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Eco-Physiological Effects Of Red Mud Waste On The Pigment Content Of A Blue-Green Alga, *Anabaena Cylindrica*, Lemm.

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ABSTRACT

The present piece of work was planned to study the impact of the RMWE / leached effluent of Alumina industry (NALCO) situated at Damonjodi, Koraput on a Blue-green alga, *Anabaena cylindrica*, Lemm and its environmental significance. The effluent of the industry was tested and found to be deadly toxic. The effluent along with the red mud wastes generated from the industry was dumped in the Red mud pond for storage and for natural evaporation. The red mud waste collected from the red mud pond was brought to the laboratory, air dried and powdered. The RMWE is almost equivalent to the leached effluent coming out from the red mud pond. The toxicity test was conducted to assess the lethal concentration values for the RMWE on this alga. Toxicity test revealed that the effluent waste coming out from the red mud pond is highly toxic in nature. The sub-lethal concentration of red mud waste extract was 0.325% (v/v) for this alga under laboratory controlled condition. During experimental period the dry weight significantly decreased with the increase in exposure period. The control alga showed normal growth during the experimental period. The total chlorophyll content decreased with the increase in exposure period. Total pheophytin content and carotenoid content followed a similar trend like total chlorophyll. No dual behavior was observed during the study. When the exposed alga was transferred to toxicant free medium, no recovery of the alga was noted. From the observed results it can be inferred that the red mud waste extracts was deadly toxic and should be allowed to leak from red mud pond. This leached red mud waste after leakage enters into the neighboring areas and enters in to the crop fields causing immense damage to the rice crop and killing the inhabitants of the crop field like blue-green alga. This alga fixes atmospheric nitrogen and a farmer friendly alga disappeared from the contaminated crop fields. All care should be taken to stop leaching from the red mud pond and the red mud waste should be carefully protected to avoid further damage to the surrounding environments.

Keywords: Alumina industry, red mud waste, RMWE, toxicity, dry weight, pigments

Introduction

Industry is responsible for creating a wide spectrum of new chemicals every year all of which eventually find their way into the environment. For most of these chemicals, not even the chemical formulae are known and much less are known about their acute, chronic or genetic impact on plants and animals. At present, the industry is the focus of attention, the world-over, as strongest polluter of the environment. Every underdeveloped or developing country aiming to be highly developed, explore nature and tried to find out methods and adopted industrial revolution, leaving behind the consequence of such trend. When the problems of environmental status and quality was taken into consideration it became apparent that, it was not enough to alleviate undesirable consequences of man's activities by technological means or to prevent them forbidding certain practices of the are of certain chemicals. Rapid industrialization and exploitation of natural resources on massive scale, accumulated undesirable substances in huge quantities in the environmental segments, thus, pollute the environment significantly affecting all microbial flora and fauna, plants, animals including man. The field under study refers to the Alumina industry specifically to Mining and Refinery complex, NALCO,

Damanjodi in the district of Koraput, Orissa, India. For analysis of various factors of pollution in alumina industry it becomes imperative for us to discuss the manufacturing process from ore mining to refining in brief with reference to NALCO. The alumina is extracted in two important stages i.e. one during mining operation & the second from the ore refinery. Installation of National Aluminium Co. Limited (NALCO) is a major step towards self sufficiency in good quality of Alumina production. After all the stages of reactions of bauxite refining process the red mud is finally generated. For 8 lakhs MT alumina production 10 lakh MT of red mud is generated and is being canalized in to red mud pond by 2nd stage pump in slurry form at the rate of 370m^3/ hr with 150gps solid consistency and 2.3gpl Na_2O the dry red mud flow in to the Red Mud Pond is 110MT/hr. For storing red mud waste a pond was constructed near refining complex towards the west side of the industry surrounded by natural hills on all sides. At the opening side a dam is constructed by the company to store the red mud waste.

The industry under study:

(Photographs (Source-Google earth): Showing the location of the industry, red mud pond and lechtes from the RMP and affected areas.)

