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Toxicological Impacts Of Cadmium Chloride On The Photosynthetic Efficiency of a Cyanobacterium *In Vivo* Under Controlled Conditions.

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Highlights

- Experiments were conducted at sub-lethal (MAC) and lethal concentrations of cadmium chloride to assess the toxicological impacts of cadmium chloride on the blue-green alga, *Anabaena cylindrica*.
- The exposed alga could accumulate significant amount residual cadmium in its body within 15days of exposure.
- Significant depletion in pigment content (Total Chlorophyll and Total pheophytin) was noted in the exposed alga at higher concentrations of Cadmium chloride compared to control alga.
- With the increase in residual cadmium content the pigment content significantly declined at lethal concentrations.
- Significant recovery of the exposed alga was noted after a prolonged exposure period (60days-4 times more than the exposure period).
- This alga can be used in biological treatment protocol for treating the paper mill effluent waste.

Abstract

The total chlorophyll content increased within 15 days of exposure in the control set and the value increased further on 15th day of recovery. A maximum of 7.3% increase was recorded on 12th day and on 15th day 3.9% increase over the control value was marked in conc. X set. When the exposed alga was transferred to toxicant free medium, the chlorophyll content increased insignificantly. A maximum of 25.6% decrease was recorded on 12th day and on 15th day 35.3% decrease over the control value was marked in conc. Y set. A maximum of 89% decrease was recorded on 12th day and on 15th day 97.1% decrease over the control value was marked in conc. Z set. The percent decrease increased with the increase in exposure period, where a positive correlation was marked. A maximum of 97.06% decrease was recorded on 15th day of exposure in conc. Z set. No significant recovery was recorded in conc. Y set and no recovery was marked in concentration Z set, when the exposed alga was transferred to toxicant free nutrient medium, rather higher depletion was noted. At conc. X, the carotenoid content increased at all exposure periods, when compared to the control value except on 3rd day. Insignificant partial recovery was recorded in conc X and no recovery was marked in conc.Y, when the exposed alga was transferred to toxicant free, nutrient medium. In case of conc.-Z, highly significant decrease in the carotenoid level was noted. In conc. X, the ratio value was significantly high when compared to control value at all exposure periods and recovery periods. Interestingly, the ratio value decreased in conc. Y at all exposure and recovery periods when compared to control and conc.-X. From the data, it was evident that the ratio value can be an indicator of stress in plant systems. The residual cadmium level increased with the increase in exposure period in all three tested concentrations. During recovery period partial recovery was noted. Prolonged recovery period indicated reversal of inhibition. The residual cadmium level decreased significantly after 30days of recovery and 60days of recovery.

Key words: Cadmium, Cyanobacterium, Pigment, Chlorophyll, Pheophytin, Residual cadmium

Introduction

Heavy metal pollution caused by Pulp and Paper industries is a direct threat to the very existence of aquatic plant and animal life in nature. Pulp and Paper mills are the major players to contribute heavy metals like mercury, cadmium, copper, arsenic and lead, polluting nearby water bodies and affecting aquatic flora and fauna. The paper mills use huge amount of fresh water drawn from water bodies in the manufacturing process and discharge significant amount of effluent as waste. Chmielowska-Bak *et al.,* (2021) reported that "contamination of the environment with metals, their adverse impact on plant performance and transmission to the human food chain through crops and vegetables are important concerns worldwide". Dey *et al*. (2018) indicated that in India, more than 55% of the mills do not have adequate effluent treatment facilities and also do not adopt modern treatment technologies. The paper mill effluents are discharged from the industry into the environment in and around surrounding the water bodies after simple physical and chemical treatments (Tripathy *et al*., 2021) indicating the need of a biological treatment. Dixit *et al*., (2018) reported presence of mercury, cadmium and lead in the final discharged Paper mill effluent. It is not possible to eliminate waste generation by the system (Kaur *et al.,* 2021) but cleaner & environment friendly technology can be used and periodically positive modifications in the technology or alterations in the treatment technology should be adopted.

