IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Impact Of Cadmium Chloride On The ATPase Activity Of A Blue-Green Alga And Its Eco-Toxicological Significance.

Saroj Kumar Mishra* and A. K. Panigrahi,

Laboratory of Environmental Toxicology, Department of Botany

Berhampur University, BERHAMPUR-760007, Odisha. India.

*Department of Botany, Karilopatna College, Karilopatna, Kendrapada-754223, Odisha.

Highlights

- Graded series of concentrations of the cadmium chloride was prepared to evaluate the toxic effects of the cadmium chloride on the blue-green alga, *Anabaena cylindrica*. The sub-lethal and lethal concentration values of $CdCl_2$ were determined and experiments were conducted to see the impact of cadmium chloride on the BGA.
- With the increase in the concentration of cadmium chloride the percent survival decreased and hundred percent death was noticed at 1.88mg.l^{-1} of cadmium chloride with in a period of 15 days.
- The alga showed stimulation of enzyme activity at sub-lethal concentration of cadmium chloride.
- The ATPase activity significantly depleted at higher concentration of cadmium chloride.
- This dual behaviour of the toxicant can be exploited for phytoremediation studies.

Abstract

The ATPase activity showed normal behavior in the control set, where as , in cadmium chloride exposure, the alga showed dual behavior. The ATPase enzyme activity showed a maximum of 6.7% increase at concentration-X of cadmium chloride on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 12.3% increase was noted on 15th day of recovery. At concentration-Y, a maximum of 6.4% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 18.7% decrease was noted on 15th day of recovery. At concentration-Z, a maximum of 91.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 99.02% 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. 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. No recovery was noted in all the two higher (Y and Z) concentrations of cadmium chloride selected. At sub-lethal concentration of the toxicant, cadmium chloride stimulation of the enzyme activity and at higher concentrations of cadmium chloride inhibition of ATPase activity was observed. This dual behavior of the toxicant can be exploited for phytoremediation studies.

Keywords: Cadmium, BGA, ATPase activity, Stimulation, Inhibition

Introduction

Heavy metals in soil occur in various forms, each possessing different mobilities and phyto-availabilities (Alloway, 1990). Areas near industrial establishments may be enriched by metals via aerial deposition in particulate form. These particulates may come from varied sources such as automobile emission, combustion of fossil fuel (Particibly coal), smelting and refining of metal ores and other. Because, these particulates arose from varying thermal conditions and matrices, they come in various forms i.e. chloride, sulfate, carbonates, oxides etc. Excessive levels of metals in the soil can lead to elevated uptake by plants. Adverse consequences may ensure, such as phyto-toxicity or quality deterioration of edible portions from metal enrichment (Chlopecka, 1996). The same author indicted the existence of poor correlation between total metal content in the soil and plant uptake, whereas, better correlations have been observed for extractable form of metal (Xian, 1989). Commercial plastics use heavy metals in their formulation (Bode, 1992 Wagner *et al.*, 1992). Heavy metals are added to plastics for a number of reasons, namely as: stabilizers, plasticizers, antioxidants, colourants and fire retardants (Bode, 1992 & Wagner *et al.*, 1992). Cadmium is widely used as a colourant and plasticizer (Tamaddon and Hogland, 1993 ; Bergback et al,.,1994) and is often used in percent amounts in PVC, Bode(1992) reports that they found Cd at levels up to 4% in PVC. Others metals used in high concentrations in plastics formulations include Sb, Hg, Pb, Zn, Cr and Cu (Bode, 1992). The rapid increase in contamination of aquatic environments with pesticide and industrial pollutants in recent years has resulted in an escalation of scientific interests in the biological effects of pollution. Because of the importance of crop in the aquatic environment, any deleterious effect of the toxicant on this is likely to be reflected on the entire ecosystem. Considerable information are available pertaining to residual toxicity levels in fresh water, estuarine and marine fishes but relatively very little work has been done on the mechanism of toxic action of mercurial compounds especially on studies concerning active transport across cellular membranes. It has been reported that a significant depression of (Na^+, K^+) ATPase activity is associated with excessive absorption of mercury (Jackim, 1974) and also suggested that the derangement of normal monovalent cation exchange induced by mercurial compounds across membranes. Although investigations have been conducted on blue-green algae (Sahu, 1987; Rath, 1991) and higher terrestrial vertebrates few researchers have studied the effects of mercury on the enzyme activity of fish (Armstrong, 1979; Passino, 1981; Panigrahi, 1980; Panigrahi, 1985 and Samant, 1989). Harichandan *et al.* (2003) and Patra *et al.*, (2004 and 2005) reported the impact of cadmium chloride on the physiology, haematology and ATPase activity of a freshwater fish under laboratory controlled conditions. Harichandan *et al.* (2003) reported experimental residual cadmium accumulation in different organs of cadmium exposed fresh water fish and opined that fish can accumulate significant amount of cadmium in their tissues. The above authors reported that the environmentally availability of cadmium in different plants; plant parts, animals available in the contaminated area. The same author also reported that this environmental cadmium can be dangerously toxic to crop plants and organisms available in the crop fields.

