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Toxicological Effects Of Cadmium Chloride On The Biomolecular Content Of A Blue-Green Alga Under Controlled Conditions.

Mishra*, S. K. and Panigrahi, A. K. ,
Laboratory of Environmental Toxicology,
Department of Botany & Environmental Sciences, Berhampur University,
BERHAMPUR-760007, Odisha. India.

Abstract

Keeping in view; 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 inhabiting organisms in the crop fields, this project was masterminded to evaluate the eco-toxicological effects of Cadmium metal in the form of Cadmium chloride on the toxicity, effect on the physiological & biochemical parameters on 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. It was observed that at very low concentrations the toxicant is deadly toxic and affects the BGA. The changes in DNA content in the exposed alga was significant when compared to control set. In conc. X, a maximum of 3% increase in DNA content over the control value was recorded on 15th day of exposure and 4.4% increase on 15th day of recovery. In conc.Y, a maximum of 38.6% decrease over the control value was recorded on 15th day of exposure and 47.2% decrease on 15th day of recovery. In conc. Z, the DNA content steadily declined showing a negative correlation. A maximum of 95.5% decrease was recorded on 15th day of exposure. In conc. Z, maximum depletion in DNA amount was noted during recovery period indicating permanent damage caused to the system. In conc-X, the recorded RNA amount was more than the control value at all exposure and recovery periods. In concentration Y, the RNA content increased with the increase in exposure period. The RNA content also increased with the increase in recovery period. When compared to respective control values, the RNA quantity was less and significant. The exposed alga was transferred to toxicant free medium, no recovery was marked rather the RNA amount further depleted showing death of algal cells. The protein content significantly decreased in the exposed alga when compared to control alga. When the exposed alga was transferred to toxicant free medium, no recovery was marked rather the protein amount was at not detectable level. When the exposed algae were transferred to toxicant free medium, significant recovery was recorded in conc. X, partial recovery was marked in concentration Y and no recovery was noted in Conc. Z. In concentration X, a maximum of 38.9% decrease over the control value was recorded on 15th day of exposure and 35.4% increase on 15th day of recovery. In concentration Y, a maximum of 59.7% decrease over the control value was recorded on 15th day of exposure and 63.6% decrease on 15th day of recovery. In conc. Z, the protein content steadily declined showing a negative correlation. A maximum of 100% decrease was recorded on 15th day of exposure and 100% decrease was recorded on 15th day of recovery. Partial recovery by 3.5% was recorded in conc. X, after 15 days of recovery, when the exposed alga was transferred to toxicant free nutrient medium. In case of conc. Y, recovery was not marked rather further depletion was seen. In conc. Z, maximum depletion in protein amount was noted during recovery period indicating permanent damage caused to the system. The FAA content followed the same trend like protein content change and RNA content change.

Keywords: Cadmium chloride, Toxicity, DNA, RNA, Protein, FAA, BGA,

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 on long term exposure to low concentrations of this element. 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 biomolecular content 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. The pollutant, Experiments were conducted at MAC, LC_{10} and LC_{50} values to understand the impact of $CdCl_2$ on the changes in biomolecular content like DNA, RNA, Protein and FAA of control and cadmium chloride exposed alga at different exposure and recovery periods.

Materials & Methods

Anabaena cylindrica, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. Allen and Arnon's (1955, a) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) 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 (intensity of 2400±200Lux) and temperature (maintained at 28± 2^0C) with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. 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. To study for different biochemical parameters the algal cultures both control and exposed were terminated by centrifuging at 20^0C and at 5000 rpm for 10 minutes. The pellet of algal mass was washed thoroughly in double distilled water and centrifuges again to get pure algal material free from any adsorbed substances. The algal cells were grinded with 5 ml of 70% ethanol (V / V) in a micro-tissue homogenizer to extract free amino acids. The extract was centrifuged and the supernatant was taken for estimation of amino acids, by ninhydrin reagent method following the procedure of Lee and Takahasi (1966). The residue was washed with 5 ml of 5% cold TCA [Trichloro acetic acid (W / V)] and centrifuged. The residue was treated with 5 ml of 10% TCA (W / V) and stirred well. It was heated in a boiling water bath for 30 minutes, cooled and centrifuged. The supernatant was taken for estimation of nucleic acids and from the residue the protein content was estimated. Total DNA in the supernatant was measured by diphenylamine reaction method (Herbert et al., 1971) and RNA by Orcinol reagent method of Volkin and Cohn (1954). The TCA precipitated protein of the above residue was measured by the method of Lowery *et al*, (1951) and Biuret test (Oser, 1965).The free amino acid, DNA, RNA and protein contents were measured by computing the optical density value from the respective standard graphs prepared from standard amino acid (Glycine), DNA, RNA and Protein (BSA) procured from Sigma Chemical Company, USA. The experiments were performed in triplicate and the data were expressed as the mean of 3 observations involving DNA, RNA, & Protein as mg / 100 ml algal culture and Amino acid as µg / 100 ml algal culture. The obtained data was statistically analyzed.

