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EFFECT OF MERCURY AND CADIMIUM ON THE ENZYME ACTIVITY OF HYDRILLA PLANT

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ABSTRACT

The study of toxicity of metals on the enzyme activity in the Hydrilla plant indicated that in general there is induction or inhibition in enzyme activity. The enzymes studied here in relation Hg and Cd toxicity like catalase, peroxidase and polyphenol oxidase found in the plant responded differently to the metal treatment. Catalase and polyphenol oxidase showed a decrease in the activity whereas peroxidase enzymes were stimulated by the metals. When the metals were treated along with growth hormone Kinetin, there was substantial recovery of the enzyme activity in the test plant. Other enzymes like acid phosphatase, acid/alkaline protease, acid /alkaline prophophatase and RNase were also studied to correlate the decrease in the molecular content in the test plant responded a decline in its activity with the metal treatment. However, the addition of Kinetin to the metals ($HgCl_2$ and $CdSo_4$) showed a recovery in the enzyme activity in the hydrilla plant .All these observations indicates that the aquatic plant hydrilla can be used as an indicator plant to assess the metal toxicity in the aquatic system.

KEY WORDS

Mercury, Cadimium, Enzymes, Hydrilla

INTRODUCTION

Life depends on a complex network of chemical reactions brought about by a specific enzyme. The enzymes are the primary instruments for the expression of gene action. Enzymes are of biological importance in metabolic function, which have the most highly specialized class of proteins. The change in metabolism can well be correlated with the disturbance in behavior and action of the enzymes. 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 nonfunctional 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.

Induction of catalase activity is brought about when toxic does of various metals are present (Van Assche and Clijsters, 1990). Taking into consideration the effect of the metal application, some workers have reported an induction of catalase in response to various heavy metals (Mukharji and Das Gupta, 1972; Subhadra et al., 1991). There are reports that cadmium decreased the activity of catalase, an antioxidant enzyme in germinating seedlings of mung bean *phaseolus vilgaris*. (Somasekharaiah et al., 1992). A decline

in catalase activity was also observed in rhy leaves when exposed to cadmium (Streh et al., 1993). Catalase activity increased significantly in *Posidonia oceanica* following 48h exposure to low concentrations of $HgCl_2$ (0.01 µg L^{-1} and 0.1 µg L^{-1}) as compared to controls (Ferrat et al., 2002b).

Increase in Peroxidase has been observed in *Glycine max* (Lee et al., 1976) and *Phaseolus Vulgaris* (Van Assche et al., 1988) in response to cadmium. Reddy and Prasad (1992) observed an increased peroxidase *Oryza sativa* treated with different concentrations of cadmium. Karataglis et al., (1991) reported that increasing concentrations of copper, zinc, lead, nickel, chromium caused complex changes in the forms of isoperxidases in the seedlings of wheat. In unicellular green alga *Scenedesmus subspicatus* Reinhold et al., (1994) observed observed a significant rise in peroxidase activity in response to cadmium. Patro et al.,(2001), reported that all concentrations of the effluents found to have strong effect on the activity of peroxidase in the leaves of *Oryza sativa L*. In *Ceratophyllum demersum* mercury induced oxidative stress increased anti-oxidant enzymes like peroxidase. Rama Devi and Prasad, 1998, Sandalio et al., 2001 Metwally, 2003, 2004; Gallego et al., 1996; Balestrasse et al., 2003). Mukherji and Maitra (1976) have demonstrated the stimulated activities of peroxidase under conditions of lead toxicity of growth of rice seedlings. Very low cadmium levels in vitro have shown to stimulate the activities of certain enzymes like peroxidase, acid phosphatase etc. (Ernst 1980, Shah and Dubey, 1997).

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^{-1} 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.(1,5,10.25mgL^{-1}) 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 amelioratine effects of growth regulators, Kintein (5mg^{-1}) on toxic effects were assessed by adding 2ml in each concentrations of Cd and Hg.

The enzymes like Catalase, Peroxydase, Poly Phenyl oxidase (Kar and Mishra, 1976), Acid Phosphatase (Sacher and De Leo, 1977) Acid/Alkaline Protease (Moore and Stein, 1948) were estimated following standard procedures.

RESULTS

The data pertaining to the effect of different concentrations of mercury and cadmium on the various enzyme activities and their percent increase/decrease was depicted in **Table No. 1 and 2**.The catalase enzyme activity was least effected and the highest percent decrease was only up to 5.28% in case of Hg and 7.69% in case of Cd.

