STUDIES ON FUNGAL DECOMPOSITION OF SAWDUST IN LAGOS LAGOON

by

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C E R T I F I C A T I O N

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ABSTRACT

The fungi isolated from Lagos Lagoon and decomposing sawdust in the lagoon included Aspergillus flavus, Aspergillus fumigatus, Aspergillus giganteus, Aspergillus niger, Aspergillus tamarii, Candida famata, Cladosporium oxysporum, Curvularia geniculata and Debaryomyces hansenii. Others were Fusarium solani, Geotrichum candidum, Mucor haemalis, Paecilomyces variotii, Penicillium species Trichoderma aureoviride and an unidentified basidiomycete.

The fungal population of Lagos Lagoon varied with season, the population being highest in the rainy season (March - October) when there was increased organic matter content (188 mg/l), greater turbidity (0.3m) and reduced salinity (2.0‰).

There was a positive correlation between suspended matter and fungal count of the lagoon. The relation between salinity and turbidity with fungal count on the other hand showed a negative correlation.

Most of the fungi isolated from decomposing sawdust in the lagoon were euryhaline, growing at a wide range of salinities (0-34‰).

Sporulation of the fungal isolates decreased with increase in salinity, the decrease being more marked in media containing over 1.7% NaCl (equivalent to 50% sea water in total ionic concentration).

Spore germination of the fungal isolates was poor in sterile distilled water and lagoon water of 21.0‰ salinity.

Conidia of Aspergillus flavus, Cladosporium oxysporum and Trichoderma aureoviride germinated in aqueous extracts of Khaya

ivorensis sawdust, whereas only the conidia of Trichoderma aureoviride germinated in aqueous extracts of the sawdust of Mitragyna ciliata and Triplochiton scleroxylon. Although germination of conidia of Aspergillus giganteus was inhibited in aqueous sawdust extracts of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon, addition of nutrients to the extract improved germination.

Mineral salts - $CaCl_2$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$ and NaCl had varied effects on conidia germination of Aspergillus flavus, A. giganteus, Cladosporium oxysporum and Trichoderma aureoviride. While KH_2PO_4 and NaCl individually supported germination of A. giganteus, $CaCl_2$ and $MgSO_4.7H_2O$ inhibited germination of all the fungi.

Cellulolytic activities of Aspergillus flavus, A. giganteus, A. niger, Cladosporium oxysporum, Geotrichum candidum, Paecilomyces variotii and a basidiomycete isolate were observed when grown on colloidal cellulose medium. In addition, Aspergillus giganteus and Cladosporium oxysporum caused significant weight loss of Whatman No. 1 filter paper in mineral salts medium. However, media containing cellulose extracted from sawdust did not support growth of the fungi.

There was a significant loss of weight of sawdust inoculated with Aspergillus flavus, A. giganteus, Cladosporium oxysporum and Trichcderma aureoviride. Addition of glucose or peptone to the medium enhanced fungal utilization of sawdust.

Lignolytic activities were not detected when the fungal isolates were inoculated onto media containing lignin.

TABLE OF CONTENTS

I. INTRODUCTION

The Lagos Lagoon is part of the West African lagoon system along the Guinea Coast (Hill and Webb, 1958). The lagoon system consists principally of three large areas of water: the Nakone Lagoon in the Republic of Benin, and the Lagos and Lekki lagoons in south-west Nigeria (Olaniyan, 1969).

The productivity of this lagoon system is of immense importance to communities living along the lagoons and some of the factors influencing productivity have been studied. One of the major biological problems facing the Lagos Lagoon is pollution. Ekundayo (1977) considered the major causes to consist of untreated sewage discharged into the lagoon at a site near the Carter Bridge, sawdust from sawmills located along the lagoon, petroleum products (crude petroleum and petrochemicals), washings from tankers and ships at Apapa terminals, waste products from various industries, and waste heat from cooling waters of Ijora power plants. Because of the high frequency and large quantities of some of the pollutants (Akpata, 1975), and because of the restricted nature of the lagoon, pollution of Lagos Lagoon is rather severe.

Biologically, pollution resulting from waste disposal may influence living organisms in a number of ways, and the individual effect may not be the same for all phyla (Perkins, 1974). Perkins (op. cit.) postulated that suspended solids may have ecological influence on organisms in several ways such as,

(a) mechanical or abrasive action (e.g. clogging of gills, and irritation of tissue)

(b) blanketing action or sedimentation

(c) reduction of light penetration

(d) availability as a surface for growth of bacteria and fungi

(e) adsorption and/or absorption of various chemicals by particulate matter making them inaccessible to micro-organisms, and

(f) reduction of temperature fluctuations.

Nelsen-Smith (1973) stated that the usual and most obvious effect of pollution is the reduction in diversity of organisms in the habitat. The organisms which can tolerate the toxicant proliferate and may then exclude species over which they have been given competitive advantage.

Solid wood wastes are known to be highly destructive to habitats in coastal waters where logging activities are practised (NAS, 1975). Floating logs moving to the mills exude substances into the water and create pollution

problems. Leachates from wood and bark, especially of certain species of trees, are quite toxic to most marine organisms, particularly in their egg and larval stages (Waldichuk, 1977). Wood retained for long periods in water decay releasing decomposition products into the water. Logs are constantly floated on the Lagos Lagoon to sawmills at Okobaba where they are sawn into various products. Some wood-attacking fungi present on trees in the forest will be transported on the timber. The survival of these terrestrial species in the lagoon depends on the physico-chemical conditions of the new environment. The decomposition activity of the surviving species will be augmented by that of wood decomposing mycoflora of the lagoon.

Many of the fungi found growing on wood in British waters occur only occasionally and are often restricted to certain areas (Byrne, 1971). The factors affecting the distribution of fungi in water include salinity, temperature and the availability of suitable substrate (Alexopoulos, 1962; Byrne and Jones, 1975; Dzawachiszwili, Landau, Newcomer and Plunkett, 1964; Gilbert and Lovelock, 1975; Johnson and Sparrow, 1961; Mitchell, 1968; and Ritchie, 1957, 1959 and 1960). Johnson and Sparrow (1961) reported that the less saline the water, the more numerous and diversified are the

Phycomycetes. Hence they observed that Pythium and saprolegniaceous fungi are collected in estuarine water of less than $5^{o}/oo$ salinity. Alexopoulos (1962) reported that some species of Saprolegniaceae usually present in freshwater may live in brackish water of estuaries as well, when salinity does not exceed $0.28^{o}/oo$. Borut and Johnson (1962) observed that spore germination of some terrestrial fungi (e.g. Gliocladium fimbriatum Gilman et Abbott and Trichoderma lignorum (Tode) Harz) except Curvularia pallescens Boedijn, was inhibited in sea water. Harrison and Jones (1975) observed that culture media containing 10-20% sea water inhibited asexual sporulation and also prevented the normal development of sexual structures in some saprolegniaceous fungi. A repression of asexual sporulation in Saprolegnia ferax (Gruith) Thuret occurred in brackish water ($7.09^{o}/oo$ salinity) even though there was excellent vegetative growth. Normal oogonial development occurred only in zero and ten per cent sea water for most species while Protoachlya paradoxa Coker produced oospheres in 20% sea water. Harrison and Jones (1975) suggested that both types of reproduction possibly respond to a similar series of events which in part is governed by environmental parameters such as concentration of inorganic salts in the culture medium. Saprolegniaceous fungi appear to be more restricted

in their ecological habitats than the Pythiaceae, being highly sensitive to even very low concentrations of salt. A concentration of 0.3% NaCl is sufficient to prevent normal development. This may explain why so few members of the genera Saprolegnia, Achlya, Isoachlya and Protoachlya are found in the sea (Johnson and Sparrow, 1961).

Byrne and Jones (1975) investigated the effect of salinity and temperature on spore germination in several terrestrial, freshwater and marine fungi. Spore germination in terrestrial fungi decreased with increasing salinities and the inhibitory effect was more marked at low temperatures. The sporangiospores of Mucor haemalis Wehimer did not germinate at salinities above 40% at any temperature. Lowering the temperature lowered percentage germination and reduced salinity tolerance. Ascospores of Chaetomium globosum Kunze ex Fr. (a terrestrial Ascomycete) were also intolerant of increasing salinities and did not germinate at salinities above 60% sea water. The terrestrial imperfect fungus, Penicillium notatum Westling showed maximum conidia germination at 30°C on distilled-water agar. Percentage germination decreased to 25% with increasing salinities up to 100% sea water agar. Penicillium notatum, however, tolerated salinity better at 10°C and germination was high on 100% sea water agar.

Byrne and Jones (1975) also studied freshwater fungi. For Tetracladium setigerum (Grove) Ingold (a Deuteromycete), a broad tolerance to salinity at 20°C and 25°C was observed. At 15°C, conidia germination was little affected by salinity up to 40% sea water agar, above which inhibition of spore germination was observed. At 10°C total inhibition occurred on 40% sea water agar. The marine fungi investigated by Byrne and Jones (1975) showed a wide tolerance to salinity at each temperature. A 100% ascospore germination was recorded for Corollospora maritima Werdermann at each salinity (zero - 100% sea water agar) at 10°C and 15°C. For Zalerion maritimum (Linder) Anastasiou (a Deuteromycete), spore germination was significantly reduced by low temperature and 100% sea water. Byrne and Jones (1975) concluded that several factors may be responsible for the exclusion of terrestrial fungi from the marine environment. Such factors include the effect of salinity on vegetative growth and reproduction, and non-availability of suitable substrates for colonization. High rate of germination may explain why some fungi are so successful in colonizing freshly submerged timber in the sea (Meyers and Reynolds, 1960).

Marine fungi have been classified into two major categories, stenohaline and euryhaline, depending upon their tolerance to salinity (Johnson and Sparrow, 1961; Ritchie, 1960). Stenohaline fungi are those which require

a salinity of sea water (above 30°/oo) whereas euryhaline fungi tolerate a wide range of salinity.

The activity of the fungi would be greatly influenced by oxygen available in the habitat. Fundamentally, the concentration of oxygen dissolved in sea water is inversely dependent upon salinity and temperature, and is normally 80% of the concentration in freshwater at the same temperature (Perkins, 1974). In polluted estuaries, the oxygen concentration falls and the medium may become anaerobic under extreme conditions. In an unpolluted estuary, the decomposition of organic matter can produce local oxygen deficiencies in the bottom water.

Olaniyan (1969) observed that in the lagoons of southwest Nigeria, oxygen content is affected by physical factors such as temperature and salt content, both being inversely proportional to oxygen content. According to him, the western transect of the lagoon consists of narrow creeks, and low oxygen concentration was observed in the rainy season in this area whereas a high oxygen content was recorded in the eastern transect at the same time due probably to the presence of open water. He further stated that the abundance of phytoplankton and consequential high photosynthetic activity could have raised the oxygen content of the water in the eastern transect. The level of dissolved oxygen in an estuary is definitely of fundamental importance

to its inhabitants, although response of individual species may be highly variable.

Nelsen-Smith (1973) observed that high temperatures and low oxygen tensions combine to increase the effect of any unfavourable discharge in an estuary. Raabe (1965) determined the Biochemical Oxygen Demand (B.O.D) of lignin in natural waters and the extent of its degradation under these conditions, and also explored the inter-relationships of B.O.D. and degradation. He reported that the B.O.D of a stream is related to the concentration of carbohydrates, the fraction of lignin components oxidizable, and possibly to other slowly decomposable organic matter. He observed that the components of the first stage of the B.O.D were indicative of the oxidation of wood sugars and other readily decomposable materials.

Although it is considered that estuarine water temperatures are controlled largely by the temperatures of the sea and run-off water, this is strictly true only for those small estuaries which have little development of sand and mud flats. Furthermore, lagoon water temperature is influenced more by insolation than by air temperature (Olaniyan, 1969). He observed that dry season and rainy season temperatures overlap by only $0.5^{o}C$ in the lagoon system of south-west Nigeria. Because the difference between

the lowest and highest temperatures is only about 5°C, Olaniyan (1969) thought that it is unlikely that such temperature difference will constitute an important ecological factor in a tropical lagoon. Temperature changes may influence the permeability of cell walls to their surrounding water. Consequently Verwey (1957) advised the study of influence of temperature changes on osmoregulation. However, this is of no importance in tropical waters which show very narrow temperature range throughout the year. Thus, problems of osmoregulation will be influenced more by salinity and the presence of a wide variety of anions and cations in water. Freyre (1973) observed that changes in Mg/Ca ratio caused a reduction in the number of the freshwater Argentine silverfish (Basilichthys bonariensis).

A total of 82 fungi have been observed growing on various substrates in British waters (Fazzani, Furtado, Eaton and Jones, 1975). Of the number, 58 species comprising 40 Ascomycetes, 16 Deuteromycetes and two Basidiomycetes have been found growing on wood. Furthermore, results of studies of lignicolous fungi found on Scots pine (Pinus sylvestris Linn.) test-blocks from two different sites in the United Kingdom indicated a greater range of marine lignicolous fungi at the less polluted estuarine site which had a higher mean annual temperature. Six species each of

Deuteromycetes and Ascomycetes were isolated. Lignicolous freshwater fungi included several aquatic Hyphomycetes and a range of terrestrial species. Also, the dissimilarity in the fungal flora observed on test-blocks in cooling towers and nearby river intake points was attributed mainly to water temperature and aeration differences between the two environments. Jones (1972) observed many aquatic Hyphomycetes growing on timber. Collins and Willoughby (1962) working on a lake in England isolated some members of Phycomycetes, Saprolegnia species, as well as a large number of fungi having septate mycelium without reproductive structures or clamp connections.

Dzawachiszwili, Landau, Newcomer and Plunkett (1964) observed a definite progressive decrease in colony size of a fungus as the NaCl content of the media increased. Also, the intensity of pigmentation of some fungi, of the genera, Monosporium and Trichophyton increased with increasing NaCl concentration in the culture medium. The substitution of certain osmotically active materials isotonic with sea water, other than NaCl, has been observed to result in a pattern of growth similar to that obtained with media prepared with artificial sea water (Ritchie and Jacobsohn, 1963). Some marine fungi grow optimally on distilled water medium (Gustafsson and Fries, 1956) and some terrestrial isolates grow well on sea water media; however, tolerance

to salinity is influenced by temperature and nutrition in some fungi (Ritchie, 1959).

Mitchell and Wirsen (1968) found that a specific antagonistic microflora was developed in response to the inoculation of non-marine fungus into natural sea water. The predominant micro-organisms were bacteria and they apparently killed the non-marine fungus by enzymatically lysing the cell walls. Thus, it would appear that several factors - physical, chemical and biological - influence the viability of fungi in a tropical lagoon.

After sawing the timber at the sawmills, sawdust which is the waste product is dumped into the Lagos Lagoon. The reduction in dissolved oxygen content due to the increased organic matter (sawdust) in Lagos Lagoon, as well as seasonal variation in rainfall may influence the survival, growth and reproduction of fungi decomposing sawdust in the lagoon. The microflora involved in the decomposition of the sawdust probably include, as in the case of the unsawn log, terrestrial forms infecting the trees in the forest before logging, and also micro-organisms introduced during processing of the logs at the sawmills. Furthermore, the logs may be affected by freshwater species invading the floating logs in rivers which convey them to the lagoon, as well as by brackish water species which are normal inhabitants of the lagoon.

There are several reports (Basham, Mook and Davidson, 1953; Gibbs, 1978; Santra and Nandi, 1975; White, 1953) on fungal infection of forest trees. These fungi constitute part of the microflora decomposing logs and possibly sawdust in the Lagos Lagoon. Olofinboba (1969) reported fungal infection of timber, Antiaris africana Engl., within ten days after felling. Umezurike (1969) isolated several fungi including Botryodiplodia theobromae Pat., Fusarium solani (Mart.) Sacc., and Aspergillus chevalieri (Mang.) Thom et Church from decaying wood of Bombax buonopozense P. Beauv. Compared with the terrestrial habitat, less attention has been paid to fungi decomposing submerged wood in the aquatic environment and a thorough scrutiny of the available literature yielded very limited report on work concerning sawdust decomposition.

Meyers and Reynolds (1960) observed an abundance of Ascomycetes and Deuteromycetes on Basswood (Tilia americana Linn.) and yellow pine (Pinus palustris Mill) panels submerged in marine water. They observed that dominant fungal biotas present on submerged wood differ between localities. Fazzani, Furtado, Eaton and Jones (1975) reported the frequent occurrence of basidiomycete infections (white and brown rots) in cooling tower timbers and isolated species of Poria, Peniophora and Lentinus. They also reported that the fungal flora on wood from the sea water-influenced towers

showed marked differences from those of brackish and river waters. Kirk (1969) submerged panels of birch (Betula papyrifera Marsh) and pine (Pinus ponderosa Dougl.) in an estuary for three to five months and isolated the aquatic Hyphomycetes, Clavatospora stellatacula Kirk, Flagellospora sp. and Tetraploa aristata Berk et Br. Goter (1978) recovered ten wood-inhabiting marine fungi from submerged wood on the Atlantic coast of South Africa. The flora comprised six Ascomycetes (Ceriosporopsis halima Linder, Corollospora maritima Werdermann, Halosphaeria appendiculata Linder, H. mediosetigera Cribb et Cribb, H. circumvestita Kohl., Lulworthia floridana Meyers), one Basidiomycete (Nia sp.) and three Deuteromycetes (Alternaria maritima Sutherland, Culcitalna achraspora Meyers et Moore, Zalerion maritimum (Linder) Anastasiou). He reported that some marine fungi prefer to colonize certain kinds of wood. A wider range of fungi was trapped on wood of Eucalyptus saligna Sm. than on wood of Pinus patula Schlecht et Cham.

Various methods have been employed in studying aquatic wood decomposing fungi. Baiting method (e.g. Johnson and Sparrow, 1961; Meyers and Reynolds, 1960) involves anchoring untreated wood panels in the inshore neritic zone. They suggested the use of thicker panels for long periods of submersion. The submerged panels are transferred onto

laboratory media to observe fungal growth. The fungus is reinoculated onto wood to observe symptons of decay. Fazzani, Furtado, Eaton and Jones (1975) reported that the periods of submersion of the test-blocks depend upon the ambient temperature of the water and to certain extent the organisms under study. In temperate waters marine fungi will colonize timber in about 12 - 18 weeks (Jones, 1963) but in tropical waters colonization may occur in about one week (Jones, 1968).

Staining procedure has been used to study wood decomposition. Santra and Nandi (1975) subjected wood blocks of Casuarina equisetifolia Forst. to fungal attack by Fomes durissimus Lloyd and examined the wood at intervals. The blocks were softened by boiling in water first and then kept in a mixture of glycerine and methylated spirit (1:1). Differential staining by safranin and picro-aniline-blue (Cartwright, 1929) and safranin-fast green were found to be very suitable for detecting the fungal hyphae from the woody host tissues. The changes that take place in lignin and cellulose contents of wood were studied qualitatively by microchemical tests on the basis of relative colour intensities in sound and partially decayed wood. Lignin was tested by 'Maule treatment' showing lighter red colour - reactions in partially decayed wood while sound wood showed darker red colour - reactions. There was gradual reduction in

lignin content in both primary and secondary walls of all the wood elements. Staining reactions with iodine - potassium - iodide indicated the presence of cellulosic materials mostly in the secondary walls of different wood elements of normal wood. During the process of decay, cellulose was also consumed from the wood elements but the destruction was much less severe than that suffered by the lignified tissues. Safranin - fast green staining reactions also corroborated the above findings. Fomes durissimus consumes mainly the lignified portion of the host wood but simultaneous utilization of cellulosic materials also takes place during the process of decay.

Leightley and Eaton (1976) carried out a direct study of infected wood by means of a scanning electron microscope. Meyers and Reynolds (1960) studied changes in sterilized thin portions of wood inoculated with Halosphaeria mediosetigera (Ascomycetes) by means of a microscope. The fungus formed cavities in tracheid walls, caused the soft rot of wood and degraded cell wall lignocellulose material. Umezurike (1969) inoculated sterile wood blocks of Bombax buonopozense with fungi and studied hyphal growth. He reported that in sections of the blocks, hyphae of Gliocladium roseum Bain. were chiefly in ray cells.

Aspergillus chevalieri and Trichoderma lignorum grew longitudinally in the lumina of vessels and tracheids. Only Botryodiplodia theobromae and Fusarium solani showed substantial growth both longitudinally and radially. B. theobromae penetrated the tracheid walls frequently and its hyphae developed appressoria. Elongated cavities with pointed ends were also observed in the secondary walls of tracheid cells in the neighbourhood of vessels heavily infected with B. theobromae. The cavities were frequently in chains and contained hyaline hyphae that stained deeply. The walls of heavily infected vessels were almost completely disorganized. These observations indicated cellulolytic ability of B. theobromae growing in the wood. Santra and Nandi (1975) performed microscopic studies of wood decay of Casuarina equisetifolia by Fomes durissimus. They reported that in early stages of decay, hyphae were mostly in the lumina of the vessels and almost absent in other wood elements. These hyphae of diameter 2.5 - 5.0 µm., were slightly thick-walled and light yellow in colour. The hyphae passed through the lumina of vessels without apparent resistance in the longitudinal direction forming narrow branches (1.0 - 1.5 µm diameter) in lateral directions which invaded the adjoining wood elements. Hyphae penetration was mainly through pits and also directly through the

cell wall, often forming bore-holes. The smooth and rounded contours of the bore-holes and pits through which hyphae passed radially indicated that cell wall penetration is due to enzymic activity of the fungus and not mechanical force. As decay progressed the walls of the pits were broken down due to depletion of lignin. Ultimately, almost all the tissues of wood became affected, and vessels became filled with wefts of hyphae, with considerable accumulation of gum in ray cells and in some fibres. With further progress of decay, as a result of cell wall thinning and tissue disintegration, lysis of host tissues started and neighbouring lysis zones often became united, forming spongy areas between the wood fibres. Successful decomposition of the chemical components of cell wall is due to the hyphae growing inside the lumen of the cell. The initial delignification process resulted in demasking of the microfibrils; the process slowed down at the complex middle lamella but this was ultimately destroyed at a very advanced stage of decay.

The role of procaryotes in the biodegradation of timber has long been considered negligible. Within the last decade, however, their importance has been recognized especially in conditions unfavourable to fungi, e.g. water logging and anaerobic situations, which favour bacterial attack (Gilbert and Lovelock, 1975). Bacillus polymyxa has been implicated

in the attack of timber (Fazzani, Furtado, Eaton and Jones, 1975). B. polymyxa was reported to hydrolyse pectin and hemicellulose compounds abundant in the middle lamella of cell walls. Information on the activities of bacteria in the breakdown of timber in aquatic environments is, however, limited.

Browning (1963) reported that wood (sawdust) generally consists of carbohydrates which include cellulose, hemicellulose, pectic substances and sugar; lignin which is an aromatic material having phenolic hydroxy groups; and extraneous components including proteinaceous substances, fats and resins (Fig. 1). Cellulose and lignin are the major wood components making up about 60% and 30% of wood respectively, depending on the type of wood. Gymnosperm wood has higher lignin content than angiosperm wood.

Cellulose is a carbohydrate composed of glucose units bound together in a long, linear chain by β linkages at carbon atoms 1 and 4 of the sugar molecule. Thus, cellulose is a polymer of β, 1 - 4 glucose units; it is crystalline and insoluble in water. Because of its specific physical structure cellulose is resistant to most enzymes and chemical reagents (Waksman, 1957). Cellulose is resistant to various oxidizing agents and is hydrolysed only by concentrated acids. However, cellulose is broken down by some bacteria, fungi, actinomycetes and lower animals

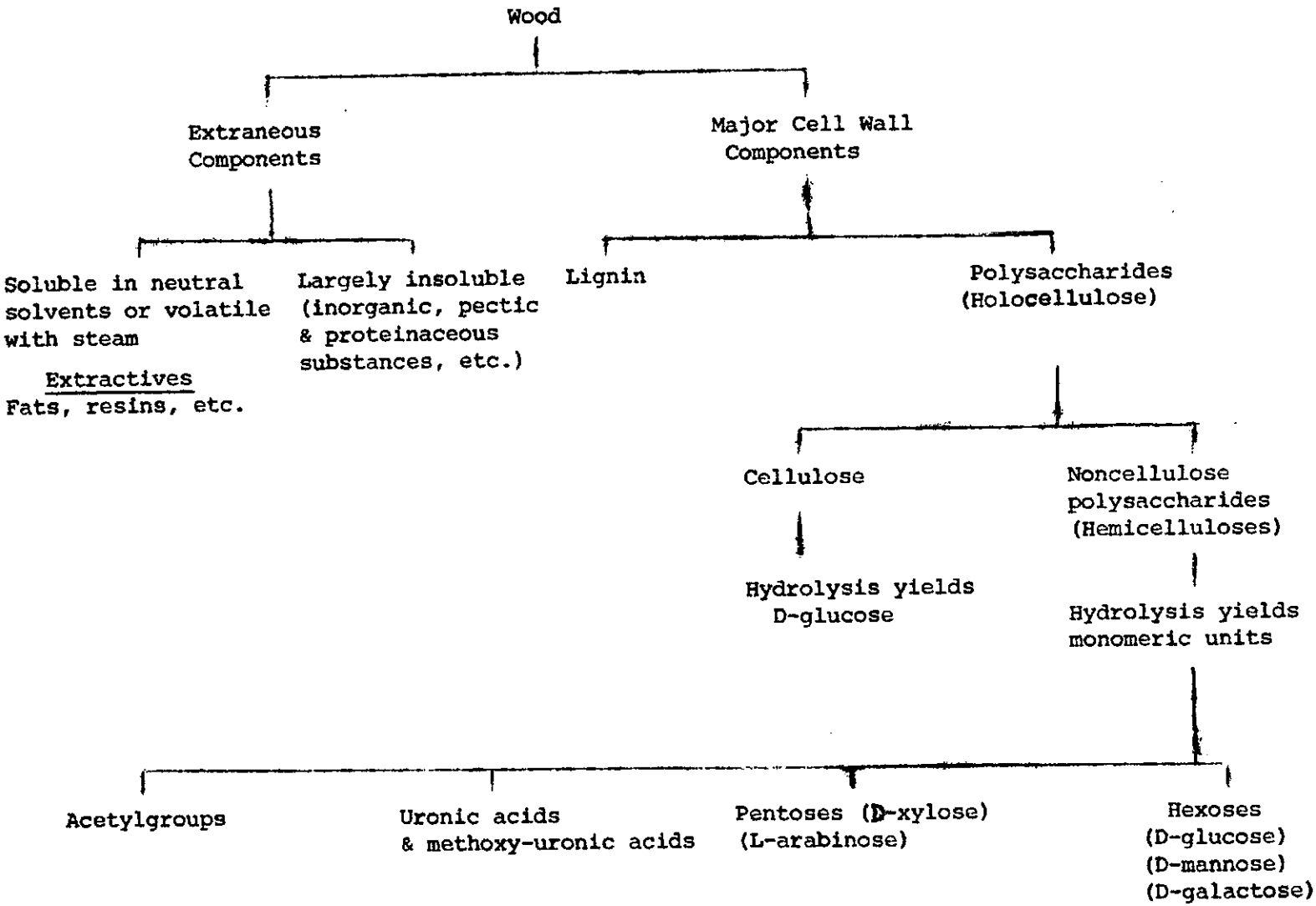


Fig. I. Idealized outline of wood components.
From Browning, B.L. (1963). The Chemistry of Wood.

(Protozoa and insects).

The initial step in cellulose destruction is the enzymatic hydrolysis of the polymer (Alexander, 1961). The enzyme or enzyme complex has been named cellulase. Cellulase catalyzes the conversion of insoluble cellulose into simple, water-soluble products. The hydrolysis leads to the liberation of mono- or di -saccharides. Then aerobes metabolize the simple sugar to CO_2 while anaerobes produce organic acids and alcohols. The microbial cell is impermeable to the cellulose molecule so the organism must excrete extracellular enzymes in order to make the carbon source available. The extracellular catalyst acts hydrolytically converting the insoluble material to soluble sugars which penetrate the cell membrane. Cellulases catalyze the hydrolysis of a class of compounds known as β-1, 4 - glucosides. They include cellobiose, cellotriose, cellotetraose, and cellulose, the molecules containing, two, three, four and many glucose units respectively (Alexander, 1961). He also reported that cellobiose is not susceptible to enzymatic cleavage by cellulases. The enzyme (cellobiase) which breaks down cellobiose is a β - glucosidase that is, an enzyme which hydrolyses β - glucosides. (Jurasek, Colvin and Whitaker, 1967) reported that the Commission on Enzymes of the

International Union of Biochemistry (1961) listed 'cellulase' as the trivial name for β-1, 4 - glucan 4 - glucanohydrolases, i.e. enzymes which hydrolyse β-1, 4 - glucans at linkages which are not restricted to terminal linkages. Thus, enzymes which degrade β - 1, 4 - glucans by splitting off glucose residues would be designated β - 1, 4 - glucohydrolases while those enzymes splitting off cellobiose would be β - 1, 4 - glucan cellobiohydrolases. Also, β - glucosidase is the trivial name for β - D - glucosidase glucohydrolases; these are a heterogenous group of enzymes. The systematic name specified one but not all the operations of an enzyme. For example, a hydrolase enzyme may also have transferase activity. This type of classification leaves room for cellulase enzymes to split other types of linkages.

Levinson, Mandels and Reese (1951) suggested that cellulose hydrolysis to the cellobiose level requires two steps. One is a reaction catalyzed by an enzyme, C_1, which yields long, linear chains composed of many glucose units. The enzyme of the second step, Cx, hydrolyses the cellulose derivatives formed by C_1. Some fungi produce Cx cellulase and β - glucosidase, while others are able to produce C_1 cellulase as well (Reese, Siu and Levinson, 1950). On the other hand, certain fungi, including most members of the

Phycomycetes, produce only β - glucosidase (Reese and Levinson, 1952). Many phytopathogens produce a distinct C_1 cellulase which hydrolyses cellulose to soluble polyanhydroglucose chains which can be acted upon by other kinds of cellulases, Cx cellulase and cellobiase (β - glucosidase) respectively (Olutiola and Cole, 1977). The hydrolysis of insoluble cellulose by culture filtrates of Botryodiplodia theobromae was enhanced by the presence of a C_1 enzyme (Umezurike, 1970). Trichoderma viride Pers. et Fr. was reported to be able to produce C_1 enzyme (Selby and Maitland, 1967). The production of C_1 enzyme has been associated with several fungi including Chrysosporium pruinosus (Gilman et Abbott) Carmichael (Rosenberg, 1978) and Aspergillus tamarii Kita (Olutiola and Cole, 1976). However, Talaromyces emersonii Stolk synthesized the C_x enzyme but not C_1 enzyme (Oso, 1978); this was evident as the fungus could not degrade filter paper whereas its cell-free filtrate hydrolysed soluble carboxymethylcellulose to reducing sugars. Romanelli, Houston and Barnett (1975) reported that Sporotrichum thermophile Apinis hydrolysed 56% of the cellulose in a culture medium in 72 hours. Li, Flora and King (1965) fractionated the cellulase of Trichoderma viride into three parts: (a) a 'hydrocellulase' fraction with high activity toward Avicel (microcrystalline

cellulose), (b) an 'endoglucanase' fraction which is essentially random in action, and (c) an 'exoglucanase' fraction, which unlike the endoglucanase degrades the soluble oligoglucosides (cellobiose) by cleaving glucose units from the non-reducing end. Eriksson (1978) studied the enzyme mechanisms involved in cellulose hydrolysis by the rot fungus, Sporotrichum pulverulentum Novobranova. Five endoglucanases were produced by the fungus. The individual endo - 1, 4 - β - glucanases have been identified and characterized with respect to their molecular activities. An exo - 1, 4 - β - glucanase has also been identified in the culture solution of S. pulverulentum and has been separated from the endoglucanases. A strong synergistic response between the five endo - 1, 4 - β - glucanases and the exo - 1, 4 - β - glucanase was observed when these enzymes were allowed to degrade dewaxed cotton. No synergism was observed if an acid swollen cellulose was used. This supports the hypothesis that the endoglucanases attack at random the β - 1, 4 - glucosidic linkages along the cellulose chain, producing non-reducing termini which are the substrate for the exoenzyme. One or several 1, 4 - β - glucosidases also belong to the hydrolytic group of cellulose-metabolizing enzymes. This enzyme regulates the flow of glucose from cellulose. Apart from the hydrolytic enzyme discussed so far, Eriksson (1978) also isolated an oxidative

enzyme of importance for cellulose degradation. The oxidizing enzyme is a haemoprotein and can oxidize cellobiose to cellobionic acid.

Lignin is a three dimensional random polymer of phenylpropane alcohols (Rosenberg, 1978). Unlike cellulose, it is an aromatic macromolecule. Gould (1966) reported that spruce lignin consists of repeating units each comprising 16 guaiacylpropane units and two p - hydroxyphenylpropane units. The repeating unit has four carbon atoms which can be sulphonated without previous hydrolysis. The repeating units are bound to each other by ether bonds that are partially broken by hydrolysis. They are further bound to carbohydrates by four ether bonds. Oglesby, Christman and Driver (1967) stated that softwood (gymnosperm or conifer) has higher lignin content than hardwood (angiosperm). Lignin of softwood contains primarily guaiacylpropane units while hardwood contains both guaiacyl-propane and syringylpropane units. Also, angiosperm wood is more susceptible to attack due to the location of lignin in the middle lamellae of the tissue rather than concentration in the cell walls as is the case with the lignin of conifers. Lignin would thus be more available to the exoenzymes of the fungi degrading angiosperm wood.

Gadd (1957) stated that the main components of wood - carbohydrates and lignin - are both too highly polymeric to be capable of penetration of cell membrane. In order that such substances be made available to participate in the metabolism of a micro-organism, they first have to be broken down by extracellular enzymes. The primary or first attack is carried out by rot fungi (Basidiomycetes). Only at a later stage does the wood become available to other fungi and bacteria which complete the wood destruction (Gadd, 1957). The action of enzymes on lignin is an oxidative process unlike cellulose decomposing enzymes which are mainly hydrolytic. Fahraeus (1952) reported that the activity of lignin decomposing fungi is connected with the ability of the mycelium to form enzymes of the phenol oxidase type. He isolated two enzymes, tyrosinase and laccase, from Hymenomycetes. Both enzymes catalyze the oxidation of di- and poly-phenols with two hydroxyl groups in the ortho position e.g. catechol, pyrogallol and gallic acid. Laccase had no action on monophenols but tyrosinase catalyzed their oxidation. While tyrosinase does not attack hydroquinone and p - phenylenediamine, laccase catalyzes their oxidation. Tyrosinase and laccase probably have different physiological functions. Wood-rotting fungi secrete mostly laccase. But different parts of Psalliota bispora Lange form different phenol oxidases (Fahraeus, 1952).

Numerous Basidiomycetes secrete phenol oxidase when cultivated on ordinary laboratory media (Lindeberg and Holm, 1952). Polyporus species appear to form laccase but no tyrosinase when grown in ordinary laboratory media, thus, Fahraeus (1952) suggested that enzymatic activity may depend on the kind of medium used. Also, laccase is formed in large quantities after growth has ceased and autolytic processes set in, then certain compounds, e.g. amino acids are liberated which may promote production of laccase. Fahraeus (1952) further reported that laccase occurred both intra - and extra-cellularly, whereas tyrosinase was found only in intracellular enzymes. Boletus versipellis Fr. contained tyrosinase. Lactarius deliciosus Fr. contained laccase both in fruit bodies and in mycelium. Marasmius species produce tyrosinase and laccase enzymes (Fahraeus, 1952). Oglesby, Christman and Driver (1967) reported that a few species of fungi producing phenolases also produce a peroxidase. They suggested that lignolytic enzymes may also function in the detoxification of a number of materials in heartwood which would otherwise cause uncoupling of oxidative phosphorylation. Peroxidases are appropriate enzymes for fungi living in heartwood because they do not compete with intercellular substrate oxidations for oxygen.

Several fungi, all Basidiomycetes, have been associated with wood decomposition or lignin oxidation. Basham, Mook and Davidson (1953) investigating the decay of balsam fir (Abies balsamea (Linn.) Mill.) isolated several Basidiomycetes including Stereum sanguinolentum Alb. et Schw. ex Fries, Corticium galactinum (Fries) Burt, Fomes pini (Thore) Lloyd, Polyporus balsameus Peck and Poria subacida PK. Sacc. White (1951) observed Corticium galactinum as an important pathogen of forest trees in Canada. White (1953) reported that Fomes pini was responsible for 90% of all losses in trunks and butts of living trees of white pine (Pinus strobus Linn.). Gadd (1957) reported that lignin is consumed by Polyporus abietinus, Trametes pini (Thore) Fr. and Fomes annosus Fr.

Gadd (1957) recognized two groups of rot fungi: destructive rots which consume carbohydrates almost exclusively, and corrosive rots which consume both carbohydrate and lignin. He reported that when Polyporus abietinus and Fomes annosus develop on wood, the lignin is consumed at about the same rate as the carbohydrates. It may be that the aromatic substances in the wood have been altered so that they do not appear as lignin. Gadd (1957) reported that monomeric aromatic compounds e.g. vanillin are formed during breakdown of lignin. Guaiacol, tetra - guaiaquinone and catechol originate when butt rot attacks

the lignin in spruce wood. He proposed the following path of action of phenolases: vanillin → chatecol → guaiacol → tetraguaiaquinone. Hence these substances are found in rot damaged wood. He explained the development of colour in rot damaged wood to be due to the formation of humin-like substances from lignin. Gadd (1957) stated that monomeric aromatic compounds are toxic to the fungi thus the function of the enzyme system is to neutralize these substances by means of a process of oxidation and polymerization resulting in the huminization of wood. Craigie and McLachlan (1964) noted the production of humic substances by micro-organisms growing on simple carbon sources. Considering the size of humic acid molecules, it seems that only humic precursors, probably phenolic or quinonoid in nature, are formed inside the cell. Following excretion, these intermediates could be oxidatively polymerized with the resultant formation of humic acid. Gadd (1957) stated that in wood decay, there is weight loss due to the consumption of carbohydrates by the fungi as well as disappearance of part of the lignin in the form of water-soluble humin like substances.

Extracellular phenolic enzymes, e.g. laccase, bring about oxidation and polymerization of simple aromatic compounds (Gadd, 1957). Also, since cell membrane does not

transmit high-polymer substances, it means that the new substances formed are no longer of any importance for the intercellular metabolism of the fungi. Therefore, Gadd (1957) assumed that the main function of laccase is not to serve the assimilation of nutrients, but to neutralize some substance which is toxic to the fungi. He suggested that some fungi by use of other enzymes could utilize part of the lignin.

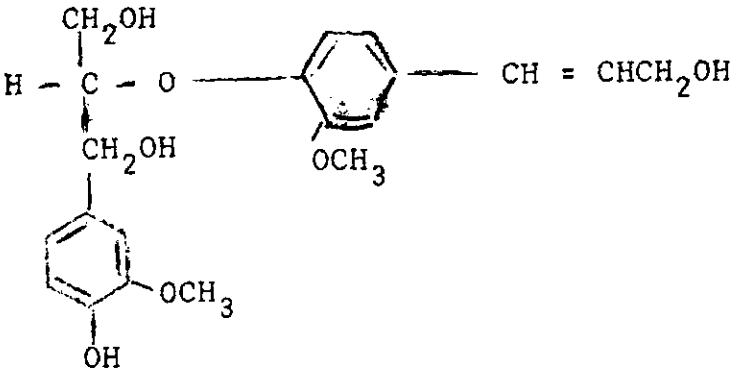
Work by Isikawa, Shubert and Nord (1963a,b) indicates that the first step in lignin biodegradation is an attack on the guaiacyl-type monomeric units, resulting in the liberation of guaiacylglycerol-β- coniferyl ether. Also produced are a number of phenylpropanoid compounds presumably produced from guaiacylglycerol-β- coniferyl ether, and existing as intermediates in subsequent degradation to benzyl derivatives which are ultimately oxidized to CO_2 and/or compounds in the citric acid cycle. On the pathways employed by micro-organisms for converting guaiacylglycerol-β - coniferyl ether units to simple benzene derivatives, one possibility is conversion to vanillic acid through coniferaldehyde and vanillin. Alternatively, guaiacylglycerol - β - coniferyl ether units may be converted via guaiacyl - pyruvic acid, β - oxyconiferyl alcohol and coniferyl alcohol to methoxy gentistic acid. From the low yields

of degradation products obtained and oxygen uptake data observed, it is apparent that lignolytic organisms do not terminate their degradation of the substrate with the formation of guaiacylpyruvic acid or vanillic acid (Sarkanen, 1967). He further indicated how protocatechuic acid and/or gentistic acid could be formed from 3-methoxy - 4 - hydroxyphenylpyruvic acid and then subsequently degraded along pathways consistent with theories of aromatic ring metabolism (Fig.2). Gadd (1957) observed that lignin in decayed wood has solubility characteristics quite different from those of undamaged wood and that lignin is transformed during decay. He also showed that a demethoxylation takes place during wood decay.