Chemical industry in India has grown up phenomenally since independence. There is to-day about 4000 chemical factories in India. They release large quantities of chemicals in the form of gas, liquid and solid wastes, into the environment. Many of these chemicals are toxic and create pollution problem. The problem of toxic hazard has already reached alarming proportions in this country and is bound to grow with increasing industrialization. India is the larges manufacturer of pesticides in the whole of South Asia and Africa. As many as 139 organic chemicals, heavy metals like zinc, lead, chromium, copper, mercury and various other compounds are used in the manufacture of dyes only. Sundaresan *et al*. (1983) have given the growth of industries dealing with toxic chemicals and generating toxic and hazardous wastes during 1950s, 1970s and 1980s, in India. Industries which are known to produce potentially toxic and hazardous wastes are pesticides, dyes and pigments, organo-chemicals, fertilizers, non-ferrous metals, steel and chlor-alkali plants. The major foci of such industries in India are Bombay, Calcutta, Kanpur, Delhi, Chandigarh, Jamshedpur, Bokaro, Hyderabad, Vishakhapatnam, Madras, Baroda, Cochin etc. The wastes from the industry are generally disposed by land filling or released into water bodies. Generally the toxic effluents from industry are neutralized before their discharge, but still they contain substantial amount of toxic substances that can cause pollution. At present it is believed that rivers are most severely polluted by industries followed by estuaries, lakes and ocean in declining order (Sahu & Panigrahi,2003).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 o long term exposure to low concentrations of this element. The discovery that Cadmium pollution from a base metal mining and smelting complex could cause series illness and possible death in a local community has led to widespread public anxiety (Kuboi *et al*, 1987). Although industrial operations are major sources of Cadmium, many countries now show concern that disposal of metal which sewage sludge on land may adversely affect the fertility of the soil and render plants a health hazards if consumed by man and animals. Nevertheless, the increasing awareness of the political hazards of cadmium contamination should not obscure the fact that cadmium is present in natural ecosystems and a ubiquitous element in all living organisms. For the environmental impact of cadmium to be assessed, major steps in the bio-geo-chemical pathway must be outlined and gaps in our knowledge identified for future research undertakings. 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. This piece of work was aimed at finding out the impact of cadmium chloride a pollutant member in the effluent on the pigment content of a cyanobacterium in vivo and to find out the possible use of this alga in biological treatment protocol.

Materials & Methods

Toxicity study: For toxicity study of the pollutant, cadmium chloride of pure grade (Analytical pure grade) was prepared by dissolving in distilled water. A graded series of concentrations of the toxicant ranging from 0.1mg.l^{-1} to 2.0mg.l^{-1} (V/V) was prepared in different experimental conical flasks and UV sterilized in a Laminar Airflow. The dilutions were made with the nutrient medium. One ml of unialgal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100 ml of solution, inside the inoculating chamber. The toxicant was diluted with sterilized nutrient medium. The homogenized algae were inoculated and the flasks were kept on culture racks. The number of individual cells of the algae present in one ml of the culture medium after micro-tissue homogenization was counted under the microscope. The inoculated flasks

were kept inside the culture room at 28 ± 2^0C and under 14 hours illumination at the intensity of 2400 ± 200Lux and were shaken periodically daily to avoid clumping of cells. The test algae were exposed for a period of 15 days in different test media after which their survival and mortality percentages were calculated by counting the number of cells present in one ml of the test solution after micro-tissue homogenization. From this, different mortality percentage and the lethal concentration values, maximum allowable concentration (MAC) were determined. From the toxicity testing as described by Sahu (1987), the marked X, Y and Z concentrations of the toxicants were selected for future experiments.

Anabaena cylindrica,Lemm is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga (BGA) belongs to the family Nostocaceae, can fix atmospheric nitrogen under aerobic condition. Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) and adopted by Sahu (1987) was most suitable basic culture for the growth of the test organisms. The pigment contents of both control and exposed alga were estimated and calculated following the method described by Vernon (1960). The alga exposed in culture flasks were carefully removed and centrifuged in high speed centrifuge and the pellet was taken in a Klein's apparatus (digester) with acid digestion mixture (Conc. Sulphuric acid and Conc. Nitric acid, 1:1 ratio) and the sample was digested till the whole plant samples were fully digested (Wantorp and Dyfverman, 1955). Minimum two cycles were maintained for al samples. After digestion, the digested extract was cooled to room temperature. After cooling the cadmium content of the digested extract was estimated by using Atomic Absorption Spectrophotometer following the procedure of Yoshida *et al*. (1976). The obtained data was statistically analyzed.

