IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Experimental Study Of Effluent Contained Solid Waste Of A Chlor-Alkali Industry On A Blue-Green Alga.

Dharmendra N. Padhy and A. K. Panigrahi,
Environmental Toxicology Laboratory, Department of Botany,
Berhampur University, BERHAMPUR-760 007, Odisha, India.

Abstract

The present study was planned to find out the impact of effluent contained solid waste of a Chlor-alkali industry on a blue-green alga under laboratory controlled conditions. The effluent waste contained significant amount of elemental mercury at the site of outlet. The mercury content decreased with the increase in distance of the effluent canal. Minimum amount of mercury was found at the collection site and the value was much more than the stipulated limit prescribed by Pollution Control Board. The effluent contained solid waste significantly affected the growth of the alga under laboratory controlled conditions. Experiments were conducted at sub-lethal and lethal concentration values. The alga showed better growth at sub-lethal concentration of the effluent waste and at lethal concentration of the effluent waste inhibition of growth of the alga was observed. The alga could recover significantly at sub-lethal concentration of the toxicant. However, at higher concentration significant recovery was not marked after 15days of recovery. When the recovery period was extended for a period of 45days, the alga could recover to its pre-test activity. The effluent needs to be treated physically, chemically and biologically and after proper treatment the effluent can only be discharged in to the natural environment for protection of crop field inhabiting economically important organism.

Keywords: Chlor-alkali industry, Solid waste, Effluent, Mercury, BGA, Growth, Production.

Introduction

The analysis of the effluent in the past indicated the highly toxic nature of the effluent and its impact on the environmental parameters. The present study indicated that the effluent discharged from the industry, effluent in the effluent canal, solid waste dumping site, effluent soaking pond near the river bank, leaching effluent and sites where effluent joins the river is highly contaminated with mercury contained effluent. Significant level of mercury was detected in all aquatic and terrestrial samples tested. The effluent significantly altered the physico-chemical properties of the water bodies impacting the physico-chemical parameters. Presence of elemental mercury in effluent and solid waste can be methylated by bacteria to ionic and inorganic forms which can pose a serious threat to the environment and available biota at the contaminated site. Many workers including Gorden and Prouse (1973), Dustan *et al*. (1975) and Gaur & Kumar (1981) have observed a stimulation of algal growth by crude oils and hydrocarbons. Rath *et al*. (1986), Sahu (1987), Sahu *et al*. (1988) and Shaw *et al*. (1988) observed stimulation by different mercurial compounds however the mechanism of growth stimulation cannot easily be explained. Such stimulation might be due to the presence of some growth regulating compounds (Gorden and Prouse, 1973) and / or trace elements (Hufford, 1971). The effluent of the chlor-alkali industry may only contain an insignificant or negligible amount of trace elements or growth regulating compounds in it. Hence we do not agree at this stage with the reports of Hufford (1971) supported by Gorden and Prouse (1973) and Dustan *et al*. (1975). Many reports (Padhy & Panigrahi, 2016, 2021 and Padhy & Panigrahi, 2023) are available pertaining to the impact of inorganic

mercury, organic mercury and effluent of the industry on growth and photosynthetic efficiency of alga and crop plants inhabiting crop fields. The present study aims at understanding the impact of industrial effluent containing solid waste on a blue-green alga under laboratory controlled conditions.

Materials & Methods

Location of industry and study sites:

(Location of industry, Effluent stocking pond & Rushikulya river. [Source: Data Basin])

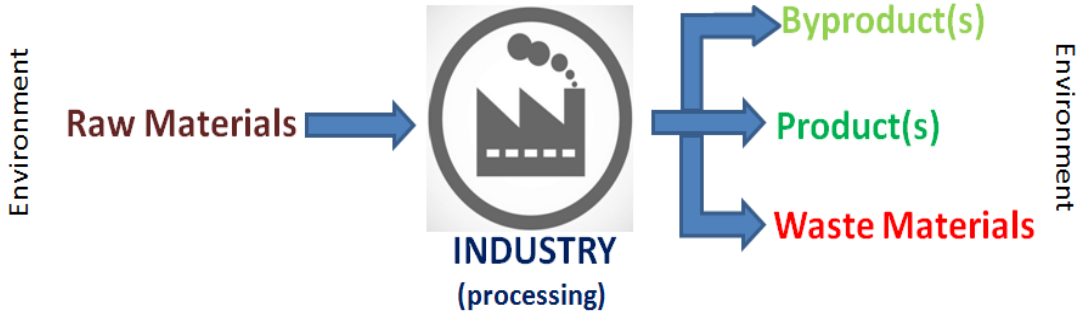
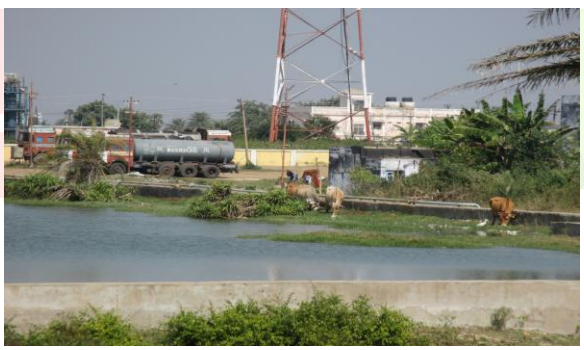


