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Research Article

ECO-TOXICOLOGICAL IMPACT OF INDUSTRIAL EFFLUENT WASTE ON THE PHOTOSYNTHETIC EFFICIENCY ONA RICE FIELD INHABITING BLUE-GREEN ALGA UNDER LABORATORY CONTROLLED CONDITIONS

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A R T I C L E I N F O

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A B S T R A C T

Present study aims at understanding the impact of mercury contained industrial effluent along with solid waste on the pigment content and photosynthetic efficiency of a blue-green alga inhabiting crop fields under laboratory controlled conditions. With the increase in exposure period, on 15th day, the chlorophyll content decreased when compared to control value. The depletion in chlorophyll content on 15th day of exposure and non recovery on 15th day of recovery indicated stress. The increment on initial days of exposure can be inferred to be an impact of initial excitement because of the mercury contained toxicant. At this stage, it is premature to say that mercury at lower concentration caused stimulation or regulation in growth of the alga. The phaeophytin content increased with the increase in exposure period up to 12th day of exposure, when compared to respective control value. But on 15th day of exposure, the pigment content decreased when compared to control value. During recovery period even after 15 days of recovery, the exposed alga could not recover to its control value. These observations indicated that mercury contained toxicant might have shown an initial induction on the alga but at higher exposure period and higher concentration showed inhibitory effect. The increase in phaeophytin on 12th day of exposure was neither indicative of stimulation nor a case of induction, as both can be a possibility at the early stages of inoculation but not at the middle of the experiment or exposure. The observed results clearly indicated that the observed stimulation in photosynthetic rate was probably a case of excitation at the initial stages of the exposure but not an indication of stimulation or regulation. This experiment has shown the dichotomous behaviour of the toxicant, where stimulation at lower concentration and inhibition at higher concentration was observed. At all concentrations, inhibition in respiration rate was noted. Higher concentration of the toxicant induced inhibition due to accumulation of mercury in the algal body.

INTRODUCTION

Rapid industrialization to meet the demand of mankind has resulted in the rise of levels of pollution. To encounter this and to preserve the high quality of the environment new concept so called "Cleaner Production / Zero discharge" for waste minimization is being introduced, and technology was designed to prevent waste emission at the source of generation itself (Uwadiae et al., 2011). Developing countries like India and some others are now heavily industrialized to meet its own demands with most priorities to supply other financing countries worldwide. Chlor-alkali industries were a great concern all over the world because of release of elemental mercury along with its effluent and air exhaust from the cell house. The leaked mercury gets evaporated and the evaporated mercury from the cells during electrolysis operation gets precipitated on the surrounding environment and finally finds its way into water bodies. The precipitated mercury on flora and fauna gets washed by rain water and finds ints into water bodies and crop fields. Leakage of mercury and washings from the cell house containing mercury comes out in the effluent canal along with the effluent which is stored in the open stocking pond. This area is meant for air drying of the effluent naturally under sun. Chemicals' leaching from the stocking pond contaminates river water ultimately polluting the estuary and the sea. Solid waste which was collected from the effluent canal and dumped near the bank of the river gets drained and mixed with the river water during rainy season, heavily contaminating the river water, estuary and ultimately Bay of bengal. Reports are available indicating heavy pollution in all most all the river systems by industries in India due to improper management of the discharged chemicals and other materials (Modak et al., 1990 and Lokhande et al., 2011). The effluent of the industry containing significant

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amount of mercury has been continuously contaminating the river and the sea over the last 45 years and showed its damaging effect on the environmental segments even after the change of technology in 2012, from mercury cell process to membrane technology. Mercury pollution is a serious scenario all over the world (Panigrahi, 1980; Shaw et al., 1985 and 1986; Li et al. 2009, Padhy & Panigrahi, 2016, 2021 and Padhy & Panigrahi, 2023a,b). High pollutant load from the chlor-alkali industry directly discharged in to aquatic environments contaminate it and the surrounding land mass (Hall et al., 1987; Sahu et al., 1986). Percolation and leaching of the waste chemicals along with toxic metals caused ground water pollution thus contaminating the ground water source to a greater extent. According to Mwinyihija (2011) pollutants are the key factors that damage the ecology of the receiving terrestrial and the aquatic system of the vicinity of the discharge point from the industry.

