Bull. Environm. Contam. Toxicol. 28, 172–180 (1982)

Effect of Chemical Speciation on the Accumulation of Cadmium by the Caddisfly, *Hydropsyche* sp.

Steven A. Dressing,[1] Richard P. Maas,[1] and Charles M. Weiss

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, NC 27514

Cadmium is one of the more toxic heavy metal pollutants in the aquatic environment. Discharges from the electroplating, battery (MCCAULL 1971), and aircraft (LIEBER and WELSCH 1954) industries have greatly augmented levels of cadmium in natural waterways. Increased recognition of the need for a comprehensive and continuous water quality monitoring system for heavy metals became manifest upon the passage of the Safe Drinking Water Act (P.L. 93-523) in 1974. Two major problems have appeared in the design of monitoring systems for heavy metals: (a) periodic grab sampling schemes do not account for the large time variability of trace metal concentrations (ANDELMAN 1973); (b) the chemical speciation of the metal and its availability to organisms may be more important than the total metal concentration in the system (JACKSON and MORGAN 1978).

Positive associations have been shown among metal concentrations in sediments, water and biota in a variety of aquatic environments (DEAN 1974; MATHIS and CUMMINGS 1973; WILHM 1970; MINOGUE 1972), thus stimulating research exploring the feasibility of using aquatic organisms to monitor heavy metal concentrations in streams (NEHRING 1976). Organism-water concentration factors determined under field conditions have been shown to produce analytical results as reliable as water sampling for up to six months, indicating that normal fluctuations in physical and chemical water parameters do not reduce the reliability or accuracy of biological monitoring of heavy metals (NEHRING et al. 1979).

In addition to water metal concentrations, factors such as organism feeding type (SMOCK 1979), degree of organism-sediment contact (KORMANDY 1965), and organism size (KORMANDY 1965; SHUMAN et al. 1977) can influence metal concentrations in benthic macroinvertebrates. Recent work suggests that metal uptake by aquatic organisms may be influenced by the chemical form of the metal (DODGE and THEIS 1979; POLDOSKI 1979). DODGE and THEIS (1979) showed that the copper uptake by a chironomid species is significantly inhibited by copper complexation with glycine. POLDOSKI (1979) determined cadmium accumulation in Daphnia magna to be strongly dependent upon the type of complexing agent present.

Relevant studies of organism metal uptake as a function of metal speciation for the purpose of advancing the state-of-the-art in biological monitoring of heavy metal pollution should simulate natural systems to the extent possible. Chelators such as

[1] Present address: North Carolina State University, National Water Quality Evaluation Project, Suite 209, 1300 St. Mary's St., Raleigh, NC 27605

0007-4861/82/0028-0172 $01.80

ethylenediaminetetraacetic acid (EDTA), though effective complexing agents, are not commonly found in aquatic systems. Nitrilotriacetic acid (NTA) is a synthetic chelator of interest to the detergent industry as a partial substitute for phosphate builders (ERICKSON et al. 1970). Though banned from detergent use in the U.S. because of potential health hazard (THOM 1971), most research has shown NTA to be relatively safe (ERICKSON et al. 1970; BARICA et al. 1973; SPRAGUE 1968). Much work has indicated NTA and its metal complexes are eventually biodegradable, (SWISHER et al. 1967; SWISHER et al. 1973; THOMPSON and DUTHIE 1968) and researchers have theorized that the resistance to biodegradation of some metal-NTA complexes is due to the toxicity of the released metal (FIRESTONE and TIEDJE 1975).

Larvae of the caddisfly, Hydropsyche sp., or very similar species are abundant in many larger, fast-flowing streams (SMOCK 1979) and hold potential as biological monitors of stream cadmium contamination (SHUMAN et al. 1977). The present study determined the effect of NTA chelation on cadmium uptake by Hydropsyche sp. to better characterize the applicability of caddisfly larvae as biological monitors of cadmium pollution.

EXPERIMENTAL

Laboratory experiments were conducted in glass aquaria with silicon cement seals. Aquaria were thoroughly cleaned with 10% HCl and soaked in distilled-deionized water before each experiment. Nytex™ 0.5 mm mesh bags were suspended in the media with twine from a plastic-coated wire grid mounted on top of each aquarium. A loose cover of sheet plastic prevented substantial evaporation of media, while not inhibiting aeration. The fast flowing waters of the caddisfly habitat were simulated with mixing from magnetic stir plates and stir bars.

