THE UNIVERSITY OF ASTON IN BIRMINGHAM

# THE ECOLOGY OF PAPER MILL BY-PRODUCT AND ITS EVALUATION AS A CASING MEDIUM IN THE CULTURE OF AGARICUS BISPORUS (LANGE) PILAT.

BY

STEPHEN GRENVILLE YEO B.Sc.

Being a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

June 1980

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## SUMMARY

Effluent from pulp and paper production at the Kemsley mill of Bowaters U.K. Paper Company Limited passes through two treatment stages before its discharge into the Swale estuary. Suspended material removed during treatment is deposited on wasteground as a thin sludge. The solids it contains are mainly wood components lost during pulp production, whilst it also has a high salt content, derived from chemicals used in pulping processes. After deposition the sludge undergoes an ageing process during which it dries out and its salt content is reduced. This ageing can be reproduced and accelerated by improved drainage under controlled conditions.

The paper mill sludge was investigated as a casing medium in the culture of Agaricus bisporus (Lange) Pilat, the cultivated mushroom. It was unsuitable up to one year from deposition due largely to the inhibitory effect of its salt content on fruiting. Material eighteen months or more in age gave yields comparable to standard peat casing. Before use as a casing the material must be shredded to a satisfactory structure, neutralised with chalk, and pasteurised to eliminate organisms harmful to the crop. The prepared medium has a high water holding capacity and a structure resilient to management procedures, important requirements of a good casing.

A passive movement of salts from the compost to the casing was shown to occur during culture, capable of enhancing the natural decline in cropping if sufficiently great. The ions chloride, potassium, sodium and sulphate were shown to be responsible, their damaging effects being due to high conductivity created in the casing. Studies of elements available during culture suggested phosphate availability in the compost could limit crop potential, whilst iron released by mycelium of A.bisporus in the casing may be utilised by associated micro-organisms.

## KEY WORDS

PAPER MILL SLUDGE

ECOLOGY

CASING LAYER

AGARICUS BISPORUS

## ACKNOWLEDGEMENTS

I would like to thank Dr. W.A. Hayes for the award of a post-graduate research studentship and his guidance and supervision of the research. Bowaters U.K. Paper Company Limited and Bowaters Technical Services Limited provided samples and invaluable help in the conduct of this research, and their assistance is gratefully acknowledged. My thanks are also due to W. Darlington and Sons Limited, Angmering for conducting commercial-scale culture trials, and Mr. J.H. Williams, Department of Geological Sciences, University of Aston for performing atomic absorption analyses. I am grateful to Dr. Williams of Soil Science, A.D.A.S., Wolverhampton for providing facilities for particle size analysis, and determining moisture retention properties of casing materials. I would like to acknowledge my colleagues at the mushroom science unit, K.J. Jakeman, P.A. Cresswell and M.J. Mosley for their co-operation and useful discussion during my studies. Finally my thanks to M. Whitehouse for typing my thesis.

## DECLARATION

Work described in this thesis is the result of my own investigations except where reference is made to published material or where assistance is acknowledged. This work has not been submitted for any other award.

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June, 1980.

CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF PLATES

## LIST OF APPENDICES

# INTRODUCTION

# INTRODUCTION

The earliest records of paper production date back to over one thousand years ago from the Orient, and seven hundred years ago from Europe. The modern process of paper production originated about one hundred years ago. Since that time the size of manufacturing plants has grown, working methods have improved and a higher level of control in production is now possible. However, the basic process has not undergone any major changes, the de-barked wood being de-fibred by mechanical or chemical means to produce pulp, which is refined and carried in aqueous suspension to the paper machines, where water removal produces the paper sheet.

Water plays a major role in the process of paper production, and nearby water courses receive liquid effluents discharged from mills. The availability of suitable outlets for mill effluents determines the location of pulp and paper mills. No river system in the United Kingdom is capable of receiving the volume of liquid effluent discharged by a pulp and paper mill, and as a result all mills are situated adjacent to the coast. The paper industry is now more acutely aware of the pollution problems created by its disposal of solid and liquid effluents, but faces mounting pressure from government bodies to reduce its pollution load to the environment.

Pollution caused by the pulp and paper industry is of three types, water, air and soil pollution.

The water used for processing and discharged into the receiving waters may contain dissolved organic compounds and chemicals from the pulping process, fibres, fillers and additives, dyes, bark, ash and lime. The dissolved compounds originate mainly in the pulping process while suspended solids are present in effluents from practically every step in the process. A water pollution index based on suspended solids, is used throughout the world as an indicator of polluted conditions. In 1970 the pulp and paper industry discharged a total of 3.8 million tonnes of suspended solids (OECD, 1973). This may represent in some countries as much as 75% of the total discharged by industry. As well as suspended solids, water pollution arises from highly toxic compounds resulting from pulping of the raw materials (Mueller and Walden, 1974) and coloured materials stemming mainly from the lignins of wood.

Atmospheric pollution arising from chemicals emitted by the pulp industry is caused by particulate matter and odorous gases including sulphur dioxide. Sulphur is present in practically all chemical pulping processes and around one third of the sulphur lost in the process is emitted in gaseous form.

Soil pollution is caused by lime sludge, ash, and mud from water treatment plants which are disposed of on waste land. The bark content of wood amounts to between 8% and 13% by weight, and in the pulping industry 70% to 80% of de-barking is undertaken at the mill site. Waste bark therefore poses an additional disposal problem.

Disposal of the large quantities of sludge that result from efforts to reduce the volume of suspended solids being discharged with waste water effluents is an increasingly important problem.

In the United Kingdom the prevention of water pollution is administered by regional river authorities. These have responsibility for all aspects of the hydrological cycle in their respective areas. The standards for effluent control which are generally applied are 30 mg./L. maximum suspended solids and 20 mg./L. maximum B.O.D. (OECD, 1973). However, it is expected that future requirements will be more stringent.

Pulp and paper mills can reduce the polluting load to receiving water courses by minimising the quantities of solid matter and soluble, oxygen-consuming substances in their waste water. The basis for the present research stems from the observations of staff at the Kemsley mill of Bowaters U.K. Limited near Sittingbourne in Kent. It was noted that a variety of fleshy Basidiomycete fungi grew readily on solids separated from mill waste water following their deposition on nearby wasteland. Hayes (1975) in an unpublished report detailed results of laboratory-scale trials with the solids. He utilised samples which had been deposited for varying lengths of time as the base for a medium used for 'capping' or 'casing' mushroom beds in commercial cultivation. In the commercial production of the cultivated mushroom, Agaricus bisporus (Lange) Pilat, a top layer of a suitable material is placed over the colonised compost substrate to induce the fruitbodies to develop. In the United Kingdom the conventional casing material is a mixture of peat and chalk.

Hayes indicated that solids aged for a year or more had physical characteristics similar to peat and supported satisfactory vegetative growth and fruitbody development of A.bisporus.

As a result of these findings the present study was aimed at achieving two objectives. Firstly, to define the gross chemical, physical and biological nature of the solid effluent from the Kemsley mill, and secondly to evaluate the potential of the deposited solids as a replacement for peat in the culture of A.bisporus. In order to achieve these objectives it was necessary not only to understand the factors governing the suitability of the material as a casing medium, but also to obtain more definitive information on the biological, chemical and physical requirements for production of A.bisporus fruitbodies in the casing layer.

The continuous production of a replacement for peat in mushroom culture is particularly relevant to the United Kingdom which imports the majority of its peat from Eire. Peat resources in Eire are diminishing and supplies are becoming increasingly difficult to obtain.

# SECTION 1

# BACKGROUND INFORMATION ON PULP AND PAPER PRODUCTION AND MUSHROOM CULTIVATION

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## 1.1 PULP AND PAPER PRODUCTION.

### 1.1.1 Manufacture of paper products.

The manufacture of paper products from wood can be broadly sub-divided into two stages. The first is the production of pulp from a variety of hard and soft woods, in which cellulose fibres are removed from associated lignins, hemi-cellulose and other wood components, and maintained in aqueous suspension before use in the paper making process.

The production of paper sheet from pulp is the second stage in the operation. This may be carried out at the same mill which produces the pulp, or a condensed pulp may be exported to paper mills in other countries for production of paper sheet. In 1975 the United Kingdom imported 46.5% of the pulp used in its paper mills (Anon, 1977). In making paper sheet from pulp, the fresh or resuspended pulp is diluted to approximately 99% water, refined and passed through several stages of dewatering on conveyors in order to produce base paper.

Generally pulp and paper mills can be classified according to product manufactured and production method used.

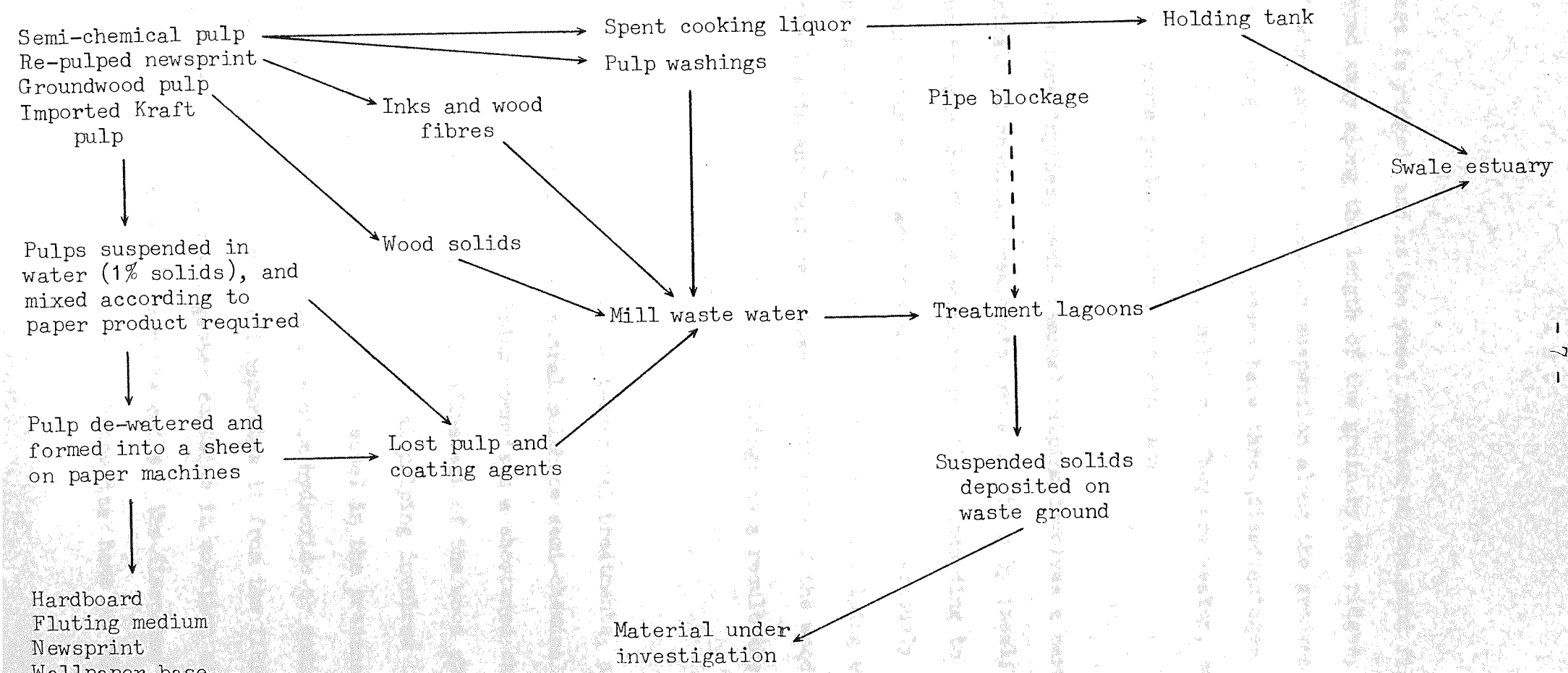
The first distinction can be made between mills which produce pulp and those which produce paper sheet from pulp. Integrated mills combine both operations. Pulp mills are classified according to the method used for pulp manufacture, viz. mechanical, chemical or semi-chemical, with several sub-divisions of each. Paper mills are categorized according to product manufactured, which may vary from low quality newsprint sheet and chipboards to the highest quality, coated writing papers.

### 1.1.2 Pulp and paper production at Kemsley Mill, Sittingbourne.

The Bowaters U.K. mill at Kemsley is an integrated mill which produces pulp by mechanical and semi-chemical processes, together with a de-inking plant for the re-pulping of waste newsprint. Bleached, Kraft (chemical) pulp is imported from Scandinavia for the production of high quality papers. The pulps supply paper machines which manufacture a variety of paper products. Pulp and paper production at Kemsley is summarised in Figure 1.1 (overleaf).

The term mechanical pulping embraces some of the oldest pulping methods, as well as more up to date processes such as thermo-mechanical pulping which are the result of improved technology and are likely to undergo an increase in usage. At Kemsley mechanical pulp is produced by the groundwood process. Here a variety of local hardwood and some softwood logs, previously de-barked, are lowered in wire cages onto a large granite wheel which is impregnated on the surface with carborundum.

FIGURE 1.1 PULP AND PAPER PRODUCTION AT KEMSLEY SHOWING ORIGINS OF MILL EFFLUENTS.



The surface is ridged, and as the wheel rotates so the wood fibres are stripped away along the length of the grain by the ridges, the fibres flowing away to an aqueous suspension along the grooves. Pulps made from groundwood processes have three disadvantages when compared with those from chemical pulping. They are weaker, coarser, and tend to become yellow after a certain time.

As the name implies, semi-chemical pulping involves a series of mechanical and chemical treatments, none of which by itself is sufficient to make the fibres separate readily. According to the order and importance of each treatment such pulp is variously termed semi-chemical, chemi-groundwood etc.. Semi-chemical pulping enables more of the lignin and hemi-cellulose constituents of the wood to be retained in the pulp than in chemical pulping; as a result the pulp is often termed 'high-yield' pulp.

One of the more commonly used semi-chemical treatments, and the one employed at Kemsley, is the neutral sulphite semi-chemical pulping process (NSSC). NSSC pulping comprises a shortened chemical pulping stage followed by mechanical refinement of the wood chips to complete the fibre separation. Chemical pulping involves the cooking of wood chips in a high pressure vessel in the presence of a suitable cooking liquor (usually sodium hydroxide or sodium sulphite). This process softens the lignin and separates it from the fibre. As a result the liquor leaving the digester contains in solution almost all of the non-cellulosic material from the chips. The dissolved lignins give the spent liquor a dark colour and hence its name of black liquor.

In NSSC pulping the cooking materials are sodium sulphite, with sodium carbonate as a neutral buffer. This cooking liquor is highly specific for lignin. The cooking time is usually four hours at a pressure of 100 to 120 p.s.i.. Following this treatment the liquor is drained from the digester and the chips pass to mechanical refiners to separate the fibres from the pulp.

Processes for recovery of chemicals lost via the black liquor exist for chemical pulping systems. In the NSSC system the heat content of the liquor is too low to make this recovery economically viable. Thus the waste liquor at Kemsley represents a major pollution hazard owing to its extremely high B.O.D. value, frequently around 20,000.

The de-inking plant utilises spent newsprint which is re-pulped mechanically following its de-inking treatment. The pulp produced is of a fairly low quality because the cellulose fibres lose some of their strength on re-pulping.

Though a variety of products are manufactured at Kemsley, whatever the finished material, the change from raw pulp to end product is essentially the same. The starting pulp is suspended in water at approximately 1% solids. It is refined several times to remove grit and dirt and then forced at high pressure through a long, narrow slit across vacuum drying units which reduce the moisture content to about 88%.

At this stage the fibres will bind together as a thin sheet and are picked up on a receiving conveyor belt. This belt moves over large bronze rollers some of which remove moisture by compression. More moisture is removed by transfer through a heated chamber, and the formed, dried sheet is collected on a roller running against the last roller on the paper making machine.

Depending upon the requirements of the finished product the sheet may pass through coating stages as well before it is dispatched. Coating agents can be pigments or resins. In pigment coating which is the method adopted at Kemsley, the paper is coated with a mixture of pigment and adhesive. Pigments used at Kemsley are Kaolin (china clay) and calcium carbonate, whilst starch and sizes serve as adhesives.

### 1.1.3 Effluents from pulp and paper manufacture at the Kemsley mill.

The major wastes from mechanical pulping at Kemsley are suspended solids in the form of wood fibres and associated wood components. Effluent from mechanical pulping processes contain relatively little in the way of dissolved substances capable of depleting oxygen levels (Cawley and Minch, 1963).

As with mechanical pulping, the effluents from paper making processes contribute relatively small quantities to B.O.D. loads, compared with chemical pulping. At Kemsley, the waste from paper production is the solids lost in the process.

It is estimated that 2% of the pulp that is supplied to the machines is lost in the system and appears in the effluent. A proportion of the agents used in paper coating (kaolin, chalk, starch and sizes), is similarly lost during paper manufacture.

Effluent from the semi-chemical pulping plant at Kemsley is the spent cooking or sulphite liquor. This contains the chemicals used in pulping, the dissolved wood components, and those fibres lost in the refining stage.

The organics which are dissolved in the spent liquor from chemical and semi-chemical pulping give the effluent very high B.O.D. values. One of the major components of semi-chemical waste is the lignosulphonates formed during the chemical extraction of the wood. White rot fungi which are capable of degrading protolignin in wood cannot utilise lignosulphonates (Shibamato, Fukuzumi, Mikawa and Hayashi, 1960), and the compounds degrade slowly in water courses, depleting oxygen reserves.

As most semi-chemical plants have no systems for recovery of chemicals from their waste, the B.O.D. load to receiving waters can be very high.

### 1.1.4 Treatment and disposal of mill effluents at Kemsley.

The effluent from semi-chemical pulping at Kemsley undergoes no treatment before its disposal.

The waste liquor is pumped directly to a holding tank, and is discharged for two hours into the Swale estuary at high tide.

The remainder of the mill's liquid effluent passes through two treatment stages before its discharge into the Swale. This effluent comprises the mill waste water, containing the effluents from groundwood pulping, de-inking and paper production. Though the spent pulping liquor is normally kept separate from the remainder of the mill waste, a small proportion is washed into it during refinement of the semi-chemical pulp. Also, when the pipe carrying the spent liquor to the holding tank becomes overloaded or blocked, an overflow pipe carries the liquor into the mill waste water.

The mill waste water passes through three treatment lagoons where its content of suspended solids and oxygen depleting substances is reduced before its discharge into the Swale. Figure 1.2 shows the layout of the treatment site at Kemsley, and the adjoining wasteland where solids removed from the mill effluent are deposited.

About six million gallons of liquid effluent are pumped daily into the primary treatment lagoon, which is shown in plate 1.1. In this lagoon suspended solids settle out from the effluent, as well as floating to the surface of the lagoon where they form a crust (see plate 1.2). The solids are removed daily by dredging and are deposited as a thin sludge on the adjoining wasteland as depicted in plate 1.3.

FIGURE 1.2 THE EFFLUENT TREATMENT AND PMS DEPOSITION SITE ADJOINING THE KEMSLEY MILL.

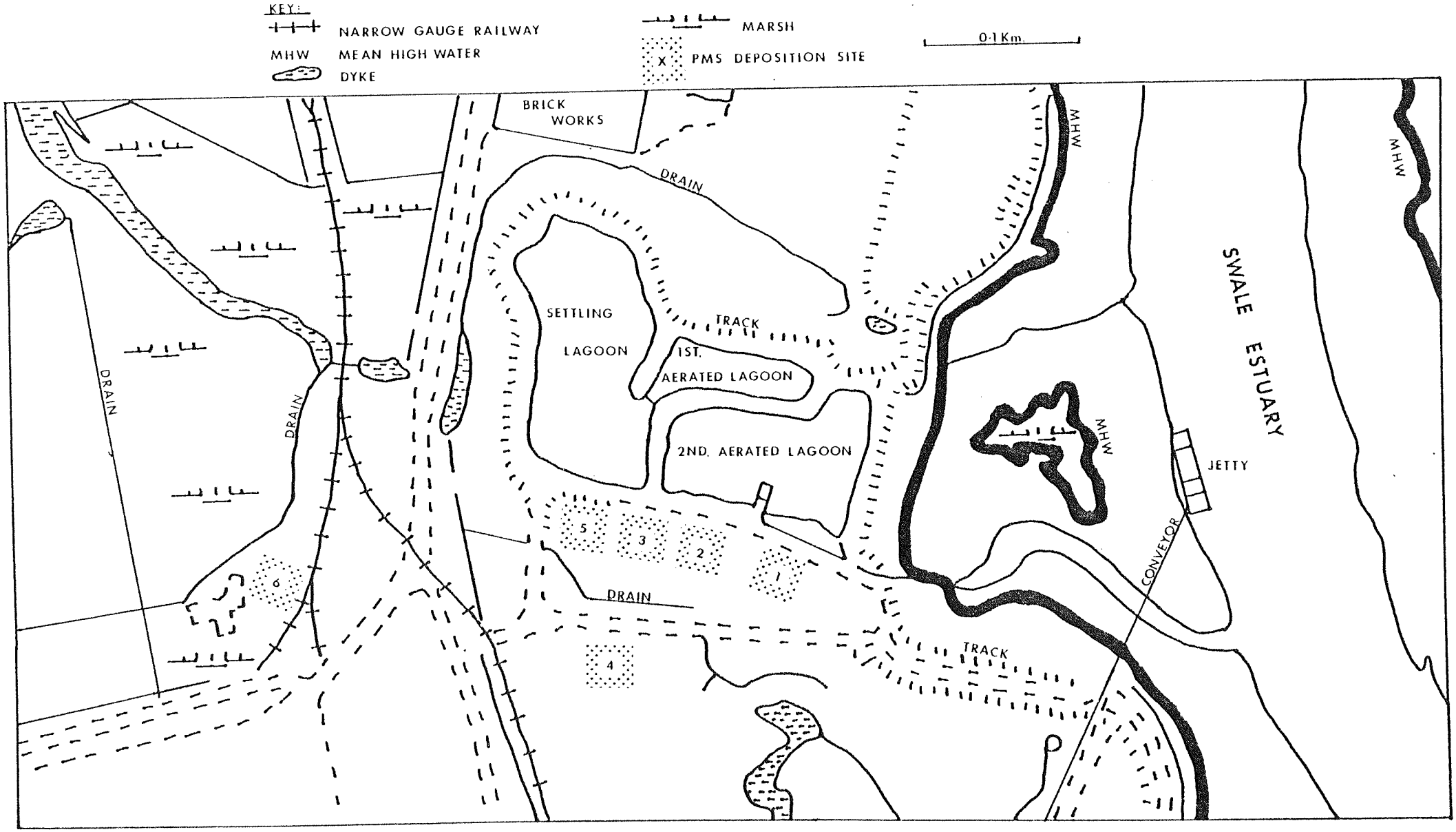


PLATE 1.1 THE PRIMARY TREATMENT OR SETTLING LAGOON.

PLATE 1.2 FORMATION OF A CRUST OF SOLID WASTE ON THE SETTLING LAGOON SURFACE.

PLATE 1.3 DEPOSITION OF DREDGED SLUDGE ON WASTELAND.

PLATE 1.4 SECONDARY TREATMENT OF THE MILL EFFLUENT IN ONE OF THE AERATED LAGOONS.

The liquid effluent, still containing some solids, flows across the primary treatment lagoon and passes through two aerated lagoons where its B.O.D. is reduced by biological oxidation of dissolved organics. Plate 1.4 shows effluent under treatment in one of the aerated lagoons. The treated waste is discharged continuously from the second aerated lagoon into the Swale estuary.

The suspended solids which are removed from the effluent in the first lagoon is the material under investigation in this study (see figure 1.1).

### 1.1.5 Possible means of utilising pulp and paper mill sludge.

Most paper mill sludges are thin, aqueous suspensions of waste materials such as pulp screenings and fibres, broke washings, clays, dyestuffs and inks. Their makeup depends on the type of mill and the products made. Sludges are hard to de-water mechanically because of the hydrous nature of the pulp fibres they contain, and following de-watering the sludge may still have a consistency of only 15% - 50% solids (NCASI, 1969). Disposal of sludge by combustion is therefore seldom convenient or economical and is not widespread (Coogan and Stoval , 1965; NCASI, 1969). Where it is practiced auxillary fuel is often needed to dry the sludge cake and maintain good combustion, while clinker from clays causes fouling of furnace grates (Aspitarte, Rosenfeld, Smale and Amberg, 1973).

Most mills practice landfill disposal of sludge. However, it is becoming increasingly difficult to obtain and maintain ample sites for disposal (Harkin, Crawford and McCoy, 1974).

Problems such as odour, and questions of soil stability and land use induce regulatory bodies to request that companies discontinue disposal even on available sites. There is a need therefore for new and effective means of sludge disposal, and numerous methods have been investigated.

Aspitarte et al. (1973) and Dolar, Boyle and Keeney (1972) investigated incorporation of sludge into soil as an amendment. Aspitarte et al. showed incorporation of sludge at low levels (100 - 200 tons/acre) gave satisfactory corn and bean yields provided sufficient nitrogen was added. A high level of incorporation (600 tons/acre) required a year of fallow preceding crop planting. Dolar et al. investigated yield and mineral nutrition of oats, Avena sativa, on soils with sludge from a variety of sources added at 2.5% and 10%. At the high incorporation rate, plant growth was diminished even with sufficient fertiliser application. Lower addition rates gave satisfactory results, though sludges with high C : N ratios required nitrogen supplementation.

Aspitarte et al. investigated hydromulching of sludge for soil stabilisation. Mulch is a slurry generally used for grass planting. They found hydromulches of sludge or a sludge and bark mixture were equivalent to the wood fibre product control. Sludge/perlite and sludge/vermiculite mixtures proved to be satisfactory potting media for containerised production of several plant species.

Aspitarte et al. showed dried sludge to be a useful absorbing material for oil-spill clean up and to have application as a bedding material for cattle.

They also showed sludge was not toxic to ruminants when used as a feed supplement up to a level of 15%, though it had a low feeding value. Millet, Baker, Satter, McGovern and Dinius (1973) found rumen digestibilities of 45% - 60% and exceptionally 90% for fibrous residues generated during pulp manufacture.

Söderhjelm (1976) reported on incorporation of sludge into grass silage processes, finding it useful as a binder. Aspitarte et al., however, found sludge alone was difficult to break down compared with regular feed ensiling materials.

Söderhjelm (1976) suggested production of the sweetener xylitol from hardwood sludge may be possible. He also reported on the successful incorporation of sludge into bricks, cement tiles and plastic products. Kirsten and Porschmann (1978) described the utilisation of pulp and paper sludge in the brickwork industry.

Despite this wide range of possibilities, no satisfactory solution has materialised to the general problem of sludge disposal. The observations of Hayes (1975) are the first report of its possible utilisation in mushroom cultivation.

## 1.2 MUSHROOM CULTIVATION.

### 1.2.1 Origin and spread of mushroom culture.

Artificial culture of Agaricus mushrooms originated in France around 1650 when it was noted these mushrooms were frequently obtained on discarded compost which had been used for melon crops. The first guide to mushroom growing was written in 1707 by the French botanist J.P. Tournefort, and he is also credited as the first user of a top layer or casing soil in mushroom culture.

In the 18th and 19th centuries mushroom cultivation was established in North America, Great Britain and other West European countries. In the early part of this century cultivation increased steadily, though there was a decline at the time of the second world war. Thereafter mushroom cultivation spread worldwide and production has increased tremendously as culture techniques have improved. Following World War II mushroom cultivation expanded in Western Europe and the U.S.A.. Though cultivation of Agaricus bisporus was introduced to Japan in 1910 (Kim, 1978), establishment of mushroom growing in Asia, as well as Eastern Europe and New Zealand was achieved between 1950 and 1960 (Hayes, 1978). Between 1960 and 1970 mushroom growing underwent an expansion in most producing countries, South American countries established small industries, and in Asia, Korea, Japan and Taiwan were established as major producers. Since 1970, African countries, China, Malaysia, India and Pakistan have all established small industries.

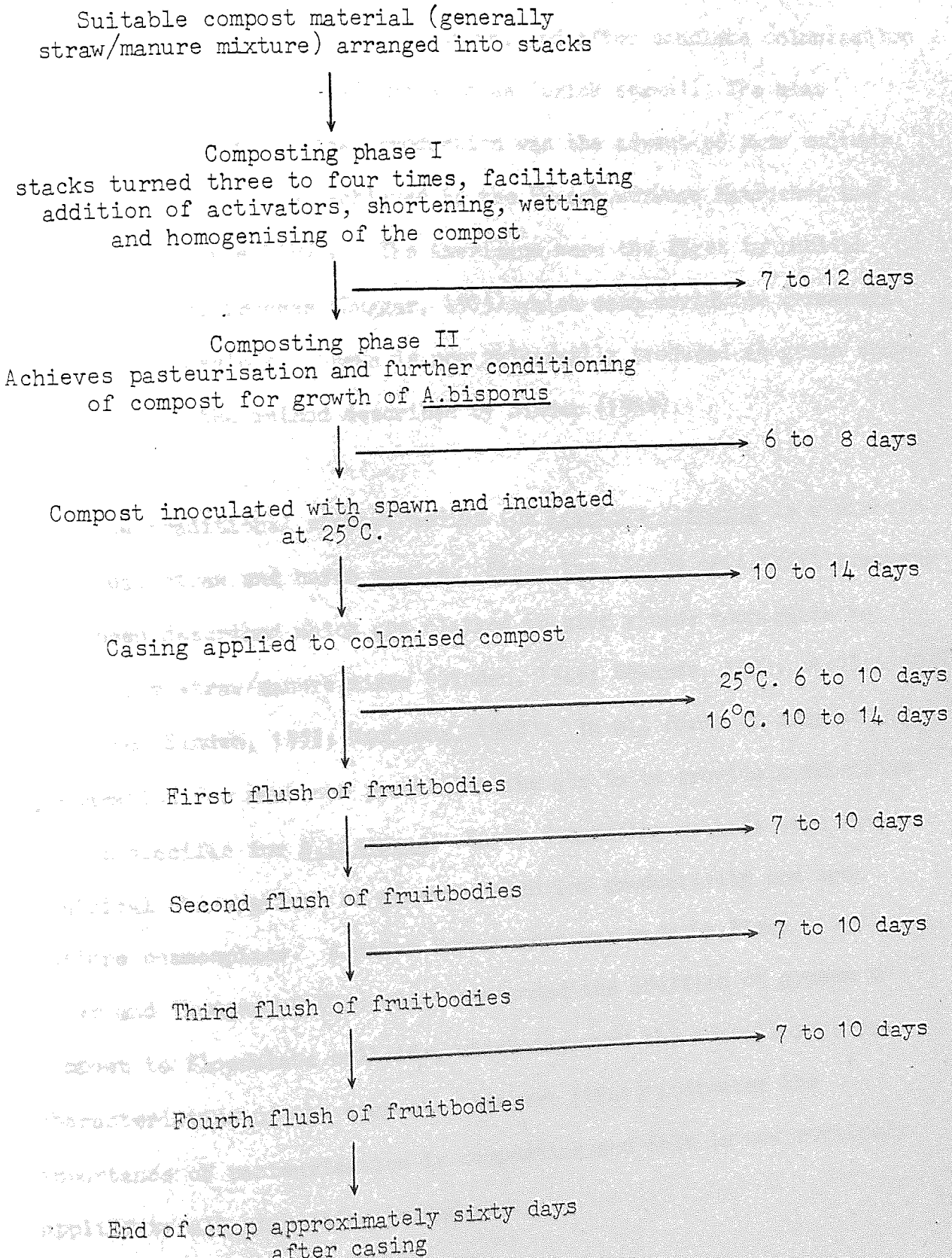
It was estimated by Delcaire (1978) that world production of fresh Agaricus mushrooms in 1978 was approximately 800,000 tons.

## 1.2.2 Culture systems and developments in mushroom production.

Modern day cultivation of Agaricus bisporus involves the preparation of a suitable substrate or compost, the inoculation of the prepared compost with mycelium grown axenically on cereal grain and termed spawn, followed by an incubation period in which the compost is colonised by the growing mycelium. A top layer of soil, peat or another suitable material is then placed over the compost surface and this casing layer in combination with environmental control, induces the switch from vegetative to reproductive growth, and the production of fruitbodies. Figure 1.3 (overleaf) outlines the procedures in mushroom production. Tournefort (1707) is credited as the first user of a casing layer in mushroom culture, and since his early writings many advances have been made in the artificial production of the cultivated mushroom.

The most important advances have been made in the use and preparation of raw materials. Originally, mushroom spawn was prepared from soil where wild mushrooms would grow, and was termed 'virgin spawn'. After the beds were fully penetrated by mycelium from the soil, and when the compost had failed to yield further mushrooms, the compost was dried or used fresh to inoculate new beds; this spawn was known as 'flake spawn'.

FIGURE 1.3 OUTLINE OF PROCEDURES IN MUSHROOM CULTURE.



In England another method of spawn production became popular. A mass of horse and cow manure and loam was mixed with water, turned out into a layer two inches thick, and cut into pieces. When half-dry these pieces were inoculated with old spawn, and after complete colonisation the entire piece was dried and sold as 'brick spawn'. The most important advance in spawn production was the advent of pure culture spawn. This was first achieved by the French workers Matruchot and Constantin in the 1890's. The Americans were the first to publish details of the process (Duggar, 1905) which made worldwide commercial production possible. Spawn is now universally produced as grain spawn according to the method described by Sinden (1932).

The traditional compost medium for Agaricus bisporus is a mixture of cereal straw and horse manure. Since the 1940's artificial composts have been described which are claimed to give yields comparable to those from straw/manure mixes (Sinden, 1946; Edwards, 1950; Stoller 1953; Yoder and Sinden, 1953; MacCanna, 1969). In all forms of compost preparation for mushroom production the aim is to provide a nutritive medium specific for A.bisporus. Early composting methods were based on empirical observations of growers, with low productivity and crop failure commonplace. A major improvement was made by Pizer (1937) and Pizer and Thompson (1938) who recommended the addition of gypsum to compost to flocculate colloids, which improved the overall physical characteristics of the compost. Lambert (1941) indicated the importance of pasteurisation in composting and this is now routinely applied to all composting procedures.

A good degree of control over the composting process was introduced by the work of Sinden and Hauser (1950, 1953) in the short composting procedure, and the long method described by Rasumssen (1963).

Until the middle of the present century a variety of soils were used as casing materials in mushroom culture. The type and nature of the soil were found by experience to greatly influence management practices, and soils were shown to be a common source of the pests and pathogens to which the mushroom crop is susceptible. The advent of soil pasteurisation around the turn of the century was a major step forward. The work of Bewley (1938, 1948) and Stoller (1952c) led to Edwards and Flegg demonstrating that fibrous peat was a suitable casing medium, Edwards (1954b). Since then peat neutralised with chalk has been adopted as a casing in much of Europe and recently in the U.S.A., owing to its uniformity and relative freedom from disease and pest organisms.

The mushroom was originally an outdoor crop unprotected from the changes of climate. The current methods for mushroom production, cave culture and mushroom house culture, have given control over the environment and greatly improved growing conditions.

In the United Kingdom the first serious text on mushroom culture was that of Abercombie two hundred years ago. He wrote that great quantities of mushrooms were raised for the markets in glasshouses, barns and in ridge beds in the open. Even as late as 1930 the majority of mushrooms in England were still grown in the open or on the floor of glasshouses.

In 1934 Henry and Herman Knaust in New York pioneered the two-zone tray system when they described pasteurisation in wooden trays in one house, and cropping of mushrooms in the same tray in another house. The system was introduced to England in 1947 by Sinden. Nowadays the majority of mushrooms in the U.K. are produced by the tray system, though cultivation in disposable containers such as plastic sacks has gained support in recent years.

General descriptions of mushroom culture from Tournefort's work to the present day are given in: Tournefort (1707), Abercombie (1817), Callow (1831), Robinson (1870, 1891), LaChaume (1882), Duggar (1905), Falconner (1910), Lambert (1938), Bewley and Harnett (1938), Monro (1948), Rettew and Thompson (1948), Kligman (1950), Stoller (1954), Sarazin (1955), Singer (1961), Atkins (1972, 1974), Hayes and Nair (1975), Chang and Hayes (eds., 1978).

### 1.2.3 Cultural practice from the time of casing.

Following casing the mycelium of A.bisporus will start to colonise the casing soil from the underlying compost. The air temperature in the house is maintained at 25°C., the same as for development in the compost and optimal for vegetative growth. Ventilation is not applied as high carbon dioxide levels favour vegetative development. As soon as the mycelium begins to appear at the casing surface the temperature is lowered to between 13°C. and 16°C. as higher temperatures inhibit 'pinning' or development of fruitbody initials. At the same time fresh air is introduced to reduce the carbon dioxide level in the air above the beds to below 0.1%.

Above this value abnormal or no fruiting will occur. The optimal temperatures and carbon dioxide levels for fruiting vary slightly according to the strain of A.bisporus being grown, but the figures quoted are the generally employed optima. Relative humidity of the air is maintained between 80% and 90% depending on the air flow rate. It is necessary to water the casing to replace that lost by evaporation and to the developing fruitbodies. This commences when necessary after cooling though not when fruitbody initials or pins are present as these are likely to die back if watered.

Cropping of mushrooms proceeds at approximately seven to ten day intervals, the first flush or crop of mushrooms appearing about twenty days after casing. This phenomenon of flushing is characteristic of all strains of A.bisporus.

## SECTION 2

# REVIEW OF LITERATURE RELEVANT TO THE CASING LAYER

## SECTION 2 REVIEW OF LITERATURE RELEVANT TO THE CASING LAYER.

### 2.1 FUNCTION OF THE CASING LAYER.

The application of a top layer or casing layer to compost colonised by the mycelium of Agaricus bisporus (spawn-run compost) induces the switch from vegetative to reproductive growth and hence the production of fruitbodies. Not all casing materials experimented with have proved successful in providing the required conditions to support adequate numbers and weight of fruitbodies for commercial purposes. Though production of fruitbodies on spawn-run compost has been reported by Eger (1961), and Flegg (1960) obtained yields 50% of normal by simply watering the surface of spawn-run compost, yields from uncased compost are rare and wholly unsatisfactory. Under conditions of commercial culture a casing layer must be applied to obtain an adequate yield of mushrooms.

### 2.2 PROPERTIES OF THE CASING LAYER AND RELATIONSHIPS TO FRUITBODY PRODUCTION.

#### 2.2.1 Physical properties.

The physical properties of a material which affect its suitability as a casing include water holding capacity, and the closely related properties of pore space and particle size. Lambert and Humfeld (1939) reported that heavy-textured casing soils such as clay or clay loam supported better yields of mushrooms than sandy soils.

They explained their results by indicating that a uniform moisture content was easier to maintain in heavy soils than sandy soils. They also warned of the difficulties in using heavy soils, and the possibility of 'panning' with these materials on watering. The first attempt to investigate the influence of water-holding properties of casing soils on yield was by Bels-Koning (1950). Using a wide variety of casing materials she concluded that the higher the water holding capacity the better the yield, provided the material allowed sufficient aeration of the compost. Bels-Koning's results are in agreement with those of Lambert and Humfeld though these writers did not mention water holding capacity. Stoller (1952b) working with peat and soil casings supported the view of Bels-Koning. Gierszynski (1974b) working with Polish peats, sand and clay concluded that a high water holding capacity was the most important character of a good casing soil. Edwards and Flegg (1953a) indicated that under conditions of high evaporative rate peat/vermiculite casing mixtures cropped better if applied to the beds wet (42% moisture) rather than dry (17% moisture). Flegg (1959) indicated that a high water holding capacity was necessary in a casing used under conditions of quick drying so that there is a large reserve of water to counteract the loss. Under conditions of slow evaporation from the casing surface such as those in cave culture, there is less need for a material with a high water holding capacity. Water requirements of casing were expressed by Edwards and Flegg (1952) in terms of soil moisture tension. Using a clay loam, a sandy soil and a soil/peat mixture they obtained best yields when the soils were kept within the range p F 2.7 - 3.9. Where the moisture tension rose above p F 4.0 yields were practically nil, suggesting there is a point analogous to the plant wilting point above which sporophore formation does not occur.

Flegg (1957, 1958) demonstrated that a low moisture stress was required for fruiting of A.bisporus and that that condition is supplied by the casing, which has a high moisture content and low soluble salt content. Reeve, Backes and Schramer (1959) using a mineral soil concluded that a soil moisture content between 60% and 90% was optimal for cropping.

Bels-Koning (1950) indicated it is necessary to have a casing structure which allows good aeration of the underlying compost. She also demonstrated the importance of regulating management procedures such as watering to maintain an open structure for any casing. Her work also emphasised the effect of casing permeability on the micro-climate over the beds. Lambert (1933) and Mader (1943) had previously shown the inhibitory effect of carbon dioxide and other compost volatiles respectively on fruiting, and Bels-Koning (1950) indicated the importance of sufficient aeration through the casing to prevent accumulation of these substances. Lambert and Humfeld (1939) observed an adverse effect on yield where soil disintegrated on watering to form a crust over the surface of the beds. Flegg (1953) suggested their results were due to reduced pore space of the casing. Chapius and Courtieu (1950) reported that a fine soil caused anaerobic conditions in the compost and reduced yield. Edwards (1952) found that a clay subsoil sieved to contain lumps one quarter of an inch to three quarters of an inch yielded better than the same soil sieved to one quarter of an inch or a 1 : 1 mixture of these grades.

Using peat, sand and vermiculite mixtures, Edwards and Flegg (1953b) found a significant relationship between the free pore-space of the mixtures when air-dry and the number of mushrooms obtained, a greater number being obtained with increasing pore-space. A similar relationship between the free pore-space when wet and yield was not quite significant.

Lambert (1933) and Stoller (1952b) demonstrated the adverse effects of high carbon dioxide concentrations in the air above the beds on fruiting, explaining the requirement for sufficient aeration and a permanent structure for casing material indicated by the work cited above.

Flegg (1953) showed management procedures including compaction of casing by tapping and watering rate reduced pore space. He also accounted for carbon dioxide transfer from compost to the air above by gaseous diffusion through the casing, underlining the importance of adequate pore space supplied by soil with a stable structure.

MacCanna and Flannagan (1972) described a casing technique termed 'spawned casing', which involved the addition of spawn-run compost to peat/chalk casing prior to its application to the beds. They obtained more rapid colonisation of the casing by the growing mycelium and earlier and more uniform cropping, which they attributed to the assistance given to the compost mycelium in colonising the casing by the spawn-run compost added.

Nair and Hayes (1974, 1975) utilising MacCanna and Flannagan's technique and using woven glass fibre as a control for the spawn-run compost, suggested that enhanced aeration of the casing brought about by their treatments was reflected in an increased activity of aerobic Pseudomonad bacteria in the casing, and a higher rate of water loss from their treated casings compared with peat alone. Their work underlines the importance of casing structure and pore space in relation to the activity of Pseudomonads shown to be associated with fruiting (Hayes, Randle and Last, 1969).

Recently, Visscher (1975) demonstrated that the more compact a casing soil the better the yield, provided the casing was watered heavily during vegetative growth and that the upper surface of the casing was raked up before induction of the fruiting stage. He suggested his results fitted in with the accepted views on pore space and ventilation as a situation of good ventilation was restored prior to fruiting. He further suggested that by compacting casing during mycelial colonisation the higher carbon dioxide levels which built up therein favoured more vigorous and better vegetative growth, leading to better cropping.

The depth to which a casing soil is applied to beds can be relevant to the physical conditions within the casing layer. This aspect of culture has received much attention and many reports are seemingly conflicting. According to Robinson (1891) the market gardeners who raised mushrooms in England and the U.S.A. prior to 1890 applied a rich garden loam in a layer two to three inches deep.

Jackson (1909) and Duggar (1915) pointed out that the customary depth was giving way to the thinner casing adopted by the French around 1885. In Germany Passecker (1932) recommended the use of a sandy loam to a depth of one inch. Lambert and Humfeld (1939) indicated that growers in America at that time preferred a casing from three quarters of an inch to one and a quarter inches deep. From their own experimentation Lambert and Humfeld found that a casing one inch deep was sufficient to maintain a proper moisture content and did not reduce yield, whilst Edwards (1954a) using a peat/vermiculite casing obtained no difference in yield with casing depths of one and a quarter inches and two inches. In 1955 Edwards reported a depth of five centimetres gave the highest yield with a peat/sand/vermiculite mixture. Using a casing of peat and lump chalk Chakravarty (1976) obtained a reduction in yield at a depth of three centimetres compared with a casing four and a half centimetres deep.

The casing layer has traditionally been thought of as inert in terms of nutrition of the growing mycelium and fruitbodies. Though a nutritional role of the casing may exist (Hayes, 1972) the importance of the casing as a physical substrate for the stage of growth leading to fruiting known as stranding is still accepted. Hein (1930a,b) and Sarazin (1953) observed that fruiting was closely associated with stranding in the casing.

Styer (1930) showed that mycelial strands of the cultivated mushroom developed readily in very wet media.

He suggested that as stranding necessarily precedes fruiting, and fruiting results from watering of the casing, then there is a relationship between stranding and reduction of aeration due to watering. Hein (1930b) agreed with Styer's observation of increased stranding in wet media. Flegg (1963a) supported the results of Hein and Styer but indicated mycelial strand development is inhibited when the high water content of the medium results in anaerobiosis.

Garrett (1954, 1956) supported the view of Hein (1930b) that the function of strands was to transport food from the nutrient rich compost to the developing fruitbodies. Garrett (1960) also observed that strands are formed only in media having a low content of free nutrients. Mathew (1961) investigated Garrett's observations and reported that at low nutrient concentrations mycelial growth was sparse but strand development conspicuous, while at higher nutrient concentrations the reverse was true. He suggested that the low nutrient content of casing was vital to the development of mycelial strands.

By shaking up compost prior to casing, and the casing after mycelial colonisation from undisturbed compost, Flegg (1967) demonstrated that stranding readily re-developed in the casing but not in the compost. He obtained no crop reduction by disturbing the compost in this way and concluded stranding in the casing was essential to fruiting but that in compost was not a necessary requirement.

Research has shown that a high water holding capacity is required in a casing material. A high casing moisture content is especially important under conditions where evaporative loss from the casing is high. The reserve of water combats this loss as well as supplying the developing fruitbodies. In addition, a wet medium provides optimal conditions for strand development in A.bisporus, an important requirement for a good yield of fruitbodies.

Though a good casing must have a high water holding capacity, this property must be coupled with a stable, open structure which can withstand management procedures such as watering and harvesting. An open structure allows carbon dioxide produced in the compost to diffuse away, whilst providing conditions favourable to development of aerobic bacteria associated with fruiting.

The requirement of a casing to provide adequate aeration to the compost may explain the apparent contradictions in the optimum depth to which the casing should be applied. Different casing types have been used in studies on casing depth, and these different media will have had varying structures, providing different aeration rates to the compost. In addition, different depths of compost may require different casing depths. The optimum depth for any casing is therefore likely to vary according to cultural conditions.

### 2.2.2 Chemical properties.

Mushroom growers have for a long time recognised the effect of casing soil reaction or p H on yield of mushrooms.

For many years the French mixed powdered sedimentary limestone from their growing caves into their casing soil to raise its p H (LaChaume, 1882). In England the custom mentioned by Ware (1935) of mixing one half of a bushel of lime with each cubic yard of casing soil probably dates back to the end of the last century.

Earliest recommendations specifying minimum, optimum and maximum p H values for casings stemmed from the work of Lambert and Humfeld (1939). The p H range covered by their investigation was 4.4 to 8.7. They obtained low values by addition of sulphuric acid and high values by adding calcium hydroxide. Subsequently they used naturally acidic soils as controls for their acid additions. They concluded that a range from 5.5 to 8.0 was suitable, with an optimum of 7.6. Their results demonstrated that using excess calcium hydroxide as a buffer could be injurious to yield, whereas addition of excess calcium carbonate was not so.

Pizer and Leaver (1947) obtained an increase in yield with the addition of chalk to a soil of p H 5.4. Courtieu (1949) found the best p H range for his casing was between 7.2 and 8.2. Bels-Koning (1950) by adding phosphate buffers to soil concluded a p H between 8.0 and 9.0 was most favourable for mushroom production. De Kleermaeker (1953) using a variety of clay soils concluded the optimum p H for cropping was 8.0 to 8.2, and that p H is probably more important than structure and water holding capacity. Allison and Kneebone (1963) concluded that within a range of 5.5 to 7.5, p H is not a limiting factor in the suitability of a soil for production of mushrooms.

Park, Kim, Park and Kwack (1971) obtained highest yield with a casing soil of p H 7.5. Table 2.1 shows the casing p H recommended in different studies.

Edwards and Flegg (1952) reported a gradual decrease in casing p H during cropping from initial values of 7.5, they also supported Lambert and Humfeld's findings that high additions of calcium hydroxide could result in crop losses whereas high levels of calcium carbonate were not deleterious. They related the damaging effect of calcium hydroxide to the initially high p H (12.3) it causes in casing when first added. Allison and Kneebone (1963) reported that casing p H tended to decrease during cropping if initially above 6.0, but increase if initially below 6.0, suggesting a release of weakly acidic metabolites.

Courtieu (1949) concluded that a good casing soil should contain 2.0% to 5.0% active calcium. Park et al. (1971) suggested calcium in the casing layer had a stimulatory effect on yield other than via p H . Stoller (1952b) discussed the role of calcium in casing soils. He indicated that by supplying a surplus of calcium, replacement of hydrogen ions by the calcium ions occurs in the micelles of the soil complex. Soils rich in calcium form a better crumb structure than those containing hydrogen ions in excess, and this better structure favours both a high moisture capacity and good aeration of the soil.

From the data available, soils of very high or low p H are seen to be unsuitable as casings and a p H of 7.5 appears to the the optimum.

TABLE 2.1 STUDIES ON THE OPTIMUM p.H OF CASING USED IN MUSHROOM CULTURE.

| Authors | p.H. of casing optimal for yield |
|---|---|
| Lambert and Humfeld (1933) | 5.5 - 8.0 suitable, optimum at 7.6 |
| Courtieu (1949) | 7.2 - 8.2 |
| Bels-Koning (1950) | 8.0 - 9.0 |
| De Kleermaeker (1953) | 8.0 - 8.2 |
| Allison and Kneebone (1963) | 5.5 - 7.5 |
| Park, Kim, Park and Kwack (1971) | 7.5 |

There is little danger of adding too much calcium as carbonate, but there is a danger of producing too high a p H by adding calcium hydroxide.

Chemical properties of the casing apart from p H and calcium status have not been examined in as much depth as physical and biological properties. This probably results from early failures to obtain correlation between some of the more common soil constituents such as potassium and phosphorus and cropping behaviour (Pizer and Leaver, 1947).

Though Pizer and Leaver found no correlation between available potassium in casing and yield, Stoller (1952b) suggested potassium in casing while not a limiting factor may contribute to higher yields. He based his conclusion on the observation that mushrooms contained about the same amount of potash whether grown on compost devoid of potash or one supplied with normal amounts. He decided that the mushrooms grown on the former probably derived the potash from the casing.

It has been shown (Anon, 1949) that an excess of magnesium has a detrimental effect on mushroom production. Gandy (1953) suggested that materials such as vermiculite and gypsum which can contain a high level of magnesium would be unsuitable as a casing in whole or part.

Hayes (1972) indicated that the availability of iron in the casing was important in the formation of fruitbodies.

He suggested that iron is likely to be relatively unavailable in a calcareous situation such as the casing layer, and may limit cropping potential. Stoller (1952b) suggested the cation-exchange capacity of the casing layer may determine its suitability. He surmised that exchange capacity affected the activity of the mycelium in the casing, and the p H of the casing material.

Stoller (1952c) found that non-fibrous peat or that from deeper horizons of a bog and hence further decomposed, was more suitable as a casing than fibrous peat. However, his experimentation with fibrous peat was limited. He also suggested that the large quantity of nitrogen present in peat was unavailable to the mushroom mycelium, as the results of Courtieu (1949) demonstrated too much nitrogen in casing stimulates the development of many mushrooms, which, however, do not reach maturity. Courtieu states 0.007% to 0.018% organic nitrogen to be the optimal range for cropping. Gierszynski (1974a,b) supported Stoller's findings in reporting peat from lower horizons in a bog was more suitable as a casing than that from nearer the surface. He suggested this was because the organic matter was decomposed to a greater extent in low peat. He also indicated that after securing the optimum water holding capacity of casing, the suitability in terms of production increased with higher contents of total nitrogen and organic matter, especially if the organic matter was of the type encountered in low peat.

Whilst high casing levels of magnesium and nitrogen can be damaging to yield, apart from the work of Hayes (1972) there is nothing to suggest that unavailability of casing constituents can limit cropping potential. Several workers have examined the addition of minerals to the casing as nutrient supplements.

Stoller (1952c) added soluble nitrogen salts (1% salt or 0.15% Nitrogen) to peat casing and found such a large quantity of available nitrogen inhibited fructification.. In the same paper Stoller reported on his addition of various fungicides to peat casing with a view to controlling brown plaster mould (Papulosporia byssinia). He found a slight increase in yield by adding Dithane D-14 which is the sodium salt of bis-dithiocarbamate. He suggested that compounds generating sulphides such as Dithane D-14 may promote a greater production of sporophores by making the soil a better reducing medium. To test his idea Stoller added slag from an iron company to peat casing. The slag readily generated hydrogen sulphide on addition to peat and an increase in yield was noted with its use, though it had the disadvantage of increasing evaporative rate from the casing. Stoller also noted an increase in yield on the addition of potash or gypsum to peat casing but no increase with the addition of superphosphate. The use of gypsum had a floculating effect on the wet peat coming directly from the bog.

Edwards (1953a) experimenting with compost additives, omitted the usual trace metals from some of his treatments.

He then investigated the effect of spraying the cased beds with 10% solutions of urea, superphosphate, sodium chloride and a trace element mixture. The urea application gave a slightly lower yield, whilst those trays sprayed with sodium chloride gave a slightly higher yield than the controls. Addition of superphosphate and trace elements had no effect.

Flegg (1957, 1958) followed up the work of Edwards (1953a) and Stoller (1952c). He examined the addition of substances to casing to test two hypotheses. The first was the suggested relation of fruiting to the low nutritional status of the casing, and the second, the view held by many growers at the time, that addition of salt (sodium chloride) to casing during cropping reduced disease incidence and produced larger mushrooms. On addition of several relatively simple organic nutrients to the casing Flegg noted no detrimental effect on yield or number of mushrooms produced. He concluded his result did not support the idea that initiation of fruiting is related to the difference in nutritional status between compost and casing layer. He formed a similar conclusion from the addition of inorganic salts but noted that as the quantity of any salt added was increased, so fewer primordia formed, but those forming developed into larger mushrooms. The total yield at high salt levels was reduced. These observations led to a series of papers by Flegg (1960, 1961a,b,c) in which he demonstrated there was a natural accumulation of soluble salts in the casing from the underlying compost, and that high levels of soluble salts in the casing were detrimental to fruitbody formation.

Reeve, Backes, Murphy, Schramer and Vollbrecht (1959) also followed up the mushrooms growers practice of adding salt to the casing. They added potassium chloride, sodium chloride, potassium carbonate and sodium sulphate at a rate of 40 pounds per 1000 sq.ft. to a mineral soil and noted a reduction in yield in each case, but without significant influence on size of mushrooms. High rates of potassium chloride addition to a muck soil drastically reduced yield. Their results are in agreement with the work of Flegg on soluble salt concentrations.

Research into the mineral constituents of casings and any role they may have in cropping potential is limited. However, the results that are available suggest the casing has little or no nutritional function in the development of A.bisporus.

Addition of salts to the casing has shown that high salinity depresses fruitbody formation. The casing provides a medium low in soluble salts, enabling good yields to be obtained. Though there is a natural movement of salts from the compost to the casing during culture, this has not been shown to reduce yields.

### 2.2.3 The gaseous environment of the casing layer.

Since the damaging effects of high carbon dioxide levels on fruiting were first noted by Lambert in 1933, the growing mycelium of A.bisporus has been shown to produce a number of gaseous metabolites which can affect fruiting.

Earliest work on the gaseous environment of the casing concentrated on the action of carbon dioxide on fruiting, and some workers (Long and Jacobs, 1969; Tschierpe, 1972) consider this gas to be the only one linked with fruitbody development in the casing.

Lambert (1933) measured the carbon dioxide levels in bell jars placed over mushrooms developing on a standard bed. He found an accumulation of 5.0% or more of carbon dioxide in the air caused abnormal growth, stunting and even death of mushrooms. Approximately 1.0% carbon dioxide was the lowest concentration that was noticeably injurious. From his results Lambert indicated the need for adequate ventilation in growing houses.

Stoller (1952a) confirmed the injurious effect of carbon dioxide on sporophore growth and emphasised the importance of sufficient air movement above mushroom beds, especially when they were cased with peat, which restricted air movement in the casing more than loam did.

Tschierpe (1959a) examined the carbon dioxide levels in the compost atmosphere and in the air above the beds in a commercial growing house. He suggested that after casing and subsequent ventilation there builds up a clear division between air in the compost and the air of the growing room. Before casing the carbon dioxide concentration in the beds was four to five times higher than in the air, but by first pinhead formation the ratio had risen to ten or twelve to one. From this he suggested there was a connection between sporophore formation and a carbon dioxide partial pressure gradient in the casing layer.

Tschierpe (1959b) tested his hypothesis by applying air containing different levels of carbon dioxide to newly cased boxes. Concentrations between 0.3% and 10.0% induced the morphological abnormalities described by Lambert (1933) and Stoller (1952a). Above 11.0% no fruitbodies formed. Between 0.5% and 1.0% the mycelium penetrated the soil but did not fruit. He interpreted his results as adding support to his idea that a partial pressure gradient of carbon dioxide is developed in the casing and induces fruiting. Tschierpe and Sinden (1964, 1965) demonstrated that 0.01% to 0.03% carbon dioxide in the air above the casing produced maximal fruitbody initiation for the strains investigated. These workers also showed that strands which developed to the casing surface under high carbon dioxide concentrations could be induced to form initials if the carbon dioxide level was reduced to the range 0.01% to 0.03%.

Thomas, Mullins and Block (1964) investigated primordium formation in Petri-dish culture and suggested a carbon dioxide gradient in the casing was not required for fruitbody initiation.

By forcing different levels of carbon dioxide down through cultures in sealed growth chambers, Long and Jacobs (1969) also doubted the requirement of a carbon dioxide gradient for fruiting, as this was obtained in a situation where a constant concentration was present throughout the casing depth. They applied different carbon dioxide levels to sterile and non-sterile casing and obtained no fruiting with sterile casing.

They found hyphal growth to be proportional to carbon dioxide levels between 0 and 104 ppm in non-sterile casing. Between 104 and 1000 ppm a retardation of strand growth occurred, coinciding with production of initials. Long and Jacobs suggested fruiting was linked with competitive inhibition by micro-organisms of vegetative growth of the mushroom mycelium. They suggested the micro-organisms might act by interfering with carbon dioxide fixation by the mycelium over the range 104 - 1000 ppm carbon dioxide.

Tschierpe (1972) reviewed the effect of carbon dioxide levels on cropping and indicated the requirement of cooling and adequate ventilation of the growing house to counteract the production of heat and carbon dioxide in the compost. He also showed that as the temperature is dropped prior to fruiting, the solubility of carbon dioxide in the water of the compost and casing increases, thus reinforcing the drop in partial pressure gradient of carbon dioxide which he linked with fructification.

Nair and Hayes (1974, 1975) indicated that the work on carbon dioxide and fruiting had been concerned with the partial pressure of the gaseous phase. They suggested the chemical breakdown products of carbon dioxide could be of equal importance in fruiting. By adding bicarbonate as sodium bicarbonate to the casing layer they investigated its effect on the bacteria associated with fruiting (Hayes, Randle and Last, 1969) and on the number of sporophores formed. They found a significant increase in the number of Pseudomonad bacteria in sodium bicarbonate amended casing compared with sodium chloride treated or unamended casings.

The maximum numbers were at 1000 ppm of sodium bicarbonate, ten days from casing. There was also an increase in the number of sporophores but this was not significant.

Nair, Short and Hayes (1976) summarised the theoretical activities of the chemical breakdown products of carbon dioxide in the casing layer and suggested results from studies on the gaseous environment of the casing could lead to selection of an ideal casing material.

Hayes and Nair (1976) showed the activity of a selected Pseudomonas isolate from casing soil, when inoculated onto sterile casing could be increased by its culture in a carbon dioxide enriched atmosphere, suggesting an indirect effect of carbon dioxide on fruiting capacity.

By comparing adpressed and non-adpressed casings, Visscher (1975) concluded that a high carbon dioxide concentration in the casing during vegetative growth gave a better yield provided that good aeration of the casing was restored prior to fructification by raking the casing surface.

Ten years after the pioneering work of Lambert on carbon dioxide and fruiting, Mader (1943) conducted experiments which led him to conclude that an unidentified gaseous substance or substances other than carbon dioxide must exist that inhibited fruiting when present in sufficient quantities.

Mader was able to remove these substances by recirculating air from growing chambers through alkaline potassium permanganate solutions, mineral oil, and activated charcoal, which suggested they belonged to the class of unsaturated hydrocarbons. He did not determine their origin but assumed it to be from the metabolism of the mushrooms themselves or that of the compost microflora.

Middlebrook and Storey (1950) compared crop production in three types of growing house, and concluded differences were due to variation in atmospheric make-up of the houses. They suggested the mushroom mycelium produced a volatile inhibitor, which accumulated to varying extents in the different buildings.

Stoller (1952a) described stem elongation and cap retardation of sporophores when mushrooms were growing thickly on the beds or when peat casing was used in conjunction with restricted air movement in the house. In both circumstances he recorded no detectable carbon dioxide and inferred a volatile substance was responsible for the abnormalities. He hypothesised that this volatile was emitted from the mycelium in the compost and casing and accumulated in the situations described. Stoller postulated that the oxidising intensity of this substance was such that it prevented development of the thickenings at hyphal tips from which sporophores formed, and he suggested one of the functions of the casing was to provide an alkaline, oxygenated medium for the destruction of this volatile. He described the action of the volatile as similar to that of a plant hormone, with different effects at different concentrations.

Sinden, in work reported by Schisler (1957), found that when he circulated air back into growing chambers without first modifying it, no sporophores developed. When the recirculated air was passed through charcoal normal fruiting occurred, but this was not so when the air was passed through soda lime. He concluded the gaseous inhibitor was a substance other than carbon dioxide. From his research Schisler (1957) came to the same conclusions as Stoller regarding the role of the casing in providing a barrier to the unknown volatile, and allowing a required concentration to develop for fruiting.

The identity of metabolites other than carbon dioxide involved in mushroom development was theoretical until 1963, when Lockard and Kneebone identified ethylene, acetaldehyde, acetone, ethyl alcohol and ethyl acetate as metabolites of mushroom mycelium. These findings were confirmed by the work of Tschierpe and Sinden (1965) and Richter (1967). These workers also showed ethanol to be a product of the anaerobic metabolism of Agaricus bisporus, whilst Tschierpe and Sinden (1965) showed acetone to be the main volatile metabolite produced under aerobic conditions. Tschierpe and Sinden (1965) applied acetone, ethyl alcohol, acetaldehyde and ethyl acetate to non-sterile cultures in a continuous air stream. They did not detect any effect on fruiting or strand formation.

As well as the first formed ideas of concentration effect to explain the possible role of metabolites in fruiting of A.bisporus, evidence had built up which suggested an indirect action of the volatiles on the microflora associated with fruiting.

Hayes, Randle and Last (1969) showed an increase in numbers of stimulatory bacteria when these were cultured on a carbon-free liquid medium exposed to atmospheres enriched with ethanol, ethyl acetate, acetone or an undefined mixture of metabolites emitted from growing cultures of A.bisporus. This suggested these bacteria could utilise the volatiles as their sole carbon source, and Hayes et al. found it possible to select for stimulatory bacteria in mixed bacterial populations by exposing them to these volatile chemicals.

Eger (1972) suggested that the two hypotheses on fruiting at the time, namely the action of stimulatory bacteria and that of carbon dioxide and volatile metabolites, were compatible within a single hypothesis, as bacteria that initiate fruiting could utilise these volatiles as carbon sources. She suggested acetone may be the key substance in fruiting but provided no experimental evidence to support her suggestion.

Visscher (1975) obtained higher yields by compressing casing prior to aeration and cooling. He suggested the casing acts as a barrier to volatile metabolites which stimulate greater bacterial activity in compressed casing, this leading to higher yields.

Hayes and Nair (1976) demonstrated that in sterile peat casing the activity of a Pseudomonas sp. isolated from casing was greatly increased by its culture in atmospheres enriched with carbon dioxide, ethanol and volatiles emitted from mushroom mycelium growing on compost.

Turner, Wright, Ward, Osborne and Self (1975) demonstrated a rise in ethylene production coinciding with fruiting. More recently, Ward, Turner and Osborne (1978) identified the growing mushroom mycelium as the source of ethylene, but could find no direct regulatory role of ethylene on the growth or development of A.bisporus.

Research has demonstrated the damaging effects of carbon dioxide on fruiting of A.bisporus. During cropping, ventilation must be applied to growing houses to reduce carbon dioxide levels in the air below 0.3%. Carbon dioxide concentrations between 0.01% and 0.1% have been suggested as optimal for fruiting by Tschierpe and Sinden (1964, 1965) and Long and Jacobs (1969). Though Tschierpe (1959a,b) demonstrated a carbon dioxide partial pressure gradient exists between the compost and the air above the casing, Thomas, Mullins and Block (1964) and Long and Jacobs (1969) have shown this gradient is not required for fruiting, so long as the carbon dioxide level at the site of fruiting is sufficiently low.

Unidentified volatile metabolites produced by the mycelium of A.bisporus or the compost microflora have been claimed to inhibit fruiting. There is little direct evidence to support this suggestion. A.bisporus has been shown to produce acetaldehyde, acetone, ethylene, ethyl acetate and ethyl alcohol, and Schisler (1957) has suggested these volatiles may act in a hormone-like fashion, inducing fruiting at certain concentrations. Again, this idea lacks adequate experimental support, whilst Ward et al. (1978) could find no direct effect of ethylene on A.bisporus.

Hayes, Randle and Last (1969) drew attention to possible interactions of metabolites of A.bisporus with bacteria associated with fruiting. Certain of the metabolites can act as carbon sources for these bacteria. The work of Nair and Hayes has shown bicarbonate added to casing can stimulate greater activity of the same bacteria, suggesting soluble products of carbon dioxide may control numbers of stimulatory bacteria. It is possible then that there is an association between metabolites of A.bisporus and fruiting via an indirect effect on bacterial populations.

### 2.2.4 Biological properties.

From their research at Wye College, Kent, Pizer and Leaver (1947) concluded that cropping of A.bisporus was not affected by organisms in the casing unless they had very specific soil requirements. They drew their conclusion from a series of experiments in which soils of known cropping potential were placed in two layers on the bed, one on top of the other. In all cases the top layer determined the crop harvested. However, from 1959 to 1962, Eger published a series of papers containing some facts and hypotheses on the action of casing bacteria on sporophore initiation in the cultivated mushroom. In 1961 she reported on the results of experiments she conducted with her 'Halbenshcalentest'. This involved placing a casing material alongside colonised compost, each occupying half the area of a glass Petri dish. She observed that when sterile casing was used no fruitbodies developed, as they did when untreated casing was used. Also in 1961, Urayama obtained increased mycelial density and production of fruitbodies by spraying a suspension of Bacillus psilocybe 1 onto casing in a normal culture situation.

Initiation of fruiting also occurred sooner.

Since these early observations a number of workers have confirmed the association of micro-organisms with fruiting, and explored the role played by these organisms in the initiation process.

O'Donoghue (1963) reported that three species of Actinomycetes belonging to the genus Streptomyces caused fruiting in grain spawn cultures of A.bisporus when these organisms were present as chance contaminants.

In 1969 Hayes, Randle and Last described an apparatus in which pure cultures of A.bisporus were maintained on composted media in filtered atmospheres, free from toxic concentrations of carbon dioxide and contaminating micro-organisms. When grown on compost alone, cultures of A.bisporus did not produce sporophores. Their formation was stimulated by a cover of unsterilised peat/chalk casing. Autoclaving, or fumigating the casing with propylene oxide decreased populations of bacteria and prevented fruiting. When micro-organisms isolated from casing on commercial beds were added to sterile casing fruiting was promoted. The stimulatory bacteria were identified as Pseudomonas putida and group IV Pseudomonads. Hume and Hayes (1972) consistently obtained high numbers of primordia when agar plugs containing Pseudomonas putida were inoculated onto agar cultures of A.bisporus, the primordia developing in close proximity to the bacterial inocula. Hayes (1972) found that primordium formation in Petri dish cultures could be inhibited by adding metal binding agents such as E.D.T.A. to the agar.

He found that the addition of only one metal, iron, would restore primordium formation, and went on to suggest that bacteria stimulating fruiting may act by releasing iron bound in the casing layer.

Arrold (1972) using the two-phase Petri dish system of Hume and Hayes (1972), confirmed the ability of Pseudomonas putida to cause fruiting. His isolate, taken from casing on a commercial bed, initiated primordia in ten strains of A.bisporus. Eger (1972) observed some inducing activity of eight fluorescent Pseudomonads on A.bisporus strain 310a, but not on strain 71a.

Park and Agnihotri (1969a) suggested bacterial metabolites stimulated the formation of sporophores in A.bisporus. In a second paper Park and Agnihotri (1969b) reported on successful sporophore initiation under aseptic conditions by the addition of such diverse substances as biotin, oxalic acid, giberellic acid and others. Eger (1972) using biotin with strains 310a and 71a could not reproduce Park and Agnihotri's results.

By spraying spores of a range of bacteria and yeasts onto spawned compost before casing, and one week later on the casing surface, Curto and Favelli (1972) recorded an increase in mycelial density, earlier initiation of fruiting and higher total yield. The supplementation of spawned compost with freeze-dried cultures of the alga Scendesmus quadricauda at casing caused a fifty per cent. increase in yield.

Margheri and Vassilacikis (1977) showed that unbroken algal cells and crude extracts induce mycelial differentiation, and sometimes sporophore

Giovannozzi-Sermanni, Grappelli, Cacciatori and Pietrosanti (1975) claimed whole cells and medium extract of an Arthrobacter sp. influenced sporophore formation of A.bisporus.

In 1961 Eger noted that sterile casing containing activated charcoal would support fruiting in much the same way as non-sterile casing. She suggested that the activated charcoal absorbed inhibitory compounds normally utilised by the soil microflora.

This work was not followed up until 1974 when Long and Jacobs confirmed Eger's results with activated charcoal. They studied control of basidiocarp initiation by micro-organisms and carbon dioxide in the casing layer, using the same apparatus as for their earlier work on carbon dioxide (Long and Jacobs 1969). Fruitbodies were not produced in sterilised materials except when activated charcoal was included in the casing. In this case responses to carbon dioxide were similar to those obtained in unsterilised casing where carpogenesis was associated with a check of hyphal growth over a restricted carbon dioxide range (100 - 1000 ppm.). Long and Jacobs suggested that bacteria may act in a similar fashion to activated charcoal in absorbing leaked nutrients from around growing hyphal tips, and under associated conditions of low carbon dioxide tensions, the mycelium is 'starved' into fruitbody formation. They considered it unlikely that metabolites other than carbon dioxide were involved in fruiting as these were continually washed down and out of their apparatus by the direction of air flow.