It has been reported that mercury stress in plants decreases the chlorophyll content and damages leaf (Dunagan et al., 2007). Reports were concluding a significant depletion in crop yield and quality due to mercury exposure (Singh et al., 2015; Han et al., 2002). Heavy metals like mercury impacts change in biochemical processes in plants, including enzyme and antioxidant production, protein mobilization and photosynthesis. Enzyme destabilization, PS II abruption and disfunctioning of electron transport chain and mineral metabolism could be a possibility due to heavy metal stress in plants (Seneviratne et al., 2019). Plants uptake methylated mercury through their roots and store in their body preventing its way out (Tangahu et al., 2011; Patra and Sharma, 2000). Plant cells have mercury sensitive aquaporins (protein channels) that allow membrane transportation of mercury across the cells (Esteban et al., 2008). Studies have been conducted in pea seeds that exposure to mercuric chloride can induce closure of water channels (aquaporins) prior to growth (Kshetrimayum et al., 2017). The inhibitory effect vanished when mercury scavenging agents like dithiothreitol and β-mercaptoethanol were given along with HgCl2. Higher amount of mercury is reported in roots than aerial system (Ling et al., 2010). Plant leaves have also been reported to absorb mercury apart from roots (Ericksen and Gustin, 2004; Fay and Gustin, 2007). It has maximum adverse effects on plant physiology like inhibition of mitotic cell division in root tips, reduction in lateral root development, deformed synthesis of cell wall (Abraham and Damodharan, 2012; Eun et al., 2000) and reports suggest inhibition of auxin transportation to the roots retarding root growth (Chen et al., 2015).

The present study aims at understanding the impact of mercury contained industrial effluent along with solid waste on the pigment content and photosynthetic efficiency of a blue-green alga inhabiting crop fields under laboratory controlled conditions.

MATERIALS & METHODS

Blue-green alga (Cyanobacteria): Anabaena cylindrica Lemm. 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 CO2 and evolve O2 where as heterocysts are unable to fix CO2 or evolve O2 but can fix nitrogen under aerobic condition. The akinetes are perennating spores that develop between vegetative cells and heterocysts and obtain fixed carbon and nitrogen from them. The alga is an inhabitant of the crop fields and fixes atmospheric nitrogen and the alga is a known biofertilizer. All the glass wares used for the experiment were Corning or Vensil make. Standard pure grade chemicals were used for the experiments. The glass wares were autoclaved for surface sterilization and after autoclaving the glass wares were kept in hot air oven to dry. Homogenized BGA was inoculated in laminar air flow to avoid any contamination. Experiments were conducted in sterilized culture room and in algal culture rack.

Culture medium: Allen and Arnon's nitrogen free medium (Allen & Arnon, 1955) with microelements suggested by Fogg (1949) modified by Pattnaik (1964) was used as the culture medium. 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± 20C. 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.

Industry under study

M/s. Jayashree chemicals Pvt. Ltd. at Ganjam, Odisha is a Chlor-alkali industry located 40km away from Berhampur town and 30 km away from Berhampur University near NH-16 on Chennei – Kolkatta corridor. This industry is situated on the bank of Rushikulya River and Rushikulya estuary (19°22'48"N & 85°03'10"E). This industry was producing tonnes of caustic soda (NaOH), hydrochloric acid (HCl), Liquified chlorine (Cl2) and sodium hypochlorite (NaClO).

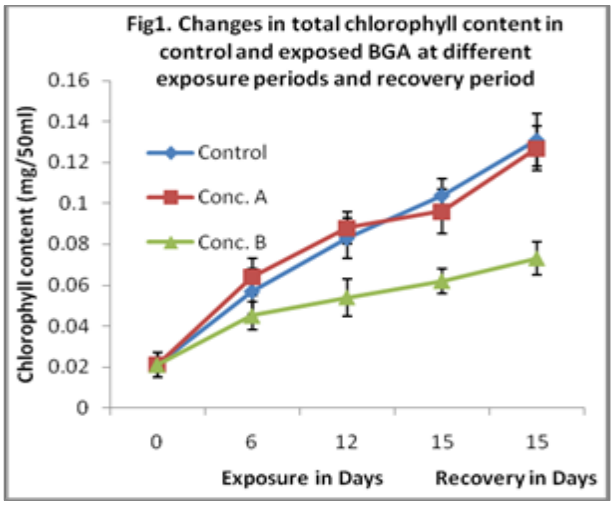
Photosynthetic efficiency study and Pigment analysis:

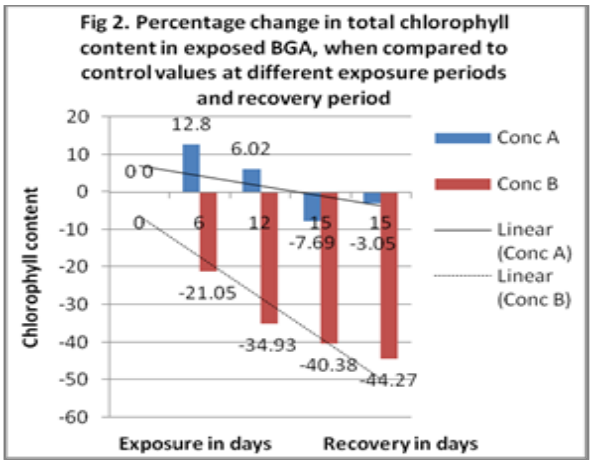
To study the pigments (total chlorophyll, total phaeophytin and carotenoid content), the pigment from the alga was extracted in 10 ml of cold 80% acetone with a pre-chilled micro-tissue homogenizer, centrifuged at 8000rpm for 10 minutes and the supernatant was taken and the extinction values at 475, 649 and 665nm were recorded to estimate total carotenoid and total chlorophyll, respectively. From the above supernatant, 5 ml was pipetted into a stoppered test tube, a pinch of oxalic acid was added, shaken thoroughly and was kept in a refrigerator overnight for estimation of phaeophytin. The total phaeophytin was measured by recording the extinction values of the extract at 655 and 666 nm. The amount of total chlorophyll and total phaeophytin was calculated by using the formula given by Vernon (1960). The evolution of carbon dioxide due to respiration and evolution of O2 in Photosynthesis of control and exposed alga were measured manometrically with the help of a Photo-warburg's apparatus (New Paul, India) following the procedure of Hannan and Patouillet (1972) and Oser (1965). Rate of photosynthesis was expressed in l of O2 evolved hr-1 g-1 of the plant and respiration rate was expressed in l of O2