Figure showing the Outline of industrial inputs and outputs.



(Chlor-alkali industry at Ganjam, Odisha; Sample collection site at the effluent canal)

(Sample collection sites; Diversion of effluent canal underground in to River Rushikulya)

This industry is located (19°22'48"N & 85°03'10"E) on the bank of Rushikulya river. This industry was producing tonnes of chlorine (Cl_2) gas, caustic soda (NaOH), hydrochloric acid (HCl) and sodium hypochlorite (NaClO).

Blue-green alga (Cyanobacteria): *Anabaena cylindrica* Lemm.

The alga is an inhabitant of the crop fields and fixes atmospheric nitrogen and the alga is a known biofertilizer. ***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 (Stewart, 1976). The akinetes are perennating spores that develop between vegetative cells and heterocysts and obtain fixed carbon and nitrogen from them.

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.

Determination of lethal concentration values: Graded series of concentrations of the effluent waste was prepared and sterilized by UV radiation. Gradation of the waste was prepared by diluting with algal culture medium. Unialgal culture of *Anabaena* was prepared, homogenized with a micro tissue homogenizer and one ml of the homogenized inoculums was inoculated into the prepared concentration flasks. The optical density and dry weight was measured at 24hrs interval after inoculation up to 96hrs. From the optical density and dry weight value, the lethal concentration values were calculated after statistical analysis. Future experiments were conducted at LC_{00} (MAC value - Maximum Allowable Concentration) as concentration-A and LC_{50} values as concentration-B, to find out the effects of effluent of the chlor-alkali industry on the growth performance of the alga.

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± 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.

Growth study: Growth was measured by optical density method. The control and exposed alga was homogenized by a micro tissue homogenizer and the homogenized algal culture was taken and optical density was measured at 530nm in a Spectrophotometer. Dry weight was measure by filtering the whole culture solution through a pre-weighed Whatman filter paper, dried in an oven at 70^{o}C for 24hrs. Final weight of the filter paper with dried alga was taken in a single pan electrical balance (Dhona make) and the dry weight of the alga was calculated.

Mercury content was determined by digesting the effluent sample with acid digestion mixture in Bethge's apparatus as outlined by Wanntorp & Dyfverman (1955) and the digested sample was analyzed in a Mercury Analyzer (ECIL, 1981). The observed data was statistically analyzed to find out the levels of significance.

Results

The effluent of the Chlor-alkali industry contained 0.250 ± 0.021mg/L in summer days and 0.233 ± 0.019mg/L during rainy season.

Toxicity testing: It was a well established fact that the concentration of a chemical decides toxicity and supports the idea that no chemical is toxic at lower or sub-lethal concentrations and all chemicals are toxic in higher concentrations. Different concentrations of a chemical indicate the toxicity level of a chemical for a particular species or organism. The toxicity values will vary from genus to genus, from species to species and from variety to variety. Hence, it was of utmost importance to find out the toxicity level of each chemical for a species before initiating any experiment which deals with the concentration of a chemical. In the present study, graded series of the chemical (high range, macro range and micro range) was prepared and each dilution was made with the selected culture medium in sterilized 100ml conical culture flasks with cotton plug. Homogenised BGA was inoculated to the control and toxicant exposed conical flasks in a Laminar air flow and aseptic condition was maintained throughout the experimental period. The optical density and dry weight was measured at 3days interval up to 15days of exposure. The optical density values and dry weight data was interpolated and regression analysis was carried out. From the regression curve, the toxicity values were deduced. The below Table 1 indicated the toxicity values of the toxicant on a selected blue-green alga (Cyanobacteria).

