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Toxicity Identification Evaluations (TIEs) of Freshwater Samples Using a Metal Chelating Resin

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Identification of toxicants in environmental samples is performed through the toxicity identification evaluation (TIE), a tiered process of physical and chemical manipulations combined with toxicity tests. One component of the TIE process is the assessment of toxicity caused by metals. For freshwater the U.S. Environmental Protection Agency (USEPA 1992, 1993) has recommended a standard approach using chelation with either ethylenediaminetetraacetate (EDTA) or sodium thiosulfate performed in a serial dilution. The use of ion-exchange resins was not recommended due to, among other reasons, wide pH changes in the treated water. Ion-exchange resins have successfully been used in saltwater and sediment TIEs by USEPA (1996) and Burgess *et al* (1997, 2000). The strong buffering capacity of seawater minimizes the activity of the resin treatment and pH changes less readily. The freshwater EPA methods require knowledge of the toxicity of the water in question based on preliminary testing with the selected test organism and water. A dilution series test is then conducted with the chelating agent and the water in question. Due to the flexibility in the EPA methods, phases I and II, the specific approach varies greatly but the methods in general require several rounds of testing and therefore a relatively large volume of water. The present TIE method was developed during a study of contaminants and their toxicity in small urban streams in the Puget Sound Region of Washington State. The study design called for the use of an automatic stormwater sampler and concurrent chemistry analysis. Hence, prior knowledge of possible toxicity could not be obtained and the water volume available for toxicity testing was limited, precluding the larger water volume required by the dilution series approach of the EPA methods. Therefore, a method for metal removal was developed treating water with batches of Chelex-100 ion-exchange resin. As opposed to chelation with EDTA or sodium thiosulfate, no preliminary testing was required and the water in question was tested with two treatments only: untreated and treated with Chelex-100 ion-exchange resin. The batch method was selected in an effort to leave the turbidity unchanged. TIE manipulations may cause changes in water parameters other than the one targeted. Consequently, the following water parameters were monitored at test initiation: pH, alkalinity, turbidity, dissolved oxygen and hardness, and during the tests: pH and dissolved oxygen. The TIE method was developed using creek samples fortified with five common toxic metals: Cd, Cu, Ni, Pb and Zn. Three chronic

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toxicity tests were conducted on two of the metals, Cd and Cu, with the alga *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum)*, the duckweed *Lemna minor* and the waterflea *Ceriodaphnia dubia.*

When sample volume constraints in the study design precludes EDTA or sodium thiosulfate as called for in the TIE protocols, the present method using batches of Chelex-100 ion-exchange resin provides an alternative that is easy to conduct and requires a smaller volume of water.

MATERIALS AND METHODS

Analytical-grade Chelex-100 ion-exchange resin (Sigma-Aldrich Chemical Co, St. Louis, MO, USA), 50-100 mesh, in the sodium form was used in units of 1.0 g (as weighed from the original container) per 100 mL of buffer or water sample. The selection of Chelex-100 resin was based on prior work by Buckley (1985). Chelex-100 resin is a styrene-divinylbenzene copolymer containing paired iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions. It has a very strong attraction for transition metals. Before usage the resin was treated with a 0.75 M acetate buffer solution according to Buckley (1985) to control for increases in pH and alkalinity. The strength of the acetate buffer solution depends on the pH of the water to be treated. In the present work, 0.75 M acetate buffer solution was prepared from 51 g sodium acetate trihydrate, 21.9 mL glacial acetic acid diluted to 1 L with deionized water (DW). Ten g of resin per 1 L of treated water sample was used to conduct the toxicity tests. Batches of 6.0 - 7.0 g Chelex-100 resin were added to 600 - 700 mL 0.75 M buffer in 1 L beakers. The resin buffer suspension was mixed for 30 min on a magnetic-mixer. The resin was recovered on a S&S no. 404 filter (Schreicher & Schuell MicroScience, Rivera Beach Fl, USA) and rinsed for 30 min with more than 3 L of DW. The resin on the filter was drained on paper towels for approximately 1 min before being rinsed into a 1 L wide mouth high-density polyethylene Nalgene bottle. Until usage the next day, 2/3 of the DW was replaced three times over 24 hr and the bottle stored in a refrigerator. The resin can be kept longer than 24 hr, however, holding time was not investigated. Just prior to test initiation the resin was recovered on a S&S filter and drained on paper towels for approximately 2 min. The resin was rinsed into the 1 L wide mouth high-density polyethylene Nalgene bottle using 600 - 700 mL of the water sample to be treated and the bottle was tumbled for 45 min. For multiple tests, the treated water batches were combined in a large beaker prior to test initiations. The resin was siphoned out and the hardness of the treated water was restored to a value similar to the untreated water with $CaCl_2$.

