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Survival of diatoms *Synedra, Gomphonema* and *Fragilaria* species in nature and in presence of different chemical and physical stress factors

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Abstract

The diatoms *Synedra*, *Gomphonema* (both epiphytic on *Pithophora oedogonia* filaments cells) and *Fragilaria* species (planktonic) occurring in the same pond exhibited similar population density change as the pond's diurnal water temperature (PDWT) change, monthwise, throughout the year. They all had the highest natural population densities in July, August (rainy season, when PDWT range between 25.0- 27.6 ^{0}C) followed by in March, April (spring, when PDWT range between 18.5- 23.8 ^{0}C); and the lowest in June (summer, when PDWT was 24.6- 28.8 ^{0}C) followed by in January (winter, when PDWT was 12.1- 14.6 ^{0}C), indicating that they were very temperature sensitive. All 3 diatoms survived short, differently, even at the lowest level of different chemical or physical stress factors used such as 1- 200 ppm of mercury, copper, cobalt, nickel or zinc; 5- 2000 ppm of gammaxine, captan, DDT, 2, 4-D, urea or thiourea; 1- 100 % of petrol, diesel, kerosene, benzene, toluene, phenol or brassica oil; 5- 100 % of sewage water; 2- 6 % agarized solid pond water, 0.2- 1 mol/L NaCl- containing pond water, 5- 60 min blot dryness; or 0.96- 11.52 kJm^{-2} of UV light. They all tolerated darkness, low light intensities (of 2 and 10 µmol m^{-2} s^{-1}) and detergent (of even 10,000 ppm level) for considerable time periods, but died rapid in absence of all nutrients (in double distilled water).

Key words: Diatoms, chemical factors, physical factors, survival

Introduction

Very little is known on the survival of diatoms with respect to seasonal changes in nature and under stress conditions in culture. On intertidal mudflats, seasonal variations in the composition, density and diversity of epipelic diatom assemblages occurred in response to temperature change, interspecific competition, irradiance level and

exposure to desiccation (Admiraal *et al.* 1984; Aktan and Aykulu 2005). In Tisza River, different epiphytic diatoms *viz. Achnanthidium, Amphora, Cocconeis, Diatoma* and *Nitzschia* dominated differently in different seasons (Szabo *et al.* 2005). A marked seasonal variability of epilithic diatoms, ranging a low level in winter to high level in spring, was observed by Totti *et al.* (2007). The herbicide atrazine inhibited photosynthesis in *Cyclotella meneghiniana* (Millie and Hersh 1987) and herbicides isoproturon and s-metolachlor lowered chlorophyll c concentration and live cell density of *Eolimna minima* and *Navicula reichardtiana* (Debenest *et al.* 2009). In *Navicula grimmei* and *Nitzschia palea,* cells gliding period, gliding cell percentage and speed fell under water stress, pH extremes, temperature extremes, UV exposure, and darkness, and in presence of heavy metals or organic substances (Gupta and Agrawal 2007a). Elevated concentrations of copper, zinc or cadmium caused abnormal outline morphology and ornamentation deformities in several diatoms (Tapia 2008).

The present study reports (i) the natural population density of 3 diatoms *viz. Synedra, Gomphonema* and *Fragilaria* species (occurring in the same pond) monthwise with respect to pond's diurnal water temperature change throughout an experimental year, and (ii) the survival of all 3 diatoms in culture with respect to different chemical or physical stress factors such as heavy metals (mercury, copper, cobalt, nickel, zinc), organic substances (gammaxine, captan, DDT, 2, 4-D), urea, thiourea, hydrocarbons and petrochemicals (petrol, diesel, kerosene, benzene, toluene, phenol), brassica oil, sewage

water, detergent, physical and physiological water stress, UV light, darkness and low light, and absence of all nutrients.

Material and Methods

Diatoms *Synedra* and *Gomphonema* (both epiphytic on green alga *Pithophora oedogonia* filaments cells and *Fragilaria* species (as filament, planktonic, occurred throughout year in the same cemented pond in our Department, University campus, Allahabad.

