



Eco-Toxicological Effects Of Cadmium Chloride On The Growth Of a Blue-Green Alga Under Laboratory Controlled Conditions.

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Highlights

- Graded series of concentrations of the cadmium chloride was prepared to evaluate the toxic effects of the cadmium chloride on the blue-green alga, *Anabaena cylindrica*.
- With the increase in the concentration of cadmium chloride the percent survival decreased and hundred percent deaths were noticed at 1.88 mg.l⁻¹ of cadmium chloride with in a period of 15 days.
- The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.31mg.l⁻¹.
- The optical density study and the dry weight analysis indicated that the toxicant cadmium in the form of cadmium chloride is deadly toxic.
- Complete bleaching of the exposed algal mass inside the test solution was observed from 3rd day of exposure period onwards at higher concentrations.
- During recovery studies, gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 30 days of recovery period.

Abstract

It was observed that at very low concentrations the toxicant is deadly toxic and affects the BGA. With the increase in the concentration of cadmium chloride the percent survival of the exposed alga decreased and hundred percent death was noticed at 1.88 mg.l⁻¹ of cadmium chloride with in a period of 15 days. The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.31mg.l⁻¹. The lethal concentration values for 15 days of exposure periods have been outlined below. The LC₁₀ value was 0.48 mg. l⁻¹. The LC₅₀ value was 0.78 mg l⁻¹. LC₉₀ value was 1.22 mg l⁻¹ and LC₁₀₀ value was 1.88 mg l⁻¹, for this particular alga, *Anabaena cylindrica*. The control set showed 100% survival. Out of the above concentrations, LC₀₀ or PS₁₀₀ as safe MAC value of 0.31 mg.l⁻¹ was selected as 'X'; LC₅₀ or PS₅₀ of 0.78 mg.l⁻¹ was selected as 'Y' and LC₉₀ or PS₁₀ value of 1.22 mg.l⁻¹ was selected as 'Z' for conducting future experiments. The optical density study and the dry weight analysis indicated that the toxicant cadmium in the form of cadmium chloride is deadly toxic. Cadmium did not show any dichotomous behavior. In recovery studies, no significant recovery was seen at lower concentration and no recovery at all at higher concentration indicated that cadmium caused irreparable damage to the exposed system. Complete bleaching of the algal mass inside the test solution (Z) was observed from 3rd day of exposure period onwards. Gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 30 days of recovery period. These particles grew in size with time. It was probably due to the appearance of photosynthetic pigments which disappeared due to the heavy metal (Cadmium) stress on the alga. Slowly the entire white mass got converted into a blue-green mass with the increase in recovery period (60 days of recovery).

Keywords: Cadmium chloride, Toxicity, Growth, Blue-green alga,

Introduction

Cadmium was recognized many years ago to be a highly toxic element but it was not until comparatively recently that concern began to be expressed over the possible effects on human health of long term exposure to low concentrations of this element. The discovery that Cadmium pollution from a base metal mining and smelting complex could cause serious illness and possible death in a local community has led to widespread public anxiety. The concern over the public health implications of cadmium pollution has resulted in a considerable amount of research being carried out by regional and national laboratories and field stations throughout the world. The most notable aspects of the geochemistry of Cadmium with regard to its rock, soil, plant & animal relationship is its low concentrations in the Earth's crust. Consequently, soil and plant contents of this element are generally low. Plants and animals are unlikely to have evolved mechanisms to cope with relatively high concentrations of Cadmium, since these rarely occur in nature. However, with the increasing production of Cadmium, pollution will assume a greater significance for soil plant animal pathways. The discharge of industrial wastes of some industry containing cadmium compounds, entry of these chemicals into water bodies of the locality along with the irrigated waters or run-off waters in the rainy season; availability of cadmium in the air and consequent precipitation and entry of rain run-off water into water bodies and crop fields and their possible effect on the blue-green algae inhabiting in crop fields, this project was masterminded to evaluate the eco-toxicological effects of cadmium metal in the form of Cadmium chloride on the toxicity and effect on the growth of a blue-green alga inhabiting crop fields and acting as a bio-fertilizer fixing atmospheric nitrogen and increasing the fertility of the crop field soil.