Five singular metals were used in the present study: Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (Table 1). Cd and Zn were introduced as chloride salts and obtained from Fisher Scientific (Fair Lawn, NJ, USA) and EM Science (Cherry Hill, NJ, USA), respectively. Cu and Ni were introduced as sulfate salts and both were obtained from Baker Chemical Co. (Phillipsburg, NJ, USA). Pb was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and introduced as nitrate salt. Metal

concentrations were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Themo Jarrell-Ash ICP61 (USEPA 1990). The water samples were digested with nitric and hydrochloric acids and heated to ionize elements associated with metal complexes and particulate matter. Results were reported as total metals. The method detection limits were 3 µg/L for Cd, 4 µg/L for Cu, 20 µg/L for Ni, 30 µg/L for Pb and 5 µg/L for Zn. Elution with acid was not performed on the resin batch samples. Turbidity was measured by the nephelometic method using a Hach 2100AN Turbidimeter (APHA 1998). The detection limit was 0.5 NTU.

Continuous in-house axenic cultures of *Raphidocelis subcapitata* were raised in synthetic algal assay medium (AAM) prepared according to USEPA (1994) and modified by the addition of 11 vitamins (Goulden *et al.* 1982). The *Raphidocelis* microplate chronic toxicity test was conducted as outlined in Environment Canada (1992). The *Raphidocelis* flask chronic test was conducted as outlined in USEPA (1994). Continuous in-house cultures of *Lemna minor* were raised in synthetic Hoagland's medium prepared according to ASTM (1991), modified by omitting sucrose, yeast extract and bactotryptone and filtered to 0.45µm. The *Lemna* chronic toxicity test was conducted according to ASTM (1991) modified by static-renewal procedure and use of Hoagland's medium at 10% of full strength. Continuous in-house cultures of *Ceriodaphnia dubia* were raised in static-renewal mass cultures and fed daily with the green algae *Raphidocelis subcapitata* (1 mL @ $36x10^6$ cells per mL) and a diet of YT with a final solids concentration of 7-12 mg/L (USEPA 1994). The *Ceriodaphnia* chronic toxicity test was conducted as outlined in USEPA (1994). Each of the chronic toxicity tests with *R. subcapitata*, *L. minor* and *C. dubia* were tested with the following treatments: in-house control, untreated creek water, Chelex-100 resin treated creek water, metal fortified creek water and metal fortified creek water treated with Chelex-100 resin.

The statistical analyses were conducted using the Toxcalc statistical program (Tidepool Scientific Software 1992-1994). The program is specifically developed for analysis of toxicity tests and includes Shapiro-Wilk's test, Bartlett's test, F-test and t-tests.

RESULTS AND DISCUSSION

Ion-exchange columns with different types of resins have frequently been used to separate metals from water samples (Pakalns *et al.* 1978; Florence *et al.* 1992). However, in the freshwater TIE procedures published by USEPA (1992, 1993) the recommended methods were chelation with either EDTA or sodium thiosulfate. Ion-exchange resins were considered more difficult to use due to the non-chemical specific property of the resins and wide pH changes in post-column treated water (USEPA 1993). However, the first shortcoming is also shared with the recommended metal complexors, EDTA and sodium thiosulfate, and the latter shortcoming has been addressed by washing the ion-exchange resin with buffer (Buckley 1985). Therefore, the use of Chelex ion-exchange resin appears to be an attractive alternative with the added advantages of being a simple test method and

Table 1. Concentrations of five common metals in metal fortified creek water and post Chelex-100 ion exchange resin treatment.

	Metal Concentrations, µg/L		
Metal	Metal Fortified	Metal Fortified & Chelex Treatment	% Metal Reduction
Cd	53.1	5.4	90
Cd	53.2	7.6	86
Cd	54.5	3.3	94
Cd	55.0	< 3.0	> 95
Cd	231	5.8	97
Cd	269	13.0	95
Cd	275	13.0	95
Cu	60.9	21.7	64
Cu	116	14	88
Cu	118	30.5	74
Cu	130	34.8	73
Cu	141	12	91
Cu	175	31.3	82
Cu	307	20.3	93
Cu	363	54	85
Cu	427	22.4	95
Ni	411	< 5	> 99
Ni	419	< 5	> 99
Ni	711	< 5	> 99
Pb	258	155	40
Pb	471	185	61
Pb	869	60	93
Zn	237	18	92
Zn	395	7.9	98
Zn	772	7.6	99

