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PHYSIO-CHEMICAL STUDIES OF THE SOIL FROM SAND DUNES OF PUSHKAR AND ESTIMATION OF TEMPERATURE TOLERANT MICRO-FLORAL ISOLATES

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Short Profile

Manisha Chotrani is working as an Assistant Professor at Department of Botany in Mithibai College, Bhaktivedanta Marg, Vile Parle (W)Mumbai.

ABSTRACT:
Pushkar, a renowned tourist place situated in the heart of the state of Rajasthan, 11 km from the holy city of Ajmer, falls in the arid zone of Thar Desert. The present study focusing on temperature variations on soil micro-flora was conducted at five different sand dune sites for a period of one year, taking into record the ecological and the soil physio-chemical parameters. Soil was treated in vitro up to temperatures higher than what the micro-community in this region is generally exposed to, under natural circumstances for isolation of thermophiles. Soil algae and fungi were isolated using Modified Bristol's Sodium Nitrate solution and PDA with 0.5 % chloramphenicol respectively. The reduction in the number of fungal isolates at gradually increasing temperature was rapidly achieved than algal isolates. The number of algal isolates came down from 25 to 1 while the number of fungal isolates came down from 20 to nil at gradually increasing temperature initiating from 45 to 70oC.

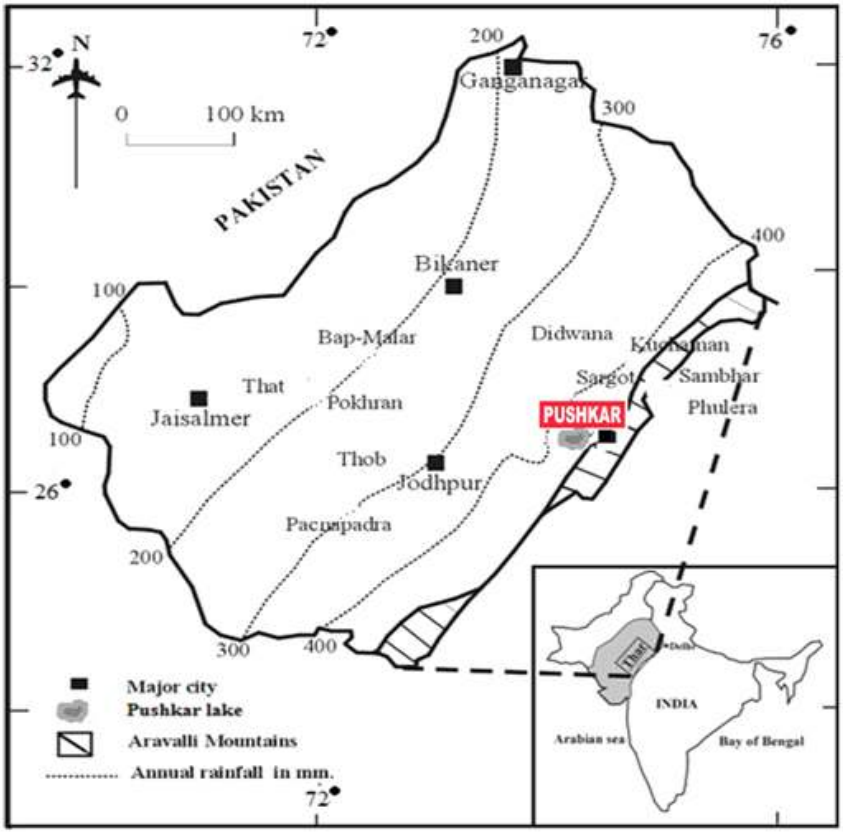
KEYWORDS
sand dunes, thermophiles, micro-flora.

INTRODUCTION

Pushkar (Latitude (DMS): 26° 30' N and Longitude (DMS): 74°32'E is located in the state of Rajasthan and is near to holy city- Ajmer (Figure 1 shows the arid and semi arid zones of India).

From Ajmer, Pushkar is just 11 kilometers away. It's spread over an area of 16.34 sq. km and is 486 m above sea level. A small part of the Ajmer district (9%) falls in the arid zone. Pushkar region of Ajmer comes under this 9% part. The rest 91% comes under semi-arid zone. Ajmer actually lies at the transition zone of arid and semi-arid regions of the Thar. Hence, Pushkar becomes a site of ecological curiosity.

