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Employment of CEPEX Enclosures for Monitoring Toxicity of Hg and Zn on *in Situ* Structural and Functional Characteristics of Algal Communities of River Ganga in Varanasi, India

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Effects of Hg and Zn on *in situ* nitrogen fixation, autotrophic index, pigment diversity, $^{14}CO_2$ uptake, and change in algal community structure of Ganges water have been studied for the first time using CEPEX chambers in aquatic ecosystem of India. A concentration-dependent decrease in *in situ* nitrogenase activity of Ganges water with Hg and Zn has been noticed. No ethylene production was observed at 0.8 μg/ml of Hg. However, an increase in the autotrophic index was observed in CEPEX enclosures treated with Hg and Zn. The AI value was maximum at 0.8 μg/ml Hg after an incubation of 15 days. An increase in pigment diversity also followed the pattern of AI with the test metals used. Inhibition of $^{14}CO_2$ uptake of phytoplankton of Ganges water was maximum at 0.8 μg/ml Hg (79%) followed by Zn (69%). Carbon fixation showed an increase for 1 hr, after which no appreciable change was noticed. Maximum inhibition of algal number was observed at 0.8 μg/ml Hg followed by 8.0 μg/ml of Zn in the CEPEX chamber. Members of Chlorophyceae showed more tolerance than Cyanophyceae and Bacillariophyceae. The filamentous forms were more tolerant to Hg and Zn. In contrast, unicellular forms were more sensitive to Hg. The test of significance (ANOVA) showed that metal-induced variations in pigment diversity, the autotrophic index, and the $^{14}CO_2$ uptake were highly significant ($P < 0.001$). © 1990 Academic Press, Inc.

INTRODUCTION

Heavy metals are ubiquitous in the biosphere, where they occur as part of the natural background of chemicals to which human beings and the biota are exposed. However, industrial uses of metals and other domestic processes like burning of fossil fuels, incineration of wastes, automobile exhausts, smelting, use of sewage sludge as landfill materials, and fertilizer have introduced substantial amounts of potentially toxic heavy metals into the food chains. Consequently such anthropogenic emissions have elevated the body burden of heavy metals. Phytoplankton are a group of organisms inseparable from interacting communities which are subjected to such unfavorable alterations of the aquatic environment. It has been generally recognized that changes in the community structure of phytoplankton species may affect the aquatic food web and influence the distribution and abundance of higher organisms. There are many practical advantages in using phytoplankton for multispecies toxicity tests. One obvious reason is that phytoplankton multiply so rapidly that the effects of the heavy metals on growth and other physiological processes can be tested on several generations without having to wait years for the results.

For this reason, laboratory ecotoxicological studies involving single algal species and heavy metals have assumed considerable importance, although information so generated fails to give the exact picture of the ensuing changes in aquatic algal communities undergoing heavy metal stress (Rai *et al.*, 1981a; Whitton, 1984). It is for

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this reason that the use of multispecies toxicity test over single algal species has been favored in both synthetic and field microcosms. More recently, microcosm tests have been suggested for evaluating the effects of toxicants on important properties and behaviors of entire communities of ecosystems (Cairns, 1980; Giesey and Odum, 1980). Microcosm tests have the advantages of providing data on the response of many species simultaneously, including the effects of toxicants on interactions between species or species and their environments.

River Ganga drains an area of 861,404 km^2, accounting for 40% of the irrigated land, and sustains 37% of the Indian population. Changes in algal community structure due to sewage pollution have already been reported (Rai, 1978), and Mathur *et al.* (1987) have reported the presence of heavy metals in this river system. Thus, because the Ganga is the life line of the Indian people and nothing is known about changes in algal organisms as influenced by heavy metals in simulated ecosystems, the present study was undertaken to evaluate for the first time the toxicity of Hg and Zn on (a) changes in algal communities at different concentrations of Hg and Zn, (b) the autotrophic index, (c) *in situ* nitrogen fixation, (d) the $^{14}CO_2$ uptake of phytoplankton, and (e) pigment diversity.