Results

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, LC_{00} or PS_{100} as safe MAC value of 0.31 $mg.l^{-1}$ was selected as 'X'; LC_{50} or PS_{50} of 0.78 $mg.l^{-1}$ was selected as 'Y' and LC_{90} or PS_{10} value of 1.22 $mg.l^{-1}$was selected as 'Z' for conducting future experiments. Fig.1 shows changes in DNA content in control and cadmium chloride exposed blue-green alga at different days of exposure and recovery. The DNA content showed a consistent positive increase from 0.52 $\pm$ 0.06 to 1.32 $\pm$ 0.14 mg / 50 ml culture within a period of 15 days. The DNA content further increased to 1.80 $\pm$ 0.11 mg / 50 ml culture on 15^{th} day of recovery. The increase in DNA content indicated increase in growth might be by cell multiplication. In concentration X, the DNA content increased with the increase in exposure period. The DNA content increased at all exposure and recovery periods, when compared to respective control values (Fig, 1 & 2). The DNA content increased from 0.52 $\pm$ 0.06 to 1.36 $\pm$ 0.09 mg / 50 ml culture on 15^{th} day of exposure and to 1.88 $\pm$ 0.14 mg / 50 ml culture on 15^{th} day of recovery (i.e. 30 days from the day of inoculation). In concentration Y, the DNA content increased with the increase in exposure period. The DNA content increased with the increase in exposure period and recovery period. When compared to respective control values, the DNA quantity was far less and significant (Fig.1). The DNA content increased from 0.52 $\pm$ 0.06 to 0.81 $\pm$ 0.03 mg / 50 ml culture on 15^{th} day of exposure and further increased to 0.95 $\pm$ 0.09 mg / 50 ml culture on 15^{th} day of recovery. Whereas, in concentration Z, the DNA content decreased steadily and significantly from 0.52 $\pm$ 0.06 to 0.06 $\pm$ 0.02 mg / 50 ml culture on 15^{th} day of exposure (Fig.1). The exposed alga was transferred to toxicant free medium, no recovery was marked rather

the DNA amount further depleted showing death of algal cells. When the exposed algae were transferred to toxicant free medium, significant recovery was recorded in concentration X, partial recovery was marked in concentration Y and no recovery was noted in Conc. Z (Fig.2). In concentration X, a maximum of 3% increase over the control value was recorded on 15th day of exposure and 4.4% increase on 15th day of recovery. In concentration Y, a maximum of 38.6% decrease over the control value was recorded on 15th day of exposure and 47.2% decrease on 15th day of recovery. In concentration Z, the DNA content steadily declined showing a negative correlation. A maximum of 95.5% decrease was recorded on 15th day of exposure. A maximum of 4.4% recovery was recorded in concentration X, after 15 days of recovery, when the exposed alga was transferred to toxicant free nutrient medium. In case of concentration Y, recovery was noted rather further depletion was seen. In conc. Z, maximum depletion in DNA amount was noted during recovery period indicating permanent damage caused to the system (Fig.3). The correlation coefficient analysis between days of exposure and DNA content indicated the existence of a significant positive correlation in control ($r = 0.986$, $p \geq 0.001$); in Conc. X ($r = 0.989$, $p \geq 0.001$) and in Conc. Y ($r = 0.889$, $p \geq 0.01$) and a significant negative correlation in conc. Z ($r = -0.928$, $p \geq 0.01$). The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns.

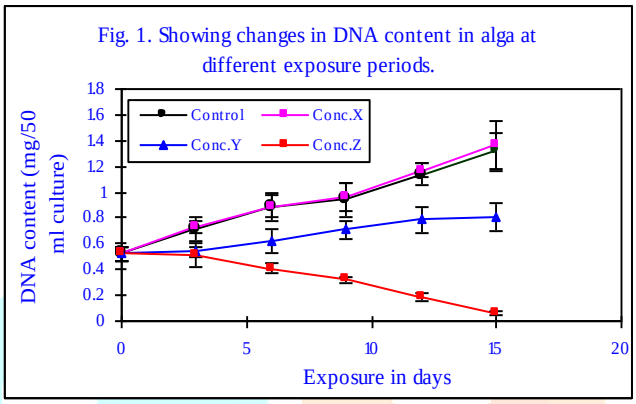


Fig. 1. Showing changes in DNA content in alga at different exposure periods.

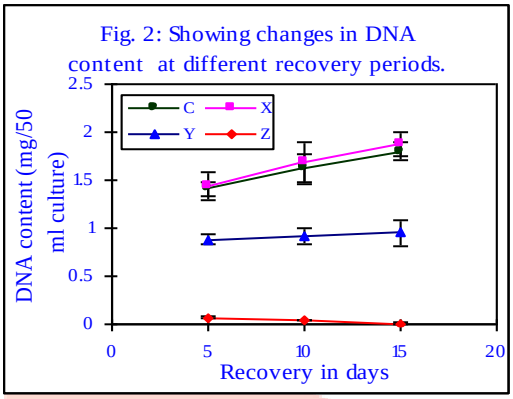


Fig. 2: Showing changes in DNA content at different recovery periods.

Fig.4 shows changes in RNA content in control and cadmium chloride exposed blue-green alga at different days of exposure and recovery. The RNA content showed a consistent positive increase from 0.38 $\pm$ 0.05 to 1.22 $\pm$ 0.08 mg / 50 ml culture within a period of 15 days. The RNA content further increased to 2.10 $\pm$ 0.12 mg / 50 ml culture on 15th day of recovery. The increase in RNA content indicated increase in growth. In concentration X, the RNA content increased with the increase in exposure period. The RNA content increased at all exposure and recovery periods, when compared to respective control values (Fig.5).