Results

The total chlorophyll content of the control and cadmium chloride exposed alga, at different days of exposure and recovery were presented in Fig.2 & 2a. The total chlorophyll content increased from 0.025±0.003 to 0.102±0.016mg /50 ml culture within 15 days of exposure in the control set and the value increased to 0.134±0.011mg/50 ml culture on 15th day of recovery. The total chlorophyll content increased significantly ($p \geq 0.01$) in the exposed set (Conc-X), the value increased from 0.025±0.003 to 0.106±0.01mg/50 ml culture on 15th day of exposure. A maximum of 7.3% increase was recorded on 12th day and on 15th day 3.9% increase over the control value was marked (Fig.2) in conc. X set. When the exposed alga was transferred to toxicant free medium the chlorophyll content increased from 0.106±0.01 mg/50 ml culture to 0.130±0.008 mg /50 ml culture after 15 days of recovery in conc-X set (Fig.2). The total chlorophyll content increased from 0.025±0.003 to 0.102±0.016mg /50 ml culture within 15 days of exposure in the control set and the value increased to 0.134±0.011 mg/50 ml culture on 15th day of recovery. The total chlorophyll content increased significantly ($p \geq 0.05$) in the exposed set (Conc.-Y), the value increased from 0.025±0.003 to 0.066±0.009mg/50 ml culture on 15th day of exposure. A maximum of 25.6% decrease was recorded on 12th day and on 15th day 35.3% decrease over the control value was marked (Fig.2a) in conc. Y set. When the exposed alga was transferred to toxicant free medium the chlorophyll content increased from 0.066±0.009mg/50 ml culture to 0.085±0.005mg /50 ml culture after 15 days of recovery (Fig.2). The total chlorophyll content decreased significantly ($p \geq 0.05$) in the exposed set (Conc-Z), the value decreased from 0.025±0.003 to 0.003± 0.001mg /50 ml culture on 15th day of exposure. A maximum of 89% decrease was recorded on 12th day and on 15th day 97.1% decrease over the control value was marked (Fig.2) in conc. Z set. When the exposed alga was transferred to toxicant free medium the chlorophyll content further decreased from 0.003±0.001mg /50 ml culture to 0.001±0.0005mg /50 ml culture after 15 days of recovery (Fig.2 & 2a). Conc. X set showed an increase in total chlorophyll content at all exposure periods (Fig.2).

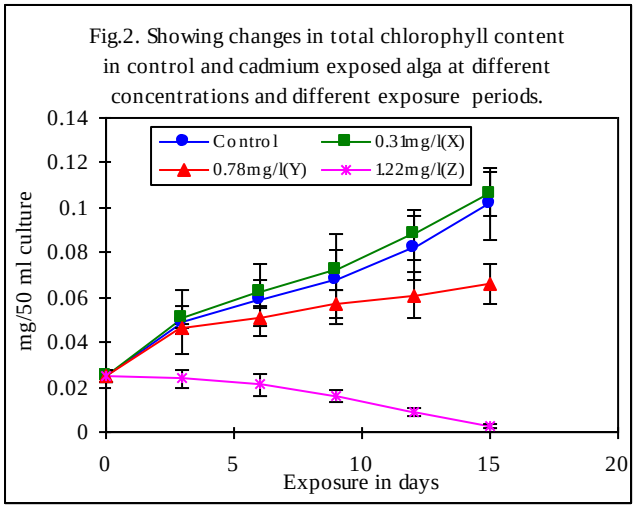


Fig.2. Showing changes in total chlorophyll content in control and cadmium exposed alga at different concentrations and different exposure periods.

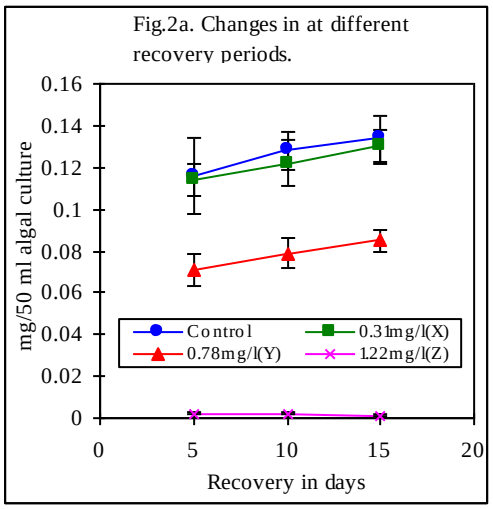
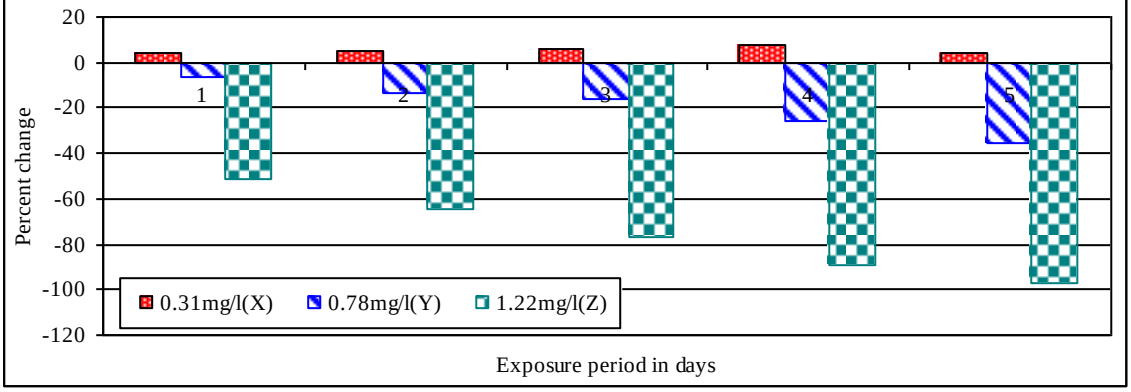


Fig.2a. Changes in at different recovery periods.