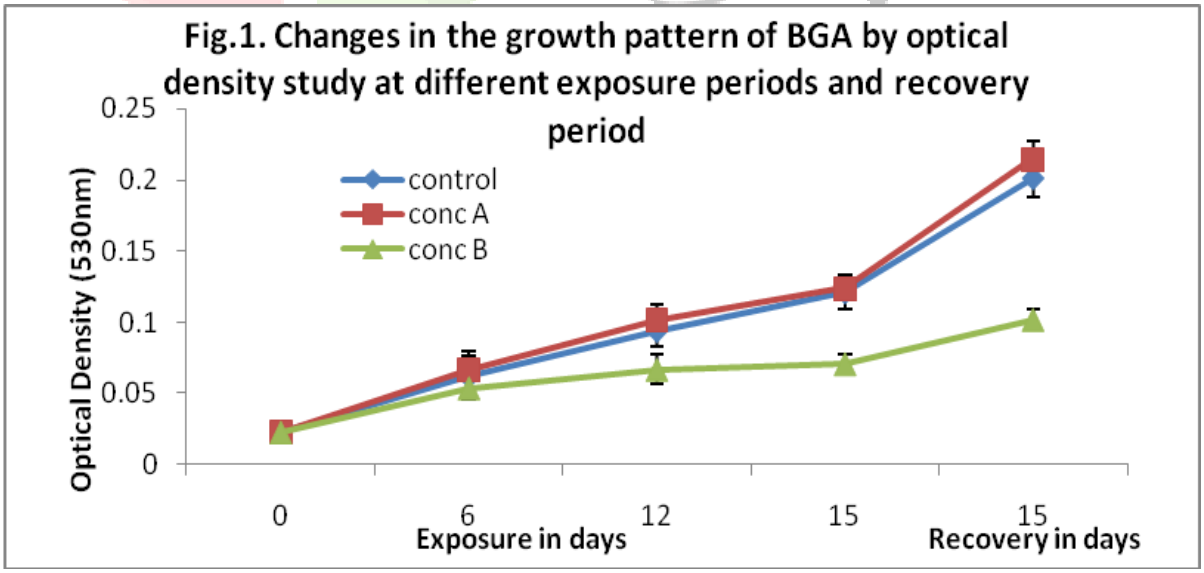
Table 1. Toxicity values for toxicity testing for BGA analysis

Sl No.	Lethal concentration values (LC)	Select concentration for the experiments.	Toxicant concentration (%)
1	LC_{00}	Concentration-A	0.38
2	LC_{10}	==	0.43
3	LC_{50}	Concentration-B	0.64
4	LC_{90}	==	1.12
5	LC_{100}	==	1.31

The above table indicated that 0.38% of the toxicant was the sub-lethal concentration or Maximum allowable concentration (MAC) for the tested alga for a period of 15days and names as concentration-A. The above table also indicated that 0.43% of the toxicant was the LC_{10} for the tested alga for a period of 15days. The above table also indicated that 0.64% of the toxicant was the LC_{50} for the tested alga for a period of 15days and named as concentration-B for all experimental work. The toxicant at 1.12% acted as LC_{90} dose and 1.31% toxicant was treated as $LC_{100.}$ 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 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 toxicity and growth of BGA for studying the impact mercury contained waste.

On exposure of the BGA to different concentration of effluent at 0^{th}, 6^{th}, 12^{th} and 15^{th} days' duration growth pattern was measured by measuring optical density at 530 nm and compared with controlled condition (Figure-1). Optical density (OD) value of the cultured increased with the increase in exposure time. OD during 0^{th} day of exposure in both control and effluent conc is 0.023 after inoculation. On the 6^{th} day of exposure OD was 0.067 in conc. A, which was high as compared to OD 0.062 in control. In conc. B it was 0.054 which was less than the control value. On the 12^{th} day of exposure OD was 0.102 in conc. A which was high as compared to OD 0.094 in control (Fig.1). In conc. B it was 0.067 which is less than the control value. On the 15^{th} day of exposure OD was 0.124 in conc. A, which was high when compared to OD 0.121 in controlled condition. In conc. B it was 0.071 which is less than the control value. OD value recorded on the 15^{th} day of recovery was 0.215 in conc. A, which was high as compared to controlled OD of 0.201. In conc. B it was 0.102 which is less than the controlled value. Percent change in OD in effluent exposed BGA at different exposure and recovery periods has been drawn in Figure-1. The percent change in OD in conc. A are 8.06%, 8.5% and 2.5% on 6^{th}, 12^{th} and 15^{th} day exposure respectively and 6.9 during 15^{th} day of recovery period. Percent change in OD in conc. B were -12.9%, -28.7% and -41.3% on 6^{th}, 12^{th} and 15^{th} day exposure and -49.3% on 15^{th} day of recovery (Fig.2).



