THE COLONISATION OF PAINT FILMS BY MICROORGANISMS IN THE UK AND NORWAY.

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ABSTRACT

This work was undertaken in order to study the colonisation of paint films by microorganisms. Quantitative data derived from a range of analytical techniques has provided information on surface changes occurring in the paint films during colonisation in the field and during laboratory exposure experiments.

Exposure trials, took place at four different sites, two in Norway and two in the UK. The sites were situated in Sandefjord and Bergen and in Preston and Blackley near Manchester, with the panels facing North at an angle of forty-five degrees. The results obtained from these studies indicate that the range of microorganisms found on the panels exposed at the locations were very similar, suggesting that there was no great difference encountered in the airborne flora as indicated by settle plates at the sites investigated. Painted panels made from Spruce were found to be more heavily colonised than those made from calcium silicate or aluminium. The formulation biocide was seen to be effective in the vermiculite bed system against *Aureobasidium pullulans*.

The Scanning Electron Microscope (SEM) and Environmental Scanning Electron Microscope (ESEM) provided visual evidence that in the case of *Aureobasidium pullulans,* fungal hyphae penetrated the paint film by the dissolution of the paint binder rather than disruption of the paint films by the growth of microorganisms through the paint film from below. Spruce panels that had been gamma irradiated showed a similar surface colonisation pattern to those untreated.

Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI TOF MS) showed that the technique was able to distinguish between spectra generated by different genera of fungi and between spectra generated by different species of the same genus. It is considered that the technique may provide evidence to confirm or refute the nominally common taxonomic status of fungal isolates from different sites.

The work undertaken using the Talysurf™, shows that the technique is suitable for detecting changes in the surface topography of unprotected paint films. The results from work undertaken with films that had been subject to prolonged exposure at the Preston site and within a vermiculite bed system confirm this.

Comparisons of the Fourier transform infrared spectroscopy (FTIR) spectra obtained for exposed and non-exposed paint films suggested that the exposure had no effect on the overall composition of the paint film.

To my Mum, Dad and Grandma

who have helped and encouraged me throughout my education

and to Andrew.

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GLOSSARY

The six paints designated A to F represent the following paints.

A - Pure acrylic paint containing fungicide

B - Pure acrylic paint containing no fungicide

C - Hybrid acrylic paint containing fungicide

D- Hybrid acrylic paint containing no fungicide

E - High solid alkyd paint containing no fungicide

F - Alkyd paint containing no fungicide

The quantifiable parameters for the Talysurf™ (Digitalsurf, 1997).

Sa – The arithmetic mean- The average roughness of the sample being measured

Sq – The quadratic mean

St – The total height of the sample, from the highest to the lowest point (St = Sp +Sv)

Sp – The highest peak over that calculated as the mean peak

Sv – The lowest valley under that calculated as the mean valley

Ssk – Skewness –Indicates the symmetry of the depth distribution.

Sku – Kurtosis – measures the sharpness and the shape of the distribution curve.

Sz – The mean value of the five highest and five lowest points on the sample.

Glossary of Terms.

AFM	Atomic Force Microscopy
API	Analytical Profile Index
α-CHCA	α-cyano-4-hydroxycinnamic acid
CMA	Corn Meal Agar
CMBT	5-chloro-2-mercaptobenzothiazole
EPS	Extracellular Polymeric Substances
FTIR	Fourier Transform Infrared Spectroscopy
ESEM	Environmental Scanning Electron Microscopy
GSED	Gaseous Secondary Electron Detector
HEC	Hydroxy Ethyl Cellulose
HEUR	A combination of two urethane thickeners
HPLC	High Performance Liquid Chromatography
IBRG	International Biodeterioration Research group.

IPBC	3- Iodo-propynyl-butyl-carbamate
MALDI TOF MS	Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry.
n.d	No date available.
PSE	Plane Square Edge
RMS	Root Mean Square
SEM	Scanning Electron Microscopy
Sp.	Species (singular)
Spp.	Species (plural)
SRM	Surface Roughness Measurement

CHAPTER 1.

GENERAL INTRODUCTION

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1.1 Rationale for the following study.

This study was undertaken in order to investigate the colonisation of paint films by microorganisms. It was also undertaken to provide information of any deleterious effects that colonisers might have upon the structure and composition of defined paint coatings. It has been established that paint surfaces may be colonised by microorganisms from the air but equally from the material that they are coating, this has been recorded extensively by Morton, 1975 and Kelly, 1983. The coatings were therefore applied to three different substrata, wood, aluminium and Masterclad™, a calcium silicate building board.

The climate of a region, whether urban or rural, will influence the level of natural biodegradation occurring in that region. This, in turn, will determine the microbiological content, and to some extent, the organic and inorganic chemical content of air arriving at exposed surfaces. The microbiological content of air is the source of microorganisms responsible for the colonisation of exposed surfaces and therefore any biodeterioration effects to those surfaces. This in fact justified the need for field trials, which would provide information on the nature of microbial colonisers and the influence of weather conditions on their incidence. It was also considered necessary to conduct a series of laboratory studies to evaluate and quantify the effects of microbial colonisation.

Microorganisms that form resident communities at surfaces are known as biofilms. This investigation was conducted to provide a better understanding of the nature of biofilms at the solid/air interface. There is a wealth of published information on biofilms formed at the solid/liquid interface in the aquatic environment. The role of extracellular polymeric substances (EPS) and the microorganisms, which produce it, have been studied extensively. Whilst there is information available on the range of microorganisms found on exposed surfaces, there is little information available on the colonisation sequence and mechanism employed to establish biofilms at the solid/air interface, or on the adherence mechanisms and spatial relationships of microorganisms in this situation.

It was envisaged that quantitative data derived from a range of analytical techniques would provide information on morphological and chemical changes occurring in the

paint film during the colonisation and weathering / laboratory exposure processes. A novel analytical aid to fungal identification was also considered worthy of further investigation, this being, Matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI TOF MS)

An important reason for undertaking the work was to establish the efficacy of the biocides that are incorporated into the paint formulations.

1.2 An Introduction to Constructional Materials.

One of the oldest constructional materials known to mankind is timber, it was used in the days of primitive man and its use throughout the world is unlikely to diminish in the foreseeable future (Berry, 1994). Its major use in the UK is for structural, semi-structural and decorative purposes in the building trade. It is used for instance in the construction of roofs, floors, doors and windows (Scott, 1968). In the Scandinavian countries, especially Norway, entire houses are constructed from wood as masonry built houses prove to be more costly.

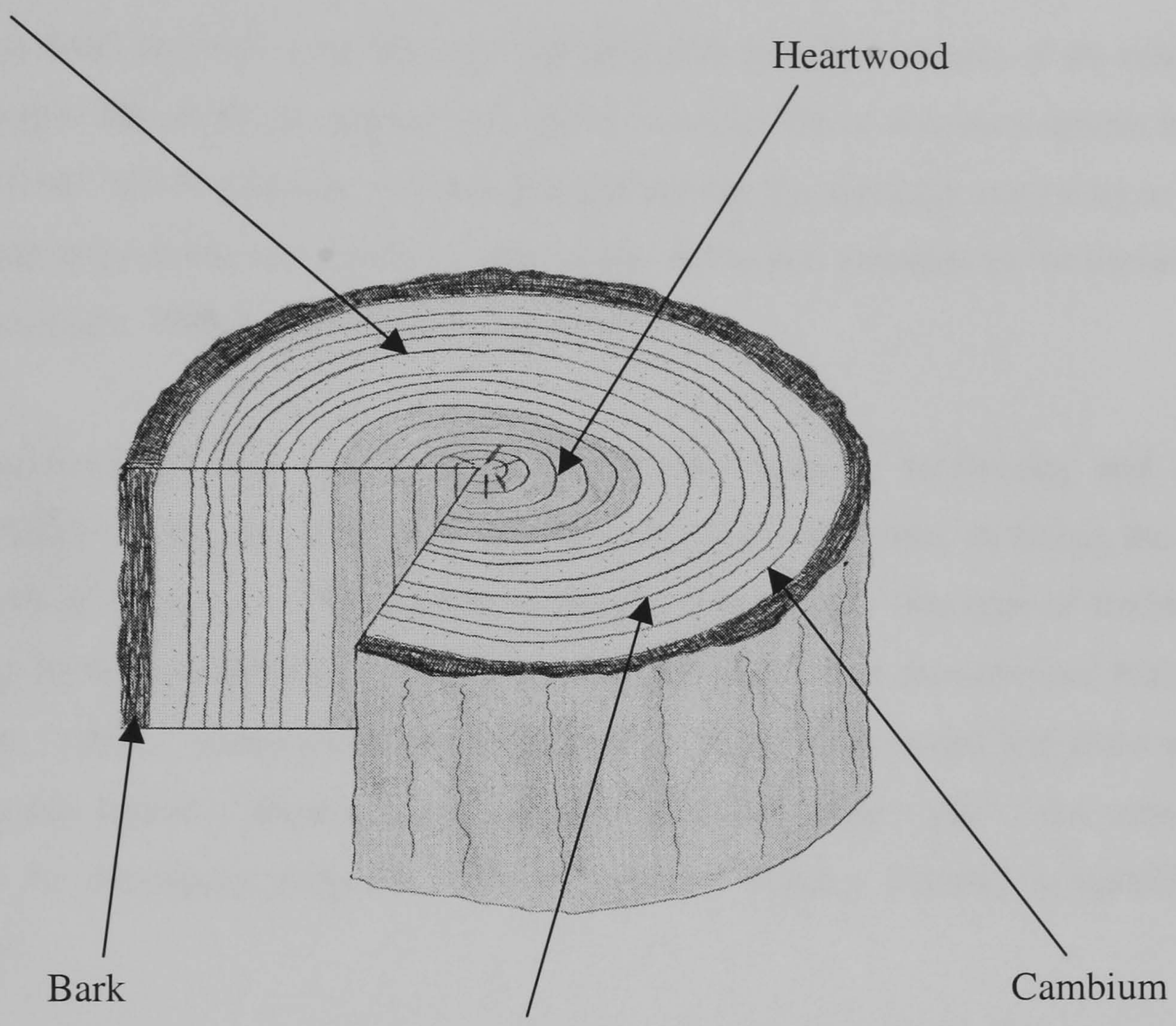
Wood, the substance that timber is composed of, can be defined as a cellular organic material (AITC, 1994) consisting of numerous microscopic cells, the principal components of which are cellulose and lignin (Berry, 1994). The older wood, at the centre of the trunk, is known as the heartwood. This is usually darker in colour than the outer section of the trunk, which is known as sapwood (Figure 1.1).

As trees grow they retain moisture, which saturates most of the cell walls and either fills or partly fills the cells in the sapwood (Scott, 1968). The heartwood has a reduced porosity, which increases its resistance to swelling and shrinkage that occurs with changes in moisture content. Thus, it is more stable and as a result is more resistant to fungal and insect attack than sapwood (Richardson, 1993).

Earlywood (AITC, 1994), also known as springwood (Levin, 1971) forms in the Spring when growth is at its greatest. This causes thin walled cells to be formed and tends to create light coloured bands. Latewood, or summerwood appears as darker bands due to the formation of thicker cell walls in the Summer when the growth is relatively slow

(Markussen, 1999 personal communication). These bands, which form the annual rings, are particularly evident in climates where the temperature limits the growing season of the tree (AITC, 1994).

Figure 1.1 A simplified cross-section of a tree (adapted from Henderson, 1961).



The felling of trees for construction purposes takes place at different times of the year in different countries. In Norway, the preferred felling season is Winter when there is little content in the phloem of the tree. This means that the tree produces a better quality timber in that it is less susceptible to fungal attack when felled (Markussen, 1999 personal communication). The UK tends to import timber from overseas, as the quality is better than home-grown timber due to its ability to withstand deterioration longer (Scott, 1968).

After felling, the timber is stacked carefully leaving air spaces between each plank and is seasoned in this way before use. Seasoning can take place in this natural (air)

seasoning manner or by artificial (kiln) seasoning. Natural seasoning relies on air to reduce the moisture content of the timber, although this method is cheap to perform it leaves the timber susceptible to attack and does not dry the timber sufficiently enough to be used in joinery. Artificial seasoning is carried out in a chamber with controlled heating and humidity so that the timber can be dried as required; hence this method is deemed the better of the two (Scott, 1968).

The manner in which wood is sawn can affect the moisture content of the timber. Using edge grained sawing or quarter sawing the swelling due to moisture uptake in the wood is as little as 4% whereas with flat grained sawing the swelling can be up to 8%. Thus the preferred method of sawing is the former as the less moisture in the timber the better (Markussen, 1999 personal communication).

Commercial timbers can be divided into two groups, hardwoods and softwoods. Softwood timbers originate from members of the conifer class, including the pines, firs, spruces and larches. The majority of building work uses this type of timber due to it being strong and durable and having a straight grain. It is also cheaper than hardwood (Scott, 1968). Hardwood timbers come from trees of the broad leaf class and include the oaks, beeches, chestnuts and mahoganies. The timber from these trees is usually used for decorative purposes such as furniture, wooden flooring or panelling (Scott, 1968).

When a house is built, the window joinery, cladding and any other external timbers will be subjected to persistent wetting by rain and snow unless the architecture is such that they are protected by the roof overhang or deep reveals (Berry, 1994). In Norway, for example the weather is often severe with a lot of snow in the South during Winter months. The traditional style of architecture is shown in Plate 1.1 where the house has a pronounced roof overhang. This practical design is under threat because of the demand for less traditional houses with more a 'modern' appearance.

Plate 1.1 A typical Norwegian property in Sandefjord.

The majority of the timber used for the construction of both British and Norwegian houses is Norway Spruce; otherwise known as White Fir or the European Spruce (*Picea abies* Clifford, 1957).

1.3 A brief introduction to surface coatings

Exposed surfaces are subject to weathering. The effects of weathering include the rusting of iron and the physical deterioration of a variety of surfaces (Turner, 1988). The exposed surface of timber will be subject to weathering. However, because of its nutritional value it will also be attacked by biological agencies including insects and microorganisms, principally the fungi (Markussen, 1999 personal communication). In order to minimise the damage to these timber surfaces and many others various coatings are applied to them for their protection. These coatings can also be used to decorate the

surface, adding colour or smoothing out any roughness or irregularities created during manufacture (Turner, 1988). Thus, the function of a surface coating is two-fold, providing the solution to both aesthetic and protective problems (Lambourne, 1987). There are a number of surface coatings, which convey both of these functions, these include: wallpaper, plastic sheet, chrome and silver plating and, the most versatile of all, paint.

1.4 Paint

Paint can be defined as 'a fluid material which, when spread over a surface in a thin layer, will form a solid, cohesive and adherent film' (Morgans, 1990). The term paint, describes a variety of materials including enamels, undercoats, varnishes and lacquers (Turner, 1988). It can be used to cover any material, no matter how large or irregular in shape (Lambourne, 1987).

1.5 The history of paint.

About twenty-five thousand years ago primitive man was credited with making the first paints. These men were cave dwellers and hunters who were thought to be inspired by the rock formations of their cave walls and by the animals that they hunted (Lambourne, 1987). Various chemical analyses of cave paintings found at Altamira (Spain) and in Lascaux (France) indicate that the main pigments used by these Palaeolithic artists were based upon iron and manganese oxides. These provided the three basic colours found in many cave paintings, i.e. black, red and yellow. Other colours are thought to have originated from burnt wood (brown) and white from chalk. These pigments were ground into a fine powder using a pestle and mortar made from naturally hollowed stones as the mortar, with bones as the pestle. They were probably mixed with water, bone marrow, animal fats, egg white or vegetable sugars to form paints. The application of these paints is thought to be by finger dabbing or using crudely made pads or brushes made from animal fur, hair or moss.

The Egyptians, during the period between 3000-600 BC, developed their own art of paint making. They created a wider range of colour pigments including a range of blues made from lapis lazuli (a sodium silicate) and from azurite, which is chemically similar

to malachite. The Greeks and the Romans during the period 600 BC to 400 AD, appreciated the fact that paint could be used not only to decorate surfaces, but also to preserve them. Varnishes incorporating drying oils were introduced throughout this period, but were not recognised in Europe until the thirteenth century.

Throughout the middle ages most painting on wood was protected by varnishing, the varnish was made by dissolving suitable resins in hot linseed, hempseed or walnut oil; which all tend to darken with time. By the late 18th century the demand for paints of all types had increased to such an extent that it was worthwhile for people to go into business, manufacturing paints and varnishes for others to use.

The industrial revolution had a major effect on the development of the paint industry, creating a demand for paint for a number of purposes. The increased use of iron and steel for construction and in engineering resulted in the development of lead and zinc based paints which were used to delay or prevent rusting and corrosion (Lambourne, 1987). This was later to be regarded as the beginning of the modern paint industry (Morgans, 1990).

1.6 The composition of modern day paints

There are many different types of paints manufactured today. However, they all contain the same primary constituents, namely; a matrix or binder, pigments and extenders (which confer colour and build) and finally, a solvent. The solvent is either organic in nature, for a solvent based paint, or water, for a water based or latex emulsion paint (Banov, 1978). In addition to these common components there are also a number of other ingredients, which make up about five percent of the total system. These include emulsifiers, biocides, plasticisers, colloids and thickeners (usually cellulose based) (Bravery, 1987). Not all paints, however, contain all of the ingredients. Gloss paints for example, do not contain extenders, which are coarse particle inorganic materials (Lambourne, 1987), whereas matt paints do. Paints can also be classified as either being alkyd or acrylic in nature. An alkyd paint is usually an oil based paint and is made from a synthetic resin, made by reacting a drying oil with a hard synthetic material. An acrylic paint is usually a water based paint. The binder in this case is made from a synthetic polymer (B.S.2015, 1992).

Pigments are employed primarily to conceal the surface to which the paint is applied. They can, however, serve as corrosion inhibitors (red lead) or serve as barriers to prevent the deleterious effect of UV radiation on the substratum. Pigments can serve to:

1. Completely cover the underlying surface.
2. Partially cover the surface (when the coating is made translucent by using various opalescent materials).
3. Alter the transparency of the coating for example by using small amounts of iron oxide in a varnish to create a brown, red or yellow stain.

There are many different types of pigments although in general they can be classified into two groups, the white and the coloured pigments. The white pigments are generally used for complete cover of the underlying surface, whereas the coloured pigments often have reduced covering capacity and are therefore used in conjunction with a white pigment to achieve the desired effect. Some examples of the white pigments include titanium dioxide, zinc sulphide and zinc oxide. Titanium dioxide has been the dominant white pigment throughout the paint industry since about 1932. Normal titanium dioxide has a hiding power of 147 square feet per pound, which far exceeds the other pigments mentioned; zinc sulphide has the highest covering power after titanium dioxide, having a value of 58 square feet per pound (Banov, 1978).

Extender pigments are used as an economical way to provide body and bulk to a surface coating. Examples of extender pigments include; chalk (calcium carbonate), clay (aluminosilicate), talc (magnesium silicate) and silica. Without the addition of extender pigments, the coating would be runny and give an uneven cover due to a lack of body. In such a case there would also be a certain amount of free binder that would cause the dried film to have a very glossy appearance.

The general function of a solvent is to interact with the binder to allow it to spread across the desired surface, as the binder is too viscous to adhere to the surface. The binder or film former provides the basis of the continuous film, sealing, therefore protecting the surface to which the paint has been applied (Lambourne, 1987). It may be a vegetable oil or synthetic resin (Banov, 1978). Solvents, however, do more than simply thin the coating, they 'help to wet the surface and contribute to adhesion by

penetrating into the pores and crevices and taking the paint with them' (Banov, 1978). They also have a role in a process known as levelling (making sure a uniformly thick film is created). If the solvent evaporates too quickly from the coating undesirable brush marks will remain and a crust of dried paint may form on the surface, leaving the underlying material vulnerable to damage. There are many different types of solvent, one of the most widely used groups are the hydrocarbons, either aliphatic or aromatic types. Others include esters, acetates, ketones and glycol ethers.

Table 1.1 shows the formulation of one of the paint models supplied for the experiments undertaken in this work. It is a pure water-based acrylic paint produced by the Norwegian company Jotun A/s.

Table 1.1 An example of a pure acrylic paint formulation as used in this work.

(The ingredients are listed in the order in which they are added).

Exact percentages for the formulation and the nature of the HEUR thickener are not available to this report due to confidentiality.

Ingredient	Function	Formula in %
Water	Thinner	14.5
Isothiazolinone / formaldehyde-releaser	In-can preservative	0.2
Hydroxy Ethyl Cellulose (HEC)	Thickener	0.3
Ammonia	pH stabiliser	0.01
Silicone polymer	Defoamer	0.2
Sodium salt of carboxylate polyelectrolyte	Dispersion agent	0.4
Titanium dioxide (TiO_2)	Pigment	27.0
Acrylic polymer	Binder	51.0
Water	Thinner for washing	2.5
Monopropylene glycol	Anti-frosting agent	2.5
Iodo-propynyl-butyl-carbamate (IPBC)	Fungicide	0.6
HEUR	Thickener	0.6
Silicone polymer	Defoamer	0.2
		100.1

Data provided by Jotun A/s

It can be seen from Table 1.1 that water is the first ingredient listed to be added to the paint. This is used to create a pigment base for the paint before the binder is added. The in-can preservative is added early in the paint manufacture process because there is a possibility that some of the raw materials may contain microorganisms. These could include mains water, the binders and any other water-containing ingredients; frequent contaminants are Gram negative rods in these cases. Pigments and other powders may also introduce microorganisms, especially *Bacillus* species and fungal spores. HEC (Hydroxy Ethyl Cellulose) is a thickener usually containing an enzyme inhibitor. The main functions of the thickener are to keep the paint stable as the pigments are added, prevent sedimentation and give the paint the required viscosity. Ammonia water is added to the system causing the thickener to swell creating a more viscous solution. As the thickener and dispersing agents will cause foaming, a defoamer is added. A dispersing agent is added to disperse the titanium dioxide. The dispersing agent imparts the surface of the pigment with a slight electrical charge, which keeps the titanium dioxide particles in the dispersed phase. The grade of titanium dioxide used is also important, the better the quality the longer the coating keeps its colour and the longer it takes before chalking. At this point in the manufacturing process a high-speed impeller blade is used to mix in the acrylic binder. A little water is added for washing and a little mono-propylene glycol for protection against frost and for levelling and extending the 'open time' of the paint. The non-metallic broad spectrum fungicide, (IPBC) is added to the paint. The urethane thickeners designated HEUR are used to thicken the polymer and to maintain the viscosity of the paint in the can. Finally a little more defoamer is added before the paint is filled in cans (Markussen, 1999 personal communication).

The ingredients of a paint in-can must remain stable for a long period of time. They must not interact chemically with each other. Once a paint is applied to a surface, the drying process does not consist solely of evaporation. There are two mechanisms involved in the drying process; one that involves chemical reactions and the one that does not (Turner, 1967). In the case of the process 'drying without a chemical reaction' or 'lacquer drying' the paint dries solely by evaporation. Examples of paints drying by this method include nitrocellulose lacquers and decorative emulsion paints (Turner, 1967). Where drying involves chemical reactions, films containing cross-linked polymers as film formers are produced. The paint usually contains certain linear (or lightly branched) polymers dissolved in the solvent. After the paint has been applied to

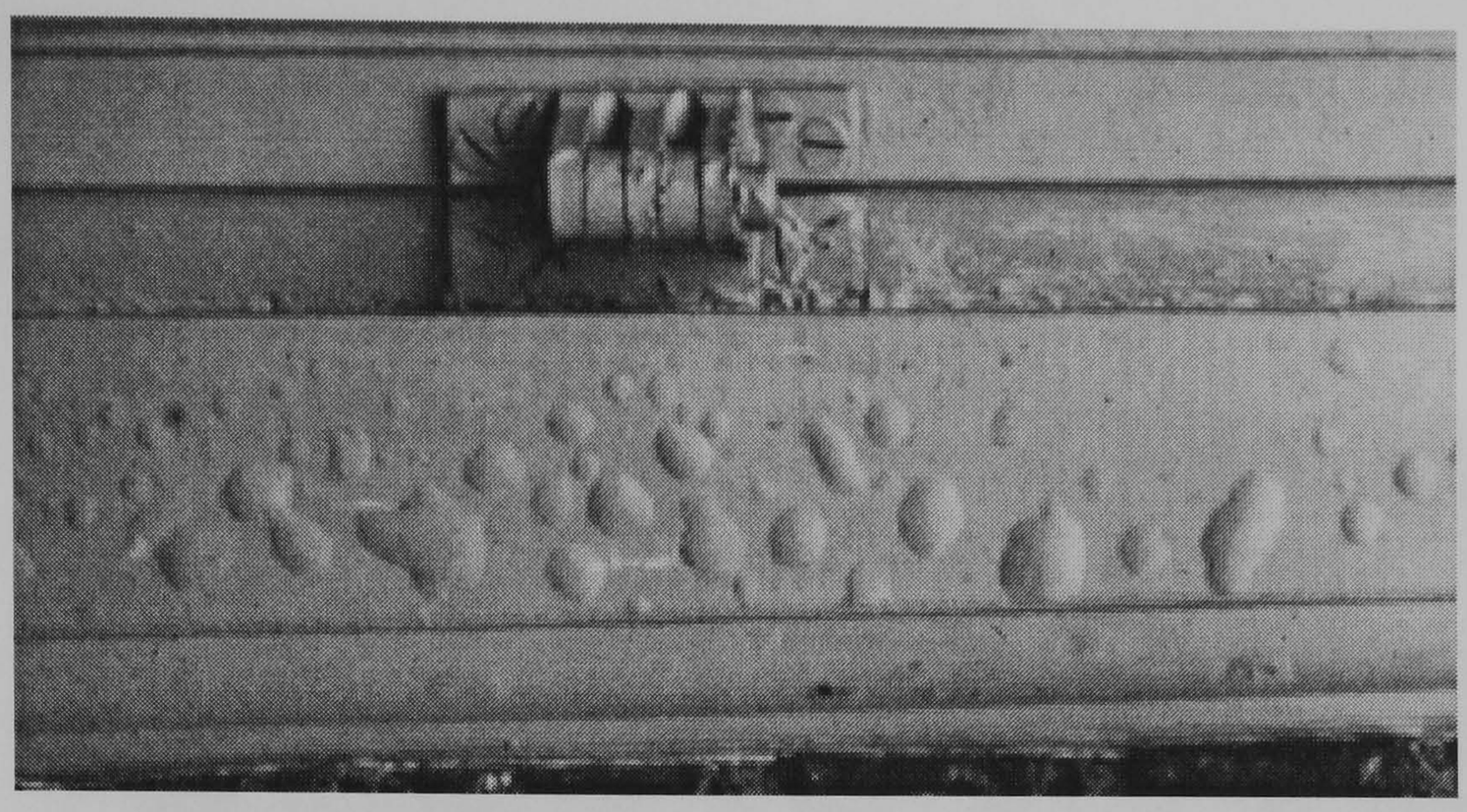
a surface, the cross-linking process takes place via a chemical reaction. Paints that dry by such a chemical reaction usually do so either by using oxygen as the chemical reactant or by reactions that occur between the ingredients in the paint. Whilst in the can, the lid protects the liquid paint from air and prevents subsequent film formation. If air enters the can a skin usually forms on the surface of the paint preventing further air from coming into contact with it. Assuming that the air has been excluded from the can a reaction only occurs when the paint is applied to a surface. As the solvents evaporate the process of cross-linking begins, the low molecular weight linear polymers in the paint are converted into a hard, tough cross-linked film. This process is slow at room temperature because the components of air must penetrate the film before full hardening can take place and the chemical reaction continues after the paint becomes 'touch dry'.

Some paints are known as 'two pack paints' they are purchased in two packs, which when mixed produce an exothermal reaction providing temperatures that are high enough to produce a stoving or enamel finish. Such paints require careful measuring of the components and expertise in their application (Turner, 1967).

1.7 Physical problems encountered with paint films.

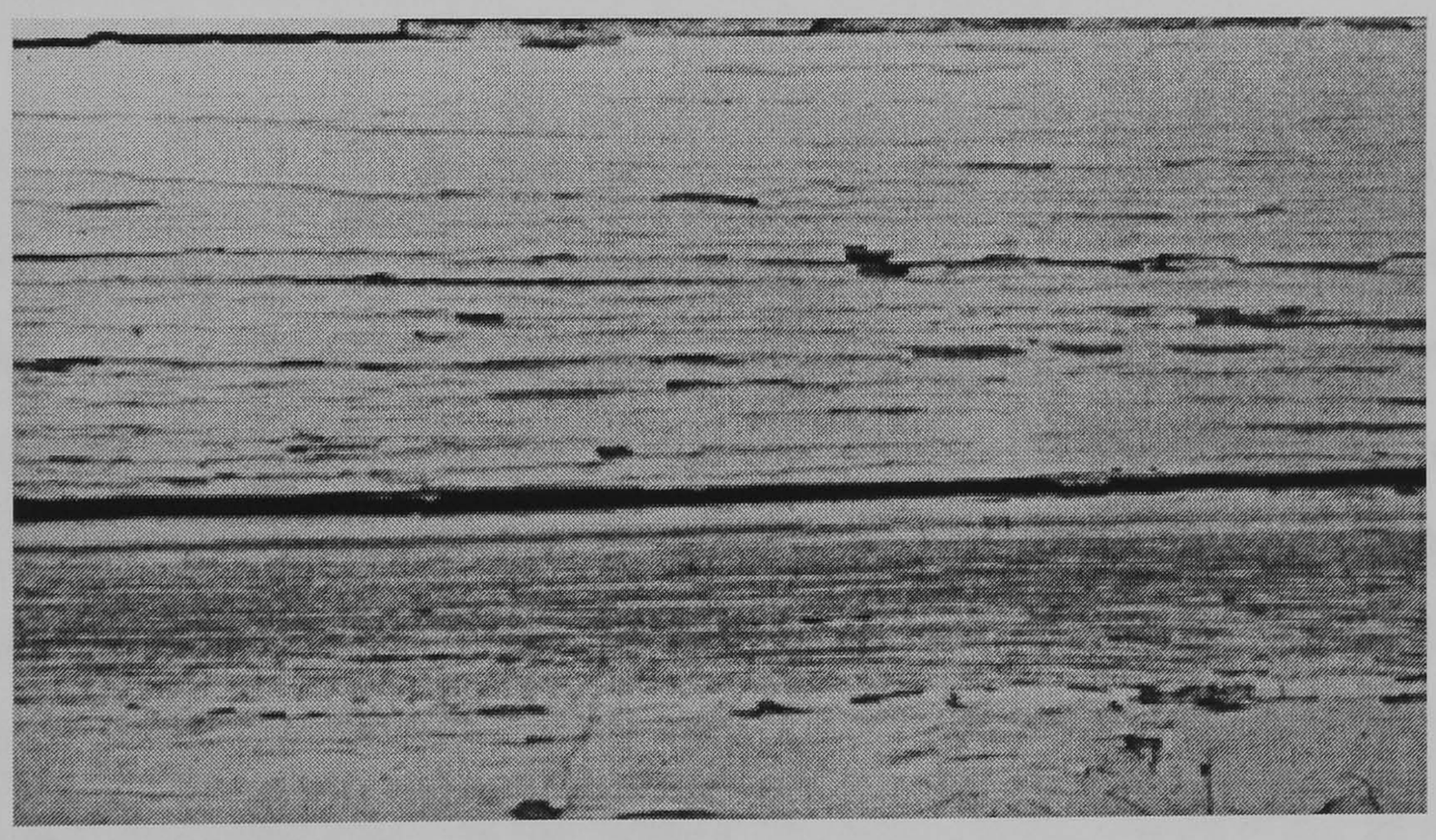
The majority of coatings have been designed for durability (Banov, 1978). This, however, does not necessarily protect them against deterioration or weathering (Schnell, 1996). The main problems identified with the deterioration of films include blistering, flaking, the appearance of mildew and discolouration (Banov, 1978). Exposure to water appears to be a major contributory factor in deterioration, cheaper formulations of paint being more susceptible than better quality ones (Weismantel, 1981). Blistering (Figure 1.2) is caused by water from the atmosphere dissolving in soluble substances in the film, thus forming the liquid section of an osmotic cell. Rainwater for example, as it collects on the surface is drawn into the liquid phase of the paint via a pressure gradient. As with any osmotic situation, a membrane must separate the first solution from the incoming water. A thin elastic paint film serves this purpose, hence the creation of a blister. Blisters will not occur if the strength of adhesion of the coating to the substrate is greater than the pressure exerted by the growing cell containing the water. They can be prevented by ensuring that the primer-topcoat system keeps out water and that the adhesion of primer to topcoat and primer to substrate are strong (Banov, 1978).

Figure 1.2 Blistering occurring on a painted wooden surface (Drummond, 1962).



Flaking (Figure 1.3) is caused by moisture entering at the joints and working under the coating's surface, causing it to peel off in sheets around the joints.

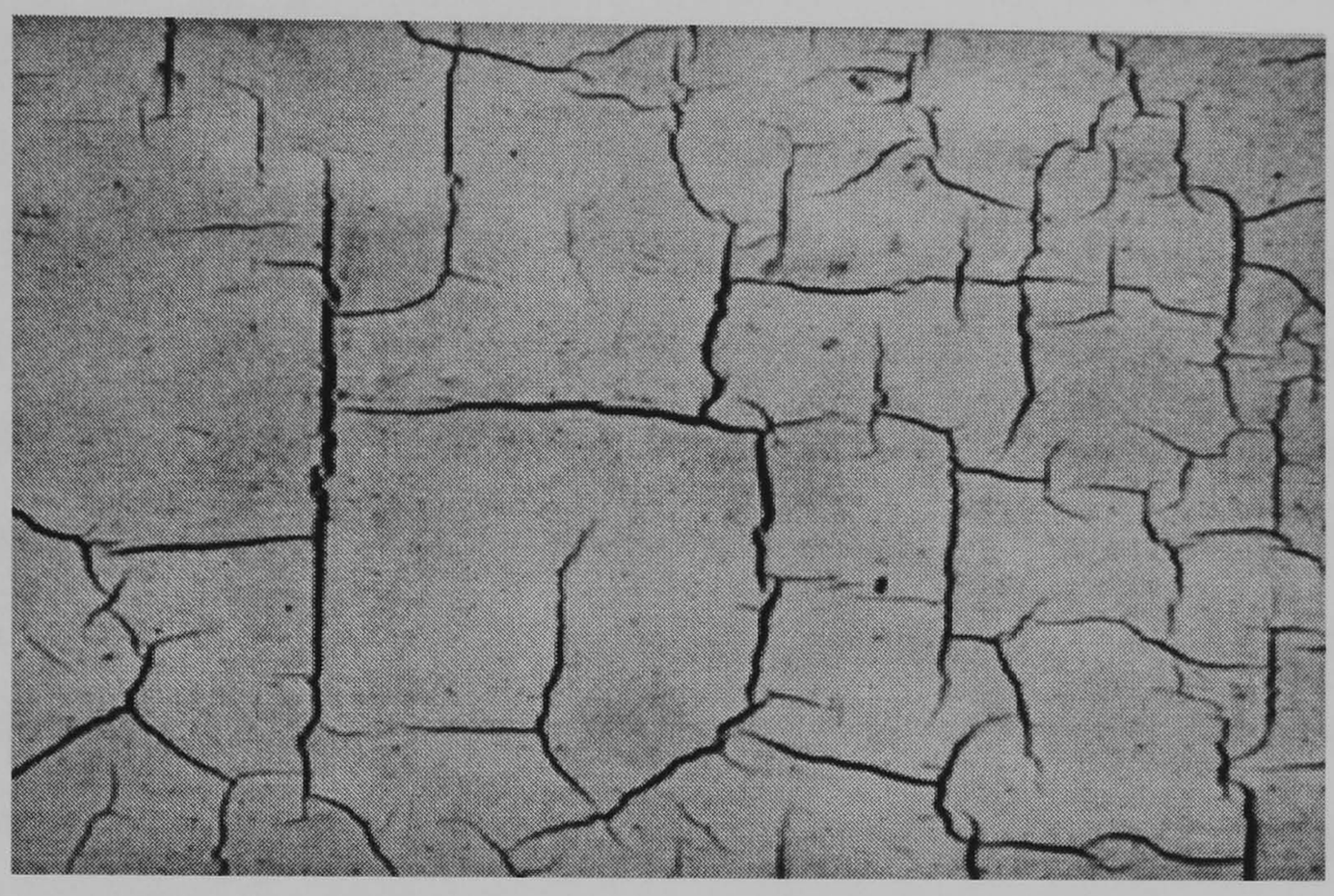
Figure 1.3 Flaking occurring on a painted wooden surface due to moisture entering the timber once the paint has hardened (Drummond, 1962).



Mildew, is usually attributed to dirt, but in fact it is the growth and reproduction of fungi deposited on the surface from the air, resulting in black or brown 'stains' (Banov, 1978). Discolouration may also occur on painted wooden surfaces, especially those of Redwood and Cedar. The soluble dyes contained within the wood form a solution with the water and diffuse to the surface creating pink or brown spots or streaks (Banov, 1978). 'Checking' is the term used when a paint film begins to break up. Initial surface

disruption is termed crazing, which progresses to cracking (Figure 1.4) as the process becomes visible to the naked eye. The term crocodiling refers to a particular pattern of cracking (Drummond, 1962).

Figure 1.4 Cracking occurring on a painted wooden surface.



1.8 Biological problems (biodeterioration) with paint films

Biodeterioration can be defined as 'the deterioration of materials of economic importance by microorganisms' (Huek, 1965). The attachment of bacteria and fungi to the surface of paint films is known as defacement. The economic loss attributed to microbial attack of this kind was estimated in excess of one million US dollars per year in 1981 (Winters, 1981). The initial defacement of painted surfaces, especially wooden ones is associated with the presence of water, twenty per-cent moisture is required in wood before fungal growth occurs (Berry, 1994). The primary class of microorganisms involved in paint film attack is the fungi (Downey, 1995). Microbial growth is common in exterior paints (Hueck-van der Plas, 1968) but fungal growth also occurs in interior situations where there is high humidity (Barry, 1978).

1.8.1. Organisms involved in the biodeterioration process

It was reported in 1986 by Seal and Morton that fungi isolated from paint films included; *Alternaria tenuis, Aspergillus flavus, Cladosporium herbarum* and *Trichoderma reesei.* The International Biodeterioration Research Group (IBRG) have collated information on paint spoilage organisms, some examples of fungi isolated include: *Alternaria alternata*, *Cladosporium* species, *Penicillium* species, *Phoma violacae, Stachybotrys chartarum* and *Aureobasidium pullulans* (Downey, 1995). The fungus *Penicillium canescens* has also been classified as a dominant organism found on paint (Onions *et al.*, 1981). Bravery in 1987 indicated that *Aureobasidium pullulans* was considered as the single most important main fungal coloniser of painted surfaces, as it was found in ninety-five percent of recorded cases. The success of *Aureobasidium pullulans* as a coloniser of painted surfaces has been attributed to the fact that it is able to utilise a wide range of sugars and celluloses, further more it is reported to be able to withstand a wide range of environmental conditions and even produces antibiotic compounds *in situ* (Winters, 1981).

Bacteria and yeasts are considered to be important contributors to film degradation, colonising paint films where there are high moisture levels (Olson *et al.*, 1986). Work in America has indicated that bacteria may not only be important colonisers, but may also be essential precursors for the invasion of external films by microfungi by aiding in adhesion and germination of fungal spores (Schmitt, 1974; Winters *et al.*, 1975). Grant *et al.*, (1993) indicated that there was evidence of bacteria and yeasts being tolerant to some paint biocides and subsequently may appear as early colonisers of certain fungicidal coatings. Exposure trials in the United States indicated *Bacillus cereus, Micrococcus albus* and *Alcaligenes recti* were some of the bacterial colonisers of paint films (Ross *et al.*, 1968).

Algae are seen to colonise external surfaces of buildings and monuments when the conditions are damp, light and warm (Morton, 1987; Morton, 1979; Springle, 1975; Whitely, 1973). Growth is most pronounced in the Spring and Autumn, but rapidly decreases when the surfaces become dry (Richardson, 1973). Once dead, the algae are a major source of dirt, contributing to the nutrients required for the growth of lichens, mosses and higher plants capable of corrosive activity and therefore more extensive

damage (Morton, 1987). Evidence of physical or biochemical erosion by algae is tenuous, although in some cases algae are considered to be corrosive because of their ability to produce organic acids (Degelius, 1962). Wee and Lee (1980) stated that algae only grow outdoors and usually on paint that has been applied to a masonry substratum, therefore the major consequences of algal growth are cracking, flaking and disfigurement. Penetration of paint films by lichen rhizoids may result in disruption and detachment of the paint film from its surface (Bravery, 1987). Examples of algae isolated from paint films include *Oscillatoria* spp, *Chlorella* spp., *Nostoc* spp., *Pleurococcus* spp. and *Trentepohlia* spp. (Seal and Morton, 1986; Downey, 1995).

The term 'biofilm' has until fairly recently been associated with the growth of microorganisms at solid surfaces in contact with water (Hamilton, 1993; Marples, 1993; Morton, 2000; Surman, 1994 and Trulear and Characklis, 1982). However, biofilms can also be found at water-air and solid-air interfaces (Flemming *et al.*, 2000). Biofilms are ubiquitously distributed within natural soil and aquatic environments, plant tissues, animals and man, as well as in filters, reservoirs, pipelines, ships' hulls, heat exchangers, separation membranes and medical devices (Costerton *et al.*, 1987; Costerton *et al.*, 1999; Flemming *et al.*, 2000 and Flemming and Schaule, 1996). It has been suggested by Wimpenny (2000) that there are as many definitions of biofilm as there are scientists working within the field. A reasonable definition should incorporate the idea of a surface on or at which microbes proliferate; the unifying effect of extracellular polymers, which envelop and protect the microbial colonies forming and the sense of community with the implication of emergent properties (Wimpenny, 2000). The simple definition of a biofilm as a collection of microorganisms and their extracellular products bound to a solid surface (Hamilton, 1993), fits surface coatings very easily and the concept of interactions occurring between members of this air/solid interface consortium is not a difficult one to accept.

1.9 Previous field trials.

Many field trials have been carried out over the years in many different countries (Bravery, 1987; Buckman and Stitt 1957; Drescher, 1958; Drisko and Crilly, 1974; Gillatt and Tracey 1987; Grant *et al.*, 1986; Heaton *et al.*, 1991; Holbrow, 1984; Jakubowski *et al.*, 1983; Meier, 1954 ; Schmitt, 1974; O'Neill *et al.*, 1977; O'Neill,

1988; Ross, 1963; Rothwell, 1958; Trueblood *et al.,* 1957; Wee, 1988). The trend has been to use panels made usually from Norway spruce, which have usually been painted twice using a paintbrush, often with a two day drying period between coats (Bardage, 1997). Exterior trials are usually conducted with panels placed on racks. The panels are invariably placed at an angle of forty-five degrees in the racks, which face South because it has been observed that the colonisation sequence occurs more rapidly than those facing North. In Norway, North facing panels tend to attract more algae, whereas with South facing panels the majority of colonisation is by fungi, possibly due to the prevailing climatic conditions, for example, a higher UV-light exposure (Bjurman, 1999; Fjelde, 1999 personal communication; Bravery, 1987). Previous field trials conducted at Sandefjord, in Norway, have indicated that one of the major colonisers of both North and South facing painted surfaces is the fungus *Aureobasidium pullulans* (Fjelde, 1999 personal communication). Trials at the Paint Research Association, Teddington, UK indicated that the occurrence of *Aureobasidium pullulans* was identical on both North and South facing panels although at rural sites the occurrence was at its greatest. A list of the major colonising fungi was compiled, with *Epicoccum nigrum* being the one with the greatest incidence (Kelly, 1999). *Aureobasidium pullulans* was featured fourth on the list contradicting most, if not all, of the other trials that have taken place (Bravery, 1987; Goll and Coffey, 1948; Goll, 1956; Haensler, 1920; Klens *et al.*, 1956; Ross *et a*l., 1968). It was concluded at this trial that both the site of exposure and the substratum used are critical. The results of a comparative study of painted wood, calcium silicate and metal panels showed that most microbial growth occurred on the surface of the wooden panel followed by the calcium silicate panels, with the least growth occurring on the metal panel surface (Kelly, 1999).

1.10 Treatment of colonised surfaces.

No general treatment for colonised paint surfaces has, as yet been established; the consensus of opinion is that it would be better to prevent colonisation rather than to treat any adverse effects resulting from colonisation at a later date. It is possible to wash off some of the organisms using special 'biocidal' washes. Other techniques include abrasive treatment, which often exacerbates the problem, for example when the filamentous green alga *Trentepholia odorata* is removed by scouring (Wee and Lee, 1980).

1.11 Role of Biocides

The term biocide came into popular use in the 1950's. The literal translation of the word comes from the Greek *bios* meaning life and the Latin *cida* meaning to kill. There have been many definitions of the term including Block (1991) who defines biocide as 'a substance that kills all living organisms, pathogenic and non-pathogenic'. He included in his definition bacterial endospores. The definition is not universally accepted especially within medical disciplines.

Antimicrobial agents may be divided into two types, the microbiocides and the microbiostats. Microbiocides kill microorganisms whereas microbiostats simply inhibit the multiplication of microorganisms. Whether the action of a substance is microbiocidal or microbiostatic depends on the concentration at which it is used (Paulus, 1993). Microbiocides are only effective against microorganisms, i.e. bacteria, fungi, yeasts, algae and lichens. It has been suggested that their chemical composition relates to their antimicrobial effect (Paulus, 1993). Experience has shown that the Gram negative organisms with their extra membrane, (especially *Pseudomonads*), are more resistant than the Gram positive organisms (Paulus, 1993).

Traditionally biocides were composed of heavy metal containing compounds such as phenylmercuric acetate (pma) and tributyltin (tbt) compounds. These gave a broad spectrum of activity to both in-can and film preservative action. The problems associated with these compounds, however, were their short-term effectiveness and their high toxicity. These products have been replaced with non-metallic products with a low toxicity and a higher selectivity of target organisms. Disadvantages with the new products include the increased cost and the fact that the metallic based products had a better all round performance (Greenhalgh, 1996).

The following are the desired properties of a paint film biocide (Downey, 1995):

- Broad spectrum of activity: the product should be effective against the full range of microorganisms that may land on the paint.
- No effects on paint appearance, such as yellowing or chalking of the dried film on exposure. Chalking is the formation of a friable, powdery coating on the surface of

the paint. It is caused by the disintegration of the binding medium usually due to weathering; it results in the fading of coloured paints.

- Cost effective at the recommended level of use.
- Low water solubility: if the material is too water soluble, it may wash out of the paint.
- Ease of formulation.
- Low odour.
- Low toxicity.
- Environmentally friendly.
- Preferably non-mercurial and non-stannous.

There are many products sold as paint film biocides on the market. Most offer fungicidal properties others offer algicidal properties. The majority of products, however, contain a single active ingredient, although there are products with more than one active ingredient on the market.

No paint preservatives offer indefinite protection to the paints. They fail due to a number of reasons. These include leaching: the loss of the active ingredient from the paint film on exposure to rain and photo-oxidation, the inactivation of the active ingredient on exposure to ultraviolet light (Downey, 1995).

Appropriate in-can preservatives include formaldehyde releasing compounds, carbonic acid esters, amides and phenylmercury acetate. Some examples of paint film fungicides / algicides include the amide N-(-4-bromo-2-methylphenyl)-2-chloracetamide (BMPCA), the carbamate, 3-iodopropargyl-N-butylcarbamate (IPBC) and the heterocyclic N,S-compound, 2-*n*-octyl-4-isothiazolin-3-one (OIT) (Paulus, 1993). Carbamates, for example, IPBC, the fungicide incorporated into the paint formulations used in this study, work by disrupting microtubule assembly, probably by preventing the recycling of actin and tubulin, thus, causing the arrest of nuclear division in fungal cells (Greenhalgh, 2002 personal communication).

1.12 The exposure sites and paints to be used in the field trials for this work.

It was decided by the sponsors that four exposure sites would be used. There were to be two in Norway (Sandefjord and Bergen) and two in the UK (Preston and Blackley). The Norwegian sites belong to Jotun, Blackley is Avecia's head quarters and the University is based in Preston. The six paints were also chosen by the sponsors. These were a pure acrylic white paint containing fungicide (Paint A); a pure white acrylic paint containing no fungicide (Paint B); a hybrid acrylic white paint containing fungicide (Paint C); a hybrid white acrylic paint containing no fungicide (Paint D); a high solid alkyd (Paint E) and a solid alkyd (Paint F) white paint both containing no fungicide.

1.13 Aims of the study

The original aims of this study were:

1. To investigate the nature and the sequence of microbial colonisation onto the surfaces of painted panels exposed at selected sites in Norway and in the United Kingdom.
2. To investigate the mechanisms of colonisation employed by microorganisms colonising surfaces at the solid air interface.
3. To establish the effect of the colonisation process upon the physical nature and the chemical composition of the paint films.
4. To investigate the weather and environmental effects of the regions upon the physical nature and the chemical composition of the paint films.
5. To investigate the effectiveness of the formulation biocide upon the colonisation of the surfaces exposed to microorganisms.

The outlines of the chapters presented below indicate an account of the experimental procedures carried out during this investigation.

Chapter 2:- Presents an account of the exposure trials both in the UK and in Norway.
It was necessary to devise an exposure trial sequence which would provide information on the microbiological colonisation of the paint films using appropriate sampling methods whilst taking into account data recorded on the weather conditions prevailing at the time.

Chapter 3:- Presents the work undertaken using MALDI TOF MS.
Matrix Assisted Laser Desorption /Ionisation Time-of-Flight Mass Spectrometry (MALDI TOF MS) was employed as a means of confirming common genera and species of the colonising microorganisms.

Chapter 4:- Presents an account of Surface Characterisation.
SEM and AFM were used in an attempt to visualise microorganisms *in situ* on exposed panels and on panels inoculated within a vermiculite bed system in the laboratory. Surface Roughness Measurement (SRM) and AFM were used to investigate the physical/topographical changes in the paint surfaces as a result of exposure and as a result of microbial colonisation, whilst FTIR spectrometry was employed to investigate chemical changes in the composition of the paint films.

Chapter 5:- Presents the General Discussion.

CHAPTER 2.

EXPOSURE TRIALS

	Contents	Pages
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2.1 Introduction.

The four exposure sites were chosen by the sponsors, these were Bergen and Sandefjord in Norway and Preston and Manchester within the UK, shown in Figure 2.1.

Figure 2.1 A section of a map of Europe showing the locations of the exposure sites.

(Adapted from AND Map graphics 1999)

Sandefjord is in the South East of Norway. It used to be one of the important whaling communities within Norway, with the majority of the population working within the whaling trade. Today, it is small community, with a population of 37,600 that is known for its shipping, technology, farming and commerce (Sandefjord Tourist Information, 2000). The actual exposure site is at the Jotun A/s paint factory at Vindal, which is approximately 2 Km in a southerly direction from Sandefjord centre, latitude 5942N and longitude 1012E (Willett, 1987). The site is situated in very close proximity to the sea and is surrounded by two paint factories, a factory manufacturing omega-3-fatty acids and a factory manufacturing polyester / fibreglass pipelines, none of which should affect the air micro flora (Schmidt, 2002 personal communication). It had an average rainfall of 939.2 mm in 1999 (based on data supplied by Jotun A/s).

Bergen, Norway's second largest city (Isaacs, 1987), is situated on the South West Coast of Norway, latitude 6023N and longitude 0520E. It was one of the most important ports in Norway with large fishing and whaling industries. Today it is still an important port, however, the whaling industry has declined. This densely populated city (225,000; Bergen Tourist Information , 2001) has a large tourist industry despite an average rainfall of 2300mm, based on 1998 precipitation data (Anon, 1999). The exposure site at Jotun is on the roof of their laboratories, which are situated about two kilometres South of the city centre. The usual orientation for panels exposed at this site, is South-facing at an angle, from the horizontal, of 45^{o} or 90^{o}. In the present trials, however, it was decided to expose the panels facing North at 45^{o} since the other three exposure sites had panels exposed this way.

In Preston, Lancashire, the exposure site was in a garden about one mile from the University, at Walton-le-Dale. The area has no large industrial factories creating pollution in the vicinity, although there is a brewery and a composting plant less than 10 Km away, which may affect the diversity of the air micro flora. Preston, based on the borough council figures of 2001, has a population of 135,700, it lies about 32 Km from the West Coast of England, at latitude 5346N and longitude 0242W (Willett, 1987). It had an average rainfall in 1999 of 1076.7mm based on readings taken at Moor Park Observatory, Preston.

Blackley, a district of Greater Manchester, latitude 5330N and longitude 0215W (Willett, 1987) is the home of Avecia Ltd. It is a densely populated area on the outskirts of Manchester, which has a population over 2.5 million and is in close proximity to the M62. The average rainfall in 1998 was 850mm. The exposure site at Avecia Ltd. is situated behind the research laboratories close to the boundaries of the site near to the road and a hedgerow. As with the other exposure trials the panels were exposed on racks that were subsequently fixed onto the existing racks at Avecia.

The nature of the panels to be exposed at each site was decided upon by the sponsors. They were spruce with aluminium and calcium silicate panels as controls. The orientation of the panels was forty-five degrees from the horizontal facing North due to the orientation of the existing racks at Sandefjord and Blackley.

Spruce was chosen because it is the primary source of timber for Norwegian architecture. Aluminium was chosen to act as a control because aluminium is chemically inert, therefore it does not promote microbiological growth (ASTM, 1996). The calcium silicate (Masterclad™) panel is a flat external Class O building board, which is a fully weather resistant calcium silicate board reinforced with selective fibres and fillers. It is formulated without asbestos or any other inorganic fibre. The board itself is pale grey in colour and has a smooth surface on either side. It can be used in external wall constructions and can easily be coated with paint or any other external applied finishes. Masterclad™ was chosen over Masterboard™, which is also a calcium silicate Class O building board, because the latter is only suitable for internal and semi-exposed applications (Cape Boards, 1997).

To determine whether organisms isolated from the panels originated from the environment rather than from the spruce panel itself, a number of panels were gamma irradiated at 25 kGy. Previous use of gamma irradiation has determined that a dose of 15 kGy is sufficient to inactivate a large number of wood biodeteriogens, including fungi and bacteria and that a dose of 25 kGy is recommended to inactivate human pathogens (Pointing, 1998).

To eliminate the possibility of microorganisms originating from the paint itself, samples of each of the paints, were placed onto malt agar, nutrient agar and R2A agar (Appendix

B) and incubated at both room temperature and 30°C. The results of this were deemed satisfactory for the exposure trials to commence.

The aims throughout this section of the work were as follows:

1. To assess the effect of the substratum on the nature of colonising microbes.
2. To assess whether there is a seasonal effect on the nature of colonisers isolated.
3. To assess whether employing a series of sacrificial panels provides evidence of colonisation pattern or biocide efficacy.
4. To investigate the effect of the location of the panels on the spectrum of organisms isolated.
5. To investigate whether the nature of the surface of the paint, (gloss versus matt finish) affected the spectrum of organisms isolated.

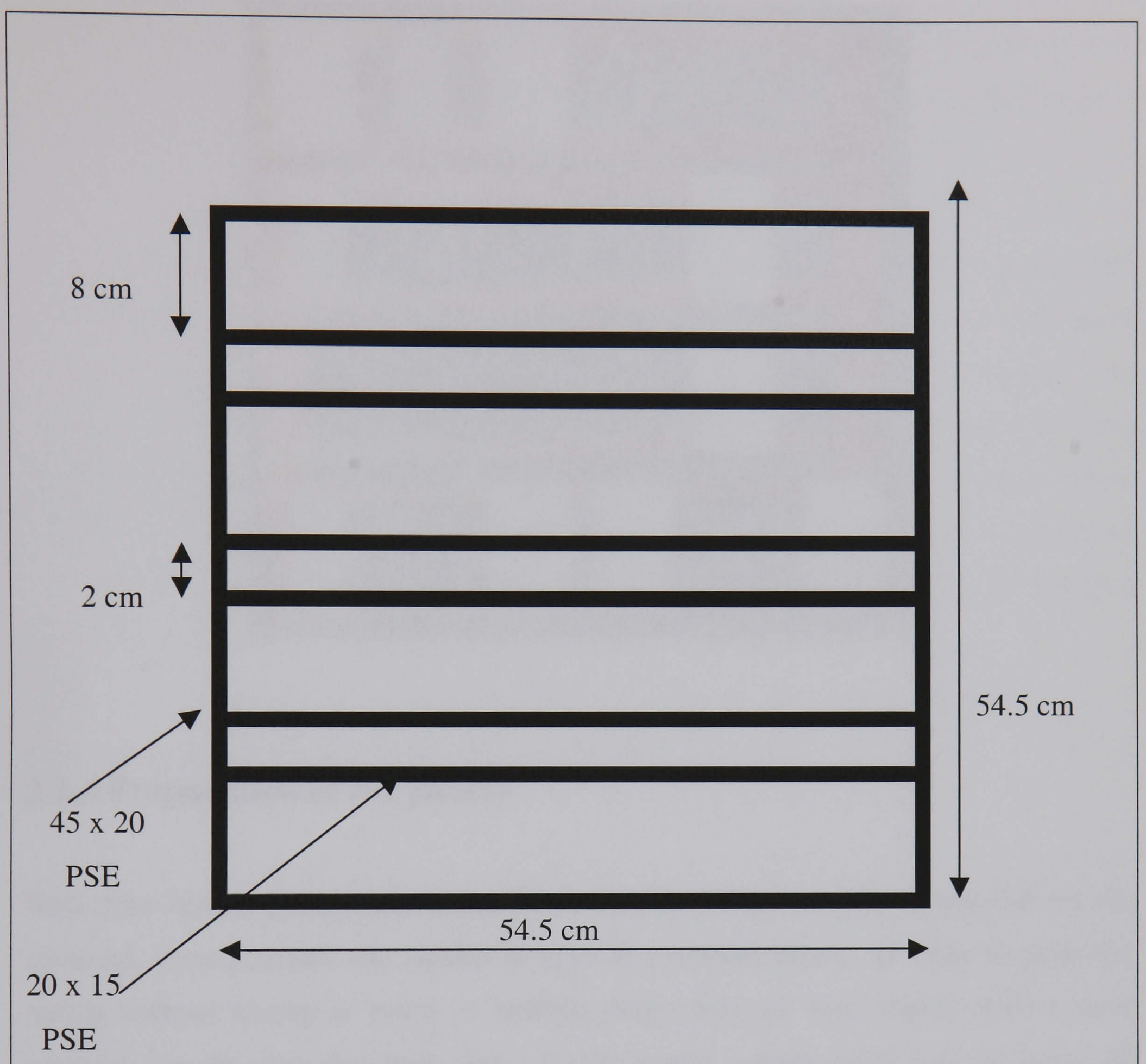
2.2 Preparation for exposure trials

2.2.1 Construction of free standing panel racks

The panels used during the course of this investigation were not the standard sized panels normally used by the paint industry for this type of work i.e. 300mm, 400mm or 500mm in length. Non-standard sized panels were used (Section 2.2.2) and in order to expose them at existing sites in Norway and Manchester and in Preston ten panel racks were constructed, which could be used at each venue. They were designed so that they fitted onto the existing racks at Bergen, Sandefjord and Manchester.

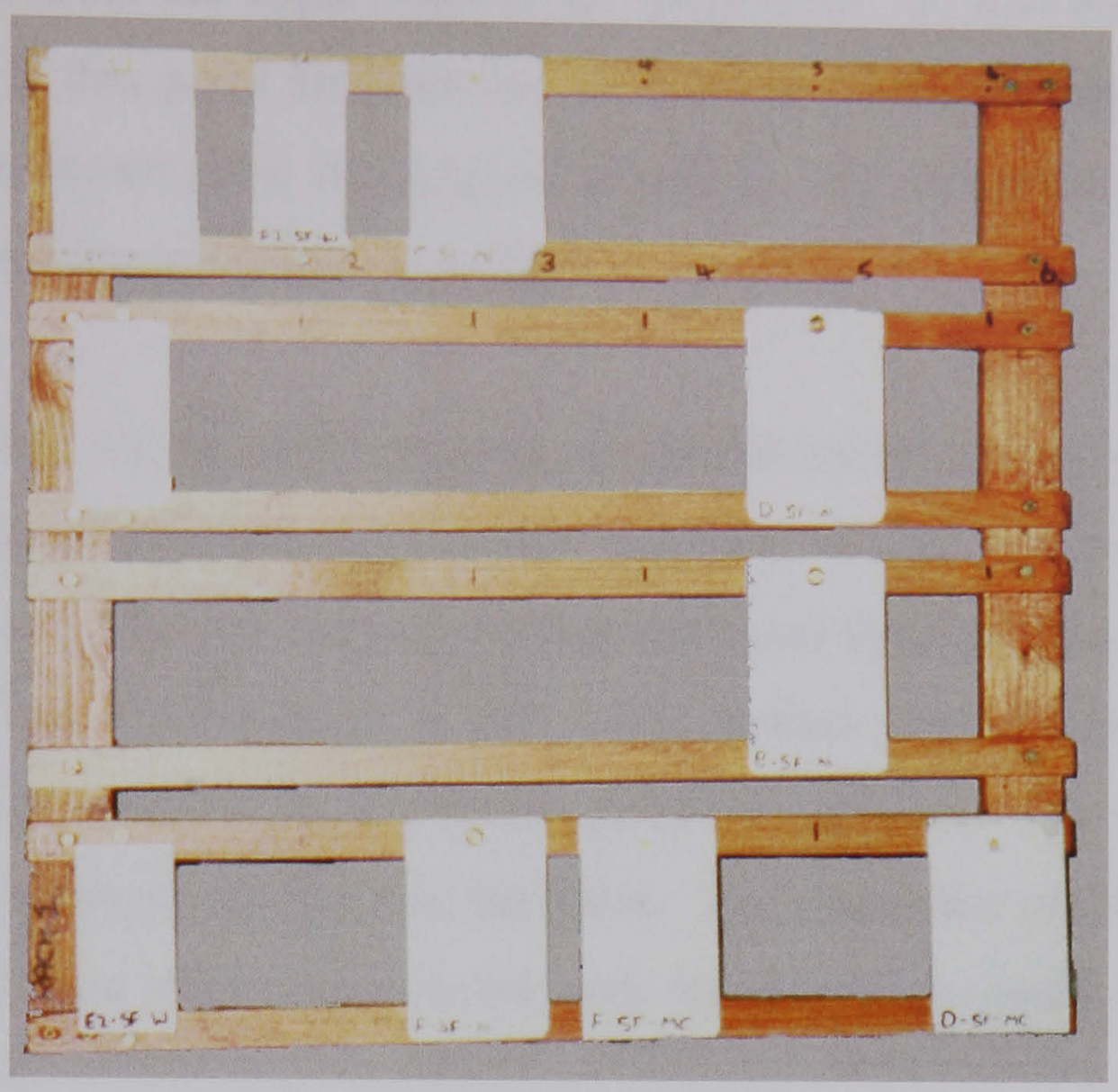
Panel racks were constructed using two different sizes of plane square edge (PSE) timber, 45mm x 20mm and 20mm x 15mm (Figure 2.2 and Plate 2.1). The racks were then coated twice with Cuprinol™ wood preserver. The racks were designed to hold twenty-four panels in total, the centre of each panel positioned so that six panels could be situated equidistantly on each of the rows of the rack (Plate 2.1). No diffusion of the Cuprinol™ into the test panels was possible since all unexposed panel surfaces in contact with it are sealed with aluminium paint.

Figure 2.2 The structure of the panel racks.



PSE (plane square edge) represents the type of timber and the figures represent the dimensions of the timber

Plate 2.1 A panel rack supporting various panels



2.2.2 Preparation of the panels

Knot free Spruce panels measuring 5 cm by 10 cm by 1.8 cm, designated by the sponsors, were prepared and sanded to provide a smooth finish. In order to paint the panels without having to resort to holding them brass (or zinc plated) eyelets were screwed into the two end-grain edges of the panels, which could then be held and painted as follows:

Paints A to D were applied separately by brush to one of the exposed surfaces of the panels. Two coats were applied allowing twelve hours between coats. Two further paints E and F, had an undercoat applied before they were painted, again with two coats. In all panels the exposed surface and lateral side edges were painted. The unexposed panel surfaces and the top and bottom end grained edges were sealed by applying two coats of aluminium paint. The panels were labelled on the underside with black enamel paint and on the upper edge with a black marker pen.

Aluminium panels were cut to size (6 cm by 12 cm) and the edges trimmed to provide a smooth surface. The aluminium was cleaned with white spirit to remove any dust

particles and grease prior to painting. These panels were very thin and had a hole in the centre about 1cm from the upper edge in the shape of a 'Q' (the makers mark). The panel was held at this point between the thumb and forefinger whilst painting took place, a little aluminium paint was applied around the 'Q' shaped hole to ensure there was no bare aluminium substratum showing.

The calcium silicate, Masterclad™ was cut to size (10 cm by 12 cm) using a jigsaw and then sanded off to give smooth edges before painting. A hole was drilled in the centre of the panel about 1cm from the top to allow the panel to be attached to the rack. As calcium silicate has a naturally high pH it was wetted with distilled water and left outdoors overnight to lower the pH before paint was applied. The desired paint was applied on the uppermost surface and the sides. The remainder of the panel including the hole that had been drilled was coated with the aluminium paint to avoid moisture entering the panel.

2.2.3 Attachment of the panels to the panel racks

The spruce panels were attached to the panel racks using brass eyelets. Two marker holes were made in the frame at designated points on the rack using an awl. Number six, three-quarter inch brass screws were passed through the eyelets at either end of the panel and placed in the holes in the rack before being screwed in.

For the aluminium panels a single marker hole was made in the panel rack. A brass washer was placed over the 'Q' shaped hole and a number six, three-quarter inch brass screw inserted through this in line with the marker hole. The panels were then screwed tightly onto the rack.

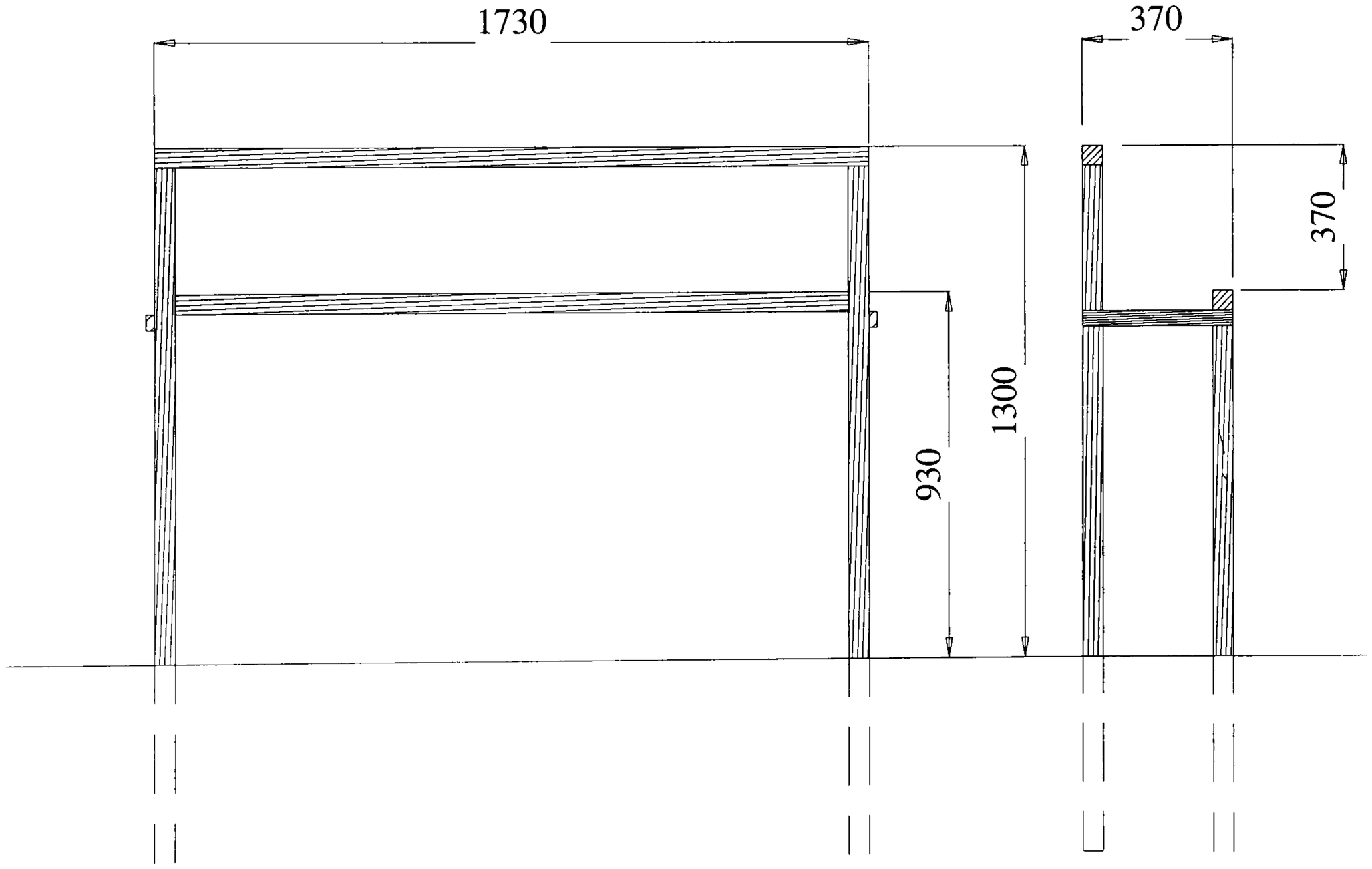
For the calcium silicate panels, a hole was drilled through each panel, a marker hole was made in the frame of the panel rack, a size six, three-quarter inch brass screw placed through the drilled hole into the marker hole and the panels were screwed onto the rack.

2.2.4 Standing rack-holders

The racks at Bergen, Sandefjord and Blackley are standing racks designed to slip panels between the runs, these are set at a forty-five degree angle facing North. To expose the panels at these sites the panel rack constructed as in Section 2.2.1 containing the required panels was attached to the wooden frame of the standing rack using screws and tie wraps.

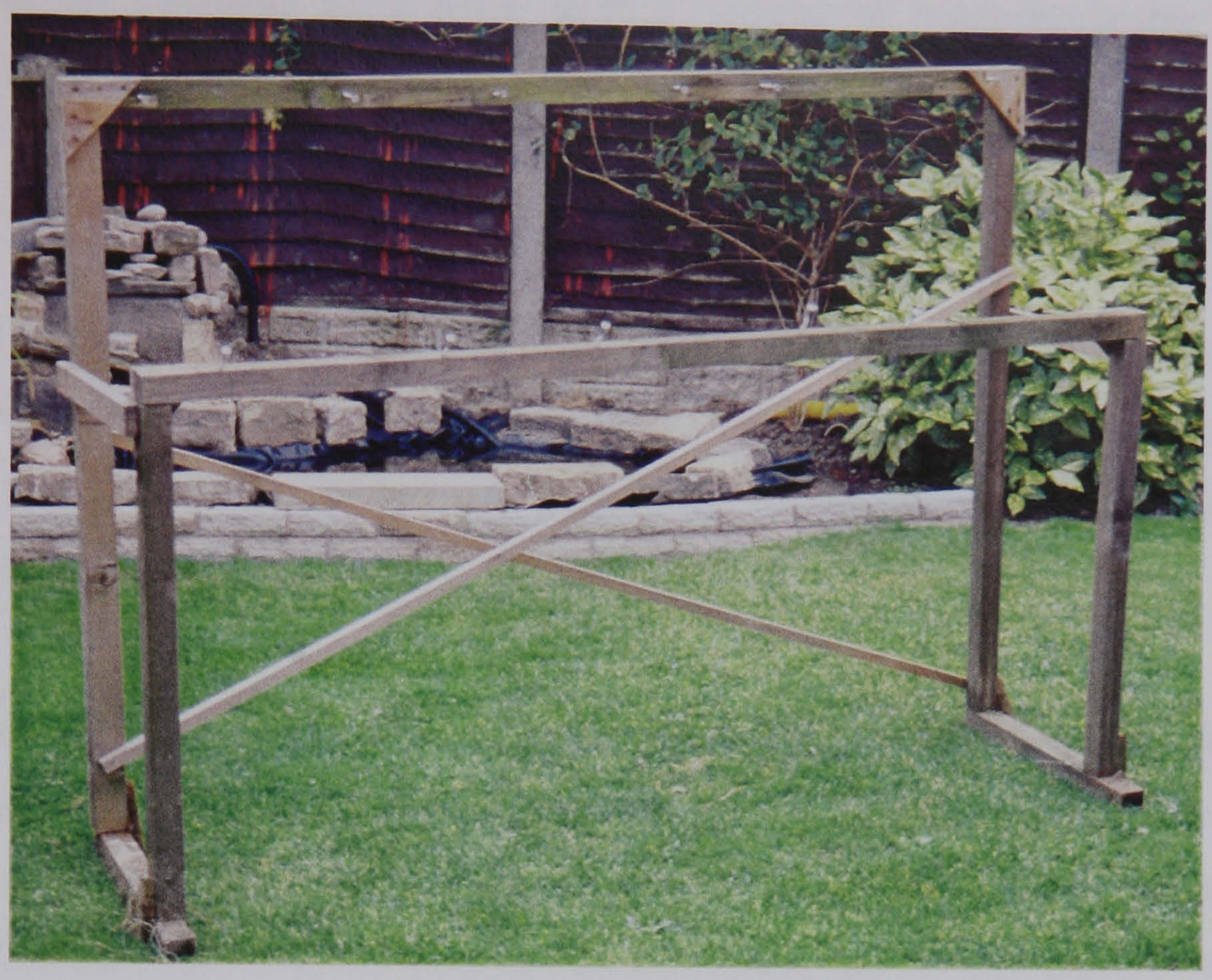
At the Walton-le-Dale site in Preston, a standing rack holder (Figure 2.3 and Plate 2.2) had to be built to hold the panel racks at a forty-five degree angle, facing North. As the standing rack holders in Sandefjord, Norway and Blackley UK, stand at a metre above the ground surface the rack to be built also had to stand at a metre from the ground.

Figure 2.3 The rack-holder constructed at Walton-le- Dale, Preston.



The racks were fixed onto the frame using two nails per panel rack as supports along the bottom edge of the frame and tie wraps to hold the racks in place.

Plate 2.2 The standing rack holder.



2.2.5 Weather data recordings

Throughout all the exposure trials the rainfall, temperature and percentage relative humidity were recorded at each site. The rainfall for the Preston exposure was recorded at the Jeremiah Horrocks Observatory Meteorological Station, Moor Park, Preston by Dr. K. Robinson, the observatory superintendent, on a daily basis. The temperature was recorded using a standard minimum/maximum aluminium thermometer in Walton-le-Dale and the humidity was recorded using a basic greenhouse/outdoor humidity dial at Walton-le-Dale. The Meteorological Office recorded the data for the exposure at Blackley and the data from Sandefjord and Bergen, was sent to the UK by the sponsors, having been recorded at the sites by computers.

2.2.6 Microbial taxonomy

Throughout the exposure trials there were a number of microorganisms to identify including bacteria, fungi algae and yeasts.

Bacteria / Yeasts

In order to identify the bacteria to genus level the standard diagnostic tests were used as follows: A Gram reaction, Hanging drop preparation, Oxidase test and the Catalase test. The yeasts were identified using their morphology before the genus or species could be determined.

Once the genus had been identified using Cowan & Steel's manual for the identification of medical bacteria (Cowan, 1993) the Analytical Profile Index (API) kits were used in conjunction with the APILAB computer software, except when using the API Coryne kit where the six figure number obtained through using the kit was faxed to bioMerieux for their identification.

The API kits consist generally of a strip containing twenty miniature biochemical tests, which when the unknown bacterium is used with one of these kits, produces a six figure number which can be used to identify the microorganism. The following API kits were used throughout the exposure work:

The API STAPH was used to identify *Staphylococci* and *Micrococci,*
The API 20E was used to identify Gram negative species of *Enterobacteriaceae*
The API 20NE was used to identify Gram negative non-Enterobacteriaceae
The API Coryne was used to identify Gram Positive *Corynebacteria* and coryne-like microorganisms,
The API 20C AUX was used to identify yeasts.

On one occasion a different identification system was used, known as BIOLOG. This technique was deemed very subjective and not very reliable in this work so the API technique was used throughout.

Fungi

Fungal isolates were identified with the aid of Professor Glyn Morton, through their morphology. Smith's Introduction to Industrial Mycology (Onions *et al.*, 1981), Introduction to food-borne fungi (Samson *et al.* 1984), A Manual of Soil Fungi (Gilman, 1966) and Modern Mycology (Deacon, 1997) were used in the identifications.

Algae

Algal colonies were identified with the aid of Professor Glyn Morton and 'A beginners guide to fresh water algae' (Belcher, 1977).

2.3 Exposure trials at Sandefjord, Norway.

The exposure trials at this site were conducted in:

September / October 1999.

July 2000

September / October 2000

June - August 2001

2.3.1 Materials and Methods

The September / October 1999 exposure

Panels were prepared as described in Section 2.2.1. The number and nature of the panels that were exposed during this trial are shown in Table 2.1.

Table 2.1 The six paint coatings, A to F and the substance of the panels that were exposed at Sandefjord between September and October 1999.

Paint	Spruce 1	Spruce 2	S1	S2	S3	S4	S5	Aluminium	Calcium silicate
A	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*
E	*	*	*	*	*	*	*	*	*
F	*	*	*	*	*	*	*	*	*

S1 to S5 = The sacrificial panels exposed as described in Table 2.2

* = Exposed panels

The experiment was designed so that some panels were exposed on the racks from the beginning of the experiment, whilst others were exposed during the time span of the experiment (Table 2.2). In order to achieve this the twelve spruce panels designated spruce 1 and 2, the aluminium and calcium silicate panels (Table 2.1) were exposed on the racks on Monday 13th September 1999 for a month's duration. The sacrificial panels

were exposed on the racks on the dates shown in Table 2.2. The experimental protocol was such that the panels that were to be exposed for twelve hours, for example, were placed onto the rack twelve hours before all the panels were removed from the rack.

Table 2.2 The exposure times and dates for the panels exposed between September and October 1999 at Sandefjord.

Sacrificial Panels	Date Exposed	Exposure Time
S1	27th September 1999	2 weeks
S2	4th October 1999	1 week
S3	10th October 1999	24 hours
S4	11th October 1999	12 hours
S5	12th October 1999 8am GMT	7 hours

2.3.2 Treatment of the panels.

Panels that had been exposed for their allotted time were taken into the laboratory at Sandefjord where they were swabbed using sterile 0.09% saline. The tip of each swab was dipped in the saline contained in a sterile universal bottle for fifteen seconds before being applied to the panels. The swabbing procedure took place in a lamina flow cabinet and followed a set routine consisting of vertical and diagonal strokes whilst rotating the swab. The resulting swab was returned to the sterile saline solution for transportation back to the UK for further analysis. The aluminium, calcium silicate and spruce 1 panels were returned to the racks for the remainder of the exposure period, whilst the sacrificial panels were placed separately into clean plastic food bags for transportation back to the UK.

On their return to the UK, the universals containing the swabs were vortexed for fifteen seconds. 0.5 ml of the saline solution was then surface-spread onto the following media: malt extract agar, nutrient agar, Difco algal agar and R2A media (Appendix A and B). The plates were left to dry for thirty minutes before they were inverted and incubated at

30°C for four to seven days. The algal plates were left for a further month under fluorescent light at room temperature. After incubation the number of isolates obtained on each medium was counted using a colony counter and distinct isolates were sub-cultured for identification.

As the aim of the experiment was to isolate the types of microorganisms which have been found to colonise paint films by other workers and in order to minimise the potentially extensive isolation program it was decided to a) use an incubation temperature of 30°C and b) to use standard media for the isolation of fungi and heterotrophic bacteria. A temperature of 30°C is suitable for the isolation of environmental mesophilic bacteria and microthermophilous bacteria. Psychrophilic bacteria are considered unlikely to attack paint films, as are thermophilic forms in European climates, this is also true of fungi and yeasts as recorded in the literature (Morton, 2002 personal communication ; Schmidt 2002 personal communication).

