but the extent of this species-dependent binding makes a difference

between the above identified Group1 and Group2 species. The developmental stage of fungal fruiting bodies contributed further to the observed THg variations in the fungal tissues. There was a consistently significant and species-independent decrease in THg content from an early developmental stage to well-grown and old fruiting bodies: premature and mature ($P < 0.0001$), premature and old ($P < 0.0001$), and mature and old ($P < 0.0016$) (Table 5.16). Also, by performing ANOVA, the significant difference of THg concentrations among the three developmental stages and type of body part (cap, stalk) was strongly supported (F-value > F-critical, P-value < 0.0001), see Table 5.17. While type of body part and developmental stage interaction was not significantly supported (F-value < F-critical, P-value = 0.00678). For example, *Lactarius vellerus* from mainland had THg concentrations of about 339, 624, and 1082 ppb, dw in caps of stages III, II, and I, respectively (ANOVA: F-value > F critical, P-value < 0.0001) (Figure 5.13; Tables 5.18 - 5.19). Type of body part and developmental stage interaction of *Lactarius vellerus*, as described earlier for all data, was not significant (F-value < F-critical, P-value = 0.1376) (Table 5.19). In contrast, elemental S and N concentrations did not change significantly during the successive stages of the fruiting bodies growth (ANOVA: F-value < F critical) (Figures 5.14 -5.15), as already observed by Dikeman et al. (2005), who further noted a general decrease in protein content but an increase in chitin and carbohydrates with increased developmental stage. Earlier, Kosson & Bakowski (1984) reported the amount of total protein concentration to be highest at the emergence stage of growth of the fruiting body. Likewise, this would also be the time for highest S-amino-acid concentrations. The decreasing THg concentrations with increasing developmental

stage would thus be related to a systematic, growth-dependent change that is influenced by substrate allocation from the fungal mycelium to the fruiting body. The mechanisms by which Hg is translocated from soil via mycelium network to the fungal fruiting bodies remains to be explored.

Table 5.15. Summary of regression results of $\log_{10}$(THg concentration (ppb, dw)) in the fruiting bodies in relation to the independent variables.

Regression variable	**Reg. coef.**	**Std. error**	**Std. coef.**	**t-value**	**P-value**	**R^2**	**Partial corr.**
Intercept	2.2	0.05	2.2	47.1	<0.0001		
Cortinarius	0.8	0.04	0.7	19.7	<0.0001	0.145	0.8
Bankera	0.8	0.06	0.4	13.6	<0.0001	0.247	0.6
Developmental stage	0.2	0.02	0.2	8.4	<0.0001	0.353	0.4
Cantharellus	-0.6	0.07	-0.3	-8.2	<0.0001	0.418	0.4
Total S	0.8	0.11	0.2	7.6	<0.0001	0.715	0.4
Cap	0.1	0.03	0.1	4.5	<0.0001	**0.731**	0.3
Lactarius	0.3	0.06	0.2	5.6	<0.0001	0.741	0.3
Russula	0.4	0.10	0.1	4.4	<0.0001	0.753	0.2
Amanita	0.2	0.06	0.1	3.5	<0.0005	0.762	0.2
Location *	0.1	0.03	0.1	3.2	<0.0013	**0.771**	0.2

* Location as a dummy variable was coded -1 for mainland and 0 for island and coast. This means having fungal fruiting bodies from mainland caused THg concentrations to be decreased by 0.1 units.
The line bellow the cap variable shows that adding other variables such as *Lactarius*, *Russula*, *Amanita*, and location the R^2 slightly increases (from 0.731 to 0.771).

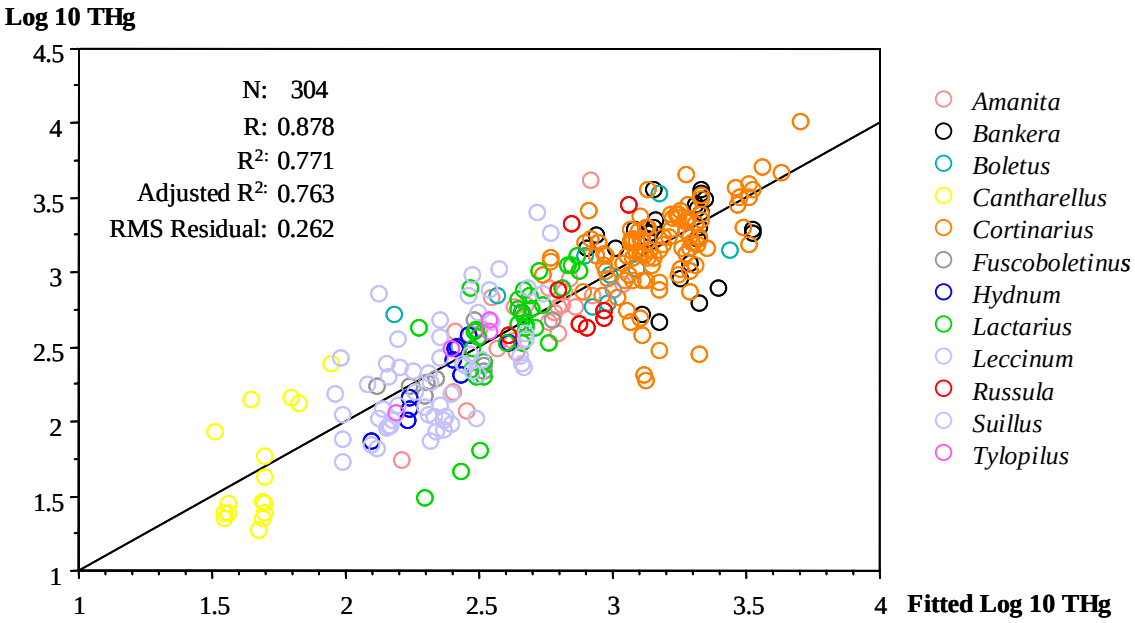


Figure 5.12. Bi-variate scattergram of $\log_{10}$(THg concentrations (ppb, dw)) versus Fitted log10 (THg concentrations (ppb, dw)) in the fruiting bodies, by genus (Fitted or predicted value is the value of dependent variable that is estimated based on the regression equation. The regression line is the best fitted-line that is drawn through the data points).

Another explanation that may contribute to the decreasing trend of THg concentration with developmental stage would be the aforementioned process of Hg^{2+} reduction to $Hg^{(0)}$. This process would be accelerated by the lying of fungal tissues by which Hg^{2+} reductase enzymes (if present) would intermingle with Hg^{2+} carrying amino acids ((Silver & Phung 2005; Wiatrowski et al. 2006). Whether this process actually occurs as part of the changing tissue dynamics of the growing fruiting body or as part of the later decaying process of fungal fruiting bodies remains to be explored.

Table 5.16. Comparison of THg concentrations (ppb, dw) of the three developmental stages.

Fruiting bodies	DF	Mean diff.	t-value	P-value
Total fruiting bodies				
mature vs old	537	272.5	3.172	0.0016
premature vs mature	585	537.6	5.941	<0.0001
Premature vs old	326	810.1	6.574	<0.0001
Fruiting bodies of *Lactarius vellerus*				
mature vs old	34	228.9	3.631	0.0009
premature vs mature	25	349.2	3.361	0.0025
Premature vs old	27	578.1	5.984	<0.0001

Table 5.17. ANOVA table for THg concentrations (ppb, dw) of the fungal fruiting bodies from the three study locations (island, coast, and mainland) based on the type of body part and developmental stage (Figure 5.13).

Source of variance	DF	SS	MS	F-value	P-value
Type of body part	1	25,064,213.3	25,064,213.3	25.5	<0.0001
Developmental stage	2	49,679,797.0	24,839,898.5	25.3	<0.0001
Type of body part x developmental stage	2	3,911,348.4	1,955,674.2	2.0	0.1376
Residual	671	659,595,946.0	983,004.4		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,1,671}$ = 3.84; F-value > F-critical
F (critical) $_{0.05,2,671}$ = 2.99; F-value > F-critical
Type of body part: cap, stalk; Developemental stage: (early, mature, senescent)

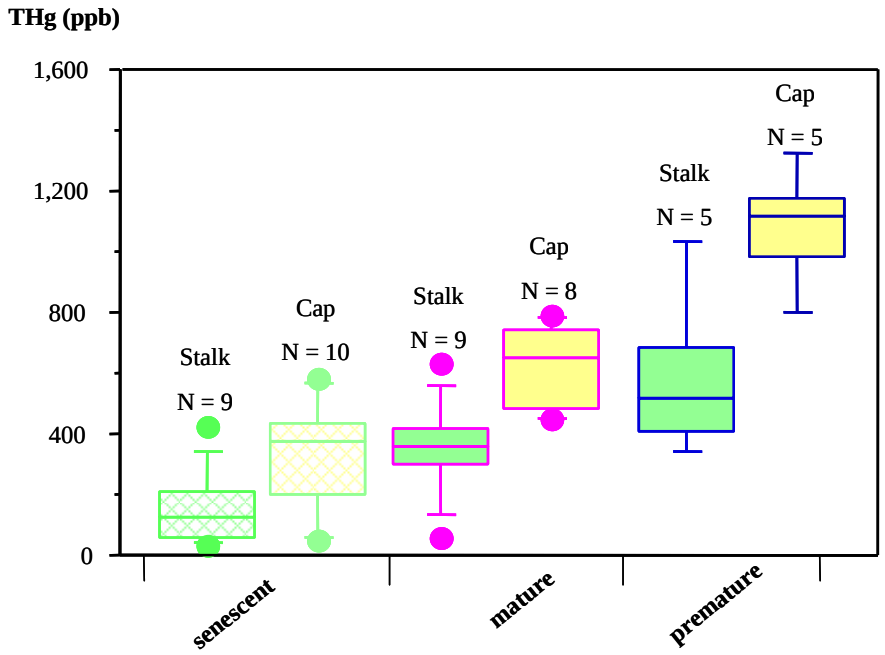


Figure 5.13. THg concentrations (ppb, dw) of the three developmental stages of *Lactarius vellereus* from mainland.

Table 5.18. THg concentrations (ppb, dw) and elemental S and N contents (%) of the three developmental stages of the *Lactarius vellereus* from mainland.

Fruiting bodies	Elemental contents *	N	Mean	Std. dev.	Std. error	Min	Max
Stage I: not fully-developed specimens							
Cap	THg (ppb)	5	1082	188	84	802	1323
	S %	5	0.1	0.03	0.01	0.09	0.2
	N %	5	4.4	0.7	0.3	3.2	5.0
Stalk	THg	5	578	270	121	341	1035
	S %	5	0.1	0.05	0.02	0.09	0.2
	N %	5	3.8	1.4	0.6	2.3	5.6
Stage II: fully-developed young specimens							
Cap	THg (ppb)	8	624	138	49	450	793
	S %	6	0.2	0.03	0.01	0.1	0.2
	N %	6	4.730	1.2	0.5	2.4	5.8
Stalk	THg	9	354	153	51	57	637
	S %	6	0.1	0.02	0.007	0.1	0.1
	N %	6	3.1	1.03	0.4	2.4	5.2
Stage III: fully-developed old specimens							
Cap	THg (ppb)	10	339	181	57	46	587
	S %	5	0.2	0.04	0.02	0.1	0.2
	N %	5	4.4	0.9	0.4	2.9	5.1
Stalk	THg (ppb)	9	155	125	42	31	423
	S %	6	0.1	0.02	0.007	0.12	0.1
	N %	6	3.4	0.9	0.4	2.7	4.9

* Mean THg concentration (ppb, dw), and total S and N percentages (%)

Table 5.19. ANOVA table for THg concentrations (ppb, dw) of *Lactarius vellereus* from mainland (Figure 5.13).

Source of variance	DF	SS	MS	F-value	P-value
Type of body part	1	1,085,120.7	1,085,120.7	36.9	<0.0001
Developmental stage	2	2,241,594.6	1,120,797.3	38.2	<0.0001
Type of body part x developmental stage	2	169,247.0	84,623.5	2.9	0.0678
Residual	40	1,175,084.3	29,377.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,1,40}$ = 4.08; F-value > F-critical
F (critical) $_{0.05,2,40}$ = 3.23; F-value > F-critical
Type of body part: cap, stalk; developemental stage: (early, mature, senescent)

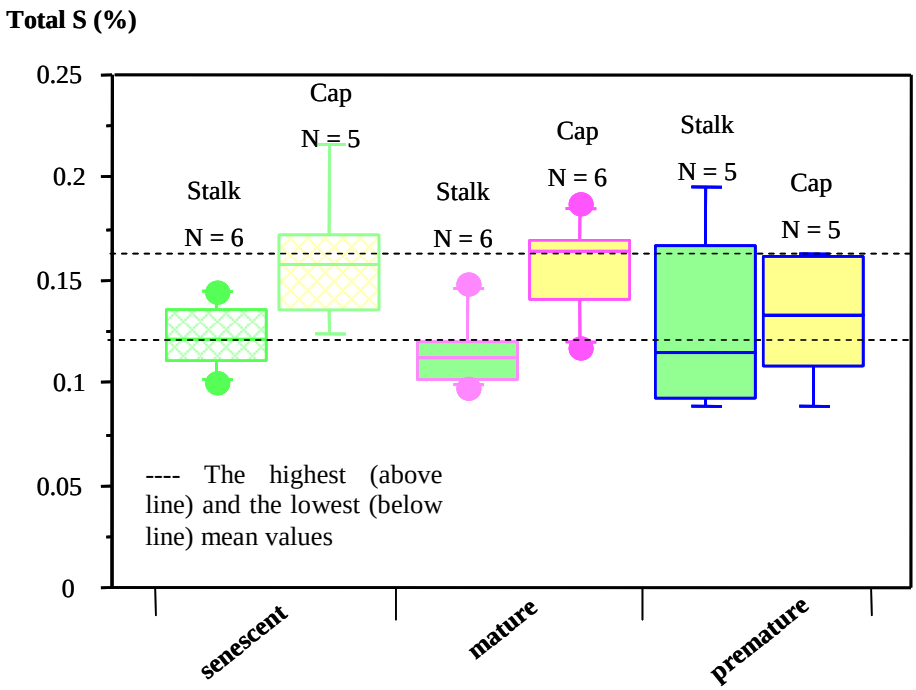


Figure 5.14. Total S (%) contents of the three developmental stages of *Lactarius vellerus* from mainland.

Table 5.20. ANOVA table for total S contents (%) of *Lactarius vellereus* from mainland (Figure 5.14).

Source of variance	DF	SS	MS	F-value	P-value
Type of body part	1	0.006	0.006	6.8	0.0148
Developmental stage	2	0.000	0.000	0.3	0.7539
Type of body part x developmental stage	2	0.002	0.001	1.4	0.2602
Residual	27	0.023	0.001		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,1,27}$ = 4.21; F-value < F-critical
F (critical) $_{0.05,2,27}$ = 3.35; F-value < F-critical
Type of body part: cap, stalk; developemental stage: (early, mature, senescent)

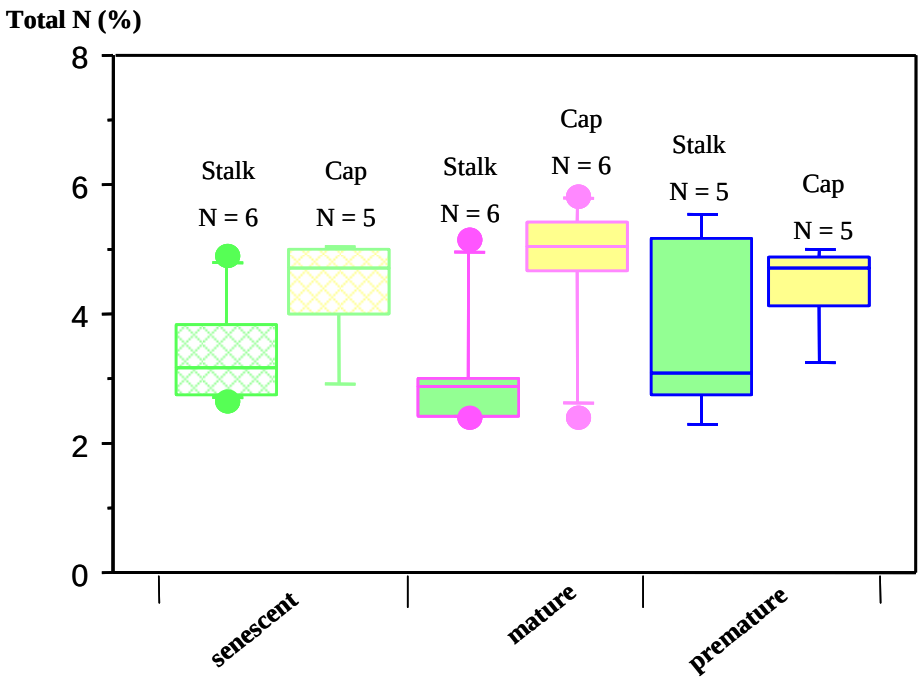


Figure 5.15. Total N (%) contents of the three developmental stages of *Lactarius vellerus* from mainland.

Table 5.21. ANOVA table for total N contents (%) of *Lactarius vellereus* from mainland (Figure 5.15).

Source of variance	DF	SS	MS	F-value	P-value
Type of body part	1	3.1	3.1	0.1	0.7022
Developmental stage	2	2.2	1.1	0.1	0.9497
Type of body part x developmental stage	2	52.2	26.1	1.3	0.3018
Residual	27	562.2	20.8		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,1,27}$ = 4.21; F-value < F-critical
F (critical) $_{0.05,2,27}$ = 3.35; F-value < F-critical
Type of body part: cap, stalk; developemental stage: (early, mature, senescent)

Differences in allocating Hg-binding substrates to the fruiting body were not only related to developmental stage but also to location within the fungal fruiting body, such that there are significant cap/stalk differences in Hg concentrations. Kojo

& Lodenius (1989) suggested that there is, furthermore, an uneven distribution of thiol groups within the cap, decreasing from center to margin, and from plait to cuticle (see also Minagawa et al. 1980). In all developmental stages in this study, THg was found to be higher in caps than in stalks (Figure 5.16). The THg cap / stalk ratios were not significantly different among fungal genus at P-value of 0.0294 (ANOVA: F-value > F-critical; P-value < 0.0001) (Table 5.22). In general, the average cap to stalk ratio of THg concentrations was 1.8:1. The average cap to stalk ratio was highest for *Boletus* and *Suillus* at 2.2:1 and *Leccinum* at 2.1:1. The ratio of 1.9:1 was found for *Amanita*, *Leccinum*, and *Russula*. The ratio was slightly lower for *Chantarrellus*, *Cortinarius*, *Fuscoboletus*, and *Hydnum:* 1.4 - 1.6:1. For species, the highest ratio was 2.9:1 for *Boletus edulis* and *Boletus separans*, and the lowest value was 1:1 for *Cantharellus cibarius*, *Lactarius camphoratus* and *Russula* sp. (Figure 5.16). In *Boletus separans*, the THg was twice as high in the spore-bearing parts than in the flesh of the cap and in the stalk. For the rest of the species/genera, the THg cap/stalk ratio varied from 1.4-2.4:1 (Figure 5.16). Also, in Figure 5.17 the bi-variate scattergram of THg concentration in cap versus THg concentration of stalk of fungal genera showed the cap and stalk concentrations were correlated and caps had higher THg concentrations than stalk ($R^2 = 0.865$).

In total, the multiple regression analysis accounted for 73-77 % of the observed THg variations. The location variable, island, coast, and mainland, also contributed to the overall explanation of the THg concentration variations of the fruiting bodies, but this variable was not as significant as species, S content of tissue, developmental stage, and fungal tissue type (cap versus stalk). The above results

suggested that fungal THg concentrations depended more on tissue-internal factors than on external factors. A part of this result could be due to insufficient sample size from the island (N = 88) as compared to the coast (N = 351), and mainland (N = 288).

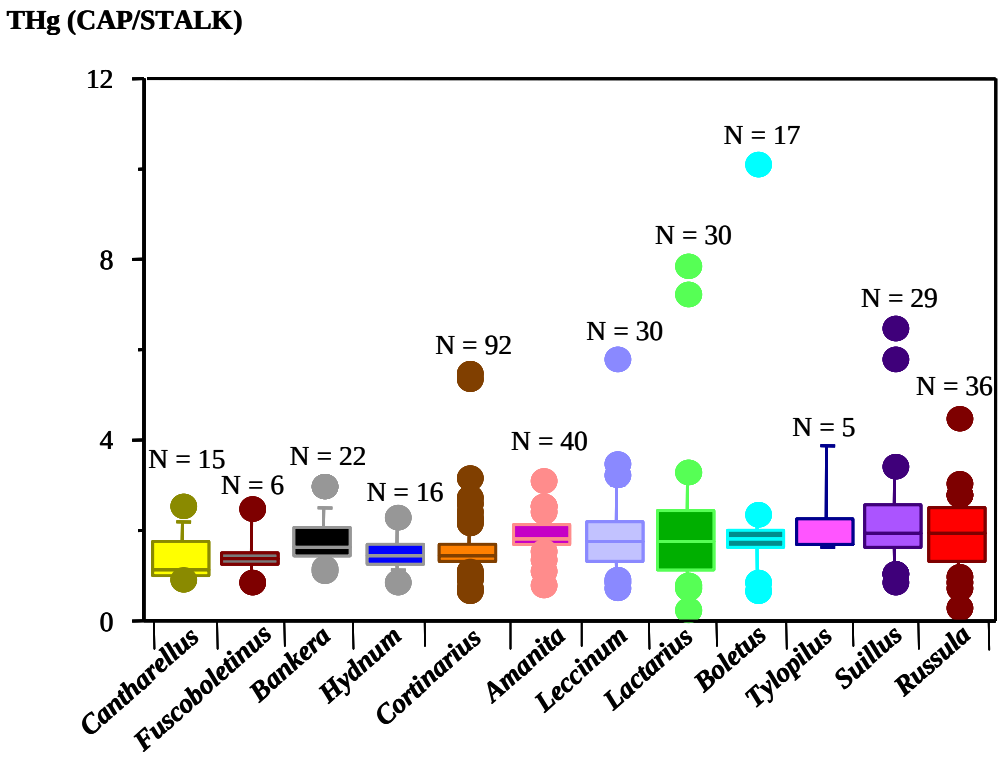


Figure 5.16. THg cap/ stalk ratios, by genus from the three study locations (island, coast, and mainland).

Table 5.22. ANOVA table for THg cap /stalk ratios of fungal genus from the three study locations (island, coast, and mainland) (Figure 5.16).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	21.057	1.914	1.983	0.0294
Residual	324	312.772	0.965		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F (critical) $_{0.05,11,324}$ = 1.83; F-value > F-critical

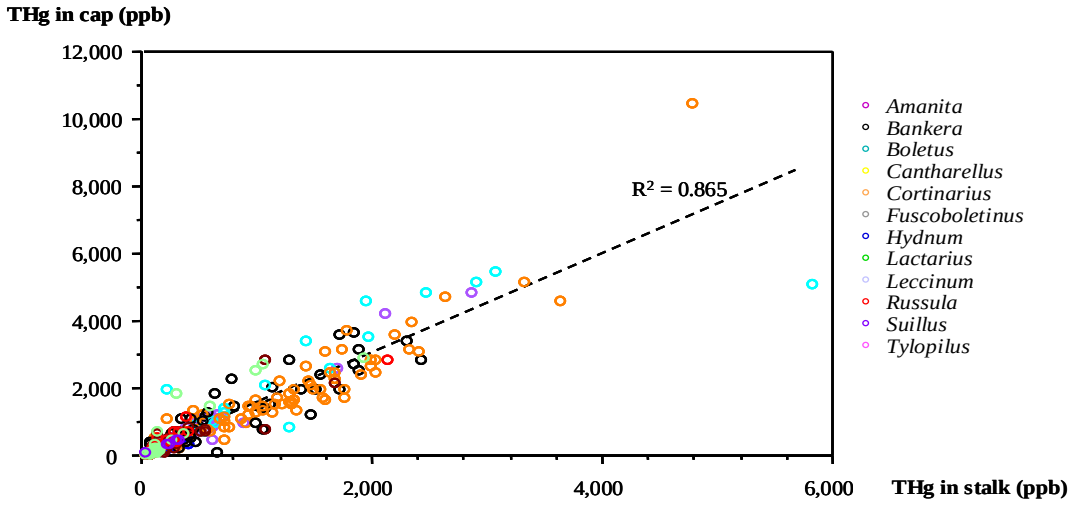


Figure 5.17. Bi-variate scattergram of THg concentrations (ppb, dw) in caps versus THg concentrations (ppb, dw) in stalks, by genus.

CONCLUSIONS

THg concentrations in fungal fruiting bodies varied by taxonomical group (family, genus, and species), and by age, type of body part, elemental S content, and location (island, coast and mainland). Not fully-developed and fully-developed specimens had higher THg concentrations than older specimens, with higher concentrations for caps than for stalks. Elemental S and N contents of the fungal fruiting bodies were positively correlated to the N and S contents, respectively, with two species groups dominating: Group1 with high and Group 2 with low Hg concentrations. The best-fitted model explained 73 % THg concentrations variations in the fungal fruiting bodies, using genus, fungal elemental S content, and developmental stages as independent variables. Adding location (island, mainland shore, interior) as predictor variables increased the explanations only in a minor way, up to 77%.

CHAPTER 6: THg IN MOSS

INTRODUCTION

According to Hall & Louis (2004), mosses are important components of THg retention and cycling in the forest floor. In contrast to fresh forest litter, mosses tend to have elevated THg concentrations within their green tissues (Table 6.1), and retain Hg rather strongly, requiring strong acids and high temperatures for analytical release (Lodenius et al. 2003). On account of this, *Sphagnum* species and *Pleurozium schreberi* have been found to be very effective in bio-monitoring THg in terrestrial environments (Harmens et al. 2004; Lodenius et al. 2003). Moore et al. (1995) reported that THg concentrations in plant tissues increase in the order: wood < grasses and herbs < tree and shrub leaves << mosses < fungal fruiting bodies. Altogether, mossy carpets tend to shield the underlying soil from direct absorption of air pollutants (Gustin et al. 2004).

Mossy carpets are generally not obscured by fresh or decaying forest litter. This is either due to a low leaf-litter inputs where mosses tend to grow, or due to direct incorporation and ingestion of leaf litter into the expanding moss layer (Morrison 2003). THg therefore accumulates in mosses either through retention of precipitation and/or throughfall, through retention of Hg released from decaying leaves and twigs within the moss carpet, or through upward absorption of soil moisture. To what extent leaf litter contributes to the growth of moss, and to what extent the moss itself actively or passively encourages fungal growth and leaf-litter decay and Hg absorption from decaying litter within the mossy carpets is not known.

