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Toxicity of $CdCl_2$, CdEDTA, $CuCl_2$, and CuEDTA to Marine Invertebrates

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Trace metals in sea water are mainly associated with chlorides and other inorganic complexes but also may be associated with organic ligands (Blutstein and Smith 1978; Engel and Fowler 1979; Engel et al. 1981; Mantoura et al. 1978). It is of interest to know if organic complexation affords protection by reducing accumulation and lethality of metals to marine invertebrates.

In fresh water, humic acid reduces the lethality of copper to juvenile Atlantic salmon. The incipient lethal levels for Cu were 25, 90 and 165 ug/L with humic acid at 0, 5 and 10 mg/L, respectively (Zitko et al. 1973). Increasing salinity or increasing concentrations of nitrilotriacetic acid (NTA) reduced the toxicity of Cd to grass shrimp (Sundra et al. 1978). Humic acid reduced the acute and chronic toxicity of Cu but increased the toxicity of Cd to Daphnia (Winner 1984).

Chelation of cadmium with ethylenediaminetetraacetic acid (EDTA) reduced the 14 d bioconcentration factor for Cd by 45% for the marine polychaete, Nereis virens, and by 25% for shrimp, Pandalus montagui, (Ray et al. 1979) and by 45% for clams, Macoma balthica (McLeese and Ray 1984). Accumulation of Cd by the American oyster was reduced by up to 70% when Cd was complexed with EDTA, NTA, or humic acid (Hung 1982). Also, decreased accumulation of Cd in the presence of chelators was found for diatoms (Cossa 1976), phytoplankton (Härdstedt-Roméo and Gnassia-Barelli 1980) and barnacles (Rainbow et al. 1980). However, the opposite effect has been reported for mussels (George and Coombs 1977) and dinoflagellates (Prévot and Soyer 1978). With these exceptions, it appears that uptake of Cd by marine invertebrates is reduced when Cd is complexed with organic ligands. Humic acid, at least up to 0.75 mg/L, did not have a significant effect on the bioaccumulation of Cu or Cd from fresh water by Daphnia (Winner 1984).

The objective of this paper is to determine if toxicities of Cd and Cu to selected marine invertebrates are altered when the metals are complexed with EDTA. EDTA was chosen as a model for natural chelating agents.

MATERIALS AND METHODS

The lethality of Cd and Cu in chloride and EDTA forms was determined in static tests. Each concentration was tested with 4 to 6 animals in a glass beaker containing 1 L of test solution. The test solutions were maintained at 10°C, aerated gently, and were renewed every 48 h. Tests were terminated at 144 h. Cd and Cu chlorides were obtained from Fisher Chemical Co. and Cd and Cu disodium EDTA salts were obtained from K and K Laboratories, Plainsview, N. Y.

The test animals were clams, *Macoma balthica*, with valve length of about 1 cm; shrimp, *Crangon septemspinosa*, about 2 g each, *Pandalus montagui*, 1.0 to 1.6 g and polychaete worms, *Nereis virens*, 1.6 to 2.3 g. They were collected near St. Andrews, N. B. and were held at 10°C for at least 1 wk before testing.

Test solutions were sampled at 0, 24, 48 h (before and after solution change) and at 96 h (before the second solution change). The metals were measured by using graphite furnace atomic absorption spectroscopy techniques. Precision and accuracy of the analyses were confirmed against standard (NBS) reference material #1643 (water). The means of the measured concentrations of the metals, based on 4 to 12 samples for each concentration, were within ± 5% of the nominal concentrations.

Times to 50% mortality (LT_{50}) and 95% confidence limits at the various lethal concentrations of the metal were estimated by probit analysis (Litchfield 1949). The 144 h LC_{50}'s (concentrations that result in 50% mortality in specified time) were estimated as the geometric mean of the highest concentration with less than and the lowest concentration with more than 50% mortality at the specified time or were estimated from lethality lines where LT_{50}'s were plotted against test concentration on double logarithmic paper.

RESULTS AND DISCUSSION

When exposed to $CdCl_2$, *Macoma*, *Crangon* and *Pandalus* were killed at Cd levels of about 5.0, 2.0, and 2.5 mg/L and higher, respectively. When exposed to CdEDTA, *Macoma* survived for 144 h at the highest level tested, 50 mgCd/L, and *Crangon* survived for 144 h at 10 mgCd/L. However, there was no difference in lethality of Cd to *Pandalus* whether it was added as $CdCl_2$ or as CdEDTA, (Table 1).

Crangon, *Pandalus* and *Nereis* survived much higher levels of Cu when it was added as CuEDTA rather than $CuCl_2$. However, there was no difference in Cu lethality to *Macoma* (Table 2).