MATERIALS AND METHODS

For this study glass CEPEX (Controlled Ecosystem Pollution Experiment) chambers with the support of an iron angle were made. The size of each CEPEX chamber was $4 \times 2 \times 1.5$ ft. The joints of chamber were sealed to make it waterproof. It was placed in the Ganges water about 7 m away from the bank. The chambers were placed in water in such a way that the edge of each chamber was always 0.5 ft above the water surface. These chambers were kept submerged by erecting parallel and horizontal bamboos in the flowing water. Chambers were filled with river water. One chamber was kept as control, and the others (in duplicate) were spiked with different doses of Hg and Zn (0.2, 0.4, 0.6, 0.8 μg/ml Hg and 2.0, 4.0, 6.0, 8.0 μg/ml Zn). Algal and water samples taken from both control and experimental chambers at regular intervals were analyzed with respect to the following parameters: pigment diversity, autotrophic index (AI), $^{14}CO_2$ uptake, *in situ* nitrogen fixing potential, and qualitative and quantitative estimate of algae.

For collection of algae, 2 liters of a water sample was passed through a 47-mm, 0.45-μm filter paper by applying about 0.3 atmospheric vacuum pressure. The algae so obtained were counted with a hemocytometer and expressed as number of individuals per milliliter. Systematic analysis was accomplished using standard taxonomic keys such as Hustedt (1930), Fritsch (1935), Tiffany and Britton (1952), Desikachary (1959), Randhawa (1959), Phillipose (1967).

In situ nitrogen fixation was measured with specially fabricated equipment (Fig. 1) fitted with 6 bottles of 275-ml capacity each, illuminated with tube light, and cooled by continuously flowing Ganges water supplied by a centrifugal pump. This assembly was kept rotating continuously. In a bottle containing 250 ml water sample, 10% of acetylene gas was injected by means of an airtight syringe. The bottles were sealed with airtight stoppers and parafilm. After an incubation of 1 hr, reaction was terminated by injection of 50% (w/v) trichloroacetic acid (Riddolls, 1985). The nitrogenase activity was measured by the acetylene–ethylene assay method (Stewart *et al.*, 1968) after the bottles were taken to the laboratory.

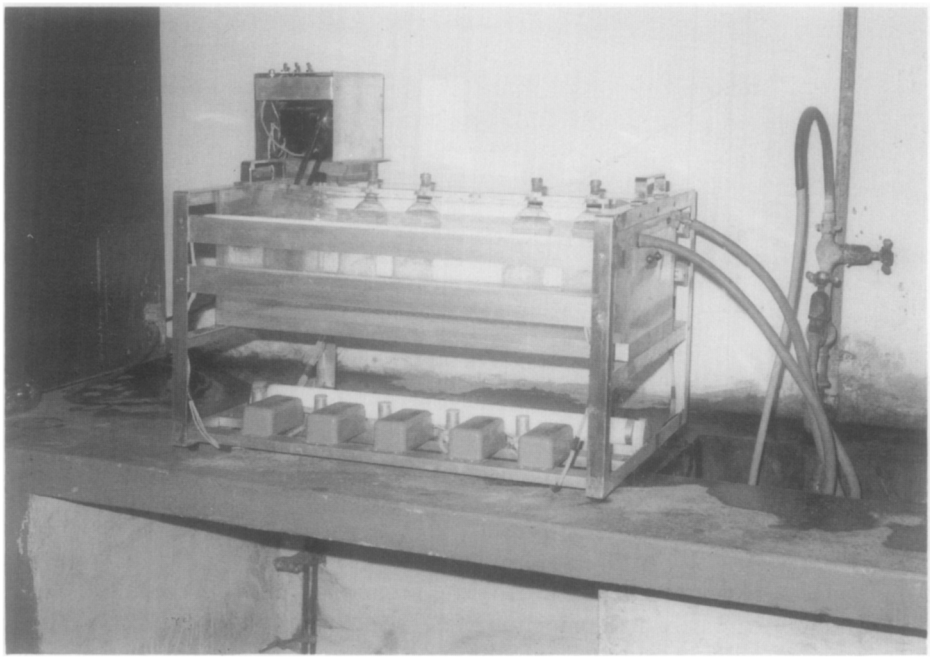

FIG. 1. Equipment for *in situ* nitrogenase assay.