In principle, mosses would offer a very different C substrate and substrate conditions for mycelia growth than the decaying forest litter, which, in turn, could affect fungal Hg uptake. In the area of this study, four mosses were common, namely *Pleurozium schreberi*, *Polytrichum juniperinum*, *Sphagnum* sp. and *Ptilium crista-castrensis*. Specific characteristics and habits of these mosses are summarized in Tables II C- II D (Appendix II).

The purpose of this chapter is to document elemental concentrations of Hg, S, N, and C in 4 moss species common to the sampling areas of this study, growing in close proximity to the fungal samples that were used to generate the results of Chapter 5. Moss samples were taken to determine whether the presence or absence of moss adjacent to or around the fungal samples would have a measurable influence on the THg concentrations within the sampled fruiting body. Attention in this chapter focussed on three objectives:

- to document and compare THg concentrations in common moss tissues, by species and location (island, coast, and mainland);
- to determine the general association pattern between elemental Hg and elemental S, N, and C contents within the moss tissues; and
- to derive a predictor model for THg concentrations in the mossy tissues as affected by species type and elemental composition in the moss and in the substrates attached to the moss.

METHODS

The spreadsheet containing the data for elemental Hg, S, N and C contents in soil, fungal and mossy samples as described in Chapter 4, and summarized in Figure 4.9, was used for the analysis of this Chapter. Again, elemental Hg, S, N and C of the moss species were plotted by way of box plots, by species and location, and the test statistics were conducted by ANOVA for each box plot. The analysis was followed by multiple regression analysis. In this analysis, THg concentrations of mosses as dependent variable were related to several independent variables such as THg concentrations of underlying soil, elemental S contents of mosses and underlying soil, and moss species. More detailed explanation of data analysis is described in Chapter 4.

RESULTS AND DISCUSSION

171 samples of moss common across the study area were collected. There were 71 *Sphagnum* sp. samples, 46 *Ptilium crista-castrensis* samples, 28 *Polytrichum juniperinum* samples, and 25 *Pleurozium schreberi* samples. THg concentrations were found to vary by species (ANOVA: F-value > F-critical, P-value < 0.0001) (Figure 6.1; Table 6.1). *Pleurozium schreberi* and *Polytrichum juniperinum* had the higher mean THg concentrations at 267 and 185 ppb, dw, respectively. *Sphagnum* sp. and *Ptilium crista-castrensis* had the lower mean THg concentrations at 152 and 79 ppb, dw, respectively.

The mean THg concentrations of *Pleurozium schreberi*, *Polytrichum juniperinum*, and *Sphagnum* sp. were similar to what has already been reported elsewhere (Table 6.2), i.e., from other studies, THg concentrations from about 50 to 280 ppb, dw for *Sphagnum* sp., based on changes from year to year. However lower THg concentrations than this study, were reported for *Pleurozium schreberi* (50 – 200 ppb, dw) and *Polytrichum juniperinum* (50 – 200 ppb, dw) (Table 6.2).

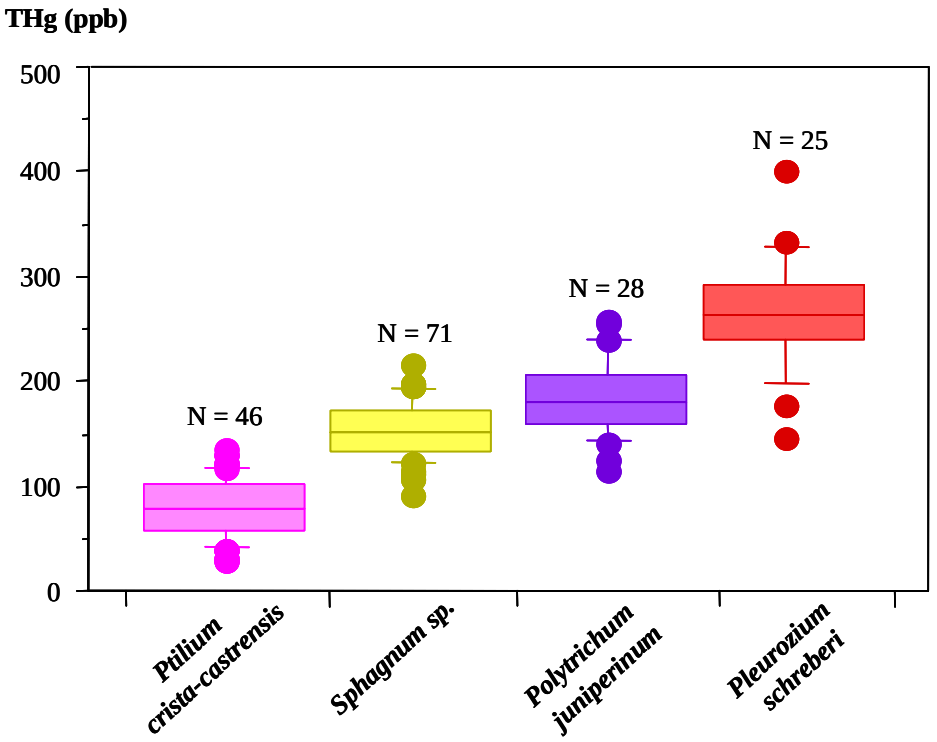


Figure 6.1. THg concentrations (ppb, dw), by moss species.

Table 6.1. ANOVA table for THg concentrations of moss species from the three study locations (island, coast, and mainland) (Figure 6.).

Source of variance	DF	SS	MS	F-value	P-value
Moss species	3	726,049.3	242,016.4	208.1	<0.0001
Residual	166	193,075.0	1,163.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,166}$ = 2.60; F-value > F-critical

The high THg concentrations in the *Pleurozium schreberi* may be explained by:

- its slow-growing but carpet-forming habit on moist and slowly decaying forest litter that could provide Hg for direct absorption,
- the presence of this carpet under dense and dark canopies where the absence of light would limit the extent of the photochemical reductions of Hg^{2+} to $Hg^{(0)}$, and
- the dripping of Hg-enriched throughfall from the forest canopy, where the primary source of this Hg would be due to atmospheric deposition, with the rough canopy conditions being more effective in sequestering atmospheric Hg than the smooth surface conditions of open fields and bogs.

Pleurozium schreberi appears to have a high capacity for rain and throughfall absorption, which also results in fairly high rates of THg absorption (Carter & Arocena 2000). In contrast, *Polytrichum juniperinum* tends to grow in less shaded and dryer micro-sites underneath forest canopies (Carter & Arocena 2000). Having a transportation system in *Polytrichum juniperinum* (Carter & Arocena 2000) eases the transport and accumulation of Hg in the tissue as well as losses of reduced Hg from the upper parts. Also, the green stem of this species grows much longer than other moss species and that could cause Hg dilution with age. S*phagnum* sp. tends to grow under fairly open conditions, often with direct exposure to sunlight, reduction and re-emission of Hg could happen on the surface of this moss tissue. *Ptilium crista-castensis* usually grows on humus, logs, and on the base of trees and absorb lower amount of Hg. *Polytrichum juniperinum* and *Ptilium crista-castrensis* do not have any

strong capacity to retain water. However these two species, compared to *Pleurozium schreberi*, are more limited in this way and rely on moisture and nutrient uptake from the soil underneath. The physiology and habit of mosses presumably contribute to the extent of Hg absorption by moss species. This resulted in categorizing moss species as *Pleurozium schreberi* with high THg concentration, *Polytrichum juniperinum* and *Sphagnum* sp. as intermediate and *Ptilium crista-castrensis* with low THg concentration.

According to the literature, THg concentrations in mosses vary widely, depending on study, species type, and location, or distance from a Hg pollution source (Table 6.2). For example, *Aulacomnium androgynum* that adheres to rocks has high to low THg concentration in its tissue with increasing distances from mining sites (Qiu et al. 2005). Carpi et al. (1994) reported Hg concentrations in *Sphagnum* sp. higher than 200 ppb, dw in the vicinity of an incinerator, dropping to about 100 ppb, dw at greater distances from the source. Lowest THg concentrations (50 ppb, dw for *Sphagnum* sp. and 100 ppb, dw for *Pleurozium schreberi* and *Polytrichum juniperinum)* occurred in years with low Hg deposition via precipitation (Roos-Barraclough et al. 2002).

Based on the sampling procedure for this study, THg concentrations of mosses represented the average concentrations of the whole 5 – 10 cm green portion of the moss stem. However different parts of the moss tissue may have affected the results reported by other authors in Table 6.2. For instance, Gramatica et al. (2006) reported an average THg concentration of 90 ppb, dw for the newly developed green part of *Pleurozium schreberi*. Matilainen et al. (2001) reported a concentration of 284 ppb,

dw in young moss tissue (1 cm stem length). MeHg, a component of THg, has been reported to accumulate at the top of the tissues of *Pleurozium schreberi* and *Sphagnum* sp. In *Pleurozium schreberi*, about 64 % of the accumulated Hg in the upper stem was determined to be MeHg.

Table 6.2. THg concentrations (ppb, dw) of mosses and other plant tissue from different studies. Data from other studies, data from this and other studies; and data from this study.

Plant tissue	≤ 10 ppb	11-50 ppb	50-100 ppb	100-200 ppb	200-300 ppb	Reference
Bryophytes						
Hylocomium slendens						(Berg et al. 2006; Gramatica et al. 2006; Roos-Barraclough et al. 2002)
Pleurozium schreberi						(Evans & Hutchinson 1996; Hall & Louis 2004; Moore et al. 1995; Roos-Barraclough et al. 2002; Sarkela & Nuorteva 1987)[1,2]
Polytrichum juniperinum						(Hall & Louis 2004; Roos-Barraclough et al. 2002)
Ptilium crista-castrensis						This study
Sphagnum sp.						(Carpi et al. 1994; Hall & Louis 2004; Moore et al. 1995)
Lichen						(Hall & Louis 2004; Moore et al. 1995)
Shrubs and herbs						
Alder leaves						(Hall & Louis 2004)
Blueberry leaves						(Hall & Louis 2004)

Table 6.2. Continued…

Plant tissue	≤ 10	11-50	50-100	100-200	200-300	Reference
	ppb	ppb	ppb	ppb	ppb	
Shrubs and herbs						
Bunchberry plants						(Hall & Louis 2004)
Sedge						(Moore et al. 1995)
Labrador tea leaves						(Hall & Louis 2004)
Other litter components						
Litter of the forest floor						(Ericksen et al. 2003; Grigal 2003; Hall & Louis 2004; Lee & Iverfeldt 1991; St Louis et al. 2001)
Fallen coniferous needles and light debris						(Grigal 2003)
Fallen deciduous leaves						(Grigal 2003)
Living birch leaves						(Hall & Louis 2004)
Living pine needles						(Grigal 2003; Hall & Louis 2004)
Living wood blocks						(Grigal 2003; Hall & Louis 2004)
Old wood						(Hall & Louis 2004)
Roots						(Hall & Louis 2004)

[1] Some studies are based on transplanted moss tissue to elevated sites, [2] some authors did not mention the specific species from *Pleurozium.*

Laboratory experiments with one-time exposure of air-borne Hg have shown that mosses absorb Hg readily: THg concentrations decrease after exposure because of:

(1) Growth dilution: *Pleurozium schreberi* growing on soil spiked with ^{203}Hg absorbed this Hg readily, but

(2) High precipitation rates following the initial exposure also decreased the THg concentrations either through leaching and/or by way of Hg^{2+} to $Hg^{(0)}$ reduction (Matilainen et al. 2001).

Mean THg concentrations in the four moss species did not differ significantly by location (ANOVA: P-value = 0.027) (Figure 6.2; Tables 6.3 – 6.4). The test statistics (ANOVA) showed that by excluding *Pleurozium schreberi* (insufficient sample size from island and coast) and *sphagnum* sp. (insufficient sample size from coast), the probability of having different mean THg concentrations in mosses from the three locations was not strong (P-value = 0.027). The insufficient moss sampling on Grand Manan Island and coast of the above moss species was because of not fungi – moss association in island and coast, compared to inland location. In general, however, fog, cloudiness and low temperatures in coastal and high elevation forests are associated with increased Hg retention by mosses (Berg et al. 2006; Evans & Hutchinson 1996).

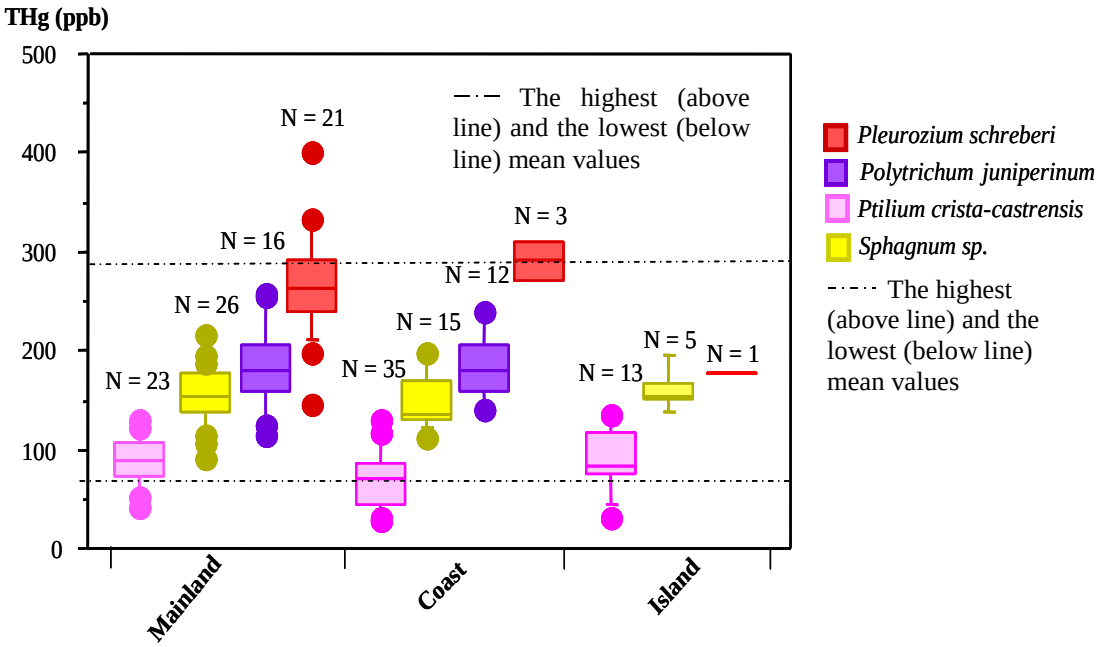


Figure 6.2. THg concentrations (ppb, dw) in mosses, by species and location (island, coast, and mainland).

Table 6.3. THg concentrations (ppb, dw) of mosses, by species and location (island, coast, and mainland).

Species and location	N	Mean ppb	Std. Dev.	Std. Error	Min ppb	Max ppb
Pleurozium schreberi	25	267	54	11	147	400
Island	1	178	N/D	N/D	178	178
Coast	3	290	27	16	263	316
Mainland	21	268	54	12	147	400
Polytrichum juniperinum	28	185	37	7	113	258
Coast	12	185	34	10	141	239
Mainland	16	184	40	10	113	258
Ptilium crista-castrensis	71	79	28	3	28	137
Island	13	89	30	8	32	137
Coast	35	70	27	5	28	130
Mainland	23	88	25	5	41	131
Sphagnum sp.	46	152	27	4	91	215
Iisland	5	160	21	9	139	196
Coast	15	147	27	7	113	197
Mainland	26	153	28	5	91	215

N/D: No Data

Table 6.4. ANOVA table for THg concentrations (ppb, dw) of mosses from the three study locations (island, coast, and mainland) (Figure 6.2).

Source of variance	**DF**	**SS**	**MS**	**F-value**	**P-value**
Location *	2	22,942.0	11,471.0	3.753	0.027
Residual	96	293,427.5	3,056.5		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,2,96}$ = 3.15; F-value > F-critical
Location: island, coast, mainland
* Due to insufficient sample size *Pleurozium schreberi* and *Sphagnum* sp. were excluded from the analysis of this table.

Mean elemental N and C contents did not differ significantly by moss species (ANOVA: P-value (N) = 0.0029, P-value (C) = 0.2799). While mean elemental S contents were significantly different among moss species (ANOVA: F-value > F-critical, P-value <0.0001) (Figures 6.3 – 6.5; Tables 6.5 – 6.8). Mean S, N and C contents of the moss species were 0.15, 1.71 and 50 %, respectively. *Pleurozium schreberi* had the highest total S and N contents at about 0.18 and 1.96. *Ptilium crista-castrensis* and *Sphagnum* sp., had the lowest S and N contents of about 0.14 and 1.54 %, respectively. Vingiani et al. (2004) reported a very high value of 2.6 % for S content, a 2 % value for N content, and a 43 % for C content for *Sphagnum capillifolium.* Bi-variate scattergrams of THg, S, N, and C showed that THg and S contents were related to one another, with the stronger correlations noted for *Sphagnum* sp. (R^2= 0.462) and *Polytrichum juniperinum* (R^2= 0.234), and the weaker correlations noted for *Pleurozium schreberi* (R^2= 0.023) and *Ptilium crista-castrensis* (R^2=0.151) (Figure 6.6). Scattergrams of THg and N, and THg and C showed no correlations between THg and N (R^2= 0.005) and C (R^2= 0.003) contents (Figures 6.7 – 6.8). The THg and N and THg and C scattergrams within species also showed no

correlations (not presented here). Overall, elemental N and C contents of moss species were not significantly correlated with Hg retention in mossy tissue.

Table 6.5. Total S, N and C contents (%) and THg/S and THg/C (ppm) of mosses, by species.

Species	N	Elemental contents	Mean	Std. dev.	Std. error	Min	Max
Pleurozium schreberi	25	S %	0.18	0.04	0.01	0.11	0.26
		N %	2.0	0.5	0.1	1.0	3.0
		C %	49	4	1	39	55
		THg/S (ppm)	151	36	7	76	216
		THg/C (ppm)	0.6	0.1	0.0	0.3	0.7
Polytrichum juniperinum	28	S %	0.17	0.03	0.01	0.12	0.26
		N %	1.6	0.5	0.1	1.0	3.0
		C %	51	4	1	40	55
		THg/S (ppm)	112	21	4	76	157
		THg/C (ppm)	0.4	0.1	0.0	0.2	0.6
Ptilium crista-castrensis	71	S %	0.14	0.03	0.00	0.10	0.25
		N %	1.8	0.5	0.1	1.0	3.0
		C %	50	4	0	42	56
		THg/S (ppm)	59	19	2	23	94
		THg/C (ppm)	0.2	0.1	0.0	0.1	0.3
Sphagnum sp.	46	S %	0.15	0.03	0.01	0.10	0.31
		N %	1.5	0.4	0.1	1.0	2.9
		C %	50	4	1	43	57
		THg/S (ppm)	100	15	2	70	134
		THg/C (ppm)	0.3	0.1	0.0	0.2	0.4

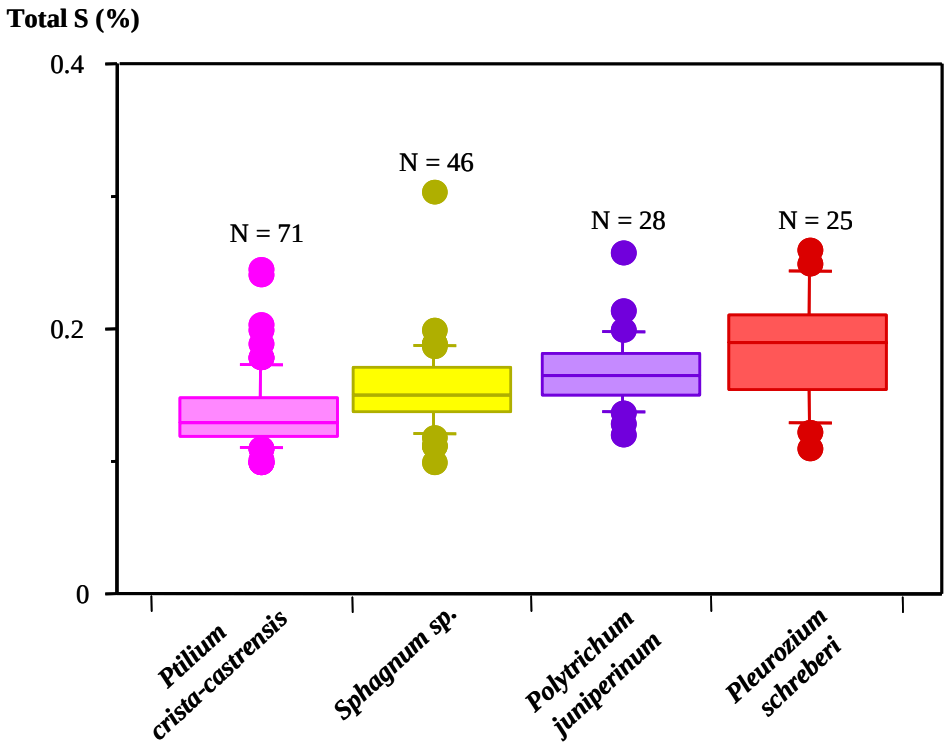


Figure 6.3. Total S content (%) in moss tissue, by species.

Table 6.6. ANOVA table for total S contents (%) of mosses from the three study locations (island, coast, and mainland) (Figure 6.3).

Source of variance	DF	SS	MS	F-value	P-value
moss species	3	0.05	0.02	15.636	<0.0001
Residual	166	0.17	0.001		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,166}$ = 2.60; F-value > F-critical

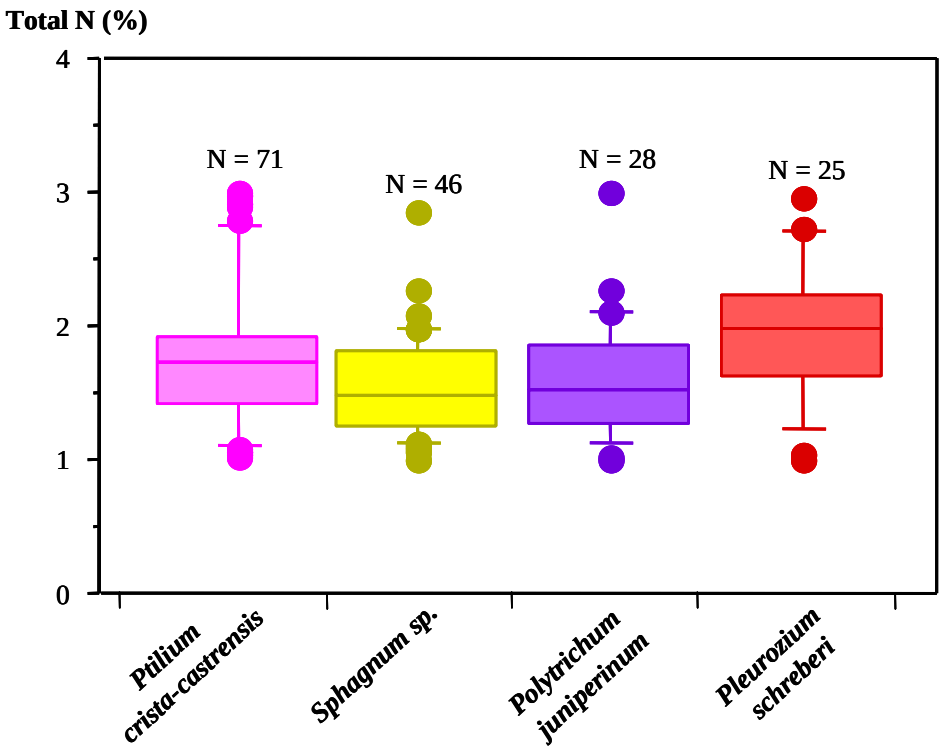


Figure 6.4. Total N content (%) in moss tissue, by species.

Table 6.7. ANOVA table for total N contents (%) of mosses from the three study locations (island, coast, and mainland) (Figure 6.4).

Source of variance	DF	SS	MS	F-value	P-value
moss species	3	3.3	1.1	4.853	0.0029
Residual	166	37.6	0.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,166}$ = 2.60; F-value > F-critical

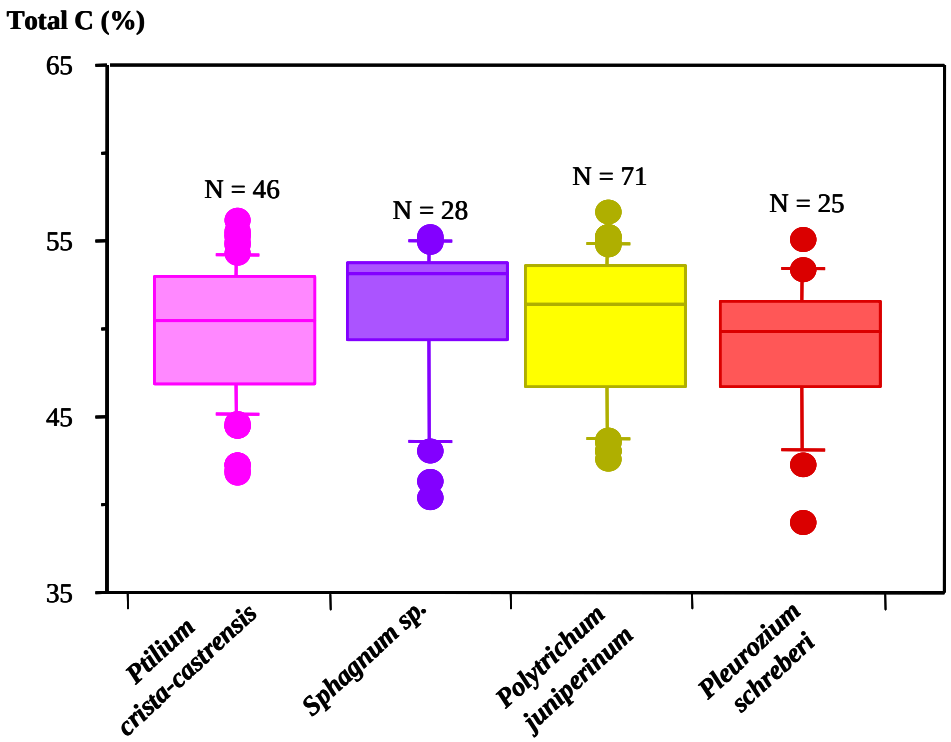


Figure 6.5. Total C content (%) in moss tissue, by species.

Table 6.8. ANOVA table for total C contents (%) of mosses from the three study locations (island, coast, and mainland) (Figure 6.5).