FIGURE - 1
MAP OF THAR DESERT, INDIA. SHOWING PUSHKAR
(Site of study Highlighted in red)



Temperature is one the most important environmental factors, and because of its ease of measurement a vast body of knowledge exists concerning its effects on living organisms. The majority of organisms are adapted to live in a moderate range of temperature. High temperature environments are relatively uncommon. The mean maximum temperatures at the study site during summers range from 45-50oC. Efforts have been made to study the growth response of micro flora at temperatures higher than what the micro community in this region is generally exposed to under natural circumstances. From the viewpoint of applied ecology, an understanding of the tolerance limit of the soil micro flora is essential. Species diversity is lower at high temperatures than at low temperatures, and at very high temperatures only single species may be found. The species found at high temperatures are not necessarily struggling to survive, but may actually be optimally adapted to such high temperatures. The approximate upper limit of temperature for algae is between 55-60oC, for fungi it is 60-62oC , for blue

green algae it is 70-75oC and for bacteria it ranges from 70 to >90oC. The significance of such data in terms of ecosystem function is clear: as the temperature increases the population structure becomes simpler. In any given soil there is a diversity of microorganisms with a diversity of temperature limitations. Each microbial group functions over a range of temperature in which its members grow, and an optimum temperature range in which they grow best. The range of soil temperature across which microorganisms can grow forms a continuum.

Hence, the present work is demonstrating the study of temperature stress on soil micro flora inhabiting sand dunes of Pushkar.

Although the words 'High Temperature' will often be used in this paper without qualification, the viewpoint of the observer or the group of soil micro organisms under discussion will often determine if a given temperature is to be considered high. Thus, a temperature of 45oC will be considered critically high while referring to some forms while for others it can be the minimum temperature regime for initiating growth. The sole aim of this part of work would comprise of treating the soil samples at various temperature regimes and observing the biota at different temperatures.

METHODOLOGY:

For soil sampling, a sterile disposable plastic spatula (surface sterilized with 70% ethanol) was used to scoop surface samples of the soil, from five sites of investigations, which are as follows:

1) Neela Sewari
2) Ganahera
3) Foothills of Savitri Pahad
4) Pushkar Fair ground
5) Motisar

ECOCLIMATIC STUDIES: the mean monthly minimum and maximum temperature (oC), Relative humidity (%), monthly rainfall (mm) was obtained for every month for the span of one year.

SOIL PHYSICAL PARAMETERS: Physical parameters of the soil such as colour, texture (standard Sieving Method), Soil Moisture (Blakemore et al. 1987), water-holding capacity (Pandey et al. 1968 and Misra et al. 1970), pore space (Narayan and Shah, 1966) were determined.

SOIL CHEMICAL PARAMETERS: Chemical parameters of the soil such as soil pH (Jackson 1973), soil temperature, soil organic matter (Blakemore et al. 1987) electrical conductivity (Systronic Conductivity Meter Model 302), soil Calcium, sodium by Digital Flame Spectrophotometer (Harron, Webster and Cairns, 1983), soil nitrogen by Kjeldhal Method by Jackson (1973), soil phosphorous by Sodium Bi-carbonate Method (Olsen et.al, 1954 and soil potassium by Digital Flame Spectrophotometer (Metson, 1956) were determined.

ALGAL STUDIES: For studying the effect of high temperature on soil algae, the soil samples were incubated to different temperature regimes under controlled conditions. Each soil sample was crumbled to allow the heat to penetrate evenly and thoroughly.Samples for the heat treatment were put on flat heat resistant trays, which were placed in a drying oven and were heated to the temperature of 45oC, 50oC, 55oC, 60oC, 65oC and 70oC. Culturing soil algae by Moist Soil Method (John, 1942; Willson and

Forest, 1957 and Forest 1959) followed this. Modified Bristol's Sodium Nitrate Solution (Bristol, 1920 and Allen, 1949) was used for algal culturing.

Identification of the algae was done using Bruhl and Biswas,(1926), Smith (1950), Korshikov (1953), Desikachari (1959), Fritsch (1961), Tiffany and Briton (1962), Prescott (1962, 1970), Chapman (1962), Brown and Bold (1964), Kantz and Bold(1964), Smith and Bold (1966), Philipose (1967), Grover and Bold (1969), Archibold and Bold(1970), Lee and Bold (1974), Patel and Isabella (1977) and Cramer (1983).