Couvy (1976) using similar equipment to Long and Jacobs, obtained a similar effect when activated charcoal was incorporated into sterile casing. She was of the opinion that the charcoal removed inhibiting substances elaborated by the mycelium itself.

The work of Eger (1961), Hayes et al. (1969) and Long and Jacobs (1974) has shown that fruiting of A.bisporus does not occur in sterile casing. Non-sterile casing supports the growth of Pseudomonas putida and group IV Pseudomonads which are required for fruiting to occur. Though some strains of A.bisporus will produce primordia in axenic culture on agar media (Wood, 1976), the only reliable reports of fruiting in sterilised casing are when activated charcoal has been included in the medium. This has led to suggestions that stimulatory bacteria act by absorbing substances inhibitory to fruiting, whilst Hayes (1972) suggested bacteria may release iron required for fruiting. Though the requirement of bacteria in the casing for fruiting has been demonstrated, their involvement in the process remains unclear.

In addition to supporting an appropriate microbial flora, one of the biological requisites of a good casing medium is that it be free from disease and pest organisms which can result in considerable crop losses.

Casing materials, especially soils, are a source of bacteria and fungi pathogenic to A.bisporus (Kneebone and Merek, 1958); Atkins and Atkins, 1971).

The bacterial pathogens Pseudomonas tolaasi and Pseudomonas agarici which cause bacterial blotch and drippy gill can be introduced via the casing; whilst the most destructive fungal pathogens which can be present in casing media include Dactylium dendroides, Mycogone perniciosa and Verticillium fungicola. Casings can also harbour saprophytic fungi which compete with the growing mycelium of A.bisporus. Amongst the more troublesome are Geotrichum sp., Papulosporia byssinia and Trichoderma viride.

In addition to microbial pathogens and competitors, casing media can introduce insect and other pests of the cultivated mushroom. The larvae of flies belonging to the families Cecidomyiidae, Lycoriidae and Phoridae feed on the mycelium and fruitbodies.

Mycophagous nematodes such as Ditylenchus myceliophagus and Aphelenchoides composticola are common inhabitants of agricultural soils (Sinden, 1971) and can destroy the mycelium in a mushroom bed in a short time.

Pests of the mushroom crop can act as vectors for pathogens of A.bisporus (Cross and Jacobs, 1969; Hussey, Read and Hesling, 1969).

Good summaries of diseases and pests of the cultivated mushroom are given by Carpentier (1971), Atkins (1974) and Vedder (1974).

Since no casing medium is completely free from disease or pest organisms, various physical and chemical treatments are applied to casing to control pests and pathogens. In turn these treatments can provide secondary problems for the grower, who must gauge their use against an overall effect on yield.

Since the advent of peat as a casing material in this country it has not been general practice for growers to pasteurise their casings, as peat is relatively free from pathogens. In the United States where peat has only recently been used in any quantity, most growers pasteurise their casings prior to application. Baker (1967) indicated most soil-borne plant pathogens can be eliminated by a steam/air treatment of 140°F. for 30 minutes. Schisler and Wuest (1971) in a review of pasteurisation techniques discussed the advantages and difficulties associated with dry heat, moist heat and chemical treatment of casings. They recommended steam treatment of casing at 180°F. for 30 minutes to provide a margin of safety in achieving kill off of pest and disease organisms. They pointed out that some soil fumigants can be highly selective in regard to pests killed.

Hayes (1971) suggested methyl bromide fumigation as an alternative to steam pasteurisation of casing. He indicated steam treatment of peat/chalk casing at 76°C. for 30 minutes gave slight but consistent yield reductions compared with untreated casing. He advocated the use of methyl bromide as it is toxic to pests and fungal pathogens, but has a relatively low toxicity to bacteria, some of which are associated with fruiting.

Disease outbreaks on farms are often combated by the application of chemical agents to the beds. Bech and Rasumssen (1967) reported no reduction in yield with application of formaldehyde, vapam, chloropicrin or basamid to peat/sand casing. Wuest, Cole and Patton (1972) and Holmes (1972) however, noted yield reductions with high dose rates of the systemic fungicide benomyl.

Wyatt (1978) found that incorporation of insecticides into casing could result in yield reductions in early flushes which were usually compensated for by heavier cropping when the insecticide effect had diminished.

Work by Cayrol and his colleagues had culminated in the marketing of a product termed 'Royal 300' (Cayrol, Franowski, Lanièce, d'Hardemere and Talon, 1978) which is used in the same way as mushroom spawn. This material is an inoculum of the nematode-trapping fungus Arthrobotrys robusta, which reduces populations of mycophagous nematodes in casing and compost and can give an increase in yield.

### 2.2.5 The casing layer in the nutrition of A.bisporus.

The nutritional requirements of the vegetative mycelium of A.bisporus have been studied largely by submerged culture techniques (Hebert and Heim, 1909, 1911; Styer, 1928, 1930; Treschow, 1944; Fraser, 1956).

Lindberg (1950) extracted polyphenol oxidases from vegetative mycelium, rhizomorphs and fruitbodies. He showed that the mycelium produced laccase and the fruitbodies a carbon monoxide sensitive polyphenoloxidase, whilst the rhizomorphs produced both enzymes.

Gerrits (1969) showed that the major fraction of compost lignin was consumed between spawning and the appearance of the first pinheads, whereas alpha-cellulose and pentosan decreased slowly during spawn-run, but were utilised more rapidly during cropping.

In more recent studies, Turner (1969, 1974) demonstrated that laccase activity characterises the vegetative stage and tyrosinase activity develops at the time of initiation of fruitbodies, suggesting different nutritional requirements of the two phases. Wood and Goodenough (1977) confirmed Turner's observations on laccase activity, and showed cellulase activity in the compost increased at fruiting.

Though differences in nutritional requirements of the vegetative and reproductive phases exist, it is a widely held belief that the nutrient source for all stages of the life cycle is the compost. The fact that the casing is comparatively lower in available nutrients than the compost has been postulated as one of its properties responsible for initiating fruiting (Mathew, 1961; Flegg, 1963; Long and Jacobs, 1974). Gerrits (1969) supported the view that the compost alone supplies nutrients to A.bisporus, suggesting even the water utilised by the fruitbodies is taken from the compost.

This belief appears to be supported by evidence provided by Lambert (1963) and Nielsen and Rasmussen (1963) who demonstrated that the mycelium of A.bisporus could transport soluble nutrients over long distances at considerable speed. However, the work of Reeve, Backes and Schramer (1959) and Flegg (1967) shows that by applying more water to the casing the water content of the fruitbodies is raised, indicating fruitbodies take water from the casing layer.

Direct investigation of a role of the casing in the nutrition of any stages of development is limited. Bels-Koning (1950) concluded that as fruitbodies would develop with ground brick as a casing, then the casing had no nutritive role in fruiting.

Several workers have investigated the addition of organic and inorganic nutrients to the casing (Stoller, 1952c; Edwards, 1953a; Reeve, Backes, Murphy, Schramer and Vollbrecht, 1959; Flegg 1957, 1958). None of the investigators obtained results which suggested a nutritional role of the casing.

Hayes (1972) suggested the casing may have an indirect role in the nutrition of A.bisporus in supplying iron made available by bacteria.

With the exception of water, which fruitbodies can take from the casing, research to date suggests that all developmental stages of A.bisporus obtain their nutrients from the compost.

## 2.3 CASING TYPES, TECHNIQUES AND MANAGEMENT.

### 2.3.1 Casing types.

From the time of the earliest artificial culture of A.bisporus until as recently as forty years ago, the type of casing applied to mushroom beds depended to a large extent on local resources and past experience with available soils. Early French growers included the sedimentary limestone from the walls of their caves in their casings (LaChaume, 1882) whilst in England it was traditional to use a good loam soil (Robinson, 1870).

For the past thirty years growers in the U.K. have used Sphagnum peat as a standard casing, but in other producing countries a variety of casing materials are still used. The type of casing employed in the major producing countries is determined to some extent by the type of growing situation employed. In countries such as the U.K. and U.S.A. where house culture is the rule, then a casing such as peat which has a high water-holding capacity is favoured. In the cave culture systems of France and Italy where evaporative loss from casing is less, then lighter soils can be employed as casings.

Table 2.2 shows the casing types employed in the major producing countries at the present time.

Serious research into the role of the casing in mushroom culture was scarce until the late 1930's, but since then study of the casing has intensified.

TABLE 2.2 CASING MATERIALS USED IN THE MAJOR MUSHROOM PRODUCING COUNTRIES.

| Country | Type of casing used |
|---|---|
| U.K. | Moss peat/chalk |
| U.S.A. | Local subsoils; peat/chalk |
| France | Local subsoil mixtures |
| Holland | Black peat/moss peat/marl/sand |
| Denmark, Poland, Russia | Peat/chalk |
| Taiwan | Clayloam subsoil/chalk |
| Korea | Clayloam subsoil/hydrated lime |

Sources: Chang and Hayes (1978), Vedder (1978).

Many of the early investigations were aimed at understanding the properties of a good casing layer, and diverse materials were used to achieve this objective.

From 1936 to 1938 Bewley and his colleagues at the experimental research station at Chestnut compared the performance of a variety of materials as casings. They used four soils of different texture, granite chips, stones, sand, peat and mixtures of soil with the previous four. They considered heavy, clay or silt soils free from plant roots to be the most suitable casings. It was found that the addition of peat to soil gave an increased yield, whilst stones and granite chips gave low yields.

Bels-Koning (1950) reported on the use of marl and a mixture of loamy garden soil and peat in an investigation of the water supply and climate of casing. She also used ground bricks with phosphate buffer, vermiculite/dusarite mixtures, and vermiculite/peat mixtures to examine p H effects. Fruitbodies were formed on all casings. Finally she compared vermiculite, brick, cloth and filter paper to study the nutritional role of the casing. The production with the latter two was less than with garden soil, whilst the others gave equal or better yields.

Stoller (1952c) discussed work that commenced in 1947 on the potential of peat from Duluth, Minnesota as a casing. The work was initiated due to the scarcity of loam in the Duluth area. He concluded that fibrous peat was not a suitable casing, but non-fibrous peat gave good yields when screened and neutralised.

De Kleermaeker (1953) compared seven different clays and vermiculite in an investigation of casing water relations and p.H .

Between 1950 and 1954 Edwards and Flegg successfully used vermiculite, sand, peat, local soil and mixtures of the four to examine the physical properties of the casing layer which regulate cropping. An indirect result of their research, but a very important one, was that Sphagnum peat was adopted as the standard casing in the U.K..

O'Donoghue (1962) found Phragmites peat to be comparable in performance to Sphagnum peat.

The aim of much of the recent research involving different casing types has been the commercial evaluation of possible casing media. In countries with established mushroom industries much research has been aimed at finding alternative casings to peat or soil. In countries which are developing their mushroom industries experimentation to find suitable local casing materials has been undertaken.

Barnard (1974) reported on trials with sedge peat as compared with Sphagnum peat. The sedge peat gave a larger yield and more even cropping early on but tended to compress with watering. He also studied perlite, vermiculite and polyeurathane crumb foam as possible alternatives to peat. Water management problems were encountered with these materials though yields were not far below the peat control with perlite, suggesting that a manufactured, inert casing material may be a possibility.

Aaron (1975) compared pine bark and an equal parts mixture of peat and pine bark, with peat as a control. Comparable yields were obtained though some fears were expressed about the phytotoxicity of the volatile oils in the bark. Bowden and Allen (1978) used bark from a variety of sources as a casing on its own or in combination with peat. They obtained yields comparable to peat in both cases, but noted that bark casing tended to dry out more rapidly than peat.

Stoller (1978) discussed his successful use of a casing comprised of shredded newspaper, powdered, activated carbon, limestone and gypsum.

Nair (1977) and Stoller (1979) discussed the re-use of spent compost as a casing. They demonstrated removal of the high soluble salt content of the material must be achieved, indicating weathering or centrifugal washing were suitable techniques. Bowden and Allen (1978) used spent compost and spent casing mixed with two types of bark and two grades of peat, as casing materials. In all cases yields were below conventional peat. They suggested leaching of the compost would be a help but doubted the uniformity of leached compost as a casing.

Mantel (1973) indicated that spent compost when properly treated was successfully used as a casing soil on Indian farms.

Hayes and Shandilya (1977) examined the suitability of a range of materials readily available in India as casings. Farm yard manure, clay soil, loam soil, forest soil and spent compost were compared. Farm yard manure and loam soil were found to be most satisfactory.

Experimentation with different casing materials has led to an understanding of the physical requirements of a good casing layer. The work of Edwards and Flegg between 1950 and 1954 resulted in the adoption of peat as casing in the U.K., and more recently in the U.S.A.. Whilst materials such as spent compost and wood bark show some promise as casings, recent research has not discovered a replacement for conventional materials.

### 2.3.2 Casing techniques and management.

It is the case that in most of the major mushroom producing countries mushroom growers do not adopt a set pattern of casing technique and management. This is because differences between growing houses make this impractical and because individual growers tend to find a system which gives good results and then adhere to that sometimes without fully understanding how they achieve those results. The way a casing material is prepared and managed after application has a considerable bearing on its performance in terms of yield.

Research has elucidated some of the effects of casing management on yield, and defined optimal environmental conditions for culture. It has also highlighted the different reactions to management exhibited by different casings.

Many casing materials must be neutralised and wetted before use. A study by Ganney and Richardson (1974) of twelve different peat casings used in the U.K. highlighted the variability of commercial casing mixes.

They found considerable variation in the ratio of peat to chalk or lime, moisture content of casing, and quantity of casing applied to 1000 sq. ft. of bed.

In the U.K. it is not usual for the standard peat/chalk casing to be pasteurised before its application. In some countries where soils are commonly used such as the U.S.A., steam/air pasteurisation is practiced. Schisler and Wuest (1971) reviewed the pasteurisation of casing soils with dry heat, steam and chemical fumigants. They recommended steam treatment rather than dry heat owing to the difficulty in obtaining an even temperature in a soil mass with dry heat. Chemical fumigants do not alter soil structure or release toxic elements as steam treatment may do, but they may be highly selective in regard to pests killed, toxic to man, and the soil may require prolonged aeration prior to application.

In commercial practice the casing layer is normally applied when the compost is fully run with mycelium. The work of Edwards (1952, 1953b) and MacCanna (1972) support this practice and demonstrates a yield reduction with earlier casing. However, some workers (Rasmussen, Slack and Mitchell, 1970; Dawson, 1977; Kitaev, Bubnova, Shelashova and Brisov, 1977) claim that casing at the time of spawning gives earlier cropping and comparable yields with later casing. San Antonio (1969) has suggested varying the time of casing after spawning as a means of regulating crop production, since earlier casing gives earlier cropping and sometimes a smaller yield in first flush.

Visscher (1975) reported that by compressing casing at application, and then raking the surface up prior to aeration of the house, increases in yield could be obtained.

The act of watering the casing layer to replace water lost during culture can have a variety of effects on the growth and yield of the mushroom. Several workers have reported that watering rate affects yield (Edwards and Flegg, 1953a; Reeve, Backes and Schramer, 1959; Kindt, 1965; Flegg, 1967). Reeve et al. and Flegg both found increasing the amount of water applied to the casing significantly raised the water content of sporophores. Furthermore, Flegg found the increased yield could be accounted for by the increased water content of the sporophores.

Edwards (1953b) showed the onset of fruiting was delayed on beds which received their first watering twenty five days after casing, compared with those watered earlier. Edwards and Flegg (1953a) demonstrated that the rate of watering during the mycelial colonisation of the casing could affect the degree of segregation of some soils. Heavy watering tended to compact soils with a less stable structure.

Flegg (1963a) described how peat casing which was kept dry resulted in the growth of only fine hyphae whereas a wet casing stimulated thick mycelial strands as well as finer hyphae. This was confirmed by Flegg (1974). In the same paper Flegg reviewed the effects of watering on the mushroom hyphae and sporophores and suggested that balance data of water lost and applied can be used to calculate the amount of water required to maintain both compost and casing at the optimum water content.

Flegg (1963b) pointed out that hand watering of the casing layer at two to three day intervals resulted in a wetting and drying cycle. He described a system of capillary watering of the casing by means of wicks placed over the casing with their free ends in a water reservoir. This maintained the casing moisture content between narrow limits and resulted in yields comparable with those from hand watered beds.

The effect of temperature on the start of cropping and on yield has been studied by Flegg and Gandy (1963), Storey (1965), Allen (1967) and Flegg (1968, 1970). They found that for maximum yield and earliest cropping the air temperature during the period of sporophore initiation after casing should be about 15 - 18°C.. Higher or lower temperatures than the optimum during the two weeks after casing delayed the onset of fruiting, the extent of the delay being about one day for every difference in air temperature of 5.5°C. (Flegg, 1968). Hauser and Sinden (1964) claimed that altering the air temperature by 1°C. some days before the start of cropping can advance or delay the crop by one to two days. Flegg (1972) demonstrated that sporophore initiation was earlier at 22°C. than at 16°C. but at the higher temperature many of the newly formed sporophores died or developed slowly. Young sporophores were particularly sensitive when between 2 and 10 mm. in diameter. The time taken to grow from a size of 10 mm. to the cup stage of maturity was decreased by one day for each rise in temperature of 4.6°C..

The relative humidity of the air immediately above the casing is an important factor in controlling cropping.

Flegg and Gandy (1963) showed that at high relative humidities (80% - 90%) the onset of fruiting tended to be earlier and the weight of mushrooms greater than at low humidities (40% - 50%). Storey (1965) produced evidence to suggest that at very high humidities when evaporation from the casing layer is much reduced and water condenses from the air onto the casing and developing fruitbodies, then cropping may be delayed and yields reduced.

Variability in management procedures has been nullified to a large extent in Holland as a result of the development of a strong co-operative organisation. This co-operative has introduced a high degree of uniformity into the design of their mushroom houses, coupled with mechanisation in management. They also supply a ready mixed casing, and their policies lead to a uniformity of growing methods.

The adoption of a pre-prepared standard casing in the U.K. would aid standardisation of management procedures.

# SECTION 3

# MATERIALS AND METHODS

# SECTION 3 MATERIALS AND METHODS.

## 3.1 INTRODUCTION.

Review of literature relevant to the casing layer has shown that fruiting in A.bisporus is regulated by a large number of factors. For a casing medium to support commercially acceptable yields it must have the correct physical and chemical requirements, as well as providing an environment which supports micro-organisms associated with fruiting. Survey of research into casing types showed that at the present time few promising alternatives exist to the standard peat casing used in the U.K., even though peat reserves in Eire are very limited.

Paper mill sludge (PMS) from the Kemsley mill was shown by Hayes (1975) to be suitable as a casing, but variable in performance with its time from deposition. Thus a programme of research was planned to define the composition and physical nature of the sludge with time from deposition, coupled with changes in populations of micro-organisms, higher plants and animals. Following definition of the ecology of PMS trials were undertaken to determine its suitability as a replacement casing for peat. The effect of its composition and biological nature on growth and fruiting of A.bisporus were investigated to define the optimum condition and management of the material as a casing. Chemical and mycological aspects of the research are presented in the present study; bacteriological studies were done by Cresswell (pers. comm.).

## 3.2 APPRAISAL AND SAMPLING OF PAPER MILL SLUDGE (PMS) DEPOSITS AT KEMSLEY.

### 3.2.1 Ageing of sludge deposits.

No chronological record of sludge deposition had been kept at the landfill site at Kemsley. Therefore in April 1976 a plan of the deposition area was drawn up (Figure 1.2) in conjunction with Kemsley mill staff, showing the approximate ages of sludge deposits existing at the time. This plan was used as a guide for the initial on-site monitoring work and sample collection.

### 3.2.2 Monitoring of higher plants and fungi present on deposited PMS.

In agreement with staff at Kemsley, six sites representing sludge 0, 4, 7, 14, 18 and 36 months after deposition were set aside in April 1976 for monitoring of higher plant and fungal populations over a period of five months. These sites were designated 1 - 6 respectively (see Figure 1.2). Higher fungi colonise the deposited sludge some three to four months after its deposition, and by 3 years after tipping the solids are completely colonised by higher plants. Sites 1 to 6 were chosen to give a representative spread of aged solids from the time of initial colonisation by fleshy fungi until the point of dominance by green plants. The study period was chosen to coincide with the time of year when the largest number of species of both higher fungi and plants are evident.

Sites 1 - 6 were all of comparable dimensions, approximately 900 $m^2$. in area.

In view of the limited time available for recording at each time of monitoring, direct counting of individual fungal fruitbodies and plants was not possible. Four arbitary categories were chosen to represent the relative abundance of fungi and plants at each site and between different sites. These categories were designated as

(i) Abundant

(ii) Frequent

(iii) Occasional

(iv) Rare

Species of higher fungi were identified by reference to Dennis (1960), Lange and Hora (1965), Watling (1973) and Orton and Watling (in press).

Species of higher plants were identified by reference to Clapham, Tutin and Warburg (1968) and Martin (1969).

3.2.3 Sampling of deposited sludge for laboratory analysis.

Samples of variously aged sludge deposits were taken using a soil auger (Engineering Laboratory Equipment Limited). A small undisturbed sample tube was driven into the sludge using a jarring link, and the tube provided a core of sludge 15 cm. in length.

Samples for analysis were taken from within 3 cm. either side of the mid point of the core. The aluminium sample tubes were sterilised by autoclaving at 121°C. for 15 minutes prior to use, so as not to influence microbial analyses of the samples.

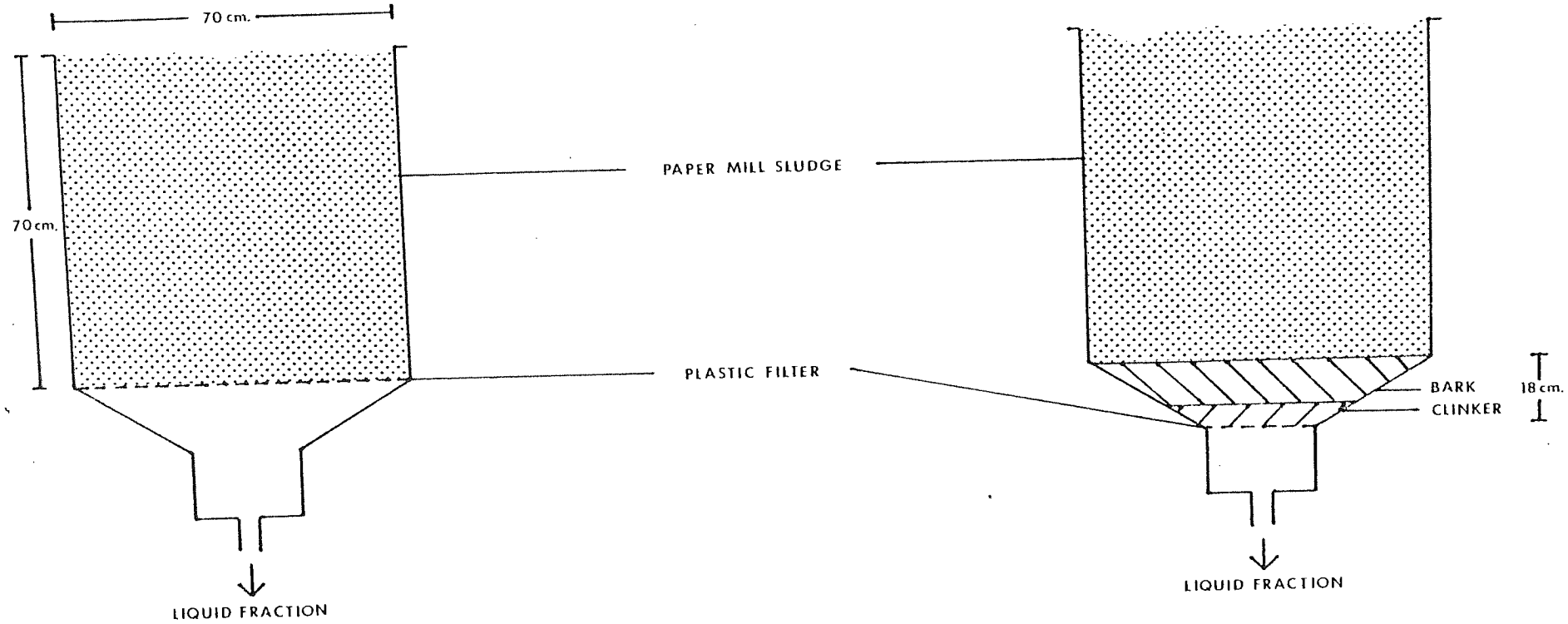
In most cases it was found that sludge deposits were not deeper than 0.5 metres. Below this depth previously deposited sludge or waste ground were usually encountered. Thus core samples were taken of the top 15 cm. and then from a depth of 30 cm. to 45 cm.. Core samples were stored in situ. at 4°C. whilst analyses were completed.

## 3.3 ACCELERATED DRAINAGE OF FRESHLY DEPOSITED PMS.

A sample of freshly deposited sludge was transported to the University of Aston where it was subjected to different drainage treatments aimed at accelerating the on-site ageing process of the deposited waste.

Two 70 L. polypropylene funnels (Materials Handling Products Limited) were supported upright in a dexion frame (see Figure 3.1). The funnels were fitted with plastic filters constructed from plastic sheet, which was bored at regular intervals with a 10 mm. cork borer. In addition, one of the funnels was fitted with a filter medium comprised of processed pine and spruce bark which is removed from logs at the Kemsley site, plus clinker waste from the mill boilers.

FIGURE 3.1 DRAINAGE OF FRESHLY EXCAVATED PMS AT ASTON UNIVERSITY.



The drainage treatments were:

1. Raw sludge drained through plastic filter.

2. Raw sludge drained through plastic filter + bark/clinker filter. The bark/clinker filter consisted of 6 Kg. fresh clinker in a layer 6 cm. deep directly above the plastic filter, with 5 Kg. of bark in a layer 12 cm. deep above the clinker.

## 3.4 LABORATORY EVALUATION OF PMS AS A CASING IN MUSHROOM CULTURE.

### 3.4.1 Source and preparation of substrates.

Commercially produced compost was obtained either already colonised by A.bisporus, or after pasteurisation. In the latter case the compost was inoculated with a commercially manufactured grain inoculum (spawn) of A.bisporus, at the rate of 10g. spawn to 1 Kg. compost, and then incubated at 25°C. until completely colonised. Unless stated a white strain, 649, of A.bisporus was used in growing trials.

Medium grade Irish Sphagnum peat was obtained in bale form and peat casing was prepared by wetting the peat to approximately 70% moisture by weight, and then mixing 4 parts wetted peat to 1 part chalk by weight, giving a casing p H of 7.5.

Paper mill sludge (PMS) was forced through a 10 mm. mesh sieve to give a standard particle size range of 10 mm. to dust for its evaluation as a casing material. Its moisture content was then raised to 70% by weight, and sufficient chalk was added to give a p H of 7.5.

Early cultural studies demonstrated the need to pasteurise PMS casings. An apparatus was devised for the pasteurisation of casing with a steam/air mixture. This apparatus, depicted in plate 3.1, allowed temperatures up to 80°C. to be attained rapidly in a casing sample, and maintained with good accuracy. A laboratory autoclave was used to provide steam, which was passed into a steel drum. Air, supplied by an Edwards air pump at the fixed rate of 5 L./min., was introduced into the bottom of the drum where it mixed with the steam supply. The drum was sealed save for a circular aperture at the top, which was ringed by a rubber gasket. The casing sample was placed in a soil pasteurising bucket (Nobles, Wellingborough Limited) which fitted tightly round the gasket. The steam/air mixture passed through the casing via a perforated, steel cone in the bottom of the bucket. The temperature of the mixture was adjusted by means of a bleed pipe on the steam line. When a maximum of 6 Kg. of casing were placed in the bucket, the temperature of the whole sample was raised to that required fifteen minutes from the introduction of the steam/air mixture. From the work of Baker (1967) and Schisler and Wuest (1971) a pasteurisation treatment of 65°C. for one hour was chosen. The period of one hour was timed from the moment the whole sample reached 65°C..

PLATE 3.1 THE LABORATORY STEAM/AIR PASTEURISATION APPARATUS.

By positioning thermometers at six points throughout the sample, the casing temperature during pasteurisation was found not to deviate from 65°C. by more than 1°C. at any one point.

### 3.4.2 The culture cabinets.

When colonised by the mycelium of A.bisporus compost was filled at the rate of 1 Kg. into 18.5 cm. x 18.5 cm. polypropylene pots or 500g. into 13 cm. x 13 cm. polypropylene pots, which had previously been sterilised at 121°C. for 15 minutes. The compost was then covered to a depth of 3 cm. with either peat or a test casing material. The pots were placed in specially constructed culture cabinets (see plate 3.2) which were designed to maintain a uniform environment during culture. Each culture cabinet consisted of a fibre glass tray filled with sand, on top of which was placed a wooden frame to support the growing pots. The whole was covered by a rectangular perspex hood fitted with electrical air heating cables. By adjustment of the air heating cables and addition of water to the sand, the environment within the growth cabinets was maintained at 25°C. $\pm$ 1°C. and 85% - 90% relative humidity until the mycelium reached the surface of the casing. Thereafter aeration and a temperature of 16°C. were provided by introducing previously cooled air into the centre of the cabinet. Relative humidity was maintained at 75% - 80%, and water was applied to the casing surface as required.

Figure 3.2 shows the plan layout of growing pots or units within a cabinet.

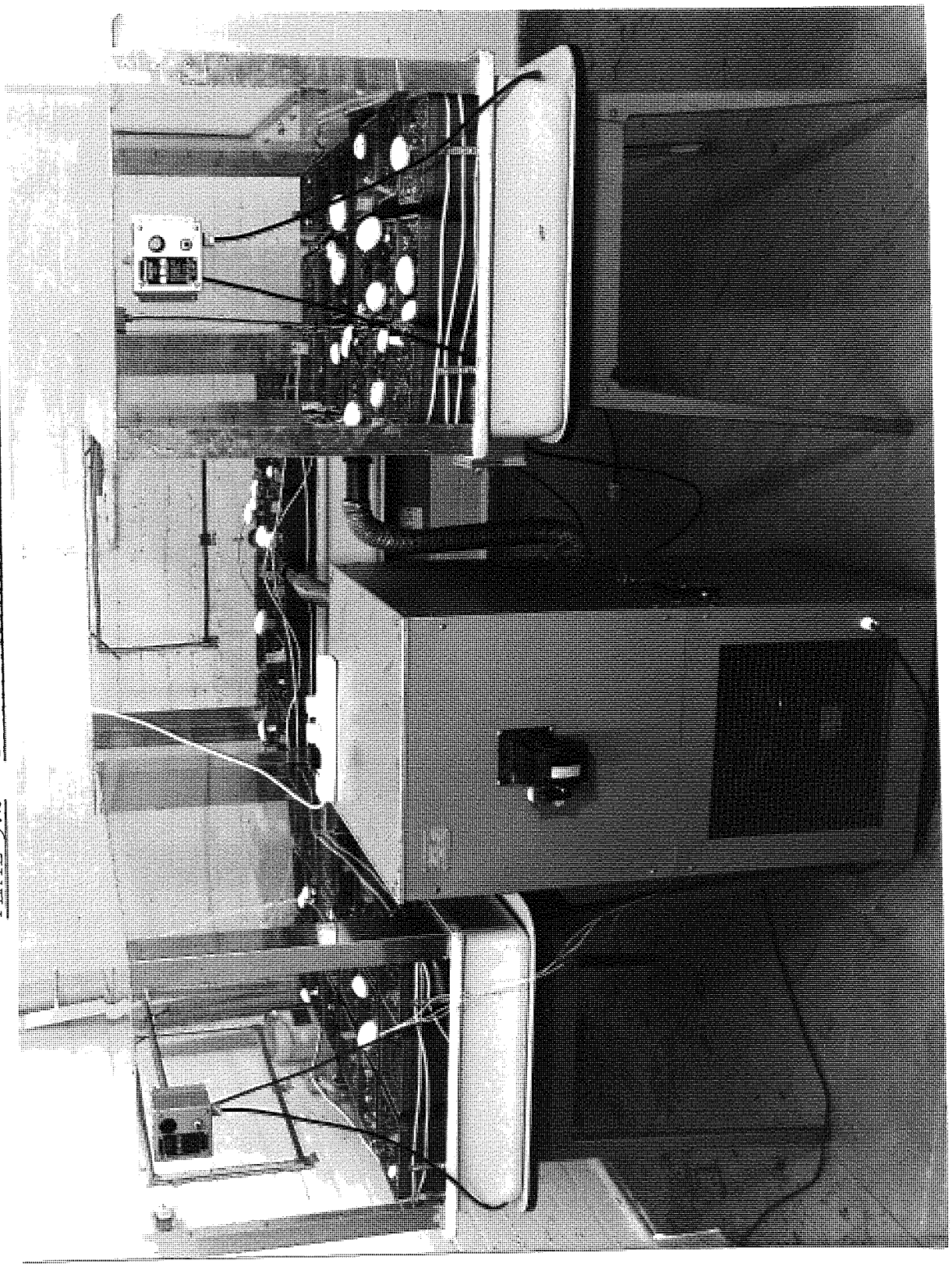


PLATE 3.2 THE LABORATORY CULTURE CABINETS.

FIGURE 3.2 PLAN LAYOUT OF 13 cm.$^2$ CULTURE UNITS WITHIN A CULTURE CABINET.

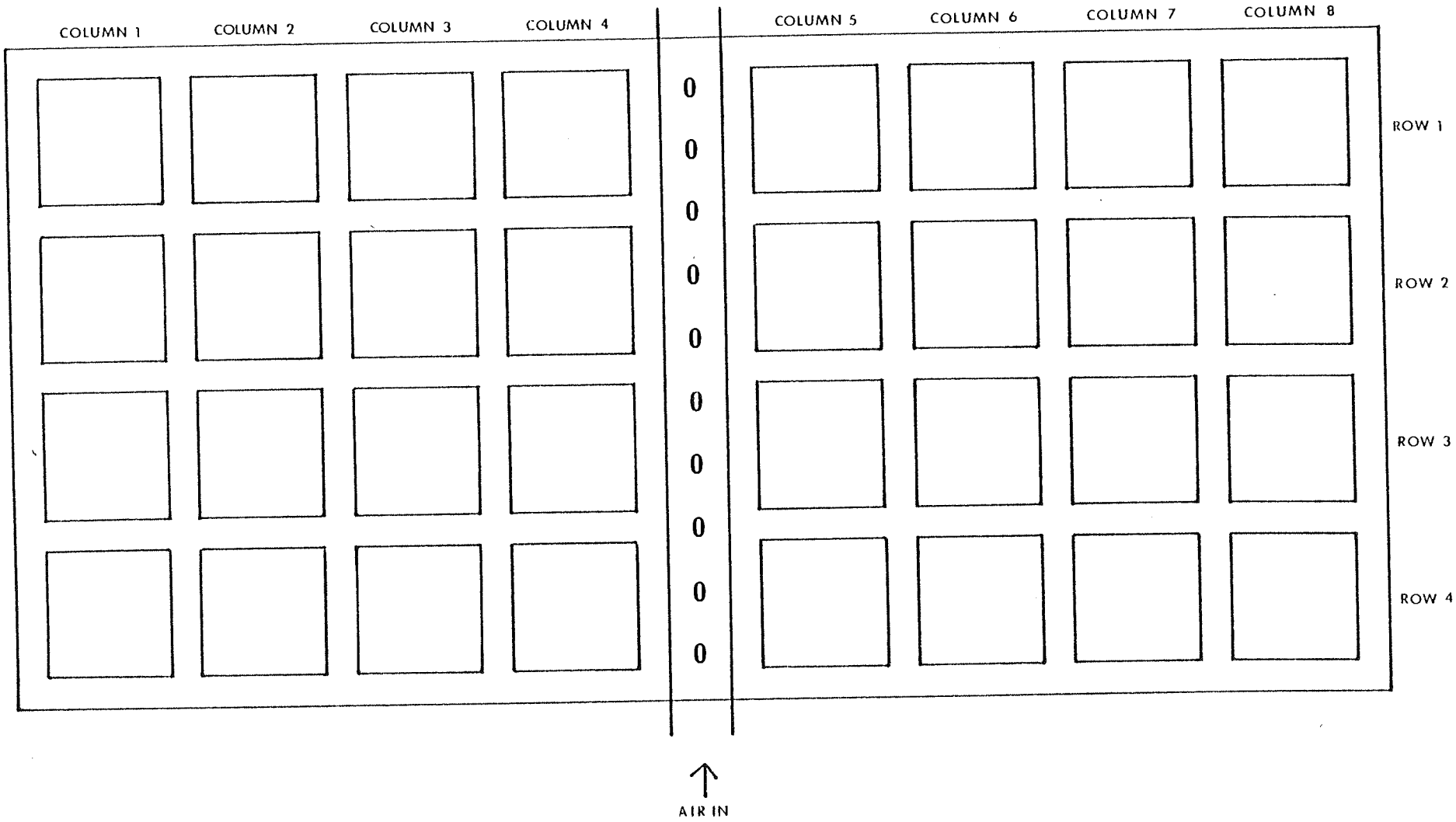


PLATE 3.3 LABORATORY APPARATUS FOR AXENIC CULTURE OF A.BISPORUS

1. AIR FILTRATION AND HUMIDIFICATION.

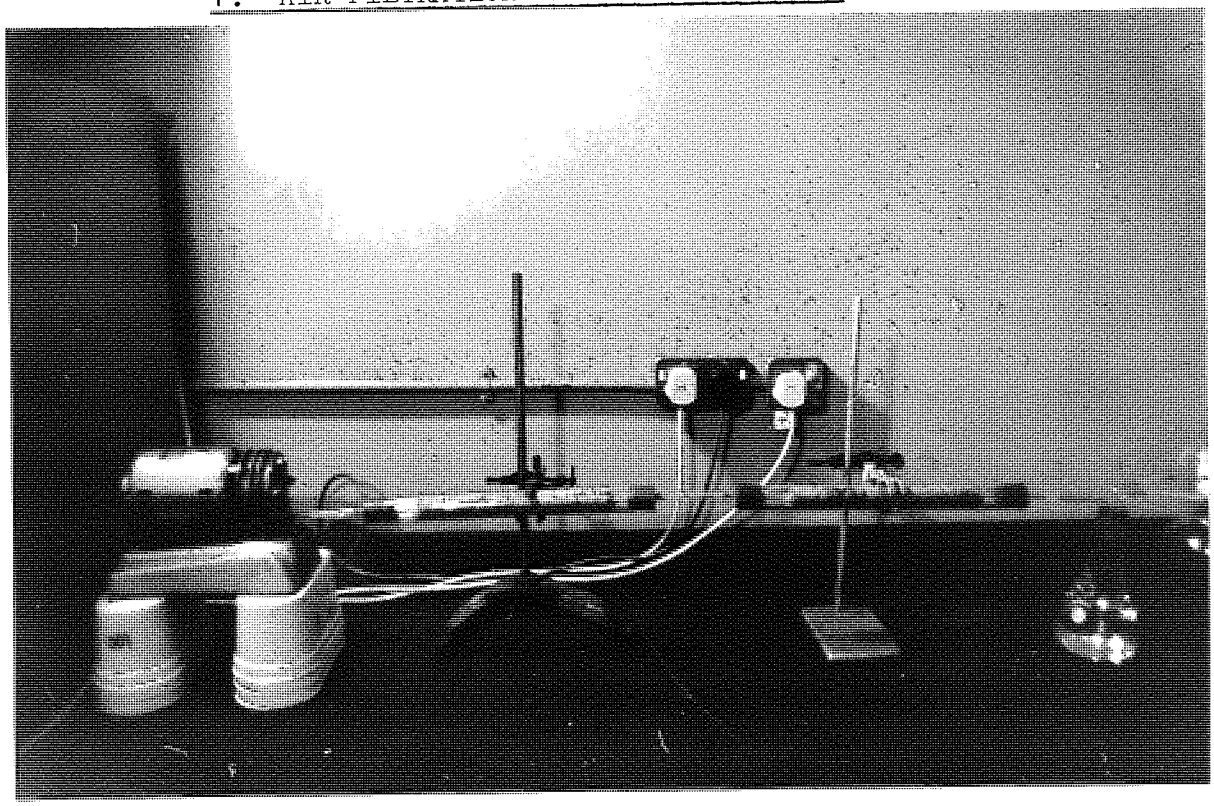


PLATE 3.4 LABORATORY APPARATUS FOR AXENIC CULTURE OF A.BISPORUS

2. THE CULTURE FLASKS.

This air passed through two flasks containing sterile, de-ionised water to maintain a satisfactory humidity in the culture flasks. The air pump provided two air changes in each flask per day.

The joining of five replicate flasks together allowed their removal at intervals during experiments without affecting conditions in other flasks, and provided a satisfactory number of replicates for analyses. Before substrates were analysed, they were plated onto malt and nutrient agar to confirm the required culture conditions had been maintained. Less than 3% of flasks became contaminated during culture.

Later studies involved the use of all possible combinations of pasteurised and sterilised substrates, with A.bisporus present and absent. For this a large number of flasks were used and three modifications to the apparatus were required. A larger air supply was provided by an air compressor, and was filtered through a sterile Whatman gamma - 12 filter as this reduced air flow to a lesser extent than cotton wool filters. Finally, the cotton wool plugs in the flasks were covered with molten wax which on hardening prevented air loss from individual flasks. Without this final amendment, air flow through the last two flasks in each series was inadequate.

### 3.4.4 Recording and harvesting procedures.

Three parametres were recorded from each growing pot or unit during all evaluation trials:

(1) The number of pinheads or fruitbody initials formed prior to the first flush or crop of fruitbodies.

(2) The number of fruitbodies harvested.

(3) The weight of fruitbodies harvested.

A pinhead was defined as a solid mycelial aggregate of diameter greater than 2 mm.. Counts were done five days after the appearance of the first initials to ensure that no further pinheads were still to develop. Only those initials which were visible without disturbing the casing were counted. Counts of fruitbody initials were only performed in trials where the rate of mycelial colonisation of the casing was the same in all treatments. Where this was not so, pinheads tended to develop below the surface in some treatments and thus gave erroneously low results. It was not possible to accurately count the initials which developed prior to the second and subsequent flushes as harvesting procedures caused the casing surface to become uneven.

When grown under commercial conditions the cultivated mushroom, A.bisporus, produces fruitbodies in successive crops separated by fairly regular intervals, each crop being referred to as a flush or break. In this study the number of mushrooms harvested and yield of mushrooms are given at five days after the end of the first flush and at ten day intervals thereafter. In so doing the cumulative results approximate closely to the flushing pattern, and statistical analysis of the data was facilitated at regular intervals. Yield is expressed as Kg. fresh fruitbodies per Kg. fresh compost.

Fruitbodies were harvested at the open cup stage of maturity. An open cup in this study follows the definition of Flegg and Gandy (1956), which covers the developmental range from the time of a visible break occuring in the veil covering the lamellae, until the outer surface of the pileus is still just visible from directly below. Plate 3.5 shows the two extremes of the range defined as open cup. The developmental time from the early stage to the later one was approximately twenty four hours, which facilitated a regular programme of harvesting once every day.

Fruitbodies were weighed after trimming off the base of the stipe at the soil line.

### 3.4.5 Sampling of substrates for analytical purposes.

Where required three cores were taken of the casing from each pot with a 10 mm. diameter core sampler, and combined to give a sample for subsequent analysis.

Compost samples were obtained by carefully removing the casing and thoroughly mixing the underlying compost before removal of a suitable quantity for analysis.

### 3.4.6 Measurement of sporophore characters.

#### (i) Dry weight

Individual sporophores of known fresh weight were dried to constant weight at 105°C..

PLATE 3.5 THE LIMITS OF FRUITBODY DEVELOPMENT COVERED BY THE RANGE 'OPEN CUP'.

(ii) Whiteness (% reflectance)

The whiteness of the pilei of harvested sporophores was determined according to the method of Gormley and MacCanna (1967):

Percentage white light reflected from the pileus surface of fruitbodies was measured using a mark III reflectometer head (Evans Electroselenium Limited - EEL) connected to a unigalvo 20 (EEL). The instrument had a measuring aperture of 13 mm. and a light spot of 8.5 mm.. The galvanometer was calibrated to 100% reflectance against a block of magnesium carbonate, and to 0% reflectance by removing the lead to the reflectometer head. Reflectance readings were taken at the centre of the upper surface of the pileus as this was the only site which was flat enough to preclude entry of extraneous light during measurements. Fruitbodies with dimpled or uneven pilei were not used in reflectance studies.

(iii) Sporophore dimensions

Determination of sporophore dimensions followed the method described by Flegg and Gandy (1956). They defined a normal sporophore as lying between set limits for the ratios of pileus diameter to total fruitbody height, and pileus diameter to stipe width.

In this study, pileus diameter was determined by taking the mean of two measurements made at right angles to one another. Similarly, two measurements of stipe width made at right angles to one another were used to obtain a mean result for that parameter. Measurement of stipe diameter was made at the mid point of the stipe. All sporophore dimensions were of fruitbodies harvested at the open cup stage.

## 3.5 COMMERCIAL-SCALE EVALUATION OF PMS AS A CASING MATERIAL.

Two growing trials were done at the research and development section of W. Darlington and Sons Limited, Angmering, Sussex. Each growing unit comprised a commercial-scale wooden tray 13.1 m. x 19.7 m.. Casing was applied to the surface of colonised compost to a depth of 4 cm.. A.bisporus strain 649 was used in both trials, and PMS was prepared as in 3.4.1. PMS casing was applied untreated, pasteurised using steam/air, or fumigated with methyl bromide.

Casing was pasteurised by passing a mixture of steam and air up through 100 Kg. of material in a polypropylene bin. This was achieved by supporting the casing on a steel mesh and running a line of combined steam and air into the container underneath the mesh. The mixture then percolated up through the material. A temperature of 65°C. for one hour was maintained throughout the sample.

Fumigation with methyl bromide was done in a sealed polypropylene bin. 100 Kg. of casing were fumigated at the minimal concentration x time product of 600 oz. hours per 1000 cu. ft. at a temperature of 70°F., as recommended by Tunney (1972).

Peat control casing was prepared as the standard mixture at the research division with the following composition:

6 parts peat : 1 part chalk by volume + water to 68% - 70% moisture.

Monitoring and management of the trials was according to standard practice at the development section.

## 3.6 STATISTICAL ANALYSIS OF RESULTS.

Two statistical tests were used for analysis of data from culture trials:

### 3.6.1 Analysis of variance. (Bishop, 1971)

All results were subjected to analysis of variance. This test showed whether any significant difference existed among treatment means. It did not reveal where the significant difference was.

The variance ratio (F) was calculated as follows:

$$F = \frac{\text{between treatments mean square}}{\text{error mean square}}$$

3.6.2 Least significant difference. (Chou, 1975)

When the analysis of variance indicated the existence of a significant difference, estimation of the least significant difference (LSD) was used to locate which of the means caused the difference. LSD is defined as the smallest difference which can exist between two significantly different means. It was calculated as follows:

$$LSD = \sqrt{\frac{2}{n} \times EMS \times F_{n_1\ n_2}}$$

$n$ = number of replicates

EMS = error mean square

$F_{n_1\ n_2}$ = F value at $P = 0.05$, where $n_1 = 1$ and $n_2$ = degrees of freedom for the error.

## 3.7 ANALYTICAL TECHNIQUES.

Results from analytical procedures are the means of three separate determinations in 3.7.3 and 3.7.5, and five separate determinations for other techniques.

### 3.7.1 Dry weight.

Between 10g. and 15g. of fresh sample were weighed into a weighed, dry beaker and dried to constant weight at 105°C..

The beaker plus dried sample were cooled in a desiccator and weighed.

$$\text{Dry weight \%} = \frac{\text{oven-dry weight (g.)} \times 100}{\text{initial sample weight (g.)}}$$

3.7.2 Pore space and water holding capacity.

The method for pore space and water holding capacity was adapted from Flegg (1951).

Between 25g. and 40g. of fresh substrate were loosely filled to a depth of 10 cm. into a glass cylinder, covered at one end with a double thickness of fine mesh nylon gauze. The glass cylinder was of known dry weight. The cylinder was then immersed in water up to the level of the substrate and allowed to stand for two hours. A weighed, dry plastic cap was then fitted to the bottom of the cylinder under water and the cylinder removed and weighed. From the weighings so far was calculated a value for the saturated weight of the sample. The plastic cap was then removed and the sample allowed to drain freely until no further water was lost. The cylinder plus sample were then weighed and a value for the drained weight of the sample was calculated. Finally the cylinder plus drained sample were dried to constant weight at 105°C. and a value for the dry weight of the sample determined.

$$\text{Pore space \%} = \frac{(\text{saturated wt. (g.)} - \text{fresh wt. (g.)}) \times 100}{\text{drained wt. (g.)}}$$

$$\text{Water holding capacity \%} = \frac{(\text{drained wt. (g.)} - \text{dry wt. (g.)}) \times 100}{\text{drained wt. (g.)}}$$

3.7.3 Moisture retention properties.

The retention of water by casing materials was measured at three different suction pressures corresponding to p F values of 0, 1.7 and 4.2. Maximum moisture retention at p F 0 is identical with the value for water holding capacity as determined in 3.7.2. Measurement of moisture retention at p F 1.7 was according to the sand-box method described by Stackman, Walk and Van der Horst (1969). The sample was pre-soaked to its water holding capacity and then placed on a sand bath designed to supply a suction pressure equivalent to 50 cm. of water. Suction was continued until the point was reached when the sample sucked back a small weight of water, indicating it had reached its maximum moisture retention at that pressure. This point was determined by daily dry weight measurements of the sample under suction.

Determination of moisture retention at a pressure of 15 bars (p F 4.2) was achieved using a pressure membrane apparatus. Gaseous nitrogen was forced down through the untreated sample at a pressure of 15 bars. The sample was housed in a brass cylinder with a porous pot base to permit drainage of water out of the sample. The treatment was continued until no further water was lost from the sample.

The maximum moisture retention figures are expressed as the dry weight (3.7.1) of the sample after treatment.

3.7.4 Particle size distribution.

Particle size distribution of mill sludge was determined following destruction of the organic matter by ashing at 400°C. for ten hours. The method then followed that given in MAFF (1972).

10g. of ashed material were suspended in 500 ml. of de-ionised water containing 10 ml. of dispersing reagent. The dispersing reagent was prepared by dissolving 50g. of sodium hexametaphosphate and 7g. of anhydrous sodium carbonate in 1 L. of de-ionised water. The weight of the residue from 10 ml. of dispersing reagent was determined by evaporating the reagent to dryness on a hot plate.

The suspended material was allowed to stand overnight at 20°C. in a graduated cylinder. The contents of the cylinder were then thoroughly mixed for 30 seconds, and the 20 ml. sampling pipette was lowered to touch the surface of the liquid. 20 ml. volumes of the suspension were removed at times t (4 minutes 19 seconds), and T (7 hours 12 minutes) from time of agitation at a depth of 9 cm.. These samples were evaporated to dryness and the weight of the residues used to calculate particle size distribution on a weight basis:

(i) % particles less than 2 μm. in diameter =

$$\frac{100\ (25 \times \text{wt. residue (g.) at T})}{\text{original sample wt. (g.)}}$$

(ii) % particles 2 μm. - 20 μm. in diameter =

$$\frac{100\ (25 \times \text{wt. residue (g.) at t})}{\text{original sample wt. (g.)}}$$

(iii) % particles greater than 20 μm. in diameter =

100 - (i) + (ii).

3.7.5 Characterisation of clay minerals.

Clay minerals used as casing amendments were analysed by powder X-ray diffraction, using a Philips X-ray diffractometer model PW 1012/30. Conditions of machine operation were as follows:

| | | |
|---|---|---|
| Sample preparation | : | Untreated sample was compacted into an aluminium sample holder. |
| Radiation | : | Cobalt Kα with an Iron β filter. |
| X-ray tube settings | : | 30 mA. and 30 Kv. |
| Starting angle of scan | : | $3^{\circ}$; sample scanned through $60^{\circ}$. |
| Scanning speed | : | $1^{\circ}$ 2θ/minute. |
| Chart speed | : | 10 mm./minute. |

Identification of the minerals present was achieved by referring the peak heights obtained on the chart to the ASTM index.

### 3.7.6 Organic nitrogen.

Organic nitrogen was determined by the micro-Kjeldahl method, according to Markham (1942). The catalyst used for digestion was a mixture of potassium sulphate, copper sulphate and selenium at a ratio of 32 : 5 : 1 by weight.

0.2g. of finely ground, dry sample were placed in a digestion flask and 3 ml. of concentrated sulphuric acid plus the tip of a spatula full of catalyst were added. The mixture was heated on a digestion rack until the digest became colourless or pale green, and then further heated for one hour. After cooling the digest was washed into a Markham still with de-ionised water and made alkaline by the addition of 5 ml. of 40% NaOH (g/L.).

The ammonia released was collected in 10 ml. saturated boric acid solution, which was then titrated against 0.01 N. HCl, using Tashiro's indicator (methyl red 2.0g./L.; methylene blue 1.0g./L.; in absolute alcohol).

Organic nitrogen was calculated as: $\frac{\text{titre} \times 0.014}{0.2} = \text{g. \% N.}$

### 3.7.7 Ammonium and nitrate plus nitrite nitrogen.

Inorganic forms of nitrogen were extracted from fresh material according to the method in Allen *et al.* (1974):

25g. of fresh sample were shaken with 200 ml. 6% NaCl in a sealed container at 100 rpm for thirty minutes. The suspension was filtered through a No. 1 Whatman filter and 150 ml. of the filtrate was removed for determination of inorganic nitrogen.

Determination of ammonium and nitrate plus nitrite nitrogen was by the distillation method described in Allen et al. (1974), using a macro-Kjeldahl apparatus.

150 ml. of NaCl extract were transferred to the distillation flask and 0.2g. MgO were added. 50 ml. were distilled over and collected in 10 ml. saturated boric acid solution. The flask was then removed, 0.4g. Devarda's alloy (Approximately 45% Al, 50% Cu, 5% Zn) were added to the extract, and the flask immediately replaced. A further 50 ml. were distilled over and collected in 10 ml. saturated boric acid solution. The collected distillates were titrated against M/140 HCl using Tashiro's indicator (see 3.7.6). Ammonium nitrogen was determined from the first collected distillate and nitrate plus nitrite nitrogen from the second distillate.

$$\text{Extractable } NH_4^+ - N\ (\mu g./g.) = \frac{\text{Titre (ml.)} \times \text{extractant volume (ml.)} \times 10}{\text{aliquot (ml.)} \times \text{sample wt. (g.)}}$$

$$\text{Extractable } NO_3^- + NO_2^- - N\ (\mu g./g.)$$

Results were corrected for dilution and dry weight of the original sample.

3.7.8 Organic carbon.

Organic carbon was determined according to the wet oxidation/titration method of Tinsley (1950).

The 0.0675 M. dichromate mixture was prepared by adding 19.86g. $K_2Cr_2O_7$ and 200 ml. $H_3PO_4$ (s.g. 1.75) to 400 ml. $H_2SO_4$ and diluting to 1 litre with de-ionised water. 0.4 M. ferrous ammonium sulphate was prepared by dissolving 156.86g. $(NH_4)_2SO_4.FeSO_4.6H_2O$ in 100 ml. de-ionised water. 20 ml. of conc. $H_2SO_4$ were then added and the volume made up to 1 litre with de-ionised water. The indicator solution was 5g. $BaCl_2.2H_2O$ and 0.3g. barium diphenylamine-sulphonate dissolved in 100 ml. de-ionised water.

0.05g. of dried (105°C.), finely ground sample were weighed into a 250 ml. pyrex conical flask. 25 ml. of chromic acid mixture were added and the whole boiled for 1 hour, using cold finger condensers in the neck of the flask. The mixture was allowed to cool and then diluted with 100 ml. de-ionised water. 5 ml. of indicator solution were added and unused dichromate was titrated with ferrous ammonium sulphate solution. A further 2.5 ml. of dichromate mixture were added when the first colour change occurred and the titration was completed dropwise.

If T ml. ferrous ammonium sulphate were used in the titration, then

$$C\% = \frac{(27.5 - T)\ \text{ml.} \times 0.12}{\text{sample wt. (g.)}}$$

3.7.9 Carbon : Nitrogen ratio.

C : N ratio was calculated from the values obtained for organic carbon in 3.7.8 and organic nitrogen in 3.7.6.

Calculation:

$$\text{C : N ratio} = \frac{\text{g. organic carbon/100g. dry sample}}{\text{g. organic nitrogen/100g. dry sample}}$$

3.7.10 Determination of organic components.

Organic and mineral components of variously aged sludge samples were determined by a stepwise technique obtained by adapting the methods in Chang (1967), and Ellis, Matrone and Maynard (1946). The six fractions determined were:

(i) Water solubles
(ii) Ethanol-benzene solubles
(iii) Hemi-cellulose
(iv) Cellulose
(v) Ash content
(vi) Lignin

0.8 - 1.2g. of finely ground, dried sample were weighed onto a previously dried and weighed Whatman No. 41 ashless filter paper. The paper was then folded to enclose the sample, secured with a small paper clip and weighed after drying overnight at 105°C..

For fractions (i) - (v) the percentage in the initial sample was determined by the loss in dry weight at 105°C. of the filter plus sample after the extraction treatments applied. Percentage lignin was estimated as the dry material remaining after treatments for removal of fractions (i) - (v).

Extraction procedures:

(i) Water solubles

The water soluble fraction was removed by continuous extraction of the samples with de-ionised water in a soxhlet thimble for four hours. The method was adapted from that described in Chang (1967) for extraction of ethanol solubles.

(ii) Ethanol-benzene solubles

Removal of the ethanol-benzene soluble fraction was by the method of Chang (1967) except that absolute ethanol : benzene (1 : 2 by volume) was used for extraction instead of absolute ethanol. Extraction was achieved by continuous washing of the samples with 650 ml. of the ethanol-benzene mixture in a soxhlet thimble for four hours. Extracted samples were allowed to air-dry to permit evaporation of the extractant before being dried at 105°C..

(iii) Hemi-cellulose

Hemi-cellulose was removed by the KOH method of Chang (1967). After removal of the ethanol-benzene soluble fraction, the dried sample was carefully scraped off the filter paper using a steel spatula into a 250 ml. pyrex conical flask. 100 ml. of 24% KOH (w/v) were added and the sample shaken on an orbital shaking tray for four hours at 25°C.. The extracted sample was then filtered through a dried, weighed No. 41 Whatman filter paper. The filter plus sample were then dried at 105°C. to determine weight loss.

(iv) Cellulose

Removal of cellulose from the samples was by the method of Ellis, Matrone and Maynard (1946), with omission of the pepsin pre-digestion stage (Morrison, 1972).

After extraction with KOH, the dried sample was carefully scraped off the filter paper into a 500 ml. round-bottomed flask. 150 ml. 5% (w/v) $H_2SO_4$ were added and the digest was refluxed with gentle heat for one hour. The mixture was then cooled and filtered through a Whatman No. 1 filter paper. The residue was washed three times with 30 ml. of hot de-ionised water, air-dried and carefully scraped into a 250 ml. pyrex conical flask. 20 ml. 72% $H_2SO_4$ (w/v) were added and the mixture held at 20°C. for two hours with occasional, gentle agitation.

125 ml. de-ionised water were slowly added to the digest which was then filtered through a Whatman No. 1 filter paper, and washed with 20 ml. hot de-ionised water. The air-dried residue was carefully scraped into a 500 ml. round-bottomed flask and 150 ml. 3% $H_2SO_4$ (w/v) were added. The mixture was refluxed with gentle heat for two hours. After cooling the digest was filtered through a previously dried and weighed Whatman No. 41 filter paper and then washed three times with 20 ml. hot de-ionised water. The filter plus sample were then dried in an oven at 105°C..

(v) Ash content

Ash content of the sample after cellulose extraction was determined by ashing the dried filter plus sample at 500°C. for six hours (3.7.11). The final result was corrected for the ash content of the filter paper by ashing a filter paper separately.

(vi) Lignin

Lignin content of the initial sample was estimated as the dry weight after cellulose extraction minus the weight of ash (Ellis, Matrone and Maynard, 1946).

3.7.11 Ash content.

0.8 - 1.2g. of dried, finely ground sample in a dry porcelain basin of known weight was placed in a muffle furnace previously heated to 500°C.. The sample was heated for six hours, removed from the furnace and transferred to a desiccator. The cooled basin plus sample were weighed and ash content of the original sample calculated from loss on ignition.

$$\% \text{ ash} = 100 - \frac{(\text{original sample wt. (g.)} - \text{wt. after ignition (g.)})}{\text{original sample wt. (g.)}}$$

3.7.12 p H .

A suspension of 3g. fresh sample in 30 ml. de-ionised water was prepared and gently agitated for five seconds. The suspension was allowed to stand for one hour in a water bath at 20°C.. The p H of the suspension was then determined at 20°C. using a glass electrode connected to a Pye Unicam model 291 mk. II p H meter.

3.7.13 Electrical conductivity.

Conductivity or specific conductance is the reciprocal of the resistance offered by a solution with platinium electrodes immersed in it each 1 cm. square and 1 cm. apart. Electrical conductivity of a soil is therefore a measure of its dissolved salt content.

A variety of methods have been used by different authors for measuring conductivity of soils and similar materials, the soil to extractant ratio, extractant type, and pre-treatment of the soil being the variables. Three different techniques were compared in a preliminary investigation using the materials under examination in this study, according to the methods described in Baker (1957), Flegg (1958) and MAFF (1972). A summary of the methods and the results are presented in appendix 3.2. Problems were encountered with re-wetting peat casing in the method of MAFF. The method in Baker gave results comparable to the simple substrate-water suspension method of Flegg, which was adapted for use in this study. This method had the advantages of being rapid (large numbers of determinations were frequently required), and allowing p H to be determined on the same suspension.

Procedure:

3g. fresh sample were weighed into a clean, dry 100 ml. beaker and 30 ml. de-ionised water were added. The mixture was allowed to stand in a water bath at 20°C. for one hour, after which the conductivity of the suspension was measured using a portable conductivity measuring bridge type MC3 (Electronic Instruments Limited). The result was corrected for dilution and dry weight of the sample, and conductivity expressed as μmhos/cm..

3.7.14 Extraction of available elements.

Owing to difficulties in re-wetting substrates analysed in this study, all materials were extracted in a fresh condition.

In addition, it has been shown that drying can increase the levels of some available elements (Sherman and Harmer, 1943; Jackson, 1958). Determination of individual elements in extracts was as in 3.7.16. Results were corrected for dilution and dry weight of the extracted sample.

(i) Water extractable elements.

The readily soluble anions chloride and sulphate were determined on water extracts. In addition water was used as an extractant for availbale elements in some mushroom culture studies.

10g. of fresh sample were weighed into a 250 ml. conical flask. 100 ml. of de-ionised water were added and the suspension was shaken on an orbital shaking tray at 100 rpm for six hours. The mixture was then filtered through a Whatman No. 1 filter paper and collected in a 100 ml. volumetric flask. Sequential additions of small volumes of de-ionised water were made to the filtered solids until the volume in the volumetric flask was made up to 100 ml..

(ii) Ammonium acetate extractable elements.

Levels of available cations were determined on ammonium acetate extracts.

Whilst no one extractant is satisfactory for all elements, ammonium acetate is considered to be suitable for a wide range of cations by Allen et al. (1974), and the method given by those authors was followed:

25g. of fresh substrate were weighed into a 250 ml. beaker. 200 ml. M. $NH_4OAc$ (p H 7.0) were added, the mixture stirred and allowed to stand for ten minutes. The mixture was then filtered through a Whatman No. 1 filter paper and the filtrate collected in a 250 ml. volumetric flask. The substrate remaining on the paper was washed with a further 50 ml. M. $NH_4OAc$, which was allowed to filter. The filtered solids were then leached with successive small additions of M. $NH_4OAc$ until the volume of filtrate collected was 250 ml..

M. $NH_4OAc$ was prepared by adding 575 ml. glacial HOAc and 600 ml. 0.880 $NH_3$ to 300 ml. de-ionised water. The solution was mixed thoroughly and diluted to ten litres with de-ionised water.

Owing to the high calcium carbonate content of casing mixtures, these were extracted with M. $NH_4OAc$ at a p H of 9.0 as relatively little calcium carbonate is dissolved at this p H (Allen et al., 1974). Excessively high calcium levels can interfere with determination of other elements in solution by atomic absorption spectrophotometry.

M. $NH_4OAc$ at p H 9.0 was prepared by taking 740 ml. $NH_3$ solution instead of 600 ml., but otherwise proceeding as for $NH_4OAc$ at p H 7.0.

(iii) Acetic acid extractable phosphate.

Available phosphate levels in sludge samples were determined on acetic acid extracts.

The method given in Allen et al. (1974) was followed. Acetic acid 2.5% (v/v) was prepared by diluting 250 ml. glacial HOAc to 10 litres with de-ionised water. The extraction procedure followed was as for M. $NH_4OAc$.

(iv) Potassium pyrophosphate extractable iron.

Using ammonium acetate results obtained for available iron were rather low considering the total values (Figure 4.14). Allen et al. (1974) considered other extractants to be more suitable for available iron, and as this element was studied in some detail during culture trials, extracts were made with potassium pyrophosphate. This extractant was recommended for available iron by Bascomb (1968), as it caused least breakdown of organic material.

10g. of fresh sample were weighed into a 250 ml. conical flask and 100 ml. 0.1 M. potassium pyrophosphate (p H 10.0) were added.

The procedure was then as for water extractable elements.

3.7.15 Total elemental levels.

Total levels of elements were determined on mixed acid digests. The method followed that given in Allen et al. (1974) with the omission of conc. $H_2SO_4$ from the digestion mixture. This omission facilitated the determination of sulphate on the digests and prevented the formation of sulphate compounds of relatively insoluble elements, which would have interfered with their determination by atomic absorption spectrophotometry.

0.18g. - 0.22g. of finely ground, dried (105°C.) sample were weighed into a 100 ml. Kjeldahl flask. 5 ml. conc. $HNO_3$ were added to the sample, which was allowed to stand for one hour. After that time 1 ml. 60% $HClO_4$ was added and digestion was proceeded at moderate heat, increasing later. The digestion was continued for fifteen minutes after the appearance of white fumes. The digest was allowed to cool for 30 minutes, and then 20 ml. de-ionised water were added and the digest boiled for ten minutes to bring iron fully into solution. The cooled digest was filtered through a Whatman No. 1 filter paper and made up to 50 ml. volume with de-ionised water. Individual elements were determined in the digest as in 3.7.16.

## 3.7.16 Estimation of individual elements.

### (i) Cations

Levels of cations in prepared solutions were determined by atomic absorption spectrophotometry, using a Perkin Elmer atomic absorption spectrophotometer type 460. The operating conditions for the determination of each element are given in appendix 3.3.

Sodium and potassium levels were also determined by flame emmission photometry using an Evans Electroselenium Limited flame photometer mark II.

### (ii) Chloride

Chloride levels were determined by automatic colourimetric titration using a type 92 chloride meter (Evans Electroselenium Limited). The machine operates via a current flowing between two silver electrodes immersed in the sample solution. The current flow precipitates chloride as silver chloride. On precipitation of all of the chloride there is a marked change in electrical conductivity of the solution which is registered by two sensing electrodes. The sensing electrodes stop an automatic timer which is calibrated in mg. Cl/L..

10 ml. sample were placed in a small beaker. To the sample were added 2 ml. acid buffer and 5 drops of gelatine indicator solution. The electrodes were immersed in the solution which was stirred by a small magnetic bar, and the current applied.

The acid buffer was prepared by adding 46 ml. glacial HOAc and 3 ml. conc. $HNO_3$ (s. g. 1.42) to 500 ml. de-ionised water. The mixture was made up to 1 L. with further de-ionised water.

The thymol blue gelatine indicator solution was prepared by adding 7.5g. gelatine to 250 ml. de-ionised water and slowly bringing to the boil. 100 ml. 0.5% (w/v) thymol blue in methanol were slowly added, followed by 0.5g. thymol as a preservative. The whole was made up to 500 ml. with methanol.

(iii) Phosphate

Phosphate was determined on a Technicon auto analyser using the method of Lundgren (1960). In this method total inorganic phosphates in solution are converted to orthophosphate by means of hydrolysis with 10 N. sulphuric acid. The phosphate concentration is then determined by the reduction of phosphomolybdic acid with aminonaptholsuphonic acid.

Reagents were prepared as follows:

Aminonaptholsulphonic acid: 120g. $NaHSO_3$ and 4g. $Na_2SO_3$ were dissolved in 800 ml. warm de-ionised water. 2g. 1 - amino - 2 - napthol - 4 - sulphonic acid ($C_{10}H_9NO_4S$) were added and the solution mixed well. The mixture was diluted to 1 litre with de-ionised water.

Ammonium molybdate reagent: 10g. $(NH_4)_6\ Mo_7O_{24}.4H_2O$ were dissolved in 1 L. 1.2 N. $H_2SO_4$.

(iv) Sulphate

Levels of sulphate in sample solutions were determined on a Technicon auto analyser using an automated procedure adapted from the method described by APHA (1965). In this method the sample is reacted with barium chloride in an acid medium to form barium sulphate. At low concentrations this sulphate precipitate exists as a suspension of fine particles. The turbidity of the suspension was measured colourimetrically at 520 nm. according to the method of Rossum and Villaruz (1961).

Reagents were prepared as follows:

Buffer (p H 10.1): 6.75g. $NH_4Cl$ were dissolved in 500 ml. de-ionised water. 57 ml. conc. $NH_4OH$ were added and the solution diluted to 1 L. with de-ionised water.

Buffered E.D.T.A.: 40g. tetrasodium E.D.T.A. were dissolved in 1 L. buffer.

Barium chloride reagent:

Solution A: 1g. powdered U.S.P. gelatin was dissolved in 400 ml. de-ionised water. Two crystals of thymol were dissolved in the solution which was made up to 1 L. with de-ionised water and allowed to stand for two days.

Solution B: 20g. $BaCl_2$ and 60 ml. N. HCl were added to 300 ml. de-ionised water.

Solutions A and B were combined and diluted to 1 L. with de-ionised water.

### 3.7.17 Cation exchange capacity.

The cation exchange capacity of substrates was determined by the method in Allen *et al*. (1974).

25g. of fresh sample were extracted with 250 ml. M. ammonium acetate (p H 7.0 or 9.0) as described in 3.7.14. Excess extractant was removed from the residue by repeated washings with industrial alcohol, 60% (v/v). To indicate when the alcohol washing was complete, 10 ml. 10% $NH_4Cl$ (w/v) were added to the first portion of industrial alcohol. The filtrate was continuously tested with $AgNO_3$ solution until no trace of chloride was detected. The residue was then leached with successive additions of 30 ml. 5% KCl (w/v) to displace absorbed $NH_4^+$ - N. Leaching was continued until 250 ml. had been collected. The $NH_4^+$ - N content in the leachate was determined by distillation as described in 3.7.6.

