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SOME EFFECTS OF BIOLOGICAL AND PHYSICAL PROCESSES ON SOIL AGGREGATE STABILITY

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ABSTRACT

The effects of biological and physical processes on the aggregate stability of some weakly structured arable and pasture soils were investigated. Preliminary investigations showed significant correlations between soil organic matter and both wet sieving and turbidimetric methods of aggregate stability; the latter method was chosen on grounds of convenience.

Scanning electron microscope pictures showed the presence of both fungi and bacteria when soils were incubated. Growth of fungi, estimated by ergosterol measurement, correspond to temporary stability increases, which could be explained by retention of soil particles within the reticulum of fungal hyphae. The effect disappeared as the fungi were destroyed and replaced by bacteria and actinomycetes. Effects caused by fungi were examined separately, using vancomycin to inhibit bacterial growth, and bacterial effects by using cycloheximide to eliminate fungi. Bacterial growth had little direct effect in stabilising soil aggregates; periodate oxidation showed that polysaccharides produced by bacteria are mainly responsible.

To examine the contribution of physical processes to increased stability in remoulded soils biological processes were eliminated by sterilisation. Thixotropic changes made a contribution to age hardening in remoulded aggregates similar in magnitude to biological processes. Thixotropic changes were reversible and accompanied by soil strength and matric water potential changes. Polysaccharides did not contribute to thixotropic aging processes.

Remoulded soils were subjected to wetting/drying and freezing/thawing cycles. After 3 to 6 cycles the stability of both sterilised and unsteriliserd soils recovered to that of natural aggregates, suggesting a contribution by thixotropy. Repeated weathering cycles decreased the stability of unsterilised, and more so sterilised, field aggregates suggesting that in the former, bond reformation due to biological activity counteracted the destruction caused by wetting/drying and freezing/thawing.

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1. INTRODUCTION

Intensified arable cultivation has been accompanied by two related major problems. These are the loss of organic matter and deterioration of the physical properties of soils. However, it was not until the introduction of heavy machinery in farming systems that concern was raised for the adverse effects that this continuous arable cultivation might be having on soil structure due in part to decline in organic matter levels. Problems associated with intensive arable cultivation were highlighted in a report by Agricultural Advisory Council (1970).

According to this report "Organic matter declines with a reduction of grass rotations and will tend to stabilise at a new lower level according to the cropping system; whether or not this level is satisfactory to retain good soil structure will depend on soil type, cropping practice and cultivation requirements. On inherently unstable soil the ploughing of grassland and adoption of an all arable rotation with its lower organic matter can create serious structural problems".pp 42.

A large number of surveys carried out to investigate the effects of cultivation on soil organic matter and structure have produced evidence which supports these conclusions of the Agricultural Advisory Council (Emmond, 1971; Low, 1972; Tisdall and Oades, 1979, 1980a, 1980b). In Britain several studies have shown that pasture phases of rotation restore organic matter levels of soils with resultant improvement of structure (Clement, 1975; Eagle, 1975; Chaney and Swift,

1984). However, these traditional improvements of structural properties by introduction of pasture are becoming increasingly difficult. For example, in the highly productive areas of Britain, management of the soil is closely related to the economics within which the land is farmed. In East Anglia high land prices have prompted production of cash crops, leading to intensive cultivation and exclusion of grass leys from farming practice (Davies, 1975). In such areas a knowledge of whether soil suffering from physical damage could be expected to recover without a lengthy period under grass could be useful.

To define those soils which are at risk because of loss of organic matter and structural deterioration, aggregate stability measurements have been employed (Marshall and Quirk,1950; Bryan 1968, 1974; Low,1972; Greenland et al., 1975; Grieve, 1979a; Morgan,1979; Hadas and Wolf,1983; Kemper et al., 1985). However, limited success in developing a universal relationship between aggregate stability and soil physical behaviour has been mainly due to lack of joint consideration of both biological and physical processes involved in formation and destruction of soil aggregates.

A major proportion of primary soil particles associate in various arrangements to form aggregates. These particles are cemented by organic and inorganic bonding agents. Organic bonding agents comprise soil organic matter and its constituents, ranging from fresh plant, animal and microbial residues, through ephemeral products of microbial decomposition to fairly stable amorphous brown to black decomposed material (humus). This composition changes from time to time depending on soil biological

activities; which in turn are influenced by weather and soil physical manipulation.

The biological processes, which are dependent on substrate availability and most active in pasture soils, reach a state of dynamic equilibrium and play a dominant role in achieving aggregate stability (Swaby, 1949; Harris et al., 1966). Roots and fungal hyphae contribute to stability by enmeshing soil particles. Adhesive substances produced through biological activity include both transient and more persistent materials often associated with polyvalent metal cations (Tisdall and Oades, 1982). Of these substances, humic materials have been closely studied in relation to soil aggregate stabilisation (Greenland, 1965; Mortland, 1970; Theng, 1979; Greenland and Hayes, 1981).

Polysaccharides are one of the major active groups of compounds within soil organic matter whose contribution to aggregate stability has not yet been fully evaluated. This is mainly due to problems involved in their characterisation (Swincer et al., 1969; Greenland and Oades, 1975; Cheshire, 1979) and in procedures of evaluation of their role in aggregate stabilisation (Mehta et al., 1960; Stefanson, 1971; Cheshire et al., 1983, 1984). The binding properties of polysaccharides enable them to form soil aggregates, but because they are degraded by microorganisms, they cannot be solely reponsible for long term stability observed in pastures. The stability of soil aggregates measured at any given time appears to be a result of the combined mechanical action of the microorganisms and their products of decomposition. This interaction needs to be assessed.

The effects of cultivation on soil structure are a manifestation of two processes. Firstly, the mechanical pulverisation of the soil crumbs exposes organic matter previously inaccessible to microbial oxidation. This gives rise to the depletion of the gluing materials holding the particles together and subsequent decline in aggregate stability. Secondly, cultivation ruptures the cementing bonds within the aggregates which results in immediate loss of cohesion or rigidity between particles within the aggregates. The extensive investigation of the biological and chemical processes in soil aggregation, as influenced by cultivation has not been accompanied by comparable studies of the physical processes.

Physical manipulation of the soil result in changes in the organisation of the particles. This reorganisation of soil particles has been studied in detail by civil engineers. Increase in strength of clays over months or years following remoulding have been observed by civil engineers and recognised as thixotropic changes by Seed and Chan (1957, 1959) and Mitchell (1960). The latter author hypothesised re-orientation of the clay particles by externally applied shearing forces into a more uniform parallel arrangement. In this more ordered structure, clay platelets can slip past one another and the matric water potential is maximum. When the shearing force is removed and the system comes to rest, thermal oscillations tend to randomise the orientation of the clay particles. The resulting disorder imparts rigidity to the system, and at the same time the matric water potential decreases as the water structure becomes modified by hydration processes and by redistribution of ions in the double layer surrounding the clay surfaces.

Arya and Blake (1972) suggested that thixotropy is important in cultivated soils, and its effects could explain observations of poorer tilth when rototilling is followed immediately by heavy rain compared with delayed rain (Page et al., 1946) and of increases in stability during aging of freshly tilled soil (Kemper and Rosenau, 1984) and artificial aggregates in the absence of biological activity (Blake and Gilman, 1970; Utomo and Dexter, 1981b). Apart from the work of Blake and associates (Blake and Gilman, 1970; Arya and Blake, 1972; Schweikle, Blake and Arya, 1974) and Utomo and Dexter (1981b) thixotropy has not been applied to the study of aggregates from cultivated soils. A major proportion of this thesis will examine the contribution of thixotropy to stabilisation of soils known to lose structure after continued cultivation.

Wetting and drying and freezing and thawing, like forces associated with tillage or raindrop impact, cause changes in the organisation of soil constituents. These factors may decrease or increase the degree of organisation of soil constituents or disperse the clay particles. Since the early work of McHenry and Russell (1943) intermittent work has been undertaken to investigate the effects of weathering cycles on soil aggregate stability. The accumulating reports have shown that wetting and drying and freezing and thawing result in structural breakdown on one hand, and structural regeneration on the other (Willis, 1955; Richardson, 1976; Utomo and Dexter, 1982). However, most studies have failed to recognise that soil responses observed with wetting and drying and freezing and thawing may not be constant with time, as these are sensitive to cropping history of the

soil; and contradictory conclusions have been drawn concerning the effects of these weathering cycles on aggregate stability. Much information could have been gained from most studies if the workers had recognised that some changes induced by weather fluctuations were partly due to biological activity and others due to physical processes.

The relative contribution of biological and physical processes to aggregate stability, as well as their interaction are assessed, and the size and importance of the effects are evaluated in cultivated and non-cultivated soils. These processes are examined under four headings:-

1. The effects of cropping practices,
2. The effects of biological activity,
3. The effects of thixotropic hardening and
4. The effects of wetting and drying and freezing and thawing cycles.

From the agricultural land management viewpoint information on these factors is important in the formulation of cultivation practices which would result in good agricultural returns with minimal soil structural deterioration. Such practices are especially necessary where weakly structured soils, similar to the Bromyard, Stirling and Dreghorn soils chosen for the present study, are encountered.

2. LITERATURE REVIEW

Previous work in the field of soil aggregation relationship with biological and physical processes relevant to the present study falls into five categories. These concern soil aggregation and aggregate stability; effects of various cropping practices, organic matter and microbial activity, age hardening (thixotropic) processes and weathering cycles. Since this review covers such a wide field of work only subjects which are of direct relevance to this study are examined in depth, whereas other aspects, no less important themselves but of peripheral interest are dealt with less fully.

2.1 Soil Aggregation and Aggregate Stability

Soil structure is strictly a field term used to describe the overall arrangement or aggregation of the soil constituents. Structure has been defined in a number of ways. Bradfield (1950), Low (1962), and Marshall (1962) defined structure as 'referring to the arrangement of the soil particles in soil profile'. By this definition there is no such thing as structureless soil, any process which alters the arrangement of the soil constituents is altering the soil structure. Brewer (1964) defined soil structure as 'the physical constitution of a soil material as expressed by the size, shape and arrangement of the solid particles and voids, including both the primary particles to form compound particles, and the compound particles themselves'.

Russell (1971) defined soil structure from the viewpoint of crop production. According to his definition, the term structure may be used

to cover a group of properties largely concerned with pore space distribution in the soil. The coarse pores control the spaces into which roots grow, the permeability of rain or irrigation and aeration of the soil when wet but well drained. The medium size pores affect the supply of water to the roots, and the finest pores the ease of cultivation. In his definition, structural pores are referred to as 'all pores that are wider than those pores in the soil when well puddled and at maximum bulk density; that is when all particles are in closest packing'.

Russell (1971) cited four ways in which structural pores are created in the field. These are:

(i) Shrinkage of soil on drying, causing cracks to open up the body of the soil.

(ii) Pressure of the plant roots, either by growing into unchannelled soil, or by swelling upon water uptake after growing into existing channels. Open channels are also left behind when roots die and decompose.

(iii) The burrowing action of soil fauna, such as earth worms.

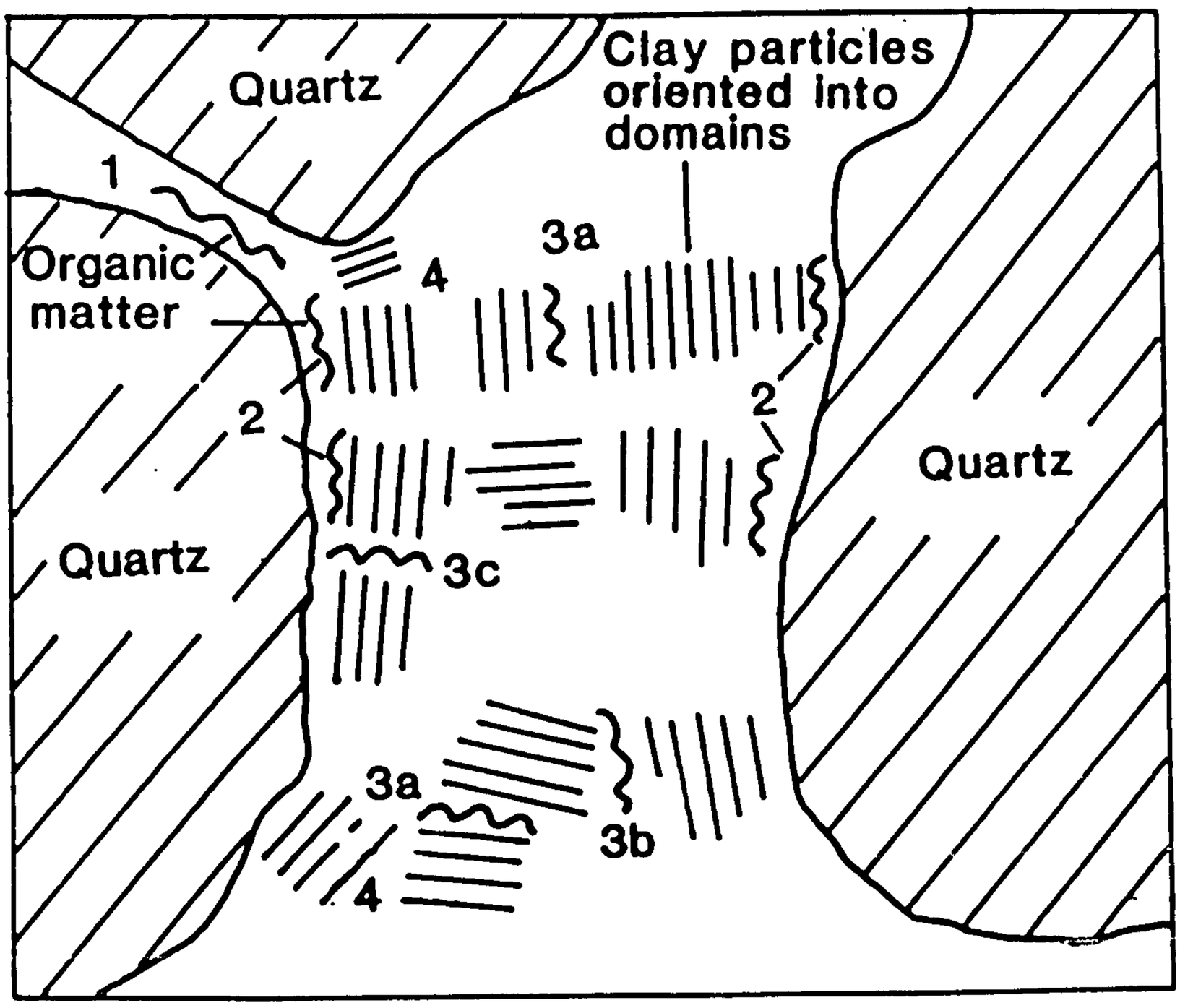
(iv) Cultivation of the soil, during seedbed preparation.

These four mechanisms together with others such as freezing and thawing, result in the formation of soil aggregates. In considering soil structure therefore, soil particles refer not only to the individual mechanical separates such as sand, silt and clay, but also to the aggregates or structural elements which have been formed by aggregation of smaller mechanical fractions.

A model proposed by Emerson (1959) illustrated in Figure 2.1 provides a basis for considering how the particles are held together so that they behave as a unit. According to this model, clay is envisaged to be arranged in domains or packets of oriented crystals, and these domains are attached to one another and to the larger particles of silt and sand by bonds as indicated in the diagram. The link may be electrostatic as between positive edge charges on one domain and the negatively charged face of another at 4. Organic matter also provides the link amongst particles as at 1, 2, and 3. The overall structure of the soil may therefore be visualised as the result of the arrangement and bonding of the individual particles in a structural unit. The individual particles in a structural unit are attached more strongly to each other than to adjacent particles, thus allowing development of different structural pores (Brewer, 1964; Greenland, 1977).

The above discussion points to the two major aspects of soil aggregation; i.e. coagulation and pressure. The pressure produces more intimate contact between particles so that the cementing influences of the binding agents are rendered more effective. The genesis and evolution of structure through time therefore involves among others, the cation effects, clay particle interactions in relation to moisture and clay-organic matter interaction. The role of these constituents will be included in the discussion of processes and mechanisms of soil aggregate formation and degradation.

One very important aspect of soil structure which influences the response and behaviour of soil in relation to stresses induced by



Bond-types

1. Quartz-organic-quartz

2. Quartz-organic-domain

3. Domain-organic -domain : a. face-face

b. edge-face

c. edge-edge

4. Domain-domain, edge-face

Figure 2.1 Emerson's (1959) model of a soil aggregate

cultivation or raindrop impact is the stability of the aggregates against dispersion under conditions of stress. Soil aggregate stability is usually measured in the field and laboratory as the resistance of a sample of aggregates towards a standardised breakdown force. Since soils are most liable to degradation in the wet state (Agricultural Advisory Council, 1970) most tests of aggregate stability determination involve wetting the sample of aggregates. Aggregate stability is thus a measure of resistance to both wetting and mechanical dispersion.

2.2. Evaluation of Soil Aggregate Stability

There are many methods reported in the literature for determination of aggregate stability. Each method of measurement is usually designed to meet the requirements of the study in hand, and consequently the results cannot be universally comparable (Bryan, 1971; Grieve, 1975). Methods of aggregate stability determination fall under two broad classes. The first group of tests subject aggregates to forces designed to simulate those occurring in the field. In this group the nature of the forces applied during testing depends on the investigator's perception of the natural phenomenon which he wishes to simulate, as well as the equipment available and the mode of its employment. The second minor group of tests uses salt solutions to apply physico chemical effects to the clay components of aggregates.

2.2.1 Wet sieving

This is the classical and still most prevalent procedure for testing the water stability of soil aggregates. The technique was

developed by Tuilin (1928) and had since then been used extensively in the USA and USSR. Basically the test involves sieving a representative sample of air-dried aggregates under water in controlled uniform manner. The resulting aggregate size distribution at the end of a specified period is used as an index of stability. The more the aggregates in the larger size classes, the lower the breakdown and the higher the stability.

To obtain meaningful results using wet sieving methods a large number of potential variables are kept constant during the investigation. These include sieve size and number, aggregate size, pretreatment of aggregates, duration of the sieving, number of oscillations per unit time and the liquid media. These variations can conveniently be treated under four headings:

1. The nature of the aggregates used
2. The wetting procedure
3. The forces applied, and
4. The final expression of the stability.

2.2.1.1 The Nature of the Aggregate Used

Most frequently aggregates to be used in wet sieving tests are obtained by sieving air-dried soil to different size fractions. De Leenheer and De Boodt (1967) mechanised the process by crushing dry soil in a mill device. The plates of the mill were adjusted 8 mm apart and this was the largest size of aggregate obtained. An earlier study by Brewer (1964) distinguished between the surface characteristics of structural peds and those obtained by fragmentation. His results raised

a question of whether such aggregates obtained by the milling process of De Leenheer and De Boodt (1967) conform to the definition of natural peds. Significant differences in surface characteristics of disturbed and undisturbed aggregates have recently been observed by Powers and Skidmore (1984).

The size fraction of aggregates used in various studies have varied from whole soil sample to fractions obtained by sieving. Williams and Cooke (1961) found that aggregate stability was very high if smaller aggregates (0.5 mm) were used, and that the stability decreased with increasing size up to 4 mm. They used 4 - 6 mm aggregates in their test. Silanpaä (1967) used aggregates less than 6 mm; Hamblin and Greenland (1977) and Grieve (1979, 1980) used 2 - 4 mm aggregates in their different stability tests.

Recently there has been a shift towards using microaggregates for stability determination. This is based on the assumptions that microaggregates (2 μm to 0.5 mm) form the structural building blocks of soil aggregates, i.e. they form nuclei of aggregation. It has been pointed out by Middleton (1930), that this microaggregate fraction is the one transported during erosion when the soil aggregates are dispersed. These size fractions have been used by Richardson (1976); Reid and Goss (1981); Reid et al., (1982) and Utomo and Dexter (1982) in different studies because their stability is important in consideration of soil structure. Organic matter, which is universally recognised as a stabilising agent, has been shown to be associated with these microaggregates (Tisdall and Oades, 1982; Oades, 1984).

2.2.1.2 The Wetting Procedure

Wet sieving tests, like other usually used tests in aggregate stability determination, involve wetting of aggregates prior to mechanical dispersion. Wetting of aggregates may cause their collapse as the bonding substances dissolve and as the clay swells and disperses. Non-uniform wetting results in one part of the aggregate swelling more than the other, and the resulting stress may fracture the aggregate. These effects are compounded by very high pressures in the occluded air, when aggregates are rapidly wetted, which may fracture aggregates as it escapes.

Emerson (1954) criticised the rapid wetting procedures on theoretical grounds. He further contested that rainfall intensity in the British Isles seldom exceeds 2 mm hr^{-1}, and that this wetting rate causes little aggregate breakdown. The problems of aggregate disruption upon rapid wetting have been overcome by pre-wetting samples prior to wet-sieving. Samples have been wet by capillary action (Silanpaä, 1967), or by capillary action against tension (Williams et al., 1966; Grieve, 1979, 1980) or under vacuum (Pannebokke and Quirk, 1957; Hofman and De Leenheer, 1975). Kemper and Koch (1966) compared different pre-wetting tests and their effects on aggregate stability, and obtained higher stability values with wetting under suction as shown in Table 2.1.

Despite theoretical objections against rapid wetting tests, these have been widely used to study the effects of cultivation, and have been useful in discriminating between soils of different cropping histories

(Low, 1972). Comparison of slow and rapid wetting tests have shown the greater discriminating power of rapid wetting tests (Williams et al., 1966; Grieve, 1979).

The role of wetting in aggregate stability test depends on the field process to be simulated. If wetting in the field is liable to be rapid as is the case with bare soils subjected to heavy rain, disruption of the aggregates by water entry becomes an important process of aggregate disruption.

Table 2.1. Effect of methods of wetting on the % of aggregates stable in water. (Source: Kemper and Koch, 1966).

Soils	Wetting rapidly by immersion	Wetting slowly under suction	Wetting while evacuated
Average of 16 soils from western USA	41	74	70
Average of 4 stable soils from eastern USA	83	98	99

2.2.1.3 The Mechanical Force Applied

The second force imposed on the aggregates during wet sieving is the mechanical abrasion caused by the impact of the aggregates with one another and the sieve during agitation under water. This force can be varied, either by altering the length of time of sieving or the amplitude of the sieving stroke. Low (1954) suggested 500 strokes as a convenient

norm. The length of the sieving time may be varied according to the stability and the nature of the soils under investigation (Williams et al., 1966). Tinsley and Coutts (1967) used horizontal motion of the sieves during stability tests, and this is obviously in contrast with the rest of the wet sieving methods.

2.2.1.4 The Final Expression of the Stability

The final source of variation in wet sieving tests occur in the manner of calculation and presentation of results. Aggregate size distribution, the most commonly used, is not a satisfactory measure of stability as for most comparisons a single figure expression is desirable. Bryan (1971) reviewed a number of ways in which these indices can be expressed, such as the percentage by weight of water stable aggregates greater than a given size (0.25, 0.5, 1.0, 2.0 or 3.0 mm). If a uniformly sized aggregate fraction is used for the test, then this percentage is an expression of the number that have not been disintegrated by the action of the sieving.

Bryan (1969) examined the efficiency of different measures for predicting soil loss determined on a laboratory rainfall simulator. He concluded from a series of experiments that complex measures which give the complete distribution such as mean weight diameter (De Leenheer and De Boodt, 1967), are not worth the time and effort involved in their calculation, as most efficient measures were found to be simplest ones, the percentage by weight of water stable aggregates greater than 3 mm diameter being the only most completely reliable index of soil erodibility (Bryan, 1968, 1969, 1971, 1976).

Many subsequent studies that followed Bryan's work used single figure indices of stability, although the aggregate size fraction chosen differed amongst various studies. Grieve (1979) used percentage greater than 1.4 mm in wet sieving tests, and found this very reproducible in comparing aggregate stability amongst sites with different cropping histories. In most recent studies using micro-aggregation techniques of stability determination, the percentage size greater than 0.25 mm and 0.5 mm have been widely used (e.g. Reid and Goss, 1981; Cheshire et al., 1983).

2.2.2 Dispersion Measurement

The micro-aggregation or dispersion technique developed by Middleton (1930), has since been used intermittently as an index of aggregate stability. His technique basically involves end-over-end shaking of a known amount of soil in distilled water in a sedimentation cylinder 20 times and then determining the amount of material smaller than 50 μm from the settling rate. The ratio of this to the amount determined through dispersion in particle size analysis was called the dispersion ratio.

The size fraction in the suspension is estimated from their rate of sedimentation using Stokes' Law. Yoder (1936) criticised the dispersion method on the grounds that particles settling cannot be assumed to be doing so at the same velocity, as these have varying densities, and also that particles that are smooth and spherical are not likely to be found in soils. He further argued that whereas some soils

have stable aggregates greater than 0.5 mm, Stokes' Law has the maximum limit at 0.5 mm. Despite such theoretical objections by Yoder (1936) tests using dispersion principles have been used widely to study amongst others the effects various cultivation practices and discriminated amongst soil with different cropping histories (Kolodny and Neal, 1941; Williams et al., 1966; Reid et al., 1982).

In early aggregate stability determinations, percentage dispersion was estimated by using the pipette method (Kolodny and Neal, 1941). Recently dispersion has been estimated by turbidimeters and absorptiometers. The advantage of these techniques is in that settling times for estimation of the desired size dispersed can be varied depending on the ratios of soil to water and the temperature at which determinations are made. Thus the main advantage of this method is its flexibility and adaptability and it has steadily gained favour.

Williams et al. (1966) dispersed 0.25 g aggregates in 20 ml water using an end-over-end shaking device at 13 rpm. The suspensions were allowed to stand until all particles of diameter greater than 2μm had settled to a level below which a light beam would pass. The % light transmission was determined with a turbidimeter. Light transmission was measured again after additional shaking and the stability expressed as the ratio of the second to the first reading. The shaking period could be varied according to the stability of the samples being investigated, and rapid wetting and slow wetting procedures could be applied. Thus the wetting as well as the breakdown procedures conform to the outline given for wet sieving.

In their tests Williams et al. (1966) found that when dealing with high stability soils, the second shaking was not necessary, and by omitting this step, their results were not altered. Edwards and Bremner (1967) demonstrated that the dispersion of the clay fraction from a number of soils was almost complete using prolonged shaking in water as it was when using ultrasonic dispersion over much shorter time period. They recommended that for simplicity and for routine laboratory and field analysis, where the number of pieces of equipment has to be reduced and the simplest means applied, the sonication step could be omitted.

An absolute measurement of soil structure stability was devised by North (1976) using a controlled application of known dispersive energies derived from a calibrated ultrasonic probe. The method entails dispersing air-dried crumbs (<2 mm) placed in a glass cell with 50 ml water, by sonicating the sample at different power levels. A graph plotting dispersive energy against percentage weight fraction <2 μm gives the dispersion characteristic. The energy at which secondary disaggregation begins is a practical measure of the soil susceptibility to natural disruption and represents an absolute measure of soil stability.

In further experiments of soil structural stability, North (1979) assessed the sensitivity of the ultrasonic method in detecting those differences between soils attributed to their contrasting manegement behaviour and cultivation history. When paired soils from fields with known problems were compared to those free from problems were examined, the ultrasonic method failed to reflect management experience.

Problematic soils sometimes yielded higher stability values than their rarely problematic counterparts. He acknowledged the fact that ultrasonic measurement of stability to dispersion is of little value in identifying management potential, but can be of great value where studies are concerned only with mechanisms of aggregate formation and breakdown.

Cheshire et al. (1983) used a method following that of North (1979). In their test the ultrasonic disintegrator was operated at 18 khz to achieve total dispersion of the aggregates. To make treatments comparable the untreated and sonicated samples were also subjected to 2 x 25 minutes shaking in an end-over-end shaker prior to turbidimetric measurement. The levels of dispersion caused by various treatments were expressed as percentage of the value obtained for sonicated soil.

Dispersion methods can be of value in identification of soils liable to erosion (Middleton, 1930; Morgan, 1979). However, the methods have been criticised by Bryan (1971, 1976) in that they involve complex and time consuming calculations of the dispersion ratios, used as indices of erodibility. Nontheless, the introduction of spectrophotometers and absorption meters have lent the method reputability especially in the study of soil micro-aggregation. Micro-aggregates are important in determining the physical behaviour of soils (Oades, 1984); they are the building blocks of aggregates, thus their stability is important. Tests which employ measurement of aggregation of the micro-aggregates are therefore useful, in that these aggregates are involved in soil processes such as crusting, and are the most transportable fraction during erosion by water and wind. What is required for standardisation of these techniques is adoption of single figure indices of stability measurement.

2.2.3. Water Drop Method

This method was first proposed by McCalla (1944). The method is designed to study the breakdown of surface soils under raindrop impact. In his method aggregates were placed on a 1 mm mesh sieve and subjected to the impact of falling drops from a burette placed at a standard height of 30 cm. The number of drops (4.7 mm) needed to wash the aggregates through the sieve was used as an index of stability. Low (1954) and Grieve (1979a) used drops of mass 0.1 g falling from 1.0 m height. With a fall from 1.0 m the impact kinetic energy of each drop (0.1 g) is 9.8 x 10^3 ergs (Low, 1954). In SI units this is 9.8 x 10^{-4} joules. Grieve (1979a) obtained maximum impact of the drop by shielding the drops with a 15 cm diameter perspex tubing through which the falling drops travelled. 100 drops were used for each test, i.e. per aggregate. Single drop stability tests would seem to be most appropriate way to characterise the resistance of soil structural units, as it is this method that comes nearest to mimicking natural erosion mechanisms. When, however, these individual units are lumped together into a plot size system it is far from clear how the stability of individual aggregates against drop impact influences erosion. Whilst its use is not disputed in studies of, for example, surface crusting of agricultural soils, stability to drop impact was judged unsuitable in the present study especially where stability of a large number of samples from different experimental treatments was to be determined.

Rainfall simulation is another group of similar tests, in that, soil aggregates are subjected to falling drops. These methods have been

applied mostly in the fields of geomorphology. In these tests the stability of aggregates subjected to standard simulated rainfall is used as an index of soil erodibility (Bryan, 1968, 1969, 1974, 1976; De Boodt et al., 1974; Soil Sci. Soc. Amer., 1979; Morgan, 1979; Imerson and Vis, 1984). Rainfall simulators operate on the same principles, the variables being the drop sizes, the height of fall of the drops and their energy. These are variable parameters, and are pre-determined by the specific needs of individual projects and the nature of the rainfall of localities under investigation.

2.2.4 Tests Using Forces Designed to Break Intra-Aggregate Bonds.

Emerson (1954) measured the stability of soil aggregates by determining the concentration of NaCl that caused the aggregates to disperse and render them impermeable. Aggregates were leached with a 1N NaCl solution to replace exchangeable cations present. These were then percolated with increasingly dilute NaCl and changes in permeability noted. The concentration of NaCl solution which caused complete dispersion of the aggregates and reduced permeability to zero was used as index of stability. The lower the concentration of NaCl therefore, the higher the stability would be. A modification of this method (Dettman and Emerson, 1959) required only one concentration of NaCl. This was chosen so that floculation was avoided in the case of known stable soils and produced a significant decrease in permeability of known unstable soils. An index of stability was obtained by calculating the ratio of the initial permeability of the aggregates to the final permeability. This method was further used by Williams et al. (1966) and the

modification made in their study only affected the expression of the results and the principle of the test was unchanged.

Proponents working along the lines of Emerson's tests claim that they hold the advantage in terms of the accuracy of their tests. The tests assessing the specific bond strength such as those of Emerson (1954) claim to test the strength of the bonds that are significant in aggregate stabilisation. However, as has been pointed out the mechanisms for aggregate stabilisation are highly complex and not yet fully understood. It is thus difficult to see in what way a test measuring the strength of a particular bond type can give a true representation of the stability of an aggregate. The stability of aggregates may include important contributions from either mechanical entanglement by filamentous organisms, or cementation of soil particles by gums produced by the organisms. Tests based on Emerson's method may therefore be of value in evaluating the contribution of the latter mechanisms, if such binding actions predominate at that particular time. The method may be of some value in categorising the mechanisms of aggregate stabilisation and breakdown, but it has been felt inferior to the other group for the purposes of the present study.

2.2.5. Methods Based on Pore Volume Changes

Williams and Cooke (1961) developed a method for estimating the instability of the soil crumbs by wetting them in narrow tubes and measuring the loss in pore space when the aggregates slaked. Williams (1963) improved the technique using mechanical vibration to reduce the

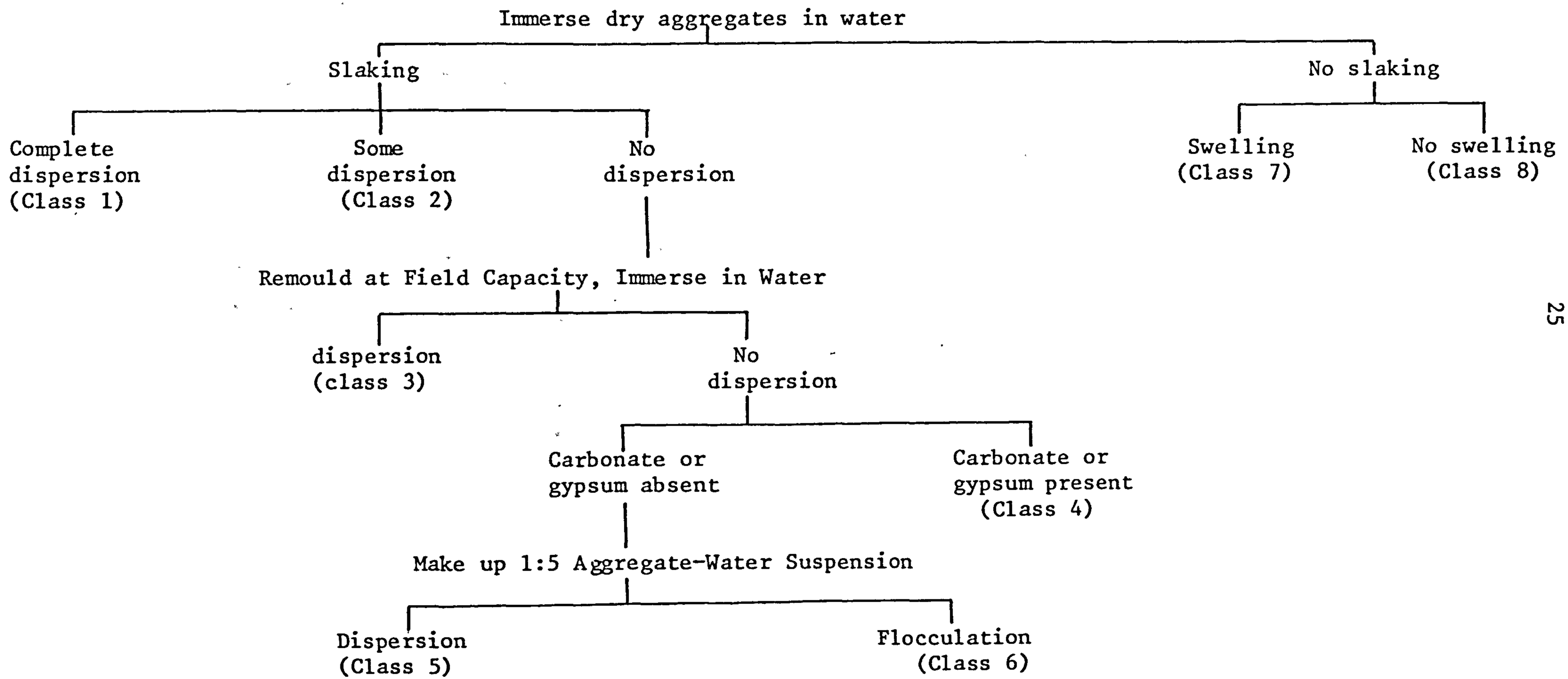
mutual adhesion of the crumbs. The water remaining in the coarse pores between the aggregates was extracted by applying low suction, and its volume compared with the volume extracted in the same manner from soil pre-stabilised with perspex.

Grieve (1975, 1979a) made further improvements on the method. In his tests, the mechanical disturbance force was provided by an ultrasonic tank. He used both tension and rapid wetting tests. The index of stability is the volume of inter-aggregate pore space determined as the volume of water drained between two tension values which were obtained by examining the water release curves for stable aggregates. Grieve omitted the pre-stabilisation of the aggregates (Williams, 1963) on the grounds that pre-stabilised aggregates became visually smaller and more rounded than natural aggregates, and also because this correction was found to increase variability of the results and not to decrease it. These pore volume tests are basically slaking tests in which the % loss in pore space is taken as an index of structural stability.

2.2.6 Stability Class from Slaking, Swelling and Dispersion

Emerson (1967) proposed a classification of soil aggregates into eight classes based on the coherence of the aggregates in water. Greenland et al. (1975) modified this test and examined the structural stability of surface soils with the aim of distinguishing soils likely to have problems in the field (Figure 2.2). The results of the slaking test are derived from the behaviour of soil aggregates when immersed in water. Two main processes operate to cause disruption of the clod into

Figure 2.2 Classification of Aggregates According to Cohesion in Water (Source: Emerson, 1967; Greenland et al. 1975).



smaller units when dried clods are wetted. The rapid intake of water causes unequal swelling throughout the clod, which fractures and fragments the clod along cleavage planes. The sorption of water into capillaries results first in compression of the occluded air and finally in explosion within the clod as the pressure of the entrapped air exceeds the cohesion of the particles.

The two main forces involved in the process of water attack on dry cohesive aggregates are the driving force of water entry, which is determined by the affinity of the internal surfaces for water and the cohesive forces holding particles in the aggregate together. As water enters into the clod, and the affinity of soil surfaces for water exceeds the cohesive forces, the aggregates lose their cohesion and the cementing bonds are ruptured. Prior to water entry, there is both free and adsorbed air in the pores, water releases the adsorbed air, which is added to the free air in the pores. If there is no space to escape, pressure builds up within the clod due to compression of the air (Kemper et al., 1985). The size of the pores and the rate at which the cohesive bonds are destroyed in relation to the speed of water penetration determines to a great extent the type of slaking action to occur. If the rate of bond destruction is equal to or greater than that of water penetration, there is orderly, progressive slaking of the aggregates, and if the rate is less than that of water entry and there is no build up of pressure due to entrapped air because of large pores; there will be no breakdown of the aggregates.

Most of the information on the extent of the effects of air entrapment on aggregate disruption is gained from studies in which the stability of aggregates prewetted under suction, capillary action or vacuum were compared to those directly immersed in water (Yoder, 1936; Kemper and Koch, 1966). However, there is some evidence to suggest that entrapped air may be an aid to disintegration rather than the principal cause (Panebokke and Quirk, 1957; Chaney, 1978). In their studies these authors recognise differential swelling in aggregates containing clay and the energy of hydration on wetting as being of primary importance in the disintegration of the soil aggregates. Chaney (1978) concluded from his slaking observation tests that when an aggregate is wetted, hydration by a polar solvent such as water together with any swelling which occurs weaken the structure of the aggregate and the pressure of entrapped air escaping provides the small amount of energy required for the sub-aggregates to break away from the severely weakened aggregate. His conclusions were drawn from comparisons of behaviour of aggregates wetted with water (polar solvent) or with non-polar solvent such as paraffin and ethanol, either under vacuum or at atmospheric pressure.

It can be concluded from the studies referred to above that the breakdown of soil crumbs can be caused by (1) stresses and strains set by unequal swelling due to soil heterogeneity and non-uniform wetting; (2) reduction in cohesion with increasing moisture content; (3) compression of entrapped air and (4) dispersion of the cementing materials. These factors are influenced by the initial moisture content of the soil (Kemper and Rosenau, 1984; Kemper et al., 1985) and the method and speed of wetting (Emerson, 1954).

2.2.7. Summary

All methods which are usually used in aggregate stability determination are of value in that they yield results which distinguish soil aggregates from localities with known structural problems and those from well structured permanent pasture and forest soils. However, as mentioned earlier in this section and by many more other researchers in this field, the final choice of the method is determined by the nature of the problem being examined.

The present study has its main interest in the stability of micro-aggregates, produced by cultivations, and the macro-aggregates resulting from binding of micro-aggregates. Thus two techniques namely wet sieving and turbidimetry, can be of value in this context. The results previously obtained by workers using these two techniques have been variable, mainly because there has been little or no standardisation of the dispersion procedures in the case of wet sieving and turbidimetry and the measurement of the resulting turbidity in the case of dispersion techniques. A method which attempts to overcome these shortcomings will be presented and evaluated in the present study.

2.3. Effects of Cropping Practices on Aggregate Stability

Many agricultural practices affect soil structure. Farm implements and animals compact the soil (Soane et al., 1972; Cary and Hayden, 1974). Tillage loosens the soil by breaking it into aggregates of the desired sizes, whilst on the other hand it exposes the soil to

direct raindrop impact. Farming practices directly or indirectly affect soil aggregate stability through their influence on the organic matter content of the soils (Grieve, 1979b, 1980; Chaney and Swift, 1984). Changes in management practices affect organic matter in two ways; by altering the annual input of organic matter and by altering the rate of organic matter decomposition. The flush in decomposition rate when the soil is tilled or disturbed is well known (Rovira and Graecen, 1957). This disturbance of the soil has been shown to cause more surfaces of the previously inaccessible substrates to become available for microbial growth and to kill part of the biomass thus depleting the organic matter content of the soil (Adu and Oades, 1978; Lynch and Panting, 1980; Carter and Rennie, 1982).

The effect of grassland is universally accepted as a beneficial one, both in the development and stabilisation of soil aggregates and replenishment of organic matter. Low (1955) observed large improvements in structural stability following periods under grass leys. He pointed out that such restoration of soil structure stability is a slow process and depending on the soil mechanical composition it may take from 5 to 50 years under grass before an old arable soil attains the structure of a grass pasture soil.

Williams and Cooke (1961) examined the effects of grass residues and farmyard manure on soil structure. Although they found that farmyard manure improved the water stability of the aggregates of arable soil, such improvements were not as high as under permanent grass pasture. These treatments were also shown to improve the permeability and

compaction of the clods. Although all grasses have the capability to regenerate soil structure, their relative effects differ significantly. In the studies by Reid and Goss (1981) growth of perennial ryegrass and lucerne for 42 days resulted in increased aggregate stability, and this stabilisation was associated with polysaccharide materials produced in the rhizosphere. Growth of maize, tomato and wheat on the other hand decreased the stability of fresh aggregates. They concluded from their results that the growth and activities of living roots may be a major factor in controlling the overall direction and magnitude of changes in aggregate stability under arable or ley crops.

In Australia, Tisdall and Oades (1979, 1980a, 1980b) in a series of experiments studied the effects of root systems of rye-grasses on stabilisation of soil aggregates and how these ryegrasses could be managed to maximise their effects. Their studies emphasised the role of biological processes in aggregate stabilisation. They found that the root system of ryegrass was more effective than that of white clover in stabilisation of soil aggregates mainly because the ryegrass roots supported larger population of vesicular arbuscular mycorrhizal hyphae in the soil. Fifty years of crop rotations decreased the stability of macro-aggregates (>250 μm diameter) mainly because these rotations decreased the length of roots and hyphae and the percentage total organic matter in the soil. Management of ryegrasses such as clipping or occasionally cutting was shown to improve their effects because this treatment stimulates the growth of hyphae.

In Britain experiments have been carried out over 30 to 40 years by four different organisations studying the effect of ley and arable cropping systems. Factors which have been measured include organic matter, water stable aggregation, crusting strength, polysaccharide content, effects of soil conditions etc. Significant losses of organic matter have been observed at Rothamsted when permanent pasture fields were put into cropping (Cooke, 1967). In ADAS ley fertility experiments (Eagle, 1975) a three year ley maintained organic matter levels at three sites only, and the 9 year ley gave marked accumulation, but this was lost in the subsequent three year cropping at all but two sites. Other similar experiments which showed deterioration of soil structure and fertility of the soils as organic matter was depleted through continuous arable cultivation were undertaken at Jealott's Hill (Low, 1975) and at Grassland Research Institute, Hurley, (Clement, 1975).

The factors responsible for development, stabilisation and degradation of soil structure under different farming and cropping practices are fairly well documented. Periods of grass, or additions of organic matter in the form of farmyard manure are the principal agents of improvement. However in modern farming especially in the temperate zones and other developed countries, such practices are increasingly becoming impossible. The Strutt Report (MAFF 1970) pointed out that cultivation is an increasing factor in structure degradation. This is mainly due to the need to achieve particular planting and harvesting dates in an increasingly busy farm year. Machinery is therefore used on land which is far too wet to resist deformation. The results are structural

failures manifested as compaction in or beneath the plough layer and crusting which cause restrictions in crop growth and depress yields.

2.4. Biological Processes and Soil Aggregate Stability

2.4.1 The Influence of Soil Organic Matter on Aggregate Stability.

The diversity of soil constituents implicated in aggregate formation, degradation and stabilisation has given rise to a great number of investigations. Clay, humic substances, polysaccharides and inorganic cements, especially colloidal forms of oxides and hydroxides of metals such as iron and aluminium, have been proposed as constituents which stabilise soil aggregates. (Kemper and Koch, 1966; Deshpande et al., 1968; Giovaninni and Sequi, 1976; Hamblin and Davies, 1977; Hamblin and Greenland, 1977). Of these many constituents studied, organic matter is recognised as the most important aggregate stabilising agent (Greenland, 1971; Russell, 1973; Allison, 1973).

The main input of organic matter in the soil is through addition of plant and animal debris. Another source is the indigenous microbial population which contributes by excretion of organic compounds and the tissue of micro-organisms upon death. Fresh organic debris recently added to the soil still retains many characteristics of the original material. As this tissue is decomposed by microorganisms many changes occur and a large number of products are formed, some of which are persistent and some transient.

The organic matter in soils has been classified by Jenkinson and Rayner (1977) into five categories: decomposable plant material (DPM), resistant plant material (RPM), soil biomass (BIO), physically and chemically stabilised organic matter (POM and COM respectively). It can therefore be seen that organic matter consists of a whole series of products which range from recognisable plant and animal tissue through ephemeral compounds of decomposition to highly decomposed products. This process is known as humification, the final product being stable amorphous brown to black material usually referred to as 'humus'. The greater part of organic matter in soils occurs in the form of clay-organic complexes (Greenland, 1965a; Mortland, 1970; Theng, 1979) and complexes of iron and aluminium which have also been shown by several studies to be important in aggregate stabilisation (Hamblin, 1977; Hamblin and Greenland, 1977; Turchenek and Oades, 1979; Shanmuganathan and Oades, 1982).

Although most of the organic matter in soils exists in the form of POM and COM, these are not the forms necessarily important in soil aggregate stabilisation. The most active materials promoting soil aggregation and stabilisation are polyuronides and polysaccharides formed by microbial decomposition of plant and root debris (Greenland and Hayes, 1981). These substances are readily subject to biological degradation and subsequently have short half lives (Jenkinson and Rayner, 1977). Recently organic binding agents actively involved in aggregate stabilisation have been treated under three classes by Tisdall and Oades (1982). Their groupings were determined on the basis of age and degradation of the organic matter and not on the proportion of the

chemically defined components. These classes are temporary binding agents such as roots and fungal hyphae; transient binding agents such as polysaccharides, produced by microbial transformation of various organic materials or associated with roots and the microbial biomass in the rhizosphere; and persistent binding agents consisting of degraded aromatic materials associated with metal cations in the form of organo-mineral complexes.

The relationship between organic matter and soil structure stability can be visualised as in Figure 2.3. The decomposition of fresh organic matter in the form of roots and plant residues by soil organisms gives rise to polymeric compounds such as polyuronides, which have the ability of cementing elementary soil particles such as clay, silt and sand. These polymeric compounds are subject to further decomposition; thus structural stability formed tends to decrease with time, unless fresh supplies of organic matter are available. Telfair et al. (1957) concluded that the stable forms of organic matter such as 'humus' occurring in complexes with the clay acts only as a filler and is of secondary importance in structural stabilisation. It does however contribute to soil fertility both by decomposition, through nitrogen release and by holding a variety of cations, eg Ca^{++} and NH_4^{++}, as a result of high ion exchange capacity.

Grieve (1980) studied aggregate stability status of sandy soils from four sites in Eastern Fife, Scotland using different stability test procedures. Both the stability test procedures (inter-aggregate pore volume change and wet sieving) revealed highly significant differences in

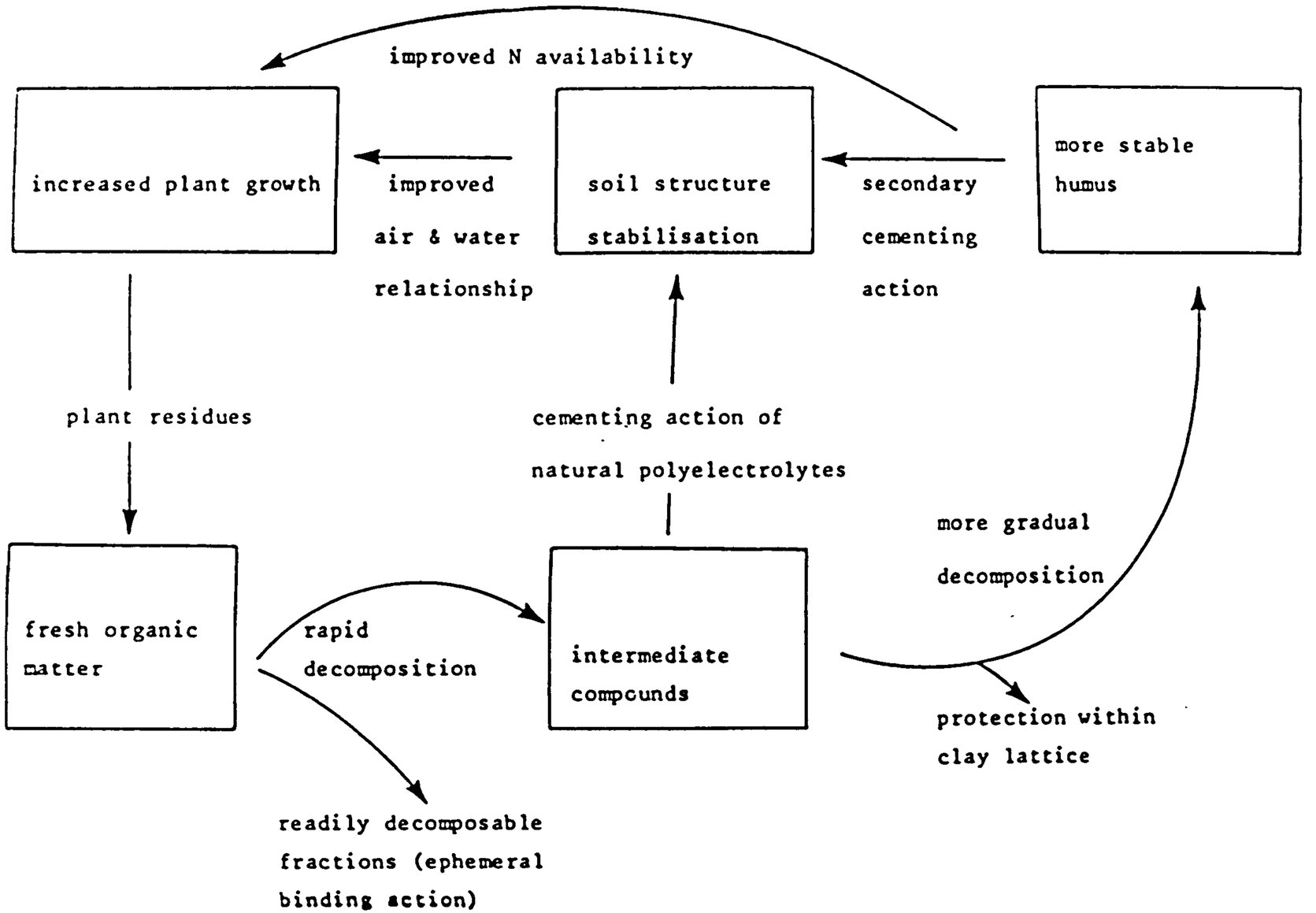


Figure 2.3 Soil structure stability and organic matter relationships (Source : Telfair et al., 1957)

aggregate stability amongst the sites, and these differences were closely related to organic matter contents of the sites, resulting from cultivation histories. Chaney and Swift (1984) assessed the influence of organic matter on aggregate stability of 126 British soils. Aggregate stability was determined by wet sieving procedure, as this was found to be most suitable in terms of reproducibility, and the results were expressed as mean weight diameter (MWD). Highly significant relationships were found between aggregate stability and organic matter. They, however, noted that total organic matter alone may not be sufficient to explain variations in aggregate stability. Chaney and Swift further correlated aggregate stability with various organic matter constituents for different soil groups, and obtained significant correlations between aggregate stability and all different soil organic matter constituents, particularly carbohydrates and pyrophosphate and sodium hydroxide extractable humic materials.

Correlations between organic matter and aggregate stability are not always simple. (Tisdall and Oades, 1982). There are several reasons for the inconsistencies revealed by the studies of aggregate stabilisation by organic matter. These may result because:

a) Only part of the organic matter may be responsible for water stable aggregation.

b) There is a level of organic matter content above which there is no further water stable aggregation.

c) Organic materials are not the only major binding agents.

d) It is the disposition rather than the type or amount of organic matter content which is important.

e) Some of the water stability in virgin soils may be related to physical factors such as particle reorganisation associated with the first disturbances of virgin soil.

f) Composition of the organic matter is not constant and can vary seasonally.

2.4.2. The Contribution of Microorganisms in Aggregate Stability

The preceding discussion presented a generalised over-view of the effect of soil organic matter on aggregate stabilisation. Numerous investigators have demonstrated an improvement in soil aggregation following organic matter applications. However, many studies suggest that this ability of complex organic materials to bind soil particles is a function of decomposition of the residues by soil organisms, rather than the presence of the organic materials themselves.

Martin and Waksman (1940) demonstrated that when a microbial energy source such as sucrose is added to the soil, and the soil is sterilised, no improvement in soil aggregation took place. When the sterilised soil is later re-inoculated with fresh soil, or with microbes, Martin and Waksman observed a marked increase in aggregation when the same soil was re-incubated. Subsequently Martin et al. (1959) observed that incorporation of soil organic residues possessing little or no innate soil binding power is followed invariably by increases in soil aggregation in the presence of but not the absence of microorganisms. Thus Baver et al. (1972) wrote 'The incorporation of organic matter in

the soil brings into the picture the activities of soil microorganisms, fungi, actinomycetes, bacteria and yeasts. Organic material itself without biological transformation has little if any effect on soil structure. Microorganisms without organic materials as source of energy are ineffective in producing soil aggregation'. pp. 155.

Changes in aggregation taking place after incorporation of organic materials have been related directly to intense microbial decomposition of the substrate and the resulting proliferation of the microbial population. These changes depend on the nature of the organic residues and subsequently their rate of decomposition (McCalla, 1945; Adu and Oades, 1978; Tisdall and Oades, 1982).

The above observations point to the fact that upon incorporation of organic substances into the soil, microorganisms proliferate. Fungi and actinomycetes produce mycelia. The metabolic processes of the microorganisms synthesize complex organic molecules, and the organisms or products of decomposition give rise to stable aggregates. Aggregate stability is therefore a result of:

a) The mechanical binding action of the cells and filaments of the organisms.

b) The cementation effects of the products of microbial synthesis.

2.4.3. The Mechanical Binding Action of the Cells and Filaments of the Organisms

Numerous investigators have evaluated the aggregating effects of various microorganisms. Studies of pure and mixed cultures have shown that aggregate stabilisation depends upon the type of organisms and the presence of both a source of energy and nitrogen for microbial metabolisms. Others have gone further to show that the aggregating power of organisms may vary with species within a given type (Harris et al., 1966). It can be concluded from these studies that conditions which favour high activity usually result in increased rate of aggregation due to rapid conversion of organic materials to organic cements and fungal mycelia.

Diverse bacteria, fungi and actinomycetes are capable of binding soil particles into stable aggregates, but their aggregating ability differ widely. McCalla (1945, 1946) found that the order of aggregating effectiveness was fungi > streptomycetes > certain bacteria > certain rhizobia > yeast > other bacteria. He concluded that increased structural stability resulting from microbial activity is temporary, and that it remains as long as the stabilising decomposition products existed in the soil. He also observed that the presence of competing organisms of low stabilising power reduced the effectiveness of microbial groups with high stabilising power, and that the kind of energy source supplied was an important contributing factor. A similar order of stabilising effects of microorganisms was observed by Swaby (1949).

Harris et al. (1963) studied the role of microorganisms on aggregate stabilisation of artificial aggregates under sterile and non-sterile conditions. They observed that little or no change in stability occurred in sterilised aggregates and attributed the changes which took place in non-sterile aggregates to microbial activity. Harris et al. (1963) also noticed that anaerobic conditions during incubation resulted in the largest increase in aggregate stability, and that such resulting stability was persistent throughout the incubation period. They attributed this maintenance of stability under anaerobic conditions to the fact that anaerobic microflora rapidly metabolised the sucrose to form organic aggregating substances during the initial incubation period, but were unable to utilise these substances as sources of energy. The decline in stability under aerobic conditions was found to result from utilisation of the metabolic products (aggregating agents produced during the initial stages) during the subsequent period of incubation.

In further studies (Harris et al., 1964) found that aggregate stabilisation was a function of microbial synthesis of soil binding agents rather than the presence of individual microorganisms. They observed that fungal stabilisation of artificial aggregates (< 0.5 mm) into crumbs (2 mm) was mainly through mechanical binding action of the filaments. The initial stages of stabilisation of aggregates by bacteria involved the binding of soil particles into small aggregates (< 2 mm). The aggregate status of the soil did not correspond to the total microbial numbers present in the soil. Harris et al. (1964) concluded that the majority of the indigenous population of microorganisms were not capable of affecting aggregation, and that aggregation is mostly a

function of microbial synthesis. Their hypothesis was supported by large increases in bacterial numbers which preceded increases in soil aggregation. They concluded that bacteria were more important than fungi in the primary stabilisation of soil aggregates during the initial stages of incubation.

Bond and Harris (1964) studied the effects of fungi, actinomycetes and bacteria in both pure and mixed cultures, and found that mixed cultures were more effective in stabilising soil aggregates. They observed that the role of filamentous organisms tended to be ephemeral because disaggregation followed degradation of hyphae. Scanning electron microscopy revealed that stable aggregates extending down the profile were always associated with intense proliferation of fungal mycelia. The resulting stable structures formed sand caps. In the layers beneath these sand caps mycelia and slime of similar appearance to that of mycelia were found. Angular, thick walled mycelia with slimy surfaces was found to be most effective in aggregate stabilisation. Bond and Harris (1964) found appreciable amounts of angular fungal hyphae and minute debris within the water-stable aggregates after a period of 4 - 6 years under pasture. They recommended that 4 - 6 years of pasture was necessary in the red brown earth soils of Australia to have sufficient fungal mycelia to ensure maintenance of good soil structure. This period of time was recommended because Bond and Harris found no appreciable increases in mycelia and structure stability beyond this period.

Filamentous fungi are capable of binding soil particles into stable aggregates because particles of soil adhere to a mucilage on the surface of the hyphae (Oades, 1984) and also because the reticulum of

hyphal filaments enmesh the soil particles. Griffiths (1965) and Tisdall and Oades (1982) concluded that in most soils the effects of fungal hyphae would be ephemeral, depending on the presence of readily decomposable material and other organisms known to degrade fungal hyphae. Thus Griffiths (1965) concluded that apart from the direct effect of fungi on soil structure in the presence of fresh organic materials, fungi may indirectly contribute to soil stabilisation through serving as substrate to bacteria during subsequent incubation periods, leading therefore to production of cements capable of stabilising soil aggregates.

The mechanisms of aggregate stabilisation by soil microorganisms can be summarised as in Figure 2.4. According to this figure Lynch (1983) visualised larger aggregates as resulting from mechanical binding of soil particles by filaments of fungi, and from the cementing action resulting from microbial polysaccharides. Microorganisms have the potential to bind soil particles both by mechanical entanglement and adhesion of extracellular polymers. Part of the organic matter is protected within the larger sand and silt sized particles. This organic matter may directly adhere to the soil particles in different positions as shown by Emerson (1959); or from complexes with colloidal clay (Theng, 1979) thus lending it is persistence in soil aggregates and longer aggregating effects (Tisdall and Oades, 1982; Oades, 1984).

These discussions point to the fact that microbial stabilisation of soil aggregates varies with time, depending on the interaction of

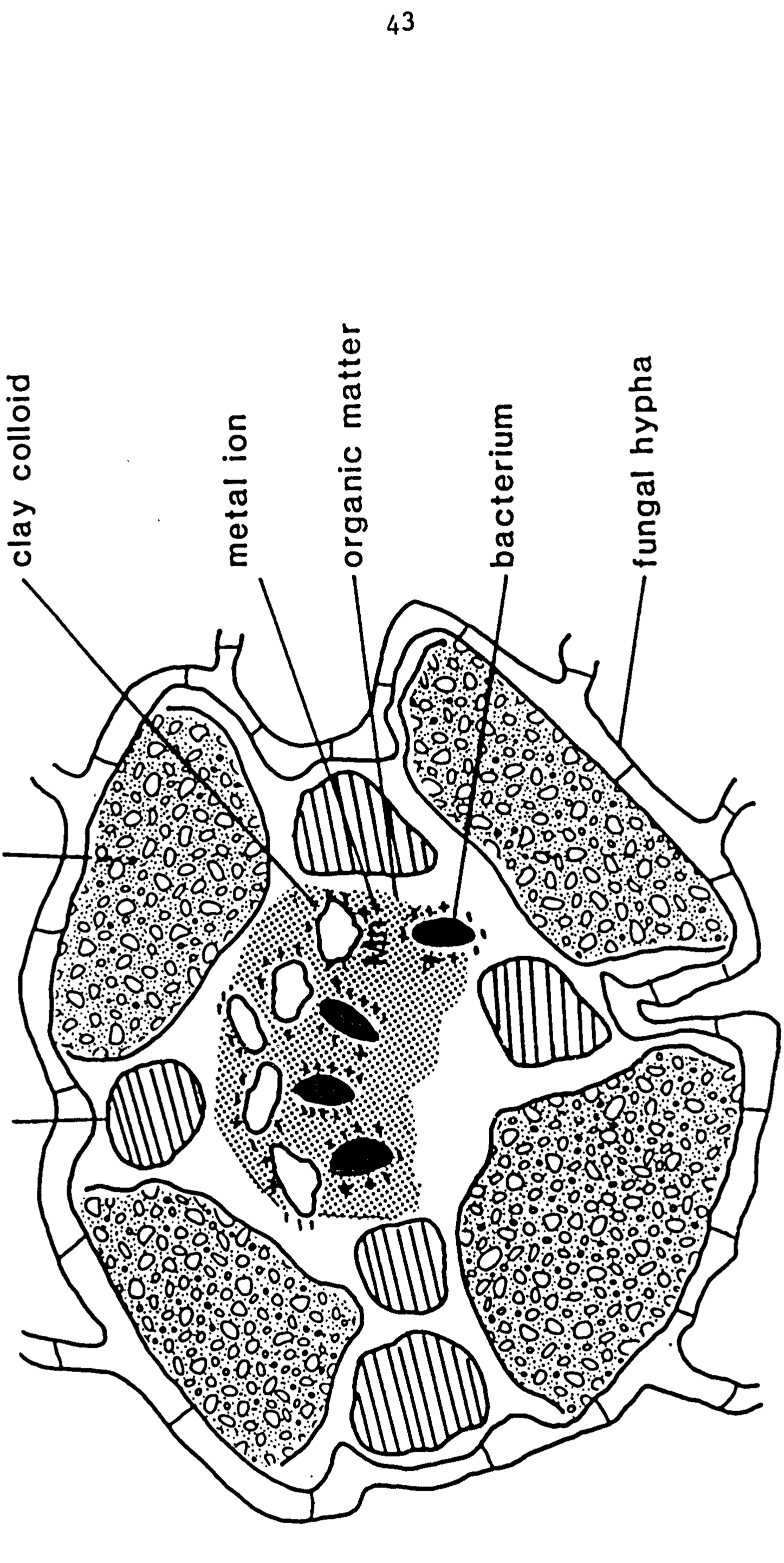


Figure 2.4 Hypothetical spatial arrangement of microorganisms in relation to soil particles (Source : Lynch, 1983)

different organisms in the soil. Many studies have shown mechanical stabilisation of the fungi to be temporary, because other organisms in the soil utilise the fungi as source of energy (Griffiths, 1965; Griffiths and Jones, 1965). Whilst this fact was noticed by Swaby (1949) little attention has been given to this aspect of microbial interactions in studies of aggregate stabilisation. Much of the information on the importance of microbial interaction has accumulated from microbial ecological studies (Potgieter and Alexander, 1966; Hsu and Lockwood, 1969; Ko and Lockwood, 1970; Griffin, 1972) and those studies aimed at measuring soil microbial mass (Gray and Williams, 1971; Parkinson et al., 1971; Jenkinson et al., 1976; Jenkinson and Ladd, 1981; Waid, 1984).

In the absence of suitable conditions for growth in the soil, fungi have a low potential for survival (Ko and Lockwood, 1967; Steiner and Lockwood, 1970), and fungal cell wall material becomes autolysed when severe nutrient depletion levels are reached in the soil, or when competition becomes stringent. Also actinomycetes and bacteria produce enzymes capable of degrading hyphal cell walls (Mitchell and Alexander, 1963; Jones and Webley, 1968). The activity of lytic enzymes (β - D - glucosidase and chitinase) have been related with susceptibility of hyphae to lysis (Ko and Lockwood, 1970). Addition of chitin to the soil has been shown to stimulate actinomycetes and to suppress development of fungi. Mitchell (1963) and Jones and Webbley (1968) have shown that cell wall material of fungi stimulates streptomycetal growth. Chitin is a constituent of most fungal cell walls (Foster and Webber, 1960) and many studies have shown that chitin in fungal cell walls is the

main source of energy for actinomycetes (Williams, and Robinson, 1981; Goodfellow and Williams, 1983). Chitinase has therefore been used as an index of fungal growth (Ride and Drysdale, 1972). This process of degradation of fungal mycelia by other organisms is an important process in aggregate stabilisation, because whilst mechanical stabilisation is lost, other mechanisms of aggregate stabilisation such as production of gummy substances are promoted.