requiring less sample volume for the evaluation. The effectiveness of the treatment was evaluated using five metals commonly found in the environment (Table 1). The Chelex-100 resin has a stated binding capacity of 0.4 meq/mL or approximately 0.7 meq/g. Hence, the 6 to 7 g resin used in this study had a binding capacity of 4.2 – 4.9 meq. Based on the relatively large amount of resin and fortified metal concentrations with a maximum of 0.02 meq, the binding capacity of the resin was never exceeded. The order of selectivity for cation metals given by the manufacturer is: $Cu^{2+} > Pb^{2+} > Fe^{3+} > Al^{3+} > Cr^{3+} > Ni^{2+} > Zn^{2+} > Ag^{2+} > Co^{2+} > Cd^{2+} > Fe^{2+} > Mg^{2+} > Ba^{2+} > Ca^{2+}$. Metal absorption is a function of pH with very low absorption at pH 2 and maximum at pH 4 remaining high through pH 6.5. The Chelex treatment reduced the concentrations of Cd, Ni and Zn by at least 86% (Table 1). These removal rates were similar to or better than the rates measured in batch samples and columns using 8-hydroxyquinoline or Chelex-100 resins in seawater and distilled water (Burgess *et al.* 1997). The reduction of Cu ranged from 64% to 95% and was negatively correlated with the turbidity of the water sample ($r = -0.83$) indicating that the presence of particulate or organic matter reduced the efficiency of the resin. The reduction of Pb ranged from 40% to 93%. Studies performed with lake water have found a negative correlation between the dialyzable proportion of Cu and Pb with organic carbon (TOC) values (Salbu and Steinnes 1995). In addition to the colloidal organic

Table 2. pH measurements in untreated and Chelex-100 ion-exchange resin treated creek water samples from nine 7-day chronic toxicity tests with *Ceriodaphnia dubia*.

Time of Analysis	pH Range in Control & Post- Chelex Treatments	Percent of Control ± SD Post Chelex Treatment	Metal Fortified Post Chelex Treatment
0hr	7.40 – 8.05	96.6 ± 1.7	98.0 ± 1.0
24hr	8.12 – 8.31	99.4 ± 0.2	99.4 ± 0.4
0hr	7.52 – 8.06	96.7 ± 1.1	97.1 ± 1.4
24hr	7.68 – 8.30	98.9 ± 0.8	98.7 ± 0.8
0hr	7.34 – 8.19	96.3 ± 2.2	98.2 ± 1.3
24hr	8.00 – 8.29	99.3 ± 0.3	99.4 ± 0.3
0hr	7.34 – 8.03	97.1 ± 0.7	97.4 ± 2.4
24hr	7.94 – 8.12	99.2 ± 0.3	99.3 ± 0.3
0hr	7.42 – 7.96	97.7 ± 1.4	98.5 ± 1.3
24hr	7.96 – 8.15	99.5 ± 0.2	99.7 ± 0.2
0hr	7.63 – 8.24	96.6 ± 1.5	95.9 ± 1.9
24hr	8.26 – 8.44	99.0 ± 0.2	98.9 ± 0.2
0hr	7.44 – 8.05	97.4 ± 0.8	97.7 ± 0.6
24hr	7.63 – 8.07	99.2 ± 0.4	99.0 ± 0.4
0hr	7.50 – 7.97	97.9 ± 1.2	98.0 ± 0.8
24hr	7.85 – 8.00	99.0 ± 0.1	99.1 ± 0.3
0hr	7.45 – 8.31	94.3 ± 2.4	94.3 ± 1.6
24hr	8.35 – 8.52	98.9 ± 0.3	98.7 ± 0.4

complexes, Pb was found to form particulate-bindings. In the TIE study by Burgess *et al.* (2000) the presence of resin in the sediment lowered the concentrations of Cu in the overlying water but only after 24 hr, whereas the Pb concentrations in the water remained, but were random, likely due to the concentrations being close to instrument detection limit. Both metals were considered to be particle-active which may explain the unusual distributions compared to the more soluble metals Cd, Ni and Zn.

The effort of the present study focused on an alternative method to the EDTA and sodium thiosulfate TIE phase I methods controlling the pH and leaving the turbidity unchanged; hence the TIE phase II verification of metals by post-treatment elution of the resin was not performed.

One of the main problems often associated with using resins in a TIE process is large pH changes measured in post-treatment water (USEPA 1993). In the present study, pH was measured daily in the newly prepared solutions (0 hr) and in the 24-hr old solutions in nine 7-day chronic toxicity tests with *C. dubia*. As shown in Table 2, the acetate buffer washing of the resin prior to test initiation eliminated the large pH changes in the post-treatment waters and the pH remained unchanged in the two post-Chelex treatments when compared to the control.