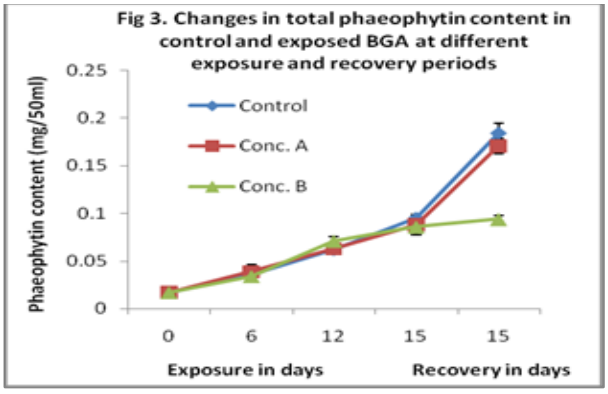
consumed hr-1 g-1 of leaf of the seedling. All the obtained values were statistically analyzed to assess the levels of significance.

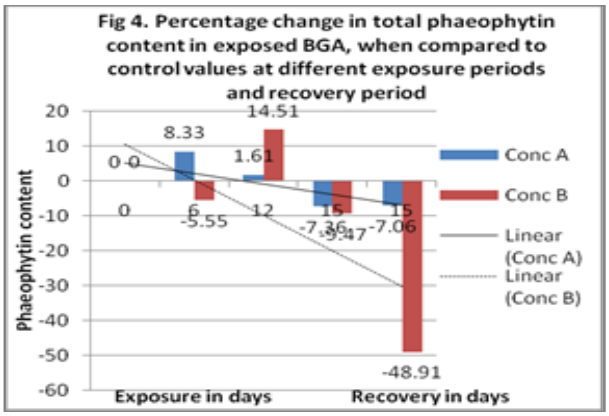
RESULTS

From the toxicity test the deduced value for the tested alga for a period of 15days was 0.38% of the toxicant as the sub-lethal concentration or Maximum allowable concentration (MAC) named as concentration-A, 0.43% of the toxicant as the LC10 lethal concentration named as concentration-B and 0.64% of the toxicant as the LC50 lethal concentration named as concentration-C. The toxicant at 1.12% acted as LC90 dose and 1.31% toxicant was treated as LC100. From the above toxicity test and the table cited above, Concentration-A (i.e. -0.38% toxicant) and concentration-B (i.e.-0.64% toxicant) were selected for conducting experiments along with a standard control (Reference) for all sets where no toxicant was inoculated. Present study was conducted for impact assessment of effluent contained solid waste from the contaminated site on a selected BGA. Approach was made for different accountabilities like Pigment studies and photosynthetic efficiency study of BGA for studying the impact mercury contained waste. Changes in total chlorophyll content (mg/50ml culture) in control and exposed BGA at different exposure and recovery periods have been shown in Figure 28. Total chlorophyll content during 0th day of exposure in both control and effluent concentration was 0.021mg. Total chlorophyll content in conc. A was 0.064mg/50ml culture on 6th day of exposure which is high as compared to 0.057mg in controlled condition. In case of concentration A, the total chlorophyll showed an increase over the control value up to 12th day of exposure. With the increase in exposure period, on 15th day, the chlorophyll content decreased when compared to control value. The depletion in chlorophyll content on 15th day of exposure and non recovery on 15th day of recovery indicated stress. The increment on initial days of exposure can be inferred to be an impact of initial excitement because of the mercury contained toxicant. At this stage, it is premature to say that mercury at lower concentration caused stimulation or regulation in growth of the alga. Because on 15th day of exposure and recovery, the chlorophyll values were much less which can be related to the inhibition caused by the toxicant. The inhibition on 15th day of exposure can be correlated with the residual mercury burden on the BGA body itself. In conc. B it was 0.045mg/50ml culture which is less than the control value.