Increased interest in the role of microorganisms in soil structural stabilisation and nutrient and energy flow relationships in natural environments emphasises the need for simple and objective methods for studying microbial populations in soils (Gray and Williams, 1971; Parkinson et al., 1971; Jenkinson and Ladd, 1981; Waid, 1984). Qualitative studies employing observational techniques such as electron microscopes (Gray, 1967; Foster and Rovira, 1973; Foster, 1979; Tisdall and Oades, 1979) and staining techniques (Jones and Mollinson, 1948; Babuik and Paul, 1970; Frankland, 1974; Jenkinson et al., 1976) have improved the knowledge of the diverse morphological and biochemical composition of soil microorganisms.

Despite such notable advances in qualitative methods, the problem of quantitative analysis remains. Quantitative studies are complicated because microbial cells are commonly attached to surfaces where they live. This problem has been eased by development of indirect methods of estimation of microbial population (Anderson and Domsch, 1973, 1978). Physiological approaches involving rates of consumption or production of chemical substances have commonly been used (Jenkinson et al., 1976; Adu and Oades, 1978; Jenkinson and Oades, 1979; Tate and Jenkinson, 1982).

Other approaches to estimation of microbial population involves measurement of biochemical components of microbial cells such as chitin (Ride and Drysdale, 1972, Wu and Stahman, 1975). Chitin, a polymer of N-acetyl glucosamine is a structural constituent of most fungal cell walls and occurs in complexes with protein (Foster and Webber, 1960). Calorimetric methods of estimation of N-glucosamine released from chitin breakdown by KOH are available. However, utilisation of chitin as an index of fungal growth in soils has several disadvantages. Chitin content of most fungal hyphae vary according to species and conditions of growth (Foster and Webber, 1960; Swift, 1973). Chitin occurs in complexes with protein and long assay periods are required in analysis and estimation. Soils contain chitinous insect exoskeletons, which could interfere with fungal chitin analysis.

An Ergosterol measurement technique to quantify fungal growth has been developed by Seitz and co-workers at the US Grain Marketing Research Laboratory (Seitz et al., 1977, 1979). The technique was developed to monitor fungal invasion in stored grains, and has since not been tested in monitoring fungal growth and evolution in other materials. An attempt was made in the present study to assess suitability of this technique to monitor fungal growth in soils.

2.4.4. Aggregate Stabilisation by Products of Microbial Synthesis.

When organic matter from plants and animals is attacked by microorganisms the decomposition products, together with secretion from living organisms provide materials capable of cementing soil elementary

particles together. Figure 2.4 has shown how the microbial cells are held to soil particles, thus they are well positioned for the metabolic products which they release during their digestion of organic substrates to interact with the soil colloidal adsorbents. Final products of microbial decomposition are polymeric compounds such as humic substances and polysaccharides which markedly influence soil structure (Hayes and Swift, 1978). There has been widespread acceptance for a long time of the role of humic materials in stabilising soil aggregates, although the problem of understanding their mechanisms of stabilisation remains (Kononova, 1961; Theng, 1979)

Approximately 5 to 16% of the soil organic matter is in the form of carbohydrates (Gupta et al., 1963). Microorganisms play an important role in the carbohydrate cycle. Bacteria, actinomycetes and fungi are chiefly responsible for decomposing plant and animal remains. The microorganisms synthesise polysaccharides and other carbohydrates in the cells, frequently as major metabolic products. A large proportion of soil polysaccharides can only be recovered after harsh treatment of the soil with chemicals (Cheshire, 1979). Therefore great difficulty is experienced in isolating them in the exact form that they exist in the soil (Tinsley and Salem, 1961; Gupta, 1967; Swincer et al., 1968; Greenland and Oades, 1975). These problems of defining and measuring their relevant properties have given rise to difficulties of assessing their role in formation and stabilisation of soil aggregates. More comprehensive accounts of the amount and nature of polysaccharides in soil, together with their isolation and identification, have been given by Cheshire (1979).

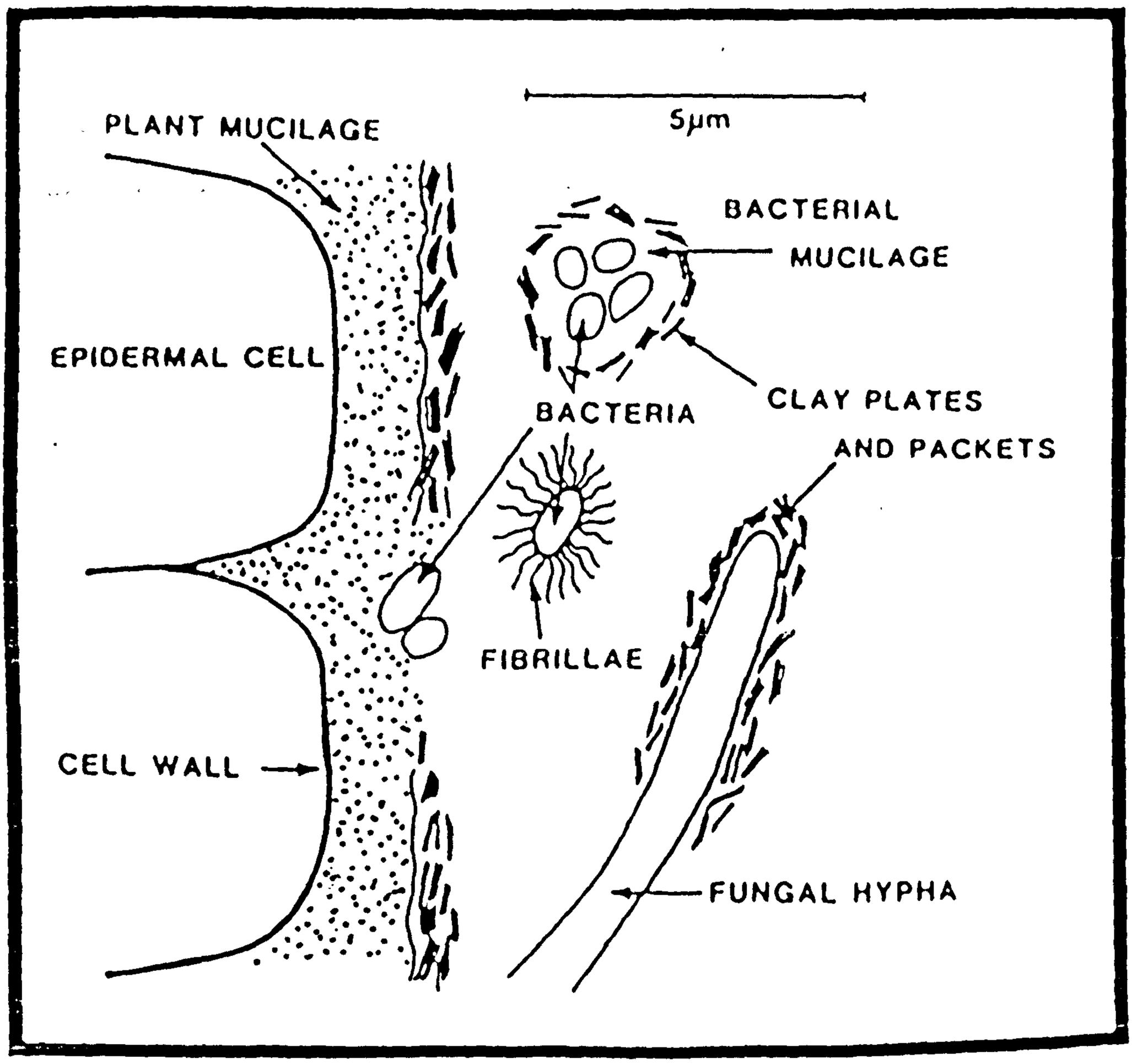


Figure 2.5 Interaction of clay with mucilages (Source: Oades, 1984)

Polysaccharides involved in soil aggregation are mucilagenous substances of microbial and plant origin (Rennie et al., 1954; Chesters et al., 1957; Acton et al., 1963). Several studies have been undertaken towards understanding their mechanisms of aggregate stabilisation. Aspiras et al. (1971) and Oades (1984) suggest that soil particles adhere to these mucilagenous substances as in Figure 2.5. Greenland (1963, 1971) suggested that stabilisation by polymers involves formation of bonds (bridges) between soil particles or group of particles making up an aggregate. Having linear, flexible chain structures, polysaccharides are well suited to form a large number of interparticle bonds giving rise to an overall stable association, although the individual bonds may themselves be relatively weak. Adsorption of anionic polymers by clay minerals can occur through cationic bridge linkages (Greenland 1965). This involvement of polyvalent cations has been evidenced by destruction of aggregate stability when soils were treated with periodate and complexing reagents such as pyrophosphate (Stefanson, 1971; Hamblin and Greenland, 1977). Formation of organo-mineral complexes and their role in soil aggregate stabilisation have been reviewed several times (Edwards and Bremner, 1967; Mortland, 1970; Hamblin, 1977; Turchenek and Oades, 1978; Lowe, 1978; Tisdall and Oades, 1982).

The role of polysaccharides in soil aggregate stabilisation is usually based upon the following observations:

a) When polysaccharides are added to the soil there is an increase in the amount and stability of the aggregates (Martin, 1945, 1946; Rennie et al., 1954).

b) There are many organisms in the soil capable of producing polysaccharides during the process of metabolising energy sources (Lynch, 1983b).

c) Polysaccharides can be extracted from the soil. Estimates have been made to indicate that 5 to 16 percent of soil organic matter consists of plant or microbial polysaccharides (Cheshire, 1979).

d) There is statistical correlation between aggregation and polysaccharide content of soil (Cheshire et al., 1984).

e) When polysaccharides are removed from the soil by chemical treatment there is a decrease in aggregation (Cheshire et al., 1983).

Substantial work has been carried out following the work of Martin and co-workers on the role of polysaccharides in soil aggregate stabilisation. Most workers applied additive procedures to evaluate their role in stabilisation. Additive procedures employ the innoculation of soil with organisms capable of producing polysaccharides or addition of polysaccharides obtained from cultures of microorganisms in the soil. Subtractive procedures on the other hand involve the use of chemical treatments known to destroy polysaccharides such as periodate (Bobbit, 1958). A measure of their importance is indicated by the resulting stability when this is compared with untreated soil. This procedure has found most application in the study of the role of polysaccharides in soil structure.

Periodate oxidation is one of the most widely used procedures for the extraction of polysaccharide fraction of organic matter from the soil. Oxidation with periodate results in 1, 2 glycol scission and has

been widely used in carbohydrate chemistry (Bobbit, 1958). Bobbit (1958) pointed out that the selectivity of the periodate reaction is in that periodate oxidations proceed quickly and quantitatively in a homogeneous reaction mixture. Thus aliquots of the reaction mixture can be removed periodically during the reaction and analysed for periodate consumption, and if this is plotted against time, the rate curve should display a constant maximum at 48 hours reaction time in a homogeneous reaction. However, in heterogeneous material, such as soil, side reactions will offset the levelling off at maximum and the oxidation may proceed for long periods (Cheshire et al., 1983, 1984).

Mehta et al. (1960) applied the principle of periodate oxidation to soil systems maintaining that due to the specifity of the reaction, soil polysaccharides would be preferentially degraded with dilute solutions. They found that artificial aggregates stabilised with a variety of polysaccharides were degraded when subjected to oxidation with 0.01 M sodium periodate followed by extraction with sodium tetraborate. This treatment did not degrade the stability of field aggregates, and therefore Mehta et al. (1960) concluded that polysaccharides were not important in the stabilisation of field aggregates.

The ability of soil aggregates to withstand periodate oxidation was found by Greenland et al. (1961, 1962) to depend on the history of the site as well as the depth from which the samples were taken. The stability of the soils was determined by a modified permeability technique of Dettman and Emerson (1959) prior to and after treatment with periodate and borate. Aggregates from soils which have been under

pasture for many years were only slightly affected. Those from non-calcareous soils which have been continuously cropped or under pasture for relatively short periods were destroyed by this treatment, as did also subsurface samples from all sites. Greenland et al.(1962) also observed that the response to periodate treatment differed with soil type. The treatment reduced the stability of solonized brown soil aggregates less than that of red brown earth, while rendzina aggregates were completely destroyed. They suggested that the resistance of the brown earth might have resulted from inhibition of periodate cleavage by the presence of $CaCO_3$ in this soil.

Clapp and Emerson (1965 a,b) re-examined the qualitative and quantitative properties of the periodate oxidation method in combination with sodium-saturation-dispersion procedures similar to the one used by Greenland et al. (1962). The stability of crumbs from pasture soils was reduced by periodate followed by borate treatment. This treatment gave complete dispersion of aggregates from cultivated plots. All the aggregates were destroyed by pyrophosphate treatment. Crumbs from wooded sites showed extreme resistance to periodate-borate treatment. The inhibition of the periodate oxidation on samples from Houston black clay was found to result from the presence of $CaCO_3$ in these aggregates, and this explains the resistance of the Swiss braunerde and forest soils dealt with in the work of Mehta et al. (1960) which also contain $CaCO_3$. Replacement of the Ca from the clay results in double layer swelling (Rimmer and Greenland, 1976) which would result in rapid diffusion of periodate into the crumbs.

In the work of Stefanson (1968, 1971) a combination of periodate and borate reduced the relative stability of aggregates taken from all pasture and arable plots more than did the individual treatments on similar group of aggregates. The results obtained by Stefanson showed that with increasing years of pasture, the periodate-sensitive materials were less important as stabilisers, and this well agrees with the previous findings by Greenland et al. (1962) and Hamblin and Greenland (1977). Pyrophosphate treatment used to remove inhibition of periodate action by the presence of $CaCO_3$ resulted in total loss of stability of all soils. Although it has previously been suggested by Greenland et al. (1961) and shown by Bond and Harris (1964) that other mechanisms such as fungal entanglement may result in resistance to periodate treatment, treatments employed by Stefanson (1971) did not reveal these mechanisms. Relative increase in stability during the summer has been attributed to increased microbial activity (Stefanson, 1968) however the seasonal trend in stability established from various crop-pasture rotations was not visible after periodate-pyrophosphate treatments.

The most recent detailed work on periodate extraction of soil polysaccharides has been carried out by Cheshire et al. (1983). Their work was initiated to re-evaluate the role of polysaccharide in soil aggregate stabilisation, to extract and quantify the amount of polysaccharide in soils, and relate it to stability and to characterise the soil polysaccharide fraction. The chemical treatment employed in their study was similar to that of Clapp and Emerson (1965). The difference was in the use of micro-aggregates (thus lessening excess

consumption) and the length of time. The amount of polysaccharide material extracted was measured by acid hydrolysis (Cheshire, 1979). Soil from a field which had been under grass for a long period was treated with 0.02 N $NaIO_4$ for up to 1176 hours, and this was followed by 0.1 M $Na_2B_4O_7$ for 6 hours. Treatment for 6 hours with $NaIO_4$ left about 70% of the sugars in the residue, and 45 hours reduced the sugars to 45%. There was a linear relationship between residual sugars and aggregate stability (determined by turbidimetry). Increasing the concentration of $NaIO_4$ to 0.05 M, and treating the soil for 6 hours reduced sugar content of the residue to 50% and to 25% after 48 hours.

In subsequent studies using 15 soils, Cheshire et al. (1984) examined the relationship between water stability of micro-aggregates and the residual carbohydrate content. The effect of periodate oxidation on soil carbohydrate was found to continue throughout the duration of the treatment and was accompanied by a progressive disruption of soil structure. From the highly significant relationship they found between residual polysaccharide in the soil and the residual stability, Cheshire et al. (1984) suggested that loss of stability would proceed until the polysaccharide content dropped to zero. They found the overall mean loss of stability was 79% when all the polysaccharide was removed from the soil. Additional treatment of the soil with pyrophosphate did not have much effect. When Countesswell series soil which had been oxidised with periodate for 168 hours was treated with 0.1 M pyrophosphate for 24 hours they found only a small increase in aggregate disruption (74 - 77%) and the carbohydrate decreased slightly from 6 to 4.5 mg g^{-1}.

The above discussions point to the fact that the role of microbial polysaccharides in the formation of stable aggregates has been the subject of investigations extending over many years. Despite this, there are still many objections to the polysaccharide theory of soil aggregation. Relationships between polysaccharides and aggregation are certainly more complex than envisaged and in certain soils (e.g. old grassland and forest soils) they do not appear to play a significant role (Greenland et al., 1962; Griffiths and Burns, 1972). Where polysaccharides exert an influence on soil aggregation, as for example in young grass leys (Greenland et al., 1961) the effects are ephemeral. This accords well with the ease with which polysaccharides can be degraded by microorganisms (McCalla, 1946; Harris et al., 1963; Martin, 1971). Much information can be gained by assessing the role of polysaccharides along the lines of Cheshire et al. (1983, 1984), whilst simultaneously taking account of processes which alter their composition and contribution from time to time such as physical manipulation of the soil, the effects of weathering cycles and the interaction of the different microorganisms in the soil environment.

2.5. Thixotropic Hardening Process in Soils

2.5.1. Soil Aggregation and Clay Content

Study and general observations have long shown that clay content is one of the major factors in soil aggregation (Sideri, 1936). However, there are still conflicting ideas on the role and significance of clay in stabilisation of soil aggregates (Kemper and Koch, 1966; Grieve, 1979b;

Chaney and Swift, 1984). Martin et al. (1955) proposed that clay is the predominant binding agent in soil aggregation and that organic materials do not act primarily to hold clay, silt and sand grains together, but rather their chief role may be to modify forces by which clay particles are attracted to one another.

The structure of the clays (Buckman and Brady, 1969; Smart and Tovey, 1982) gives rise to electrical charges. These are negative on the sheet surfaces but can consist of both positive and negative charges at the edges. Thus clay has an affinity for cations and anions. There are also attraction and repulsion forces between clay particles. Clay particles may flocculate and stick together by means of the attraction forces between edge and one face and these take place according to the hypothesis put forward by Emerson (1959) and illustrated in Figure 2.1. Cohesive forces between clay particles may further involve: (i) linkage by chains of water dipoles (ii) bridging between clay particles by long-chain organic molecules and (iii) cross-bridging and sharing of intercrystalline ionic forces and interaction of exchangeable cations between oriented clay particles. Clay forms a continuous network in most soils that enmeshes and binds silt and sand grains together. The exchangeable ions and charges on the surface of the clay particles interact with water molecules between the surfaces; the binding force increases as the neighbouring clay particles assume preferred orientations. Therefore any process conducive to producing preferred orientation helps to increase aggregate stability. One such process is the swelling and shrinkage action of the 2:1 clay minerals, which have an expanding lattice. This movement of particles greatly improves the

chances of getting the necessary orientation for the formation of clay-organic complexes.

McHenry and Russell (1943) studied the effects of clay content on aggregate stability by examining a series of samples prepared with sand and clay in varying percentages. Their results shown in Figure 2.6 indicate that a positive correlation exists between clay content and aggregate stability. Without smoothing the curve, discontinuity resulted at 30% clay content. They attributed this discontinuity to the change in dominance of two distinct processes involved. They hypothesised that this discontinuity represents the region where clay particles having established themselves at all possible contact points on the sand particles, find no centre for orientation and hence agglomerate amongst themselves with resultant aggregates composed of clay micelles, rather than the more desirable sand-clay aggregates. They further showed that as the amount of silt increases, the stability increases with higher increases in clay content because silt increases the number of large orientation centres. The clay agglomeration does not occur in silt-clay mixtures as the large number of silt particles provides large centres for orientation of the clay micelles.

Relations between clay and aggregate stability have been examined by Kemper and Koch (1966) for 500 soils from the western USA and Canada. They concluded that the stability due to clay arises internally from bonds between clay plate domains and other particles. However sodium in the exchange complex can make the clay unstable. Also if the clays are arranged in an open structure they may collapse under shear, causing landslides in sensitive clays (Mitchell, 1960).

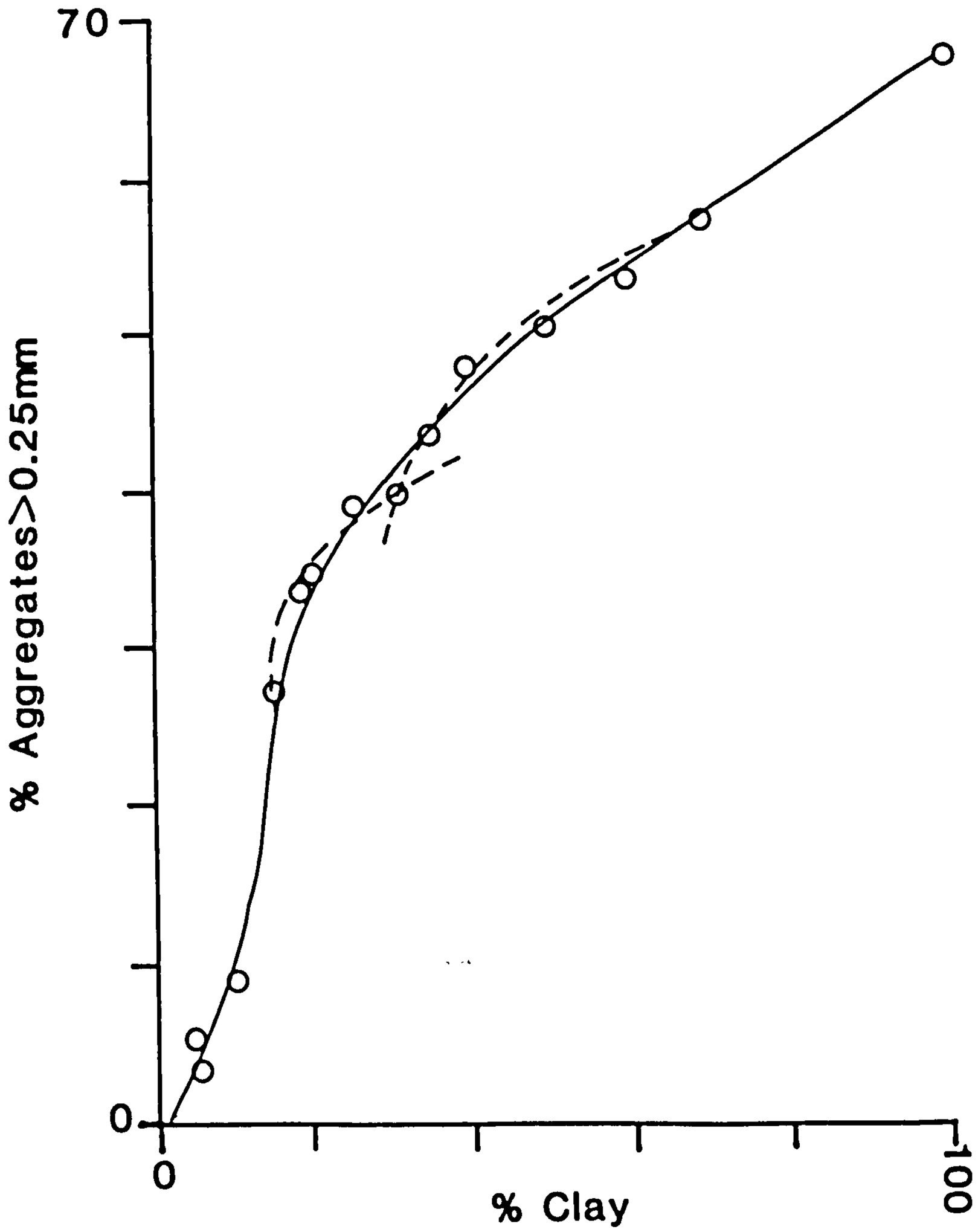


Figure 2.6 The influence of clay content on aggregation of Bentonite-sand mixtures (Source: McHenry and Russell, 1943)

Baver et al. (1972) observed a high degree of correlation between the amount of clay in soils and the percentage of aggregates larger than 0.05 mm. They suggested that the cementation effects of clay were more pronounced with smaller aggregates. Another important aspect noted was that there was a higher correlation between clay content and aggregation as the amount of organic matter decreased. Recently studies by Shanmuganathan and Oades (1982), showed that the amount of dispersible clay in soils controls various physical and mechanical properties of soils such as swelling, porosity, water retention capacity, friability and modulus of rupture. Shanmuganathan and Oades (1982) observed that maximum compaction occurred when water content at moulding was near the plastic limit. They concluded that at this water content shearing forces caused particle re-orientation, increasing the contact between particles and reducing porosity.

The above discussion indicates the awareness of the role of clay by researchers working with agricultural soils. However, the mechanisms involved in aggregation by clay are not yet fully understood by agriculturalists. Cultivation or remoulding of these agricultural soils usually results in a loss of their strength and this has been attributed to the breakdown of connecting links and cementing bonds between soil particles (Koenigs, 1963). Aggregation of soil particles results in discrete domains of the order of few millimetres which are of agricultural significance. Thus the stability of the resulting aggregates at any given time is the result of opposing constructive and destructive influences that proceed more or less simultaneously and continuously (Blake and Gilman, 1970). The activity and behaviour of

clay in clay-water systems in influencing the soil structure has been observed by civil engineers, and the process was termed 'Thixotropy'.

2.5.2. Thixotropic Behaviour of Soils

Freundlich (1935) used the term 'thixotropy' to describe isothermal gel-sol-gel transformations in colloidal suspensions induced by shearing and subsequent rest. Skempton and Northey (1952) studied thixotropic effects in remoulded natural clays. Their study was aimed at determining the extent to which thixotropic hardening could contribute to the sensitivity of the clays. When remoulded soil was allowed to rest, at constant water content, it regained part or all of its strength. They proposed the term 'part thixotropy' for the partial regain in strength and 'pure thixotropy' for the total strength regain.

Mitchell (1960) defined thixotropy as an isothermal, reversible, time dependent process occurring under conditions of constant composition and volume whereby a material stiffens while at rest, and softens upon remoulding. He further suggested that the change in the energy status resulting from particle re-orientation during remoulding may also be attributed or related to thixotropic processes. When a thixotropic soil is remoulded or compacted, a part of the shearing energy is used to disperse the platy clay particles into uniform parallel arrangement (Figure 2.7). Upon remoulding the externally applied stresses assist the repulsive force between particles in producing a dispersed system. At stage (a) the system has a lower strength, as the particles easily slide past one another. Moulding thus results in the breaking of cementing and

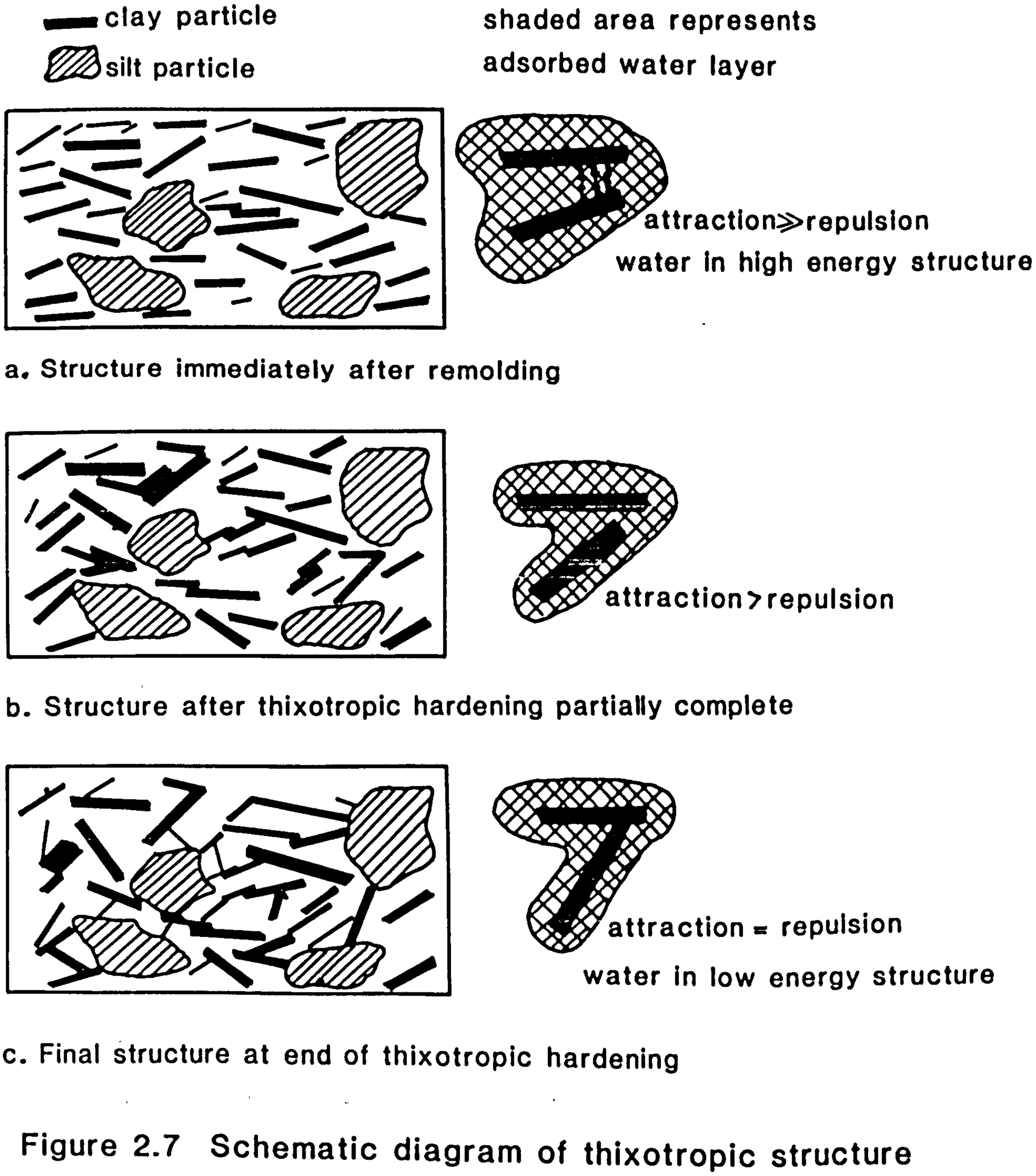


Figure 2.7 Schematic diagram of thixotropic structure change in a fine grained soil (Source: Mitchell, 1960)

linking bonds such as those resulting from hydration of ions, osmotic swelling, the edge to plate attraction bonds and the bonds of organic matter (Koenigs , 1963).

As the shearing stops, the structure attempts to adjust itself into a lower free energy following the trend proposed by Skempton and Northey (1952), i.e. there is a partial regain in strength as at (b) and subsequently a total regain in strength (c). Through these stages the net repulsive forces become progressively smaller, whereas the attractive forces are building up. At completion of the process the attractive forces exceed the repulsive forces.

Mitchell (1960) based his hypothesis of thixotropy in soils on the assumption that the internal energy and stress conditions in a thixotropic soil immediately after remoulding are not at equilibrium. Thus the soil undergoes spontaneous structural changes during resting period, as shown by Day (1955). The hypothesis applies to soils in which thixotropy is primarily a structural effect i.e. property changes with time result from changes in factors such as particle re-arrangement, adsorbed water structure and distribution of ions in the fluid phase.

The ease with which structural change may be accomplished differs from soil to soil, and in direct response to soil composition, and the environmental conditions at the time of formation. The water content also plays an important role during remoulding and subsequently during aging.

Based on these observations, Mitchell (1960) postulated that a soil will exhibit thixotropic behaviour if the following conditions are met:

(i) The net interparticle force balance is such that the soil will flocculate if given the chance.

(ii) The flocculation tendency is not strong enough, to prevent dispersion by mechanical action such as shearing or stirring the soil.

2.5.3. Thixotropic Strength Changes in Soils.

The mechanical strength of soil is important from both the standpoint of seedling emergence and root growth, and soil tillage. It influences the energy consumption in cultivations, the intensity of compaction caused by trafficking and the efficiency of cultivations (Utomo and Dexter, 1981b; O'Sullivan and Ball, 1982). Strength is imparted to the soil by cohesive forces between particles and by frictional resistance met by particles that are forced to slide over one another, or move away from interlocked positions during moulding (Baver et al., 1972; Marshall and Holmes, 1979). Shear strength, S, of a soil is the maximum internal resistance of the soil to the movement of its particles and is expressed by Coulomb's Law:

$$S = C + \tan \phi \, P$$

where C is the cohesion, P is the effective pressure normal to the shear plane, and tan ϕ is the coefficient of friction, where ϕ is the angle of friction. The frictional component is usually affected by physical factors such as the sliding of particles over one another and the

interlocking of soil particles (Cashen, 1966). The magnitude of the cohesive component is mainly affected by water content as the latter controls the distance between particles and the attractive forces associated with them (Koenigs , 1963). The application of compressive forces to the soil mass will increase cohesion by bringing about particle orientation thereby decreasing the spacing between particles and affecting both the attractive and repulsive forces.

Soil can deform by shear, compression or a combination of the two. Shear deformation is a process traditionally studied in soil mechanics because it has overriding influences on the stability of civil engineering structures such as foundations and slopes. Failure by compression is however characteristic of agricultural top soils, and has usually been referred to as compaction.

Many techniques and varieties of instrumentation have evolved for the characterisation of soil strength. Strength measurements have been used for characterisation of soil crusts (Hegarty and Royle, 1978; Page and Hole, 1977; Page, 1979); compaction of top agricultural soils resulting from heavy machinery, (Braunack and Dexter, 1978; Pidgeon and Soane, 1978); and strength and crop emergence relationships, (Ball and O'Sullivan, 1982). Strength changes observed and measured on agricultural top soils have,however, seldom been related to or explained in terms of thixotropic behaviour as has been done for civil engineering structures.

Unconfined compressive strength measurements of samples moulded and stored in tight containers to eliminate water loss were made by Moretto (1948). Increases of 445% in unconfined compressive strength were reported due to aging of Laurentian clay and lesser increases were reported for several other soils. Moisture changes of 1% were considered insignificant and the increases in strength were believed to be due to slow physico-chemical reactions which took place during the aging period.

Seed and Chan (1957) showed that compacted clays may exhibit appreciable thixotropic strength gain with time. Normal triaxial compression tests were carried out on unconsolidated specimens after different periods of storage at constant water contents between plastic and liquid limits. They found that for the majority of natural clay deposits remoulding caused a pronounced reduction in strength even though the composition of the soil was unchanged. Seed and Chan, used the term 'sensitivity' to express these changes:-

$$\text{Sensitivity} = \frac{\text{Strength of undisturbed material}}{\text{Strength of remoulded material}}$$

The use of sensitivity factors as index of thixotropy allows for comparisons of soils with different composition. Descriptive terms in Table 2.2 have been used to classify clays with different sensitivities:

Table 2.2 Classification of sensitivity values.(Source:Seed and Chan, 1957).

Sensitivity	Type of clay
1-0	insensitive
1-2	low sensitivity
2-4	medium sensitivity
4-8	sensitive
8-16	extra sensitive
16	quick clays

Seed and Chan (1957) also showed that the method of compaction has a pronounced effect on the properties of soil compacted wet of the optimum, especially for those soils very susceptible to structural change during shear.

Mitchell (1960) and subsequently (1976) using strength measurement tests on remoulded soils, investigated the reversibility of thixotropy, the effects of initial structure on thixotropic behaviour of compacted clays and the methods of compaction. The results of thixotropic hardening process are summarized in Figure 2.8. In its undisturbed state the material has shear strength, stability or matric water potential value C. When tested at the same rate of shear immediately after remoulding, shear strength is reduced to a value C_r. If the material is then allowed to remain under constant external conditions without any change in composition, the strength C_f will gradually increase and after sufficient length of time the original strength C will be regained. The aged strength of compacted clay at any

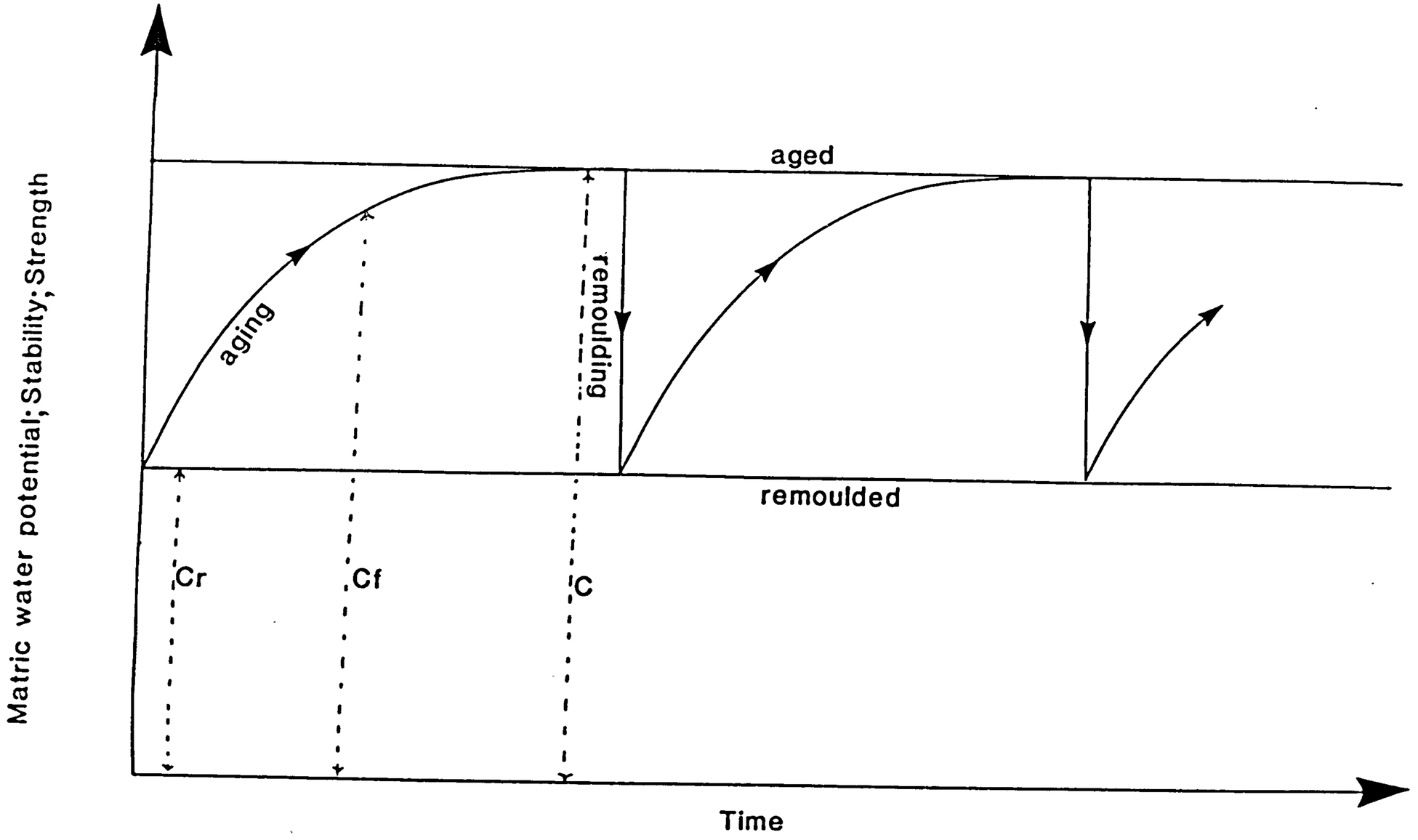


Figure 2.8 Properties of a thixotropic soil (Source: Mitchell, 1960)

time during aging depends on the water content at which the strength was measured.

Considering the effects of initial structure on the thixotropic behaviour of compacted clays, Mitchell (1960) hypothesised that if thixotropic changes are caused by dispersion-flocculation phenomena, it would be expected that the relation of the structure of soil immediately after compaction to the structure that would exist if all particles were free to choose their own positions would influence the magnitude of thixotropic property changes. If structure is initially strongly flocculated, and resistant to imposed external strain, then negligible thixotropic effect would result since this structure is one of equilibrium. If however, the structure and inter-particle forces are such that the equilibrium is changed by externally applied strain, then the new structure will seek equilibrium through flocculation during the aging, thus showing thixotropic behaviour. The results with Vicksburg silty clay showed that thixotropic effects are maximum at intermediate water contents and low or negligible at low and high water contents. This was found to be so because when this soil was compacted dry of optimum it exhibited a flocculated structure, and when at higher moisture contents than the optimum the structure dispersed when subjected to large shear strain and thus responded to the method of compaction. Non-shear straining methods, such as static compaction, however, did not disperse the soil. At high moisture contents the material disperses of its own accord due to double layer repulsion. At low contents the material is strongly flocculated due to double layer water deficiency (Kemper and Rosenau, 1984).

Mitchell's data clarified the role of water content in thixotropic behaviour. Water content is primarily important in determining the status of the inter-particle forces. Skempton and Northey (1952) indicated that for five different clays aged for 100 days, three showed an increase in thixotropy with increasing water content and two passed through maximum at or near the liquid limit and then dropped off with further water content increase.

Utomo and Dexter (1981c) translated these findings of civil engineers (working with purely clay systems) to agricultural soils, to explain the strength changes taking place following cultivation of these soils. By working at moisture contents between plastic and liquid limit, strength increase during aging of untreated, sterilised and soil pre-treated to remove organic matter was characterised by penetrometer resistance, tensile strength and compression resistance measurements. Aging of the remoulded Urrbrae fine sandy loam, Strathalbyn sandy loam and Waco clay, resulted in increased resistance to probe penetration. This change in strength was also observed in oxidised and sterilised samples. It was observed that in oxidised soils there was an initial increase in strength and a subsequent large increase during longer aging time periods. They proposed that the initial increase should be attributed to truly thixotropic process, and the second increase resulted from inter-particle cementation, especially in the absence of organic matter. These increases in strength resulting from aging also resulted in an increase in crushing and compression resistance of the aggregates.

2.5.4 Effects of Aging on Water Potential Changes

Soil matric water potential is governed by pore size distribution (Marshall and Holmes, 1979; Hillel, 1982). Any change in pore water stress during shear or with time reflects a change in the free energy of the system as well as the changes in the effective stress. Changes in energy in turn reflect changes in particle arrangement, water structure and electrolyte distribution within the system (Mitchell, 1960).

Several studies have shown that there is a decrease in pore pressure, or increase in pore water tension with time after compaction or remoulding. Day (1955) studied the effect of shear on the forces of cohesion between clay particles through measurement of the intensity of water absorptive forces. A measure of these forces was obtained by determination of the soil moisture tension using a simple tensiometer. Saturated clay samples at water content near plastic limit were allowed to equilibrate with the tensiometer, the suction was noted and then the sample was stirred with a motor driven mixer. The results indicated that tension falls to a minimum value after stirring and then gradually returns to nearly the initial value, and the process is repeatable. Although Day (1955) did not make any strength change measurements during aging, he observed that the stirring action produced a decrease in viscosity and that the gel slowly returned to its original state as tension was restored. This observation is in agreement with subsequent studies by Seed and Chan (1957, 1959); Mitchell (1960, 1976) and Utomo and Dexter (1981c). The phenomena of decreased tension or increased pore pressure occurred in a variety of different clays, thus Day (1955) concluded that this phenomena is of quite general occurrence.

Seed and Chan (1959) studied the significance of pore water pressure as one of the factors affecting strength characteristics of the soils during aging. Their tests were aimed at determining whether the difference resistances to deformation by specimens having different structures are due primarily to differences in pore-water pressures development in the samples or to some inherent characteristics of the soil structure itself.

They demonstrated the stress-deformation relationship and pore water pressures developed during undrained tests on samples of silty clay having the same density and water content, but different structures. The results also showed that during the early stages of the test the pore-water pressures in samples compacted wet of optimum (dispersed structure) are considerably greater than those in the samples compacted dry of optimum (flocculated structure). This characteristic influence of the initial structure on pore water pressure changes observed by Seed and Chan (1959) was consistent with their observations of strength changes during aging (Seed and Chan, 1957). Mitchell (1960) measured pore pressure in order to investigate its changes with time and to ascertain whether or not sufficient structural change took place during aging to be reflected in pore water pressure during shear between aged and freshly prepared samples. Samples were compacted by kneading compaction at 21.7% water content, wrapped in rubber membrane and aged for 37 days. At each testing time, some samples were tested without disturbance, whilst others were remoulded and recompacted to the same density and then tested.

The results obtained showed that the initially aged samples had lower pore pressure than the freshly re-compacted ones. He attributed these differences to the concept of development of more flocculated particle orientation and more ordered water structure with time in a thixotropic soil as shown in Figure 2.7 and these results are in agreement with those of Seed and Chan (1959).

Cashen (1966) studied the effects of shear and aging on matric water potential in thixotropic and dilatent materials. He observed that the matric potential of water in gels of five-sixths neutralised aluminium montmorillonite, and aluminium kaolinite changed during periods of shear and periods of rest. Shear increased the potential of water in bentonite, and kaolinite gels, but decreased the potential in kaolinite compacted wet of optimum. It was observed that in dilatent pastes of silt size particles, in which the value of electrical charges are small, the pressure deficiency can reach large values after an increase in pore space. It was also noted that there was an initial increase in suction to a maximum and subsequent decrease. This fall in suction after attainment of the maximum, was attributed to the fact that a random structure was first formed, but that swollen units or domains later contract, with subsequent decrease in suction. The reversibility of the process was also observed in all tests.

Schweikle et al. (1974) investigated changes in matric water potential on remoulded aggregates, which were obtained by remoulding puddled soil to pass a 0.3 mm sieve. Samples were treated with H_2O_2 to destroy organic matter, or sterilised by heating to 350^{o}C. These

treatments were imposed in order to separate the non-biological activities which contributed to age hardening process. Soil matric water potential increased with time when the pulverised soils were moistened with deionised water. The reversibility of the process was examined, using soils previously sterilised and those treated with H_2O_2. Although the soils used here were not pure clays as used previously by civil engineers, the results of the work of Schweikle et al. (1974) agreed well with the findings of Cashen (1966); Seed and Chan (1957); and Mitchell (1960).

Similar tension measurements were carried out by Utomo and Dexter (1981c) using remoulded agricultural soils to investigate the occurrence of thixotropic changes in these soils. Samples of the Urrbrae (fine sandy loam) and Waco (self-mulching montmorillonite black earth) were remoulded at 20% and 65% water contents respectively compacted, and aged at constant temperature in closed containers. Their results showed that aging resulted in a more negative value of matric potential and that when these samples were remoulded the matric water potential became less negative, thus the process observed was repeated under constant volume, moisture and temperature conditions, and satisfied requirements of thixotropy outlined by Mitchell (1960).

2.5.5. Aggregate Stability Changes With Aging

Blake and Gilman (1970) were the first to investigate the changes in aggregate stability of artificial aggregates during aging. Their investigation was carried out using a clay and a loam soil.

The artificial aggregates were obtained by pulverisation of field aggregates and remoulding them in a mortar mixer. Aggregates 3 mm diameter were obtained from remoulded soil and aged in jars held at ±0.5°C, to inhibit biological activity during aging. The aggregate stability, determined by wet sieving, was shown to increase during aging. This increase in stability occurred at constant moisture and temperature, and in the absence of biological activities, therefore was assumed to result from thixotropic hardening processes. They noted that organic matter could also participate in some of the physical processes of aggregate stabilisation.

Aggregate stability regain in moist soil following tillage have been observed by Utomo and Dexter (1981b) and Kemper and Rosenau (1984). The latter workers observed that stability regain was slow in dry soil and continued over longer periods of time. With increase in water content, however, the changes were more rapid and the process of stability regain reached completion over shorter time periods. Whilst the changes observed by Kemper and Rosenau are similar to those of other workers, who have shown that moist soil loses strength(cohesion)upon tillage or moulding, and recovers when allowed to rest or age at intermediate moisture content (Mitchell, 1960; Utomo and Dexter, 1981b); Kemper and Rosenau (1984) did not attribute these changes to thixotropic processes.

The preceding review points to the fact that most previous research on 'thixotropic behaviour' of soils has been confined to civil engineering. As civil engineers are interested in the stabilisation of

structures such as road embankments, with clay as the most important component, their examination of thixotropic behaviour in soils was performed on 'purely' clay systems. Little work has been carried out to investigate the occurrence of thixotropy in agricultural soils. These soils are remoulded by farm implements during tillage operations. Agriculturists are not interested in purely clay systems, but work with soils of different textural classes and at moisture contents between plastic and liquid limit, at which these soils can be worked with safety. Blake and Gilman (1970) and Utomo and Dexter (1981c), provided a basis for research in this line, and their work needs substantiation with more agricultural soils from different localities and under different cropping systems.

2.6 Effects of Weathering Cycles on Aggregate Stability

Weather fluctuations under natural conditions in the form of rain, wind and frost play a major role in determining the effects of vegetation, soil organisms and cultivation on soil aggregation, aggregate stabilisation and degradation. Weather variables such as moisture and temperature directly influence the formation and destruction of soil aggregates. Soil conditions such as 'tilth' (Utomo and Dexter, 1981a; Powers and Skidmore, 1984) and biological activity (Birch, 1958; Adu and Oades, 1978) influenced by weathering cycles have been extensively studied but the resulting reports have been conflicting.

2.6.1 Alternate Wetting and Drying Cycles

Under field conditions soil in the undisturbed state or when disturbed by cultivations continually undergoes wetting by rainfall or condensation and drying by solar radiation or wind. These cycles of wetting and drying have been related to the formation of soil aggregates, and thus Russell (1957) quoted by Utomo and Dexter (1981a) wrote:

> "For making the crumbs, the operative agents are climatic, and the purpose of cultivation is to put the soil into such condition that they can act most effectively....the alternate wetting and drying effected by rain and wind prompts the formation of stable crumbs; the resulting swelling and contractions cause cracks which weaken the clods and if now they are struck by a hoe or harrow while just sufficiently moist they fall into smaller fragments which can be worked down".pp. 173-174.

In his earlier work Russell (1938) argued that wetting probably only destroys aggregates because:

(a) Aggregates may be unstable in water and thus disperse into microaggregates and primary particles.

(b) Secondary effects such as uneven swelling and entrapped air may cause aggregate break-down and

(c) Aggregates may be degraded mechanically by rainfall impact.

Drying, on the other hand, was found to effect aggregation and Russell (1938) suggested that this is due to dehydration of aggregating cements which increase the inherent stability of the aggregates. Although drying does cause aggregation, it increases the disruptive forces of water slaking when overdry samples are wetted. However, this has been found to be related to the method of wetting (Panebokke and Quirk, 1957).

These introductory remarks point to the fact that aggregates that are stable are continually produced, and at the same time degraded by weather induced processes. Thus the effects of alternate wetting and drying under natural conditions are of a complex nature, and conflicting results have been obtained by several researchers in this field.

McHenry and Russell (1943) found that water stable aggregation of mixtures of sand and bentonite clay increased with each alternate wetting and drying up to about 20 cycles and declined with subsequent cycles. It was suggested that each drying caused further orientation of water dipoles so that the water stability of the mixture increased. The subsequent decrease observed could not be explained, and McHenry and Russell (1943) suggested that other factors besides moisture changes werre probably involved. Similar results were obtained by Willis (1955).

Cycles of wetting and drying cause successive flushes in C and N (Birch, 1958, 1960; Agarwal et al., 1971). Birch (1960) found that each successive cycle causes a slightly smaller flush. The size of the flush was positively related to the humus content, the dryness of the

soil and the length of time the soil has remained dry. Wetting and drying physically disrupts some of otherwise stable organic matter, causing exposure of new surfaces for microbial attack (Rovira and Graecen, 1957). Some supporting evidence of this theory is provided by the findings of Soulides and Allison (1961) and Adu and Oades (1978).

Soulides and Allison (1961) found that after three cycles of wetting and drying there was a decrease in water stable aggregates. Although they did not relate this decline in aggregate stability to microbial activity, it is evident from their results that there was a decrease in bacterial population with repeated wetting and drying, and the decline was less in frozen and thawed samples. Soulides and Allison (1961) concluded that the increased decomposition of soil organic matter following wetting and drying is due primarily to the release of nutrients especially energy sources which could be readily oxidized by the soil flora. Whether these developing microflora were capable of utilising the substrate released upon wetting and drying and produced aggregate stabilising agents is not clear from their results.

Richardson (1976) observed that wetting and drying puddled and remoulded soil increased the stability after 3 cycles to slightly above that of non-cycled soil. This initial recovery was not altered with additional cycles of wetting and drying. Tisdall et al.(1978) studied the effects of wetting and drying in soil aggregates (2-4 mm) amended with organic substrates. Wetting and drying caused a decrease in stability of all soils, and the decline was more severe in sterilised aggregates.

Utomo and Dexter (1982) examined the effects of wetting and drying cycles on the stability of both field and artificial aggregates and as in the work of Tisdall et al. (1978) found that wetting and drying cycles resulted in a decrease in the proportion of water stable aggregates of natural non-tilled soil. However, when aggregates obtained from tilled plots and by remoulding were subjected to wetting and drying, there was an initial increase in stability (Utomo and Dexter, 1982). In remoulded aggregates the increase in stability took place even when the aggregates were wetted with a sterilising solution. This increase was attributed to thixotropic changes by Utomo and Dexter. This process did not take place in natural non-moulded tilled aggregates, which had already reached thixotropic equilibrium, therefore their stability showed a steady decline with further wetting and drying cycles.

The work of Tisdall et al. (1978) and Utomo and Dexter (1982) suggests that in non-sterile soils, microbial activity would compensate for the bonds destroyed by stresses resulting from wetting and drying. This microbial bond reformation process would be absent in sterilised soils, as shown by the larger amount of decreases in stability with additional wetting and drying cycles. However, whether such initial increases in stability observed in non-sterilised, tilled and moulded aggregates (Utomo and Dexter, 1982) would be maintained subsequently is not apparent from their results. These workers confined their work to 6 to 8 cycles of wetting and drying,. although it was earlier shown by McHenry and Russell (1943) and Willis (1955), that the initial increase in stability observed with wetting and drying followed by a decline, when additional cycles were imposed on the aggregates.

2.6.2. Effects of Freezing and Thawing

Farmers in the temperate regions have long noticed the effects of frost action upon soil tilth when clay soils are ploughed in the autumn or late winter (Bisal and Nielsen, 1967; Baver et al., 1972). Autumn ploughed clays are friable and easily tilled in the spring. The nature of the effects of frost on soil aggregation and related properties is a complex one. The action of frost differs according to the soil type, organic matter content, the condition of freezing and thawing and water content at the time of freezing (Logsdail and Webber, 1959; Williams, 1968; Baver et al., 1972; Russell, 1973; Chamberlain and Gow, 1979).

Freezing may cause either aggregation or dispersion, and the action depends mainly on the nature of water crystalisation (Williams, 1968). Slow cooling results in the formation of large ice-crystals in the tension free pore spaces. Since there is about 9 percent volume increase when water changes to ice, development of ice-crystals gives rise to high pressures within the soil lumps, and this has loosening effects. This slow ice development has also been shown to result in dehydration of the soil mass as water is pulled from the clay particles towards the ice-crystals. Aggregation during freezing therefore results from combined effects of ice-crystal pressure and dehydration. Rapid freezing on the other hand causes the formation of many small crystals (Williams, 1968) which have dispersive effects influencing structure and permeability of soils (Benoit and Bornstein, 1970; Benoit, 1973).

Conflicting reports have been made concerning the actual effect of freezing and thawing on soil aggregate stability. Slater and Hopp (1949) questioned whether frost ever promotes good structure. They noted that when clay soils are ploughed in the fall they are frequently friable and easily tilled in spring. The frost action fractures intractable lumps and clods. By the same token bonds within the aggregates are likewise destroyed. The result is that the soil while apparently in good condition because of its freedom from clods, actually has so little water stability remaining that the favourable tilth produced by land preparation is not retained throughout the growing season. They found that repeated freezing and thawing decreased water stability of aggregates especially at high moisture contents and suggested that the soil moisture content at the time of freezing, the effect of freezing and thawing at a given soil moisture and the interaction of these factors should be considered in evaluating the effect of freezing on soil structure.

Chepil (1954) assessed the effects of natural weathering processes on soil structure and its erodibility by wind, using samples collected seasonally from plots either under crop cover or partly under crop cover. Frost action tended to break down the coarse water stable aggregates and at the same time tended to consolidate the finest fraction to intermediate sizes of water stable aggregates (Silanpaä and Webber, 1961). Bisal and Nielsen (1967) studied the effects of frost action on soils of the Great Plains of Canada. Laboratory as well as field experiments were undertaken to investigate the influence of frost and moisture content at the time of freezing on the aggregate size

distribution. At low moisture levels the clay soils showed some breakdown from frost action, but at high levels the frost had consolidating effects. Frost action improved the structure of the loam under both field and laboratory conditions. The fine sandy loam was disintegrated by frost action. These complex results led them to conclude that no general statement of the effects of freezing and thawing on aggregate size can be made that is applicable to all the soils they investigated. The results mostly depended on the moisture content at the time of freezing and the methods of drying after freezing.

The differences in magnitude of freezing effect on different soils (Ceraltzi, 1956; Bisal and Nielsen, 1967) have been summarised by Williams (1968) as in Table 2.3. Williams (1968) carried out calorimetric study of the freezing of a variety of soils. This allowed for calculation of the amount of water left unfrozen in soil at various below zero temperatures, which subsequently allowed the establishment of the relationships between moisture content and suction characteristics of soils. The water left unfrozen when the soils were subjected to freezing was found to influence the effective stresses resulting upon ice formation. To study this process, Williams used samples in which one part was frozen and the other not. He determined dry density and water content of the part without ice. He observed that decrease of water content and consolidation of the ice-free part occurred, and calculated the effective stresses and suction resulting in unconfined samples. Thus when soils are frozen under natural conditions, there is migration of water from the lower horizons to the upper ones when ice is forming (Ceraltzi, 1956).

Table 2.3. Magnitude of the freezing effect for different soils. (Source: Williams, 1968).

General soil type	$p_i - p_w = \frac{2\sigma_{iw}}{r_c}$ kg cm^{-2}
Coarse sands, or coarser material only	0
Medium and fine sands, or coarse silty sands....	0-0,075
Medium silts, or mixed soils with small amounts <0,006 mm particle diameter	0,075-0,15
Largely fine silts, or silts with some clays....	0,015-0,5
Silty clays	0,5 - 2,0
Clays	>2

Where p_i = pressure of ice
p_w = pressure of water
σ_{iw} = surface tension ice-water
r_c = radius equivalent to size of largest continuous openings through soil pore system.

This table illustrates the pressure differences $p_i - p_w$ that must occur between the ice and water phases for various soil at the frost line when this is penetrating the soil. The pressure of the ice and of the water also depend on the factors such as over-burden and ground water level.

The difference $p_i - p_w$ shows the relative magnitude of the freezing effect for different soils. If there is no overburden or other pressure except atmospheric acting on the ice, then $p_i - p_w$ is equal to the negative pore water pressure at the frost line. If the pore water pressure were atmospheric then $p_i - p_w$ would be equal to the maximum heaving pressure at the frost line. Note that because the grain size composition is not related in a simple manner to the value r_c the figures given should be regarded as illustrations only. This migration has consolidating effects in lower layers due to suction whilst in the

upper layers structure may disperse upon thawing, and this mainly depends upon the inherent stability of the soil at the time of freezing. Takagi (1979) called this mechanism of freezing 'Segregation Freezing'.

The effective stress equation (Williams, 1968) after Skempton (1961) has been used to quantify the consolidation effects of ice lens formation upon freezing.

$$\sigma^1 = \sigma - u$$

Where σ^1 = effective stress, σ = total stress and u = pore water pressure. According to this equation, a change in pore water pressure results in a change of effective stress. The pressure p_i on the ice of a frozen soil, equals the total stress σ. The pressure difference $p_i - p_w$ is an effective stress, and is therefore a consolidating effect associated with freezing. Immediately below the frost line, in the unfrozen soil there is a layer subject to an effective stress approaching that represented by $p_i - p_w = 2\sigma$ iw/rc, (Table 2.1); whose thickness depends on the rate at which freezing occurs and permeability.

After the penetration of ice through the soil has occurred, a progressively high effective stress is developed as the temperature falls. In predominantly silty soils, ice then fills the pores fairly uniformly and any consolidation occurring would appear to be limited to local micro-structural aggregations in which small particles separated by only small volumes of unfrozen water are drawn closer together (Chamberlain and Gow, 1979). In clayey soils the unfrozen clay layers present between ice lenses at relatively high temperature are substantially consolidated (the effective stress reaching several kg m^{-2}) before the ice penetrates the pores of these layers. Structural

changes have been shown to occur in clays even after this penetration (Chamberlain and Gow, 1979). Anderson and Hoekstra (1966) quoted by Williams (1968), have shown that water is removed from the interlamellar spaces of the clay mineral particles at temperatures of $-5^{o}C$ and lower. After thawing however, the water draining from the clay does not become reassociated with the particles in the original manner. The void ratio of the clay and its saturation moisture content are therefore decreased and the freezing process is thus to be regarded as a pre-consolidation. The effective stress developed by freezing to temperatures of $-5^{o}C$ may be large (about 6 kg cm^{-2} in clays) and the resulting consolidation correspondingly significant.

Richardson (1976) studied the effects of freezing and thawing on a fine sandy loam obtained from Holland, Lincolnshire, which was severely puddled and reconstituted. After 3 freezing and thawing cycles stability of the soil reached a maximum value, greater than the untreated soil collected from the same field. The effects of freezing and thawing upon increased stability was found to be more prominent in soil of high organic matter. SEM pictures showed finer particles in frozen and thawed soil which were orientated parallel to each other, producing platy micro-aggregates up to $\pm$ 50 µm in diameter. Similar structural changes were observed by Chamberlain and Gow (1979).

The above review points to the fact that both wetting and drying and freezing and thawing result in the formation of water stable aggregates but that the aggregates produced are of temporary nature and not very stable unless sufficient organic materials are presented to stabilise them. The depletion of these organic materials resulting from

exposure to microbial attack during repeated wetting and drying and freezing results in cancellation of the initial ameliorative effects and subsequent decline in stability. The response to weathering action appears to differ amongst soil textural classes and within these classes according to management practices imposed on the soils. The conflicting results obtained by various researchers within this field might be explained partly by the fact that they did not differentiate between contribution from purely biological processes and those which are truly physical in nature. The work of Tisdall et al. (1978) and Utomo and Dexter (1981a, 1982) has shed some light into the separate contribution of these processes. Dalrymple and Jim (1984) suggested that the reorganization of the clay fraction influenced by the isotropic stresses resulting from wetting and drying may be an important process in the formation of soil structural peds. This process is analogous to thixotropy, but little or no work assessing thixotropic aspects related to wetting and drying has been reported.

3. MATERIALS AND METHODS

3.1. Soil Characteristics.

Soil samples were obtained from five sites on three soil series in England and Scotland. Some properties of the soils are given in Table 3.1. Particle size analysis was determined by the pipette method and plastic limit moisture content by the Casagrande method (British Standard Institution, 1975). Organic carbon content was determined by Walkley-Black method (Hesse, 1971). These soils have good agricultural potential, but continuous cultivation depletes the organic matter, and the consequent loss of stability gives rise to structural defects which can limit crop yields, particularly in years of adverse weather. These soils are weakly structured and susceptible to surface capping by heavy rain, or mechanical damage if worked when wet.

The Stirling series soils are non-calcareous gleys formed from late Glacial estuarine and raised beach silts and clays (Ragg and Claydon, 1973). Soil samples from Carse of Stirling were obtained from five fields with different cropping histories on West Drip Farm (National Grid Reference NS 757956). Stirling 1 field had been under grass for at least 80 years, Stirling 2 for about 20 years and Stirling 3, Stirling 4 and Stirling 5 had been under alternating arable cultivation (6 - 7 years) and Timothy grass ley (7 - 8 years).

The Bromyard soils were obtained from Rosemaund Experimental Husbandry Farm (National Grid Reference SO 564480) near Hereford, through ADAS. Samples were collected from adjacent fields (Bromyard 1, Bromyard 2) with contrasting cultivation histories. These are argillic brown earths (Hollis and Hodgson, 1974). The Dreghorn is a freely drained brown forest soil formed from raised beach sands and gravels, and carboniferous sediments and basic lavas (Ragg et al., 1978; Bown and Shipley, 1982). The samples were obtained from a locality near the University which has been under pasture for over 40 years (National Grid Reference NS 804961).

Table 3.1 Selected properties of 0 - 150 mm soil zone

Soil series	% Clay (<2μm)	% Silt (2-63μm)	% Sand (63μm-2mm)	% Organic carbon	Plastic limit	pH	Cultivation history
Stirling 1	34.1	9.5	56.4	11.5	34.7	5.1	Permanent pasture
Stirling 2	29.8	18.9	51.3	7.1	25.3	5.1	Permanent pasture
Stirling 3	30.2	16.7	53.1	5.6	27.4	5.6	Alternating pasture & arable
Stirling 4	30.7	22.6	46.6	5.6	27.6	5.6	Alternating pasture & arable
Stirling 5	24.3	22.1	54.5	5.1	24.9	6.3	Alternating pasture & arable
Bromyard 1	19.6	70.6	9.8	3.7	31.7	6.8	Permanent pasture
Bromyard 2	14.4	74.5	11.1	2.0	25.5	7.1	Continuous arable
Dreghorn	19.4	16.1	64.5	7.0	29.2	5.3	Permanent pasture

3.2 Aggregate Stability Determination

3.2.1 Wet Sieving

The wet sieving test adopted in the present study is that of Grieve (1979a) with some minor modifications. The apparatus is shown in Figure 3.1. An electric motor is mounted on a platform over a large basin (bucket). The specifications of the apparatus were essential to permit comparison with the results of previous workers. These relate to the speed of sieving and the amplitude of the sieving cycle. Kemper (1965) pointed out that a speed of 30 or 35 rpm and amplitude of 38 mm have usually been used. The platform height and the sieve holder were adjusted to ensure that the sieve neither touched the sink at the base of the cycle nor emerged from the water at the top.

The motor used was geared at 35 rpm. A metal disc (to which a sieve holder is attached) is bolted to the gear-box. The distance of 19 mm from the bolt centre to the centre of the rotation allows a total vertical displacement of the sieve through 38 cm.