Life depends on a complex network of chemical reactions brought about by specific enzymes. The enzymes are the primary instruments for the expression of gene action. Enzymes are of biological importance in metabolic functions, which have the most highly specialized class of proteins. The change in metabolism can well be correlated with the disturbance in behaviour and action of the enzymes (Panigrahi, 1984). The reduction in active metabolism has been correlated with the toxic effective different types of toxicants. The disturbance in enzymatic action can be related to the specific action of certain toxicants by inhibiting the enzymes to act, bind the active sites of the enzyme and enzymes become non functional due to disintegration. Inhibition of enzymes by chemical agents may be reversible or irreversible, competitive or noncompetitive. There are many different mechanisms through which thiol groups of enzymes can be acted upon by heavy metals. The importance of enzymatic studies in the system lies in the functional interpretation of the pollutant causing different types of disorders. Membranes play a role in the structure and function of the cell and its organelles. One of the way by which membranes can affect cellular metabolism is by interacting with enzymes. Some membrane function as the organizing matrix for enzyme systems, the micro environment in all these cases at or near the membrane surface may modulate the activities of the enzymes located in the region. The discharge of industrial effluent waste of Paper 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 consequently to crop fields; and this project was masterminded to evaluate the impact of Cadmium chloride on the toxicity and on a key and vital enzyme in metabolism of a blue-green alga inhabiting crop fields.

Materials & Methods

Anabaena cylindrica, Lemm. is photo-autotrophic, unbranched, filamentous, heterocystous, blue-green alga belonging to the family Nostocaceae. It shows three different types of cells viz. vegetative cells, heterocysts and akinetes. The spores and vegetative cells are always cylindrical in shape. The vegetative cells fix CO_2 and evolve O_2 where as heterocysts are unable to fix CO_2 or evolve O_2 but can fix nitrogen under aerobic condition.

Allen and Arnon's (1955, a) nitrogen free medium with trace elements of Fogg (1949) as modified by Pattnaik (1964) was most suitable for the organism. It was used as the basic culture solution in all the experiments in the present study. The experimental algal cultures were grown under controlled conditions of light and temperature inside a culture room. The culture flasks were kept in series on a culture rack, of glass plate with iron frame. Light was provided by means of white fluorescent tubes, connected at the backside of glass plate of each rack, which illuminates the upper glass surface at the intensity of 2400±200Lux, with 14 hours photoperiod and 10 hours nyctoperiod to allow the alga to grow photo-autotrophically. Temperature was regulated in the culture room and was maintained at 28± 2^0C. The culture flasks were regularly hand shaken twice a day to avoid clumping of the cells as well as their adhesion to the wall of the conical flasks.

The pollutant, Cadmium was prepared by taking standard Cadmium metal and dissolved in acid. A graded series of concentrations of cadmium chloride ranging from 0.1mg l^{-1} to 2.0mg l^{-1} (V/V) was prepared in different experimental conical flasks. The dilutions were made with the nutrient medium. One ml of unialglal, axenic, homogenised culture was inoculated in each 150 ml flask containing 100 ml of solution, inside the inoculating chamber. The number of individual cells of the algae present in one ml of the culture medium after micro-tissue homogenisation was counted under the microscope. The test algae were exposed for a period of 15 days in different test medium after which their survival and mortality percentage were calculated by counting the number of cells present in one ml of the test solution after micro-tissue homogenization. From this, different survival percentage and mortality percentage, the lethal concentration (MAC) value were determined.