Part of the phytoplankton sample collected by filtration was used for pigment diversity. The latter was calculated as the ratio of carotenoid to chlorophyll *a* (Margalef, 1958). The autotrophic index was calculated by measuring the planktonic biomass (dry weight) as well as chlorophyll *a* from a known amount of sample following the method of Weber and McFarland (1969).

$$\text{Autotrophic Index (AI)} = \frac{\text{Biomass (dry weight) organic matter}}{\text{Chlorophyll } a}$$

For $^{14}CO_2$ uptake, the phytoplankton from the filter paper were transferred into the scintillation vials containing 1.0 ml filtered water and 0.2 ml $NaH^{14}CO_3$. The $^{14}CO_2$ uptake by algal suspension was stopped by adding 0.2 ml 50% acetic acid. The reaction was terminated at different time intervals. Scintillation cocktail (5.0 ml) was added to the resulting suspension. Air was bubbled for 5 min. Counting was done in a Beckman Model LS-7000 liquid scintillation counter and the rate of $^{14}CO_2$ uptake was expressed in cpm (counts per minute).

Analysis of Variance (ANOVA). The data for autotrophic index, pigment diversity, and $^{14}CO_2$ uptake (Tables 1, 2, and 3) were verified and the variance ratio (F) was calculated by the equation

$$F = \frac{\text{Treatment mean square}}{\text{Residual mean square}}.$$

RESULTS

Effects of Hg and Zn on the *in situ* nitrogen fixing potential of Ganges water are summarized in Fig. 2. A concentration-dependent decrease in the nitrogenase activity

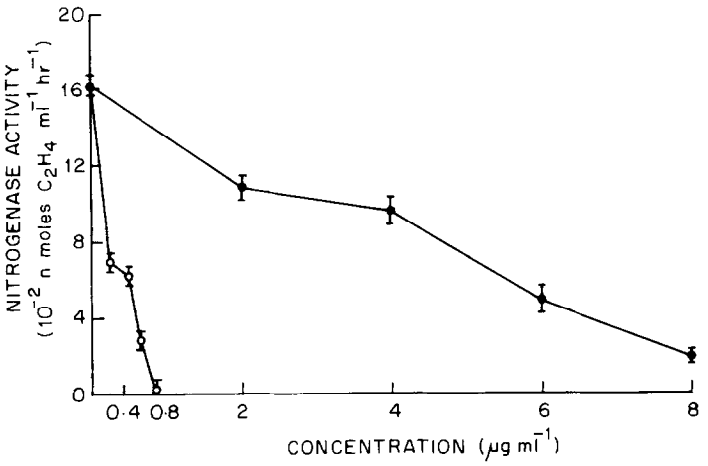

FIG. 2. Effects of different concentrations of heavy metals on nitrogenase activity. Zn (●) and Hg (○).

of this water has been observed. No ethylene production was observed at 0.8 μg/ml of Hg and approximately 80% reduction of N_2ase activity was observed at 8.0 μg/ml of Zn, whereas only 50 and 30% inhibition of *in situ* N_2ase activity was observed, respectively, at 4.0 μg/ml Zn and 0.4 μg/ml Hg.

The two-factor ANOVA showed that the metal-induced autotrophic index varied significantly with respect to time ($F_{4,16} = 12.28$, $P < 0.001$) and treatment ($F_{4,16} = 9.37$, $P < 0.001$). A high autotrophic index was noted with 0.8 μg/ml Hg and 8.0 μg/ml of Zn (Table 1). A concentration-dependent increase in the autotrophic index value was found for both mercury and zinc. A significant increase in the autotrophic index with test metals was noted only after 6 days, although some increase in the autotrophic index was evident from the third day of experiment.

The two-factor ANOVA showed that metal-induced variation in pigment diversity varied significantly with respect to time ($F_{4,16} = 11.8$, $P < 0.001$) and treatment ($F_{4,16} = 27.8$, $P < 0.001$). Effects of Hg and Zn on pigment diversity of algal planktons of river Ganga are summarized in Table 2. Pigment diversity followed the pattern of the autotrophic index. It did not show a sudden increase as noted for autotrophic index values. A concentration-dependent increase in pigment diversity with both mercury and zinc was observed. Maximum pigment diversity was noted at 0.8 μg/ml Hg followed by 0.4 μg/ml Hg and 8.0 and 4.0 μg/ml of Zn after 15 days. An increase in pigment diversity occurred with increasing incubation time in both treated CEPEX chambers and control ones.