Monthly pond's diurnal water temperature and natural population density of diatoms: The diurnal water temperature of the pond was measured weekly and the extreme value of the weekly readings of a month was given for each month of an experimental year from June 2009 to May 2010 (Table 1). In natural population of diatoms, quantification of population density was difficult and therefore approximate evaluation (signs -, +, +-, ++, ++- and so on) had to be used to express their increasing number. The population density of each diatom was observed weekly and the mean value of the weekly readings of a whole month was given for each month of an experimental year (Table 1).

***Survival of diatoms in control cultures or in presence of different chemical or physical factors*:** Filaments of *P. oedogonia* having attached with epiphytic *Synedra and Gomphonema* cells along with *Fragilaria* species filaments in centrifuged pond water were inoculated in 10 mL of sterilized pond water (having pH 7.8 prior to autoclave) and kept in culture chamber at the temperature of 25 ± 1 ^{0}C and white light intensity of 40 µmol m^{-2} s^{-1} under a 16/ 8

h light- dark cycle. All 3 diatoms were observed in culture till they survived (the survival period, Table 2). Dead diatoms cells appeared hyaline, having cytoplasmic shrinkage or ruptured wall.

***Heavy metals*:** Cu, Zn, Hg, Ni, CO added as copper sulfate (99 %; *Merck*, India), zinc sulfate (99 %), mercuric dichloride (98 %; both *SD Fine Comp.*, India), nickel sulfate (99 %; *Merck*, India), or cobalt dinitrate (97 %; *BDH*, India) were dissolved and individually mixed with pond's water (pH adjusted prior to autoclaving to 7.8) to a final concentration of 1- 200 ppm. Inoculated culture tubes were maintained in the culture chamber.

***Organic substances, urea, thiourea*:** Gammaxine (5 % malathion; *Pentapharma*, India), captan (50 %; *Rallis*, India), DDT (10 %; Governmental supply, India), 2, 4-D (86 %; *Tropical Agro*, India), urea (nitrogen 46 %; *Vijaypur Fertilizer Comp.*, India) or thiourea (99 %; *Merck*, India) were added to the pond's water (pH adjusted prior to autoclaving to 7.8) to a final concentrations of 5- 2,000 ppm. All inoculated culture tubes were placed in the culture chamber.

***Hydrocarbons, petrochemicals, brassica oil*:** Petrol, diesel, or kerosene (100 %; Governmental supply, India), benzene (99.7 %; *Merck*, India), toluene (99 %, *Ranbaxy*, India), phenol (99.5 %; *Sisco Research Lab*, India), or brassica oil (100 % pure mustard oil, *Adani Wilmar Ltd*, India) were added separately to sterilized pond water to prepare a 1 to 50 % suspension or solution, or were used as such (100 %) without any dilution. The pH was adjusted to 7.8.

***Sewage water*:** It was collected from an open sewage tunnel at Allahabad city. Its physico- chemical properties were

assessed in *Water Analysis Laboratory, Indian Farmers Fertilizer Cooperative* (Allahabad) and were reported to be: pH 7.98, total hardness 298 ppm, turbidity 172 nephelometric turbidity units, conductivity 1465 mW/ cm, and (in ppm) chloride 136, sulfate 136, nitrate 22.68, free ammonia 11, iron 10.2, total inorganic phosphate 5, sulfide 3.5, zinc 1.69, dissolved oxygen 1.2, copper 0.72, chromium 0.386, nickel 0.168, lead 0.126, manganese 0.112, BOD 50.7.

***Detergent*:** Surf Excel powder (*Hindustan Lever*, India) was added into sterilized pond water so as to prepare 1-10,000 ppm solutions (pH adjusted to 7.8).

***Physical and physiological water stress*:** Diatoms were exposed to water stress either by spreading them on solid pond's water containing 2, 4, or 6 % agar or inoculating them in liquid pond water with 0.2- 1 mol/ L NaCl. Another part of diatoms was completely blotted-dried for 5- 60 min and then inoculated into sterilized pond water and placed in the culture chamber.