(Photographs: NALCO industry, Red mud pond and lechate leaking from the dam site)

The mines and refinery complex of NALCO, Damonjodi is situated at Similiguda block, under Potangi tahasil in the district of Koraput, Odisha state, India. From the district head quarter Koraput, it is 38 kilometers towards south-east on road, i.e. 27kms towards south in NH-43 up to Similiguda junction and further 11kms towards east on project road. It is 60 kms from Jeypore, the oldest city of Koraput district. Damonjodi is at a highest of about 1300 mts. from sea level, located at latitude 18^0-6'-18^0-58' towards North and longitude 82^0.57'- 83^0.04' East. The area enjoys an annual rainfall of 1723-1855 mm. The area enjoys a modest climate with little high rainfall when compared to other areas of Koraput district. Panda *et al*., (2017, 2018) reported that the red mud waste is deadly toxic with following composition: Chemical properties: Typical = Al_2O_3(%)- 98.7; Na_2O(%)- 0.38 ; Fe_2O_3(%)- 0.01; SiO_2(%)- 0.012; and CaO(%)-0.042. Alumina hydrate: Physical properties: Typical:LOI (110-1000^{o}C)%-34-36, Moisture-3-6; Granulometry: Typical-45Micron(%)-3-6. Chemical properties: Typical = Al_2O_3 (%)- 65±0.5, Na_2O(%) Total-0.23-0.30, Na_2O (%) Soluble-0.015-0.025, SiO_2(%)-0.007-0.010, Fe_2O_3(%)- 0.006-0.008 & Hydrate Content- 99.0%.

Keeping in view; the discharge of red mud effluents of the industry into red mud pond and leaking of lechate chemicals from the red mud pond of the industry in to the environment and its entry into nearby crop fields drew our attention. During rainy season entry of these chemicals and lechate along with runoff water and over flow of runoff water of the paddy fields, their entry into fresh water bodies like fish ponds, canals, rivers and the water reservoir of the Upper Kolab hydroelectric cum irrigation project, this project was planned. During field study it was observed that the lechate chemicals enter in to the surrounding crop fields,

where rice is cultivated. The farmers complained that the red mud waste lechates destroyed their crop production and the rice colour changed to brown with filthy smell. We visited the crop fields of the contaminated site nearer to the red mud pond. We observed that the complain raised by the farmers was right. We entered in to the crop fields and found that no blue-green algae were found. We enquired that earlier days prior to establishment of the industry blue-green scums were seen in the crop fields during cultivation time. But after establishment of the industry and red mud pond, the Blue-green scum disappeared. Basing on the field observation, this project was aimed to evaluate the impact of the leached waste of Red Mud Pond / red mud waste extract (prepared in the laboratory on the growth and pigment content of a blue-green alga under laboratory controlled conditions.

Materials & Methods

Anabaena cylindrica, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO_2 and evolve O_2 where as heterocysts are unable to fix CO_2 or evolve O_2 but can fix nitrogen under aerobic condition. Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) was most suitable for the organism. It was used as the basic culture solution in all the experiments in the present study. The experimental algal cultures were grown under controlled conditions of light and temperature inside a culture room. The culture flasks were kept in series on a culture rack, of glass plate with iron frame. Light was provided by means of white fluorescent tubes, connected at the backside of glass plate of each rack, which illuminates the upper glass surface at the intensity of 2400±200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and was maintained at 28± 2^0C. The culture flasks were regularly hand shaken twice a day to avoid clumping of the cells as well as their adhesion to the wall of the conical flasks.

The pollutant: Red Mud Waste from Red Mud Pond.

The red mud collected from RM pond is brought to the laboratory. The slurry was air dried, powdered and sieved. Red mud waste powder was kept in plastic containers for laboratory experimental study. A known (2kg.) quantity of red mud dried powder is mixed with known amount (2lit.) of distilled water in 5liter glass jar and stirred for 15 days with the help of Remi stirrer with medium speed for one hour and allowed to rest for 2hours. The same process was continued for the whole day. It was allowed to settle over night. The process of stirring was repeated for 15days. After 15days the supernatant was decanted and filtered through a Tea strainer (plastic filter) to remove visible suspended particles. It was kept inside a refrigerator for future use. On the day of use the supernatant extract was again passed through a multi-sieve soaking and filtering system and the obtained extract is the extracted leached chemical of the waste, which is used for the experiments. This is the red mud waste extract (RMWE) and used as the toxicant for tests.

