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TOXICITY OF SIXTY-THREE METALS AND METALLOIDS TO *HYALELLA AZTECA* AT TWO LEVELS OF WATER HARDNESS

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Abstract—The toxicity of all atomically stable metals in the periodic table, excluding Na, Mg, K, and Ca, was measured in one-week exposures using the freshwater amphipod *Hyalella azteca* in both Lake Ontario, Canada, and soft water (10% Lake Ontario). Metals were added as atomic absorption standards (63 metals), and also as anion salts for 10 metals. Lethal concentrations resulting in 50% mortality (LC50s) were obtained for 48 of the metals tested; the rest were not toxic at 1,000 μg/L. The most toxic metals on a molar basis were Cd, Ag, Pb, Hg, Cr (anion), and Tl, with nominal LC50s ranging from 5 to 58 nmol/L (1 to 58 nmol/L measured). These metals were followed by U, Co, Os, Se (anion), Pt, Lu, Cu, Ce, Zn, Pr, Ni, and Yb with nominal LC50s ranging from 225 to 1,500 nmol/L (88–1,300 nmol/L measured). Most metals were similarly or slightly more toxic in soft water, but Al, Cr, Ge, Pb, and U were >17-fold more toxic in soft water; Pd was less toxic in soft water. Atomic absorption (AA) standards of As and Se in acid had similar toxicity as anions, Sb was more toxic as the AA standard, and Cr and Mn were more toxic as anions. One-week LC50s for *H. azteca* correlate strongly with three-week LC50s and three-week effect concentrations resulting in 50% reduction in reproduction (EC50s) in *Daphnia magna*.

Keywords—Metals Toxicity *Hyalella azteca* Periodic table

INTRODUCTION

Under the Canadian Environmental Protection Act, 1999 [1], 23,000 substances on Canada's Domestic Substances List (DSL) must be categorized by 2006. Categorization (as defined in the Canadian Environmental Protection Act) involves evaluation of the substances on the basis of their persistence, bioaccumulation, and inherent toxicity. Substances that meet specified criteria for inherent toxicity, as well as either persistence or bioaccumulation, will undergo screening assessments. For the 1,500 mostly metal-containing inorganic substances and organic metal salts on the DSL, toxicity is a key determinant of the outcome of categorization. A common ion approach will be used to increase the efficiency of the process. For example, for all copper-containing substances that are water-soluble and fully dissociate, toxicity will be estimated on the basis of lethal concentrations resulting in 50% mortality (LC50s) for dissolved forms of the Cu ion [2]. Although categorization of substances on the DSL for inherent toxicity ideally should use both aquatic (including benthic) and terrestrial species, an overwhelming majority of experimental ecotoxicological data has been obtained in tests with aquatic species. In addition, virtually all of the quantitative structure-activity relationship estimates (as well as experimental toxicity data) have been generated employing external effect concentrations in the aquatic environment. Therefore, the aquatic compartment, applying external median lethal (LC50) or effective (EC50) concentrations, has been used systematically to categorize the substances on the DSL [2].

The inherent toxicity criterion is extremely important in this exercise. The term inherent toxicity, as applied to the assessment of substances in a regulatory-legal milieu, can be defined as ''the degree of being poisonous'' [3]. In the present context, acute toxicity testing to aquatic organisms is the approach selected to assess inherent toxicity due to the paucity of published data for toxicity of inorganic substances and organic metal salts on the DSL. Application of the same test to the majority of substances being categorized (i.e., in well-controlled and well-documented experimental conditions) allows the determination of the relative toxicity or potency of these chemicals. The categorization of inherent toxicity is based on a criterion of 1 mg/L for acute LC50 values (dissolved forms of the metal ion in the present case). This numerical cut-off is in agreement with some well-recognized international initiatives, such as the Organization for Economic Cooperation and Development's Screening Information Data Set [2]. To assist in the categorization process, two federal government laboratories (one using *Hyalella* and another using *Daphnia*) have conducted tests for acute lethality using standard methodologies approved by the Canadian stakeholders interested in the process [2]. In this paper, we present the results of a suite of aquatic toxicity tests performed by the National Water Research Institute (Burlington, ON, Canada). The toxicity of all atomically stable metals in the periodic table (in this paper we use the term metals loosely to include metalloids such as As and Se) was determined in one-week toxicity tests conducted using the freshwater amphipod *Hyalella azteca* (Crustacea) in both Burlington City tap (Lake Ontario, Canada) and soft (10% tap) water. The only metals excluded from this study were those that do not possess stable isotopes (Tc, Pm, and elements with atomic numbers above 83, other than U and Th) and the major ions Ca, Mg, Na, and K. The latter four ions are present in excess of 1 mg/L in tap water and are considered of little toxicological interest.

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A one-week toxicity test with young *Hyalella*, including feeding, was used because 48-h acute tests without food are difficult to conduct with young *Hyalella*, which swim and stick to the surface if not fed. The one-week test corresponds to the first week of a four-week or 10-week chronic test, therefore, allowing data from the first week of chronic tests to be compiled together with and compared to data from one-week tests using the same or other substances. The time interval between one-week and four to 10-week chronic toxicity tests is shorter than between 48- or 96-h acute tests and four to 10-week chronic tests, and one-week toxicity is presumed more likely to be proportional to (and hence predictive of) chronic toxicity; the one-week test fits well within a 7-d work week (tests can be set up any day of the week, maximizing the number of tests that can be run). Reproduction in *Hyalella* usually begins after about five to six weeks in chronic toxicity tests initiated with less than one-week-old animals, and 10-week tests are sufficient to obtain reliable estimates of the effects of toxic substances on reproduction [4–6]. Metals were tested at concentrations of up to 1,000 (soft water) or 3,150 (tap water) μg/L. A one-week test represents roughly one-tenth the time required for measuring reproduction, although individual amphipods can live for many months in the laboratory. Compared to typical tests conducted with *Daphnia magna* (acute test = 2 d, reproduction test = 21 d [7]), this 7-d test still should be considered an acute test.

The data presented here provide a single set of toxicity values for all metals at two levels of water hardness for a single test species collected under identical conditions. Although these data were collected specifically for categorization of substances on Canada's DSL, they also provide a useful overview of metal toxicity to *Hyalella*, including rarely studied metals, and indicate which metals are highly toxic and perhaps deserve further scrutiny.

METHODS

Hyalella used for toxicity tests originated from Valens Conservation Area (ON, Canada), in 1985 and were cultured as described in Borgmann et al. [8]. Culture water was dechlorinated Burlington City tap (Lake Ontario, Canada) water (hardness 124 mg/L, carbonate alkalinity 84 mg/L, Ca 35 mg/L, Mg 8.7 mg/L, Na 13 mg/L, K 1.6 mg/L, SO_4 32 mg/L, Cl 25 mg/L, and dissolved organic carbon [DOC] 1.1 mg/L from January 2001 to October 2003, $n = 69$, coefficient of variation = 3–12%, except DOC, which was 74%). Culturing and toxicity tests were conducted in an incubator at 24 to 25°C under a 16:8-h light:dark photoperiod. Culture water was renewed and young separated from adults weekly on Mondays. Toxicity tests were set up Tuesday to Friday, making the initial age of the test animals 1 to 11 d at the start of the test.