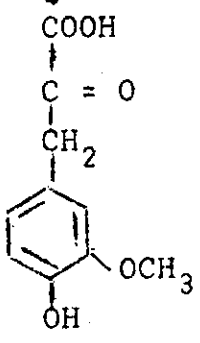
The direct methylation and oxidative degradation of the lignin preparations, however, gave only degradation acids derived from originally phenolic units (Chang, Cowling and Brown, 1975). Also the molecular weight of lignin *in situ* is much higher than in the isolated lignins, and its content of free phenolic hydroxyl groups may be even less than in the preparations. Chang *et al.* (1975) stated that the content of free phenolic hydroxyl groups in lignin is a highly significant result of the process of dehydrogenative polymerization by which the lignin has been formed. Their

FIG. 2

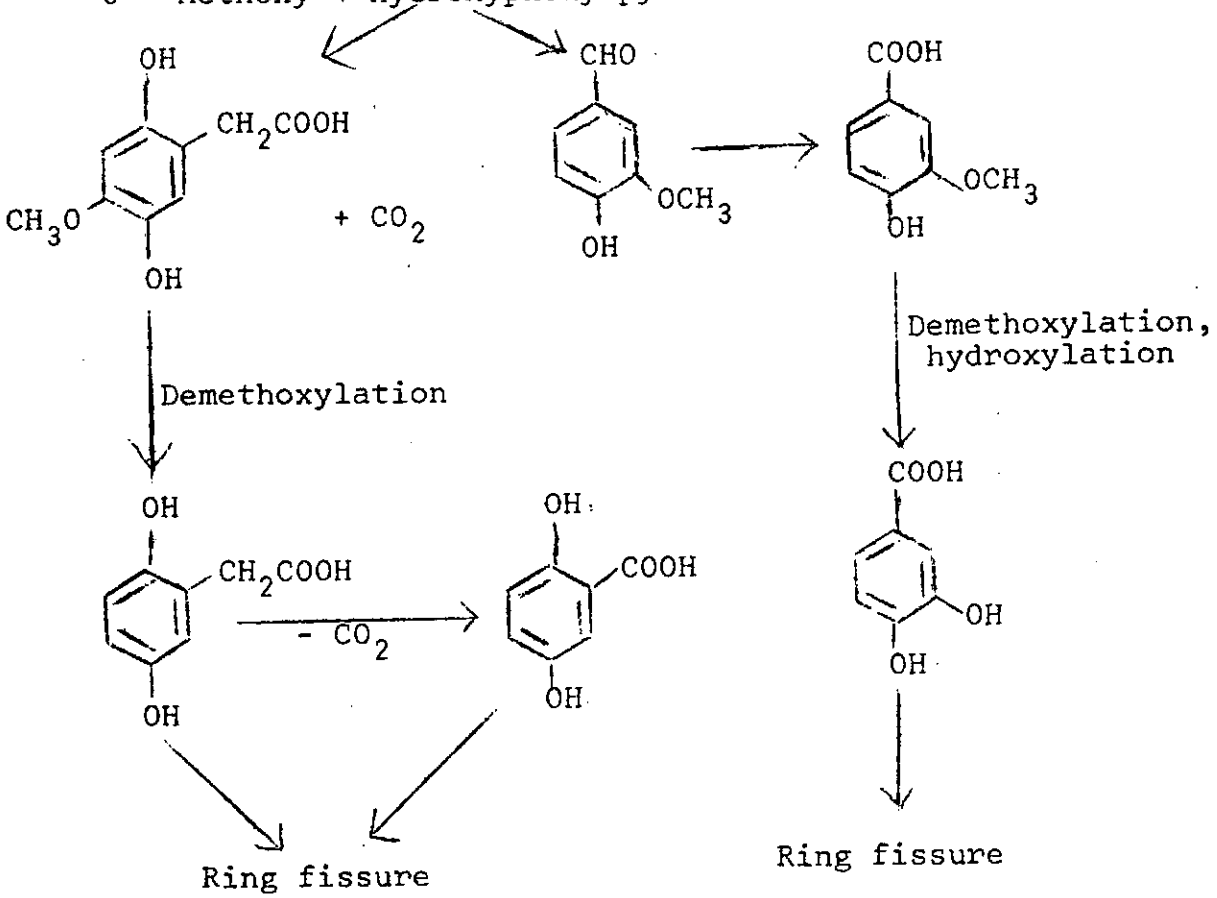
Possible pathways of aromatic ring metabolism by white-rot fungi (From Sarkanen, K.V., 1967).



Guaiacylglycerol - β - coniferyl ether units



3 - Methoxy-4-hydroxyphenylpyruvic acid



results, which are similar to those of Erickson and Miksche (1972) and Sarkanen (1971), support the idea that lignins are the products of a continuous dehydrogenation process.

It was demonstrated by Varadi (1972) that a wide variety of phenols repressed the production of cellulases and xylanases in the fungi Schizophyllum commune Fr. and Chaetomium globosum. Ander and Eriksson (1976) showed that kraft lignin and phenols decreased endoglucanase synthesis due to the absence of phenol oxidase production in a mutant of Sporotrichum pulverulentum, while kraft lignin even stimulated endo-glucanase production in the wild-type. They explained that phenol oxidases may thus function in regulating the production of both lignin - and polysaccharide - degrading enzymes by oxidation of lignin and lignin - related phenols, acting as repressors of endo-glucanase production, when Sporotrichum pulverulentum was growing on wood.

Since the initial attack on lignin macromolecule involves extracellular reactions, a variety of products will be available for uptake by other micro-organisms present in the vicinity. It is known that bacteria oxidize phenol in the following sequence: phenol → catechol → O benzoquinone → 5 hydroxiquinone → β - adipinic acid → acetic acid → formic acid, and a similar process may occur

in the case of rot fungi (Gadd, 1957). Henderson (1957) demonstrated that soil fungi, of the genera, Haplographium, Hormodendrum and Penicillium, are capable of converting ortho -, meta - , and para - methoxy benzoic acid to the corresponding monohydroxy - benzoic acid and that Penicillium and Hormodendrum further metabolized p - hydroxy-benzoic acid via protocatechuic acid. Gadd (1957) noted that where living substances are involved, it must be presumed that a great number of reactions take place simultaneously. The microbiological decomposition of wood is thus a complicated process.

The rate of decomposition of the sawdust in the lagoon will also be a function of factors inherent in the wood itself besides external factors. Considerable variation exists among tree species in their resistance to decay. Table I provides an indication of the variability in resistance among local timbers to decay.

TABLE I

Heartwood durability of some West African Timber species reported by indicated authors.

Species	Durability Rating	Reference
Afromorsia elata Harms	Very resistant	Kukachka (1962)
Carapa guineensis Aubl.	Durable to very durable	" "
Chlorophora excelsa (Welw.) Benth & Hook.	Very resistant	" "
Khaya ivorensis Chev.	Moderately resistant	" "
Nauclea diderrichii (De Wild.) Merrill	Very durable	Savory (1954)
Terrietia utilis Sprague	Non-durable	" "
Tectonia grandis Linn.	Durable	Kukachka(1962)
Terminalia superba Engl. and Diels.	Non-durable	" "
Triplochiton scleroxylon K. Schum.	Non-resistant	" "

Toxic extractable substances deposited during the formation of heartwood are the principal source of decay resistance in wood (Hawley, Fleck and Richards, 1924). They have demonstrated that resistance to decay of heartwood is greatly reduced by extraction with hot water or organic

solvents. The toxic heartwood extractives are mainly flavonoids (e.g. quercetin and robinetin), stilbenes (e.g. pinosylvin monomethylether and pinosylvin dimethylether), terpenoids (e.g. carvacrol, p - methoxycarvacrol, p - methoxythymol and hydrothymoquinone) and tropolenes (e.g. β - thujaplicin, a - thujapliclinol, β - thujapliclinol, pygmaein and nootkatin) (Scheffer and Cowling, 1966).

The decay-inhibitory extractives of the more durable woods provide protection for many years. But the protection is not permanent; the compound involved can be inactivated by denaturation or lost from the wood over a period of time. The rate of change or loss varies with the species of the wood and the mechanism of modification or loss involved. It is believed that microbial degradation of the extractives could also occur. MacLean and Gardener (1956) and Scheffer (1957) in fact attributed such chemical changes to non-decay fungi that invade the heartwood of certain Western red cedar trees. Several fungal species have also been shown to reduce markedly the toxicity of natural and synthetic phenolic compounds (Duncan and Deverall, 1964; Lyr, 1962).

Durable woods can also lose decay resistance over the years as a result of leaching away of natural preservatives. Such an event should be expected to occur more rapidly in

marine and freshwater environment.

In addition to the toxic extractives which undoubtedly play a dominant role in decay resistance, other mechanisms of natural resistance to microbial deterioration operate in wood. Lignification of wood cell walls is by far the most important non-toxic factor that contributes to the natural resistance of wood to microbial deterioration. Lignin acts as a physical barrier preventing the polysaccharide-splitting enzymes of many organisms from reaching a sufficient number of glycosidic bonds in the polysaccharide to permit sufficient hydrolysis, in cases in which the organism does not possess appropriate enzymes to depolymerize lignin, or at least disrupt its association with the polysaccharides (Pew, 1957).

Another important factor is the crystallinity of the cellulose. It is known that cellulose molecules exist in wood in both the crystalline core and the paracrystalline cortex of the microfibrils. Reese (1956) has suggested that because cellulolytic enzymes are proteins of large particle size, they cannot penetrate the crystalline core of the microfibrils. For this reason their activities are confined to cellulose molecules exposed on the surface of the crystallites. The higher the proportion of

crystalline cellulose present, the smaller will be the accessible molecular surface, and hence the more resistant the tissue will be to microbial deterioration.

Merrill and Cowling (1965) have reported that a low nitrogen content in wood increases its resistance to decay. However, a wide variety of decay and staining fungi have been shown to develop preferentially in tissues that have comparatively high nitrogen content.

In the event of successful decomposition, there is conversion of dead organic matter leading eventually to its complete mineralization, the regeneration of nutrient compounds into forms essential for plant food, the synthesis of organic matter from inorganic compounds and direct participation in a series of food chains (Kriss, Mishustina, Mitskevich and Zemtsova, 1967); all these constitute the manifold activity of micro-organisms in the creation of the biological and, in particular, the fishery production of the world's seas and oceans.

The presence of sawdust in the Lagos Lagoon provides an environment for studying the activities of fungi in decomposition process in an aquatic medium. Isolation, identification and physiological studies of these organisms will be carried out. In addition, probable fungal succession on decomposing sawdust will be investigated. The results obtained may provide information on the role of fungi in the mineralization of wood and wood materials.

II. MATERIALS AND GENERAL METHODS

(i) MATERIALS

(a) Lagoon Water Samples

The main sampling station was the Okobaba Sawmill (Plate 1) where sawdust and wood shavings are constantly discharged into the Lagos Lagoon (Fig.3). Water samples were also taken from a second site about 1km off the University of Lagos jetty and comparatively freer from sawdust. This area is referred to in the text as 'mid-lagoon area'.

Trips to each sampling station were made in the Department of Biological Sciences motor boat, 'Unilag II'. Samples were collected from January 1977 to March 1978 at bi-monthly intervals, and usually between 9.30 and 11.30 am. The boat was not available, due to mechanical fault, in August and October, 1977 and samples could not therefore be obtained from the mid-lagoon site in those months.

Wide-mouthed 250 ml pre-sterilized ground-glass sampling bottles were used to collect samples, for microbial analysis, at 5-10 cm below water surface in the lagoon. The bottle was filled to 3/4 capacity to allow for aeration.

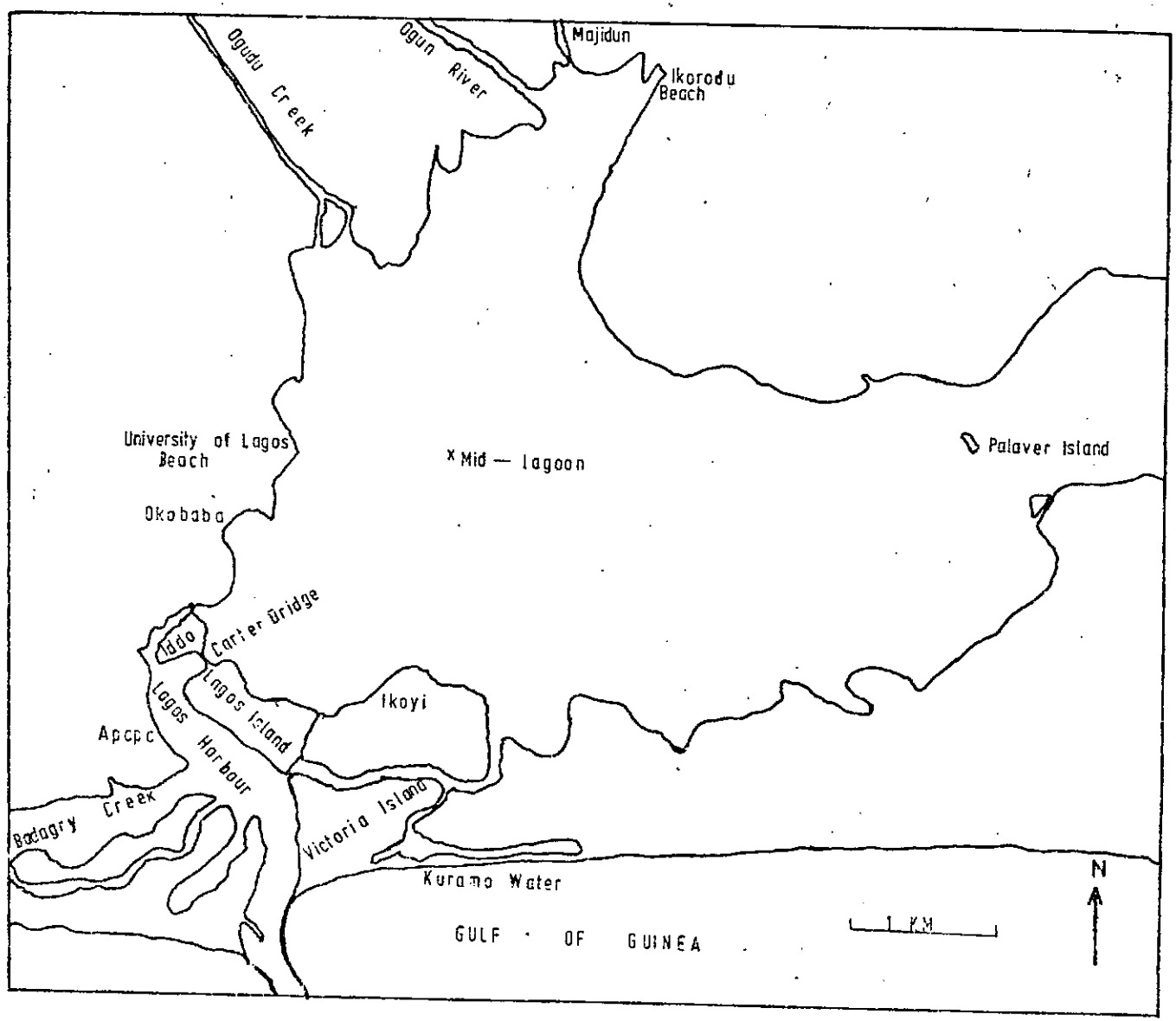
PLATE 1

Photograph showing the Okobaba Sawmill station, Lagos Lagoon. Note the heaps of sawdust along the bank of the lagoon, and the floating logs kept in the 'log pond'

FIG. 3

Map of Lagos Lagoon showing sampling stations.

x = sampling station in the mid-lagoon.


Ogudu Creek
Ogun River
Majidun
Ikorodu Beach
University of Lagos Beach
Mid — lagoon
Palaver Island
Okobaba
Carter Bridge
Iddo
Lagos Island
Ikoyi
Apapa
Lagos Harbour
Victoria Island
Badagry Creek
Kuramo Water
GULF OF GUINEA
1 KM
N

At each sampling station, one clean Winchester bottle was also filled with water which was used for chemical analysis.

(b) Decomposing sawdust

Decomposing sawdust from which sawdust - attacking fungi were isolated was obtained from the Okobaba Station.

(c) Fresh sawdust

Fresh sawdust used for trapping lagoon fungi, preparation of sawdust-ammonium agar, and sawdust medium, estimation of sugar, cellulose and lignin content of sawdust, determination of fungal deteriorating activities, and used as growth medium for a basidiomycete isolate was obtained from either the Okobaba sawmill or specially prepared from three timber species. The specially prepared sawdust came from planks of Khaya ivorensis A. Chev., Mitragyna ciliata Aubr. et Pell and Triplochiton scleroxylon K. Schum. purchased from the Okobaba sawmill. The identification of the wood pieces was confirmed by the Forestry Research Institute of Nigeria, Ibadan, Nigeria. Wood shavings were made from the planks and dried in an oven at 80°C for three days. The dried shavings were then ground into dust with a bench mill obtained from Brook Motors Limited, Huddersfield, England. The sawdust was sieved with a 0.5mm mesh before use.

Sawdust obtained from the Okobaba sawmill was similarly dried and sieved before use.

(d) Bacterial cultures

Stock cultures of isolates of bacteria from the lagoon water were maintained on Nutrient agar slopes in McCartney tubes and covered with a film of sterile paraffin and stored at 4°C in the refrigerator.

(e) Fungal cultures

Stock cultures of Aspergillus flavus Link ex Fr., Aspergillus fumigatus Fres., Aspergillus giganteus Wehmer, Aspergillus niger Van Tiegh., Aspergillus tamarii Kita, Cladosporium oxysporum Berk et Curt., Curvularia geniculata (Tracy et Earle) Boedijn, Geotrichum candidum Link ex Pers., Paecilomyces variotii Bainier, Trichoderma aureoviride Rifai aggr., Candida famata and Debaryomyces hansenii and a non-fruiting basidiomycete isolated from lagoon water, baits and decomposing sawdust employed in studies described in the thesis were maintained on slants of Potato Dextrose Agar in Mc Cartney tubes and stored at 4°C in the refrigerator.

(ii) GENERAL METHODS

(a) Determination of Physico-chemical Parameters of the lagoon water.

1. Temperature

The temperature of lagoon water and the temperature of the air above the sampling station were measured with a mercury bulb thermometer.

2. Hydrogen-ion concentration (pH)

The pH of each water sample was determined in the laboratory using an electrical pH meter (SIEBOLD).

3. Salinity

For salinity determinations, 10ml of lagoon water were titrated against 27.09 g/l silver nitrate, using potassium chromate indicator (Harvey, 1945). At least three titrations were recorded for each water sample.

4. Dissolved oxygen (D.O)

Two 200-ml bottles were filled to the brim at each sampling station. The contents of one white and one amber ampoule (B.D.H. Chemicals Ltd., England) were poured into each bottle to fix the D.O. in the water. The white ampoule contained 0.2g manganous chloride while the amber ampoule produced 36% (w/v) potassium oxide in water. Later in the laboratory the mixture was acidified with 2ml concentrated sulphuric acid and the D.O. content was estimated using a Lovibond comparator (Winkler method).

5. Transparency / Turbidity

A Secchi disk was lowered into the lagoon about ten metres offshore. The point at which the disk disappeared was noted on the Secchi line. The depth was

estimated by measuring the distance from the top of the disk to the mark on the line (Olaniyan, 1975).

6. Sulphide Content

About 100 ml water sample were collected in a 250-ml bottle. A piece of lead acetate paper was put over the rim of the bottle such that the paper was in contact with the air over the water sample. Blackening of the paper indicated production of hydrogen sulphide gas by the micro-organisms in the water (Standard Method, 1971).

7. Sulphate Content

Quantitative estimation of the sulphate content of each water sample was carried out by gravimetric method (Standard Methods, 1971). The water sample was passed through a Whatman No. 1 filter paper and 200 ml of the filtrate were acidified with 5 ml of dilute hydrochloric acid. The solution was brought to the boil before 10ml of 10% barium chloride solution were added and left to stand for at least 30 minutes. The supernatant solution was decanted into a Whatman No. 42 Ashless filter paper. The precipitate formed was also poured and washed with distilled water into the filter paper. Excess barium chloride was washed off the filter paper with distilled water. The filter paper and barium sulphate precipitate were ashed in a crucible of known weight in the fume

chamber, cooled and put in a desiccator. The crucible with content were weighed on a Mettler balance, and the weight of the precipitate determined. The sulphate content of the water sample was calculated using the formula: $\frac{mgBaSO_4 \times K}{ml\ sample} = mg/l\ SO_4^{=}$,

Where K = 411.5.

8. Suspended organic matter

One litre of lagoon water was passed through a Whatman No. 1 filter paper pre-dried to constant weight. The filter paper with its contents were put into the oven at 80°C and dried to constant weight. The difference in weight represented the dry weight of the suspended matter in the water.

9. Calcium and Magnesium content

The following reagents were used for the determination of the calcium and magnesium content of each water sample: Buffer solution, of pH 10, was prepared with 6.75g ammonium chloride (analytical reagent) and 57ml concentrated ammonia. The solution was diluted to 100ml in a volumetric flask. The buffer is best for the formation of the metal complex with EDTA (Vogel, 1960). A solution of 0.01M EDTA was used for the titration. Two indicators, murexide and Eriochrome, were employed. Murexide is ammonium purpurate ($C_8H_8N_6O_6.H_2O$), and

Erichrome Black T is sodium hydroxy naphthylazo naphthol sulphonic acid ($C_{20}\ H_{12}\ N_3\ Na\ O_7S$).

Determination of calcium and magnesium content was carried out by measuring 10ml of filtered water sample into an Erlenmeyer flask. Five millilitres of the pH 10 buffer solution were added to the water and a few drops of Eriochrome indicator were put into the solution which was titrated against 0.01M EDTA. A blue end point indicated the volume of water equivalent to the total calcium and magnesium present. At least three readings were taken. The amount of calcium alone was obtained by repeating the titration using a few granules of murexide indicator. The difference in volume between the Eriochrome and murexide titrations was used in calculating the amount of magnesium present in the water sample:

$$\frac{\text{Vol. EDTA x concentration}}{\text{Vol. water}} = \frac{\text{Burette reading x 0.01}}{10}$$

$$= X\ M\ Ca^{++} \text{ in solution}$$

Atomic wt. Ca^{++} = 40

X x At. wt. Ca^{++} = $40X$ g/l Ca^{++}

Since 1mg/l = 1 ppm

1g/l = 1000 ppm

$\therefore$ $40X \times 1000 = Y$ ppm Ca^{++} in water sample.

The same calculation was applied to magnesium content using At. wt. of 27.

10. Copper and Iron content

The concentrations of copper and iron in each water sample were determined by the use of an Atomic Absorption Spectrophotometer (A.A.S.). De-ionized distilled water was used for the preparation of all reagents, solutions and calibration standards. Stock copper and iron solutions were prepared according to Standard Methods (1971). A series of standard metal solutions containing 1 - 100 µg/l was prepared by appropriate dilution of the stock metal solution. The test water samples were similarly diluted. The standards needed for the working curves were run on the A.A.S. and the concentrations of the standards were plotted against absorbance. The prepared water samples were also run on the A.A.S. and the absorbance recorded. By extrapolation on the standard curve, the concentration of copper or iron in the water samples was determined. At least three readings were recorded on each occasion.

11. Tide

The height of the tide at the time of sampling was recorded from Tide Tables of the Nigerian Navy(1977,1978), containing tidal predictions for Lagos, Escravos bar, Forcados bar, Bonny Town and Calabar.

(b) Assessment of Bacterial content of the lagoon

1. Total Viable Aerobic Plate Count

Commercial (Oxoid) preparation of Nutrient agar was used for counting the total aerobic bacteria in the water samples.

Twenty-eight grams of dehydrated Nutrient agar were reconstituted by dissolving in 100ml lagoon water. The medium was autoclaved at 1.1 kg/cm^2 for 15 minutes and dispensed in approximately 20ml volumes into sterile Petri dishes and left to solidify. A drop of water sample, 0.1 ml in volume, was placed on each agar plate and spread with a sterile L-bent glass rod. Duplicate plates were incubated at 22 $\pm$ 2 ^{o}C for 72 hours to isolate heterotrophic bacteria, and another set of two plates incubated at 37 $\pm$ 2 ^{o}C for 48 hours to isolate members of the Enterobacteriaceae. The viable counts represented the total aerobic bacteria in the water sample.

2. Coliform population

Eosin methylene blue (EMB) agar, a medium selective for coliforms was used. The EMB agar was prepared by dissolving 37.5g EMB agar (Difco) in 1000ml distilled water. One tenth millilitre of the 10^{o} or 10^{1} diluted lagoon water sample was spread on the surface. Three replicates were prepared for each water sample and incubated at $37^{o}C$ for 48 hours. The number of each type of colony was counted

and the number of organisms (coliforms) per ml of undiluted water sample was calculated.

3. Gram stain reaction of isolated species

A thin smear of a representative of each colonial type of bacteria was made on a grease-free glass slide and stained with methyl violet solution (0.5g methyl violet in 100 ml distilled water) for 20 seconds. The stain was washed off with water and the smear treated with iodine solution (1g iodine and 2g potassium iodide in 100ml distilled water) for one minute. The cells were washed with 95% alcohol until the iodine solution stopped running off, then rinsed with water and finally counterstained with 1% aqueous safranin solution for 30 seconds. The safranin was washed off with water and the smear allowed to dry before microscopic examination under oil immersion. Species with purple or violet stained cells were recorded as Gram positive while those with cells showing red colouration were judged to be Gram negative.

4. Characterization of isolated bacteria

The various bacteria were distinguished on the basis of form and colour of colony on Eosin Methylene Blue or Nutrient agar, Gram stain reaction, and morphology of the cells.

(c) Isolation of fungi from lagoon and decomposing sawdust

I. Direct microscopic examination of decomposing sawdust particles.

Sterile wide-mouth sampling bottles were used to collect samples from the heaps of decomposing sawdust along the bank of the lagoon at Okobaba. Sawdust particles removed with a pair of sterile forceps were spread on clean glass slides and stained with cotton blue in lactophenol. The slides were then examined under the microscope for the presence of fungi.

II. Media for fungal isolation and sporulation

1. Corn Meal Agar (CMA)

CMA	15g
Lagoon water	1000ml

(The Oxoid manual, 1972)

2. Czapek Dox Agar (CDA)

Sucrose	30g
Dipotassium hydrogen phosphate	1g
Sodium nitrate	3g
Magnesium sulphate	0.5g
Potassium chloride	0.5g
Ferrous sulphate	0.01g
Agar	12g
Distilled water	1000ml

(Collins, C.H. and Lyne, P.M., 1970)

3. Czapek Dox - Ammonium Agar

Ammonium chloride	2.0g
CDA less sodium nitrate	1000ml

4. Lagoon Water Agar (LWA)

Agar	20g
Lagoon water	1000ml

5. Leonard's Agar (LA)

Peptone	0.625g
Potassium dihydrogen phosphate	1.25g
Malt extract	6.25g
Maltose	6.25g
Magnesium sulphate	0.625g
Agar	20g
Lagoon water	1000ml

(Bridge Cooke, W.M., 1973)

6. Malt Extract Agar (MEA)

Malt extract	40g
Agar	15g
Lagoon water	1000ml

(Collins, CH. and Lyne, P.M., 1970)

7. Neopeptone Dextrose Agar (NDA)

Neopeptone	5g
Dextrose	10g
Agar	20g
Lagoon water	1000ml

(Bridge Cooke, W.M., 1973)

8. Potato Dextrose Agar (PDA)

PDA 39g

Lagoon water 1000ml

(The Oxoid Manual, 1972)

9. Yeast Extract Agar (YEA)

YEA 23g

Lagoon water 1000ml

(The Oxoid Manual, 1972)

10. Sawdust medium

This medium was specifically used for a basidiomycete which did not fructify on the other media.

Twenty grams sawdust from the sawmill station and 100ml tap water were mixed in a sampling bottle and autoclaved at 1.1 kg/cm^2 for 30 minutes. The sterile sawdust was inoculated with the mycelium growing on PDA and incubated at 27 $\pm$ 2°C for two months. The culture was moistened at 4 day intervals with sterile distilled water during incubation.

III. Isolation Techniques

Four methods were used in isolating fungi from Lagos Lagoon.

1. Poured plate method

Seven media - Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Corn Meal Agar

(CMA), Czapek Dox Agar (CDA), Neopeptone Dextrose Agar (NDA) and Leonard's Agar were used for the preliminary investigation while two of them, PDA and MEA were used for subsequent isolations. Undiluted as well as 10^{-1} and 10^{-2} dilutions of lagoon water samples were analysed. One millilitre of each dilution was put into a sterile Petri dish and about 20ml of cool molten agar added. The lagoon water and molten agar were mixed by a circular motion of the plate before the agar set. There were three replicates for each medium. The plates were incubated at room temperature for 5 days after which the number of colonies was counted and the species identified.

Isolations were made from water samples from both the sawmill and mid-lagoon sites.

2. Spread plate method

Petri plates of the seven previously mentioned media were prepared and used after they had solidified. Using a sterile pipette, 0.1ml of either the undiluted or 10^{-1} and 10^{-2} dilutions of water sample was inoculated onto the agar plate and spread with a bent glass rod. There were three replicates for each water dilution. All inoculated plates were incubated at room temperature for 5 days, after which the number of colonies was counted and the species identified.

3. 'Seeding' plate method

The fungal flora of the decomposing sawdust were also investigated by placing bits of sawdust from the lagoon on Petri plates of either Leonard's Agar, NDA, PDA or MEA. The plates were incubated at room temperature for 7 days and fungi which developed were identified.

4. Baiting methods

Two types of baits, mini-wood blocks and sawdust particles, were used to trap fungi from lagoon water samples.

(4a) Mini-wood blocks

Shavings of wood, each measuring 10 x 10 x 1 mm, serving as mini-wood blocks, were wrapped in aluminium foil and sterilized by autoclaving at 1.1 kg/cm^2 for 30 minutes. Three or four of the pieces of shavings were put into each of several sterile Petri dishes. Approximately 20ml of lagoon water collected from the Okobaba sawmill station were poured into each dish and the dishes incubated at room temperature. Samples of the shavings were withdrawn after 4, 10 and 21 days, respectively, placed on PDA plates, and incubated at room temperature for 7 days. Species of fungi developing from the baits were recorded. Control plates were inoculated with baits suspended in sterile distilled water.

(4b) Sawdust

About 2g of sawdust in a nylon mesh and suspending in a previously sterilized 250 ml graduated Erlenmeyer flask filled with lagoon water (up to 200 ml mark) served as the second bait. The flasks were incubated at room temperature so that lagoon fungi could grow on the sawdust baits. Samples of the bags were removed at intervals of 3, 5 or 10 days and some of the sawdust transferred with a sterile pair of forceps onto three types of agar media, PDA, MEA and sawdust extract agar. The 'seeded' plates were incubated at room temperature for 7 days after which the number of each fungal species was recorded. At each sampling time, three replicate plates were prepared for each medium. Control plates were inoculated with baits suspended in sterile distilled water.

IV. Identification of fungal isolates.

The Saccardo System of Classification (Barnett and Hunter, 1972) was used. The primary basis of the system is the morphology of the reproductive apparatus and colour of the spores. Dimensions of fungal structures were measured with an eye-piece micrometer. The morphology of the mycelium was also used in distinguishing the 'advanced' fungal classes. Pure cultures of each fungus were sent to the Commonwealth Mycological Institute, Kew, England and to

Centraalbureau voor Schimmelcultures, BAARN (Netherlands) for confirmation of identifications. Yeasts were identified at the National Collection of Yeast Cultures, Nutfield, Surrey, England.

(d) Media for physiological studies of isolated fungi.

1. Synthetic media

The ability of the fungi isolated from decomposing sawdust to use various carbon and nitrogen sources was studied using synthetic media. The basal medium to which test carbon and nitrogen compounds, at desired concentrations were added consisted of:

Dipotassium hydrogen phosphate ..	1g
Magnesium sulphate	0.5g
Potassium chloride	0.5g
Ferrous sulphate	0.01g
Distilled water	1000ml

2. Sawdust Extract Agar.

Sawdust extract (2%)	1000ml
Agar	20g

The sawdust extract was prepared by heating in a water bath of a temperature of 100°C, 50g sawdust in 1000ml distilled water. The liquid was passed through Whatman No. 1 filter paper. Some distilled water was put into the sawdust again, shaken and filtered so as to bring

the volume of the filtrate to 1000ml. This constituted 5% aqueous sawdust extract from which further lower dilutions were prepared.

3. Sawdust Extract - Sodium nitrate Broth

Sodium nitrate 3g

Sawdust extract (1%) 1000ml

Basal medium ..... salts only.

Each salt was weighed into a separate Erlenmeyer flask and dissolved in 10ml 1% sawdust extract. The solutions were pooled and made up to 1000ml with 1% sawdust extract.

4. Sawdust - Ammonium Agar.

Sawdust 10g

Ammonium sulphate 2g

Basal medium 1000ml

Sawdust was used as the sole carbon source. It was sieved with a 0.5mm - mesh to remove the larger particles before use.

5. Carboxymethylcellulose Agar.

Carboxymethylcellulose powder 10g

Agar 15g

Distilled water 1000ml

The cellulose powder was mixed with a few millilitres of distilled water to form a smooth paste and then stirred into the remaining distilled water. The molten agar medium was poured thinly into Petri dishes so that any clearing of the medium due to cellulolytic enzymes could be easily detected.

6. Carboxymethylcellulose with mineral salts.

Carboxymethylcellulose powder	10g
Sodium nitrate	3g
Basal medium	1000ml

The medium was dispensed in 20ml aliquots into 50ml Erlenmeyer flasks.

7. Carboxymethylcellulose with Ammonium.

Carboxymethylcellulose powder	10g
Ammonium sulphate	2g
Basal medium	1000ml

8. Phosphoric acid-swelled cellulose.

Prepared with 5g cellulose powder, 60ml concentrated phosphoric acid, and distilled water (Montenecourt, B.G. and Eveleigh, D.E., 1977).

The cellulose powder was gradually mixed with 20ml distilled water to a smooth paste and 30ml concentrated phosphoric acid were added slowly while stirring. The mixture was left for about 10 minutes on a Gallenkamp magnetic

stirrer regulator hotplate. Then the remaining 30ml phosphoric acid was added slowly while stirring as above. The mixture was poured into 800ml distilled water stirring all the time. Later the swollen cellulose now in colloidal state was allowed to sediment and the supernatant was discarded. The cellulose was washed in several changes of distilled water so as to remove traces of the phosphoric acid, and possibly the glucose. Washing was stopped when the pH of the preparation became 7.0.

9. Filter paper medium.

Ammonium sulphate	2g
Magnesium sulphate	0.15g
Dipotassium hydrogen phosphate	0.15g
Potassium chloride	0.6g
Filter paper disks	2g
Distilled water	1000ml

(Park, D., 1976)

The medium was dispensed in 50ml aliquots into 250ml Erlennmeyer flasks, and one disk of filter paper, 0.1g dry weight, was put into each flask before autoclaving.

10. Colloidal Cellulose Agar.

Phosphoric-acid-swelled cellulose (2.5%)	40ml
Dipotassium hydrogen phosphate	0.1g
Magnesium sulphate	0.05g

Potassium chloride 0.05g

Ferrous sulphate 0.001g

Agar 0.6g

Distilled water 60ml

11. Lignin Agar.

Lignin 5g

Agar 15g

Distilled water 1000ml

Lignin extracted from sawdust and Commercial lignin obtained from Gurr Ltd., London, England, were used separately in preparing two lignin agar media. The pH of the medium was adjusted with either 0.1N sodium hydroxide solution, or 0.1N hydrochloric acid before autoclaving.

Lignin was extracted from sawdust previously dried at 105°C to constant weight. Fifteen grams of the sawdust was put into a Soxhlet apparatus and extracted with 800ml ether for 10 hours. It was next extracted with 900 ml of a 2 : 1 mixture of benzene and absolute ethanol for 10 hours. The sawdust residue was washed with water and dried to constant weight to obtain percentage extractives in the sample. The sawdust residue was treated with 70% H_2SO_4 in a 5-litre flask to remove the constituent carbohydrates. About 3,000ml distilled water was used to dilute the mixture

and a reflux condenser connected to the flask so that the mixture could be heated under constant volume. Heating was carried out on a heating mantle for 3 hours. The mixture was left overnight to settle and the supernatant decanted. The lignin which formed the residue was dried at 105^{o}C to constant weight.

12. Lignin-Glucose Agar.

Lignin 5g

Glucose 0.1g

Agar 15g

Distilled water 1000ml

Glucose was dissolved in 100ml distilled water and autoclaved at 0.4kg/cm^2 for 10 minutes. Lignin was gradually mixed with 900ml distilled water and the agar was added before autoclaving at 1.1 kg/cm^2 for 15 minutes. The sterile glucose solution was gradually poured into the molten lignin agar mixture aseptically in the inoculating room and the two were thoroughly mixed by a gentle swirling or rotation of the flask.

13. Lignin-Glucose-Salts Agar.

Lignin 5g

Glucose 0.1g

Agar 15g

Basal medium 1000ml

The medium was prepared as described for Lignin-Glucose-Agar except that the basal medium was used instead of distilled water.

(e) Sterilization

All media were sterilized in an autoclave at 1.1 kg/cm^2 for 15 minutes except otherwise stated.

Glassware were soaked in antiseptic (Izal) solution overnight. They were washed with detergent (Omo) and rinsed in several changes of tap water and finally with distilled water. The glassware were arranged on the drain board to drip off and dry. The Petri dishes were fitted with lids and packed in metal canisters. Pipettes were also packed in canisters. Other glassware were wrapped in aluminium foil and all were sterilized in a hot air oven at 160^{o}C for 3 hours holding time. The glassware were allowed to cool before removal.

The inside of the inoculating chamber was wiped with methylated spirit and then sprayed with a solution of 5% phenol in 70% alcohol. Similar sterilization procedures were used for the anaerobic incubator.

The inoculating room was sterilized by cleaning the floor with antiseptic (Dettol) solution and the room sprayed with 1% antiseptic solution after which the ultra-violet light was turned on for at least one hour before use.

Inoculating loops were dipped into methylated spirit and flamed until red hot. Bent glass rods used for spreading inocula, and metal cork borer used for cutting mycelium disks from Petri plates were sterilized by dipping in methylated spirit and flaming.

(f) Inoculation of fungal cultures.

Agar disks (obtained with a 5mm diameter cork borer) from the edge of actively growing cultures of the fungus on PDA were used.

(g) Incubation of cultures.

1. Types of culture.

Two types of culture media were used: stationary solid or broth culture media, and also liquid culture media agitated continuously on a culture shaker (Model HT Infors AG CH - 4015 from Basel, West Germany).

2. Temperature of incubation.

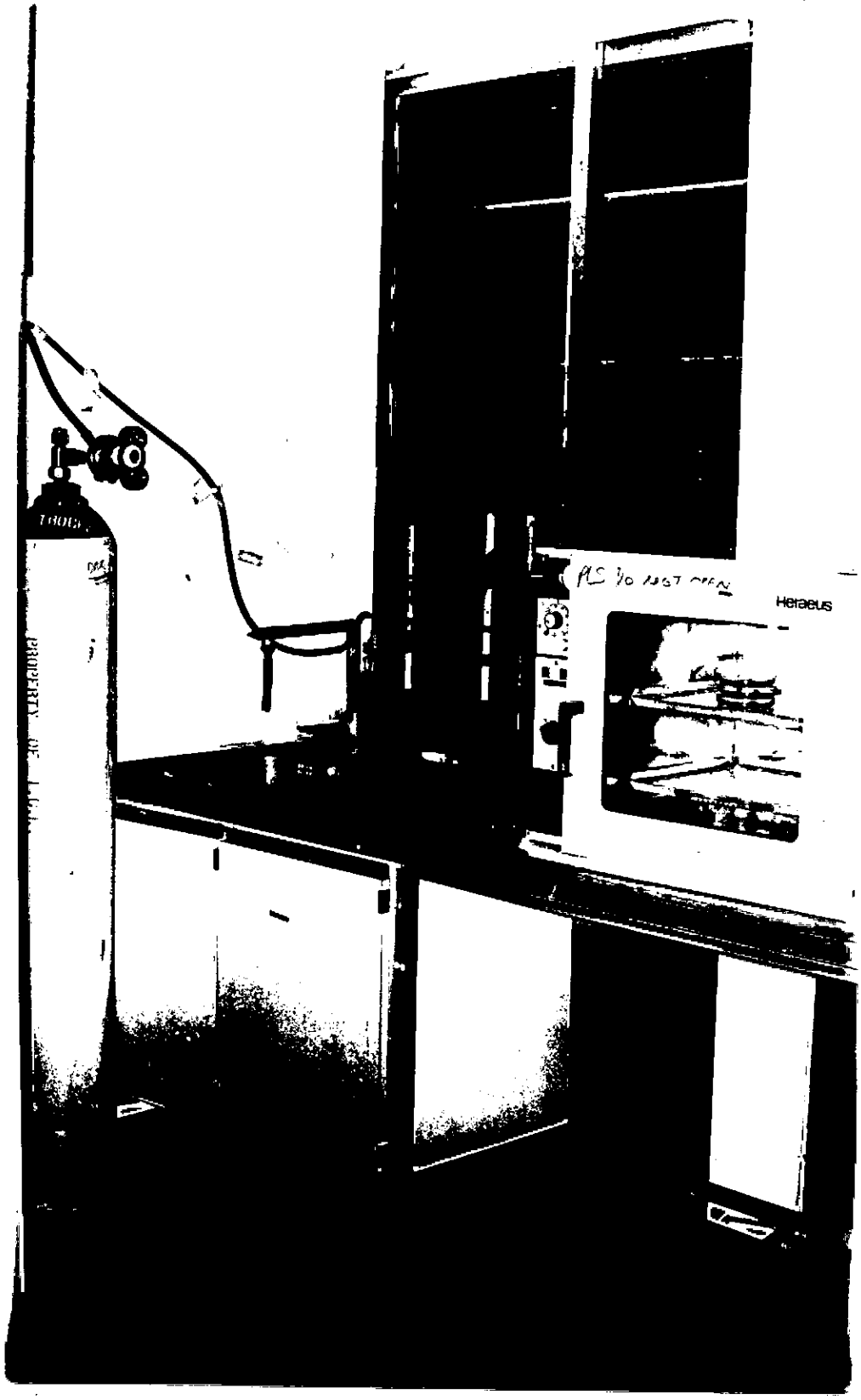
Cultures were incubated at room temperature except otherwise stated. A maximum and minimum thermometer kept besides the cultures recorded a temperature range of 26 to 31^{o}C.

