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The interdependence of watermoulds occurring in water and soil habitats affecting their population density, distribution and periodicity

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Abstract: Studies on watermoulds – particularly isolation, taxonomic criteria, occurrence and phenology - have been conducted by several aquatic mycologists in different geographical regions of the world. However, the ecology of fungi, particularly watermoulds, has received very little attention. In this contribution, the occurrence, seasonal periodicity, population density and distribution of watermoulds in water bodies and soils in different geographical areas of the globe, are reviewed. Various types of water and soil sampling techniques and the quality of the resulting data are also reviewed. Data from different geographical areas of the globe indicate that distinct eccentric and centric Occurrence Zones of the world may be identified for the members of saprolegniaceae, and an altitude-latitude relationship has also been elucidated. On the basis of the occurrence and phenology of watermoulds in aquatic and terrestrial conditions, the interdependence between aquatic and terrestrial conditions is also discussed.

Resumen: Varios micólogos acuáticos han llevado a cabo estudios sobre los mohos acuáticos –en particular el aislamiento, los criterios taxonómicos, la presencia y la fenología–en diferentes regiones geográficas del mundo. No obstante, la ecología de hongos, en particular de los mohos acuáticos, ha recibido muy poca atención. En esta contribución se revisan la presencia, la periodicidad estacional, la densidad poblacional y la distribución de mohos acuáticos en cuerpos de agua y en suelos de diferentes áreas geográficas del planeta. También se revisan varios tipos de técnicas de muestreo de suelo y agua, así como la calidad de los datos resultantes. Los datos procedentes de diferentes áreas geográficas del orbe indican que es posible identificar Zonas de Presencia excéntricas y céntricas para los miembros de saprolegniaceae, y asimismo permitieron dilucidar una relación altitud-latitud. Con base en la presencia y en la fenología de hongos acuáticos en condiciones acuáticas y terrestres, también se discute la interdependencia entre condiciones acuáticas y terrestres.

Resumo: Vários micologistas aquáticos têm levado a cabo vários estudos sobre os bolores aquáticos - em particular o isolamento, os critérios taxonómicos, ocorrência e fenologia – em diferentes regiões geográficas do mundo. Contudo, a ecologia dos fungos, particularmente dos bolores, tem sido objecto de muito pouca atenção. Nesta contribuição, revê-se a ocorrência, periodicidades estacional, densidade populacional e distribuição dos bolores nos cursos de água e solos em diferentes áreas geográficas do globo. Reviram-se, igualmente, várias técnicas de amostragem da água e do solo bem como a qualidade dos dados resultantes. Os dados de diferentes áreas geográficas do globo indicaram que distintas Zonas de Ocorrência, excêntricas e cêntricas do mundo, podem ser identificadas por membros das saprolegniaceae, e do mesmo modo, esclarecer relações de altitude-latitude. Com base na ocorrência e fenologia dos fungos em condições aquáticas e terrestres, discute-se a interdependência entre as condições aquáticas e terrestres.

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Key words: Altitude-latitude relationship, distribution, occurrence zones, population density, seasonal periodicity, watermoulds.

Introduction

Studies on watermoulds – particularly isolation, taxonomic criteria, occurrence and phenology – have been conducted by several aquatic mycologists in different geographical regions of the globe (Dick & Newby 1961; Dick 1966, 1968; Forbes 1935; Harvey 1925a,b; Khulbe & Bhargava 1977; Khulbe 1980; Liu & Volz 1976; Lund 1934, 1978; Marano & Steciow 2006; Milanez 1967; Nasser 2003; Paliwal & Sati 2009; Prabhuji 1979, 1984a,b, 2010; Prabhuji *et al.* 1984; Prasad *et al.* 2009; Sati & Mer 1989; Schoenlein-Crusius *et al.* 1999; Srivastava 1967a,b; Willoughby 1962, Zeigler 1958). Prabhuji (2005) has recently reviewed the literature on the occurrence and phenology of oomycetes, particularly members of the saprolegniaceae, and observed an interesting relationship between the geographical conditions of the area and the type of oöspore developed in saprolegniaceous fungi. He has also discussed the reasons behind the differences in isolation of watermoulds observed when they are obtained from water bodies and from soil in nearby areas.

Studies of the ecology of fungi, particularly water moulds, have been carried out with little attention paid to the ecological aspects. The field aspects of the ecology of watermoulds have lagged behind laboratory investigations of such parameters as the effects of temperature, nutrition, and pH on growth and reproduction. The emphasis has been on laboratory analyses in the development of knowledge of the ecology of watermoulds, with the least attention paid to the ecology for some time.

There is a vast array of work by aquatic mycologists on the occurrence, seasonal periodicity, population density and distribution of water moulds in water bodies and soils in different geographical areas of the globe using various sampling techniques. These investigations, together with the sampling techniques, are reviewed here to present a comprehensive account of ecological studies on watermoulds.

Sampling techniques

Watermoulds have been isolated from water – mostly stagnant water – and from inundated, wet or dry soils, and therefore, sampling techniques must be considered separately for water bodies and soils.

Water sampling

The first collections of specimens of watermoulds were from fish and floating dead flies (Ledermüeller 1760; Schränk 1789; Spallanzani 1777; Wrisberg 1765). Most of the aquatic mycologists working on watermoulds prefer the simple aliquot water sampling technique in which 1-2 litres of water is collected from the sampling site, thoroughly mixed, and 10-15 ml aliquots are placed in different Petri dishes, baited with boiled hempseed cotyledons. These are incubated for 2 - 4 days to observe the growth of watermould hyphae (Khulbe 1980; Lund 1978; Milanez 1967; Prabhuji 2005; Srivastava 1967a, b).

