INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY
Volume: 2: Issue-4: Oct - Dec -2011 IJABPT ISSN 0976-4550

AN EXPERIMENTAL INVESTIGATION ON GROWTH STIMULATION (+) AND INHIBITION (-) OF ALGAE (*OSCILLATORIA.CHLORINA AND SCENEDESMUS.QUADRICAUDA*) TREATED WITH PULP AND PAPER MILL EFFLUENTS

*M.K.Saikia, **S.Kalita, [1]G.C.Sarma

*Asstt. Prof. of Botany, Dhing College: Nagaon(Assam),Pin-782123
**Professor, Dept. of Environmental Science, Gauhati University: Guwahati-14 (Assam)
[1]Curator, Department of Botany, Gauhati University, Guwahati-14(Assam)

ABSTRACT : The growth stimulation (+) and inhibition (-) of algae treated with pulp and paper mill effluent was investigated in laboratory culture condition. The result revealed that the effect of paper mill effluent on algae was species specific. Effluents were stimulatory as well as inhibitory to the growth of test algae . Oscillatoria chlorina tolerated higher concentration where as Scenedesmus quadricauda tolerated lower concentration of effluent. At 100% effluent concentration growth was retarded and inhibition was the result. During investigation significant algal growth was observed between concentration and treatment days of which 21 days incubation period was considered as final yield

Key words: Pulp and paper mill, Oscillatoria chlorina, Scenedesmus quadricauda, stimulation, inhibition.

INTRODUCTION

The pulp and paper industry has historically been considered a major consumer of natural resources and energy, including water, and a significant contributor of pollutant discharges to environment (Rigol et al., 2003). Pulp and paper mill liberates heavily loaded waste in to surrounding environment (Anonymus, 1999; Baruah and Das, 2001) which are mainly arising out from pulping and bleaching process. Due to higher chemical diversity of pulp mill effluents the recipient watercourses may be highly affected. Among aquatic organisms algae are very sensitive to pollutants discharge than other organisms. Algae show some adverse effect of chemicals more quickly than other organisms (Barbour et al, 1999). Depending on toxicity of pollutants, the growth of algae can be either inhibited or stimulated. Primary production, concentration of dissolved oxygen, photosynthetic pigments, and biochemical compounds and biomass may also get affected.

A wide range of toxicity tests have been developed in recent years to predict the probable effect of effluents on aquatic ecosystem utilizing different organisms such as algae, crustaceans, mollusks and fish (Walsh et al. 1980). Among this algal toxicity test are increasingly becoming an indispensable part of test batteries in water pollution monitoring because firstly algae are the primary producers of food chain, and secondly they are more sensitive to contaminants than fish or invertebrates (Wong, 1995). The nature of the effluents can be assayed by algae since the response can be measured in terms of biomass production or through metabolic response generated (APHA, 1998). Due to its low cost, simplicity and accuracy, the algal bioassay has been extensively utilized and several reports have been published in different countries of the world.

The algal assay procedure has been included in Standard Methods (APHA; 1989; U.S.EPA, 2002) which has been adopted worldwide. Although the algal assay largely confined to eutrophication potential and nutrients limitation studies as proposed by Skulberg (1962), attempts have also been made to evaluate the toxicity of complex industrial waste. To name a few were Gopinathan et al., 1994; Eloranta, 1994; Wong, 1995; Kobbia et al., 1995; Dash and Mishra, 1999; Tang et al., 2000; Reemol, 2004; Nicolas and John, 2006; Palma et al., 2008, Hall et al., 2009.

