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Toxicological Impacts Of Cadmium Chloride On Nitrate Reductase Activity and Nitrogenase Activity Of A Blue-Green Alga *In Vivo.*

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Abstract

The toxicant cadmium chloride showed all the features of heavy metal toxicity on a blue-green alga, where the metal showed dual behavior like stimulation and good recovery at sub-lethal concentration and inhibition followed by no recovery at higher lethal concentrations. The present piece of work aims at understanding the impact of cadmium chloride on the activities of two important enzymes like nitrate reductase and nitrogenase of a blue-green alga *in vivo.* The Nitrate reductase activity of the control set remained static within the standard deviation range with the increase in exposure period. At sub-lethal conc.-X, a maximum of 27.08% increase in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 18.37% increase was noted on 15th day of recovery. At higher lethal concentrations of cadmium chloride significant depletion in the enzyme activity was observed showing inhibitory action of the toxicant cadmium chloride. The nitrogenase activity of the control set remained static within the standard deviation range with the increase in exposure period. At sub-lethal conc.-X, a maximum of 5.05% decrease in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 7.87% decrease was noted on 15th day of recovery. At higher concentration, a maximum of 90.8% decrease in enzyme activity was marked on 9th day of exposure and with the increase in exposure period 100% decrease was noted and when the exposed alga was transferred to toxicant free medium. 100% decrease in the enzyme activity was noted on 15th day of recovery indicating no recovery was noted. The toxicant, Cadmium chloride significantly affected both the enzymes and the regulatory action of both the enzymes was lost in the process and the exposed alga could not resist the heavy metal toxicant and ultimately died at higher concentrations.

Key words: Cadmium chloride, Blue-green alga, Nitrate-reductase activity, Nitrogenase activity.

Introduction

The paper mill effluents are discharged from the industry directly into the aquatic environment after physical and chemical treatments (Tripathy *et al.*, 2021). The effluent of the industry is toxic, which is indicating the need of a biological treatment before discharge in to the environment. Tripathy *et al.*, (2021) reported presence of mercury, cadmium and lead in the final discharged Paper mill effluent after treatment. As per technology, waste generation is a must and it is not possible to eliminate waste generation by the system (Kaur *et al.*, 2021). Cadmium is an effective inhibitor of chlorophyll biosynthesis (Stobart *et al.*, 1985), Photosynthesis (Weigel, 1985), respiration, nitrogen fixation (Sahu *et al*, 1988) and the activities of several enzymes (Bishnoi *et al.*, 1993). Mishra and Panigrahi (2023a, b) reported that cadmium chloride significantly affected the pigment content and photosynthetic efficiency of BGA inhabiting crop fields and the metal is deadly toxic. Mishra and Panigrahi (2023c, d) also reported the effects caused by cadmium chloride on the nitrogen metabolism and respiratory physiology of the same blue-green alga under laboratory controlled conditions. Nitrate reductase is one of the most important enzymes in the assimilation of exogenous nitrate, the predominant form of nitrogen available to green plants growing in soil. Activity of this enzyme in plants gives

a good estimate of the nitrogen status of the plant and is very often correlated with growth and yield. Nitrogenase is the key enzyme for biological nitrogen fixation. Nitrogenase converts nitrogen to ammonia. It is present in some prokaryotes. Nitrogenase catalyses the conversion of nitrogen in to ammonia. The reduction of dinitrogen to ammonia by living organisms is called biological nitrogen fixation. Ammonia is the first stable product in nitrogen fixation. Nitrogenase acts as a catalyst and reduces the activation energy. This project was planned to evaluate the eco-physiological impacts of a heavy metal, Cadmium chloride on the nitrogen metabolism and its interaction on some important enzymes of a blue-green alga inhabiting crop fields.

Materials & Methods

Lethal concentration values as deduced by Mishra and Panigrahi (2023a) pertaining to toxicity study was taken as such for conducting the experiments. One ml of unialgal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100ml of cadmium chloride 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 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 and the 15d exposed alga was allowed to recover in nutrient fresh medium without cadmium chloride.. From the toxicity testing as described by Mishra and Panigrahi (2023b), the marked X, Y and Z concentrations of the toxicants were selected for future experiments.