Source of variance	DF	SS	MS	F-value	P-value
moss species	3	57.799	19.266	1.289	0.2799
Residual	166	2,480.743	14.944		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,166}$ = 2.60; F-value < F-critical

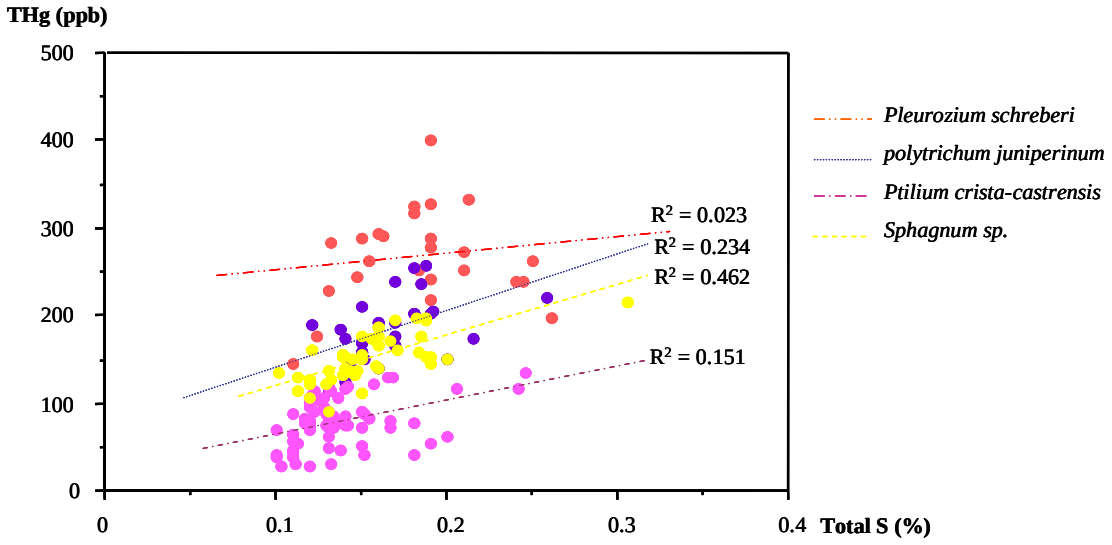


Figure 6.6. Bi-variate scattergram of THg concentrations (ppb, dw) versus total S (%) in mosses, by species.

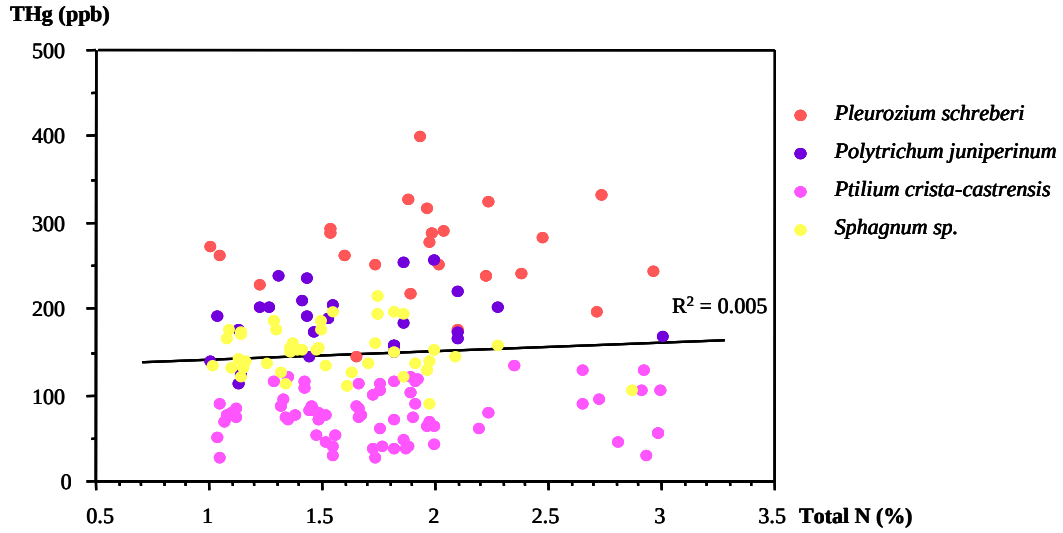


Figure 6.7. Bi-variate scattergram of THg concentrations (ppb, dw) versus total N (%) in mosses, by moss species.

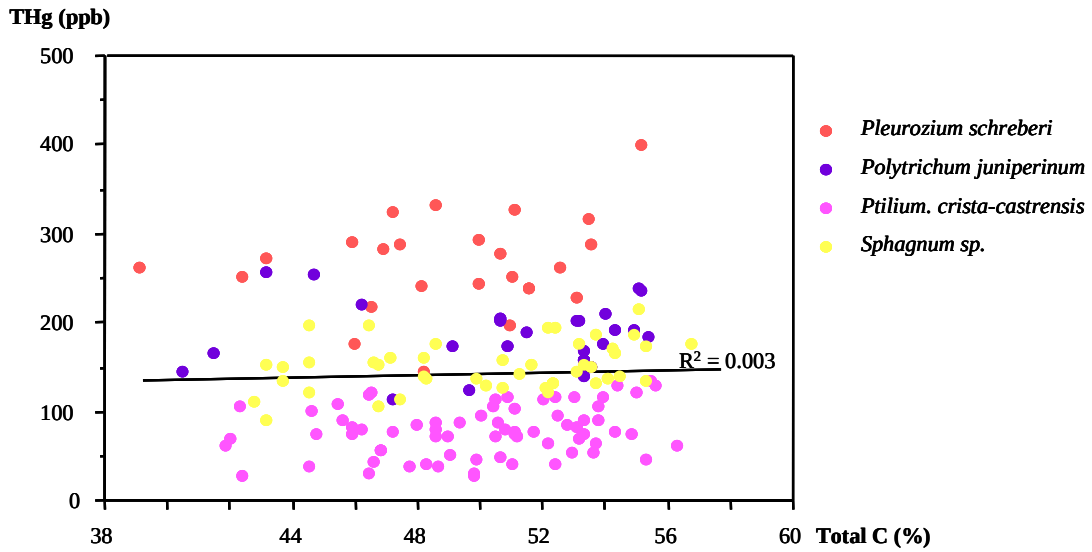


Figure 6.8. Bi-variate scattergram of THg concentrations (ppb, dw) versus total C (%) in mosses, by moss species.

The THg concentrations within the four moss species were positively correlated to the THg concentrations in the soil substrate (F-layer of the forest floor) below the moss layer, with slopes increasing *Ptilium crista-castrensis* (R^2=0.197) to *Sphagnum* sp. (R^2=0.317), *Polytrichum juniperinum* (R^2=0.688), and *Pleurozium schreberi* (R^2=0.553). This is also shown in Figure 6.9 by way of plotting the THg/C ratio of the moss versus the THg/C ratio of the soil substrate below the moss.

Similar, but less definitive, results were obtained by plotting the THg/S ratio of moss versus the THg/S ratio of the soil substrate in Figure 6.10. Here, the strongest correlation was again obtained for *Pleurozium schreberi* (R^2= 0.634). For *Sphagnum* sp. (R^2= 0.276) and *Ptilium crista-castrensis* (R^2= 0.237), the correlations were weaker, and absent altogether for *Polytrichum juniperinum* (R^2= 0.018).

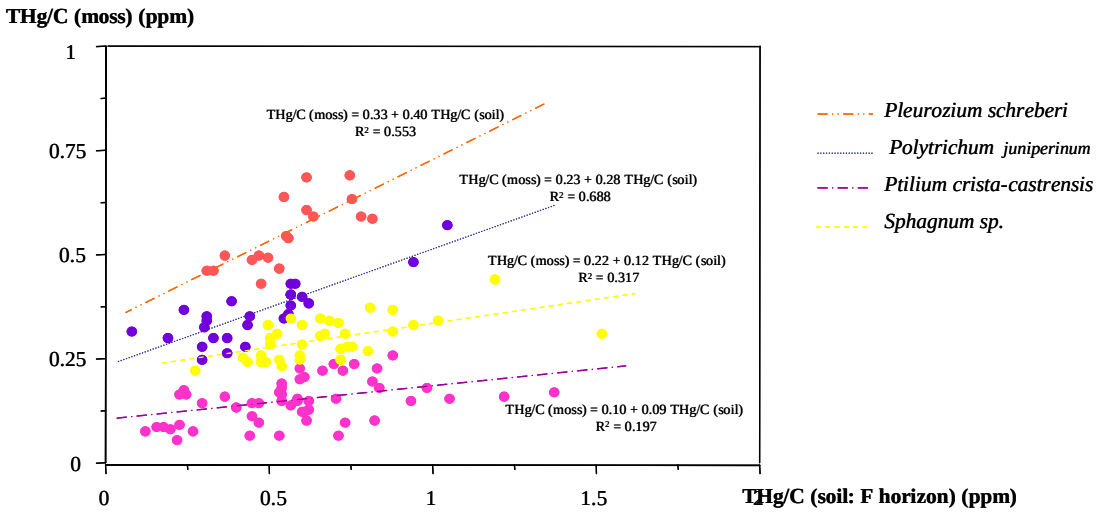


Figure 6.9. Bi-variate scattergram of THg/C (ppm) in mosses versus THg/C (ppm) in F-layer of organic soil, by moss species.

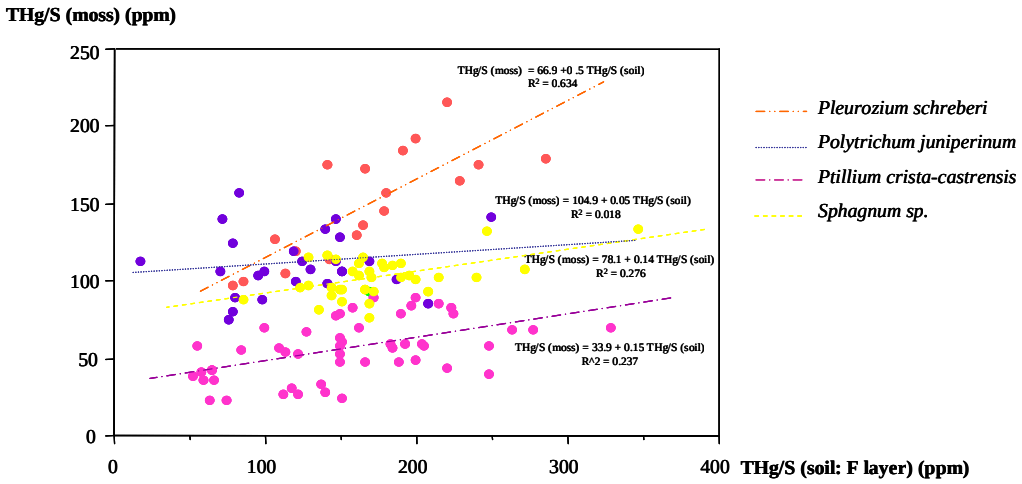


Figure 6.10. Bi-variate scattergram of THg/S (ppm) in mosses versus THg/S (ppm) F-layer of organic soil, by moss species.

The above results suggest that the THg concentrations in the mosses of our sampling areas are likely related to the THg contents of the underlying substrate, which is likely also influenced by continued THg inputs from precipitation or the

canopy via throughfall drip (Gramatica et al. 2006). Mosses, once they receive and accumulate this Hg, tend to pass some of this to the underlying forest floor. In this, the S content of both the mosses and the underlying substrate likely affect the degree of overall Hg retention and release. The lack of correlation between THg/S for *Polytrichum juniperinum* and soil substrate is probably obscured, because of four outliers represented by relatively high THg/S ratios in the moss at low THg/S ratios (<100 ppb, dw S/ %S) of the soil substrate.

Multiple regression analysis was used to determine which variables significantly affect the overall THg concentration variations in the sampled moss tissues in a significant way. The variables included in this analysis were: the elemental S, N, and C contents within the moss tissues and within the underlying soil substrate; length of the moss tissue; and moss species. The best-fitted regression result is summarized in Figure 6.11 and Table 6.9.

Table 6.9. Summary of regression results of THg concentration (ppb, dw) in mosses in relation to the independent variables.

Regression variable	**Reg. coef.**	**Std. error**	**Std. coef.**	**t-value**	**p-value**	**R^2**	**Partial corr.**
Intercept	206.6	15.3	206.6	13.5	<0.0001		
Ptilium crista-castrensis	-178.8	7.7	-1.2	-23.3	<0.0001	0.556	-0.9
Sphagnum sp.	-112.7	7.7	-0.7	-14.6	<0.0001	0.693	-0.8
Polytrichum juniperinum	-69.8	7.5	-0.4	-9.3	<0.0001	0.79	-0.6
THg (F-layer)	0.2	0.0	0.2	6.9	<0.0001	0.879	0.5
Total S (%) (moss)	339.0	76.2	0.2	4.5	<0.0001	0.888	0.4
Total S (%) (F-layer)	-243.2	56.0	-0.2	-4.4	<0.0001	0.903	-0.4

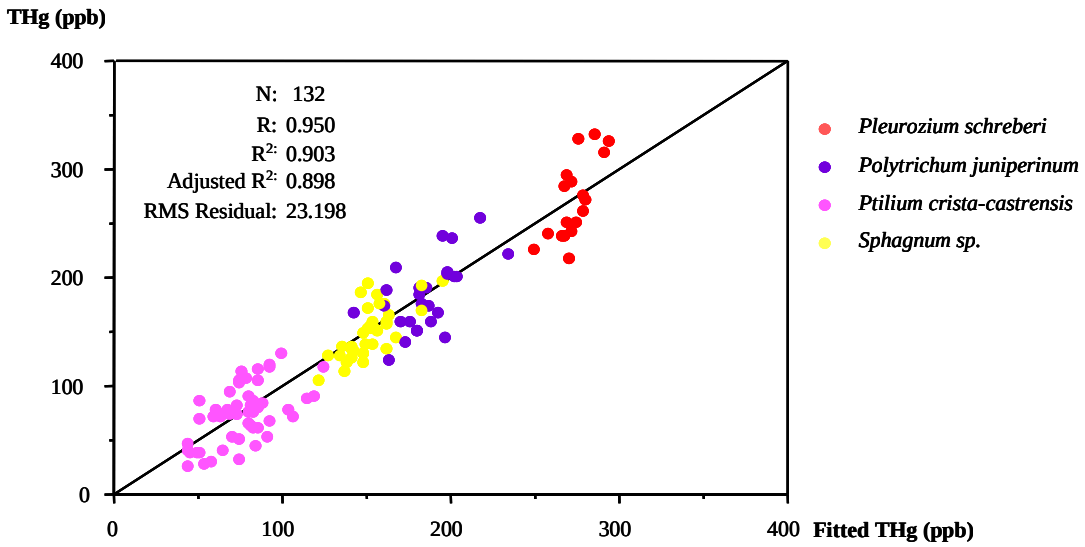


Figure 6.11. Bi-variate scattergram of THg concentrations (ppb, dw) versus fitted THg concentration (ppb, dw) in mosses, by species (Fitted or predicted value is the value of dependent variable that is estimated based on the regression equation. The regression line is the best fitted-line that is drawn through the data points).

Clearly, the best predictor for THg in mossy tissues is the moss species itself, with *Polytrichum juniperinum* (Regression Coefficient = -70), *Sphagnum* sp. (Regression Coefficient = -113), and *Ptilium crista-castrensis* (Regression Coefficient = -179). All of these regression coefficients have a negative sign, indicating that, the contrast between these species and high THg-loading *Pleurozium schreberi*. The next important variable was the THg content of the forest floor underneath the moss layers (regression coefficient = 0.17) followed by the elemental S content of that layer (regression coefficient = - 243) and of the moss tissue itself (regression coefficient = 339).

Apart from the species-affected contributions to the overall THg concentrations in the moss tissues, it is evident that greater THg concentrations in the forest floor are associated with greater THg concentrations in the moss tissues. In

part, higher S concentration of the moss tissues lead to higher THg concentrations as well, while higher S concentrations in the underlying forest floor imply less transfer from the forest floor to the moss.

CHAPTER 7: THg IN THE FOREST FLOOR (L-, F-, H-LAYERS) AND THE UNDERLYING MINERAL SOIL (A-LAYERS)

INTRODUCTION

Mercury accumulates in the forest floor and in the mineral soil below because of a variety of processes:

1) directly or indirectly by way of atmospheric deposition (precipitation, snow, fog, dust, and gaseous absorption) coupled with canopy interception and subsequent throughfall and litterfall;
2) through the weathering of Hg-containing soil minerals; and
3) through Hg recycling, via root uptake, litterfall, and organic matter deposition; this recycling can have many pathways, via uptake by vascular plants, mosses, lichens, and fungi.

As fresh forest litter decomposes, one can expect that the rate of Hg turn-over is considerably slower than the rate of C, and N turn-over, thereby leading to generally increased Hg concentration with advancing state of decay, which would be least in the litter layer (L-layer: first layer or organic soil), higher in the fermentation layer (F-layer: second layer of organic soil), higher yet in the humification layer (H-layer: third layer of organic soil), and highest in the fully humified organic matter of the underlying mineral soil layers such as the organically enriched A-layer. The objectives of this Chapter are:

- to document changes THg concentrations in L-, F-, H-, and A- layers of soil samples in relation to the elemental S, N, and N contents of these layers, and other soil attributes such as presence or absence of mosses on top of the soil, the depth of the organic litter layers, and their locations (island, coast, inland);
- to check whether the trends observed for the study areas match trends already reported in the literature; and
- to derive a model to predict likely THg concentrations in the L-, F-, H-, and A- layers and subsoil layers based on soil layer type, and other soil attributes, through regression analysis.

The pattern of investigation of this Chapter is similar to that of Chapters 5 (Hg in fungal fruiting bodies) and 6 (Hg in moss species common to the study area).

METHODS

The finalized data spreadsheet for elemental Hg, S, N and C contents of the soil layers along with other measured and recorded variables described in Chapter 4 was used again for data analysis. The soil related data are summarized by mean value, standard deviation, standard error, and range in Table III A (Appendix III). Elemental Hg, S, N and C concentrations within the individual soil samples were plotted by way of box plots, by soil layer (L, F, H, A) and location (island, coast, mainland), and the test statistics were conducted by ANOVA, also see Chapter 4. THg concentrations of the soil were also graphed versus S, N and C soil concentrations, by soil layer. Average THg/C, THg/N ratios were plotted against average C/N ratio to determine the extent to which these concentrations and ratios are correlated with one another.

Simple and multiple regression analyses (explained in Chapter 4) were then performed to determine which soil attributes influence the observed soil THg concentration variations the most. In this analysis, THg concentrations of substrate (soil and mosses) as dependent variable were related to several independent variables such as soil layer type, elemental S and C of soil, soil thickness, moss species, and location.

RESULTS AND DISCUSSION

THg concentrations of 659 soil samples were determined: 211 L- samples, 272 F- samples, 35 H-samples, and 136 A- samples. Mean THg concentrations of soil were significantly different among soil layers (ANOVA: F-value > F-critical, $P < 0.0001$) (Figure 7.1; Table 7.1). The organic L-, F-, and H- soil layers had higher THg concentrations than the underlying A–layer (ANOVA: F-value> F-critical, P-value < 0.0001) (Figure 7.1; Table 7.2), with the H- and F-layers averaging THg concentrations at 299 and 260 ppb, dw, respectively. The L-layer had a mean THg concentration of 141 ppb, dw. The A-layer had a mean concentration of 110 ppb, dw.

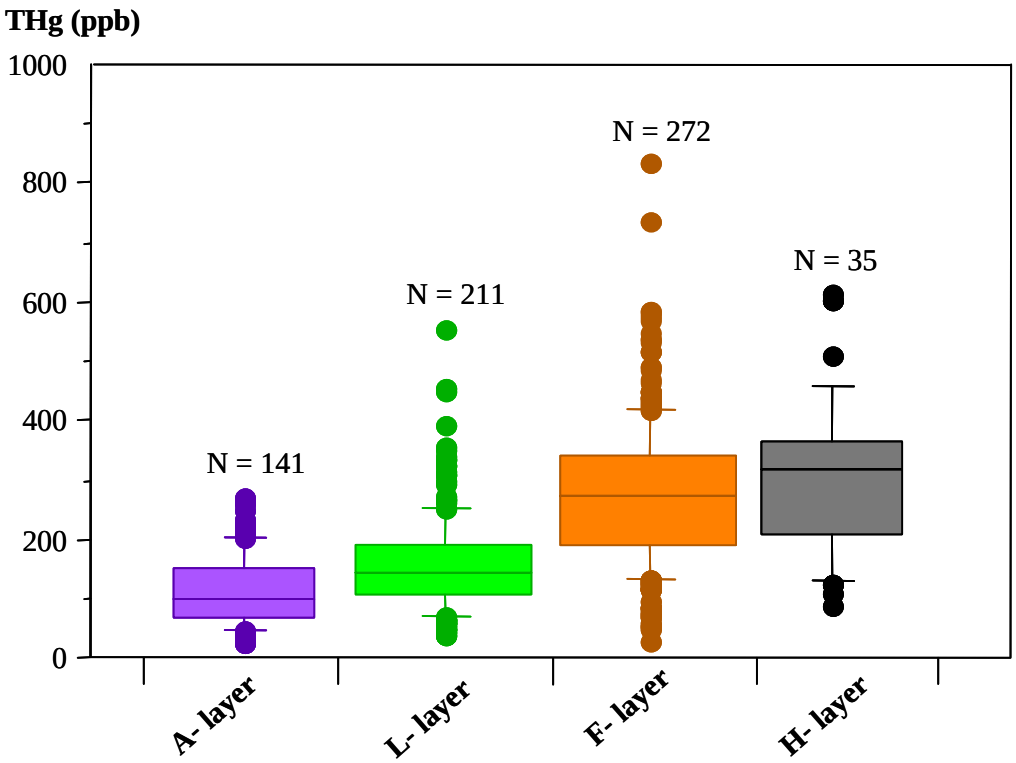


Figure 7.1. THg concentrations (ppb, dw) in soil, by soil layer.

Table 7.1. ANOVA table for THg concentrations of soil layers from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Soil layer	3	3,143,629.4	1,047,876.5	102.802	<0.0001
Residual	655	6,676,521.7	10,193.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,655}$ = 2.60; F-value > F-critical
Soil layer: L, F, H, A

Table 7.2. ANOVA table for THg concentrations (ppb, dw) of organic and mineral soil from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-Value	P-value
Organic / mineral soils	1	1,196,167.2	1,196,167.2	91.127	<0.0001
Residual	657	8,623,984.0	13,126.3		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,1,657}$ = 3.84; F-value > F-critical

In general, THg concentrations followed the order A-layer < L-layer < F-layer < H-layer. A-layer THg concentrations were likely lowest because of its high mineral content: most Hg in this layer would be associated with its organic matter content, or Carbon (C) content (see below). In terms of sampling location, overall soil layers THg concentrations varied such that THg (island) > THg (coast) > THg (mainland). In particular, soil THg concentrations averaged 190 ppb, dw on Grand Manan Island, with a highest mean concentrations of about 270 ppb, dw; in contrast, average THg concentrations were 180 and 170 ppb, dw at the coast and mainland locations, respectively. The test statistics (ANOVA) showed that soil layer and location had significant influences on THg concentrations of soil (F-value > F-critical, P-value < 0.0001) but the soil layer type and location interaction was not significant (P-value = 0.0029) (Table 7.3). This sequence was likely due to the general atmospheric deposition gradient regarding precipitation (mm/year), and wet deposition fluxes (kg/ha/yr) of H, N, S, Ca, Mg, K across the sampling areas (NB Environment Report, see also websites), being highest over Grand Manan Island, lower along the coast, and lowest at the mainland location. At each sampling locations (island, coast, and mainland), THg concentrations increased consistently from the A- to the L-, F- and H-layers (Figure 7.2).

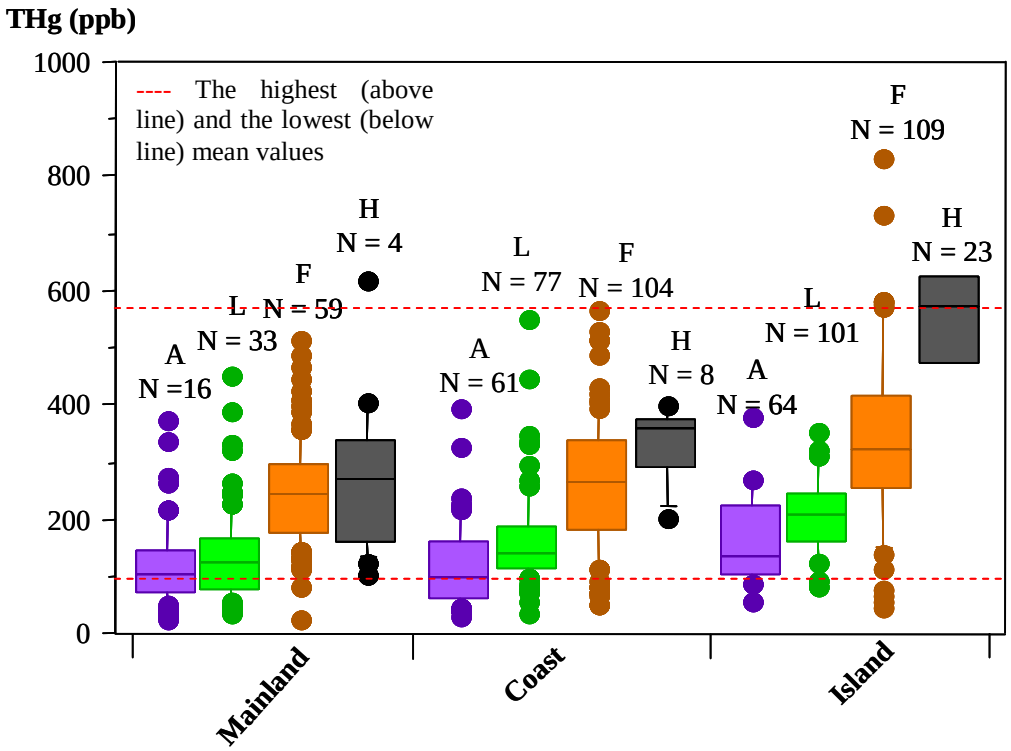


Figure 7.2. THg concentrations (ppb, dw) in soil, by soil layer and location (island, coast, and mainland).

Table 7.3. ANOVA table for THg concentrations of soil from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Soil layer	3	2,763,217.1	921,072.4	101.745	<0.0001
Location	2	563,168.0	281,584.0	31.105	<0.0001
Soil horizon x Location	6	182,065.3	30,344.2	3.352	0.0029
Residual	647	5,857,106.1	9,052.7		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,2,647}$ = 2.2.99; F-value > F-critical
F(critical) $_{0.05,3,647}$ = 2.60; F-value > F-critical
F(critical) $_{0.05,6,647}$ = 2.09; F-value > F-critical
Soil layer: L, F, H, A; location: island, coast, mainland

The ANOVA tables and comparison of THg concentrations of soil layers from the three study locations are shown in Tables 7.4 – 7.9. The significant effect of location on the variation of THg concentrations in L-, F-, and H- layers is shown in Tables 7.4, 7.6, and 7.8 (P-value < 0.0001). A consistent decrease of THg content was for L-layer from island to coast (P-value < 0.0001) (Table 7.5). THg concentrations in F- layer significantly decreased from island to coast to mainland: island versus coast (P-value = 0.0001), island versus mainland (P-value < 0.0001) (Table 7.7). For H-layer the significant effect of location on the variation of THg concentrations was for island versus mainland (P-value = 0.00002) (Table 7.9).