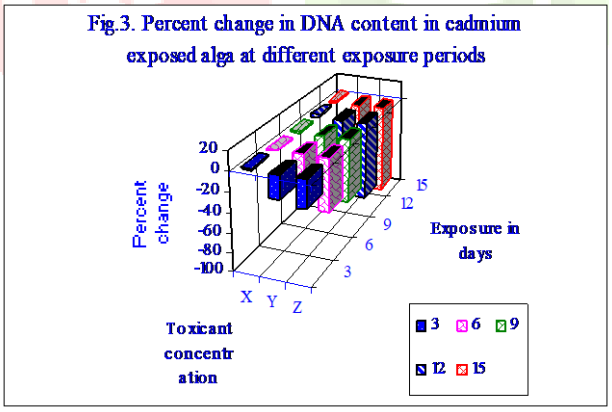


Fig.3. Percent change in DNA content in cadmium exposed alga at different exposure periods

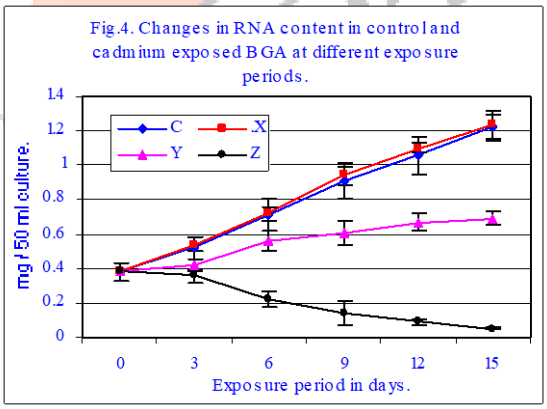


Fig.4. Changes in RNA content in control and cadmium exposed BGA at different exposure periods.

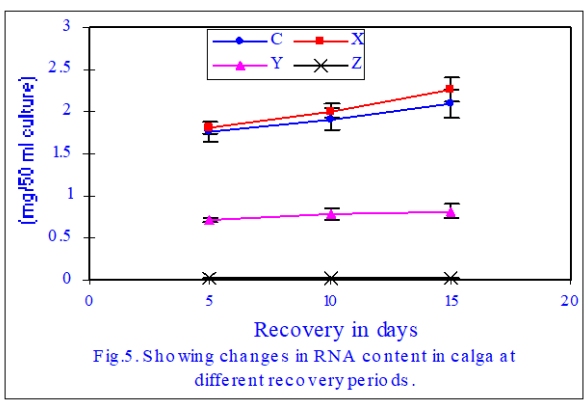


Fig.5. Showing changes in RNA content in calga at different recovery periods.

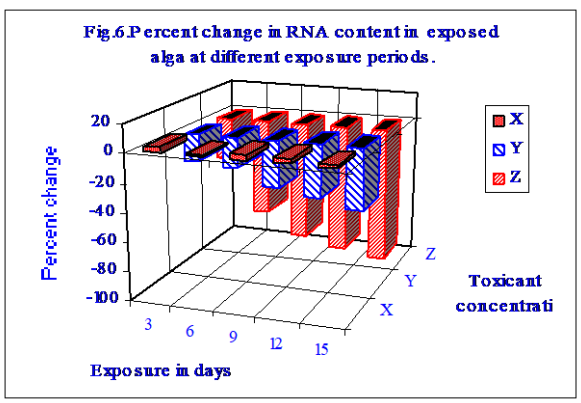
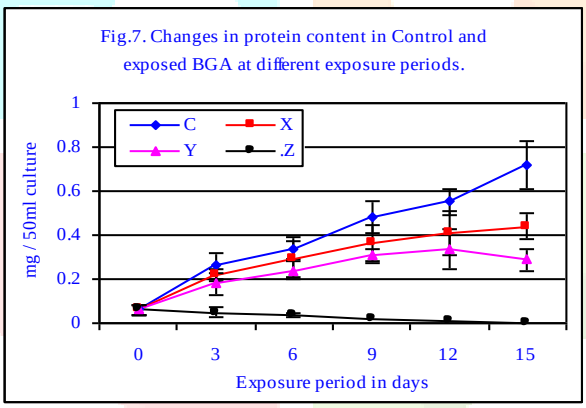


Fig.6 Percent change in RNA content in exposed alga at different exposure periods.

