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Studies on the Ability of Water Hyacinth (*Eichhornia crassipes*) to Bioconcentrate and Biomonitor Aquatic Mercury

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ABSTRACT

*Water hyacinth (*Eichhornia crassipes, *Mart solms) plants were employed to assess bioconcentration and genotoxicity of aquatic mercury. Plants were exposed to water contaminated with mercuric chloride (MC) or phenyl mercuric acetate (PMA) at 0·001 to 1·0 mg litre*$^{-1}$*, or mercury contaminated effluent from a chloralkali plant for various periods of 4 to 96 h. Root samples taken after 4, 8, 12, 24, 48, 72 and 96 h of exposure were analysed for bioconcentration of mercury spectrophotometrically, and the root meristems were fixed in aceto-ethanol for cytological analysis to determine the frequencies of cells with micronuclei (MNC). Ethyl methane sulfonate and tap water served as positive and negative controls, respectively. The results indicated that bioconcentration of mercury in root tissue was both time- and concentration-dependent, providing evidence that water hyacinth is a good absorbant of aquatic mercury. The frequency of root meristematic cells with MNC followed a concentration-response. The findings indicate the potential of water hyacinth plants for* in situ *monitoring and for mitigation of aquatic mercury pollution.*

INTRODUCTION

Industrial wastes and discharges have been recognised as one of the major sources of toxic chemicals present in the environment. Whereas it is almost

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impossible to separate industrial growth from the ensuing environmental pollution, the latter can be minimised through cost effective approaches of environmental management consisting of three basic components: pollution identification, pollution assessment and pollution control. Water hyacinth, *Eichhornia crassipes* (Mart solms), a prolific aquatic weed of world wide distribution, has shown some promise in the biological management of aquatic pollutants because of the plant's ability to absorb and concentrate certain chemicals, including heavy metals (Wolverton & McDonald, 1979). Furthermore, it has been recently shown that this plant may be used as a bioassay to monitor low levels of aquatic cadmium (Rosas *et al.*, 1984) and mercury (Panda *et al.*, 1988) on the basis of induction of micronuclei (MNC) in the root meristematic cells, a genotoxic endpoint. With a view to exploit this plant for environmental management and control of mercury pollution, the objectives of this present paper are to assess the ability, as well as limitation, of water hyacinth plants to bioconcentrate mercury present in different forms, organic and inorganic; and to determine whether the frequencies of root meristematic cells with MNC are correlated with the levels of root mercury (bioconcentrated mercury) and aquatic mercury.

MATERIAL AND METHODS

Free floating plants of water hyacinth were obtained from a local unpolluted pond near Berhampur. The plants were thoroughly washed and maintained in culture, in cement tanks, 92 cm long, 53 cm wide and 28 cm deep, in the garden of the department for at least a week before the experiments. Experiments were conducted in the garden under natural conditions, 13/11 h light/dark cycle and $32 \pm 2^{\circ}C$ temperature, using young plants of uniform size having a good number of healthy roots. For each exposure, eight to ten plants were placed in plastic buckets, diameter 24 cm and depth 24 cm, containing 8 litres of the experimental solutions. Prior to use, the buckets were washed overnight with 10% HNO_3. Aqueous solutions of chemicals in tap water (pH 7) were used in the experiments. Ethyl methanesulfonate (EMS, Sigma, USA) was used as the positive control. Two mercury compounds; namely, mercuric chloride (MC, Loba-Chemie, Bombay) and phenyl mercuric acetate (PMA, Merck, West Germany), and mercury contaminated effluent obtained from the chloralkali plant (M/s. Jayashri Chemicals Pvt. Ltd, Ganjam, Orissa) were tested. The characteristics of the effluent were (μg at litre^{-1}): dissolved oxygen 1·653 mg litre^{-1}, NO_3-N 0·094, NO_2-N 0·789, PO_4-P 10·578, silicate 141·27, salinity 22‰ and pH 7·5. This effluent, containing mercury concentration 1 mg litre^{-1}, was toxic to water