To obtain quantitative data, Ulken & Sparrow (1968) used the Most Probable Number (MPN) method to estimate the numbers of saprolegniaceous fungi in water samples, but the resulting data were highly variable and inconclusive. It is still doubtful whether the MPN method can produce consistent, reliable data. Park (1972) tried to collect quantitative data on the occurrence of zoosporic fungi by dilution plating and particle plating. However, neither the dilution (1:1,00,000 to 1:7,00,000) nor the particle plating approach yielded members of the saprolegniaceae. While investigating the occurrence of saprolegniaceous fungi in a river system, Ho (1975) prepared discs of hempseed extract agar, put these into Petri dishes containing water samples and incubated them at 18 - 22 ºC. The number of such discs that supported growth of watermould hyphae was taken as the "isolation percentage". Similarly, in a study of the frequency of watermoulds in a Newfoundland river, Maestres (1977) centrifuged 20 litres of river water during each sampling, and the concentrate was used to prepare various dilutions with sterile distilled water. She plated these dilutions on nutrient agar, incubated the plates at 18 - 20 ºC and analyzed the yield quantitatively to relate approximate number of propagules in the original volume of water sample. The results, however, could not be considered reliably quantitative.

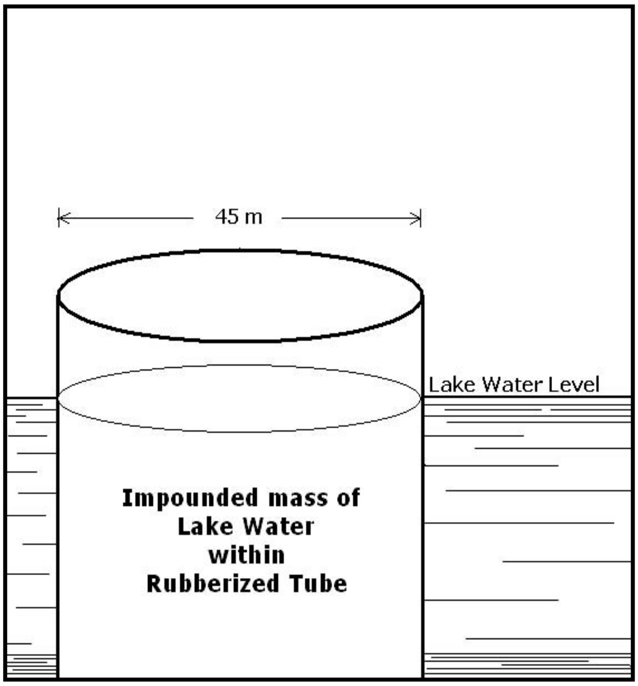

Fig. 1. Diagram showing Willoughby's method of sampling within a permanently marked water area.

Willoughby (1962, 1965) collected a large volume of water (5 - 10 liters) that was shaken thoroughly, and aliquots were mixed with molten oatmeal agar and poured into sterile Petri dishes. As soon as the medium solidified, it was cut into eight equal parts; each section was placed in a new Petri dish containing sterile distilled water and incubated for 24 - 48 h. The edge of each part, following incubation, developed a fringe of watermould mycelia. Willoughby observed that in each agar section the morphology of the mycelium was consistent over the entire surface of that section, indicating that the fringe mycelium came from a single propagule. In cases where more than one species was isolated on a single section, Willoughby introduced a "correction factor" (the number of sections that could be expected to harbor more than one propagule) which he developed and tested using the spores of a non-sexual *Saprolegnia* and claimed to be able to calculate with reasonable accuracy the number of propagules per liter of water sample. Later, Thakurji & Dayal (1966) combined Willoughby's quantitative technique with sample dilution.

Suzuki (1960a, b), working on quantifying the frequency of species of saprolegniaceae in terms of the numbers of zoospores per unit volume of water and sediment samples, used a different, but erroneous, method. He floated three hempseed halves on the water/sediment sample placed in Petri dishes. Following incubation for 2 - 4 days, hyphae on each of the hempseed half were counted under the assumption that the number of hyphae corresponded to the number of zoospores in a sample. However, branching and sporulation in these fungi is so frequent that it is erroneous to consider that each hyphal thread represents single germinated encysted zoospore, or that branching did not take place during the entire 2 - 4 day period of incubation.

Willoughby (1974) employed a different technique for sampling lakes and ponds in a permanently marked area to assess the seasonal periodicity of watermoulds. He took a rubberized tube (45 m in diameter) and positioned it vertically in a shallow lake to impound a large mass of water (although, given the impractical nature of such a large structure, the size has been suggested to be 4.5 m), and anchored it within the bottom mud so that no water might enter from that end (Fig. 1). This sampling method was supposed to be ideal for sampling within a permanently marked area; however, the preliminary data indicated that, with time, dissimilarity developed between the population density of watermoulds within and outside the tube. Differences may have arisen due to a lack of natural flow of lake water within the area enclosed by the tube.

To overcome problems with assessing the distribution of watermoulds within various vertical strata of a water body, Johannes (1957) devised a novel bait submergence technique. He stuck ant eggs onto thick paper slips covered with a paraffin layer and attached these slips at measured distances on a pole. The poles were submerged upright in such a fashion as to position the eggs at various depth intervals. Following an average submergence time of five days, the eggs were removed and transferred to sterile Petri dishes containing sterile distilled water and observed for growth of watermoulds.

The simple aliquot water sampling technique, adopted by aquatic mycologists from the very beginning, is still the best water sampling technique. Other techniques, such as that suggested by Willoughby (1974) for a defined water volume, have been found to suffer from several errors and may not yield reliable results. However, the method adopted by Johannes (1957) to assess the distribution of watermoulds in vertical strata of water bodies is reliable enough and should be adopted by the aquatic mycologists in future. Many of the techniques given by various aquatic mycologists to quantify numbers of watermould propagules in a water body are thought to provide unreliable data, except for the technique adopted by Willoughby (1962, 1965).