The peroxydase enzyme was elevated with the increase in Hg and Cd treatment and highest %increase was seen in case of 25 mg Hg treatment for 10 days. The polyphenol oxidase enzyme showed a decrease in activity with the increase in metal concentration and the maximum decrease was seen in case of 25 mg Cd treatment for 10 days. The results obtained with enzymes like Peroxydase and Poly phenyl oxidase was statistically significant. The Acid Phosphatease enzyme showed an increase in activity with the increase in metal concentration but the trend was not statistically significant.The Acid protease activity showed a decreasing trend with the increase in metal concentration and the highest decrease (71.25%) was seen in case of 25 mg Hg treatment for 5 days.

DISCUSSION

The catalase activity decreased following exposure to elevated cadmium concentrations (Shim et al., 2003; Dalurzo et al., 1997; 2001; Fornazier et al., 2002) Romero – Puertas et al., (2004) studied the involvement of H_2O_2 and O_2 in the signaling events that lead to the variation of the transcript levels of catalase in Pea plants under cadmium stress. The activity of several enzymes like RNase, catalase etc., also decrease due to toxic action of chromium compounds (Panda et al., 1997).

In the present investigation catalase showed a decreased trend in response to increasing concentrations of mercury and cadmium. There was a –ve correlation between various concentrations of both metals and the enzyme activity in *Hydrilla* plant after 5 and 10 days of interval. Similar decreasing trend also been observed when treated with metals and kinetin. Compared to metals the activity of catalase is more expressed in presence of kinetin.

Peroxidase is present in grana and stroma lamellae (Henry, 1974, 1975.a.b. Henry et al., 1981.b) and thus may be closely associated with the changes in chloroplast including variation in chlorophyllII Pigment. Peroxidase is considered as a reliable indicator of various physiological processes. Some workers observed a marked change in peroxidase activity during root initiation and considered as an indicator of the process (Gasper et al., 1982, 1985; Moncousin 1986; Moncousin et al., (1987). There are also reports of other metals inducing peroxidase activity viz. mercury in *Oryza sativa*, mercury in *Lemna minor* (Subhadra et al.1991), choromium in *Eichhornia crassipes* (Satyakala and Jamil, 1993).

In the present investigation there was induction of peroxidase activity in *Hydrilla* plant in response to both mercury and cadmium after 5 to 10 days of treatment, though there were some differences in degree of induction. There was a +ve correlation between induction of peroxidase and metal concentration both for mercury and cadmium.

TABLE NO: 1: EFFECT OF DIFFERENT CONCENTRATIONS OF $HgCl_2$ AND $CdSO_4$ ON VARIOUS ENZYME ACTIVITIES OF HYDRILLA Sp. (EACH DATUM IS THE MEAN OF SIX REPLICATES) (Values in parentheses indicate the percent increase / decrease in content from control values)

Treatment	Metal Conc.	CATALASE In µ moles of H_2O_2 utilized / min-1 g-1 fresh weight / min-1		PEROXIDASE In absorbance (A) units		POLYPHENOL OXIDASE In absorbance (A) units		ACID PHOSPHATASE In absorbance (A) units		ACID PROTEASE In absorbance (A) units	
		5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
$HgCl_2$	Control	119.25	119.18	1.88	1.89	1.24	1.25	1.18	1.18	1.6	1.6
	1mg	118.23 (-0.855)	117.89 (-2.591)	2.02 (7.446)	2.01 (6.349)	1.09 (-12.096)	1.08 (-13.6)	1.03 (12.711)	1.01 (-14.406)	1.04 (-35)	1.03 (-35.625)
	5mg	116.16 (-2.591)	115.07 (-3.448)	3.04 (61.702)	3.01 (59.259)	1.02 (-17.741)	0.96 (-23.2)	2.09 (77.118)	2.06 (74.576)	1.01 (-36.875)	0.98 (-38.75)
	10mg	114.04 (-4.368)	113.98 (-4.363)	3.8 (106.914)	3.71 (96.296)	0.96 (-22.580)	0.90 (-28)	1.94 (64.406)	1.84 (55.932)	0.82 (-48.750)	0.99 (-51.25)
	25mg	113.05 (-5.199)	112.88 (-5.286)	4.12 (119.148)	4.08 (115.873)	0.80 (-35.483)	0.76 (-39.2)	2.48 (110.169)	2.46 (108.474)	0.46 (-71.250)	0.68 (-57.5)
	r Value	-0.893*	-0.858*	0.868*	0.889*	-0.924**	-0.888*	0.849*	0.853*	-0.868*	-0.767^{Ns}
$CdSO_4$	Control	119.24	119.19	1.89	1.88	1.24	1.25	1.18	1.18	1.6	1.6
	1mg	117.18 (-1.727)	117.18 (-1.686)	1.92 (1.587)	1.89 (0.531)	1.11 (-10.483)	1.09 (-12.8)	1.06 (-10.169)	1.02 (-13.559)	1.63 (1.875)	1.60 (0.00)
	5mg	115.14 (-3.438)	115.01 (-3.507)	2.11 (11.640)	1.90 (1.063)	0.96 (-22.580)	0.84 (-32.8)	2.06 (74.576)	2.03 (72.033)	1.48 (-7.5)	1.46 (-8.75)
	10mg	111.98 (-6.088)	111.08 (-6.804)	3.29 (74.074)	3.08 (63.829)	0.78 (-37.096)	0.72 (-42.4)	1.05 (-11.016)	1.03 (-12.711)	0.98 (-38.75)	0.96 (-46)
	25mg	110.46 (-7.363)	110.02 (-7.693)	4.06 (114.814)	4.01 (113.297)	0.45 (-63.709)	0.36 (-71.2)	2.20 (86.440)	2.11 (78.813)	0.88 (-45)	0.85 (-46.875)
	r Value	-0.900*	-0.883*	0.958**	0.962**	-0.977***	-0.957**	0.643^{Ns}	0.617^{Ns}	-0.897*	-0.902*