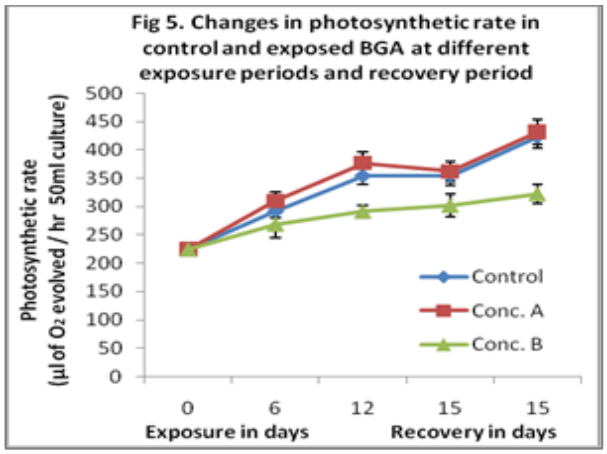


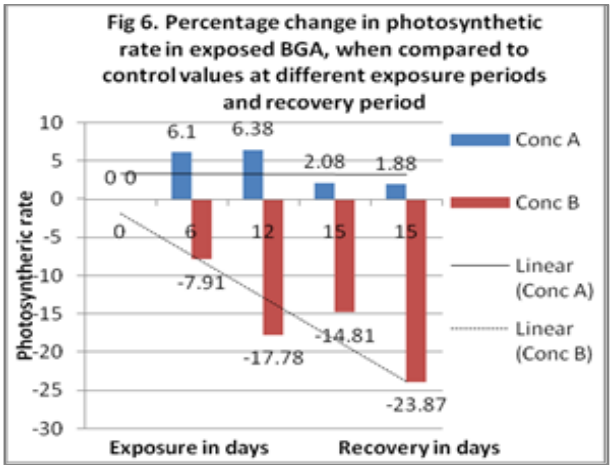


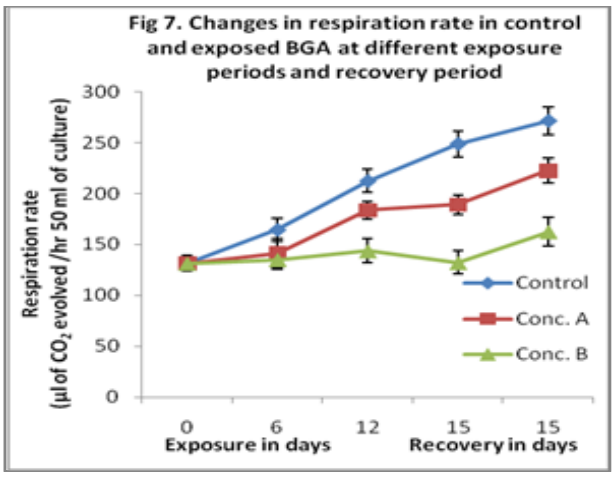


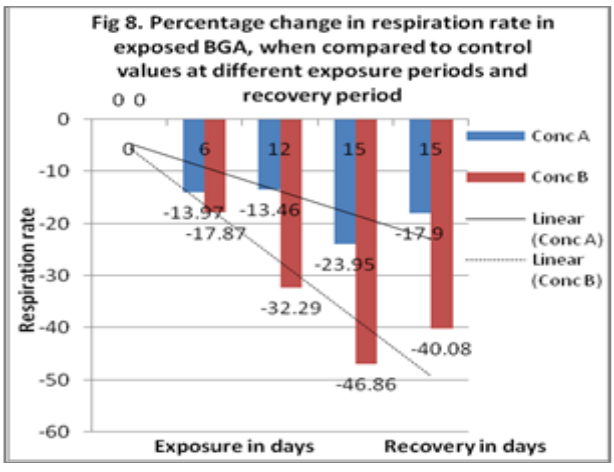
On the 12th day of exposure total chlorophyll content was 0.088mg/50ml culture in conc. A which was high as compared to 0.083mg/50ml culture in controlled condition. In conc. B it was 0.054mg/50ml culture which was less than the control value. On the 15th day of exposure chlorophyll content was 0.096mg/50ml culture in conc. A which was low as compared to 0.104mg/50ml culture in controlled condition. In conc. B it was 0.062mg/50ml culture which is less than the control value. Total chlorophyll content recorded on the 15th day of recovery was 0.127mg/50ml culture in conc. A, which was low as compared to controlled 0.131mg/50ml culture. In conc. B it was 0.073mg/50ml culture which was less than the controlled value (Fig.1). At higher exposure period and at higher toxicant concentration inhibition of chlorophyll synthesis or destruction chlorophyll pigment can be expected. Non recovery of the exposed system during the recovery period indicated permanent damage caused to the BGA system by the toxicant. Percent change in total chlorophyll content in effluent exposed BGA at different exposure and recovery periods has been drawn in Figure-2. The percent change in total chlorophyll content in conc. A are 12.28%, 6.02% and -