Soil sampling

The earliest investigation on the collection and isolation of watermoulds from soils is that of Raper (1928), following Harvey (1925), who tried to determine the abundance of these fungi in a given area and volume of soil. He simply prepared suspension of known quantities of soil with distilled water and, following the settling of soil particles, baited the supernatant liquid for isolates. He obtained data showing the frequency with which particular species were isolated from various sites.

Dick & Newby (1961) and Dick (1962), investigating the distribution and abundance of saprolegniaceae in the soils of southeastern England, developed a "quadrat sampling" method. A quadrat with an area of 90 cm^2 was permanently fixed on a particular soil sampling area, and divided it into 16 square subdivisions. From each subdivision soil samples were taken from five fixed points and baited for watermoulds by conventional methods. The terms "species totals" and "quadrat totals", introduced by Dick & Newby (1961), have been used to denote "the sum of records for species for given quadrat area" and "the sum of the counts for all species recorded from a given quadrat area", respectively. Prabhuji (1979) has also employed this method for his studies on some lower fungi occurring in soils of Gorakhpur (India).

Dick (1966) developed a technique for quantitatively determining the watermould populations in soil, based on fractionation of the samples. A soil sample was mixed with water, stirred mechanically, and three portions were selected for plating and baiting: the coarse plant material, supernatant fluid, and the slurry. The supernatant portion yielded a species composition unlike that recovered from the slurry; by contrast, the spectrum of watermould species found in the coarse plant material was very similar to that in the baited slurry.

Occurrence, distribution and periodicity

The occurrence, distribution and periodicity of watermoulds have been studied in aquatic as well as in terrestrial habitats from different areas of the world (Johnson *et al.* 2002; Khulbe & Bhargava 1977; Liu & Volz 1976; Lund 1978; Manoharachary 1979a, b; Marano & Steciow 2006; Mer *et al.* 1980; Nasser 2003; Paliwal & Sati 2009; Prabhuji 1984a, b; Prabhuji 2005; Prasad *et al.* 2009). The occurrence, periodicity and distributional data of watermoulds for these two different habitats vary significantly (Fig. 2 A-D) and, therefore, merit separate discussion. However, it is also important to discuss the interrelationship of the variations observed in aquatic and terrestrial habitats.

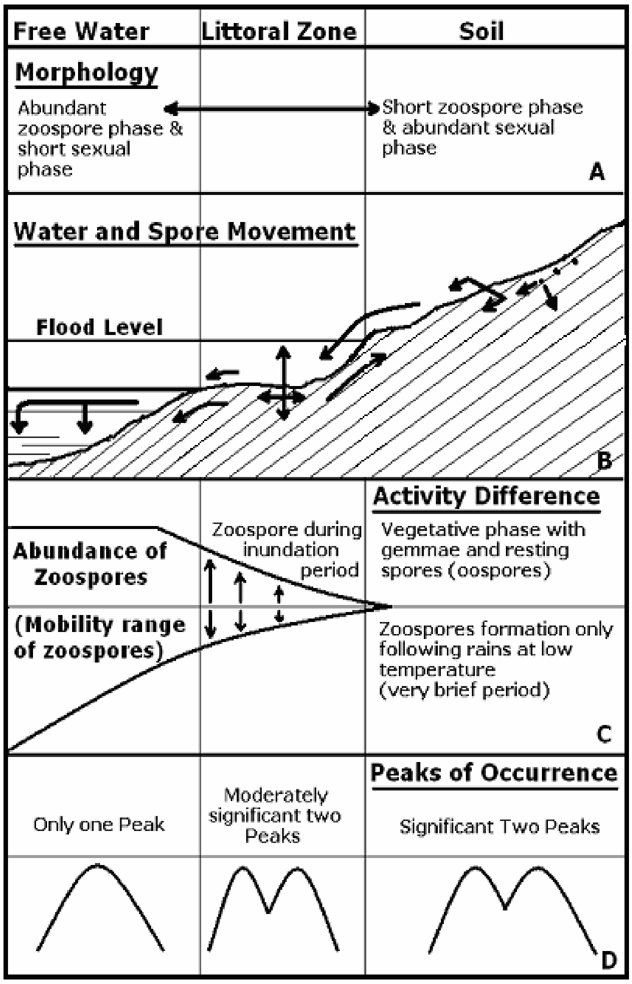

Fig. 2 A-D. Occurrence and distribution of watermoulds in water bodies, littoral zones and in soils.

A: Morphology of propagules in free water and in soils.
B: Movement of zoospores in different water situations.
C: Difference in zoospore activity in different water situations.
D: Peaks of occurrence of watermoulds in different situations.

Periodicity refers to the seasonal variation in the occurrence of watermoulds with respect to fluctuations of temperature, pH, light, nutrients and other physico-chemical factors in aquatic and terrestrial habitats. Attempts to interpret or compare and contrast the various existing reports on periodicity are limited often by incomplete accounts of sampling and lack of statistical analyses, among others. For example, most reports do not mention the latitude or altitude of the sampling sites. Nevertheless, the importance of these parameters has been established (Prabhuji 2005).