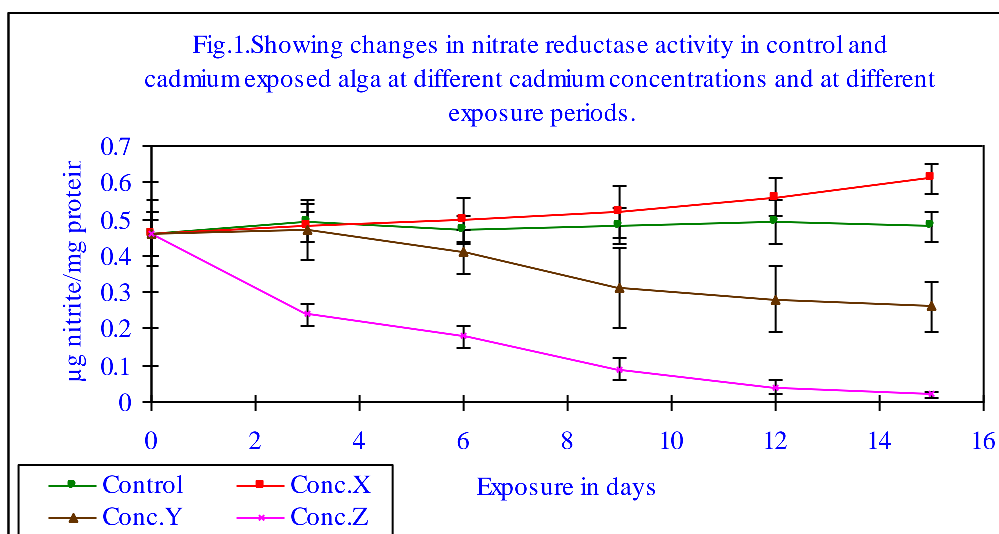
Anabaena cylindrica, Lemm is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga (BGA) belongs to the family Nostocaceae. Allen and Arnon's (1955) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) and adopted by Sahu (1987) was most suitable basic culture for the growth of the test organisms.

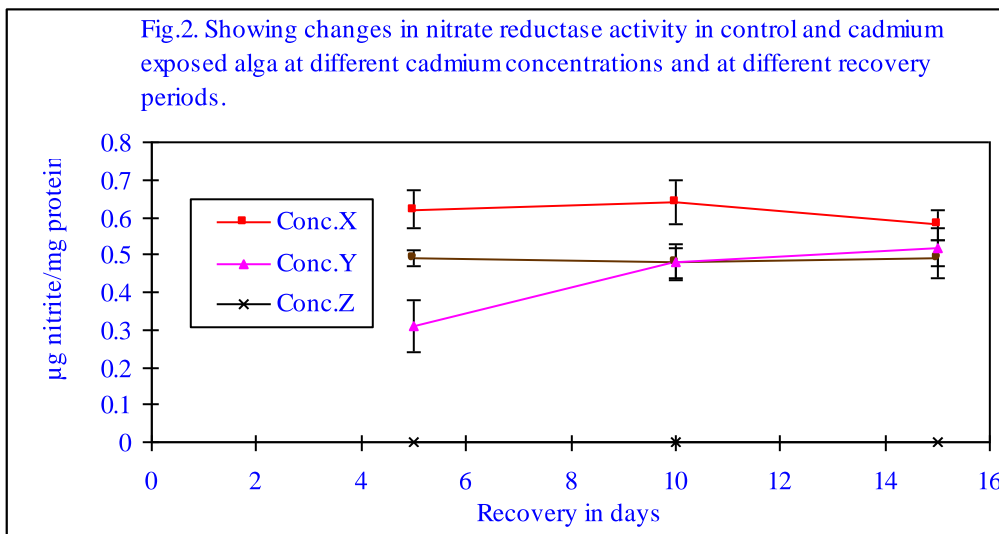
Nitrate reductase activity and Nitrogenase activity measurements:

The assay of nitrate reductase activity was carried out by the colorimetric method of Joy and Hageman (1966) and Snell and Snell (1949). The assay mixture contained 2 ml of 0.1 M potassium phosphate buffer (pH, 7.5), 0.2ml of 4 mM $NaNO_2$, 0.2ml of mM methyl violegen, freshly prepared 50mM sodium dithionite and 0.2ml of of enzyme extracted from the sample. The reaction was initiated by adding slowly 0.4ml of sodium dithionite to the incubation mixture. Few drops of liquid paraffin were placed on the surface of the incubation mixture to prevent the atmospheric oxidation of sodium dithionite. The reaction mixture was terminated by vigorous shaking till the blue colour of reduced violegen had disappeared. The incubation mixture was pipetted to colour development reagent (1% W/V) Sulphanilamide and 0.01% (W/V) N-(1-naphthyl) ethylene diamine hydrochloride (2ml each). The amount of decrease of NO_2^- in the incubation mixture against blank was expressed as µg nitrite /mg of protein. The protein content of the alga was determined by following the procedure mentioned in biochemical studies. The data were computed and expressed in terms of µg nitrite / mg of protein. Nitrate reductase activity of both control and exposed cultures was determined. The exposed algae were transferred to cadmium free nutrient medium, to study the percent of recovery of enzyme activity of the alga. Percent change in enzyme activity in exposed cultures was calculated over the control value, at all exposure periods and recovery periods. Control and experimental flasks were maintained in culture room. Three different concentrations named X, Y and Z were maintained in experimental flasks. At 3d interval five sets of culture were taken and the cultures were transferred to previously sterilized vizzy bottles (Air-tight). The vizzy bottles were incubated in the culture rooms. Nitrogenase activity was measured by the acetylene reduction technique (Hardy *et al*., 1968). Experiments were initiated by the addition of calculated amount of acetylene (Collected in balloons). Gas tight syringes were used to insert and remove samples from the vizzy bottles. Ethylene and acetylene concentrations were measured by a Gas Chromatograph. At the conclusion of the analysis, the volume of the gas phase and the dry weight of the algae were determined. The nitrogenase activity was expressed as n mol of C_2 H_4 (Ethylene) production g^{-1} dry weight. Nitrogenase activity was determined both in control and exposed cultures. After 15 days of exposure, the exposed algae were transferred to cadmium free nutrient medium, to study the extent of recovery. Percent change in enzyme activity was computed in exposed and recovery algae over the respective control value, at all exposure and recovery periods. The obtained values were statistically analyzed to find out the levels of significance.

Results:

Fig.1 shows the changes in Nitrate reductase activity in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The Nitrate reductase activity of the control set remained static within the standard deviation range with the increase in exposure period. The value ranged within 0.46±0.0605µg nitrite mg^{-1} protein to 0.49±0.05µg nitrite mg^{-1} protein during exposure and recovery periods in the control set. In concentration X, the value showed significant increase at all exposure and recovery periods, when compared to the control value. The value increased from 0.46 ± 0.0605µg nitrite mg^{-1} protein to 0.61 ± 0.4µg nitrite mg^{-1} protein on 15th day exposure and from 0.46 ± 0.0605µg nitrite mg^{-1} protein to 0.58 ± 0.04µg nitrite mg^{-1} protein 15th day of recovery, respectively (Fig.1). In concentration Y, the nitrate reductase activity increased from 0.46 ± 0.0605µg nitrite mg^{-1} protein to 0.47 ± 0.05µg nitrite mg^{-1} protein on 3rd day of exposure and it decreased steadily from 0.46 ± 0.0605µg nitrite mg^{-1} protein to 0.26 ± 0.08 µg nitrite mg^{-1} protein and the enzyme activity on 15th day of exposure. When the exposed alga was transferred to toxicant free medium for recovery studies, the enzyme activity increased from 0.26 ± 0.0805µg nitrite mg^{-1} protein to 0.52 ± 0.05µg nitrite mg^{-1} protein on 15th day of recovery. In concentration Z, the reductase activity decreased from 0.46 ± 0.0605µg nitrite mg^{-1} protein to 0.02 ± 0.01µg nitrite mg^{-1} protein on 15th day of exposure. When the exposed alga was transferred to toxicant free medium, no recovery was marked rather further depletion in the enzyme activity was marked. The activity depleted further and the activity was not within detectable range (Fig.2). This non recovery indicated that the toxicant cadmium chloride is deadly toxic and recovery was not possible even if the conditions were made favorable for the cadmium exposed alga. At concentration-X, a maximum of 27.08% increase in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 18.37% increase was noted on 15th day of recovery. At concentration-Y, a maximum of 45.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 6.12% increase was noted on 15th day of recovery indicating a partial recovery. At concentration-Z, a maximum of 95.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 100% decrease in the enzyme activity was noted on 15th day of recovery. At concentration-X, little increment was noted at all exposure and recovery periods except on the 3rd day, where an insignificant decrease was noted. However, these increments were well within the standard deviation range, when compared to control set. Hence, it was not possible to say that the increment caused in the parameter was due to the toxicant, Cadmium chloride. Basing on this type of insignificant increase, it was also not possible to indicate that the toxicant showed dual behavior in its action. The recovery in concentration-Y is interesting as a significant recovery was noted. In case of conc. Y, 45.8% decrease on 15th day of exposure and 6.12% increase on 15th day of recovery, showed 52% recovery which was significant. No recovery in concentrations-Z was noted (Fig.3). The data also indicated that this enzyme was probably responsible for low rate of NO production. The correlation coefficient analysis between days of exposure and nitrate reductase activity indicated the existence of no significant correlation in control (p = NS) and in Conc. X positive correlation ($r = 0.981$, $p \geq 0.01$) and in conc. Y negative correlation ($r = -0.972$, $p \geq 0.05$) and a negative significant ($r = -0.986$, $p \geq 0.01$) correlation in Conc. Z. The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns.





