Indian J. Fish., 37 (2) : 163 - 165 (1990)

VOLATILIZATION OF INORGANIC MERCURY BY *ISOCHRYSIS GALBANA* PARKE FROM AQUATIC SYSTEM

S. V. ALAVANDI*, P. KALADHARAN AND V. K. PILLAI
Central Marine Fisheries Research Institute, Cochin 682 031, India

ABSTRACT

The loss of mercury added extraneously as an inorganic source to the algal cultures (*Isochrysis* sp.) and to the seawater containing *in situ* phytoplankton populations off Cochin was determined quantitatively. Such loss of mercury was attributed to uptake as well as volatilisation of mercury (Hg^0) by the activity of phytoplankton and micro - organisms. The rate of removal of mercury in the culture system was 54% per day and that by *in situ* phytoplankton was 38%.

Mercury gained its importance as a major hazardous pollutant since the Minamata Bay incident. Mercury is known to get transformed through biological or nonbiological means into toxic organic forms like methyl and dimethyl mercury (Brinckman and Iverson, 1975; Wood, 1974) or into volatile forms. Volatilization of mercury involves formation of Hg^0, which is less toxic than the other forms of mercury, a process termed as detoxification (Fenchel and Blackburn, 1979). Mercury resistant micro - organisms isolated from various environments have been found to transform mercury compounds into elemental (Hg^0) mercury (Magos *et al.*, 1964; Wood, 1974). Volatilization of organic and inorganic forms of mercury mediated by bacteria has been extensively reported (Bhattacharya and Mandal, 1989; Nakamura *et al.*, 1988; Nakamura, 1989; Nelson *et al.*, 1973; Sprangler *et al.*, 1973; Summers and Lewis, 1973). Effect of mercury on the phytoplankton has been exhaustively studied (Davies, 1978; Nuzzy, 1972). Algae (Ben-Bassat and Mayer, 1975) and Dunaliella tertiolecta (Betz, 1977) are also known to volatilize mercury.

Here we report volatilization of inorganic mercury by (1) axenic culture of *Isochrysis galbana* Parke, a unicellular marine microalga and (2) *in situ* population of phytoplankton and micro - organisms in the natural sea water.

Pure culture of *Isochrysis galbana* Parke was maintained in filtered sea water (through GF/C glass fibre filter, 0.45 µm pore size), supplemented with Miquells solution (Sol. A : 20.2 g KNO_3 in 100 ml distilled water and Sol. B : 4 g $CaCl_2$, 2 g $FeCl_2$, 4 g $NaHPO_4$, 2 ml conc. HCl in 100 ml distilled water; 0.55 ml and 0.5 ml each of solutions A and B respectively were added to one litre sea water) and 10 µg/l each of Biotin and Vitamin B-12. The pH was adjusted to 7.8 and salinity to 32-33 ppt. The culture was grown under 15,000 lux 12/12 light/dark regime at 30°C (Joseph, 1983). Growth of the culture was recorded by haemocytometer. Lc_{50} of mercury for *Isochrysis galbana* Parke was 25 ppb (Kaladharan *et al.*, 1989). For volatilization experiments, to a log phase culture of one litre of the alga, a sublethal dose of 20 µg of mercury in the form of $HgCl_2$ was added (Stock sol. of $HgCl_2$: 0.1354 g in 100 ml distilled water = 1 mg/ml of Hg; working sol. : 1 ml of stock solution

* Present address : Central Institute of Brackishwater Aquaculture, Madras, India.

diluted to 100 ml = 10 µg/ml of Hg). The culture was continuously aerated by blowing mercury free air obtained by passing through a 5% $KMnO_4$ sol. The rate of volatilization of mercury was measured by estimating the amount of mercury remaining in the whole medium and the culture supernatant (obtained by centrifugation at 11,000 g at 4°C for 10 min) at periodic intervals upto 48 hr. The difference of Hg concentration in the whole culture and the culture supernatant was attributed to uptake (bioaccumulation) by the cells of the alga.

