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EFFECT OF MERCURY AND CADIMIUM ON THE BIOCHEMICAL PARAMETERS OF HYDRILLA PLANT

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ABSTRACT

The metal phytotoxicity was examined taking two non-essential elements like mercury and cadmium on the aquatic hydrophytes like *Hydrilla* plant. Though the plants show toxicity symptoms in response to metal, they too are seen to develop resistance to the toxicity. To explore the possible recovery of the phytotoxicity of the test plant, growth hormone, kinetin was supplemented with the heavy metals in the experimentation. The total chlorophyll content in the test plant showed a decrease in content with the treatment of $Hgcl_2$ and $CdSO_4$ but, when these metal compounds were supplemented with kinetin showed significant recovery in the chlorophyll content. It was also observed that, mercury pronounced the level of toxicity in comparison to cadmium. In bimolecular studies like protein, Amino acid, sugar and nucleic acids (DNA and RNA) were studied. Protein, Sugar and DNA content in the treated plant showed a decrease in the act treatment and overall it was concentration dependant. In this case also $Hgcl_2$ with Kinetin and $CdSO_4$ with Kinetin reduced the toxicity of the metal to some extent in the plant. With reference to the days /periods of study, 10 days interval pronounced more stress conditions in the test plant, there by overall decline in the biochemical parameters.

Keywords: Mercury, Cadmium, Hydrilla, Biochemistry

INTRODUCTION

Industrial emissions of metals such as aluminum, antimony, arsenic, cadmium, cobalt, copper, chromium, iron, leadmanganese, mercury, molybdnum, nickel, selenium, vanadium, zinc etc, are primarily responsible for the overall metal pollution(Nriagu,1988), of these a few metals such as cobalt, manganese, iron, copper, selenium, molybdenum, nickel are essential elements, whereas aluminum, arsenic, cadmium, chromium, lead, mercury are non-essential (Foy et al,1978; Woolhouse, 1983; Baker and Walker 1990; Prasad,1997). Essential elements at high concentrations and non- essential elements even at low concentrations are toxic (Berry and Wallace,1981); Baker and walker; (1989); Mehera and Farrago,(1994) Metals in the environment operate as stress factors causing physiological changes (strain)and in doing so reduce vigor, or in the extreme case cause death (Levitt,1980).The persistence of metals in the environment and their presence in a variety of organic and inorganic forms result in their becoming incorporated into biological cycles. Environment toxicity of metals stem from their phototoxic and genotoxic effects in plants in addition to other organisms. Heavy metal pollution can bring about severe phyototoxicity that may be manifested at different levels of organization ranging from molecular level to organism or at

population level, Trace metals namely Al, As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Zn have been considered to be major environment pollutants and their phytotoxicity is well established (Prasad,1997). After mercury enters into aquatic system, it undergoes chemical conversion from one form to the other in a cyclic manner. (Jonasson and Boyle, 1971). Mercury is a unique pollutant and its oscillates between all the phases of the environment (Brosset, 1981) and (Nelson et al, 1971). Monomethyl mercury accumulates in the food chain, e.g. algae, planktons, fish and other higher aquatic and terrestrial organisms including man at the top (Shaw, 1987). Thus, there is proportionately more mercury in algae than in the water in which they live yet more in fish that feed upon the algae; etc. Finally bacteria and consequent decay promote the conversion of mercury into methyl mercury. Cadmium was recognized many years ago to be a highly toxic element but it was not until recently that concern began to be expressed over the possible effects on human health of long term exposure to low concentrations of this element. Cadmium pollution from metal mining and smelting complex, caused series of illness and possible death in a local community has led to widespread public anxiety (Kabayashi and Hagino,1978).

The main course of cadmium is through discharge of effluents from industries, such as electroplating, paints, plastic, battery, zinc mining and refining, because of its high toxicity. Interest in cadmium contamination began after the outbreak of itai-itai diseases in Japan. Cadmium is extremely toxic to organisms because its inhibits a large number of metabolic enzyme system, forms complexes with aminoacids, peptides and proteins. It also affects the conformation of polyriboadenylic acid and the physical properties of DNA (Conway, 1978). Toxic effects on growth may be studied by examining macromolecules involved in growth, such as DNA, RNA and protein (Barron and Adelman, 1984). However, toxicant-induced changes in macromolecular content (RNA, DNA and protein) and RNA/DNA, RNA/protein, and protein/DNA ratios have received very little attention in fish (Barron and Adelman, 1984).

