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Bioaccumulation of Mercury and its Effect on Protein Metabolism of the Water Hyacinth Weevil *Neochetina eichhornae* (Warner)

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Water hyacinth growth is kept under control by the weed eating weevils, Neochetina eichhornae (Warner) and N. bruchi (Hustache). Water hyacinth is generally considered an aquatic weed because it clogs waterways, although it has recently been evaluated as a potential biological filter for sewage effluents (Wolverton et al. 1978; Kaiser Jamil et al. 1987). Mercury and mercurial compounds became widely known as toxic substances only after their effects received world wide attention due to the repeated outbreak of the epidemic Minamata disease in Japan (Tsubaki 1977; Takeuchi et al. 1959) and in Iraq (Bakir et al. 1973). Recently, increasing concerns stem from the wide existence of mercury contamination (D'Itri 1972) and this has stimulated renewed interest in various facets of environmental pollution of mercury. Advances have been made in our knowledge of mercury contamination in the aquatic environment and its mode of interaction with various biological systems.

Water hyacinth plants are continuously subjected to contamination by mercury and other heavy metals from industrial effluents and fertilizer impurities (Wolverton and McDonald 1975; Kaiser Jamil et al. 1985; Muramato and Oki 1983). Little is known about the interaction of heavymetals/plants/insects. Kay and Haller (1986) studied the influence of heavy metals as Pb, Cd and Cu on feeding behaviour of the weevil N.eichhornae. In an earlier publication we reported that Cd, Zn and Mn altered the protein and nucleic acid content of the weevil N.eichhornae (Saber Hussain and Kaiser Jamil 1989). Enhanced reproductive activity of N.bruchi fed on water hyacinth plants grown in polluted waters was also reported by Kaiser Jamil and Jyothi (1988).

Aminotransferases are generally considered as indices for metabolic disturbances during Hg exposure. These enzymes are key enzymes for transamination, i.e., transfer of amino group from amino acid to keto acids which are ubiquitous in insects (Rockstein 1965). The objective of the present study was to examine the bioaccumulation of mercury through the food chain in Neochetina eichhornae and its impact on protein metabolism.

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MATERIALS AND METHODS

Water hyacinth Eichhornia crassibes (Mart) Solms plants were grown in a laboratory pond. From this culture the plants selected for experimental purposes were approximately of the same age, with the same number of leaves and similar root mass to ensure uniform absorption areas. Two plants were introduced into each of 2 l capacity beakers containing either 25, 50 or 75 ppm of mercuric chloride solution in 1 l of tap water. Plants were kept for one week in this solution in a greenhouse at 28 ± 2°C at 60-70% RH. Leaves from these Hg-treated plants were taken for metal analyses and were provided to the weevils as food for a period of one week. Insects released on plants without mercury treatment were used as controls. After one week of feeding the insects were taken for metal analyses and for estimation of biochemical parameters. All experiments were repeated 3 times often in triplicate each.

The plant leaves and insects were washed with distilled water twice, dried in separate containers at 80°C for 48 hours in an oven and ground to powder in a grinder. The samples were weighed and digested with a mixture (1:3) of concentrated H_2SO_4, concentrated HNO_3, and then oxidized with 6% potassium permanganate solution. After digestion the samples were filtered and analyzed for mercury content using an inductively coupled plasma-atomic emission spectrometer (ICP-AES).

The insects fed on Hg-treated leaves for one week were taken for biochemical measurements as follows: Protein estimation was done by Lowry's method (Lowry et al. 1951). Free amino acids were determined by using Ninhydrin reagent (Moore and Stein 1954). Alanine and asparate aminotransferase (AIAT and AAT) activity were estimated as described by Bergmeyer and Bernt (1965).

Data were subjected to analysis of variance (ANOVA). The data are expressed as the mean ± standard error. The level of statistical significance employed in all cases was $P < 0.01$.

RESULTS AND DISCUSSION

Accumulation of mercury in the various pathways of the trophic chain is presented in Table 1. After one week the uptake of Hg by the plants from the 25, 50 and 75 ppm concentrations was about 70-75%. At 25 ppm the concentration of Hg in leaves was 220.8 ug/100 mg, at 50 ppm 382.18 ug/100 mg and at 75 ppm it was 597.72 ug/100 mg. Thus, a concentration dependent accumulation of Hg in water hyacinth leaves was found over a period of one week.