FUNGAL STUDIES:For studying the effect of high temperature on soil fungi, the soil from all five sand dunes was homogenized well. 10 gm of each of the soil was transferred into 100 ml of sterile water. The contents of the flask were vigorously mixed using shaker.

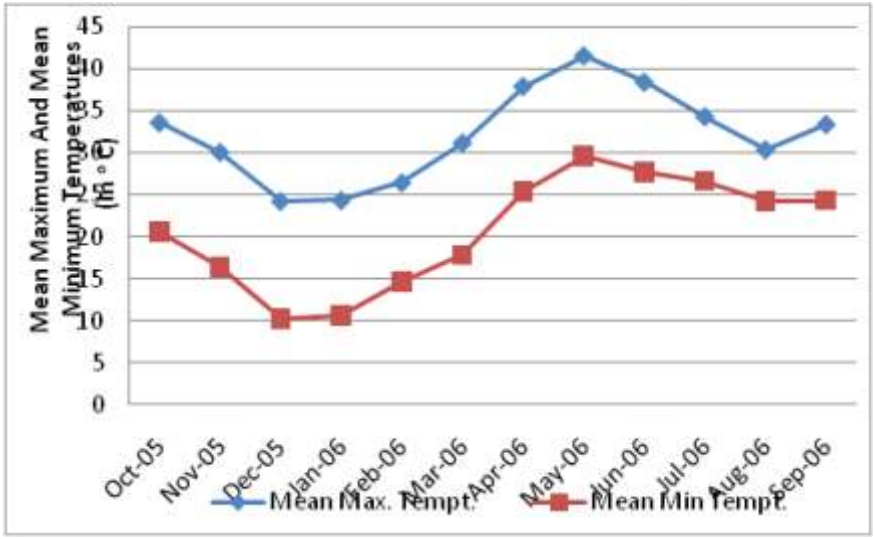
10 ml from each of the five flasks was transferred into another 6 flasks, which were labeledwith different temperature regimes (45°C, 50°C, 55°C, 60°C, 65°C and 70°C). The flasks were heated in a water bath.This process was repeated for soil from all 5 sites.The flasks were allowed to cool to 40 oC. Then, 1 ml is pipetted out from each of the heat treated flasks, serially diluted (10 fold) and transferred aseptically to 2 replicas each containing peptone dextrose agar medium plus rose Bengal and streptomycin or aureomycin (Martin, 1950 and Johnson, 1957)and incubation was at ambient temperature for seven days.

Petri dishes were covered in plastic bags to avoid moisture loss and incubated in upright position at 25oC in dark. After 24 hours of incubation up to the period when no new fungal colonies appeared, the representative members of fungal colonies were identified.

Standard procedures based on colony, spore and structural morphology were followed for identification of fungal isolates at the generic level using Jensen (1912), Clements and Shear(1931), Bessey (1951), Gilman (1966), Barnet (1967) and Domsch et al. (1980), Subramanian (1982,1986) and Bilgrami et al. (1991).

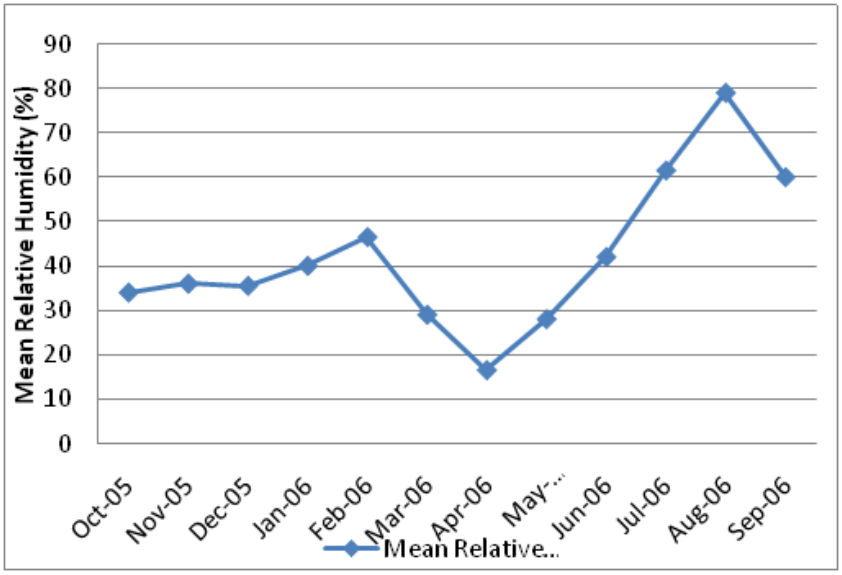
RESULTS AND DISCUSSIONS:

GRAPH – 1
MEAN MAXIMUM AND MEAN MINIMUM TEMPERATURES
(IN OC) FROM OCTOBER 2005 TO SEPTEMBER 2006



GRAPH – 2

MEAN RELATIVE HUMIDITY (%) OF PUSHKAR, DURING THE PERIOD OF STUDY (OCT. 2005 TO SEPT. 2006)



GRAPH – 3

MEAN ANNUAL RAINFALL (mm) IN PUSHKAR DURING THE STUDY PERIOD (OCTOBER 2005 TO SEPTEMBER 2006)

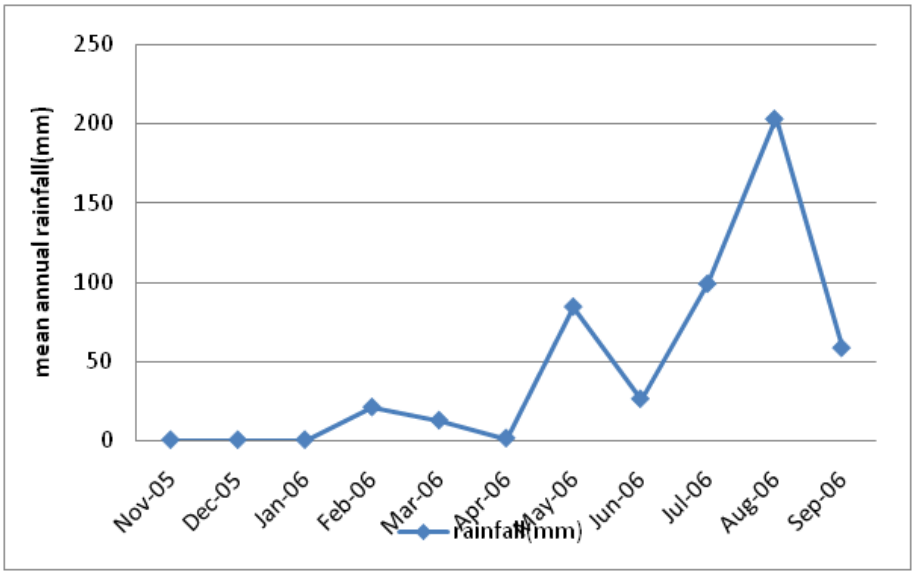


TABLE 1:
PHYSICAL PARAMETERS (COLOUR, TEXTURE, PORE SPACE) OF THE SOIL FROM FIVE STUDY SITES*

Physical Parameters			Study Sites				
			1	2	3	4	5
1	Colour		Dark brown	Yellowish brown	Light brown	Light brown	Dark brown
2	Texture	% Coarse sand	14.50	19.29	15.05	12.20	15.50
		% Fine sand	72.00	62.25	70.15	47.75	68.25
		% Clay + silt	10.75	15.24	10.12	40.25	16.40
3	Pore Space (%)		48	44	42	48	43

* The five sites of study 1)Neela sevri, 2)Ganahera, 3) Foothills of savitri pahad, 4) Annual Fair Ground, 5)Motisar.

TABLE 2:
AVERAGE WATER HOLDING CAPACITY (%) OF THE SOIL SAMPLES FROM FIVE SITES* OF STUDY

Months	Water Holding Capacity (W.H.C) in %				
	Site 1	Site 2	Site 3	Site 4	Site 5
October 2005	24.7	26	25	24	23
November 2005	25	21	23	22.5	25
December 2005	22.5	24.5	23.4	25.5	22
January 2006	26.4	24	24.6	25	26
February 2006	24	23	23.2	23	24
March 2006	22	21	23.5	20	21.5
April 2006	21.5	20	19.5	19	21.5
May 2006	18	17	18.4	16	17
June 2006	17	19.5	18	15.5	16.5
July 2006	27	24	29	24	25
August 2006	28	26	28	27	27
September 2006	28	24	27	25	28

* The five sites of study 1) Neela sevri, 2)Ganahera, 3) Foothills of savitri pahad, 4) Annual Fair Ground, 5)Motisar.