If T ml. of N/100 HCl were required for titration then:

$$\text{C.E.C. (me./100g.)} = \frac{\text{T (ml.)} \times \text{final leachate vol. (ml.)}}{\text{aliquot (ml.)} \times \text{sample wt. (g.)}}$$

The results were corrected for dry weight of the initial sample.

3.7.18 Estimation of fungal populations.

(i) Preparation and formulation of agar media.

Malt extract agar

Boots pure malt extract 20.0g.; oxoid agar No. 3 15.0g.; Rose bengal solution (0.3g./L.) 100 ml.; de-ionised water to 1 litre.

The whole was autoclaved at 121°C. for five minutes, dispensed into 100 ml. volumes and then sterilised by autoclaving at 121°C. for fifteen minutes.

Paper mill sludge (PMS) extract agar

Extract agars were prepared from sludge deposited for 0, 6, 18 and 36 months. Core samples taken from the surface were used to prepare the extracts for the three latter samples. In the case of sludge at deposition (0 months) 1 Kg. of sludge as deposited was used. The method of James (1958) was followed to prepare the extracts:

1 Kg. of fresh PMS was autoclaved with 1 litre of de-ionised water at 121°C. for twenty minutes. The liquid was strained through fine cotton muslin. To the filtrate were added: oxoid agar No. 3 15.0g.; Rose bengal solution (0.3g./L.) 100 ml.; de-ionised water to 1 litre.

The whole was autoclaved at 121°C. for five minutes, dispensed into 100 ml. volumes and then sterilised by autoclaving at 121°C. for fifteen minutes.

Cellulose agar

Cellulose agar was prepared according to the method of Eggins and Pugh (1962):

Ammonium sulphate 0.5g.; L - Asparagine 0.5g.; potassium dihydrogen phosphate 1.0g.; potassium chloride 0.5g.; magnesium sulphate ($.7H_2O$) 0.2g.; calcium chloride 0.1g.; difco yeast extract 0.5g.; oxoid agar No. 3 15.0g.; ball milled cellulose 10.0g.; Rose bengal solution (0.3g./L.) 100 ml.; de-ionised water to 1 litre.

The cellulose was prepared as a 4% suspension in water of Whatman's standard grade cellulose powder for chromatography, ball milled for 72 hours.

The whole was autoclaved at 121°C. for five minutes, dispensed into 100 ml. volumes and then sterilised by autoclaving at 121°C. for fifteen minutes.

Before use the agars were melted at 121°C. for five minutes and cooled to 60°C.. A solution of chlortetracycline previously sterilised by filtering through a 0.2μ seitz filter, was added at the rate of 1 ml. of solution to 99 ml. of cooled, molten agar to give a final concentration of 6μg. of chlortetracycline per ml. agar. Chlortetracycline in combination with rose bengal was used to suppress bacterial growth in the agar cultures. Rose bengal was also added to restrict the spreading of fungal colonies (Hayes, 1969).

(ii) Isolation of fungi

The number of viable fungal propagules occuring in deposited sludge samples was determined using the dilution plate count method.

10g. of fresh PMS were added to 100 ml. of quarter strength Ringer's solution. The suspension was shaken on an end over end shaker at 100 rpm for five minutes. A series of ten fold dilutions of the suspension were made by pipetting 1 ml. aliquots into 9 ml. of sterile, quarter strength Ringer's solution. (Prior to each pipetting operation the liquid to be transferred was sucked into and blown out of the pipette ten times in order to saturate the absorption sites on the pipette wall, and to ensure further dispersion). 1 ml. aliquots of selected dilutions were transferred to 7.5 cm. diameter Petri dishes and covered with 9 ml. of molten agar. The whole was thoroughly mixed by gentle swirling and allowed to set.

Five replicate dilution series were prepared for each original sample to provide five replicates of each plate at each dilution.

Plates were incubated at 25°, 45° or 52°C. and observed daily for development of fungal colonies.

Two days after the appearance of the first colonies, the plates were examined for colony numbers and species of fungi present.

(iii) Identification of fungal isolates.

Fungal isolates were identified by reference to Barnett and Hunter (1972), Cooney and Emerson (1964), Gilman (1957), Haard (1968), Raper and Fennel (1965), Raper and Thom (1968) and Rifai (1969).

# SECTION 4

# EXPERIMENTAL AND RESULTS

## SECTION 4 EXPERIMENTAL AND RESULTS.

### 4.1 VISUAL APPRAISAL OF PMS DEPOSITS AT THE KEMSLEY MILL.

Mill effluent which has been removed from the primary treatment or settling lagoon is deposited as a semi-solid sludge on waste ground adjoining the mill complex (plate 4.1). Following its deposition the sludge dries out and becomes reasonably firm after about six months. As the sludge dries it contracts, and by four months large fissures appear in its surface. These eventually develop into trenches several feet deep (plate 4.2), which remain comparatively moist even when the surface dries out during the summer months.

The fruitbodies of higher fungi develop on the sludge beds some three to six months after their deposition (plate 4.3). They are present throughout the year, but are most abundant during the spring and autumn months.

Higher fungi disappear from the waste fourteen to sixteen months from its deposition and shortly before colonisation by green plants commences.

Grasses are frequently the primary plant colonisers of the solids, but a wide range of species soon develop. The diversity of plant types decreases after the initial colonisation, sludge three or more years in age being overgrown by grasses, reeds and a few bushes (plate 4.4).

PLATE 4.1 SLUDGE FRESHLY DEPOSITED AT THE KEMSLEY SITE.

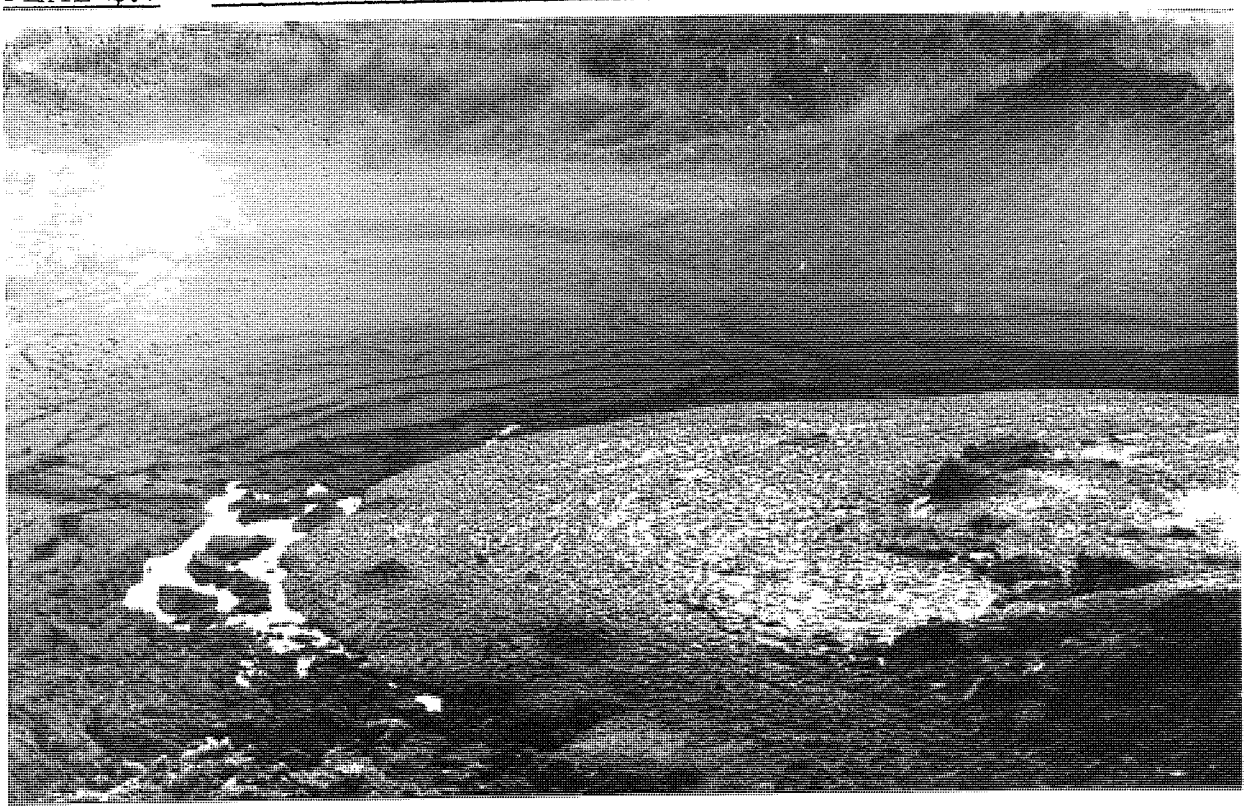


PLATE 4.2 AN AGED SLUDGE DEPOSIT SHOWING THE DEVELOPMENT OF DEEP TRENCHES.

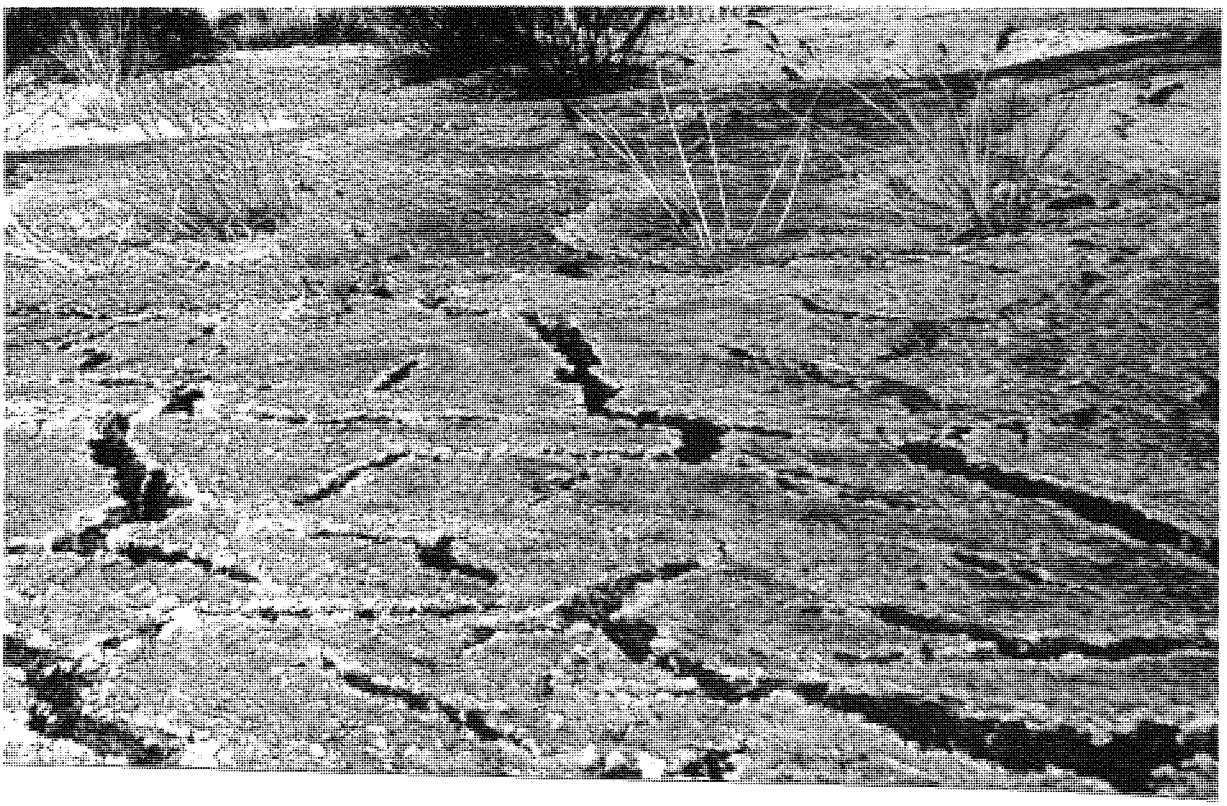


PLATE 4.3 FRUITBODIES OF STROPHARIA SEMIGLOBATA GROWING ON AGED SLUDGE.

PLATE 4.4 A SLUDGE DEPOSITION SITE LARGELY OVERGROWN BY HIGHER PLANTS.

## 4.2 PHYSICAL AND CHEMICAL ANALYSES OF PMS SAMPLES.

Samples of freshly deposited and aged PMS were taken in April 1976 for physical and chemical analyses. Core samples were taken at two depths from aged sludge deposits as described in 3.2.3. PMS deposits one and a half, three, six, nine, twelve, eighteen and thirty six months in age were sampled.

For the determination of particle size distribution, organic components and individual elements, fresh sludge and top cores from the samples six, eighteen and thirty six months after deposition only were used.

### 4.2.1 Dry weight.

The dry weight of PMS freshly excavated from the settling lagoon was approximately 8%, but rapid drying out caused it to increase to 16% two months after deposition, and approximately 25% at twelve months (figure 4.1). As would be expected the top 15 cm. of the sludge beds were drier than the deeper samples, though the difference was generally only a few per cent..

### 4.2.2 p H .

The p H of PMS as excavated from the settling lagoon was 6.3. Up to nine months after its deposition there was little change in the p H value of the sludge, but thereafter it declined steadily, falling below 4.0 by the time the deposits had aged for eighteen months (figure 4.2).

FIGURE 4.1 DRY WEIGHT OF PMS WITH TIME FROM DEPOSITION.

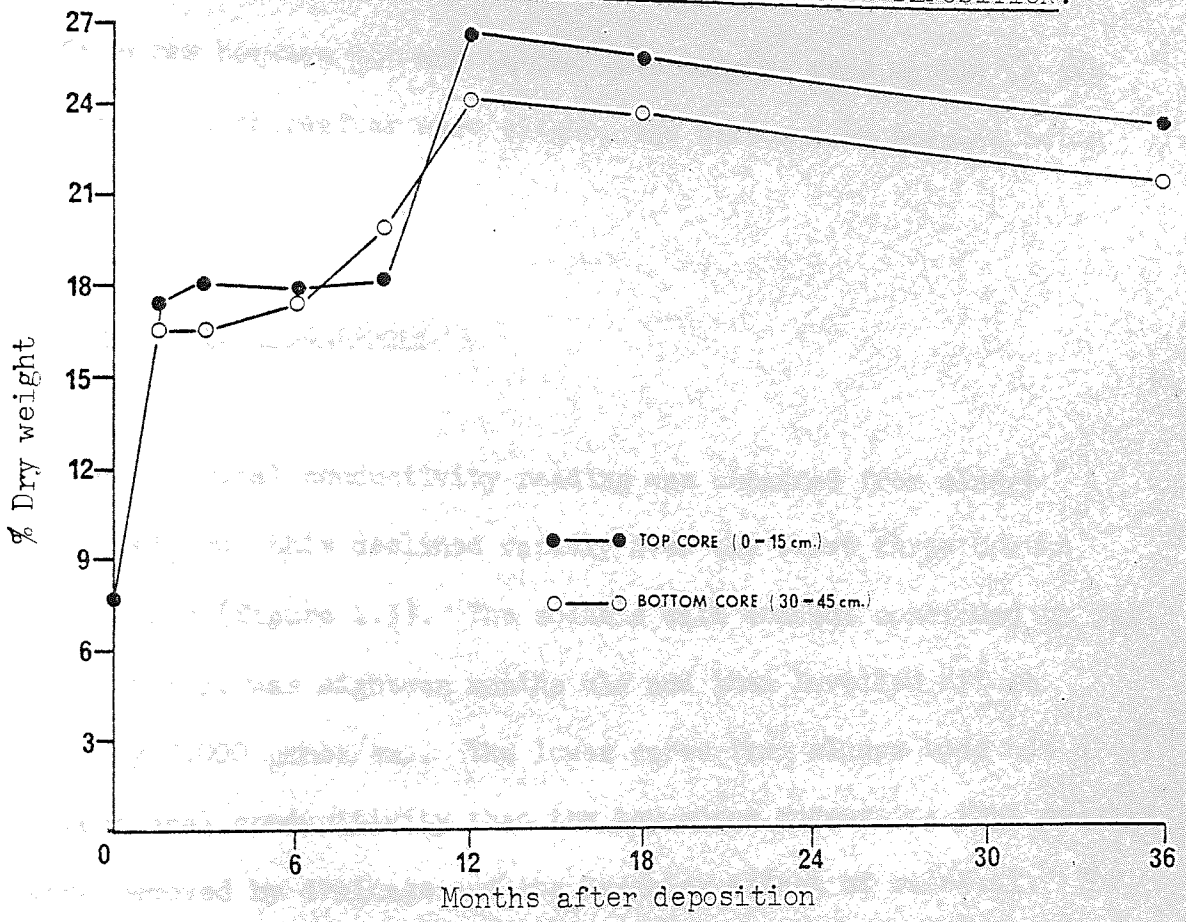
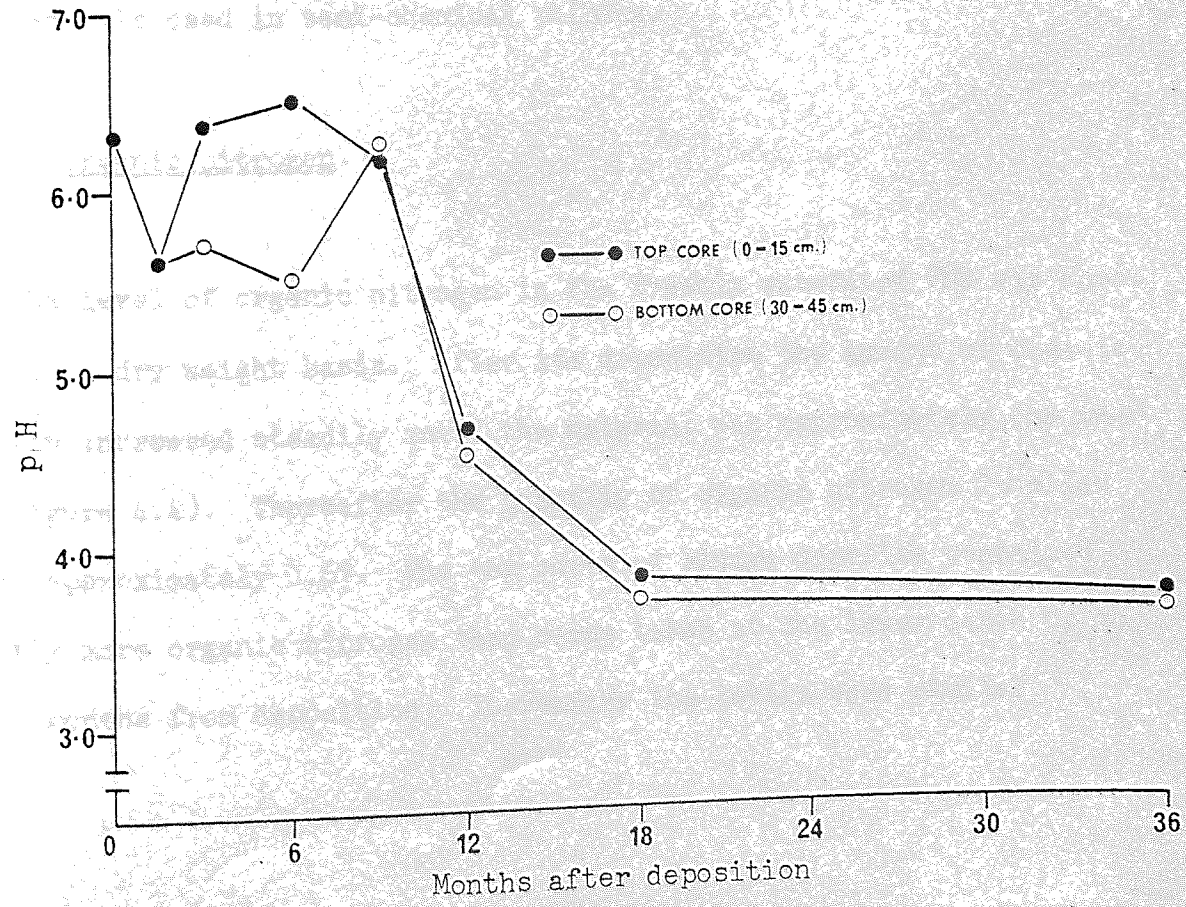



FIGURE 4.2 p H OF PMS WITH TIME FROM DEPOSITION.



Differences between top and bottom core samples were marked in the early stages, but thereafter were slight, the bottom core samples being generally lower.

### 4.2.3 Electrical conductivity.

A high elctrical conductivity reading was obtained from sludge when excavated, but this declined rapidly over the first three months after deposition (figure 4.3). The soluble salt content continued to fall until the material was eighteen months old and then levelled off at approximately 2,000 μmhos/cm.. The lower cores from sludge beds had a higher electrical conducitivity than the top cores suggesting that soluble salts were removed by drainage and the leaching effect of rainfall. The source of the high salt content of the waste sludge was presumed to be the chemicals used in semi-chemical pulping.

### 4.2.4 Organic nitrogen.

The level of organic nitrogen in the freshly excavated PMS was about 0.27% on a dry weight basis. After its deposition the amount of organic nitrogen increased steadily until the material was approximately one year old (figure 4.4). Thereafter the quantity of organic nitrogen levelled off at approximately 0.6%. The top cores of sludge deposits contained slightly more organic nitrogen than cores taken at the lower depth up to twelve months from deposition. Thereafter the levels were similar.

FIGURE 4.3 ELECTRICAL CONDUCTIVITY OF PMS WITH TIME FROM DEPOSITION.

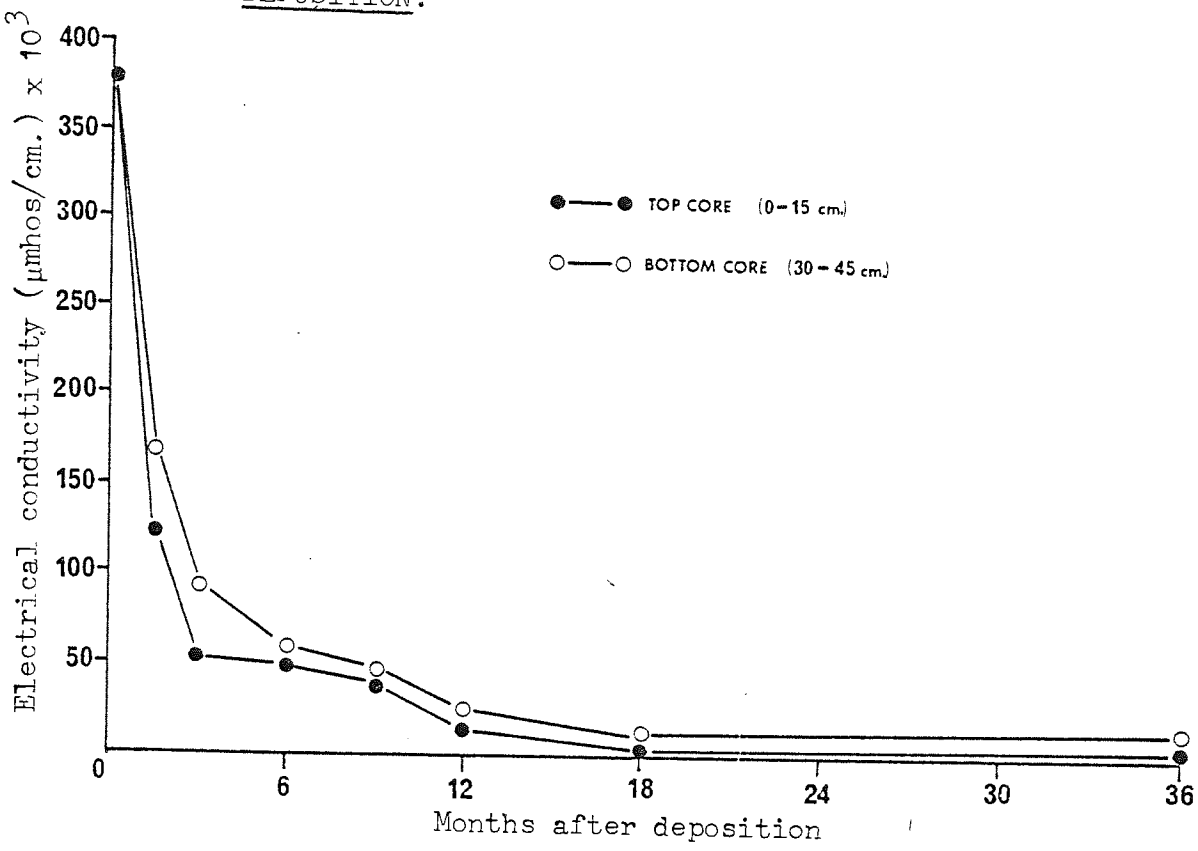
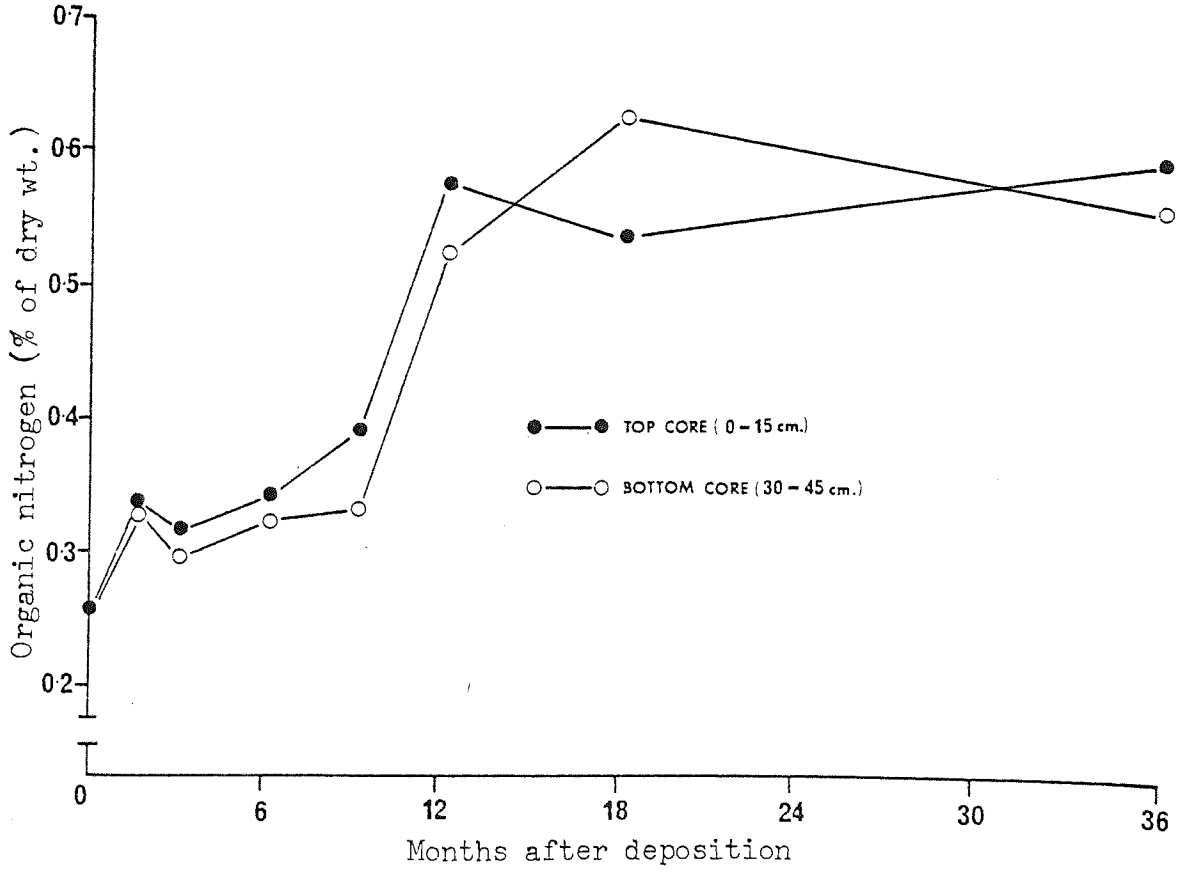



FIGURE 4.4 ORGANIC NITROGEN CONTENT OF PMS WITH TIME FROM DEPOSITION.



### 4.2.5 Organic carbon.

Levels of organic carbon in PMS following its deposition are given in figure 4.5. The quantity of organic carbon declined from 42.5% (dry weight) in the raw sludge to 38% by twelve months after tipping, and remained at that level in subsequent samples. There was no real trend when the levels of organic carbon in top and bottom cores from the sample sludge deposits were compared.

The overall pattern for organic carbon was the reverse of that for organic nitrogen.

### 4.2.6 Carbon : Nitrogen ratio.

Estimates of C : N ratio (figure 4.6) reflect changes in organic carbon and nitrogen levels, decreasing from 165 : 1 at deposition to 65 : 1 in samples taken at twelve months and later. Differences between top and bottom core samples were relatively minor.

### 4.2.7 Ash content.

The ash content of PMS increased from 17.7% (dry weight) in the fresh material, to approximately 28% eighteen months after deposition and remained the same thereafter (figure 4.7). There were no consistent differences in the relative ash contents of samples from the two different depths.

FIGURE 4.5 ORGANIC CARBON CONTENT OF PMS WITH TIME FROM DEPOSITION.

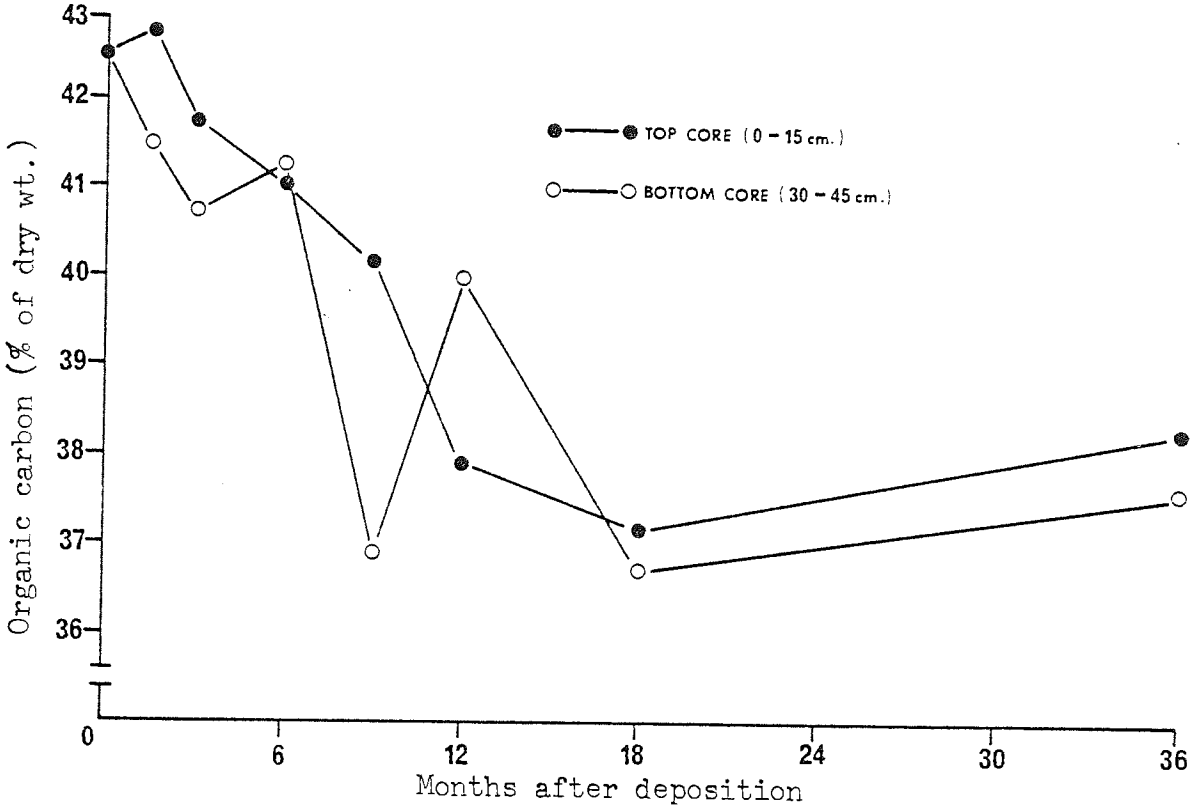
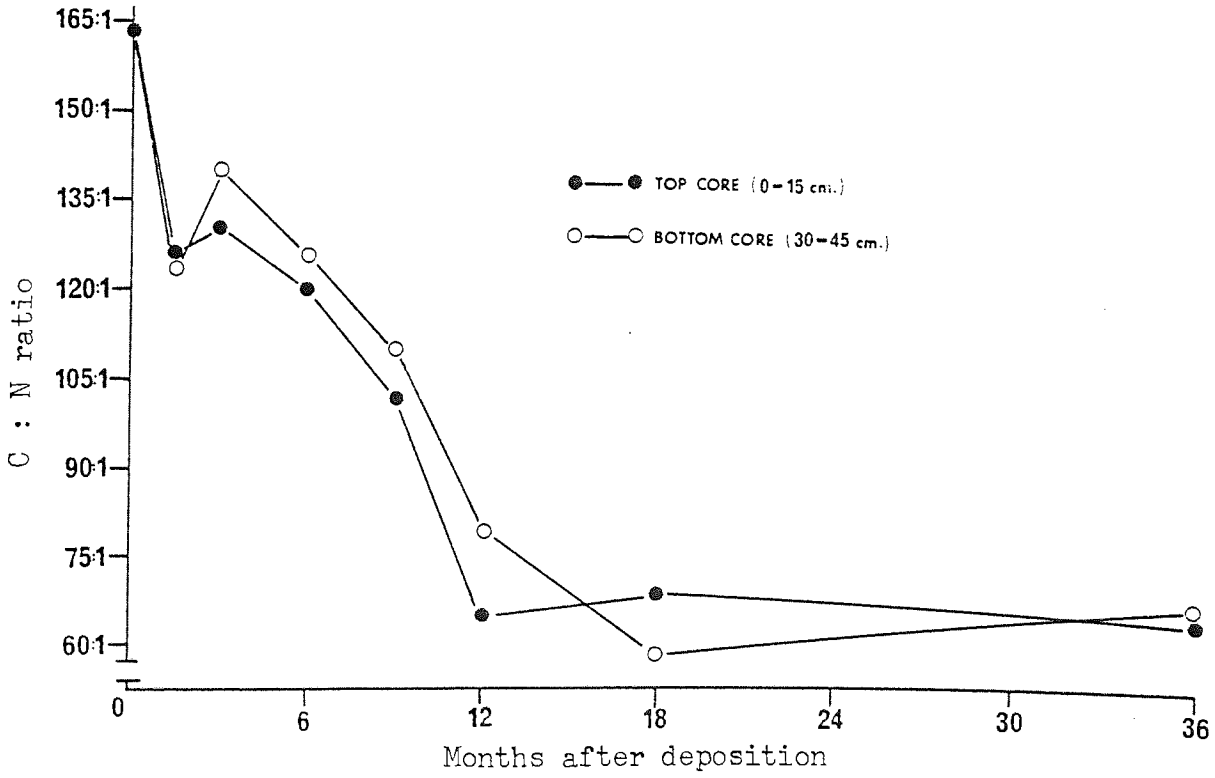



FIGURE 4.6 CARBON TO NITROGEN RATIO OF PMS WITH TIME FROM DEPOSITION.



### 4.2.8 Water holding capacity.

The water holding capacity of PMS showed little change with time after its deposition (figure 4.8). In fresh sludge and that six, eighteen, and thirty six months in age, water holding capacity was between 76% and 81%.

### 4.2.9 Particle size distribution.

In all of the samples analysed the majority of the mineral particles on a weight basis fell into the range 2 - 20 μm. in diameter, with least in the range below 2 μm. (figure 4.9).

### 4.2.10 Organic constituents.

On a dry weight basis over 35% of the sludge as excavated was determined to be cellulose. The next largest organic component was lignin, at 22%. Other fractions, hemi-cellulose, ethanol/benzene solubles, and water solubles were all between 6% and 8.5% of the dry weight (figure 4.10). The ash content remaining after extraction of the first five fractions was a few per cent. below the figures obtained in 4.2.7 as some soluble minerals were removed by previous extractions.

With time after deposition, the cellulose content of PMS declined steadily to around 33%. Levels of water solubles and ethanol/benzene solubles also fell slightly below their original value.

FIGURE 4.7 ASH CONTENT OF PMS WITH TIME FROM DEPOSITION.

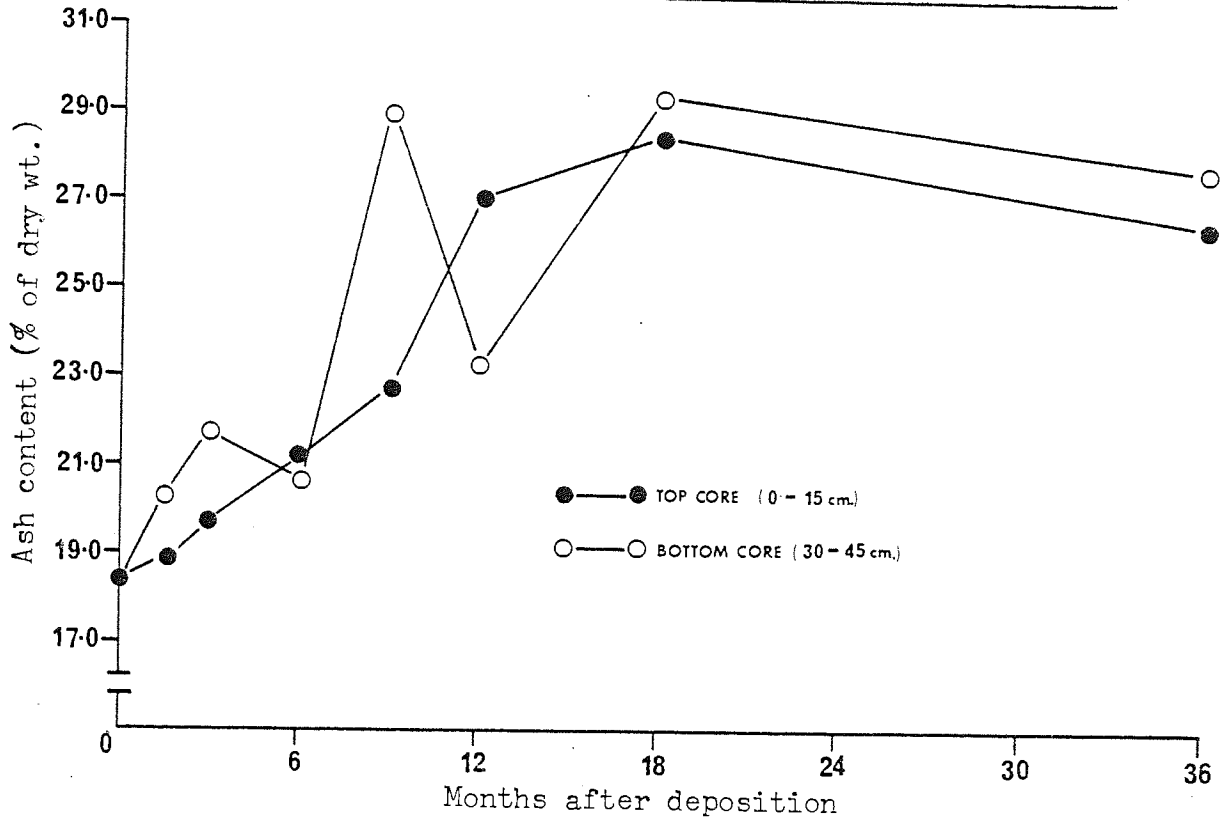



FIGURE 4.8 WATER HOLDING CAPACITY OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

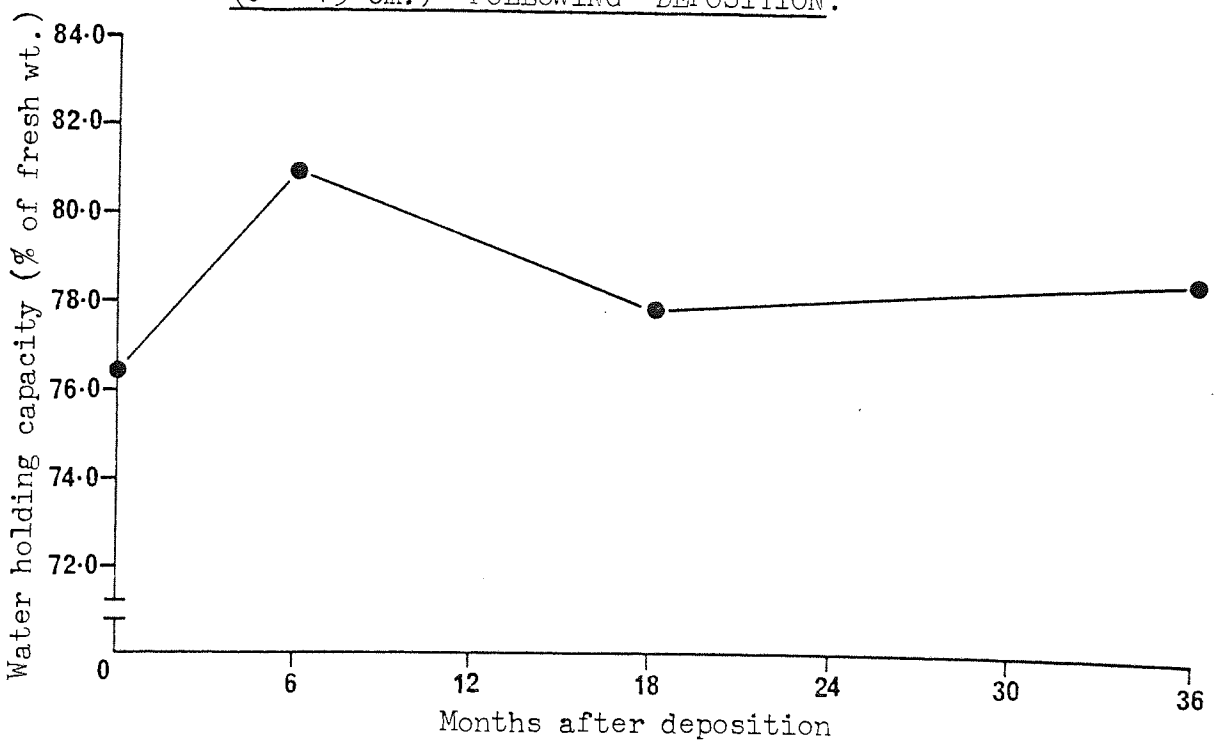


FIGURE 4.9 PARTICLE SIZE DISTRIBUTION OF MINERAL COMPONENT OF PMS SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

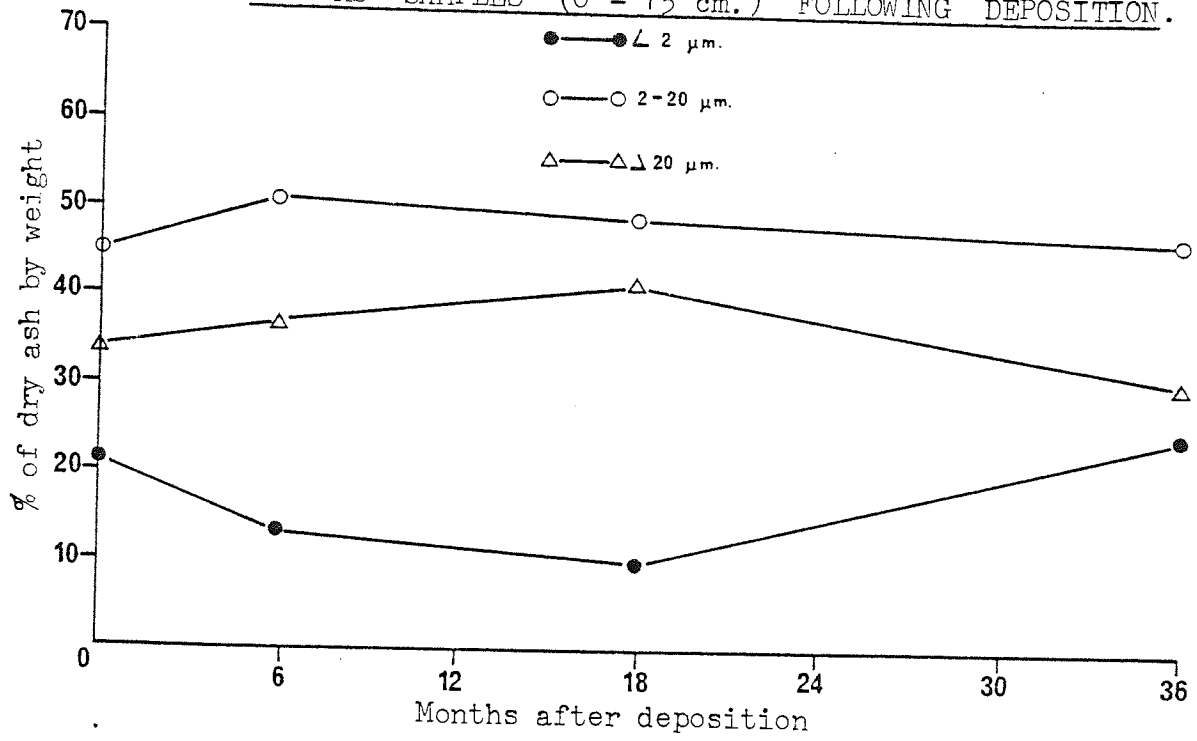
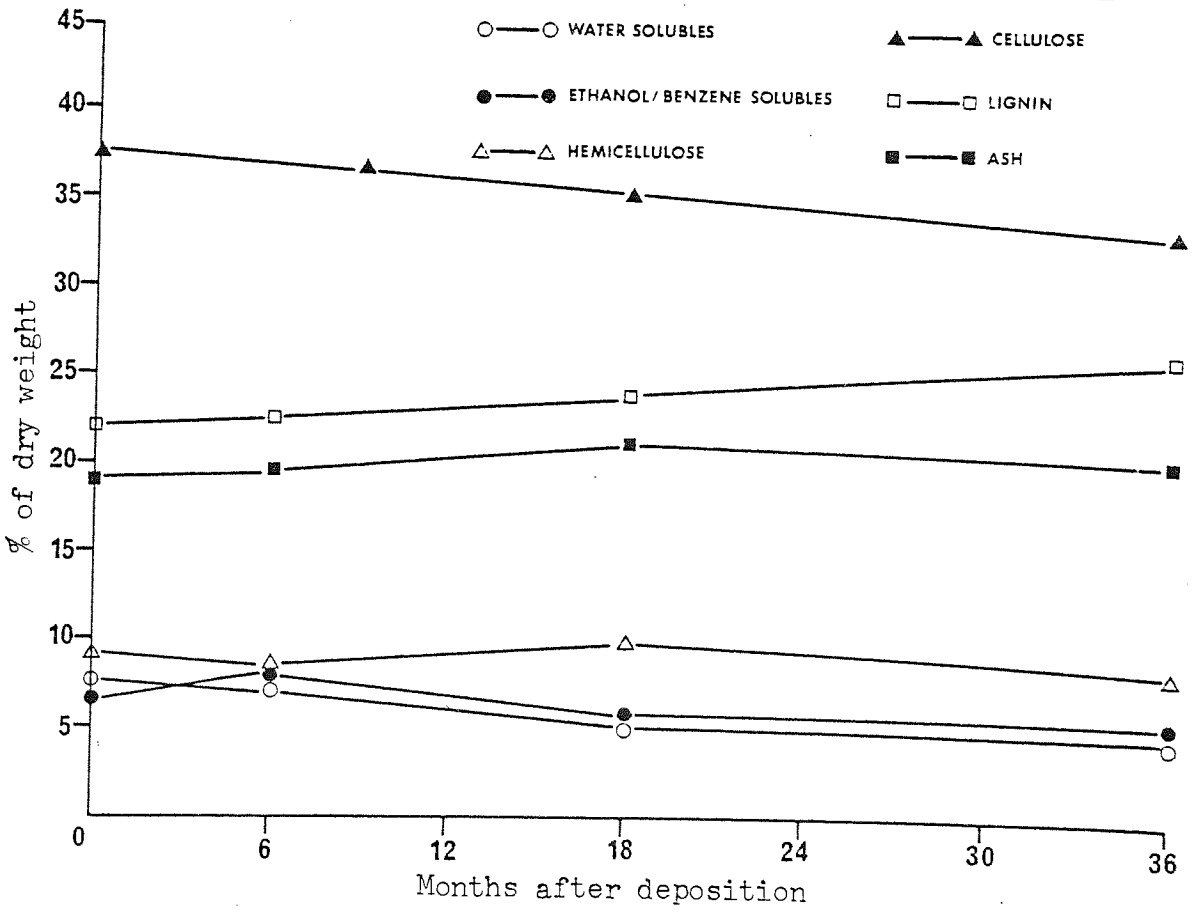



FIGURE 4.10 ORGANIC AND MINERAL FRACTIONATION OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.



The level of hemi-cellulose remained relatively stable, just below 10% of the dry weight. The value for lignin showed an apparent increase but this is most likely to indicate a relatively higher proportion of a fixed lignin content owing to the decline in the amounts of other fractions.

### 4.2.11 Levels of individual elements.

The levels of individual elements in fresh PMS and three samples after its deposition are given in figures 4.11 to 4.22.

The elements which were most abundant in the fresh sludge were calcium, magnesium, sodium, sulphate and iron, with sodium and sulphate being present in the largest quantities. Lead, mercury, cadmium, chromium and nickel were all below detectable levels in mixed-acid digestions.

Calcium, magnesium and sodium were relatively available compared with the other elements present in the fresh sludge (table 4.1). These elements showed a marked decline in concentration and availability following sludge deposition (table 4.1), whilst levels of the readily soluble ions chloride and sulphate also fell. Other elements showed no real trends in concentration with ageing of the sludge deposits.

The overall changes which occurred in the composition of the sludge after deposition are summarised in table 4.2.

TABLE 4.1 PERCENTAGE AVAILABILITY OF INDIVIDUAL ELEMENTS IN FRESHLY EXCAVATED AND AGED PMS.

| | % Availability | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Element | Ca | Cu | Fe* | K | Mg | Mn | Na | $PO_4$** | Zn |
| Fresh PMS | 80.0 | 0.5 | 31.0 | 11.8 | 50.8 | 19.6 | 85.4 | 15.3 | 13.2 |
| Aged PMS (36 months) | 65.9 | 0.4 | 27.7 | 15.9 | 9.3 | 12.2 | 33.3 | 10.1 | 8.7 |

$$\% \text{ availability estimated as } 100 \times \frac{\text{ammonium acetate extractable levels}}{\text{levels in mixed-acid digestion}}$$

*Availability based on pyrophosphate extraction.

**Availability based on acetic acid extraction.

TABLE 4.2 CHANGES IN COMPOSITION OF PMS FROM THE KEMSLEY MILL FOLLOWING ITS DEPOSITION.

| Dry weight | Ash content | Organic nitrogen | Organic carbon | C : N ratio | Cellulose | p.H. | Electrical conductivity | Individual elements (Ca Cl Mg Na $SO_4$) |
|---|---|---|---|---|---|---|---|---|
| + | + | + | - | - | - | - | - | - |

+ Denotes an increase following deposition.

- Denotes a decrease following deposition.

FIGURE 4.11 CALCIUM CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

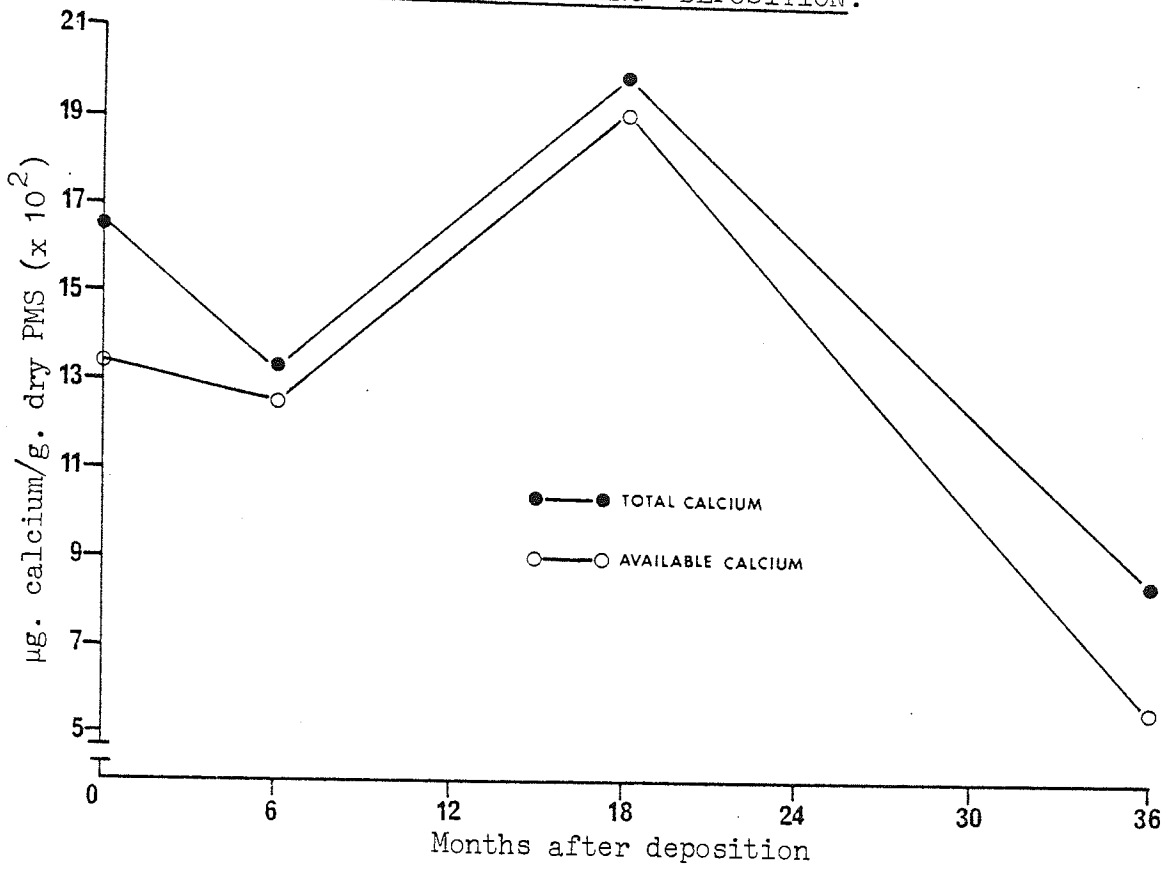



FIGURE 4.12 WATER SOLUBLE CHLORIDE LEVELS IN PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

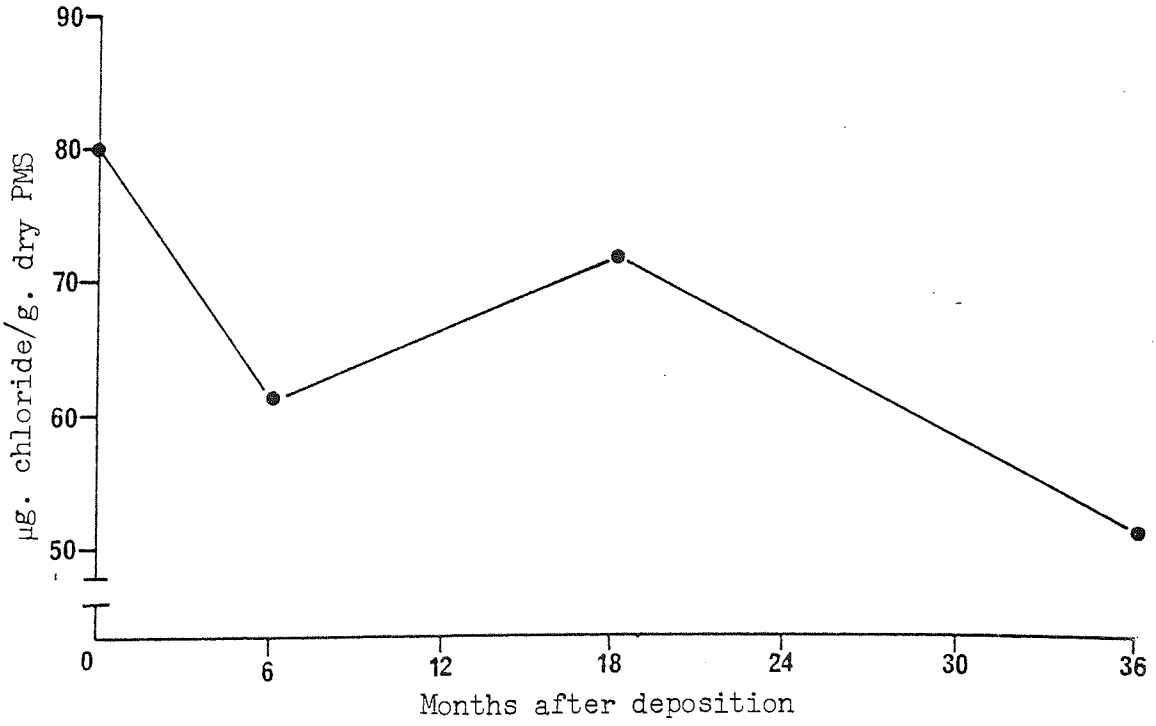


FIGURE 4.13 COPPER CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

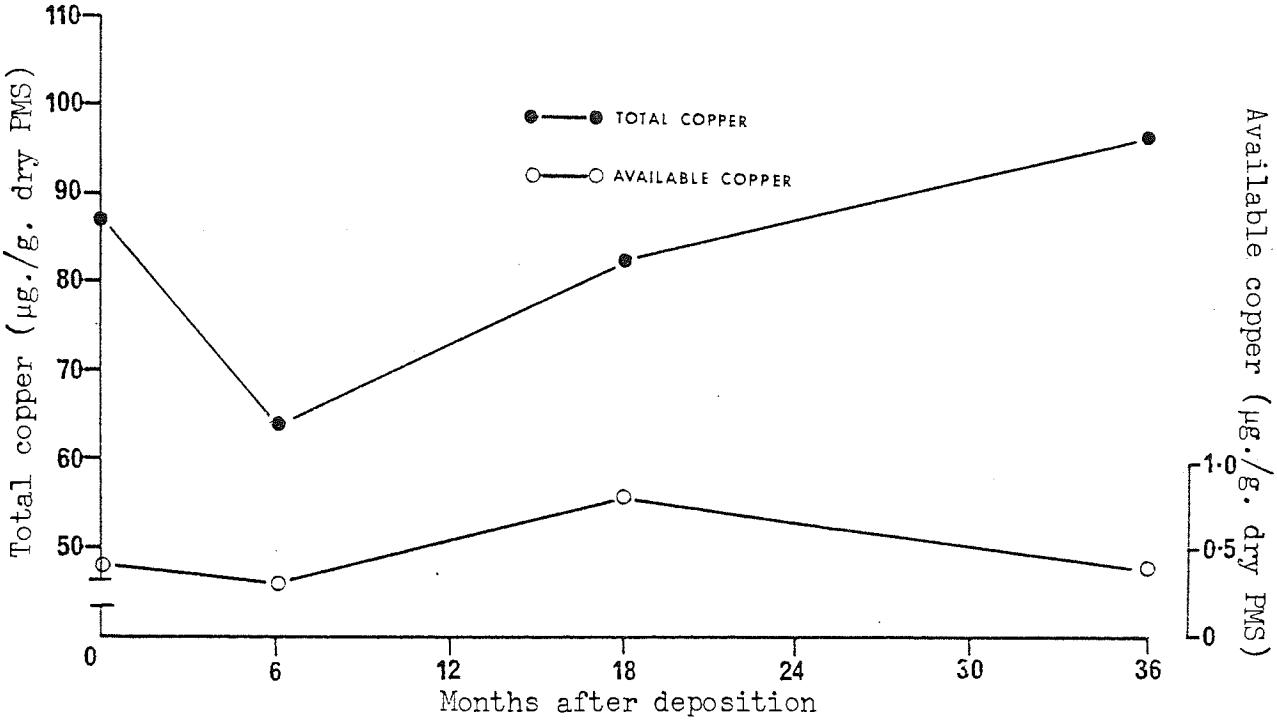



FIGURE 4.14 IRON CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

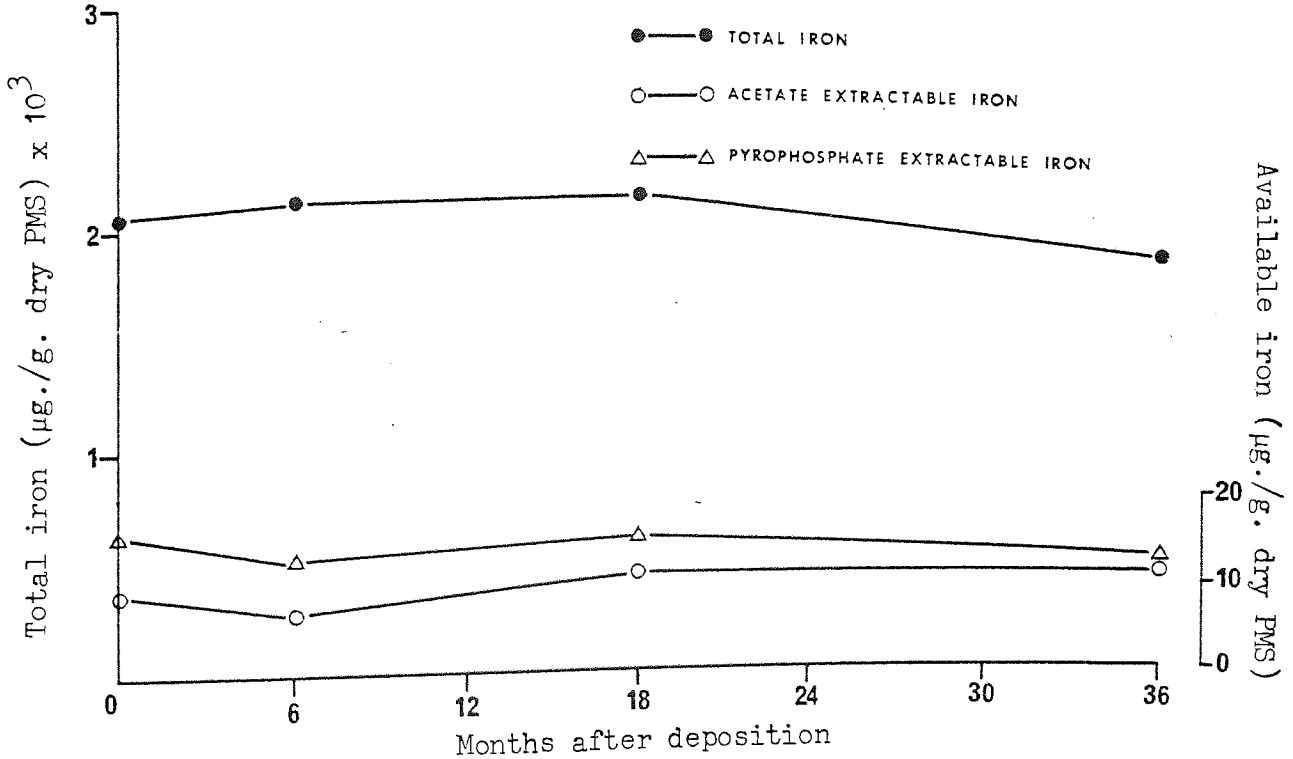


FIGURE 4.15 MANGANESE CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

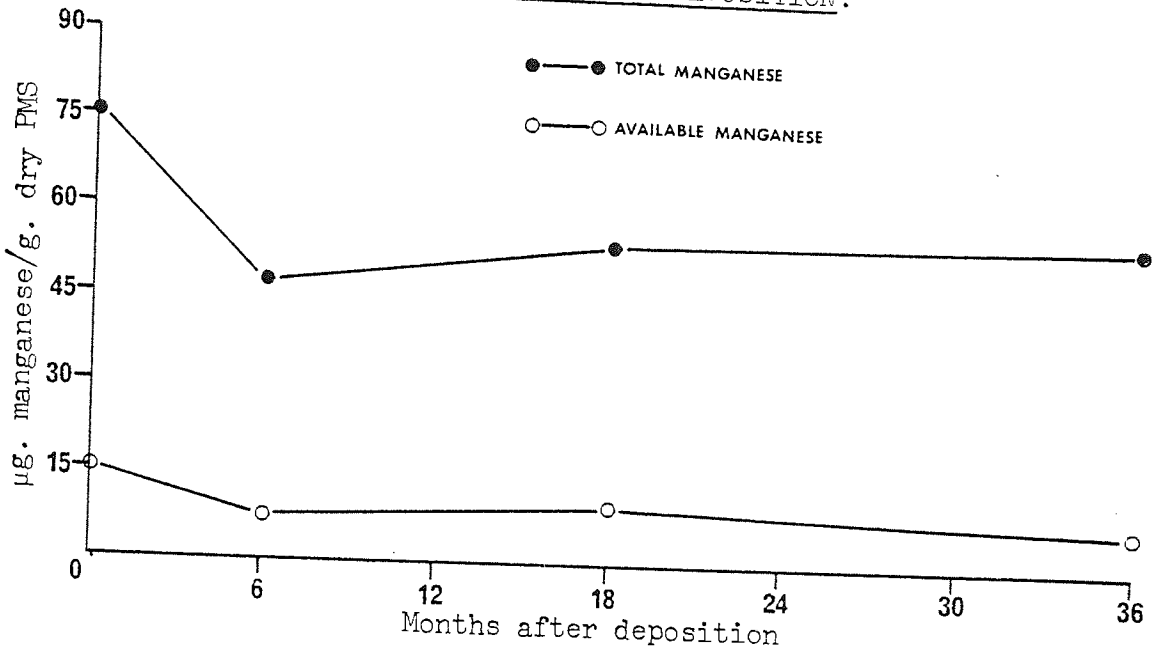



FIGURE 4.16 MAGNESIUM CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

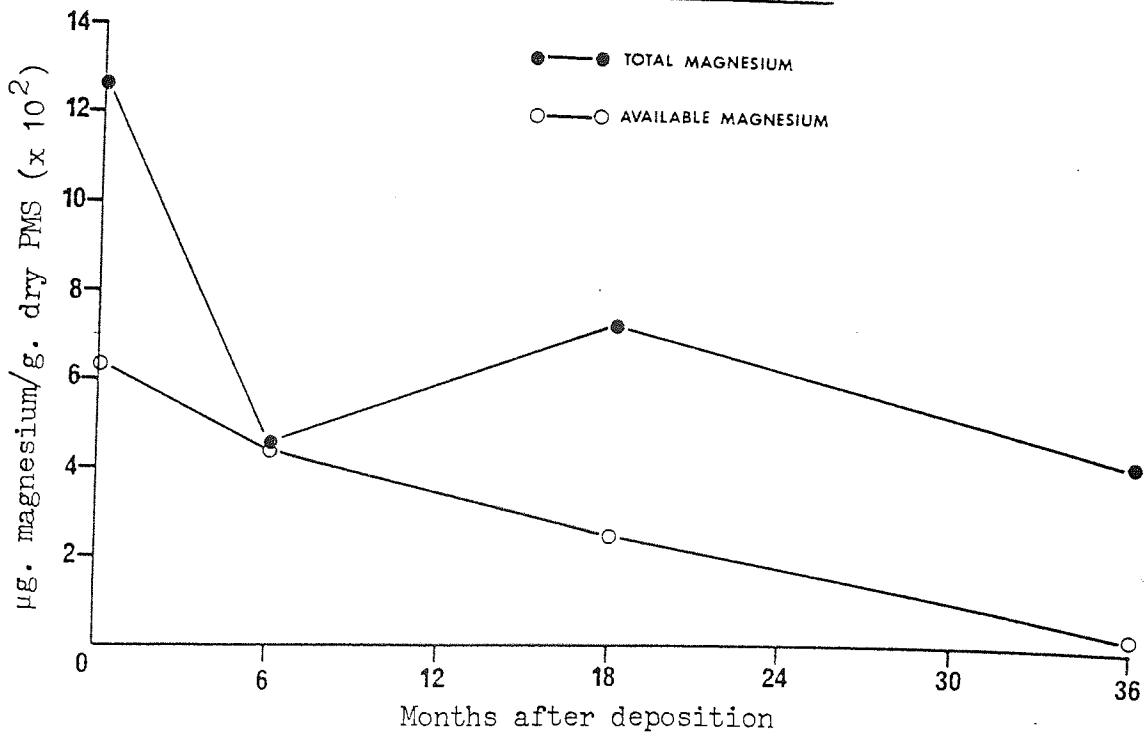


FIGURE 4.17 INORGANIC NITROGEN CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

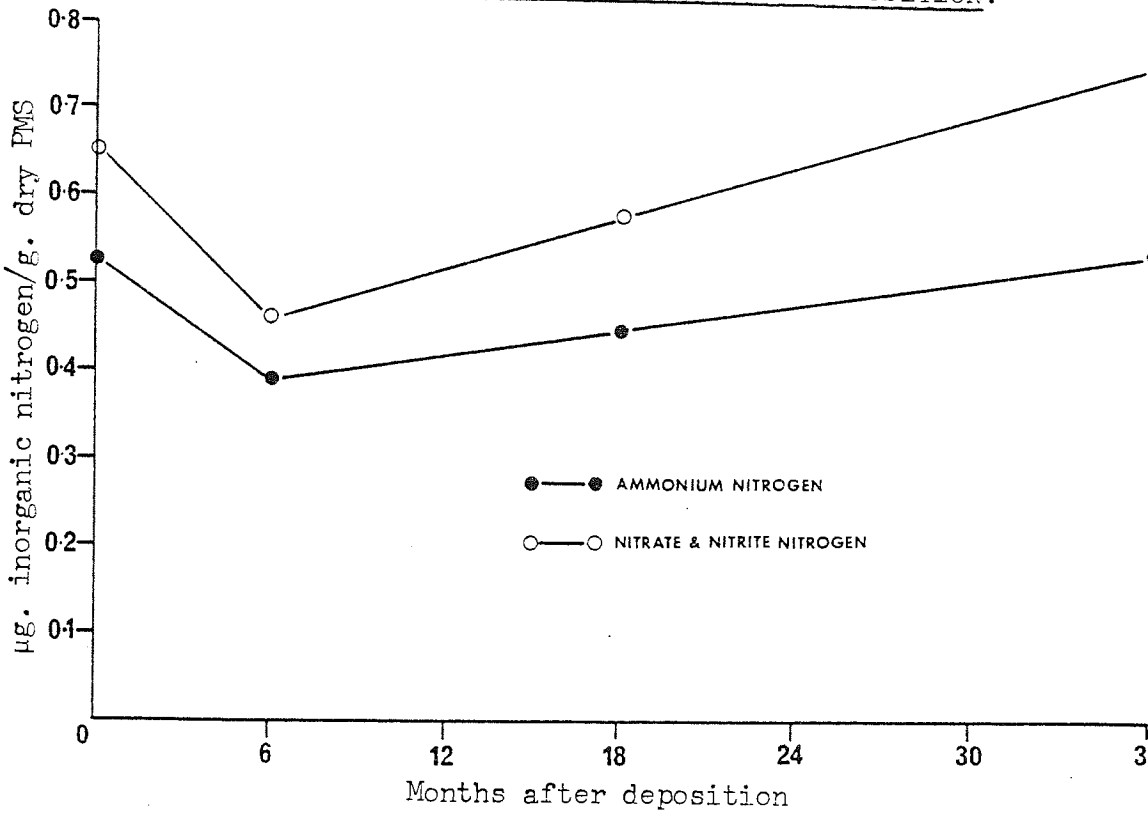



FIGURE 4.18 PHOSPHATE CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

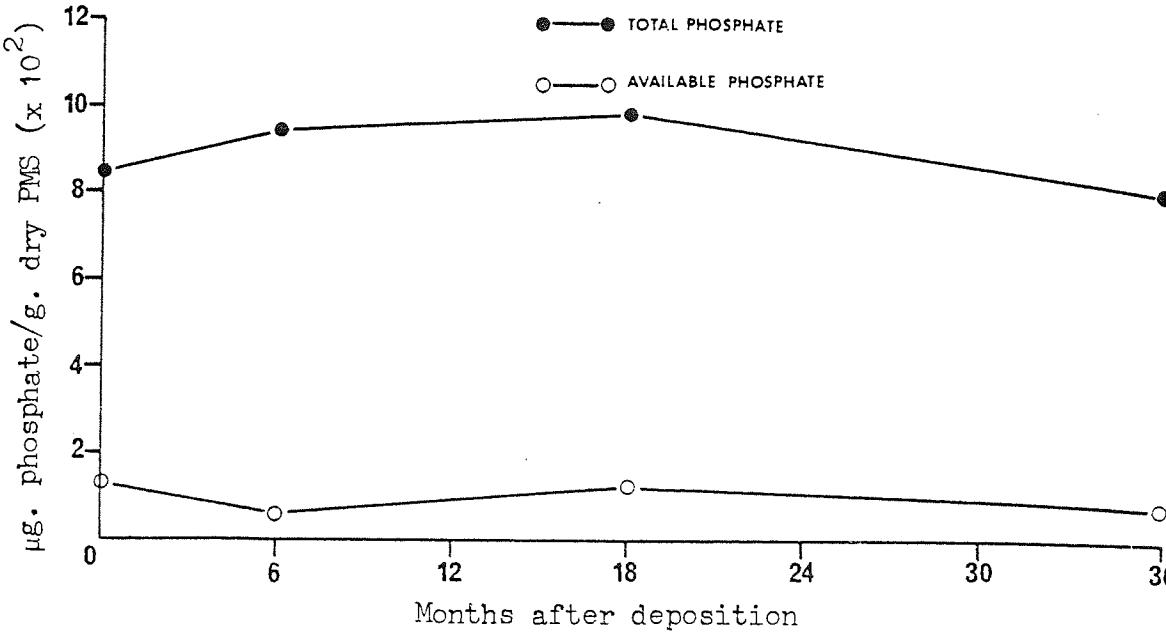


FIGURE 4.19 POTASSIUM CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

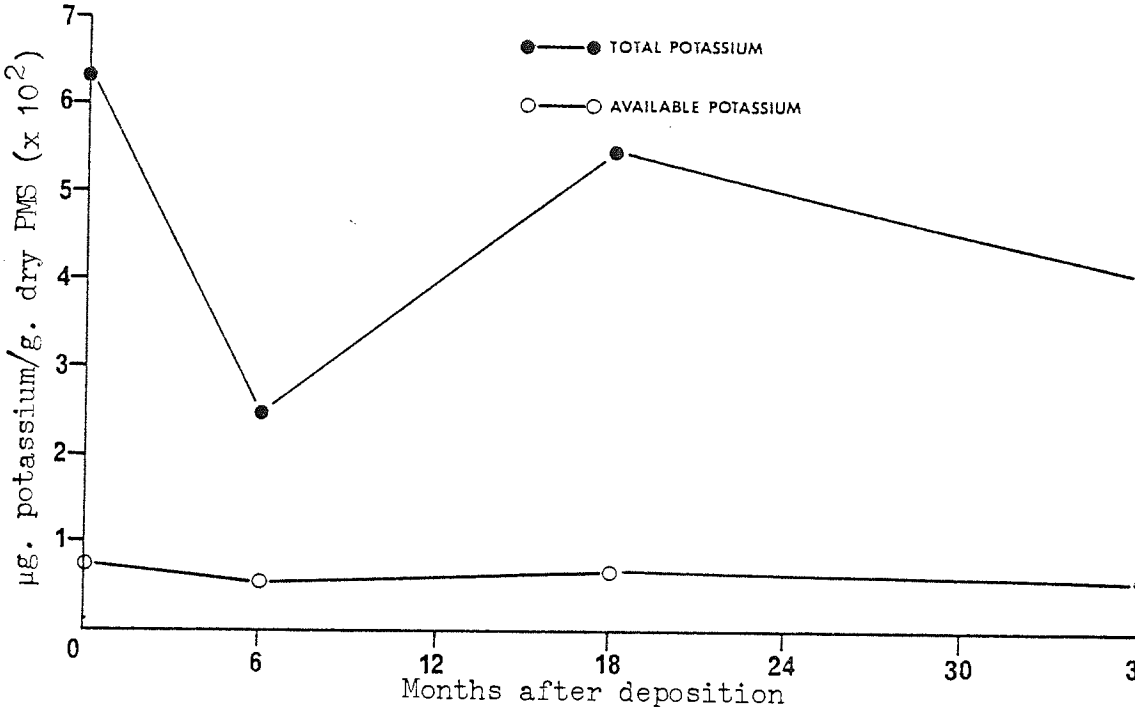



FIGURE 4.20 SODIUM CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

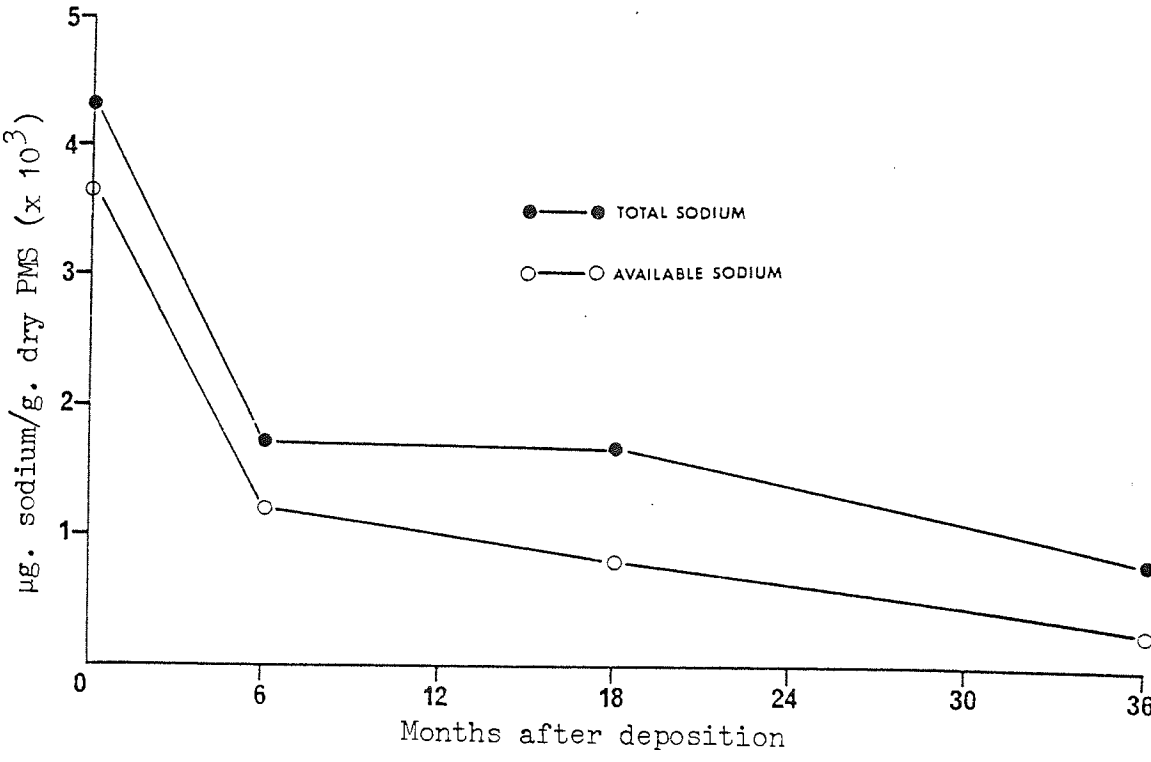


FIGURE 4.21 WATER SOLUBLE SULPHATE LEVELS IN PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.