Inoculation: One ml of unialglal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100 ml of solution, inside the inoculating chamber. The number of individual cells of the algae present in one ml of the culture medium after micro-tissue homogenization was counted under the microscope. The test algae were exposed for a period of 15 days in different test medium after which their survival and mortality percentage were calculated by counting the number of cells present in one ml of the test solution after micro-tissue homogenization. From this, different survival percentage and mortality percentage, the lethal concentration value were determined. Growth measurement was done by recording the dry wt of the alga per 100 ml culture. Exponentially growing algal suspension of same age, volume and biomass was inoculated initially into the experimental flasks. Dry weight of the alga in the culture flasks was estimated centrifuging in a refrigerated centrifuge (High speed centrifuge, Remi) at 8000 rpm for 10 minutes. The algal pellet was transferred to a pre-weighed glass cover slip. It was dried in an oven at 60^0C for 24 hours, cooled in desiccators and the final weight of the glass cover slip was recorded in a single pan electric balance. The data were expressed as mean of 5 samples ± standard deviation in mg / 100 ml culture. The amount of total chlorophyll, phaeophytin and carotenoid content present in the alga was estimated and calculated by following the procedure of Vernon (1960) and Davies (1976). The obtained data were expressed as the mean ± standard deviation as µg/50ml algal culture.

Results

A graded series of concentrations of the toxicant, Red mud waste extract was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. Unialgal, axenic culture of *Anabaena cylindrica*, Lemm was inoculated and the survival percentage was determined. The following concentrations were selected for further detailed studies pertaining to the effects of Red mud waste extract on the blue-green alga.

Table - A: Toxicity table showing deduced lethal concentration values after 15 days of exposure.

Lethal Concentration (LC)	Toxicant Concentration (%) v/v	Percent Survival (PS)
LC_0	2.45%	PS_{100}
LC_{10}(A)	3.0%	PS_{90}
LC_{50}(B)	5.0%	PS_{50}
LC_{90}(C)	7.5%	PS_{10}
LC_{100}	9.2%	PS_0

In the above cited tables LC_{10}, LC_{50} and LC_{90} were expressed as A, B & C respectively and 'Con.' stands for control. Table - A indicates that with the increase in concentration of the toxicant (Red mud waste extract) the survival percent decreased significantly showing a negative correlation. The above three lethal concentrations were chosen to study the differential effects of different concentrations of the toxicant (Red mud waste extract) on the blue-green alga, *Anabaena cylindrica*, Lemm. The below data showed the changes in growth pattern of the alga, when exposed to different sub-lethal concentrations of Red mud waste extract at different days of exposure and recovery. The toxicant did not bring about any significant morphometric change in the exposed alga, as observed by the naked eye. However, at higher concentration of the toxicant, bleaching of the algal mass was observed. Control and 'A' experimental flasks did not show any change except a normal growth, with the increase in exposure period and concentrations of the toxicant. An initial bleaching of the filaments leading to drastic decline in growth was marked. The bleached condition continued till the exposed algal materials were transferred to toxicant free nutrient medium. At higher concentrations of the toxicant the BGA mass along with its mucilaginous sheath was covered by light yellowish brown colour deposits. After 85-105 days of recovery, tiny blue-green particles started making their appearance, in the white turbid mass, the tiny particles grew in size, the blue-green colour was due to the appearance of photosynthetic pigment, which disappeared due to the toxicant stress on the organism at lower concentration of the red mud waste extract. Slowly the entire white mass got converted into a blue-green mass, which did not show the normal growth and function. The appearance of blue-green colour in recovery studies can be correlated with the accumulation and effects of the toxicants in exposure period and subsequent excretion or degradation in recovery period. At higher exposure period and at higher concentrations of the toxicant, red mud waste extract, even after 120 days of continuous flux recovery, no recovery was marked. The coloured turbid mass remained as such for a long time and later this mass started degradation leading total disintegration of the algal mass. This indicated total death of the organism due to acute toxic nature of the toxicant. Probably detoxification was not caused by the exudates of BGA in the recovery experimental flasks.

