VOLUME 2 ISSUE 1 2022

INTERNATIONAL JOURNAL OF BIOLOGICAL AND ENVIRONMENTAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Biological and Environmental Investigations
Contents available at Journals Home Page: www.ijbei.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Managing Editors: Dr. Abhishek Kumar & Dr. Abhai K. Srivastava
Published by: Saran Publications, Gorakhpur, India
ISSN: 2583-1690

Impact of Effluent of a Chlor-Alkali Industry on the Photosynthetic Efficiency of Crop Field Inhabiting Cyanobacteria

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Received: 22nd May, 2022; **Accepted:** 18th June, 2022; **Published online:** 30th June, 2022

https://doi.org/10.33745/ijbei.2022.v02i01.006

Abstract: The Chlor-alkali industry located at Ganjam, Odisha, India caused aquatic and terrestrial mercury contamination by its effluent and solid waste discharges discharged from the industry containing metallic mercury. The Rushikulya river water and the surrounding area were contaminated, which affected the flora and fauna of the area ultimately affecting live flora and fauna of Bay of Bengal. Toxicity study revealed the acute toxic nature of the effluent. At sub-lethal concentration of the effluent, either no impact or little increment in growth values (dry weight) was noted in case the tested alga available in the contaminated sites. At higher concentrations of the effluent, inhibition of growth was noted in the effluent exposed alga. The pigment contents like chlorophyll, phaeophytin, carotene and c-phycocyanin content significantly decreased as compared to the control algal pigments. It was observed that at sub-lethal concentration of the effluent, growth enhancement was noted but with the increase in effluent concentration growth declined. The pigments initially increased at sub-lethal concentrations and with the increase in effluent concentration the pigments significantly decreased indicating toxic stress. Analysis indicated that with the increase in exposure period the residual accumulation of mercury increased significantly showing a positive correlation ($r=0.924$; $P<0.01$) at all exposure periods. The change in pigment contents was significant. The data indicated that higher residual mercury in Blue Green Algae (BGA) impacted the pigments of the exposed BGA and at low residual mercury concentrations, increase in pigments was noted. The effluent of the chlor-alkali industry is deadly toxic and contained significant amount of mercury which can influence the physiological activity and biochemical metabolism.

Keywords: Chlor-alkali industry, Effluent, Mercury, Growth, Algal pigments, Blue Green Algae

Citation: Prusti Jhilli Prabha and Panigrahi A.K.: Impact of effluent of a chlor-alkali industry on the photosynthetic efficiency of crop field inhabiting cyanobacteria. Intern. J. Biol. Environ. Invest. 2(1): 51-59, 2022.
https://doi.org/10.33745/ijbei.2022.v02i01.006

Introduction

Chlor-alkali industries operating with mercury cell process discharges huge amount of mercury as unseen waste from the electrolysis cell house during operation as mercury vapor, during washing of the cells and cell house, as spilled mercury during handling and also from hydrolysis chamber followed by from compression chamber during washing into the aquatic and terrestrial environment. Caustic–chlorine industries are the important and main sources of mercury discharge

and its availability in the surrounding environment (Fimreite, 1970; Hartung and Dinman, 1974). The industry discharges huge amount of mercury in the effluent. Mercury settles to the bottom of the effluent canal along with other debris released after washing. It was observed that the total mercury estimated in the effluent was high and contained mainly three types of mercury. The mercury speciation study revealed that the effluent was having more amount of metallic mercury (67.2%), less of inorganic mercury (29.4%) and least amount of organic mercury (3.4%). The mercury speciation study revealed that the solid waste present on the top soil was having more amount of metallic mercury (65.3%), less of inorganic mercury (31.6%) and least amount of organic mercury (3.1%). The bottom solid waste contained 23.6% metallic mercury, 61.5% inorganic mercury and 14.9% organic mercury (Prusti and Panigrahi, 2022). It is a well known fact that higher concentration of any chemical can kill the live flora and fauna but at sub-lethal concentrations / doses visible effects may not be seen but in the long run, the impact can be seen by way of chronic depletion of ecosystem structure, composition, function, metabolic activity of live flora and fauna and loss of biodiversity. The industry discharges its effluent into a stocking pond and then into Rushikulya river. The effluent lechate enters into nearby crop fields. In addition, the effluent mixed river water is used for irrigation of crop fields, where nitrogen fixing BGA inhabits. Non availability of Blue Green Algae (BGA) in the contaminated crop fields is a sign of deterioration of agricultural field ecosystems. The present study aims at understanding the impact of mercury as a pollutant in the effluent on growth and pigment content of BGA and residual mercury accumulation in BGA, *Westiellopsis prolifica*, Janet.