2.3.3 Air sampling in Sandefjord, Norway.

Air sampling at regular intervals during the exposure period would have been the most appropriate action to take, however, this was not possible since attendance at the sites was limited. It was decided, therefore, to sample at the end of the exposure period so the resultant flora isolated are limited to these occasions but nevertheless provide representative flora for the region.

Two petri-dishes each containing malt agar, R2A media, Difco algal media and nutrient agar were exposed and placed at ground level at the foot of the panels racks for a period of thirty minutes at the end of the exposure period. After the allotted time the plates were sealed using Parafilm and packed for transportation back to the UK.

Throughout the journey back to the UK, (48 hours in total) the plates were incubating at ambient temperature. In the UK they were left to incubate at 30°C for two days before being placed in the fridge to prevent further growth. The colonies obtained were subsequently sub-cultured for identification.

The July 2000 exposure

There were already a number of panels remaining on the racks from the initial exposure, between September and October 1999, those labelled spruce 1 and the aluminium and calcium silicate panels. For this exposure, sacrificial panels were sent to Norway for the staff at Jotun to expose. The experimental procedure was as in the previous exposure trial with the panels being exposed for one month commencing on 3rd July 2000, the exposure dates are shown in Table 2.3.

Table 2.3 The exposure times and dates for the panels exposed throughout July 2000 at Sandefjord.

Sacrificial Panels	Exposure Date	Exposure Time
S1	10th July 2000	3 weeks
S2	17th July 2000	2 weeks
S3	24th July 2000	1 week
S4	28th July 2000	3 days
S5	31st July 2000	1 day

The panels that had been exposed for their allotted duration were, in this section of the exposure experiment, swabbed at the site in the manner described in Section 2.3.2. The swab-transport medium in this case it was sterile Calgon Ringers, rather than 0.09% sterile saline solution. On their return to the UK the swabs were treated and processed as described in Section 2.3.2. The air sampling at this exposure site was conducted in an identical manner to that used in the previous exposure trial described in Section 2.3.3.

The September / October 2000 exposure

The exposure trial, which took place between September and October 2000, commenced on the 21st September 2000, again as in previous trials, for a month, with

the Jotun staff exposing the panels. The spruce 1 panels, aluminium and calcium silicate panels remained on the racks from the previous exposure. The sacrificial panels in this exposure experiment, however, were only for paints A and B, which were exposed on the dates shown in Table 2.4.

Table 2.4 The exposure dates and times for the panels exposed between September and October 2000 in Sandefjord.

Sacrificial Panels	Date Exposed	Exposure Time
S1	21ST September 2000	1 month
S2	5th October 2000	2 weeks
S3	12th October 2000	1 week
S4	16th October 2000	3 days
S5	18th October 2000	1 day
S6	19th October 2000	6 hours

This exposure trial was unlike the previous ones, in that the sacrificial panels were returned to the UK by post. This meant that no air settle plates were obtained and the panels that remained exposed were not sampled. On delivery to the laboratory the procedure used to swab and process the panels was as used in Section 2.2.2, again using Calgon Ringers as the transport medium for the swab.

The June / August 2001 exposure

The final exposure took place between June and August 2001. In this exposure the panels were exposed for a total of eight weeks commencing on 11th June 2001, the dates of exposure can be found in Table 2.5.

Table 2.5 The exposure dates and times for the panels exposed between June and August 2001 at Sandefjord.

Sacrificial Panel	Exposure Date	Exposure Time
S1	11th June 2001	8 weeks
S2	25th June 2001	6 weeks
S3	8th July 2001	4 weeks
S4	16th July 2001	3 weeks
S5	23rd July 2001	2 weeks
S6	30th July 2001	1 week
S7	4th August 2001	3 days
S8	6th August 2001	1 day

As in the previous exposure work, the panels were returned to the UK by post, this time; however, all the panels on the rack were returned, as this was the final exposure at this site. Once in the UK the panels were swabbed and analysed as in previous exposure trials, described in Section 2.3.2, using sterile Calgon Ringers. As in the exposure that took place between September and October 2000 no air settle plates were used.

2.3.4 Results

The September / October 1999 exposure

Air micro flora

Microorganisms isolated from the air settle plates exposed at the end of the first exposure at Sandefjord between September / October 1999 can be found in Table 2.6.

Table 2.6 The microorganisms isolated from the air settle plates exposed at Sandefjord in October 1999.

FUNGI	BACTERIA	YEAST	ALGAE
Alternaria alternata	*Bacillus sp.*	*Cryptococcus laurentii*	*Chlorella* sp.
Alternaria tenuis	*Pseudomonas fluorescens*		*Chlorococcus* sp.
Aureobasidium pullulans	*Sarcina sp.*		*Stichococcus* sp.
Cladosporium cladosporioides	*Staphylococcus lentus*		
Fusarium oxysporum			
Mycelium sterilium – pink			
Penicillium aureogriseum			
Penicillium chrysogenum			

Exposed panels

The results of this work are shown in Tables 2.7, 2.8 and 2.9. Tables 2.7 and 2.8 list the microorganisms isolated from sacrificial panels that were coated with the various paints. Table 2.9 lists the isolates obtained from the three different panels (i.e. spruce, aluminium and calcium silicate), which were exposed on the 13th September 1999.

The isolation of microorganisms from the sacrificial panels does not seem to follow any particular trend. There are fungi, bacteria and yeasts present on all of the paints. Bacteria were isolated less frequently during the sampling programme than yeasts or fungi and they seem to be distributed randomly between the sacrificial panels rather than colonising a particular paint.

Table 2.7 Colonisation of the sacrificial panels painted with paints A to C from the September / October 1999 exposure at Sandefjord.

	Paint A					Paint B					Paint C				
Organisms	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5
FUNGI															
Aureobasidium pullulans								■		■					
Fusarium oxysporum										■	■				
Mycelium sterilium – white					■										
Penicillium aureogriseum				■											■
BACTERIA															
Bacillus sp.				■					■				■		
Sarcina sp.				■											
YEASTS															
Cryptococcus laurentii	■	■	■		■	■	■	■	■	■	■				
Rhodotorula rubra				■	■	■								■	

S1 to S5 represent the order of the sacrificial panels as described in Table 2.2. The shading represents the presence of a particular microorganism on the panel

Table 2.8 Colonisation of the sacrificial panels painted with paints D to F from the September / October 1999 exposure at Sandefjord.

Organism	Paint D					Paint E					Paint F				
	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5
FUNGI															
Aureobasidium pullulans		■	■				■	■	■	■		■	■	■	■
Cephalosporium sp.							■								
Cladosporium cladosporioides												■	■		
Penicillium chrysogenum						■									
BACTERIA															
Bacillus sp.									■						
Sarcina sp.							■	■							■
YEASTS															
Cryptococcus humicolus											■	■			
Cryptococcus laurentii	■		■					■	■		■		■	■	
Rhodotorula rubra	■										■	■			■

S1 to S5 represent the order of the sacrificial panels as described in Table 2.2. The shading represents the presence of a particular microorganism on the panel.

From the results presented in Table 2.9, it can be seen that the spruce panels produced the greatest variety of isolates. There does not appear to be a great deal of growth recorded on the aluminium panels, except for *Aureobasidium pullulans* which was found on paints C to E, together with *Rhodotorula rubra* and *Cryptococcus laurentii*. More isolates were obtained from the calcium silicate panels (10) than from the aluminium panels (8), but not as many as found on the spruce panels (21).

Table 2.9 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed between September and October 1999 at Sandefjord.

Organisms	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Alternaria alternata																		
Alternaria tenuis																		
Aureobasidium pullulans																		
Cephalosporium sp.																		
Chaetomium sp.																		
Cladosporium cladosporioides																		
Fusarium oxysporum																		
Mycelium sterilium – white																		
Unidentified Phycomycete																		
Penicillium aureogriseum																		
Penicillium chrysogenum																		
Penicillium citrinum																		
Penicillium notatum																		
Stemphyllium sp.																		
BACTERIA																		
Bacillus sp.																		
Pseudomonas fluorescens																		
Sarcina sp.																		
Streptomyces sp.																		
YEASTS																		
Cryptococcus humicolus																		
Cryptococcus laurentii																		
Rhodotorula rubra																		
ALGAE																		
Chlorococcus sp.																		
Stichococcus sp.																		

The letters A to F in Table 2.9 represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

Plates 2.3 and 2.4 are photomicrographs of one of the spruce panels painted with paint B; Plate 2.5 is a photomicrograph of a panel painted with paint F, a gloss paint. It can be seen from these plates that algae and fungi are colonising the panels after one month's exposure and there appear to be many hyphal branches spreading across the gloss paint.

Plate 2.3 A spruce panel painted with the acrylic paint without fungicide after a month's exposure (x100) at Sandefjord.

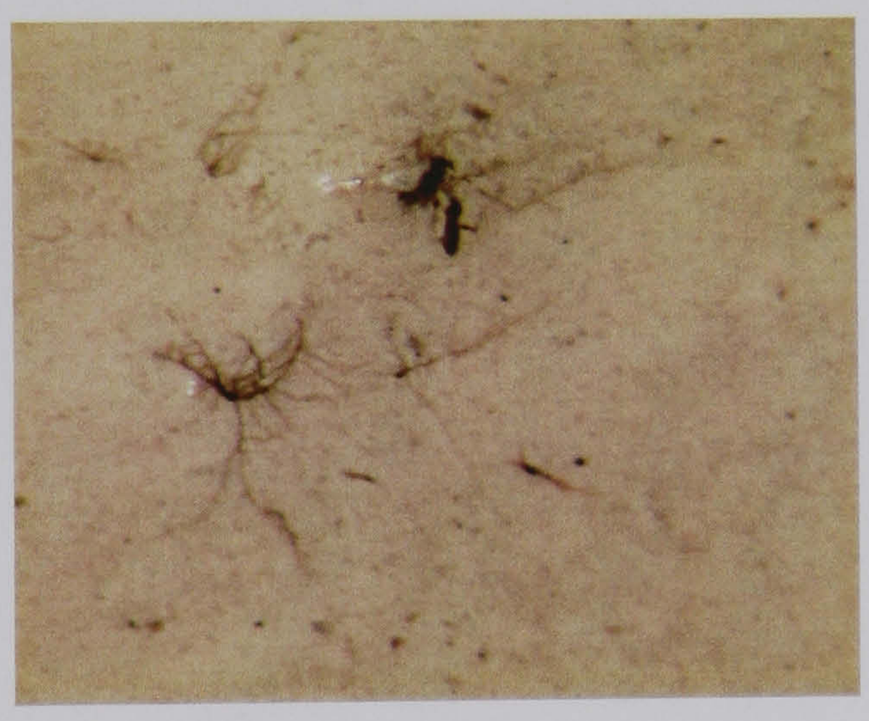


Plate 2.4 A spruce panel painted with the acrylic paint without fungicide after a month's exposure (x100) at Sandefjord.

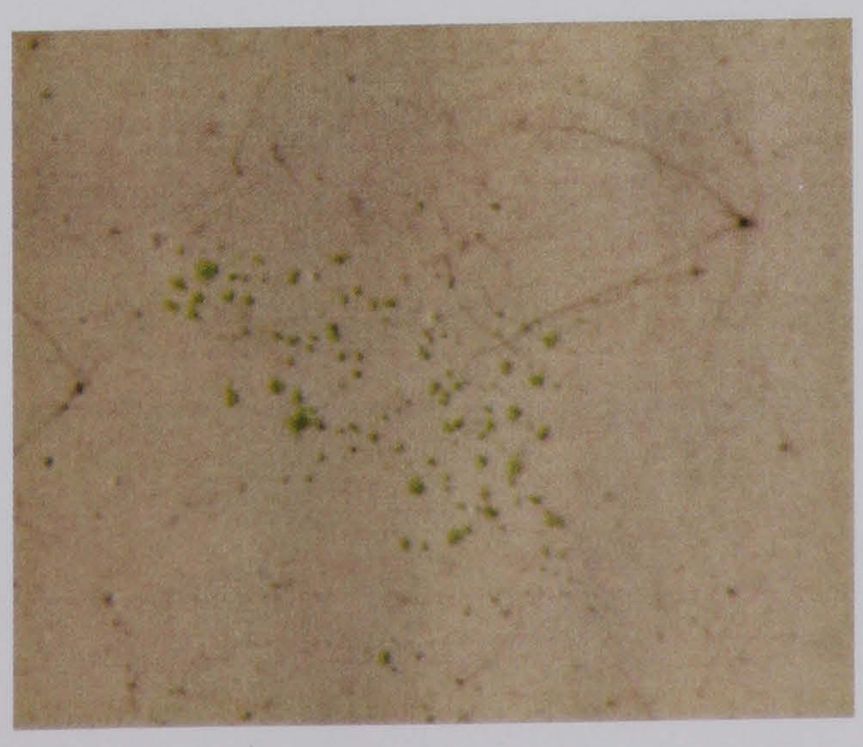
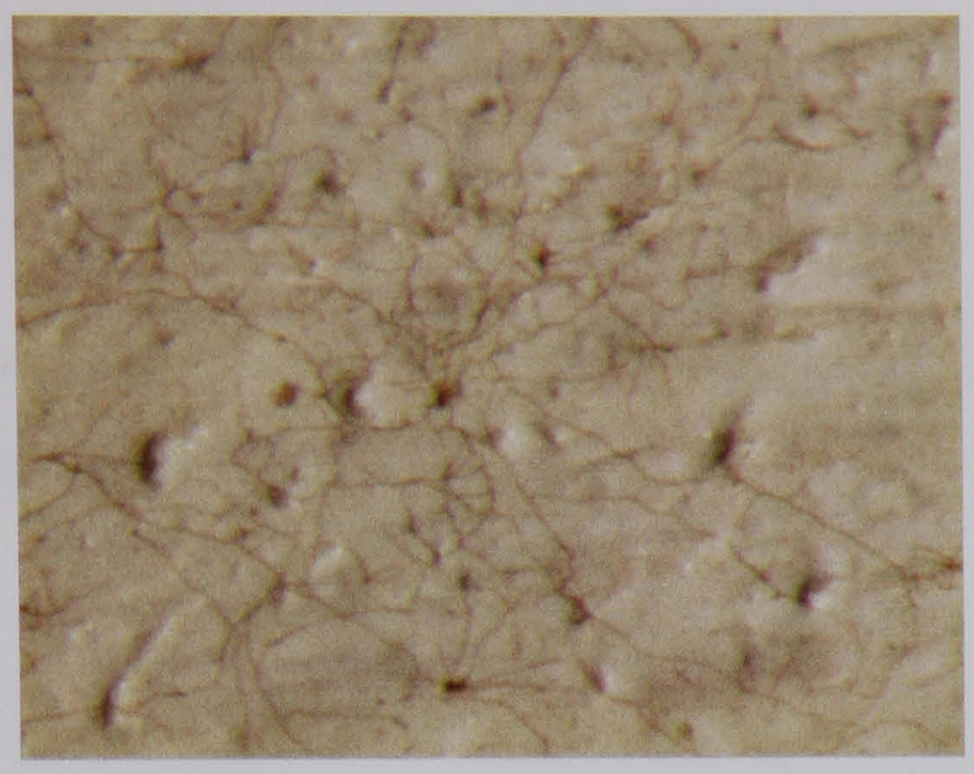


Plate 2.5 A spruce panel painted with the alkyd paint without fungicide after a month's exposure (x100) at Sandefjord.



The weather data

The weather data recorded throughout the exposure is presented in Appendix C:1.

The July 2000 exposure

Air micro flora

Microorganisms isolated from the air settle plates exposed at the end of the Sandefjord exposure in July 2000 can be found in Table 2.10.

Table 2.10 The microorganisms isolated from the air settle plates exposed at Sandefjord in July 2000.

FUNGI	BACTERIA	YEASTS	ALGAE
Alternaria alternata	*Bacillus sp.*	*Rhodotorula rubra*	*Chlorococcus sp.*
Aureobasidium pullulans	*Cellulomonas sp.*		*Stichococcus sp.*
Mycelium sterilium – white	*Pseudomonas fluorescens*		
Penicillium chrysogenum	*Staphylococcus cohnii cohnii*		
Penicillium simplicissimum	*Staphylococcus xylosus*		

Exposed panels

The results recorded from the exposed panels have been condensed into Tables 2.11, 2.12 and 2.13. Table 2.11 shows the results from the sacrificial panels painted with paint A to C, Table 2.12 shows the results from the sacrificial panels painted with paint D to F and the final table, Table 2.13 shows the organisms isolated from the surface of the three different panel materials.

From Table 2.11 and 2.12 it can be seen that a range of ten fungi and five bacteria were isolated, two yeasts and no algae. There does not appear to be any relevant pattern to the colonisation. The predominant organisms here were *Alternaria alternata* (paints D, E and F), *Aspergillus fumigatus*, a white sterile mycelium, *Cellulomonas* sp. and *Aeromonas hydrophila.*

Table 2.11 Colonisation of the sacrificial panels painted with paints A to C during the July 2000 exposure at Sandefjord.

Organisms	Paint A					Paint B					Paint C				
	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5
FUNGI															
Alternaria tenuis				■	■										
Aspergillus fumigatus	■	■	■	■	■				■		■	■	■	■	■
Aureobasidium pullulans		■			■						■			■	
Chaetomium globosum												■	■		
Fusarium oxysporum				■											
Mycelium sterilium - pink		■					■		■						
Mycelium sterilium – white	■		■	■		■			■		■	■	■	■	
Penicillium chrysogenum				■											
BACTERIA															
Aeromonas hydrophila						■	■		■		■		■	■	■
Bacillus sp.		■	■												
Burkholderia cepacia															■
Cellulomonas sp.	■	■	■		■	■			■			■	■		
Sarcina sp.												■	■	■	
YEASTS															
Rhodotorula mucilaginosa		■	■		■	■						■	■	■	
Rhodotorula rubra	■					■									

S1 to S5 represent the order of the sacrificial panels as described in Table 2.3. The shading represents the presence of a particular microorganism on the panel.

Table 2.12 Colonisation of the sacrificial panels painted with paints D to F during the July 2000 exposure at Sandefjord.

Organisms	Paint D					Paint E					Paint F				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
FUNGI															
Alternaria alternata															
Aspergillus fumigatus															
Aspergillus ustus															
Aureobasidium pullulans															
Mycelium sterilium – pink															
Mycelium sterilium – white															
BACTERIA															
Aeromonas hydrophila															
Cellulomonas sp.															
Sarcina sp.															
YEASTS															
Cryptococcus laurentii															
Rhodotorula mucilaginosa															
Rhodotorula rubra															

S1 to S5 represent the order of the sacrificial panels as described in Table 2.3.
The shading represents the presence of a particular microorganism on the panel.

From Table 2.13 it can be seen that a similar range of microorganisms to those found on the sacrificial panels are found on the three different materials. In this exposure more isolates were obtained from the spruce panels (19) than the calcium silicate (13) and the aluminium coated panels (10).

Table 2.13 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed at the July 2000 exposure at Sandefjord.

Organisms	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Alternaria alternata																		
Alternaria tenuis																		
Aspergillus fumigatus																		
Aspergillus niger																		
Aspergillus ustus																		
Aureobasidium pullulans																		
Chaetomium globosum																		
Fusarium oxysporum																		
Mycelium sterilium – pink																		
Mycelium sterilium – white																		
Penicillium chrysogenum																		
Phoma sp.																		
BACTERIA																		
Aeromonas hydrophila																		
Bacillus sp.																		
Burkholderia cepacia																		
Cellulomonas sp.																		
Pseudomonas fluorescens																		
Sarcina sp.																		
YEASTS																		
Cryptococcus laurentii																		
Rhodotorula rubra																		
Rhodotorula mucilaginosa																		

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

The weather data

The weather data recorded during this exposure can be found in Appendix C:2.

September / October 2000 exposure

Exposed panels

The exposure trial between September and October 2000 consisted only of an evaluation of the sacrificial panels coated with two paints, paint A and paint B, the pure acrylic paint. From Table 2.14, which shows the results of this trial, it can be seen that fungi, bacteria, algae and yeasts have been isolated. There is little difference in the number of isolations obtained from the paint containing fungicide (paint A) which has ten microorganisms isolated from it and that without (paint B) which has fourteen microorganisms isolated from it.

Table 2.14 Colonisation of the sacrificial panels painted with paints A and B from the September / October 2000 exposure at Sandefjord.

	Paint A						Paint B					
Organisms	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
FUNGI												
Alternaria alternata							■		■	■		
Aspergillus fumigatus			■	■						■		
Aspergillus niger										■		
Mycelium sterilium – white	■	■					■	■				
Penicillium citrinum						■	■					
Penicillium simplicissimum		■	■								■	
BACTERIA												
Aureobacterium spp.							■	■		■		■
Bacillus sp.	■	■			■		■		■	■		
Chryseomonas luteola	■	■			■		■					
Staphylococcus lentus	■								■	■		
Streptomyces sp.									■	■		
YEASTS												
Rhodotorula glutinis	■									■	■	■
Rhodotorula rubra	■	■				■	■	■	■	■	■	
ALGAE												
Stichococcus sp.					■	■			■	■	■	■

S1 to S6 represent the order of the sacrificial panels as described in Table 2.4.

The shading represents the presence of a particular microorganism on the panel.

The weather data

The weather data recorded throughout the exposure can be found in Appendix C:3.

June / August 2001 exposure

Exposed panels

The isolates obtained from the sacrificial panels for the exposure between June and August 2001 are shown in Table 2.15. From these it can be seen that a greater diversity of microorganisms (16 in total) were isolated from the surface of the paint without the fungicide than from that with fungicide. The organisms isolated include *Aureobasidium pullulans* and a pink mycelium sterilium. There were two bacteria and four fungi isolated from the panels painted with paint A, with fungicide, and four bacteria, ten fungi and two yeasts from paint B, both exposed for four weeks.

Table 2.15 Colonisation of the sacrificial panels from the exposure between June and August 2001 at Sandefjord.

Organisms	Paint A								Paint B							
	S1	S2	S3	S4	S5	S6	S7	S8	S1	S2	S3	S4	S5	S6	S7	S8
FUNGI																
Acremonium strictum															▒	
Alternaria alternata	▒								▒	▒						
Alternaria tenuis												▒				
Aspergillus fumigatus																▒
Aureobasidium pullulans			▒						▒	▒	▒	▒		▒		
Cephalosporium sp.														▒		
Cladosporium cladosporioides										▒						
Mycelium sterilium – pink	▒					▒	▒		▒	▒	▒					▒
Mycelium sterilium – white		▒									▒					
Penicillium chrysogenum										▒						
BACTERIA																
Bacillus sp.							▒		▒	▒				▒		
Burkholderia cepacia															▒	▒
Cellulomonas sp.						▒	▒							▒		
Sarcina sp.															▒	
YEASTS																
Rhodotorula mucilaginosa											▒					
Rhodotorula rubra											▒	▒				

S1 to S8 represent the order of the sacrificial panels as described in Table 2.5.

The shading represents the presence of a particular microorganism on the panel.

Table 2.16 presents the isolates from the surface of all of the exposed panels. It can be seen that a greater variety of microorganisms were isolated from the surface of the spruce panels (19) than the other two materials; 14 on the aluminium and 11 on the calcium silicate. The predominant organisms were *Aureobasidium pullulans*, a pink mycelium sterilium, a white mycelium sterilium, *Rhodotorula rubra*, *Cellulomonas* sp., *Bacillus* species and *Stichococcus* species.

Table 2.16 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed at the June / August 2001 exposure at Sandefjord.

Organisms	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Acremonium strictum																		
Alternaria alternata																		
Alternaria tenuis																		
Aspergillus fumigatus																		
Aureobasidium pullulans																		
Cephalosporium sp.																		
Cladosporium cladosporioides																		
Fusarium oxysporum																		
Mycelium sterilium – pink																		
Mycelium sterilium – white																		
Mycelium sterilium – white 2																		
Penicillium chrysogenum																		
Penicillium citrinum																		
Trichoderma viride																		
BACTERIA																		
Bacillus sp.																		
Burkholderia cepacia																		
Cellulomonas sp.																		
Sarcina sp.																		
YEASTS																		
Rhodotorula mucilaginosa																		
Rhodotorula rubra																		
ALGAE																		
Stichococcus sp.																		

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.
'M' represents the calcium silicate painted panels.

A summation of the microorganisms isolated from the four separate exposures that took place in Sandefjord can be found in Appendix D:1 and 2. From this, the number of times each microorganism was isolated, was expressed as a percentage of the number of occasions sampled. This shows clearly that *Aureobasidium pullulans* was the most frequently isolated organism from this site.

Plates 2.6 to 2.8 are photographs of the three different panels painted with paints A and B. From these it can be seen that the spruce panel painted with paint A is relatively clean, whereas the panel painted with paint B is heavily colonised in comparison. The calcium silicate panels have very little visible growth on them compared to the spruce panels, although there is evidence of some growth. The aluminium panels show very few signs of microbial growth.

Plate 2.6 Spruce panels painted with paint A and B after exposure for twenty-three months at Sandefjord.

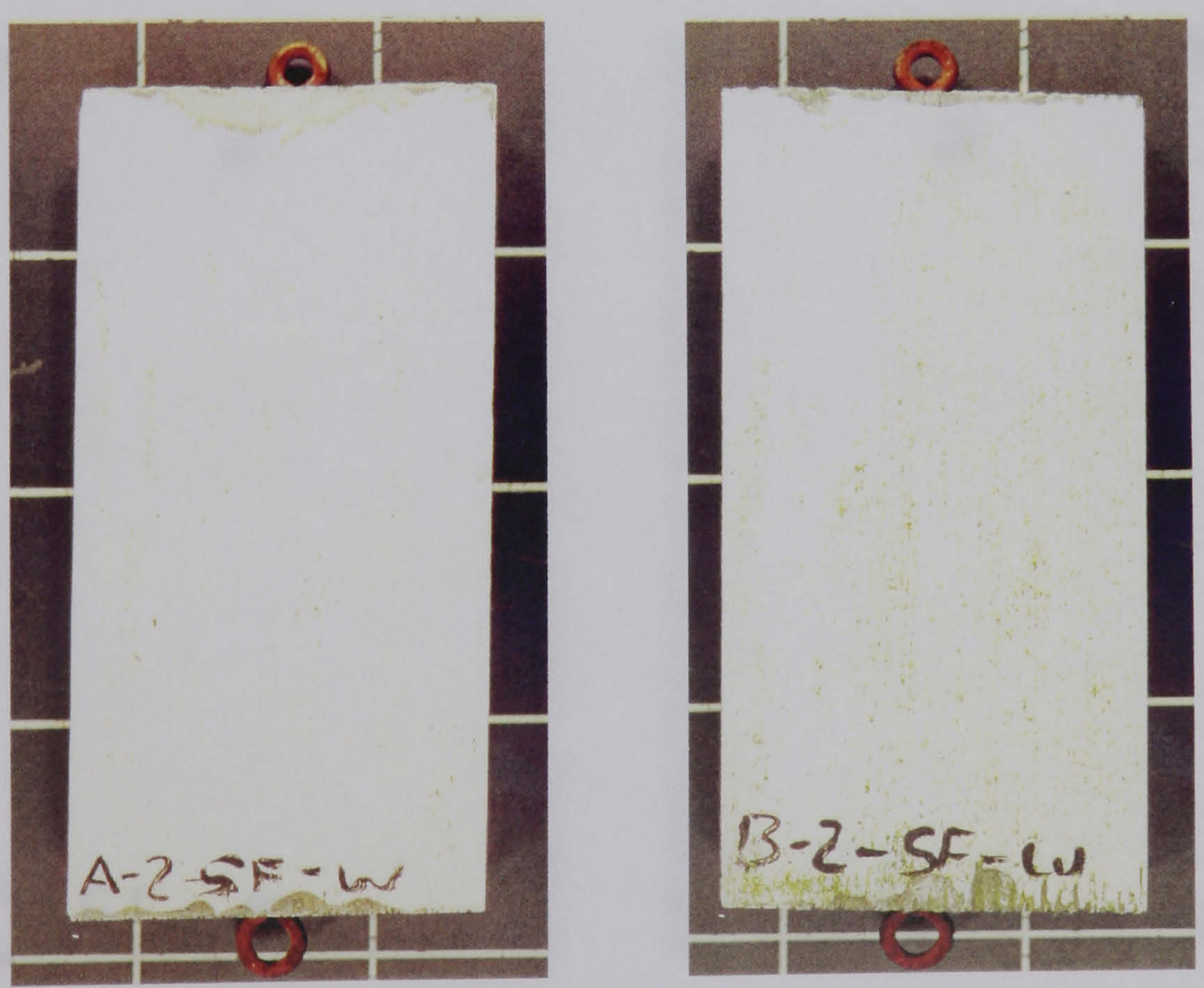


Plate 2.7 Calcium silicate panels painted with paint A and B after exposure for twenty-three months at Sandefjord.

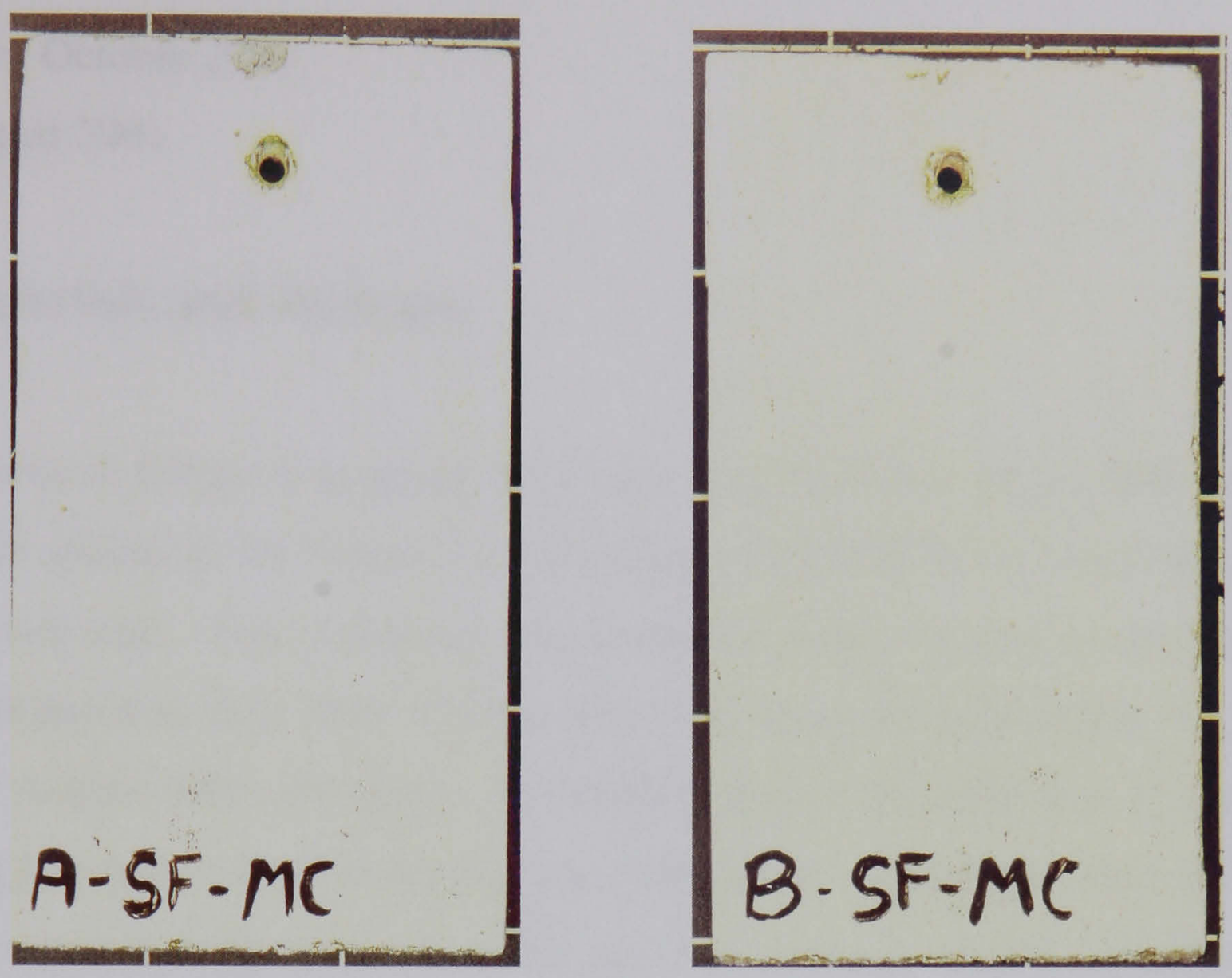


Plate 2.8 Aluminium panels painted with paint A and B after exposure for twenty-three months at Sandefjord.

The weather data

The weather data recorded during the exposure period can be found in Appendix C: 4.

2.4 Exposure trials at Bergen

The exposure trials at this site were conducted in:

July 2000

September / October 2000

June / August 2001

2.4.1 Materials and Methods

As attendance at Bergen throughout the exposure period was not possible, pre-prepared panels were mailed to the Bergen site and exposed by staff of the Jotun laboratories for each exposure trial. This researcher was present in Bergen to process the panels, which had been exposed in July 2000. On the other two occasions (September / October 2000 and June / August 2001) the panels were mailed back to the laboratory at the University of Central Lancashire in Preston and processed there. During the visit to Bergen the microbial content of the air at the Jotun site was assessed, on the occasions when the panels were mailed back the air micro flora was not sampled.

The July 2000 exposure

The panels for the exposure in July 2000 were prepared as in Section 2.2.2. The designation and nature of the panels exposed is shown in Table 2.17. Gamma-irradiated panels were included in the exposures at this site.

Table 2.17 The six paint coatings and the substance of the panels, which were exposed at Bergen during July 2000.

Paint	Spruce 1	Spruce 2	S1	S2	S3	S4	S5	A	M	Gamma
A	*	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*	*	*	*
C	*	*						*	*	*
D	*	*						*	*	*
E	*	*						*	*	*
F	*	*						*	*	*

S = Sacrificial panel

* = Exposed panel

A = Aluminium panel

M = calcium silicate panel

The experimental protocol was the same as that used for the Sandefjord exposure trial, described in Section 2.3.1. The twelve spruce panels (Spruce 1 and 2 in Table 2.17), the aluminium, calcium silicate and gamma irradiated spruce panels were exposed on the racks initially. They were exposed on July 6th 2000 for a month's duration. The exposure dates for the sacrificial panels are recorded in Table 2.18.

Table 2.18 The exposure times and dates for the panels exposed at Bergen in July 2000.

Sacrificial Panels	Dates Exposed	Exposure Time
S1	6th July 2000	3 weeks
S2	12th July 2000	2 weeks
S3	20th July 2000	1 week
S4	24th July 2000	3 days
S5	26th July 2000	1 day

Panels that had been exposed for their allotted time were removed from the racks and put into cold storage until they were collected a day later. The panels were swabbed using sterile alginate swabs and sterile Calgon Ringers solution in the manner described in Section 2.3.2. The resulting swab was returned to the sterile Ringers solution for transportation back to the UK for processing as described in Section 2.3.2.

The air micro flora was sampled by exposing two plates each of malt agar; Difco algal media and nutrient agar were exposed at the foot of the panel rack holder for a period of thirty minutes. After this time the petri dishes were sealed using Parafilm and packed for transportation back to the UK.

Throughout the journey back to the UK the plates were incubating at ambient temperature. Once back in the laboratory they were incubated at 30°C for four to seven days before being placed in the refrigerator to prevent further growth of the isolates. The colonies obtained were subsequently sub-cultured for identification. The algal plates were incubated on the windowsill of the laboratory under a lamp for a period of four weeks.

The September / October 2000 exposure

For the exposure between September / October 2000 there were already a number of panels remaining on the racks, these were the panels labelled as spruce 1 and spruce 2, aluminium (A) and calcium silicate (M). The staff at Jotun exposed the panels for a total period of one month commencing on 21st September 2000; the exposure dates for the sacrificial panels are shown in Table 2.19.

Table 2.19 Exposure dates and times for the panels exposed at Bergen between September and October 2000.

Sacrificial Panels	Date Exposed	Exposure Time
S1	21st September 2000	1 month
S2	5th October 2000	2 weeks
S3	12th October 2000	1 week
S4	16th October 2000	3 days
S5	18th October 2000	1 day
S6	19th October 2000	6 hours

Unlike the previous exposure at Bergen in July 2000, the sacrificial panels were returned to the UK by post. The air micro flora was not sampled and the calcium silicate, aluminium, gamma-irradiated and spruce 1 and 2 panels were also not sampled. On the sacrificial panels' delivery to the laboratory, they were swabbed and processed

as described in Section 2.3.2, again using Calgon Ringers solution as the transport medium for the swab.

The June / August 2001 exposure

The final exposure in Bergen was that between June and August. As in the previous exposure there were a number of panels already on the rack; these were the panels labelled as spruce 1 and 2, aluminium (A) and calcium silicate (M). The panels were exposed by Jotun staff for a total period of eight weeks commencing on June 8th 2001. The resulting exposure dates for the sacrificial panels are shown in Table 2.20. After the allotted time period, all of the panels exposed on the racks were returned to the UK by post and processed as in the previous exposure trials (see Section 2.3.2).

Table 2.20 Exposure dates and times for the panels exposed at Bergen between June and August 2001.

Sacrificial Panel	Exposure Date	Exposure Time
S1	19th June 2001	8 weeks
S2	3rd July 2001	6 weeks
S3	17th July 2001	4 weeks
S4	23rd July 2001	3 weeks
S5	31st July 2001	2 weeks
S6	1st August 2001	1 week
S7	5th August 2001	3 days
S8	8th August 2001	1 day

2.4.2 Results

The July 2000 exposure

Air micro flora

Microorganisms isolated from the air settle plates from the end of the July 2000 exposure at Bergen can be found in Table 2.21.

Table 2.21 The microorganisms isolated from the air settle plates exposed at Bergen in July 2000.

FUNGI	BACTERIA	YEASTS	ALGAE
Alternaria alternata	*Aeromonas hydrophila*	*Rhodotorula mucilaginosa*	*Chlorococcus sp.*
Aspergillus fumigatus	*Burkholderia cepacia*	*Rhodotorula rubra*	*Stichococcus sp.*
Aspergillus niger	*Sarcina sp.*		
Mycelium sterilium - white			

Exposed panels

The colonies isolated from the panels are depicted in Tables 2.22 and 2.23. Table 2.22 shows the colonies isolated from the sacrificial panels and Table 2.23 shows those isolated from the paint films coating the three different panel materials.

Table 2.22 shows that the major isolate, was *Aspergillus fumigatus,* as it was found on all of the sacrificial panels. The fungus, mycelium sterilium was only found on the panels painted with the paint without fungicide, as was the *Sarcina*. *Aeromonas hydrophila* and *Cellulomonas* sp. were found on both types of paint. There was little visual difference between the isolates obtained from the two paints, however, three different isolates were identified from paint A whereas seven isolates were identified from paint B.

Table 2.22 Colonisation of the sacrificial panels painted with paints A and B from the exposure between June and July 2000 at Bergen

Organism	Paint A						Paint B					
	S 1	S 2	S 3	S 4	S 5	S 6	S 1	S 2	S 3	S 4	S 5	S 6
FUNGI												
Aspergillus fumigatus												
Aspergillus niger												
Mycelium sterilium- white												
BACTERIA												
Aeromonas hydrophila												
Cellulomonas sp.												
Sarcina sp.												
YEAST												
Rhodotorula rubra												

S1 to S6 represent the order of the sacrificial panels as described in Table 2.18.
The shading represents the presence of a particular microorganism on the panel.

From Table 2.23 it can be seen that the predominant isolate found on the panels was *Aspergillus fumigatus.* It was isolated from all the panel types and all of the paints except the gamma-irradiated spruce panel painted with paint F. *Aeromonas hydrophila* and *Cellulomonas* species were isolated from many of the paints. From this table it can also be seen that paint B had a greater number of isolates identified than any of the other paints. It can also be observed that the spruce panels had a greater diversity of organisms isolated from them (11) followed by the gamma-irradiated spruce panels (5), the aluminium (4) and the calcium silicate panels (3) suggesting that the panel material affects the level of isolation.

Table 2.23 The microorganisms found on the three different panel materials for each of the six paints exposed at the July 2000 exposure at Bergen.

Organism	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
FUNGI																								
Alternaria alternata																								
Aspergillus fumigatus																								
Aspergillus niger																								
Aureobasidium pullulans																								
Cephalosporium sp.																								
Mycelium sterilium- white																								
BACTERIA																								
Aeromonas hydrophila																								
Cellulomonas sp.																								
Pseudomonas sp.																								
Sarcina sp.																								
YEASTS																								
Rhodotorula rubra																								

The letters A to F represent the six paints

'S' represents the spruce painted panels

'A' represents the aluminium painted panels

'M' represents the calcium silicate painted panels

'G' represents the gamma-irradiated spruce painted panels

The shading represents the presence of a particular microorganism on the panel.

The weather data

The weather data recorded throughout the exposure period is displayed in Appendix C:5.

The September / October 2000 exposure

The results of the exposures between September and October 2000 are summarised in Table 2.24. From this it can be seen that *Aspergillus fumigatus* occurs on both paint A and B. *Burkholderia cepacia* was present on three of the sacrificial panels painted with paint B, but was not isolated from the panels painted with paint A. The yeasts that were isolated are from the same genus and appear to colonise both of the paints.

Table 2.24 Colonisation of the sacrificial panels painted with paints A and B from the exposure at Bergen between September and October 2000.

	Paint A						Paint B					
Organism	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
FUNGI												
Aspergillus fumigatus	■		■	■	■	■		■	■	■	■	■
Aspergillus niger									■			
Aureobasidium pullulans			■				■				■	
Penicillium citrinum											■	
BACTERIA												
Burkholderia cepacia							■	■	■		■	■
Micrococcus spp.			■									
Pseudomonas fluorescens					■		■					
Streptomyces sp.			■									
YEASTS												
Cryptococcus uniguttulatus											■	
Rhodotorula mucilaginosa	■			■			■					■
Rhodotorula rubra		■										

S1 to S6 represent the order of the sacrificial panels as described in Table 2.19.

The shading represents the presence of a particular microorganism on the panel.

The weather data

The weather data recorded at this time can be seen in Appendix C:6.

The June / August 2001

The results of the final exposure at Bergen are displayed in Tables 2.25 and 2.26. Table 2.25 shows the results of the isolations from the sacrificial panels. Paint A, despite the fact that it contains a biocide provided a greater number of isolates (10) than paint B (4). The predominant organisms isolated were *Bacillus* species and *Aureobasidium pullulans*.

Table 2.25 Colonisation of the sacrificial panels painted with paints A and B from the exposure at Bergen between June and August 2001.

Organisms	Paint A								Paint B							
	S1	S2	S3	S4	S5	S6	S7	S8	S1	S2	S3	S4	S5	S6	S7	S8
FUNGI																
Aspergillus niger																
Aureobasidium pullulans																
Cladosporium cladosporioides																
Mycelium sterilium - pink																
Mycelium sterilium - White1.																
Mycelium sterilium -White 2																
Penicillium brevi-compactum																
Penicillium chrysogenum																
Penicillium citrinum																
BACTERIA																
Bacillus sp																
YEASTS																
Rhodotorula rubra																

S1 to S8 represent the order of the sacrificial panels as described in Table 2.20.

The shading represents the presence of a particular microorganism on the panel.

Table 2.26 shows that *Aureobasidium pullulans* and *Bacillus* species are major isolates on many of the panels and the spruce panels are more heavily colonised than the other substrata.

Table 2.26 The microorganisms found on the three different panel materials for each of the six paints exposed between June / August 2001 at Bergen.

Organisms	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
FUNGI																								
Alternaria alternata																								
Aspergillus niger																								
Aureobasidium pullulans																								
Cephalosporium sp.																								
Chaetomium globosum																								
Cladosporium cladosporioides																								
Fusarium oxysporum																								
Mycelium sterilium - pink																								
Mycelium Sterilium – white 1																								
Mycelium Sterilium – white 2																								
Penicillium brevi-compactum																								
Penicillium chrysogenum																								
Penicillium citrinum																								
Stemphylium botryosum																								
BACTERIA																								
Bacillus sp.																								
Staphylococcus capitis																								
Unidentified G+ rod																								

Table Continued. Organisms	A S A M G	B S A M G	C S A M G	D S A M G	E S A M G	F S A M G
YEASTS						
Cryptococcus laurentii						
Rhodotorula mucilaginosa						
Rhodotorula rubra						
ALGAE						
Stichococcus sp.						

The shading represents the presence of a particular microorganism on the panel.

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

'G' represents the gamma irradiated spruce painted panel

G+ represents a Gram Positive organism.

The weather data

The weather data recorded throughout the exposure period can be found in Appendix C:7.

A summation of the number of microorganisms isolated at each of the separate exposures at Bergen can be found in Appendix D:2. From this it can be observed that *Aspergillus fumigatus* is the microorganism to be isolated most frequently (51%), followed by *Aureobasidium pullulans* (47%) and a species of *Bacillus* (34%).

2.5 Exposure trials at Preston

The exposure trials at this site were carried out in:

September / October 1999,

April / May 2000,

February / March 2001,

June / August 2001

2.5.1 Materials and Methods

The September / October 1999 exposure

The panels for the September / October 1999 exposure were prepared as described in Section 2.3.1. As with the exposures that took place in Sandefjord, (Section 2.3) some panels were exposed on the racks from the beginning of the experiment (Table 2.27).

Table 2.27 The six paint coatings and the materials of the panels that were exposed at Preston between September / October 1999.

Paint	Spruce 1	Spruce 2	S1	S2	S3	S4	S5	aluminium	calcium silicate
A	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*	*	*
C	*	*						*	*
D	*	*						*	*
E	*	*						*	*
F	*	*						*	*

The twelve spruce panels (spruce 1 and 2 in Table 2.27), the aluminium and calcium silicate panels were exposed on the racks initially. They were exposed on Sunday 19th September 1999 for a month's duration. The remaining panels were placed on the racks on the dates shown in Table 2.28.

Table 2.28 The exposure times and dates for the panels exposed at Preston between September and October 1999.

Sacrificial Panels	Date Exposed	Exposure Time
S1	26th September 1999	2 weeks
S2	10th October 1999	1 week
S3	17th October 1999	24 hours
S4	23rd October 1999	12 hours
S5	24th October 1999 8am GMT	6 hours
S6	24th October 1999 11am GMT	3 hours

Panels that had been exposed for their allotted duration were taken indoors where they were swabbed in an identical manner to that described in Section 2.3.2, although not in a lamina flow cabinet. After incubation the number of each isolate obtained was recorded using a colony counter and distinct isolates were sub-cultured for identification.

Two petri –plates, each containing malt agar, Difco algal media and nutrient agar were exposed and placed at ground level at the foot of the panel racks for a period of thirty minutes. After the allotted time period the plates were sealed using Parafilm and incubated at 30°C for four to seven days before being placed in the fridge to prevent further growth. The distinct colonies obtained were subsequently sub-cultured for identification.

The April / May 2000 exposure

For the exposure between April and May 2000 some panels remained on the racks from the previous exposure, these were the calcium silicate, aluminium and spruce 1 and 2 panels (Table 2.27). The experimental procedure was the same as that used in the trials in September / October 1999. The initial sacrificial panels were exposed on April 7th

2000, as were a series of gamma irradiated spruce panels. The remaining sacrificial panels were added to the racks on the dates shown in Table 2.29.

Table 2.29 The exposure times and dates for the panels exposed at Preston between April and May 2000.

Sacrificial Panels	Date Exposed	Exposure Time
S1	7th April 2000	5 weeks
S2	21st April 2000	3 weeks
S3	3rd May 2000	1 week
S4	9th May 2000	1 day
S5	10th May 2000	6 hours

Panels that had been exposed for their allotted time were removed from the racks and swabbed as in the previous exposure before incubation and analysis. The air micro flora was sampled using two replicate petri dishes each containing malt agar, Difco algal medium, R2A agar andor nutrient agar. These were exposed at the foot of the panel rack holder for a period of thirty minutes, after which the petri dishes were sealed using Parafilm and then incubated at 30°C for four to seven days.

The February / March 2000 exposure

The exposure between February and March 2001 commenced on February 19th 2001, with the sacrificial panels being exposed for a period of four weeks. The remaining sacrificial panels were exposed on the following dates; 5th March, 12th March, 18th March and the 20th March. After the panels had been exposed for their allotted duration they were removed from the racks and analysed using the sterile swabbing procedure as in the previous exposure trials.

The June / August 2001 exposure

For the final exposure, between June and August 2001, the spruce 1 and 2 panels, the aluminium, gamma irradiated spruce and calcium silicate panels were already present on the racks as in previous exposures. On this occasion three sacrificial panels were exposed, commencing on 9th June 2001, the second sacrificial panel was placed on the rack on 30th June 2001 and the third on 21st July 2001. After the panels had been exposed for their allotted time, a maximum of eight weeks, they were all removed from the racks (August 4th 2001) and swabbed as in previous exposures. For this exposure no settle plates were exposed.

2.5.2 Results

The September / October 1999 exposure

Air micro flora

Microorganisms isolated from the air settle plates exposed at the end of the exposure that took place between September and October 1999 at Preston can be found in Table 2.30.

Table 2.30 The microorganisms isolated from the air settle plates exposed at Preston in October 1999.

FUNGI	BACTERIA	YEASTS	ALGAE
Aspergillus sp.	*Actinomycete sp.*	*Cryptococcus laurentii*	*Chlorococcus sp.*
Cephalosporium sp.	*Bacillus* sp.	*Rhodotorula rubra*	*Stichococcus sp.*
Mycelium sterilium	*Cellulomonas sp.*	*Saccharomyces cerevisiae*	
Trichoderma sp.	*Pseudomonas sp.*		
	Staphylococcus lentus		

Exposed panels

The results of this work are shown in Tables 2.31 and 2.32. Table 2.31 lists the microorganisms isolated from the sacrificial panels coated with paints A and B. It can be seen that more fungi than bacteria were isolated from paint B and more bacteria from paint A, however a greater variety (12) of isolates were obtained from paint B than paint A (9).

Table 2.31 The sacrificial panels painted with paints A and B from the September / October 1999 exposure at Preston.

	Paint A						Paint B					
Organism	S	S	S	S	S	S	S	S	S	S	S	S
FUNGI												
Alternaria alternata												
Fusarium culmorum												
Mycelium sterilium (P34)												
Mycelium sterilium (P35)												
Mycelium sterilium (P37)												
Mycelium sterilium (P41)												
Mycelium sterilium (P63)												
Penicillium aureogriseum												
Penicillium chrysogenum												
BACTERIA												
Cellulomonas sp.												
Sarcina sp.												
Unidentified G+ rod												
YEASTS												
Cryptococcus laurentii												
Rhodotorula rubra												
Saccharomyces cerevisiae												
ALGAE												
Chlorella sp.												
Stichococcus sp.												

S1 to S6 represent the order of the sacrificial panels as described in Table 2.28.

The shading represents the presence of a particular microorganism on the panel.

G+ represents a Gram Positive bacterium.

Table 2.32 shows that *Pseudomonas fluorescens* was a frequent isolate throughout the survey, except on the panels coated with paint F and that the spruce panels had the most organisms isolated from them (31), followed by the calcium silicate (17) then the aluminium panels (11).

2.32 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed between September and October 1999 at Preston.

Organism	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Absidia sp.																		
Alternaria alternata																		
Aspergillus fumigatus																		
Aureobasidium pullulans																		
Cephalosporium sp.																		
Chaetomium sp.																		
Cladosporium cladosporioides																		
Curvularia sp.																		
Dematiaceous hyphomycete																		
Fusarium culmorum																		
Fusarium oxysporum																		
Mycelium sterilium - pink																		
Mycelium sterilium white																		
Mycelium sterilium P34																		
Mycelium sterilium P35																		
Mycelium sterilium P37																		
Mycelium sterilium P41																		
Mycelium sterilium P63																		
Penicillium aureogriseum																		
Penicillium chrysogenum																		
Penicillium citrinum																		
Phoma sp.																		

Table Continued.	A			B			C			D			E			F		
Organism	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
BACTERIA																		
Bacillus sp.																		
Cellulomonas sp.																		
Pseudomonas fluorescens																		
Sarcina sp.																		
Streptomyces sp.																		
Unidentified G+ coccus																		
Unidentified G+ rod																		
YEASTS																		
Cryptococcus laurentii																		
Rhodotorula mucilaginosa																		
Rhodotorula rubra																		
Saccharomyces cerevisiae																		
ALGAE																		
Chlorella sp.																		
Stichococcus sp.																		

The shading represents the presence of a particular microorganism on the panel.

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

G+ represents a Gram Positive bacterium.

<u>The weather data</u>

The weather data recorded throughout the period of exposure can be found in Appendix C:7.

The April / May 2000 exposure

Air micro flora

Microorganisms isolated from the air settle plates from the end of this exposure can be found in Table 2.33

Table 2.33 The microorganisms isolated from the air settle plates exposed at Preston in May 2000.

FUNGI	BACTERIA	YEASTS	ALGAE
Cephalosporium sp.	*Cellulomonas sp.*	*Rhodotorula mucilaginosa*	*Chlorococcus sp.*
Mycelium sterilium - white	*Bacillus* sp.	*Rhodotorula rubra*	*Stichococcus sp*
	Sarcina sp.		
	Streptomyces sp.		

Exposed panels

The organisms isolated from the panels can be seen in Tables 2.34 and 2.35. Table 2.34 shows the isolates obtained from the sacrificial panels. From the results it can be seen that *Aspergillus fumigatus* was a frequent isolate on paint B, the bacterium *Cellulomonas* sp. was isolated from all of the sacrificial panels for both of the paints and *Rhodotorula glutinis* was the only yeast isolated and that was from a panel painted with paint A.

Table 2.34 Colonisation of the sacrificial panels painted with paint A and B from the April /May 2000 exposure at Preston.

	Paint A					Paint B				
Organism	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
FUNGI										
Aspergillus fumigatus										
Aspergillus niger										
Aspergillus ustus										
Mycelium sterilium (P6)										
Phoma sp.										
BACTERIA										
Cellulomonas sp.										
YEASTS										
Rhodotorula glutinis										

S1 to S5 represent the order of the sacrificial panels as described in Table 2.29. The shading represents the presence of a particular microorganism on the panel.

Table 2.35 shows the isolates obtained from the surface of the different panel materials. It can be seen that *Aspergillus fumigatus*, *Cellulomonas* sp., *Aeromonas hydrophila*, *Rhodotorula glutinis* and the mycelium sterilium (P6) were isolated frequently throughout the survey.

Table 2.35 The microorganisms found on the three different panel materials for each of the six paints exposed at the April / May 2000 exposure at Preston.

Organisms	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
FUNGI																								
Alternaria alternata																								
Aspergillus fumigatus																								
Aspergillus niger																								
Aspergillus ustus																								
Aureobasidium pullulans																								
Cladosporium cladosporioides																								
Fusarium oxysporum																								
Mycelium sterilium (P6)																								
Phoma sp.																								
Trichoderma viride																								
BACTERIA																								
Aeromonas hydrophila																								
Cellulomonas sp.																								
YEASTS																								
Rhodotorula glutinis																								
Rhodotorula rubra																								
ALGAE																								
Stichococcus sp.																								

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

'G' represents the gamma irradiated spruce panels.

The weather data

The weather data recorded throughout this exposure period can be seen in Appendix C:9.

The February / March 2001 exposure

Air micro flora

Microorganisms isolated from the air settle plates at the end of this exposure trial can be found in Table 2.36.

Table 2.36 The microorganisms isolated from the air settle plates exposed at Preston between February and March 2001.

FUNGI	BACTERIA	YEASTS	ALGAE
Alternaria alternata	*Bacillus sp.*	*Rhodotorula mucilaginosa*	*Chlorococcus sp.*
Aspergillus fumigatus	*Staphylococcus cohnii cohnii*	*Rhodotorula rubra*	*Stichococcus sp.*
Penicillium chrysogenum			

Exposed panels

The organisms isolated from the panels at the Preston exposure between February and March 2001 can be seen in Tables 2.37 and 2.38. From the sacrificial panels coated with paints A and B in Table 2.37 it can be seen that a white mycelium sterilium was isolated from both paints as was a species of *Chlorella*. *Brevibacterium* sp., *Staphylococcus cohnii cohnii* and *Hansenula polymorpha* were isolated more frequently from paint A. The greatest variety of isolates was obtained from paint B (17), which was biocide free, compared with paint A, which had 13 isolated from it.

Table 2.37 Colonisation of the sacrificial panels painted with paints A and B from the exposure between February and March 2001 at Preston.

	Paint A					Paint B				
Organism	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
FUNGI										
Alternaria alternata		■	■				■	■		■
Aspergillus fumigatus				■						
Aureobasidium pullulans	■						■			
Chaetomium sp.		■		■						
Cladosporium cladosporioides								■		
Dematiaceous hyphomycete									■	
Mycelium sterilium - white		■	■				■	■	■	■
Paecilomyces marquandii					■				■	
Penicillium chrysogenum								■	■	
BACTERIA										
Arthrobacter sp.									■	
Brevibacterium spp.	■			■				■	■	
Chryseomonas luteola		■								
Staphylococcus cohnii cohnii		■	■	■			■			
Streptomyces sp.			■							
YEASTS										
Candida ciferii							■			
Candida utilis		■								
Cryptococcus humicolus								■		■
Hansenula polymorpha	■	■	■	■			■	■		
Rhodotorula mucilaginosa							■			
Rhodotorula rubra									■	
ALGAE										
Chlorella sp,		■	■	■	■		■	■	■	■
Stichococcus sp.						■				

S1 to S5 represent the order of the sacrificial panels.

The shading represents the presence of a particular microorganism on the panel.

Table 2.38 shows the isolates obtained from the paint films of the various panels. From this it can be seen that *Cladosporium cladosporioides*, the white mycelium sterilium, *Alternaria alternata*, *Aureobasidium pullulans, Arthrobacter sp.*, *Brevibacterium sp.*, *Staphylococcus cohnii cohnii*, *Hansenula polymorpha, Stichococcus sp. and Chlorella sp.* were the most frequently isolated microorganisms at this exposure site.

2.38 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed between February and March 2001 at Preston.

Organisms	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
FUNGI																								
Alternaria alternata																								
Aspergillus fumigatus																								
Aspergillus niger																								
Aureobasidium pullulans																								
Cephalosporium sp.																								
Chaetomium sp.																								
Cladosporium cladosporioides																								
Dematiaceous hyphomycete																								
Mycelium sterilium – white																								
Paecilomyces marquandii																								
Penicillium chrysogenum																								
Penicillium simplicissimum																								
BACTERIA																								
Arthrobacter sp.																								
Bacillus sp.																								
Brevibacterium sp.																								
Chryseomonas luteola																								
Pseudomonas fluorescens																								
Pseudomonas paucimobilis																								
Staphylococcus cohnii cohnii																								
Streptomyces sp.																								

Table Continued.																							
	A				B				C				D				E				F		
Organisms	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
YEAST																							
Candida ciferrii																							
Candida utilis																							
Cryptococcus albidus																							
Cryptococcus humicolus																							
Hansenula polymorpha																							
Rhodotorula mucilaginosa																							
Rhodotorula rubra																							
ALGAE																							
Chlorella sp.																							
Stichococcus sp.																							

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

'G' represents the gamma irradiated spruce panels.

<u>The weather data</u>

The weather data recorded at the time of the exposure can be found in Appendix C:10.

The June / August 2001 exposure

The results of the colonisation of the panels during the exposure between June and August 2001 are shown in Tables 2.39 and 2.40. Table 2.39 shows that there are fewer isolates occurring on paint A (14) than paint B (9).

Table 2.39 Colonisation of the sacrificial panels painted with paints A and B from the exposure between June and August 2001 at Preston.

	Paint A			Paint B		
Organisms	S1	S2	S3	S1	S2	S3
FUNGI						
Aspergillus fumigatus						
Aspergillus niger						
Aureobasidium pullulans						
Mycelium sterilium - Pink						
Mycelium sterilium – white						
Penicillium brevi-compactum						
Penicillium citrinum						
Phialophora sp.						
Puecilomyces variotii						
BACTERIA						
Bacillus sp.						
Micrococcus spp.						
Staphylococcus capitis						
Staphylococcus lentus						
YEAST						
Saccharomyces cerevisiae						
ALGAE						
Stichococcus sp.						

S1 to S3 represent the order of the sacrificial panels.

The shading represents the presence of a particular microorganism on the panel.

Table 2.40 shows that the most frequent isolates obtained from the panels were *Aureobasidium pullulans*, the pink mycelium sterilium, *Bacillus* species, *Saccharomyces cerevisiae* and the algae. It can also be seen from this table that the gamma irradiated panels produced fewer isolates (12) than the other types of panel, the standard spruce panel having 21 different isolates; calcium silicate, 14 and aluminium, 15.

Table 2.40 The microorganisms found on the three different panel materials for each of the six paints exposed between June and August 2001 at Preston.

Organisms	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
FUNGI																								
Alternaria alternata																								
Alternaria tenuis																								
Aspergillus fumigatus																								
Aspergillus niger																								
Aureobasidium pullulans																								
Chaetomium globosum																								
Curvularia sp.																								
Fusarium oxysporum																								
Mycelium sterilium -Pink																								
Mycelium sterilium – white																								
Penicillium brevi-compactum																								
Penicillium canescens																								
Penicillium citrinum																								
Phialophora sp.																								
Phoma sp.																								
Puecilomyces variotii																								
Trichoderma viride																								
BACTERIA																								
Bacillus sp.																								
Micrococcus spp.																								
Pantoea spp.																								
Pseudomonas fluorescens																								
Staphylococcus capitis																								

Table Continued. Organisms	A				B				C				D				E				F			
	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G	S	A	M	G
Staphylococcus lentus																								
Serratia ficaria																								
Vibrio fluvalis																								
YEAST																								
Saccharomyces cerevisiae																								
ALGAE																								
Chlorella sp.																								
Stichococcus sp.																								

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

As with the previous exposure sites, a summation of the microorganisms isolated at Preston and the percentage frequency in which they occur can be found in Appendix D:3. From this it can be seen that a species of *Chlorella* is the most frequently isolated organism (38%) closely followed by *Aureobasidium pullulans* (35%) and a white mycelium sterilium and a species of *Stichococcus* (34%).

Plates 2.9 to 2.11 show various panels at the end of their exposure before they were swabbed.

Plate 2.9 shows the two non-fungicide containing acrylic paints (paints B and D) painted onto the spruce panels after their full exposure period. Inspection of the panels with a low power stereoscopic microscope showed that the panels were colonised by algae and fungi and that there was surface debris present. This degree of colonisation was not apparent on the paints containing fungicide (paints A and C).

Plate 2.9 The two non-fungicide containing acrylic paints after twenty-three months exposure at Preston.

(Paint B)

(Paint D)

Plate 2.10 shows the photographs of the gamma irradiated panels painted with paints B, C and D. It can be observed from these photographs that paint C, containing fungicide does not appear to be heavily colonised, (this was also noticed in paint A). Paints B and D, however are more heavily colonised, paint D more so than paint B. The major isolates from the panels were fungi and algae, which was concentrated on the bottom edge of the panels, rather than throughout the panel. *Aureobasidium pullulans* was also observed to be a major coloniser of the panels (Table 2.40).

Plate 2.10 Gamma irradiated spruce panels painted with paints B, C and D after twenty-three months exposure at Preston.

(Paint B) (Paint C) (Paint D)

Plate 2.11 shows the photographs of the Masterclad™ panels painted with paints A, B and C. The panel painted with paint A (pure acrylic containing fungicide) is colonised with what appeared to be fungi, although surface debris, could also be observed. The panel painted with paint B, which is the pure acrylic paint that does not contain a fungicide, appears to be heavily colonised with fungi. Paint C, the hybrid acrylic paint containing fungicide appears to be less heavily colonised than paint A, although in places flaking of the paint can be observed.

Plate 2.11 Calcium silicate panels painted with paints A, B and C after Twenty-three months exposure at Preston.

(Paint A) (Paint B) (Paint C)

The weather data

The weather data recorded at Preston during the June / August exposure trial can be found in Appendix C:11.

2.6 Exposure trials at Blackley, Greater Manchester

The exposure trials at this site were conducted in:

April / May 2000,

February / March 2001,

November 2001.

2.6.1 Materials and Methods

The April / May exposure

Panels were prepared as described in Section 2.2.2. The number and nature of panels exposed initially (April 2000) are shown in Table 2.41. The experimental protocol was similar in nature to those conducted at other exposure sites. The spruce, aluminium and calcium silicate (Masterclad™) panels were exposed initially on April 11th 2000 and the sacrificial panels were placed on the racks on the dates shown in Table 2.42.

Table 2.41 The six paint coatings, A to F and the substance of the panels that were exposed at Blackley in April / May 2000.

Paint	Spruce 1	Spruce 2	S1	S2	S3	S4	S5	Aluminium	Calcium Silicate
A	*	*	*	*	*	*	*	*	*
B	*	*	*	*	*	*	*	*	*
C	*	*						*	*
D	*	*						*	*
E	*	*						*	*
F	*	*						*	*

S = Sacrificial panel.

* = exposed panel.

Table 2.42 The exposure times and dates for the panels exposed between April / May 2000 at Blackley.

Sacrificial panels	Date Exposed	Exposure Time
S1	11th April 2000	3 weeks
S2	25th April 2000	2 weeks
S3	2nd May 2000	1 week
S4	8th May 2000	3 days
S5	11th May 2000	3 hours

Panels that had been exposed for their allotted time were removed from the racks and swabbed and processed as described in Section 2.3.2. The air micro flora were sampled using a series of two plates each of malt agar, R2A agar, Difco algal media and nutrient agar, which were exposed at the foot of the panel racks for a period of thirty minutes. After the allotted time the petri-dishes were sealed using Parafilm before being incubated at 30°C for four to seven days, or in the case of the algal plates, left under a lamp on the windowsill for four weeks.

The February / March exposure

The following exposure trial was conducted between February and March 2001. For this trial the aluminium, calcium silicate and spruce panels labelled spruce '1' remained on the panel rack from the previous exposure. The sacrificial panels were exposed on the dates shown in Table 2.43.

Table 2.43 The exposure times and dates for the panels exposed between February / March 2001 at Blackley.

Sacrificial Panels	Date Exposed	Exposure Time
S1	20th February 2001	4 weeks
S2	6th March 2001	2 weeks
S3	13th March 2001	1 week
S4	19th March 2001	3 days
S5	21st march 2001	1 day
S6	22nd March 2001	3 hours

After the allotted period of time the panels were treated in a similar manner to those in the September / October exposure and as described in Section 2.3.2.

The November 2001 exposure

In November of 2001 the remaining panels were removed from the racks at Blackley and swabbed as in all the other exposure trials. As this was the conclusion of the exposure trials at this site, the air micro flora was not sampled.

2.4.2 Results

The April / May 2000 exposure

Air micro flora

The results of the Manchester exposure trial in April / May 2000 can be seen in Tables 2.45 and 2.46. The microorganisms in the air recorded at the end of the exposure can be found in Table 2.44.

Table 2.44 The microorganisms isolated from the air settle plates exposed at Blackley in May 2000.

FUNGI	BACTERIA	YEASTS	ALGAE
Alternaria alternata	*Aeromonas hydrophila*	*Rhodotorula rubra*	*Chlorella sp.*
Aspergillus flavus	*Bacillus sp.*		*Chlorococcus sp.*
Aureobasidium pullulans	*Cellulomonas* sp.		*Stichococcus sp.*
Cephalosporium sp.	Unidentified G+ rod		
Mycelium sterilium - White			
Penicillium chrysogenum			

Exposed panels

The organisms found on the sacrificial panels coated with paints A and B are listed in Table 2.45. It can be seen that there was a combination of yeasts, bacteria, algae and fungi isolated from the panels. *Cellulomonas* species, *Staphylococcus lentus*, a species of *Bacillus* and *Rhodotorula rubra* were the major isolates obtained from the surface of paint A, which contains fungicide, but not from paint B which does not contain fungicide. There were no fungi isolated from paint A and only a few from paint B.

There were twice as many isolates obtained for paint B (10) than from paint A which contained the fungicide.

Table 2.45 Colonisation of the sacrificial panels painted with paints A and B from the exposure between April / May 2000 at Blackley.

	Paint A					Paint B				
Organism	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
FUNGI										
Aspergillus fumigatus							■		■	
Mycelium Sterilium- white						■				
Penicillium chrysogenum									■	
Penicillium simplicissimum										■
BACTERIA										
Aeromonas hydrophila									■	
Arthrobacter sp.							■		■	
Bacillus sp.	■	■	■							
Cellulomonas sp.	■		■	■	■	■			■	
Staphylococcus lentus	■	■	■							
Unidentified G+ rod										■
YEAST										
Rhodotorula mucilaginosa						■				
Rhodotorula rubra	■		■	■	■					
ALGAE										
Chlorella sp.							■	■		■
Stichococcus sp.			■							

S1 to S5 represent the order of the sacrificial panels.

The shading represents the presence of a particular microorganism on the panel.

G+ represents an unidentified Gram Positive bacterium.

Table 2.46 shows the isolates obtained from the surface of the paint films of the three different panel materials. It can be seen that there were very few fungi isolated from paint A on any of the materials, but there were three different bacterial isolates identified, a yeast and two species of algae. There were fungi and yeasts, bacteria and algae isolated from paints B and C. Paint D supported very few organisms and paints E and F were shown to support bacteria, fungi, yeasts and algae. Throughout this study the calcium silicate panels provided the least number of isolations (11) whereas the aluminium panels supported 12 and the spruce panels supported 20 different isolates.

Table 2.46 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed between April and May 2000 at Blackley.

	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
Organism																		
FUNGI																		
Alternaria alternata																		
Aspergillus fumigatus																		
Aureobasidium pullulans																		
Cephalosporium sp.																		
Cladosporium cladosporioides																		
Mycelium sterilium -pink																		
Mycelium sterilium - white																		
Penicillium chrysogenum																		
Penicillium simplicissimum																		
BACTERIA																		
Aeromonas hydrophila																		
Arthrobacter sp.																		
Aureobacterium sp.																		
Bacillus sp.																		
Cellulomonas sp.																		
Staphylococcus lentus																		
Staphylococcus xylosus																		
Streptomyces sp.																		
Unidentified G+ rod																		
YEAST																		
Rhodotorula mucilaginosa																		
Rhodotorula rubra																		
ALGAE																		
Chlorella sp.																		
Stichococcus sp.																		

G+ represents a Gram Positive Rod.

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

The weather data

The weather data taken at the time of exposure can be found in Appendix C:12.

The February / March 2001 exposure

Air micro flora

The microorganisms isolated from the atmosphere at the end of this exposure can be found in Table 2.47.

Table 2.47 The microorganisms isolated from the air settle plates exposed at Blackley in March 2001.

FUNGI	BACTERIA	YEAST	ALGAE
Aspergillus fumigatus	*Bacillus sp.*	*Rhodococcus spp.*	*Chlorella sp.*
Mycelium sterilium- pink	*Serratia liquefaciens*	*Rhodotorula glutinis*	*Chlorococcus* sp.
Mycelium sterilium- white	*Staphylococcus cohnii cohnii*		*Stichococcus sp.*
Penicillium chrysogenum	*Staphylococcus cohnii ureal*		*Tolypothrix* sp.
Penicillium expansum	*Streptomyces sp.*		

Exposed panels

It can be seen from Table 2.48 showing the sacrificial panels that there does not appear to be any major differences in the colonisation pattern between the paint containing fungicide (paint A) which provided 14 isolates and that which does not (paint B) which provided 10 isolates. An interesting observation, however, is the fact that only two bacteria were isolated from paint B, whereas seven different genera were isolated from paint A.

Table 2.48 Colonisation of the sacrificial panels painted with paints A and B from the exposure between February and March 2001 at Blackley.

	Paint A					Paint B				
Organism	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
FUNGI										
Aspergillus fumigatus			■		■		■	■		
Aureobasidium pullulans								■		■
Cylindrocarpon candidum	■	■			■	■				■
Mycelium sterilium- white						■		■		
Penicillium spinulosum	■									
BACTERIA										
Aeromonas hydrophila		■	■							
Arthrobacter sp.			■							
Bacillus sp						■		■		
Burkholderia cepacia			■							
Cellulomonas sp.	■		■		■					
Chryseomonas luteola			■							
Corynebacterium stratiatum										■
Non fermenter sp.					■					
Staphylococcus epidermidis	■									
YEAST										
Cryptococcus laurentii	■							■		
Rhodotorula rubra	■	■						■		
ALGAE	■							■		
Chlorella sp.										■
Stichococcus sp.			■	■						

S1 to S5 represent the order of the sacrificial panels.

The shading represents the presence of a particular microorganism on the panel.

Table 2.49 shows the isolates obtained from the paint films coating the panel materials. It can be seen that the spruce panels are the most heavily colonised (21 isolates) followed by the calcium silicate (15 isolates) and aluminium panels (11 isolates). It can also be seen that the two alkyd paints (paints E and F) are the least heavily colonised.

Table 2.49 The microorganisms found on the surface of the three different panel materials coated with each of the six paints exposed between February and March 2001 at Blackley.

Organism	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Aspergillus fumigatus																		
Aureobasidium pullulans																		
Chaetomium globosum																		
Cylindrocarpon candidum																		
Mycelium sterilium - Pink																		
Mycelium sterilium - white																		
Penicillium expansum																		
Penicillium spinulosum																		
Phoma herbarum																		
Trichoderma koningii																		
Trichoderma viride																		
BACTERIA																		
Aeromonas hydrophila																		
Arthrobacter sp.																		
Bacillus sp.																		
Burkholderia cepacia																		
Cellulomonas sp.																		
Chryseomonas luteola																		
Corynebacterium stratiatum																		
Non fermenter sp.																		
Staphylococcus epidermidis																		
Streptomyces sp.																		
YEASTS																		
Cryptococcus humicolus																		
Cryptococcus laurentii																		
Rhodotorula mucilaginosa																		
Rhodotorula rubra																		
ALGAE																		
Chlorella sp.																		
Stichococcus sp.																		

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

The weather data

The weather data recorded during this exposure period can be found in Appendix C:13.

The November 2001 exposure

Exposed panels

No sacrificial panels were exposed on the racks in November 2001, although the existing panels were removed. The organisms isolated from these remaining panels can be found in Table 2.50. It can be seen from Table 2.50 that *Aureobasidium pullulans* was the most frequent microorganism isolated; it was found on all of the panels except one (the calcium silicate panel coated with paint D). The fungus designated as a sterile white mycelium was also isolated frequently. There does not appear to be a marked difference between the isolates obtained from the three panel materials. Fewer microorganisms were isolated from the films coating the aluminium panels (5 isolates) than the other two materials, which provided 19 and 24 isolates.

Table 2.50 The microorganisms found on the three different panel materials for each of the six paints exposed at the end of the exposure trial in November 2001 at Blackley.

Organism	A			B			C			D			E			F		
	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M	S	A	M
FUNGI																		
Alternaria alternata																		
Alternaria tenuis																		
Aspergillus fumigatus																		
Aspergillus niger																		
Aureobasidium pullulans																		
Cephalosporium sp.																		
Chaetomium globosum																		
Cladosporium cladosporioides																		
Fusarium oxysporum																		
Gliocladium sp.																		
Mycelium sterilium - Grey																		
Mycelium sterilium - Pink																		
Mycelium sterilium - White																		
Penicillium chrysogenum																		
Penicillium citrinum																		
Penicillium expansum																		
BACTERIA																		
Bacillus sp.																		
Cellulomonas sp.																		
Sarcina sp.																		
Staphylococcus capitis																		
Staphylococcus lentus																		
Streptomyces sp.																		
YEASTS																		
Rhodotorula mucilaginosa																		
Rhodotorula rubra																		
ALGAE																		
Chlorella sp.																		
Stichococcus sp.																		

The letters A to F represent the six paints.

'S' represents the spruce painted panels.

'A' represents the aluminium painted panels.

'M' represents the calcium silicate painted panels.

A summation of the frequency of microorganisms isolated from the separate exposures at Blackley can be found in Appendix D:4. From this it can be seen that *Aureobasidium pullulans* was the most frequently isolated microorganism (55.4%) followed by a white mycelium sterilium (39.2%).

As with the other exposure sites photographs were taken of some of the panels before they were swabbed, these can be seen in Plates 2.12 to 2.14. Plate 2.12 shows the spruce panels painted with paints B, C and D. From these it can be observed that the paints that did not contain the fungicide (paints B and D) are the ones that are the most colonised. Paint C, (and paint A, which is not shown), were colonised at the base of the panel.

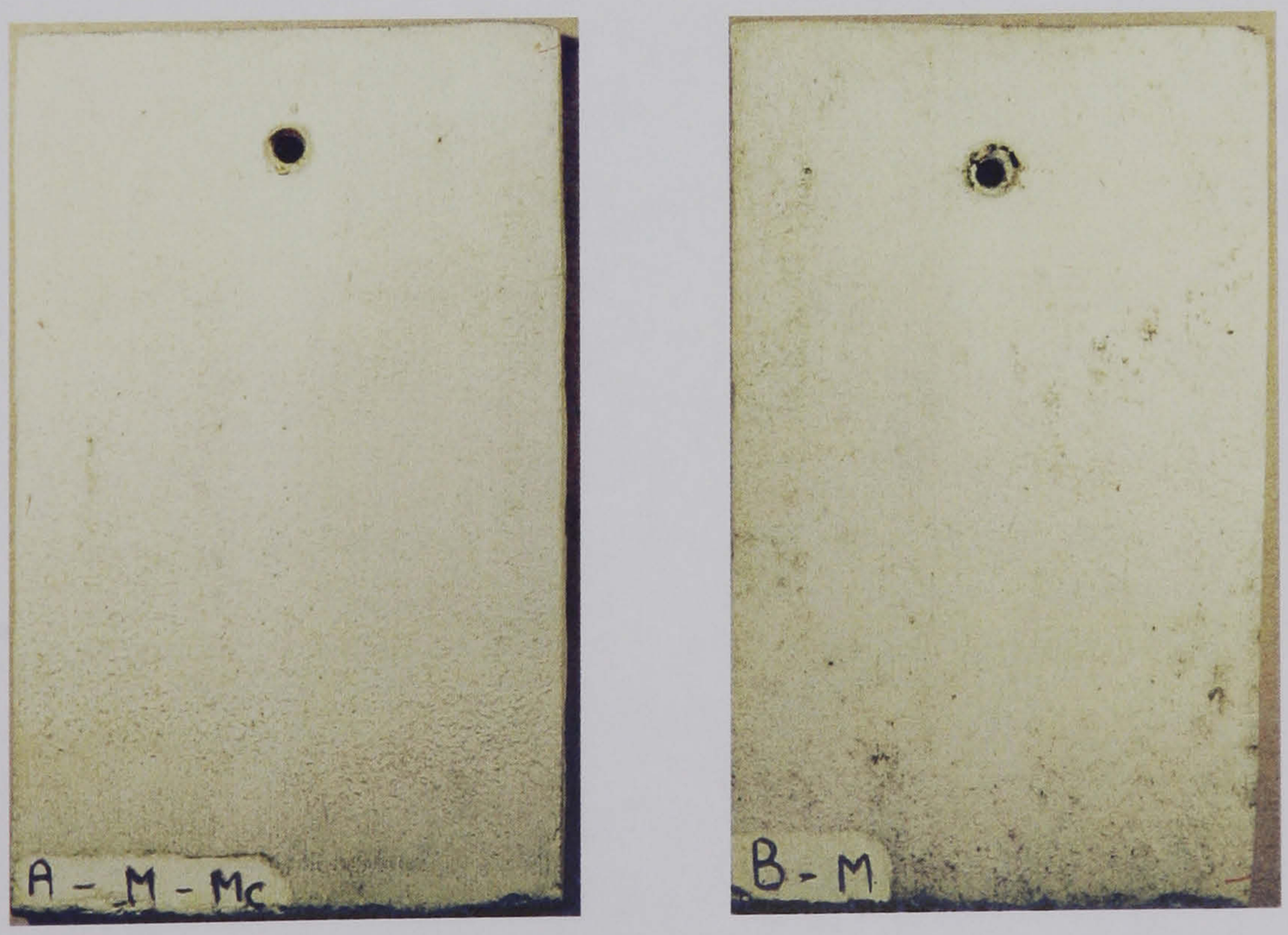
Plate 2.13 shows the photographs of the Masterclad™ panels painted with paints A and B, from these it can be observed that both panels have been colonised, however the panel coated with paint B, (not containing fungicide), appears to be more heavily colonised than paint A, which contains fungicide.

