THE IMPACT OF LEAD ON GROWTH, CHLOROPHYLL AND PRODUCTIVITY POTENTIALS OF *TETRASELMIS GRACILIS* (Kylin)

ABSTRACT

The impact of varying concentrations of lead, one among the major pollutants in aquatic environment, on a tetraflagellate *Tetraselmis gracilis* was studied. Though the pollutant inhibited cell synthesis and reduced chlorophyll concentration the impact was the maximum on primary organic production. Even at a very low concentration of 0.001 ppm the cut in potential production was estimated to be 97%.

INCREASED industrialization and domestic exercises have accelerated the deposition of heavy metals into Cochin estuarine ecosystem where the major primary producers are planktonic algae. The response of these autotrophic flora to the metal pollutants varies with species and many of them are generally very much sensitive. The heavy metals inhibit the growth and production of planktonic algae. Since any effect on the lowest level of food-chain will have consequences on higher trophic levels planktonic algae are quite suitable for the assessment of the effect of toxic substances.

The impact of heavy metals such as Cu, Hg, Mn, Cd, Zn etc. has been studied using algae as test organisms. Very little has been done on the impact of lead which is one among the major pollutants in the environment. Megalhaes *et al.* (1993) performed radiotracer studies of lead accumulation by *Padina gymnospora*, a brown seaweed. There are a few studies, carried out elsewhere (Schulze and Brand, 1978; Silverberg *et. al.*, 1977) on the impact of lead on microalgae. But no work has been done on the effect of lead on *Tetraselmis gracilis* (Kylin).

The alga used for the impact study was *Tetraselmis gracilis*, a green tetraflagellate isolated from the Cochin estuary by exploiting their phototactic movements and maintained in Walne's medium. Stock solution of 1000 ppm of lead was prepared from analytical reagent salt of lead.

One and a half litres each of Walne's medium was taken in six 2 litre capacity conical flasks. One of these was taken as control. To the remaining five flasks suitable aliquots of stock solutions of lead were added so as to contain 0.001 ppm., 0.01 ppm., 0.1 ppm., 1 ppm. and 10 ppm. lead respectively. A known volume of *Tetraselmis gracilis* culture (30 ml) with known concentration was added to each flask. These flasks were exposed to intermittent light (fluorescent tube) and dark periods of 12 hrs each.

Initial concentrations of cell numbers, oxygen and pigment were estimated. Besides cell concentration, oxygen evolution and pigment concentrations in all the six flasks were estimated at regular intervals. Cell concentration was determined using a haemocytometer, chlorophyll *a* concentration was estimated spectrophotometrically and primary production by Light and Dark bottle oxygen method (Strickland and Parsons, 1972).

This study has sought to understand the impact of a heavy metal on the growth, photosynthetic pigment and productivity of aquatic flora in Cochin estuary. Among heavy metals, lead has been chosen for the experiment as it is a common pollutant, the ecotoxicology of which has not been done much. *Tetraselmis gracilis*, a tetraflagellate has been selected as the test organism since it is a common primary producer in the estuary. The impact of varying concentrations of lead, ie, 0.001, 0.01, 0.1, 1.0 and 10 ppm were studied against uniform concentration of test organisms. The results were presented in Tables 1-3.

The initial concentration of the algae in the control and other containers with lead were 15,000 cells/ml each. In the control and upto 0.1 ppm lead the growth rate was found to be more or less uniform. Infact the pollutants at lower concentrations appear to enhance the growth rate. At higher concentrations the growth was found to be inhibited very much. With 1ppm lead on the second day the growth was only about 38% of that in the control. Hence the inhibition is 62%. Consequently the inhibition rate was found to be decreasing indicating the adaptation of the algae to the pollutant. Thus the inhibition of 62% observed on the second day was gradually decreased to 9% on the sixteenth day.

With 10 ppm lead the inhibition on the growth as measured by the cell concentrations was 75% on the second day as against 62% observed against 1 ppm of lead. On the fourth day the inhibition was decreased to 37%. The algae appeared to have tolerated the toxicity to some extent as indicated by the increase in the cell numbers (Table 1).

The initial concentration of chlorophyll *a* in all the flasks was 61.4 µg/l. After two days in control it was increased to 160.2 µg/l. With 0.001 ppm lead, the corresponding value for chlorophyll was 164.4 µg/l. With higher concentration of lead, a gradual decrease in chlorophyll is observed. The lower concentration appear to have no significant impact on chlorophyll *a*. With regard to chlorophyll *a* there was an initial inhibition. Thus on the second day with 0.01 ppm the inhibition of chlorophyll *a* was 27%, for 0.1 ppm lead it was 50% and for 10 ppm lead the inhibition was 30%. On subsequent days chlorophyll concentration was found to be enhanced (Table 2).