Aquatic habitats

Forbes (1935) and Waterhouse (1942), working on the periodicity of watermoulds in temperate waters (without mentioning the temperature range), recorded the maximum yield of watermoulds during winter (December - February) and minimum during summer (May - June). Similarly, Srivastava (1967b), working on the ecology of aquatic fungi of Gorakhpur (India), also recorded only one peak of occurrence (Fig. 3), during winter (December - January). However, Khulbe & Bhargava (1977), studying the distribution and seasonal periodicity of watermoulds in some lakes in Nainital hills (India), reported maximum numbers of watermoulds during summer (May - June) at 17.5 - 23.5 °C (average 20.5 °C). The most important consideration here is that the summer temperature of the hills corresponds with the winter temperature of the plains; therefore, a logical identity does exist among all these observations. By contrast, Perrott (1960) and Roberts (1963) found two periods of maximum yield: one in autumn and the other in spring. Stpiczyńska-Tober (1965) recorded the members of saprolegniaceae in two rivers in Poland and found more representatives of this family in samples from water at -2 to -5 °C than in those taken when the temperature was 18-30 °C. Rooney & McKnight (1972), in a report of collections of saprolegnians in Utah Lake (USA), found the number and frequency of these fungi to increase with the seasonal rise in water temperature, beginning with the onset of the ice-free period in May. Muhsin (1977) isolated and identified 20 species of watermoulds in river waters in Iraq and found January to be the peak month for frequency and diversity of species, with the lowest frequency occurring in summer. Investigating a 32-km sector of a Japanese river, Okane (1978) noted that the number of watermould spores per sample increased during the late spring and in the autumn, but declined throughout the summer and winter months. Al-Saadi *et al.* (1979) traced annual fluctuations in the frequency of watermoulds and phytoplankton recovered from an Iraqi river and found that during the period when the phytoplankters were at their maximum, the frequency of saprolegniaceous fungi was correspondingly lowest and *vice-versa.* They concluded that the watermoulds were dependent on dead phytoplankters as a nutrient source, which accounted for the increase of fungi following the decline of the phytoplankton.

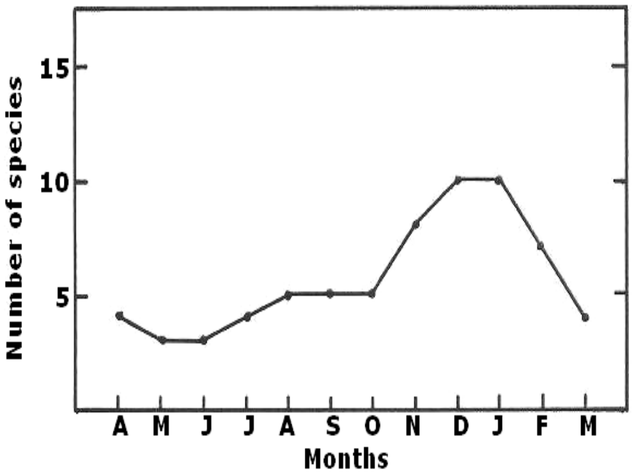

Fig. 3. Monthly periodicity of watermoulds in water bodies (after Srivastava 1967b).

Roberts (1963) reported that watermoulds in aquatic ecosystems were distributed according to hydrogen ion concentration and arranged them in three groups: acid (pH 5.6 - 7.0), alkaline (7.0 - 8.8) and neutral (5.6 - 8.8). Lund (1934) and Srivastava (1967a,b) also classified watermoulds based on water pH. Several other aquatic mycologists have also noted the significance of hydrogen ion concentration on the occurrence and distribution of watermoulds in water bodies (Ergaskev & Kirgizbaeva 1978; Florinskaya 1969; Hasija & Batra 1978; Milanez 1966; Suzuki & Nimura 1960; Zaborowska 1965). However, pH may not be the only factor which determines the distribution of aquatic fungi. Roberts (1963) has pointed out that the watermoulds live on debris in the water and the type of decaying vegetation found in acid waters as opposed to that in alkaline waters may be the deciding feature in their distribution. In acid conditions, watermoulds may play a more important role in the decomposition of organic matter.

The debris in water, although consisting mainly of decaying vegetation, also contains animal remains. Only in certain cases, however, sample collections have been made mostly or exclusively from only one of the two types of organic matter; for example, Cutter (1941) collected *Aphanomyces helicoides* (= *A. laevis*) and *A. amphigynus* from insect exuviae. Thus, it may be that the animal populations of a habitat also affect the distribution pattern of watermoulds.

Stoll (1936), reporting on the occurrence and distribution of watermoulds with respect to temperature, stated that there were fewer species of these fungi in colder waters than in warmer ones. His conclusions supported the views of Lund (1934) that species of *Achlya* thrive best in cool temperatures and exhibit poor performance in warm waters. Ho (1975) has also recognized a correlation between water temperature and isolation frequency of watermoulds. Zeigler (1958) recorded temperatures extant at the time of sampling and found that these correlated with the oöspore type of the recovered species. Zeigler (1962) subsequently analyzed the isolated species of watermoulds during his studies and indicated that the optimum temperature range for species with centric and sub-centric oöspores was 16 - 19 ºC, whereas that for eccentric oöspore forms was 19 - 30 ºC. Srivastava (1967a) grouped watermoulds into 'low temperature species' and 'constant species', and most of the members were eccentric oöspore forms. Khulbe & Bhargava (1977) recognized four clusters of saprolegniaceous forms in the hilly lakes of India, viz., constant species in samples, collected at 10 - 28.5 ºC; low temperature species, most frequent in waters at 10 - 18 ºC; moderate temperature species, sampled at 18 - 22 ºC; and high temperature species, sampled at 24 - 28.5 ºC. Eccentric oöspore species were found more frequently in subtropical lakes and centric oöspore species in temperate lakes (Khulbe & Bharagava 1977). However, Mer *et al.* (1980), studying the seasonal periodicity of watermoulds in lakes and soils of the same geographical and climatic area as Khulbe & Bharagava (1977), recognized only three groups: low temperature species (below 18 ºC); moderate temperature species (18.1 - 26.8 ºC) and constant species (8 - 26.8 ºC); and classified them into aquatic, amphibious and terrestrial (from soil samples) species. Furthermore, they reported that terrestrial forms showed two maxima, one in the spring and the other in the rainy season. They also observed that the centric oöspore forms were dominant in lakes, whereas eccentric forms dominated in soils. Maestres (1977) isolated several watermould species from Newfoundland and placed them into three categories: cold, warm, and intermediate temperature species. All the collected species had centric oospores: no species producing eccentric oöspores appeared in Maestres' collections (Maestres 1977). Johnson *et al.* (2002) have suggested that it cannot be concluded from Maestres' report that eccentric oöspore forms are absent from cold water environments, and suggest that there is ample evidence from numerous collections that eccentric-egged forms occur in glacier-fed streams and other bodies of cold water in Iceland (Howard *et al.* 1970). More recently Prabhuji (2005) suggested that the centric and sub-centric oöspore forms dominate in temperate regions, and eccentric oöspore forms dominate in tropical regions of the globe.