Materials & Methods

Anabaena cylindrica, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO₂ and evolve O₂ whereas heterocysts are unable to fix CO₂ or evolve O₂ but can fix nitrogen under aerobic condition (Stewart, 1976).

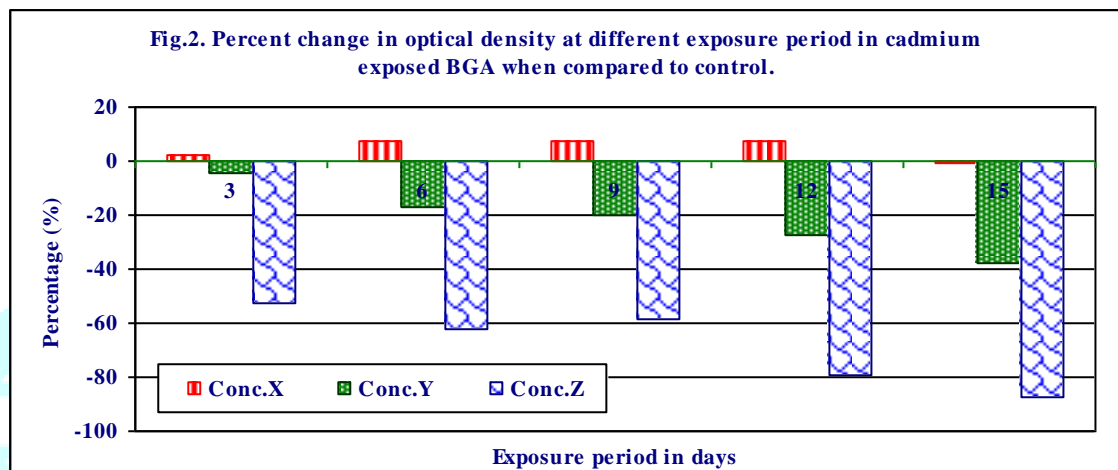
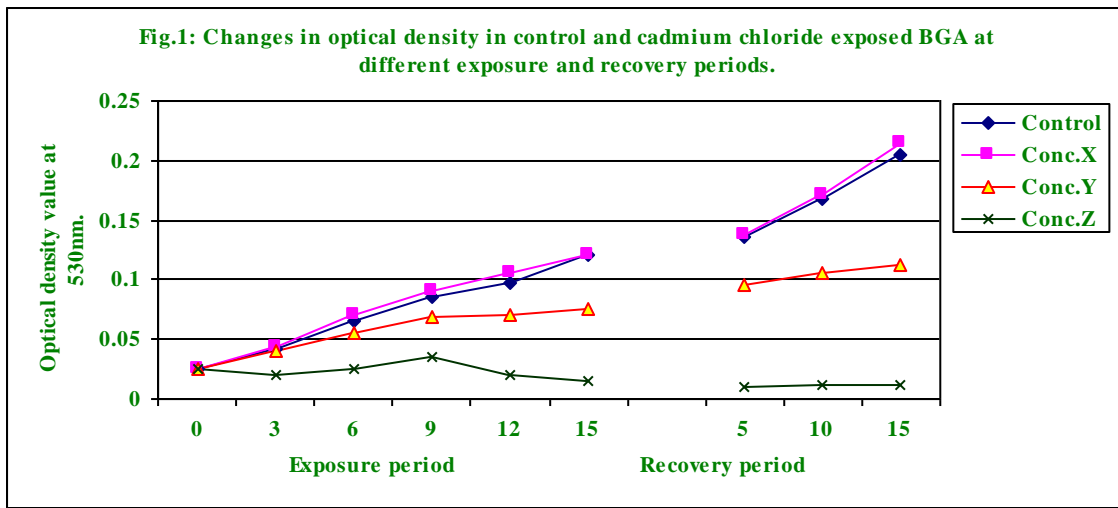
Allen and Arnon's (1955, a) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) was most suitable for the organism. It was used as the basic culture solution in all the experiments in the present study. The experimental algal cultures were grown under controlled conditions of light and temperature inside a culture room. The culture flasks were kept in series on a culture rack, of glass plate with iron frame. Light was provided by means of white fluorescent tubes, connected at the backside of glass plate of each rack, which illuminates the upper glass surface at the intensity of 2400±200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and was maintained at 28± 2°C. The culture flasks were regularly hand shaken twice a day to avoid clumping of the cells as well as their adhesion to the wall of the conical flasks. The pollutant, Cadmium was prepared by taking standard Cadmium metal and dissolved in acid. A graded series of concentrations of cadmium chloride ranging from 0.1mg l⁻¹ to 2.0mg l⁻¹ (V/V) was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. One ml of unialgal, axenic, homogenised culture was inoculated in each 150 ml flask containing 100 ml of solution, inside the inoculating chamber. The number of individual cells of the algae present in one ml of the culture medium after micro-tissue homogenisation was counted under the microscope. The test algae were exposed for a period of 15 days in different test medium after which their survival and mortality percentage were calculated by counting the number of cells present in one ml of the test solution after micro-tissue homogenization. From this, different survival percentage and mortality percentage, the lethal concentration (MAC) value were determined.