Table 7.4. ANOVA table for THg concentrations of L-layer from the three study locations (island, coast, and mainland).

Source	DF	SS	MS	F-value	P-value
Location	2	143,936.6	71,968.3	12.343	< 0.0001
Residual	208	1,212,803.0	5,830.8		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,2,208}$ = 2.2.99; F-value > F-critical

Table 7.5. Comparison of THg concentrations (ppb, dw) of L- layer from the three study locations (island, coast, and mainland).

Location	Mean Diff.	DF	t-value	P-value
Island vs coast	43.734	108	2.676	0.0086
Coast vs mainland	30.406	176	2.581	0.0107
Island vs mainland	74.14	132	5.108	< 0.0001

DF: degree of freedom; Mean Diff.: Mean difference

Table 7.6. ANOVA table for THg concentrations of F-layer from the three study locations (island, coast, and mainland).

Source	DF	SS	MS	F-value	P-value
Location	2	370,463.5	185,231.7	14.054	< 0.0001
Residual	269	3,545,435.8	13,180.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,2,269}$ = 2.2.99; F-value > F-critical

Table 7.7. Comparison of THg concentrations (ppb, dw) of F- layer from the three study locations (island, coast, and mainland).

Location	Mean Diff.	DF	t-value	P-value
Island vs coast	82.84	160	3.897	0.0001
Coast vs mainland	11.367	211	0.83	0.4074
Island vs mainland	94.207	167	5.003	< 0.0001

DF: degree of freedom; Mean Diff.: Mean difference

Table 7.8. ANOVA table for THg concentrations of H-layer from the three study locations (island, coast, and mainland).

Source	DF	SS	MS	F-value	P-value
Location	2	275,522.3	137,761.1	11.803	0.0001
Residual	32	373,499.9	11,671.9		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,2,32}$ = 3.31; F-value > F-critical

Table 7.9. Comparison of THg concentrations (ppb, dw) of H- layer from the three study locations (island, coast, and mainland).

Location	Mean Diff.	DF	t-value	P-value
Island vs coast	217.741	10	4.598	0.001
Coast vs mainland	64.922	29	1.451	0.1576
Island vs mainland	282.662	25	4.458	0.0002

DF: degree of freedom; Mean Diff.: Mean difference

According to the literature, THg concentrations in soil substrates tend to vary widely, as summarized in Table 7.10. Some of these variations are due to the nature of the studies, which were often conducted in areas with past Hg exposure. In studies that deal with Hg speciation, most of the Hg stored in mineral soils, and poorly drained soils in particular, is in the form of highly insoluble HgS (Revis et al. 1990). However, small amounts are also found in the form of $Hg^{(0)}$, MeHg compounds, and organically complexed Hg^{2+} (Revis et al. 1990). Typically, in this study THg concentrations drop from top-soils to sub-soils by 1 to 2 orders of magnitude, thereby suggesting that Hg in soils is more derived from surface sources than underlying geological sources coupled with soil weathering. Exceptions to this occur in areas underlain by Hg-containing heavy-metal ore bodies. In fact, analyzing the spatial distribution pattern of Hg in sub-soils above such ore bodies has become an important component of geological prospecting procedures to locate and define the extent and heavy-metal content of these geological formations containing these ore bodies (Revis et al. 1990).

Table 7.10. Comparison of THg concentrations (ppb, dw) in soil, by soil type and soil layer from different studies. Data from other studies, data from this and other studies; and data from this study.

Soil layer	≤ 50	50-100	100-200	200-300	≥ 300	Reference
	ppb	ppb	ppb	ppb	ppb	
Podzol soil						
O (F)						(Eriksson 2002; Gladkova & Malinina 2005; Lindqvist et al. 1991)
H						
E (Ae)						
B1 (upper 10cm)						
B2 (10-30 cm depth)						
BC (down to 60 cm)						
C						
Litter (Hardwood stand)						(Taylor et al. 2005)
O						
Upland soil *						
L & F: 0-3.5 cm						(Schwesig et al. 1999)
H: 3.5-7.5 cm						
Ahe: 7.5-20 cm						
B_1:20-40 cm						
B_2: 40-60 cm						
C: 60-80 cm						
Floodplain **						
Forest floor (0-5 cm) & mineral soil (5-30)	Site 1		Site 2		Site 3	(Revis et al. 1990)
Fen *						
Peat-like mor: 0-20 cm						(Eriksson 2002; Schwesig et al. 1999)
Peat-like mor: 20-40 cm						
Peat-like mor: <50 cm						
Ah: 50 – 90 cm						
C: 90-100						
Wet slope						
Peat-like mor: 0-20 cm						(Eriksson 2002)
Peat-like mor: 20-40 cm						
Ah: 50 – 90 cm						

* Affected site by atmospheric Hg emission

** Polluted sites where 90 % of THg in the soil was in a form of HgS.

In terms of S, N, and C contents, there was a significant difference among soil layers (ANOVA: F-value > F-critical, P-value < 0.0001) (Figures 7.3 – 7.5; Tables 7.11 – 7.13). Particularly, there were large differences between S, N, and C contents of the organic (L, F, H) and mineral soil layers of this study (ANOVA: F-value> F-critical, P-value < 0.0001) (Tables 7.14 – 7.16): for the organic layers, C/N/S ratios generally varied in the proportions of 300:10:1, respectively, with a C content at 46 %, which is somewhat less than the 58% C content of pure and fully humified soil organic matter (Bohn et al. 1985). Typical S, N, and C % values in mineral soil were 1 and 0.1 to 0.05 %, respectively (Bohn et al. 1985, Gladkova et al. 2005), but the C, N and S % values of the soils of this study were 10 and to 0.5 and 0.05 %, respectively. Generally, organic matter levels in the A-layers drop, by definition, from the Ah-layer to the Ahe-layer and then to the fully leached Ae-layer. Organic matter is introduced into minerals soils by bio-mixing, root growth and decay, and soil eluviation, or leaching of dissolved organic matter from the upper soil layers into the lower soil layers (Bohn et al. 1985). Figures 7.3, 7.4, and 7.5 show how the S, N, and C % values vary from low to high across the soil layers, with the A-layers generally having the least S, N and C concentrations. The H-layer has lower C concentrations than the F-layer, which also tends to have lower C % values than the L-layer. This sequence is likely dictated by an increased inclusion of mineral soil particles in the H-layer.

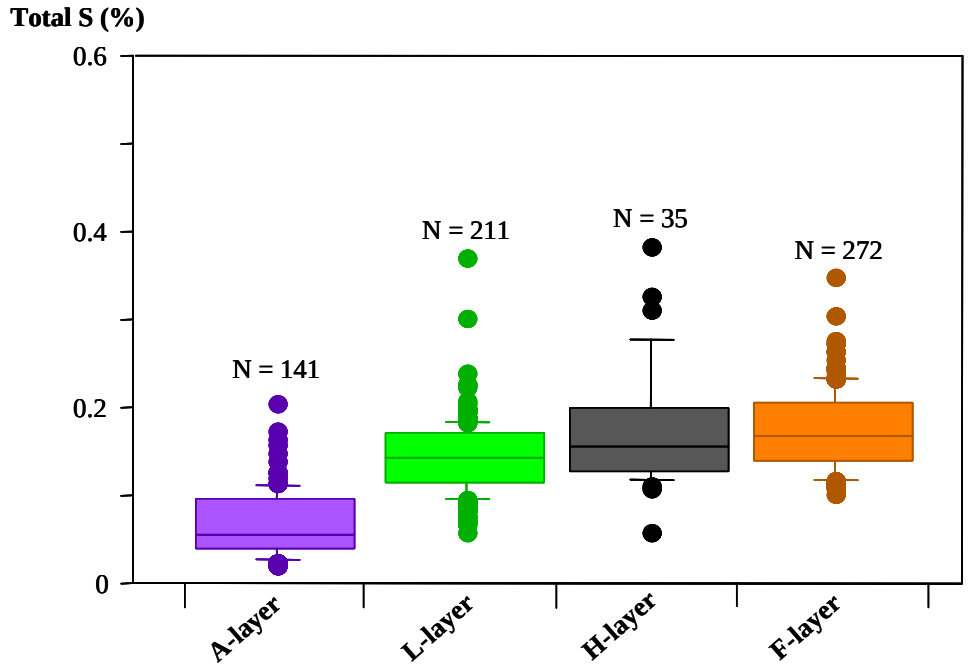


Figure 7.3. Total S content (%) in soil, by soil layer.

Table 7.11. ANOVA table for total S contents (%) of soil layers from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Soil layer	3	1.1	0.4	203.981	<0.0001
Residual	655	1.2	0.0		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,655}$ = 2.60; F-value > F-critical
Soil layer: L, F, H, A

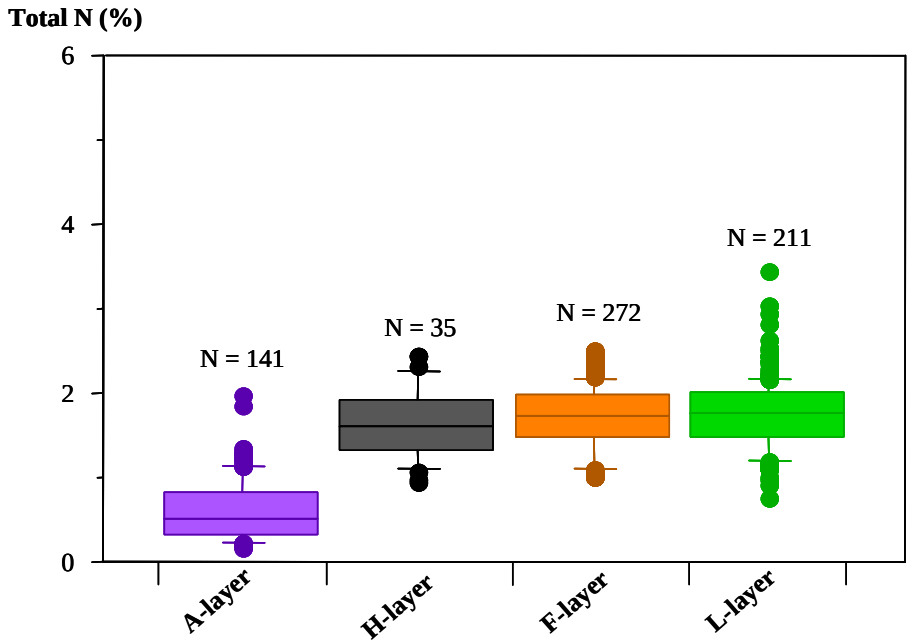


Figure 7.4. Total N content (%) in soil, by soil layer.

Table 7.12. ANOVA table for total N contents (%) of soil layers from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Soil layer	3	137.1	45.7	309.671	<0.0001
Residual	655	96.6	0.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,655}$ = 2.60; F-value > F-critical
Soil layer: L, F, H, A

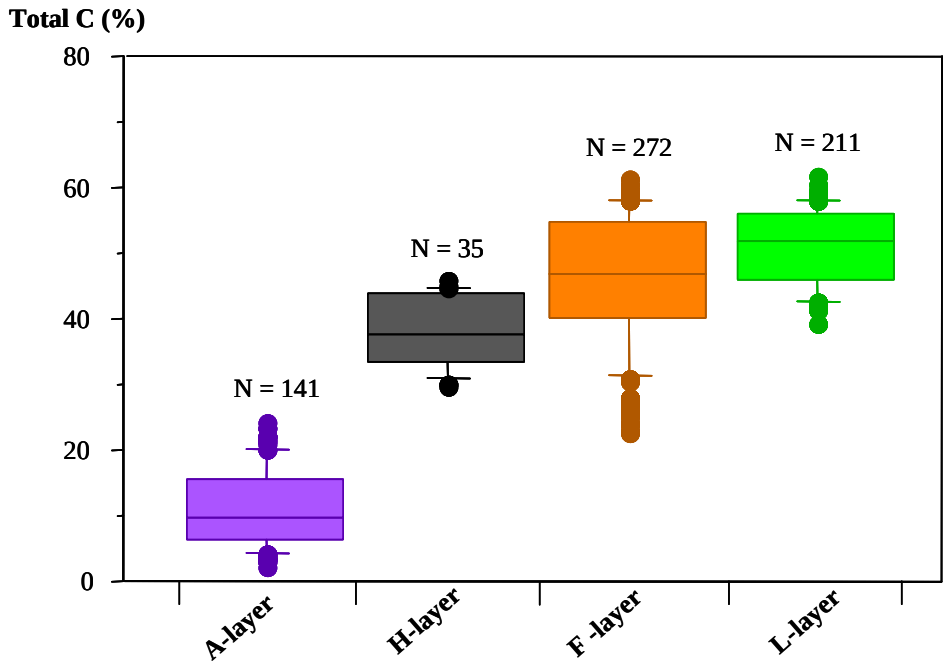


Figure 7.5. Total C content (%) in soil, by soil layer.

Table 7.13. ANOVA table for total C contents (%) of soil layers from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Soil layer	3	151,768.4	50,589.5	869.592	<0.0001
Residual	655	38,105.4	58.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,3,655}$ = 2.60; F-value > F-critical
Soil layer: L, F, H, A

Table 7.14. ANOVA table for total S contents (%) of organic and mineral soil from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Organic / mineral soils	1	1.0	1.0	495.487	<0.0001
Residual	657	1.3	0.0		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,1,657}$ = 2.60; F-value > F-critical

Table 7.15. ANOVA table for total N contents (%) of organic and mineral soil from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Organic / mineral soils	1	136.6	136.6	923.359	<0.0001
Residual	657	97.2	0.1		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,1,657}$ = 2.60; F-value > F-critical
Oranic soil: L-, F-, H- layers; mineal soil: A- layer

Table 7.16. ANOVA table for total C contents (%) of organic and mineral soil from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Organic / mineral soils	1	145,946.5	145,946.5	2,182.9	<0.0001
Residual	657	43,927.3	66.9		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square
F(critical) $_{0.05,1,657}$ = 2.60; F-value > F-critical
Oranic soil: L-, F-, H- layers; mineal soil: A- layer

THg concentrations in the L-, F-, H-, and A-layers were weakly correlated with S, N, and C contents (Figures 7.6 – 7.8). THg and S correlation were weak (R^2= 0.153) across the soil layers (Figure 7.6). THg and N correlations was much stronger for L- and A- layers (R^2 = 0.202) than for F- and H- layers (R^2 = - 0.058), and THg and C correlations was stronger for F-, H-, and A- layers (R^2 = 0.416) than for L-layers (R^2 = - 0.053). The stronger correlation between THg and S than N and C underscores the observation that S contributes, at least in part, to Hg retention within the L-, F-, H-, and A-layers, mostly involving Hg-S-organic matter bonds in humic materials.

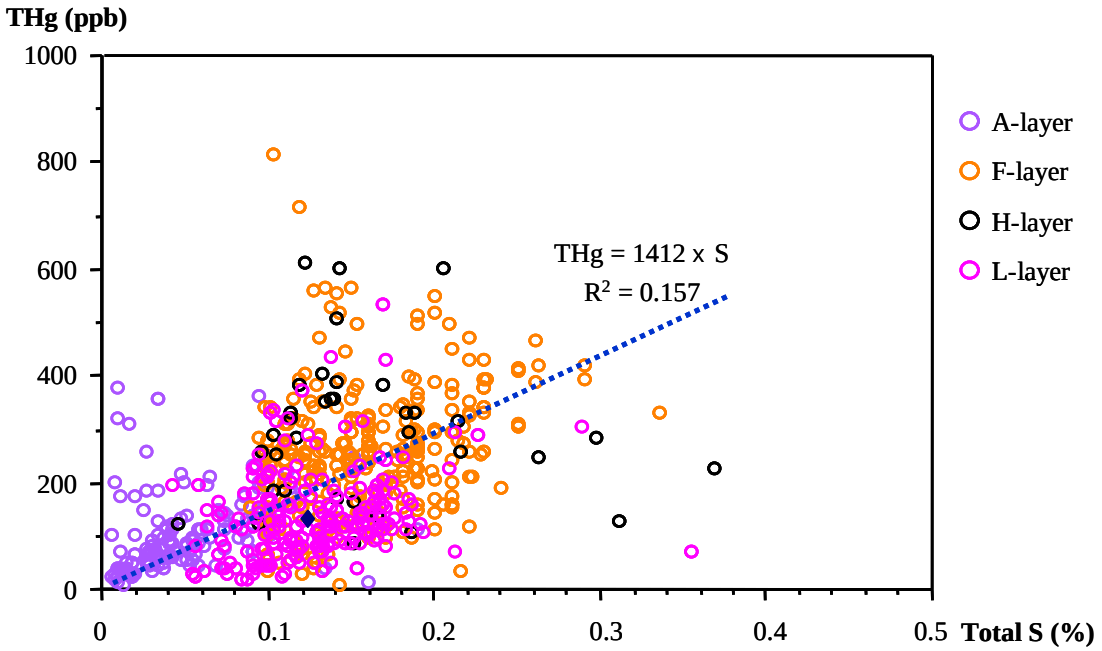


Figure 7.6. Bi-variate scattergram of THg concentrations (ppb, dw) versus total S content (%) in the soil.

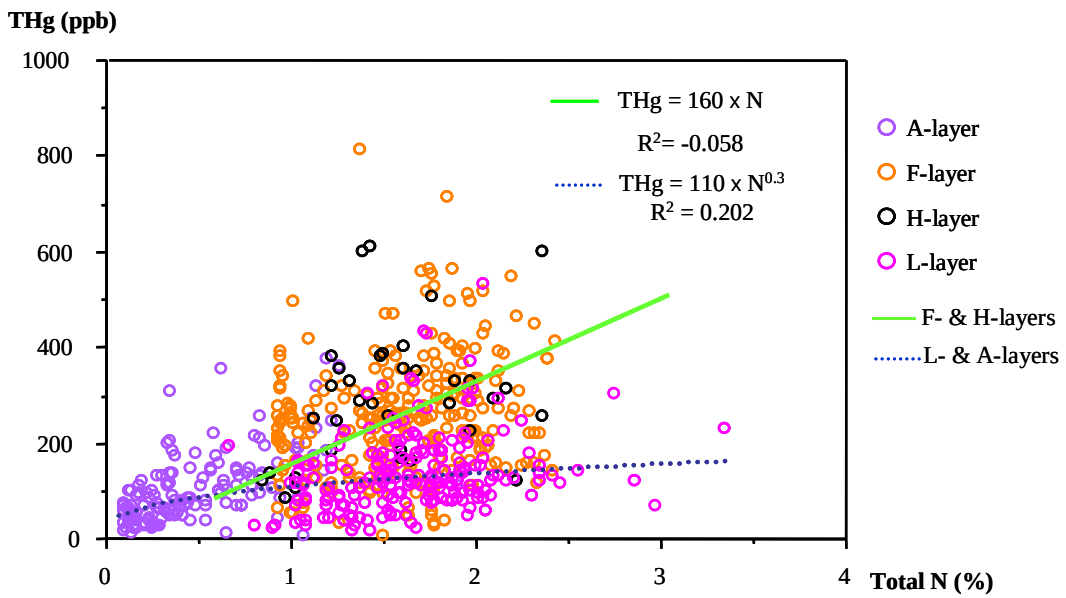


Figure 7.7. Bi-variate scattergram of THg concentrations (ppb, dw) versus total N content (%) in the soil (R^2 or coefficient of determination is defined as (1-SSE/SST) where SSE is an explained sum of squares and SST is the total sum of squares. As directed above the minus R^2 is as the result of high variability of residuals around the trendline, compared to the overall variability. In this case, the relationship between Y and X are very week and negligible).

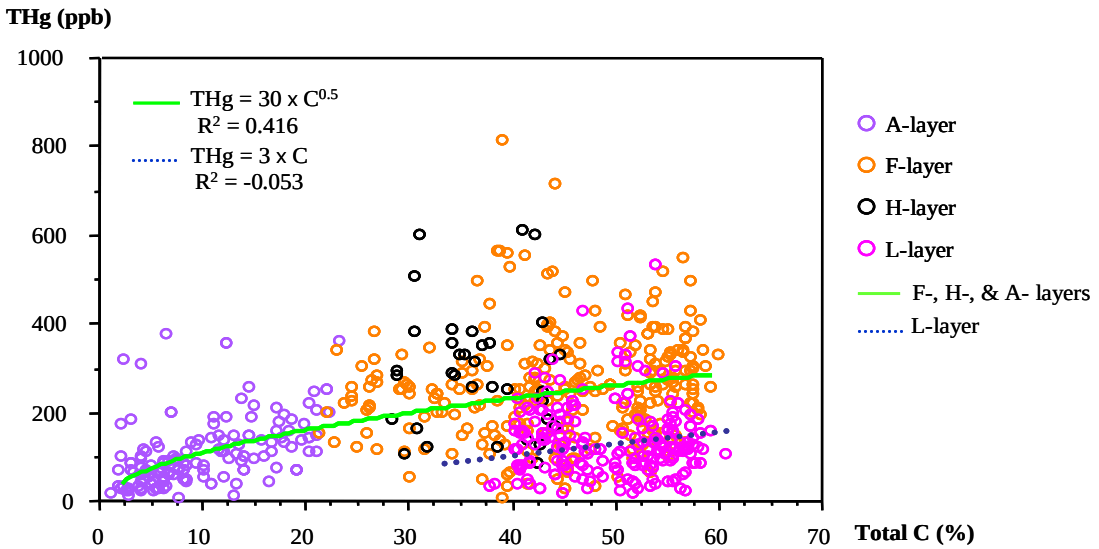


Figure 7.8. Bi-variate scattergram of THg concentrations (ppb, dw) versus total C content (%) in the soil (R^2 or coefficient of determination is defined as (1-SSE/SST) where SSE is an explained sum of squares and SST is the total sum of squares. As directed above the minus R^2 is as the result of high variability of residuals around the trendline, compared to the overall variability. In this case, the relationship between Y and X are very week and negligible).

The S content of the L-, F-, H-, and A-layers is in itself were correlated with the N and C concentrations of these layers, as demonstrated by the bi-variate S versus C and S versus N plots in Figures 7.9 and 7.10. In turn, N is also closely related to C (Figure 7. 11). All of these correlations are simply due to the general make-up of fresh to humified soil organic matter, with similar correlations existing for plant organic matter in general.

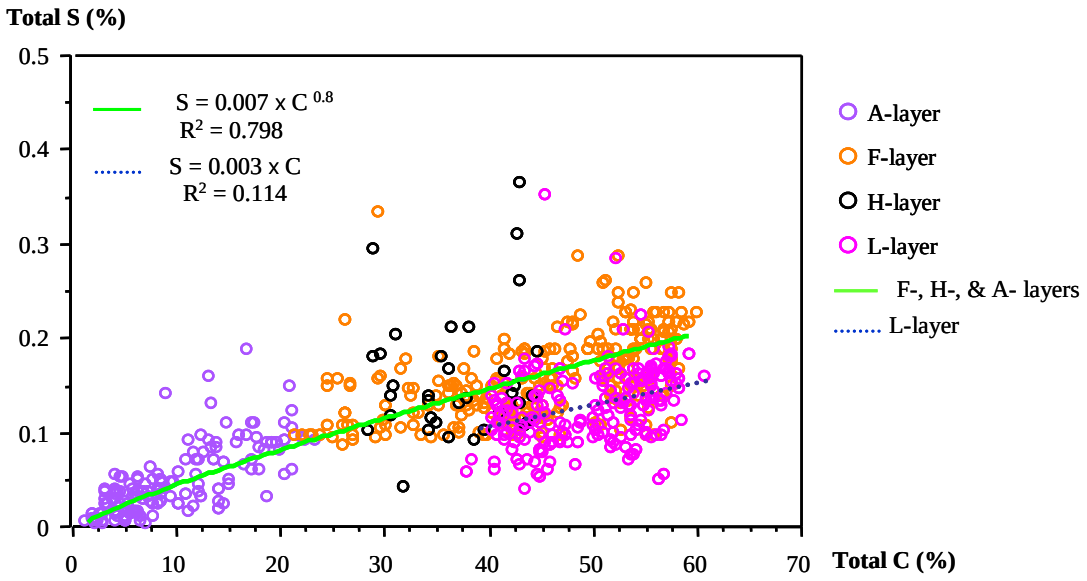


Figure 7.9. Bi-variate scattergram of total S content (%) versus total C content (%) in the soil.

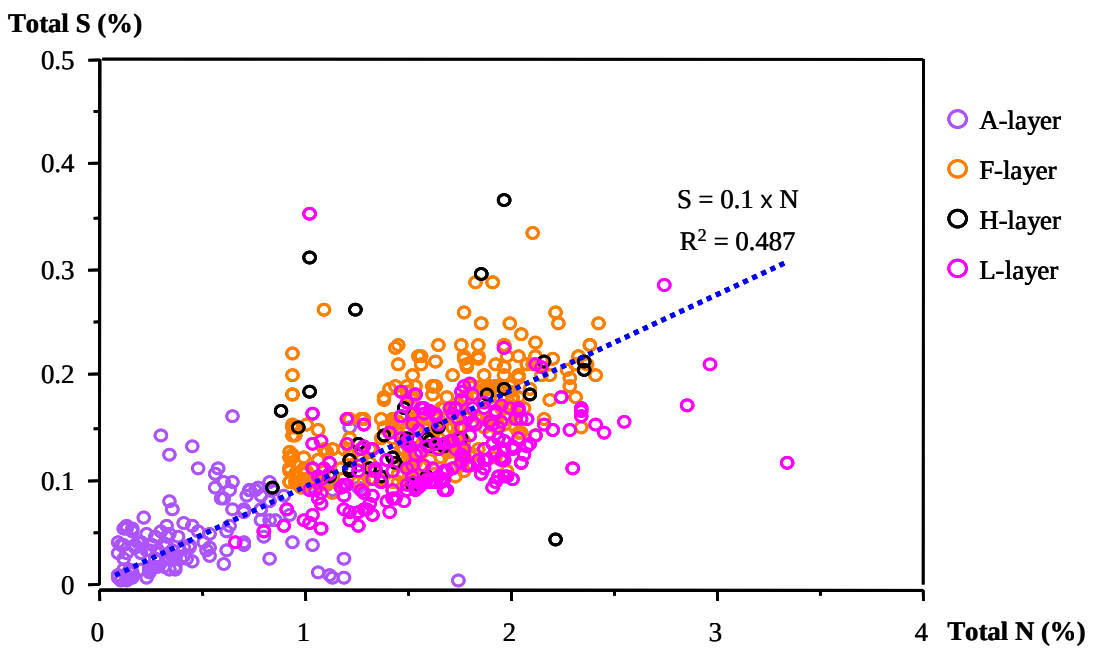


Figure 7.10. Bi-variate scattergram of total S content (%) versus total N content (%) in the soil.