The sieve holder was constructed from 12 mm x 3 mm brass strip bolted at the lower end to a sieve frame with the mesh removed. If sieves are banked in the usual manner as in all sieving tests, the adjustment of the level of water in sink must be such that the uppermost sieve just reaches it, but the sample does not emerge. For the purpose of the present study, the simple index of Low (1954) was used. The percentage retained by the 1.4 mm sieve was used as index of stability, thus simplifying the analyses of the results.

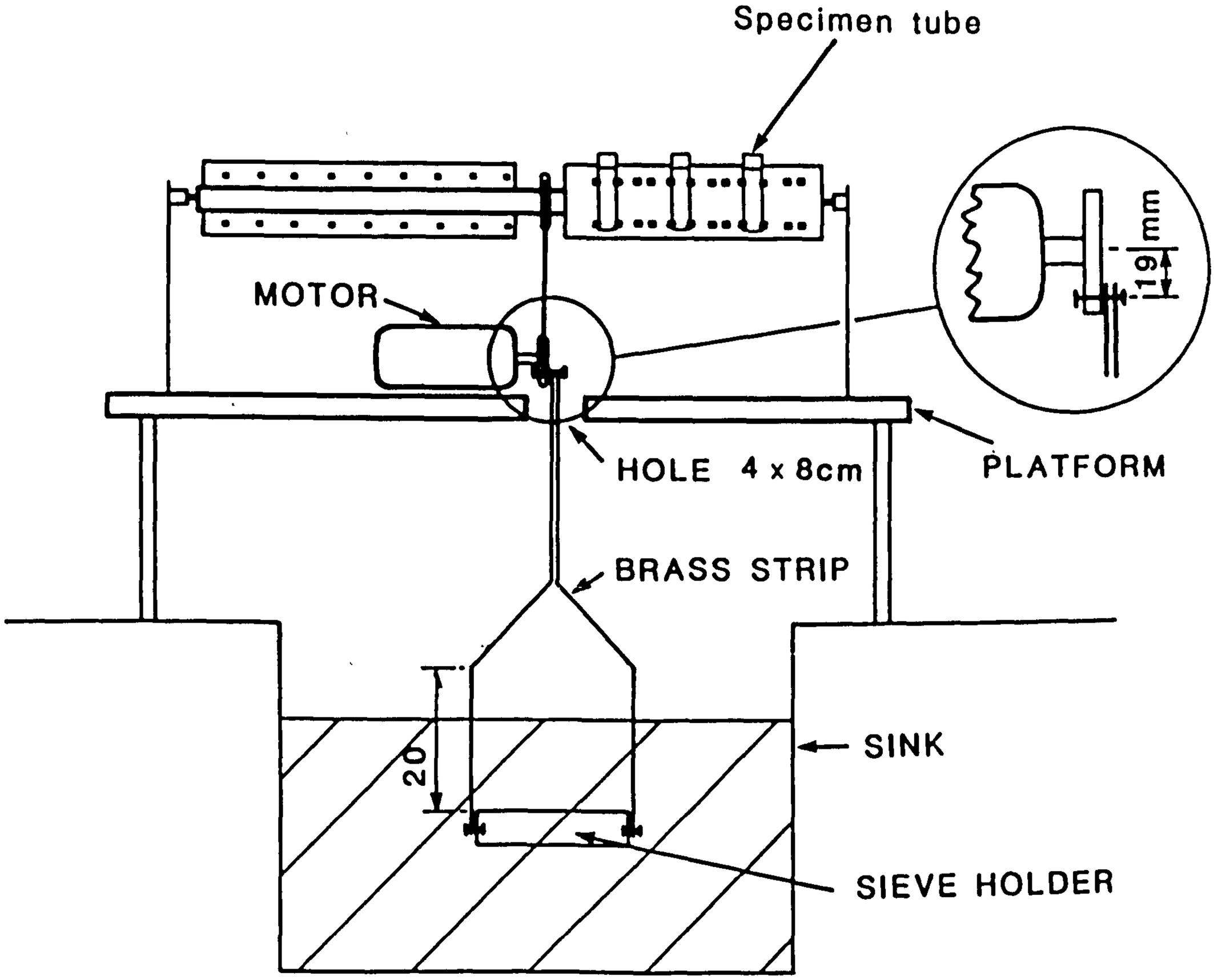


Figure 3.1 Diagram of apparatus used in wet sieving and end -over-end shaking.

Air-dried soil samples obtained from five adjacent fields on West Drip Farm were mildly sieved to obtain 2 - 4 mm diameter aggregates. The aggregates were then spread on a tray, and stones and roots removed. 50g samples obtained by quartering were used for each test. The 50g (W1) samples were either directly transferred to the sieve shaker or pre-wet as in Figure 3.2. Pre-wetting of the sample prior to mechanical dispersion was achieved by allowing the sample to take up water by capillary action from a body of water overnight. Similar pre-wetting procedure was used by Silanpaä (1967) and Grieve (1975). The cloth was finely woven cotton and had a pathwidth of 20 cm. The factor which controls the wetting rate here is the size of the capillary forces in the cloth and not those in the soil hence the wetting rate is independent of the soil. For flood wetting test the air dry sample was directly placed on the sieve under water and immediately shaken. The procedure was modified from that used by Grieve (1979a) in which the air-dry aggregates were allowed to wet completely before shaking by standing them for 20 minutes under water.

The aggregates were sieved for 15 minutes in water at $20 \pm 0.5^{o}C$. The portion collected on the 1.4 mm sieve was transferred without loss to a 500 ml beaker. After standing for 30 minutes the water was decanted and the soil dried overnight at $105^{o}C$ in the oven and weighed (W2). As all the stones were carefully removed before sieving there was no need for correction for them. The stability index was calculated as:

$$\% \text{ Water Stable Aggregates} > 1.4 \text{ mm} = \frac{W2 \times 100}{W1}$$

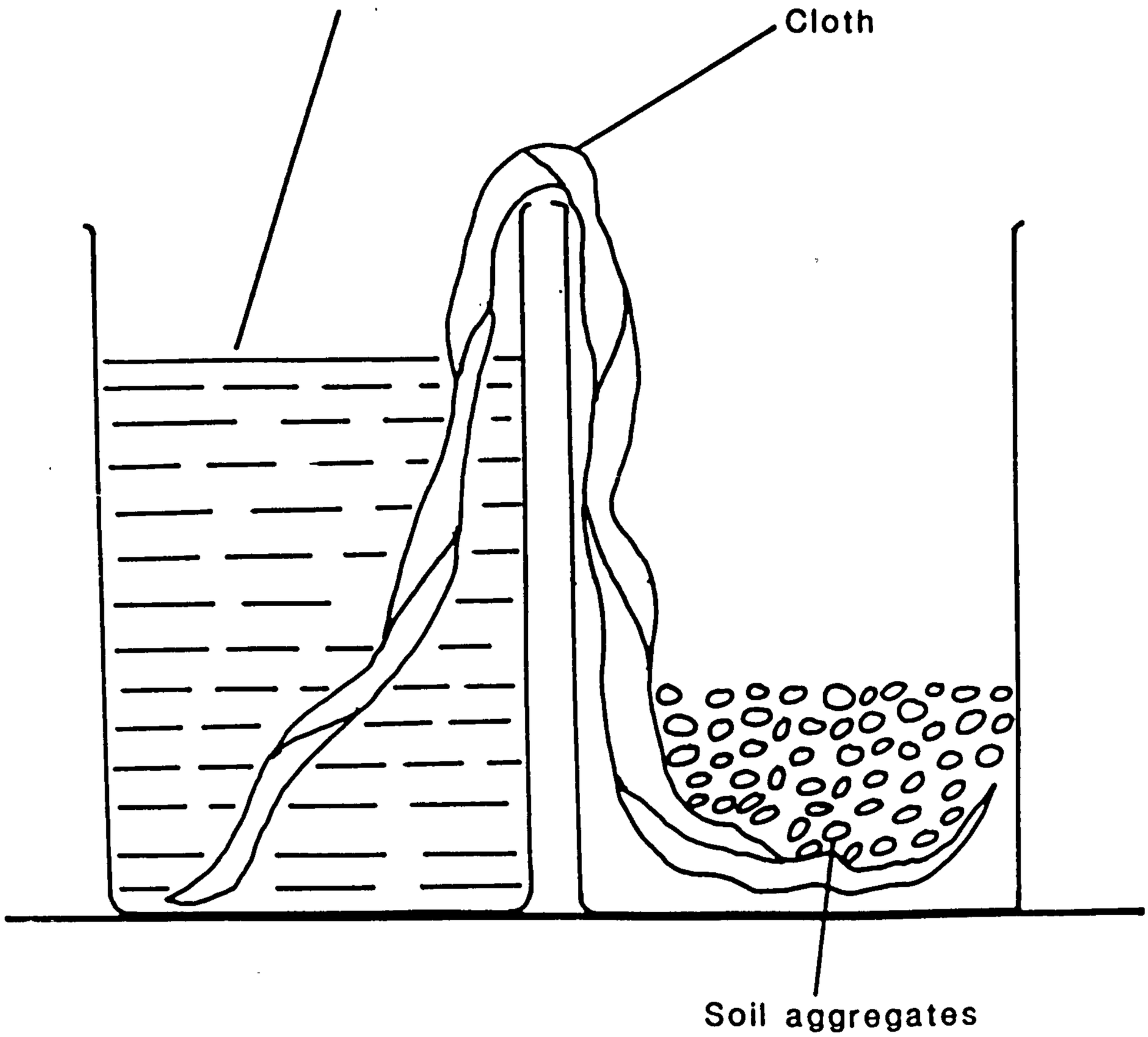


Figure 3.2 Apparatus for slow wetting of soils. (Silanpaä, 1967).

3.2.2 Turbidimetry Test

The method used for turbidimetry test was a modification of that of Williams et al. (1966). 2 - 4 mm aggregates obtained by mild sieving of air dry aggregates were weighed to obtain 0.25g portions. These were directly immersed in water or pre-wetted by capillary action as in Figure 3.2 prior to shaking.

Dry or pre-wet samples were placed in 30 ml of de-ionised water in specimen tubes 2 cm diameter and 7 cm length. The tubes were turned end-over- end at 35 rpm for 7 minutes, using the same drive mechanism as in the wet sieving test (Fig. 3.1). 10 ml of the suspension was immediately drawn off from halfway depth of the specimen tube, transferred to a spectrophotometer cell and allowed to settle for 2 min. % Light transmission was read on a SP6-350 spectrophotometer (Pye Unicam Ltd., Cambridge) at 630 nm using a red photocell. The instrument was calibrated to 100% light transmission with de-ionised water before each individual determination.

3.3 Effects of Microbial Activity on Aggregate Stability

3.3.1 Test on Selectivity of Antibiotics.

Treatments of Dreghorn pasture soil with bactericides (vancomycin or streptomycin; and a combination of the two) and a fungicide, (cycloheximide) respectively were tested to assess the relative effectiveness of the antibiotics in inhibiting the growth and activity of bacteria and fungi, and to determine the strength (doses) to be applied to achieve total inhibition. 2 - 4 mm aggregates were obtained by mild

sieving of air-dry soil. 1 kg and 1 g portions of the aggregates were treated with:

(i) 100 mg Streptomycin sulphate (Sigma Chemical Company, Poole)

(ii) 100 mg Vancomycin (Sigma Chemical Company, Poole)

(iii) Streptomycin and Vancomycin at 50 mg of each, and

(iv) 100 mg Cycloheximide (Aldrich Chemical Company,Milwaukee, Wisconsin, USA).

The antibiotics were mixed with talc powder (BDH Chemicals Ltd., Poole) at 5 g/g soil to facilitate thorough dispersion. The mixing was done with dry plastic containers. The aggregates were then moistened to plastic limit moisture by capillary action. The control sample was moistened with de-ionised water only. The samples sealed in containers were incubated at 20 ± 2^{o} C for a period of 15 days. Sub-samples were taken at 5 days interval to check on the possibility of microbial growth, which would indicate depreciation of antibiotic activity. Bacterial growth was checked by plating on nutrient agar, and fungal growth monitored by visual inspection of the plates and scanning electron microscopy (SEM).

The results of the experiment (Table 3.2) indicate that vancomycin alone, and vancomycin plus streptomycin completely inhibited bacterial growth, at the concentration used. Streptomycin alone showed signs of depreciation in effect as counts were shown to increase with subsequent period of incubation. On comparing the plates treated with vancomycin, streptomycin and vancomycin plus streptomycin, there were signs of depressed fungal growth in the latter two treatments, however it was not

Table 3.2 Test for selectivity of inhibitors on bacterial growth. Results give average number of colonies ± standard error (x 10^4) per gram of soil for 10 replicate plates.

Treatment	Concentration of inhibitor	Incubation period (days)		
		0	5	15
Control		7.8±2.3	30.6±5.9	59.8±5.1
Streptomycin	a	6.2±1.5	14.3±2.5	17.8±3.2
	b	2.4±1.2	0.4±0.1	0
Vancomycin	a	0	0	0
	b	0	0	0
Streptomycin + Vancomycin	a	0	0	0
	b	0	0	0
Cycloheximide	a	10.6±3.2	48.5±4.3	55.8±6.8
	b	11.3±2.4	50.6±5.1	55.4±5.6

a = 100 mg kg^{-1} b = 100 mg g^{-1}

clear whether the presence of streptomycin had some effects on fungal growth. Fungal growth was most vigorous on vancomycin treated soil, and this treatment was adopted in subsequent experiments intended to inhibit bacterial growth.

Samples treated with cycloheximide were examined with SEM to check fungal growth. This treatment produced total inhibition of fungal growth and this was evident throughout incubation period. Anderson and Domsch (1973) achieved total inhibition of fungi when samples were treated with 500 ppm of cycloheximide. Lynch (1983), however found that when used over long incubation periods, the effects of cycloheximide declined. 1.5 mg ml^{-1} of cycloheximide did not kill the fungi, although it did decrease their maximum specific growth rate. Lynch cautioned that care must be exercised when using antibiotics, because some antibiotics promote processes they are intended to inhibit and that effective doses should be determined before detailed experiments are carried out (Lynch, 1983). Neither the antifungal nor the antibacterial agents used in the present study affected the growth of actinomycetes, which tended to appear during later stages of incubation as revealed by SEM.

Mixing the antibiotics with talc was found unsuitable because talc was not soluble in water. The undissolved talc gave problems during stability determinations especially when using turbidimetric light transmission technique. Thus in subsequent experiments, the antibiotics were dissolved in water and soil aggegates moistened with the solutions.

3.3.2 The Relative Contributions of Fungi and Bacteria to Aggregate Stabilisation

Soils of the Stirling 1 and 3; Bromyard 1 and 2 and Dreghorn air dried at room temperature were sieved to obtain 2 - 4 mm aggregates. The samples were treated as follows:

3.3.2.1 Moistening with de-ionised water.

1 kg of aggregates from each of the 5 sites was allowed to take up water by capillary action (Sillanpaä, 1967). Moist aggregates were spread in a flat plastic container to form a bed 1 cm thick, wrapped in plastic bags and incubated at 20 ± 2^{o}C.

3.3.2.2 Moistening with Vancomycin Solution.

1 kg of aggregates were moistened (as in 3.3.2.1) with a solution containing vancomycin (100 mg g^{-1} soil to inhibit bacterial growth). The antibiotic was dissolved in the amount of water that will give moistening to plastic limit for each soil.

3.3.2.3 Moistening with Cycloheximide Solution.

A kilogram of soil was moistened with a solution of cycloheximide (100 mg g^{-1} soil) to prevent fungal growth. The wetting procedure was essentially as in 3.3.2.1.

3.3.2.4 Moistening with a Sterilising Solution.

Whereas vancomycin and cycloheximide treatments inhibited bacterial and fungal growth respectively, treatment with a sterilising solution was intended to inhibit all biological processes in the soil samples. 1 kg portions of soil aggregates were wetted to their plastic limit moisture content with a sterilising solution containing 0.5 g sodium azide (B D H Chemicals Ltd., Poole) and 0.5 g mercuric chloride (Fisons Scientific Apparatus, Loughborough). This sterilisation treatment was used by Tisdall et.al. (1978).

3.3.3 Incubation and Sampling

Containers from all four treatments sealed in plastic bags were incubated at $20\pm2^{o}C$ for a period of 30 days. Sub-samples were removed from each tray at 3 day intervals over a period of 15 days, and subsequently at 5 day intervals. These samples were used for aggregate stability determination, examination by scanning electron microscope (SEM), measurement of the fungi by ergosterol determination, enumeration of the bacterial growth and assessment of the contribution of polysaccharides to aggregate stabilisation.

3.3.4 Aggregate Stability Determination

Aggregate stability changes during incubation of soil aggregates treated with different antibiotics (as above) was determined using turbidimetric technique. Wet sieving was found unsuitable for stability

determination in the present study mainly because of the amount of sample used, and the length of sieving time involved. Stability was determined on 8 replicates (0.25 g each) from each treatment using the rapid wetting procedure, whereby aggregates which had been dried over phosphorus pentoxide were directly immersed in water, and shaken end-over-end using the shaking device as in Figure 3.1.

3.3.5 Examination of Soil Aggregates with Scanning Electron Microscope.

Individual aggregates obtained from the four treatments were examined with SEM to assess the mechanism of stabilisation by the filamentous organisms, the association of bacterial products of decomposition with soil particles and to establish micromorphological changes taking place during incubation. Immediately after removal from the trays, the samples were plunged into liquid nitrogen and subsequently freeze-dried under vacuum overnight (Smart and Tovey, 1982). Freeze-dried samples were mounted on E.M. aluminium stubs with silver conducting paint (E.M. Scope Laboratories Ltd., Ashford) and kept in a desiccator containing phosphorous pentoxide to prevent moisture gain during drying of the adhesive. The samples mounted on stubs were coated with gold under vacuum in an S150 sputter coater and examined with an I.S.I. 60 SEM (International Scientific Instruments) at various magnifications. Photographs were taken at 2 or 3 places per stub to allow for larger view of the sample.

3.3.6 Measurement of Fungal Growth by High Pressure Liquid Chromatography (HPLC).

This technique was adopted from cereal chemistry and has never previously been used in soils. The technique used was developed by Seitz et al. (1977) as an indicator of fungal invasion in stored grains. Seitz et al. (1979) improved and modified this technique for routine laboratory work. The technique comprises basically two major procedures, the extraction of ergosterol and its estimation by HPLC.

25 g of soil (2 - 4 mm) from each of the incubated trays (removed at each sampling day) was extracted with 100 ml of methanol (MeOH) in 125 ml plastic bottles. A mechanical shaker was used to disperse the aggregates and dislodge the fungal mycelia and to grind them in the process. The shaking continued for 30 minutes. The methanol extract with additional 25 ml MeOH used to rinse the bottle were transferred to 100 ml centrifuge tubes and centrifuged for 5 min. at 2000 rpm. The supernatant was decanted and the residue resuspended with further 25 ml MeOH, shaken by hand for 30 seconds and centrifuged again.

The two supernatants were combined in 500 ml round bottom flasks, mixed with 10 g potassium hydroxide and 25 ml ethanol and refluxed for 30 minutes. This saponification stage is used to separate ergosterol from other sterols such as stigmasterol, present in higher plants, which are found in the saponifiable fraction (Fieser and Fieser, 1944). The saponified mixture was cooled with 30 ml of deionised water and transferred to a separating flask. After cooling the mixture was

extracted with 50 ml, followed by another 25 ml of petroleum ether (Bpt 60 - 80^{o} C). The petroleum ether extracts were combined in 250 ml round bottom flasks and evaporated on a warm steam bath, with a gentle flow of nitrogen gas. The containers were covered with aluminium foil to minimise irradiation. (Irradiation of ergosterol by uv light converts it to vitamin D_2). The residue was dissolved in 2.5 ml methylene chloride: isopropanol (99: 1 v/v). The final solution was kept in specimen bottles, in the dark until further use.

The HPLC apparatus comprised of an Altex model 110A pump with a load of 3000 psi and a flow rate of 1.7 ml min^{-1} (Altex, Berkeley, California, USA); the Holochrome variable wavelength detector set at 282 nm and 0.1 absorption units full scale (AUFS) (Gilson, France SA, Villiers Le Bel, France); 5 µ ODS-Hypersil Column (1.5 mm i.d. and 10 mm o.d.), (Shandon Southern Products Ltd., Cheshire) and a model 2800 recorder (Bryans Southern Instruments Ltd., Micham).

The instrument was calibrated with ergosterol standard prior to running soil extracts. Ergosterol (85%) (Aldrich Chemical Company Ltd., Gillingham) was recrystallised twice from absolute ethanol and freeze-dried overnight. 22 mg of recrystallised purified ergosterol was dissolved in 10 ml methylene chloride: isopropanol (99: 1 v/v) and diluted to make standards for calibration between 0.1 and 22 µg range of the detector.

10 µl of the final soil extract dissolved in methylene chloride: isopropanol was injected on to the column. Using a reversed phase

system, 95% MeOH and 5% water as the eluent, ergosterol was detected at the 9th minute. The extra guard column used for extra cleaning of the petroleum ether extract prior to injection on to the column by Seitz et al. (1977) was found unnecessary as the extracts were free from soil particules. Ergosterol content was estimated using the above calibration curve. Some of the soil extract eluted at 9th minute was collected for verification of the presence of ergosterol in the soil extracts. Mass spectrometer determinations coupled with true mass determinations confirmed the presence of ergosterol in the soil extracts.

The results of True Mass determination on the ergosterol standard and the soil extract were compared (Appendix 1). Impurities in the soil extract resulted in an error of 6.6 ppm in the atomic mass of the ergosterol and this was not unexpected especially when dealing with soils. The efficiency of the methanol extraction technique and measurement of the ergosterol as an index of fungal growth in soils was assessed. Known amounts of the ergosterol standard were thoroughly mixed with 25 g portions of soil. The Bromyard, the Stirling and Dreghorn soils were used to allow for comparison amongst the textural soil classes. Soils were moistened with a sterilising solution containing sodium azide and mercuric chloride at 0.5 mgg^{-1} soil of each. This treatment inhibited biological activities thus the ergosterol to be extracted was that which was added to the soil. The soils were incubated for 3 days to allow for complete dispersion of the ergosterol into the soil aggregates. The ergosterol was extracted from the samples as before, and the amount recovered was expressed as % of the original.

The recovery of ergosterol which had been added to fungus-free ground soil was 90.8 ± 1.63% for Stirling 1 soil, 97.3 ± 0.92% for Bromyard 1 and 96.0 ± 6.50% for Dreghorn. Thus the method extracted 94.7 ± 2.43%. The results from soil extracts in which a mechanical shaker was used to disperse the soil well agreed with those of Seitz et al. (1979) in which a Waring blender method recovered 93 ± 5% of the ergosterol which had been added to fungus-free grains.

3.3.7 Enumeration of Bacterial Population

Bacterial population evolving during incubation of selectively treated soil aggregates was estimated by the dilution plate count technique (Atlas and Bartha, 1981). 0.22 g soil aggregates were dispersed in 20 ml of autoclaved deionised water using a Whirlimixer (Fisons Scientific Apparatus, Loughborough). 0.1 ml solution was diluted with 9.9 ml autoclaved water and again mixed by aid of the vibrator. 1 ml of the final solution was mixed with autoclaved nutrient agar (± 45^{o}C) by gently moving the petridish forwards backwards and sideways. Gray and Williams (1971) stated that mixing nutrient media with the soil suspension by rotation of the petridish results in organisms concentrated to the periphery and making counting impossible.

The media used was nutrient agar (Oxoid Ltd., Basingstoke), with composition per litre of:

Lab-Lemco Powder	1.0 g
Yeast Extract	2.0 g
Peptone	5.0 g
Sodium Chloride	5.0 g
Agar No. 3	15.0 g

28 g of the above mixture was mixed with 1 litre of deionised water and completely dissolved by autoclaving in 15 ml glass tubes at 121^{o}C for 20 min. The agar in glass tubes was then cooled to 45^{o}C in a warm bath and subsequently the contents of each tube were transferred to one petridish, thus giving all petridishes an equal amount of agar (15 ml). When the agar had cooled and set, the plates were packed upside down into stacks of 5, and incubated at 20 ± 2^{o}C. Counting was done on each plate after 2 days with the aid of a binocular microscope. Plates with counts more than 200 were discarded because counting was unreliable due to the majority of organisms forming clumps. 10 replicate plates were prepared for each soil sample on the sampling day.

3.3.8 Periodate Treatment

The periodate treatment of soil aggregate (2-4 mm) from all treatments above, was carried out on a tension plate apparatus (Figure 3.3). The tension plate was constructed by using a 7 cm diameter Buchner funnel with a Whatman No. 52 Glass fibre filter. The funnel was connected to a polythene tube reservoir which was clamped vertically. Suctions of 102 mm of water (h) were easily obtained, (=1kPa). 2 g of aggregates at 20 ± 2^{o}C were treated with 60 ml solutions of 0.05 M NaCl for 48 hours, followed by 0.05 M $NaIO_4$ for 48 hours and then 0.1 M $Na_2B_4O_7$ (pH 9.3) for 48 hours. This treatment was previously used by Cheshire et al. (1983, 1984). Their treatment was longer than that used by Clapp and Emerson (1965) and Stefanson (1971). At the end of each individual treatment, the solution was drawn off by applying suction at the end of the tube and washing the sample with 2 x 50 ml portions of deionised water.

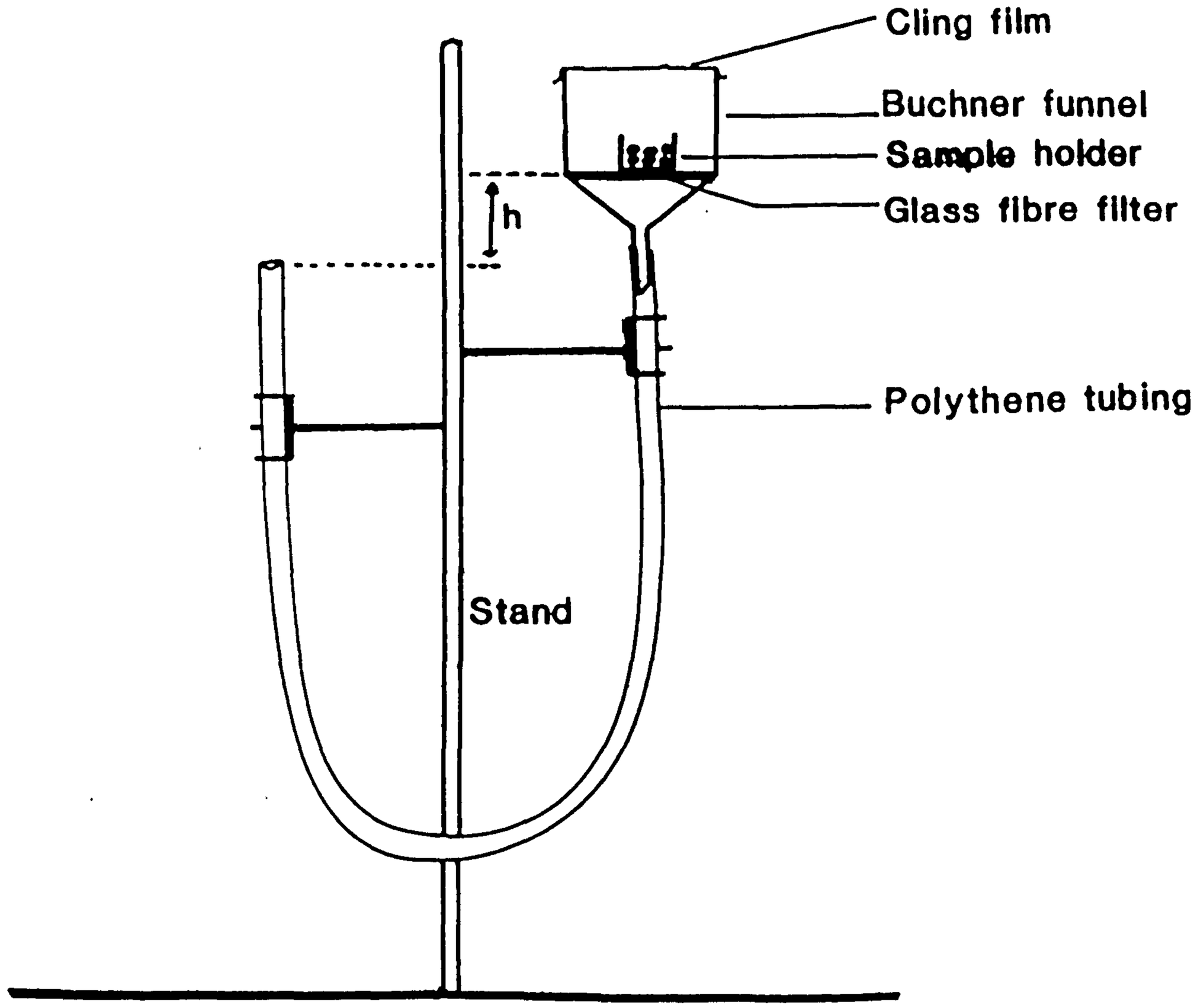


Figure 3.3 Tension plate for periodate leaching

h=tension(cm water).

After completion of the treatment, the aggregates on the glass fibre filters were transferred to a desiccator over phosphorus pentoxide and dried overnight. They were then taken for stability measurement by the turbidimetric method. This treatment was carried out at suitable intervals throughout the incubation period for each soil treatment.

3.4. Age Hardening of Moulded Soil Aggregates.

Three measures (aggregate stability, water potential and probe penetration resistance) were employed to characterise thixotropic age hardening processes with three soil textural classes. The Stirling 1 and 3; Bromyard 1 and 2; and the Dreghorn soils were used (Table 3.1).

3.4.1. Preparation of Moulded (Artificial) Aggregates

3.4.1.1 Moistening with Deionised Water

Artificial aggregates were prepared from field soil that had been air-dried. The macro-aggregates were crushed to pass a 0.25 mm sieve. The pulverised soils were moistened with deionised water to moisture content equivalent to their plastic limit. Remoulding was done by hand until the soil was as homogeneous as possible. The remoulded soils were allowed to equilibrate overnight, then again remoulded and rolled into balls of 10-20 mm diameter, by rolling between the hands for three minutes. The stability (0 day) was determined on one ball from which 8 replicate samples of 0.25 g was obtained and the rest of the balls were aged in desiccators over a layer of water, for a period of 15 days. At 3

day intervals, the aggregate stability was determined for each soil, using the turbidimetry technique.

3.4.1.2 Sterilisation and Periodate Oxidation

In order to determine the separate contribution of microbial activity and periodate-labile materials to the increase in aggregate stability after remoulding, samples were sterilised and oxidised prior to remoulding and incubation. The sterilising solution contained 0.5 mg sodium azide and 0.5 mg mercuric chloride for each gram of soil (Tisdall et al., 1978).

To degrade the organic materials, in particular the polysaccharide fraction, of the soils, samples were treated with standard periodate technique as in 3.3.8 (Cheshire et al., 1983, 1984). Both sterilised and periodate treated soils were moulded with deionised water or sterilising solution and incubated as before. Aggregate stability changes were measured at three day intervals using turbidimetry.

3.4.2 Water Potential Changes

Soil matric water potential is governed by soil pore size distribution (Marshall and Holmes, 1979; Hillel, 1982). In order to test the hypothesis that thixotropic hardening is associated with particle re-orientation, (which would modify the interparticle pore sizes) measurements were made of soil water matric potential as a function of time after remoulding. Samples of Stirling, Dreghorn and

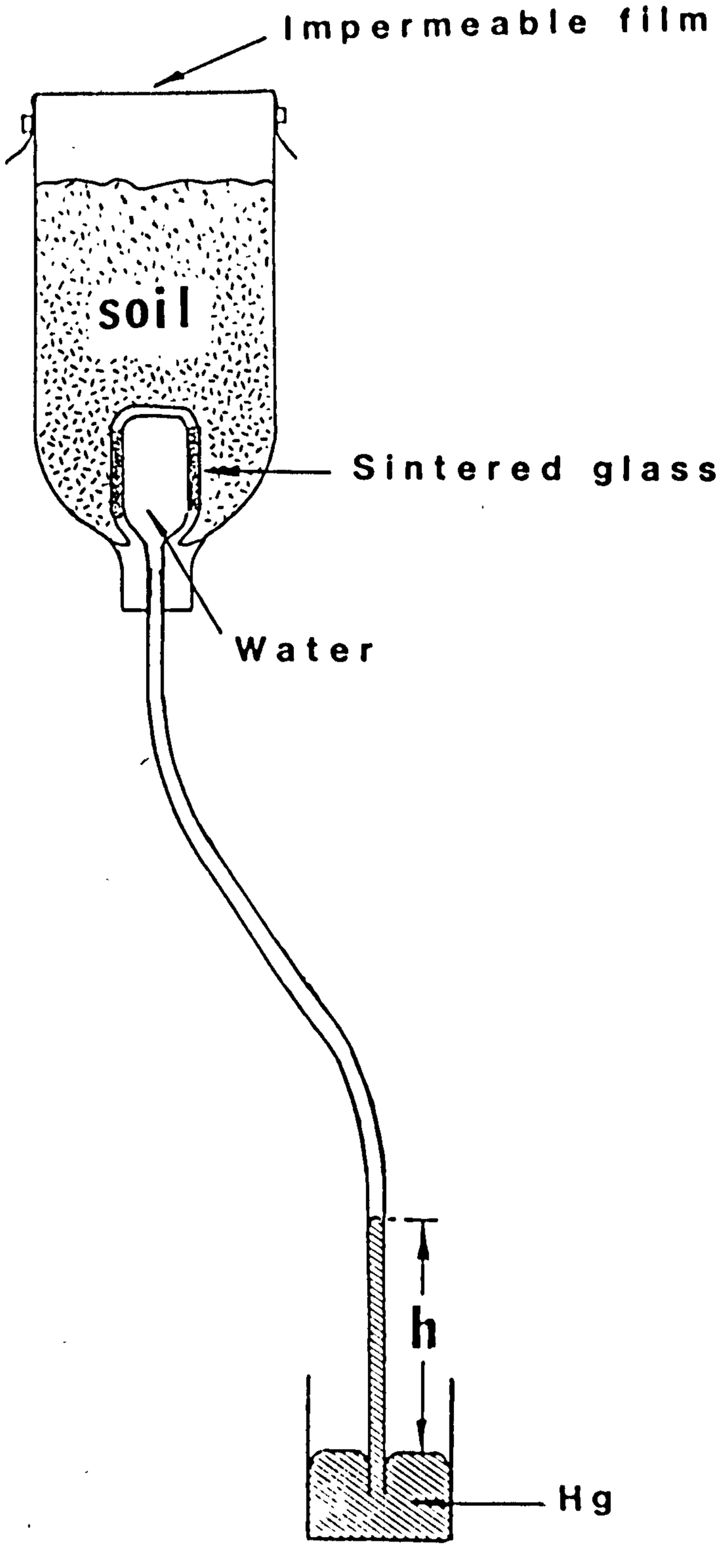


Figure 3.4 Apparatus used to measure matric water potential

Bromyard soils puddled and remoulded to plastic limit moisture contents with either deionised water or sterilising solution were subjected to tension measurements. The contribution of organic materials was again separated by the periodate treatment of the aggregate prior to subjection to tension measurements.

Equilibrated soil was remoulded and packed into a tensiometer cup which could contain up to 30 ml of soil (Figure 3.4). The soil was compacted manually (kneading compaction) until it formed a compact mass with no visible spaces within the cup. The sintered glass thimble was filled with deionised water and connected by transparent polythene tubing to a mercury reservoir, taking care that air bubbles were excluded. The tops of the cups were sealed with cling film to make them air tight. Three samples were prepared for each soil, and the cups were stored at 20 $\pm$ 2^{o}C for 15 days. Suction of the water was measured by the mercury rise in the manometer at one day intervals. At the end of the 15 day period, the soils were remoulded whilst still in the containers and the measurements were repeated as above.

3.4.3. Measurement of Soil Strength

Bromyard and Stirling soils were used for this test. The samples were moulded with sterilising solution containing 1 mg per gram mercuric chloride. Sodium azide was omitted because metal containers were used in ageing the samples. After equilibrating overnight, these samples were remoulded and compressed into metal compartments. The containers were wrapped with cling film and placed in an airtight plastic food container

and kept at 20 $\pm$ 2^{o}C. Soil strength was measured with a motor driven laboratory penetrometer. The probe had a diameter of 2.4 mm and a total tip angle of 60^{o}. This size and shape is commonly used in root growth studies.

The force required to penetrate the soil was measured by means of a JJT 500 Testing Machine (J.J. Lloyd Instruments Ltd., Southampton). The probe was mounted on a 5N load cell and driven downward at 50 mm min^{-1}, and the force/distance diagram was drawn on an XY plotter. One measurement was done on each compartment, and there were five measurements per soil at each sampling day. The strength was calculated as resistance to probe penetration, Qp, using the equation of Utomo and Dexter (1981c).

$$Qp = 4F/\pi d^2$$

where F is the force required to penetrate the sample at depth of 4 cm and d is the diameter of the probe. Testing was done immediately after remoulding and packing (0 day) and subsequently at three day intervals for a period of 18 days.

At the end of the 18 day period, the samples were taken out, remoulded and pressed back into compartments, and the penetration test repeated for another 18 days. The samples were treated with periodate to destroy organic matter at the end of the 2nd 18 day period. After the treatment, these were dried at 20^{o}C until at moisture content equivalent to their plastic limit. The soils were then remoulded, and

pressed back into compartments and measurement of penetration repeated. Tests were done for 18 days, then remoulded, then taken again for another 18 days.

3.5 Weathering Cycles.

The soils used in this experiment are those in Table 3.1. The experiment was designed to examine the effects of wetting and drying; and freezing and thawing cycles on aggregate stability of field (natural) and moulded (artificial) aggregates. In addition to the artificial weathering cycles, natural weathering cycles were examined using the Stirling soils.

3.5.1. Wetting and Drying Cycles

3.5.1.1 Artificial Wetting and Drying of Field Aggregates.

Aggregates of 2-4 mm diameter were obtained by mild sieving of air-dried field aggregates, obtained from arable tilled, arable non-tilled and pasture plots of Stirling and Bromyard soils. For the Dreghorn soil the aggregates were obtained only from permanent pasture. The aggregates were brought to equilibrium moisture content on sintered glass funnels kept at 20 ± 2^{o}C and sealed with cling film. The samples were wet at 102 mm (=1 kPa) suction with either deionised water or sterilising solution. The bed of aggregates, (ca. 5 mm thick) was contained in baskets made by gluing polythene plastic tubing (3 cm i.d. and 2 cm height) to a glass fibre filter which facilitated contact with the sinter and non-destructive handling of the aggregates during changing for wetting and drying.

The samples were maintained at -1 kPa (102 mm suction) for 3 days to attain equilibrium moisture (checked by weighing). The samples were then subjected to wetting and drying between -1 kPa and -10 kPa on sintered glass funnels, or between -1 kPa on sintered glass funnels and -100 MPa over saturated lithium nitrate ($LiNO_3 3H_2O$) solution (Fisons, Loughborough) (Newman, 1983). Each cycle consisted of wetting for 16 hours on sintered glass funnels at -1 kPa and 8 hours drying over $LiNO_3$ then re-wetting. Control sample was kept at -1 kPa throughout the wetting and drying programme. At the end of each pre-determined number of wetting and drying cycles, the aggregates were dried overnight in desiccators over phosphorous pentoxide, and aggregate stability determined on 8 replicate samples (0.25 g) by turbidimetry method.

3.5.1.2 Effects of Sterilisation on Field Aggregates during Wetting and Drying Cycles.

In this experiment, the reservoirs of the tension wetting apparatus contained sterilising solutions. These solutions contained 0.5 mg of NaN_3 and 0.5 mg of $HgCl_2$ per gram of soil to be contained on the sintered glass funnel. After equilibrating for 3 days, at -1 kPa, the control sample was left at -1 MPa and others subjected to wetting and drying cycles between -1 kPa and-100 MPa. Aggregate stability was determined again at the end of each pre-determined number of wetting and drying cycles.

3.5.2 Effects of Field Tillage.

Samples of Stirling 3 tilled and non-tilled plots were obtained, air-dried and sieved to obtain 2-4 mm aggregates. Tillage on one half of the plot was done by the farmer in November 1983, and a sample was obtained immediately after tillage. Another sample was obtained from the other half of the same field which had been left fallow. Aggregates were equilibrated on sintered glass funnels at -1 kPa for 3 days and subsequently subjected to wetting and drying cycles with either deionised water or sterilising solutions as before, and aggregate stability changes measured using turbidimetry technique.

3.5.3 Wetting and Drying of Moulded Aggregates

Moulded aggregates were obtained by the following procedure: Field aggregates obtained by sieving air-dried soil were crushed to pass a 0.25 mm sieve. These were moulded with deionised water to plastic limit moisture contents. After standing overnight, they were remoulded with hand and rolled into balls of 10—20 mm diameter. The balls were aged for two weeks at 20 ± 2^{o}C to allow for thixotropic and biological hardening effects to reach equilibrium.

3.5.3.1 Wetting with deionised water.

Wetting with deionised water and drying was performed initially over a range of matric water potentials. Wetting and drying at -1, -5, and -10 kPa was on sintered glass funnels and -100 MPa over lithium

nitrate. Subsequently the samples were wetted at -1 kPa and dried at -100 MPa for 3, 6, 9, 12 and 15 cycles. At the end of each wetting and drying cycle programme, the balls were dried over phosphorus pentoxide for two days. Each ball was then divided to obtain 8 replicates of 0.25g for aggregate stability measurements using turbidimetry.

3.5.3.2. Wetting with a Sterilised Solution

The soils were moistened to their plastic limit water contents with a sterilising solution containing 0.5 mg NaN_3 and 0.5 mg $HgCl_2$ for each gram of soil. After equilibrating overnight, the soils were remoulded, and rolled into balls as before. The balls were incubated in a desiccator containing a sterilising solution for two weeks to allow for thixotropic aging, and were then subjected to wetting and drying as above. For this treatment, the sintered glass funnels were connected to reservoirs of NaN_3 and $HgCl_2$ solutions. The stability was again determined by turbidimetry on 8 replicates obtained by sub-dividing balls dried over phosphorous pentoxide.

3.5.4. Measurement of the Amount of Wetting and Drying

Natural wetting and drying of soils was simulated in the laboratory using both natural aggregates, 2 - 4 mm diameter obtained by mild sieving of air-dry soil, and artificial aggregates, obtained by pulverising natural aggregates and remoulding with deionised water or sterilising solution and making balls of 10 - 20 diameter as before.

The amount of wetting and drying (ΔW) undergone by samples at each one of wetting and drying cycles was estimated by measuring gravimetric water content changes resulting from wetting and drying. Water content was determined after wetting for 16 hours at -1 kPa on sintered glass funnels and after drying for 8 hours over lithium nitrate. These measurements of water content changes were used to estimate both cumulative wetting and drying ($\Sigma\Delta W$) and the final water content at the end of each cycle (% w/w). The amount of wetting and drying (ΔW) for any cycle was calculated by the method of Utomo and Dexter (1981a):

$$\Delta W_i = \left| Wmax_{(i-1)} - Wmin_{(i-1)} \right| + \left| Wmax_{(i)} - Wmin_{(i-1)} \right|$$

The cumulative amount of wetting and drying $\sum_j \Delta W$ which the sample had undergone between 0 and j day cycles was calculated as:

$$\sum_j \Delta W = (\Delta W_1 + \Delta W_2 + ..\Delta W_j) - j(4\sigma/\sqrt{\pi k})$$

The final term $j\ (4\sigma/\sqrt{\pi k})$ is an error term which allows for sampling variation expected to result from non-uniform wetting and drying related to moisture release characteristics of different soils. j = number of cycles, k = number of samples on which measurements were taken; W_{max} and W_{min} represent wet and dry weights respectively and σ is the standard deviation in the amount of wetting and drying for any number of cycles.

3.5.5 Freezing and Thawing of Field Aggregates

In order to allow for comparisons between the effects of wetting and drying and freezing and thawing, similar samples, obtained in the same manner and pre-treated the same way were used for this experiment. 2 - 4 mm aggregates were moistened to equilibrium moisture content with either deionised water or sterilising solution. These were then removed whilst in the baskets made from plastic tubing and subjected to freezing and thawing cycles. Freezing was done overnight in deep freeze at $-10^{o}C$ and thawing in desiccators with a layer of deionised water or sterilising solution kept at $20 \pm 2^{o}C$. After completion of a pre-determined number of cycles soils were removed and dried over P_2O_5 overnight. Stability was again determined using turbidimetry.

3.5.6 Freezing and Thawing of Moulded Aggregates

Moulded aggregates were obtained by remoulding pulverised soil with deionised water or sterilising solution as before. The balls (10 - 20 mm) were aged for 14 days, after which they were subjected to freezing at $10^{o}C$ and thawing at $20 \pm 2^{o}C$. At the end of each cycle, the balls were dried overnight over P_2O_5 and then sub-sampled by picking apart into individual aggregates, which were further dried over P_2O_5 overnight to allow for complete drying before stability determination.

3.5.6.1 Effects of Size of Moulded Aggregates During Freezing and Thawing

Remoulded aggregates (balls 10 - 20 mm diameter) were aged for 14 days as before. At the end of the aging period these balls were sub-divided into 4 - 6 mm aggregate sizes and then subjected to freezing and thawing cycles as before.

3.5.7 Seasonal Variation in Aggregate Stability of Stirling Soils

Seasonal changes in aggregate stability as affected by natural weathering cycles in the field was followed using samples of the Stirling soils from West Drip Farm. Samples from arable non-tilled and pasture plots were used in this experiment. Part of Stirling 3 arable plot received first tillage in November 1983 to a depth of ± 150 mm, and the second implement pass was in March 1984.

2 kg samples were obtained randomly from 15 cm depth in November 1983, i.e. immediately after cultivation, and subsequently at monthly intervals up to December 1984. The samples were air dried at room temperature, and sieved to obtain 2 - 4 mm aggregates, and stones and roots were removed. Aggregate stability was determined on 24 replicates from each field using turbidimetry. Part of the sample from each field was subjected to standard periodate treatment as previously described and the aggregate stability subsequently determined by turbidimetry on aggregates dried over phosphorous pentoxide.

4. RESULTS AND DISCUSSIONS

4.1 Preliminary Experiment to Study Factors Affecting Aggregate Stability in Soils.

An experiment was carried out using the five Stirling soils (Table 3.1) to compare the two methods of aggregate stability determination and to use these methods to assess the relationship between cropping practices and aggregate stability and the relationship between some soil constituents and aggregate stability.

4.1.1 A Comparison of Aggregate Stability Tests

Two methods were examined for assessing soil aggregate stability, namely wet sieving and turbidimetry. The results from turbidimetry are plotted in Figure 4.1 against the results from wet sieving tests, using both slow and rapid wetting procedures. The correlation coefficient of 0.98 (Table 4.1) indicates that turbidimetry and wet sieving are closely related, reflecting the similarity of the wetting processes used. Figure 4.1 however, hints that the relationship between the two methods with flood wetting may not be a simple linear one. Using capillary wetting procedure a correlation coefficient of 0.96 (Table 4.1) was obtained between turbidimetry and wet sieving. Figure 4.1 also shows that in wet sieving there are highly significant differences between the stability of aggregate pre-wetted by capillary action and those rapidly immersed in water prior to shaking. For the turbidimetry test, the two wetting procedures did not produce significant differences in stability. In both

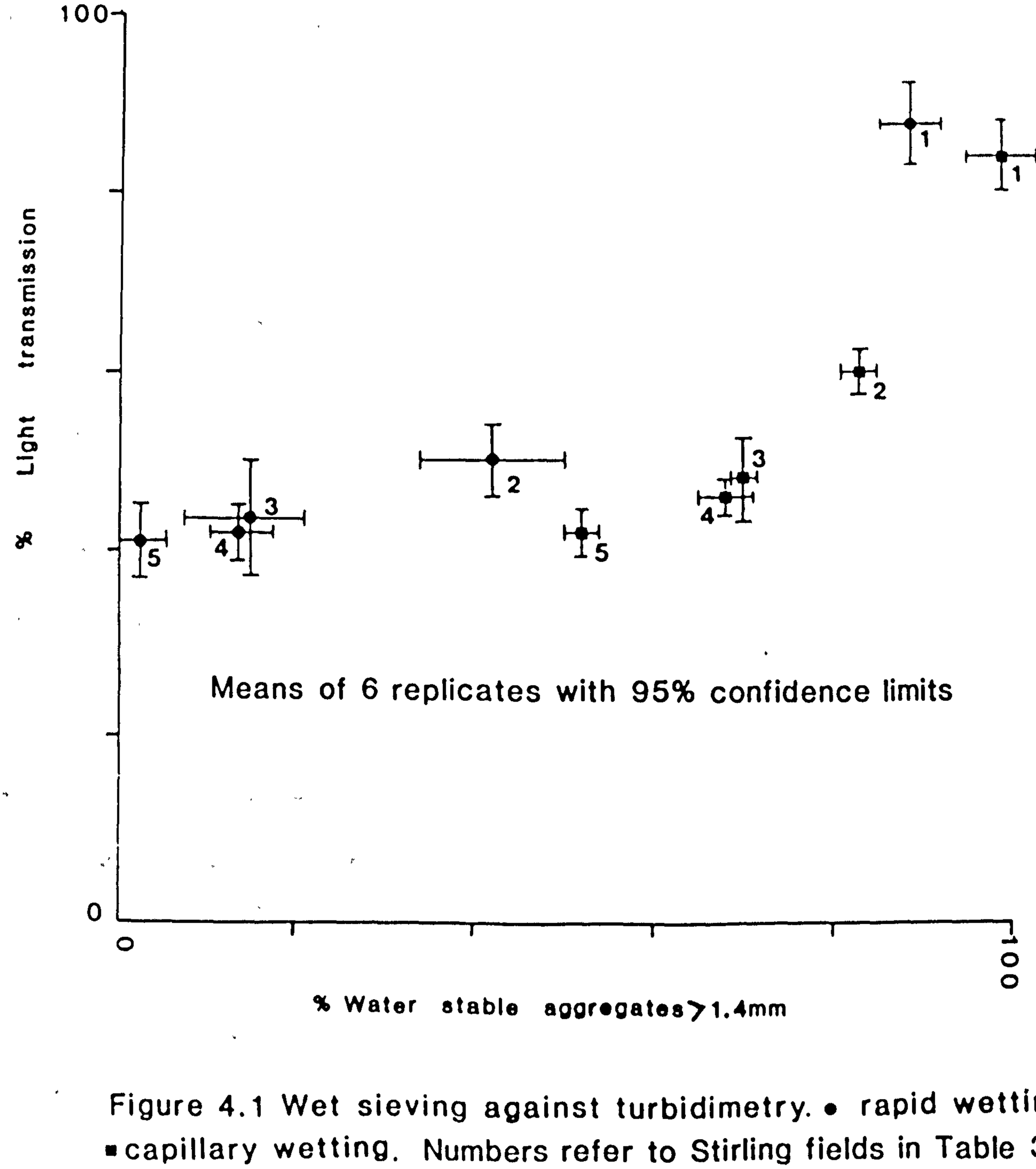


Figure 4.1 Wet sieving against turbidimetry. • rapid wetting, ▪ capillary wetting. Numbers refer to Stirling fields in Table 3.1

turbidimetry and wet sieving, there were highly significant correlations between the stability values obtained using both wetting procedures as shown in Table 4.1 which explains the similarity in the two methods used in differentiating between soils of low inherent stability from those with high stability as resulting from management practices on these soils.

The insignificant differences of the aggregate stability values obtained using both wetting procedures in turbidimetry are surprising. When dry aggregates were directly immersed in water (flood wetting) a series of air bubbles could be seen escaping from the aggregates, the aggregates became swollen, and some of those from cultivated plots parted along cracks which formed but did not collapse, unless they were agitated. The Stirling 1 soil aggregates remained intact even after 7 minules end-over-end shaking. Whilst stability test employing rapid wetting procedures have been criticised (Emerson, 1954) the results obtained in the present study using turbidimetry with rapid wetting hints that air entrapment may not be the primary cause of slaking on rapid wetting, but that other processes such as differential swelling in aggregates containing clay and the energy of hydration upon water entry may be the major contributing factors to aggregate slaking.

When aggregates which were pre-wetted by capillary action were immersed in water, there were no immediate signs of slaking, and most of the aggregates from both pasture and arable plots remained intact throughout the shaking period. Surprisingly in the turbidimetry method, whether the aggregates slaked or did not slake (rapid and capillary wetting respectively) there was resultant turbidity with end-over-end

shaking. This suggests that dispersion of the clay is the major process by which these aggregates are being degraded during shaking in water. This clay sized fraction which is being dispersed from the aggregates during shaking plays a major role in soil degradation process (Middleton, 1930; Morgan, 1979; Hudson, 1981). These results indicate how the turbidimetry method is very close to simulating field conditions of how aggregates are disintegrated by water. These results also point to the limitation of the wet sieving method in studying mechanisms of aggregation and probably soil erodibility, and other related problems of soil structural degradation, such as formation of crusts (McIntyre, 1958; Agassi et al., 1985; Ben-Hur et al., 1985).

4.1.2 Relationships of Soil Constituents to Aggregate Stability.

Several analyses were carried out on the five Stirling soils to see if there was any particular soil constituent which was closely related to the obtained values of aggregate stability from wet sieving and turbidimetry tests. The measurements were: - organic carbon, plastic limit, percentage clay, silt and sand. The results of these determinations are given in Table 4.1.

Differences among the five fields in aggregate stability (Figure 4.1) were most closely related to differences in organic carbon content which in turn reflected cropping history of the soils (Table 3.1). This demonstrates the role of organic compounds in stabilising soil aggregates

Table 4.1 Correlation Coefficients of Aggregate Stability Measurements and Soil Properties

	Turbidimetry, flood wetting	Turbidimetry capillary wetting	Wet sieving flood wetting	Wet sieving capillary wetting	Organic carbon	Plastic Limit	% Clay	% Silt
Turbidimetry Capillary wetting	0.97**							
Wet sieving, flood wetting	0.98**	0.99**						
Wet sieving, capillary wetting	0.84	0.95**	0.90*					
Organic carbon	0.98**	0.99**	0.97**	0.82				
Plastic Limit	0.92*	0.85	0.84	0.77	0.86			
% Clay	0.76	0.78	0.79	0.93*	0.67	0.81		
% Silt	-0.89*	-0.90*	-0.87	-0.84	-0.92*	-0.87	-0.69	
% Sand	0.63	0.50	0.57	0.37	0.73	0.55	0.12	0.80

* $P<0.05$ **$P<0.01$

(Emerson, 1959; Greenland, 1971; Russell, 1973; Allison, 1973) but there was a difference between slow and rapid wetting in wet sieving test. The correlation between organic carbon and wet sieving stability after capillary wetting was not significant. All the other stability test procedures gave highly significant correlation coefficients with organic carbon. These results reflect the role of organic compounds in stabilising soil aggregates through formation of inter-particle bonds and by reducing the wetting rate and therefore breakdown forces associated with water entry.

When the relationship between organic carbon and aggregate stability by turbidimetry was examined using all soils in Table 3.1, the correlation coefficient of 0.75 ($P<0.05$) was obtained. This result shows that even when soils with a wide range of textures are examined, organic carbon is still the major constituent influencing aggregate stability. The results however indicate that in different localities and in various soil types, particular organic matter constituents may have varying influences (Chaney and Swift, 1984). For example Bromyard 1 soil which had been under pasture for a long period (Table 3.1) had organic carbon content of 3.7% and was more stable than the Stirling arable plots, which had carbon content of 5 to 7%. Although no attempt was made in the present study to fractionate the organic matter in these soils, other investigators (Hamblin and Greenland, 1977; Hamblin and Davies, 1977; Cheshire et al., 1983; Chaney and Swift, 1984) have shown significant correlations between particular organic matter fractions (polysaccharides and humic materials) and aggregate stability.

Fairly high correlations were obtained between clay and aggregate stability with all test procedures for the Stirling soils (Table 4.1), but the only significant correlation coefficient ($p < 0.05$) was obtained with wet sieving after capillary wetting. In flood wetting methods the influence of organic matter on the rate of wetting probably masks any effects of clay content on aggregate stability. When texture was varied by including the Bromyard and Dreghorn soils, (Table 3.1) the apparent influence of clay on aggregate stability was greatly reduced, and the correlation coefficient obtained between turbidimetry stability and clay was 0.25.

Plastic limit moisture content correlated significantly only with aggregate stability measured by the turbidimetry test employing rapid wetting procedure. The relationship between these two however does not permit clear inferences on any causal connection. Variations in turbidimetry results reflect differences in organic matter, and organic matter may also be influencing plastic limit. Clay and organic matter interact to control plastic limit moisture content and the relationship was found to be highly significant at $p<0.01$ when examined by correlation and regression analysis for the Stirling soils. Similar interactions between organic matter and clay in influencing plastic limit moisture content have been observed by Archer (1975).

4.1.3 Discussion

When soils in Table 3.1 are ranked in order of decreasing aggregate stability the order can be seen to be approximately the same as

the order if the soils are ranked according to their history of cultivation. This supports the generally held view that aggregate stability declines under arable cultivation (Clarke and Marshall, 1947; Low, 1955, 1972; Clement, 1975; Eagle, 1975). Cultivation depletes the organic matter content of the soils due to microbial oxidation (Lynch and Panting, 1980) and causes physical destruction of the soil aggregates (Rovira and Graecen, 1957). When soils are cultivated, the physical breakdown of the aggregates promotes the interaction between microorganisms and organic substrates initially protected within the clay lattices. This leads to increased microbial oxidation of the substrate and subsequent depletion of the organic matter store of the soils. Thus the lower levels of stability of the soils in Table 3.1 which have been under continuous or alternating pasture and arable cultivation is a result of the interaction of the two processes referred to above.

The other major differences among the sites as noticed in the soils during sampling was the root density which differed considerably between the pasture and arable plots. The higher stability of the Bromyard 1, Stirling 1 soil aggregates well agree with the views of Russell (1971, 1973) that plant roots are of primary importance in the formation of a stable soil structure. Although the other fields were sampled during the pasture phase of rotation (Stirling 3 and 4 were under Timothy grass ley at the time of sampling) the root density was low and the roots did not penetrate to greater depth as compared to the Stirling 1 and 2 plots. The other striking feature of the soils was in the size of the clods that were sampled from arable and pasture plots. For example a large clod from the Stirling 5 arable plot was over 25 cm in

diameter. A comparison of this soil with the Stirling 1 soil which contained granular aggregates up to 1 cm diameter, illustrates the effect that permanent grass can have on improving structure. The largest clods of the arable plots were probably a result of compaction due to overtilling and machinery traffic on moist soil during the cropping season.

The differences in magnitude of the effect of roots of different crops on aggregate stabilisation is well established, and the results obtained here are in agreement with those of previous workers (Williams and Cooke, 1961; Emmond, 1971; Tisdall and Oades, 1979, 1980a, 1980b; Reid and Goss, 1981). This ability of roots in stabilising aggregates is two-fold. Plant roots, especially those of perennial grasses support a large population of microorganisms such as bacteria, fungi and actinomycetes which produce mechanical or chemical stabilisation of the soil aggregates, and roots themselves produce mucigel to which soil particles adhere (Tisdall and Oades, 1982; Oades, 1984). On the other hand roots mechanically force larger lumps apart during growing season, whilst at the same time they cause dehydration of the soil mass, thus forcing the finer aggregates into closer packing (Russell, 1971; Reid et al., 1982). Upon dying, roots leave behind a porous structure and the decomposed residues provide substrate for microbial population. Thus roots are important in structural stabilisation but also affect other soil properties beneficial to soil productivity.

The incorporation of pastures in farm management as evidenced by the results obtained in the present study should therefore be

encouraged. However, it should be noted that recovery of structure under pasture is not a simple matter. Low (1955) reported that periods between 5 and 50 years, depending on soil texture, were required to restore the stability of old arable soils to levels comparable with those under permanent grass. This is evidenced by the results of Stirling 2 soil, which has been under 20 years of pasture, but still significantly below that of Stirling 1, in both organic matter content and aggregate stability.

Using the Stirling series soils, there was a strongest relationship between clay content and aggregate stability by wet sieving applying capillary wetting procedure (Table 4.1). In flood wetting tests the effects of clay content on aggregate stability are masked by the influence of organic matter. Grieve (1975) found that the severity of flood wetting tests was more pronounced in soils of higher clay contents and that soils with ca. 12% clay did not suffer much from flood wetting tests. Chaney and Swift (1984) using soils with narrow range of textures found no significant correlation between clay content and aggregate stability.

The results obtained in Table 4.1 are elusive in that, when the relationship between clay content and aggregate stability was examined using soils with a wide range of textures the correlation coefficient obtained was too small and in no way approached significance. In earlier work (McHenry and Russell, 1943; Clarke and Marshall, 1947; Kemper and Koch, 1966) it was concluded that clay is the major constituent influencing aggregate stability. It is thus surprising in that there

seems to be no agreement in the results available so far as to the influence of clay on aggregate stability. The results obtained in the present study however hint that generalisation made as to the influence of clay on aggregate stability should be confined to textural classes and that the wetting procedure used to derive the relationship should be specified, as the latter gives different emphasis to the effects of organic matter.

Whilst other properties such as sand (Williams, 1970) and silt (Thomasson, 1978) have been cited as soil constituents which contribute largely to aggregate stability, these did not make any significant contribution in the present study. The significant correlation obtained between plastic limit moisture content and aggregate stability is explained by the relationship between this property and the clay plus the organic matter which are both reflected in the stability of aggregates as measured by turbidimetry. The plastic limit test is important in that it yields valuable information on the maximum moisture content at which the soil may be safely cultivated. The Agricultural Advisory Council Report (1970) suggested that soils should not be cultivated at moisture contents greatly in excess of plastic limit. Thus Stirling 1 field can be safely cultivated at moisture content more than 9.8% greater than Stirling 5, field. The significance of such reduction in the latitude of cultivation has been stressed by Russell (1971), particularly for crops such as sugar beet which are harvested late in the year.

4.1.4 Assessment of Merits of Tests

The two methods used to test aggregate stability in this study are consistent with each other in reflecting the cultivation histories of the fields, and the selection between them appears to be a matter of convenience. Although aggregate stability measurements by wet sieving after slow wetting are useful for demonstrating small differences between soils of low stability, such as those of Stirling 3, 4 and 5, for most purposes the most useful test for aggregate stability is turbidimetry. Each turbidimetry test requires 0.25 g soil and occupies only 9 minutes with simple apparatus. Replication is easy with turbidimetry. By contrast, wet sieving requires 50 g soil per replicate and even with flood wetting takes 15 minutes plus drying time (usually overnight). Tests involving prewetting of samples require even more time.

The turbidimetry method is the most sensitive of the methods in that changes of particle concentration, as measured by differences in light intensity can be detected with a far greater degree of accuracy than by weighing. Although the use of turbidimetry with rapid wetting procedures has been shown to have larger technique variability (Williams et al., 1966) and this drawback was found to occur commonly in other tests employing rapid wetting procedures (Grieve, 1979a), the error bars in Figure 4.1 indicate that turbidimetry has the advantage of better reproducibility.

The two methods tried gave results which distinguished soil aggregates from fields with known structural problems and those from well

structured permanent pasture, however they both suffer from being semiquantitative. It was decided to use turbidimetry in all further experiments, mainly because it gave more reproducible results and distinguished between all soils tested, and because it uses less soil and takes less time therefore allowing better replication. It was also thought that turbidimetry would be appropriate for evaluating stability of reformed aggregates and of soils subjected to various chemical treatments.

4.2 Effects of Microbial Activity on Aggregate Stability

The contribution due to microbial population, in particular fungi and bacteria to changes in aggregate stability was examined by laboratory incubation experiments. Results from Bromyard 1 (pasture) and 2 (arable); Stirling 1 (pasture) and 3 (arable) and Dreghorn (pasture) soils (Table 3.1) are presented.

4.2.1 Incubation of Soil Moistened with Deionised Water

4.2.1.1 Aggregate Stability Change

Field aggregates (2-4 mm) from five soils which differ in aggregate stability due to cultivation history (Table 3.1) were moistened with deionised water and incubated aerobically at constant temperature. Aggregate stability was determined on 8 replicates from each soil using turbidimetry with rapid wetting over a period of 30 days.