In case of concentration Y, the total chlorophyll content though increased on 3rd day onwards, when compared to 0 day value but the chlorophyll amount was far less than control and conc. X and a maximum of 35.3% decrease was recorded, when compared to the control value. The percent decrease increased with the increase in exposure period, where a positive correlation was marked. A maximum of 97.06% decrease was recorded on 15th day of exposure in conc. Z (1.22 mg of cadmium as cadmium chloride / l^{-1}) set (Fig.3).

Fig.3; Percent changes Chlorophyll content in exposed alga compared to control alga at different exposure periods.(1=3,2=6,3=9,4=12,5=15 days)



The correlation coefficient analysis between days of exposure verses total chlorophyll indicated the existence of significant positive correlation in control ($r = 0.991$, $p \geq 0.001$) and Conc. X ($r = 0.994$, $p \geq 0.001$). In case of Conc. Y, a positive correlation was marked between the chlorophyll content and days of exposure ($r = 0.911$, $p \geq 0.01$). A negative correlation ($r = -0.806$, $p \geq 0.05$) was marked in Conc. Z. The two way analysis of variance ratio test based on the data, indicated the existence of significant difference between rows and significant difference between columns. The total chlorophyll present in control, exposed and recovered alga was clearly indicated in Fig. 2a. The correlation coefficient value of total chlorophyll with days of exposure was significant at $p \geq 0.001$ level for control and X concentration of the toxicant, i.e. the total chlorophyll amount increased significantly with the increase in days of exposure at control, and "X" concentration, whereas at Z concentration it was not significant. With the increase in concentration, the total chlorophyll content decreased on all days of exposure but on 6th, 12th and 15th day the values were significant at $p \geq 0.05$ levels. The percent change value of total chlorophyll increased with the increase in days of exposure at X concentration but the values were not statistically significant, whereas this value decreased with days of exposure in Y concentration and significantly decreased in Z concentration. With the increase in concentration the percent change value of total chlorophyll showed gradual decrease on all days of exposure which was statistically significant at $p \geq 0.001$ levels except on 3rd day where it was significant at 0.01 levels. In recovery studies, it was clearly observed that no recovery was marked in conc. X, Y and Z set. The chlorophyll content was drastically affected in cadmium chloride exposed alga compared to control alga.

Fig.4 indicated the changes in total phaeophytin content in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The total phaeophytin content increased from 0.018±0.003 to 0.092±0.004mg/50 ml culture in a period of 15 days. The value further increased from 0.092±0.004 to 0.184±0.011mg /50 ml culture in 15 days of recovery. The phaeophytin content interestingly declined from control value at all exposure and recovery periods in conc-X. No doubt, the phaeophytin content increased from 0.018±0.003 to 0.089±0.009mg /50 ml culture on 15th day of exposure and the same value increased from 0.089±0.009 to 0.176±0.008mg /50 ml culture, showing a positive correlation after 15 days of recovery ($p \geq 0.01$), with the increase in recovery period, like the control set but the values were interestingly less than the respective control value for each exposure and recovery period (Fig.4).

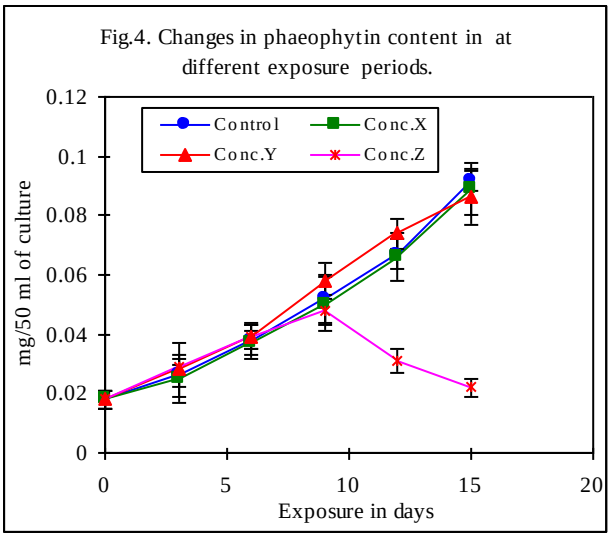


Fig.4. Changes in phaeophytin content in at different exposure periods.