Most experiments were conducted using atomic absorption standards containing 1 g metal/L. This was less expensive than purchasing metals salts, and the stock solutions can be expected to contain fully dissolved metals. A list of all standards used in the toxicity tests is provided in Table 1. For selected metals commonly present as oxy-anions (As, Cr, Mn, Mo, Sb, Se, Sn, Te, V, and W), tests were repeated using Na salts (i.e., sodium arsenate, $Na_2HAsO_4 \cdot 7H_2O$; chromate, Na_2CrO_4; permanganate, $NaMnO_4$ solution in water; molybdate, $Na_2MoO_4 \cdot 2H_2O$; antimonate, $NaSbO_3$; selenate, Na_2SeO_4; stannate, $Na_2SnO_3 \cdot 3H_2O$; tellurite, Na_2TeO_3; orthovanadate, Na_3VO_4; tungstate, $Na_2WO_4 \cdot 2H_2O$). Anion salt stocks were made up to 0.1 g/L expressed as mass of metal.

Table 1. List of metal standards and preservative used in toxicity tests

Preservative	Preservative concn. (%)	Metal standards used
None (water)	—	B, Nb[a]
HCl	1	Cs, Hf,[a] Li, Rb
HCl	2	Al, Au, Ba, Be, Bi, Ce, Dy, Er, Eu, Fe, Ga, Gd, Ho, In, La, Lu, Mo, Nd, Pr, Re, Sc, Se, Sm, Sr, Tb, Th, Tm, V, Y, Yb
HCl	5	Pd, Pt, Zr[a]
HCl	8	Sn[a]
HCl	10	Ir, Os, Rh, Ru
HCl	20	Sb, Te,[b] Ti
HNO_3	1	Ge, Tl[a]
HNO_3	2	Ag, As, Cd, Co, Cr, Cu, Mn, Ni, Pb, U, Zn
HNO_3	3.2	Hg[a]
HNO_3/HF	1/0.1	Ta[a]
KOH	2	Te[ab], W[a]

[a] Obtained from Sigma-Aldrich (Oakville, ON, Canada); all others from Delta Scientific (Mississauga, ON, Canada).

[b] Both Te standards preserved in HCl and KOH were tested.

To test a large number of metals with a minimum of test treatments (i.e., the fewest number of redundant tests at 0% and 100% mortality), the basic experimental design was modified from the classical toxicity test. Instead of testing one or a few metals at many concentrations at once, a large number of substances were tested simultaneously at one concentration only (either the maximum concentration of interest, or a concentration close to the predicted toxic threshold) in the first experiment. The concentration of each substance was then either increased or decreased in the next experiment, depending on whether mortality was observed. This procedure was repeated until the toxic range was covered for each substance, or until the substance was demonstrated to cause less than 50% mortality at the highest concentration of interest. Repeat tests were then conducted on either side of the LC50. If estimates of survival from replicate test containers for each concentration near the LC50 differed by more than 25%, tests were repeated giving a total of up to 2 to 5 replicates per test concentration. The number of concentrations tested was reduced from the usual 10, 18, 32, 56, 100 logarithmic series to 10, 32, 100. This provided a less precise estimate of the LC50, but allowed determination of LC50s for a much larger number of metals within a shorter time frame. Chronic toxicity test data already were available for a number of metals in tap water (Co, Cu, Hg, Mn, Ni, Pb, Tl, Zn). Data from the first week of these chronic tests were extracted and used to compute one-week LC50s. For these metals in tap water, the more usual concentration series (10, 18, 32, 56, 100) was used.

Tests were static, nonrenewal, one-week exposures conducted in 400 ml of test water in 500-ml polyethylene cups (snap-top specimen containers). Relatively large test volumes were used in order to reduce the surface area:volume ratio and decrease potential adsorption, and also to reduce pipetting variability from handling small volumes of stock solutions. Two sets of experiments were run, one in tap (Lake Ontario) water (see above for chemistry), and another in soft water consisting of 10% tap water and 90% Milli-Q® (Millipore, Bedford, MA, USA) deionized water (soft water measured hardness 18 mg/L, carbonate alkalinity 14 mg/L, Ca 5.6 mg/L, Mg 0.90

Table 2. Number of readings, pH, and conductivity (μS/cm^2) at the end of one-week exposures for test solutions made with metal standards or anion salts. Values are listed separately for controls and standards with different acid concentrations

Sample	*n*	pH	Range	Conductivity	Range
Soft (10% tap) water					
Controls	142	7.37	(6.79–7.84)	46	(34–70)
0–5% Standards[a]	448	7.39	(6.44–8.52)	66	(35–235)
8–10% HCl standards	20	7.71	(7.26–8.16)	235	(50–360)
20% HCl standards	18	8.27	(7.67–8.68)	447	(222–614)
Tap water (Lake Ontario, Canada)					
Controls	109	8.39	(8.09–8.84)	311	(288–345)
0–5% Standards[a]	384	8.21	(7.23–8.83)	345	(288–958)
8–10% HCl standards	47	8.30	(7.82–8.93)	515	(296–1,290)
20% HCl standards	23	8.46	(7.93–8.98)	730	(303–1,670)

[a] Includes HCl, HNO_3, and KOH standards as well as anion salts without acid.

mg/L, Na 1.4 mg/L, K 0.15 mg/L, SO_4 3.4 mg/L, Cl 2.5 mg/L, and DOC 0.28 mg/L, *n* = 17, coefficient of variation = 5–11%, except for Ca 45%, alkalinity 51% and DOC 69%). Calcium and alkalinity in the soft water were more variable than the other ions and ranged from 10 to 20% of expected concentrations because an airstone was inadvertently used to aerate the test water. Soft water tests were designed to simulate a reasonable worst-case condition for Canada (e.g., dilute waters of the Canadian Shield with a low DOC content), while still falling within the range tolerated by *Hyalella*. A solution of 19 parts 1 M $NaHCO_3$ plus 1 part 1 M KOH (similar to the Na:K ratio of the test water) was used to neutralize excess acid in the metal standards and control pH. Sufficient buffer to control pH, if required, was added first, followed by addition of the metal standard. This resulted in better survival of acid-controls than adding the metal solution first. Buffer was not added for tests with anion salts. Neutralization was required primarily for tests conducted in soft water. Acid-controls consisted of acid and neutralizing solution additions equal to the amount added in the tests with acidified metal standards. Survival in acid-controls was 82% of survival in soft water controls at the highest metal concentration (1,000 μg/L) for metal stocks supplied in 10% HCl, however, this dropped to 32% at 1,000 μg/L for metal stocks supplied in 20% HCl. Hence, toxicity measured at 1,000 μg/L for Sb, Te (in HCl), and Ti in soft water partly may be caused by the acid and not the metal. Following addition of neutralizing buffer and metal, the water was aerated gently overnight to allow equilibration of pH and CO_2, and any rapid changes in metal speciation that might occur. Initial pH and conductivity were then measured. A piece of 2.5 × 2.5-cm cotton gauze and 2.5-mg Tetra-Min® (Ulrich Baensch, Melle, Germany) fish food flakes were then added to each container, followed by addition of 15 young *Hyalella*. Test containers were not aerated during the test. An additional 2.5 mg of food was provided midweek. After 7 d, the pH, conductivity, and ammonia and oxygen concentrations were measured, and the number of survivors was counted.