***UV light*:** Diatoms placed in 10 mL sterile pond water, spread in open Petri dishes (diameter 90 mm) were exposed to UV light from a *Philips* germicidal lamp (main output at 254 nm and a fluence rate of 3.2 W/ m^2). The energy fluence of UV light which was obtained by increasing the time of exposure from 5 to 60 min ranged from 0.96 to 11.52 kJ/ m^2. After irradiation, the materials were centrifuged, transferred to fresh pond water and kept in darkness for 1 d to avoid photoreactivation and then maintained in the culture chamber.

***Darkness, low light intensity*:** Diatoms were separately exposed to white light intensity of 2 and 10 μmol m^{-2} s^{-1} by adjusting the distance of inoculated culture tubes from the light

source. The light intensity was measured using a lux meter (*Lutron Electronics,* USA) and converted into μmol m^{-2} s^{-1}. Some of the inoculated culture tubes were wrapped in black paper and kept in the dark. The survival period was determined as usual.

Results and Discussion

***Monthly pond's diurnal water temperature and natural population density of diatoms*:** *Synedra, Gomphonema* and *Fragilaria* species survived in the pond water throughout the year and tolerated a wide range of pond's diurnal water temperature (PDWT) change occurring in the year. They all exhibited a similar natural population density change with the monthwise change in PDWT (Table 1). All 3 diatoms bloom in July, August (rainy season) when PDWT range between 25.0- 27.6 ^{0}C, and this was only after a drop of 1.2 ^{0}C in pond's day water temperature of 28.8 ^{0}C in June (summer) when population densities of all 3 diatoms were at their lowest levels (Table 1) indicating that how much sensitive was the diatoms cell division to a slight change in temperature. Similar temperature change also bring about a tremendous increase in survivability of green alga *P. oedogonia* (to which *Synedra* and *Gomphonema* cells were attached) and *Cladophora glomerata* occurring in the same pond (Gupta & Agrawal 2007b). The peak diatom epiphyte (*Cocconeis placentula*) density co-occurred with peak *Cladophora* biomass to which the diatom was epiphyte (Malkin *et al.* 2009). The population densities of all 3 present diatoms declined with the fall in PDWT after August and reached to very low levels in January (winter, when PDWT

was 12.1- 14.6 ^{0}C), next to the lowest levels occurring in June (summer, when PDWT was 24.6- 28.8 ^{0}C, Table 1). With an increase in PDWT after January, the population densities of all 3 diatoms increased and reached to about ¾ levels of their maximum levels (occurring in rainy season) in March, April (spring, when PDWT range between 18.5- 23.8 ^{0}C). The population densities of all 3 diatoms in March, April (spring) were close to those in October (autumn, having PDWT of 18.3- 23.2 ^{0}C, almost identical to that of spring, Table 1). With the further increase in PDWT to 22.4- 27.9 ^{0}C in May (the onset of summer), the population densities of all three diatoms declined and reached to their lowest levels in June (summer). Thus all 3 diatoms population densities were at their maximum levels in rainy season followed by in spring; and at their lowest levels in summer followed by in winter. In a dam reservoir, overall phytoplankton density (including that of diatoms) was low during the fall and winter months and started to become abundant in the spring (Akbay *et al.*1999).

Table 1. Influence of monthly variations in pond's diurnal water temperature on the natural population density of *Synedra, Gomphonema* and *Fragilaria* species [a]

Month	Diurnal water temperature [b]	Natural population density of 3 diatoms [c]
June 2009	24.6- 28.8	-
July	25.3- 27.6	+ + + + +

August	25.0- 27.0	+ + + + +
September	22.6- 24.9	+ + + +
October	18.3- 23.2	+ + + -
November	17.4- 21.6	+ + +
December	15.4- 19.0	+ +
January	12.1- 14.6	+
February	13.6- 17.0	+ -
March	18.5- 20.8	+ + + -
April	20.1- 23.8	+ + + -
May 2010	22.4- 27.9	+ + -

[a] All 3 diatoms have the same population density change month wise throughout the experimental year.
[b] Extreme value of weekly readings of a month.
[c] Mean value of weekly observations of a month for all months of an experimental year. In natural population, quantification was difficult and therefore approximate evaluation (signs -, +, + -, + +, + + -, + + +, + + + - and so on) had to be used to express their increasing number.