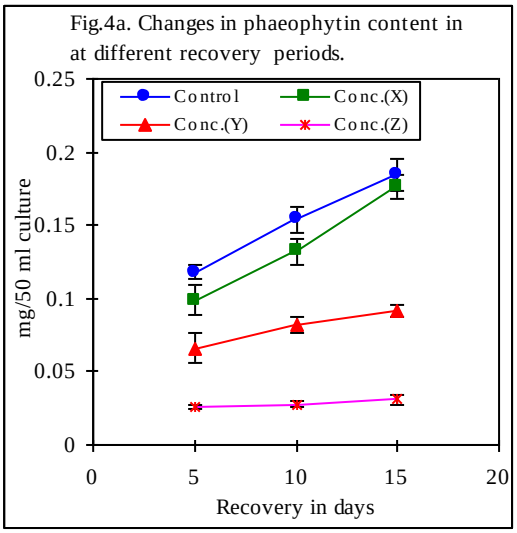


Fig.4a. Changes in phaeophytin content in at different recovery periods.

In conc.Y, the phaeophytin content increased from 0.018±0.003 to 0.074±0.005mg/50 ml culture on 12th day of exposure showing more than the control and conc. X set and than the value significantly declined with the increase in exposure period. The value declined from 0.074±0.005 to 0.086±0.009mg/50 ml culture on 15th day of exposure. When the exposed alga of conc. 'Y' was transferred to toxicant free nutrient medium, no recovery was altogether marked. Rather the values further depleted to 0.066±0.01mg/50 ml culture, on 5th day of recovery, indicating total damage and destruction of the pigment (Fig.4a). At higher recovery periods, the phaeophytin content increased from 0.066±0.01 to 0.092±0.004mg/50ml culture.

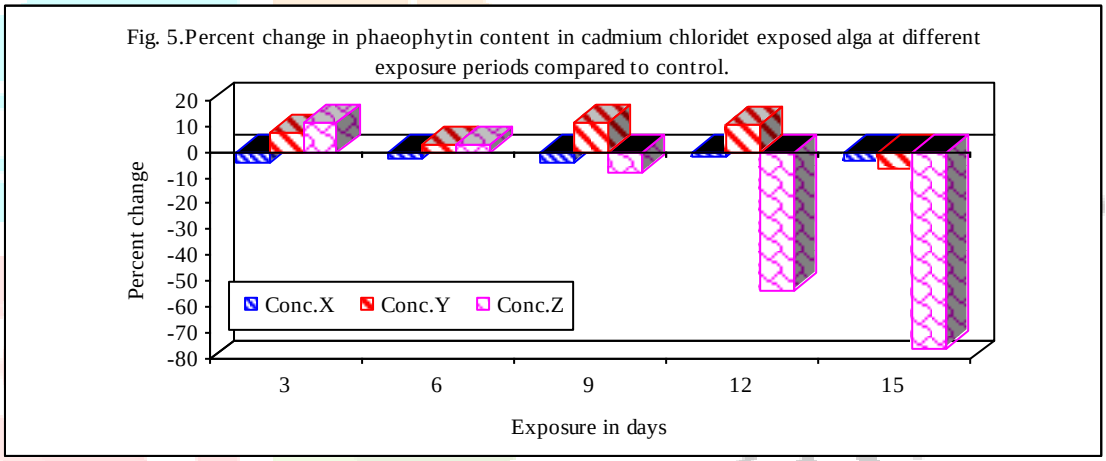


Fig. 5.Percent change in phaeophytin content in cadmium chloridet exposed alga at different exposure periods compared to control.

In conc. Z, the phaeophytin content increased from 0.018±0.003 to 0.048±0.005mg /50 ml culture on 9th day of exposure showing less than the control and conc. X and Y sets and than the value significantly declined with the increase in exposure period. The value declined from 0.048± 0.005 to 0.022±0.003mg /50 ml culture on 15th day of exposure. When the exposed alga of conc.Z was transferred to toxicant free nutrient medium, partial insignificant recovery was marked. The values increased to 0.031±0.003mg/50 ml culture, on 15th day of recovery, indicating total damage and destruction of the pigment. Fig. 4 indicated the percent change in phaeophytin content in the exposed alga at different exposure periods when compared to the control set. The phaeophytin content decreased by 2.6% on 6th day of exposure and than partial recovery up to 12th day of exposure and 3.3% decrease on 15th day of exposure was recorded in concentration X (Fig.4a). The phaeophytin content increased by 11.5% on 9th day of exposure and than decreased on 12th day of exposure showing 10.5% increase over control value and with the increase in exposure period, 6.5% decrease on 15th day of exposure was recorded in conc. Y. When the exposed alga was transferred to toxicant free medium, instead of showing any recovery, further significant depletion in the pigment content was recorded and a maximum of 50% decrease was noted when compared to control.

