

MICROBIAL QUALITY OF HEMP (*Cannabis sativa* L.) AND FLAX
(*Linum usitatissimum* L.) FROM PLANTS TO THERMAL INSULATION

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ACADEMIC DISSERTATION

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To my family

Microbial quality of hemp (*Cannabis sativa* L.) and flax (*Linum usitatissimum* L.) from plants to thermal insulation

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Abstract

Flax and hemp have traditionally been used mainly for textiles and yarns, but recently interest has also been focused on non-textile applications. For example bast fibrous thermal insulations are considered to be ecologically advantageous, which is one of their most important advantages in competition.

Microbial quality throughout the whole processing chain of bast fibres has not previously been studied. Especially in the Nordic climates, aerial humidity during the harvest season is often high. This can cause problems for harvesting, retting, storing and processing of the crop. This study concentrates on the microbial quality and possible microbial risks in the primary production chain of hemp and flax fibres and fibrous insulation products.

In order to be able to utilize hemp and flax fibres in industrial applications, the bast fibres must be separated from the rest of the plant. Non-cellulosic components can be removed with various pretreatment processes. Detachment of fibres has usually been achieved by dew retting, water retting or microbial treatments with enzymes, followed by mechanical extraction processes. There is a certain risk of microbial contamination associated with the pretreatment processes. In milling, pieces of stem are crushed in a hammer mill and separated by screening. Steam explosion (STEX) is a process in which the sample is treated with steam at high temperature and pressure. In this study, STEX was used as a reference method, in order to determine whether it is effective against possible fungal growth on fibre materials. On the basis of the results obtained in this study, dry conditions during the growing season caused smaller height and diameter of the plants and increased the dry weight content of the matured stalk. High aerial humidity encouraged the growth of microbes in plants. In both plants studied, the mould and bacterial contents on stalks increased at the end of the growing season and during frost retting in the winter. However, mechanically fractionated fibres of hemp harvested after early frost or in spring had the lowest amount of moulds. Therefore by processing and mechanical separation, it is possible to produce fibres containing somewhat less moulds and bacteria than the whole stem or the plant in the field. Enzymatic treatment encouraged the growth of moulds in fibres, the mould content being 500 times higher after the enzymatic treatment than before it. Steam explosion reduced the amount of moulds in fibres. Dry thermal treatment did not markedly reduce the amount of microbes.

Emissions from building materials have been measured with field sampling from e.g. water-damaged buildings, and with special emission measurement chambers in laboratories. However, only little attention has been focused on the interdisciplinary approach. In this project an emission measurement chamber was developed which was suitable for both mat type and loose fill type insulations and capable of interdisciplinary sampling. In this study, all insulations made of flax and hemp fibres contained microbes, the amounts of which increased in very moist conditions. The highest amounts of fungal emissions were in the range of 10^3 – 10^5 cfu m⁻³ from the flax and hemp insulations at 90% RH of air. After drying of moulded insulations at 30% RH, the amounts of emitted moulds were in all cases higher compared to the emissions at 90% RH before drying.

The most common fungi in bast fibres were *Penicillium* and *Rhizopus*. The widest variety of different fungi was in the untreated hemp and linseed fibres and in the commercial loose-fill flax insulation. *Penicillium*, *Rhizopus* and *Paecilomyces* were the most tolerant to steam explosion. *Bipolaris* and *Alternaria* were absent in enzymatically treated hemp samples, whereas *Penicillium* grew well during the enzymatic treatment. According to the literature, the most common fungi in building materials and indoor air are *Penicillium*, *Aspergillus* and *Cladosporium*, which were all found in some of the bast fibre materials in this study. Microbes of e.g. the genera *Stachybotrys*, *Chaetomium* and *Fusarium*, found in flax and linseed samples in this study, may be a sign of moisture damage. Hygrothermal loads in building structures may lead to degradation failure of building materials, as well as to poor indoor air quality. As hygroscopic materials, hemp and flax adsorb moisture from the surroundings if the vapour pressure is lower within the material. Capillary absorption of water in building materials results in rapid fungal contamination.

Some fungal diseases can be controlled by treatment with fungicides or by crop rotation. The use of disease-resistant cultivars can increase the possibilities of growing hemp and flax. New resistant cultivars should still be developed. However, as organic materials, hemp and flax fibres contain high levels of nutrients, and therefore none of the current methods offers a perfect and permanently hygienic result. However, it is advantageous that the amount of microbes can be decreased by the processing methods, and that the environmental conditions can be regulated so that microbial growth is not possible or is slower.

Key words: Microbial quality, linseed, flax, hemp, bast fibre, thermal insulation, emission, mould.

Foreword

I acknowledge Professor Jukka Ahokas, the director of our institute, for providing me the opportunity to carry out this work at the Department of Agrotechnology. I am grateful to professor Aarne Pehkonen for his expertise and knowledge and for generating several agrofibres projects which gave me the possibility to carry out my investigations. Docent Mikko Hautala helped with problems and ideas concerning physics, for which I am grateful. Professor Anna-Maija Sjöberg encouraged and motivated me throughout the study, and helped in scientific writing with her experience and expertise, for which I am most thankful.

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Helsinki, June 2006

Minna Nykter

Abbreviations

a_w	water activity
cfu	colony forming unit
dw	dry weight (oven dry mass) [g or kg]
MVOC	microbial volatile organic compound
RH	relative humidity of air [%]
STEX	steam explosion
TGY agar	tryptone-glucose-yeast agar
VOC	volatile organic compound
w.b.	wet basis, related to actual (“wet”) mass

List of original publications

- I Koivula, M.*, Kymäläinen, H.-R., Vanne, L., Levo, S., Pehkonen, A. & Sjöberg, A.-M. Microbial quality of linseed and fibre hemp plants during growing and harvest seasons. *Agricultural and Food Science* 13 (2004): 327-337.
- II Virta J., Koivula M.*, Hussein T., Koponen S., Hakkarainen H., Kymäläinen H.-R., Hämeri K., Kulmala M. & Hautala M. Emissions from thermal insulations – Part 1: Development and characteristics of the test apparatus. *Building and Environment* 40 (2005): 797-802.
- III Koivula M.*, Kymäläinen H.-R., Virta J., Hakkarainen H., Hussein T., Komulainen J., Koponen H., Hautala M., Hämeri K., Kanerva P., Pehkonen A. & Sjöberg A.-M. Emissions from thermal insulations – Part 2: Evaluation of emissions from organic and inorganic insulations. *Building and Environment* 40 (2005): 803-814.
- IV Kymäläinen, H.-R., Nykter, M.*, Hautala, M. & Sjöberg, A.-M. Hygienic quality of stem fractions of mechanically processed fibre hemp and linseed. *Agricultural and Food Science* 14 (2005): 143-153.
- V Nykter, M.*, Kymäläinen, H.-R., Thomsen, A.B., Lilholt, H., Koponen, H. Sjöberg, A.-M. & Thygesen, A. Effects of thermal and enzymatic treatments and harvesting time on the microbial quality and chemical composition of fibre hemp (*Cannabis sativa* L.). Submitted.

The author's contribution in the original papers

- I Minna Nykter* (Koivula) is the corresponding author, designed the experiments, performed the sampling and most of the measurements, analysed the data, interpreted the results and wrote the paper.
- II Minna Nykter* (Koivula) designed the microbiological experiments and performed the microbiological measurements, interpreted the microbiological results and wrote parts of the paper.
- III Minna Nykter* (Koivula) is the corresponding author, designed the microbiological experiments and performed the microbiological measurements, analysed part of the data, interpreted the results and wrote most of the paper.
- IV Minna Nykter* designed and performed part of the measurements, interpreted some of the results and wrote parts of the paper.
- V Minna Nykter* is the corresponding author, designed the microbiological experiments, performed part of the measurements, interpreted a significant part of the results and wrote a significant part of the paper.

* During this study the author's surname changed from Koivula to Nykter. Thus the first three publications are authored by Koivula and the last two by Nykter.

1 Introduction

1.1 *Botanical and morphological features and chemical composition of hemp and flax fibres*

1.1.1 *Hemp and flax plants*

Fibre hemp, *Cannabis sativa* L. belongs to the family *Cannabinaceae*. According to Small and Cronquist (1976), *C. sativa* comprises two subspecies, which are further divided into two varieties. According to Schultes et al. (1974), *Cannabis* is divided into three species (*C. sativa*, *C. indica* and *C. ruderalis*), each of which has its own varieties. *Cannabis* is grown for three purposes: fibre hemp is used for production of bark fibres, oil hemp for production of hemp oil and drug hemp is used for production of psychoactive resin. The present study focuses on fibre hemp.

Fibre hemp is an annual herb plant ranging in height from 1.2 to 5 m and with a diameter from 4 to 20 mm. It is a dioecious plant, although occasionally monoecious plants are also found. Before flowering, male and female plants are indistinguishable from each other, although they may differ somewhat in growth habit such as height and extent of branching. Female plants are often shorter and have more branches than male plants. The male flowers hang in long and loose, multibranched and clustered panicles, which can be up to 30 cm long, whereas female flowers are tightly crowded in the axils (Clarke 1999). The male plants grow more quickly, which leads to uneven maturity at harvest (Berger 1969).

Flax *Linum usitatissimum* L., belongs to the family *Linaceae*. Cultivars that are cultivated for fibre are called fibre flax or flax, and cultivars that are cultivated for oilseeds are called linseed, oilseed flax or flax. In the present study, the term “linseed” means oilseed varieties and “flax” refers to both types. There are also combined cultivars that are cultivated for both fibre and oilseeds.

Flax plants are annual bast fibre plants that grow to a height from 40 cm to nearly one meter, with a stalk diameter of 1-2 mm (Sultana 1992a). The linseed types are bushy and often bear basal branches arising just above the surface of the soil. Flax varieties can be distinguished by the colour of the flowers. They can range from a dark to a very light blue, white or pale pink.

1.1.2 Structure and chemical composition of hemp and flax stems

Hemp and flax stems (Figure 1) consist of two different components: the bark including fibre bundles, and the core consisting of wood cells (Ranalli 1999). These components differ from each other in their chemical composition. The outer layer of stem, the epidermis, is covered by a surface layer, the cuticulum. Cuticulum protects the plant from drying, and it consists of waxy matter (Haudek and Viti 1978). Epidermis consists of colour-binding media and stomata (pores), through which the plant ventilates and regulates evaporation (Haudek and Viti 1978).

The bast component of hemp includes elementary fibres: outer primary and inner secondary fibres. Both are thick-walled, with a high content of cellulose and a low content of hemicellulose and lignin (Ranalli 1999). Primary bast fibres are 5-40 mm long and heterogeneous, whereas secondary bast fibres are smaller and uniformly about 2 mm long (Ranalli 1999). The primary fibre bundles contain 2-20 and the secondary fibre bundles over 40 individual cells (Haudek and Viti 1978).

Under the outer layer of the flax stem there is the bark layer. This contains 20-50 fibre bundles, each of which contains 10-40 elementary fibres of single cells (Peters 1963, Berger 1969, Haudek and Viti 1978). The bark layer contains chlorophyll. During growth of the plant there is a cambium, which distinguishes the bast fibre layer from the wooden layer (Haudek and Viti 1978).

The woody core of hemp, xylem, consists of parenchyma and vessels, which both have transport functions; and libriform fibres (core fibres), which give the plant rigidity and strength (Ranalli 1999). The xylem of flax consists of strong-walled wood cells, which are short and thick. The core is the innermost layer of the xylem, consisting of loosely

packed core cells (Haudek and Viti 1978). Core fibres are short and thin-walled, and their chemical composition is close to that of hardwood: about 40% cellulose, 20% hemicellulose and 20% lignin (Ranalli 1999). Pieces of the woody part of the flax and hemp stem are called shives. In the middle of the stem there is the lumen (cavity).

Structures of flax and hemp stems are presented in Figure 1.

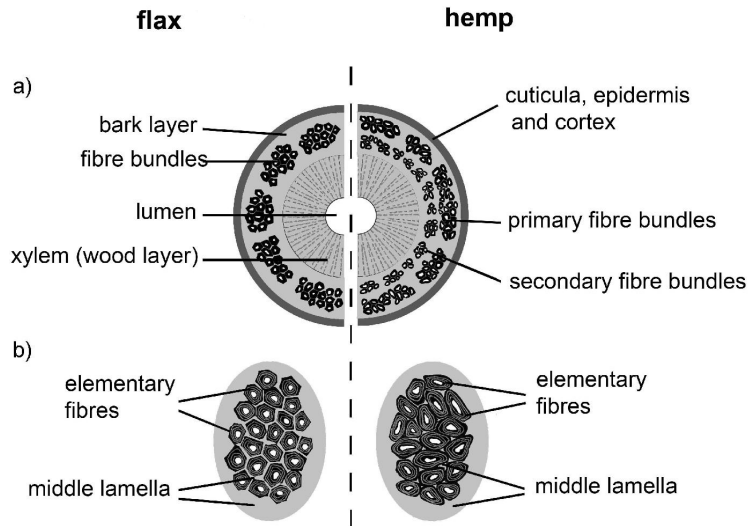


Figure 1. Structures of flax and hemp stems. The true diameter of flax stem is 1-2 mm and of hemp stem 4-20mm. 1a) cross-section of stems, 1b) fibre bundles (Härkäsalmi 2006).

The elementary fibres (single cells) of both plants consist of layers (Figure 2). The elementary fibre of flax is cylindrical in shape and it ends in a sharp point, whereas the end of hemp fibre is not sharp.

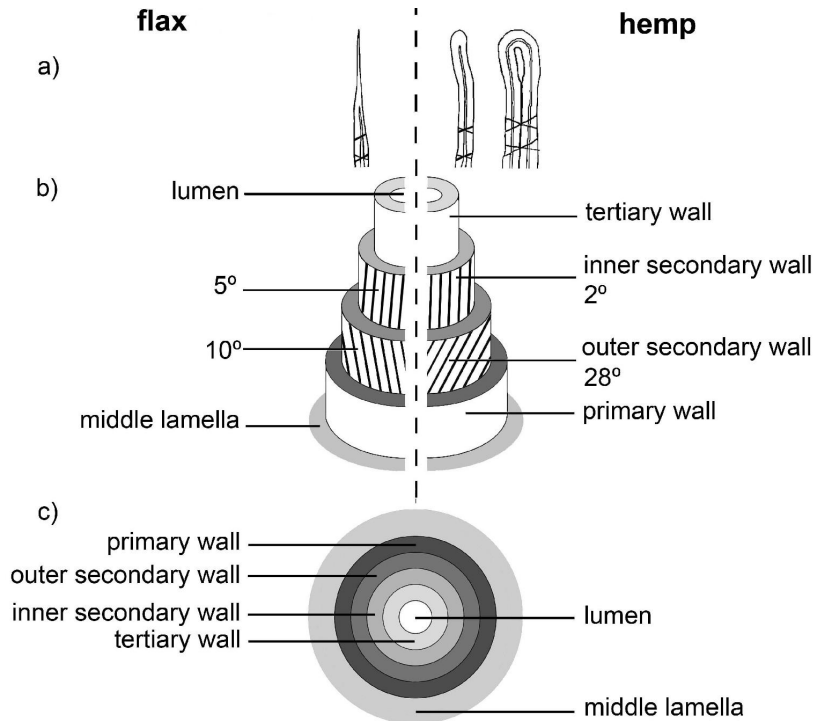


Figure 2. Structure of the elementary fibres of flax and hemp. 2a) ends of the fibres, 2b) longitudinal section of fibres, 2c) cross-section of fibres (Härkäsalmi 2006).

Flax plants consist of cellulose, hemicelluloses, pectins, lignin, fat and wax, and some water soluble matter. The chemical composition of hemp is similar, but the amount of lignin is greater than in flax. There is some variation in the data given in the literature (e.g. Peters 1967, Haudek and Viti 1978, Kozłowski et al. 1997), due to e.g. differences in retting degree and processing and research methods. In both hemp and flax the greatest fractions are cellulose ($\approx 60\text{-}70\%$) and hemicellulose ($\approx 15\text{-}19\%$). Both plants contain approximately 2-4% lignin, 2-4% pectin and 1-2% fat and wax. The distribution between the chemical components is different in bast fibres and shives (Table 5 in Kymäläinen 2004). The bast fibres contain more cellulose, whereas the shives contain more lignin and hemicellulose and less cellulose (e.g. Thomsen et al. 2005).