The observed values for aggregate stability changes in field aggregates following incubation are summarised graphically in Figure

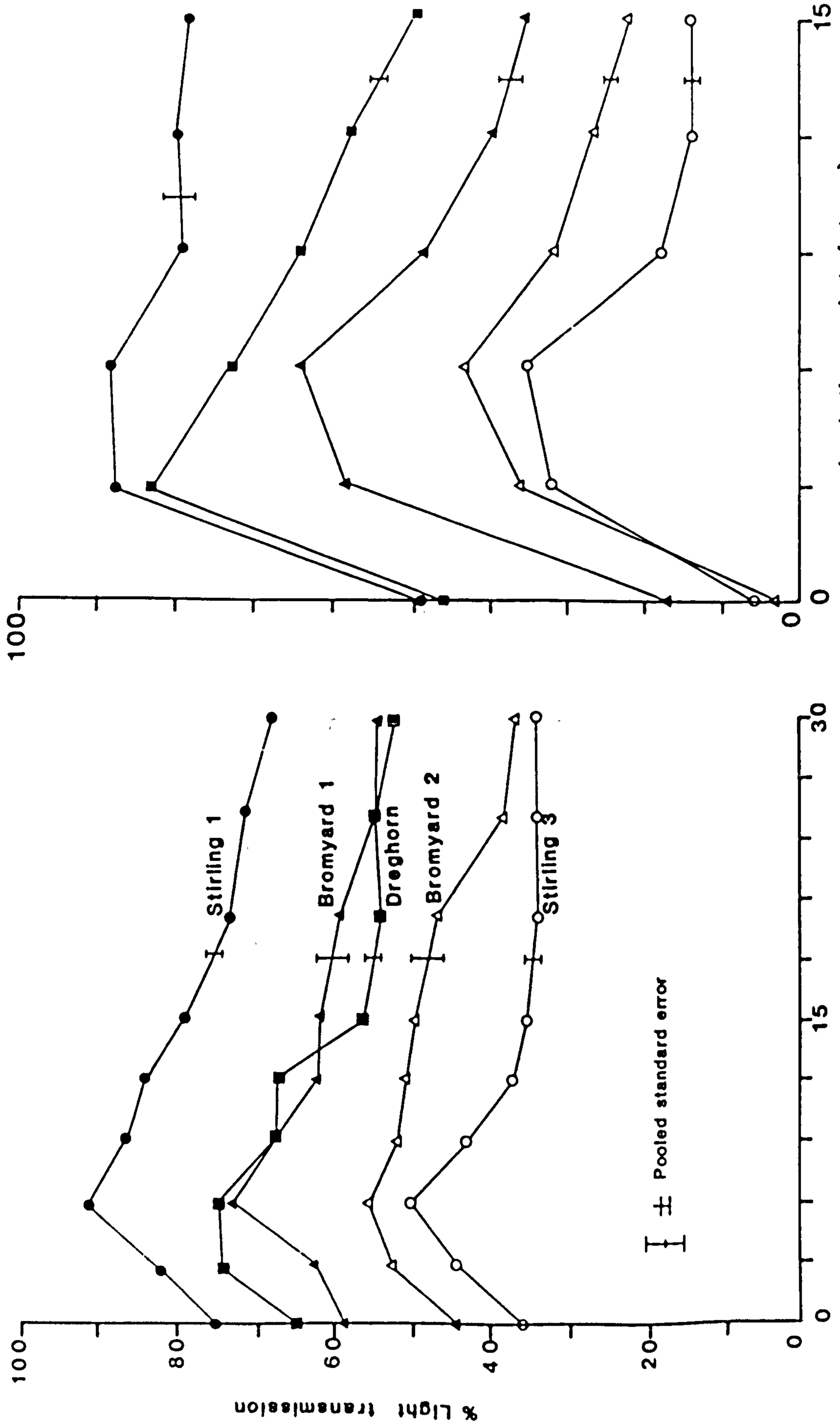


Figure 4.2a Stability change in field aggregates moistened with de-ionised water

Figure 4.2b Stability change in moulded aggregates moistened with de-ionised water

4.2(a). Inspection of the data reveal the maintenance of the order of natural aggregate stability among the soils, which is a direct result of management history of these soils as has been emphasised in section 4.1. When field aggregates were crushed and moulded, their initial stability fell as shown in Figure 4.2(b). This reduction in initial stability upon moulding is a simulation of loss of stability under field conditions when soils are manipulated by cultivation implements or the action of soil fauna. Processes and mechanisms involved in the observed changes of stability of moulded aggregates will be discussed fully in section 4.3.

When both field and moulded aggregates were incubated under similar conditions, (Figure 4.2 (a) and (b)) there was an initial increase in stability of these aggregates during the first 6 days. This increase occurred in parallel with visible fungal growth. Thereafter, the stability declined and this was accompanied by visible deterioration of the fungal mycelia. This was therefore investigated further by SEM. The stability of field aggregates fell back to levels slightly below that at 0 day during a period of 15 days, and subsequently the aggregates maintained a fairly steady level until the end of the 30 day incubation period.

4.2.1.2 <u>Microbial Interactions as Revealed by SEM</u>

Aggregates removed from the incubated soils at different days were examined with SEM. This was carried out to confirm the visual observations made of the initial flush of fungal hyphae and subsequent decline thereof. It was also aimed at establishing the succession of

microbial populations and the possible mechanisms involved in their stabilisation of soil aggregates. Typical micrographs presented here refer only to one of the soils, an example from the Bromyard series.

Plate 1a, a picture of sterilised control soil after 3 days incubation, shows the soil mineral particles and their orientation and no growth of microorganisms. After 3 days of incubation, the unsterilised soil exhibited initial fungal growth (Plate 1b). The period between 4 and 6 days is characterised by prolific growth of fungal hyphae (Plate 2a) forming a reticulum of threads confering maximum stability on the soil aggregates as shown in Figure 4.2. Development of fungal sporangia (Plate 2b) as well as the release of spores could be observed (Plate 3a).

Two types of mycelia are seen in these micrographs. There are mycelia with sticky surfaces, with adhering clay particles. This material to which clay particles are firmly attached has been identified as polysaccharides by heavy metal staining of ultrathin sections (Tisdall and Oades, 1979; Foster, 1981). Bacteria can be seen on the fungal mycelia, linked by thinner threads running throughout the sample (Plate 3b). These threads, produced by the drying process, represent bacterial polysaccharides , which are also contributing to increased aggregate stability. Similar bacterial polysaccharides have been observed adhering to fine silt particles by Fehrmann and Weaver (1978) using SEM. The other type of mycelia visible in the micrographs does not adsorb clay particles. It is therefore composed of the wax-like material described by Bond and Harris (1964).

The period of 15 to 20 days is characterised by predominance of actinomycetes (Plate 4a). Actinomycetes have been classified as feeble competitors (Alexander, 1961) but they are capable of utilising complex organic substrates such as chitin (Williams and Robinson, 1981). These organisms flourish on chitin rich media (Williams and Davies, 1965) and thus the deterioration of chitin rich fungal cell walls is attributed to lysis by actinomycetes. Bacteria also take part in this destruction of fungal mycelia (Mitchell and Alexander, 1963). In addition to lysis, grazing organisms also caused the depletion of fungal mycelia (Plate 4b). Organisms such as nematodes have been indicated as predators of fungi (Griffin, 1972). The combined effects of mycolysis and the grazing effect result in degraded and empty channels representing depleted hyphae (Plate 5a). The fungal cell walls have been digested by lytic enzymes produced by antagonistic bacteria and actinomycetes (Mitchell and Alexander, 1963) or due to autolysis by the fungi themselves resulting from nutrient deprivation (Hsu and Lockwood, 1971).

Towards the end of the 30 day incubation period all the fungal and actinomycetal mycelia were lysed, subsequently only spores of these organisms survived (Plate 5b). Survival of the spores and their response to fungistasis have been studied by Steiner and Lockwood (1970). Spores undergo characteristic morphological modifications such as formation of clamydospores, with thick double cell walls and development of sclerotia (Alexander, 1961). To ensure continuity of the fungi, and to avoid wastage of spores, spores do not germinate until a critical nutrient level exists in the soil (Ko and Lockwood, 1970). Thus spores in Plate 5b will remain dormant until a time when the nutrients in the soil are replenished and conditions made conducive for growth.

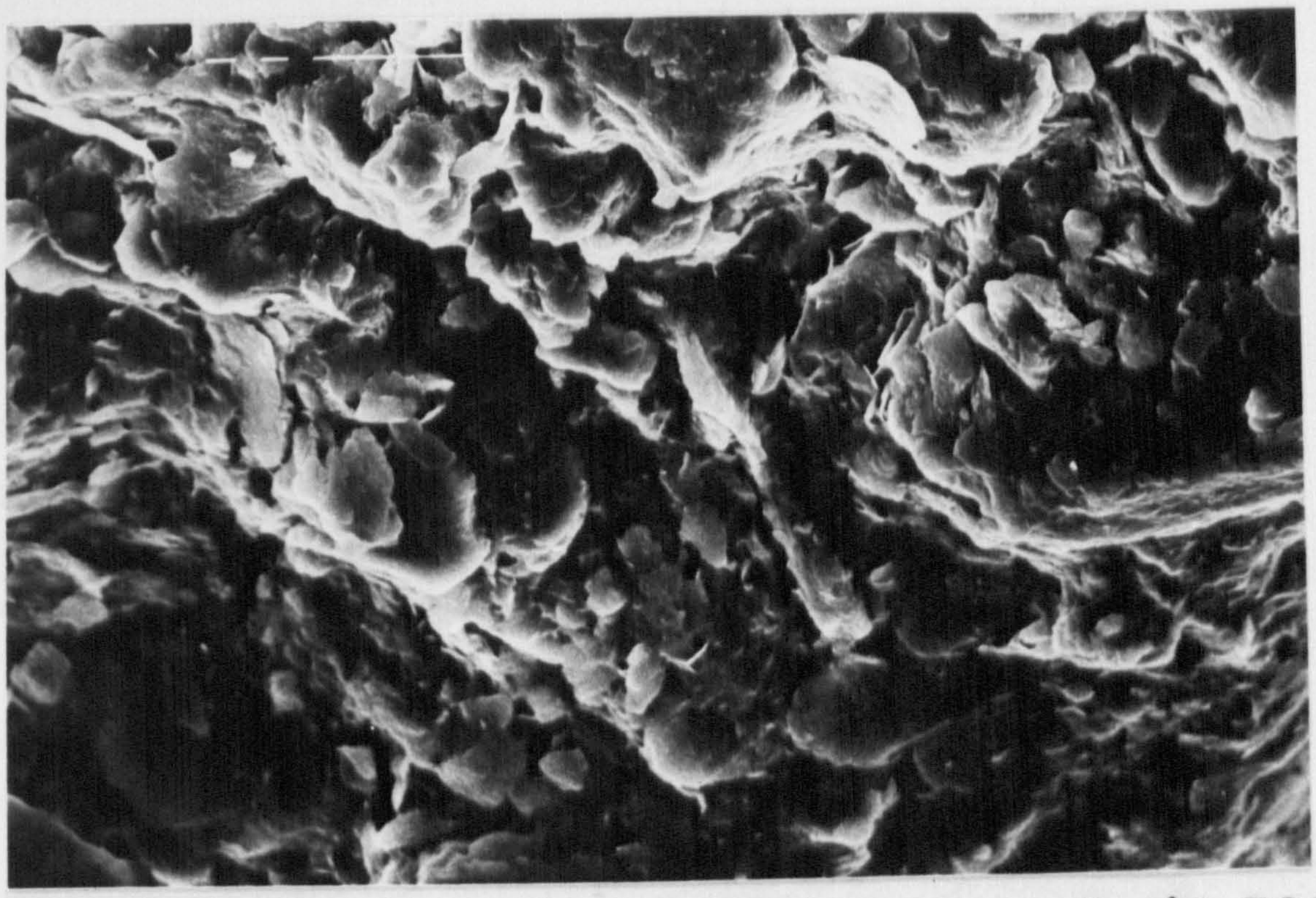


Plate 1a Sterilised physically stable soil (1 700x)

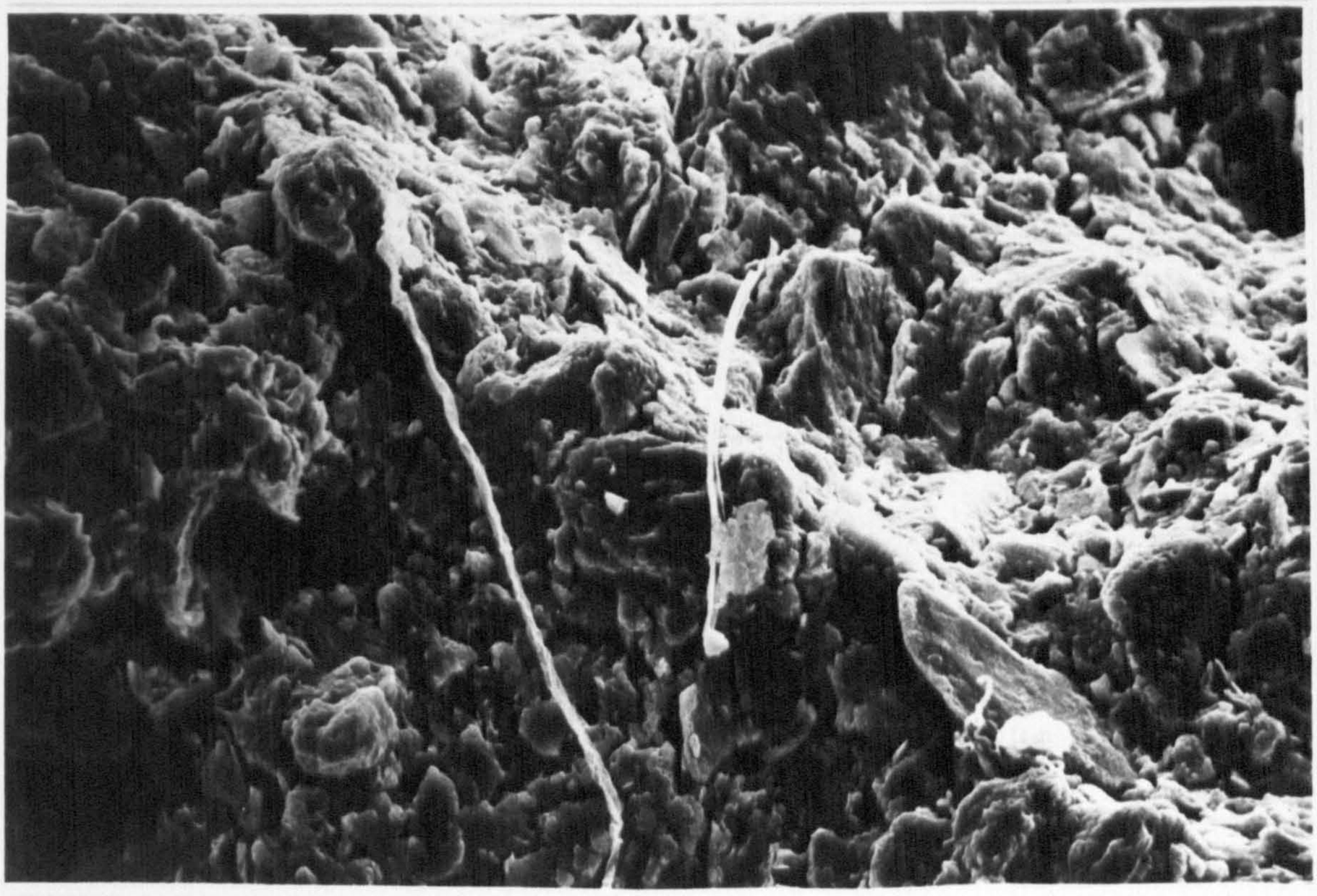


Plate 1b Initial fungal growth on soil moistened with de-ionised water (850x)

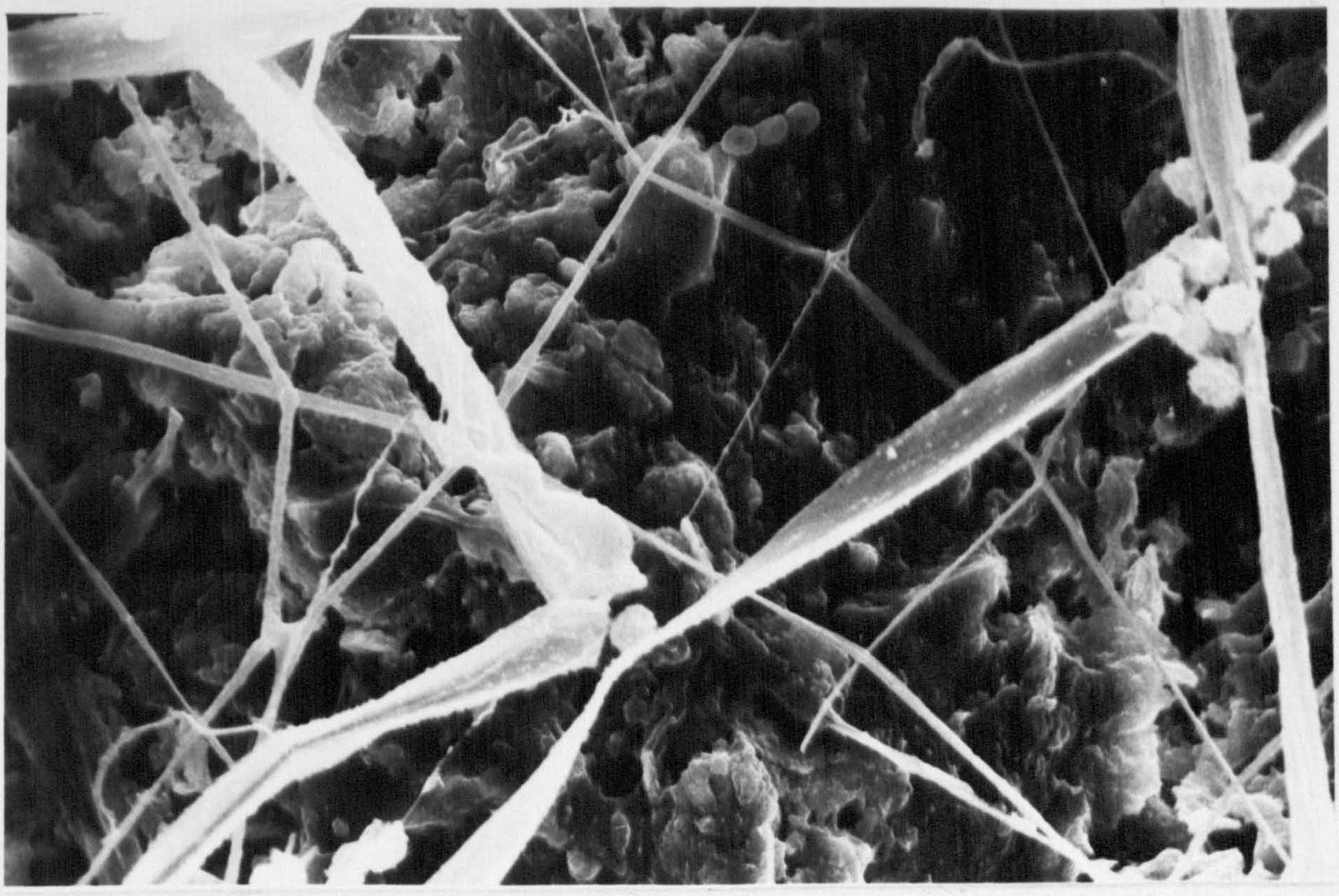


Plate 2a Development of fungal and bacterial growth after 6 days (1 100x)

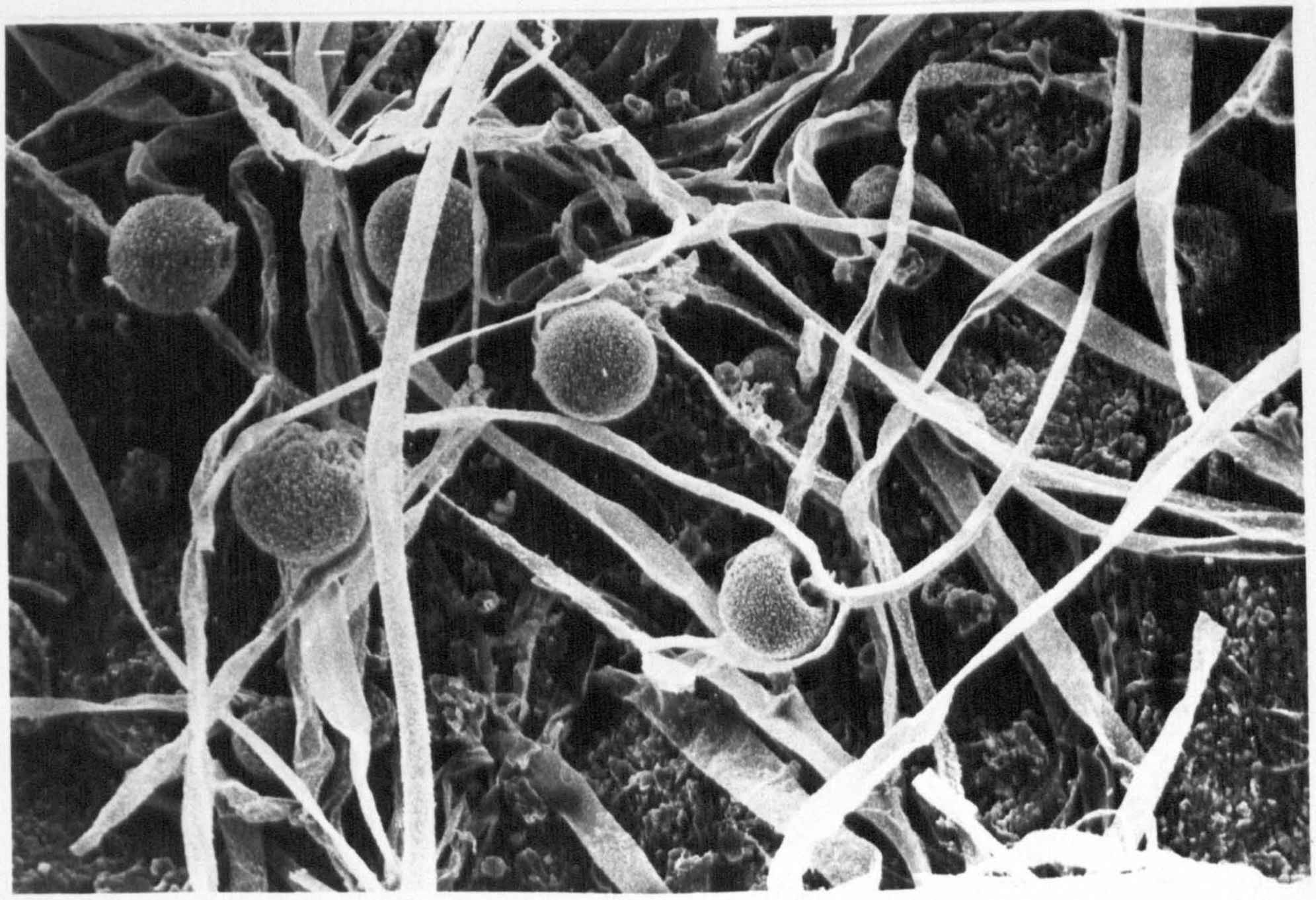


Plate 2b Matured hyphae and sporangia (300x)

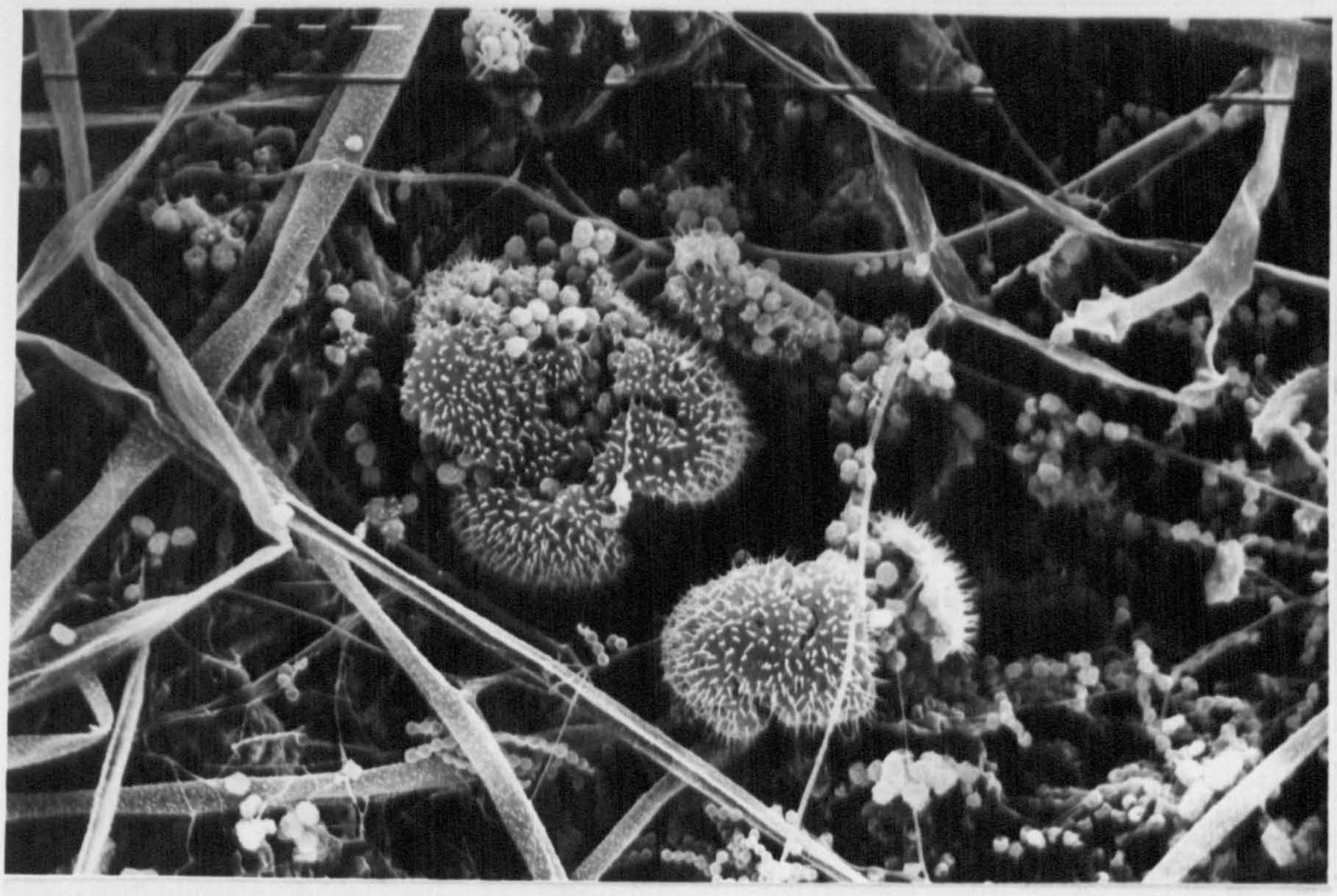


Plate 3a Matured sporangia burst and release the spores (500x)

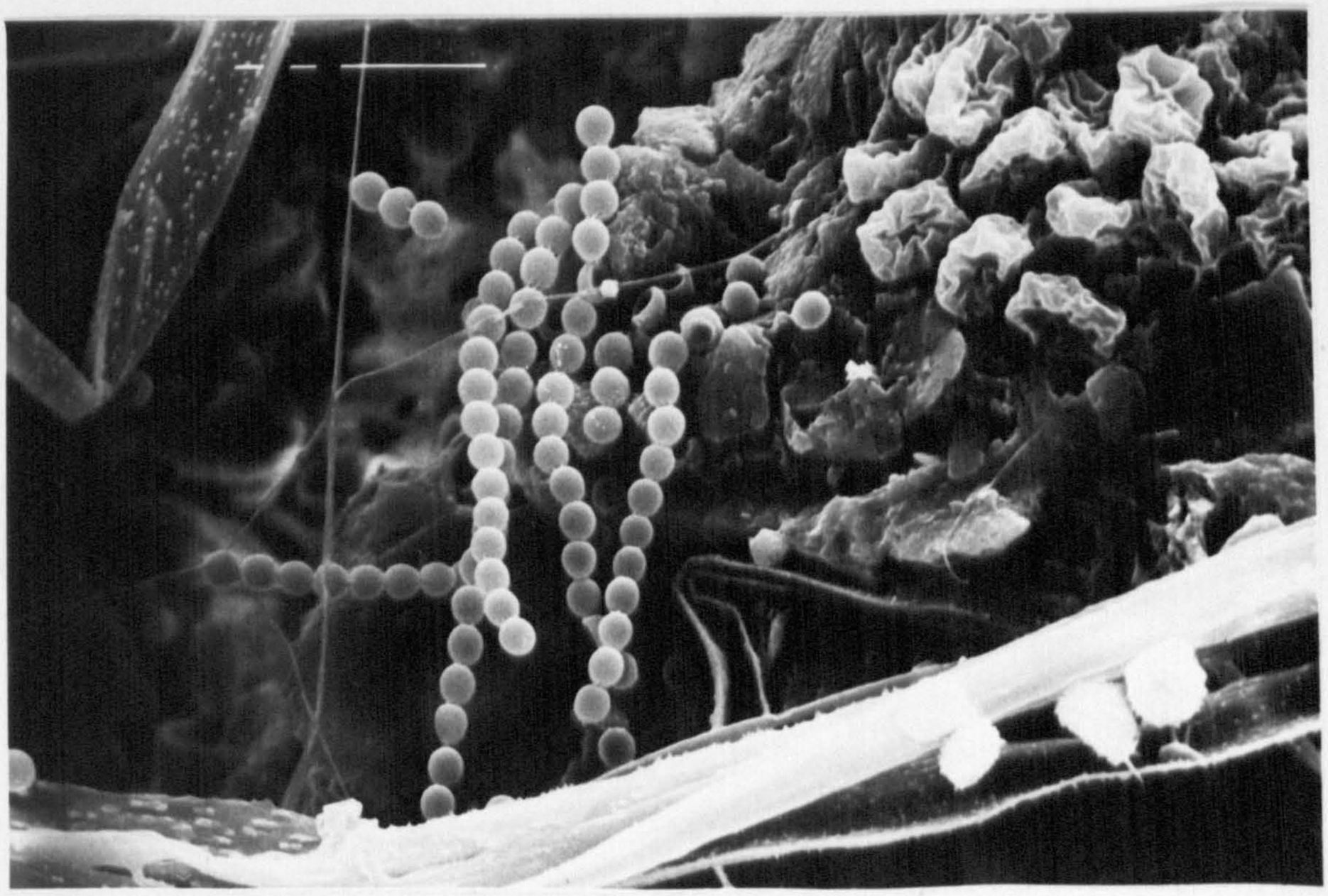


Plate 3b Characteristic spores of Penicillium sp, bacteria and polysaccharides after 9 days (1 500x)

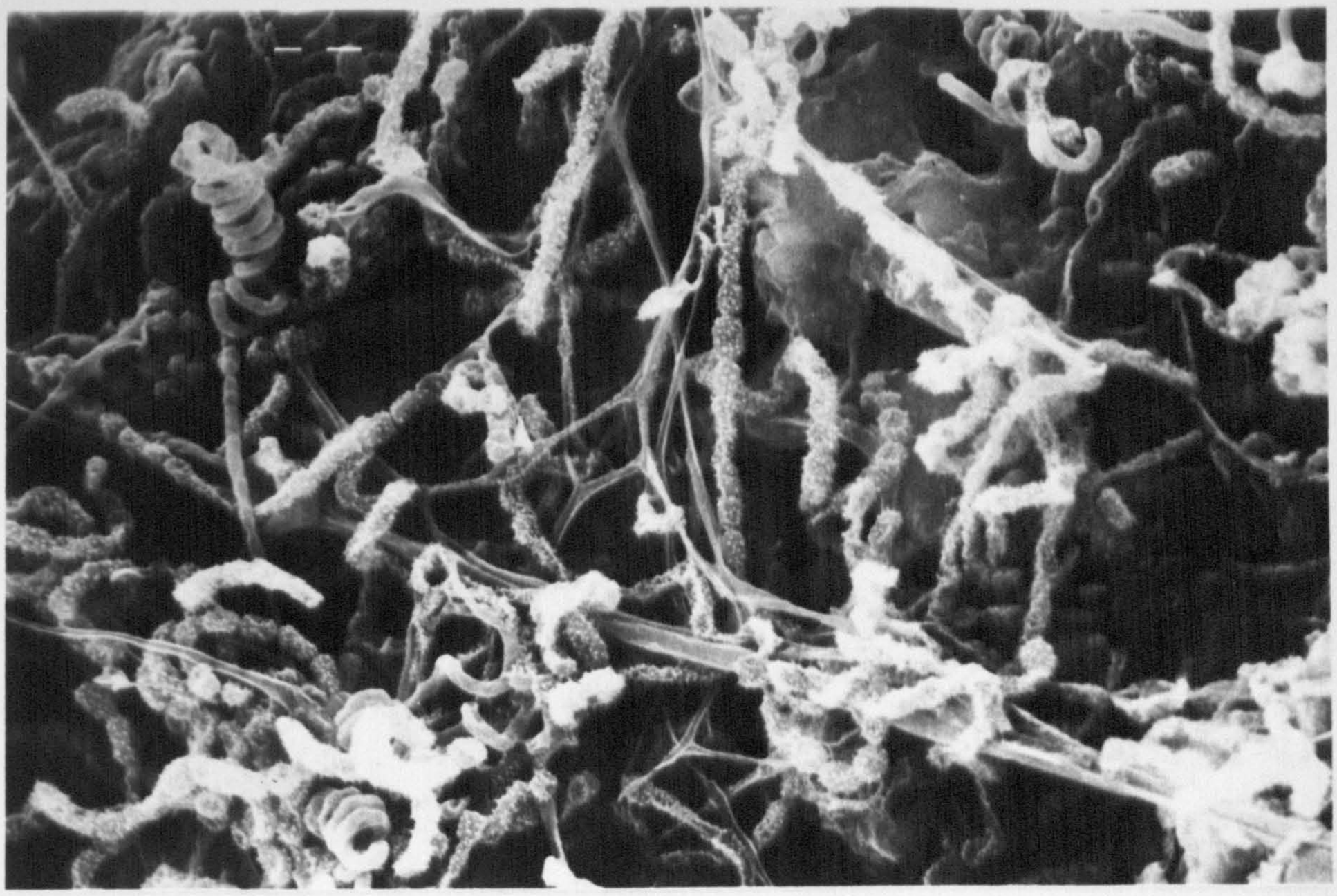


Plate 4a Fungi, bacteria and actinomycetes after 15 days incubation (3 000x)

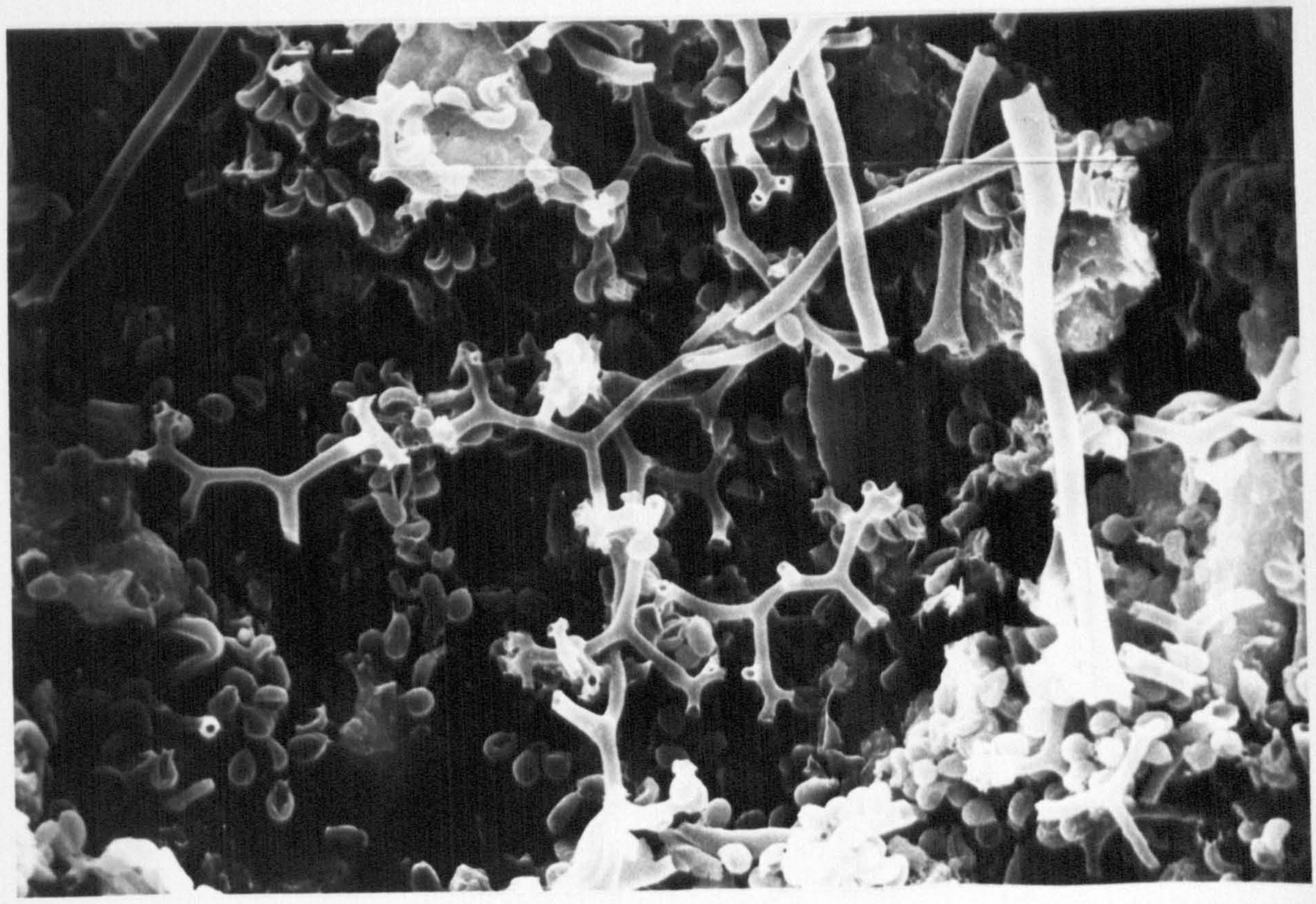


Plate 4b Damage to hyphae by unknown grazing organism (25 days incubation) (2 000x)

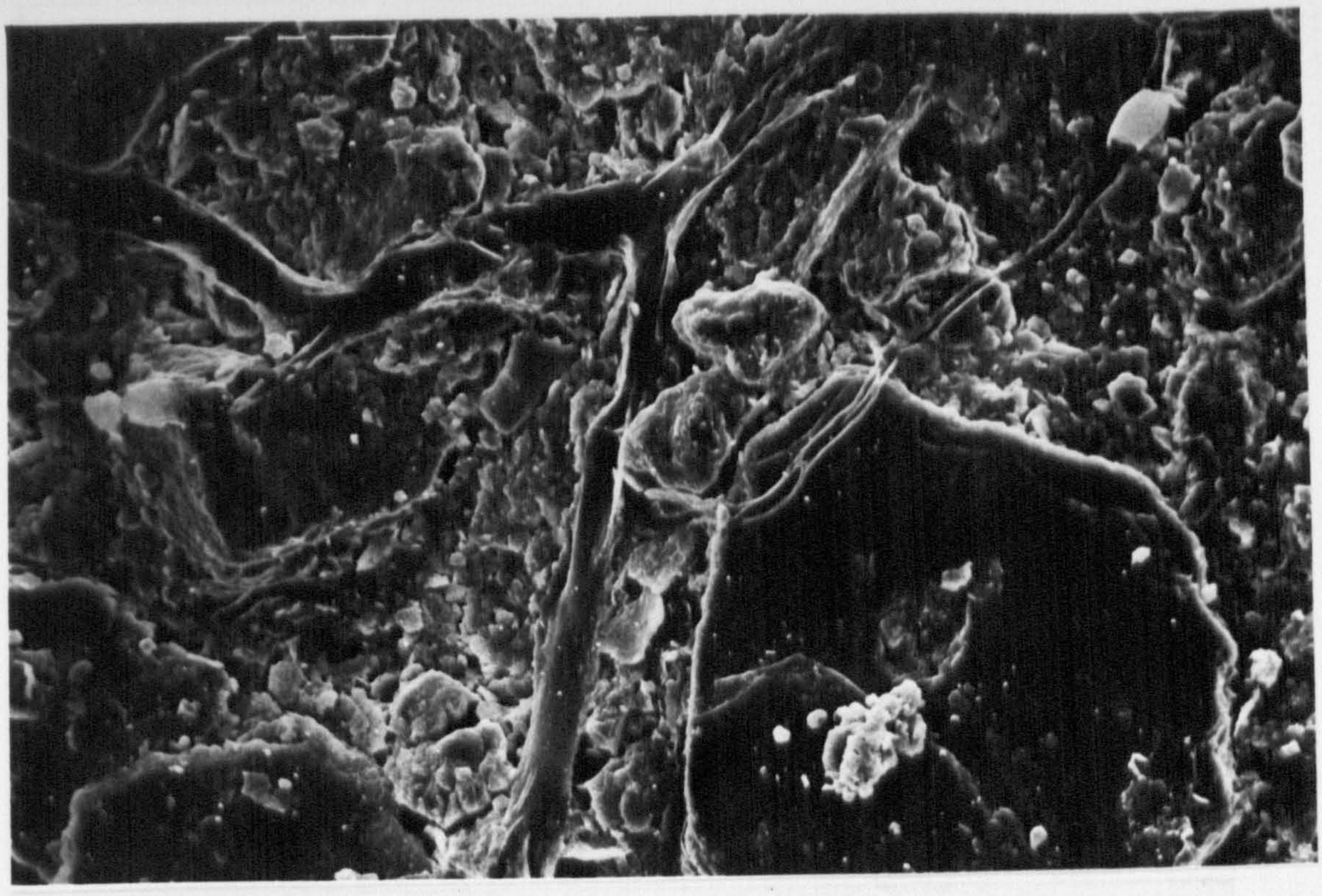


Plate 5a Ruptured and empty fungal hyphae

(30 days incubation) (500x)

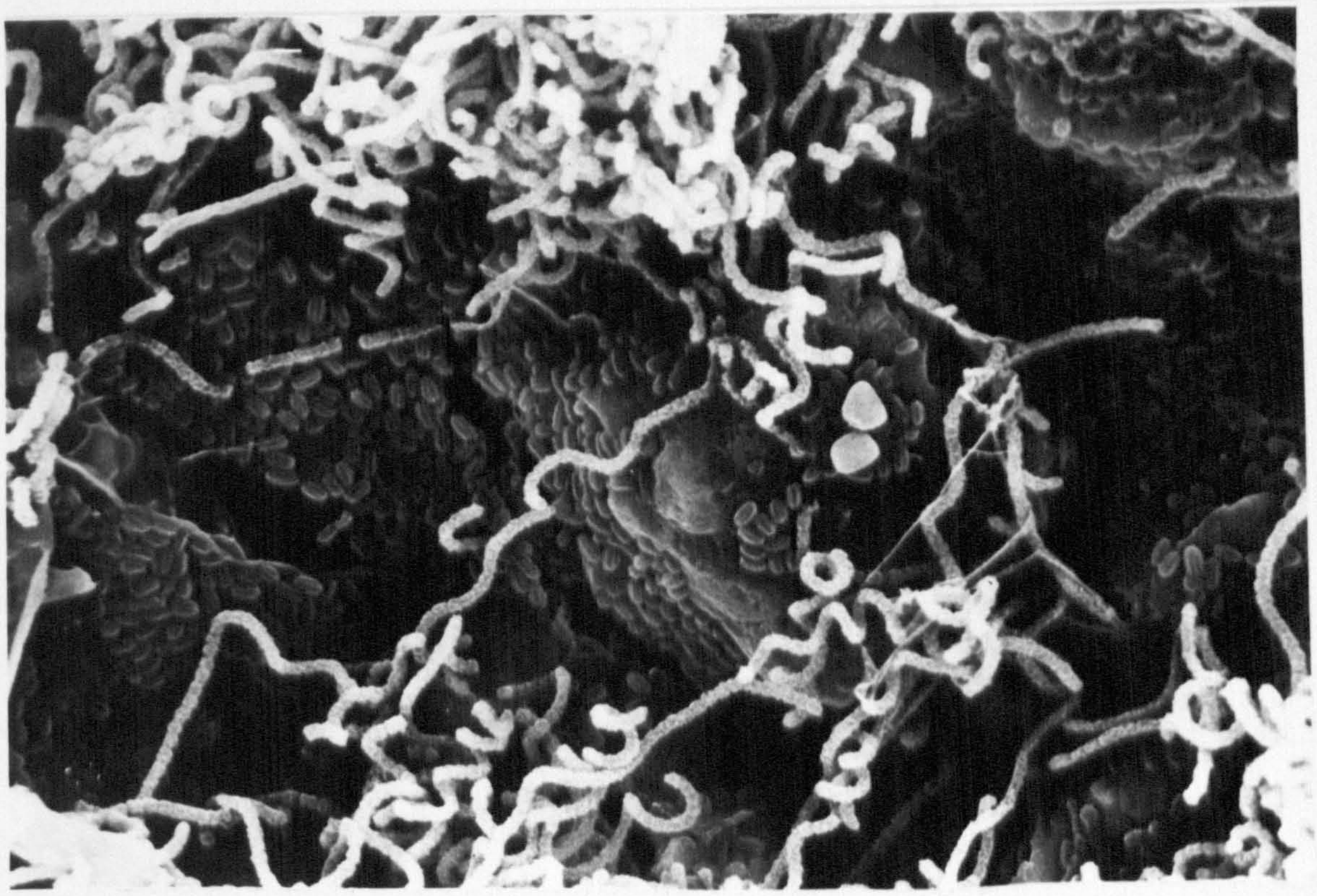


Plate 5b Disappearance of fungal and actinomycetal hyphae, spores and conidia remain after 30 days incubation (2 000x)

4.2.1.3 Measurement of Microbial Population Evolving During Incubation

The impressions gained from SEM pictures were refined by measurements of fungal growth and bacterial numbers. Fungal growth (determined by ergosterol measurement, Appendix I) reached a maximum after the first 6 days, and declined thereafter and this happened within all soils (Figure 4.3(a)). The highest ergosterol yields occurred in soils with high organic matter content (Table 3.1). This high activity of fungi in pasture soils accords with previous incubation studies which indicated that in the presence of readily decomposable substrate in soils, fungal proliferation reaches maximum levels (Tisdall and Oades, 1982). Adu and Oades (1978b) obtained similar pattern of fungal growth in soils incubated with labelled ^{14}C-glucose. In their study fungal growth was estimated as fungal pieces by method of Jones and Mollinson (1948). This period of maximum development of fungi corresponds to attainment of maximum aggregate stability in all soils as shown in Figure 4.2 and the decline in ergosterol paralleled a decline in aggregate stability.

Bacterial numbers on the other hand showed a steady development and reached a peak by the end of the 30 day incubation period as in Figure 4.3(b). As opposed to fungi, bacterial numbers did not bear any clear relationship to aggregate stability developing over 30 days. The absence of relationship between bacterial numbers and aggregate stability suggest that bacteria stabilise aggregates by some other forms of mechanisms rather than mechanical binding action. To investigate this

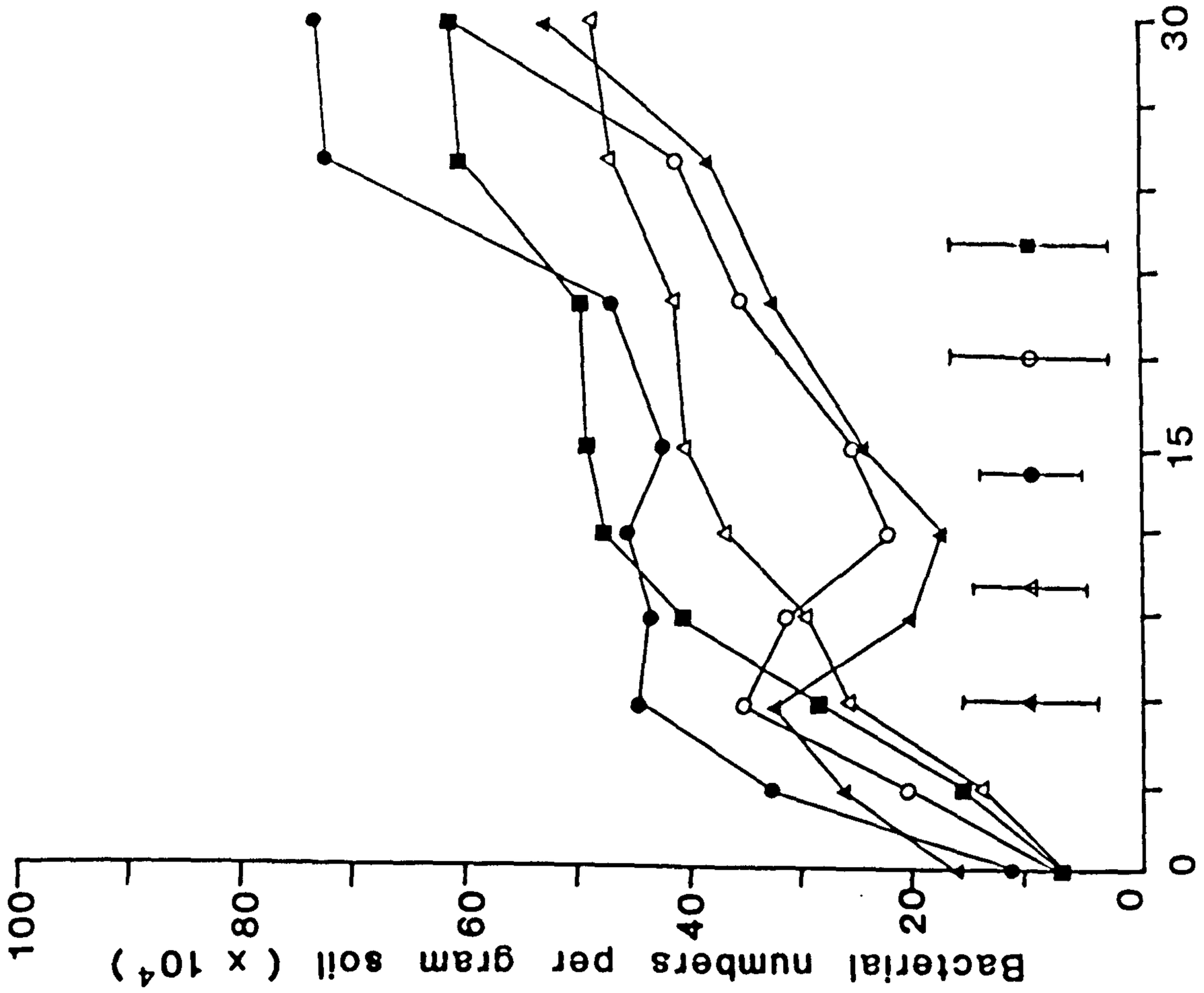


Figure 4.3a Fungal growth in soils moistened with de-ionised water (symbols as in Fig.4.2) (Single determinations).

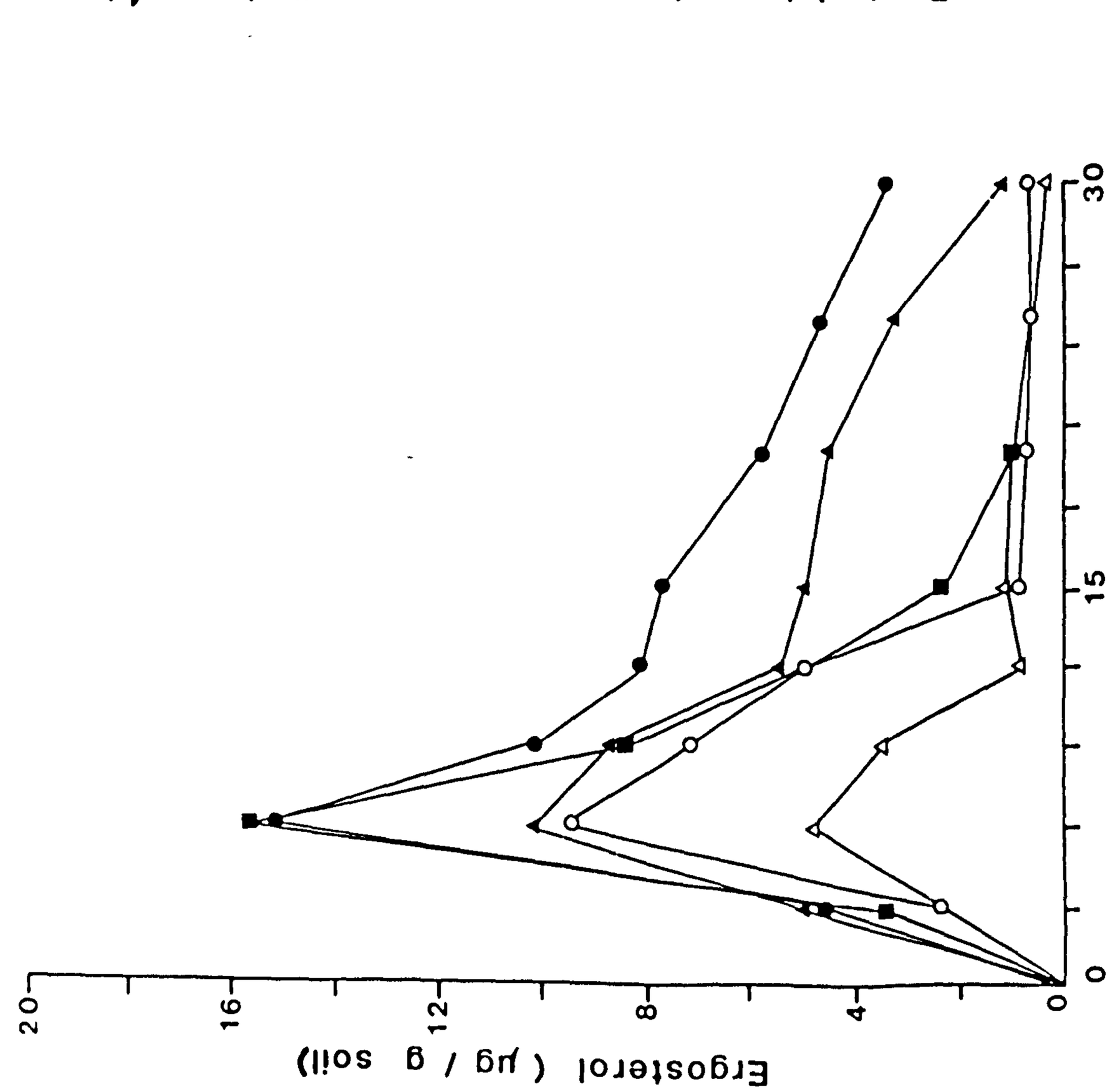


Figure 4.3b Bacterial growth in soils moistened with de-ionised water (symbols as in Fig.4.2)

further, the soil aggregates were treated with periodate to oxidise polysaccharides and the results are discussed later in this section.

When fungal and bacterial growth pattern curves are compared (Figures 4.3(a) and (b)) it becomes evident that fungal decline after 6 day period is accompanied by bacterial increase. Thus bacteria are proliferating at the expense of fungi. This pattern can be explained in terms of the process of fungistasis as propounded by Lockwood and co-workers (Lockwood, 1960; Lingappa and Lockwood, 1962; Ko and Lockwood, 1970). Evidence from their studies and other similiar ones (Griffin, 1972) suggest degradation of fungal cell walls by other organisms such as bacteria and actinomycetes or from autolysis. This process of fungal degradation has been revealed by SEM pictures in the present study.

4.2.2 Incubation of Soil Moistened with Vancomycin Solution

4.2.2.1 Aggregate Stability Change

When field aggregates were moistened with a solution of vancomycin to inhibit bacterial growth, aggregate stability increased over the first 6 days of incubation (Figure 4.4). There were big increases in aggregate stability associated with vigorous growth of fungi especially in the absence of bacteria (confirmed by plate counts). The stability obtained with this treatment was higher than the other three treatments evidencing the superiority of fungal mycelia in forming highly

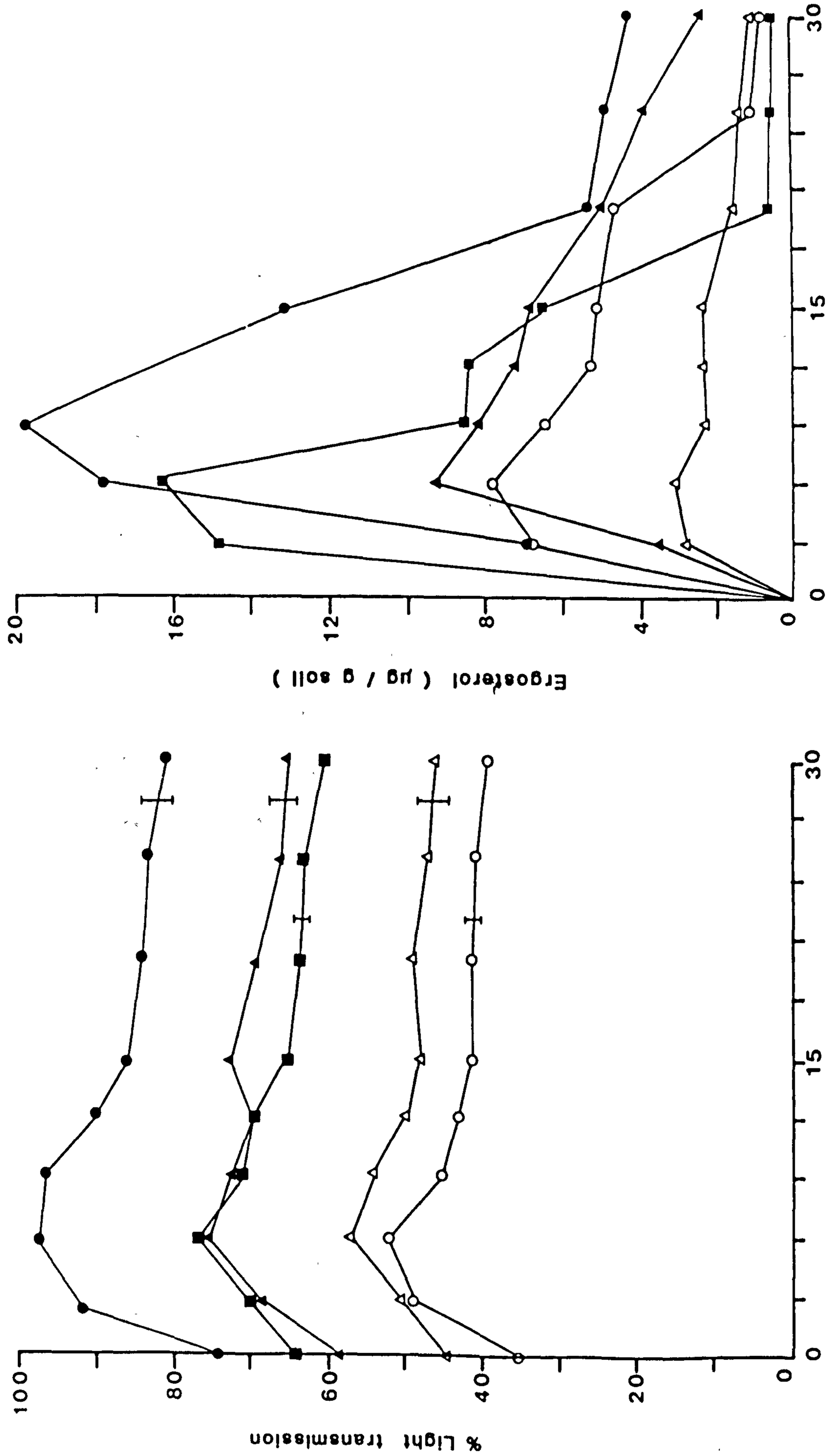


Figure 4.4 Aggregate stability change in soils treated with vancomycin (Symbols as in Fig. 4.2)

Figure 4.5 Fungal growth in soils treated with vancomycin (Symbols as in Fig. 4.2) (Single determinations).

stable aggregates. With subsequent incubation period there was only a slight decline, followed by a constant level of stability of the aggregates.

When aggregates were inspected with the naked eye, they appeared to be covered with hyphal filaments, and these were identified as filaments of actinomycetes with the aid of SEM. Thus the vancomycin treatment did not affect the actinomycetes, which have the ability to decompose fungal mycelia in search of chitin, (Goodfellow and Williams, 1983), and they replaced fungi in these soils. The decline of fungi was monitored by ergosterol measurements. Actinomycetes have been found to be second to fungi in stabilisation ability of soil aggregates (McCalla, 1946; Swaby, 1949) thus the persistence in stability of aggregates treated with vancomycin is attributed to the mechanical binding effects of these organisms. Actinomycetes have frailer filaments thus they have lower stabilising power than fungi.

4.2.2.2 Fungal Growth in Vancomycin Treated Soils

In the absence of bacterial growth resulting from this treatment, high yields of ergosterol were obtained with all but Bromyard 2 soil within the first 3 to 15 days (Figure 4.5). The ergosterol content was highest in the Stirling 1, Dreghorn and Bromyard 1 soils, which are pasture soils and have high organic matter contents. This initial high yield can partly be explained especially in the pasture soils by the abundance of readily decomposable substrate, and by the absence of competition for substrate when the bacterial growth is inhibited.

The remarkable decline in the ergosterol content of soils treated with vancomycin to inhibit bacterial growth was initially not expected. However, SEM revealed that this treatment did not affect the growth of actinomycetes, which proliferated during later stages of incubation. These organisms are slow growers and feeble competitors, thus they do not participate during the initial stages of incubation, when substrate is abundant and competition high. These organisms however, are capable of utilising a large variety of complex substrates such as chitin and other nitrogen containing substrates. Enough evidence is now available to show that actinomycetes feed on fungal mycelia, and the decline of ergosterol is evidence of fungal decline through being utilised by actinomycetes as nutrient source.

4.2.3 Incubation of Soil Moistened with Cycloheximide Solution

4.2.3.1 Aggregate Stability Change

When fungal growth was inhibited with cycloheximide, aggregate stability increased over the first 3 or 6 days as shown in Figure 4.6. For the Bromyard and Stirling arable soils the increase in stability took place over the initial 6 days of incubation, whereas in the pasture soils (Stirling 1 and Bromyard 1) the increase occurred within the first 3 days and for the Dreghorn, the increase was insignificant. The increases in stability observed with this treatment were smaller than in soils moistened with deionised water or vancomycin solution. These initial increases in stability were followed by a decline to stability levels which did not change significantly during subsequent period of

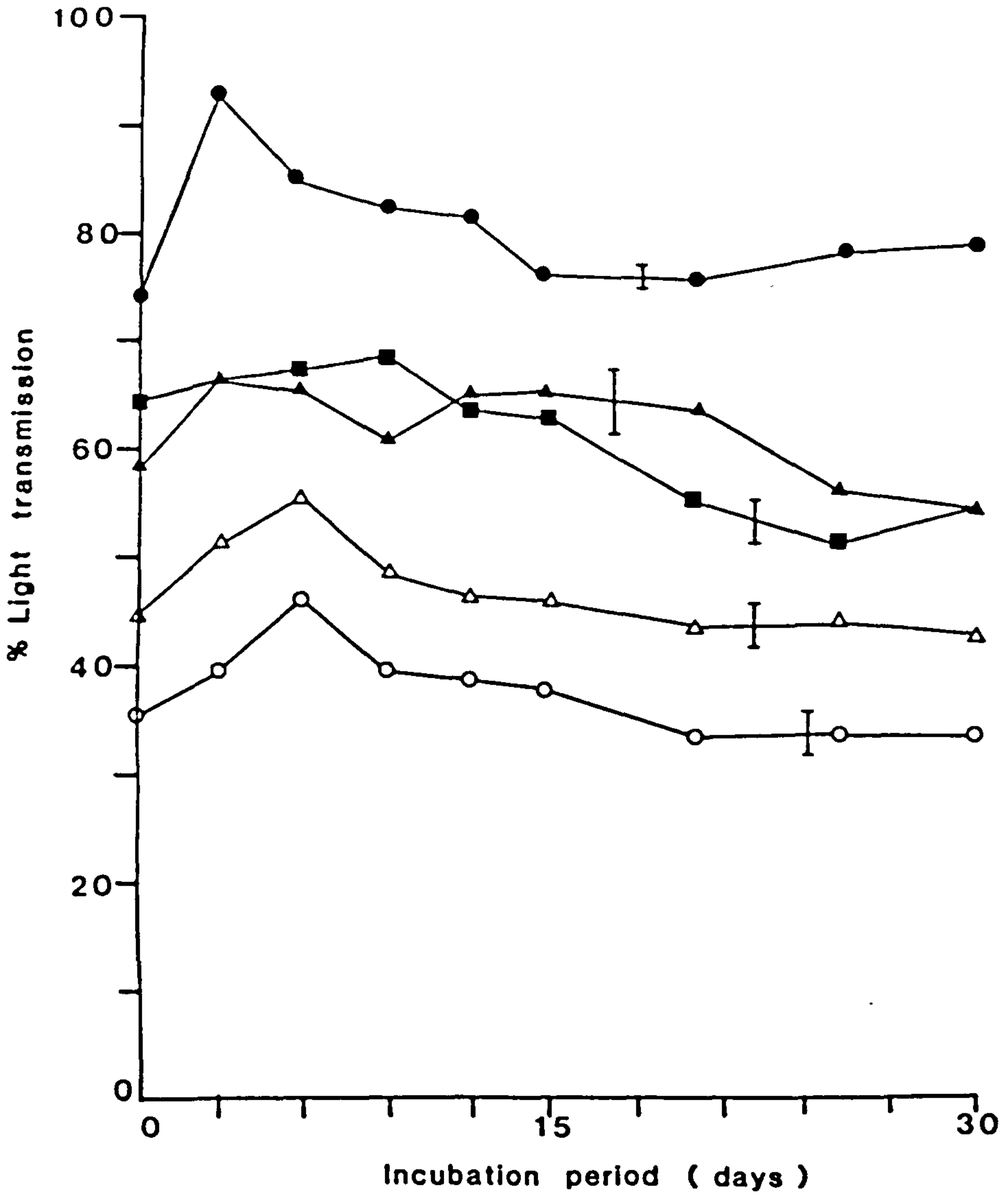


Figure 4.6 Stability change in soils treated with cycloheximide (Symbols as in Fig. 4.2)

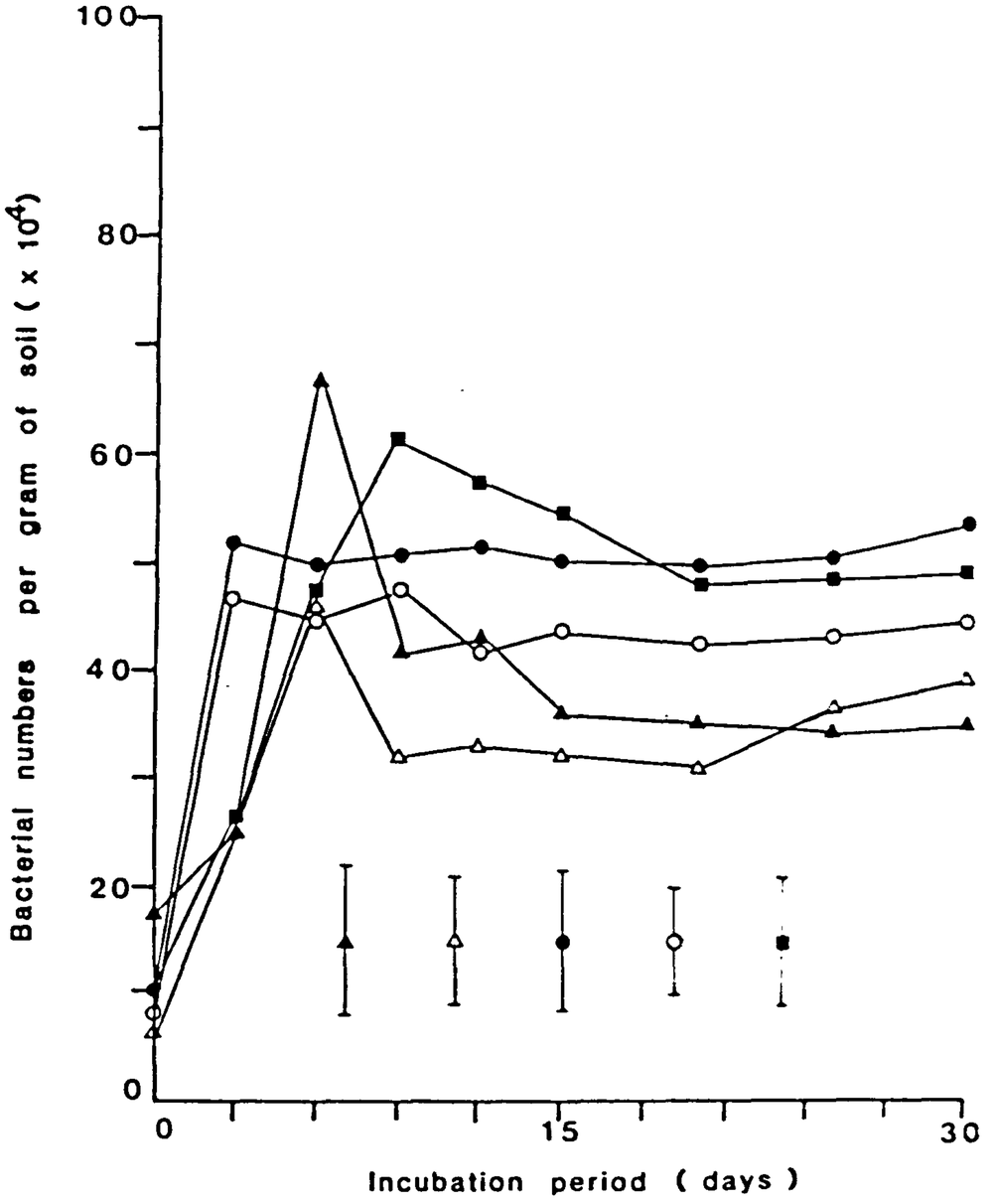


Figure 4.7 Bacterial growth in soils treated with cycloheximide (Symbols as in Fig. 4.2)

incubation. The stability levels established over 30 days of incubation were much the same as those of the initial period (0 day).