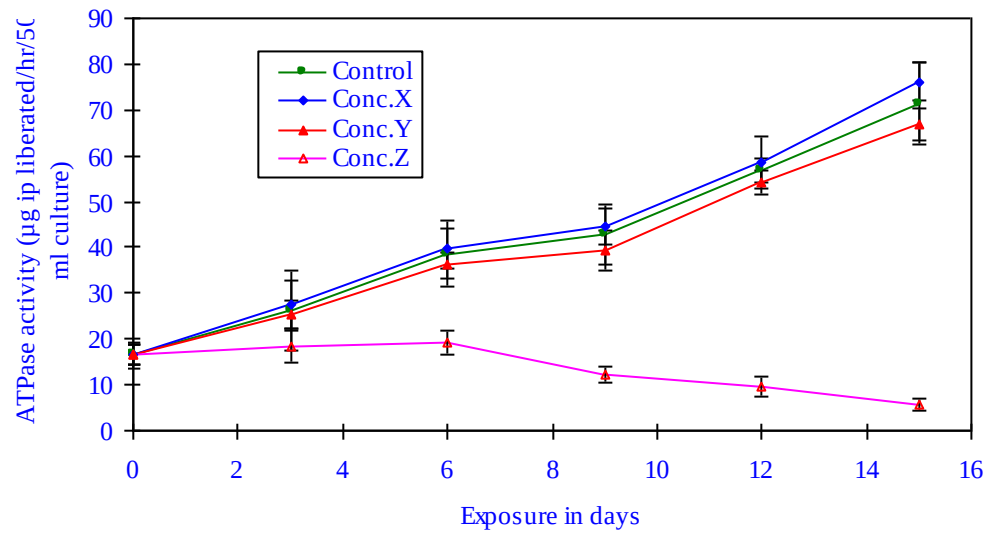
The enzyme extraction was done by homogenizing the algal pellet thoroughly with the help of a pre-chilled micro tissue homogenizer in 10 ml of 0.1 M Acetate buffer, (Sodium acetate and acetic acid), pH 5.6. All enzyme extraction procedures were carried out at 2 to 4^0C. The crude homogenates were centrifuged at 10,000 rpm for 20 minutes at 2^0C and the supernatant fraction was used as the source of enzyme for the assay of ATPase. ATPase activity was assayed by a modified method of Parida and Mishra adopted by Shaw (1987). The assay mixture consisted of 2 ml of 0.1 M acetate buffer (pH 5.6), 1 ml of $MgCl_2$ (5 µmol.), 1 ml of ATP (Sigma Chemical, Co., USA) solution in buffer (5 µmol neutralized disodium ATP salt / ml) and 1 ml of the enzyme extract (sample). This assay mixture was incubated at 37^0C for 30 minutes and then the reaction was stopped by adding 1 ml of 20% Perchloric acid ($HClO_4$). It was done for control as well as experimental set. Simultaneously a "ZERO" time control was prepared by adding 20% $HClO_4$ (the terminating reagent) to the reaction mixture immediately before the addition of the enzyme extract. The assay mixtures were then transferred for 30 minutes to a refrigerator at 5^0C to allow complete precipitation. The precipitate was then precipitated in a low speed centrifuge at 4,000 rpm for 5 minutes. The supernatant was taken for the estimation of total ATPase activity, which was determined by measuring the amount of inorganic phosphate produced when ATP to ADP. ip. produced as a result of the cleavage of ATP to ADP by molybdenum blue method described by Martineck (1970) was adopted. ATPase activity was expressed as µg i.p. liberated / hr /100 mg dry wt of alga. The obtained data was statistically analyzed.

Results

The control set showed 100% survival. The same data can also be interpreted as 10% survival at 1.22mg l^{-1}, 50% survival at 0.78 mg l^{-1}, 90% survival at 0.48 mg l^{-1}, and 100% survival at 0.31mg l^{-1} was marked. Out of the above concentrations, LC_{00} or PS_{100} as safe MAC value of 0.31mg l^{-1} was selected as 'X'; LC_{50} or PS_{50} of 0.78mg l^{-1} was selected as 'Y' and LC_{90} or PS_{10} value of 1.22mg $l^{-1}$was selected as 'Z' for conducting future experiments. Fig.1 shows the changes in total ATPase activity in control and cadmium chloride exposed blue-green alga at different exposure and recovery periods. The total ATPase activity of the control set increased with the increase in exposure period. The value increased from 16.5 ± 2.8 to 71.4 ± 2.8µg i.p. liberated hr^{-1} 50ml culture on 15^{th} day exposure of the control set. The value further increased to 112.6 ± 4.8µg i.p. liberated hr^{-1} 50ml culture on 15^{th} day of recovery (Fig.1). In concentration X, the value showed significant increase at all exposure and recovery periods, when compared to the control value. The value increased from 16.5 ± 2.8 to 76.2 ± 4.1µg i.p. liberated hr^{-1} 50ml culture on 15^{th} day exposure and from 76.2 ± 4.1 to 126.4 ± 5.9µg i.p. liberated hr^{-1} 50ml culture 15^{th} day of recovery, respectively (Fig.1). In concentration Y, the ATPase activity increased from 16.5 ± 2.8 to 66.8 ± 3.6µg i.p. liberated hr^{-1} 50ml culture on 15^{th} day of exposure and the enzyme activity increased from 66.8 ± 3.6 to 91.5 ± 2.9µg i.p.