The toxicity of pulp and paper mill wastes has been demonstration many times in the literature (Fujiya, 1961); most of this work has dealt solely with the lethal & sub-lethal effect of the waste on fish populations. Limited papers have reported on the toxic effect of these wastes on other members of aquatic communities. In Assam two large paper mills are in operation. Although much work has been done in Assam on systematic of algae in lentic and lotic environment, but very little imformation available in paper mill polluted habitat. Considering the above the present study was undertaken. The present study was conducted to evaluate the growth stimulation (+) and inhibition (-) potentials of Nagaon Paper Mill effluent on two species of algae namely *Oscillatoria chlorina Kuetz* and *Scenedesmus quadricauda Smith* in laboratory bioassay condition. The test compound selected for present study includes waste effluent of Nagaon Paper Mill (NPM)
Methodology

The effluents samples were collected from the outlet of Nagaon Paper Millwere analyzed as per methodology of APHA (1989), Trivedy and Goel (1986), Golteman et al. (1978), Greenberg et al. (1985) and ready to use for bioassay. The isolation and identification of test algae was done following Lackey's (1938) ,Desikachay (1959; Prescott (1951); Smith, (1950). The collected algal species were uniculturally grown in laboratory condition and used for bioassay. The Culture media used were BG-11(Stainer et al, 1971) for *Oscillatoria chlorina* and Ward and Parish(1982) for *Scenedesmus quadricauda*. The bioassay of test algae was conducted as per bacteriological techniques (Stein, 1973; Gopinathon, 1982; APHA, 1980; Ward and Parish, 1982 and Parsons et al, 1984). The algal growth in terms of Chlorophyll a, carotene were analyzed spectrophotometrically in a UV-VIS Spectrophotometer Model 117 as per methods given by parsons et al. (1984) after 5 days interval for a period of 21 days .The biomass was measured by taking the dry wt. of harvested filaments on 24 days when decline phase starts and expressed as mg/ml of algal suspension. The growth of 21st day was taken as final yield of the experiment.

RESULTS AND DISCUSSION

The growth response of Oscillatoria chlorina Kuetz. Ex. Gomont, Scenedesmus quadricauda Smith treated in culture media supplemented with different concentrations of Nagaon Paper Mill effluent has been shown in Table 1 and 2 while their (%) percentage stimulation (+) or inhibition (-) of growth in terms of Chlorophyll a content over the control at 21 (twenty one) days optimum growth phase period has been shown in Figure. 1. The individual growth response of algae in terms of chlorophyll contented various concentration of PME were given in Figure-2-3. The Chlorophyll a, Carotene and Biomass content of Oscillatoria chlorina was increased with increasing the concentrations of paper mill effluent. Highest amount of chlorophyll a (2.85 mg/ml), Carotenoid (1394 mg/ml) and biomass (1762 mg/ml) content of O.chlorina was recorded at 80% effluent concentration enriched with basal medium(Table-1). The lowest amount of chlorophyll a (1.07 mg/ml), Carotenoid (0.625mg/ml) and biomass (0.768mg/ml) was recorded at 100% effluent concentration without basal medium (Table-1).

Table: 1. % Stimulation (+) and Inhibition (-) of chlorophyll 'a', Carotene & biomass content of Oscillatoria chlorina at 21 days optimal growth period.

Effl.Conc. (%)	*Oscillatoria chlorina Kuetz. Ex. Gomont*								
	Chlorophyll a (mg/ml)			Biomass (mg/ml)			Caroteniods (mg/ml)		
	Mean	%(+)	%(-)	Mean	%(+)	%(-)	Mean	%(+)	%(-)
BM+0	1.57	-	-	1013.00	-	-	0845.00	-	-
BM+20	2.06	+31.21	-	1258.00	+24.18	-	0962.00	+13.86	-
BM+40	1.50	+59.24	-	1436.00	+41.75	-	1042.00	+23.31	-
BM+60	2.83	+80.25	-	1694.00	+67.22	-	1335.00	+57.98	-
BM+80	2.85	+81.52	-	1762.00	+73.93	-	1394.00	+64.47	-
100%	1.07	-	-31.84	0768.00	-	-24.18	0625.00	-	-26.03