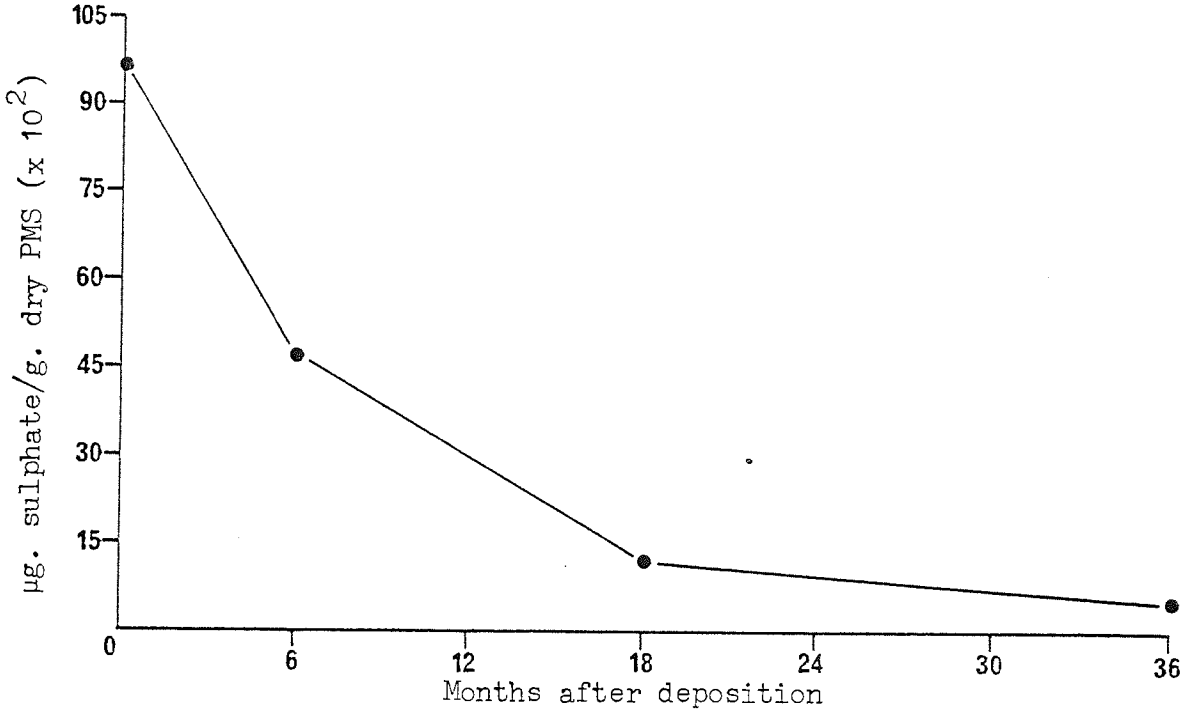
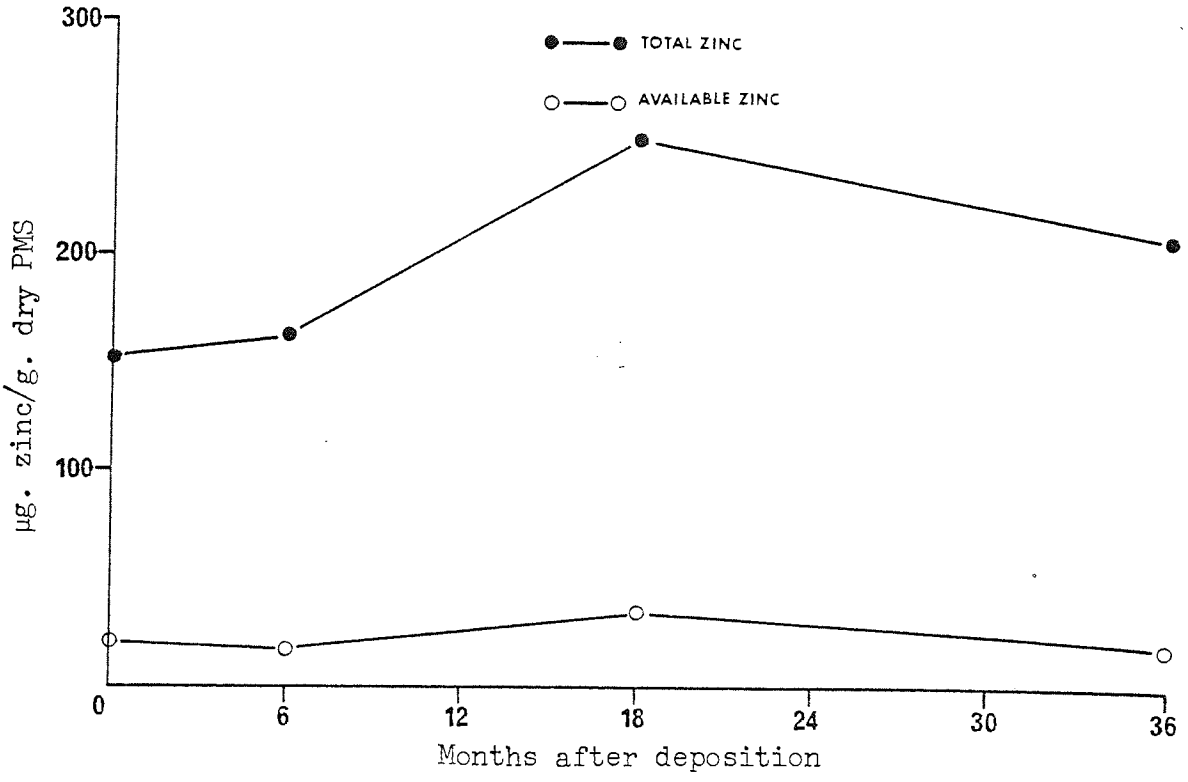



FIGURE 4.22 ZINC CONTENT OF PMS TOP CORE SAMPLES (0 - 15 cm.) FOLLOWING DEPOSITION.



## 4.3 COLONISATION OF DEPOSITED PMS BY FUNGI AND PLANTS.

### 4.3.1 Occurrence of higher fungi and plants on sludge deposits.

The relative abundance of higher fungi and plants was recorded at chosen sites, numbered 1 to 6 (see figure 1.2). The results are given in tables 4.3 and 4.4.

The appearance of fruitbodies of higher fungi was influenced primarily by the time of the year, most being evident in the autumn months Fruitbodies were present on the solids on all three of the recording dates. Comparing sites on the October recording date when the majority of the fungal fruitbodies were evident, it can be seen that the sludge beds were colonised by higher fungi some four months after their deposition. The diversity of species present soon increased and remained high until the waste was approximately one year old. By the time the deposits had matured for some eighteen months the number and diversity of fungal fruitbodies was very low or they were absent.

Two Ascomycetes, Peziza badia and Nectria sp., and the Basidiomycete Stropharia semiglobata were the first species which appeared on deposited sludge and amongst the last to disappear. They were the most abundant species during the recording period, and accounted for six of the seven records from all sites on the April date.

TABLE 4.3 RELATIVE ABUNDANCE OF HIGHER FUNGI ON PMS DEPOSITS FROM APRIL TO SEPTEMBER, 1976.

KEY:

| | | | |
|---|---|---|---|
| A | Abundant | O | Occasional |
| F | Frequent | R | Rare |

| Site number and approximate age of PMS on 28.4.76 | Species of higher fungi recorded. | Date of recording 28.4.76 | 24.7.76 | 13.9.76 |
|---|---|---|---|---|
| 1 Freshly deposited | Nectria sp. | - | - | R |
| | Peziza badia | - | - | O |
| | Stropharia semiglobata | - | - | O |
| 2 4 months | Coprinus impexi stirps | - | F | - |
| | Coprinus insignis | - | R | - |
| | Coprinus picaceus | - | R | - |
| | Coprinus sterquilinus stirps | - | O | - |
| | Nectria sp. | O | O | O |
| | Panellus mitis | - | - | O |
| | Peziza badia | F | - | F |
| | Scutellinia scutellata | - | - | O |
| | Stropharia semiglobata | O | A | O |
| | Tremella encephala | - | - | R |
| | Volvariella speciosa | - | O | - |

TABLE 4.3 (Continued)

| Site number and approximate age of PMS on 28.4.76 | Species of higher fungi recorded | Date of recording 28.4.76 | 24.7.76 | 13.9.76 |
|---|---|---|---|---|
| 3 7 months | Coprinus comatus | - | - | R |
| | Coprinus impexi stirps | O | O | - |
| | Coprinus micaceus stirps | - | - | O |
| | Hypholoma fasiculare | - | R | A |
| | Irpex sp. | - | - | O |
| | Peziza badia | - | R | - |
| | Psathyrella sp. | - | - | R |
| | Stropharia semiglobata | F | - | O |
| 4 14 months | Nectria sp. | R | - | - |
| | Panellus mitis | - | - | O |
| | Stropharia semiglobata | O | - | - |
| 5 18 months | No higher fungi present | | | |
| 6 36 months | | | | |

TABLE 4.4 RELATIVE ABUNDANCE OF HIGHER PLANTS ON PMS DEPOSITS FROM APRIL TO SEPTEMBER, 1976.

KEY:

| | | | |
|---|---|---|---|
| A | Abundant | O | Occasional |
| F | Frequent | R | Rare |

| Site number and approximate age of PMS on 28.4.76 | Species of higher plants recorded | Date of recording 28.4.76 | 24.7.76 | 13.9.76 |
|---|---|---|---|---|
| 1 Freshly deposited | | | | |
| 2 4 months | No higher plants present | | | |
| 3 7 months | | | | |
| 4 14 months | Anthemis cotula | - | - | O |
| | Anthoxanthum odoratum | - | R | F |
| | Cirsium arvense | - | R | F |
| | Holcus lanatus | - | R | F |
| | Phragmites communis | - | - | O |
| | Senecio jacobaea | - | - | O |
| | Solanum dulcamara | - | R | O |

TABLE 4.4 (Continued)

| Site number and approximate age of PMS on 28.4.76 | Species of higher plants recorded | Date of recording 28.4.76 | 24.7.76 | 13.9.76 |
|---|---|---|---|---|
| 5 18 months | Anthemis cotula | O | F | F |
| | Aster tripolium | O | O | O |
| | Chamaenerion angustifolium | F | O | O |
| | Cirsium arvense | R | R | O |
| | Cirsium vulgare | - | - | R |
| | Holcus lanatus | O | O | O |
| | Phragmites communis | - | - | R |
| | Rumex longifolius | - | O | O |
| | Sambucus sp. | - | - | R |
| | Senecio jacobaea | R | R | R |
| | Senecio vulgaris | - | - | R |
| | Sisymbrium officinale | - | O | O |
| | Solanum dulcamara | R | O | O |
| | Solanum nigrum | - | O | O |
| | Sonchus asper | - | R | O |
| | Tussilago farfara | - | O | O |

TABLE 4.4 (Continued)

| Site number and approximate age of PMS on 28.4.76 | Species of higher plants recorded | Date of recording 28.4.76 | 24.7.76 | 13.9.76 |
|---|---|---|---|---|
| 6<br>36 months | Anthoxanthum odoratum | O | A | A |
| | Atriplex hasta | - | - | R |
| | Holcus lanatus | O | A | A |
| | Phragmites communis | O | F | O |
| | Plantago sp. | - | - | R |
| | Salix cinerea | O | O | O |

The occurrence of other species was more sporadic, and seemed to be affected more by the time of year than age of sludge deposits. For example, Coprinus insignis, Coprinus picaceus and Volvariella speciosa occurred at site 2 in July but not at site 1 in April, though the deposits were the same age at those times.

As with higher fungi, the greatest number and diversity of green plants was recorded at the time of year when they are most evident in their normal environments. This is the time when most plants are in flower, and is between the months of June and October.

Excluding the seasonal effect on the records, the solids were colonised about eighteen months after their deposition, at a time when the occurrence of fungal fruitbodies had ceased or was at a very low level The widest range of species occurred soon after the initial colonisation, and by the time the sludge beds were approximately three years or more in age, they were completely overgrown by grasses, with a lower diversity of other plant life.

The grasses scented vernal grass, Anthoxanthum odoratum, and Yorkshire fog, Holcus lanatus, along with creeping thistle, Cirsium arvense, and woody nightshade, Solanum dulcamara, were among the first species to appear on the solids. Material three years in age was largely overgrown by A.odoratum and H.lanatus, with the common reed, Phragmites communis, and sallow, Salix cinerea, occasional.

Between these two times, a large number of species were evident in the summer and early autumn, with stinking chamomile, Anthemis cotula, and rose bay willow herb, Chamaenerion angustifolium, being among the more prevalent.

4.3.2 Isolation of micro-fungi from PMS samples.

It was decided to adopt an indirect method of studying fungal populations, so that a reasonable number of samples could be analysed soon after they were taken. The three commonly used indirect methods of studying soil fungi are the soil dilution, Warcup and Waksman plates. All of these techniques tend to favour fast growing and heavily sporing species, and give a similar picture of the fungal flora. The soil dilution plate method was chosen as this is the only technique which afford any opportunity to estimate the number of fungal propagules per known weight of sample.

In order to standardise on procedures, preliminary tests were done on samples of fresh sludge, and material one year in age. Dilutions of $10^{-1}$, $10^{-3}$, $10^{-5}$ and $10^{-7}$g. of fresh sample per ml. of suspending medium were prepared. No fungi were isolated from the fresh sludge, but countable and distinct fungal colonies were obtained from the year old sample at the dilution of $10^{-5}$. This dilution rate was therefore adopted for the investigation of fungal populations, the isolations being carried out as in 3.7.18, at 25°, 45° and 52°C..

Isolations were made onto three different agar media to see if the nutrients available in the isolation medium greatly affected the type of species isolated. Malt agar is a commonly employed medium for the isolation of most soil fungi. It is a nutritionally complete medium. Cellulose agar (Eggins and Pugh, 1962) was chosen as the cellulose provided a complex nutrient source, and one which is present in large quantities in the sludge (4.2.10). Finally an extract agar was prepared from each sample studied to provide a nutrient source which would detect any nutritional influences specific to the waste.

Figure 4.23 gives the mesophilic fungal population in the sludge following deposition. No fungi were isolated from the fresh material, but the waste was rapidly colonised, fungal numbers reaching a peak six months after its deposition. Thereafter the population declined, falling below half its maximum by eighteen months.

Generally a larger number of fungal colonies developed on malt agar than on cellulose agar, while the least number occurred on the extract agars. Almost without exception, a greater number of fungal colonies developed on plates prepared from top core samples compared with lower cores, irrespective of time after deposition and agar medium used.

The three agar media used in the study had little qualitative effect on fungal species isolated (appendices 4.1 to 4.3). Figure 4.24 shows the relative abundance of mesophilic fungi in PMS samples, determined from isolations onto malt agar.

FIGURE 4.23 NUMBERS OF MESOPHILIC FUNGI IN PMS TOP CORES (0 - 15 cm.) AND BOTTOM CORES (30 - 45 cm.) WITH TIME FROM DEPOSITION.

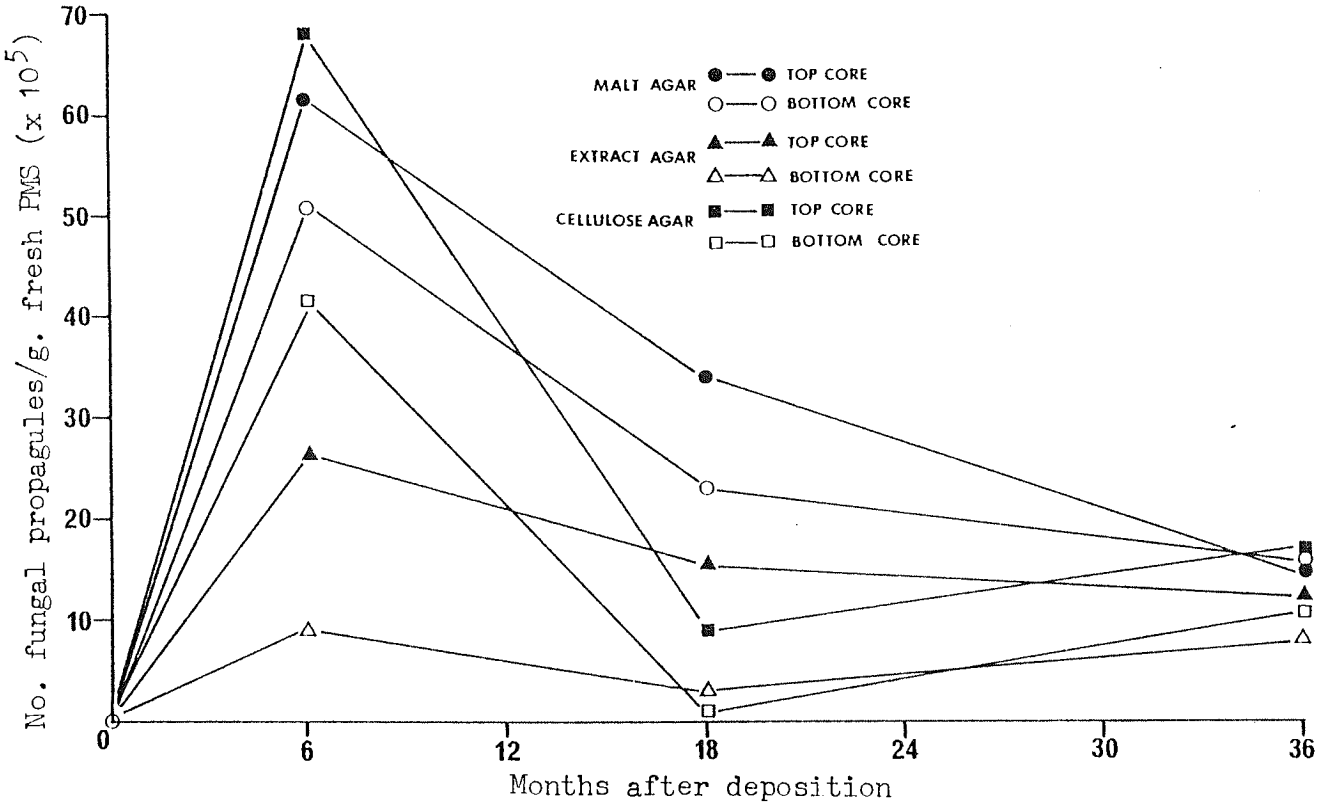
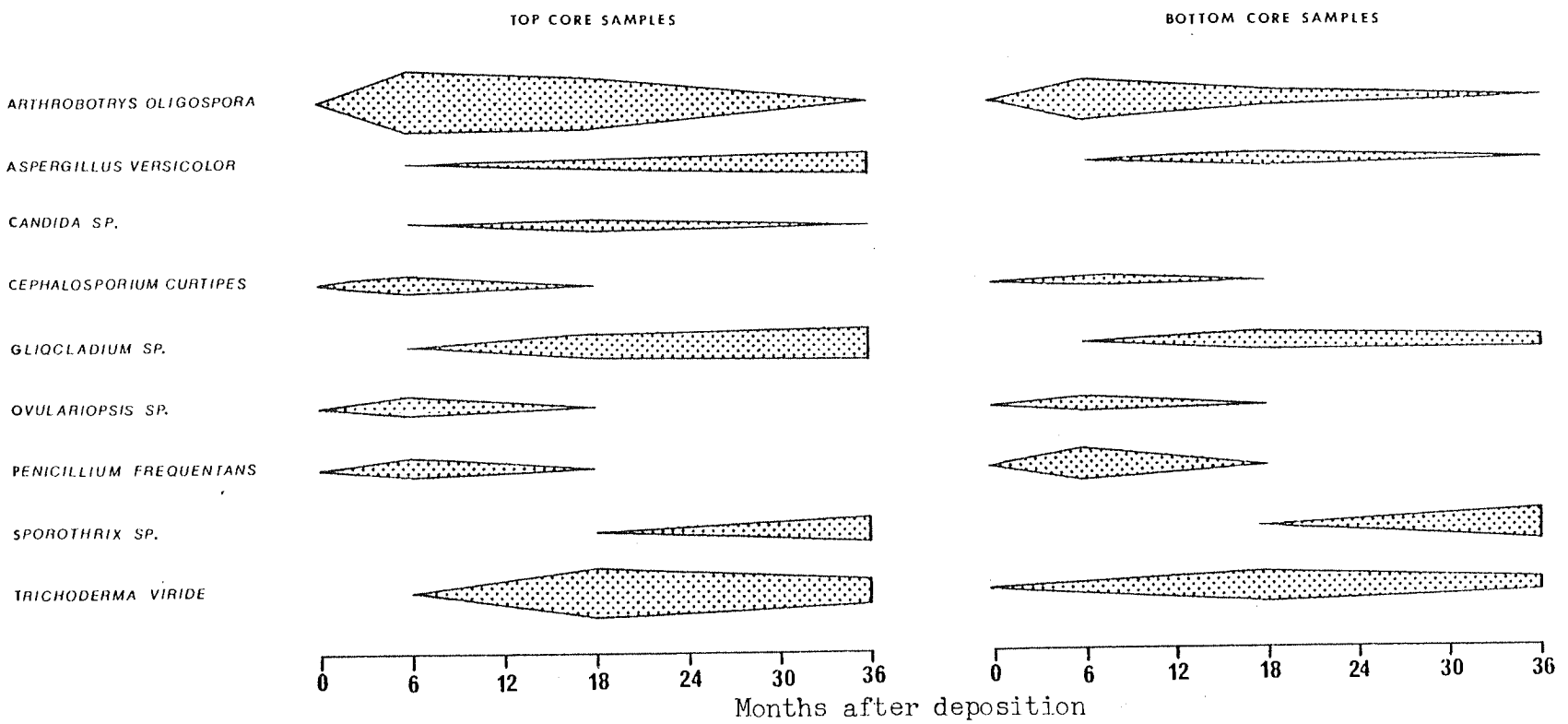


FIGURE 4.24 RELATIVE ABUNDANCE OF MESOPHILIC FUNGI IN PMS DEPOSITS.

Represents 30 x $10^5$ propagules/g. fresh PMS



Though fungi were more abundant in top core samples than lower ones, the individual species and pattern of their occurrence were very similar at both depths. The only major difference was the presence of Candida sp. in top core samples, which was not isolated from corresponding bottom cores. Up to six months from sludge deposition, Arthrobotrys oligospora, Cephalosporium curtipes, Ovulariopsis sp. and Penicillium frequentans were the most abundant species. Apart from A.oligospora, these species disappeared after eighteen months, Gliocladium sp., Sporothrix sp. and Trichoderma viride becoming dominant in old deposits.

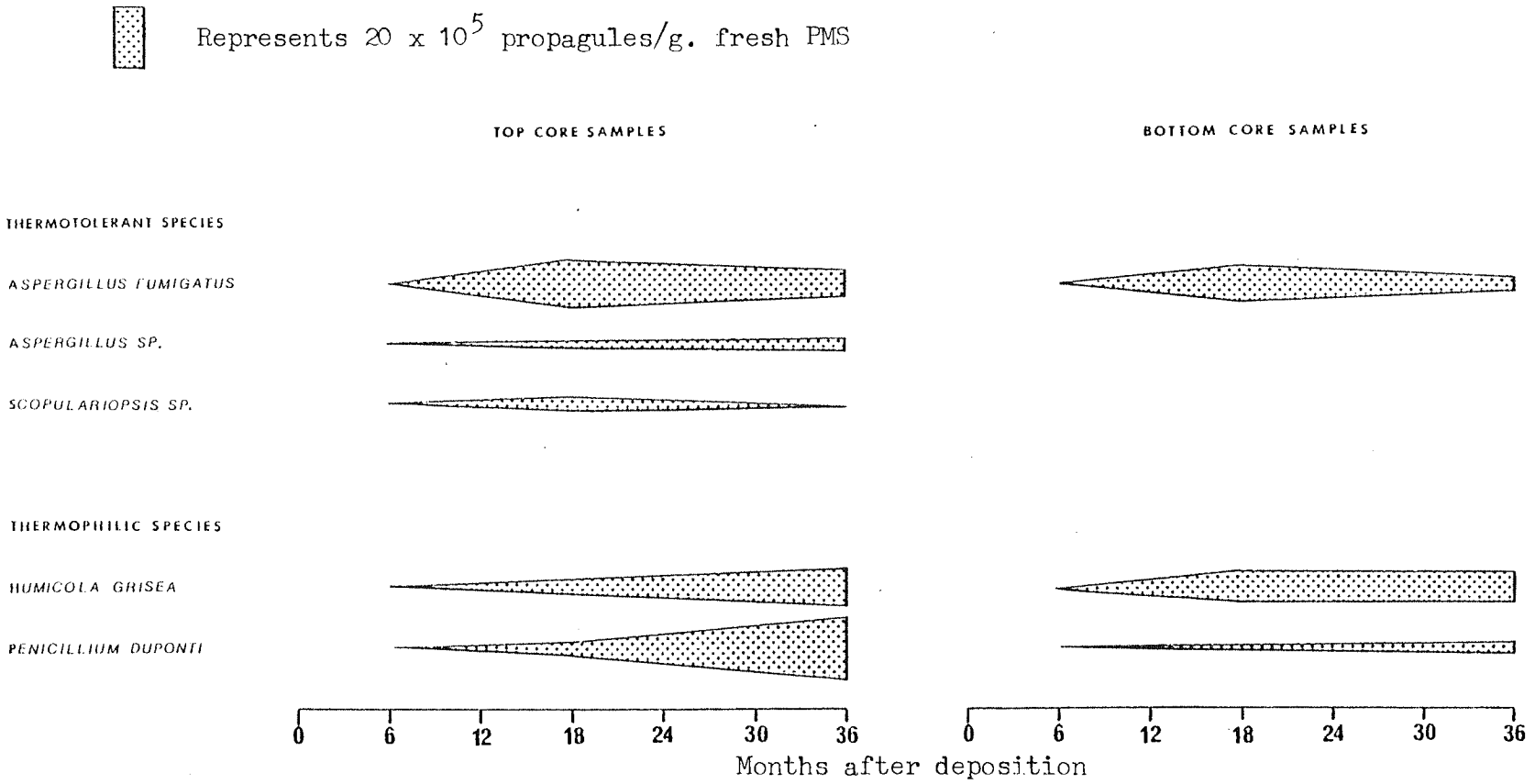
Thermotolerant and thermophilic fungi were not isolated from fresh sludge or the six month old sample (figure 4.25). The thermotolerant species isolated, Aspergillus fumigatus, Aspergillus sp. and Scopulariopsis sp., were most abundant in sludge eighteen months old. A.fumigatus was the dominant species, and the only one isolated from bottom core samples.

The thermophiles Humicola grisea and Penicillium duponti were most prevalent in sludge deposited for three years. H.grisea was equally abundant in top and bottom core samples, whilst P.duponti was more frequently isolated from the shallower depth.

## 4.4 DRAINAGE OF PMS AT ASTON UNIVERSITY.

At the Kemsley mill site drainage and leaching of PMS deposits occur rather slowly.

FIGURE 4.25 RELATIVE ABUNDANCE OF THERMOTOLERANT AND THERMOPHILIC FUNGI IN PMS DEPOSITS.



Two other mill wastes, bark removed from logs and clinker from the boilers, are dumped in the vicinity of the mill complex, and were investigated as a filter medium which could accelerate drainage of the sludge.

Drainage through a bark/clinker filter and simple drainage without the filter medium were compared in modified funnels (described in 3.3) exposed on the roof of the George Alexander Laboratories, University of Aston. Dry weight, p H , electrical conducitivity and fungal populations were monitored at regular intervals. Parallel measurements of bacterial populations were carried out by Cresswell ( pers. comm.).

After an initial settling, the solids assumed a depth of approximatel 70 cm.. Samples for analyses were taken from near to the surface (10 cm. deep) and near to the bottom of the sludge (60 cm. deep). Samples were taken using a 15 mm. diameter core sampler.

4.4.1 Dry weight, p.H. and electrical conductivity of drained samples.

Drainage was improved by the incorporation of a bark/clinker filter (figure 4.26). As would be expected, both samples dried out most rapidly near the surface. Some fifteen months after setting up of the comparison, the material drained through the filter had reached a uniform moisture content throughout its depth, whereas PMS allowed simply to drain remained wetter at the lower depth.

The p H of both sludge samples declined rapidly from two months after commencement of drainage (figure 4.27). The rate of decline was slightly greater where the sludge was filtered. In both treatments the material was more acidic at the 60 cm. depth than at 10 cm., over the first year of monitoring. After fifteen months, sludge drained through the bark/clinker filter had reached a uniform p H .

There was a rapid decline in elctrical conductivity of PMS in both treatments (figure 4.28), which was slightly more rapid where the filter was used. A higher value was obtained in both treatments at the greater depth of 60 cm..

Compared with samples from the Kemsley site (figure 4.1), the rate of drying out was more rapid where PMS was drained though bark and clinker. Otherwise the drying pattern was very similar.

The electrical conductivity of PMS in both drainage funnels declined more rapidly over the first three months than in sludge deposited at the mill (figures 4.3 and 4.28).

The fall in PMS p H which was recorded at Kemsley (figure 4.2) commenced six months earlier in both drainage treatments. Where the bark/clinker filter was used, the sludge p H fell below 4.0 after twelv months, compared with eighteen months in deposits at Kemsley.

FIGURE 4.26 DRY WEIGHT OF PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.

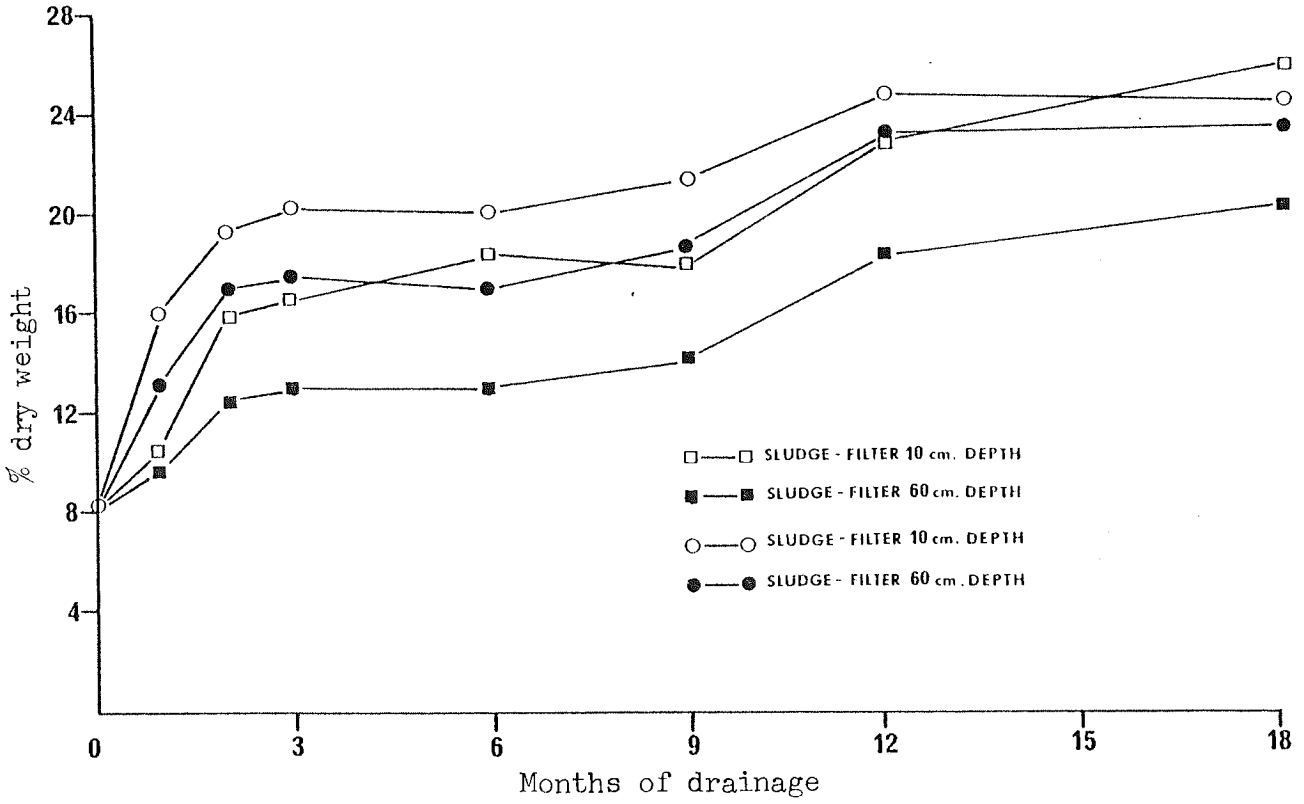


FIGURE 4.27 p H OF PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.

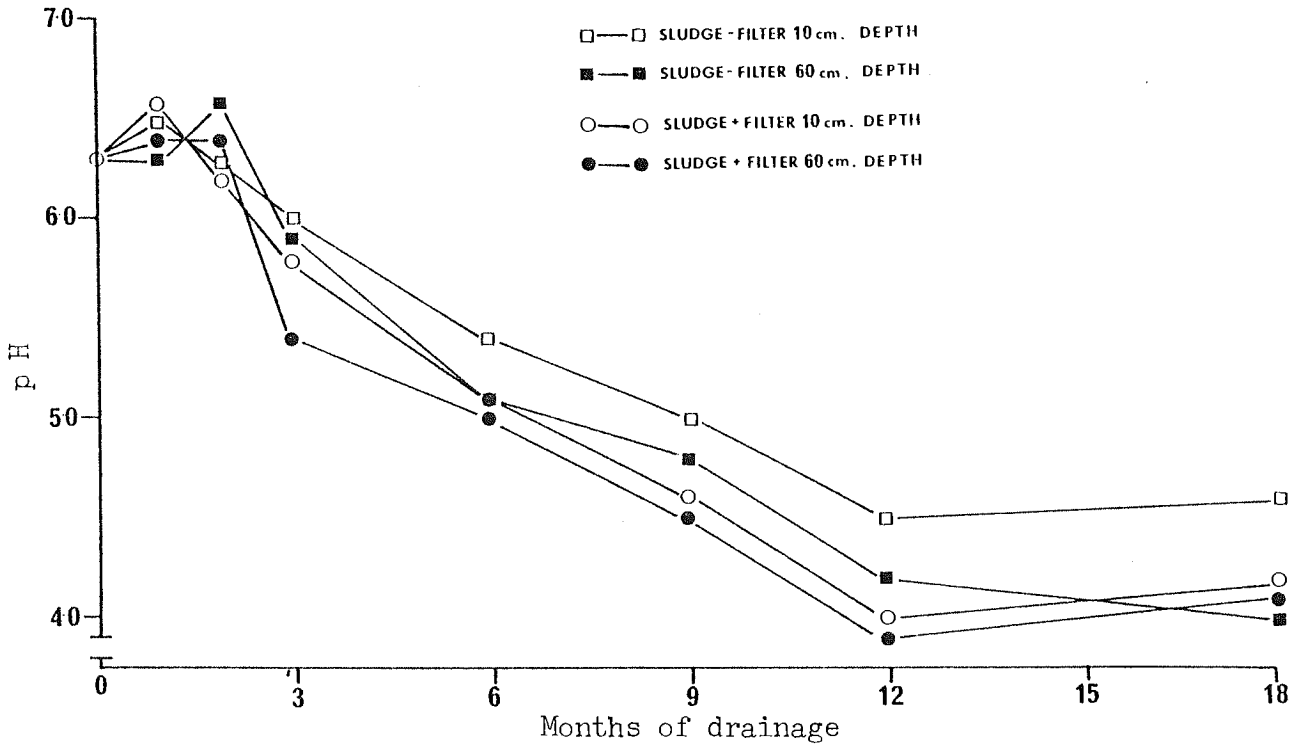
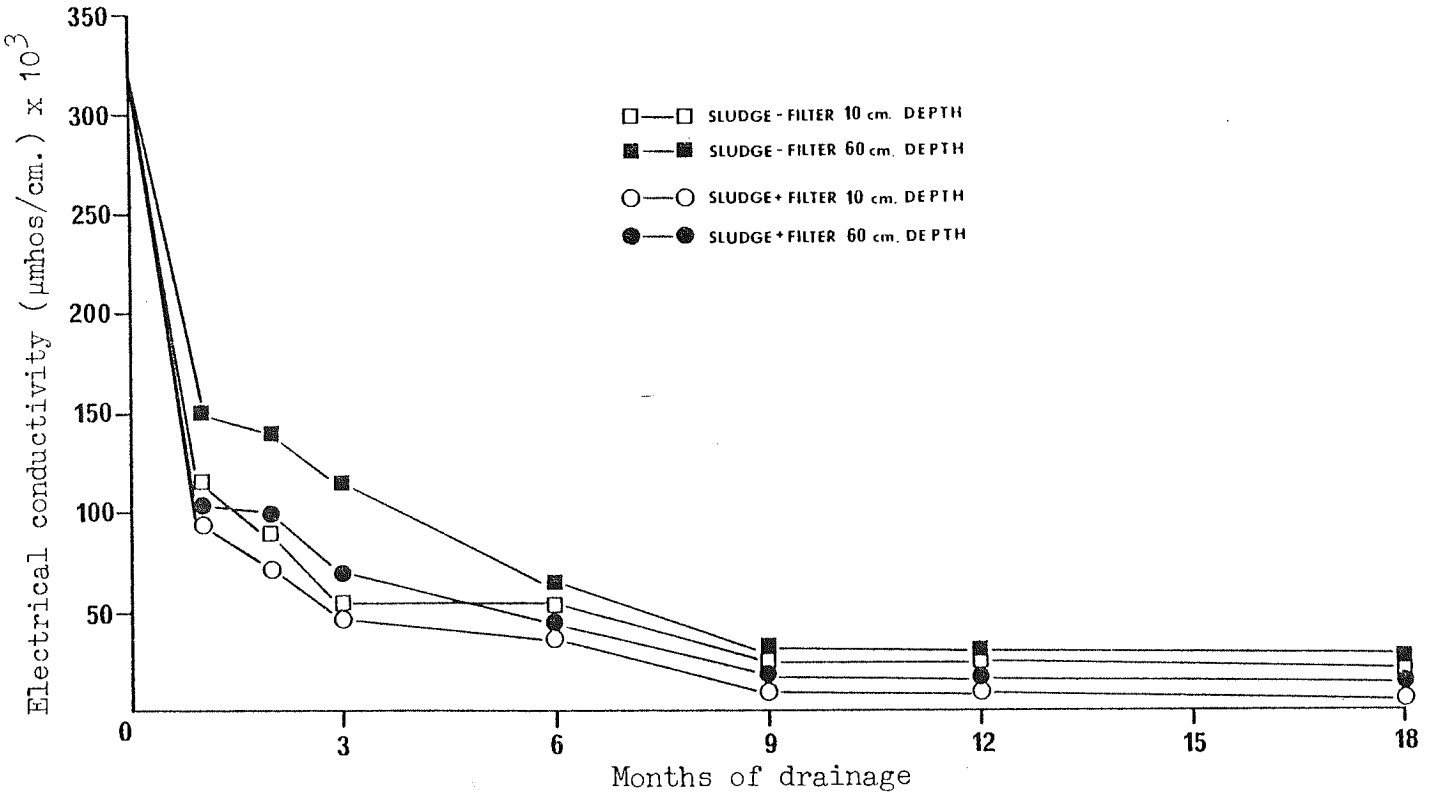


FIGURE 4.28 ELECTRICAL CONDUCTIVITY OF PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.



## 4.4.2 Estimation of fungal populations.

Numbers of mesophilic fungi isolated from the drained sludges are given in figure 4.29. Fungal populations increased rapidly to reach a peak after six months where PMS was drained through the filter medium. After that time the population declined slowly. Where no filter was used, the fungal population initially rose sharply then continued to increase slowly to a peak by twelve months. Thereafter the numbers fell slightly. Fungal populations were higher in the sludge drained through the filter up to six months from commencement of the experiment. Over the first twelve months, the highest number of fungal propagules was isolated from the shallow depth of 10 cm., in both treatments. After sixteen months of drainage fungal populations were the same at both depths in sludge drained through the filter medium.

The relative abundance of mesophilic fungi in the drained samples is given in figures 4.30 and 4.31. The same species of fungi were recorded from sludge in both funnels. As in the sludge deposits at Kemsley, the sampling depths had no effect on the range of species isolated.

In both sludge samples, Cephalosporium curtipes, Meria sp., Monocillium sp. and a member of the Mycelia sterilia were the most abundant species over the first few months. Aspergillus versicolor, Gliocladium sp., Penicillium frequentans and Trichoderma viride replaced these species as the dominant group by eighteen months.

FIGURE 4.29 NUMBERS OF MESOPHILIC FUNGI IN PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.

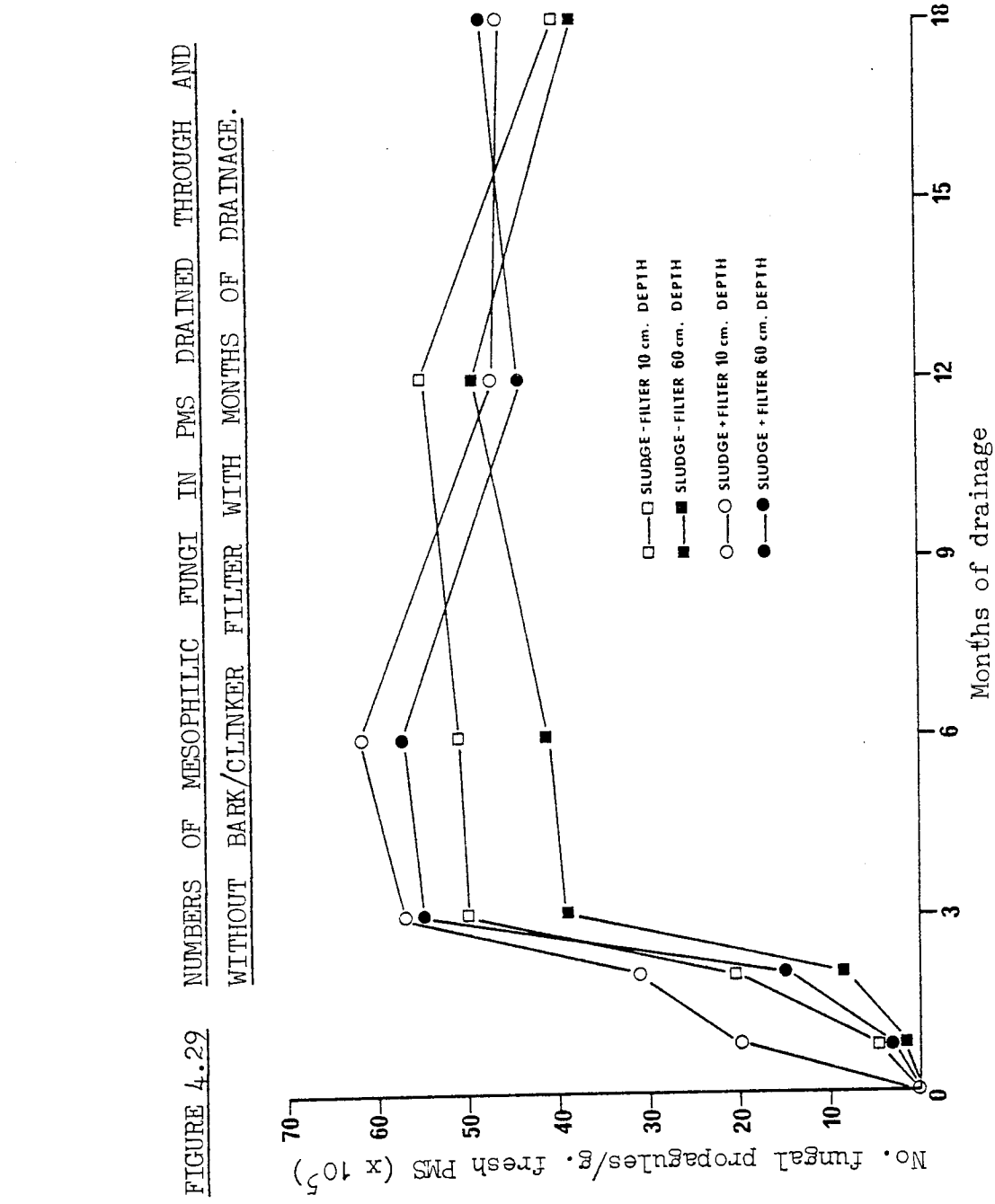


FIGURE 4.30 RELATIVE ABUNDANCE OF MESOPHILIC FUNGI AT 10 cm. DEPTH IN PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.

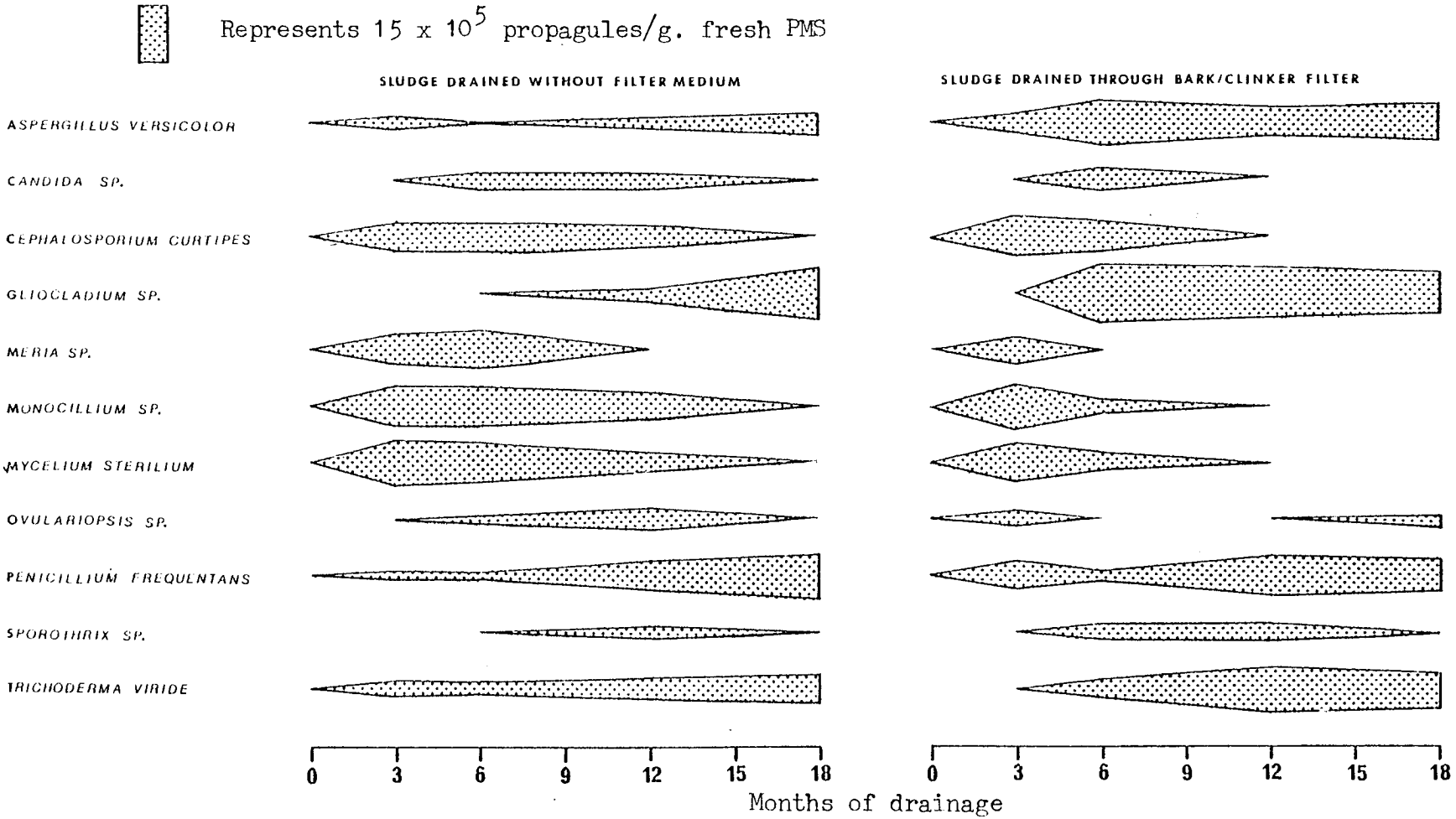
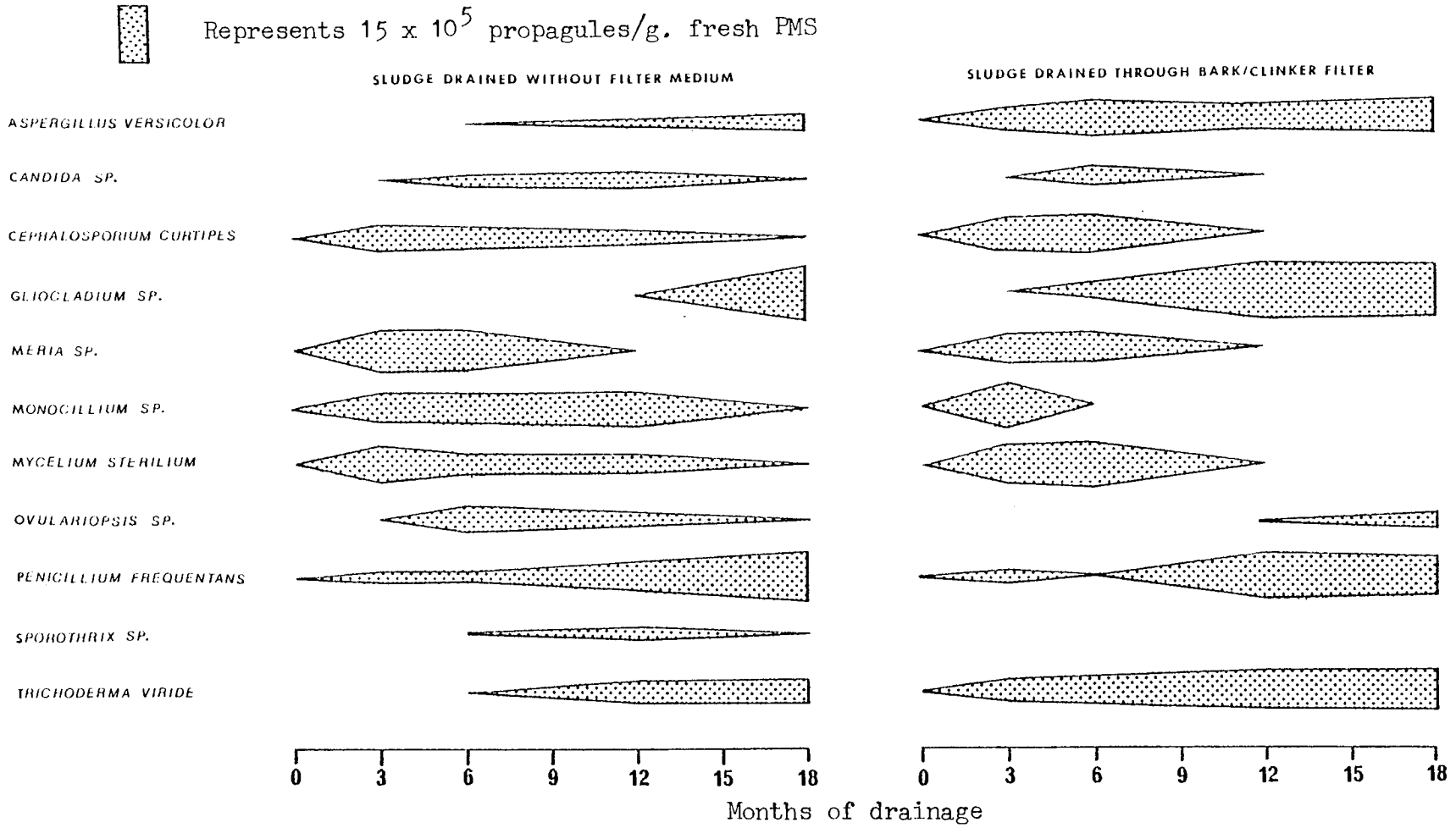


FIGURE 4.31 RELATIVE ABUNDANCE OF MESOPHILIC FUNGI AT 60 cm. DEPTH IN PMS DRAINED THROUGH AND WITHOUT BARK/CLINKER FILTER WITH MONTHS OF DRAINAGE.



The most noticeable difference between the two drainage treatments was that the initially dominant group of fungi gave way more gradually to the second group where sludge was drained without the filter medium. Where a filter was used the initially abundant species disappeared after six to twelve months, but were present for up to eighteen months with no filter. In addition the second dominant group of fungi appeared three to six months earlier where sludge was drained through the filter medium.

Of the species isolated from the Kemsley samples (figure 4.24), only Arthrobotrys oligospora did not occur in the drained sludges. However, three of the initially abundant species in the drainage treatments, Meria sp., Monocillium sp. and Mycelium sterlilium, were not isolated from sludge deposited at Kemsley. In both drained and deposited sludge, Cephalosporium curtipes and Ovulariopsis sp. were most abundant in the early months, whilst Gliocladium sp. and Trichoderma viride became more prevalent later on. Penicillium frequentans was most numerous after six months in samples from Kemsley, but was most abundant after one year in the drained sludges.

## 4.5 EVALUATION OF PMS AS A CASING IN MUSHROOM CULTURE.

It was shown by Hayes (1975) that PMS should be considered as a possible alternative to peat as a casing medium in mushroom culture. However, PMS was only suitable if left for a protracted period following its deposition.

This section describes five laboratory-scale and two commercial-scale experiments aimed at assessing the potential of PMS as a casing material when compared with a standard peat casing soil.

## 4.5.1 The behaviour of PMS as a casing medium with time after its deposition.

Five sludge deposits of different age were sampled to provide casing media, which were compared with a peat control. The age, p H and electrical conductivity of the samples were as follows:

| PMS sample (time after deposition) | p H | Electrical conductivity (µmhos/cm.) |
|---|---|---|
| 4 months | 6.3 | 50000 |
| 12 months | 5.9 | 26090 |
| 18 months | 5.2 | 13421 |
| 20 months | 5.4 | 12730 |
| 36 months | 4.1 | 7692 |

The four month old sludge was chosen as it was the earliest time that the material appeared dry enough to be prepared as a casing medium (see 3.4.1), and corresponded to the time when fungal fruitbodies first appeared on the deposits. The year old sample was chosen as this was when the occurrence of fungal fruitbodies was greatest. At eighteen and twenty months after its deposition, higher fungi had disappeared from the sludge, and green plants begun to colonise.

Finally, a sample of three year old waste was chosen to represent the material following its overgrowth by higher plants.

Only the four month old sample proved difficult to prepare as a casing, owing to its high moisture content. The fruiting characteristics and yield from the different treatments differed markedly and confirmed the results of Hayes (1975). The number of fruitbody initials or pinheads formed prior to the first flush could be related to the electrical conductivity of the casing treatment (table 4.5), more developing as the soluble salt content declined. All PMS based casings except the thirty six month treatment produced significantly fewer initials than the control.

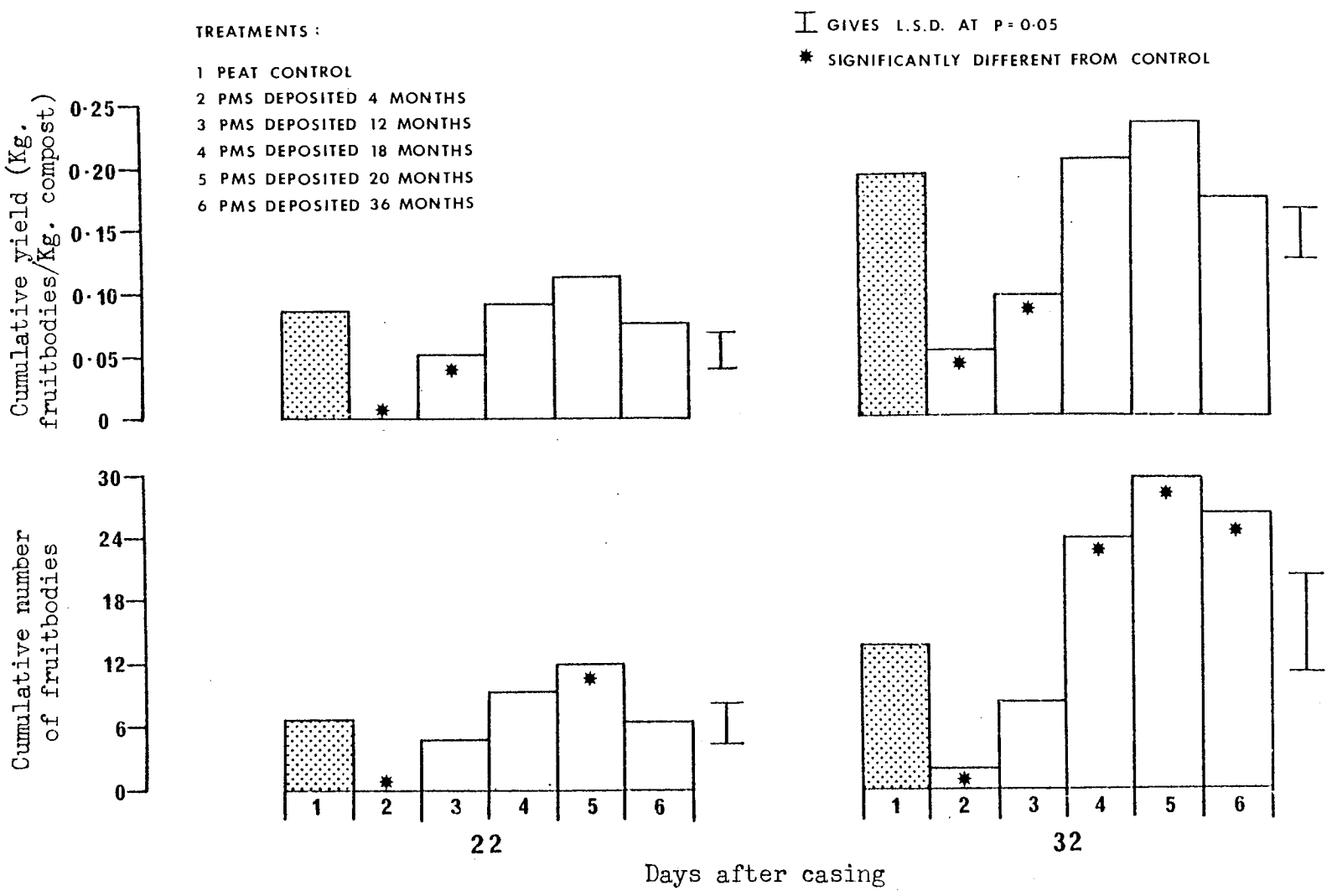
TABLE 4.5 CASING CONDUCTIVITY COMPARED WITH NUMBER OF INITIALS FORMED ON PEAT AND PMS BASED CASINGS.

| Casing treatment | Casing conductivity (μmhos/cm.) | Mean number of initials |
|---|---|---|
| Peat control | 2857 | 147.7 |
| PMS 4 months after deposition | 54861 | 57.3 |
| PMS 12 months after deposition | 22941 | 63.5 |
| PMS 18 months after deposition | 9842 | 70.7 |
| PMS 20 months after deposition | 9714 | 92.7 |
| PMS 36 months after deposition | 7143 | 126.3 |
| L.S.D. (P = 0.05) | - | 38.8 |

The numbers and yield of fruitbodies produced from each treatment also differed according to the age of PMS used (figure 4.32). The four and twelve month samples cropped poorly compared with the peat control. The eighteen and twenty month old samples gave yields and numbers of mushrooms higher than peat whilst the thirty six month sample produced more mushrooms, but gave a lower yield. Statistical analysis of the results (appendix 4.4 and figure 4.32) showed the control treatment to be significantly higher yielding than the four and twelve month treatments, and to produce significantly more fruitbodies than the four month old sample. The yields from the eighteen and twenty month treatments did not differ significantly from the control, whilst the eighteen, twenty and thirty six month samples produced significantly more fruitbodies than peat. On comparing the different PMS casings, there was no significant difference between the eighteen, twenty and thirty six month samples, all of which were superior to the two other treatments.

This trial had to be curtailed at the beginning of the third flush owing to the appearance of diseased fruitbodies on the twenty and thirty six month treatments, and the peat control. Isolations made from infected fruitbodies onto 2% malt agar showed that the infection was caused by a Gliocladium species, identified as a member of the G. delequescens series by reference to Raper and Thom (1968), and identical to the Gliocladium sp. isolated previously from PMS (4.3 and 4.4). By spraying an axenic spore suspension of the organism onto healthy, developing fruitbodies the disease symptoms could be re-created.

FIGURE 4.32 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PEAT AND PMS BASED CASINGS.



The causal Gliocladium sp. is previously unrecorded as a pathogen of A.bisporus. The disease symptoms are summarised below, together with a description of colony and microscopic characters of the pathogen.

'Growth of the causal organism on the surface of the casing is generally the first indication of the disease. The fungus often spreads from an old, undeveloped pinhead onto the casing material in the form of a white, fluffy mycelium. This mycelium soon turns bright green in colour as spores are formed. Before fruitbodies have reached the cup stage of maturity they tend to be overwhelmed by the spreading mycelium of the pathogen (see plate 4.5). The young fruitbody is soon covered in a luxuriant weft of hyphae which rapidly turns green. Sporophores developed to the cup stage and beyond are most frequently attacked at the base of the stipe. There is a dark discolouration and cracking of the tissue at the site of infection, the initially fluffy, off-white mycelium of the pathogen becoming evident a few days later. Growth of the pathogen onto the pileus and lamellae follows infection of the stipe and sometimes engulfs the entire fruitbody (plate 4.6). Less frequently, primary infection of mature fruitbodies is a dark blotching of the surface of the pileus, the sites of infection soon coalescing to cause gross tissue damage (see plate 4.7).

Gliocladium sp. grows readily on 2% (w/v) malt agar at 25°C... The colony is initially grey, but turns dark green in three to four days as the conidia are formed. The conidia are borne on long, branched conidiophores.

There is a single cluster of sterigmata at the tip of each branch, which produce conidia successively to form a short chain (see plate 4.8). The proximity of the sterigmata soon results in the coalescence of conidial chains to form mucilagenous drops, and eventually large spheres of conidia are enveloped in slime, as shown in plate 4.9. The conidia are bright green, smooth and eliptical, measuring 3.75μ - 6.5μ in length, by 2.5μ - 4.5μ wide.'

In addition to the infections with Gliocladium sp., fruitbodies of a member of the Coprinus impexi stirps were regularly formed on the four month old PMS casing, and the presence of large numbers of nematodes was observed on all PMS casings. By reference to Hussey, Read and Hesling (1969) the nematodes were identified as belonging to the order Rhabditida, non-mycophagic types. Nevertheless, all nematodes are discouraged in the commercial situation as there is evidence (Steiner, 1933) that Rhabditid nematodes can transport the bacterium Pseudomonas tolaasi, the cause of bacterial blotch in mushrooms. It is probable that any nematodes in mushroom beds can act as vectors of micro-organisms.

### 4.5.2 Variability of PMS as a casing medium.

Following results from the previous trial, it was decided to use PMS eighteen months after deposition in future evaluations.