7.69% on 6th, 12th and 15th day exposure respectively and -3.05% during 15th day of recovery period. Percent change in chlorophyll content in conc. B has decreased significantly which are -21.05%, -36.93% and -40.38% on 6th, 12th and 15th day exposure and -44.27% on 15th day of recovery. Change in total phaeophytin content in control and exposed BGA at different exposure and recovery periods is shown in Fig.3. Total phaeophytin content during 0d day of exposure in both control and effluent concentration was 0.017mg/50ml culture. Total phaeophytin content in conc. A was 0.039mg/50ml culture on 6th day of exposure which is high as compared to 0.036mg/50ml culture in controlled condition. In conc. B it was 0.034mg/50ml culture which is less than the control value. On the 12th day of exposure phaeophytin was 0.063mg/50ml culture in conc. A which was high as compared to 0.062mg/50ml culture in controlled condition. In conc. B it was 0.071mg/50ml culture which is higher than the control value. On the 15th day of exposure phaeophytin content was 0.088mg/50ml culture in conc. A which was low as compared to 0.095mg/50ml culture in controlled condition. In conc. B it was 0.086mg/50ml culture which is less than the control value. Total phaeophytin content recorded on the 15th day of recovery was 0.171mg/50ml culture in conc. A which was low as compared to controlled 0.184mg/50ml culture. In conc. B it was 0.094mg/50ml culture which is less than the controlled value (Fig.3). The figure indicated that the phaeophytin content increased with the increase in exposure period up to 12th day of exposure, when compared to respective control value. But on 15th day of exposure, the pigment content decreased when compared to control value. During recovery period even after 15 days of recovery, the exposed alga could not recover to its control value. These observations indicated that mercury contained toxicant might have shown an initial induction on the alga but at higher exposure period and higher concentration showed inhibitory effect. The increase in phaeophytin on 12th day of exposure was neither indicative of stimulation nor a case of induction, as both can be a possibility at the early stages of inoculation but not at the middle of the experiment or exposure. On 15th day of exposure significant decline in the parameter and non recovery in recovery period indicated damage to the pigment system caused by the mercury contained toxicant. Percent change in total phaeophytin content in effluent exposed BGA at different exposure and recovery periods has been drawn in Figure-4. The percent change in total phaeophytin content in conc. A are 8.33%, 1.61% and -7.36% on 6th, 12th and 15th day exposure respectively and -7.06% during 15th day of recovery period. Percent change in total phaeophytin content in concentration-B are -5.6%, 14.5% and -9.5% on 6th, 12th and 15th day of exposure and -48.9% on 15th day of recovery (Fig.4). Change in photosynthetic rate (µl of O2 evolved hr-1 50 ml culture) in control and exposed BGA at different exposure and recovery periods is shown in Figure-5. Photosynthetic rate during 0th day of exposure in both control and effluent concentration was 224.5µl of O2 evolved hr-1 50 ml culture. Photosynthetic rate in conc. A was 309.6µl on 6th day of exposure which was high as compared to 291.8µl of O2 evolved hr-1 50 ml culture in controlled condition. In conc. B it was 268.7µl of O2 evolved hr-1 50 ml culture which was less than the control value.









On the 12th day of exposure photosynthetic rate was 376.8µl of O2 evolved hr-1 50 ml culture in conc. A which was high as compared to 354.2 µl in controlled condition. In conc. B it was 291.2µl of O2 evolved hr-1 50 ml culture which was lower than the control value. On the 15th day of exposure photosynthetic rate was 361.8 µl in conc. A which was high as compared to 354.4µl of O2 evolved hr-1 50 ml culture in controlled condition. In conc. B it was 301.9µl of O2 evolved hr-1 50 ml culture which was less than the control value. Photosynthetic rate recorded on the 15th day of recovery was 431.5µl of O2 evolved hr-1 50 ml culture in conc. A which was high as compared to controlled 423.5µl of O2 evolved hr-1 50 ml culture. In conc. B it was 322.4µl of O2 evolved hr-1 50 ml culture which was less than the controlled value. Fig. 35 clearly indicated that lower concentration of the toxicant and lower exposure period, the toxicant induced stimulation but in the same concentration, at higher exposure period induced inhibition. Inhibition at higher exposure was probably due to residual accumulation of mercury in the alga. Higher exposure period and higher concentration of the toxicant probably caused harm to the alga by way of interference in the metabolic activity and consequently physiological activity. This depletion was probably due to inhibition caused by mercury in the alga. The control alga showed normal activity. Percent change in photosynthetic rate in effluent exposed BGA at different exposure and recovery periods has been drawn in Figure-6. The percent change in photosynthetic rate in conc. A are 6.1%, 6.4% and 2.1% on 6th, 12th and 15th day exposure respectively and 1.9% during 15th day of recovery period. Percent change in photosynthetic rate in conc. B were -7.9%, -17.8% and -14.8% on 6th, 12th and 15th day of exposure and -23.87% on 15th day of recovery (Fig.6). Change in respiration rate (µl of CO2 evolved hr-1 50 ml culture) in control and exposed BGA at different exposure and recovery periods is shown in Figure-7. Respiration rate during 0th day of exposure in both control and effluent concentration was 131.4µl of CO2 evolved hr-1 50 ml culture. Respiration rate in conc. A was 141.6 µl of CO2 evolved hr-1 50 ml culture on 6th day of exposure which was low as compared to 164.6µl of CO2 evolved hr-1 50 ml culture in controlled condition. In conc. B it was 135.2µl of CO2 evolved hr-1 50 ml culture which was less than the control value. On the 12th day of exposure respiration rate was 183.8µl of CO2 evolved hr-1 50 ml culture in conc. A, which was low as compared to 212.4µl of CO2 evolved hr-1 50 ml culture in controlled condition. In conc. B it was 143.8µl of CO2 evolved hr-1 50 ml culture which was lower than the control value. On the 15th day of exposure respiration rate was 189.2µl of CO2 evolved hr-1 50 ml culture in conc. A, which was low as compared to 248.8µl of CO2 evolved hr-1 50 ml culture in controlled condition. In conc. B it was 132.2µl of CO2 evolved hr-1 50 ml culture which was less than the control value. Respiration rate recorded on the 15th day of recovery was 222.8µl of CO2 evolved hr-1 50 ml culture in conc. A which was low as compared to controlled 271.4µl of CO2 evolved hr-1 50 ml culture. In conc. B it was 132.2µl of CO2 evolved hr-1 50 ml culture which was less than the controlled value. No stimulation was observed in respiration rate of the exposed alga when compared to control alga. At all concentrations, inhibition in respiration rate was noted. Higher concentration of the toxicant induced inhibition due to accumulation of mercury in the algal body. Percent change in respiration rate in effluent exposed BGA at different exposure and recovery periods has been drawn in Fig.-8. The percent change in respiration rate in conc. A are -13.97%, -13.5% and -23.95% on 6th, 12th and 15th day exposure respectively and -17.9% during 15th day of recovery period. Percent change in respiration rate in conc. B are -17.9%, -32.3% and -46.9% on 6th, 12th and 15th day exposure and -40.08 on 15th day of recovery. The statistical analyses indicated that there exists significant difference between rows and columns, where different exposure periods showed variation and different concentrations of the toxicant also showed variation.