liberated hr^{-1} 50ml culture on 15^{th} day of recovery. In concentration Z, the ATPase activity increased from 16.5 ± 2.8 to 19.2 ± 2.1µg i.p. liberated hr^{-1} 50ml culture on 6^{th} day of exposure and the enzyme activity decreased from 19.2 ± 2.1 to 5.8 ± 0.9µg i.p. liberated hr^{-1} 50ml culture on 15^{th} 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 depleted to 1.1 ± 0.2µg i.p. liberated hr^{-1} 50ml culture on 15^{th} day of recovery (Fig.1). This non recovery indicated that

Fig.1. Showing changes in ATPase activity of control and cadmium exposed alga at different concentrations and at different exposure and recovery periods.



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 6.7% increase in the enzyme activity was marked on 15^{th} day of exposure and when the exposed alga was transferred to toxicant free medium 12.3% increase was noted on 15^{th} day of recovery. At concentration-Y, a maximum of 6.4% decrease in enzyme activity was marked on 15^{th} day of exposure and when the exposed alga was transferred to toxicant free medium 18.7% decrease was noted on 15^{th} day of recovery. At concentration-Z, a maximum of 91.8% decrease in enzyme activity was marked on 15^{th} day of exposure and when the exposed alga was transferred to toxicant free medium 99.02% decrease in the enzyme activity was noted on 15^{th} day of recovery.

Fig. 2. Showing changes in ATPase activity of control and cadmium exposed alga at different concentrations and at different exposure and recovery periods.

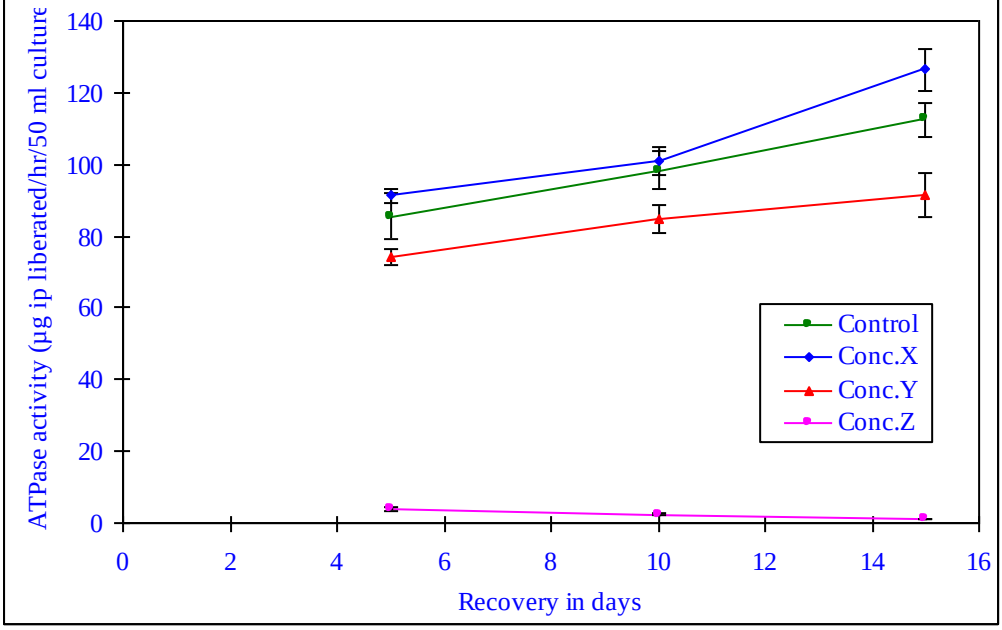
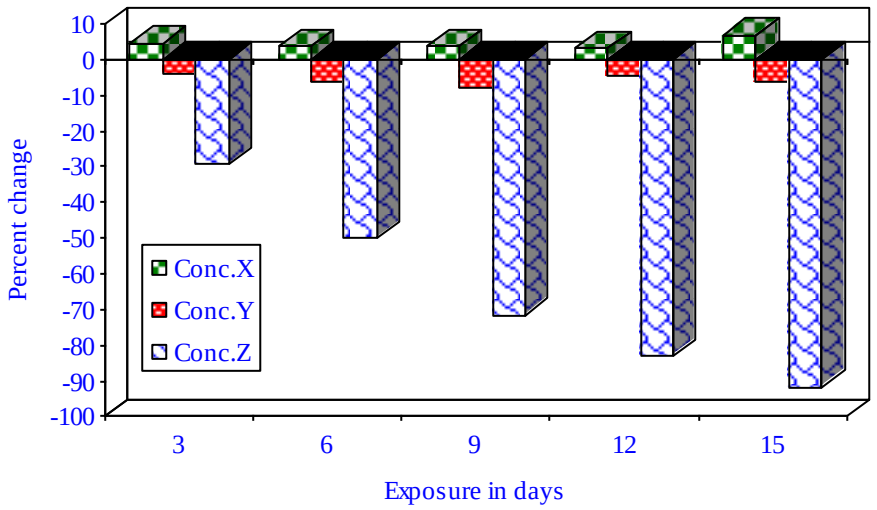


Fig. 3: Percent change in ATPase activity of cadmium exposed alga at different exposure periods when compared to control.