Three samples of PMS were obtained from separate deposits at the Kemsley mill, all the deposits being approximately eighteen months old. The three samples were used as casing media in comparison with a peat control.

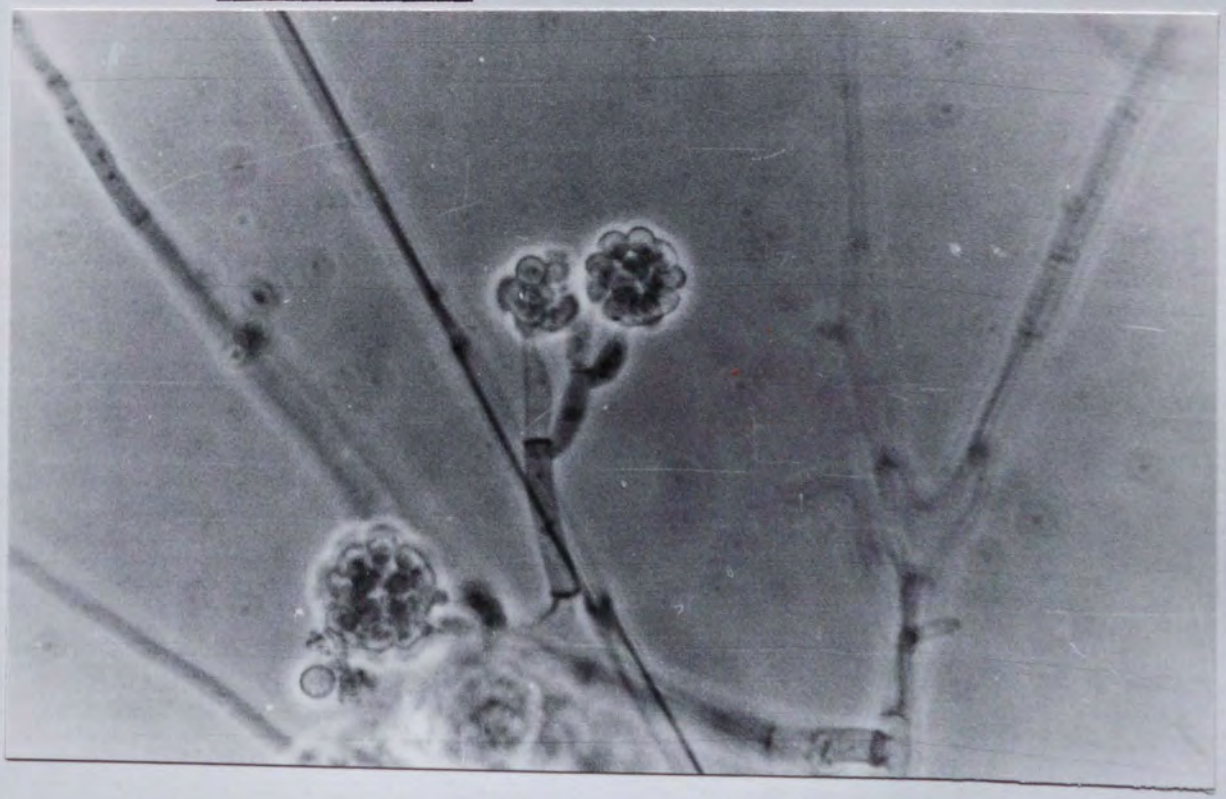
PLATE 4.5 A YOUNG FRUITBODY OVERGROWN BY THE MYCELIUM OF GLIOCLADIUM SP..

PLATE 4.6 MATURE FRUITBODIES ENGULFED BY A HEAVY INFECTION OF GLIOCLADIUM SP..

PLATE 4.7 HEAVY BLOTCHING OF FRUITBODIES CAUSED BY GLIOCLADIUM DISEASE.

PLATE 4.8 A SINGLE CONIDIOPHORE OF GLIOCLADIUM SP..

PLATE 4.9 THE FORMATION OF SLIME HEADS ON CONIDIOPHORE OF GLIOCLADIUM SP..



p H , dry weight and electrical conductivity of the three samples were measured before their use, and were also determined on the casings during the course of the trial. Values for the three PMS samples were as follows:

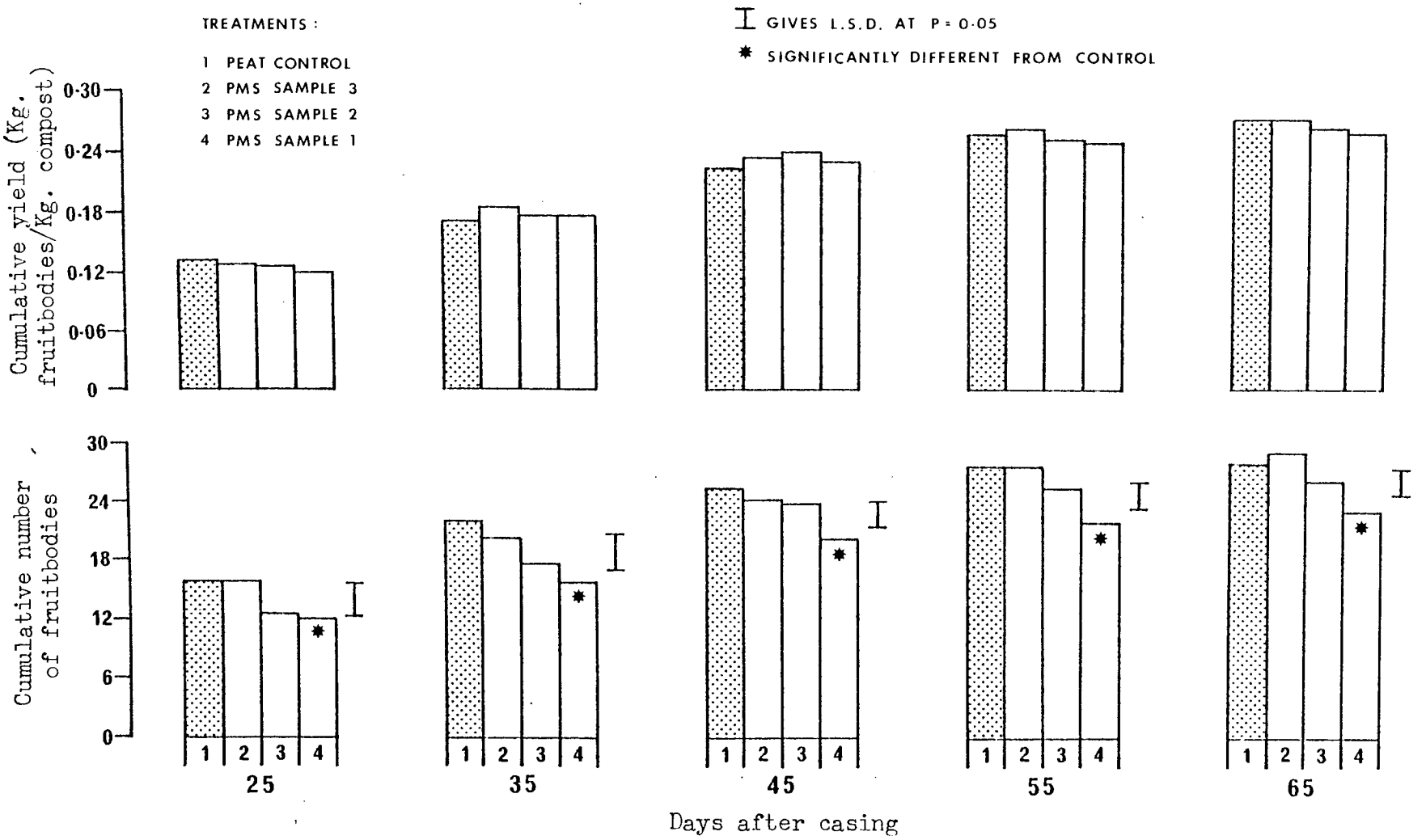
| PMS sample | p H | Electrical conductivity (μmhos/cm.) | % Dry weight |
|---|---|---|---|
| 1 | 4.8 | 17893 | 28.0 |
| 2 | 6.9 | 8433 | 31.9 |
| 3 | 5.5 | 4102 | 25.6 |

The number of fruitbody initials which developed prior to the first flush could be related to the electrical conductivities of the casings (table 4.6). The number declined as the conductivity value increased. Significantly fewer initials were formed on PMS sample 1 compared with the other casings, and on PMS 2 compared with PMS 1 and the peat control.

The number of fruitbodies that developed on the four casings followed the same trend as for number of initials and casing conductivity (see figure 4.33). Significantly fewer sporophores were formed on PMS casing 1 compared with peat and PMS 3 throughout the trial, and PMS 2 at day forty five and after. There were no significant differences between the other treatments.

The yield of fruitbodies from the four casings did not differ significantly (appendix 4.5).

FIGURE 4.33 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM A PEAT CONTROL AND THREE SAMPLES OF PMS EIGHTEEN MONTHS AFTER DEPOSITION.



It can therefore be concluded that the fruitbodies which developed on PMS sample 1 were significantly heavier than those from the other casing treatments.

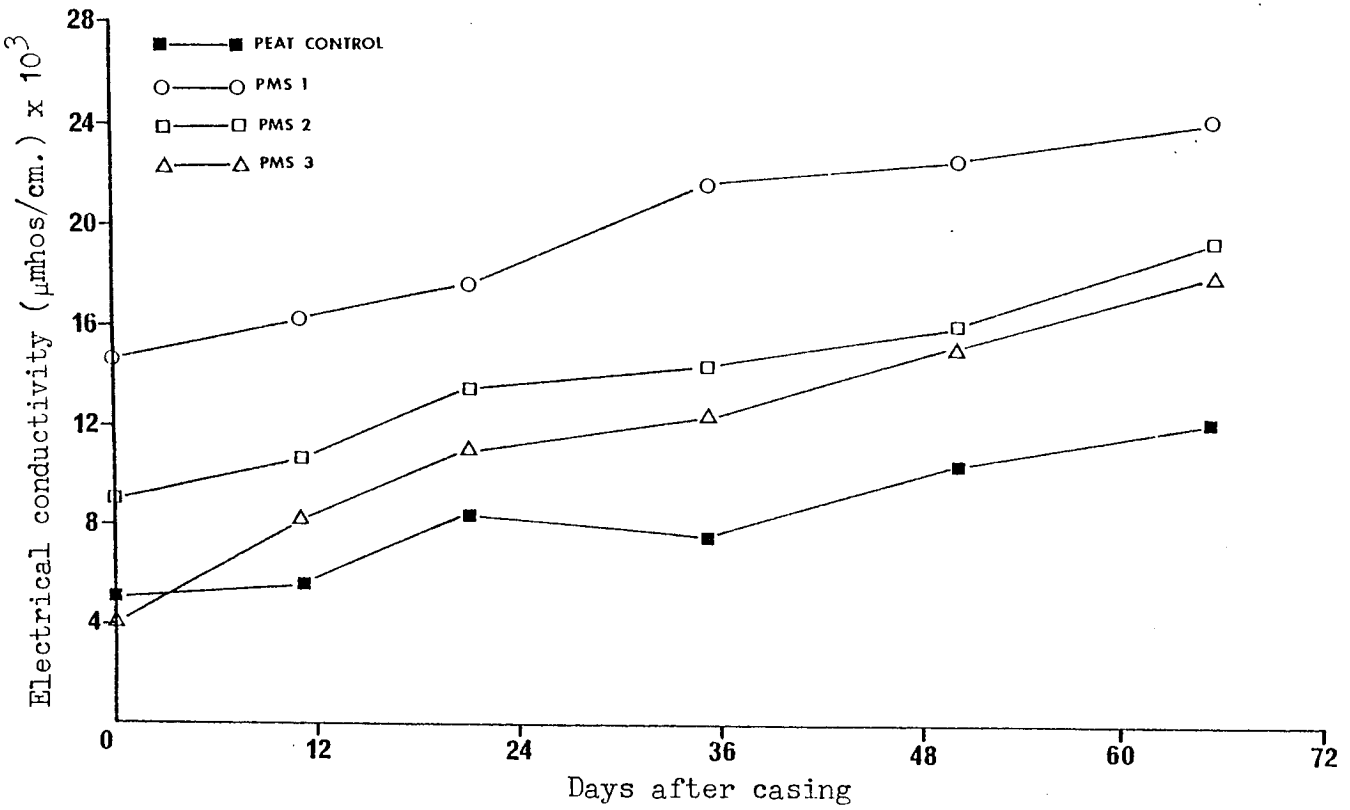
TABLE 4.6 NUMBER OF INITIALS FORMED ON PEAT AND PMS CASINGS 1, 2 AND 3, COMPARED WITH CASING CONDUCTIVITY.

| Casing treatment | Casing conductivity (µmhos/cm.) | Mean number of initials |
|---|---|---|
| PMS 1 | 18092 | 120.0 |
| PMS 2 | 8136 | 142.0 |
| PMS 3 | 4227 | 168.0 |
| Peat control | 2913 | 171.0 |
| L.S.D. (P = 0.05) | - | 17.3 |

Figure 4.34 shows that there was an accumulation of soluble salts in all four casings. The rate of salt build up was less in peat than in the PMS treatments, the final conductivity of peat being two thirds to a half of that in the sludge based casings. Salt accumulation was more rapid in PMS 3 than PMS 1, which was initially more saline than PMS 3.

The Gliocladium infection described in 4.5.1, also occurred on PMS casings in this trial, though the disease was not so severe. Rhabditid nematodes were also observed on PMS casings 1 and 3 in relatively large numbers. In subsequent trials PMS was pasteurised before use in order to eliminate both Gliocladium sp. and nematodes (see 3.4.1).

FIGURE 4.34 ELECTRICAL CONDUCTIVITY OF CASINGS BASED ON PEAT AND PMS SAMPLES EIGHTEEN MONTHS AFTER DEPOSITION WITH TIME FROM CASING.



## 4.5.3 Neutralising requirements of PMS casing.

In subsequent experimental work it was planned to study the effects of elemental content and particle size of PMS casing on fruiting of A.bisporus. In mushroom production in the U.K. peat based casing soil is neutralised with chalk from a variety of sources, and with a range of particle sizes. Chalks from different sources can contain quite different elements as impurities (Hancock, 1975), whilst different particle sizes confer different structural characteristics to casing. In view of these considerations it was decided to use 'Morden R' chalk as a standard in all future trials. This chalk undergoes refinement and purification as well as being a finely ground material (Gibson, 1975).

The p H values obtained by the addition of 'Morden R' chalk at different rates to peat and PMS samples 2 and 3 used in the previous trial are given in appendix 4.6. Compared with peat both PMS samples required the addition of less chalk to achieve a p H of 7.5. The addition rate of 1 part chalk to 8 parts PMS by weight was subsequently adopted as the standard for PMS.

A culture trial was carried out to determine whether PMS required neutralisation with chalk to act as a satisfactory casing. Chalk was added at the rate of 1 : 8 by weight to pasteurised sample 3, as the control treatment. Samples 2 and 3 were used pasteurised, but without added chalk.

Sample 2 had a p H of 6.9 which is close to that normally aimed at in a casing, whilst the p H of sample 3 was 5.5, lower than generally considered satisfactory for a casing. Sample 3 with chalk added prior to pasteurisation was included to assess the effect of pasteurising ready neutralised casing.

The addition of chalk to PMS 3 raised its conductivity from *circa* 4 x $10^3$ µmhos/cm. to *circa* 8 x $10^3$ µmhos/cm. (figure 4.35). The conductivity of all casings increased steadily during culture. Addition of chalk at the ratio of 1 part chalk to 8 parts PMS maintained a satisfactory casing p H throughout the trial. However, the p H of non-neutralised sample 2 declined to 5.6 by day seventy, while PMS 3 remained just above 5.5.

The mycelium grew more slowly into non-neutralised casings than when chalk was added. Thus it was not possible to accurately compare the number of initials formed prior to the first flush.

Analysis of yield and numbers of mushrooms produced on the four casings (appendix 4.7 and figure 4.36) showed no significant difference between PMS neutralised before or after pasteurisation. Both neutralised casings were significantly superior to samples 2 and 3 unadjusted. In subsequent evaluations, PMS was neutralised with chalk prior to its pasteurisation.

No incidence of *Gliocladium* disease or presence of nematodes were recorded during the trial.

FIGURE 4.35 p H AND ELECTRICAL CONDUCTIVITY OF NEUTRALISED AND NON-NEUTRALISED PMS CASINGS WITH TIME FROM CASING.

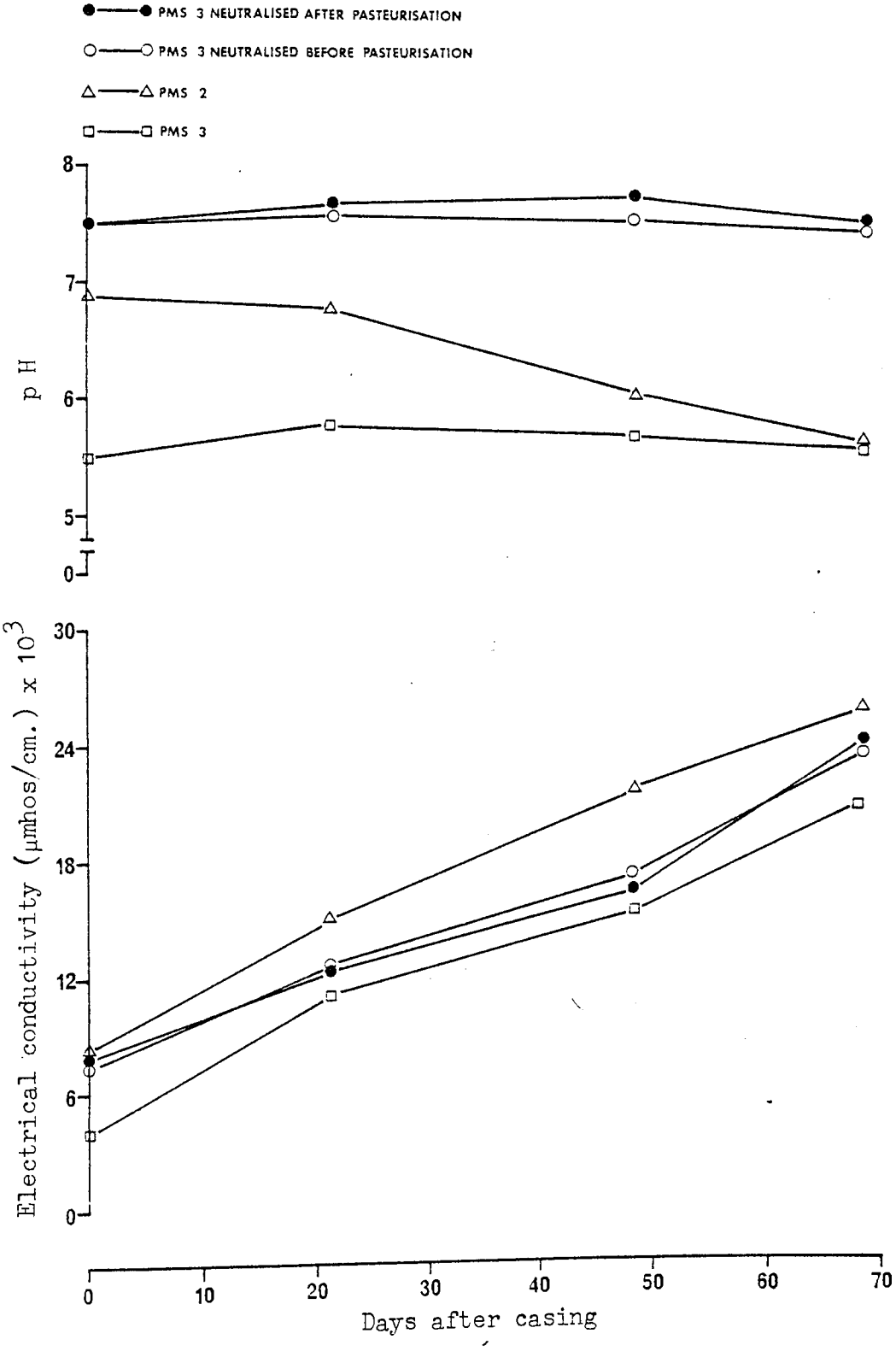
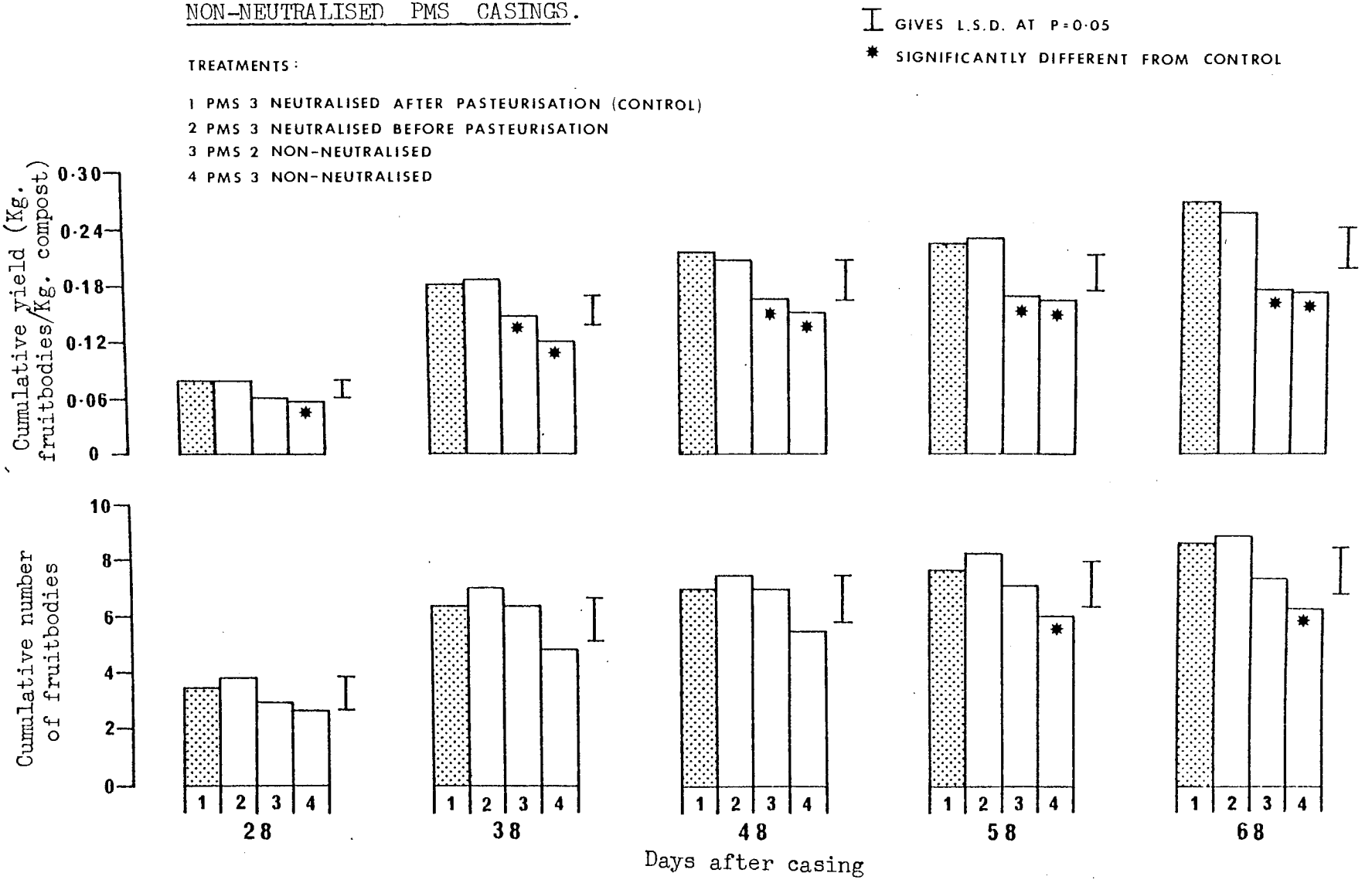


FIGURE 4.36 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM NEUTRALISED AND NON-NEUTRALISED PMS CASINGS.



## 4.5.4 Evaluation of PMS as a casing material under commercial conditions.

The evaluation of PMS as a casing on commercial-scale growing units was done at the research and development section of W. Darlington and Sons Limited, Angmering, Sussex. This section reports on the results of two trials conducted at the unit, using 2.2 sq. m. trays. In the first trial only one third of a growing house was available, but the second trial occupied a full house and was used to repeat some of the treatments from the first, with the degree of replication increased from two to eight.

As well as comparing peat and PMS, the first trial was used to compare PMS pasteurised with steam/air with non-pasteurised material. Though steam/air mixtures are used in the U.S.A. for pasteurisation of casing soils, fumigation of soils with various chemicals is an alternative method (Schisler and Wuest, 1971; Berkum and Hoestra, 1979). Hayes (1971) suggested fumigation of peat casing with the soil fumigant methyl bromide could be advantageous since bacterial activity was stimulated during the fumigation procedure. This fumigant was included for comparison with the steam/air mix as a pasteurisation agent.

PMS casing was prepared as in 3.4.1 except that Duncton's chalk was added, the same as for the peat control (3.5).

In the first trial the casing treatments were PMS non-pasteurised, PMS pasteurised with steam/air and methyl bromide, and a non-pasteurised peat control.

The methyl bromide treatment was omitted in the second trial.

Electrical conductivity, levels of available elements and p H were determined on the casings in the first trial at their application and at the end of the trial.

There were no significant differences in yield from the treatments in the first trial (appendix 4.8), which are shown in figure 4.37. This was also the case in the second trial, save for a significant advantage with the non-pasteurised PMS at day twenty four (appendix 4.9 and figure 4.38). This advantage was due to earlier development of fruitbodies on that casing prior to the first flush.

The mean yields obtained in both trials were satisfactory by commercial standards:

| | |
|---|---|
| Peat | 18.2 and 18.4 Kg./sq. m. |
| PMS non-pasteurised | 16.1 and 17.1 Kg./sq. m. |
| PMS steam/air pasteurised | 16.9 and 19.4 Kg./sq. m. |
| PMS methyl bromide pasteurised | 14.6 Kg./sq. m. |

No high incidence of disease or pest organisms was recorded by Darlington's staff monitoring the trials.

The p H of all four casings in the first trial fell slightly during culture, but remained at acceptable levels.

FIGURE 4.37 CUMULATIVE YIELDS FROM THE FIRST COMMERCIAL-SCALE COMPARISON OF PEAT WITH PMS CASINGS.

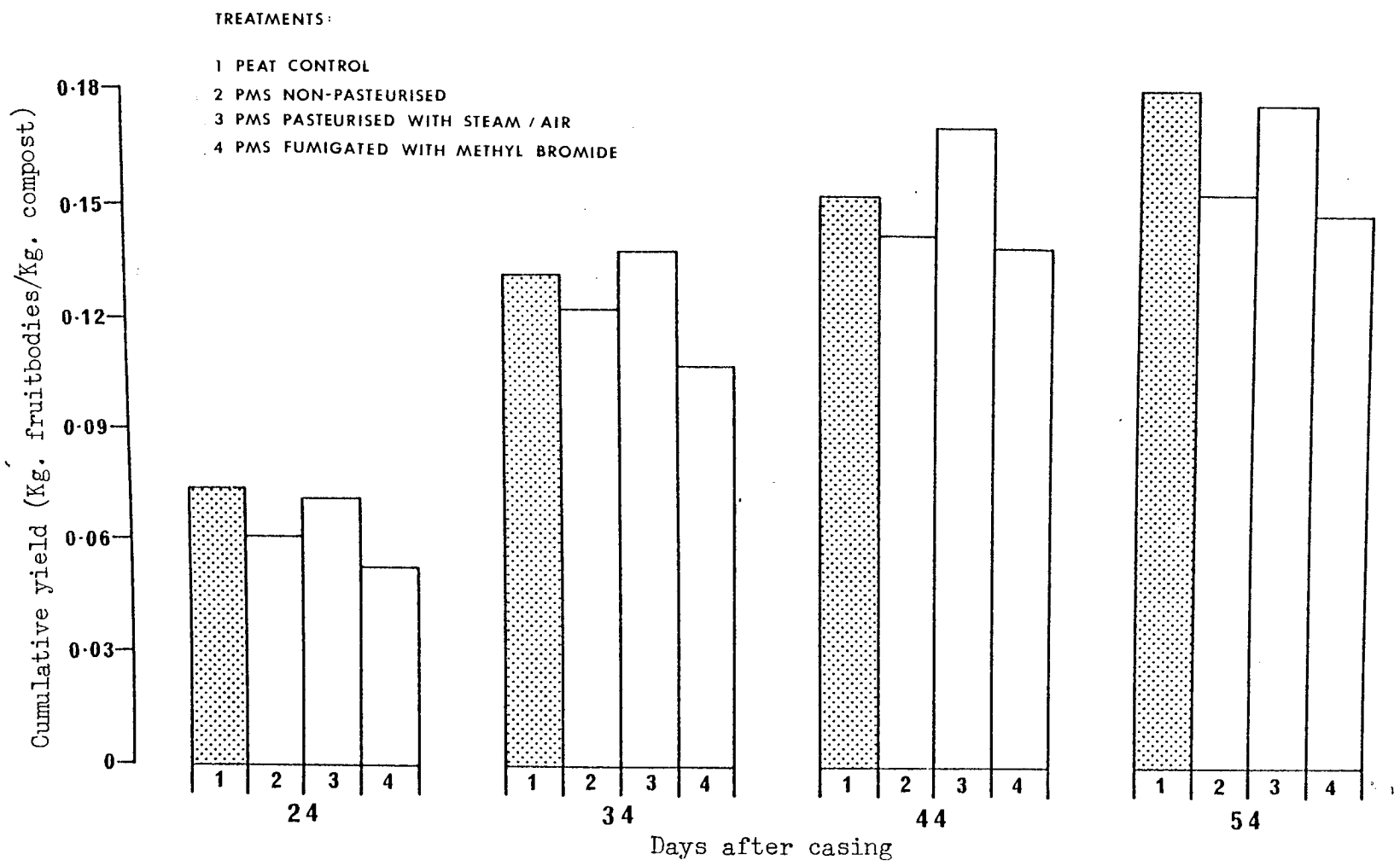
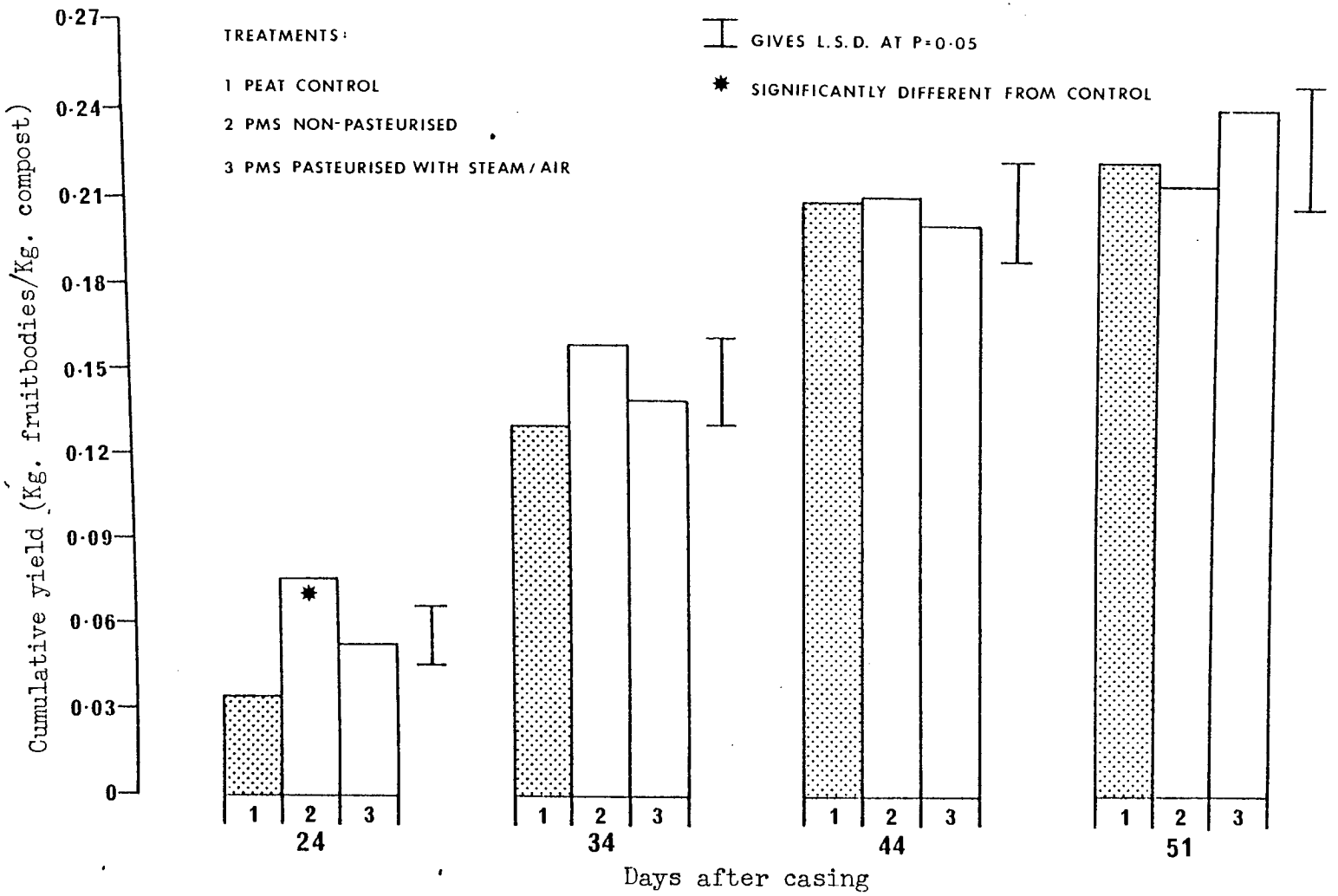


FIGURE 4.38 CUMULATIVE YIELDS FROM THE SECOND COMMERCIAL-SCAIE COMPARISON OF PEAT WITH PMS CASINGS.



As in previous trials the electrical conductivity of all casings increased after their application (appendix 4.10). However, the rate of salt accumulation in the peat control was much greater than in the PMS treatments.

The levels of ions extracted with ammonium acetate (3.7.14) from casings in trial one are given in table 4.7.

The initial levels of available ions in peat and PMS casings were quite similar save for the higher sodium and sulphate content of PMS, which is derived from the spent pulping liquor at Kemsley. The initial sulphate content of PMS was ten times higher than in peat, but fifty four days from casing the level in all treatments was around $6 \times 10^3$ µg./g. dry casing, reflecting a much greater rate of increase in peat. A similar pattern was recorded with sodium. Initially three and a half times higher in PMS treatments, the level in all casings was around $0.7 \times 10^3$ µg./g. by the end of the trial.

Chloride and potassium increased in all casing treatments, chloride attaining levels over $1.5 \times 10^3$ µg./g., and potassium $0.7 \times 10^3$ µg./g. in PMS casings, half the final concentration in peat.

Apart from slight increases in magnesium levels, no other elements showed consistent changes in concentration.

TABLE 4.7 CHANGES IN THE LEVELS OF AVAILABLE IONS IN CASINGS USED IN THE FIRST COMMERCIAL TRIAL.

| TREATMENT | DAYS AFTER CASING | AVAILABLE IONS ( µg./g. DRY CASING) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ca | Cl | Cu | Fe | K | Mg | Mn | Na | $SO_4$ | Zn |
| Peat control | 0 | 4290 | 35 | <3 | <4 | 60 | 145 | 7 | 142 | 207 | 2 |
| | 54 | 4371 | 1725 | <3 | <4 | 1557 | 225 | 8 | 763 | 5986 | 2 |
| PMS non-pasteurised | 0 | 3571 | 80 | 14 | <4 | 78 | 91 | 8 | 514 | 2019 | 18 |
| | 54 | 3486 | 1426 | 3 | 8 | 711 | 92 | 10 | 754 | 6327 | 6 |
| PMS steam/air pasteurised | 0 | 3500 | 59 | <3 | <4 | 68 | 108 | 10 | 509 | 2074 | 8 |
| | 54 | 3257 | 1097 | 5 | 7 | 751 | 117 | 14 | 751 | 6248 | 11 |
| PMS methyl bromide pasteurised | 0 | 3569 | 67 | 9 | <4 | 73 | 101 | 13 | 523 | 2138 | 12 |
| | 54 | 3817 | 1317 | 7 | 6 | 709 | 119 | 8 | 778 | 6339 | 12 |

## 4.5.5 Steam/air pasteurisation of PMS casing.

A trial on the effect of varying the duration and temperature of steam/air pasteurisation of PMS casing was carried out to define more accurately an appropriate treatment. The six treatments chosen were a non-pasteurised control and casing pasteurised with the different regimes of 50°C. for one hour, 65°C. for thirty minutes and one hour, and 80°C. for thirty minutes and one hour. The temperature range of 50°C. to 80°C. was selected after reference to Baker (1957) and Schisler and Wuest (1971).

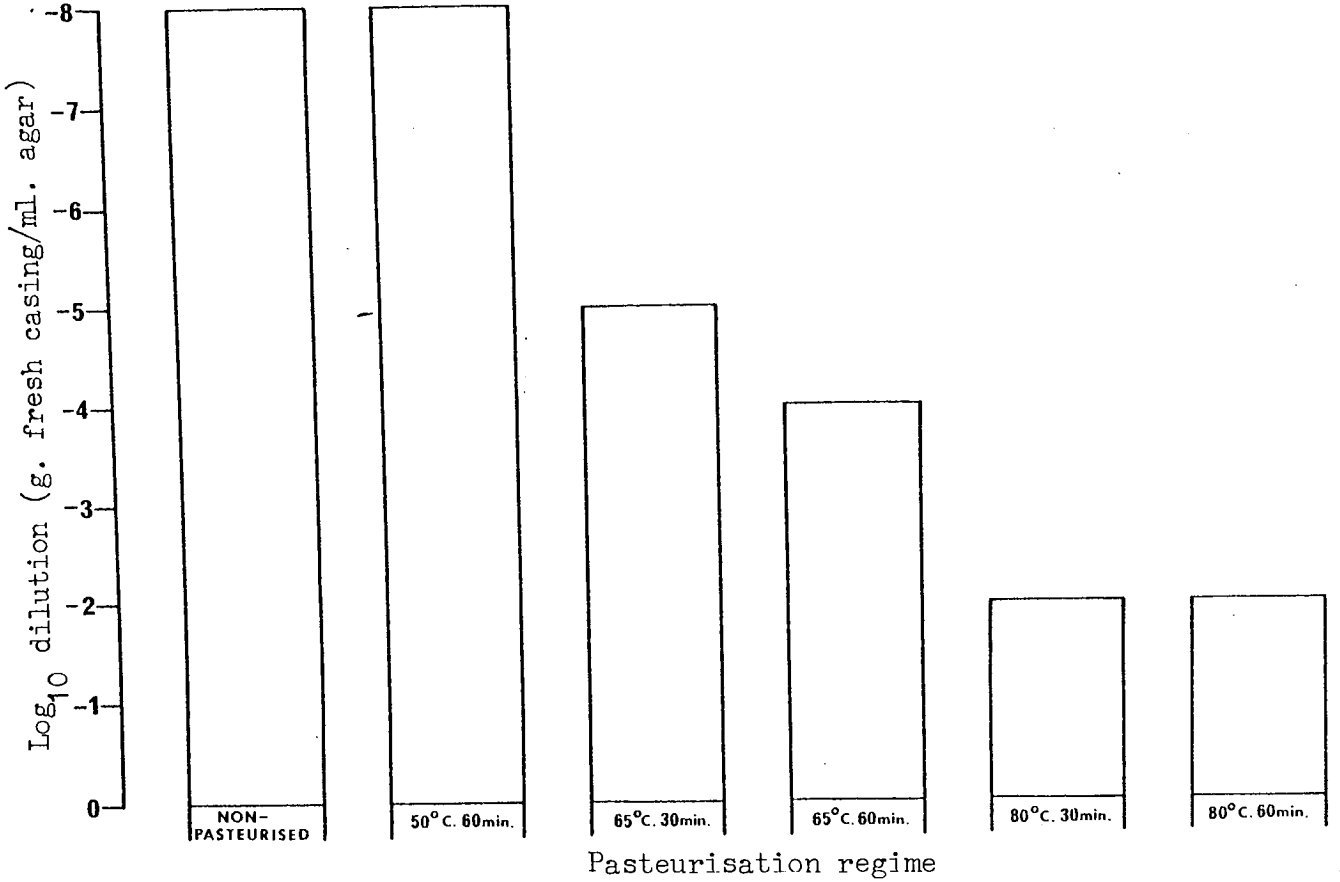
Dry weight, p H , electrical conductivity and numbers of viable fungal propagules were determined on the casing treatments immediately after pasteurisation and compared with the untreated control. Changes in numbers of bacteria and nematodes were estimated in parallel studies by Cresswell (pers. comm.).

The p H and electrical conductivity of the casing were not altered by any of the pasteurisation treatments, but its moisture content was raised slightly by pasteurisation at 50°C. for one hour (appendix 4.11).

Fungal populations were estimated as in 3.7.18 except that isolations were made onto malt agar at $10^{-2}$ to $10^{-8}$ g. fresh casing/ml. agar, and plates incubated at 25°C. only.

Figure 4.39 shows the lowest dilution at which no viable propagules were isolated.

FIGURE 4.39 THE LOWEST DILUTION AT WHICH NO VIABLE FUNGAL PROPAGULES WERE ISOLATED FROM PMS CASING FOLLOWING PASTEURISATION WITH DIFFERENT STEAM/AIR REGIMES.



No reduction in fungal numbers was indicated following pasteurisation at 50°C. for one hour. Treatment at 65°C. for thirty minutes reduced the number of viable propagules from $10^6$ - $10^7$ /g. fresh casing to $10^3$ - $10^4$ /g., and 65°C. for one hour gave a further reduction to $10^2$ - $10^3$ /g.. The pasteurisation regimes at 80°C. both reduced viable fungal propagules below 10/g. fresh casing.

The fungal species which were isolated from the casings are given in appendix 4.12. No fungi were isolated from the two casings treated at 80°C.. Only four species were isolated from the non-pasteurised control, Gliocladium sp., Meria sp., Mucor sp. and Trichoderma viride. The same species, plus Aspergillus versicolor were isolated from casing pasteurised at 50°C. for one hour. All of these fungi were previously isolated from PMS in 4.3 and 4.4. Aspergillus versicolor and Meria sp. were also isolated following treatment at 65°C., but the other three fungi were absent. A member of the Mycelia sterilia isolated in 4.4, plus Aureobasidium sp. and Helicosporium sp. were isolated after pasteurisation at 65°C.. The latter two species were not previously isolated from PMS. Pasteurisation at 50°C. for one hour did not alter the composition of the fungal flora, but treatment at 65°C. for thirty minutes and one hour did so, and eliminated the Gliocladium sp. responsible for infections in previous trials. Treatment of casing at 80°C. reduced fungal numbers to very low levels, with none being isolated at the dilutions used.

When the casings were used in a culture trial less initials formed on all of the pasteurised treatments compared with the control, though the differences were not significant (table 4.8).

FIGURE 4.40 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING FOLLOWING ITS PASTEURISATION WITH DIFFERENT STEAM/AIR REGIMES.

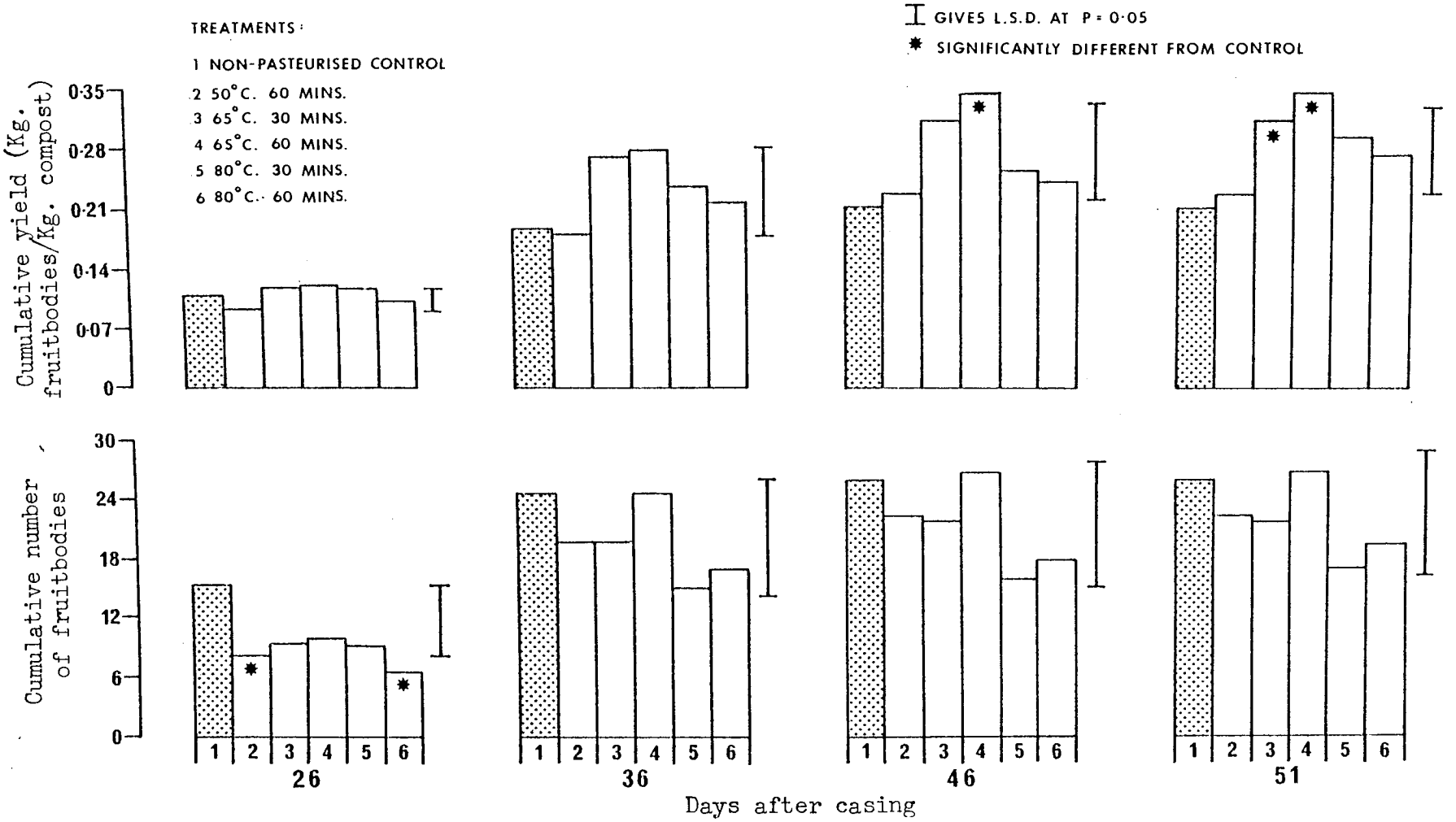


TABLE 4.8 EFFECT OF DIFFERENT STEAM/AIR PASTURISATION REGIMES ON THE NUMBER OF INITIALS FORMED ON PMS CASING.

| Casing treatment | Mean number of initials |
|---|---|
| Non-pasteurised control | 53.0 |
| 50°C. for 60 minutes | 30.0 |
| 65°C. for 30 minutes | 34.0 |
| 65°C. for 60 minutes | 30.3 |
| 80°C. for 30 minutes | 23.7 |
| 80°C. for 60 minutes | 29.3 |
| L.S.D. (P = 0.05) | 30.1 |

There was a reduction in the number of fruitbodies which developed on all pasteurised casings during the first flush, which was significant or close to significance in all cases (appendix 4.13 and figure 4.40). Following the first flush, the reduction in numbers from pasteurised casings was maintained save for that treated at 65°C. for one hour, which was comparable to the control. The statistical variation between treatments fell steadily after the first flush (appendix 4.13).

The appearance of fruitbodies on casings treated at 80°C. was delayed after the second flush and consequently yield data (figure 4.40) is expressed fifty one days from casing as well as at forty six days.

Comparing the yields from the six treatments (figure 4.40), the two casings treated at 65°C. gave the highest yields, with the two 80°C. treatments the next most productive, while the control and 50°C. treatment gave the lowest yields. By day forty six the yield from casing pasteurised at 65°C. for one hour was significantly higher than that from the control, as was that from casing treated at 65°C. for thirty minutes by day fifty one. Yield from the longer treatment at 65°C. was significantly higher than that from the 50°C. regime throughout the trial. There were no other significant yield differences.

Infection of fruitbodies with the Gliocladium sp. described in 4.5.1 occurred in this trial, the yield from the six treatments corresponding to the severity of the disease. The infection was relatively severe on the control and 50°C. treatment. It occurred to a lesser extent on casings pasteurised at 80°C., whilst no infection was noted on casings treated at 65°C.

Table 4.9 summarises the effects of the pasteurisation regimes on PMS casing.

## 4.5.6 Interaction of fungi isolated from PMS with A.bisporus during culture.

Competitive inhibition between fungi or fungistasis is a phenomenon thought to occur in natural environments (Dobbs and Hinson, 1953; Garrett, 1963; Smith 1973).

TABLE 4.9 SUMMARY OF EFFECTS OF DIFFERENT STEAM/AIR TREATMENTS ON PMS CASING.

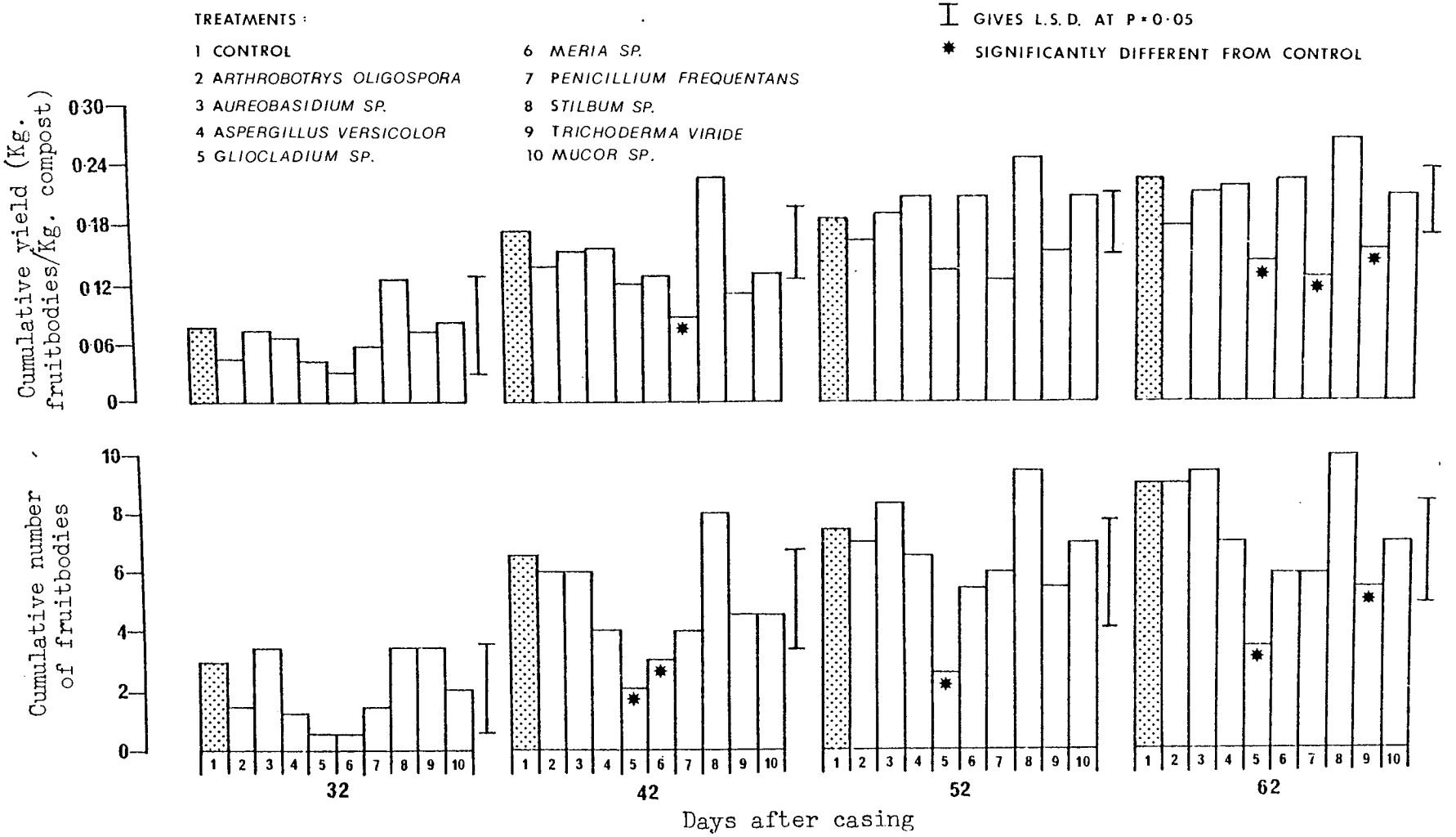
| | | Pasteurisation regime | | | | |
|---|---|---|---|---|---|---|
| | Non-pasteurised control | 50°C. 60 mins. | 65°C. 30 mins. | 65°C. 60 mins. | 80°C. 30 mins. | 80°C. 60 mins. |
| Casing p H | 7.8 | No effect | | | | |
| Casing conductivity ($\mu$mhos/cm.) x $10^3$ | 5.6 | No effect | | | | |
| Casing moisture content (%) | 70.9 | Increased to 75.3 | No effect | | | |
| Number of viable fungal propagules/g. fresh casing | $10^6 - 10^7$ | No effect | Reduced to $10^3 - 10^4$ | Reduced to $10^2 - 10^3$ | Reduced below 10 | Reduced below 10 |
| Number of initials | 53.0 | Reduced to 25.0 to 35.0 by all treatments | | | | |
| Number of fruitbodies | - | Reduced by all treatments except 65°C. for 60 mins. after first flush | | | | |
| Yield | - | 65°C. treatments > 80°C. treatments > 50°C. treatment and control. Corresponded to incidence of *Gliocladium* infection. | | | | |
| Incidence of *Gliocladium* disease | + + + | + + + | - | - | + | + |

Moulds competitive with A.bisporus are commonly encountered in commercial production, with Ostracoderma sp., Scopulariopsis fimicola and Trichoderma spp. being among the more troublesome in the U.K.. A total of nine of the micro-fungi most frequently isolated from PMS in 4.3 and 4.4 were selected to determine their compatability with A.bisporus in the casing layer.

PMS casing was prepared as previously but was not pasteurised. Six hundred gramme samples were placed in one litre wide-mouth, round bottomed flasks and sterilised by autoclaving at 121°C. for fifteen minutes on three successive days. The sterile casings were then inoculated with individual isolates using six agar plugs from three week old cultures. The casings were incubated for three weeks at 25°C. to allow development of the fungi throughout the samples. After that time each treated casing was used to case colonised compost, along with a control casing pasteurised at 65°C. for one hour.

The results (figure 4.41 and appendix 4.14) show four of the nine isolates examined had a significant effect on the productivity of the crop. Three of the fungi, Gliocladium sp., Penicillium frequentans and Trichoderma viride, reduced both the yield and number of fruitbodies by one third to a half of the control. Gliocladium sp. was particularly inhibitory, reducing the number of fruitbodies by two thirds, with several diseased fruitbodies being recorded. Inoculation of casing with Meria sp. resulted in development of a third fewer fruitbodies, but no reduction in yield. Yield was not significantly increased by any of isolates tested.

FIGURE 4.41 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING FOLLOWING ITS COLONISATION BY FUNGI ISOLATED FROM PMS.



## 4.6 STRUCTURAL REQUIREMENTS AND MANAGEMENT OF PMS WHEN USED AS A CASING MEDIUM.

Previous work on casing structure and management was reviewed in section two. It was concluded that a good casing requires a permanent structure which is sufficiently open to allow adequate gaseous diffusion from the compost. Studies on casing moisture content have shown that a high water holding capacity is favourable, especially under conditions where evaporative loss from the casing is high. The management of a casing, in particular watering procedure, is however still regarded as something of an art in mushroom growing.

Since PMS is structurally manufactured as a casing, this facilitates varying the composition of the final product. It was decided to optimise the structure of PMS casing and then gain some information on its performance under different regimes of watering.

### 4.6.1 The effect of varying the maximum particle size of PMS casing.

In previous trials the structure of PMS was obtained by forcing the solids through a ten millimetre mesh sieve. In this trial the material was forced through mesh sizes of five, ten and twenty millimetres before neutralising with 'Morden R' chalk as previously. The use of this fine grade of chalk minimised any effect on the particle size ranges created. Peat casing was included as a control treatment. A.bisporus strain 21 series was used in this trial.

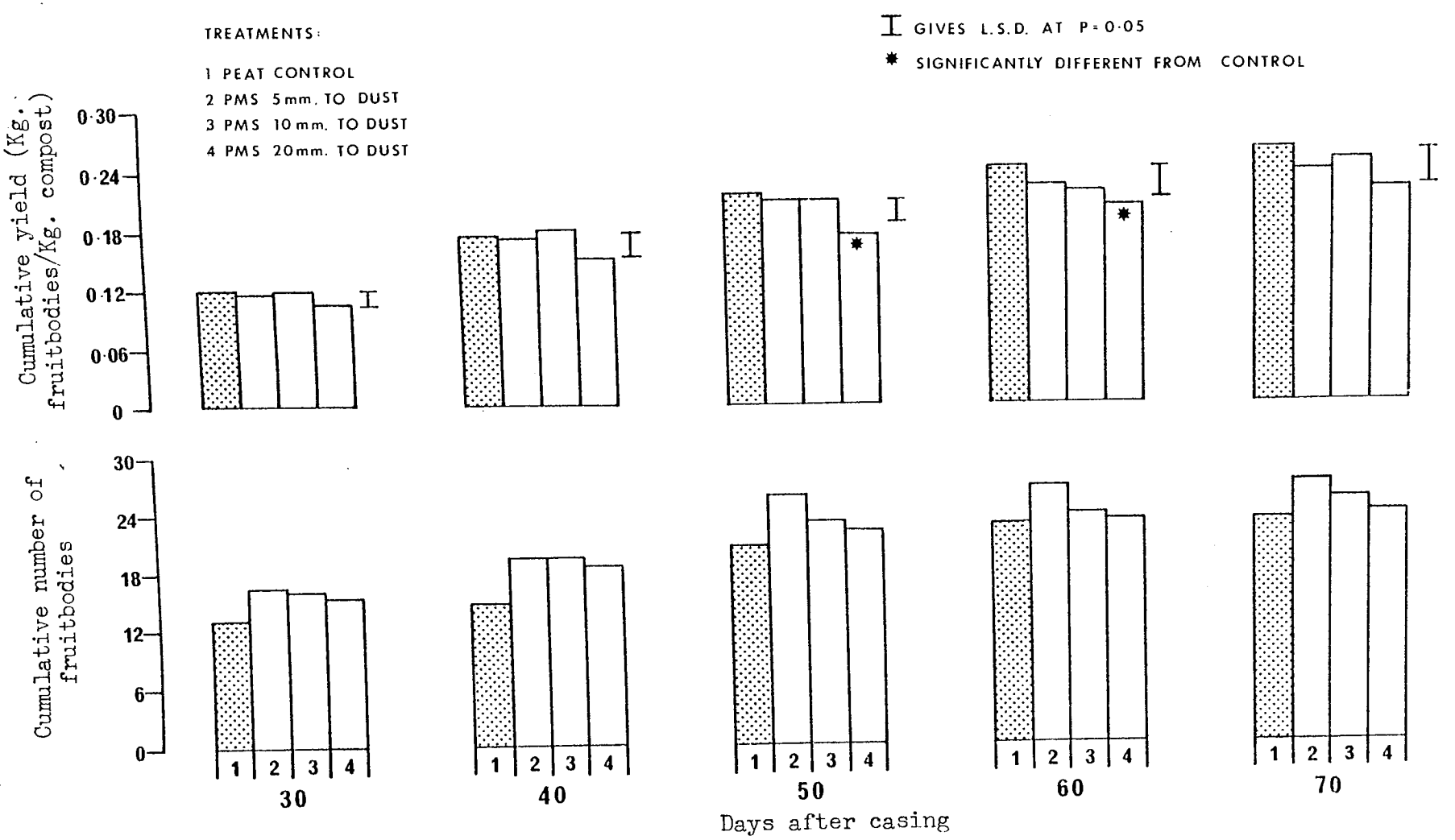
There were no significant differences between the number of fruitbody initials which formed on the four casings (table 4.10) as well as the number of fruitbodies which developed during the trial (appendix 4.15 and figure 4.42). Slightly fewer fruitbodies were formed on the peat control compared with PMS treatments but this effect was not significant.

TABLE 4.10 NUMBER OF FRUITBODY INITIALS FORMED ON PMS CASING OF DIFFERENT GRADES COMPARED WITH A PEAT CONTROL.

| Casing treatment | Mean number of initials |
|---|---|
| Peat control | 135.8 |
| PMS 5 mm. to dust | 128.5 |
| PMS 10mm. to dust | 120.3 |
| PMS 20mm. to dust | 122.0 |
| L.S.D. (P = 0.05) | 36.0 |

PMS screened through the twenty millimetre mesh sieve tended to produce lighter fruitbodies than the other three casings, this effect being reflected in a consistently lower yield than that from the peat control. The difference was close to or above significance at all intervals from casing (appendix 4.15 and figure 4.42). There were no other significant differences in yield.

FIGURE 4.42 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM A PEAT CONTROL AND PMS CASING WITH A MAXIMUM PARTICLE SIZE OF FIVE, TEN AND TWENTY MILLIMETRES.



4.6.2 Alteration of the particle size range of PMS casing within the limits of ten millimetres to dust.

In earlier experiments it was noted that preparation of PMS by sieving through a ten millimetre mesh did not always give a uniform casing structure. The moisture content of the material as excavated affected the particle size range obtained after sieving. When the PMS was relatively dry it tended to break up more on sieving, giving a higher proportion of fine particles than when the sample was initially quite wet.

It was decided to determine the effect of different grades of PMS casing in the range ten millimetres to dust on the yield and number of fruitbodies produced. Since Rao and Block (1963) had suggested casing structure could affect the shape of fruitbodies which formed, the dimensions of fruitbodies harvested in the first two flushes were determined using the method of Flegg and Gandy (1956), given in 3.4.6. Fruitbody size and dry weight were also recorded.

PMS was prepared as usual by sieving through a ten millimetre mesh sieve. This material was then air-dried for forty eight hours and five different grades were prepared by sieving the dried sample through a block of sieves, agitated on an Endecott's test sieve shaker, EEL 2 mk. 11. When sufficient material of each grade had been obtained, the samples were re-wetted by soaking overnight, drained to 70% moisture, and neutralised with 'Morden R' chalk. Pore space and water holding capacity of the different grades used are shown overleaf.

| Treatment | Particle size range (mm.) | % pore space | Water holding capacity (% of fresh wt.) |
|---|---|---|---|
| 1 | 10.0 - dust (Control) | 44.0 | 73.6 |
| 2 | < 1.0 | 37.0 | 71.7 |
| 3 | 1.0 - 3.35 | 42.0 | 75.5 |
| 4 | 3.35 - 5.0 | 44.0 | 70.3 |
| 5 | 5.0 - 6.3 | 51.0 | 71.4 |
| 6 | 6.3 - 10.0 | 58.0 | 70.2 |

Despite variations in the casing structure and pore space, no differences in yield, the number of initials and fruitbodies formed, and the size, dimensions and dry weight of fruitbodies were obtained (tables 4.11 and 4.12, appendix 4.16 and figure 4.43).

TABLE 4.11 NUMBER OF FRUITBODY INITIALS FORMED ON PMS CASING OF DIFFERENT GRADES IN THE RANGE TEN MILLIMETRES TO DUST.

| Casing treatment | Mean number of initials |
|---|---|
| Control (10 mm. - dust) | 44.8 |
| < 1.0 mm. | 32.8 |
| 1.0 - 3.35 mm. | 52.8 |
| 3.35 - 5.0 mm. | 35.2 |
| 5.0 - 6.3 mm. | 31.8 |
| 6.3 - 10.0 mm. | 43.2 |
| L.S.D. (P = 0.05) | 23.0 |

TABLE 4.12 SIZE, DRY WEIGHT AND DIMENSIONS OF FRUITBODIES HARVESTED FROM PMS CASING OF DIFFERENT GRADES IN THE PARTICLE SIZE RANGE OF TEN MILLIMETRES TO DUST.

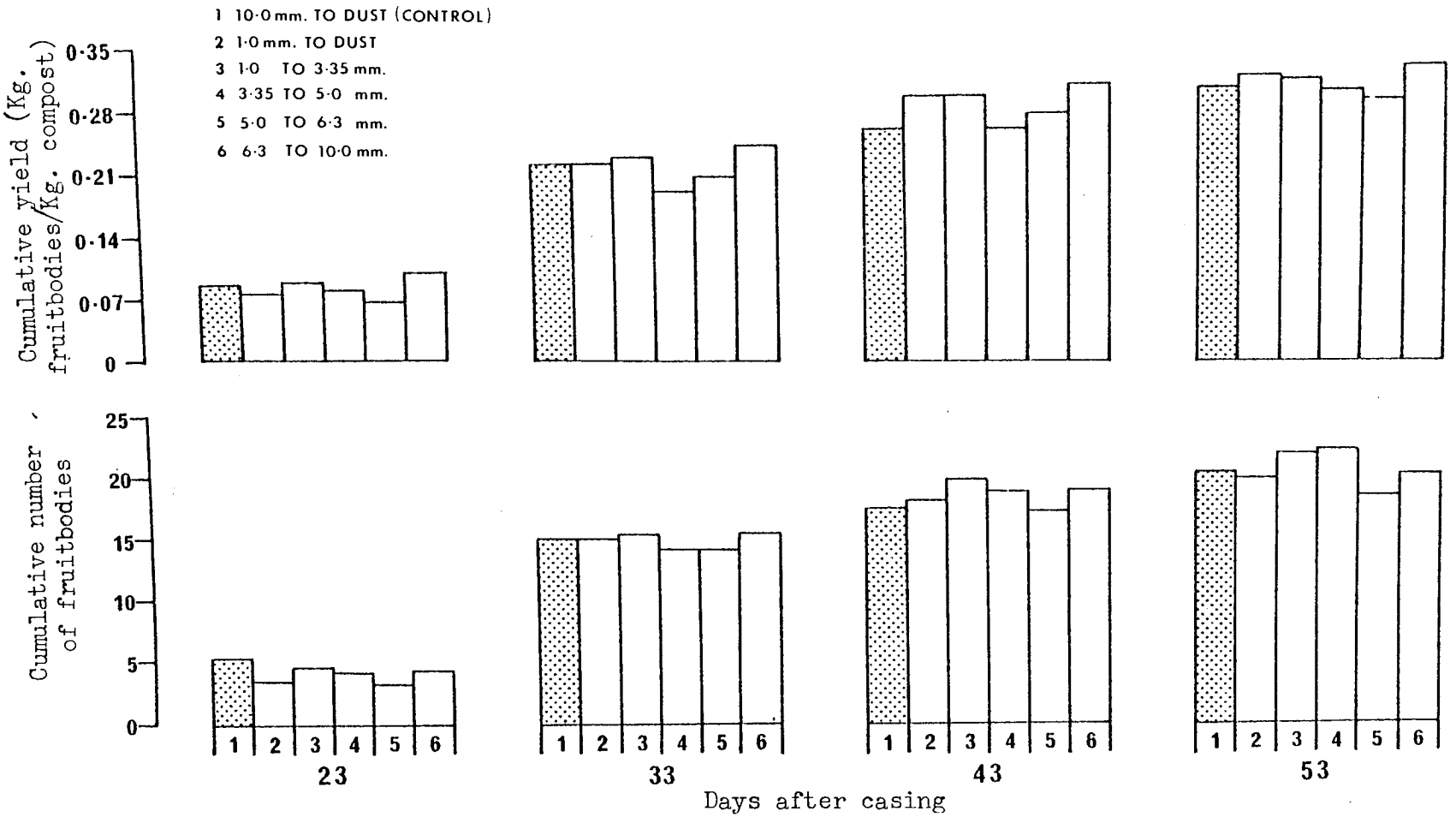
| Casing treatment | Fresh weight (g.) | | % dry weight | | Pileus diameter + total height | | Pileus diameter ÷ stipe width | | Stipe length (mm.) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| Control (10 mm. - dust) | 9.65 | 7.89 | 9.43 | 8.36 | 0.97 | 0.84 | 3.95 | 3.65 | 3.30 | 3.71 |
| < 1.0 mm. | 12.15 | 7.73 | 9.37 | 7.68 | 0.98 | 0.80 | 3.99 | 3.43 | 3.48 | 3.22 |
| 1.0 - 3.35 mm. | 11.04 | 6.75 | 9.06 | 8.21 | 1.00 | 0.79 | 3.84 | 3.41 | 3.25 | 3.47 |
| 3.35 - 5.0 mm. | 9.91 | 6.79 | 9.40 | 9.96 | 0.99 | 0.84 | 3.83 | 3.80 | 3.27 | 3.60 |
| 5.0 - 6.3 mm. | 11.11 | 7.17 | 9.26 | 8.89 | 1.06 | 0.81 | 3.84 | 3.41 | 3.25 | 3.53 |
| 6.3 - 10.0 mm. | 11.73 | 7.84 | 9.32 | 8.24 | 1.05 | 0.85 | 3.83 | 3.49 | 3.23 | 3.53 |
| L.S.D. (P = 0.05) | 4.03 | 2.22 | 1.38 | 2.67 | 0.10 | 0.07 | 0.46 | 0.40 | 0.55 | 0.83 |

1st = Fruitbodies harvested in first flush.

2nd = Fruitbodies harvested in second flush.

Total height = total height of fruitbodies.

FIGURE 4.43 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING SCREENED TO DIFFERENT PARTICLE SIZE RANGES BETWEEN TEN MILLIMETRES AND DUST.



## 4.6.3 The reaction of peat and PMS casings to different rates of water application.

A high water holding capacity is desirable for a casing soil, especially in modern growing houses, where water loss due to evaporation can be high. The casing should also retain water applied, and prevent it from percolating through to the compost.

An experiment was designed to determine whether there were any differences between peat and PMS casings when contrasting watering regimes were applied.

PMS casing prepared by the standard method of screening from ten millimetres to dust and a peat control were compared for water holding capacity and moisture retention properties as in 3.7.2 and 3.7.3.

The casings were each used to case twelve 18.5 $cm.^2$ pots, four of each casing being subjected to a high, medium or low watering rate. Water was applied at two day intervals after casing to maintain casing moisture at approximately 80% (high), 70% (medium) and 60% (low).

The quality of fruitbodies formed in this trial was measured as dry weight content, and percentage reflectance of white light using an E.E.L. reflectometer (3.4.6). Good quality mushrooms have clean, white caps, and a high dry weight enabling them to resist bruising.