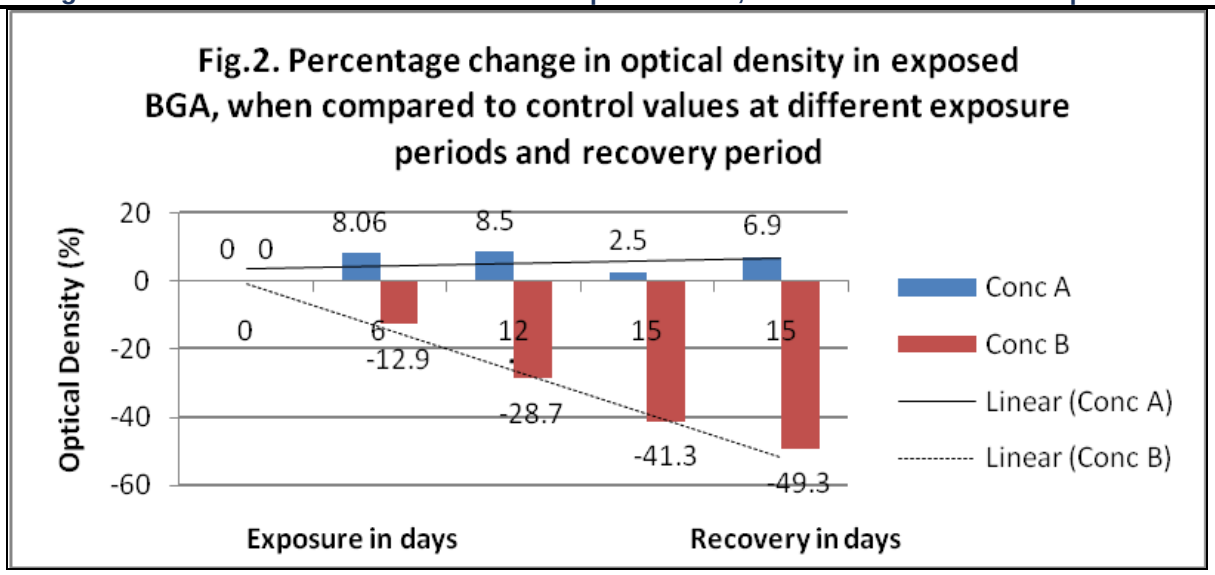
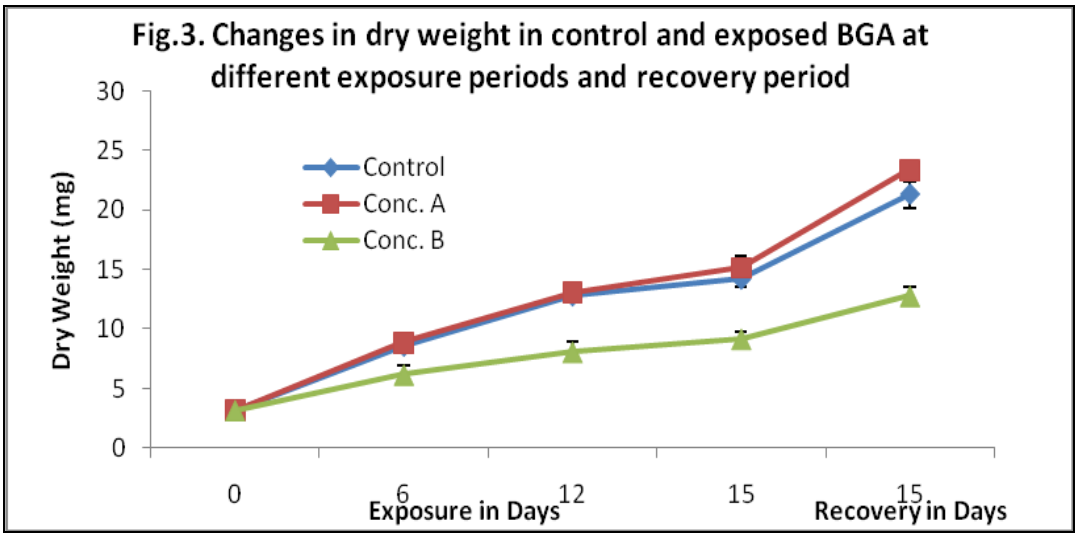