Each experiment consisted of three controls, three acid controls, if needed, and one replicate of each metal to be tested in that experiment. As many metals as possible were tested in each experiment. Depending on the test results, the concentration of each metal was either increased (if nontoxic) or decreased (if toxic) by a factor of 10 in the next experiment. Once the toxic range was bracketed, intermediate concentrations (i.e., 3.15-fold higher or lower) were tested. This procedure was repeated until each metal was tested at least twice at a concentration resulting in <25% survival, at a concentration resulting in >75% survival, and at all intermediate concentrations, relative to controls. Only data from experiments with ≥80% control survival were used. Using this procedure made it possible to cover the toxic range of a much larger number of metals than would have been possible if each metal had been tested sequentially in a concentration series.

Routine major ion analyses were performed on tap water and on each batch of soft (10% tap) water used (see above for chemistry). In addition, DOC was measured in randomly selected samples of the test containers at the end of the 7-d exposure and averaged 1.4 mg/L (*n* = 83, coefficient of variation 31%, range 0.7–3.6). Major ion and DOC analyses were conducted by the National Laboratory for Environmental Testing.

Water samples for metal analyses were not collected for tests conducted in tap water using atomic absorption (AA) standards, which were completed first, but were collected from all test solutions at the end of the 7-d exposure in tests conducted in soft water, and all tests with anion salts. Measured metal concentrations in tap water also were available for previously conducted toxicity tests with some metals. Filtered (0.45 micron) samples were collected using disposable filter cartridges (Acrodisks®, Pall Canada Limited, Mississauga, ON) attached to polypropylene syringes, acidified with high purity nitric or hydrochloric acid (or preserved with KOH), and stored in 14-ml capacity Falcon® (Becton Dickinson, Franklin Lakes, NJ, USA) polypropylene disposable round bottom tubes with snap caps. Water samples were preserved in the same acid (or KOH) and concentration that was supplied with the metal standards. Metal analyses were performed by inductively coupled plasma mass spectrometry by the National Laboratory for Environmental Testing. Quality assurance/quality control methodology included calibration checks at the initiation and completion of each run, and verification and drift standards. Certified reference standards also were included when available (about half the metals). Also included were machine blanks, sample blanks (collected at the same time as the samples), control samples (from control exposures without added metals), and acid controls (from exposures without added metal but with acid and base additions equivalent to those added along with the metals).

The concentration resulting in 50% mortality (LC50) and 95% confidence limits were computed using the Trimmed Spearman-Karber method [9]. In cases where the confidence limits could not be computed reliably (e.g., if there were no partial effect concentrations), the concentrations tested on either side of the LC50 are listed. Most curve fitting methods

Table 3. *Hyalella* one-week lethal concentrations resulting in 50% mortality ([LC50] μg/L) and confidence limits (CL) for metals in tap and soft water added as atomic absorption standards in acid (unmarked) or in base (KOH) or as anion salts

Atomic no.	Metal	Soft water (nominal) LC50	Soft water (nominal) (95% CL)	Soft water (measured) LC50	Soft water (measured) (95% CL)	Tap water (nominal) LC50	Tap water (nominal) (95% CL)	Tap water (measured) LC50	Tap water (measured) (95% CL)
3	Li	650	(447–945)	650	(456–928)	3,130	(1,743–5,622)	—	—
4	Be	120	(89–163)	67	(53–85)	240	(181–316)	—	—
5	B	2,773	(1,548–4,968)	2,935	(1,638–5,258)	>3,150	—	—	—
13	Al	186	(165–210)	89	(79–100)	>3,150	—	—	—
21	Sc	100	(76–131)	29	(25–33)	175	(100–315)	—	—
22	Ti	979	(707–1,355)	<272	—	>3,150	—	—	—
23	V	989	(616–1,588)	1,251	(790–1,980)	1,032	(675–1,577)	—	—
23	V (anion salt)	334	(247–452)	368	(271–500)	>1,000	—	—	—
24	Cr	>1,000	—	—	—	>3,150	—	—	—
24	Cr (anion salt)	2.9	(2.2–3.9)	3.1	(2.4–4.0)	159	(123–205)	137	(106–176)
25	Mn	>1,000	—	—	—	5,049[a]	(3,967–6,426)	2,729	(2,140–3,479)
25	Mn (anion salt)	181	(100–315)	92	(55–147)	774	(315–1,000)	169	(100–197)
26	Fe	>1,000	—	—	—	>3,150	—	—	—
27	Co	16	(11–23)	16	(11–23)	89[a]	(75–106)	61	(52–72)
28	Ni	77	(58–101)	75	(55–101)	147[b]	(133–162)	133	(119–147)
29	Cu	56	(32–100)	36	(21–61)	121[c]	(109–135)	90	(82–99)
30	Zn	70	(59–83)	56	(46–68)	404[c]	(366–446)	222	(201–245)
31	Ga	>1,000	—	—	—	>3,150	—	—	—
32	Ge	190	(152–236)	209	(167–261)	>3,150	—	—	—
33	As	465	(298–724)	494	(319–765)	426	(293–618)	—	—
33	As (anion salt)	596	(449–790)	581	(437–772)	484	(395–594)	483	(393–594)
34	Se	60	(32–100)	41	(24–63)	118	(88–158)	—	—
34	Se (anion salt)	49	(41–59)	43	(36–52)	432	(319–584)	371	(283–487)
37	Rb	>1,000	—	—	—	>3,150	—	—	—
38	Sr	>1,000	—	—	—	>3,150	—	—	—
39	Y	183	(136–245)	66	(44–101)	549	(394–764)	—	—
40	Zr	>1,000	—	—	—	>3,150	—	—	—
41	Nb	250	(177–354)	26	(16–43)	1,938	(1,692–2,219)	—	—
42	Mo	>1,000	—	—	—	>3,150	—	—	—
42	Mo (anion salt)	>1,000	—	—	—	>1,000	—	—	—
44	Ru	>1,000	—	—	—	>3,150	—	—	—
45	Rh	980	(648–1,482)	804	(517–1,251)	>3,150	—	—	—
46	Pd	>1,000	—	—	—	570	(443–732)	—	—
47	Ag	1.72	(1.00–3.15)	0.25	(0.07–1.00)	1.05	(0.88–1.26)	—	—
48	Cd	0.57	(0.43–0.76)	0.15	(0.12–0.19)	4.41	(3.47–5.60)	1.60	(1.21–2.11)
49	In	>1,000	—	—	—	>3,150	—	—	—
50	Sn	>1,000	—	—	—	>3,150	—	—	—
50	Sn (anion salt)	>1,000	—	—	—	>1,000	—	—	—
51	Sb	576	(460–720)	687	(553–855)	>3,150	—	—	—
51	Sb (anion salt)	>1,000	—	—	—	>1,000	—	—	—
52	Te	>1,000	—	—	—	1,519	(1,093–2,111)	—	—
52	Te (KOH)	>1,000	—	—	—	2,336	(1,747–3,124)	—	—
52	Te (anion salt)	>1,000	—	—	—	>1,000	—	—	—
55	Cs	>1,000	—	—	—	>3,150	—	—	—
56	Ba	>1,000	—	—	—	>3,150	—	—	—
57	La	229	(162–322)	18	(18–19)	1,665	(1,000–3,150)	—	—
58	Ce	131	(88–197)	32	(14–70)	651	(521–813)	—	—
59	Pr	183	(161–209)	35	(30–41)	441	(332–585)	—	—
60	Nd	337	(260–436)	55	(45–67)	511	(315–1,000)	—	—