At the end of the trial the degree of casing compaction and the extent of water drainage into the compost were determined. The degree of compaction was measured on air-dried samples removed from the top and bottom one centimetre of the casing, with a 25 mm. diameter borer. The samples were shaken through a 5.6 mm. mesh sieve placed above a 2.0 mm. mesh sieve on an Endecott's test sieve shaker. The degree of compaction was determined by the weight of casing retained in the two sieves and collecting pan.

The extent of water drainage into the compost was determined by measuring the dry weight of the casing, and the compost at depths of one, five and ten centimetres below the lower surface of the casing. Samples were taken from the centre of pots to avoid areas where water may have run down the sides.

Table 4.13 gives the moisture retention properties of the two casings. PMS casing was shown to have a high water holding capacity, but below that of peat. Its retention of moisture at p.F. values of 1.7 and 4.2 (permanent plant wilting point) were high, though again somewhat lower than peat.

The three different watering rates had quite different effects on the yield data obtained when PMS and peat casings were compared. The number of initials and fruitbodies as well as the yield from PMS casing were unaffected by the different watering regimes (table 4.14, figure 4.44). However, with peat the number of initials and fruitbodies formed at the low and high application rates were significantly below those when the casing was maintained at 70% moisture.

TABLE 4.13 WATER HOLDING CAPACITIES OF PEAT AND PMS CASINGS AT DIFFERENT MOISTURE SUCTION PRESSURES.

Water holding capacities of casings (% of fresh weight)

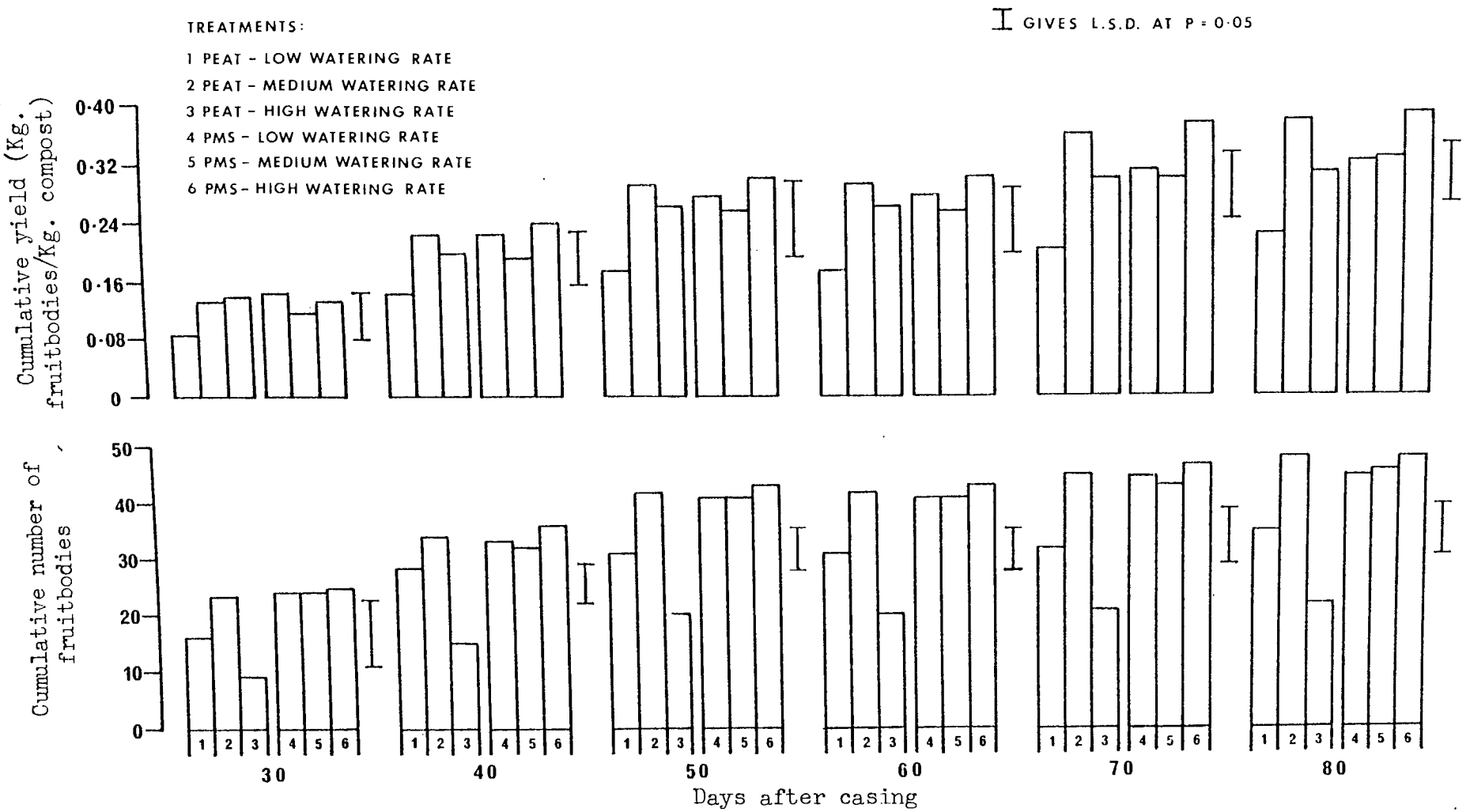
| Casing | p.F. 0 | p.F. 1.7 | p.F. 4.2 |
|---|---|---|---|
| Peat | 82.3 % | 61.7 % | 36.7 % |
| PMS | 77.8 % | 53.9 % | 26.3 % |

The least number of sporophores developed on peat casing treated at the high application rate, but those that formed were very large. As a consequence the yield, though lower than from the medium water rate was not significantly reduced (figure 4.44). With the low watering application fruitbodies that developed were small, causing a significant yield reduction compared with the medium rate.

TABLE 4.14 NUMBER OF FRUITBODY INITIALS FORMED ON PEAT AND PMS CASINGS SUBJECTED TO DIFFERENT RATES OF WATER APPLICATION.

| Casing treatment | Mean number of initials |
|---|---|
| Peat - low watering rate | 108.3 |
| Peat - medium watering rate | 141.6 |
| Peat - high watering rate | 85.7 |
| PMS - low watering rate | 132.9 |
| PMS - medium watering rate | 140.8 |
| PMS - high watering rate | 137.3 |
| L.S.D. (P = 0.05) | 30.9 |

FIGURE 4.44 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PEAT AND PMS CASINGS WATERED AT DIFFERENT RATES DURING CULTURE.



Comparing the treatments as a whole (appendix 4.17 and figure 4.44) peat watered at the low and high rates produced significantly fewer fruitbodies than the other casings, whilst peat treated at the low rate also gave a significantly lower yield. There were no other significant effects.

As the amount of water applied to the casings was increased, the dry weight of fruitbodies formed declined, irrespective of the flush. There was a trend with all treatments for the dry weight content of fruitbodies to increase in later flushes (appendix 4.18). Reflectance readings from fruitbodies were similar for peat and PMS, but in both cases were reduced considerably with the high watering rate (appendix 4.19). This treatment soaked developing mushrooms and casing alike, causing casing particles to adhere to the fruitbody surface, which soon became discoloured. In terms of quality there were no differences between the fruitbodies formed on the two casings. Regardless of the casing type, best quality mushrooms were formed with low water application, the value declining when watering rate was heavy.

During the course of the trial it was visually apparent that the greater the rate of watering, the more compacted the casing surface became. This was especially noticeable with peat. However, sieving dried casing at the end of the trial gave results which appeared to contradict this observation (appendix 4.20). Most particles from the casings treated at the low watering rate passed through the 2 mm. mesh, whilst with casing watered heavily, the majority of particles were retained by the 5.6 mm. mesh.

On examination of the sieved fractions, it was found that where both casings had been watered at the high rate, thick mycelial strands had developed. These strands had bound the casing together on drying, forming large aggregates which would not pass through the large mesh. Casings watered only lightly, had few or no strands, the particles remaining reasonably separate on drying.

Water drainage from casing into the compost was similar for both casing types, irrespective of watering rate. As would be expected, the extent of drainage into the compost increased with greater application rate (appendix 4.21).

### 4.6.4 The optimum depth for PMS casing.

In reviewing the published work on optimum casing depth (section two) it was concluded that contradictory results owe a lot to the use of different casing media. Individual casing types probably have different optimum casing depths, which are related to their water holding and structural properties. In previous trials casing was applied at the depth of three centimetres, the standard used in trials at the research unit. It was decided to compare productivity from PMS casing applied at depths of one to six centimetres.

Casing was applied to colonised compost at the six depths and the pots maintained at 25°C. until the mycelium was approaching the casing surface, when they were removed to a separate cabinet, ventilated with air at 16°C..

The time difference between removal of pots cased to one centimetre, and those at six centimetres depth was six days. A.bisporus strain L.X.10 was used in this trial.

The results (figure 4.45, appendix 4.22) show that with casing depths of one and two centimetres, yield and number of fruitbodies by day forty eight were significantly reduced compared with the standard depth of three centimetres. At this depth and above differences were not significant though there was a trend for greater numbers of fruitbodies to form with increased depth. Yield from the three centimetre deep casing was in advance of the other treatments by day forty eight. With the shallow casings (one and two centimetres deep) there was a tendency for the mycelium to cover areas of the casing surface even after cooling, resulting in the condition known as mycelial overlay or stroma. These patches of casings were afterwards unproductive. Above a depth of three centimetres fruitbody initials tended to form below the casing surface. This resulted in sporophores developing from beneath the surface and pushing up through the casing. As a result, the fruitbodies formed on these casings were dirty and of a low quality. The formation of initials deep in the casing prevented any comparison of their numbers relative to casing depth.

It was noted in the first flush that sporophores from the shallower casings had a firmer texture than those from deep casings. They also tended to bruise less readily on handling. Consequently, dry weight measurements were carried out on fruitbodies from the second flush. Table 4.15 shows that as casing depth was increased, so the dry weight content of fruitbodies fell.

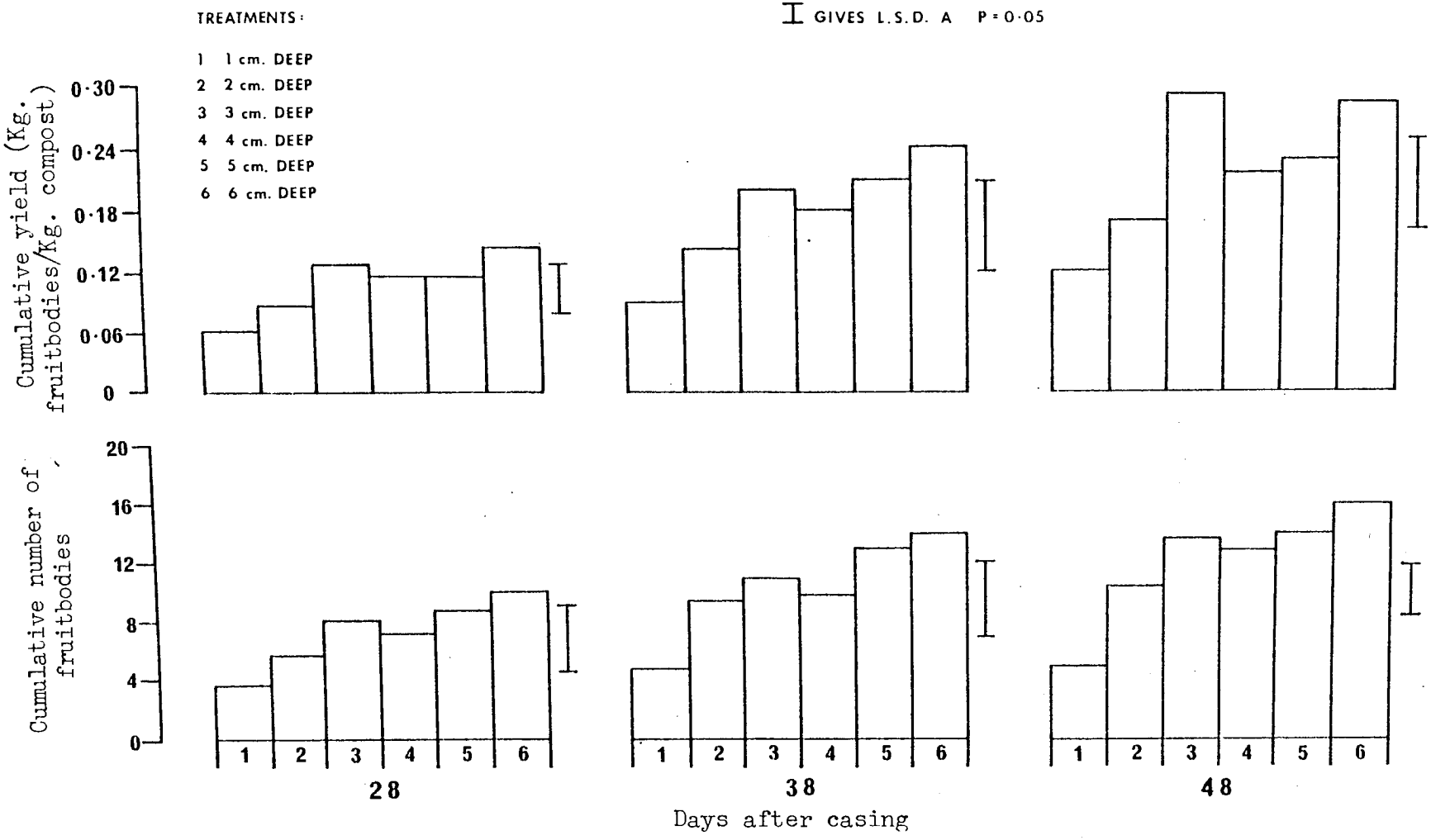
TABLE 4.15 DRY WEIGHT OF SECOND FLUSH FRUITBODIES HARVESTED FROM PMS CASING APPLIED AT DIFFERENT DEPTHS.

| Casing depth | % dry weight |
|---|---|
| 1 cm. | 11.84 (4) |
| 2 cm. | 11.01 (11) |
| 3 cm. | 11.04 (9) |
| 4 cm. | 9.60 (6) |
| 5 cm. | 9.07 (13) |
| 6 cm. | 8.40 (12) |

Figures in parentheses give the number of fruitbodies dried per treatment.

Casing was applied at the standard depth of three centimetres in subsequent trials.

FIGURE 4.45 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING APPLIED AT DIFFERENT DEPTHS.



## 4.7 AVAILABLE ELEMENTS IN THE CASING LAYER.

Initial investigation of PMS as a casing showed that the residue of salts derived from pulping operations is a very important factor in determining its usefulness as a casing medium. There is a natural accumulation of soluble salts in the casing layer with time after its application, and a series of trials were designed to determine the source of salts accumulating in PMS and peat casings and assess the importance of initial differences in salt concentration and levels of individual elements between the two materials.

### 4.7.1 Salt accumulation in PMS and peat casings.

The elements which were responsible for salt accumulation in peat and PMS casings in 4.5.4 were determined to be chloride, magnesium, potassium, sodium and sulphate. The source of these ions was investigated by chemical analyses of compost and casings with time after casing application. Levels corresponding to total and available forms of the elements were determined using ammonium acetate and mixed acid digestion (3.7.14 and 3.7.15). Chloride was omitted from the investigation as the perchloric - nitric acid digestion was not suitable for this element. Estimation of the elements in prepared extracts was as in 3.7.16.

13 $cm.^2$ pots of colonised compost were cased with PMS and peat casings. At regular intervals from casing four pots of each casing treatment were removed from the cabinet and compost and casing samples taken as in 3.4.5 for analysis.

FIGURE 4.46 LEVELS OF MAGNESIUM IN CASING AND COMPOST SUBSTRATES WITH TIME FROM CASING.

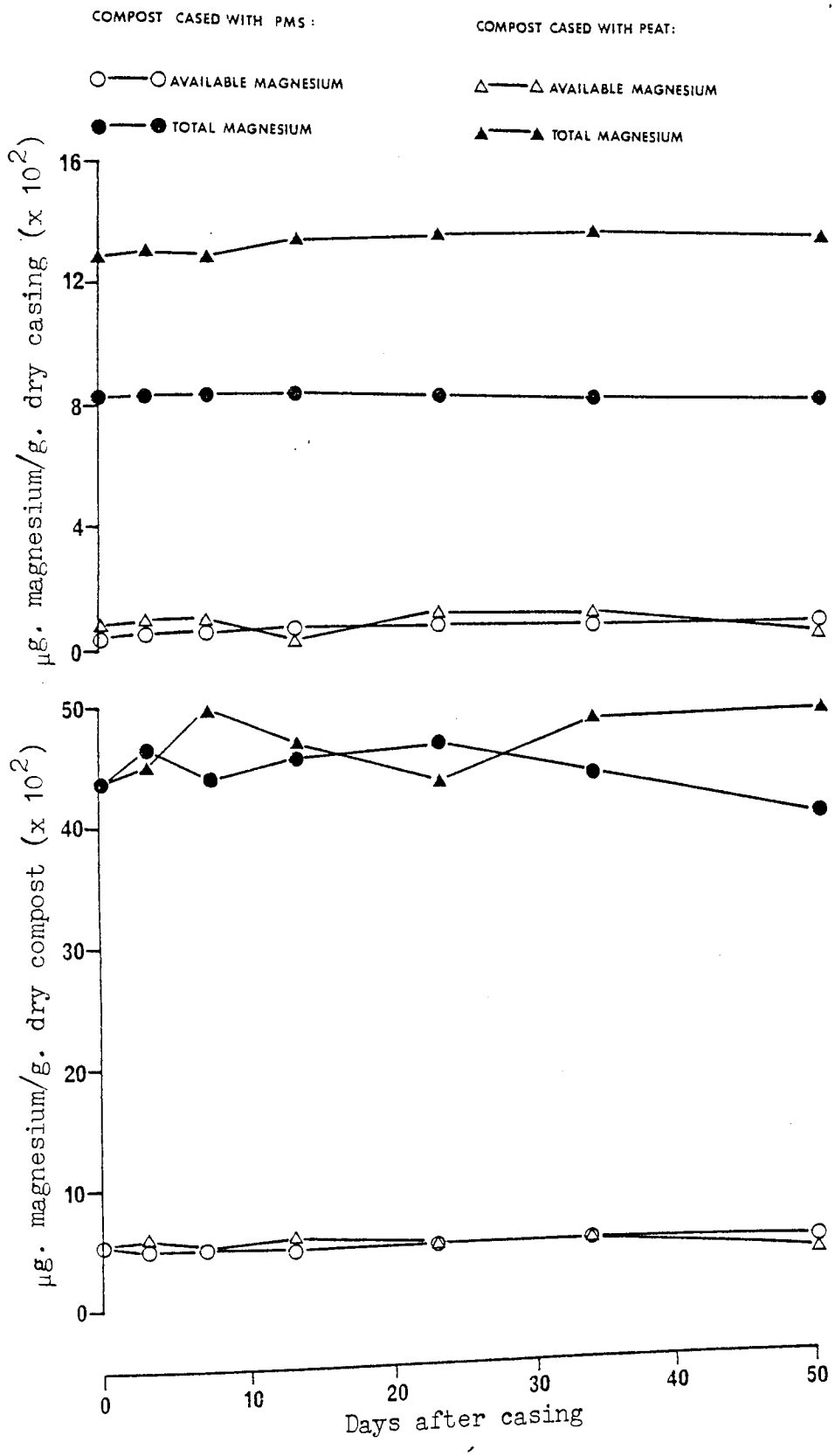


FIGURE 4.47 LEVELS OF POTASSIUM IN CASING AND COMPOST SUBSTRATES WITH TIME FROM CASING.

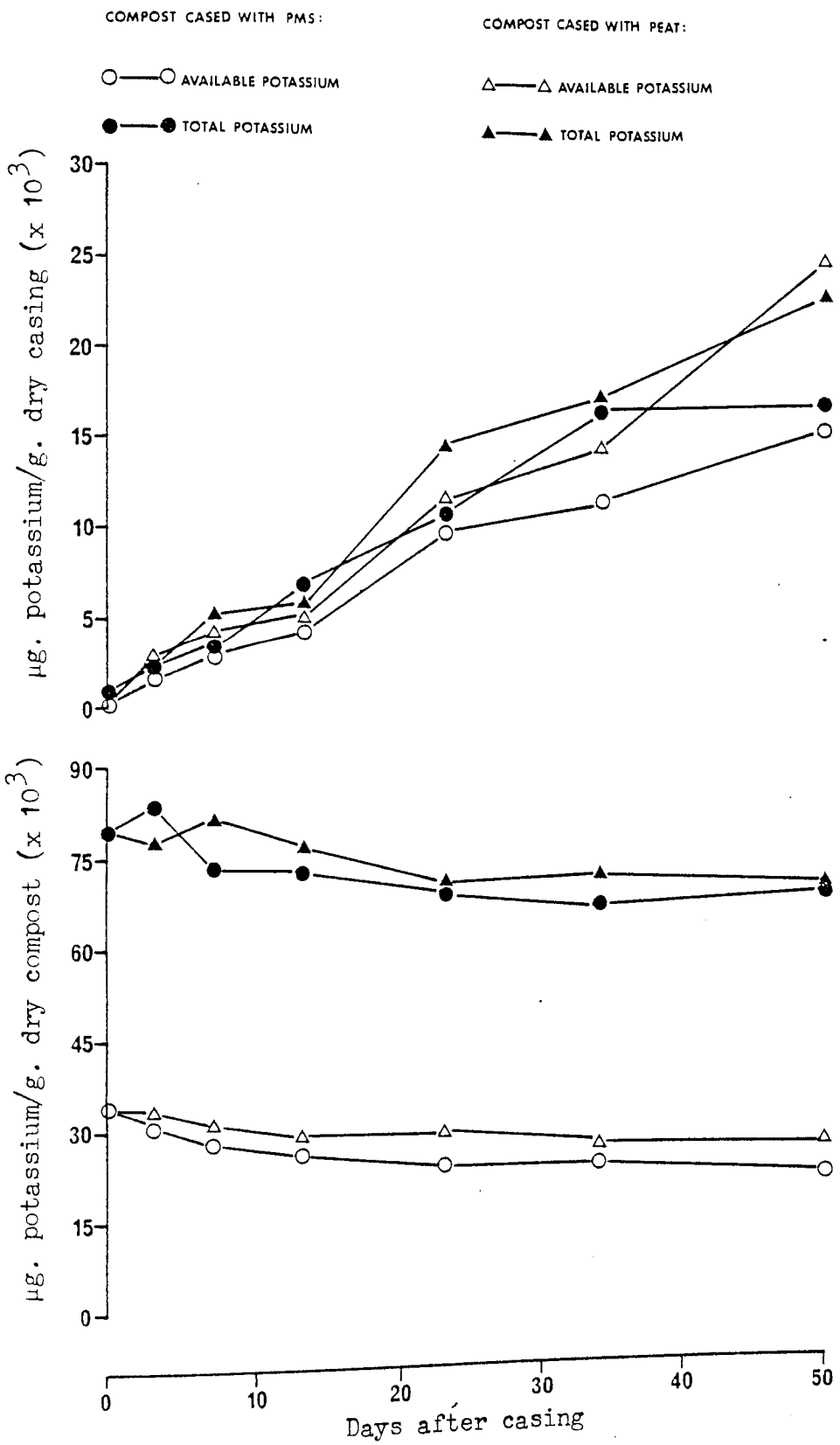


FIGURE 4.48 LEVELS OF SODIUM IN CASING AND COMPOST SUBSTRATES WITH TIME FROM CASING.

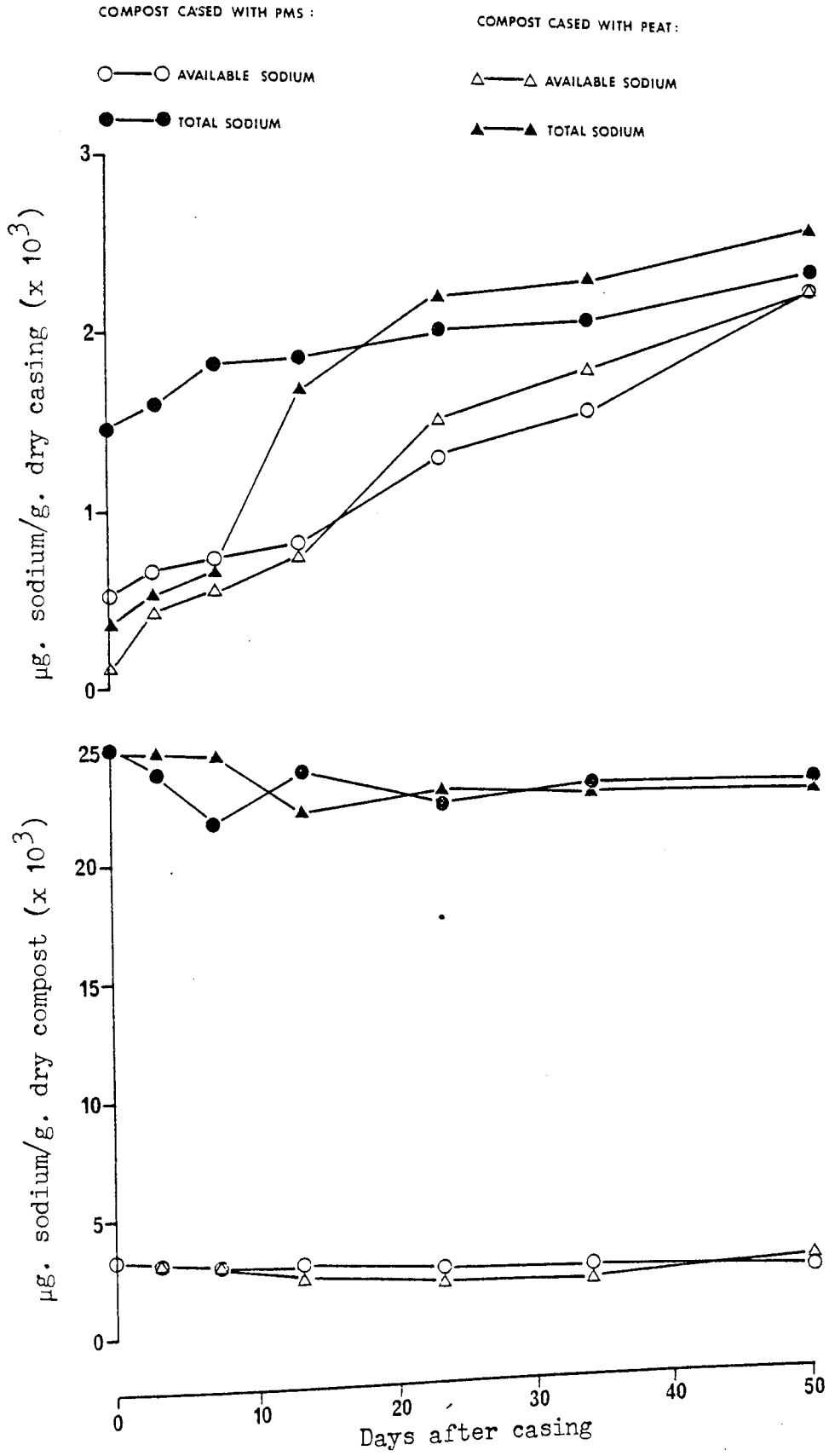
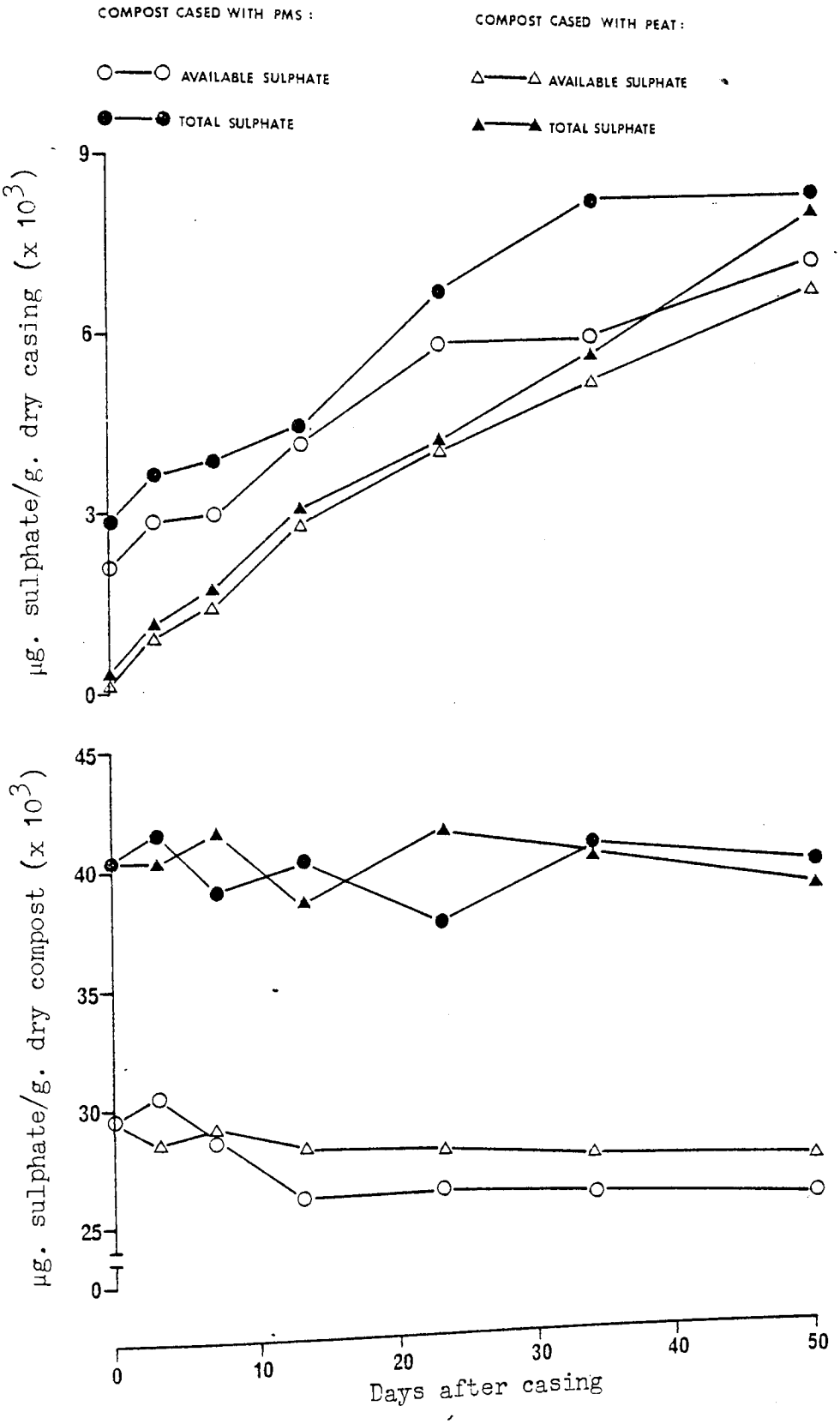


FIGURE 4.49 LEVELS OF SULPHATE IN CASING AND COMPOST SUBSTRATES WITH TIME FROM CASING.



Levels of potassium, sodium and sulphate all followed the same pattern irrespective of whether the compost was cased with peat or PMS (figures 4.47 to 4.49). Total and available levels in the casing increased linearly following its application. Conversely, in the compost both total and available forms of the elements showed a gradual decline over the same period, indicating the compost to be the source of these elements accumulating in the casing.

Levels of magnesium showed very little change with time from casing and no real trends were demonstrated (figure 4.46), suggesting minimal movement of this element from the compost to the casing.

### 4.7.2 Salts in peat and PMS and their effect on fruitbody formation and salt accumulation in casing during culture.

At an early stage in the investigations it was thought that the initially higher salt content of suitable PMS deposits reduced the number of initials and fruitbodies which formed (4.5.1 and 4.5.2). The effect of removing the excess salts from PMS on formation of initials and fruitbodies, and on the rate of salt accumulation from the compost, was examined by comparing peat and untreated PMS casing with PMS casings leached with de-ionised water.

PMS was screened and then leached for fifteen and twenty five minutes prior to neutralisation. This gave two experimental PMS casings, one with a conductivity value below that of peat, the other slightly higher.

Electrical conductivity of the casings was recorded at intervals from casing (figure 4.50). Where salinity of PMS was initially reduced, salt accumulation was more rapid than in unleached material. Salt build-up was less marked in peat than the PMS casing. Reduction of the initial salt content of PMS resulted in an increase in the number of fruitbody initials formed prior to the first flush, which was significant if the reduction was sufficiently large (appendix 4.23, table 4.16). No significant effect was noted with the leaching treatments on the number and yield of fruitbodies, though the leached casings produced more fruitbodies in the first flush compared with untreated PMS (figure 4.51).

TABLE 4.16 NUMBER OF FRUITBODY INITIALS FORMED ON PEAT AND PMS CASINGS COMPARED WITH LEACHED PMS CASINGS.

| Casing treatment | Mean number of initials |
|---|---|
| Peat control | 98.0 |
| PMS | 89.3 |
| PMS leached for 15 minutes | 143.3 |
| PMS leached for 25 minutes | 166.5* |
| L.S.D. (P = 0.05) | 58.0 |

*Significantly different from control.

The results demonstrate the reduction of initial and fruitbody numbers by the salt levels in PMS compared to standard peat casing.

FIGURE 4.50 SALT ACCUMULATION IN PEAT AND PMS CASINGS COMPARED WITH PMS CASING LEACHED WITH DE-IONISED WATER PRIOR TO APPLICATION.

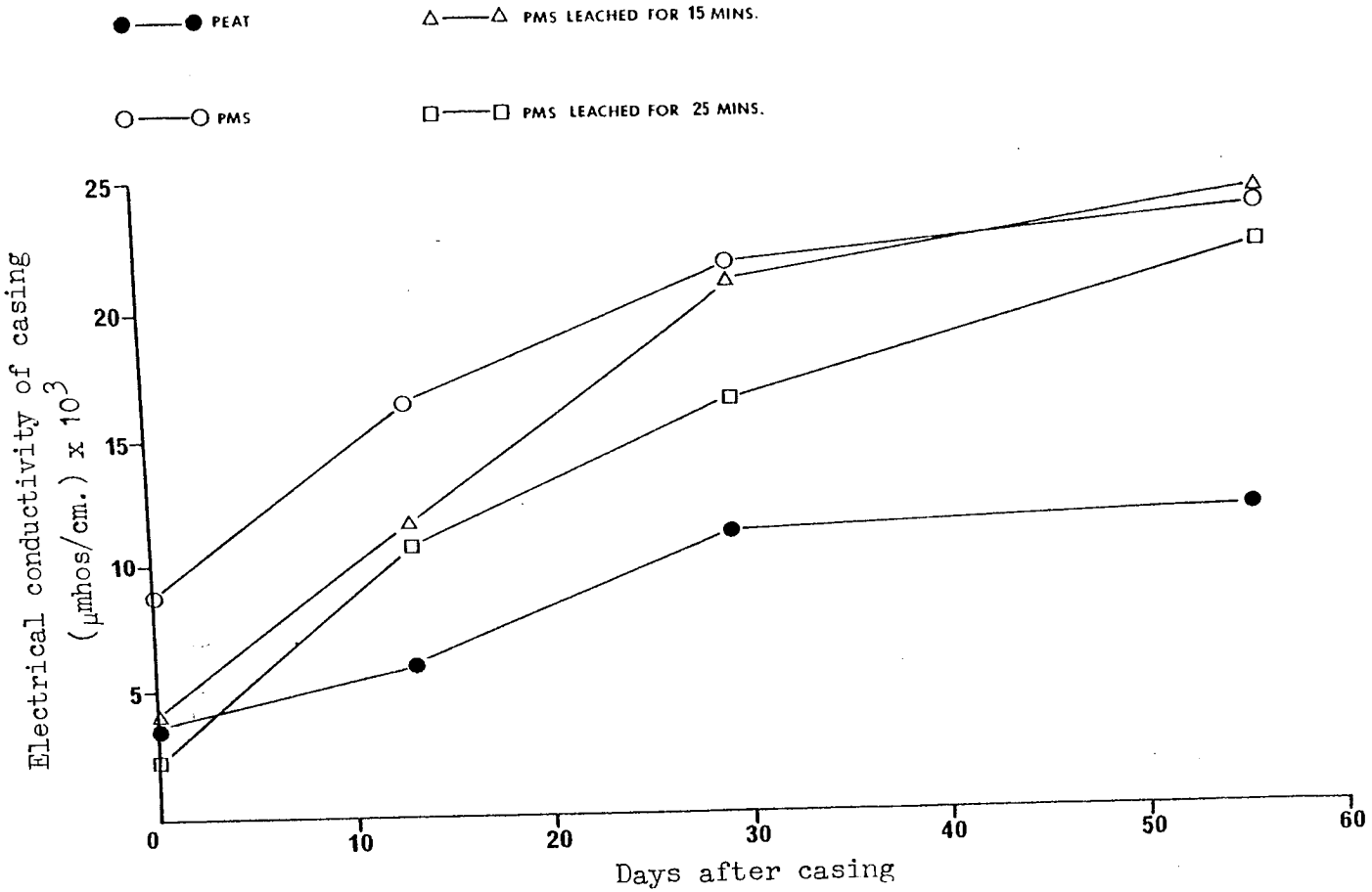
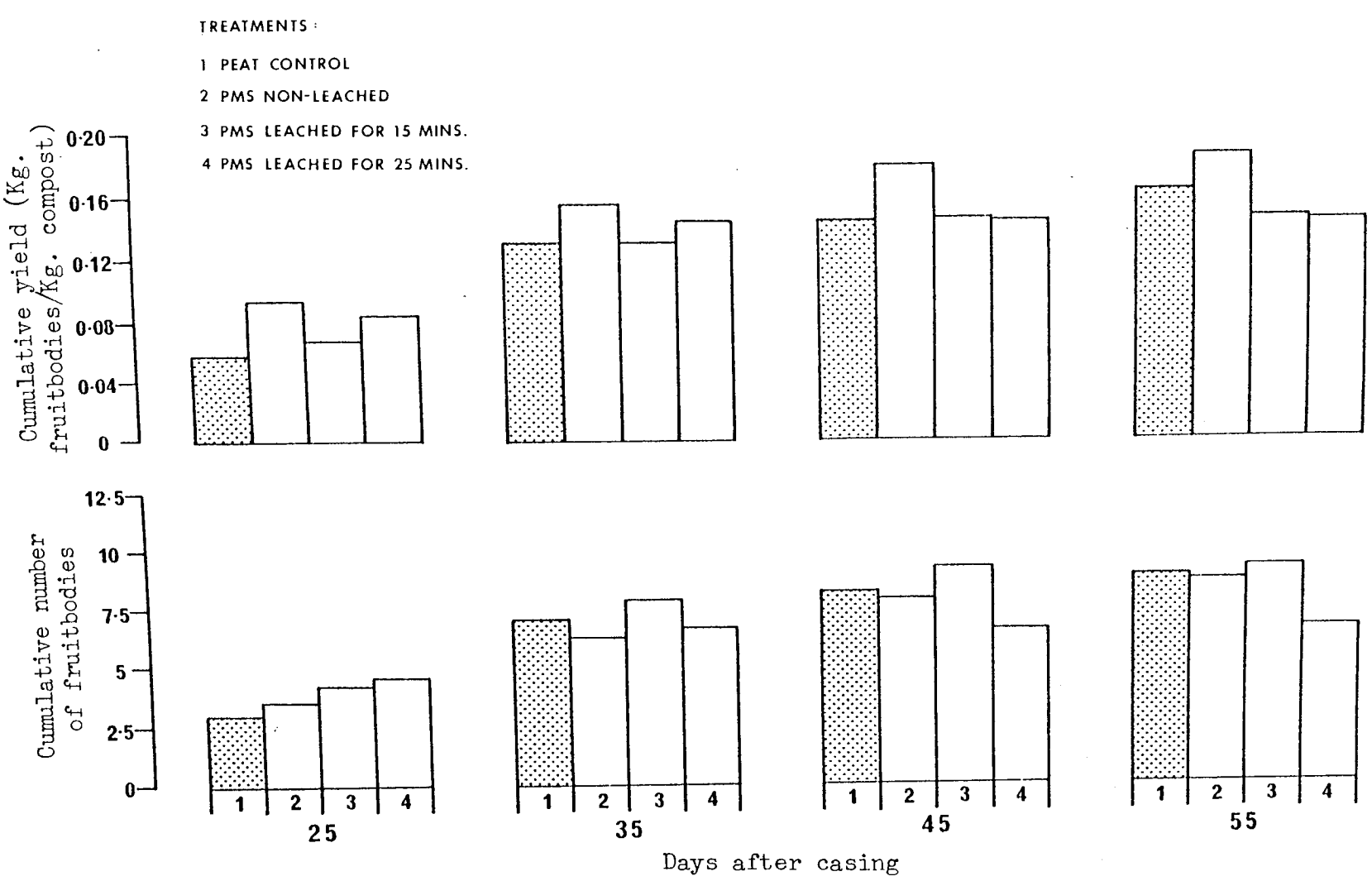


FIGURE 4.51 CUMUIATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PEAT CASING COMPARED WITH PMS CASING NON-LEACHED AND LEACHED PRIOR TO APPLICATION.



However, removal of the soluble salts from PMS before casing merely resulted in a greater rate of salt accumulation after its application to the beds.

Comparing these results with those from the previous trial, there is an apparent anomaly in the rate of salt accumulation in peat and PMS casings. In 4.7.1 it was shown that sodium, potassium and sulphate accumulated to similar concentrations in both casings. In 4.5.2 and the present trial, the conductivity of peat casing remained below that of the PMS treatments, the values at the end of culture not approaching one another as might be expected. The soluble salts which accumulate in both peat and PMS casing are derived from the compost (4.7.1). It is likely that this ion movement is a passive process resulting from the difference in salinity between compost and casing. In 4.7.1 available ions were extracted using ammonium acetate. This extractant acts by replacing and removing all ions from exchange sites within a soil complex with ammonium ions, as well as extracting those dissolved in the soil water. Measurement of electrical conductivity was done on a water suspension. Water brings into solution only those ions which are readily soluble and does not perform replacement extraction at exchange sites. It seems likely then that peat contains more sites for ionic adsorption and exchange than does PMS, and that more of the ions which accumulated in peat casing in this comparison were adsorbed by the casing complex.

This hypothesis was tested by comparing the cation exchange capacities of the two materials using the method in 3.7.17. Cation exchange capacity is a measure of the number of sites within a material where adsorption and exchange of cations can occur.

PMS casing had a cation exchange capacity of 24.9 m.eq./100g. dry casing, whilst that of peat was 70.1 m.eq./100g. dry casing, roughly two and a half times the value of PMS. This suggests that peat is capable of binding more available ions than PMS.

### 4.7.3 Salt accumulation in the casing layer and the productivity of A.bisporus.

In the present study the phenomenon of salt accumulation has been demonstrated to occur regardless of casing type, and the elements responsible for the increase in casing conductivity have been identified. Identification of the principle elements involved in salt accumulation enabled their individual effects on productivity to be determined.

Choride, magnesium, potassium, sodium, and sulphate were added to achieve concentrations in PMS casing which were encountered by day fifty in PMS casing in 4.7.1. Chloride concentration was estimated from the levels recorded in PMS casings in 4.5.4. The concentrations of the individual ions as $\mu$g./g. dry casing were: magnesium 850, sodium 2,000, chloride 3,750, sulphate 7,000 and potassium 15,000. A treatment combining the individual additions was included.

Individual ions were added either as the calcium or carbonate salts. Calcium and carbonate are both present in excess in the casing due to neutralisation with chalk and further addition is unlikely to have any effect on casing conductivity.

The salts were added at day thirteen after casing in the normal water application to the casing. Application at this time allowed mycelial colonisation of the casing to occur, as the effects of the salts on fruiting and yield were under investigation.

Addition of magnesium and sodium to the casing raised its conductivity slightly above the control value, whilst the four other treatments gave appreciable increases. The elements combined raised the casing conductivity to $48.9 \times 10^3$ μmhos/cm., the individual ions increasing the value in proportion to the quantity added (figure 4.52).

The conductivity of the control casing increased to approximately $35.0 \times 10^3$ μmhos/cm. by the end of the trial (figure 4.52). Similar rates of salt accumulation to the control were recorded in casings amended with magnesium, sodium and chloride. The salt concentrations in these casings remained roughly the same amount above the control levels as on the day of addition. Following the sulphate and potassium additions the rate of salt accumulation declined compared with the control. Where all the elements were added together casing salt levels did not increase any further, but showed a slight decline.

Additions of potassium, sulphate and the combined elements resulted in a reduction in the number of initials formed (table 4.17). The effects of the separate salt additions on the yield and number of fruitbodies harvested is shown in figure 4.53, with statistical analysis in appendix 4.24.

FIGURE 4.52 ELECTRICAL CONDUCTIVITY OF PMS CASING FOLLOWING THE ADDITION OF VARIOUS IONS THIRTEEN DAYS AFTER CASING.

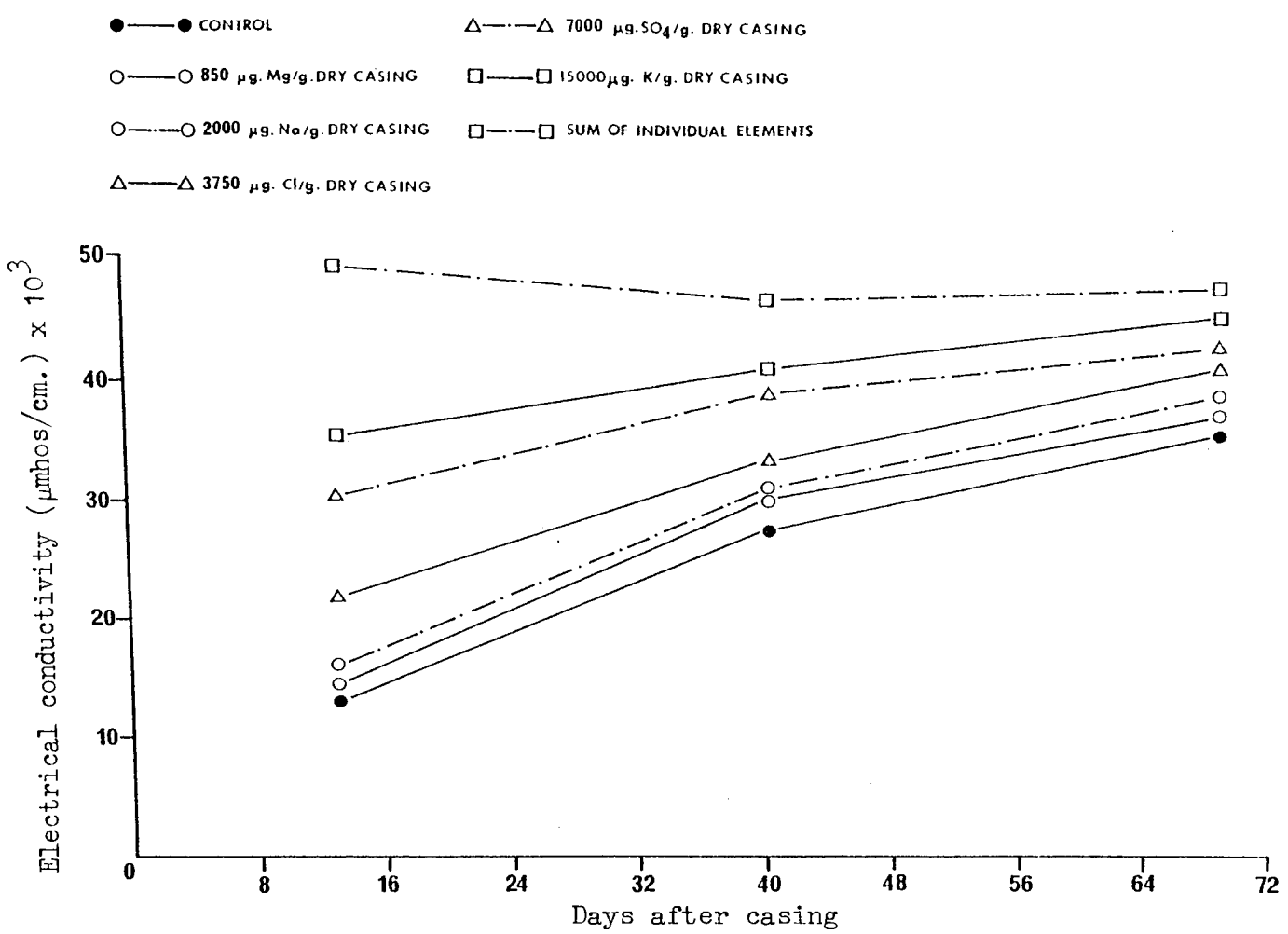


FIGURE 4.53 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING FOLLOWING THE ADDITION OF VARIOUS IONS THIRTEEN DAYS AFTER CASING.

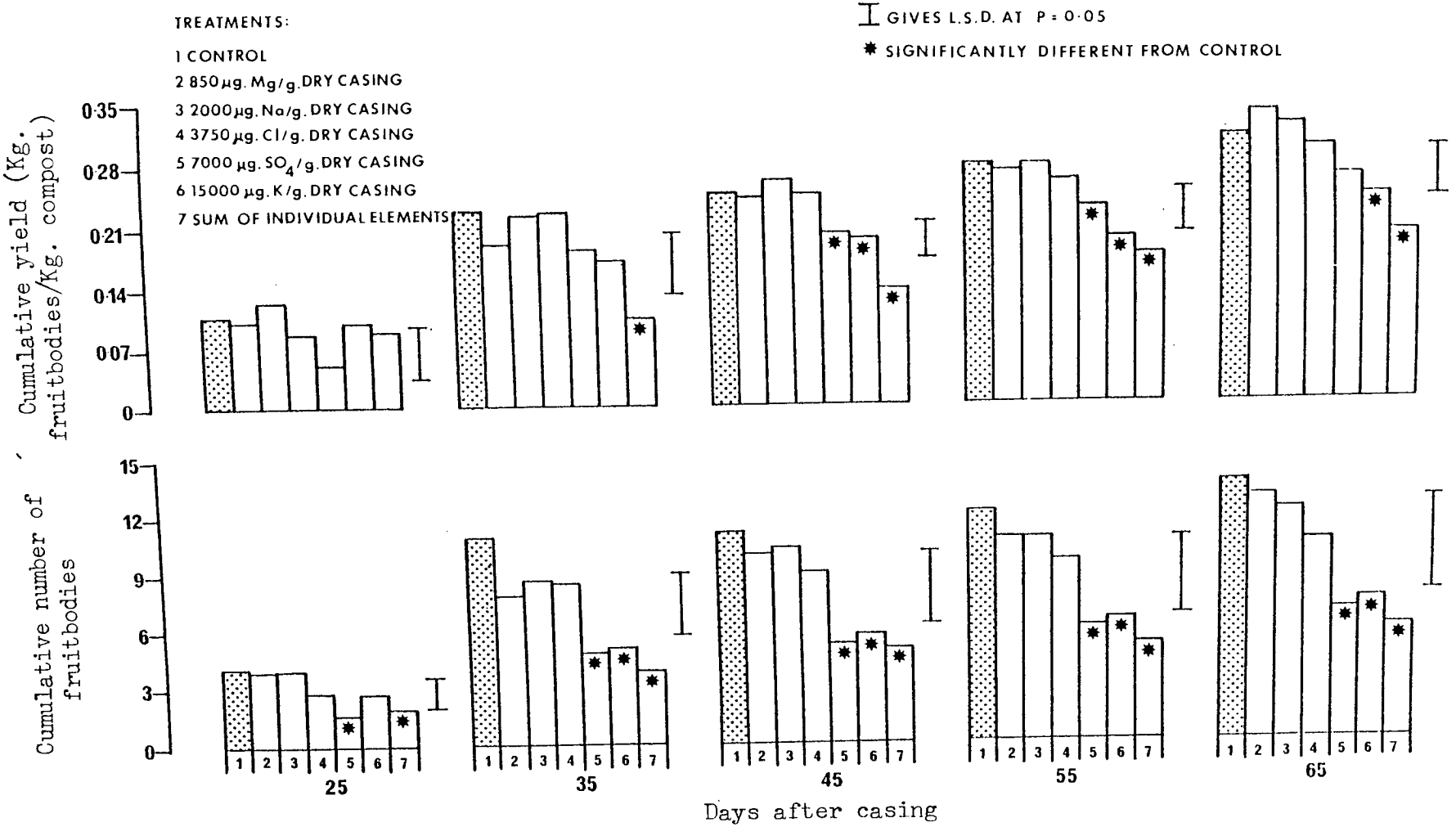


TABLE 4.17 NUMBER OF FRUITBODY INITIALS FORMED ON PMS CASING FOLLOWING THE ADDITION OF VARIOUS IONS.

| Casing treatment | Mean number of initials |
|---|---|
| Control | 108.9 |
| 850 µg. Mg/g. dry casing | 97.3 |
| 2,000 µg. Na/g. dry casing | 81.4 |
| 3,750 µg. Cl/g. dry casing | 84.6 |
| 7,000 µg. $SO_4$/g. dry casing | 69.1* |
| 15,000 µg. K/g. dry casing | 60.2* |
| Sum of individual additions | 43.7* |
| L.S.D. (P = 0.05) | 38.6 |

*Significantly different from control.

All of the additions reduced the number of fruitbodies which developed. Though additions of magnesium, sodium and chloride resulted in consistent reduction with time from casing, the results were not statistically different from the control. Reduction of numbers following potassium, sulphate and combined additions were at significant levels.

Magnesium, sodium and chloride additions had no significant effect on yield, whereas addition of potassium, sulphate and the sum of the elements gave significant reductions.

The extent of yield reduction was not as great as that with numbers, since heavier fruitbodies were formed on all addition treatments (figure 4.53).

Reduction of numbers and yield where it occurred was proportional to the conductivity created in the casing irrespective of the element added. The greater the conductivity the greater was the reduction (compare figures 4.52 and 4.53).

### 4.7.4 The relationship between salinity of the casing layer and compost density.

The previous trial demonstrated passive salt accumulation can be sufficiently great to give casing conductivities which reduce the potential yield. In the first commercial-scale comparison of peat and PMS casings (4.5.4), results suggested the density of compost beneath a given depth of casing altered the rate of salt build up in the casing. In commercial production there is a wide variation in the ratio of compost density to casing area. The effect of varying the density of compost on salt accumulation in the casing was investigated and compared with the number and yield of sporophores from the different treatments.

Colonised compost was compacted into 18.5 $cm.^2$ pots to give densities of 0.2, 0.4 and 0.6 Kg./L., the latter being the maximum attainable under laboratory conditions. The compost occupied the same volume in each treatment.

PMS casing was applied to the standard depth of three centimetres, and sampled for estimation of conductivity at intervals from application. Compost conductivities were measured at filling and at the end of the trial.

As compost density was increased the rate of salt accumulation in the casing also increased (figure 4.54). Irrespective of compost density, salt accumulation was more pronounced over the first ten days. The conductivity of the compost declined more rapidly the less densely it was compacted.

Yields per unit weight of compost from the three treatments were comparable (figure 4.55 and appendix 4.25), indicating that the higher casing conductivities which resulted from more compacted compost did not reduce the yields. On a weight per area of casing basis, the yield of fruitbodies was proportional to the weight of compost (figure 4.56). The same was true for the number of initials which developed on all treatments (table 4.18), and for fruitbody numbers with compost densities of 0.2 and 0.4 Kg./L.. In the 0.6 Kg./L. of compost treatment the number of fruitbodies which formed was only a little higher than with 0.4 Kg./L. and the difference was not significant, even though the yield was one third greater (figure 4.56).

FIGURE 4.54 ELECTRICAL CONDUCTIVITY OF CASING AND COMPOST DURING CULTURE WITH DIFFERENT DENSITIES OF COMPOST UNDER A STANDARD CASING APPLICATION.

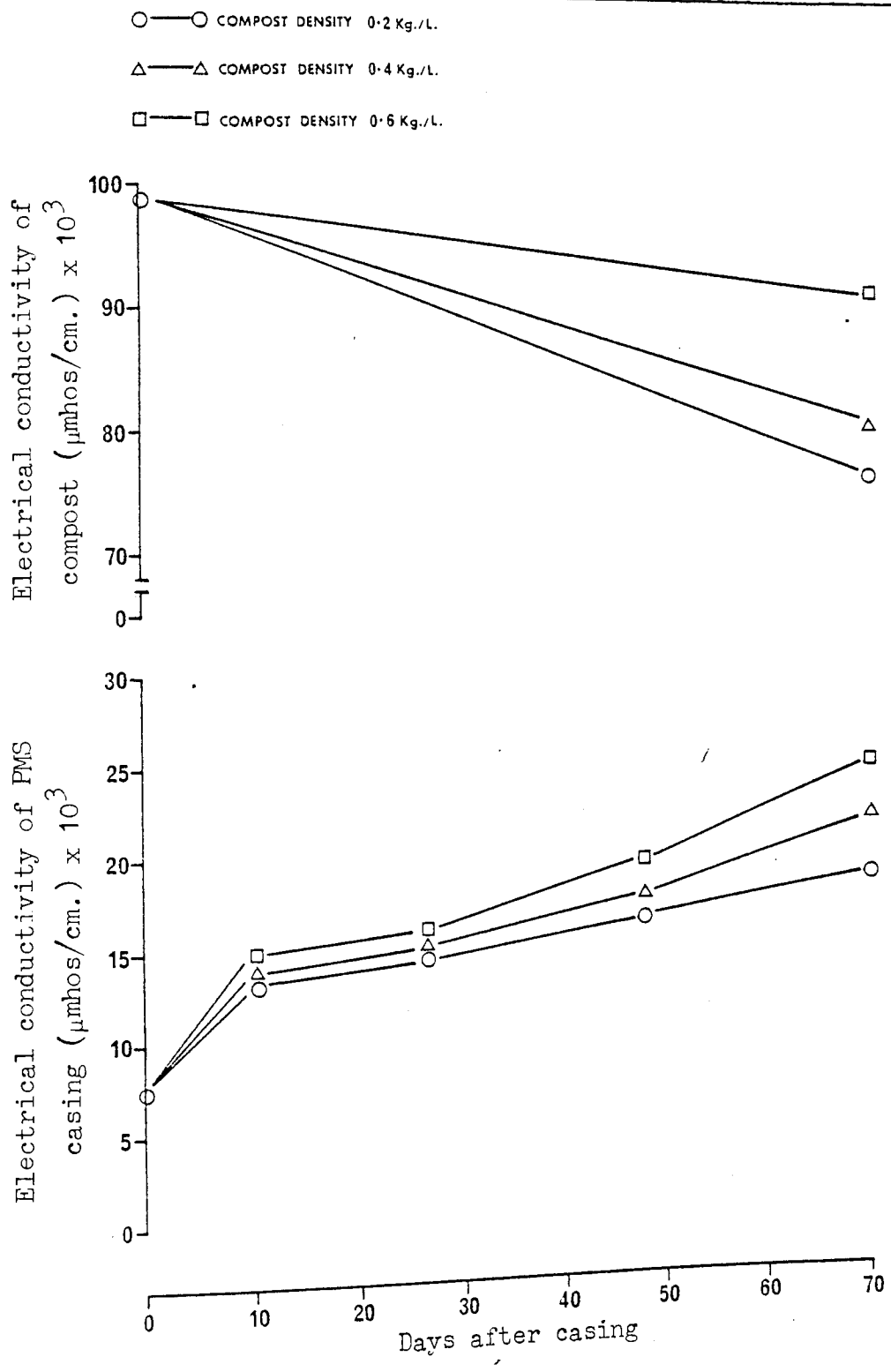


FIGURE 4.55 CUMULATIVE YIELD (Kg. FRUITBODIES/Kg. COMPOST) HARVESTED FROM A STANDARD APPLICATION OF PMS CASING TO DIFFERENT DENSITIES OF COMPOST.

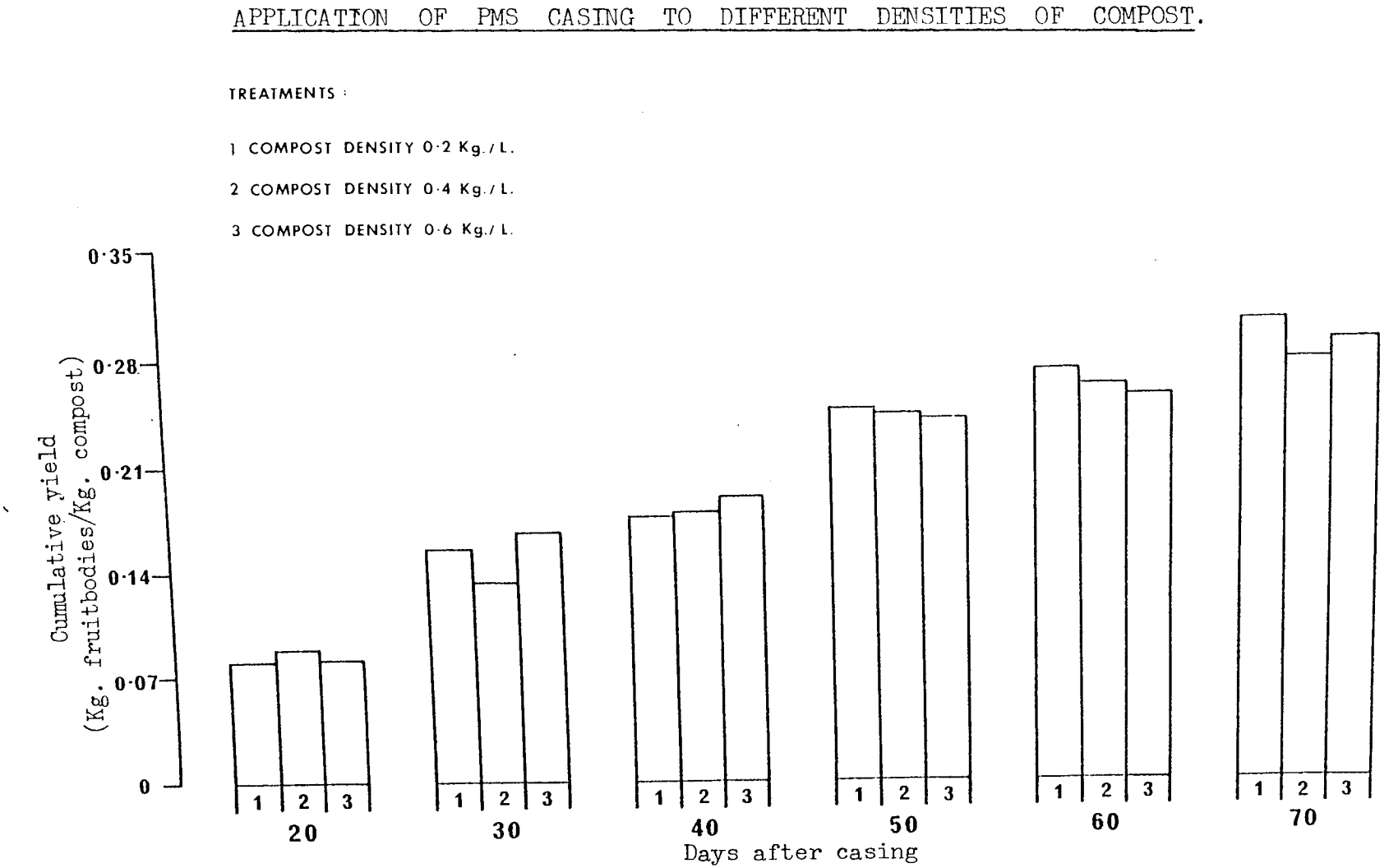


FIGURE 4.56 CUMULATIVE YIELD (Kg. FRUITBODIES/sq. m. CASING) AND NUMBER OF FRUITBODIES HARVESTED FROM A STANDARD APPLICATION OF PMS CASING TO DIFFERENT DENSITIES OF COMPOST.

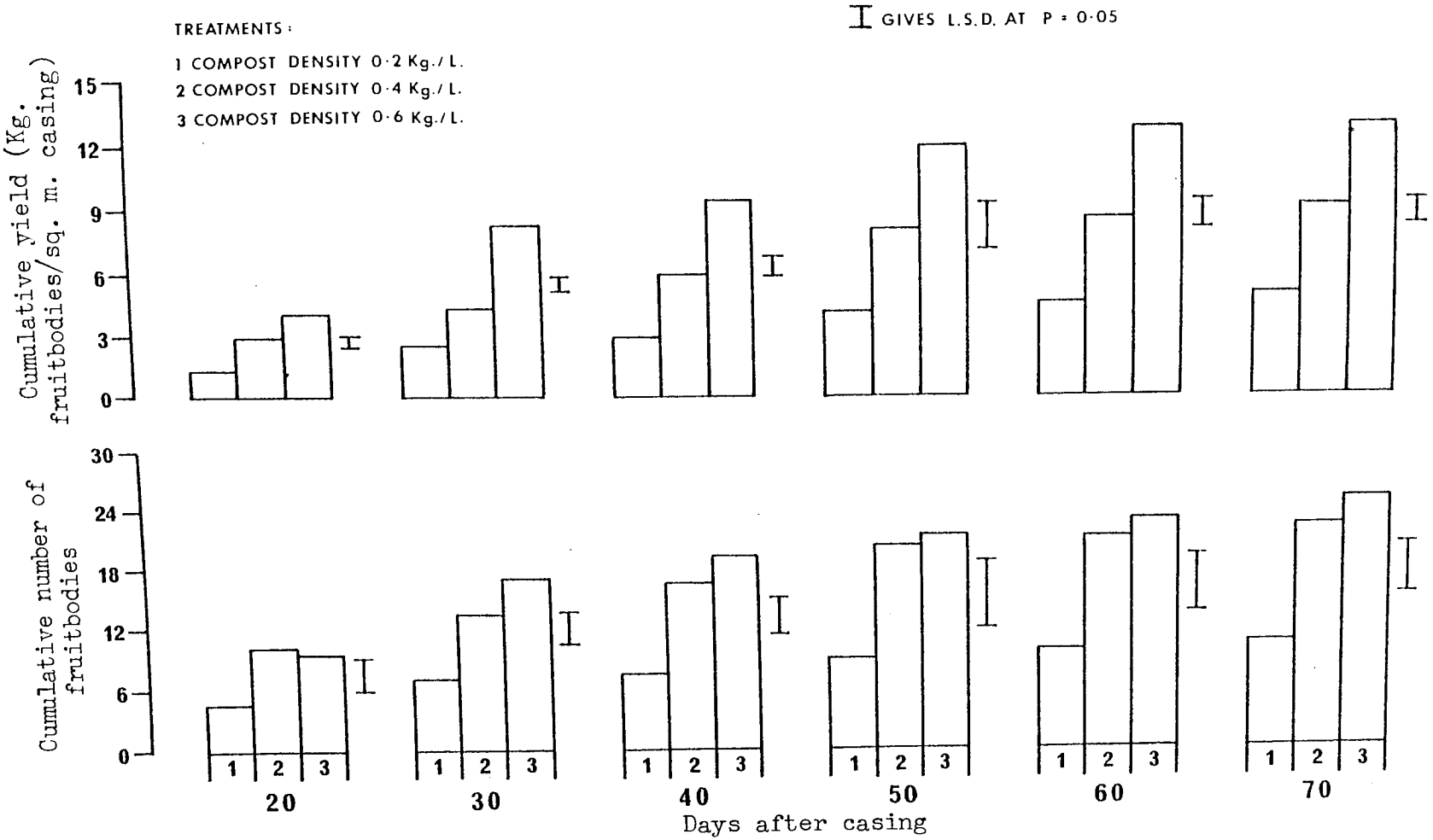


TABLE 4.18 NUMBER OF FRUITBODY INITIALS FORMED ON A STANDARD APPLICATION OF PMS CASING TO DIFFERENT DENSITIES OF COMPOST.

| Treatment | Mean number of initials |
|---|---|
| Compost density 0.2 Kg./L. | 103.4 |
| Compost density 0.4 Kg./L. | 159.1 |
| Compost density 0.6 Kg./L. | 183.7 |
| L.S.D. (P = 0.05) | 63.5 |

## 4.7.5 Possibilities of reducing salt accumulation in PMS casing.

Natural salt accumulation can reduce the number and yield of fruitbodies that develop during the culture of A.bisporus. Consequently, ways of reducing accumulation which could be applied in commerce were investigated:

### (i) Re-casing of colonised compost with fresh casing at intervals during culture.

Replacement of the original casing with fresh material at the end of the first, second and third flushes was investigated as a means of re-creating conditions of low salinity. Casing conductivity values and the yield and number of fruitbodies which developed were compared with an untreated control. Re-cased boxes were removed to a separate cabinet at 25°C. until the mycelium had colonised the fresh casing.

The vegetative mycelium readily re-colonised the fresh casing applied after the first and second flushes. Appearance of fruitbodies was approximately twenty one days from re-casing, the same period as when freshly colonised compost is cased in commercial culture. When the casing was replaced at the end of the third flush, the mycelium grew very weakly into the fresh casing, with no obvious strand development.

Figure 4.57 shows that casing conductivity increased quite rapidly after the application of fresh material. High salt levels were recorded early in the culture cycle in the control treatment, and the re-casing technique achieved the aim of reducing the conductivity well below that of the control by the time the next flush of fruitbodies appeared. However, re-casing of the compost at the end of the first flush significantly reduced the yield thereafter. Application of fresh casing after the second and third flushes had no significant effect on the number and yield of fruitbodies formed (appendix 4.26 and figure 4.58).

(ii) Addition of salt binding agents to casing.

It was demonstrated in 4.7.2 that PMS casing was less able to bind soluble ions than peat. Several salt binding agents were added to PMS in an attempt to reduce salt accumulation during culture.

FIGURE 4.57 ACCUMULATION OF SOLUBLE SALTS IN PMS CASING FOLLOWING ITS REPLACEMENT WITH FRESH MATERIAL AT INTERVALS DURING CULTURE.

