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Growth Response of the Nitrogen-Fixing Cyanobacterium *Westiellopsis prolifica* Janet to Fertilizer Factory Effluents

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In recent years, algae have been widely used as the test system for evaluating the extent of pollution caused by industrial effluents (Rai and Kumar 1976). Algae being primary producers and occurring widely in almost all aquatic habitats can, if suitably employed, serve as indicator of habitat condition (Rana et al. 1971; Palmer 1980). Various pollution tolerant strains have been isolated and growth and behaviour of these selected algal strains were studied in the laboratory to evaluate the nutrient status of industrial effluents (Rai and Kumar 1976; Adhikary 1987; Adhikary and Sahu 1988).

The fertilizer factory of Talcher (Fertilizer Corporation of India, Talcher Unit) discharges 1-3 million gallons of partially treated or untreated effluents per day into Nandira River. A number of villages and rice-fields are located on the river bank. During the rainy season,waste water enters into the surrounding rice-fields and destroys the crop. Thus it is an urgent concern to analyse the physico-chemical characteristics of the waste water discharged from the factory and to evaluate the extent of pollution caused by the waste water using a nitrogen-fixing cyanobacterium of local unpolluted rice fields as the test organism.

MATERIALS AND METHODS

The fertilizer factory, Talcher ($20^{o}57'$N, $85^{o}15'$ E) has manufactured urea and ammonia since 1979. The factory discharges effluent to Nandira River through a 3-km long open canal (Fig.1). Physico-chemical analysis of the effluent was conducted following the methods given in APHA (1989). The effluent was black in color, highly alkaline and contained objectionable amounts of total solids, ammoniacal nitrogen and oil (Table 1). Surveys of the polluted area for the occurrence of various algal species and zooplankton, mostly at three sampling sites

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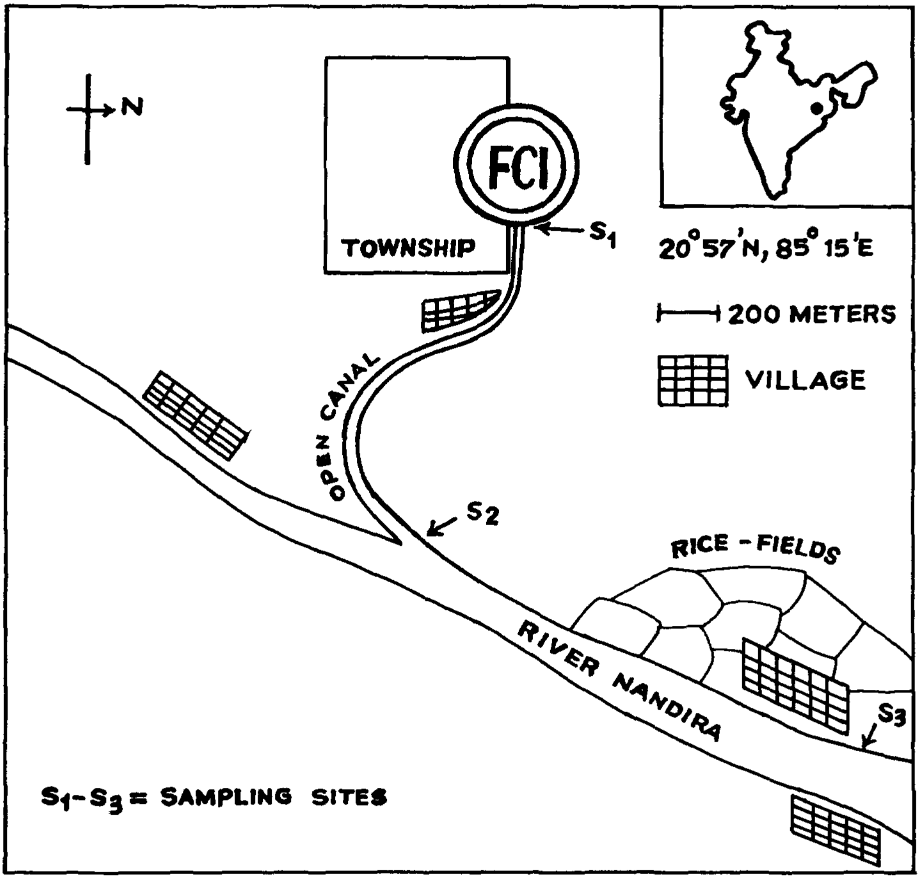


Figure 1. Study site showing positions of the Talcher fertilizer factory (Fertilizer Corporation of India, Talcher Unit) and passage of waste water from the discharge point of the factory to Nandira River.