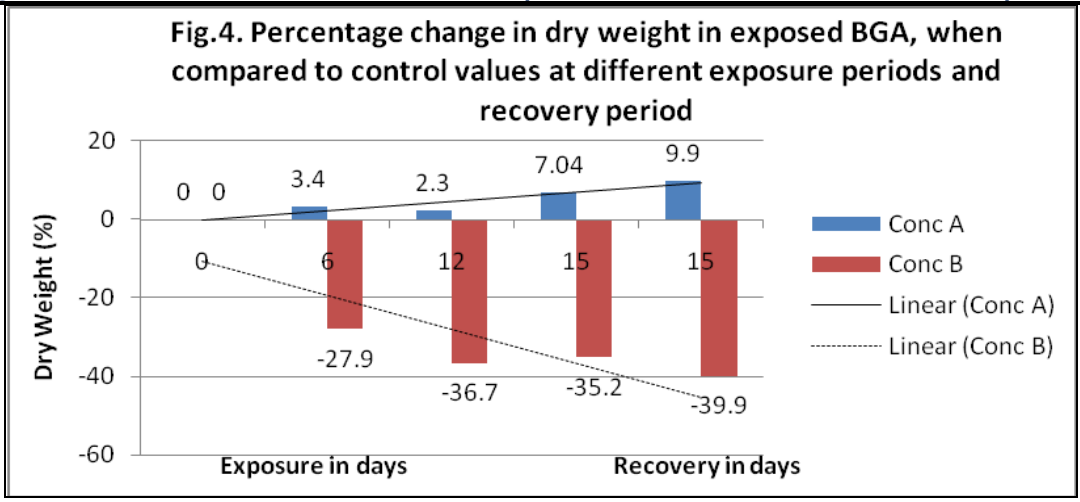
Table-2. Statistical analysis of growth pattern of BGA by optical density study at different exposure periods and recovery period.

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*			
Regression	2	0.005344	0.002672	415.3969	0.034673			
Residual	1	6.43E-06	6.43E-06					
Total	3	0.00535						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.006841	0.005479	1.24869	0.429879	-0.06277	0.076457	-0.06277	0.076457
X Variable 1	1.177908	0.144507	8.151247	0.077713	-0.65822	3.014038	-0.65822	3.014038
X Variable 2	-0.46318	0.293034	-1.58065	0.359105	-4.18653	3.260163	-4.18653	3.260163

Table-3. Statistical analysis of dry weight in control and exposed BGA at different exposure periods and recovery period

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*			
Regression	2	73.16537	36.58268	236.578	0.045924			
Residual	1	0.154633	0.154633					
Total	3	73.32						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.230809	4.520412	0.272278	0.830765	-56.2065	58.66809	-56.2065	58.66809
X Variable 1	1.216949	1.340312	0.907959	0.530687	-15.8133	18.24722	-15.8133	18.24722
X Variable 2	-0.57258	2.700453	-0.21203	0.866986	-34.8851	33.73993	-34.8851	33.73993