3. Anaerobic incubation.

The 'Heraeus" anaerobic incubator (Plate 2) and the regular metal anaerobic jar were used. Only PDA medium was employed.

PLATE 2

Photograph of 'Heraeus' Anaerobic Incubator used for growing the fungi under anaerobic conditions


AS 'lo NOT OPEN
Heraeus

3a. 'Heraeus' anaerobic incubator.

The 'Heraeus' anaerobic incubator model VT5042EK/N_2 supplied by Karl-Klob Scientific Technical Supplies, West Germany was used. The interior of the anaerobic incubator was sterilized by wiping with methylated spirit. Air was then evacuated from the incubator with a vacuum pump, after the inoculated plates had been placed in it. After evacuation, purified nitrogen gas was passed into the incubator until the pressure reached the level of atmospheric pressure. This pressure was then maintained throughout the period of incubation, and the temperature was kept at 28°C.

Purification of nitrogen gas.

Industrial nitrogen gas supplied by Industrial Gases (Nigeria) Limited, Apapa, was purified to remove any residual traces of oxygen. The gas cylinder was connected by rubber tubing to a series of three Dreschler bottles. The outlet of the last bottle in the series was connected to the anaerobic incubator. The first and second Dreschler bottles contained 50ml alkaline pyrogallol each while the third bottle contained 50ml sodium hyposulphite. The nitrogen gas was first passed through the first and second Dreschler bottles for the alkaline pyrogallol to remove any traces of oxygen in the gas while the sodium hyposulphite in the third bottle dried the gas before entering the incubator.

Alkaline pyrogallol solution.

Fifteen grams pyrogallol crystals were dissolved in 100ml of 50% sodium hydroxide solution.

Sodium hyposulphite solution.

Sodium hyposulphite crystals 1.4g

Indigo carmine 1.4g

Potassium hydroxide solution (10%)..... 100ml

3b. Preparation of anaerobic jar.

The inside of the metal anaerobic jar (Baird and Tatlock, England) was cleaned with methylated spirit and the inoculated plates were put into it. Using the Oxoid gas generating Kit, code No. BR38, an anaerobic atmosphere was created as follows: one edge of the sachet was cut with a pair of scissors and 10ml tap water added to the contents. The sachet was carefully lowered upright into the jar which was covered with a tight-fitting lid and the hydrogen and carbon dioxide gases emitting from the sachet filled the jar. The jar remained closed throughout the period of incubation of the cultures.

(h) Assessment of growth rate of mycelium.

Growth of fungi was assessed by determining the diameter of cultures on each Petri plate at desired intervals along two pre-determined diameters drawn at right angles to each other at the bottom of the dish, or by dry weight of mycelium produced in culture broth.

(i) Estimation of Reducing Sugar content of sawdust.

A stock of 10.0mg/l glucose solution was prepared and from which dilution series from 1.0 to 9.0 mg/l were made. These served as the standard solutions for the standard curve. A mixture of 25ml copper reagent A and 1ml copper reagent B was prepared just before the test was performed. A mixture of 1ml of a standard glucose solution and 1ml of copper reagent mixture was made in a large boiling tube and boiled for 20 minutes in a water bath. The mixture was cooled rapidly under running tap and 1ml of arsenomolybdate reagent added. The solution was mixed thoroughly on a Gallenkamp magnetic stirrer regulator hotplate until CO_2 effervescence ceased. The volume of the mixture was made up to 25ml with distilled water. For each glucose concentration, a second preparation was made up to a volume of 10ml with distilled water instead of 25ml. Blank tube was prepared with distilled water instead of glucose solution. A photoelectric colorimeter, model AE-11 supplied by ERMA Optical Works Limited, Tokyo, Japan, was employed for measuring the optical density (O.D.) of each standard glucose solution using a 500 - 520 mμ filter. The dial was adjusted to read 100% transmission or zero O.D. on the introduction of the blank solution into the colorimeter tube. The O.D. of the series of glucose solutions

was next recorded. Duplicate determinations of O.D for each sample was obtained, and the average of the two readings was used in a graph of glucose concentration versus O.D. Two graphs thus produced for the 10ml and 25ml solutions served as the standard curves (Fig. 4).

The same procedure was followed in preparing solutions from test specimens for O.D. determinations. If the colour was too intense giving an O.D. outside the calibration values, the test solution was diluted and the dilution factor noted. By means of the O.D. values obtained for the test specimens, the respective concentrations of reducing sugar was read from the standard curve.

1. Reagents.

The Nelson (1944) and Somogyi (1937, 1945) reagents were employed in determining the reducing sugar content of test samples.

1a. Copper reagent A.

Anhydrous sodium carbonate	25g
Potassium sodium tartrate	25g
Sodium bicarbonate	20g
Anhydrous sodium sulphate	200g
Distilled water	1000ml

The reagents were dissolved in 800ml distilled water and made up to 1000ml in distilled water.

FIG. 4

Standard curves of glucose concentration and optical density (O.D.).

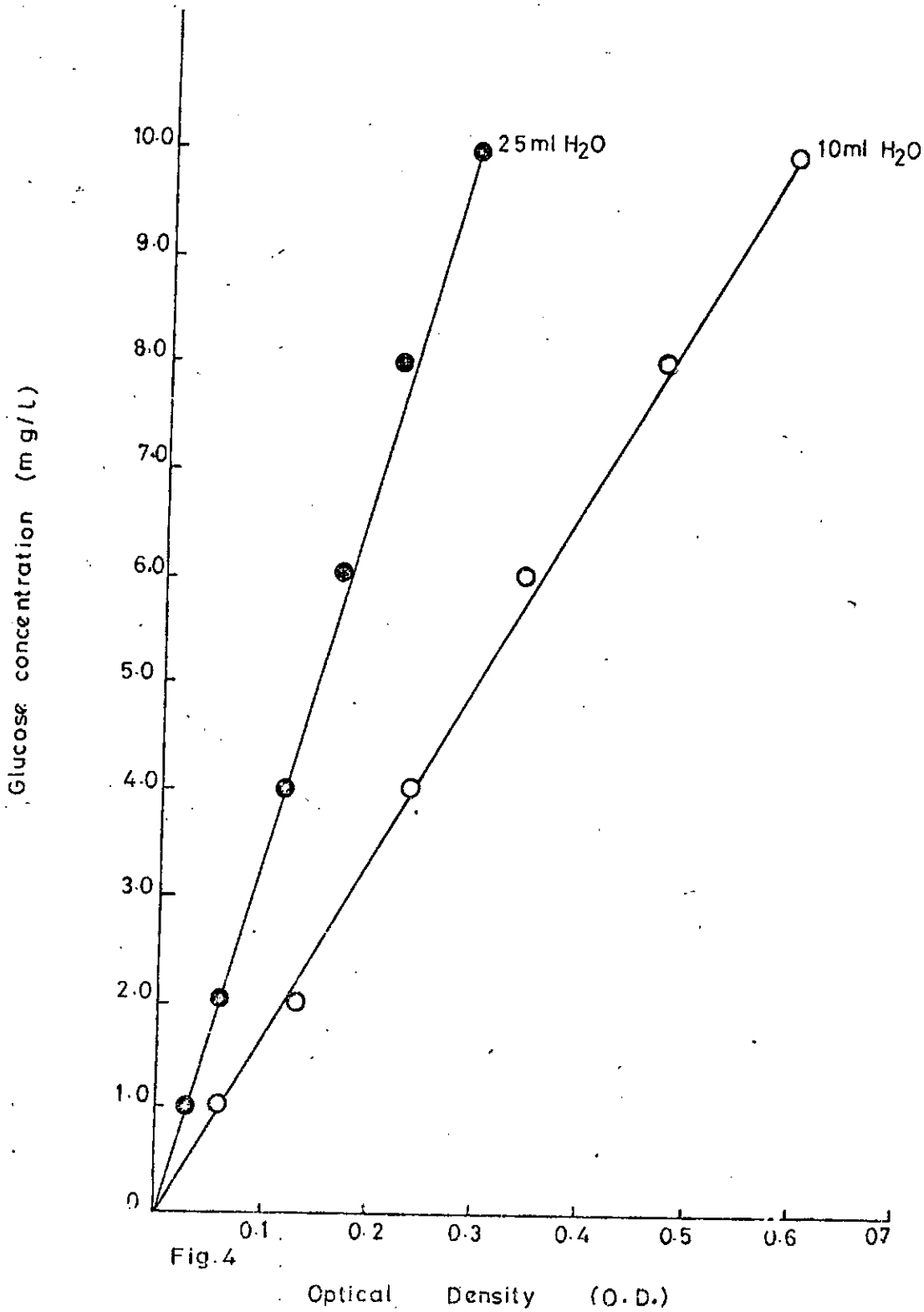

Glucose concentration (m g/L)
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
0·1
0·2
0·3
0·4
0·5
0·6
07
25ml H2O
10ml H2O
Optical Density (O.D.)

Fig. 4

1b. Copper reagent B.

$CuSO_4.5H_2O$	25g
15% conc. H_2SO_4	0.2ml
Distilled water	1000ml

1c. Arsenomolybdate reagent.

Twenty-five grams ammonium molybdate were dissolved in 450ml distilled water and 21ml concentrated H_2SO_4 then added. Three grams of sodium arsenate ($Na_2HAsO_4.7H_2O$) were dissolved in 25ml distilled water and added to the first mixture. The resulting preparation was placed in an incubator at 37°C for 2 days before storing in stoppered brown bottles at room temperature.

(j) Determination of cellulose content of sawdust.

The cellulose content of sawdust was estimated using the following method (which has been used for the determination of cellulose content of leaves) obtained from Gooday, G.W. (personal communication). The procedure required treatment of the sawdust with several chemical solutions. The residue in suspension was collected on each occasion for the next treatment by centrifuging the suspension at 3,000 r.p.m. for 30 minutes.

1. Sawdust was dried in an oven at 80°C to a constant weight.

2. One gram was boiled in 40ml of distilled water for 5 minutes and then left in the cooled water overnight on a mechanical shaker.
3. The residue was again boiled in distilled water for 5 minutes on two occasions.
4. The residue was next boiled three times, for 5 minutes on each occasion, in three changes of ethanol, followed by boiling also three times, for five minutes on each occasion in three changes of acetone.
5. The residue was dried in an oven at 80°C to constant weight.
6. The residue was then shaken for 18 hours in 5% KOH (w/v) at 28°C, boiled three times for five minutes on each occasion in three changes of 5% Acetic acid (w/v) and finally shaken overnight in 2% $KMnO_4$ (w/v).
7. The residue was washed first in distilled water and then in 50% oxalic acid (w/v).
8. The residue was boiled three times, for five minutes on each occasion, in three changes of 0.5N HCl.
9. The residue was finally washed in distilled water and dried in an oven at 80°C to constant weight.

Calculation of cellulose content of sawdust.

Wt. of dried sawdust ..... Ag

Wt. of final sawdust residue (cellulose)... Bg

Wt. of dried filter paper ..... Cg

Wt. of filter paper residue ..... Dg

Since filter paper is pure cellulose, the amount of cellulose lost during extraction procedures = (C - D)g ;

$$\text{The percentage loss} = \frac{(C - D)g}{C_g} \times \frac{100}{1} \%$$

If wt of cellulose residue from sawdust = Bg; The corrected wt. of cellulose in sawdust

$$= B + \left(100 \frac{(C - D)}{C} \times B\right) g.$$

(k) Determination of Lignin content of sawdust.

Sawdust was dried at 105°C to constant weight and 15g of it was put into a Soxhlet apparatus and extracted with 800ml ether for 10hours. It was next extracted with 900 ml of a 2 : 1 mixture of benzene and absolute ethanol for 10 hours. The sawdust residue was washed with water and dried to constant weight to obtain percentage extractives in the sample. The sawdust residue was treated with 70% H_2SO_4 in a 5 - litre flask to remove the constituent carbohydrates. About 3,000 ml distilled water was used to dilute the mixture and a reflux condenser connected to the

flask. Heating was carried out on a heating mantle for 3 hours. The mixture was left overnight to settle and the supernatant decanted. The lignin which formed the residue was dried at 105°C to constant weight.

(1) Fungus spore germination tests.

The germination of spores of some of the fungi isolated from the decomposing sawdust was tested using the 'Slide Method'. Sawdust aqueous extract was prepared as previously described and used directly or amended with either organic or inorganic compounds. Sterile Petri dishes, each containing a sterile glass slide supported on a glass V - piece over a small quantity of sterile distilled water constituted the germination chamber. Using a sterile dropper, two individual drops of spore suspension prepared with the appropriate solution and spores from 6-day old culture of the test fungus were placed on the slide and the lid of the dish replaced. After incubation for 24 hours at room temperature, germination percentage was calculated out of at least 200 observed spores. The lengths of germ tubes of 20 germinated spores were also measured with the aid of an eye-piece micrometer and the mean germ tube length for the treatment calculated. Distilled water used in place of the sawdust extract served as control.

(m) Measurement of pH of media.

The pH of culture media and germination media was measured with a SIEBOLD electrical pH meter.

(n) Sawdust decomposition tests.

The ability of the fungi to decompose sawdust was tested by inoculating sawdust in Petri dishes with the fungus. Sawdust of known timber was inoculated on each occasion by a test fungus and incubated at room temperature for 40 days. Each Petri dish contained 2g of fresh sawdust in 12ml of distilled water and the dishes with sawdust autoclaved on three consecutive days at 1.1kg/cm^2 for 20 minutes before being inoculated. Four lots of 2g fresh sawdust were dried separately at 80^{o}C for 3 days and the mean dry weight obtained represented the initial dry weight of the sawdust.

Inoculum was prepared by growing the test fungi in Mycological Peptone Broth in 500 ml Erlenmeyer flasks at room temperature for 7 days. The culture was then macerated with a microspatula. Each Petri dish was inoculated with ten loopfuls of the macerated culture and incubated at room temperature for 40 days. A few drops of sterile distilled water were added to each Petri dish, at 5-day intervals during incubation, to keep the sawdust continually moist. The dry weight of the contents of the Petri dish was determined, as described above, at the

end of the incubation period.

The loss in dry weight of the sawdust after 40 days represented the amount of sawdust broken down by the test fungus.

(o) Photomicrography.

The Zeiss photomicroscope model 47 20 45 - 9901 was used to photograph microscopic structures. The specimen for photomicrograph was mounted on a grease-free clean plain glass slide. Fungal tissues were stained with cotton blue in lactophenol. All photographs were taken with a black and white 21 DIN/ASA 100 Agfa (Germany) photographic film.

(p) Statistical Analysis.

Results were statistically analysed where appropriate.

The relations between some physico-chemical parameters of the lagoon which showed seasonal fluctuations, e.g. salinity, and fungal counts of the sampling stations were statistically tested by means of correlation coefficient (r) and by use of a t - test (t). The formulas used were:-

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{\left(\sum x^2 - \frac{(\sum x)^2}{n}\right)\left(\sum y^2 - \frac{(\sum y)^2}{n}\right)}}$$

where x = salinity, y = corresponding fungal count and n = number of observations.

$$t\text{ - statistic} = r\sqrt{\frac{n-2}{1-r^2}} \text{ with } n\text{-}2 \text{ d.f.}$$

d.f. = degrees of freedom.

The relation between days of incubation and weight of filter paper inoculated with fungi was also tested by correlation coefficient (r) and by use of a t - test. Similar statistical analyses were applied to the temperature of incubation and weight of sawdust inoculated with different fungi in the sawdust decomposition experiments (section Mi.). The same tests were performed on the relation of glucose concentrations and weights, and the relation of varying peptone concentrations and weights of sawdust. The statistical operations were accomplished using the Monroe Computer Model 1930.

III. EXPERIMENTAL DETAILS

A. BACTERIA OF SAWDUST-POLLUTED LAGOON WATER

The activies of fungi which will decompose sawdust put into the lagoon will be extensively influenced by several factors. Among the most important of these would be environmental conditions which are mainly, the physical and chemical factors. The latter includes metabolites produced by other organisms living in the habitat. Since the bacteria constitute the greatest proportion of that population, the density of bacteria in the water was studied. The procedure adopted has been fully described under Materials and Methods, and the population of the heavily polluted area was compared with that of a comparatively sawdust-free area in the lagoon.

As the bacteria were not the main subject of investigation reported in this thesis, no attempt was made at an extensive study that would have provided full details of the bacteria. The genera of the bacteria were, therefore, not established.

Water samples for the isolation of the bacteria were taken twice each month from January to April, 1977.

B. FUNGI OF SAWDUST-POLLUTED LAGOON WATER

The fungal flora of the sawdust-polluted and sawdust-free areas of the Lagoon was investigated. Since the results of other isolation studies (e.g. Gilbert and Lovelock, 1975 and Johnson and Sparrow, 1961) have shown that a better result is achieved in such studies if more than one type of medium is used, isolation was carried out in this work with several agar media, Corn-Meal, Czapek-Dox, Leonard's, Malt-Extract, Neopeptone-Dextrose and Potato-Dextrose.

Water samples for the fungal isolation were taken twice a month from January, 1977 to March, 1978.

Two sorts of isolations were also made. Isolation under aerobic conditions, and under anaerobic conditions.

C. PHYSICO-CHEMICAL PROPERTIES OF THE LAGOON WATER

The fungal and bacterial populations in the preceding experiments were not the same. The changes could be attributed to either changes in the physico-chemical properties of the lagoon or interactions among the inhabitants of the lagoon or to both. The easier of the two to study are the physico-chemical characteristics of the lagoon.

Water samples for the series of analyses were taken on days when fungal population of the lagoon was estimated. There were two samplings each month.

The numerous measurements were carried out according to the methods described earlier. Measurements were made of air temperature immediately above the lagoon, and the temperature, pH, salinity, dissolved oxygen level, sulphate content, sulphide content, calcium content, magnesium content, copper level, iron level, amount of suspended matter and transparency of the lagoon.

D. FUNGI ASSOCIATED WITH WOOD IN THE LAGOON

Naturally, not all the fungi isolated from the lagoon will be expected to be wood-decomposing species. Fungi actually associated with decomposing sawdust were isolated for subsequent investigations. Actual isolation of the wood-decomposing species was preceded by an examination of particles of decomposing sawdust under the microscope for associated fungi.

Fungal species were obtained from decomposing sawdust, sawdust baits and submerged mini-wood-blocks.

During the first isolation from the decomposing sawdust, in March 1977 two agar media were used, viz., Malt-Extract and Neopeptone-Dextrose, while on the second occasion in April 1977, three agar media were used:

Leonard's, Malt-Extract and Potato-Dextrose.

Fungi developed from particles of decomposing sawdust removed from the lagoon and placed on the different agar media. All the species were identified in most cases to the species level.

Fresh sawdust was put in nylon bags and the bags placed in lagoon water for 48 days. At desired intervals, samples were removed and 'seeded' on Potato Dextrose Agar in Petri dishes. This particular exercise permitted the study of species invading and decomposing the sawdust and the observation of possible succession of the fungi involved in the decomposition activity.

A similar experiment, providing information on both the identity of the decomposing species and the succession of the species, was carried out, using mini-wood-blocks. Samples of the submerged wood blocks were withdrawn at desired intervals and 'seeded' on Potato Dextrose Agar. The last wood blocks were removed from the lagoon water on the 30th day.

i EXPERIMENT TO INDUCE FRUITING IN A BASIDIOMYCETE

Among the fungi obtained from the baits of the preceding experiments was a basidiomycete which would not fructify on the Potato Dextrose Agar. Cultures were raised on Corn Meal Medium and Sawdust Medium and kept for two months.

(a) Corn Meal Culture

Ten grams of corn were crushed lightly, washed briefly and put into an Erlenmeyer flask. Fifty millilitres of tap water was added and boiled for 10 minutes. Excess water was decanted and the flask covered with loose cottonwool topped with aluminium foil. The flask was autoclaved at 1.1kg/cm^2 for 20 minutes. The mashed corn was inoculated heavily with the mycelium of the Basidiomycete growing on PDA and incubated at room temperature for two months. The culture was moistened with sterile distilled water at 4 days intervals during incubation.

(b) Sawdust Medium Culture

Twenty grams sawdust from the Okobaba sampling station and 100ml of tap water were mixed in a sampling bottle and autoclaved at 1.1kg/cm^2 for 30 minutes. The sterile sawdust was inoculated with the mycelium growing on PDA and incubated at room temperature for two months. The culture was moistened at 4 days intervals with sterile distilled water during incubation.

E. MAJOR COMPONENTS OF THE SAWDUST

Wood decomposing species are usually divided into two groups, the principally cellulose decomposing species and the principally lignin decomposing species (Wood, 1967).

The amounts of these two important wood components in the sawdust were therefore estimated as a guide to the extent to which sawdust would be decomposed in the lagoon in the presence of each of the two groups. In addition, the reducing sugar content and amount of extractives of the sawdust which would affect the activities of both types of decomposers were also determined.

F. GROWTH OF FUNGI ON SAWDUST EXTRACT AGAR

Some of the most frequently occurring fungal species were grown on Sawdust Extract Agar to compare their ability to grow on a sawdust medium. The fungi tested were Aspergillus flavus, Aspergillus giganteus, Aspergillus niger, Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum, Paecilomyces variotii, Penicillium funiculosum and an unidentified basidiomycete. Duplicate plates of each fungus on the Sawdust Extract Agar were incubated at 27 $\pm$ 2°C for 10 days. Growth was assessed qualitatively and any distinct cultural characteristics of the mycelia noted.

Another set of cultures were grown on Lagoon Water Agar under the same incubation conditions.

G. ASSESSMENT OF CELLULOLYTIC ACTIVITIES OF FUNGI ISOLATED FROM SAWMILL STATION IN LAGOS LAGOON

Cellulose was expected to influence greatly the decomposition of the sawdust in view of the high levels (56.65%) found in sawdust. The cellulolytic activities of the fungi isolated deserved study. The fungi employed for the series of experiments carried out were Aspergillus flavus, Aspergillus giganteus, Aspergillus niger, Cladosporium oxysporum, Geotrichum candidum, Paecilomyces variotii and the unidentified basidiomycete; and the different types of cellulose media used were:

i. Carboxymethylcellulose(CMC) Agar

The fungi were grown on carboxymethylcellulose agar and their rate of growth was assessed by measuring periodically the diameters of the cultures.

Another criterion used to compare the abilities of the different fungi was the development of a clear zone, forming a halo around the culture, caused by the breakdown of the carboxymethylccellulose by cellulases released by the growing fungus. The width of the clear zone is therefore directly proportional to the amount of enzyme produced.

ii. CMC - mineral salts agar

The basal mineral salts described in Materials and General Methods (sub-section (d) (1) were added to CMC agar and inoculated with selected fungi.

Growth was assessed by taking an average of two diameters of a colony growing on a Petri plate and the extent of clearing due to enzymatic activities on CMC-mineral salts agar was determined qualitatively.

iii. CMC Agar of different pHs

The effect of pH on the ability of each fungus to utilise CMC was tested. The pH of CMC-mineral salts agar was adjusted to 4.5, 6.0, 7.0 and 8.0 using 0.1NHCl or 0.1N NaOH solution. The culture diameters were measured periodically and cellulolytic activity, as shown by clearing of the CMC-mineral salts agar, was qualitatively assessed.

iv. CMC-NH_4^+ Agar

Some fungi utilize NH_4^+ as a nitrogen source, so $(NH_4)_2SO_4$ was added to CMC agar to observe effect of NH_4^+ on growth and the formation of cellulases by the selected fungi. The diameters of the cultures were measured at intervals and the extent of clearing of the agar recorded.

v. Colloidal Cellulose Agar

Colloidal cellulose is not as compact as CMCellulose and is thus more easily broken down by cellulolytic fungi. The selected fungi were also grown on colloidal cellulose agar to find out whether some of the fungi which were unable to breakdown CMCellulose could attack the loose molecules of colloidal cellulose.

The diameters of the cultures were used in assessing growth of the fungi on this medium and the extent of clearing of the agar in determining the extent of enzyme production.

vi. PRODUCTION OF REDUCING SUGARS

Reducing sugars, e.g. glucose, are produced when enzymes breakdown cellulose molecules. This was expected to occur also during growth of the fungi found in the previous experiments to be capable of breaking down cellulose. These fungi were grown in CMC-mineral salts broth, CMC-NH_4^+ broth and Sawdust Extract - sodium nitrate broth at 27± 2°C for 10 days. The mycelium was then separated from the medium by filtration through Whatman No. 1 filter paper and the amount of reducing sugar in the filtrate was determined by the method of Nelson (1944) and Somogyi (1937 and 1945) as described in Materials and General Methods sub-section (i).

vii. Filter paper medium

The ability of fungi to breakdown cellulose fibres of filter paper was tested. The fungi used were Aspergillus flavus, Aspergillus giganteus, Aspergillus tamarii, Cladosporium oxysporum and Curvularia geniculata. Basal mineral salts medium containing filter paper disks as the sole carbon source was used, and the cultures were incubated at 27±2°C under two different conditions. Half of the cultures were agitated during growth at 100 r.p.m.

on a shaker incubator at $27\pm2^{o}C$, while the remaining half were kept still. A total of 12 cultures was raised in the case of each fungus, six as agitated and six as still cultures. Two flasks were removed at 5 days intervals from each set for determination of ability to attack the filter paper. The filter paper in the medium was carefully removed and rinsed with water to remove any adhering mycelium. It was then put in a dish in the oven at $80^{o}C$ and dried to constant weight. The mean dry weight of each set of filter papers was recorded and the percentage weight loss was calculated. The pH and reducing sugar content of the culture medium were also measured and compared with the initial values recorded at the beginning before growth started.

H. ASSESSMENT OF LIGNOLYTIC ACTIVITIES OF THE FUNGI

Aspergillus flavus, *Aspergillus giganteus*, *Cladosporium oxysporum* and *Paecilomyces variotii* were used in this experiment. The extent of growth of the fungi on a lignin medium was a measure of the extent of its ability to attack lignin.

i. Growth on Lignin Agar

Two types of lignin media were used. The extracted lignin obtained from analytical studies on sawdust, and commercial lignin obtained from Gurr Ltd., England were used in two separate media.

The Lignin Agar was prepared as described in Materials and General Methods sub-section (d) (11). Duplicate plates of each lignin medium were inoculated with mycelial disks of each fungus growing on PDA and incubated at $28\pm2^{o}C$ for 10 days. Growth was assessed qualitatively while the morphology of the mycelium was noted in each case. The extent of a clear zone around any of the colonies due to the activity of lignin decomposing enzymes was also recorded.

ii. Growth on Lignin Agar amended with glucose and mineral salts

Glucose a more easily assimilated carbon source was added to Lignin Agar to test if the supply of a "nutrient starter" will enhance the ability of each fungus to grow on and utilize lignin as a source of energy. Growth at $28\pm2^{o}C$ was again assessed qualitatively after 10 days. The extent of production of clear zones around the colonies was also noted.

Similar experimental procedures were carried out using this time basal mineral salts medium to which lignin was added as the sole carbon source. Both extracted and commercial lignin were employed in two separate preparations.

I. SALINITY TOLERANCE OF THE FUNGI

The ability of some of the fungi to withstand the wide range of salinity occurring in Lagos Lagoon was investigated using Czapek Dox Agar (CDA) amended with different concentrations of NaCl. The fungi used were Aspergillus flavus, Aspergillus giganteus, Aspergillus tamarii, Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum, Paecilomyces variotii and Trichoderma aureoviride.

The total solute content of sea water can be obtained by dissolving 3.4g NaCl in distilled water (Dzawachiszwili, Landau, Newcomer and Plunkett, 1964). The following concentrations of NaCl were prepared:

0.0% NaCl	≒ 0‰	Salinity	≡ 0%	sea water	concentration
0.2% NaCl	= 2‰	"	≡ 6.3%	" "	"
0.4% NaCl	= 4‰	"	≡ 12.5%	" "	"
0.9% NaCl	= 9‰	"	≡ 25%	" "	"
1.7% NaCl	= 17‰	"	≡ 50%	" "	"
3.4% NaCl	= 34‰	"	≡100%	" "	"
6.8% NaCl	= 68‰	"	≡200%	" "	"

The seven solutions above were used to prepare Czapek Dox Agar (CDA). Duplicate Petri plates of each NaCl-amended CDA were inoculated with mycelium disks of the respective test fungus and incubated at $27 \pm 2^{0}C$ for 10 days during which daily measurements of colony

diameters were taken.

Sporulation of fungi growing on the NaCl-amended CDA was also observed daily and any distinct characteristics of the spores were noted. Degree of sporulation was estimated by flooding the agar surface with 10ml distilled water to dislodge the spores, and a drop of spore suspension obtained was put on a haemocytometer slide to estimate the spore density. Spore diameters were measured with the aid of an eye-piece micrometer and the mean diameter of 50 spores in each NaCl treatment was calculated.

J. GERMINATION OF FUNGAL SPORES IN SAWDUST EXTRACT

Spores are very important dispersal units of fungi. It is expected that germinating spores will initiate largely the decomposition of the sawdust put in the lagoon. During their germination and growth of the germ tubes in the 'infection court' the extracts of the sawdust would play a very deciding role. The effects of sawdust extracts of three important West African timber species were therefore studied in a series of experiments. The sawdust extracts used were obtained from three wood species, Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon. Four frequently occurring fungi on Lagos Lagoon, Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride were used as test organisms.

Extracts of sawdust of each wood species were prepared as described in Materials and General Methods sub-section (d) (2).

i. Germination in aqueous extracts of sawdust

The spores were germinated in sawdust extracts of concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0%, respectively. The spores were germinated on glass slides placed on V-shaped glass rods over moist filter paper in sterile Petri dishes (Purkayastha and Deverall, 1965). Two individual drops of sterile extract-spore suspension (about 0.1ml) were placed apart on a glass slide and the suspension incubated at $28\pm2^{o}C$ for 24 hours. At least 200 spores were examined with a microscope and percentage germination was calculated. Germination was considered to have occurred when a germ tube was perceptible. An eye-piece micrometer was used to measure the lengths of germ tubes of 20 spores in each case and the average germ tube length was recorded.

ii. Germination of fungal spores in nutrient-amended sawdust extract of Khaya ivorensis

Chemical compounds of various sorts are present in the Lagos Lagoon. It is impossible that the germinating spores will be influenced only by the sawdust extract. It should be expected that chemicals in the lagoon will influence germination thus altering the effect of sawdust extract.

Such compounds may either enhance germination or inhibit it. The extent to which carbohydrates, nitrogenous compounds and mineral salts may modify the effect of the sawdust extracts was investigated. Aqueous sawdust extract (5%) was prepared as previously described and used amended with the various compounds.

(a) Carbohydrate-amended extract

Five per cent aqueous sawdust extract of Khaya ivorensis was amended with different concentrations (0.5, 1.0 and 2.0%: w/v) of fructose, glucose, maltose and sucrose, and spores were provided by Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. Percentage germination and mean germ tube length were estimated for each treatment.

(b) Amino acid-amended extract

Five per cent aqueous sawdust extract of Khaya ivorensis was amended with different concentrations (0.1, 0.2 and 0.4%: w/v) of alanine, asparagine, glycine and leucine and used in germination tests with spores of Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. Percentage germination and mean germ tube length were calculated for each treatment.

(c) Further tests with Sawdust Extract amended with nitrogenous compounds

Complex nitrogenous compounds, viz., Casein hydrolysate, Peptone and Yeast-extract (Yeastrel) were used in further tests to investigate the effect of nitrogenous compounds. Different concentrations, 0.1, 0.2 and 0.4% (w/v) were added to 5% aqueous sawdust extract of Khaya ivorensis and used in germinating spores of Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. Percentage spore germination and mean germ tube length were determined for each fungus in each treatment.

(d) Mineral salts-amended extract

Different concentrations, 0.01, 0.02 and 0.05% (w/v) of $CaCl_2$, KH_2PO_4, $MgSO_4.7H_2O$ and NaCl were finally added to 5% aqueous extract of sawdust of Khaya ivorensis and the mixtures used in spore germination tests. Spores of Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride were germinated in the mineral salts-amended extract for 24 hours. Percentage germination and mean germ tube length were calculated as usual for each treatment.

iii. Spore germination in lagoon water

Lagoon water was sterilized by passing through Millipore filters (pore = 0.45 μm). Different concentrations, 25, 50, 75 and 100% were prepared by appropriate dilution with sterile distilled water and used in germination tests involving spores of Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. Percentage spore germination and mean germ tube length were estimated for each fungus in the different lagoon water dilutions.

K. DECOMPOSITION OF SAWDUST BY FUNGI

Sawdust of three commonly used wood species, Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon was inoculated with different fungi to investigate the ability of these fungi to decompose the sawdust. Two grams of fresh sawdust were put into a Petri dish, moistened with 12 ml distilled water and autoclaved on three consecutive days at 1.1kg/cm^2 for 20 minutes. Four Petri dishes containing 2g. of fresh sawdust were put in the oven at 80^{o}C and dried to constant weight. The mean dry weight obtained was used as the initial dry weight of the experimental sawdust. Four frequently occurring fungi in Lagos Lagoon - Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride - were used.

The fungi were cultured on PDA for 7 days after which the plates were flooded with 5ml. sterile distilled water to suspend the spores. Then Mycological Peptone medium (prepared in 50ml volumes in 250ml Erlenmeyer flasks) was inoculated with the spore suspensions. The culture flasks were incubated at $27\pm2^{o}C$ for 7 days to obtain surface mycelial mats. Working under aseptic conditions in the inoculating room, the mycelium was macerated with a sterile glass rod. Ten loopfuls of mycelial fragments were stirred into the sawdust in each Petri dish.

i. Effect of temperature on sawdust decomposition

Decomposition at four temperatures covering the range measured at the sawmill station on Lagos Lagoon was studied. The temperatures were 24, 27, 30 and $33^{o}C$. The inoculated sawdust was incubated at the different temperatures for 40 days. There were three replicates for each fungus in each temperature treatment. A few drops of sterile distilled water were added to each Petri plate at 5 days' intervals during incubation to keep the sawdust continually moist. The dry weight of the contents of the Petri dish was determined at the end of the incubation period. The loss in dry weight of the sawdust after 40 days represented the amount of sawdust broken down by the test fungus.

ii. Effect of glucose on sawdust decomposition

Different concentrations, 0.1, 0.5, 1.0 and 2.0% (w/v) of glucose were added to sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon to verify if addition of a more easily assimilated carbon source will increase decomposition of sawdust by fungi. Using the same four fungi, Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride, sterile sawdust in the Petri dishes was inoculated and incubated at 33°C for 40 days. The sawdust was again kept moist by the application of sterile glucose solution of the same concentration at 5 days' intervals. Mean dry weight of three replicates of each treatment was then calculated at the end of the incubation period.

iii. Effect of peptone on sawdust decomposition

Sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon was next amended with 0.1, 0.2, 0.4 and 0.8% (w/v) of peptone solution to investigate this time the effect of the addition of a complex nitrogenous compound (peptone) on sawdust decomposition by Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. The prepared plates were incubated at 33°C for 40 days after which the dry weight of the sawdust was determined. There were three replicates for each treatment.

IV. RESULTS

A. BACTERIA OF SAWDUST-POLLUTED LAGOON WATER

i. Bacterial population

The bacterial population was clearly far greater at the Okobaba sawmill station than at the mid-lagoon station as shown by the data in Tables 2 and 3. When the inoculated plates were incubated at 22°C, the total aerobic count of water from the mid-lagoon station was less than a fifth of that of water from the Okobaba sawmill station. For plates incubated at 37°C, the difference was even greater and the mid-lagoon aerobic population was less than a 10th of that of the sawmill station (Table 2).

There was apparently no variation in the aerobic bacterial population of each station over the period of January to April, 1977.

Table 3 also indicates the presence of a higher number of coliform bacteria in water at the Okobaba sawmill station. The coliform bacterial population at mid-lagoon ranged from 19 to 31 x 10^2 per ml, while that of water at the sawmill station ranged from 62 to 84 x 10^2 per ml.

TABLE 2

Bacterial population of Lagos Lagoon water at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Total viable aerobic count ($x10^{-5}$) per ml. on inoculated plates incubated for 72 hours at			
	22°C		37°C	
	Sawmill station sample	Mid-lagoon station sample	Sawmill station sample	Mid-lagoon station sample
1977				
January				
13	26.4	-*	>3.0	-
31	25.6	4.3	2.4	0.2
February				
16	26.6	3.8	3.0	0.2
28	25.1	4.1	>3.0	0.1
March				
16	27.2	4.6	2.1	0.2
31	28.5	4.4	2.5	0.2
April				
15	28.8	4.5	2.4	0.2
30	30.0	4.8	2.4	0.2

* No sample taken.

TABLE 3

Number ($x10^{-2}$) of coliform bacteria per ml. of lagoon water from Okobaba Sawmill and mid-lagoon stations recorded on inoculated EMB Agar plates after 24 hours at $37^{o}C$.

Date	Water sample from	
	Okobaba sawmill station	Mid-lagoon station
1977		
January		
13	62	-*
31	70	19
February		
16	73	25
28	68	29
March		
16	75	28
31	71	30
April		
15	78	29
30	84	31

* No sample taken.

ii. General characteristics of the bacteria

A list of bacteria isolated from water at the Okobaba sawmill station using EMB Agar and Nutrient Agar are presented in Tables 4 and 5. Rods constituted the predominant bacteria. All the isolates on EMB Agar, and five of the nine isolates on Nutrient Agar were rods. There were, on the other hand, approximately the same number of Gram negative and positive isolates. Seven of the isolates were Gram negative and six were Gram positive.

It was possible to separate, easily, the isolates by their distinctive cultural characteristics and the number of isolates in Tables 4 and 5 represented the number of genera present.

B. FUNGI OF SAWDUST-POLLUTED LAGOON WATER

i. Fungal population

Population studies on the fungi confirmed the findings of the bacterial studies. The presence of sawdust at the Okobaba sawmill station greatly encouraged fungal growth. The population at this site was consequently far greater than that of the mid-lagoon station when isolation was done with both Potato-Dextrose and Malt-Extract Agar (Fig. 5).

TABLE 4

Characteristics of colonies of bacterial isolates appearing on EMB Agar plates inoculated with Lagos lagoon water from Okobaba Sawmill station and incubated at 37°C for 24 hours.

Isolate	Colony form	Cell type	Gram staining reaction
A	Dark red with green metallic sheen, 2-3mm diameter, convex, smooth edge having butry consistency.	rods	-ve
B	Pink, 3mm diameter, raised, smooth edge and mucoid.	rods some in chains	-ve
C	Cream/pale pink, 3-4mm diameter, flat, spreading, undulate edge and watery.	rods	-ve
D	Pink, tiny, 1-2mm diameter, flat, smooth-edge and butry.	short rods	-ve

TABLE 5

Characteristics of colonies of bacterial isolates appearing on Nutrient Agar plates inoculated with Lagos lagoon water from Okobaba Sawmill station and incubated at 22°C for 72 hours.