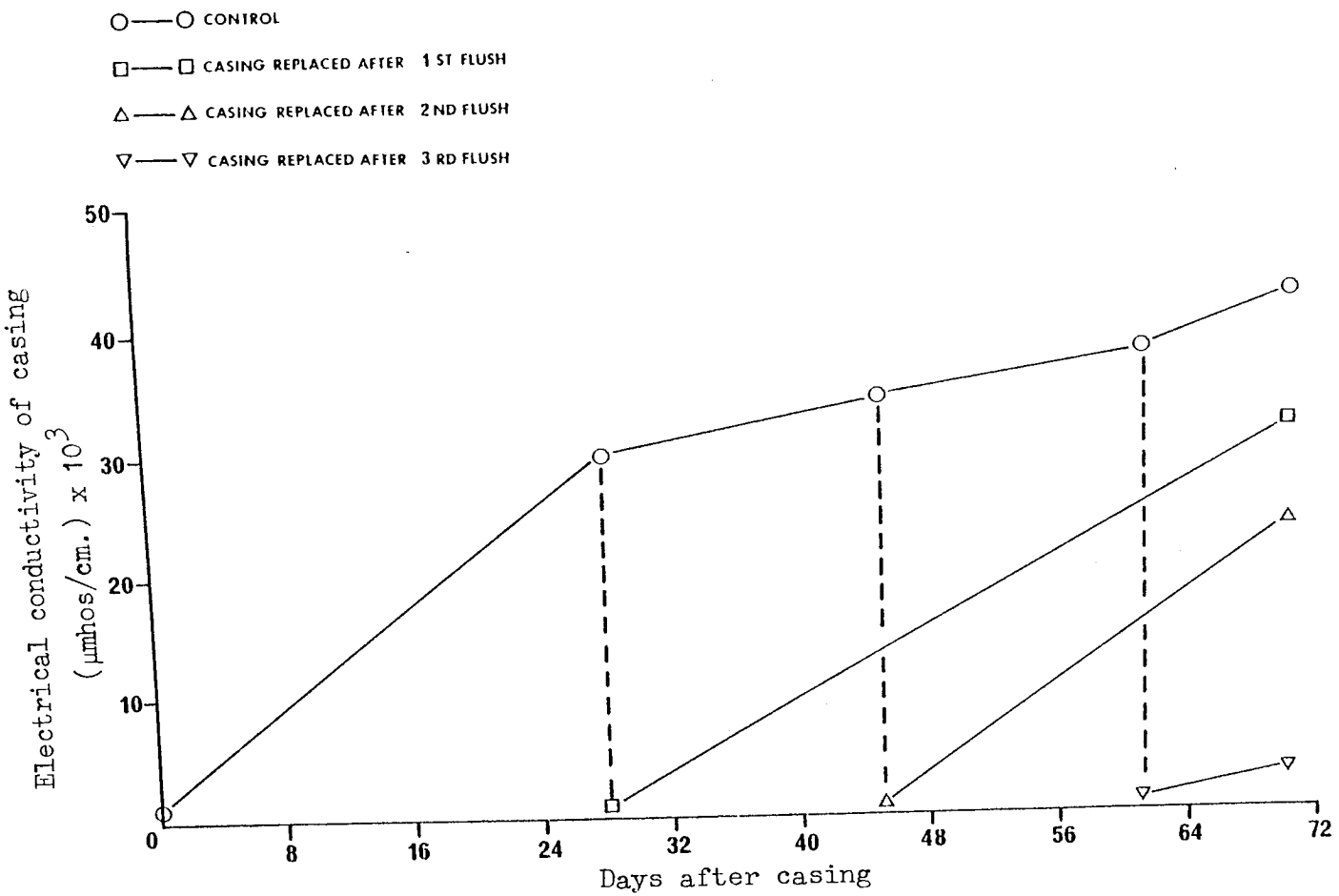
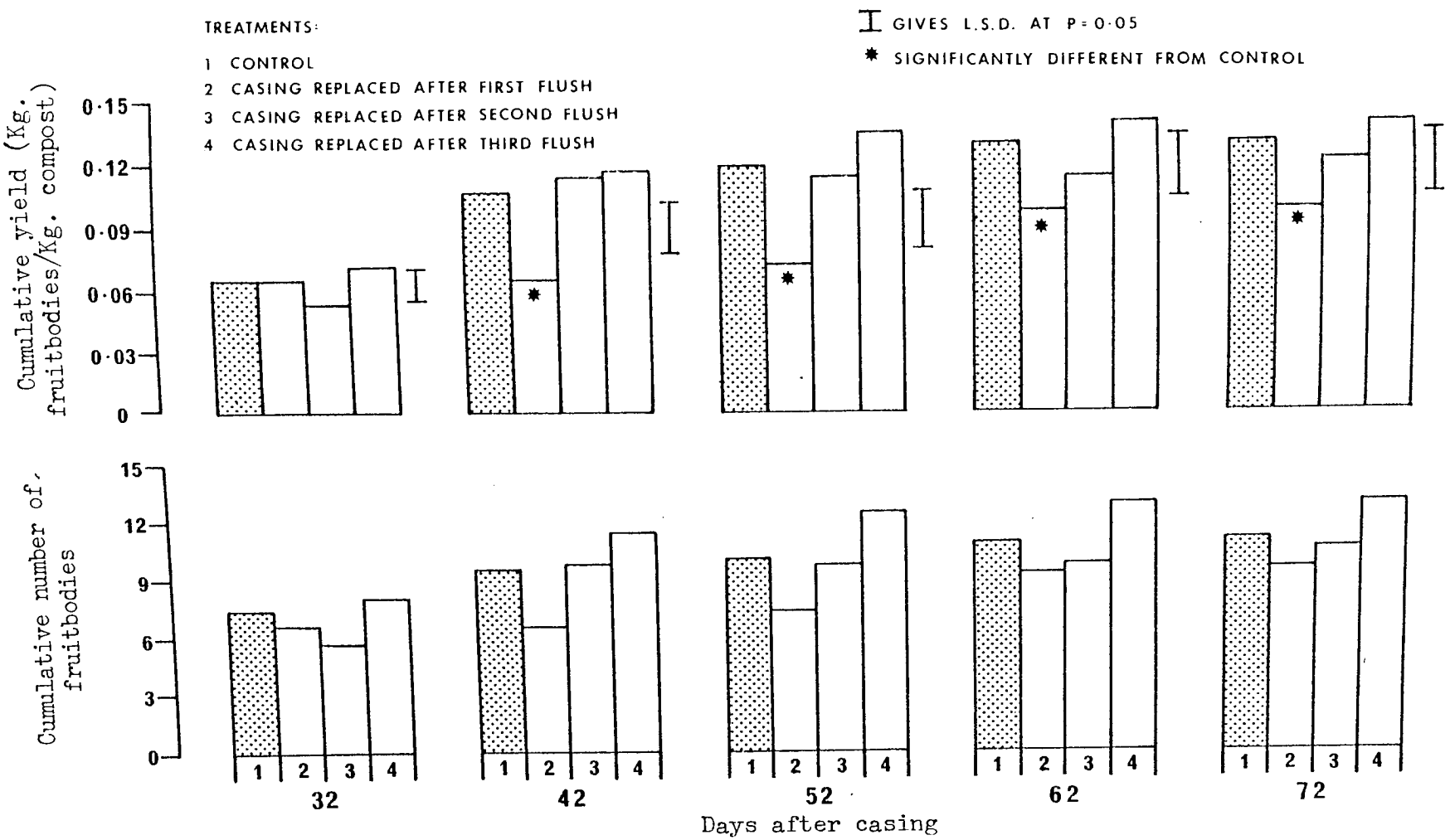


FIGURE 4.58 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING FOLLOWING ITS REPLACEMENT WITH FRESH MATERIAL AT INTERVALS DURING CULTURE.

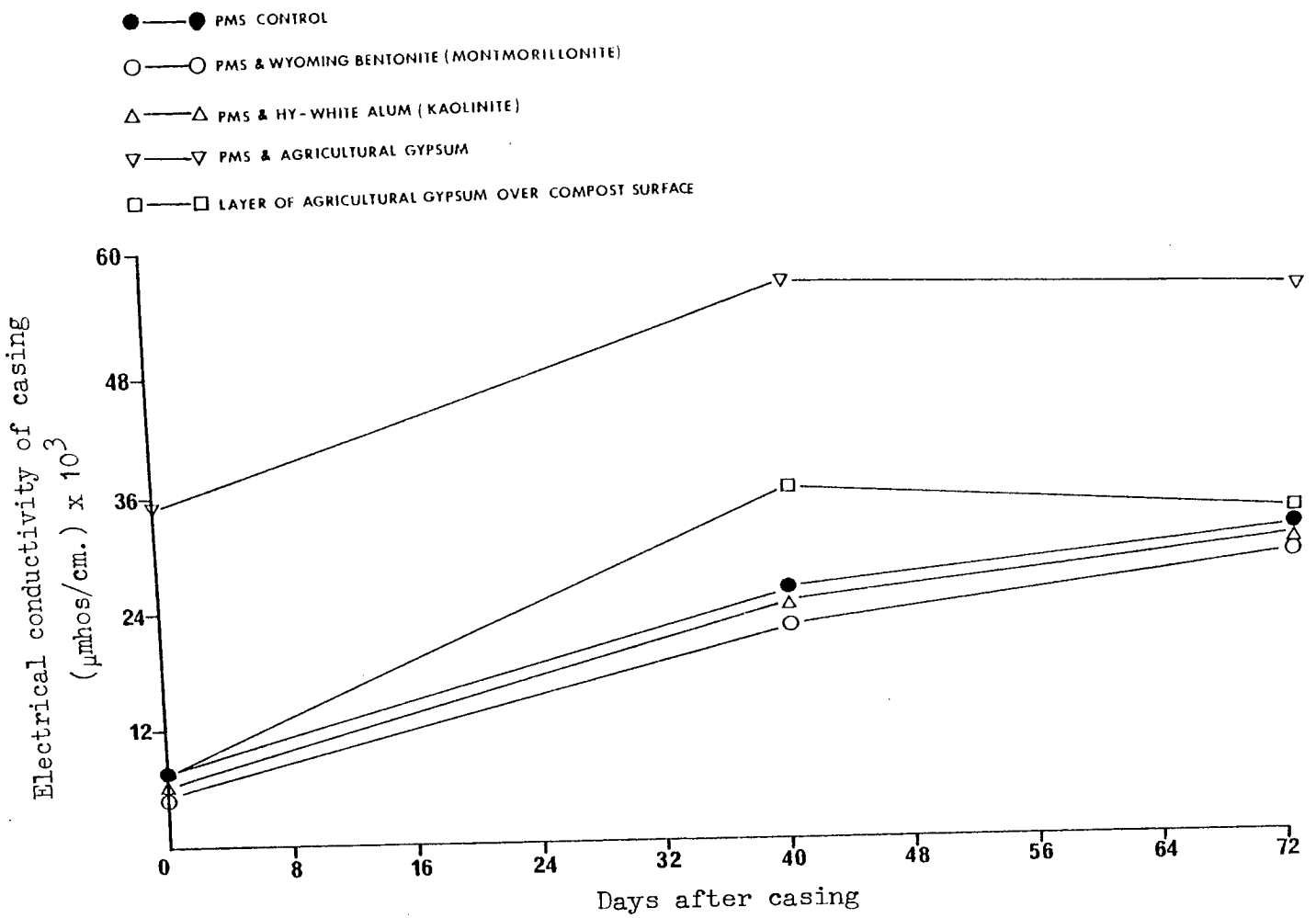


The clay minerals montmorillonite and kaolinite as well as agricultural gypsum were added to prepared PMS casing at the arbitary level of 20% by weight before its application. Montmorillonite was added in the form of Wyoming bentonite, supplied by Steetly (Berk. Limited) Mineral products division, Milton Keynes. Kaolinite was added as Hy-white Alum, supplied by English China Clays Company Limited, St. Austell, Cornwall. The minerals were subjected to x-ray diffraction analysis as in 3.7.5. Hy-white Alum was found to contain kaolinite and quartz as its major components, Wyoming bentonite was mainly a montmorillonite with sodium as the interlayer cation.

A thin, even coverage of fifty grammes of agricultural gypsum was applied to the compost surface in a separate treatment. Standard PMS casing was then applied, the gypsum layer intended to act as a barrier to salt movement.

Electrical conductivity of the casings was measured during culture (figure 4.59). The addition of both clay minerals reduced casing conductivity only marginally, montmorillonite reducing the value most. When a layer of gypsum was applied to the compost surface, the conductivity of the casing above rose more rapidly than in the control. Mixing gypsum into the casing resulted in very high conductivities.

FIGURE 4.59 SALT ACCUMULATION IN PMS CASING COMPARED WITH MATERIAL AMENDED (20% BY WEIGHT) WITH CLAY MINERALS AND AGRICULTURAL GYPSUM.



Significantly fewer initials developed following addition of montmorillonite, and gypsum as a layer over the compost (table 4.19). In the latter case it was found that a proportion of the initials developed deep in the casing, in contact with the gypsum layer.

TABLE 4.19 NUMBER OF FRUITBODY INITIALS FORMED ON PMS CASING AMENDED WITH SALT BINDING AGENTS.

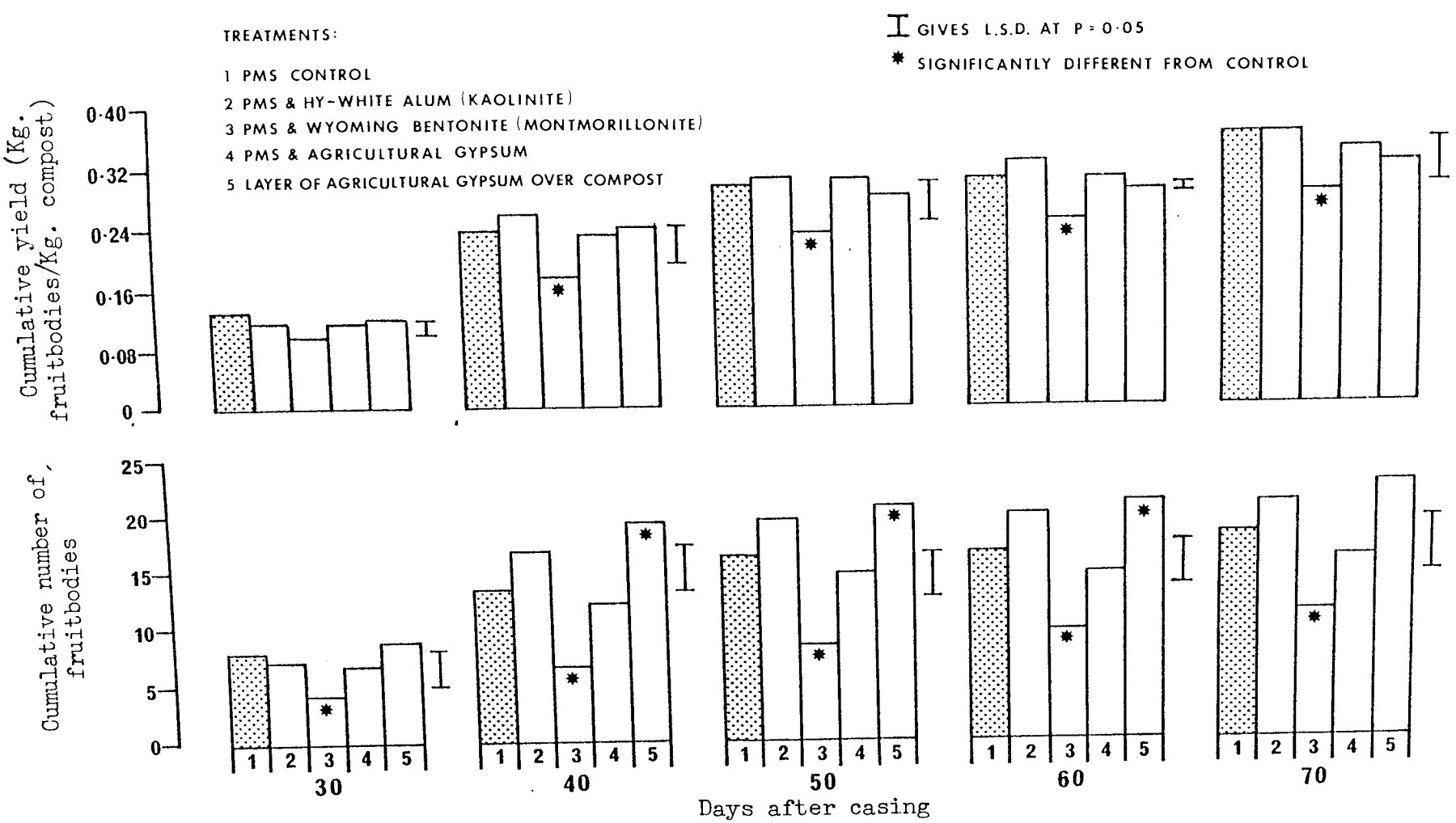
| Treatment | Mean number of initials |
|---|---|
| PMS control | 60.2 |
| PMS + Hy-white alum (kaolinite) | 62.0 |
| PMS + Wyoming bentonite (montmorillonite) | 13.0 |
| PMS + Agricultural gypsum | 53.5 |
| Agricultural gypsum as a layer over the compost surface | 33.3 |
| L.S.D. (P = 0.05) | 30.1 |

During the course of the experiment, fewer fruitbodies formed on casings amended with montmorillonite and gypsum, the reduction being significant with montmorillonite (figure 4.60, appendix 4.27). A larger number of sporophores was harvested from casings with kaolinite added, and where a layer of gypsum was applied to the compost surface. In the latter, the increase was close to or just above significance at all intervals from casing.

Fruitbodies from this treatment tended to develop deep in the casing in contact with the gypsum layer.

The only significant effect on yield was a reduction with the addition of montmorillonite up to fifty days from casing.

FIGURE 4.60 CUMULATIVE YIELD AND NUMBER OF FRUITBODIES HARVESTED FROM PMS CASING COMPARED WITH MATERIAL AMENDED WITH CLAY MINERALS AND AGRICULTURAL GYPSUM.



## 4.8 THE ROLE OF A.BISPORUS AND OTHER MICRO-ORGANISMS IN THE STATUS OF IONS IN CULTURE SUBSTRATES.

The present study has demonstrated the importance of available ions in the casing layer. The soluble salt content of PMS is an important factor in determining its suitability as a casing, whilst salt accumulation has been shown to be related to the decline in cropping during culture. It is also well known that micro-organisms play an important part in the culture of A.bisporus. In the casing layer they are required for fruitbody formation (Hayes, Randle and Last, 1969), whilst bacterial biomass provides an important nutrient source for A.bisporus in the compost (Hayes, 1969).

Studies so far have shown salts accumulating in the casing are derived from the compost (4.7.1), and it was suggested this involved a passive movement of solutes. To conclude the experimental aspects of this study an attempt was made to determine whether salt accumulation in casing was passive, or an active process involving A.bisporus or other micro-organisms present in the culture situation.

Hayes (1972) suggested that bacteria associated with fruiting might act by solubilising iron in the casing layer, making the element available for fruitbody formation. A study of the effect of A.bisporus and other micro-organisms on iron solubilisation in the casing was included in this section.

The process of salt accumulation was shown to occur in both peat and PMS casings (4.7.1). Consequently, studies on available elements in the final experiments were confined to PMS casing, the material under investigation in this study.

4.8.1 The mechanism of salt accumulation in the casing layer.

Three experimental treatments were devised to study the mechanism of salt accumulation in the casing layer:

(1) Sterilised casing applied to sterile compost.

(2) Sterilised casing applied to an axenic culture of A.bisporus in sterilised compost.

(3) Standard cultural practice.

Treatment one would show any passive salt accumulation, whilst treatment two was used to examine any role of A.bisporus in ion movement. By comparing treatments two and three the effect of the compost and casing microflora could be determined.

A flask culture apparatus was devised for maintaining the three treatment situations described under the culture conditions applied in normal practice, and is described in 3.4.3, and illustrated in plates 3.3 and 3.4.

At intervals from casing, five flasks from each treatment were emoved and the compost and casing sampled, and extracted with M. ammonium etate as in 3.7.14. Studies in 4.7.1 showed the ions which build up the highest levels in the casing (chloride, potassium, sodium and lphate) followed the same pattern of gradual accumulation from the mpost. The results also demonstrated salt accumulation to be a movement available ions. In this trial only levels of available potassium and odium were studied, reducing the time taken to analyse the large number extracts. The elements were estimated by flame emission photometry as 3.7.16.

It was decided to take advantage of the experimental treatments in his trial to examine the effect of bacteria and A.bisporus on iron lubilisation in the casing. Casing extracts were made with 0.1 M. otassium pyrophosphate as in 3.7.14, to determine the total levels of ron available. Readily available iron was determined on water extracts, repared as in 3.7.14. Iron levels in extracts were determined by tomic absorption spectrophotometry (3.7.16).

Plating out of compost and casing immediately after sampling onto gar media showed no contamination of the culture flasks had occurred. wo flushes of fruitbodies were harvested from treatment three flasks.

Figures 4.61 and 4.62 show the pattern of gradual accumulation of otassium and sodium in the casing was unaffected by the experimental reatments. Available levels in the compost showed a corresponding ecline over the same period in all treatments.

FIGURE 4.61 AMMONIUM ACETATE EXTRACTABLE POTASSIUM IN COMPOST AND PMS CASING DURING CULTURE.

TREATMENTS :

1 COMPOST AND CASING STERILE DURING CULTURE
2 AXENIC CULTURE OF A.BISPORUS
3 STANDARD CULTURE SITUATION

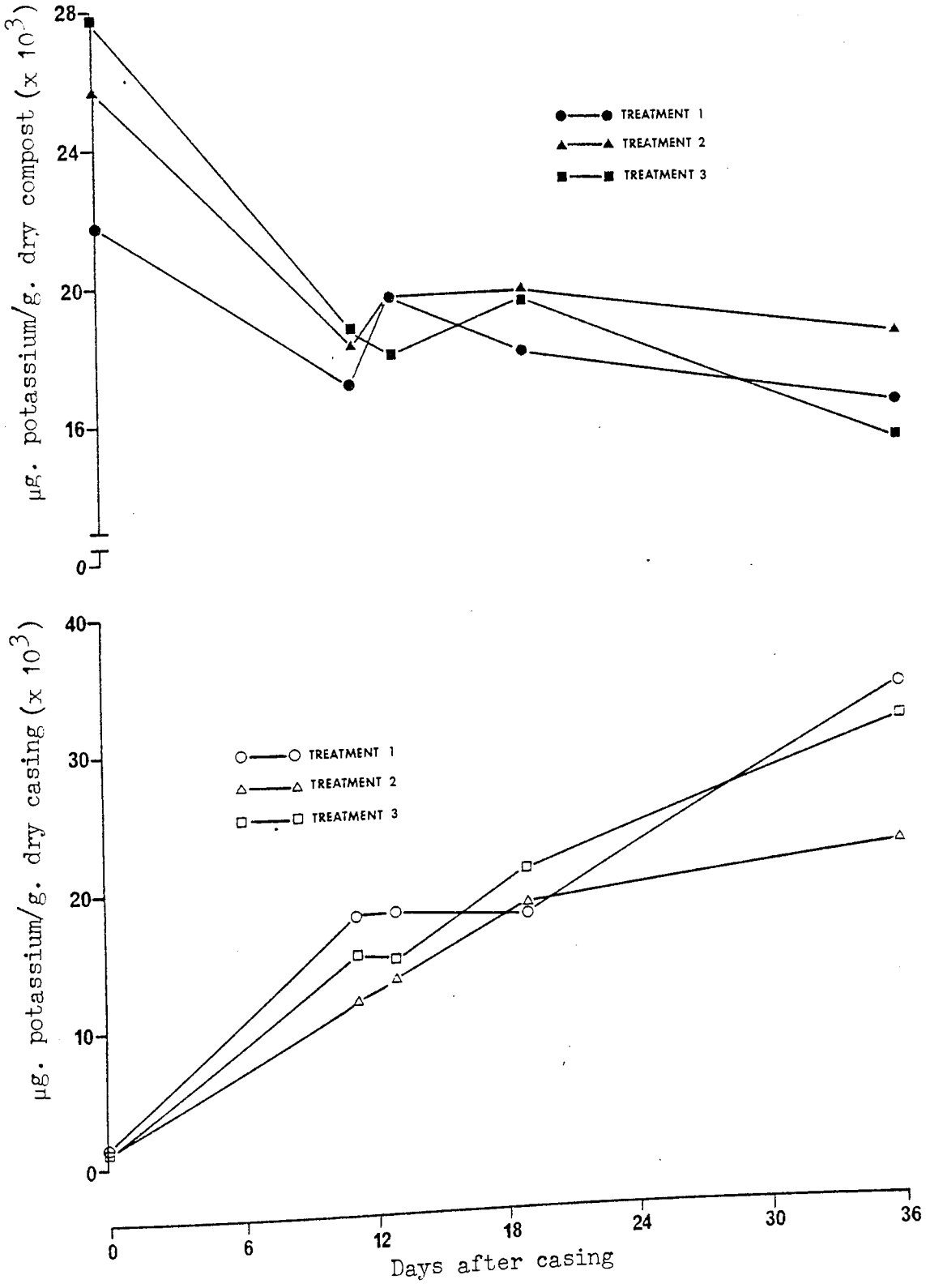


FIGURE 4.62 AMMONIUM ACETATE EXTRACTABLE SODIUM IN COMPOST AND PMS CASING DURING CULTURE.

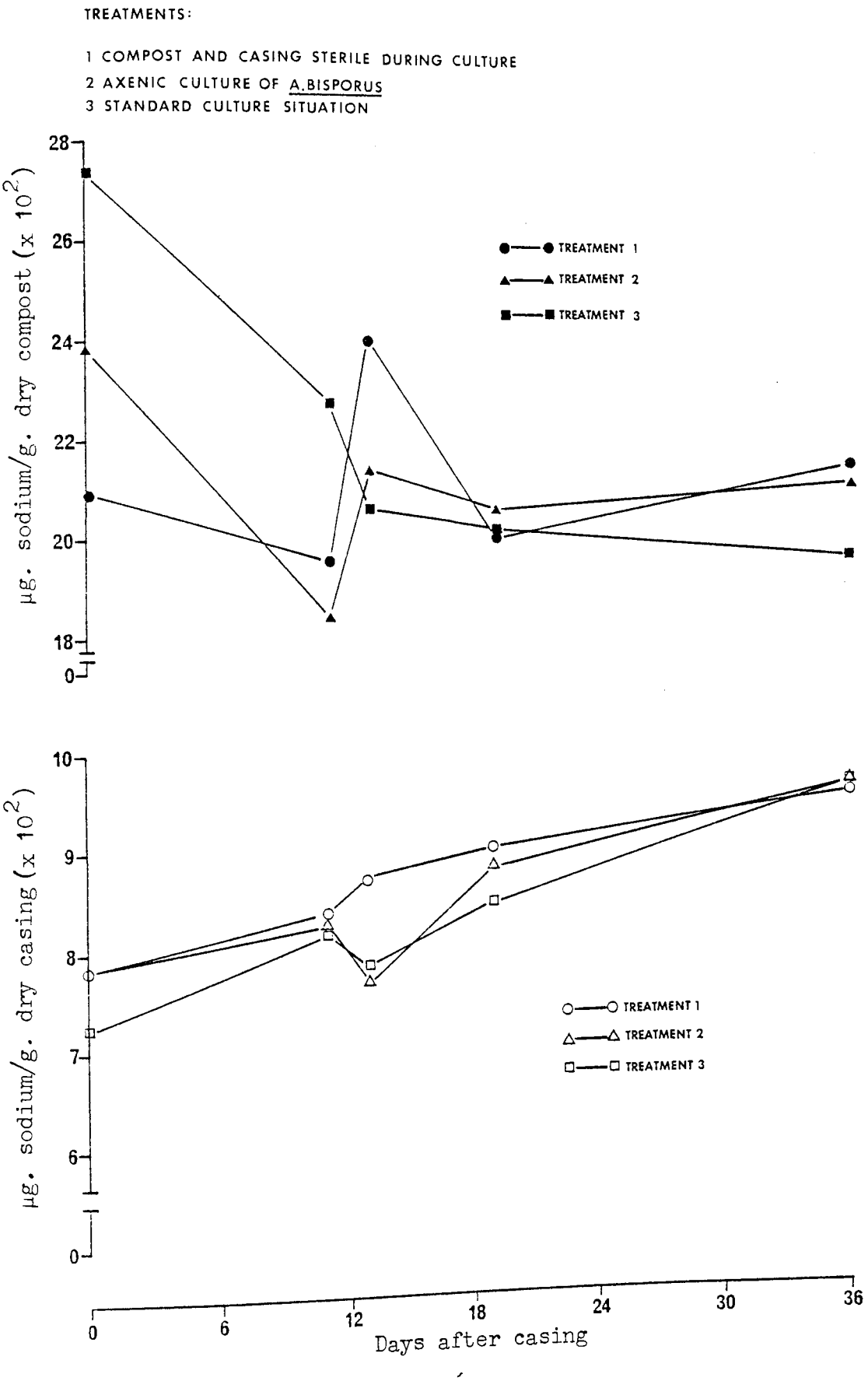
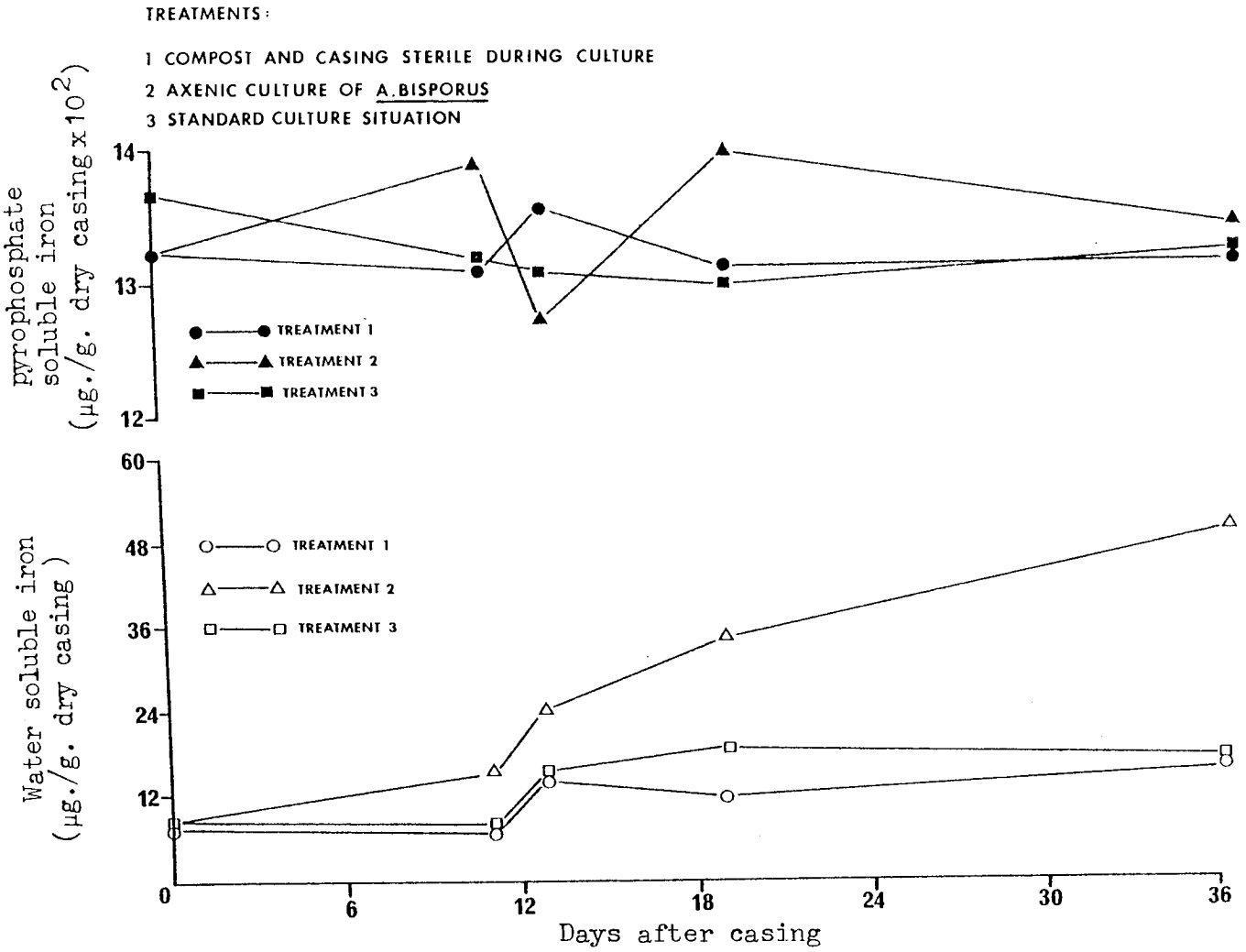


FIGURE 4.63 POTASSIUM PYROPHOSPHATE AND WATER SOLUBLE IRON IN PMS CASING DURING CULTURE.



The concentrations of potassium and sodium in the casings were a good deal lower than those which built up over the same time period in 4.7.1.

Pyrophosphate soluble iron in the casings showed no real trend with time from casing (figure 4.63). Very slight increases in water soluble iron were evident in treatments one and three, but in axenic culture of A.bisporus, water soluble iron rose markedly during culture.

### 4.8.2 Further studies on the interaction of A.bisporus and associated micro-organisms with elements available during culture.

Following the success achieved in maintaining the required culture conditions in the previous experiment, it was decided to employ the flask culture technique to further investigate any relationship of A.bisporus and associated micro-organisms with levels of available elements.

The combinations of sterilised and pasteurised compost and casing substrates were increased to include all permutations, each then being studied with A.bisporus absent and present. This gave eight treatments:

1. Compost and casing sterilised — A.bisporus absent
2. Compost and casing sterilised — A.bisporus present
3. Compost and casing pasteurised — A.bisporus absent
4. Compost and casing pasteurised — A.bisporus present
5. Compost sterilised, casing pasteurised — A.bisporus absent
6. Compost sterilised, casing pasteurised — A.bisporus present
7. Compost pasteurised, casing sterilised — A.bisporus absent
8. Compost pasteurised, casing sterilised — A.bisporus present

In the previous experiment it was noted that water soluble iron accumulated in the casing in the presence of a pure culture of A.bisporus, but not when associated micro-organisms were also present. This suggested an interaction of the casing microflora with levels of readily available iron. Consequently, a wide range of elements was studied to investigate any possible effects of compost and casing microflora on their availability.

No one extractant is suitable for the removal of available levels of a wide range of elements (Allen et al., 1974). In the culture environment readily available ions are dissolved or soluble in water . For these reasons de-ionised water was chosen as a standard extractant, following the method in 3.7.14. Levels of aluminium, cadmium, chloride, chromium, copper, iron, lithium, magnesium, manganese, nickel, phosphate, sulphate and zinc were estimated on the extracts as in 3.7.16. Potassium and sodium were excluded as they had already been studied comprehensively.

The modifications to the flask culture technique described in 3.4.3 were employed in this experiment.

Where A.bisporus was present, fruitbodies were produced in treatment four, six and eight flasks, but not treatment two flasks. The results are given in table 4.20.

TABLE 4.20 YIELD DATA FROM FLASK CULTURE TREATMENTS WITH A.BISPORUS PRESENT.

| Treatment | Days after casing | Cumulative yield and number of fruitbodies harvested | |
|---|---|---|---|
| | | Number | Yield (Kg. fruitbodies/Kg. compost) |
| 2. Compost and casing sterilised | 27 | - | - |
| | 37 | - | - |
| 4. Compost and casing pasteurised | 27 | 4 | 0.076 |
| | 37 | 6 | 0.102 |
| 6. Compost sterilised casing pasteurised | 27 | 3 | 0.063 |
| | 37 | 6 | 0.099 |
| 8. Compost pasteurised casing sterilised | 27 | - | - |
| | 37 | 2 | 0.051 |

Yield figures are the mean values from five flasks.

Of the elements studied, aluminium, cadmium, chromium, lithium, manganese, nickel and zinc were all below the limits of detection. This represented a concentration of less than 2 μg./g. of dry substrate for all elements save aluminium, where the level was 10 μg./g. dry substrate, or less.

Copper was detectable in both compost and casing extracts, but only at levels of 5 μg./g. dry substrate or less. No patterns were evident with time from casing or treatment applied.

Three of the six elements that were detectable, chloride, magnesium and sulphate, showed a pattern of steady accumulation in the casing irrespective of the treatment (figure 4.64a and b, 4.65a and b and 4.66a and b). There was an overall pattern of gradual decline in the chloride concentration in compost. Levels of magnesium and sulphate in the compost showed less well defined trends. No real pattern with compost magnesium emerged with the various treatments or time from casing. Where pasteurised compost was used, sulphate levels in compost declined steadily with time from casing. However, sterilisation of compost reduced the available sulphate content which then showed an initial increase followed by a slow decline with time after casing.

Figure 4.67a and b shows water soluble iron increased in the casing in treatments two (axenic culture of A.bisporus) and eight (where sterile casing was applied to pasteurised, colonised compost). In other casings water soluble iron levels showed no real pattern.

Concentrations of iron in the compost were low, and remained level or fell slightly after casing application, except in treatment two where there was a slight decrease. In the compost whether previously pasteurised or sterilised, following colonisation by A.bisporus the level of water soluble iron was increased. A similar effect was noted with water soluble phosphate in the compost (figure 4.68a and b). At casing available phosphate was approximately six times higher in compost when it had been colonised by A.bisporus. From the time of casing this higher level declined in the four treatments with A.bisporus present, approaching the lower constant level in uncolonised compost by the end of the trial. Water soluble phosphate was below the detectable limit of 3 μg./g. in all casing extracts.

FIGURE 4.64a WATER SOLUBLE CHLORIDE IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 1 TO 4.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST AND CASING STERILISED | A.BISPORUS ABSENT | 1 |
| | A.BISPORUS PRESENT | 2 |
| COMPOST AND CASING PASTEURISED | A.BISPORUS ABSENT | 3 |
| | A.BISPORUS PRESENT | 4 |

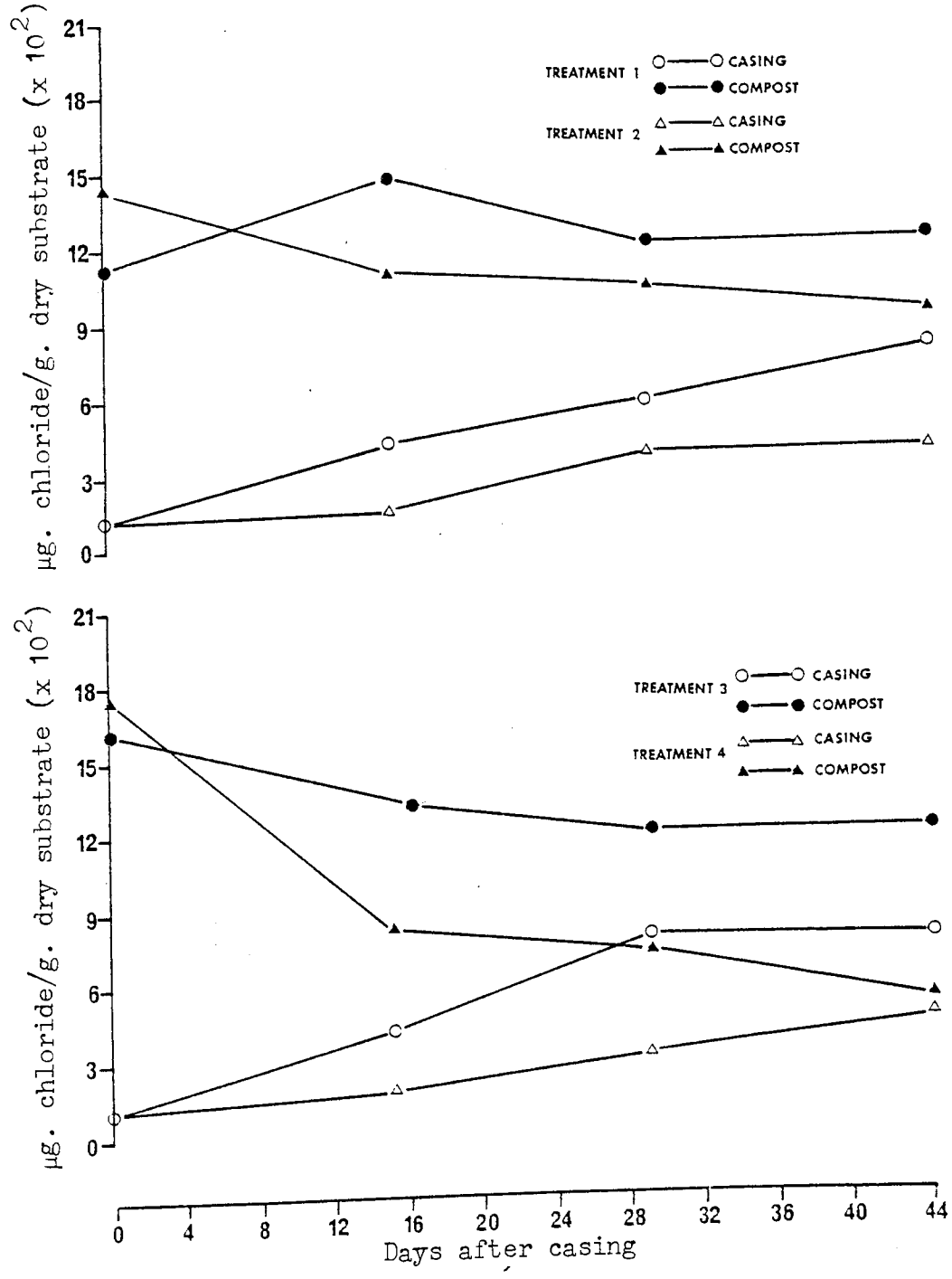


FIGURE 4.64b WATER SOLUBLE CHLORIDE IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 5 TO 8.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST STERILISED CASING PASTEURISED | A.BISPORUS ABSENT | 5 |
| | A.BISPORUS PRESENT | 6 |
| COMPOST PASTEURISED CASING STERILISED | A.BISPORUS ABSENT | 7 |
| | A.BISPORUS PRESENT | 8 |

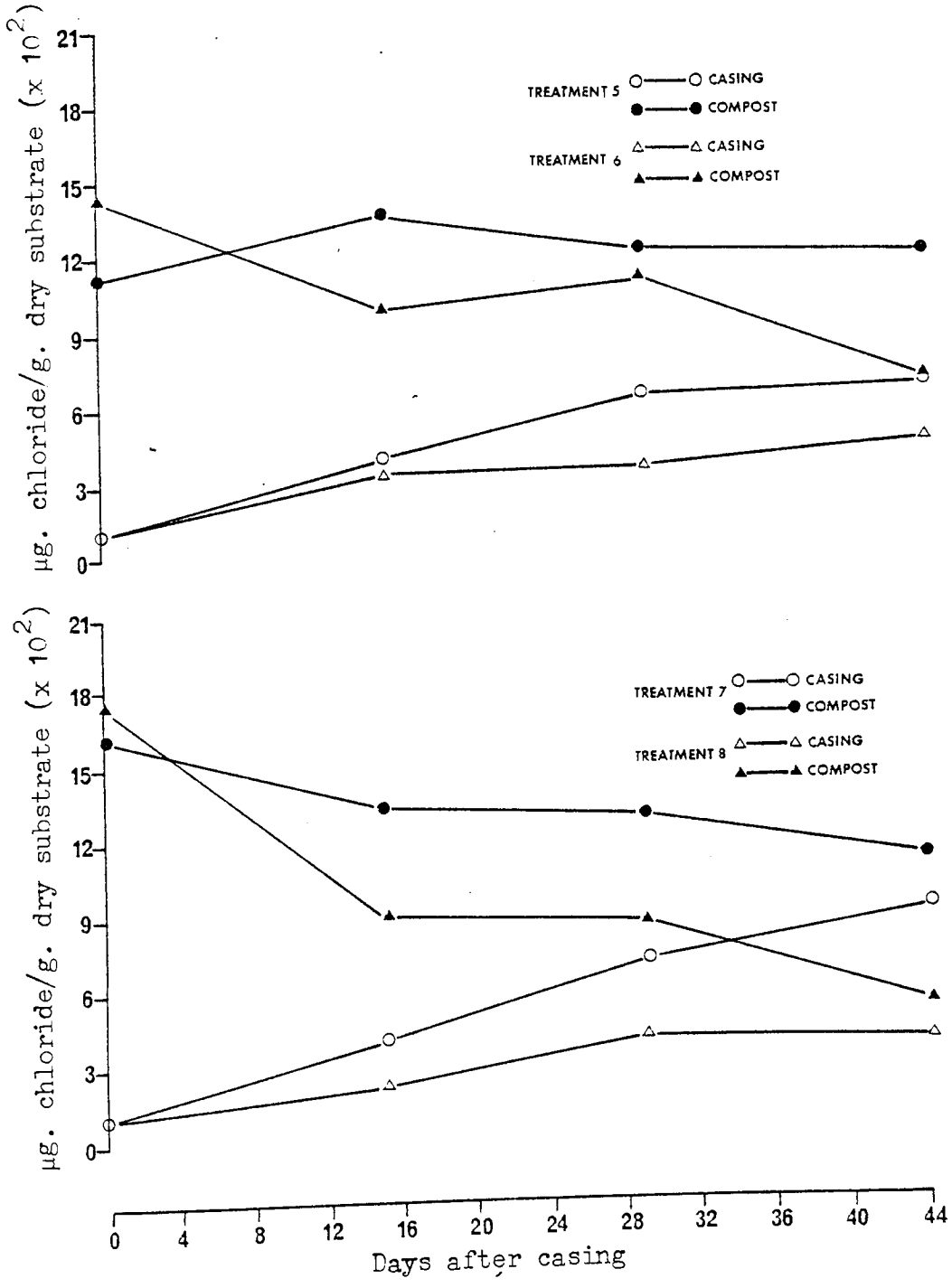


FIGURE 4.65a WATER SOLUBLE MAGNESIUM IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 1 TO 4.

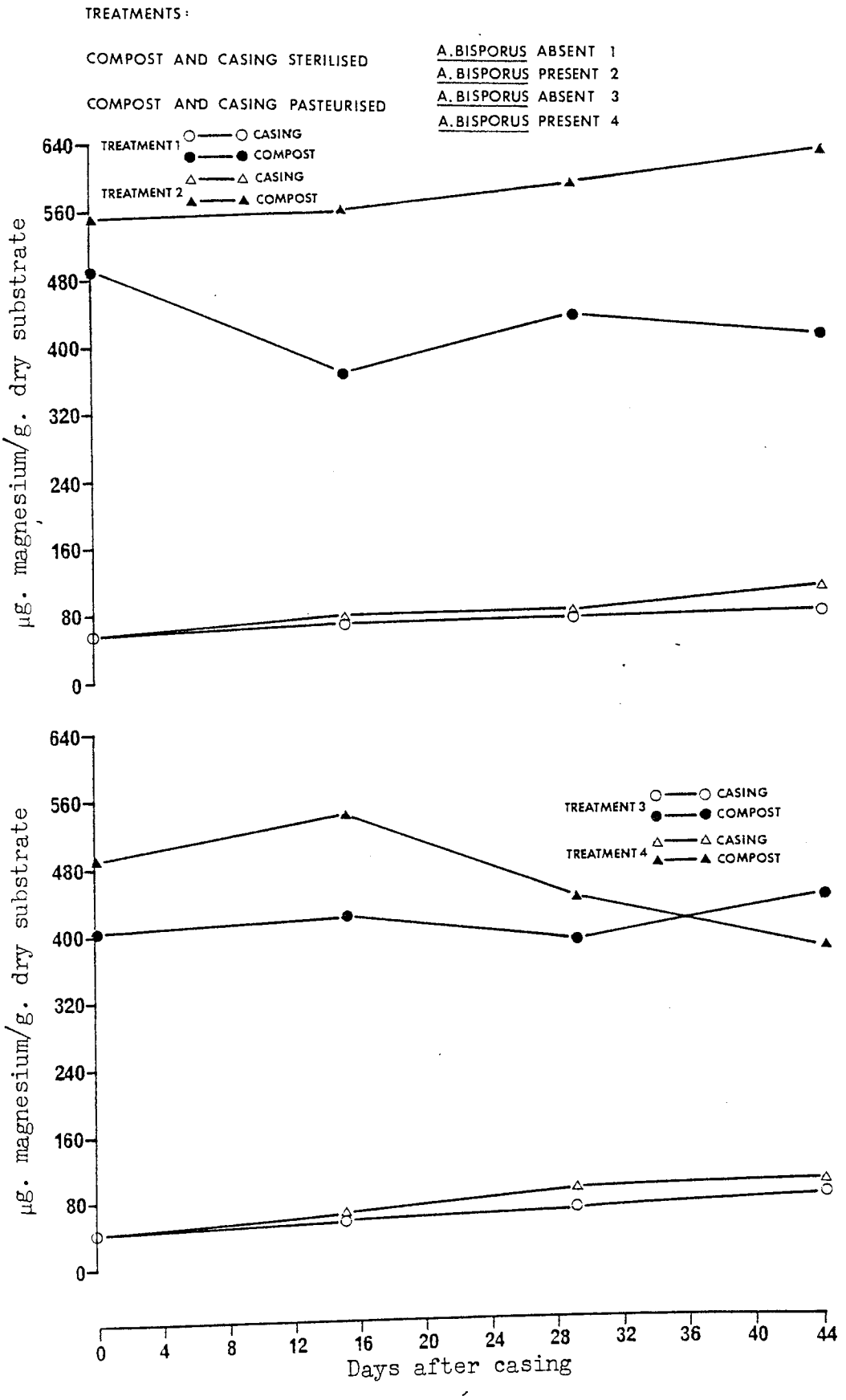


FIGURE 4.65b WATER SOLUBLE MAGNESIUM IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 5 TO 8.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST STERILISED CASING PASTEURISED | A.BISPORUS ABSENT | 5 |
| | A.BISPORUS PRESENT | 6 |
| COMPOST PASTEURISED CASING STERILISED | A.BISPORUS ABSENT | 7 |
| | A.BISPORUS PRESENT | 8 |

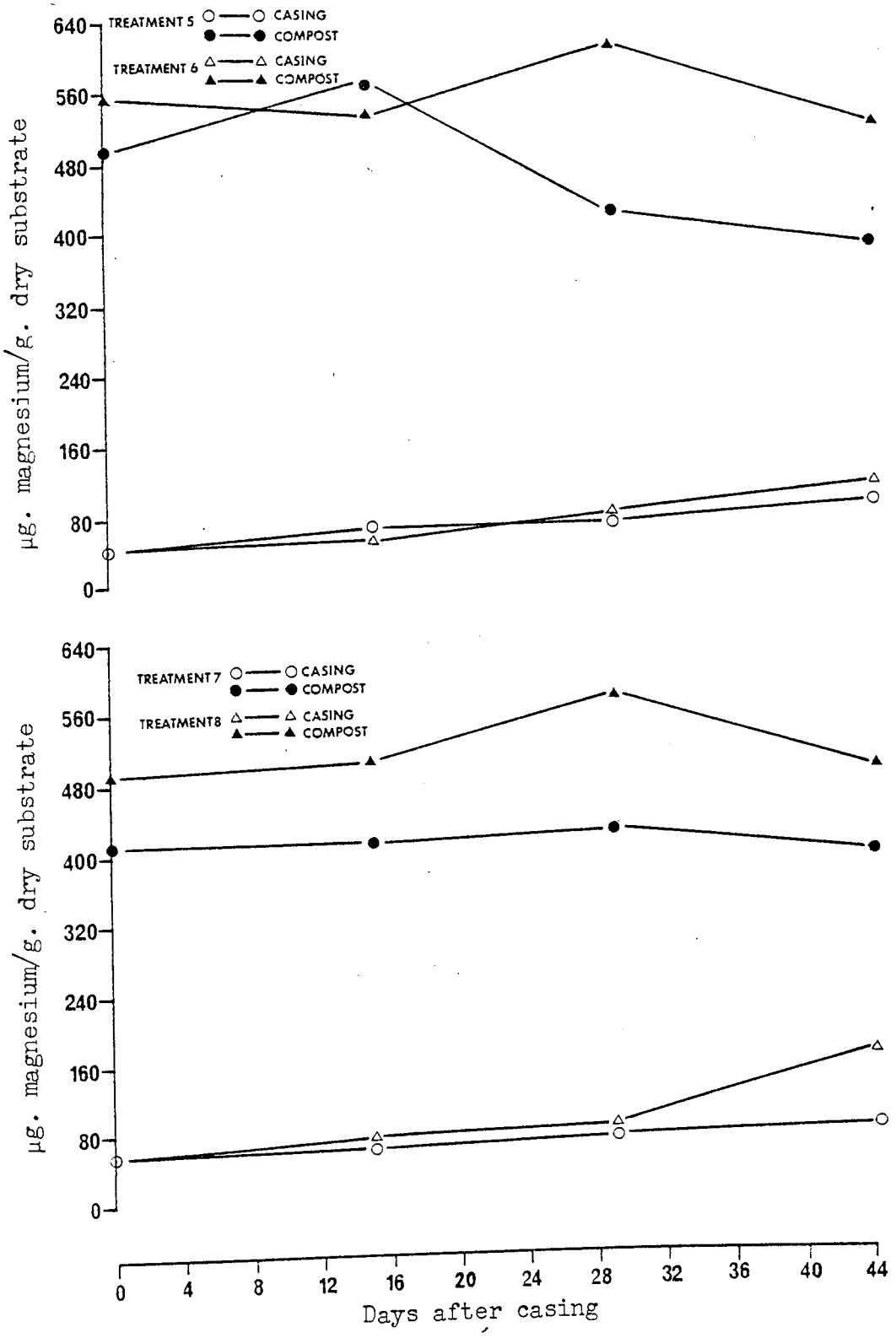


FIGURE 4.66a WATER SOLUBLE SULPHATE IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 1 TO 4.

TREATMENTS :

COMPOST AND CASING STERILISED — A. BISPORUS ABSENT 1; A. BISPORUS PRESENT 2

COMPOST AND CASING PASTEURISED — A. BISPORUS ABSENT 3; A. BISPORUS PRESENT 4

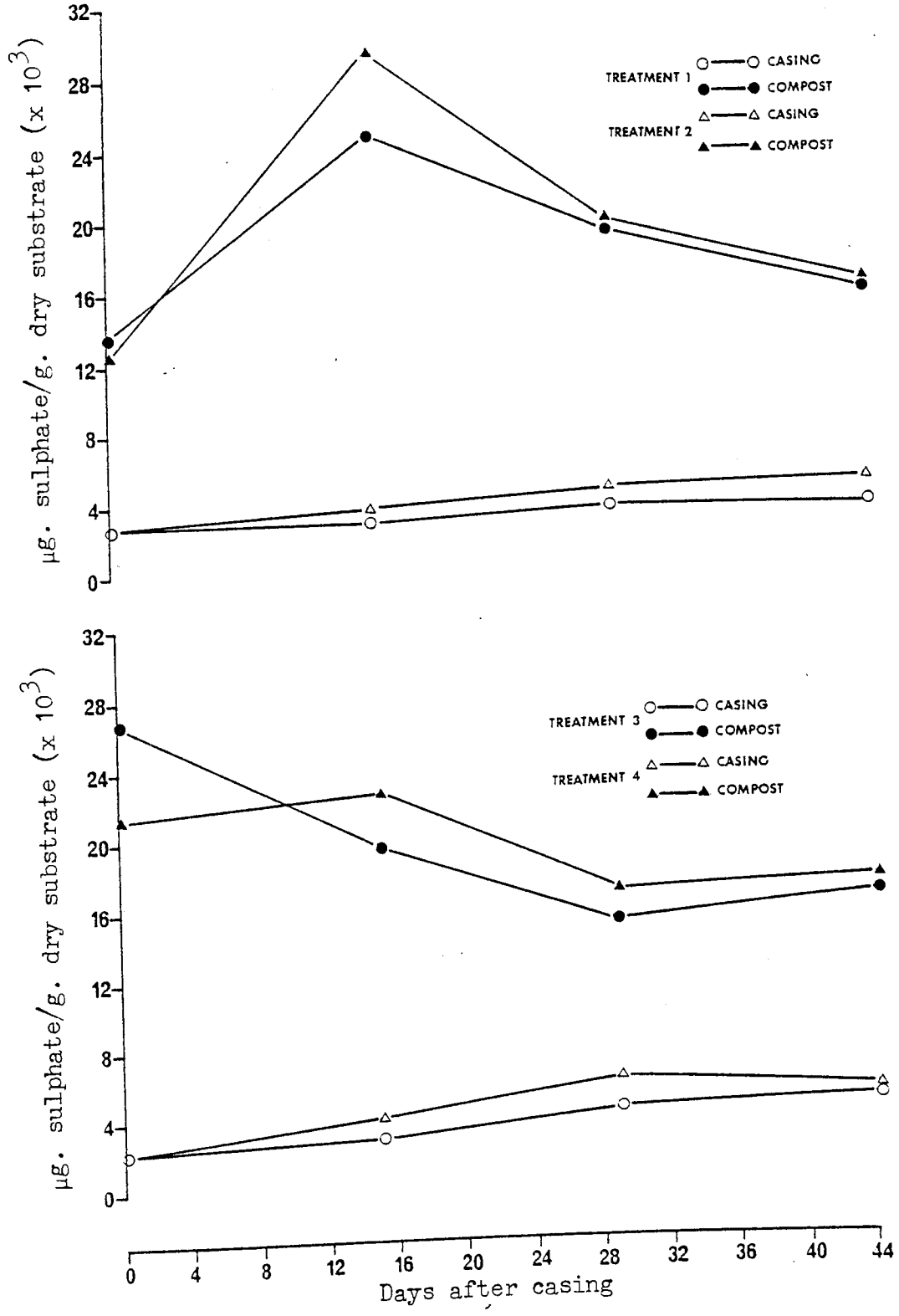


FIGURE 4.66b WATER SOLUBLE SULPHATE IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 5 TO 8.

TREATMENTS :

COMPOST STERILISED CASING PASTEURISED
A.BISPORUS ABSENT 5
A.BISPORUS PRESENT 6

COMPOST PASTEURISED CASING STERILISED
A.BISPORUS ABSENT 7
A.BISPORUS PRESENT 8

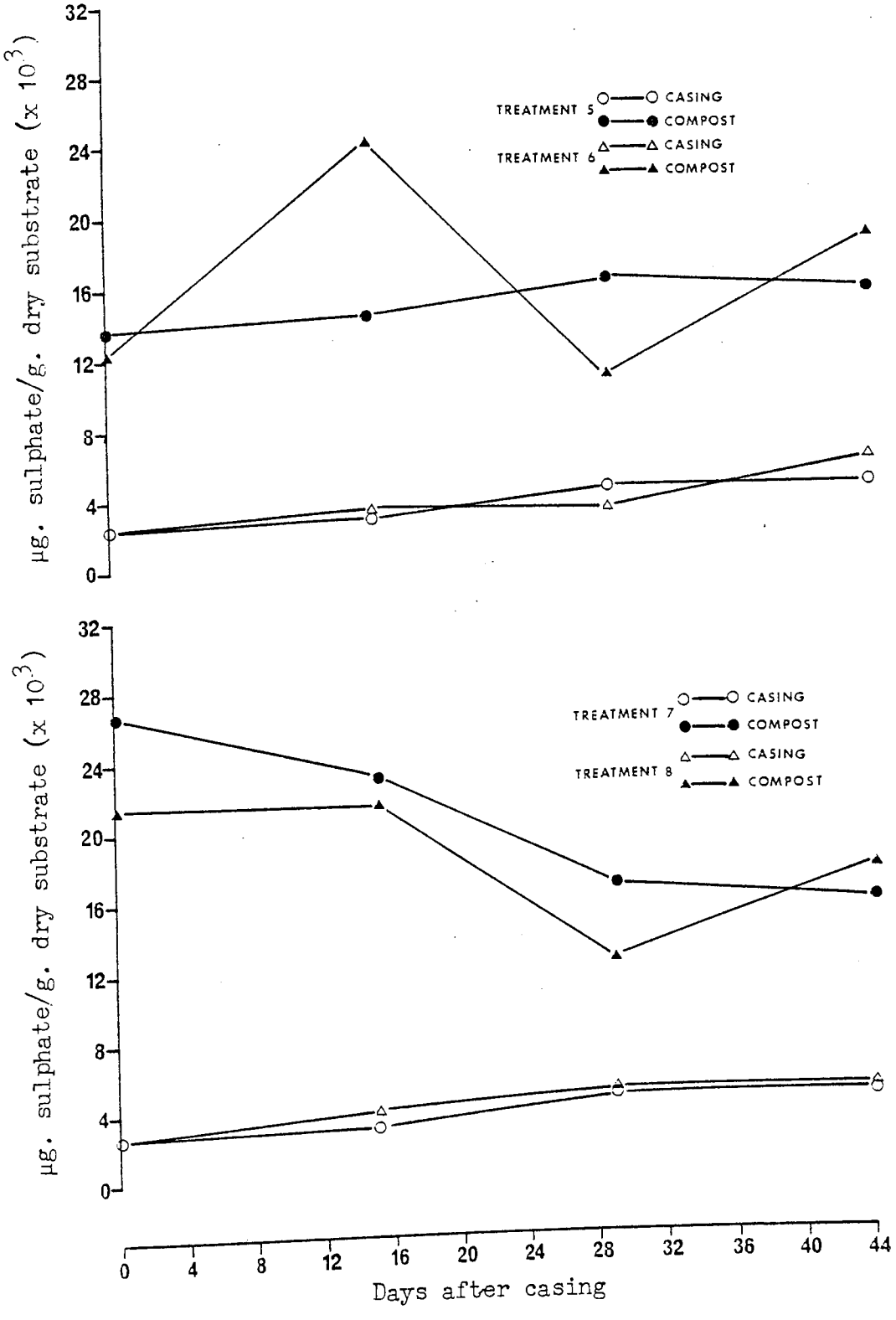


FIGURE 4.67a WATER SOLUBLE IRON IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 1 TO 4.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST AND CASING STERILISED | A.BISPORUS ABSENT | 1 |
| | A.BISPORUS PRESENT | 2 |
| COMPOST AND CASING PASTEURISED | A.BISPORUS ABSENT | 3 |
| | A.BISPORUS PRESENT | 4 |

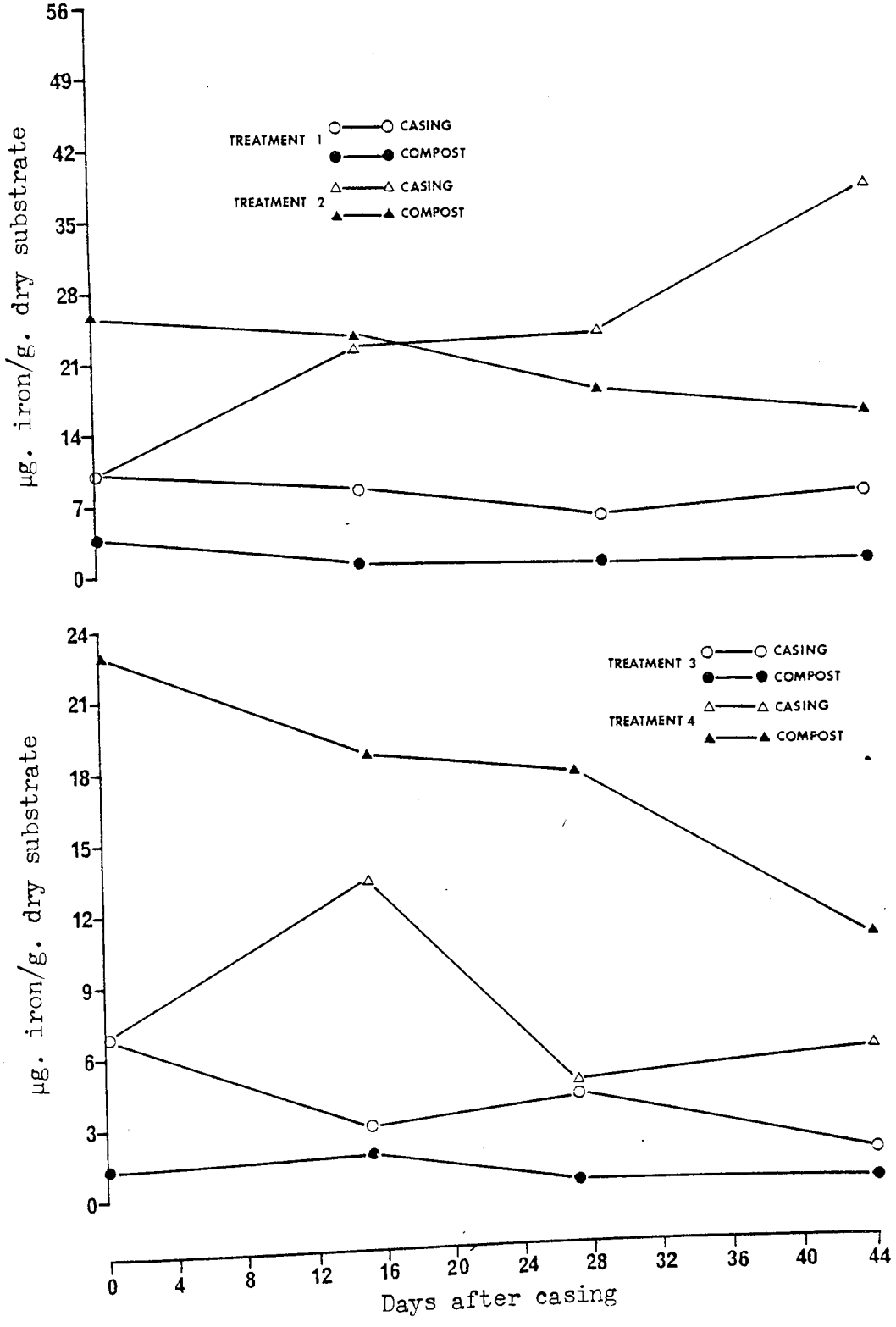


FIGURE 4.67b WATER SOLUBLE IRON IN COMPOST AND PMS CASING DURING CULTURE : TREATMENTS 5 TO 8.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST STERILISED CASING PASTEURISED | A.BISPORUS ABSENT | 5 |
| | A.BISPORUS PRESENT | 6 |
| COMPOST PASTEURISED CASING STERILISED | A.BISPORUS ABSENT | 7 |
| | A.BISPORUS PRESENT | 8 |

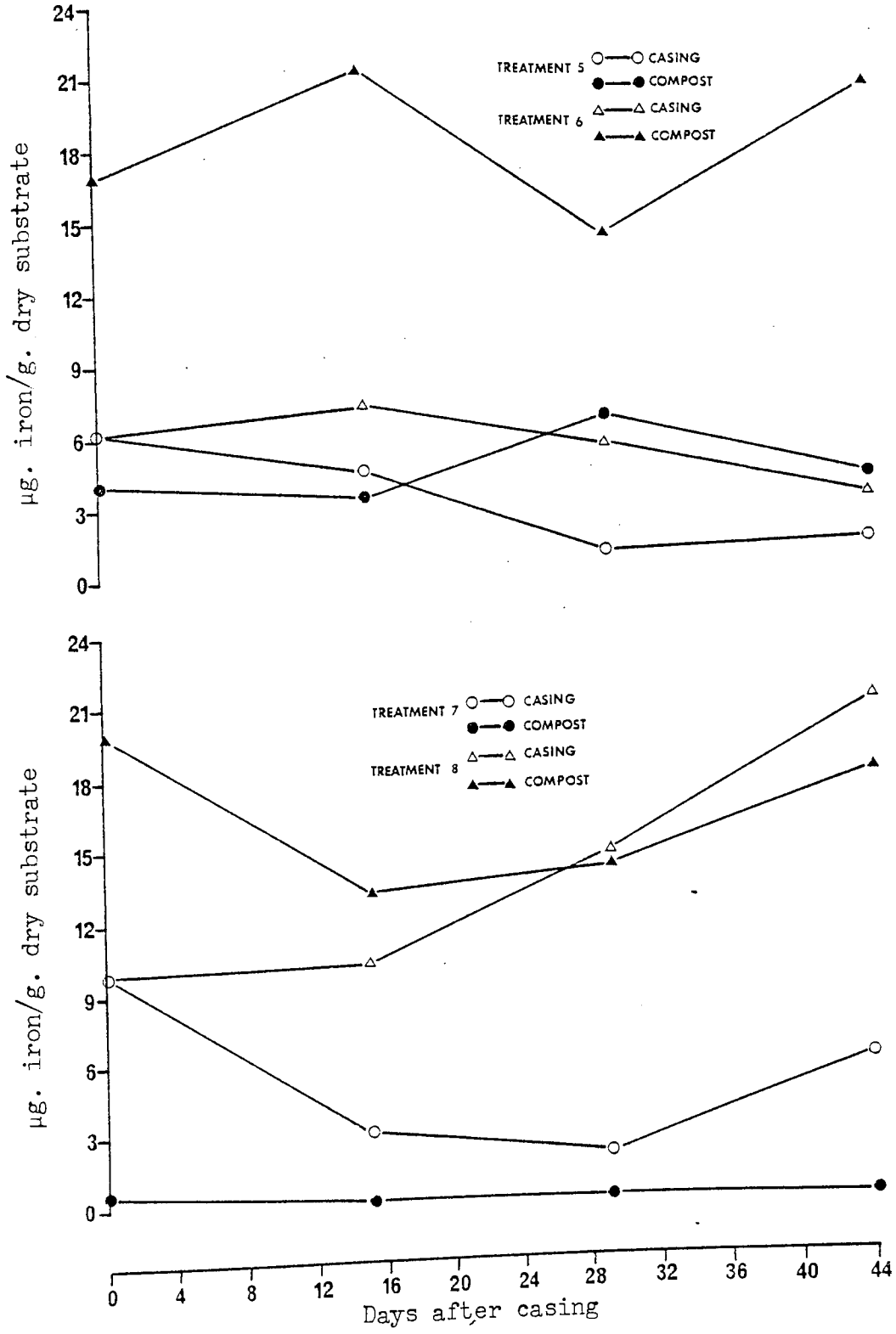


FIGURE 4.68a WATER SOLUBLE PHOSPHATE IN COMPOST DURING CULTURE : TREATMENTS 1 TO 4.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST AND CASING STERILISED | A.BISPORUS ABSENT | 1 |
| | A.BISPORUS PRESENT | 2 |
| COMPOST AND CASING PASTEURISED | A.BISPORUS ABSENT | 3 |
| | A.BISPORUS PRESENT | 4 |

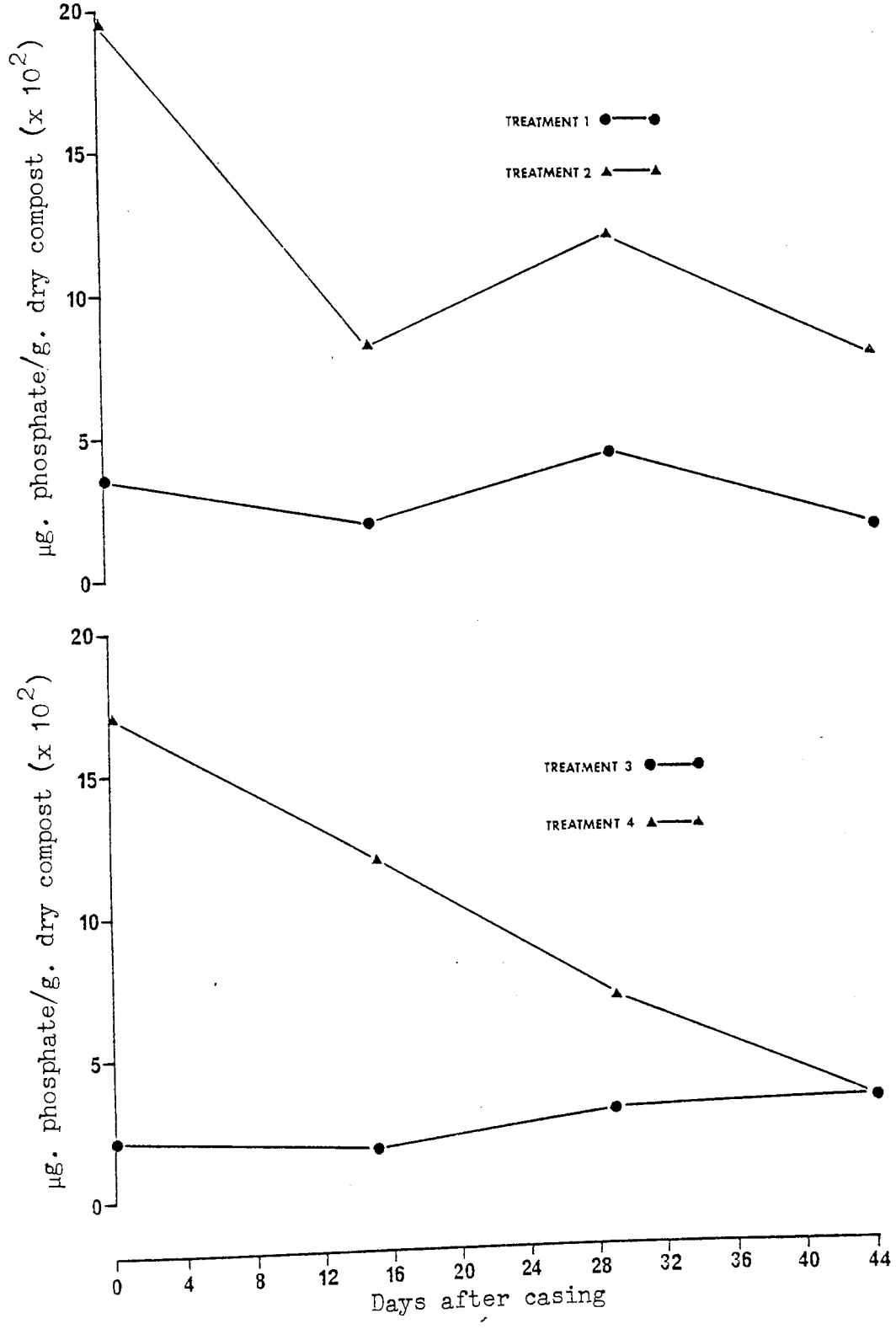
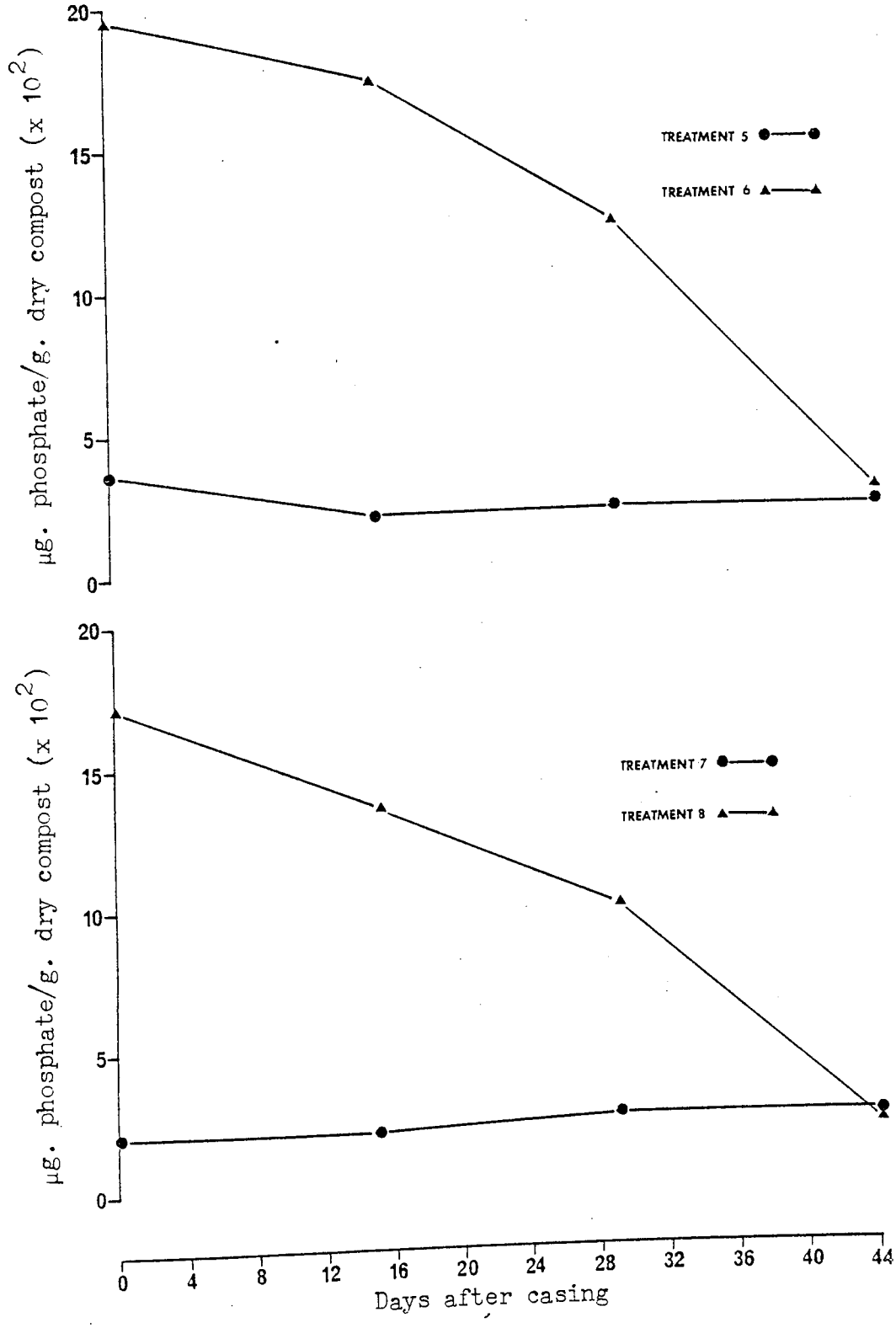


FIGURE 4.68b WATER SOLUBLE PHOSPHATE IN COMPOST DURING CULTURE : TREATMENTS 5 TO 8.