The growth pattern of control and exposed alga *Anabaena cylindrica* were analyzed by dry weight analysis. Control set showed the existence of a positive correlation with the exposure period in case of dry weight ($r = 0.995$ $p \leq 0.001$). At 3.0% Red mud waste extract, the dry weight of the exposed alga increased with the increase in exposure period, showing a significant positive correlation ($r = 0.989$, $p \leq 0.01$). But the values were much less than the control values. At LC_{10}, no significant initial increase followed by decrease in dry weight over the control value was marked at 15 d exposure period and no insignificant increase in the recovery periods was marked. On 15^{th} day of exposure, the dry weight depleted from 15.5±0.4 to 10.4±0.6mg/50ml culture, showing a maximum depletion by 32.9%. The rate of growth or increase in dry weight depleted with the increase in exposure period and in recovery period, no partial increase was marked, rather further depletion in the values were obtained and a maximum of 39.9% decrease was recorded on 15^{th} day of recovery. However, the data line did fall below the control values. At LC_{50} (B), a gradual increase in dry weight was marked with the increase in exposure period up to 15 days of exposure ($r=0.956$; $p \geq 0.01$). When compared to the control set, a significant decrease in dry weight was marked. On 15^{th} day of exposure, 57.4% decrease and on 15^{th} day of recovery, 66.7% decrease over the control value was observed. Interestingly, during recovery period, instead of showing any sign of recovery, higher percentage of decrease in the dry weight value indicated acute poisoning or stress. All the data were statistically significant. At LC_{90}, (C) significant decreasing trend was marked. The dry weight (mg) of exposed alga was significantly less than the control alga, at all exposure periods and recovery periods. The dry weight decreased from 15.5±0.4 to