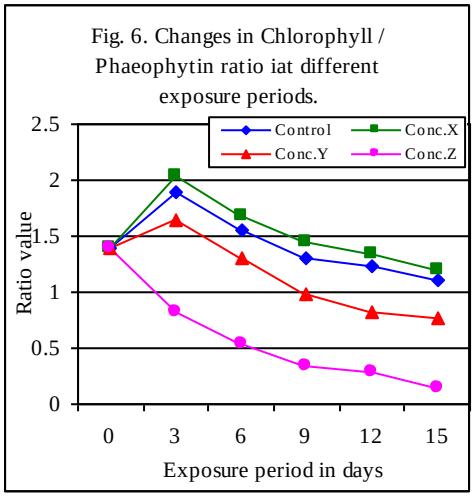


Fig. 6. Changes in Chlorophyll / Phaeophytin ratio iat different exposure periods.

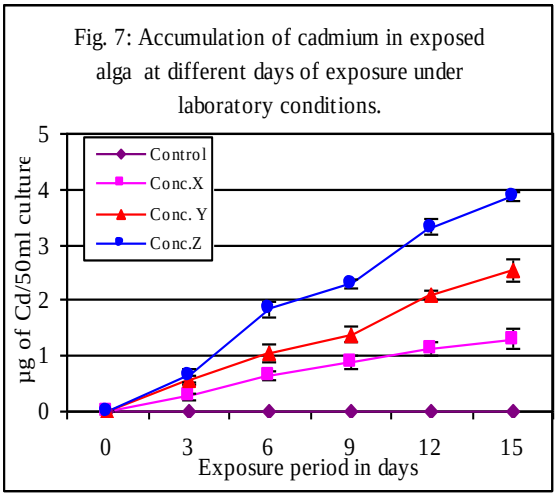


Fig. 7: Accumulation of cadmium in exposed alga at different days of exposure under laboratory conditions.

In conc. Z, 11.5% increase over the control value was recorded on 3rd day of exposure and the phaeophytin content significantly declined with the increase in exposure period and a maximum of 76.09% decrease was recorded on 15th day of exposure. No significant recovery was recorded in conc.Y set and no recovery was marked in conc. Z set, when the exposed alga was transferred to toxicant free nutrient medium, rather higher depletion was noted (Fig.5). The correlation coefficient analysis between phaeophytin content and days of exposure indicated the existence of positive and significant ($p \geq 0.001$) correlation in control ($r = 0.986$); Conc. X ($r = 0.984$) and in conc. Y ($r=0.991$). But in case of conc. Z a non-significant correlation was marked ($r = -0.924$, $p = NS$). The pigment ratio value showed an initial increase from 1.39 to 1.89 followed by linear decrease to 1.11 on 15th day of exposure and the ratio value further depleted to 0.73 when the alga was transferred to toxicant free medium during recovery studies. In conc. X, the ratio value was significantly high when compared to control value at all exposure periods and recovery periods. Interestingly, the ratio value decreased in conc. Y at all exposure and recovery periods when compared to control and conc.-X. In case of conc.-Z, further depletion of pigment ratio value was marked at all exposure and recovery periods. From the data, it was evident that the ratio value can be an indicator of stress in plant systems.

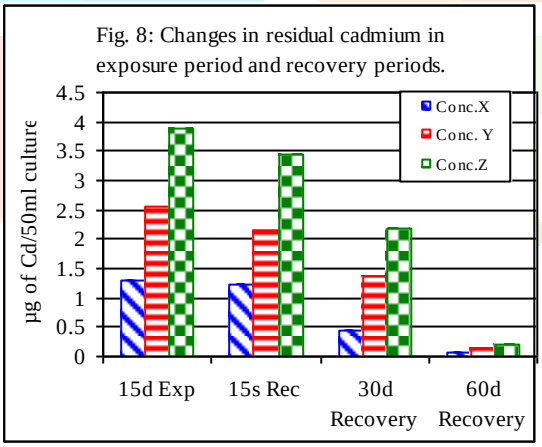


Fig. 8: Changes in residual cadmium in exposure period and recovery periods.

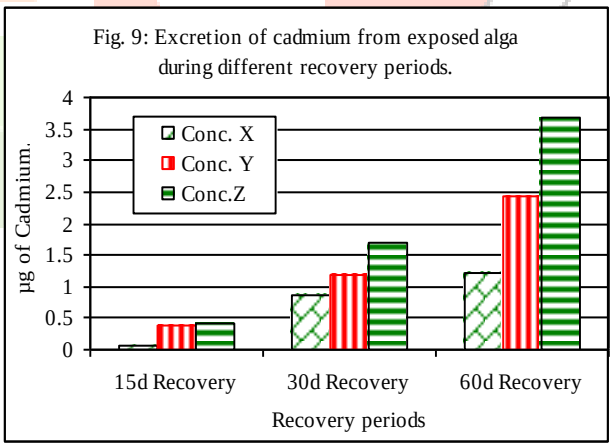


Fig. 9: Excretion of cadmium from exposed alga during different recovery periods.