The results obtained on bioaccumulation of Hg in insects are also presented in Table 1. At the 25 ppm concentration the total Hg accumulated was 15.16 ug/100 mg of leaves in male insects and 14.81 ug/100 mg in females. A similar pattern of bioaccumulation was observed at 50 and 75 ppm. In males the levels of Hg were slightly higher. However, the differences were not statistically significant. It was also observed that the feeding of insects declined as the mercury concentration increased in the plants. This avoidance mechanism of the insect to the toxicant (Hg) is a remarkable feature. This was evident from the mercury content

estimated from the insect bodies as seen in Table 1. With an increase in mercury concentrations from 25 to 75 ppm thre was a decrease (from 6.8% to 1.7%) in mercury content of the insect bodies; this was the result of less feeding by the insects (unpublished data).

Table 1. Concentration of mercury in leaves and insects after one week treatment

Parameters	Mercury concentrations			
	Control	25 ppm	50 ppm	75 ppm
Mercury concentrations in water after one week	0	6.23 ± 0.46	13.10 ± 0.29	23.67 ± 0.37
Mercury uptake by plants by difference (ppm)	0	18.77 (75%)	36.90 (73%)	51.30 (68%)
Mercury concentrations in leaves (ug/100 mg)	0	220.8 ±14.96	382.18 ±13.58	597.72 ±11.4
Mercury concentrations in insects (ug/100 mg)				
Males	0	15.16 ±0.81	14.37 ±0.48	10.12 ± 0.51
Females	0	14.81 ± 0.22	12.71 ± 0.37	9.67 ± 0.12
Percentage accumulated in insects by feeding on treated plants				
Males	0	6.8	3.6	1.7
Females	0	6.7	3.3	1.6

(ANOVA test carried out. $P < 0.01$ in all cases).

The level of total protein free amino acids and enzyme activity in control and mercury-exposed weevils are presented in Table 2.

These results indicated that the decrease in total protein was related to the concentration of metal absorbed by the insects. The changes were more significant at 25 ppm, which may be due to higher feeding rates as evidenced by their feeding marks on the plants. Feeding was found to decrease in the insects with increasing metal concentrations. The results indicate an increase in free amino acids in mercury contaminated weevils over the controls (Table 2). Two possible mechanisms for this could be predicted. Firstly, the amino acids are available in pyruvate via reaction involving TCA cycle into axaloacetate. Secondly, the presence of free amino acids indicates a possible role in their increase in the rate of gluconegenesis to act as a supplementary source of energy to meet the energy requirements under mercury stress. An increase in both the enzymes AIAT and AAT also indicates that there is more conversion of amino acids into ketoacids to be utilized for energy synthesis.

Table 2. Changes in biochemical parameters of Neochetina eichhornae (Warner) fed on mercury-treated plants

Parameters	Initial mercury concentration in water			
	Control	25 ppm	50 ppm	75 ppm
Total protein content (ug/100 mg)	3115.35 ± 71.80	2086.33 ± 93.68	2759.12 ± 36.83	2865.66 ± 35.11
Total free amino acids (ug/100 mg)	825.12 ± 25.10	985.75 ± 40.12	1210.12 ± 26.19	1250.15 ± 35.12
AIAT (u moles of pyruvate/formed/ mg/protein/hr)	12.55 ± 0.42	18.85 ± 0.25	16.17 ± 0.25	15.92 ± 0.54
AAT (u moles of pyruvate/formed/ mg/protein/hr)	10.25 ± 0.28	13.25 ± 0.31	12.21 ± 0.35	12.51 ± 0.41

(ANOVA test carried out. $P < 0.01$ in all cases)
AIAT = Alanine aminotransferase; AAT = Asparate aminotransferase

It is also evident from our results that the dearrangement in protein degradation is reflected by changes in protein composition that is protein metabolic perturbation during mercury stress. Knox and Greengard (1965) reported that aminotransferases served as a strategic link between carbohydrate and protein metabolism under environmental stress. The relatively higher AIAT and AAT actively demonstrate high adapatability of the insects to mercury toxicity. Since these enzymes are known to display the state of adaptability under stress conditions (Nichol and Rosen 1965), it is therefore concluded that heavy metals interfere in normal protein metabolism of the weevils. However, significant bioaccumulation and biochemical changes induced by heavy metals must be viewed with caution with respect to human health as some metal ions affect growth rate and the ability to assimilate food. The heavy metals that induce biochemical alterations through the food chain are poorly understood and need to be investigated more extensively.

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