Isolate	Colony form	Cell type	Gram staining reaction
A	White, wrinkled papillate surface, with crenated margin, 3-5mm diameter and membranous.	rods	+ve
B	Cream, raised with undulate margins, 2-3mm diameter, butry.	rods having central/ subterminal spores	+ve
C	White, 1mm diameter, low convex with smooth margin and granular.	staphylococci	+ve
D	Pale yellow-lemon, 2mm diameter, flat, smooth margin, butry.	streptococci 2-4 cells in chain	+ve
E	Yellow, 2-3mm diameter, convex, smooth margin, mucoid.	cocci	+ve

TABLE 5 (Cont'd)

Characteristics of colonies of bacterial isolates appearing on Nutrient Agar plates inoculated with Lagos lagoon water from the Okobaba Sawmill station and incubated at 22°C for 72 hours.

Isolate	Colony form	Cell type	Gram staining reaction
F	Pink, 2-3mm diameter, convex, smooth margin and butry.	cocci	+ve
G	Cream, 4mm diameter, flat, undulate margin and slightly mucoid.	fat rods	+ve
H	Blue/green, 4mm diameter, flat, spreading with undulate margin, watery.	long rods	-ve
I	Cream, 3-4mm diameter, low convex, smooth margin, butry.	rods	-ve

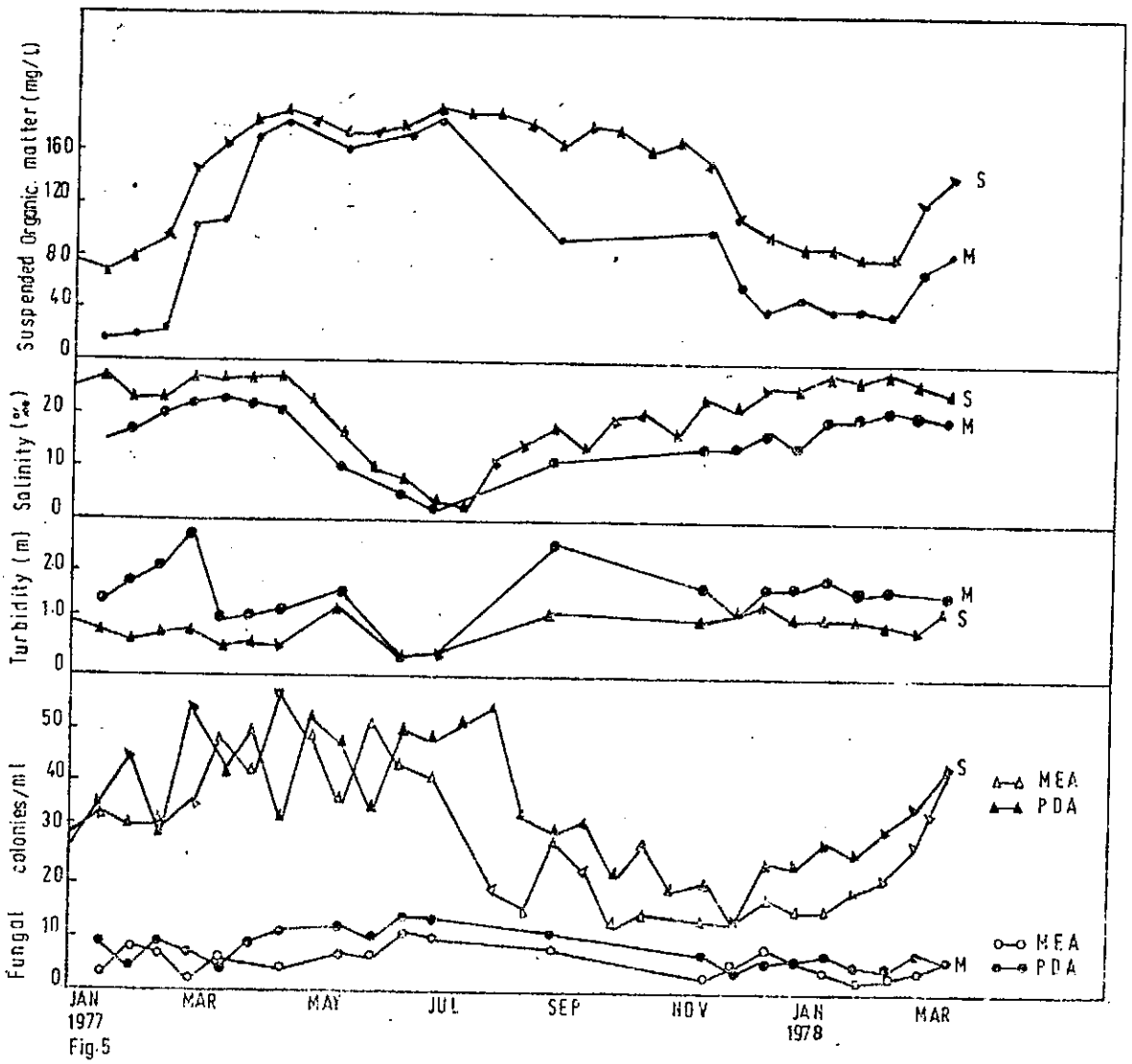


Fig.5

S = Sawmill station

M = Mid – lagoon station

There were as many as 52-56 colonies per ml. of water (March, April, May 16 and August 17, 1977) from the Okobaba sawmill station on the occasion of highest population counts in contrast to greatest number of colonies of 14 per ml. of mid-lagoon water recorded on June 29 and July 13, 1977. Curiously, Corn Meal Agar which was included during the first three months of this study was found to be extremely unsuitable. Fungal growth was so scanty that it was excluded from subsequent studies. Potato Dextrose Agar was a better medium than Malt Extract Agar as it always supported a greater number of colonies.

Fluctuation in population over the period of studies could be seen particularly clearly in the population of the Okobaba sawmill station. The population rose from January 1977 to a peak in March to August 1977, and declined from late August to reach the lowest level in November and December 1977. The population then rose till March 1978 when sampling was discontinued. The same pattern of population fluctuation was noticeable in the mycoflora of the mid-lagoon station.

There was a negative correlation between salinity and fungal count, and turbidity and fungal count of the mid-lagoon and sawmill stations (Table 6).

TABLE 6

The relation between fungal counts and varying physico-chemical parameters in Lagos Lagoon (tested with correlation coefficient (r) and t-test statistics).

Physico-chemical parameters	Sampling stations	Correlation coefficient (r)	t-test (t)
Salinity	Mid-lagoon	-0.1558	-0.6694
	Sawmill	-0.3668	-2.0864*28
Suspended matter	Mid-lagoon	0.6839	3.9771*18
	Sawmill	0.4255	2.4877*28
Turbidity	Mid-lagoon	-0.3516	-1.5937
	Sawmill	-0.5878	-3.1668*19

* Significant at 5% level with the indicated d.f.

The higher the salinity or turbidity of the lagoon, the lesser was the fungal count. However, the negative correlation was not significant at the mid-lagoon station with correlation coefficient (r) values of -0.1558 and -0.3516 (5% level and 18 d.f.), for salinity and turbidity respectively. Tabulated r-value is 0.4438 at 5% level of significance and 18 d.f. The negative correlation was significant at the sawmill station with r-values of -0.3668 and -0.5878 for salinity and turbidity respectively (tabulated r-value 0.3494 at 5% level of significance and 28 d.f.). When the t-test was applied to further verify the significance of r, the sawmill station showed significant negative correlation with t-values of -2.0864 and -3.1668 for salinity and turbidity respectively (tabulated t-value at 5% level of significance and 28 d.f. = 2.0864).

The correlation between suspended matter content and the fungal count of Lagos Lagoon at both the mid-lagoon and sawmill stations was significant (Table 6). The more the suspended matter in the lagoon, the higher was the fungal count. It is noteworthy that the relation was more significant at the mid-lagoon station.

ii. Fungal species

A full list of fungi isolated from the two stations is presented in Table 6a. The Aspergilli were the predominant fungi isolated from the Okobaba sawmill station, while a Mucor sp. occurred in greatest number in water from the mid-lagoon station. Water of the Okobaba sawmill station did not only contain a greater fungal population but also supported a greater number of species. Thus, only five species were isolated from the mid-lagoon station and 16 from the sawmill station.

A total of five Aspergillus species were found at the Okobaba sawmill station and only two at the mid-lagoon. Besides the Aspergilli, the only genera occurring at both stations were Cladosporium, Mucor and Penicillium. Seven genera found in the water of the sawmill station, Curvularia, Fusarium, Geotrichum, Paecilomyces, Trichoderma, yeast and an unidentified basidiomycete were absent in the mid-lagoon water.

The Aspergilli of the sawmill station constituted 48.9% of the total fungal population. Among the non-Aspergilli, Cladosporium, Trichoderma, Penicillium, Mucor and Curvularia, in that order, were the next important genera isolated, forming together 39.3% of the total population. In the mid-lagoon water, Mucor was the most abundant genus (51.4%) followed by Aspergillus (30.6%).

TABLE 6a

Fungi isolated from Lagos lagoon water from Okobaba Sawmill and mid-lagoon stations with Potato Dextrose Agar.

Fungal species	Okobaba Sawmill station		Mid-lagoon station	
	No. of colonies	% of total population	No. of colonies	% of total population
Aspergillus flavus Link ex Fr.	127	12.0	0	0
Aspergillus fumigatus Fres.	61	5.8	9	5.2
Aspergillus giganteus Wehmer	104	9.9	0	0
Aspergillus niger Van Tiegh.	181	17.2	44	25.4
Aspergillus tamarii Kita	42	4.0	0	0
Cladosporium oxysporum Berk. et Curt.	159	15.1	0	3.5
Curvularia geniculata (Tracy et Earle)Boedijn	55	5.2	0	0
Fusarium sp.	22	2.1	0	0
Geotrichum candidum Link ex Persoon	34	3.2	0	0
Mucor sp.	56	5.3	89	51.4

TABLE 6a(Cont'd)

Fungi isolated from Lagos lagoon water from Okobaba Sawmill and mid-lagoon stations with Potato Dextrose Agar.

Fungal species	Okobaba Sawmill station		Mid-lagoon station	
	No. of colonies	% of total population	No. of colonies	% of total population
Paecilomyces variotii Bainier	33	3.1	0	0
Penicillium species	60	5.7	25	14.5
Trichoderma aureoviride Rifai agar.	84	8.0	0	0
Yeasts	21	2.0	0	0
Basidiomycete isolate	15	1.4	0	0
Total population	1054	100.0	173	100.0

The basidiomycete isolated from the Okobaba sawmill station failed to fructify on all the media tried.

iii. Fungal species isolated under anaerobic conditions

In the preceding experiment the fungi were isolated under conditions that permitted free ingress of oxygen onto the cultures. Some of the species may be able also to grow under anaerobic conditions and might be thus more adaptable to the low oxygen conditions of the polluted areas of the lagoon. Such species were identified by incubating inoculated Potato Dextrose Agar under anaerobic condition. Fungi which grow under this condition are shown in Tables 7 and 8. Species listed in Table 7 grew on plates incubated in an anaerobic jar and fungi of Table 8 developed on plates in the Heraeus anaerobic incubator.

It was not surprising that few fungi grew under anaerobic conditicns. Mucor was the predominant fungus. It is worthy of note that the yeast, Candida famata occurred in water at the sawmill station but not in mid-lagoon water.

TABLE 7

Fungi developing on Potato Dextrose Agar inoculated with one ml. water samples from Okobaba Sawmill and mid-lagoon stations and incubated in anaerobic jar at $27\pm2^{o}C$ for 5 days.

Fungus	Fungi in sawmill station water		Fungi in mid-lagoon water	
	Number of isolates	% of total population	Number of isolates	% of total population
Aspergillus flavus	3	13.0	0	0.0
Aspergillus niger	4	17.4	2	28.6
Candida famata	3	13.0	2	28.6
Debaryomyces hansenii	5	21.7	0	0.0
Mucor sp.	7	30.4	3	42.8
Paecilomyces variotii	1	4.3	0	0.0

TABLE 8

Fungi developing on Potato Dextrose Agar inoculated with water samples from Okobaba Sawmill and mid-lagoon stations and incubated in anaerobic incubator at $27\pm2^{o}C$ for 5 days.

Fungus	Fungi in Sawmill station water		Fungi in mid-lagoon water	
	Number	% of total population	Number	% of total population
Aspergillus flavus	3	10.0	0	0
Aspergillus niger	5	16.7	4	28.6
Candida famata	3	10.0	0	0
Debaryomyces hansenii	2	6.7	2	14.3
Mucor sp.	10	33.3	8	57.1
Penicillium species	6	20.0	0	0
Trichoderma aureoviride	1	3.3	0	0

Furthermore, the absence of Aspergillus flavus, Paecilomyces variotii and Trichoderma aureoviride in the mid-lagoon water under aerobic incubation conditions (See Table 6a)has been confirmed by the anaerobic experiments.

C. PHYSICO-CHEMICAL PROPERTIES OF THE WATER AT THE TWO STATIONS

The physico-chemical properties of the water in which the bacteria and fungi were growing are given in Tables 9 to 14 and Fig. 5. The following observations were made.

i. Temperature

There were three noticeable features (Table 9). First, the temperatures of the water at both stations did not show a recognisable seasonal variation. The air temperatures in December, 1977 and January 1978, however, appeared to be lower than temperatures of other months.

Secondly, a rise in air temperature was not necessarily accompanied by a corresponding rise in water temperature and vice-versa. Thus, the water temperatures in December 1977 (26.0-29.0^{o}C) when the air temperatures were lowest (22.0-25.0^{o}C), were the same as water temperatures of September and October, 1977 (26.0-29.5^{o}C) with higher air temperatures of

TABLE 9

Temperature (°C) of air and lagoon water at Okobaba sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station		Mid-lagoon station	
	Air	Water	Air	Water
1977				
January				
13	26.5	28.0	-*	-
31	27.0	27.5	26.0	28.0
February				
16	30.5	28.5	31.5	29.2
28	29.5	28.0	28.0	30.0
March				
16	30.0	29.5	33.0	30.0
31	26.0	29.0	27.5	29.5
April				
15	30.5	29.5	29.0	30.0
30	28.0	29.0	28.5	29.0
May				
16	29.0	33.0	-	-
31	28.5	29.0	29.2	28.5
June				
15	24.0	28.0	-	-
29	28.5	27.0	27.0	27.0

* Readings not taken.

TABLE 9 (Cont'd)

Temperature (°C) of air and lagoon water at Okobaba sawmill and mid-lagoon stations on sampling days.

	Sawmill station		Mid-lagoon Station	
Date	Air	Water	Air	Water
1977				
July				
13	26.0	27.0	26.5	26.5
28	28.0	27.0	-	-
August				
17	24.5	26.0	-	-
31	29.0	25.0	-	-
September				
16	29.0	26.0	29.0	26.0
29	28.0	27.0	-	-
October				
15	28.0	27.5	-	-
31	28.5	29.5		
November				
15	26.5	29.0	-	-
30	28.0	30.0	28.5	29.5
December				
15	24.0	28.0	25.0	29.0
30	24.0	27.0	22.0	26.0

TABLE 9 (Cont'd)

Temperature (°C) of air and lagoon water at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station		Mid-lagoon station	
	Air	Water	Air	Water
1978				
January				
16	24.0	27.5	25.5	28.0
31	27.0	29.0	26.0	29.0
February				
14	29.5	30.0	31.0	30.0
28	30.5	29.5	29.5	30.0
March				
15	29.0	30.0	30.0	31.0
29	30.0	29.5	31.0	30.0
Mean	27.7	28.4	28.2	28.8
Range	24.0-30.5	25.0-33.0	22.0-33.0	26.0-31.0

28.0 and 29.0°C. It seemed that other factors were influencing the water temperature besides the air temperature.

Thirdly, there was no marked difference between the two stations with regard to both air and water temperatures. Where differences occurred they were usually only 0.5 or 1.0°C. The means for the air temperatures and those for the water temperatures were therefore close. The mean air temperatures for the sawmill station and mid-lagoon were 27.7 and 28.2°C and the mean water temperatures, 28.4 and 28.8°C, respectively.

ii. Transparency/Turbidity

Water of the sawmill station was more turbid than the mid-lagoon water (Fig. 5), and the Secchi disk could be observed at greater depths, mean of 1.5 metres, at mid-lagoon than at the sawmill station (0.9 metre). The water at both stations became very cloudy in June and July, 1977 and became generally clearer from September 1977 to March, 1978.

iii. Dissolved Oxygen (D.O.)

The results in Table 10 show the marked difference between the levels of dissolved oxygen, at each testing time, of the Okobaba sawmill and the mid-lagoon stations.

TABLE 10

Amount of dissolved oxygen (mg/l) in the Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
January		
13	0.4	*
31	0.4	0.8
February		
16	0.4	0.8
28	0.4	0.6
March		
16	0.4	0.5
31	0.4	0.6
April		
15	0.4	0.7
30	0.4	0.4
May		
31	0.4	0.4
July		
13	0.6	0.7
August		
31	0.4	-

* No determination made.

TABLE 10 (Cont'd)

Amount of dissolved oxygen (mg/l) in the Lagos Lagoon at the Okobaba Sawmill and mid-lagoon Stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
September		
16	0.5	0.6
29	0.4	-
October		
15	0.4	-
31	0.4	-
November		
15	0.4	-
30	0.4	0.6
1978		
January		
31	0.4	0.6
February		
14	0.4	0.7
28	0.4	0.4
March		
15	0.4	0.5
29	0.4	0.4
Mean	0.4	0.6
Range	0.4-0.6	0.4-0.8

There was consistently lower level of dissolved oxygen at the Okobaba sawmill station and the mean values for the sawmill and mid-lagoon stations were 0.4 and 0.6 mg/l, respectively, while the ranges for the two stations were 0.4-0.6 and 0.4-0.8 mg/l, respectively.

iv. pH

Generally, water at the two stations was slightly alkaline (Table 11). A greater pH range (pH 6.5-7.6) was recorded at the sawmill station than at mid-lagoon (pH 7.0-7.5). However, very few of the pH values at the sawmill station fell below pH 7.0, bringing the means close together: pH 7.1 and 7.2 for the sawmill station and mid-lagoon, respectively.

Any seasonal variation might have been offset by other factors, because pH recorded in months of the year which were covered in 1977 and 1978 did not show much similarity. At the sawmill station, the pHs for January, 1977 were pH 6.5 and 6.7 and for January, 1978 pH 7.5 and 7.4, and in February, 1977, pH 7.4 and 7.6 in comparison to pH 7.2 and 7.0 in February, 1978.

In mid-lagoon with less pollution from sawdust, pH of the water was lower in May-September, 1977 (pH 7.0-7.1) than in the preceding period, January-April, 1977 (pH 7.1-7.5) and the succeeding period, November, 1977 to March 1978 (pH 7.0-7.5).

TABLE 11

Hydrogen ion concentration (pH) of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
January		
13	6.5	-*
31	6.7	7.3
February		
16	7.4	7.5
28	7.6	7.5
March		
16	7.0	7.1
31	7.3	7.5
April		
15	7.6	7.3
30	7.1	7.3
May		
16	6.9	-
31	7.0	7.0

* No determination made.

TABLE 11 (Cont'd)

Hydrogen ion concentration (pH) of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
June		
15	7.5	-
29	7.1	7.0
July		
13	6.9	7.0
28	7.3	-
August		
17	7.1	-
31	6.8	-
September		
16	7.2	7.1
29	6.9	-
October		
15	7.0	-
31	7.1	-

TABLE 11 (Cont'd)

Hydrogen ion concentration (pH) of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
November		
15	7.4	-
30	7.5	7.5
December		
15	7.2	7.3
30	7.4	7.2
1978		
January		
16	7.5	7.2
31	7.4	7.2
February		
14	7.2	7.1
28	7.0	7.0
March		
15	7.1	7.2
29	7.0	7.1
Mean:	7.1	7.2
Range:	6.5-7.6	7.0-7.5

v. Salinity

The results in Fig. 5 show that salinity was greater in the water at the Okobaba sawmill station than in mid-lagoon water. From January to early May, 1977, salinity at the sawmill station was between 21.0 and 26.2‰ and at mid-lagoon from 14.5 to 21.8‰. Very low salinity was recorded at both stations from late May to September, 1977, ranging from 1.8 to 16.8‰ and 0.2 to 10.8‰ at the sawmill and mid-lagoon stations, respectively. High salinity occurred again from November, 1977 to March, 1978. The respective salinity values for the two stations rising to 16.4-28.9 and 13.9-20.9‰. There was thus a distinct uniform salinity fluctuation at both stations. The high and low salinities corresponded to the dry and rainy seasons in Lagos.

vi. Sulphides

There was a strong foul odour at the sawmill station suggestive of the presence of hydrogen sulphide gas. Lead acetate paper tests showed that whereas no hydrogen sulphide gas could be detected at mid-lagoon, the gas was detected on most occasions at the sawmill (Table 12). There was a definite trend in the production of the gas at this station.

TABLE 12

Amount of hydrogen sulphide gas in Lagos Lagoon at Okobaba Sawmill and Mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
January		
13	+++**	N.R.*
31	+++	-
February		
16	+++	-
28	+++	-
March		
16	+++	-
31	++	-
April		
15	++	-
30	++	-
May		
16	++	N.R.
31	++	-

* Not recorded.

** Degree of blackening of lead acetate paper:

-, white; +, brown; ++, black; +++, very black.

TABLE 12 (Cont'd)

Amount of hydrogen sulphide gas in Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
June		
15	++	N.R.
29	+	N.R.
July		
13	-	-
28	+	N.R.
August		
17	-	N.R.
31	-	N.R.
September		
16	-	-
29	+	N.R.
October		
15	+	N.R.
31	-	N.R.

TABLE 12 (Cont'd)

Amount of hydrogen sulphide gas in Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station	Mid-lagoon station
1977		
November		
15	++	N.R.
30	+	-
December		
15	++	-
30	++	-
1978		
January		
16	++	-
31	++	-
February		
14	++	-
28	+++	-
March		
15	+++	-
29	++	-

Higher quantities of hydrogen sulphide gas were produced in January to May, 1977 and November, 1977 to March, 1978 than in June to October, 1977.

vii. Sulphates

Barium chloride was used to precipitate the sulphate from the water samples. The results in Table 13 show that there was slightly more sulphate at the sawmill station than at mid-lagoon. A fluctuation in the amount of sulphates, especially at the Okobaba sawmill station, could be detected. The total sulphate at the sawmill station was higher from February to early June, 1977 (between 1761.2 and 2113.1 mg/l) and from December, 1977 to March, 1978 (between 1790.0 and 2310.6 mg/l), but lower from late June to November, 1977 (between 1530.8 and 1790.0 mg/l). Except for a drop in the sulphate level on March 29, 1978, water at the mid-lagoon station also followed this pattern of variation. It is noteworthy that the decrease in sulphate content of the lagoon coincided with the rainy season.

viii. Calcium and magnesium content

Similar to sulphate, calcium was found in higher quantities at the sawmill station than at mid-lagoon.

TABLE 13

Amount of sulphates in Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station		Mid-lagoon station	
	$BaSO_4$ (mg)	Total $SO_4^{=}$ (mg/l)	$BaSO_4$ (mg)	Total $SO_4^{=}$ (mg/l)
1977				
February				
16	924	1901.1	720	1481.4
28	912	1876.4	722	1485.5
March				
16	988	2032.8	849	1746.8
31	930	1913.5	841	1730.4
April				
15	991	2039.0	910	1872.3
30	1027	2113.1	913	1878.5
May				
16	856	1761.2	-*	-
31	890	1831.2	841	1730.4
June				
15	864	1777.7	-	-
29	848	1744.8	790	1625.4

* No sample taken.

TABLE 13 (Cont'd)

Amount of sulphates in Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station		Mid-lagoon station	
	$BaSO_4$ (mg)	Total $SO_4^{=}$ (mg/l)	$BaSO_4$ (mg)	Total $SO^{=}_4$ (mg/l)
1977				
July				
13	826	1699.5	788	1621.3
28	793	1631.6	-	-
August				
17	811	1668.6	-	-
31	790	1625.4	-	-
September				
16	802	1650.1	793	1631.6
29	744	1530.8	-	-
October				
15	816	1678.9	-	-
31	852	1753.0	-	-
November				
15	870	1790.0	-	-
30	836	1720.1	820	1687.2

TABLE 13 (Cont'd)

Amount of sulphates in Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Sawmill station		Mid-lagoon station	
	$BaSO_4$ (mg)	Total $SO_4^{=}$ (mg/l)	$BaSO_4$ (mg)	Total $SO_4^{=}$ (mg/l)
1977				
December				
15	911	1874.4	862	1773.6
30	906	1864.1	779	1602.8
1978				
January				
16	923	1899.1	898	1847.6
31	870	1790.0	711	1462.9
February				
14	1181	2430.0	847	1742.7
28	1091	2244.7	1007	2071.9
March				
15	991	2039.0	862	1773.6
29	1123	2310.6	692	1423.8
Mean	905.9	1864	823.4	1694.2
Range	744-1181	1530.8-2430.0	692-1007	1423.8-2071.9

A relationship between the seasons and calcium content was clearly discernable. In the dry season, from November to April, a high calcium content occurred, reaching a level of 1312 (January 13, 1977) to 2440 p.p.m. (February 28, 1978) while a low calcium content, mostly below 1000 ppm (June, July, August and September 29, 1977) was obtained in the remaining months which cover the rainy season. The cited values came from the sawmill station recordings. Data of the mid-lagoon followed similar pattern of fluctuation (Table 14).

Magnesium did not show any clear pattern either in relation to the position of the stations or to the seasons. It was observed that values for the sawmill station were sometimes greater (e.g. February 16 and 28, March 16, November 30, December 15 and 30 1977, and January 16 and 31, and March 15, 1978) or smaller than (e.g. April 15, May 31, September 16, 1977 and February 14 and March 29, 1978) or approximately the same (e.g. January 31, June 29 and December 30, 1977 and February 28, 1978) as those of the mid-lagoon station.

Naturally, in the absence of any recognisable pattern of occurrence of magnesium, the Ca^{++}/Mg^{++} ratio did not show any distinct pattern either.

TABLE 14

Calcium (Ca^{++}) and Magnesium (Mg^{++}) content of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Ca^{++} (ppm) at		Mg^{++} (ppm) at	
	Sawmill station	Mid-lagoon station	Sawmill station	Mid-lagoon station
1977				
January				
13	1312	-**	96(13.7)*	-
31	1430	1104	93.6(15.3)	96(11.5)
February				
16	1672	1300	124.8(13.4)	75.6(17.2)
28	1636	1412	92.4(17.7)	81.6(17.3)
March				
16	2400	1920	38.4(62.5)	21.4(89.7)
31	2132	1876	34.8(61.3)	38.4(48.9)
April				
15	2384	1856	45.6(52.3)	81.6(22.7)
30	1836	1552	112.8(16.3)	100.8(15.4)
May				
16	1292	-	192(6.7)	-
31	1020	970	48(21.3)	65.8(14.7)

* Figures in bracket are Ca^{++}/Mg^{++} ratio.

** No determination made.

TABLE 14 (Cont'd)

Calcium (Ca^{++}) and Magnesium (Mg^{++}) content of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Ca^{++} (ppm) at		Mg^{++} (ppm) at	
	Sawmill station	Mid-lagoon station	Sawmill station	Mid-lagoon station
1977				
June				
15	564	-	24(23.5)	-
29	456	412	16.8(27.2)	16(25.8)
July				
13	280	122	9.3(30.1)	10.4(11.7)
28	152	-	4.8(31.7)	-
August				
17	672	-	60(11.2)	-
31	936	-	57(16.3)	-
September				
16	2052	680	43.2(47.5)	50.4(13.5)
29	904	-	4.8(188.3)	-
October				
15	1132	-	21.6(52.4)	-
31	1368	-	9.6(142.5)	-

TABLE 14 (Cont'd)

Calcium (Ca^{++}) and Magnesium (Mg^{++}) content of Lagos Lagoon at Okobaba Sawmill and mid-lagoon stations on sampling days.

Date	Ca^{++} (ppm) at		Mg^{++} (ppm) at	
	Sawmill station	Mid-lagoon station	Sawmill station	Mid-lagoon station
1977				
November				
15	1432	-	16.8(85.2)	-
30	1640	1296	24(68.3)	14.4(90.0)
December				
15	1348	1120	28.8(46.8)	21.6(51.9)
30	1536	1004	21.6(71.1)	19.2(5.9)
1978				
January				
16	1724	1224	48.0(35.9)	31.2(39.2)
31	1636	1128	55.2(29.6)	43.2(26.0)
February				
14	2235	1588	93.5(23.9)	100.0(15.9)
28	2440	2012	137.7(17.7)	135.5(14.8)
March				
15	2384	1876	120.8(19.7)	111.0(16.9)
29	2056	1758	88.2(23.3)	98.6(17.8)

ix. Copper and iron content

There was no measurable level of copper in water samples collected from Okobaba sawmill and mid-lagoon stations on analysis with the Perkin Elmer Atomic Absorption Spectrophotometer.

Only traces of iron were detected, readings being about 0.2 and 0.1 ppm for Okobaba sawmill and mid-lagoon water samples, respectively.

x. Suspended organic matter

It was reasonable to expect that, the sawmill station receiving such heavy loads of sawdust must contain greater amount of suspended organic matter. Although this was so, the diferences in the corresponding values obtained for the two stations were not of the same magnitude. Thus, whereas large differences, between 51 and 59 mg/l were recorded in January to March, 1977 and 70 mg/l on September 16, 1977, very small differences, for example 8 mg/l (April 30, 1977) and 13 mg/l (July 13, 1977) occurred on other occasions. Results of suspended organic matter are presented in Fig. 5.

Suspended matter was made up of materials other than sawdust, and any variation in the levels at any one station may be caused by several factors.

The high levels of suspended organic matter in the rainy season (March to October-November) may be due to silt brought down by the swollen rivers entering the lagoon.

xi. Tides

Data for height of the tide at sampling time are presented in the Appendix. Most of the water samples for different analyses were collected during low tide (height less than 1.0 m).

D. FUNGI ASSOCIATED WITH WOOD IN THE LAGOON WATER

i. Microscopic observation of decomposing sawdust

Different types of micro-organisms were found to be associated with decomposing sawdust which were collected from the Okobaba sawmill station. Many algae, especially members of the class Cyanophyceae (blue/green) were present. Many of the algae were spherical in shape. Both the algae and the numerous protozoa observed were not identified any further. Septate fungal hyphae were found growing on the sawdust particles. In the absence of any reproductive structures, however, identification was not possible.

ii. Fungi isolated from decomposing sawdust

Table 15 shows the fungi which developed from decomposing sawdust collected from the bank at the sawmill station.

TABLE 15

Fungi developing on Malt Extract Agar and Neopeptone Dextrose Agar inoculated with decomposing sawdust from Okobaba Sawmill station and incubated at $27\pm2^{o}C$ for 7 days

Fungal species	Agar Medium	
	Malt Extract	Neopeptone Dextrose
Aspergillus flavus	++*	-
Aspergillus fumigatus	+	-
Aspergillus giganteus	+	+
Aspergillus niger	+++	++
Fusarium sp.	-	+
Paecilomyces variotii	+	-
Penicillium species	+++	-

*-, absent; +, few; ++, abundant; +++, very abundant.

Although Malt Extract Agar was a better isolation medium, using the Neopeptone Dextrose Agar in addition proved justifiable, as it supported Fusarium species which either failed to grow at all on Malt Extract Agar or was over-grown or inhibited by other fungi on the medium. The list came from test carried out in March 1977. A month later, that is April, 1977, the exercise was repeated using on that occasion three media, viz., Potato Dextrose Agar, Malt Extract Agar and Leonard's Agar. The second isolation experiment was expected to both confirm the previous findings and possibly reveal other sawdust decomposing species. It would be observed that new species were found in the second experiment. These were Aspergillus tamarii, Curvularia geniculata, Trichoderma aureoviride, and an unidentified basidiomycete.

Although, Aspergillus was the most predominant genus, and among the five species isolated, A. niger was consistently the most abundant on all the four media. It was impossible to tell from the mycelial features only, whether the basidiomycete isolate which grew on the Potato Dextrose Agar (Table 16) and the isolate obtained from the lagoon water (Table 6a) belong to the same species or even genus.

TABLE 16

Fungi developing on different agar media inoculated with decomposing sawdust from Okobaba Sawmill station and incubated at $27\pm2^{o}C$ for 7 days.

Fungal species	Agar Medium		
	Potato-Dextrose	Malt-Extract	Leonard's
Aspergillus flavus	*+	++	-
Aspergillus giganteus	++	+	-
Aspergillus niger	+++	+++	+++
Aspergillus tamarii	++	-	-
Curvularia geniculata	-	-	+
Fusarium sp.	-	+	-
Penicillium species	+	++	+
Trichoderma aureoviride	-	+	-
Basidiomycete isolate	+	-	-

*-, absent; +, few; ++, abundant; +++, very abundant.

Without any knowledge of how long the sawdust has been submerged, it is not possible to place the flora obtained in its correct position in the succession of fungi colonizing the particles. An indication of this was obtained from baiting experiments which were carried out as a sequel to this.

iii. Fungi invading sawdust baits

Samples of sterile sawdust in bags and submerged in lagoon water from the Okobaba sawmill station were withdrawn after specific periods and plated on different agar media. Species which developed on the plates inoculated with each sample are shown in Table 17. The salient features of the results of this experiment were:

(a) the number of fungi increased with time until the 10th day of baiting and then declined.

(b) Mucor sp., a rapidly growing species, was isolated only on baits kept in the water for not more than 5 days.

(c) Curvularia geniculata and Fusarium sp. occurred only on 5 and 10-day bait and never in subsequent isolations.

TABLE 17

Fungi occurring on sawdust baits after submergence for varying periods in Lagos Lagoon water from Okobaba Sawmill station.

Baiting period (days)	Fungus	Number of colonies on agar medium after 7 days		
		Potato-Dextrose	Malt-Extract	Sawdust-Extract
3	Aspergillus flavus	8	1	0
	Aspergillus niger	5	6	2
	Mucor sp.	13	7	0
	Yeast species	4	2	0
5	Aspergillus flavus	7	4	0
	Aspergillus fumigatus	6	2	0
	Aspergillus niger	10	7	2
	Cladosporium oxysporum	5	0	0
	Fusarium sp.	2	2	0
	Mucor sp.	5	0	0
	Paecilomyces variotii	3	5	0
10	Aspergillus fumigatus	6	3	0
	Aspergillus giganteus	9	4	1
	Curvularia geniculata	3	0	0
	Fusarium sp.	4	1	0
	Trichoderma aureoviride	6	2	0
	Yeast species	2	0	0

TABLE 17 (Cont'd)

Fungi occurring on sawdust baits after submergence for varying periods in Lagos Lagoon water from Okobaba Sawmill station.

Baiting period (days)	Fungus	Number of colonies on agar medium after 7 days		
		Potato-Dextrose	Malt-Extract	Sawdust-Extract
20	Aspergillus flavus	1	0	0
	Aspergillus giganteus	5	3	0
	Cladosporium oxysporum	8	1	1
	Trichoderma aureoviride	6	0	0
	Yeast species	3	1	0
30	Aspergillus giganteus	2	4	1
	Aspergillus niger	5	2	0
	Cladosporium oxysporum	7	2	0
	Trichoderma aureoviride	2	0	0
40	Aspergillus niger	4	1	0
	Cladosporium oxysporum	4	0	0
48	Aspergillus niger	2	1	0
	Cladosporium oxysporum	3	1	0
	Yeast species	3	0	0

(d) Aspergilli were always present on the baits.

(e) Aspergillus niger colonized the fresh sawdust and persisted throughout the period of 48 days.

(f) Aspergillus niger, Cladosporium oxysporum and yeasts constituted the flora of sawdust of advanced stage of decomposition.

(g) Potato Dextrose Agar was the best medium for isolation and Sawdust Extract Agar was the poorest.

iv. Fungi invading mini-wood blocks

Fairly similar results were obtained when mini-wood blocks were submerged in the water and the best medium (Potato Dextrose Agar) only was used (Table 18). Mucor sp. was an early colonizer which disappeared after only a few days. In fact, it was present only on the 3-day baits. The Aspergilli were again the most important genus and were present on all the baits. Fungi on the longest submerged, 30-day baits were Aspergillus fumigatus, Aspergillus niger, Cladosporium oxysporum and yeasts. Finally, Curvularia geniculata did not survive beyond 5 days on the baits while Fusarium sp., another short-lived species on the sawdust baits, was not isolated at all.

TABLE 18

Fungi occurring on mini-wood block baits after submergence for varying periods in Lagos lagoon water from Okobaba Sawmill station.

Baiting period (days)	Fungus	No. of colonies on Potato Dextrose Agar after 7 days
3	Aspergillus niger	3
	Mucor sp.	2
	Yeast sp.	1
5	Aspergillus flavus	2
	Aspergillus niger	4
	Cladosporium oxysporum	4
	Curvularia geniculata	1
	Penicillium funiculosum	1
10	Aspergillus flavus	3
	Aspergillus giganteus	1
	Aspergillus niger	6
	Cladosporium oxysporum	3
	Trichoderma aureoviride	1
	Yeast sp.	1

TABLE 18 (Cont'd)

Fungi occurring on mini-wood block baits after submergence for varying periods in Lagos lagoon water from Okobaba Sawmill station.

Baiting period (days)	Fungus	No. of colonies on Potato Dextrose Agar after 7 days
15	Aspergillus flavus	1
	Aspergillus niger	3
	Cladosporium oxysporum	4
	Trichoderma aureoviride	1
20	Aspergillus flavus	2
	Aspergillus fumigatus	1
	Aspergillus niger	3
	Cladosporium oxysporum	3

TABLE 18 (Cont'd)

Fungi occurring on mini-wood block baits after submergence for varying periods in Lagos Lagoon water from Okobaba Sawmill station.

Baiting period (days)	Fungus	No. of colonies on Potato-Dextrose Agar after 7 days.
25	Aspergillus fumigatus	1
	Aspergillus niger	4
	Cladosporium oxysporum	3
	Trichoderma aureoviride	1
	Yeast sp.	1
30	Aspergillus fumigatus	1
	Aspergillus niger	2
	Cladosporium oxysporum	3
	Yeast sp.	1

E. MAJOR COMPONENTS OF SAWDUST

Fungi attacking wood depend mainly on the cellulose and lignin components of the wood. The portions of these important components of the sawdust used in the baiting experiments are reported in this section.

i. Cellulose content

Three weight measurements were used in the estimation of cellulose content.

(a) Initial dry weight.

(b) Final dry weight which is the weight of cellulose in the sawdust since the series of treatments applied was designed to remove lignin and extraneous materials from the sawdust.

(c) Weight of pure cellulose (filter paper) was used as a correction factor for loses during extraction procedure for cellulose in sawdust.

Calculation of cellulose content of sawdust

Weight of dried sawdust 0.992g

" " final sawdust residue (cellulose) 0.559g

" " dried filter paper 0.997g

" " final filter paper residue .. 0.991g

Since filter paper is pure cellulose, the amount of cellulose lost during extraction procedures

= 0.997-0.991g

= 0.006g.

The percentage loss = $\frac{0.006}{0.997} \times 100\%$

= 0.602%.

If weight of cellulose residue from sawdust

= 0.559g.

Then corrected weight of cellulose in sawdust

= $0.559 + (\frac{0.602}{100} \times 0.559)g$

= 0.559 + 0.003g.

= 0.562g.

Thus, percentage of cellulose in sawdust sample = $\frac{0.562}{0.992} \times 100\%$

= 56.65%.

ii. Lignin content of sawdust sample

Weight of dried sawdust 15.180g

" " final sawdust residue (lignin) 3.559g

∴ Percentage of lignin in sawdust = $\frac{3.559}{15.180} \times 100$

= 23.45%.

iii. Reducing sugar content of sawdust extract

The reducing sugar content as determined by the Nelson (1944) and Somogyi (1937, 1945) method was 2.0mg/l for 1% sawdust extract.

iv. Amount of extractives in sawdust sample

The amount of extractives in the sawdust sample was estimated during cellulose and lignin determinations.

In the cellulose extraction, the weight of residue obtained after acetone treatment was used in calculating the amount of extractives in the sawdust sample thus:

Dry weight of sawdust 0.992g

" " " " residue.. .. .. 0.884g

∴ Weight of extractives in sawdust sample

= 0.992-0.884g

= 0.108g.

Percentage of extractives in sawdust sample

$$= \frac{0.108}{0.992} \times 100$$

$$= \underline{10.88\%}.$$

In lignin determination, the weight lost after treatment with a mixture of benzene and ethanol was equivalent to the weight of extractives in the sawdust sample:

Weight of dried sawdust 15.180g

" " sawdust residue after ben zene and ethanol treatment 13.802g

Hence weight of extractives in sawdust

= 15.180-13.802g

= 1.378g.

