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Effect of Lead Speciation on Toxicity

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Limnologists and aquatic toxicologists generally ignore the interactions between metals and ligands in aqueous systems. Chemical considerations often overlooked in metal toxicity bioassays and biogeochemical analyses of aquatic systems include ligand concentrations, pH, oxidation state, complex formation, precipitation and reaction kinetics (LEE 1973). Although hardness and alkalinity are occasionally implicated indirectly in some toxicity assays (SINLEY et al. 1974, KINKADE and ERDMAN 1975, HOWARTH and SPRAGUE 1978, MCCARTY et al. 1978) such considerations usually fail to incorporate the metal-ligand reaction mechanisms. Since the biological activity and availability of metals and ligands may be altered by chemical interactions and water systems may have unique chemical constituents, estimates of chemical speciation that incorporate general reaction mechanisms are necessary for accurate assessments of biogeochemical activity. Recent data support the hypothesis that chemical interactions influence the biochemistry and physiology of aquatic organisms (ANDERSON and MOREL 1978, JACKSON and MORGAN 1978, LELAND et al. 1978) and analyses that consider only the total concentration of a trace or toxic element in the environment may lead to seriously inaccurate interpretations of the fate of those constituents (STUMM and BILINSKI 1972, CHAPMAN 1973).

Our interest in the hypothesis that chemical speciation is a significant factor in the biogeochemistry of aquatic environments has led us to consider the possible importance of speciation in bioassay procedures. Generally, bioassays are utilized to demonstrate the effect of dilution on chemical substances and to determine environmentally safe levels of discharge. Toxic components of effluents are diluted with either receiving water or synthetic media; pH is frequently controlled by the addition of buffers.

Chemical equilibrium analysis of the interactions of all constituents in the bioassay mixture provides a relatively rapid and inexpensive method for examination of theoretical chemical speciation (STUMM and MORGAN 1970). If it is assumed that free or uncomplexed forms of the elements are the biochemically active species, experiments can be designed to fit the most important chemical boundary conditions of the reaction mixture.

The efficacy of this approach was demonstrated in our experiments on the toxicity of lead in a synthetic medium (Table 1).

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Using REDEQL2, a chemical equilibrium program (MOREL and MORGAN 1972), we calculated lead speciation at different total concentrations of lead and phosphate and at different pH levels. These calculations provided the theoretical basis for fitting the experimental design to maximum change in free lead (Pb^{+2}) concentration as a function of the phosphate, lead and pH levels.

TABLE 1

Synthetic media for toxicity bioassays.

Constituents	Concentration (M)
$Ca(NO_3)_2$	2.5×10^{-4}
$Mg(SO_4)$	1.0×10^{-4}
$Na_2(CO_3)$	1.9×10^{-4}
$K_2(PO_4)$	1.0×10^{-6}, 10^{-5}, 10^{-4}, or 10^{-3}

Table 2 contains the results of the REDEQL2 calculations for lead at pH 6 and 8 with 1, 3, and 5 mg/l total lead and 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M phosphate. We used mg/l to describe lead concentrations as this is the common terminology in aquatic literature. Molar units should be used for lead and phosphate in order to more accurately describe the stoichiometry of chemical reactions. At pH 6 very little free lead exists at high phosphate concentrations irrespective of the total lead concentration. Likewise, little free lead is predicted at any phosphate concentration at pH 8.

The hypothesis that free lead concentration reflects lead's biochemical activity better than total lead was tested with 120 hour static bioassays with the amphipod Hyallela azteca. Using a modification of PELTIER's (1978) methods, bioassays were applied with test solutions synthesized to fit the speciation patterns elucidated in the equilibrium analyses. The tests were conducted in 300 ml glass jars containing 250 ml of experimental media. The experimental media equilibrated for one week prior to experimentation; the amphipods acclimated for 24 hours in a similar, lead-free media.

TABLE 2

Free lead (Pb^{+2}) as a function of total lead and total phosphate at pH 6 and 8.

