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The pH mediated occurrence of watermoulds in soil habitat affecting their distribution

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ABSTRACT

An attempt has been made to determine a distribution pattern of watermoulds in slightly acidic (pH 6.5 – 7.0), near neutral (pH 7.0 – 7.5), slightly alkaline (pH 7.5 – 8.0) and moderately alkaline (pH 8.0 – 8.5) soils in Gorakhpur (India). The watermoulds, belonging to Saprolegniaceae and Blastocladiaceae families, have shown a variable occurrence in the soils of varying pH from slightly acidic to moderately alkaline. *Pythiopsis cymosa* has been classified under slightly acidic soil type whereas *Allomyces arbuscula*, *Allomyces moniliformis* and *Allomyces javanicus*, all belonging to family Blastocladiaceae, have been found to be distributed within slightly acidic to neutral (pH 6.5 – 7.5) soils. *Saprolegnia diclina* and *Saprolegnia ferax* were restricted to neutral to slightly alkaline (7.0 – 8.0) soil pH whereas *Achlya orion*, *Achlya diffusa* and *Brevilegnia spp.* (unidentified species of *Brevilegnia*) have been classified as constant type. The present studies indicate *Aphanomyces laevis* to be characteristic of neutral to moderately alkaline (7.0 – 8.5 pH) soils and *Brevilegnia bispora* and *Brevilegnia indica* as slightly alkaline type (7.5 – 8.0 pH). *In vitro* variable pH experiments have shown that the watermoulds itself modify the pH of the medium and, then develop different stages of their life cycle or *vice versa*. Temperature has been found to modify the pH of pure water, pond water and soil-extract water and therefore, this distinctly identifies the importance of temperature in pH mediated distribution of watermoulds.

Introduction

Lund (1934) was most instrumental in Denmark in calling attention to the importance of hydrogen-ion concentration as a factor influencing the occurrence of watermoulds in aquatic conditions. During his study Lund concluded that *Aplanes* spp., *Saprolegnia diclina* and *S. littoralis* "preferred" acid habitats, while *Achlya radiosa* alone was limited to the alkaline waters. Those species that seemed not to be influenced by pH either in their occurrence or frequency included *Achlya racemosa*, *A. colorata* and *Saprolegnia ferax*. Later, the findings of Dudka (1965) with respect to the occurrence of *A. racemosa* and *S. ferax* agreed precisely with Lund's observations.

Roberts (1963) recorded that the watermoulds were distributed according to the hydrogen-ion concentration and had arranged them in three groups, viz., acid (pH 5.6 – 7.0), alkaline (7.0 – 8.8) and neutral (5.6 – 8.8). Srivastava (1967b) has also classified the isolated watermoulds on pH profile of the water. Several other aquatic mycologists have also recorded the significance of hydrogen-ion concentration on the occurrence and distribution of watermoulds in water bodies (Zaborowska, 1965; Ergaskev and Kirgizbaeva, 1978; Florinskaya, 1969; Milanez, 1966; Hasija and Batra, 1978; Suzuki and Nimura, 1960). 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. Bacteria are more common in waters of high pH than of low pH. These, therefore, bring about the decomposition of organic matter in alkaline conditions and the 'alkaline' species of watermoulds may live on the secondary products of decomposition. In acid conditions, watermoulds probably play a more important role in the decomposition of organic matter (Prabhuji, 2011). The existing distribution and occurrence data do not permit the general conclusions that a given species of Saprolegniaceae can be expected to inhabit waters of a particular pH level. *Achlya androgyna* is a notable exception, and *Saprolegnia turfosa*, common in Scandinavian waters, appears to be another. These species are "weedy" occupants of acid bogs (*Sphagnum* spp.) in Norway and Sweden (Johnson *et al.*, 2002). Maestres (1977) believed that the influence of pH in the Newfoundland river waters, she sampled, was on reproduction and not growth (Maestres and Nolan, 1978) of these fungi.