Growth measurement was done by recording the dry wt and optical density of the alga per 100 ml culture. Exponentially growing algal suspension of same age, volume and biomass was inoculated initially into the experimental flasks. The optical density was measured by light scattering technique. Optical density measurement was carried out by withdrawing the culture under aseptic conditions on every 5th day and homogenizing it with micro-tissue homogenizer, and then noting observations in a UV-Visible spectrophotometer (Systronics, PC based, 119) at 530nm (wave length). Dry weight of the alga in the culture flasks was estimated centrifuging in a refrigerated centrifuge (High speed centrifuge, Remi) at 8000 rpm for 10 minutes. The algal pellet was transferred to a pre-weighed glass cover slip. It was dried in an oven at 60°C for 24 hours, cooled in desiccators and the final weight of the glass cover slip was recorded in a single pan electric balance (Dhona). The data were expressed as mean of 5 samples ± standard deviation in mg / 100 ml culture.

Results

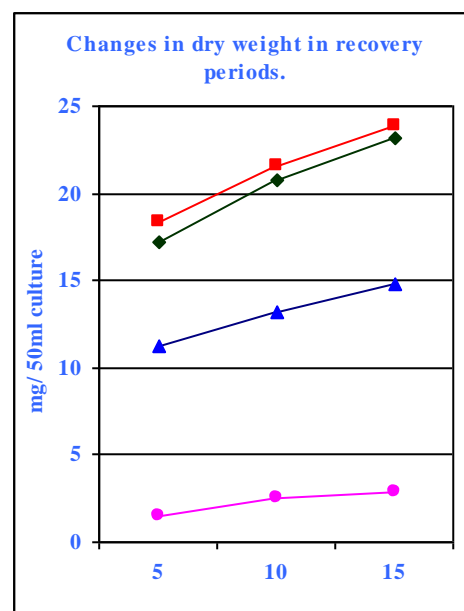
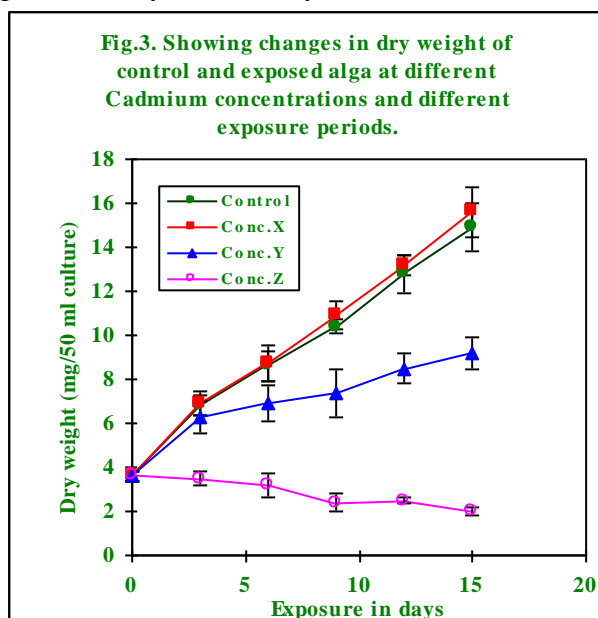
In the present investigation, a graded series of concentrations of the cadmium chloride was prepared ranging from 0.1 to 1.0mg l⁻¹(micro-range), from 1.0 to 10.0mg.l⁻¹(middle range) and from 10 to 100mg l⁻¹(High range) to evaluate the toxic effects of the cadmium chloride on the blue-green alga and to find out maximum allowable concentration of cadmium chloride for the experimental purposes. Below mentioned table (AB) explains the toxicity of the cadmium chloride to the blue-green alga, *Anabaena cylindrica*. With the increase in the concentration of the toxicant the percent survival decreased and hundred percent deaths were noticed at 1.88 mg l⁻¹ of cadmium chloride with in a period of 15 days. With the increase in exposure period the concentration of cadmium decreases at a particular lethal dose / concentration value. The maximum allowable concentration (MAC) recorded for this alga for 15 days exposure was 0.31mg l⁻¹. The lethal concentration values for 15 days of exposure periods have been outlined below. The LC₁₀ value was 0.48 mg. l⁻¹. The LC₅₀ value was 0.78mg l⁻¹. LC₉₀ value was 1.22mg l⁻¹ and LC₁₀₀ value was 1.88mg l⁻¹, for this particular alga, *Anabaena cylindrica*.