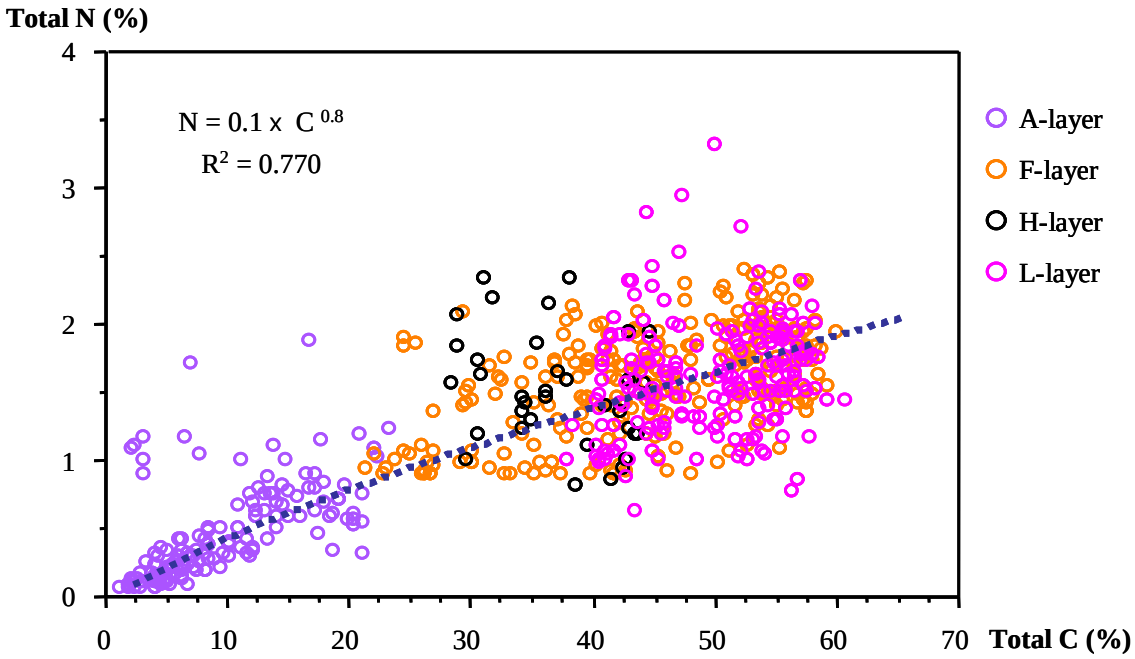


Figure 7.11. Bi-variate scattergram of total N content (%) versus total C content (%) in the soil.

Overall, the THg concentrations and the THg/C and THg/N ratios of the L-, F-, H-, and A-layers appear to be functionally related to

(1) the state of organic matter humification and

(2) the slower turnover rates of S and N versus C, and of Hg versus S and N,

Average L-, F-, H-, and A-layer values for Hg, S, N, C, THg/C, THg/N, and THg/S ratios are listed in Table 7.17 for this study, and also for a similar study conducted by Gladkova & Malinina (2005) at the Central Forest Reserve near the centre of the European part of Russia (southwestern part of the Valdai Hills; Tver region, Nelidovo and Andreapol districts; http://www.clgz.ru/geo_en.htm). Plotting the particular values of THg/N versus C/N and THg/C versus C/N revealed straight lines for each study (Figures 7.12 - 7-13). In turn, these particular equations can then be used to predict average THg concentrations in the L-, F- and H-, and A-layers,

Table 7.17. Average elemental composition of Canadian (New Brunswick) and Russian (Tver Region) soil, by soil layer.

Soil layer	Exp. THg	Cal. THg **	C	N	S	C/N	C/S	THg/C	THg/N	THg/S
	ppb	ppb	%	%	%			ppm	ppm	ppm
CANADA										
L	141	161	49.5	1.6	0.13	31.8	419	0.3	8.7	116
F	260	248	44.9	1.6	0.16	29.1	292	0.6	17.0	169
H	299	338	36.8	1.5	0.16	26.2	267	0.8	19.9	212
A	110	139	9.9	0.5	0.05	23.7	235	1.5	28.3	350
B1	90	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
B2	61	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
RUSSIA										
L	97	117	42.9	1.6	0.09	32.3	1271	0.3	7.5	130
F	268	196	37.1	1.8	0.09	24.8	1110	0.5	11.2	218
H	284	299	28.2	1.5	0.08	21.8	941	1.1	19.8	374
A1A2	79	81	4.3	0.3	0.07	16.2	164	1.9	26.1	116
A2B	34	40	1.3	0.1	0.04	15.5	89	3.0	42.1	100
B1	30	44	1.1	0.1	0.05	16.0	59	4.0	55.0	88
B2	18	23	0.5	0.1	0.04	11.9	34	4.4	45.0	56
C	7	8	0.1	N/D	0.06	N/D	3	11.4	N/D	13

* in the thee study locations of this study (Canada), all data for each layer were combined.
** Canadian THg concentrations were calculated by equation 3.
N/D: NO DATA

provided the C/N ratio is known, as follows (for the data of this study, A , L, F, and H-layers only):

THg (ppb) = TN (g/kg) (81.21- 2.26 C/N), or,

THg = TC (4.67- 0.14 C/N).

While both studies revealed straight lines for THg/N versus C/N and THg/C versus C/N, it is important to realize that the slopes of these equations differed, such that there appears to be a greater amount of THg retention versus C or N retention in the sampling areas of this study versus the sampling area of the Gladkova & Malinina

(2005) study. This difference could possibly be due to differences in the rate of organic matter decomposition such that slower rates, due to a cooler climate, would lead to lower slopes, and vice versa. Average climate conditions for the area of this study and that of Gladkova & Malinina (2005) study are as follows (Table 7.18):

Table 7.18. Climate conditions of New Brunswick and Tver Region.

Climate condition	**New Brunswick ** (Canada)**	**Tver Region ** (Russia)**
Climate	warm continental – marine climate	moderately continental
Average winter temperature (^{o}C)	(-7) - (-12)	-12
Average summer temperature (^{o}C)	+ 20	+ 22
Rainfall (mm/yr)	1000 – 1200	700
Fog event (days/summer month)	4 – 5 days *	N / C

* in the fall, fog events usually occur during night time and the duration of fog event lasts 6-8 hrs especially in Grand Manan Island and New Brunswick southern coastal regions.
** data are adapted from http://atlantic-web1.ns.ec.gc.ca/.
*** data are adapted from http://www.clgz.ru/geo_en.htm.
N / C: Not Considered as important climatic issue.

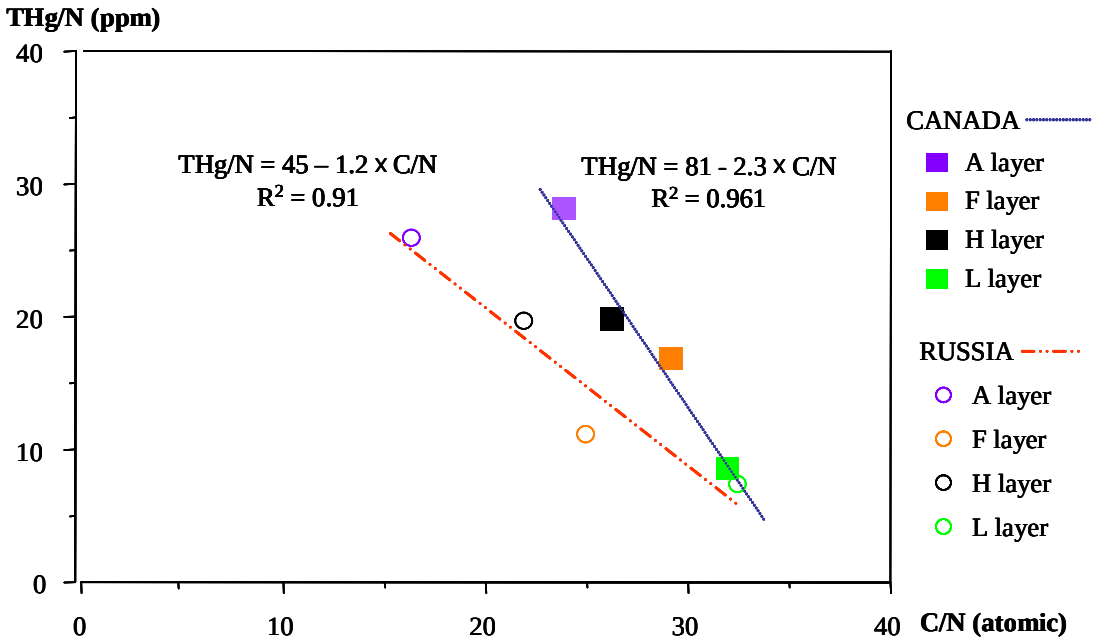


Figure 7.12. Bi-variate scattergram of THg/N (ppm) versus C/N (atomic) in Canadian and Russian podzol soil.

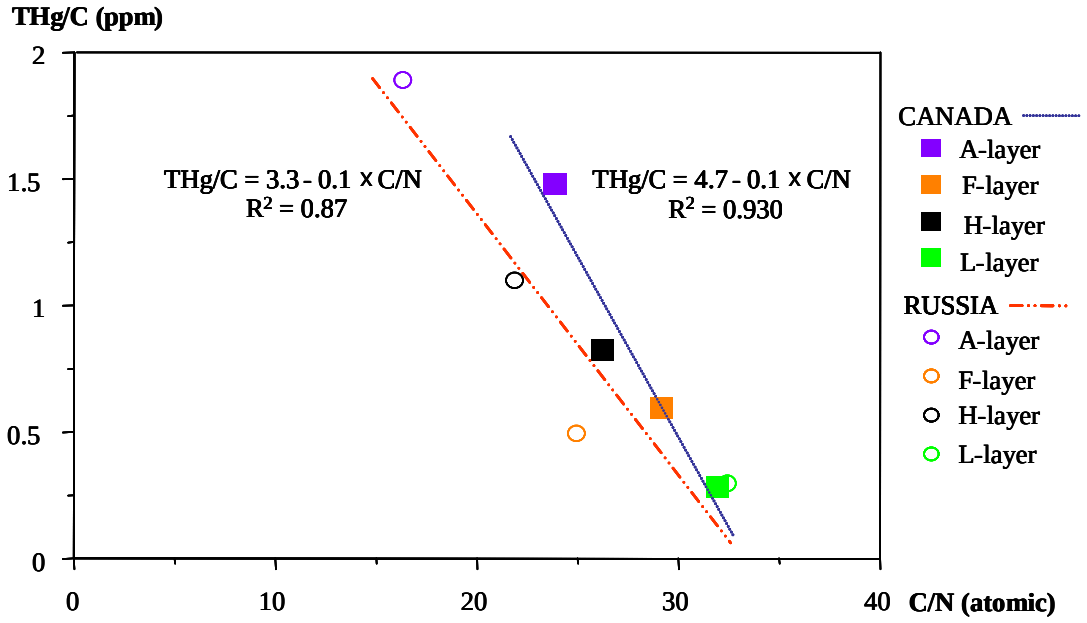


Figure 7.13. Bi-variate scattergram of THg/C (ppm) versus C/N (atomic) in Canadian and Russian podzol soil.

By using equation THg = TC (4.67- 0.14 C/N), a very close relationship was found between experimental and calculated THg concentrations in the soil (R^2 = 0.942) (Fig. 7.14, Table 7.17). This result revealed that calculated values for THg concentrations were good approximates for Hg distribution in organic soil and A-layer of mineral soil. Although this study focussed on analyzing THg in L-, F-, H-, and A-layers, to estimate the retention or loss of deposited Hg to the soil it is important to extend the analysis of THg concentrations in the lower soil layers as well. Fortunately, the Gladkova & Malinina (2005) study also included THg, C, N, and S data for these lower-lying soil layers, and the resulting values are shown in Table 7.17. Inspecting these values relative to the L-, F-, H-, and A-layers values revealed steady decreasing THg, C, and N values with increasing soil depth. At the same time, C/N and C/S ratios also decreased systematically while S values did not change much from the A- to the C- layer. In contrast, and as to be expected, the THg/C and THg/N ratios are seen to increase strongly with soil depth, while the THg/S ratio increases towards a maximum in the H-layer, and then decreases again towards the C layer. A simple and best-fitted model that quantified the listed average THg concentration variations in the mineral soil layers from A- to C- layer is given in:

$$THg = 35.074 \times (C\ \%)^{0.54},\ R^2 = 0.989 \text{ (Figure 7.15, Table 7.12)}$$

which suggests that THg in the mineral soil remains mostly associated with the organic matter fraction which in turn has its own N and S content, as opposed to the total S content of the mineral soil. In fact, the THg values in Table 7.17 do not,

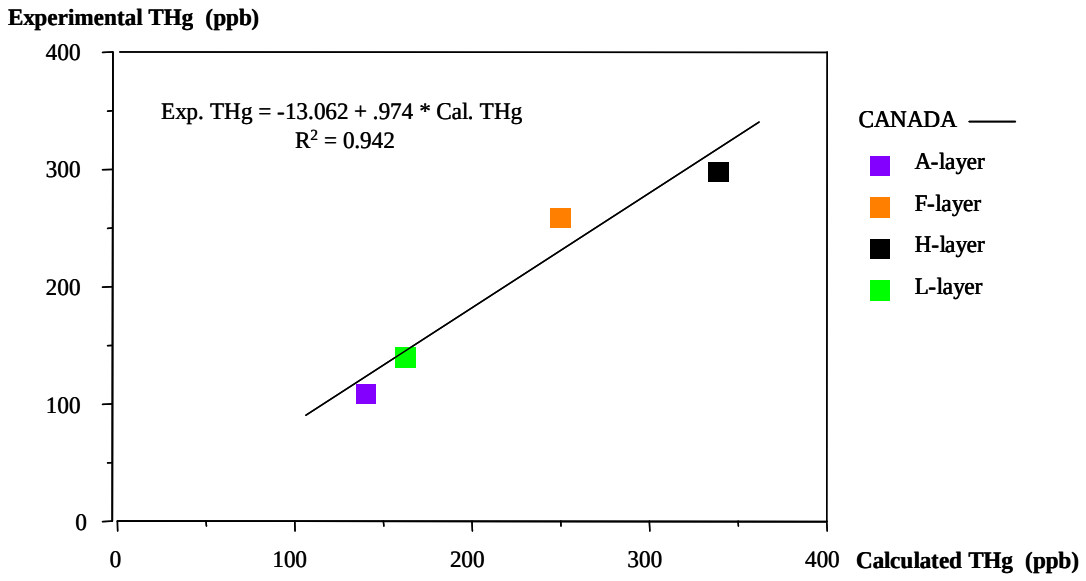


Figure 7.14. Bi-variate scattergram of experimental versus calculated THg concentrations (ppb, dw) in the Canadian podzol soil.

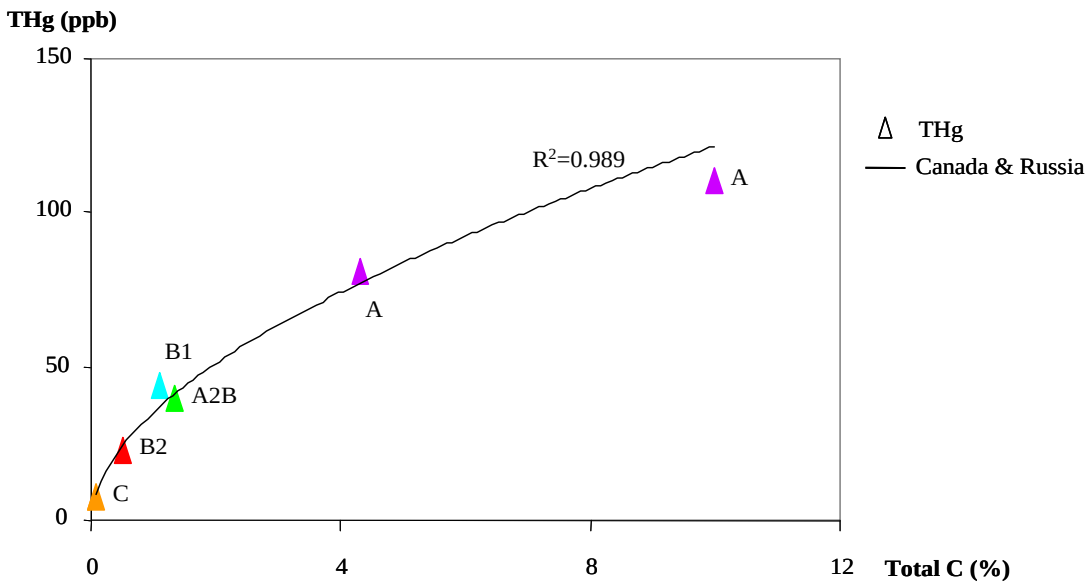


Figure 7.15. Bi-variate scattergram of THg concentration (ppb, dw) versus total C content (%) in the mineral Canadian & Russian soil.

correlate significantly to the S % content of the mineral soil layers from A- to C- (R^2 = 0.0102). This, in turn, means that organically bound S remains organically bound, even though there may be sufficient S in the soil to bind Hg as Hg-S. To some extent,

this may be due to the drainage condition of the soil: in poorly drained soil, most Hg tends to be associated with Hg-S, but this does not appear to be the case in well drained soils, where organic S is likely oxidized, thereby unable to bind Hg in the mineral Hg-S form.

Apart from soil layer type, soil THg concentrations are also affected by the soil layer depth of the forest floor: the deeper the depth, the lower is the THg concentration, as shown in Figure 7.16. Again, this likely relates to the overall turn-over rate of organic matter: deeper layers have lower turn-over rate, and therefore do not concentrate THg as much as thinner layers. In this regard, the L-, F-,H- and A-layers all follow the same curvilinear trend, except that the THg concentrations for the L-layer are about one half lower than the corresponding THg concentrations in the other three layers.

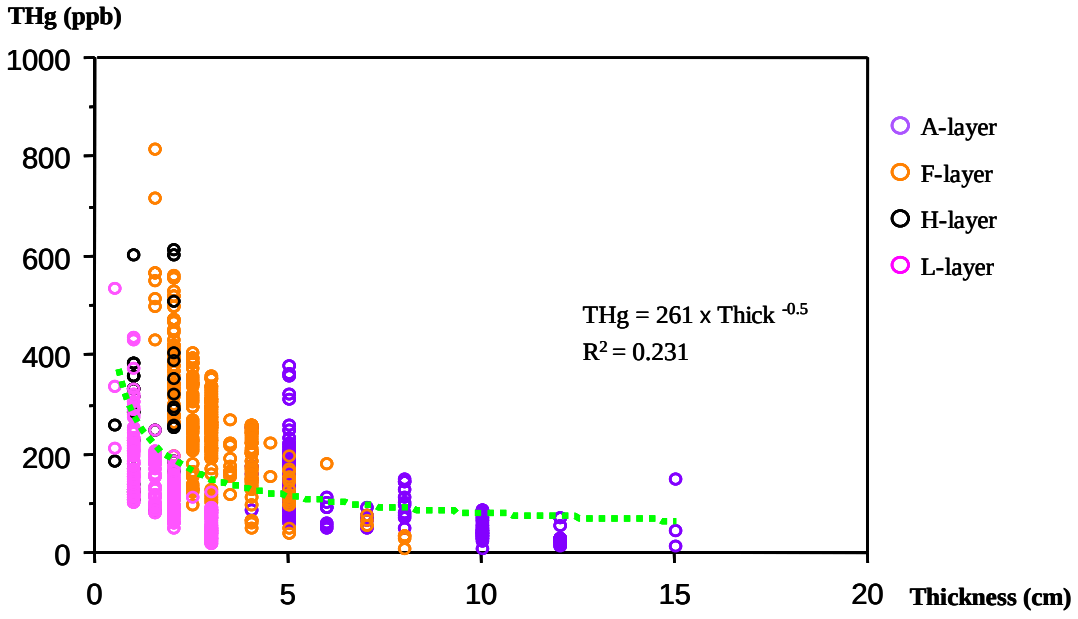


Figure 7.16. THg concentrations (ppb, dw) versus thickness (cm) of the soil layer.

Multiple regression analysis using the soil THg concentrations as a dependent variable, and soil layer type, S, N, and C concentrations in each soil sample, absence or presence of each of the four common moss species, and location (island, coast, and mainland), and soil depth as independent variables generated the best-fitting model results summarized in Table 7.19, and Figure 7.17. This model reflects that THg concentrations are higher in F- and H-layer soils than the A- and L-layer soils (Figure 7.1), increase with increasing S concentration in each soil layer (Figure 7.3), decrease with increasing C content, decrease with increasing depth of soil layers, and are enhanced by the presence of *Sphagnum* sp. and *Pleurozium schreberi* mosses. Finally, the model confirms that location contributes to soil THg concentrations in the order island > coast > mainland locations (Tables 7.3; 7.19).

Table 7.19. Summary of regression results of THg concentration (ppb, dw) in fungal substrate (soil and moss) in relation to the independent variables.

Regression variable	**Reg. coef.**	**Std. error**	**Std. coef.**	**t-value**	**p-value**	**R^2**	**Partial corr.**
Intercept	160.8	12.3	160.8	13.1	<0.0001		
F-layer	89.5	6.9	0.4	12.9	<0.0001	0.221	0.41
H-layer	137.1	14.8	0.3	9.3	<0.0001	0.321	0.31
Substrate thickness	-10.9	1.2	-0.3	-9.1	<0.0001	0.391	0.30
Location *	33.8	4.2	0.2	8.1	<0.0001	0.431	0.27
Total S (%)	588.2	76.5	0.3	7.7	<0.0001	0.454	0.26
Pleurozium schreberi	58.3	10.6	0.2	5.5	<0.0001	0.468	0.19
Sphagnum sp.	44.0	8.2	0.1	5.3	<0.0001	0.485	0.18
Total C (%)	-1.2	0.3	-0.2	-4.5	<0.0001	**0.498**	0.16

* Location as a dummy variable was coded -1 for mainland and 0 for island and coast. This means having fungal fruiting bodies from mainland caused THg concentrations to be decreased by 33.8 units.

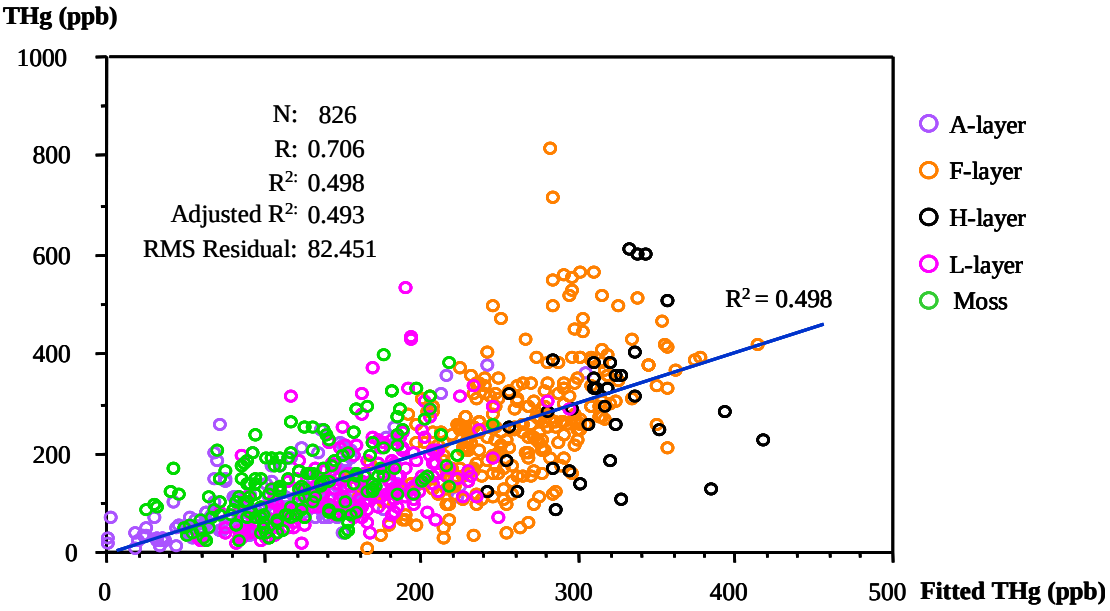


Figure 7.17. Bi-variate scattergram of THg concentration (ppb, dw) versus fitted THg concentration (ppb, dw) in the soil, by substrate layer (Fitted or predicted value is the value of dependent variable that is estimated based on the regression equation. The regression line is the best fitted-line that is drawn through the data points).

CONCLUSIONS

THg concentrations in the soils of this study and elsewhere are generally bound to soil organic matter, and generally increase with increased state of soil organic matter humification. Within the organic matter, THg is strongly correlated with the S content of the organic matter, and this remains so from the L-, F-, and H-layers to the C-layer, where THg and soil total C concentrations are generally very low. Apart from the general organic matter and organic S association, THg concentrations vary by type of moss layer on top of soil, exposure to Hg at the surface (e.g., differences in atmospheric Hg deposition by location), and soil layer thickness. Lower THg concentrations with increasing L-, F-, H- and A-layer thickness are related to lower THg, C, N, and S turn-over rates. Based on this study, at least 50 % of the THg concentrations in the L-, F-, H-, and A-layers can be accounted for by the above considerations.

In comparison, the THg concentrations in relation with the elemental S, N, and C contents of the soil layers of this study are somewhat higher than what has been reported by Gladkova & Malinina (2005) for a study area near Moscow, Russia. This difference can be explained by lower organic matter decomposition rate of the soil from this study (higher C/N ratio). Also, by aiming Russian study by Gladkova & Malinina (2005), we also concluded that THg retention in the mineral soil (A - C layers) is correlated to amount of stored organic matter i.e. C- layer has the lowest THg concentrations than B- and A- layers.

CHAPTER 8: THg CONCENTRATION IN FUNGAL FRUITING BODIES: ECOLOGICAL CONSIDERATIONS

INTRODUCTION

Organisms such as fungi extract water, mineralized carbohydrates, nutrients and heavy elements such as Hg from their substrates through their mycelium. Extracted materials are then re-synthesized and re-allocated to other parts of the mycelium, including the emerging fruiting bodies (Dighton 2003), where the particular amounts of allocated materials depend on tissue type (cap, stalk, spore-producing tissue) and developmental stage. The analysis in Chapter 5 revealed that the uptake and concentrations of Hg in fungal fruiting bodies are strongly affected by species type, which, in turn, Hg in fungal species are affected by the amount of Hg-binding S present in specific tissue types, presumably because of species-dependent S-amino-acid contents (Chapter 2). The analysis in Chapter 6 revealed similar trends for THg concentrations in mosses adjacent to fungal fruiting bodies, and Chapter 7 revealed that THg accumulations in adjacent organic and mineral soil layers also depend on the organic S content of these layers in particular. In addition, THg concentrations within these layers were found to increase with increasing state of organic matter humification (decreasing C/N ratio), which was further exemplified by the trend that highest THg concentrations would be found in rapidly decomposing and therefore thinnest F- and H-layers.