TABLE 3:
AVERAGE SOIL MOSITURE (%) OF THE SOIL SAMPLES FROM FIVE SITES* OF STUDY

Months	Soil moisture (%)				
	Site 1	Site 2	Site 3	Site 4	Site 5
October 2005	3.02	3.00	3.15	2.48	3.12
November 2005	3.19	2.86	3.40	3.00	2.98
December 2005	1.52	1.80	1.72	1.49	2.15
January 2006	1.31	1.85	1.45	1.27	1.78
February 2006	2.10	2.46	2.12	1.90	2.15
March 2006	2.23	2.30	2.29	2.56	2.48
April 2006	1.40	1.39	1.41	1.50	1.62
May 2006	1.25	1.15	1.30	1.32	1.20
June 2006	1.25	1.38	1.45	1.50	1.45
July 2006	10.3	8.56	10.12	9.8	9.30
August 2006	10.5	11.15	10.45	11.00	9.86
September 2006	12.25	12.50	12.29	11.57	12.48

*The five sites of study 1)Neela sevri, 2)Ganahera, 3) Foothills of savitri pahad, 4) Annual Fair Ground, 5)Motisar

GRAPH - 4
SOIL PH FROM SOILS OF 5 SITES AT 2 DIFFERENT DEPTHS

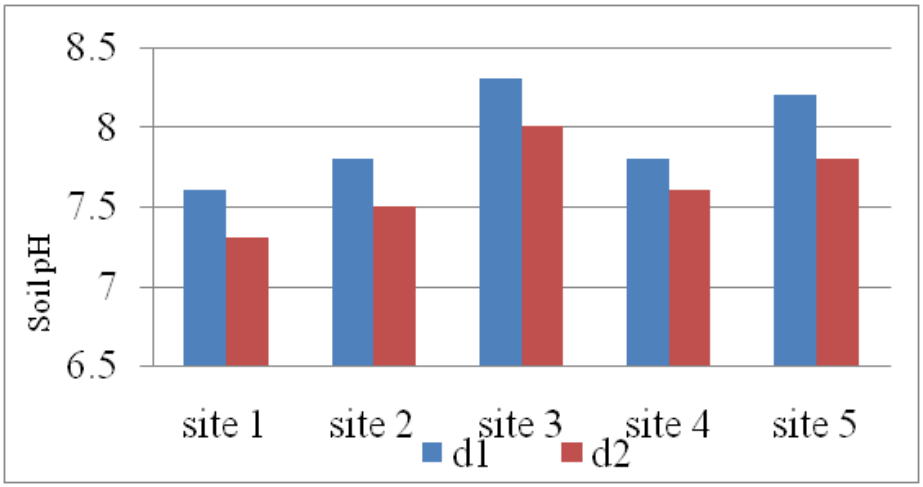


TABLE 4:
SOIL TEMPERATURES (OC) OF SAMPLES FROM FIVE STUDY SITES* (AT 2 DEPTHS d1 AND d2) FOR THE STUDY PERIOD (OCTOBER 2005-SEPTEMBER 2006)

MONTH	DEPTHS	Soil Temperatures (^{O}C)				
		site 1	site 2	site 3	site 4	site 5
October 05	d1	36	35	34	36	34
	d2	30	28	28	29	30
November 05	d1	32	31	35	30	32
	d2	29	27	28	29	27
December 05	d1	25	24	25	22	23
	d2	23	22	23	20	22
January 06	d1	25	26	24	27	25
	d2	22	23	21	25	21
February 06	d1	26	25	25	24	23
	d2	23	22	23	21	20
March 06	d1	29	30	28	29	27
	d2	25	26	24	25	23
April 06	d1	35	35	34	33	34
	d2	28	27	28	29	30
May 06	d1	43	42	43	44	42
	d2	34	35	37	33	33
June 06	d1	44	45	46	45	44
	d2	36	37	39	41	40
July 06	d1	35	36	33	36	37
	d2	34	35	36	35	36
August 06	d1	35	34	33	35	34
	d2	32	31	30	32	32
September 06	d1	34	33	34	32	33
	d2	32	31	29	32	30

d1= 20-25 cm ; d2=60-65 cm
*The five sites of study 1)Neela sevri, 2)Ganahera, 3) Foothills of savitri pahad, 4) Annual Fair Ground, 5)Motisar