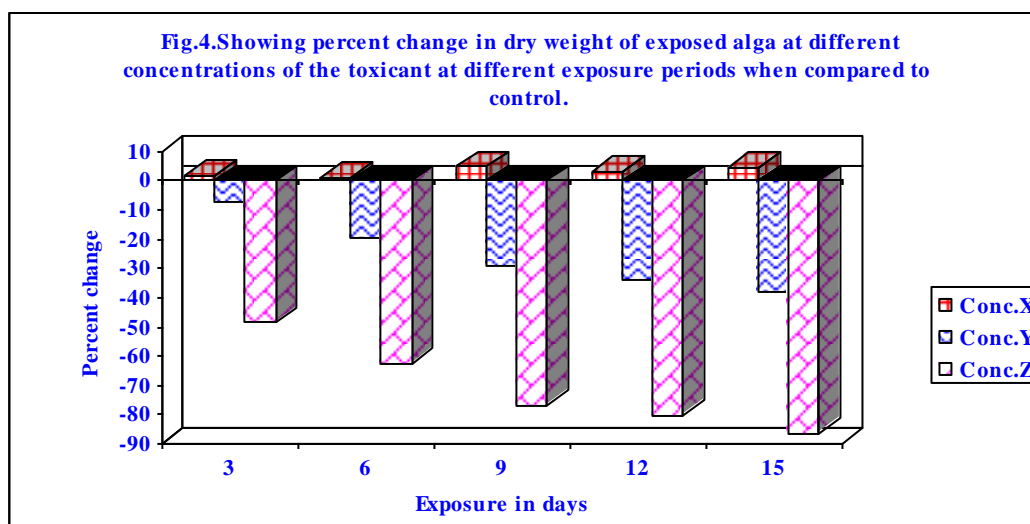
The control set showed 100% survival. The same data can also be interpreted as 10% survival at 1.22 mg l⁻¹, 50% survival at 0.78 mg l⁻¹, 90% survival at 0.48 mg l⁻¹, and 100% survival at 0.31 mg l⁻¹ was marked. Out of the above concentrations, LC₀₀ or PS₁₀₀ as safe MAC value of 0.31 mg l⁻¹ was selected as 'X'; LC₅₀ or PS₅₀ of 0.78 mg l⁻¹ was selected as 'Y' and LC₉₀ or PS₁₀ value of 1.22mg l⁻¹ was selected as 'Z' for conducting future experiments. The growth measured in terms of optical density at 540 nm of the alga exposed to different concentrations of cadmium chloride and control set at different days of exposure and recovery was presented in Fig. 1. The optical density value increased from 0.025±0.005 to 0.121±0.014 on 15th day of exposure and it increased to 0.205±0.016 on 15th day of recovery in the control set (Fig.1). The optical density value in case of set 'X' increased from 0.025±0.005 to 0.12±0.01 on 15th day of exposure and increased to 0.215±0.012 on 15th day of recovery. In optical density study the "X" concentration value was more than the control value on all days of exposure and recovery, and the two values showed a consistent significant increase with the increase in days of exposure up to 15th day (Fig.1). In case of "Y" concentration the value increased to 0.075 on 15th day of exposure. All the values were more than the control set. Where as, in case of 'Z', the optical density value initially increased from 0.025±0.005 to 0.035±0.004 on 9th day and then the value decreased with the increase on exposure period and a minimum of 0.015±0.0002 value was reached on 15th day of exposure. No change in value was marked in recovery period, rather the OD value further depleted. A maximum decrease by 0.83%, 38.02% and 87.6% was recorded on 15th day of exposure when compared to the control value in case of concentration X, Y and Z, respectively (Fig.2). The highest percent of increase in O. D. value over the control value was seen at "X" concentration on 6th day of exposure (7.58%) and highest percent decrease (38%) was seen on 15th day of exposure in "Y" concentration and a maximum of 87.6% decrease was noted at concentration "Z" on 15th day of exposure (Fig. 2). When the cadmium chloride exposed algae was transferred to toxicant free medium during recovery studies, on 15th day of recovery insignificant recovery by 4.9% was noted in concentration-X, when compared to control value. Where as, in case of concentration-Y and Z, a maximum of 45.3% and 94.1% decrease was recorded on 15th day of recovery. These two values were much more than the 15th day exposure value. This clearly indicated that the toxicant is deadly toxic and the exposed alga could not recover even during recovery period in toxicant free nutrient medium. No recovery was marked at higher concentrations of the toxicant rather further depletion in the parameters was noted indicating permanent damage caused to the exposed system. Only at sub-lethal or at lowest concentration selected (Con. X), the exposed alga indicated good recovery. On 15th day of exposure 0.8% decrease when compared to control was seen and on 15th day of recovery 4.9% increase over the control value was recorded. This increase in recovery over the decrease in exposure indicated partial recovery (Fig.2).



