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Toxicity Testing with Freshwater Algae in River Periyar (India)

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A wide range of toxicity tests have been developed in the recent decades to predict the probable effect of new chemicals and effluents on aquatic ecosystem, utilizing different organisms such as algae, crustaceans, molluscs and fish (Sprague 1973; Walsh et al. 1980; Reish and Oshida 1986). Walsh et al. (1982) observed that "in general aquatic animals are more sensitive than algae to single pollutants and heavy metals, but there is evidence that algae are more sensitive than animals to complex wastes such as industrial and municipal effluents". Kallqvist (1984) recommended algal assays as a supplement to chemical analyses to assess pollution. The parameters of response often employed in algal assays are cell counts and photosynthetic rate (Cheng and Antia 1970; Kallqvist 1984). The present investigation to determine the effect of a fertilizer factory effluent on two species of unicellular algae were conducted to predict the probable effect of continued discharge of this complex waste on the microphytic flora in River Periyar located along the South West coast of India. The algal species selected occurred frequently in the freshwater regime of the river.

MATERIALS AND METHODS

Laboratory studies were conducted on axenic cultures of two freshwater algae: Nitzschia palea (Kütz) W.Sm. and Oocystis pusilla Hansgirg. var. major Skuja, isolated from the upstream area of River Periyar. Cultures were developed according to the standard procedure (Stein 1973). The algae were maintained and tested in freshwater medium described by Ward and Parrish (1982). Algal assays were conducted to study the response of the test species to liquid wastes collected from the fertilizer factory producing nitrogen and phosphorus fertilizers located on the banks of River Periyar. The effluents were collected from the discharge point every 3 hr and mixed to get a homogeneous sample. In the laboratory the sample was filtered first through absorbent cotton and then through Whatman glass microfiber filters (GF/C) of pore size 0.45 μm to remove all suspended materials (Walsh et al. 1980).

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The filtrate was stored in clean polyethylene container and kept in refrigerator at 4°C until use. The assays were initiated within two days of storage.

The effluent was analysed immediately after filtration to estimate the following parameters: pH, ammonia, phosphate, chemical oxygen demand (COD), and fluoride. The pH value of the effluent was measured using a portable pH meter (L.G. Nester, U.S.A. phase IV). The phenolhypochlorite method of Solo'rzano (1969) was used to estimate ammonia. Phosphate was determined by the ascorbic acid method and COD was determined by the Open Reflux Method described by APHA (1985). Fluoride content was measured by complexing the acid distilled effluent (APHA 1985) with lanthanum-alizarin reagent according to the procedure described by Martin (1968).

The effluent was allowed to attain room temperature, pH was adjusted between 7.6 and 8.2 using NaOH solution and further filtered using Whatman (U.K.) GF/C filter papers (pore size 0.45 μm) to conduct the algal assays. Same quantity of macro and micro nutrients used to prepare test medium were added to the effluent samples for enrichment. The enriched effluent was diluted using test medium to get different dilutions of the effluent, keeping the concentration of added nutrients unaffected. These diluted effluent grades were used for the assays. A preliminary range finding test using 10, 25, 50, 75 and 100% effluent was conducted to choose the concentrations for definitive tests. The concentrations of 5, 10, 30, 50, 70 and 90% and 5, 10, 15, 20, 25 and 30% effluent were selected for *N. palea* and *O. pusilla* var. *major*, respectively. The maintenance medium was used as control.

Axenic cultures of the two species at exponential phase were inoculated under aseptic conditions into 75 mL each of test media in 150 mL culture flasks so as to yield $1x10^4$ cells mL^{-1}. All tests were performed in triplicate. The test cultures were exposed to illumination from daylight fluorescent lamp assembly ≃ 2000 $\mu W\ cm^{-2}$ on a 12:12 light-dark cycle at 27 ± 1°C. The cultures were shaken at 6 hr intervals on a rotary shaker at ≃ 100 rpm. Test duration was 96 hr.

Aliquots of cultures were fixed in Lugol's iodine after incubation and the cell number was counted using haemocytometer. The photosynthetic pigments were estimated by spectrophotometric method (Jeffrey and Humphrey 1975). Cultures of *N. palea* (50 mL) were filtered through Whatman GF/C filter papers (pore size 0.45 μm) and extracted in 90% acetone (Vollenweider 1974). Cultures of *O. pusilla* var. *major* were filtered (50 mL) through Sartorius (West Germany) membrane filters (pore size 0.45 μm) and extracted in dimethyl sulfoxide (DMSO) because it did not give satisfactory extraction with acetone (Burnison 1980). The amount of the pigments are reported in $\mu g\ L^{-1}$ of the culture.