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

TABLE NO: 2: EFFECT OF DIFFERENT CONCENTRATIONS OF $HgCl_2$ WITH KINETIN AND $CdSO_4$ WITH KINETIN ON VARIOUS ENZYME ACTIVITIES OF HYDRILLA Sp. (EACH DATUM IS THE MEAN OF SIX REPLICATES)(Values in parentheses indicate the percent increase / decrease in content from control values)

Treatment	Metal Conc.	CATALASE In µ moles of H_2O_2 utilized / min-1 g-1 fresh weight / min-1		PEROXIDASE In absorbance (A) units		POLYPHENOL OXIDASE In absorbance (A) units		ACID PHOSPHATASE In absorbance (A) units		ACID PROTEASE In absorbance (A) units	
		5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
$HgCl_2$ Kinetin	Control	119.43	119.25	1.9	1.9	1.25	1.26	1.19	1.19	1.7	1.7
	1mg	118.63 (-0.669)	117.95 (-1.090)	2.12 (11.578)	2.05 (7.894)	1.11 (-11.2)	1.10 (-12.698)	1.06 (-10.169)	1.04 (12.605)	1.05 (-38.235)	1.04 (-380823)
	5mg	116.56 (-2.403)	115.87 (-2.834)	3.14 (65.263)	3.11 (63.684)	1.06 (-15.2)	1.02 (-19.047)	2.11 (78.813)	2.09 (75.630)	1.02 (-40)	1.01 (-40.588)
	10mg	117.34 (-1.749)	114.02 (-4.385)	3.96 (108.421)	3.92 (106.315)	0.98 (-21.6)	0.87 (-30.952)	1.98 (67.796)	1.88 (57.983)	0.89 (-47.647)	0.86 (-49.411)
	25mg	113.35 (-5.090)	113.12 (-5.140)	4.22 (122.105)	4.18 (120)	0.82 (-34.4)	0.79 (-37.301)	2.58 (118.644)	2.56 (115.126)	0.56 (-67.058)	0.54 (-68.235)
	r Value	-0.948**	-0.878*	0.870*	0.869*	-0.940**	-0.880*	0.898*	0.870*	-0.813*	-0.813*
$CdSO_4$ with Kinetin	Control	119.25	119.28	1.90	1.90	1.25	1.26	1.19	1.19	1.7	1.7
	1mg	117.58 (-1.400)	117.43 (-1.550)	1.95 (2.631)	1.93 (1.578)	1.13 (-9.6)	1.12 (-11.111)	1.08 (-9.243)	1.03 (-13.445)	1.65 (-2.941)	1.62 (-4.7.5)
	5mg	115.34 (-3.278)	115.11 (-3.495)	2.21 (16.315)	2.19 (15.263)	0.98 (-21.6)	0.88 (-30.158)	2.09 (75.630)	2.06 (73.109)	1.58 (-7.058)	1.56 (-8.235)
	10mg	112.63 (-5.551)	111.98 (-6.120)	2.12 (11.578)	3.28 (72.631)	0.82 (-934.4)	0.76 (-39.682)	1.11 (-6.722)	1.09 (-8.403)	1.08 (-36.470)	1.06 (-37.647)
	25mg	111.56 (-6.448)	110.76 (-7.142)	4.26 (124.210)	4.11 (116.315)	0.47 (-62.4)	0.38 (-69.841)	2.24 (88.235)	2.15 (80.672)	0.98 (-42.352)	0.95 (-44.117)
	r Value	-0.884*	-0.888*	0.951**	0.966**	-0.982***	-0.967**	0.660 Ns	0.636 Ns	-0.899*	-0.902*

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

In the present study it is observed that polyphenol oxidase activity was reduced in *Hydrilla* plant in response to mercury and cadmium after 5 to 10 days of interval. There was a close-ve correlation between metal concentration applied and the decrease in the activity of the enzyme in response to both the metals. Our investigation is contradictory to the views of Garg et al., (1994) that an increase in polyphenol oxidase activity in *Marsilea minuta* under cadmium induced stress.