At concentration-X, little increment was noted at all exposure and recovery periods. 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 behaviour in its action. No recovery in all the two (Y and Z) concentrations selected was noted (Fig.20). The correlation coefficient analysis between days of exposure and ATPase activity indicated the existence of positive significant correlation in control ($r = 0.981$, $p \geq 0.001$) and in Conc. X ($r = 0.995$, $p \geq 0.001$) and in conc. Y ($r= 0.986$, $p \geq 0.01$) and a negative significant ($r = -0.932$, $p \geq 0.05$) correlation in Conc. Z. The ANOVA test indicated the existence of non-significant difference between rows and significant difference between columns.

Discussion

The wide spread occurrence, as well as certain chemical properties of chlorophyll pigments *in vivo* suggested that these pigments play an active role in photosynthesis functioning as photo-enzymes and the cadmium and mercurial compounds were toxic for the biosynthesis of chlorophyll pigments. Rath (1991) and Sahu (1987) indicated stimulation of growth, increase in pigment content, photosynthesis rate, respiration rate, and enzyme activity at lower concentrations of mercurial compounds on *W. prolifica*, Janet. Murty and Mohanty (1991) reported that mercury at a low concentration (3µm) caused an enhancement in the intensity of room temperature fluorescence, emitted by phycocyanin and induced a blue-shift in the emission peak of *Spirulina* cells indicating the alterations in the energy transfer within the phycobilisomes, whereas this phenomenon was not seen in *Anacystis*, *in vitro*. It is a common place observation that toxicity of metals showed great variations under field and laboratory conditions (Whitton, 1970). A given concentration of a metal may be more toxic to algae in the field than under laboratory conditions and vice-versa (Rai *et al*., 1981 b). Hence, it becomes explicit that laboratory based information cannot solely be used to stimulate field conditions, because many environmental and nutritional factors operate to bring about metal toxicity in field conditions (Gadd & Griffiths, 1978; Pradhan *et al.*, 2004). Eley *et al*. (1983) suggested that inhibition of photosynthesis might be responsible for growth retardation. Photosynthetic inhibition is mainly due to disturbances in the light energy trapping mechanism. The inhibition of photosynthesis and respiration in plant systems by cadmium (Mishra & Panigrahi, 2023) and mercurial compounds was reported by Sahu *et al*. (1988). In the present study it was observed that ATPase activity can be considered as indicator for assessing metal pollution. In the present study, at concentration-X, a maximum of 6.7% increase in the enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 12.3% increase was noted on 15th day of recovery. This information also prompts use to interpret that the alga could be used for phytoremediation purposes. This alga survives and removes cadmium from the medium and no interference of the metal was noticed at lower exposure period. However, the alga can accumulate the metal in long run and the absorbed and accumulated cadmium might influence all the metabolic activity of the alga. It was observed that at concentration-Y, a maximum of 6.4% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 18.7% decrease was noted on 15th day of recovery. At concentration-Z, a maximum of 91.8% decrease in enzyme activity was marked on 15th day of exposure and when the exposed alga was transferred to toxicant free medium 99.02%