The RNA content increased from 0.38 ± 0.05 to 1.24 ± 0.08 mg / 50 ml culture on 15th day of exposure and to 2.26 ± 0.14 mg / 50 ml culture on 15th day of recovery (i.e. 30 days from the day of inoculation). In concentration-X, the recorded RNA amount was more than the control value at all exposure and recovery periods. In concentration Y, the RNA content increased with the increase in exposure period. The RNA content also increased with the increase in recovery period. When compared to respective control values, the RNA quantity was less and significant (Fig.5). The RNA content increased from 0.38 ± 0.05 to 0.69 ± 0.04 mg / 50 ml culture on 15th day of exposure and further increased to 0.82 ± 0.08 mg / 50 ml culture on 15th day of recovery. Whereas, in concentration Z, the RNA content decreased steadily and significantly from 0.38 ± 0.05 to 0.05 ± 0.009 mg / 50 ml culture on 15th day of exposure (Fig.4 & 5). The exposed alga was transferred to toxicant free medium, no recovery was marked rather the RNA amount further depleted showing death of algal cells. When the exposed algae were transferred to toxicant free medium, significant recovery was recorded in concentration X, partial recovery was marked in concentration Y and no recovery was noted in Conc. Z. In concentration X, a maximum of 1.6% increase over the control value was recorded on 15th day of exposure and 7.6% increase on 15th day of recovery. In concentration Y, a maximum of 43.4% decrease over the control value was recorded on 15th day of exposure and 60.9% decrease on 15th day of recovery. In concentration Z, the RNA content steadily declined showing a negative correlation. A maximum of 95.9% decrease was recorded on 15th day of exposure and 99% decrease was recorded on 15th day of recovery. A maximum of 7.6% recovery was recorded in concentration X, after 15 days of recovery, when the exposed alga was transferred to toxicant free nutrient medium. In case of concentration Y, recovery was not marked rather further depletion was seen. In conc. Z, maximum depletion in RNA amount was noted during recovery period indicating permanent damage caused to the system (Fig.6). The correlation coefficient analysis between days of exposure and RNA content indicated the existence of a significant positive correlation in control ($r = 0.989$, $p \geq 0.001$); in Conc. X ($r = 0.992$, $p \geq 0.001$) and in Conc. Y ($r = 0.899$, $p \geq 0.01$) and a significant negative correlation in conc. Z ($r = -0.941$, $p \geq 0.01$). The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns.



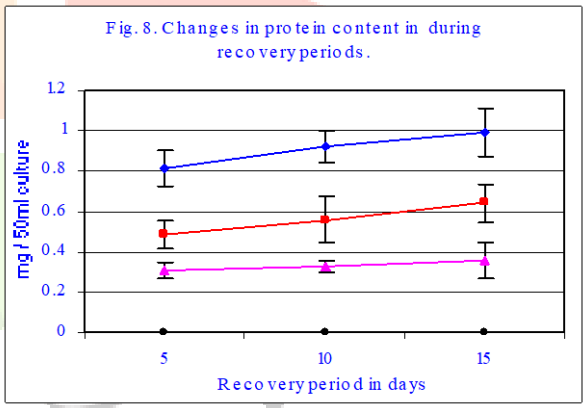
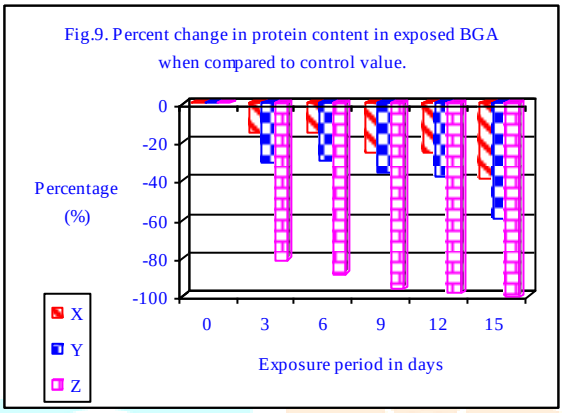
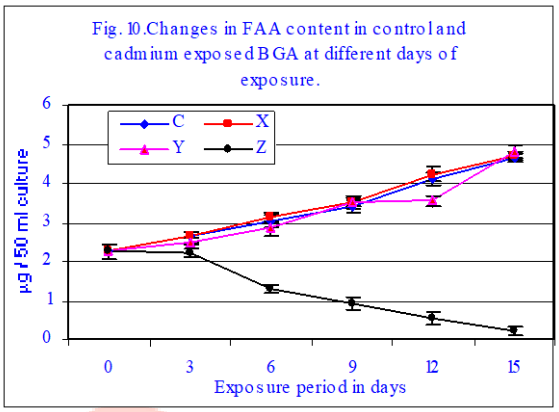
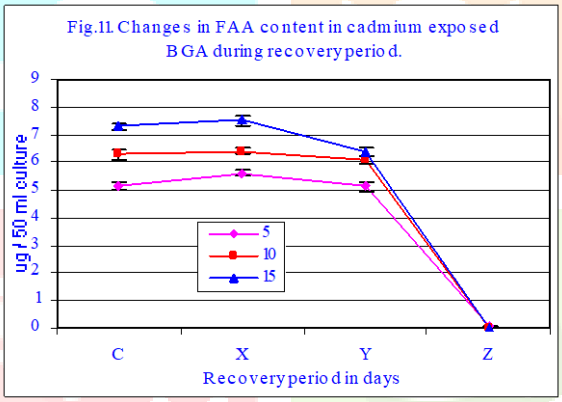