TREATMENTS:

| | | |
|---|---|---|
| COMPOST STERILISED CASING PASTEURISED | A.BISPORUS ABSENT | 5 |
| | A.BISPORUS PRESENT | 6 |
| COMPOST PASTEURISED CASING STERILISED | A.BISPORUS ABSENT | 7 |
| | A.BISPORUS PRESENT | 8 |



# SECTION 5

# DISCUSSION AND CONCLUSIONS

## SECTION 5 DISCUSSION AND CONCLUSIONS

The casing layer in the culture of Agaricus bisporus is the medium in which growth of the cultivated mushroom switches from the vegetative phase to one of reproduction. Properties of the casing relevant to fruiting in A.bisporus were discussed in section two. Though research has explained some of the factors which govern fruitbody formation and the yield harvested, the action of the casing is still not fully understood.

While a variety of media can act as casings in mushroom culture, only a few meet the requirements of commercial production. The standard material in the United Kingdom is a mixture of Sphagnum peat and chalk, the majority of the peat being imported from Eire where reserves are diminishing rapidly. A suitable replacement for peat is therefore a priority for the U.K. industry. Paper mill sludge (PMS) produced at the Kemsley mill of Bowaters U.K. Paper Company Limited was considered as a potential substitute in the present study following its successful use by Hayes (1975). In addition, the material is continually produced in sufficient quantity to make it a realistic alternative.

### 5.1 THE NATURE OF PMS PRODUCED AT KEMSLEY.

The composition of the mill effluent at Kemsley discharged via the waste water into the settling lagoon varies according to the pulp and paper processes in operation at any one time. Dredging operations remove only a small fraction of the suspended solids discharged daily into the lagoon, which consequently acts as a mixing vessel.

The result was that the samples of sludge taken from different sites in the lagoon showed little variation when analysed in 4.2 (appendix 5.1). In addition material excavated at a later time for drainage trials had a very similar composition to previous samples. Thus PMS as freshly excavated at Kemsley is reasonably uniform.

The fresh sludge had a dry weight content of approximately eight per cent., a p H between 6.6 and 6.9, and a high content of soluble salts derived mainly from the semi-chemical pulping process, and reflected in high levels of sodium and sulphate. On a dry weight basis the major component of the material was organic matter lost mainly during pulping, with cellulose and lignin the largest fractions. Paper coating agents such as clay and chalk plus the salts used in semi-chemical pulping provide the inorganic fraction of the sludge, which was eighteen per cent. of the dry weight. After sodium and sulphate the most abundant elements in PMS were iron, magnesium and calcium. Iron is unlikely to be present in any great quantity in the materials used in pulp and paper processes, and the most likely source is the steel digesters used for semi-chemical pulping. Calcium is derived from chalk used in paper coating, whilst magnesium is occasionally used as magnesium hydrosulphate for bleaching of groundwood pulp.

Following its deposition the appearance of PMS alters as it dries out, with a range of higher fungi and finally green plants colonising the deposits. During its ageing process a number of changes occurred in the composition of the sludge, which are summarised in table 4.2.

The material dried out to just under thirty per cent. dry weight over a period of one year, during which time its p H declined steadily to below 4.0. The microbial breakdown of organic carbon results in the release of carbon dioxide and organic acids, which would cause the p H to be lowered. Organic carbon was shown to decrease in PMS during that period, with cellulose being the major component broken down. The initial level of organic nitrogen in PMS was low, but increased in the waste after its deposition. This may be explained by a breakdown of other organics increasing the relative level of organic nitrogen, as well as fixation of atmospheric nitrogen by bacteria such as Azotobacter and Clostridium, which supply much of the organic nitrogen present in non-fertilised soils (Meyer, Anderson, Bohning and Fratianne, 1973). The ash content of PMS increased as organic components were degraded. There was a decline in the available forms of calcium, magnesium and sodium as well as water soluble chloride and sulphate, which with the exception of chloride are responsible for the initially high salinity of PMS. These soluble salts are largely removed from PMS deposits via drainage and leaching, this being demonstrated by the higher electrical conductivities recorded in bottom sludge cores compared with samples taken nearer to the surface, and the rapid decline in salt content of PMS following deposition.

Apart from salt content the only consistent differences in the composition of PMS at depths of approximately ten and forty centimetres were in its dry weight and content of organic nitrogen. Sludge deposits were drier nearer to the surface, as one would expect from evaporative loss.

Over the first year, PMS contained more nitrogen at the shallower depth, possibly reflecting the greater biological activity in that region demonstrated by study of fungal populations, as well as bacterial and faunal communities (Cresswell, pers. comm.).

Some four months after dumping of the sludge a range of higher fungi appeared on the waste, and were evident for some fourteen months, when colonisation of the deposits by green plants commenced. From that time PMS was covered by plant growth.

With the exception of Coprinus comatus and Stropharia semiglobata, all of the higher fungi recorded on PMS deposits are normally associated with dead wood. This result would be expected with a material mainly derived from wood. Of the higher plants which colonised the solids Atriplex hastata, Chamaenerion angustifolium, Sambucus sp. and Sisymbrium officinale are pioneer colonisers of wasteground. Other species are associable with properties of PMS. Aster tripolium and Plantago sp. are indicative of saline conditions, whilst Phragmites communis, Rumex longifolius and Solanum dulcamara are associated with wet places. Cirsium arvense is common in grass, which covers aged PMS deposits.

Physical and chemical analyses of PMS samples suggest a number of reasons why the deposits are unsuitable for plant development for the first eighteen months. Of the essential elements required in any quantity by plants, nitrogen is the only nutrient below levels generally available in organic soils (Allen et al., 1974).

Plants normally utilise inorganic nitrogen sources, in particular nitrate, which accumulates in PMS following its deposition, but is always low in concentration. PMS deposits are very wet for over a year at any appreciable depth, as well as being very compact. Oxygen availability will be low under these conditions, and unfavourable to plant development (Meyer et al., 1973). Finally, deposited sludge is increasingly saline with depth, and most plant species are intolerant of high salt levels, which reduce water availability.

It is likely that higher fungi develop on PMS much earlier than plants due to their greater ability to exploit the organic source of nitrogen. In addition fungi are more able to develop in the surface region than plants which require rooting systems to some depth.

No micro-fungi were isolated from freshly excavated sludge. Conditions in the primary lagoon where settled solids compact beneath the liquid fraction are condusive to an anaerobic environment, which would eliminate fungal development. After the sludge has been dumped micro-fungi rapidly colonise it, reaching a population peak by six months. More fungal colonies were recorded on plates prepared from top core samples compared with the lower cores. In soil environments, fungal activity is generally greatest near to the surface due to a preponderance of organic matter in that region. This is not the case in the sludge deposits, and differences are most likely to be due to lower oxygen availability in wetter bottom cores. Cresswell (pers. comm.) recorded a similar effect on the numbers of aerobic bacteria.

The use of different agar media in fungal isolations had little qualitative effect on fungal species isolated, and numerical differences (appendices 4.1 to 4.3) probably reflect the nutrient status of the different media.

The majority of the fungal species isolated were heavily sporing types likely to be encountered using the soil dilution technique. The occurrence of the nematacious Arthrobotrys oligospora corresponded to the level of the nematode population recorded by Cresswell (pers. comm.).

Survey and analysis of PMS deposited at Kemsley showed the material to undergo a number of changes in composition, with a successional colonisation by higher fungi and plants. Populations of micro-fungi, and bacteria (Cresswell, pers. comm.) reached maxima some six months after sludge deposition. It was decided to see whether the ageing process recorded at Kemsley could be reproduced in a controlled situation, since systematic deposition of PMS would have to be implemented if it was adopted as a casing base. A feature of PMS dumped at Kemsley was the long time taken for the material to drain and dry out. Hayes (1975) found PMS was unsuitable as a casing up to one year from its deposition. Improvement of the drainage of PMS was studied as a means of accelerating the ageing of the sludge.

Controlled drainage of PMS was studied in modified plastic funnels at Aston University (figure 3.1). A bark/clinker filter medium was included in a second treatment with the aim of improving drainage.

Comparison of analyses of drained samples with those from sludge deposits at Kemsley showed drainage of PMS was improved in both funnels, but more so when the filter medium was present. The enhanced drainage accelerated the process of salt removal and drying out over the first three months in particular. The p.H. of PMS drained through the filter fell below 4.0 after twelve months, six months earlier than in deposits at Kemsley. The first higher fungi which occurred on PMS deposited at Kemsley, Nectria sp., Peziza badia and Stropharia semiglobata were recorded on sludge in both drainage treatments, after six months. Coprinus impexi stirps was also recorded occasionally after that time where the filter medium was used. Estimation of the numbers of micro-fungi in drained samples resulted in the isolation of all species encountered in sludge from Kemsley, with the exception of Arthrobotrys oligospora. Correspondingly, Cresswell (pers. comm.) did not isolate any nematodes from drained material. Comparison of fungal species in the drained samples shows inclusion of the filter medium accelerated the succession of micro-fungi. Parallel isolation of bacteria from drained sludges by Cresswell showed their numbers to attain similar maxima as in deposits at Kemsley, but at an earlier time.

The ageing of PMS under controlled conditions can be readily achieved, and accelerated to some extent by improving the drainage of the sludge. The wasteground on which PMS is deposited at Kemsley acts as an inoculum for nematodes occurring in the waste, whilst restricting drainage of excavated material. The isolation of thermotolerant and thermophilic fungi from PMS gives hope that microbial acceleration of the ageing process may follow drainage of stacked sludge.

Acceleration of the ageing processes in PMS could facilitate its earlier use as a casing medium in mushroom culture.

## 5.2 INITIAL EVALUATION OF PMS AS A CASING MEDIUM.

Analysis of PMS produced at Kemsley showed the material to be variable with time from deposition. The first culture trial with PMS as a casing medium demonstrated it to be unsuitable up to one year after deposition, compared with standard peat casing. Its inferiority was related to the residue of salts derived from pulp production, though material aged for four months was also difficult to form into a satisfactory structure due to its high moisture content. PMS samples eighteen, twenty and thirty six months in age gave yields comparable to peat. All PMS based casings produced fewer fruitbody initials than peat, whilst material four and twelve months from deposition produced a lower number of fruitbodies. These effects of the high salt contents of the casings are in agreement with the work of Flegg (1961b) who obtained fewer, larger fruitbodies and depressed yields by adding soluble salts to peat casing.

A problem was encountered with the presence of large numbers of nematodes on PMS casings, as well as the occurrence of a disease of the fruitbodies on material above eighteen months in age, caused by a Gliocladium sp. previously isolated from PMS in 4.3.2.

The results of this first trial demonstrated PMS is a satisfactory casing medium but must be allowed to age following deposition before it can be used successfully.

In view of productivity and the absence of Gliocladium disease, PMS eighteen months after deposition was selected as optimal for use as a casing.

Three different PMS deposits all eighteen months from deposition were shown to vary in composition but perform similarly as casing media. No significant differences in yield were found between the three deposits or in comparison with a peat control. The number of fruitbodies formed on PMS casing was again shown to be governed by its salt content, significantly fewer forming in comparison with peat when the salt level was high.

Measurement of casing salinity during culture showed soluble salts to increase in all casings, but at a greater rate in PMS than peat, when the sludge had an initially low salt content (figure 4.34).

Though PMS eighteen months after deposition can vary, its suitability as a casing medium is not affected. The variable salt content of the material can influence the number of fruitbodies which develop as well as the rate at which soluble salts accumulate in the casing. Flegg (1961a, b,c) demonstrated a natural salt accumulation in the casing layer, but doubted that it could have any detrimental effect on the crop. The same phenomenon was recorded with PMS, but to a greater degree than with peat, warranting further investigation. The occurrence of large numbers of nematodes and infection with Gliocladium sp. in the first two trials indicated PMS must be pasteurised before it can be safely employed as a casing.

Published results on the p H of the casing layer (see table 2.1) suggest a neutral or alkaline value is optimal for fruitbody production. This is achieved with peat by the addition of chalk. The p H of suitable PMS deposits can vary between 3.7 and 6.9, the latter being close to the recommended optimum. The addition of chalk to peat and PMS samples (appendix 4.6) showed PMS required half the quantity of chalk to achieve a casing p H of 7.5. Culture studies with neutralised and non-neutralised PMS casings of p H 5.5 and 6.9 showed PMS required the addition of chalk to give a satisfactory yield.

Chalk addition not only raises the p H to 7.5, but buffers the casing at that value. The p H of non-neutralised PMS declined during culture when initially 6.9 to 5.5, and remained at 5.5 in the other treatment. Edwards and Flegg (1952) and Allison and Kneebone (1963) recorded a gradual decrease in casing p H during culture, which is presumably due to production of acidic metabolites. Thus PMS requires chalk addition as a casing to maintain a satisfactory p H.

In the evaluation of chalk addition casings were pasteurised with a steam/air mixture before use. Since no nematodes or infection with Gliocladium sp. were recorded, the treatment was further investigated in combination with commercial-scale evaluation of PMS as a casing. Pasteurisation of PMS prior to or after the addition of chalk had no effect on its performance.

Two trials under commercial-scale growing conditions at W. Darlington and Sons Limited demonstrated PMS casing to perform equally as well as the standard peat casing. The pasteurisation of PMS casing with steam/air and the fumigant methyl bromide did not alter production compared with a non-pasteurised treatment.

Consequently steam/air was adopted as the standard pasteurisation treatment since methyl bromide is toxic to man, and the casing requires ventilation prior to use.

As in laboratory trials, the electrical conductivity of casings in the first commercial-scale trial increased with time after application. However, in this instance the salinity of the peat control increased more rapidly than in the PMS treatments, all casings having a similar conductivity fifty four days from application (appendix 4.10). Flegg (1961a) suggested the source of salts accumulating in casing was the compost, accumulation occurring due to the higher salinity of the compost compared to the casing. At Darlington's, compost is compressed into trays using a hydraulic press, achieving much higher ratios of compost to casing area than in laboratory trials at Aston. It may be that under conditions of high compost density salt accumulation is more rapid and differences between peat and PMS encountered in laboratory culture are not evident.

Estimation of the levels of available elements in casings in the first commercial trial (table 4.7) showed chloride, potassium, sodium and sulphate to be involved in salt build up. Salt accumulation in peat and PMS casings was investigated in a later series of experiments, and its effect on fruitbody formation assessed.

Having settled on steam/air as the standard pasteurisation treatment for PMS casing, treatment at 50°C. for sixty minutes, 65°C. for thirty and sixty minutes, and 80°C. for thirty and sixty minutes were compared to a non-pasteurised control in order to define the optimum regime. The p H and electrical conductivity of the casings were not altered by any of the treatments, whilst moisture content was slightly raised by treatment at 50°C. only. It is probable that at this low temperature sufficient steam condensed in the casing during treatment to raise the moisture level a little.

There was a reduction in the number of fruitbody initials formed prior to the first flush, and number of fruitbodies formed during the first flush on all pasteurised casings. Thereafter the yield from the different treatments was inversely proportional to the incidence of Gliocladium infection, which was severe on the 50°C. and non-pasteurised treatments, less severe on the 80°C. treatments, and absent on the 65°C. treatments.

The aim of a pasteurisation treatment is to eliminate organisms harmful to the crop, at the same time leaving a sufficiently large microbial population to prevent re-colonisation of the casing by harmful micro-organisms (Baker, 1967; Schisler and Wuest, 1971). Estimation of fungal numbers in the casings immediately after treatment showed pasteurisation at 50°C. for one hour did not greatly reduce the fungal population. Both treatments at 65°C. halved fungal numbers, and at 80°C. less than ten viable propagules per gramme survived. The pathogenic Gliocladium sp. was not isolated following treatment at 65°C. and 80°C..

Cresswell (pers. comm.) demonstrated reductions in total bacterial numbers at 65°C. and 80°C., and reduction of the numbers of Pseudomonad bacteria at all temperatures. She also recorded the elimination of nematodes in all pasteurised casings.

It seems probable that the occurrence of Gliocladium disease resulted from inadequate pasteurisation at 50°C., and re-colonisation of over-treated casing at 80°C.. The occurrence of the infection then affected the yield according to disease severity. The reduction in the number of initials and fruitbodies on pasteurised casings in the early stages may have been due to the initial depression in Pseudomonad numbers recorded by Cresswell, some of which are associated with fruiting (Hayes et al., 1969).

Treatment of PMS casing at 65°C. achieved the required aims of pasteurisation, and 65°C. for one hour was adopted as the standard regime. The use of non-pasteurised PMS can result in infection of the crop and reduced yield.

During the culture of A.bisporus, other fungi can develop in the casing layer and reduce the yield harvested. The plaster moulds Papulosporia byssinia and Scopulariopsis fimicola, cinnamon-brown mould, Ostracoderma sp., and Trichoderma spp. are among those most commonly encountered in the U.K.. By allowing prior development of nine of the micro-fungi isolated from PMS in PMS casing before its use it was shown that Gliocladium sp., Meria sp., Penicillium frequentans and Trichoderma viride could have a deleterious effect on the mushroom crop.

Gliocladium sp., Penicillium frequentans and Trichoderma viride were the most destructive, and are recorded as producers of antibiotics active against other fungi (Brian, Curtis, Hemming and McGowan, 1946; Grove and Brian, 1951; Weindling, 1937).

Re-colonisation and dominance of casing by fungi competitive with A.bisporus can follow over pasteurisation, and the results underline the need for satisfactory treatment of PMS. Of the four inhibitory species, only Meria sp. was isolated from PMS casing pasteurised at 65°C. for sixty minutes, showing this treatment to be effective in eliminating the most destructive fungi native to PMS.

## 5.3 STRUCTURAL REQUIREMENTS AND MANAGEMENT OF PMS CASING DURING CULTURE.

Having decided upon the optimum stage after its deposition, and initial preparation of PMS as a casing medium the structural requirements and management of PMS casing were investigated.

PMS as removed from deposits at Kemsley contains many large, solid aggregates, which must be broken down before it can be used as a casing base. In initial studies PMS was prepared before chalk addition by forcing the solids through a ten millimetre mesh. When a comparison was made between PMS forced through mesh sizes of five, ten and twenty millimetres and a peat control, the two finer grades yielded as well as peat. The coarse grade however, gave fruitbodies which were smaller, reducing the yield. In addition, difficulty was encountered in preventing the coarser PMS casing from drying out during culture.

It is probable that the excessive evaporation from coarse PMS casing can reduce the size of developing fruitbodies by reducing the quantity of water available in the casing. Fruitbodies were shown to take water from the casing layer by Reeve, Backes and Schramer (1959) and Flegg (1967), and contain approximately 90% moisture. Thus PMS should be prepared as a casing base to have a particle size range of ten millimetres to dust.

Review of the literature on casing structure (2.2.1) showed the casing must allow adequate ventilation of the compost. Flegg (1953) suggested reduced pore space in fine casings did not allow this. In addition, Edwards and Flegg (1953b) found fewer fruitbodies developed on peat, sand and vermiculite mixtures as pore space was reduced. Rao and Block (1963) obtained fruitbodies with long, tapering stipes from fine grade casing, whilst those formed on coarser casings had short, straight stipes. During culture studies, preparation of PMS by sieving through a ten millimetres mesh resulted in a coarser casing as the moisture content of the sample increased. Consequently, five different grades of PMS casing in the selected range of ten millimetres to dust were compared. Though a range of pore spaces from 37% to 51% was created, the casing water holding capacities and crop production, as well as dimensions of fruitbodies formed with the different grades were not altered significantly. Thus extreme alteration of the structure of PMS casing within the limits of ten millimetres to dust does not affect the performance of the material as a casing medium.

Assuming gaseous diffusion from the compost is not impaired, a large reserve of water in the casing layer is desirable to offset loss to the fruitbodies and by evaporation (section 2.2.1).

A high water holding capacity enables the casing to retain applied water, the use of marl (Bels-Koning, 1950) and vermiculite (Barnard, 1974) proving unsuccessful since they allowed water to drain into the compost and caused the mycelium to die-back. The ability to absorb a large volume of water was one of the reasons why peat was adopted as the conventional casing in the U.K.. The water holding capacity of PMS casing was shown to be high, just a few per cent. below that of peat (table 4.13).

The rate of water application to the casing during culture varies according to the preference of the individual grower. Maintenance of PMS casing at approximately 60%, 70% or 80% moisture did not alter the yield or number of fruitbodies harvested. However, with peat both parameters were depressed at the high and low watering rates. Uptake of water by plants is dependent on the water potential of the medium they are growing in (Meyer et al., 1973). A combination of the osmotic potential of the soil solution and the matric potential (attractive forces between soil particles and water molecules) determine the soil water potential. At saturation, soil water potential is only slightly less than zero, but is in the vicinity of - 15 bars at the permanent plant wilting point. As a soil dries out matric potential contributes the major fraction to the water potential. Comparison of moisture retention by peat and PMS casing (table 4.13) showed peat to retain increasingly more water than PMS as moisture suction was increased to p F 4.2 (15 bars). Consequently increasingly less water would be available to developing fruitbodies in peat casing compared with PMS as watering rate was reduced.

It may be that the low watering rate applied in these studies caused a significant reduction in water availability with peat but not PMS casing.

In the present study greater visual compaction of peat than PMS was recorded with a high watering rate, which would enhance reduction of pore space. Edwards and Flegg (1953b) recorded the development of fewer fruitbodies as casing pore space was reduced. A similar effect was noted here with peat, fewer, larger fruitbodies developing at the high watering rate. An attempt to assess the degree of casing compaction by sieving dried samples was affected by the condition of mycelial development in the casings. Irrespective of casing type heavier stranding and development of fewer fine hyphae were recorded as watering rate was increased. These results are in agreement with published work on stranding in A.bisporus (section 2.2.1).

In conclusion, PMS compares favourably to peat in its ability to hold water, whilst the yield from PMS is less likely to be affected by extremes in water application than with peat. The extent of moisture drainage into the compost and quality of fruitbodies harvested from the two casings do not differ, and are dependent on the rate of watering. As the quantity of water applied is increased, so is the moisture content of the fruitbodies with both peat and PMS casings. This underlines the importance of water availability in the casing layer.

In reviewing the published work on casing depth (2.2.1), it was concluded that cultural conditions including compost depth, aeration through the casing and growing house environment could alter the optimum depth for a given casing type. Under culture conditions in the laboratory cabinets a casing depth below three centimetres was inadequate for PMS. No advantage was obtained with depths greater than three centimetres, whilst quality of fruitbodies was reduced above that depth. As depth of PMS casing was increased so the moisture content of fruitbodies was also raised, suggesting more water was available to the fruitbodies. In commercial production peat casing is generally applied at a depth of three to five centimetres. Thus results indicate PMS to have a similar optimum depth, though the exact value could vary according to cultural conditions.

## 5.4 THE ROLE OF ELEMENTS AVAILABLE IN THE CASING IN FRUITING OF A.BISPORUS.

Review of the literature in 2.2.2 showed that a high salinity in the casing layer was damaging to fruitbody formation in A.bisporus. Flegg (1961a) was the first to demonstrate the natural accumulation of soluble salts in the casing during culture, the same phenomenon being recorded in the present study. The salt content of PMS was shown to influence its suitability as a casing, whilst samples of PMS considered satisfactory can have varying salinities. Consequently salinity in peat and PMS casings and its effect on cropping were compared.

Earlier experiments (4.5.4) had suggested chloride, magnesium, potassium, sodium and sulphate caused casing salinity to increase by their movement from the underlying compost. This was confirmed with the omission of chloride, there being a movement of available forms of the ions rather than any change in their status. Initially higher concentrations of sodium and sulphate in PMS casing caused these ions to accumulate at a slower rate than in peat, suggesting salt build up may be a passive process due to differences in compost and casing concentrations. The contribution of magnesium to increased casing salinity was shown to be small.

Though initial differences in salts levels between PMS and peat casings can affect the rate of accumulation of individual elements, the basic process of salt build up is the same in both materials.

PMS casing usually has an initially higher salt content than peat, which was related to a reduction in the number of fruitbody initials, and less frequently the number of fruitbodies which formed. Removal of soluble salts from PMS casing resulted in a increase in the number of initials which formed but no increase in fruitbody numbers. Initial reduction of salts in PMS to a level encountered in peat resulted in an increased rate of salt accumulation. Peat casing was shown to have a greater ability than PMS to bind water soluble ions.

Reduction of the salt content of PMS prior to its use can increase the number of fruitbody initials which form, but is unlikely to increase the number of fruitbodies, since salt accumulation merely occurs at a greater rate.

Under laboratory conditions accumulation of water soluble salts occurs more rapidly in PMS than peat, which may be explained by the greater ability of peat to bind soluble ions. This would cause the osmotic potential of the casing water in PMS to be higher than in peat. Studies under commercial-scale conditions suggested this difference between peat and PMS was overcome if the ratio of compost to casing area was increased, saturating adsorption sites in peat.

By the addition of individual and combined elements to PMS casing (4.7.3) it was possible to raise the casing salinity to a level which significantly reduced the yield. $30 \times 10^3$ µmhos/cm. was the lowest inhibitory conductivity recorded, but a value between $22 \times 10^3$ and $30 \times 10^3$ µmhos/cm. may reduce yield. A concentration of $30 \times 10^3$ µmhos/cm. or greater was recorded in PMS casings on several occasions during laboratory culture studies, but never in peat. However, the salinity of both peat and PMS was shown to exceed this value under commercial culture conditions, where the compost density to casing area ratio was much greater. The inhibitory effect of different elements was proportional to the casing conductivity created rather than any specific ion effect. Under saturated conditions the water potential of a substrate is governed by the dissolved solutes (Meyer et al., 1973), and when their concentration is high water is less available to developing plants. Since the vegetative mycelium readily develops in the extremely saline compost, the results suggest that high casing conductivities reduce yield by making water less available to developing fruitbodies.

Before a yield reduction occurred a reduction in the number of fruitbodies with increasing salinity was recorded.

However, those fruitbodies which did develop were larger in size, presumably due to the greater quantity of nutrients available. Between $22 \times 10^3$ and $30 \times 10^3$ µmhos/cm. there is a point where the reduction in fruitbody numbers is no longer offset by their increase in size and a reduction in yield occurs. The exact level at which this happens will depend upon the rate of nutrient assimilation, effects of any hormonal compounds which may be released by maturing fruitbodies (Urayama, 1956; Hagimoto and Konishi, 1959; Gruen, 1963) and adverse cultural conditions such as low humidity, which could cause premature opening of fruitbodies. In addition, commercial strains of A.bisporus are specified as producing differently sized fruitbodies, which may have an effect.

In conclusion the natural accumulation of soluble salts in the casing can attain a level which enhances the decline in cropping normally encountered due to nutrient exhaustion. This condition was recorded with PMS casing but not peat in laboratory trials, but results demonstrated that with the greater compost densities which can be achieved in commercial culture, damaging levels are also recorded in peat. The damaging effect of soluble salts is probably via the rendering of casing water less available to fruitbodies.

Comparison of different compost densities under a given area of PMS casing demonstrated that increased density does result in a greater rate of salt accumulation in the casing. This was reflected by a more rapid depletion of compost salt levels the less densely it was compressed. In this trial a point was not reached where casing salinity reached a value capable of reducing yield on the basis of weight of fruitbodies to weight of compost, although this was recorded in other trials.

It may be that compost used in other experiments was more saline, resulting in a greater rate of salt build up in the casing. It was noted that the number of fruitbodies harvested with a compost density of 0.6 Kg./L. was well below the expected value of three times the number from 0.2 Kg./L.. This suggests casing salinity had reached a point where a significant depression of fruitbody numbers occurred.

Whilst compost density does affect the rate of salt accumulation in the casing, the salinity of the compost will also be an important factor.

Replacement of casing and addition of ion binding agents to PMS were investigated as methods of combating salt accumulation, which could be employed in the commercial situation. Neither technique resulted in a significant increase in yield. The replacement of casing with fresh material at intervals during culture was successful in reducing casing salinity, but the time interval before appearance of the next flush was increased by approximately ten days, with the result that one more flush developed on the control. Consequently for any advantage in yield to be achieved the loss of one flush would have to be overcome. This was not achieved but yields were comparable to the control with casing replacements after the second flush. Replacement of the casing after the first flush significantly reduced subsequent yield. A.bisporus strain 649 was used in this trial, a strain which yields most heavily in first and second flushes. Disruption of the culture at the time of greatest production is therefore unsatisfactory.

Replacement of casing with fresh material achieves the required reduction in casing salinity during culture, but the yield harvested following replacement is not sufficiently increased to compensate for the loss of one flush in the normal period of culture. Production from the compost in the re-casing evaluation was low (figure 4.58), and extension of the cropping period with a more productive compost may give some benefit following casing replacement.

Salt accumulation in PMS casing was only slightly reduced following addition of clay minerals. The addition of montmorillonite caused the casing to form into large balls and become plastic in texture, which significantly reduced yield and number of fruitbodies. As montmorillonite is the clay with the greatest ability to bind ions, the use of clays does not seem very likely to improve PMS casing. A lower addition rate or the use of degraded illite, which has an affinity for potassium (Grim, 1962) may be more successful.

Salt levels in the casing above a layer of gypsum rose more rapidly than in the control, presumably as sulphate built up at a greater rate. An interesting effect of the gypsum layer was the formation of a large number of fruitbodies in contact with the layer below the casing surface, suggesting a physical stimulus. Mixing gypsum into PMS casing apparently gave very high conductivities, but no reduction in yield was recorded. It is likely that dilution of the casing during conductivity measurement brought into solution more of the solid gypsum than was actually dissolved in the casing. Gypsum seems to be of little value as an ion binding agent.

Studies utilising the flask culture apparatus described in 3.4.3 confirmed that the movement of ions from the compost to the casing is a passive process, occurring in the absence of A.bisporus and other micro-organisms. Thus the rate of salt accumulation will be dependent on the difference in salinity between the compost and the casing layer, as well as the extent of evaporative loss from the casing surface, which will tend to draw moisture up from the compost. The ions chloride, potassium, sodium, sulphate and to a lesser extent magnesium are responsible for the increase in casing salinity owing to their relative availability in the compost. Apart from these ions, only iron and phosphate were present in detectable levels in aqueous extracts of culture substrates. However, the results did not suggest these ions accumulated passively in the casing.

A marked increase in water soluble iron in the casing was noted with the axenic culture of A.bisporus, but no such increase where other micro-organisms were present, or with sterile substrates alone (refer to figures 4.63 and 4.67a). The results suggest iron is released into the casing by the mycelium of A.bisporus in pure culture, but when other micro-organisms are present they utilise this released iron, or there is no release. Where compost was inoculated with A.bisporus there was an increase in the level of water soluble iron by the time of casing (figures 4.67a and b). Since this occurred with axenic culture of A.bisporus it can be assumed the growth of this organism was responsible. Following casing water soluble iron in the compost declined in treatments two and four, though trends were less clear in the other two treatments.

Assuming A.bisporus removed excess water soluble iron previously released into the compost, then calculations in appendix 5.2 suggest iron taken up is in excess of the requirements for growth and fruiting, and the excess may be released into the casing layer.

Stanek (1976) suggested that bacteria intimately associated with hyphae of A.bisporus in the compost were dependent for growth to some extent on nutrients released by the hyphae. It is known that bacteria initiate fruitbody formation in the casing, and are presumably in close contact with the hyphae. There may be a similar dependence of bacteria in the casing for nutrients released from the hyphae, certain of the bacteria stimulating fruiting in return.

Water soluble phosphate in the compost was shown to be increased by the activity of A.bisporus in a similar fashion to iron. Following casing water soluble phosphate declined steadily in the compost, but was below the detectable level of 3 µg./g. dry material in the casing. The results suggest a similar solubilisation of phosphorous in the compost during its colonisation by A.bisporus, which then utilises the excess during its growth in the casing. Similar calculations to those performed for iron (appendix 5.3) suggest the levels of water soluble phosphate in the compost at casing could supply much of the phosphorous required for growth and fruiting of A.bisporus in the casing. The results do not account for phosphorous present in the hyphae at the time of casing. As phosphate tends to become fixed in insoluble forms which limit its availability to plants (Allen et al., 1974), the ability of A.bisporus to utilise the forms of phosphorous present in pasteurised compost, and the quantity of suitable phosphorous sources could limit crop potential.

## 5.5 GENERAL CONCLUSIONS.

Paper mill sludge (PMS) as removed from the primary treatment lagoon at the Kemsley mill of Bowaters U.K. Paper Company Limited is a sufficiently uniform material for the purpose of its utilisation as a casing medium in mushroom culture.

Following its deposition on wasteground the material undergoes an ageing process, during which it dries out, and its soluble salt content is greatly reduced by drainage and the leaching effect of rainfall. Drainage of PMS at the Kemsley site occurs slowly, whilst the ageing process can be reproduced and accelerated by improving drainage under controlled conditions.

PMS as deposited at Kemsley is unsuitable as a casing medium in the culture of Agaricus bisporus (Lange) Pilat until approximately eighteen months in age, after which time its use gives comparable yields to those from the conventional peat casing used in the United Kingdom. Similarly aged PMS deposits at Kemsley were shown to vary, and differences could have some effect on the number of fruitbodies harvested from PMS casing. Systematic dumping of PMS under conditions of improved drainage could be employed to provide a continuous supply of a uniform material, as well as reducing the time before it could be used as a casing medium.

The preparation of PMS for use as a casing involves shredding of the solids to give a particle size of ten millimetres to dust, followed by its neutralisation with chalk and pasteurisation to eliminate organisms harmful to the crop. These processes could readily be carried out at a treatment plant, which would provide the advantage to growers of a uniform, pre-prepared material.

The physical properties of PMS casing compare favourably to peat. It has a high water holding capacity and a structure not affected by management procedures. In addition its water retention properties may make it easier to manage than peat casing.

The phenomenon of salt accumulation in the casing previously recorded in the culture of A.bisporus, was shown to occur similarly in peat and PMS. The process was demonstrated to be a passive accumulation of solutes from the more saline compost, with chloride, potassium, sodium and sulphate being the principal ions involved. This salt build up in the casing was previously not thought to be damaging to crop production. The present study has shown that it can enhance the natural decline in cropping, with the ratio of compost density to casing area and compost salinity being important factors. The damaging effect of salt accumulation is due to the creation of high casing conductivities, the contribution of individual ions being proportional to the conductivity they create. High casing conductivity probably results in moisture tension, making water less available to developing fruitbodies.

Culture of A.bisporus in controlled environments via a flask culture technique suggested the availability of phosphate in the compost could limit crop potential, whilst studies on iron solubilisation in the casing may indicate a dependence of bacteria associated with fruiting on nutrients released from hyphae in the casing.

The continuous production of large quantities of PMS coupled with the potential to reduce the time before the material is suitable as a casing base, make it a realistic alternative to peat in the commercial culture of Agaricus bisporus.

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# APPENDICES

APPENDIX 3.1 VARIATION BETWEEN IDENTICAILY TREATED CULTURE UNITS DUE TO POSITION IN A CULTURE CABINET.

Variance ratio (F) at days after casing

| | Day 23 | | Day 30 | | Day 40 | | Day 50 | |
|---|---|---|---|---|---|---|---|---|
| | Rows | Columns | Rows | Columns | Rows | Columns | Rows | Columns |
| Number of initials formed prior to first flush | 0.1 | 0.5 | - | - | - | - | - | - |
| Number of fruitbodies harvested | - | - | 0.1 | 0.4 | 0.1 | 0.2 | 0.1 | 0.9 |
| Yield (Kg. fruitbodies/Kg. compost) | - | - | 0.7 | 0.8 | 0.3 | 1.2 | 0.2 | 2.0 |

Rows : gives variation between four rows of culture units

Columns : gives variation between eight columns of culture units

(Refer to figure 3.1)

For a significant difference between rows F must exceed 3.0

For a significant difference between columns F must exceed 2.5

## APPENDIX 3.2 COMPARISON OF METHODS FOR ESTIMATING ELECTRICAL CONDUCTIVITY.

Electrical conductivity at 20°C. ( μmhos/cm. x $10^3$ - dry weight basis)

| Substrate | Baker (1957) Conductivity measured on 10g. fresh sample raised to its water holding capacity. | Flegg (1958) Suspension of 5g. fresh sample in 10 ml. distilled water. | MAFF (1972) 20 ml. air-dry sample extracted with 50 ml. satd. $CaSO_4$ solution and filtered. |
|---|---|---|---|
| PMS 18 months after deposition | 6.3 | 6.8 | 4.5 |
| Peat casing | 2.9 | 3.1 | 2.6 |
| Spawn-run compost | 91.2 | 98.5 | 87.3 |

Values are the means of five separate determinations.

APPENDIX 3.3 OPERATING CONDITIONS FOR INDIVIDUAL ELEMENTS USING PERKIN-ELMER 460 ATOMIC ABSORPTION SPECTROPHOTOMETER.

| Element | Wavelength (nm.) | Slit width (nm.) | Flame type |
|---|---|---|---|
| Aluminium | 309.3 | 0.7 | Nitrous oxide/acetylene; Reducing (rich red) |
| Cadmium | 228.8 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Calcium | 422.7 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Chromium | 357.9 | 0.7 | Air/acetylene; Reducing (rich, yellow) |
| Copper | 324.8 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Iron | 248.3 | 0.2 | Air/acetylene; Oxidising (lean, blue) |
| Lead | 283.3 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Lithium | 670.8 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Magnesium | 285.2 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Manganese | 279.5 | 0.2 | Air/acetylene; Oxidising (lean, blue) |
| Mercury | 253.7 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Nickel | 232.0 | 0.2 | Air/acetylene; Oxidising (lean, blue) |
| Potassium | 766.5 | 2.0 | Air/acetylene; Oxidising (lean, blue) |
| Sodium | 589.0 | 0.7 | Air/acetylene; Oxidising (lean, blue) |
| Zinc | 213.9 | 0.7 | Air/acetylene; Oxidising (lean, blue) |

Light source : Hollow cathode lamp in all cases.

APPENDIX 4.1 MICRO-FUNGI ISOLATED AT 25°C. FROM TOP CORE PMS SAMPLES WITH TIME FROM DEPOSITION.

Number of propagules per gramme of fresh material ($\times 10^5$)

| Fungal species | Fresh sludge | | | 6 months | | | 18 months | | | 36 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MA | CA | EA | MA | CA | EA | MA | CA | EA | MA | CA | EA |
| Arthrobotrys oligospora | – | – | – | 30.0 | 10.0 | 10.0 | 25.0 | – | – | – | – | – |
| Aspergillus versicolor | – | – | – | – | – | – | 4.0 | – | 4.0 | 5.0 | – | – |
| Candida sp. | – | – | – | – | – | – | 4.0 | 2.5 | 1.5 | – | – | – |
| Cephalosporium curtipes | – | – | – | 7.5 | – | 10.0 | – | – | – | – | – | – |
| Gliocladium sp. | – | – | – | – | – | – | 11.5 | 9.0 | 10.0 | 15.0 | – | 5.0 |
| Ovulariopsis sp. | – | – | – | 7.5 | 2.5 | – | – | – | – | – | – | – |
| Penicillium frequentans | – | – | – | 5.0 | 7.5 | 4.0 | – | – | 5.0 | – | 10.0 | – |
| Sporothrix sp. | – | – | – | – | – | – | – | – | – | 10.0 | – | – |
| Stilbum sp. | – | – | – | – | – | – | – | – | – | – | – | 10.0 |
| Trichoderma viride | – | – | – | – | – | – | 24.0 | 5.0 | 20.0 | 12.5 | 10.0 | – |

MA = Malt agar
CA = Cellulose agar
EA = PMS extract agar

APPENDIX 4.2 MICRO-FUNGI ISOLATED AT 25°C. FROM BOTTOM CORE PMS SAMPLES WITH TIME FROM DEPOSITION.

Number of propagules per gramme of fresh material ($\times 10^5$)

| Fungal species | Fresh sludge | | | 6 months | | | 18 months | | | 36 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MA | CA | EA | MA | CA | EA | MA | CA | EA | MA | CA | EA |
| Arthrobotrys oligospora | – | – | – | 20.0 | 15.0 | 2.5 | 5.0 | – | – | – | – | – |
| Aspergillus versicolor | – | – | – | – | – | – | 5.0 | – | – | – | – | – |
| Cephalosporium curtipes | – | – | – | 1.5 | 10.0 | 5.0 | – | – | – | – | – | – |
| Gliocladium sp. | – | – | – | – | – | – | 8.5 | – | 1.5 | 7.5 | – | 7.5 |
| Ovulariopsis sp. | – | – | – | 1.5 | 5.0 | – | – | – | – | – | – | – |
| Penicillium frequentans | – | – | – | 15.0 | 5.0 | – | – | – | – | – | 5.0 | – |
| Sporothrix sp. | – | – | – | – | – | – | – | – | – | 5.0 | – | – |
| Trichoderma viride | – | – | – | 5.0 | – | – | 15.0 | 1.5 | 2.5 | 5.0 | 7.5 | 1.5 |

MA = Malt agar
CA = Cellulose agar
EA = PMS extract agar

## APPENDIX 4.3 MICRO-FUNGI ISOLATED AT 45°C. AND 52°C. FROM PMS SAMPLES 18 AND 36 MONTHS AFTER DEPOSITION.

| Fungal species | Number of propagules per gramme of fresh material ($\times 10^5$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 18 months 45°C. | | | 36 months 45°C. | | | 18 months 52°C. | | | 36 months 52°C. | | |
| | MA | CA | EA | MA | CA | EA | MA | CA | EA | MA | EA | CA |
| Top cores | | | | | | | | | | | | |
| Aspergillus fumigatus | 15.0 | 10.0 | 5.0 | 7.5 | 5.0 | 5.0 | – | – | – | – | – | – |
| Aspergillus sp. | 4.0 | – | – | 4.0 | – | – | – | – | – | – | – | – |
| Humicola grisea | – | – | – | – | – | – | 8.5 | 10.0 | – | 12.5 | 5.0 | 10.0 |
| Penicillium duponti | – | – | – | – | – | – | 8.5 | – | 1.5 | 20.0 | 1.3 | 4.0* |
| Scopulariopsis sp. | 5.0 | – | – | – | – | – | – | – | – | – | – | – |
| Bottom cores | | | | | | | | | | | | |
| Aspergillus fumigatus | 12.5 | 7.5 | – | 4.0 | 1.3 | – | – | – | – | – | – | – |
| Humicola grisea | – | – | – | – | – | – | 10.0 | – | 5.0 | 10.0 | 1.5 | 8.5 |
| Penicillium duponti | – | – | – | – | – | – | 1.5 | – | – | 12.5 | 2.5 | 5.0* |

MA = Malt agar
CA = Cellulose agar
EA = PMS extract agar
* = Clearing on cellulose agar

## APPENDIX 4.4 STATISTICAL ANALYSIS OF YIELD DATA FROM THE EVALUATION OF PMS AS A CASING WITH TIME AFTER ITS DEPOSITION.

| | Variance ratio (F) at days after casing | | |
|---|---|---|---|
| | Day 14 | Day 22 | Day 32 |
| Number of initials formed prior to first flush | 13.1* | - | - |
| Number of fruitbodies harvested | - | 10.1* | 11.2* |
| Yield (Kg. fruitbodies/Kg. compost) | - | 18.3* | 26.2* |

For a significant difference F must exceed 3.1.

* Denotes a significant difference at P = 0.001

## APPENDIX 4.5 THE VARIABILITY OF PMS AS A CASING MEDIUM - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | | |
|---|---|---|---|---|---|---|
| | Day 18 | Day 25 | Day 35 | Day 45 | Day 55 | Day 65 |
| Number of initials formed prior to first flush | 12.8*** | - | - | - | - | - |
| Number of fruitbodies harvested | - | 3.5 | 5.5* | 7.7** | 8.4** | 9.6** |
| Yield (Kg. fruitbodies/Kg. compost) | - | 1.4 | 0.9 | 1.2 | 1.9 | 1.7 |

For a significant difference F must exceed 3.5.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

*** Denotes a significant difference at P = 0.001

APPENDIX 4.6 p H OF PEAT AND PMS CASINGS WITH DIFFERENT RATIOS OF CASING BASE TO CHALK.

| Casing base | Ratio wetted substrate to 'Morden R' chalk by weight | | | | |
|---|---|---|---|---|---|
| | 2 : 1 | 4 : 1 | 6 : 1 | 8 : 1 | 10 : 1 |
| Peat | 8.1 | 7.4 | 7.2 | 6.9 | 6.0 |
| PMS 2 | 8.5 | 8.1 | 7.8 | 7.6 | 7.1 |
| PMS 3 | 8.0 | 7.9 | 7.6 | 7.4 | 6.9 |

Casing materials were raised to 70% moisture by weight before addition of chalk.

APPENDIX 4.7 NEUTRALISING REQUIREMENTS OF PMS CASING - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | |
|---|---|---|---|---|---|
| | Day 28 | Day 38 | Day 48 | Day 58 | Day 68 |
| Number of fruitbodies harvested | 1.7 | 2.9 | 2.5 | 3.1* | 5.2** |
| Yield (Kg. fruitbodies/Kg. compost) | 3.2* | 7.5*** | 3.1* | 8.7*** | 10.6*** |

For a significant difference F must exceed 3.0.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

*** Denotes a significant difference at P = 0.001

APPENDIX 4.8 STATISTICAL ANALYSIS OF YIELD DATA FROM THE FIRST COMMERCIAL-SCALE TRIAL.

| | Variance ratio (F) at days after casing | | | |
|---|---|---|---|---|
| | Day 24 | Day 34 | Day 44 | Day 54 |
| Yield (Kg. fruitbodies/Kg. compost) | 2.9 | 3.3 | 5.9 | 3.5 |

For a significant difference F must exceed 6.6.

APPENDIX 4.9 STATISTICAL ANALYSIS OF YIELD DATA FROM THE SECOND COMMERCIAL-SCALE TRIAL.

| | Variance ratio (F) at days after casing | | | |
|---|---|---|---|---|
| | Day 24 | Day 34 | Day 44 | Day 51 |
| Yield (Kg. fruitbodies/Kg. compost) | 8.8* | 3.2 | 0.2 | 0.9 |

For a significant difference F must exceed 3.5.

* Denotes a significant difference at P = 0.01

APPENDIX 4.10 CHANGES IN THE p H AND ELECTRICAL CONDUCTIVITY OF CASINGS IN THE FIRST COMMERCIAL TRIAL.

| TREATMENT | DAYS AFTER CASING | p H | ELECTRICAL CONDUCTIVITY (μmhos/cm.) |
|---|---|---|---|
| Peat control | 0 | 7.9 | 3429 |
| | 54 | 7.4 | 49000 |
| PMS non-pasteurised | 0 | 7.9 | 14286 |
| | 54 | 7.6 | 51143 |
| PMS steam/air pasteurised | 0 | 7.8 | 14857 |
| | 54 | 7.6 | 59429 |
| PMS methyl/bromide pasteurised | 0 | 7.8 | 14571 |
| | 54 | 7.5 | 50571 |

APPENDIX 4.11 DRY WEIGHT, p H AND ELECTRICAL CONDUCTIVITY OF PMS CASING FOLLOWING TREATMENT WITH DIFFERENT STEAM/AIR PASTEURISATION REGIMES.

| | Non-pasteurised control | 50°C. 60 mins. | 65°C. 30 mins. | 65°C. 60 mins. | 80°C. 30 mins. | 80°C. 60 mins. |
|---|---|---|---|---|---|---|
| % dry weight | 29.1 | 24.7 | 28.2 | 30.0 | 28.6 | 28.9 |
| p H | 7.8 | 7.7 | 7.7 | 7.6 | 7.9 | 7.7 |
| Electrical conductivity ($\mu$mhos/cm.) x $10^3$ | 5.6 | 5.4 | 5.9 | 5.7 | 5.9 | 5.8 |

APPENDIX 4.12 MICRO-FUNGI ISOLATED FROM PMS CASING PASTEURISED WITH DIFFERENT STEAM/AIR REGIMES.

| Treatment and fungal species isolated | Mean number of colonies/plate with serial dilution of sample (g. fresh casing/ml. agar) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| Non-pasteurised control | | | | | | | |
| Gliocladium sp. | Plates overgrown | | | 2.3 | - | - | No fungi isolated |
| Meria sp. | | | | - | 4.5 | 0.5 | |
| Mucor sp. | | | | 0.3 | - | - | |
| Trichoderma viride | | | | 5.0 | 1.3 | 0.3 | |
| 50°C. 60 minutes | | | | | | | |
| Aspergillus versicolor | Plates overgrown | | | - | 0.3 | - | No fungi isolated |
| Gliocladium sp. | | | | 2.5 | - | - | |
| Meria sp. | | | | - | 1.3 | 0.3 | |
| Mucor sp. | | | | 1.0 | 0.5 | - | |
| Trichoderma viride | | | | 3.3 | 1.3 | - | |

APPENDIX 4.12 (Continued)

| Treatment and fungal species isolated | Mean number of colonies/plate with serial dilution of sample (g. fresh casing/ml. agar) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| 65°C. 30 minutes | | | | | | | |
| Aspergillus versicolor | 1.5 | - | - | | | | |
| Aureobasidium sp. | 17.5 | 3.5 | - | | | | |
| Helicosporium sp. | 1.8 | - | - | No fungi isolated | | | |
| Meria sp. | 16.5 | 8.5 | 0.8 | | | | |
| Mycelium sterilium | 0.5 | - | - | | | | |
| 65°C. 60 minutes | | | | | | | |
| Aspergillus versicolor | 4.3 | - | | | | | |
| Helicosporium sp. | 3.3 | - | | | | | |
| Meria sp. | 0.8 | 1.5 | | No fungi isolated | | | |
| Mycelium sterilium | 1.0 | 0.5 | | | | | |
| 80°C. 30 minutes and 80°C. 60 minutes | No fungi isolated | | | | | | |

APPENDIX 4.13 STATISTICAL ANALYSIS OF YIELD DATA FROM PMS CASING TREATED WITH DIFFERENT STEAM/AIR PASTEURISATION REGIMES.

| | Variance ratio (F) at days after casing | | | | |
|---|---|---|---|---|---|
| | Day 17 | Day 26 | Day 36 | Day 46 | Day 51 |
| Number of initials formed prior to first flush | 2.4 | - | - | - | - |
| Number of fruitbodies harvested | - | 3.8* | 2.6 | 2.4 | 1.8 |
| Yield (Kg. fruitbodies/Kg. compost) | - | 3.5* | 3.5* | 4.8* | 5.7** |

For a significant difference F must exceed 3.1.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

APPENDIX 4.14 INTERACTION OF FUNGI ISOLATED FROM PMS WITH A.BISPORUS - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | |
|---|---|---|---|---|
| | Day 32 | Day 42 | Day 52 | Day 62 |
| Number of fruitbodies harvested | 1.7 | 5.8* | 6.7* | 6.6* |
| Yield (Kg. fruitbodies/Kg. compost) | 1.3 | 5.6* | 10.7* | 9.9* |

For a significant difference F must exceed 2.5.

* Denotes a signiciant difference at P = 0.001

APPENDIX 4.15 COMPARISON OF PMS CASING OF DIFFERENT GRADES WITH A PEAT CONTROL - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | | |
|---|---|---|---|---|---|---|
| | Day 19 | Day 30 | Day 40 | Day 50 | Day 60 | Day 70 |
| Number of initials formed prior to first flush | 0.1 | - | - | - | - | - |
| Number of fruitbodies harvested | - | 1.0 | 2.1 | 1.1 | 0.7 | 0.6 |
| Yield (Kg. fruitbodies/Kg. compost) | - | 1.8 | 2.7 | 7.0* | 2.8 | 2.6 |

For a significant difference F must exceed 3.3.

* Denotes a significant difference at P = 0.01

APPENDIX 4.16 STATISTICAL ANALYSIS OF YIELD DATA FROM PMS CASING SIEVED TO DIFFERENT GRADES IN THE RANGE TEN MILLIMETRES TO DUST.

| | Variance ratio (F) at days after casing | | | | |
|---|---|---|---|---|---|
| | Day 17 | Day 23 | Day 33 | Day 43 | Day 53 |
| Number of initials formed prior to first flush | 1.1 | - | - | - | - |
| Number of fruitbodies harvested | - | 0.8 | 0.4 | 0.5 | 1.6 |
| Yield (Kg. fruitbodies/Kg. compost) | - | 1.6 | 2.0 | 1.3 | 0.5 |

For a significant difference F must exceed 2.6.

APPENDIX 4.17 APPLICATION OF WATER AT DIFFERENT RATES TO PEAT AND PMS CASING DURING CULTURE - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 21 | Day 30 | Day 40 | Day 50 | Day 60 | Day 70 | Day 80 |
| Number of initials formed prior to first flush | 6.2** | - | - | - | - | - | - |
| Number of fruitbodies harvested | - | 3.1* | 5.6** | 5.7** | 6.1** | 4.9** | 5.1** |
| Yield (Kg. fruitbodies/Kg. compost) | - | 2.6 | 3.0* | 3.2* | 3.1* | 3.5* | 4.7** |

For a significant difference F must exceed 2.9.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

APPENDIX 4.18 % DRY WEIGHT OF FRUITBODIES HARVESTED FROM PEAT AND PMS CASING WATERED AT DIFFERENT RATES.

| Days after casing | Low watering rate (casing moisture ≃ 60%) | | Medium watering rate (casing moisture ≃ 70%) | | High watering rate (casing moisture ≃ 80%) | |
|---|---|---|---|---|---|---|
| | Peat | PMS | Peat | PMS | Peat | PMS |
| 30 | 7.6 (40) | 7.5 (40) | 7.1 (40) | 7.0 (40) | 6.5 (40) | 6.3 (40) |
| 40 | 8.1 (39) | 7.7 (37) | 7.4 (38) | 7.2 (36) | 6.7 (36) | 6.2 (27) |
| 50 | 7.9 (12) | 8.0 (36) | 8.1 (31) | 7.8 (37) | 6.9 (21) | 7.0 (28) |
| 70 | 8.8 (7) | 9.2 (12) | 8.3 (8) | 8.3 (12) | 7.6 (4) | 7.1 (12) |
| 80 | 9.6 (13) | 9.1 (5) | 8.7 (11) | 8.9 (10) | 8.0 (6) | 7.4 (4) |

Figures in parentheses are the number of fruitbodies dried.

APPENDIX 4.19 REFLECTANCE READINGS FROM FRUITBODIES HARVESTED FROM PEAT AND PMS CASINGS WATERED AT DIFFERENT RATES.

| Days after casing | Low watering rate (casing moisture ≃ 60%) | | Medium watering rate (casing moisture ≃ 70%) | | High watering rate (casing moisture ≃ 80%) | |
|---|---|---|---|---|---|---|
| | Peat | PMS | Peat | PMS | Peat | PMS |
| 30 | 90% (40) | 92% (40) | 87% (40) | 89% (40) | 51% (40) | 56% (40) |
| 80 | 87% (13) | 90% (5) | 88% (11) | 86% (10) | 59% (6) | 57% (4) |

Figures in parentheses give the number of fruitbodies tested.

APPENDIX 4.20 PERCENTAGE BY WEIGHT OF DRY PEAT AND PMS CASINGS RETAINED BY DIFFERENT MESH SIZES FOLLOWING WATERING OF THE CASINGS AT DIFFERENT RATES FOR SEVENTY DAYS FROM APPLICATION.

| Sieve mesh size | Low watering rate (casing moisture ≃ 60%) | | | | Medium watering rate (casing moisture ≃ 70%) | | | | High watering rate (casing moisture ≃ 80%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peat | | PMS | | Peat | | PMS | | Peat | | PMS | |
| | U.S. | L.S. | U.S. | L.S. | U.S. | L.S. | U.S. | L.S. | U.S. | L.S. | U.S. | L.S. |
| 5.6 mm. | 9.5 | 19.6 | 26.6 | 32.8 | 39.7 | 25.6 | 48.4 | 21.3 | 62.2 | 39.6 | 46.6 | 39.0 |
| 2.0 mm. | 32.8 | 21.7 | 33.5 | 39.0 | 27.9 | 29.7 | 38.4 | 59.8 | 23.0 | 33.5 | 42.2 | 45.1 |
| <2.0 mm. | 57.8 | 58.7 | 39.9 | 33.3 | 32.4 | 44.8 | 13.2 | 18.9 | 14.9 | 27.1 | 11.3 | 16.0 |

U.S. Upper surface of casing (0 - 1 cm. depth).

L.S. Lower surface of casing (2 - 3 cm. depth).

APPENDIX 4.21 DRY WEIGHT CONTENTS OF PEAT AND PMS CASINGS AND UNDERLYING COMPOST FOLLOWING APPLICATION OF WATER AT DIFFERENT RATES FOR SEVENTY DAYS.

| | Low watering rate (casing moisture ≃ 60%) | | Medium watering rate (casing moisture ≃ 70%) | | High watering rate (casing moisture ≃ 80%) | |
|---|---|---|---|---|---|---|
| | Peat | PMS | Peat | PMS | Peat | PMS |
| Casing | 37.6% | 36.9% | 32.6% | 29.7% | 22.4% | 21.9% |
| Compost 1 cm. depth | 37.1% | 37.2% | 32.2% | 30.9% | 26.3% | 23.6% |
| Compost 5 cm. depth | 47.6% | 46.4% | 45.4% | 47.6% | 24.3% | 21.0% |
| Compost 10 cm. depth | 62.4% | 53.0% | 49.2% | 44.8% | 28.5% | 22.0% |

Values are the means from four replicates.

APPENDIX 4.22 STATISTICAL ANALYSIS OF YIELD DATA FROM PMS CASING APPLIED AT DEPTHS OF ONE TO SIX CENTIMETRES.

| | Variance ratio (F) at days after casing | | |
|---|---|---|---|
| | Day 28 | Day 38 | Day 48 |
| Number of fruitbodies harvested | 3.2* | 8.2** | 4.0* |
| Yield (Kg. fruitbodies/Kg. compost) | 3.6* | 2.6 | 5.3* |

For a significant difference F must exceed 3.1.
* Denotes a significant difference at P = 0.05
** Denotes a significant difference at P = 0.01

APPENDIX 4.23 STATISTICAL ANALYSIS OF YIELD DATA FROM PEAT AND PMS CASINGS COMPARED WITH PMS CASINGS LEACHED PRIOR TO APPLICATION.

| | Variance ratio (F) at days after casing | | | | |
|---|---|---|---|---|---|
| | Day 19 | Day 25 | Day 35 | Day 45 | Day 55 |
| Number of initials formed prior to first flush | 4.9* | - | - | - | - |
| Number of fruitbodies harvested | - | 1.7 | 0.4 | 1.4 | 1.2 |
| Yield (Kg. fruitbodies/Kg. compost) | - | 3.3 | 0.8 | 1.3 | 1.6 |

For a significant difference F must exceed 3.5.

* Denotes a significant difference at P = 0.05

APPENDIX 4.24 STATISTICAL ANALYSIS OF YIELD DATA FROM PMS CASING FOLLOWING THE ADDITION OF VARIOUS IONS.

| | Variance ratio (F) at days after casing | | | | | |
|---|---|---|---|---|---|---|
| | Day 18 | Day 25 | Day 35 | Day 45 | Day 55 | Day 65 |
| Number of initials formed prior to first flush | 13.8*** | - | - | - | - | - |
| Number of fruitbodies harvested | - | 3.3* | 7.2** | 4.8* | 7.5** | 6.6** |
| Yield (Kg. fruitbodies/Kg. compost) | - | 1.9 | 8.1** | 4.6* | 8.0** | 6.7** |

For a significant difference F must exceed 3.2.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

*** Denotes a significant difference at P = 0.001

APPENDIX 4.25 STATISTICAL ANALYSIS OF YIELD DATA FROM A STANDARD APPLICATION OF PMS CASING TO DIFFERENT DENSITIES OF COMPOST.

| | Variance ratio (F) at days after casing | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 15 | Day 20 | Day 30 | Day 40 | Day 50 | Day 60 | Day 70 |
| Number of initials formed prior to first flush | 8.1* | - | - | - | - | - | - |
| Number of fruitbodies harvested | - | 8.3* | 23.2** | 25.0** | 11.1* | 16.7** | 26.5** |
| Yield (Kg. fruitbodies/$m^2$ casing) | - | 52.7** | 178.7** | 114.5** | 35.4** | 100.4** | 142.9** |
| Yield (Kg. fruitbodies/Kg. compost) | - | 0.1 | 2.3 | 0.3 | 0.1 | 0.5 | 1.0 |

For a significant difference F must exceed 4.3.

* Denotes a significant difference at P = 0.01

** Denotes a significant difference at P = 0.001

APPENDIX 4.26 REPLACEMENT OF PMS CASING WITH FRESH MATERIAL AT INTERVALS DURING CULTURE - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | |
|---|---|---|---|---|---|
| | Day 32 | Day 42 | Day 52 | Day 62 | Day 72 |
| Number of fruitbodies harvested | 1.0 | 2.5 | 3.0 | 1.6 | 1.1 |
| Yield (Kg. fruitbodies/Kg. compost) | 2.4 | 12.9** | 11.8** | 4.5* | 4.2* |

For a significant difference F must exceed 3.0.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.001

APPENDIX 4.27 AMENDMENT OF PMS CASING WITH SALT BINDING AGENTS - STATISTICAL ANALYSIS OF YIELD DATA.

| | Variance ratio (F) at days after casing | | | | | |
|---|---|---|---|---|---|---|
| | Day 17 | Day 30 | Day 40 | Day 50 | Day 60 | Day 70 |
| Number of initials formed prior to first flush | 10.7*** | - | - | - | - | - |
| Number of fruitbodies harvested | - | 3.2* | 14.3*** | 14.8*** | 12.9*** | 6.6** |
| Yield (Kg. fruitbodies/Kg. compost) | - | 2.5 | 4.0* | 3.1* | 3.7* | 2.2 |

For a significant difference F must exceed 3.0.

* Denotes a significant difference at P = 0.05

** Denotes a significant difference at P = 0.01

*** Denotes a significant difference at P = 0.001

APPENDIX 5.1 VARIATION IN THE COMPOSITION OF PMS SAMPLES TAKEN FROM DIFFERENT AREAS OF THE PRIMARY TREATMENT LAGOON.

| Parameter | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Mean of samples | Standard deviation between samples |
|---|---|---|---|---|---|---|---|
| Dry weight (g.%) | 7.97 | 7.80 | 7.10 | 7.43 | 7.42 | 7.54 | 0.30 |
| Electrical conductivity ($\mu$mhos/cm. x $10^3$) | 378 | 384 | 342 | 353 | 368 | 365 | 16 |
| p H | 6.30 | 6.30 | 6.30 | 6.35 | 6.30 | 6.31 | 0.02 |
| Ash content (% of dry wt.) | 17.90 | 17.70 | 17.50 | 18.10 | 17.30 | 17.70 | 0.28 |
| Organic nitrogen (% of dry wt.) | 0.31 | 0.26 | 0.26 | 0.27 | 0.25 | 0.27 | 0.02 |
| Organic carbon (% of dry wt.) | 42.40 | 41.31 | 43.19 | 44.28 | 41.32 | 42.50 | 1.14 |

Standard deviation (Bishop, 1971) = $\sqrt{\left[\frac{\sum d^2}{n}\right]}$ $\sum d^2$ = Sum of squares of deviations from sample mean.
n = Number of samples.

## APPENDIX 5.2 BALANCE OF WATER SOLUBLE IRON IN COMPOST AND CASING DURING FLASK CULTURE STUDIES.

(Refer to figure 4.67a)

1. 200 g. fresh compost at casing = 80 g. dry compost
2. In treatment 4, 23 µg. water soluble Fe/g. dry compost at casing = $1.8 \times 10^{-3}$g. Fe
3. 13.0 g. fresh wt. fruitbodies harvested by day 44 = 1.3 g. dry wt. = $0.1 \times 10^{-3}$g. Fe
4. In treatment 2, the change in water soluble Fe concentration in compost = 10 µg./g. = $0.8 \times 10^{-3}$g. Fe
5. Therefore $0.8 \times 10^{-3}$g. Fe utilised by mycelial development
6. 50 g. fresh casing applied = 15 g. dry casing
7. In treatment 2, increase in water soluble Fe in casing by day 44 = 28.5 µg./g. = $0.4 \times 10^{-3}$g. Fe
8. Therefore water soluble iron in compost is in excess of iron utilised by the mycelium and fruitbodies, whilst half of the water soluble iron taken up by the mycelium could be released into the casing

Note Fe content of fruitbodies estimated as 8.5 mg./100 g. dry weight (Watt and Merrill, 1963; F.A.O., 1972)

APPENDIX 5.3 ESTIMATION OF THE CONTRIBUTION OF WATER SOLUBLE PHOSPHATE IN COMPOST TO THE PHOSPHOROUS REQUIREMENT OF FRUITBODIES AND MYCELIUM DURING FLASK CULTURE STUDIES.

(Refer to figure 4.68a)

1. 200g. fresh compost at casing = 80g. dry compost
2. In treatment 4, 1700 $\mu$g. water soluble $PO_4$/g. dry compost at casing = 0.136g. $PO_4$ = 0.044g. P
3. Concentration of water soluble $PO_4$ in compost at day 44 = 250 $\mu$g./g. = 0.0065g. P
4. Therefore a maximum of 0.038g. water soluble P were utilised by mycelium and fruitbodies
5. 13g. fresh wt. fruitbodies were harvested by day 44 = 1.3g. dry wt. = 0.013g. P
6. Therefore 0.025g. P were available to the mycelium
7. In treatment 2, change in water soluble $PO_4$ concentration = 1250 $\mu$g./g. = 0.033g. P utilised by mycelial development
8. Levels of water soluble $PO_4$ in compost at casing could supply much of the P utilised by development of mycelium and fruitbodies in the casing

Note P content of fruitbodies estimated as 1.0% of the dry weight (Watt and Merrill, 1963; F.A.O., 1972)