Materials and Methods

The chlor-alkali industry M/S Jayashree Chemicals Pvt. Ltd., is situated on the road side of NH-16 at Ganjam, Odisha. The industry is located on the Bank of River Rushikulya discharging its waste into the river directly/indirectly and nearer to Rushikulya estuary and about 1.5 km away from the sea, Bay of Bengal on the East and 30 km North of Berhampur city on the south-eastern side at 84^0 53'E longitude and 19^0 16'N latitude.

The physicochemical properties of the effluent were determined as per the standard procedures of APHA (1995). Effluent waste was collected in glass bottles from different selected sites and brought to the laboratory for analysis. Few parameters were measured with the help of field analysis kit. The water, soil and plant leaf samples were digested in a Bethge's apparatus (Wanntorp and Dyfverman, 1955) and analyzed in a Mercury Analyser-MA5800, ECIL (1981). Sahu (1987) found that Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Patnaik (1964), was most suitable for the organisms, *Westiellopsis prolifica*, Janet (BGA) and was used as the basic culture solution as diluents for all laboratory experiments in the present study. Experiments were conducted at 3day interval for 15 days and different parameters were studied in conical flasks. Graded series of concentrations of the industrial effluent were prepared using the culture medium as diluents and were expressed in terms of percentage (v/v) for toxicity testing. Toxicity of the effluent was measured following the principles adopted by Sahu (1987) and dry weight of the samples was measured by mono pan balance after drying in an oven. Plant pigment analysis was carried out adopting the procedure of Vernon (1960) and Davies (1976). Obtained values were statistically analyzed.

Results

Physicochemical properties of the effluent samples sampled from the effluent canal are as follows: Temperature- 28.6 ±1.8 C, pH- 9.5±0.2, Alkalinity- 311.2±18.5 mg/l as $CaCO_3$, Hardness- 317.5±14.6 as $CaCO_3$ mg/l, Chlorinity- 651.4±18.7 mg/l, Dissolved Oxygen- 3.8±0.2 mg/l, BOD- 28.3±9.4 mg/l, COD- 258.5±18.5 mg/l, Suspended solids- 86.8±21.4 mg/l, Total Nitrogen- 2.9±1.6 mg/l, Total Phosphorus- 0.62±0.12 mg/l and total

mercury- 0.58±0.28 mg/l. The analysis indicated that effluent which was discharged into the environment is toxic and a significant amount of mercury was available in the effluent collected from the effluent canal, much more than the stipulated limit prescribed by Environmental Protection Act, 1986 and Pollution Control Board of India. Experimental studies with BGA revealed interesting features. Toxicity study revealed that the effluent is deadly toxic. The sub-lethal concentration value of the effluent was determined to be 0.1ml/50ml culture for this alga. The LC_{00}, LC_{10}, LC_{50} ,LC_{90} and LC_{100} values of the chlor-alkali industry effluent were 0.092, 0.11, 0.21, 0.32 and 0.41ml/50 ml culture, respectively. Experiments were conducted at the above concentrations.

Figure 1 indicates the residual mercury accumulation by BGA during the experimental period. The residual mercury level showed a positive trend with the increase in exposure period and with the increase in effluent concentration. No mercury was traceable in the alga growing in the control set. At 0.1 ml of effluent/50 ml culture, the residual mercury concentration increased to 3.9±0.5 µg/g dry weight on 3rd day and the value increased to 6.1±0.6 µg/g dry weight on 9th day of exposure and residual mercury further increased to 7.4±0.8 µg/g dry weight on 15th day of exposure (Fig. 1). At 0.2 ml of effluent/50 ml culture, the residual mercury concentration increased to 4.1±0.3 µg/g dry weight on 3rd day and the value increased to 5.9±0.5 µg/g dry weight on 9th day of exposure and residual mercury further increased to 7.9±0.5 µg/g dry weight on 15th day of exposure. At 0.3 ml of effluent/50 ml culture, the residual mercury concentration increased to 3.8±0.5 µg/g dry weight on 3rd day and the value increased to 5.1±0.3 µg/g dry weight on 9th day of exposure and residual mercury further increased to 7.8±0.6 µg/g dry weight on 15th day of exposure. At 0.4 ml of effluent/50 ml culture, the residual mercury concentration increased to 4.1±0.4 µg/g dry weight on 3rd day and the value increased to 5.7±0.5 µg/g dry weight on 9th day of exposure and residual mercury further increased to 7.6±0.4 µg/g dry weight on 15th day of exposure (Fig. 1).