∴ Percentage of extractives in sawdust sample

$$= \frac{1.378}{15.180} \times 100$$

$$= \underline{9.08\%}$$

To summarise, the sawdust analysed contained these components in the following proportions:-

Cellulose	56.65%
Lignin	23.45%
Reducing sugar	2.0mg/l of 1% sawdust extract
Extractives	10.88 and 9.08%.

F. GROWTH OF SOME FUNGI ISOLATED FROM SAWDUST IN LAGOON

Growth of fungi on Sawdust Extract Agar and Lagoon Water Agar

The following fungi isolated from sawdust were tested: Aspergillus flavus, Aspergillus giganteus, Aspergillus niger, Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum, Paecilomyces variotii, Penicillium funiculosum and an unidentified basidiomycete. The fungi were inoculated onto Sawdust Extract Agar or Lagoon Water Agar and growth was estimated qualitatively to determine the ability of each fungus to utilize water extractable constituents of sawdust. All fungi except Curvularia geniculata, Geotrichum candidum and Paecilomyces variotii grew fairly well on Sawdust Extract Agar. Similarly, all the fungi had good growth on Lagoon Water Agar and Cladosporium oxysporum produced a light purple pigment on this medium (Table 19).

G. CELLULOLYTIC ACTIVITIES OF FUNGAL ISOLATES

The ability of the fungal isolates to decompose cellulose, a major component of wood, was investigated. The fungi used were Aspergillus flavus, Aspergillus giganteus, Aspergillus niger, Cladosporium oxysporum, Geotrichum candidum, Paecilomyces variotii and the unidentified basidiomycete.

TABLE 19

Growth of fungi on Sawdust Extract Agar (SEA) and Lagoon Water Agar (LWA) at 27±2°C for 10 days.

Fungus	Medium	
	SEA	LWA
Aspergillus flavus	+++	++
Aspergillus giganteus	+++	+++
Aspergillus niger	++	++
Cladosporium oxysporum	++	+++ (pigment)
Curvularia geniculata	+	+++
Fusarium solani	++	++
Geotrichum candidum	+	++
Paecilomyces variotii	+	++
Penicillium funiculosum	++	++
Basidiomycete isolate	++	++

+, poor growth; ++, fair growth; +++ good growth.

i. Growth on Carboxymethylcellulose (CMC) Agar

Growth was measured by taking an average of two diameters of the fungus colony. Results in Table 20 show that all the fungi grew sparsely and slowly on CMC Agar. Aspergillus giganteus and Cladosporium oxysporum produced slight clearing indicating ability to utilize CM cellulose.

ii. Growth on CMC-mineral salts agar

Results in Table 21 show that the addition of mineral salts containing NO_3^- as nitrogen source enhanced the growth of all the fungi on CMC Agar. However, sparse mycelium was produced on CMC-mineral salts Agar.

iii. Growth on CMC-mineral Salts Agar at different pHs

The pH of CMC-mineral salts Agar was adjusted to 4.5, 6.0, 7.0 and 8.0 (Table 22) and inoculated with some fungi to observe the effect of pH on ability to utilize CMcellulose as carbon source. The optimum pH for Aspergillus flavus on CMC-mineral salts Agar was 4.5, Aspergillus niger was from 4.5 to 6.0, while Geotrichum candidum, Paecilomyces variotii and the unidentified basidiomycete grew equally well on pH 4.5, 6.0 and 7.0.

All the fungi produced sparse mycelium at all pHs. Clearing of the medium, which indicates cellulose activity, was not observed except when A. niger was at pH 4.5 and 6.0.

TABLE 20

Growth of some fungal species on Potato Dextrose Agar (PDA) and Carboxymethylcellulose (CMC) Agar at 27±2°C for 6 days.

Fungus	Mean diameter of culture (mm) after indicated incubation period (days)											
	PDA (Control)						CMC Agar					
	1	2	3	4	5	6	1	2	3	4	5	6
Aspergillus flavus	10	25	40	60	80	*	5	10	10	12	20	20
Aspergillus giganteus	10	23	38	50	65	80	5	8	11	14	20	23
Aspergillus niger	20	25	55	80	-	-	10	20	25	30	35	40
Cladosporium oxysporum	8	24	39	51	67	78	10	16	21	28	35	41
Geotrichum candidum	10	20	35	50	70	80	5	10	15	25	35	35
Paecilomyces variotii	10	30	50	60	80	-	10	15	18	20	26	31
Basiodiomycete isolate	20	50	64	80	-	-	10	15	30	40	45	50

* 80mm.

TABLE 21

Growth of some fungal species on Potato Dextrose Agar (PDA) and Carboxymethylcellulose-mineral salts (CMC-salts) Agar at 27±2°C for 6 days.

FUNGUS	Mean diameter of culture (mm) after indicated incubation period (days)											
	PDA (Control)						CMC-salts Agar					
	1	2	3	4	5	6	1	2	3	4	5	6
Aspergillus flavus	15	25	45	80	*	-	5	10	15	25	30	35
Aspergillus giganteus	10	23	38	52	68	80	15	25	30	36	44	50
Aspergillus niger	20	55	68	80	-	-	10	20	25	35	40	50
Cladosporium oxysporum	20	25	36	50	66	80	12	18	25	35	40	50
Geotrichum candidum	10	20	35	50	65	80	5	10	20	25	35	45
Paecilomyces variotii	10	25	50	65	80	-	10	15	25	35	45	50
Basidiomycete isolate	25	55	68	80	-	-	15	25	30	45	50	55

* 80mm.

TABLE 22

Growth of some fungal species on Potato Dextrose Agar (PDA) and Carboxymethylcellulose-mineral salts (CMC-salts) Agar with adjusted pH values at 27±2°C for 6 days.

Fungus	Mean diameter of culture (mm) at indicated pH values							
	PDA (Control)				CMC-salts Agar			
	4.5	6.0	7.0	8.0	4.5	6.0	7.0	8.0
Aspergillus flavus	85	85	85	85	55	50	40	40
Aspergillus giganteus	80	85	80	80	40	45	42	40
Aspergillus niger	85	85	85	85	50	50	40	40
Cladosporium oxysporum	85	85	85	85	42	42	40	40
Geotrichum candidum	80	85	80	70	45	45	45	40
Paecilomyces variotii	85	85	85	85	40	40	40	40
Basidiomycete isolate	85	85	85	85	50	50	50	50

iv. Growth on CMC-NH_4^+ Agar

Some fungi are known to grow better in presence of NH_4^+ rather than NO_3^- as nitrogen source. $(NH_4)_2SO_4$ was therefore added to CMC Agar to see if the fungi will utilize CMC Agar more efficiently (Table 23). A. flavus, A. giganteus and A. niger showed improved growth. Growth of C. oxysporum was not enhanced, while G. candidum, P. variotii and the basidiomycete showed poor growth.

Clearing of the medium was shown by A. niger, C. oxysporum and the basidiomycete (Table 23).

v. Growth of fungi on Colloidal Cellulose Agar

When cellulose is treated with phosphoric acid the molecules become less compact and the substrate appears swollen. These less compact or loose molecules are more easily attacked by cellulose decomposing organisms (Koopmans, 1970). Thus, some fungal isolates from the sawdust decomposing site of Lagos Lagoon were inoculated into Colloidal Cellulose Agar to study the growth (Table 24).

Results in Table 25 show that all the species grew better on Colloidal Cellulose Agar than on CMC Agar (Table 20). Furthermore improved growth was observed on Colloidal Cellulose mineral salts Agar indicating that addition of mineral salts enhanced utilization of colloidal cellulose (Table 25).

All the fungal species showed clearing on Colloidal Cellulose Agar, the best being Aspergillus niger, Cladosporium oxysporum and the basidiomycete (Table 26).

TABLE 23

Growth of some fungal species isolated from Okobaba sawmill station on Carboxymethylcellulose-$(NH_4)_2SO_4$ Agar incubated at $27\pm2^{o}C$ for 6 days.

Fungus	Mean diameter of culture (mm) after indicated incubation period (days)					
	1	2	3	4	5	6
Aspergillus flavus	5	10	12	15	20	26
Aspergillus giganteus	8	15	20	25	30	37
Aspergillus niger	9	18	24	30	35	42
Cladosporium oxysporum	10	16	21	27	33	42
Geotrichum candidum	5	10	16	21	25	30
Paecilomyces variotii	7	11	15	20	24	28
Basidiomycete isolate	10	18	24	29	35	40

TABLE 24

Ability of some fungal species isolated from Okobaba sawmill station to utilize Carboxymethylcellulose-$(NH_4)_2SO4$ as sole carbon source as indicated by zones of clearing around the fungal colonies.

Fungus	Degree of clearing around fungal colony after incubation for indicated period (days)					
	1	2	3	4	5	6
Aspergillus flavus	-	-	-	-	-	+
Aspergillus giganteus	-	-	-	+	+	+
Aspergillus niger	-	-	-	+	+	++
Cladosporium oxysporum	-	-	-	+	+	++
Geotrichum candidum	-	-	-	-	-	-
Paecilomyces variotii	-	-	-	-	+	+
Basidiomycete isolate	-	-	-	+	+	++

-: No clearing

+: Poor clearing

++: Good clearing.

TABLE 25

Growth of some fungal species on Colloidal Cellulose Agar and Colloidal Cellulose-mineral salts Agar at $27\pm2^{\circ}C$ for 6 days.

Fungus	Mean diameter of culture (mm) after indicated incubation period (days)											
	Colloidal Cellulose agar						Colloidal Cellulose-mineral salts agar					
	1	2	3	4	5	6	1	2	3	4	5	6
Aspergillus flavus	5	10	15	24	31	37	5	11	18	26	33	41
Aspergillus giganteus	15	25	31	38	45	52	15	25	33	40	45	55
Aspergillus niger	10	20	26	35	41	50	12	20	26	34	43	55
Cladosporium oxysporum	12	17	25	33	40	50	14	23	31	40	47	60
Geotrichum candidum	6	10	18	24	35	45	10	15	22	30	38	50
Paecilomyces variotii	10	15	25	36	43	50	12	17	24	32	40	50
Basidiomycete isolate	15	25	32	45	50	52	15	25	33	42	50	55

TABLE 26

Degree of clearing produced around the colonies of some fungi on Colloidal Cellulose Agar and Colloidal Cellulose-mineral salts Agar at $27\pm2^{o}C$ for 6 days.

Fungus	Medium	
	Colloidal Cellulose agar	Colloidal Cellulose-mineral salts agar
Aspergillus flavus	+	++
Aspergillus giganteus	++	+++
Aspergillus niger	+++	+++
Cladosporium oxysporum	+++	+++
Geotrichum candidum	+	+
Paecilomyces variotii	+	++
Basidiomycete isolate	+++	+++

+: Poor clearing

++: Fair clearing

+++: Good clearing.

vi. Production of reducing sugars on CMC medium and Sawdust Extract broth.

Having assessed cellulolytic activity by clearing produced by extra-cellular enzymes of the fungi on cellulose agar media, the production of reducing sugars from cellulose by selected fungi on liquid media was tested.

Results in Table 27 show that except for Cladosporium oxysporum which produced 3mg/l reducing sugar in CMC-mineral salts medium after 10 days, there was no measurable amount of reducing sugar produced by the fungi growing in either CMC-NH_4^+, CMC-mineral salts or Sawdust Extract broth for 10 days.

vii. Growth of fungi on filter paper medium

Results of earlier experiments (Tables 21 and 25) indicated that the fungal isolates showed improved cellulolytic activities when mineral salts were incorporated into cellulose media. In the following experiment, filter paper (containing native cellulose fibres) was substituted for CMC or colloidal cellulose as sole carbon source in mineral salts medium and mycelial disks of fungi were inoculated into the filter paper medium. Cellulolytic activity was assessed as per cent weight loss of filter paper. The correlation between weight of filter paper and period of incubation with each fungus was tested with the correlation coefficient (r) and t-test statistics.

TABLE 27

Reducing sugar content of Carboxymethylcellulose (CMC) media and Sawdust Extract medium inoculated with some fungi at 27±2°C for 10 days.

Fungus	Media		
	CMC-NH_4^+	CMC-Salts	Sawdust Extract
Aspergillus flavus	-	-	-
Aspergillus giganteus	-	-	-
Aspergillus niger	-	-	-
Cladosporium oxysporum	-	3mg/l	-
Geotrichum candidum	-	-	-
Paecilomyces variotii	-	-	-
Basidiomycete isolate	-	-	-

Results presented in Table 28 show that Aspergillus giganteus caused the greatest weight loss of filter paper indicating highest cellulolytic activities. There was significant weight loss of filter paper on inoculation with each of the test fungi (Table 28). A. giganteus utilized the filter paper more efficiently in agitated cultures, resulting in 48.2% weight loss by the 25th day, than in still cultures in which 34.8% weight loss was recorded on the 30th day (Table 28). Statistical analysis confirmed that A. giganteus produced the most significant negative correlation (r), between weight of filter paper and period of incubation in both agitated and still cultures, with r-values of -0.9414 and -0.9836 respectively (tabulated r-value 0.3809 at 5% level of significance and 25 d.f.). On the contrary, Cladosporium oxysporum utilized the filter paper more efficiently in still cultures than in agitated cultures (Table 28) producing significant values of -22.5887 and -8.3672 respectively, as verified by the t-test (Table 28a). Tabulated t-value at 5% level of significance and 25 d.f. = 2.060.

On the pattern of attack of filter paper by the fungi, A. giganteus showed random attack all over the surface (Plate 3) while C. oxysporum attacked the filter paper from the edge (Plate 4).

TABLE 28

Effect of fungi isolated from sawdust on filter paper in mineral salts medium at 27±2°C for 30 days.

Type of culture	Fungus	% of weight loss of filter paper on incubation (in days)					
		5	10	15	20	25	30
Agitated	Aspergillus flavus	14.9	18.8	20.8	19.8	17.8	17.8
	Aspergillus giganteus	17.5	20.2	39.5	47.4	48.2	48.2
	Cladosporium oxysporum	11.8	13.7	14.7	17.6	18.6	19.6
	Curvularia geniculata	11.9	11.9	12.9	12.9	12.9	12.9
Still	Aspergillus flavus	5.0	5.9	6.9	7.9	11.9	12.9
	Aspergillus giganteus	3.6	4.5	14.3	25.0	29.5	34.8
	Cladosporium oxysporum	11.0	24.0	30.0	36.0	48.0	49.0
	Curvularia geniculata	3.0	2.0	3.0	7.0	12.0	13.0

TABLE 28a

The relation between period of incubation and weight of filter paper in mineral salts medium inoculated with different fungi (tested with correlation coefficient (r) and t-test statistics).

Type of culture	Fungal species	Correlation coefficient (r)	t-test (t)
Agitated	Aspergillus flavus	-0.6604	-3.8338*
	Aspergillus giganteus	-0.9414	-12.1670*
	Cladosporium oxysporum	-0.8869	- 8.3672*
	Curvularia geniculata	-0.6640	- 3.8716*
Still	Aspergillus flavus	-0.9565	-14.2847*
	Aspergillus giganteus	-0.9836	-23.7427*
	Cladosporium oxysporum	-0.9819	-22.5887*
	Curvularia geniculata	-0.9338	-11.3796*

* Significant at 5% level with 25 d.f.

PLATE 3

Photograph (a) showing extensive breakdown of filter paper disk after 30 days by Aspergillus giganteus growing in filter paper medium. Plate (b) shows control filter paper (x 1)

Plate 3(a)

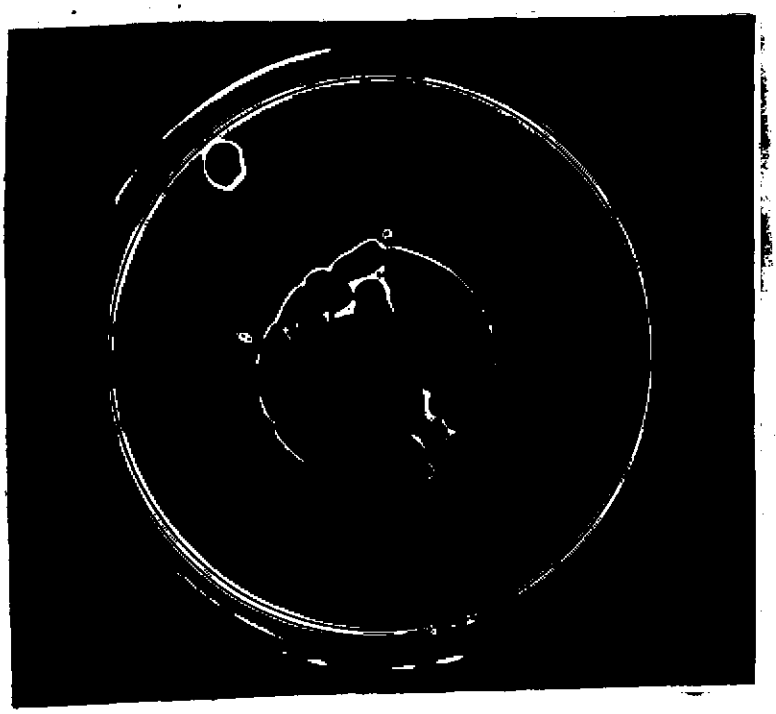


Plate 3(b)

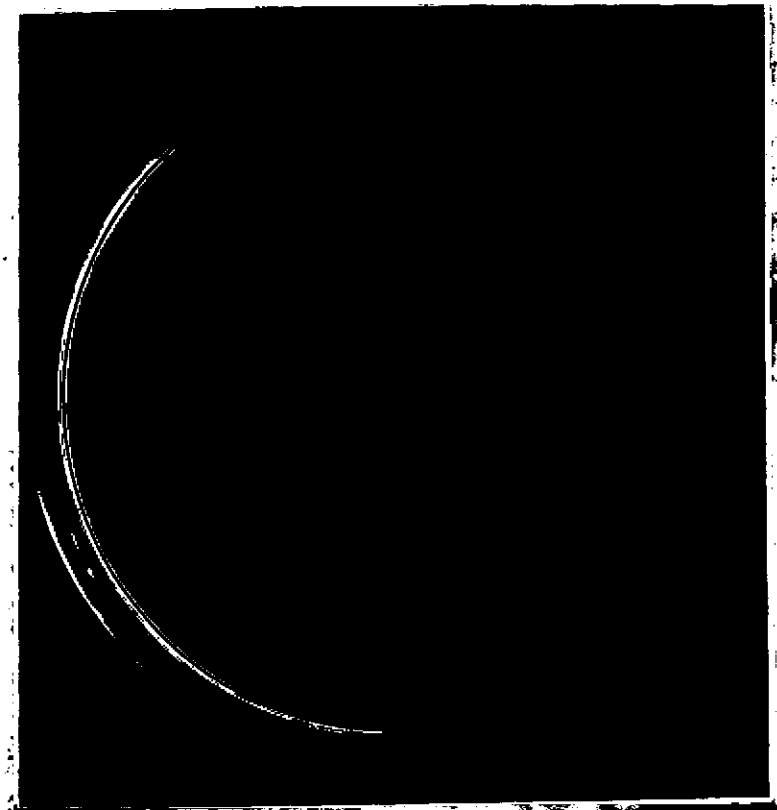


PLATE 4

Photograph (a) showing breakdown of filter paper disk especially at the edges after 30 days by Cladosporium oxysporum growing in filter paper medium. Plate (b) shows control filter paper disk (x 1)

Plate 4(a)

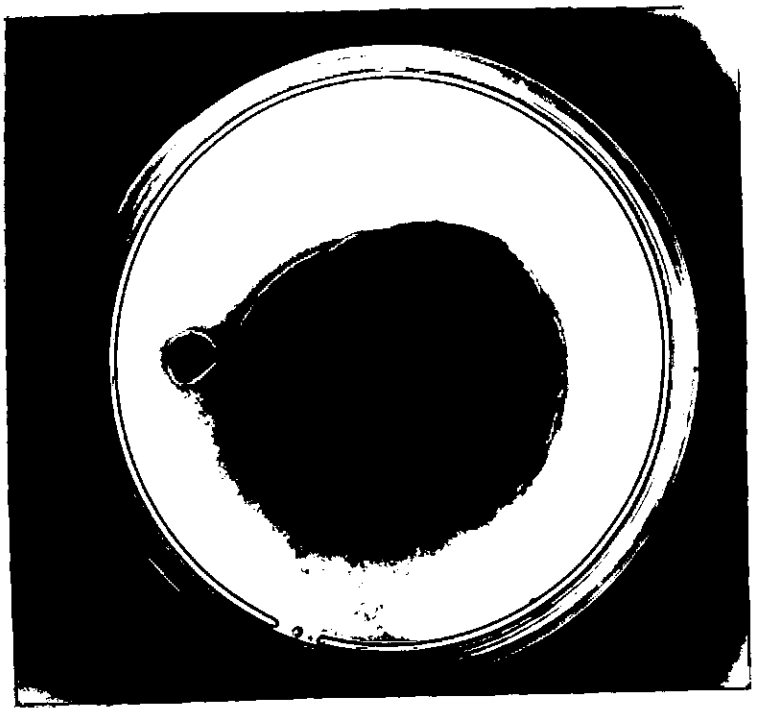
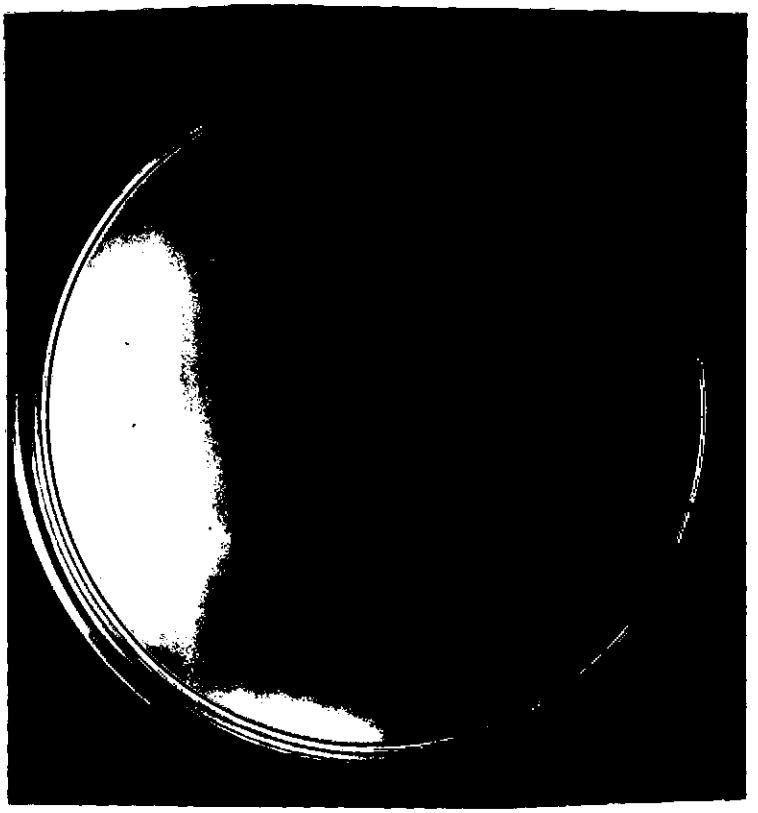


Plate 4(b)



viii. pH changes and reducing sugar concentration of filter paper medium inoculated with fungi

Since separation of fungal mycelium from filter paper was not feasible, dry weight determinations of fungal mycelia was not possible. Other parameters - pH and reducing sugars - produced as a result of cellulolytic activities of the fungi were therefore measured. Results in Table 29 show that no pH changes and no measurable amounts of reducing sugars were produced by the fungi in the filter paper medium after 20 days of incubation. However, Cladosporium oxysporum produced 2mg/l reducing sugar into the filter paper medium after 20 days of agitated incubation, and the amount of reducing sugar produced decreased with period of incubation (Table 30).

H. GROWTH OF FUNGI ON LIGNIN AGAR

The ability of some fungi isolated from the sawdust to breakdown lignin, another major component of wood, was investigated. Both the lignin sample extracted from sawdust (see section E ii) and commercial lignin were used.

Results in Table 31 show that all the fungi tested could not utilize lignin as carbon source, and there was therefore no growth on solid media containing extracted or commercial lignin.

TABLE 29

Final pH values and reducing sugar concentration of Filter paper medium inoculated with fungi at $27\pm2^{o}C$ for 20 days. (Initial pH of medium = 7.0; initial reducing sugar in medium = 0.0mg/l).

Fungus	pH values		reducing sugar (mg/l)	
	Still Culture	Agitated culture	Still culture	Agitated culture
Aspergillus flavus	7.0	7.0	0.0	0.0
Aspergillus giganteus	7.0	7.0	0.0	0.0
Cladosporium oxysporum	7.0	7.0	0.0	2.0
Curvularia geniculata	7.0	7.0	0.0	0.0

TABLE 30

Amount of reducing sugar in agitated Filter paper medium inoculated with Cladosporium oxysporum at $27\pm2^{\circ}C$ for 20 days.

Period of incubation (days)	Reducing sugar (mg/l)
0	0.0
5	4.0
10	3.0
20	2.0

TABLE 31

Growth of some fungal species on Lignin Agar media at 28±2°C for 10 days.

Fungus	Media					
	Lignin Agar		Lignin-Glucose Agar		Lignin-salts Agar	
	(a)	(b)	(a)	(b)	(a)	(b)
Aspergillus flavus	-	-	-	+	+	+
Aspergillus giganteus	-	-	-	+	-	+
Cladosporium oxysporum	-	-	+	+	+	+
Paecilomyces variotii	-	-	-	+	-	+

(a): extracted lignin

(b): commercial lignin

-: No growth

+: Sparse mycelium.

The addition of glucose and mineral salts to lignin encouraged some fungi to grow sparsely, probably utilizing only the added nutrients. However, Cladosporium oxysporum had some growth on extracted Lignin-glucose Agar and extracted Lignin-mineral salts Agar. Adjustment of Lignin Agar to different pH values did not enhance utilization of lignin. However, Aspergillus flavus and C. oxysporum grew sparsely on extracted lignin at pH 6.0 (Table 32). On media containing commercial lignin, sparse mycelial growth was produced by A. flavus and C. oxysporum at pH 4.5 and 6.0, while A. giganteus grew sparsely at pH 6.0, and Paecilomyces variotii at pH 6.0 and 7.0.

I. GROWTH AND SPORULATION AT DIFFERENT SALINITIES

A wide range of salinity was observed in Lagos Lagoon (Fig. 5). Since the fungi breaking down sawdust in the lagoon will carry out the activities under lagoon conditions, it was thought necessary to determine the effect of salinity on the growth and sporulation of the fungi. As Na^+ and Cl^- ions are the dominant ions in the sea which control the salinity of the lagoon (Akpata, 1975), NaCl was used to adjust the salinity of Czapek Dox Agar (CDA) and fungi isolated from sawdust were inoculated into the medium to observe their salinity tolerance under lagoon conditions.

TABLE 32

Growth of some fungal species on Lignin-mineral salts Agar with adjusted pH values at 28±2°C for 10 days.

Fungus	Growth at indicated pH values							
	Extracted Lignin Agar				Commercial lignin Agar			
	4.5	6.0	7.0	8.0	4.5	6.0	7.0	8.0
Aspergillus flavus	-	+	-	-	+	+	-	-
Aspergillus giganteus	-	-	-	-	-	+	-	-
Cladosporium oxysporum	-	+	-	-	+	+	-	-
Paecilomyces variotii	-	-	-	-	-	+	+	-

-: No growth

+: Sparse mycelium.

i. Growth of fungi at different salinities

Generally, there was a decrease in colony diameter with increase in salinity of the culture medium. Three patterns of growth were observed (Table 33). In the first type, colony diameter was more than 45mm (or half the diameter of the Petri plate) after 10 days on a medium containing 6.8% NaCl. This pattern was characteristic of fungi that tolerated high salinities, e.g. Aspergillus flavus. Such fungi were described as halophilic by Rheinheimer (1974). The second pattern of growth exhibited by some of the fungi was that in which colony diameter was more than 45mm after 10 days incubation in less saline media containing 1.7% NaCl. This pattern was shown by Aspergillus giganteus, Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum and Trichoderma aureoviride. This group will belong to the euryhaline fungi of Johnson and Sparrow (1961) and Ritchie (1960). The third growth pattern, which was characteristic of Aspergillus tamarii and Paecilomyces variotii, is that in which the colony diameter was less than 45mm after 10 days of incubation in media containing 1.7% NaCl. This category of fungi was described as halophobic by Rheinheimer (1974).

The last two groups could be regarded as brackish-water fungi and they are the normal fungal flora of lagoon waters.

TABLE 33

Growth of fungi on Czapek Dox Agar (CDA) amended with different concentrations of Sodium Chloride (NaCl) at 27±2°C for 10 days.

Fungus	Salinity of CDA (% NaCl)	Colony diameter (mm) at different salinties									
		Period of incubation (days)									
		1	2	3	4	5	6	7	8	9	10
Aspergillus flavus	0.0	5	10	24	40	50	68	76	90	90	90
	0.2	5	9	22	37	42	55	58	79	90	90
	0.4	5	9	21	26	32	38	45	56	68	81
	0.9	5	8	20	27	33	39	43	55	66	78
	1.7	5	10	24	27	31	35	48	53	65	76
	3.4	5	8	18	30	40	45	53	60	66	72
	6.8	5	8	18	25	31	37	42	46	50	54
Aspergillus giganteus	0.0	6	11	22	36	45	56	67	80	90	90
	0.2	6	11	20	34	45	55	60	70	81	90
	0.4	6	10	18	27	36	41	50	57	66	76
	0.9	6	9	16	25	34	40	45	50	54	60
	1.7	5	8	12	18	22	28	34	40	45	51
	3.4	5	6	9	15	20	24	29	34	40	46
	6.8	5	5	5	6	8	9	10	12	13	13
Aspergillus tamarii	0.0	6	13	29	38	50	61	70	77	82	90
	0.2	6	13	28	37	48	54	62	71	79	88
	0.4	6	10	25	33	40	46	52	57	63	72
	0.9	5	6	12	20	26	31	38	45	51	60
	1.7	5	5	10	14	17	21	25	30	36	44
	3.4	5	5	7	11	15	20	23	26	30	35
	6.8	5	5	5	8	12	14	15	16	17	17

TABLE 33 (Cont'd)

Growth of fungi on Czapek Dox Agar (CDA) amended with different concentrations of sodium chloride (NaCl) at 27±2°C for 10 days.

Fungus	Salinity of CDA (% NaCl)	Colony diameter (mm) at different salinities — Period of incubation (days)									
		1	2	3	4	5	6	7	8	9	10
Cladosporium oxysporum	0.0	5	8	13	25	36	50	61	71	80	88
	0.2	5	8	13	20	29	36	44	58	67	80
	0.4	5	7	12	20	28	36	42	56	61	70
	0.9	6	9	11	16	22	28	34	40	46	53
	1.7	6	8	13	19	25	31	35	40	44	49
	3.4	5	7	12	15	20	24	28	31	38	43
	6.8	5	5	8	13	17	21	27	32	36	41
Curvularia geniculata	0.0	10	19	27	36	46	54	65	73	81	90
	0.2	10	15	22	31	46	55	62	70	76	83
	0.4	9	12	20	29	37	42	50	58	67	75
	0.9	7	13	18	26	30	37	41	46	50	54
	1.7	5	8	11	16	23	30	35	40	43	45
	3.4	5	5	9	12	15	17	18	21	23	26
	6.8	5	5	10	12	14	15	16	16	16	16
Fusarium solani	0.0	6	15	26	35	48	60	70	78	84	90
	0.2	6	18	29	37	48	57	68	76	84	90
	0.4	6	16	25	32	41	50	59	68	76	84
	0.9	5	10	18	26	34	40	46	52	59	68
	1.7	5	7	15	20	26	31	37	43	49	55
	3.4	5	5	9	13	17	20	23	25	27	30
	6.8	5	5	7	8	10	12	14	17	19	22

TABLE 33 (Cont'd)

Growth of fungi on Czapek Dox Agar (CDA) amended with different concentrations of sodium chloride (NaCl) at 27±2°C for 10 days.

Fungus	Salinity of CDA (% NaCl)	Colony diameter (mm) at different salinities									
		Period of incubation (days)									
		1	2	3	4	5	6	7	8	9	10
Geotrichum candidum	0.0	6	9	20	28	35	40	48	55	65	78
	0.2	6	9	21	30	40	47	56	64	73	80
	0.4	6	8	14	20	29	38	45	53	60	70
	0.9	5	8	11	15	20	25	30	38	47	55
	1.7	5	5	10	16	22	30	38	46	52	59
	3.4	5	7	10	18	24	30	36	45	50	55
	6.8	5	5	8	12	14	18	19	20	22	23
Paecilomyces variotii	0.0	6	13	28	37	48	60	72	80	90	90
	0.2	6	12	18	26	36	49	61	70	78	83
	0.4	6	8	14	20	30	39	49	56	61	66
	0.9	5	6	9	14	20	26	30	33	37	42
	1.7	5	5	6	9	12	15	17	22	26	30
	3.4	5	8	11	14	15	17	19	20	20	22
	6.8	5	5	5	6	8	9	10	11	11	11
Trichoderma aureoviride	0.0	11	26	34	44	55	65	72	80	87	90
	0.2	10	24	31	40	48	57	65	71	80	90
	0.4	10	23	30	39	46	53	60	66	72	79
	0.9	8	16	23	30	35	44	52	60	70	76
	1.7	8	10	16	20	28	35	40	45	50	55
	3.4	6	8	10	14	20	24	27	30	32	35
	6.8	5	5	8	10	12	15	17	18	19	20

ii. Sporulation at different salinities

The effect of salinity on sporulation varied from one isolate to the other (Table 34).

Aspergillus flavus: The spores produced were bright-green at 0.0% NaCl, pale-yellow at low salinities, and bright-yellow in media containing 6.8% NaCl. Spore diameter was affected by the salinity of the medium, it decreased from 6.3µm in media containing no NaCl (control) to 4.7µm in media containing 6.8% NaCl. There was a decrease in sporulation with increase in salinity (Table 34).

Aspergillus giganteus: Sporulation decreased with increasing salinity of the medium and at 6.8% NaCl, sporulation became very poor. Spore diameter was 6.3µm on control cultures, and 1.6 µm on cultures maintained in media containing 6.8% NaCl (Table 34).

Aspergillus tamarii: Sporulation decreased with increase in salinity of medium (Table 34). Spore diameter in media containing no NaCl was 7.9 µm, and 1.6 µm in media containing 6.8% NaCl.

Cladosporium oxysporum: Sporulation decreased with increasing salinity of the medium (Table 34), and spores produced at salinities above 3.4% NaCl became intensely black.

TABLE 34

Sporulation of fungi on Czapek Dox Agar (CDA) amended with different concentrations of NaCl at 27±2°C for 10 days.

Fungus	Conc. of NaCl in CDA (%: w/v)	Sporulation density	Spore size (diameter in µm)
Aspergillus flavus	0.0	215.8×10^4	6.3
	0.4	187.2×10^4	6.3
	1.7	73.1×10^4	6.3
	3.4	19.4×10^4	6.3
	6.8	8.2×10^4	4.7
Aspergillus giganteus	0.0	111.4×10^4	6.3
	0.4	87.3×10^4	4.7
	1.7	56.2×10^4	3.2
	3.4	17.6×10^2	1.6
	6.8	3.2×10^2	1.6
Aspergillus tamarii	0.0	114.0×10^4	7.9
	0.4	84.1×10^4	3.2
	1.7	33.6×10^4	2.4
	3.4	5.2×10^2	1.6
	6.8	1.7×10^2	0.8
Cladosporium oxysporum	0.0	201.0×10^4	6.3* 11.1+
	0.4	174.0×10^4	6.3* 9.5+
	1.7	111.4×10^4	6.3* 7.9+
	3.4	98.2×10^4	4.7* 7.9+
	6.8	56.2×10^4	4.7* 7.9+

* microconidia

\+ macroconidia.

TABLE 34 (Cont'd)

Sporulation of fungi on Czapek Dox Agar (CDA) amended with different concentrations of NaCl at 27±2°C for 10 days.

Fungus	Conc. of NaCl in CDA (%: w/v)	Sporulation density	Spore size (diameter in µm)
Curvularia geniculata	0.0	120.0×10^4	3.2
	0.4	96.0×10^4	3.2
	1.7	81.5×10^4	3.2
	3.4	44.3×10^2	3.2
	6.8	16.8×10^2	2.4
Fusarium solani	0.0	166.3×10^4	25.0
	0.4	101.5×10^4	19.0
	1.7	86.4×10^4	15.8
	3.4	27.3×10^2	7.9
	6.8	6.8×10^2	4.7
Geotrichum candidum	0.0	108.0×10^2	7.9
	0.4	73.1×10^2	4.7
	1.7	36.0×10^2	3.2
	3.4	8.2×10^2	1.6
	6.8	0.0	0.0
Paecilomyces variotii	0.0	171.0×10^4	4.7
	0.4	83.6×10^4	4.7
	1.7	28.5×10^4	3.2
	3.4	21.3×10^2	1.6
	6.8	0.6×10^2	1.6

TABLE 34 (Cont'd)

Sporulation of fungi on Czapek Dox Agar (CDA) amended with different concentrations of NaCl at $27\pm2^{\circ}C$ for 10 days.

Fungus	Conc. of NaCl in CDA (%: w/v)	Sporulation density	Spore size (diameter in µm)
Trichoderma aureoviride	0.0	$112.6x10^4$	4.7
	0.4	$77.1x10^4$	4.7
	1.7	$40.5x10^4$	3.2
	3.4	$14.3x10^4$	2.4
	6.8	$0.8x10^4$	1.6

The maximum spore diameter in control media were 6.3 and 11.1 µm for micro- and macro-conidia respectively, while corresponding values at 6.8% NaCl were 4.7 and 7.9 µm.

Curvularia geniculata: There was a decrease in sporulation in media containing 3.4 and 6.8% NaCl (Table 34). Spore size remained the same at all salinities.

Fusarium solani: Sporulation decreased as salinity of the medium increased. The maximum spore diameter of 25.3 µm on media of 0.0% salinity decreased with increasing salinity to 7.9 µm at 3.4% NaCl concentration and 4.7 µm at 6.8% NaCl (Table 34).

Geotrichum candidum: The maximum diameter of mycelial fragments produced was from 7.9 µm in media containing no NaCl to 1.6 µm in media with 3.4% NaCl (Table 34).

Paecilomyces variotii: Sporulation was poor in media containing 3.4 and 6.8% NaCl (Table 34). Spore diameter decreased from 4.7 µm on media containing 0.0 and 0.4% NaCl to 1.6 µm in media containing 3.4 and 6.8% NaCl. The golden brown colour of spores produced in media containing no NaCl became much lighter (almost white) on media containing 6.8% NaCl.

Trichoderma aureoviride: Sporulation decreased as the salinity of medium increased (Table 34). Spore diameter was 4.7 µm in control cultures but decreased to 1.6 µm in media containing 6.8% NaCl. As the NaCl concentration of the medium increased through 1.7 to 6.8%, thick-walled intercallary and terminal cells (spores)were produced.

J. GERMINATION OF FUNGAL SPORES IN SAWDUST EXTRACT

The spores of the fungi attacking sawdust are very important as colonization bodies in view of the large numbers produced. Experiments to investigate the extent to which the extract of sawdust of three wood species would influence the germination of the asexual spores of Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride are presented in this section.

i. Germination in aqueous extract of Khaya ivorensis sawdust

The conidia of Aspergillus giganteus did not germinate in either distilled water or in any of the extract solutions (0.5-5.0%) used. The response of conidia of the remaining three fungi were:

Aspergillus flavus (Fig. 6). The conidia did not germinate in distilled water. All concentrations of the extract stimulated germination, percentage germination rising with increasing extract concentration.

FIG. 6

Germination of conidia of some fungi in different concentrations of ~~extract~~ of Khaya ivorensis sawdust.