Cellulose provides strength to the cell wall (Lennholm et al. 2003). Hemicellulose is present throughout the whole fibre, joining together cellulose and lignin (Focher 1992). Lignin increases the strength and stiffness of the cell wall, decreases its water

permeability and hinders chemical, physical and microbiological degradation (Mauseth 1988). Pectins are present in the middle lamella between cells of all types (Teleman 2003).

1.2 Fibre hemp and flax during the growing season

1.2.1 Production areas of fibre hemp and flax

Hemp and flax are grown in several different climates and areas. The main production area of hemp is the Far East (FAOSTAT 2006). In Finland fibre hemp was cultivated only in a very minor area. The worldwide growing area of linseed is much greater than that of fibre hemp or fibre flax. The world's major production areas of linseed are the Far East, Canada, USA and Europe. The main producers of fibre flax are Europe, the Russian Federation and the Far East (FAOSTAT 2006). Harvested areas of fibre hemp, linseed and fibre flax are presented in Table 1.

Table 1. Harvested areas of fibre hemp, linseed and fibre flax during the years 2000-2005.

Plant	Fibre hemp		Linseed		Flax	
	Harvested area (Ha)		Harvested area (Ha)		Harvested area (Ha)	
Year	Global^a	Finland^b	Global^a	Finland^b	Global^a	Finland^b
2000	58 350	100	2 706 500	1400	437 250	1100
2001	63 700	100	2 618 900	1600	515 300	400
2002	59 500	40	2 516 000	1400	458 300	200
2003	53 000	15	2 747 000	2000	499 550	100
2004	52 400	10	2 644 500	2050	514 200	70
2005	52 450	5	3 146 500	1800	501 250	60

^a FAOSTAT 2006

^b TIKE 2006

1.2.2 Effects of cultivation conditions on the growth of fibre hemp and flax

Environmental conditions affect the growth of fibre hemp. Humidity around the stalks makes the micro-environment suitable for many fungal pathogens. On the other hand, fungal infection may be caused if excessive lodging occurs in the field due e.g. to too high plant density (Struik et al. 2000). If suitable levels of nutrients, water and light are available, hemp can reach a height of 5 m in a four- to six-month growing season (Clarke 1999). When hemp is planted in close stands in a field, the plants do not branch, but grow as tall, thin and straight stalks. Fibre hemp produces long and wide stalks, which are grown in tight proximity. Hemp has a dual response to daylength. During the first months of growth long daylength results in more vigorous vegetative growth of the hemp. Shorter daylength at the end of the growing season encourages flowering and completion of the life cycle (Sankari and Mela 1998, Clarke 1999). Critical daylength for flowering is 12-14 hours, depending on the strain (Clarke 1999).

Hemp grows rapidly, and adequate supplies of available nitrogen are essential. According to Bócsa et al. (2000), for the production of 1 ton of hemp stems, 15-20 kg of nitrogen, 4-5 kg of phosphorus (P_2O_5) and 15-20 kg of potassium (K_2O) are needed. Although some hemp crops benefit from added nitrogen, this is not always necessary if the soil is in good condition. Excessive application of nitrogen can cause more leafy and succulent growth and increase of the stem diameter above the optimum range (Ranalli 1999), or lead to self-thinning and reduction of the number of living plants (van der Werf et al. 1994).

In order to obtain the best flax crop results, the weather conditions should be stable from germination to the end of flowering. Availability of water and sufficient temperature are also important for the stem development. Usually flax is cultivated in areas where the daily temperature remains below 30°C. The yield has been reported to be optimal at a temperature sum of about 1400 day degrees (DD) (Sultana 1992a). If the daily temperature is high (maximum > 28°C), the flax plants will remain short, which affects the fibre yield and quality. Flax is generally cultivated at more than 400 m above sea level in a cool and humid climate. The rate of growth and development varies with the age of the plant (Sultana 1992a).

Application of nitrogen, phosphorus and potassium fertilisers influences the fibre content and fibre yield of flax (Keijzer and Metz 1992). In order to obtain a yield of 7 tons of flax, 75-80 kg nitrogen per hectare is required. Of the other major chemicals, potassium oxide is often used, its requirement during flowering being greater than 140 kg/h. In addition, flax requires a remarkable amount of calcium (Sultana 1992a). Especially compared with hemp, flax is prone to lodge easily, and therefore it is recommended to provide flax crops with no more than the required amount of nitrogen fertilization (Sultana 1992a).

1.2.3 Pathogens of hemp and flax and spoilage of the crop

Environmental conditions partly regulate the growth of microbes. In favourable conditions of nutrient levels, moisture and temperature, microbes can proliferate and lead to damage of organic materials. Alternating periods of wet and dry conditions can affect the spreading of some diseases, as the wet periods encourage the formation of spores while the dry ones encourage their distribution (Mercer 1992). Prolonged wet periods and relatively high temperatures between flowering and harvest encourage development of the fungal pathogen *Alternaria linicola*. In a study by Vloutoglou et al. (1999), eight hours of leaf wetness at 25°C or ten hours at 15°C initiated infection by *Alternaria linicola*. In addition, fungal spores possibly survive the fluctuating temperature and moisture conditions (Pasanen et al. 2000a). The fungi can cause losses of dry matter by utilising the carbohydrate reserves, and they can also cause quality deterioration by destroying the structure of the fibres. Several microorganisms (e.g. the fungi *Cladosporium*, *Fusarium* and *Epicoccum*) are common on aerial plant parts (Dazzo 1980), and they can act as spoilage organisms in humid environments. Hemicellulose and pectins support a profuse growth of fungi (Sharma et al. 1992).

Fungal pathogens affecting seedlings cause damping-off. Root diseases include e.g. sore shin and root rots, and stalks diseases caused by fungi are e.g. stalk rot, stalk canker, anthracnose, wilt and blight and in leaves fungal infections cause e.g. rust, leaf spot, downy mildew and powdery mildew (literature in Tables 2 and 3).

Hemp is a rather pest-tolerant plant. Several problems arise in the case of *Cannabis*, but only rarely do these problems cause severe damage. Over 420 fungal taxa infecting *Cannabis* have been reported (McPartland 1999), although many of the names are synonyms. The most severe disease in hemp is gray mould, caused by *Botrytis cinerea*. This is a remarkable problem especially in humid regions (over 60% RH) and in cool to moderate temperatures (20-24°C) (McPartland 1999). Fungal pathogens of hemp are presented in Table 2.

Table 2. Fungal pathogens of *Cannabis* according to the literature.

Species or genus	Plant part of infection	Reference
<i>Aecidium cannabis</i>	Leaves	McPartland 1991
<i>Alternaria alternata</i>	Stalk	McPartland 1991, 1995a, 1999
<i>Alternaria solani</i>	Stalk	McPartland 1995a, 1999
<i>Alternaria longipes</i>	Stalk	McPartland 1995a, 1999
<i>Ascochyta cannabina</i>	Stalk, leaves	McPartland 1994
<i>Ascochyta prasadii</i>	Stalk, leaves	McPartland 1991, 1994
<i>Botryosphaeria obtusa</i>	Stalk	McPartland 1995b, 1999
<i>Botryosphaeria marconii</i>	Stalk	McPartland 1995b, 1999
<i>Botrytis cinerea</i>	Seedlings, stalk, flower	McPartland 1991, 1999
<i>Cercospora cannabis</i>	Leaves	McPartland 1991, 1999
<i>Cladosporium herbarum</i>	Stalk	McPartland 1991, 1995a, 1999
<i>Cladosporium cladosporioides</i>	Stalk	McPartland 1991, 1995a
<i>Cladosporium tenuissimum</i>	Stalk	McPartland 1995a
<i>Colletotrichum coccodes</i>	Stalk	McPartland 1991, 1995a, 1999
<i>Colletotrichum dematium</i>	Stalk	McPartland 1991, 1995a, 1995c, 1999
<i>Curvularia cymbopogonis</i>	Seeds, seedlings, leaves	McPartland and Cubeta 1997, McPartland 1999
<i>Curvularia lunata</i>	Leaves	McPartland 1991, 1999
<i>Cylindrosporium cannabinum</i>	Stalk	McPartland 1991
<i>Dendrophoma marconii</i>	Stalk	McPartland 1991, 1999
<i>Diaporthe arctii</i>	Stalk	McPartland 1995c
<i>Didymella arcuata</i>	Stalk, leaves	McPartland 1994
<i>Didymella cannabis</i>	Stalk, leaves	McPartland 1994, Verkley et al. 2004
<i>Epicoccum nigrum</i>	Roots, stalk	McPartland 1991
<i>Fusarium oxysporum</i> f.sp. <i>cannabis</i>	Seedlings, stalk	McPartland 1991, 1995a, 1999, Tioureaev et al. 2001, McPartland and Hillig 2004
<i>Fusarium oxysporum</i> f.sp. <i>vasinfectum</i>	Seedlings, stalk	McPartland 1991, 1995a, 1999, McPartland and Hillig 2004
<i>Fusarium solani</i>	Seedlings, roots	McPartland 1991, 1999
<i>Gibberella cyanogena</i>	Seedlings, stalk	McPartland 1991, 1995a, 1995c
<i>Gibberella zeae</i>	Seedlings, stalk	McPartland 1995a, McPartland and Cubeta 1997
<i>Glomus mosseae</i>	Roots	McPartland and Cubeta 1997
<i>Lasiodiplodia theobromae</i>	Stalk	McPartland 1991, 1995b
<i>Leptosphaeria acuta</i>	Stalk	McPartland 1991, 1994, 1995c, 1999
<i>Leptosphaeria cannabina</i>	Stalk	McPartland 1991
<i>Leptosphaeria woroninii</i>	Stalk	McPartland 1991
<i>Leptosphaerulina trifolii</i>	Leaves	McPartland 1995c, 1999
<i>Leveillula taurica</i>	Leaves	McPartland 1991, 1999

Table 2 continues...

Table 2 (continues). Fungal pathogens of *Cannabis* according to the literature.

Species or genus	Plant part of infection	Reference
<i>Micropeltopsis cannabis</i>	-	McPartland and Cubeta 1997
<i>Macrophomina phaseolina</i>	Seedlings, stalk	McPartland 1991, 1999
<i>Melanospora cannabis</i>	Stalk	McPartland 1991
<i>Ophiobolus anguillidus</i>	Stalk	McPartland 1991
<i>Ophiobolus cannabinus</i>	Stalk	McPartland 1991, 1999
<i>Orbilia luteola</i>	Stalk	McPartland and Cubeta 1997
<i>Pestalotiopsis</i>	Stalk, leaves	McPartland and Cubeta 1997
<i>Phoma exigua</i>	Stalk, leaves	McPartland 1991, 1994
<i>Phoma herbarum</i>	Stalk, leaves	McPartland 1991, 1994
<i>Phoma glomerata</i>	Stalk, leaves	McPartland 1994
<i>Phomopsis cannabina</i>	Stalk	McPartland 1983, 1991, 1999
<i>Phomopsis ganjae</i>	Leaves	McPartland 1983, 1984, 1991, 1999, McPartland and Hughes 1994
<i>Phyllachora cannabidis</i>	Leaves	McPartland 1991
<i>Phyllosticta</i>	Stalk, leaves	McPartland 1991
<i>Phymatotrichopsis omnivora</i>	Roots	McPartland 1991
<i>Pseudocercospora cannabina</i>	Leaves	McPartland 1991, 1995c, 1999
<i>Pseudoperonospora cannabina</i>	Leaves	McPartland 1991, 1999, McPartland and Cubeta 1997
<i>Pseudoperonospora humuli</i>	Leaves	McPartland 1991, 1999
<i>Pythium ultimum</i>	Seedlings	McPartland 1991, 1995a, 1999
<i>Pythium aphanidermatum</i>	Seedlings	McPartland 1991, 1999
<i>Pythium debaryanum</i>	Seedlings	McPartland 1991
<i>Rhizoctonia solani</i>	Seedlings, roots, stalk	McPartland 1991, 1999, McPartland and Cubeta 1997
<i>Schiffnerula cannabis</i>	Leaves	McPartland and Hughes 1994, McPartland 1999
<i>Sclerotinia sclerotiorum</i>	Stalk	McPartland 1991, 1995c, 1999, Bains et al. 2000
<i>Sclerotium rolfsii</i>	Roots, stalk	McPartland 1991, 1999
<i>Septoria neocannabina</i>	Seedlings, stalk, leaves	McPartland 1991, 1995a, 1999, McPartland and Cubeta 1997
<i>Septoria cannabis</i>	Seedlings, stalk, leaves	McPartland 1991, 1995d, 1999
<i>Sphaerotheca macularis</i>	Leaves	McPartland 1991, 1999, McPartland and Cubeta 1997
<i>Stemphylium botryosum</i>	Stalk, leaves	McPartland 1991, 1995a, 1995c, 1999
<i>Stemphylium cannabinum</i>	Stalk, leaves	McPartland 1991, 1995c
<i>Trichothecium roseum</i>	Leaves	McPartland 1991, 1999
<i>Uredo kriegeriana</i>	Leaves	McPartland 1991
<i>Uromyces inconspicuus</i>	Leaves	McPartland 1991
<i>Verticillium albo-atrum</i>	Stalk	McPartland 1991, 1999
<i>Verticillium dahliae</i>	Stalk	McPartland 1991, 1999

- not mentioned

Flax can be attacked by several fungi. Some of them can destroy the plants or reduce the amount and quality of the crop, whereas others are less serious. For example the wilt disease caused by *Fusarium oxysporum* f.sp. *lini* can result in up to 90% losses in yield, being one of the most severe diseases in flax crops worldwide (Kroes et al. 1998 and 1999). The serious consequences of epidemics in flax crops are often due to the very high population density. According to Vloutoglou et al. (1995b) and Harold et al. (1997), the fungal pathogen *Alternaria linicola* can overwinter in naturally infected seeds, crop debris and in weeds. The sowing of seeds infected by *Alternaria linicola* can cause poor germination and damping-off of seedlings or leaf spotting on plants (Evans et al. 1996). Fungal pathogens of flax are presented in Table 3.

Table 3. Fungal pathogens of flax according to the literature.

Species or genus	Plant part of infection	Reference
<i>Alternaria alternata</i>	Stalk, fibre (in storage)	Brown and Sharma 1984, Mercer 1992, Evans et al. 1996
<i>Alternaria lini</i>	Stalk, leaves, buds, flowers, capsules	Mercer 1992, Singh and Singh 2005
<i>Alternaria linicola</i>	Seeds, seedlings, roots, stalk, leaves	Beaudoin 1989, Hoes 1991, Rashid and Platford 1991, Mercer 1992, Mercer et al. 1992, Mercer and Ruddock 1994, Vloutoglou et al. 1995a, 1995b, Evans et al. 1996, Harold et al. 1997, McKay et al. 1999, Kroes et al. 1999, Vloutoglou et al. 1999, Singh and Singh 2005
<i>Ascochyta linicola</i>	Seedlings, stalk	Beaudoin 1989
<i>Asterocystis radialis</i>	Stalk, roots	Beaudoin 1989
<i>Aureobasidium lini</i>	Seeds, stalk, leaves, capsules	Hoes 1991, Mercer 1992
<i>Botrytis cinerea</i>	Seeds, seedlings, stalk, capsules	Brown and Sharma 1984, Bratt et al. 1988, Beaudoin 1989, Mercer 1992, Mercer et al. 1992, Mercer and Ruddock 1994, Diolez et al. 1995, Harold et al. 1997
<i>Cladosporium herbarum</i>	Stalk	Brown and Sharma 1984
<i>Colletotrichum lini</i>	Seedlings, roots, stalk, leaves	Beaudoin 1989, Hoes 1991, Mercer 1992
<i>Epicoccum nigrum</i>	Stalk	Brown and Sharma 1984

Table 3 continues...

Table 3 (continues). Fungal pathogens of flax according to the literature.