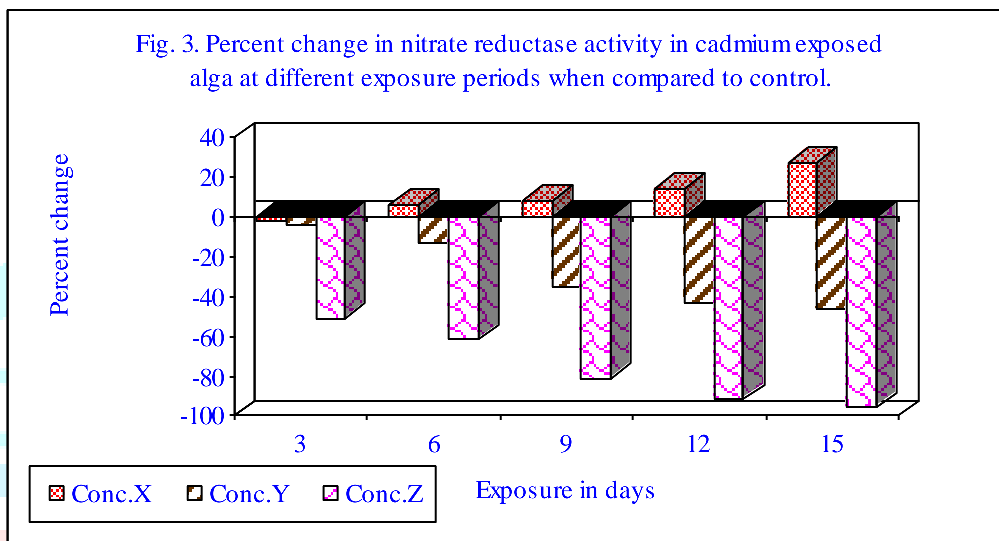
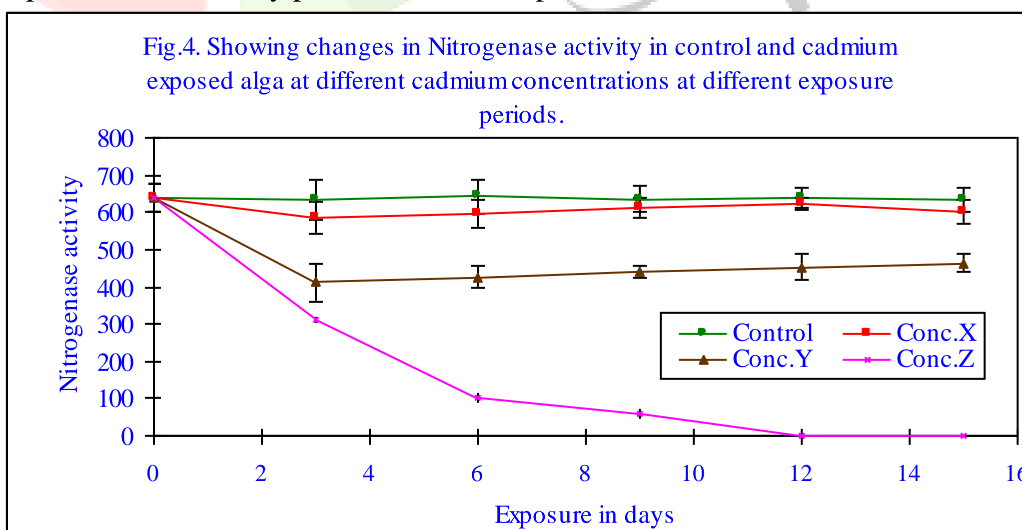
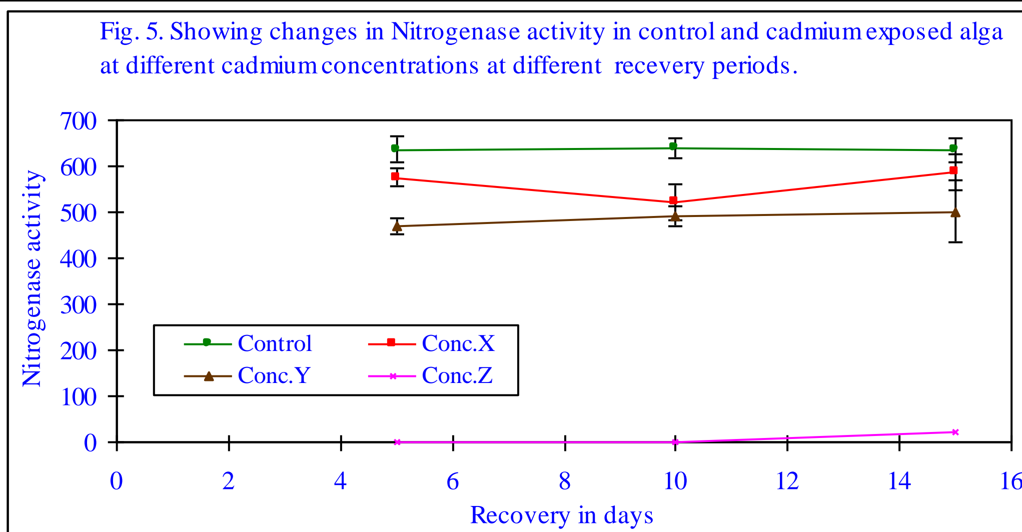
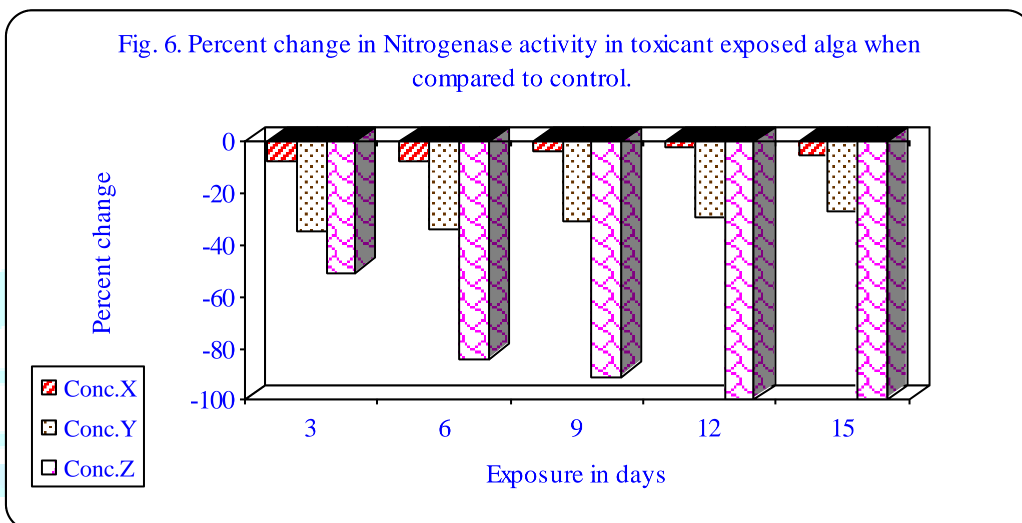


