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Eco-Toxicological Impacts Of Cadmium Chloride On The Nitrogen metabolism Of A Blue-Green Alga Under Laboratory Controlled Conditions.

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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 their possible effect on the fresh water fishes surviving in the water bodies, this project was masterminded to evaluate the eco-toxicological effects of Cadmium metal in the form of Cadmium chloride on the nitrogen metabolism 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. In case of the control set, the cellular nitrogen content increased from $2.4\pm0.3\mu$ g/50 ml culture to $11.2\pm0.4\mu$ g/50 ml culture within 15 days of exposure. The cellular nitrogen content increased to 24.6±0.3µg/50 ml culture as recorded on 15th day of recovery. The cellular nitrogen increase showed a positive correlation with the exposure period. In case of conc.- X, at all exposure periods, rise in cellular content level was marked, when compared to the control value. The increase in the cellular nitrogen content in conc. X set was negligible. Virtually there was no difference between control and Conc. X sets in cellular nitrogen content. When the exposed alga was transferred to toxicant free medium 2.44% recovery was noted, which was not significant. In conc.-Y, significant depletion in cellular nitrogen content was recorded compared to the control set. A maximum of 54.5% decrease over the control value was recorded on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, the cellular nitrogen content further depleted to 67%, compared to respective control. In case of conc.-Z, the percent decrease increased with the increase in exposure period and 100% decrease was recorded on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, no recovery was marked and the cellular nitrogen content was not within the measurable range and 100% depletion was noted. The extra-cellular nitrogen content in the control set showed an increasing trend, with the increase in exposure period. The value increased to 7.6 \pm 0.5 on 3rd day and 31.8 \pm 0.4 μ g/50 ml culture on 15th day of exposure. The extra-cellular nitrogen content in the recovery flask, increased to 62.6±0.7µg/50 ml culture on 15th day of recovery. In conc.-Y, a maximum depletion by 73.1% on 15th day of exposure and 77% decrease on 15th day of recovery was observed, when compared to its respective control value. In conc.-Z, a maximum depletion by 100% on 15th day of exposure and 100% decrease on 15th day of recovery was observed. Cadmium chloride significantly affected the nitrogen fixation by the alga. Cadmium was responsible for the depletion of nitrogen fixation affecting the biofertility of the soil.

Key words: Cadmium chloride, Blue-green alga, Cellular nitrogen, Extra-cellular nitrogen,

Introduction

Pulp & Paper Mills are the most potent polluters of aquatic environments polluting water bodies in the down stream. 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". 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. Tripathy et al., (2021) reported presence of mercury, cadmium and lead in the final discharged Paper mill effluent after physical and chemical 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). But cleaner & environment friendly recycling technology can be adopted and periodically positive modifications in the technology or alterations in the treatment technology should be planned. The solid waste and effluent from the industry are generally disposed for land filling or discharged into water bodies. Generally the toxic effluents from industry are treated before their discharge but still they contain substantial amount of toxic substances that can cause pollution. The effluent of paper mill contained significant amount of heavy metals like cadmium, mercury, lead and others. These heavy metals are known toxic metals causing immense harm to aquatic flora and fauna. Plant metabolism may be affect by Cadmium in different ways. 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). In winter wheat (Triticum *aestivum*, L. W. Hv8) Cd^{+2} caused a growth retardation and changes in ion uptake immediate target of Cd^{+2} in the cell membrane, where both the membrane composition and function can be altered and damaged (Fodor *et* al., 1994). Popovic et al., (1996) suggested several ways by which the plants can reduce these negative effect of Cd⁺². One of the possibilities suggested by them and that hypothesis has recently became very popular was that the heavy metals form chelates with sulphur rich proteins (Wagner, 1984).

The industry under base study was Paper Mill located at Jaykaypur, Rayagada, Odisha which discharges its effluent into River Nagavalli. The effluent mixed water is used by the farmers in neighboring crop fields. It is well established fact that this effluent mixed water affected the crop plant and reduced production of crop. Mishra and Panigrahi (2023a, b) reported that cadmium chloride significantly affected the pigment content, photosynthetic efficiency of BGA inhabiting crop fields and the metal is deadly toxic. This piece of work was aimed at finding out the impact of cadmium chloride on the atmospheric nitrogen fixation by the blue-green alga *in vivo*.