DISCUSSION

Pollution of mercury is one such silent epidemic that affects almost all the biota connected in a food chain of the ecosystem. Once mercury, in any form, enters the food chain, its concentration goes on increasing by many folds from the first trophic level to the last trophic level. This bioaccumulation and bioconcentration of the heavy metal, mercury showed fatality in organisms occupying at all the trophic levels and severely to the top trophic level due to biomagnification in any such food chain. As per the current scenario extra addition of mercury to the environment is least but the reports of mercury poisoning are still available. This might be due to the non degradable nature of mercury. It may remain for years as such in the environment. It can be absorbed and the metal passes from one trophic level to the other higher trophic level through food chain link up and after death of the organisms can return to the abiotic environment. Hence mercury is still available in toxic form in surroundings near the so called previously contaminating areas. Dumping mercury in large scale beyond the permissible level was so frequent in past decades that their detrimental effect is still continuing. Chlor-alkali industries using mercury cell technology had polluted the environment (Gonzalez, 1991) with most toxic substance mercury which is a neurotoxin (Chang, 1977) and is also referred as ‘quicksilver’ from the ancient times due to its mobility and unpredictable characteristics. Effluent and solid wastes from the industry are dumped nearer to the river Rushikulya, which finds its way into the river, reaching to the estuary and ultimately Bay of Bengal. Both effluent and solid waste contained Hg beyond the permissible limits prescribed by Pollution Control Boards. Bernaus et al., 2006 also reported discharge and pollution of mercury from Chlor alkali industry in Netherlands suggesting the huge concentration of mercury leading to detrimental effects on biota. The discharge of effluent into the soaking pond permits settlement of mercury in the sediments, which can be methylated by bacteria and the product is more than toxic than elemental mercury. Mercury by natural oxidation and reduction followed by its interaction with environmentally available chemicals and in presence of microbes change their status from elemental to ionic than to inorganic and organic forms. All other forms of mercury except elemental mercury can be absorbed by living organisms and can be translocated to different parts of the body and can be retained in different tissues, which will lead to residual accumulation. The transport by way of translocation to fruiting part or edible part of the plant is significant, which may lead to biomagnifications of the toxicant in the ecosystem. This accumulated mercury in life forms may find their way into human body by consumption of contaminated food, which can be disastrous. Industrial contaminated sites were profusely inhabited by microorganisms. Microbes like blue green algae,