Two reasonably extensive accounts of the distribution and frequency of watermoulds in soil in relation to pH are those of Dick (1963) and Lund (1978). The greatest frequency of occurrence of all species that Dick collected was realized by culturing soils within a pH range of 4.3 – 4.8 whereas the greatest number of isolates of species that occurred in soil were found by Lund in Danish soils having a pH range of 4.4 – 6.5. However, Prabhuji (1979), using the quadrat method of isolation of watermoulds from the environs of four lakes and ponds of Gorakhpur (India), could not provide a definite pattern of distribution of watermoulds in relation to the hydrogen-ion concentration of the soil. While the extant data on the occurrence and distribution of watermoulds in relation to pH are scanty for water as well as the soil, two inferences may be derived: firstly, most species are not commonly found in highly acid waters; and secondly, acid soils are more likely to yield a diverse flora of saprolegniaceous fungi than are alkaline ones (Johnson *et al.*, 2002).

During the course of present investigations an attempt has been made to determine a distribution pattern of watermoulds in

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slightly acidic (pH 6.5 – 7.0), near neutral (pH 7.0 – 7.5), slightly alkaline (pH 7.5 – 8.0) and moderately alkaline (pH 8.0 – 8.5) soils in Gorakhpur (India).

Materials and Methods

Sampling areas

During the present investigations, an attempt has been made to determine a distribution pattern in watermoulds in the soils of four different areas (Figs. 1, 2), viz., Environs of Ramgarh Lake with slightly acidic (pH 6.5 – 7.0), Environs of Asuran Pond with near neutral (pH 7.0 – 7.5), Environs of Turra Nalā with slightly alkaline (pH 7.5 – 8.0) and the M.G. Post Graduate College campus with moderately alkaline (pH 8.0 – 8.5) soils in Gorakhpur city (India). A special care has been taken in ensuring that no sampling site in any area may get inundated during the monsoon season affecting the sampling process.

Sampling and method of recording isolates

Each pH range included three permanently fixed sites (A, B and C) from where sampling has been done throughout the study period (November, 2010 to October 2011) using the quadrat technique (Prabhuji, 1984a). The terms 'Species Totals' and 'Occurrence Totals' have been used to denote "the sum of records for the species for given site quadrat area" and "the sum of the species totals of the particular watermould in one soil pH range" respectively.

***In vitro* pH change study**

To ascertain whether the different stages in the life cycle of certain watermoulds get modified by the pH variation, or the watermoulds itself modify the pH of the medium and, then develop the different stages of their life cycle, *in vitro* variable pH experiments have also been conducted (Fig. 3). The pH value of pure water, according to Le Châtelier's Principle, changes with the temperature (Fig. 4). Keeping in view this situation, another *in vitro* experiment on the pH of pond water and soil-extract water has been noted at 50°C and, was recorded that with the decrease of temperature by 10°C, i.e., at 40, 30, 20, 10 and 5°C; the change in temperature modifies pH value without modifying the hydrogen-ion / hydroxyl-ion concentration (Fig. 5).

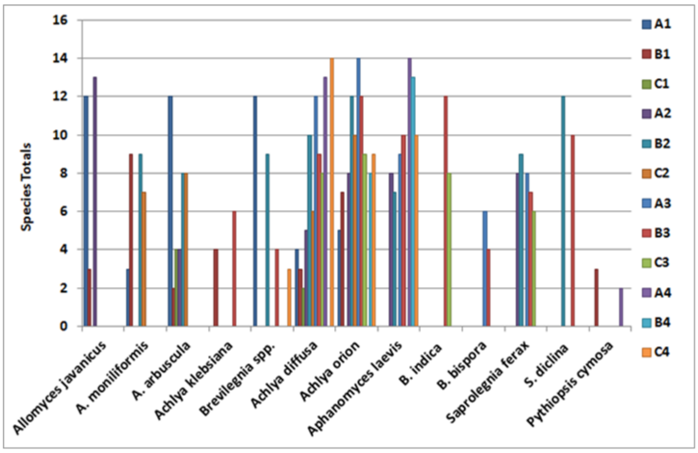

Fig. 1: Species totals of different watermoulds in permanently fixed sampling sites of four soil areas having variable pH range