The relationships between lethality of the metals as chloride or EDTA forms are illustrated further by comparison of the 144 h LC_{50}'s (Table 3).

Table 1. Time to 50% mortality (LT_{50}) and 95% confidence limits (CL) for Cd as Cl_2 or EDTA.

Cd	Macoma		Crangon		Pandalus	
	Cl_2	EDTA	Cl_2	EDTA	Cl_2	EDTA
concentration (mg/L)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)
50	-	5/6	-	80 (55-116)	-	-
25	25 (23-27)	5/6	-	130, 2/4	-	-
10	31 (25-39)	5/6	30 (23-39)	4/4	-	84 (62-113)
5	70 (41-118)	6/6	45 (35-58)	4/4	98 (75-127)	120 (100-140)
2.5	6/6[a]	5/6	100 (85-118)	4/4	135, 2/4	80 (69-92)
1.0	-	-	4/4	4/4	-	-
0.5	-	-	-	-	4/4	4/4
0.05	-	-	-	-	4/4	4/4

[a]Fraction of animals surviving at 144 h.

Table 2. Time to 50% mortality (LT_{50}) and 95% confidence limits (CL) for Cu as Cl_2 or EDTA.

Cu	Macoma		Crangon		Pandalus		Nereis	
concentration	Cl_2	EDTA	Cl_2	EDTA	Cl_2	EDTA	Cl_2	EDTA
(mg/L)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)	LT_{50} (CL) (h)
30	-	-	-	4/4	-	4/4	-	140, 2/5
20	-	65 (52-80)	-	4/4	-	2/4	-	3/5
10	120 (104-138)	100 (82-122)	-	4/4	-	3/4	<6	5/5
10	-	120 (105-137)	-	-	-	-	-	-
8	100 (86-121)	120, 3/6	-	-	-	-	-	-
8	120 (105-137)	-	-	-	-	-	-	-
5	-	6/6	-	4/4	-	3/4	<6	5/5
4	5/6[a]	-	65 (56-75)	-	<6	-	-	-
2	5/6	-	55 (17-175)	-	<6	-	-	-
1	6/6	6/6	4/4	4/4	<6	-	45 (31-65)	5/5
0.5	5/6	-	4/4	4/4	12 (6-23)	-	80 (71-90)	-
0.5	-	-	-	-	11 (7-17)	-	-	-
0.1	6/6	-	4/4	-	13 (6-23)	-	5/5	-
0.1	-	-	-	-	90 (72-112)	-	-	-
0.05	-	-	-	-	100 (88-137)	-	5/5	-
0.05	-	-	-	-	100, 2/4	-	-	-

[a]Fraction of animals surviving at 144 h.

In the chloride form, Cu is considerably more toxic than Cd to Pandalus, is slightly more toxic to Crangon but is less toxic than Cd to Macoma (Table 3). With EDTA forms there is a reversal in the order of lethality of the metals. Cu is less toxic than Cd to Pandalus and Crangon but is more toxic than Cd to Macoma.

Table 3. 144 h LC_{50}'s for $CdCl_2$, CdEDTA, $CuCl_2$ and CuEDTA.

	144 h LC_{50} (mg/L)			
Metal	Macoma	Crangon	Pandalus	Nereis
$CdCl_2$	2.8	1.9	2.1	0.28
CdEDTA	>50.0	15.8	2.1	
$CuCL_2$	6	1.4	0.05	
CuEDTA	6	>30.0	>30.0	>30.0

Reasons for chelation reducing the lethality of one but not the other metal for Pandalus and Macoma are not known. Perhaps the differences are related to species specific responses to the metals. For example, the degree to which chelation reduces accumulation of the metals by the animals might correlate with change in lethality. Chelation of Cd with EDTA reduced the bioconcentration factor and greatly reduced the toxicity of Cd for Macoma. In Pandalus the effect on the bioconcentration factor was less, a reduction of 25% compared with 45%, and toxicity was unchanged. Information on effect of chelation on bioconcentration factor of Cu for the four invertebrates is not available for comparison with the effects of chelation on lethality. However, a simple relationship between change in bioconcentration factor and change in lethality is unlikely. Humic acid did not change accumulation of Cu or Cd by Daphnia but the toxicity of Cu was reduced and of Cd was increased (Winner 1984).

This paper shows that Cd and Cu in the EDTA form are considerably less toxic to some marine invertebrates. However, the exceptions, Cd and Pandalus, Cu and Macoma, indicate that reduction in toxicity afforded by chelation with EDTA is not universal for marine invertebrates.

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