(+)= Stimulation, (-) =Inhibition

The increase or decrease of chlorophyll 'a', carotenoid and biomass content were measured and expressed as percentage(%) stimulation (+) or (%) Inhibition (-) over the control. The maximum percentage stimulation (+) of chlorophyll a (81.52%), Carotenoid (64.47 %) and biomass (73.93%) were recorded at BM+ 80 % effluent and minimum stimulation for chlorophyll a (31.21%), carotenoid (13.86%) and biomass (24.18%) were observed at BM+ 20%.effluent concentration(Table-1). Experimental results revealed that Oscillatoria chlorina exhibited a gradual stimulation of chlorophyll a, carotene and biomass content upto 80% effluent concentration. But above the 80% effluent concentration, this value decline sharply and at absolutely 100% effluent concentration no stimulation take place and inhibition (-) was the result. At 100% effluent concentration there was a marked decline of chlorophyll a (-31.84%), carotenoids (-26.03%) and biomass (-24.18 %) content over the control value.

Similarly in Scenedesmus quadricauda highest amount of chlorophyll a (3.62 mg/ml), biomass (2390.00 mg/ml) and carotene (1505.00 mg/ml) was recorded at 40% effluent concentration enriched with basal medium (Table-2). The lowest amount of chlorophyll a (0.38mg/ml), biomass (520.00mg/ml) and carotene (0450.00mg/ml) was recorded at 100 % effluent concentration without basal nutrient medium (Table-2).The highest stimulation Chlorophyll a (+81.52%); Biomass (+73.93%) and Carotenoids (+53.26%) was recorded at 40% effluent concentration with nutrient basal medium. The highest percentage inhibition (-) was recorded at effluent concentration 100% without basal medium (Table-2).The analysis of growth culture showed that maximum chlorophyll $_a$ pigment, Carotene and Biomass contents were reported on 21st day of optimum growth period. Although 20%, 40%, 60%, 80% effluent obstructed chlorophyll production till 5th day and after that a sharp increase in this pigment was observed from 9th day onwards. A steady decline of chlorophyll a was obviously seen in 100% effluents for both algae. In above experiment biomass was recorded on harvesting day (24th day) when decline phase began. Therefore the percentage of increase in terms of biomass was relatively less than the increase rate of chlorophyll.

The ANOVA result showing the variation of growth between concentration and treatment days for chlorophyll a, Carotene and biomass was given in Table-3 .The result revealed that there was significant difference of algal growth between concentration and treatment days. Maximum growth of chlorophyll a, carotene and biomass of Oscillatoria chlorina was recorded at 80% effluent whereas maximum growth of Scenedesmus quadricauda was recorded at 40% effluent concentration at 21 days incubation period.

Table: 2. % Stimulation (+) and Inhibition (-) of chlorophyll 'a' & biomass content of Scenedesmus quadricauda at 21 days optimal growth period

Effl.Conc. (%)	*Scenedesmus quadricauda Smith*								
	Chlorophyll a (mg/ml)			Biomass (mg/ml)			Carotenoids (mg/ml)		
	Mean	%(+)	%(-)	Mean	%(+)	%(-)	Mean	%(+)	%(-)
BM+0	2.12	-	-	1155.00	-	-	0980.00	-	-
BM+20	3.19	+50.47	-	1986.00	+36.47	-	1235.00	+26.02	-
BM+40	3.62	+70.75	-	2390.00	+64.26	-	1505.00	+53.26	-
BM+60	1.23	-	-41.98	1090.00	-	-25.08	0796.00	-	-18.77
BM+80	0.88	-	-58.49	840.00	-	-42.27	0678.00	-	-30.81
100%	0.38	-	-82.07	520.00	-	-64.26	0454.00	-	-53.67