Plate 2.14 shows the Masterclad™ panels painted with paints C and D, from these it can be seen that paint C is lightly colonised whereas paint D, which does not contain the fungicide, is very heavily colonised with algal and fungal colonies. The area around the hole used for attaching the panel to the rack for paint D appears to be free of surface debris, this may be due to water run off, or its position on the rack.

Plate 2.12 Spruce panels coated with paints B, C and D after nineteen months exposure at Blackley.

(Paint B) (Paint C) (Paint D)

Plate 2.13 Calcium silicate panels coated with paints A and B after nineteen months exposure at Blackley.



(Paint A) (Paint B)

Plate 2.14 Calcium silicate panels coated with paints C and D after nineteen months exposure at Blackley.

(Paint C)

(Paint D)

2.7 Discussion

The results obtained from all of the exposure sites show that a wide range of microorganisms were isolated from the panels and that many of the organisms found on the panels were also found within the air micro flora of the sites. This can be seen especially in Appendix E where all of the microorganisms isolated from both the panels and the air at each site are listed.

Any assessment of the extent of colonisation is subjective, particularly in this study where no structured numerical data are recorded. However, we can say that one surface is more extensively colonised than another surface by considering the variety of organisms isolated and the number of times isolates are recorded on the surface during the exposure trial period (Appendix D).

Exposure trials at Sandefjord

From the results of the first exposure trial at Sandefjord, which took place between September and October 1999, it was found that the air micro flora consisted predominantly of fungi, including, *Aureobasidium pullulans*, and members of the genus *Penicillium*. There were also a number of bacteria, algae and one yeast isolated (Table 2.6). Before the exposed panels were swabbed, the photographs shown in Plates 2.3 to 2.5 were taken; they show the presence of algae and fungi on the panels. From the results presented in Table 2.7 there appears to be little difference between the range of organisms isolated from paints A and B. The majority of organisms isolated from the sacrificial panels (Table 2.7 and 2.8) were also isolated from the air (Table 2.6).

From the information provided in Tables 2.7 and 2.8 the formulation fungicide does not appear to have a deleterious effect on the colonisation of paints C and D. *Aureobasidium pullulans* and the yeasts isolated from paints E and F appear to be the major colonisers of these two gloss paints. *Aureobasidium pullulans* was not isolated from the two paints that contained the fungicide (paints A and C). The results presented in Table 2.9 show the organisms isolated from the surface of each of the three types of panel. From this table it can be seen that *Aureobasidium pullulans* was isolated from the majority of the spruce panels. It can also be seen that the spruce panels produced a

greater variety of isolates (21) than the calcium silicate panels (10), which in turn are more heavily colonised than the aluminium panels (8). It is, however, too early to establish conclusively whether the type of panel material, had an effect on the initial colonisation by microorganisms or whether the type of paint (gloss or matt) is more susceptible to microbial colonisation.

The weather data recorded at the time of the exposure (Appendix C:1) showed that the temperature remained stable at about 18°C, the relative humidity fluctuated between about 60% and 100% whereas the amount of rainfall varied from day to day with an average of about 20 mm falling a day.

The results of the second exposure trial at Sandefjord, that took place in July 2000, show that microorganisms found in the air micro flora of the previous exposure were also present at this one (Table 2.6 and 2.10). The microorganisms isolated from the sacrificial panels show that the panels painted with paint A were colonised more extensively (11) than those coated with paint B (7) (Table 2.11). This is contrary to expectation, as it would be reasonable to expect that the panels coated with a non-fungicide containing paint would have more organisms on them. The results obtained for paints C and D indicate that paint D produced a similar flora (10) to paint C (9) (Tables 2.11 and 2.12). Panels coated with paints E and F (Table 2.12) produced a similar range of isolates and no more so than the matt paints, A to D.

Table 2.13 shows the microorganisms isolated from the surface of the different types of panel. Paint A produced more isolates than paint B regardless of the underlying panel material. The spruce panels were more heavily colonised than the calcium silicate or aluminium panels.

The weather data recorded throughout the exposure period (Appendix C:2) was similar to that recorded between September and October 1999 (Appendix C:1). The rainfall was 4.5 mm a day on average whereas the temperature was lower than that of the previous exposure, between 13-18°C, and the relative humidity remained between 60% and 100%.

The exposure at Sandefjord between September and October 2000 consisted of sacrificial panels for paints A and B only. From Table 2.14 it can be seen that both paint A and B provided a range of isolates, although paint B shows a greater variety of isolations (14) than paint A (8). The aluminium and calcium silicate panels were not sampled at this exposure and neither was the air micro flora.

The weather data recorded (Appendix C:3) showed a consistently low temperature (10°C), the rainfall fluctuated, although the total amount recorded during the exposure period was less than that recorded at the September / October 1999 exposure.

Photographs were taken of some of the panels from the exposure between June and August 2001, before they were swabbed, these can be seen in Plates 2.6 to 2.8. With the aid of a stereoscopic microscope algae and fungi can be seen on the spruce panels. There was an accumulation of algae towards the upper and lower edges of the spruce panels (Plate 2.6 and 2.7), probably due to the pooling effect of water on the panel and an accumulation of surface debris. This was not observed as much in the calcium silicate and aluminium panels possibly because they are more slender in nature. Fewer organisms are visible on the Masterclad™ panels and fewer still on the aluminium panels. After swabbing it could be seen that the sacrificial panels coated with paint B produced more isolates (16) than those coated with paint A (8) (Table 2.15). A comparison of the three different panel materials (Table 2.16) shows that the spruce panels produced the highest number of isolates (19), followed by the aluminium (14) and then the calcium silicate (11).

The weather data recorded at the final exposure trial at Sandefjord between June and August 2000 (Appendix C:4) showed a mean temperature of about 10°C, the relative humidity was between 80% and 100% and the rainfall totalled about 90 mm for the exposure period. The weather data recorded throughout this exposure trial was very similar to that recorded for the previous year at the same time period.

The results obtained from Sandefjord, indicate that the panels that provided the most isolates, were the spruce panels, followed by the calcium silicate and finally aluminium panels and therefore that the nature of the panel material had an effect on the colonising microbes. The nature of the surface of the paint in these trials, whether gloss or matt

finish, did not seem to affect the spectrum of organisms isolated. The results obtained by employing the series of sacrificial panels did not provide evidence of a colonisation pattern but did, however, provide information on what appear to be frequent colonisers, such as *Aureobasidium pullulans*, *Rhodotorula rubra*, and *Cellulomonas sp*. As four time periods were examined, two in the Summer months and two in the Autumn, a seasonal effect in the colonisations was looked for, but was not observed.

Exposure trials at Bergen

The first exposure trial at Bergen took place in July 2000; from the air settle plates a different range of organisms was identified compared to those in the Sandefjord exposure trials (Table 2.21). This was expected as Bergen has a different climate to Sandefjord and is more of an urban location. The Sandefjord site is in close proximity to the paint factory and woodland area, whereas the Bergen site is on the roof of the laboratories in Bergen city centre. The weather data recorded throughout the exposure (Appendix C:5) showed an average temperature and low precipitation in general with some days having up to 18 mm of rain in a day. The sacrificial panels (Table 2.22) showed that *Aspergillus fumigatus* was isolated frequently from the panels coated with paints A and B. The remainder of the sacrificial panels did not provide many isolates (Table 2.23). Table 2.23 also shows that *Aspergillus fumigatus* was a primary isolate within this exposure and it appears that the painted spruce surface was the most heavily colonised (11), followed by the gamma irradiated spruce (5), the aluminium (4) and finally the calcium silicate panels (3). As in the first Sandefjord trial, the panels coated with paint containing fungicide (paint A) have not been colonised by *Aureobasidium pullulans*, although this organism does not feature predominantly within this particular exposure.

The exposure that took place in Bergen between September and October 2000 did not include the use of air settle plates. The only information available from this trial are the results of the colonisation of the sacrificial panels (Table 2.24), which as in the previous exposure (Tables 2.22 and 2.23) show *Aspergillus fumigatus* as the major isolate, present on the majority of the sacrificial panels. The weather data for this period (Appendix C:6) showed a high precipitation level and average temperatures. This

change within the weather however, does not appear to change the colonisation of the panels in any way.

The final exposure at Bergen took place between June and August 2000. The weather during this exposure was much the same as in the previous exposures (Appendix C:7), except for the higher rainfall recorded. From the isolations obtained from the sacrificial panels (Table 2.25) it can be seen that *Aureobasidium pullulans*, is a primary coloniser on both of the paints A and B, unlike the previous trials (Tables 2.22, 2.23 and 2.24). Table 2.26, which shows the isolates obtained from the paint films of the different substrata, supports this, as *Aureobasidium pullulans* was isolated from all the surfaces of the panels, including those that were gamma irradiated. The paint films coating spruce panels produced the highest number of isolates (16) followed by the calcium silicate (12) with the aluminium (11) and gamma irradiated panels having the least number of organisms isolated from them (8).

The overall results of the exposure trials that took place at Bergen provide no evidence of a colonisation pattern or of fungicide efficacy. There is also no evidence of a seasonal effect. *Aspergillus fumigatus* was seen in two trials, September and July 2000, but not in the June to August 2001 trial. There is not enough evidence to suggest that the nature of the substratum affects the colonising microbes at this site, as only two exposures took place where the colonisation of the paints with the three underlying materials could be compared.

Exposure trials at Preston

The exposure trial that took place in Preston between September and October 1999 showed that numerous organisms were isolated from the air settle plates, including *Saccharomyces cerevisiae*, *Aspergillus* sp. and *Rhodotorula rubra* (Table 2.30). These are all organisms that were isolated at the previous exposure sites. The *Saccharomyces cerevisiae* may have been in the air due to there being a brewery less than 10 Km from the exposure site. The weather data recorded (Appendix C:8) throughout the exposure period revealed that there was a low level of precipitation, the relative humidity fell between 50% and 100% and the temperature averaged about 15°C.

From the exposed sacrificial panels coated with paints A and B (Table 2.31) it could be seen that only one fungus had been isolated from the panels coated with paint A, whereas many were isolated from paint B. No bacteria were isolated from paint B, although three were isolated from paint A. From the data in Table 2.32 it can be seen that more microorganisms were isolated from paint B than A and a similar pattern is seen from paints C and D, with the majority of microorganisms being isolated from paint D; the presence of fungicide in this exposure trial seems to be effective. From this table it can also be seen that the nature of the panel can affect the colonisation of the paint, in this case the spruce is the most heavily colonised (31), followed by the calcium silicate (17) and finally the aluminium panels (11). From these results it is not possible to establish whether the finish of the paint, matt or gloss had an effect on the isolations although it can be seen that the gloss paints are not as heavily colonised as the non-fungicide containing matt paints (Table 2.32).

The second exposure trial at Preston, between April and May 2000 showed that the air micro flora contained many of the organisms encountered in the previous trial (Table 2.30 compared to Table 2.33). The weather data recorded (Appendix C:9) revealed that the weather conditions were not unlike those of the September / October exposure (Appendix C:8). The temperature rose steadily from about 10°C to 20°C, when there was a low level of precipitation and the relative humidity ranged between 50% and 80%. From the sacrificial panels (Table 2.34) it can be seen that *Cellulomonas* sp. and *Aspergillus fumigatus* are the predominant organisms isolated from the panels. The panels painted with paint A appear to be slightly more heavily colonised than those painted with paint B, which is surprising as paint A contains fungicide and B does not. A comparison of the isolates obtained from the surface of the different panel materials (Table 2.35) shows that the spruce panels yielded the greatest diversity of microorganisms (12), followed by the aluminium (11) and the gamma irradiated spruce panels (10) and closely followed by the calcium silicate panels (9). From these results it is not possible to state whether the material of the panel influences the pattern of microorganisms isolated. These results also show that there was no difference in the level of diversity between the matt and gloss finishes.

The exposure at Preston between February and March 2001 showed that the air micro flora had not changed significantly since the previous exposure (Table 2.36). The

weather throughout the exposure period was cold with temperatures dropping below zero at times and the level of precipitation was low (Appendix C:10). This suggests that the weather did not alter the diversity of microorganisms within the air micro flora. The results of the sacrificial panels (Table 2.37) showed that both paints A and B produced isolates from swabs; with paint B providing a greater variety of organisms. Table 2.38 showed that the spruce panels provided the most diverse flora (26 isolates); followed by the calcium silicate (18) and aluminium panels (15) and finally the gamma irradiated spruce panels (14). This again suggests that the material of the panel has an effect on the colonisation of the painted surface above them.

The results of the final exposure trial at Preston, between June and August 2001 showed that paint A had a greater diversity of microorganisms isolated from it (14) than paint B (9) (Table 2.39). The material of the panel influenced the range of organisms isolated from the paint films above them (Table 2.40) i.e. the spruce panels had the most (21), followed by the aluminium (15), calcium silicate (14) and finally the gamma irradiated panels (12).

Exposure trails at Blackley

The results of the first Blackley exposure during April and May 2000 showed that the organisms isolated from the air were very similar to those found at the three previous exposure sites, (Table 2.44). These included *Alternaria alternata*, *Penicillium chrysogenum*, *Bacillus* sp., *Rhodotorula rubra* and *Stichococcus* sp. The weather data recorded (Appendix C:11) at the time show that the temperature had been increasing throughout the month peaking at 23°C and the first half of the exposure had been fairly wet and the latter half dry. The organisms isolated from the sacrificial panels (Table 2.45) showed that there were differences in the isolation pattern between paints A and B. From the organisms isolated from the different panel materials (Table 2.46) it could be observed that the spruce panels produced the most isolates (20) followed by the aluminium panels (12) and the calcium silicate panels (11).

The exposure that took place between February and March 2001 showed that the organisms isolated from the air (Table 2.47) included *Aspergillus fumigatus*, *Penicillium chrysogenum*, a *Bacillus sp.* and a *Stichococcus sp.*, i.e. similar to those

found in the previous trial (Table 2.44). The weather data recorded (Appendix C:12) at that time showed a varied temperature range, between 4°C and 15°C whereas the rainfall was fairly low with the occasional day having up to 6 mm of rain. The results from the colonisation of the sacrificial panels (Table 2.48) showed that as in previous exposures there were differences in the organisms isolated between the paint containing fungicide and the one without. The results displayed in Table 2.49 show that the gloss paints (paints E and F) provided fewer isolates than the matt paints and that of the matt paints, paints C and D were less colonised than paints A and B. Paint A had more organisms isolated from its surface than paint B; this had been seen in previous trials at other sites (Table 2.40). As with previous trials the spruce panels were more colonised (21) than the calcium silicate panels (15), which in turn were more colonised than the aluminium panels (11).

When the panels were removed from the exposure site at Manchester in November 2001 it was found that the gloss paints were less colonised than the matt paints (Table 2.50). There is no obvious fungicidal effect or colonisation pattern; *Aureobasidium pullulans* was seen to be a frequent isolate and the nature of the substratum had an effect on the colonisation of the painted surface. As in previous exposures the spruce panels produced the most isolates, followed by the calcium silicate and then the aluminium panels. From the results of these exposure trials it can be concluded that:

1. The nature of the substratum has an effect on the colonising microbes.

   It is perhaps to be expected that some nutrients may diffuse from the wood of the panels, through to the paint film. This would account for the higher incidence of microorganisms at the surface of the paint (Tables 2.9, 2.13, 2.16, 2.23, 2.26, 2.32, 2.35, 2.38, 2.40, 2.46, 2.49, 2.50).

2. There does not appear to be a seasonal effect on the organisms that were isolated.

   The weather data recorded during the various periods of exposure are such that one might expect a similar spectrum of representative microorganisms of the flora isolated at 30°C to be isolated from the panels, microorganisms that thrive under these conditions. The nature of the air micro flora will, to some extent, dictate the nature of the microorganisms isolated from the panels.

3. Employing the series of sacrificial panels did not provide any evidence of a colonisation pattern or sequence.

An unexpected result was the higher incidence of isolations obtained from paint A, which contained a fungicide. Possibly the effectiveness of the fungicide was not obvious because:

(a) Any nutrients at the surface will reduce the biocidal effect. (The presence of nutrients promoting active growth can mask the effect of biocides, which are not as effective against less active fungi. This situation will be exacerbated by the presence of dust, dirt and pollutants).

(b) The microorganisms recorded were transient flora (rather than true colonisers), which were unaffected by the biocide. (Transient flora are not in contact with the surface of the paint film for a long enough period).

(c) A low residence time of the microorganisms or

(d) The fungicide was ineffective.

The timing and duration of the exposure trials were such that it is not realistic to expect to see a discernable colonisation pattern. However, certain common genera and species were encountered throughout the study i.e. *Aureobasidium pullulans*, *Alternaria alternata*, *Penicillium chrysogenum* and *Aspergillus fumigatus*. MALDI TOF MS was used to establish whether these organisms were the same at each exposure site or whether different strains were involved.

CHAPTER 3.

THE APPLICATION OF MALDI TOF MASS SPECTROMETRY TO FUNGAL IDENTIFICATIONS.

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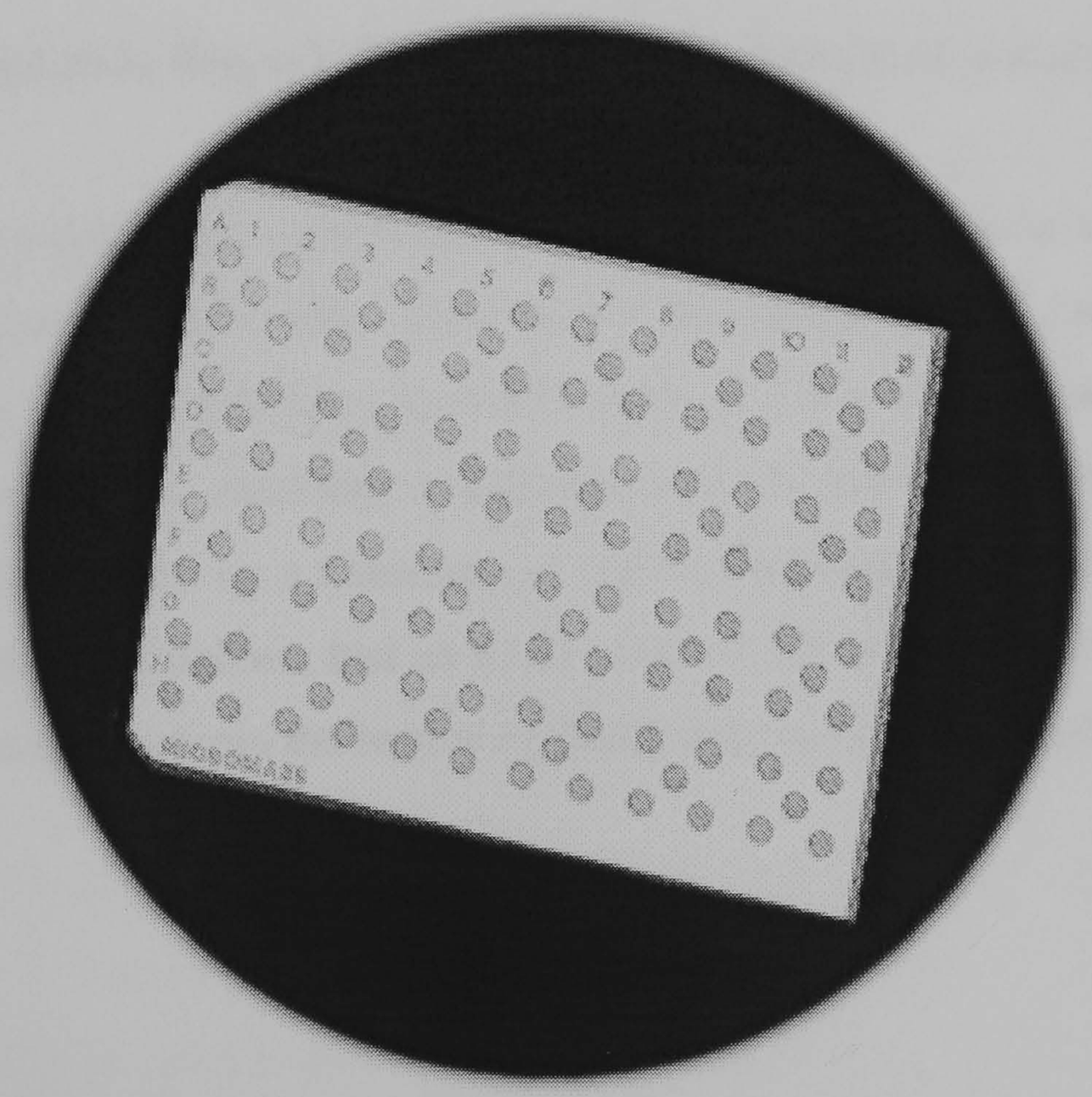
3.1 INTRODUCTION

Matrix assisted laser desorption / ionisation time of flight mass spectrometry (MALDI TOF MS) is emerging as a useful tool in bacterial classification. It was made available through Micromass UK. Ltd., Manchester, with the assistance of Dr. Therese McKenna. The technique enables ionisation of cell surface molecules to generate a mass spectrum and therefore bacteria can be classified according to their mass spectral fingerprint (Keys *et al*., 2000 ; Mckenna *et al*., 2000 ; Van baar, n.d).

Mass fingerprints of unknown bacteria can be matched reliably against databases of quality controlled reference mass spectra. The process is not time consuming, with the entire procedure being completed within minutes for each microorganism. The sample preparation is quick and easy. Intact cells from a primary culture are smeared across a stainless steel target plate (Figure 3.1) and the preparation allowed to crystallise with a UV absorbing matrix. After drying, the target is placed into the MALDI TOF mass spectrometer.

Figure 3.1 The stainless steel target plate.



The microorganisms in this matrix are illuminated with a pulse from a nitrogen laser at 337nm. The matrix absorbs energy from the laser and macromolecules from the surface are desorbed and ionised. The resulting ionised macromolecules are mass analysed and the results displayed as a plot of mass (x axis) versus abundance (y axis). The Mass-fingerprint of the test microorganism is then submitted to the MicrobeLynx™ search algorithm which challenges an appropriately selected database from a wide range of quality controlled bacterial reference spectra.

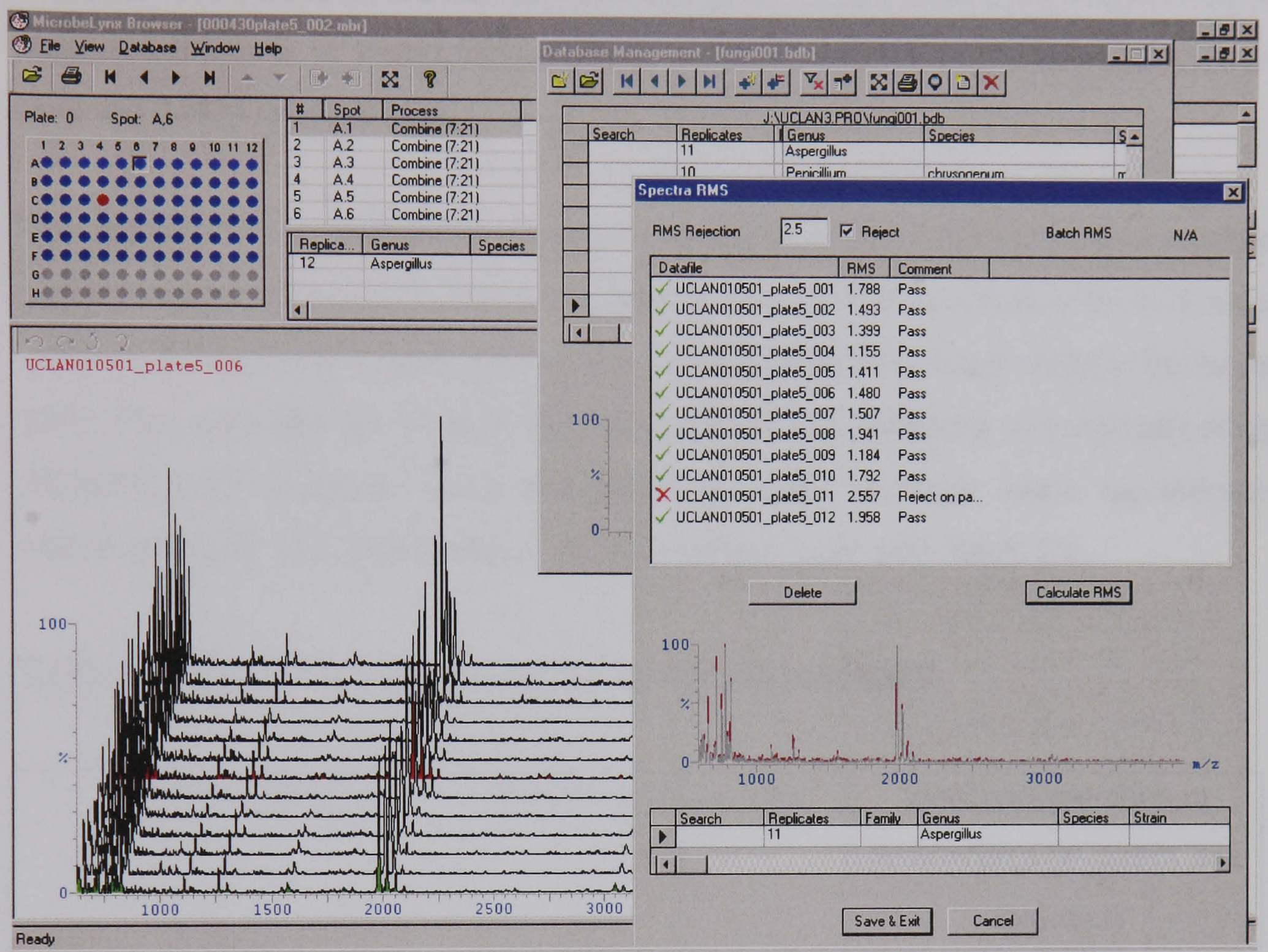
In this study MALDI TOF mass spectrometry has been used to evaluate the potential application of the technique in identifying fungi, from mycelia, spores and subtending hyphae. The fungi used included those that were isolated from the four exposure sites as described in Chapter 2. Some work has been done previously on the characterisation of fungal spores (Welham *et al.*, 2000) but none, to date, on the identification of fungi.

Whilst MALDI TOF MS is not intended to replace conventional taxonomic techniques, a database of the spectra may provide additional information on the taxonomic relationship of the isolates obtained. For the technique to be successful the mass spectral fingerprint must be reproducible using standard protocols and it must be independent of both the instrument and the operator.

3.2 MicrobeLynx Search Engine and the database creation

MicrobeLynx enables the reproducibility of replicate spectra to be compared using the root mean square (RMS) value, obtained by the comparison of each replicate in turn with the eleven other replicates. An RMS value of 3.0 is usually used to reject outliers prior to inclusion in a database. A database was created from nine tentatively named fungi, each represented by a minimum of twelve replicates. The spectra that were used to create the fungal database, had an RMS value of 2.5 or less. Figure 3.2 represents the rejection of an outlier from the twelve replicate spectra from *Aspergillus fumigatus*.

Figure 3.2 Rejection of an outlier from twelve replicate spectra from *Aspergillus Fumigatus.*



3.3 The reproducibility / reliability of MALDI TOF MS and a comparison of matrices.

3.3.1 Materials and Methods

Fungal mycelium and spores were removed aseptically using a loop from a culture of *Alternaria alternata* that had been grown on malt extract agar at 30°C for seven days. These were suspended in sterile distilled water. 1µl was applied directly to each of the twelve wells of one row of the target plate. Six of the wells were then covered with 1µl of the matrix solution α-cyano-4-hydroxycinnamic acid (α-CHCA) (Sigma) at 14mg/ml, which is usually used with Gram negative bacteria. The remaining six wells were covered with 1µl of 5-chloro-2-mercaptobenzothiazole (CMBT) (Aldrich) at

3mg/ml, (which is usually used with Gram positive bacteria) and allowed to dry prior to mass spectrometric analysis. The UV absorbing matrix solutions used (α-CHCA and CMBT), which serve to transmit the laser energy to the cells, were prepared freshly as saturated solutions in water, methanol and acetonitile (1:1:1) containing 0.1% formic acid and 0.08M crown-6 ether.

The instrument was calibrated using the average molecular weights from a standard peptide mixture (Table 3.1). The renin substrate ion at 1760 Da was used for lock mass calibration. 1μl of the peptide mixture was applied to the five single wells in the target plate. The mass spectral fingerprints were acquired and processed automatically using MicrobeLynx™ software on a MALDI linear time of flight mass spectrometer (Micromass UK, Ltd., Manchester, UK) over the m/z range 500-20000 Da.

Table 3.1 The peptide mixture used to calibrate the instrument

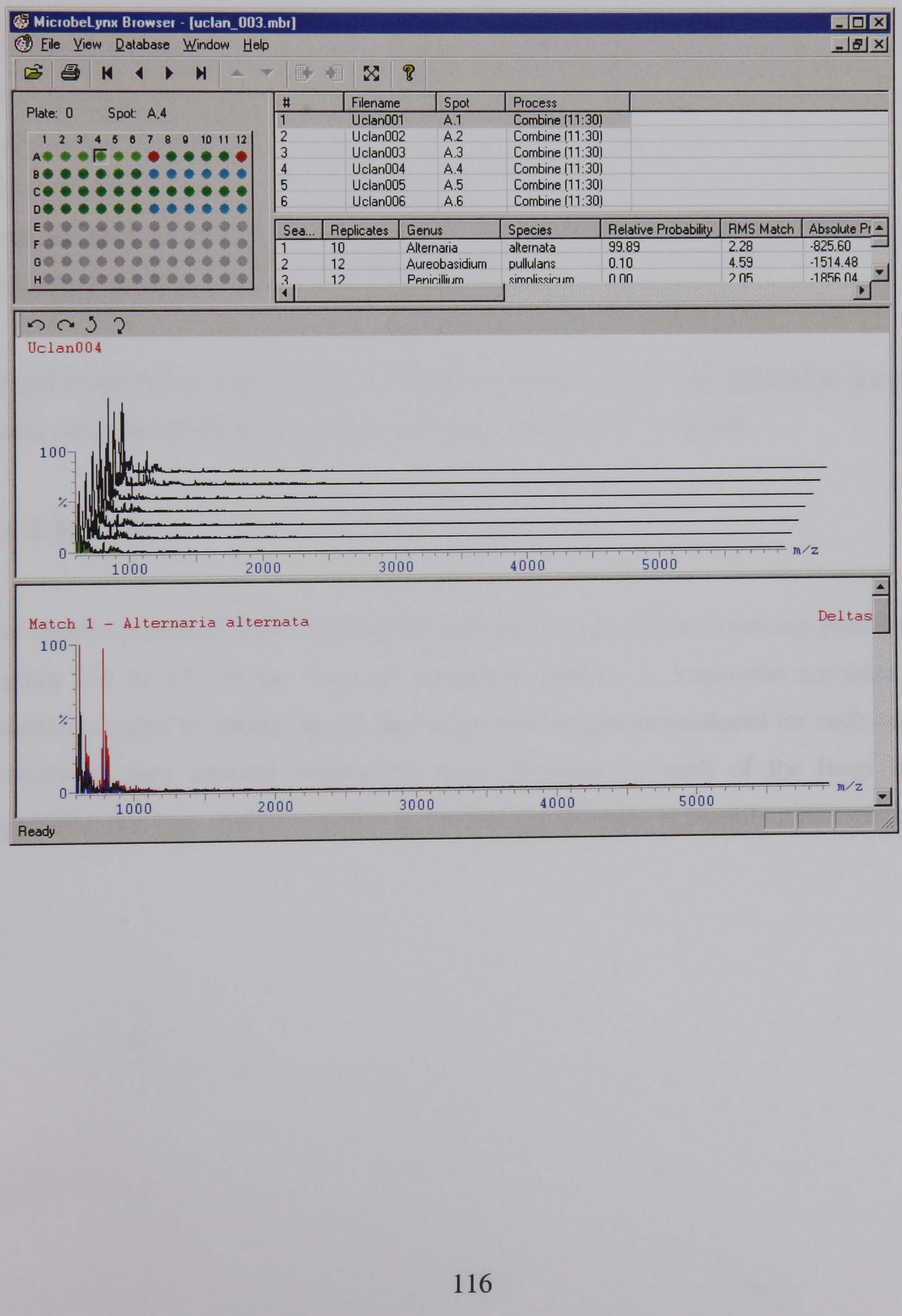
Substance name	Average molecular mass (amu)	Final concentration in protein/peptide matrix mix (pmol/μl)
Bradykinin	1060.2	1.0
Angiotensin 1	1296.5	1.0
Glu-fibrino peptide B	1570.6	1.0
Renin substrate	1759.0	1.0
ACTH (18-39 clip)	2465.7	1.0
Bovine insulin	5733.5	2.0
Ubiquitin	8564.9	10.0

3.3.2 Results.

Figure 3.3 shows the mass spectral fingerprint for *Alternaria alternata* in both CMBT, wells 7-12 and α-CHCA, wells 1-6 for rows A to D. Average spectra from the six replicates were searched against the database. Matches higher than 80% are indicated by the green wells, whilst the turquoise wells indicate matches lower than 60%. It can be seen that the computer software has identified the isolate as *Alternaria alternata* with a

99.89% certainty indicating that the instrument has the capability to 'recognise' new spectra and match them to spectra already held in the database. From these data it can be established that the α-CHCA matrix is the more informative as there was a higher degree of matches to the sample in the database, this is seen in rows 1-6 being green compared to the turquoise in rows 7-12 which contain the CMBT matrix.

Figure 3.3 Search results for *Alternaria alternata* analysed in α-CHCA and CMBT



3.4 To establish whether different genera of fungi produce different spectra

3.4.1 Materials and Methods

The five genera of fungi and pink yeast listed below, were grown on malt extract agar for seven days at 30°C.

Alternaria alternata

Aureobasidium pullulans

Fusarium oxysporum

Aspergillus fumigatus

Penicillium chrysogenum

Rhodotorula rubra

Fungal suspensions were made as described in Section 3.3.1 and applied to the target plates using an α-CHCA matrix and analysed using MALDI TOF MS.

3.4.2 Results

The spectra produced from the different genera of fungi and the yeast are presented in Figures 3.4 to 3.9, in the form of composite spectra. A composite spectrum is a spectrum created by taking the average of the twelve spectra produced for each culture. Distinctive mass spectral fingerprints were obtained for each of the fungi listed, suggesting that they could be used in a comparative database to identify unknown fungi.

Figure 3.4 The composite spectrum of *Alternaria alternata*

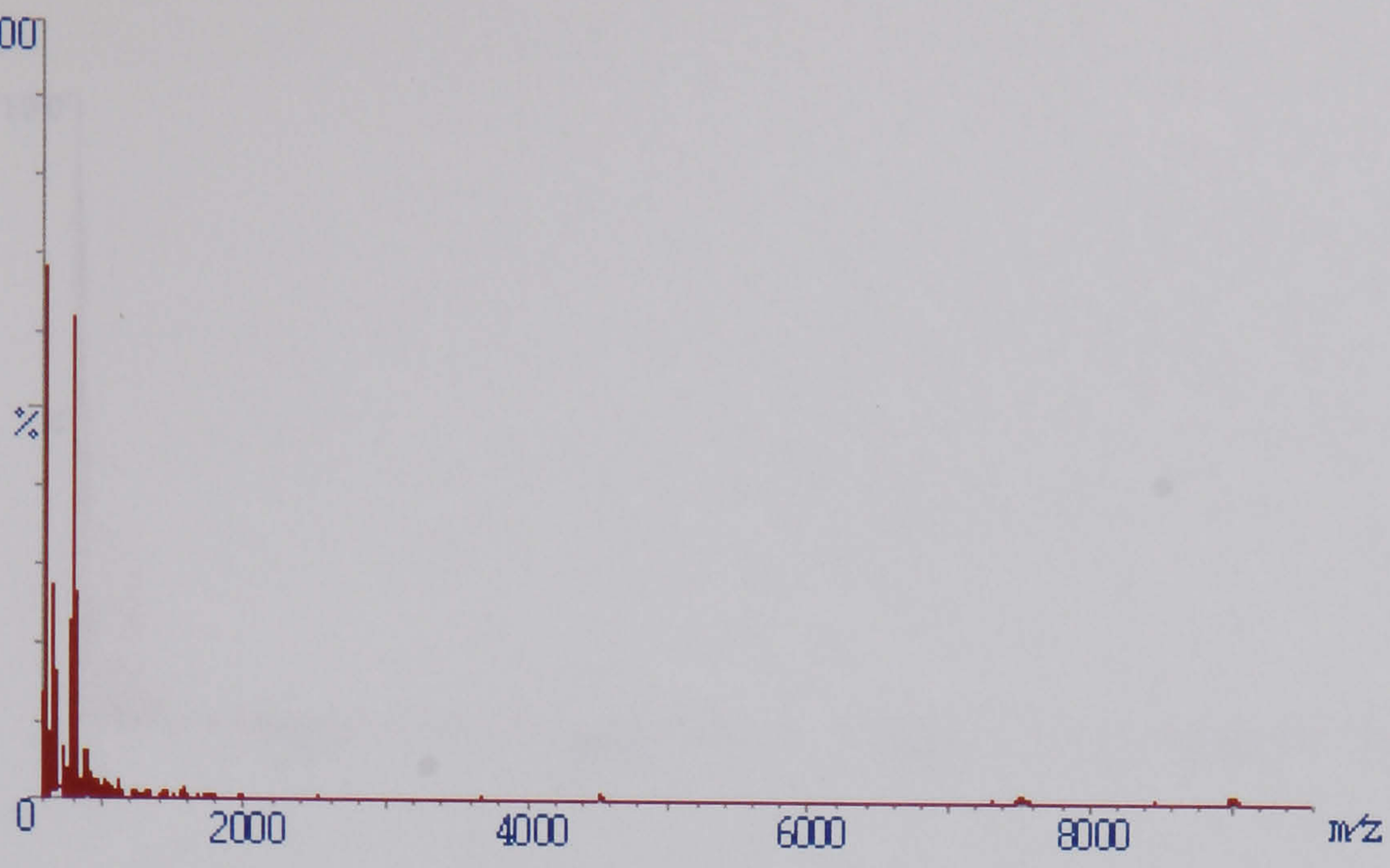


Figure 3.5 The composite spectrum of *Aureobasidium pullulans*

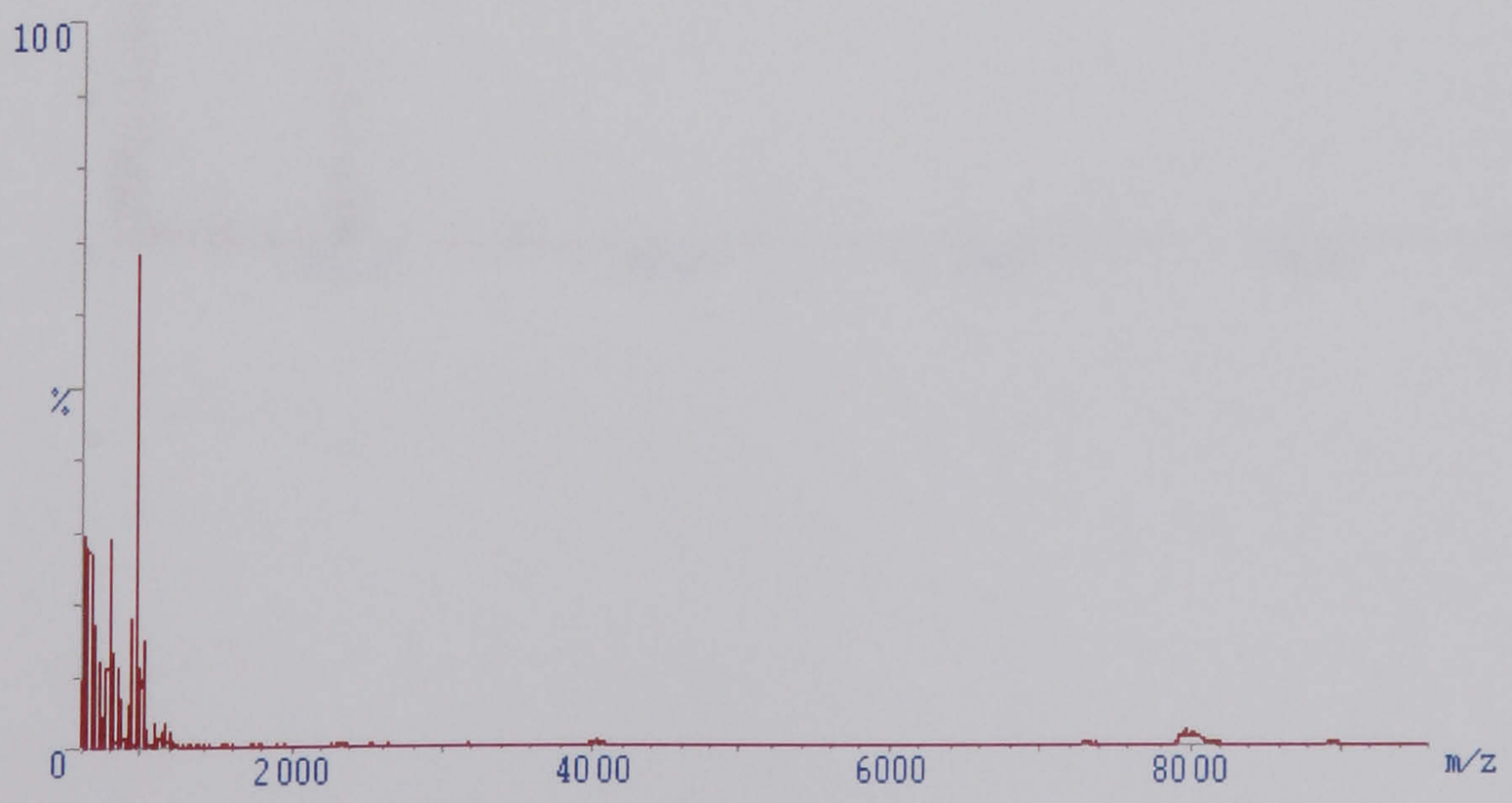


Figure 3.6 The composite spectrum of *Fusarium oxysporum*

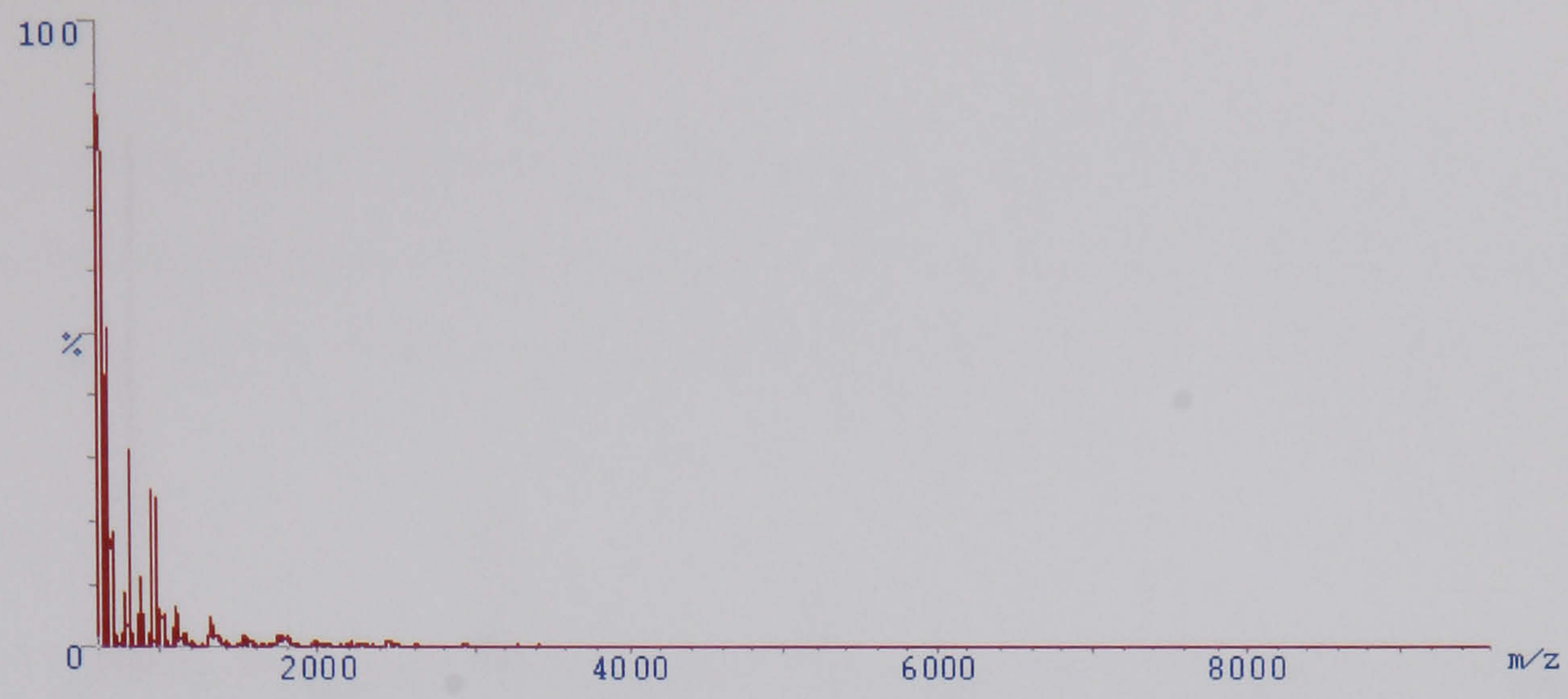


Figure 3.7 The composite spectrum of *Aspergillus fumigatus*

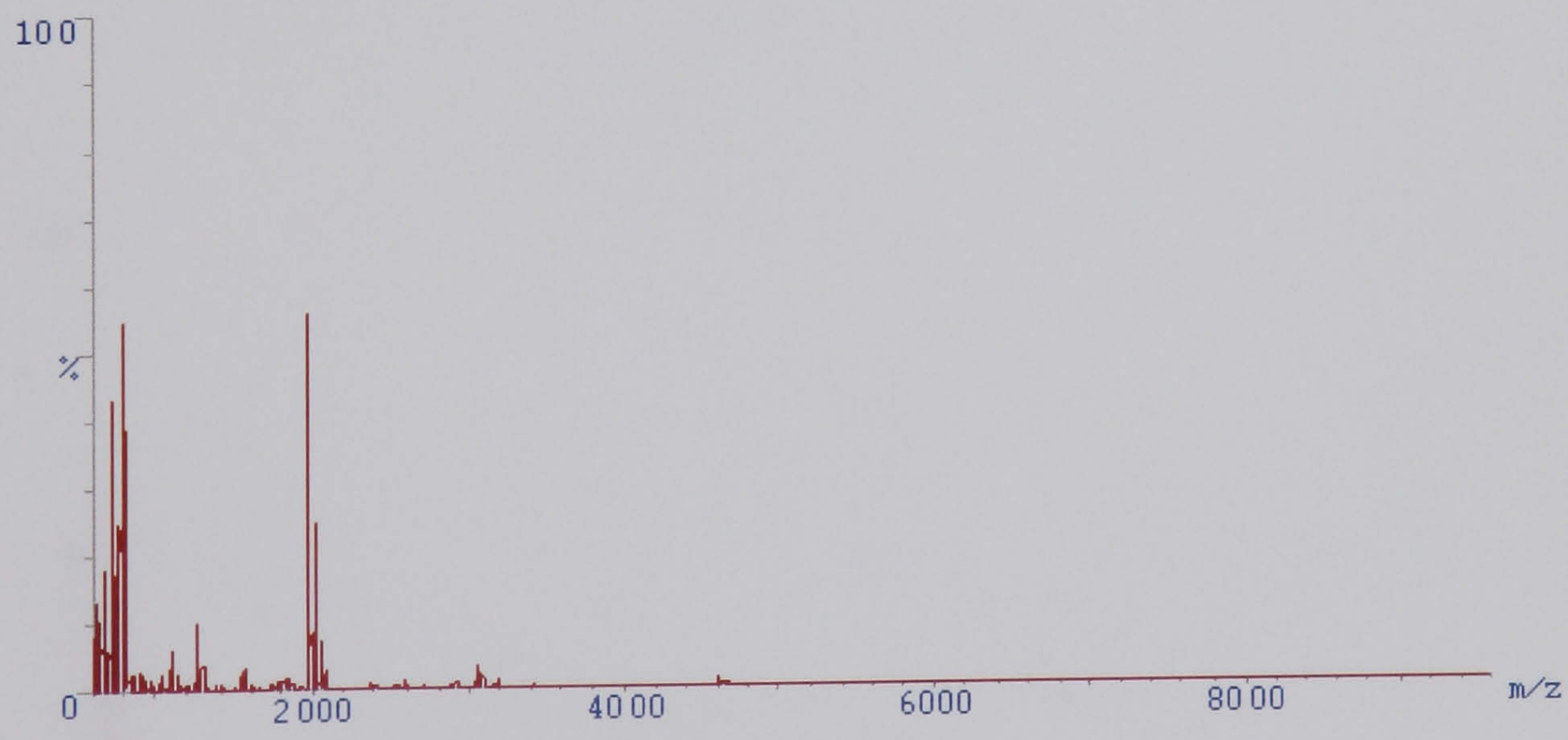


Figure 3.8 The composite spectrum of *Penicillium chrysogenum*

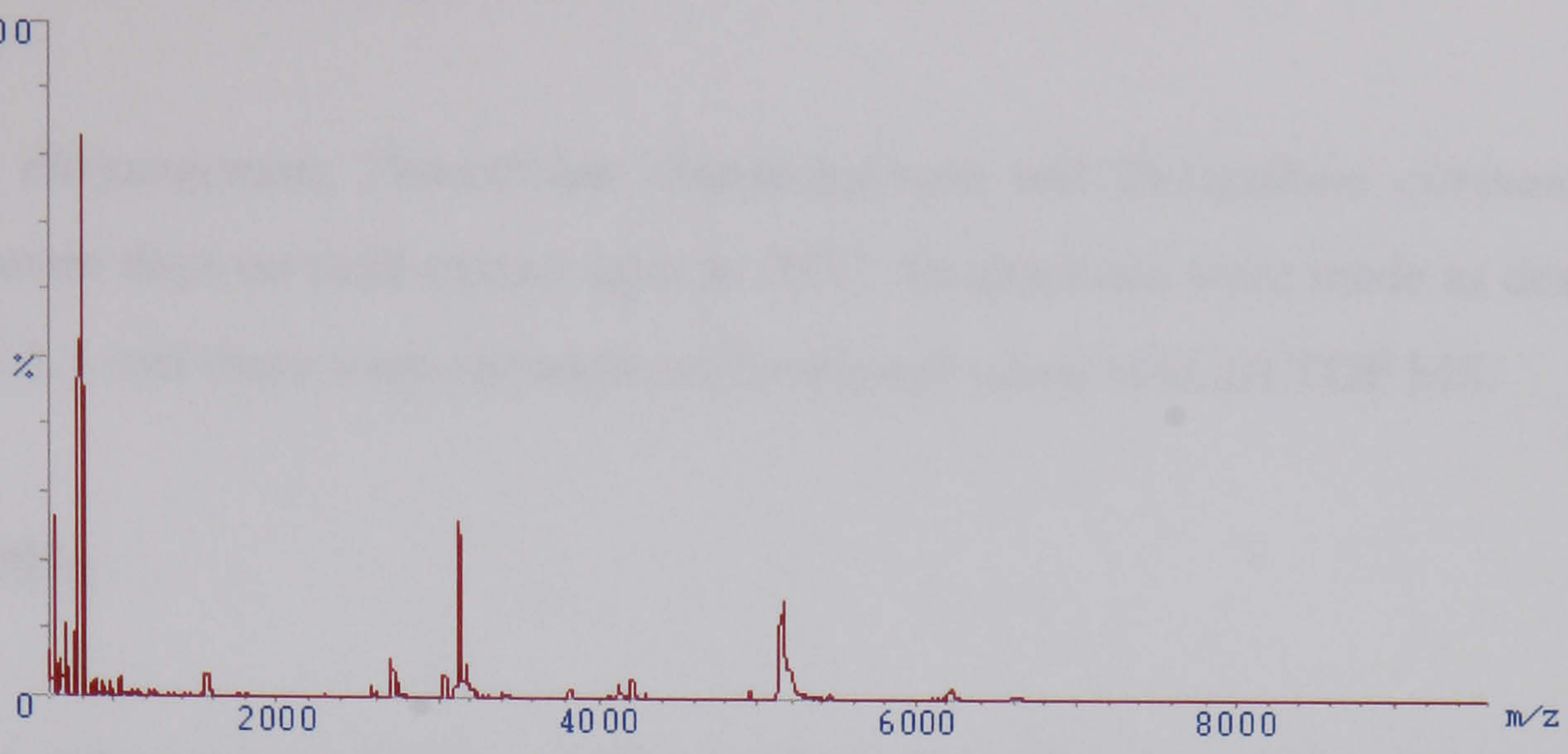
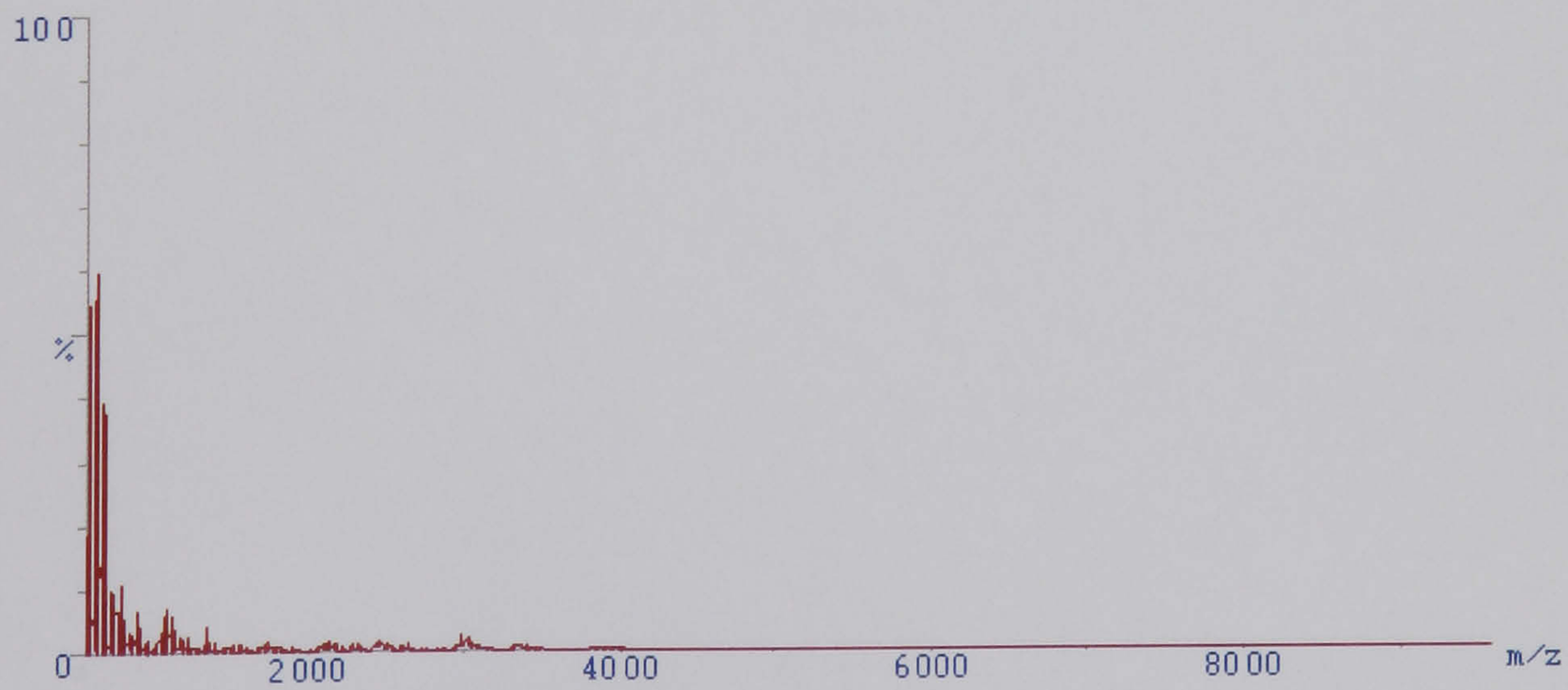


Figure 3.9 The composite spectrum of *Rhodotorula rubra*



3.5 Determination of species level using MALDI TOF MS

3.5.1 Materials and Methods

Penicillium chrysogenum, *Penicillium simplicissimum* and *Penicillium citrinum* were grown for seven days on malt extract agar at 30°C. Suspensions were made as described in Section 3.3.1 and these were subsequently analysed using MALDI TOF MS.

3.5.2 Results

The spectra presented in Figures 3.10 and 3.11 represent the composite spectra of *Penicillium simplicissimum* and *Penicillium citrinum* respectively. When these are compared with *Penicillium chrysogenum* (Figure 3.8) it can be seen that there are differences between the spectra. The spectra for *Penicillium citrinum* and *Penicillium chrysogenum,* are very similar in terms of the position of the peaks, however, it is considered that the spectra confirm their potential in fungal taxonomy.

Figure 3.10 The composite spectrum of *Penicillium simplicissimum*

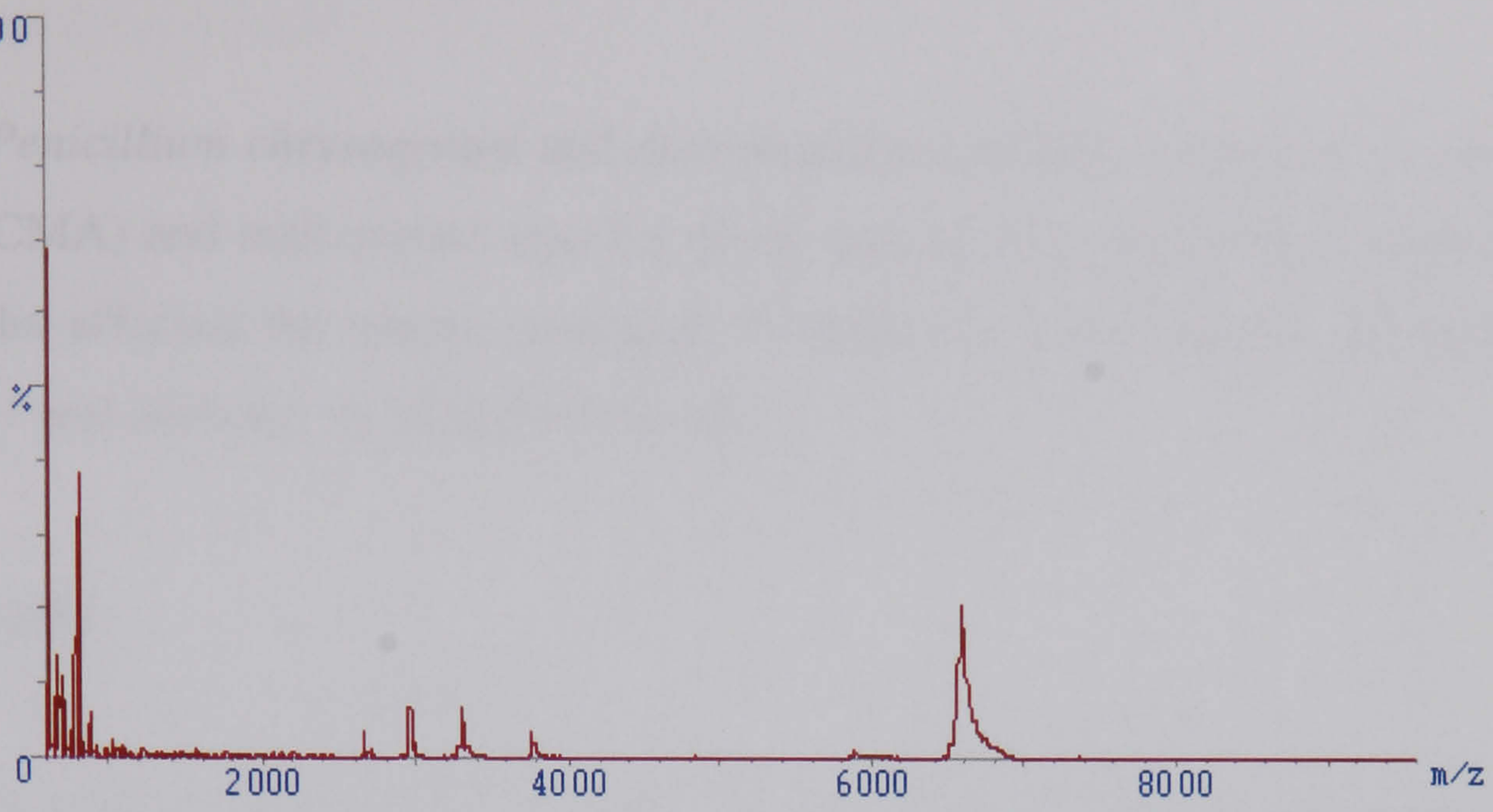
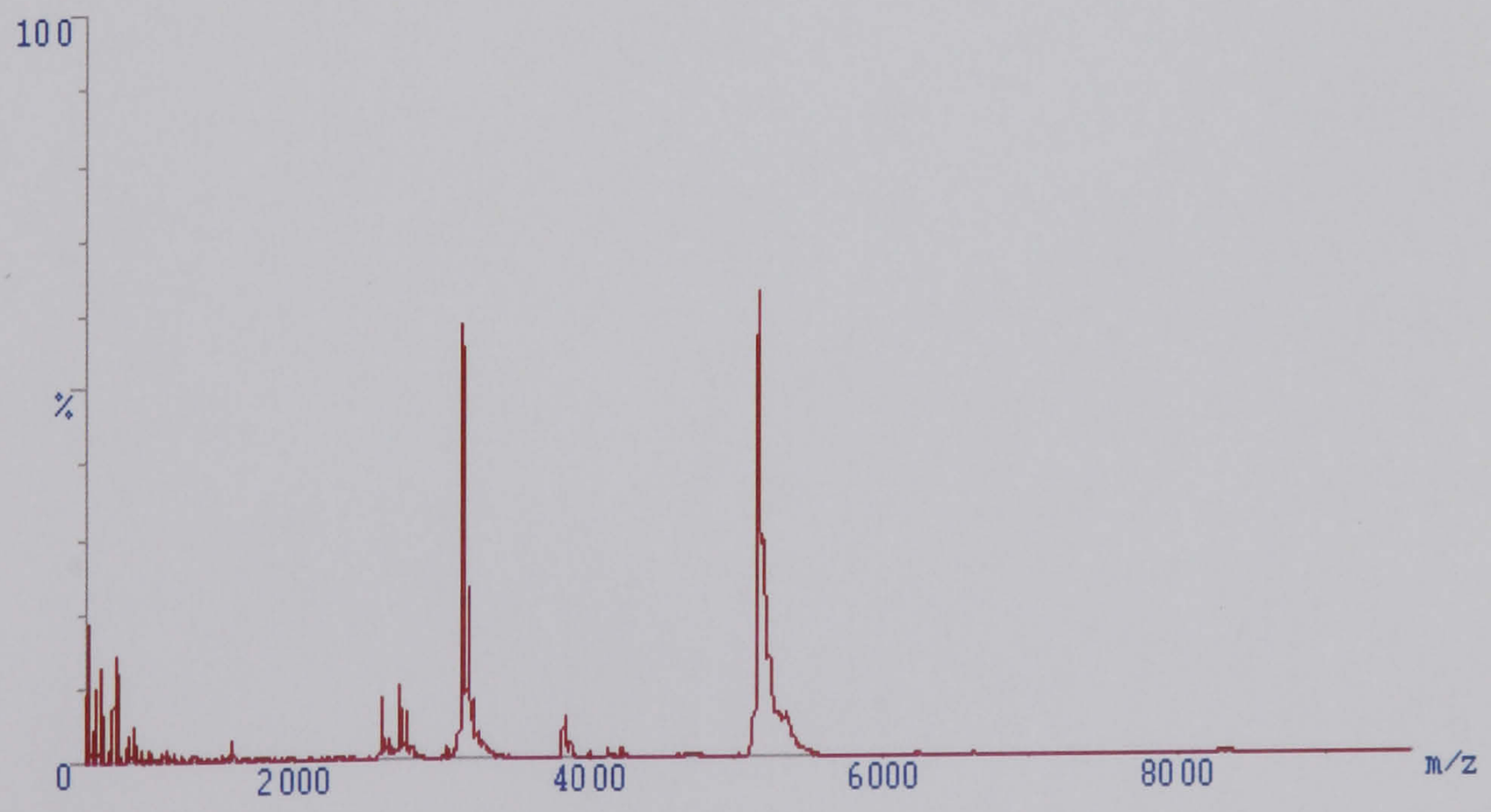


Figure 3.11 The composite spectrum of *Penicillium citrinum*



3.6 Effect of culture media on the spectra generated.

3.6.1 Materials and Methods

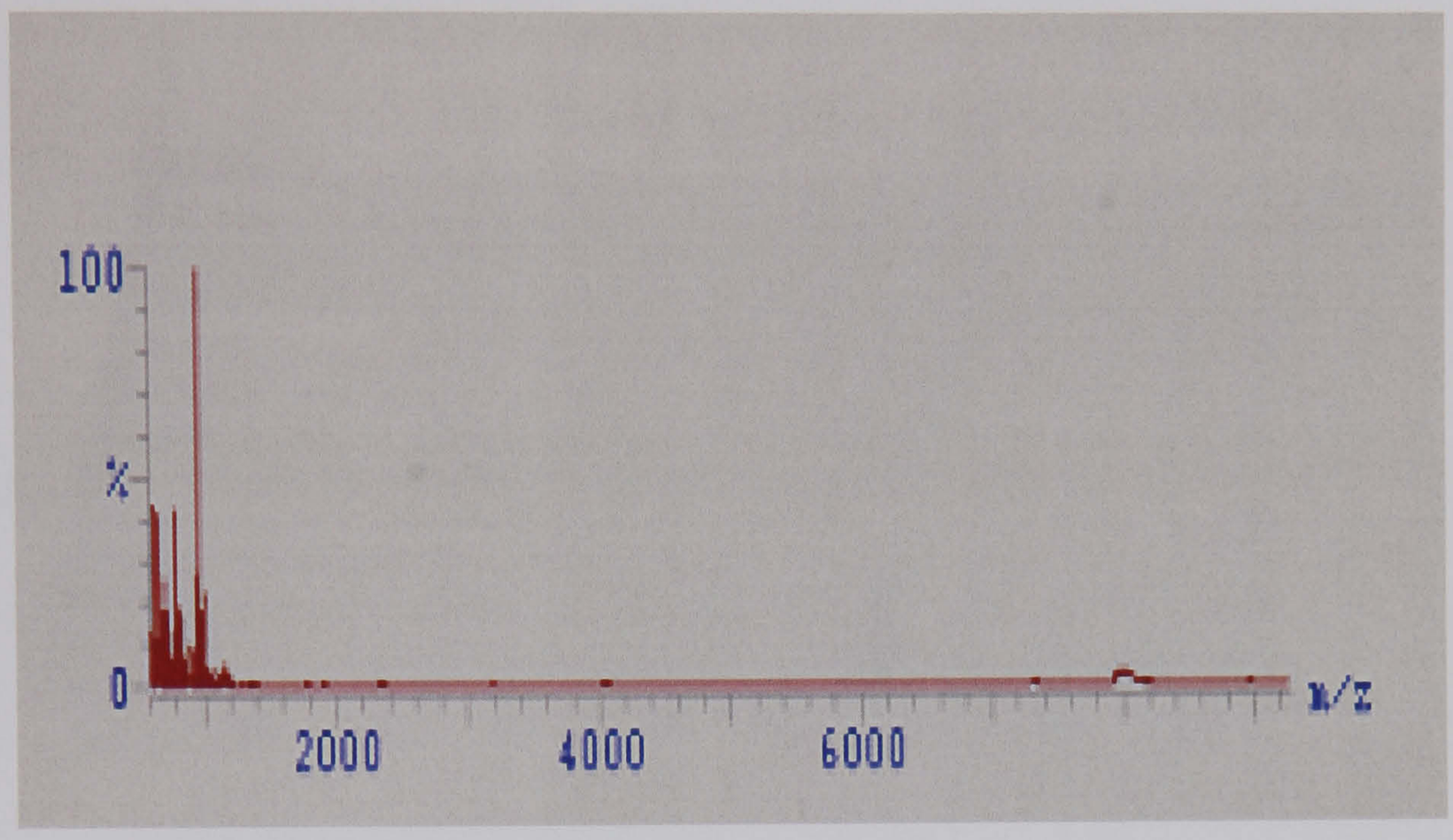
Isolates of *Penicillium chrysogenum* and *Aureobasidium pullulans* were grown on corn meal agar (CMA) and malt extract agar for seven days at 30°C to establish whether the growth media affected the spectra produced. Suspensions were made as described in Section 3.3.1 and analysed by MALDI TOF MS.

3.6.2 Results

The data shown in Figures 3.12 (a) and 3.12 (b) show the spectra produced when *Aureobasidium pullulans* and *Penicillium chrysogenum* were grown on malt extract agar. Figures 3.12 (c) and 3.12 (d) show the spectra produced when *Aureobasidium pullulans* and *Penicillium chrysogenum* isolates were grown on corn meal agar. The twelve replicates from each of the fungi grown on either medium showed good reproducibility and are within an RMS value of 2.5, which is acceptable. A comparison of the mean spectra gave an RMS value of 4.73 for *Penicillium chrysogenum* and 20.36 for *Aureobasidium pullulans*. These high values suggest that the growth medium does have an effect on the fingerprint produced, therefore the media must be standardised.

Figure 3.12 a and 3.12 b Composite spectra obtained from *Aureobasidium pullulans* and *Penicillium chrysogenum* grown on malt extract agar.

(a) *Aureobasidium pullulans*



(b) *Penicillium chrysogenum*

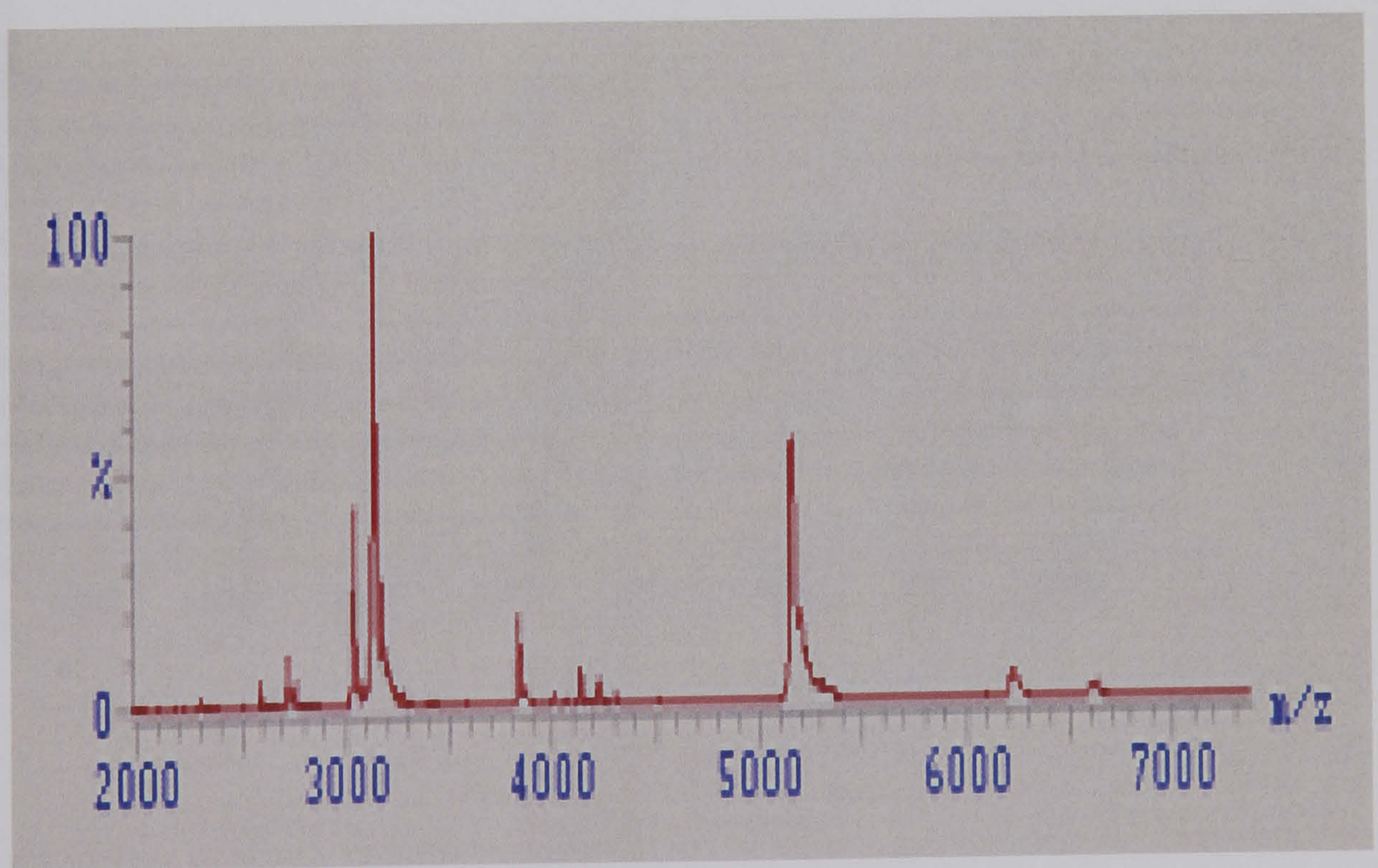
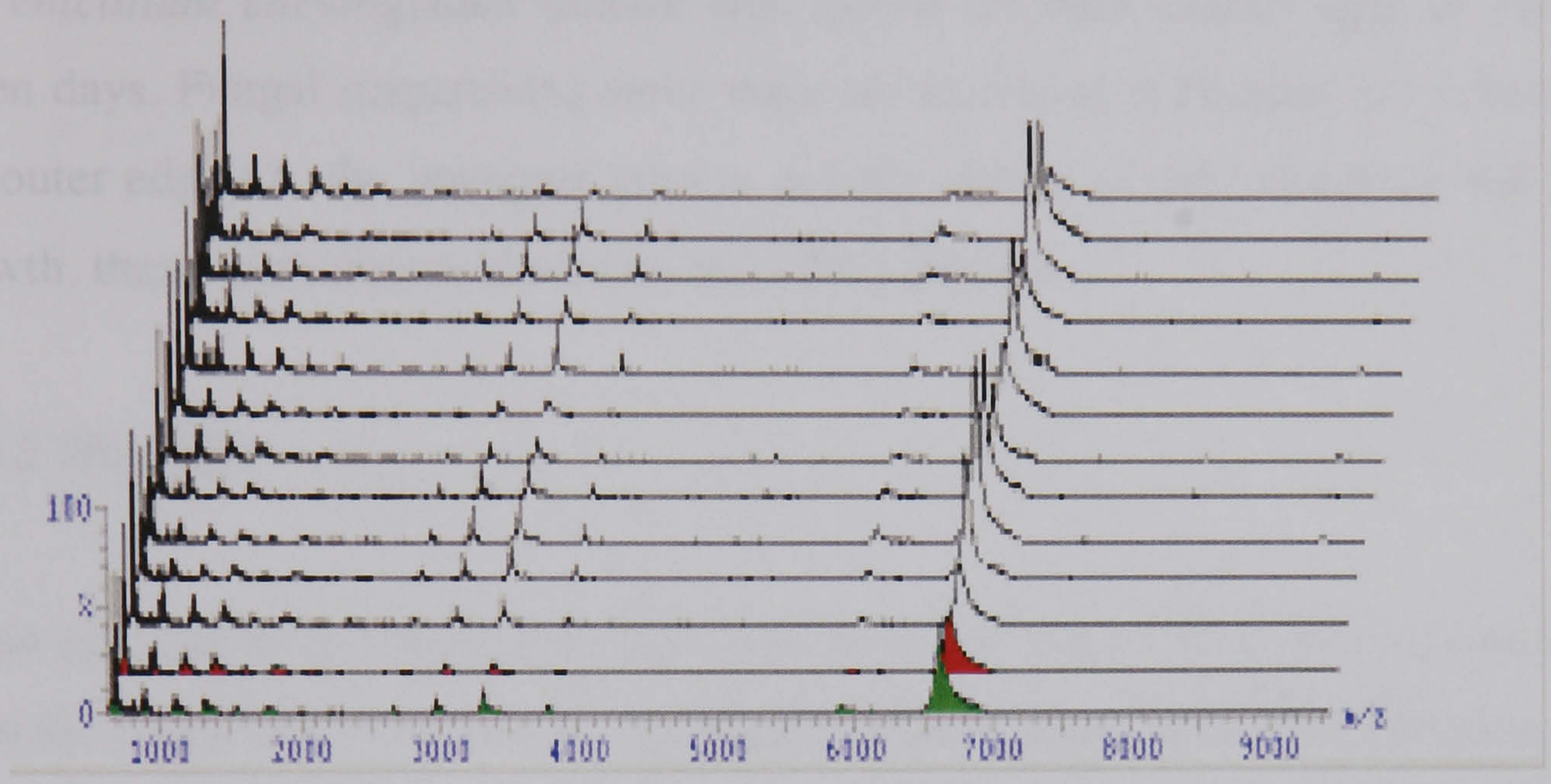
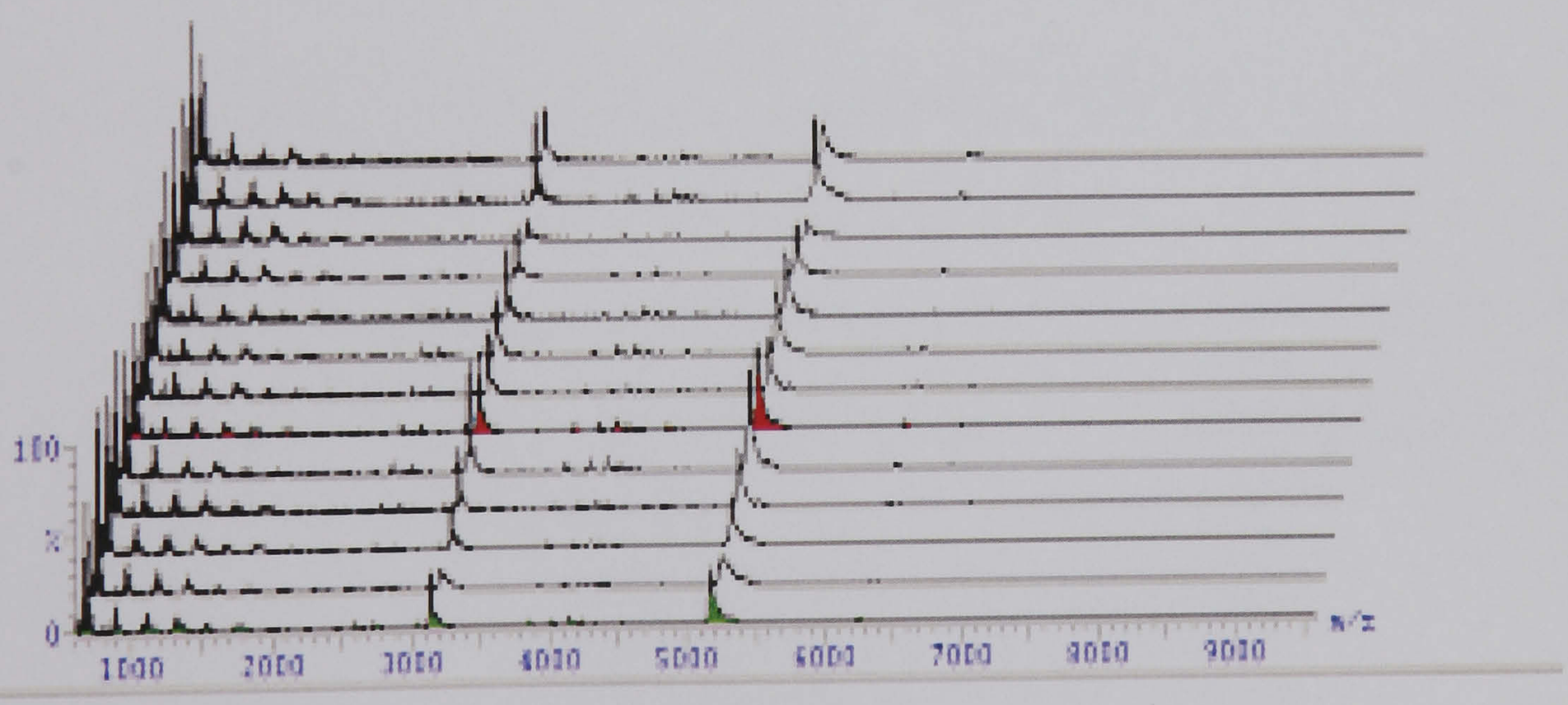


Figure 3.12c and 3.12d Replicate spectra obtained from *Aureobasidium pullulans* and *Penicillium chrysogenum* grown on corn meal agar

(c) *Aureobasidium pullulans*



(d) *Penicillium chrysogenum*



3.7 Effect of the age of the culture on the spectrum produced.

3.7.1 Materials and Methods

A *Penicillium chrysogenum* culture was grown on malt extract agar at 30°C for seven days. Fungal suspensions were made as described in Section 3.3.1 from both the outer edge i.e. the youngest growth and the centre of the culture i.e. the oldest growth, these were then analysed by MALDI TOF MS.