Table 3. Continued

Atomic no.	Metal	Soft water (nominal)		Soft water (measured)		Tap water (nominal)		Tap water (measured)	
		LC50	(95% CL)	LC50	(95% CL)	LC50	(95% CL)	LC50	(95% CL)
62	Sm	296	(231–378)	74	(57–95)	846	(603–1,188)	—	—
63	Eu	405	(239–688)	112	(69–181)	717	(535–962)	—	—
64	Gd	450	(319–636)	150	(107–209)	599	(424–845)	—	—
65	Tb	365	(252–528)	84	(58–122)	693	(455–1,054)	—	—
66	Dy	485	(140–1,676)	162	(34–769)	897	(671–1,198)	—	—
67	Ho	494	(397–614)	143	(109–188)	755	(528–1,079)	—	—
68	Er	559	(335–933)	191	(101–362)	929	(696–1,239)	—	—
69	Tm	721	(458–1,133)	0.01	(0.01–0.02)	739	(492–1,110)	—	—
70	Yb	248	(189–326)	69	(48–99)	278	(216–357)	—	—
71	Lu	120	(90–160)	29	(21–39)	1,054	(756–1,471)	—	—
72	Hf	>1,000	—	—	—	>3,150	—	—	—
73	Ta	353	(285–436)	2	(1.8–2.1)	1,977	(1,750–2,234)	—	—
74	W (KOH)	>1,000	—	—	—	>3,150	—	—	—
74	W (anion salt)	>1,000	—	—	—	>1,000	—	—	—
75	Re	>1,000	—	—	—	>3,150	—	—	—
76	Os	93	(69–125)	81	(61–108)	57	(49–67)	—	—
77	Ir	>1,000	—	—	—	>3,150	—	—	—
78	Pt	131	(102–168)	110	(86–140)	221	(165–296)	—	—
79	Au	841	(590–1,199)	446	(151–1,319)	>3,150	—	—	—
80	Hg	8.4	(6.9–10.2)	NA[d]	—	10.0[c]	(8.7–11.6)	2.1	(1.9–2.5)
81	Tl	12	(9–16)	12	(9–16)	49[e]	(45–53)	46	(42–49)
82	Pb	4.8	(3.3–7.1)	1.0	(0.7–1.5)	113[c]	(101–126)	11	(10–12)
83	Bi	722	(315–1,000)	25	(10–36)	2,543	(1,720–3,758)	—	—
90	Th	473	(303–737)	5.2	(4.4–6.2)	>3,150	—	—	—
92	U	54	(44–65)	21	(17–26)	1,651	(1,451–1,878)	—	—

[a] W. P. Norwood (National Water Research Institute, Burlington, ON, Canada, unpublished data).

[b] Data from Borgmann et al. [4].

[c] Data from Borgmann et al. [5].

[d] Not available. Hg appears to have been lost from samples upon storage.

[e] Data from Borgmann et al. [6].

Table 4. Percent survival and measured metal concentrations at 315 and 1,000 μg/L for metal solutions where lethal concentrations resulting in 50% mortality (LC50) could not be calculated (i.e., >1,000 or >3,150 μg/L values in Table 3). Survival values have not been corrected for control survival

Atomic no.	Metal	% Survival at 315 μg/L in soft water	% Survival at 1,000 μg/L in soft water	Measured metal at 315 μg/L in soft water	Measured metal at 1,000 μg/L in soft water	Survival at 1,000 μg/L in tap water
5	B	—	80[a]	—	1,058	94
13	Al	16	30[a]	150	198	95
22	Ti	80	44[a]	<272	<272	77
23	V (anion salt)	50	0[a]	336	1,187	67
24	Cr	89	71	—	63	93
25	Mn	77	67	344	595	65[a]
26	Fe		73	—	15	95
31	Ga	82	48	326	750	88
32	Ge	20	0[a]	351	—	87
37	Rb	83	64	—	970	92
38	Sr	82	87	—	1,029	93
40	Zr	60	60	20	4.2	70
42	Mo	—	97	—	1,090	87
42	Mo (anion salt)	—	97	—	937	100
44	Ru	77	63	229	688	68
45	Rh	70	43[a]	239	822	71
46	Pd	87	87	—	355	20[a]
49	In	89	87	—	10	90
50	Sn	87	60	<638	<638	87
50	Sn (anion salt)	—	97	—	<638	100
51	Sb (anion salt)	—	90	—	197	93
52	Te (anion salt)	—	90	—	265	93
52	Te (HCl)	87	47	417	1,064	73[a]
52	Te (KOH)	—	75	—	142	82[a]
55	Cs	—	89	—	1,048	77
56	Ba	84	93	—	1,102	89
72	Hf	87	90	—	2.9	93
74	W (anion salt)	—	90	—	1,058	90
74	W (KOH)	—	68	—	1,080	94
75	Re	90	80	—	1,123	86
77	Ir	—	78	—	1,107	83
79	Au	80	37[a]	22	758	77
90	Th	60	20[a]	3.3	9.1	90

[a] See Table 3 for LC50 estimate.

could not be used because mortality curves for *Hyalella* tend to be fairly steep and a concentration series with each concentration increasing by a factor of 3.15 over the previous concentration usually will result only in one partial effect concentration. The LC50s for Cu and Cd were compared to previously published values by performing linear regressions of log(LC50) against pH, log(hardness) and log(test duration) for available published data, and comparing observed LC50s in this study to predicted LC50s from the regressions based on published data.

Table 5. Ratio of the lethal concentration resulting in 50% mortality (LC50) in soft (10% tap) water divided by the LC50 in tap water for different metals

LC50 ratio	Metals
<0.6	Pd
0.6–1	Ag, As, As (anion), Os
1–2.5	Be, Cu, Dy, Er, Eu, Gd, Hg, Ho, Nd, Ni, Pr, Pt, Sc, Se, Tb, Tm, V, Yb
>1	B
2.5–10	Bi, Cd, Ce, Co, La, Li, Lu, Mn (anion), Nb, Se (anion), Sm, Ta, Tl, Y, Zn
>2.5	Au, Rh, Sb, Th, Ti, V (anion)
>10	Al (>17), Cr (anion, 54), Ge (>17), Pb (23), U (31)

RESULTS AND DISCUSSION

Addition of AA standards and neutralizing buffer contributed to conductivity somewhat, and this was most noticeable for AA standards made up in >5% HCl. The effect was proportionately greater in soft water (Table 2). Final oxygen values ranged from 7 to 10 mg/L ($n = 1,161$) and temperature from 24 to 25°C (mean 24.7, $n = 46$). Of 1,252 ammonia readings, all were <0.1 mM, except for 10 that ranged from 0.1 to 0.24 mM. In eight of these cases, survival ranged from 80 to 100%, and in the other two it was 60 to 67% (both for solutions of Ge at 3,150 μg/L in tap water with ammonia at 0.21 mM). The four-week LC50 for ammonia in tap water is about 0.95 mM [10]. Ammonia, therefore, does not appear to have contributed noticeably to toxicity.