Fig.7 indicated the changes in protein content in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The protein content increased with the increase in exposure period showing a positive correlation. The protein content showed a consistent positive increase from 0.06 ± 0.02 to 0.72 ± 0.11 mg / 50 ml culture within a period of 15 days. The protein content further increased to 0.99 ± 0.12 mg / 50 ml culture on 15th day of recovery. The increase in protein content indicated increase in growth. In concentration X, the protein content increased with the increase in exposure period. The protein content decreased at all exposure and recovery periods, when compared to respective control values (Fig.8). The protein content increased from 0.06 ± 0.02 to 0.44 ± 0.06 mg / 50 ml culture on 15th day of exposure and to 0.64 ± 0.09 mg / 50 ml culture on 15th day of recovery (i.e. 30 days from the day of inoculation). In concentration-X, the recorded protein amount was less than the control value at all exposure and recovery periods. In concentration Y, the protein content increased insignificantly with the increase in exposure period. The protein content increased with the increase in recovery period. When compared to respective control values, the protein quantity was less and significant (Fig.7). The protein content increased from 0.06 ± 0.02 to 0.29 ± 0.05 mg / 50 ml culture on 15th day of exposure and further increased to 0.36 ± 0.09 mg / 50 ml culture on 15th day of recovery. Whereas, in concentration Z, the protein content decreased steadily and significantly from 0.06 ± 0.02 to 0.01 mg / 50 ml culture on 12th day of exposure and on 15th day of exposure, the protein content was at not recordable stage (Fig.8). The exposed alga was transferred to toxicant free medium, no recovery was marked rather the protein amount was at not detectable level. When the exposed algae were transferred to toxicant free medium, significant recovery was recorded in concentration X, partial recovery was marked in concentration Y and no recovery was noted in Conc. Z. In concentration X, a maximum of 38.9% decrease over the control value was recorded on 15th day of exposure and 35.4% increase on 15th day

of recovery. In concentration Y, a maximum of 59.7% decrease over the control value was recorded on 15th day of exposure and 63.6% decrease on 15th day of recovery. In concentration Z, the protein content steadily declined showing a negative correlation. A maximum of 100% decrease was recorded on 15th day of exposure and 100% decrease was recorded on 15th day of recovery. Partial recovery by 3.5% was recorded in concentration X, after 15 days of recovery, when the exposed alga was transferred to toxicant free nutrient medium. In case of concentration Y, recovery was not marked rather further depletion was seen. In conc. Z, maximum depletion in protein amount was noted during recovery period indicating permanent damage caused to the system (Fig.9). The correlation coefficient analysis between days of exposure and protein content indicated the existence of a significant positive correlation in control ($r = 0.991$, $p \geq 0.001$); in Conc. X ($r = 0.878$, $p \geq 0.05$) and in Conc. Y ($r = 0.511$, p = NS) and a significant negative correlation in conc. Z ($r = -0.901$, $p \geq 0.05$). The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns.







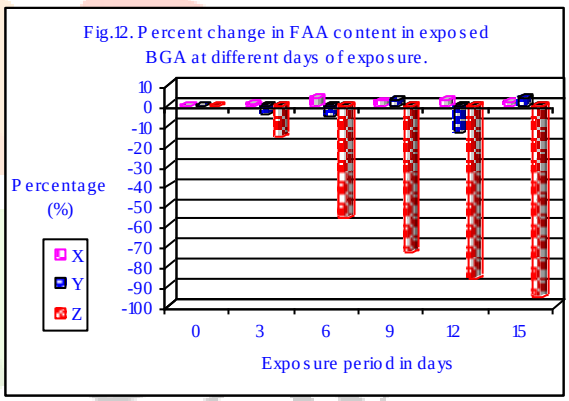


Fig.10 indicate the changes in free amino acid (FAA) content in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The free amino acid content increased from 2.26 ± 0.18 to 4.64 ± 0.11 µg / 50 ml culture within 15 days of exposure. The FAA content further increased to 7.31 ± 0.12 µg / 50 ml culture during recovery period on 15th day of recovery. In concentration X, the FAA content increased with the increase in exposure period, but all the values were more than the control values. The value increased from 2.26 ± 0.18 to 4.71 ± 0.11µg / 50 ml culture within 15 days of exposure. The FAA content increased significantly during recovery period (Fig.10) from 4.71 ± 0.11 to 7.52 ± 0.18 µg / 50 ml culture. In concentration Y, the FAA content decreased with the increase in exposure period, but all the values were less than the control values and conc. X values except on 15th day of exposure, where a significant increase was recorded. The value increased from 2.26 ± 0.18 to 4.82 ± 0.15 µg / 50 ml culture within 15 days of exposure. The FAA content increased significantly during recovery period (Fig.11) from 4.82 ± 0.15 to 6.38 ± 0.14 µg / 50 ml culture. The 15th day recovery value was less than the control and Conc. X value. In case of concentration Z, the FAA content significantly and interestingly decreased up to 15th day of exposure and all the 3rd, 6th, 9th and 12th day values were much less than the control set and conc. X and Y set values. The value decreased from 2.26 ± 0.18 to 0.22 ± 0.09 µg / 50 ml culture on 15th day of exposure in conc. Z set. With the increase in exposure period, the FAA content decreased significantly to 0.22 ± 0.09 µg / 50 ml culture on 15th day of exposure. During recovery period, the FAA content further depleted to 0.07 ± 0.02µg / 50 ml culture on 5th day of recovery and beyond 10th day recovery onwards not detectable level of FAA was found indicating total destruction of the biomolecular content (Fig.11). In concentration X, the percent increase increased and showed the maximum by 1.5% on 15th day of exposure. In concentration Y, 2.6% and 3.8% increase was recorded on 9th & 15th day of exposure, when compared to the control value and on 12th day of exposure 13.3% decrease was recorded. The FAA content showed significant decline at concentration-Z. A maximum of 95.3% decrease when compared to control was recorded on 15th day of