4.2.3.2 Bacterial Growth in Cycloheximide Treated Soils

In the absence of fungi, whose growth was inhibited by cycloheximide, there was an initial flush of bacteria which were enumerated by the dilution plate count technique on nutrient agar media. This initial flush (Figure 4.7) was double that in soils moistened with deionised water, in which fungi were first to proliferate. Apart from the Bromyard soils, which showed a fall in bacterial numbers after 6 days, all other soils reached a maximum after 3 days, and showed a constant level of development thereafter. Bacterial numbers in the Bromyard soils fell to equilibrium at 9 days, and subsequently maintained a steady level like the other soils. The actinomycetes, which contribute by their filamentous nature to the persisting non-changing stability, appeared during the period after 15 days, although it was subsequently shown that the stability resulting from bacteria is mainly through production of polysaccharides. The relationship and coexistence of bacteria and actinomycetes is however not well established and there are suggestions that bacteria are capable of lysing actinomycetes filaments (Alexander, 1961). The stability observed in Figure 4.6 is thus a result of the contribution of both bacteria and actinomycetes.

4.2.4 Aging of Soil Moistened with Sterilising Solution

When the soil was sterilised with sodium azide and mercuric chloride all biological activity was inhibited. Aggregate stability

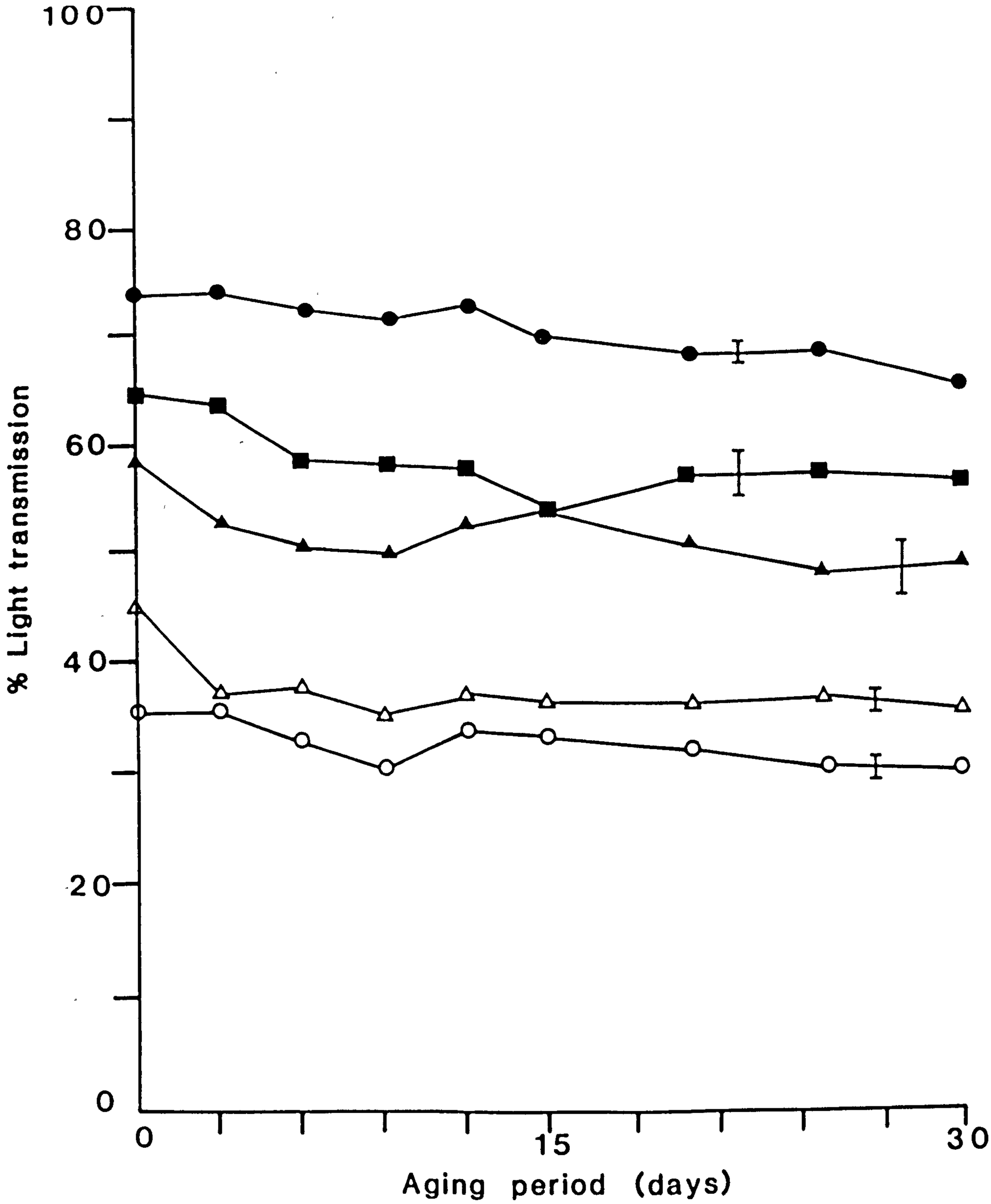


Figure 4.8 Stability of sterilised field aggregates during aging (Symbols as in Fig. 4.2)

measured over an aging period equal in length to the previous periods of incubation revealed no significant changes (Figure 4.8). The stability however remained lower than in all of the above treatments, and this contribution of biological activity in stabilising soil aggregates is evident in the present study and other previous ones (Tisdall and Oades, 1982). The success of the sterilisation treatment was confirmed by the total absence of bacterial growth using plate counts and fungal growth by ergosterol. This 'equilibrium' stability of the field aggregates obtained with little disturbance is attributed to other forms of stabilising agents, such as polysaccharides, which are in part the result of microbial activity, and this is assessed in the following discussions.

4.2.5 The Contribution of Polysaccharides in Stability of Field Aggregates

The contribution of periodate-labile organic matter (polysaccharides) in soil aggregate stabilisation during incubation of field aggregate was assessed. Soil moistened with deionised water, vancomycin, cycloheximide and sterilising solutions respectively and incubated, were subjected to periodate-borate treatment at different days throughout the incubation period.

4.2.5.1 Dispersive Effects of Sodium Ions

A preliminary experiment was carried out to assess the dispersive effects of sodium ions during the periodate-borate treatment. Aggregates

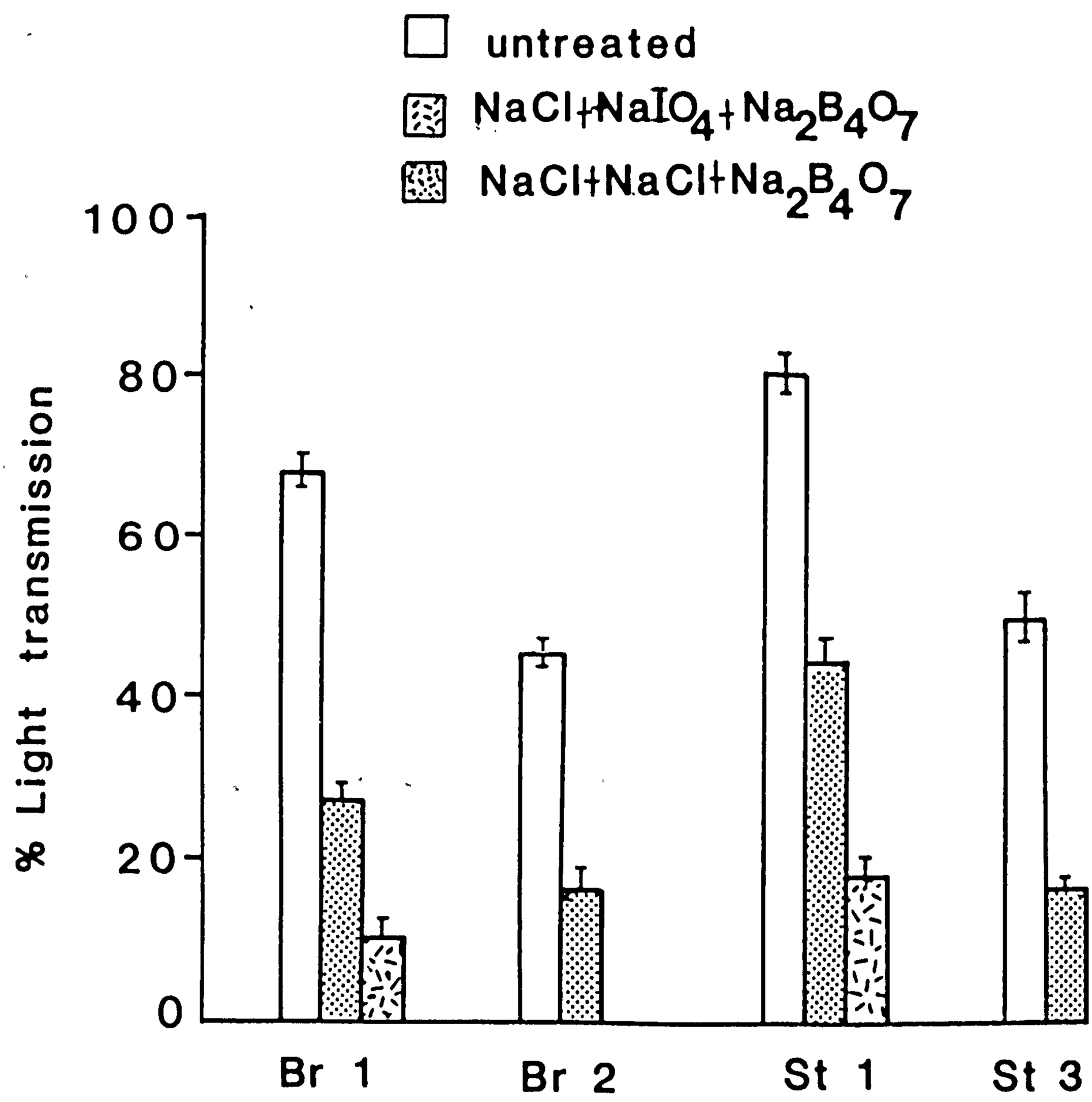


Figure 4.9 The dispersive effect of sodium ions during periodate-borate leaching

from Bromyard and Stirling soils were subjected to two treatments. Treatment 1 comprised 0.05 M NaCl, followed by 0.05 M NaCl, then 0.1 M $Na_2B_4O_7$ (pH 9.3). The second batch of aggregates were given 0.05 M NaCl, followed by 0.05 M $NaIO_4$ and lastly 0.1 M $Na_2B_4O_7$. Each treatment continued for 48 hours and the resultant stabilities were compared with those of untreated field aggregates. A substantial disaggregation of these soils was caused by sodium chloride/sodium tetraborate treatment as shown in Figure 4.9. For the Bromyard 1 and 2 soils the loss of stability was 60.3 and 64.4% respectively, and 45% for Stirling 1 and 67.3% for the Stirling 3 soil.

The periodate-borate treatment resulted in the total loss in stability of the Bromyard 2 and Stirling 3, and this accords with conclusions by Greenland et al. (1962) that polysaccharides are more important in the stabilisation of arable and young pasture soils. The periodate-borate treatment resulted in 85.3% and 77.5% disaggregation in the Bromyard 1 and Stirling 1 soils respectively. The results obtained here indicate that in addition to the loss of stability as a result of oxidation of polysaccharides by sodium-periodate-borate treatment, there is a large initial effect related to the dispersive effect of sodium ion on soil clays.

4.2.5.2 <u>Soil Moistened with Deionised Water</u>

Stability of aggregates from five sites (Figure 4.10), moistened with deionised water and incubated was greatly decreased by treatment with periodate, demonstrating the prominent role of polysaccharides

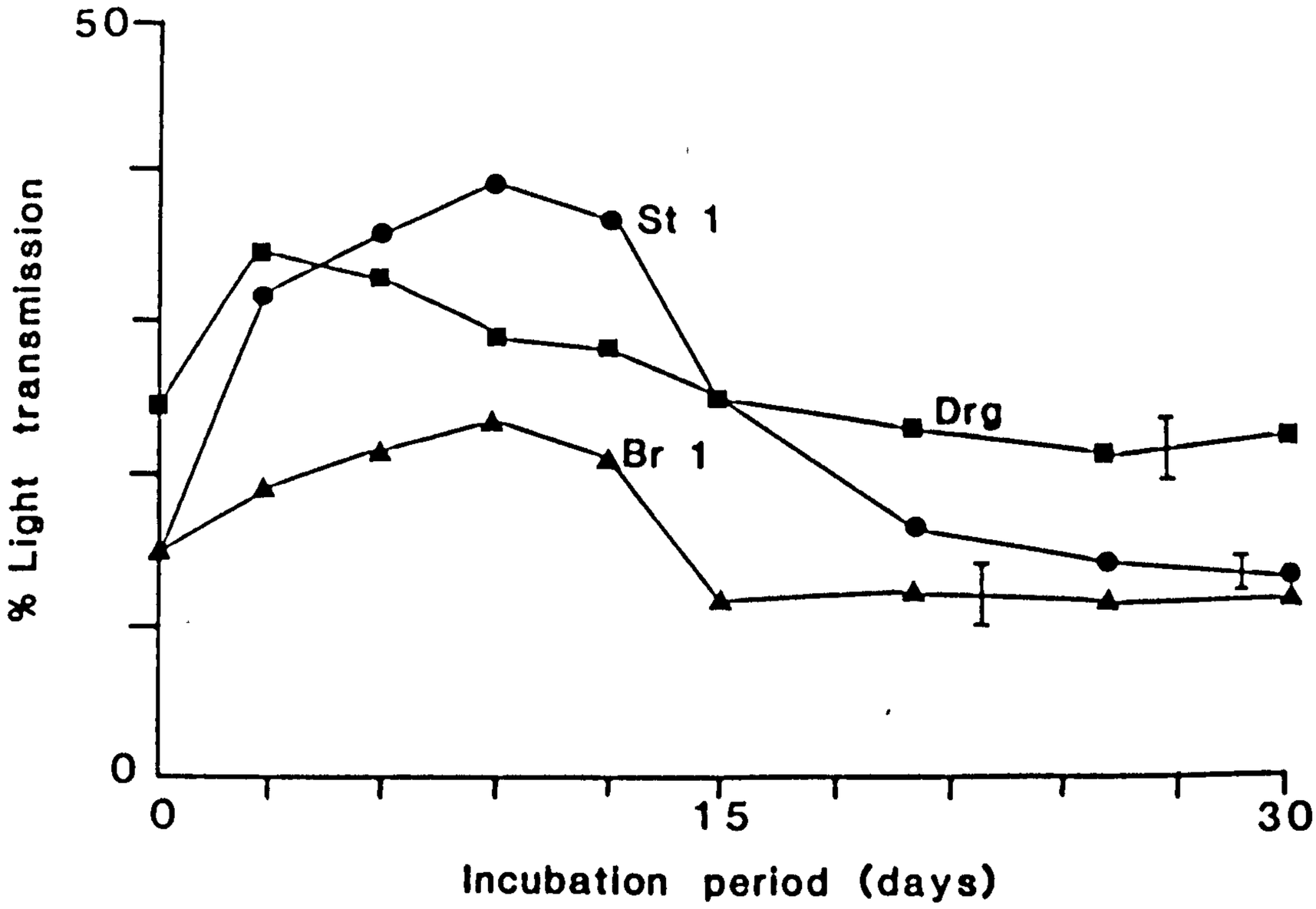


Figure 4.10 Aggregate stability change in soil moistened with de-ionised water and leached with periodate

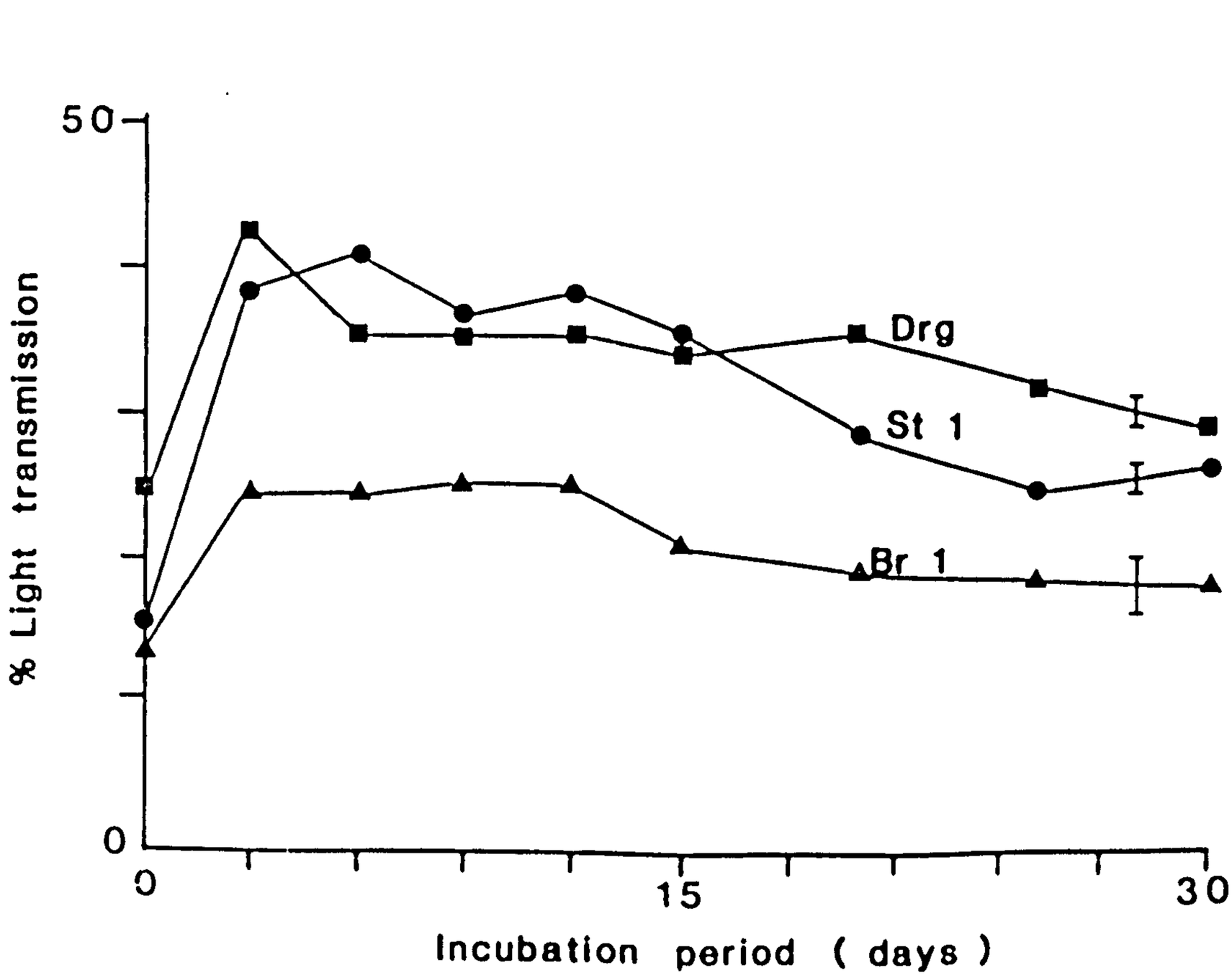


Figure 4.11 Aggregate stability change in soil treated with vancomycin and leached with periodate

throughout the period of incubation. Whereas the aggregates from pasture soils (Bromyard 1, Stirling 1 and Dreghorn) partially resisted this treatment, those from arable plots (Bromyard 2, Stirling 3) totally lost their stability after 48 hours of periodate, followed by 48 hours of borate treatment. The greater resistance to periodate treatment in pasture soils over the first 3 to 12 days shows the independent aggregating function of fungal mycelia and their resistance to periodate degradation. With subsequent period of incubation however, this resistance diminished and was paralleled by the decline and disappearance of fungal mycelia.

4.2.5.3 Soil Moistened with Vancomycin Solution

In soils treated with vancomycin to inhibit bacterial growth (Figure 4.11), the periodate resistant component increased in size as compared with Figure 4.10, when the bacteria were present, and did not decline to the same extent after 9 days. This shows that fungal and actinomycetal stabilising activity is resistant to periodate oxidation. Fungal and actinomycetal hyphae resulted in more mechanical stabilisation of the aggregate and imparted more resistance in pasture soils to periodate treatment. However, the total loss of stability in the Bromyard 2 and Stirling 3 soils shows that for these soils polysaccharides are more important than other soil stabilising agents as noted by Greenland et al.(1962) for arable soils.

4.2.5.4 Soil Moistened with Cycloheximide Solution

When soils treated with cycloheximide were subjected to periodate treatment the stability was reduced to the lowest levels in aggregates from pastures, and those from arable sites totally lost their stability (Figure 4.12). These increased reductions in stability, especially in pasture soils, show that in the absence of binding hyphae the main aggregating substances were polysaccharides. During incubation, polysaccharides of plant or microbial origin are utilised by microorganisms, and subsequently converted to microbial gums which bind the soil aggregates. Polysaccharides in soil are therefore transient, in that they are continually being produced and utilised.

4.2.5.5 Soil Moistened with Sterilising Solution

Periodate treatment removed all of the stability in Bromyard 2 and Stirling 3 arable soils sterilised and incubated (Figure 4.13). There was a low residual aggregate stability remaining in pasture soils and this was constant over the aging period. The removal of biological activity by sterilisation treatment left the soils in their stable physical state thus the soils kept their initial aggregate stability unchanged. At that equilibrium aggregate stability, pasture soils are assumed to be stabilised by fungal hyphae, polysaccharides and more humified substances, whilst polysaccharides accounted for most of the stability of the aggregates from arable soils. The residual stability of pasture soil aggregates after periodate treatment is therefore attributable to the presence in them of fungal mycelia, more humified

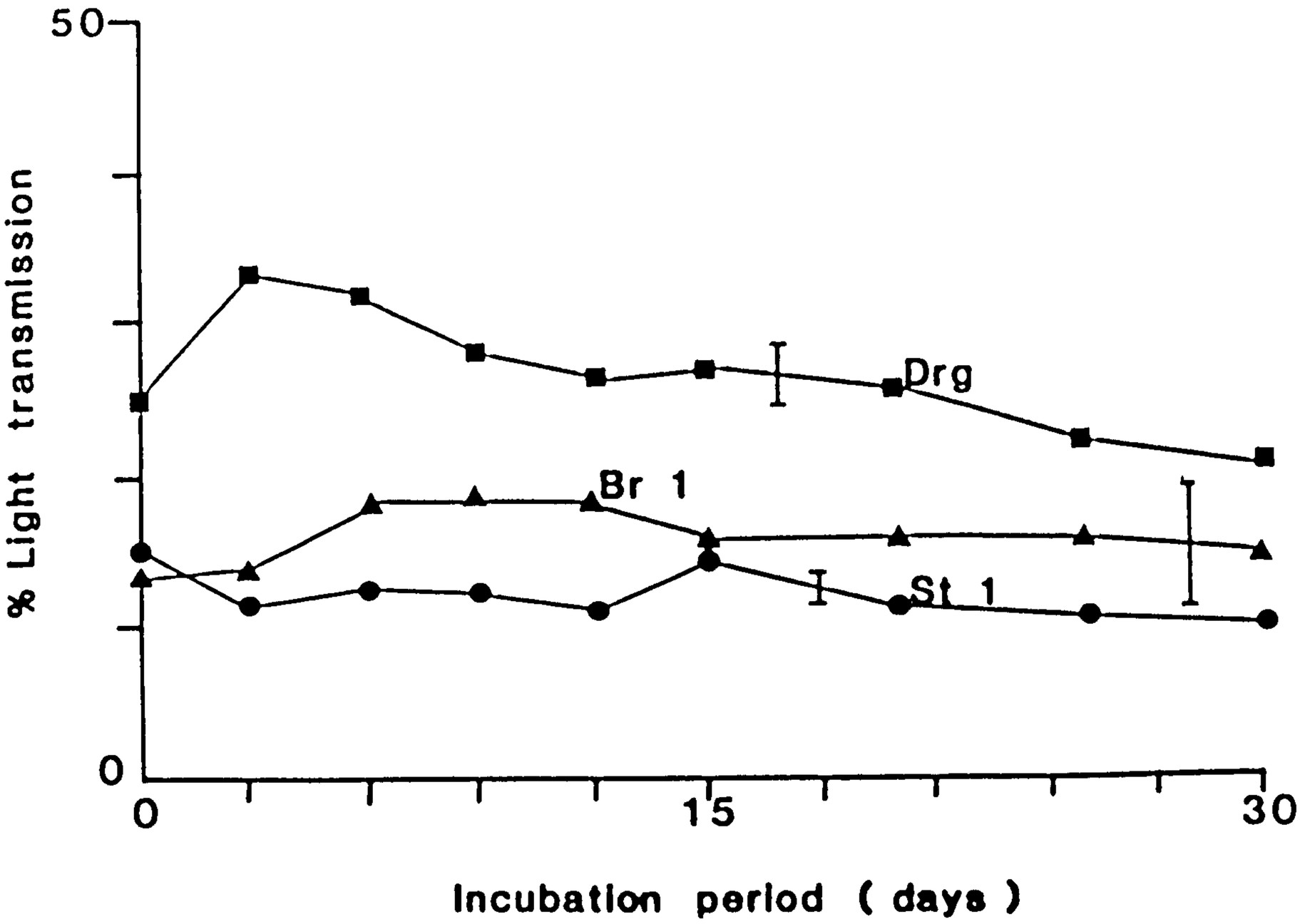


Figure 4.12 Aggregate stability change in soil treated with cycloheximide and leached with periodate

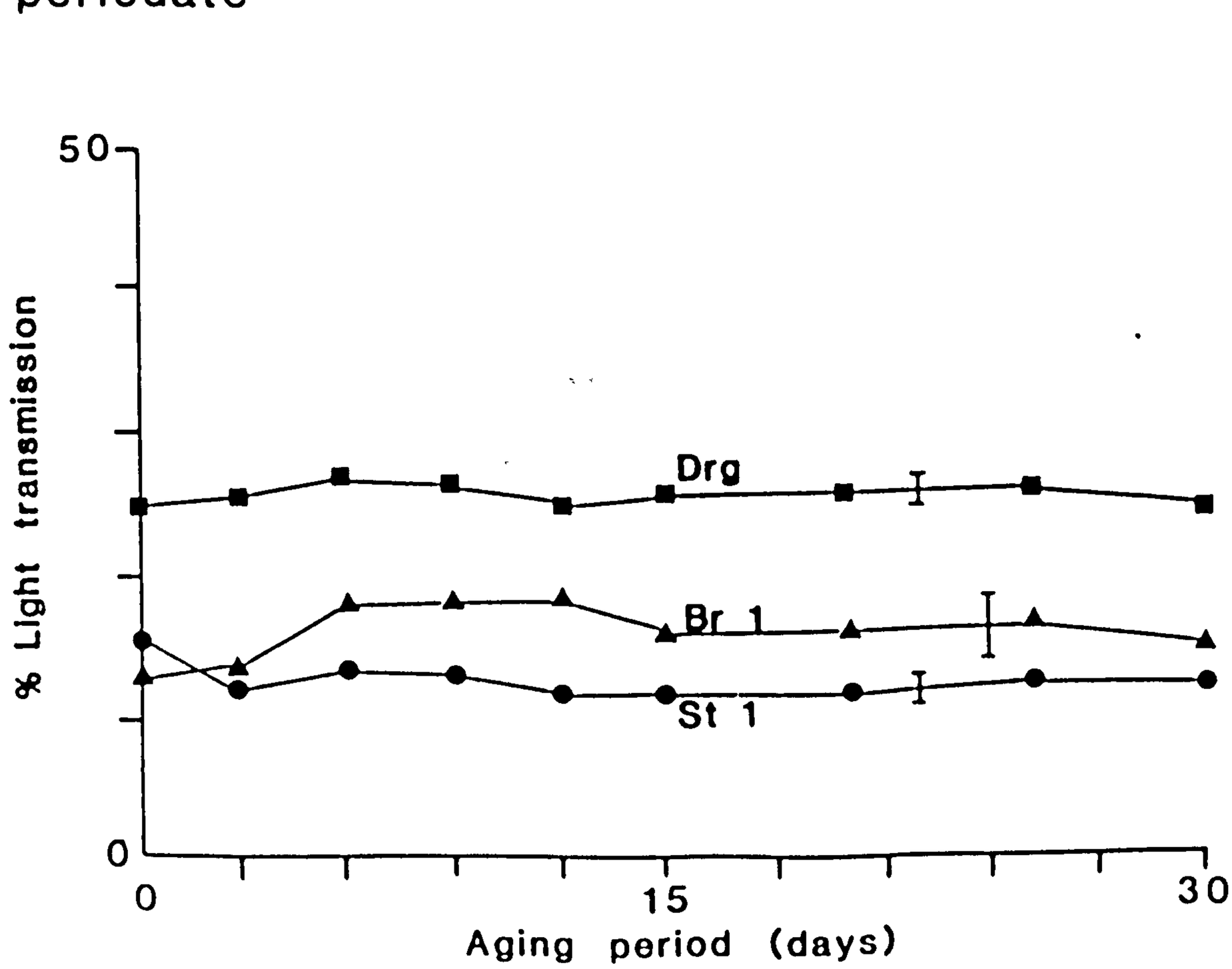


Figure 4.13 Aggregate stability of sterilised and periodate leached soil

substances and plant polysaccharides which are more resistant to periodate treatment, plus microbial polysaccharides, produced from bacterial decomposition of the fungi and other substrates which are degradable by periodate treatment.

4.2.6. Discussion

The results obtained and summarised graphically in Figure 4.2 reveal a consistent pattern of initial increase in aggregate stability and subsequent decline, and this was similar in all five soils atlthough levels of stability were clearly different. In field aggregates a steady trend in the decline in stability was observed. The persistence of stability is attributable to actinomycetal mechanical stabilisation. These results obtained under artificial laboratory conditions support the view held by some workers that the effects of fungi on aggregate stabilisation are temporary (Griffiths, 1965; Tisdall and Oades, 1982). Under field conditions fungi have been shown to build up in the soil within a few weeks or months when arable soils are left fallow or when root systems grow as the crop cover becomes established. They are therefore sensitive to management (Oades, 1984). On the other hand studies by Bond (1960), Bond and Harris (1964) and Forster (1979) have shown that in sandy soils fungal hyphae were regularly present and were major stabilising agents. They concluded that fungal mycelia were either extremely persistent or being produced continually to provide a dynamic equilibrium.

The ability of different groups of microorganisms to utilise organic substrates added to the soil and convert them into aggregate stabilising glues is well established. However, conflicting results have been reported as to which 'group' of microorganisms is important during the initial utilisation of the substrate, and subsequently important in initial stabilisation of the amended or non-amended aggregates during incubation. Harris et al. (1964) found that bacteria were more important in initial stabilisation of sucrose amended aggregates incubated aerobically. A similar pattern of microbial growth was obtained by Behera and Wagner (1974). On the other hand Adu and Oades (1978b) found that when soil aggregates amended with glucose and starch were incubated and the process of utilisation followed through a measure of CO_2 release, fungi were responsible for the initial utilisation of the substrate. This view is supported by measurements made of ergosterol and SEM pictures obtained in the present study, which show the initial flush of fungi and bacterial steady development rather than initial flush.

The speed and extent of microbial utilisation of substrates in the soil depend on either diffusion of substrate or movement of the organisms and this is supported by the results in Figure 4.2. Fungal mycelia have the potential to grow rapidly along the walls of coarse pores and even across the pores as the organisms move towards a potential substrate (Griffin, 1981). The spread of bacteria which lack hyphal systems to bridge pore spaces may be limited (Papendick and Campbell, 1981) and this will be extremely slow as reproduction leads to formation of another cell which may be pulled back within the boundary of the parent colony by surface tension (Marshall, 1968). The rapid growth of

fungi give rise to their initial stabilisation of aggregates occurring between 3 and 6 days of incubation, and subsequently the stability declines as the hyphae become degraded.

The initial increase and subsequent decline in aggregate stability similar to the one observed in Figure 4.2 has led to the dismissal of the importance of fungi in aggregate stabilisation by several researchers (e.g. Griffiths and Jones, 1965). However, it is thought here that since the death of fungi promotes the growth of other organisms such as bacteria and actinomycetes, which subsequently have long lasting effects on aggregate stability of field soils (similar to that obtained in Figure 4.2), fungi contribute indirectly to aggregate stability persisting over weeks and years in field aggregates.

Inspection of Figure 4.3 reveals that fungal decline during incubation is accompanied by proliferation of bacteria and actinomycetes. The latter population was revealed by SEM, but not quantified. Degradation of fungal mycelia as revealed by SEM and quantified by ergosterol measurements is attributable to the process of fungistasis. This process has been studied by Lockwood and co-workers (Lockwood, 1960; Lingappa and Lockwood, 1962; Ko and Lockwood, 1970). Evidence from their studies and other similar ones on the ecology and interaction of microorganisms in different habitats suggest degradation of fungal cell walls to be a result of digestion by bacteria and actinomycetes or from autolysis.

Bacteria and actinomycetes produce enzymes such as chitinases and glucanases which are able to degrade fungal cell walls (Mitchell and Alexander, 1963; Skujins et al., 1965; Potgieter and Alexander, 1966). Autolysis may be induced by nutrient deprivation, causing enzymes to dissolve cell constituents and release protoplasm which may stimulate other organisms (Lloyd and Lockwood, 1966; Ko and Lockwood, 1967; Lockwood, 1977). Such processes would have occurred during incubation of soils with or without antibiotics, and explain the shapes of the fungal and bacterial growth curves obtained in the present study. The results obtained in Figures 4.3 and 4.5 show that the more stringent the competition, the quicker the decline of the fungal mycelia. The competition which resulted during incubation can either be a competition for nutrient source which is short supply - the case in soils with low organic matter contents, - or antagonism of one species towards another which results in the inhibition of the second. The Stirling, Dreghorn and Bromyard pasture soils support large population of microorganisms, which leads to stringent levels of competition in those soils as evidenced by largest decline in the fungal contents (Figures 4.3b and 4.5).

Persistence of the stability of field aggregates (Figure 4.2) was unexpected because the fungal mycelia which stabilise the aggregates during the initial incubation period were declining at a faster rate. Thus while changes in ergosterol contents were large stability remained steady. The problem was resolved by SEM picture which revealed hyphal degradation in the form of holes and empty cells and the presence of large population of actinomycetes and bacteria. The differences in

levels of stability during the initial period of incubation (period of maximum fungal growth) and latter period of bacterial and actinomycetal proliferation (Figures 4.2, 4.4, 4.6) support the view held by some workers that fungal mycelia are superior in the ability to stabilise soil aggregates (Swaby, 1949; Bond and Harris, 1964). However, this ameliorative contribution of fungi to increased stability was short lived in the presence of antagonistic organisms such as bacteria and actinomycetes.

Assessment of the relative contribution of bacteria and actinomycetes to aggregate stability has never been a simple matter due to the difficulty of making compositional and morphological distinctions between these organisms. Most previous researchers have grouped actinomycetes with bacteria and these organisms were considered to be bacteria with the ability to form branching hyphae at some stage of their developments (Goodfellow and Williams, 1983). Williams and co-workers have studied the growth requirements and environmental conditions under which actinomycetes flourish (Williams and Davies, 1965). Their studies emphasise the point made earlier that actinomycetes flourish in chitin rich media, and fungal cell wall materials provide a rich source of substrate to these organisms (Mitchell, 1963; Skujins et al., 1965; Lloyd and Lockwood, 1966; Jones and Webbley, 1968).

This discussion points to the conclusion that actinomycetes are involved in degradation of fungal mycelia in utilising chitin rich sources. The decline of fungi in the absence of bacteria as shown in Figure 4.5 is attributable to their degradation by actinomycetes, and the

persistent stability of the aggregates in the absence of fungi and bacteria is partly a result of enmeshment by these organisms.

Periodate oxidation treatment similar to the one used in the present study has previously been used to confirm the observation made by Martin (1945) that polysaccharides are involved in the stabilisation of soil aggregates (Greenland et al., 1961, 1962; Clapp and Emerson, 1965a, 1965b; Stefanson, 1968, 1971; Tisdall and Oades, 1979, 1980; Cheshire et al., 1983, 1984). Although some caution has to be exercised in comparing absolute values of light transmission after periodate treatment with other treatments because of the dispersion effect of sodium ions on clay, (Figure 4.9), it is apparent that materials oxidised by periodate contribute a substantial part in soil aggregate stabilisation and in particular account for most of the stability in arable and young pasture soils. The results obtained here are in agreement to those of Cheshire et al. (1983) who examined the dispersive effect of the sodium ion during periodate oxidation treatment. About 40% of the disaggregation in soils dealt with in their experiment was attributable to the dispersive effect of sodium ions on the clays. They found that reduction of 10% in carbohydrate content of the soil subjected to this treatment took place.

The initial higher resistance to periodate treatment observed during incubation of the Bromyard 1, Stirling 1 and Dreghorn soils, is attributable to the mechanical enmeshment of the soil aggregates by fungal mycelia (Figures 4.10 and 4.11). When the mycelia become degraded (through being lysed by bacteria and actinomycetes) this resistance disappeared as shown by the decline in stability, but the aggregates from

pasture did not lose all of their stability. The presence in these aggregates from pastures of more resistant organic materials such as plant polymers and polysaccharides complexed with metal ions is indicated.

Similar resistance of pasture and forest soils to periodate treatment was observed by Greenland et al. (1962). This led them to conclude that, whilst polysaccharides and polyuronides are probably involved in stabilising aggregates in soils from young pastures the stability of forest soils would be mainly due to the mechanical action of fungi. The occurrence of fungi in those soils dealt with in the work of Greenland et al. (1962) was previously observed by Bond (1960) and Bond and Harris (1964). When those soils were incubated with organic substrates Harris et al. (1963) observed that the stability of their aggregates could be destroyed by periodate treatment and suggested that similar effects would correspond to those in young pastures or cultivated soils. Harris et al. (1964) concluded that the initial development of soil structure largely depends on microbial gums, which produce only a temporary effect, and that more permanent aggregation is a result of the development of a network of fungal mycelia. The present study would reverse these conclusions in that fungal huphae produce only temporary effects, and more permanent effects appear to be the result of aggregation by transient polysaccharides, which are formed over and over again in a continuing dynamic process.

In field aggregates obtained with little mechanical disturbance (such as mild sieving used to obtain 2-4 mm aggregates in this study) much of the polysaccharides associated with clays will remain

inaccessible to most bacteria until the crevices are opened by mechanical action to fungal hyphae. Fungi have the greater potential for growth and their extension through the micropores would lead to opening of the crevices during the initial incubation period, bringing most of the bacteria into contact with polysaccharides. This encapsulation of bacteria and associated polysaccharides by the clay plates partly explains why most of the polysaccharide materials are not rapidly utilised by microbial populations, and therefore part of these materials will have long lasting aggregate stabilising effects in undisturbed field soils.

The decrease in stability observed with periodate treatment of aggregate enmeshed by fungal mycelia is a complex one. Microscopic examination by Bond and Harris (1964) revealed that fungal mycelia which had the most stabilising effect were encrusted with slime material. This material was identified as polysaccharides by Tisdall and Oades (1979) and Foster (1981) using stained thin sections and transmission electron microscope (TEM). Other studies have shown that fungal mycelia contain chitin and glucan in their cell walls (Foster and Webber, 1960; Ride and Drysdale, 1972) which are polysaccharides resistant to periodate oxidation (Cheshire , 1979). Whether these polysaccharides are involved in more permanent stabilisation of the aggregates associated with fungal mycelia present under pastures as shown by Bond (1960) and Bond and Harris (1964) has yet not been determined. Most of the mycelia revealed by SEM in the present study had sticky surfaces and were encrusted with clay particles, however, it has not been possible to establish the origin of this material as in most cases, bacteria also were exposed on the

fungal filaments. Thus polysaccharides involved in stabilisation of soil aggregates can be of bacterial, fungal or plant origin especially in pasture aggregates in their natural state. However, upon incubation and microbial proliferation, microbial polysaccharides are produced, and these are periodate sensitive (Martin, 1971).

4.3 Thixotropic Changes in Moulded Soil Aggregates

This section discusses the results of three experiments carried out to assess the contribution of thixotropy and periodate-labile soil organic matter to changes in moulded soil aggregates. Thixotropic changes were estimated by measurement of aggregate stability, soil matric water potential and probe penetration resistance. These tests were carried out using Bromyard 1 and 2; Stirling 1 and 3 and Dreghorn soils at plastic limit moisture contents of the respective soils.

4.3.1 Effects of Incubation (Aging) on Aggregate Stability

4.3.1.1 Soil Moistening with Deionised Water

Field aggregates were obtained by mild sieving which resulted only in minimal physical disturbance. The stability of these field aggregates from the five soils was reduced by pulverisation and moulding. Comparing stability values for field aggregates prior to incubation (Figure 4.14 (a) 0 day),with those of moulded aggregates at 0 day (Figure 4.14 (b)) shows that stability of the latter aggregates was reduced by the following magnitudes:- Bromyard 1, 71.2%; Bromyard 2, 91.7%; Stirling 1, 30.0%; Stirling 3, 91.7% and Dreghorn, 29.2%.

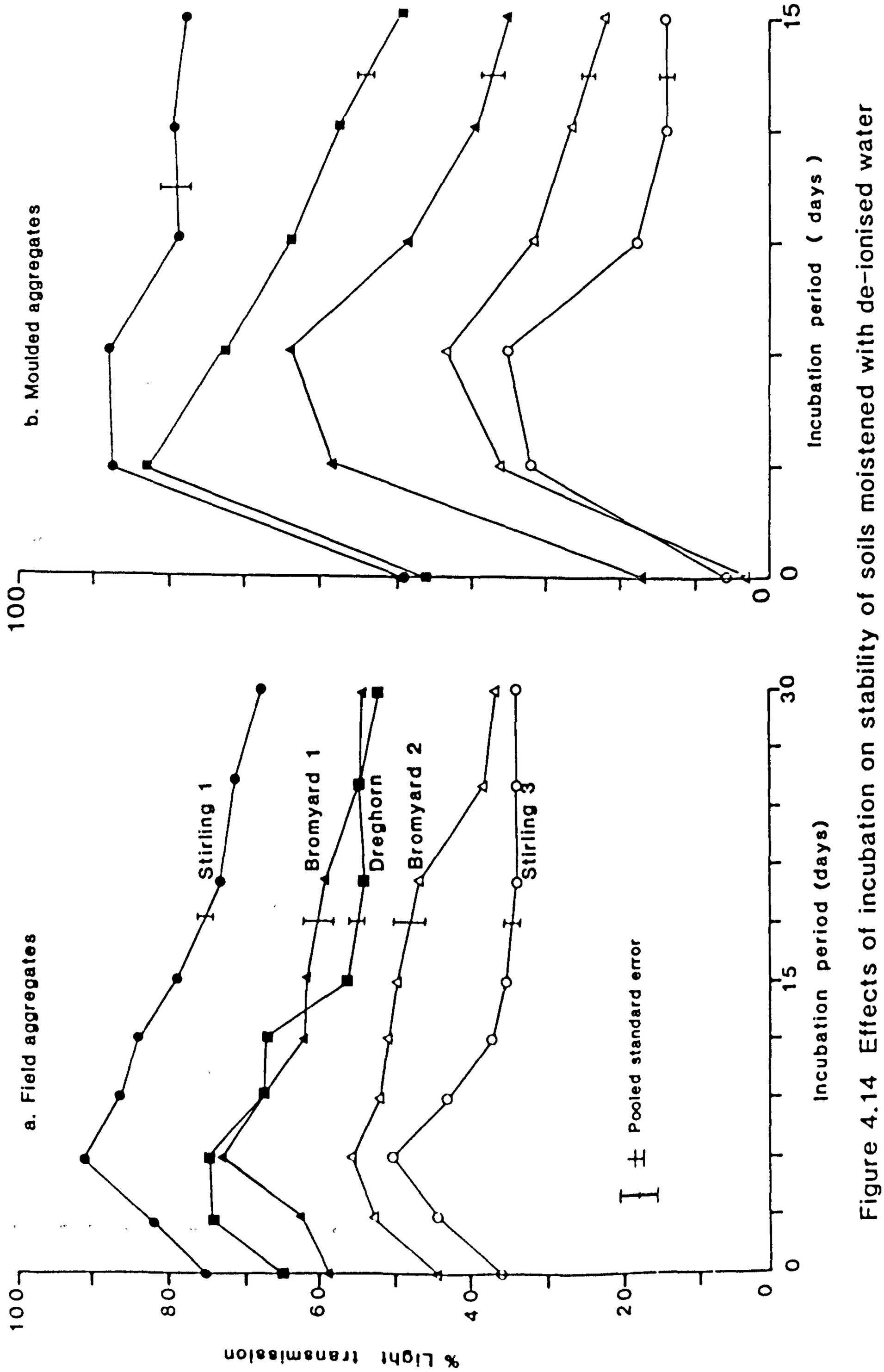


Figure 4.14 Effects of incubation on stability of soils moistened with de-ionised water

Several processes may have influenced the varying degrees of loss of stability of the respective soils, but there is evidence from these results of the greater severity of the moulding process in soils with lower organic matter contents.

When moulded aggregates were incubated under constant moisture and temperature conditions, there was an initial increase in stability varying for the different soils between 3 and 6 days as shown in Figure 4.14(b). The largest component of this initial increase in stability occurred within the first 3 days of incubation. Comparing stability changes occurring in both field and moulded aggregates (Figure 4.14) it can be seen that in the latter aggregates stability doubled during the initial 3 day period. This large stability regain brought moulded aggregates to approximately similar stability levels to their field counterparts. After reaching maximum stability over the six day period, the stability of moulded aggregates declined with subsequent incubation period. The decline in stability was more pronounced between day 6 and 9, and subsequently the decline became more gradual. The stability attained by the end of the 15 day period was higher than that of at 0 day, and this was so for all the soils.

The rise and fall of aggregate stability in both types of aggregates (Figure 4.14) suggest involvement of several mechanisms. The results obtained and discussed in section 4.2 have shown that organic matter and microbial activity may be some of the important factors contributing to the increase in stability of these aggregates. The initial increase of stability and subsequent decline thereof was shown to

be accompanied by growth and decay of fungi and periodate sensitive materials of microbial origin. However, the loss of stability occurring when field aggregates were moulded, and the large increases in stability observed when these aggregates were incubated suggest that in addition to biological contribution, there are other processes which are physical in nature also contributing to the observed changes in stability. Moulded aggregates in Figure 4.14 were incubated under constant moisture and temperature conditions, thus the additional contribution to increased stability influenced by physical manipulation would be analogous to increase in strength of moulded thixotropic soils observed by civil engineers (Mitchell, 1960, 1976). The contribution due to thixotropic processes is assessed in the experiments below.

4.3.1.2 Soil Moistening with Sterilising Solution

The contribution of microbial activity i.e. the growth of fungi or production of exudates shown to stabilise aggregates (Figure 4.14) was eliminated by soil sterilisation with solutions containing sodium azide and mercuric chloride as previously described. Figure 4.15 shows that moulding the soils with sterilising solutions resulted in a loss of stability similar in magnitude to that produced by moistening and moulding with deionised water in Figure 4.14.

By contrast to stability changes observed in Figure 4.14, the stability of moulded sterilised soils increased more gradually over the initial aging period, varying for the different soils between 3 and 9 days. It then remained constant as shown in Figure 4.15b. The constant

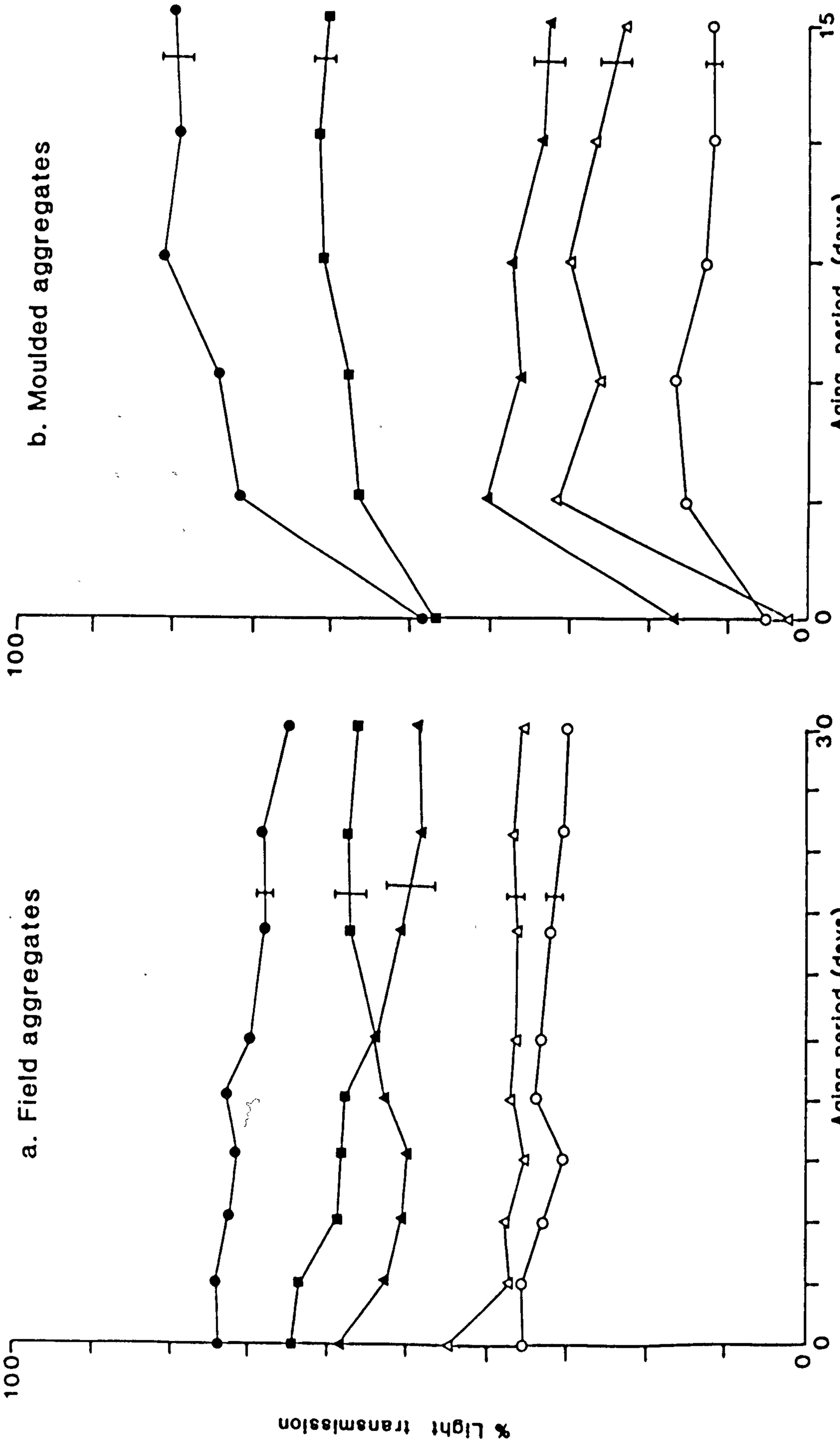


Figure 4.15 Effects of aging on the stability of sterilised soils (Symbols as in Figure 4.14)

stability levels were approximately the same as those to which soils moistened with deionised water fell back at the end of the 15 day incubation period, when the fungal growth had declined and the fungal hyphae were no longer contributing to stability.

Comparison of sterilised moulded and field aggregates (Figure 4.15) shows the latter aggregates retained most of their stability when aged for a period of 30 days. The stability of these field aggregates is that established over long periods in the field through a combination of processes, some biological, some physical. This equilibrium stability remains unchanged in the absence of biological activity or physical manipulation when these soils are aged at constant temperature and moisture under laboratory conditions. Moulding similar aggregates resulted in the observed stability changes and these changes occurred in the absence of biological activity. The loss of stability upon moulding and the regain occurring during aging are therefore consistent with mechanisms of thixotropy propounded by Mitchell (1960). This involves rearrangement of the clay particles to face to face pattern when soil is remoulded, and randomisation of the particle orientation during aging. With longer aging period however, chemical bonding may take place within the aggregates, which would also contribute to changes in stability. Such changes would be irreversible (Bjerrum and Lo, 1963).

To distinguish between purely thixotropic and chemical bonding reversibility of the observed changes in stability must be demonstrated. Sterilised moulded aggregates (Figure 4.15) aged for 15 days were remoulded. The results of stability change in moulded and remoulded

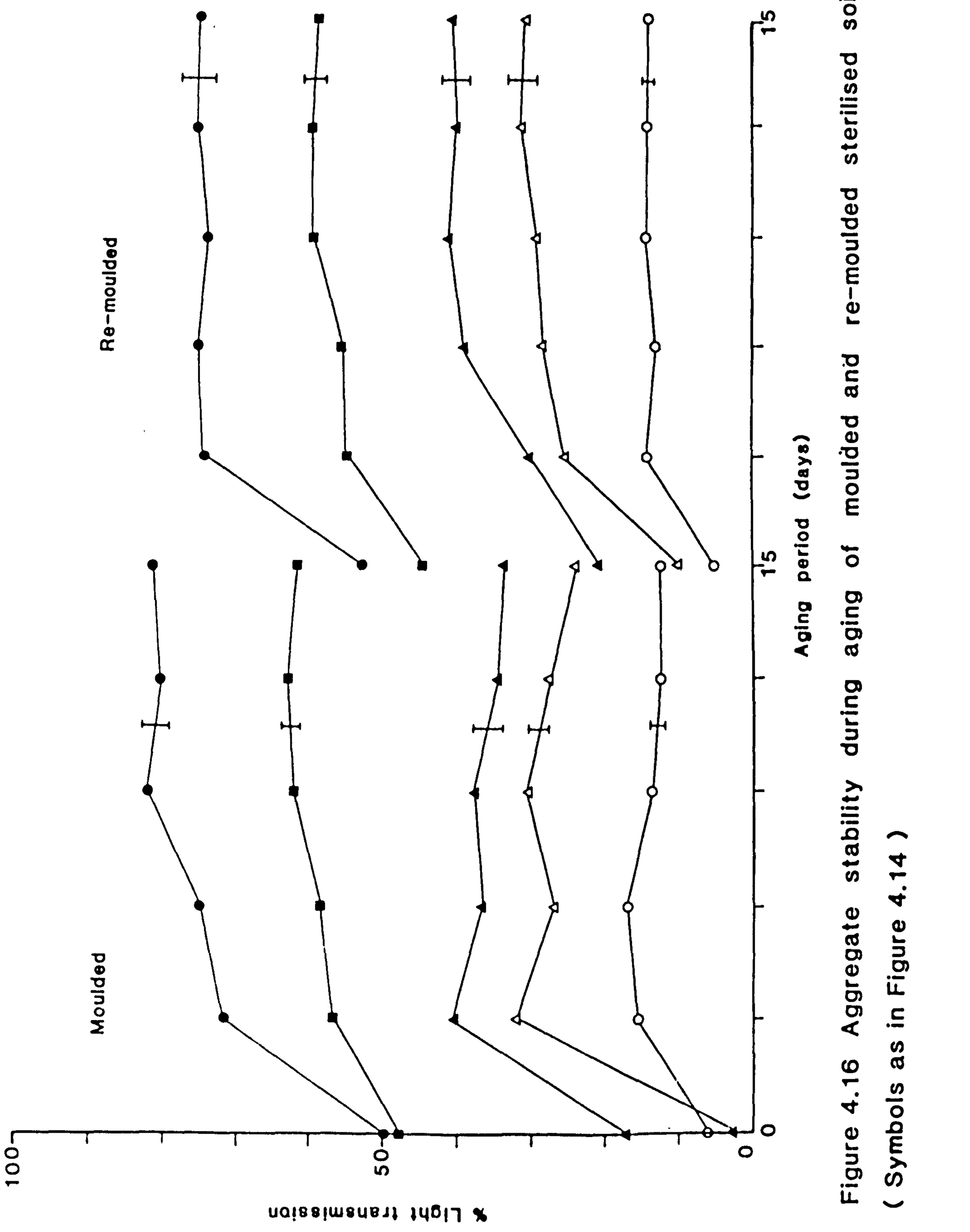


Figure 4.16 Aggregate stability during aging of moulded and re-moulded sterilised soils
(Symbols as in Figure 4.14)

aggregates are shown in Figure 4.16. When moulded and aged aggregates were remoulded, there was a reduction in stability to levels approximating those of 0 day. During aging the stability of remoulded aggregates increased over a period of 3 to 9 days and reached values approximating those during the first cycle.

Sterilisation treatment does not remove participation of organic matter in aggregate stabilisation, but only prevents its modification through biological activity during aging. Therefore the inherent organic matter contents of the respective soils would not have been altered, and could have influenced the observed changes in stability occurring in sterilised aggregates.

4.3.1.3 Stability of Soils Treated with Periodate and Moulded

In order to separate the contribution of soil organic matter, mainly the periodate-labile fraction, samples from the five soils were oxidised with periodate prior to moulding and incubation (aging) which proceeded under similar conditions as for soils moistened with deionised water and sterilisation solutions respectively. The stability of periodate treated soils, moistened with deionised water and moulded (Figure 4.17) was lower than in the other two treatments dealt with above. The reduction in stability occurring during moulding is more conspicuous with Bromyard 1, Stirling 1 and Dreghorn soils, which in Figures 4.14 and 4.16 were shown to retain a considerable amount of their stability when moulded.

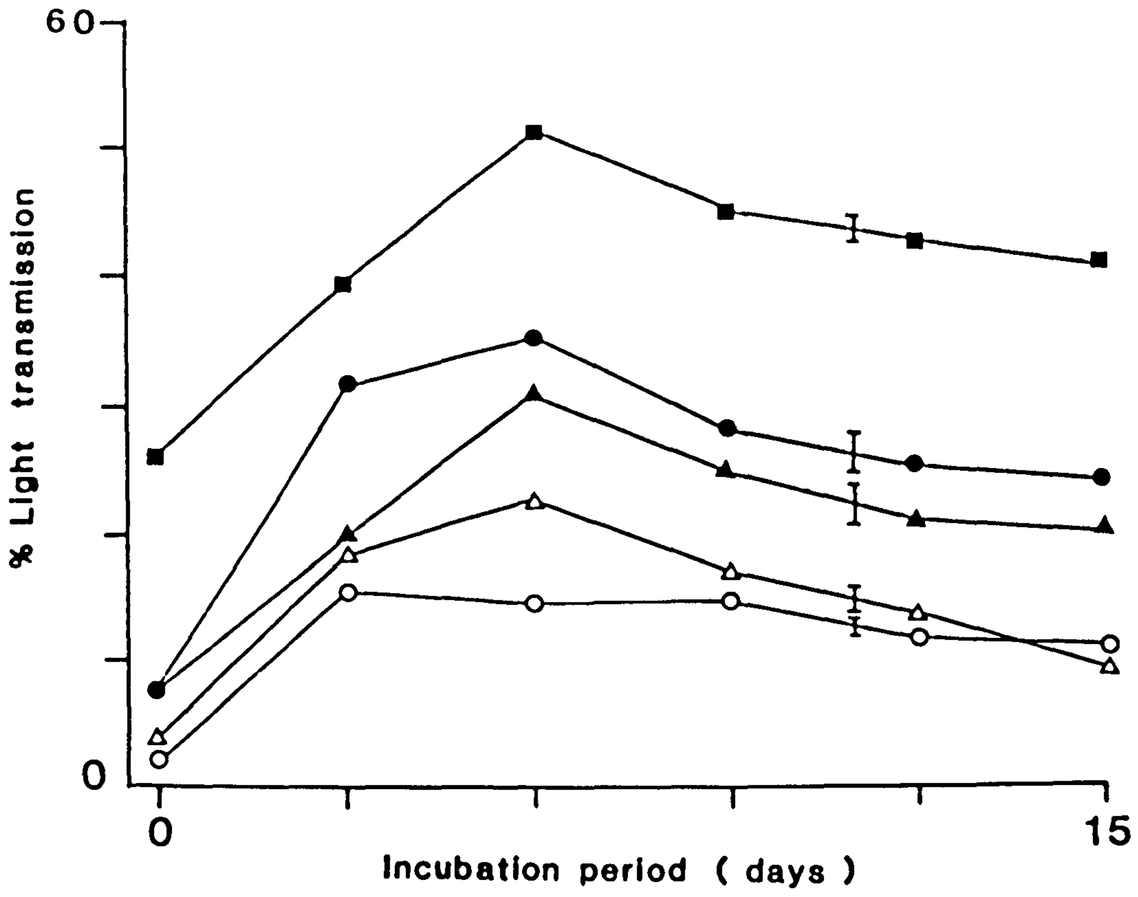


Figure 4.17 Aggregate stability during incubation of periodate treated and moulded soils (Symbols as in Fig. 4.14)

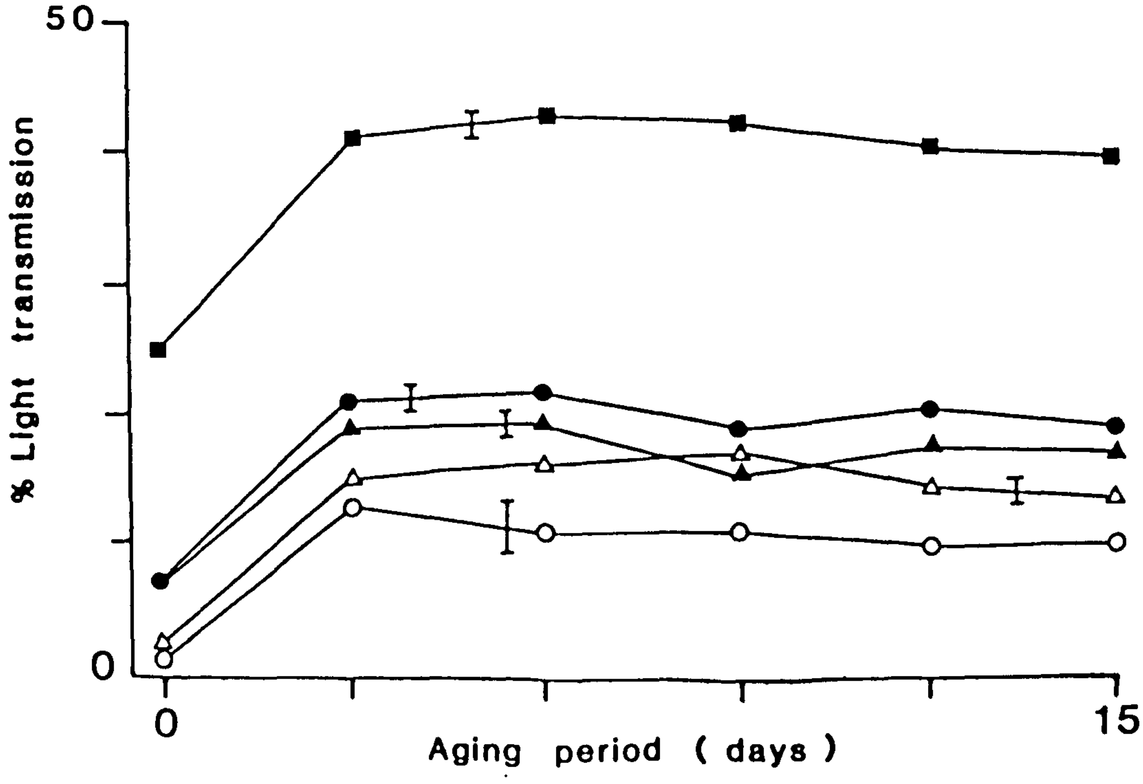


Figure 4.18 Aggregate stability during aging of periodate treated, sterilised and moulded soils (Symbols as in Fig. 4.14)

Destruction of polysaccharides by periodate, though probably not complete (Cheshire et al., 1983), removed most of the differences between paired soils of Stirling 1 and 3 and Bromyard 1 and 2. Although some caution has to be exercised when comparing values of light transmission after periodate treatment with other treatments because of the dispersion effects of sodium ions on clays (see Figure 4.9), it is apparent that the materials oxidised by periodate play a prominent role in aggregate stabilisation and in particular account for the most improved stability of pasture soils. Whilst periodate treatment removed all the stability of field aggregate from the Bromyard 2 and Stirling 3 soils (Figure 4.9) moulding and subsequent incubation resulted in recovery of part of their lost stability as shown in Figure 4.17, and this stability regain occurred in all other aggregates over a period of 6 days. This pattern of stability increase was similar to the one observed when soil aggregates moistened with deionised water, moulded and incubated were examined (Figure 4.14). During incubation of periodate treated aggregates biological activity would therefore give rise to similar increases and reductions in stability observed previously in Figure 4.14.

