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Effect of the Effluent from a Chlor-Alkali Factory on a Blue-Green Alga: Changes in the Pigment Content

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Photosynthetic pigments play the role of mediators in transfer of energy from the solar system to the plant's body. Dugdale (1975) described the growth of an algal population as being proportional to the effect of light on photosynthesis. Harriss et al.(1970) and Overnell (1975) have reported that reduction in growth of algae is because of reduction in the chlorophyll content. In other words, any effect of toxicants on growth and final yield is generally expected to be first reflected by some sort of alteration in the photosynthetic pigment composition. Innumerable literatures are available regarding the effect of different heavy metals, pesticides and insecticides on the pigment composition of algae.

Release of hazardous wastes into aquatic ecosystems produces a variety of complex responses beyond lethality to specific organisms (Christman et al., 1973). The industrial wastes discharged into the environment play a major role in polluting our valuable natural water resources. Growing awareness in this regard has led to the development of several test methods of evaluating their effects on the environment. Since the waste waters and/or the other toxicants discharged into an aquatic system generally first affect at the primary producers level, and the fact that the pigment systems are more liable to be affected first, a study of the pigment composition of algae exposed to the same may give valuable informations regarding their impact on the recipient waterbodies. Keeping these in view, the present piece of work was designed to investigate the effect of the effluent from a chlor-alkali factory at Ganjam (Shaw et al., 1986; Shaw and Panigrahi, 1986) on the pigment composition of a blue-green alga, Westiellopsis prolifica Janet. A study of growth rate of the alga exposed to the same effluent revealed that the effluent at higher concentrations was toxic in nature (Shaw, 1987).Selection of the test organism is important

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in the sense that the effluents discharged into the waterbodies ultimately, by the way of irrigation, find their way into the paddy fields in which the alga occurs as a dominant biofertilizer.

Analysis of the effluent revealed that it contained 0.465 mg/l of mercury, a highly toxic heavy metal, of which 0.055 mg/l was present in the supernatant (i.e., in soluble form) and the rest remained adhered to the suspended solids of the effluent. Other chemical characteristics of the effluent were : pH - 10.5, suspended solids - 19.3 mg/100 ml, dissolved solids - 90 mg/100 ml, biological oxygen demand (BOD) - 39.32 mg/l, chemical oxygen demand (COD) - 301.38 mg/l, chlorinity - 2000 mg Cl/l, hardness - 500 mg/l as $CaCO_3$, nitrate nitrogen 1.24 mg/l as NO_3 phosphate phosphorus - 0.308 mg/l as PO_4^{3-} and reactive silicate - 15.4 mg/l as Si.