The rate of volatilization of mercury was measured in the sea water collected from off Cochin, in the Arabian Sea. To a 20 l tank of sea water, mercuric chloride solution was added to get a concentration of 20 µg/l of mercury. The system was kept aerated continuously with mercury free air. A similar experiment with filtered (GF/C glass fibre filter, 0.45 µm pore size) sea water was also set up. The volatilization of mercury was measured by estimating mercury remaining in the sea water at periodic intervals. Extraction of mercury was done by digesting 25 ml each of whole culture of the alga and its culture supernatant and 50 ml of sea water. Mercury content was analysed by cold vapour atomic absorption spectrophotometry (Hatch and Ott, 1968). The recovery of mercury extraction was 84 per cent.

At the time of addition of mercuric chloride solution to the culture, the cell count was 3.8 X 10/ ml and at 48 hr, 2 X 10^3/ml. The volatilization experiments revealed that the amount of mercury both in the culture supernatant as well as in the whole culture decreased rapidly during the initial hours (Fig. 1), and at the same time the amount of mercury bound (bioaccumulated) by the cells of the microalga (as measured by difference in the mercury concentration in the whole culture and the culture supernatant) during the initial hours was also high (Fig. 2). After the eighth hour, the amount of mercury removed from the culture broth was slow, and it reached a minimum of 1 µg/l in 48 hrs. The amount of mercury bound (bioaccumulated) by the cells of the alga also sharply decreased from 0. 12 ng/cell in the second hr to 0.05 ng/cell at 48 hrs. It is also inferred that the amount of mercury taken up by the cells of *Isochrysis galbana* Parke is directly proportional to the amount of mercury present in the culture broth which follows the monomolecular curve (Von Slykes, 1942).

The loss of mercury from the filtered sea water accounted to 39% and 82.7% at 24 and 48 hrs respectively, though at a slower rate than the pure culture of *Isochrysis galbana* Parke. The rate of volatilization of mercury from the sea water was relatively slower than

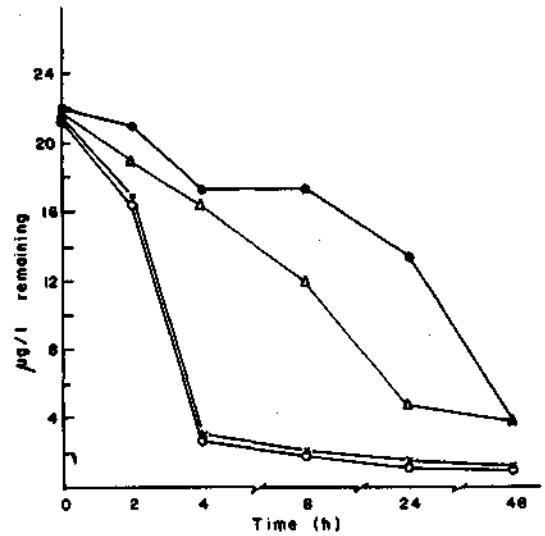

Fig. 1. Removal of mercury from sea water, filtered sea water, whole culture and culture supernatant.

in the algal culture system (Fig. 1), though more than 80% of the metal was eliminated in 48 hrs. This observation is obviously due to small numbers of phytoplankton and micro - organisms resistant to mercury, endowed with the enzymes capable of volatilizing mercury in the natural sea water. Loss of mercury from the filtered sea water, which is devoid of phytoplankton and to some extent, bacteria, can be attributed to aeration. This phenomenon has been observed earlier (Betz, 1977). Hence, aeration, augmented with microalgae like *Isochrysis galbana* Parke and other bacteria viz., *Pseudomonas, Vibrio, Moroxella, Flavobacterium, Bacillus, Micrococcus* etc., (Nakamura, 1989) with capacity to volatilise mercury will be of immence importance in the aquatic environments to reduce mercury pollution in a short duration.

The authors thank Dr. P. S. B. R. James, Director, and Mr. M. S. Rajagopalan, Head, Fisheries Environmental Management Division for encouragement.