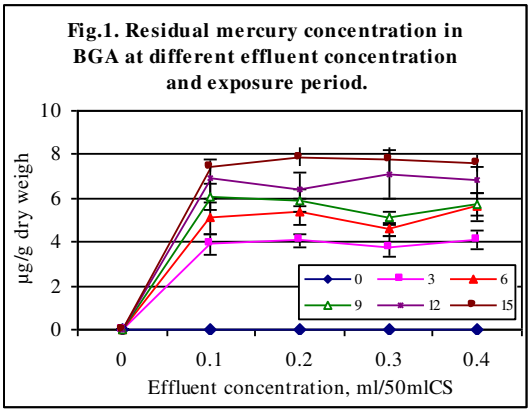


Figure 2 shows the changes in optical density of control and effluent exposed cultures at different days of exposure and at different effluent concentrations. The optical density of the homogenized culture increased with the increase in exposure period and effluent concentration except few exceptions where either decrease followed by increase or increase followed by decrease in OD value was noted. With the increase in effluent concentration no clear trend was noted. In general it can be inferred that with the increase in exposure period the optical density value increased indicating a positive growth in the control set showing a positive correlation. The observed data did not show any specific trend and the obtained values were not statistically significant as observed in regression analysis. Hence, this parameter of measuring optical density for growth studies is not acceptable as suggested by Padhy (1982) by light scattering technique.

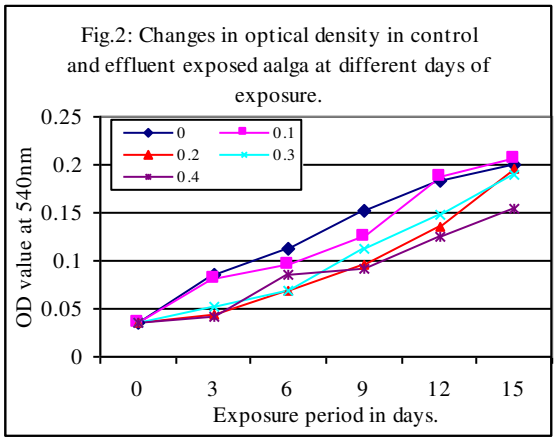


Figure 3 shows changes in dry weight as a parameter of growth studies in the control and effluent exposed BGA at different days of exposure period and at different effluent concentration. The dry weight value increased from 2.5±0.6 mg/50 ml culture (0 day) to 25.6±0.8 mg/50 ml culture (15th day) in the control set showing a positive correlation (P<0.001). The dry weight value increased from 2.5±0.6 mg/50 ml culture (0 day) to 24.8±0.7 mg/50 ml culture (15th day) in the 0.1 ml of effluent/50 ml culture set showing a positive correlation (P<0.01). The dry weight value increased from 2.5±0.6 mg/50 ml culture (0 day) to 17.4±1.1 mg/50 ml culture (15th day) in the 0.2 ml of effluent/50 ml culture set showing a positive correlation (P<0.01).

The dry weight value increased from 2.5±0.6 mg/50 ml culture (0 day) to 11.2±0.8 mg/50 ml culture (15th day) in the 0.3 ml of effluent/50 ml culture set showing a positive correlation (P<0.05).The dry weight value increased from 2.5±0.6 mg/50 ml culture (0 day) to 9.8±0.6 mg/50 ml culture (15th day) in the 0.4 ml of effluent/50 ml culture set showing a positive (P<0.05) correlation (Fig. 3). The dry weight decreased with the increase in effluent concentration at all exposure period indicating that the mercury contained effluent is toxic and can affect the growth of the exposed alga in the exposed cultures.