hyacinth plants and therefore was diluted with tap water for experimental use. The plants were exposed to tap water, EMS (0·0001–0·005 mg litre^{-1}), MC (0·0003–0·05 mg litre^{-1}), PMA (0·0005–0·03 mg litre^{-1}) or mercury contaminated diluted effluent (0·001–0·13 mg litre^{-1}) for 4–96 h. The figures in parentheses, with the exception of EMS, represent the range of mercury concentration of experimental solutions as determined by cold vapour atomic absorption spectrophotometry, as described in the next section. Experiments were conducted in three replications with a change of experimental solutions after every 24 h. The roots were removed from plants at 4, 8, 12, 24, 48, 72 and 96 h of exposure and dried at 60°C to constant weight for analysis of total mercury. Further, batches of root meristems (10 to 15) excised at the above exposure times were fixed in acetic acid: ethanol, 1:3, and preserved in 70% ethanol at 4°C for cytological analysis.

Mercury analysis

Analysis of total mercury was carried out following the procedure of Environmental Protection Agency (1976) with some modifications. Samples of experimental solutions were digested in H_2SO_4:HNO_3 (2:1 v/v) in BOD bottles at 95°C in a water bath, then oxidised with potassium permanganate and potassium persulfate. The dried root samples were first wet digested at room temperature in concentrated perchloric acid:HNO_3 (1:4 v/v) for 12–24 h until dissolution of the root samples occurred. This was followed by gentle heating in a Betheges apparatus, until all brown nitrogen dioxide fumes were driven from the solution, which indicates completion of digestion. This procedure avoids losses of mercury by volatilisation (Bull *et al.*, 1981). The root digests were oxidised by potassium permanganate. Total mercury of the digests was measured by cold vapour atomic absorption spectrophotometry after reduction with 20% $SnCl_2$, using a Mercury Analyser (MA 5800D, ECIL, India) with a detection limit of 0·02 μg. Prior to analysis, the instrument was tested for non-specific absorption. For standards ($HgCl_2$) taken at 0·02 and 0·04 ppm, the analysis gave standard deviations of $\pm 8\%$ and $\pm 5\%$, respectively, when 12 subsamples from the same solutions were analysed for mercury. The precision of analysis, expressed as the coefficient of variance (*cv*) of replicate analyses, was 1·9%.

Cytological analysis

The root meristems were processed for cytological analysis following a haematoxylin schedule (Darlington & LaCour, 1976). The temporary slides were examined under a microscope and representative observations

Fig. 1. (A) A control population of root meristematic cells of water hyacinth; (B) metaphase chromosomes and (C) cell with a micronucleus (MNC).

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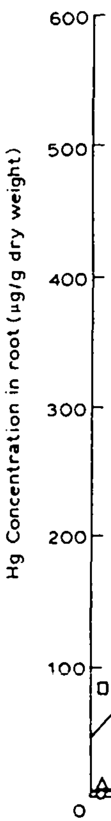

Fig. 2. Biocon
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were recorded using a Zeiss standard photomicroscope. Water hyacinth, with its many small chromosomes ($2n = 32$), was found unsuitable for analysis of chromosome aberrations or for sister chromatid exchange (Fig. 1(B)). Instead, the cytological endpoint scored was the frequency of interphase cells with micronuclei, MNC (Fig. 1(C)); the procedure has been standardised for water hyacinth (Panda *et al.*, 1988). The cytological slides were read blind and at least 5000 cells from eight to ten root meristems at each point were examined for the purpose.

