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Accumulation of Mercury by *Azolla* and Its Effect on Growth

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Although mercury and mercurial compounds had been known as toxic substances, the hazards caused by them received world-wide recognition after the outbreak of the Minamata Bay disease in Japan. Since then a lot of research has been done to discover the sources of mercury in the environment and the modes of interaction of mercury and mercurial compounds with various organisms. Jensen and Jernelov (1969), and Hamdy and Wheeler (1978) reported interaction of mercury with some sedimental bacteria in river beds and inhibition of the growth of bacteria by mercury, respectively. The effect of mercury on algae, which constitutes the base of the aquatic food chain, has also been highlighted (Hannan et al 1973; Mishra et al 1985). Agarwal and Kumar (1979) reported the toxicological effect of mercurial effluents on algae. Of the various industrial set-ups polluting the environment with mercury, chloralkali factories are the most important. Wallin (1976) reported the presence of mercury in the exhaust air, and the presence of a large amount of mercury has also been reported in the solid waste of chloralkali factories (Mishra et al 1985). The loss of mercury from the culture medium through microbial activities under laboratory conditions has been established (Lenda 1978; Ben-Bassat and Mayer 1975; Ben-Bassat and Mayer 1978; Rogers and James 1979). The present investigation deals with the effect of mercury contaminated solid wastes of chloralkali factories on the growth of Azolla (a water-weed) and loss of mercury from the medium under field conditions following its culture. It was noticed that the discharge of chloralkali greatly affected the local vegetation including cultivated cereals. An attempt was, therefore, made to reclaim the waste soil with Azolla.

MATERIALS AND METHODS

Mercury-contaminated, brine-soild wastes of a chloralkali plant, (M/s Jayashree Chemicals, Ganjam, Orissa) which are periodically removed and dumped near the effluent channel were collected in gunny bags and air-dried under field conditions for two days prior to analysis.

The pH of the waste soil was measured as 9.2 ± 0.05. The mercury content analysed by cold vapour atomic absorption technique using Mercury Analyser, Model No. MA - 5800A supplied by Electronics Corporation of India, Hyderabad was estimated to be 0.95 ± 0.03 g kg^{-1} of soil. The solid waste contained Na^+ 6g kg^{-1}, Cl-18 g kg^{-1}, k^+ 55 mg kg^{-1} and Po_4^- 60 mg kg^{-1}.

Solid waste and dry garden soil (pH 6.11 ± 0.25) were mixed throughly to a total weight of 4 kg per pot. The mixture concentrations ranged from 5% to 30% (W/W). An equal amount of water was added to all pots (soil:water = 1:2), mixed thoroughly and allowed to settle. The pots were kept in field in the month of December and January. The medium in the pots showed alkaline condition (pH 8.52 to 8.72). The water levels in the pots were maintained upto the mark. Nitrogen fixing water-weed Azolla pinnata previously cultured in trays, were removed, washed throughly, and inoculated at the rate of 15 g per pot. Sets with different combinations of the waste were inoculated at the same rate. A control set containing garden soil only was maintained for comparison. Effect of the waste on growth and uptake of mercury by Azolla and loss of mercury from the medium were studied at different time intervals of 30 and 60 days.

Growth of Azolla was monitored following measurement of fresh weight of the plant on 30 and 60 days inoculation periods. Mercury content of both Azolla and soil were determined in different sampling periods. Growth and uptake of mercury by Azolla and residual mercury levels were statistically analysed (Misra and Misra 1983). Difference from control was expressed in terms of level of significance (p) (Table IV).

RESULTS AND DISCUSSION

Mercury accumulation by Azolla from the culture medium was found both concentration and time dependent (Table 1). Mercury accumulation by Azolla increased with increasing concentration of solid waste and time. In 30 days inoculation period, uptake of mercury ranged from 2.7 ± 0.3 µg to 14.3 ± 1.5 µg g^{-1} fresh weight of Azolla grown on 5% to 30% waste soil whereas in 60 days inoculation period it ranged from 4.8 ± 1.0 µg to 18.3 ± 2.4 µg g^{-1} fresh weight. A highly significant correlation ($p < 0.001$) was obtained between concentration and mercury uptake (Table 4).

Growth of Azolla measured as fresh weight per pot decreased from 92.3 ± 11.2 g pot^{-1} in control set to 17.7 ± 2.1 g pot^{-1} in 30% waste soil during 30 days inoculation period (Table 2). At 60 days inoculation period also a decrease in growth from 150.0 ± 9.2 g pot^{-1} in control to 30.0 ± 5.9 g pot^{-1} in 30% waste soil was marked. The correlation coefficient value between waste concentration and fresh weight

of Azolla for both 30 and 60 days inoculation period were significant at $p < 0.5$ only (Table 4). Correlations between mercury uptake by Azolla and fresh weights were however highly significant ($p < 0.001$).

Table 1. Uptake of mercury in μg g-1 fresh weight of Azolla cultured in different concentrations of solid waste at different time intervals.

Days of culture	% concentration of the waste soil					
	5	10	15	20	25	30
30 days	2.7 ±0.3	4.8 ±0.8	7.5 ±1.0	9.8 ±1.1	11.7 ±1.0	14.3 ±1.5
60 days	4.8 ±1.0	7.0 ±1.3	10.2 ±1.2	12.0 ±1.7	15.4 ±2.0	18.3 ±2.4

Values are mean of 3 samples ± standard deviation

Table 2. Changes in growth of Azolla measured as fresh weight per pot culture after exposure to different concentration of the solid waste for periods of 30 days and 60 days.