Although, in the present study, the maximum day water temperature in May (27.9 ^{0}C) was close to that in July (27.6 ^{0}C), the population densities of all 3 diatoms in May were about half of that in July (Table 1) and this may probably be due to low night temperature in May (22.4 ^{0}C) than in July (25.3 ^{0}C), incidence of grazing of diatoms by herbivores (Admiraal *et al.* 1984), attack of diatoms by fungal parasites (Reynolds 1973) or by some other factors not known.

***Heavy metals and survival of diatoms*:** *Synedra, Gomphonema* and *Fragilaria* species survived in control

cultures for 97, 98, and 100 d, respectively. The survival period of all 3 diatoms fell sharp, differently, at 1 ppm (the lowest level) of different heavy metals used (*viz.* mercury, copper, cobalt, nickel, and zinc) and slowly thereafter as their concentrations increased to 200 ppm (the maximum level), indicating that all 3 diatoms were very sensitive to heavy metals even at low levels (Table 2). Metals are bound to various intracellular ligands that may control metal toxicity. The diatom *Thalassiosira nordenskioeldii* accumulated more cellular cadmium at low cadmium concentrations but the accumulation slowed down when the cadmium concentration further increased implying that cellular binding saturation had been reached (Wang and Wang 2008). The toxicity of different metals in the present study was in the order of Hg > CO ≈ Cu > Zn > Ni. *Synedra* was found to be more sensitive to all metals than equally less sensitive *Gomphonema* and *Fragilaria* species (evident particularly at lower levels of different metals, since at high levels all metals proved to be very toxic and killed all diatoms rapid, Table 2). Elimination of diatoms from metals treated streams depends upon the sensitivity of diatoms to metals; some were more sensitive than others (Medley and Clements 1998; Guasch *et al.* 2002). *Synedra ulna* was found to be more sensitive to copper than *Melosira varians* and *Diatoma vulgare* (Barranguet *et al*. 2005).

Table 2. The survival period (h, d) of *Synedra (Syn.), Gomphonema (Gom.)* and *Fragilaria (Fra.)* species under different chemical or physical stress conditions [a]

Treatment	Diatom	Chemical or physical stress conditions						
		Metals in pond water, ppm						
		1	5	10	25	50	100	200
Mercury	*Syn.*	9 d	8 d	8 d	7 d	5 d	4 d	3 d
	Gom.	25 d	21 d	17 d	13 d	8 d	5 d	3 d
	Fra.	27 d	21 d	19 d	14 d	9 d	5 d	4 d
Copper	*Syn.*	9 d	8 d	8 d	7.5 d	7 d	6 d	5 d
	Gom..	22 d	21 d	19 d	13 d	11.5 d	10 d	6 d
	Fra.	25 d	21 d	19 d	13 d	13 d	11 d	7.5 d
Cobalt	*Syn.*	9 d	9 d	8 d	8 d	7 d	6 d	6 d
	Gom.	30 d	28 d	26 d	16 d	9 d	7 d	7 d
	Fra.	30 d	29 d	27 d	16 d	9 d	7.5 d	7 d
Nickel	*Syn.*	12 d	11d	10 d	10 d	9 d	8 d	7 d
	Gom.	48 d	45 d	40 d	33 d	31 d	18 d	11 d
	Fra.	48 d	46 d	42 d	35 d	31 d	18 d	10 d

Zinc	*Syn.*	12 d	11 d	10 d	9 d	9 d	8 d	7 d
	Gom.	25 d	22 d	16 d	16 d	14 d	10 d	10 d
	Fra.	25 d	20 d	16 d	16 d	14 d	12 d	12 d