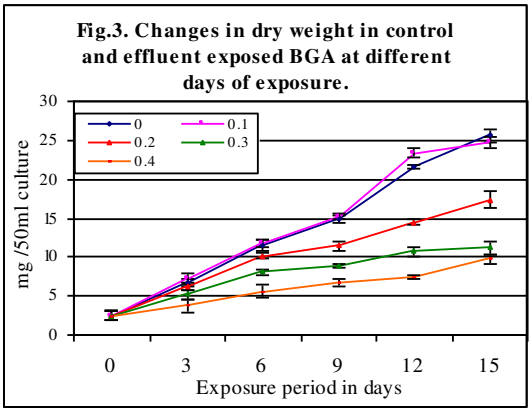
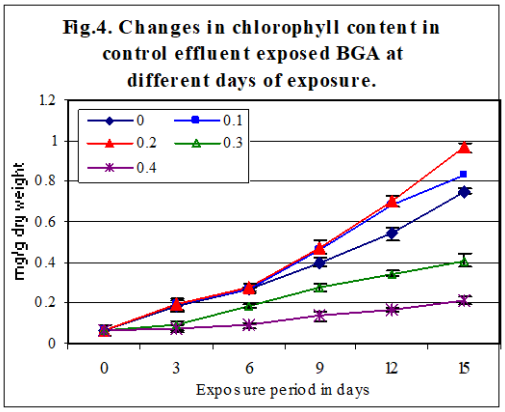


Figure 4 showed changes in total chlorophyll content as a parameter of growth and metabolic studies in the control and effluent exposed BGA at different days of exposure period and at different



effluent concentrations. The chlorophyll content increased from 0.065±0.024 mg/g dry weight (0 day) to 0.752±0.014 mg/g dry weight (15th day) in the control set showing a positive correlation (P<0.001) in the control set. The total chlorophyll content increased from 0.065±0.024 mg/g dry weight (0 day) to 0.827±0.021 mg/g dry weight (15th day) in the 0.1 ml of effluent/50 ml culture set showing a positive correlation (P<0.001). The total chlorophyll content increased from 0.065± 0.024 mg/g dry weight (0 day) to 0.966±0.031 mg/g dry weight (15th day) in the 0.2 ml of effluent/50 ml culture set showing a highly significant positive correlation (P<0.001). The total chlorophyll content increased gradually from 0.065±0.024 mg/g dry weight (0 day) to 0.408±0.024 mg/g dry weight (15th day) in the 0.3 ml of effluent/50 ml culture set showing a significant positive correlation (P<0.05). The total chlorophyll content increased gradually from 0.065±0.024 mg/g dry weight (0 day) to 0.214±0.018 mg/g dry weight (15th day) in the 0.4 ml of effluent/50 ml culture set showing a significant positive correlation (P<0.05). The total chlorophyll content increased at 0.1 and 0.2 ml/50 ml culture solution of effluent concentration at all exposure period compared to control values and the values were more than the control values. But at 0.3 and 0.4 ml of effluent/50 ml culture the total chlorophyll content of the exposed alga was significantly less than the respective control values (Fig. 4). The total chlorophyll content decreased with the increase in effluent concentration at higher doses at all exposure periods indicating that the mercury either

interfered in the chlorophyll biosynthesis process reducing chlorophyll amount or degraded the pigment. Mercury contained effluent is toxic and can affect the growth of the exposed alga in the exposed cultures.