Both alkalinity and hardness were affected by the Chelex treatment. Alkalinity was reduced by approximately 30 percent (69.01 ± 6.21%, n = 22) and hardness was reduced to 0 mg/L as $CaCO_3$. Alkalinity can be restored by the addition of

Table 3. Comparison of Chelex-100 resin control with untreated control from chronic toxicity tests using creek water and *R. subcapitata*, *L. minor* and *C. dubia*.

Untreated Control	Treated Control	% of Untreated Control	Untreated Control	Treated Control	% of Untreated Control
Raphidocelis subcapitata (growth)**			*Lemna minor* (growth)		
381	372	97.6	32.39	33.81	104.4
256	230	89.8*	23.89	25.25	105.7
407	393	96.6	29.05	30.59	105.3
382	355	92.9	29.14	31.13	106.8
374	362	96.8	29.44	28.24	95.9
379	353	93.1	29.05	30.58	105.3
361	340	94.2	33.42	33.73	100.9
348	388	111.5	23.32	24.07	103.2
261	217	83.1*	31.57	32.88	104.1
218	205	94.0	26.86	29.16	108.6
185	221	119.5	30.57	32.47	106.2
152	154	101.3	31.57	32.88	104.1
169	188	111.2	28.58	30.39	106.3
187	186	99.5	26.12	23.95	91.7*
n = 14	Mean ± SD = 98.7 ± 9.6		28.10	30.75	109.4
Ceriodaphnia dubia (reproduction)			29.41	28.79	97.9
27.8	27.8	100.0	26.36	25.40	96.4
28.8	28.1	97.6	19.31	19.15	99.2
25.8	25.8	100.0	26.34	24.82	94.2
28.3	30.6	108.1	33.47	31.43	93.9
29.2	29.3	100.3	n = 20	Mean ± SD = 102.0 ± 5.3	
31.2	33.2	106.4			
29.0	29.7	102.4			
26.8	30.5	113.8			
n = 8	Mean ± SD = 103.6 ± 5.4				

Data in the table are endpoint values in untreated and Chelex-100 ion-exchange resin treated control, and the treated endpoint value as a percentage of the untreated value. *R. subcapitata* mean cell counts x 10^4, *C. dubia* mean number of offspring per female and *L. minor* mean growth (mg). * Significantly ($p < 0.05$) lower growth than in the controls. ** *R. subcapitata* tests with cells counts > 340 were conducted in Erlenmeyer flasks according to USEPA (1994).

$NaHCO_3$ and hardness by the addition of $CaSO_4$ and $MgSO_4$ or by the more readily dissolved $CaCl_2$. In the present toxicity tests, hardness was restored to pretreatment values by the addition of $CaCl_2$. The post-treatment alkalinity values were left unadjusted because differences were minor and the adjustments were deemed unnecessary. At the onset of the TIE method development, the manipulations were aimed at not changing the turbidity of the water sample due to preliminary testing which indicated that toxicity could be associated with particulate matter. Hence, a resin column was not used in favor of batch treatment. Repeated measurements taken in an untreated creek water sample and in a Chelex batch-treated subsample found no significant difference in turbidity (2-tailed t-test, $p > 0.05$) ($n=10$; 8.78 ± 0.48 vs 8.92 ± 0.32 NTU). A comparison based on single measurements taken from several different creek samples found similar turbidity values in the pre- and post-Chelex treatments. A mean value of $93.7 \pm 23.3\%$ was calculated by taking the turbidity in the Chelex treatment as a percentage of the turbidity in the untreated water over 32 single sampling events.

The key to useable data from a TIE is to obtain non-toxic blanks (USEPA 1993). Because TIE manipulations can have different effects on organisms, chronic toxicity tests were conducted with three different organisms: the alga *Raphidocelis subcapitata*, the monocot *Lemna minor,* and the crustacean *Ceriodaphnia dubia*. The treatment control was non-toxic (one-tailed t- test, $p > 0.05$) in 39 of 42 toxicity tests (92.9%) (Table 3). Two tests with *R. subcapitata* and one with *L. minor* had significantly reduced growth (t-test, $p < 0.05$) in the treatment control with a reduction ranging between 9.3 and 16.9%. For each test, a comparison was performed on the endpoint values of the untreated and Chelex treated controls by taking the Chelex control treatment value as a percentage of

Table 4. *Raphidocelis subcapitata* cell counts and metal concentrations before and after treatment with Chelex-100 ion-exchange resin. Tests performed in creek water.