3.7.2 Results

It can be seen from Figure 3.13 that the sample of *Penicillium chrysogenum* taken from the centre of the culture on the plate and the sample taken from the outer edge, show similar spectra. The region from 2500-6500 Da has been expanded so this can be observed more easily. The RMS values calculated for these samples compared to data already in the database can be seen in Table 3.2. The data show that the age of the culture does not affect the spectrum produced.

Figure 3.13 Averaged spectra produced from *Penicillium chrysogenum* cultures of different ages

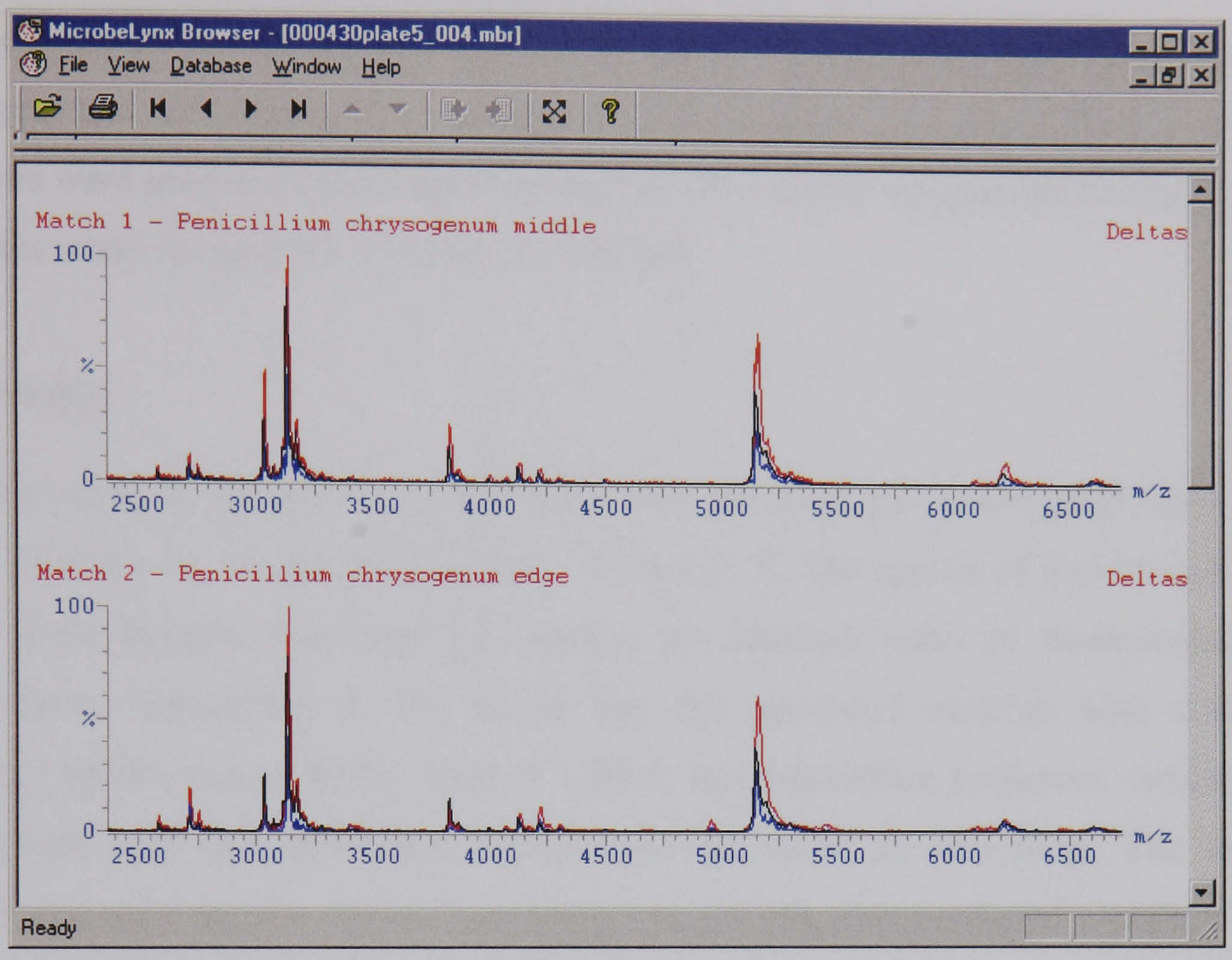


Table 3.2 The RMS values obtained by comparison of old and new growth of *Penicillium chrysogenum*

	Database entries	
	RMS old growth	RMS new growth
Penicillium chrysogenum (new growth)	1.60	1.04
Penicillium chrysogenum (old growth)	0.83	1.61

3.8. Reproducibility with time and the location of sample collection

3.8.1 Materials and Methods

In order to compare the spectra of strains of *Aureobasidium pullulans* from Manchester and Bergen and of *Aspergillus fumigatus* from Preston and Sandefjord. Fungal suspensions were prepared as described in Section 3.3.1 and were applied directly to the target plates ready for analysis by MALDI TOF MS.

3.8.2 Results

The spectra obtained for *Aureobasidium pullulans* and *Aspergillus fumigatus* from the different locations can be found in Figures 3.14 and 3.15. The spectra of *Aureobasidium pullulans* from Bergen, were searched against the database entry of *Aureobasidium pullulans* from Sandefjord. It was found that this produced matches with a high probability (100%) and an RMS value of 1.16 to *Aureobasidium pullulans*, indicating that there was very little difference between the spectra of the two fungi. The same procedure was used for the *Aspergillus fumigatus* isolates, this produced a probability match of 96.73% with an RMS value of 3.98, which is high to be a perfect match, but indicates high similarities between the two spectra.

Figure 3.14 *Aureobasidium pullulans* isolated from Sandefjord (row A1-12) and Bergen (row B1-12) analysed in November 2000 and matched against the database

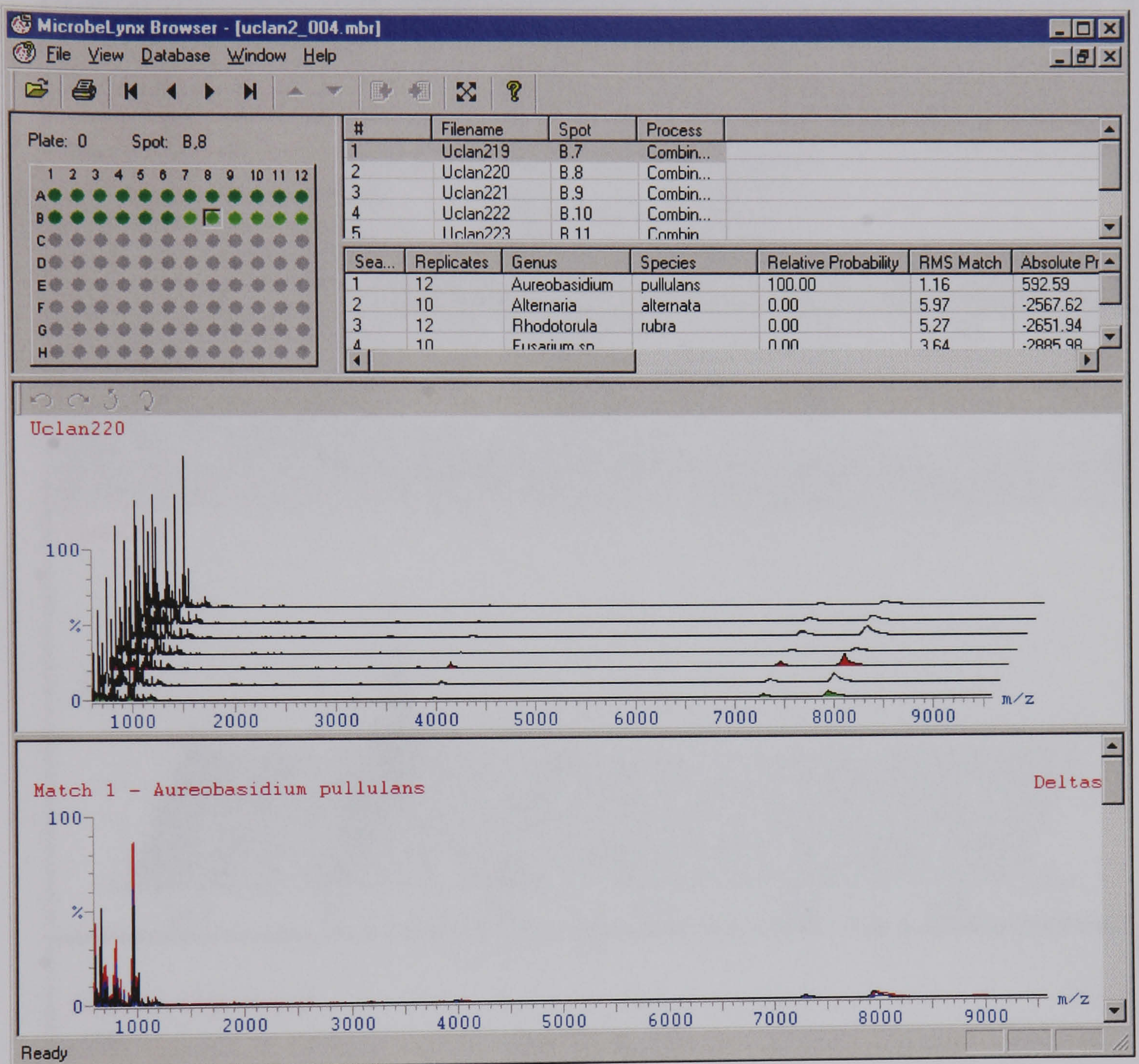
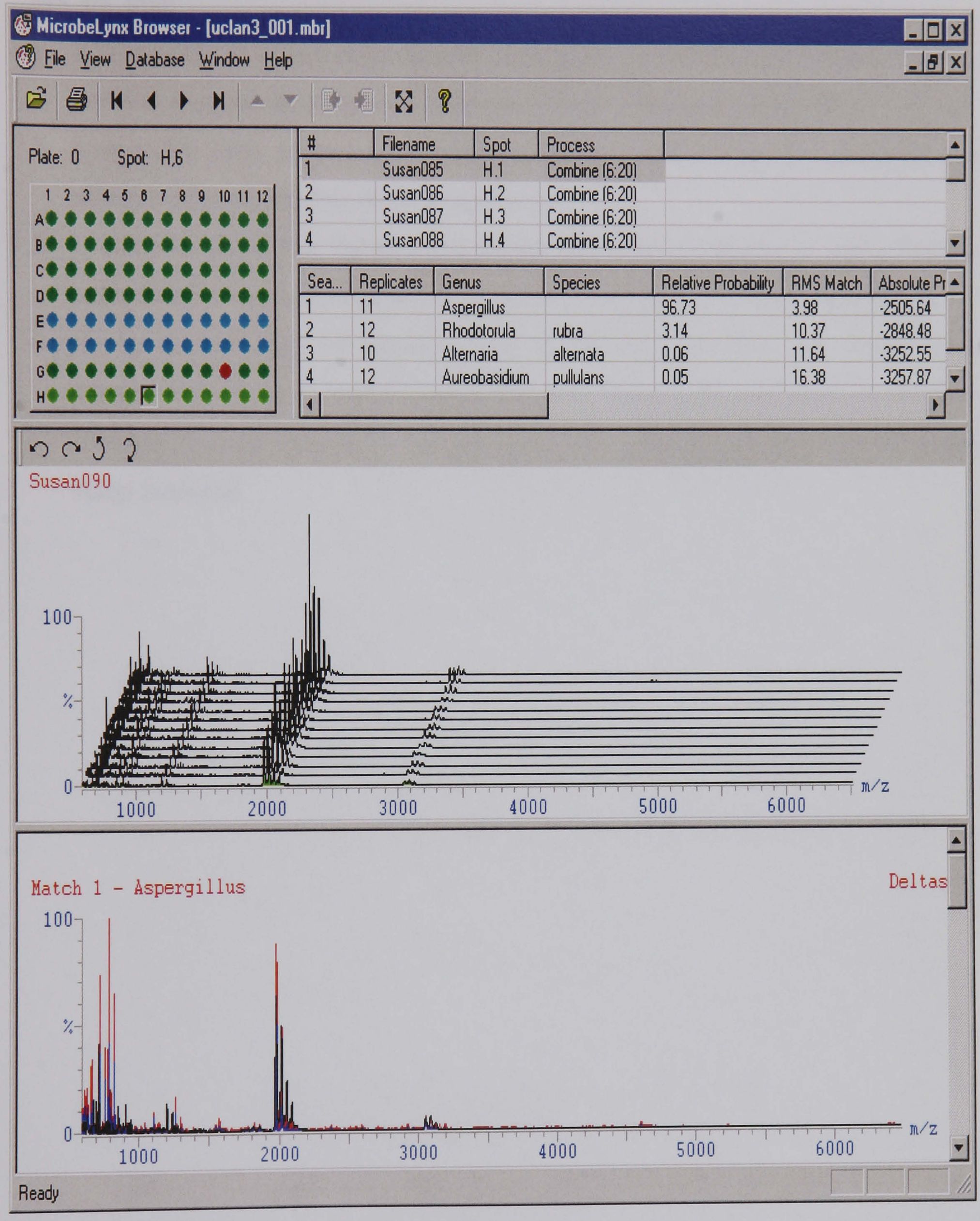


Figure 3.15 The averaged spectrum of *Aspergillus fumigatus* analysed in February 2001 matched against the database.



3.9 Conclusions.

The overall conclusions that can be made from work undertaken with MALDI TOF MS are, that:

1. It is possible to obtain reproducible fingerprints or spectra from fungal cultures, and that they can be matched to reference spectra within the database.
2. Distinctive mass spectra were produced for the different genera analysed, and also for different species of the same genus.
3. It was considered that the growth medium had to be standardised, with all of the subsequent analyses performed using malt extract agar as the growth medium.
4. It was decided that the reproducibility within the replicates, determined by a comparison of each sample spectrum, was acceptable and that of the two matrices evaluated the α-CHCA produced more informative spectra for all of the fungi analysed.

CHAPTER 4.

SURFACE CHARACTERISATION.

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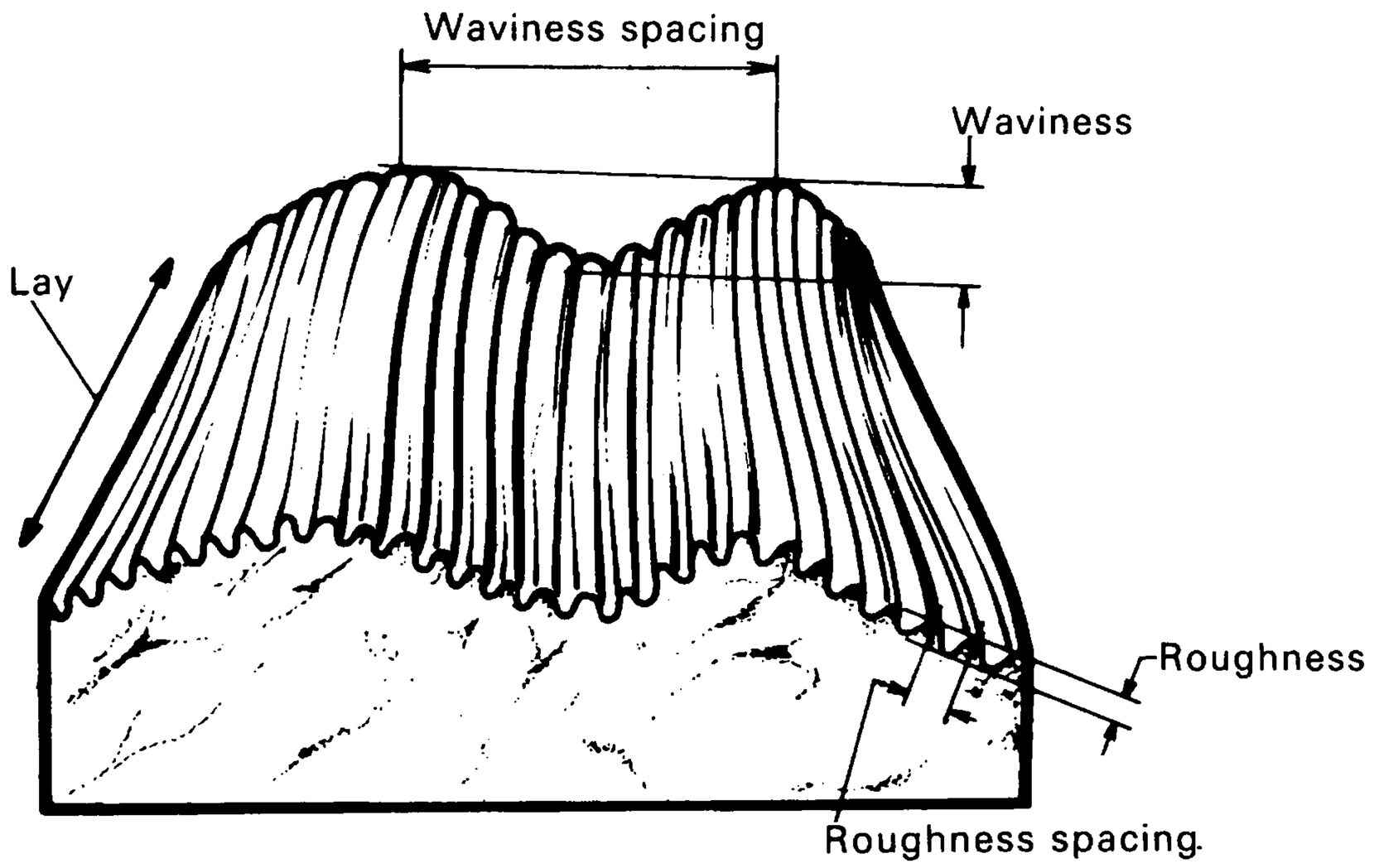
Chapter 4: Surface Characterisation.

4.1 Introduction.

A surface can be defined as the outside of an object, either the uppermost area or the sides (Anon, 1984). Everything has a surface, and surfaces can range from rough to smooth. In many engineering applications surface texture is closely related to function, particularly when one surface comes into moving contact with another surface, for example, a shaft and its bearing (Dagnall, 1980). Surface texture is often associated with how rough or how smooth a surface is. Roughness and waviness when considered together are considered to constitute the texture of a surface (Figure 4.1)

Figure 4.1 The relationship between roughness and waviness (Dagnall, 1980).

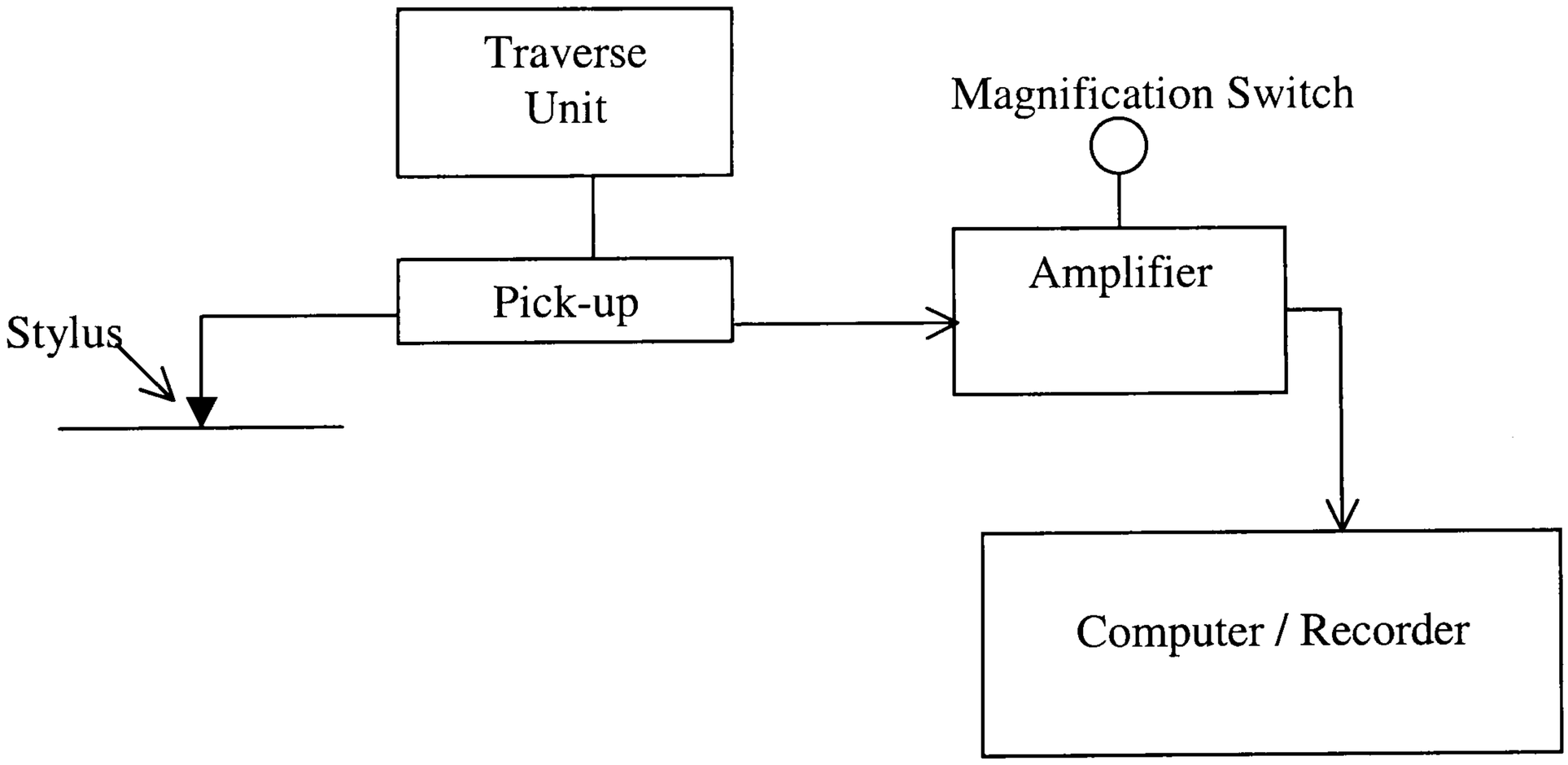


There are various methods for characterising surfaces; these include Surface Roughness Measurement (SRM) by using a Talysurf™ machine, for example; Scanning Electron Microscopy (SEM); Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Force Microscopy (AFM). Each method characterises a surface in a different manner, for example a Talysurf™ examines surface topography, SEM and AFM both examine

surfaces at a high level of magnification and FTIR establishes the chemical composition of the surface in question.

The quantification of surface roughness has been an essential feature within the field of engineering for many years. From as early as 1918, the growing interest in the role played by surface scratches and machining marks in metal fatigue, particularly in the aviation industry, was increasing in importance. By 1929 a simple surface texture recorder was available (Dagnall, 1980). In response to the need for more advanced instruments R.E. Reason began a research project in 1935 into the nature and measurement of surface finishes (Dagnall, 1980). As a result of this work the production of the first 'Talysurf™' instrument began in 1942. The basic components of the Talysurf™ are shown in Figure 4.2. The stylus is traversed across the surface and the pick-up converts the vertical movements into an electrical signal, which is amplified and used to operate a recorder, or the data are fed into a computer.

Figure 4.2 The major components of a stylus based surface texture/roughness measurement instrument (adapted from Dagnall, 1980).



A Talysurf™ is used mainly for evaluation work (Nevelos *et al.*, 1999 ; Chenggui *et al.*, 2000 ; Lee *et al.*, 2000) and for measuring the topography of nominally flat surfaces of engineering products or coatings (Hansen, 1972 ; Gledhill *et al.*, 2001). In this investigation it has been used to characterise and quantify the surface topography of

painted surfaces. A Talysurf™ 4, manufactured by Rank Taylor Hobson has been employed throughout this work. It has been adapted by the inclusion of a computer controlled translation stage, which permits the collection of surface data via a computer. It offers a resolution of 0.5 μm in both the 'x' and 'y' axes and can in principle be used to measure sample areas up to 100mm^2. However, specimens are restricted to being no more than 10mm^2 in practice due to the practical considerations of data storage and collection time. The data is analysed in the 'Mountains Map' programme, produced by the French company Digitalsurf. The topography of the surface can be displayed in a number of ways, including a 3D image, known as a 'meshed axonometric diagram'; a 'pseudo-colour image' and a 'contour diagram'. Using this software a list of 'parameters' can be obtained, as shown below. Parameters are simply a numerical description of the feature being measured (Dagnall, 1980).

The quantifiable parameters obtained from the system (Digitalsurf, 1997).

The parameters are as follows:

Sa – The arithmetic mean

(i.e. The average roughness of the sample being measured; in this case the painted surface on the wooden panel)

Sq – The quadratic mean

(i.e. the root mean square (rms) of the sample. This is obtained by squaring each value for the peaks and troughs in the given sample and then taking the square root of the mean of them)

St – The total height of the sample, from the highest to the lowest point (St = Sp +Sv)

Sp – The highest peak over that calculated as the mean peak

Sv – The lowest valley under that calculated as the mean valley

Ssk – Skewness –Indicates the symmetry of the depth distribution within a standard amplitude statistical distribution curve (Figure 4.3). The direction of the skew depends on whether the bulk of the material is above the mean line (a negative skew) indicating that the surface is mainly composed of a plateau and shallow valleys, or below the mean line (a positive skew) indicating a surface with numerous peaks on a substratum.

Sku – Kurtosis – measures the sharpness and the shape of the distribution curve.

Sz – The mean value of the five highest and five lowest points on the sample.

Figure 4.3 Positive and negative skewness.

Ordinate height distribution.

Profile of surface

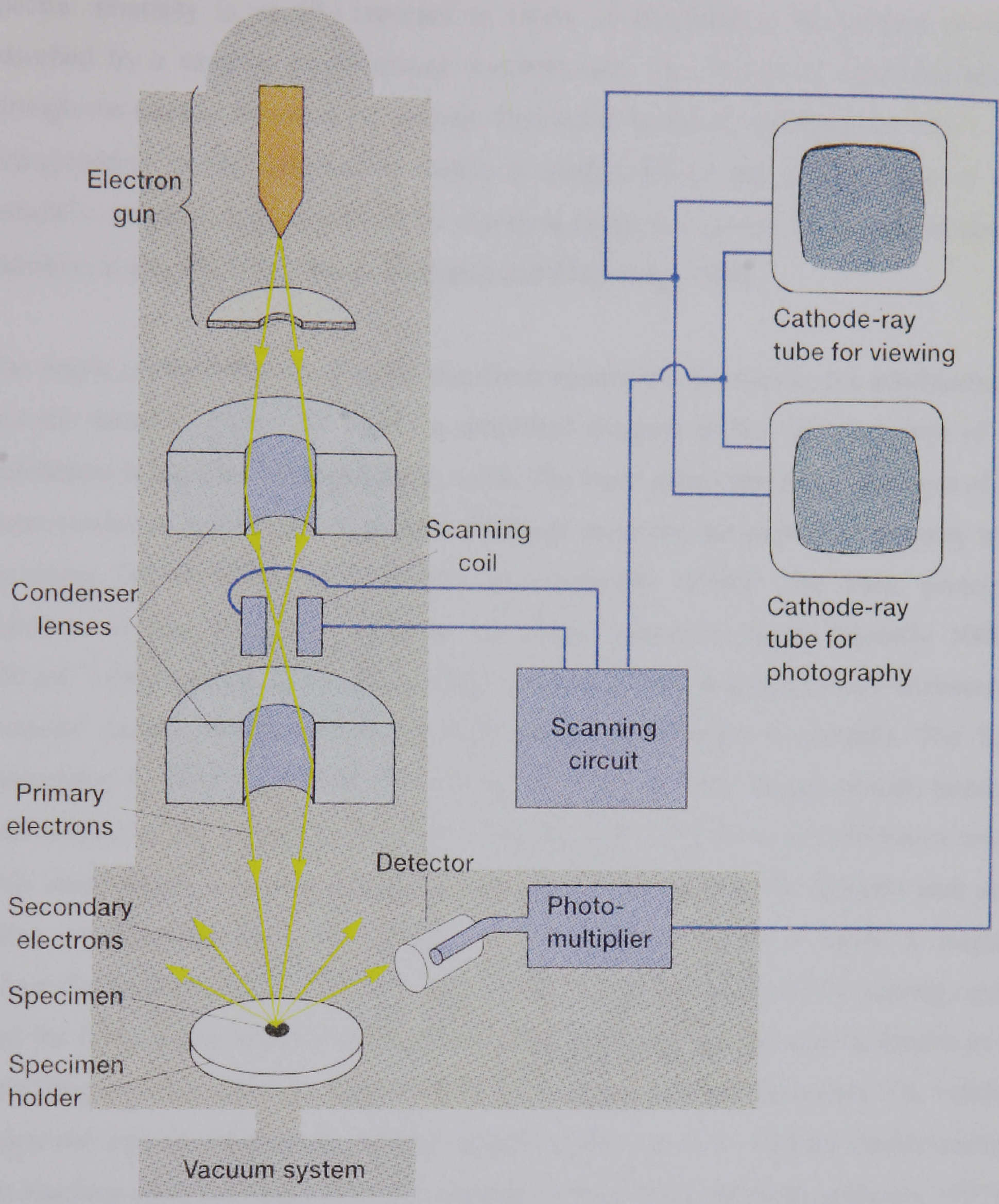
The Scanning Electron Microscope (SEM) is able to produce a seemingly vivid detailed three dimensional picture of a specimen surface over a wide range of magnifications from as low as 15x up to about 100,000x (Hayat, 1978; Madigan, *et al.*, 1997 ; Campbell, 1993). The SEM differs from TEM in that it produces an image from electrons emitted, by an object's surface, rather than from transmitted electrons. The SEM is being used routinely within the fields of microbiology and engineering for example, including looking at paint films (Cai *et al.*, 2001 ; Videla *et al.*, 2000 ; Hakkardinen *et al.*, 2000 ; Zanardini *et al.*, 2000 ; Surman *et al.*, 1996 ; Schutz *et al.*, 1997).

The SEM does have a number of limitations. The specimen must be conductive, therefore it is coated with a thin layer of a conductive metal, (usually gold) and it must be able to endure very low pressures, as it must be placed in a vacuum environment before examination can take place (Hayat, 1978).

The SEM (Figure 4.4) produces a narrow beam of electrons from an electron gun at one end of a vacuum column. This is scanned back and forth across the specimen surface placed at the far end of the column. When the beam strikes a particular area on the specimen surface atoms discharge a tiny shower of electrons, known as secondary electrons. These secondary electrons are trapped by a special detector where they strike

a scintillator causing light flashes to be emitted. The light flashes are converted to an electrical current and amplified by a photomultiplier. The signal is sent to a cathode-ray tube where an image is produced which can be viewed or photographed. The number of secondary electrons reaching the detector is dependent on the nature of the specimen, if the electron beam strikes a raised area, a large number of secondary electrons enter the detector, whereas with a depression fewer secondary electrons reach the detector. This means that raised areas on the specimen appear lighter on the image than depressed areas (Prescott et al., 1996 ; Hayat, 1978 ; Thornton, 1968 ; Oatley, 1972).

Figure 4.4 The Scanning Electron Microscope (Prescott *et al.*, 1996).



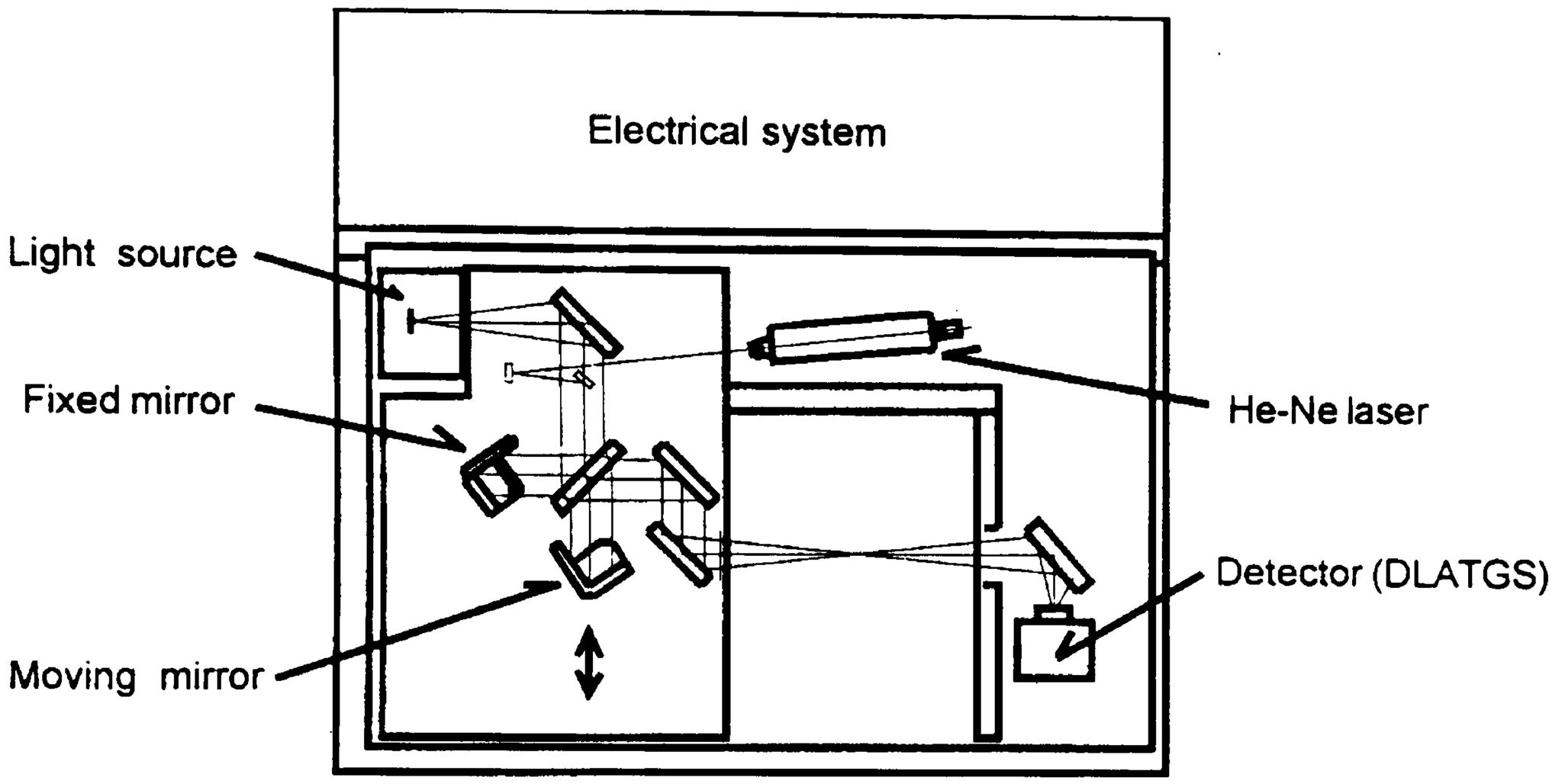
The Environmental Scanning Electron microscope (ESEM) differs from SEM in the fact that untreated wet specimens can be viewed under saturated water vapour pressure. This is made possible by the use of a differential pumping system, which allows the specimen to be maintained at a relatively high pressure (5-10 Torr) whilst the column and the gun are maintained at a high vacuum (10^{-5} to 10^{-7} Torr)

Fourier transform infrared (FTIR) spectrometers record the interaction of infra red (IR) radiation with a sample, thus measuring the frequencies at which the sample absorbs the

radiation and the intensities of the absorptions (Tapper, 1998). The intensity and frequency of sample absorptions are depicted in two-dimensional plots known as spectra. Intensity is usually reported in terms of absorbance, the amount of light adsorbed by a sample, or percentage transmittance, the amount of light that passes through the sample. Frequency is usually depicted in terms of wavenumbers (cm^{-1}). The determination of the frequencies within a sample allows the identification of that sample's chemical composition, since chemical functional groups are known to absorb radiation at specific frequencies (Williams and Flemming, 1989).

The major components of a Fourier transform system are the source, the interferometer and the detector; Figure 4.5 shows a simplified diagram of the optical system of the instrument being used throughout this work. The basic principles and advantages of the instrumentation and theory of modern FTIR-spectroscopy are explained in many texts including Diem (1994) and Griffiths and deHaseth (1986). The basic principle, however, is that the light (covering the whole frequency range, typically 5000 - 400 cm^{-1}) (Williams and Flemming, 1989) which is passed through the interferometer is focussed on the sample holder position (where the sample is placed). The light transmitted from the sample is focussed onto the detector. The change in light intensity with respect to the moving length of the moving mirror produces an interference wave. This interference wave is converted into an electrical signal by the detector and, after being amplified by the main amplifier, is converted into a digital signal. A diagram where the horizontal axis represents the light path difference between the moving mirror and the fixed mirror and the vertical axis, represents the light intensity is known as an interferogram. The intensity distribution with respect to the wavenumber (i.e. infrared spectrum) can be obtained by subjecting this interferogram to Fourier transformation, the resulting spectrum appears on the computer screen ready for printing (Jasco, 1997).

Figure 4.5 A simplified diagram of an FTIR spectrometer. (Jasco, 1997)



Infrared and FTIR spectroscopy have been applied to many areas including the studies of surface corrosion products and microbial and material analysis, including paint (Voevodin *et al.*, 2001 ; Cohen *et al.*, 2000 ; Schmalreck and Hotzel, 2000 ; Prieto *et al.*, 1999 ; Jasinski and Lob, 1988 ; Schutz *et al.*, 1999; Schmitt and Flemming, 1998). An advance on FTIR is micro-Fourier transform infrared spectroscopy, which in essence is the addition of a microscope to the FTIR system. There have been many uses for this technique, including the examination of paints and applications in forensic chemistry to examine hair, fibres and paints (Zie ba-Palus, 1999; Arnold, 1989). During the course of this investigation FTIR spectroscopy was used to detect gross chemical changes in the surface of the paints after inoculation within a vermiculite bed system and after exposure in field trials. A Golden Gate / reflectance attachment was used; the diamond tip allows observation down to about $725cm^{-1}$. The spectrum allows identification of the various functional groups in the polymeric paint binder, i.e. ester C=O at $1724cm^{-1}$, ether C-O at $1268cm^{-1}$ and $1238cm^{-1}$ and $1064cm^{-1}$ ester C-O's. Titanium dioxide (TiO_2) peaks occur well below $700cm^{-1}$ but they do impart a surface light scattering effect, which appears to be responsible for the general poor definition of the fresh paint surfaces.

In this investigation the Talysurf™ has been used to characterise and quantify the surface topography of painted surfaces, both from a vermiculite bed environment and from exterior exposure. SEM and ESEM were used primarily to investigate the

mechanism of attachment of the microorganisms after the painted panels had been exposed for six weeks. The FTIR was employed to investigate any changes in the chemical composition of the paint film due to microbial growth.

4.2 Surface Roughness Measurement - Talysurf™

4.2.1 Operation and calibration of the surface roughness measurement apparatus.

The wooden panels used throughout this Talysurf™ work had to be secure and accurately positioned on the base of the Talysurf™; therefore a panel holder was manufactured for this purpose (Appendix F).

Once the computer and the three other components of the system (the traverse unit, the amplifier and the pen recorder) of the Talysurf™ have been switched on, the icon Peak v1.5 (Talysurf™) is selected from the desktop on the computer to run the software. It is recommended that the stylus position be maintained at the same co-ordinates on the traverse unit for each related scan, (usually 50,000μm by 50,000μm) although this can be altered in the programme. The sensitivity range should be set at '1' i.e. 1/10 or 0.1mm, this means that the maximum sample area available can be analysed. The higher the sensitivity setting, the smaller the area is to be looked at, the principle is similar to a microscope the higher the magnification the less of the sample that can be seen.

In order to calibrate the machine, a calibration panel is provided. The panel, which is made from plastic has three grooves etched onto its clear glass surface. When the stylus has been lowered onto the panel to the left-hand side of these grooves, calibration may begin by initiating a sweep to the right. The computer programme requires the value on the calibration panel, which represents the depth of the three grooves, to be the same as in its programme. In this case the figure had to be adjusted to 2.29.

Appropriate settings must be chosen to select the size of the area to be sampled. The surface to be scanned should be placed in the chamber and the stylus lowered into position. The scan area for the majority of this work was $1.8mm^2$, as $1mm^2$ was found to be too small an area, therefore, the computer values were set at x = 180, y = 180 with interval spacing or resolution of 10μm. To reduce the amount of vibration on the stylus

head, the door to the chamber must be closed before the scan commences. An ADC check must be done and the scan saved in binary format for a map of the topography to be produced.

4.2.2 The Vermiculite bed system.

The vermiculite bed technique is a method for creating a high humidity environment within a sealed container. Previous uses of the vermiculite technique have included the testing of biocides against surface growth of algae and fungi (Morton, 1987 ; Grant and Bravery, 1981a, b). In this case the vermiculite bed technique was used to assess the deteriogenic properties of various fungi when inoculated onto painted wooden panels.

4.3 The effect of the vermiculite bed system on coated wooden panels.

4.3.1 Materials and Methods

Twelve panels of wood measuring 68mm x 44mm x 20mm were sanded to give a smooth surface. Six panels, with the grain of the wood running longitudinally, were given two coats of a pure acrylic paint and six were given two coats of hybrid acrylic paint allowing twenty-four hours in air, at room temperature, between coats. Once they were dry, the surfaces of the painted panels were then characterised using the Talysurf™ using a scan area of $1mm^2$.

Four plastic boxes measuring 160mm x 100mm x 45mm in depth, were cleaned with 100% ethanol. 50g of autoclaved vermiculite was placed in each box and 150ml of distilled water added and allowed to absorb into the vermiculite for forty-eight hours, at room temperature, before three of the painted wooden panels were added to each box.

The original intention of this experiment was to spray painted panels with selected microorganisms whilst they were in the vermiculite bed system and to assess the biodeterioration effect of the microorganisms against un-inoculated controls. However, after the 48 hours it was noticed that despite the attempts to maintain sterile conditions all of the painted panels in all of the boxes including the intended controls had become contaminated. It was decided nevertheless to assess the effect of the contaminants on the

surface of the paint films, so they were left for a further two weeks in the vermiculite at room temperature. The panels were then placed in a refrigerator at 4°C to retard further growth of the contaminants. The surfaces of the panels were then wiped clean, using a tissue and 1% Chloros and measured again using the Talysurf™. A panel was swabbed with cotton swabs moistened with ¼ strength Ringers solution, the swabs were applied to the surface of malt extract and nutrient agar, in order to culture and identify the contaminating microorganisms.

4.3.2 Results

Table 4.1 lists the values of a range of surface roughness parameters evaluated from the same area of a sample painted with paint D, (a hybrid paint without fungicide), before and after inclusion in the vermiculite bed system. Noticeable differences in all of the parameters are evident. The topographic images obtained, although not presented, were also inspected and were found to be quite different to the original images, in that, features on the original images could not be identified on the subsequent images.

Table 4.1 The parameters of the samples taken before and after incubation.

Parameter	Hybrid paint without fungicide before vermiculite treatment	Hybrid paint without fungicide after vermiculite treatment
Sa	18.3µm	11.4µm
Sq	21.2µm	15µm
Sp	27.3µm	19.8µm
Sv	58.5µm	70.4µm
St	85.7µm	90.1µm
Ssk	-0.741	-1.56
Sku	2.29	5.43
Sz	78.8µm	80.7µm

For Parameter definitions see Glossary.

The microorganisms contaminating the panel which was sampled were identified as a species of *Bacillus*, a species of *Aspergillus* and two yeasts.

4.3.3 Discussion

The parameterisation of the 'before' and 'after' data (Table 4.1) and the visual inspection of the recorded topography, suggested either; that a different part of the topography of the sample had been inspected following the vermiculite bed treatment, or the topography had notably altered. The replacement of the panel, on the panel holder of the Talysurf™ was achieved by aligning two of the faces of the panel against the two datum strips situated on the panel holder (Appendix F). It was realised that the end grain on the panel, on which the paint was applied, had been left exposed. Although the two faces of the panel had been correctly positioned on the datum strips, this did not relocate the Talysurf™ stylus to its original start position, principally because the panel had swollen in the latitudinal direction. A second possible contribution to the changes in the parameterisation could have been due to the cleaning of the panel prior to the measurements being taken. In addition to these problems, relocation of the samples will also be subject to small random experimental errors in the relocation process. For example, due to the slight differences in the way the sample was placed in the panel holder, each of these issues was investigated.

4.4 Assessment of the reliability and repeatability of the Talysurf™ measurements.

Three experiments were conducted for this assessment:

Evaluation of the accuracy of the relocation procedure under dry conditions,

Evaluation of the accuracy of the relocation procedure under humid conditions and

The effect of cleaning on 'Parameter' values

4.4.1 Evaluation of the accuracy of the relocation procedure under dry conditions.

4.4.1.1 Materials and Methods

One wooden panel measuring 70mm by 50mm by 15mm was coated twice with a pure acrylic paint using a paint brush. The end grains of the panels were sealed using an aluminium based paint, as recommended by the sponsors. Five pin-marks, each in the order of 30μm deep, were pierced into the paint surface using a sharp needle. Only three of the pin-marks in the form of a triangle were used, however, in this section of the experiment, the remaining pin-marks were for use in the experiment described in Section 1.4.2. This triangle of pin-marks was situated in the centre of the area to be scanned by the Talysurf™, so that its position on the axes could be monitored throughout the experiment. The pin-marks were then scanned using Talysurf™ co-ordinates 50,000μm x 50,000μm with a scanning area of 1.8mm^2 and sample resolution of 10μm. The topography of the panel in the vicinity of the pin-marks was measured five times, on each occasion the panel was removed and replaced. The instrument was also re-calibrated for each measurement to enable any in the calibration process and effects due to replacement to be identified.

4.4.1.2 Results

Examination of Table 4.2 shows some variation in the parameters despite the precision of the relocation. The reasons for these variations may include; slight differences generated by the re-calibration process or the influence of repositioning in the z plane, as a levelling process is performed in the programme. Minor changes may be attributed to the sample being damaged due to the contact with the stylus, it can be noticed that the Sa value falls as the test progresses. Examination of the pin-marks showed that the 'y' axis replacement was acceptable, the 'x' axis replacement was not as accurate, but still considered acceptable (within 0.1mm) (Table 4.3).

Table 4.2 The parameters of the five replacement tests in a dry environment.

Test No.	Sa µm	Sq µm	Sp µm	Sv µm	St µm	SSk	Sku	Sz µm
1	9.70	18.00	22.80	134.00	157.00	-3.64	18.20	145.00
2	8.54	16.80	21.30	125.00	146.00	-3.66	18.40	137.00
3	8.23	16.30	21.50	134.00	155.00	-3.86	20.80	143.00
4	8.48	17.00	23.10	137.00	160.00	-3.84	20.40	153.00
5	8.25	16.50	21.40	131.00	152.00	-3.87	20.60	142.00
Standard Deviation	0.61	0.66	0.86	4.55	5.34	0.11	1.27	5.83

For Parameter definitions see Glossary.

Table 4.3 The pin-mark positions on the axes after replacement.

Test number	'Y' axis (mm)			'X' axis (mm)		
1	0.48	0.78	1.10	0.70	1.30	0.25
2	0.48	0.78	1.10	0.70	1.20	0.20
3	0.48	0.78	1.10	0.60	1.20	0.20
4	0.48	0.78	1.10	0.60	1.20	0.20
5	0.48	0.78	1.10	0.60	1.20	0.20

The meshed axonomeric diagram and pseudo-colour image for test number three can be seen in Figures 4.6 and 4.7. These show clearly the position of the three pin-marks in the surface of the paint.

Figure 4.6 The meshed axonomeric diagram for the relocation in dry conditions of test number three.

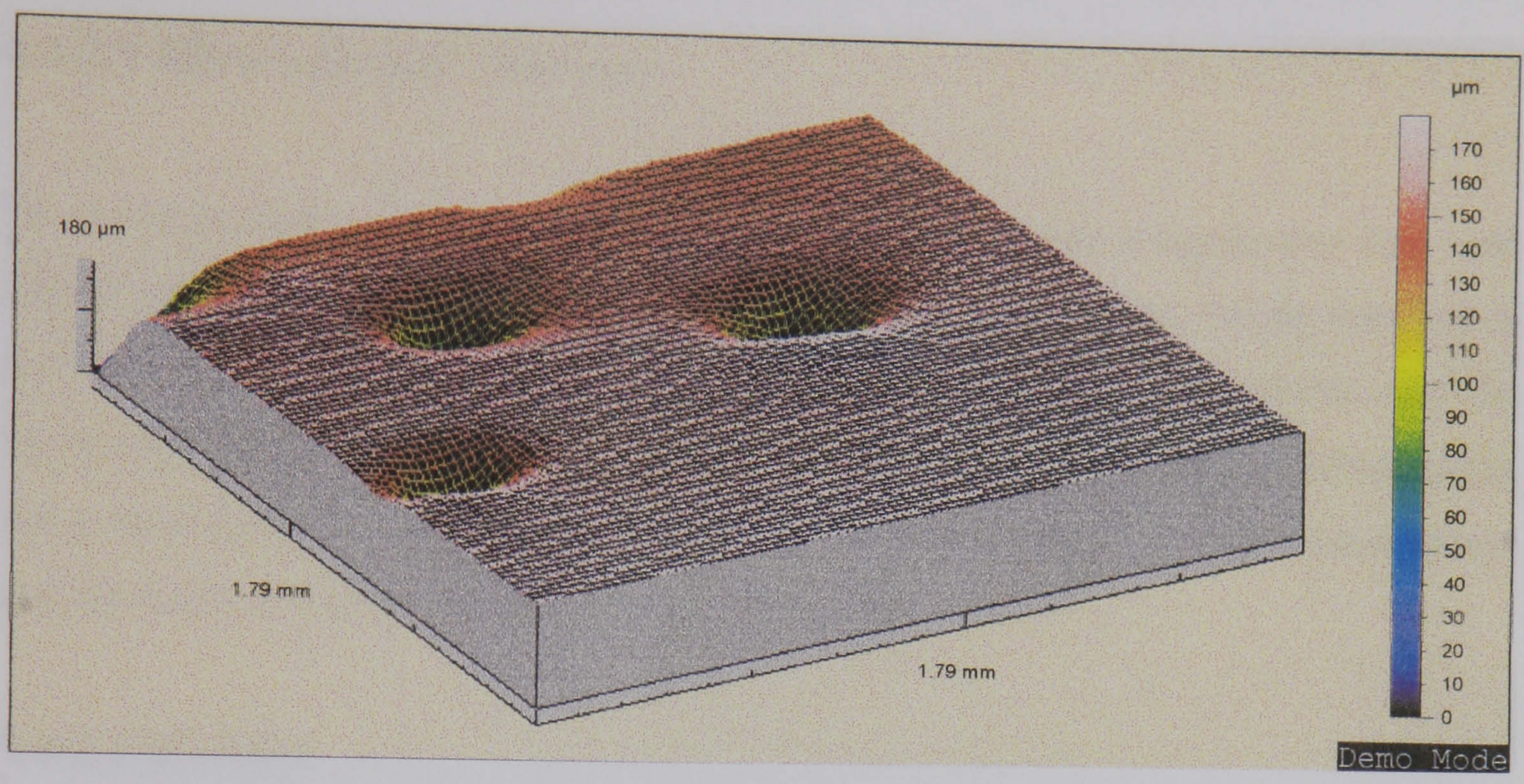
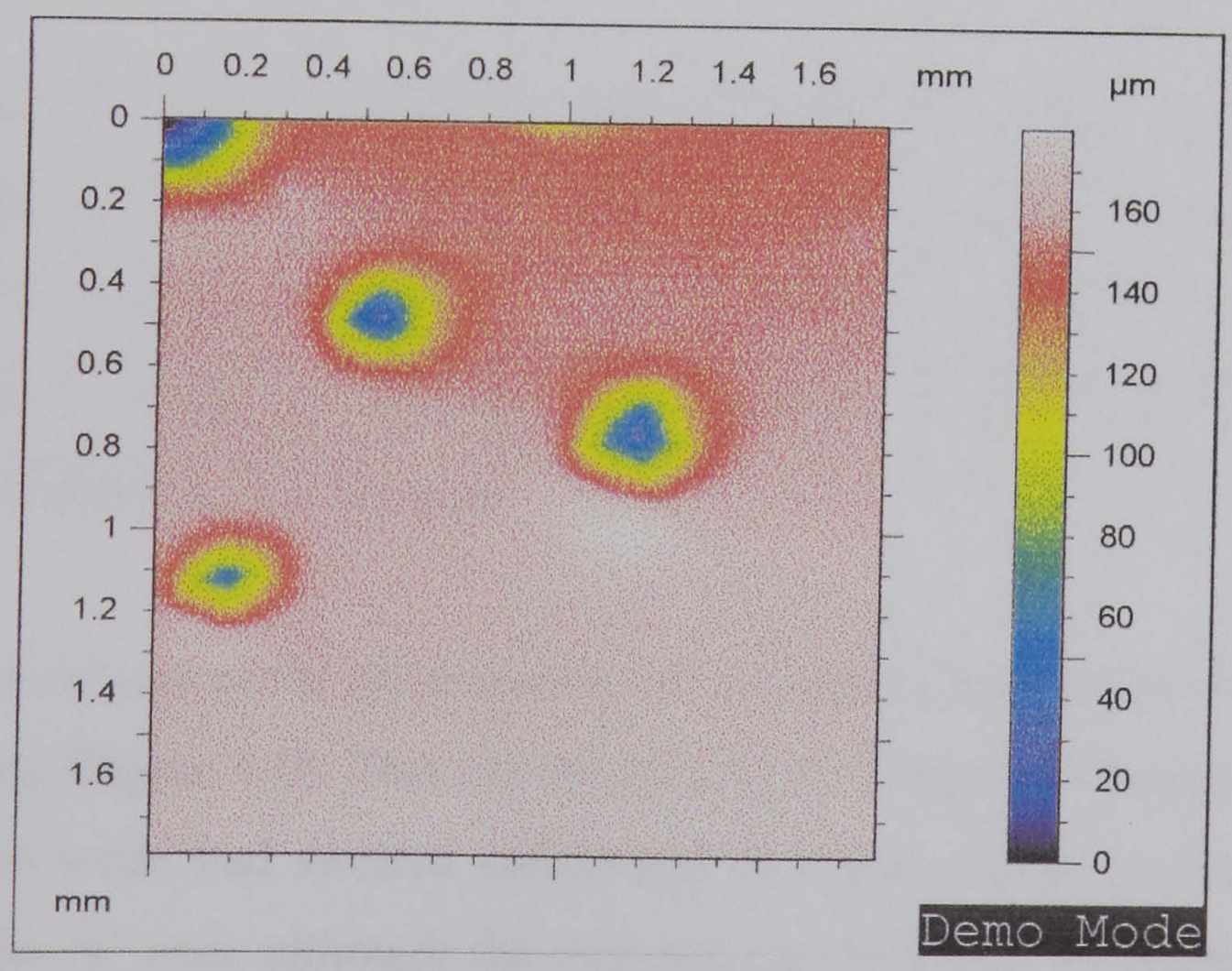


Figure 4.7 The Pseudo-colour image for the relocation in dry conditions of test number three.



4.4.2 Evaluation of the accuracy of the relocation procedure under humid conditions.

4.4.2.1 Materials and Methods

The same painted panel used in section 4.4.1 was placed into a vermiculite bed, with 20g of vermiculite and 50ml of distilled water. After three hours, twenty-four hours, four days and one month, the panel was removed from the vermiculite and the topography was measured using the Talysurf™. For each time period the measurement using the Talysurf™ was repeated twice, the panel was removed from the panel holder and the machine was re-calibrated between each scan.

4.4.2.2 Results.

Table 4.4 The parameters after one month's duration for the panels within the sterile vermiculite bed system.

Test No.	Sa	Sq	Sp	Sv	St	SSk	Sku	Sz
3 hrs	5.78	12	18	105	123	-4.23	24.4	111
24 hrs	6.49	13.5	19.2	116	135	-4.09	22.9	122
96 hrs	7.75	14.9	22.2	123	145	-3.52	18.2	132
1 mth	3.94	8.09	18.1	105	124	-5.51	49.6	106

For Parameter definitions see Glossary.

In the humid environment the relocation to the pin-marks was not as accurate as in the dry environment (Figure 4.6). This can be seen in the images obtained, after four days (Figure 4.8) the wood had swelled sufficiently to cause a 0.5mm discrepancy within relocation in the 'y' axis, although the original area that was measured could still be clearly seen, as it remained within the overlap. After a month (Figure 4.9) in the humid environment of the vermiculite bed, the wood had swelled so that the discrepancy in the 'y' direction was over 1mm, there was no detectable change in the values obtained in the x-axis. The area of three pin-marks, that was measured initially, was partly visible;

the five pin-marks however, could be seen after this period of exposure indicating a slight problem with the relocation to the measurement site. The swelling of the wood was measured using a pair of callipers. After one month the panel of wood had expanded by approximately 1mm perpendicular to the grain. It was found that the swelling effect could be reduced by placing a piece of plastic between the vermiculite and the panel, the swelling was seen to reduce by fifty percent.

Figure 4.8 The meshed axonomeric diagram of the relocation after four days in humid conditions.

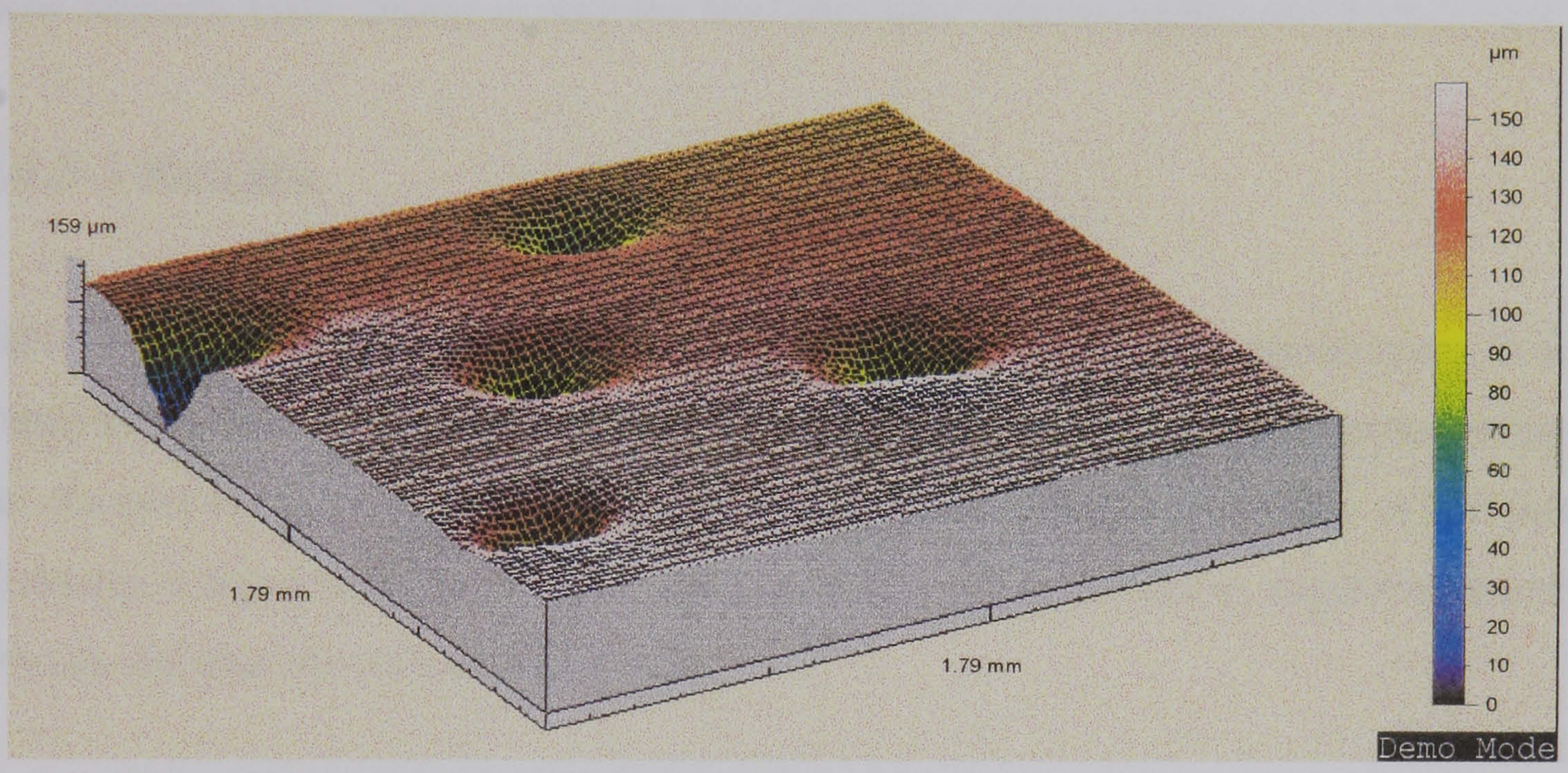
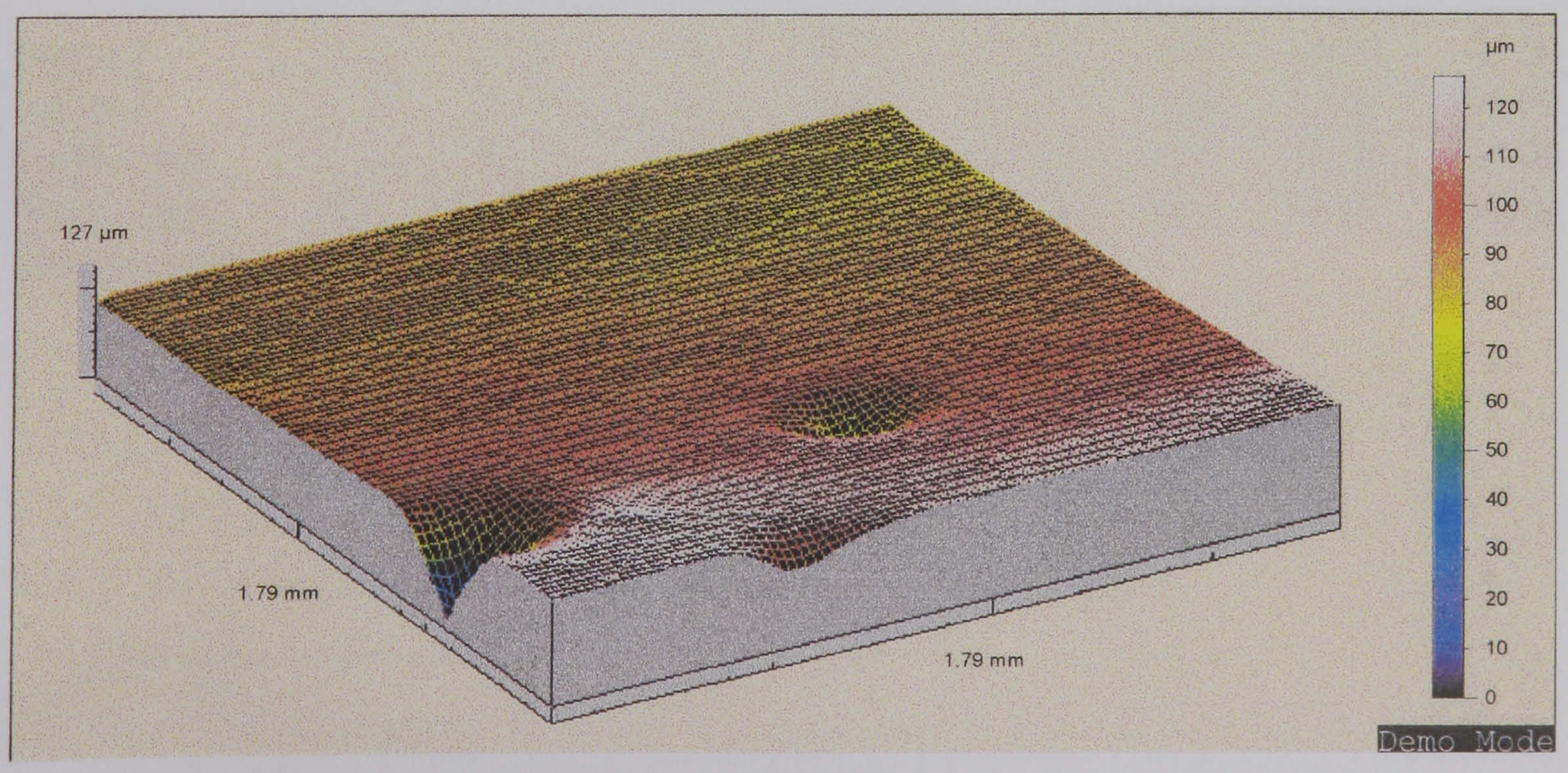


Figure 4.9 The meshed axonomeric diagram of the relocation after four weeks in humid conditions.



4.4.3 Effect of cleaning on 'parameter' values.

4.4.3.1 Materials and Methods

One painted panel, similar to that used in 4.4.1, was prepared and its topography recorded twice at the same location. The panel was then removed from the Talysurf™ panel holder, cleaned lightly using a piece of tissue and a solution of 1% Chloros, remounted in the holder and its topography re-measured. The panel was then subjected to a second cleaning regime, this time a 'heavy' clean by scrubbing vigorously with the same solution and material as used for the light clean, and its topography re-measured.

4.4.3.3 Results.

Table 4.5 summarises the data from the tests. From these cleaning experiments, it was found that a light clean with 1% chloros did not notably change the parameterisation of the cleaned zone, suggesting that it did not alter the surface topography of the paint. However, a heavy scrub did lead to a change in the parameterisation, suggesting that the process did alter the surface topography.

Table 4.5 The parameters obtained from the cleaning experiment.

Parameters	Sa µm	Sq µm	Sp µm	Sv µm	St µm	Ssk	Sku	Sz µm
Initial 1	11.00	18.30	41.80	137.00	179.00	-3.09	16.00	165.00
Initial 2	11.10	18.40	41.50	140.00	181.00	-3.06	15.90	168.00
Light clean	11.00	18.20	35.90	138.00	174.00	-3.07	16.10	164.00
Heavy clean	9.37	15.20	31.70	116.00	147.00	-3.01	16.00	138.00

For Parameter definitions see Glossary.

4.5 Changes in the surface topography due to the presence of microorganisms.

4.5.1 Materials and Methods

Five panels of wood measuring 70mm by 50mm by 15mm were sanded and prepared as follows: panels 1 and 2 were coated twice using a pure acrylic paint containing a fungicide (paint A). Panels 3 and 4 were coated twice using a pure acrylic paint which did not contain a fungicide (paint B) and panel 5 was coated with a high solid alkyd gloss paint also without a fungicide (paint F). In each case the end grains and the underside of the panel were sealed using the aluminium based paint recommended by the sponsors. Their topographic profiles at four separate positions were recorded. This was achieved by first sampling two areas at the co-ordinates 50,000μm by 50,000μm and at the coordinates 55,000μm by 55,000μm. The panel was then rotated through one hundred and eighty degrees and the sampling repeated, resulting in four sampled areas per panel. Panels 1, 3 and 5 were inoculated with a suspension of *Aureobasidium pullulans* using an airbrush and allowed to incubate for twenty weeks at 23°C within a vermiculite bed system. Panels 2 and 4 were attached to the panel rack at Preston facing North for the same period of time. After which the samples were removed and axonomeric representations of their surfaces were re-measured.

4.5.2 Results.

The panel painted with paint 'A' having been exposed at Preston

An examination of the images obtained from those panels that had been exposed on the racks at Preston (Figures 4.10 and 4.11) shows that paint A, the pure acrylic paint containing a fungicide, did not show any obvious differences in the topography between the before and after images. It is evident however, that the stylus did not return to the original position, nevertheless, the two images do overlap; so recognisable features within the image can be identified (point A). From the parameters of one of the co-ordinates sampled shown in Table 4.6 it can be interpreted that in general the surface of the paint film has become smoother, i.e. a negative Ssk value was recorded after the

exposure period. Surface debris including microorganisms accumulating in the troughs of the grain of the wood, may contribute to this effect.

Table 4.6 The parameters of the unexposed and exposed painted surfaces for the pure acrylic paint containing fungicide.

Parameters	Parameters before exposure	Parameters after exposure
Sa	4.93µm	4.8µm
Sq	6.22µm	5.55µm
Sp	18.9µm	22.3µm
Sv	16.5µm	19.6µm
St	35.4µm	41.9µm
Ssk	0.62	-0.263
Sku	3.06	2.62
Sz	32.2µm	35.9µm

For Parameter definitions see Glossary.

Figure 4.10 The meshed axonomeric diagram of Paint A before exposure at Preston.

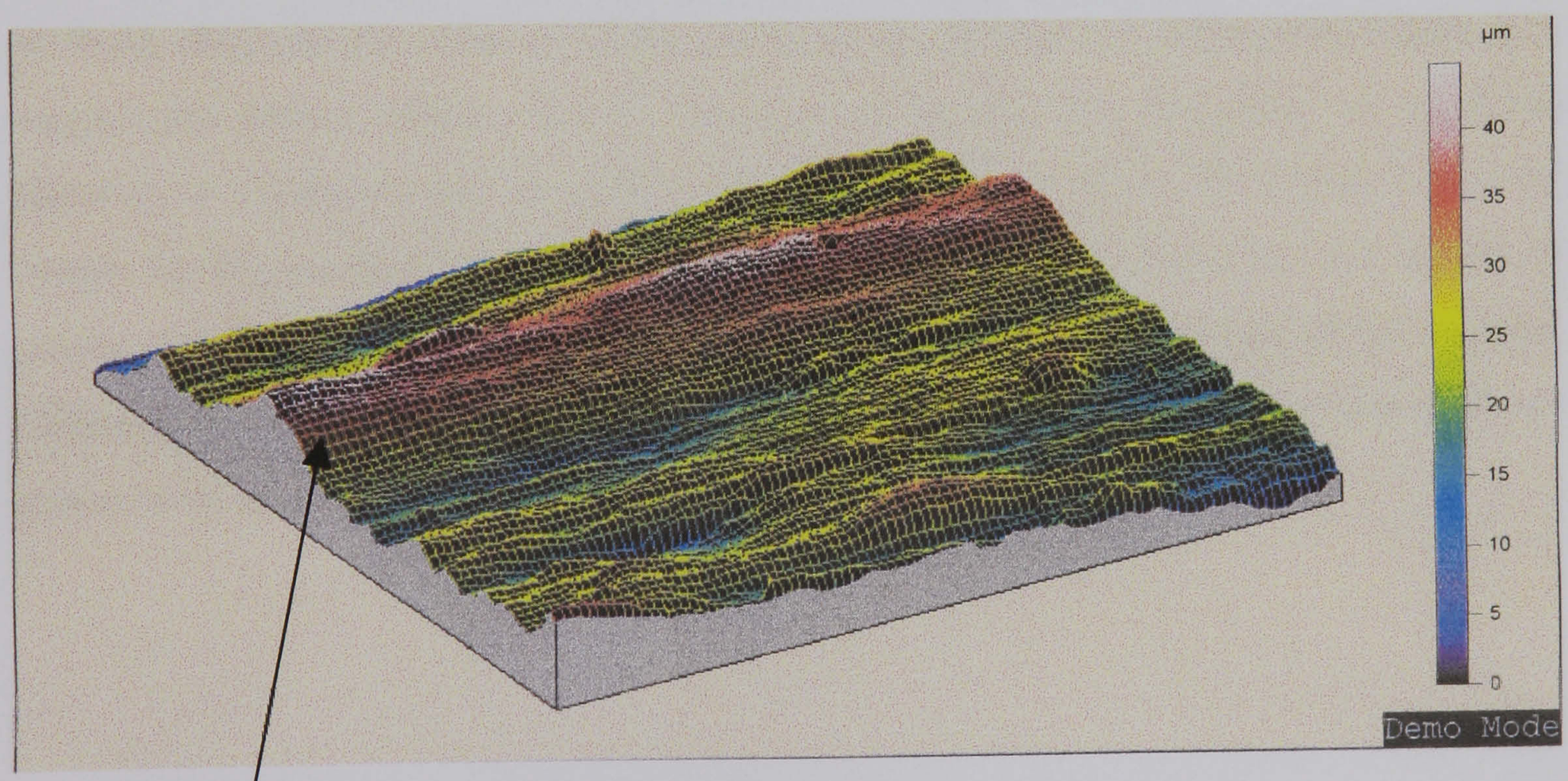
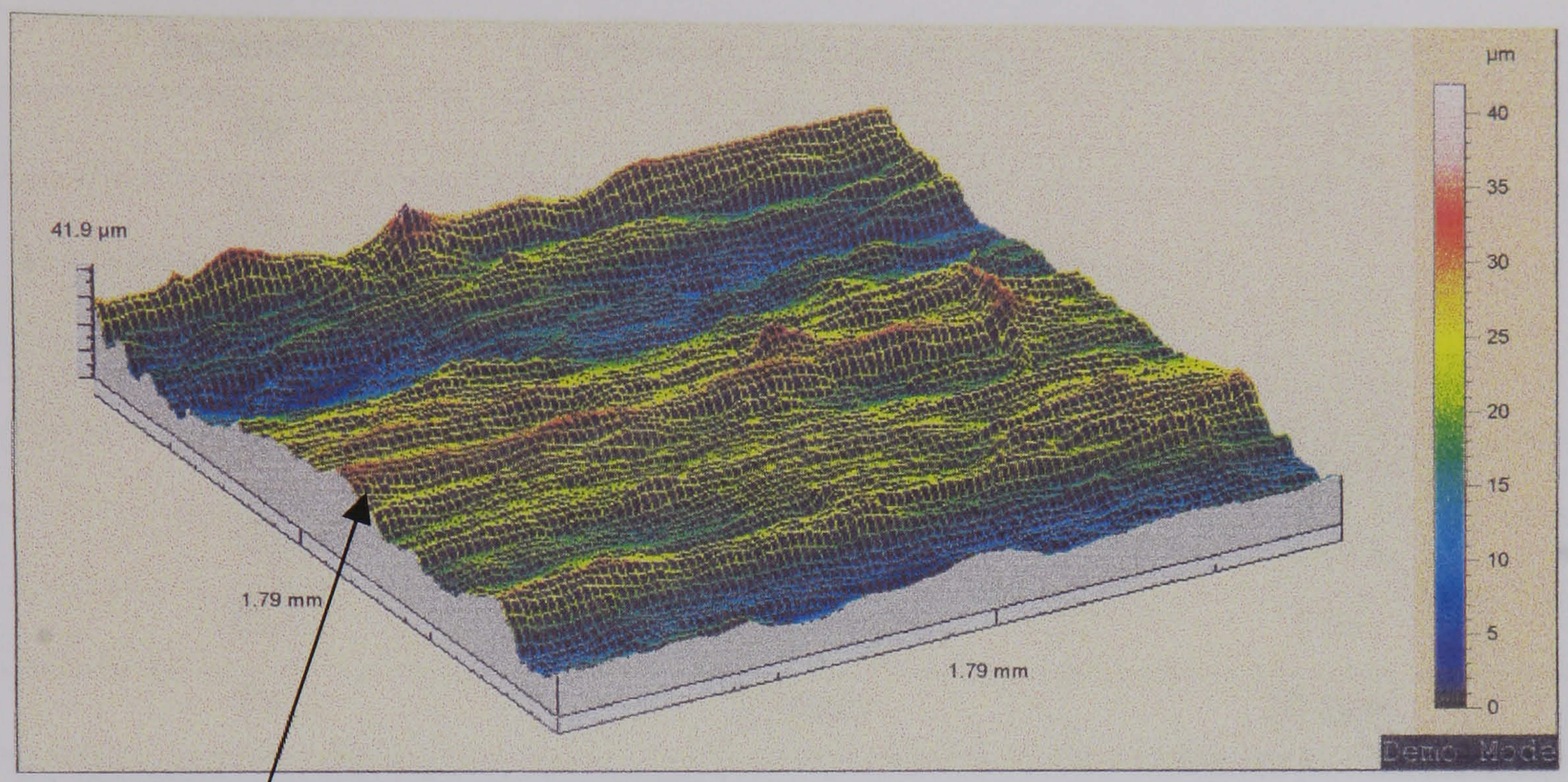


Figure 4.11 The meshed axonomeric diagram of Paint A after twenty weeks of exposure at Preston.



The panel painted with paint B having been exposed at Preston

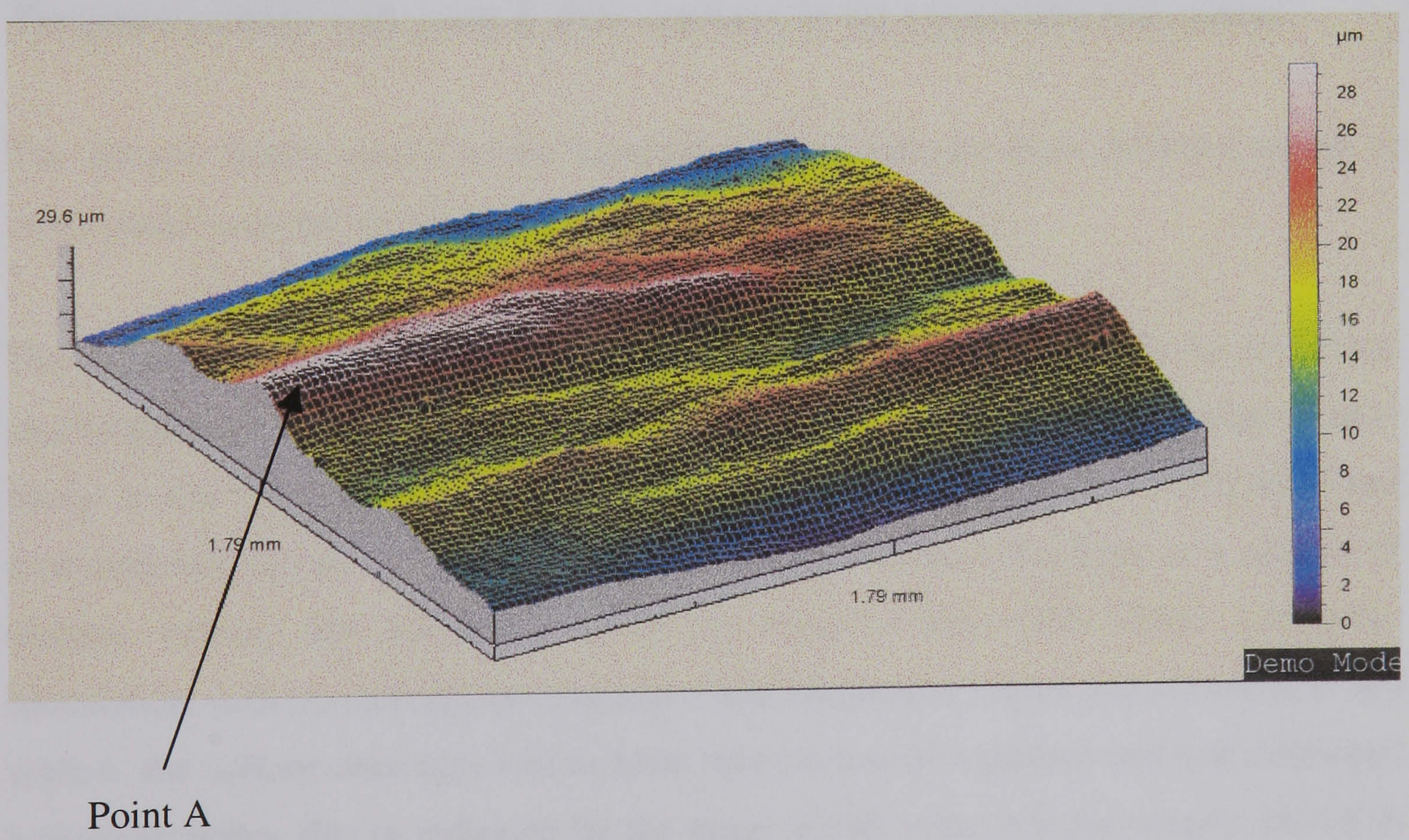
Similar observations could be made for paint B (the pure paint containing no fungicide) to those obtained for paint A,. After the exposure period, the parameters (Table 4.7) suggest the surface appears to have become a little smoother in nature, i.e. a plateau effect or a predominance of shallow troughs, indicated by the Sv value. From the images in Figures 4.12 and 4.13 it appears that the troughs have become deeper in places, depicted by 'point A', which represent the same point on each of the Figures. Although there are differences in the images before and after exposure there does not appear to be any evidence of areas of degradation.