The exponential growth constant k' was computed from the cell count using the formula

$k' = \ln (N_{t_1} / N_o) / t_1 - t_o$. Where N_{t_1} = final cell count, N_o = initial cell count, $t_1 - t_o$ = period of exposure in days (Reynolds 1984). k' was also computed from photosynthetic pigments by substituting N_{t_1} and N_o in the above equation by P_{t_1} and P_o (where P_{t_1} is the final amount of the pigment and P_o is the initial pigment content. The significance of k' was tested by Student's t-test (Snedecor and Cochran 1967).

EC_{50} were obtained graphically by plotting effluent concentration against percentage inhibition of growth (cell count) on a semi-logarithmic graph (Walsh 1987). The values of EC_{50} were used to compute the 7-day, 10-yr low-flow volume (Q_r) required for safety in the river receiving this effluent, following the relation given by Walsh et al. (1982).

$$0.01 \times EC_{50} = \frac{Q_w}{Q_r + Q_w} \times 100$$

Therefore $$Q_r = Q_w \left(\frac{10^4}{EC_{50}} - 1\right)$$

Where Q_w = volume of discharge of effluent, Q_r = the 7-day, 10-yr low-flow volume of the receiving water and 0.01 = a safety factor currently used by US EPA in instream waste concentration calculations for issuance of discharge permits.

In order to assess the recovery of the test species after 96-hr exposure to the effluent, in each case, 1 mL of the culture was transferred aseptically to 75 mL of sterilized control medium contained in 150 mL flasks. These resuspension cultures were incubated for a period of 9 days and harvested to determine the cell number. The growth rates were compared by Student's t-test.

RESULTS AND DISCUSSION

The characteristics of the effluent collected for the assays is given in Table 1.

Table 1. Analytical data of effluent collected for algal assays

pH	Color	COD	Ammonia	Phosphate	Fluoride
4.88	Pale Yellow	110 mg L^{-1}	3 mg L^{-1}	266.6 mg L^{-1}	79 mg L^{-1}

The preliminary range finding test revealed that the effluent was inhibitory to the growth of the test species and their relative tolerance varied. The effluent significantly inhibited the rate of cell multiplication and production of chlorophyll *a*, *c*, and carotenoids of *N. palea* at all concentrations of the effluent tested (Table 2). Inhibition of growth (EC_{50}) occurred at 74% concentration of the effluent (Figure 1).

Table 2. Mean growth rate (k') of *Nitzschia palea* in different effluent concentrations after 96-hr exposure (definitive test).

Effluent %	Mean growth rate (k')			
	Cell number	Chloro-phyll *a*	Chloro-phyll *c*	Carote-noids
0	1.106	1.106	1.106	1.106
5	1.053*	1.090*	1.105	1.074*
10	1.052*	1.088*	1.084*	1.075*
30	1.046*	1.060*	1.075*	1.050*
50	1.004*	1.018*	1.029*	0.996*
70	0.956*	0.920*	0.936*	0.922*
90	0.722*	0.582*	0.578*	0.547*

*t value significant at 5% level

Unlike that of *N. palea*, the growth of *O. pusilla* var. *major* as indicated by the cell number was stimulated by 5% effluent and at 10%, the rate of growth was similar to that of the control. As the concentration increased the growth rate declined. However, the amount of chlorophyll *a* was found to be significantly low at 5% effluent. The other pigments i.e. chlorophyll *b* and carotenoids exhibited similar trend as that of cell count (Table 3). Fifty percent of growth was observed at 21% concentration of the effluent (Figure 2).

The results suggest that the response to a particular industrial effluent is dependent upon species as well as concentration. Walsh et al. (1980; 1982) have observed that phytoplankton respond to industrial effluents either by stimulation only or by stimulation at low concentration and inhibition at higher. The industrial effluents affect primary production either by increased light attenuation or direct phytotoxicity or physiological stress (Stockner and Cliff 1976). Gaur and Kumar (1981) reported that growth regulators in the effluents when present at a particular concentration can enhance growth rate. It is pointed out that the bioactivity of an effluent is related to the interactions of its various components in relation to its physical