The correlation coefficient analysis indicated the existence of positive significant correlation between days of exposure and optical density values in control ($r = 0.988, p \leq 0.01$), Conc. X ($r = 0.994, p \leq 0.001$), and Conc. Y ($r = 0.966, p \leq 0.05$), however, negative non-significant correlation was observed in Conc. Z ($r = -0.412; p = NS$). The two way analysis of variance ratio test of Table-1, pertaining to optical density indicated that there exists a significant difference between columns and significant difference between rows.

The data presented in Fig- 3 depict the change in dry weight of the alga *Anabaena cylindrica*, at different days of exposure and recovery, when exposed to different concentrations of cadmium chloride. The dry weight increased from $3.6 \pm 0.4 \text{mg}$ to $14.9 \pm 0.7 \text{mg}$ on 15th day of exposure and the dry weight further increased to $23.2 \pm 1.4 \text{mg}$ on 15th day of recovery in the control set. At concentration-X, the dry weight increased from $3.6 \pm 0.4 \text{mg}$ to $15.6 \pm 1.1 \text{mg}$ on 15th day of exposure and the dry weight further increased to $23.8 \pm 0.9 \text{mg}$ on 15th day of recovery.





At concentration-Y, the dry weight increased from 3.6 ± 0.4 mg to 9.2 ± 0.5 mg on 15th day of exposure and the dry weight further increased to 14.8 ± 0.5 mg on 15th day of recovery. At concentration-Z, the dry weight decreased from 3.6 ± 0.4 mg to 2.0 ± 0.4 mg on 15th day of exposure and the dry weight increased to 2.9 ± 0.2 mg on 15th day of recovery (Fig.-3). At concentration “X” the dry matter value was greater than the control value on all days of exposure and recovery. Higher rate of growth was marked in case of concentration “X” than “Y and Z”. Highest increase in percentage of growth (4.8%) was marked on 9th day of exposure over the control value (Fig. 4). The “Y” concentration showed a significant decreasing trend in growth when compared to control and concentration “X”, in which a maximum decrease of 38.3% was marked on 15th day of exposure. Whereas, an increasing trend was marked up to 15th day of exposure when compared to 0 day value and with the increase in exposure period the dry weight of the alga increased and then the value further increased during recovery period when compared to 15th day exposure value, however all the values were much less when compared to control and concentration-X. In case of concentration-Z, the dry weight value did not show any increase when compared to 0 day value. Rather the dry weight decreased with the increase in exposure period. All the exposed values were significantly less than the control, concentration-X and concentration-Y. A maximum of 86.6% decrease when compared to control value was noted indicating severe damage caused to the exposed system. A significant dual behavior of the toxicant on the exposed alga was clearly evident from the obtained data (Fig. 3 & 4).