Materials & Methods

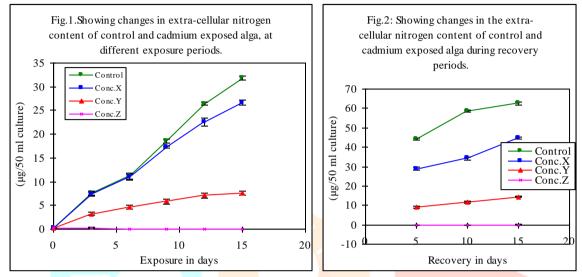
Lethal concentration values were taken from the publication of Mishra and Panigrahi (2023a) pertaining to toxicity study: One ml of unialgal, axenic, homogenized culture was inoculated in each 150 ml flask containing 100ml of cadmium chloride solution, inside the inoculating chamber. The toxicant was 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 $28 \pm 2^{\circ}$ C 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. Cellular and extra-cellular Kjeldahal nitrogen, were estimated by Kjeldahal Nesslerization method following the protocol of Herbert *et al.* (1971). The algal culture was centrifuged in a refrigerated centrifuge at 20^oC and 5000 rpm for 10 minutes. From the supernatant the extra-cellular nitrogen was estimated and cellular nitrogen was extracted by digestion from the whole algal material present in 100 ml culture. The experiments were performed in triplicate and the data were expressed as the mean of three observations involving cellular nitrogen and extra-cellular nitrogen as $\mu g/100$ ml algal culture. The obtained value from cellular and extra-cellular value and is expressed as $\mu g / 100$ ml of total algal culture. The obtained values were statistically analyzed to find out the levels of significance.

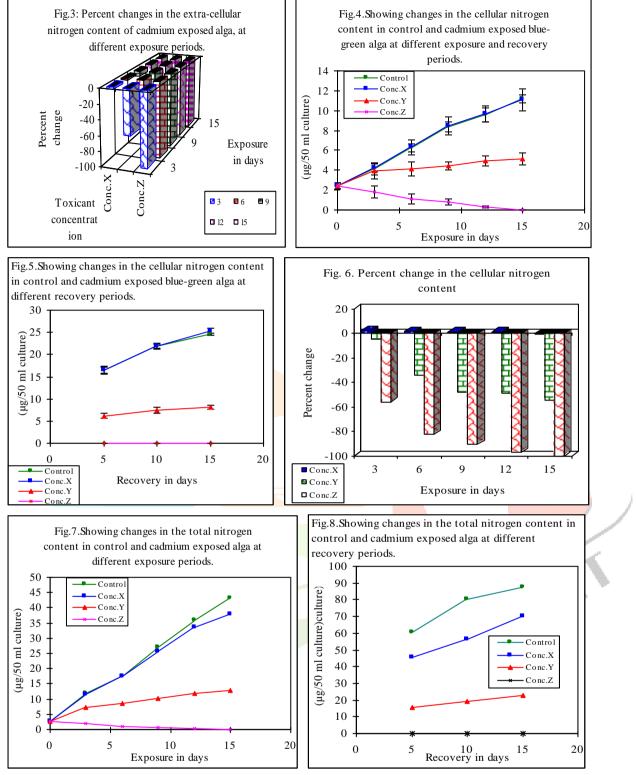
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Results

Fig.1 indicated the changes on extra-cellular nitrogen content in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The extra-cellular nitrogen was $0.20\pm0.04\mu g / 50$ ml culture in the inoculation day, as the selected nutrient media is free from nitrogen, which is more suitable for the growth of heterocystous, nitrogen fixing blue-green alga. The extra-cellular nitrogen content in the control set showed an increasing trend, with the increase in exposure period. The value increased to 7.6 ± 0.5 on 3^{rd} day, 11.2 ± 0.6 on 6^{th} day, 18.5 ± 0.4 on 9^{th} day, 26.4 ± 0.3 on 12^{th} day and $31.8\pm0.4\mu g / 50$ ml culture on 15^{th} day of exposure. The extra-cellular nitrogen content in the recovery flask, increased to $62.6\pm0.7\mu g / 50$ ml culture on 15^{th} day of recovery. This value was the total amount of extra-cellular nitrogen fixed by the blue-green alga, which has been exuded to the medium (Fig.2).