Species or genus	Plant part of infection	Reference
<i>Fusarium avenaceum</i>	Seeds, seedlings, roots	Mercer 1992
<i>Fusarium culmorum</i>	Stalk, roots	Brown and Sharma 1984, Mercer 1992
<i>Fusarium oxysporum</i> f. sp. <i>lini</i>	Seedlings, stalk, leaves	Beaudoin 1989, Hoes 1991, Rashid and Platford 1991, Mercer 1992, Mercer et al. 1992, Höper et al. 1995, Kenaschuk et al. 1996, Serra-Wittling et al. 1996, Kroes et al. 1998, Kenaschuk and Rashid 1998, 1999, Kroes et al. 1999, Rowland et al. 2002a, 2002b, 2002c, 2002d, Duguid et al. 2004
<i>Fusarium roseum</i>	Seedlings	Beaudoin 1989
<i>Melampsora lini</i>	Stalk, leaves, capsules	Saharan & Singh 1985, Beaudoin 1989, Hoes 1991, Mercer 1992, Kenaschuk et al. 1996, Kenaschuk and Rashid 1998, 1999, Rowland et al. 2002a, 2002b, 2002c, 2002d, Duguid et al. 2004
<i>Mucor</i>	Stalk	Mercer 1992
<i>Mycosphaerella linicola</i>	Seedlings, stalk, leaves, capsules	Mercer et al. 1992
<i>Oidium lini</i>	The whole plant	Mercer 1992, Mercer et al. 1992, Mercer and Ruddock 1994, Rashid and Duguid 2005
<i>Penicillium</i>	Stalk	Brown and Sharma 1984
<i>Phoma exigua</i> var. <i>linicola</i>	Seedlings, roots, stalk	Hoes 1991, Mercer 1992, Decognet et al. 1994, Mercer and Ruddock 1994
<i>Phytophthora nicotianae</i> var. <i>paracitica</i>	Roots	Mihail 1993
<i>Polyspora lini</i>	Stalk	Beaudoin 1989
<i>Pythium</i>	Seedlings, roots, stalk	Beaudoin 1989, Hoes 1991, Mercer 1992, Mihail 1993
<i>Rhizoctonia solani</i>	Seedlings, roots, stalk	Hoes 1991, Mercer 1992
<i>Selenophoma linicola</i>	Roots	Hoes 1991
<i>Septoria linicola</i>	Leaves	Hoes 1991, Mercer 1992, Halley et al. 2003
<i>Sclerotinia sclerotiorum</i>	Stalk	Mederick and Piening 1982, Mitchell et al. 1986, Beaudoin 1989, Hoes 1991, Rashid and Platford 1991, Mercer 1992, Mercer et al. 1992
<i>Thielaviopsis basicola</i>	Seedlings, roots, stalk	Beaudoin 1989, Hoes 1991, Mercer 1992
<i>Verticillium dahliae</i>	-	Mercer et al. 1992
- not mentioned		

In addition to fungi, bacteria, viruses, mycoplasma-like organisms, insects and other plants also cause problems in hemp and flax. For example bacterial blight, striatura ulcerosa and wildfire caused by *Pseudomonas syringae* are common diseases in hemp. Leaf spot and blight can be caused by bacterial infection (McPartland 1999). A weak

bacterial attack can actually increase yield of flax straw and capsules, but severe attack can reduce straw and seed yields (Mercer 1992). At least five viruses cause problems in hemp: the hemp streak virus (HSV), alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), arabis mosaic virus (ArMV) and hemp mosaic virus (HMV) (McPartland 1999). In flax, at least four viruses cause problems: aster yellows virus (AYV), oat blue dwarf virus (OBDV), curly top virus (CTV) and beet pseudo-yellow virus (BPYV) (Mercer 1992). Almost 300 insects have been described on *Cannabis*, but only very few of them cause severe crop losses. The most serious insect pests injuring hemp are stem borers, especially caterpillars of European corn borers (*Ostrinia nubilalis*) and hemp borers (*Grapholita delineana*) (McPartland 1996). In addition, dozens of leaf-eating or flower- and seed-damaging caterpillars have been reported on *Cannabis*. Two sorts of insects are especially dangerous for flax: flea beetles and thrips (Beaudoin 1989). Several beetle larvae also bore stalks and roots and eat leaves and flowering tops of hemp and flax (Mercer 1992, McPartland 1999). Leafhoppers and grasshoppers cause only minor damage to hemp, whereas bugs can cause more serious damage. The most noteworthy non-insect pests of hemp are mites, the most serious being spider mites. Birds and rabbits can cause problems in flax cultivation. Nematodes can also be a serious problem in hemp and flax, infesting the roots. Incorrectly balanced nutrients can cause deficiency symptoms (e.g. Mercer 1992, McPartland 1999).

1.3 Effects of processing on microbiological quality and fibre properties

1.3.1 Processing phases of bast fibrous raw materials and thermal insulations

The bast fibres must be separated from the rest of the plant in order to be able to utilize hemp and flax fibres. This separation has usually been achieved by dew retting or water retting, followed by mechanical extraction processes.

In the production chain of bast fibrous thermal insulations, the processing steps depend on the structure of the insulation produced. Different additives (e.g. fire-retarding and anti-fungal agents) are added into the preprocessed bast fibre raw material. After that, in the production of loose fill insulation, no further production steps in addition to drying and packaging are needed. In the production of mat type insulations, the fibre material must be scribbled and binding agent must be added in order to obtain consistent structure and sufficient density. The mat type insulations are heated and dried before packaging (Rissanen et al. 1998, Kauriinvaaha et al. 2001).

1.3.2 Retting

Retting can be described as a partial degradation of the tissues of the stem, allowing the fibre bundles to be separated from the stem. The retting process and degree of retting affect the colour of the fibres, separation of the fibre bundles, chemical composition of the fibres, fibre strength and straw yield (Pallesen 1996). During retting the components that bind the fibre bundles to the other plant tissues are broken down. This enzymatic action may be derived from the activity of microorganisms (Easson and Molloy, 1996). Several retting enzymes (e.g. pectinases, xylanase and cellulase) produced by colonizing fungi have been detected from flax stems (Brown and Sharma 1984).

Traditional dew-retting on the field is based on naturally occurring fungi, whereas water retting is based on the presence of bacteria (Easson and Molloy 1996). In dew-retting, fungi degrade the epidermis and thin-walled and unlignified parenchyma cell walls.

Highly cellulosic fibres are less easily degraded by dew-retting microorganisms than the surrounding pectin- and hemicellulose-rich tissues (Akin et al. 1996). Straw yields decline over the retting period, due to the loss of dry matter caused by the microorganisms breaking down the water-soluble and hemicellulosic substances (Pallesen 1996). In water-retting the bast fibre bundles are freed from the surrounding tissue by the combined action of moisture and bacteria. During water-retting, fibre release is primarily caused by anaerobic, pectin-decomposing bacteria (Sharma et al. 1992).

In the dry-line method (or frost-retting), seeds are harvested in autumn and the rest of the plant is left in the field to be stand-retted and dried during the winter (Pasila 2004). Frost-retting results in over-retted fibres that are very absorbent in the case of hemp, and relatively resistant to moulding due to the decrease of nutrients during retting (Kymäläinen and Pasila 2000, Kymäläinen et al. 2001). Frost-retting has previously been used as a low-cost way to produce porous hemp and flax fibre material e.g. for insulations, filters and packages (Pasila 2004).

In order to obtain better processibility of flax, non-cellulosic components can be removed with pretreatment processes. A conventional pretreatment in strongly alkaline conditions is often considered environmentally harmful. Microbial treatments with enzymes are alternatives to strong alkaline treatments (Bhattacharya and Shah 2004). In order to obtain good removal of non-cellulosic impurities, the enzyme preparations should include hemicellulase and pectinase activities. Both bacteria and fungi produce pectinase enzymes. For the retting process, especially polygalacturonase, lyases and esterases are important, but in addition xylanases and cellulases have been shown to contribute to the fibre release (Sharma et al. 1992). Weight losses occur due to the removal of impurities from the fibres as a result of enzymatic activity (Bhattacharya and Shah 2004). According to Bhattacharya and Shah (2004), flax fabric behaves differently with enzymes in different reaction conditions. They also observed that with removal of hemicelluloses and other impurities, the moisture content of flax fibres decreased. They suggested that enzymatic treatment is more beneficial compared to conventional alkaline treatment for removing non-cellulosics from flax.

Hobson et al. (2001) studied the differences in yield and fibre properties of mechanically decorticated retted and unretted hemp fibres. Extracting fibres from unretted stems requires more vigorous treatments than from retted stems, and this may lead to unacceptable reduction in strength and length of the fibre bundles. Hobson et al. (2001) found the yield, length distribution and strength of fibre to be the same for both retted and unretted fibres. However, unretted fibre was coarser and contained more impurities. Unretted fibre had a lower amount of fungi than retted fibre. In a study by Pallesen (1996), fibres were more easily purified from the shives at a moderate degree of retting, but the over-retted fibres were more difficult to clean. According to Sultana (1992b), woody materials remain firmly attached to the fibre in under-retted flax.

Effects of microbial contamination associated with the retting process have been presented in some previous studies. However, the main focus has been on other aspects (e.g. Hepworth et al. 2000, Hobson et al. 2001, Hautala et al. 2004). According to Hepworth et al. (2000), the retting process causes microbiological contamination of the fibres as a side effect.

1.3.3 Harvesting

The traditional method for harvesting of flax is pulling. Green flax is first pulled and positioned in long swaths in which the stems are laid crosswise on the ground, where they are left until the moisture content is below 15% (Sultana, 1992a). Flax may also be harvested by straight combining or by cutting with a swather and threshing with a combine. After drying, the flax is usually packed into round bales. There is some risk of spoilage of the crop due to possible rain during the field drying. In addition, damp fibres can be twisted round all possible axles in the harvesters, and are more difficult to process due to their higher friction coefficient (Pasila 1998). Flax can be harvested during different stages of the maturation period depending on the desired quality of the harvested fibre and whether or not the seeds will be harvested. The sooner the crop is harvested, the finer is the fibre (Klemola and Kuikka, 1991). Flax used for water-retting is generally harvested green.

1.3.4 Drying and storage

Especially in the Nordic climates, the harvest season in autumn is often rainy and the air humidity is high. This can cause problems for harvesting, retting, storage and processing of the crop. The moisture content of the harvested crop may be as high as 75-80% in the autumn (Bruce et al. 2005). Drying is needed in order to avoid spoilage of the crop because of moulding. If the moisture content is higher than 16% (w.b.) there is a risk that the retting process will continue during storage, resulting in fibre quality deterioration (Sultana 1992a).

In dry line method the fibre crop is harvested in spring period, which is in Finland usually drier than the autumn period. According to Pasila (2004), the moisture content of hemp yield was 8-10% (w.b.) in May 1999, and the fibre crop harvested in spring needed no drying. This makes the dry line method advantageous.

According to a study of Bruce et al. (2005), if leaves and heads are stripped from the stems before cutting, the stems dry significantly faster than unstripped ones. The retting also affects drying; retted stems dry at least four times faster than unretted ones. If the swath of stems is too dense, the air cannot circulate well and the drying may be slower. Turning of a partially dried swath is effective in promoting drying. During baling of retted crop the heads of hemp tend to remain damp and cause partial spoilage of the baled crop.

1.3.5 Fractionation

In order to be able to utilize hemp and flax fibres in industrial applications, the fibres must first be separated from the rest of the plant. The bast fibre is the main fraction in the production of fibrous thermal insulations, whereas shive is usually considered as a residue or by-product. The structure of the flax and hemp stems makes it possible to separate the fibres from the inner wooden part with a mechanical process.

Scutching means preparation of straw into stricks. It was previously carried out manually, using a comb for separating the capsules from the stems, a wooden hammer for crushing the stems and a vertical board with a blade for removal of shives. The invention of the scutching mill and the scutching machine have greatly improved the process. The divider and the breaker consist of several toothed wheels, positioned under and above the swath (Sultana 1992b).

Hobson et al. (2001) separated hemp fibres from the stems with a decorticator with fluted rollers developed for flax. The machine was first made suitable for extracting fibre from retted hemp stems, and with further modifications it was used to extract fibres from unretted hemp fibres.

In insulation materials, relatively short fibres are used. The length of fibres in insulation mats is usually less than 15 cm (Kauriinvaaha et al. 2001), and for loose-fill insulations the length of fibres is even shorter. Milling with a hammer mill is a common method for the production of short fibres (e.g. Sultana 1992a and 1992b, Pallesen 1998, Pasila et al. 1998, Kauriinvaaha et al. 2001). In the milling process, pieces of stem are crushed in a hammer mill. This process makes it easier to mix a sample evenly, or to separate fibres and shives mechanically. In smaller pieces, the lignocellulose material is more easily penetrated by chemicals and can thus be submitted to modification or chemical analysis (Thomsen et al. 2005). In the crushing process the fibres and shives are mixed together, and can be separated by screening.

There are only a few reports available about quantitative information on the microbial quality of fractionated stems of bast fibre plants. However, several research groups have studied the microbial contamination of dust and possible health effects in processing of flax or hemp (e.g. Buick and Magee 1999, Fishwick et al. 2001). Buick and Magee (1999) studied flax dust in the preparing area of a flax mill. According to these authors, byssinotic symptoms are more common in the earlier stages of production, where the concentrations of dust are the highest. In their study, flax dust was contaminated by gram-positive and gram-negative bacteria (on average $2.2 \cdot 10^9$ cfu g⁻¹) and by fungi. Species of gram negative bacteria were similar to those contaminating cotton. Fishwick et al. (2001) studied the dust in a hemp processing plant. In their study, the respirable amounts of bacteria were $3.4 \cdot 10^5$ cfu m⁻³, fungi $1.6 \cdot 10^5$ cfu m⁻³ and actinomycetes

$5.7 \cdot 10^4$ cfu m^{-3} in personal sampling of the employees. In air samples taken with an Andersen sampler the amount of actinomycetes was approximately $1.4 \cdot 10^5$ cfu m^{-3} . In a sampling time of 15 seconds, the plates were overloaded with either fungal or bacterial growth. From the results of countable samples the authors suggested that there was between a two- and six-fold increase in fungal counts when the production was at full capacity compared to limited production of hemp fibres.

1.3.6 Thermal treatments

During the manufacturing process of thermal insulations, the insulation mats are heated (Rissanen et al. 1998). Heating can prevent the growth of microorganisms due to the denaturation of proteins, which leads to destruction of enzyme activities and enzyme-controlled metabolism in microorganisms. Effectiveness of the heating depends on e.g. the type of microbe, temperature and the length of the heating time (Fellows 2000).

Steam explosion (STEX) is a process in which the sample is treated with steam at high temperature and pressure. It was developed for board production, to defibrate wood into fibres (Vignon et al. 1995). It is used for the separation of lignocellulosic components such as cellulose, hemicellulose, pectin and lignin (Vignon et al. 1996). Fibres are both chemically modified and mechanically defibrated in the STEX process. Vignon et al. (1995) reported that the STEX process is a suitable treatment for woody hemp core, which can be purified to provide chemical grade cellulose. STEX treatment is suitable for the processing of semi-retted hemp fibres (Garcia-Jaldon et al. 1998), and also for flax fibres intended for textiles and composites (Kessler et al. 1998).

1.4 *Microbial quality of fibrous thermal insulations*

1.4.1 *Microbial growth on fibrous thermal insulations*

The moisture requirements for fungal growth in fibrous building materials have been reported in the literature (e.g. Grant et al. 1989, Pasanen et al. 1992, Ezeonu et al. 1994, Chang et al. 1995, Foarde et al. 1996). According to these studies, the limit value of the equilibrium relative humidity of the material for fungal growth in building materials is between 70% and 90% depending on the materials and the fungal species. In a study by Grant et al. (1989), fungi were grouped as primary, secondary or tertiary colonizers of building materials. Primary colonizers, e.g. *Aspergillus* and *Penicillium*, are capable of growth at a water activity (a_w) of less than 0.8, secondary colonizers, e.g. *Cladosporium*, grow at water activity levels of 0.8-0.9, and tertiary colonizers, e.g. *Fusarium* and *Stachybotrys* require a water activity over 0.9. According to Pasanen et al. (1991), fungal growth is predominately controlled by the moisture content of the substrate, and RH of the air has only an indirect influence. They found that if water is available on the surface, fungi may grow at very low levels of air humidity. Therefore, fungal growth on damp building materials is possible even when the air humidity is low. On the other hand, Viitanen and Ritschkoff (1990) suggested that the relative humidity of air is more important than the water content of the growth media for spore germination and mycelial growth of moulds.