Fig.4 showed the changes in nitrogenase activity in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The nitrogenase activity of the control set remained static within the standard deviation range with the increase in exposure period. The value ranged within 634 ± 17nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight to 642 ± 18nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight during exposure and recovery periods in the control set. In concentration X, the value showed significant decrease at all exposure and recovery periods, when compared to the control value.







The value decreased from 638 ± 22nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight to 602 ± 11nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight on 15th day exposure and from 638 ± 11 to 586 ± 18 nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight 15th day of recovery, respectively (Fig.5). In concentration Y, the nitrogenase activity decreased steadily and significantly from 638 ± 22 nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight to 464 ± 9nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight and the enzyme activity on 15th day of exposure. When the exposed alga was transferred to toxicant free medium for recovery studies, the enzyme activity increased from 464 ± 9nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight to 502 ± 17nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight on 15th day of recovery. In concentration Z, the nitrogenase activity decreased from 638 ± 22nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight to 58 ± 16nmoles of C_2H_4 production hr^{-1} g^{-1} dry weight on 9th day of exposure. With the increase in exposure period no enzyme activity was recorded. When the exposed alga was transferred to toxicant free medium, no recovery was marked rather further depletion in the enzyme activity was marked. The activity depleted further and the activity was not within detectable range (Fig.5). This non recovery indicated that the toxicant cadmium chloride is deadly toxic and recovery was not possible even if the conditions were made favorable for the cadmium exposed alga. At concentration-X, a maximum of 5.05% decrease in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 7.87% decrease was noted on 15th day of recovery. At concentration-Y, a maximum of 26.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 21.07% decrease was noted on 15th day of recovery indicating no recovery. At concentration-Z, a maximum of 90.8% decrease in enzyme activity was marked on 9th day of exposure and with the increase in exposure period 100% decrease was noted and when the exposed alga was transferred to toxicant free medium 100% decrease in the enzyme activity was noted on 15th day of recovery. At concentration-X, little depletion was noted at all exposure and recovery periods. Hence, it was possible to say that the depletion caused in the parameter was due to the toxicant, Cadmium chloride. Basing on this type of significant decrease, it was also possible to indicate that the toxicant did not show dual behaviour in its action. The recovery in concentration-Y is interesting as a significant recovery was not noted. In case of conc. Y, 26.8% decrease on 15th day of exposure and 21.07% decrease on 15th day of recovery, showed 5.8% recovery which was not significant. No recovery in concentrations-Z was noted (Fig.6). The correlation coefficient analysis between days of exposure and nitrogenase activity indicated the existence of no significant

correlation in control (p = NS) and in Conc. X no correlation (p = NS) and in conc. Y negative data but no correlation (p = NS) and a negative significant ($r = -0.981$, $p \geq 0.01$) correlation in Conc. Z. The ANOVA test indicated the existence of non-significant difference between rows and non-significant difference between columns.