Table 4.7 The parameters of the unexposed and exposed painted surfaces for the pure acrylic paint containing no fungicide.

Parameters	Parameters before exposure	Parameters after exposure
Sa	3.99µm	4.27µm
Sq	5.09µm	4.91µm
Sp	12.5µm	10.9µm
Sv	17µm	10.8µm
St	29.6µm	21.7µm
Ssk	-0.209	-0.158
Sku	2.83	1.91
Sz	26.4µm	20.1µm

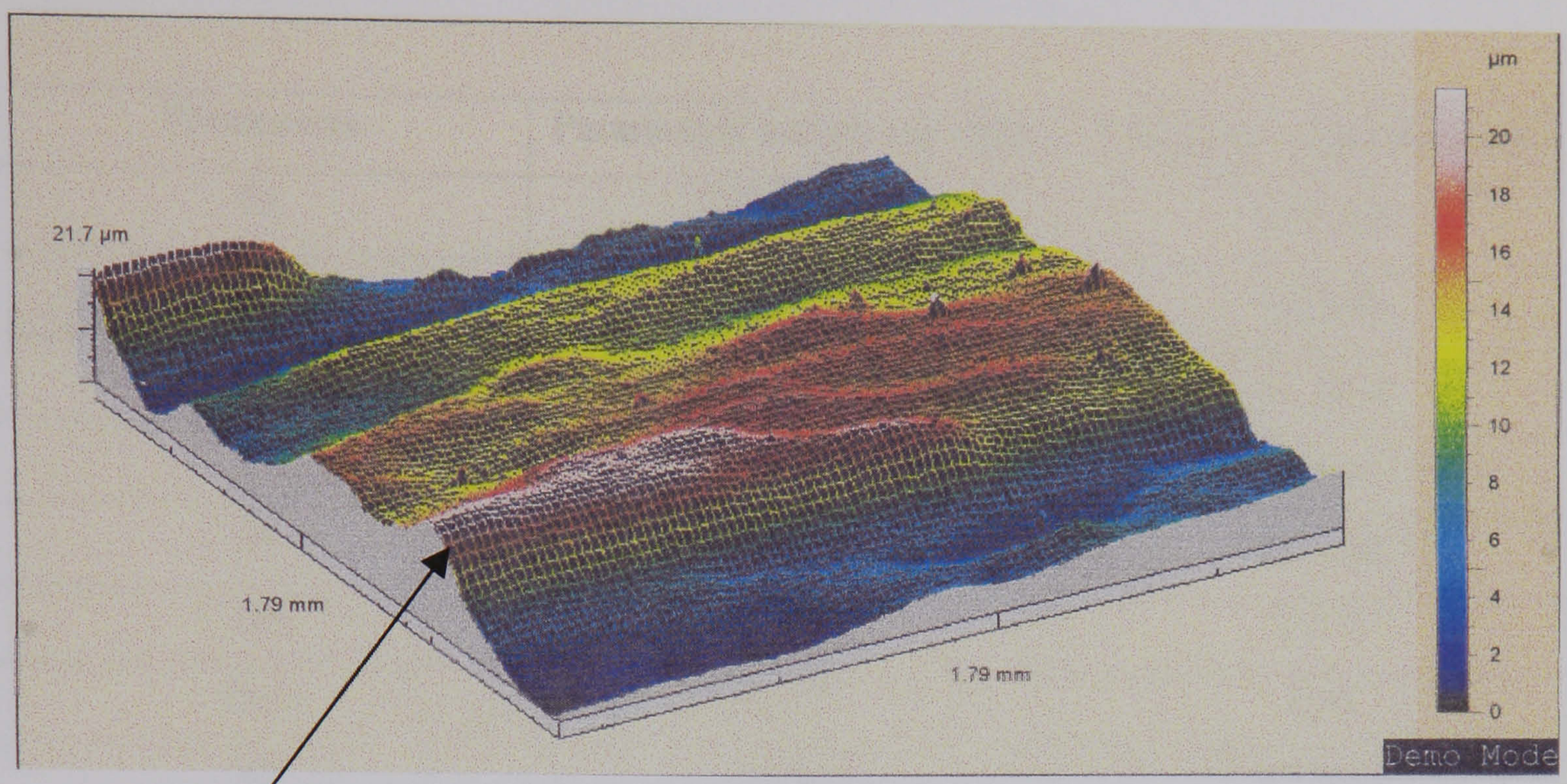
For Parameter definitions see Glossary.

Figure 4.12 The meshed axonomeric diagram of Paint B before exposure at Preston.



Point A

Figure 4.13 The meshed axonomeric diagram of Paint B after twenty weeks exposure at Preston.



The panel painted with paint A after exposure in the vermiculite bed system

The painted panels placed in the vermiculite bed treatment show different results to those panels exposed at the site in Preston.

The panel painted with the pure acrylic paint containing fungicide (paint A) which was inoculated with *Aureobasidium pullulans,* appeared to have changed very slightly after being in the vermiculite bed system for twenty weeks. Before the vermiculite bed treatment the surface of the painted panel appeared to have the form of a plateau of shallow valleys, this was indicated by the negative Ssk value (Table 4.8). After inoculation with *Aureobasidium pullulans* and incubation within the vermiculite bed system, the surface area appeared to have become less of a plateau and had developed numerous peaks, this is indicated by the positive Ssk value and the images shown in Figures 4.14 and 4.15, where the position marked point A on each of the figures, corresponds to the same position on the painted surface. The remaining parameters do not indicate any obvious differences between the two images.

Table 4.8 The parameters of the unexposed and exposed painted surfaces for the pure acrylic paint containing fungicide inoculated with *Aureobasidium pullulans*.

Parameters	Parameters before exposure	Parameters after exposure
Sa	6.13µm	5.01µm
Sq	7.37µm	6.17µm
Sp	16.6µm	17.5µm
Sv	16.7µm	16.4µm
St	33.3µm	33.8µm
Ssk	-0.176	0.139
Sku	2.12	2.58
Sz	30.4µm	32.2µm

For Parameter definitions see Glossary.

Figure 4.14 Meshed axonomeric diagram of paint A before inoculation with *Aureobasidium pullulans*.

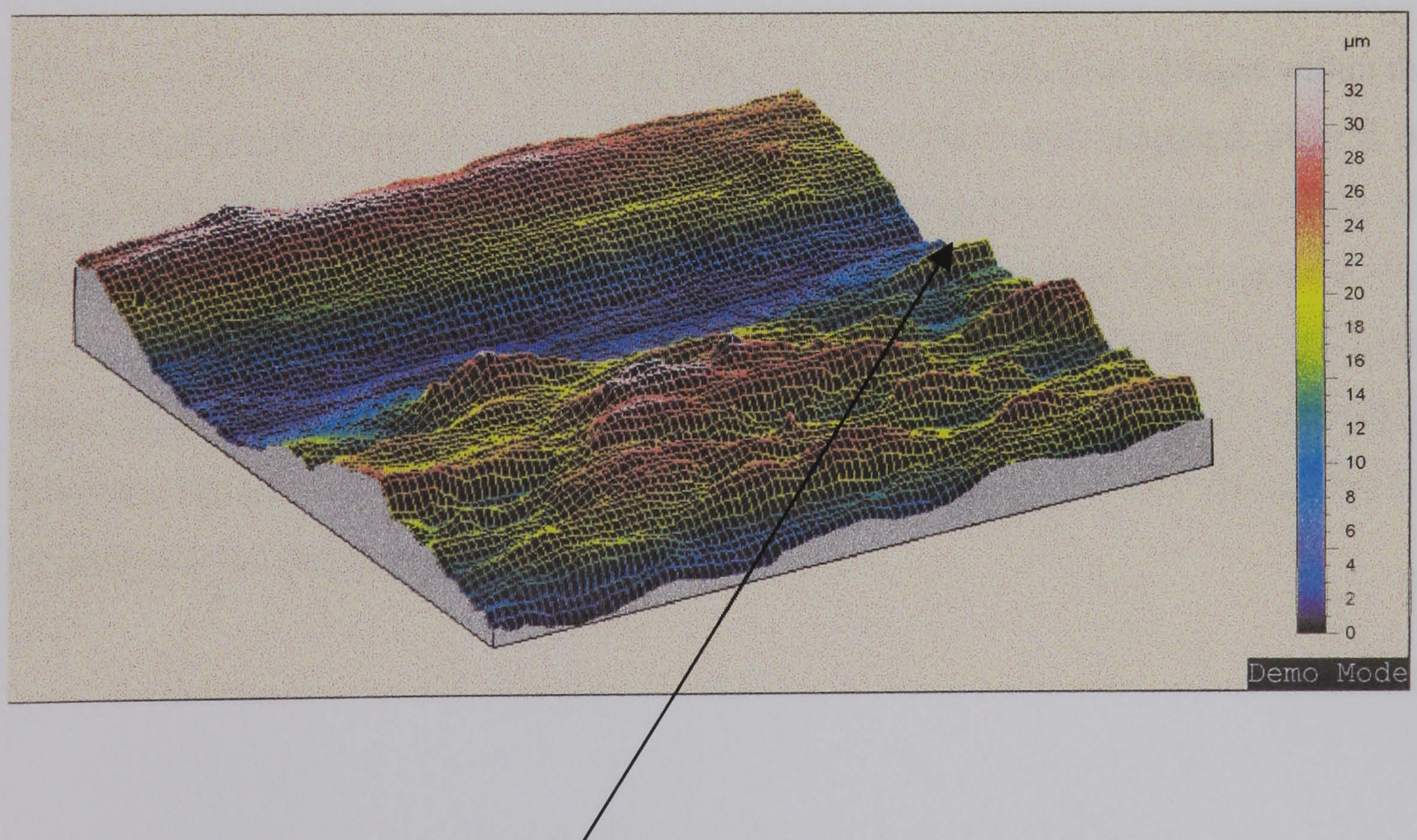
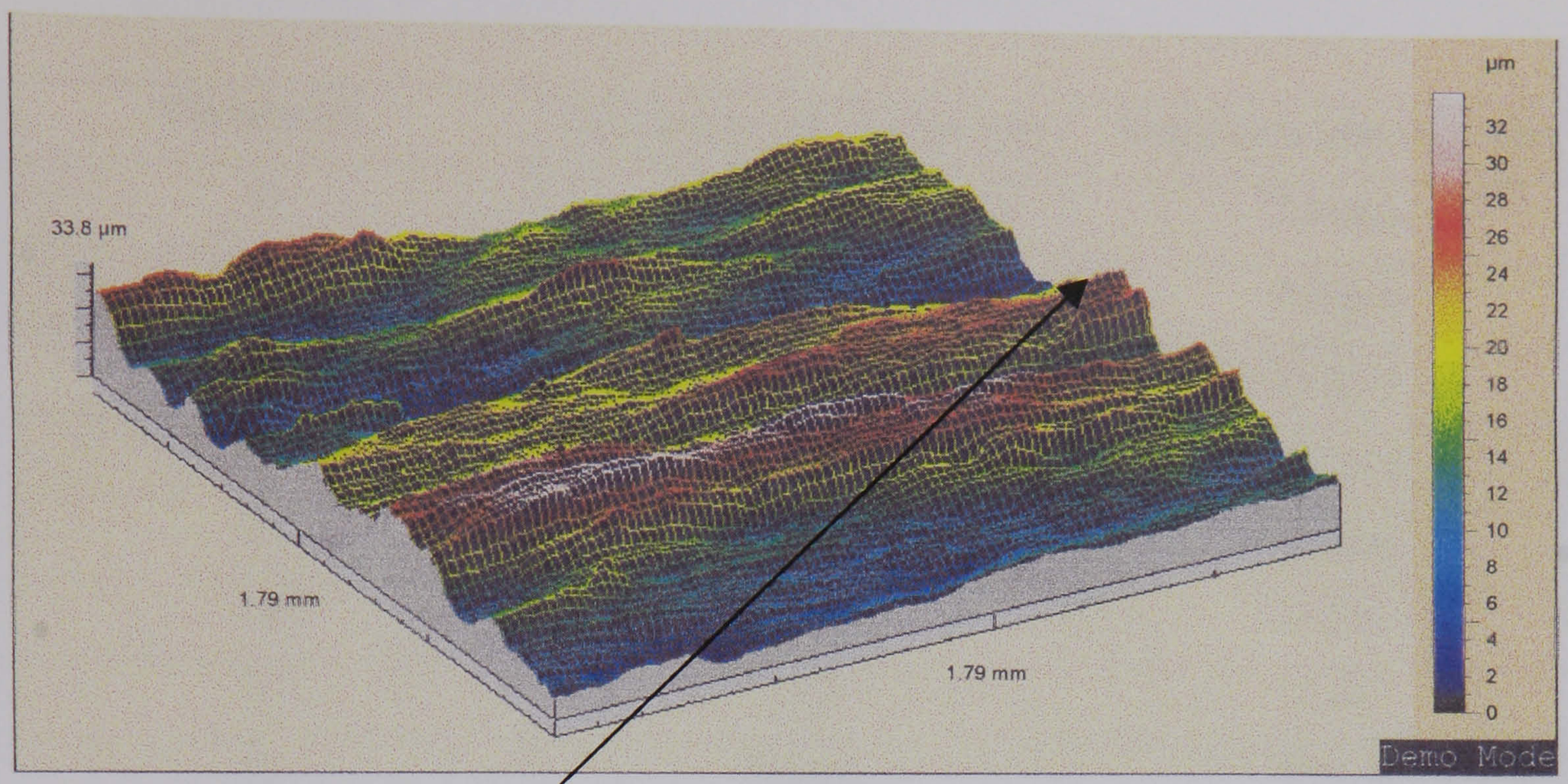


Figure 4.15 Meshed axonomeric diagram of paint A after inoculation with *Aureobasidium pullulans* and twenty weeks in the Vermiculite bed system.



The panel painted with paint B after exposure in the vermiculite bed system

The pure acrylic paint without fungicide (paint B), shows no obvious changes within the surface topography when the images in Figures 4.16 and 4.17 are compared, point A on Figure 4.16 relates to point A on Figure 4.17. The parameters, however, in Table 4.9 show that there are some changes in the data relating to the surface after exposure. The mean roughness values (Sa and Sq) have obviously altered as has the St value, this value, after exposure, indicates that the total height of the sample area has decreased, however, the Ssk value indicates that the area has altered, from being a plateau, to being an area with numerous peaks.

Table 4.9 The parameters of the unexposed and exposed painted surfaces for the pure acrylic paint containing no fungicide inoculated with *Aureobasidium pullulans*.

Parameters	Parameters before exposure	Parameters after exposure
Sa	15.2µm	7.16µm
Sq	19.1µm	9.66µm
Sp	40.2µm	33.3µm
Sv	68.1µm	40.5µm
St	108µm	73.8µm
Ssk	-0.743	0.325
Sku	3.59	4.33
Sz	106µm	70.6µm

For Parameter definitions see Glossary.

Figure 4.16 Meshed axonomeric diagram of paint B before inoculation with *Aureobasidium pullulans* and incubation in the vermiculite bed system.

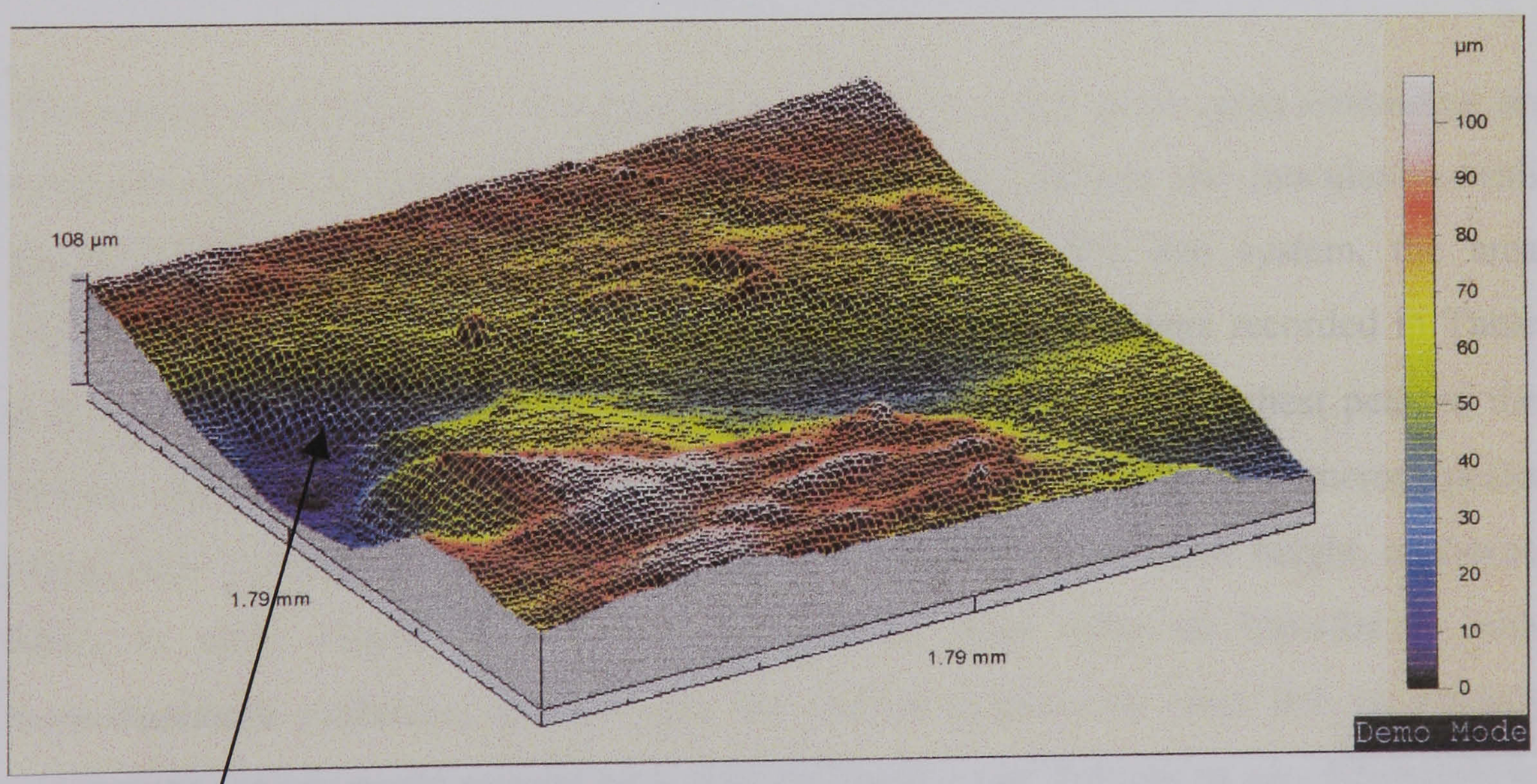
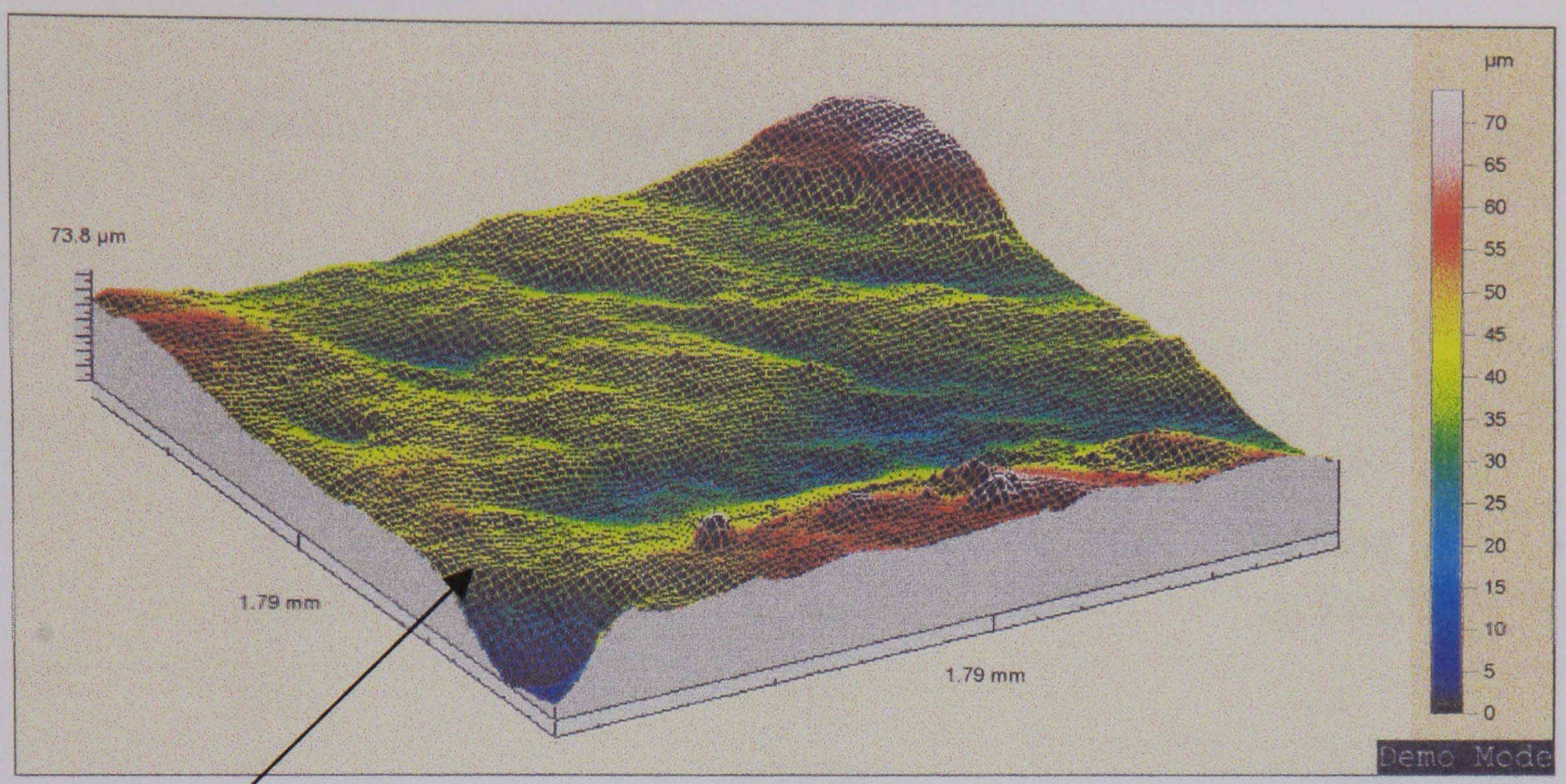


Figure 4.17 Meshed axonomeric diagram of paint B after inoculation with *Aureobasidium pullulans* and incubation in the vermiculite bed system.



Point A

The panel painted with paint F having been exposed in the vermiculite bed system

The surface topography of the panel painted with the alkyd gloss paint containing no fungicide (paint F), seems to have altered drastically. Before the inoculation with *Aureobasidium pullulans* and incubation in the vermiculite bed system, the area appeared to be plateau-like in nature (Figure 4.18). The parameters recorded in Table 4.10 indicate that the surface was quite flat, only 17µm from the highest peak to the lowest trough. The Ssk value indicates that the surface was made up of numerous peaks rather than a plateau, however, as these peaks are all about the same height, a plateau effect is seen. Figure 4.19 shows the painted panel after its inoculation with *Aureobasidium pullulans*, in this case the surface appears to have lost its smooth appearance, or any peaks present have become more magnified due to the differences in the St values (17µm and 37.7µm). The negative Ssk value suggests that the area has become more plateau-like, however, the other parameters suggest that the area has a rougher topography, which cannot be explained.

Table 4.10 The parameters of the unexposed and exposed painted surfaces for the alkyd gloss paint containing no fungicide inoculated with *Aureobasidium pullulans*.

Parameters	Parameters before exposure	Parameters after exposure
Sa	1.14µm	5.53µm
Sq	1.47µm	6.77µm
Sp	8.95µm	19.3µm
Sv	8.09µm	18.4µm
St	17µm	37.7µm
Ssk	0.515	-0.0336
Sku	4.66	2.4
Sz	11.8µm	33.7µm

For Parameter definitions see Glossary.

Figure 4.18 Meshed axonomeric diagram showing paint F before inoculation with *Aureobasidium pullulans* and incubation within the vermiculite bed system.

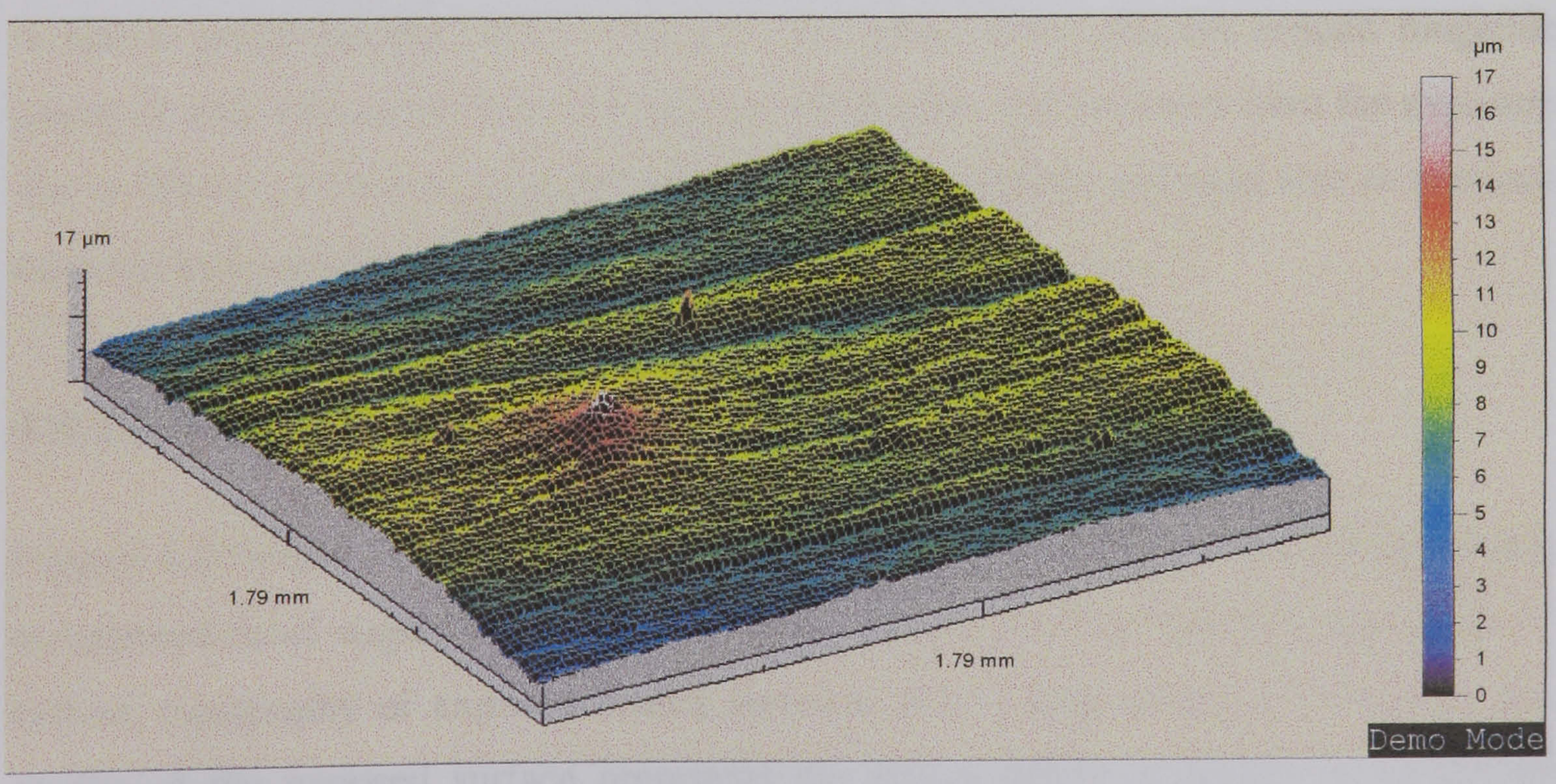
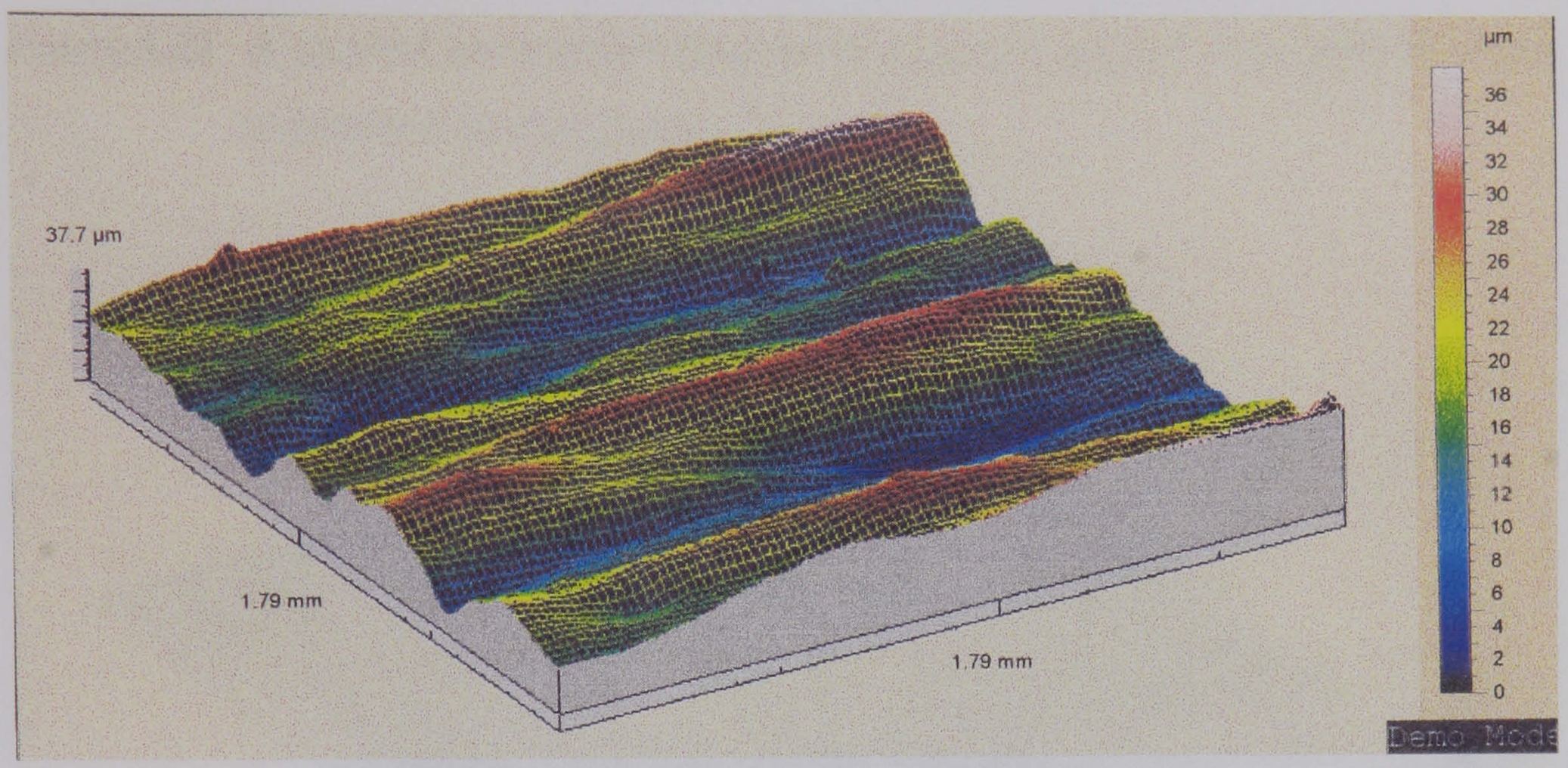


Figure 4.19 Meshed axonomeric diagram showing paint F after inoculation with *Aureobasidium pullulans* and incubation within the vermiculite bed system.



4.6 To investigate the effects of long-term exposure

4.6.1 Materials and Methods

A spruce panel painted with a hybrid acrylic paint, which did not contain fungicide (paint D) and which had been exposed for 30 months, was removed from the exposure site at Preston. This was analysed using the same procedure and scan size as previous measurements obtained using the Talysurf™.

4.6.2 Results

Figures 4.20 and 4.21 show the meshed axonomeric profiles of an unexposed panel and an exposed panel respectively. These indicate that it is possible to detect changes in the surface topography of exposed coated surfaces after a long period of exposure. The profile of the exposed surface represents the accumulation of debris and microbial biomass on the surface. Figure 4.22 represents the profile of the surface after light cleaning with 1% Chloros. A change in the roughness of the profile of the surface is evident. The exposed painted surface also exhibited an interesting pattern of fungal

colonisation. Low power microscopic examination (x100) of the panel (Figure 4.23) showed that fungal hyphae of *Aureobasidium pullulans* traversed along the peaks of the grain of the wood, rather than in the troughs as might be expected.

Figure 4.20 A meshed axonomeric diagram of a surface painted with paint D before exposure at the Preston site.

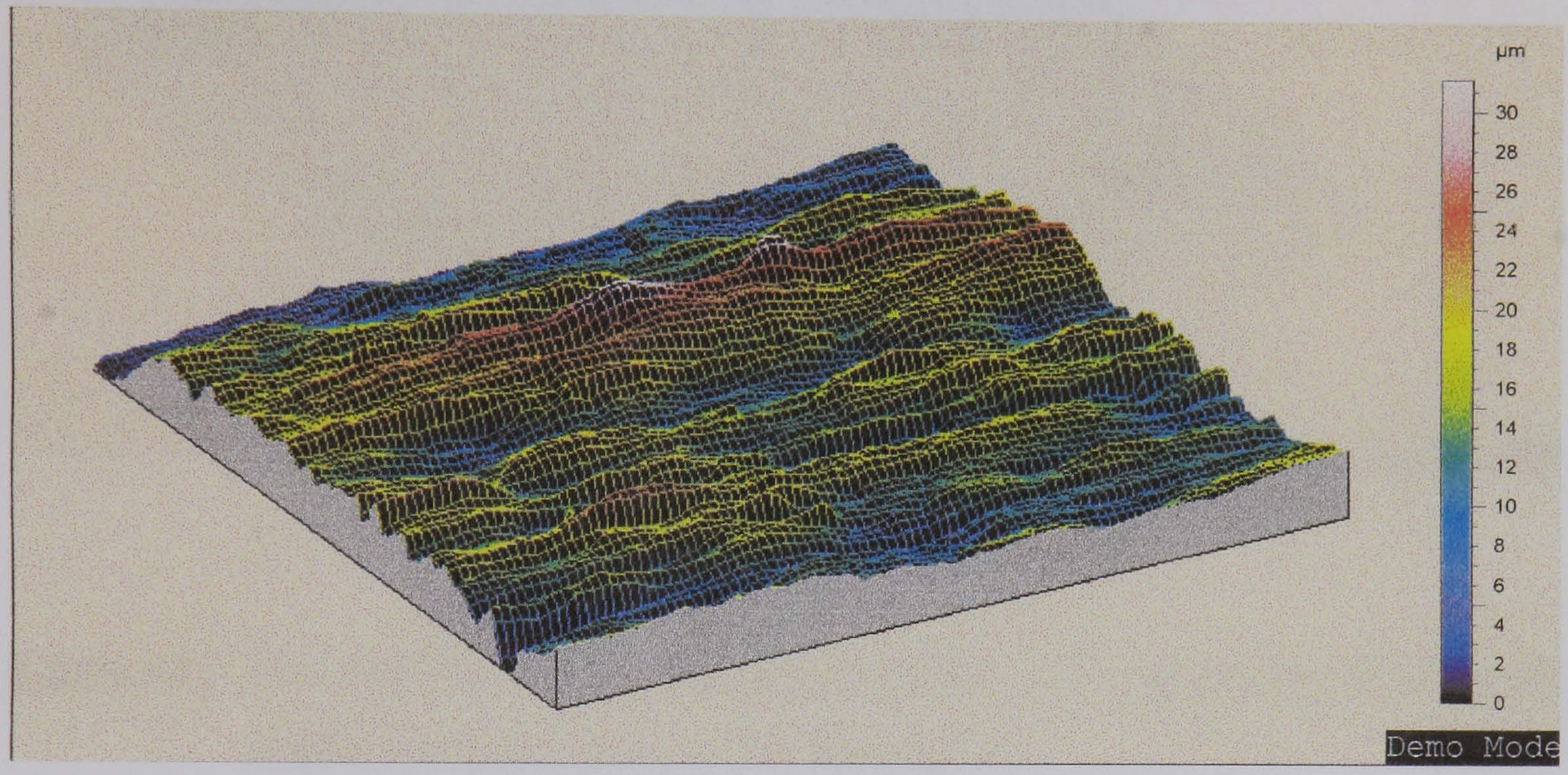


Figure 4.21 A meshed axonomeric diagram of an exposed painted surface painted with paint D

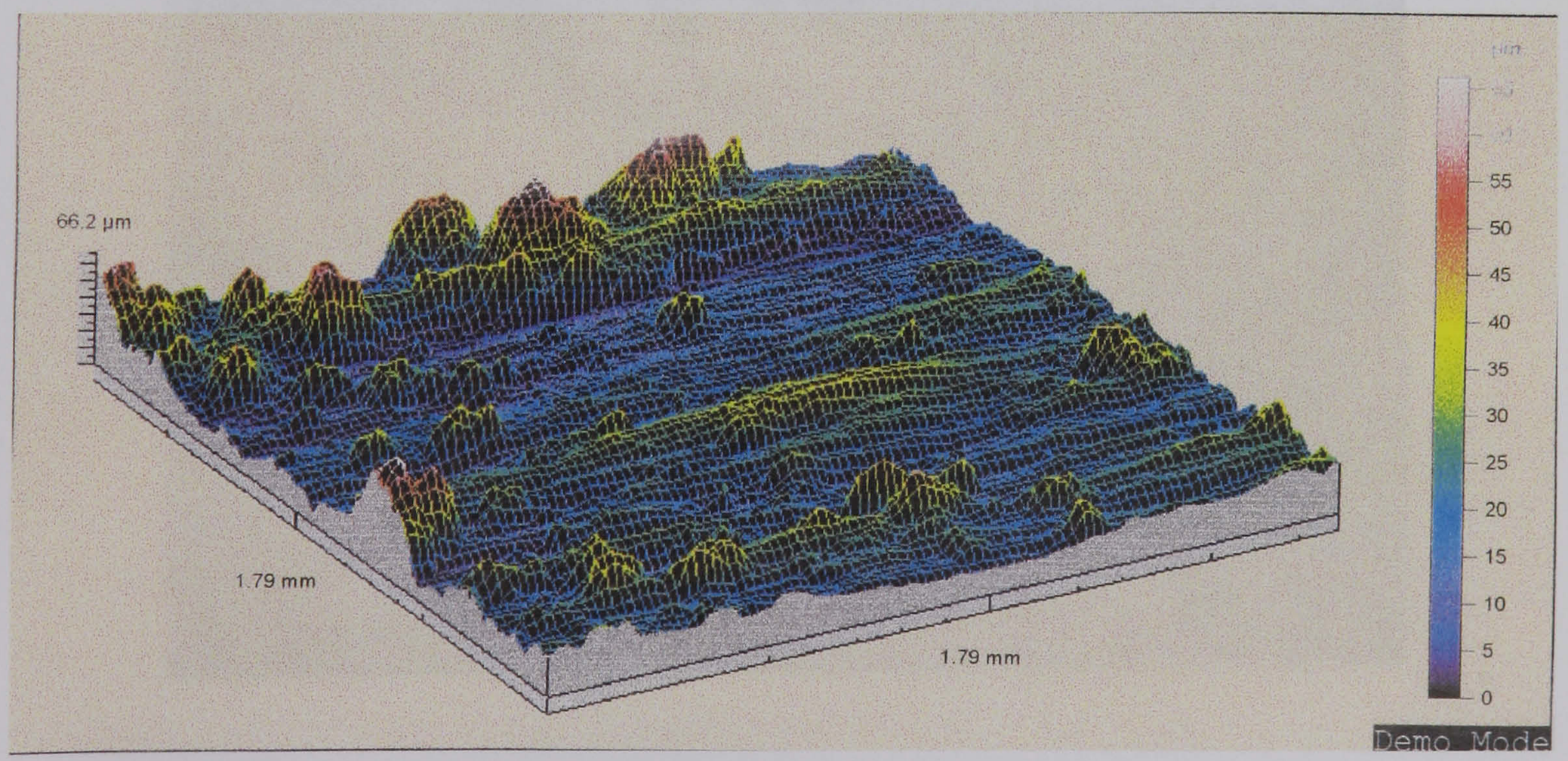


Figure 4.22 A meshed axonomeric diagram of the exposed painted surface after light cleaning.

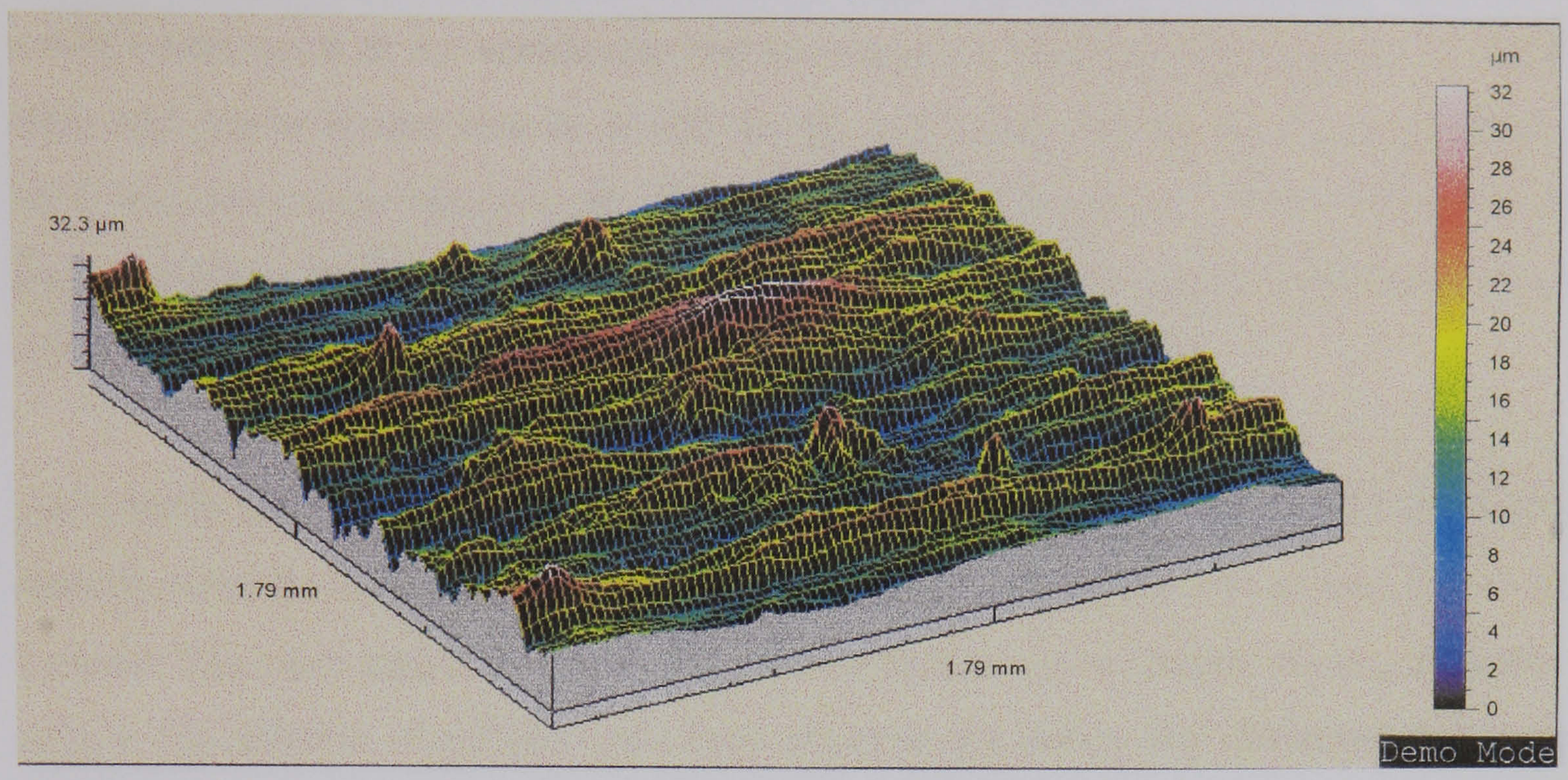


Figure 4.23 An image of the exposed painted surface showing the growth of fungal hyphae along the 'peaks' of the wood grain.

4.7 Summary.

As a result of this work it is considered that any panels placed into a humid environment, such as the vermiculite bed technique, should be coated at least twice and their end grains should also be sealed. In dry conditions (Section 4.4.1) no problems with relocation were experienced. Placing the wooden panels on a sheet of plastic (to avoid contact with the moist vermiculite in the humid environment) was necessary to achieve acceptable relocation (Section 4.4.2).

As wooden panels exposed to microorganisms in the laboratory could not be placed directly onto the Talysurf™, because they would contaminate the stylus, they had to be cleaned. The most appropriate solution to this problem was to cleanse the panels lightly with a 1% solution of Chloros and allow them to dry before any measurements were taken.

The relatively short experiment in terms of exposure, i.e. over twenty weeks, showed that there was no obvious differences in the surface topography between the exposed and unexposed panels painted with paint A. This can be seen in Table 4.6 and Figures 4.12 and 4.13 where, for example, the value Sa, the arithmetic mean, is 4.93µm before exposure and 4.8µm after. There were however, measurable changes in the parameters measured for panels painted with paint B, which did not contain fungicide (Table 4.7 and Figures 4.14 and 4.15). After exposure it was noticed that the troughs of the grain of the wood had deepened slightly, this can be seen in the Sa values of 3.99µm and 4.27µm for the before and after exposure parameters. This could be due to a number of factors, there is a possibility of microbial degradation, weathering, ageing of the paint film or the fact that surface debris, including microorganisms, were accumulating on the close peaks of the surface, thus making the troughs appear deeper.

The results of the twenty-week vermiculite bed experiment are different results to the ones obtained for the panels which were exposed. The surface of the panel painted with paint A, the pure acrylic paint containing fungicide, is seen to have changed from being a relatively flat surface, to one containing small peaks. This could be attributed to the uneven accumulation of *Aureobasidium pullulans* on the surface, or by the slight disruption of the paint film by the cleaning process, resulting in an uneven surface.

Paint B, the pure acrylic paint containing no fungicide, showed definite changes in its topography after inoculation when the recorded parameters were compared. The mean roughness values (Sa) changed considerably, indicating that the total height of the sample area had decreased. The Ssk value, however, indicates that the area has altered from a plateau region to an area with numerous peaks. A possible explanation for this is that the area had developed more peaks, but the height of these peaks was not great. This could be due to either the effect of cleaning or the effect that the growth of *Aureobasidium pullulans* had on the profile of the paint film. The gloss paint, paint F showed marked differences between the before and after images (Table 4.10 and Figures 4.18 and 4.19). Before the inoculation with *Aureobasidium pullulans,* the surface area appeared to be flat, although in fact it was made up of numerous small peaks. After the inoculation the surface topography appeared to be stretched. This could be due to increased water content as the panel was covered in tiny water droplets when it was removed from the vermiculite bed system, or again due to the presence of the *Aureobasidium pullulans*.

The analysis of the surface of the painted panel that was exposed at Preston for thirty months (Figures 4.20 and 4.21) was found to have changed drastically, in that it became a surface scattered with numerous small peaks, which were attributed to surface debris including microorganisms. It was therefore concluded from this and Figure 4.22, which had been lightly cleaned that it is possible to detect long-term changes in topography using the Talysurf™.

4.8 Scanning Electron Microscopy (SEM) and Environmental Scanning Electron microscopy (ESEM)

4.8.1 Materials and Methods

This work was undertaken in the laboratories of AVECIA Ltd. at Blackley in Manchester under the direction of Geoff Poszar. The microscope used was a Philips XL30 SFEG. Spruce panels coated with paints A and B and an aluminium panel coated with paint F that had been exposed at Preston for six weeks and two years respectively, were visualised using the SEM.

Initially the SEM was used at low voltage and pressure without the sample material having being sputter coated. Further samples were given a 5nm sputtering of platinum palladium applied by using a Cressington 208 sputter coater.

ESEM images were also obtained by the kind assistance of two electron microscope suppliers.

Images were obtained as a result of a visit to Leeds University where Mr. David Beamer demonstrated the use of their FE1 XL30 ESEM-TMP Scanning electron microscope system. Appendix G provides further information on the specific application.

Images were also obtained as a result of a visit to the JEOL applications centre (Welwyn Garden City). The machine used was the JSM-5610LV.

The surfaces in these two latter cases examined were the pure acrylic paints (A and B) i.e. containing, and not containing fungicide, that had been exposed at Preston for two months.

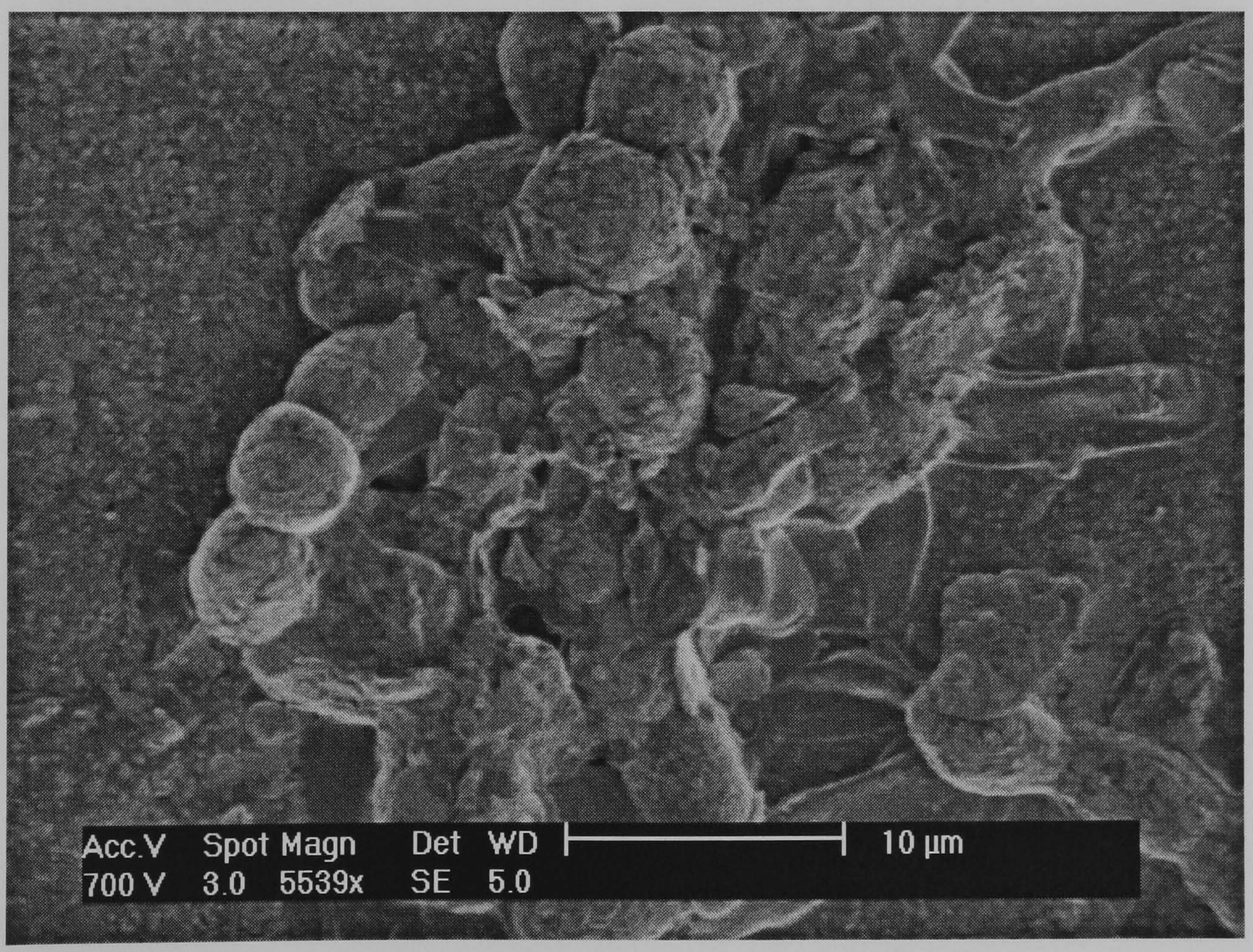
4.8.2 Results

SEM - Philips XL30 SFEG and sputter coating applications: AVECIA Ltd.

Images were obtained of *Aureobasidium pullulans* that was colonising the surface of a spruce panel coated with the non-fungicide containing acrylic paint (paint B), which had been exposed at Preston for a period of six weeks. These can be observed in Figures 4.24 to 4.41.

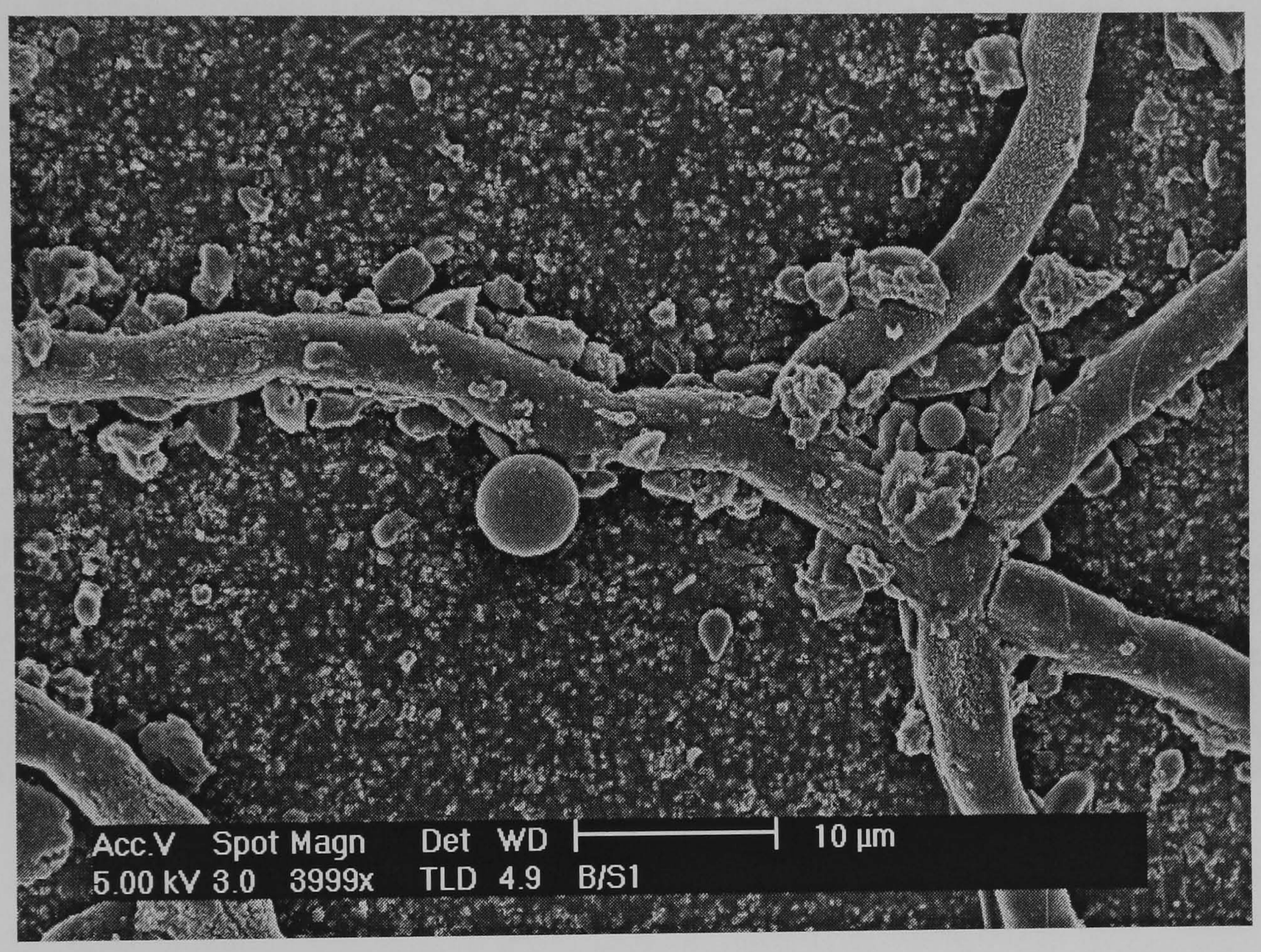
The SEM photomicrograph (Figure 4.24) shows a cluster of spores on the surface of the paint, hyphae are also evident. The resolution, however, even without sputtering shows all aggregation and the beginnings of hyphal foraging. No evidence of hyphal penetration is visible.

Figure 4.24 A low voltage (700V) image of *Aureobasidium pullulans* colonising the surface of the non-fungicide containing pure acrylic paint (Paint B). Despite not being sputter-coated cells are visible in a hydrated state.



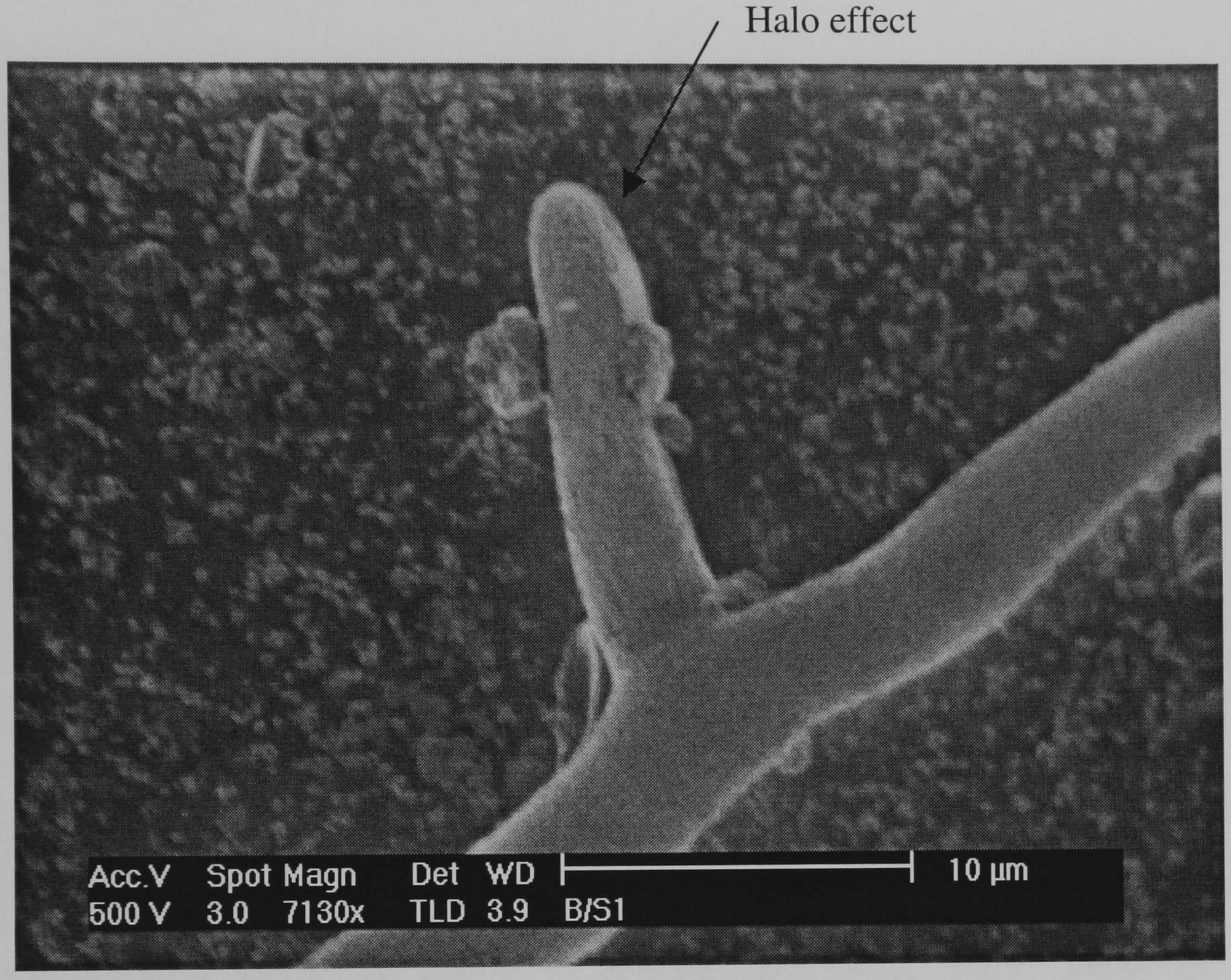
In Figure 4.25 the hyphae are well defined and a single centrally positioned spore is visible. The granular background of titanium dioxide is uniform and shows no evidence of disruption.

Figure 4.25 A high voltage (5.00Kv), sputtered image of a branched mycelium with what is considered to be surface debris in the background. This accumulation of non-biotic material can contribute significantly to the deformation of a paint surface.



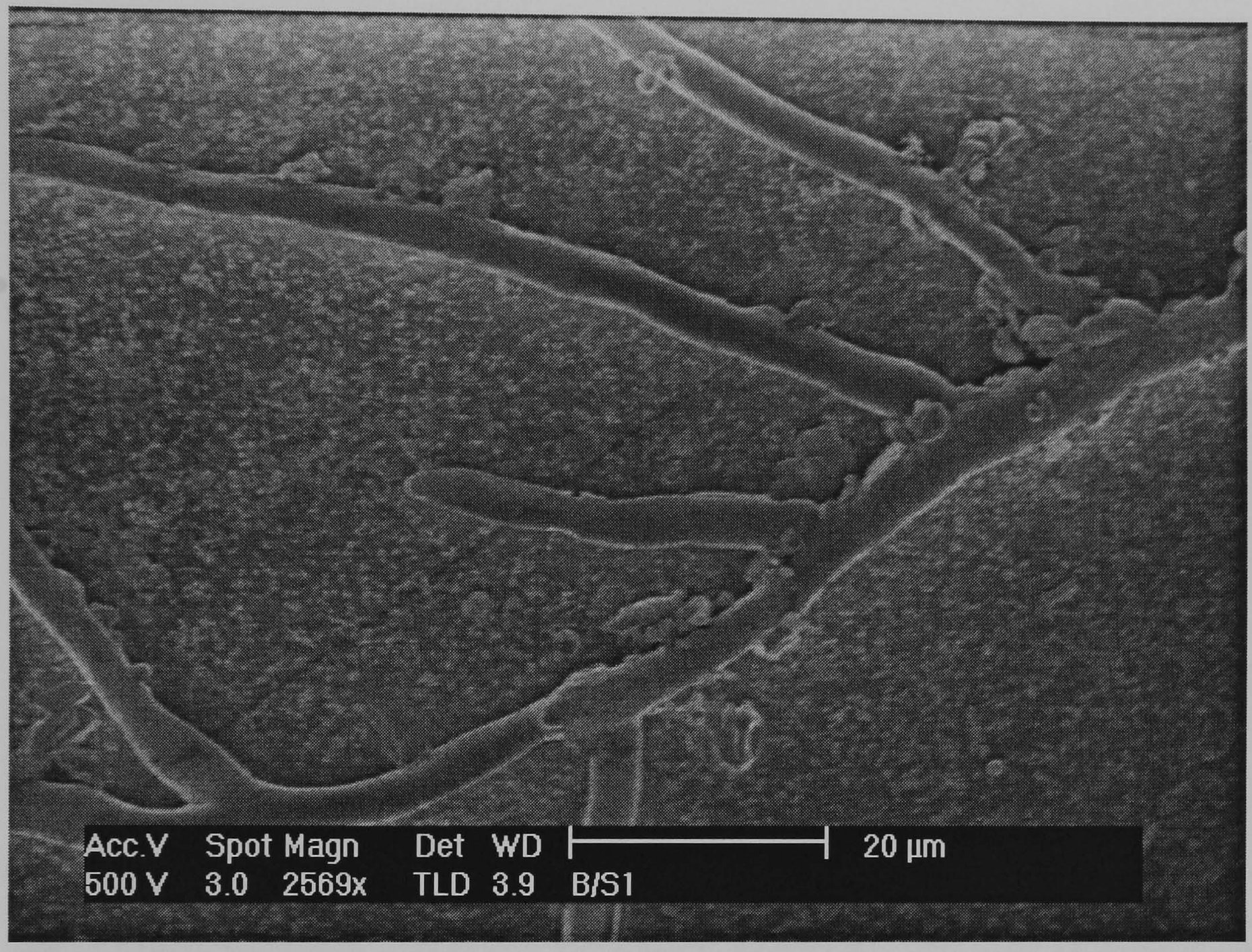
The halo effect in Figure 4.26 is the result of extracellular enzymic activity around the periphery of the hyphal tip, indicating the dissolution of the binder matrix.

4.26 A hyphal tip with a 'halo' effect and attached debris.



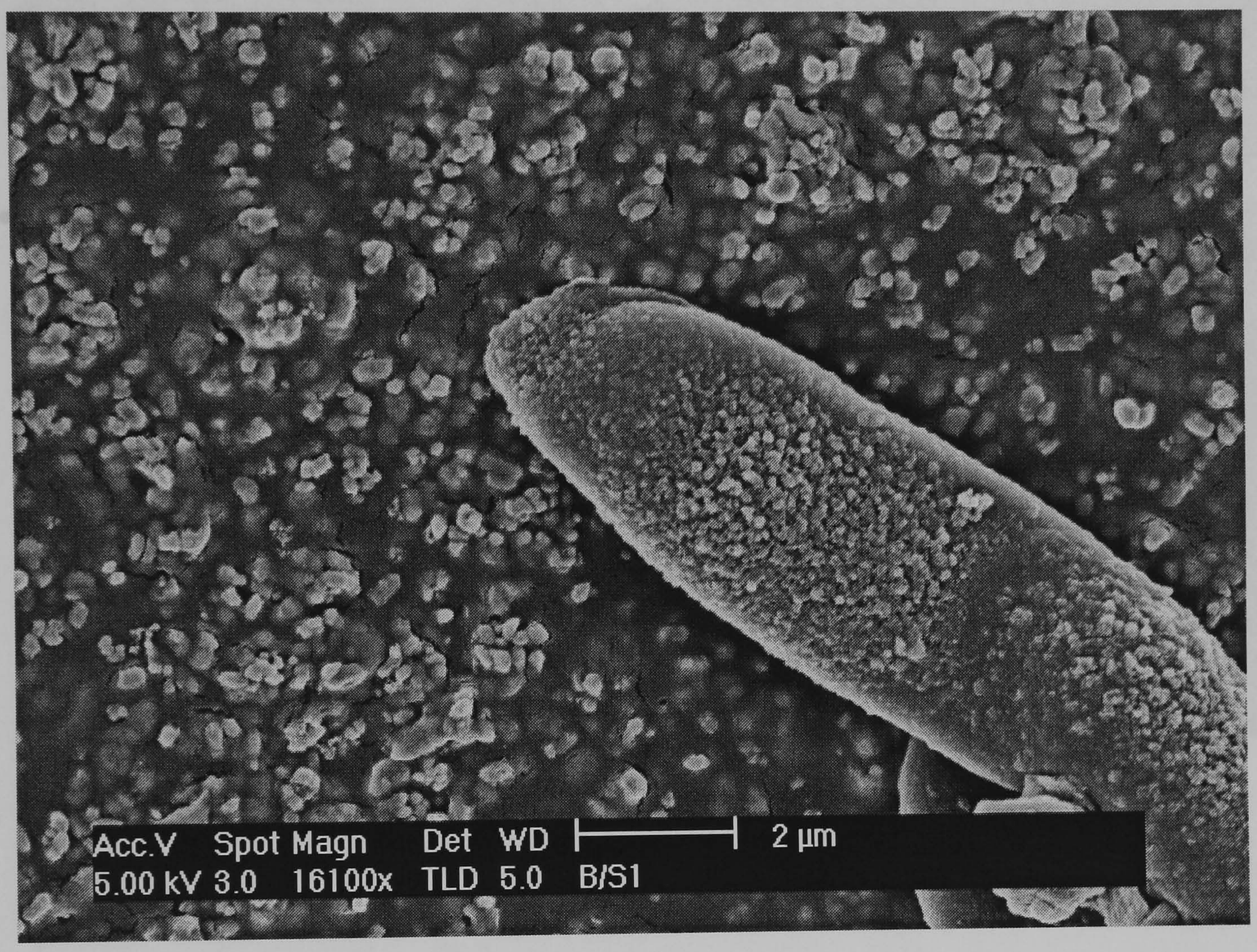
The micrograph in Figure 4.27 shows hyphal side initials issuing from a main hyphal strand. The hyphae are superficial at this stage but there is evidence of the halo effect at the edges of this mycelium.

Figure 4.27 Hyphal tips without the 'Halo' effect



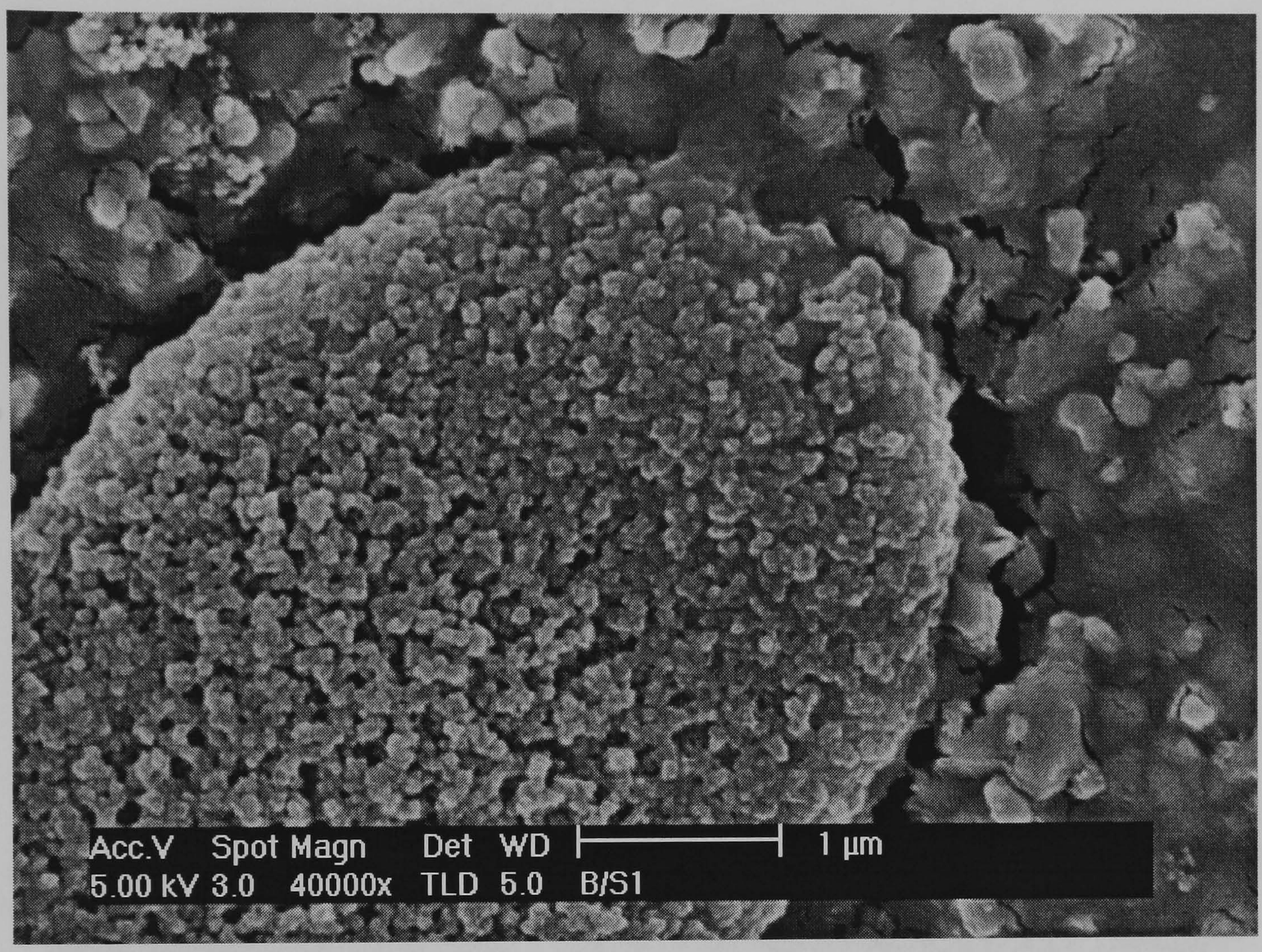
The micrograph in Figure 4.28 shows a hyphal tip, the halo effect is not well marked in this image and the titanium dioxide pigment is still bound by the binder matrix. The fine granules on the surface of the hyphal tip may be due to the disruption of the chitin of the cell wall.

Figure 4.28 Hyphal tip without the 'halo' effect



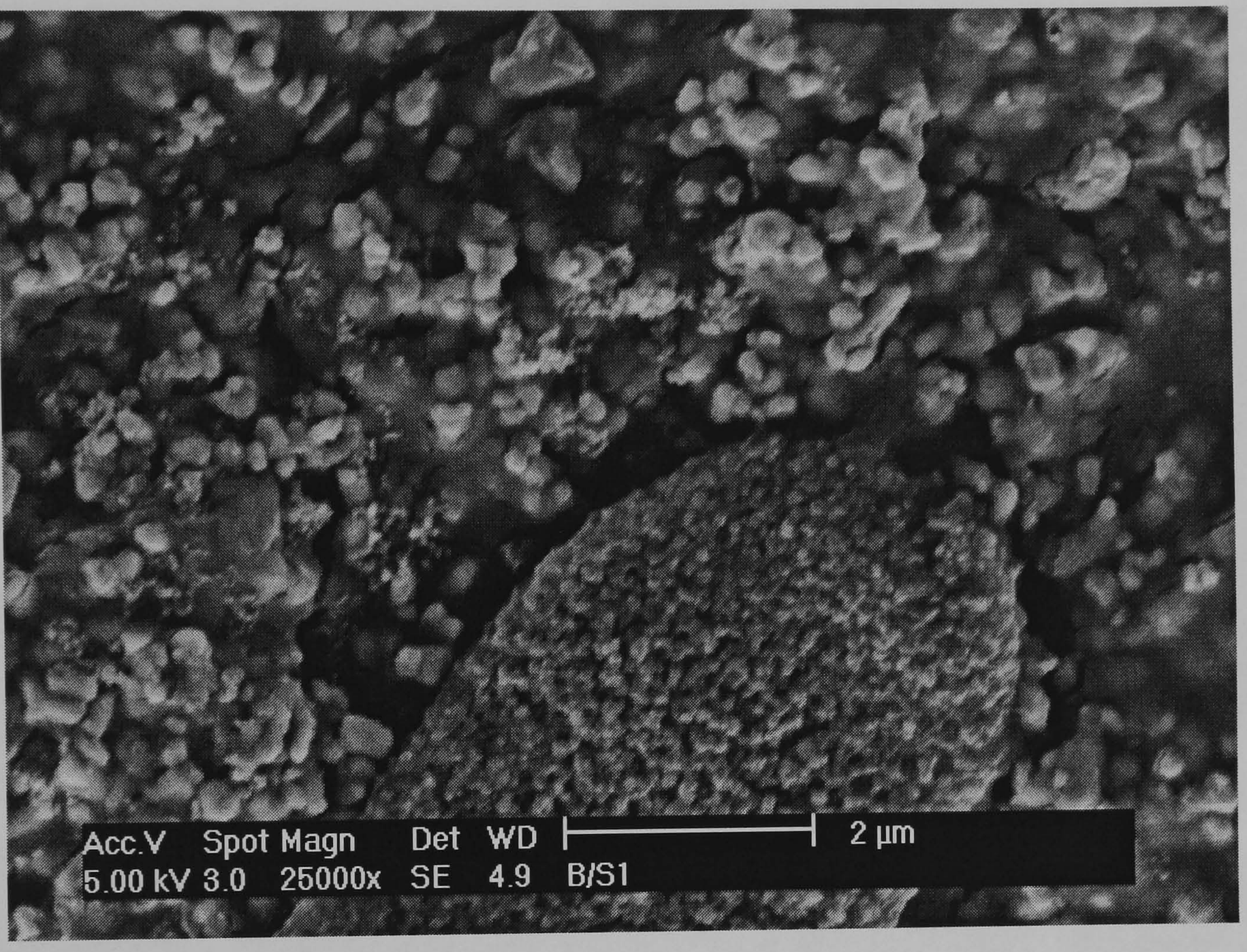
In the micrograph (Figure 4.29) there is clear cracking of the matrix material. The granular appearance of the hyphal tip is attributed to disruption of the hyphal wall.

Figure 4.29 Hyphal tip showing cracking of the paint film.



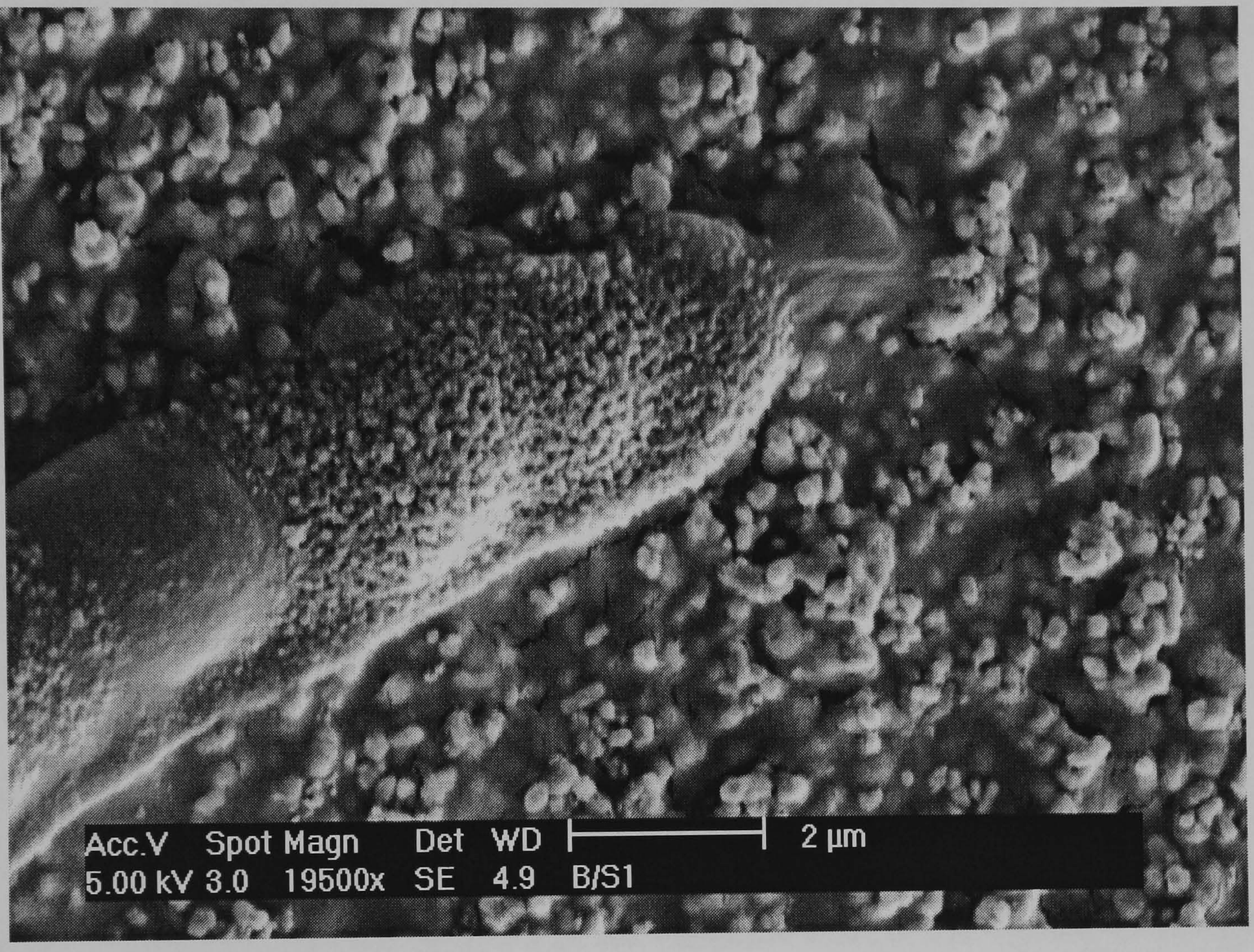
There is a clear margin visible between the titanium dioxide-bound particles and the hyphal tip in Figure 4.30. The cracking of the film is spreading from around the tip into the surrounding film.