Figure 5 shows changes in total pheophytin content as a parameter of metabolic studies in the control and effluent exposed BGA at different days of exposure period and at different effluent concentrations. The total pheophytin increased from 0.082±0.031 mg/g dry weight (0 day) to 0.957±0.061 mg/g dry weight (15th day) in the control set showing a positive correlation (P<0.001). The total pheophytin content increased from 0.082±0.031 mg/g dry weight (0 day) to 1.025±0.081 mg/g dry weight (15th day) in the 0.1 ml of effluent/50 ml culture set showing a positive correlation (P<0.001). The total pheophytin content increased from 0.082±0.031 mg/g dry weight (0 day) to 1.213±0.074 mg/g dry weight (15th day) in the 0.2 ml of effluent/50 ml culture set showing a highly significant positive correlation (P<0.001). The total pheophytin content increased gradually from 0.082±0.031 mg/g dry weight (0 day) to 1.146±0.065 mg/g dry weight (15th day) in the 0.3 ml of effluent/50 ml culture set showing a significant positive correlation (P<0.001). The total pheophytin content increased gradually from 0.082±0.031 mg/g dry weight (0 day) to 0.894±0.045 mg/g dry weight (15th day) in the 0.4 ml of effluent/50 ml culture set showing a significant positive correlation (P<0.01). The total pheophytin content increased at 0.1, 02 and 0.3 ml/50 ml culture solution of effluent concentration at all exposure period compared to control values and the values were more than the control values. But at 0.4 ml of effluent/50 ml culture the total pheophytin content of the exposed alga was significantly less than the control value (Fig. 5). The total pheophytin content decreased with the increase in effluent concentration at higher doses at all exposure periods indicating that the mercury either interfered in the pheophytin synthesis process reducing chlorophyll amount or degraded the pigment. Mercury contained effluent is toxic and can affect the growth of the exposed alga in the exposed cultures.

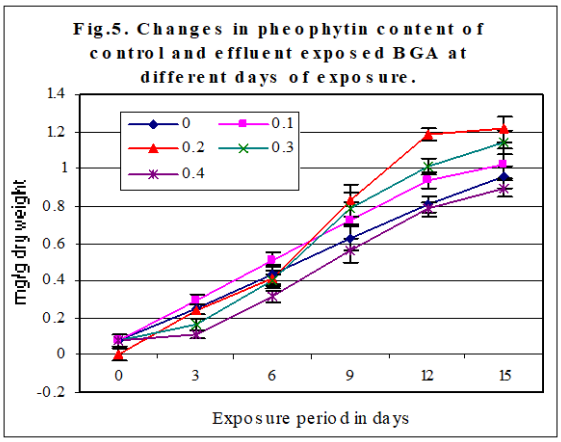


Figure 6 illustrates changes in total carotene content as a parameter of metabolic studies in the control and effluent exposed BGA at different days of exposure period and at different effluent concentrations. The carotene content increased from 0.0002 mg/g dry weight (0 day) to 0.0022 mg/g dry weight (15th day) in the control set showing a positive correlation (P<0.01) in the control set. The total carotene content increased from 0.0002 mg/g dry weight (0 day) to 0.0019 mg/g dry weight (15th day) in the 0.1 ml of effluent/50 ml culture set showing a positive correlation (P<0.05). The total carotene content increased from 0.0002 mg/g dry weight (0 day) to 0.00091 mg/g dry weight (15th day) in the 0.2 ml of effluent/50 ml culture set showing a positive correlation (P<0.05). The total carotene content increased from 0.0002 mg/g dry weight (0 day) to 0.00057 mg/g dry weight (15th day) in the 0.3 ml of effluent/50 ml culture set showing positive correlation. The total carotene content increased gradually from 0.0002 mg/g dry weight (0 day) to 0.00029 mg/g dry weight (6th day) in the 0.4 ml of effluent/50 ml culture set and with the increase in exposure period the total carotene content significantly decreased showing a non-significant correlation (P=NS). The total carotene content initially increased and then decreased at 0.1 and 0.2 ml/50 ml culture solution of effluent concentration at all exposure period compared to control values and the values were more than the control values. But at 0.3 and 0.4 ml of effluent/50

ml culture the total carotene content of the exposed alga was significantly less than the respective control values (Fig. 6). The total carotene content decreased with the increase in effluent concentration at higher doses at all exposure periods indicating that the mercury degraded the pigment. Mercury contained effluent is toxic and can affect the growth of the exposed alga in the exposed cultures.