decrease in the enzyme activity was noted on 15th day of recovery. Gould (1975) reported that mercury influences the photosynthetic capacity of the algae by inhibiting the electron inhibitor. There were also a few reports regarding the stimulating nature of some toxicants at lower concentrations. An increase in the oxygen evolution rate at lower concentration of mercury has been reported by Sahu *et al*. (1988). De Filippis and Pallaghy (1976 a) hypothesized that the enhancement might be related to the lowering of the concentration of the heavy metals in the cells as they induced to divide. Matsumoto *et al*. (1971) showed that mitochondrial enzyme activities and respiratory activities increased at lower concentrations of the toxicant. The activities declined significantly with the increase in the concentrations of the toxicant and the exposure period (Webb, 1966). Shaw (1987) reported the dual behavior of mercurial compounds on the growth of BGA and confirmed the dichotomous behaviour of the toxicants on living systems (Sahu, 1987 & Rath, 1991). Cadmium is extremely toxic to organisms because it inhibits a large number of metabolic enzyme system, complexes with amino acids, peptides, and proteins and affects the conformation of polyriboadenylic acid and the physical properties of DNA (Conway, 1978). Algae bioaccumulate cadmium (Kelly and Whitton, 1989; Pradhan *et al.*, 2004 Misra & Panigrahi, 2023) and bioaccumulation ratios of about 10^4 have been reported in freshwater (Conway, 1978) and marine (Cossa, 1976) diatoms. Although species-specific differences in sensitivity to Cadmium exist among algae (De Noyelles *et al*., 1980, Zhang *et al*., 1992) and at least one algae a *Euglena gracilis*, is known to have Cadmium resistant strains. Cadmium is generally very toxic to algae. For example, $2\mu g.l^{-1}$ Cadmium decreased chlorophyll by about 10% (Nalewajko, 1995). Cell suspension cultures of tomato were found to be more sensitive to excess copper than to similar concentrations of Cadmium, which was explained by the lack of synthesis of copper binding peptides by copper exposed cells. Indeed, these cells developed tolerance to 1mM Cadmium after sub-culturing with 100mM Cadmium induced the formation of cadmium binding particles (Inouhe *et al*., 1991) in cell suspension cultures of *Rawolfia serpentina.* Grill *et al*., (1985) demonstrated the induction of heavy metal binding peptides of general structure (YEC) G not only by Cadmium but also by other heavy metal ions, such as copper, phytochelations (Galli *et al*., 1996). Grill *et al*., (1989), showed that these peptides are synthesized from glutathione (GSH). Correspondingly, cell suspension cultures of *Datura innoxia (*Jackson *et al*., 1992*)* and maize roots showed a depletion of the GSH content after exposure to Cadmium. The function of phyto-chelations in the differential tolerance of plants to heavy metals however is controversial and needs further confirmation by repeated experimentation on different systems. 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 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. Besides, sulphur is a structural constituent of amino acids and several co-enzymes and prosthetic groups such as ferrodoxine, which are important for nitrogen assimilation (Petrovic and Kastori, 1994).

The change in enzyme activity affects energy metabolism and also the cation transport across the membranes (Henderson *et al*., 1979). The main role of Na^+, K^+-ATPase is of course, the active exchange transport, with the hydrolysis of ATP, sodium ion and potassium ion movement takes place. The decrease in ATP and the increase in i. p. and ADP affect the energy metabolism. Rath *et al*. (1986 a) reported an enhance in total ATPase activity at very lower concentrations of $HgCl_2$ and Emisan-6, whereas, at higher concentrations of the toxicant, a significant inhibition in ATPase activity was marked. Matsumoto *et al*. (1971) showed that there was an increase in activity at lower concentrations of the toxicant. Mercurials stimulate the enzyme activity, especially at low concentrations, the action-concentration curves being biophasic (Webb, 1966). Pradhan *et al.*, (2004) reported that Polis and Meyerhof first observed the stimulation of Ca^{++}-activated myosin ATPase by PM (Phenyl mercury). It was attempted to explain the stimulation of ribonuclease by p-MB as due to a reaction with RNA, this favouring in some manner the enzymatic hydrolysis, and detected spectral changes upon mixing RNA and p-MB (p. Mercuribenzoate). The stimulation of ATPase by mercurials has generally been explained (Pradhan *et al.*, 2004) since the report in terms of differently located SH groups around the active center. Enzymes may be protected against heavy metals by (1) substrates; (2) co-enzymes; (3) metal ion co-factors; (4) reversible inhibitors and (5) thiols or other complexes (Webb, 1966). Protection may occur by two general mechanisms: reaction of the protector with the enzyme to block off the mercurial or reaction of the protector with the mercurial. The failure to achieve protection may be due to an inadequate concentration of the protector, too low a relative affinity of the enzyme for the protector, or a long incubation where in equilibrium is reached and yet the substance

examined may participate in the reaction and interact with the enzyme in the same way as effective protectors. So definite protection allows one to make reasonable assumption that the mercurial binds somewhere in the region occupied by the protector (Webb, 1966). Metallic cations are highly essential for many enzymes to achieve their catalytic activity. These cations maintain the acid base balance; and are also highly essential for the ionic composition of the body fluids. Disturbance in electrolyte concentration are quite common in toxicity. Different studies on elecrolyte change resulting from toxic status have produced a variety of diverse effects, because of differential size of toxic actions in the body. The reaction of mercury compounds with sulfahydryl groups has been well established. A considerable decrease in succinic dehydrogenase activity of the mitochondrial fraction was reported. Bruin (1976) reported that cation movements within tissue cells originate from toxic effects on cellular membranes. Fungicides of diverse chemical structure are capable of changing mitochondria function, thereby making the membrane permeable to cation. Such cations are the mobile co-factors which either firmly bound to metal are called metaloenzymes or metal activated enzymes. It was reported that decrease in plasma calcium and an increase in plasma sodium in winter flounder, when exposed to mercury chloride at the concentrations of 10 and 20 ppb Hg level. Mercurials have long been recognized as agents which interact with the poison proteins in general and enzymes in particulars (Fox *et al*., 1975). The observed result in the present study agrees with the findings of 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.