The two-factor ANOVA showed that metal-induced inhibition of $^{14}CO_2$ uptake varied more significantly with treatment ($F_{4,8} = 21.35$, $P < 0.001$) than with time ($F_{2,8} = 8.04$, $P < 0.025$). Inhibition of $^{14}CO_2$ uptake of phytoplankton of Ganges water was maximum at 0.8 μg/ml Hg (79%) followed by 8.0 μg/ml Zn (69%) (Table 3). The inhibition showed sudden increases for 1 hr, after which it did not show an appreciable change and stabilized after 2 hr. Maximum carbon fixation by phytoplankton of Ganges water was observed up to 2 hr only.

The inhibition of algal genera and species by test metals (Tables 4 and 5) began after the metals were mixed into the CEPEX chamber and was found to be pronounced

TABLE 1

EFFECTS OF DIFFERENT CONCENTRATIONS OF Hg AND Zn ON AUTOTROPHIC INDEX OF GANGES WATER USING CEPEX CHAMBER

Concentration (μg/ml)	Number of days					
	0	3	6	9	12	15
Control	12.93 ± 0.18	12.00 ± 0.13	12.95 ± 0.12	12.98 ± 0.11	13.00 ± 0.15	13.12 ± 0.14
Mercury						
0.4	12.93 ± 0.18	17.86 ± 0.11	20.32 ± 0.09	36.63 ± 0.10	58.54 ± 0.11	62.75 ± 0.17
0.8	12.93 ± 0.18	19.90 ± 0.15	28.32 ± 0.11	42.00 ± 0.10	65.75 ± 0.13	66.00 ± 0.15
Zinc						
4.0	12.93 ± 0.18	13.90 ± 0.09	16.73 ± 0.14	24.64 ± 0.11	32.08 ± 0.11	45.00 ± 0.11
8.0	12.93 ± 0.18	14.45 ± 0.11	18.45 ± 0.13	28.00 ± 0.12	38.54 ± 0.12	54.05 ± 0.12

Note: ANOVA: $F_{\text{time } 4,16} = 12.28$, $P < 0.001$; $F_{\text{treatment } 4,16} = 9.37$, $P < 0.001$.

TABLE 2

EFFECTS OF DIFFERENT CONCENTRATIONS OF Hg AND Zn ON PIGMENT DIVERSITY OF GANGES WATER USING CEPEX CHAMBER

Concentration (μg/ml)	Number of days					
	0	3	6	9	12	15
Control	0.51 ± 0.03	0.50 ± 0.02	0.51 ± 0.04	0.54 ± 0.05	0.52 ± 0.03	0.53 ± 0.03
Mercury						
0.4	0.51 ± 0.03	0.98 ± 0.04	0.92 ± 0.03	1.06 ± 0.03	1.20 ± 0.02	1.40 ± 0.02
0.8	0.51 ± 0.03	0.88 ± 0.03	1.20 ± 0.03	1.26 ± 0.04	1.48 ± 0.04	1.50 ± 0.03
Zinc						
4.0	0.51 ± 0.03	0.58 ± 0.02	0.60 ± 0.03	0.85 ± 0.03	1.00 ± 0.07	1.10 ± 0.05
8.0	0.51 ± 0.03	0.62 ± 0.03	0.62 ± 0.04	0.93 ± 0.03	1.20 ± 0.03	1.20 ± 0.05

Note: ANOVA: $F_{\text{time } 4,16} = 11.8$, $P < 0.001$; $F_{\text{treatment } 4,16} = 27.0$, $P < 0.001$.

TABLE 3

EFFECTS OF DIFFERENT CONCENTRATIONS OF Hg AND Zn ON *IN SITU* $^{14}CO_2$ UPTAKE OF GANGES WATER USING CEPEX CHAMBER

Concentration (μg/ml)	$^{14}CO_2$ uptake (cpm $\times 10^3$)						
	0 hr	0.5 hr	% inhibition	1 hr	% inhibition	2 hr	% inhibition
Control	0.101 ± 0.013	28.866 ± 0.012	—	48.703 ± 0.016	—	54.222 ± 0.011	—
Mercury							
0.4	0.101 ± 0.013	13.675 ± 0.011	52.6	26.546 ± 0.012	45.4	28.756 ± 0.014	46.9
0.8	0.101 ± 0.013	7.323 ± 0.009	74.6	11.304 ± 0.010	76.7	11.386 ± 0.012	79.0
Zinc							
4.0	0.101 ± 0.013	22.756 ± 0.016	21.1	31.948 ± 0.008	34.4	35.000 ± 0.008	35.4
8.0	0.101 ± 0.013	14.691 ± 0.013	49.1	15.852 ± 0.013	67.4	16.684 ± 0.011	69.2

Note: ANOVA: $F_{\text{time } 2,8} = 8.04$, $P < 0.025$; $F_{\text{treatment } 4,8} = 21.35$, $P < 0.001$.