(S 1,point of discharge of the effluent from the factory; S 2 ,the site where effluent canal mixes with Nandira River and S 3, 2-km away in the down stream from the above confluence point) were carried out throughout the years 1988 and 1989.

The nitrogen-fixing cyanobacterium, Westiellopsis prolifica Janet occurring predominantly in the rice-field soils of Orissa (Adhikary and Pattnaik 1979) was employed as the test organism to evaluate the nutrient status of the fertilizer factory effluent. Experiments were conducted using axenic cultures of W. prolifica grown in Allen and Arnon's nitrogen free medium (1955) with the micronutrients as used by Fogg (1949) at 28 $\pm$ 2 ^{o}C and 2,400 lux light intensity. Growth was estimated by measuring O.D. of the culture suspension at 760 nm in Spekol (Carlzeiss) spectrophotometer. Chlorophyll and

Table 1. Physico-chemical characteristics of Talcher fertilizer factory (FCI, Talcher, India) effluent. The waste water was collected from the effluent discharge point of the factory and from Nandira River at site 1 and site 2, respectively. All values except pH and temperature are in mg/L ; collected on 29th March 1989.

Characteristics		Effluent from discharge point of the factory (site 1)	Effluent mixed with Nandira River water (site 2)
Color		Blackish	Blackish
Odor		Ammoniacal	Ammoniacal
pH		10.5	10.0
Temperature (at site 1), ^{o}C		48.0	35.0
Total suspended solids	(mg/L)	2260.0	1830.0
Biochemical oxygen demand	(mg/L)	91.5	82.0
Chemical oxygen demand	(mg/L)	520.0	220.0
Dissolved oxygen	(mg/L)	2.2	2.8
Ammoniacal nitrogen	(mg/L)	850.0	590.0
Nitrate nitrogen	(mg/L)	105.0	26.0
Urea	(mg/L)	590.0	480.0
Phenol	(mg/L)	156.0	90.0
Oil and grease	(mg/L)	30.0	22.0
Chloride	(mg/L)	360.0	280.0
Sulphate	(mg/L)	168.0	148.0
Calcium	(mg/L)	35.0	44.5
Magnesium	(mg/L)	15.0	24.0
Phosphorus	(mg/L)	4.5	4.5

carotenoid pigments were extracted with 80 % acetone and quantitatively determined following Mackinney (1941) and Davies (1976) respectively. Change in morphological features and survivability of the cyanobacterium was studied in agar plates containing varying concentrations of the industrial effluent (1, 5, 10, 20, 30, 40, 50, 70 and 100 %, v/v), prepared in Allen and Arnon's nitrogen free medium. Aliquots of 1.0 mL diluted suspension of the organism was spread aseptically on each of the agar plates and incubated under light in the culture room. After 10 d of growth, colonies were counted and survival data were calculated taking survival on control plates as 100 %. Total nitrogen content of certain cultures were estimated by nesslerization method (Herbert et al. 1971). The amount of nitrogen fixed was determined after substracting the nitrogen present in the medium and inoculum.

RESULTS AND DISCUSSION

The fertilizer factory at Talcher discharges millons of gallons of untreated waste water every day into Nandira River. The effluent was highly alkaline (pH > 10) and contained excessive amounts of total solids, oil and ammoniacal nitrogen (Table 1). No algal growth was visible either at the effluent discharge point (site 1) or in the open canal which mixes with Nandira River (site 2). In the down stream (site 3) only a few organisms, *Gloeocapsa*, *Chroococcus*, *Lyngbya*, *Oscillatoria*, *Chlorella*, *Scenedesmus*, *Coelosphaerium*, *Cylindrocystis*, *Hydrodictyon* and *Melosira* appeared in the river bed (Table 2). Most of these organisms are found to possess a well defined sheath around their cells/trichome. Such ensheathed forms of cyanobacteria which have also been reported from various other industrial waste polluted water bodies (Adhikary 1987; Adhikary and Sahu 1988), might be tolerant of the waste water due to possession of such adaptive morphological features. However, none of the heterocystous cyanobacteria were recorded either in the River Nandira or in the adjoining rice fields which are periodically overflooded with the effluent mixed water.