Results and Discussion

Hydrogen-ion concentration and distribution

The watermoulds, belonging to Saprolegniaceae and Blastocladiaceae families, have shown a variable occurrence in the soils of varying pH from slightly acidic to moderately alkaline (Fig. 1). However, the data from each site (A, B and C in each pH range of soil) indicates a distinct distribution pattern of watermoulds within soils of different pH (Fig. 2). Based on these data these watermoulds may be classified into slightly acidic, slightly acidic to neutral, neutral to slightly alkaline, neutral to moderately alkaline, constant and slightly alkaline types (Table 1).

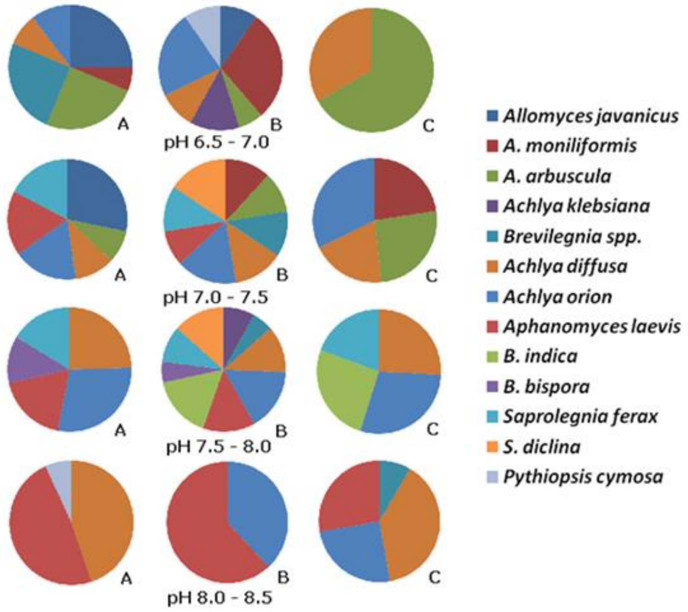

Fig. 2: Variable occurrence of watermoulds at each sampling site

Lund (1934) recorded *Pythiopsis humphreyana* from water samples with pH range of 3.2 – 5.4 and placed it in highly acidic type. During the present study *Pythiopsis cymosa* has been classified under slightly acidic soil type because its more species totals has been recorded in 6.5 – 7.0 pH range of soils, although it has also been recorded in moderately alkaline (pH 8.0 – 8.5) soil. However, Staniak (1971) has found no distributional patterns in watermoulds with respect to pH.

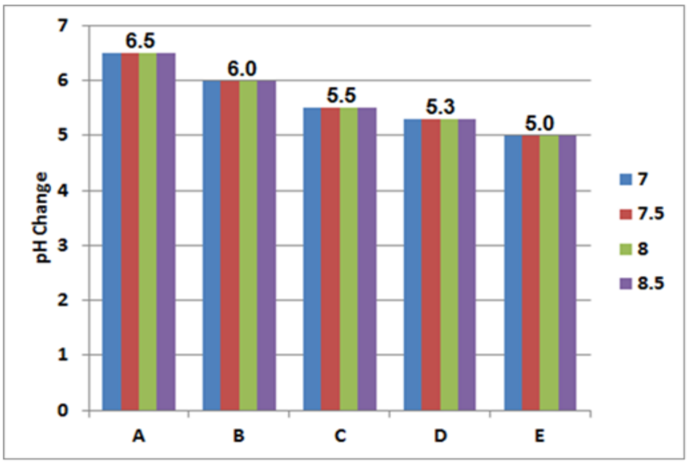

Fig. 3: Changes in pH with different stages of life cycle in *A. diffusa*, *A. orion*, *Allomyces arbuscula*, and *A. recurvus*.
A: Vegetative hyphal growth initiation.
B: Full grown hyphae and formation of zoosporangia.
C: Formation of antheridial branches.
D: Oögonial initiation.
E. Maturation of oögonia and attachment of antheridial branches.
(Chaturvedi, 2009; Singh, 2009; Prabhuji, 2010)