Acknowledgement

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

Declarations

AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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.

Alloway, B. J. (1990): (Ed) Heavy metals in soils. John Wiley and Sons Inc., New York. pp-100-124.

Armstrong, F.A. (1979): Effects of mercury compounds. In: Nriagu, J.O. (ed.). The Biogeochemistry of mercury in the environment. North Holland, Biomedical Press, 657.

Bergback, B., Anderberg, S. and Lohm, U. (1994): Accumulated environmental impact: the case of Cadmium in Sweden. Sci. Total Environ., 145:13-28.

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.

Bode, P. (1992): The use of INAA for the determination of trace elements, in particular Cadmium, in plastics in relation to enforcement of pollution standards. J. Radiational. Nuclear Chem., 167:361-367.

Bruin, A. De. (1976): Nucleic acid metabolism. In: Biochemical toxicity of environmental agents. Ed. Bruin, A.. De, pp-605-655. Elsevier/ North Holland Biomedical press, The Netherlands.

Chlopecka, A. (1996): Forms of Cd, Cu, Pb and Zn in soil and their uptake by cereal crops, when applied jointed as carbonates. Water, Air and Soil Pollution, 87:297-309.

Conway, H. L. (1978): Sorption of arsenic and Cadmium and their effect on growth, micronutrient utilization and photosynthetic pigment composition of *Asterionella formosa.* J. Fish. Res. Bd. Can., 35:286-294.

Cossa, D. (1976): Sorption of Cadmium by a population of the diatom, *Phaeodactylum tricornutum* in culture. Mar. Biol., 34:163-167.

De Filippis, L. F. and Pallaghy, C. K. (1976 a): The effects of sub-lethal concentrations of mercury and zinc on *Chlorella*. 1. Growth characteristics and uptake of metals. Z. Pflanzen-physiol. Bd., 78:197-207.

De Noyelles, F. Jr.; Knoechel, R.; Reinke, D.; Treanor, D, and Altenhoffer, C. (1980): Continuous culturing of neutral phytoplankton communities in the experimental Lakes, area: Effects of enclosure *in situ* incubation, light, Phosphorus and Cadmium. Can. J. Fish. Aquat. Sci., 37:424-433.

Eley, J. H., J. F. Mc Connell and R. H. Catlett (1983): Inhibition of metribuzin on growth and Photosynthesis of the blue-green alga *Anacystis nidulans*. Environ. Exp. Bot., 23:365-368.

Fodor, E.; Erdei, L. and Szabo-Nagy, A.(1994): The effect of Cadmium stress on the plasma membrane of wheat root. Biol. Plant., 36 (Suppl): 362.

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.

Fox, J. H., K. Patel-Mandlik and M. M. Cohen (1975): Comparative effects of organic and inorganic mercury on brain slice respiration and metabolism. J. Neurochemistry, 24:757

Gadd, G. M. and Griffith, A. J. (1978): Microorganism and metal toxicity. Microbial Ecol., 4:303-317.

Galli, U.; Schuepp, H. and Brunold, C. (1996): Thiols in Cadmium and Copper treated maize (*Zea mays*, L.). Planta, 198:139-143.

Gould, J. M. (1975): Inhibition of photosystem II-dependent phosphorylation in chloroplasts by mercurials. Biochem. Biophys. Res. Commun., 64:673-680.

Grill, E.; Winnalker ,E.L. and Zenk, M. H. (1985): Phytochelatins: The principal heavy metal complexing peptides of higher plants. Science,230: 674-676.

Grill, E.; Loftler, S.; Winnacker, E. L. and Zenk, M. H. (1989): Phytochelatins - the heavy metals binding peptides of plants are synthesized from glutathione by a specific ϒ-glutanyl cysteine dipeptidyl transpeptidase (Phytochelatinsynthase). Proc. Natl. Acad. Sci. USA.86:6838-6842.

Harichandan, N., Beura, B.K. and Panigrahi, A. K. (2003): Toxicological effects of cadmium chloride on a fresh water fish and its ecological significance. Indian J. Environ. & Ecoplan., 7(1):63-70.

Henderson, G. R., Huang, W. and Askari, A. (1979): Transport ATPase- the different modes of inhibition of the enzyme by various mercury compounds. Biochem. Pharmacol., 28:429-433.