MATERIALS AND METHODS

TEST MATERIAL

The submerged rooted macrophyte *Hydrilla Verticillata casp* is an aquatic weed belonging to family Hydrocharitaceae.It grows abundantly in lakes, ponds and ditches of tropical and temperature climates. The coarsely serrated leaves of this plant occur in whorls of three to eight and have characteristic spines on the underside of the midrib. *Hydrilla* produces reproductive propagates called turions and tubers. Turions are compact dormant buds that are produced in leaf axils and fall from the plant, when they are mature. Tubers are formed terminally on rhizomes and can be found up to a foot deep in the sediment. A pond near A.S.College, Balia Dist: Jajpur (Orissa) was selected for the collection of *Hydrilla verticillata* plants. Samples of the plant were obtained from the site and grown in experimental ponds near the college premises.

TEST CHEMICALS

The chemicals used in the present study were of the purest grade available and were obtained from renowned laboratories. Mercuric chloride [Hg Cl_2] (M.W-272)] and cadmium sulphate, [3 $CdSO_4$, $8H_2O$ (M.W. 769.51)] were used at the test chemicals. Different concentration of the test chemicals were prepared by using double distilled water as the solvent. The selected concentration of the test chemicals used in the present study for both Mercury and cadmium were 1, 5, 10, 25mg/L for experimentations.

EXPERIMENTAL SETUP

Hydrilla growth in experimental tanks were collected in the form of sample and after acclimatization that would be subjected to tested in four different concentrations.(1,5,10.25mg/L) of Cd and Hg for 5 days and 10 days separately. Various physiological and biochemical parameters of the plant were measured in response to the metal treatment. After assessing the toxic effects of Cd and Hg at various selected concentration on the test plant, an attempt would be made to study the ameliorative effects of growth regulators, Kinetin (5mg/L)on toxic effects were assessed by adding

2ml in each concentrations of Cd and Hg. The biochemical parameters like Chlorophyll (Arnon,1949), Protein (Lowary et al.,1957), Amino Acids (Moore and Stein,1948), Sugar (Yoshida et al,1972), DNA and RNA (Schmeider,1957) of the Hydrilla Plant were estimated following the standard procedure.

RESULTS

The results obtained after the experiment was given Table No.1 and 2. The chlorophyll content expressed in mg. g-1 showed a decreasing trend with the increase in the heavy metal treatment. The maximum percent decrease was found to be 39.13% in case of 25mg Hg (10Days) and 40.47% in case of Cd (10 days).When the Kinetin was supplemented with heavy metals, there was significant recovery in the chlorophyll content. The protein and sugar content was worst effected among all the biochemical parameters studied. The highest decrease was in case of Cd (10 days) which was up to 64.28% in case of protein and it was 72% in case of Sugar content. The least affected biomolecule was found to be DNA content. However, when the Kinetin was supplemented with the heavy metal treatment, the recovery in biochemical parameters like protein, Sugar, DNA and RNA was statistically significant.

TABLE NO -1

EFFECT OF $HgCl_2$ AND $CdSO_4$ ON THE PIGMENT CONTENT AND BIOCHEMICAL PARAMETERS OF HYDRILLSp. AFTER 5 AND 10 DAYS OF INTERVAL (EACH DATA IS REPLICATED FIVE TIMES)

(Figures in parentheses represent the percent increase / decrease in content from control values)