exposure. When the exposed alga was transferred to toxicant free medium, the FAA content further depleted and 98.6% was recorded on 5th day of recovery and 100% decrease was recorded on 10th day onwards. When the exposed alga was transferred to toxicant free media, a partial recovery was marked in concentration X and no recovery was marked in conc. Y and Z. Rather the value further depleted and a maximum of 100% decrease was recorded on 15th day of recovery (Fig.12). The correlation coefficient analysis between days of exposure and FAA content indicated the existence of a significant positive correlation in control ($r = 0.989$, $p \geq 0.01$); in Conc. X ($r = 0.969$, $p \geq 0.05$) and in Conc. Y ($r = 0.972$, $p \geq 0.05$) and a significant negative correlation in conc. Z ($r = -0.991$, $p \geq 0.001$). The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns. From the obtained data, it was clear that cadmium chloride is deadly toxic and the availability of this heavy metal in the environment is dangerous. Hence, no waste containing cadmium is allowed to be dumped in the natural environments both aquatic and terrestrial.

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. This piece of work strongly agrees with the findings of the above authors. Probably the result indicated a new line of thinking, which can become a possibility in case of heterogeneous toxicants, where synergistic and antagonistic effects were expected. Here, it can be presumed that chemicals present other than cadmium probably act as a masking agent on cadmium, reducing the toxicity in turn, showing variation in the observed data. More work is essential on different live systems to confirm the synergistic and antagonistic characteristic features of the mixture toxicants. Change in nucleic acids and protein content occasionally serve as a diagnostic criterion of early poisoning. Good number of publications has described alterations in the level of DNA, RNA, protein and FAA under the influence of toxic chemicals. Effect of toxicants on macromolecules, is often due to an indirect action on nucleic acid and protein synthesis since a toxicant, that interferes with energy yielding reactions is indirectly an inhibitor of synthesis of DNA, RNA and protein. A toxicant is probably acting in such a manner that it inhibits the synthesis of all four macromolecules by comparable levels in dose response experiments (Holbrook, 1980). A considerable dose-dependent reduction in the RNA, DNA, protein and amino acid content was clearly observed in this study, also confirms the action of the toxicant on macromolecular synthesis. The decrease was more pronounced with time and significantly correlated with waste soil combinations. Tewari *et al*. (1990) reported the inhibition of lipid, protein and carotenoids in *Codium* and length, biomass, dry weight, protein, carbohydrate, chlorophyll-a and carotenoids in *Ulva*, near the effluent discharge point of a chlor-alkali industry, with a biodeposition of 0.10 - 0.38 µg Hg g $^{-1}$ dry algae in *Codium* and 0.14 - 0.23 µg Hg g $^{-1}$ dry algae in *Ulva*, respectively, from the sea water containing 0.70 - 0.85 µg Hg l^{-1}. Sahu (1987) & Rath (1991) indicated that pertaining to mercurial poisoning (both inorganic and organic forms) at very low concentrations, the toxicant induced stimulation, and at higher concentrations it induced inhibition to the macromolecular metabolism. Toxicants induced stimulation of nucleic acids and protein synthesis indicated an acceleration of cellular metabolism and growth. De Flilippis and Pallaghy (1976a) found similar stimulative action of the toxicants at lower concentration. In this present finding the same type of observation was prominent, which showed statistical significance at $p \leq 0.001$ level in case of DNA, RNA and protein and at $p \leq 0.01$ level in amino acid content. But the effect at higher concentration of the toxicant did not show negative correlation with the residual mercury content. Since DNA is the chemical estimate of cell numbers (Bruin, 1976), its increase suggests more number of cells in algal culture and its decline suggests loss of cell from the algal culture, which reflects a possible interaction of the pollutants from the solid waste extract on the organism. The total RNA content of algae was found to be more in comparison to DNA and protein of algal culture, which is similar with the findings of Frenkel and Randles (1982) suggested that methyl mercury specifically stimulated RNA synthesis by RNA polymerase I and III, in a study of nucleic acid synthesis, *in vitro*, in isolated nuclei. In this study the protein and the free amino acid also showed a stimulatory effect in lower concentration of the toxicant and a decreased value in higher concentration of the toxicant. In control as well as exposed, the amount of amino acid is more compared to protein amount. Shaw (1987) reported a decline in free amino acid content in exposed algal system, when compared to control, which he opined that due to rapid transcription during the exposure period, might be another reason for the low levels of FAA. It was reported an increase in the FAA levels in algae and rice seedlings, respectively, exposed to solid waste of a chlor-alkali industry containing mercury. This increase was correlated with the amino acids released as a result of breakdown of protein. Our result agrees with their findings but we do not agree with the stimulatory effect as seen in mercury poisoning. Cadmium caused inhibitoty effect in the exposed alga. Passow (1970) reported that mercury blocks glucose permeability. Stein (1967) reported that many but by no means all, -SH reagents inhibit glucose transfer. The change in glycogen content is mostly related with the photosynthesis and respiration as glycogen is the reserved starch in case of

blue-green algae. Just like photosynthesis and pigment content it showed an identical trend, which was stimulatory at very lower concentrations but showed decline in value in higher concentration. From the above findings it is obvious that though the stimulatory effect was only due to the mercury content present in the solid waste extract in lower concentrations. The depletion in higher concentrations was not at all related significantly with residual mercury and cannot be attributable to only mercury. Along with mercury, the solid waste extract contained high amount of sodium, chloride, phosphate etc. which might be affecting the different parameters along with the heavy metal, mercury. In the present study we have taken only a singular chemical, Cadmium chloride. The observed effects were only due to cadmium present in the exposed cultures in addition to nutrient medium. In absence of any recovery it can be inferred that cadmium affects DNA synthesis, RNA synthesis and interferes in protein biosynthesis. In addition cadmium probably caused proteolysis. As observed from the results, it is imperative that cadmium is deadly toxic and should not be allowed or discharged in to the aquatic ecosystems.