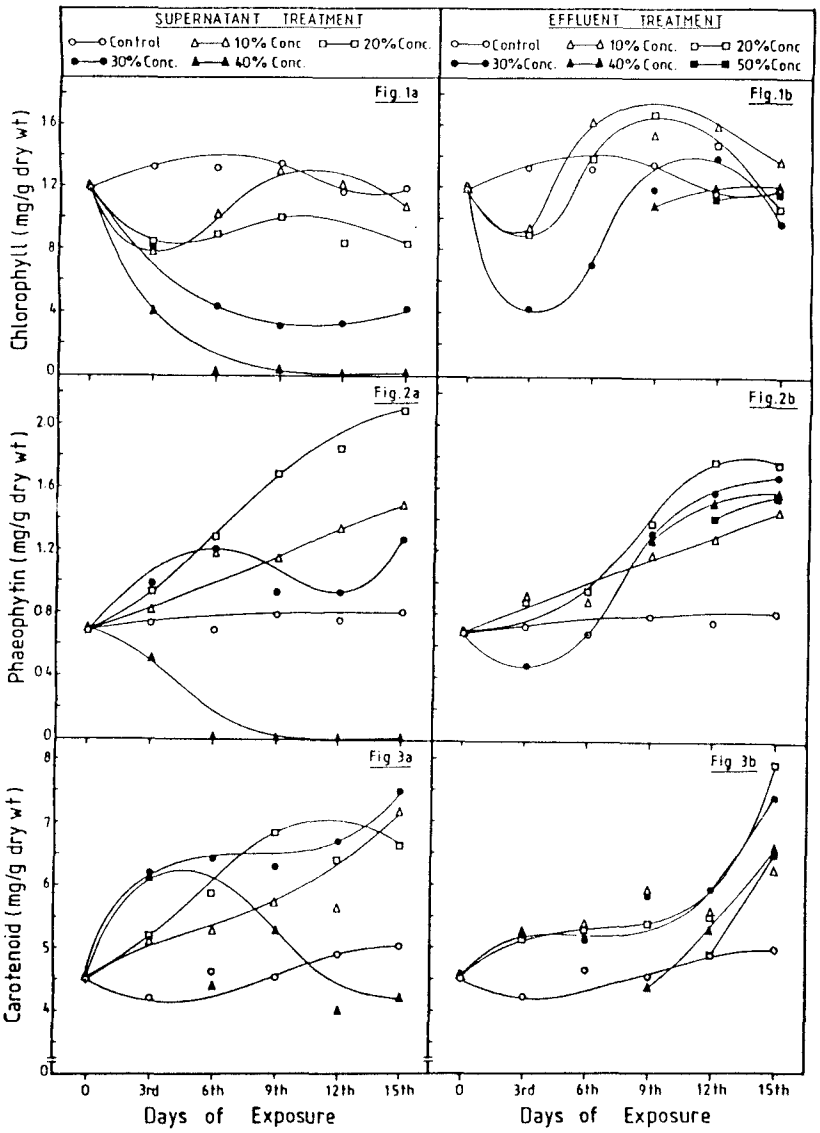
MATERIALS AND METHODS

The effluent was used in two ways for the experiment; firstly, it was used as such, and secondly, the suspended solids of the effluent were allowed to settle down the clear supernatant thus obtained was used. Graded concentrations of the effluent and the supernatant were prepared by using the sterilized culture medium (Patnaik,1964) as diluent and expressed as percent v/v. The total volume of each concentration was 50 ml. The graded concentrations prepared for the effluent were 10, 20, 30, 40 and 50%. For the supernatant, concentrations only up to 40% were used since the alga was not found to grow in higher concentrations. Culture without the effluent or the supernatant was taken as control. One ml of the homogenized axenic culture of the alga, optical density adjusted to 0.8 at 530 nm,was inoculated to each of the flasks. The vessels were plugged and incubated at 27 ± 2°C with an alternate 12 hrs photo (2,500 ± 200 lux) and 12 hrs dark period, and hand shaken twice daily to prevent any adherence of the algal cells to the wall of the vessels. For each concentration five vessels were inoculated and thus there were 5 sets of culture vessels. Estimation of different pigments content of the alga in each concentration, including the control, was carried out at the end of 3rd, 6th, 9th, 12th and 15th day of exposure by removing one complete set of the culture flasks on those days.

Pigments were extracted by grinding the algal material in 80% acetone with the help of a mortar and pestle in dark. The amount of total chlorophyll and phaeophytin was estimated by the procedure of Liasen-Jansen and Jensen (1971). The experiment was reported in terms of mg of pigments per gram dry wt of the algal tissue.