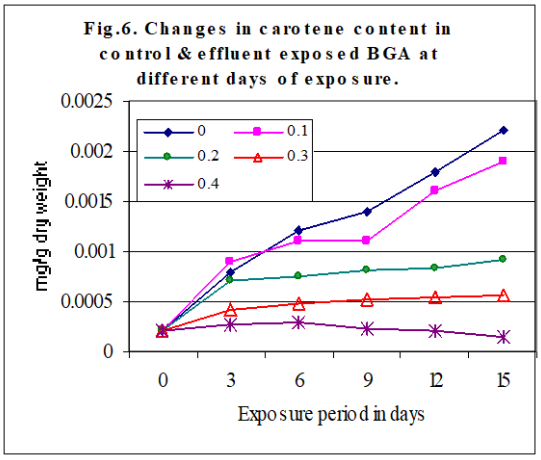


Figure 7 indicates the changes in c-phycocyanin content in control and effluent exposed alga at different exposure periods. Significant increase in c-phycocyanin content was observed in effluent exposed alga compared to control alga at lower effluent concentrations. At higher doses of effluent the pigment content decreased in the effluent exposed alga. At 0.3 ml of effluent in 50 ml culture, the pigment content showed highest increase and at 0.4 ml of effluent in 50 ml culture maximum decrease was noted.

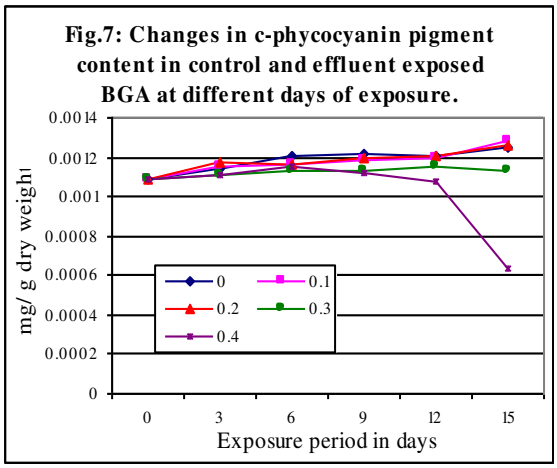
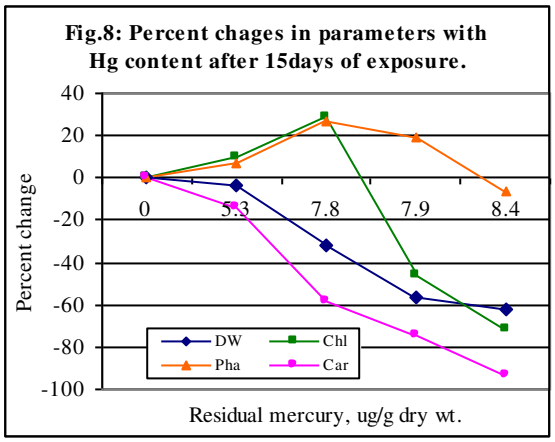


Figure 8 depicts the per cent changes in pigment content in the effluent exposed alga compared to their respective control value and residual mercury accumulation. At 0.1 ml and 0.2 ml of effluent /50 ml culture, increase in chlorophyll and phaeophytin content was noted and at the same concentration of the effluent dry weight and carotene content decreased. In other doses of effluent all studied parameters decreased significantly. Figure 8 shows clearly that at lower residual mercury concentration and at lower exposure period, the chlorophyll and phaeophytin content increased and at higher residual mercury concentration and at higher exposure period, the parameters significantly depleted. All the obtained values were statistically significant.



Discussion

Mercury is a persistent chemical and can remain in the environment for a pretty long time in some form or other. The temperature of the effluent is important when discharged into aquatic water bodies. Maintenance of pH to a particular range is important since all the biochemical activities depend on pH of the environment. Suspended materials play the role of scavenger for the particular metals in aquatic systems. The suspended particles in water bodies reduce solar energy penetration and reduce photosynthetic activity in case of phytoplankton and all aquatic plants. They cause ecological imbalance by mechanical abrasive actions, blanketing action and sedimentation, reduction of light penetration, availability as a surface for growth of bacteria and

fungi, reduction of temperature fluctuation and absorption of various chemicals, specifically the heavy metals. Higher loads of suspended solids cause a significant deterioration in the survival conditions for aquatic organisms. Salinity and hardness, which may together be represented as dissolved solids, play an important role in distribution and maintenance of many organisms in the estuary. Mercury gets into the environment and gets circulated in all environmental segments through mercury cycle. Prusti and Panigrahi (2017a) reported residual mercury level in the plants collected from the contaminated site where the solid waste was dumped and the effluent soaking pit is located. They also reported that the residual mercury availability in plants was due to mercury absorption from the effluent and leached chemicals from the effluent stocking pond. Prusti and Panigrahi (2017b) indicated that this alga could be used for phycoremediation of mercury contained waste. The same authors used effluent direct and amended effluent, solid waste extract and amended solid waste extract for reclamation studies. Interesting results were reported and the data indicated that all growth parameters and photosynthetic pigments showed increase in values compared to control values, higher exposure period values and higher residual mercury concentration values. The same authors opined that mercury at lower concentration stimulates/ enhances growth and higher pigment values were obtained but at higher doses and higher residual mercury concentration, the growth was inhibited and pigments significantly declined.