(+)= Stimulation, (-) =Inhibition

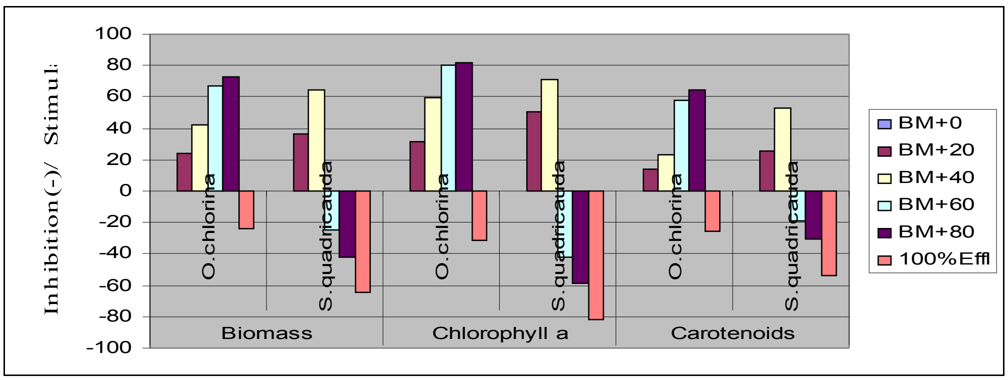

Figure: 1. Percent Stimulation (+) and Inhibition (-) of biomass, chlorophyll a and carotenoids content Oscillatoria chlorina and Scenedesmus quadricauda after 21 days incubation period.

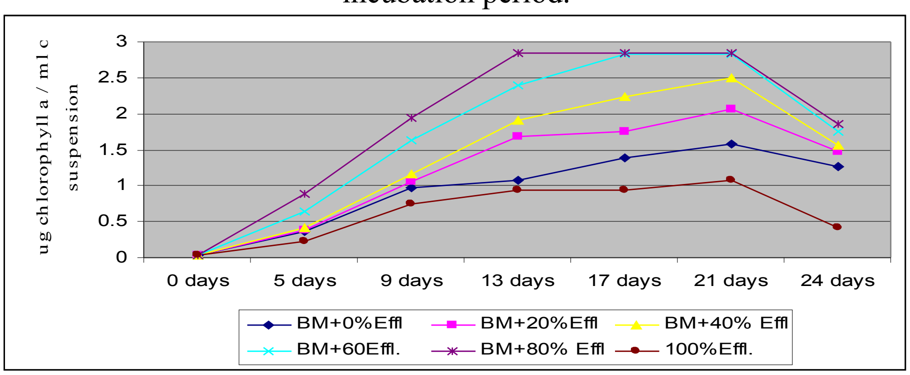

Figure: 2. Chlorophyll a content during the growth of O.chlorina treated with paper mill effluent enriched with basal medium.

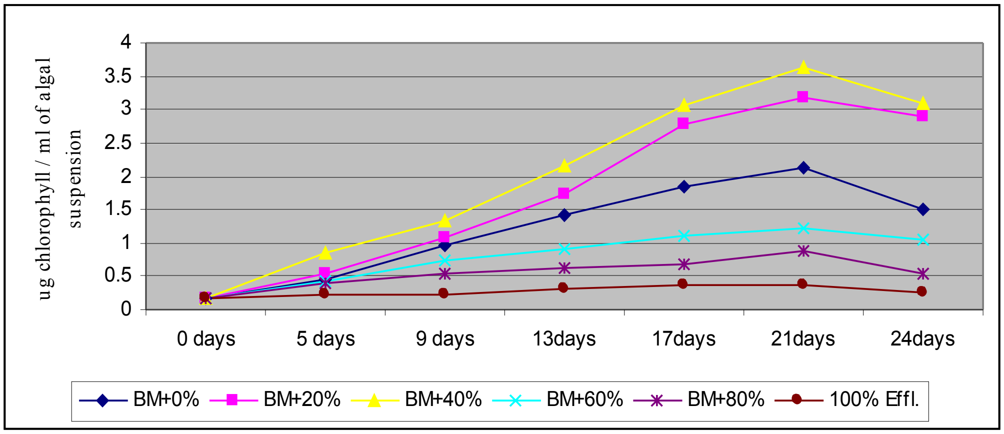

Figure: 3. Chlorophyll a content during the growth of S. quadricauda treated with paper mill effluent enriched with basal medium

The result of the experiment showed that paper mill effluents were stimulatory as well as inhibitory to the growth of test organisms. An inhibitory (-) effect on the growth was also noticed at 100% effluents. Our findings slightly vary with the findings of Palria (1991), Adhikari (1997). Dash and Mishra (1999) who demonstrated enhanced growth of algae at 100% waste water when enriched with basal nutrient medium.