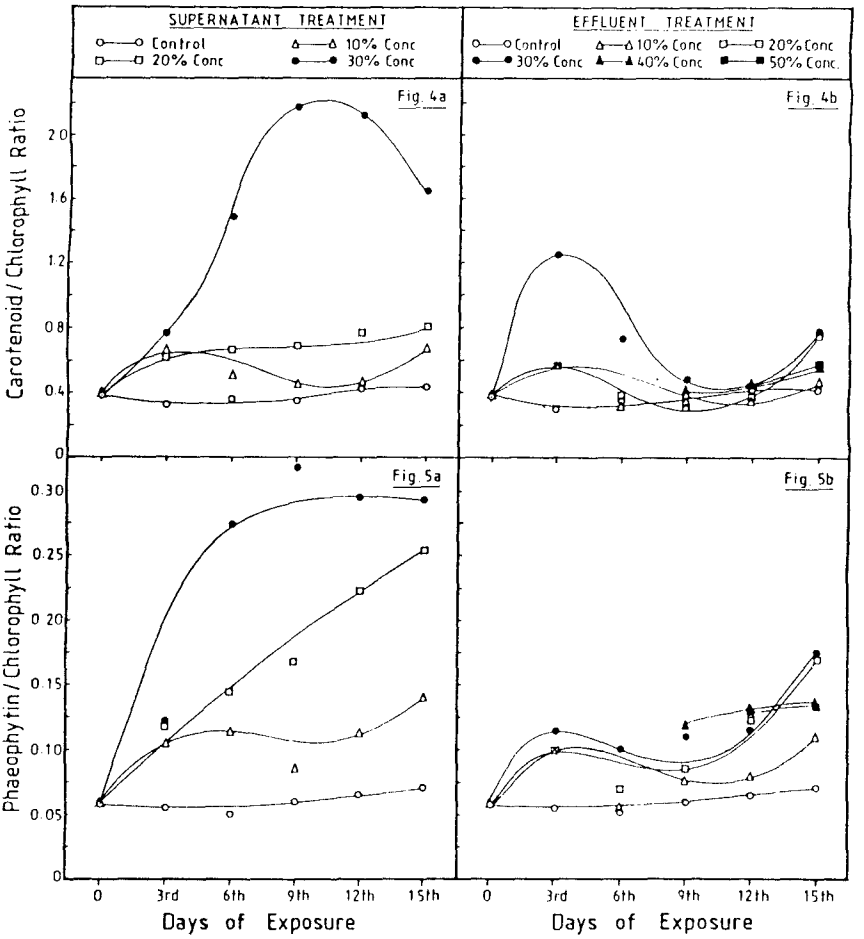
RESULTS AND DISCUSSION

The level of chlorophyll decreased progressively with increase in concentration of the supernatant (Fig.1a). In 10 and 20%



Figures 1-3: Changes in the pigments content of the alga, Westiellopsis prolifica Janet,exposed to different concentrations of the supernatant of the effluent as a whole for different days.

concentrations of the supernatant, on the 3rd day, the chlorophyll content of the alga decreased to 40 and 38% respectively over the control (Table 1). Later on the chlorophyll level increased, but on longer exposure the level again decreased. In 30% treatment the level of chlorophyll went on declining till the 9th day (78% over the control) and then increased a little, probably due to the development of some sort of resistance to the stress. In 40% treatment the chlorophyll content of the alga touched the zero mark.



Figures 4 & 5: Changes in carotenoid/chlorophyll and phaeophytin/chlorophyll ratios of the alga, Westiellopsis prolifica Janet, exposed to different concentrations of the supernatant of the effluent and the effluent as a whole for different days.

The effluent, in contrast, showed a marked increase in the level of chlorophyll (Fig. 1b and Table 1); particularly 10% concentration was found to be highly stimulative. Although 20 and 30% concentrations were also greately stimulative, the level of chlorophyll in those concentrations decreased slightly over the control on the 15th day of exposure. It is interesting to note that the chlorophyll content of the alga in 40 and 50% concentrations was more or less same to that of the control culture even after the 15th day of exposure though the chlorophyll content of the alga in 20 and 30% treatment declined to 11 and 18% respectively over the control. The level of pigment could not be measured until the 9th day in case of 40% effluent and until the 12th dat in case of 50% effluent treatment since the algal materials had, so to say, disappeared from the culture medium for 6 days in case of the former and for 9 days in

case of the latter (Shaw, 1987). Later on the alga reappeared in granular form, probably after developing some sort of barrier (Shaw, 1987) which might also be responsible for the higher level of chlorophyll than in 20 and 30% treatment, as observed on the 15th day of exposure.

Phaeophytin (Fig. 2a and 2b) and carotenoid (Fig. 3a and 3b) content of the algal culture showed a slight increase with increase in the age. The increase, however, was much more pronounced in the treated cases, particularly in the supernatant treated cases (Table 1). Their level increased with increase in concentration of the supernatant as well as the days of exposure. Nevertheless, at the higher concentrations a decline was

Table 1. Percent increase/decrease (over control of the same day) in chlorophyll, phaeophytin and carotenoid content of the alga, Westiellopsis prolifica Janet, exposed to different concentrations of the supernatant of the effluent and the effluent as a whole for different days.