Höhnk (1935) proposed the grouping of genera by sporulation types on the basis of his studies on distribution of watermoulds in different habitat types in the Weser River Basin, between Bremerhaven and Bremer, West Germany. He developed the scheme by correlating isolates of members of saprolegniaceae with five habitat types: (i) tidal water; (ii) river and adjacent stream water (its tributaries); (iii) ditch water; (iv) soil from the river shore-line; and (v) soil from fields bordering the river. The five groupings (or distribution) of genera were :

(i) *Pythiopsis* (ii) *Saprolegnia, Isoachlya, Leptolegnia* (iii) *Achlya, Aphanomyces, Plectospira* (iv) *Thraustotheca, Calyptralegnia, Brevilegnia* (v) *Aplanes* and *Geolegnia.*

Höhnk & Bock (1954) were the first to relate the distribution and abundance of species of saprolegniaceae to the depth of water in freshwater temperate lakes and ponds. They sampled lake water at various depths and observed that the species they collected, could be distributed into three groups as proposed by Höhnk (1935) on the basis of the manner of sporulation. These groups are: (i) The *Saprolegnia* type (species of *Saprolegnia*, *Isoachlya* and *Leptolegnia*) (ii) The *Achlya* type (species of *Achlya* and *Aphanomyces*) (iii) The *Dictyuchus* type.

The representatives of group (i) were limited to the upper half of the lake's water column; group (ii) was distributed throughout the lake at all depths; and group (iii) occurred only from the surface to a depth of about two meters. By contrast, Suzuki & Hatakeyama (1960) found that the spores from the species of *Achlya*, *Saprolegnia*, *Aphanomyces*, *Leptolegnia*, and *Thraustotheca* were distributed uniformly within the water column due to water circulation, results that are in some disagreement with those of Höhnk & Bock (1954).

In studies on the distribution and abundance of watermoulds in a shallow lake of Japan, Suzuki (1961c) obtained variable results and reported a curious pattern of diurnal migration of planonts. The number of zoospores found in samples taken in the morning and evening of a clear day was approximately the same at the surface, bottom and middle region of the lake. He suggested that the zoospores occurring in these horizontal regions had migrated up from the bottom layers of water. At night, the highest concentration of watermould zoospores was in surface waters. Samples taken on cloudy days indicated that the motile spores (zoospores) were concentrated at the lake surface throughout the day. During prolonged periods of rain watermould propagules were evenly distributed in the lake from surface to the bottom. Suzuki (1961c) speculated that the oxygen level in water drove the diurnal vertical migration of the zoospores of watermoulds. He further stated that during the summer and winter there was no diurnal migration of watermould zoospores in the shallow lakes. Clausz (1970) studied a somewhat deeper lake than that explored by Suzuki, and reported a non-random distribution of watermoulds. Assuming the nutrient sources in the water to be adequate, Clausz (1970) attributed the observed vertical distribution to the influence of oxygen level.

The mineral content of water bodies has also been found to affect the watermould populations and to regulate their distribution significantly. Suzuki & Nimura (1961a) reported that out of a group of five lakes, three species of watermoulds – *Saprolegnia* sp. (unidentified), *Saprolegnia diclina* and *Aphanomyces* sp. (unidentified) – were found only in a lake having no detectable levels of iron, manganese, calcium, chloride or sulphate. Suzuki & Nimura (1962) analyzed the watermould populations and hydrochemical characteristics of a cluster of three lakes connected by a common watercourse that were rich in sulphate, chloride and calcium, but deficient in nitrate and phosphate. Each lake harboured the spores of water moulds but the species composition differed significantly among the three bodies of water.

Terrestrial habitats

Harvey (1925) was the first to study watermoulds occurring in the soil. Later, Raper (1928) tried to determine the abundance of watermoulds in a given surface area and volume of soil, and reported the number of times particular species were isolated from different sites. Cook & Morgan (1934), using Harvey's methods, collected a large number of species and concluded that the members of the family saprolegniaceae as a group were primarily terrestrial, and only a few members adapted to a completely aquatic environment. Wolf (1939), on the basis of his isolations, concluded that the species of *Brevilegnia* are presumed to be limited to soil habitats. Prabhuji (1984a, b, 2005) also support the contention of Wolf (1939) that *Brevilegnia* is a terrestrial genus. By contrast, Johnson *et al.* (2002) did not agree on the basis of their studies in Iceland.

Dick & Newby (1961) employed a quadrat technique for monthly sampling of permanently marked soil areas for the presence of members of the saprolegniaceae, and demonstrated their occurrence in a number of different types of soil. They found that there is a seasonal fluctuation in the frequency of these fungi, with maxima in spring and autumn; and that the fluctuations are independent of the relative frequencies of species isolated from any given quadrat, and of the identity of the species recorded. The samplings showed that the saprolegniaceous flora was relatively constant at each site and more than half of the species isolated (Dick 1962) were in an “aggregate pattern”, that is, they occurred repeatedly in the same part of the quadrat over two-and-half-years. More recently Prabhuji (1984a, b), employing the same sampling method as Dick (1962), found results similar to those of Dick & Newby (1961) and Dick (1962) in certain soils of Gorakhpur in India (Fig. 4).