TABLE 4

CHANGES IN ALGAL GENERA AT DIFFERENT CONCENTRATIONS OF Hg IN CEPEX CHAMBER

	Number of algal cells/filament/colony × 10^4 liter^{-1}			
	Control		0.4 μg/ml	0.8 μg/ml
Name of algae	0 day	15 days	15 days	15 days
Chlorophyceae	25.625	26.534	11.413 (56.9)	4.484 (83.1)
Chlorella vulgaris	0.968	1.680	0.620 (63.1)	0.280 (83.3)
Coelastrum lanceolatum	0.021	0.035	0.008 (77.1)	— (100.0)
Microspora	12.321	11.960	5.482 (54.1)	2.850 (76.1)
Pediastrum simplex	1.125	2.090	0.625 (70.1)	— (100.0)
Pediastrum sp.	1.084	1.007	0.410 (59.2)	— (100.0)
Scenedesmus	1.345	1.643	0.400 (75.6)	0.004 (99.7)
Spirogyra	3.120	4.007	1.600 (60.0)	0.430 (89.2)
Ulothrix	4.520	3.300	1.825 (44.7)	0.820 (75.1)
Zygnema	1.221	1.312	0.643 (50.9)	0.200 (84.7)
Cyanophyceae	29.793	31.233	11.499 (63.1)	4.195 (86.5)
Anacystis	0.565	0.800	0.250 (69.9)	— (100.0)
Anabaena	8.435	9.643	2.900 (66.4)	0.400 (95.8)
Lyngbya	3.820	3.320	1.500 (54.8)	0.900 (72.8)
Merismopedia minima	3.670	4.624	0.845 (81.7)	— (100.0)
Oscillatoria formosa	12.867	13.206	5.804 (56.0)	2.800 (78.8)
Phormidium	0.261	0.300	0.120 (60.0)	0.075 (75.0)
Spirulina	0.175	0.340	0.080 (76.4)	0.019 (94.4)
Bacillariophyceae	44.660	38.616	13.812 (64.2)	5.330 (86.2)
Anorthoneis excentrica	1.811	1.806	— (100.0)	— (100.0)
Cylindrotheca	11.761	8.650	4.000 (53.7)	1.600 (81.5)
Gyrosigma	13.186	10.210	1.852 (81.8)	0.460 (95.4)
Nitzschia	6.161	6.000	2.960 (50.6)	1.254 (79.1)
Fragilaria	12.741	11.950	5.000 (58.1)	2.016 (83.1)

Note. Values in parentheses are % inhibition.

until the 15th day. The decline in algal number was concentration dependent and metal specific. A species-specific interaction was also noted during the present investigation. Maximum inhibition of algal number occurred at 0.8 μg/ml Hg followed by 8.0 μg/ml Zn. The Chlorophyceae members exhibited more tolerance than Cyanophyceae and Bacillariophyceae to both the test metals. The filamentous forms like *Microspora, Hormidium, Oscillatoria formosa, Oscillatoria* sp., *Spirulina, Spirogyra, Ulothrix,* and *Zygnema* showed more resistance toward Hg and Zn than the unicellular forms like *Chlorella vulgaris, Chlorella* sp., *Pediastrum simplex, Anacystis, Merismopedia minima, Cylindrotheca, Gyrosigma, Nitzschia,* and *Navicula. Anorthoneis excentrica* was very sensitive to Hg, as there was complete disappearance of this alga on the 6th day at 0.4 μg/ml Hg. Diatoms in general were very sensitive, followed by blue-greens and green algae against Hg. However, *Lyngbya* followed by *Microspora, Zygnema,* and *Oscillatoria formosa* showed much tolerance for Hg. Likewise, zinc was also very toxic to diatoms, followed by blue-green and green algae. In contrast, zinc was less toxic to filamentous forms like *Microspora, Zygnema,* and *Ulothrix.* Approximately 21% inhibition of *Phormidium* was observed after 15 days of incubation at 8.0 μg/ml zinc.