Figure 4.30 Halo effect at hyphal tip indicating breakdown of the binder.



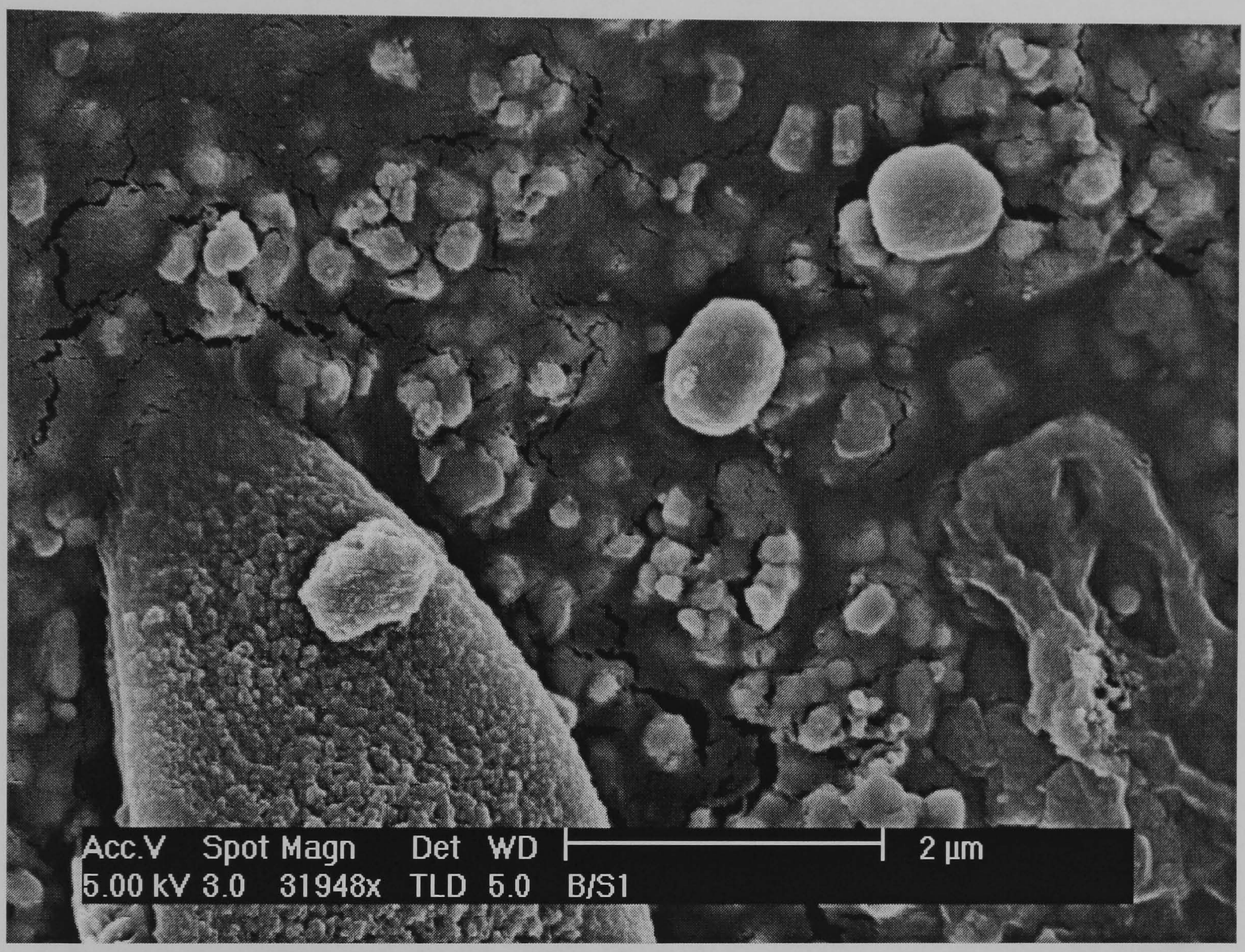
The photomicrograph in Figure 4.31 shows a hyphal tip secreting EPS, onto the surface of the film, onto the binder and titanium dioxide particles. The granular nature of the surface of the tip may be due to the drying of EPS at the hyphal surface.

Figure 4.31 Hyphal tip with EPS production



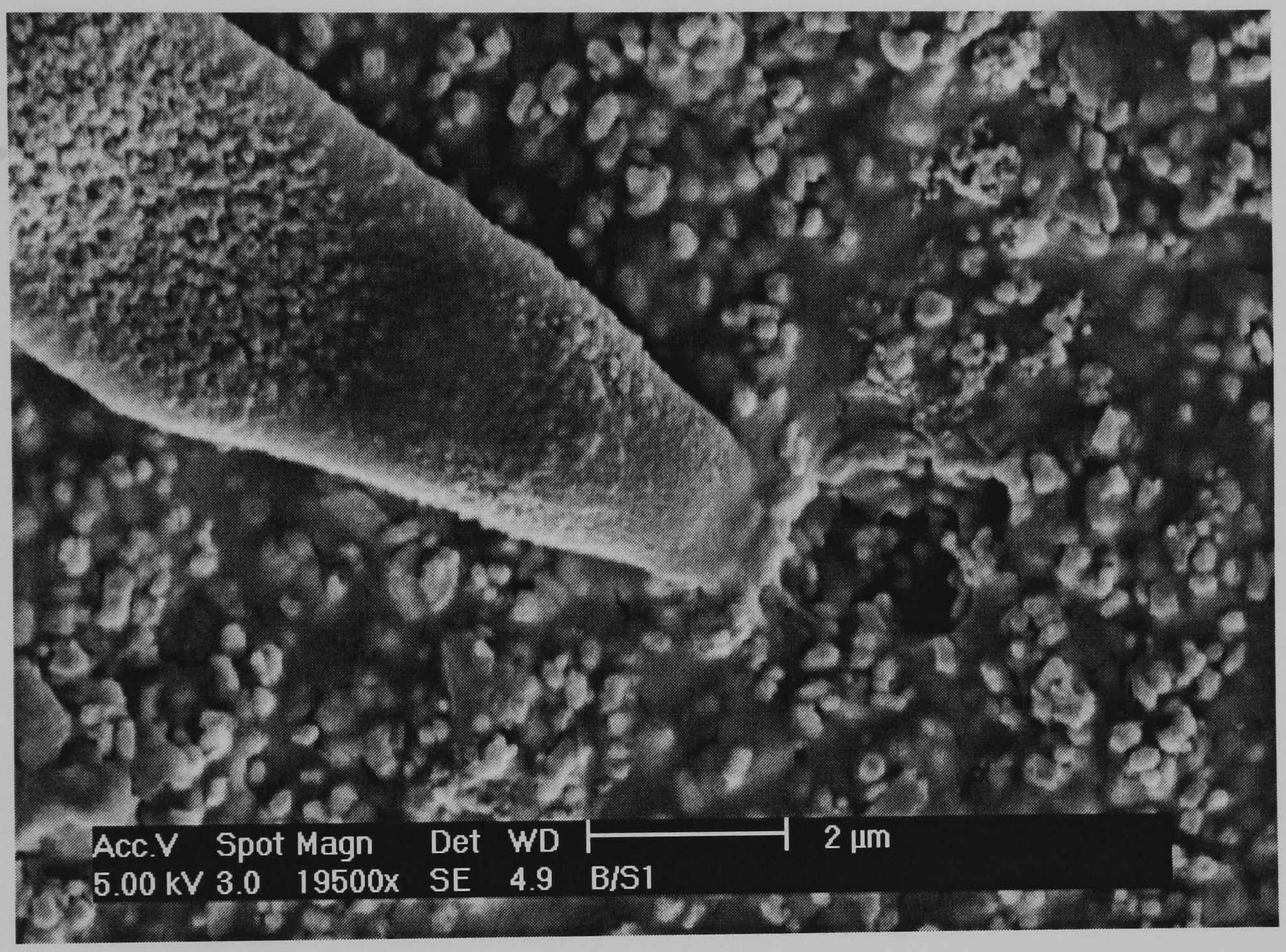
The larger particles are considered to be dust or larger aggregates of the pigment in Figure 4.32.

Figure 4.32 Hyphal tip with EPS production confluent with the paint film. The breakdown / cracking of the paint film can be observed



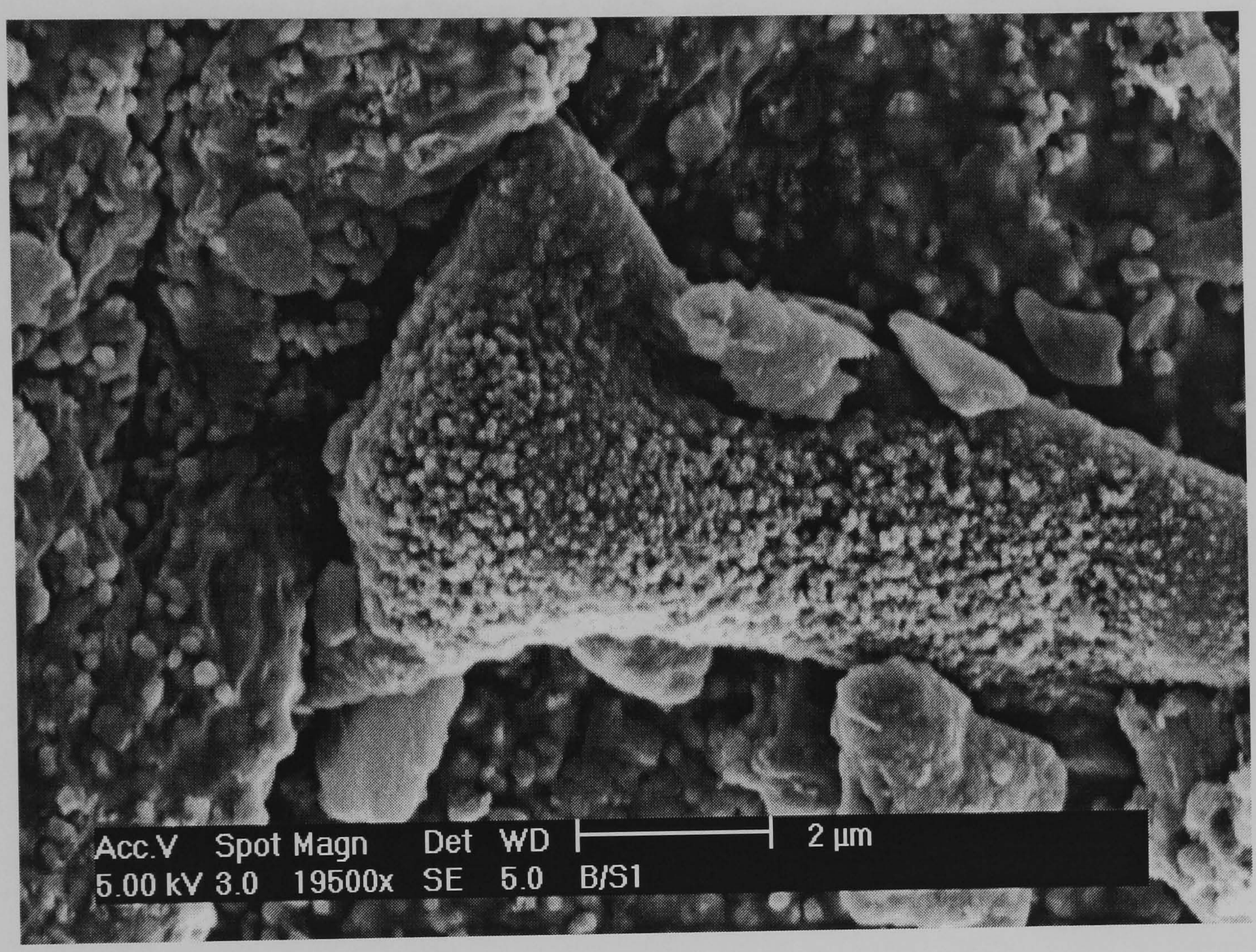
In Figure 4.33 the hyphal tip appears to have come away from the surface pulling a portion of the binder and titanium dioxide particles with it, leaving a hole in the surface and giving the hyphal tip its fringe-like appearance.

Figure 4.33 A hyphal tip associated with the surface of the film. There is evidence of EPS production at the tip and dissolution of the binder occurring near the tip leading to cavity formation.



In Figure 4.34 the club-like nature has larger particles of debris on the surface. The granular nature of the surface of the hyphae is considered to be due to the wall and EPS drying out, rather than the presence of bacterial contaminants.

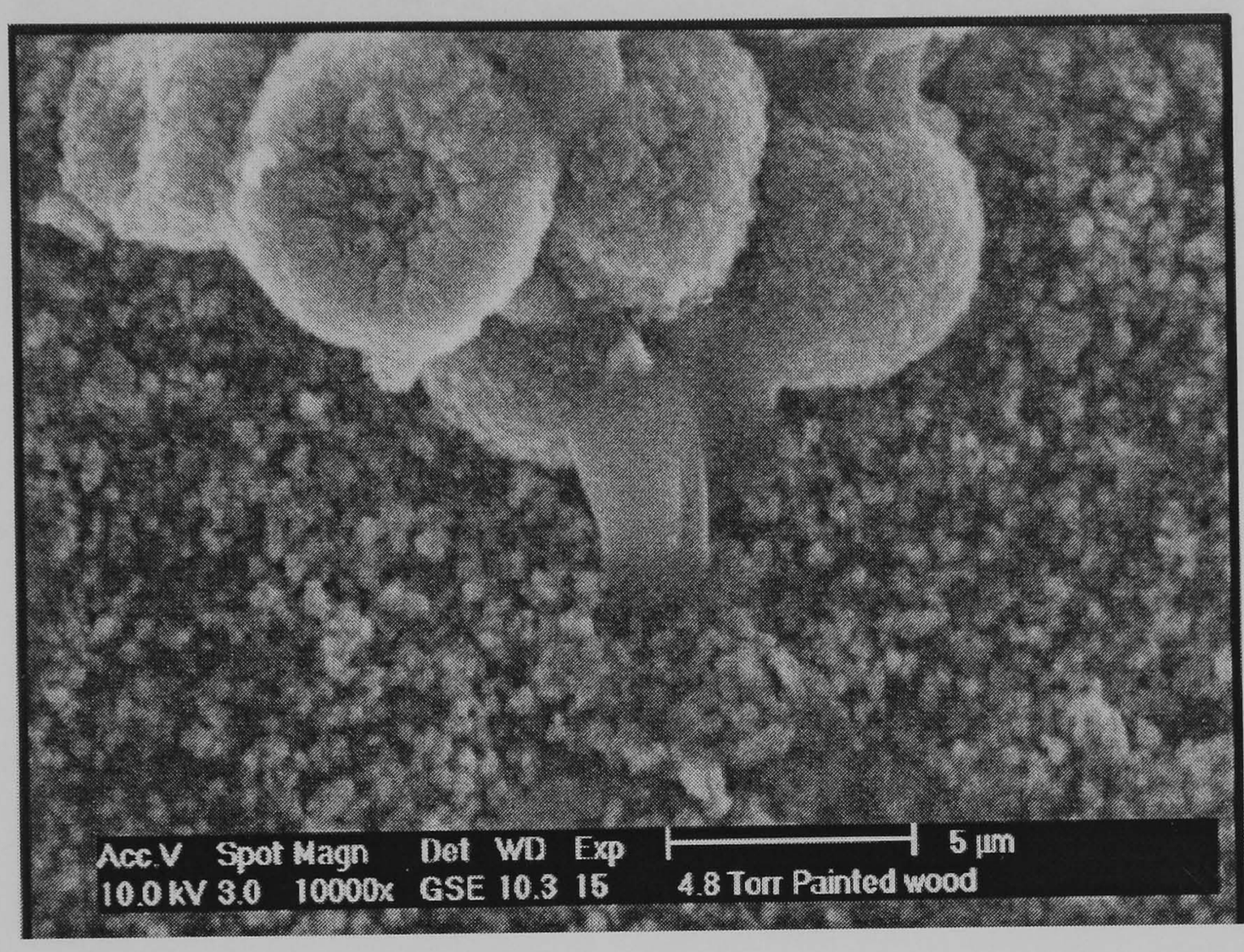
Figure 4.34 A club shaped hypha with a branch which appears to be penetrating into the paint film. The resulting loss of integrity allows ingress of water that is in part responsible for blistering and flaking.



The results obtained from the FEI XL30 ESEM

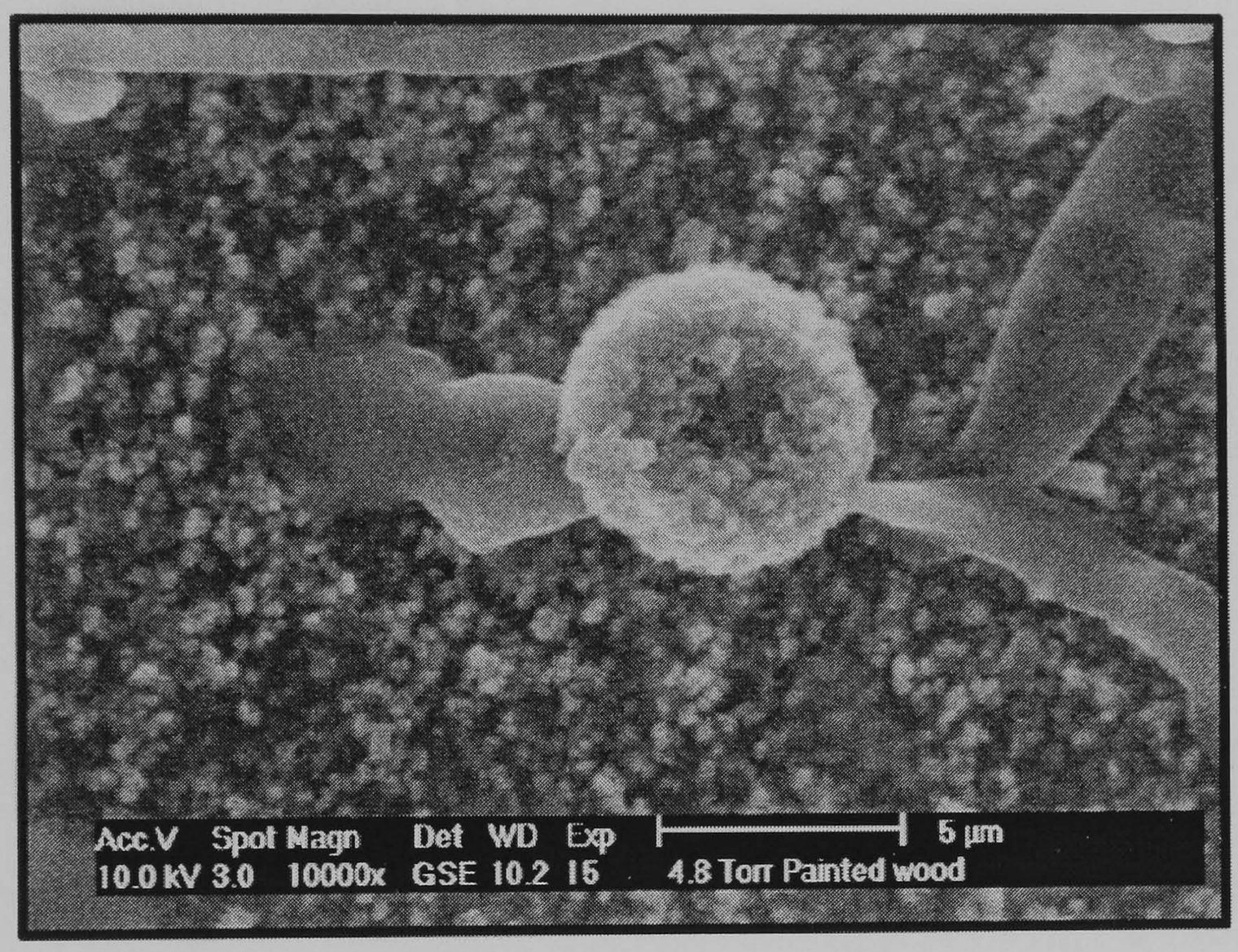
A cluster of spores is visible in the photomicrograph represented by Figure 4.35. The 'dimple' effect visible in two of the spores is a typical effect of dehydration. There is also evidence of spore germination with the issuing hypha penetrating the film.

Figure 4.35 A cluster of cells and hyphal penetration of the paint film.



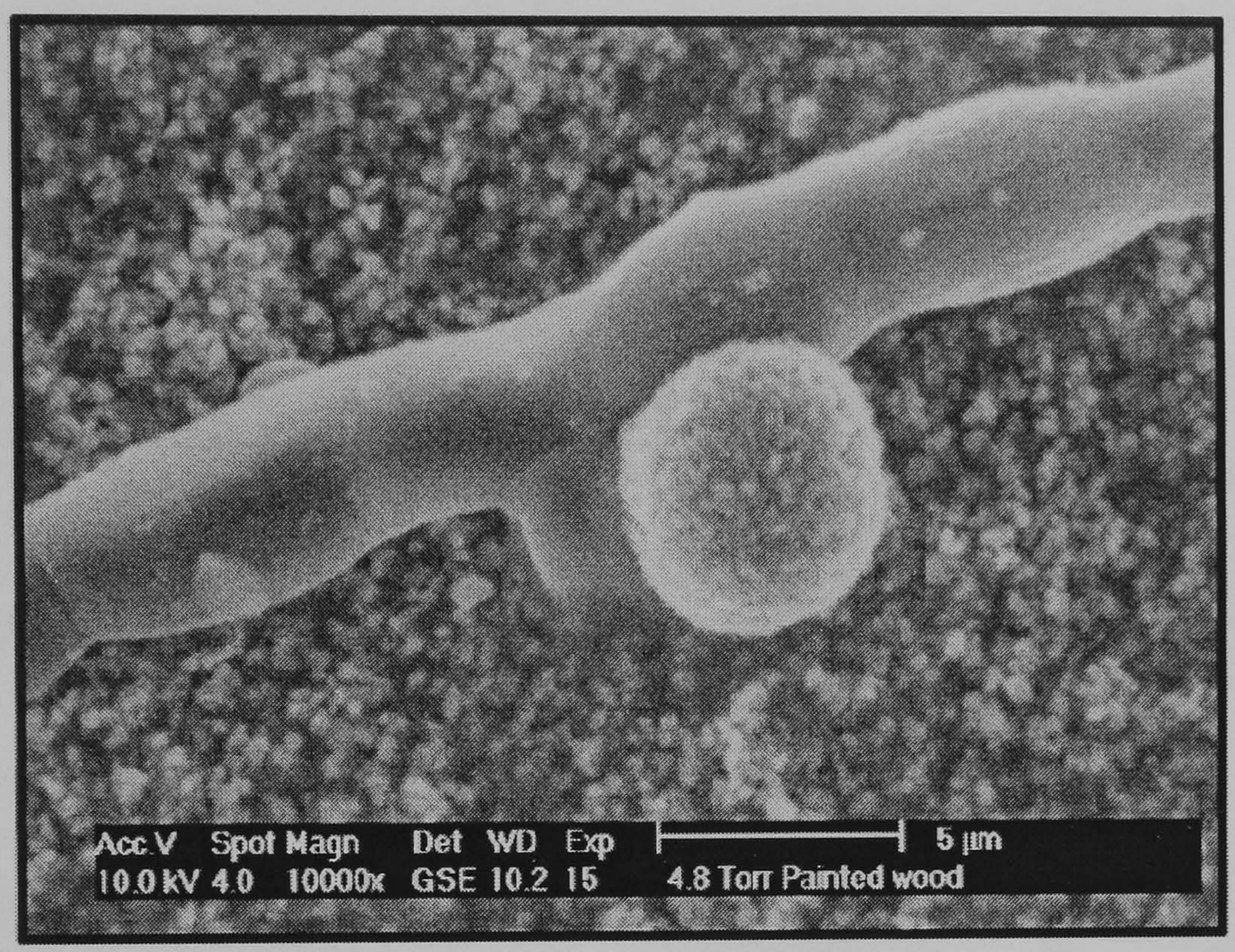
A spore, which is granular in appearance, is producing hyphae, one of which (left) is actively penetrating the paint film (Figure 4.36). The ability of newly emerged hyphae to penetrate is a feature of a successful coloniser.

Figure 4.36 The hypha on the left ,which is in association with the paint film shows a similar association to that seen in Figure 4.33.



In this photomicrograph (Figure 4.37) a side branch of the mycelium and a single spore are evident. The surface of the spore, unlike the hyphae, has a granular appearance. There is no obvious disruption of the surface of the paint film, however, there is evidence of penetration.

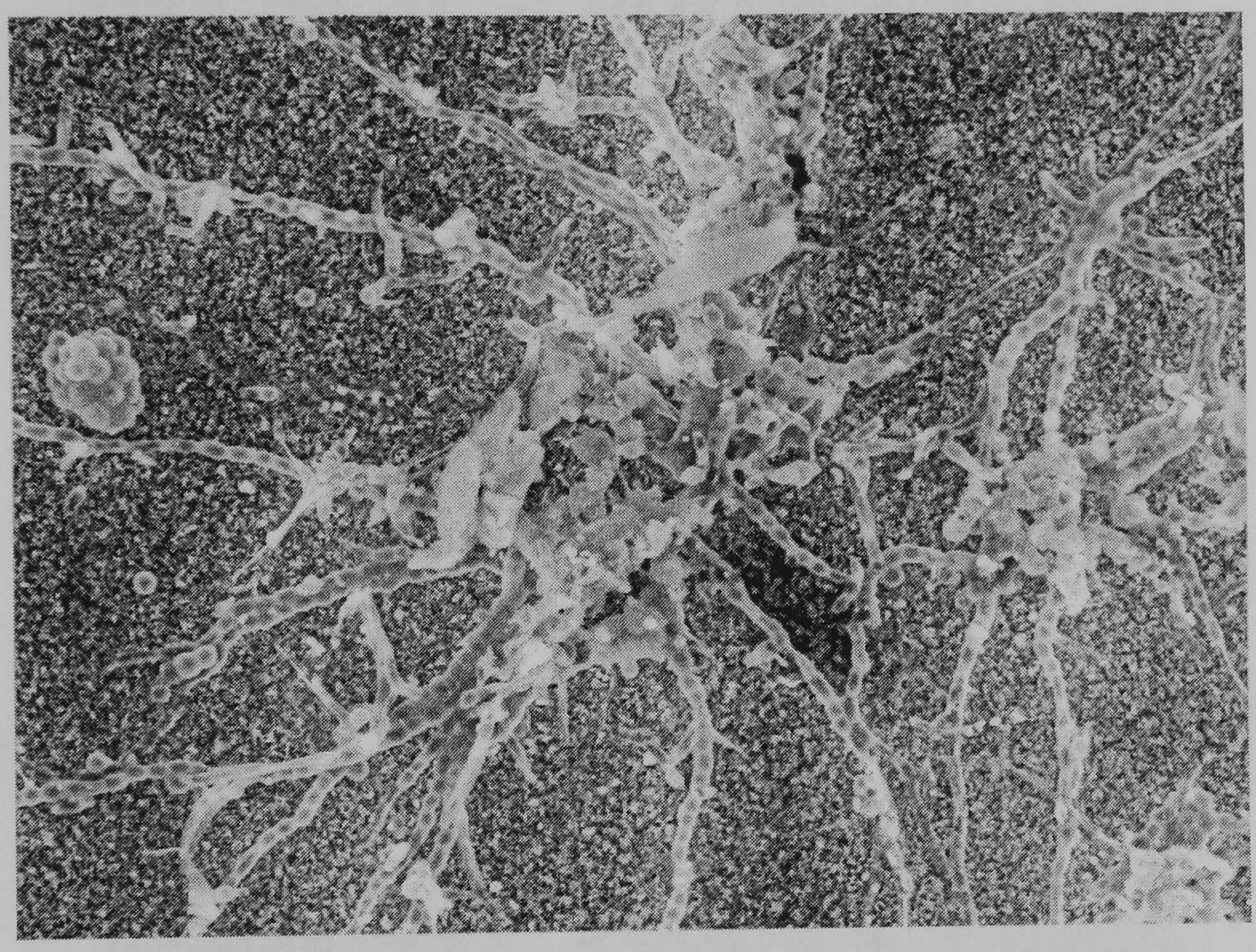
Figure 4.37 A similar effect to that seen in Figure 4.36.



The results obtained from the JSM-5610LV ESEM

The micrograph in Figure 4.38 shows hyphae emanating from a central point on the film. This is a typical colonisation pattern in *Aureobasidium*. The hyphae show the yeast-like nature of this fungus with the formation of the pseudo-mycelium characteristic of this organism.

Figure 4.38 *Aureobasidium pullulans* growing on a non-fungicide containing pure acrylic paint. There is obvious surface colonisation rather than the organism growing through the paint film.



Hyphal segments of a pseudomycelium with foraging lateral offshoots can be seen in Figure 4.39. There are no signs of disruption of the film by this superficial growth. However, the hyphae are actively growing indicating the availability of nutrients.

Figure 4.39 The mycelium of *Aureobasidium pullulans* with lateral branches probing the surface of the paint (x1500).

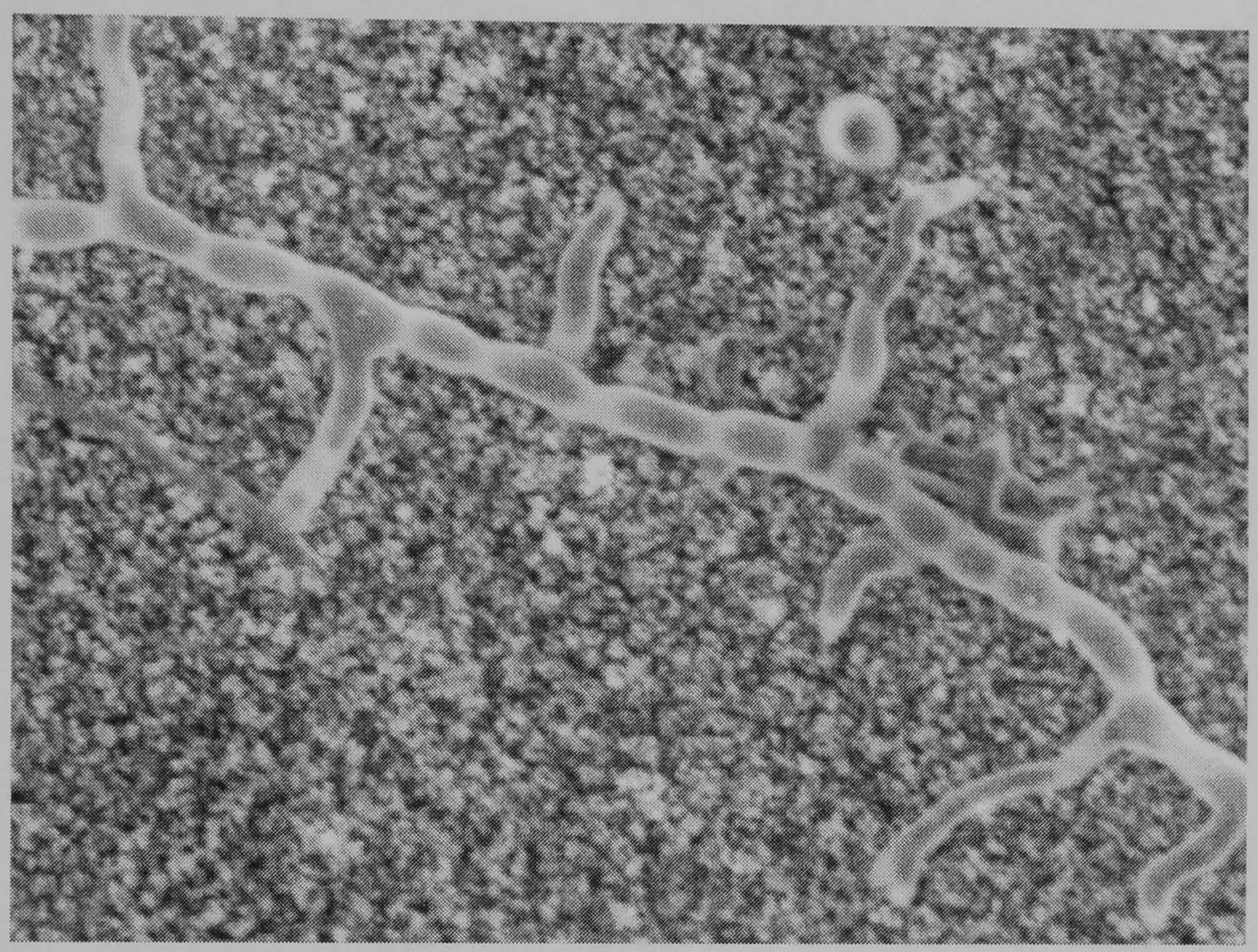
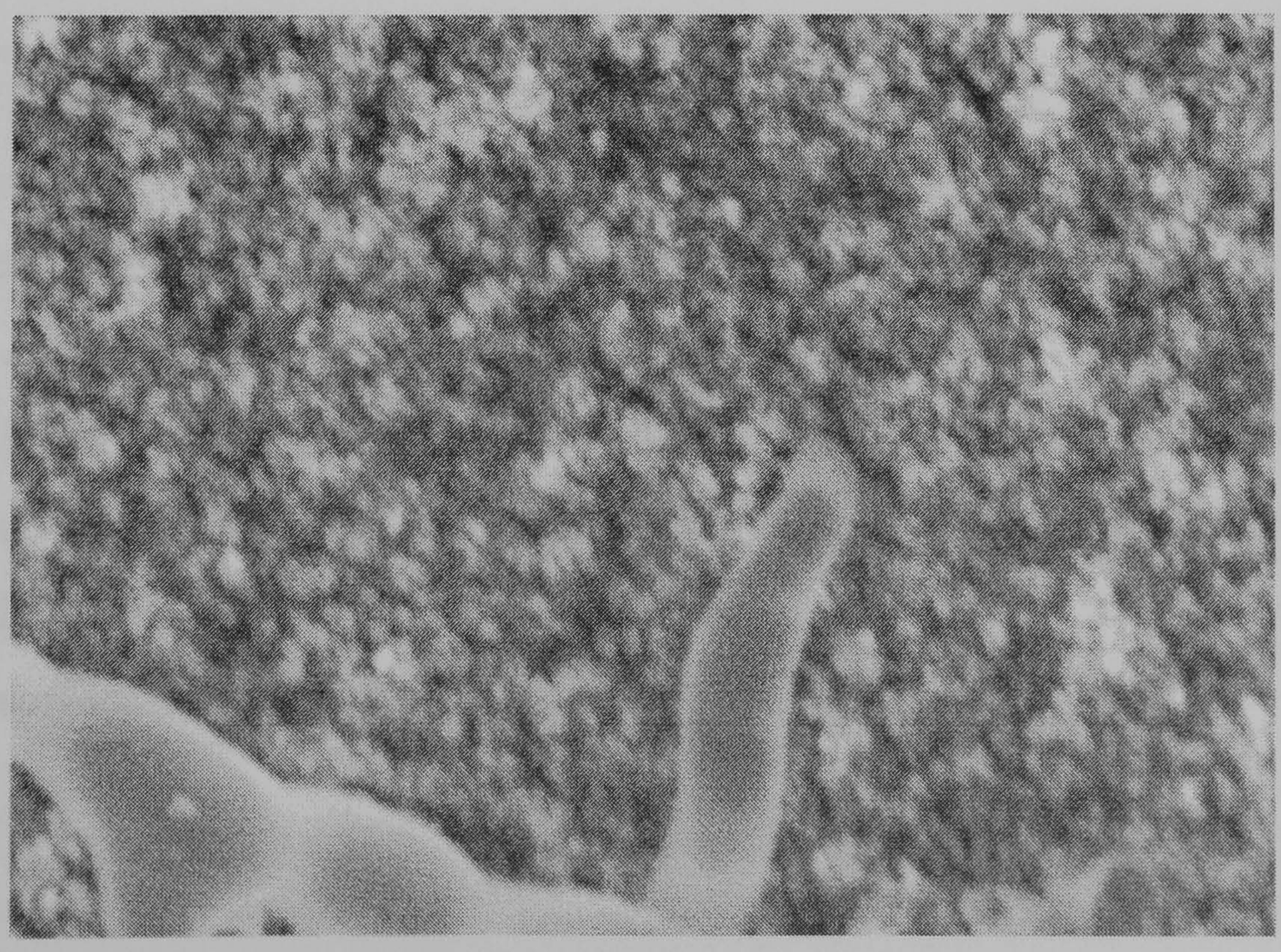
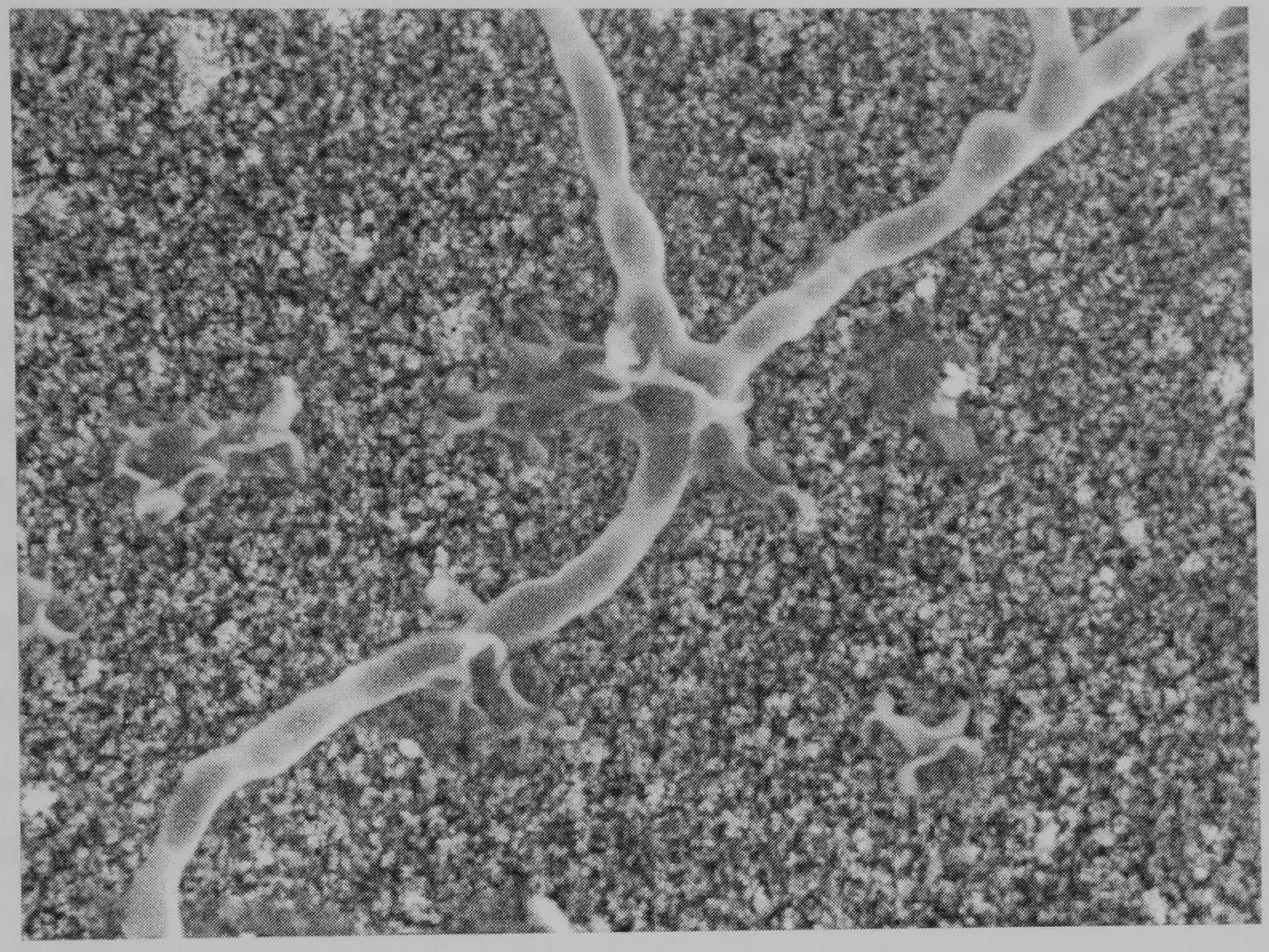


Figure 4.40 A higher magnified image of the lateral hypha seen in Figure 4.39 (x4000).



The micrograph in Figure 4.41 shows a branched hyphal strand which appears to be constricting to produce cells. Centrally there is evidence of EPS production, serving to anchor the hyphae to the substratum.

Figure 4.41 A branched hypha with what looks like EPS secreted at the centre (x1500).



4.8.3 Summary

The SEM images (Figures 4.24 to 4.34) have shown that interactions between the hyphal tips of *Aureobasidium pullulans* and the paint film are such that the hyphae may initiate the breakdown of the binder.

The ESEM images (Figures 4.35 to 4.41) obtained show the quality of ESEM and how these images confirm the predominance of *Aureobasidium pullulans* on panels of this age. They also confirm the surface colonisation and penetration, rather than disruption of the paint surface from below.

4.9 The use of FTIR spectroscopy to characterise paint films

4.9.1 Materials and Methods

A paint film of approximately 0.5mm in depth was required for the Jasco model FT/IR 400 with a Golden Gate appliance. This was achieved by coating a piece of acetate sheet measuring 4cm x 2cm four times with the appropriate paint using a brush. The coated piece of acetate could then be placed directly in the chamber of the FTIR for analysis.

Three films were prepared for each of the six paints. These were analysed using the FTIR and the spectra recorded. Three plastic boxes containing vermiculite were prepared as in Section 4.3.1. The vermiculite was covered with a clean piece of acetate to avoid the vermiculite particles spreading over the painted surfaces. One set of each of the six paints was placed into each of the three boxes. The paint films in the first box were not inoculated, they acted as the controls. The paint films in the second box were inoculated with a suspension of *Penicillium chrysogenum* and those in the third box with *Alternaria alternata*. All eighteen paint films were analysed approximately every four weeks over a six month period. The machine was used in reflectance mode using the FTIR with a scan range of 2000 to 700cm^{-1}.

In order to investigate the effect of prolonged exposure on the chemical composition of a paint film the following procedure was followed. A small panel measuring 1cm x 1cm x 0.5cm was cut from a spruce panel which had been painted with paint B, the non-fungicide containing pure acrylic paint. This acted as the control and was duly analysed using the FTIR.

A sample measuring 1cm x 1cm x 0.5cm was cut from a spruce panel that had been exposed at the Preston site for twenty-three months, i.e. from September 1999 to August 2001. This sample was also analysed after the surface had been lightly cleaned to remove surface debris.

All of the raw materials of the acrylic paints were also analysed using the FTIR spectrometer.

4.9.2 Results

From the spectra obtained for the control paints which had been in the vermiculite bed, it was evident that there had been no changes in the chemical composition of the paint. The peaks had not shifted position or altered in their appearance. It was also recorded that the paints that had been inoculated with *Penicillium chrysogenum* and *Alternaria alternata* showed no obvious changes throughout the six month period. Spectra recorded after three months exposure showed an increase in the sharpness of the peaks obtained for the inoculated paints in the vermiculite beds (Figures 4.42 and 4.43).

One observation after the third month of exposure showed a considerable increase in the sharpness of the peaks recorded for all of the inoculated paints. This can be seen in Figures 4.44 and 4.45 which show the FTIR traces of the biocide free acrylic paint (Paint B) recorded before and after inoculation with *Penicillium chrysogenum*.

This is most likely due to the following:

(i) The removal of trace organic materials from the paint surface or,

(ii) The removal or flattening of the titanium dioxide particles in the surface, the specific nature of which is responsible for the high diffuse reflectance of the paint surface.

Figure 4.44 shows the FTIR spectrum of the spruce panel painted with the hybrid paint containing no fungicide. When this spectrum is compared with the spectrum shown in Figure 4.45 which shows the same type of paint after twenty-three months exposed at Preston, it can be seen that there is little difference between the two, other than what appears to be a slight amount of sharpening up around peak 1.

The resulting spectra from the raw materials of the paint can be found in appendix H. The titanium dioxide trace shows no significant peaks in the 2000-700cm^{-1} regions although there are various sharp, but low intensity peaks that are considered to be OH bends and the titanium dioxide overtones. This region is clearly distinguishable on the trace of the paint between 2000 and 1500cm^{-1}. The cellulose thickener (HEC) shows no OH bends due to the fact that the OH of cellulose is found at about 1600cm^{-1}, therefore it can be deduced that this molecule has either been ethylated or methylated. The

propylene glycol (anti-frosting agent) contains an OH bend between peaks five and ten, due to the nature of propylene glycol it will have evaporated from a well cured paint so would not be very distinguishable in the spectrum of the entire paint. The defoamer is made from a short polymer, it can be seen that peak number five is Si-CH_3 and that peak number six, Si-O, is present in the spectrum for the entire paint as is peak number seven. The acrylic thickeners show numerous polyester and polyether bends, although thickener one has less polyether in it than thickener two. Various sections of these spectra can also be identified with the spectrum for the entire paint. The binder for the pure acrylic paint is shown in Appendix H:2 and the hybrid in Appendix H:3 these show both ester and ether links. The majority of these spectra can be recognised in the spectrum for the entire paint.

Figure 4.42 The FTIR trace of the un-inoculated paint.

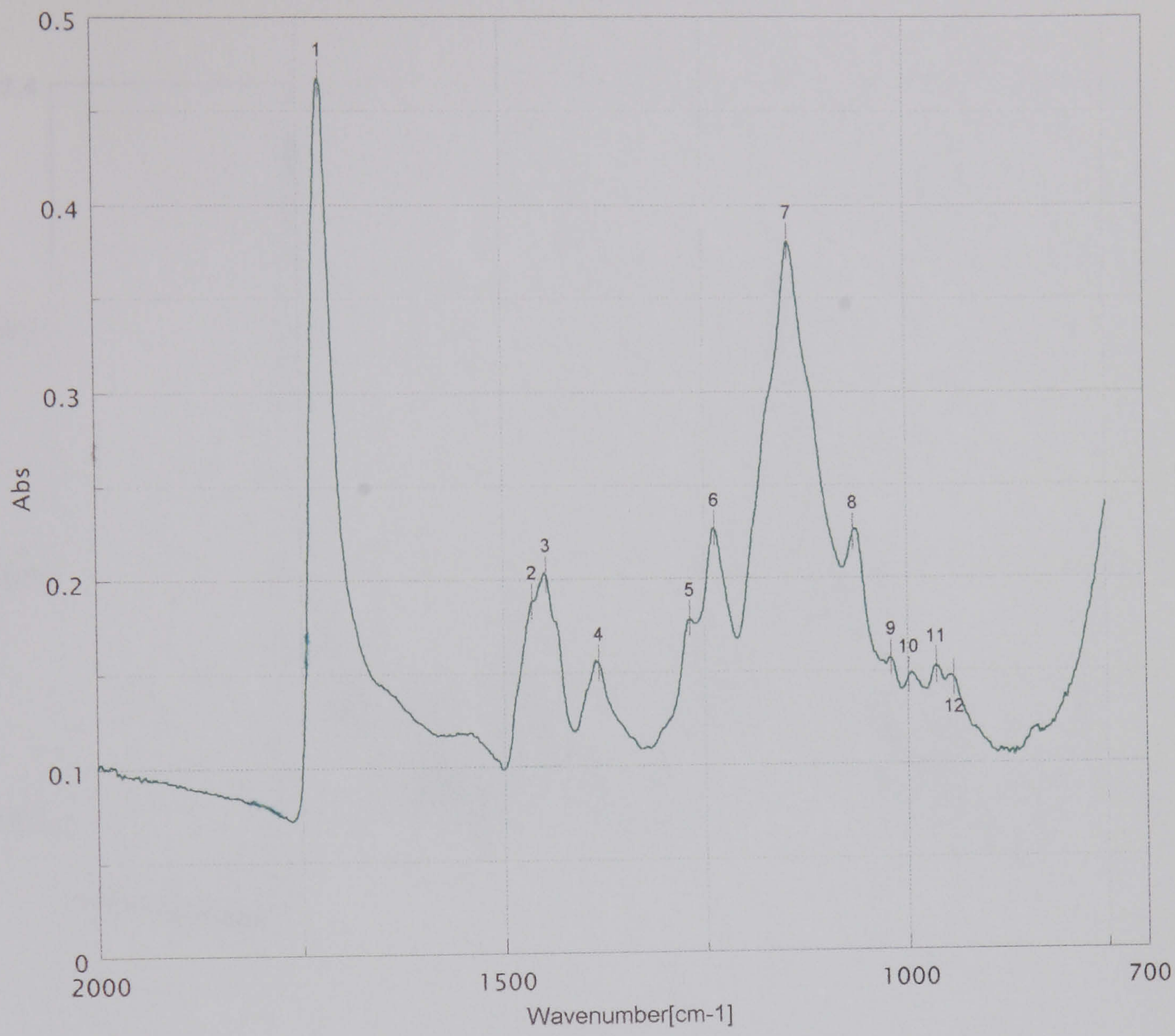


Figure 4.43 The FTIR trace of the paint film inoculated with *Penicillium chrysogenum* in a vermiculite bed system after five months.

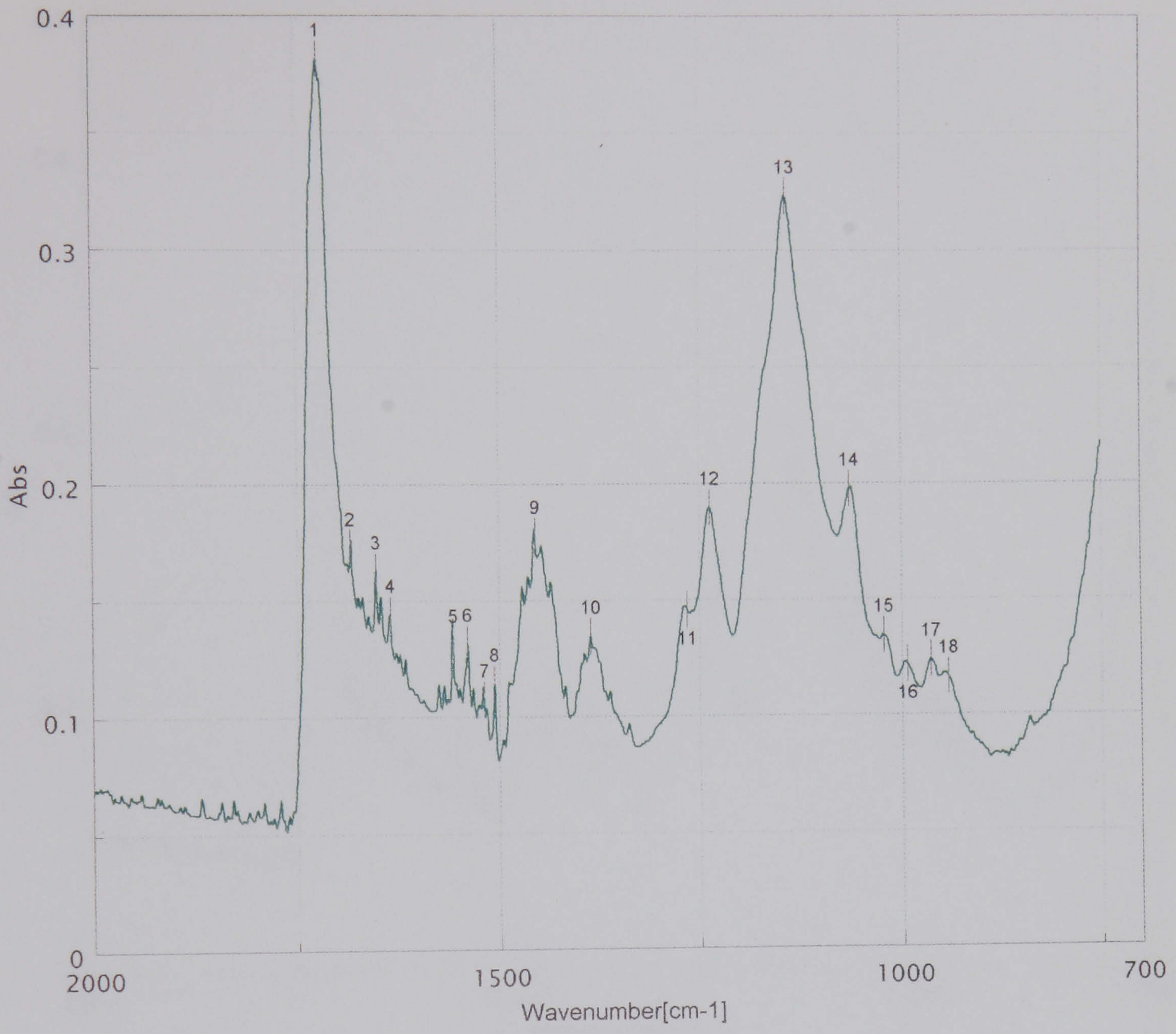


Figure 4.44 The FTIR spectrum of the spruce panel painted with the hybrid paint containing no fungicide before exposure at Preston.

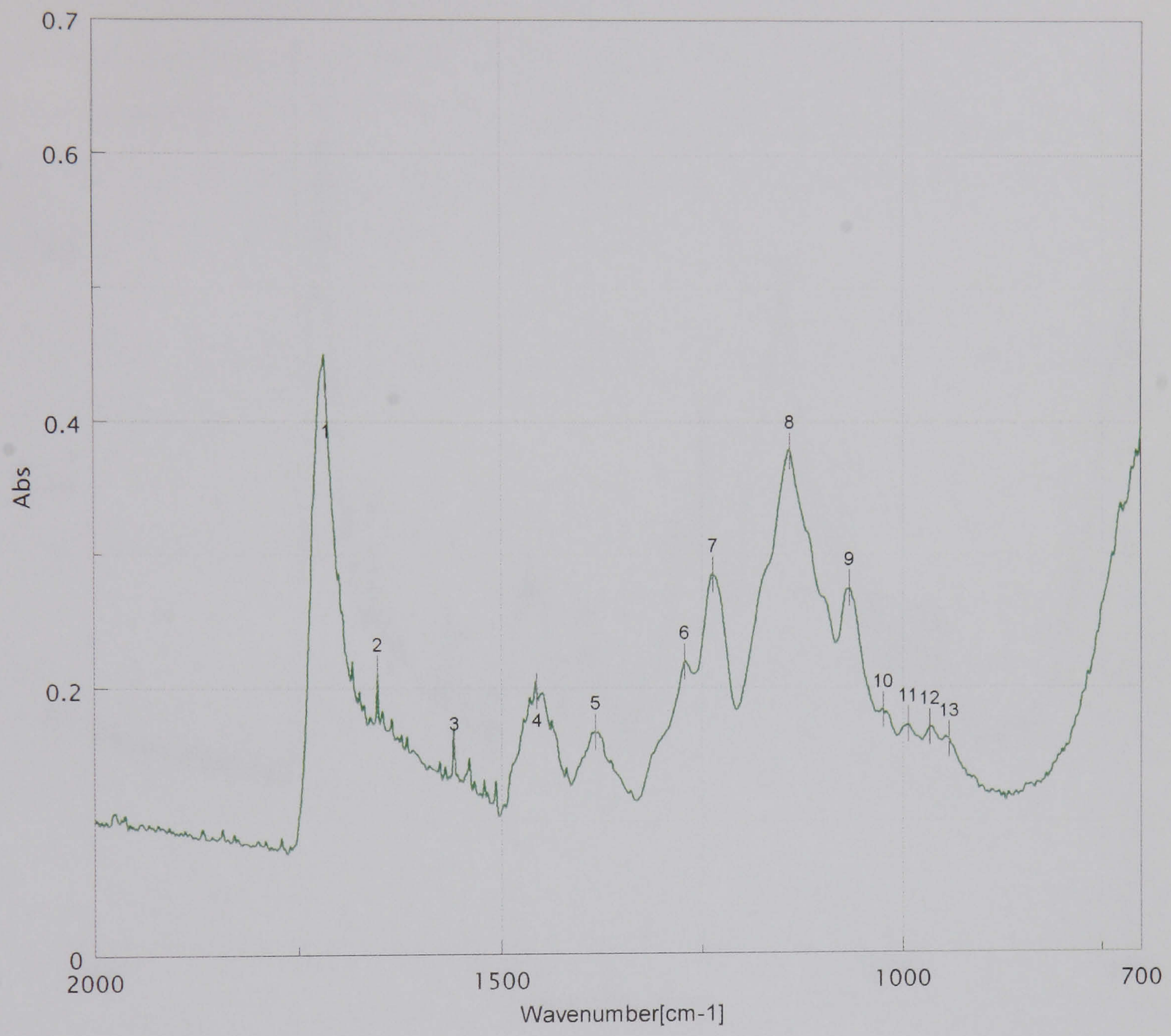
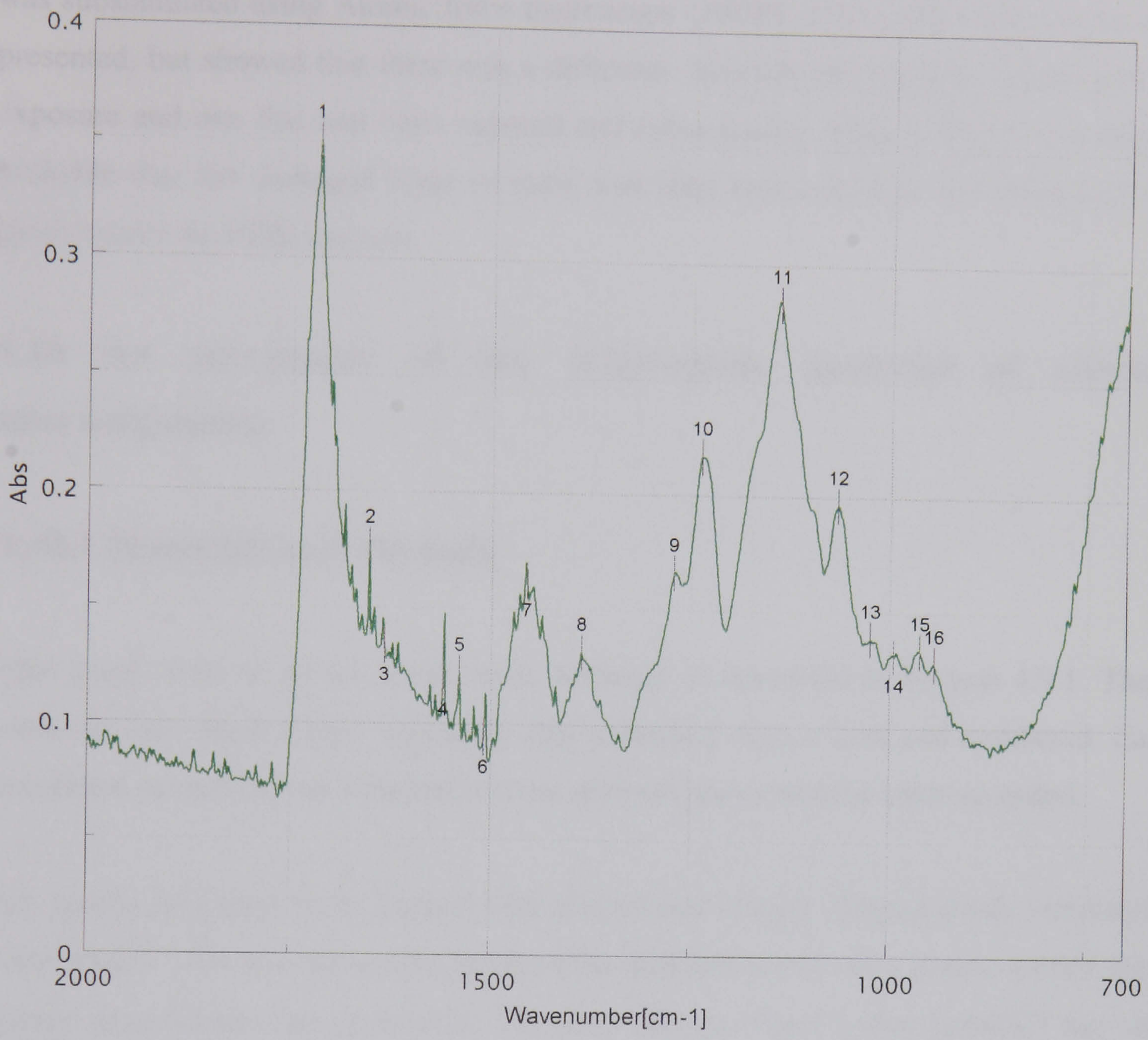


Figure 4.45 The FTIR spectrum of the spruce panel painted with the hybrid paint containing no fungicide after twenty-three months exposed at Preston.



4.9.3 Discussion

From the results of the FTIR work it can be seen that there is a similarity between the spectra of the raw materials and that of the entire paint, which is what would be expected.

The series of experiments within the vermiculite bed showed that the overall composition of the paints did not alter, but there was a sharpening of the peaks (Figures 4.42 and 4.43). This was accounted for by either, the removal of trace organic materials or the removal / flattening of the titanium dioxide particles in the surface of the paint. The painted spruce panel that was analysed after twenty-three months (Figure 4.45) also

showed no apparent changes in the composition of its surface. It was considered that any changes in the surface chemistry of the paint film, which may have occurred as a result of exposure, may have been removed or obscured by the cleaning process. This was substantiated using Atomic force microscopy (AFM). The results from this are not presented, but showed that there was a difference between the image of a panel before exposure and one that had been exposed and subsequently cleaned, thus it was highly probable that the damaged layer of paint had been removed from the surface of the panel before the FTIR analysis.

4.10 An assessment of the deteriogenic potential of selected microorganisms

4.10.1 Materials and Methods

Thin paint films of all six paints were prepared as described in Section 4.9.1. These were cut into small pieces measuring approximately 4cm x 2cm and numbered. Each numbered paint film was weighed to three decimal places and the value recorded.

Six plastic seed trays were cleaned with ethanol and to each 250g of sterile vermiculite were added. This was dampened using sterile distilled water and a plastic acetate sheet placed directly over the vermiculite. The trays were enclosed within Sterilin™ bags and sealed using a giant paperclip. They were allowed to stand for twenty-four hours at room temperature to equilibrate.

To five of the trays, six pre-weighed paint films of the six paints were added, i.e. thirty-six films in total. To the remaining tray three paint films were added. The trays were then treated as follows:

Tray 1: This was resealed to act as a control (Figure 4.46).

Tray 2: A 5ml turbid suspension of *Aureobasidium pullulans* in sterile Ringers was sprayed onto the paint films using an airbrush with propane propellant, the bag was then resealed.

Tray 3: A 5ml turbid spore suspension of *Aspergillus fumigatus* in sterile Ringers was sprayed onto the paint films using an airbrush with propane propellant, the bag was then resealed.

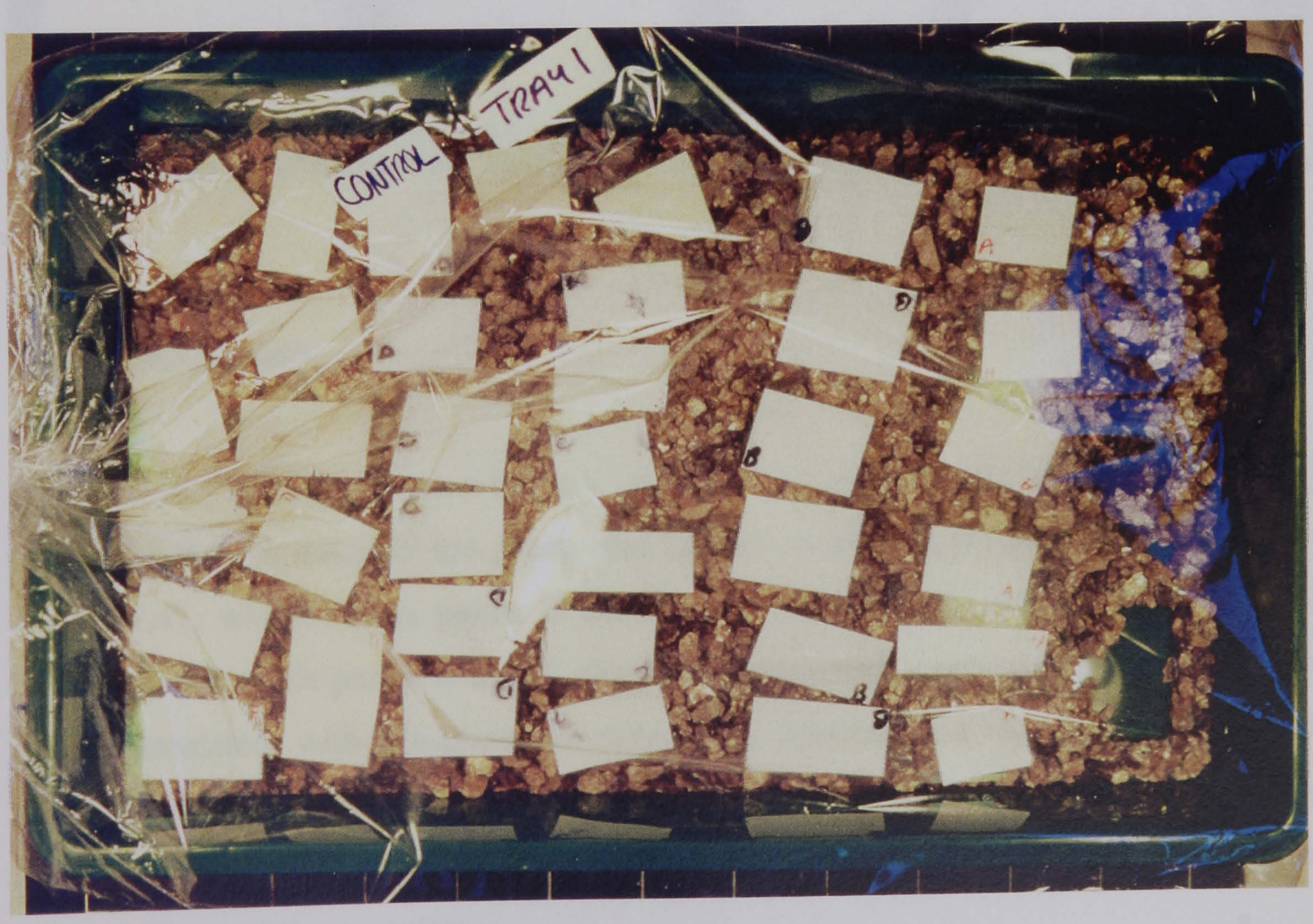
Tray 4: Sterile Ringers was sprayed onto the paint films using the airbrush with propane propellant and the bag was then resealed. This acted as a control of the airbrush propellant and the Ringers.

Tray 5: A 5ml turbid suspension of *Rhodotorula rubra* in Ringers was sprayed onto the paint films using an airbrush with propane propellant and the bag was then resealed.

Tray 6: A 5ml turbid suspension of *Rhodotorula mucilaginosa* in Ringers was sprayed onto the paint films using an airbrush with propane propellant and the bag was then resealed.

All of the trays were then placed on a shelf at room temperature for five months. Throughout this period of time, approximately every four weeks, the paint films were removed from the trays to be re-weighed. In order to do this all of the paint films were allowed to stand for ten minutes at room temperature to dry before being re-weighed, ten minutes being the time required for any water droplets to have dried on the surface of the paint film.

Figure 4.46 The vermiculite bed system containing the control paint films.



4.10.2 Results

The tables in Appendix I present the results of these experiments. It can be seen that there was no obvious difference between any of the initial weights and the final weights. The paint films inoculated with *Aureobasidium pullulans* showed that this microorganism grew on paints B, D, E and F and not on A or C, which both contained fungicide. Figure 4.47 shows the paint films A to D inoculated with *Aureobasidium pullulans*, displaying this.

Figure 4.47 Paints A to D after inoculation with *Aureobasidium pullulans.*

4.10.3 Summary

From the results of this section of the work it can be seen that *Aureobasidium pullulans* is readily able to colonise the paints that do not contain fungicide. This is shown in Figure 4.47, which shows paints B and D (the non-fungicide containing paints) are colonised whilst the paints A and C, the paints containing fungicide are not. The paint films inoculated with *Rhodotorula rubra* and *Rhodotorula mucilaginosa* became contaminated with *Aspergillus fumigatus*. The results of the weight loss experiments (Appendix I) do not provide evidence of weight loss resulting from inoculating the paint films with any of the organisms.

CHAPTER 5.

GENERAL DISCUSSION

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5.1 The Exposure trials

When the many aspects of this work are examined there are a number of observations that can be made. The results of the exposure trials are interesting in that many of the microorganisms, such as *Aureobasidium pullulans*, *Alternaria alternata,* the *Penicillium* species, *Micrococcus* species, and *Chlorella* species isolated at the sites investigated in this study are reported to have been isolated in previous exposure work (Barry, 1978 ; Bravery, 1987 ; Downey, 1995 ; Ross *et al.*, 1968 ; Seal and Morton, 1986 ; Winters, 1981). Many of these organisms have been isolated from paint films since at least 1968 (Ross *et al.*, 1968).

5.1.1 The exposures that took place at Sandefjord

From the results of the first exposure that took place at Sandefjord, between September and October 1999 it was evident that there was little difference between the range of isolates obtained from the panels coated with the paints containing fungicide, and those that did not (Tables 2.7 and 2.8). From Table 2.9 it is apparent that the films coating the spruce panels produced the most varied flora followed by the paint films on the calcium silicate and finally the films coating the aluminium panels. The gloss paints and the matt paints produced a similar range of isolates.

From the second exposure at Sandefjord, that took place in July 2000 it can be seen from the results presented in Tables 2.11 and 2.12 that the panels coated with paint A provided a similar number of different isolates as paint B. Paints C and D provided a similar range of organisms as paints E and F. Table 2.13 shows that all of the paints covering the different panel materials were similarly colonised. Unlike the previous exposure (September / October 1999), however, the gloss coated paints produced a less varied range of isolates.

The third exposure at Sandefjord, that took place between September and October 2000, showed that paint B yielded more isolates than paint A (Table 2.14). These were the only results obtained for this exposure; the calcium silicate and aluminium panels were not swabbed.

The results of the final exposure, which took place between June and August 2001, are shown in Table 2.15. It can be seen that over the period of time that the sacrificial panels were exposed, the non-fungicide containing paint yielded the highest range of isolates. Table 2.16 confirms that panels coated with paint B produced more isolates than panels coated with paint A. Table 2.16 also shows that a similar spectrum of isolates were obtained from the other paints.

As four exposures took place at Sandefjord throughout the entire exposure period of thirty-five months evidence of seasonal effects were examined. There did not, however, appear to be any direct evidence of seasonal effects in that common microorganisms were identified at each exposure; for example *Aureobasidium pullulans*, *Penicillium chrysogenum* and a species of *Bacillus*.

5.1.2 The exposures that took place at Bergen.

From the results of the first exposure trial that took place at Bergen in July 2000 it can be seen that the sacrificial panels coated with paint A supported fewer isolates than those coated with B (Table 2.22). From Table 2.23 it can be seen that *Aspergillus fumigatus* was a frequent isolate and that the panels coated with paints containing fungicide, had fewer microorganisms isolated from them than those painted with non-fungicide containing paints. It can also be seen that the gloss paints had fewer organisms isolated from them than the matt paints. It can also be seen at this exposure that the gamma-irradiated panels have very few organisms isolated from them. The material of the panel is shown to affect the number of isolates obtained, in that the majority of organisms were isolated from the spruce panels followed by the aluminium panels and finally the calcium silicate, although there was only one microorganism difference.

The exposure that took place between September and October 2000 showed that the sacrificial panels painted with paints A and B (Table 2.24) had a similar number of microorganisms isolated from them, with *Aspergillus fumigatus* being the most frequent isolate as was in the case of the July 2000 exposure at Bergen.

The final exposure that took place between June and August 2001 showed that *Aureobasidium pullulans* was a frequent isolate from the sacrificial panels painted with paints A and B. From these results it can also be seen that paint A appeared to have more organisms isolated from it than paint B (Table 2.25). From the results displayed in Table 2.26 it can be seen that the non-fungicide containing paints had more organisms isolated from them than the fungicide containing paints. From these results it can also be seen that there is no obvious difference, in terms of isolates obtained, between panels painted with gloss paints and those painted with matt paints. The panel material is shown to affect the number of microorganisms isolated at this exposure, with the majority of organisms found on spruce, followed by aluminium panels, the gamma irradiated panels and then the calcium silicate panels.

5.1.3 The exposures that took place at Preston.

From the first exposure trial that took place in Preston, between September and October 1999 it can be seen that the panels painted with paints A and B (Table 2.31) had a similar number of microorganisms isolated from them. Table 2.32 shows that the panel material influenced the isolates obtained, with the majority of organisms being isolated from the paint covering the spruce panels followed by calcium silicate and aluminium panels.

The results of the exposure between April and May 2000, showed that on the sacrificial panels a species of *Cellulomonas* was isolated frequently (Table 2.34), and that the panels painted with paints A and B had a similar numbers of microorganisms isolated from them. From Table 2.35 it can be seen that the *Cellulomonas* species, *Rhodotorula glutinis*, *Aspergillus fumigatus* and the mycelium sterilium (P6) were frequent isolates. These results also show that all of the coated panels were colonised to similar levels and that the three different materials do not seem to influence the range of isolates obtained. At this particular exposure, gamma irradiated panels were introduced, these were found to have as many organisms isolated from them as the other panel types.

The results of the exposure that took place between February and March 2001 show that for the sacrificial panels (Table 2.37) there were no obvious differences between the

colonisation of the two paints (paints A and B). Table 2.38 shows that the material of the panel influences the isolates obtained.

The results of the final exposure that took place in Preston between June and August 2001 are shown in Table 2.39 and 2.40. It is clear that there was little difference in the isolates obtained from the two paints, A and B (Table 2.39). The results presented in Table 2.40 showed that the material of a panel had an effect on the isolates obtained, with the spruce panels being the most heavily colonised followed by the calcium silicate and finally the aluminium panels. From these results it can also be seen that the gloss paints were colonised to a lesser extent than the matt paints and that gamma irradiating the panels reduced the number of organisms isolated from the paints.

5.1.4 The exposures that took place at Blackley, Manchester

From the results of the first exposure at Manchester which took place between April and May 2000 it was evident from the sacrificial panels (Table 2.45) that there was little difference in the isolates obtained from the panels painted with the two paints, A and B. The results from Table 2.46 show that the spruce panels produced more isolates than the calcium silicate and aluminium panels, thus the material of the panel does effect the colonisation of the panels. From these results it is also evident that the matt and gloss paints produced a similar range of isolates.

The results of the exposure which took place between February and March 2001 shows that the sacrificial panels painted with paints A and B (Table 2.48) produced a similar numbers of isolates. Table 2.49 shows that the substratum effects the number of isolates obtained, with the spruce panels being the most heavily colonised followed by the calcium silicate and the aluminium panels.

The panels were simply removed from the racks at Blackley in November 2001, with no sacrificial panels being exposed, as it was not thought possible to examine all of the panels from all of the four sites at the same time. From the removed panels that were sampled, it was seen that the gloss paints had fewer isolates identified from them than the matt paints and that the material of the panel affected the number of isolations. The

spruce panels had the most isolates identified from them, followed by the calcium silicate and the aluminium panels (Table 2.50).

5.1.5 A summary of the exposures

Common isolates

Appendix E shows the occurrence of microorganisms isolated during the investigation at the various sites during the twenty-three month exposure period. Certain fungi are clearly more frequently isolated, for example *Alternaria alternata*, *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Fusarium oxysporum* and *Penicillium chrysogenum.* This is also recorded in Tables 2.9, 2.26, 2.40 and 2.50.

The fungi isolated in these experiments are compared with fungi, isolated by other researchers. Kelly, 1999 reported that *Epicoccum nigrum*, *Cladosporium herbarum*, *Alternaria alternata*, *Aureobasidium pullulans*, *Penicillium chrysogenum*, *Cladosporium cladosporioides* and *Penicillium brevi-compactum* were some of the major fungi isolated from paint surfaces, most of which were also isolated in the present study.

Jakubowski *et al.*, (1983) recorded that *Aureobasidium pullulans* and *Alternaria* species were the dominant causative agents of degradation of exterior paint films, both of which were isolated at all four exposure sites in this trial. Other fungi to isolated by this author in 1983 were *Geotrichum* species, *Fusarium* species and *Scopulariopsis* species, of which only *Fusarium* species were isolated during this study.

Barry, (1978), isolated *Cladosporium cladosporioides*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Stemphylium species, Acremonium sp*ecies, *Aureobasidium pullulans*, *Phoma violacea* and *Aspergillus versicolor* from paint films. It can be seen from the information in Appendix E that most of these fungi were also isolated in these exposures.

Drescher, 1958 recorded the isolation of *Alternaria dianthicola*, *Aspergillus flavus*, *Cladosporium* species, *Cladosporium sphaerospermum*, *Cephalosporium carpogenum*, *Penicillium oxalicum*, *Aureobasidium pullulans* and *Stemphylium consortiale* from emulsion paints at six different locations in the United States. The results of the work in the United States and in this study show that *Aureobasidium pullulans* was the fungus isolated most frequently.

Gillatt and Tracey, 1987 recognised that fungi were the main deteriogens isolated from coatings, especially fungi in the genera *Aureobasidium*, *Cladosporium*, *Aspergillus* and *Penicillium*. Goll and Coffey (1948) and Rothwell (1958) indicated that *Aureobasidium pullulans* and *Cladosporium* species were two of the main genera involved in the deterioration process. Seal and Morton in 1986 reported that fungi isolated from paint films included; *Alternaria tenuis*, *Aspergillus flavus*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Fusarium oxysporum*, *Paecilomyces varioti* and *Trichoderma reesei*. The International Biodeterioration Research Group (Downey, 1995), report that *Alternaria alternata*, *Cladosporium* species, *Penicillium* species, *Phoma violacea*, *Stachybotrys chartarum* and *Aureobasidium pullulans* were amongst the fungi isolated from paint films. Gaylarde and Morton, 1999 reported that *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Exophiala*, *Fusarium*, *Geomyces*, *Mucor*, *Penicillium*, *Stachybotrys*, *Stemphylium*, *Trametes*, *Trichoderma*, *Ulocladium*, and *Verticillium* were involved in the deterioration of painted surfaces. Onions *et al.*, 1981 states that *Aureobasidium pullulans*, *Penicillium canescens* and *Phoma herbarum* were major paint film deteriogens. Many of these fungi mentioned were isolated from the painted panels that were exposed at the various sites during these exposures.

In summation, the fungi isolated at the four sites in this exposure trial were comparable to those fungi isolated by other workers throughout the years, even though the majority of the exposures cited were for longer periods of time than the ones that took place at Sandefjord, Bergen, Preston and Blackley. This could be due to the fact that the microflora of the air of the regions, the weather conditions and the nature of the exposed paint films were similar.