Although inorganic materials do not naturally contain nutrients for fungi, fungal growth has also been reported in inorganic building materials such as fibreglass or rock wool insulations, mineral insulation, ceramic products, plastics and gypsum board. Ezeonu et al. (1994) identified *Acremonium* sp., *Alternaria* sp., *Aspergillus* spp, *Aureobasidium* sp., *Cladosporium* spp., *Curvularia* sp., *Exophila* sp., *Fusarium* sp., *Paecilomyces* spp., *Penicillium* spp., *Rhizopus* sp. and *Rhodotorula* sp. in new fibreglass insulations. Hyvärinen et al. (2002) found that the main fungal taxa in paper insulation from water-damaged buildings were *Penicillium* spp., yeasts, non-sporulating moulds and *Cladosporium* spp., and in mineral insulation from water-damaged buildings *Penicillium* spp., non-sporulating moulds, yeasts, *Acremonium* spp. and *Aspergillus* spp.

Reponen et al. (1996) used in their experiments fungal strains of *Aspergillus* spp., *Cladosporium* sp. and *Penicillium* spp. previously isolated from mouldy building materials. Pasanen et al. (1992) identified e.g. *Penicillium* spp., yeasts, *Cladosporium* spp. and *Aspergillus* spp. from building materials such as acoustic fibre board and wallpaper. Species of mycotoxin-producing moulds in fibrous materials include e.g. *Alternaria* (Evans et al. 1996), *Aspergillus* (Etzel 2002), *Fusarium* (Committee on Environmental Health 1998, Etzel 2002), *Trichoderma* (Committee on Environmental Health 1998), *Stachybotrys* (Jarvis et al. 1996, Committee on Environmental Health 1998, Jarvis et al. 1998, Andersen and Nissen 2000, Etzel 2002), *Chaetomium* (Andersen and Nissen 2000) and *Memnionella* (Jarvis et al. 1996, Jarvis et al. 1998, Andersen and Nissen 2000).

Klamer et al. (2004) proposed that even small amounts of moisture can lead to the deterioration of flax insulation materials. They observed fungal growth in paper and flax insulations at all moisture levels tested. The large amount of boric acid in the paper insulation was insufficient to prevent fungal growth (Klamer et al. 2004). Klamer et al. (2004) suggested that boric acid would not prevent fungal growth in insulation materials when major water damage has occurred. Pessi et al. (2002) did not suspect bacteria other than actinomycetes to have originated from the mineral wool thermal insulation. A fungal colonization can be detected if the amount of moulds as cfu/g is one hundred times higher than in the original material. Limits for microbial quality of fibre materials have been given e.g. in the legislation for thermal insulations (STM 2003).

1.4.2 Research methods of microbial growth on building materials

According to the literature, culturable microorganisms from building material samples are cultivated by dilution using several different media (Table 4).

Table 4. Growth media used in determining or isolating culturable microorganisms from building materials.

Microbe	Media	Reference
Fungi	DG18 agar	Andersson et al. 1997, Korpi et al. 1998, Pasanen et al. 2000a, Pasanen et al. 2000b, Hyvärinen et al. 2002
	Malt extract agar	Pasanen et al. 1992, Andersson et al. 1997, Korpi et al. 1998, Gravesen et al. 1999, Miller et al. 2000, Pasanen et al. 2000a, Pasanen et al. 2000b, Pessi et al. 2000, Hyvärinen et al. 2002, Pessi et al. 2002
	Czapek yeast extract agar	Andersson et al. 1997, Gravesen et al. 1999, Miller et al. 2000
	Glycerol nitrate agar G25N	Andersson et al. 1997
	Corn meal agar	Andersson et al. 1997
	Oat meal agar	Gravesen et al. 1999
	Mycological agar	Ezeonu et al. 1994
	V8 agar	Gravesen et al. 1999, Klammer et al. 2004
	Yeast extract sucrose agar	Gravesen et al. 1999
	Creatine sucrose agar	Gravesen et al. 1999
	Gummed paper technique	Boutin-Forzano et al. 2004
Bacteria	Tryptone-yeast extract agar	Pasanen et al. 2000a, Pasanen et al. 2000b, Hyvärinen et al. 2002
	Tryptone-yeast-glucose agar	Korpi et al. 1998, Pessi et al. 2000, Pessi et al. 2002
Mesophilic actinomycetes	Caseinate-propionate agar	Pasanen et al. 1992
Thermotolerant actinomycetes	Half-strength nutrient agar	Pasanen et al. 1992

Mathematical modelling has also been used in prediction of fungal growth or toxin production in buildings (Clarke et al. 1999, Rowan et al. 1999, Pessi et al. 2000). Clarke et al. (1999) and Rowan et al. (1999) described mathematically the critical limits for fungal growth in terms of growth limit curves (defining the minimum combination of temperature and relative humidity at which growth will occur) using the Environmental Systems Performance (ESP-r) computer-based program. Pessi et al. (2000) used microbial contaminations of the insulations, other microbial sources and environmental variables as explaining factors in order to analyze indoor air spores using Generalized Linear Mixed Models (GLMM).

1.4.3 Emission measurements from fibres or fibrous thermal insulations

Air is a combination of physical, chemical and biological factors. Many kinds of measurement methods have been designed and constructed to investigate different pollution processes of indoor air. Several investigators have focused on VOCs (Volatile Organic Compounds) (e.g. Korpi et al. 1998, Kelman et al. 1999) or particle emissions (e.g. Kildesø et al. 2003) from building materials, and some on microbial emissions (e.g. Korpi et al. 1998, Górny et al. 2001, Górny et al. 2002, Kildesø et al. 2003).

Emissions from building materials have been measured with field sampling from e.g. water-damaged buildings, and with special emission measurement chambers in laboratories. Górny et al. (2001) and (2002) used an aerosolization chamber for the emission measurements. Fungal spores were released from the contaminated material by passing clean air over the surface. Górny et al. (2001) and (2002) measured the concentration of released spores with an optical particle counter. Kildesø et al. (2003) used a specially designed small chamber which was placed on the wet building material. Fungal spores and other particles were released by well-controlled airflow from rotating nozzles. Korpi et al. (1998) studied the fungal concentrations and MVOC emissions from moulded building materials in air-tight glass chambers in different RH levels.

In different studies, microbial emissions have been collected with the Andersen N6 impactor (Pessi et al. 2000, Korhonen et al. 2000, Górný et al. 2001, Górný et al. 2002, Pessi et al. 2002, Shelton et al. 2002) and the Reuter Centrifugal Sampler (Miller et al. 2000). Media used have been e.g. malt extract agars (Korhonen et al. 2000, Pessi et al. 2000, Pessi et al. 2002, Shelton et al. 2002), DG8 agar (Korhonen et al. 2000), tryptone-yeast-glucose agars (Korhonen et al. 2000, Pessi et al. 2000, Pessi et al. 2002) and rose bengal agar (Miller et al. 2000, Shelton et al. 2002).

1.5 Summary of the literature and the importance of this study

Flax has been used mainly for the production of linen cloth and yarns since ancient times. Fibre hemp is also one of the oldest economical plants, which has been used in several different applications. The cultivation of hemp decreased during the 1960s because of strong competition with cotton and synthetic fibres, and confusion of fibre hemp with marijuana varieties has also hindered its widespread cultivation (Forapani et al. 2001). Recently investigations of bast fibre plants have been focused on non-textile applications. Bast fibres may be used for building materials, e.g. thermal insulations (Oosten 1989, Ringleb and Schulz 1996, Kauriinvaaha et al. 2001), packaging materials (Oosten 1989, Tavisto et al. 2001) and composites (e.g. Oosten 1989, Rowell 1995, Hepworth et al. 2000, Hautala et al. 2004). In this study, the focus is especially on fibrous thermal insulations.

Only a few reports are available combining both cultivation of hemp or flax and the observation of microbiological and meteorological data. The quality of fibre material can vary greatly from one growing season to another, due to different environmental conditions during the growing seasons, harvesting and storage. It is important to be able to know whether the microbiological quality of the raw material is high enough to be used in technological applications. Therefore in this study the microbiological quality of the fibre material was examined throughout the primary production chain.

In the manufacturing of technological products (e.g. thermal insulations, packaging materials, composites and textiles), several different processing phases and methods are used. However, in this study the main focus is on the primary production, in which the raw material from bast fibre plants is harvested and fractionated so that the different fractions of the plants can be used separately. In this study the effects of the production chain phases of fibre hemp and flax on the microbiological quality of the fibre material were examined. Scientifically reported cultivation of the plants was combined with the monitoring of meteorological data. Different harvesting times, retting degrees and thermal treatments were compared. Especially the effect of frost retting over winter (dry line method) on the microbiological quality is important. The distribution of microbes to different fragments in fractionation was measured.

Emissions from different building materials have been studied widely. However, only little attention has been focused on the interdisciplinary approach or on microbial emissions. In this study an emission measurement chamber capable of measuring microbes, volatile organic compounds (VOCs) and particles in different atmospheric conditions was developed.

Steam explosion has previously been used for the processing of semi-retted hemp fibres (Garcia-Jaldon et al. 1998) and for flax fibres intended for textiles and composites (Kessler et al. 1998). In this study, STEX was used as a reference method, in order to determine whether it is effective against possible fungal growth in fibre materials.

The microbiological quality throughout the whole processing chain has not been studied previously. This study provides information hitherto lacking in the literature concerning the microbiological quality and possible microbial risk conditions in the production chain of hemp and flax fibres and fibrous insulation products.

2 Aims of the study

In this study typical phases of the production chain of fibre hemp and flax from field to thermal insulations were examined, focusing on microbial quality.

The specific aims of the study were:

1. To evaluate the effect of different atmospheric conditions on the growth and microbial quality of the stems and to investigate the microbial quality of hemp and flax during the growing season (I).
2. To evaluate the efficiency of frost, fractionation, and different thermal and enzymatic treatments on the microbial quality of fibre material (I, IV, V).
3. To evaluate the microbial quality of and emissions from fibrous thermal insulation materials in different moisture conditions (II, III).
4. To develop monitoring techniques, processes and equipment and to evaluate the microbial quality of flax and hemp fibres in thermal insulations (III, IV, V).

3 Materials and methods

3.1 Materials and treatments

During this project hemp and linseed were cultivated in Siuntio at the research farm of the University of Helsinki and processed at the Department of Agrotechnology. The phases of growing and fractionation are presented in Figure 3. Enzymatic treatment and steam explosion were performed in Risø National Laboratory, Denmark.

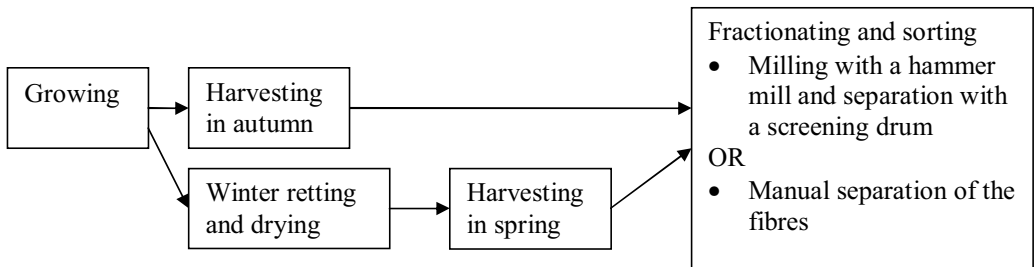


Figure 3. Phases of growing and fractionation.

In this study several different fractions and products of fibre hemp and flax were studied, and some reference materials were examined. The raw materials, fractions/products, treatments and examined quality features used in this study are presented in Table 5.

Table 5. The raw materials, fractions/products, treatments and methods used in this study. I, II, III, IV and V are the original publications.

Raw material	Fraction / product	Treatments	Examined quality features	
Fibre hemp (I, III, IV, V)	Fibre (III, IV, V)	Thermal treatments (V)	Amount of microbes (V)	
			Genera of fungi (V)	
			Chemical composition (V) *	
		Separated manually (V)	Enzymatic treatments (V)	Amount of microbes (V)
				Genera of fungi (V)
				Chemical composition (V) *
	Separated by fractionation (IV)	Steam explosion (V)	Amount of microbes (V)	
			Genera of fungi (V)	
			Chemical composition (V) *	
Shive (IV)	Separated by fractionation (IV)	Amount of microbes (IV) Ash content (IV) *		
Stems (I)	Cut and separated manually (I)	Amount of microbes (I) Genera of fungi (I)		
Linseed (I, III, IV)	Fibre (III, IV)	Separated by fractionation (IV)	Microbial emissions (III)	
			Particle emissions (III) *	
			VOCs (III) *	
			Amount of microbes (III)	
			Genera of fungi (III)	
	Ash content (III, IV) *			
Shive (IV)	Separated by fractionation (IV)	Amount of microbes (IV) Ash content (IV) *		
Stems (I)	Cut and separated manually (I)	Amount of microbes (I) Genera of fungi (I)		
Flax (III)	Commercial insulations (III)	Produced commercially	Microbial emissions (III)	
			Particle emissions (III) *	
			VOCs (III) *	
			Amount of microbes (III)	
			Genera of fungi (III)	
Glass, stone, recycled paper (III)	Commercial insulations (III)	Produced commercially	Microbial emissions (III)	
			Particle emissions (III) *	
			VOCs (III) *	
			Amount of microbes (III)	
			Genera of fungi (III)	
Ash content (III) *				

* Not included in this thesis.

3.1.1 Plant varieties and growing conditions

Fibre hemp (*C. sativa* L., variety Uso 31) and linseed (*L. usitatissimum* L., variety Helmi) used in the study (I, III-V) were cultivated in Siuntio, southern Finland, during the years 2001-2002. The cultivation was carried out with the procedure commonly used in Finland (Sankari and Mela 1998, Hongisto et al. 2000). Linseed was sown at a seed density of 68 kg ha⁻¹ and hemp at approximately 30 kg ha⁻¹. The soil type was silty clay with pH 6.1 and a phosphorus balance of 8.0 mg l⁻¹. The weather conditions in Finland varied significantly in different years and the date of sowing depended on the weather during spring. During the growing season of 2001 the conditions were mostly humid, warm during the daytime and rather cold during the night. The growing season of 2002 was dryer and warmer than the growing season of 2001; reported in detail in (I). The sowing and harvesting dates, amount of fertilization, length of the growing seasons and sum of effective temperature are presented in Table 6.

Table 6. The sowing and harvesting dates, amount of fertilization, length of the growing seasons and sum of effective temperature.

Year	Plant	Sowing date	Harvesting date	Fertilization	Length of the growing season*	Sum of effective temperature**
2001	Hemp	17 th May	14 th May 2002	140 kg/ha N 21 kg/ha P	181 days (22 nd Apr. – 19 th Oct.)	1575°C
	Linseed	17 th May	Not harvested	70 kg/ha N 10.5 kg/ha P	181 days (22 nd Apr. – 19 th Oct.)	1575°C
2002	Hemp	25 th April	19 th Sept. 2002 10 th Oct. 2002 6 th May 2003	92 kg/ha N 12 kg/ha P	153 days (20 th Apr. – 19 th Sept.)	1595°C
	Linseed	26 th April	12 th Sept. 2002	60 kg/ha N 9 kg/ha P	153 days (20 th Apr. – 19 th Sept.)	1595°C

* normally 175 days in this cultivation area.

** normally 1400-1500°C in this cultivation area.

3.1.2 Measurement of meteorological conditions

The daily standard meteorological data (air humidity and temperature) were recorded (I) with Vaisala HMP 35D PT100 and Vaisala HMP 35D HUMICAP instruments at the Metsähovi Research Station of the Finnish Geodetic Institute, Kirkkonummi, Finland. The meteorological conditions in the middle of the hemp field were also checked regularly in order to observe the microclimate in the field among stalks (I). During the summer of 2001 the meteorological data in the field was recorded with a thermohygrograph (Lambrecht, Germany) and during the summer of 2002 with a Data Logger (Tinytag Ultra, Gemini, England). The field measurements were carried out at a height of 20 cm above the ground.