Mercury (Hg) is a contaminant found in ecosystems globally. Although inorganic and insoluble Hg has relatively low bioavailability, methyl mercury (MeHg) is a neurotoxic chemical which can biomagnify rapidly to elevated concentrations in biota such that concentrations in top trophic predators can threaten both ecological and human health (Boudou *et al*., 1991; Mason *et al*., 1995). Mercury can be biomagnified at all levels of aquatic food chains (Mason *et al*., 1995), and high levels of mercury have been detected in fish, especially in the organic form. This imposes a great threat on human health through seafood consumption (Wu and Wang, 2012). The same author also pointed out that toxic responses are specific to metals and species, but the sub-cellular basis underlying such inter-species and inter-metal differences was not clear. Photosynthetic pigments of the plant system play a vital role in trapping solar energy and CO_2 fixation (Kashyap and Gupta, 1981). The chlorophylls have long been recognized as the primary light acceptors in plants and they are invariably present in every organism, which carries out photosynthesis with absorption of CO_2 and evolution of molecular oxygen. Rai and Khatonair (1980) and Rai *et al.* (1981) reported that heavy metals reduce chlorophyll content and inhibit photosynthesis. The phaeophytin content in the leaf of plant has an important role for the fact that any unfavourable change in the environment inhabited by the plant is reflected through a change in phaeophytin level. Chlorophylls are known to be converted to phaeophytins as a consequence of exposure to weak acids, by replacement of Mg^{2+} with two atoms of hydrogen and thereby changing the spectral properties. Carotenoids play a vital role as a protector of photosynthetic tissues against photosensitized oxidation. Here, in our experiment also there was increase in chlorophyll and phaeophytin content with the decrease in carotenoid content. Wu and Wang (2012) studied the accumulation, sub-cellular distribution and toxicity of inorganic mercury and methyl mercury in marine phytoplankton. Mercury exerts its toxicity by binding with sulphydryl groups and producing oxidative stress. Significant amount of mercury was recorded in the effluent and also in the BGA as residual Hg after absorption from the culture medium. Mercury might be getting attached to -SH groups, glutathiones and form a complex, thereby inhibiting chlorophyll biosynthesis in exposed BGA. A significant volume of information is available on the pollution of the surrounding biota due to the discharges of gaseous exhausts, solid wastes and effluents discharged from chlor-alkali industries (Suckcharoen and Nourteva, 1982; Shaw *et al*.,

1986). Wiener *et al*. (2012) indicated a severe pollution of the surrounding environment, which had received in past, the waste discharge from caustic soda factories. Reports of mercury dispersion and contamination of Lake Superior region (Glass *et al*., 1986), and eight northern Minnesota lakes (Sorensen *et al*., 1990) were available. It is time to save the existing flora and fauna at the contaminated sites, river and estuary. Due to change in technology, now no more mercury is available but the dumped mercury as solid waste needs attention. The industry people instead of recycling mercury removing all the top soil up to the depth of 10 feet and shifting to some other place and use these solid wastes for construction of roads. Now the problem is the new dumping sites, where mercury gets exposed and chances of evaporation of mercury and leaching of mercury to neighboring areas can not be ruled out. Non-availability of mercury in the area might be due to change in technology or washing of all discharged mercury into Bay of Bengal or a major portion of discharged mercury was recovered by following recycling technology.

Acknowledgements

Authors wish to thank the Head, Department of Botany and authorities of Berhampur University for providing laboratory and library facilities. JP Prusti wishes to thank DST, Govt. of India for providing INSPIRE fellowship. A.K. Panigrahi is thankful to UGC, New Delhi for Emeritus Professor Fellowship.

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