0.6±0.3mg/ 50ml culture after 15 days of exposure, showing a maximum of 96.1% depletion when compared to the control value. When the exposed alga was transferred to toxicant free medium, instead of showing any recovery, the dry weight further depleted from 24.3±0.7 to 0.2±0.1mg/50ml culture after 15 days of recovery, where a maximum of 99.2% decrease was noted. Non-significant negative correlation exists between exposure period and dry weight of the alga (r = -0.513; p = NS), at LC_{90} of Red mud waste extract. A maximum of 96.1% decrease on 15th day of exposure and 99.2% decrease on 15th day of recovery over the control value was marked. The correlation coefficient analysis between days of exposure and dry weight of the control and exposed alga, exposed to different concentrations indicated the existence of a positive significant correlation in control (r = 0.995; p≥0.001), Conc. A (r = 0.989, p ≥ 0.01) and Conc. B (r = 0.956; p≥0.01). A negative but no significant correlation (r = - 0.513; p= NS) existed between days of exposure and dry weight of the alga in Conc. C (7.5%). The percent change in dry weight showed all positive and highly significant correlation coefficient values except in case of conc. A. During recovery studies, no recovery was marked in all the three concentrations. No recovery during recovery studies clearly indicated that the damage caused by the toxicant to the system was a permanent one. No temporary depression or inhibition might have taken place in the exposed system like other toxicants. This clearly indicated the acute toxic nature of the toxicant, red mud waste extract drawn from the red mud waste. The two-way analysis of variance ratio test based on the data of dry weight indicated the existence of significant difference between rows and columns. Control set showed a regular trend of increase in chlorophyll content with the exposure period. The total chlorophyll content increased from 0.027±0.004 to 0.108±0.009 mg / 50ml culture within 15 days of exposure and the pigment further increased to 0.151±0.010 mg /50 ml culture in the next 15 days of recovery. However, at LC_{10} (3.0%) no initial increase in chlorophyll content was marked over the control value but the value declined with the increase in exposure period. The total chlorophyll content decreased from 0.108±0.009 to 0.088±0.009mg/50ml culture within 15 days of exposure and the pigment further increased to 0.128±0.014mg/50ml culture in the next 15 days of recovery. The chlorophyll content increased during the recovery period but the values were less than the control values. At 5.0% (LC_{50}) of RMWE, the chlorophyll content depleted significantly when compared to control values at all exposure periods. The total chlorophyll content decreased from 0.108±0.009 to 0.053±0.004 mg / 50ml culture within 15 days of exposure and the pigment further increased to 0.064±0.004 mg /50 ml culture in the next 15 days of recovery. However, the percent change in chlorophyll content with the increase in exposure period showed a positive correlation. With the increase in exposure period, the chlorophyll content increased. In contrast, at 7.5% (LC_{90}) after exposure, the chlorophyll content reduced drastically, even the values were much less than the inoculated value of '0' day of exposure. The total chlorophyll content decreased from 0.108±0.009 to 0.007±0.002mg/50ml culture within 15 days of exposure and the pigment further decreased to 0.011±0.005mg/50 ml culture in the next 15 days of recovery. When the exposed alga was transferred to toxicant free nutrient medium, the exposed alga (Conc. C) could not recover altogether to its control value. The total chlorophyll content declined by 18.5%, 50.9% and 93.5% after 15 days of exposure, at conc. A, B and C, respectively. When the exposed alga was transferred to toxicant free medium, no significant recovery in the chlorophyll content was marked. On 15th day of recovery, 15.2%, 57.6% and 92.7% decrease, when compared to respective control value was marked. On 5th day of recovery, no recovery was noted. On 15th day of recovery, 3.3% and 0.8% recovery was noted at Conc. A and C where as, at Conc. B, no recovery was seen. The percent increase / decrease of chlorophyll content of the exposed alga, exposed to various concentrations of Red mud waste extract, when compared to its control value. At 3.0%, a significant decrease up to 15th day of exposure was marked. At this concentration, significant decreasing trend was recorded, but a maximum of 18.5% decrease was recorded on 15th day of exposure. No recovery was marked. However, at 3.0 & 7.5% of Red mud waste extract, a negative correlation between exposure period and chlorophyll content was marked. With the increase in exposure period, a highest 50.9% decrease on 15th day of exposure at LC_{50} (5.0%) and 93.5% decrease on 15th day of exposure at LC_{90} (7.5%) was observed. Maximum decrease by 95.9% was recorded on 5th day of recovery, showing permanent damage to the system. The correlation coefficient analysis between days of exposure verses total Chlorophyll indicated the existence of significant positive correlation in control (r = 0.997, p≥0.001), Conc. A (r = 0.989, p≥ 0.01) and Conc. B (r = 0.971, p≥0.01). A negative correlation (r=-0.925, p≥0.05) was marked in Conc. C. The correlation coefficient analysis between percent change in chlorophyll content verses days of exposure were all positive significant (p ≥ 0.01) in Conc. A & B but in case Conc. C , highly significant (p≤0.001) correlation was marked. In case of Conc. A, a maximum of 18.5%, in Conc. B 50.9% and in case of Conc. C, a maximum of 93.5% decrease, over the control value was marked. When the exposed alga was transferred to toxicant free medium for recovery studies, no recovery was marked. This non-recovery or higher rate of inhibition during recovery period indicates the acute toxic ness of the toxicant used for the study. The two-way analysis of variance ratio test indicated the existence of significant difference between rows and non-significant difference between

columns). Spectral analysis of the acetone pigment extract was carried out to study the possible shift of peak of the pigments and behavior of the spectra. Control pigment extract showed a normal spectrum. However, in the exposed pigment extracts interesting changes were noted. No clear shift in peak and drastic depletion in peak height were marked in all the three selected concentrations, when compared to the control value. The optical density value declined at all wavelengths, when compared to the optical density of the control pigment extract.