3.1.3 Sampling of the plants and handling of field samples

The microbiological samples from the field were taken 2-4 times a month during the growing seasons (I). The head and the lower part of the plants were handled separately. The distance between the head and lower parts was approximately 20-40 cm in linseed, and 100-300 cm in hemp. All heads of the plants were combined and cut into 2-5 cm pieces, some of which were randomly taken for further treatments. The same procedure was carried out with the lower parts of the plants. The dry weight of the plants was measured by drying a separate, weighed sample in a kiln (24 hours, 104°C).

3.1.4 Harvesting of stalks

Stalks were harvested by cutting with garden shears, threshing or mowing at around midday of the harvest day (I, III-V). Stalks were transported to a drying room ($T = 50^{\circ}\text{C}$, $t = 7$ days) approximately 3-4 hours after the harvesting. The dried stems were stored in a storage room ($T \sim 18^{\circ}\text{C}$) sealed in a plastic foil wrapping. Spring-harvested hemp in (IV) was round-baled after mowing, and stored in a storage room ($T \sim 18^{\circ}\text{C}$) sealed in a plastic foil wrapping.

3.1.5 Fractionation of hemp and flax plants

In this study, a hammer mill, a long continuous-type screening drum and a sieve vibrator were used for fractionation (III, IV). The stems, cut from the field and dried, were milled with a hammer mill. The fractions were separated in an air stream with a cyclone. The fibres in the milled material were separated from the shive with a screening drum, and cleaned by screening with a sieve vibrator. Samples were randomly picked from the fraction container. The dried, unprocessed stalk and five fractions (fibre, shive, dust, leaves and seeds or seed capsules) of hemp and linseed were studied. The technological processes are presented in detail in (IV).

3.1.6 Enzymatic treatment

The enzymatic treatment (V) was carried out as described by Madsen et al. (2003) but with higher dry matter consistency. In the enzymatic treatment four bottles ($V=5$ l), two for hemp before frost (BF) and two for hemp after early frost (AF), were used with 60-120 g of hemp fibres in each bottle. A solution (3.33 L/100 g fibres) of 0.05 M acetate buffer (pH 5.0) and Pectinex (1 g enzyme solution/100 g fibres) was used. The enzymatic treatment was performed at 23°C for 24 h, after which the fibres were rinsed three times with distilled water and dried at 50°C for 40 h. The enzymatic method is presented in detail in (V).

3.1.7 Thermal treatments

The steam explosion treatment (V) was carried out with 35 g dried material. The temperature was increased to 185°C with steam addition to a pressure of 11.3 bar. The steaming was continued for 2 minutes. The fibres were separated from the suspension by filtration, rinsed with tap water and dried at 50°C for 16 hours. The dry matter contents were determined from 0.5 g samples, using a halogen moisture analyzer (Mettler-Toledo) with a drying temperature of 105°C. The steam explosion method is presented in detail in (V).

The effect of dry heating (V) on the microbiological quality of the samples was measured by heating the samples in an oven. Three temperatures (80°C, 100°C, 120°C) and five durations (5 min, 10 min, 20 min, 40 min and 60 min) were used in the thermal treatments. The time-temperature combinations were selected to represent possible treatments in e.g. the manufacture of thermal insulations. Five replicate treatments were performed for each combination.

3.1.8 *Chemical composition*

In addition to the microbial measurements, the effects of the enzymatic and thermal treatments on chemical composition were also measured, and the methods and results can be examined in detail in (V). However, in this thesis the focus is on microbial measurements.

3.1.9 *Thermal insulation samples for emission measurements*

In this study (III), eight insulation materials were examined, six of which were organic and two inorganic insulations. Five of the insulation materials were manufactured from bast fibre materials. Some of the insulations were of mat type, others were loose-fill type. Six of the materials were commercial and two were bast fibre raw materials without additives. The insulation materials are presented in Table 7, and reported in detail in (III).

Table 7. Raw materials, insulation types and amount of products in the emission tests.

Raw material	Type of insulation	Number of products
Flax fibre + additives	Insulation mat	2
Flax fibre + additives	Loose-fill	1
Linseed fibre, no additives	Loose-fill	1
Hemp fibre, no additives	Loose-fill	1
Recycled wood + additives	Loose-fill	1
Inorganic fibres (glass, stone) + additives	Insulation mat	2

3.2 Development of measurement techniques and equipment

Hygicult[®] plates were selected for microbial analyses because they were easy to use and readily portable. Hygicult[®] slides are validated against swabbing and control plate methods (Salo et al. 2000 and 2002) and can therefore be reliably used as microbiological media.

Different harvesting techniques, fractionating processes and equipment have been tested and compared at the Department of Agrotechnology (e.g. Kymäläinen 2004, Pasila 2004). Therefore well-designed and workable methods could be selected for this study. Because well known methods and equipment were used, it was possible to evaluate the real risk points of the processing chain. Some details had to be modified in order to make the process chain suitable for microbiological sampling.

An apparatus suitable for both mat type and loose fill type insulations and capable of interdisciplinary sampling was needed for emission measurements. The emission chamber was developed in cooperation with the Laboratory of Structural Engineering and Building Physics at Helsinki University of Technology (main responsibility), the Department of Physical Sciences at Helsinki University and the Finnish Institute of Occupational Health. The size of the chamber was selected so that it was suitable for investigation of various applications in the chamber. During the design and construction, the critical aim was to maintain uniform and controlled conditions within the chamber. The development process and evaluation of the control of conditions in the emission chamber are presented in Figure 4 and in detail in (II).

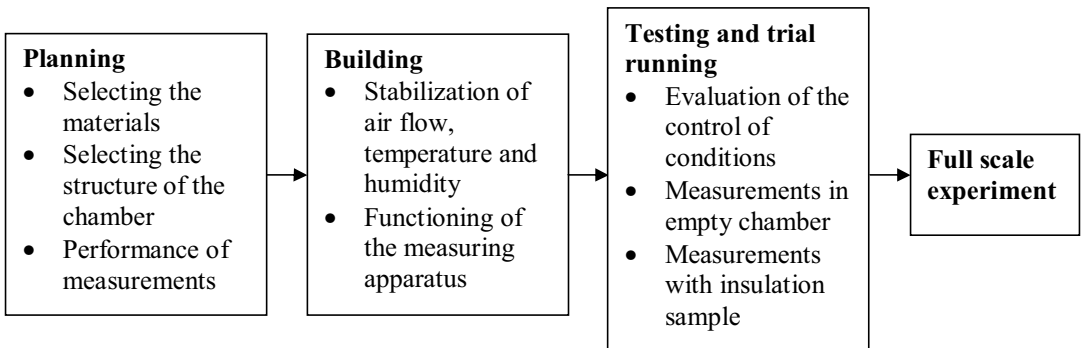


Figure 4. Development process of the emission chamber and evaluation of the control of conditions.

The emission apparatus consisted of four parts: an air output unit (oilless compressor), a purification unit (filters), a moisturizing unit and an emission chamber. The apparatus was mainly composed of low-emission materials such as stainless steel, glass, Teflon and gaskets. The chamber was 1.80 m high and its diameter was 0.32 m. Relative humidity, temperature, airflow and pressure in the chamber were controlled. The system had a running control with a limit value alarm. The chamber and its peripherals are depicted in Figure 5. More profound technological information concerning the emission apparatus is presented in detail in (II).

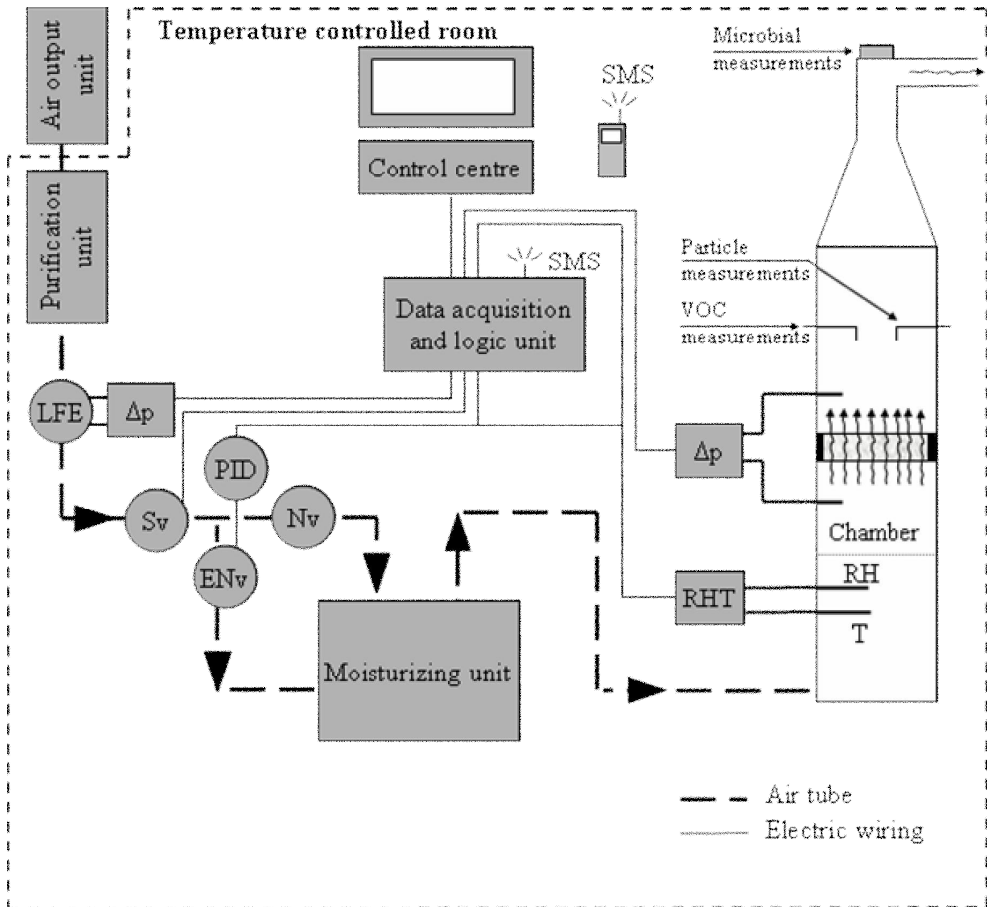


Figure 5. Experimental apparatus for measuring emissions from thermal insulations. Abbreviations: LFE is a laminar flow element, S_v , EN_v and N_v are valves, PID is a proportional integral derivate controller, SMS is Short Message Service, RHT is a sensor for relative humidity and temperature (modified from Figure 1 in II / Virta et al. 2005).

3.3 Microbiological measurements

3.3.1 Plants and insulations

Samples ($m = 5-10$ g) were extracted for 30 minutes with 100 ml sterile salt solution (9 mg NaCl ml^{-1}) and then homogenized (5 minutes, 230 rpm) with a Stomacher 400 Circulator (Merck Eurolab).

The microbiological analyses were carried out using Hygicult[®] plates (Orion Diagnostica) (I, III-V). The media used was Hygicult[®] Y&F for analysing yeasts and fungi and Hygicult[®] TPC for analysing total bacterial counts. The compositions, pH values and incubation conditions of the Hygicult[®] plates are presented in Table 8. Serial dilution was used to make the amount of colonies per plate countable. Plate Count Agar (Difco 0479) supplemented with 0.05% cycloheximide and Potato Dextrose Agar (Difco 0013) with 0.01% chloramphenicol and chlortetracycline (I) were also used.

Table 8. Use, compositions, pH values and incubation conditions of the Hygicult[®] plates.

Plate	Use	Composition	pH value	Incubation
Hygicult [®] Y&F	Yeasts and moulds	Malt agar, yeast extract, dextrose, agar, chloramphenicol, gentamycine sulphate	5.0-5.5	Room temperature (22-25°C), 5 days
Hygicult [®] TPC	Total bacterial count, yeasts, moulds	Tryptose, yeast extract, dextrose, agar, lecithine, tween 80, neutralizing components	7.0-7.4	Room temperature (22-25°C), 3 days

In addition to the microbial contents, the taxa of the moulds were determined (I, III, V). The media used were Potato Dextrose Agar (Difco 0013) with 0.01% chloramphenicol and chlortetracycline (incubation for 7 days at 25°C) (I) and water agar (incubation for 14 days at 25°C) (III, V). The moulds were identified to genus level by microscopy.

3.3.2 *Air samples from the field*

The amount of microbes in field air (I) was measured with an MAS-100 sampler (Merck Eurolab). The growth media used were plate count agar and malt agar.

3.3.3 *Measurement of emissions*

Microbial samples were collected using an MAS 100 air sampler with tryptone-yeast-glucose agar and malt extract agar Petri dishes. The airflow in the chamber was 100 l/min during the sampling. After sampling, the airflow was decreased to 4 l/min. The Petri dishes were incubated at room temperature (22–24°C) for 4–5 days, after which the amounts of moulds, yeast and bacterial colonies were counted. The detection limit for this method is 5 cfu m⁻³.

In addition to the microbial emissions, volatile organic compounds (VOCs) and aerosol particles were also measured, and the methods and results are presented in detail in (II, III). However, in this thesis only the microbial emissions are considered.

3.4 *Statistical analyses*

Bivariate correlation analysis (Pearson's correlation coefficients, two-tailed test of significance) of the SPSS statistical tool (Norusis 2004) was used to examine the possible correlation of the microbial contents between the growing seasons (I) and the possible correlation between moulds, yeasts and bacteria in different fractions, and between ash and microbial contents (IV). Linear regression analysis of the SPSS statistical tool was used to examine the possible effect of microbial emissions on emissions of VOCs and particles (III). Variance analysis (one-way ANOVA with Tukey's post hoc test of significance) of the SPSS statistical tool was used to examine differences between the microbial contents and ash of the fractions (IV). One-way ANOVA with Tukey's test from the SPSS statistical tool was used to examine the possible effects of the different treatments on the microbial contents of the fibres (V).

4 Results

4.1 *Effects of environmental conditions on the growth*

The growing season of 2002 was warmer and drier than that of 2001. The air humidity in the middle of the hemp field was approximately 10 % higher than that measured outside the field. In 2001 the hemp stems reached a height of over 3.5 m and a maximum diameter of 4 cm. In 2002 the highest hemp plants reached only a height of 2 m and the diameter was only approximately 2 cm. In 2001 the linseed stems reached a height of 50 cm, but in 2002 they were only 30 cm high.

As can be seen in Table 9, the dry weight of the hemp at the end of the growing season was higher in 2002 than in 2001. At the end of the growing season of 2001 there was a noticeable difference between the dry weights of the head and the lower part of the hemp. In linseed the dry weight at the end of the growing season of 2002 was noticeably higher in the head than in the lower part of the plant (I).

Table 9. The dry weights (mean of two measurements) of hemp and linseed at the beginning and at the end of the growing seasons of 2001 and 2002 (I).

Examined parts	Growing season of 2001		Growing season of 2002	
	Beginning	End	Beginning	End
Head of the hemp	25%	60%	20%	78%
Lower part of the hemp	20%	30%	20%	70%
Head of the linseed	26%	70%	25%	80%
Lower part of the linseed	23%	70%	30%	50%

4.2 Microbial quality of the plants and the fibre material

4.2.1 Microbial growth during the growing season

Total bacterial count and the amount of moulds (cfu g_{dw}⁻¹) in hemp varied between different parts of the plant. The difference between the top and lower parts of linseed was smaller than in hemp. In the case of both hemp and linseed the mould and bacterial contents (cfu g_{dw}⁻¹) increased at the end of the growing season of 2001 when the plants started to mature. During the growing season of 2002 the increase in mould and bacterial contents was also evident, but occurred more evenly throughout the whole growing season (Figures 6 and 7) (I).

The differences between lowest and highest bacterial contents in both hemp and linseed were significant: in hemp from $3.0 \cdot 10^2$ to $3.4 \cdot 10^9$ cfu g_{dw}⁻¹ (2001) and from $9.1 \cdot 10^3$ to $3.6 \cdot 10^7$ cfu g_{dw}⁻¹ (2002) and in linseed from $9.0 \cdot 10^4$ to $7.9 \cdot 10^9$ cfu g_{dw}⁻¹ (2001) and from $1.0 \cdot 10^2$ to $6.4 \cdot 10^9$ cfu g_{dw}⁻¹ (2002). In the growing season of 2001, the bacterial counts reached higher levels than in 2002. The maximum bacterial content was higher in linseed than in hemp in both growing seasons.