Allomyces arbuscula, *Allomyces moniliformis* and *Allomyces javanicus*, all belonging to family Blastocladiaceae, have been found to be distributed within slightly acidic to neutral (pH 6.5 – 7.5) soils during the present investigations. Prabhuji (1979) has also recorded the occurrence of *Allomyces arbuscula*, *Allomyces moniliformis* and *Allomyces javanicus* in the soil samples with 6.5 – 8.0 pH range almost in accordance with the present investigations.

Table 1: Occurrence of watermoulds in the soils of variable pH range

Name of the watermoulds with soil category	Occurrence Totals*			
	1	2	3	4
Slightly acidic				
Pythiopsis cymosa	03	-	-	02
Slightly acidic to neutral				
Allomyces arbuscula	18	19	-	-
Allomyces moniliformis	12	16	-	-
Allomyces javanicus	15	13	-	-
Neutral to slightly alkaline				
Saprolegnia diclina	-	12	10	-
Saprolegnia ferax	-	16	22	-
Neutral to moderately alkaline				
Aphanomyces laevis	-	17	17	37
Constant type				
Achlya orion	12	30	35	17
Achlya diffusa	09	21	29	27
Brevilegnia spp.	12	09	04	03
Slightly alkaline				
Achlya klebsiana	04	-	06	-
Brevilegnia bispora	-	-	10	-
Brevilegnia indica	-	-	20	-

*Occurrence Totals are the sum of Species Totals of sites in each pH range.

pH ranges **1**: 6.5 – 7.0; **2**: 7.0 – 7.5; **3**: 7.5 – 8.0; and **4**: 8.0 – 8.5

The distribution patterns of *Saprolegnia diclina* (at pH 3.2 – 5.4 by Lund, 1934; at 8.0 – 8.5 by Stpiczynska, 1962; at neutral pH by Roberts, 1963; at 8.0 by Stpiczynska-Tober, 1965; at 5.5 – 6.5 by Zeborowska, 1965 and at 6.5 – 7.0 by Srivastava, 1967b) and *Saprolegnia ferax* (at pH 3.2 – 9.0 by Lund, 1934; at 6.0 – 9.0 by Stpiczynska, 1962; at alkaline pH by Roberts, 1963; at 7.0 – 8.0 by Stpiczynska-Tober, 1965 and at 6.5 – 8.8 by Srivastava, 1967b) have been recorded within the water bodies having variable pH range. The present investigation indicates that these watermoulds were restricted to neutral to slightly alkaline (7.0 – 8.0) soil pH which is in accordance with Stpiczynska-Tober (1965) and Srivastava (1967b). *Achlya orion*, *Achlya diffusa* and *Brevilegnia spp.* (unidentified species of *Brevilegnia*) have been classified as constant type during the present study, occurring in all types of soil reaction (pH 6.5 – 8.5) having maximum occurrence totals within 7.5 – 8.0 pH range except *Brevilegnia spp.* (pH 6.5 – 7.0) whereas *Achlya klebsiana* has been a slightly alkaline (pH 7.5 – 8.0) type. In water bodies Lund (1934) recorded *Achlya klebsiana* from highly acidic (pH 3.2 – 5.4) condition and Srivastava (1967b) found *Achlya diffusa* and *Achlya orion* from 6.5 – 7.5 pH range; and *Achlya klebsiana* characteristic of 6.4 – 8.8 pH range. During his studies on Danish soils, Lund (1978) found the isolated species of *Achlya* and *Saprolegnia* characteristic of 4.4 – 6.5 pH range. These observations by Lund (1978) seem to agree in general with the data of Dick (1963).