bacteria and fungus grow in the contaminated sites and can resist worse effects of the contaminants (Dranguet et al., 2017). Heavy metal affects the BGA by different aspects like growth, physiology, biochemical components, etc. (Ahsan et al., 2007). There is a decrease in the pigment content of the alga when exposed to higher concentration of the toxicant with exposure period. Similar results were reported by Knowles and Zingmark (1978) where mercury containing toxicant affected the growth of Nitrschia sp. significantly. Lower concentration (MAC) of the toxicant for a small exposure period increases the pigment content. Initial increment in pigmentation might be due to impact of initial excitement in the alga due to mercury containing toxicant. The review made by Whitton (1970), Gadd and 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. Mishra et al. (1985b) reported that mercury affects the nucleic acid, protein and nitrogen content in BGA. Exposure of alga to toxicant containing mercury severely affects the physiology of the alga. Photosynthetic pigments are associated with energy production and CO2 fixation (Kashyap and Gupta, 1981). Chlorophylls are the primary light acceptors involved in transducing light energy into chemical energy. Carotenoids assist chlorophyll in photosynthesis as well as protect other pigments (Zakar et al., 2016). At lower concentration exposure there was marked increase in chlorophyll, phaeophytin and carotenoid content. This reveals the stimulatory effect of toxicant at lower concentration with small period of exposure. Disruption of pigments or inhibition of pigment synthesis resulted at higher concentration. Rai et al. (1981a) reported similar reports of stimulation at lower concentration by Chlorella vulgaris. Reports of Mishra et al. (1985a) shown similar decrease in pigment content of algae cultured in solid waste from a chlor alkali industry, with a crop plant, with increase in concentration of the waste soil and exposure period which was also significantly correlated with mercury uptake by alga. Wide spread occurrence of chlorophyll pigments suggest that chlorophyll play a vital role in photosynthesis and acts as photo enzymes (Rabinowitch and Govindjee, 1965). Mercury playing a detrimental role interferes with biosynthesis of chlorophyll and ultimately affects the rate of photosynthesis. This may affect the gross productivity of the exposed alga and will seriously affect the physiology of the alga. Data in the present study showed that there is increased amount of chlorophyll at the initial exposure periods at lower concentration and at higher concentration there is inhibitory effect. De Filippis and Pallaghy (1976) observed reduction in chlorophyll content in Chlorella treated with zinc and mercury. A similar trend was observed in phaeophytin content. Stimulation at lower toxicant concentration and inhibitory at higher toxicant concentration is the case. There observed an increase in phaeophytin content at higher concentration during 12th day exposure may be a presumption that conversion of chlorophyll into phaeophytin occurred. Similar case was reported by Singh and Singh (1984) where chlorophylls were converted to phaeophytin as a consequence of exposure to weak acids by replacement of Mg2+ with two atoms of hydrogen. Another vital photosynthetic pigment is carotenoid playing significant role in protecting photosynthetic tissues against photosensitized oxidation. Exposure of mercury containing toxicant to alga at higher concentration decreases the carotenoid content. In conc.-A, the pigment level increased at all exposure periods, when compared to control value but in conc. B the pigment values were much less than the control and conc. A values. This decrease in carotenoid content in algal cells exposed to higher concentration of mercurial toxicant induces heavy metal stress that lead to decrease in protection from the stress to the photosynthetic tissue. The ratio of chlorophyll to carotenoid signifies a strong parameter to indicate unfavorable condition for algal growth (Rai et al., 1981a). The observed results indicated the toxic nature of the mercury contained effluent on the alga. From the data, it looked like the use of the information for growth stimulation at sub-lethal concentration of the effluent in the crop fields, where the alga can be used as biofertilizer. But in my opinion, diluted effluent use will lead to accumulation of mercury in all life forms and ultimately the crop plant will accumulate mercury and finally mercury will reach to human beings, which will be hazardous. Best way can be reclamation by using biological agents rather than chemical treatment or dilution. Dilution is no solution for pollution, as evident from the observations of the present study. Change in photosynthetic rate as well as respiration rate was calculated in the exposed alga. At lower concentration (A) of the toxicant and lower exposure period, the toxicant induced stimulation but in the same concentration, at higher exposure period induced inhibition. Rath (1984), Sahu (1987) and Shaw (1987) indicated stimulation of growth, increase in pigment content, photosynthetic rate, respiration rate and enzyme activity at lower concentrations of mercurial compounds on Westiellopsis prolifica, Janet. Similar effects have also been shown in the current piece of work on Anabaena cylindrica Lemm. Overnell (1975) showed that light induced oxygen evolution from the freshwater species Chlamydomonas reinhardii were very sensitive to cadmium, methyl mercury and lead. Levels of 0.8 and 1.69 mg/L Hg reduced photosynthetic oxygen evolution by 50% and 90% respectively (Brown and Rattigan, 1979). Inhibition of photosynthesis and respiration in plants by mercurial compounds was reported by Sahu et al. (1988). A report of Gould (1975) indicated that inhibition of photosynthesis by mercury might be due to inhibition of electron inhibitor. Inhibition at higher exposure was probably due to residual accumulation of mercury in the alga. Higher exposure period and higher concentration of the toxicant probably caused harm to the alga by way of interference in the metabolic activity and consequently physiological activity. This depletion was probably due to inhibition caused by mercury in the alga. Similar reports were shown by Agarwal and Kumar (1978) in Chlorella. Percent change in photosynthetic rate showed the toxicant at lower concentration stimulates the photosynthetic rate in the initial periods of exposure and on prolonged exposure at same concentration inhibits photosynthetic rate. At higher concentration photosynthetic rate decreases. This is due to inhibitory effect of the toxicant on photosynthetic pigments at higher exposure and prolonged exposure periods. Eley et al. (1983) suggested inhibition of photosynthesis is mainly due to disturbances in light energy trapping mechanism. The effect of mercury containing toxicant on respiration rate was showing peculiar outcome. No stimulation was observed in respiration rate of the exposed alga when compared to control alga. At all concentrations, inhibition in respiration rate was noted. Higher concentration of the toxicant induced inhibition due to accumulation of mercury in