		Total lead (mg/l)		
		1	3	5
pH	Phosphate (M)	Free lead (mg/l)		
6	1×10^{-3}	0.02	0.02	0.03
	1×10^{-4}	0.12	0.11	0.11
	1×10^{-5}	0.60	1.05	2.24
	1×10^{-6}	1.00	2.75	2.76
8	1×10^{-3}	0.00	0.00	0.01
	1×10^{-4}	0.02	0.02	0.02
	1×10^{-5}	0.09	0.11	0.11
	1×10^{-6}	0.11	0.11	0.11

RESULTS AND DISCUSSION

Table 3 presents the mortality rate data for the first series of assays performed at pH 6. A graphical illustration of the mortality data for the maximum lead concentration (5 mg/l) at three phosphate concentrations is presented in Figure 1. This graph clearly demonstrates the detoxifying effect of increasing phosphate concentrations. The bioassay results at pH 8 reveal essentially the same pattern; that is, the highest mortality rates are associated with the highest free lead concentrations as predicted by equilibrium calculations. Although the mortality rates in the highest (10^{-3} M) phosphate concentrations do not fit the expected pattern, the high control mortalities suggest that physiological stress is associated with that particular synthetic media composition.

TABLE 3

Mortality (%) at pH 6.

Pb mg/ℓ	Phosphate (M)	Hours 12	24	36	48	60	72	84	96	108	120
0	1×10^{-3}	0	0	0	0	10	20	20	40	40	40
0	1×10^{-4}	0	0	0	0	0	9	9	9	18	18
0	1×10^{-5}	9	9	9	18	18	36	36	36	36	45
0	1×10^{-6}	0	0	0	0	8	33	33	33	33	33
1	1×10^{-3}	0	10	10	10	10	10	10	10	20	20
1	1×10^{-4}	0	0	0	0	0	0	8	25	25	25
1	1×10^{-5}	0	0	0	0	0	9	9	9	18	18
1	1×10^{-6}	8	50	83	91	91	91	91	91	91	91
3	1×10^{-3}	0	10	20	20	20	30	40	40	40	40
3	1×10^{-4}	0	0	0	0	9	9	9	9	9	9
3	1×10^{-5}	9	9	9	9	9	9	9	18	18	27
3	1×10^{-6}	8	58	83	91	91	100	100	100	100	100
5	1×10^{-3}	0	0	10	20	20	20	30	30	30	30
5	1×10^{-4}	0	0	0	0	0	0	8	8	16	25
5	1×10^{-5}	0	18	36	72	81	90	90	90	90	100
5	1×10^{-6}	0	16	75	100	100	100	100	100	100	100

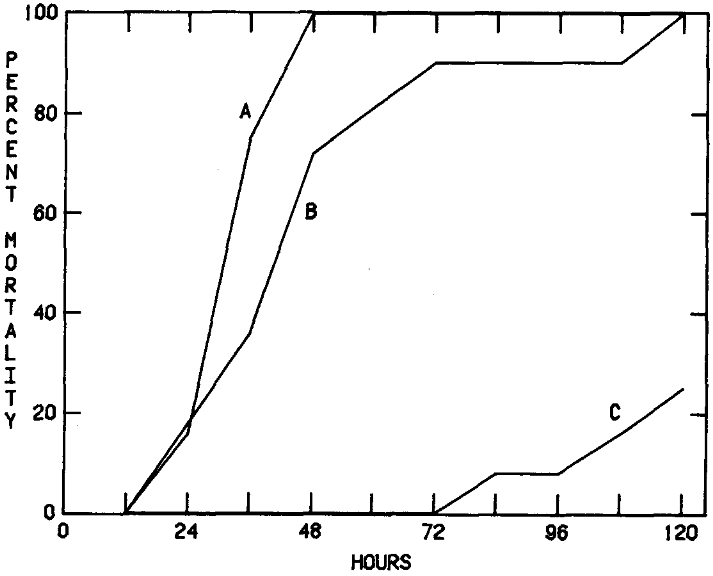

Figure 1. Percent mortality of Hyallela azteca at a lead concentration of 5 ppm and pH 6; Phosphate molarity of A, 10^{-6}; B, 10^{-5}; C, 10^{-4}.

In spite of the limitations of chemical and kinetic information, chemical equilibrium models provide a powerful tool for the examination of the effects of chemical interactions in aqueous systems. This is especially true in toxicity assays and in analyses of biogeochemical processes where alterations in the chemical composition of the medium may affect the chemical and biochemical activity of the elements. Support for the hypothesis that the free, uncomplexed chemical components are the most biochemically active species in aqueous chemical mixtures provides a valuable aid to the interpretation of the results of the theoretical speciation calculations.

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