Dick (1963) reported that wet soil was richer in saprolegniaceae than dry soil. Later, Dick (1966) isolated many species of *Saprolegnia* and *Achlya* from the sites in areas liable to inundation. Confirming his earlier contention and discussing the distribution of aquatic oomycetes, Dick (1968) has shown conclusively that oomycetes have a definite affinity with water and has indicated that the aquatic environment is required for propagule germination, vegetative growth, sporulation, and the dissemination of propagules. Prabhuji (1984a, b) has also isolated mostly saprolegniaceous species from soil samples with high moisture content, and has reported that dry soils yield mostly species belonging to the Pythiaceae and Blastocladiaceae. Apinis (1960 and 1964) also concluded that water-

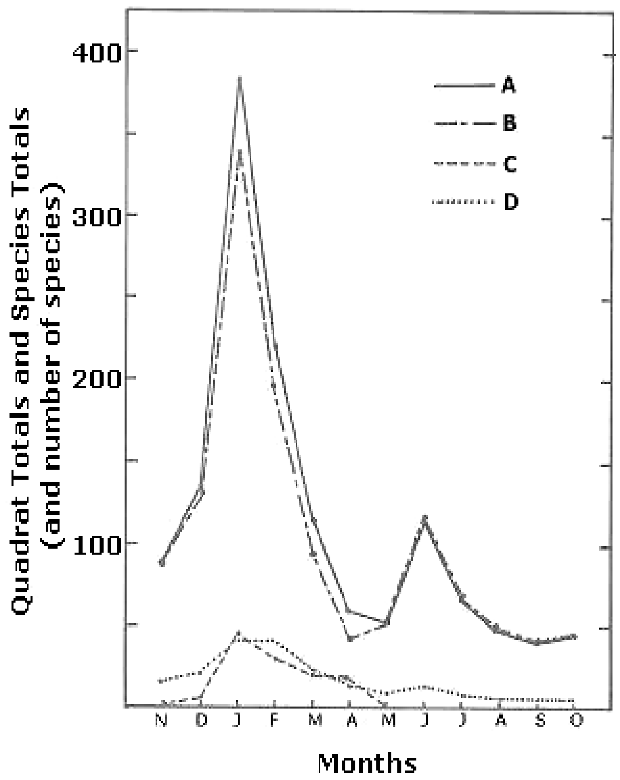

Fig. 4. Monthly periodicity of watermoulds in soils (after Prabhuji 1984a).

moulds were confined largely to marshes, swamps and water-logged soils. In an investigation of the water-moulds of cultivated and uncultivated soils in Denmark, Lund (1978) reported that wet soils were richer in these fungi than the dry habitats.

Dick & Newby (1961) suggested that there can be a relatively constant flora of watermoulds in any given soil site, as well as seasonal fluctuations in the frequency of appearance of individual species making up the composite population. Data of Dick (1962) and Prabhuji (1984a, b) show that even over distances of a few centimeters the composition of the watermould mycoflora in soil varies significantly. A correlation also appears to exist (Dick 1966) between distribution patterns of individual species and the topography of the sampling site. Some species have been found to be restricted to higher ground, others to low lying regions, and still others appeared to be distributed without any regard to slope (Dick 1966).

Another important pattern of distribution has been found to exist at the terrestrial – aquatic interfaces. O'Sullivan (1965) sampled in a temperate region along transects marking a gradient of sites from strictly exposed to dryness to continuously submerged. Following analysis of data, she proposed six narrow categories of species. These were:

(i) Aquatic: collected only from water.
(ii) Aquatic and damp terrestrial: isolated from water and closely adjacent soil.
(iii) Aquatic and general terrestrial: found in water and soil from the water's edge to the maximum transect distance upland.
(iv) Damp terrestrial: species found in the soil only, but near the water's edge.
(v) Terrestrial: occurring only in soil but not at the maximum distance from the water line.
(vi) Distant terrestrial: collected only from soil exclusively at distant upland points.

As O'Sullivan pointed out, her data suggest that Höhnk's (1935) groupings based on sporulation type and habitat oversimplified what is likely a very complex series of distribution patterns.

By selecting four different types of sites – seldom or never inundated, poorly drained, subject to flooding, and permanently waterlogged – Dick (1966) isolated and identified 32 species of saprolegniaceae. The wetter sites harbored the greatest diversity of species, but there was no pattern of "distribution of genera" among the soils, as proposed by Höhnk (1935) in his studies on water bodies. Dick (1971), on the basis of his studies, concluded that the saprolegniaceae as a group are best thought of as fungi characteristic of the emergent littoral zone (centered at the water line) and the lentic/littoral zone.

Seasonal periodicity, distribution and oöspore type

Ziegler (1958) was the first to suggest that a seasonal periodicity of saprolegniaceae could be recognized if the oöspore structure was taken into account, a hypothesis later examined in depth by Hughes (1959, 1962). Earlier, Coker (1923) had encountered, but did not recognize this pattern. Hughes (1962) concluded that the species with eccentric oöspores do not exhibit a seasonal periodicity, whereas those with centric or sub-centric oöspores clearly do. However, not all species of saprolegniaceae are expected to universally exhibit a relationship between oöspore type and month or season of occurrence, and there are several studies to support this contention (Dick & Newby 1961; Dick 1966, 1968; Khulbe & Bhargava 1977; Liu & Volz 1976; Lund 1978; Milanez 1967; Prabhuji 1979; Srivastava 1967 a, b).