Table 2. Occurrence of different organisms in the Talcher fertilizer factory (FCI, Talcher, India) effluent polluted area for the period from November 1987 to March 1989.

Organism	Sampling sites		
	S 1	S 2	S 3
Algae			
Chroococcus minutus	-	+	++
Gloeocapsa sp.	-	+	++
Lyngbya majuscula	-	+	++
Oscillatoria formosa	-	-	+
Coelosphaerium sp.	-	-	+
Cylindrocystis sp.	-	-	+
Scenedesmus quadricauda	-	-	+
Chlorella pyrenoidosa	-	-	+
Hydrodictyon reticulatum	-	-	+
Melosira granulata	-	-	+
Protozoa			
Glaucoma sp.	-	+	++
Rotifera			
Platyias sp.	-	+	+
Copepoda			
Cyclops sp.	+	+	-

Table 3. Effect of different concentrations of the effluent* of the Fertilizer Factory (FCI, Talcher, India) on the survival and morphological features of *Westiellopsis prolifica*. Cultures were grown at 28 $\pm$ 2°C under 2,400 lux light intensity up to 15 days

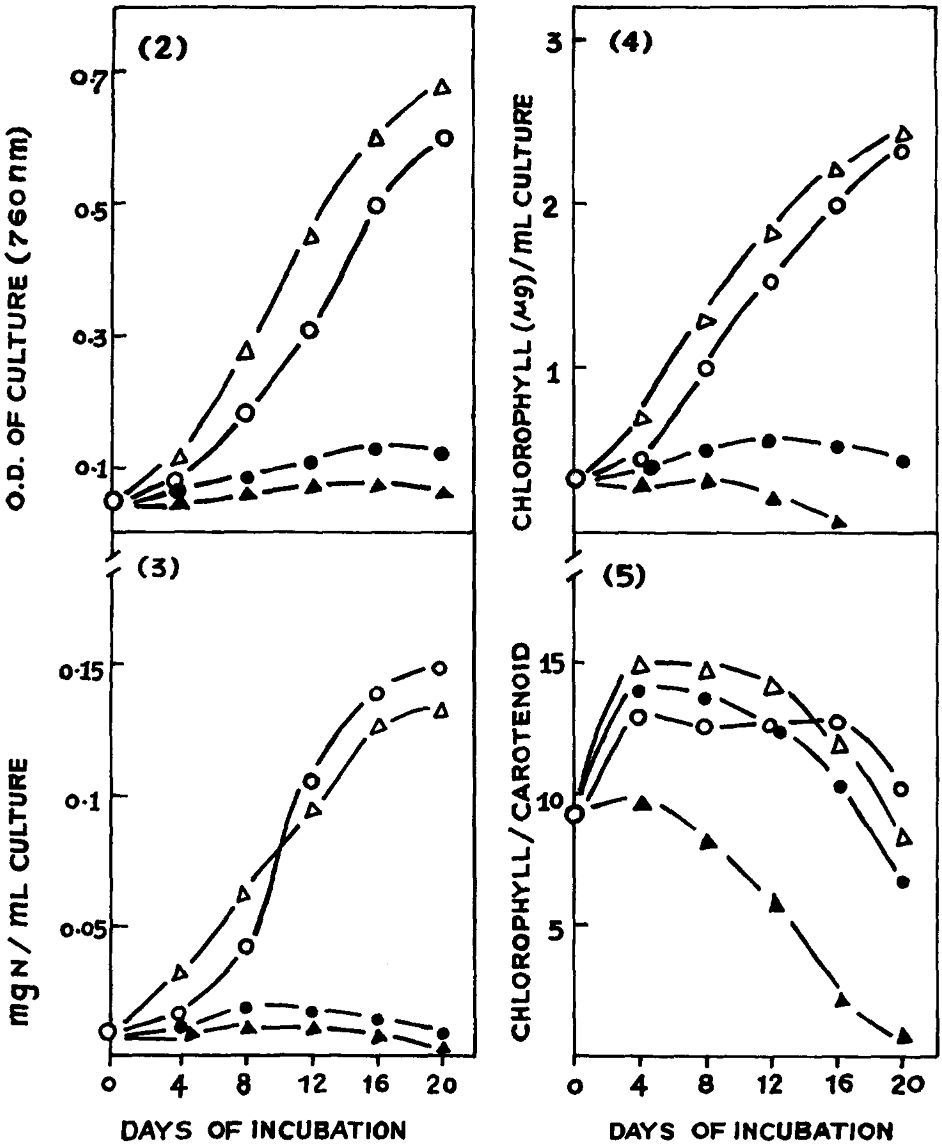
Effluent concentration (% , v/v)	Survival** (%)	Heterocyst	Branching	
			Prostrate filaments	Erect branches
0 (control)	100	4.2 $\pm$ 0.8 ***	Barrel shaped cells, as long as broad ****	Profuse branching
1	100	Few in number	Barrel shaped cells, as long as broad	Profuse branching
5	100	Few in number, granular	Barrel shaped cells, as long as broad	Reduced in length
10	98	Few in number, granular	Barrel shaped cells, as long as broad	Reduced in number and length
20	85	Rarely occur	Cells enlarged and granular	Few in number and suppressed
30	62	Rarely occur	Cells enlarged and granular	Few in number and suppressed
40	45	Suppressed	Cells enlarged , with branch initials	Suppressed
50	12	Suppressed	Cells enlarged, with few branch initials	Absent
70 and 100	0	No filamentous structure ; cells clumped and bleached		