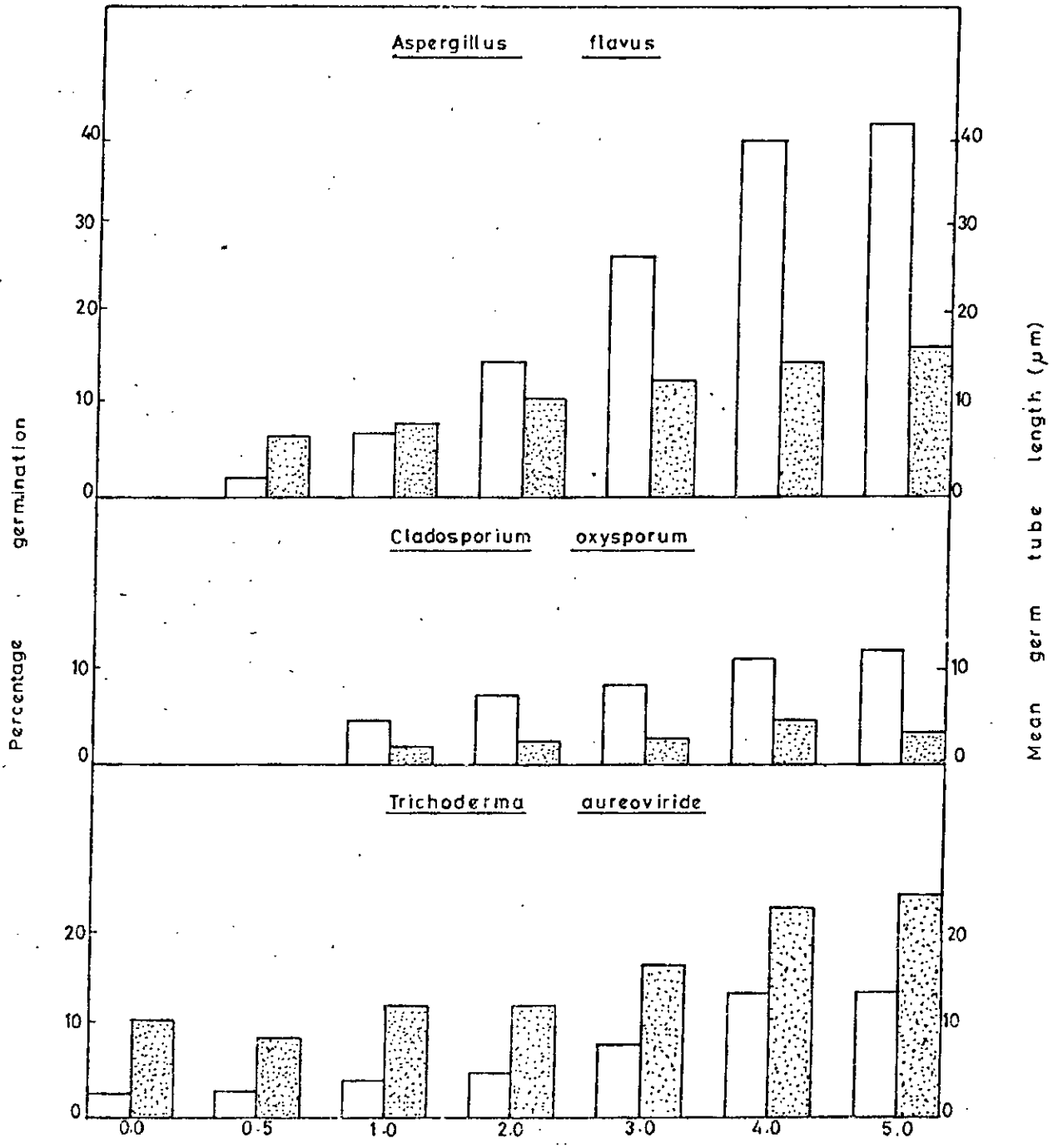

Aspergillus flavus
Cladosporium oxysporum
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
0
10
20
30
40
0.0
0.5
1.0
2.0
3.0
4.0
5.0
Concentration of extract of sawdust of Khaya ivorensis (% : v/v)
Percentage germination
Mean germ tube length (µm)

Fig. 6

The 5.0% extract, therefore, gave the highest germination of 42.0% and the germinated conidia (Plate 5) had the longest germ tubes, a mean of 16.8 µm.

Cladosporium oxysporum (Fig. 6). The conidia of C. oxysporum did not also germinate in distilled water. Sawdust extract of sufficiently high concentration stimulated germination. Thus, germination occurred at extract concentrations of 1.0-5.0%, but not at 0.5%. The higher the extract concentration the greater the amount of germination. The best germination (13%) and longest germ tubes (mean length: 4.4 µm) occurred in the 5.0% extract solution.

Trichoderma aureoviride (Fig. 6). The conidia germinated in both distilled water and in the extracts. Extracts encouraged greater germination than did distilled water. The best germination was recorded in 4.0-5.0% extract.

ii. Germination in aqueous extract of Mitragyna ciliata sawdust

The conidia of Aspergillus flavus, Aspergillus giganteus and Cladosporium oxysporum germinated very poorly in the extract (Fig. 7). The best germination did not exceed 5.0%.

PLATE 5

Photomicrograph of germinating conidia of Aspergillus flavus (X5000)

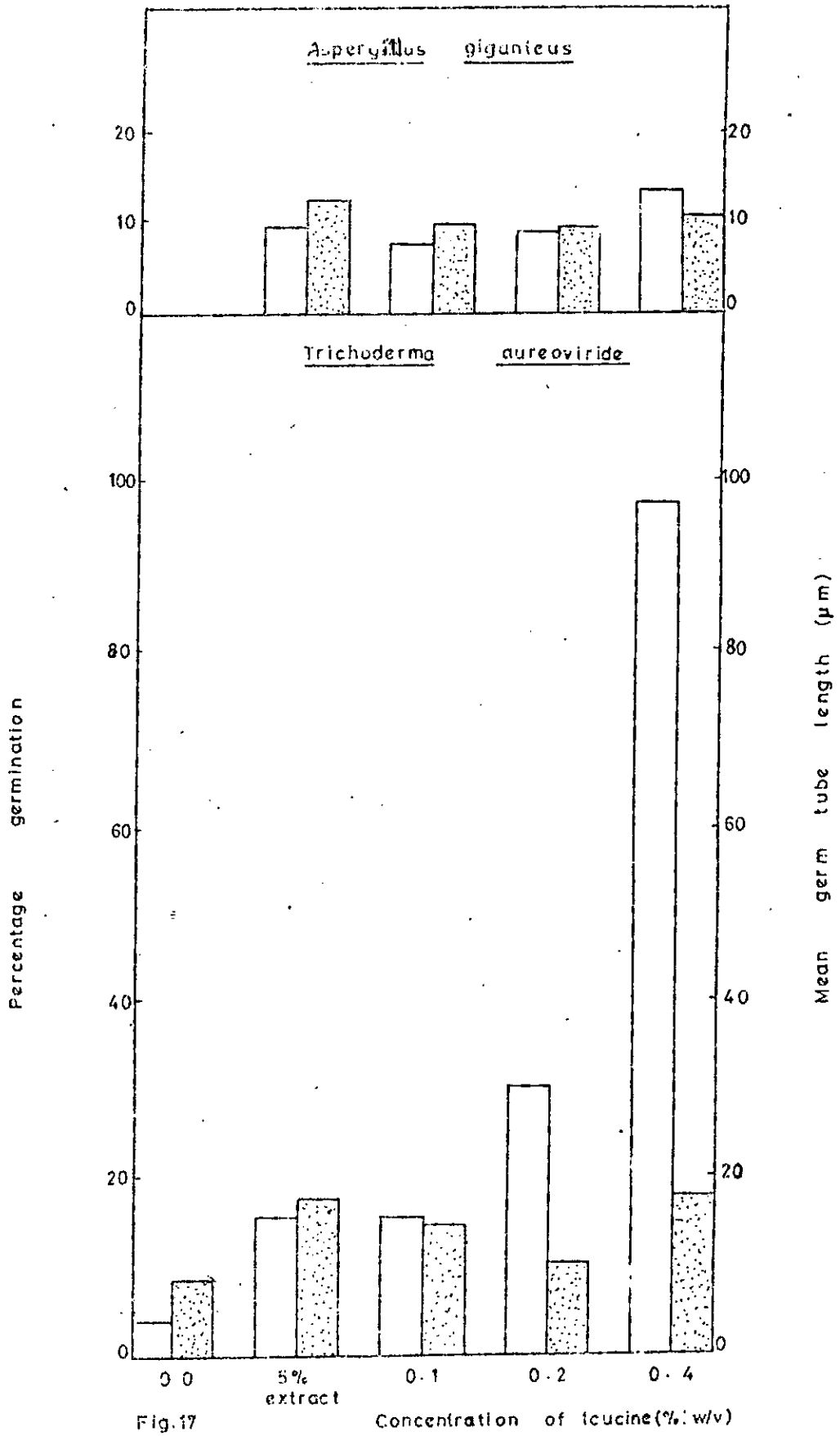

Aspergillus giganteus
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
0
10
20
40
60
80
100
0 0
5% extract
0.1
0.2
0.4
Concentration of leucine (%: w/v)
Percentage germination
Mean germ tube length (µm)

Fig.17

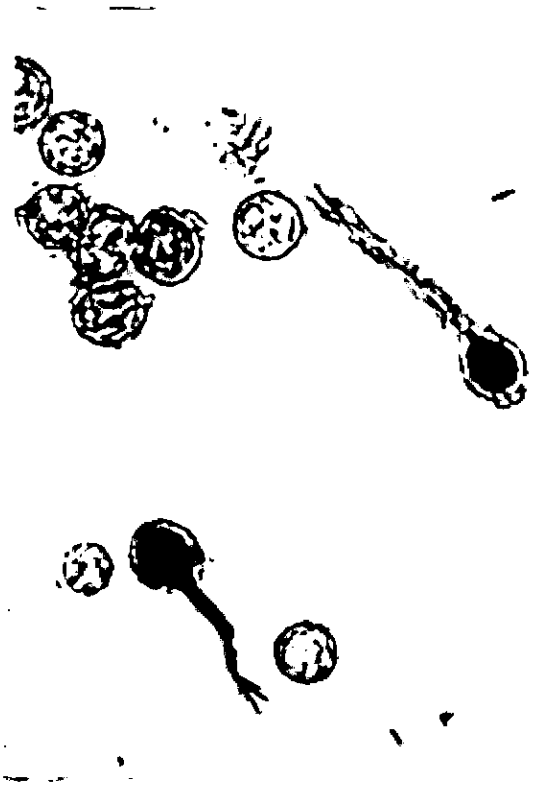


FIG. 7

Germination of conidia of some fungi in different concentrations of extract of Mitragyna ciliata sawdust.

The highest germination in A. flavus (4.3%), A. giganteus (3.5%) and C. oxysporum (2.0%) occurred in 4.0, 3.0 and 1.0% extract solutions, respectively.

Germination of Trichoderma aureoviride conidia was fairly high (36.0 and 33.0%) in 1.0 and 2.0% extract solutions respectively, but low (0.5-4.0%) at extract concentrations of 3.0-5.0% respectively (Fig. 7). However, the spores which were 3.16 µm in diameter became swollen (Plate 6) to 8.06 µm even in those concentrations of extract (2.0-4.0%) which did not support appreciable germ tube production (Fig. 8).

iii. Germination in aqueous extract of Triplochiton scleroxylon sawdust

Conidia of Aspergillus giganteus and Cladosporium oxysporum did not germinate in both distilled water and all the extract solutions.

Conidia of Aspergillus flavus were weakly stimulated (2.5 and 2.0% germination) by extract concentrations of 4.0 and 5.0%. Lower extract concentrations did not support any germination (Fig. 9).

Conidia of Trichoderma aureoviride, on the other hand, germinated at all extract concentrations, and did so very well (41.0%) at 5.0% extract concentration (Fig. 9). There was also moderate germination (2.0-28.0%) at 1.0-4.0% extract concentrations.

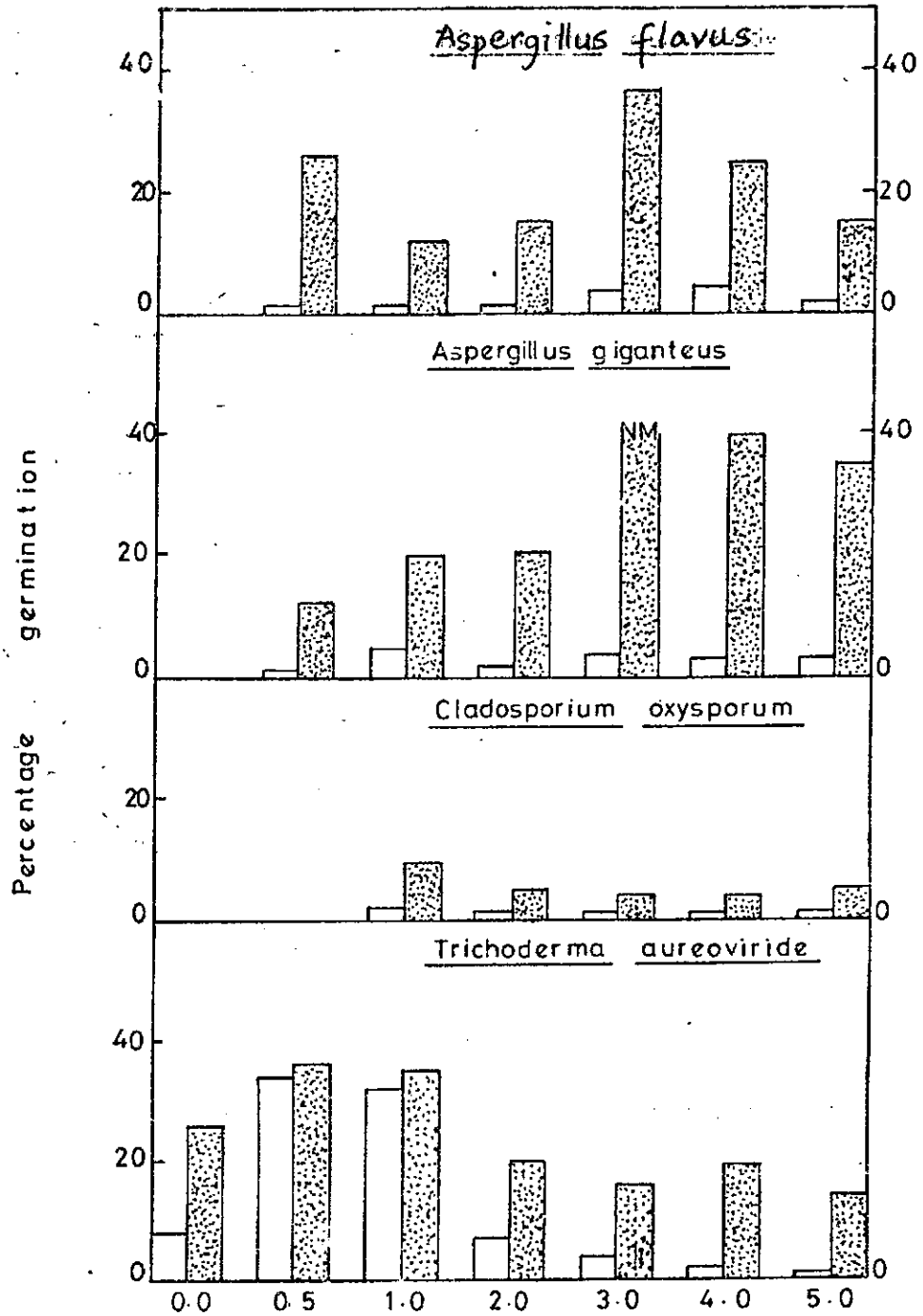

Aspergillus flavus
Aspergillus giganteus
Cladosporium oxysporum
Trichoderma aureoviride
NM
40
20
0
Percentage germination
Mean germ tube length (µm)
0·0
0·5
1·0
2·0
3·0
4·0
5·0
Concentration of extract of Mitragyna ciliata (%: v/v)
Percentage germination
Mean germ tube length (µm)
NM Not measurable

Fig.7

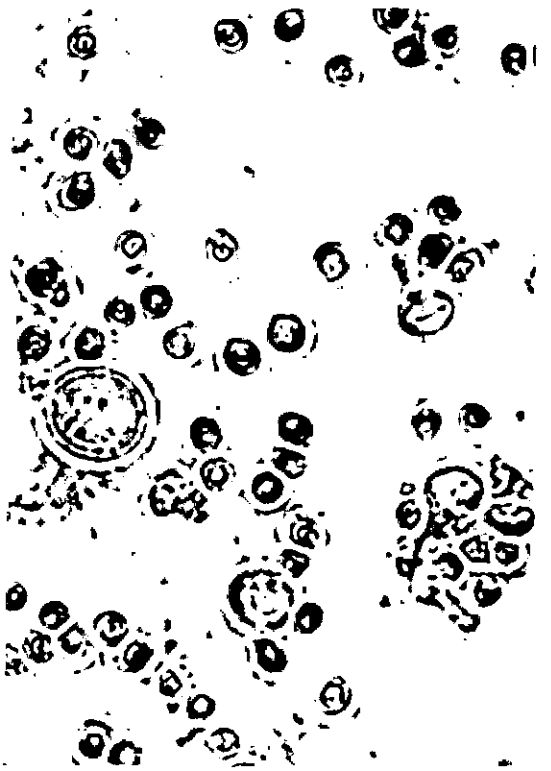


PLATE 6

Photomicrograph of conidia of Trichoderma aureoviride showing the extensive swelling of the conidia in 3.0% extract of Mitragyna ciliata sawdust (X5000)

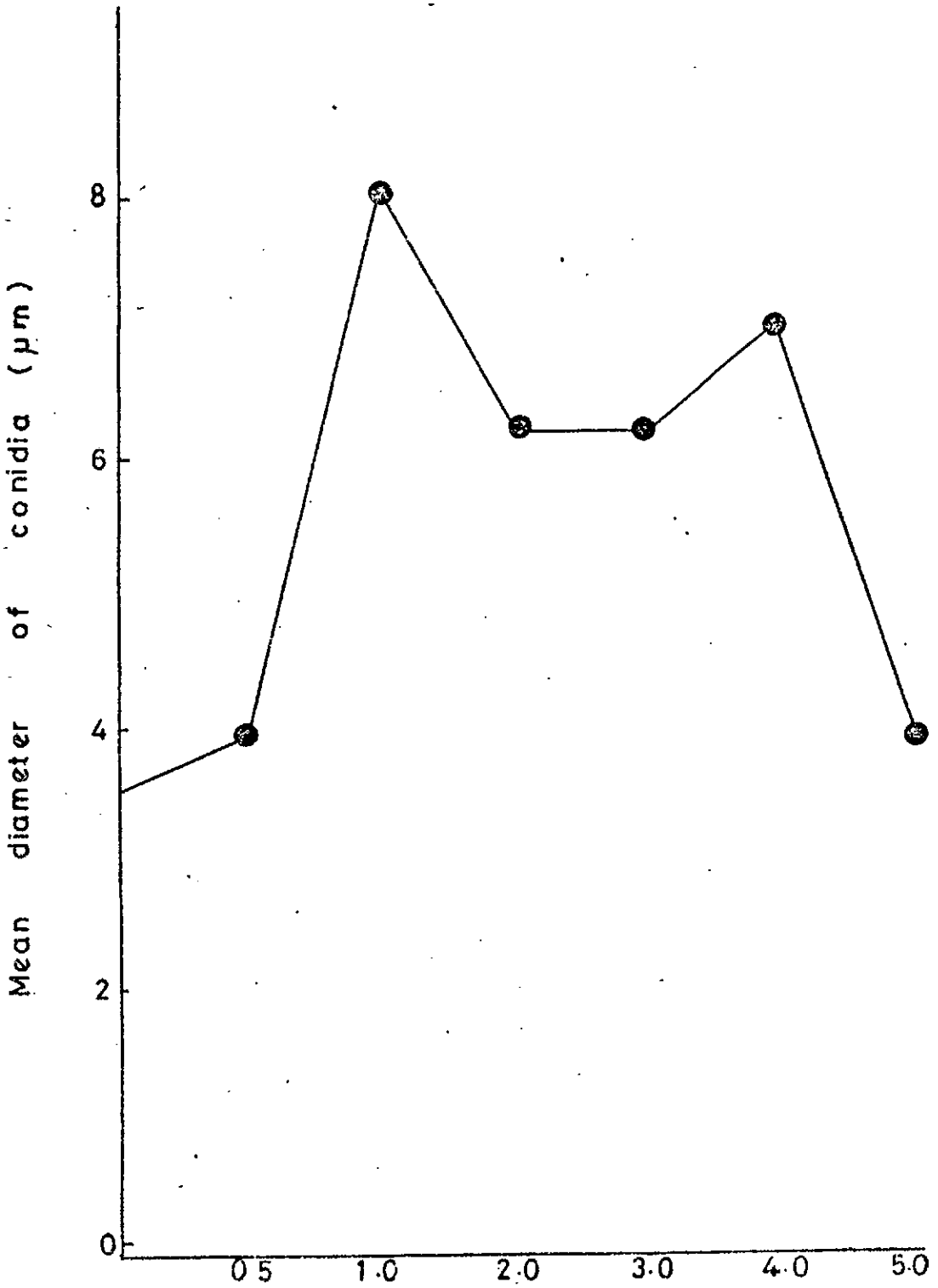


Fig 8

Concentration of sawdust extract of Mitragyna ciliatg

FIG. 8

Swelling of conidia of Trichoderma aureoviride in different concentrations of extract of Mitragyna ciliata sawdust.

FIG. 9

Germination of conidia of some fungi in different concentrations of extract of Triplochiton scleroxylon sawdust.

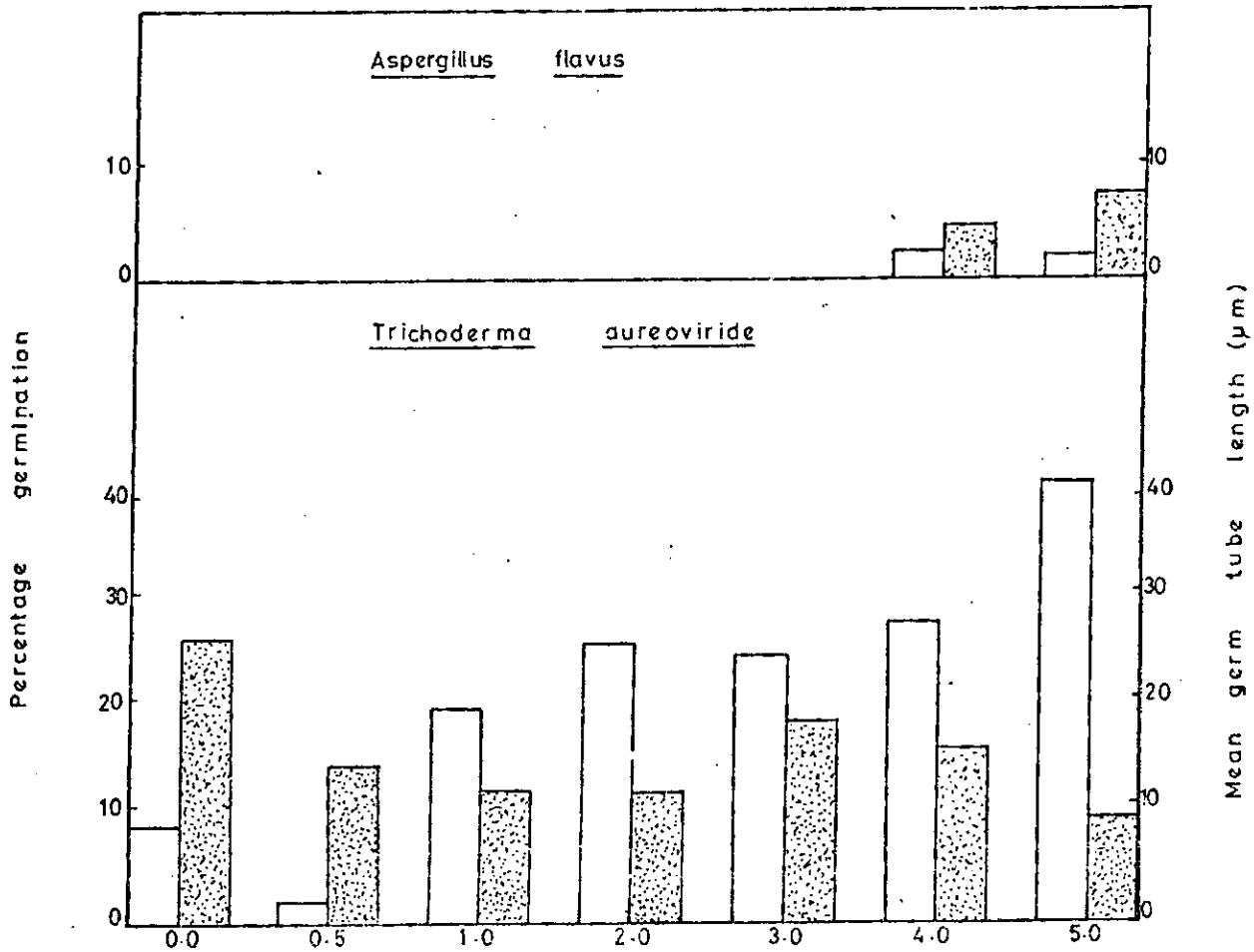


Fig.9 Concentration of extract of sawdust of Triplochiton scleroxylon (% : v/v)

Percentage germination

Mean germ tube length (µm)

iv. Germination in nutrient-amended extract of Khaya ivorensis sawdust

The possibility of germination being raised in the extracts of the sawdust in the lagoon by nutrients in the environment was verified in a series of experiments employing 5% aqueous extract of Khaya ivorensis and conidia of species which germinated very poorly or not at all in the extract of sawdust of Khaya ivorensis.

(a) Carbohydrate-amended extract

Results of experiments carried out with extract amended with different concentrations (0.5-2.0%: w/v) of fructose, glucose, maltose and sucrose are presented in Figs. 10 to 13.

Fructose. Fructose highly stimulated conidia of Cladosporium oxysporum at concentrations of 1.0 and 2.0% (Fig. 10), but ineffective at all concentrations against Aspergillus giganteus and Trichoderma aureoviride conidia (Fig. 10).

Glucose. Conidia of Cladosporium oxysporum germinated very well (up to 92% germination) at all glucose concentrations (Fig. 11) while those of Aspergillus giganteus and Trichoderma aureoviride were only stimulated by 2.0% glucose resulting in 16% and 27% germination respectively (Fig. 11).

FIG. 10

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of fructose.

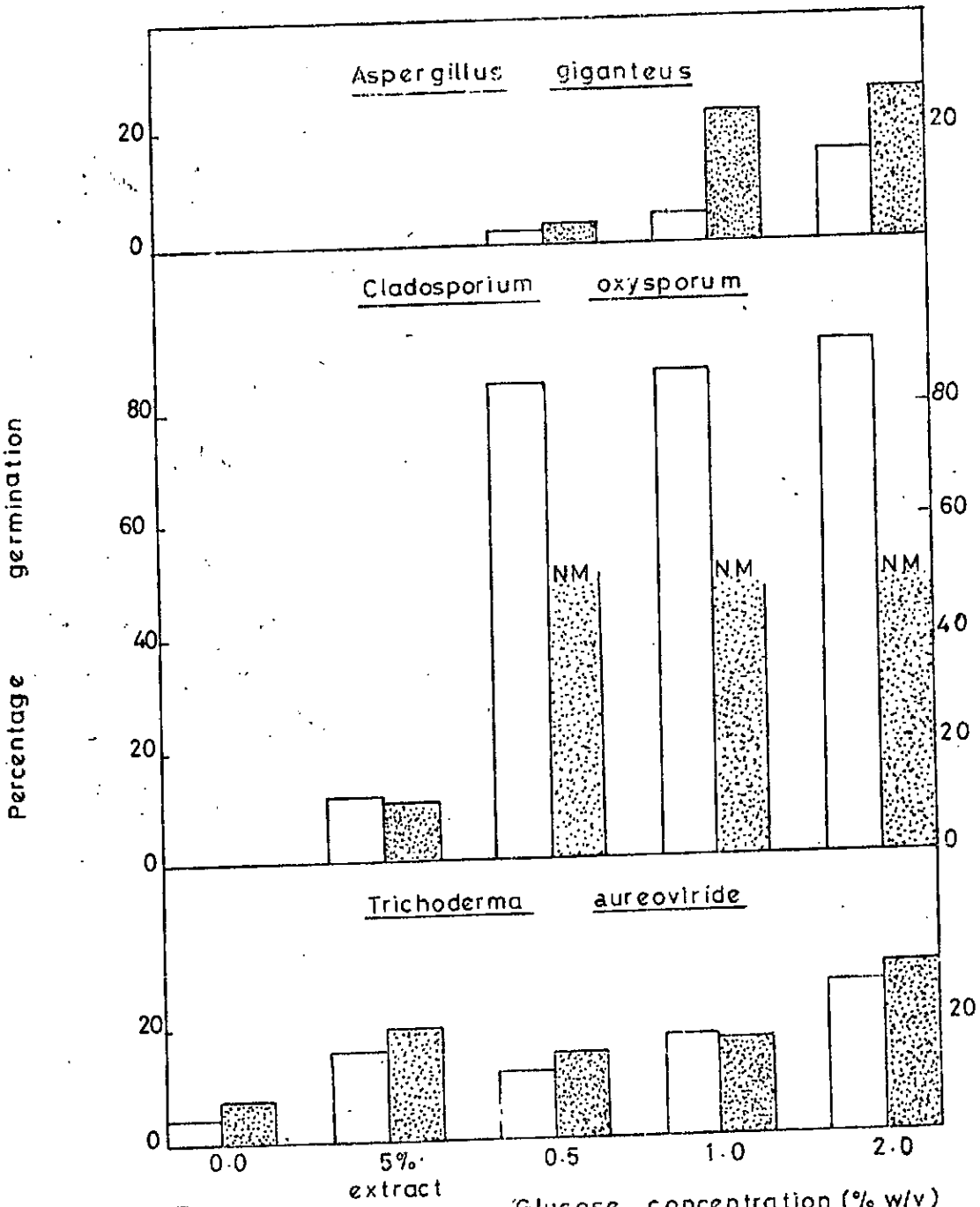


Fig. 11

Percentage germination

Mean germ tube length (µm)

NM Not measurable

FIG. 11

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of glucose.

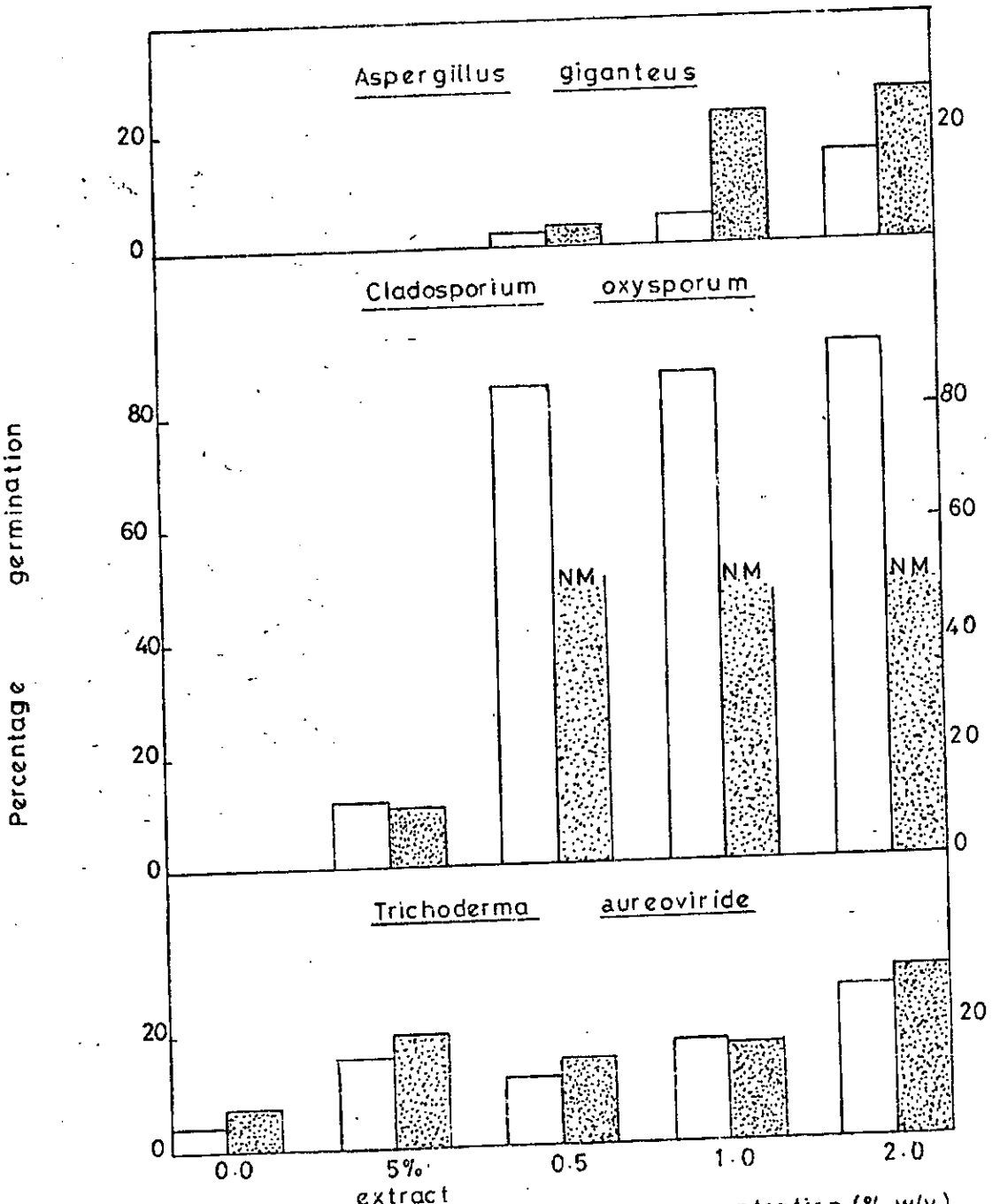


Fig. 11

Percentage germination

Mean germ tube length (µm)

NM Not measurable

Maltose. The addition of maltose to 5.0% Khaya ivorensis sawdust extract did not effect any appreciable change in the germination of conidia of Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride (Fig. 12). The germ tube length of Aspergillus giganteus was not affected by maltose (Fig. 12) while those of Cladosporium oxysporum decreased from 10.8 µm in the unamended extract to 4.0 µm in 1.0% maltose (Fig. 12), and germ tube lengths of conidia of Trichoderma aureoviride decreased from 20.0 µm in unamended extract to 10.1 µm in 1.0% maltose (Fig. 12). Thus, while maltose did not affect percentage germination of the test fungus, it tended to suppress germ tube elongation in C. oxysporum and T. aureoviride.

Sucrose. Increased concentrations of sucrose brought about slight increases in percentage germination of conidia and length of germ tubes produced by Aspergillus giganteus (Fig. 13). Sucrose did not affect germination and germ tube elongation of T. aureoviride, but suppressed germ tube elongation in C. oxysporum (Fig. 13).

(b) Amino acid-amended extract

Alanine. Increased concentrations of alanine stimulated the germination of conidia of Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride (Fig. 14).

FIG. 12

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of maltose.

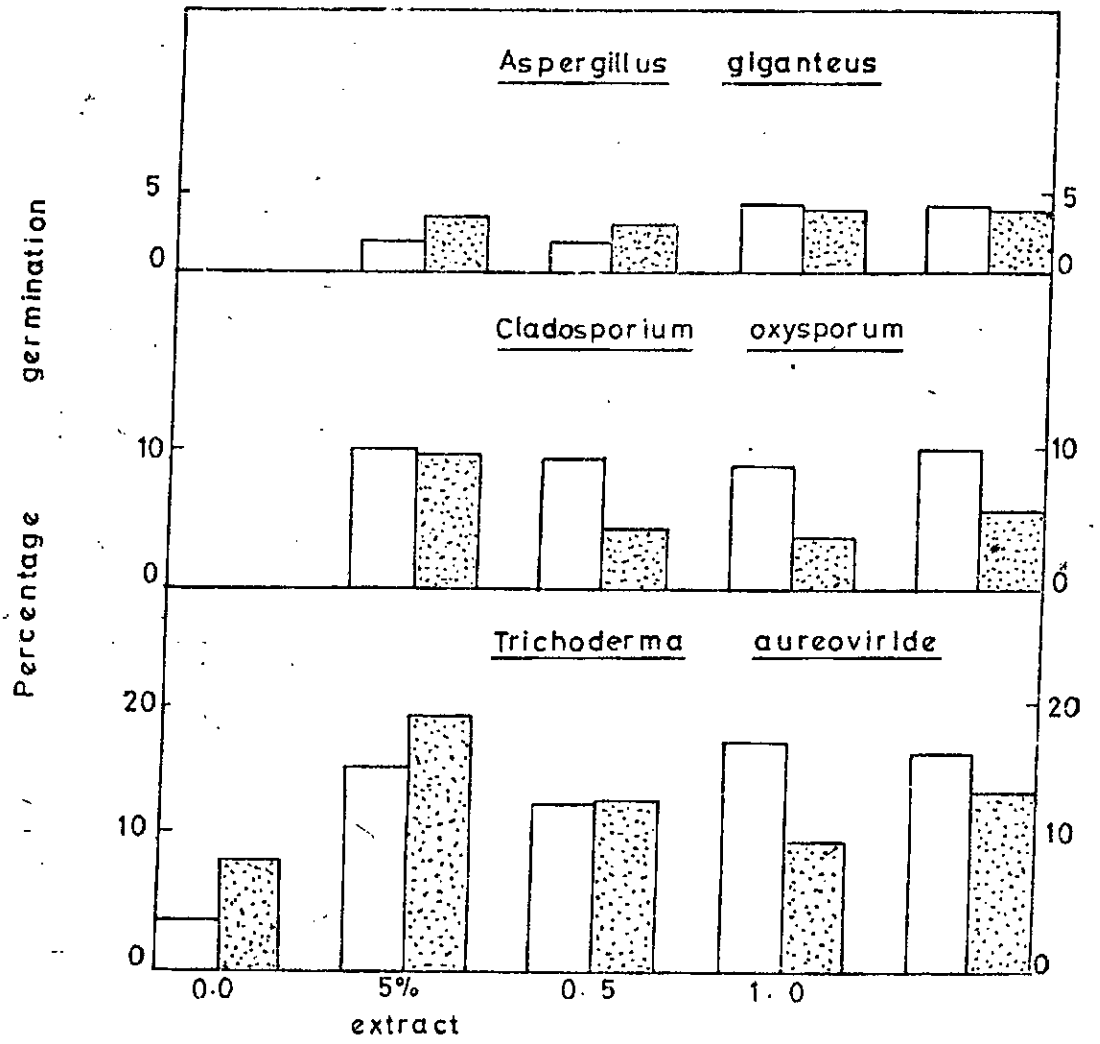

Aspergillus giganteus
Cladosporium oxysporum
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
0
5
10
20
0·0
5% extract
0·5
1·0
Concentration of maltose (%: w/v)
Percentage germination
Mean germ tube length (µm)

Fig.12

FIG. 13

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of sucrose.

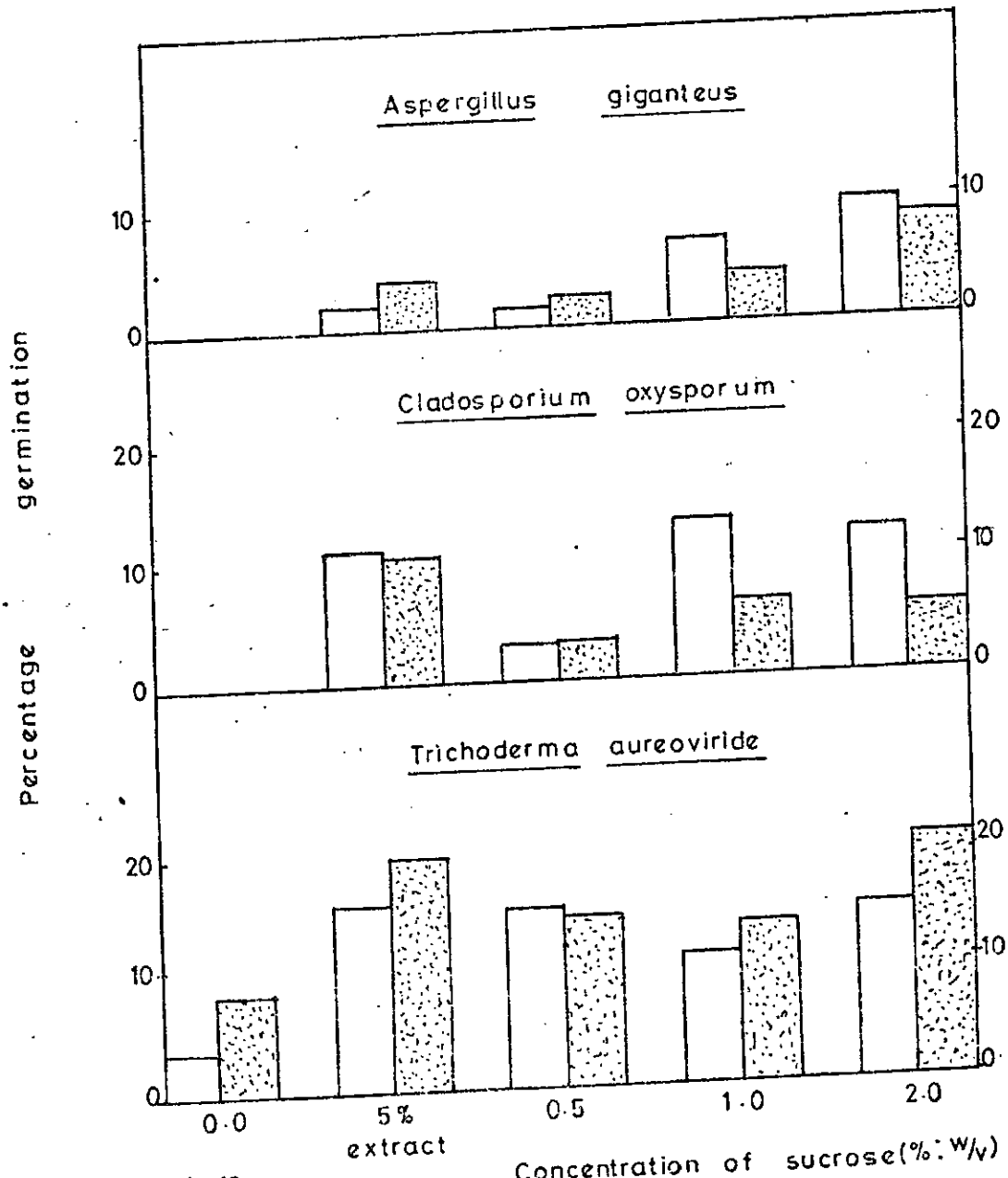


Fig.13

Percentage germination

Mean germ tube length (µm)

FIG. 14

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of alanine.