Change in dry weight in control and exposed BGA at different exposure and recovery periods is shown in Figure-3. Dry weight during 0^{th} day of exposure in both control and effluent concentration was 3.2mg. Dry weight of the exposed alga in conc. A is 8.9 mg on 6^{th} day of exposure which was high as compared to dry weight 8.6 mg in controlled condition. On the 12^{th} day of exposure dry weight was 13.1 mg in conc. A which is high as compared to dry weight 12.8mg in controlled condition. In conc. B it was 8.1mg which is less than the control value. On the 15^{th} day of exposure weight was 15.2mg in conc. A which is high as compared to weight 14.2mg in controlled condition. In conc. B it was 9.2mg which is less than the control value. Dry weight recorded on the 15^{th} day of recovery was 23.4mg in conc. A which was high as compared to controlled weight 21.3mg. In conc. B it was 12.8mg which is less than the controlled value (Fig.3). The dry weight of the exposed BGA culture showed an increase in dry weight in all exposure periods when compared to respective control values. With the increase in toxicant concentration, the dry weight decreased when compared to respective control values. This experiment has shown the dichotomous behavior of the toxicant, where stimulation at lower concentration and inhibition at higher concentration was observed. The experiment with optical density analysis indicated a similar feature but with fluctuations of values in optical density measurements. Dead cells and debris can also scatter more light increasing the turbidity of the medium, which can show higher values of optical density. Hence optical density values can be considered as an indicative parameter to show growth of BGA but under no circumstances can be considered as authentic indicator. However, the dry weight measurement to test growth is a confirmative parameter to measure growth and any conclusion basing on the trend supports the idea that mercury contained toxicant can stimulate growth at lower concentrations (less than MAC value) and inhibit growth at higher concentration. Percent change in dry weight in effluent exposed BGA at different exposure and recovery periods has been shown in Figure-3. The percent change in dry weight in conc A are 3.4%, 2.3% and 7.04% on 6^{th}, 12^{th} and 15^{th} day exposure respectively and 9.9% during 15^{th} day of recovery period. Percent change in dry weight in conc B are -27.9%, -36.7% and -35.2% on 6^{th}, 12^{th} and 15^{th} day exposure and -39.9% on 15^{th} day of recovery. Statistical analysis indicated very interesting information (Fig.4). The regression analysis of optical density and dry weight data indicated the existence of significant positive correlation in the control and conc. A sets with the increase in exposure period. In case of conc. B set, insignificant positive correlation was observed. ANOVA analysis of the whole tabulated data indicated that there was significant difference between rows and insignificant difference between columns (Table-3). This statistical analysis also indicated the similarity in the trend of data between control and concentration-A set. Concentration-B set was much different in its action when compared to control and concentration-A. The obtained information separates the actions and behavior of alga in conc. B from the data seen in case of conc. A. It can be concluded that concentration-A can be used as a safe concentration over concentration-B and Concentration-A can be recommended as the dose at which the effluent can be diluted minimum before discharge of the effluent into the environment.

Discussion

Microbes play an important role in the movement of mercury in nature, especially in the soil, sediments and aqueous environments. Microbes play a crucial role in methylation of mercury in to its organic derivatives in natural environments. The main result of microbial action on mercury seems to be its volatilization, whether it involves reduction of the mercuric ion or methyl or phenyl mercury compounds to volatile Hg^{o}, or whether it involves conversion of the mercuric ion to dimethyl mercury or of the phenyl mercuric ion to diphenyl mercury. The mercuric ion (Hg^{2+}) may be methylated by bacteria and fungi to give methyl mercury [$(CH_3)Hg^{+}$], which is water soluble. Some bacteria may further methylate methyl mercury and convert it to

