Microbiological Effects of Metal Ions in Chesapeake Bay Water and Sediment

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Metal ions enter Chesapeake Bay in varying amounts and from a variety of sources. Sources traced to human activities provide at least one half of the Cr, Cu, Cd, and Pb entering the estuary, while at the present time the human contribution to the input of Mn, Fe, Co, Ni, and Zn is somewhat less (HELZ, 1976). Furthermore, it has been established that the impact of such inputs could be felt not only on a local scale but on a regional one as well. In order to ascertain the potential effect of high concentrations of metal ions on the microflora of Chesapeake Bay, an investigation was undertaken to measure the effects of metals on microbial photosynthesis, nitrification, and glucose oxidation in Chesapeake Bay water and sediment. Aerobic, heterotrophic bacterial populations showing resistance to metal ions in water and sediment samples collected from Colgate Creek, (Baltimore Harbor), an area of high metal stress (VILLA and JOHNSON, 1974), and a station in the middle of the ship channel near Chesapeake Beach, an area near the center of the Bay containing much lower metal concentrations than Baltimore Harbor (HELZ, 1976) were enumerated.

The effect of metals on photosynthesis was determined by measuring changes in uptake of ^{14}C labelled Na_2CO_3 by unialgal suspensions of Dunaliella sp. and Chlorella sp., and by the nannoplankton present in samples of surface water collected from the Chesapeake Beach sampling station. Suspensions of algae were grown under constant illumination in Bold's Basal Medium (BOLD, 1942), amended with 5.0 g NaCl/l, 1.5 g $MgSO_4 \cdot 7H_2O$/l, and 0.16 g KCl/l, to a cell density of approximately 3 x 10^4 cells/ml, as determined by direct counting with a hemocytometer. Uptake of $^{14}CO_3{}^{2-}$ was determined according to the procedure outlined in Standard Methods for the Examination of Water and Wastewater (1971), with appropriate modification for smaller volumes of sample.

Suspensions of algae were amended with 0, 25, or 100 ppm of Cd^{2+}, Co^{2+}, Cr^{3+}, or Pb^{2+}, or 0, 2.5, or 10 ppm of Hg^{2+}. The metals were added as sterile, concentrated solutions of $CdSO_4 \cdot 8H_2O$, $CoSO_4 \cdot 7H_2O$, $CrCl_3 \cdot 6H_2O$, $Pb(NO_3)_2$, or $HgCl_2$.

To test the effect of metal ions on nitrification, 100 ml of water collected at the Chesapeake Beach station were placed in a 250-ml Erlenmeyer flask to which had been added 100 ppm NH_4Cl-N and 0 or 100 ppm of Cr^{3+}, Co^{2+}, Cd^{2+}, Pb^{2+}, or 0 or 10 ppm Hg^{2+}. The flasks were incubated at room temperature on a rotary shaker

Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 1

table operated at 150 rpm. Samples were taken upon initiation of the experiment and at recorded intervals thereafter for analysis of NH_4^+ (WILSON and KNIGHT, 1952) and NO_2^- (MONTGOMERY and DYMOCK, 1961).

The effect of metals on glucose oxidation by the Chesapeake Bay microorganisms present in the water or sediment samples was determined following the method of BUDDEMEYER (1974) for continuous and cumulative measurement of $^{14}CO_2$ release from labelled substrates. Water or sediment collected from Colgate Creek or Chesapeake Beach was amended with 0.01% glucose (0.5 µCi ^{14}C) and 0, 10, or 100 ppm of Cr^{3+}, Cd^{2+}, Co^{2+}, Pb^{2+}, or 0, 1, or 10 ppm Hg^{2+}.

Enumerations of metal-resistant bacteria were done on a routine sampling basis from October 1975 to May 1976 (see NELSON and COLWELL, 1975). A medium different from that of NELSON and COLWELL (1975) was employed, and its formulation is as follows: NaCl, 5.0 g; KCl, 0.10 g; $MgSO_4$, 1.5 g; Bacto Peptone (Difco), 1.0 g; Bacto Yeast Extract (Difco), 1.0 g; Bacto Agar (Difco), 20.0 g; and distilled water, 1,000 ml. Immediately before pouring of the plates, 100 ppm Cr^{3+}, Co^{2+}, Cd^{2+}, Pb^{2+}, or Hg^{2+} was added to the medium. Plates containing no metal were used to determine the total, viable, aerobic, heterotrophic count (TVC).

Table 1 shows results of experiments designed to measure the effects of metal ions on photosynthesis. Addition of Cr^{3+}, Cd^{2+}, or Co^{2+} to suspensions of *Dunaliella* did not inhibit ^{14}C uptake, whereas uptake was decreased when Pb^{2+} or Hg^{2+} were added. *Chlorella*, on the other hand, was inhibited by 25 ppm Cd^{2+}, but not by 100 ppm. There is no readily apparent explanation for this interesting and reproducible observation. The addition of Hg^{2+} also caused a decrease in photosynthesis by *Chlorella*, with 100 ppm causing cessation of photosynthesis. In samples of Chesapeake Bay water, $^{14}CO_2$ uptake was inhibited by Cd^{2+}, Co^{2+}, and Hg^{2+}, but not by Cr^{3+} or Pb^{2+}. Although there are several possible explanations for the different patterns of response to the several ions by the various samples, the most likely is that neither *Dunaliella* nor *Chlorella* was the dominant photosynthetic organism in the water samples. At any rate, it is obvious that high concentrations of metals can have deleterious effects on primary production in Chesapeake Bay. Whether such damage would be temporary or more long lasting or whether chronic changes could be effected upon prolonged exposure to lower levels of these metals remains to be determined.