Changes in the total phaeophytin content of *Anabaena cylindrica* after exposure to different concentrations of toxicant, Red mud waste extract, at different days of exposure and recovery. Control set showed a positive correlation with the increase in exposure period and showed a steady increase after the very first day of inoculation for the entire period of exposure. Identical increase in phaeophytin content was marked in case of LC_{10} (A) & LC_{50} (B) of Red mud waste extract, whereas C (LC_{90}) showed drastic decline in phaeophytin content at all exposure periods. In all the exposed flasks, decline in phaeophytin content was observed when compared to the control values. The total phaeophytin content decreased from 0.099±0.004 to 0.059±0.005mg/50ml culture within 15 days of exposure and the pigment further increased to 0.101±0.004 mg /50 ml culture in the next 15 days of recovery in Conc. A. The total phaeophytin content decreased from 0.099±0.004 to 0.042±0.005 mg / 50ml culture within 15 days of exposure and the pigment further increased to 0.074±0.003 mg /50 ml culture in the next 15 days of recovery in Conc. B. The total phaeophytin content decreased from 0.099±0.004 to 0.015±0.004 mg / 50ml culture within 15 days of exposure and the pigment further decreased and in the next 15 days of recovery increased to 0.015±0.003 mg /50 ml culture in Conc. C. The total phaeophytin content decreased from 0.099±0.004 to 0.059±0.005 mg / 50ml culture within 15 days of exposure and the pigment further increased to 0.101±0.004 mg /50 ml culture in the next 15 days of recovery in Conc. A. No significant recovery was marked, when the exposed algae were transferred to toxicant free nutrient medium, except in conc. A, where a maximum of 5.1% recovery, when compared to 15d exposure value was marked. At 3.0% RMWE, 40.4% decrease in phaeophytin content was marked on 15th day of exposure over the control value. At 5.0% toxicant concentration 57.6% decrease and at 7.5% toxicant concentration, 84.8% decrease in phaeophytin content was marked over the control value on 15th day of exposure. The percent decrease in phaeophytin content increased with the increase in exposure period and increase in toxicant concentration. No significant recovery was marked in any experimental exposed cultures. When the exposed alga in Conc. A, B, & C were transferred to toxicant free medium, instead of showing any positive recovery, the depletion of phaeophytin content further increased and a maximum of 52.6% in conc. B and 90.4% in conc. C was recorded after 15 days of recovery. The correlation coefficient analysis between phaeophytin content and days of exposure indicated the existence of positive and highly significant ($p \geq 0.001$) correlation in control, conc. A and in conc. B significant correlation ($p \geq 0.01$) was marked. But in case of conc. C a non-significant negative correlation was marked in concentration C (p=NS). The percent change in phaeophytin content showed the existence of negative significant correlation in all the three concentrations (A, B, C) studied. The ANOVA test indicated the existence of significant difference between rows and non-significant difference between columns.

The changes in the carotenoid content of *Anabaena* after exposure to different concentrations of Red mud waste extract at different days of exposure and recovery showed interesting results. Control set showed a steady increase in carotenoid content with the increase in exposure period, showing a positive ($r=0.996$, $p \geq 0.001$) correlation. The carotenoid content in the control set increased from 0.0022±0.0006 to 0.0121±0.0010mg/50ml culture within a period of 15 days showing a positive increase. During recovery period the pigment content further increased steadily and a maximum of 0.0174±0.0011mg/50 ml culture was recorded on 15th day of recovery. The carotenoid content in the Conc. A set increased from 0.0022±0.0006 to 0.0109±0.0007 mg / 50ml culture within a period of 15 days showing a positive increase. The carotenoid content was more than the control value up to 9th day of exposure. After 9th day, the pigment content declined, when compared to control. And on 15th day of exposure, the pigment content declined to 0.0109±0.0007mg/50ml culture. During recovery period the pigment content further increased steadily and a maximum of 0.0132±0.0008mg/50ml culture was recorded on 15th day of recovery. The carotenoid content in the Conc. B set increased from 0.0022±0.0006 to 0.0048±0.000mg/ 50ml culture within a period of 15 days showing a positive increase. The carotenoid content was less than the control value at all exposure periods. On 15th day of exposure, the pigment content declined from 0.0121±0.0010 to 0.0109±0.0007mg/50ml culture. During recovery period the pigment content further decreased and a maximum of 0.0079 ± 0.0010 mg / 50 ml culture was recorded on 15th day of recovery. The carotenoid content in the Conc. C set decreased from 0.0121±0.0010 to 0.0007±0.0002mg/50ml culture within a period of 15 days showing a significant decrease. The carotenoid content was significantly less than the control value at all exposure periods. On 15th day of exposure, the pigment content declined from 0.0121±0.0010 to 0.0007±0.0002mg/50ml culture. During recovery period the pigment content further decreased and a maximum of 0.0003± 0.0001mg/50 ml culture