Statistical analysis

The data on cells with MNC were tested statistically to determine the levels of significance, using the table of Kastenbaum and Bowman (1970). Analysis

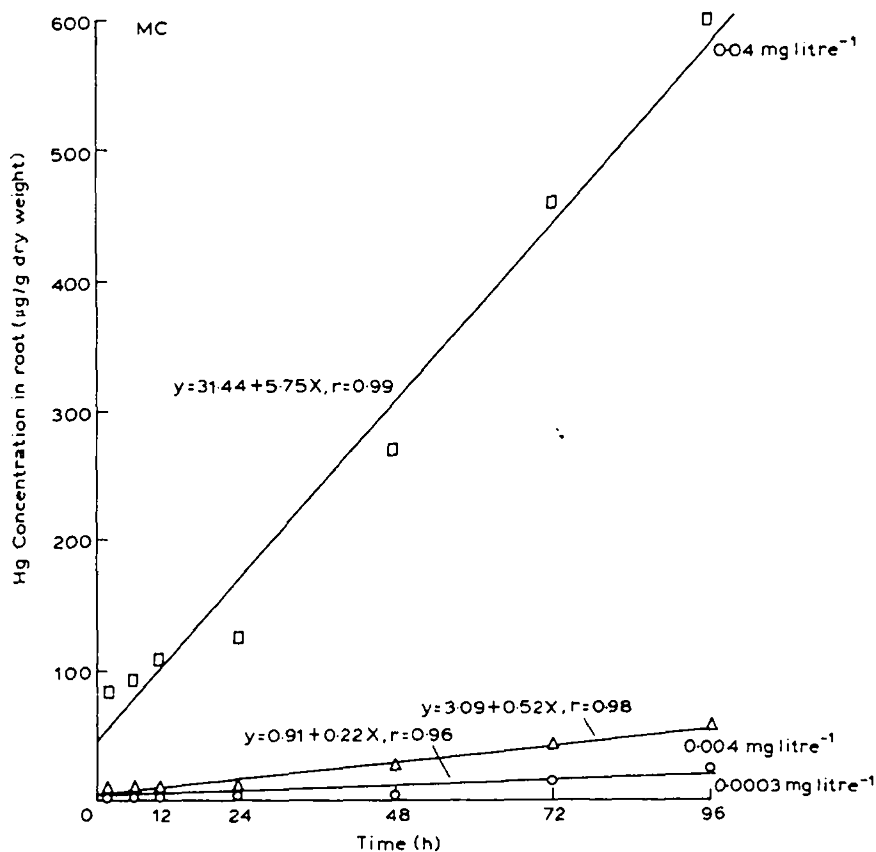

Fig. 2. Bioconcentration of Hg in root tissue as a function of concentration of Hg in experimental solution and exposure time, the source of the metal being MC.

of regression, coefficients of variance and squares of the correlation coefficient were obtained following standard methods (Gomez & Gomez, 1984).

RESULTS

The results indicate a time- and concentration-dependent increase of bioconcentrated mercury in the root tissue of water hyacinth plants (Figs 2 to 4). Regression analysis further indicated that the correlation between mercury concentration in the experimental solution and that of the root tissue was highly significant ($r = 0{\cdot}99$, $P \leq 0{\cdot}01$). For the sake of brevity the data on MNC at 96 h exposure only are presented (Table 1). In general, the induction of MNC followed a concentration-response with respect to both