Periodate treated soils, sterilised and moulded (Figure 4.18) behaved in a manner similar to sterilised soils in Figure 4.16. Aging of these soils resulted in initial increase in stability occurring over a period of 3 days, and the stability remained constant thereafter. This initial increase in stability occurred in the absence of the biological activity, and is consistent with findings of Blake and Gilman (1970) and Utomo and Dexter (1981c) in whose experiments the contribution of organic matter was removed by soil treatment with hydrogen peroxide.

4.3.2 Effects of Aging on Matric Water Potential Changes

Soil water matric potential is governed by the pore size distribution. In order to further test the hypothesis that thixotropic hardening is associated with soil particles re-orientation following shear, measurements were made of soil matric water potential as a function of time after moulding. Changes in soil matric water potential during aging process in moulded sterilised soils of the Bromyard 1 and 2; Stirling 1 and 3 and Dreghorn series were measured using a simple tensiometer as described in methods section. Figure 4.19 shows the decrease in water potential after moulding the sterilised soils into the tensiometer cup. It should be mentioned here that the soil was packed by kneading compaction with hand, and that results may vary depending on the method of compaction applied (Seed and Chan, 1957, 1959).

The less negative matric water potential values obtained with all soils sheared and moistened to plastic limit moisture contents is a result of a change from a flocculated to a dispersed structure. Thus the initial suction at 0 day is consistent with the concept of more dispersed structure and non-equilibrium of water molecules associated with the soil particles (Mitchell, 1960; Cashen, 1966). Maximum values of soil water tension were achieved during aging which varied for the different soils from 2 to 8 days. The time to reach equilibrium increased in the following order, Stirling 1, Dreghorn, Stirling 3, Bromyard 1 and Bromyard 2. Apart from the anomalous position of the Dreghorn soil this is in decreasing order of the clay content. Maximum values were reached in eight days or less, and then remained constant. After 15 days the

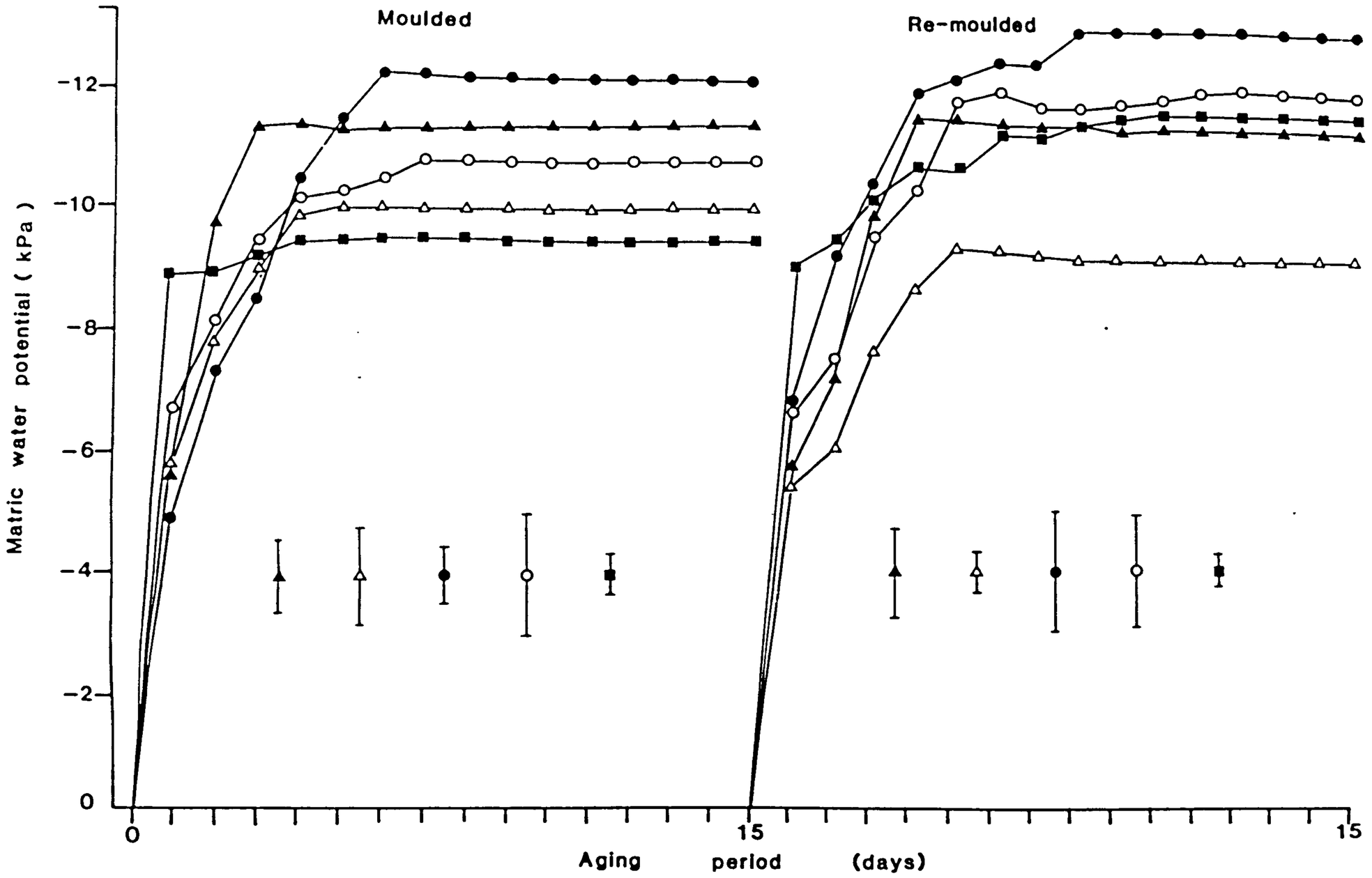


Figure 4.19 Matric water potential changes during aging of sterilised soils (Symbols as in Fig. 4.14)

soils were remoulded *in situ*; the matric potentials temporarily increased, then fell over a period of approximately eight days to values comparable to those attained before as in Figure 4.19. As in Figure 4.18 it is possible that polysaccharides might have taken part in the processes.

The contribution and interference of organic matter in particle re-organisation was removed by treatment of the soils with periodate prior to tension measurements. After oxidation of the organic matter with periodate the sterilised soils showed a rather slower development of minimum water potential, but no great differences in the maximum tension reached as shown in Figure 4.20. In the absence of organic matter the more clayey samples of Stirling 1 and 3, attained highest suction values, and this accords with the findings of McHenry and Russell (1943) and Koenigs (1963) that in the absence of organic matter which only acts to modify the forces of attraction between the clay particles, clay has higher affinity for water molecules and subsequently higher mobility.

Remoulding at the end of the 15 day aging period showed the reversibility and reproducibility of the changes in matric water potential as shown in Figure 4.20. The results obtained in Figures 4.19 and 4.20 are virtually the same indicating that polysaccharides play little or no part in the reversible thixotropic processes in soil aggregates. The matric water potential results obtained with soil treated with periodate well agree with those of Schweikle et al. (1974) and Utomo and Dexter (1981c) in which measurements were made on samples pre-treated with H_2O_2 to remove organic matter.

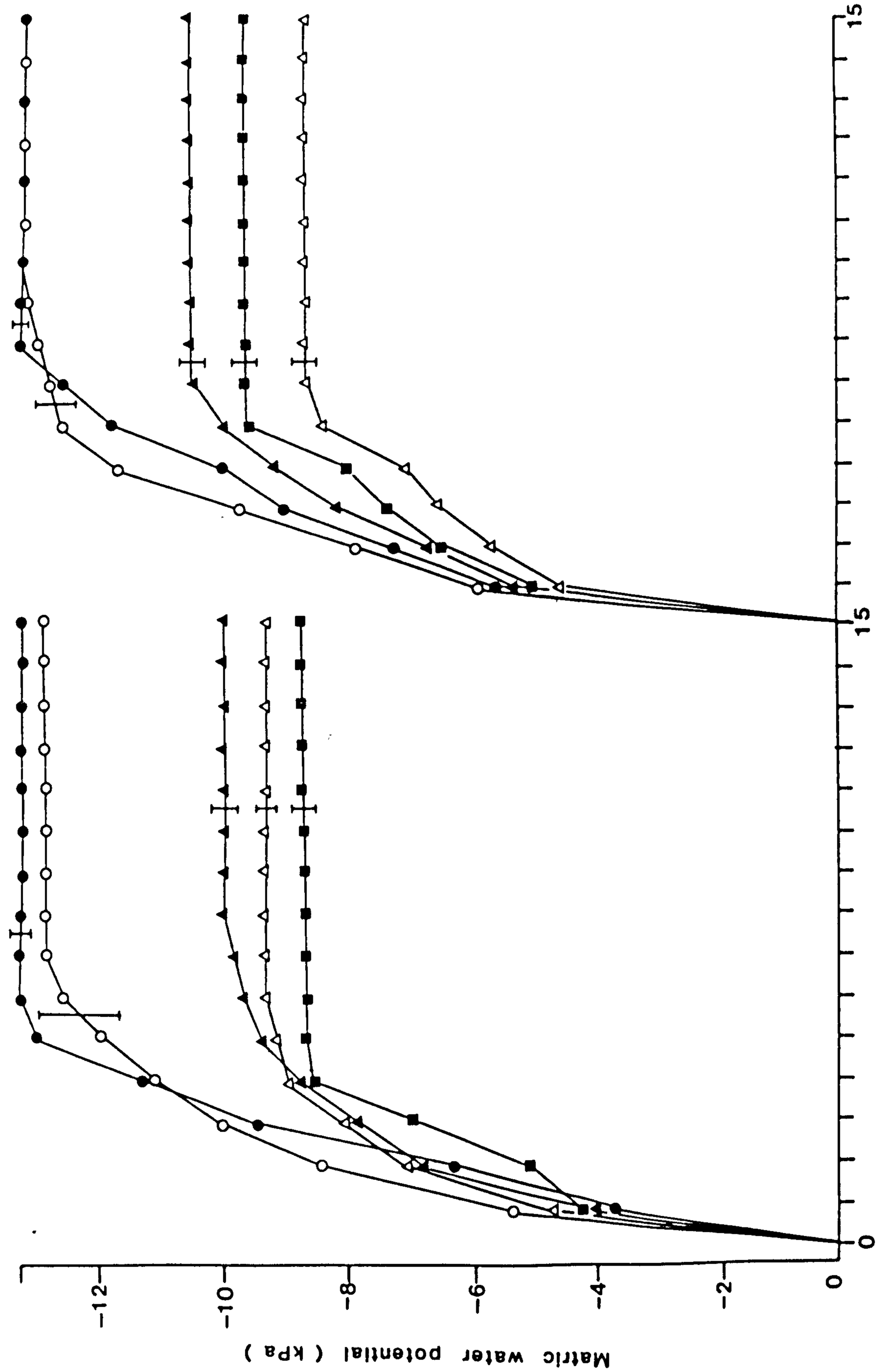


Figure 4.20 Matric water potential changes during aging of periodate treated and sterilised soils (Symbols as in Fig. 4.14)

4.3.3. Effects of Aging on Soil Strength

It was observed during aging of soil samples in stability and tension experiments that the soils attained maximum strength over a period of 15 days. These soils were kept at constant moisture thus the resultant strength attained was attributed to thixotropic hardening. This strengthening of the soil was noticeable in tension tests and gave rise to difficulty in removing the soils from the tensiometer cups at the end of each aging cycle. These observations suggested the use of strength changes as an additional measure of thixotropic behaviour in these soils. The soils were prepared as previously described in the methods section, and probe penetration resistance measures were made of both sterilised and periodate treated sterilised soils.

Aging of moulded Stirling 1 and 3 and Bromyard 1 and 2 soils increased the resistance of the soils to probe penetration (Figure 4.21). In these soils (sterilised with mercuric chloride to inhibit biological activity), strength regain proceeded throughout most of the 18 day aging period, but appears to become constant after 15 days. The behaviour of the low organic soils approximated those of the high organic matter contents as can be seen by the similarities of the curves in the Figure. However, these results show that the lower organic soils maintained higher penetration resistance throughout aging. When the samples were remoulded after 18 days aging period, their strength fell to about the same levels as those of (0 day) samples, and subsequently recovered in strengths to probe penetration resistance values approximating those of the initial aging cycle, over another 18 day aging period. The water content changes determined for each sampling day

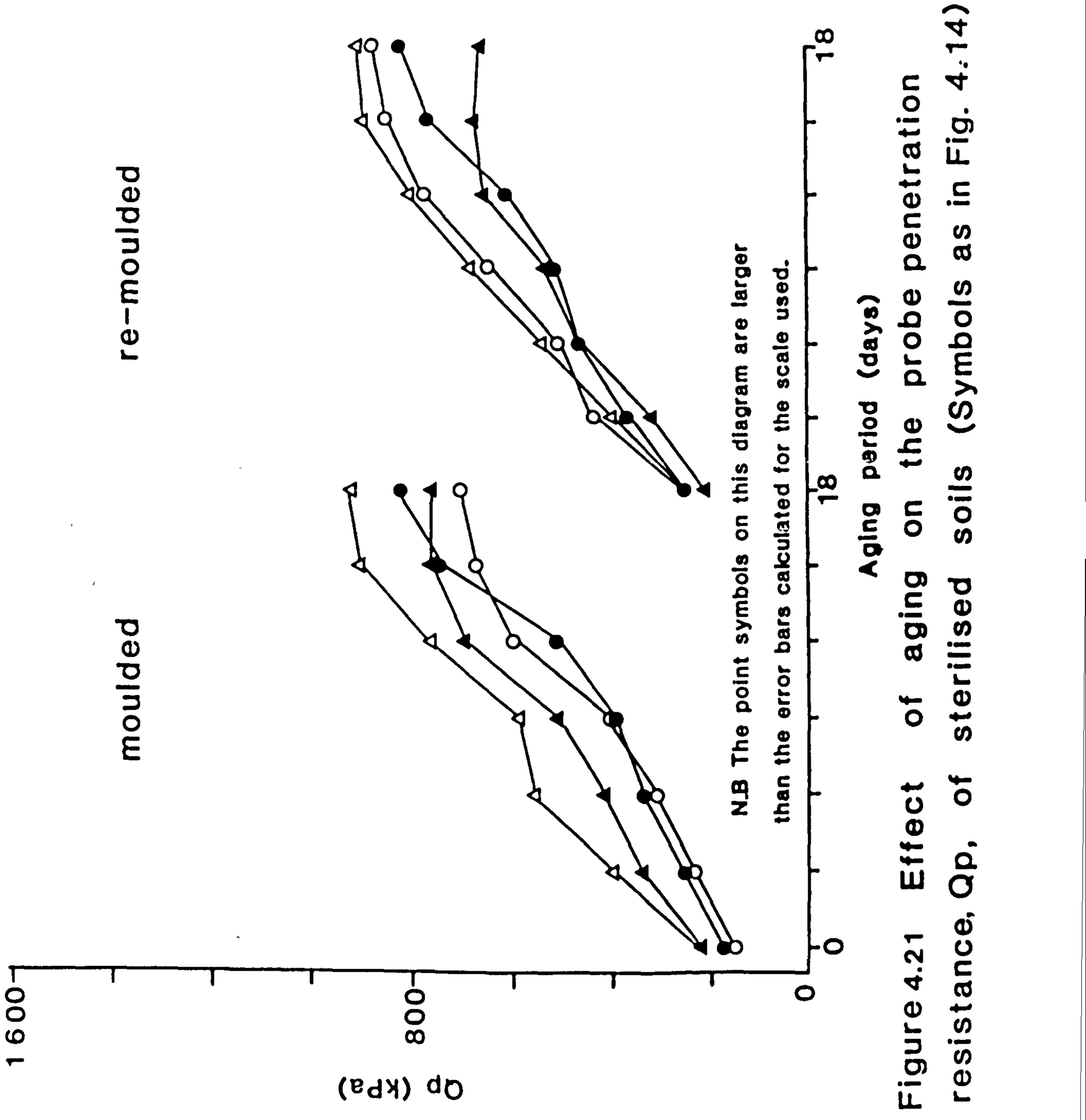


Figure 4.21 Effect of aging on the probe penetration resistance, Qp, of sterilised soils (Symbols as in Fig. 4.14)

during aging were negligible for all soils, and thus strength changes that are observed here were not the result of decrease of water content (which has a very large effect on soil strength) but were the result of thixotropic changes.

After completion of the second 18 day aging period, the same samples were treated with periodate to remove organic matter prior to aging and strength measurements. The results are shown in Figure 4.22 for the Bromyard and Stirling soils. The strength of oxidised and sterilised soils for non-aged samples (0 day) is similar to that of sterilised soils and this shows the reproducibility of the method of compaction used. These samples were packed by kneading compaction by hand. Whilst the trend in strength regain followed that of sterilised soils, oxidised soils increased in strength twice as much as sterilised soils over the 18 day aging period.

Similar large increases in strength of soils treated with hydrogen peroxide to destroy organic matter have been observed by Utomo and Dexter (1981c). This led them to conclude that in addition to the thixotropic process, there was an additional component of strength regain resulting from particle cementation. When in the present investigations the aged samples were remoulded at the end of the first 18 day aging cycle, these soils lost their resistance to penetration to levels approximating that of 0 day. Chemical forms of hardening may take place during aging of soil aggregates such as the cementing bonds discussed by Bjerrum and Lo (1963) and the conversion of montmorillonite to the aluminium form (Mathers et al., 1955). Rupture of these bonds on

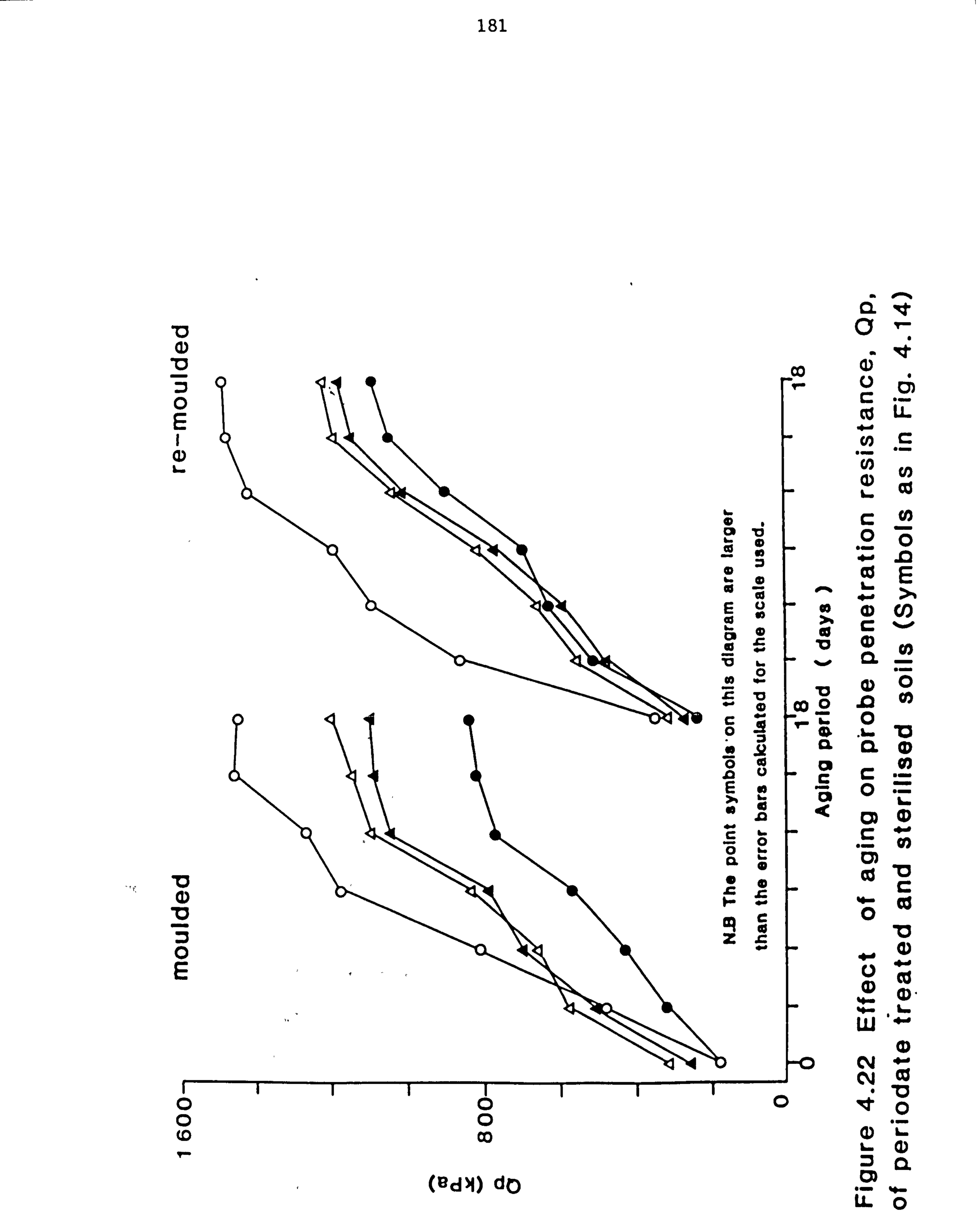


Figure 4.22 Effect of aging on pr probe penetration resistance, of periodate treated and sterilised soils (Symbols as in Fig. 4

remoulding would not be reversible. The loss in strength obtained upon remoulding therefore show that the strength regain obtained over the 18 day aging period is truly a result of thixotropic hardening, without need to invoke the cementation process envisaged by Utomo and Dexter (1981c). Apart from the Stirling 1 soil in which the strength regain was much higher during the second 18 day aging period, all the other soils regained their resistance to levels similar to the initial establishment which took place during the first aging cycle. The water contents of soils were kept constant and the difference between penetration resistance obtained for Stirling 1 soil during the first and second aging cycles could be the result of compression during packing of the soil in containers.

4.3.4 Discussion

Moulding undisturbed Bromyard, Stirling and Dreghorn soils resulted in a loss of part of their aggregate stability and strength (probe penetration resistance) and an increase in their matric water potentials. The loss in stability and strength observed immediately after the soils were moulded is consistent with the ideas of Mitchell (1960) and Schweikle et al. (1974), who suggested that shearing or moulding a soil rearranged the platy clay particles into a more uniform parallel arrangement. This increases the repulsive forces between particles resulting from double layer interaction. This face to face arrangement of the clay particles and reduced cohesion among them leaves them in a position where they can slip past one another with ease. In addition moulding results in the breaking of inorganic and organic particle

interconnecting and cementing bonds, which would have been established over long periods in the field from a combination of chemical, physical and biological processes (Martin et al., 1955; Bjerrum and Lo, 1963).

Aging moulded or remoulded soil resulted in increase in aggregate stability, probe penetration resistance and water tension, and these changes were observed within all five soils dealt with. In the sterilised soils the initial increase in stability in every case was less than the unsterilised soil, and after a period varying for different soils between 3 and 9 days remained constant. Biological activity therefore accounted for part of the initial increase in stability and the subsequent decline observed in the unsterilised soils. These biological effects on stability have been discussed in reviews by Martin et al. (1955), Harris et al. (1966), and Tisdall and Oades (1982), and the contribution made by fungi, bacteria and actinomycetes to the differences between sterilised and unsterilised soils dealt with in section 4.2.

The aging phenomena observed in sterilised soils in which biological activity did not participate is interpreted to be analogous to cases observed by civil engineers, who studied the process of thixotropy in pure clay systems (Seed and Chan, 1957, 1959; Mitchell, 1960, 1976). Their studies suggested that at low water contents the soil is strongly flocculated due to double layer water deficiency, and therefore thixotropic process would be negligible. On the other hand at high water contents, the soil disperses on its own accord from double layer repulsion (Koenigs, 1963) and consequently thixotropic process would be at its minimum. The effects of water content on the rate at which

cohesion increases during aging in disturbed soil would be compatible with the explanation of the bonding mechanisms in terms of slightly soluble components diffusing to and cementing points of contacts between particles. Such movements would be negligible in dry soil. With only one molecular layer of water on their mineral surfaces, soils were found to increase their cohesion over longer periods than when wetter (Kemper and Rosenau, 1984).

Moretto (1948) and Skempton and Northey (1952) found that thixotropic strength regain of natural clays decreased with decreasing water content from liquid limit. The latter authors even suggested that thixotropic strength regain at water content close to or near plastic limit might be zero. Mitchell (1960) and Utomo and Dexter (1981c) on the other hand found that thixotropic strength regain in compacted soils occurred at water contents below plastic limits. The results obtained in the present study with soils moistened to their plastic limit moisture contents are in agreement with conclusions from Mitchell's work in that in these soils at intermediate water contents the structure is dispersed through shear strain or moulding, and when this shear is released the soil is able to flocculate on its own accord with a consequent stability and strength increase.

Kemper and Rosenau (1984) also noted that freshly cultivated soil had aggregates with lower stability than the undisturbed one. When the disturbed aggregates were aged at different water contents, their stability and modulus of rupture increased. The changes were much slower in air-dry soil, and increased with increases in water content. It was

suggested that cohesional forces due to water were large enough to provide a major portion of the stability measured in those soils. Although their results are explained in terms of mineral to mineral contact and subsequent migration of bonding agents to points of contact (which would be negligible in dry soil) occurring during aging, which is consistent to thixotropic changes observed in the present study and others (Blake and Gillman, 1970), Kemper and Rosenau did not recognise those changes to be due to thixotropy. Similarly, Skinner (1979) observed increases in stability of sterilised and unsterilised moulded aggregates, but was unable to explain the change taking place in sterilised aggregates. The stability regain in irradiated artificial aggregates observed by Skinner could be explained in terms of thixotropy.

The change in particle orientation produced by moulding would be expected to result in initial non-equilibrium of water molecules (Seed and Chan, 1957). Day and Ripple (1966), Mitchell (1960), Schweikle et al. (1974), and Utomo and Dexter (1981c) have shown that the free energy of pore water decreases with time and subsequently increases on shearing. The observed increases in pore water pressure have been attributed to spontaneous shifting of the particles into positions of reduced potential energy with aging under the influence of thermal oscillations. As the structure adjusted itself to the lower energy status, a decrease in pore water pressure was observed. The results obtained for the Bromyard, Stirling and Dreghorn soils (Figure 4.19 and 4.20) show that moulding and remoulding of these soils at their plastic limit moisture contents results in a high relative free energy of the system which leads to increased matric water potential and that this water potential declines with aging.

If the stability, strength and tension regain observed in the present study and the explanation that such changes are of thixotropic origin is correct, then these changes with aging should be independent of other processes which could contribute to such regains during aging such as the particle cementation (Bjerrum and Lo, 1963; Utomo and Dexter, 1981c) and the formation of bonds by organic matter. Destruction of organic matter by periodate oxidation yielded results similar to those of Schweikle et al. (1974) and Utomo and Dexter (1981c) and this shows that organic matter does not participate directly in thixotropy, but may only modify the process due to its colloidal properties and its close associations with the clay.

Previous work in the field of soil tillage research has not yet been able to establish or develop a unique relationship between aggregate stability and soil strength (Chorley, 1959; Towner, 1973; Al-Durrah and Bradford, 1982). The similarity in development of aggregate stability and probe penetration resistance obtained for all soils in the present study indicates that together with the increase in aggregate stability resulting from cohesion forces, there is a corresponding increase in soil strength. Most of previous work by civil engineers has concentrated on quantification of soil strength resulting from thixotropic hardening (e.g. Mitchell, 1960).

Apart from the work of Blake and Gilman (1970), Arya and Blake (1972), Schweikle et al. (1974), and Utomo and Dexter (1981b) who have shown that thixotropic hardening processes are prominent in agricultural soils following cultivations (tillage), these processes have been

neglected in the field of soil tillage research. The moulding of aggregates by kneading compaction employed in the present study although more severe in extent than field soil manipulation, is analogous to the shearing of soil masses by swelling, shrinkage by surface tension forces resulting from drying, by soil fauna activity and most importantly by tillage implements. The recovery in these agricultural soils with time after 'tillage' suggests that simply allowing a soil to rest a few days after tillage would increase its water stability and aggregate strength. These changes would result in decrease in the susceptibility of the soil to damaging effects of erosion by water and compaction by tillage implements or trampling by farm animals. Similar conclusions have been arrived at by Kemper and Rosenau (1984) who wrote 'soils will disintegrate and slump less if a few days are allowed for freshly cultivated soils to regain their solid phase cohesion before they are saturated by irrigation'.

4.4 Effects of Weathering Cycles on Soil Aggregation

Effects of wetting and drying and freezing and thawing on the stability of aggregates produced by laboratory manipulation or field tillage were studied. Experiments were carried out using the Dreghorn series and the paired soils of Bromyard and Stirling series. All soils were moistened with the amount of water equivalent to their plastic limit moisture contents, and thus the assessment of effects of wetting and drying and freezing and thawing proceeded at moisture content levels at which the respective soils are in danger of damage if worked. Changes in aggregate stability were assessed by turbidimetry, and microstructural changes at the surface of the aggregates by scanning electron microscope (SEM).

4.4.1 Changes in Aggregate Stability Induced by Wetting and Drying Cycles

A preliminary experiment was carried out using the Bromyard paired soils to assess the effects of different matric water potential on the stability of artificial aggregates and cumulative water content changes during wetting and drying. Air dried aggregates of Bromyard 1 and Bromyard 2 were ground to pass a 0.25 mm sieve, moistened with deionised water and incubated for a period of two weeks. This preliminary two weeks aging period was intended to allow for completion of thixotropic hardening effects. Utomo and Dexter (1982) found that, without this initial aging period, inconsistent results were obtained and it was not possible to separate the effects of wetting and drying cycles from the thixotropic effects.

The first impression gained from the results in Table 4.2 is that wetting and drying gives rise to formation of water stable aggregates. Secondly it can be seen that the more drastic the wetting and drying the higher the stability attained by the aggregates. When the soils were wetted to -1kPa and oven dried at 60^{o}C there was a greater cumulative amount of wetting and drying, and the aggregates formed had the highest stability. The third observation of interest in Table 4.2 is the significant water stable aggregation which has occurred in the control samples. These control samples were maintained at constant tension (-1kPa) throughout the six day period during which the other samples underwent six cycles of wetting and drying.

Table 4.2 Effects of different matric water potential on the % stability S of reformed aggregates and cumulative water content change $\Sigma\Delta W$ during six cycles of wetting and drying.

Matric Water Potentials	Bromyard 1		Bromyard 2	
	S	$\Sigma\Delta W$	S	$\Sigma\Delta W$
Control -1kPa	46.1	0.50	38.3	0.80
-1 & -10kPa	50.4	0.12	41.2	0.14
-1 & -100MPa	59.3	2.30	46.5	1.70
-10 kPa & -100MPa	42.8	0.06	34.4	0.08
-1kPa & Oven drying (60°C)	64.4	4.30	50.7	5.01
-1kPa & 20°C	58.5	3.11	47.8	4.30
-10kPa & Oven drying	45.7	0.31	35.6	0.23
-10kPa & 20°C	45.6	0.43	35.4	0.44

Removing the samples from a desiccator containing a layer of water to maintain constant moisture conditions after two weeks aging period, and placing them on sintered glass funnels kept at -1kPa resulted in the gain in moisture content (Table 4.2). During wetting, the originally randomly oriented clay domains swell by inbibing interlamellar water and push against each other, the overall effect of which is formation of microstructure that maintains the relative position of the domains, unless the water is removed by drying. These movements would have caused significant preferred orientation of the clay domains, and therefore the increase in stability of aggregates which were kept at -1kPa is probably a result of further thixotropic hardening processes. The results obtained in section 4.3 (Figure 4.21) hinted that thixotropic hardening would continue to increase beyond the period of 15 days chosen for the present study. These results suggest that additional thixotropic hardening occurs as a result of stresses set up in the soil by wetting and drying in samples subjected to wetting and drying cycles.

The highest stability levels attained with wetting and drying between -1kPa and oven drying at 60^{o}C suggest that more drastic wetting and drying is accompanied by generation of higher mechanical stresses within the soil. These stresses can generate planes of weakness within the soil mass which in turn provides initial faces of soil aggregates. Drying which extends into high suction can cause maximum particle interlocking due to the degree of dehydration. The resultant differences in stability obtained when these soil aggregates were subjected to wetting and drying at different matric water potentials show that the greater the changes in water content, the greater are the stresses

produced by wetting and drying. This relationship between aggregate stability and amount of wetting and drying was assessed using the Bromyard and Stirling paired soils.

Dry soils were sieved to obtain 2-4 mm diameter equilibrated at -1kP and later subjected to wetting and drying cycles between -1kPa and -100MPa. Cumulative amount of wetting and drying, $\in\Delta W$, was estimated from the amounts of wetting and drying, ΔW, determined for each cycle of wetting and drying. The results of changes in aggregate stability are plotted against cumulative wetting and drying in Figure 4.23(a). The number of wetting and drying cycles to which the samples were subjected in Figure 4.23(a) increases from left to right in the order 0,3,6,9,12 and 15. Figure 4.23(a) shows that the stability of the field aggregates decreases as the cumulative amount of wetting and drying increases.

The strength of these approximately linear negative relationships between aggregate stability and cumulative amount of wetting and drying was assessed by regression analysis, and the results are presented in Table 4.3, Column A. The highly significant negative correlation coefficients obtained for these field aggregates suggest that the frequency of wetting and drying has a great influence on the stability of soils. The repeated microcrack formation resulting from repeated wetting and drying partly explains the decline in stability observed, however, it should be noted that other changes which are of biological origin may also contribute to these observed changes.

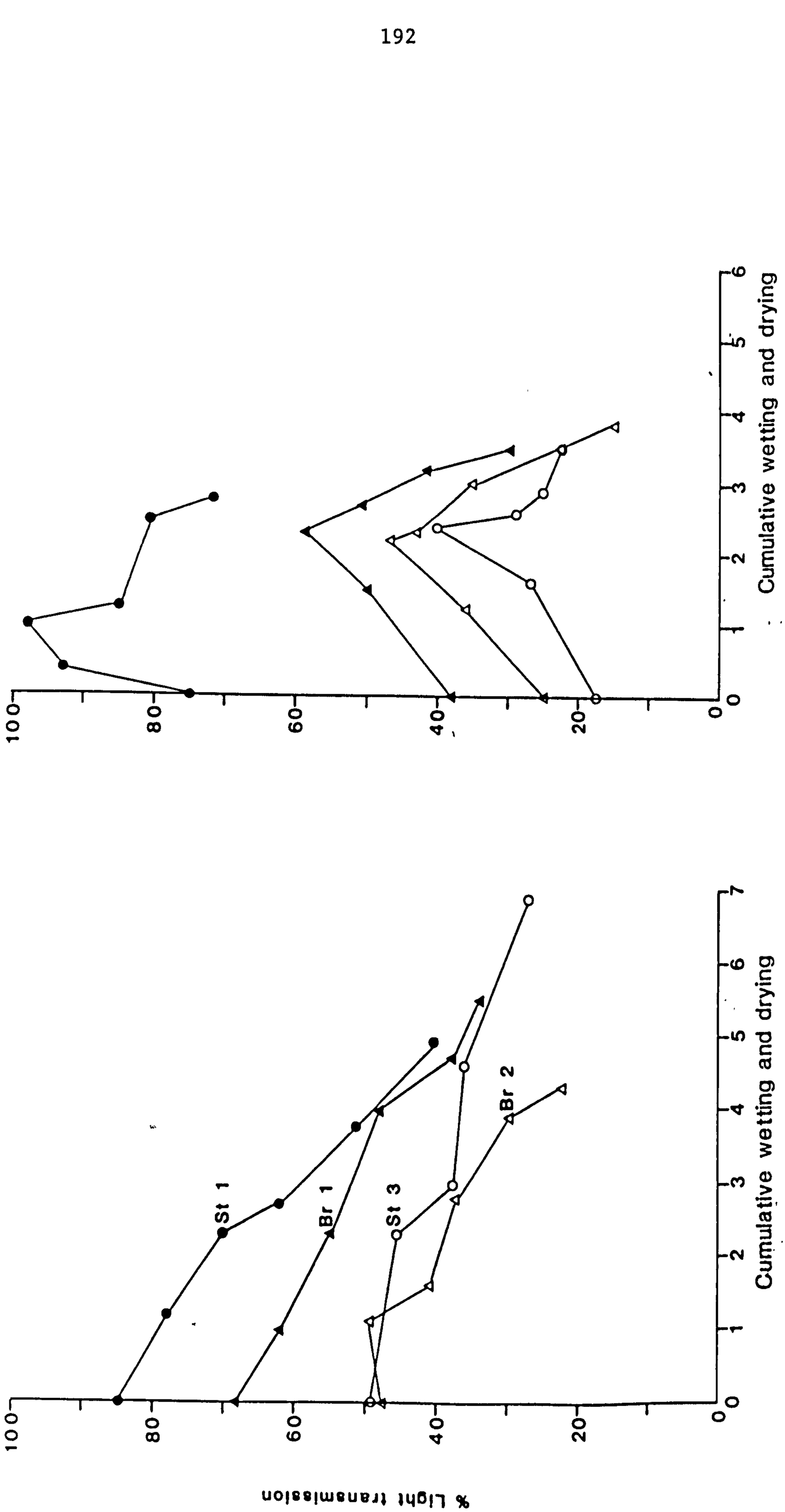


Figure 4.23a Effect of Cumulative amount of wetting and drying on the stability of field aggregates

Figure 4.23b Effect of Cumulative amount of wetting and drying on aggregation of dispersed soils

Table 4.3 Correlation coefficients between aggregate stability and cumulative amount of wetting and drying. A, Field Aggregates (3 to 15 cycles); B, Artificial Aggregates (3 to 15 cycles); C, Artificial Aggregates (6 to 15 cycles).

Soil	A	B	C
Bromyard 1	-0.979**	-0.725 NS	-0.987*
Bromyard 2	-0.969**	-0.819*	-0.999*
Stirling 1	-0.998**	-0.887*	-0.903*
Stirling 2	-0.904*	-0.231 NS	-0.899*

NS = Not Significant, (* $p<0.05$) (** $p<0.01$).

Whilst the statistical testing revealed a linear relationship between aggregate stability and cumulative amount of wetting and drying, subjection of these soils to additional 15 cycles of wetting and drying showed that the decline in stability levels off after 20 cycles. Thus with additional cycles, the amount of wetting and drying increases but the stability reached a minimum and then remained constant. The stability results therefore tended to conform to a linear relationship only over a limited initial range. It should be noted however that this levelling off might reside within limitations of the technique used to asses stability.

When remoulded aggregates (10 - 20 mm diameter balls) from both the Bromyard and Stirling paired soils were subjected to wetting and drying in the same manner as field aggregates a more complex relationship was obtained between aggregate stability and cumulative amount of wetting and drying. Figure 4.23(b) shows the results of aggregate stability plotted against cumulative amount of wetting and drying. Inspection of this Figure reveals that the stability of remoulded aggregates at the end of the 2 weeks incubation period is lower than that of field aggregates from which these remoulded aggregates were formed. The initial aging period was imposed upon these reformed aggregates to allow for completion of thixotropic hardening processes and the results have been discussed in detail in section 4.3. Thus without any wetting and drying cycles, the stability of reformed aggregates was far below that field agggegate at the end of the aging period.

Wetting and drying of remoulded aggregates resulted in an increase in stability over the initial six cycles and subsequently there was a decline in stability similar to but steeper than that of field aggregates. As suggested by results in Table 4.2 in which control samples increased in stability without being subjected to wetting and drying cycles, further thixotropic changes could partly account for the observed increase in stability in these moulded aggregates which took place during wetting and drying. Figure 4.23(b) shows that stability of the aggregates increased and later declined, whereas the cumulative amount of wetting and drying increased continually with increasing wetting and drying cycles. The complexity of the relationships between these two parameters lies within this initial increase in stability, and it was therefore decided to treat the results in two parts.

Regression analysis was utilised to assess the strength of the relationship between aggregate stability and cumulative amount of wetting and drying and the results are presented in Table 4.3 columns B and C. These columns represent treatment of data obtained when wetting and drying was between 3 and 15 cycles and 6 and 15 cycles respectively. For moulded aggregates there is an initial recovery in stability during the 3 to 6 cycles period to levels similar to those of their field counterparts, and thereafter stability declines. When all cycles between 3 and 15 were included, calculation of correlation coefficients indicated no significance for the relationship between stability and cumulative wetting and drying for Bromyard 1 and Stirling 3, (Column B). If the initial period of recovery was omitted, the decline in stability from 6 to 15 cycles was shown to be significantly correlated to cumulative wetting and drying (Column C).

Comparing the aggregate stability values obtained for Bromyard and Stirling aggregates in Figures 4.24 and 4.25 shows that the stability decline occurring in reformed aggregates with repeated wetting and drying cycles from the 6th to the 15th cycle parallels and approximates to that of field aggregates, which indicates that the process and mechanism of structural degradation involved is similar in both these types of aggregates. Further inspection of Figure 4.23 shows that the higher values of cumulative amount of wetting and drying were obtained in field aggregates than in moulded aggregates. This observation is attributed to the inherent porous structure of field aggregates as compared to compact moulded aggregates. The former structure would take up or lose more water during wetting and drying respectively. Size of the aggregates could also have contributed to the differences in amount of wetting and drying, in that field aggregates were 2 - 4 mm and moulded aggregates 10 - 20 mm diameter.

Having established the possible processes and mechanisms involved during wetting and drying, the preceding experiments were repeated under non-sterile and sterile conditions, using both field and reformed aggregates to allow for separation of processes that are physical or biological contributing to stability changes in the soil. The results of field aggregates are plotted together with those of moulded aggregates.

Field aggregates and artificial aggregates of the Bromyard 1 and 2, Stirling 1 and 3 and Dreghorn soil series, obtained by mild sieving were subjected to 3,6,9,12 and 15 cycles of wetting at -1kPa and drying at -100 MPa and the results are illustrated in Figures 4.24 to 4.28. The

results from field and moulded aggregates, the latter obtained by pulverisation of field aggregates and reconstitution, have been plotted together to simplify comparisons between these aggregates for the individual soils. Moulded aggregates obtained by crushing 2 - 4 mm natural aggregates to pass a 0.25 mm sieve, and reconstituting these into balls of 10 - 20 mm diameter, were aged for a period of 15 days before being subjected to wetting and drying cycles. The stability changes taking place during aging of these balls have been discussed in section 4.3. This initial 15 day aging period allows the thixotropic hardening effects to occur. These thixotropic effects gave rise to the non-microbial stability changes as observed with soil samples which had been reconstituted.

Inspection of Figures 4.24, 4.25 and 4.26 shows that the stability of aggregates from moulded soils was much lower than that of field aggregates before wetting and drying. This indicates that moulding destroys much of the previous bonding within the aggregates. Similar decreases in aggregate stability following tillage were observed by Rovira and Graecen (1957) and Kemper and Rosenau (1984). The stability of non-tilled field aggregates obtained by mild sieving of air-dried soil was decreased by repeated wetting and drying cycles. The decline in stability continued over 15 cycles of wetting and drying and did not appear to be entirely completed. It was observed during preliminary experiments that after 20 cycles of wetting and drying, there were no more significant changes in aggregate stability, and subsequently experiments continued only up to 15 cycles, because the study has its main interest in changes following immediately after tillage operations,

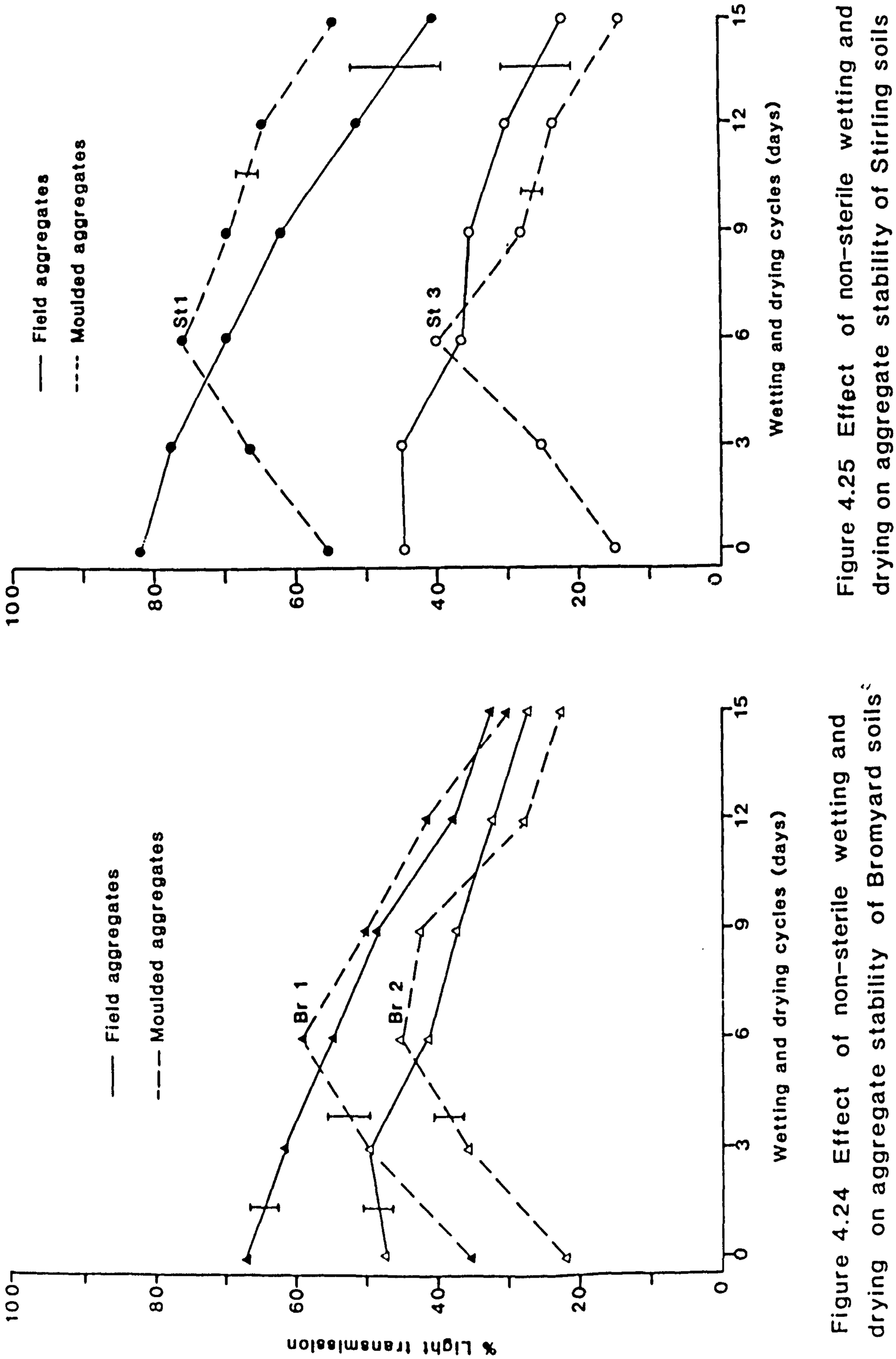


Figure 4.25 Effect of non-sterile wetting and drying on aggregate stability of Stirling soils

Figure 4.24 Effect of non-sterile wetting and drying on aggregate stability of Bromyard soils

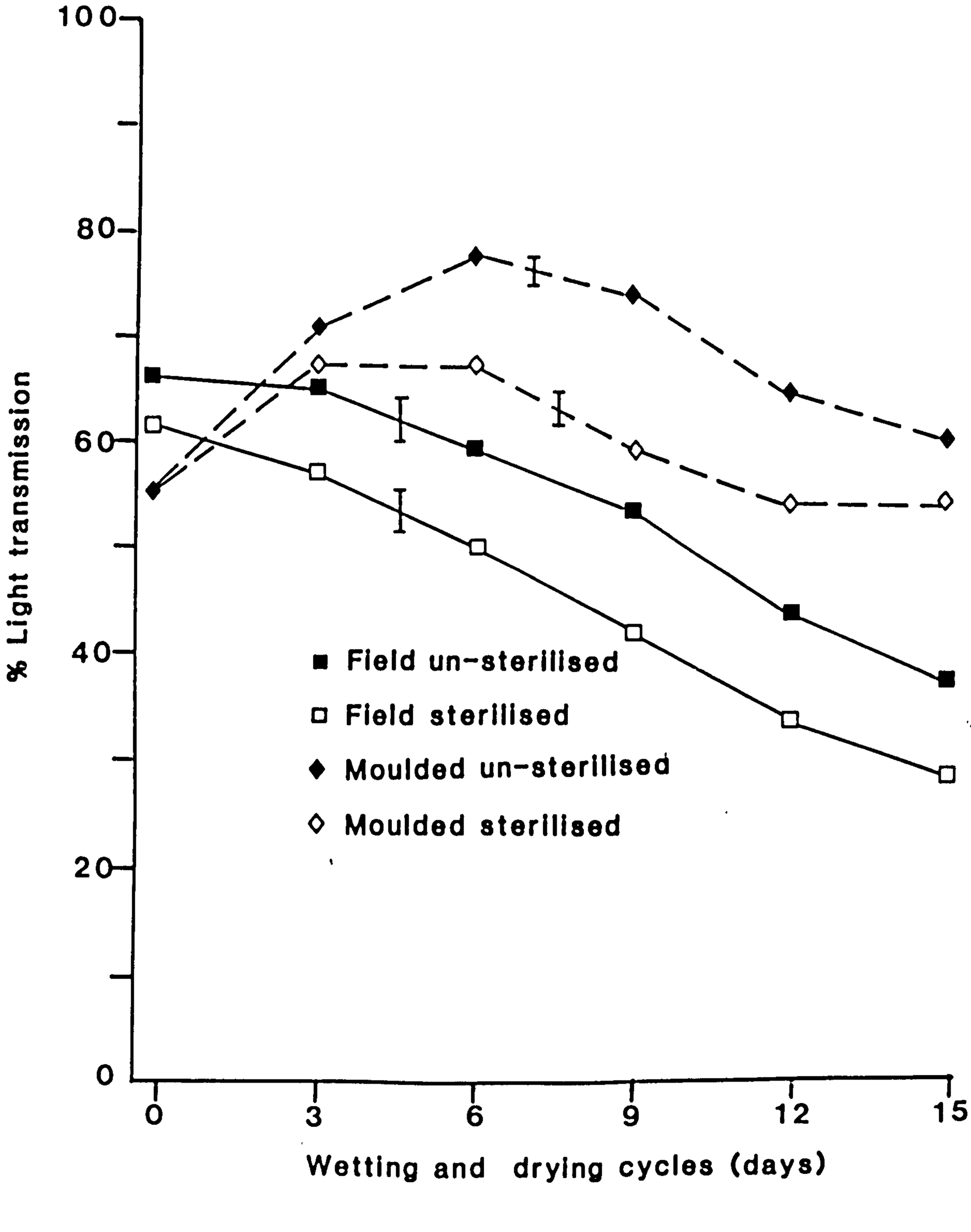


Figure 4.26 Effect of wetting and drying on aggregate stabilty of Dreghorn soil

and it was considered that the greater part of the change would have taken place in this time.

Figures 4.26, 4.27 and 4.28 represent the results in which aggregates were wetted with sterilising solutions containing 0.5 mg of sodium azide and 0.5 mg mercuric chloride per gram of soil. Wetting and drying of sterilised natural aggregates resulted in a decrease in stability. The decrease persisted with increasing numbers of wetting and drying cycles and the sterilised aggregates maintained lower stability levels to those of non-sterilised counterparts throughout (e.g. compare Figure 4.24 and 4.27). The differences between stability levels of the non-sterilised and sterilised aggregates suggest that microbial activity is imparting to non-sterilised soils additional resistance to the forces associated with wetting and drying.

In contrast to field aggregates, moulded aggregates increased in stability with increasing number of wetting and drying cycles up to 6 cycles and the stability declined thereafter with repeated cycles up to 15 cycles as shown in Figures 4.24, 4.25 and 4.26. The initial increase in stability also occurred with wetting and drying of those aggregates moistened with sterilising solution (Figures 4.26, 4.27 and 4.28). The results obtained here support the proposed mechanisms by which wetting and drying cause opposing processes of age hardening due to thixotropic process, and a decline in stability due to cracking as discussed earlier in this section. The parellel evolution of change in stability is evident from comparisons of the aggregate stability values in non-sterilised soils in Figures 4.24, 4.25, 4.26 and those of sterilised

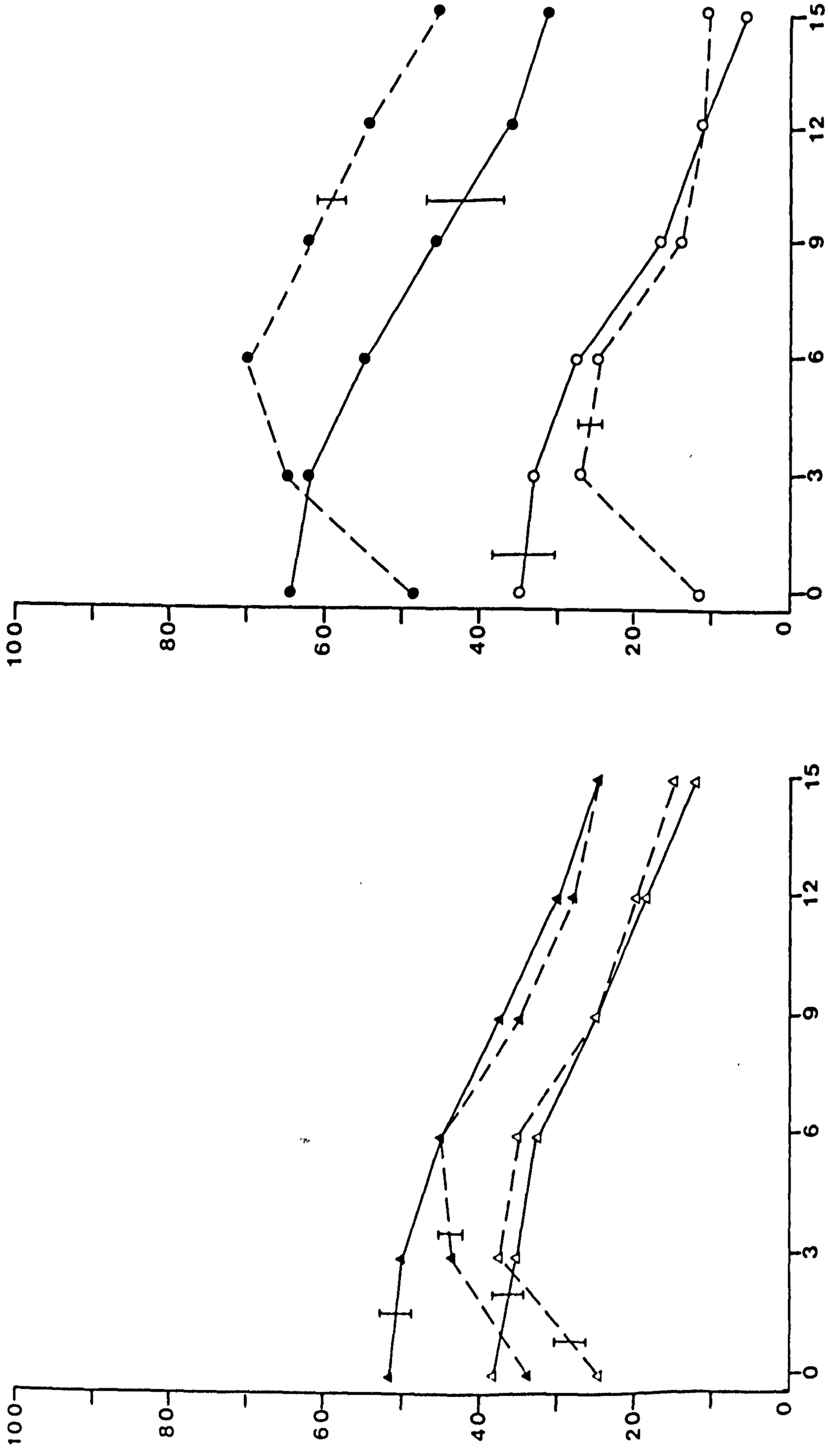


Figure 4.28 Effect of sterile wetting and drying on aggregate stability of Stirling soils (Symbols as in Fig. 4.25)

Figure 4.27 Effect of sterile wetting and drying on aggregate stability of Bromyard soils (Symbols as in Fig. 4.24)

soils in Figures 4.26, 4.27 and 4.28. The higher levels of stability attained by non-sterilised aggregates demonstrate the important contribution of soil microbial activity in imparting water stability aggregates.

4.4.2 Changes in Aggregate Stability Induced by Freezing and Thawing Cycles.

Whilst there appears to be no agreement in the results obtained from freezing and thawing experiments in the literature, some workers have suggested that these processes affect stability by the same mechanisms as wetting and drying (e.g. Richardson, 1976). To allow for comparisons between the effects of wetting and drying and freezing and thawing experiments of the latter processes were performed using soils pre-treated as in the wetting and drying experiments. Some of the field aggregates 2 - 4 mm were subjected to freezing and thawing cycles, and part of these were ground to pass 0.25 mm sieve, and moulded into balls of 10 - 20 mm diameter, which were aged for 15 days, and subsequently subjected to freezing and thawing cycles.

Moistening field aggregates of the Bromyard 1 and 2, Stirling 1 and 3 and Dreghorn soils with deionised water to plastic limit moisture content and subjecting them to freezing and thawing did not result in appreciable stability changes. Figures 4.29, 4.30 and 4.31 show that natural field aggregates (2 - 4 mm diameter) frozen for 16 hours at $-10^{o}C$ and thawed for 8 hours under moist conditions in a desiccator containing a layer of deionised water, and the process repeated up to 15

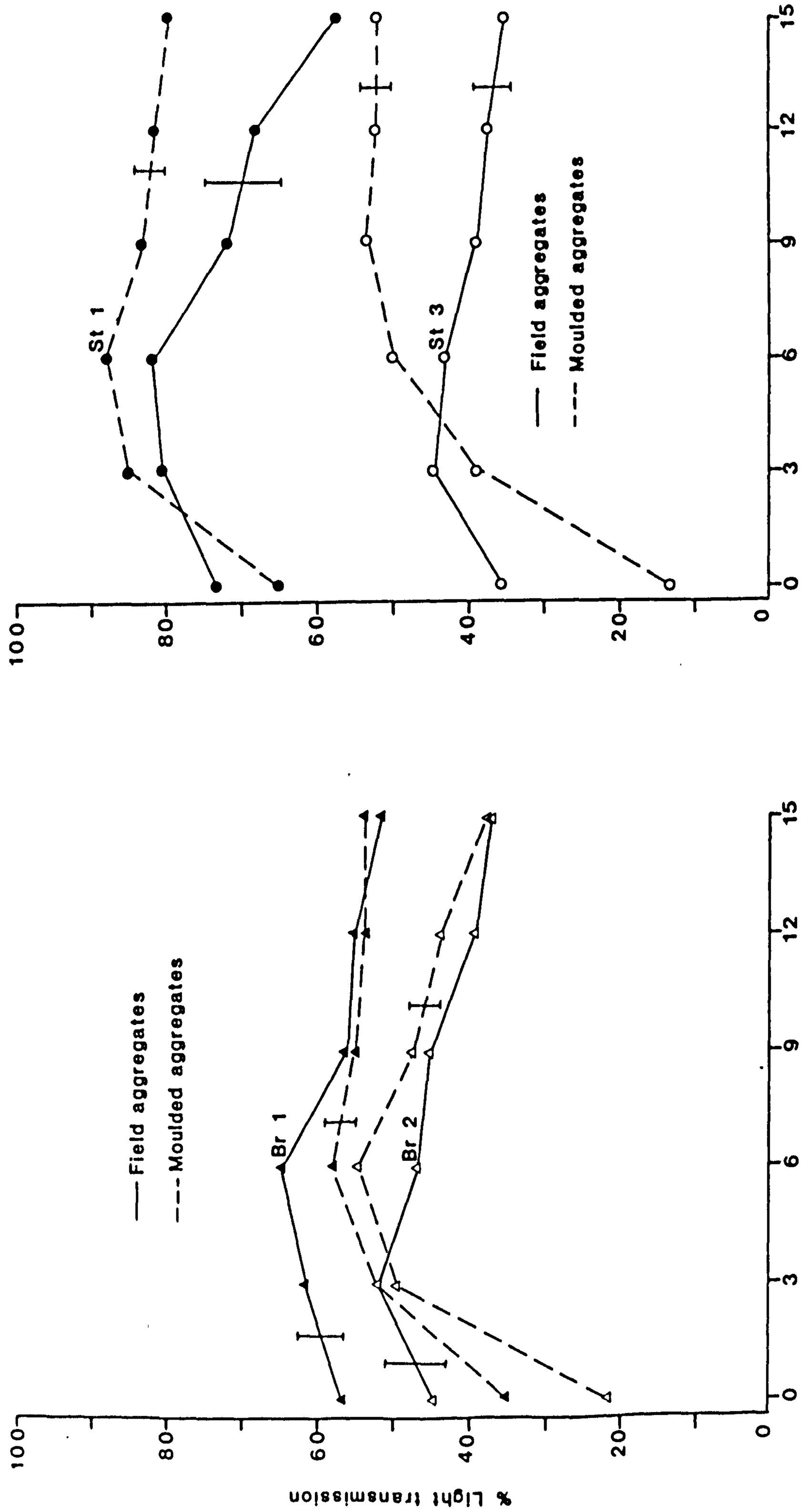


Figure 4.29 Effect of non-sterile freezing and thawing on aggregate stability of Bromyard soils

Figure 4.30 Effect of non-sterile freezing and thawing on aggregate stability of Stirling soils

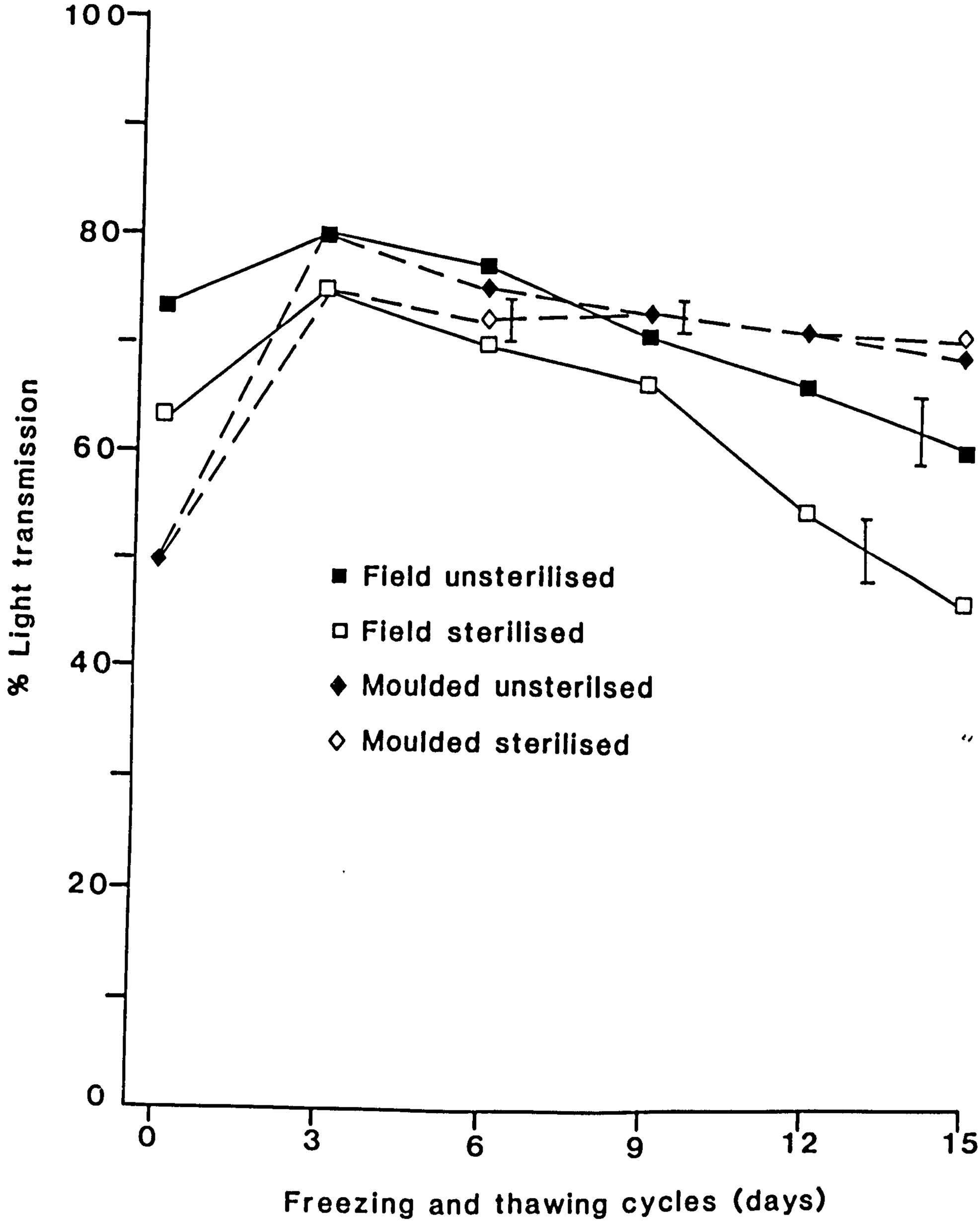


Figure 4.31 Effect of freezing and thawing on aggregate stability of Dreghorn soil

days, changed only slightly as compared to samples subjected to wetting and drying cycles. A small increase in stability during the initial 3 to 6 cycles varying for the different soils was followed by a gradual decline. Apart from the Stirling 1 and Dreghorn soils the decline in stability in other soils was very small and values approximating the initial stability (0 day) were reached after 15 cycles of freezing and thawing.