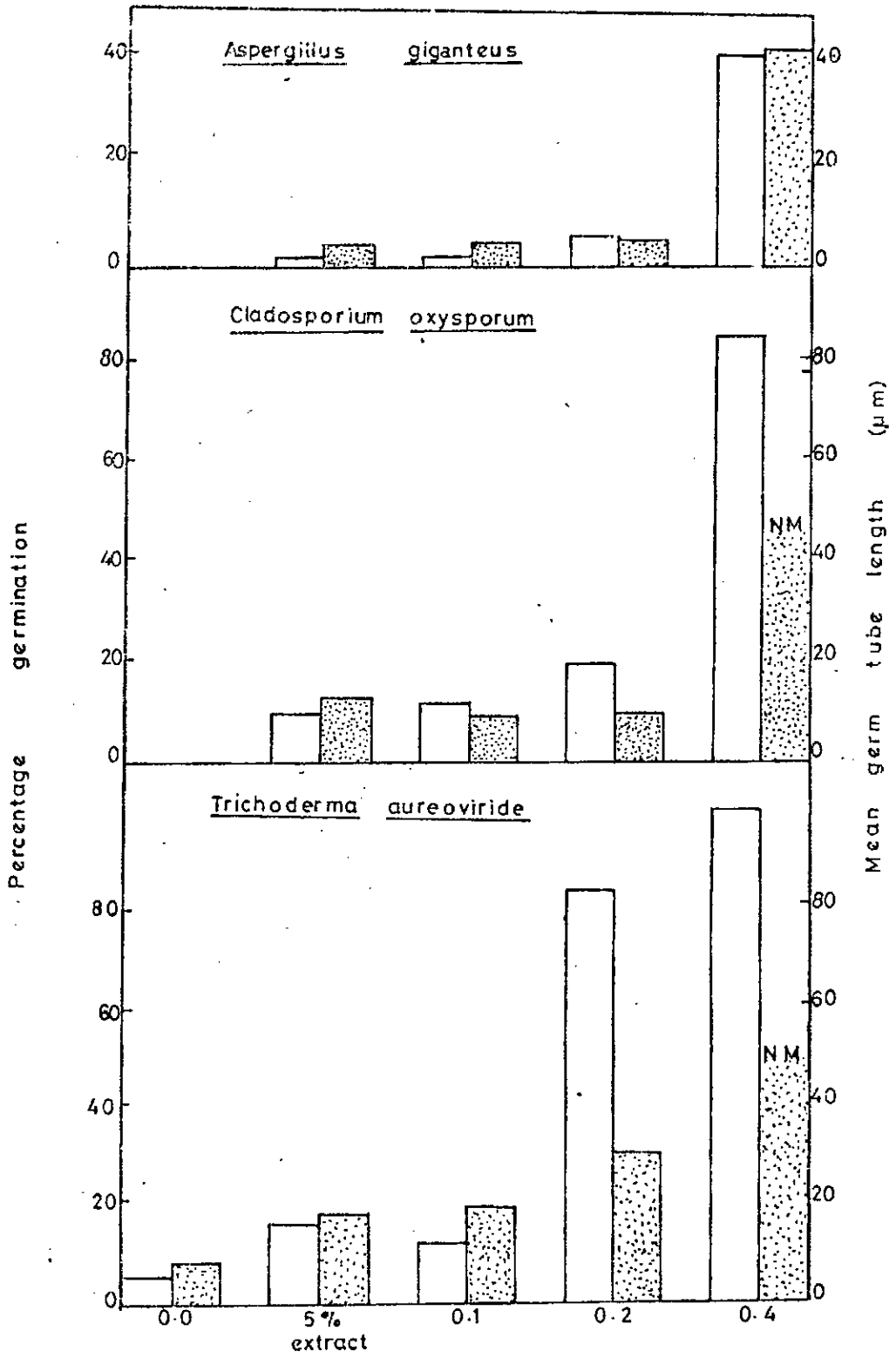


Fig. 14

Percentage germination

Mean germ tube length (µm)

NM Not measurable

The germ tubes of all three species also increased with increasing concentrations of alanine in the medium.

Asparagine. The effect of asparagine on C. oxysporum and T. aureoviride was similar to that reported for alanine. Furthermore, 0.2% asparagine improved percentage germination of conidia of A. giganteus six fold and germ tube elongation three fold (Fig. 15).

Glycine. At some concentrations (0.2 and 0.4%), glycine decreased germination in A. giganteus and T. aureoviride and suppressed germ tube length (Fig. 16). However, 0.4% glycine improved germination of conidia of C. oxysporum and also increased germ tube length slightly (Fig. 16).

Leucine. Leucine had no significant effect on the germination of the fungi used. However, at 0.4%, germination of conidia of T. aureoviride was enhanced (Fig. 17).

(c) Nitrogenous compounds-amended extract

Because conidia of Aspergillus giganteus did not germinate in unamended extract of sawdust of Khaya ivorensis, and addition of some amino acids- asparagine, glycine and leucine - did not improve germination, more complex nitrogenous compounds were added to aqueous extract of Khaya ivorensis sawdust to study their effect on conidial germination of A. giganteus.

FIG. 15

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of asparagine.

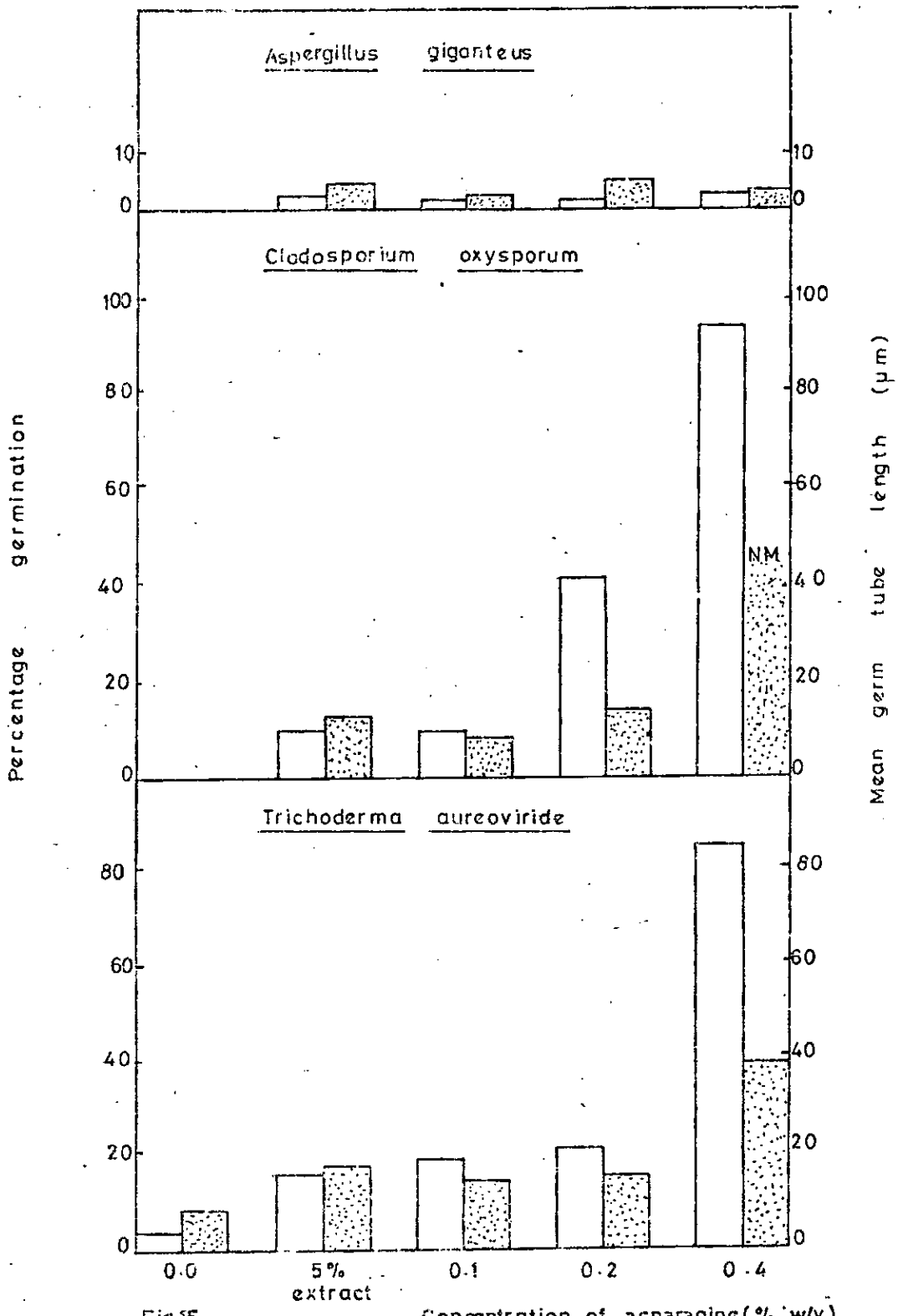


Fig.15

Percentage germination

Mean germ tube length (µm)

NM Not measurable

FIG. 16

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of glycine.

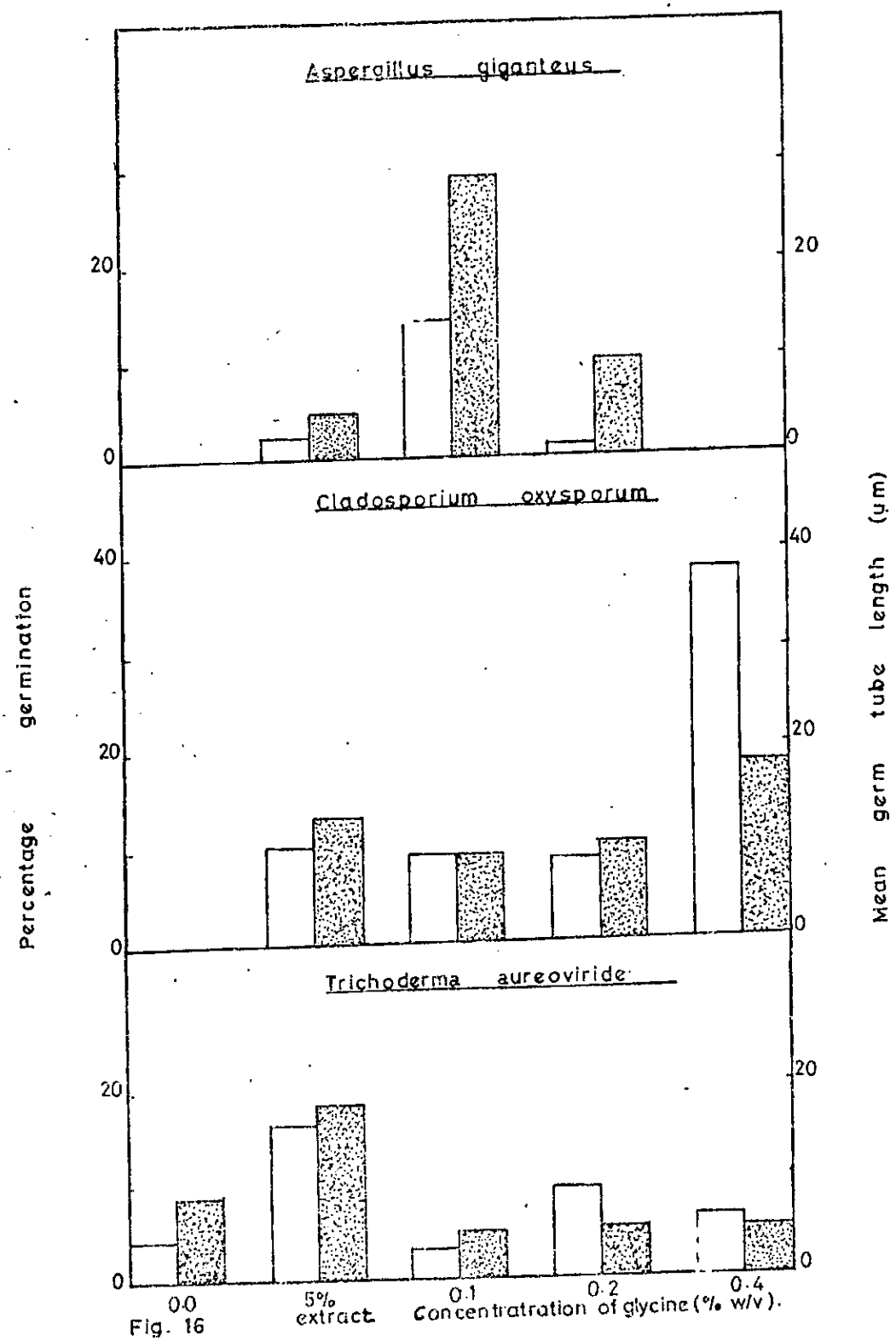

Aspergillus giganteus
Cladosporium oxysporum
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
0
20
40
0.0
5% extract
0.1
0.2
0.4
Concentration of glycine (% w/v)
Percentage germination
Mean germ tube length (µm)

Fig. 16

FIG. 17

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of leucine.

Results in Fig. 18 show that lower concentrations (0.1 and 0.2%) of Yeastrel inhibited germination of conidia of A. giganteus while a higher concentration (0.4%) supported germination. Casein hydrolysate produced improved germination (up to 66%) and longer germ tubes (up to 39.0 μm) at higher concentrations (0.2 and 0.4%) of the N-compound. Peptone similarly enhanced germination. At higher concentrations peptone in particular produced significant increases in conidia germination and germ tube length (Fig. 18).

(d) Inorganic salt-amended extract

Some major inorganic salts shown by Akpata (1975) to be present in Lagos Lagoon water were added to extract of Khaya ivorensis sawdust to study the germination of fungal spores under lagoon conditions. $CaCl_2$. The conidia of Aspergillus flavus and Cladosporium oxysporum did not germinate in $CaCl_2$-amended extract. For Aspergillus giganteus and Trichoderma aureoviride, conidia germination decreased with increasing concentration of $CaCl_2$ (Fig. 19).

KH_2PO_4. Conidia of A. flavus, C. oxysporum and T. aureoviride did not germinate or germinated very poorly in KH_2PO_4 solutions. However, conidia of A. giganteus had good germination (78-89%) and very long germ tubes (Fig. 20) in Khaya ivorensis extract to which different concentrations of KH_2PO_4 were added.

FIG. 18

Germination of conidia of Aspergillus giganteus in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of nitrogenous compounds

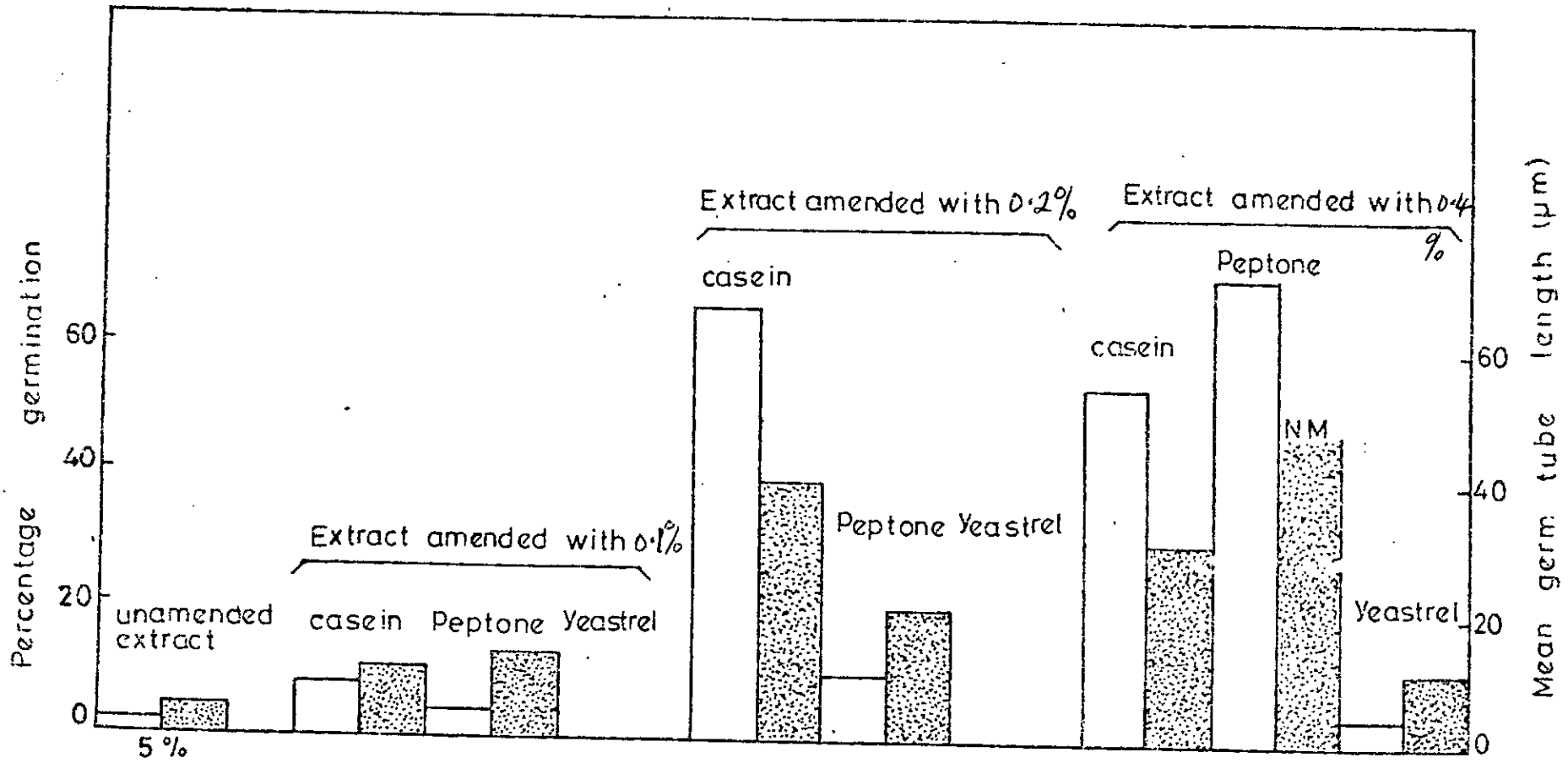

Percentage germination
Mean germ tube length (μm)
60
40
20
0
unamended extract
5 %
Extract amended with 0·1%
casein
Peptone
Yeastrel
Extract amended with 0·2%
casein
Peptone
Yeastrel
Extract amended with 0·4 %
casein
Peptone
NM
Yeastrel
Percentage germination
Mean germ tube length (μm)

Fig. 19

FIG. 19

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of $CaCl_2$

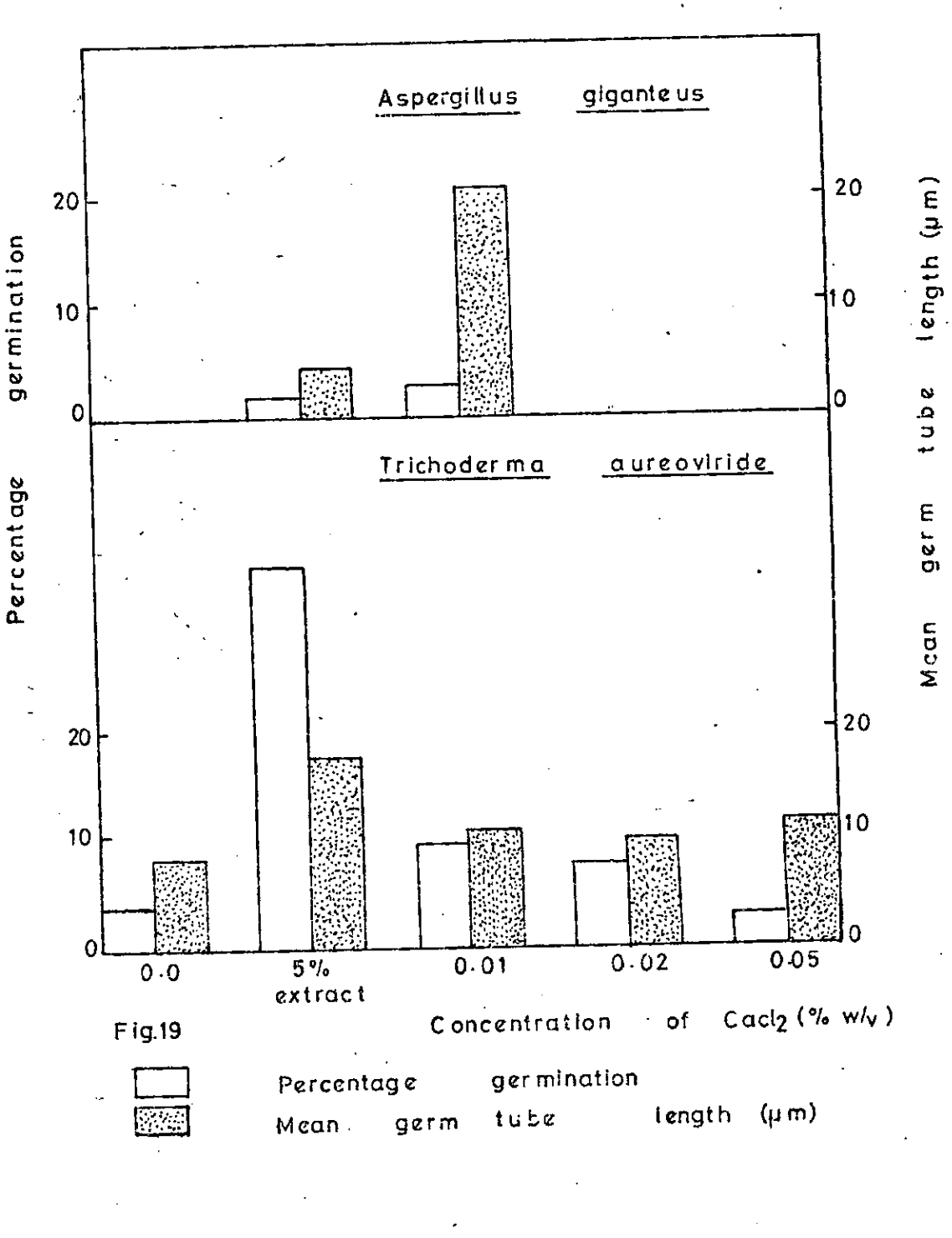

Aspergillus giganteus
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
20
10
0
0·0
5% extract
0·01
0·02
0·05
Concentration of Cacl2 (% w/v)
Percentage germination
Mean germ tube length (µm)

Fig.19

FIG. 20

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of KH_2PO_4

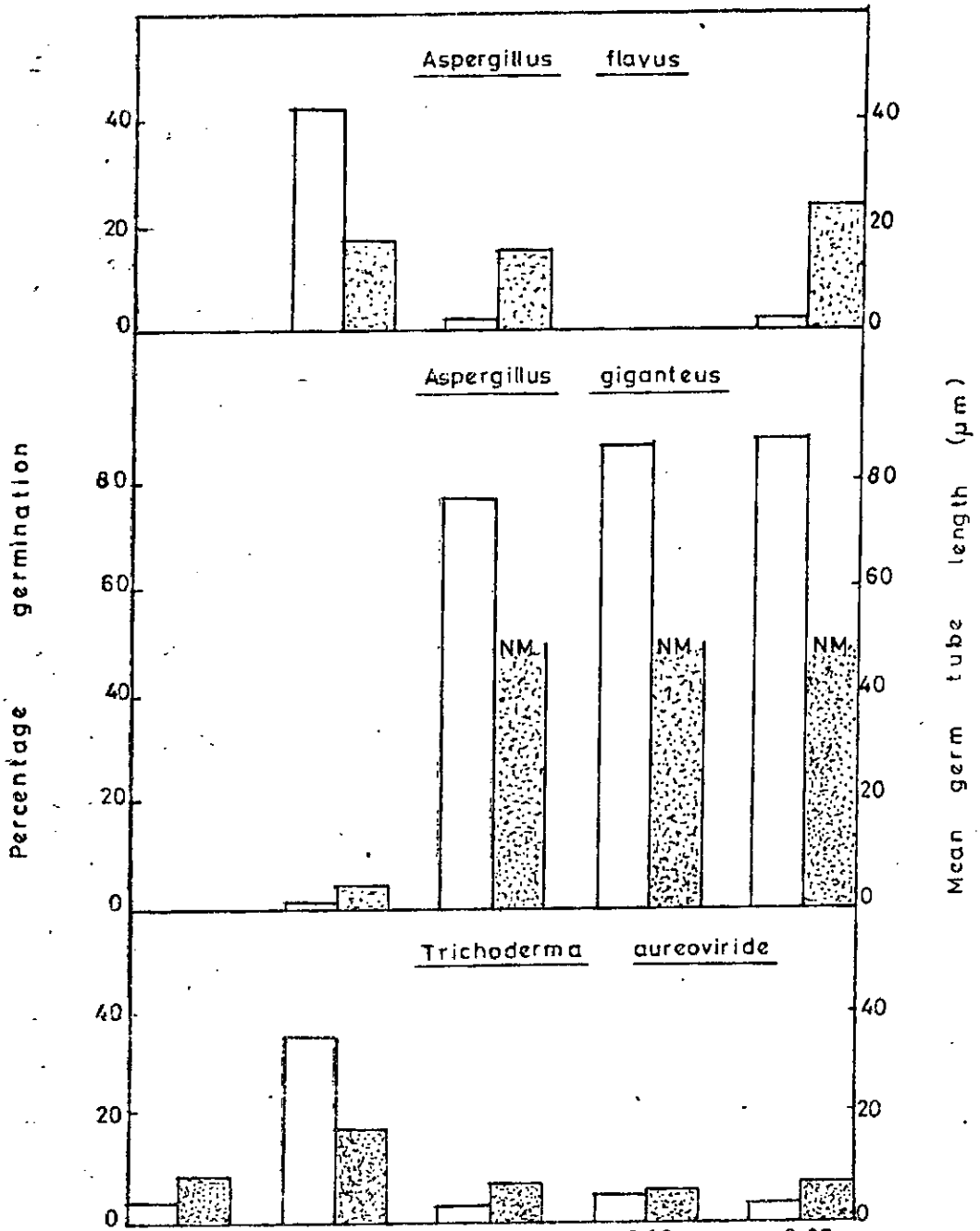


Fig. 20

Percentage germination

Mean germ tube length (µm)

NM Not measurable

$MgSO_4.7H_2O$. Conidia of Aspergillus flavus did not germinate in presence of $MgSO_4.7H_2O$. There was inhibition of germination of conidia of A. giganteus, Cladosporium oxysporum and Trichoderma aureoviride as the concentration of $MgSO_4.7H_2O$ increased (Fig.21). NaCl. Conidia of A. flavus and C. oxysporum did not germinate in presence of NaCl. For T. aureoviride, germination and germ tube length decreased with increasing concentration of NaCl (Fig. 22). Although low concentrations (0.01 and 0.02%) of NaCl improved conidia germination and germ tube length in Aspergillus giganteus (Fig. 22), higher concentrations inhibited germination and germ tube length.

v. Spore germination in lagoon water

Fungal spores were suspended in different concentrations of sterile lagoon water to study germination in the lagoon.

Except for Aspergillus flavus which had only 9% maximum germination and germination decreased with increasing concentration of lagoon water, other fungal species either did not or germinated very poorly in lagoon water (Fig. 23).

FIG. 21

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of $MgSO_4.7H_2O$.

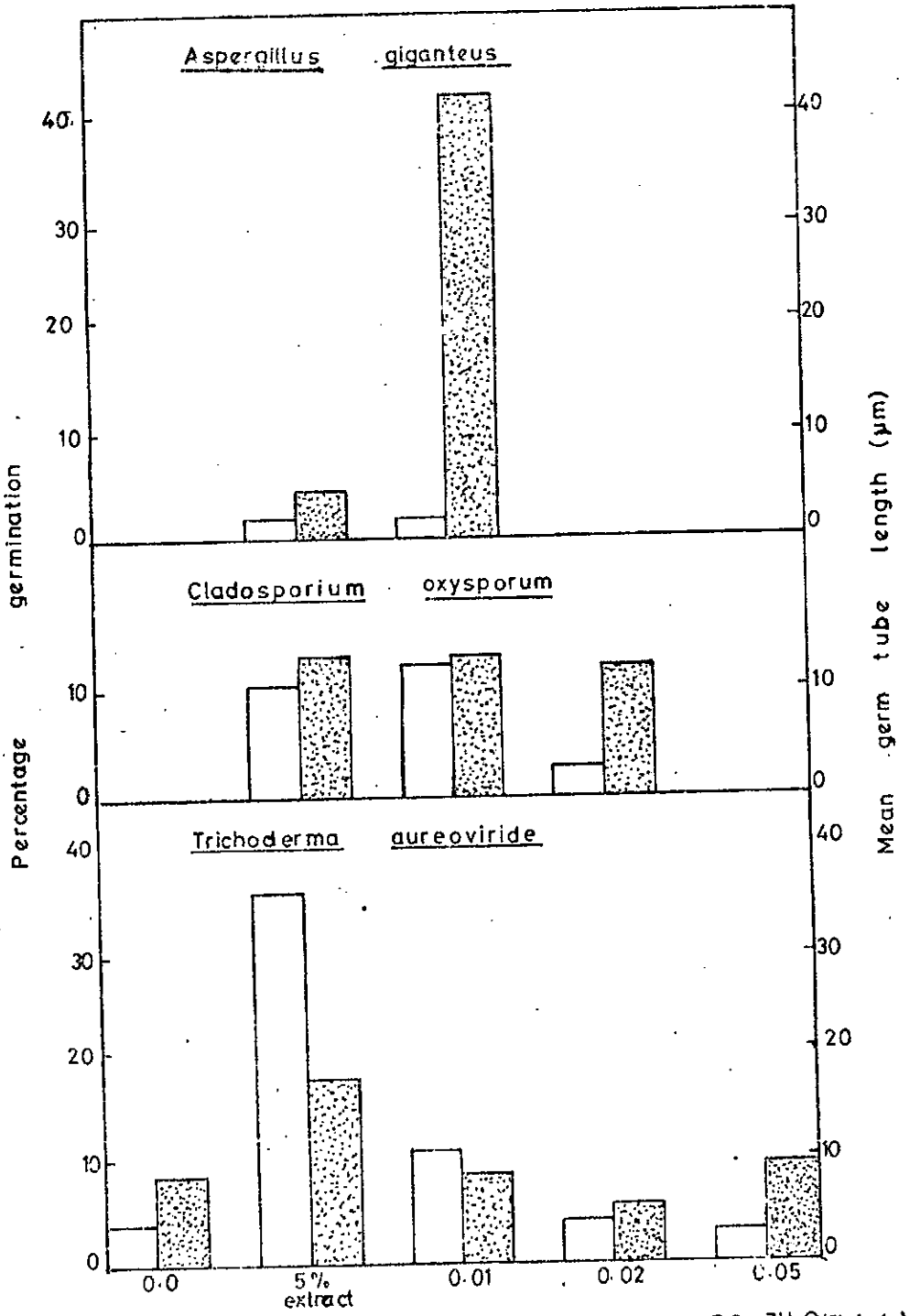

Aspergillus giganteus
Cladosporium oxysporum
Trichoderma aureoviride
Percentage germination
Mean germ tube length (µm)
0
10
20
30
40
0·0
5% extract
0·01
0·02
0·05
Concentration of $MgSO_4 \cdot 7H_2O$(% :w/v)
Percentage germination
Mean germ tube length (µm)

Fig. 21.

FIG. 22

Germination of conidia of some fungi in 5% aqueous extract of Khaya ivorensis sawdust amended with different concentrations of NaCl.

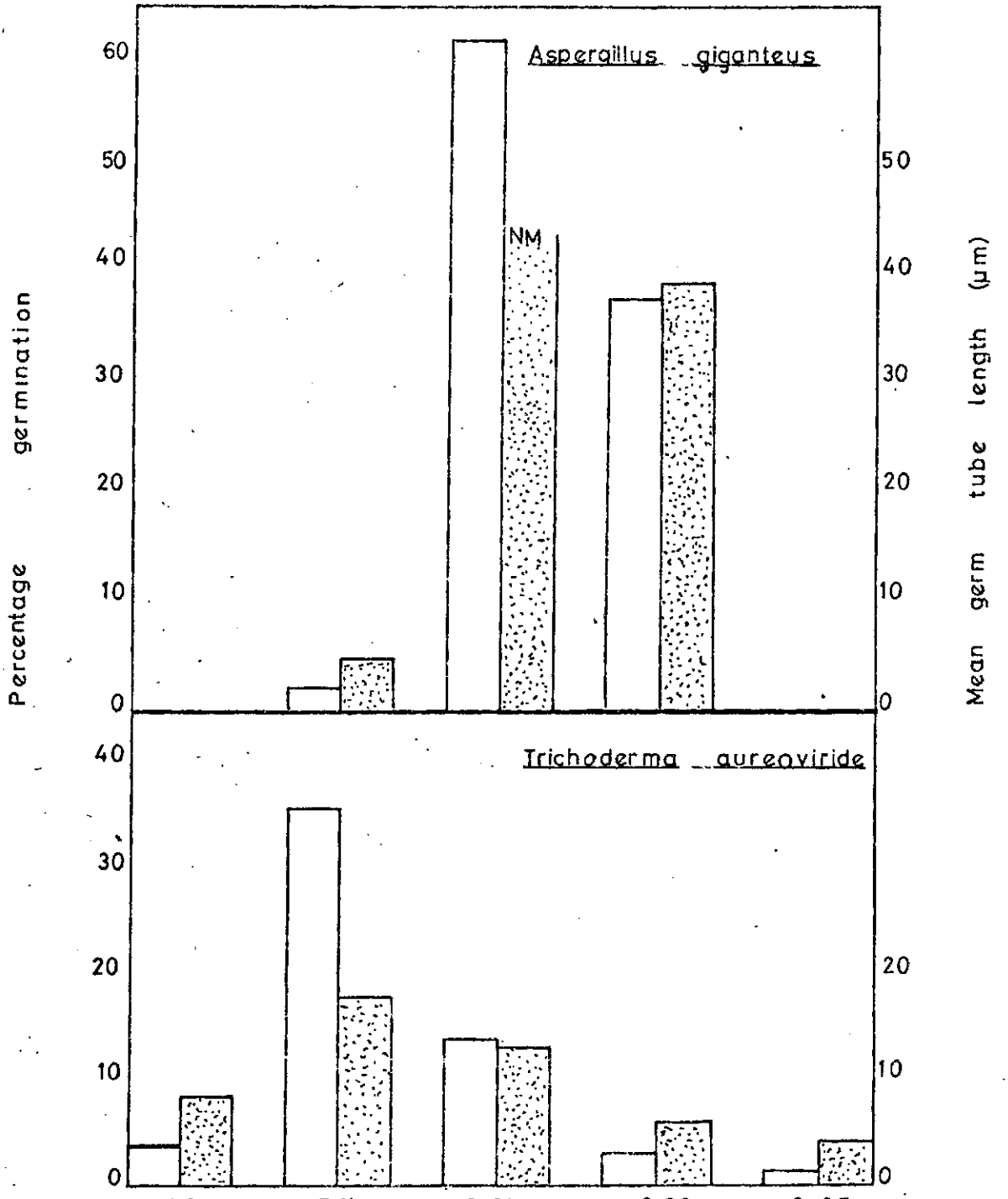

Aspergillus giganteus
Trichoderma aureoviride
NM
Percentage germination
Mean germ tube length (µm)
60
50
40
30
20
10
0
0·0
5 % extract
0·01
0·02
0·05
Concentration of Nacl (% : w/v)
Percentage germination
Mean germ tube length (µm)
NM Not measurable

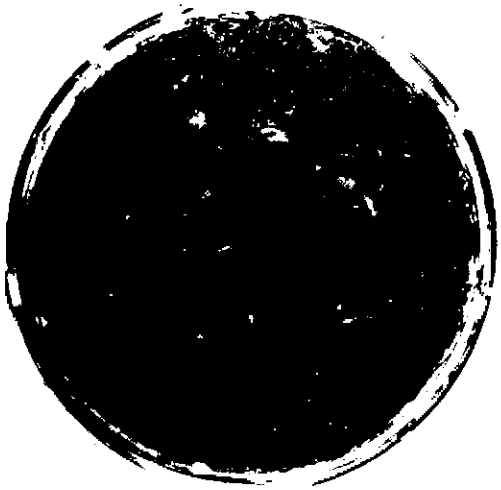
Fig 22.

FIG. 23

Germination of conidia of some fungi in different concentrations of lagoon water

Plate 7

Plate 8



K. DECOMPOSITION OF SAWDUST BY FUNGI

Sawdust particles of three popular wood species, Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon, were inoculated with four frequently occurring fungi in the Lagos Lagoon to study their ability to decompose sawdust. Decomposition was assessed as loss in dry weight of sawdust after incubation with each of the fungi for 40 days. The fungi tested were Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride. The results of the correlation coefficient (r) and t-test statistics are presented in Tables 37a, 40a and 43a.

i. Effect of temperature on sawdust decomposition

During the period of this study the lagoon water temperature at the mid-lagoon and sawmill stations were recorded and found to vary between 22 and 33°C (Table 9).

Water temperature at the sawmill station varied between 24.0 and 30.5°C (Table 9). The inoculated plates of sawdust were therefore incubated at 24, 27, 30 and 33°C to study the effect of temperature on the ability of each fungus to decompose sawdust.

There was negative correlation between temperature and weight in each of the sawdust samples. The higher the temperature, the greater was the loss of weight of sawdust.

Khaya ivorensis sawdust

When inoculated with the fungal species and incubated at 24°C, only Aspergillus giganteus caused any appreciable loss (1.7%) in dry weight.

At 27°C, all the fungi had good growth (Plates 7 and 8) and caused significant decrease in the dry weight of the sawdust. Similar results were obtained at 30°C for sawdust inoculated with A. flavus, A. giganteus or Cladosporium oxysporum. Only A. flavus and C. oxysporum decreased the dry weight of the sawdust at 33°C (Table 35).

Although there was a negative correlation between temperature and weight in each of the sawdust samples, the negative correlation was not significant when Khaya ivorensis sawdust was inoculated with A. flavus or Trichoderma aureoviride. The calculated r-values of -0.2764 and -0.1509 respectively were obtained as compared with tabulated r-value of 0.5760 at 5% level of significance and 10 d.f. The negative correlation was however highly significant when K. ivorensis sawdust was inoculated with A. giganteus and Cladosporium oxysporum with r-values of -0.7766 and -0.6960 respectively. When the t-test was applied to further verify the significance of r, A. giganteus and C. oxysporum showed significant negative correlation with t-values of -3.8978 and -3.0652 respectively (tabulated t-value at 5% level of significance and 10 d.f. = 2.764). The negative correlation of the two other fungal species, A. flavus and T. aureoviride was not significant as shown by the t-test (Table 37a).

Mitragyna ciliata sawdust

The relation between temperature and weight of sawdust of Mitragyna ciliata showed negative correlation when inoculated with each of the four fungal isolates (Table 36), but none of the correlations was significant (Table 37a).

PLATE 7

Photograph showing Aspergillus flavus growing on Khaya ivorensis sawdust after 40 days at 27°C (x0.65).
Note large mycelial colonies

PLATE 8

Photograph showing Aspergillus giganteus growing on Khaya ivorensis sawdust after 40 days at 27°C. (x0.65)

TABLE 35

Changes in dry weight of Khaya ivorensis sawdust inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride and incubated at different temperatures for 40 days.