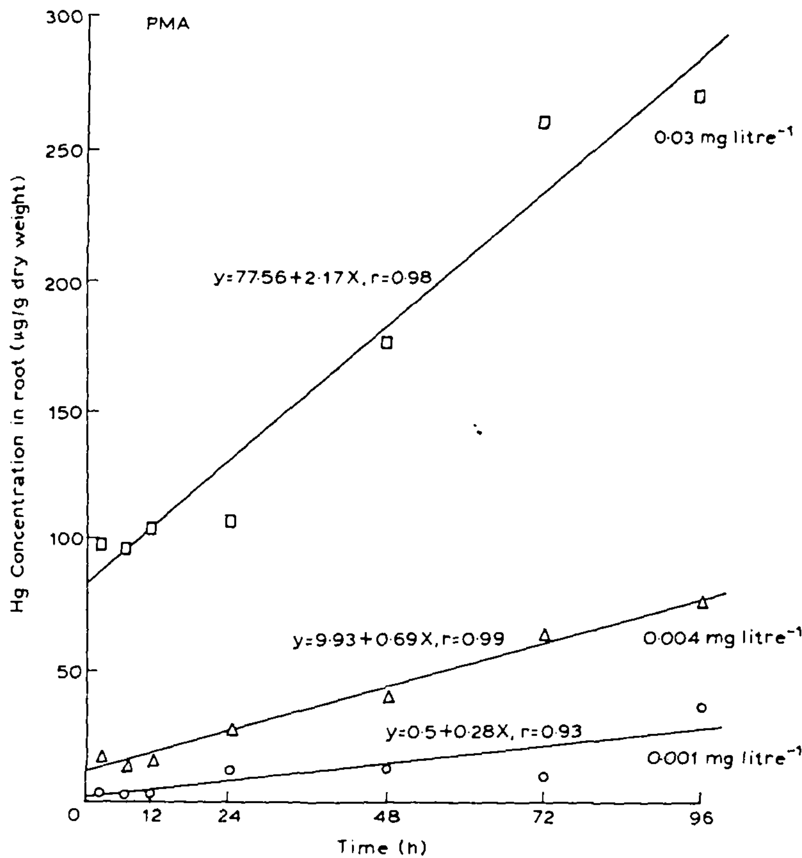

Fig. 3. Bioconcentration of Hg in root tissue as a function of concentration of Hg in experimental solution and exposure time, the source of the metal being PMA.

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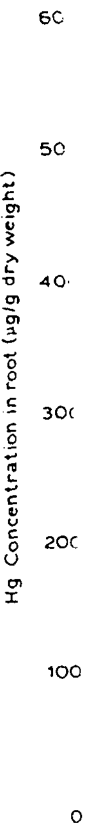

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mercury in experimental solution and bioconcentrated mercury in root tissue. The squares of coefficient of correlation are presented in Table 2. EMS induced MNC at a statistically significant level at all the concentrations tested ($r = 0{\cdot}77$, $P \leq 0{\cdot}01$). MC induced MNC at significant levels at 0·007 and 0·04 mg litre^{-1} and PMA at 0·001 and 0·004 mg litre^{-1}. At higher concentrations both of the mercury compounds were cytotoxic. The effluent significantly induced MNC at 0·005 and 0·075 mg litre^{-1} concentrations of mercury. The effluent was found to be toxic at a mercury concentration of 0·13 mg litre^{-1}. Table 3 summarises the threshold assessment values for MC, PMA and effluent mercury, such as highest ineffective concentration tested (HICT), lowest effective concentration tested (LECT) and gross toxicity concentrations tested (GTCT) with respect to the levels of mercury concentration in experimental solution, bioconcentrated