Field aggregates moistened with sterilising solution and frozen and thawed under sterile conditions (Figures 4.31, 4.32 and 4.33) increased in stability with initial 3 cycles of freezing and thawing. Thereafter, there was a decline in stability over succeeding cycles and significantly lower values than those in non-sterile treatment were obtained after 15 cycles. The differences in stability level in non-sterilised and sterilised aggregates show the importance of microbial activity which would replace fractured bonds from the disruptive forces generated by freezing. Resumption of microbial activity would be expected during thawing in non-sterilised soils.

The results of aggregate stability change in moulded aggregates plotted together with those of field aggregates in Figures 4.31, 4.32 and 4.33 show that moulded aggregates regained stability during the initial 3 to 6 cycles of freezing and thawing. The increase in stability observed was about twice that obtained when similar samples were subjected to wetting and drying. The recovery in stability of artificial aggregates after 3 cycles of freezing and thawing resulted in attainment of stability values approximately the same as field aggregates or in some

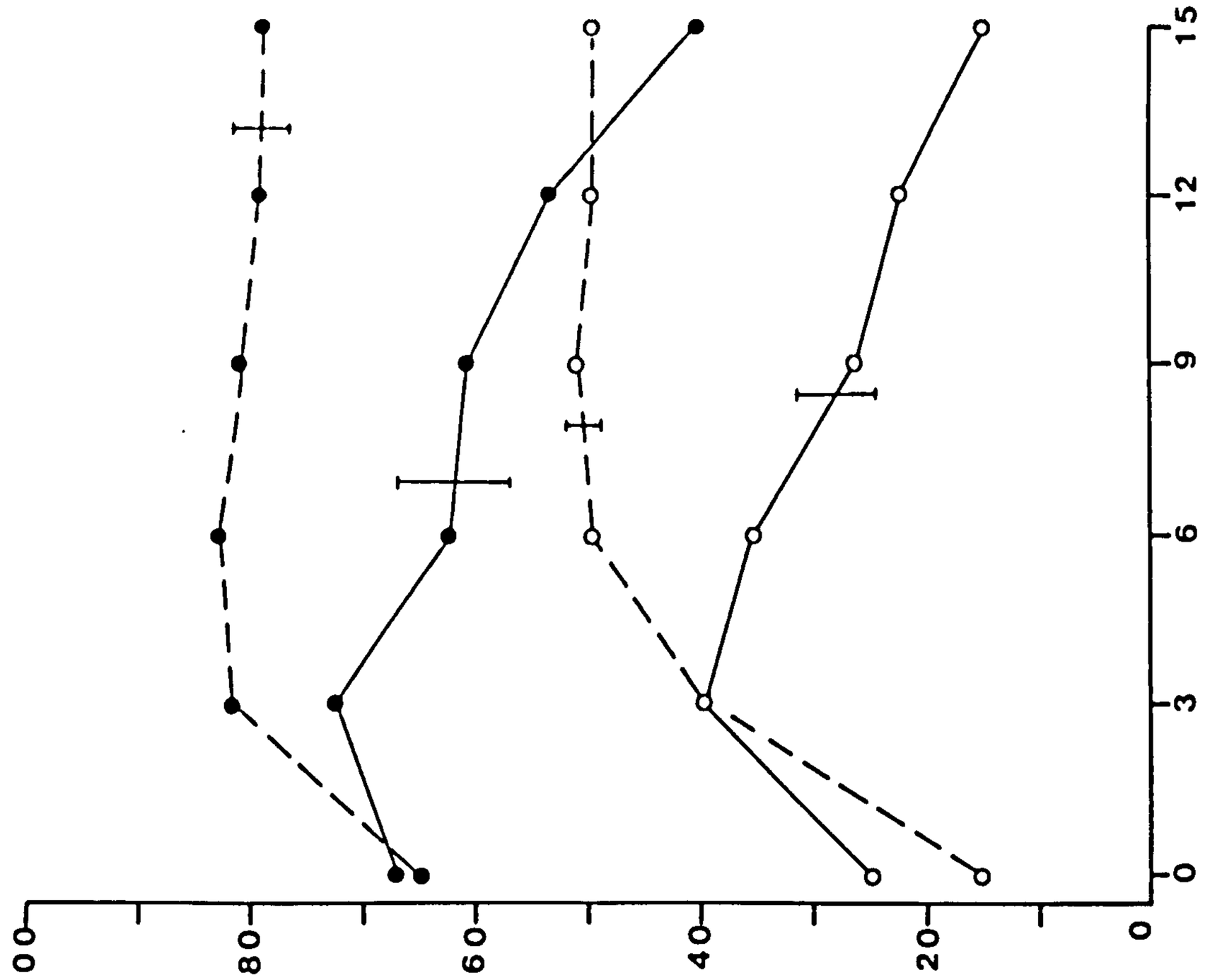


Figure 4.33 Effect of sterile freezing and thawing on aggregate stability of Stirling soils (Symbols as in Fig. 4.30)

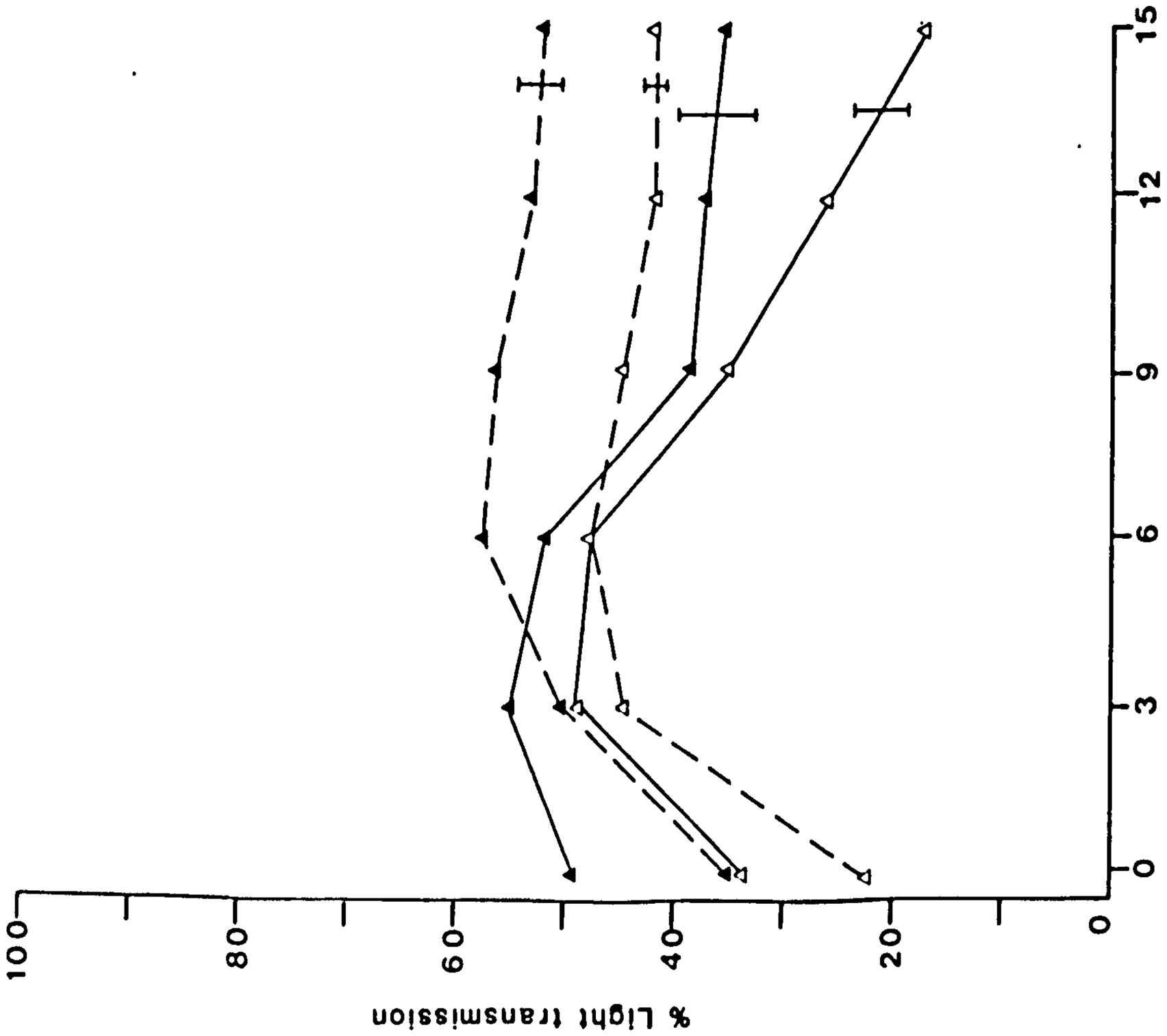


Figure 4.32 Effect of sterile freezing and thawing on aggregate stability of Bromyard soils (Symbols as in Fig. 4.29)

cases to slightly higher values. As opposed to all other treatments such as wetting and drying for example, the stability reached after 3 cycles of freezing and thawing of the moulded aggregates, was maintained with subsequent freezing and thawing cycles.

It can be seen from these results of freezing and thawing of moulded aggregates that sterilised soils showed similar behaviour to non-sterilised ones. The increase in stability observed in these sterilised moulded aggregates, in which biological activity did not contribute suggest that stresses generated by freezing forces the aggregates into closest possible packing, and cause interlocking of the clay plates which had already established desired contacts through re-orientation which took place during thixotropic hardening. The results therefore suggest that the stability increase in artificial aggregates with freezing and thawing is a further manifestation of the thixotropic hardening process.

Silanpaä (1961) and Williams (1968) suggested that the aggregate structure (e.g. porous or compact), the size of the aggregates, the moisture content as well as the rate of freezing all interact to influence the ultimate effects of freezing and thawing on aggregate stability. An experiment was conducted using the Bromyard and Stirling paired soils to assess the influence of aggregate size during freezing and thawing of moulded aggregates. Reformed balls (10 - 20 mm), aged as before, were subdivided to give aggregates 4 - 6 mm diameter. These sub-aggregates were allowed to recover for 3 days at constant moisture and subsequently subjected to freezing and thawing cycles as before.

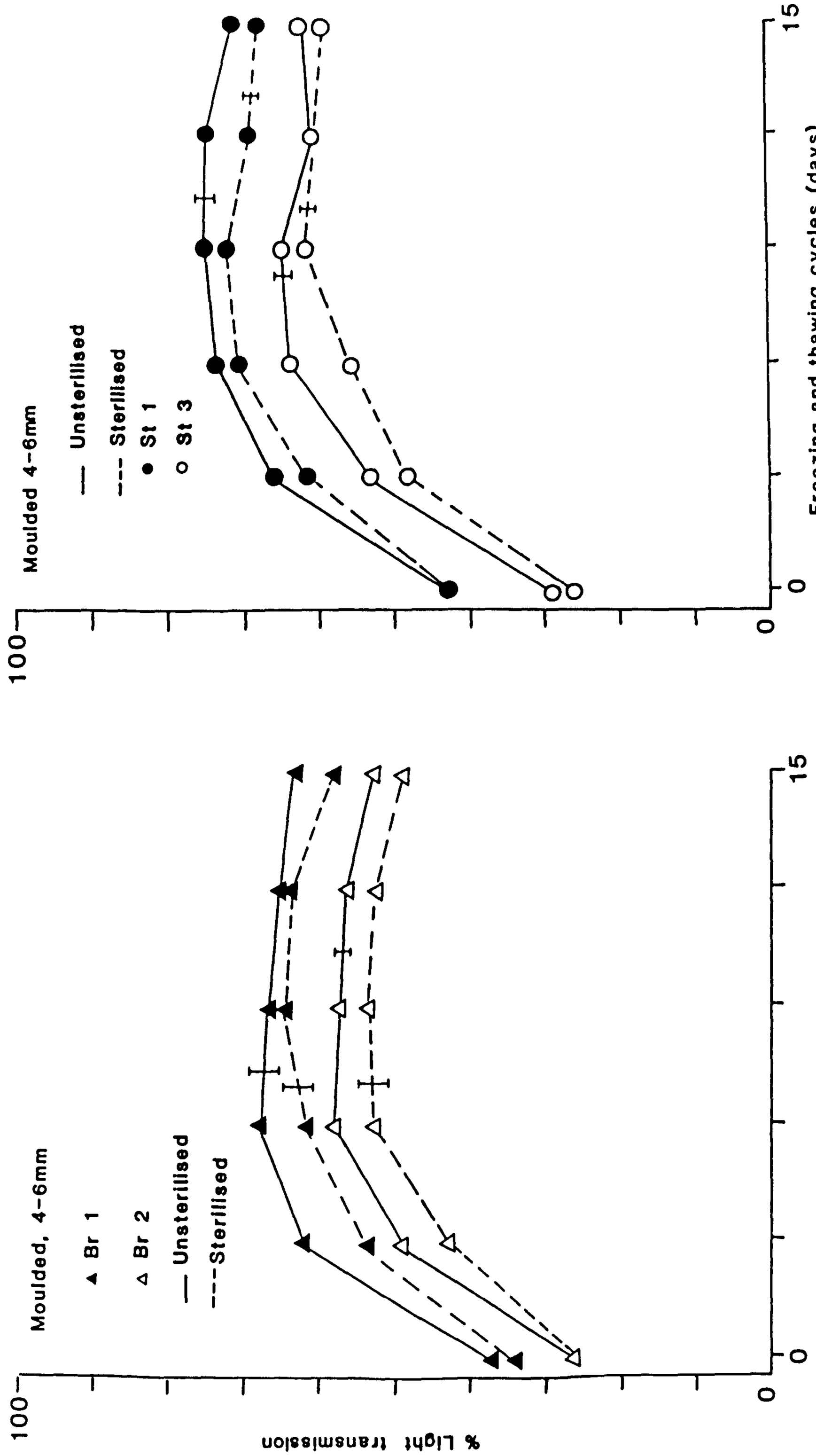


Figure 4.35 Effect of freezing and thawing on the stability of Stirling moulded aggregates

Figure 4.34 Effect of freezing and thawing on the stability of Bromyard moulded aggregates

Figure 4.34 shows that the Bromyard 1 and 2 aggregates increased in stability over the first 3 to 6 cycles, and subsequently remained constant with further cycles of freezing and thawing. The increases were parallel to those attained when the moulded balls of 10 - 20 mm diameter were frozen and thawed under similar conditions and for the same number of cycles. Similar results were obtained for the Stirling soils (Figure 4.35).

As opposed to field aggregates in which stability declined with freezing and thawing under sterile conditions, moulded aggregates reduced in size from 10 - 20 mm to 4 - 6 mm diameter increased in stability and the inrease in sterilised aggregates paralleled that of non-sterilised ones. The results show that the stability of sterilised soils maintained lower levels to those of non-sterile ones. On comparing the results obtained with 10 - 20 mm and 4 - 6 mm aggregates, of both non-sterilised soils, it can be seen that slightly higher values of stability were obtained with the smaller of aggregates. However for the Stirling 1 soil the recovery brought the 4 - 6 mm aggregates to similar levels of stability obtained with field aggregates 2 - 4 mm diameter. The similarity in the trends of stability development, and the approximately similar values obtained show that size did not significantly influence the effects of freezing and thawing, but that the results were most influenced by the moulding prior to freezing and thawing cycles.

4.4.3 Micromorphological Studies by SEM

The general picture gained from the results of wetting and drying and freezing and thawing especially with moulded aggregates, suggests the

gain in stability to be a result of combined physical and biological processes, however with a larger contribution from the former process as evidenced by results obtained in sterilisation treatments. In order to gain more information on these physical changes, samples were examined with SEM. Scanning electron micrographs obtained revealed striking microstructural changes at the surface of the aggregates.

Plate 6a shows the much greater disorder in the particle arrangement for the newly moulded soil which has not been subjected to wetting and drying cycles. The most striking microstructural differences in non-cycled soils can be seen by comparing Plate 6a to Plate 6b and 7a to 7b. Inspection of Plates 6b and 8a reveal the much greater degree of orientation of the particles in frozen and thawed soil than in wetted and dried soil. The platy structure resulting from freezing and thawing was obvious on removal of the aggregates from the freezing chamber and in most cases plates of thickness ca. 1 mm could be measured. SEM revealed particles at the surface of cycled aggregates arranged in steplike fashion mainly in one direction and which appeared to be tightly held together. Cracks and pores were more obvious in samples subjected to repeated cycles of freezing and thawing, as in Plates 8b, 9a and 9b, and minor cracking resulted from the stresses caused by wetting and drying (Plate 7a).

The SEM picture obtained here revealed the surface morphological changes taking place in these aggregates, at magnifications which could only detect the arrangement of particles in the sand and silt size ranges. Therefore the disordered structure referred to here, does not

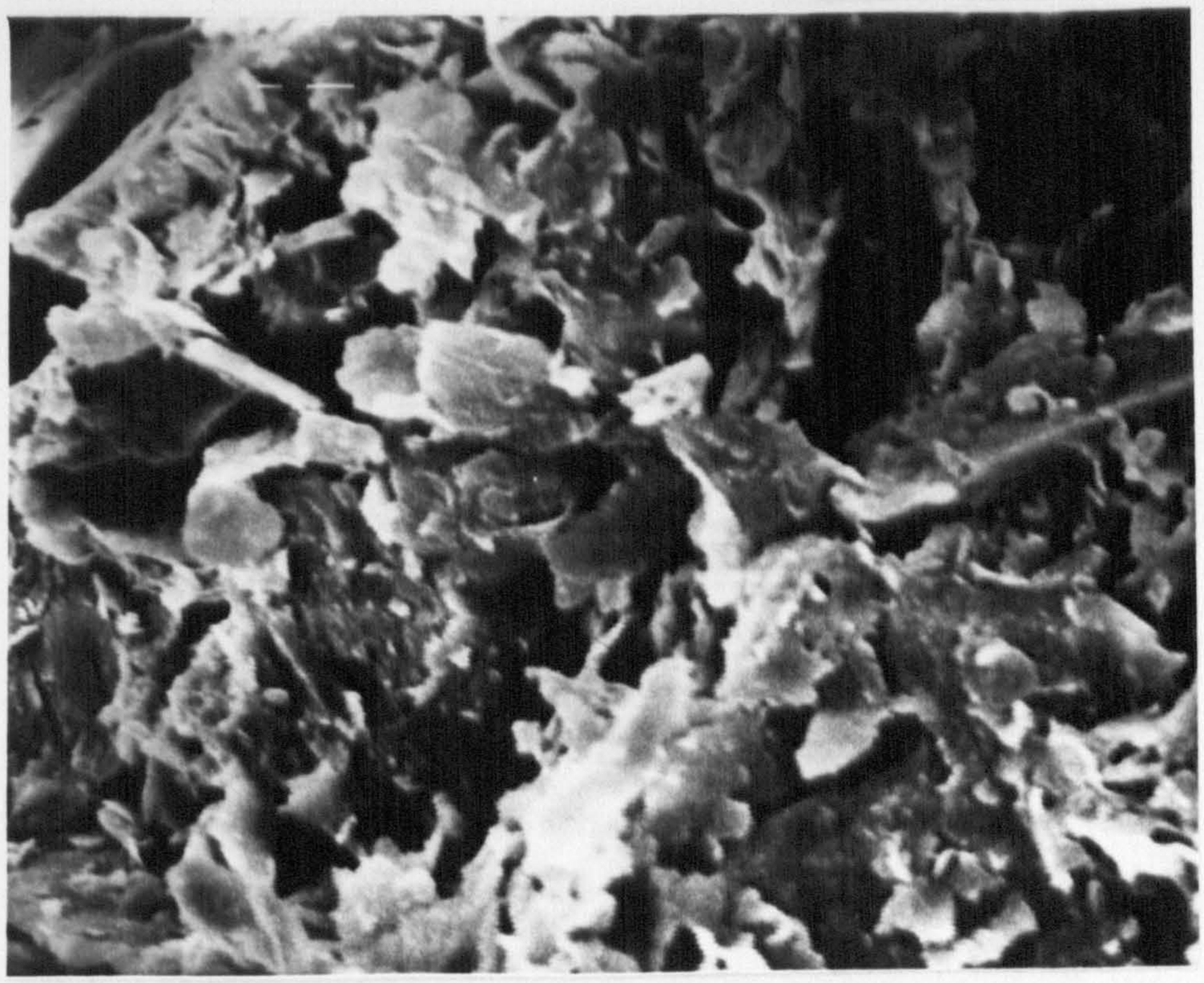


Plate 6a Non-cycled soil (4 500x)

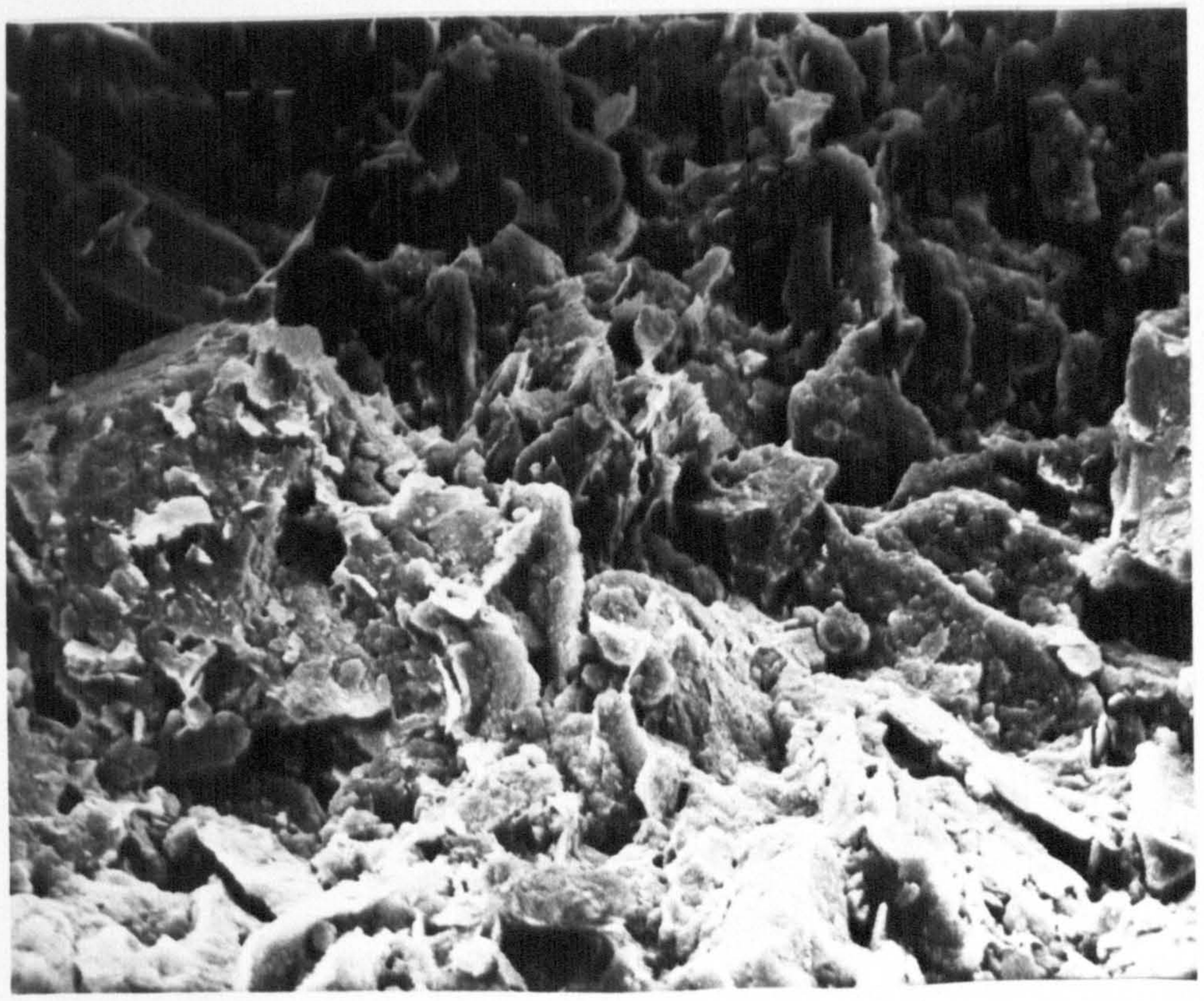


Plate 6b 3 cycles wetting and drying (2 000x)

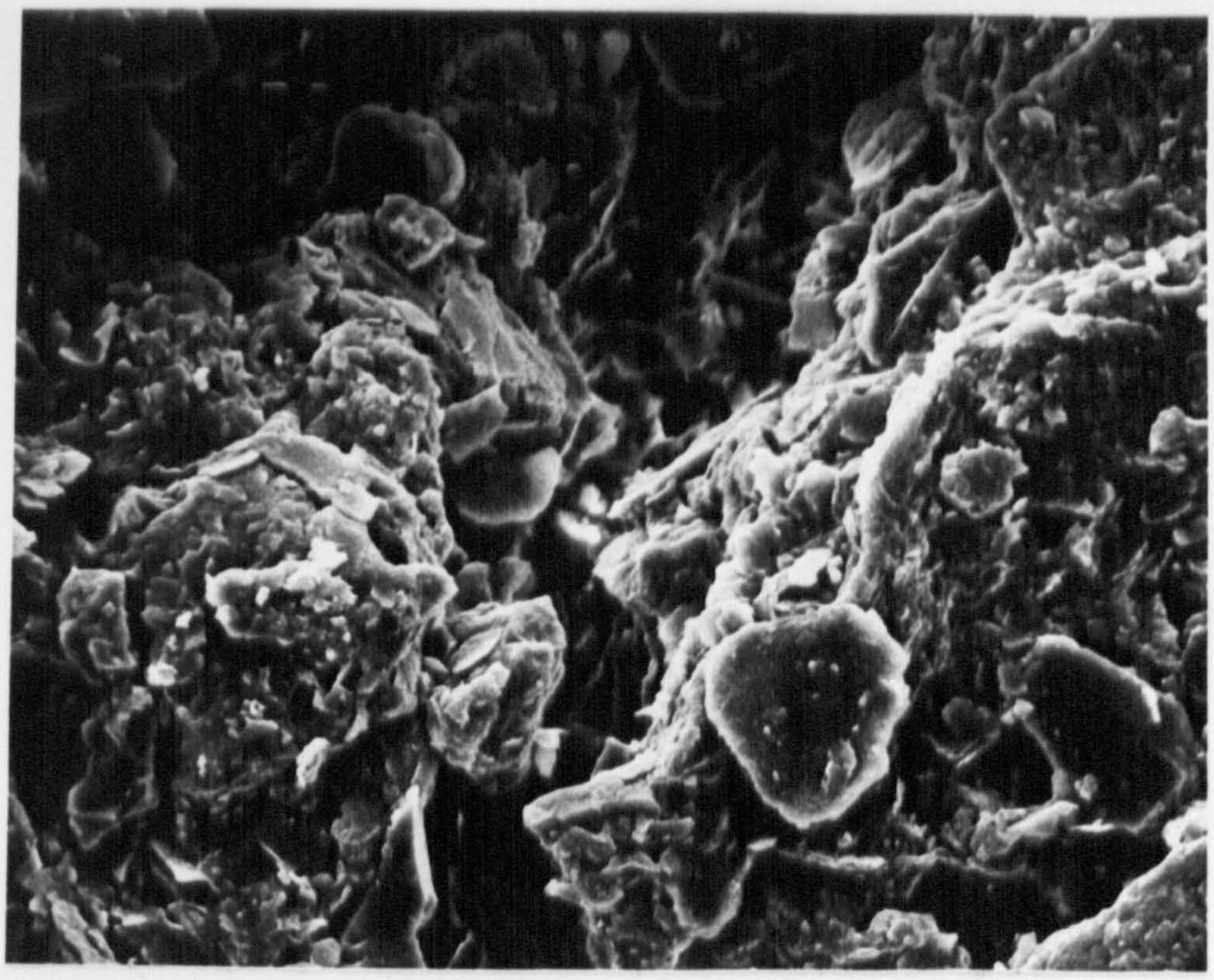


Plate 7a 6 cycles wetting and drying (2 000x)

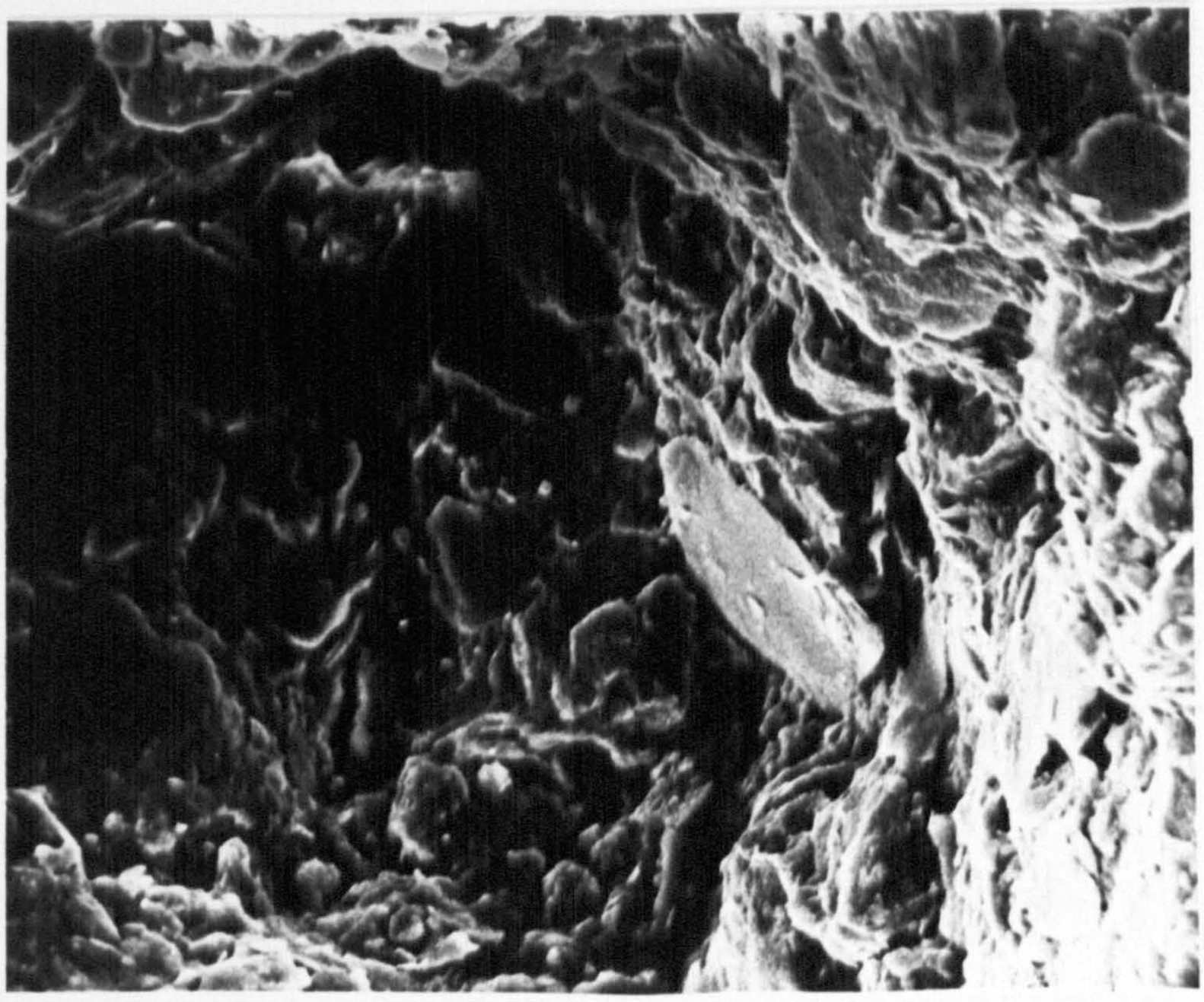


Plate 7b 9 cycles wetting and drying (2 000x)

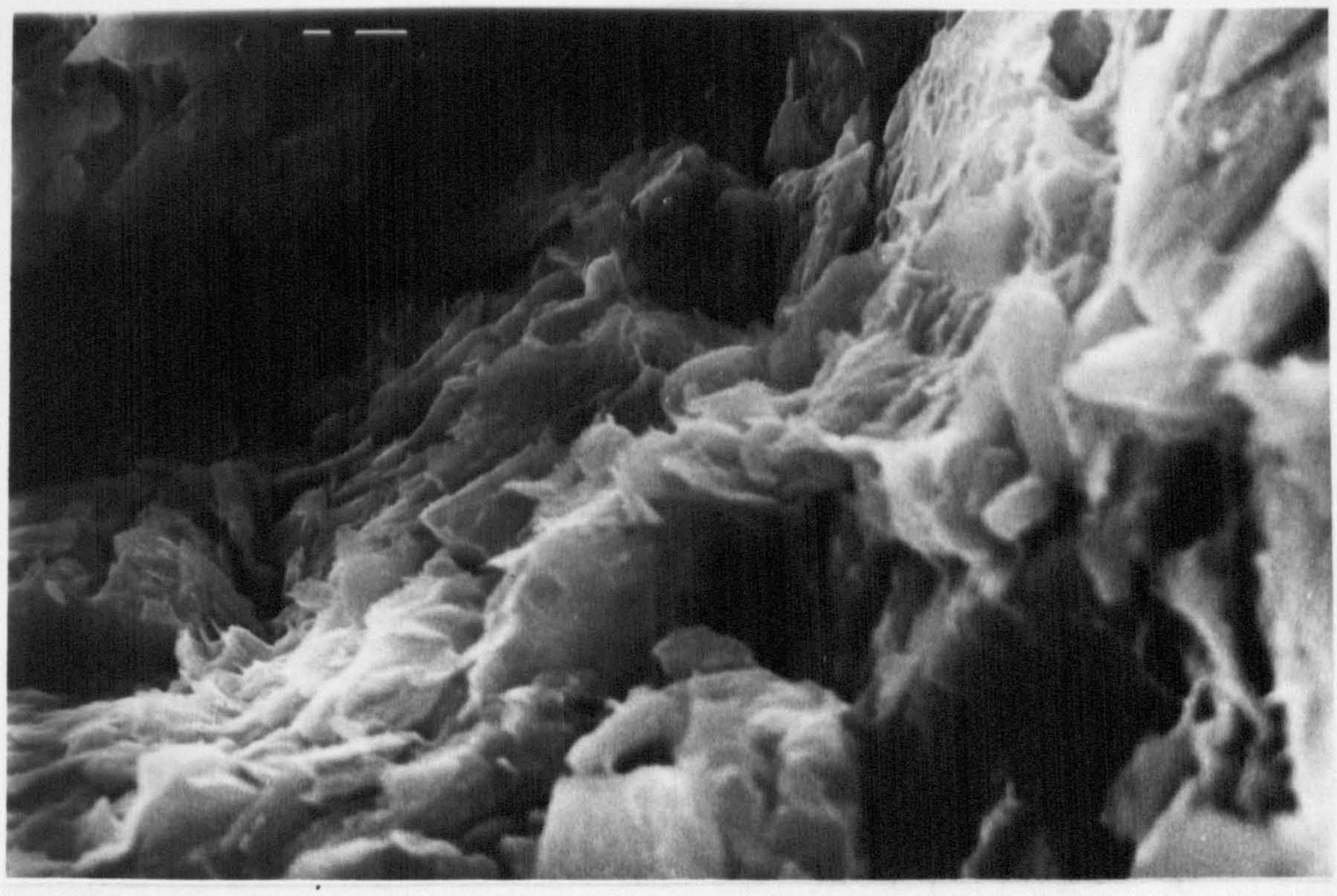


Plate 8a 3 cycles freezing and thawing (5 000x)

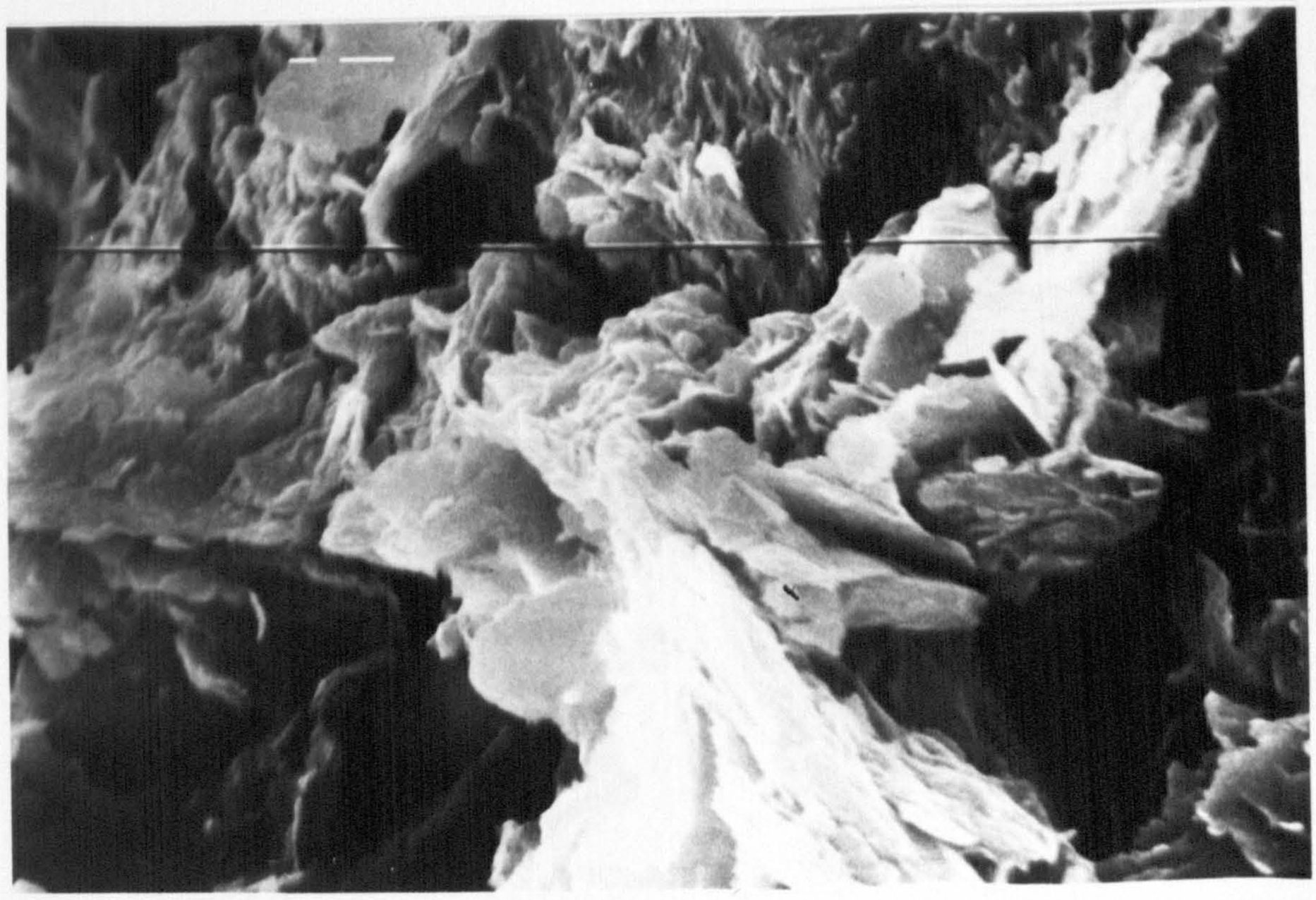


Plate 8b 6 cycles freezing and thawing (5 000x)

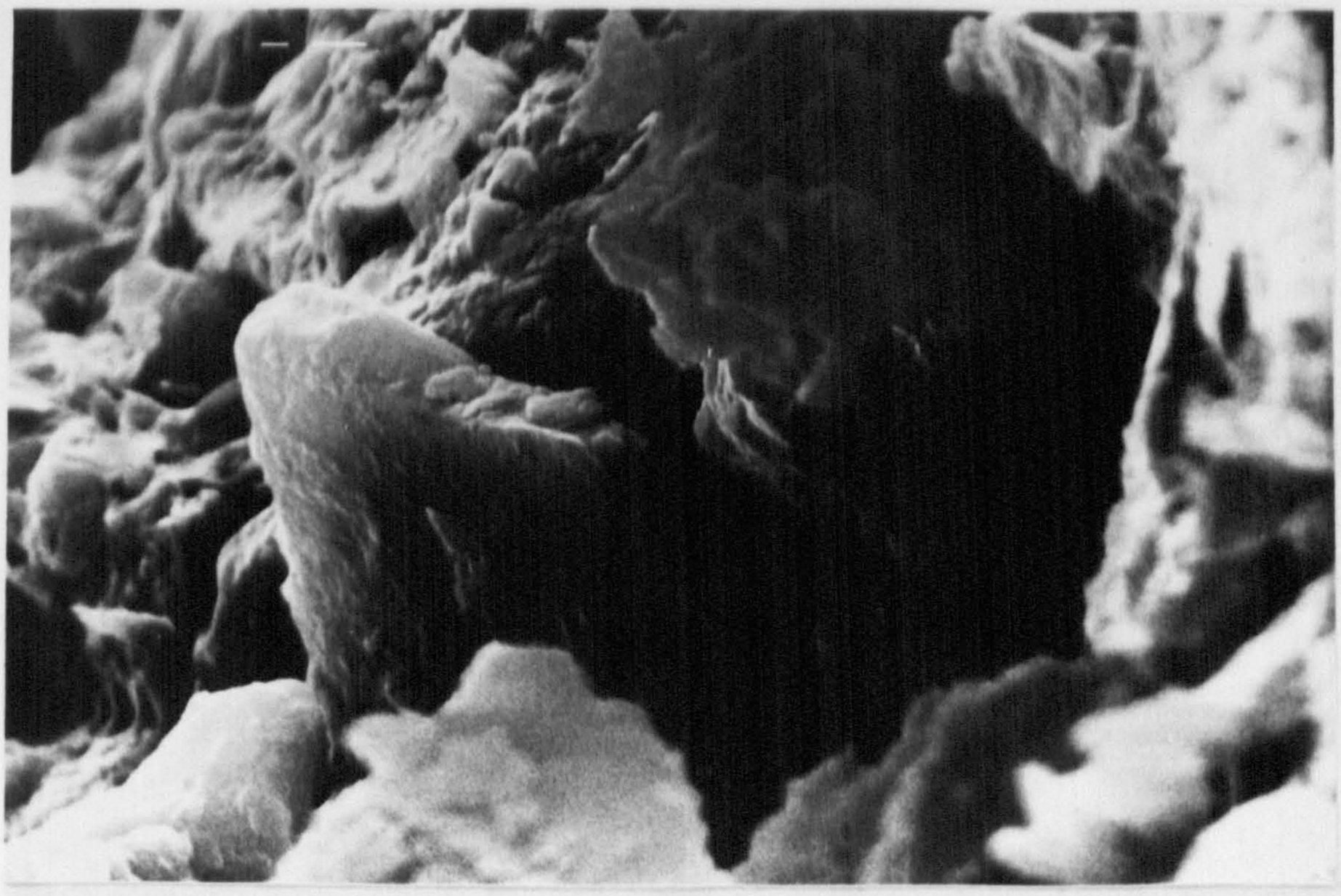


Plate 9a 9 cycles freezing and thawing (5 000x)

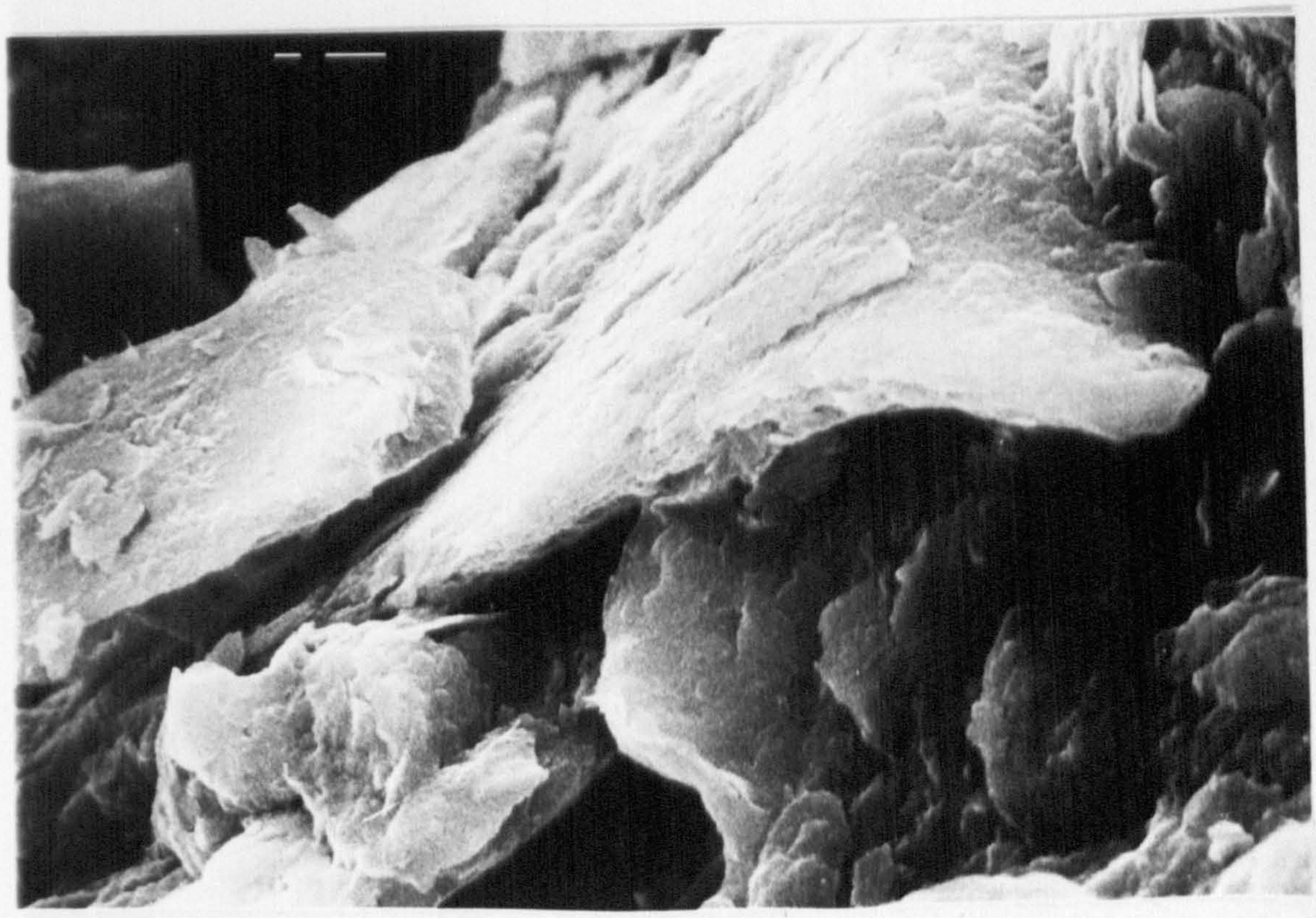


Plate 9a 12 cycles freezing and thawing (6 000x)

imply that involved when clay domains move into interlocking positions, but only macroscopic scale structural appearance.

4.4.4 Seasonal Variations in Aggregate Stability of Stirling Soils.

The aim of this investigation was to relate some of the responses previously noted in laboratory experiments of soil aggregates to environmental conditions and to compare the effects of laboratory treatments with those of natural (field) practices. Of main interest here were changes in aggregate stability induced by tillage operations and seasonal weather variations.

Samples were obtained from West Drip Farm (Stirling series) at monthly intervals for a period of one year (November 1983 to December 1984), during which time some of the plots were tilled for seedbed preparation and for sowing. In addition monthly temperature and rainfall values for the Carse of Stirling were obtained through meteorological offices in Edinburgh and an indication of the snow and frost cycles were given.

In November 1983, part of Stirling 3 field (3A) received its 3rd year cultivation after previously being under timothy grass ley (7 to 8 years). After tillage (November, 1983) with a tined cultivator, samples obtained immediately from the top $\pm$ 150 mm had the lowest stability as shown in Figure 4.36. The loss in stability resulting from field tillage compares well with that caused by laboratory moulding of moist soils, in which the loss of stability has been shown to result from disorganisation

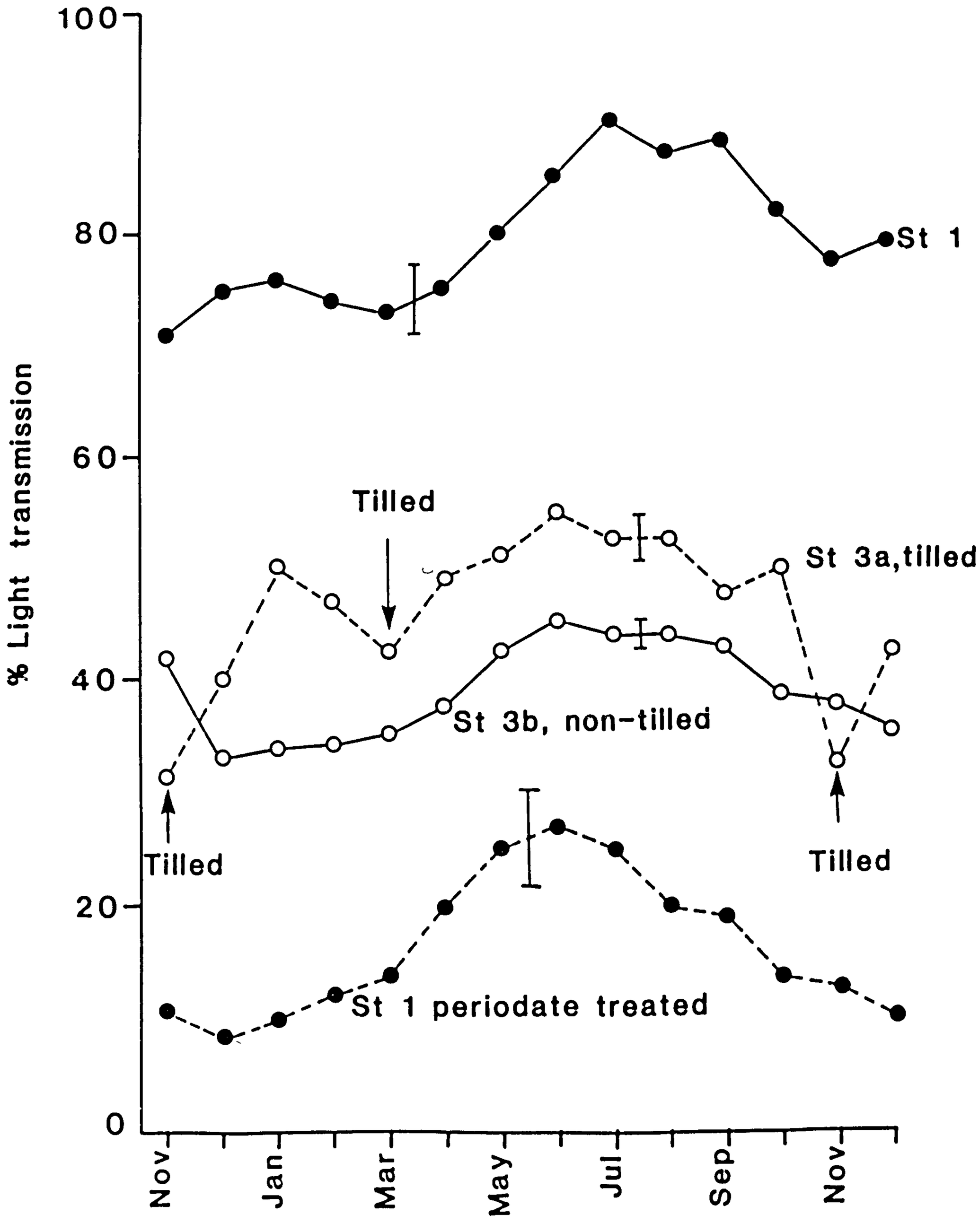


Figure 4.36 Seasonal variation in aggregate stability of Stirling soils

of platy clay particles, the difference being only that of magnitude. Losses in slaking resistance similar to the one observed in this field tillage application have been observed in other studies (Kemper and Rosenau, 1984). Whilst the present study and that of Utomo and Dexter (1981b) on the Red brown earths of Australia recognise these changes in stability to be due to thixotropic process, Kemper and Rosenau (1984) did not implicate thixotropy in changes they observed when moist soil was subjected to tillage, nevertheless their results are consistent with thixotropic changes observed in this study.

Aggregate stability in the tilled field (3A) recovered and reached a higher value by January 1984. The period of regain in stability occurred during the period of heavy snow fall in the Central Scotland area. The thawing period occurred between January and March, and aggregate stability in the tilled field declined during this period and reached a minimum when it was tilled again in March, 1984. In the non-tilled part of the Stirling 3 field (3B) and the Stirling 1 field which was under pasture (Table 3.1) the stability was at its minimum during the winter months. The stability of soils from all the plots (St 1, St 3A and St 3B) increased throughout the spring and reached a maximum during the summer, and subsequently declined towards the end of Autumn 1984. This stability evolution is consistent with findings of Stefanson (1968, 1971) and Imeson and Vis (1984). The lowest stability was again obtained within the 3A field in November 1984 immediately after tillage, and this stability was being regained when the field was sampled in December 1984.

Subjecting the samples to periodate-borate treatment resulted in a total loss of the stability in tilled and non-tilled arable Stirling 3 soil and reduced that of Stirling 1 significantly. The periodate resistant component in the Stirling 1 soil was higher during spring-summer months and lower during autumn-winter months. These results emphasise the established point that polysaccharides whilst contributing a large part of the stability of old pastures are entirely responsible for the stabilisation of arable and virgin pasture soils (Greenland et al., 1962; Hamblin and Greenland, 1977). Sensitivity of soil aggregates to periodate-borate treatment with seasonal changes have been attributed to changes in amount and composition of organic matter (Greenland et al., 1962). Increased microbial activity which has been shown to occur during spring - summer months (Campbell and Biederbeck, 1982) partly explains the higher stability values obtained in these soils, and higher resistance to periodate treatment occurring in the Stirling 1 soil, during which time there was a flush of fungal growth, as revealed by SEM (Plate 10a) and which is at its minimum during winter months (Plate 10b). Higher temperature attained during the period between May and September 1984, and the long dry spell may also partly explain the higher stability values during this period.

Some of the field aggregates from Stirling 3 plot were subjected to wetting and drying cycles as before, to compare the effects of the laboratory moulding treatment to those of field tillage. Samples from the tilled plot (Stirling 3A) were obtained immediately after tillage in November 1983, which was performed with a tined cultivator to a depth of $\pm$ 150 mm. Also treated were samples from the non-tilled part of the plot

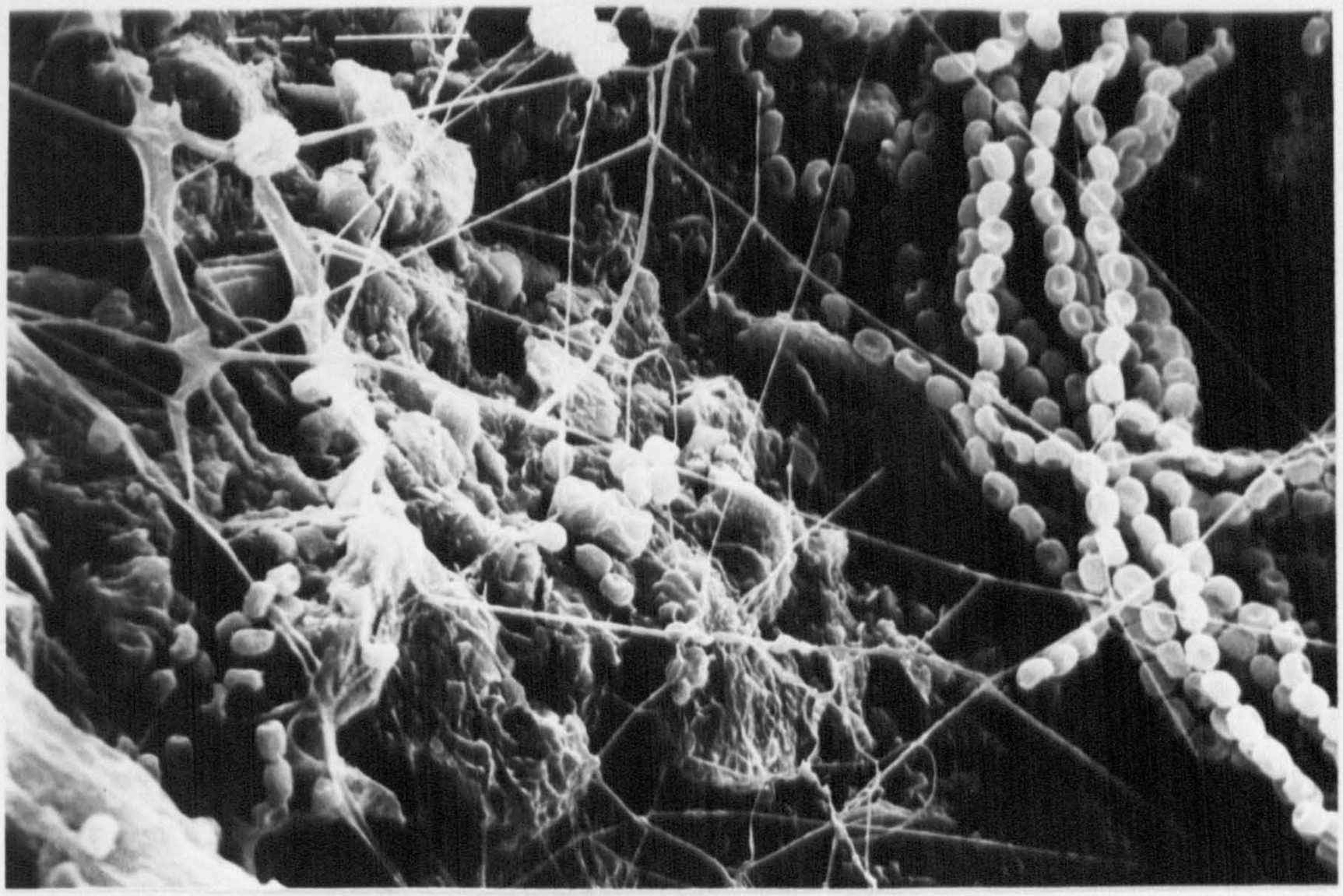


Plate 10a Stirling 1 soil obtained in the summer (1 400x)

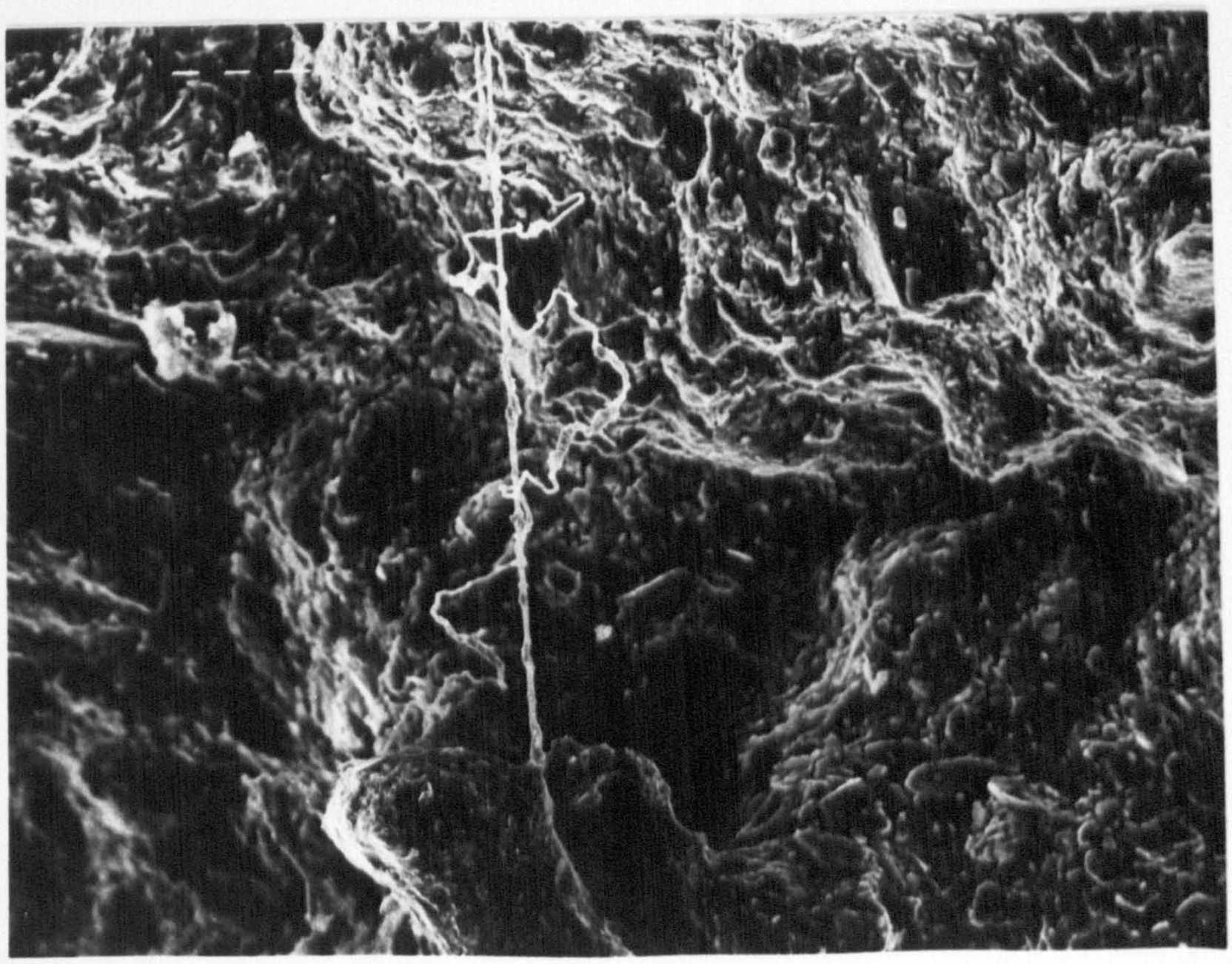


Plate 10b Stirling 1 soil obtained in winter showing development of a platy micro-structure (300x)

(Stirling 3B). Prior to wetting and drying the non-tilled soil had aggregates with higher stability than those from the tilled plot (Figure 4.36 and 4.37). This indicates that tillage like moulding destroys much of the inter particles bonds within the aggregates.

Wetting and drying under non-sterilised conditions increased the stability of field tilled aggregates to levels higher than that of their non-tilled counterparts. This increase occurred during the initial 6 cycles but decreased with further cycles. This initial increase is attributable to the bond reformation process resulting from microbial activity. As suggested by Rovira and Graecen (1957) tillage results in physical disturbance of the soil and also exposes new surfaces to microbial attack. The newly exposed substrate will give rise to microbial flushes during wetting and drying producing aggregating effects. Such flushes are however transient (Birch, 1958), depending on substrate abundance. With repeated cycles therefore, the substrate becomes depleted and bond destruction processes are in excess of bond reformation processes, with a resultant decrease in stability of aggregates from tilled plots. The stability of aggregates from the non-tilled plot (Stirling 3B) were reduced by wetting and drying cycles, and this decrease is consistent with the result obtained earlier in this section with aggregates from other soils (Bromyard 1 and 2; Stirling 1 and Dreghorn).

Wetting and drying with a sterilising solution resulted in a decrease in stability of aggregates from tilled and non-tilled plots (Figure 4.37) and the results suggest that in the absence of biological

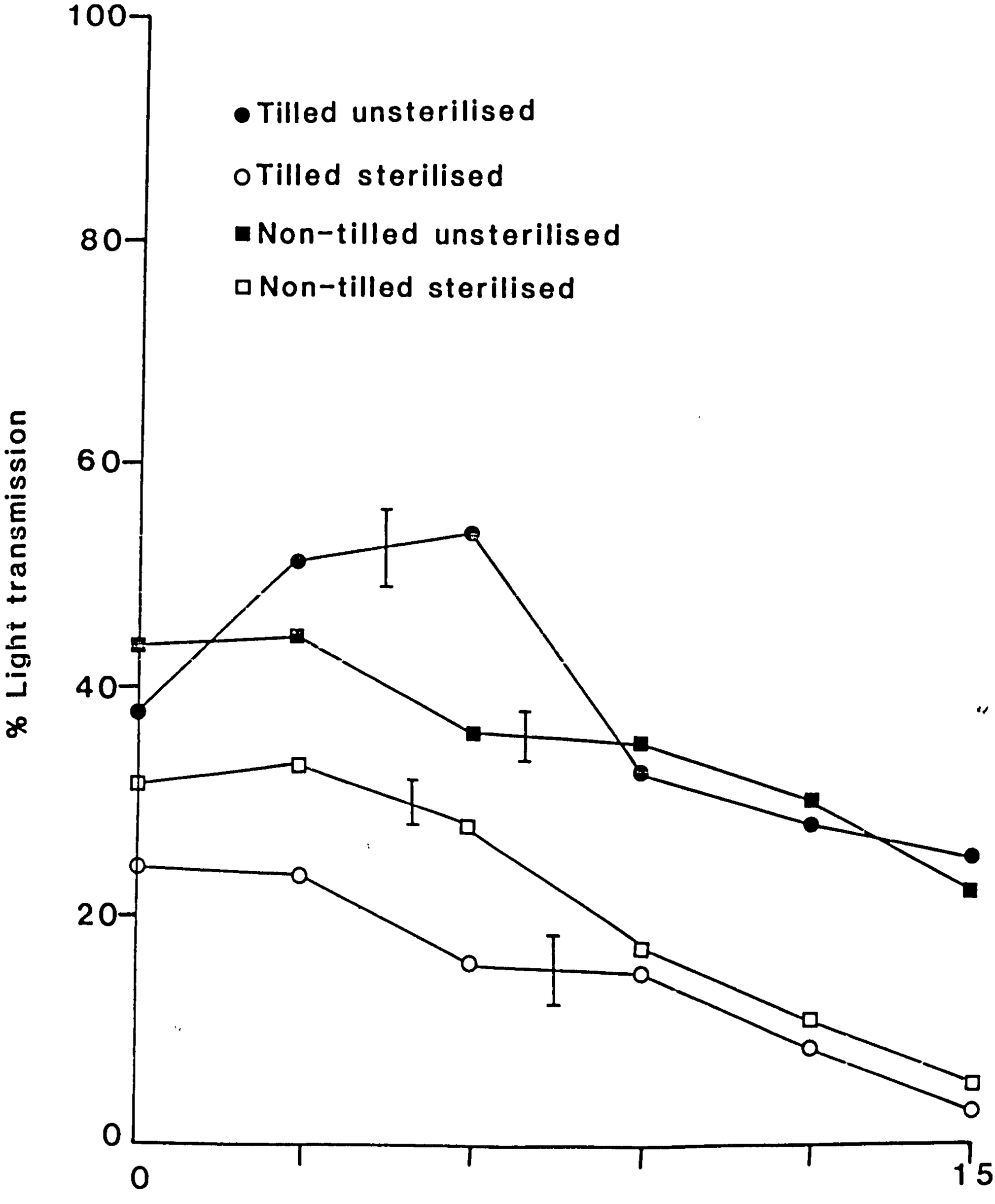


Figure 4.37 Effect of wetting and drying on stability of Stirling 3 natural aggregates

activity, the bond destruction process resulting from the fracturing effects of wetting and drying is not counteracted. These results further evidence the fact that the observed initial increase in stability of aggregates from tilled plot were mainly due to biological activity and well agree with findings of Tisdall et al. (1978).