TABLE 5

CHANGES IN ALGAL GENERA AT DIFFERENT CONCENTRATIONS OF Zn IN CEPEX CHAMBER

	Number of algal cells/filament/colony × 10^4 liter^{-1}			
	0 Control		4.0 μg/ml	8.0 μg/ml
Name of algae	0 day	15 days	15 days	15 days
Chlorophyceae	25.863	26.393	14.596 (44.9)	12.008 (54.5)
Chlorella vulgaris	0.869	0.880	0.305 (65.2)	0.219 (75.1)
Coelastrum lanceolatum	0.012	0.015	— (100.0)	— (100.0)
Microspora	13.014	13.130	6.987 (46.7)	6.000 (54.3)
Pediastrum simplex	2.540	2.582	1.314 (49.1)	1.000 (61.9)
Scenedesmus bicaudatus	2.456	2.520	1.000 (60.3)	0.960 (61.9)
Ulothrix	4.852	4.855	3.285 (32.3)	2.504 (48.4)
Zygnema	2.120	2.411	1.705 (29.2)	1.325 (45.4)
Cyanophyceae	29.112	28.729	16.432 (42.8)	14.042 (51.5)
Anabaena	6.720	6.730	3.856 (42.7)	3.206 (52.3)
Anacystis	0.600	0.636	— (100.0)	— (100.0)
Lyngbya	4.201	4.205	2.856 (32.0)	2.500 (40.5)
Nostoc linckia	1.175	1.250	0.823 (34.1)	0.475 (62.5)
Oscillatoria formosa	11.861	11.864	6.205 (47.7)	5.404 (54.4)
Oscillatoria sp.	3.931	3.410	2.120 (37.8)	1.956 (42.6)
Phormidium	0.624	0.634	0.572 (09.7)	0.501 (20.9)
Bacillariophyceae	42.261	41.134	19.344 (52.9)	14.840 (68.7)
Anorthoneis excentrica	0.934	1.002	0.090 (91.0)	— (100.0)
Cylindrotheca	10.809	9.751	6.889 (30.1)	5.840 (40.1)
Gyrosigma	14.250	14.080	5.550 (60.9)	5.000 (64.4)
Navicula	10.120	10.101	4.066 (59.7)	— (100.0)
Nitzschia	6.148	6.200	2.879 (53.5)	2.000 (67.7)

Note. Values in parentheses are % inhibition.

DISCUSSION

The use of algae in monitoring heavy metal toxicity is increasing because of their ubiquity in aquatic environments where they influence and/or are influenced by most aquatic processes. Algae incorporate solar energy into biomass, produce oxygen that is dissolved in water and used by aquatic organisms, function critically in cycling and mineralization of chemical elements, and serve as food for herbivorous animals. Thus a wealth of literature dealing with the disastrous impact of heavy metals on taxonomic diversity and production of algae has been published (Rai *et al.*, 1981a; Stokes, 1983; Davies, 1983; Whitton, 1984; Wong *et al.*, 1986).

Some heavy metals, e.g., zinc, copper, and molybdenum, are required in trace amounts by algae for various physiological and biochemical processes (Round, 1973). Others comprising mercury, cadmium, lead, etc., include those whose significance or requirement in metabolism is not known. In general, all metals are toxic to algae at higher concentrations. Pollution phycologists have encountered difficulties in interpreting field and laboratory bioassay results because the toxicity of metals varies under field and laboratory conditions. Studies conducted on zinc and other metals have suggested that several factors of the aquatic environment, notably pH, hardness, alkalinity, salinity, dissolve oxygen, temperature, chelators, phosphate, and calcium,

TABLE 6

CHANGES IN COMMUNITY STRUCTURE OF DIFFERENT ALGAL GROUPS AT DIFFERENT CONCENTRATIONS OF TEST METALS IN CEPEX STUDY

	Number of algal cells/filaments/colonies $\times 10^4$ liter^{-1} on 15th day		
Algal group	Control	0.4 μg Hg ml^{-1}	0.8 μg Hg ml^{-1}
Chlorophyceae	26.534	11.413 (56.9)	4.484 (83.1)
Cyanophyceae	31.233	11.499 (63.1)	4.195 (86.5)
Bacillariophyceae	38.616	13.812 (64.2)	5.330 (86.2)
	Control	4.0 μg Zn ml^{-1}	8.0 μg Zn ml^{-1}
Chlorophyceae	26.393	14.596 (44.9)	12.008 (54.5)
Cyanophyceae	28.729	16.432 (42.8)	14.042 (51.5)
Bacillariophyceae	41.134	19.344 (52.9)	12.840 (68.7)

Note. Values in parentheses are % inhibition.

influence metal toxicity (Harding and Whitton, 1977; Say and Whitton, 1977; Gadd and Griffiths, 1978; Rai and Kumar, 1980).