Organic substances, urea, thiourea in pond water, ppm

		5	25	50	100	250	500	1000	2000
Gammaxine	*Syn.*	6 d	5 d	3.5 d	3 d	60 h	42 h	36 h	24 h
	Gom.	10 d	6 d	4.5 d	4 d	64 h	48 h	50 h	27 h
	Fra.	11 d	6 d	4.5 d	4 d	64 h	48 h	36 h	27 h
Captan	*Syn.*	9 d	8 d	8 d	8 d	8 d	7 d	6 d	5 d
	Gom.	31 d	27 d	23 d	17 d	12 d	11.5 d	10 d	7.5 d
	Fra.	30 d	26 d	21 d	17 d	12.5 d	11 d	10 d	7 d
DDT	*Syn.*	8 d	8 d	7 d	7 d	7 d	7 d	6 d	6 d
	Gom.	40 d	37 d	33 d	26 d	23 d	13 d	8 d	7 d
	Fra.	41 d	37 d	34 d	27 d	24 d	13 d	8.5 d	6 d

2, 4- D	*Syn.*	15 d	12 d	10 d	8.5 d	8 d	7 d	6.5 d	6 d
	Gom.	48 d	46 d	40 d	35 d	30 d	20 d	10 d	8 d
	Fra.	49 d	47 d	40 d	36 d	32 d	21 d	11 d	9 d
Urea	*Syn.*	16 d	12 d	11 d	11 d	10 d	9 d	8 d	8 d
	Gom.	50 d	45 d	43 d	35 d	33 d	25 d	15 d	10 d
	Fra.	50 d	46 d	43 d	36 d	33 d	26 d	15.5 d	11 d
Thiourea	*Syn.*	5 d	4 d	3 d	2 d	45 h	40 h	36 h	24 h
	Gom.	10 d	5 d	5 d	4 d	52 h	48 h	40 h	30 h
	Fra.	11 d	6 d	5 d	4 d	53 h	50 h	40 h	30 h

Hydrocarbons, petrochemicals, brassica oil in pond water, %

		1	10	25	50	100
Petrol	*Syn.*	10 d	4 d	3 d	3 d	1 d
	Gym.	21 d	16 d	11 d	6 d	3 d
	Fra.	22 d	16 d	11 d	7 d	5 d
Diesel	*Syn.*	9 d	3 d	2 d	1 d	0.5 d

	Gom.	15 d	12 d	7 d	5 d	3 d
	Fra.	16 d	12 d	8 d	6 d	2 d
Kerosene	*Syn.*	5 d	4 d	3 d	2.5 d	12 h
	Gom.	6 d	4 d	4 d	2.5 d	8 h
	Fra.	6 d	5 d	4 d	2.5 d	12 h
Benzene	*Syn.*	4 d	3 d	48 h	30 h	24 h
	Gom.	4.5 d	3.5 d	52 h	48 h	24 h
	Fra.	4.5 d	3.5 d	52 h	48 h	24 h
Toluene	*Syn.*	3 d	48 h	35 h	24 h	8 h
	Gom.	3.5 d	50 h	35 h	24 h	8 h
	Fra.	3.5 d	50 h	37 h	24 h	8 h
Phenol	*Syn.*	5 d	4 d	60 h	28 h	24 h
	Gom.	5.5 d	5 d	72 h	28 h	24 h
	Fra.	6 d	5.5 d	72 h	28 h	24 h
Brassica	*Syn.*	7.5 d	5 d	62 h	48 h	24 h
oil	*Gom.*	8 d	6 d	72 h	50 h	24 h
	Fra.	8.5 d	6 d	72 h	50 h	26 h

Sewage water, %, in pond water

		5	25	50	100
	Syn.	20 d	14.5 d	7 d	6 d
	Gom.	21 d	16 d	8 d	5 d
	Fra.	22 d	16.5 d	8 d	5 d

Detergent in pond water, ppm

		1	100	1,000	10,000
Surf Excel	*Syn.*	50 d	40 d	38 d	25 d
	Gom.	48 d	35 d	30 d	22 d
	Fra.	48 d	35 d	32 d	24 d