Conc.of the	Supernatant Treatment					Effluent Treatment				
Effluent/	3rd	6th	9th	12th	15th	3rd	6th	9th	12th	15th
Supernatant	(days of exposure)					(days of exposure)				
					Chlorophyll					
10%	-40	-22	-3	+2	-10	-30	+22	+16	+33	+15
20%	-38	-33	-25	-30	-30	-31	+5	+23	+25	-11
30%	-39	-67	-78	-73	-64	-68	-47	-11	+19	-18
40%	-69	-100	-100	-100	-100	-	-	-20	+2	+1
50%	-	-	-	-	-	-	-	-	-3	-2
					Phaeophytin					
10%	+15	+71	+42	+79	+83	+26	+28	+49	+72	+80
20%	+32	+86	+113	+143	+157	+24	+38	+75	+137	+117
30%	+35	+73	+17	+24	+56	-33	-1	+67	+111	+107
40%	-29	-100	-100	-100	-100	-	-	+62	+103	+95
50%	-	-	-	-	-	-	-	-	+91	+93
					Carotenoid					
10%	+21	+14	+27	+15	+44	+25	+15	+28	+14	+25
20%	+22	+28	+51	+31	+33	+23	+14	+18	+12	+58
30%	+47	+39	+39	+37	+40	+24	+10	+29	+21	+48
40%	+45	-5	+17	-18	-16	-	-	-3	+9	+32
50%	-	-	-	-	-	-	-	-	00	+30

+ and - signs represent increase and decrease, respectively over the control value

also observed, probably because of the breakdown of those pigments at higher concentrations. The phaeophytin was found to be more susceptible than the carotenoid. The effluent as a whole also increased the level of both the pigments significantly. Although, like the supernatant treatment, maximum increase in their level was experienced in 20% concentration, but unlike it, no disintigration of the pigments was observed at the higher concentrations showing somewhat lesser toxic effect of the effluent as a whole.

An increase in the carotenoid/chlorophyll ratio was observed in 30% concentration in both the supernatant as well as the effluent treatment (Fig. 4a and 4b). In other concentrations no marked increase in the ratio was observed. Nonetheless, the ratios for the treatment were higher than the ratios for the control. At lower concentrations of the supernatant and at both lower and higher concentrations of the effluent there occurred no increase in the ratio, must be because of the increase in the level of chlorophyll concomitant with increase in the carotenoid level. At the higher concentration (40%) of the supernatant, degradation of the chlorophyll occurred and hence it was not possible to calculate the ratio.

Phaeophytin/chlorophyll ratio showed a sharp increase in 20 and 30% of the supernatant treatment (Fig. 5a). In 10% treatment the ratio increased slightly. In 40% treatment no ratio could be calculated because of the degradation of the pigments. Effluent treatment also revealed a higher phaeophytin/chlorophyll ratio (Fig.5b) though not as high as obtained with the supernatant treatment.

The pigments are known to participate in generation of energy for carbon dioxide fixation (Kashyyap and Gupta, 1981). The chlorophylls have long been recognized as the primary light acceptor in plants and they are invariably present in every organism which carries out photosynthesis with absorption of carbon dioxide and evolution of molecular oxygen. Chlorophyll is organized in plants in small photosynthetic units containing a few hundred molecules, and the function of most of the chlorophyll ("antenna" chlorophyll) appears to be to gather light, whilst a small proportion acts as the primary reaction centre where light conversion occurs. Carotenoids on the other hand not only help in photosynthesis but also protect the photosynthetic tissue against photosensitized oxidation.

Decrease in the level of chlorophyll in algae and other plants exposed to different toxicants have been reported by various workers. Geike (1977) reported decrease in the chlorophyll level in two algae exposed to mercury. Filippis and Pallaghy (1976) observed reduction in the chlorophyll content in Chlorella treated with zinc and mercury. Mercury related decrease of the chlorophyll levels in some aquatic plants was reported by De et al. (1985) and Mhatre and Chaphekar (1984,1985) . De et al. (1985) hypothesized that the decrease in the chlorophyll level was as a result of increase in the chlorophyllase activity. On the other hand, Matson et al. (1972) reported that the decrease in the chlorophyll content in the freshwater alga Ankistrodemus braunii exposed to mercuric chloride and methyl mercury was due to inhibition of biosynthesis of chlorophyll and lipids, especially galactolipids.