* Different concentrations of the effluent were prepared in basal inorganic medium and adjusted to pH 7.5 by aseptic addition of N/10 HCl.

** Survival in presence of different concentrations of the effluent was computed taking the survival in the control cultures as 100.

***Frequency of heterocysts (number of cells between two successive heterocysts).

****Barrel shaped cells which are about as long as broad (14.6 - 16.1 μ long and 11.0 - 12.1 μ broad)



Figures 2-5. Growth (2), nitrogen fixation (3), chlorophyll content (4) and chlorophyll-carotenoids pigment ratio (5) of Westiellopsis prolifica in presence of fertilizer factory effluent.

○, control (c) ; ●, c+ 2 g/L KNO_3 ; △, c + 10 % (v/v) effluent ; ▲, c+ 50 % (v/v) effluent.

In order to evaluate the toxic effect of effluents on the nitrogen economy of rice-fields, the nitrogen-fixing cyanobacterium, Westiellopsis prolifica, commonly occurring in this region (Adhikary and Pattnaik 1979) was used as the test organism. The cyanobacterium could not survive in presence of more then 50 % (v/v) neutralized waste water of the industry. Normal filaments of the organism were found in presence of up to 10 % (v/v) of the waste water in the culture medium. With the increase in concentration of the effluent in the medium, heterocysts occur rarely, the cells of the prostrate filaments became enlarged and the number and length of erect branches decreased considerably. With further increase of the effluent proportion in the culture, filamentous structure of the organism was lost, cells became clumped, disorganized and bleached (Table 3).

In liquid culture, W. prolifica grew and fixed nitrogen appreciably in the basal nitrogen free medium up to 20 d (Fig. 2 and 3). The growth rate was further enhanced when the medium contained 10 % (v/v) of the fertilizer factory effluent. But when the basal medium was supplemented with KNO_3 or 50 % (v/v) of the effluent, growth was inhibited. However, nitrogen-fixing capacity of the organism was decreased over control even in presence of 10 % (v/v) of the effluent (Fig. 3). It is well known that growth of nitrogen-fixing cyanobacteria are suppressed or reduced by supplementing combined nitrogen into the culture medium (Fogg et al. 1973). Reduction in growth and nitrogen fixing capacity and suppression of heterocysts and erect branches in presence of higher concentrations of the waste water proves that the effluent discharged outside of the factory is nitrogen rich. It was also observed that chlorophyll content of W. prolifica increased in presence of 10 % (v/v) effluent in the culture medium over control (Fig. 4). But in presence of KNO_3 or higher concentrations of the effluent (50 %, v/v) in the medium, chlorophyll content per cell decreased significantly and after 12 d of incubation, the cells became colorless. Decrease in the carotenoid content of the cells in presence of combined nitrogen sources followed a similar trend but the degradation was slow in comparison with the chlorophyll value (Fig. 5). This shows that the nitrogen rich fertilizer factory effluent affects the photosynthetic system by degrading the pigment content of the cells thereby making the photosynthetic organisms unable to thrive in the waste water of the industry.

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