With the increase in exposure period the residual cadmium content increased in the exposed alga showing a positive correlation. Fig. 7 showed the residual accumulation of cadmium in control and exposed blue-green alga at different exposure periods. Residual cadmium accumulation was not observed in the control set indicating the non contamination of the control set. In case of conc. X, the residual accumulation of cadmium increased with the increase in exposure period. The amount of cadmium accumulated in the conc. X exposed alga was 0.28±0.06µg/50ml culture after 3rd day of exposure and 1.31±0.18µg/50ml culture after 15th day of exposure. In case of conc. Y, the residual accumulation of cadmium increased with the increase in exposure period showing a significant positive correlation. The amount of cadmium accumulated in the conc. Y exposed alga was 0.58±0.08µg/50ml culture after 3rd day of exposure and 2.54±0.21µg/50ml culture after 15th day of exposure. The amount of cadmium accumulated in the conc. Z exposed alga was 0.65±0.11µg/50ml culture after 3rd day of exposure and 3.88± 0.09µg/50ml culture after 15th day of exposure (Fig. 7).

On 15th day of exposure, the exposed alga could accumulate 1.31±0.18µg/50ml culture and in case of conc.X after 15days of recovery the residual cadmium level decreased to 1.24µg/50ml culture. The recovery period was extended for a period of 30days and the residual cadmium further depleted to 0.45µg/50ml culture. On further extension of recovery period to 60days, 4 times more than the exposure period, the residual level significantly decreased to 0.08µg/50ml culture showing a significant amount residual cadmium excretion (Fig.8). After 15 days of exposure, the exposed alga could accumulate 2.54±0.21µg/50ml culture and in case

of conc. Y after 15days of recovery the residual cadmium level decreased to 2.14µg/50ml culture. The recovery period was extended for a period of 30days and the residual cadmium further depleted to 1.35µg/50ml culture. On further extension of recovery period to 60days, 4 times more than the exposure period, the residual level significantly decreased to 0.12µg/50ml culture showing a significant amount residual cadmium excretion (Fig.8). After 15 days of exposure, the exposed alga could accumulate 3.88± 0.09µg/50ml culture and in case of conc. Z after 15days of recovery the residual cadmium level decreased insignificantly to 3.46µg/50ml culture. The recovery period was extended for a period of 30days and the residual cadmium further depleted to 2.18µg/50ml culture. On further extension of recovery period to 60days, 4 times more than the exposure period, the residual level significantly decreased to 0.19µg/50ml culture showing a significant amount residual cadmium excretion (Fig.8). In case of conc. X set - 0.07µg of cadmium got excreted after 15days of recovery; 0.86µg of cadmium was excreted after 30days of recovery; 1.23µg of cadmium was excreted after 60days of recovery. In case of conc. Y set - 0.4µg of cadmium got excreted after 15days of recovery; 1.19µg of cadmium was excreted after 30days of recovery; 2.42µg of cadmium was excreted after 60days of recovery. In case of conc. Z set - 0.42µg of cadmium got excreted after 15days of recovery; 1.7µg of cadmium was excreted after 30days of recovery and 3.69µg of cadmium was excreted after 60days of recovery (Fig.9). At higher recovery period, the removal of cadmium metal from the plant body supported the survivability of the alga. After 30d exposure green dot like structures appeared and after 60days of exposure the whole white turbid mass turned green due to appearance of pigments. Appearance of pigments indicated revival of the exposed alga during recovery period.