From an ecological perspective, it would appear that a number of fungal species act as local Hg concentrators, by sequestering Hg from surrounding decaying soil organic matter, and allocating some of this to their fruiting bodies. Therefore, it is of general interest:

- to determine to what extent THg concentrations in fungi correlate with THg concentrations in the soil,
- to isolate simple indicators such as species type, tissue type, soil type, and soil elemental content, as best THg concentrations predictors,
- to determine the overall THg soil-fungus bioconcentration factor (BCF) for specific fungi, and to determine those factors that might possibly affect BCF,
- to estimate the quantities of THg that are present in the soils of our sampling areas, and the likely portion of this Hg that is sequestered by fungi on a per hectare per year basis, and
- to determine whether the THg so accumulated will either return to the forest floor following fungal decay, or whether this Hg is transferred to other species by way of mushroom feeding, or lost to the atmosphere through a bio-chemical Hg^{2+} - Hg^{0} reduction process.

METHODS

Data for the elemental Hg, S, N and C contents of fungal fruiting bodies and corresponding soil and moss samples along with other measured and recorded variables describing substrate type (soil layer type, thickness, location, surface

conditions, etc.) and fungal tissue type (cap, stalk, state of development, species type) were again used for data analysis. Also included in the analysis were composite variables such as elemental ratios (C/N, THg/S, THg/N, and THg/C), and the THg bio-concentration factors BCFs (THg in fungi / THg in F-layer soil). ANOVA was used to examine the significant effect of fungal genus on BCF values. Simple and multiple regression analyses (described in Chapter 4) were then performed to determine which fungal, moss and soil attributes influenced the observed fungal THg concentrations and bio-concentration factors the most. Some of the data variables, especially THg and BCF, were log-transformed (ie. linearized), to optimize the final regression results.

RESULTS AND DISCUSSION

Plotting THg/C in fungi versus THg/C in soils in Figure 8.1 (specifically the F-layer soil) revealed positive correlations between these ratios by species, especially for species with a sufficiently large sample size, as is the case for *Bankera carnosa*. In contrast, the correlation between THg/S for *Fuscoboletinus viscidus* and soil was weakened by three outliers of high fungal THg/S ratios at low-layer THg/S ratios. Examining this systematically for the THg/C, THg/N, and THg/S ratios in soil and in fungi by species produced the results listed in Table 8.1. Apparently, the Hg fungal-soil associations were species dependent.

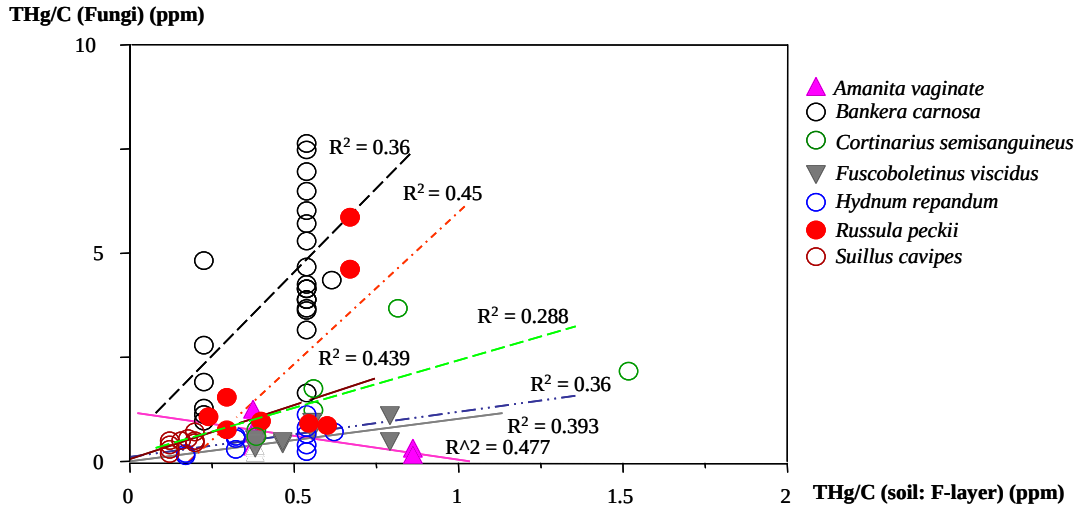


Figure 8.1. Bi-variate scattergram of THg/C in the fruiting bodies versus THg/C in the F-layer of organic soil.

Table 8.1. THg/S, THg/N, and THg/C (ppm) of some fruiting bodies and corresponding F-layers.

Fungal species	N	THg/S			THg/N			THg/C		
		Mean	Std. dev.	Std. error	Mean	Std. dev.	Std. error	Mean	Std. dev.	Std. error
		ppm			ppm			ppm		
Amanita vaginata	6	122	71	29	4	3	1.0	0.5	0.4	0.2
F-layer	13	136	51	14	15	6	1.6	0.6	0.2	0.1
Bankera carnosa	25	1129	422	84	56	21	4.2	4.1	1.9	0.4
F-layer	42	147	48	7	15	4	0.6	0.6	0.2	0.0
Cortinarius semisa-nguineus	6	226	138	56	14	10	4.0	1.0	0.8	0.3
F-layer	24	171	94	19	17	10	2.1	0.7	0.4	0.1
Fuscobo-letinus viscidus	7	118	60	23	6	4	1.3	0.5	0.3	0.1
F-layer	7	123	34	13	13	3	1.1	0.5	0.2	0.1
Hydnum repandum	11	222	105	32	7	4	1.2	0.5	0.3	0.1
F-layer	28	131	60	11	11	4	0.8	0.4	0.2	0.0
Russula peckii	6	360	351	143	18	22	9.1	1.8	2.0	0.8
F-layer	35	165	57	10	20	10	1.6	0.6	0.2	0.0
Suillus cavipes	9	129	41	14	6	2	0.6	0.5	0.1	0.1
F-layer	22	74	42	9	8	4	0.8	0.2	0.2	0.0

The results also were interpreted in terms of mycelial growth habits, by species, for those cases for which mycelial growth information exists (Tables II A - II C: Appendix II). For example, *Cantharellus cibarius*, the lowest Hg containing species, has a short-lived mycelium (not more than 2 months old), mostly known to grow in the upper 5 cm of top forest floor (i.e., the L-layer) (Danell and Eaker 1992). Hg uptake by *Russula* sp. is likely limited due to short-ranging extrametrical mycelia (fungal hyphae that emanate out from a mycorrhizal root and are important for fungal foraging in the soil) and high fruiting bodies reproduction (Peter et al. 2001). In

contrast, *Suillus* sp. and *Cortinarius* sp. with 2 to 6 times higher THg concentrations develop wider extrametrical mycelia than *Russula* sp. (Koide et al. 2005; Leeke et al. 2004). In particular, the mycelium of *Cortinarius* sp. mostly grows in the Hg-enriched F-layer, with Hg concentrations up to 260 ppb, dw or higher (Koide et al. 205). Root tips covered with *Cortinarius acutus* hyphae are most frequent within the F-, H- and A-layers of podsolic soils (Landeweert et al. 2003), generating fungal Hg concentrations of about 1000 ppb, dw. *Suillus* sp. develop an extensive extraradical mycelium (fungal hypea that are expanded through the soil and are responsible for nutrient acquisition) rarely found in the F-layer, but preferably grow in the mineral soil, specifically in B-layer soil (Landeweerk et al. 2003). The mycelium of the species, *Amanita vaginata,* with the lowest Hg concentrations from this genus, also develops in B-layer soil while *Amanita virosa*, a species with 3x higher Hg concentrations, has its mycelium distributed in F-layer soil (Dickie et al. 2002; Koide et al. 2005). In general, mycelial development of ECM fungi depends in part on the rooting habit of the host species.

Examining the THg concentration data across species by way of multiple regression analysis, using all the available data including tissue type, state of fruiting body development, location of sampling areas, soil layer type, and elemental contents in soils and fungal tissues, revealed that the soil-based data contribute significantly to the overall assessment of the THg variations within the fungal fruiting bodies (Figure 8.2; Table 8.2:). The most significant soil variables that entered the list of significant predictor variables were $\log_{10}$(C, F-layer), $\log_{10}$(S, F-layer), and $\log_{10}$(THg, F-layer). Specifically, higher C and Hg concentrations were found to increase the overall

transfer of Hg from the soil to the fungal fruiting bodies, while higher S concentrations is the soil generated the opposite trend.

Table 8.2. Summary of regression results of $\log_{10}$(THg concentration (ppb, dw)) in the fruiting bodies in relation to the independent variables.

Regression variable	Reg. coef.	Std. error	Std. coef.	t-value	p-value	R^2	Partial corr.
Cortinarius	0.7	0.04	0.64	17.85	<0.0001	0.145	0.15
Bankera	0.8	0.06	0.43	13.64	<0.0001	0.247	0.04
Development stage	0.2	0.03	0.27	8.93	<0.0001	0.353	0.11
Cantharellus	-0.6	0.08	-0.28	-7.78	<0.0001	0.418	-0.07
$\log_{10}$(C, F-layer)	**0.9**	**0.12**	**0.15**	**7.36**	**<0.0001**	**0.459**	**0.002**
$\log_{10}$(S, F-layer)	**-0.7**	**0.13**	**-0.15**	**-5.70**	**<0.0001**	**0.475**	**-0.01**
$\log_{10}$(S, fungus)	0.5	0.10	0.18	5.17	<0.0001	0.739	0.02
$\log_{10}$(THg, F-layer)	**0.4**	**0.08**	**0.14**	**4.94**	**<0.0001**	**0.749**	**0.02**
Lactarius	0.3	0.06	0.16	4.57	<0.0001	0.769	-0.01
Cap (0), stalk (1)	-0.1	0.03	-0.13	-4.07	<0.0001	0.784	-0.02

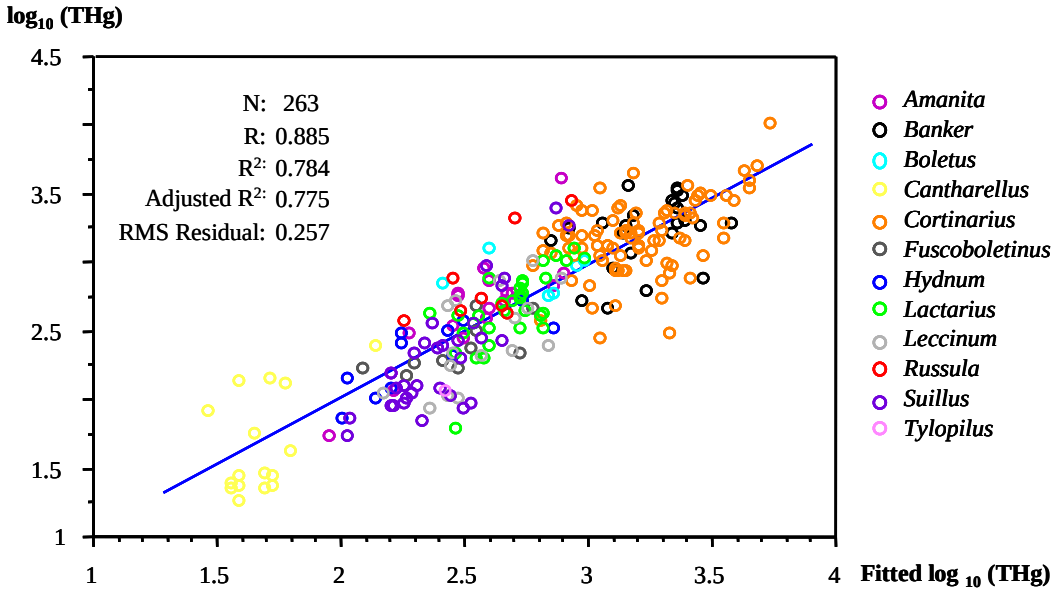


Figure 8.2. Bi-variate scattergram of THg concentration (ppb, dw) versus fittedTHg concentration (ppb, dw) in the fruiting bodies, by genus (Fitted or predicted value is the value of dependent variable that is estimated based on the regression equation. The regression line is the best fitted-line that is drawn through the data points).

As found in Chapter 5, species within the genera *Bankera* and *Cortinarius* continued to contribute strongly and positively to the variations of the THg concentrations in this extended regression analysis, although *Lactarius* did so in moderate proportions and *Cantharellus* contributed to these variations strongly but negatively. The other fungal species had THg concentrations close to the overall average concentration and therefore did not contribute much to the overall THg concentration variations. The developmental stage of fungal growth also accounted for a part of the overall THg variations such that THg concentrations decreased from premature (not fully-developed) to mature (fully-developed) and senescent (old fully-developed). Similarly, tissue type also influenced the results with THg concentrations being lower in stalks than in caps. Including the total S concentration of the fungal tissues improved the proportion of the explained THg concentrations (R^2 value) from 0.5 to 0.7: Hg retention in the fungal tissues increased with increasing S content of the same tissue. Including the $\log_{10}$(S), $\log_{10}$(C) and $\log_{10}$(THg) of the F-layer was found to increase the proportion of the explained THg concentrations even further, such that THg concentrations in the fungal tissues increased when total C and THg concentrations of the F-layer increased, whereas the THg concentrations in the fungal tissue decreased when the total S concentrations of the F-layer increased. This supported that there was a persistent affinity between S and Hg, with higher THg concentrations in fungal tissues expected to occur when the S concentrations of these tissues were high and when the S concentrations of the mycelial substrate were low. High THg concentrations within the mycelial substrates would further encourage mycelial Hg uptake. Finally, the location factor also contributed to the overall THg

concentration variations, decreasing from the island location to the coast and to the mainland, as already discussed in Chapter 7. The best-fitted model explained 80 % variations of the THg concentration variations in the fungal tissues.

Concentration ratios of THg in fungal tissues and the underlying F-later were used to estimate the overall soil-fungus bio-concentration factor BCF. The resulting BCF values for individual samples ranged from 0.1 to 45 (Table 8.3), with the highest average values for *Bankera* (BCF: 9), *Boletus* (BCF: 8), and *Cortinarious* (BCF: 6) (Table 8.3: Fig. 8.3). ANOVA showed that Log_{10}(BCF) values were significantly different among fungal genus (F-value > F-critical, P-value < 0.0001) (Table 8.4). *Cortinarius stillatitius* had the highest average BCF of 12. *Boletus edulis* and *Bankera carnosa* had a somewhat lower mean BCF value of 9, while *Cortinarius armillatus* had a BCF value of 8. These species were therefore relatively strong Hg accumulators. *Amanita flavoconia, Amanita muscaria, Amanita virosa, Boletus separans, Cortinarius acutus, Cortinarius pholidius, Cortinarius gentilis, Cortinarius semisanguineus, Lactarius vellereus, Leccinum holopus, Russula peckii, Suillus cavipes,* and *Suillus grevillei* with BCF values between "2-5" were intermediate Hg accumulators. Fungi with BCF ≤ 1 were considered as low Hg accumulators. These fungi species were *Russula* sp., *Cantharellus cibarius, and Tylopilus plumbeoviolaceus* (BCF: 0.2), *Cantharellus tubaeformis* (BCF: 0.6), and *Amanita vaginata, Amanita fulva,* and *Fuscoboletinus viscidus, Hydnum repandum, Lactarius camphorates, and Leccinum scabrum* with BCF = 1. Low Hg uptake has been confirmed for *Cantharellus cibarius* (Fischer et al. 1995).

Table 8.3. Bioconcentration factors (BCFs) of the fruiting bodies, by genus.

Species	N	Mean	Min	Max	Std. dev.	Std. error
Amanita	72	2.4	0.2	12.6	2.4	0.3
Bankera	42	8.6	3.0	18.6	3.6	0.6
Boletus	25	8.1	2.2	20.6	5.4	1.1
Cantharellus	28	0.4	0.1	1.2	0.3	0.1
Cortinarius	148	6.4	0.5	45.0	6.2	0.5
Fuscoboletinus	10	1	0.5	1.9	0.4	0.1
Hydnum	28	1.4	0.5	3.6	0.7	0.1
Lactarius	44	2.2	0.1	6.9	1.6	0.2
Leccinum	36	1.6	0.2	4.4	1.3	0.2
Russula	66	1.1	0.1	7.5	1.5	0.2
Suillus	60	2.2	0.1	8.1	1.9	0.2
Tylopilus	2	0.2	0.1	0.3	0.1	0.1

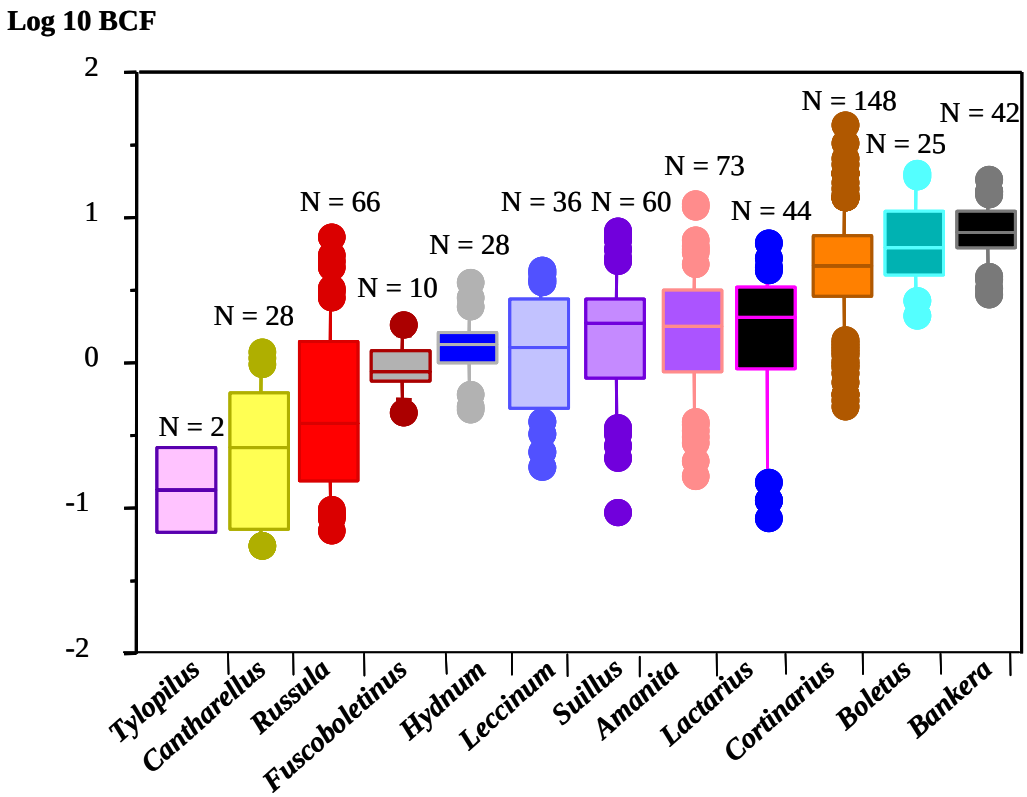


Figure 8.3. Log_{10}(BCFs (Bioconcentration Factors)) of the fruiting bodies, by genus.

Table 8.4. ANOVA table for Log_{10}(BCF) values fungal genus from the three study locations (island, coast, and mainland).

Source of variance	DF	SS	MS	F-value	P-value
Fungal genus	11	98.9	9.0	55.1	<0.0001
Residual	549	89.5	0.2		

DF: degree of freedom; SS: Sum of Squares; MS: Mean Square F
F(critical) $_{0.05,11,549}$ = 1.83; F-value > F-critical

In this study, by measuring total Hg contents of the fungal fruiting bodies, the amount of Hg species (inorganic Hg: Hg^{2+}, Hg^0 and organic Hg: CH_3Hg^+ (MeHg)) was not specified. While in the literature, Hg uptake was sometimes differentiated by inorganic Hg versus MeHg uptake. For example, *Boletus edulis*, *Leccinum scabrum*, and *Amanita muscaria* taked up MeHg with a BCF value of 3 to 10, while inorganic Hg uptake had a BCF value of about 1 (Fischer et al. 1995). Fischer et al. (1995) also reported that MeHg/THg ratio in fungal fruiting bodies ranged from 0.70 to 19. Specifically, this ratio increased with developmental stage of the fruiting body, from MeHg/THg = 0.4:1 at emergence to 0.80:1 and 0.9:1 at maturity in *Amanita muscaria*.

The result from multiple regression analysis for log_{10}(BCF) in Table 8.5 and Figure 8.4 showed that about 70 % of these variations were accounted for by fungal genus, developmental stage (not fully-developed > fully-developed > old), type of body part (cap > stalk), a surrounding presence of *Polytrichum juniperinum* and *Pleurozium schreberi*, and location (island > coast > mainland). Again, the genera *Cantharellus* and *Russula* ranked as low BCF species, while *Cortinarius*, *Bankera* and *Boletus* ranked as high BCF species. The results also confirmed that increased S

concentrations in the soil reduced BCF. In addition, the *Polytrichum juniperinum* and *Pleurozium schreberi* mosses appeared to facilitate the fungal uptake of Hg.

Table 8.5. Summary of regression results of log $_{10}$(BCF) of the fruiting bodies in relation to the independent variables.

Regression variable	**Reg. coef.**	**Std. error**	**Std. coef.**	**t-value**	**P-value**	**R^2**	**Partial corr.**
Intercept	0.8	0.10	0.83	7.99	<0.0001		
Developmental stage	0.3	0.03	0.27	7.81	<0.0001	0.114	0.408
Cantharellus	-0.8	0.11	-0.29	-7.41	<0.0001	0.245	-0.388
Cortinarius	0.3	0.05	0.25	6.75	<0.0001	0.338	0.358
Russula	-0.4	0.06	-0.24	-6.69	<0.0001	0.429	-0.355
Bankera	0.6	0.11	0.21	5.94	<0.0001	0.52	0.320
Cap (0), stalk (1)	-0.2	0.04	-0.19	-5.74	<0.0001	0.556	0.310
Location *	0.3	0.05	0.21	5.31	<0.0001	0.582	0.289
Polytrichum juniperinum	0.3	0.06	0.18	4.92	<0.0001	0.617	0.269
Boletus	0.9	0.18	0.16	4.86	<0.0001	0.641	0.266
Total S (F-layer)	-1.7	0.46	-0.14	-3.70	0.0003	0.654	-0.205
Pleurozium schreberi	0.2	0.06	0.13	3.41	0.0007	0.666	0.190

* Location as a dummy variable was coded -1 for mainland and 0 for island and coast. This means having fungal fruiting bodies from mainland caused THg concentrations to be decreased by 33.8 units.

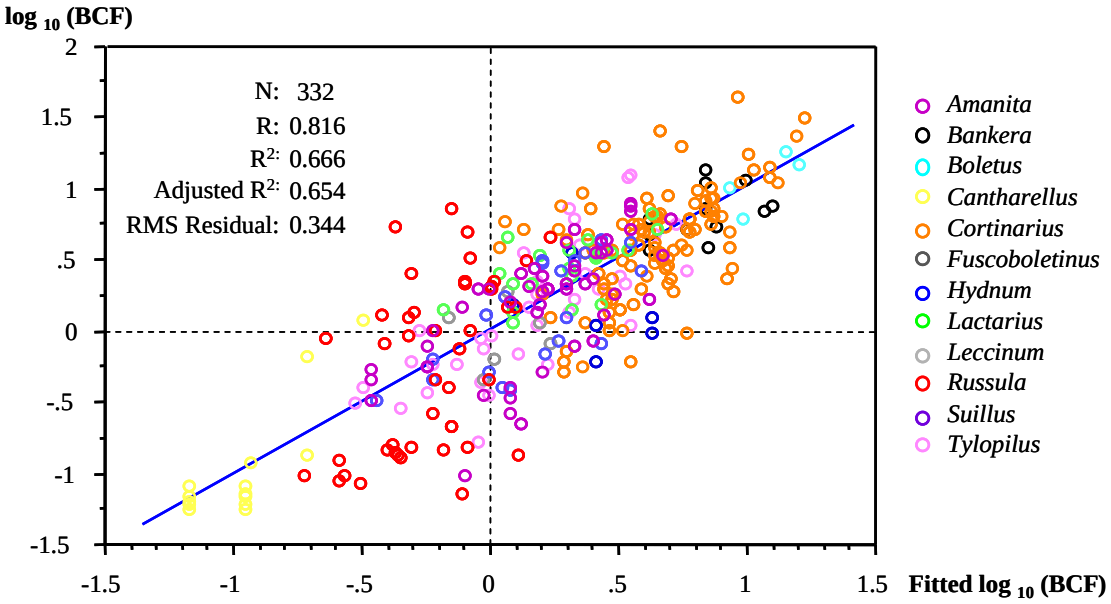


Figure 8.4. Bi-variate scattergram of log 10 (BCF) versus fitted log 10 (BCF) of the fruiting bodies, by genus (Fitted or predicted value is the value of dependent variable that is estimated based on the regression equation. The regression line is the best fitted-line that is drawn through the data points).

On average, the total biomass in fungal fruiting varied from about 100 to 1000 g/ha/yr, see Table 8.6. Using 313 g/ha/yr as an average dry weight per hectare for total amount of fruiting body biomass (Table 8.6), and an average THg concentration of 822 ppb, dw (this study) generated an estimate of 0.26 mg of THg sequestered by fungal fruiting bodies per hectare per year. In comparison, THg retention in the litter layer that included mosses, leaves, needles and woody debris amounted to 7.4 g/ha, and this number increases to about 100 g/ha in B-layer soil (Table 8.7). The above result implied that fungal fruiting bodies contributed very little to Hg extraction from any soil layer even though some of the fungal species were Hg hyper-accumulators. In addition, the annual rate of fungal Hg extraction from the soil is about 1000 x less than what is deposited annually on the forest by way of atmospheric deposition and/or canopy throughfall (Table 8.8).

Table 8.6. Mass of Hg input to the forest floor (mg/ha/yr) from the fully-developed fruiting bodies.

Plot	Plot size	Year	Forest type	Total samples	Weight	Mass THg per ha
N	m^2	N		N	g/ha	mg/ha/yr
6	256	3	Hardwood	35	824	**0.68**
4	1000	1	Hardwood	18	325	**0.27**
1	2000	2	Hardwood	27	122	**0.10**
3	2000	2	Mixedwood/softwood	57	258	**0.21**
3	1905	1	Mixedwood/softwood	114	1081	**0.89**
2	1340	5	Softwood	65	175	**0.14**
4	2000	2	Softwood	50	226	**0.19**
1	1000	1	Mixed hardwood	16	289	**0.24**
1	1000	2	Mixed hardwood	22	199	**0.16**
1	1000	2	Mixed hardwood	13	117	**0.10**
1	1000	2	Mixed hardwood	26	235	**0.19**
1	1000	3	Mixed hardwood	25	151	**0.12**
1	1000	3	Mixed hardwood	28	169	**0.14**
1	1000	3	Mixed hardwood	36	217	**0.18**
Average						**0.26**

Average mushroom weight (this study), wet: 13.44 g; dry:1.81 g
Average mushroom Thg concentration, dry weight (this study): 822 ppb, dw

Table 8.7. Turn-over rate of Hg from the fruiting bodies of fungal mass into the soil.