Discussion

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 nitrogen fixing blue-green algae for long term exposure to low concentrations of this element. Nitrate reductase (NR) a key enzyme for nitrogen assimilation in multiple organisms (Chamizo-Ampudis *et al*., 2017) which catalyzes the nitrate to nitrite reduction in plant cell cytoplasm (Kolbert *et al*., 2008). The same author also indicated that nitrate its main substrate has been shown to be necessary for cellular-signalling and commonly distributed in multiple plant tissues. This enzyme also functions as an important enzymatic source of nitric oxide (NO) which then regulates plant growth and resistance to biotic and abiotic stresses. Nitrogenase is an enzyme responsible for catalyzing nitrogen fixation, which is the reduction of nitrogen (N_2) to ammonia (NH_3) and a process vital to sustaining life on Earth. Nitrate reductase (NR) catalyzes the first reaction in nitrate assimilation, the reduction of nitrate to nitrite. Nitrate reductase requires molybdenum (Mo) as cofactor. Nitrate reductase in higher plants is proposed to be a homodimer, with two identical subunits joined and held together by the Mo cofactor. Nitrate reductase (NR) is regulated at the transcriptional and translational levels induced by light, nitrate, and possibly a negative feedback mechanism. First, nitrate assimilation is initiated by the uptake of nitrate from the root system, reduced to nitrite by nitrate reductase, and then nitrite is reduced to ammonia by nitrite reductase. Ammonia then goes into the GS-GOGAT pathway to be incorporated into amino acids. When the plant is under stress, instead of reducing nitrate via NR to be incorporated into amino acids, the nitrate is reduced to nitric oxide which can have many damaging effects on the plant. Thus, the importance of regulating nitrate reductase activity is to limit the amount of nitric oxide being produced (Taiz *et al.,* 2014). In the present investigation interesting results were obtained which directly affected the nitrogen metabolism of the alga and significantly influenced the nitrogen balance of the crop fields. The Nitrate reductase activity of the control set remained static within the standard deviation range with the increase in exposure period. In conc.-X, the value showed significant increase at all exposure and recovery periods, when compared to the control value. In conc.-Y, the nitrate reductase activity decreased steadily up to 15th day of exposure. In conc.- Z, the reductase activity decreased on 15th day of exposure. When the exposed alga was transferred to toxicant free medium, no recovery was marked rather further depletion in the enzyme activity was marked. The activity depleted further and the activity was not within detectable range. This non recovery indicated that the toxicant cadmium chloride is deadly toxic and recovery was not possible even if the conditions were made favorable for the cadmium exposed alga. At conc.-X, a maximum of 27.08% increase in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 18.37% increase was noted on 15th day of recovery. At conc.-Y, a maximum of 45.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 6.12% increase was noted on 15th day of recovery indicating a partial recovery. At conc.-Z, a maximum of 95.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 100% decrease in the enzyme activity was noted on 15th day of recovery. At concentration-X, little increment was noted at all exposure and recovery periods except on the 3rd day, where an insignificant decrease was noted. However, these increments were well within the standard deviation range, when compared to control set. Hence, it was not possible to say that the increment caused in the parameter was due to the toxicant, Cadmium chloride. Basing on this type of insignificant increase, it was also not possible to indicate that the toxicant showed dual behavior in its action. The data also indicated that this enzyme was probably responsible for low rate of NO production. The nitrogenase activity of the control set remained static within the standard deviation range with the increase in exposure period. At conc.-X, a maximum of 5.05% decrease in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 7.87% decrease was noted on 15th day of recovery. 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possible to indicate that the toxicant did not show dual behaviour in its action. The nitrogenase enzyme requires a high input of energy in the form of ATP. ATP is used to transfer hydrogen atoms to dinitrogen during biological nitrogen fixation. The activator of enzyme nitrogenase is molybdenum. Nitrogenase is essential during nitrogen fixation and it catalyses the conversion of atmospheric nitrogen into ammonia. Molybdenum also activates another essential enzyme in nitrogen metabolism, nitrate reductase. Nitrogenase is a complex, bacterial enzyme that catalyzes the ATP-dependent reduction of dinitrogen (N_2) to ammonia (NH_3). In its most prevalent form, it consists of two proteins, the catalytic molybdenum-iron protein (MoFeP) and its specific reductase, the iron protein (FeP). During the process of biological nitrogen fixation, the enzyme nitrogenase catalyzes the ATP-dependent reduction of dinitrogen to ammonia. Nitrogenase consists of two component metalloproteins, the iron (Fe) protein and the molybdenum-iron (MoFe) protein; the Fe protein mediates the coupling of ATP hydrolysis to interprotein electron transfer, whereas the active site of the MoFe protein contains the polynuclear FeMo cofactor, a species composed of seven iron atoms, one molybdenum atom, nine sulfur atoms, an interstitial light atom, and one homocitrate molecule. This Perspective provides an overview of biological nitrogen fixation and introduces three contributions to this special feature that address central aspects of the mechanism and assembly of nitrogenises (Howard & Rees, 2006). From the obtained data, it was clear that cadmium chloride is deadly toxic and significantly affects the biological nitrogen fixation by the alga affecting the biofertility of crop field soils and the availability of this heavy metal in the environment is dangerous. Hence, no industrial waste containing cadmium or any other heavy metal should be allowed to be discharged in to natural environments both aquatic and terrestrial.