Inouhe, M.; Mitsumure, M.; Tohoyama, H.; Joho, M. and Murayama, T. (1991): Contribution of cell wall and metal binding peptides to Cadmium and Cu tolerance in suspension cultured cells of tomato. Bot. Mag. Tokyo,104:217-229.

Jackim, E. (1974): Enzyme response to metals in fish. In: Pollution and Physiology of Marine Organisms. Ed. By F. J. Vernberg and W. B. Vernberg, Academic Press, London, 59.

Jackson, P. J.; Delhaize, E. & Kuske, C. R. (1992): Biosynthesis of metabolic roles of Cystines (ϒ-EC)n G and their precursors in *Datura innoxia*. Plant soil.146:281-289.

Kelly, M. G. and Whitton, B. A.(1989): Inter-specific difference in Zinc, Cadmium and Pb accumulation by freshwater algae and bryophytes. Hydrobiologia, 175,1-11.

Martineck, R. G. (1970): Review of methods for determining inorganic phosphorus in biological fluids. J. Am. Med. Technol., 32:337.

Matsumoto, H., N. Wakiuchi and E. Takashasi (1971): Changes of some mitochondrial enzyme activities of cucumber leaves during ammonium toxicity. Physiol. Plant., 25:353-357.

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.

Murthy, S. D. S. and Mohanty, P. (1991): Plant Cell Physiol., 32(2):231.* Cross Reference.

Nalewajko, C. (1995): Effects of Cadmium and metal contaminated sediments on photosynthesis, heterotrophy and phosphate uptake in Mackenzie river delta phytoplankton.Chemosphere,30(7):1401-1414.

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

Petrovic, N. and Kastori, R.(1994): Effect of sulphur on nitrogen assimilation in young sugar beet plants (*Beta vulgaris*,L.).Biol. plant., 36(Suppl.):201.

Popovic, M.; Kevresan, S.; Kandrac, J. & Nikolic, J. (1996): The role of sulphur in detoxification of Cadmium in young sugar beet plants. Biologia Plantarum, 38(2):281-287.

Pradhan, P. K.; A. Sahu and A. K. Panigrahi (2004): Eco-toxicological effects of solid waste extract of a Chlor-alkali industry on the ATPase activity and ion content of a cyanobacterium. The Ekologia, 2 (1-2): 49-56.

Rath, P. (1984): Toxicological effects of pesticides on a blue-green algae, *Westiellopsis prolifica* Janet. Ph. D. thesis, Berhampur University, India.

Rath, P., Misra, B.N. and Panigrahi, A. K.(1986): Effect of both inorganic and organic mercury on ATPase activity of *Westiellopsis prolifica*, Janet. Environ. Pollut. Ser. A., 42,143-149.

Rath, S. C. (1991): Toxicological effects of a mercury contained toxicant on *Anabaena cylindrica*, L. and its ecological implications. Ph. D. Thesis, Berhampur University, India.

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

Sahu, A.; B. P. Shaw; A. K. Panigrahi and B. N. Misra (1986): Effect of phenyl mercuric acetate on ATPase activity of a blue-green alga, *Westiellopsis prolifica*, Janet.Microb. Lett., 33, 45-50.

Shaw, B. P. (1987): Eco-physiological studies of industrial effluent of a chlor-alkali factory on biosystems. Ph. D. thesis, Berhampur University, Orissa, India.

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.

Tamaddon, F. and Hogland, W. (1993): Review of Cadmium in plastic waste in Sweden. Water Manage. Res., 11:287-295.

Wagner, J. P.; El-Ayyoubi, M. A.; Konzen, R. B. and Krohn, J. L. (1992): Quantitative identification of antimony, barium, Cadmium and Tin during controlled combustion of plastics. Polymer plastics Technol. Engineer, 31:73-101.

Webb, J. L. (1966): Enzymes and metabolic inhibitors. 1st ed., Vol. II,p.729. Academic Press, New York.

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

Whitton, B. A. (1970): Toxicity of heavy metal to fresh water algae: A review. Phykos, 9:116-125.

Xian, X. and Shokohifad, G. I. (1989): Effect of pH on chemical forms and plant availability of Cadmium, Zinc and Lead on polluted soils. Water, Air, Soil Pollut., 45:2655-273.

Zhang, M.; Wang, J. and Bao, J. (1992): Study on the relationship between speciation of heavy metals and their eco-toxicity. I. Toxicity of Copper, Cadmium, Lead and Zinc in seawater to three marine algae in the presence of different complexation agents. Chinese J. Ocean. and Limnol**.,** 10:215-222.