Like most of the exposures performed by other workers, *Aureobasidium pullulans* was found to be one of the most frequent isolates, especially in Sandefjord (Appendix D:1) and Blackley (Appendix D:4). In this study *Aureobasidium pullulans* was not, however, always the most frequent isolate, *Aspergillus fumigatus* was the most frequent in Bergen (Appendix D:2) and *Chlorella* species in Preston (Appendix D:3).

A comparison of the performance of the paints in terms of surface colonisation.

The pure acrylic paints containing (paint A) and not-containing fungicide (paint B).

The comparisons between paint A and paint B show that a greater diversity of microorganisms were isolated from paint B, which did not contain fungicide in its formulation (Tables 2.14, 2.15, 2.22, 2.24, 2.31, 2.37 and 2.45). In Tables 2.7, 2.11, 2.25, 2.34 and 2.39 paint A is seen to have a greater diversity of microorganisms isolated from its surface than paint B. It would be expected to see a greater diversity on paint B as it is a formulation that did not contain a fungicide. From Plate 2.6 it can be seen that the surface of the panel coated with paint A appears to be less colonised by algae than the surface of paint B, where they occur at the top and bottom of the panel (a pooling effect) and over the surface of the panel. It is suggested that the fungicide is effective against biodeteriogenic fungi only, rather than transient forms or algae.

The hybrid acrylic paints containing (Paint C) and not-containing fungicide (Paint D).

In the two exposures at Sandefjord the results for sacrificial panels of paints C and D, show that in both cases there was only one additional microorganism on paint C than paint D (Tables 2.2.7, 2.8, 2.11 and 2.12). This difference is considered to be too small to make a judgement about the effectiveness of the biocide in this hybrid paint. From Tables 2.13, 2.16, 2.23 and 2.32 it can be seen that the number of microorganisms isolated from the spruce panels for paint C and paint D are the same. In Table 2.26, however, which records the isolates obtained from Bergen, two isolates are recorded for paint C and six for paint D. This pattern, where paint D supports a greater diversity of microorganisms is also seen in Tables 2.35, 2.49 and 2.50. Tables 2.9, 2.38, 2.40 and 2.46 show that paint C supported a greater diversity of microorganisms that paint D. This is an unexpected result.

The performance of the pure acrylic in relation to the hybrid acrylic paints.

A direct comparison between the diversity of microorganisms isolated from paints A and C shows that in the majority of cases paint A produced more microorganisms than paint C (Table 2.7, 2.11, 2.13, 2.23, 2.26, 2.32, 2.35, 2.38, 2.40 and 2.49). In the other panels there was either an equal number of isolations on both paints or fewer isolates on paint A than paint C (Tables 2.9, 2.16, 2.46 and 2.50).

A direct comparison between the diversity of microorganisms isolated from paints B and D shows that in the majority of cases paint B produced more isolates than paint D (Tables 2.7 and 2.8, 2.9, 2.16, 2.23, 2.26, 2.32, 2.38, 2.46 and 2.49). In the other panels there was an equal number of isolations on the paints, or fewer isolated on paint B than D (Tables 2.11 and 2.12, 2.13, 2.35, 2.40 and 2.50).

Visual comparisons of the degree of colonisation of the two paint types showed that the hybrid paint (D) was less colonised than the pure biocide-free paint (B) when coated onto spruce panels (Plate 2.9). On calcium silicate panels (Plate 2.11) it can be seen that paint B was the most heavily colonised followed by paint A and then paint C. Plate 2.12 shows paints B, C and D coated onto spruce panels, from these images it can be seen that both paint B and D are heavily colonised in comparison to paint C, however, it is not possible to distinguish which paint was the least susceptible to colonisation by microorganisms. Plate 2.13 shows paints A, B, C and D coated on calcium silicate panels, it is possible to see in this case that paint D is more heavily colonised than paint B, however, paints A and C appear to be similarly colonised and therefore it is not possible to distinguish between the susceptibility of these two paints.

These comparisons show that generally the hybrid acrylic paint, when coated on to spruce panels is less susceptible to microbial colonisation than pure acrylic paint.

The gloss paints.

The roughness of a surface is considered to influence the initial colonisation of a surface. During the course of this investigation matt and gloss surfaces were exposed. In all of the exposures except one (Table 2.9), it can be seen that the gloss paints were less colonised than the matt paints (Tables 2.13, 2.16, 2.23, 2.26, 2.32, 2.35, 2.38, 2.40 2.49 and 2.50. Whitely, (1973) and Rusche *et al.*, (1977) discussed that the properties of a paint film and its environment govern its susceptibility to biodeterioration by certain microorganisms. Wright, (1986) stated that textured masonry paints and low gloss paints encourage attachment and oil based alkyd paints are more resistant to growth. The results obtained in these exposures comply with this theory, in that gloss paints are less susceptible to microbial colonisation than matt paints.

Fungicide

There was little difference recorded between the range of isolates obtained from biocide-free and biocide-containing paint. It is considered that the biocide may not be effective against organisms which are simply present at the surface rather than actively degrading the paint film i.e. the biocide is a non-leaching biocide.

Material of the panel

The material of the panel does seem to influence the range of microorganisms isolated from the overlying paint film. In the majority of cases it was found that the panels made of spruce produced the most varied flora, followed by the calcium silicate and finally the aluminium (Tables 2.9, 2.13, 2.26, 2.32, 2.38, 2.49 and 2.50). There were, however, some exposures where the aluminium panels supported a greater diversity of microorganisms than the calcium silicate panels (Tables 2.16, 2.23, 2.35, 2.40 and 2.46) although in the majority of cases it was one microorganism difference which is negligible. These observations are consistent with previous exposure trials performed by Kelly (1999), where similar results were recorded. It is reasonable to suggest that wood contains nutrients, which may diffuse through to the surface of the overlying paint film to promote microbiological growth.

Spruce panels in relation to gamma-irradiated spruce panels.

Spruce panels were gamma-irradiated in order to establish whether microorganisms were growing from within the spruce panel through the paint film and therefore increasing the number of microorganisms isolated. Tables 2.23, 2.26, 2.38 and 2.40 all show that the incidence of microorganisms is approximately half that found on the non-irradiated spruce panels. Table 2.35 shows that the diversity of microorganisms isolated were very similar, although, fewer isolates were obtained from the gamma-irradiated spruce panels. The SEM and ESEM images show that penetration of the paint film on spruce panels was from above rather than from within the spruce panels. This effect, is attributed to the radiation reducing the number of nutrients available in the spruce.

Seasonal isolations

When the information in Appendix D is examined it can be seen that there does not appear to be any marked seasonal effects on the range of microorganisms isolated from the panels, at the various times of the year when the sampling took place. The term seasonal was used because some of the exposures took place in February (Winter), some in April (Spring) some in June (Summer) and some in September (Autumn). However, not all of the seasons were recorded at all of the sites. In Norway, no recordings were taken between October and April because of the severe weather conditions, which prevail during these months.

Weather records

These show that the weather recorded was similar at the various times of the year at all of the sites. This data is recorded in Appendix C:1 to C:13 and Appendix J. The weather data collected throughout the exposures in Norway, between June and October, was very similar (Appendix D:1 and D:2). In the UK, there was a more marked difference between the temperatures and level of precipitation between the Autumn and winter months and the Spring and Summer months (Appendix D:3 and D:4).

5.2 The application of MALDI TOF MS

As many of the fungi which were isolated during the course of this investigation were common to all four sites, for example, *Aureobasidium pullulans* and *Alternaria alternata* inevitably the question arises as to the taxonomic relationships of these common isolates to each other. Other than the use of DNA profiling for example the genetic relationship of these organisms can not be addressed. The classification of fungi by cataloguing their various morphological features relies on the experience of the taxonomist and this is a subjective process.

As a result of the work of this investigation the potential role of MALDI TOF MS as an aid to fungal taxonomy has been assessed, and the following points are made:

1. Reproducible mass spectra or 'fingerprints' can be generated for fungi.
2. These fingerprints are peculiar or specific to pure cultures of fungi.
3. The spectra are such that they can be used to distinguish between genera of fungi (Figures 3.4 to 3.8) studied and more interestingly between species of the same genus (Figures 3.8, 3.10 and 3.11)

The above findings are the result of a limited amount of preliminary work based on the results obtained after standardising media and matrices. As an aid to fungal taxonomy it is considered more fully in this application. The availability of well-documented cultures from culture collections, could be used in this work. The implications of its use in identifying target organisms e.g. *Aureobasidium pullulans* may be of interest to biocide manufacturers.

5.3 Surface Characterisation

5.3.1 Talysurf™

The results of the work undertaken using the Talysurf™ instrument (section 4.2 to 4.7) establish that this type of instrument is suitable for providing topographic profiles of paint films applied to wood, the grain of the wood beneath the film providing a suitable profile for this stylus-based device. The results show clearly that the instrument can

detect differences in surface profiles which result from colonisation by microorganisms during the exposure of the paint films to the environment or in a vermiculite bed system.

Figures 4.10, 4.11, 4.12 and 4.13 show spruce panels coated with paints A and B before and after exposure on the panel racks at Preston for a duration of twenty weeks. On comparison of Figures 4.10 and 4.11 it can be observed that after the exposure period paint A had not obviously altered in its topography. On comparison of Figures 4.12 and 4.13 it can be seen that the troughs in the surface appears to have become deeper, however, Table 4.7 indicates that the surface has in fact become smoother in nature. This could be attributed to an accumulation of surface debris, which would include microorganisms.

Figures 4.14 to 4.19, show spruce panels coated with paints A, B and F before and after exposure within the vermiculite bed system.

Panels coated with paint A (Figures 4.14 and 4.15) show a change in the recorded topography, from being plateau-like in nature to a surface recording the presence of small peaks. This change is reflected in the Ssk value which has changed from –0.176 to +0.139 (Table 4.8).

Figures 4.16 and 4.17 show that the meshed axonomeric images obtained for paint B show no obvious changes in the nature of the surface of the paint film. The data in Table 4.9, however, indicate that a change had occurred, in that the parameter referred to as the 'total height' of the specimen (St) changed from 108 to 73.8 i.e. the total height value had decreased. A decrease in the Ssk value once again points to a change from a 'plateau' to a profile comprised of numerous peaks.

Figures 4.18 and 4.19 represent the meshed axonomeric images obtained for paint F before and after inclusion in the vermiculite bed system. The changes in the topography are very marked, however, the parameters and the visual observations are conflicting.

Figures 4.20 and 4.21 show meshed axonomeric diagrams of a spruce panel coated with paint D before exposure and after exposure for a duration of thirty months at Preston. It

can be seen from these images that the surface of the paint film has altered drastically. On comparison of the left edge of the paint film it can be seen that the surface of the grain has changed from smooth to a jagged appearance suggesting changes in the actual surface of the paint film have taken place.

The changes from a recorded plateau-like profile to one comprised of small peaks can be accounted for as follows:

1. The surface is disrupted by microbial degradation causing the appearance of peaks.
2. The accumulation of *Aureobasidium pullulans* at the surface.

The paint films which are formed above the grain of the wood often show an undulate pattern of peaks and troughs. Figure 4.23 which is a photomicrograph of the exposed spruce panel coated with paint D shows the hyphae of *Aureobasidium pullulans* growing along the peaks of these undulations, rather than in the troughs as one might expect i.e. following a route offering protection and possibly moisture. Perhaps the peaks are more hydrophilic in nature than the troughs as a result of greater surface energy in these regions relative to the troughs, or perhaps the peaks provide regions which are higher in nutritional value, because they have a thinner paint film coating them, allowing nutrients to diffuse through from the paint below.

5.4 The Microscopical analysis

From the images obtained using the SEM at AVECIA Ltd and the two ESEM's it was possible to confirm that *Aureobasidium pullulans* was in fact a coloniser of the paint films. The image in Figure 4.33 shows a spruce panel coated with a non-fungicide containing paint (paint B) that had been exposed for six weeks only. It is possible to see EPS production at a hyphal tip, which appears to be initiating cavity formation. Figures 4.34 and 4.35 show *Aureobasidium pullulans* penetrating the paint film from above, which substantiates the theory that in this case *Aureobasidium pullulans* has been colonising the surface of the paints rather than disrupting the paint film from beneath.

5.5 FTIR

The work undertaken using FTIR shows that the chemical composition at the surface of the paint films did not change after inclusion in the vermiculite bed system for six months, or after exposure at Preston for a period of thirty months (Figures 4.44 and 4.45). There was, however, a sharpening of the peaks after three months in the vermiculite bed system (Figures 4.42 and 4.43). This was thought to be due to either the removal of trace organic materials from the paint surface or the flattening of the titanium dioxide particles in the surface film, the specific nature of which is responsible for the high diffuse reflectance of the paint surface.

Cleaning the films prior to analysis (to remove surface debris) may have removed chemical components which might otherwise have been detected. The application of FTIR as a method for detecting changes in the composition of exposed surfaces of paint films does not seem to be an appropriate technique to use in work of this kind.

By comparing the FTIR spectra of the individual components of the paint with the spectra obtained for the entire formulation before and after exposure it was hoped that those components which were susceptible to degradation might be identified. Since the spectra of the paint films did not suggest that any change had taken place this exercise was not considered relevant.

5.6 An assessment of the deteriogenic potential of selected microorganisms

The major result from this experiment was in the observation that only the non-fungicide containing paint films inoculated with *Aureobasidium pullulans* and *Aspergillus fumigatus,* were shown to be colonised. This can be seen in Figure 4.47, where paints A and C are clear, whereas paints B and D have been colonised with *Aureobasidium pullulans*. This suggests that the fungicide has been effective against *Aureobasidium pullulans*, which is deemed as one of the predominant colonisers of paint (Bravery, 1987 ; Downey, 1995). The fact that the fungicide has been found to be effective against these fungi suggests that during the exposure trials the fungi found on

the panels painted with fungicide containing paints were not actually colonising them, but simply present at the time of sampling.

5.7 Methods for quantifying the level of fouling of surfaces.

The only generally accepted method for quantifying the extent of the fungal colonisation of surfaces is ergosterol analysis. Ergosterol is uniquely present in the cell membranes of filamentous fungi and is routinely used to determine fungal growth as its presence can be detected before any visual fungal growth is seen (Kelly, 1999). The technique involves the extraction of ergosterol from the sample by either High Performance Liquid Chromatography (HPLC) (Gardner *et al.*, (1993) ; Montgomery *et al.*, (2000)) or by gas chromatography- mass spectrometry (Axelsson *et al.*, (1995).

Other methods, including ATP assays (Pelkonen and Tenno (1993); Van der Kooij *et al.*, (1995) ; Vrouwenvelder *et al.*, (1998) ; Horiuchi *et al.*, (2002)), vital staining (Decho and Kawaguchi, (1999) ; Auschill *et al.*, (2001) ; Matharu *et al.*, (2001); Neu *et al.*, (2002)) and other fluorochrome applications have not proved completely successful for the quantification of surface fungal growth or their recognition in fungal biofilms (Roberts *et al.*, 1999).

SEM and ESEM studies undertaken during the course of this investigation (Figures 4.24 to 4.41) do provide evidence of surface attachment and penetration, but do not provide qualitative evaluations.

Fungal growth on surfaces is significantly effected and influenced by the availability of nutrients. The morphology of fungal isolates in oligotrophic and copiotrophic situations differ considerably. This will also effect their metabolic activities as biodeteriogens since co-metabolic effects (Kay, 1992) of fungi can play a significant role in the biodeterioration of surface coatings.

5.8 Future work.

Future work should include more of the MALDI TOF MS technique. Initially a database of professionally identified fungi should be created which can be expanded if the technique proves to be reliable. To determine the reliability of the technique different genera and species of fungi should be analysed to create a broader range of microorganisms.

An investigation of a single site with regular sampling intervals would be advantageous to provide a truer picture of true colonisation patterns and any seasonal effects.

The use of AFM and a confocal laser microscopy may be advantageous to investigate the spatial relationships between the microorganisms in biofilms.

The use of ergosterol assays to quantify the level of fungal growth on the paint films may be used in conjunction with the sampling techniques already employed.

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APPENDICES

APPENDIX A: Formulation for Difco algal medium

The recipe for one litre of the Difco algal medium is as follows:

Sodium nitrate	($NaNo_3$)	1.0g
Ammonium chloride	(NH_4Cl)	0.05g
Calcium chloride	($CaCl_2.2H_2O$)	0.074g
Magnesium sulphate	($MgSo_4.7H_2O$)	1.026g
Dipotassium phosphate	(K_2KPO_4)	0.25g
Ferric chloride	($FeCl_3$)	0.003g
Agar no. 2		2%

The media should be autoclaved at 120 PSI for twenty minutes

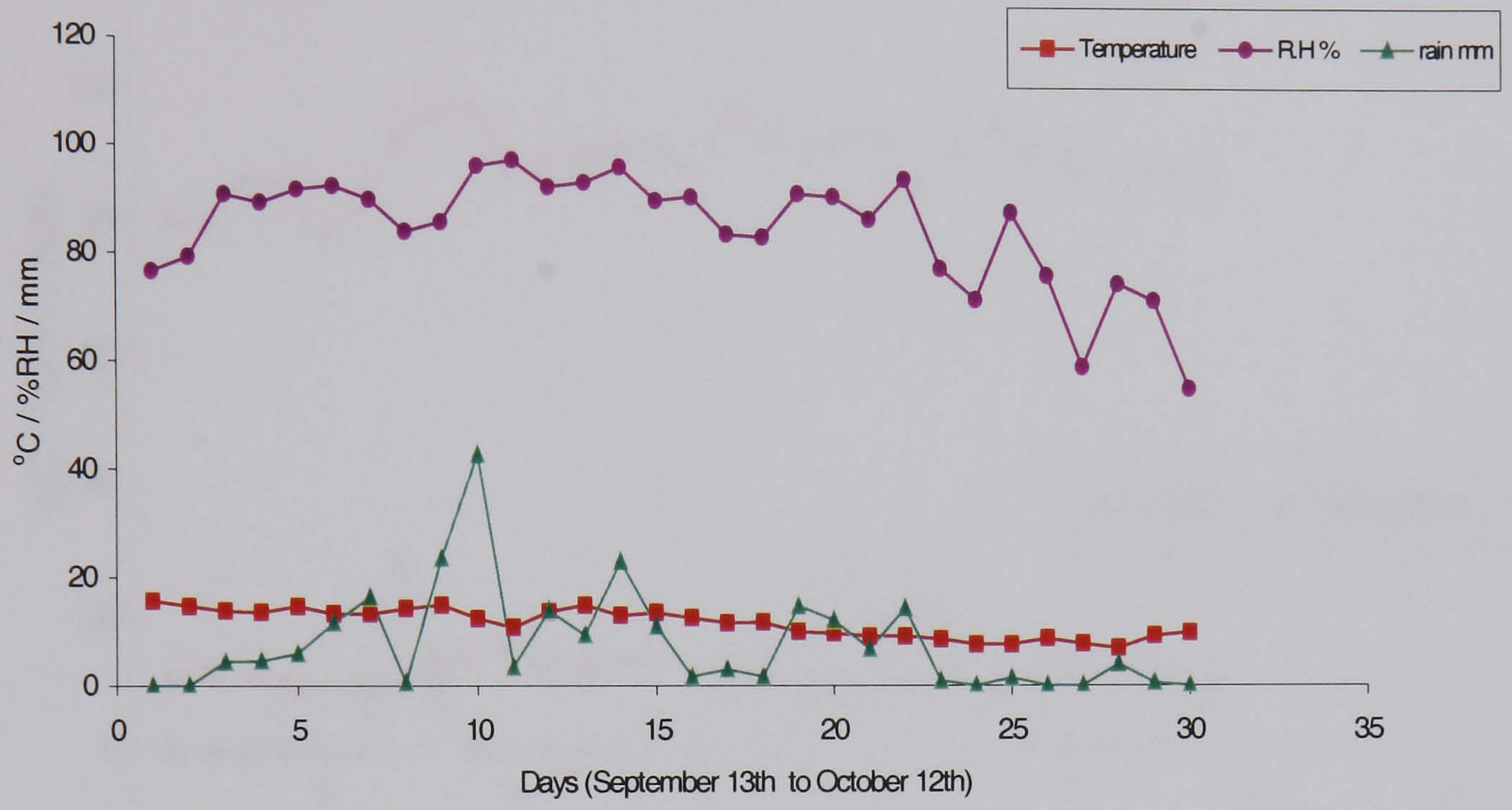
APPENDIX B: Formulation for R2A medium

The recipe for one litre of the R2A media (Reasoner & Geldreich (1985) is as follows:

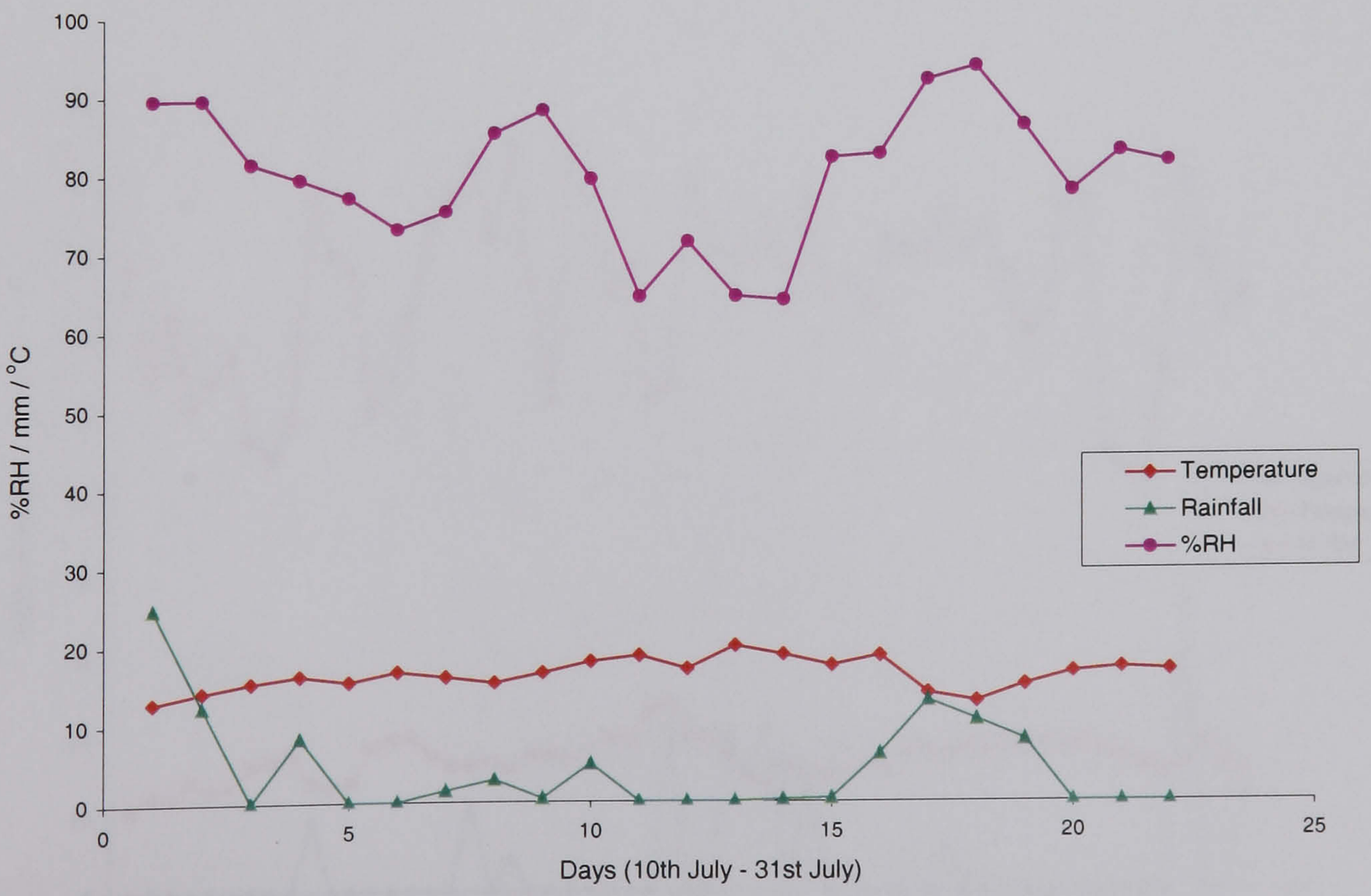
Yeast extract	0.5g
Difco protease peptone no.3	0.5g
Casamino acids	0.5g
Glucose	0.5g
Soluble starch	0.5g
Sodium pyruvate	0.5g
K_2HPO_4	0.3g
$MgSO_4.7H_2O$	0.05g
Agar no.2	15g

APPENDIX C: The weather data recorded over the exposure period for the four sites.

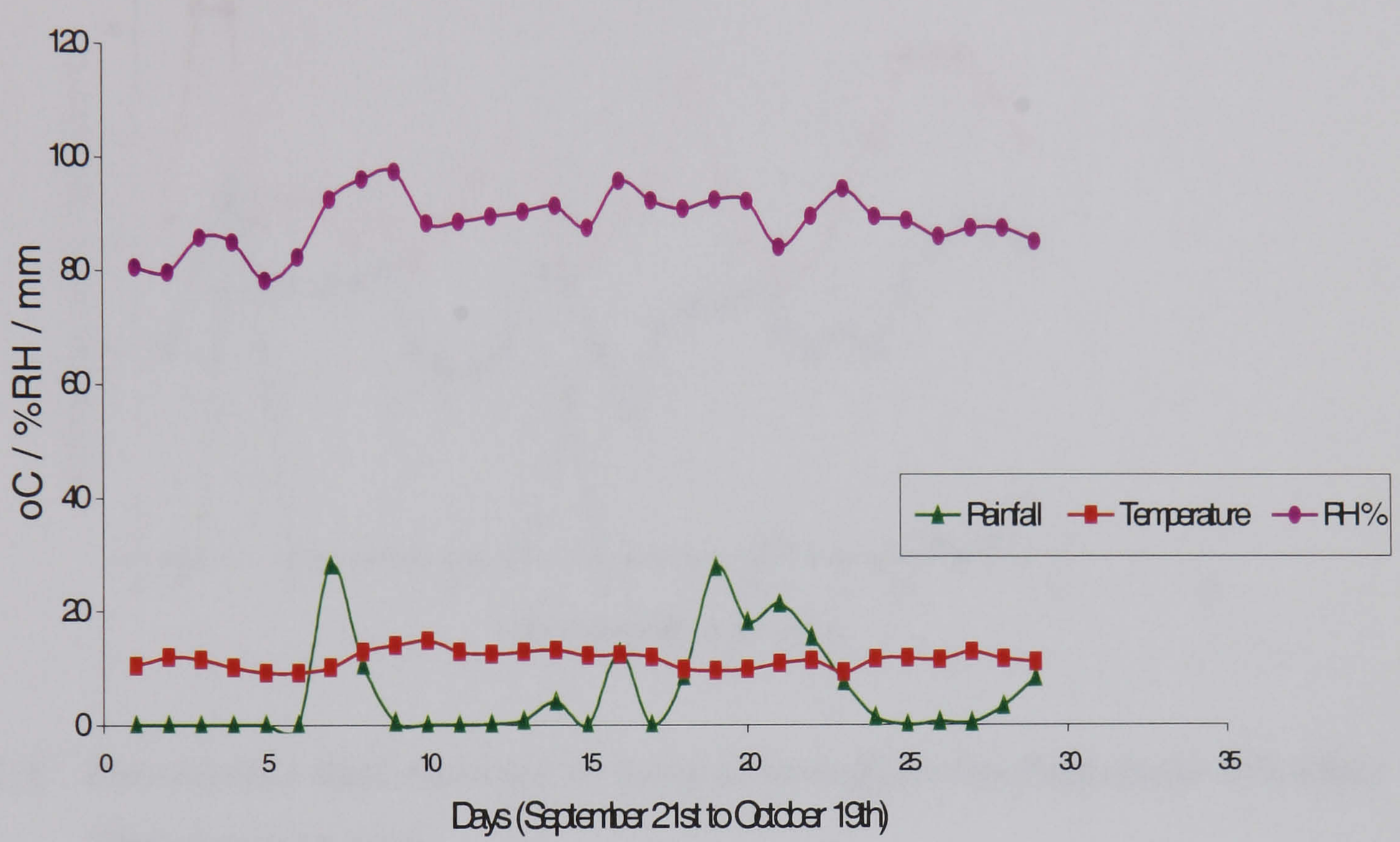
C:1 The weather data recorded for the period of exposure between September and October 1999 at Sandefjord



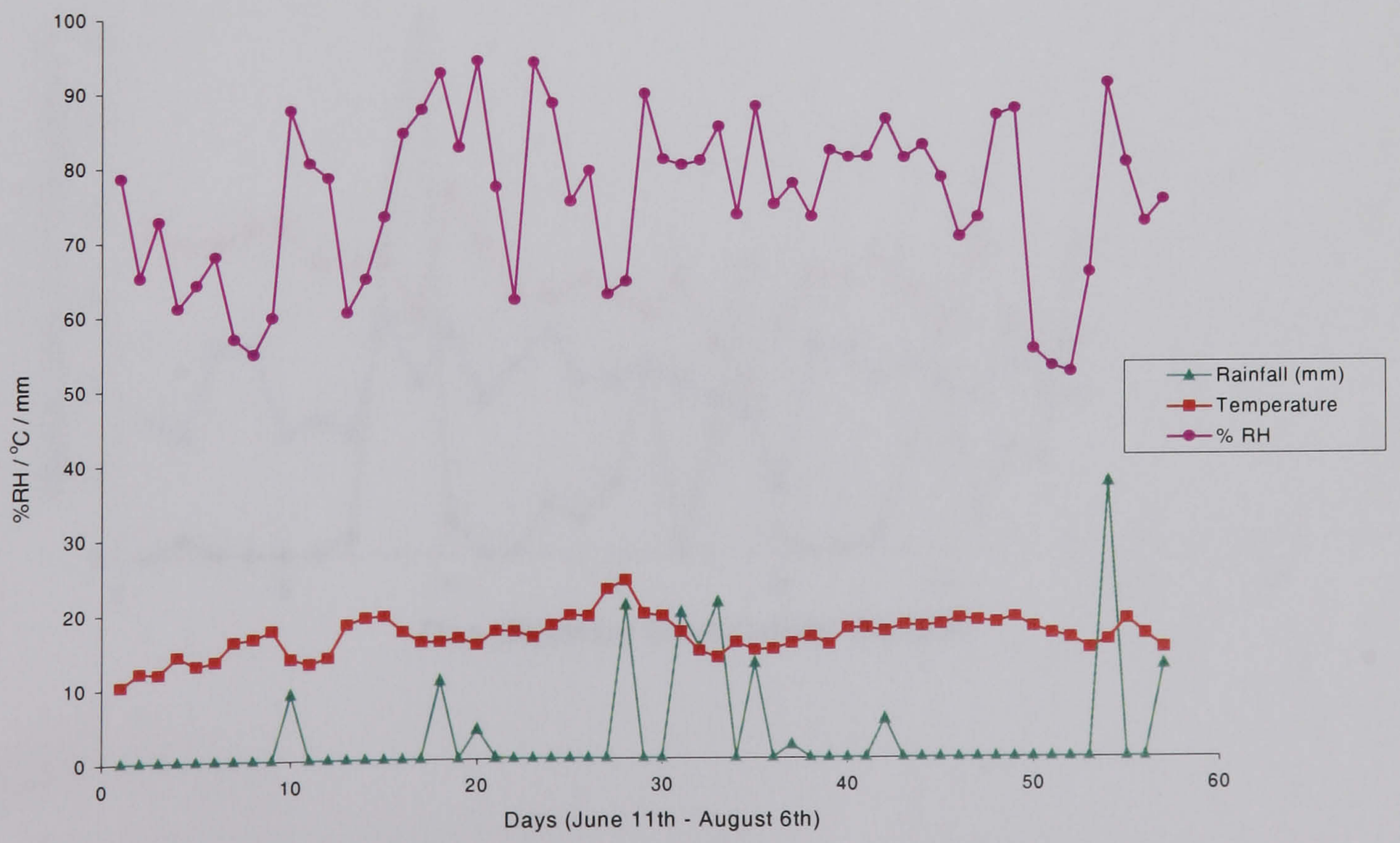
C:2 The weather data recorded for the period of exposure during July 2000 at Sandefjord.



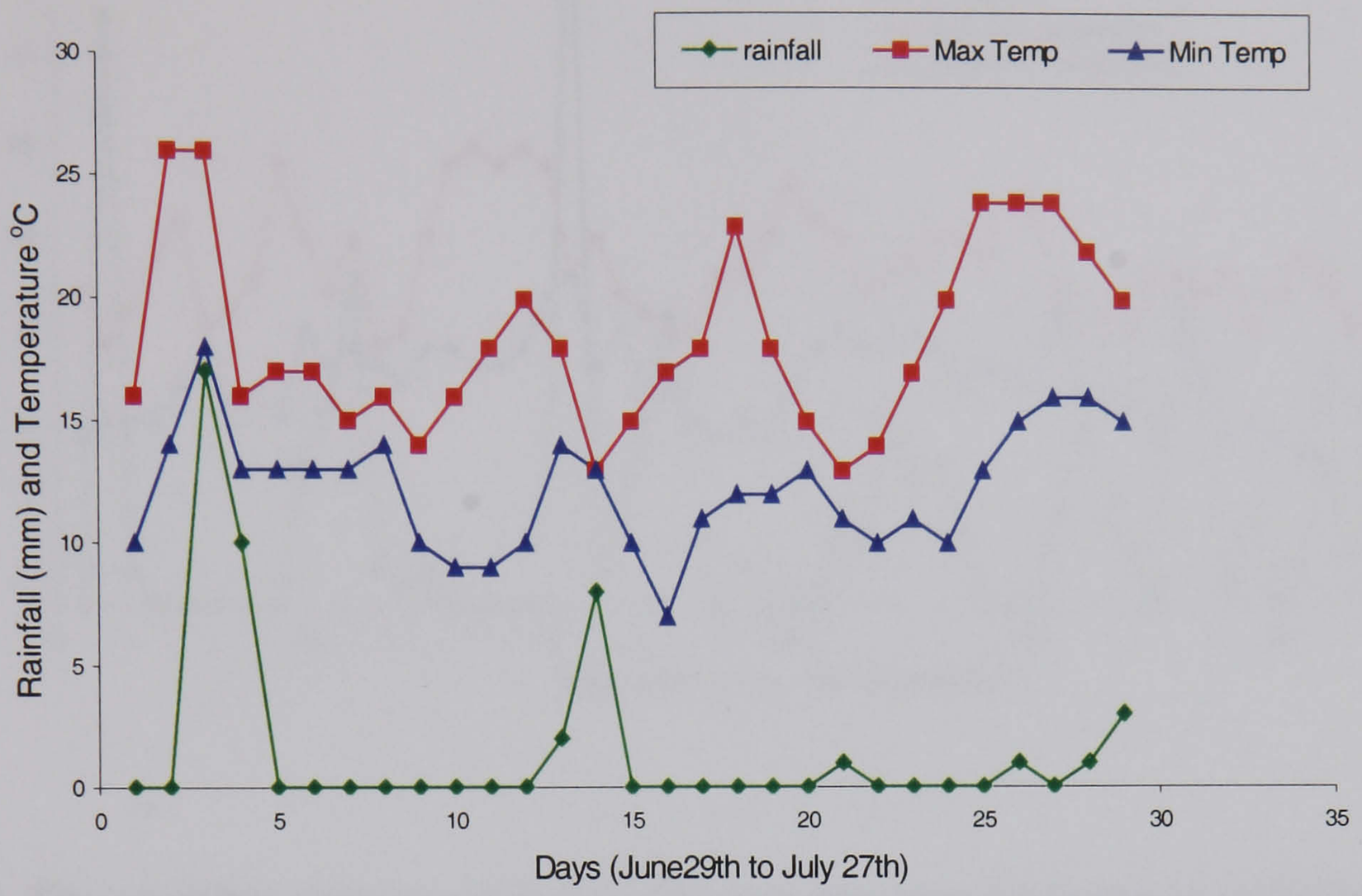
C:3 The weather data recorded for the period of exposure between September and October 2000 at Sandefjord.



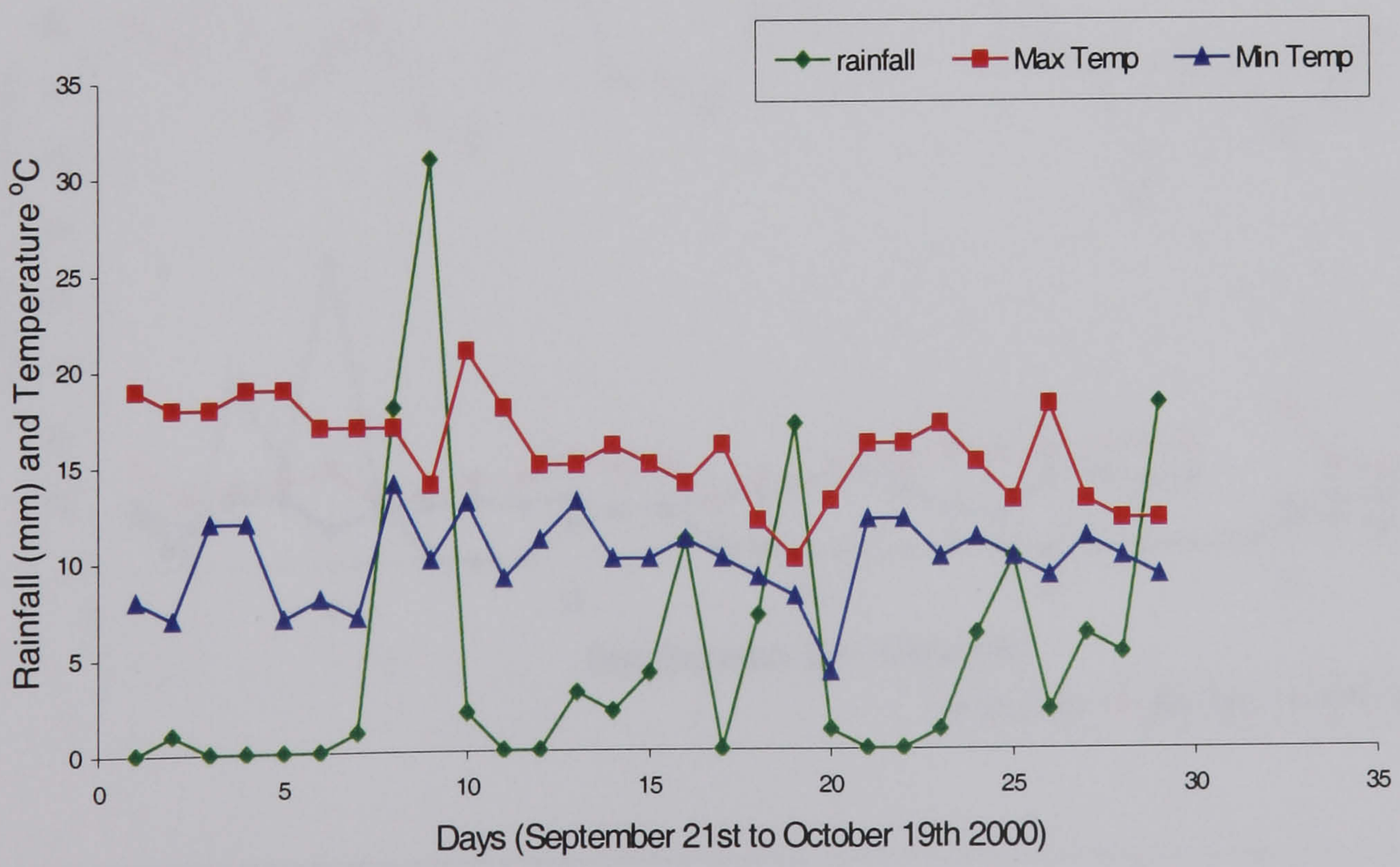
C:4 The weather data recorded at Sandefjord between June and August 2001



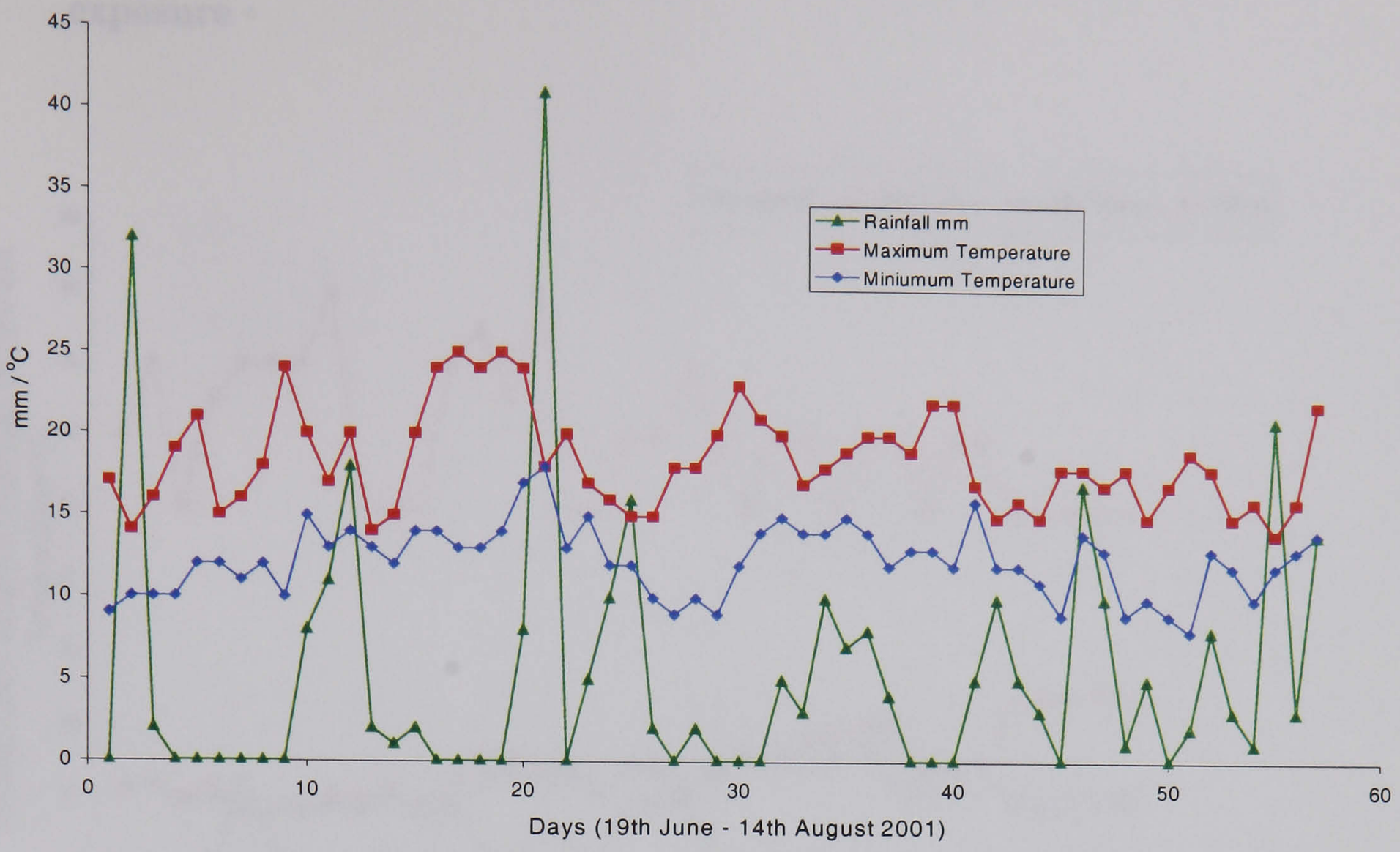
C:5 The weather data recorded at Bergen for the exposure between June and July 2000



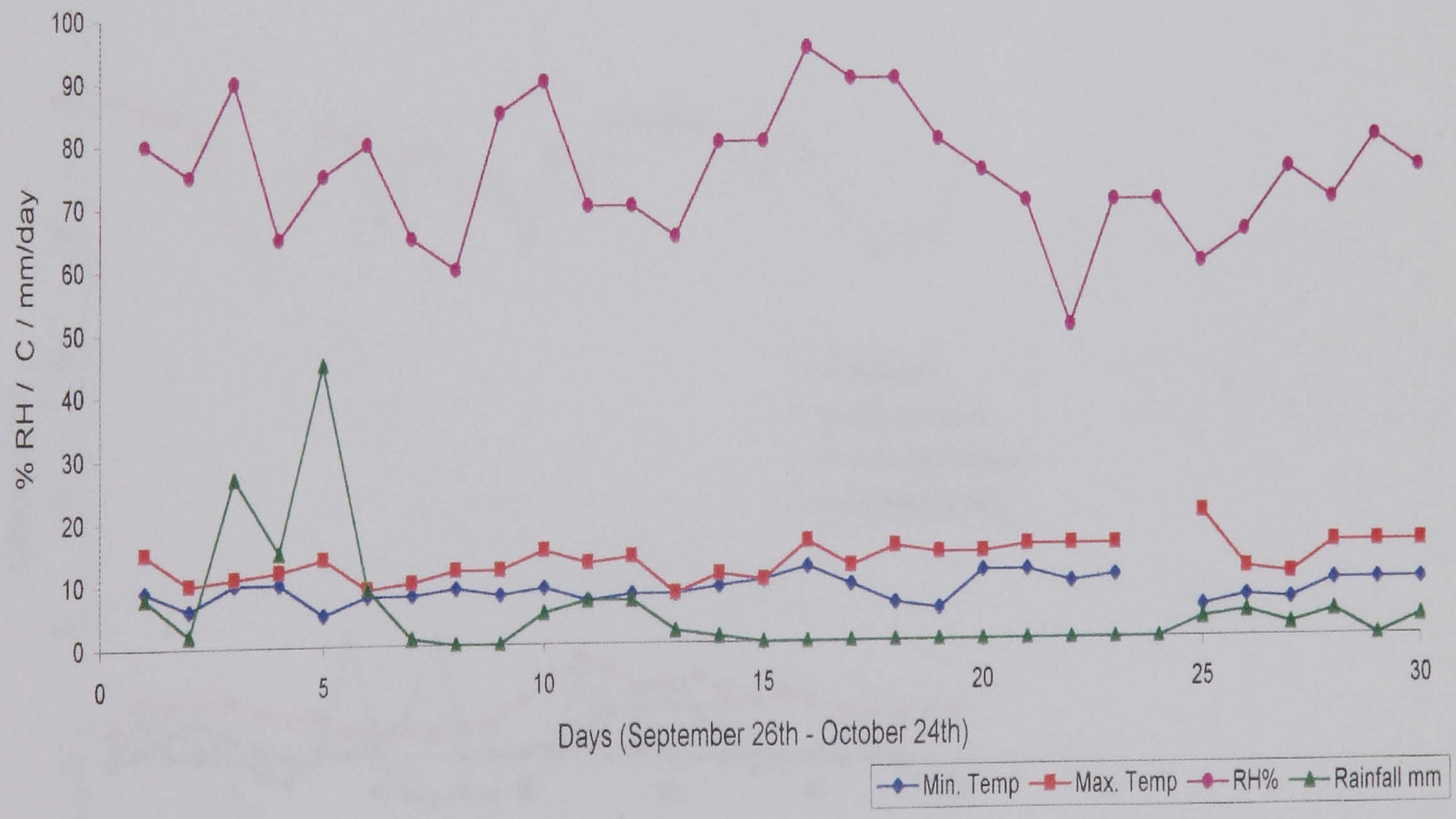
C:6 The weather data recorded at Bergen throughout the September / October 2000 exposure trial.



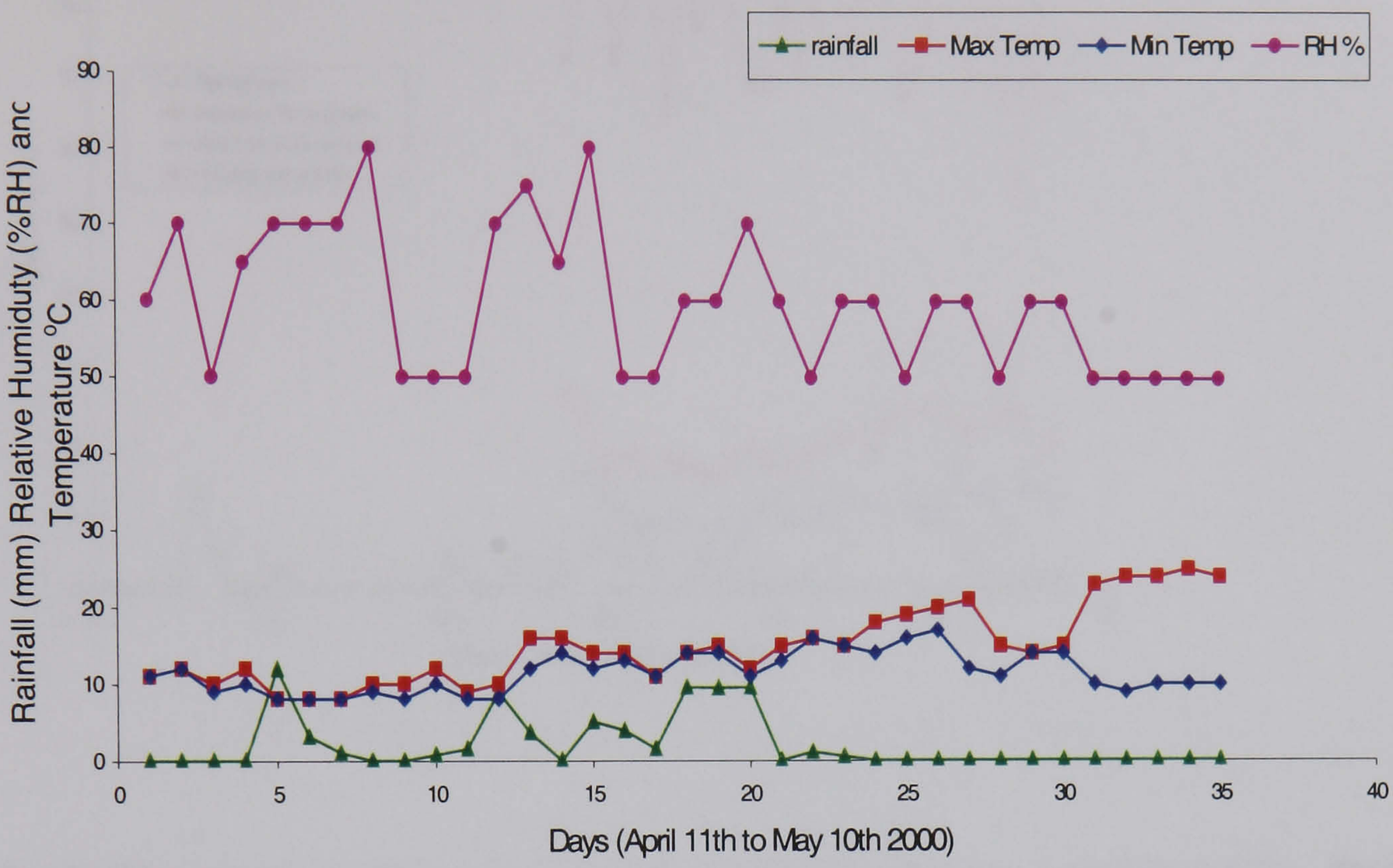
C:7 The weather data recorded in Bergen between June and August 2001



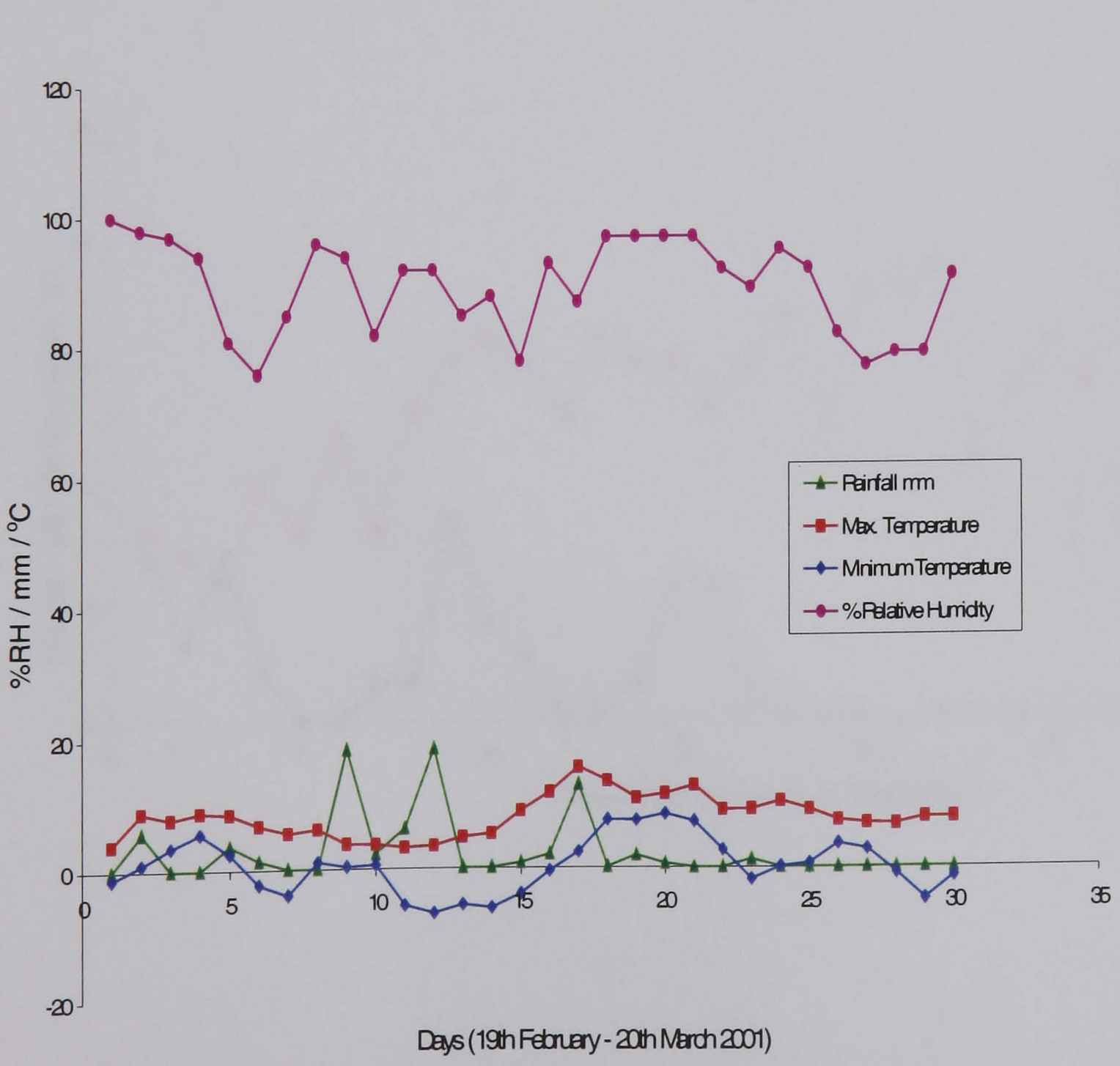
C:8 The weather data recorded at Preston between September and October 1999



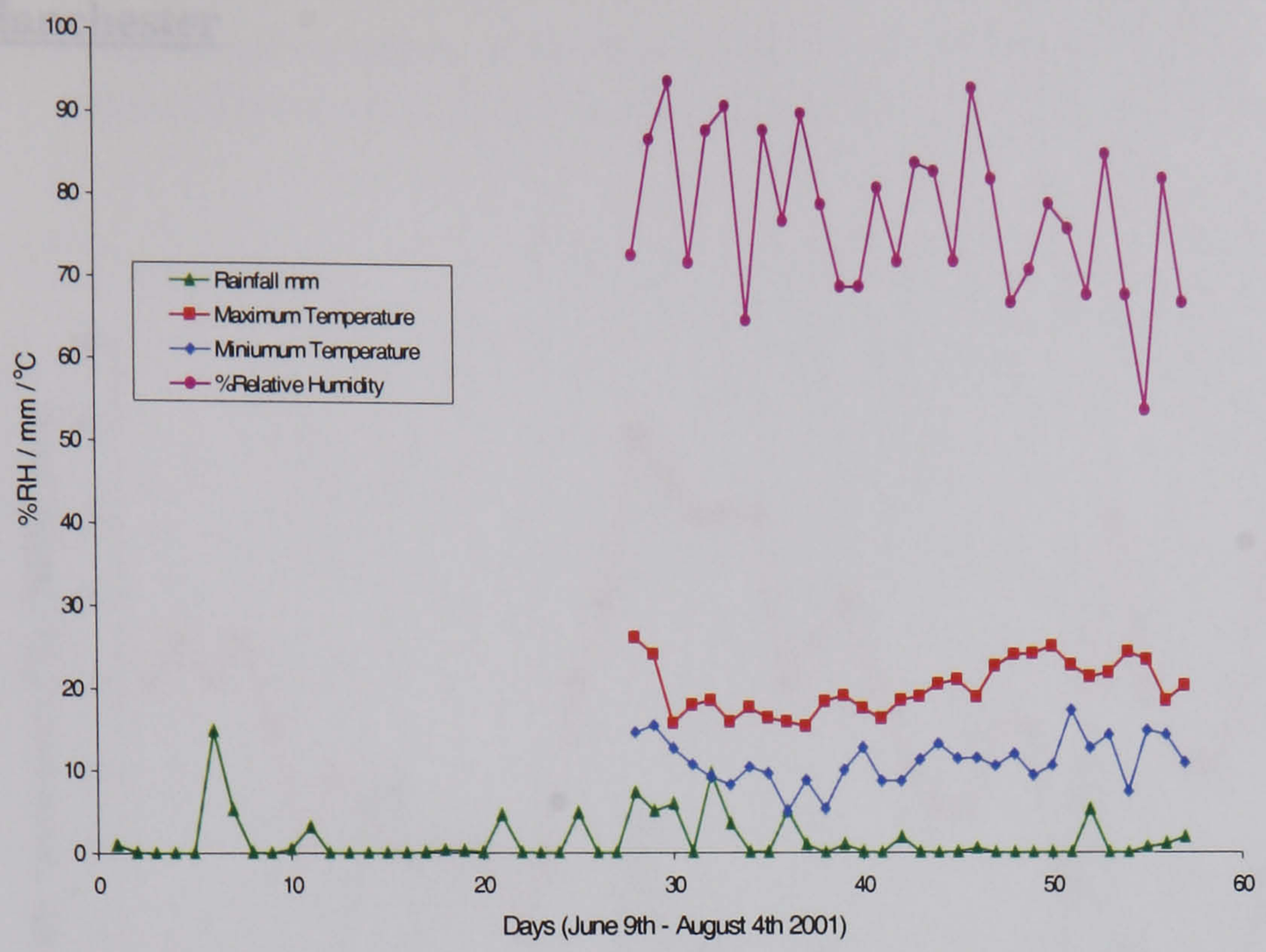
C:9 The weather data recorded at Preston throughout the April / May 2000 exposure



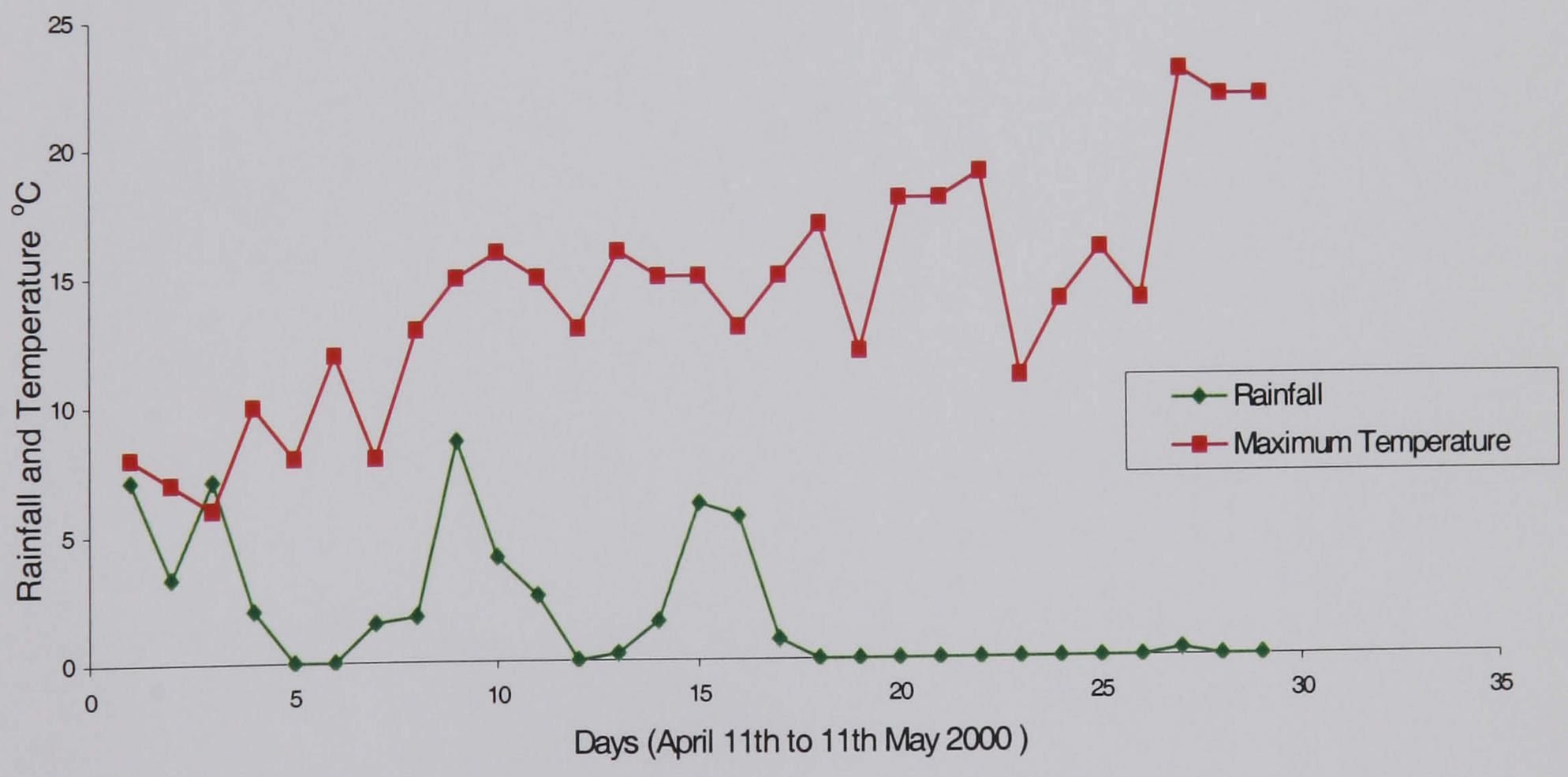
C:10 The weather data recorded at Preston between February and March 2001



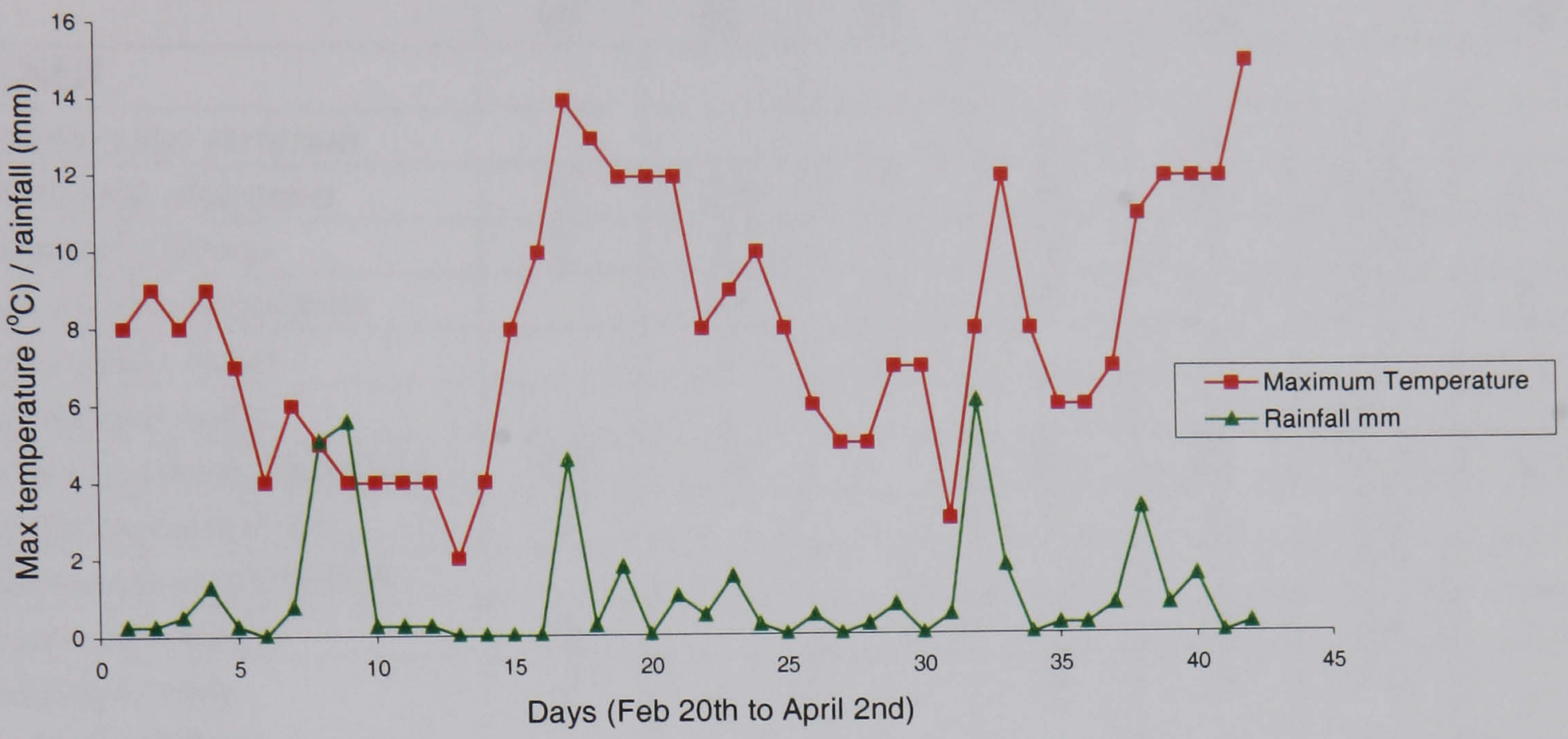
C:11 The weather data recorded at Preston between June and August 2001



C:12 The weather data taken in Manchester between April and May 2000



C:13 The weather data recorded during the February / March exposure at Manchester



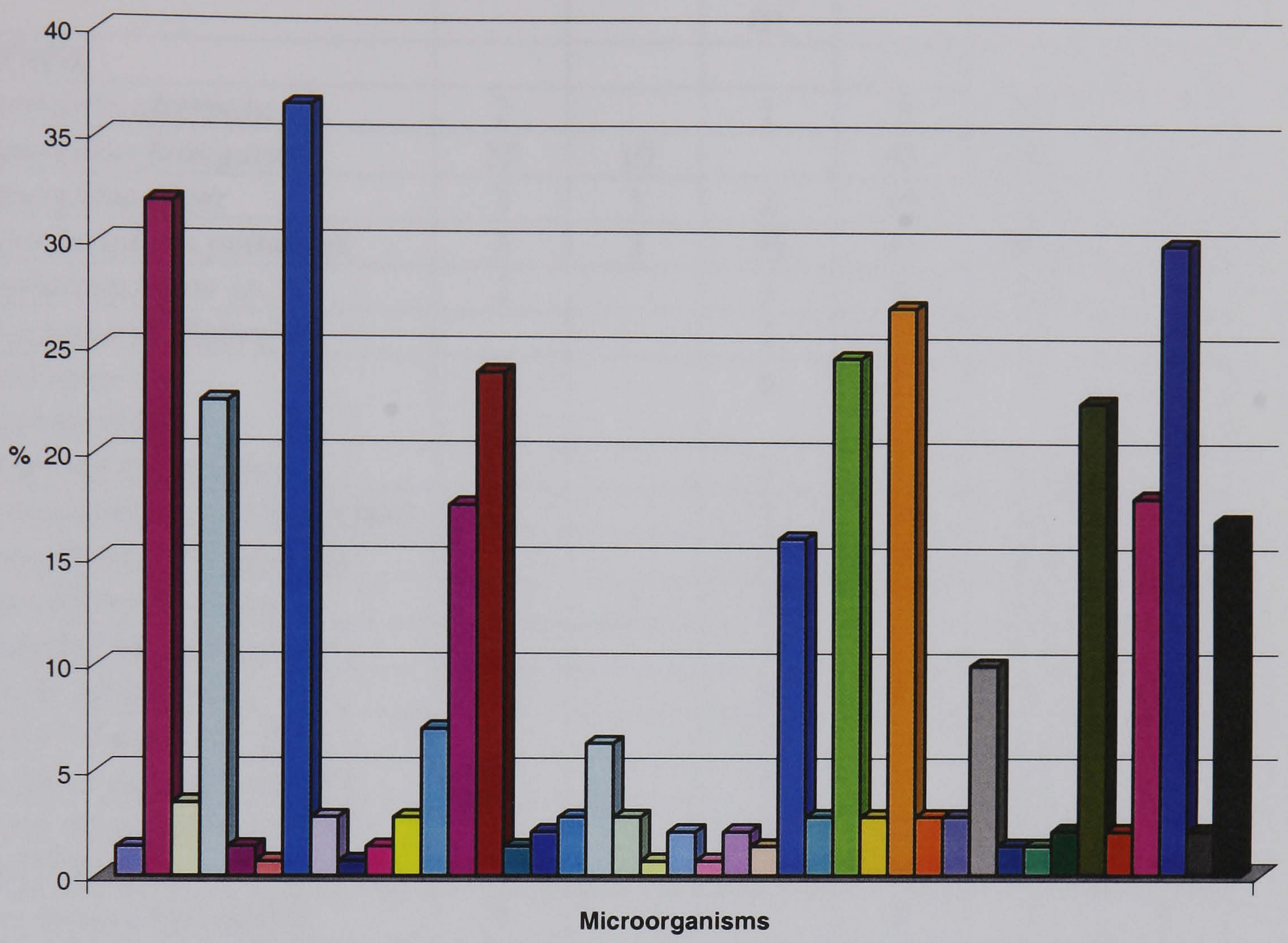
APPENDIX D: The microorganisms isolated at each of the four exposure sites.

D:1a The total number of microorganisms isolated from each of the exposures at Sandefjord and the subsequent percentage isolation of that organism.

Organisms	Sept 99	July 00	Sept 00	June 01	Total / 142	%	Rank 1-10
FUNGI							
Acremonium strictum				2	2	1.4	
Alternaria alternata	5	29	3	9	46	32.0	2
Alternaria tenuis	2	1		2	5	3.5	
Aspergillus fumigatus		23	3	6	32	22.5	7
Aspergillus niger		1	1		2	1.4	
Aspergillus ustus		1			1	0.7	
Aureobasidium pullulans	22	9		21	52	36.6	1
Cephalosporium sp..	3			1	4	2.8	
Chaetomium globosum		1			1	0.7	
Chaetomium sp.	2				2	1.4	
Cladosporium cladosporioides	2			2	4	2.8	
Fusarium oxysporum	4	3		3	10	7.0	
Mycelium sterilium - pink		6		19	25	17.6	10
Mycelium sterilium – white	4	17	4	9	34	23.9	6
Mycelium sterilium – white 2				2	2	1.4	
Unidentified Phycomycete	3				3	2.1	
Penicillium aureogriseum	4				4	2.8	
Penicillium chrysogenum	5	1		3	9	6.3	
Penicillium citrinum	1		2	1	4	2.8	
Penicillium notatum	1				1	0.7	
Penicillium simplicissimum			3		3	2.1	
Phoma sp.		1			1	0.7	
Stemphylium sp.	3				3	2.1	
Trichoderma viride				2	2	1.4	
BACTERIA							
Aeromonas hydrophila		23			23	16.0	
Aureobacterium sp.			4		4	2.8	
Bacillus sp.	12	2	5	16	35	24.6	5
Burkholderia cepacia		1		3	4	2.8	
Cellulomonas sp.		24		15	39	27.0	4
Chryseomonas luteola			4		4	2.8	
Pseudomonas fluorescens	2	2			4	2.8	
Sarcina sp.	7	7		1	15	10.0	
Staphylococcus lentus			2		2	1.4	
Streptomyces sp.	1		1		2	1.4	

Table continued.							
Organisms	Sept 99	July 00	Sept 00	June 01	Total / 142	%	Rank 1-10
YEASTS							
Cryptococcus humicolus	3				3	2.1	
Cryptococcus laurentii	28	4			32	22.5	7
Rhodotorula glutinis			3		3	2.1	
Rhodotorula mucilaginosa		23		3	26	18.0	9
Rhodotorula rubra	*16*	*5*	*7*	*15*	*43*	*30.0*	*3*
ALGAE							
Chlorococcus sp.	3				3	2.1	
Stichococcus sp.	5		5	14	24	16.9	

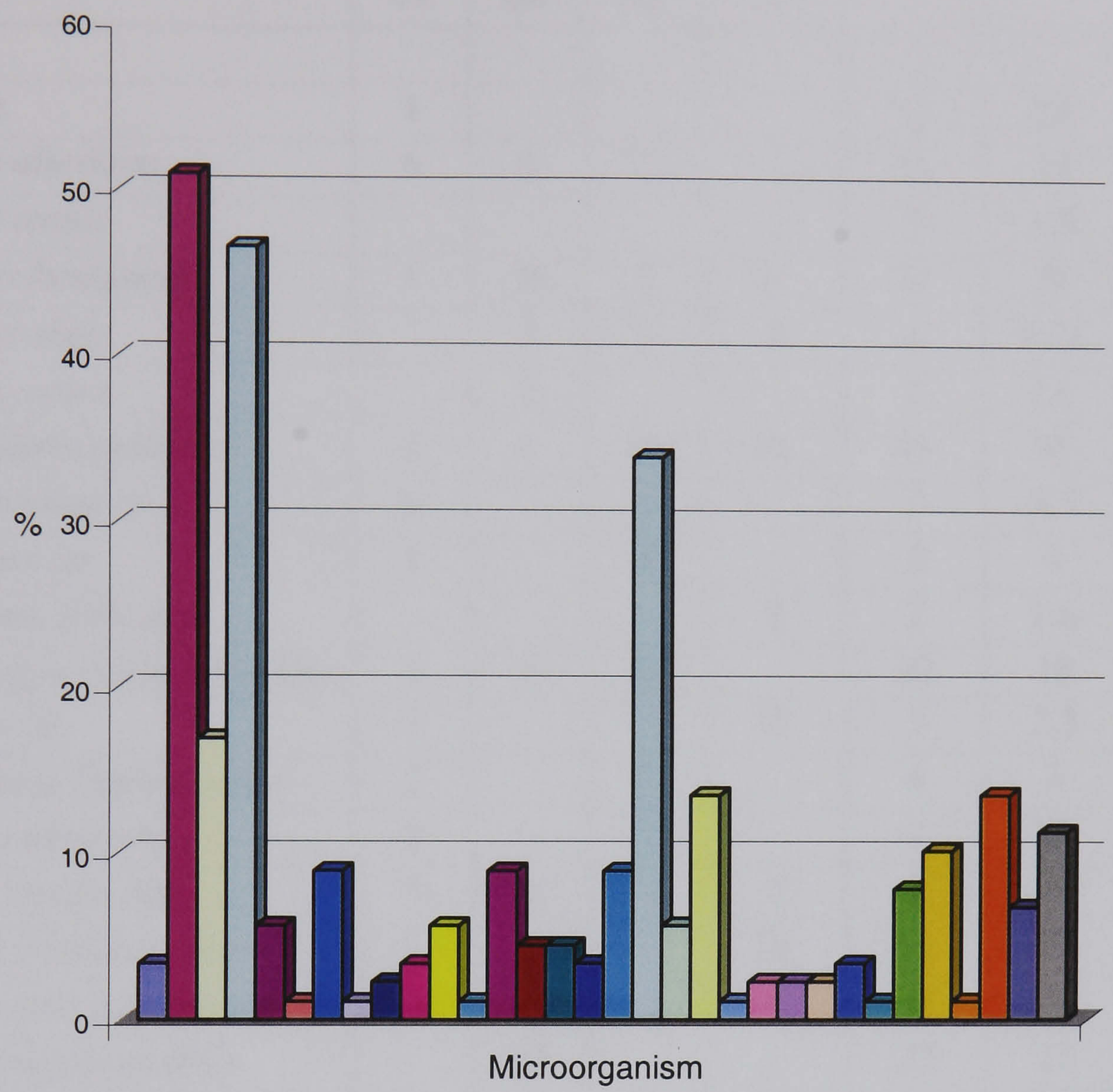
APPENDIX D:1b The percentage of microorganisms isolated at Sandefjord during the four exposures that took place



APPENDIX D:2a The total number of microorganisms isolated from each of the exposures at Bergen and the subsequent percentage isolation of that organism.

Organisms	July 00	Sept 01	June-Aug 01	Total	%	Rank 1-10
FUNGI						
Alternaria alternata	2		1	3	3.4	
Aspergillus fumigatus	35	10		45	51	1
Aspergillus niger	2	1	2	15	17	4
Aureobasidium pullulans	3	3	35	41	46.6	2
Cephalosporium sp.	2		3	5	5.7	
Chaetomium globosum			1	1	1.1	
Cladosporium cladosporioides			8	8	9	
Fusarium oxysporum			1	1	1.1	
Penicillium brevi-compactum			2	2	2.3	
Penicillium chrysogenum			3	3	3.4	
Penicillium citrinum		1	4	5	5.7	
Stemphylium botryosum			1	1	1.1	
Sterile Mycelium 1	2		6	8	9	
Sterile Mycelium - pink			4	4	4.5	
Sterile mycelium- white 1	3		1	4	4.5	
Sterile Mycelium- white 2			3	3	3.4	
BACTERIA						
Aeromonas hydrophila	8			8	9	9
Bacillus sp.			30	30	34	3
Burkholderia cepacia		5		5	5.7	
Cellulomonas sp.	12			12	13.6	5
Micrococcus spp.		1		1	1.1	
Pseudomonas sp.	2			2	2.3	
Pseudomonas fluorescens		2		2	2.3	
Sarcina sp.	2			2	2.3	
Staphylococcus capitis			2	3	3.4	
Streptomyces sp.		1		1	1.1	
Unidentified G+ rod			7	7	7.9	10
YEASTS						
Cryptococcus laurentii			9	9	10.2	8
Cryptococcus uniguttulatus		1		1	1.1	
Rhodotorula mucilaginosa		5	7	12	13.6	5
Rhodotorula rubra	2	1	3	6	6.8	
ALGAE						
Stichococcus sp.			10	10	11.4	7

APPENDIX D:2b The percentage of microorganisms isolated at Bergen during the three exposures that took place.