Discussion

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 (Jennett & Wixson, 1975; Trollope & Evans, 1976; and Say *et al*., I & II, 1977). The uptake and accumulation of cadmium by algae consists of two phases (1) adsorption of cadmium to the cellular walls and (2) penetration of cadmium into the cell. Since very little was known regarding the effects of sub-lethal concentrations of cadmium as well as mercury contained solid waste on the physiology of the freshwater blue-green alga, it was not possible to predict the detailed action on blue-green algal systems. The selectivity in mercury accumulation by plant cell might be a distinctive property of mercury including high mortality and direct uptake by the surfaces being tight bound to the acidic groups of the cell wall. A concentration and exposure time period dependent mercury uptake by blue-green algae have been observed by Pradhan *et al* (2005) and Rath *et al*. (1983 a). Shaw (1987) reported effect of mercury contained industrial effluents on blue-green algae and opined that heavy metals behave drastically in a very different fashion in presence of other environmental chemicals, excluding heavy metals, and an antagonistic effect clearly prevailed with the mercury action in presence of other chemicals. Photosynthetic pigments are known to participate in generation of energy and CO_2 fixation (Kashyap & Gupta, 1981). The chlorophylls have long been recognized as the primary light acceptors, a small portion of which acts as the primary reaction centre where light conversion occurs. Carotenoids, not only help in photosynthesis, by transferring light energy but also protect the other photosynthetic pigments, preventing photo-oxidation and providing light shielding (Krinsky, 1966). Decrease in the level of chlorophyll in algae and other plants exposed to different toxicants have been reported. Geike (1977) reported decrease in the chlorophyll level in algae exposed in mercury. De Filippis and Pallaghy (1976 a, b) observed reduction in the chlorophyll content in *Chlorella* treated with zinc and mercury. Rai *et al*. (1981 a, b) reported a reduction in chlorophyll content of *Chlorella vulgaris*, when exposed to $HgCl_2$ between 100-1000µg/lt concentration, for 3 weeks. De *et al*. (1985) suggested that 20 mg/lt concentration of $HgCl_2$ decreased chlorophyll content of *Pistia stratiotes*, when exposed for 2 days. The decrease in chlorophyll level was a result of increase in the chlorophyllase activity. The wide spread occurrence, as well as certain chemical properties of chlorophyll pigments *in vivo* suggested that these pigments play an active role in photosynthesis functioning as photo-enzymes (Rabinowitch & Govindjee, 1973) and the mercurial compounds were toxic for the biosynthesis of chlorophyll pigments. Results obtained here are peculiar. In toxicological studies, involving algae, estimation of phaeophytin content serves as an important tool, since any unfavourable change in the environment or the effect of the toxicant is reflected by the change in its 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 (Singh & Singh, 1984). Degradation to phaeophytin might be the first step towards the breakdown of chlorophyll, which was evident from the increased levels of phaeophytin in the treated cultures. In this investigation at higher concentrations of the toxicants phaeophytin

content increased confirming the above presumption. Carotenoid play a vital role as a protector of photosynthetic tissues against photosensitised oxidation. The decrease in carotenoid content in algal cells exposed to heavy metal stress lead to a decrease in protection from the stress to the photosynthetic tissue. The ratio of chlorophyll to carotenoid has long been identified as a valuable parameter for defining environmental conditions unfavorable for algal growth. When the nutrient in the medium are exhausted or a toxicant was introduced into the medium the ratio increased due to decrease in the chlorophyll content (Rai *et al*., 1981 b). Increased ratio indicated inhibition of chlorophyll biosynthesis, inactivation of enzyme systems and disruption of many physiological and biochemical processes (Sorentino, 1979). The level of significance indicated a steady increase in chlorophyll, carotenoid and phaeophytin content with the increase in residual mercury only in lower concentrations of SWE. In higher concentrations of Hg in SWE, the decrease did show statistical significance. Rath (1984); Rath *et al*, 1983a,b;1985; Sahu (1987); Sahu & Panigrahi (2002) and Shaw (1987) indicated stimulation of growth, increase in pigment content, photosynthesis rate, respiration rate, and enzyme activity at lower concentrations of mercurial compounds on *W. prolifica*, Janet. Mercury at relatively low concentrations also affects the energy transfer by selectively affecting the phycocyanin in the phycobilisomes of intact cells of *Spirulina*, which was reported by Murty and Mohanty (1991). The photosynthetic efficiency of phycocyanin equals to that of chlorophyll-a was reported earlier. Murty and Mohanty (1991) reported that mercury at a low concentration (3 µm) caused an enhancement in the intensity of room temperature fluorescence, emitted by phycocyanin and induced a blue-shift in the emission peak of *Spirulina* cells indicating the alterations in the energy transfer within the phycobilisomes, whereas this phenomenon was not seen in *Anacystis*, *in vitro*. It is a common place observation that toxicity of metals showed great variations under field and laboratory conditions (Whitton, 1970). A given concentration of a metal may be more toxic to algae in the field than under laboratory conditions and vice-versa (Rai *et al*., 1981 b). Hence, it becomes explicit that laboratory based information cannot solely be used to stimulate field conditions, because many environmental and nutritional factors operate to bring about metal toxicity in field conditions (Gadd & Griffiths, 1978). The study indicated that cadmium could affect the blue-green alga drastically causing irreparable damage. Prolonged recovery period almost 4 times more than the exposure period helped to recover the impact of cadmium. At higher recovery period, the removal of cadmium metal from the plant body supported the survivability of the alga. After 30d exposure green dot like structures appeared and after 60days of exposure the whole white turbid mass turned green due to appearance of pigments. Appearance of pigments indicated revival of the exposed alga during recovery period. Cadmium should not be allowed to escape and be available in the environment for protection of flora, fauna, human beings and the environment.

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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.