Days of culture	% concentration of the solid waste						
	0	5	10	15	20	25	30
30 days	92.3 ±11.2	38.7 ±2.3	35.3 ±6.4	32.0 ±7.6	26.0 ±5.6	21.0 ±1.0	17.7 ±2.1
60 days	150.0 ±9.2	62.0 ±5.5	55.7 ±11.9	45.7 ±4.9	39.7 ±3.8	34.3 ±2.5	30.0 ±5.9

Values are mean of 3 samples ± standard deviation

One of the important changes observed in the waste soil combination following culture of Azolla was loss of mercury from the culture pot. Since waste soil contains 950 mg of mercury kg-1, mercury contents were respectively 19, 38, 57, 76, 95 and 114 mg pot-1 in 5%, 10%, 15%, 20%, 25% and 30% combinations (Table 3). Mercury taken up by Azolla from the culture pots ranged from 0.1 mg pot-1 to 0.3 mg pot-1 in 30 days and 0.3 mg pot-1 to 0.55 mg pot-1 in 60 days inoculation periods. Residual mercury levels of the soil after inoculation of Azolla for 60 days were estimated to be 12.1 mg to 51.9 mg pot-1 in 5% to 30% waste soil combinations, respectively. So, the unaccounted mercury from the culture

pot ranged from 6.6 mg pot-1 in 5% to 61.55 mg pot-1 in 30% waste soil combination and the total loss of mercury, along with those taken up by Azolla ranged from 6.9 mg pot-1 to 62.1 in 5% to 30% combinations of the waste soil (Table 3). Percent loss mercury from the waste soil combinations were 36.31% in 5% to 54.47% in 30% waste soil combination. A highly significant correlation ($p < 0.001$) was obtained between intial mercury content in pots with total loss of mercury. The correlation between percent loss of mercury and waste soil was highly significant ($p < 0.001$) (Table 4).

Table 3. Loss of mercury mg pot-1 from waste soil concentrations following culture of Azolla for 60 days.

Variables	% of waste soil concentrations					
	5	10	15	20	25	30
Total Hg present (mg pot-1 in soil)	19.0	38.0	57.0	76.0	95.0	114.0
Residual Hg in soil after culture of Azolla for 60 days (mg pot-1)	12.1	22.4	32.3	39.8	47.0	51.9
Total loss of Hg (mg pot-1)	6.9	15.6	24.7	36.2	48.0	62.1
Hg taken up by Azolla (mg pot-1) 30 days	0.1	0.18	0.23	0.24	0.25	0.3
Hg taken up by Azolla (mg pot-1) 60 days	0.30	0.4	0.47	0.5	0.53	0.55
Direct loss from the soil (mg pot-1)	6.6	15.2	24.23	35.7	47.47	61.55
% Loss of Hg	36.31	41.05	43.33	47.63	50.53	54.47

Table 4. Bivariate correlation coefficient values.

Variables	Correlation coefficient 'r'	Degrees of freedom	Significance trend p <
Concentration Vs mercury uptake by Azolla	a = + 0.999	5	0.001
	b = + 0.999	5	0.001
Concentration Vs growth	a = - 0.829	6	0.001
	b = - 0.803	6	0.05
Mercury uptake Vs growth	a = - 0.993	5	0.001
	b = - 0.991	5	0.001

Table 4. contd.

Variables	Correlation coefficient 'r'	Degrees of freedom	Significance trend p <
Mercury content Vs loss of mercury	+ 0.996	5	0.001
Percent waste soil Vs percent loss of mercury	+ 0.988	5	0.001

a. Inoculation period for 30 days
b. Inoculation period for 60 days

The effects of the toxicity of mercury on producer organisms and its subsequent transfer in the trophic chain, have been a matter of great concern to ecologists. When subjected to a mercurial habitat, concentration and time-dependent accumulation of mercury by algae has been reported (Mishra et al 1985). Wallin (1976) reported the accumulation also by moss of the mercury of industrial origin. The fact, that heavy metals and mercury were found in the grain and straw of cereals cultivated in contaminated soil (Dudas and Pawluk 1977; Kelly et al 1979), indicates that various plants have the ability to accumulate mercury. Siegel et al (1977) further reported selectivity in mercury-uptake by plants. It is reported here that the uptake of mercury by Azolla depends both on time and concentration of the element in the medium.

One of the important changes following the culture of Azolla was the loss of mercury from the mercurial waste soil. After 60 days of culture (12.1 mg pot-1 to 51.9 mg pot-1, for 5% to 30% concentrations), the total loss of mercury along with that accumulated by Azolla ranged from 36.31% in 5% waste soil combination to 54.47% in 30%. Ben-Bassat and Mayer (1975) reported significant loss of mercury from the culture medium containing Chlorella, which was enhanced with illumination (Ben-Bassat and Mayer 1978). However, at the present stage of investigation it is difficult to assess the definite role played by Azolla in the loss of mercury, although a significant and positive correlation was found between concentration of the waste soil and mercury uptake and mercury content and loss of mercury (Table 4).

Besides mercury accumulation, a significant decline in the growth of Azolla has been observed in culture pots containing waste soil. Growth declined with increase in concentration and exposure period. In support of this result, Kelly et al (1979) and Miles and Parker (1979) have reported retardation in the growth of plant species. Increasing concentration of Cd and Hg causes decrease in the growth of bacteria (Hamdy and Wheeler 1978). Further, heavy metals including mercury

have a retrograde effect on the growth of the plants. From the highly significant correlations between mercury uptake and growth Azolla, it can be inferred that mercury plays an important role in growth retardation of the waterweed. It is pertinent to note that mercury is converted into a volatile form by the direct or indirect action of Azolla. This could be attributed to either a humic acid mediated abiotic process as shown by Alberts et al (1974) or a microbial activity stimulated by substances that Azolla releases to the culture medium. In addition, mercury taken up by Azolla may be converted into a volatile form.

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