The difference between the lowest and the highest mould contents in both hemp and linseed was considerable, especially in the growing season of 2001 (in hemp from $1.0 \cdot 10^2$ to $1.9 \cdot 10^7$ cfu g_{dw}⁻¹ (2001) and from $5.8 \cdot 10^3$ to $5.2 \cdot 10^5$ cfu g_{dw}⁻¹ (2002) and in linseed from $3.0 \cdot 10^3$ to $2.4 \cdot 10^7$ cfu g_{dw}⁻¹ (2001) and from $8.7 \cdot 10^3$ to $6.9 \cdot 10^6$ cfu g_{dw}⁻¹ (2002)). The mould contents also reached a higher level in the growing season of 2001 than in 2002.

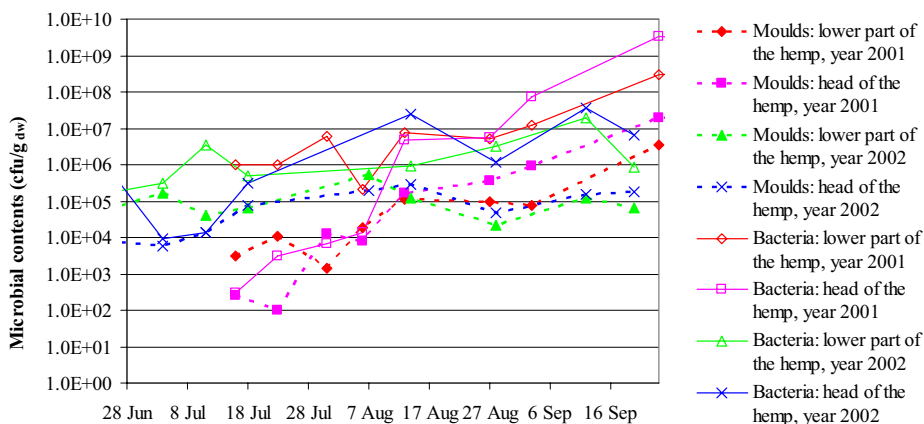


Figure 6. Microbial contents (moulds and bacteria) in hemp during the growing seasons of 2001 and 2002 (Modified from Figures 2 and 3 in I).

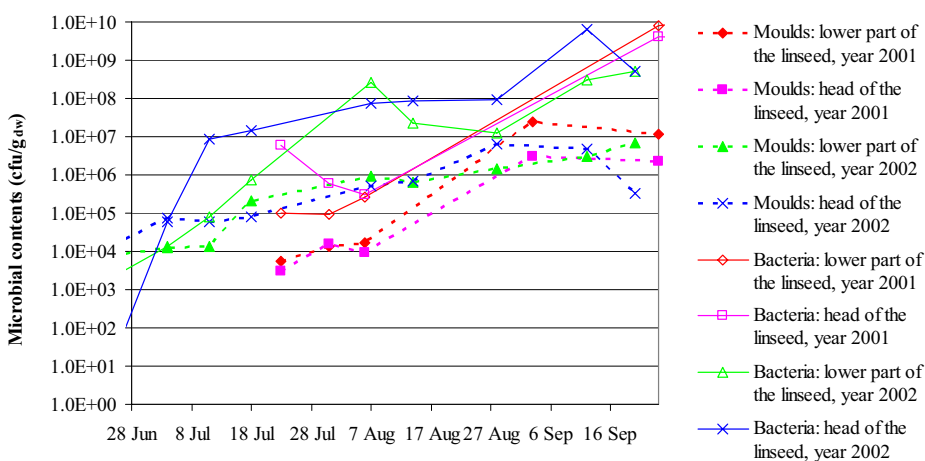


Figure 7. Microbial contents (moulds and bacteria) in linseed during the growing seasons of 2001 and 2002 (Modified from Figures 4 and 5 in I).

The amounts of microbes in field air during the growing season of 2001 varied from $1.6 \cdot 10^2$ to $1.6 \cdot 10^3$ cfu m⁻³, which corresponds to the normal summertime outdoor air quality in Finland. Fungal counts in the air during the growing season of 2002 were generally higher than $2.5 \cdot 10^5$ cfu m⁻³ (I).

4.2.2 Effects of frost on microbial growth

Before early frost, the amounts of the moulds, yeasts and total bacteria were higher in linseed fractions than in hemp fractions ($p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively). The amounts of moulds, bacteria and yeasts of standing hemp stems in the field increased during the winter (I, IV, V). The amounts of moulds and bacteria in hemp and linseed stems and fibres in autumn (before frost) and in spring are presented in Figure 8.

The amounts of bacteria in flax and hemp harvested at different times differed statistically significantly from each other ($p < 0.05$). During the winter and spring period there was a clear increase in the amount of bacteria, especially in the stems but also in the fibre and shive fractions (see Figure 8 in this study and Figure 3 in IV).

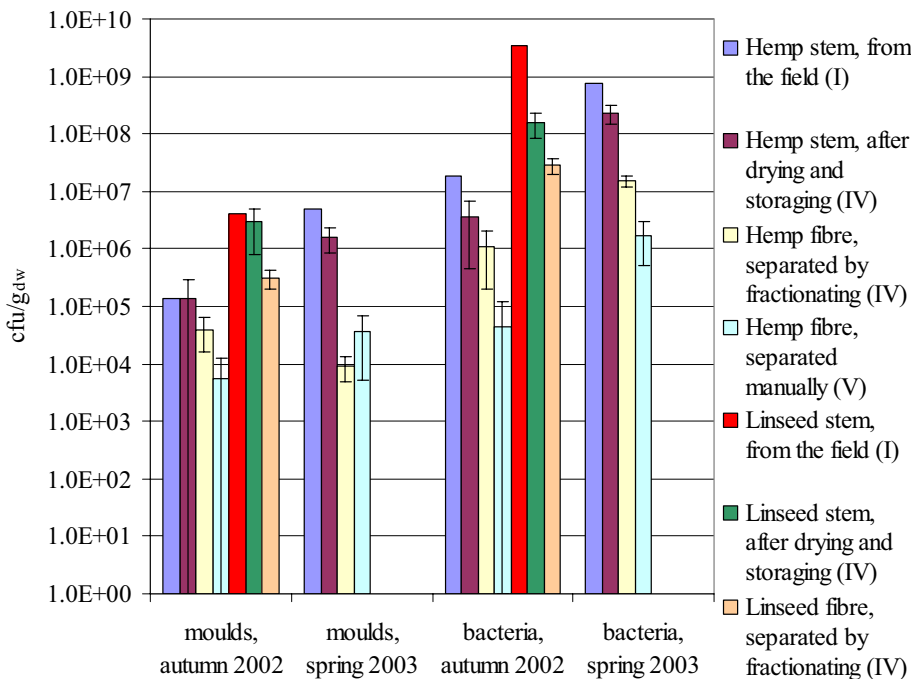


Figure 8. Amounts of moulds and bacteria in hemp and linseed stems and fibres harvested in autumn 2002 or in spring 2003. Columns indicate means and bars (\pm stdev) standard deviations of means of five replicates (Data from I, IV and V).

4.2.3 *Effects of fractionation on microbial quality*

The microbial contents of fractions of fibre hemp and linseed varied between 10^3 and 10^9 cfu g_{dw}^{-1} . The mechanically fractionated fibre of hemp harvested after early frost or in spring had the lowest amount of moulds. The difference between the fractions harvested before and after early frost was statistically significant ($p < 0.05$) (IV). In (V) both mould contents and bacterial contents of manually separated fibres were higher in the spring than in the autumn (Figure 8). There were less yeasts in the fibre and shive than in the stems ($p < 0.01$). The amount of total bacteria was lower in fibre and shive than in stems of linseed and hemp before frost and in spring-harvested hemp ($p < 0.01$) (Figure 8). The amounts of moulds, yeasts and total bacteria were at the same level in the linseed stems cut before threshing of the seed ($2.9 \cdot 10^6$, $3.6 \cdot 10^6$ and $1.6 \cdot 10^8$ cfu g_{dw}^{-1} for moulds, yeasts and bacteria, respectively) and in the stems collected from the windrow after threshing ($1.5 \cdot 10^6$, $4.2 \cdot 10^6$ and $9.5 \cdot 10^7$ cfu g_{dw}^{-1} for moulds, yeasts and bacteria, respectively). The mould contents of the fibre and shive fractions were lower than that of stem ($p < 0.001$) (IV) (stem and fibre fractions are presented in Figure 8).

4.2.4 *Effects of enzymatic and heating treatments on microbial quality*

Enzymatic treatment encouraged the growth of moulds in fibres harvested both before frost and after frost ($p < 0.05$), the mould content increasing from $5.5 \cdot 10^3$ to $5.1 \cdot 10^6$ cfu g_{dw}^{-1} in hemp fibres harvested before frost and from $3.4 \cdot 10^2$ to $9.7 \cdot 10^4$ cfu g_{dw}^{-1} in hemp fibres harvested after frost. Enzymatic treatment did not have a clear effect on bacterial growth (see Figures 9 and 10).

Steam explosion alone or in combination with the enzymatic treatment appeared to reduce the amount of moulds in fibres, although this difference was not statistically significant ($p > 0.05$) (Figure 9). The mould content in fibres harvested before frost was $5.5 \cdot 10^3$, $3.0 \cdot 10^2$ and $1.3 \cdot 10^2$ cfu g_{dw}^{-1} in untreated, steam exploded and enzyme treated + steam exploded samples, respectively, and in fibres harvested after frost $3.4 \cdot 10^2$, $3.0 \cdot 10^2$ and $6.0 \cdot 10^1$ cfu g_{dw}^{-1} in untreated, steam exploded and enzyme treated + steam exploded samples, respectively. The bacterial content decreased by about an order of

magnitude in the steam explosion (Figure 10 and Figure 1 in V). Dry thermal treatment with the time-temperature variations used did not appear to reduce markedly the amount of colonizing moulds and bacteria (Figures 11 and 12). There were no statistically significant differences between different temperatures or heating durations ($p>0.05$) (V).

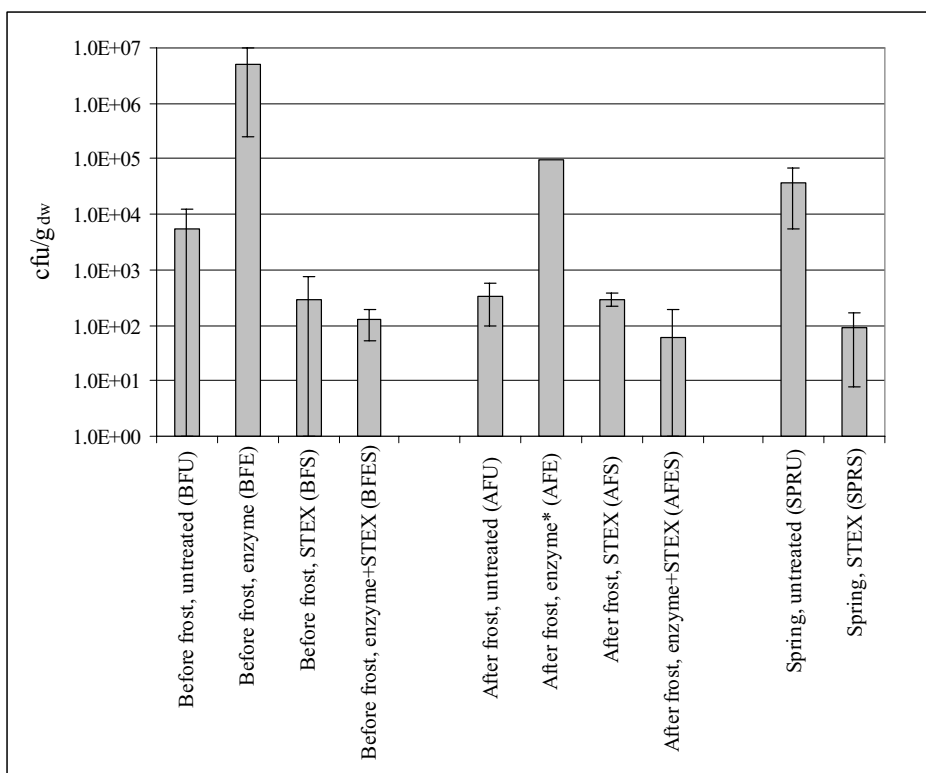


Figure 9. Mould content on the hemp fibres after frost retting, enzyme treatment and steam explosion. Columns indicate means and bars (\pm SE) standard errors of means of five replicates. STEX = steam explosion (*only one replicate).

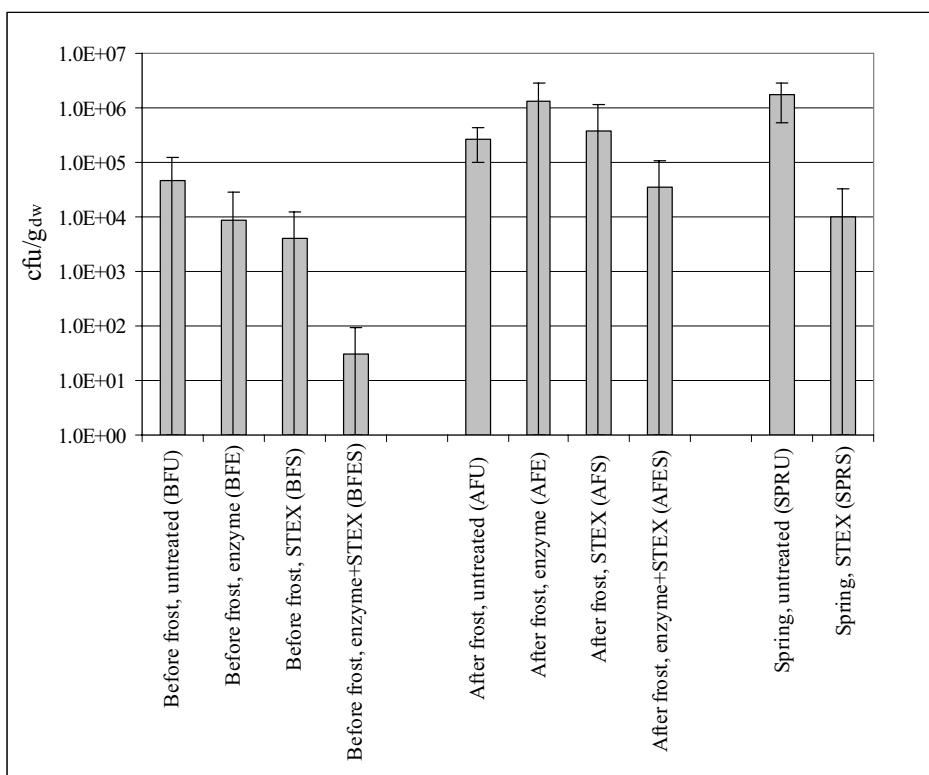


Figure 10. Bacterial content on the hemp fibres after frost retting, enzyme treatment and steam explosion. Columns indicate means and bars (\pm SE) standard errors of means of five replicates. STEX = steam explosion.