Soil layer	Depth	Density	THg/C	C	Mass THg in soil	Turn-over rate *	Turn-over rate **
	cm	g/cm^3	ppm	%	g/ha	yr	yr
L	2	0.1	0.3	49.5	3.0	11,423	20
F	3	0.1	0.6	44.9	8.1	31,085	35
H	1	0.1	0.8	36.8	2.9	11,323	39
Ae	9	1.3	2.7	3.3	105.2	404,773	154
Ahe	8	1.2	1.2	15.8	189.1	727,373	311
Ah	6	1.1	1.2	7.1	58.7	225,783	257
B1	20	1.3	4	1.1	114.4	440,000	301
B2	30	1.5	4.4	0.5	99.0	380,769	261
C	30	1.7	11.4	0.1	58.1	223,615	153

* Turn-over rate of Hg from mass of fungal fruiting bodies to the soil,
** Turn-over rate of Hg from throughfall and litter fall input (38**µ**g/m^2/yr) to the soil.

Table 8.8. Estimates of THg input and output in forests and terrestrial watersheds.

Type of Hg input	Rate µg/m²/yr	Rate mg/ha/yr	Type of Hg output	Rate mg/ha/yr
Precipitation[1,2]	0.6-1	60	Soil sequestration	50
Fog[3]	3.5-435*		Streamflow	20
Throughfall[1]	17	170 } 380	Foliar reemission**	2
Litterfall[1]	21	210	Uncertain volatilization	320

[1] (Grigal 2002), [2] (Environment Canada), [3] (Ritchie et al. 2006)
* (ng/L), ** Foliar re-emission is set as a small portion of total uncertain volatilization

Although fungal Hg extraction rates from the soil may be insignificant on an annual per hectare basis, fungi would still influence soil-sequestered Hg into the local food chain, especially if fungal Hg hyper-accumulators are eaten by slugs, mites, squirrels, mice, voles, gophers and deer (Hiol et al. 1994; North et al. 1997; Toljander et al. 2006). People consuming Hg hypo-accumulators are somewhat less at risk, because of larger body weight, and significant Hg losses during meal preparations (about 70 %) (Svoboda et al. 2002).

CHAPTER 9: SUMMARY

OVERALL RESEARCH RESULTS

The results of this research are now summarized by transforming the regression results of Chapters 6, 7 and 8 into simple models (Table 9.1), useful for estimating:

1. the THg concentrations in the cap and stalk tissues of the fungal fruiting bodies of this study (Model 1, Chapter 8);
2. the THg concentrations in the green parts of the mosses of this study (Model 2, Chapter 6);
3. the THg concentrations in the L, F, H, and A soil layers of this study (Model 3, Chapter 7); and
4. the fungal Hg bio-concentration factor for this study (Model 4, Chapter 8).

Table 9.1. Model summary.

Model	Dependent variable	Chapter	R^2	RMS Residual
1	$\log_{10}$(THg in fungi)	8	0.781	0.262
2	THg (moss)	6	0.903	23.198
3	THg (substrate)	7	0.498	82.451
4	Log_{10} (BCF)	8	0.666	0.344

Models 2 and 3 are directly generated from the corresponding multi-variate regression tables following the useful pattern, i.e.

$$\hat{Y} = \beta_0 + \sum \beta_j X_j + \text{error} \qquad j = 1, 2, \ldots, n$$

where $\hat{Y}$ is the estimated value of Y, Xj is a specific value of the jth independent variable, β_0 is the intercept, β_j are the regression coefficients, and n is the total number of variables that make significant contributions to the estimation of Y. Since THg concentrations of fungal fruiting bodies were scattered in a wide range, there were non-linear relationship between dependent variable and independent variables. The non-linear model was described as

$$\hat{Y} = C\, X_j^{\,\beta 0} \qquad j = 1, 2, \ldots, n$$

Logarithm transformation normalized the data and linear relationships were found between dependent and independent variables of models 1 and 4 according to the following equation:

$$\text{Log}_{10}(\hat{Y}) = \text{Log}(C) + \beta_0 (\text{Log}_{10}(X(j)) \qquad j = 1, 2, \ldots, n$$

As such, these models are written as follows:

<u>Model 1</u> Log_{10} (THg in fungi) = 0.7 × *Cortinarius* + 0.8 × *Bankera* + 0.2 × DS – 0.6 × *Cantharellus* + 0.9 × $\log_{10}$ (C, F-layer) – 0.8 × $\log_{10}$(S, F-layer) + 0.5 × $\log_{10}$(C, fungi) + 0.4 × $\log_{10}$(THg, F-layer) + 0.3 × *Lactarius* – 0.1 × Type of body part (cap versus stalk), and

Model 2 THg (moss) = 206.6 – 178.8 *Ptilium crista-castrensis* – 112.7 *Sphagnum* sp. – 69.8 *Polytrichum juniperinum* + 0.2 THg (F-layer) + 339.0 Total S (moss) – 243.2 TS (F-layer),

Model 3 THg (substrate) = 160.8 + 89.5 × F-layer identifier + 137.1 × H-layer identifier - 10.9 × Substrate thickness (cm) + 558.2 × Total S + 58.3 × *Pleurozium schreberi* + 44 × *Sphagnum* sp. -1.2 × TC,

Model 5 Log_{10} (BCF) = 0.8 + 0.3 × DS- 0.8 × *Cantharellus* + 0.3 × *Cortinarius* - 0.4 × *Russula* + 0.6 × *Bankera* + 0.2 × Type of body part (cap versus stalk) + 0.3 × Location (island, coast and mainland) + 0.3 × *Polytrichum juniperinum* + 0.9 *Boletus* - 1.7 × TS(F-layer) + 0.2 × *Pleurozium schreberi.*

Overall, most of the THg concentrations in fungal fruiting bodies were simply related to developmental and species-specific variations, so that environmental variables, whether these are related to the soil, moss or location attributes, only play a minor numerical role. This being so makes it difficult to use fungi as indicators of environmental Hg pollution, especially when spatial gradients of Hg pollution locally or across a region are not significantly different.

ORIGINAL CONTRIBUTION

1. This thesis was the fist to present and examine details about the variations of THg concentrations in the fruiting bodies of wild-growing ECM fungi and

their surrounding substrates (mosses, soils), at three study locations (island, coast, and mainland)

2. In this study, about 80 % of the THg concentration variations in the fungal fruiting bodies were successfully explained by: fungal taxonomical group, development stage, cap/stalk component, and elemental S, N, and C contents in the fungal tissue. Location, soil and moss attributes associated with fungal samples only explained very small portion of the observed THg concentration variations in the fungal fruiting bodies.
3. THg concentrations in fungal tissues differed strongly by species, being highest in the genera *Boletus*, *Bankera* and *Cortinarius*, and lowest in the genera *Cantharellus*, and *Russula.*
4. To some extent, THg concentrations in fungal tissues are related to the total S content of these tissues which, in turn, are hypothesized to be related to species-specific amino-acid composition differences.
5. While THg concentrations are highest in the slowly decomposing portions of the forest floor (L and H-layers, especially in layers with a low C/N ratio), and least in the mineral soil underneath the forest floor, THg amounts were considerably higher in the mineral soil than in the forest floor.
6. No previous study examining the influence of surrounding mosses on THg concentrations in fungal fruiting bodies could be found. In this study, fungal fruiting bodies surrounded by mosses with higher Hg levels have higher Hg concentrations.

7. The total uptake rate of Hg from the forest floor and/or underlying soil is negligible compared to the incoming Hg amounts of wet and dry atmospheric deposition alone. In addition, on a per hectare bases, Hg relocation from the soil below to the top of the forest floor by fungal sequestration does not constitute a numerically significant ecological pathway for intra-soil Hg transport.

SUGGESTIONS FOR FUTURE WORK

1. Further studies should be conducted to study the effect of the developmental stage of fungal growth on Hg storage and retention:
   - whether the declining Hg concentrations with increasing developmental stage of the fruiting body is simply due to growth dilution, or a change of substrate mycelium allocation to the fruiting bodies, e.g., from a high protein allocation at first to a high carbohydrate allocation thereafter; and
   - whether the declining Hg concentrations is due to Hg^{2+} reducing enzymes that would be become more active as cellular tissues of the fungal fruiting bodies start to disintegrate.
2. It is important to determine the portion of accumulated MeHg in fungal fruiting bodies, because MeHg bio-accumulates more intensively than mercury in its inorganic Hg^{2+} form.
3. The above models should be verified by another sampling study, to see whether the variables identified above as independent variables prove themselves as good predictor variables, or not.

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APPENDIX I

TOTAL NITROGEN (N) AND AMINO ACID COMPOSITION OF THE FUNGAL FRUITING BODIES

Table I A. Total nitrogen (N) and amino acid composition (%) of the fruiting bodies of some fungal species.

	Total protein								Total N
	Agaricus bisporus *	*Amanita muscaria*	*Amanita muscaria*	*Amanita muscaria*	*Pleurotus ostreatus**	*Pleurotus ostreatus**	*Suillus granulatus*	*Boletus pruinatus* *	*Agaricus bisporus* *
Amino acids	**Ref. 1**	**Ref. 4**	**Ref. 5**	**Ref. 5**	**Ref. 1**	**Ref. 1**	**Ref. 3**	**Ref. 1**	**Ref. 1**
Nutritional valued amino acids									
Lysine	8.4	7.5	7.7	18	7.2	6.7	0.6	2.6	6.2
Threonine	5.9	7.4	5.4	6.6	4.8	6.8	1.4	5	3.5
Valine	3.6	6.6	4.4	5.1	5	7.3	1.4	6	3.6
Methionine	**1.0**	**2.0**	**1.0**	**2.2**	**1.7**	**1.1**	**2.2**	**1.5**	**1.3**
Cystine	**1.1**	**0.4**	**1.3**	**0.9**	**0.8**	**1.0**	N/D	N/D	N/D
Isoleucine	5.1	3.1	1.3	3.2	5.8	6.7	N/D	N/D	2.7
Leucine	7.6	5	7.2	5.8	8.2	7.3	2.9	8.4	4.8
Tyrosine	4.2	N/D	0.26	N/D	3.5	5.2	2.1	3.4	2.3
Phenylalanine	4.7	1	1.5	1.5	4.3	5.4	1.2	NA	5.3
Tryptophan	2.2	N/D	N/D	N/D	1.5	N/D	1.4	2.9	N/D
Other selected essential amino acids									
Alamine	5.8	4.9	9.4	5.9	6.2	6.6	1.7	11.5	6.6
Arginine	8.8	3.8	2.2	4.1	6.2	5.9	1.9	0	5.9
Glycine	3.6	17	14	13	4.6	5.3	1.2	6.1	5.3
Histidine	2.8	N/D	N/D	N/D	2	2.7	0.3	N/D	2.7
Aspartate	8.1	15	9.7	12	9.3	6.5	2.4	4.9	6.5
Glutamate	16.2	11	9.2	22	17.7	5.4	3.5	15.4	5.4
Proline	6.1	8.9	2.1	4.3	6.2	5.5	1.2	NA	5.5
Serine	5.2	8.2	10	6.6	4.7	5.5	1.4	7.4	5.5
Valine	N/D	6.6	4.4	5.1	N/D	N/D	1.4	N/D	N/D
Isoleucine	N/D	N/D	N/D	N/D	N/D	N/D	2	N/D	N/D

Table I A. Continued…

Total amino acids										
Amino acids	***Hydnum repandum*** **Ref. 4**	***Lentinula edodes**** **Ref. 1**	***Pleurotus ostreatus**** **Ref. 1**	***Lactarius* sp.** **Ref. 2**	***Suillus cavipes*** **Ref. 2**	***Suillus* sp.** **Ref. 2**	***Russula hiemisilvae*** **Ref. 2**	***Cantharellus cibarius*** **Ref. 4**	***Russula rosacea*** **Ref. 6**	***Russula hiemisilvae*** **Ref. 2**
Nutritional valued amino acids										
Lysine	4.2	5	5.4-6.4	0.0	3.3	N/D	N/D	3.9	1.0	N/D
Threonine	4.4	5.6	4.7-5.3	11	NA	NA	4.1	3.6	N/D	4.1
Valine	3.9	3.8	4.3-5.2	11.4	8.0	NA	NA	2.8	N/D	NA
Methionine	**1.0**	**2.2**	**1.5-2.3**	**2.1**	**2.31**	**0.96**	**0.1**	**1.1**	N/D	**0.1**
Cystine	**0.8**	**3.4**	**1.2-1.7**	N/D	N/D	N/D	N/D	**0.6**	N/D	N/D
Isoleucine	3.2	3.3	3.9-4.7	N/D	N/D	N/D	N/D	2.6	N/D	N/D
Leucine	14.5	6.4	6.3-7.3	15.9	10.6	0.46	N/D	13.4	1.34	N/D
Tyrosine	3.7	2.6	3.6-4.6	2.3	4.8	NA	N/D	2.1	N/D	N/D
Phenylalanine	3.4	3.8	3.8-4.7	3.09	3.3	0.74	0.1	2.5	0.97	0.1
Tryptophan	1.4	1.9	1.1-1.6	5.34	2.2	NA	N/D	1.1	N/D	N/D
Other selected essential amino acids										
Alamine	4.9	5.3	6.0-8.3	22.6	NA	N/D	N/D	3.8	N/D	N/D
Arginine	4.4	5.7	7.0-11.5	N/D	9.0	10.0	N/D	4.1	1.0	N/D
Glycine	3.6	4.7	4.4-4.8	N/D	8.4	0.57	N/D	2.9	N/D	N/D
Histidine	1.6	3	3.6-4.3	N/D	N/D	N/D	N/D	1.3	0.5	N/D
Aspartate	8.5	10.2	9.2-12.1	N/D	10	NA	4.8	6.4	N/D	4.8
Glutamate	8.5	20.9	13.1-16.6	24.1	NA	NA	30.4	8.1	N/D	30.4
Proline	N/D	3.9	3.6-4.8	N/D	NA	NA	NA	0	N/D	N/D
Serine	4.8	5.7	3.5-6.0	N/D	8.9	23	0.4	3.5	N/D	0.4
Valine	3.9	N/D	N/D	N/D	N/D	N/D	N/D	0	N/D	N/D
Isoleucine	3.2	N/D	N/D	N/D	N/D	N/D	N/D	2.6	N/D	N/D

* data from fresh samples, N/D: No Data. Reference: Ref. 1: (Bernas et al. 2006), Ref. 2: (Mdachi et al. 2004), Ref. 3: (Miletic et al. 1990), Ref. 4: (Surinrut et al. 1987), Ref. 5: (Colline-Hansen et al. 2002), and Ref. 6: (Vetter 1993).

APPENDIX II

TAXONOMICAL ORDER OF THE STUDIED FUNGI, AND HABIT, CHARACHTERISTIC, AND IMAGES OF THE FUNGAL FRUITING BODIES AND MOSSES

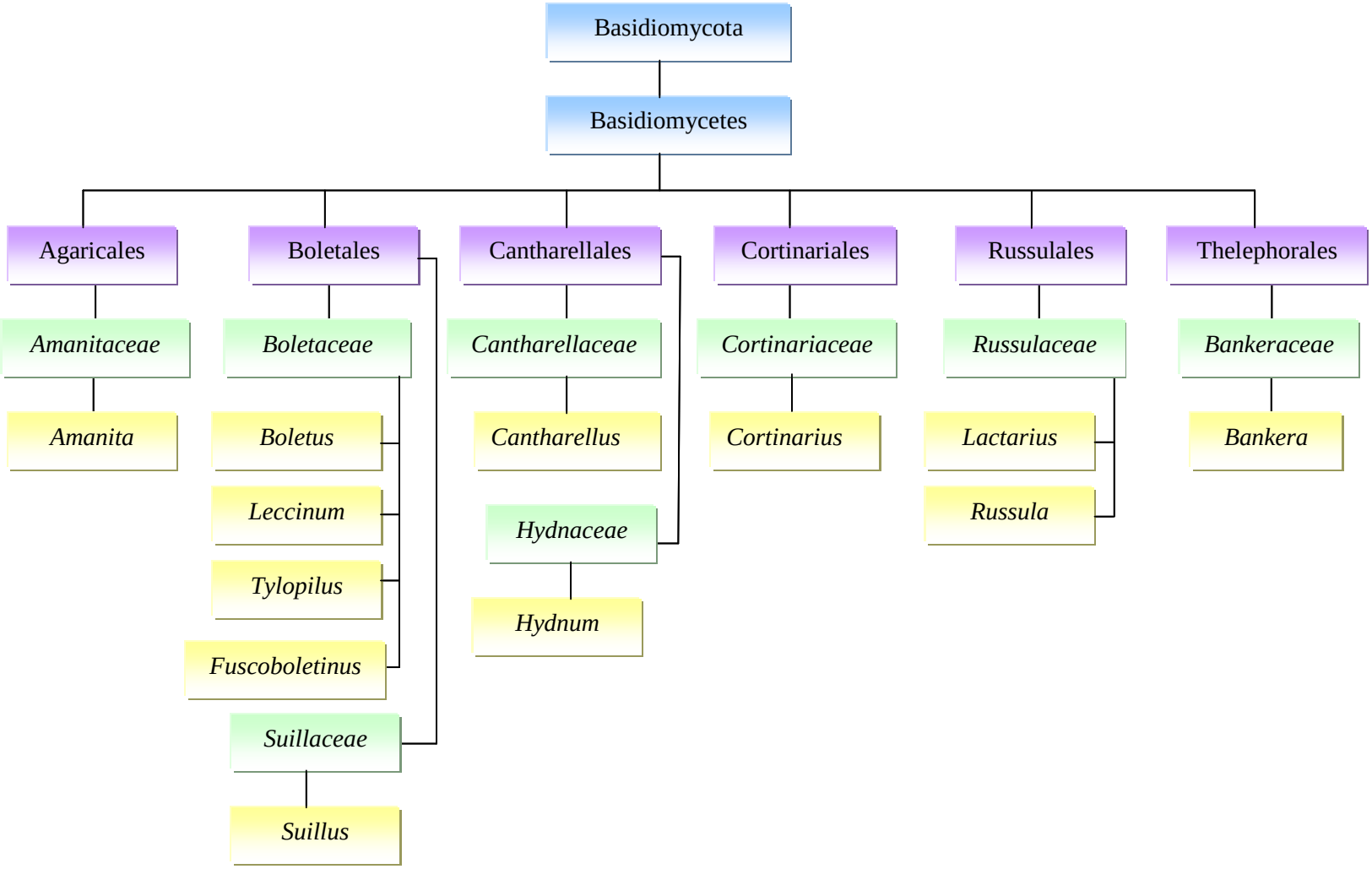


Figure II A. Taxonomical order of studied fungi.

Table II A. Attributes of the fungal fruiting bodies.

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Amanitaceae	*Amanita*	Late-stage, midway [1], mycelium grow in forest floor (5-10 cm depth) [2], and usually grows in Coniferous forest					
		A. flavoconia Atk.	2-8 cm across, convex to flat, yellow-orange, scattered yellow cottony patches on the top. Free white gills	3- 12 cm long, 0.6 cm thick, pale, with a yellow volva ring, swollen base	Ectomycorrhizal: pine[1], oak [1, 3], and birch [3]	Not-edible	
		A. fulva (Schaeff.) Fr.	4-10 cm across, convex orange-brown, pale by age, smooth, with the central bump, white and smooth flesh and Free white gills	7-15 cm long, 0.4 cm thick, tawny brown, smooth; with a tawny brown volva at the base	Ectomycorrhizal: pine [1], oak [1, 4], spruce [5], birch [6], and beech.	Not-edible	
		A. muscaria var. formosa (Pers.:Fr.) Bert. in DeCh.	5-30 cm broad, yellow-orange, watery, with white warts on the top	5-15 cm long, 0.5-1 cm thick, shaggy, a white ring at the top and concrete bands at the bulbous base	Ectomycorrhizal: Pine [1,6], fir[2], Birch [6], spruce[6], larch, aspen, hemlock, oak, beech	Not-edible	
		A. vaginata var. vaginata (Bull.:Fr) Vitt.	3-10 cm broad, grayish brown, lined margin, central bump. Free white, and close gills	5-15 cm long, 0.5-1.5 cm thick, grayish, smooth, with a grayish pale volva at the base	Ectomycorrhizal: Pine[1], oak[1, 4], spruce[5] , aspen, beech, hemlock	Not-edible, mycelium grows in B layer [7], very low mycelium in F-layer [8].	

Table II A. Continued…

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Amanitaceae	*Amanita*	*A.virosa* Secr.	5-30 cm broad, yellow-orange, watery, white warts on the top	5-15 cm long, 0.5-1 cm thick, shaggy, a white ring at the top and concrete bands at the bulbous base	Ectomycorrhizal: birch	Not-edible, grow at the forest edge, very low mycelium in F-layer [8].	
Bankeraceae	*Bankera*	*Bankera carnosa* (Banker) Snell, Dick & Taussig	3-10 cm broad, light grayish-brown. With spines pale gray, up to 4 mm long	2-7 cm long, 0.5-2.0 cm thick, concolorous with the cap, smooth.	Ectomycorrhizal, fir or birch.	Not-edible	
Boletaceae	*Boletus*	Late stage, grow in old growth forest [North].					
		Boletus edulis Bull.:Fr.	8-30 cm cross, 3-6 cm thick, fleshy, light brown to dark brown, leathery white pores and flesh changing to yellow-olive, not burnish	8-20 cm long, 4-8 cm thick, white to pale yellow and club-shape	Ectomycorrhizal oak [10], birch [10,6], spruce [6], pine[14], beech[10], and aspen, hemlock, fir, chestnut, larch, hazelnut	Edible, grow on grass & tree debris [10], and Loamy sand, sandy loam soil	
		Boletus separans *Pk.*	5-20 cm broad, fleshy, olive brown to red brown, dry and wrinkled, convex, dense and small yellow pores, white flesh and not bruising after cutting	6-15 cm long, 1-4 cm thick, slightly reddish, dry, not even	Ectomycorrhizal: oak, pine	Edible	

Table II A. Continued…

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Boletaceae	*Fuscob-oletinus*	*Fuscoboletinus viscidus* (L.:Fr. & Hök) Grund & KA Harrison	4-9 cm broad, fleshy, convex, viscid, dark brown, changing to bluish-green where bruised	4-8 cm long, 8-15 mm thick, brown, with a viscid annulus	Ectomycorrhizal: larch	Edible	
	Leccinum	*Leccinum holopus* (Rost.) Watling var. americanum AH Sm. & Thiers	5-15 cm broad, convex, white becoming pink or green, viscid by age. White Flesh, turing pink by cutting	6-15 cm long, 1-1.5 cm thick, white and is covered by blackish brown scurfy dots, White depressed tubes then brownish	Ectomycorrhizal: birch [6].	Edible, grow at the edge of swamps, bogs, mosses and coniferous.	
		Leccinum scabrum (Bull.:Fr.) S.F.Grey	5-15 cm broad, smooth to velvety, convex to plane, tan to smoky or reddish-brown. White flesh, not staining. White-grayish tubes, staining darker. Tiny, round pores	4-15 cm long, smoky white/gray covered by vertically raised black dots. The spore print is brown to olive-brown	Ectomycorrhizal: Birch [6], beech, aspen, chestnut, Hazelnut	Edible, grow on boggy area close to mosses	
	Tylopilus	*Tylopilus plumbeoviolaceus* (Snell & Dick) Sing.	4-15 cm broad, violet with pinkish pores, flat		Ectomycorrhizal: beech and oak [3], aspen [3], hickory [3]	Not-edible, associates just with deciduous forest	

Table II A. Continued…

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Suillaceae	*Suillus*	Grow at forest edges usually in upper slope acidic poor soil [13], exteramatrical mycelium with hydrophobic network & minor component of the root-tips[11], mycelium growgh into mineral soil (B-layer) but not in F-layer [8, 12]					
		Suillus cavipes (Opat.) Smith & Thiers	3-15 cm broad, reddish, smooth and shinny, and the skin is easily peeled off. Yellow round pores turning olive, and with a yellow partial ring	2-6 cm long, 0.6-1.5 thick and reddish brown	Ectomycorrhizal: larch [14,15, 16]	Edible, displaces other species [14], Late-stage [15]	
		Suillus grevillei (Kl.) Sing.	3-10 cm broad, brown and convex to plane covered by reddish or white hairs and fibers, and with a white partial veil .Yellowish open angular pores	2-8 cm long, 0.5-1.5 think, and brown, smooth toward the top and hairy close to bottom, and hallowed at the base	Ectomycorrhizal: fir [15], larch [15,16], alder [15]	Edible, Late-stage [15], comprises with other ECM species, small and short lived mycelium, don't recolonized with the same root tips, intract with other mycelium from different species [14]	13
Cantharellaceae	*Cantharellus*	*Cantharellus cibarius* Fr.	2-10 cm across, vase-shape fleshy cap with wavy margin, wrinkled false gills underneath the cap, running down to the stalk, white flesh	2-4 cm long, .5 cm thick, Short and very smooth, pretty the same color as cap	Ectomycorrhizal: pine [6], spruce [6], birch [6]	Edible, grow on rich humus ususually in young growth forest [9]	14

Table II A. Continued…

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Cantharellaceae	*Canthar-ellus*	*Cantharellus tubaeformis* Fr.	20-80 mm broad, yellow-brown, funnel-shaped. Gills yellowish to gray-violet	25-80 mm long. 4-10 mm thick, yellow, smooth	Ectomycorrhizal: coniferous	Edible, grow in young growth forest [9]	
Cortinariaceae	*Cortina-rius*	Late stage [15], not laboratory culturing [11], has much higher above-ground fruiting bodies than the mass at root tips [17], mostly colonized at root tips than as excessive mycelium in mesh bag [17], grow into B layer [7], A & O layers [13] of mineral soil, and F-layer of organic soil [8]					
		Cortinarius acutus Fr.	10-25 mm, conical or bell-shaped, orange-brown, striate	25-60 mm long, 1-4 mm thick, smooth or with white bands.	Ectomycorrhizal: Spruce [5, 18, 2], and balsam fir [1]	Not-edible, grow in coastal conifer on mosses [2], grow in lower slope in forest interior [13], mycelium grow in O & A-layers [12]	
		Cortinarius armillatus (Fr.) Fr.	4-25 cm broad, fibred, red break or cinnamon brown. Free, wide, pale rusty gills covered by cortina	4-15 cm long, brownish, wider and swollen at the base, several irregular red rings	Ectomycorrhizal: hemlock [19,20], pine [19, 20], birch [6, 20], fir [20], and spruce [20]	Edible, grow in higher slope, in forest interior [13]	