Significant reduction in nitrogenase activity such as that observed in the present study can be explained in the following ways. Metals may be toxic to nitrogenase in many different ways: (i) there can be direct action on enzyme complex, or (ii) there may be an effect on the supply of ATP or reductant pool which is a prerequisite for enzyme activity. Since photosynthesis is the main source of ATP and reductant, inhibition of this process can bring about a reduction in ATP and reductant. High-level inhibition of $^{14}CO_2$ uptake by test metals seems to have direct bearing on nitrogenase activity. The metal-induced inhibition of carbon fixation followed the same sequence as that observed for nitrogenase, but the level of inhibition was higher for carbon fixation than for nitrogenase. Present observations supported the findings of Blinn *et al.* (1977), who found significant inhibition of phytoplankton productivity following Hg treatment in a CEPEX study of Lake Arizona.

The autotrophic index is defined as the ratio between the dry weight of biomass and chlorophyll *a* (Weber and McFarland, 1969). The observations of Rai *et al.* (1981b) and De Filippis and Pallaghy (1976) on zinc and mercury using *C. vulgaris* have indicated severe reduction in chlorophyll *a* content following supplementation of these metals. Thus any decrease in chlorophyll content will lead to an increased autotrophic index value. The maximum autotrophic index value as noted for Hg followed by Zn in CEPEX chambers could be due to the high toxicity of Hg and Zn, respectively, against chlorophyll *a*, thus leading to an increase in the autotrophic index.

Pigment diversity follows the same pattern as the autotrophic index. The ratio of carotenoid to chlorophyll *a* has long been suggested to be a good tool for monitoring pollution conditions in aquatic ecosystems (Margalef, 1958). The utility of this ratio has been confirmed by Rai *et al.* (1981b) while working on the toxicity of zinc, mercury, and methylmercury on *C. vulgaris*. A change in pigment diversity such as that observed in the present study could be due to inhibition of chlorophyll *a* by Hg and Zn. Metal toxicity again followed the trend of the autotrophic index.

The data listed in Table 6 on the toxicity of metals to algae in a field microcosm are interesting from many angles. Present observations involving zinc and phytoplankton from River Ganga are in essential agreement with those of Whitton (1970), who found diatoms to be more sensitive to copper, zinc, and lead. Whitton (1970) reported that *Ulothrix* and *Microspora* species were tolerant to copper, zinc, and lead. The low percentage inhibition of *Ulothrix* and *Microspora* by test metals used in the present study further attests to their tolerant nature. These findings nevertheless fit well with those of Patrick (1949) dealing with reduction in species diversity under high pollution conditions. Three algal genera, *Coelastrum, Anacystis,* and *Anorthoneis,* were found to be highly sensitive to Hg and Zn. Of these, *Anorthoneis* and *Coelastrum* seem to be highly sensitive to Hg and Zn.

All the parameters studied in the CEPEX under metal-stressed conditions testify to the toxic behaviors of Hg and Zn. Looking at the sensitivity of all the structural and functional parameters, it becomes evident that $^{14}CO_2$ uptake is the more sensitive parameter in biomonitoring of heavy metal toxicity in the field. The present study lends further support to the views of Rai and co-workers (see Rai and Raizada, 1985;1988) and confirms that $^{14}CO_2$ uptake could be a reliable parameter in monitoring metal toxicity in natural ecosystem.

CONCLUSION

A concentration-dependent reduction in AI, pigment diversity, algal community structure, $^{14}CO_2$ uptake, and N_2ase activity in a natural aquatic system in India has been reported for the first time. This study suggested that $^{14}CO_2$ fixation could be used as sensitive parameter for biomonitoring of metal toxicity, although this requires confirmation from different aquatic systems with varied physicochemical and biological characteristics.

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