Complete bleaching of the algal mass inside the test solution (Z) was observed from 3rd day of exposure period onwards. Gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 30 days of recovery period. Unlike of mercury poisoning case where recovery or tiny particles appear from 12th day of exposure itself and become dominating blue-green during recovery period at lower concentration of mercury, where as in cadmium poisoning such a case was not observed. These particles grew in size with time. It was probably due to the appearance of photosynthetic pigments which disappeared due to the heavy metal (Cadmium) stress on the alga. Slowly the entire white mass got converted into a blue-green mass with the increase in recovery period (60 days of recovery). At “Z” (1.22mg / l) concentration (LC₉₀) a drastic decrease in dry weight value was noticed on 9th day of exposure and afterwards it showed continuous decrease up to 15th day of exposure (Fig. 4), however, the values were far less than control and concentration- “X & Y”. The dry wt of control, “X” and “Y” concentration increased significantly with the increase in days of exposure, which were significant. No recovery was marked. Rather the values were much less than the exposure value showing drastic depletion in the parameter. The dry weight values decreased in conc. Z. significantly at all exposure days at $p < 0.01$ level except on 3rd day with the increase in concentration of the toxicant. The percent change value of the dry weight over its control also increased insignificantly with increase in days of exposure at concentration “X” and the increase was not consistent. At concentration “Y” the dry weight value increased with the increase in exposure period and the marked values were less than the control and concentration-X values at all exposure and recovery periods. The percent change in dry weight in concentration ‘Y’ showed an increasing trend up to 15th day of exposure and then declined during recovery period. However, the dry weight values in conc. X were significantly higher than the control value. However, in case of concentration Y, the dry weight value showed no increase rather consistent decrease was noted. Than with the increase in recovery period the dry weight increased and a maximum of 38.3% decrease was recorded on 15th day of exposure and 36.2% decrease was noted on 15th day of recovery (Fig. 4). The dry weight significantly decreased and showed a maximum decrease by 86.5% on 15th day of exposure and when the exposed alga

was transferred to toxicant free medium further depletion in dry weight was noted at initial recovery period. With the increase in recovery period, partial recovery was noted and the percent recovery was not significant (Fig.4). The recovery values of different days of exposure were quite prominent from Fig.3 & 4. In "X" concentration highest percent of recovery was observed in exposed alga when exposed to toxicant free culture media on 10th day of recovery. With the increase in days of recovery the percent recovery value became less and it attained the lowest value after 15th day of recovery. In case of concentration-Y, a maximum of 2% recovery was noted. But in case of "Z" concentration zero percent recovery was seen and the dry weight gradually decreased up to 5th day of recovery and after 10th day of recovery little increment was noted. The correlation coefficient analysis between days of exposure and dry weight of the control and exposed alga, exposed to different concentrations of cadmium chloride indicated the existence of a positive significant correlation in control ($r = 0.991$; $p \leq 0.001$) and Conc. X ($r = 0.997$, $p \leq 0.001$). In case of concentration-Y, a positive correlation was noted ($r = 0.976$, $p \leq 0.01$). A negative and significant correlation ($r = -0.829$; $p \leq 0.05$) existed between days of exposure and dry weight of the alga in Conc. Z.. The two way analysis of variance ratio test indicated the existence of significant difference between rows and non-significant difference between columns.