Initial gross productivity of the microalgae is estimated to be 0.06 mgC/l/hr and the net productivity is 0.03 mgC/l/hr. The production gradually increased to 3.22 mgC/l/hr on the 12th day and thereafter showed a decrease in production. More or less the same trend is exhibited in all other microalgae culture with varying concentration of lead. In all the cases the maximum productivity was recorded on the 12th day. The difference was observed only in the magnitude of production. With increasing concentration of lead the productivity showed a progressive decrease. When maximum production of 3.22 mgC/l/hr was obtained for the control, the values slightly decreased to 3.11 mgC/l/hr for 0.001 ppm, 0.25 mgC/l/hr for 0.01 ppm, 0.1mgC/l/hr for 0.1 ppm, and 0.23mgC/l/hr for 10 ppm lead. The culture with 10 ppm lead was found to be affected most as projected by comparatively very low values (Table 3). The results indicate that the productivity potential of *Tetraselmis gracilis* is inhibited very much with varying concentrations of lead.

In control the initial net production was 0.03 mgC/l/hr. A lag in the productivity was observed on the second day as the production being 0.05 mgC/l/hr. Then on the fifth day there was an increase of productivity, the value being 0.13 mgC/l/hr. On the twelfth day the peak production of 3.22 mgC/l/hr was recorded and on the nineteenth day a decrease in the production (0.12mgC/1/hr) obtained indicating

TABLE 1. Effect of Lead on Growth of *Tetraselmis gracilis* (cells/ml.)

No. of days	Control	0.001 ppm	0.01 ppm	0.1 ppm	1 ppm	10 ppm
0	15,000	15,000	15,000	15,000	15,000	15,000
2	40,000	30,000	25,000	25,000	15,000	10,000
4	40,000	48,000	25,000	25,000	25,000	25,000
5	43,000	48,000	33,000	33,000	30,000	25,000
6	53,000	45,000	40,000	40,000	40,000	28,000
8	63,000	50,000	58,000	43,000	43,000	33,000
9	80,000	93,000	60,000	60,000	58,000	57,000
10	70,000	68,000	75,000	75,000	75,000	50,000
11	83,000	90,000	75,000	75,000	75,000	73,000
12	135,000	105,000	115,000	145,000	90,000	62,000
13	115,000	123,000	87,000	95,000	95,000	75,000
16	115,000	250,000	90,000	125,000	105,000	46,000
19	158,000	263,000	225,000	155,000	125,000	—

the stationary phase. A substantial arrest in the productivity potential was observed on the twelfth day when the production in the control was 3.22 mgC/l/hr. With a lower concentration of 0.001 ppm lead practically there was no change in production.

TABLE 2. Effect of Lead on Chlorophyll *a* Concentration (μg/l)

No. of days	Control	0.001 ppm	0.01 ppm	0.1 ppm	1 ppm	10 ppm
0	61.41	61.41	61.41	61.41	61.41	61.41
2	160.2	164.4	118.5	80.4	112.9	—
6	329.7	431	378.9	338.8	547.4	364.5
12	380	318.7	348	400.16	451.13	320.4
19	200	255.1	285.6	366.3	251	101

On the twelfth day with 0.001 ppm lead the production was reduced to 97%. With 0.01 ppm the production was reduced to 9% of that in the control, showing an inhibition of 91% of the production. With 0.1 ppm lead the net production recorded was only 7%, the percentage of inhibition was 93%. With 1 ppm lead the production was only 4%, the inhibition being 96%. In the case 10 ppm lead there was practically no production at all:

TABLE 3. Effect of Lead on Primary Productivity (mgC/l/hr.)

No. of days	Control	0.001 ppm	0.01 ppm	0.1 ppm	1 ppm	10 ppm
0	0.03	0.03	0.03	0.03	0.03	0.03
2	0.05	—	0.03	0.01	0.14	—
5	0.13	0.9	0.33	0.09	0.17	0.04
12	3.22	3.11	0.28	0.23	0.14	—
19	0.12	0.11	—	0.09	0.072	—

Since the microalgae forms the first link in the estuarine food chain, any amount of inhibition or reduction on the productivity potential induced by the heavy metal pollutant would affect the magnitude of the entire renewable fishery recourses in the ecosystem. As any effect on the lowest level of the food chain will also have consequences on the other trophic levels, micro-algae, especially as the one which is used as a test organism for the present study is very suitable for the determination of the impact of toxic substance on the aquatic environment. Micro-algae accumulate pollutants especially some trace metals from the surrounding waters and these metals in turn are integrated to the higher trophic levels through the food chain. The rate of accumulation varies with the species. Once the algal species with high rate of accumulation of pollutants are identified it would be a significant step towards the detoxification of natural waters.

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