Table: 3 ANOVA results showing the effect of paper mill effluents on Chlorophyll a, biomass and carotenoid contents of Oscillatoria chlorina and Scenedesmus quadricauda between concentrations.

A] ANOVA Summary for Chlorophyll a [*Oscillatoriiia chlorina]*

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	10.81	5	2.162	6.54 0	0.003
Within concentration	9.918	30	0.33.6		
Total	20.73	35			

B] ANOVA Summary for Carotenoids [*Oscillatoria chlorina*]

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	28,728788	1	28,728788	12.1 1	0.004
Within concentration	28,47055	12	2,372546		
Total	57.199344	13			

C] ANOVA Summary for Biomass [*Oscillatoria.chlorina*]

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	39.2486	1	39.2486	11.7 8	0.004
Within concentration	39.9819	12	3.3318		
Total	79.2305	13			

A] ANOVA Summary for Chlorophyll a [*Scenedesmus quadricauda*]

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	62.5829	1	62.5829	12.03	0.004
Within concentration	62.41	12	5.2008		
Total	124.9929	13			

B] ANOVA Summary for Carotenoids[*Scenedesmus quadricauda*]

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	17.56608	1	17.56608	14.83	0.002
Within concentration	14.212037	12	1.184336		
Total	31.778117	13			

C] ANOVA Summary for Biomass [*Scenedesmus .quadricauda*]

	Sum of Square	df	Mean Square	F	P
Treatment[between concentration]	35.84	1	35.84	15.96	0.001
Within concentration	26.9428	12	2.2452		
Total	62.7828	13			

P<5% significant difference; P>5% not significant difference

Many workers noticed negative impact of higher concentrations of various industrial effluents on algae. Higher concentration inhibited pigment production due to absorption of bio-available contaminants by algal cells from the effluents. Similar observations of enhancement of pigment content in lower concentrations of effluents and decrease in higher concentration was recorded by Smith et al., (1988), Kobbia et al., (1995); Aidar et al.,(1997), Bindu Alex (2005). Reemol (2004) reported a stimulatory and inhibitory effect of distillery effluent on algal growth at higher and lower concentrations respectively. Similar observation was recorded in present studies.

Table: 4 Chemical composition of Nagaon Paper Mill effluent used for conducting algal Bioassay experiment.

Parameters	Value	Parameters	Value
Temperature ^{O}C	32	Biochemical Oxygen Demand(BOD)mg/mL	418.00
pH	8.9	Chemical Oxygen Demand(COD)mg/mL	594.00
Conductivity. mmho/cm	155	Alkalinity,mg/mL	363
Total Suspended Solids (TSS) mg/L	4.05	Total Nitrogen(TN) in ppm	4.60
Total Dissolved Solids(TDS)mg/mL	1369	Total phosphorus(TP) in ppm	1.14
Total Solids(TS)	1788	Sulphate in ppm	186
Turbidity,NTU	124	Total Hardness in ppm	637
Dissolve Oxygen(DO) mg/mL	0.62		

In the present study blue green algae (O.chlorina) tolerated higher concentration of paper mill effluent and showed remarkable growth in higher concentration in comparision to green algae (S.quadricauda). The enhanced growth of blue green algae in paper mill effluent may be due to excess amount oxidizable organic matter, nutrients, Low DO, high P^H, temperature which may favour the growth of Cyanophycean algae. In our study blue green algae have showed positive correlation with the above parameters except DO. The above findings clearly suggest that the effects of paper mill effluents are species dependent. Some species have better adaptability to higher concentration but some were not.
Experimental investigation by Adhikari (1997) demonstrated that Oscillatoria chlorina can grow well in organically polluted water and can successfully reduce the level of COD in industrial effluents. In our study the luxuriant growth of Oscillatoria chlorina was noticed in paper mill effluent both in field as well as in laboratory .Therefore these species could be utilized for biological treatment of paper mill effluents and also used for abatement studies.