We obtained LC50s and corresponding confidence limits for 48 of the 63 metals tested, either as AA standards or anions in either soft or tap water (Table 3). The LC50s for the other metals were >1,000 μg/L in soft water or >3,150 μg/L in tap water (Table 4). The LC50s in tap water usually were either similar to (0.6–2.5-fold) those in soft water, or slightly higher (2.5–10-fold, Table 5). Some metals, including the AA standards of Al, Ge, Pb, and U, and the anion form of Cr, were much more toxic (>17–54-fold) in soft water. Palladium was more toxic in tap (LC50 = 570 μg/L) than in soft water (>1,000 μg/L). When this unusual observation was first made

Table 6. Comparison of atomic absorption (AA) standards in acid and anion (KOH standards or anion salts[a]) lethal concentrations resulting in 50% mortality (LC50, μg metal/L) based on nominal and measured (in parentheses) concentrations

	Soft water		Tap water	
Metal	Acid AA standard LC50	Anion LC50	Acid AA standard LC50	Anion LC50
Similar toxicity				
Se	60 (41)	49 (43)	118	432 (371)
As	465 (494)	596 (581)	426	484 (483)
More toxic as AA standard				
Sb	576 (687)	>1,000	>3,150	>1,000
More toxic as anion				
Cr	>1,000	2.9 (3.1)	>3,150	159 (137)
Mn	>1,000	181 (92)	5,049 (2,729)	774 (169)
More toxic as anion in soft water only				
V	989 (1,251)	334 (368)	1,032	>1,000
Low toxicity for both				
Mo	>1,000	>1,000	>3,150	>1,000
Se	>1,000	>1,000	>3,150	>1,000
Te	>1,000	>1,000	1,519	>1,000
Te (KOH)	—	>1,000	—	2,336
W	—	>1,000	—	>1,000
W (KOH)	—	>1,000	—	>3,150

[a] Na_XMO_Y, Y = 3 (Sb, Sn, Te) and 4 (all other salts); valence = 4 (Sn, Te), 5 (Sb, V), 6 (Se, As, Cr, Mo, W), or 7 (Mn).

for Pd, another experiment was set up with two replicates of Pd at 1,000 μg/L in both tap and soft water, measured simultaneously using the same batch of test animals. This verified that survival was greater in soft water (9 and 13 survivors out of 15) than in tap water (one and three survivors).

The LC50 confidence limits provided in Table 3 should be viewed as an approximate guide to data reliability only. When confidence limits are computed using a standard test procedure (i.e., a concentration range with all concentrations tested simultaneously), the limits provide a measure or reliability for that specific test, and not for the chemical in question. True confidence limits should be computed by repeating the test multiple times, estimating the LC50 for each test separately, and computing the mean and standard deviation of those

Table 7. Comparison of metal toxicity (lower of the lethal concentration resulting in 50% mortality [LC50] in soft and tap water) on a mass and molar basis grouped according to metal solubility (measured metal in solution recovered at the end of the one-week exposure)

Rank	LC50	<10% Recovery	10–75% Recovery	>75% Recovery
Mass basis				
1	<3.2 μg/L	—	Ag, Cd	Cr (anion salt)
2	3.2–10 μg/L	—	Hg, Pb	—
3	10–32 μg/L	—	—	Co, Tl
4	32–100 μg/L	—	Cu, Se, U	Ni, Os, Se (anion salt), Zn
5	100–320 μg/L	La	Al, Be, Ce, Lu, Mn (anion salt), Nb, Pr, Sc, Sm, Y, Yb	Ge, Pt
6	320–1,000 μg/L	Bi, Ta, Th, Ti, Tm	Au, Dy, Er, Eu, Gd, Ho, Nd, Pd, Tb	As, As (anion salt), Li, Rh, Sb, V, V (anion salt)
7	>1,000 μg/L	Cr, Fe, Hf, In, Sn, Sn (anion salt), Zr	Mn, Ru, Sb (anion salt), Te (anion salt), Te (KOH)	B, Ba, Cs, Ga, Ir, Mo, Mo (anion salt), Rb, Re, Sr, Te (HCl), W (anion salt), W (KOH)
Molar basis				
1	<16 nmol/L	—	Ag, Cd	—
2	16–50 nmol/L	—	Hg, Pb	—
3	50–160 nmol/L	—	—	Cr (anion salt), T1
4	160–500 nmol/L	—	U	Co, Os
5	500–1,600 nmol/L	—	Ce, Cu, Lu, Pr, Se, Yb	Ni, Pt, Se (anion salt), Zn
6	1,600–5,000 nmol/L	Bi, La, Ta, Th, Tm	Au, Dy, Er, Eu, Gd, Ho, Mn (anion salt), Nb, Nd, Sc, Sm, Tb, Y	Ge, Sb
7	>5,000 nmol/L	Cr, Fe, Hf, In, Sn, Sn (anion salt), Ti, Zr	Al, Be, Mn, Pd, Ru, Sb (anion salt), Te (anion salt), Te (KOH)	As, As (anion salt), B, Ba, Cs, Ga, Ir, Li, Mo, Mo (anion salt), Rb, Re, Rh, Sr, Te (HCl), V, V (anion salt), W (anion salt), W (KOH)

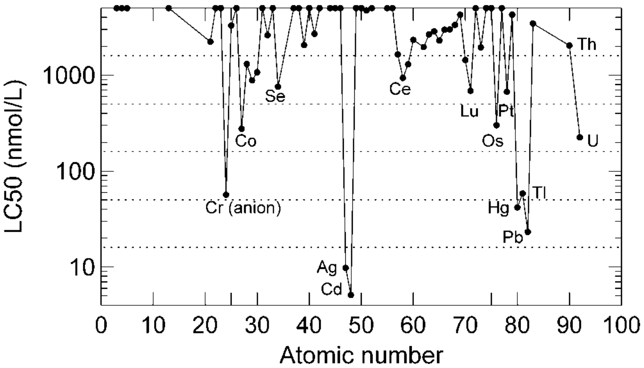

Fig. 1. One-week lethal concentrations resulting in 50% mortality ([LC50] lower value of tap and soft water) for metal toxicity to *Hyalella azteca* plotted against atomic number. All metals with LC50s above 5,000 μmol/L are shown along the top of the figure. In cases where tests were conducted using both the anion salt and the atomic absorption standard, the more toxic form is shown. The equivalent LC50s for all 63 metals expressed as μg/L are listed in Table 3.

LC50s. In the present study, multiple tests were conducted, but only one or a few concentrations of each metal were included in each test. The data were then pooled and analyzed as if they had all been obtained from a single test. This provides an approximate guide to data reliability, but the statistical procedures were not really designed to evaluate results from the novel test procedure used in this study, as necessitated by the large number of metals tested.