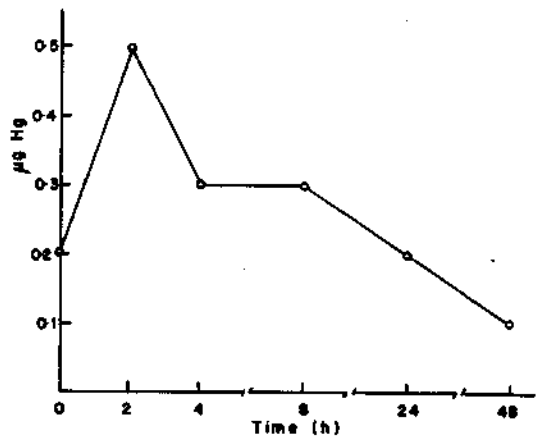

Fig. 2. Amount of mercury bound by the cells of *Isochrysis galbana* Parke in 1 l culture.

References

Ben-Bassat, D., and A. M. Mayer 1975. Volatilization of mercury by algae. *Physiol. Plant*, 33 : 128-1342. Betz, M. 1977. Investigations of simultaneous uptake and release of mercury by *Dunaliella tertiolecta* volatilize mercury. *Mar. Biol.*, 41 : 89-97. Bhattacharya, G., and A. Mandal, 1989. Volatilization of mercury by soil bacterium *Azotobacter lwoffi* GBI. *Indian J. Exp. Biol.*, 25 : 347-348. Brinckman, F. E., and W. P. Iverson 1975. Chemical and bacterial cycling of heavy metals in the coastal environment. *Am. Chem. Soc., Symp. Ser.*, 18 : 319-342. Davies, A. G. 1978. Pollution studies with marine plankton Part II. Heavy Metals. *Adv. Mar. Biol.*, 15 : 381-508. Fenchel, T., and T. H. Blackburn 1979. *Bacteria and Mineral Cycling*. Academic Press, London, 225 pp. Hatch, W. R., and W. L. Ott 1968. Determination of sub micro gram quantities of mercury by atomic absorption spectrophotometry. *Anal. Chem.*, 40 : 2085-2087. Joseph, K. J. 1983. Culture and growth kinetics of selected nanoplankters, Ph. D. Thesis, Cochin University of Science and Technology. Kaladharan, P., S. V. Alavandi and V. K. Pillai 1989. Effect of sublethal concentration of mercury on the photosynthetic oxygen release by *Isochrysis galbana* Parke. *Proc. National Conf. Coastal Zone Mangmts., Cochin*, (in press). Magos, L, A. A. Tuffery, and T. W. Clarkson, 1964. Volatilization of mercury by bacteria. *British J. Ind. Med.*, 21 : 294-298. Nakamura, K. 1989. Volatilization of fluorescein mercuric acetate by marine bacteria from Minamata Bay. *Bull. Environ. Contam. Toxicol.*, 42 : 785-790. Nakamura, K., T. Sakata and H. Nakahara 1988. Volatilization of mercury compounds by methyl mercury volatilizing bacteria in Minamata Bay sediment. *Bull.Environ. Contam. Toxicol.*, 41 : 651-656. Nelson, J. D., W. Blair, F. E. Brinckman, R. R. Colwell and W. P. Iverson 1973. Biodegradation of phenyl mercuric acetate by mercury resistant bacteria. *Appl. Microbiol.*, 26 : 321-326. Nuzzi, R. 1972. Toxicity of mercury to phytoplankton. *Nature*, 237 : 38-40. Sprangkler, W. J., J. L. Spigarelli, J. M. Rose, H. M. Miller 1973. Methyl mercury : Bacterial degradation in late sediments. *Science*, 1980 : 192-193. Summers, A. O. and E. Lewis 1973. Volatilization of mercuric chloride by mercury resistant plasmid bearing strains of *E. coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Bacteriol.*, 112 : 1070-1072. Von Slykes, D. D. 1942. The kinetics of hydrolytic enzymes and their bearing on methods for measuring enzyme activity. In : *Advances in Enzymology*, Vol. II. Interscience Publishers Inc., New York, pp 33-47. Wood J. M. 1974. Biological cycles for toxic elements in the environment. Science, 183 : 1049-1052.