TABLE 5:
CHEMICAL PROPERTIES OF THE SOIL FROM FIVE STUDY SITES*

Chemical parameters		site 1	site2	site 3	site 4	site 5
1	Soil Organic Matter (%)	1.4	1.7	1.5	0.98	1.5
2	Electrical conductivity (mmhos/cm)	0.62	0.68	0.70	0.65	0.80
3	Calcium (meq/l)	63	62	60	45	64
4	Sodium (meq/l)	54	53	64	40	62
5	Potassium as K_2O (Kg/ha)	220	245	270	190	310
6	Available Phosphorous as P_2O_5 (Kg/ha)	20	21	33	17	23
7	Available Nitrogen(Kg/ha)	345.25	456.56	560.0	245.0	578,50

* The five sites of study 1)Neela sevri, 2)Ganahera, 3) Foothills of savitri pahad, 4) Annual Pushkar Fair Ground, 5) Motisar

TABLE – 6:
EFFECT OF HIGH TEMPERATURE ON THE NUMBER OF ALGAL AND FUNGAL ISOLATES IN THE SOIL SAMPLE

Temperature regime	Number of algal isolates	Number of fungal isolates
45 °C	25	20
50 °C	15	11
55 °C	10	9
60 °C	7	4
65 °C	2	1
70 °C	1	_

TABLE 7:
Algal isolates at gradually increasing high temperature

Tempt.	Number of Algal Isolates	Genera identified
45 °C	25	*Synechococcus , Dactylococcopsis, Navicula, Nitzschia, Tetradendron, Spinoclosterium,Spirulina,Phacus,Gloeocapsa Haplosiphon,Pleodorina, Scenedesmus, Glaucocystis,Synechocystis, Calothrix, Pleurochloris, Tertaspora, Navicula, Cylindrospermum, Aphanocapsa, Nostoc, Oscillatoria, Anabaena,Plectonema, Microcystis*
50 °C	15	*Synechococcus,Phacus,Gloeocapsa, Haplosiphon,Pleodorina, Scenedesmus, Glaucocystis,Synechocystis, Cylindrospermum, Navicula, Dactylococcopsis,Nitzschia, Tetradendron, Spinoclosterium,Dactylococcopsis*
55 °C	10	*Nitzschia, Tetradendron, Spinoclosterium,Dactylococcopsis, Nostoc, Oscillatoria, Anabaena,Plectonema, Calothrix, Pleurochloris*
60 °C	7	*Cylindrospermum, Aphanocapsa, Nostoc, Synechococcus , Dactylococcopsis, Navicula, Anabaena*
65 °C	2	*Cylindrospermum, Aphanocapsa*
70 °C	1	*Cylindrospermum*

TABLE 8:
Fungal isolates at gradually increasing high temperature

Tempt.	Number of Fungal Isolates	**Genera identified**
45 °C	20	*Rhizopus, Aspergillus, Penicillium, Candida, Paecilomyces, Chaetomium, Talaromyces, Thermomyces, Eurotium,Neosartorya, Achyla, Alternaria, Fusarium, Arthrinium, Trichoderma, Circinella, Pythium, Mucor, Blackeslea, Torula*
50 °C	11	*Penicillium, Candida, Paecilomyces, Chaetomium, Talaromyces, Thermomyces, Eurotium,Neosartorya, Curvularia*
55 °C	9	*Penicillium, Paecilomyces, Chaetomium,Neosartorya, Eurotium,Circenella, Curvularia, Blakeslea, Pestalotiopsis*
60 °C	4	*Talaromyces, Thermomyces, Eurotium,Neosartorya*
65 °C	1	*Pestalotiopsis*
70 °C	0	None