Toxicity of anion salts compared to AA standards

A number of metals commonly are present in the environment as anions under oxic conditions at pH 7 to 8 (e.g., $HAsO_4^{2-}$, CrO_4^{2-}, SeO_3^{2-}) [11]. These metals are not always the same forms as expected at low pH values in acidified AA standards. Although the anion may be favored thermodynamically, the conversion to the thermodynamically stable form could be a slow process and may not be completed within the first few days of the experiment. Therefore, it is possible that use of AA standards could under- or overestimate toxicity observed under natural conditions for these metals. To investigate this possibility, the toxicity of several anion salts also was tested and compared to the toxicity of the AA standards. The toxicity of metal anions, if measurable, usually was similar to or greater than that of the AA standards in acid (Table 6). The metalloids As and Se demonstrated similar toxicity as AA standards and anions. Chromium and Mn were much more toxic as anions than as AA standards. Vanadium was more toxic in anionic form, but only in soft water. A number of metals (Mo, Sn, Te, W) were relatively nontoxic regardless of their ionic form. Antimony (Sb) was the only metal clearly more toxic as the AA standard. However, the AA standard of Sb was preserved in 20% HCl, the highest acid concentration (Table 1), and required considerable neutralization. Some of this toxicity may have been associated with the acid preservative, rather than the metal itself. Similarly, the slightly higher toxicity of the acid AA standard of Te compared to the KOH standard (Table 6), although not statistically significant (Table 3), might have been associated with the much higher (20%) amount of acid compared to the amount of base (2%) required in the preservative (Table 1). The toxic AA standards of Se, As, Cr, Mn, and V (Table 6) were all preserved in low acid (2%), and the acid likely did not affect toxicity. The similar toxicity between anions and AA standards in acid observed for a number of these metals implies that either their toxicity is equivalent or that the conversion from one ionic form to the other occurs sufficiently rapidly that differential toxicity is not observed in the one-week tests. Overall, anion toxicity, if measurable, usually was greater in soft than in tap water (except for As), analogous to the situation for most AA standards (Table 6).

Relative toxicity ranking

The ranking of metal toxicity can be done on either a mass or molar basis. Most published data report metal toxicity on a mass basis (i.e., μg/L). Furthermore, metal AA standards are produced and sold on a mass basis. Toxicity tests in this study, therefore, were set up and the results compared to other published data, using mass units. Ranking on a mass basis also is more relevant from an environmental hazard classification perspective, because chemicals are shipped and regulated on a mass basis. However, ranking by molar units is more relevant on a chemical stoichiometric basis. Either way, Ag and Cd are the most toxic metals, followed by Hg, Pb, and then Tl (Table 7). The anion of Cr, on the other hand, is extremely toxic on a mass basis, but slightly less so on a molar basis. This occurs because it is a much lighter (52 g/mol) element that Ag, Cd, Hg, Pb, or Tl (108–204 g/mol). Similarly, Al and Be, two very light elements (27 and 9 g/mol), are much more toxic (rank 5) on a mass than on a molar (rank 7) basis (Table 7). Cobalt, Cu, Ni, Se, Zn (59–79 g/mol), and several other less toxic metals also are slightly less toxic on a molar basis, relative to the heavier elements. On the scientifically more relevant molar basis, Ag and Cd are the metals most toxic to *Hyalella*, followed by Pb, Hg, Tl, and Cr (anion). The LC50s range from 5 to 58 nmol/L nominal, or 1 to 58 nmol/L based on final measured concentrations. Next most toxic are Co, Os, and U with LC50s between 225 and 490 nmol/L nominal, or 88 and 430 nmol/L measured. These metals are followed by Se, Ce, Lu, and Pt and then Ni, Cu, Zn, Pr, and Yb, with LC50s ranging from 670 to 1,500 nmol/L nominal or 160 to 1,300 nmol/L measured (Fig. 1, Table 7). This list includes most of the commonly studied metals, but it also includes a number of less well-studied metals (e.g., Os, U, Ce, Lu, Pt, Pr, and Yb).

Metal toxicity expressed as metal added (nominal) is affected by solubility. The more toxic metals were all either soluble (>75% recovery at the end of the one-week exposure) or partly soluble (10–75% recovery). None of the sparingly soluble metals (<10% recovery) were extremely toxic (Table 7).

Comparison to other published LC50s for Hyalella

Four- to 14-d LC50s for metal toxicity to *Hyalella* measured in other studies compare favorably with those reported here, especially for Cd and Cu that have been studied most extensively (Table 8). Nickel and Se appeared to be slightly more toxic in the present study than in others, but in both cases there was only one other study with an LC50 for >4 d. Both test duration and water chemistry can affect toxicity. The hardness effect is well-studied for metals but is not necessarily due to Ca or Mg ions; it could be due to any associated cations (e.g., K^+ in the case of Tl toxicity [6]), or even anions (e.g., $SO4^{2-}$ in the case of selenate [12]), which increase in concentration roughly in proportion with hardness. It also partly can be the result of increased carbonate or hydroxide complexation and, hence, reduced metal bioavailability at the higher alkalinity associated with increased hardness [13]. A de-

Table 8. Comparison of published data on 4- to 14-d lethal concentrations resulting in 50% mortality (LC50, μg/L) for toxicity of metal cations and the selenate anion to *Hyalella azteca* at different hardnesses and pH (uncertain values followed by ?)