In concentration- Y, at all exposure periods, the extra-cellular exudate nitrogen content was less than the control value and conc. X value. The value increased from 0.2 ± 0.04 to 7.6 ± 0.4 µg / 50 ml culture on 15th day of exposure and the value reached maximum to $14.4\pm0.3\mu g/50$ ml culture on 15^{th} day of recovery (Fig.1). In case of concentration-Z, an insignificant amount of nitrogen was fixed on 3^{rd} day $0.3\pm0.1\mu$ g / 50 ml culture. With the increase in exposure period the exposed alga could not fix any more nitrogen and the amount if at all fixed was not detectable. When the exposed alga was transferred to toxicant free medium, no recovery was marked indicating total damage done by the toxicant, Cadmium chloride. These values were much less when compared to the control values. In concentration- X, at all exposure periods, the extra-cellular exudate nitrogen content was less than the control value. The value increased from 0.2 ± 0.04 to $26.6\pm0.5\mu g$ / 50 ml culture on 15th day of exposure and the value reached maximum to 44.8±0.6µg / 50 ml culture on 15th day of recovery (Fig.2). With the increase in exposure period, the extra-cellular nitrogen availability in the flask reduced and on 15th day of exposure, no extra-cellular nitrogen was recorded in the culture flask (Fig.3). When the exposed alga was transferred to toxicant free medium for recovery, no recovery was marked. In concentration-X, a maximum depletion by 16.35% on 15th day of exposure and 28.4% decrease on 15th day of recovery was observed, when compared to its respective control value. In concentration-Y, a maximum depletion by 73.1% on 15th day of exposure and 77% decrease on 15th day of recovery was observed, when compared to its respective control value. In concentration-Z, a maximum depletion by 100% on 15th day of exposure and 100% decrease on 15th day of recovery was observed, when compared to its respective control value.



No recovery was marked in all the three exposed sets (Fig.3). The correlation coefficient analysis between days of exposure and extra-cellular nitrogen content indicated the existence of a positive correlation in control (r = 0.984, $p \ge 0.01$), in Conc. X (r = 0.980, $p \ge 0.01$) and in conc. Y (r = 0.912, $p \ge 0.05$). A non-significant correlation (r = -0.119, p = NS) in Conc. Z was marked. The two way of analysis of variance ratio test indicated the existence of significant difference between rows and a significant difference between columns.

Fig.4 showed changes in cellular nitrogen content and cadmium chloride exposed blue-green alga at different days of exposure and recovery. In case of the control set, the cellular nitrogen content increased from $2.4\pm0.3\mu g / 50$ ml culture to $11.2\pm0.4 \mu g / 50$ ml culture within 15 days of exposure. The cellular nitrogen content increased to $24.6\pm0.3 \mu g / 50$ ml culture as recorded on 15^{th} day of recovery. The cellular nitrogen increase showed a positive correlation with the exposure period (Fig.4). In concentration 'X' significant higher values up to 12^{th} day of exposure, when compared to control was recorded. At higher exposure periods insignificant decrease in the parameter was observed. The cellular nitrogen content increased from $2.4\pm0.3\mu g / 50$ ml culture on 12^{th} day of exposure and then the value reached to $11.1\pm1.1\mu g / 50$ ml culture on 15^{th} day of exposure. The cellular nitrogen content increased from $2.4\pm0.3\mu g / 50$ ml culture on 15^{th} day of exposure and then the value reached to $11.1\pm1.1\mu g / 50$ ml culture on 15^{th} day of exposure. The cellular nitrogen content in concentration-X was much higher