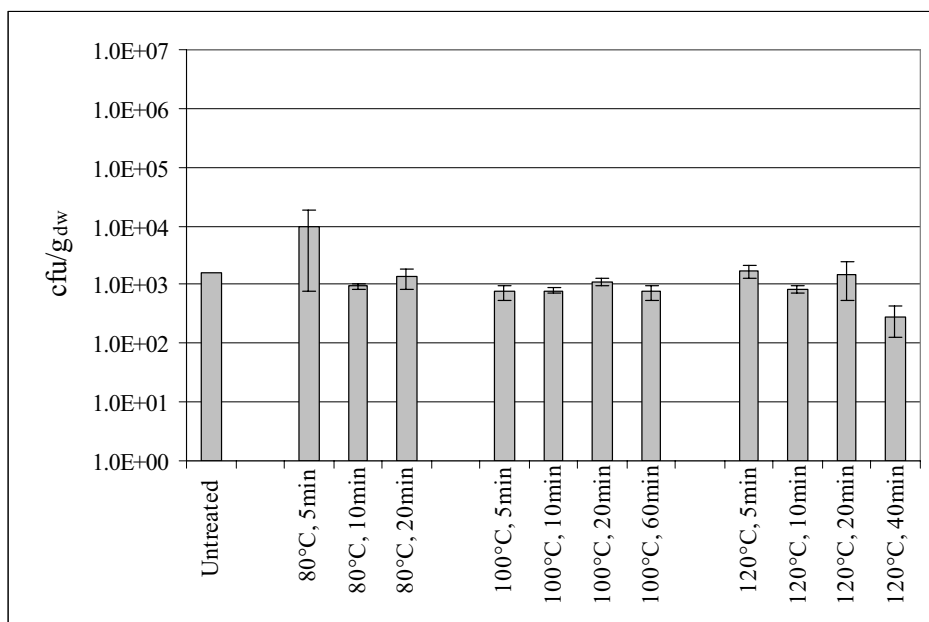


Figure 11. Mould content on the hemp fibres after thermal treatments. Columns indicate means and bars (\pm SE) standard errors of means of five replicates.

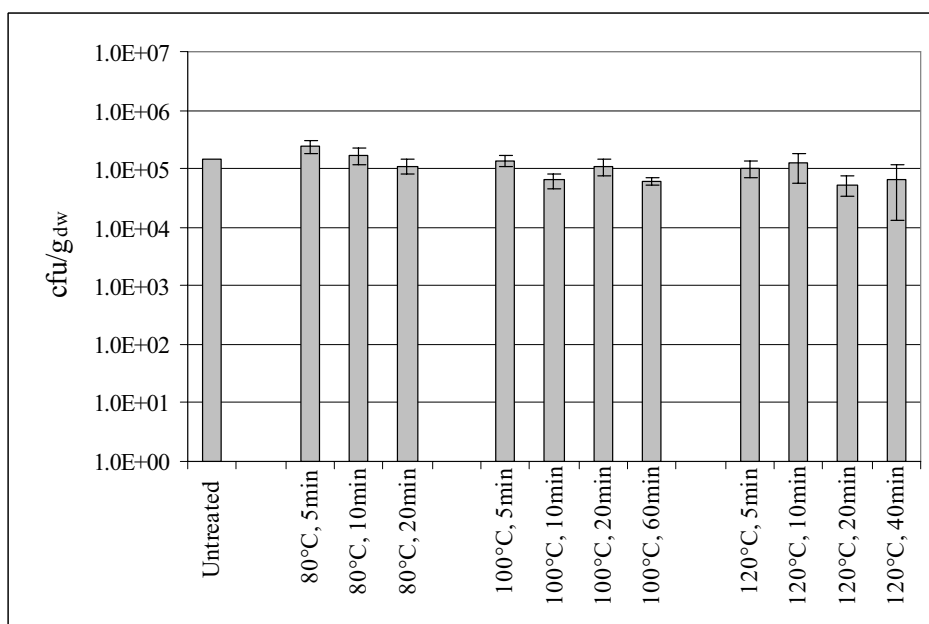


Figure 12. Bacterial content on the hemp fibres after thermal treatments. Columns indicate means and bars (\pm SE) standard errors of means of five replicates.

4.3 Microbial growth and emissions from thermal insulations

The amounts of microbes in the room-dry flax and hemp insulations varied greatly (Table 10). The lowest microbial contents were in the commercial products and the highest contents in linseed fibre without additives. As can be seen in Table 10, the amount of moulds increased in very moist conditions (90% RH). Excessive growth of moulds prevented the calculation of bacterial colonies.

Table 10. Microbial contents of the insulations before and after the emission chamber measurement.

	Linseed fibre	Hemp fibre	Commercial flax mat insulation	Commercial loose-fill flax insulation
Microbial content before tests (cfu g _{dw} ⁻¹)				
Moulds	1.6*10 ⁵	9.0*10 ³	1.4*10 ³	9.8*10 ²
Bacteria	2.6*10 ⁷	1.5*10 ⁷	1.4*10 ⁴	1.1*10 ⁶
Microbial content after the test at 80% RH (cfu g _{dw} ⁻¹)				
Moulds	-	4.3*10 ³	UD	3.9*10 ²
Bacteria	-	1.1*10 ⁶	1.1*10 ⁴	2.9*10 ⁵
Microbial content after the test at 90% RH and drying at 30% RH (cfu g _{dw} ⁻¹)				
Moulds	1.5*10 ⁶	2.1*10 ⁷	4.5*10 ^{7a}	1.5*10 ⁷
Bacteria	4.8*10 ⁶	ND	ND ^a	ND

- Test was not performed.

UD Under the detection limit (approximately 1.8*10² cfu g_{dw}⁻¹)

ND Not detected, due to excessive mould growth on the slide

^a Measurement after the test in 90% RH, no drying in 30% RH

In a properly cleaned empty chamber, the numbers of moulds, yeasts, and bacteria were ≤ 25 cfu m⁻³ (II). Stability of conditions in the chamber was attained. At 90% RH of air, the maximum amounts of fungal emissions were in the range of 10³–10⁵ cfu m⁻³ in the case of the flax and hemp insulations (Figure 13). After drying at 30% RH, the amounts

of emitted moulds were in all cases higher on day 27 compared to the emissions at 90% RH on day 21. The amounts of microbial emissions from stone wool, glass wool and recycled paper insulations were below 10^2 cfu m^{-3} even at 90% RH. Equally low values were obtained from the commercial flax sheet 1 in both 30% and 80% RH and from the hemp fibre and recycled wood in 80% RH. The commercial loose-fill flax emitted less than $4.0 \cdot 10^2$ cfu m^{-3} moulds in 80% RH. The amounts of yeasts and bacteria did not increase markedly in the air of the chamber at 30%, 80% or 90% RH (III).

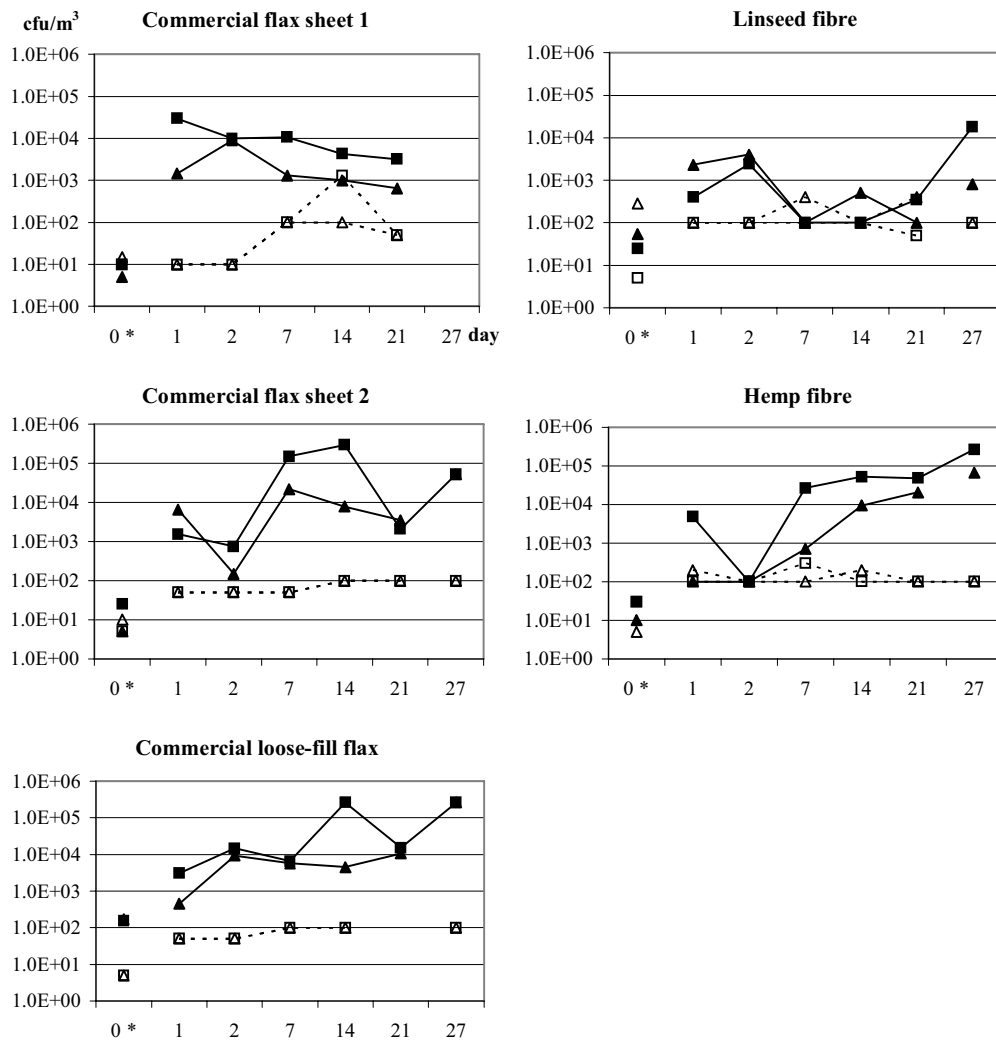


Figure 13. Microbial emissions (cfu m^{-3} of air) of the bast fibre insulations at 90 % RH of air. —■— moulds on malt agar plate, --□-- yeasts and bacteria on malt agar plate, —▲— moulds on TGY agar plate, --△-- bacteria on TGY agar plate. (Figure 2 in III).

* day 0 = empty chamber measurement

4.4 Fungal taxa from fibres and fibrous thermal insulations

The recognized taxa of fungi in the fibres and the insulations are presented in Table 11. In addition to the fungi, *Streptomyces* was recognized in all *Linum* materials (III) and in untreated hemp fibres harvested before frost or in spring (V). The most common genera of fungi were *Penicillium* and *Rhizopus*, both recognized in almost all hemp samples even after the treatments (V). The widest variety of different taxa was identified from the untreated hemp and linseed fibres and from the commercial loose-fill flax insulation (III, V). *Penicillium*, *Rhizopus* and *Paecilomyces* spp. were the most tolerant to steam explosion. However, the number of visible colonies on plates was low on the steam exploded samples compared to that on the other samples. *Bipolaris* and *Alternaria* were absent in the enzymatically treated hemp samples, but *Penicillium* grew well during the enzymatic treatment.

Table 11. Fungal taxa recognized from the samples. I-V are the original publications. U=untreated, E=enzymatic treatment, S=steam explosion ES=combined enzymatic treatment and steam explosion.

Sample	Linseed fibre	Hemp fibre	Insulation																				
			Harvested before frost (BF)						Harvested after frost (AF)						Harvested in spring (SPR)		Flax	Recycled wood	Inorganic				
			U	E	S	ES	U	E	S	ES	U	S	U	S									
<i>Acromonium</i>	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Alternaria</i>	I, III	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Arthrinium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus</i>	-	V	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bipolaris</i>	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cladosporium</i>	I, III	I, V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Epicoccum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Fusarium</i>	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Mucorales</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Myxomycetes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paecilomyces</i>	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium</i>	-	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
<i>Rhizopus</i>	-	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
<i>Sordariaceae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Stachybotrys</i>	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ulocladium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown fungus	I, III	I, V	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacterial growth	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- Not detected.

5 Discussion

In this study the microbial quality of hemp and flax was measured throughout the processing chain. Although some factors cannot be regulated (temperature and humidity during the growing season), others can be controlled. Because the natural bast fibres and fibrous products are organic materials prone to microbial growth and deterioration, attention must be paid to the cleanliness and control of conditions during their processing, storage and use. In addition, agricultural and processing machines are potential contamination sources for the plant materials being processed.

Thermal insulation is a material which must be produced with relatively low costs. Therefore expensive and time-demanding process steps cannot be used, because they would increase the price of the insulation. Inorganic insulations are cheaper and they contain less nutrients for microbes, compared with insulations produced from organic bast fibres. However, there is increasing interest in renewable materials and other ecological values (e.g. Bócsa et al. 2000, Nova-Institut 2003). Thermal bast fibre insulations are often considered to be an ecological alternative, which has been evaluated to be their most important advantage in competition (Rissanen et al. 1998). Furthermore, use of fibres from plants cultivated primarily for their seeds can provide some extra income for the farmer.

5.1 Effects of environmental conditions on growth and microbial quality during the growing season

The draught during the growing season of 2002 affected growth of the plants. The effects of dry conditions can be seen e.g. in the smaller height and diameter of the plants and in the increased dry weight content of the matured stalk. According to Struik et al. (2000), hemp is sensitive to poor soil structure and shortage or excess of water, especially during the early stages of growth. Reduced fertilization, especially nitrogen, can also have had an effect on the size of the plants (van der Werf et al. 1994, Ranalli 1999).

High humidity of air during the growing season of 2001 encouraged the growth of both moulds and other microbes in plants. During the summer of 2001 the air humidity was high at the time when the plants were mature and no longer growing. High moisture content of the plants at harvesting (especially of hemp in the growing season of 2001, the moisture content of the lower part being 70%) may have affected the quality properties due to increased microbial activity. Moisture contents over 16% lead to continued retting of fibres (Sultana 1992a). Therefore all harvested fibre material should be dried in order to avoid spoilage of the crop. Costs of drying and transportation have been mentioned as a disadvantage of plants with a high moisture content (Amaducci et al. 2000). The same microorganisms that help to break down the complex plant materials in the natural cycling of organic matter may give rise to spoilage problems e.g. during storage (Adams and Moss 2002).

In this study, total microbial contents varied between different parts of the plant, the difference between the top and lower parts being smaller in linseed than in hemp. This is understandable, because the difference in height between the two plants is considerable.

In addition to fungal growth, production of mycotoxin is also heavily influenced by environmental factors, especially temperature and water activity. According to a study by Krogh (1984), a pronounced variation in mycotoxin content was evident even in the same crop from the same area, compared on an annual basis, as a combined effect of the environmental factors.

Frost-retting of hemp can result in over-retted fibres that are very absorbent, and relatively resistant to moulds due to removal of nutrients during retting (Kymäläinen and Pasila 2000, Kymäläinen et al. 2001). Accordingly, in (IV) the mould content appeared to be lower in spring-harvested fibres separated by milling than in fibres harvested in the autumn. However, the mould content of stalks increased during the winter. In this respect, the manually separated fibres of (V) appear to behave more like the stems than the mechanically milled fibres, with regard the amount of moulds.

Frost retting of the hemp stems in the field makes peeling and defibration easier by decreasing binding between xylem and cortex. According to the results concerning chemical composition in (V), minerals (ash) and water-extractables were removed during frost-retting and the cellulose fraction of fibres increased.

Frost for any long period can destroy young hemp plants (Berger 1969). Early frost was also found to be a problem in the case of kenaf because it killed the crop before the harvest and the plants were infected by fungi (Ramaswamy et al. 1999).

5.2 Effects of fractionation on microbial quality

The amount of moulds in the cut, dried hemp stems was at the same level as in the living plants at the end of the growing season in autumn. By processing and mechanical separation, it was possible to produce fibre containing somewhat less moulds and bacteria than the whole stem or the plant in the field. Frost decreased the amount of total bacteria in those parts of hemp containing tissues of outer layers. The outer surface of a plant may prevent microbial invasion of the tissues in living plants (Adams and Moss 2002), and it may have affected the lower microbial content of the fibre and shive fractions compared to the whole stalk.

The microbial contents of the fractions of fibre hemp and linseed varied between 10^3 and 10^9 cfu g_{dw}⁻¹. Levels exceeding 10^5 cfu g⁻¹ may be too high for certain applications such as building materials (STM 2003). The fibre of hemp harvested after early frost or in spring had the lowest amount of moulds, but during winter and spring the amount of bacteria and yeasts increased in hemp.

5.3 Effects of enzymatic and heating treatments on microbial quality

The mould content was 500 times higher after the enzymatic treatment than before it. It is possible that 24 hours in moist and warm conditions encouraged the growth of moulds. However, not all the fungi appeared to be tolerant to the enzymatic treatment, which decreased the variety of different taxa of fungi more than the frost period (V).