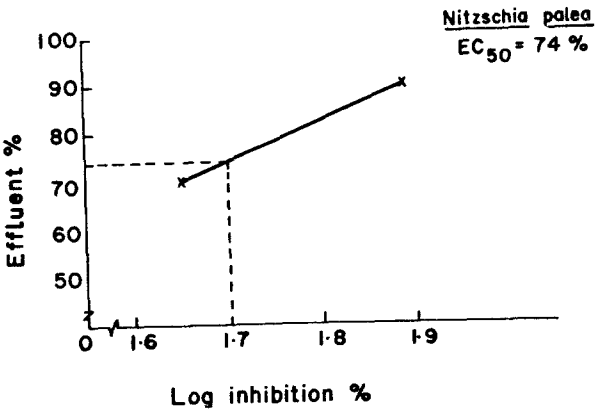

Figure 1. Effect of effluent on growth of Nitzschia palea in axenic culture.

properties (Walsh and Merrill 1984). Inhibition of growth and reproduction may be brought about by sublethal effects on metabolism.

Table 3. Mean growth rate (k') of Oocystis pusilla var. major in different effluent concentration after 96-hr exposure (definitive test)

Effluent %	Mean growth rate (k')			
	Cell number	Chloro-phyll a	Chloro-phyll b	Carote-noids
0	0.862	0.862	0.861	0.862
5	0.965*	0.706*	1.032*	0.889*
10	0.806	0.547*	0.858	0.780*
15	0.762*	0.523*	0.808	0.740*
20	0.698*	0.479*	0.782*	0.693*
25	0.627*	0.441*	0.676*	0.646*
30	0.568*	0.349*	0.684*	0.603*

*t value significant at 5% level.

As computed from the EC_{50} values of these species, this effluent which is being discharged into River Periyar from the fertilizer factory at a rate of 36,000 $m^3 day^{-1}$ requires a minimum volume (Q_r) of 4.80 to 17.11 Mm^3 day^{-1} of water in the river to dilute it to safe level. During the sampling year 1986, the lowest 7-day volume of water recorded in River Periyar was 1.10 Mm^3 day^{-1} in the month of April (premonsoon season). In the decade 1977-1986 the lowest discharge rate observed was 0.58 Mm^3 day^{-1} in the month of February (PWD 1982;

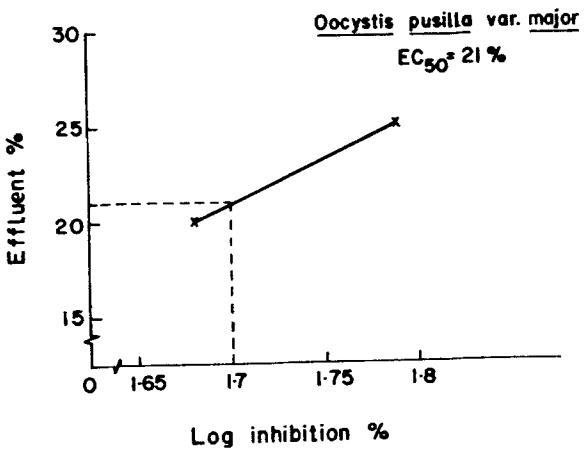

Figure 2. Effect of effluent on growth of Oocystis pusilla var. major in axenic culture.

1986). These data clearly reveal that the discharge in River Periyar during premonsoon months is inadequate to effect dilution of the effluent to safe level. It should be mentioned that the effluent used for the assay forms only a minor fraction of the total industrial waste entering the river.

The toxic effect of the effluent on the species tested was a temporary response at least at the lower concentrations tested. N. palea exposed to 90% effluent recovered growth when resuspended in control medium. O. pusilla var. major showed similar behaviour at 25% effluent (Table 4).

Table 4. Mean growth rate (k') of Nitzschia palea and Oocystis pusilla var. major in resuspension cultures.

Nitzschia palea		Oocystis pusilla var. major	
Effluent %	Mean k'	Effluent %	Mean k'
0	0.556	0	0.656
5	0.562	5	0.663
10	0.562	10	0.679
30	0.567	15	0.633
50	0.550	20	0.628
70	0.569	25	0.569*
90	0.510*	30	0.544*

*t value significant at 5% level

The situation in River Periyar is that the phytoplankton

community is constantly subject to the effluents from the factories which is likely to be diluted to safe levels only during monsoon and postmonsoon seasons. During premonsoon period neither the river discharge nor the tidal influx in the vicinity of industrial installations can restore the water to normal conditions as is evidenced by this algal toxicity assay.

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