Prabhuji (2005) has suggested that there is a relationship between the geographical distribution of saprolegniaceae and oöspore type. Prabhuji (1984a) had pointed to the dominance of the members of saprolegniaceae with eccentric oöspores in the tropics. Perusal of earlier records, particularly for the occurrence of eccentric and centric/sub-centric forms of saprolegniaceae, reveals a very interesting and characteristic pattern of global distribution of saprolegniaceae. For example, studies made in the Indian subcontinent (Prabhuji 1984 a; Srivastava 1967a, b) and Brazil (Milanez 1967, 1968 1970; Milanez & Beneke 1968), both within tropical regions of the globe, have shown a distinct dominance of eccentric forms over centric and sub-centric forms. By contrast, investigations conducted in United Kingdom (Dick & Newby 1961; Dick 1966), Canada (Dick 1971), Iceland (Howard *et al.* 1970), United States of America (Miller & Ristanovic 1969) and Denmark (Lund 1934, 1978),

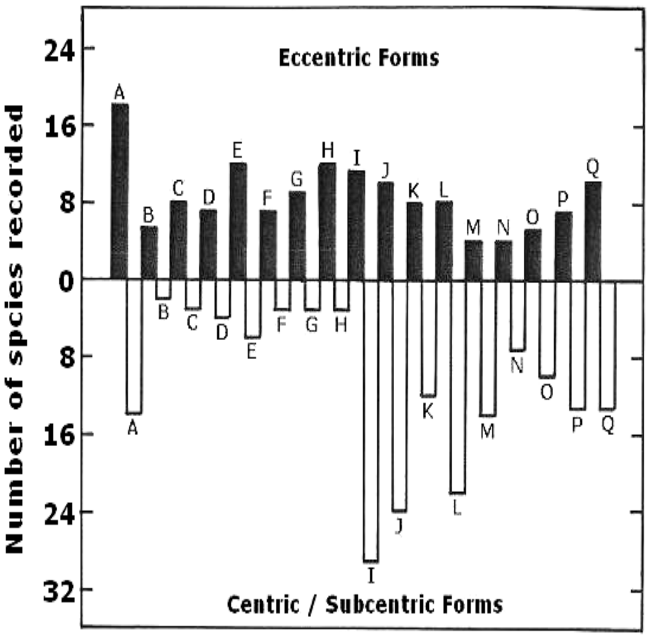

Fig. 5 A-Q. Occurrence of eccentric and centric/sub-centric forms of Saprolegniaceae in different geographical areas of the world by different aquatic mycologists.

A: Srivastava (1967a)
B: Srivastava (1967b)
C: Prabhuji (1984a)
D: Prabhuji(1984b)
E: Milanez (1967)
F: Milanez (1968)
G: Milanez (1970)
H: Milanez & Beneke (1968)
I: Dick & Newby (1961)
J: Dick (1966)
K: Dick (1971)
L: Howard *et al.* (1970)
M: Miller & Ristanovic (1969)
N: Lund (1934)
O: Lund (1978)
P: Khulbe & Bhargava (1977)
Q: Khulbe (1980)

which represent temperate regions of the world, indicate dominance of centric and sub-centric forms over the eccentric forms of saprolegniaceae (Fig. 5). Based on such studies, a distinct eccentric and centric (including sub-centric forms) "Occurrence Zones" of the world may be identified for the members of saprolegniaceae (Fig. 6). Further studies in other tropical and temperate regions may yield data that further confirm this hypothesis.

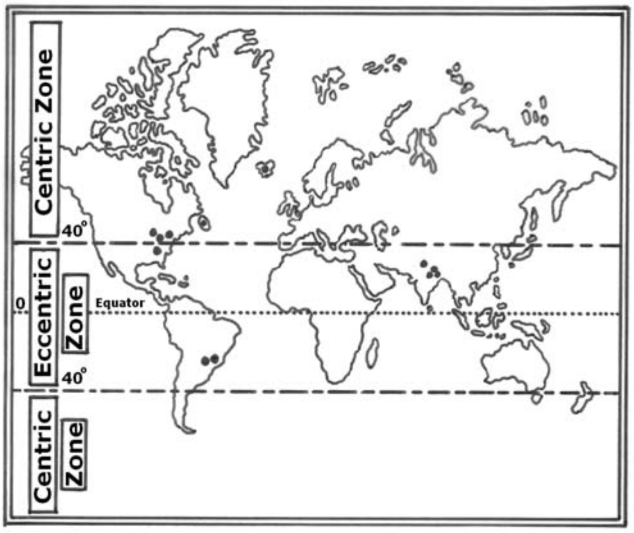

Fig. 6. Different "Occurrence Zones" for the members of saprolegniaceae of the world.

Studies conducted in the hills of Nainital in India (Khulbe & Bhargava 1977; Khulbe 1980; Mer *et al.* 1980) at 1300 - 1700 m above sea level, have shown the dominance of centric and sub-centric forms of saprolegniaceae over eccentric forms. By virtue of being in the "eccentric zone" of the world (Fig. 6), data for the Indian subcontinent are expected to exhibit dominance of eccentric forms, and therefore, such observations raise a question on the validity of the present contention. However, if we consider climatic changes in accordance with the change in altitude, we can expect that at an altitude of 1,000 - 3,000 m above sea level the conditions would be identical to the temperate region, irrespective of the geographic zone. Thus, the distribution for the "centric zone" should be observed at an altitude range of 1300 - 1700 m above sea level. This suggests another pattern, the "altitude-latitude relationship" in the distribution of saprolegniaceae in relation to oöspore type (Fig. 7). If the altitude-latitude relationship is correct for the distribution of higher plants or forests, the same should be valid for the