was recorded on 15th day of recovery. At higher concentration (7.5%) of Red mud waste extract, a drastic decline in the pigment content was marked, when compared to the respective control values. Non-significant partial recovery in the rate, over the 15 day value was marked in B, whereas in conc. A & C, no recovery was marked, rather the values were much less when compared to inoculation day value. With the increase in concentration of the toxicant, the carotenoid content decreased, showing a negative correlation. No significant recovery was marked, showing a total destruction of the pigment in exposed alga. A maximum of 9.9% decrease on 15th day in 3.0% toxicant (A) concentration, 60.3% decrease on 15th day in 5.0% toxicant concentration (B) and 94.2% decrease on 15th day in 7.5% toxicant concentration (C) was recorded. When the system was allowed to recover in toxicant free nutrient medium, the carotenoid pigment level further depleted. Maximum 24.1%, 54.6% and 98.3% was recorded on 15th day of recovery at A, B, & C toxicant concentration, respectively. The correlation coefficient analysis between carotenoid content of control and exposed alga verses days of exposure indicated the existence of a positive correlation and the values were highly significant ($p \geq 0.001$), in control, Conc. A and Conc. B. A negative and significant ($r = -0.855$, $p \leq 0.05$) correlation was marked in Conc. C. The percent change in carotenoid content versus days of exposure in Conc. A , showed a non-significant negative correlation and in case of Conc. B and C, showed a negative but significant ($p \leq 0.05$) correlation. The ANOVA test indicated the existence of significant difference between rows and columns. The chlorophyll / phaeophytin ratio value initially increased up to third day and in higher exposure period, the ratio value decreased with the increase in exposure and recovery period. In case of conc. A, the ratio value showed an identical trend with that of control, however, the ratio values in conc. A. was much higher when compared to control value. In case of conc. B. the ratio value showed a similar trend with conc. A., but the ratio values were much less than control and Conc. A. Interestingly, in case of conc. C., the ratio value significantly declined with the increase in exposure period and also during recovery period. During recovery period, we could not observe any type of significant recovery in exposed blue-green alga. Significant increase in ratio value in conc. A and B indicated stress and the decrease in the ratio value indicated the toxic nature of the toxicant and the possible effect of the toxicant, red mud waste extract on the pigment content of the exposed alga.

Discussion

Algae are more sensitive than any other plants to complex wastes such as industrial and municipal effluents. Their use in bioassays is of ecological significance, since algae are the dominant primary producers in aquatic environments. A wide range of toxicity tests has been developed in the recent decades to predict the probable effects of new chemicals and industrial wastes in aquatic ecosystems utilizing different organisms such as algae, crustaceans, mollusks and fish (Miller *et al*., 1978). Kamp-Nielson (1971) demonstrated a time dependent effect of $HgCl_2$ (added to 30µg/liter) on the photosynthesis of *Chlorella pyrenoidosa*. Algae, the most important primary producers of the aquatic environments have received least attention. Very few references are available particularly on the toxicity effects and physiological changes induced by alumina industry waste on algae. The review made by Whitton (1970), Gadd & Griffiths (1978) and Sorentino (1979) on impact and effect of heavy metals on algae added a lot of information to the literature of algal toxicology. Algae have been shown to concentrate heavy metals to a larger extent (Say *et al*., I & II, 1977). Information's are available pertaining to the toxicity of mercury in the form of metal, mercury based pesticides, industrial wastes containing mercury etc. on fresh water blue-green algae (Rai *et al*., 1981a, b; Sahu, 1987). Agarwal & Kumar (1978) showed decrease in growth of *Chlorella* sp when exposed to mercurial effluent and solid wastes indicating toxic nature of mercury on the organism. A liquid industrial waste may affect the algal growth in any of three ways: stimulation, inhibition and stimulation at lower concentrations but inhibition at higher concentrations (Walsh & Alexander, 1980; Sahu, 1987). The enhancements of growth, heterocyst frequency and nitrogen fixation at lower doses of Furadon (0.75µg / ml of Carbofuran) have also been reported earlier. The present investigation did not agree with the above conclusions. But such stimulation in the growth cannot be easily explained at this stage of the study. Sahu (1987) attributed the reason for stimulation, for the presence of some growth regulating compounds and/or trace elements in the crude oil. Some suggested uptake and metabolisation of the constituent as the probable mechanism for growth stimulation.