Nitrification, as measured by loss of NH_4^+ and accumulation of NO_2^-, was completely inhibited by all of the metals tested. Nitrite appeared after 16 days in the Chesapeake Bay water samples treated with NH_4^+ but to which no metals were added. At day 30, the NH_4^+ and NO_2^- were no longer detectable, presumably having been converted to NO_3^-. Nitrite was not observed in any of the samples to which metal ion had been added, nor did any of the NH_4^+ disappear except for a very small amount accounted for by volatilization.

TABLE 1

Effect of metal ions on $^{14}CO_2$ uptake by algal suspensions and microorganisms present in samples of Chesapeake Bay water.

		Metal ion added									
		Cr^{3+}		Cd^{2+}		Co^{2+}		Pb^{2+}		Hg^{2+}	
Sample	0	25	100	25	100	25	100	25	100	2.5	10.0
	CPM/ml	Per cent of CPM with no metal added									
<u>Dunaliella</u>	1.8E4*	100	100	100	100	100	100	75	53	24	22
<u>Chlorella</u>	1.6E3	100	100	87	100	100	100	100	100	34	0
Chesapeake Bay water samples	9.3E2	100	100	67	48	93	60	100	100	8	4

*1.8×10^4

The effect of metal ions on ^{14}C glucose oxidation by microorganisms present in samples of water and sediment collected at the Chesapeake Beach and Colgate Creek sampling stations is shown in Table 2. The amount of inhibition was, in general, greater at the higher metal concentrations, and the presence of metals caused greater inhibition in samples collected from the unpolluted Chesapeake Beach site than in samples from Colgate Creek.

TABLE 2

Per cent inhibition of C-14 glucose oxidation caused by addition of metal ions to water and sediment samples collected from several areas of Chesapeake Bay.

		Chesapeake Beach		Colgate Creek	
Metal	Added ppm	Water	Sediment	Water	Sediment
Co^{2+}	10	72	67	47	100
	100	80	84	73	100
Cr^{3+}	10	35	0	29	0
	100	88	0	94	12
Pb^{2+}	10	23	73	64	19
	100	81	30	72	67
Cd^{2+}	10	84	59	68	0
	100	92	90	94	51
Hg^{2+}	10	90	0	97	0
	100	94	88	98	0

The fact that there was less inhibition by metals of microbial glucose oxidation in Colgate Creek than at Chesapeake Beach suggests that the microorganisms present in Colgate Creek are metal-resistant. From the data given in Table 3, it is also suggested that a higher proportion of heterotrophic microorganisms in Colgate Creek, where a great deal of metal input occurs, is resistant to metals than in the relatively clean site at Chesapeake Beach. In Table 3 are given 60 sets of data (Colgate Creek vs. Chesapeake Beach). Twenty-two of these were either non-comparable or the same at both stations. Of the remaining 38 pairs of observations, 23, or 61%, were higher at Colgate Creek, indicating the presence of a metal-resistant microbial population.

The values given for percentage of metal-resistant microorganisms at Colgate Creek showed significantly greater variation than those calculated for the Chesapeake Beach samples, most probably due to the greater variation in temperature, salinity, dissolved oxygen, nutrient levels, etc., in Colgate Creek. Earlier studies of Colgate Creek showed a correlation between percentage of organisms resistant to Hg^{2+} and turbidity and dissolved oxygen (NELSON and COLWELL, 1975).

From the results of this study, it is concluded that high levels of metal ions can cause acute perturbations in the microbial

TABLE 3

Per cent of heterotrophic organisms (TVC) resistant to metal ions.[1]

	Sediment						Water					
	Oct	Nov	Dec	Mar	Apr	May	Oct	Nov	Dec	Mar	Apr	May
						Colgate Creek						
Pb^{2+}	15	25	100	60	100	36	14	10	89	65	96	22
Cd^{2+}	35	<1	<1	<1	<1	<1	23	<1	<1	<1	<1	<1
Hg^{2+}	99	46	100	12	7	2	57	40	100	17	5	<1
Cr^{3+}	5	-	40	8	66	77	<1	-	<1	<1	-	60
Co^{2+}	2	34	4	8	23	<1	<1	43	27	<1	4	45
						Chesapeake Beach						
Pb^{2+}	75	21	72	100	100	100	20	14	-	20	33	<1
Cd^{2+}	<1	<1	<7	<1	-	<1	<1	<1	-	<1	<1	<1
Hg^{2+}	69	2	<7	<1	8	<1	10	<1	-	<1	<1	<1
Cr^{3+}	52	-	<7	14	45	100	40	-	-	<1	13	61
Co^{2+}	<1	18	<7	23	38	<1	27	<1	-	2	<1	<1

[1]The concentration employed was 100 ppm, except in the case of Hg^{2+} which was 10 ppm.

ecology of Chesapeake Bay. Whether long term exposure to lower concentrations of metal ions will cause related, chronic disturbances is presently under study in our laboratory.

ACKNOWLEDGMENTS

The authors thank the Captain and crew of the R/V RIDGLEY WARFIELD for their assistance in the field work. We gratefully acknowledge the excellent technical assistance of Mary Baldini. This work was supported by Sea Grant Project No. 04-5-158-11 and NSF Grant No. ENV76-08310.

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