dimethyl mercury, which is volatile and escapes into the air. Upon weathering, mercuric sulphide (cinnabar, HgS) is converted to mercuric sulphate and becomes disseminated in soil and water. Bacteria, fungi, and humic acid reduce Hg^{2+} and cause a wider range of distribution. Methyl mercury, as well as phenyl mercury, may again be enzymatically reduced to volatile Hg^{o} by bacteria. This causes detoxification of soil. Phenyl-mercury, which is usually anthropogenic in origin, may be reduced by soil bacteria and converted to diphenyl mercury. Biogenic H_2S may convert the mercuric ion to HgS, again under anaerobic conditions. Accumulation of heavy metals in agriculture soils has become a major concern for food crop production. Of these metals, mercury is recognized as one of the most hazardous elements, which is not essential for plant growth. Since mercury is known to be easily taken up by plants and translocated within the plant (Sahu, 1987), a clear understanding of its bioavailability to plants is essential for reducing the mercury entry into the food chain with potentially harmful effects on human health. It is well known that mercury concentrations in plant tissues are directly related to the concentration of plant available mercury in soil (Shaw, 1987). Wiener *et al.*, (2012) studied the risks of mercury in yellow perch a species important in trophic transfer of methyl mercury in the Laurentian Great Lakes region. Wu and Wang (2012) studied the accumulation, sub-cellular distribution and toxicity of inorganic mercury and methylmercury in marine phytoplankton. Mercury exerts its toxicity by binding with sulphydryl groups and producing oxidative stress. Different algae employ different physiological strategies and exhibit different sensitivities to mercury exposure. It is known that phytoplankton use at least three strategies to alleviate mercury toxicity. Heavy metal toxicity is common near chlor-alkali industries due to release of mercury from industry. Mercury is persistent heavy metal mostly studied because of its non degradability and global distribution. Heavy metal affects the BGA by different aspects like growth, physiology, biochemical components, etc.. It is obvious that algae growing in heavy metal polluted water show great capacity to withstand metals. There is a dichotomous role shown by the toxicant where stimulation at lower concentration and inhibition at higher concentration was observed. Studies have reported that toxicants at low concentration stimulates metabolism and at higher concentration it shows inhibitory effects (Rath, 1991). The toxicity of any chemical is dependent on the dose or concentration of the chemical. At very low concentration the chemicals are found to be nontoxic and can act as an essential substance for growth and development. It can be inferred that toxicity is determined by the concentration of the chemical. The present study has also focused on determining the lethal concentration of the toxicant and MAC (maximum allowable concentration) values. Sub-lethal concentration or MAC value for this present study was 0.38% of the toxicant. Common usage of lethal concentration values in toxicity testing usually interpreted by statistical analysis, described as LC_{50} which is determined from the line of best fit at the 50% mortality value. Toxicological studies are conducted to trace the nature of adverse effects and correlate response to dose. Early studies on eco-toxicological effects of effluent and solid waste from chlor-alkali industry on BGA were reported (Panigrahi, 1980; Shaw, 1987; Sahu, 1987 and Rath, 1991) showing similar trend to the present work. There is a concentration and time dependent increase in the accumulation of mercury by algae. Positive correlation between mercury uptake and concentration of the waste at different time intervals is also noticed. In the present investigation it was observed that at low concentration of the toxicant growth of the alga increased up to a short exposure periods. The percent change in OD in conc. A (lower conc) were 8.06, 8.5 and 2.5 on 6^{th}, 12^{th} and 15^{th} day exposure respectively and 6.9 during 15^{th} day of recovery period. Percent change in OD in conc. B were -12.9, -28.7 and -41.3 on 6^{th}, 12^{th} and 15^{th} day exposure and -49.3 on 15^{th} day of recovery. Growth of the alga in terms of calculating OD value may not be used as authentic indicator of growth. As in pollution studies there occurs death of algal cells during testing and dead cells, debris can scatter light interfering with light transmission and there will be fluctuation on OD values. However growth of the alga can be indicated more strongly by dry weight measurement. In this piece of work percent change in dry weight in concentration-A were 3.4, 2.3 and 7.04 on 6^{th}, 12^{th} and 15^{th} day exposure respectively and 9.9 during 15^{th} day of recovery period. Percent change in dry weight in conc B were -27.9, -36.7 and -35.2 on 6^{th}, 12^{th} and 15^{th} day exposure and -39.9 on 15^{th} day of recovery. The dry weight of the exposed BGA culture showed an increase in dry weight in all exposure periods when compared to respective control values. With the increase in toxicant concentration, the dry weight decreased when compared to respective control values. This experiment has shown the dichotomous behaviour 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).

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 algae (Sahu, 1987). 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. Growth rate studies by optical density method showed inconsistent data in exposed cultures. However, consistency was observed in the dry weight measurement studies. Dry weight has been considered by good number of workers as a parameter of growth. The change observed in optical density study exactly does not reflect the real changes induced by the pollutant, but an approximation can be made out of this data. Since growth is a summation of all cellular metabolisms, any inhibition of growth reflects toxic effects on a number of metabolic processes. Also, the use of growth rates allows one to observe, if the bioassay organism has the capability of recovery from the toxic effect, over extended periods of time. There is the disadvantage that as the algal cells increases in number, the concentration of the toxicant per cell decreases from the original value (Trevors and Vedelago, 1985). Walsh & Alexander (1980) demonstrated that algal species that were sensitive to pesticides in monoculture were less sensitive in the presence of resistant species, perhaps because, the resistant species grew quickly and absorbed the pesticide, thus, reducing its concentration in growth medium. Industrial waste caused a reversal in species numerical dominance in mixed algal cultures.

Acknowledgement

The authors wish to thank the Head of Botany department and authorities of Berhampur University, Berhampur, Odisha, India for providing the laboratory and library facilities. The financial support in the form of INSPIRE fellowship (DST, New-Delhi) to D. N. Padhy is thankfully acknowledged.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

References;

Allen, M. B. and Arnon, D. I. (1955): Studies on nitrogen fixing blue-green algae, growth and nitrogen fixation by *Anabaena cylindrica,* Lemm. Pl. Physiol. Lancaster.,30,366-372.

Dunstan, W. M.; L. P. Atkinson and J. Natoli (1975): Mar. Biol., 31:305. (Cross reference)

ECIL (Electronic Corporation of India Limited) (1981): Analytical methods for determination of mercury with mercury analyser, MA 5800 A.

Fogg, G. E. (1949): Growth and heterocyst production in *Anabaena cylindrica,* Lemm. II. in relation to carbon and nitrogen metabolism. Ann. Bot. N. S., 13:241-259.