In the control set, the growth rate showed an initial increasing trend and static growth rate with the increase in exposure period up to 15th day of exposure was marked. During recovery period, significant variation in growth rate was observed. In conc. X, the growth rate values were more than the growth rate values of the control set except 12th day of exposure, where a slight decrease in growth rate was marked and on the 15th day of exposure increase was noted. Higher growth rate in conc. X. was marked during recovery period, when compared to control set except on 15th day of recovery. In conc. Y, an initial increase on 3rd day of exposure and afterwards with the increase in exposure period decrease in growth rate was marked. When the exposed alga was transferred to toxicant free medium, the growth rate significantly declined when compared to control and conc. X set. With the increase in exposure period, the growth rate showed all negative values indicating death of the cells. No recovery was marked in conc. Y. In case of concentration-Z, negative values were obtained at initial periods of exposure and an insignificant value at higher exposure periods. When the exposed alga was transferred to toxicant free medium, an initial negative value followed by significant fall in the growth rate was marked (Fig.5). The growth rate pattern ($\Delta N/\Delta t$) computed from optical density values at different days of exposure showed, no change in case of control up to 15th day of exposure and "X" concentration showed a constant increase up to 15th day by exposure. In "Z" concentration it showed an increase up to 3rd day of exposure and then declined up to 15th day of exposure. With the increase in toxicant concentration, the optical density values decreased non-significantly at different days of exposure.

Discussion

Generally, algae are more sensitive than animals to complex wastes such as industrial and municipal effluents. Their use in bioassays is of ecological significance, since algae are the dominant primary producers in aquatic environments. A wide range of toxicity tests has been developed in the recent decades to predict the probable effects of new chemicals and industrial wastes in aquatic ecosystems utilizing different organisms such as algae, crustaceans, mollusks and fish (Miller *et al.*, 1978). Kamp-Nielson (1971) demonstrated a time dependent effect of HgCl₂ (added to 30 µg / liter) on the photosynthesis of *Chlorella pyrenoidosa*. Shaw (1987) reported that the algae *Westiellopsis prolifica*, Janet, could tolerate only 40% concentration of the supernatant effluent of a Chlor-alkali industry. Algae, the most important primary producers of the aquatic environments have received least attention. Very few references are available particularly on the toxicity effects and physiological changes induced by heavy metals on algae. The review made by Whitton (1970), Gadd & Griffiths (1978) and Sorentino (1979) on impact and effect of heavy metals on algae added a lot of information to the literature of algal toxicology. Algae have been shown to concentrate heavy metals to a larger extent (Say *et al.*, I & II, 1977). Information's are available pertaining to the toxicity of mercury in the form of metal, mercury based pesticides, industrial wastes containing mercury etc. on fresh water blue-green algae (Rai *et al.*, 1981a, b; Shaw, 1987; Sahu, 1987; Rath, 1984; Rath, 1991; Dash, 1991 and Sahu, 1998). Agarwal & Kumar (1978) showed decrease in growth of *Chlorella* sp when exposed to mercurial effluent and solid wastes indicating toxic nature of mercury on the organism. A liquid industrial waste may affect the algal growth in any of three ways: stimulation, inhibition and stimulation at lower concentrations but inhibition at higher concentrations (Walsh & Alexander, 1980; Sahu, 1987; Shaw, 1987 and Rath, 1991). The enhancements of growth, heterocyst frequency and nitrogen fixation at lower doses of Furadon (0.75µg / ml of Carbofuran) have also been reported earlier. The present investigation did not agree with the above conclusions. But such stimulation in the growth cannot be easily explained at this stage of the study. Sahu (1987) and Shaw (1987) attributed the reason for stimulation, for

the presence of some growth regulating compounds and/or trace elements in the crude oil. Some suggested uptake and metabolism of the constituent as the probable mechanism for growth stimulation.