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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no conflicts of interest.

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 Sri Saroj K. Mishra laboratory analysis and related experimental work, calculation and finalization of data. Mishra contributed reagents and glassware.

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.

Bishnoi, N. R., Sheoran, I. S., & Singh, R.: (1993): Influence of Cadmium and nickel on photosynthesis and water relations in wheat leaves of different insertion level. Photosynthetica, 28:473-479.

Chamizo-Ampudia, A., SanzLuque, E. Llamas, A. Galvan A. and Ferrandez, E. (2017): Nitarte reduactase regulates plant nitric oxide homeostatsis.Trend Plant Sci,22,163-164. doi.10.1016/j.tplants.2016.12.001

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.

Hardy, R. W. F., Holstel, R. D., Jackson, E. K. and Burns, R. C. (1968): Determination of nitrate reductase activity. Plant Physiol., 43:1185.

Joy, K. W. and Hageman, R.H. (1966): Biochem. J., 100:263.

Kolbert, Z., Bartha, B. and Ersei, L.(2008). Exogenous auxin induced NO synthesis is nitrate reductase –associated in Arabidopsis thaliana root primordia. Journal of plant Physiology, 165,967-975. doi:10.1016/jplph.2007.07.019

Kaur, B., Garg, R. K., Singh, A. P., (2021): Treatment of Wastewater from Pulp and Paper Mill using Coagulation and Flocculation. J. Environ. Treat. Tech., 9(1): 158-163. https://doi.org/10.47277/JETT/9(1)163

Mishra, S. K. and A. K. Panigrahi (2023a): Eco-toxicological effects of Cadmium chloride on the growth of a blue-green alga under laboratory controlled conditions. IJCRT, 11(10):b859-b867.

Mishra, S. K. and A. K. Panigrahi (2023b): Toxicological impacts of Cadmium chloride on the photosynthetic efficiency of a cyanobacterium *in vivo* under controlled conditions. IJCRT, 11(10):c934-c943.

Mishra, S. K. and A. K. Panigrahi (2023c): Eco-toxicological impacts of cadmium chloride on the nitrogen metabolism of a blue-green alga under laboratory controlled conditions. IJCRT, 11(11):c232-c240

Mishra, S. K. and A. K. Panigrahi (2023d): Toxicological impacts of cadmium chloride on the primary productivity of a blue-green alga in vivo under controlled conditions. Int.J. of Current Advanced Research, 12(10): 2567-2573.

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

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 & its ecological implications.Ph.D. Thesis. Berhampur University, India.

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.

Snell, F. D. and Snell, C. T. (1949): In colorimetric methods of analysis.IIIEd.,2:804. D. Van Nostrand Company. Inc. Princeton, New Jercy & New York.

Stobart, A. K.; Griffiths, W. T.; Ameen Bukhariu, I. and Sher wood, R. P. (1985): The Effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant., 63:293-298.

Taiz L, Zeiger E, Moller IM, Murphy A (2014). Plant Physiology and Development (6 ed.). Massachusetts: Sinauer Associates, Inc. p. 356. ISBN 978-1-60535-353-1.

Tripathy A. P., Dixit, P. K., Panigrahi, A. K. (2021) Impact of effluent of Pulp & Paper industry on the flora of river basin at JayKaypur, Odisha, India and its ecological implications. Environmental Research, 204 (2022): 111769.

Weigel, H. J.(1985): The effect of Cd^{2+} on photosynthetic reactions of mesophyll protoplasts. Physiol. Plant., 63:192-200.