Treatment	Metal Conc.	CHLOROPHILL mg g^{-1}		PROTEIN mg g^{-1}		AMINO ACID mg g^{-1}		SUGAR mg g^{-1}		DNA mg g^{-1}		RNA mg g^{-1}	
		5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
$HgCl_2$	Control	0.45	0.46	3.6	3.6	5.1	5.1	1.5	1.6	8.5	8.5	1.16	1.16
	1mg	0.38 (-15.555)	0.37 (-19.565)	2.9 (-19.444)	2.8 (-22.222)	5.62 (10.196)	5.52 (8.235)	1.42 (-5.333)	1.30 (-18.750)	8.1 (-4.705)	8.15 (-4.117)	1.32 (12.068)	1.31 (12.931)
	5mg	0.36 (-20)	0.35 (-23.913)	2.5 (-30.555)	2.4 (-33.333)	5.90 (15.686)	5.82 (14.117)	1.18 (-21.333)	1.03 (-35.625)	8.05 (-5.294)	8.02 (-5.647)	1.42 (22.413)	1.41 (21.551)
	10mg	0.33 (-26.666)	0.32 (-30.434)	2.1 (-41.666)	1.9 (-47.222)	6.52 (27.843)	6.41 (25.686)	0.74 (-50.666)	0.72 (-55.00)	7.5 (-11.764)	7.4 (12.941)	1.62 (39.655)	1.58 (36.206)
	25mg	0.29 (-35.555)	0.28 (-39.130)	1.6 (-55.555)	1.4 (-61.111)	6.93 (35.882)	6.75 (32.352)	0.55 (-63.333)	0.51 (-68.125)	7.3 (-14.117)	7.2 (-15.294)	1.66 (43.103)	1.62 (39.655)
	r Value	-0.867*	-0.830*	-0.891*	-0.885*	0.914*	0.913*	-0.924**	-0.893*	-0.892*	-0.898*	0.857*	0.850*
$CdSO_4$	Control	0.45	0.46	3.5	3.5	5.1	5.2	1.5	1.5	8.62	8.59	1.17	1.18
	1mg	0.39 (-13.333)	0.38 (-17.391)	2.82 (-19.428)	2.71 (-22.571)	5.68 (11.372)	5.59 (7.5)	1.33 (-11.333)	1.29 (-14)	8.38 (-2.784)	8.24 (-4.074)	1.28 (9.401)	1.31 (11.016)
	5mg	0.35 (-22.222)	0.34 (-26.087)	2.51 (-28.285)	2.49 (-28.857)	6.15 (20.588)	6.05 (16.346)	1.13 (-24.666)	1.11 (-26)	8.26 (-4.176)	8.19 (-4.656)	1.11 (20.512)	1.43 (21.186)
	10mg	0.30 (-33.333)	0.32 (-30.434)	2.21 (-36.857)	2.19 (-37.428)	6.55 (28.431)	6.45 (24.038)	0.62 (-58.666)	0.55 (-63.333)	8.16 (-5.336)	8.09 (-5.820)	1.58 (35.042)	1.60 (35.593)
	25mg	0.27 (-40)	0.296 (-43.478)	1.65 (-52.857)	1.25 (-64.285)	7.05 (38.235)	6.95 (33.653)	0.45 (-70)	0.42 (-72)	8.12 (-5.800)	8.07 (-6.053)	1.67 (42.735)	1.69 (43.220)
	r Value	-0.872*	-0.876*	-0.902*	-0.937**	0.904*	0.929**	-0.910*	-0.893*	-0.774^{Ns}	-0.682^{Ns}	0.901*	0.896*

(*- Significant at P ≤ 0.05, **- Significant at P ≤ 0.01, ***- Significant at P ≤ 0.001, NS- Not-Significant.)

TABLE NO - 2

EFFECT OF $HgCl_2$ WITH KINETIN AND $CdSO_4$ WITH KINETIN ON THE PIGMENT CONTENT AND BIOCHEMICAL PARAMETERS OF HYDRILLA Sp. AFTER 5 AND 10 DAYS OF INTERVAL (EACH DATA IS REPLICATED FIVE TIMES)

(Figures in parentheses represent the percent increase / decrease in content from control values)