Toxicological studies involve the science of poisons, their effects, antidotes and detection. Toxicity is the ability of a chemical molecule or compound to produce injury once it reaches a susceptible site in or on the body of the organism. In toxicity testing the laboratory bioassay is generally the most favored because experimental conditions can be controlled and the response of test organisms observed or monitored to a greater degree. Effects on organisms are generally categorized into those causing: a) direct lethal toxicity and b) sub-lethal disruption of behavioral or physiological or biochemical activities. Quantitatively lethal effects can be defined as those responses that occur when physical or chemical agents interfere with cellular and sub-cellular processes in the organism to such an extent that death follows directly. In comparison, sub-lethal effects are those that disrupt physiological or behavioral activities but do not cause immediate mortality although death may follow because of interference with feeding, abnormal growth or behavior, lesser ability to colonize or other direct causes and effects. Measurements of lethality are frequently used to derive "safe" levels of exposure to toxicants. The assumptions adopted in lethality measurement are not well supported empirically and as an alternative, the use of chronic, sub-lethal tests may be more appropriate. Sub-lethal measurements are considered suitable for predicting safe level of toxicants. Toxicity tests were designed to find out safe level of toxicants and different lethal concentration values for a particular organism or for different types of organisms. The toxicity value varies from organism to organism. The present study indicated that cadmium is deadly toxic as observed from toxicity test. Dry weight measurement and optical density measurements were considered as the parameter of growth. Sahu (1987) reported that algal systems behaved very differently towards light scattering in presence of different stresses. Optical density of the homogenized medium of the culture has been considered as a growth parameter in normal studies but in pollution studies, in presence of pollutants a consistent data in optical density was never obtained. Rath (1991) indicated the idea that in presence of a pollutant, the deflection in optical density may not be exactly due to the algal growth or increase, it may also be due to the pollutant and dead cells present, as it deflects the light from the original path of penetration. The changes observed in optical density study exactly do not reflect the real changes induced by the pollutant, but an approximation can be made out of this data. Growth is a summation of all cellular metabolisms. So, any inhibition of growth reflects toxic effects on a number of metabolic processes. The paper mill effluent contained a significant amount of cadmium in its effluent. When the effluent was taken for toxicity study, it was observed that along with the accumulation of cadmium from the effluent waste it was difficult to ignore a possible accumulation of other ions from the effluent that might have played certain role in growth acceleration in lower concentrations and retardation in higher concentrations. In the present study, we find higher deposition of sodium ion in concentration X than concentration Y set, when compared to the control value. In case of potassium ion significant decrease in ion content was marked. It was also confirmed by earlier workers, who reported that mercury appears to be less toxic in media with a high concentration of dissolved salts. To confirm the effect of mercury in a combined form on the growth of the alga, the dry weight and optical density measurements are not enough and basing on these data, no clear cut presumption can be made. So further pigment particularly chlorophyll, physiological, biochemical and enzymological studies can reveal the detailed mechanism of the effect of the pollutant on algal system in greater details. In the present study the toxicant, cadmium chloride showed stimulation at sub-lethal concentration and inhibition at higher concentration, showing dichotomous behavior. Stimulation at very low concentration of cadmium chloride and appearance of chlorophyll during recovery period indicated that the alga at toxic environment could avoid the stress by some mechanism and in favorable conditions recovered fully. The recovery period was much more than exposure period.

Cadmium as cadmium chloride did not show any dichotomous behavior like mercury. In recovery studies, no significant recovery was observed at lower concentration and no recovery at all at higher concentration indicated that cadmium caused irreparable damage to the exposed system. Complete bleaching of the algal mass inside the test solution (Z) was observed from 3rd day of exposure period onwards indicating the impact of cadmium chloride. Gradually tiny blue-green particles started making their appearance in the white turbid mass as observed in the naked eye after 30 days of recovery period. This recovery was probably due to excretion of cadmium from the algal body and depletion of heavy metal impact on the alga. These particles grew in size with time. It was probably due to the appearance of photosynthetic pigments which disappeared due to the heavy metal (Cadmium) stress on the alga. Slowly the entire white mass got converted into a blue-green mass with the increase in recovery period (60 days of recovery). Further physiological and biochemical studies pertaining to impact of cadmium on the BGA will help to understand the impact and mechanism of impact of cadmium.

Acknowledgement

The authors wish to thank the Head, Department of Botany and authorities of Berhampur University, Odisha, India for permitting the use of the research laboratory and library facilities.

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Declarations**AUTHOR CONTRIBUTION STATEMENT**

Prof. A.K. Panigrahi: Conceptualization, planning and execution of the project, field visit, original draft preparation, supervision, reviewing and editing. Research work conducted by scholar – Saroj K. Misra paper mill effluent collection , analysis and related experimental work. Misra contributed reagents, glassware, field related work, calculation and finalization of data.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