the algal body. This finding is a deviation to Matsumoto et al. (1971) suggesting increase in mitochondrial enzyme activities and respiration rate at lower concentration of the toxicant. The reason might be the concentration of the mercury in the toxicant and other chemicals in the toxicant mask the effect of mercury. Growth is a summation of all cellular metabolisms. Any inhibition of growth reflects toxic effect on a number of metabolic processes. In the present study along with the accumulation of mercury from the waste extract it is difficult to ignore a possible accumulation of other ions from the extract that might play certain role in growth acceleration in lower concentrations and retardation in higher concentration. Accumulation of mercury in biotic system also leads to biomagnifications. Wallin (1976) reported mercury accumulation in grasses, earthworm and small animals from discharges of Chlor-alkali industry which was dependent on availability of Hg and distance from the source. Heavy metal like mercury is also accumulated in the crops and vegetables grown in nearby agricultural fields (Fleming and Parle, 1977, Hirota et al., 1983 and Browne & Fang, 1978). Photosynthetic pigments of the plant systems play a vital role in trapping solar energy. The pigments are known to participate in generation of energy for CO2 fixation (Kashyap and Gupta, 1981). Shaw (1987) reported that with the waste treatment, the chlorophyll content increased to a great extent in lower concentrations and virtually no decrease in the level in higher concentration. Zingmark & Miller (1975) and De Filippis and Pallaghy (1976) reported that heavy metals inhibit photosynthesis. De Filippis and Pallaghy (1976), Rai and Khatoniar (1980) and Rai et al. (1981b) reported that heavy metals reduce chlorophyll content. De et al. (1985) suggested that at 20ppm the Hg(II) lowered the chlorophyll by decreasing the synthesis of chlorophyll, as well as possibly by increasing the synthesis of chlorophyll as well as possibly by increasing chlorophyllase activity in Pistia. De Filippis and Pallaghy (1976b) reported that all the heavy metal solutions inhibited the rate of chlorophyll synthesis in the cultures, with PMA and ZnCl2 causing the greatest and least inhibition respectively. Conway (1978) found a significant lowering of pigment content in Asterionella formosa after addition of cadmium. Many studies on lead point to its weak toxic effect on photosynthesis, respiration and cell division of various algae. Shaw (1987) opined that when mercury has been reported to be toxic, an increase in the level of chlorophyll in the alga exposed to the toxicant highly concentrated with mercury could hardly be explained, which we support and also agree. During the experimental period, bleaching of the toxicant exposed algal filaments was marked at higher concentrations, when compared to control algal filaments. At lower concentration of the toxicant, stimulatory growth was noticed, when compared to the control set. Mercury accumulated in the plants or producers was probably by absorption by the algal cell. Transfer of accumulated mercury to consumers in food chain is a result of biosorption of Hg. BGA can absorb considerable amount of Hg. This uptake and accumulation of Hg by algae occurs in two steps. First, mercury gets adsorbed to the cell walls, second, absorption or entry of mercury into the cell (Riley and Roth, 1971). This clearly indicated that the observed stimulation in photosynthetic rate was probably a case of excitation at the initial stages of the exposure but not an indication of stimulation or regulation. This experiment has shown the dichotomous behavior of the toxicant, where stimulation at lower concentration and inhibition at higher concentration was observed. This result is in accordance to the reports of Sahu (1987), Rath (1984) and Shaw (1987). At low concentration of solid waste extract from chlor alkali industry showed stimulation on growth of different blue green algae (Sahu and Panigrahi, 2002). The same authors opined that with the increase in residual mercury concentration the growth decreased significantly. In the present study, at higher exposure period, depletion in growth rate was observed and the observed impact was due to mercury contained effluent waste. The present study clearly indicated the impact of mercury contained effluent waste on the growth and photosynthetic efficiency of the alga tested. Unlike the reports of Gaur & Kumar (1981), here, we have observed an increase in the final yield following effluent treatment. Since, an increase in the final yield in the effluent treated alga was observed in the present study, the possibility of stimulation either by the absorbed metal or by some other mechanisms looks more appropriate than the metabolization of the effluent with mercury. The only speculation left, to account for the enhancement; by Gaur & Kumar (1981) was the likely presence of some growth regulator(s), which might have influenced climax of the test alga. This type of speculation is not acceptable at this stage and is not valid for this type of effluent treatment, where most of the fractional constituents at higher concentrations, are independent poisons/toxicants and in combination might show antagonistic or synergistic effects. The peculiar behavior of the algal organisms under stress to avoid the stress is an interesting feature in toxicological studies. Due to exudation, the medium might be changing or the exuding chemicals might be reacting with mercury and the other chemicals of the effluent forming a hard cyst, which must be providing an adhering surface for the heavy metal. The cyst might be restricting the heavy metal's entry into the cell, due to the formation of a barrier. It has been reported that with the increase in exposure period, the mercury concentration increased in exposed alga (Padhy & Panigrahi, 2021,2023).

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