The media consisted of a phosphate buffer, calcium chloride, potassium chloride, magnesium sulfate, potassium bicarbonate, sodium chloride, nitrilotriacetic acid (NTA), and cadmium chloride (Table 1). In addition to its potential for use in detergents, NTA was chosen as the chelator because it creates a better cadmium metal buffer system as compared with EDTA. In the well-buffered region of the cadmium-NTA titration curve small errors in NTA additions do not cause large changes in Cd speciation. This condition is a prerequisite for determining pCd (-log free Cd activity) from calculations where all chemical species are not directly analyzed. Varying concentrations of cadmium and NTA were used to provide a wide concentration range of total cadmium with various percentages present as the free and NTA-bound forms. Four liters of media were added to each aquarium and the pH adjusted using either sodium hydroxide or hydrochloric acid. The media were allowed to equilibrate for twenty-four hours prior to the addition of organisms. Ambient temperature and a twelve-hour light/dark cycle were maintained throughout the experiments.

Larvae of the caddisfly Hydropsyche sp. (Trichoptera: Hydropsychidae) were collected from streams containing essentially baseline metal levels (Eno River and Morgan Creek, North Carolina) and transported to the laboratory in Nalgene™ buckets. At the time of stream collection, some organisms were placed with stream water into

seven-ml glass vials and frozen for later analysis to determine initial organism cadmium concentrations.

TABLE I

Composition of Aquaria Media

Aquarium Number	-log Measured Molar Concentration*						Measured pH±0.1	Measured D.O. (mg/l)
	Cd	NTA†	Ca	Cl	SO_4	PO_4		
1	8.65	10.0	5.90	3.54	4.38	3.66	6.4	7.3
2	8.33	7.00	5.49	3.53	4.37	3.66	6.1	7.8
3	7.94	7.00	6.12	3.58	4.33	3.65	6.7	7.3
4	5.98	6.00	5.43	3.56	4.33	3.63	6.6	8.0
5	8.16	8.00	5.82	3.67	4.32	3.64	7.0	7.8
6	7.20	7.00	5.76	3.69	4.30	3.65	7.0	7.8
7	7.00	6.00	4.56	3.69	4.34	3.66	7.0	7.8
8	8.74	7.00	5.83	3.68	4.34	3.65	7.0	7.8
9	5.93	5.00	5.60	3.66	4.28	3.64	7.1	7.8
10	8.45	none	6.00	3.65	4.34	3.64	6.9	7.8
11	6.08	6.00	5.86	3.58	4.34	3.68	7.2	8.8
12	7.46	9.00	6.00	3.58	4.34	3.70	7.1	9.5
13	7.89	6.00	6.00	3.58	4.34	3.72	7.1	9.2
14	8.50	none	5.70	3.58	4.34	3.70	6.9	9.4
15	6.10	6.00	5.76	3.58	4.34	3.68	7.2	9.0

*For all Aquaria: $10^{-4.0}$ M K, $10^{-4.3}$ M HCO_3 and Mg were added.
†Added Concentration.

To prevent predation in laboratory tests, one organism was placed in each nylon bag with extra organisms placed directly in the aquaria. To test the role of active sorption as opposed to surface adsorption and ion exchange as an important mode of accumulation, some of the organisms were killed prior to placing them in the aquaria. No food was added to the aquaria. Organism and water samples were taken daily during the experiments. Organisms were taken from the aquaria using Teflon-coated forceps and placed in seven-ml glass vials containing a small amount of aquaria media. The dead organisms were also collected from the aquaria during the first five days of exposure. Both organism and water samples were immediately frozen at -28°C. Temperature and pH were determined daily while dissolved oxygen (D.O.) was determined at the end of each test. Experiments were typically run for six to twelve days to insure equilibrium between organisms and media.

Thawed organisms were inspected for damage under a dissecting microscope and only intact organisms were selected for analysis. These were dried at 60°C for five hours, cooled and individually tared. Organism samples were then digested overnight in 1+1 purified nitric acid at 60°C.

Cadmium was determined in both water and organism samples using either flame or graphite furnace atomic absorption spectro-

photometry (AAS) depending on the cadmium concentration of the sample. Ortho-phosphate, chloride and sulfate were determined using EPA approved methods (U.S. ENVIRONMENTAL PROTECTION AGENCY 1979). NTA was determined in a separate test by the zinc-zincon method (U.S. ENVIRONMENTAL PROTECTION AGENCY 1979).

Cadmium concentrations in organisms are expressed as micrograms cadmium per gram of organism (μg/g) dry weight. The REDEQL2 equilibrium computer model (MOREL and MORGAN 1972) was used to calculate the negative logarithm of the free cadmium ion concentration (pCd). Measured values of total Cd, calcium, phosphate, chloride and sulfate concentrations; measured pH; and added NTA, potassium, magnesium and bicarbonate concentrations were used in REDEQL2 (Table I). Sodium was used for the purpose of charge balance, and a pCO_2 of 3.5 was assumed for all calculations. Redox conditions, as indicated by electron activity (pE), were determined from dissolved oxygen measurements.