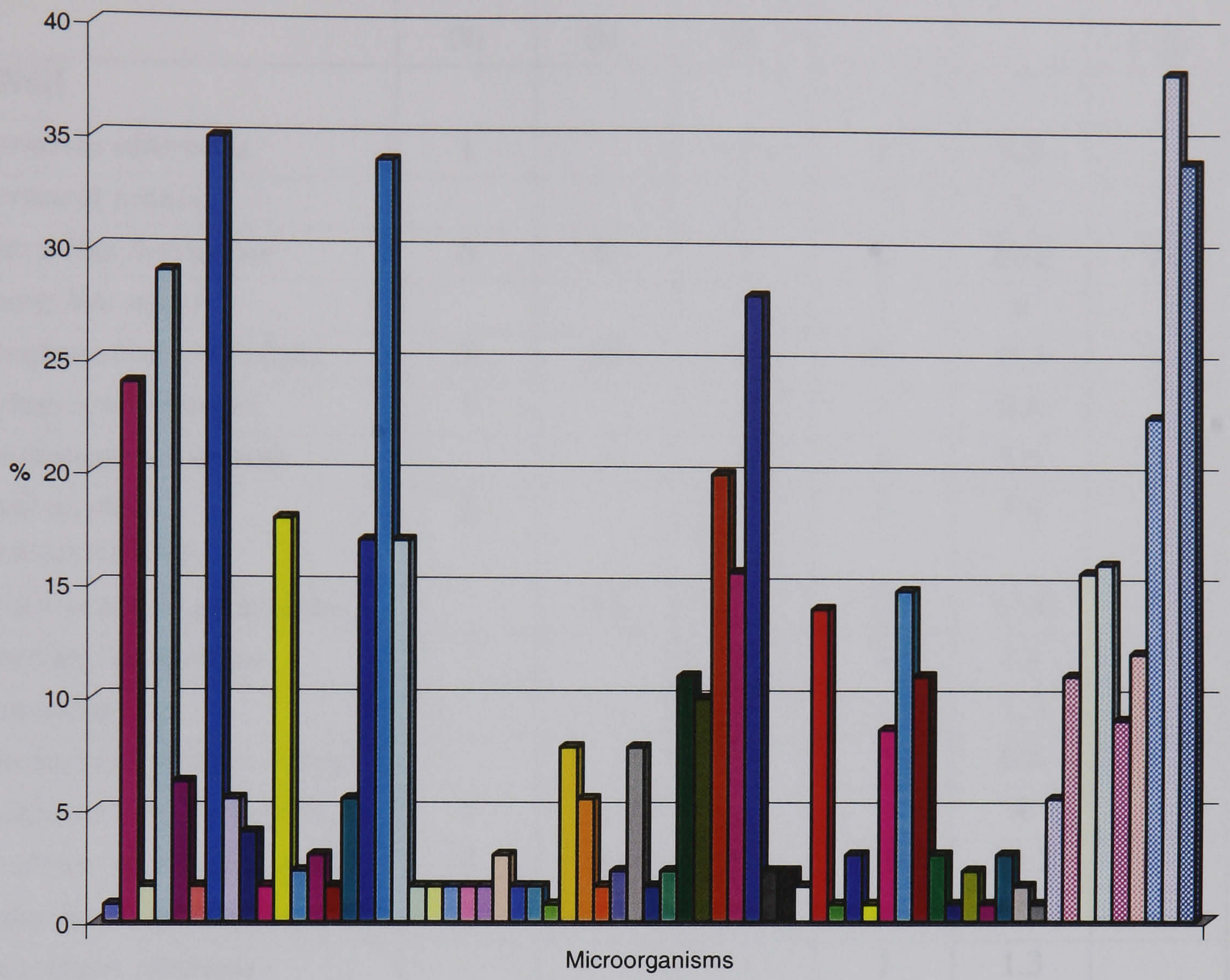
- *Alternaria alternata*
- *Aspergillus fumigatus*
- *Aspergillus niger*
- *Aureobasidium pullulans*
- *Cephalosporium sp.*
- *Chaetomium globosum*
- *Cladosporium cladosporioides*
- *Fusarium oxysporum*
- *Penicillium brevi-compactum*
- *Penicillium chrysogenum*
- *Penicillium citrinum*
- *Stemphylium botryosum*
- Sterile Mycelium 1
- Sterile Mycelium - pink
- Sterile Mycelium - white 1
- Sterile Mycelium - white 2
- *Aeromonas hydrophila*
- *Bacillus sp.*
- *Burkholderia cepacia*
- *Cellulomonas sp.*
- *Micrococcus spp.*
- *Pseudomonas sp.*
- *Pseudomonas fluorescens*
- *Sarcina sp.*
- *Staphylococcus capitis*
- *Streptomyces sp.*
- Unidentified G+ rod
- *Cryptococcus laurentii*
- *Cryptococcus uniguttulatus*
- *Rhodotorula mucilaginosa*
- *Rhodotorula rubra*
- *Stichococcus sp.*

APPENDIX D:3a The total number of microorganisms isolated from each of the exposures at Preston and the subsequent percentage isolation of that organism.

Organism	Sept 99	Apr 00	Feb 01	June 01	Total	%	Rank 1-10
FUNGI							
Absidia sp.	1				1	0.8	
Alternaria alternata	6	2	22	1	31	24	7
Alternaria tenuis				2	2	1.6	
Aspergillus fumigatus	1	28	2	6	37	29	5
Aspergillus niger		5	1	2	8	6.25	
Aspergillus ustus		2			2	1.6	
Aureobasidium pullulans	2	3	19	21	45	35	2
Cephalosporium sp.	6		1		7	5.5	
Chaetomium sp.	1		4		5	4	
Chaetomium globosum				2	2	1.6	
Cladosporium cladosporioides	1	2	20		23	18	10
Curvularia sp.	1			2	3	2.3	
Dematiaceous hyphomycete	2		2		4	3	
Fusarium culmorum	2				2	1.6	
Fusarium oxysporum	5	1		1	7	5.5	
Mycelium sterilium - pink	1			21	22	17	
Mycelium sterilium white	4		28	12	44	34	3
Mycelium sterilium (P6)		22			22	17	
Mycelium sterilium P34	2				2	1.6	
Mycelium sterilium P35	2				2	1.6	
Mycelium sterilium P37	2				2	1.6	
Mycelium sterilium P41	2				2	1.6	
Mycelium sterilium P63	2				2	1.6	
Paecilomyces marquandii			4		4	3	
Penicillium brevi-compactum				2	2	1.6	
Penicillium aureogriseum	2				2	1.6	
Penicillium canescens				1	1	0.8	
Penicillium chrysogenum	5		5		10	7.8	
Penicillium citrinum	1			6	7	5.5	
Penicillium simplicissimum			2		2	1.6	
Phialophora sp.				3	3	2.3	
Phoma sp.	1	8		1	10	7.8	
Puecilomyces variotii				2	2	1.6	
Trichoderma viride		2		1	3	2.3	

Table continued. Organism	Sept 99	Apr 00	Feb 01	June 01	Total	%	Rank 1-10
BACTERIA							
Aeromonas hydrophila		14			14	11	
Arthrobacter sp.			13		13	10	
Bacillus sp.	1		4	21	26	20	9
Brevibacterium sp.			20		20	15.6	
Cellulomonas sp.	2	34			36	28	6
Chryseomonas luteola			3		3	2.3	
Micrococcus spp.				3	3	2.3	
Pantoea spp.				2	2	1.6	
Pseudomonas fluorescens	12		3	3	18	14	
Pseudomonas paucimobilis			1		1	0.8	
Sarcina sp.	4				4	3	
Serratia ficaria				1	1	0.8	
Staphylococcus capitis				11	11	8.6	
Staphylococcus cohnii cohnii			19		19	14.8	
Staphylococcus lentus				14	14	11	
Streptomyces sp.	1		3		4	3	
Unidentified G+ coccus	1				1	0.8	
Unidentified G+ rod	3				3	2.3	
Vibrio fluvalis				1	1	0.8	
YEASTS							
Candida ciferrii			4		4	3	
Candida utilis			2		2	1.6	
Cryptococcus albidus			1		1	0.8	
Cryptococcus humicolus			7		7	5.5	
Cryptococcus laurentii	14				14	11	
Hansenula polymorpha			20		20	15.6	
Rhodotorula glutinis		21			21	16	
Rhodotorula mucilaginosa	4		8		12	9	
Rhodotorula rubra	7	2	6		15	12	
Saccharomyces cerevisiae	15			14	29	22.6	8
ALGAE							
Chlorella sp.	14		22	13	49	38	1
Stichococcus sp.	13	7	12	12	44	34	3

APPENDIX D:3b The percentage of microorganisms isolated at Preston during the twenty-three month exposure period



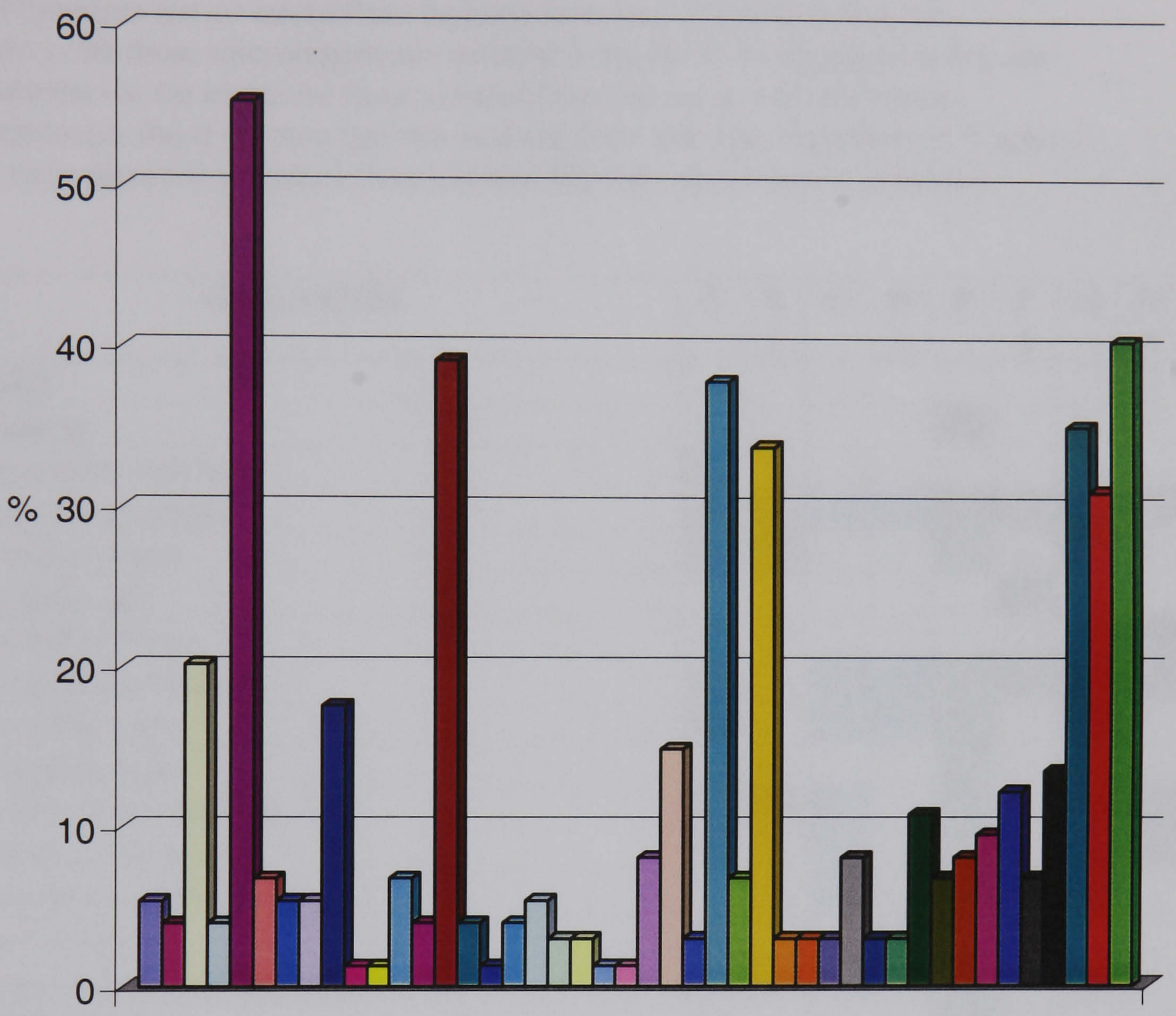
- *Absidia sp.*
- *Alternaria alternata*
- *Alternaria tenuis*
- *Aspergillus fumigatus*
- *Aspergillus niger*
- *Aspergillus ustus*
- *Aureobasidium pullulans*
- *Cephalosporium sp.*
- *Chaetomim sp.*
- *Chaetomium globosum*
- *Cladosporium cladosporioides*
- *Curvularia sp.*
- *Dematеatous hyphomycete*
- *Fusarium culmorum*
- *Fusarium oxysporum*
- Mycelium sterilium - pink
- Mycelium sterilium - white
- Mycelium sterilium - P6
- Mycelium sterilium - P34
- Mycelium sterilium - P35
- Mycelium sterilium - P37
- Mycelium sterilium - P41
- Mycelium sterilium - P63
- *Paecilomyces marquandii*
- *Penicillium brevi-compactum*
- *Penicillium aureogriseum*
- *Penicillium canescens*
- *Penicillium chrysogenum*
- *Penicillium citrinum*
- *Penicillium simplicissimum*
- *Phialophora sp.*
- *Phoma sp.*
- *Puecilomyces variotii*
- *Trichoderma viride*
- *Aeromonas hydrophila*
- *Arthrobacter sp.*
- *Bacillus sp.*
- *Brevibacterium sp.*
- *Cellulomonas sp.*
- *Chryseomyces luteola*
- *Micrococcus spp.*
- *Pantoea spp.*
- *Pseudomonas fluorescens*
- *Pseudomonas paucimobilis*
- *Sarcina sp.*
- *Serratia ficaria*
- *Staphylococcus capitis*
- *Staphylococcus cohnii cohnii*
- *Staphylococcus lentus*
- *Streptomyces sp.*
- Unidentified G+ coccus
- Unidentified G+ rod
- *Vibrio fluvalis*
- *Candida ciferrii*
- *Candida utilis*
- *Cryprococcus albidus*
- *Cryptococcus humicolus*
- *Cryptococcus laurentii*
- *Hansenula polymorpha*
- *Rhodotorula glutinis*
- *Rhodotorula mucilaginosa*
- *Rhodotorula rubra*
- *Saccharomyces cerevisiae*
- *Chlorella sp.*
- *Stichococcus sp.*

APPENDIX D:4a The total number of microorganisms isolated from each of the exposures at Blackley and the subsequent percentage isolation of that organism

Organism	Apr 00	Feb 01	Nov 01	Total	%	Rank 1-10
FUNGI						
Alternaria alternata	1		3	4	5.4	
Alternaria tenuis			3	3	4	
Aspergillus fumigatus	6	6	3	15	20.2	8
Aspergillus niger			3	3	4	
Aureobasidium pullulans	5	19	17	41	55.4	1
Cephalosporium sp.1	1		4	5	6.8	
Chaetomium globosum		1	3	4	5.4	
Cladosporium Cladosporioides	3		1	4	5.4	
Cylindrocarpon candidum		13		13	17.6	9
Fusarium oxysporum			1	1	1.3	
Gliocladium sp.			1	1	1.3	
Mycelium sterilium - Grey			5	5	6.8	
Mycelium sterilium -pink	1	1	1	3	4	
Mycelium sterilium - white	6	10	13	29	39.2	3
Penicillium chrysogenum	2		1	3	4	
Penicillium citrinum			1	1	1.3	
Penicillium expansum		1	2	3	4	
Penicillium simplicissimum	4			4	5.4	
Penicillium spinulosum		2		2	3	
Phoma herbarum		2		2	3	
Trichoderma koningii		1		1	1.3	
Trichoderma viride		1		1	1.3	

Table continued.						
Organism	Apr 00	Feb 01	Nov 01	Total	%	Rank 1-10
BACTERIA						
Aeromonas hydrophila	2	4		6	8.1	
Arthrobacter sp.	9	2		11	14.9	10
Aureobacterium sp.	2			2	3	
Bacillus sp.	18	7	3	28	37.9	4
Burkholderia cepacia		5		5	6.8	
Cellulomonas sp.	20	4	1	25	33.8	6
Chryseomonas luteola		2		2	3	
Corynebacterium stratiatum		2		2	3	
Non fermenter sp.		2		2	3	
Sarcina sp.			6	6	8.1	
Staphylococcus epidermidis		2		2	3	
Staphylococcus capitis			2	2	3	
Staphylococcus lentus	4		4	8	10.8	
Staphylococcus xylosus	5			5	6.8	
Streptomyces sp.	1	3	2	6	8.1	
Unidentified G+ rod	7			7	9.5	
YEAST						
Cryptococcus ciferii		9		9	12.2	
Cryptococcus laurentii		5		5	6.8	
Rhodotorula mucilaginosa	2	5	3	10	13.5	
Rhodotorula rubra	11	9	6	26	35.1	5
ALGAE						
Chlorella sp.	9	8	6	23	31	7
Stichococcus sp.	8	12	10	30	40.5	2

APPENDIX D:4b The percentage of microorganisms isolated at Blackley during the nineteen month exposure period.



- *Alternaria alternata*
- *Alternaria tenuis*
- *Aspergillus fumigatus*
- *Aspergillus niger*
- *Aureobasidium pullulans*
- *Cephalosporium sp.*
- *Chaetomium globosum*
- *Cladosporium cladosporioides*
- *Cylindrocarpon candidum*
- *Fusarium oxysporum*
- *Gliocladium sp.*
- *Mycelium sterilium - grey*
- *Mycelium sterilium - pink*
- *Mycelium sterilium - white*
- *Penicillium chrysogenum*
- *Penicillium citrinum*
- *Penicillium expansum*
- *Penicillium simplicissimum*
- *Penicillium spinulosum*
- *Phoma herbarum*
- *Trichoderma koningii*
- *Trichoderma viride*
- *Aeromonas hydrophila*
- *Arthrobacter sp.*
- *Aureobacterium sp.*
- *Bacillus sp.*
- *Burkholderia cepacia*
- *Cellulomonas sp.*
- *Chryseomonas luteola*
- *Corynebacterium stratiatum*
- *Non fermenter sp.*
- *Sarcina sp.*
- *Staphylococcus epidermidis*
- *Staphylococcus capitis*
- *Staphylococcus lentus*
- *Staphylococcus xylosus*
- *Streptomyces sp.*
- Unidentified G+ rod
- *Cryptococcus ciferii*
- *Cryptococcus ciferii*
- *Rhodotorula mucilaginosa*
- *Rhodotorula rubra*
- *Chlorella sp.*
- *Stichococcus sp.*

APPENDIX E: Microorganisms isolated from each of the sites throughout the twenty-three months of exposures.

S represents those microorganisms isolated from the four exposures at Sandefjord.
SA represents the air micro flora isolated from the exposures at Sandefjord.
B represents those microorganisms isolated from the three exposures at Bergen.
BA represents the air micro flora isolated from the exposure at Bergen.
P represents those microorganisms isolated from the four exposures at Preston.
PA represents the air micro flora isolated from the exposures at Preston.
M represents those microorganisms isolated from the four exposures at Blackley
MA represents the air micro flora isolated from the exposures at Blackley.

ORGANISM	S	SA	B	BA	P	PA	M	MA
FUNGI								
Absidia sp.								
Acremonium strictum								
Alternaria alternata								
Alternaria tenuis								
Aspergillus sp.								
Aspergillus flavus								
Aspergillus fumigatus								
Aspergillus niger								
Aspergillus ustus								
Aureobasidium pullulans								
Cephalosporium sp.								
Chaetomium globosum								
Chaetomium sp.								
Cladosporium cladosporioides								
Curvularia sp.								
Cylindrocarpon candidum								
Dematiaceous hyphomycete								
Fusarium culmorum								
Fusarium oxysporum								
Gliocladium sp.								
Mycelium sterilium								
Mycelium sterilium 1								
Mycelium sterilium P6								
Mycelium sterilium P34								
Mycelium sterilium P35								
Mycelium sterilium P37								
Mycelium sterilium P41								
Mycelium sterilium P63								
Mycelium sterilium - grey								
Mycelium sterilium – pink								
Mycelium sterilium – white								

Table Continued

ORGANISM	S	SA	B	BA	P	PA	M	MA
Mycelium sterilium – white 2								
Paecilomyces marquandii								
Penicillium aureogriseum								
Penicillium brevi-compactum								
Penicillium canescens								
Penicillium citrinum								
Penicillium chrysogenum								
Penicillium expansum								
Penicillium notatum								
Penicillium simplicissimum								
Penicillium spinulosum								
Phialophora sp.								
Phoma herbarum								
Phoma sp.								
Puecilomyces variotii								
Stemphyllium botryosum								
Stemphyllium sp.								
Trichoderma sp.								
Trichoderma koningii								
Trichoderma viride								
Unidentified Phycomycete								

ORGANISM	S	SA	B	BA	P	PA	M	MA
BACTERIA								
Actinomycete sp.								
Aeromonas hydrophila								
Arthrobacter sp.								
Aureobacterium spp.								
Bacillus sp.								
Brevibacterium sp.								
Burkholderia cepacia								
Cellulomonas sp.								
Chryseomonas luteola								
Corynebacterium stratiatum								
Non fermenter sp.								
Micrococcus spp.								
Pantoea spp.								
Pseudomonas fluorescens								
Pseudomonas paucimobilis								
Pseudomonas sp.								
Sarcina sp.								
Serratia ficaria								
Serratia liquefaciens								
Staphylococcus capitis								

Table continued

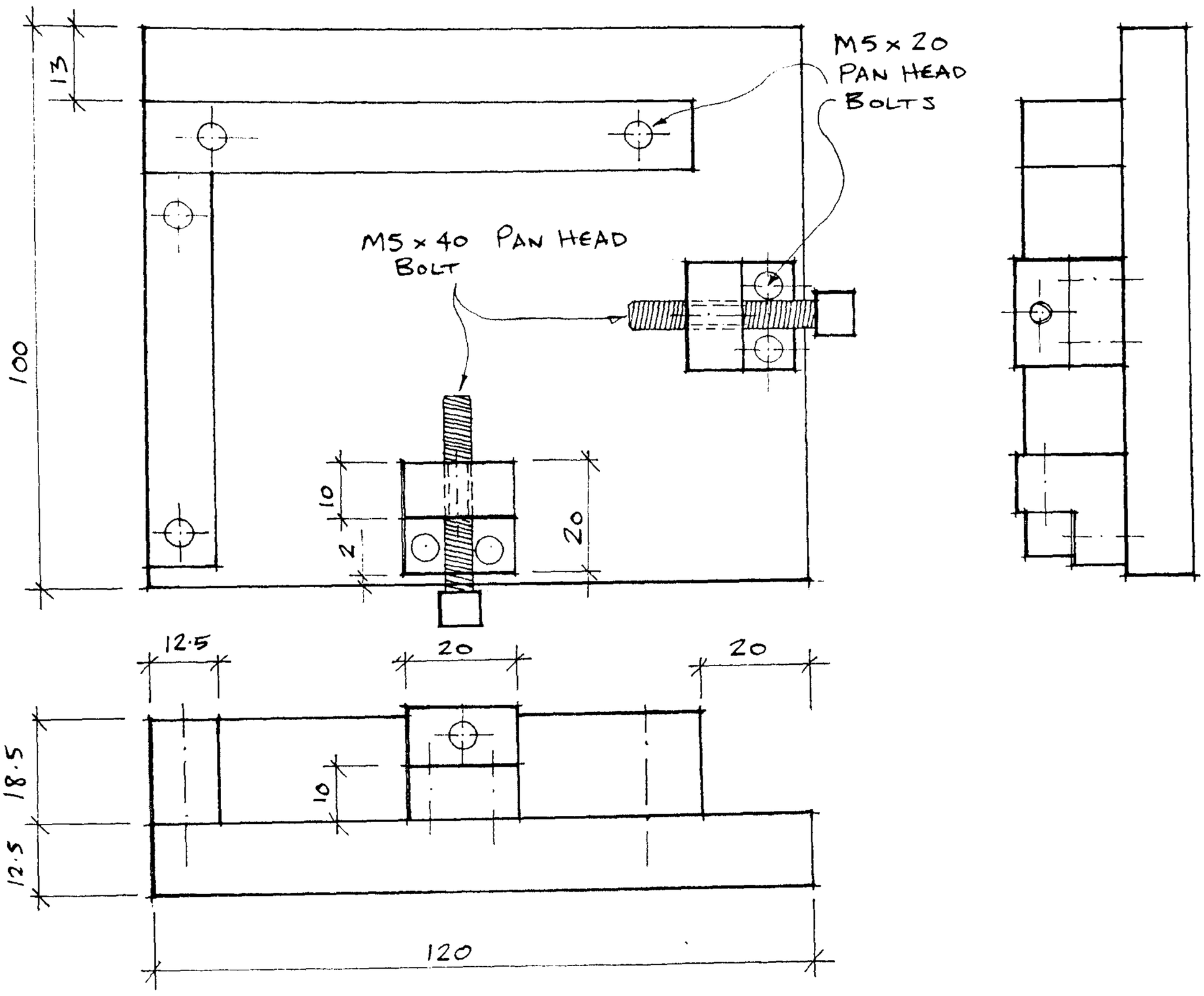
ORGANISM	S	SA	B	BA	P	PA	M	MA
Staphylococcus cohnii cohnii								
Staphylococcus cohnii ureal								
Staphylococcus epidermidis								
Staphylococcus lentus								
Staphylococcus xylosus								
Streptomyces sp.								
Unidentified G+ rod								
Unidentified G+ coccus								
Vibrio fluvalis								

ORGANISM	S	SA	B	BA	P	PA	M	MA
YEASTS								
Candida ciferrii								
Candida utilis								
Cryptococcus albidus								
Cryptococcus laurentii								
Cryptococcus humicolus								
Cryptococcus uniguttulatus								
Hansenula polymorpha								
Rhodococcus spp.								
Rhodotorula glutinis								
Rhodotorula mucilaginosa								
Rhodotorula rubra								
Saccharomyces cerevisiae								

ORGANISM	S	SA	B	BA	P	PA	M	MA
ALGAE								
Chlorella sp.								
Chlorococcus sp.								
Stichococcus sp.								
Tolypothrix sp.								

APPENDIX F: The panel holder for the Talysurf™

Below is an engineering drawing used for the manufacture of the panel holder, all of the measurements are in mm.



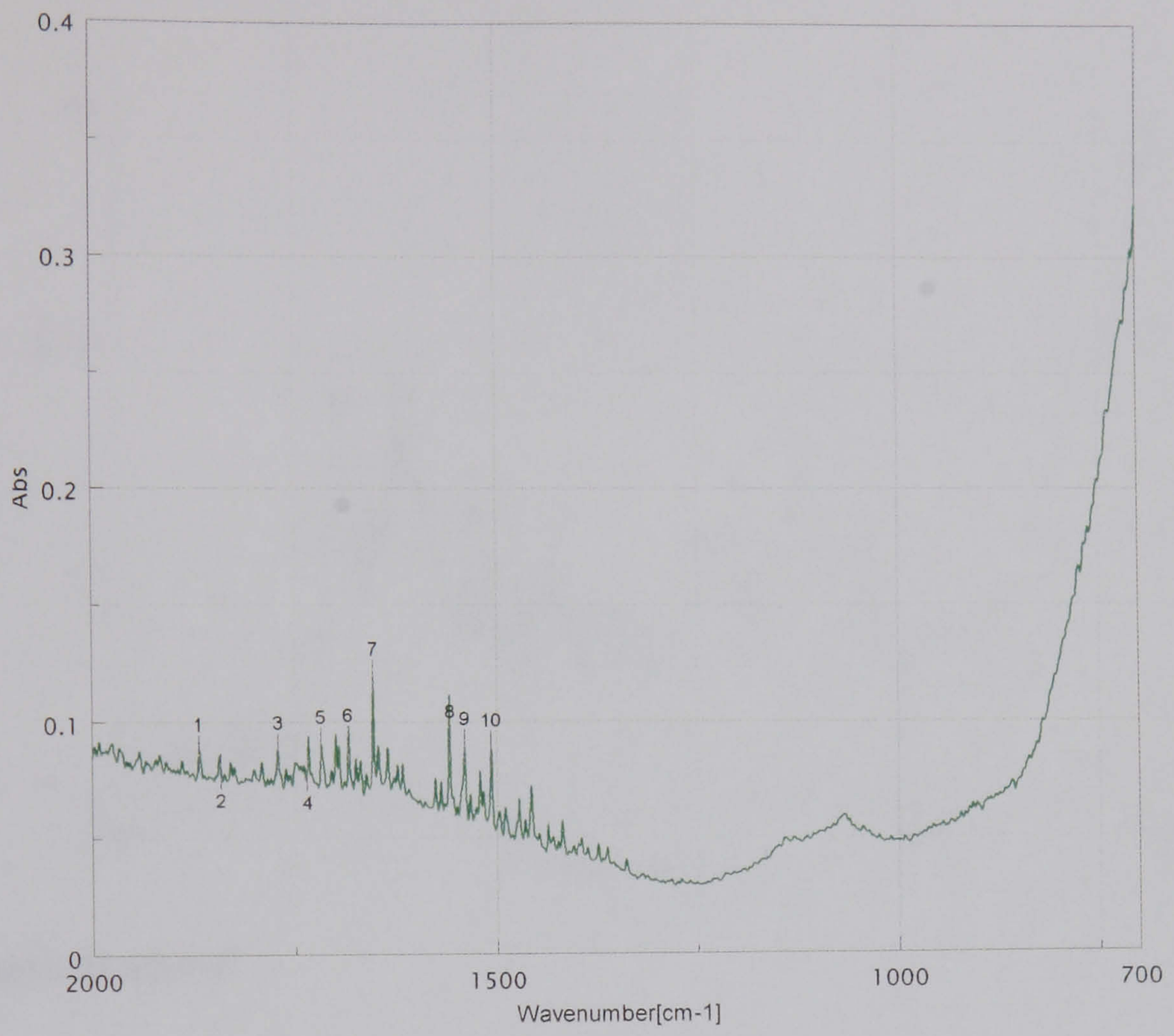
APPENDIX G: The application of the FEI XL30 ESEM (Taken directly from the FEI Company Applications Report)

The equipment used was a FEI XL30 ESEM-TMP tungsten gun Environmental Scanning Electron Microscope.

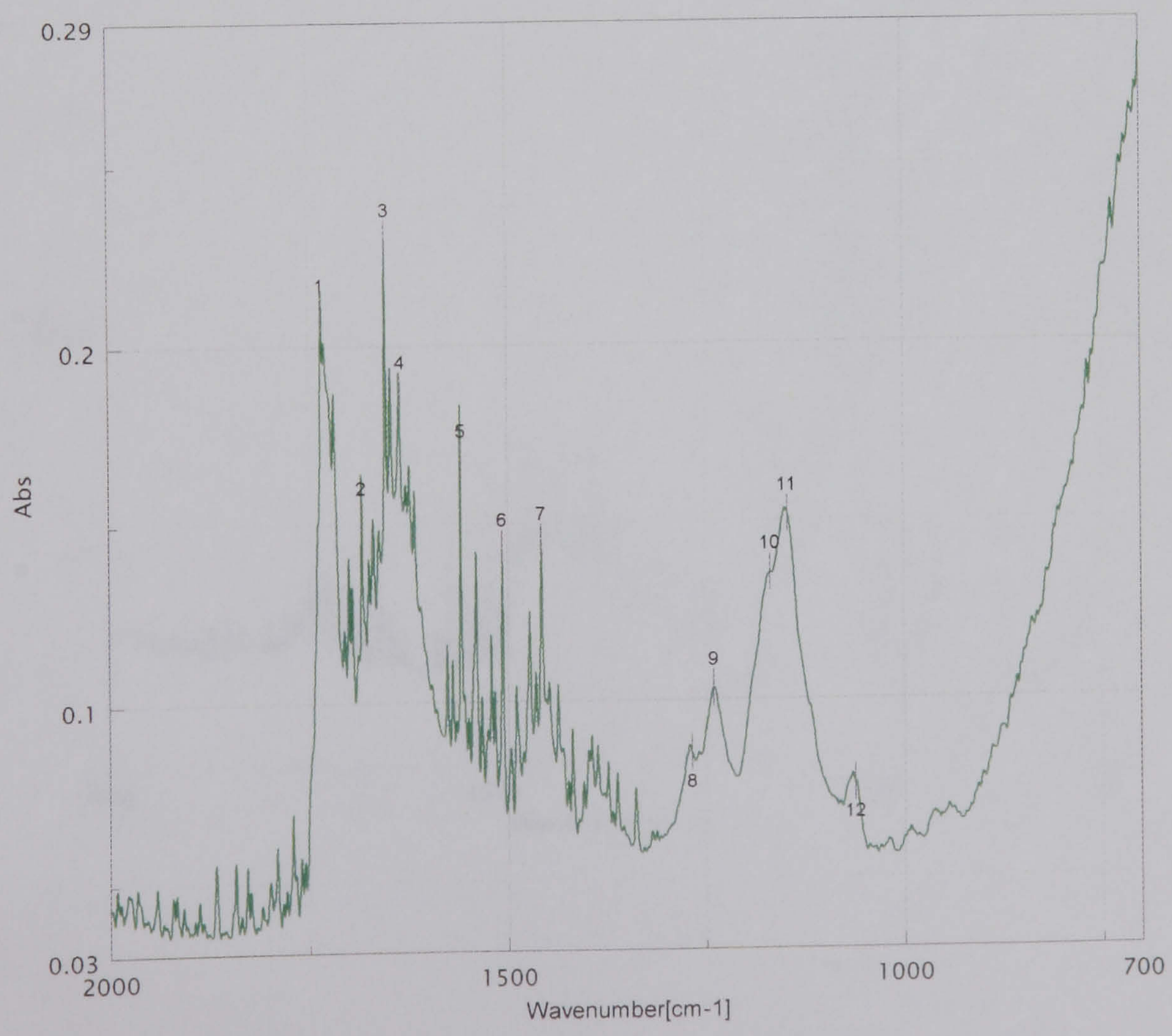
The wet specimens were mounted on 10mm stubs for use on the Peltier cooling stage. Digital images were recorded and stored on floppy disc. All image processing, analysis and printing was done off line via the XL-Docu software which is standard with the XL30 ESEM-TMP. Since the specimens were wet, the instrument was configured in the ESEM mode using the Gaseous Secondary Electron Detector (GSED) incorporating a second differential pumping aperture. Excess water was removed by controlled evaporation leaving the specimens in a fully hydrated state. The atmosphere in the specimen chamber becomes ionised due to the interaction of the electron beam and the gas. The positive ions are attracted to the specimen and neutralise the beam-induced charge build up. The environmental electrons produced due to interaction of secondary electrons with the gas, cascade towards the GSED and form the basis of the secondary electron signal amplification. (FEI patent). The high vacuum in the lectron optical column is maintained due to the use of double differential pumping apertures with intermediate pumping (FEI patent)

APPENDIX H: The FTIR spectra of the raw paint materials

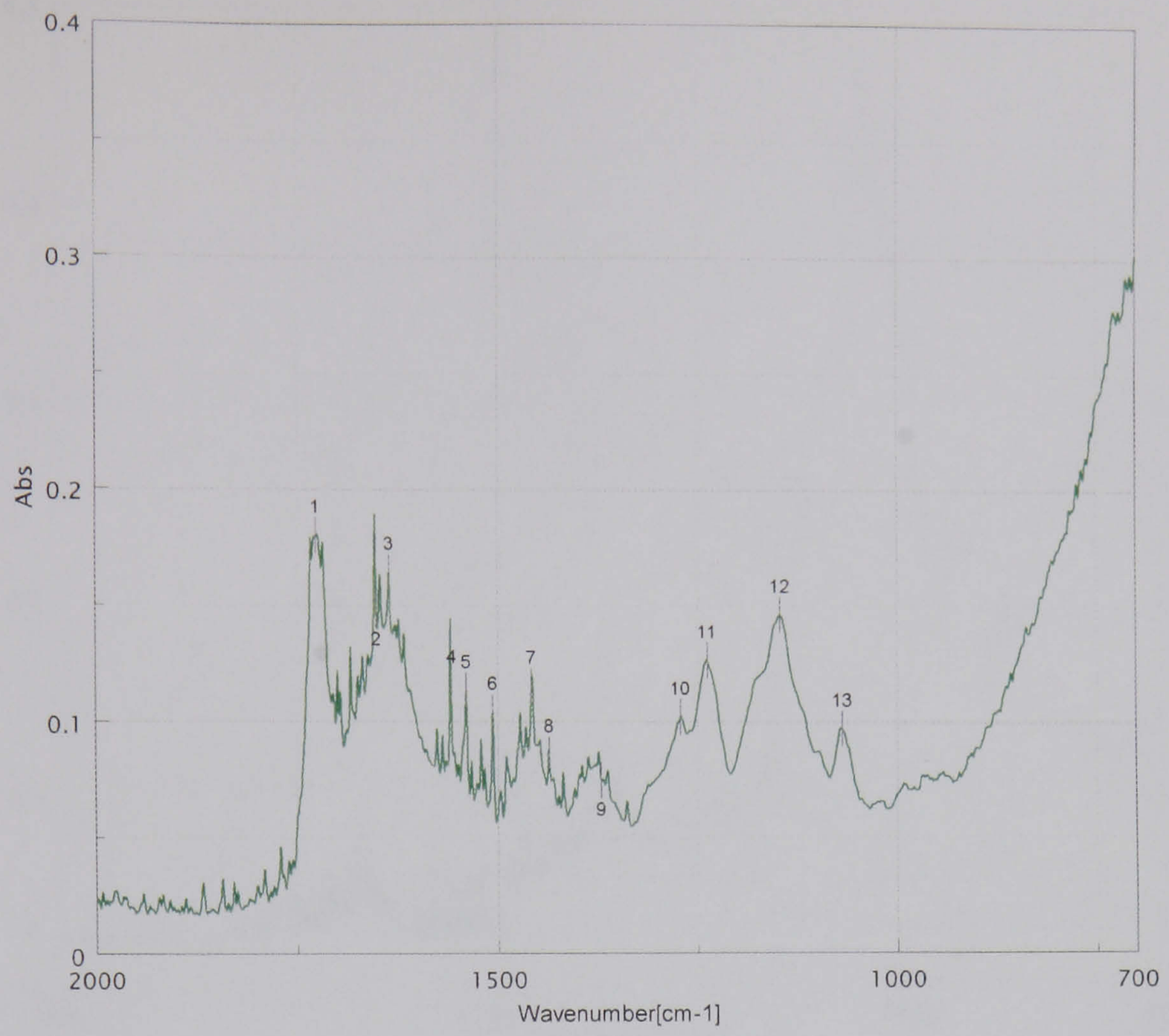
H:1. Titanium dioxide



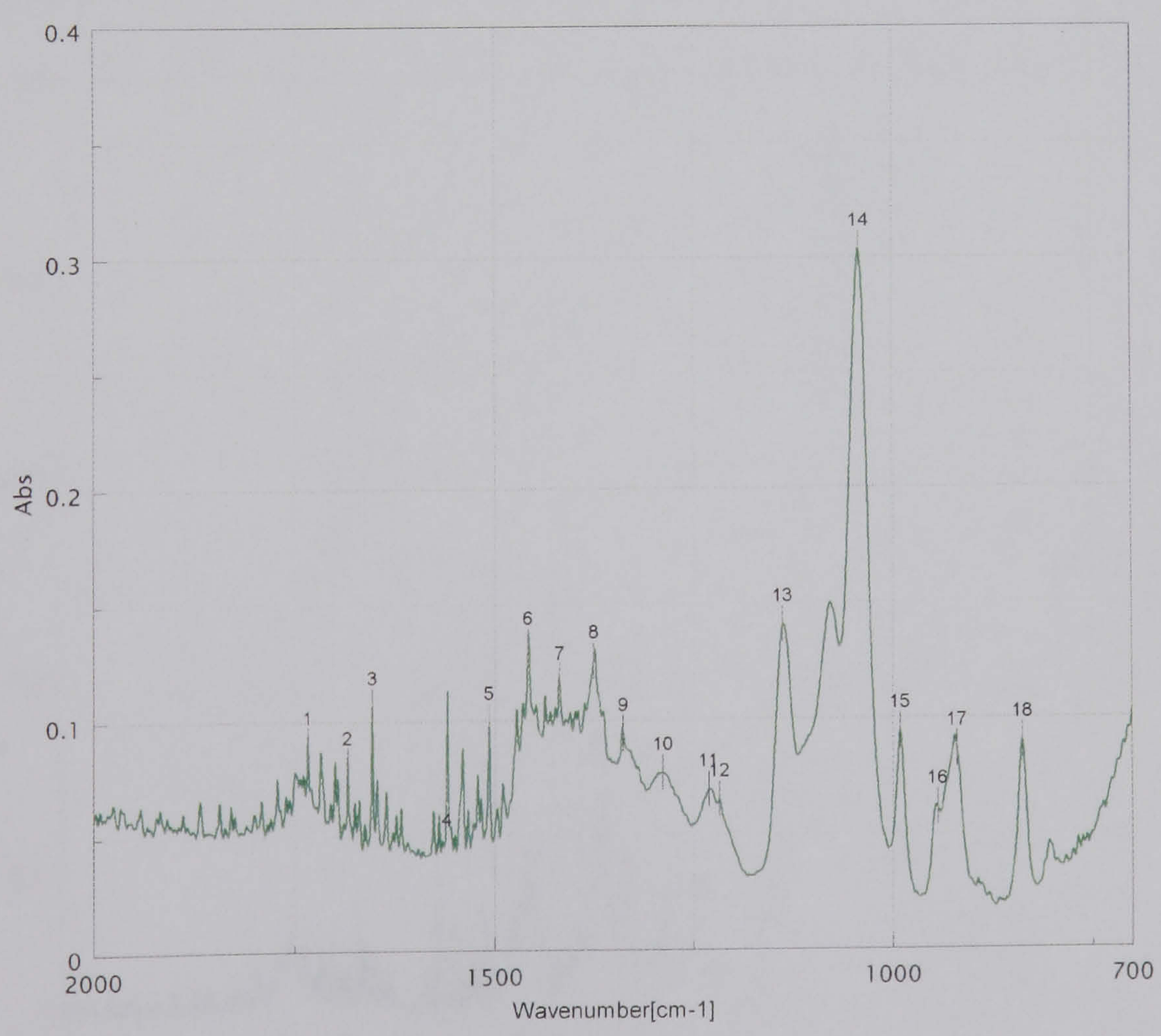
H:2 The binder for the pure acrylic paint



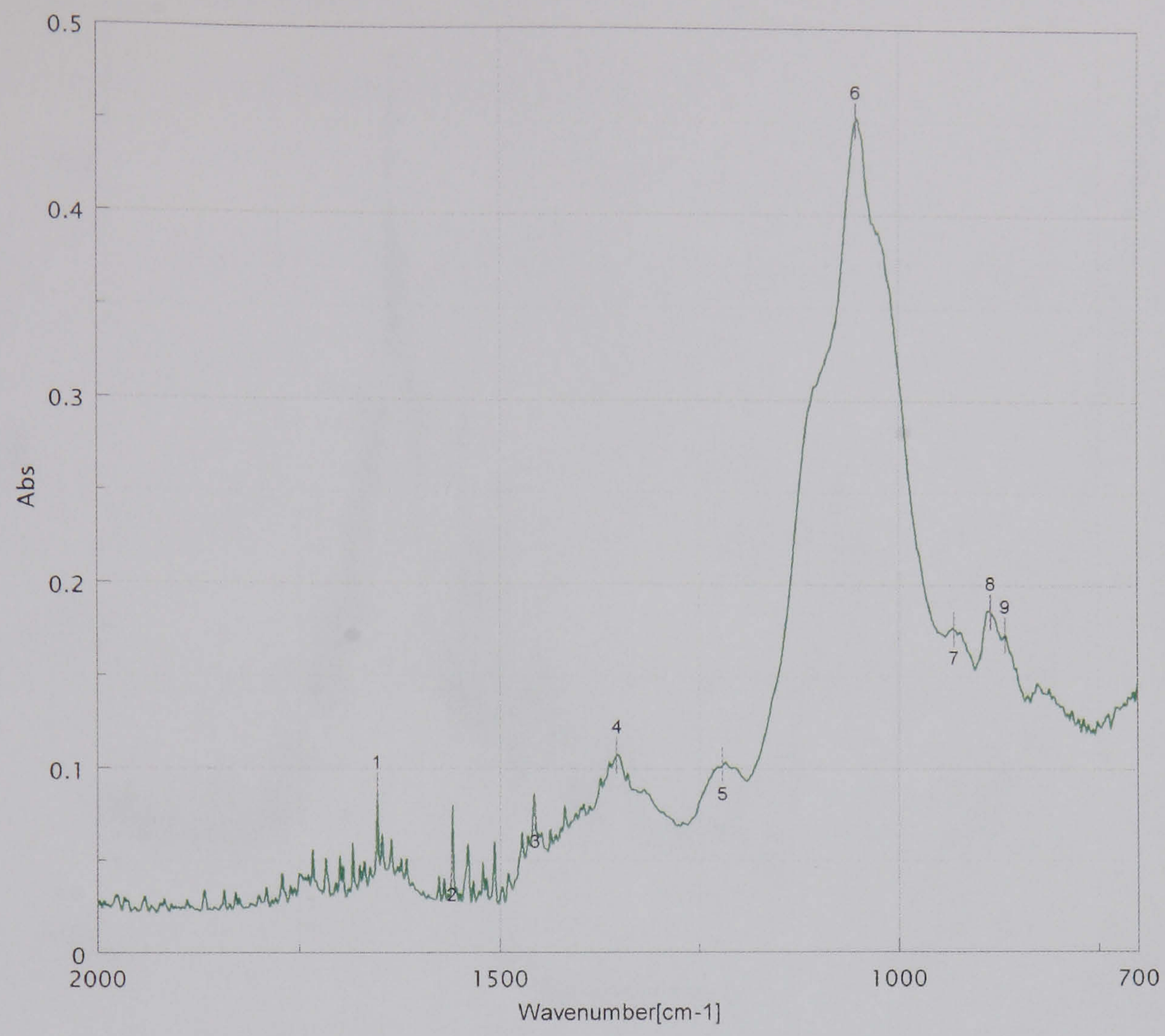
H:3 The binder for the hybrid acrylic paint



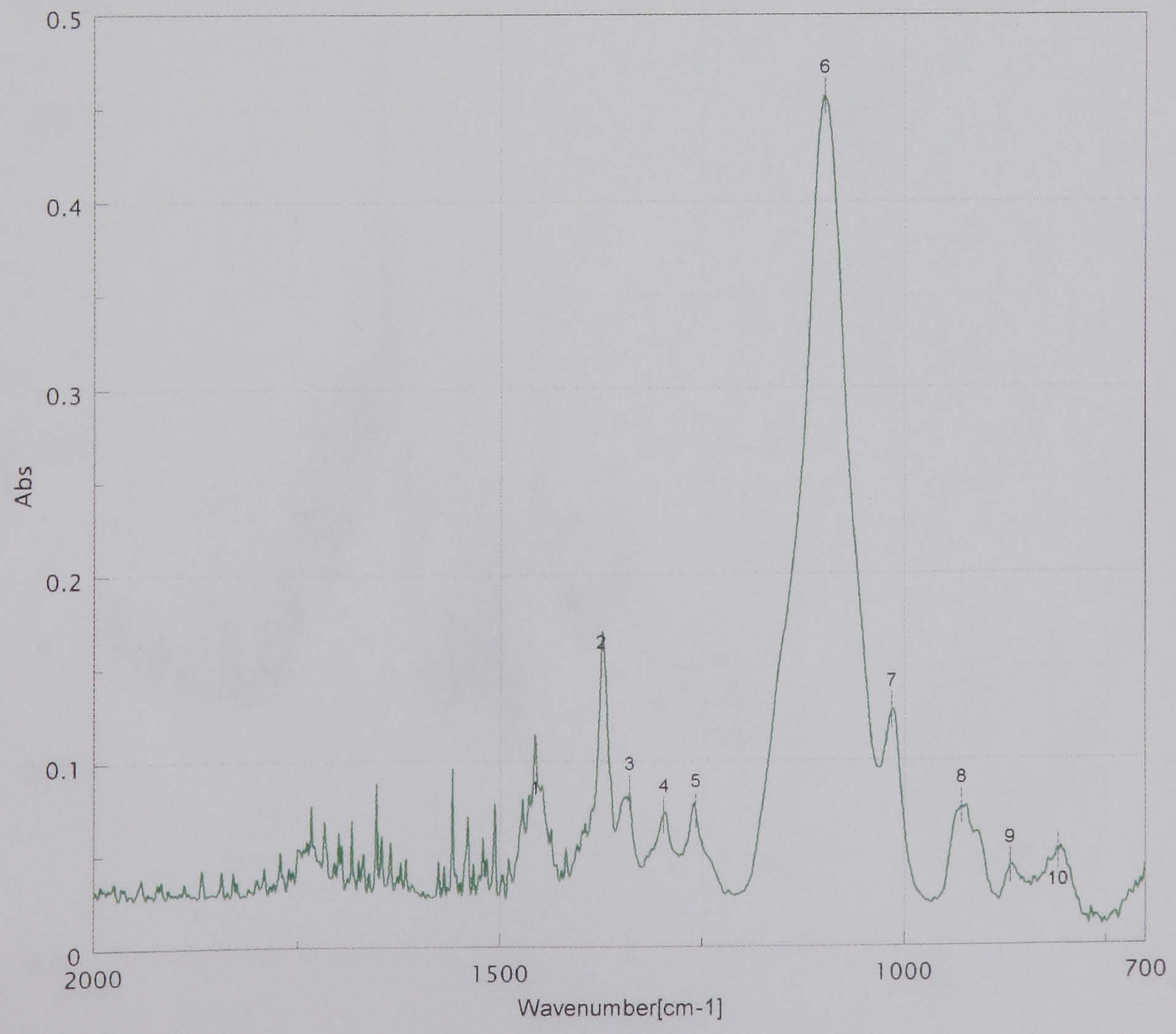
H:4 Propylene glycol



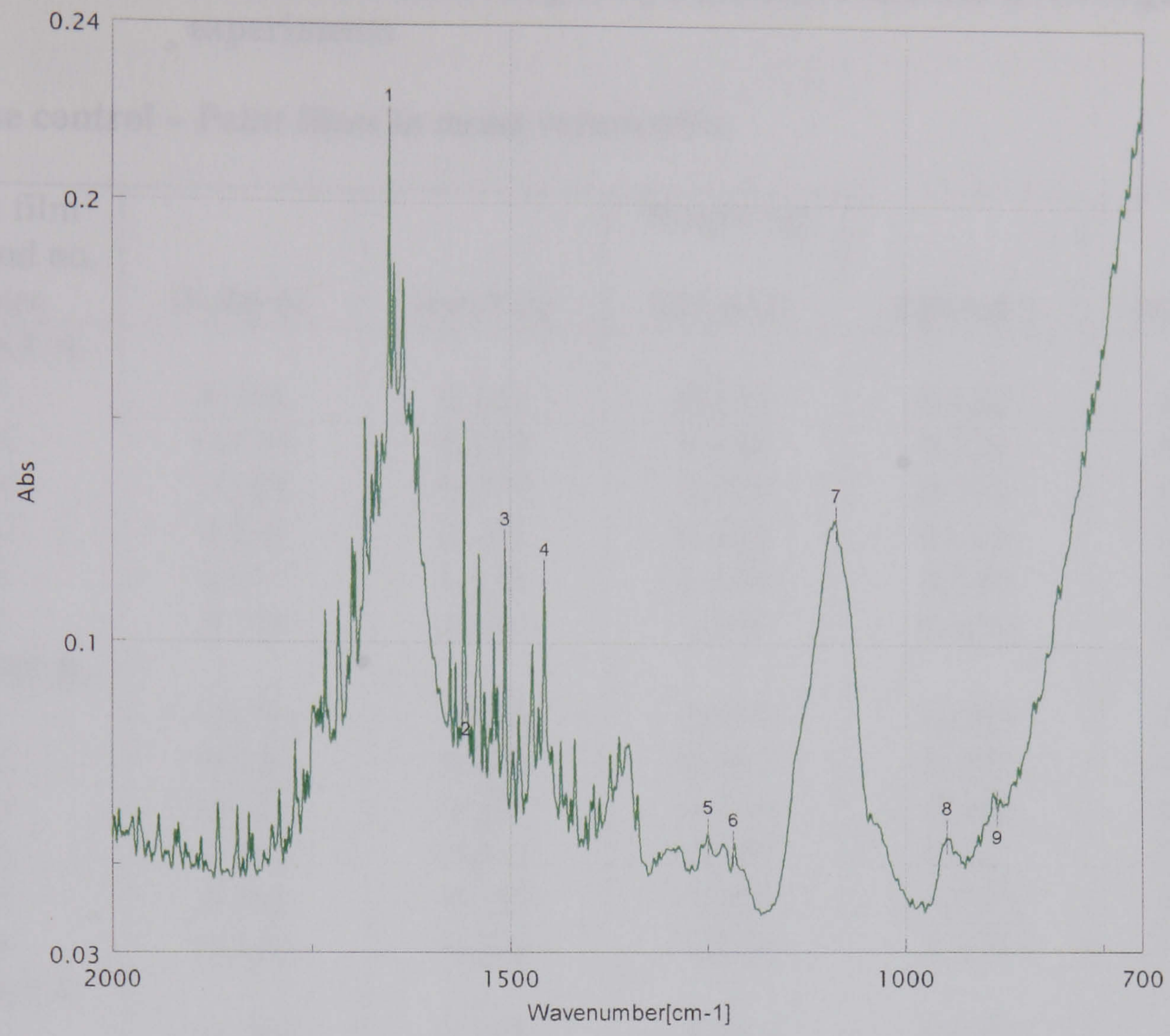
H:5 Cellulose thickener



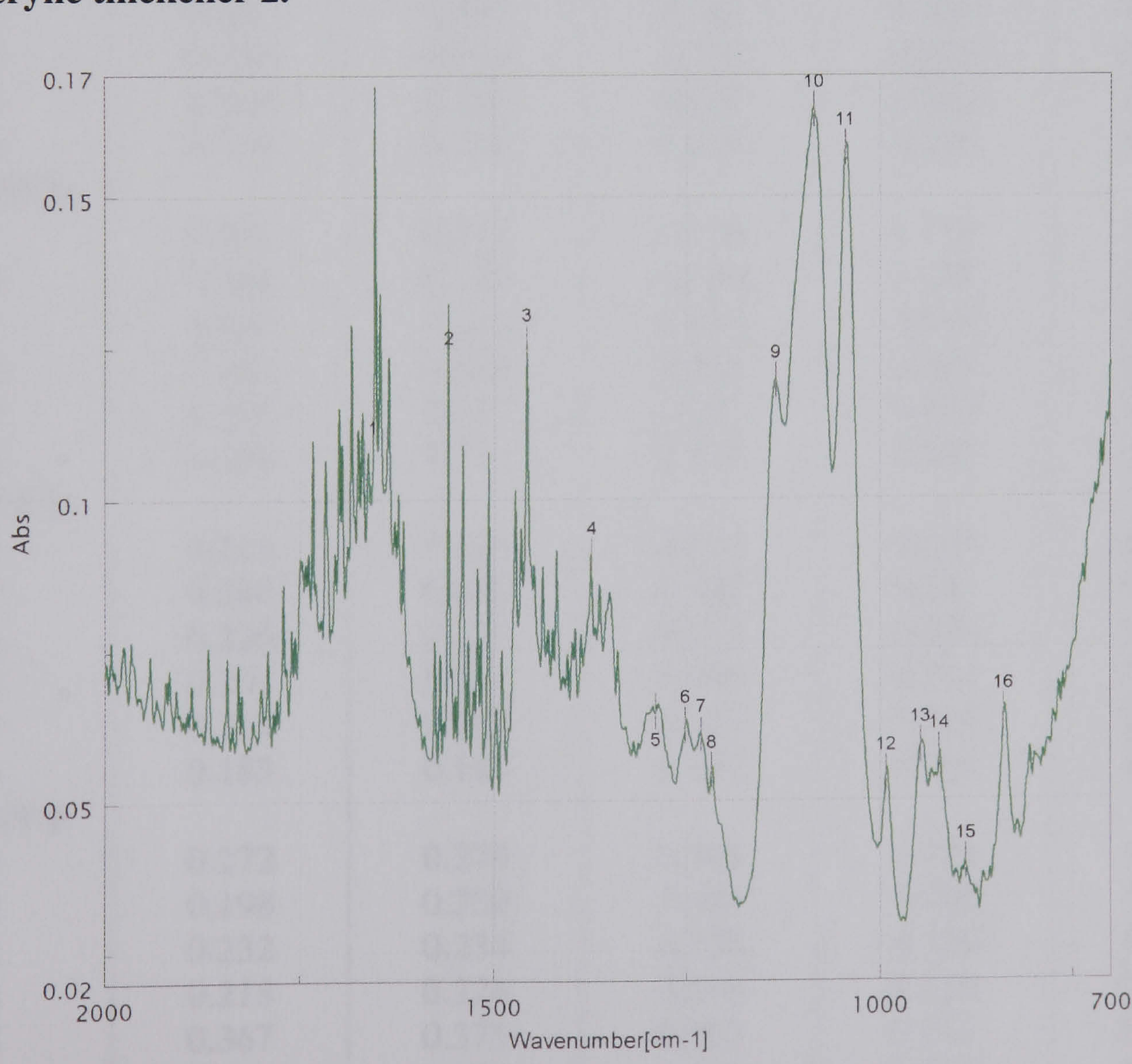
H:6 Defoamer



H:7 Acrylic thickener 1.



H:8 Acrylic thickener 2.



APPENDIX I: Results of the deteriogenic potentials of selected microorganisms experiments

I:1 The control – Paint films in moist vermiculite.

Paint film type and no. Dates	Weight (g) 04/06/01	05/07/01	07/08/01	08/09/01	05/11/01
PAINT A					
1	0.121	0.122	0.122	0.122	0.120
2	0.133	0.133	0.134	0.134	0.132
3	0.168	0.179	0.171	0.171	0.166
4	0.182	0.192	0.182	0.183	0.180
6	0.137	0.139	0.139	0.137	0.136
7	0.155	0.160	0.156	0.156	0.150
PAINT B					
1	0.178	0.180	0.178	0.180	0.179
2	0.184	0.188	0.182	0.185	0.183
3	0.238	0.238	0.239	0.240	0.236
4	0.240	0.245	0.239	0.241	0.236
5	0.191	0.193	0.191	0.192	0.191
6	0.232	0.243	0.236	0.236	0.233
PAINT C					
1	0.241	0.271	0.247	0.247	0.238
2	0.206	0.222	0.208	0.210	0.203
3	0.163	0.178	0.163	0.163	0.159
4	0.226	0.246	0.227	0.228	0.223
5	0.204	0.244	0.204	0.206	0.201
6	0.188	0.225	0.188	0.189	0.185
PAINT D					
1	0.158	0.177	0.158	0.158	0.160
2	0.148	0.170	0.148	0.148	0.150
3	0.194	0.238	0.192	0.190	0.189
4	0.168	0.199	0.168	0.168	0.167
5	0.135	0.150	0.135	0.135	0.137
6	0.184	0.228	0.185	0.185	0.185
PAINT E					
1	0.213	0.214	0.214	0.214	0.212
2	0.240	0.242	0.242	0.241	0.239
3	0.226	0.227	0.225	0.228	0.226
4	0.201	0.202	0.200	0.202	0.199
5	0.217	0.222	0.217	0.218	0.217
6	0.183	0.187	0.182	0.182	0.180
PAINT F					
1	0.272	0.279	0.269	0.273	0.273
2	0.198	0.200	0.198	0.198	0.196
3	0.232	0.234	0.232	0.232	0.229
4	0.218	0.224	0.218	0.219	0.216
5	0.367	0.375	0.365	0.366	0.366
6	0.246	0.252	0.246	0.252	0.246

I:2 Paint films inoculated with *Aureobasidium pullulans*.

Paint film type and no. Dates	04/06/01	05/07/01	Weight (g) 07/08/01	08/09/01	05/11/01
PAINT A					
8	0.186	0.187	0.186	0.186	0.187
9	0.202	0.214	0.204	0.204	0.202
10	0.192	0.190	0.192	0.193	0.193
11	0.212	0.211	0.212	0.213	0.214
12	0.135	0.132	0.135	0.134	0.134
13	0.226	0.226	0.226	0.226	0.227
PAINT B					
7	0.231	0.234	0.233	0.235	0.323
8	0.182	0.184	0.183	0.185	0.185
9	0.211	0.214	0.212	0.215	0.218
10	0.213	0.214	0.211	0.219	0.216
11	0.168	0.169	0.169	0.169	0.168
12	0.246	0.247	0.247	0.249	0.247
PAINT C					
7	0.199	0.200	0.196	0.199	0.200
8	0.180	0.184	0.178	0.184	0.182
9	0.185	0.187	0.185	0.187	0.187
10	0.164	0.163	0.162	0.165	0.165
11	0.161	0.161	0.160	0.162	0.164
12	0.123	0.123	0.122	0.122	0.124
PAINT D					
7	0.189	0.200	0.196	0.194	0.193
8	0.172	0.178	0.175	0.178	0.174
9	0.172	0.180	0.173	0.172	0.174
10	0.163	0.168	0.165	0.164	0.164
11	0.176	0.179	0.176	0.175	0.175
12	0.162	0.165	0.165	0.164	0.163
PAINT E					
7	0.267	0.269	0.267	0.268	0.268
8	0.237	0.238	0.237	0.237	0.237
9	0.209	0.209	0.209	0.209	0.209
10	0.310	0.309	0.310	0.310	0.310
11	0.255	0.254	0.254	0.254	0.254
12	0.251	0.251	0.250	0.250	0.251
PAINT F					
7	0.263	0.266	0.264	0.264	0.264
8	0.303	0.306	0.303	0.303	0.303
9	0.302	0.303	0.302	0.302	0.302
10	0.320	0.324	0.324	0.324	0.323
11	0.320	0.323	0.322	0.322	0.321
12	0.276	0.277	0.276	0.275	0.274

I:3 Paint films inoculated with *Aspergillus fumigatus*.

Paint film type and no. Dates	04/06/01	05/07/01	Weight (g) 07/08/01	08/09/01	05/11/01
PAINT A					
14	0.203	0.203	0.200	0.200	0.198
15	0.175	0.179	0.176	0.175	0.168
16	0.178	0.208	0.203	0.202	0.194
17	0.157	0.157	0.155	0.155	0.151
18	0.181	0.183	0.180	0.180	0.179
19	0.172	0.177	0.174	0.172	0.168
PAINT B					
13	0.238	0.242	0.240	0.240	0.240
14	0.199	0.201	0.200	0.200	0.200
15	0.191	0.192	0.192	0.192	0.193
16	0.343	0.345	0.344	0.345	0.347
17	0.249	0.254	0.250	0.250	0.252
18	0.209	0.213	0.212	0.212	0.210
PAINT C					
13	0.130	0.134	0.128	0.130	0.136
14	0.147	0.156	0.146	0.147	0.150
15	0.192	0.215	0.189	0.192	0.199
16	0.162	0.183	0.167	0.167	0.167
17	0.172	0.194	0.173	0.175	0.187
18	0.154	0.164	0.154	0.154	0.155
PAINT D					
13	0.142	0.153	0.144	0.145	0.153
14	0.181	0.206	0.181	0.189	0.190
15	0.190	0.196	0.189	0.190	0.197
16	0.156	0.165	0.158	0.160	0.162
17	0.145	0.172	0.146	0.146	0.147
18	0.194	0.232	0.197	0.197	0.198
PAINT E					
13	0.174	0.176	0.172	0.174	0.176
14	0.174	0.184	0.173	0.174	0.178
15	0.160	0.178	0.160	0.160	0.163
16	0.230	0.238	0.229	0.300	0.236
17	0.208	0.218	0.209	0.209	0.209
18	0.261	0.271	0.262	0.262	0.264
PAINT F					
13	0.151	0.151	0.151	0.152	0.153
14	0.357	0.367	0.359	0.359	0.365
15	0.169	0.176	0.171	0.171	0.172
16	0.202	0.216	0.202	0.202	0.202
17	0.310	0.330	0.310	0.312	0.316
18	0.160	0.170	0.161	0.162	0.169

I:4 Paint films inoculated with the air propellant only.

Paint film type and no. Dates	Weight (g) 04/06/01	05/07/01	07/08/01	08/09/01	05/11/01
PAINT A					
20	0.243	0.245	0.245	0.242	0.241
21	0.210	0.210	0.210	0.208	0.206
22	0.280	0.280	0.281	0.280	0.276
PAINT B					
19	0.196	0.196	0.198	0.198	0.195
20	0.196	0.197	0.200	0.201	0.197
43	0.288	0.288	0.290	0.289	0.285
PAINT C					
37	0.186	0.189	0.188	0.184	0.182
50	0.128	0.130	0.129	0.127	0.125
60	0.202	0.203	0.204	0.203	0.198
PAINT D					
41	0.158	0.173	0.160	0.157	0.157
42	0.150	0.156	0.156	0.150	0.150
44	0.126	0.125	0.127	0.127	0.126
PAINT E					
36	0.170	0.176	0.180	0.180	0.178
37	0.227	0.227	0.229	0.230	0.228
38	0.200	0.200	0.200	0.200	0.197
PAINT F					
19	0.184	0.183	0.183	0.184	0.178
20	0.301	0.300	0.300	0.300	0.298
43	0.231	0.231	0.321	0.231	0.229

I:5 Paint films inoculated with *Rhodotorula rubra.*

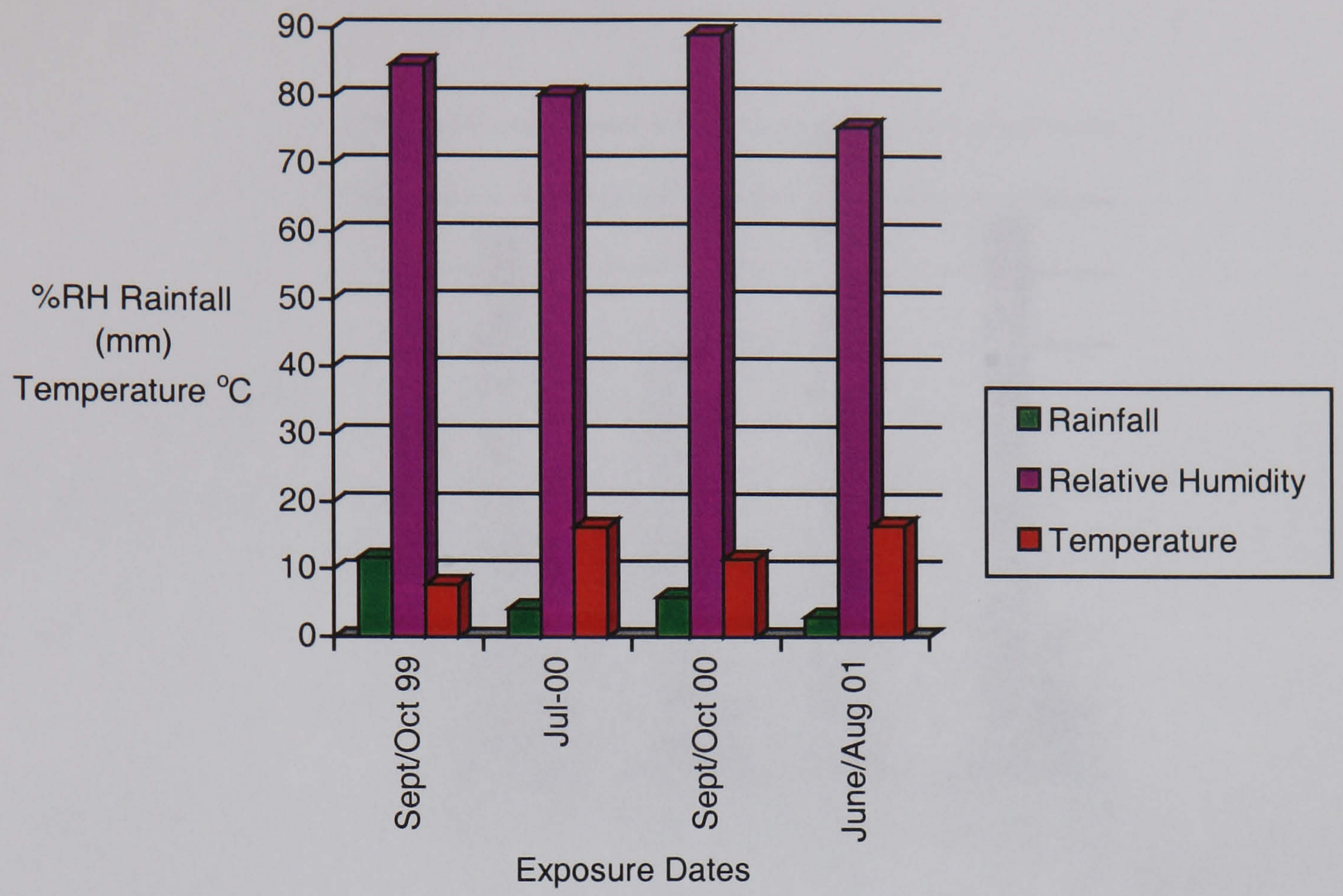
Paint film type and no. Dates	04/06/01	05/07/01	Weight (g) 07/08/01	08/09/01	05/11/01
PAINT A					
23	0.150	0.149	0.142	-	0.148
24	0.173	0.174	0.172	-	0.173
25	0.138	0.141	0.138	-	0.140
26	0.164	0.164	0.164	-	0.165
27	0.240	0.243	0.239	-	0.245
28	0.161	0.161	0.160	-	0.164
PAINT B					
21	0.238	0.240	0.238	-	0.230
22	0.219	0.233	0.218	-	0.224
23	0.191	0.192	0.190	-	0.193
24	0.199	0.202	0.197	-	0.201
25	0.198	0.202	0.198	-	0.202
26	0.230	0.232	0.230	-	0.235
PAINT C					
44	0.155	0.155	0.156	-	0.156
52	0.186	0.182	0.184	-	0.184
31	0.183	0.183	0.183	-	0.187
58	0.170	0.170	0.170	-	0.173
21	0.168	0.166	0.169	-	0.170
47	0.123	0.125	0.128	-	0.130
PAINT D					
20	0.152	0.154	0.151	0.150	0.150
21	0.179	0.181	0.179	0.179	0.181
22	0.165	0.167	0.163	0.164	0.165
23	0.190	0.197	0.193	0.196	0.197
24	0.135	0.141	0.134	0.134	0.134
25	0.151	0.156	0.149	0.149	0.149
PAINT E					
20	0.134	0.135	0.134	Contaminated	Contaminated
21	0.290	0.290	0.290	Contaminated	Contaminated
22	0.242	0.242	0.242	Contaminated	Contaminated
23	0.259	0.260	0.258	Contaminated	Contaminated
24	0.265	0.266	0.266	Contaminated	Contaminated
25	0.305	0.306	0.303	Contaminated	Contaminated
PAINT F					
21	0.251	0.254	0.253	Contaminated	Contaminated
22	0.241	0.245	0.242	Contaminated	Contaminated
23	0.236	0.238	0.236	Contaminated	Contaminated
24	0.232	0.237	0.236	Contaminated	Contaminated
25	0.189	0.191	0.190	Contaminated	Contaminated
26	0.174	0.175	0.172	Contaminated	Contaminated

- represents the paint films that were not re-weighed at this time.

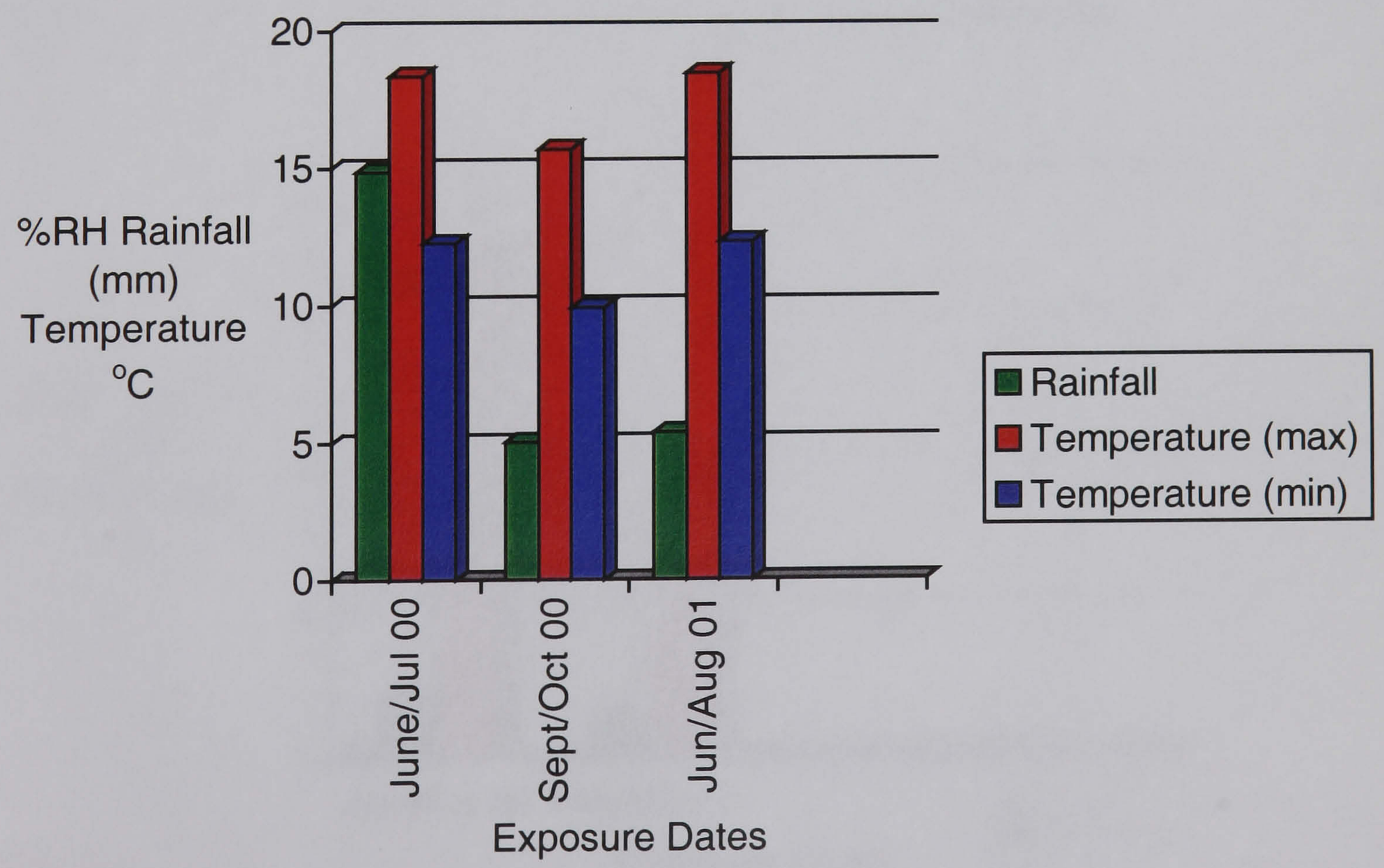
I:6 Paint films inoculated with *Rhodotorula mucilaginosa.*

Paint film type and no. Dates	04/06/01	05/07/01	Weight (g) 07/08/01	08/09/01	05/11/01
PAINT A					
29	0.190	0.196	0.190	Contaminated	Contaminated
30	0.255	0.270	0.255	Contaminated	Contaminated
31	0.243	0.261	0.244	Contaminated	Contaminated
32	0.205	0.218	0.207	Contaminated	Contaminated
33	0.233	0.244	0.236	Contaminated	Contaminated
34	0.230	0.235	0.230	Contaminated	Contaminated
PAINT B					
27	0.192	0.201	0.196	Contaminated	Contaminated
28	0.212	0.217	0.213	Contaminated	Contaminated
29	0.252	0.259	0.252	Contaminated	Contaminated
31	0.206	0.214	0.210	Contaminated	Contaminated
32	0.167	0.169	0.168	Contaminated	Contaminated
33	0.236	0.191	0.192	Contaminated	Contaminated
PAINT C					
22	0.206	0.213	0.206	Contaminated	Contaminated
53	0.168	0.172	0.169	Contaminated	Contaminated
33	0.174	0.183	0.175	Contaminated	Contaminated
39	0.237	0.267	0.237	Contaminated	Contaminated
23	0.213	0.227	0.214	Contaminated	Contaminated
24	0.184	0.194	0.186	Contaminated	Contaminated
PAINT D					
26	0.188	0.203	0.191	Contaminated	Contaminated
27	0.174	0.203	0.174	Contaminated	Contaminated
28	0.159	0.167	0.160	Contaminated	Contaminated
29	0.177	0.199	0.178	Contaminated	Contaminated
30	0.156	0.171	0.157	Contaminated	Contaminated
31	0.184	0.195	0.185	Contaminated	Contaminated
PAINT E					
26	0.256	0.265	0.260	Contaminated	Contaminated
27	0.197	0.199	0.197	Contaminated	Contaminated
28	0.277	0.280	0.278	Contaminated	Contaminated
29	0.297	0.302	0.298	Contaminated	Contaminated
30	0.336	0.342	0.336	Contaminated	Contaminated
31	0.250	0.252	0.250	Contaminated	Contaminated
PAINT F					
27	0.176	0.171	0.177	Contaminated	Contaminated
28	0.245	0.247	0.245	Contaminated	Contaminated
29	0.231	0.236	0.231	Contaminated	Contaminated
30	0.263	0.268	0.264	Contaminated	Contaminated
31	0.203	0.205	0.204	Contaminated	Contaminated
32	0.236	0.238	0.238	Contaminated	Contaminated

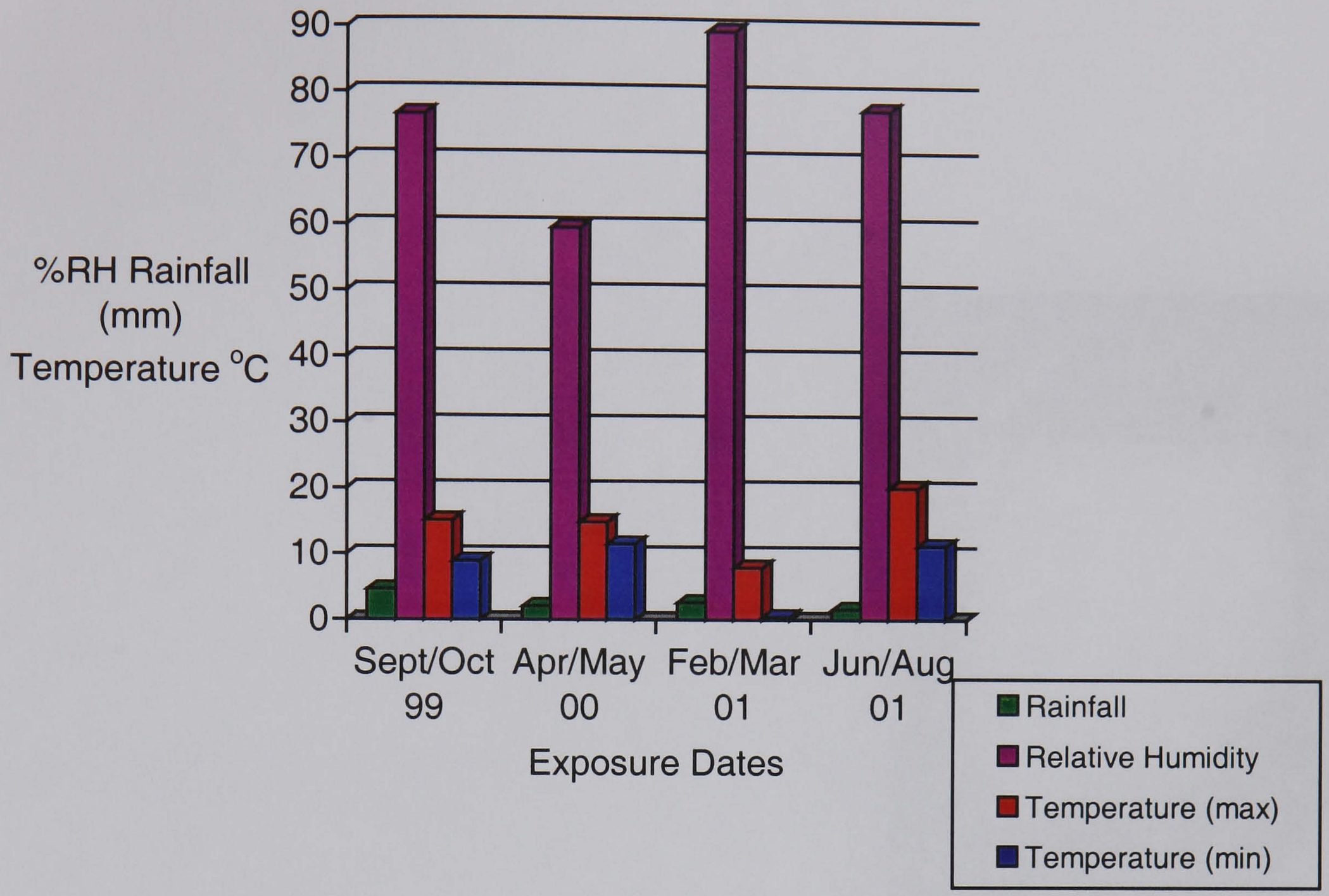
APPENDIX J:1 The weather data from the four exposures at Sandefjord.



APPENDIX J:2 The weather data from the three exposures at Bergen.



APPENDIX J:3 The weather data from the four exposures at Preston.



APPENDIX J:4 The weather data from the two exposures at Blackley