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 in 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. 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. 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. The most notable aspects of the geo chemistry of Cadmium with regard to its rock soil plant animal relationships is its low concentrations in the Earth’s crust. Consequently, soil and plant contents of this element are generally low except where soils are formed on rocks with anomalously high concentrations of the metal, such as Black Shales of where pollution has occurred. 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.

Heavy metal contamination caused by either natural processes or by human activities is one of the most serious eco-toxicological problems (Reedy and Prasad, 1990). Since, plants function as the principal entry point of heavy metals into the food chain leading to animals and man(Rauser,1990),the agricultural use of Cadmium containing fertilizers and of Cu as a fungicide is of major concern. Whereas, it is well established fact that Cadmium is more toxic to man and other mammals than Cupper, the differential toxicity of these two heavy metals for plants is not clear (Galli *et al.*, 1996). The use of phosphate fertilizers will invariably increase at least to a slight extent, the Cadmium concentration in soils used for commercial agriculture as long as the accumulation exceeds the amount removed by crops harvested and leached from the plough layer (Alloway, 1990 and Singh, 1994). Application of Cadmium containing fertilizers may not appreciably increase the plant cadmium concentration at present, but low annual application may result in elevated cadmium concentrations in the cultivated layer, especially where high Cadmium fertilizers are used. Some of

the Cadmium added to soils will invariably be removed by the crop or by leaching with the former playing a more important role. The amounts of cadmium removed by the crop depend on the crop species or variety grown, the Cadmium concentration of the fertilizer used, the prevailing soil conditions and the magnitude of yields. Cadmium removal through leaching is generally of less importance than through crop removal although reliable values are still missing (Jeng and Singh,1995). There was growing concern that the soil microbial community may not be adequately protected from the effects of metals in soils receiving sewage sludge. The main sources of Cadmium in streams are effluents from industries such as electroplating, paints, plastic, battery and zinc mining and refining. Because of its high toxicity, most countries include Cadmium among the "Priority pollutatnts" requiring suitable treatment prior discharge into the environment (Puranik *et al.*, 1995). The United States Environmental Protection Agency limits Cadmium levels in drinking water to 0.001 mg/l. In India, the permissible concentration of Cadmium in the industrial effluents discharged into inland surface waters is 0.1 mg. l^{-1}. At present a variety of physico-chemical processes one employed to treat Cadmium containing effluents. These processes, however prove expensive when situations involving high volume and low metal concentration (typically less than 50mg / l) are encountered (Puranik *et al*, 1995). Various microorganisms are known to adsorb metals from dilute solutions and concentrate them several fold by the process of biosorption. The use of dead cell mass in metal sorption can be of great interest because of the large variety and low cost of these biological materials. Heavy metals ions in small quantities are required for various physiological process and the normal functions of cells in plants and animals. Elevated levels of such metal ions are generally toxic and cause major damage to cells. In addition to the utilization of metal ions as essential elements, adjustment of intracellular levels of free ions by binding to macromolecules or other mechanisms is indispensable if cells are to protect themselves against excessive metal ions or changes in levels of such ions in the environment (Webb, 1987 & Mehra and Winge,1991). Two different classes of cytoplasmic molecules that participate in binding of metal ions and thus, in resistance to metal ions have been identified in various plant and animal cells (Inouhe *et al.*, 1996). Animal cells produce heavy metal binding protections known as metallothioneins (Grill *et al.*1985). Both types molecules are rich in cysteine residues as metal-binding sites but they are very different from each other in that the former are synthesized via an m-RNA transcript, while the latter are generated from GSH by PC synthase (Scheller *et al.* 1987, Grill *et al.*,1989). Inouhe *et al*., (1996) opined that it was important to determine, why such different systems became established and are exploited by plant and animal cells.