Activity of polyphenol oxidase increased into a lot by the addition of kinetin with heavy metals and was observed in *Hydrilla* plant after 5 to 10 days of interval. As far as *Hydrilla* plant is concerned polyphenol oxidase can safely be used as an indicator of mercury and cadmium toxicity.

A rise in acid phosphate activity was observed in *Glycine max* in response tyo cadmium (Lee et al., 1976). *Medicago polymorpha* in response to drought stress. (Ehsanpour et al., 2003). *Trticum aestivum* in response to chromium (Sharma and Sharma, 1996). On the other hand an inhibition of acid phosphatase activity was observed by Kong and Chen, (1995) in response to Al and Zn, and Rajni et al., (1991) in response to Cu, Zn, Hg in potato tubers are available.

In *Hydrilla* at low concentrations of mercury and cadmium levels (5 mg L^{-1}) stimulate the activity of acid phosphatase. Whereas at higher concentrations of both metals (10mg L^{-1}) have inhibitory to thjis enzyme. Further increasing the concentrations of both metals (25mg L^{-1}) the activity of acid phosphatase again increases after 5 to 10 days of interval. This observations was supported by Ernst, (1980); Shah and Dubey,(1997); Kumar and Banerjii, (1992).

Similar trend was also observed when the test plant was both metals and kinetin. There was a +ve correlation between mercury and enzyme activity and mercury and kinetin and enzyme activity both after 5 to 10 days of treatment but a non-significant correlation was observed between the enzyme activity with cadmium and enzyme activity with cadmium and kinetin. The trends in acid phosphatase activity in response to metals shows that the enzyme cannot be used as a general indicator of metal toxicity as its responses are dependent on species and organ.

Sneh Lata(1989) on *Phaseolus aureus* had shown that cadmium influences the activity of protease which seem to inhibit the seedling growth. Cadmium inhibits protease activity in germinating pea seeds was reported by Bansal et al.,(2001). The protease enzyme inhibition with $HgCl_2$ was also observed in germinating mungbean seeds. (Yamaoka et al., 1990). Oats (Drivdhal and Thimann, 1978). Common bean (Rascusen and foote, 1970), Soybean (Ragster and Chrispeels, 1979) and Agave (Du Toit, 1976). In *Hydrilla* we report the presence of two sulfhydryl protease, one alkaline with pH optimum of 8.0 and the other a highly acidic one, with optimum pH of 3.0. Activity of acidic and alkaline protease were inhibited by the response of heavy metals in Kalachoe leaves. (Jasrai et al., 1992). In the present investigation an attempt is made to analyse the activity of both acidic protease and alkaline protease in *Hydrilla* plant. There was a significant decrease in the activity of both acidic and alkaline protease *Hydrilla* in response to both mercury (after 5 and 10 days) of interval. But a non-significant correlation was observed after 10 days of interval with mercury treatment.

In the present investigation the acid pyrophsphatase showed decreasing trend in response to both the metal and enzyme activity after 5 and 10 days interval. Similar trend exists when treated with metals and kinetin and showed non-significant correlation between the enzyme and metal with kinetin.

The alkaline pyrophosphatase enzyme also showed a decreased trend in response to both the

metals and there was a –ve correlation and enzyme activity after 5 days of treatment but after 10 days non-significant correlation exists between them. In response to metals and kinetin similar decreasing trend also observed in *Hydrilla* and showed a –ve correlation between enzyme activity and metal with kinetin after 5 days of treatment but the non-significant correlation was existing after 10 days of interval. Thus inorganic pyrophosphatase can be used as an indicator of metal toxicity response.

Change in synthesis or alterations in levels of RNase may be involved in the regulation of RNA content. To explore the second possibility RNase was included in the present investigation. There was a decline in the RNase activity in *Hydrilla* in response to both mercury and cadmium after 5 and 10 days of interval. A non-significant correlation exists between mercury and enzyme activity after subsequent days but a close –ve significant correlation exists between cadmium and enzyme acivity.

Induction of kinetin to the metals showed similar trend in *Hydrilla* plant and a non-significant correlation exists between enzyme activity and mercury with kinetin, but with cadmium and kinetin a significant close –ve correlation exist after 5 and 10 days of interval. Compared to only metal treatment RNase activity is more pronounced when treated with metal and kinetin *Hydrilla* after 5 and 10 days of interval. It may be concluded that RNase may be involved in the regulation of RNA content in *Hydrilla* in response to metals under study.

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