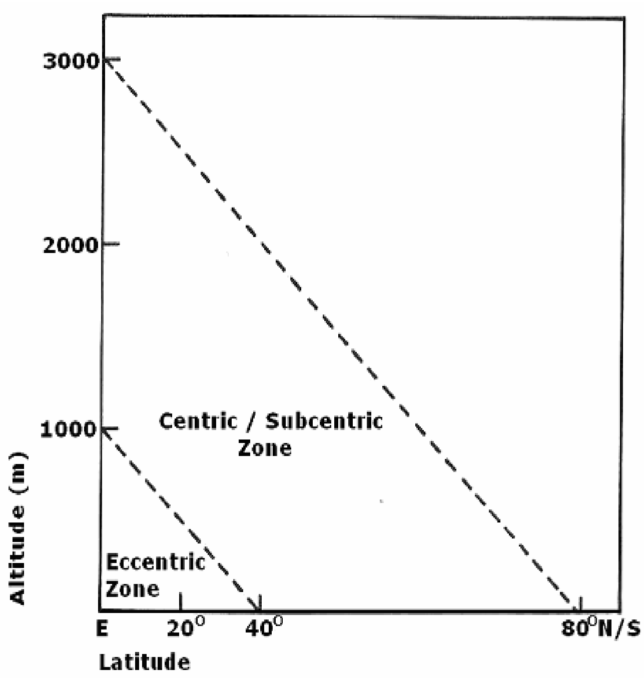

Fig. 7. The altitude-latitude relationship in the distribution of saprolegniaceae in relation to their oöspore type.

distribution of watermoulds in relation to their oöspore type.

Interdependence of aquatic and terrestrial habitats

The Oomycetes have a definite affinity with water. Dick (1968), discussing the distribution of biflagellate members (aquatic oomycetes), has shown that these fungi are affected by water in four ways: (i) through effects of water on germination of propagules; (ii) requirement of water for vegetative growth; (iii) influence of water on sporulation; and (iv) the role of water in propagule dissemination. It is accepted that zoospores (evanescent propagules) provide the major inoculum for the isolation of watermoulds either from water or from soil. Dick (1966) concluded that the resting propagules (oöspores, gemmae, etc.) are initially responsible for almost all isolations of watermoulds from soil; which on getting adequate moisture and optimal temperature germinate within 2 - 4 hours and develop into zoospores. However, it is very difficult to identify the type of propagules present during the isolation of watermoulds, particularly from soils. For example, Warcup (1950) isolated *Thraustotheca* sp. (a member of saprolegniaceae) from hyphal fragments present in the soil.

Fundamentally, aquatic habitats provide suitable conditions – with adequate moisture and optimal temperature – for watermoulds and, in turn, they produce evanescent propagules (zoospores) in luxuriance. In addition, because they are less likely to experience unfavourable conditions, watermoulds in aquatic habitats develop the least amount of resting propagules such as gemmae and oöspores. In contrast, the soil habitat, although it contains a good amount of organic matter, lacks adequate moisture and suitable temperature except during the rainy season, resulting in the development of a good amount of resting propagules (gemmae and oöspores) and fewer zoospores (Fig. 2A). During the rainy season, with adequate moisture and low temperature, the resting propagules in soils germinate and produce an abundance of zoospores, which are mobile within the water in soil pores; and are carried down slope towards a stagnant or flowing water source. When soils are flooded or inundated, a similar phenomenon operates in terrestrial and the littoral zones, although for a short duration (Fig. 2 B, C).

Isolation of watermoulds from water bodies and from soil presents two very different situations. Water bodies can be divided into two types – those that are large enough to have flowing water and those that are smaller and have stagnant condition. In the tropics optimal temperatures are reached during December - January (winter season) when the asexual sporulation is at its peak, resulting into abundance of zoospores exhibiting maximum number of isolations (types and frequency). Later, following a high temperature period in May - June (summer season), a slightly low temperature condition is experienced in July - August (monsoon season); however, the temperature is not optimal like December - January. Therefore, only a few members (only constant and moderate temperature species) sporulate and result in a few isolations. Furthermore, the high speed of water-flow in large water bodies during the rainy season (sometimes due to floods) and a high bacterial density in stagnant water bodies can also become limiting factors. Thus, only one significant "peak" (during December - January) develops.

In soil, the first situation (maximum isolations during December - January) is the same as in the water bodies, but during the monsoon season (during July - August) it is not. The soil contains a variety of oömycetes in the form of resting propagules – the oöspores. Dick (1968) has considered the oöspores (contained within oögonia) as a non-dispersed, semi-permanent source of evanescent propagules – the zoospores and called it "a zoospore bank" in soil. During rains the mature resting oöspores get the germination-stimulus of rains, and such temporary flushing would favour oöspore germination and zoospore production and its release. In the soil environment, oöspore dispersion over less than a 5 mm radius would be enough to provide required inoculum's density for the production of adequate amount of zoospores for watermould growth (Dick 1968). This causes a sudden surge of zoospore production from the resting propagules (oöspores) of a variety of oömycetes present within the soil and results in significant isolations from soil during July - August, forming a second "peak" of occurrence. However, this peak is less prominent than that of December - January, as shown in Fig. 2 D. Because they are thick-walled resting structures in soil, the oöspores, would not be affected by those limiting factors (particularly the presence of high bacterial density in soil) which normally affect the thin-walled motile zoospores and vegetative structures in water bodies.

Conclusions

Various water and soil sampling techniques, adopted by different scientists working in the field of water moulds, have been discussed critically to assess their merits and shortcomings. Many of the traditional water and soil sampling methods were found to yield ecologically viable results, although some are either unfeasible or fail to provide statistically significant data. The methods adopted by various aquatic mycologists for quantitative assessment of watermould propagules in water samples, were not found to provide reliable data. By contrast, quantitative methods applied to soil samples appear to yield reliable data. These findings point to the need for further work to develop new methods that yield reliable quantitative data from water as well as soil samples. The occurrence, distribution and periodicity of watermoulds in water bodies and soil was also been reviewed, along with altitudinal and global distribution patterns in relation to oöspore type. Further studies made in different geographical regions of the globe would definitely strengthen or modify these observations.

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