Results obtained here are peculiar. With the supernatant an intial stimulation and then reduction in the chlorophyll content at higher concentrations was marked. However, with the effluent

treatment though the chlorophyll content increased to a great extent at lower concentrations, virtually no decrese in the level at higher concentrations was marked. Working with the same alga Rath (1984) reported a decrease in the chlorophyll content with increase in the mercuric chloride and Emisan-6 concentration. Thus when mercury has been reported to be toxic, an increase in the level of chlorophyll in the alga exposed to the effluent heavily contaminated with mercury can hardly be explained. In fact, increase in the chlorophyll content in any plant system exposed to any toxicant is yet to be reported.

In ecological or toxicological studies involving algae, estimation of the phaeophytin content serves as an important tool since any unfavourable change in the environment, or the effect of the toxicants is reflected by the change in its level in the algae. Chlorophylls are known to be converted to phaeophytins as a consequence of exposure to weak acids by replacement of Mg^{2+} with two atoms of hydrogen and thereby changing the spectral properties (Rao and LeBlanc, 1966; Singh and Singh, 1084). Degradation to phaeophytin might be the first step towards the breakdown of chlorophyll. This is evident from the increased levels of phaeophytin in the treated cultures. At higher concentrations of the toxicants phaeophytin is also broken down as is the case with 40% supernatant treatment. Increased ratios of chlorophyll to phaeophytin may be taken as a warning towards possible pollution of the aquatic system.

Role of carotene as a protector of photosynthetic tissues against photosensitized oxidation is well known. Increase in the carotenoid content of the alga on exposure to the effluent, or the supernatant, as has been reported under the present investigation, may be related to the possible role of this pigment in protecting the photosynthetic tissues under stress. However, so far no such role of the pigment has been reported.
The ratio of chlorophyll to carotenoid has been identified as a valuable parameter for defining environmental conditions unfavourable for algal growth. When the nutrients in the medium are exhausted, or a toxicant is introduced, the ratio rises due to decrease in the chlorophyll content (Rai et al., 1981). Increased ratio indicated inhibition of chlorophyll biosynthesis, inactivation of enzyme systems and disruption of many physiological and biochemical processess (Filippis and Pallaghy, 1976; Leland et al., 1979; Sorentino, 1979).

Although a remarkable amount of mercury, much more than the reported lethal dose of 0.04 mg/l to the alga under study, was present in the effluent, it was not found to play any role in determining the behaviour of the effluent towards the alga. This is evident from the fact that despite heavy mercury content the effluent as a whole was much less toxic, or brought much less alteration in the pigments content of the alga than the supernatant containing only a fraction of the total mercury present in the effluent. It appears that the toxic character of the effluent was being determined by some substance other

than mercury present in soluble form, and that the toxicity of the same was being counteracted by the residues present in the effluent resulting in greater toxic nature of the supernatant than the effluent as a whole. The same is also supported from the fact that 40% supernatant, which is lethal, contained only 0.02 mg Hg per litre in contrast to the reported lethal dose of 0.04 mg Hg per litre. The stimulative nature of the effluent was probably in some way associated with the residues present.

From the study it is concluded that the effluent is not fit to be discharged as such because: 1) The level of mercury exceeds much more than the stipulated limit of 0.01 mg/l set by the Central Board for the Prevention and Control of Water Pollution, India. 2) The supernatant is highly toxic in nature. On getting discharged into an aquatic system, the residues will settle down leaving the supernatant in the water column to show its action. The same has been reported by Shaw (1987). This study coupled with the report of Shaw (1987) that 50% of the alga died at 34.8% and 29.6% supernatant concentrations in 24 hrs and 48 hrs respectively sugfgests a rigorous treatment of the effluent before it is discharged into the environment.

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