than the control value at all exposure periods except 15th day. When exposed alga was transferred to toxicant free medium, significant increase in cellular nitrogen content was recorded, where maximum value of $25.2\pm0.8 \ \mu\text{g}$ / 50 ml culture was recorded, which was also more than the 15th day recovery value (Fig.5). In concentration 'Y' significant lower values up to 15th day of exposure, when compared to control was recorded. The cellular nitrogen content increased from $2.4\pm0.3 \mu g / 50$ ml culture to $4.9\pm0.5 \mu g / 50$ ml culture on 12^{th} day of exposure and then the value reached to $5.1\pm0.6\mu\text{g}$ / 50 ml culture on 15^{th} day of exposure. The cellular nitrogen content in concentration-X was much lower than the control and conc. X value at all exposure periods. When the exposed alga was transferred to toxicant free medium, significant increase in cellular nitrogen content was recorded, where maximum value of 8.1±0.5µg / 50 ml culture was recorded on the 15th day of recovery. In concentration-Z, the cellular nitrogen content decreased significantly from 2.4 ± 0.3 to $0.3\pm0.1\mu$ g / 50 ml culture on 12th day of exposure. At higher exposure periods (15th day), the cellular nitrogen content was not detectable. When the exposed alga was transferred to toxicant free medium for recovery studies, no trace of cellular nitrogen was detected. These values were less than the respective control values and concentration X and Y set values. In case of concentration X, at all exposure periods, rise in cellular content level was marked, when compared to the control value. On 12th day of exposure, a maximum of 1.04% increase over the control value was marked (Fig.6). The increase in the cellular nitrogen content in conc. X set is negligible. On 15th day of exposure, 0.89% decrease was recorded, which was also insignificant. Virtually there was no difference between control and Conc. X sets. When the exposed alga was transferred to toxicant free medium 2.44% recovery was noted, which was not significant. In concentration Y, significant depletion in cellular nitrogen content was recorded when compared to the control set. The percent decrease showed a positive correlation value with the exposure period. A maximum of 54.5% decrease over the control value was recorded on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, the cellular nitrogen content further depleted to 67%, when compared to respective control. In case of concentration-Z, the percent decrease increased with the increase in exposure period and 100% decrease was recorded on 15th day of exposure and when the exposed alga was transferred to toxicant free medium, no recovery was marked and the cellular nitrogen content was not within the measurable range and 100% depletion was noted. In case of concentration A, insignificant recovery was marked and the changes in recovery values were not significant and the values come within the standard deviation range. But in case of concentration- Y and Z, significant depression and no recovery was recorded (Fig.6). The correlation coefficient analysis between days of exposure and cellular nitrogen content showed the existence of a significant positive correlation in control (r = 0.987, $p \ge 0.01$); in Conc. X (r = 0.989, $p \ge 0.01$) and in conc. Y $(r = 0.924, p \ge 0.05)$. A significant negative $(r = -0.905, p \ge 0.05)$ correlation in Conc. Z was marked. The two way analysis of variance ratio test indicated the existence of non-significant difference between rows and significant differences between columns. Fig.7 indicated that changes in total nitrogen (cellular nitrogen + extra-cellular nitrogen) fixed by the blue-green alga in the control set and cadmium chloride exposed set at different exposure and recovery period. The total nitrogen content increased from 2.6 to 43.0 µg / 50 ml culture on 15th day of exposure and it increased to 87.2 μ g / 50 ml culture on 15th day of recovery (Fig.8). In concentration X, the total nitrogen content increased from 2.6 to 37.7µg / 50 ml culture, as recorded on 15th day of exposure and the value significantly increased to 70µg / 50 ml culture on 15th day of recovery. All the values in concentration X, at different exposure periods, were much less than the respective control values. In concentration Y, the total nitrogen content increased from 2.6 to 12.7µg / 50 ml culture, as recorded on 15th day of exposure and the value insignificantly increased to 22.5µg / 50 ml culture on 15th day of recovery. All the values in concentration Y, at different exposure periods, were much less than the respective control values and concentration-X values. In concentration-Z, the total nitrogen content decreased from 2.6 to 0.3 μ g / 50 ml culture on 12th day and the value decreased significantly to zero 15th day of exposure and when the exposed alga was transferred to toxicant free medium for recovery studies, no recovery was noted (Fig.7). In conc.-X, lower percentage of decrease was recorded at all exposure periods, when compared to the control value and 12.3% decrease was recorded on 15th day of exposure. In conc.-Y, higher percentage of decrease was recorded at all exposure periods, when compared to the control value and 70.5% decrease was recorded on 15th day of exposure. In concentration-Z, 100% decrease was recorded on 15th day of exposure, when compared to the control value. With the increase in exposure period, the percent decrease of total nitrogen content increased significantly, showing a positive correlation ((Fig.8). The correlation coefficient analysis between days of exposure and total nitrogen content showed the existence of a significant positive correlation $(r = 0.984, p \ge 0.001)$ in control and a positive correlation $(r = 0.979, p \ge 0.001)$ in conc.-X was marked. A non significant correlation exists in Conc.-Y. Where as, a non-significant correlation (r = 0.541, p = NS) existed in conc.-Z. The ANOVA test indicated the existence of a non-significant difference between rows and a significant difference between columns.