Lund (1934) found *Aphanomyces laevis* in water samples with 7.0 – 9.0 (constant alkaline) pH range and Perrott (1960) recorded it in neutral acidic (6.4 – 7.0) pH; whereas Srivastava (1967b) found this watermould in neutral alkaline (6.4 – 8.8) pH of water samples. However, Stpiczynska, (1962), Roberts (1963) and Stpiczynska-Tober (1965) have also recorded its significant occurrence from water samples having pH range of 7.0, 8.0 – 8.5 and 7.0 – 8.0 respectively. The present studies indicate *Aphanomyces laevis* characteristic of neutral to moderately alkaline (7.0 – 8.5 pH) soils which is in agreement with Lund (1934), Stpiczynska, (1962), Roberts (1963), Stpiczynska-Tober (1965) and Srivastava (1967b). Milanez (1966) pointed out that *Saprolegnia ferax* and *Aphanomyces laevis* are not restricted to any particular pH range, however, earlier data (Johnson *et al*., 2002) suggested that these two species are distributed generally in low acid-alkaline waters.

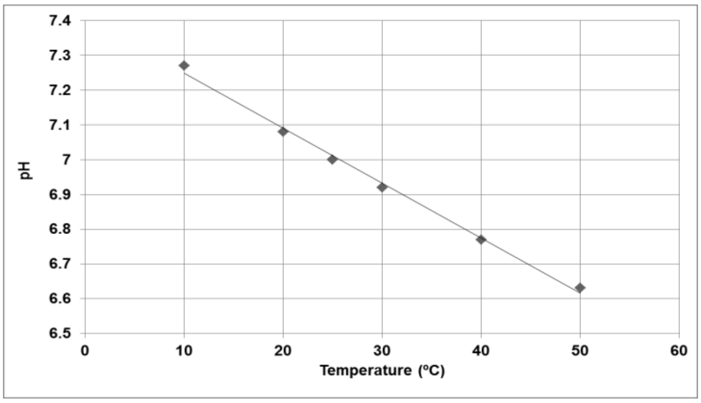

Fig. 4: Change in pH of pure distilled water with the change in temperature

Brevilegnia bispora and *Brevilegnia indica* have been classified as slightly alkaline type as they were isolated from soil samples with pH range of 7.5 – 8.0. Prabhuji (1979) has recorded the occurrence of *Brevilegnia subclavata* in soils of 7.5 – 8.0 pH range and *Brevilegnia indica* has originally been isolated from alkaline soil (Prabhuji and Sinha, 1993). Khulbe (1980) has recorded a species of *Brevilegnia*, viz., *Brevilegnia linearis* from water samples with 7.3 – 8.8 pH range.

Hydrogen-ion concentration and the life cycle pattern

Very little work has been done by the aquatic mycologists to assess the possible relationships between hydrogen-ion-concentration (pH) and the sexual reproduction in water moulds. The information available indicates that slightly acidic medium has a favourable effect on the formation of sexual apparatus in the members of Saprolegniaceae and also has a positive influence on their (sex cells) maturation (Ziegler, 1950; Ziegler and Linthicum, 1950). I would like to indicate towards the erroneous observation of Lounsbury (1930), in which he indicated that not all species of Saprolegniaceae respond so expressively to this factor. In *Protoachlya paradoxa*, he reported that neither increasing nor decreasing the acidity of the growth medium could induce mycelium to develop sex cells.