	Metal Concentrations, µg/L		Mean ± SD Cell Count x 10^4		
	Fortified	Metal Fortified & Chelex Treatment	Control	Metal Fortified	Metal Fortified & Chelex Treatment
Cd	231	5.8	261 ± 16	25 ± 3*	220 ± 2*
Cd	275	13.0	218 ± 11	17 ± 2*	199 ± 11
Cd	269	13.0	185 ± 11	11 ± 1*	173 ± 3
Cu	427	22.4	154 ± 5	2 ± 1*	144 ± 4*
Cu	363	54.0	169 ± 7	2 ± 1*	153 ± 1*
Cu	307	20.3	187 ± 16	2 ± 0*	169 ± 12

* Significantly lower cell count ($p < 0.05$) than the untreated control.

Table 5. *Lemna minor* mean growth and metal concentrations before and after treatment with Chelex-100 ion-exchange resin. Tests performed in creek water.

	Metal Concentrations, µg/L		Mean ± SD growth, mg		
	Fortified	Metal Fortified & Chelex Treatment	Control	Metal Fortified	Metal Fortified & Chelex Treatment
Cd	231	5.8	26.12 ± 0.66	19.83 ± 0.84*	25.51 ± 1.88
Cd	275	13.0	28.10 ± 2.40	16.27 ± 0.87*	30.20 ± 2.44
Cd	269	13.0	29.41 ± 2.61	17.80 ± 2.04*	29.93 ± 1.22
Cu	427	22.4	19.31 ± 1.36	5.04 ± 0.48*	19.81 ± 1.08
Cu	363	54	26.34 ± 2.43	5.74 ± 0.22*	27.45 ± 2.53
Cu	307	20.3	33.47 ± 3.53	8.24 ± 0.46*	34.72 ± 2.81

* Significantly lower growth ($p < 0.05$) than the untreated control.

Table 6. *Ceriodaphnia dubia* mean number of offspring per female and metal concentrations before and after treatment with Chelex-100 ion-exchange resin. Tests performed in creek water.

	Metal Concentrations, µg/L		Mean ± SD Reproduction per Female		
	Fortified	Metal Fortified & Chelex Treatment	Control	Metal Fortified	Metal Fortified & Chelex Treatment
Cd	53.2	7.6	28.8 ± 2.7	All Dead	27.4 ± 2.9
Cd	55.0	< 3.0	25.8 ± 6.4	All Dead	23.9 ± 4.0
Cd	54.5	3.3	28.3 ± 4.5	All Dead	30.1 ± 2.8
Cu	141	12	31.2 ± 2.1	All Dead	32.1 ± 4.0
Cu	118	30.5	29.0 ± 2.0	All Dead	23.7 ± 3.6*
Cu	116	14	26.8 ± 1.8	All Dead	30.2 ± 1.5

* Significantly lower reproduction ($p < 0.05$) than the untreated control.

the untreated water value. The following mean values were calculated: 98.7 ± 9.6% (*R. subcapitata*, n = 14), 102.3 ± 5.0% (*L. minor*, n = 20) and 103.6 ± 5.4% (*C. dubia*, n = 8).

Chronic toxicity tests were conducted on creek samples fortified with either Cd or Cu. The treatment with Chelex-100 resin reduced the concentrations of metals in the tests by between 74 - 97%. The remaining fraction of the fortified metal probably had formed strong complexes with inorganic and organic ligands in the creek water and was therefore not removed by the Chelex treatment. Generally, the unremoved, complexed metal was not bioavailable or the metal concentration had been reduced, in most cases, to non-toxic levels. Overall, the Chelex resin treatment significantly increased the endpoint values when compared to the metal fortified treatment values in all tests to levels equal to, and in most cases not significantly different from, the endpoint values in the untreated control water. All fortified concentrations were highly toxic to the three organisms (Tables 4, 5 and 6). In all but four of the tests the toxic metal concentration was reduced by the Chelex treatment to levels causing no significant difference (t-test, $p > 0.05$) in the given endpoint when compared to the control. Of those four tests, the three conducted with *R. subcapitata* had a growth increase ranging between 87 - 99% when compared to the metal fortified treatment. However, the relatively low standard deviation surrounding the mean counts may have caused the significant difference.

The present study provides an additional metal chelation method to those already recommended in EPA TIE documents for investigating the presence of toxic metals in an environmental sample. The method presented here uses limited volumes of water and provides an easy comparison between the untreated toxic sample with the Chelex-100 resin treated sample. Hence, the users may find the present method suitable for phase I manipulation when the objective is to narrow down the number of possible toxic contaminants.

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