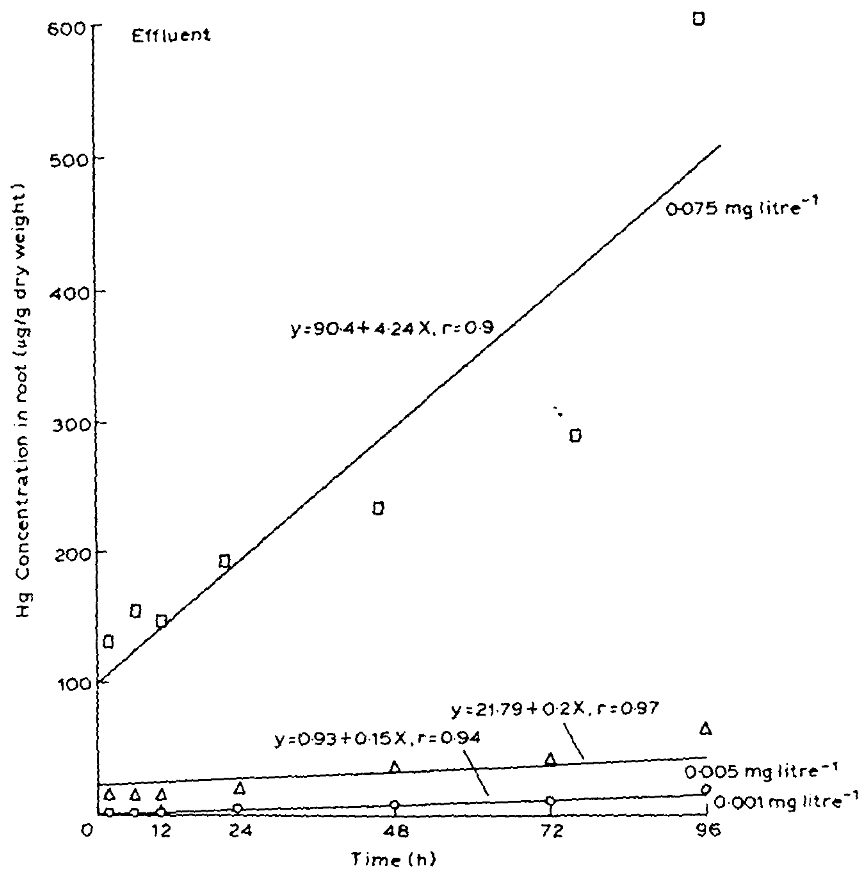

Fig. 4. Bioconcentration of Hg in root tissue as a function of concentration of Hg in experimental solution and exposure time, the source of the metal being the effluent from the chloralkali plant.

TABLE 1

Bioconcentration of Mercury in Root and Frequency of Cells with MNC in the Root Meristems in Water Hyacinth Following 96 h of Continuous Exposure to Water Contaminated with EMS, MC, PMA or Effluent

Chemical	*Concentration (mg litre⁻¹)*	*Root Hg (dw) (μg g⁻¹ ± SD)*	*Total cells scored*	*MI*	*Cells with MNC*	*MNC/1 000 cells*
EMS	0 (control)		6 565		1	0·15
	0·000 1		6 645		12	1·8*
	0·000 5		6 612		22	3·33**
	0·001		7 480		40	5·34**
	0·005		6 577		40	6·08**
MC	0 (control)	2·69 ± 0·58	7 151	8·73	3	0·58
	0·000 3	24·1 ± 0·64	8 015	6·58	3	0·37
	0·004	54·76 ± 14·67	5 611	5·55	3	0·53
	0·007	76·77 ± 7·22	4 207	3·8	10	2·38*
	0·04	600·00 ± 20·2	5 097	2·2	23	4·51**
	0·05	960·18 ± 87·59	Toxic[a]	—	—	—
PMA	0 (control)	3·70 ± 0·89	6 816	7·22	2	0·29
	0·000 5	2·62 ± 0·95	7 814	3·94	5	0·63
	0·001	14·47 ± 2·57	6 989	4·02	12	1·72*
	0·004	75·76 ± 8·45	8 083	3·11	21	2·6**
	0·03	272·16 ± 9·8	Toxic	—	—	—
Effluent	0·000 2 (control)	2·46 ± 0·36	8 420	5·64	0	0
	0·001	18·38 ± 2·11	6 754	8·87	1	0·14
	0·005	64·47 ± 2·65	7 432	7·32	7	0·94*
	0·075	606·67 ± 46·18	6 407	7·77	17	2·65**
	0·13	946·2 ± 15·3	Toxic	—	—	—

Significantly different from control at 0·05 (*) and 0·01 (**) levels; MI: mitotic index.
[a] Toxicity was evident by nuclear pycnosis and difficulty in maceration of root meristems.

TABLE 2

The Squares of Correlation Coefficients (r^2) between the Frequencies of MNC and Aquatic Hg/Root Hg and Between Root Hg and Aquatic Hg, Calculated after 96 h of Exposure of Water Hyacinth to MC, PMA and Effluent

Name of the test chemical		*Aquatic Hg*	*Root Hg*
MC	MNC	0·71**	0·65**
	Root Hg	0·98**	—
PMA	MNC	0·84**	0·69**
	Root Hg	0·99**	—
Effluent	MNC	0·95**	0·95**
	Root Hg	0·99**	—

'r' is significant at 0·05 (*) and 0·01 (**) levels.