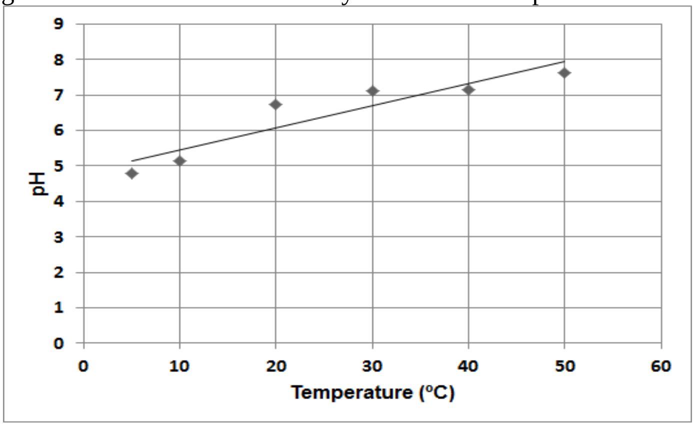

Fig. 5: Change in pH of pond / soil-extract water with the change in temperature

During our study on the possible relationship between sexual reproduction in water moulds and the hydrogen-ion-concentration of the substrate (Chaturvedi, 2009; Singh, 2009; Prabhuji, 2010) certain surprising and peculiar results have been recorded. The cultures (*Achlya orion*, *A. diffusa*, and some members of family: Blastocladiaceae, viz., *Allomyces arbuscula* and *A. recurvus*), initially placed at any range of pH (6.5 to 8.5), modified the pH of the medium to 6.5 while the vegetative hyphae started growing. With the full-grown hyphae and formation of zoosporangia, pH is changed to 6.0. The pH value changed to 5.5 when the cultures started developing antheridial branches and with the oögonial initiation this value is changed to 5.3. However, with the maturation of oögonia and attachment of antheridial branches the pH has finally changed to 5.0 and stabilized (Fig. 3). This gives a clear indication that the cultures, by themselves, modified the pH of the medium and simultaneously, corresponding with it, the sexual reproductive structures developed or *vice versa*. The possibility remains, which is to be studied, regarding secretion of certain biochemicals by the water moulds during the process of its development that, in turn, modifies the pH of the substrate.

Johnson *et al*. (2002) were of the opinion that such discordant distributional data may signify wide tolerance to pH levels on the part of these fungi. The earlier reports (Chaturvedi, 2009; Singh, 2009; Prabhuji, 2010) have indicated that the watermoulds themselves modify the pH of the substrate with the change in different stages in their life cycle. There may be a strong possibility that the watermould species modify the pH of their surrounding and the same species is isolated from the soils of different pH range depending upon the stage of its life cycle (types of propagules) from which the isolation is made.

According to Le Châtelier's Principle, if a change is made to the conditions of a reaction in dynamic equilibrium, the position of equilibrium moves to counter the change made. Hence, if the temperature of pure water is increased, the equilibrium will move to lower the temperature again. It will do that by absorbing the extra heat which means that the forward reaction will be favoured, and more hydrogen ions and hydroxide ions will be formed. The effect of that is to increase the value of Kw as temperature increases and pH value of pure distilled water decreases giving an inverse correlation (Fig. 4). However, in natural pond water and soil-extract water the temperature variations have also been found to modify the pH and it was recorded to be directly proportional to the temperature change (Fig. 5). Such changes in soil pH in accordance with seasonal temperature change have also been recorded while sampling at the sites throughout the year (November, 2010 to October 2011) during the present investigations. This distinctly identifies the importance of temperature in pH mediated distribution of watermoulds as has been described for global distribution of these fungi in tropical and temperate zones on the basis of their oöspore types (Prabhuji, 2011).

Conclusions

The watermoulds, belonging to families Saprolegniaceae and Blastocladiaceae, have shown a variable occurrence in the soils of varying pH from slightly acidic to moderately alkaline. In vitro variable pH experiments have shown that the watermoulds itself modify the pH of the medium and, then develop different stages of their life cycle or *vice versa*. Temperature has been found to modify the pH of pure water, pond water and soil-extract water and therefore, the importance of temperature in pH mediated distribution of watermoulds is significant as has been described earlier for global distribution of these fungi in tropical and temperate zones on the basis of their oöspore types.

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