The toxic effects of Cadmium on plants have been described (Boddi *et al*., 1995) and characterized in many physiological processes (Marschner,1983). Among these, the chlorosis of leaves (Bishoni *et al*., 1993; Ferretti *et al*.,1993 and Siedlecka and Bazynski,1993) and the inhibition of various reactions of photosynthesis (Baszynski *et al*.,1980) have been described. Boddi *et al*., (1995) raised the question, whether the Cd^{2+} inhibits directly the chlorophyll biosynthesis and several reactions of photosynthesis or if it interacts with other metabolic processes and acts indirectly. Cadmium^{2+} might induce iron deficiency (Marschner, 1983) which later causes the above mentioned symptoms (Siedlecka and Baszynski, 1993). A further possibility is that the cadmium^{2+} stimulates decomposition processes in the plants, resulting in the decrease of the chlorophyll contents and changes of the photosynthetic apparatus. Specific inhibitory effects of the Cd^{2+} have been observed by Boddi *et al*.,(1995), on the synthesis of 5-aminolevulinic acid and on the Pchlide photoreduction into chlide. Boddi *et al*., (1995) opined based on his observations that the reaction of Cd^{2+} was concluded to interact with essential thiol groups of enzymes involved in those reactions. The inactivation of the tennary complex of the Pchlide-NADPH oxidoreductase complex was indicated by a blue shift of the absorption-maximum of dark grown leaves, if shifted from 650nm to 630-635 nm (Stobart *et at*.,1985). The concern for the transfer of the Cadmium within the food chain is greater than for other potentially toxic elements. This is due to trace principal factors: high toxicity, long time of retention in the human body and high mobility in the environment. Cadmium may be the more toxic heavy metal for plants at concentrations lower than for other metals, inhibiting the synthesis of chlorophyll in barley and beans, the growth of sugar beet plant and the photosynthetic activity of tomato. Chlorophyll and carotenoid content are parameters that allow to study the incidence of different Cadmium treatments on the growth and development of tomato plants (Gil *et al*.,1995). The uptake of Cadmium by plant roots and its subsequent translocation to the stem and leaves is influenced by several factors, including plant genetics, Addition of Ammonium and phosphate fertilizers tend to increase plant Cd concentration (Choudhury *et al*., 1994). Phytochelatins (Pcs) are low molecular weight peptides enzymatically produced by the plants under the action of a specific phytochelatin synthase, in response to excessive uptake of heavy metals (Grill *et al*.,1989, Scheller *et al*.,1987). They are cystein rich peptides with general stricture (γ-Glu-cys) in Gly (n=2 to 11) capable of binding metal ions via thiolate coordination. This reaction leads to the formation of an intracellular compound between a metal ion and PCs (Grill *et al.*, 1985 &1987). These inducible metal binding complexes act as an effective heavy metal

detoxifying mechanism, because they sequester metal ions in a form, which limits damage to metabolic processes (Meharg, 1994; Gekeler *et al*., 1988). Among the metal ions capable of inducing phytochelatin synthesis in plant cells, cadmium represents the best activator of the enzyme (Grill *et al*., 1985, 1987). Knowledge of the reactions of heavy metals with soils is necessary to accurately assess the future problems of food production and quality and to plant adequate counter measures. At moderate concentrations of heavy metals above the deficiency range, most natural soils act as a repository or sink for metals, without any effect of the metals on soil biological behavior. In recent decades, there has been growing concern about the increasing concentrations of heavy metals in soils, because of the relative ease of their transfer from soil to plants (Carrillo Gonzalez *et al*., 1996). Thus the ability to predict metal concentrations in soils is vital to the adequate regulation of contaminated waste disposal and to present excessive uptake of heavy metals by plant s and their transport to ground water. However, the extent of transfer of metals from soil to plants not only depends on plant species and the kind of metal involved, but also on such factors as soil solution composition, soil type, distribution of metals between solid and solution phase and chemical reactions controlling the mobility of heavy metals (Carrillo-Gonzaliez *et al*.,1996). A thorough knowledge of basic mechanisms by which toxic agents impair organisms is fundamental to any eco-toxicological approach. The toxicity of the metal was probably detoxified due to the presence of the masking agents in the waste or the nutrients present in the medium or the exudates of the blue-green alga. The initial depression of the parameters of the exposed blue-green alga was probably due to the stress or sudden change of the environment, which probably changed slowly due to the modification of the environment either due to formation of complexes in the medium or the chelating effect caused due to interaction or might be due to the non-availability of toxic chemicals, indicating either acclimatization or adaptation of the blue-green alga to the changed medium. The variance ratio test, pertaining to the parameters linked to the nitrogen fixation and from the correlation matrix, it can be hinted that the extra-cellular products of the blue-green alga probably helped to change the nature and toxicity of the toxicants. It was clearly observed from the above correlation analysis that very low concentrations of the mercury stimulate growth, by increasing different parameters like dry wt., optical density, pigment contents, biochemical variations etc. and high concentration of the waste / toxicant inhibited growth to a large extent, damaging the live system. Detoxification mechanisms can involve storage of metals at inactive sites within organisms on a temporary or more permanent basis. Temporary storage is generally by binding of metals to proteins, polysaccharides and amino acids in soft tissues or in body fluids. So it can be suggested that the waste of the industry containing cadmium should be diluted sufficiently, before its discharge to outside environment either to be used in the crop fields for irrigation purpose or for any other purpose. It can be concluded from this piece of investigation that during the life span of 15 days the amount of cadmium accumulated was not that much enough to show its effect, nullifying the antagonistic effect of other chemicals and the effect of cadmium was masked in the presence of those other chemicals. But in a long run, the amount of accumulated cadmium will be very high in the BGA, which will bioconcentrate and biomagnify through different trophic levels and will represent a potential hazard ultimately to man. Before the oncoming of such a situation, the people as well as the Government should be cautious enough to take major steps to avoid the disaster which may be caused in the near future. In addition, the blue-green algae can be used as an agent, which can remove toxicants from a contaminated area and simultaneously can act as a bio-fertilizer.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

Author contribution staement

Prof. A.K. Panigrahi: Conceptualization, planning and execution of the project, supervision, original draft preparation, reviewing and editing. Research work conducted by Sri Saroj K. Mishra analysis and related experimental work.

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