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Effect of Zinc Chloro Complexes to Photoluminescent Bacteria: Dependence of Toxicity on Metal Speciation

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Environmental problems are multifold and complex, especially those arising from the disposal of hazardous materials. Therefore, a pressing requirement at present is to identify and assess the toxicity of such substances. Classically, chemical analyses have been the only technique used to identify contaminants in a water ecosystem. Sensitive analytical methodologies are available for determining total concentration of many contaminants and interpretation of the concentration data is relatively straightforward. However, these analysis have some important limitations when employed alone to assess the potential for toxicity. Traditionally, toxicant levels in water effluents and other sources have been estimated by bioassays employing micro and macrovertebrates. Lately, there has been an increased tendency to use microbial systems for screening toxicants as an alternative to tests with animals. Microbial assays are simple, rapid, inexpensive and reproducible. Of all the microbial systems, the Microtox test developed by (Bulich et al. 1981) is the most widely applied. Because the general presence of metals in our environment, studies of their toxicity have become important. In this context, the Microtox® assay has been used to screen the toxicity of some metallic salts (Rib6 et al. 1989; Hinwood and McCormick 1987; Dutka and Kwan 1981). In this method toxicity is measured in a 2% NaCl medium using the luminescent marine *Vibrio fischeri* When the bacteria are exposed to a toxic sample the emitted light is reduced in relation to the potency of the sample. The EC50, the effective concentration of toxicant that causes a light reduction of 50%, can be calculated.

In chloride containing media, most of the metals form chlorocomplexes (Baes and Mesmer 1976; Hinwood and McCormick 1987; Sillen and Mat-tell 1982; Stumm and Morgan 1996). The different metal speciation forms generally exhibit different physico-chemical properties and, therefore, may exert different effects on biological organisms. Recently, the influence of NaCl concentration on toxicity of some metallic salts has been reported (Villaescusa et al. 1996; 1998).

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In this study, zinc toxicity to luminescent bacteria was tested as a function of sodium chloride concentration. The results have been treated statistically, together with previously reported values for lead, cadmium and nickel in the same medium, to correlate the toxicity as a function of chloro-metal speciation complexes.

MATERIALS AND METHODS

The freeze-dried luminescent bacterium, *Vibrio fischeri,* and the reconstitution solution were supplied by AZUR Environmental. Zinc chloride and sodium chloride were reagent grade and were purchased from Merck (Darmstadt, Germany). Dilution solutions of different ionic strength were prepared by dissolving NaCl in pure water from a Milli-Q system (Millipore, Bedford, MA, USA).

The tests were performed using the Microtox Model 500 Toxicity Analyzer system from AZUR Environmental. The total metal concentration of solutions was determined with an Atomic Absorption Spectrophotometer Varian Techtron AA-1275/1475 (Springvale, Australia). The pH of solutions was monitored with a Crison Digilab 517 pHmeter.

Sample preparation. In order to ascertain the most suitable range of Zn(ll) concentration for toxicity determination, preliminary tests for each medium were conducted. Solutions of different concentration of $ZnCI_2$ in NaCl (0.34 to 1.02 mol/L) were used to evaluate the different toxicity of metal - chloride speciation complexes to the photoluminescent bacteria. The pH range in all the solutions was 5.0 to 6.0. At this pH there is neither formation of hydrocomplexes nor precipitation for zinc in the studied medium (Baes and Mesmer 1976).

EC50 determination. The EC50 values were obtained by following the experimental procedure described elsewhere (Villaescusa et al. 1996; 1997). The effective concentration was determined by using the GAMMA (Γ) function which is defined as the ratio of light lost to light remaining (Rib6 et al. 1987) and when plotting the log Γ vs log [metal] straight lines were obtained. The EC50 value is the concentration at which Γ=1.

Zn(II) species distribution diagrams. Species distribution diagrams for Zn(ll) species, at different total Zn(ll) concentration in different NaCl concentration (0.34-1.02 M) at pH 5-6 were obtained using equilibrium constants given in the literature (Sillen and Mar-tell 1982; Baes and Mesmer 1976) and a specially made computer program (Simple Equilibrium Diagrams TRITA-00K-3010, version IBMPC-F5a, Royal Institute of Technology, Stockholm, Sweden).

RESULTS AND DISCUSSION

The EC50 values obtained for Zn(ll) solutions at different sodium chloride concentrations for exposure times of 5 and 15 min, as well as the relative standard deviation (RSD) and the number of determinations (n), are presented in Table 1. If we compare these results with those found in literature (Hinwood and McCormick 1987, Carlson-Ekvall and Morrison 1995) there are some differences due presumably to the different experimental conditions and procedure employed.