Table II A. Continued…

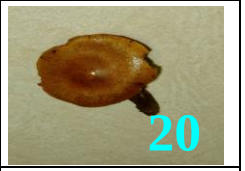
Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Cortinariaceae	*Cortinar -ius*	*Cortinarius pholidius* (Fr.:Fr.) Fr.	30-80 mm broad, gray-brown to yellow-brown, densely scaly	50-120 mm long, 10-15 mm thick, grayish brown, with brown scales like the cap	Ectomycorrhizal: spruce, birch	Not-edible	N / A
		Cortinarius gentilis (Fr.) Fr.	20-45 mm broad, reddish brown, silky, yellow at the margin. Gill brownish orange	45-65 mm long, 4-8 mm thick, pale yellow at first but later reddish brown	Ectomycorrhizal: spruce	Not-edible (poisonous)	 20
		Cortinarius semisanguineus (Fr.) Gill.	2-8 cm cross, silky, olive brown, convex to flat, dark central bump, red blood, dense, attached gills covered by yellow Cortina	2-5 cm long, 1.5-2 cm thick, pale yellow. Grow alone or in-group from summer to late fall.	Ectomycorrhizal: pine [1, 6, 19 ,20], oak [1], spruce [5, 20, 6], (pine/oak) [1], hemlock [19,20], fir [20], birch [20]	Non-edible, used as wool dyer	21
		Cortinarius stillatitius Fr.	30-80 mm, gray-brown, viscid	60-90 mm long, 10-15 mm thick, with a pale violet slimy covering	Ectomycorrhizal: spruce, fir	Not-edible	22
Hydnaceae	*Hydnum*	*Hydnum repandum* (L.) Fr.	2-8 cm broad, pale orange, wavy margin. orange teeth or spins hanging downward from cap	2-5 cm long, 0.5 cm thick, pretty similar colour as cap	Ectomycorrhizal: pine [1,19], Hazelnut, oak [1], (pine/oak) [1], hemlock [19], Douglas fir), beech	Edible	23

Table II A. Continued…

Family	Genus	Species	Cap	Stalk	Mycorrhizal association	Other attributes	Image
Russulaceae	*Lactarius*	Late-stage [15]					
		Lactarius camphoratus (Fr.) Fr.	Odor milky cap, 1-6 cm broad, reddish brown, convex with a central bump, attached reddish brown gills running down the stalk, pink to whitish flesh, not staining after cutting	1.5-6 cm long, .4-1 cm thick, hairy down the base, reddish brown	Ectomycorrhizal: pine [1,6], oak [1], (pine/oak) [1], spruce [6], birch [6]	Edible	
		Lactarius vellereus (Fr.) Fr.	8-30 mm broad, convex-depressed, velvety, white to tan. Latex white, drying yellowish	20-60 mm long, 20-50 mm thick, dry, whitish	Ectomycorrhizal: pine [1], oak [1], (pine/oak) [1],	Not-edible, used as medicine in cancer treatment	
	Russula	Grow throughout the fruiting season [1], Late-stage [21], not laboratory culturing extraradical mycelium [11], mostly colonized at root tips than as excessive mycelium in meshbag [17] Ectomycorrhizal: spruce[5], pine[1, 19, 2], oak[1,4], hemlock[19]					
		Russula peckii Singer	2-6 cm broad, pink reddish smooth, convex to central depressed, white flesh, close, broad and white gills, ovoidal spores, white (A-B) spore prints, skin is peeled about half way	4.5-10 cm long, 5-2.5 cm thick, pinkish with white flesh	Ectomycorrhizal: pine [1], oak [1],	Non-edible, taste mild to bitter, grow in mixedwood forests	

N / A: Not Available

References: Ref. 1: (Palmer et al. 1994), Ref. 2: (Wehmeyer 1950), Ref. 3 (Lincoff & National Audubon Society 1981), Ref. 4: (Schmit et al. 1999), Ref. 5: (Peter et al. 2001), Ref. 6: (Taylor et al. 2003), Ref. 7: (Dickie et al. 2002), Ref. 8: (Koide et al. 2005), Ref. 9: (North et al. 1997), Ref. 10: (Wang et al. 1995), Ref. 11: (Leake et al. 2004), Ref. 12: (Landeweert et al. 2003), Ref. 13: (Toljander et al. 2006), Ref. 14: (Zhou & Hogetsu 2002), Ref. 15: (Jumpponen et al. 2002), Ref. 16: (Molina & Pacific Northwest Research Station (Portland 1993), Ref. 17: (Kjoller 2006), Ref. 18: (Wehmeyer 1950), Ref. 19: (Durall et al. 1999), Ref. 20: (Kranabetter & Kroeger 2001)

Table II B. Sources of adapted fungi images from Table II A.

Image*	Reference	Copy right	Date adapted	Time
1	http://www.pbase.com/comafungi/image/50735121	© Dianna Smith	February 6, 2007	11:00 AM
2	pkaminski.homestead.com/page2.html	© 2003-Pamela's Mushrooms	February 16, 2007	2:00 PM
3	plantbio.berkeley.edu/~bruns/tour/fungi1.html	Not specified	February 16, 2007	2:00 PM
4	digilander.libero.it/.../funghi/a_battarrae.html	©A. Biondo R. Marchi e P. Stivala	February 16, 2007	2:05 PM
5	http://kinoko-ya.jp/01eng/amanita_virosa.htm	© Hiroshi Takahashi	February 16, 2007	2:05 PM
7	www.cegep-sept-iles.qc.ca/.../Bolets_A-R.htm	© Raymond Boyer	February 16, 2007	2:10 PM
8	http://www.pbase.com/comafungi/image/50735121	© Dianna Smith	February 16, 2007	2:10 PM
12	www.njmyco.org/Tylopilus%20plumbeoviolaceus%2...	© Sue Hopkins	February 16, 2007	2:10 PM
13	site.voila.fr/.../suillus_cavipes_jland.html	© Jacques Landry	February 16, 2007	2:20 PM
14	www.mykoweb.com/picpages/misc_photos.html	© Michael Wood	February 16, 2007	2:20 PM
16	www.uoguelph.ca/~gbarron/MISC2003/ctubaefo.htm	Not specified	February 16, 2007	2:30 PM
17	http://site.voila.fr/cmaq1c/mycotheque_c/cortinarius_acutus_anders.html	© 2004- Yves Deneyer	February 16, 2007	2:30 PM
22	http://users.skynet.be/bs133881/champis/cortinarius_stillatitius_(yd)_1.htm	© 2006- Yves Deneyer	February 16, 2007	2:30 PM
24	http://sienet.luontonetti.com/fi/sivut/lactarius_camphoratus.htm	© Raija Tuomainen	February 16, 2007	2:35 PM

* Images taken by this author are not included in this table.

Table II C. Habit and specific characteristics of moss species.

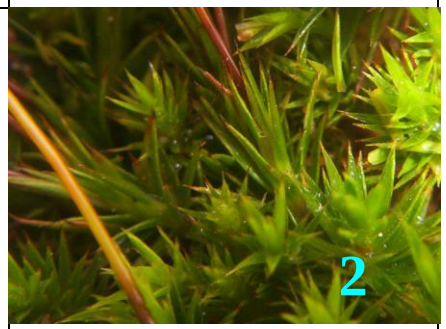
Bryophyte	Habit	Specific characteristics	Image
Pleurozium schreberi (Feather moss)	Grows on humus, mineral soil and rock, and moist well drained sites under softwood & mixwood; very shade tolerant; occurs in closed to semi-open coniferous woods, on damp ground, swamps, or margins of bogs; pH sensitive (PH≥ 5); forms carpets after the canopy closure; strong competitor against other species; grows throughout the year depending on location and climate.	High moisture absorbance from soil to tissue; capturing rainfall and throughfall as primary source for water and nutrients; has a lower photosynthesis rate than *Ptilium;* soil underneath generally is less podsolic than underneath *Ptilium*: Ae layer thin.	
Polytrichum juniperinum (hair cap moss)	Grows on humus, mineral soils and rocks; on moist, well-drained sites under softwoods and mixwoods; on stumps, banks, trailsides, and dry open woods; frequent after fire or logging; most common on dry, exposed, and acidic sites.	Relies on soil as the primary source of essential nutrients and moisture; has a transport system for interior nutrient and water movement; length of stem generally longer than other mosses.	
Ptilium crista-castrensis (Plume moss)	Grows on humus, moist-well drained sites under softwoods & mixwoods, sometimes on logs, boulders and tree bases.	Has low demand for nutrients; has a higher photosynthesis rate than *Pleurozium*; associated with a high intensity of podzolization: thick Ae layer and Al and Fe enriched B layers underneath.	

Table II C. Continued…

Bryophyte	Habit	Specific characteristics	Image
Sphagnum sp.	Grows among and on rocks in wet areas and bogs, under softwoods; forms extensive carpets; associated with acidic sites and high precipitation rates.	Highly characteristic of upland areas with shallow surface water, including pits in areas next to bogs and swamps.	

References: (Carter & Arocena 2000) and Arp 2002

Table II D. Sources of moss images from Table II D.

Image	Reference	Copyright	Adapted date	Time
1	http://www.drehwald.info/wallpaper/wallpaper2.htm	© 2005-2006 Uwe Drehwald	Feb. 8, 2007	10:00 AM
2	http://calphotos.berkeley.edu/cgi/img_query?query_src=dl&seq_num=192233&one=T	© 2006 Robert Svensson	Feb. 8, 2007	10:10 AM
3	http://www.sbf.ulaval.ca/lapointem/Floraweb_Kucyniak_2005/pages/Kucyniak_2005-38.htm	© 2005 Martine Lapointe	Feb. 8, 2007	10:12 AM
4	http://www.sbf.ulaval.ca/lapointem/Floraweb_Kucyniak_2005/pages/Kucyniak_2005-40.htm	© 2005 Martine Lapointe	Feb. 8, 2007	10:12 AM

APPENDIX III

THG CONCENTRATIONS OF SOIL LAYERS AND MOSSES, AND THG CONCENTRATIONS, BIOCONCENTRATION (BCF) VALUES, AND CAP/STALK RATIOS OF FUNGAL FRUITING BODIES

Table III A. THg concentrations (ppb, dw) of soil layers and mosses.

Substrate type	Depth* cm	N	Mean ppb, dw	Std. dev.	Std. error	Min ppb, dw	Max ppb, dw
All data		829	182	116	4	12	818
Moss	7	170	144	75	6	28	400
island		19	112	44	10	32	196
coast		65	119	67	8	28	316
mainland		86	170	78	8	41	400
All data (L-layer)	2	211	141	80	6	19	536
island		33	192	67	12	66	339
coast		77	149	83	9	19	536
mainland		101	118	74	7	23	439
All data (F-layer)	3	272	260	120	7	12	818
island		59	329	158	21	33	818
coast		103	246	112	11	36	553
mainland		110	235	87	8	12	501
All data (H-layer)	1	35	299	138	23	87	616
island		4	534	98	49	406	616
coast		8	317	67	24	188	388
mainland		23	252	119	25	87	604
All data (A-layer)	7	141	110	73	6	13	382
island		16	151	86	22	44	364
coast		61	104	72	9	18	382
mainland		64	106	69	9	13	357

* Depth refers to soil thickness or moss height.

Table III B. THg concentrations (ppb, dw), cap/stalk ratios, and BCF values of studied fungal fruiting bodies.

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Ectomycorrhizal fungi							
Total fruiting bodies (THg (ppb))	all data	727	822	1029	38	3	10475
	the whole body	50	439	632	89	3	2494
	cap	339	1055	1230	67	12	10475
	stalk	338	643	774	42	12	5805
	cap/stalk	336	1.8	1.0	0.1	0.2	10.1
	BCF	561	3.7	4.6	0.2	0.1	45.0
Fungal families							
Amanitaceae		84	712	783	85	30	4855
Bankeraceae		44	1761	853	129	308	3698
Boletaceae		124	895	1242	112	30	5805
Cantharellaceae		30	74	66	12	12	249
Cortinariaceae		192	1336	1201	87	98	10475
Hydnaceae		28	249	121	23	76	546
Russulaceae		163	346	438	34	3	2860
Suillaceae		62	431	662	84	30	2927
Fungal genera							
Amanita							
THg (ppb)	all data	84	712	783	85	30	4855
	the whole body	5	552	424	190	30	1164
	cap	39	928	963	154	119	4855
	stalk	40	520	543	86	56	2856
	cap/stalk	40	1.9	0.4	0.1	0.8	3.1
	BCF	72	2.4	2.4	0.3	0.2	12.6

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Bankera							
THg (ppb)	all data	44	1761	853	129	308	3698
	cap	22	2202	856	182	628	3698
	stalk	22	1319	592	126	308	2425
	cap/stalk	22	1.8	0.5	0.1	1.2	3.0
	BCF	42	8.6	3.6	0.6	3.0	18.6
Boletus							
	all data	36	2161	1622	270	200	5805
	the whole body	1	2494				
	cap	18	2665	1739	410	760	5520
	stalk	17	1607	1388	337	200	5805
	cap/stalk	17	2.2	2.1	0.5	0.7	10.1
	BCF	25	8.1	5.4	1.1	2.2	20.6
Cantharellus							
THg (ppb)	all data	30	74	66	12	12	249
	cap	15	91	83	21	12	249
	stalk	15	56	39	10	12	139
	cap/stalk	15	1.4	0.5	0.1	1.0	2.5
	BCF	28	0.4	0.3	0.1	0.1	1.2
Cortinarius							
THg (ppb)	all data	192	1336	1201	87	98	10475
	the whole body	8	972	656	232	128	2402
	cap	92	1629	1452	151	146	10475
	stalk	92	1075	848	88	98	4774
	cap/stalk	92	1.6	0.7	0.1	0.7	5.5
	BCF	148	6.4	6.2	0.5	0.5	45.0

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Fuscoboletinus							
THg (ppb)	all data	12	326	193	56	152	768
	cap	6	392	232	95	152	768
	stalk	6	260	133	54	172	506
	cap/stalk	6	1.5	0.5	0.2	0.9	2.5
	BCF	10	1.0	0.4	0.1	0.5	1.9
Hydnum							
THg (ppb)	all data	28	249	121	23	76	546
	cap	14	298	127	34	98	546
	stalk	14	201	96	26	76	386
	cap/stalk	14	1.5	0.4	0.1	0.9	2.3
	BCF	28	1.4	0.7	0.1	0.5	3.6
Lactarius							
THg (ppb)	all data	71	482	443	53	17	2145
	the whole body	10	652	780	247	63	2145
	cap	31	548	375	67	28	1323
	stalk	30	358	330	60	17	1457
	cap/stalk	30	2.1	1.7	0.3	0.2	7.8
	BCF	44	2.2	1.6	0.2	0.1	6.9
Leccinum							
THg (ppb)	all data	66	399	489	60	50	2876
	the whole body	6	176	66	27	114	295
	cap	30	531	607	111	75	2876
	stalk	30	312	362	66	50	1662
	cap/stalk	30	1.9	1.0	0.2	0.7	5.8
	BCF	36	1.6	1.3	0.2	0.2	4.4

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Russula							
THg (ppb)	all data	92	242	406	42	3	2860
	the whole body	20	67	83	19	3	353
	cap	36	380	513	85	21	2860
	stalk	36	200	351	58	19	2125
	cap/stalk	36	1.9	0.8	0.1	0.3	4.5
	BCF	66	1.1	1.5	0.2	0.1	7.5
Suillus							
THg (ppb)	all data	62	431	662	84	30	2927
	the whole body						
	cap	31	610	819	147	72	2927
	stalk	31	253	393	71	30	1919
	cap/stalk	29	2.2	1.2	0.2	0.9	6.5
	BCF	60	2.2	1.9	0.2	0.1	8.1
Tylopilus							
THg (ppb)	all data	10	297	156	49	30	530
	the whole body						
	cap	5	377	161	72	117	530
	stalk	5	216	111	50	30	317
	cap/stalk	5	2.1	1.0	0.4	1.6	3.8
	BCF	2	0.2	0.1	0.1	0.1	0.3
Fungal species							
Amanita flavoconia							
THg (ppb)	all data	35	1015	1090	184	124	4855
	the whole body	4	683	356	178	405	1164
	cap	15	1354	1418	366	252	4855
	stalk	16	780	766	191	124	2856

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Amanita flavoconia	cap/stalk	16	1.7	0.3	0.1	1.1	2.3
	BCF	31	3.0	3.1	0.6	0.3	12.6
Amanita fulva	all data	16	490	349	87	130	1220
THg (ppb)	cap	8	661	387	137	253	1220
	stalk	8	318	207	73	130	642
	cap/stalk	8	2.1	0.3	0.1	1.9	2.6
	BCF	14	1.4	1.3	0.3	0.3	5.0
Amanita muscaria							
THg (ppb)	all data	12	601	295	85	169	1233
	cap	6	820	241	98	523	1233
	stalk	6	383	135	55	169	577
	cap/stalk	6	2.3	0.5	0.2	1.7	3.1
	BCF	6	2.9	2.0	0.8	0.6	5.8
Amanita vaginata							
THg (ppb)	all data	13	266	219	61	30	761
	the whole body	1	30				
	cap	6	380	249	102	119	761
	stalk	6	190	138	56	56	417
	cap/stalk	6	2.1	0.3	0.1	1.7	2.6
	BCF	13	1.2	1.1	0.3	0.2	3.3
Amanita virosa							
THg (ppb, dw)	all data	8	719	225	80	473	1082
	cap	4	850	268	134	473	1082
	stalk	4	589	31	15	544	614
	cap/stalk	4	1.5	0.5	0.2	0.8	1.8
	BCF	8	3.0	0.9	0.3	2.0	4.6

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Bankera carnosa							
THg (ppb)	all data	44	1761	853	129	308	3698
	cap	22	2202	856	182	628	3698
	stalk	22	1319	592	126	308	2425
	cap/stalk	22	1.8	0.5	0.1	1.2	3.0
	BCF	42	8.6	3.6	0.6	3.0	18.6
Boletus edulis							
THg (ppb)	all data	23	2838	1653	345	459	5805
	the whole body	1	2494			2494	2494
	cap	11	3583	1627	490	918	5520
	stalk	11	2125	1478	446	459	5805
	cap/stalk	11	1.9	0.4	0.1	0.9	2.4
	BCF	19	9.4	5.6	1.3	2.2	20.6
Boletus separans							
THg (ppb)	all data	13	962	482	134	200	2025
	cap	7	1222	436	165	760	2025
	stalk	6	657	351	143	200	1276
	cap/stalk	6	2.9	3.6	1.5	0.7	10.1
	BCF	6	4.2	1.4	0.6	2.8	6.2
Cantharellus cibarius							
THg (ppb)	all data	20	46	55	12	12	249
	cap	10	52	71	22	12	249
	stalk	10	40	37	12	12	139
	cap/stalk	10	1.1	0.3	0.1	1.0	1.8
	BCF	18	0.2	0.3	0.1	0.1	1.2

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Cantharellus tubaeformis	all data	10	128	53	17	68	222
THg (ppb)	cap	5	169	40	18	133	222
	stalk	5	87	19	9	68	119
	cap/stalk	5	2.0	0.4	0.2	1.6	2.5
	BCF	10	0.6	0.3	0.1	0.3	1.1
Cortinarius acutus		8	972	656	232	128	2402
THg (ppb, dw)	the whole body	8	972	656	232	128	2402
	BCF	6	4	3	1	1	9
Cortinarius armillatus							
THg (ppb)	all data	110	1696	973	93	183	5174
	cap	55	2000	1099	148	262	5174
	stalk	55	1392	716	97	183	3618
	cap/stalk	55	1.5	0.3	0.0	1.0	3.2
	BCF	94	7.3	5.7	0.6	0.6	32.8
Cortinarius gentilis							
THg (ppb)	all data	6	918	323	132	519	1274
	cap	3	1204	67	39	1139	1274
	stalk	3	632	99	57	519	706
	cap/stalk	3	2.0	0.4	0.3	1.7	2.5
	BCF	6	4.1	2.7	1.1	2.1	9.3
Cortinarius pholidius							
THg (ppb)	all data	4	826	310	155	468	1108
	cap	2	1085	33	23	1062	1108
	stalk	2	568	142	101	468	669
	cap/stalk		2	2.0	0.4	0.3	1.7
	BCF	4	3.5	1.3	0.7	2.0	4.7

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Cortinarius semisanguineus							
THg (ppb)	all data	52	381	218	30	98	1144
	cap	26	473	238	47	146	1144
	stalk	26	289	151	30	98	699
	cap/stalk	26	1.9	1.2	0.2	0.7	5.5
	BCF	26	2.0	1.1	0.2	0.5	5.5
Cortinarius stillatitius							
THg (ppb)	all data	12	2804	2621	757	1197	10475
	cap	6	3636	3398	1387	1552	10475
	stalk	6	1973	1381	564	1197	4774
	cap/stalk	6	1.7	0.4	0.2	1.1	2.2
	BCF	12	12.2	11.3	3.3	3.7	45.0
Fuscoboletinus viscidus							
THg (ppb)	all data	12	326	193	56	152	768
	cap	6	392	232	95	152	768
	stalk	6	260	133	54	172	506
	cap/stalk	6	1.5	0.5	0.2	0.9	2.5
	BCF	10	1.0	0.4	0.1	0.5	1.9
Hydnum repandum							
THg (ppb)	all data	28	249	121	23	76	546
	cap	14	298	127	34	98	546
	stalk	14	201	96	26	76	386
	cap/stalk	14	1.5	0.4	0.1	0.9	2.3
	BCF	28	1.4	0.7	0.1	0.5	3.6

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Lactarius camphoratus							
THg (ppb)	all data	19	608	674	155	17	2145
	the whole body	5	1042	944	422	159	2145
	cap	7	436	496	187	28	1223
	stalk	7	469	557	211	17	1457
	cap/stalk	7	1.1	0.5	0.2	0.8	2.2
	BCF	10	1.4	1.6	0.5	0.1	3.4
Lactarius vellereus							
THg (ppb)	all data	52	437	318	44	31	1323
	the whole body	5	263	313	140	63	809
	cap	24	580	338	69	46	1323
	stalk	23	325	233	49	31	1035
	cap/stalk	23	2.4	1.8	0.4	0.2	7.8
	BCF	34	2.4	1.5	0.3	0.3	6.9
Leccinum holopus							
THg (ppb)	all data	50	431	540	76	50	2876
	cap	25	559	656	131	75	2876
	stalk	25	302	362	72	50	1662
	cap/stalk	25	2.0	1.0	0.2	0.9	5.8
	BCF	28	1.7	1.3	0.2	0.2	4.4
Leccinum scabrum							
THg (ppb)	all data	16	301	266	66	113	1069
	the whole body	6	176	66	27	114	295
	cap	5	388	250	112	205	792
	stalk	5	364	399	178	113	1069
	cap/stalk	5	1.4	0.5	0.2	0.7	2.0
	BCF	8	1.3	1.3	0.4	0.3	3.7

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Russula peckii							
THg (ppb)	all data	43	447	521	79	17	2860
	the whole body	3	46	33	19	17	82
	cap	20	633	575	129	148	2860
	stalk	20	321	437	98	53	2125
	cap/stalk	20	2.4	0.7	0.2	1.3	4.5
	BCF	37	1.8	1.7	0.3	0.2	7.5
Russula sp.							
THg (ppb)	all data	49	62	66	9	3	353
	the whole body	17	71	90	22	3	353
	cap	16	65	64	16	21	285
	stalk	16	48	29	7	19	134
	cap/stalk	16	1.3	0.5	0.1	0.3	2.5
	BCF	29	0.2	0.1	0.0	0.1	0.5
Suillus cavipes							
THg (ppb)	all data	22	202	100	21	104	509
	cap	11	271	93	28	161	509
	stalk	11	134	44	13	104	257
	cap/stalk	11	2.1	0.6	0.2	1.4	3.4
	BCF	22	2.6	1.3	0.3	0.9	6.3
Suillus grevillei							
THg (ppb)	all data	40	557	796	126	30	2927
	cap	20	796	975	218	72	2927
	stalk	20	318	480	107	30	1919
	cap/stalk	18	2.3	1.5	0.4	0.9	6.5
	BCF	38	2.0	2.1	0.3	0.1	8.1

Table III B. Continued…

Fungal taxonomical order	Type	N	Mean	Std. dev.	Std. error	Min	Max
Tylopilus plumbeoviolacus							
THg (ppb)	all data	10	297	156	49	30	530
	cap	5	377	161	72	117	530
	stalk	5	216	111	50	30	317
	cap/stalk	5	2.1	1.0	0.4	1.6	3.8
	BCF	2	0.2	0.1	0.1	0.1	0.3
Saphrophytic fungi							
Total fruiting bodies THg (ppb)	all data	11	736	751	226	120	2420
Psathyrella foenisecii (Fr.) A.H.S.							
THg (ppb)	the whole body	4	253	218	109	120	578
Marasmius oreades (Bolt. Ex Fr.) Fr.							
THg (ppb)	the whole body	5	963	970	434	146	2420
Entoloma strictius (Pk.) Sacc.							
THg (ppb)	the whole body	2	1133	473	335	798	1467

GLOSSARY

Basidiomycete: fungi that produce fruiting body making basidia (spore-bearing tissues), in units containing four spores. In mature mushrooms, basidia located underneath the cap, either within pores as in the *Boletaceae*, or on gills in families such as *Amanitaceae and Cortinariaceae.*

Bioconcentration: refers to the process that produces higher concentrations of a substance in the body of an organism than in its trophic substrate.

Cortina: refers to the cotton webby form of the partial veil that covers not fully-developed spore-bearing parts of the cap and continues to the upper part of stalk.

Cystine: a S-containing amino acid, important in the retention of Hg in organic substrates**.**

Ectomycorrhizal (ECM) fungi: fungi that produce an inter-cellular mantle (also called Hartig net) of hyphae within root cortex of their host trees.

Metallothionines (MTs): refer to low-molecular weight cystine-rich proteins.

Methionine: a S-containing amino acid, important in the retention of Hg in organic substrates.

Mycorrhiza: refers to the symbiosis of fungi with roots.

Mycelium: refers to the network of filamentous hyphae of fungi.

Sporophore (also called basidiocarp, or mushroom): the visible above-ground part of the fruiting body of fungi.

Thallus: the filamentous part of fungi (mycelium), this part does not have an organized structure such as the sporophore.

Volva: ruptured membrane remaining when the fungal fruiting body grows out of its initial veil.

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Nasr, M., Ritchie, C. D., and P. A., Arp. 2005. Atmospheric Hg deposition and terrestrial Hg pathways in coastal catchments of the Bay of Fundy, Canada. ICHMET XIII International Conference on Heavy Metals in the Environment, Ed. Trindade R. B.E., Melamed, R., Sobral, L. G. S., Barbosa, J., P., B. Rio de Janeiro, Brazil. p. 268.

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