The colour of the soils from five sites shows variations ranking from dark brown, yellow brown to light brown. Soils from foothills of Savitri Pahad and from that of Pushkar Annual fairground have light brown coloured soil, while soils from Neela Sevri and Motisor were dark brown. Soil from Ganahera showed yellow brown colour. The textural studies on soils from five sites shows that the average % of coarse silt in all soil is 15.30%, the % of fine sand is 64.08% and the % of silt and clay comes out to be 18.55%. Thus overall the soil is sandy loam. The average pore space % for the soils from five sites comes out to be 45%.The average water holding capacity ranges from 16 to 24 %, being lowest for soil from Annual Pushkar fair ground. The soil moisture percentage ranges from a minimum of 1.27 (Pushkar annual fairground) to a maximum of 12.50 at Ganahera. The soil moisture % fluctuates seasonally, being high during monsoon season and hitting minimum during summer months (April, May and June)

Soil pH studies have shown that the soil is alkaline. And with depth the soil pH is seen to fall down a bit. The pH of soil from Motisor and foothills of Savitri pahad are more alkaline than that of Ganahera, Neela sevri and Pushkar annual fair ground. Highest soil temperatures were recorded during the months May and June and minimum during December and January. The soil temperature reduced with depth. Lowest soil organic matter (%) was recorded from Pushkar annual fair ground and highest from Ganahera. Total soluble salts were detected to be more in soil from Motisar and least in soil from Neela sevri. Soil sodium and calcium estimation showed that soil from Motisar is rich in Sodium as well as Calcium. Soil phosphorous was detected to be lowest for soils from annual Pushkar annual fair ground, while nitrogen in soil was highest for soil from Motisar. The number of algal isolates obtained were in the order: Cyanophyta>Chlorophyta>Bacillariophyta>Euglenophyta=Pyrophyta. The number of fungal isolates were in the order:Fungi Imperfecti> Phycomycetes> Ascomycetes.The heat treatment had a significant effect on species number. The mean number of algal and fungal isolates came down at temperatures higher than 45oC. The number of fungi declined more rapidly at higher temperatures than algae.

The present investigation lies in line with the findings of Sharma et al. (2009), with respect to the predominance of Chlorophycean group over Bacillariophyta and Euglenophyta isolates. The monthly variation of microbial communities finds its results in line with the conclusions of the studies done by Freeman et al. (2009) on the 'barren' desert soil which state that presence of photoautotrophs and microbes varies seasonally as well as annually and the microbial photosynthesis provides a significant input of carbon to the soil. It is a matter of fact that the duration and direction of sunlight varies during the year affecting photosynthesis of the microbial Photoautotrophs in the soil and hence their population and diversity. The present study falls in line with the findings of Barcenas et al. (2009), who drew a relationship between high temperature heat treatment and decline in the number of algal and fungal flora. In their studies the decline rate of algae and fungi at high temperature was more than that of bacteria. The present investigation on heat tolerance of soil micro flora is in conformity of these findings. The present study reports dominance of Cyanophycean alga over other groups of alga viz. Cholorophyceae, Bacillariophyceae, Euglenophyceae and Pyrophyceae. The present study reports a total of 37 algal isolates out of which 19 belong to Cyanophyta. This is in accordance with the findings of Langhans, Storm & Schwabe (2009). Similar conclusions were drawn by Budel et al. (2009) from their study on South African soil which is classified as highly diverse drylands, sharing the environmental features of the five study sites at Pushkar, in the present work in terms of dryness and restricted rainfall. The present study done on extreme environments revealing microbial diversity in algal groups corresponds to the unexpected phylogenetic diversity of microbial autotrophs done in the arid soils of Antarctica by Shmidt et al (2010).

CONCLUSIONS:

It can be concluded from the present investigation on the micro- floral diversity in the arid soils from sand dunes of Pushkar region, that the soils in this region are not deficient in micro floral diversity. It is the scarcity of water together with the arid eco-climatic factors prevalent in the region, which delimit the growth of micro flora in the dune soil. It will be reasonable to say, that from the foregoing discussion, the picture emerges of soil from the sand dunes in Pushkar region, as a rich reservoir of different algae, fungiand with gradually increasing temperature treatment the number of algal and fungal isolates tend to decrease. This decline is significant in case of fungal isolates.

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