REFERENCES

Anonymous, 1999: Corncern over pollution caused by Nagaon Paper Mill (NPM). *The Assam Tribune, 3rd, Jan/1999.*

Baruah, B. K. and Das, M. 2001: Study on plankton as indicator and index of pollution in aquatic ecosystem receiving paper mill effluent. *Ind. J. Env. Sci. Vol.5 No.1*, pp.41-46.

Rigol. A., Lacorte. S., Barcelo D. (2003): Sample handling and analytical protocol for analysis of resin acids in process waters and effluents from pulp and paper mills. *Trends in Analytical Chemistry* .22(10): pp.738-749.

Walsh, E.B. 1980: Ecological effects of waste water: Applied limnology and pollutant effects (second edition). Chapman and Hall, London.

Barbour, M.T.; Gerritsen, J.; Snyder, B.D.; Stribling, J. B. 1999: Rapid Bioassessment Protocols for Use in streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. Second Edition. U.S. Environmental

Wong, S.L. 1995: "Algal Assay Approaches to pollution studies in Aquatic Systems", in B.C. Rana (ed.), Pollution and Bio-monitoring. Tata McGrew-Hill publishing Company Ltd., New delhi, pp. 26-51.

APHA, 1998: Standard Methods for the examination of Water and waste water, *American Public Health Association*, Washinton, D.C.

APHA, 1989: Standard Methods for Examination of Water and waste water. *American Public Health Association* (17th edition). Washington D.C

US. EPA .2002: Biological assessment and criteria: Critical Component of Water Quality Programme. EPA 822-F-02-006.

Skulberg,O.M, 1962: Algal problems related to the eutrophication of European water supplier and a bioassay method to assess fertilizing influences of pollution of inland waters.In: Algae and Man.ed.by. D.F.Jackson, 262-299.Plenum Press, New York.

Gopinathan, C. P., V.K. Pillai and J.X. Rodrigo, 1994: Influence of thermal effluents on the growth characteristics of phytoplankton in the waters of Tuticorin Bay. In: *Nutrients and bioactive substance in aquatic organisms* , Society of Fisheries Technology-India, 235-246

Wong, S.L. 1995: "Algal Assay Approaches to pollution studies in Aquatic Systems", in B.C. Rana (ed.), Pollution and Bio-monitoring. Tata McGrew-Hill publishing Company Ltd., New delhi, pp. 26-51.

Eloranta, P. 1994: Phytoplankton recovery after cease of sulphite pulp mill effluent load into a water course. *Verh Int Ver Limnol*. .25:pp1526-9.
Kobbia, I.A., R.M.Met wall and H.M.EI-Adel, 1995: Influence of water effluents of soap and oil factory at Benha on Nile phytoplankton communities. *Egypt. jour. Bot*.35 (1):pp 45-57.
Dash, A.K. and Mishra , P.C. 1999: Growth response of the blue green alga, *Westiellopsis prolifica* in sewage enriched paper mill waste water. *Rev.Int.Contam. Ambient*, 15(2):pp79-83.
Tang, G., Readford, K., Harris, S. 2000: Algal bioassay on the fairway pond of wakehurst Golf Course .In: UTS Freshwater Ecology Report. Dept. of Environ. Sci. University of Technology, Sydney.
Reemol,S.A.,2004: Studies on toxicity of industrial effluents on phytoplankters. Ph.D. Thesis M.G.University, Kottayam.pp.92-93
Hall, T.J., Ragsdale, R.L., Arthurs W.J., Ikoma, J., Borton, D.L. 2009: A long term multitrophic level study to assess pulp and paper mill effluents on aquatic communities in four US receiving waters: characteristics of the study streams, sample sites, mills, and mill effluents. *Integr Environ Assess Manag* 5(2):pp199-218.
Nicolas, V and John, I. 2006: A preliminary study of toxicity by bioassay of the waste of pulp and paper production units. Ecological Risk A M S. *Springer journal,* pp (307-314).