Temperature of incubation (°C)	Initial dry wt. of K. ivorensis (mg)	Mean dry wt. (mg) of K. ivorensis inoculated with				% loss in dry wt. of K. ivorensis inoculated with			
		A	B	C	D	A	B	C	D
24	177.5	174.4	177.5	177.5	177.4	1.7	0.0	0.0	0.1
27	177.5	170.2	174.6	165.5	172.5	4.1	1.6	6.8	2.8
30	177.5	171.3	171.5	166.8	176.5	3.5	3.4	6.0	0.6
33	177.5	168.5	176.5	165.5	177.0	5.1	0.6	6.8	0.3

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 36

Changes in dry weight of Mitragyna ciliata sawdust inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride and incubated at different temperatures for 40 days.

Temperature of incubation (°C)	Initial dry wt. of M.ciliata (mg)	Mean dry wt. (mg) of M. ciliata inoculated with				% loss in dry wt. of M. ciliata inoculated with			
		A	B	C	D	A	B	C	D
24	186.0	185.3	184.7	186.0	185.0	0.4	0.5	0.0	0.5
27	186.0	183.7	182.7	186.0	184.0	1.2	1.8	0.0	1.1
30	186.0	182.3	180.3	185.3	183.0	2.0	3.1	0.4	1.6
33	186.0	181.3	180.3	181.7	181.0	2.5	3.1	2.3	2.7

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

Triplochiton scleroxylon sawdust

Results in Table 37 show the loss in dry weight of the sawdust caused by each fungus. Significant negative correlation occurred between temperature and dry weight of sawdust inoculated with Aspergillus flavus, A. giganteus or Trichoderma aureoviride (Table 37a).

ii. Effect of glucose on sawdust decomposition

It is expected that nutrients such as glucose will be available at the sawdust decomposing site in Lagos Lagoon. The possibility of decomposition of sawdust being increased in the lagoon by nutrients (e.g. glucose) was verified by addition of different concentrations (0.1, 0.5, 1.0 and 2.0%) of glucose to sawdust of three wood species used for the temperature experiment (section Ki).

Sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon

On amendment of the sawdust of the three wood species with different concentrations of glucose solution (up to 2.0%), utilization by the fungal species, as measured by loss in dry weight of sawdust, was greatly enhanced (Tables 38 to 40).

There was significant negative correlation between varying glucose concentrations and dry weights of K. ivorensis sawdust inoculated with each of the four fungal species. Aspergillus flavus showed the most significant effect (Table 40a)

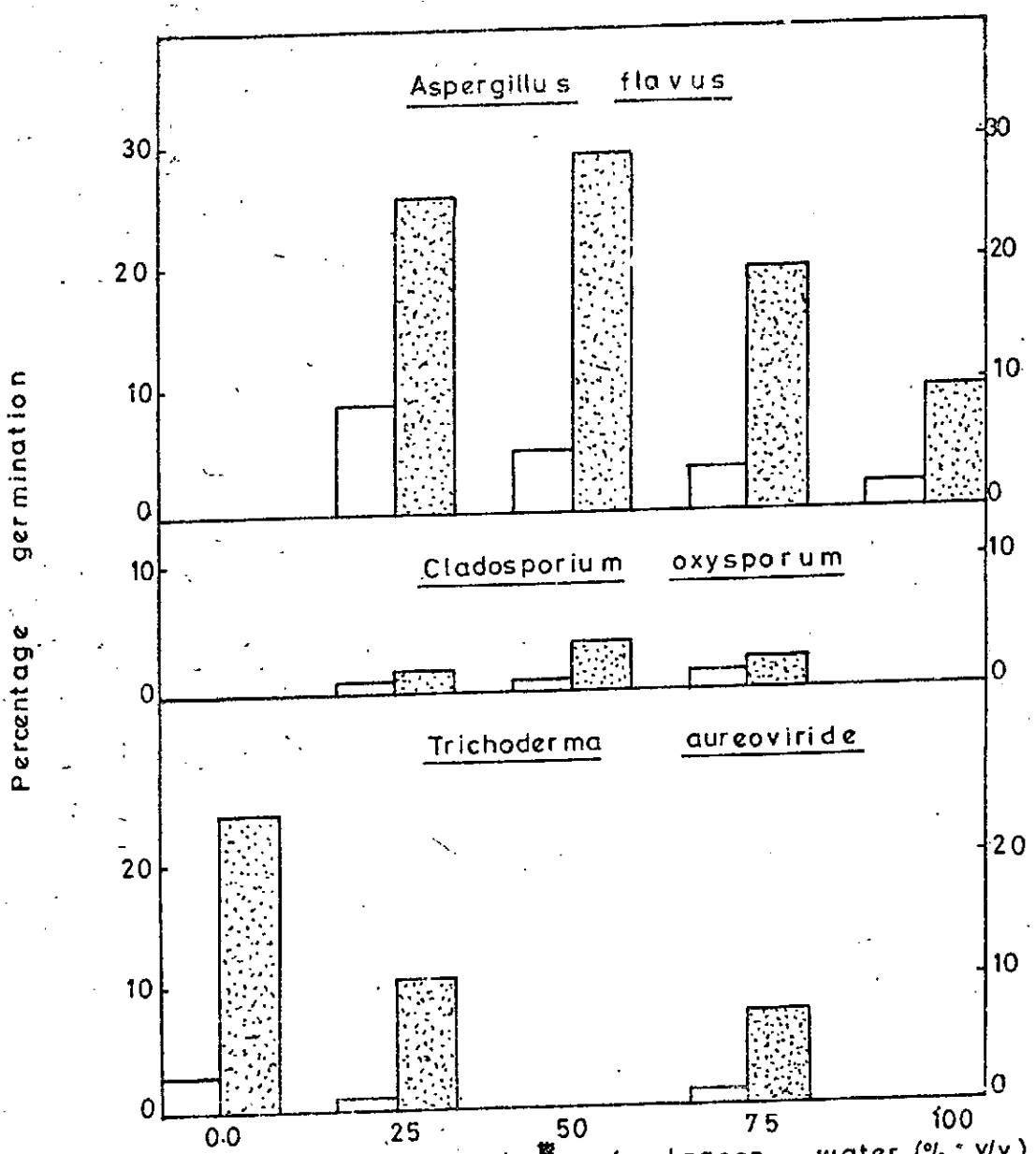


Fig.23

Percentage germination

Mean germ tube length (µm)

* Lagoon water of salinity 21.0‰ was diluted with sterile distilled water

TABLE 37a

The relation between temperature and weight of sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon inoculated with different fungi (tested with correlation coefficient (r) and t-test statistics).

Wood species	Fungal species	Correlation coefficient (r)	t-test (t)
Khaya ivorensis	Aspergillus flavus	-0.2764	-0.9093
	Aspergillus giganteus	-0.7766	-3.8978*
	Cladosporium oxysporum	-0.6960	-3.0652*
	Trichoderma aureoviride	-0.1509	-0.4826
Mitragyna ciliata	Aspergillus flavus	-0.4476	-1.5830
	Aspergillus giganteus	-0.5173	-1.9114
	Cladosporium oxysporum	-0.4674	-1.6719
	Trichoderma aureoviride	-0.4630	-1.6519
Triplochiton scleroxylon	Aspergillus flavus	-0.7631	-3.7342*
	Aspergillus giganteus	-0.6565	-2.6112
	Cladosporium oxysporum	-0.4466	-1.5784
	Trichoderma aureoviride	-0.7446	-3.5282*

* Significant at 5% level with 10 d.f.

TABLE 38

Changes in dry weight of Khaya ivorensis sawdust amended with different concentrations of glucose solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Glucose concentration (%: w/v)	Initial dry wt. of K.ivorensis (mg)	Mean dry wt. (mg) of K.ivorensis inoculated with				% loss in dry wt. of K.ivorensis inoculated with			
		A	B	C	D	A	B	C	D
0.0	177.5	168.3	171.5	165.6	172.7	5.2	3.4	6.7	2.7
0.1	177.5	165.7	170.5	164.9	172.1	6.6	3.9	7.1	3.0
0.5	177.5	164.4	169.2	163.5	171.5	7.4	4.7	7.9	3.4
1.0	177.5	163.7	167.5	162.3	170.7	7.8	5.6	8.6	3.8
2.0	177.5	162.5	165.3	161.0	169.8	8.5	6.9	9.3	4.3

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 39

Changes in dry weight of Mitragyna ciliata sawdust amended with different concentrations of glucose solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Glucose concentration (%: w/v)	Initial dry wt. of M.ciliata	Mean dry wt. (mg) of M.ciliata inoculated with				% loss in dry wt. M.ciliata inoculated with			
		A	B	C	D	A	B	C	D
0.0	186.0	181.2	180.4	181.9	180.6	2.6	3.0	2.2	2.9
0.1	186.0	181.0	179.8	181.1	179.9	2.7	3.3	2.6	3.3
0.5	186.0	178.9	179.2	179.9	179.4	3.8	3.7	3.3	3.5
1.0	186.0	176.4	177.6	179.0	178.7	5.2	4.5	3.8	3.9
2.0	186.0	174.7	176.3	177.6	177.9	6.1	5.2	4.5	4.4

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 40

Changes in dry weight of Triplochiton scleroxylon sawdust amended with different concentrations of glucose solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Glucose concentration (%: w/v)	Initial dry wt. of T.scleroxylon (mg)	Mean dry wt. (mg) of T. scleroxylon inoculated with				% loss in dry wt. of T.scleroxylon inoculated with			
		A	B	C	D	A	B	C	D
0.0	184.0	175.3	177.6	178.2	178.5	4.7	3.5	3.2	3.0
0.1	184.0	175.0	176.9	177.2	177.4	4.9	3.9	3.7	3.6
0.5	184.0	173.0	175.8	176.1	176.9	6.0	4.5	4.3	3.9
1.0	184.0	170.6	174.0	174.8	176.0	7.3	5.4	5.0	4.3
2.0	184.0	179.1	172.0	173.5	174.9	8.1	6.5	5.7	4.9

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 40a

The relation between varying glucose concentrations and weight of sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon inoculated with different fungi (tested with correlation coefficient (r) and t-test statistics).

Wood species	Fungal species	Correlation coefficient (r)	t-test (t)
Khaya ivorensis	Aspergillus flavus	-0.9833	-19.4876*
	Aspergillus giganteus	-0.8301	- 5.3694*
	Cladosporium oxysporum	-0.9606	-12.4663*
	Trichoderma aureoviride	-0.9655	-13.3698*
Mitragyna ciliata	Aspergillus flavus	-0.9780	-16.9179*
	Aspergillus giganteus	-0.9669	-13.6657*
	Cladosporium oxysporum	-0.9667	-13.6279*
	Trichoderma aureoviride	-0.2735	- 1.0254
Triplochiton scleroxylon	Aspergillus flavus	-0.9886	-23.6345*
	Aspergillus giganteus	-0.4084	- 1.6132
	Cladosporium oxysporum	-0.9609	-12.5056*
	Trichoderma aureoviride	-0.9509	-11.0769*

* Significant at 5% level with 10 d.f.

The same pattern of significant negative correlation was obtained for Mitragyna ciliata sawdust except when inoculated with Trichoderma aureoviride (Table 40a).

Sawdust of Triplochiton scleroxylon also produced the same pattern of negatively significant correlation coefficient with the most significant effect being obtained on inoculation with A. flavus (Table 40a).

iii. Effect of peptone on sawdust decomposition

That the addition of a nitrogen source will enhance the decomposition of sawdust by fungi in the lagoon was verified by adding different concentrations of peptone (0.1, 0.2, 0.4 and 0.8%) to the sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon.

The results (Tables 41-43) were similar to those obtained for glucose-amended sawdust. More significant weight losses were obtained in the peptone-amended sawdust media than in those amended with glucose. The three wood species showed marked negative correlation between varying peptone concentrations and dry weights of sawdust when inoculated with each of Aspergillus flavus, A. giganteus, Cladosporium oxysporum and Trichoderma aureoviride. The negative correlations were also highly significant as shown by the t-test (Table 43a).

TABLE 41

Changes in dry weight of Khaya ivorensis sawdust amended with different concentrations of peptone solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Peptone concentration (%: w/v)	Initial dry wt. of K.ivorensis (mg)	Mean dry wt. (mg) of K.ivorensis inoculated with				% loss in dry wt. of K.ivorensis inoculated with			
		A	B	C	D	A	B	C	D
0.0	177.5	168.3	171.5	165.6	172.7	5.2	3.4	6.7	2.7
0.1	177.5	167.0	170.6	164.7	172.3	5.9	3.9	7.2	2.9
0.2	177.5	166.5	169.4	163.9	171.8	6.2	4.6	7.7	3.2
0.4	177.5	165.4	168.5	162.6	171.2	6.8	5.1	8.4	3.5
0.8	177.5	163.8	167.1	162.0	170.5	7.7	5.8	8.7	3.9

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 42

Changes in dry weight of Mitragyna ciliata sawdust amended with different concentrations of peptone solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Peptone concentration (%: w/v)	Initial dry wt. of M. ciliata	Mean dry wt. (mg) of M. ciliata inoculated with				% loss in dry wt. of M. ciliata inoculated with			
		A	B	C	D	A	B	C	D
0.0	186.0	181.2	180.4	181.9	180.6	2.6	3.0	2.2	2.9
0.1	186.0	179.9	179.2	181.3	180.2	3.3	3.7	2.5	3.1
0.2	186.0	178.5	178.8	180.0	180.0	4.0	3.9	3.2	3.2
0.4	186.0	176.2	177.9	179.0	179.2	5.3	4.4	3.8	3.7
0.8	186.0	175.4	176.1	177.4	178.6	5.6	5.3	4.6	4.0

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 43

Changes in dry weight of Triplochiton scleroxylon sawdust amended with different concentrations of peptone solution and inoculated with Aspergillus flavus, Aspergillus giganteus, Cladosporium oxysporum and Trichoderma aureoviride at 33°C for 40 days.

Peptone concentration (%: w/v)	Initial dry wt. of T.scleroxylon (mg)	Mean dry wt. (mg) of T. scleroxylon inoculated with				% loss in dry wt of T.scleroxylon inoculated with			
		A	B	C	D	A	B	C	D
0.0	184.0	175.8	177.6	178.2	178.5	4.7	3.5	3.2	3.0
0.1	184.0	174.2	177.0	177.7	177.6	5.3	3.8	3.4	3.5
0.2	184.0	173.5	176.2	176.9	177.0	5.7	4.2	3.9	3.8
0.4	184.0	172.2	174.9	175.8	176.4	6.4	4.9	4.5	4.1
0.8	184.0	171.3	173.5	174.4	175.2	6.9	5.7	5.2	4.8

A - Aspergillus flavus

B - Aspergillus giganteus

C - Cladosporium oxysporum

D - Trichoderma aureoviride.

Mean dry weights are averages of three replicates.

TABLE 43a

The relation between varying peptone concentrations and weight of sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon inoculated with different fungi (tested with correlation coefficient (r) and t-test statistics)

Wood species	Fungal species	Correlation coefficient (r)	t-test (t)
Khaya ivorensis	Aspergillus flavus	-0.9646	-13.1900*
	Aspergillus giganteus	-0.9729	-15.1614*
	Cladosporium oxysporum	-0.9283	- 8.9999*
	Trichoderma aureoviride	-0.9671	-13.7085*
Mitragyna ciliata	Aspergillus flavus	-0.9802	-17.8378*
	Aspergillus giganteus	-0.9267	- 8.8889*
	Cladosporium oxysporum	-0.9749	-15.7934*
	Trichoderma aureoviride	-0.9622	-12.7352*
Triplochiton scleroxylon	Aspergillus flavus	-0.9459	-10.5162*
	Aspergillus giganteus	-0.9804	-17.9488*
	Cladosporium oxysporum	-0.9847	-20.3497*
	Trichoderma aureoviride	-0.9674	-13.7624*

* Significant at 5% level with 10 d.f.

V. DISCUSSION

The physico-chemical features of the Lagos Lagoon were studied and most of the features showed seasonal variation. Marked seasonal fluctuation in the salinity of the Lagos Lagoon has been reported (Akpata and Ekundayo, 1978; Ezenwa, 1978; Hill and Webb, 1958; Olaniyan, 1969; Oyenekan, 1975). It is generally agreed that the high salinity during the dry season (November - March) is due to the entrance of sea water through the harbour mouth into the lagoon and the low salinity in the rainy season to influx of freshwater from rivers into the lagoon. Hill and Webb (1958) and Akpata, Ekundayo, Fashina and Chukwura (1979) ascribed the increased turbidity of the lagoon in the rainy season partly to the influx of turbid river waters to the Lagos Lagoon. Furthermore the nutrients derived in the mangrove ecosystem are washed during the tidal cycles into the lagoon and during the vertical mixing which follows the nutrients are retained within the lagoon environment.

The temperature of the lagoon was generally above 27^{o}C except in August when there was a drop to 25^{o}C and 26^{o}C. Hill and Webb (1958) observed that the temperature at the Lagos harbour ranged from 25^{o}C in August to 29^{o}C in March and explained that cooler river water during the rainy season gradually effects a drop in the harbour temperature.

The generally high bacterial counts obtained during the studies show the level of pollution in Lagos Lagoon. The high organic matter content at the sawmill station provided suitable substrate for the heterotrophic bacteria, hence higher bacterial counts were generally obtained at the sawmill station.

Gram positive and Gram negative bacteria constituted the bacterial flora of Lagos Lagoon. Akpata and Ekundayo (1978) isolated Gram negative bacteria of intestinal origin from the lagoon. Similarly, Collins (1960) isolated Gram negative bacteria and Fondén (1969) heterotrophic bacteria in lakes. Carpenter (1977) stated that water containing organic matter supports more luxuriant microbial population than pure water. He reported bacteria found in water to include organisms of the Pseudomonas fluorescens type and other chromogenic (violet, red, yellow) rods, coliforms e.g. Escherichia coli, Proteus species, spore-formers, and cocci (white, yellow, pink).

Sawdust is discharged in large quantities into the Lagos Lagoon (Plate 1) but it is by no means the only organic matter deposited in the lagoon. The inhabitants of Okobaba and the Lagos City Council dispose of domestic wastes into the lagoon thus increasing the organic matter content.

Akpata and Ekundayo (1978) reported the presence of faecal pollution in most parts of Lagos Lagoon and that the level of faecal organisms was influenced by the salinity of water at the sampling stations.

Counts of fungal population were generally high. Higher populations were obtained in sawmill water containing sawdust than in sawdust-free water at the mid-lagoon (Tables 6a and 7).

Seasonal fluctuations of fungal population were noticeable. Higher counts were obtained in the rainy season (April - October) when the nutrients washed into the lagoon increased its organic matter content providing increased nutrients for micro-organisms. There was a simultaneous decrease during this period in salinity making the lagoon favourable for the rapid multiplication of brackish water micro-organisms. Hill and Webb (1958) reported the influx to the Lagos Lagoon of turbid river waters rich in mineral salts during the early rains. Willoughby (1965) observed that fungal spores of terrestrial origin were washed into the lakes of the English Lake District following heavy rainfall, and spores were present in greater concentrations at the lake margin than at the lake centre. Dawson (1966) stated that the general prevalence of fungi in aquatic environments was markedly influenced by salinity, the fungal population being

inversely proportional to the salinity of the water. Siepmann(1959) noted that certain filamentous fungi showed seasonal fluctuation in populations in Weser River sediments.

As a result of discharge from pulp mills or saw mills containing particulate wood wastes, sludge beds of sawdust may form (Waldichuk, 1977). These decompose, utilizing oxygen from the sediments and overlying water, and often release gases, mainly methane, but sometimes also highly toxic hydrogen sulphide (Waldichuk, op.cit.). In the study reported here, hydrogen sulphide (H_2S) was consistently detectable at the sawmill station indicating the activities of anaerobic micro-organisms. Also, dissolved oxygen content ($\leq$ 0.4mg/l) was lower at the sawmill station than in the mid-lagoon (0.4-0.8 mg/l). Concentrations of H_2S of a few tenths of a milligram per litre cause an objectionable rotten egg odour (Standard Methods, 1971). Gorham (1957) stated that anaerobic decomposition frequently produced H_2S in organic sediments, the gas could diffuse into the overlying water and become oxidized to sulphuric acid (H_2SO_4) when oxygen was present.

Perkins (1974) explained that regeneration of oxygen in estuaries was brought about by mixing with oxygenated waters from rivers or the sea, direct re-aeration from the air, and by the photosynthetic activity of plants. When the water is turbid, the last process is of minor importance,

compared with re-aeration from the air. Perkins (1974) related Secchi disk readings to the amount of sediment in suspension, and reported that the suspended material present in most estuarine waters ensured that nearly all the light which penetrated was absorbed in the surface 1-2m. The low turbidity recorded at the mid-lagoon station in this study may have created more favourable conditions for photosynthetic activities which would in turn increase the dissolved oxygen content of the water. Also, the inflow of oxygenated water from River Ogun into the mid-lagoon station would increase the oxygen content of the water at that station.

Raabe (1968) noted that lignin discharged to a stream had a high colouring effect and it introduced a large organic load. Sawdust discharged into the lagoon will probably have similar effects on the lagoon.

The most predominant genus of fungi at the sawmill station was Aspergillus. TeStrake (1959) reported that molds occur in brackish waters up to 3.5‰ salinity. Several workers (Alasoadura, 1968; Anastasiou, 1961; Elliot, 1930; Hasija and Batra 1978; Jones, 1962; Johnson and Sparrow, 1961; Kolmeyer, 1960; Meyers 1953 and 1957; Park, 1972; Vishniac 1956) have reported isolation of fungi from fresh, brackish and marine waters.

Roth, Orpurt and Ahearn (1964) isolated species of Aspergillus and Penicillium from a subtropical marine

environment. Phycomycetes were isolated infrequently and were of low population density. They also obtained a large spectrum of filamentous Fungi Imperfecti and stated that the distribution of the fungi implied either a transitory or permanent marine life cycle. Wood (1967) stated that there was ample evidence of the presence of terrestrial fungi such as Aspergillus and Penicillium in estuarine waters and sediments.

Apart from the Aspergillus species - A. flavus, A. fumigatus, A. giganteus, A. niger and A. tamarii - other fungi isolated from the sawdust station were Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum, Mucor species, Paecilomyces variotii, Penicillium species, and Trichoderma aureoviride. The yeasts isolated included Candida famata and Debaryomyces hansenii. An unidentified basidiomycete forming clamp connections was part of the mycoflora.

Borut and Johnson (1962) working on estuarine sediments isolated members of the genera Aspergillus, Fusarium, Mucor, Penicillium and Trichoderma. Also, Cladosporium herbarum (Pers.) Link, Curvularia geniculata, Geotrichum candidum, Paecilomyces flavescens (Brown and Smith) and P. puntonii (Vuill) Nann.

Christensen and Whittingham (1965) worked on the microfungi of swamps in Wisconsin and they isolated

Geotrichum candidum, Cladosporium herbarum, Paecilomyces carneus, Mucor sp., Trichoderma sp. and yeasts.

Working under conditions of very low oxygen tension obtained by passing air through a set up that would rid the air of its oxygen, Aspergillus niger, Candida famata, Debaryomyces hansenii and Mucor species were isolated from water samples of the sawmill station of Lagos Lagoon. Considering the fact that similar results were obtained by Christensen and Whittingham (1965) it would appear that fungi can become adapted to low oxygen situations.

The presence of the unidentified basidiomycete in the sawmill sample and not in the sawdust-free lagoon water may be explained by the cellulose and lignin decomposing ability associated with Basidiomycetes. Rosenberg (1978) reported lignocellulose decomposition by some members of Basidiomycetes, e.g. Chrysosporium pruinosus and Sporotrichum pulverulentum. Lignin decomposition has often been associated with white-rot fungi (Chang, 1967; Eriksson, 1978; Fergus, 1969; Gadd, 1957; White, 1953).

That more diversified genera of fungi were isolated from the sawmill station than in mid-lagoon may be due to the higher organic matter content at the sawmill station, thus providing more nutrients for the fungi.

Baiting technique has been used for isolation of aquatic fungi (Borut and Johnson, 1962; Fazzani et al.,

1975; Johnson and Sparrow, 1961; Meyers, 1953; Roth, et al.1964). Wood blocks or cellulose sponges have also been used by these workers for isolating cellulose-decomposing fungi. However, since decomposition of sawdust in the Lagos Lagoon is the subject for study in the work reported here, sawdust wrapped in nylon mesh was used as bait for the lagoon fungi.

The initial increase in fungal population observed on sawdust bait may be due to the presence of soluble sugars and other water-soluble extractives of the wood components. This fact may explain the presence of Mucor species as first colonizers even though these species are known to be incapable of cellulose or lignocellulose degradation (Rosenberg, 1978). On the ecological succession of fungi on plant remains in the soil, Garrett (1951) observed that the "sugar" forms appeared first and the "lignin-decomposing" types last. The persistence of Aspergillus species on the sawdust bait may be due to their ability to utilize sugars and cellulose components of wood (Millner, Marsh, Snowden and Parr, 1977; Olutiola and Cole, 1977; Rosenberg, 1978). A. niger, Cladosporium oxysporum and yeasts formed the mycoflora of sawdust at advanced stages of decomposition. Several species of Cladosporium have been collected from estuarine sediments (Borut and Johnson, 1962;

Roth et al., 1964) and there are reports of yeasts in brackish waters (Buck, 1978).

The major components of sawdust available for microbial decomposition were analysed as cellulose (56.65%), lignin (22.45%), reducing sugars (2.0mg/l) and extractives constituted about 10%. Browning (1963) stated that cellulose was the major wood component, being approximately one-half of the wood weight. Stephens and Heichel (1975) reported that the proportion of cellulose in plant tissue varied both with type of tissue and plant as well as with the method of extraction. They further reported that most angiosperms have an alpha-cellulose (i.e. lignin-free cellulosic material consisting of long chains of (β-1,4 - glucose molecules) content above 40%.

Browning (1963) explained that some of the sulphonated lignin (Klason lignin) dissolved in sulphuric acid used in lignin determination and as such only part of the lignin content is obtained. The lignin content of softwood (gymnosperms) varies from 17.6% in Populus tremuloides (Aspen) to 37.7% in African pencil-cedar (Browning, op.cit.) Lignin contents of various Nigerian woods varied from 19.4 to 33.3% (Odeyemi, 1968). Thus the lignin content (23.45%) obtained for the sawdust sample agreed with results of previous analyses by Odeyemi (op.cit.).

Extractives constituted about 10% of sawdust sample. Odeyemi (1968) recovered values of 4.4 - 11.9% for Nigerian woods. Extractives influence the physical properties of wood and where phenolic compounds are present, they lend resistance to fungal and insect attach (Browning, 1963).

The detection of 2.0mg/l reducing sugar in sawdust extract indicates that a readily utilizable carbon source is available for micro-organisms for growth. This will probably explain the high microbial population at the sawmill station on Lagos Lagoon. Olofinboba (1969) showed that the wood of Antiaris africana contained various sugars including fructose, glucose, maltose and sucrose.

The microbial decomposition of wood has been reported by several workers (Grant and Savoury, 1969; Gilbert and Lovelock, 1975; Olofinboba, 1969; Santra and Nandi, 1975a,b; Savoury, 1954; Umezurike, 1969).

The fungi employed in this study showed good growth on sawdust extract and lagoon water agar indicating their ability to grow in the lagoon and to utilize water soluble extracts of the sawdust in the lagoon for growth. Arinze (1978) reported that Botryodiplodia theobromae degraded the extracts (phenol, phenol derivatives and

furanoterpenoids) of Ipomoea batatas L et Lam.

Aspergillus flavus, A. giganteus, A. niger, Cladosporium oxysporum, Geotrichum candidum, Paecilomyces variotii and the unidentified basidiomycete utilized colloidal cellulose. All the fungi also utilized carboxymethylcellulose in mineral salts medium thus confirming the production of C_x cellulase by the fungi. The fact that reducing sugars produced from the breakdown of CMcellulose were not always detected in the culture medium can be explained if it is assumed that the fungi made use of the sugars in their metabolic activities.

Several reports (Olutiola, 1976; Reese and Levinson, 1952; Rosenberg, 1978; Umezurike, 1969) have been published of cellulase production by Aspergillus species. Olutiola and Cole (1976 and 1977) reported that the cellulase complex of A. tamarii and A. Sydowi had C_1, C_x cellulase and cellobiase activities. Ability to hydrolyse CMcellulose indicates C_x cellulase activity (Olutiola and Cole, 1977) while ability to utilize insoluble cellulosic substrates indicates that the organism produces C_1 cellulase (Selby and Maitland, 1967; Whitney Chapman and Heale, 1969). Aspergillus giganteus and Cladosporium oxysporum utilized filter paper (insoluble cellulose) as sole carbon source. This shows

that the fungi produced C_1 cellulase. Similar results have been obtained for Cladosporium cucumerinum Ell et Arth (Husain and Rich, 1958).

The fungi were unable to utilize lignin, showing their inability to produce lignolytic enzymes. The white-rot fungi, members of the class Basidiomycetes, are the only group reported to have lignolytic activities (Gadd, 1957; Rosenberg, 1978). Moreover, the plate assay method was employed in the present study, but it is known nowadays that detection of phenol oxidase activities (Lindeberg and Holm, 1952) and use of carbon-14-labelled techniques (Crawford and Crawford, 1976) provide more sensitive assessment of lignolytic activities in fungi.

Many of the lagoon isolates - Aspergillus giganteus, Cladosporium oxysporum, Curvularia geniculata, Fusarium solani, Geotrichum candidum and Trichoderma aureoviride - tolerated a wide range of salinities up to 3.4% NaCl for growth. Thus they belong to the group described as euryhaline by Johnson and Sparrow (1961) and Ritchie (1960). The salinity of the lagoon ranged from 0.2‰ in the rainy season to 29.0‰ in the dry season. This makes the lagoon freshwater during the rainy season and becoming brackish in the dry season (Moore, 1961). It would seem that euryhaline fungi are capable of growth

throughout the year in the lagoon environment (of 0.2 - 29.0‰ salinity). In presence of ample nutrients, fungi readily adapt to a salinity level far in excess of that which they can tolerate in lower nutrient environment (Borut and Johnson, 1962). Furthermore, the higher microbial populations recorded at the sawmill station could be rightly attributed to the higher organic matter content at this station. Dzawachiszwili et al. (1964) reported that the tolerance of fungi to various concentrations of sea water or NaCl can be influenced by temperature. At increased temperature some fungi attain optimum growth at higher salinities (Ritchie, 1957, 1959). But Johnson and Sparrow (1961) observed a reverse situation in other fungi. In the tropical conditions of the Lagos Lagoon, temperature was generally high, up to 33°C in the dry season with a mean of 28.4 - 28.8°C. Thus for the fungi isolated from Lagos Lagoon, if Ritchie's assertion applies, the high temperatures of the water probably increase the salinity tolerance of the fungi.

Aspergillus flavus showed the greatest tolerance to salinity by growing at 6.8% NaCl. Thus A. flavus falls in the halophilic group described by Rheinheimer (1974).

Aspergillus tamarii and Paecilomyces variotii demonstrated poor growth and sporulation at low salinity

of 1.7% NaCl. Organisms with such characteristics were described as halophobic by Rheinheimer (1974). This observation explains the seasonal appearance of these fungi in Lagos Lagoon. A. tamarii and P. variotii constituted only 4.0 and 3.1% respectively of the aerobic population of micro-organisms isolated from the sawmill station. So, it may be that these two fungi appear in the lagoon during the rainy season when low salinities 0-17‰ are observed in the Lagos Lagoon. These halophobic fungi are probably freshwater forms brought by the rivers or washed from the soil by the rains into the lagoon.

The importance of spores in the life cycle of the fungus and the part they play in the spread of economically important fungi need no emphasis (Ekundayo, 1965). The spore, however, must germinate in order to be effective. This important process of germination was therefore investigated in the present studies.

The conidia of Aspergillus flavus, A. giganteus and Cladosporium oxysporum did not germinate in sterile distilled water. There are numerous reports of fungus spores which do not germinate in distilled water in absence of exogenous nutrients (Broadfoot, 1926; Cochrane, 1958; Gottlieb, 1950; Gould and Shaw, 1969). Similarly in lagoon water only A. flavus germinated while spores of the other

fungi did not. Byrne and Jones (1975) reported that spore germination in Gliocladium fimbriatum, Paecilomyces puntonii and Trichoderma lignorum was inhibited in sea water, and Dzawachiszwili et al. (1964) reported that filter-sterilized sea water maintained its inhibitory effect while autoclaved sea water lost this property. In the study reported here filter-sterilized lagoon water was used.

It is interesting to note that Aspergillus flavus spores germinated in sawdust extracts of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon but germination was inhibited by $CaCl_2$, KH_2PO_4, $MgSO_4.7H_2O$ or NaCl. It could be inferred that poor germination of A. flavus in lagoon water may be due to the inhibitory effects of these inorganic constituents which are present in lagoon water (Akpata et al., 1979). Harrison and Jones (1975) reported the supression of germination of some fungal spores by Ca^{++} ions.

Germination of Cladosporium oxysporum conidia was inhibited in lagoon water and in all the mineral salt solutions tested, and low concentrations of NaCl stimulated conidia germination in Trichoderma aureoviride. It thus appears that specific inorganic salts had specific effects on individual fungi.

The ability of fungal isolates to decompose sawdust was investigated. There was weight loss of sawdust inoculated with either Aspergillus flavus, A. giganteus, Cladosporium oxysporum or Trichoderma aureoviride. This observation indicates the production of enzymes capable of decomposing wood components by the fungi. Husain and Rich (1958) reported that Cladosporium cucumerinum produced extracellular cellulase on filter paper, wood cellulose or CM cellulose. It thus seems that cellulase production is common within the genus Cladosporium. There are also reports of cellulase production by Trichoderma (Li et al. 1965; Mandels and Reese, 1957; Selby and Maitland, 1967; Wood, 1968).

There was a correlation between temperature and weight loss of sawdust inoculated with different fungi. It thus seems that moderately high temperatures, e.g. 33^{o}C favour sawdust utilization by the fungi. The correlation between temperature and loss of weight may be explained by increased metabolic activities at such temperatures (Rheinheimer, 1974). Furthermore, Rosenberg (1978) reported that for some micro-organisms, specific growth rates increased with increasing temperature optima.

The addition of glucose (carbon source) or peptone (nitrogen and carbon source) enhanced sawdust

decomposition. Romanelli et al.(1975) reported that addition of organic carbon and nitrogen sources to a medium containing cellulose and mineral salts markedly increased the rate of cellulose degradation by fungi. Co-metabolism, a process whereby decomposition is achieved through the synergistic ability of microbial enzyme systems attacking the growth-promoting metabolite and the non-utilized co-metabolite in the habitat, has been proposed by Slater and Somerville (1979) as a means of decomposing organic matter by microbial communities under natural conditions. Many micro-organisms growing at the expense of one substrate may also have the capacity to transform one or more compounds in the habitat. Co-metabolism may also occur through the activity of enzymes not directly associated with the catabolism of the growth substrate. Thus, although lignolytic activity has not been detected in any of the fungal isolates, it is possible that lignin may be decomposed by co-metabolism. Since cellulose and lignin do not occur in the pure state in the lagoon, and isolated lignin has not been shown to be degraded significantly by any organism (Rosenberg, 1978), decomposition of sawdust, particularly the lignin component, in the lagoon will best be accomplished by the microbial communities through co-metabolism.

The basidiomycete isolated in this study did not fructify on the laboratory media and cultural conditions employed in the isolation studies. However, lignin decomposition is always associated with Basidiomycetes. Eriksson (1978) proposed that lignin decomposing enzymes act through the cellulose pathway.

The three wood species employed for studies on sawdust decomposition - Khaya ivorensis, Mitragyna ciliata and Tripochiton scleroxylon - were the most commonly demanded wood species at the sawmill stations and as such of economic importance. Moreover, Irvine (1961) reported that Khaya (African mahogany), Mitragyna (Poplar) and Triplochiton (Obeche) are of great commercial values for furniture and boat building. These wood species also contribute largely to the sawdust heaps in the lagoon. The fact that each fungal isolate was able to decompose the sawdust of individual wood species in the present studies is an indication that decomposition of the sawdust in Lagos Lagoon is partly or wholly by micro-organisms.

In conclusion it must be emphasized that biodeterioration of organic matter is an applied ecological problem, involving a wide spectrum of micro-organisms in the habitat. The interactions between these microbial species, and their patterns of distribution may be important factors in determining rates and patterns of decomposition (Anderson and Mac Fadyen, 1976).

VI. SUMMARY

The fungus flora of the sawdust disposal area of the Lagos Lagoon was investigated. There was seasonal variation in the population of fungi in the lagoon, more fungi being isolated in the rainy season.

Some physico-chemical parameters (e.g. salinity, turbidity and suspended matter) of the Lagos Lagoon showed seasonal fluctuations, but pH, temperature and magnesium content were relatively stable.

There was a significant positive correlation between suspended matter content and fungal count of the lagoon.

There was a negative correlation between both salinity and turbidity of the lagoon and the fungal count at the sampling stations.

The fungi isolated from the lagoon included Aspergillus flavus, A. fumigatus, A. giganteus, A. niger, A. tamarii, Candida famata, Cladosporium oxysporum, Curvularia geniculata and Debaryomyces hansenii. Others were Fusarium solani, Geotrichum candidum, Mucor haemalis, Paecilomyces variotii, Penicillium species, Trichoderma aureoviride and an unidentified basidiomycete. These fungal genera were also isolated from decomposing sawdust in the lagoon.

Most of the fungal isolates grew at a wide range of salinities (0-34‰). Aspergillus flavus tolerated up to 68‰ salinity.

Sporulation of the fungi decreased with increase in the salinity of the culture medium.

Conidia germination was variable in aqueous extracts of sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon. Conidia germination was enhanced in extracts amended with nutrients (carbon and nitrogen).

Addition of $CaCl_2$ or $MgSO_4.7H_2O$ to sawdust extract reduced conidia germination in Aspergillus flavus, A. giganteus, Cladosporium oxysporum and Trichoderma aureoviride. Good germination of A. giganteus occurred in extracts amended with KH_2PO_4 or NaCl.

Conidia germination was poor in distilled water and lagoon water of 21.0‰ salinity.

Studies on ecological succession of fungi on sawdust showed that the Mucorales were the first invaders and they disappeared after 5 days. Aspergillus niger, Cladosporium oxysporum and yeasts constituted the mycoflora of sawdust at advanced stages of decomposition.

Chemical analysis of sawdust showed that it contained cellulose (56.65%), lignin (23.45%), extractives (10%) and reducing sugar (2.0mg/l).

Some of the fungal isolates - Aspergillus flavus, A. giganteus, A. niger, Cladosporium oxysporum, Geotrichum candidum, Paecilomyces variotii and an unidentified basidiomycete - grew on colloidal cellulose medium, whilst A. flavus, A. giganteus, A. niger, Cladosporium oxysporum and the basidiomycete grew on carboxymethylcellulose agar.

Aspergillus flavus, A. giganteus, Cladosporium oxysporum and Curvularia geniculata caused significant weight loss of filter paper in mineral salts medium.

Cladosporium oxysporum utilized the filter paper more efficiently in still cultures than in agitated cultures.

None of the fungal isolates grew on media containing lignin as sole carbon source.

There was a positive correlation between temperature of incubation (24, 27, 30 and 33^{o}C) and weight loss of sawdust of Khaya ivorensis and Triplochiton scleroxylon inoculated with Aspergillus giganteus or Cladosporium oxysporum. Similar results were obtained for the weight loss of sawdust of Khaya ivorensis, Mitragyna ciliata and Triplochiton scleroxylon amended with glucose or peptone and inoculated with Aspergillus flavus, A. giganteus, Cladosporium oxysporum or Trichoderma aureoviride.

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IX APPENDIX

Height of the tide (m) at the Lagos harbour at different sampling times.

Date	Sampling time (a.m.)	*Height of tide (m)
1977		
January		
13	9.50	0.1
31	10.30	0.0
February		
16	9.50	0.1
28	9.45	0.2
March		
16	8.30	0.1
31	8.50	0.1
April		
15	8.30	0.1
30	8.40	0.1
May		
16	11.30	0.1
31	11.30	0.0
June		
15	9.25	0.1
29	10.30	0.0

* Obtained from "Tidal Tables" 1977 and 1978. Nigerian Naval Headquarters, Lagos.

APPENDIX (Cont'd)

Height of the tide (m) at the Lagos harbour at different sampling times.

Date	Sampling time (a.m.)	*Height of tides (m)
1977		
July		
13	10.30	0.2
28	10.00	0.0
August		
17	10.10	0.1
31	10.00	0.1
September		
16	10.15	0.1
29	9.30	0.0
October		
15	9.25	0.1
31	9.40	0.0
November		
15	9.00	1.0
30	9.00	0.9
December		
15	8.55	0.9
30	9.15	0.8

APPENDIX (Cont'd)

Height of the tide (m) at the Lagos harbour at different sampling times.

Date	Sampling time (a.m.)	*Height of tide (m)
1978		
January		
16	10.45	0.6
31	9.40	0.7
February		
14	9.30	0.6
28	9.00	0.8
March		
15	9.10	0.7
29	8.50	0.8