In Table 1, two trends can be observed, there is a clear increase of EC50 values as the sodium chloride concentration is increased and an increase of toxicity with the exposure time. Both effects have been reported in previous metal toxicity determination studies (Babich and Stotzky 1978, 1979, 1981; Carlson-Ekvall and Morrison 1995; Hahne and Kroontje 1973; Hinwood and McCormick 1987; Villaescusa et al. 1996, 1998;). The former could be explained by taking into account the metal species distribution at different chloride concentration. The percentages of each species were calculated from zinc species distribution diagrams at the different ionic strengths and presented in Table 2. The second effect is due to the slow response of the bacteria to Zn(ll) toxicity in NaCl medium. This behavior is common for the response of the test organism to heavy metals (Rib6 et al. 1989). In this context, some experiments were carried out at longer exposure time and after 25 minutes a constant EC50 value was obtained.

Table 1. The EC50 values of zinc in different NaCl concentrations at pH= 5.0-6.0

NaCl (mol/L)	EC50-5 min (mg/L Zn(II))	RSD[a] (%)	n[b]	EC50-15 min (mg/L Zn(II))	RSD (%)	n
0.34	7.55	6.1	3	1.27	9.9	4
0.51	29.04	2.5	5	2.44	16.3	3
0.68	46.24	0.5	3	6.47	4.3	3
0.85	82.07	6.3	6	15.73	7.5	7
1.02	148.31	12.9	5	26.88	4.0	5

[a] Relative standard deviation : [b] Number of determinations.

In order to correlate the percentages of chloro-zinc complexes to the experimentally determined toxicities a statistical treatment was performed. The same procedure has also been applied to previous toxicity

determinations carried out in our laboratory for cadmium, lead and nickel under the same experimental conditions (Villaescusa et al. 1996; 1998). In all cases the pH range used was 5-6 to avoid either precipitation or hydrocomplexes formation (Baes and Mesmer 1976). A summary of the statistical correlations found for all these metals is shown in Table 3.

Table 2. Zn(ll) species percentage in different NaCl concentrations

NaCl (mol/L)	Zn^{2+} (%)	$ZnCl^{+}$ (%)	$ZnCl_2$ (%)	$ZnCl_3^{-}$ (%)	$ZnCl_4^{2-}$ (%)
0.34	81.74	14.14	2.85	0.95	0.31
0.51	76.60	15.83	4.27	2.13	1.15
0.68	71.31	16.63	5.51	3.66	2.87
0.85	65.62	16.79	6.51	5.41	5.67
1.02	59.54	16.42	7.24	7.22	9.57

Table 3. Pearson matrix correlation coefficients of aqueous metal speciation as a function of toxicity given as EC50-5 min and EC50-15 min (mg/L M(ll))

	Pb^{2+}	$PbCl^{+}$	$PbCl_2$	$PbCl_3^{-}$	$PbCl_4^{2-}$
Pb					
EC50-5	-0.908	-0.985	0	0.905	0.969
EC50-15	-0.771	-0.867	0	0.744	0.848
	Cd^{2+}	$CdCl^{+}$	$CdCl_2$	$CdCl_3^{-}$	$CdCl_4^{2-}$
Cd					
EC50-5	-0.892	-0.951	0.879	0.971	*
EC50-15	-0.833	-0.908	0.817	0.936	*
	Ni^{2+}	$NiCl^{+}$	$NiCl_2$	$NiCl_3^{-}$	$NiCl_4^{2-}$
Ni					
EC50-5	-0.919	*	0.919	*	*
EC50-15	-0.986	*	0.986	*	*
	Zn^{2+}	$ZnCl^{+}$	$ZnCl_2$	$ZnCl_3^{-}$	$ZnCl_4^{2-}$
Zn					
EC50-5	-0.969	0.626	0.919	0.976	0.996
EC50-15	-0.959	0.581	0.903	0.971	0.997

*indicates that the corresponding aqueous species is not present in the database used

The results presented in Table 3 have been evaluated taking into account that the ionic medium (NaCl) itself has no direct effect on the toxicity. This conclusion is based on similar phenol toxicity values reported in literature, under the same experimental procedures and NaCl concentration rate

used in this work (Hinwood and McCormick 1987; Villaescusa et al. 1996). Based on that, it is concluded that differences observed in toxicity values of Zn in Table 2 and correlations for all the metals presented in Table 3 are due to changes in the aqueous metal speciation. For all metals studied the results showed that when increasing the chloride concentration ions decreased the toxicity with the free ion being the most toxic form. In Table 3 it can be seen that EC50 and the free metal ion fraction show a clear negative correlation coefficient. In addition, the highest chloride complexed species show in all cases a clear positive correlation coefficient.

From the experimental results obtained by the authors in previous and the present work for the different metals studied Pb(II) was found to be the most toxic metal followed by Zn(II), Cd(II) and Ni(II). The The data also demonstrated that toxicity has a great dependence on the aqueous speciation in a sodium chloride medium. Therefore, for the metals studied, their total concentration in the aqueous solution does not give by its own a reliable indication of the toxicity of the sample. In other words, when complexing agents are present in the medium the observed toxicity has been found to be lowered with respect to the case where metal is mainly in its free form.

The use of 2% NaCl in the standard Microtox test procedure will then result, in cases as the ones studied in this work, in a toxicity determination that may not correspond to the one of the original sample. For this reason we postulate the necessity, in these cases, of developing a method of toxicity determination that should use a non-complexing medium (i.e. perchlorate).

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