Treatment	Metal Conc.	CHLOROPHILL mg g^{-1}		PROTEIN mg g^{-1}		AMINO ACID mg g^{-1}		SUGAR mg g^{-1}		DNA mg g^{-1}		RNA mg g^{-1}	
		5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
$HgCl_2$ With Kinetin	Control	0.46	0.47	3.7	3.7	5.2	5.2	1.6	1.7	8.6	8.6	1.18	1.18
	1mg	0.42 (-8.695)	0.40 (-14.893)	3.2 (-13.513)	3.1 (-16.216)	5.8 (11.538)	5.7 (9.615)	1.44 (-10)	1.32 (-22.352)	8.2 (-4.651)	8.2 (-4.651)	1.32 (11.864)	1.32 (11.864)
	5mg	0.40 (-13.043)	0.37 (-21.276)	2.8 (-24.324)	2.6 (-29.729)	6.1 (17.307)	6.0 (15.384)	1.21 (-24.375)	1.05 (-38.235)	8.0 (-6.976)	8.1 (-5.813)	1.42 (20.338)	1.42 (20.338)
	10mg	0.38 (-17.391)	0.32 (-31.914)	2.3 (-37.837)	2.2 (-40.540)	6.7 (28.846)	6.5 (25)	0.84 (-47.5)	0.82 (-51.764)	7.8 (-9.302)	7.6 (-11.627)	1.64 (38.983)	1.64 (38.983)
	25mg	0.32 (-30.434)	0.30 (-36.170)	1.8 (-51.351)	1.7 (-54.054)	7.1 (36.538)	6.9 (32.692)	0.65 (-59.375)	0.61 (-64.117)	7.5 (-12.790)	7.4 (-13.953)	1.58 (19.696)	1.58 (19.696)
	r Value	-0.956**	-0.835*	-0.923**	-0.899*	0.901*	0.912*	-0.918*	-0.866*	-0.895*	-0.890*	0.856*	0.753^{Ns}
$CdSO_4$ With Kinetin	Control	0.46	0.47	3.6	3.6	5.2	5.3	1.61	1.52	8.65	8.58	1.18	1.19
	1mg	0.41 (-10.869)	0.40 (-14.893)	3.1 (-13.888)	3.0 (-16.666)	5.7 (9.615)	5.6 (5.660)	1.38 (-14.285)	1.35 (-11.184)	8.42 (-2.658)	8.28 (-3.496)	1.32 (11.864)	1.35 (13.445)
	5mg	0.38 (-17.391)	0.36 (-23.404)	2.7 (-25)	2.6 (-27.777)	6.2 (19.230)	5.2 (-1.886)	1.15 (-28.571)	1.12 (-26.315)	8.31 (-3.930)	8.19 (-4.545)	1.45 (22.881)	1.47 (23.529)
	10mg	0.34 (-26.087)	0.31 (-34.042)	2.2 (-38.888)	2.0 (-44.444)	6.6 (26.923)	6.5 (22.641)	0.81 (-49.689)	0.72 (-52.631)	8.21 (-5.086)	8.17 (-4.778)	1.62 (37.288)	1.63 (36.974)
	25mg	0.28 (-39.130)	0.24 (-48.936)	1.6 (-55.555)	1.5 (-58.333)	7.1 (36.538)	7.0 (32.075)	0.65 (-59.627)	0.60 (-60.526)	8.19 (-5.317)	8.16 (-4.895)	1.71 (44.915)	1.73 (45.378)
	r Value	-0.941**	-0.926**	-0.937**	-0.913*	0.912*	0.895*	-0.896*	-0.892*	-0.753^{Ns}	-0.610^{Ns}	0.890*	0.890*

(*- Significant at P ≤ 0.05, **- Significant at P ≤ 0.01, ***- Significant at P ≤ 0.001, NS- Non-Significant.)

DISCUSSION

Cadmium accumulation severely decreased the chlorophyll contents in *Hydrilla vertivillata. (*Garg et al., 1997). Chlorophyll contents decreased with an increased concentration of cadmium in different varieties of sugarcane (Radha et al., 2006). In the present investigation it is found that increased concentration of cadmium caused significant loss of chlorophyll pigments and there was a –ve correlation between the chlorophyll content and cadmium after 5 and 10 days of treatment. Under the present investigation chlorophyll content being reduced under various concentrations of cadmium in *Hydrilla* plant but by the addition of phytohormone kinetin the chlorophyll content is increased into some extent after 5 and 10 days of intervals. The fact that both mercury and cadmium cause the decrease in chlorophyll content in general provides support to the trend observed by earlier workers. De Filippies and Pallaghy (1976 b); and Mohapatra et al., (1997) observed that the protein content decreases with corresponding increase in concentration of mercury. Some workers reported an increase in protein content in response to cadmium (Thompson and Couture 1991; Narwal and Singh, 1993, Kavita et al., 1997). Decline in protein content in response to application of other metals was also reported by a number of workers (Mishra, 1992; Shukla and Pandev, 1993). Radha et al., (2006) reported that protein contents decreased with an increase in cadmium supply to the varieties of sugarcane .Kalita et al., (1993) also supports this effect that in presence of cadmium inhibition of protein synthesis takes place. According to Singh et al., (2001) the excess levels of Cd also decreased the concentrations of soluble protein.