Physical, physiological water stress

Agarized solid pond water, %

	2	4	6
Syn.	27.5 d	21.5 d	20 d

Gom.	28 d	21 d	18 d
Fra.	28 d	21.5 d	19 d

Salinized liquid pond water, NaCl mol/ L

	0.2	0.4	0.6	0.8	1.0
Syn.	8 d	5 d	5 d	4 d	2.5 d
Gom.	10.5 d	8 d	5 d	5 d	3 d
Fra.	11 d	8 d	5 d	5 d	3 d

Blot dryness, min

	5	10	20	30	60
Syn.	20 d	15 d	13 d	10 d	6 d
Gom.	18 d	16 d	12 d	7 d	5 d
Fra.	17 d	16 d	12 d	6 d	4 d

UV light, kJm^{-2}

0.96	1.92	2.88	3.84	5.76	11.52

Syn.	9 d	9 d	5 d	5 d	4 d	20 h
Gom.	11 d	8.5 d	7 d	5 d	4.5 d	14 h
Fra.	11 d	8.5 d	7 d	5 d	4.5 d	14 h

	Visible light intensity, μmol m^{-2} s^{-1}		
	0	2	10
Syn.	64 d	70 d	82 d
Gom.	60 d	72 d	82 d
Fra.	62 d	70 d	84 d

[a] The survival periods of control *Synedra, Gomphonema* and *Fragilaria* (with no stress condition) were 97, 98 and 100 d, respectively.

***Organic substances, urea, and thiourea and survival of diatoms*:** The survival period of all 3 diatoms decreased sharp at 5 ppm (the lowest level) of any of organic substances used (gammaxine, captan, DDT, 2, 4-D), urea, or thiourea, and slowly thereafter as the concentration of these substances increased to 2,000 ppm (the highest level used, Table 2). Thus, the present diatoms were very sensitive to these substances even at their lowest level used. Likewise, chronic atrazine exposure significantly reduced the growth rate of *Craticula cuspidata* only during the first day of treatment, and no significant effect was

detected throughout the remainder of the 67 d period (Nelson *et al.* 1999). Out of these substances, gammaxine and thiourea proved to be the most toxic to the present diatoms, while 2, 4-D and urea, the least (Table 2). Chlorophenyl dimethylurea at 2 ppm prevented diatoms from developing (Maloney 1958). Oliviera and Antia (1986) found urea to be one of the best sources of nitrogen for diatoms, but in the present study, any of all concentrations of urea used decreased the survival period of all 3 diatoms (Table 2).

***Hydrocarbon, petrochemical, brassica oil and survival of diatoms*:** Petrol, diesel, kerosene, benzene, toluene, phenol, and brassica oil, all, at 1 % level decreased the survival period of all 3 diatoms sharp, but that of *Synedra* more than of *Gomphonema* and *Fragilaria* species equally less, indicating their levels of survivability (Table 2). High concentrations of all of these substances were very toxic to all 3 diatoms and killed them rapid. Among them toluene was the most toxic while petrol the least (Table 2). Phenol was found to be algicidal (Palmer 1956) and toluene acts as a barrier between the air and the surface of the alga and acts as accelerator of toxin release from *Microcystis* cells to their environment (Chantara *et al.* 2004). Petroleum products produced a significant reduction of algal growth (Vandermeulen and Ahorn 1976).

***Sewage water and survival of diatoms*:** Even 5 % of sewage water decreased the survival period of all 3 diatoms sharp (to 20- 22 d than of control diatoms surviving for 97- 100 d, Table 2). In 100 % sewage water, all 3 diatoms died by 5, 6 d. They all seem to be more or less equally sensitive to sewage water (Table 2). Diatom

distribution and decline in the diatom community depends on the degree of organic pollution of water by sewage drains (Dakshini and Soni 1982; Newall and Walsh 2005). However, some diatom species flourished in a microecosystem collected from deposits near a municipal sewage outfall (Admiraal 1977; Watanabe 2006). Sewage water decreased the survival of many blue- green and green algae (Agrawal & Gupta 2009).