TABLE 3
Threshold Assessment Values for Mercury in Water Hyacinth: Lowest Effective Concentration Tested (LECT). Highest Ineffective Concentration Tested (HICT) for Induction of MNC and Gross Toxicity Concentration Tested (GTCT) as Indicated by Cytotoxic Symptoms as Evident from Nuclear Pycnosis and Difficulty in Maceration of Root Meristems

Name of the chemical	*LECT*		*HICT*		*GTCT*	
	Water (*mg litre*$^{-1}$)	*Root Hg* (*dry wt*) ($\mu g\ g^{-1}$)	*Water* (*mg litre*$^{-1}$)	*Root Hg* (*dry wt*) ($\mu g\ g^{-1}$)	*Water* (*mg litre*$^{-1}$)	*Root Hg* (*dry wt*) ($\mu g\ g^{-1}$)
MC	0·007	76·77	0·004	54·76	0·05	960·18
PMA	0·001	14·47	0·000 5	2·62	0·03	272·16
MMC*	0·001	—	0·000 5	—	0·05	—
Effluent	0·005	64·47	0·001	8·38	0·13	946·2

* MMC: methyl mercuric chloride (data adopted from Panda *et al.*, 1988).

mercury in root tissue and induction of MNC in root meristematic cells at significant levels. By all counts the order of toxicity to water hyacinth was PMA > effluent > MC.

DISCUSSION

Water hyacinth is known to absorb and concentrate a variety of metals which include lead, cadmium, mercury, copper, nickel etc. (Wolverton & McDonald, 1978; Muramoto & Oki, 1983; Lee & Hardy, 1987). The ability of water hyacinth to bioconcentrate metals in its roots has been attributed to the occurrence of certain metal binding complexes (Fujita & Kawanishi, 1986). The present results have also shown that water hyacinth is a good absorber and accumulator of mercury, irrespective of the species, inorganic and organic. In view of its possible practical utility, the plant's ability to absorb mercury from the industrial effluent is noteworthy. With the knowledge that water hyacinth can withstand a wide range of pH 5–8 (Jamil *et al.*, 1987) and salinity up to 6‰ (Muramoto & Oki, 1988) it seems possible that the plants might be put to use for removal of mercury from industrial effluents.

The present study further underlines the utility of water hyacinth to biomonitor aquatic mercury. Heavy metals, such as cadmium and mercury, are known to induce MNC in the root meristematic cells of water hyacinth through impairment of spindle function in mitosis (Rosas *et al.*, 1984; Panda *et al.*, 1988). The use of the MNC assay as a genotoxic endpoint has been

suggested for biomonitoring purposes (Panda *et al.*, 1989). The frequencies of cells with MNC in the root meristems of water hyacinth, which were concentration-dependent, showed correlations at significant levels between aquatic and root-bioconcentrated mercury (Table 2). The threshold assessment values determined on the basis of induction of MNC (Table 3) are indicative of the relative toxicities of mercury tested in different forms. Of these, the LECT values, such as 0·007, 0·001 and 0·005 mg litre^{-1}, indicate the detection limits of water hyacinth MNC assay for inorganic, organic and effluent mercury, respectively, It is of interest to note that the effluent mercury (species not known) behaved somewhat differently to either MC or PMA. This difference may be attributed to some of the physico-chemical factors associated with the industrial effluent, which warrant further investigation. The family of curves plotted for bioconcentration of mercury in root versus time (Figs 2 to 4) can be useful, particularly, to monitor lower levels of aquatic mercury which might escape detection by the MNC assay. The present experiments thus provide evidence that water hyacinth can not only bioconcentrate but also biomonitor aquatic mercury, and therefore can play a useful role in environmental management of mercury pollution.

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