The number of fungal colonies was small on the steam exploded samples compared to that on the other samples. STEX treatment also reduced the amount of different taxa in the samples. Dry heating had no effect on the amount of microbes on the fibres. Hemp fibres have favourable thermal insulation properties (Wieland et al. 2000). It is possible that the time used in the present experiments was not long enough to heat the whole material to a temperature high enough to kill the viable moulds and spores. However, longer heating periods or higher temperatures may not be possible in e.g. insulation manufacturing. According to the results of this study, it is evident that the thermal effect in the manufacturing process of the insulations is not sufficiently effective in destroying microbes and spores.

According to the results of chemical composition in (V), mainly water-extractives, minerals (ash) and pectins were removed and the cellulose fraction increased during the enzymatic treatment. During steam explosion, minerals (ash) and water-extractives were removed to a similar extent as in enzymatic treatment. In addition, hemicelluloses and pectins were extracted in steam explosion. Because hemicellulose and pectins support a profuse growth of fungi (Sharma et al. 1992), steam explosion can improve the resistance of stems to moulds due to removal of those nutrients. Thorough rinsing or washing after steam explosion might help to purify the fibres and improve the tolerance to microbial growth.

5.4 Microbial growth and emissions from thermal insulations

Several studies have shown that moisture damage has an effect on microbiological growth in different building materials (e.g. Pasanen et al. 2000a, Kildesø et al. 2003). According to Nevalainen et al. (1998), moisture problems have been reported in 55% of residential houses in Finland. Fungi usually enter the buildings through heating, ventilation or air conditioning systems, through doors and windows and as contaminants on building materials. Moisture damage in buildings has been shown to be associated with several health effects. Fungal growth on building materials and fungal emissions to the indoor air can be hazardous, and must therefore be limited or prevented. The most important way to achieve this is the prevention of high moisture conditions in building materials and in indoor air. Choosing building materials that are less susceptible to fungal growth is another way to minimize fungal growth in buildings. The problem is that microorganisms can colonize and multiply on building materials if sufficient nutrients and moisture are available. Inorganic materials can also be colonized by microbes, because the surfaces in buildings are normally covered with dust and organic debris, which contains suitable amounts of nutrients for microorganisms (Korpi et al. 1997). Moulds need only very minor amounts of nutrients, for example from building material components or accumulated dust. According to Pasanen et al. (1991), even a short period of favourable conditions was sufficient to initiate fungal growth.

In this study, all insulations made of flax and hemp fibres contained microbes, the amounts of which increased in very moist conditions. The reason for microbial growth in the bast fibrous insulations of this study can be the moisture load during the growing seasons as well as the environmental conditions in the emission chamber. Anti-moulding agents, fungicides, fire-retarding agents and other additives in commercial bast fibre insulation products may decrease the amount of microbes in the insulations, which was observed as somewhat lower microbial contents of the dry, unmoistured commercial insulations compared with the fibre raw material without additives. However, according to the results of the chamber test at 90% RH, no significant difference was observed between the microbial emissions of the commercial bast fibre insulations and of the fibres without additives. After drying of the insulations after the test in 90% RH, the microbes were increasingly detached from the bast fibre insulations.

This may be due to real increase of the microbes, the existing microbes may have detached more easily due to the lower moisture content of the materials, or spore generation may have resulted from the decrease of RH. Similarly, in a study by Korpi et al. (1998), the levels of fungi increased under drying conditions compared with the levels at the beginning of the incubation period.

According to this study, some of the microbes attached to the insulation material were released from the insulation due to the movement of air, which resulted in microbial emissions. In lower relative humidities of air (30% and 80%), the amount of microbes in insulations decreased in some materials. This may be a result of detachment of microbes from the insulation materials. In addition, lower relative humidities are not as favourable for fungal reproduction as the relative humidity of 90%. According to Górný et al. (2001), the fungal species, air velocity above the surface, texture of the surface and vibration of the contaminated material affect the release of fungal spores. In their experiment the release rate was usually highest during the first several minutes of exposure to air currents and mechanical vibration.

In a study by Hyvärinen et al. (2002) the highest fungal concentrations were observed in wooden and paper materials, and the lowest concentrations in mineral insulation materials, among others. However, no agro fibre insulations were included in their study. In a study by Ritschkoff et al. (2000), several building materials (e.g. glass wool insulation) were susceptible to mould growth in humidities higher than 90% RH and at temperatures above +15°C. However, the origin and raw material of the building materials affected the moulding tendency. In the stone-based materials the critical humidity level and required exposure time were higher than in the wood-based materials. In our study the insulation materials were new and unused, and there was no accumulation of dust and other nutrients. Cellulose may support the growth of microbes in organic materials (Tye et al. 1980). Damp building materials containing cellulose are particularly susceptible to colonization by *Stachybotrys* and *Chaetomium* species (Andersen and Nissen 2000), because of the strong cellulolytic activities of those species (Udagawa 1984). In this study the wood-based insulation contained many inorganic additives, which may have prevented the fungal growth. Glass wool and stone wool are nutrient-poor materials and do not support the growth of microbes (Tye et al. 1980). However, used inorganic insulations have also been found to yield a variety of

fungi (Pasanen et al. 1992, Ezeonu et al. 1994, Reiman et al. 2004). In a study by Gravesen et al. (1999), water-damaged, aged organic materials containing cellulose were most vulnerable to moulding. A correlation between moisture content and the amount of fungi was reported in particle and wood boards by Pasanen et al. (2000a). This correlation was also observed in the present study. According to STM (2003), a fungal colonization can be detected if the amount of moulds (cfu g^{-1}) is 100 times higher than in the original material. In this study this trend was seen in bast fibre insulations in 90% RH.

Górny et al. (2002) found that in addition to spores, a significant amount of immunologically reactive particles, considerably smaller than spores, was released from surfaces contaminated with fungi. They suggested that future fungal spore investigations should include the quantitation of smaller fungal fragments. In our study, in addition to the microbiological sampling, particles smaller than fungal spores were also detected.

5.5 Fungal taxa in bast fibres and fibrous thermal insulations

The genera of the moulds recognized from the hemp and linseed samples included genera common in outdoor air and in soil. Among the recognized taxa of fungi there are some that contain toxin-producing species (e.g. Evans et al 1996, Jarvis et al. 1996 and 1998, Committee on Environmental Health 1998, Andersen and Nissen 2000, Etzel 2002). *Penicillium* species are active pathogens of some plants, but their dominant role is in saprophytic habitats, especially in cooler climates (Pitt and Udagawa 1984). Common fungal colonisers and plant pathogens according to the literature, *Alternaria*, *Cladosporium*, *Fusarium* and *Epicoccum*, were found from several bast fibre samples in this study.

The widest variety of different fungi was in untreated fibre samples from different levels of frost retting. *Penicillium*, *Rhizopus* and *Paecilomyces* were the most tolerant fungi to steam explosion. *Bipolaris* and *Alternaria* were absent in the enzymatically treated samples, even though enzymatic treatment increased the total amount of moulds.

The most common fungi in building materials and indoor air are *Penicillium*, *Aspergillus* and *Cladosporium* (STM 2003), which were all found in some of the bast fibre materials in this study. According to STM (2003), microbes of e.g. the genera *Stachybotrys*, *Chaetomium* and *Fusarium*, which were also found in flax samples in this study, may be a sign of moisture damage.

5.6 Evaluation of the research methods

In this study it was possible to combine specific meteorological data with data from controlled cultivation in field conditions. The whole production chain and all steps could be studied. The quality of the raw material and the growing and storage history were known. Microbiological methods and equipment were suitable for both field sampling and also for accurate laboratory measurements. For isolation of fungi from wet environments, water agar or malt extract agar can be used. For drier conditions, DG18 and malt extract agar are recommended (Samson et al. 1994). In this study, malt extract agar was selected for the emission measurements, and water agar was used for the recognition of mould genera. Steam explosion (STEX) appeared to be effective against viable microbes. The use of STEEX with fibres intended for use in technological applications might help to maintain better microbiological quality of the products.

The emission chamber is capable of detecting microbes, particles and VOCs from several different materials and it can be modified to use different measurement equipments. With the measuring system developed in this project it can reliably be used in several applications and research fields. Using the present measurement setup, the emission chamber can be considered to be physically, microbiologically and chemically clean. The risk of microbial contamination in the chamber e.g. due to air leakage during sampling, contamination of the moisturizing unit or settling of pollutants onto the inner surfaces of the chamber during drying should be eliminated.

5.7 Challenges for production of hemp and flax fibres and thermal insulations from bast fibres

5.7.1 From the point of view of the farmer

Fungal diseases in flax crops are often due to the very high crop density or lodging. Flax has a proclivity for lodging when cultivated with excess nitrogen, and it is recommended to fertilize the flax crop with no more than the required amount of nitrogen (Sultana 1992a).

Some fungal diseases can be controlled by treatment with fungicides (e.g. Kroes et al. 1999, Halley et al. 2003). The seeds can be treated with different fungicides or by surface sterilization, which are effective against the damping off diseases. However, some seed-borne pathogens (e.g. *Alternaria linicola*) are not always destroyed by surface sterilization or other seed treatments (Harold et al. 1997, Kroes et al. 1999). The severity of Pasm, caused by *Septoria linicola*, can be reduced with azoxystrobin and sulphur fungicides (Halley et al. 2003). However, some fungal diseases are hard to control (Beaudoin 1989). Chemical pre-harvest retting with glyphosate has proved to be fungitoxic *in vitro*, but in a study by Brown and Sharma (1984) it had only a very slight effect on fungi colonizing flax. Crop rotation is effective against some fungal pathogens. According to Kok et al. (1994), fibre hemp suppressed two major soil pathogens (*Verticillium dahliae* and *Meloidogyne chitwoodi*), and therefore may improve soil health and be used in crop rotation. Hemp also suppresses weeds rather efficiently (Van der Werf 1994) and it can be cultivated as a shelter crop.

Soil suppressiveness to diseases is defined as the ability of a soil to reduce disease severity, and can be induced by biotic or abiotic soil properties. Clay minerals and soil pH can affect the suppressiveness of soil to fungal diseases. In a study by Höper et al. (1995), pure clay minerals (e.g. kaolinite) were added to the soil and the pH value was increased by liming. The additions modified several physicochemical and biological properties, and the soil suppressiveness to *Fusarium* wilt of flax was significantly

increased. According to Serra-Wittling et al. (1996), the addition of municipal solid waste compost also increased soil suppressiveness to *Fusarium* wilt.

There is a significant risk of spoilage of the crop when the weather is rainy and the air humidity is high during the harvest season. Problems concerning microbial growth can occur during the harvesting, retting, storage and processing of the crop. The high moisture content of the crop must be decreased to no more than 16% (w.b.) (Sultana 1992a). Drying is usually needed in order to avoid moulding. In addition, storage in humid and rainy conditions may cause water intake both from the air and from free water, and should be avoided.

The use of disease-resistant cultivars can increase the possibilities of growing hemp and flax. Several studies have been published concerning the resistance of different flax cultivars to certain fungal pathogens (e.g. Mercer and Ruddock 1994, Kenaschuk et al. 1996, Kenaschuk and Rashid 1998 and 1999, Kroes et al. 1999, Rowland et al. 2002a, 2002b, 2002c and 2002d, Halley et al. 2003, Duguid et al. 2004, Rashid and Duguid 2005). New resistant cultivars are still required particularly for Nordic climates.

5.7.2 From the point of view of the insulation manufacturer

The main technical requirements for thermal insulations are thermal resistance, fire performance and moisture behaviour (Nelson 1990). However, there are several secondary and supportive functions, one of which is the ability of the insulation to promote high quality of indoor air.

In the production of bast fibre insulations, attention should be paid to retting degree, amount of shives and other impurities, moisture content and microbial content of the bast fibres used as raw material. Consistent quality, sufficient density, elasticity and sagging of the insulations are also important factors (Kauriinvaaha et al. 2001).

The shive content of insulations can be up to 5 % (Nova-Institut 2003). The bast fibres contain more cellulose, whereas the shives contain more lignin and hemicellulose (e.g. Thomsen et al. 2005). Because hemicellulose and pectins support the growth of fungi

(Sharma et al. 1992), separation of fibres from hemicellulose- and pectin-rich fractions would decrease the tendency to moulding.

According to the recommendations of the Ministry of Social Affairs and Health in Finland (STM 2003), the amount of fungal spores in building materials should not exceed 10^4 cfu g^{-1} and the amount of bacteria should be below 10^5 cfu g^{-1} . During the growing season of 2001 the mould content in stems was higher than this limit value. For that reason, further development of processing methods or treatments must be carried out in order to reduce the microbial counts in fibres.

Dry heating in the manufacturing process of the insulations is not sufficiently effective in destroying microbes and spores. Therefore anti-moulding agents must be added to the products in order to prevent microbial growth.

5.7.3 From the point of view of the constructor

Use of bast fibrous thermal insulations in building structures is presented in Kauriinvaha et al. (2001). Hygrothermal loads in building structures may lead to degradation failure of building materials, as well as to poor indoor air quality. Moisture damage in buildings can be a consequence of leakage in structures, e.g. in window frames, or of insufficient insulation of pipes or washing facilities and should be avoided.

Storage of insulations in humid conditions, for example uncovered in wall structures or in the construction sites during periods of rain, the most probably causes water intake both from the air and from free water. Capillary absorption of water in building materials results in rapid fungal contamination (Pasanen et al. 2000a). In addition, being hygroscopic materials, hemp and flax adsorb moisture from the surroundings if the vapour pressure is lower within the material than in the ambient air.

6 Conclusions

1. Atmospheric conditions affected the growth and microbial quality of linseed and hemp. In both hemp and linseed the mould and bacterial contents ($\text{cfu g}_{\text{dw}}^{-1}$) increased at the end of the growing season and during the winter. In this study, dry growing conditions resulted in smaller height and diameter of the plants and increased dry weight of the matured stalk. High humidity of air encouraged the growth of microbes in plants. The genera of moulds recognized from hemp and linseed samples during the growing season included *Cladosporium*, *Fusarium*, *Penicillium*, *Mucor* and *Alternaria*.
2. During the winter and spring period there was a clear increase in the amount of moulds, bacteria and yeasts, especially in the standing stems in the field but also in the fibre and shive fractions. According to this study, it is possible to produce fibres with lower microbial contents compared to the unprocessed stems by mechanical fractionation. Retting of hemp fibres with enzymatic treatment encouraged the growth of moulds. Steam explosion (STEX) decreased the total amount of moulds in fibres. Dry thermal treatment up to 60 minutes at 100°C or 40 minutes at 120°C did not reduce the amount of colonizing moulds and bacteria. The most common genera of fungi in fractionated hemp and linseed samples were *Alternaria* and *Cladosporium*, as well as *Penicillium* in hemp samples. Enzymatic treatment and STEX treatment reduced the variety of taxa in the samples, *Penicillium* and *Rhizopus* were the most tolerant to steam explosion and enzymatic treatment.
3. All insulations made of flax and hemp fibres contained microbes. Untreated hemp and linseed fibres and the commercial loose-fill flax insulation contained the widest variety of different fungi. Amounts of microbes and microbial emissions to the air increased in very moist conditions (90% RH of air). There was no significant difference between the microbial emissions from the commercial bast fibre insulations and from the bast fibres without additives. Drying of moist, moulded bast fibre insulations increased the microbial emissions. The amounts of moulds in inorganic insulations and recycled paper

were low even in humid conditions. Equally low values were obtained from the bast fibrous materials in lower humidities (at 30% and 80% RH of air). The emissions of yeasts and bacteria did not increase markedly in different moisture conditions. In linseed and hemp fibres used as insulations *Alternaria* and *Cladosporium* were the most common fungi, whereas in commercial flax insulations the dominating taxa were *Aspergillus* and *Penicillium*.

4. With a monitoring system including techniques and equipment developed in this study, it is possible to monitor and partly control the microbiological quality of the raw materials and the products made of natural fibres. The emission chamber developed in this study is suitable for detecting microbes, particles and VOCs from several different materials. Because hemp and flax fibres contain considerable amounts of nutrients, none of the current methods offers a perfect and permanently hygienic result. However, the amount of microbes can be decreased with processing methods or with additives to the products, and the environmental conditions can be regulated so that growth is not possible or is slower. This is advantageous even though a totally and permanently hygienic status cannot be achieved.

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