Test duration (days)	Hardness (mg/L)	Alkalinity (mg/L)	pH	Ag	Al	Cd	Cu	Hg	Mn	Ni	Pb	Se (anion)	U	Zn	Reference
4	6–10	9–21	6.9–8.0	—	—	—	66	—	—	—	—	—	—	—	[15]
4	6–28	8–18	5.5–7.7	—	—	2.8	—	—	—	—	—	—	—	—	[16]
4	9	—	6.4	—	—	—	—	4	—	—	—	—	—	—	[17]
4	10	8	7.0	—	—	3.8	—	—	—	—	—	—	—	—	[18]
4	10–15	10–22	6.9–7.5	6.8	—	—	—	—	—	—	—	—	—	—	[19]
4	15.3	5.2	5.0	—	>1,000	12	—	—	—	—	10	—	—	—	[20]
4	15.3	5.2	5.5	—	>400	16	—	—	—	—	21	—	—	—	[20]
4	15.3	5.2	6.0	—	>400	33	—	—	—	—	18	—	—	—	[20]
4	26	40	8	—	—	—	—	—	3,000	—	—	—	—	—	[21]
4	34	31	7.1	—	—	8	—	—	—	—	—	—	—	—	[22]
4	35.2	32.3	7.7	1.9	—	—	—	—	—	—	—	—	—	—	[23]
4	52?	—	6.7	—	—	—	—	—	—	—	—	741	—	—	[24]
4	80	80	8.3	—	—	—	—	—	8,600	—	—	—	—	—	[21]
4	80–124	8	7.0	—	—	6–12	—	—	—	—	—	—	—	—	[18]
4	90	—	7.4–8.1	—	—	6.5–14	34–53	—	—	—	—	—	—	200–350	[25]
4	98	64	7.7–8.0	—	—	—	—	—	—	3,045	—	—	—	—	[26]
4	100	60	—	—	—	—	—	—	—	—	—	—	—	436	[27]
4	120–140	75–100	7.5–8.5	—	—	13	210	—	—	3,620	—	—	—	—	[28]
4	133	302	8.6	—	—	—	—	—	—	—	—	1,868	—	—	[29]
4	143	—	7.4–8.2	—	—	—	—	—	—	—	—	1,350–3,580	—	—	[12]
4	164	164	8.4	—	—	—	—	—	13,700	—	—	—	—	—	[21]
4	185–379	8	7.0	—	—	12–55	—	—	—	—	—	—	—	—	[18]
4	280–300	225–245	6–6.5	—	—	230	17	—	—	2,000	<90	—	—	1,200	[30]
4	280–300	225–245	7–7.5	—	—	<25	24	—	—	1,900	>5,400	—	—	1,500	[30]
4	280–300	225–245	8–8.5	—	—	5	87	—	—	890	>5,400	—	—	290	[30]
7	6–10	9–21	6.9–8.0	—	—	—	53	—	—	—	—	—	—	—	[15]
7	6–28	8–18	5.5–7.7	—	—	1.7		—	—	—	—	—	—	—	[16]
7	18	14	7.4	1.72 (0.25)[a]	186 (89)	0.57 (0.15)	56 (36)	8.4	>1,000	77 (75)	4.8 (1.0)	49 (43)	54 (21)	70 (56)	This study
7	124	84	8.3	1.05	>3,150	4.41 (1.60)	121 (90)	10 (2.1)	5,049 (2,729)	147 (133)	113 (11)	432 (371)	1,651	404 (222)	This study
10	6–10	9–21	6.9–8.0	—	—	—	67	—	—	—	—	—	—	—	[15]
10	6–28	8–18	5.5–7.7	—	—	1.2	—	—	—	—	—	—	—	—	[16]
10	<10	<10	6.9–7.0	—	—	—	42	—	—	—	—	—	—	—	[31]
10	10–15	10–22	6.9–7.5	5.8	—	—	—	—	—	—	—	—	—	—	[19]
10	34?	31?	6.8?	—	—	—	59	—	—	—	—	—	—	—	[32]
10	34	31	7.1	—	—	<2.8	—	—	—	—	—	—	—	—	[22]
10	22–64	22–63	7.4–8.2	—	—	—	92–143	—	—	—	—	—	—	—	[31]
10	44	45	7.3	—	—	—	31	—	—	—	—	—	—	—	[33]
10	44–47	45–46	6.7–7.4	—	—	—	—	—	—	780	—	—	—	—	[34]
10	44–47	45–46	6.7–7.4	—	—	—	—	—	—	—	<16	—	—	73	[35]
10	133	302	8.6	—	—	—	—	—	—	—	—	1,135	—	—	[29]
14	6–10	9–21	6.9–8.0	—	—	—	44	—	—	—	—	—	—	—	[15]
14	6–28	8–18	5.5–7.7	—	—	0.65	—	—	—	—	—	—	—	—	[16]
14	98	64	7.7–8.0	—	—	—	—	—	—	>120	—	—	—	—	[26]
14	157	137	7.91	—	—	—	—	—	—	—	—	—	1,520	—	[36]

[a] Nominal (measured values in parentheses).

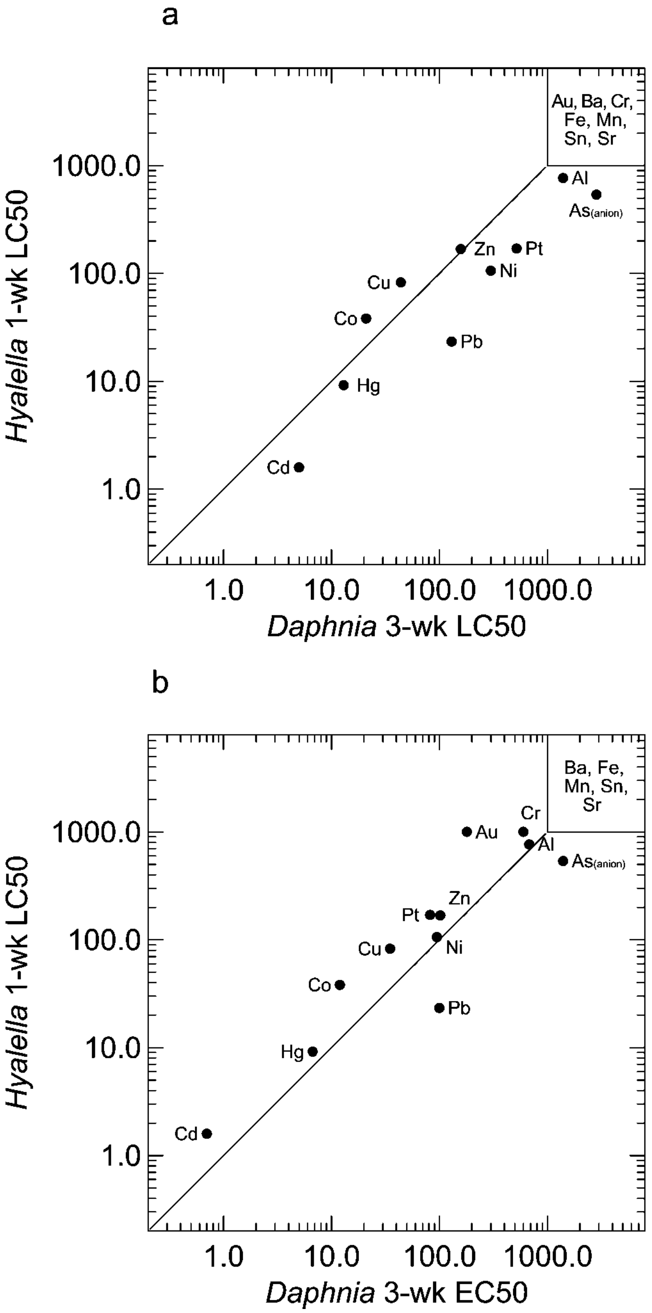

Fig. 2. Comparison of one-week lethal concentrations resulting in 50% mortality ([LC50] μg/L, geometric mean of soft and tap water values) for *Hyalella azteca* with (**a**) three-week LC50s and (**b**) three-week EC50s for reproduction in *Daphnia magna* in Lake Superior (Canada) water [7]. The geometric means of LC50s for *Hyalella* in soft and tap water were used for this comparison because Lake Superior water chemistry is approximately equal to the geometric mean of the soft and tap water used in this study. Metals for which LC50s or EC50s for both species exceed 1,000 μg/L are listed in the upper right-hand corner.

tailed quantitative comparison of results obtained in this study with published values is difficult for most metals due to the low number of published values and the range of water chemistry and exposure times. However, for Cu and Cd, the most extensively studied metals, regressions of log(LC50) against pH, log(hardness) and log(test duration) were performed using only previously published data (i.e., excluding the present data, omitting values reported only as less than, and using the average hardness or LC50 where a range is given). This produced

$$\log(\mathrm{LC50_{Cd}}) = 3.790 + 0.8233 \log(\mathrm{hardness}) - 0.4417\ \mathrm{pH} - 1.835 \log(\mathrm{days\ duration}) \quad \mathrm{and}$$

$$\log(\mathrm{LC50_{Cu}}) = -1.228 + 0.3998\ \mathrm{pH}$$

with r^2 values of 0.8216 for Cd and 0.5342 for Cu. The above coefficients for hardness, pH, and exposure time all were significant at $p < 0.01$ (hardness and exposure time were not significant for Cu, $p > 0.4$). Using these relationships, the ratios of the observed (data from this study) to predicted (from regression of literature values) LC50, based on measured concentrations at the end of the exposure in the present study, were 0.15 and 0.81 for Cd and 0.67 and 0.73 for Cu for soft and tap water, respectively. Based on nominal LC50s, the ratios were 0.56 and 2.23 for Cd and 1.04 and 0.98 for Cu for soft and tap water, respectively. Hence, the LC50s measured in this study (especially the nominal values) are close to previously reported values for Cd and Cu for *Hyalella*.