Similar trend has been discussed by Garg et al.(1977) in *Hydrilla verticillata.* Neelu et al.(2000) also supported this view that total soluble protein of leaf, stem and root suffered a pronounced loss with increasing concentration of cadmium. The fact that both mercury and cadmium cause a decrease in protein content in general provides support to the trend observed by earlier workers in which a decrease was seen in protein content in response to metal application. There was a negative correlation between the increase in metal concentration and the decrease in protein levels both after 5 and 10 days of treatment to *Hydrilla* plant in response to both the metals. The changes in the content of protein are usually associated with the addition of kinetin in culture medium. Gupta et al.(2003) reported that protein content was increased by the application of cytokinin into the culture medium. Similar trend has been reported by Koshy et al.,(2001) in *Azolla filiculoides*. Narwal and Singh, (1993) observed an increase in amino acid content in Maize plants in response to cadmium however the same authors observed a decrease in the α – amino nitrogen content in response to the zinc. Chromium induced biochemical changes with reference to amino acids in the seedling of *phaseolus mungo* (Mahadeswarswamy and Theresa, 1992). Excess cellular concentrations of cadmium either inhibit the utilization of amino acids or promote protein hydrolysis, thus affecting the normal balance of cellular proteins (Tandon and Srivastava. 2004).

In the present investigation Cd treated seedlings also contained higher amounts of free amino acids as has been observed in different parts of plants under stressful conditions (Reddy and Vora, 1995; Dubey and Rani, 1989; Alia and Saradhi. 1991; Dubey and Pessarakli, 1995; Shah and Dubey, 1995). As far as effect of mercury and cadmium on amino acid content of *Hydrilla* plant is concerned, it can be said that both of them causing opposite effect in the plant after 5 and 10 days of interval. Addition of kinetin in both metals promotes the amino acid contents in some extent in *Hydrilla* plant after 5 and 10 days of intervals and this was supported by Gupta et al., (2003) and suggested that amino acid content showed an increasing trend in the test plant in response to mercury and cadmium with kinetin bearing a +ve correlation with the increasing concentrations of the metals. Greger and Johansson, (1992) observed that cadmium caused a diminished carbohydrate concentration in sugar beet (*Beta Vulgaris*), Malik et al., (1992) observed that increased accumulation of cadmium in leaves caused a reduction in carbohydrate reserve in wheat seedlings. Moya et al., (1993) reported an inhibition of transport of carbohydrate reserves from the rice seeds from which the rest of the plants were developing when

treated with cadmium. Narwal and Singh, (1993) noted that both cadmium and other metals decreased reducing, non-reducing and total sugars of maize grown in soil treated with the said metals. According to Satyakala and Jamil, (1997) *Pistia stratiotes I* plants when treated with 5-100 ppm of copper and cadmium solutions for 72h. showed decrease in sugar content. In the response of mercury and cadmium there was a steady decrease in the sugar content in *Hydrilla.*

Gupta et al., (2003) observed that treatment with kinetin promotes the sugar content in wheat genotypes. In the present investigation there was also increase in sugar content to some extent by the treatment of kinetin after 5 and 10 days of interval and showed a –ve correlation between sugar and metals with kinetin in *Hydrilla plant. The sugar level in Hydrilla* plant got elevated further when the metals (Hg and Cd) were supplemented with kinetin. The present investigation supporting the views of earlier workers as the DNA content decreased with the increase in mercury and cadmium concentration. Duan et al,(1992) observed an increase followed by decreasing trend in Vica faba in the content of RNA in response to Cadmium. Angadi et el ,(1996) observed that increase in RNA I response to cadmium and there was a decrease at higher concentration of the metal. However, De Filipps and Pallaghy (1976b) reported contradictory findings in Chlorella.

CONCLUSIONS

The plants show toxicity symptoms in response to heavy metal (Hg. and Cd,) but are seen to develop resistance to the toxicity when kinetin was given to them along with metals. The total chlorophyll content in the test plant showed a decrease in content with the treatment of $Hgcl_2$ and $CdSO_4$ but, when these metal compounds were supplemented with kinetin showed significant recovery in the chlorophyll content. It was also observed that, mercury pronounced the level of toxicity in comparison to cadmium. In bimolecular studies, Protein, Sugar and DNA content in the treated plant showed a decrease in contents wit and overall it was concentration dependant. In this case also $Hgcl_2$ with Kinetin and $CdSO_4$ with Kinetin reduced the toxicity of the metal to some extent in the plant. With reference to the days /periods of study, 10 days interval pronounced more stress conditions in the test plant, there by overall decline in the biochemical parameters.

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