Toxicological studies involve the science of poisons, their effects, antidotes and detection. Toxicity is the ability of a chemical molecule or compound to produce injury once it reaches a susceptible site in or on the body of the organism. In toxicity testing the laboratory bioassay in generally the most favored because experimental conditions can be controlled and the response of test organisms observed or monitored to a greater degree. Effects on organisms are generally categorized into those causing: a) direct lethal toxicity and b) sub-lethal disruption of behavioral or physiological or biochemical activities. Quantitatively lethal effects can be defined as those responses that occur when physical or chemical agents interfere with cellular and sub-

cellular processes in the organism to such an extent that death follows directly. In comparison, sub-lethal effects are those that disrupt physiological or behavioral activities but do not cause immediate mortality although death may follow because of interference with feeding, abnormal growth or behavior, lesser ability to colonize or other direct causes and effects. Measurements of lethality are frequently used to derive "safe" levels of exposure to toxicants. The assumptions adopted in lethality measurement are not well supported empirically and as an alternative, the use of chronic, sub-lethal tests may be more appropriate. Sub-lethal measurements are considered suitable for predicting safe level of toxicants. Toxicity tests were designed to find out safe level of toxicants and different lethal concentration values for a particular organism or for different types of organisms. The toxicity value varies from organism to organism. The present study indicated that cadmium is deadly toxic as observed from toxicity test. Dry weight measurement and optical density measurements were considered as the parameter of growth. Sahu (1987) reported that algal systems behaved very differently towards light scattering in presence of different stresses. Growth is a summation of all cellular metabolisms. So, any inhibition of growth reflects toxic effects on a number of metabolic processes. The paper mill effluent contained a significant amount of cadmium in its effluent. When the effluent was taken for toxicity study, it was observed that along with the accumulation of cadmium from the effluent waste it was difficult to ignore a possible accumulation of other ions from the effluent that might have played certain role in growth acceleration in lower concentrations and retardation in higher concentrations. It was also confirmed by earlier workers, who reported that mercury appears to be less toxic in media with a high concentration of dissolved salts. To confirm the effect of mercury in a combined form on the growth of the alga, the dry weight and optical density measurements are not enough and basing on these data, no clear cut presumption can be made. So further pigment particularly chlorophyll, physiological, biochemical and enzymological studies can reveal the detailed mechanism of the effect of the pollutant on algal system in greater details. In the present study the toxicant, cadmium chloride showed stimulation at sub-lethal concentration and inhibition at higher concentration, showing dichotomous behavior. Stimulation at very low concentration of cadmium chloride and appearance of chlorophyll during recovery period indicated that the alga at toxic environment could avoid the stress by some mechanism and in favorable conditions recovered fully. The recovery period was much more than exposure period. Red mud waste extract did not show any dichotomous behavior like mercury. In recovery studies, no significant recovery was observed at lower concentration and no recovery at all at higher concentration indicated that Red mud waste extract caused irreparable damage to the exposed system. Complete bleaching of the algal mass inside the test solution (C) was observed from 3rd day of exposure period onwards indicating the impact of cadmium chloride. Gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 45 days of recovery period. This recovery was probably due to excretion of absorbed waste from the algal body and depletion of chemical impact on the alga. These particles grew in size with time. It was probably due to the appearance of photosynthetic pigments which disappeared due to the Red mud waste extract stress on the alga. Slowly the entire white mass got converted into a blue-green mass with the increase in recovery period (120 days of recovery). Further physiological and biochemical studies pertaining to impact of Red mud waste extract on the BGA will help to understand the impact and mechanism of impact of red mud waste.

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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT
Prof. A.K. Panigrahi & Dr. Alaka Sahu for Conceptualization, planning and execution of the project, field visit, original draft preparation, supervision, reviewing and editing. Research work conducted by Sri Jayaram Bishoyie - analysis and related experimental work. Bishoyi contributed reagents, glassware, field related work, calculation and finalization of the data.

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