Comparison of metal toxicity to Hyalella *and* Daphnia

Relatively few databases report the toxicity of many metals for the same species in the same test medium, but one of the most extensive is the study of metal toxicity to *Daphnia magna* in Lake Superior (MN, USA) water [7]. The Lake Superior water chemistry (hardness 45.3, alkalinity 42.3, Ca 13.7 mg/L, Mg 3.2 mg/L, pH 7.74) was similar to the geometric mean of the tap and soft waters used in this study (hardness 46.8, alkalinity 34.2, Ca 14.0 mg/L, Mg 2.8 mg/L, pH 7.87). Consequently, the geometric mean of the metal toxicity to *Hyalella* in soft and tap water was computed for comparison with toxicity to *Daphnia*. Biesinger and Christenson [7] reported toxicity at three weeks for more metals than at 48 h. Using their three-week data, the trend in metal toxicity was very similar between the two species, with *Hyalella* one-week LC50s usually falling slightly under the *Daphnia* three-week LC50s (except for Co, Cu, and Zn, Fig. 2a) and slightly above the *Daphnia* three-week EC50 for reproductive impairment (except for As and Pb, Fig. 2). The rank order of toxicity was the same for Cd < Hg < Co < Cu < Zn in both species. Nickel and Pt toxicity were close to that of Zn, although the exact ranking varied between *Daphnia* and *Hyalella*, and between the LC50 and EC50. Lead, however, appears to be more toxic to *Hyalella* (ranked between Hg and Co) than to *Daphnia* (toxicity similar to Zn).

The correlation between *Hyalella* and *Daphnia* is not as close if the 48-h *Daphnia* LC50s are used in the comparison instead of the three-week LC50s. For example, the relative toxicity ranking of Cd and Hg is now reversed (Fig. 3). The 48-h LC50 for Cu without food (9.8 μg/L) is particularly low for *Daphnia*, compared to the 48-h LC50 with food (60 μg/L, Fig. 3), the three-week LC50 (44 μg/L), or even the three-week threshold for reproductive impairment (a 16% drop in reproduction at 22 μg/L [7]). Similarly, the one-week LC50s for *Hyalella* in tap water did not correlate extremely well with the 48-h LC50s for *Daphnia* in hard (240 mg $CaCO_3$/L) water as reported by Khangarot and Ray [14], especially for Cd (Fig. 3). The acute-chronic ratios in the LC50s are quite variable in *Daphnia*, especially for acute tests without food [7], suggesting that 48-h tests with *Daphnia* should be interpreted with caution when used to estimate potential chronic effects.

The overall similarity in metal toxicity to *Hyalella* (one-week LC50s) and *Daphnia* (three-week LC50s or EC50s), both among the most sensitive of aquatic organisms to toxic chem-

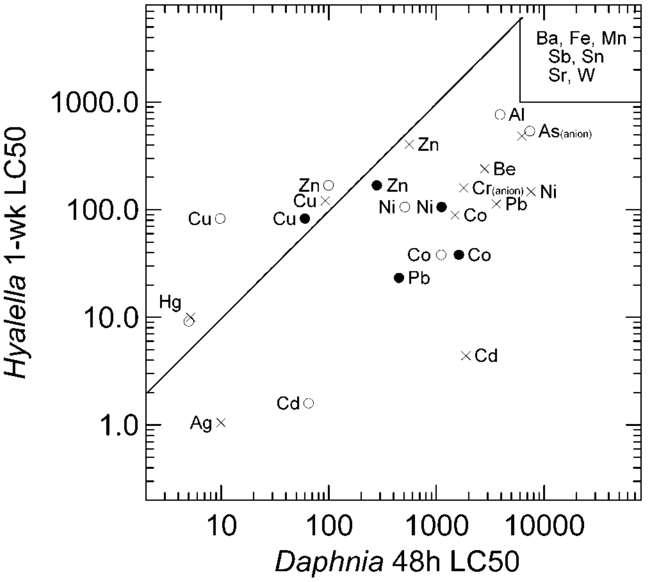

Fig. 3. Comparison of one-week lethal concentrations resulting in 50% mortality ([LC50] µg/L, geometric mean of soft and tap water values) for *Hyalella azteca* with 48-h LC50s for *Daphnia magna* in Lake Superior (Canada) water with (●) or without (○) food [7], and one-week LC50s (tap water) for *Hyalella* with 48-h LC50s for *Daphnia* in hard well water (crosses) [14]. Metals for which LC50s exceed 1,000 (*Hyalella*) or 6,000 (*Daphnia*) µg/L are listed in the upper right-hand corner.

icals, suggests that the data presented here should be a useful guide to the relative toxicity of metals to sensitive crustaceans in general.

CONCLUSION

The toxicity of 63 metals to *Hyalella* was determined in one-week tests in both Lake Ontario (tap) and soft (10% tap) water. The most toxic metals on a molar basis were Cd, Ag, Pb, Hg, Cr (anion), and Tl, followed by U, Co, Os, Se (anion), Pt, Lu, Cu, Ce, Zn, Pr, Ni, and Yb. Most metals were similarly or more toxic in soft water, but Pd was more toxic in tap water. The LC50s for *Hyalella* correlate strongly with three-week LC50s and three-week EC50s for reproduction in *D. magna*.

All 63 of the metals tested are constituents of one or more of the 1,500 inorganic substances and organic metal salts on Canada's Domestic Substances List. For metal-containing substances that are water-soluble and fully dissociate, toxicity will be estimated based on the LC50 for the metal that, most of the time, will be the chemical entity of concern in these substances. An LC50 of <1 mg/L for *Hyalella*, or any other aquatic species, is one of the triggers that will determine if that substance must undergo a screening assessment under the Canadian Environmental Protection Act. The LC50s for 49 of the metals tested were below 1 mg/L in either tap or soft water, for metals tested either as AA standards or as anion salts. Substances containing these metals, therefore, have the potential for being classified as inherently toxic.

Although these data were collected primarily for the purpose of categorizing substances on the DSL, they also provide a useful overview of the relative toxicity of metals to *Hyalella* that can be used for comparison to other species, for identification of metals potentially contributing to toxicity in environmental samples, or for modeling studies relating physical–chemical properties of metals to toxicity.

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