Discussion

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 Rath et al. (1983 a). Stimulation in nitrogen fixing ability at low concentrations of various toxicants have been reported by several authors. Wurtsbaugh and Apperson (1978) reported an increase in nitrogen fixation with some insecticides. A species dependent resistance towards heavy metals with respect to nitrogen fixation was observed earlier. It was studied that the effects of different pesticides on eight asymbiotic cyanophyceae species and a blue-green phycobiont of a lichen which has nitrogen fixing capacity and observed two patterns of response: viz (a) in some cases an initial period of depression was succeeded by increased activity in nitrogen fixing ability; and (b) an initial decrease was observed in other cases with a subsequent decrease in nitrogen fixation as a function of time. Shaw et al. (1989) reported a decrease in nitrogen fixation capacity of BGA, W. prolifica, Janet, when exposed to the effluent of a chlor-alkali industry. Rath (1984) described a decrease in the cellular Kjeldhal nitrogen level with an increase in the concentration of HgCl₂. It was reported a decrease in algal nitrogen content with the increase in waste soil concentrations from a chlor-alkali industry and exposure period. The solid waste is loaded with pollutants like Hg, Na⁺ and Cl⁻ in very high concentrations. Stratton et al. (1979) stated that mercuric ion was toxic towards Anabaena inaqualis's growth, photosynthesis and nitrogenase activity to be the primary site of toxicity (Kamp-Nielson, 1971 and Passow, 1970). In the present study, however, cadmium with other chemicals does not appear to play any significant role in its highest concentration applied, to inhibit the nitrogen fixation capacity of the BGA, though, in lower concentrations it showed a stimulatory effect which was correlated with the amount of mercury present in the medium. The industrial waste is a heterogeneous compound and its extract containing an objectionable amount of cadmium is deadly toxic in nature. This toxicity may be due to the presence of cadmium or due to the presence of some other substances; the derogatory effect of which on living organisms in the ecosystem cannot be overruled, because this waste may show antagonistic or synergistic effect in field conditions rather than in laboratory controlled conditions. On the basis of experimental evidences for the existence of various cadmium and mercury species in the environment (Nriagu & Davidon, 1986), elemental Hg and methylmercury (dimethyl and monomethyl) are the principal candidates for volatilisation (emission and/or reemission) into the atmosphere. Total vapor-phase Hg fluxes from the agricultural and forest soils were found to be smaller than those from the surface of a lake. Previous studies confirmed that the exposed system recovered only partially, thereby indicating a possible damage caused due to the toxicant. The alga accumulated mercury from the solid waste extract and also volatilised mercury, either from the toxicant contained medium or from the accumulated mercury (Sahu and Panigrahi, 2003). This double action of the alga (cyanobacterium) is a subject of much interest; as it can be used as a detoxifier of the mercury contained environments, like mercury resistant bacteria and fungi, De Filippis and Pallaghy (1976 c) observed that in mercury stressed Chlorella cultures, 25% of mercury released was due to ethylene generation and the remainder mercury lost is due to a mercury reducing enzyme. Mishra and Panigrahi (2023 a, b) reported the impact of cadmium chloride on the toxicity, growth and photosynthetic efficiency of a blue-green alga in vivo. The authors reported that the heavy metal cadmium induced stimulation at sub-lethal concentrations of the toxicant. They also added that the metal showed dual behavior towards the alga; stimulation at lower concentrations and inhibition at higher concentrations. Such a dual behavior was not marked in the present study. Any insignificant increase or decrease at maximum allowable concentration in this study can not be attributed to the stimulatory activity of the metal. The heavy metal is toxic and can induce damaging effects on any plant or animal system.

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 subcellular 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. Cadmium has an estimated crustal abundance of between 0.15 $\mu g.g^{-1}$ and $0.2\mu g.g^{-1}$ & thus occurs in sixty seventh position in an order of relative abundance. These low contents of Cadmium in igneous rocks accounts for its low crustal abundance. With the exception of ore bodies, trace elements occur as impurities in primary minerals, which form igneous rocks. They are substituted for one of the major ions in the crust of a mineral without significantly attaining the internal structure.

Accumulation of heavy metals in agriculture soils has become a major concern for food crop production. Of these metals, Cadmium is recognized as one of the most hazardous elements, which is not essential for plant growth. Since, Cadmium is known to be easily taken up by plants and translocated within the plant (John *et al.*, 1992; Turner, 1973) a clear understanding of its bioavailabililty to plants is essential for reducing cadmium entry into the food chain with potentially harmful effects on human health. It is well known that cadmium concentrations in plant tissues is directly related to the concentration of plant available cadmium in soil (Mench *et al.*,1989). However, a number of soil factors can alter cadmium uptake and accumulation in plants. Soil cadmium speciation (Soltanpour,1991), Soil pH (Xian and shokohifad,1989) and soil organic matter concentration (Street *et al.*,1977)are factors most frequently observed to affect Cd availability to plants. It has also been reported that concentration of cadmium in plants varies among species and cultures (Page *et al.*,1981; Cieslinski *et al.*,1996). It was observed in the present study that the toxicant cadmium chloride significantly affected the rate of nitrogen production and significant decrease in extracellular nitrogen thereby reducing the biofertility of the soil. Hence, the effluent should be treated chemically and biologically to remove heavy metals from the effluent and then discharge the heavy metals free effluent into water bodies.

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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 analysis and related experimental work. Mishra contributed reagents, glassware, field related work, calculation and finalization of data.

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