Gaur, J. P. and H. D. Kumar (1981) Environ. Pollut., Ser.A., 25:77 (Cross reference)

Gordon, D. C. Jr and N. J. Prouse (1973): Mar.Biol., 22:329. (Cross reference)

Hufford, G. L. (1971): The biological response of oil in the marine environment, a review. Background report, Washington, D. C., U. S. Coast guard Oceanographic Unit, Office of Research and Development, U. S. Coast guard Headquarters.

Padhy, D.N. and A. K. Panigrahi (2016): Analysis of effluent of a Chlor-alkali industry and bioconcentration of mercury in some selected plants collected from the contaminated area at Ganjam, Odisha. Natl. J. of Life Sciences, 13(1):05-10.

Padhy, D.N. and A. K. Panigrahi (2021): Identification, purification and characterization of mercury resistant bacteria from Chlor-alkali industrial effluent. Eco. Env. Cons., 27 (October Supplement issue):pp-S400- S406.

Padhy, D.N. and A. K. Panigrahi (2023): Eco-toxicological study of industrial effluent and solid waste of a Chlor-alkali industry on the aquatic and terrestrial environments of the contaminated site at Ganjam, Odisha, India. IJCRT, 11(9),a659-a667.

Pattnaik, H. (1964): Studies on nitrogen fixation by *Westiellopsis prolifica,* Janet, Ph. D. Thesis, University of London, UK.

Rath, P. (1984): Toxicological effects of pesticides on a blue-green algae, *Westiellopsis prolifica* Janet. Ph. D. thesis, Berhampur University, India.

Rath, P., Misra, B.N. and Panigrahi, A. K.(1986): Effect of both inorganic and organic mercury on ATPase activity of *Westiellopsis prolifica*, Janet. Environ. Pollut. Ser. A., 42,143-149.

Rath, S. C. (1991): Toxicological effects of a mercury contained toxicant on *Anabaena cylindrica*, L. and its ecological implications. Ph. D. Thesis, Berhampur University, India.

Sahu, A. (1987): Toxicological effects of a pesticide on a blue-green alga : III. Effect of PMA on a blue-green alga, *Westiellopsis prolifica* Janet. and its ecological implications. Ph. D. Thesis, Berhampur University, India.

Sahu, A.; B. P. Shaw; A. K. Panigrahi and B. N. Misra (1986): Effect of phenyl mercuric acetate on ATPase activity of a blue-green alga, *Westiellopsis prolifica*, Janet.Microb. Lett., 33, 45-50.

Sahu, A.; B. P. Shaw; A. K. Panigrahi and B. N. Misra (1988): Effects of phenyl mercuric acetate on oxygen evolution in a nitrogen fixing blue-green alga, *Westiellopsis prolifica*, Janet. Microb. Lett., 37, 119-123.

Sahu, A. and A. K. Panigrahi (2002): Eco- toxicological effects of a chlor-alkali industry effluent on a cyanobacterium and its possible detoxification. In: Algological Research in India, P-351-430 (Ed. N Anand) Published by Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

Shaw, B. P. (1987): Eco-physiological studies of industrial effluent of a chlor-alkali factory on biosystems. Ph. D. thesis, Berhampur University, Orissa, India.

Shaw, B. P., A. Sahu, S. B. Choudhury & A. K. Panigrahi, (1988): Mercury in the Rushikulya river estuary. Mar. Poll. Bull., 19(5): 223-234.

Trevors, J. T. and H. Vedelago (1985): Bull. Environ. Contam. Toxicol., 34:696. (Cross reference)

Walsh, G. E. and S. V. Alexander (1980): A marine algal bioassay method: result with pesticides and industrial wastes. Water, Air and Soil Pollution, 13:45-55.

Wantorp, H. and A. Dyfverman (1955): Identification and determination of mercury in biological materials. Arkiv. fur Kemi., 9(2), 7-12.

Wiener, J. G.; Sandheinrich, M. B.; Bhavsar, S. P.; Bohr, J. R., Evers, D. C.; Monson, B. A. & Schrank, C. S. (2012): Toxicological significance of mercury in yellow perch in the Laurentian Great Lakes region. Environ. Pollut., 161:350-357.

Wu, Y. and Wang, W-X. (2012): Accumulation, sub-cellular distribution and toxicity of inorganic mercury and methyl mercury in marine phytoplankton. Environ. Pollut., 159, 3097-3105.