***Detergent and survival of diatoms*:** Detergents represent one of the newer sources of phosphorus for waters that receive treated or untreated sewage of cities and towns. In the present study, at 1 ppm of detergent, all 3 diatoms survived by 48, 50 d (than of control diatoms surviving for 97- 100 d) and at 20,000 ppm by 22- 25 d (Table 2), thus present diatoms can tolerate wide range of detergent concentrations for considerable time periods. All 3 diatoms seem to be more or less similarly sensitive to detergent (Table 2). Maloney (1966) observed an increased growth of *Chlorella* sp. by sodium triphosphate, an ingredient of synthetic detergent. However, detergent stopped diatom movement (Drum and Hopkins 1966).

***Physical and physiological water stress and survival of diatoms*:** Water stress of any type, even at the initial level (*e.g.* 2 % agarized solid pond water, 0.2 mol/ L NaCl- containing liquid pond water, or 5 min blot dryness of diatoms) decreased the survival period of all 3 diatoms (differently, more by salinized medium than by blot dryness or agarized medium, Table 2). All 3 diatoms seem to be more or less equally sensitive to any type, extent of water stress (Table 2). Density of diatoms was found to be lower on desiccated cobbles (Benenati *et al.*

1998). *Navicula pygmaea* survived partial desiccation more completely than *N. salinarum* (Admiraal *et al.* 1984). Approximate molar equivalent of diatoms has been found to be 0.1- 0.19 of NaCl (Übeleis 1957). In plasmolyzed diatom cells, movement continued until protoplast withdrew from the raphe system (Drum and Hopkins 1966). In saline streams, Round (1964) found immobile forms of diatom species which normally were mobile.

***UV light and survival of diatoms*:** All 3 diatoms were very sensitive to UV light and has survival period reduced to 9, 11 d at even the lowest UV dose administered (O.96 kJ m^{-2}) than of control diatoms surviving between 97-100 d (Table 2). The survival period of all 3 diatoms decreased slowly, and more or less similarly, with the increase in UV dose (Table 2). UV light inhibited survival in *Skeletonema costatum* (Wei *et al.* 2004).

***Darkness, low light intensity and survival of diatoms*:** All 3 diatoms survived appreciable the long time periods of 60- 64 d in darkness; 70, 72 d in low light intensity of 2 μmol m^{-2} s^{-1}; and of 82, 84 d in low light intensity of 10 μmol m^{-2} s^{-1} (Table 2). They all were almost equally tolerant to any of light conditions. The pond water from which the present diatoms were collected was almost covered by *Salvinia* plants growing on the water surface (which cut most of the sunlight) and that's why the diatoms were growing in natural dim light conditions and were tolerant to darkness and dim light conditions for long time period. Smayda and Mitchell-Innes (1974) observed that 7 of the 9 diatoms retained their viability for 90 days in the dark following removal from the euphotic zone. The cells of the

Anaulus australis were able to survive for up to 75 days in the dark (Du Preez and Bate 1992). The growth of *Cocconeis* sp. was not inhibited in low light intensity of < 4 μmol m^{-2} s^{-1} (Watson *et al.* 2004). However, the cells of *Navicula grimmei* and *Nitzschia palea* growing on moist soil surface and exposed to direct sunlight survived darkness only for 2.5 days (Gupta and Agrawal 2007a).

Absence of all nutrients and survival of diatoms: *Synedra, Gomphonema* and *Fragilaria* species survived absence of all nutrients in the medium for not more than 6.5, 8, and 8.5 d, respectively, and thus were very sensitive to absence of nutrients. *Navicula grimmei* and *Nitzschia palea* cells stopped gliding quickly within 1 h and died within 4 h in double- distilled water (Gupta and Agrawal 2007a). *Craticula* spp. and *Nitzschia* spp. moved well for 2- 5 h in distilled water with < 30- 40 % loss in either speed or percentage of motile cells (Cohn and Disparti 1994). Lack of nitrogen or phosphorus in culture media inhibited growth in *Achnanthes brevipes* (Guerrini *et al.* 2000).

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