4.4.5 Discussion

The differences in the proportion of water stable aggregates between soils obtained from pasture plots and those from arable plots (Table 3.1) show that continuous cultivation of field soils gives rise to structural deterioration. The decrease in aggregate stability is usually associated with a decrease in organic matter in the soils (Low, 1954; Clement, 1975; Eagle, 1975; Chaney and Swift, 1984), and physical disruption.

Bonds such as those associated with biological acitivity (see section 4.2) or chemical and physical bonds discussed in section 4.3 can be destroyed by physical manipulation of the soil. Direct physical disruption of the inter-aggregate bonds similar to the ones observed in the present study have been observed with soil tillage (Rovira and Graecen, 1957; Utomo and Dexter, 1981a; Kemper and Rosenau, 1984). Other forms of physical disruption such as slaking are associated with rapid wetting of air-dried aggregates (Emerson, 1954; Panabokke and Quirk, 1957; Kemper and Koch, 1966). This latter form of physical disruption is an important process by which structure of field soils exposed by cultivations or tillage may be destroyed.

Changes in aggregate stability observed with laboratory simulated tillage and artificial wetting and drying cycles in the present study may be due either to a change in the organisation of the soil constituents of the aggregates or the relative proportion and composition of the organic constituents. Changes in the organisation of the soil constituents including both organic and inorganic may be brought about by wetting and forces associated with moulding. These factors may cause a decrease in the degree of organisation of soil particles with the aggregates and dispersion of the clay particles. Tillage induced responses such as flushes of microbial growth (Birch, 1958; Agarwal et al., 1971; Tisdall et al., 1978) and changes in aggregate stability (Utomo and Dexter, 1982) during wetting and drying of soil have been observed. Some tillage induced responses may be complex in which increases in stability and decreases can take place which may not be evident if the time intervals chosen are too long or the period of observation is too short. Failure to recognise these facts can lead to erroneous conclusions about the cause and effect relationships.

The initial increases in the stability of moulded aggregates observed with the initial wetting and drying as shown in Figures 4.24, 4.25 and 4.26 suggest simultaneous involvement of several mechanisms and processes, which act individually or in combination in any one aggregate. The extent of influence of those several mechanisms seems to depend largely on the initial state of the aggregates at the time observations are made, i.e. whether aggregates are physically disturbed or non-disturbed.

Initially with wetting and drying mechanical stresses are generated within the soil resulting in creation of planes of weakness. The swelling behaviour of the clay minerals present as well as the organic matter content probably influence the extent of the swelling process. The microcracks formed give rise to initial faces of aggregation. The results obtained in Table 4.2 show that the more drastic the wetting and drying, i.e. the greater the changes in cumulative amount of wetting and drying, the more water stable aggregates are produced during the initial cycles of wetting and drying.

Baver et al. (1972) suggested that wetting and drying alone does not result in water stable aggregates, and that there must be another process responsible for stabilisation of the aggregates involving microbial products or other soil constituents. The results obtained for non-sterilised and sterilised aggregates in the present study evidence the importance of microbial activity in forming water stable aggregates. That physical disruption associated with wetting and drying of soil aggregates stimulates microbial activity has been established (Birch, 1958; Adu and Oades, 1978). The detrimental effect of restriction of microbial activity by the sterilisation treatment is more obvious in both field and artificial aggregates and the results obtained have indicated the lower stability levels attained in sterilised aggregates. Flushes in microbial activity associated with wetting and drying in non-sterilised soils could have resulted in the reformation of organic bonds which have been destroyed by tillage, as in the case of moulded aggregates, however this increase is transient as evidenced by the parallel subsequent decline in stability of both field and moulded aggregates (Figures 4.24, 4.25, 4.26).

A stability increase in tilled aggregates was obtained by Rovira and Graecen (1957) after one cycle of wetting and drying. Wetting and drying of tilled aggregates or reformed aggregates resulted in increase in stability after 3 cycles (Richardson, 1976) and 6 cycles Utomo and Dexter (1982). The results obtained in the work of Utomo and Dexter (1982) led them to conclude that the decline in stability observed in the work of Tisdall et al. (1978) was mainly because their tillage treatment, which involved mild sieving of aggregates to pass a 4 mm sieve, did not provide sufficient mechanical disturbance to make organic matter protected within the clay lattice available for microbial attack.

In the work of Tisdall et al. (1978) wetting and drying caused a significant decrease in the stability of aggregates of all the soils under sterile condition. They concluded that disruption of aggregates by the physical action of wetting and drying caused more decrease in stability of the aggregates in the absence than in the presence of microbial activity. Microorganisms active in non-sterile soils can produce new bonds from the decomposable organic material in the soil which compensate partially for those broken physically.

The decline in aggregate stability occurring in both non-sterile and sterile soils observed with repeated cycles of wetting and drying in the present study suggest that in non-sterile aggregates the contribution of microbial activity would have been virtually absent in these aggregates, so that the destruction of bonds far exceeded their formation. In the work of Utomo and Dexter (1982) increase in stability was observed with six cycles of wetting and drying but they did not

continue to investigate whether the stability attained would later be destroyed through destruction by both physical processes or microbial degradation of the binding agents. During the initial wetting and drying, the abundance of substrate results in flushes of microbial activity and production of aggregate binding agents. With repeated cycles however, the substrates become depleted and subsequently the microorganisms utilise their products of decomposition as source of energy, and subsequently the bonds reformation reaches its lowest levels.

Fluctuations in moisture content of the soil caused by wetting and drying does result in particle movements. The finer particles are probably the most active fraction in the aggregates and their orientation and disorientation influence the stability of the aggregates. The contribution of colloidal clay in formation of aggregates is well established (Emerson, 1959), and the activity and mobility of this colloidal fraction in soil-water systems have been studied by civil engineers and the process involved recognised as thixotropy (e.g. Mitchell, 1960). In Table 4.2 it has been shown that when aggregates maintained at constant moisture in desiccators were transferred to sintered glass funnels and kept at -1kPa for 6 days there was further increase in stability. Without being subjected to wetting and drying cycles there was a slight gain in moisture content, and stability, and such gains evidence additional thixotropic process resulting from changes in the moisture tension in the aggregates. Changes in the moisture stress cause particle movements and therefore their orientation, and the changes observed during the initial wetting and drying especially in artificial aggregates under both non-sterile and sterile conditions

suggest contribution due to thixotropic hardening process. Thixotropic changes are reversible, thus with repeated wetting and drying the contacts established between the particles may be overcome as disorientation takes place induced by repeated shifting of the particles. This would be more severe in the absence of microbial bonds, hence the lower stability levels of sterilised soils. The initial increase of stability observed with wetting and drying of reconstituted aggregates by Richardson (1976) might well have been a manifestation of thixotropic hardening.

Using field aggregates 2 - 4 mm diameter showed that freezing and thawing did not result in any signficant increase or decrease in stability of soils under non-sterilised conditions (Figures 4.30, 4.31, 4.32). When soils were moistened with sterilising solution repeated freezing and thawing caused a decrease in aggregate stability and this occurred in all soils (Figures 4.32, 4.33, 4.34). In those field aggregates which were moistened with deionised water or sterilising solution whilst at their maximum natural stability (stable porous structure) freezing would result in formation of the ice from the outside, but since the soil has an open structure at this stage the process of ice development would be completed without much stress being created by migration of the water from the clay particle sites to the freezing front. It can thus be said in these field aggregates (2 - 4 mm) the action of ice formation is evenly distributed within the aggregate, thus the resultant expansion in volume (ca.9%) would result in fracturing. In non-sterilised soils, the smaller masses pushed apart can still retain their integrity because of replenishment of the broken bonds

by microbial activity during thawing. The absence of microbial activity in sterilised soils would have resulted in the loss in stability observed.

It is suggested in this study that while freezing (at -10°C) acts as a temporary sterilant, it does not kill the microorganisms, therefore with thawing at 20 ± 2°C microbial activity would resume and production of aggregate binding agents continue. Replenishment of the broken bonds by microbial acitivity is consistent with the results obtained in non-sterilised soils. Reports on the influence of freezing on soil microorganisms are scarce. Soulides and Allison (1961) claim an action similar to that of drying, i.e. an increased mineralisation of organic matter as a result of freezing and thawing, the difference being largely one of degree. In their experiments intermittent drying caused a large decrease in bacterial growth whereas freezing only resulted in smaller changes. McCalla (1945) found that freezing had no effect or only an insignificant one on microbial activity.

In remoulded aggregates (10 - 20 mm soil balls) it can be assumed that since freezing proceeds gradually from the outside of the balls, there is migration of water from the inside of the ball towards the outside where ice formation is taking place. This dehydration process forces the clay particles into closest possible packing with resultant interlocking of the platelets and resultant increase in stability. When the aggregates of Bromyard and Stirling paired soils were subdivided to 4 - 6 mm, to reduce the influence due to size, again the remoulded aggregates increased in stability with initial freezing and thawing cycles, and the stability was maintained over 15 cycles in both

non-sterilised and sterilised aggregates.

The observed differences in behaviour of field and moulded aggregates during initial cycles of wetting and drying and freezing and thawing, and the similar pattern of stability evolution soon after the moulded aggregates have regained their stability to that of field counterparts suggest that the initial increase is mainly through the contribution of thixotropy. The results also show that microbial activity is important, and the size of this contribution is more evident with repeated wetting and drying or freezing and thawing beyond six cycles. It can therefore be concluded that the action of freezing on aggregate stabilisation is mainly a physical process, the action of which depends on the initial structure at the time of freezing, which would control the nature of ice crystals formed, and subsequently their influence on the aggregate structure.

The results obtained from the monthly observed stability changes in the field (Figure 4.36) are in agreement with the view held that aggregate stability at any given time of the year is a function of aggregate formation and degradation processes. In general the number of macrostable aggregates increases in spring to a maximum in summer and then decreases throughout the autumn to a minimum in winter (Rennie et al., 1954). Increased aggregation in spring is associated with high production of microbial aggregating agents resulting from climatic conditions favourable for high microbial activity and the presence of supply of readily available organic materials (Harris et al., 1966). At the other times of the year degradation processes may predominate. In

addition there were decreases in stability from time to time, (Figure 4.36) resulting from tillage operations, and this occurred according to the mechanisms discussed in the work of Utomo and Dexter (1981a); and in this thesis. Intensive rainfall may cause serious aggregate disruption if it occurs directly after soils are exposed by tillage.

5. IMPLICATIONS AND CONCLUSIONS

The experiments described in this thesis produced results which are significant in three spheres of interest, that of soil structural management, that of soil aggregation dynamics and that of soil microbial dynamics. For ease of reference these aspects will be discussed under four headings as laid out in section 1.

5.1 Effects of Cropping Practices

The experiments in this section were carried out to examine methods of aggregate stability evaluation and to use these to assess the effects of various cropping practices on soil aggregate stability, and to study the relationships between soil constituents and aggregate stability. The two methods used to evaluate stability i.e. wet sieving and turbidimetry were consistent with each other in reflecting the cultivation histories of the fields. However, turbidimetry had more advantages over wet sieving in terms of rapidity and reproducibility, and also in studies of mechanisms of aggregation, aggregate stabilisation and degradation.

Turbidimetry may hold further advantage over wet sieving in assessment of erodibility of soils under field conditions, where the stability of microaggregates is important. Microaggregates are formed from the association of the clay fraction and organic materials, and the stability of this association determines the resistance of soil aggregates to erosive forces. Dispersion and transportation of the

microaggregates are the two active processes in soil structural deterioration (manifested as surface sealing, crusting and compaction); measurement of their stability is therefore important in assessment of susceptibility of soils to degradation.

Improvement of soil organic matter content and aggregate stability as a result of introduction of grassland does appear to be the general phenomenon, but the various crops differ in their effects. Superiority of perennial grass over timothy grass has been emphasised by results from the Stirling soils (Table 3.1). Inclusion of grass leys in farm management as a means of improving fertility and physical properties of these soils has been underway at Rosemaund and West Drip farms, and the beneficial effects of this practice on soil structure are emphasised by the results obtained in this study. But whether such a practice can be applied at other localities much depends on the economics within which the land is farmed.

The significant correlation coefficient obtained between organic carbon and aggregate stability evidences the importance of organic matter in stabilisation of soil aggregates. However, the results suggested that particular components of organic matter may be more important than total organic matter in stabilisation of aggregates of various soil types and under different localities and climatic conditions. Whilst cultivation may improve soil aeration and the interaction of soil constituents, it results in increased organic matter oxidation due to microbial activity, and depletion of aggregate binding agents. Subsequently, a decline in the resistance of soil aggregates to dispersive forces occurs. The reduction

in aggregate stability of the Stirling and Bromyard arable soils makes them susceptible to structural damage resulting from vehicular compaction, surface sealing and crusting (Burnham and Mackney, 1964; Mackney and Burnham, 1966; Hodgson, 1972; Soane et al., 1972; Page, 1983). This structural damage could give rise to problems of seedling emergence, root penetration, reduced infiltration capacity and increased surface run-off, leading to the loss of surface agricultural soil and a decline in the productivity of the land (Hodges, 1978).

These problems are common to most silty soils under arable farming in parts of England and Wales (Agricultural Advisory Council, 1970; Davies, 1975; Greenland et al., 1975, Hamblin, 1977; Hamblin and Davies, 1977). In most areas where these soils are farmed high land prices have prompted production of cash crops, and exclusion of grass leys from farming practices. In East Anglia adjustment of cropping system to include grass is usually unacceptable where the crop gives high returns. In such areas adoption of practices to preserve soil structure without inclusion of lengthy grass periods have been attempted. Synthetic conditioners have been tested on some of these soils (Page, 1979, 1980). In the present study, "physical" or "natural conditioning" i.e. the effects of aging and weathering cycles were examined, and the extent to which these processes could improve and restore structure to damaged soils was assessed.

5.2. Effects of Biological Activity

Inspection of the literature up to the recent reviews by Waid (1984) and Lynch (1984) shows that the relative quantitative roles of

microorganisms in soil aggregate stabilisation and nutrient cycling are still not well understood, mainly because of problems involved in their study. The difficulty of estimation of microbial population as well as complexity arising from ecological associations of various organisms are formidable problems in assessment of their precise roles. Most recent studies of microbial aggregate stabilisation have neglected the contribution made by actinomycetes, and this has mainly resulted from problems of detecting physiological and morphological differences between these organisms and bacteria. These problems have partly been overcome in the present work by application of selective antibiotic inhibition technique and the use of SEM.

The experiments conducted in this section have emphasised the superiority of fungal mechanical stabilisation of soil aggregates. The action of fungi was, however, found to be temporary because fungal mycelia soon became degraded through being utilised by bacteria and actinomycetes. Under field conditions, particularly in pastures, where readily usable substrate is continually being replenished through the decay of plant and animal debris, continuous fungal growth may give rise to permanent stabilisation of soil aggregates. Fungi are sensitive to management, and do not appear to be very active in stabilisation of aggregates from arable soils which are continually disturbed by tillage operations.

Bacterial action in aggregate stabilisation has been shown to be mainly due to production of polysaccharides. These materials were produced during active bacterial growth which resulted in depletion of

fungal mycelia and disappearance of mechanical stabilisation of the soil aggregates. The contribution of polysaccharides was assessed by periodate treatment. Although there has been controversy as to the actual role of polysaccharides in stabilisation of field aggregates, the present study has emphasised the point made by Cheshire et al. (1983) that polysaccharides may be more important than presently considered in stabilisation of soil aggregates.

The resistance of aggregates enmeshed with fungal mycelia, observed at some stages of the present study has led some workers to conclude that polysaccharides were less important in stabilisation of field aggregates. However, the reduced resistance of the pasture aggregates (Bromyard 1, Stirling 1, Dreghorn) to periodate treatment when the fungi had declined shows that polysaccharides contribute a substantial part of their stability. Erroneous conclusions on the role of polysaccharides appear to have resulted from the use of shorter time periodate treatments than was originally suggested by Bobbit (1958). There has not yet been success in treating soils with polysaccharides extracts to improve stability, but practices such as straw inoculation with microbes capable of producing these materials, and burying it in the soil holds a promise in this line of research (Lynch, 1983; Lynch and Elliot, 1983). Microbial stabilisation of soil aggregates is sensitive to management. Practices which improve soil organic matter will improve microbial activity, and subsequently production of aggregate stabilising materials.

5.3. Effects of Thixotropic Hardening Process

Many soils contain free carbonates, iron oxide, alumina, organic matter, etc., which may precipitate at interparticle contacts and act as cementing agents. In addition there are adsorbed water layers which have a high viscosity near particle surfaces and thus are responsible for a strong adhesion between mineral grains at the points of contact between particles. This organisation of soil constituents evolving over long periods under natural field conditions results in the formation of stable aggregates. If the soils remain undisturbed as is the case when they are under pastures, the aggregates will reach an equilibrium stability.

Physical manipulation of the soil, resulting from swelling, shrinkage, soil fauna activity and most importantly by tillage tools cause rupture of the existing inter-aggregate bonds and the stability and strength to drop. The extent to which the stability and strength of the soil will drop will, among other things, depend on the method and degree of manipulation, and the water content at the time of manipulation. Civil engineers have termed the ratio of peak undisturbed strength to the moulded strength as sensitivity. Thus more sensitive soils such as those with low organic matter contents would suffer more structural damage from tillage operations.

Whilst continuous arable cultivation over long periods is associated with a decline in organic matter and subsequent decrease in the stability of soil aggregates, tillage, like laboratory moulding, has

been shown to result in immediate reduction in stability and strength of the aggregates. Tillage and moulding decrease the stability of aggregates due to breaking of the connecting inter-particle bonds, and at the same time exposes the initially inaccessible organic matter to microbial oxidation. This increased microbial oxidation would result in depletion of the organic matter store of the soils and at the same time production of aggregate binding agents.

When moulded and remoulded aggregates were incubated under non-sterilised and sterilised conditions, there were initial increases in aggregate stability and strength. Undisturbed field aggregates moistened with sterilising solution did not change during aging, but sterilised moulded ones changed. The observed changes in the latter situation evidence the fact that thixotropic changes are taking place when moulded aggregates are left to rest under conditions of constant temperature and moisture. The changes taking place in sterilised aggregates were shown to be reversible, and the results are therefore in agreement with the mechanisms of thixotropy propounded by civil engineers.

The initially higher stability of non-sterilised moulded aggregates during incubation suggests an additional contribution due to microbial activity. In these aggregates, pulverisation and moulding would have prompted a flush in microbial activity and production of stabilising agents. However, such flushes are ephemeral as has been shown by the results in section 4.2, and the stability of these aggregates fell back to those of their sterilised counterparts after 2 weeks.

The findings of this study together with those of other workers suggest that some soils will recover part or all of their stability a few days after tillage. The length of time of recovery will, among other things, depend on the soil type, moisture content and cultivation history of the soil. Although the relationship between clay and aggregate stability (section 4.1) was not found to be significant when soils with a wide range of textures were examined, the activity of the colloidal clay and its ability to form interparticle bonds in sand-silt mixtures has been confirmed by civil engineers, and the present results suggest that the samples with higher clay content would exhibit more thixotropic characteristics. Therefore clay becomes more important in regeneration of disturbed soil fabrics, mainly because of its colloidal properties and mobility in soil-water systems.

Allowing soils to rest a few days after tillage would therefore impart some resistance to dispersion when exposed to second implement passes or destructive forces associated with raindrop impact. Adoption of farm practices which would allow such spacing between tillage operations and irrigation or anticipated rain are conservative to structure, and would be desirable where soils exhibiting thixotropic behaviour are encountered.

5.4. Effects of Wetting and Drying and Freezing and Thawing Cycles

The results obtained for the effects of wetting and drying and freezing and thawing on aggregate stability are consistent with the concept of equilibrium states propounded by Utomo and Dexter (1982).

According to this hypothesis, for any soil composition and environmental conditions a soil will have a range of proportions of water stable aggregates. Soil with less than the appropriate equilibrium value will increase the proportion, and soil which has more than the appropriate value will decrease the proportion. The former case would have taken place in moulded and tilled aggregates, which had their stability initially reduced, and the latter would have occurred in field non-tilled aggregates.

The increases in stability of moulded aggregates observed with initial wetting and drying and freezing and thawing were shown to be associated with biological activity and thixotropic hardening. The decreases were mainly due to the fracturing effects resulting from mechanical stresses induced by wetting and drying and freezing and thawing. The speed and extent of approach to equilibrium stability therefore appears to be through a combination of mechanisms, some biological, some thixotropic, and these processes are largely influenced by soil type, cropping history and tillage.

Many previous studies have emphasised the utilisation of weathering cycles in weakening the furrow slice, and thus reducing the energy consumption costs in seedbed preparation (Utomo and Dexter, 1981a, 1981b). Most information pertaining to this action of weather was obtained by assessment of the aggregate size distribution of soils and this aspect was termed tilth mellowing (Russell, 1973; Utomo and Dexter, 1981a; Voorhees and Lindstrom, 1984). This practice is more common in

the temperate zones, and farmers on the Carse of Stirling have emphasised the utilisation of frost action in making autumn ploughed clays more friable when they are worked in the spring. The Stirling series soils, having typical characteristics of a surface-water-gley become massive, sticky and structureless upon wetting. On drying, the soils shrink into large, almost perfect prismatic structural units, attaining maximum hardness during the dry summer months. These soils are therefore difficult to cultivate unless at the correct time and with the correct moisture content. Where such intractable soils are experienced, consideration of climate-soil interaction plays an important role in planning cultivation operations.

Whereas the present study was confined to the action of weathering cycles on soil aggregate stabilisation the importance of weather in formation of field structural types cannot be overlooked. (Russell, 1973; Davies et al., 1982). Stresses set up within the soil mass by freezing, swelling upon wetting, and shrinkage resulting from drying (dehydration) cause soil particle movements relative to each other and this constant reorientation should afford better opportunities for suitable conditions for aggregation to occur.

It is common knowledge that wetting and drying and to a lesser extent, freezing and thawing, enhance C and N mineralisation (Birch, 1958; Campbell, 1979). Such microbial respiration flushes are important in soil fertility, whilst on the other hand the flush of microbial growth would give rise to aggregate stabilisation. These facts are borne out by the lower stability obtained when soil aggregates were wetted and dried and frozen and thawed under sterilised conditions.

The results obtained suggest that the ameliorative effects of wetting and drying and freezing and thawing are ephemeral and that with extensive and intensive weathering action the structure may deteriorate. E.g. whilst the furrow slice weakening due to frost action is important, the intensive and extensive action gives rise to dispersion effect and problems of impermeability can result during thawing. It would therefore appear a useful approach to adopt management practices that would reduce the severity of frost action, whilst still allowing for the benefits gained. The use of stable mulches and rough surfaces would result in discontinuities in ice development, thus would be useful in reduction of its severity. Also good drainage would reduce the problem of puddling during thawing (Benoit and Bornstein, 1970; Chamberlain and Gow, 1979).

5.4. Implications for Future Research

In terms of future research, the results of this work indicate that there are some areas which need re-evaluation in order to improve the understanding of mechanisms and processes of soil aggregation, stabilisation and degradation. Physical and biological processes influencing soil structure may be studied within the domains of soil microbiology, soil physics, soil chemistry and soil tillage research. It would be desirable to adopt interdisciplinary approaches for acquisition of better understanding of the interaction of these processes and their influence on soil structure. Whilst keeping this approach in mind, other areas also need further investigation.

1. The microaggregation technique of stability measurement used in the present study is of value in assessment of mechanisms involved in

aggregate stabilisation (assuming that stable aggregates are a result of the clay-organic matter interaction). The technique yields reproducible results and allows for rapid determinations, however the use of light transmission as an index of stability is semi-quantitative. Development of a more quantitative measure of soil aggregate stability based on the use of ultrasonics (North, 1976) probably avoiding the use of aqueous suspensions would be more appropriate.

2. The ergosterol measurement as index of fungal growth has been adopted from cereal chemistry and tested in soil for the first time, using five soils from three soils series. The method was found reliable, rapid and reproducible with these soils. It would be a useful thing to test this method using a large variety of soils obtained from a large variety of management practices and also soils amended with organic substrates.

3. Much work is needed in identifying soils which show thixotropic behaviour when subjected to tillage, as this information can be of value in planning farm operations. It would also be useful to assess thixotropic behaviour using soils of a large variability in clay contents, as in the present study the clay content of the samples was restricted to between 15 and 35%.

4. Much information can be gained about the interaction of weather and soils under arable cultivation by identifying thixotropic components of the observed changes in aggregate stability due to wetting and drying and freezing and thawing which in the present study were only inferred.

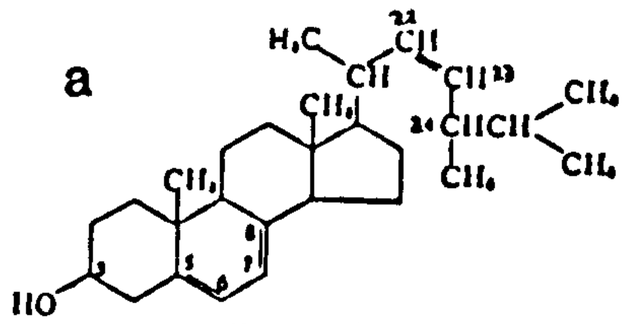
6. APPENDIX

HPLC Ergosterol Measurement

Ergosterol's characteristic UV absorption, which differs from those of higher plant sterols such as sitosterol and stigmasterol lends it advantage in the detection of ergosterol in other materials. Because ergosterol has a conjugated pair of double bonds at 5 and 6 and 7 and 8 (Figure I.1(a), it absorbs UV light strongly between 240 and 300 nm, and this characteristic UV absorption differentiates it from non-absorbing cholesterol and stigmasterol. Ergosterol is found in the nonsaponifiable fraction and this lends the technique further advantage in that ergosterol can be separated from other saponifiable sterols. The technique is however not free from problems in that ergosterol occurs in small quantities and is always accompanied by related sterols. The other problem is that ergosterol is unstable and light sensitive, and changes to calciferol or vitamin D_2 upon irradiation. Care should therefore be exercised during extraction stages to keep the extract away from direct radiation and to maintain oxygen free conditions.

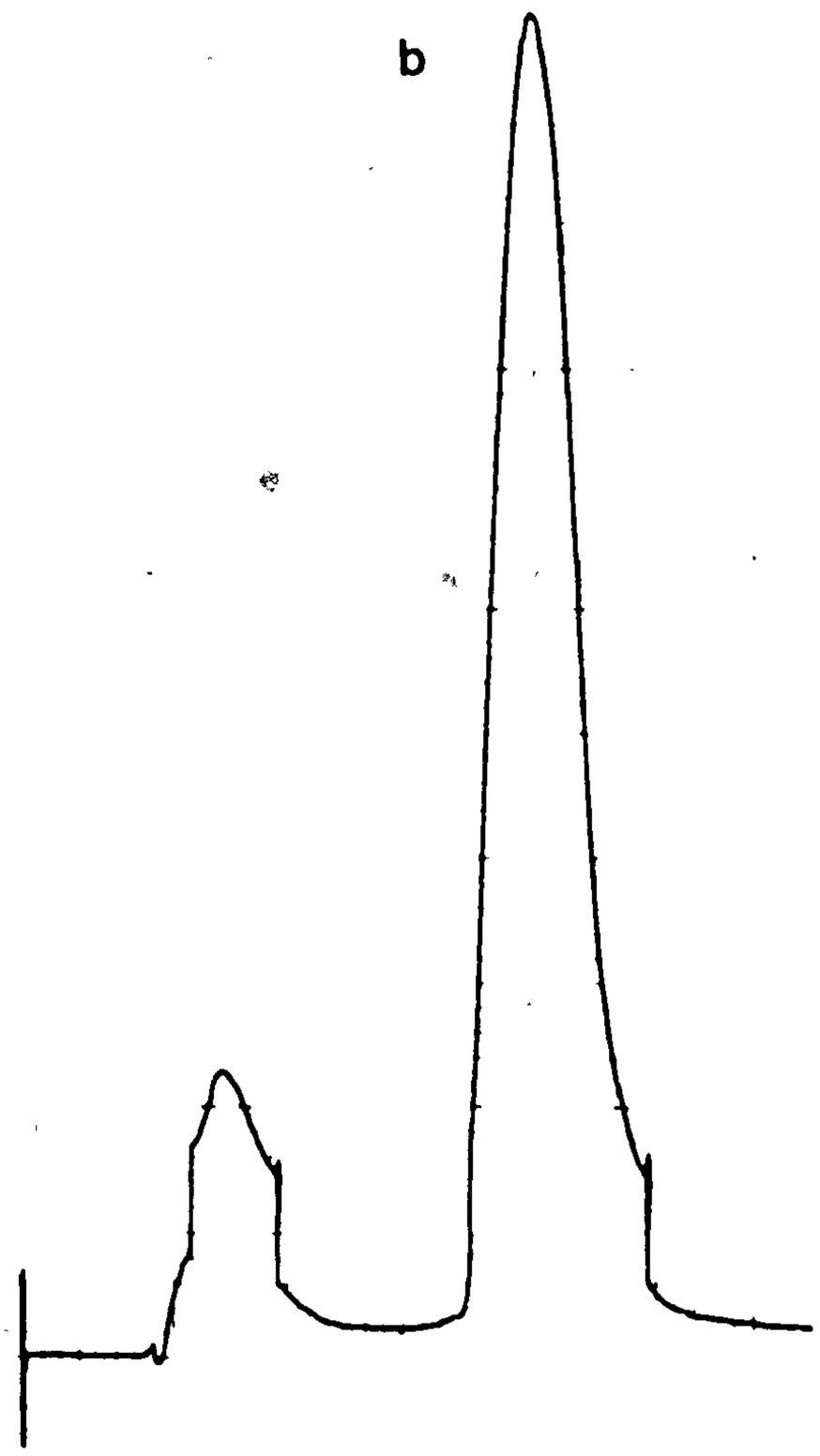
Ergosterol standard (85%) recrystallised twice from absolute ethanol and dried under vacuum was dissolved in methylene-dichloride: isopropanol (99: 1 v/v) and 10 µl portion of the solution injected into the HPLC. With the HPLC machine conditions set as described in methods section, ergosterol was eluted at between 9 and 10th minutes and typical high pressure liquid chromatogram obtained for the standard is represented in Figure I.1(b). A soil extract obtained as described in

a



Ergosterol
(m.p. 163°, $[\alpha]_D$ −133°,
abs. max. 2800 Å)

b



c

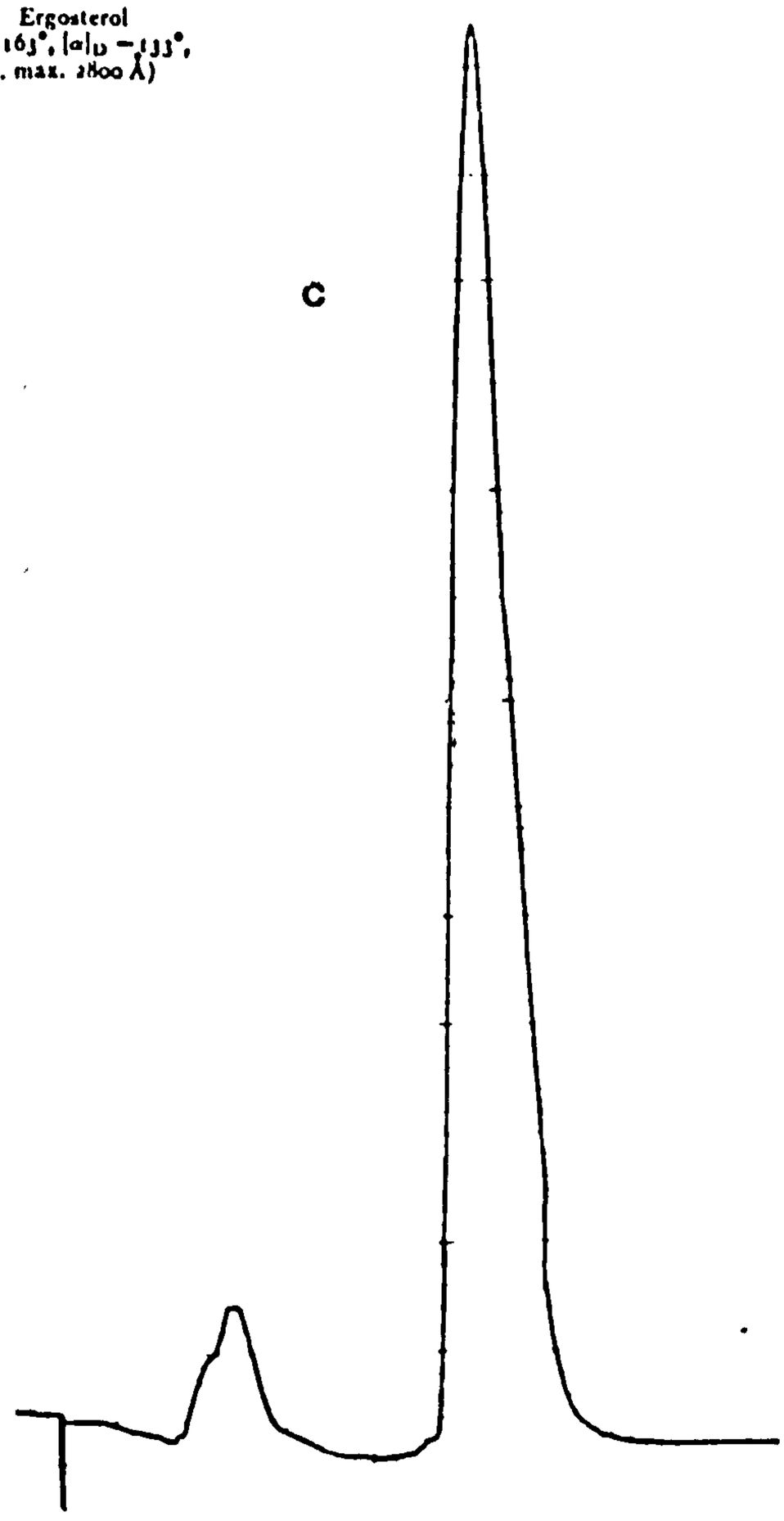


Figure I.1 Structure (a) and characteristic chromatograms from ergosterol standard (b) and soil extract (c). With detector set at 282nm and 0.1 AUFS, and the chart speed of 5mm/min, ergosterol was eluted between 9&10th minutes. The amount of ergosterol is calculated from the area under the peak.

the method section was also run with HPLC and the characteristic chromatogram in Figure I.1(c) similar to that for the standard was obtained.

Portions of the solutions of the standard and the soil extract injected into the HPLC were cut off during elution, and mass spectrometric determinations made on these to characterise the ergosterol and confirm the presence of ergosterol in the soil extract. These portions were concentrated by making several injections and repeatedly cutting off the eluent between 8 and 11 minutes. The final volumes obtained for the standard and the soil extract were freeze-dried overnight and the residues examined using a JEOL JM5 - D100 mass spectrometer. Typical mass spectroscopic results obtained for the standard and the soil extract are presented in Table I.1 and I.2 respectively.

At 70^{o}C the ergosterol standard molecule was split according to Table I.1. When several of the prominent peaks are plotted together ergosterol (M/E 396) yielded the strongest peak. Other peaks of intensity lower to that of ergosterol were also detected as shown in the plot. At 70^{o}C mass spectrographic determinations of the soil extract did not give any evidence of the presence of ergosterol (Table I.2(a)). At 80^{o}C the ergosterol was detected and examination of data in Table I.2 (b) and comparison of this data with that obtained from the standard show a close similarity of the products under examination. However, when prominent peaks from the soil extract spectrographic results are plotted together, it can be seen that other peaks stronger than that of

ergosterol are present. The peak obtained at 396 (Int. 56.2) shows unequivocally the presence of ergosterol in the soil extract.

The impressions gained from Table I.2b were refined by accurate mass determination made on the soil extract residue obtained as described above, and the results are given in Table I.3.

Table I.3. Accurate mass spectroscopic determinations of soil extract.

Measured Value (M/E)	Calculated Value (M/E)	Error
396.3419	396.3393	6.6 ppm

The figure obtained for the measured mass value closely agrees with that of the calculated mass with an error of 6.6 ppm. The soil extract compound had molecular structure of C = 28; H = 44 and O = 1 and this is similar to that of ergosterol as represented in Figure I.1 (a) and this confirms that what was obtained and measured from the soil extract was ergosterol. The results obtained here however hint that some impurities not detected by HPLC set at conditions as described were present in the soil extract, and this was not unexpected, especially when dealing with soil.

APPENDIX TABLE I.1 ERGOSTEROL STANDARD AT 70° C.

30 MAY 84.
ERGOSTEROL /3
LM CORRN= -181
BASE PK=45% FSD
TIC/PKS=98409/371

M/E	INT	M/E	INT	M/E	INT	M/E	INT	M/E	INT
28	35.5	29	8.4	30	1.2	31	0.6	32	10.5
34	0.5	36	0.4	38	1.6	39	5.7	40	0.9R
41	20.9	42	3.2	43	33.6	44	4.8	45	15.7
46	2.7	51	3.1	52	0.7R	53	4.3	54	1.0R
55	38.5	56	7.9	57	26.6	58	1.4	59	2.1
60	7.4	61	0.6	63	1.2R	64	0.8R	65	3.7
66	1.2	67	18.1	68	4.3	69	57.2	70	8.4
71	15.0	72	0.9	73	4.5	74	2.0	75	0.8
76	2.2	77	9.4	78	2.6	79	13.0	80	1.3
81	29.6	82	7.9	83	29.1R	84	4.1	85	6.0
86	1.5	87	1.6	88	0.9	89	1.8	90	0.7
91	19.1	92	4.9	93	13.2	94	4.2R	95	19.1
96	4.6	97	11.5	98	3.3	99	2.3	100	0.8R
102	1.4	102	2.3	104	2.5	105	18.9	106	3.3
107	16.5	108	4.5	109	18.1	110	4.1	111	6.3
112	2.8	113	2.3R	114	0.5R	115	6.5	116	3.1
117	10.2	118	4.7R	119	17.0	120	4.1	121	7.9
122	2.5	123	7.9	124	4.6	125	11.2	126	2.1
127	4.0	128	12.3	129	15.0	130	3.3	131	15.0
132	4.3	133	13.0	134	2.9	135	7.9	136	2.9
137	4.3	138	1.5	139	3.5	140	1.7	141	13.6
142	7.9	143	29.6	144	10.2	145	27.8	146	6.6
147	16.5	148	3.8	149	38.5	150	3.1	151	3.5R
152	2.0R	153	4.9R	154	2.8	155	12.0R	156	6.5
157	34.3	158	14.2	159	25.2R	160	4.9	161	5.6
162	2.3R	163	4.1	164	1.7R	165	5.9	166	2.1
167	5.3	168	3.4	169	12.1	170	7.4	171	19.1
172	3.9	173	10.0	174	2.8	175	12.4	176	3.5
177	6.0R	178	5.7	179	4.5	180	2.8	181	5.2
182	4.3	183	12.3	184	5.7	185	15.3	186	13.7
187	6.0	188	4.3	189	4.5	190	1.6R	191	2.8
192	1.9R	193	1.9	194	2.3	195	6.0	196	3.8
197	15.3	198	5.2	199	14.3	200	3.3	201	3.0
202	1.5	203	1.9	204	1.1	205	4.9	206	4.0
207	5.3	208	1.2	209	6.5	210	4.0	211	26.6
212	5.8	213	13.2	214	2.8	215	4.3	216	2.1
217	8.8	218	2.2	219	1.2	220	0.9	221	1.6
222	7.9	223	8.6	224	4.5	225	7.6	226	1.9
227	9.4	228	3.1	229	5.8	230	2.5	231	3.1
232	1.3	233	1.2R	234	0.9	235	1.2R	236	1.2
237	7.6	238	4.3	239	12.0	240	3.7	241	2.2
242	1.9	243	2.9	244	3.5	245	4.7	246	2.6
247	1.2	249	1.6	250	1.2	251	11.5	252	4.5
253	45.3	254	11.5	255	6.2	256	3.3	257	3.5
258	1.8	259	1.5R	260	1.4	261	0.4	262	3.9
263	1.1	265	3.6	266	0.9R	267	2.7	268	1.8R
269	5.1	270	2.8	271	29.1	272	7.6	273	3.4
274	0.8	275	0.7	277	0.5R	278	0.9	279	2.3
280	1.8	281	1.6	282	1.2	283	1.6	284	1.0R
285	1.1	286	0.7	287	0.8R	291	0.8	293	2.1
294	1.2	295	1.0R	296	0.6R	297	1.7	298	0.4
299	1.2R	300	1.9	301	0.7R	302	0.6R	307	0.8
308	0.7	309	2.5	310	0.8	311	1.5	312	0.6

/ Continued

APPENDIX TABLE I.1 (CONT.)

M/E	INT	M/E	INT	M/E	INT	M/E	INT	M/E	INT
313	0.7	314	1.2	315	0.9	321	0.4	322	0.5
323	1.1	324	0.5	325	0.7R	326	0.6	330	0.4
333	0.5	334	0.4	335	3.3	336	1.2	337	38.8
338	13.6	339	5.1	340	1.4	341	0.7	342	0.9
345	0.5	349	0.5R	350	0.6R	351	0.5	352	0.6
353	1.1	354	0.9R	355	0.8	357	0.5	360	0.4
361	1.4	363	73.0	364	23.4	365	7.1	366	1.7
367	1.8	368	2.4	369	1.5	370	0.4R	376	2.5
377	2.3R	378	12.7	379	5.4	380	2.8	381	3.7R
382	1.7	383	1.5	384	1.4	385	0.4	386	0.4
392	0.4	393	0.4	394	2.8	395	2.4	396	100.0
397	30.7	398	10.6	399	2.8	400	2.6	401	0.9R
410	0.8	412	2.3	413	1.0				

A PLOT OF 15 STRONGEST PEAKS FROM TABLE I.1

ERGOSTEROL /RL
LM CORRN= -181
BASE PK=45%FSD
TIC/PKS=98409/371

M/E	INT.	0 1 2 3 4 5 6 7 8 9 10
28	35.5	I- - - - - - - - - - -
43	33.6	I- - - - - - - - - - -
55	38.5	I- - - - - - - - - - -
69	57.2	I- - - - - - - - - - -
81	29.6	I- - - - - - - - - - -
83	29.1	I- - - - - - - - - - -
143	29.6	I- - - - - - - - - - -
149	38.5	I- - - - - - - - - - -
157	34.3	I- - - - - - - - - - -
253	45.3	I- - - - - - - - - - -
271	29.1	I- - - - - - - - - - -
337	38.8	I- - - - - - - - - - -
369	73.0	I- - - - - - - - - - -
396	100.0	I- - - - - - - - - - -
397	30.7	I- - - - - - - - - - -

APPENDIX TABLE I.2a SOIL EXTRACT AT 70°C.

15 OCT 1984

ERGOSTEROL 2/RL
LM CORRN=+326
BASE PK= 34%FSD
TIC/PKS=94343/437

M/E	INT	M/E	INT	M/E	INT	M/F	INT	M/E	INT
28	20.0	29	24.7	38	2.6	31	5.0	32	8.5
36	2.2	37	2.8	38	2.4	39	10.4	40	4.6
41	63.2	42	11.0	43	91.4	44	6.2R	45	31.6
52	2.8	47	1.8	49	2.2	50	3.5	51	5.3
46	3.0	53	8.9	54	7.9	55	80.6	56	26.9
52	100.0	58	6.7	59	5.4	60	5.0	61	2.6
62	2.7	63	3.3	64	3.3	65	5.9R	66	3.8
67	28.8	68	8.8	69	63.2	70	23.3	71	56.7
72	5.6	73	6.8	74	3.5	75	2.6	76	5.2
77	8.5	78	3.7	79	12.0	80	7.1	81	41.0
82	18.7	83	44.1	84	14.7	85	37.1	86	4.4
87	3.5	88	3.1R	89	2.8	90	2.0	91	11.0
92	5.3	93	12.2	94	7.9R	95	39.2R	96	17.3
97	42.1	98	13.3	99	17.8	100	3.8	101	3.9
102	3.6	103	3.2	104	4.4	105	12.3R	106	5.9
107	13.4	108	7.0R	109	26.2R	110	12.7	111	26.9
112	10.9	113	14.7	114	2.8	115	7.1	116	3.6
117	5.6	118	3.6	119	13.4	120	7.3	121	8.6
122	6.0	123	18.9	124	9.5	125	18.7	126	9.0
127	10.7	128	5.5	129	7.1	130	4.6	131	6.8
132	3.8	133	9.4	134	5.3	135	8.3	136	6.0
137	11.9	138	6.9	139	10.6	140	6.3	141	9.5
142	4.2	143	6.3	144	4.2	145	9.0	146	3.9
147	7.3	148	4.0	149	20.7	150	5.4	151	10.3
152	6.2	153	9.0	154	5.7	155	8.8	156	4.4
157	6.9	158	4.2	159	8.3	160	4.0	161	4.4
162	4.2	163	7.7	164	4.0	165	8.4	166	4.6R
167	6.9	168	5.4	169	6.8	170	3.6	171	5.6
172	2.8	173	4.8	174	3.2	175	5.6	176	4.1
177	6.5	178	5.9	179	7.3	180	4.2	181	7.1
182	5.6	183	6.2	184	3.6	185	5.6	186	6.5
187	4.8	188	3.3	189	5.0	190	3.4	191	8.3
192	5.0	193	7.7	194	5.6	195	6.4	196	4.4
197	6.2	198	3.8	199	5.3	200	3.3	201	5.0
202	3.5	203	5.0	204	3.5	205	5.9	206	5.4
207	5.0	208	4.7	209	5.3	210	5.8	211	2.8
212	3.5	213	4.0	214	2.8	215	3.3	216	5.1
217	4.4	218	5.0	219	6.8	220	3.5	221	3.2
222	4.4R	223	4.8	224	4.4	225	5.0	226	4.3
227	3.5	228	3.3	229	3.4	230	2.5	231	4.2
232	4.4	233	4.1	234	3.6	235	3.9	236	2.8
237	4.0	238	4.2	239	4.6	240	2.8	241	4.1
242	2.6	243	2.8	244	2.8	245	3.1	246	5.0
247	4.0	248	3.5	249	3.6	250	4.1	251	3.3
252	5.0	253	6.7	254	3.8	255	3.2	256	3.3R
257	3.2	258	2.8	259	3.4	260	3.6	261	4.1R
262	4.7	263	3.8	264	3.8	265	3.2	266	4.4
267	4.2	268	3.5	269	2.4	270	2.3	271	3.0
272	2.7	273	3.4	274	3.3	275	3.0	276	4.0
277	4.0	278	2.7	279	4.0	280	4.4	281	2.6
282	3.2	283	2.9	284	2.7	285	2.7	286	3.2
287	2.8	288	3.8	289	2.9	290	3.1	291	2.5
292	3.5	293	2.3	294	3.8	295	2.8	296	3.0
297	2.5	298	2.5	299	2.8	300	2.4	301	3.1
302	3.2	303	2.5	304	2.6	305	3.2	306	3.5

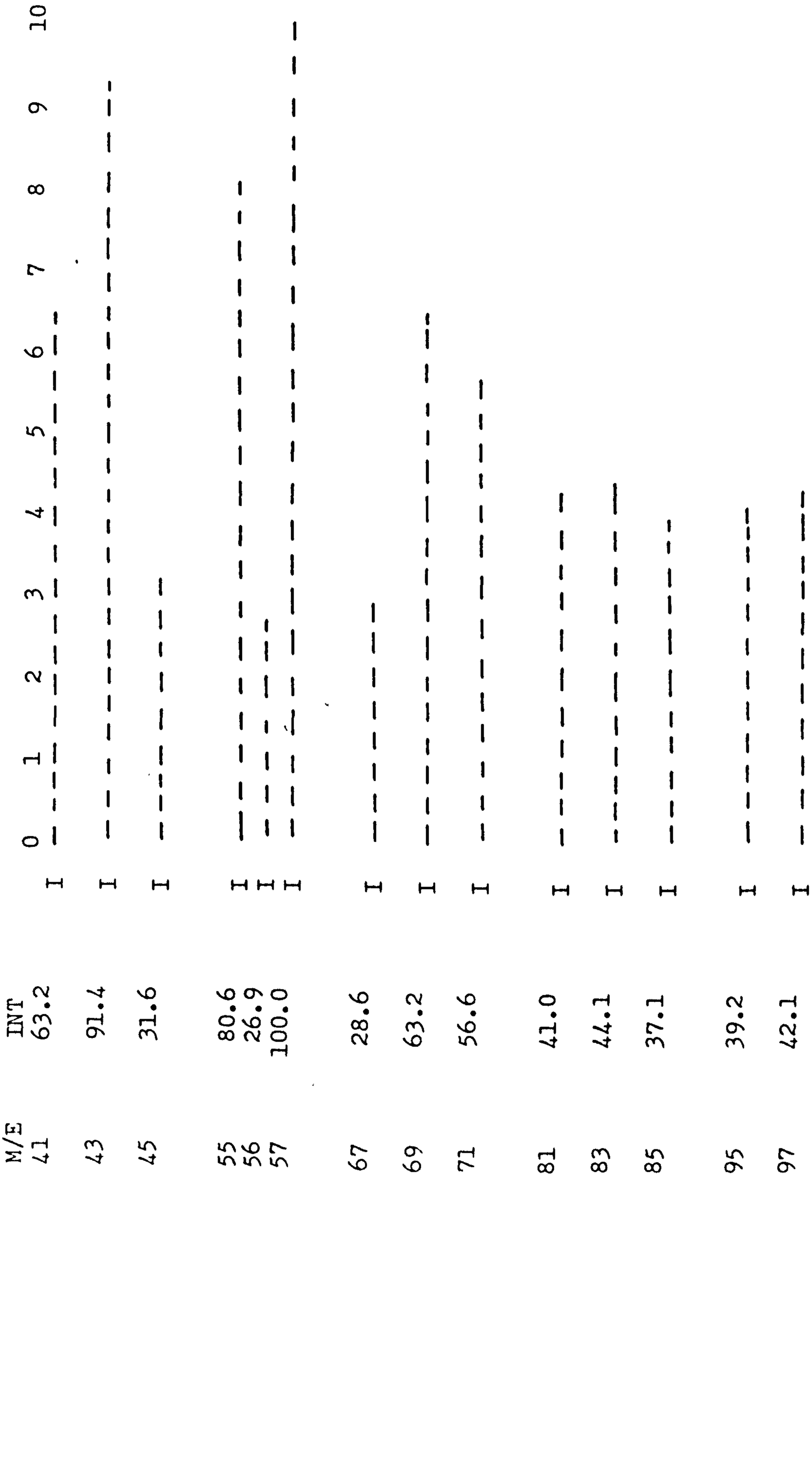
/CONTINUED

APPENDIX TABLE I.2a (CONT.)

M/E	INT	M/E	INT	M/E	INT	M/E	INT	M/E	INT
307	2.9	308	3.3	309	3.4	310	2.8	311	2.2
312	2.4	313	2.3	314	2.5	315	2.5	316	2.8
317	2.6	318	3.1	319	3.2	320	3.3	321	2.6
322	3.1	323	3.4	324	2.7R	325	2.1	326	2.2
327	2.3	328	2.8	329	2.6	330	2.7	331	3.0
332	2.8	333	2.3R	334	3.0	335	2.8	336	2.8
337	3.8	338	2.8	339	2.2	340	2.8	341	2.2
342	2.7	343	2.5	344	3.6	345	2.8	346	2.5
347	2.8	348	2.8	349	2.0	350	2.8	351	2.6
352	2.3	353	2.5	354	2.7R	355	2.6	356	2.8
357	2.2	358	3.2	359	2.7	360	3.3R	361	2.6
362	2.8	363	6.0	364	4.4	365	3.3	366	2.9
367	2.7	368	2.8	369	2.3	370	3.1	371	2.9
372	3.4	373	3.2	374	3.2	375	2.8	376	3.4
377	3.1	378	4.9	379	3.2	380	3.3	381	2.7
382	2.8	383	2.3	384	2.8	385	2.4	386	3.6
387	3.4	388	3.8	389	2.5	390	3.8	391	2.4
392	3.4	393	2.5R	394	2.8	395	2.0R	396	9.4
397	4.2	398	4.0	399	3.0	400	4.5	401	3.1
402	3.2	403	2.8R	404	3.0	405	2.5	406	3.0
407	2.5	408	2.6	409	2.1	410	2.6	411	2.4
412	3.2	413	2.5	414	3.4	415	2.7	416	3.1
417	2.3	418	3.0	419	2.5	420	2.8R	421	2.0
422	2.4	424	2.7	425	2.2	426	2.5	427	2.3
428	3.2	429	2.4	430	2.5	432	2.7	434	2.7
435	2.2	439	2.2	440	2.4	441	2.2	442	2.5
444	2.5	445	2.1	446	2.4	447	2.0	448	2.4R
454	2.0	456	2.2	462	2.2				

A PLOT OF 15 STRONGEST PEAKS FROM TABLE I.2a

ERGOSTEROL 2/2
LM CORRN=+326
BASE PK=34%FSD
TIC/PKS=94343/437



APPENDIX TABLE I.2b SOIL EXTRACT AT 80°C

15 OCT 1984
ERGOSTEROL 2/3
LM CORRN=+566
BASE PK= 41%FSD
TIC/PKS= 135836/489

M/E	INT	M/E	INT	M/E	INT	M/E	INT	M/E	INT
28	15.8	29	31.8	30	2.4	31	3.1	32	6.0
36	2.0	37	1.9	38	2.9	39	14.8	40	3.3
41	75.6	42	11.6	43	92.2	44	6.6R	45	19.8
46	1.9	47	1.7	50	3.1	51	5.3	52	2.8
53	10.2	54	7.0	55	100.0	56	23.9	57	79.8
58	6.6	59	4.9	60	6.9	61	2.4	62	1.5
63	2.6	64	2.4	65	6.1	66	3.8	67	36.1
68	13.1	69	89.8	70	23.5	71	52.8	72	4.8
73	6.4	74	3.7	75	2.9	76	4.0	77	12.2
78	4.4	79	19.8	80	7.3	81	54.2	82	19.8R
83	53.2	84	13.4	85	33.6	86	4.1	87	3.6
88	2.1	89	2.8	90	2.0	91	18.9	92	5.7R
93	22.5	94	9.5R	95	42.5R	96	17.3	97	42.5R
98	13.2R	99	15.5	100	2.9	101	3.7	102	2.8
103	3.9	104	4.5	105	19.4	106	6.9	107	17.4R
108	8.1R	109	30.5R	110	13.4	111	26.4	112	8.9
113	11.4	114	2.9	115	10.4	116	5.8	117	10.2
118	6.8R	119	19.6R	120	6.1	121	12.5	122	5.8
123	18.2	124	11.9	125	22.3	126	6.6	127	9.2
128	10.0	129	13.9	130	5.4	131	14.4	132	6.1
133	13.6	134	6.2	135	11.2	136	4.9	137	13.8
138	7.1	139	11.5	140	6.1	141	11.5	142	7.0
143	19.4	144	7.5	145	19.3	146	5.7R	147	11.6R
148	4.5	149	17.4	150	5.3	151	9.7	152	6.8
153	8.1	154	6.7	155	10.4	156	6.1	157	17.6
158	9.4	159	17.1	160	4.5	161	7.5R	162	3.9
163	7.5	164	4.7	165	9.6	166	5.4	167	7.7
168	5.3	169	9.9	170	5.5	171	11.2	172	4.5
173	7.7	174	3.7	175	9.6	176	4.5	177	7.4
178	5.4	179	7.5	180	4.7	181	7.5	182	6.8
183	9.4	184	4.7	185	9.0	186	7.4	187	5.4
188	3.3	189	5.5	190	4.0	191	7.4	192	5.1
193	5.8	194	4.5	195	6.2	196	6.5	197	11.6
198	4.7	199	9.6	200	4.1	201	4.7	202	3.4
203	4.1	204	3.4	205	5.6	206	4.4	207	4.5
208	4.0	209	6.3	210	5.0	211	13.3R	212	5.7
213	7.5	214	3.6	215	5.4	216	3.6	217	6.7
218	6.1	219	7.1	220	4.6	221	5.2	222	4.3
223	6.5	224	5.7	225	6.4	226	5.1	227	7.0
228	3.6	229	4.4	230	3.7	231	4.5	232	3.6
233	4.1	234	3.1	235	4.5	236	3.6	237	6.3
238	6.3	239	6.3	240	3.8	241	3.3	242	2.8
243	3.4	244	3.1	245	4.0	246	3.9	247	3.6
248	3.1	249	3.3	250	3.9	251	7.5	252	6.3
253	27.6	254	7.3	255	5.2	256	3.6	257	4.0
258	3.1	259	3.6	260	3.4	261	3.1	262	4.7
263	4.3	264	3.6R	265	4.5	266	4.1	267	4.3
268	2.9	269	4.5	270	3.2	271	11.6	272	4.1
273	3.6	274	2.9	275	3.3	276	2.7	277	3.1
278	3.3	279	4.2	280	4.2	281	3.5	282	3.1

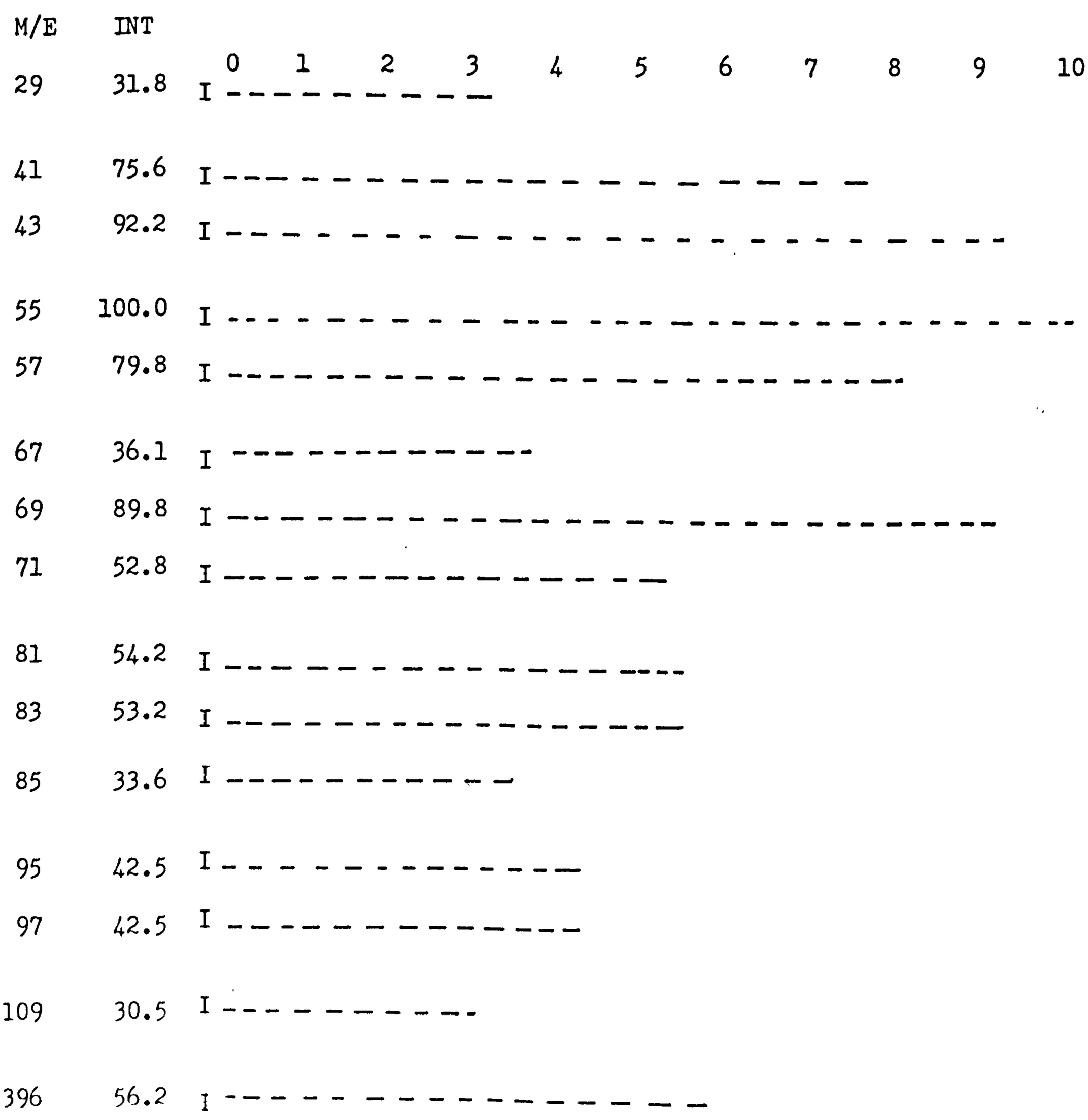
/ CONTINUED

APPENDIX TABLE I.2b (CONT.)

M/E	INT	M/E	INT	M/E	INT	M/E	INT	M/E	INT
283	2.9	284	2.8	285	2.9	286	2.3	287	2.9
288	2.8	289	2.9	290	2.7	291	3.5	292	2.9
293	3.2	294	3.3	295	3.5	296	2.8	297	2.6
298	2.4	299	2.2	300	2.9	301	3.0	302	2.8
303	2.8	304	2.6	305	2.6	306	3.3	307	2.9
308	3.0	309	3.1	310	2.6	311	2.3	312	2.2
313	2.8	314	2.7	315	2.5	316	3.0	317	2.7
318	2.8	319	2.6	320	2.8	321	2.9	322	3.1
323	3.3	324	2.5	325	2.8R	326	2.2	327	2.4
328	2.2	329	2.4	330	1.9	331	2.3R	332	2.3
333	2.7	334	2.7	335	3.6	336	3.3	337	14.8
338	7.2	339	3.5	340	2.9R	341	2.4	342	2.6
343	2.6	344	2.9	345	2.7	346	2.7	347	2.8
348	3.0	349	2.6	350	3.1	351	2.8	352	2.3R
353	2.6	354	2.6	355	2.2	356	2.4	357	2.2
358	2.9	359	2.6	360	2.7	361	3.1	362	3.1
363	29.1	364	10.9	365	4.0	366	2.9R	367	2.8
368	3.1	369	2.4	370	2.4	371	2.1	372	3.0
373	2.3	374	2.7	375	2.3	376	4.7	377	3.5
378	11.6	379	5.7	380	3.3	381	3.3	382	3.0
383	2.8	384	3.1	385	2.6R	386	3.9	387	2.7
388	2.4	389	2.3	390	2.4	391	2.1	392	2.3
393	2.6	394	3.9	395	2.9	396	56.2	397	18.9
398	9.4	399	3.5	400	5.5	401	3.0	402	2.9
403	2.4	404	2.5	405	2.2	406	2.9	407	2.7
408	2.5	409	1.9	410	2.7	411	2.4R	412	2.8
413	2.2	414	3.3	415	2.4	416	2.7	417	2.4
418	2.4	419	2.3	420	2.1	421	2.1	422	2.1
423	1.9	424	2.8	425	2.2	426	2.8	427	2.1
428	3.4	429	2.4R	430	2.3	431	1.9	432	2.4R
433	1.9	434	2.5	435	2.0	436	1.9	437	2.3
438	2.1	439	2.0	440	2.5	441	2.3	442	2.2R
443	2.1	444	2.2	445	2.1	446	2.4	447	1.8
448	2.6	449	2.1	450	2.2	451	1.7	452	2.1
454	2.4	455	1.8	456	2.2	457	1.7	458	2.0
458	1.9	460	2.2	461	1.9	462	2.2	463	1.9
464	1.9	466	1.9R	468	1.9	469	1.9R	470	2.0
471	1.8	472	1.9	473	1.8	474	2.1	475	1.9
476	2.0	477	1.8	478	1.9R	480	1.8	482	2.1R
484	1.8	485	1.7	486	1.8	487	1.8	488	1.7
489	1.7	490	1.9	491	1.7	492	1.8	494	1.8
496	1.8	498	1.8	504	1.8				

A PLOT OF 15 STRONGEST PEAKS FROM TABLE I.2b

ERGOSTEROL 2/RL
LM CORRN=+566
BASE PK=41%FSD
TIC/PKS=135836/489



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