RESULTS AND DISCUSSION

When the cadmium content of Hydropsyche sp. was examined with respect to exposure time it was noted that the organisms achieved equilibrium with the synthetic metal solution within three to five days of initial exposure (Figure 1). On this basis mean organism Cd concentrations were calculated from samples collected on day three through day twelve of exposure. Table II shows the mean standard deviation for the test species cadmium content in each experimental aquarium. Also shown in Table II are the measured total cadmium (Cd_T) concentration in each exposure, the free Cd ion concentration (pCd) as calculated from REDEQL2, and the calculated percentage of free cadmium in each aquarium. Hand calculations of pCd for aquaria exposed to other temperatures were compared to REDEQL2 calculations at 25°C to determine whether the temperature differences significantly affected pCd values. Temperature effects on pCd were found not to be significant in these experiments, and thus REDEQL2 calculations were used without temperature correction.

A separate test showed that NTA biodegradation was unlikely in these experiments as initial (Day 0) and final (Day 12) day samples had identical NTA concentrations (2.8 mg/l). Though the zinc-zincon method was used near the generally accepted detection limit for NTA, the calibration curve was linear to 0.5 mg/l NTA.

Dead organisms were collected from the aquaria and analyzed for Cd content to provide information concerning possible modes of cadmium uptake by Hydropsyche sp.. The results are shown in Table III along with Cd concentrations of living organisms in the same aquaria. Though the data are not conclusive, there is evidence that living organisms accumulate Cd to a greater degree than dead organisms, especially at higher Cd exposures. Dead organism cadmium content averaged only 60 percent that of living organisms. This suggests that active sorption of metals, possibly through gill membranes, may play an important role in cadmium accumulation by Hydropsyche sp..

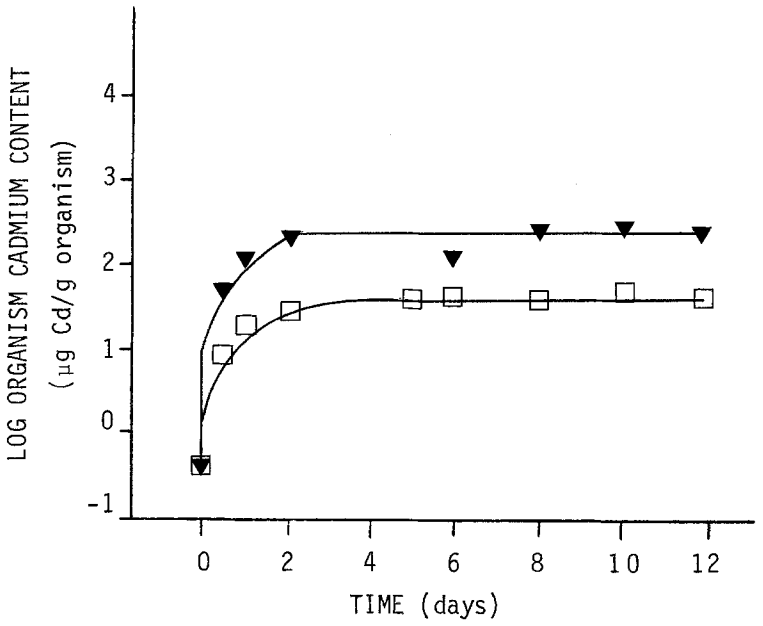

FIGURE 1. Cadmium concentration in Hydropsyche sp. with respect to length of exposure for Aquarium No. 12 (▼) and Aquarium No. 15 (□).

Equilibrium values for Cd concentration of Hydropsyche sp. (Table II) are plotted versus mean total water cadmium concentrations and versus free cadmium (Table II) in Figures 2 and 3, respectively. A strong linear correlation ($r^2 = 0.80$, $p \leq 0.001$) was found between Hydropsyche sp. and total water cadmium concentrations of the experimental media. To test the experimental reproducibility, media in aquaria nos. 11 and 15 were prepared almost identically. As shown in Figure 2 the mean equilibrium Cd organism concentrations for the aquaria were remarkably close indicating good reproducibility.

Figure 3 shows that the linear relationship between equilibrium Hydropsyche sp. Cd concentration and free Cd ion concentration ($r^2 = 0.31$, $p = 0.03$) is significantly weaker than between Hydropsyche sp. and total Cd concentration ($r^2 = 0.80$, $p \leq 0.001$). If the chemical speciation of the cadmium had no effect on its accumulation by Hydropsyche sp., it might be expected that there would be no significant linear relationship between free cadmium and Hydropsyche sp. concentrations since the relationship between free and total Cd was randomized by varying the percentage of total Cd present as the free ion (Table II). However, the free and total Cd are not totally independent variables since, while the fraction of the total Cd present as free ion can be varied, the free ion concentration can never exceed the total Cd concentration. For this reason the experiments can not be completely randomized, and some

TABLE II

Cadmium Concentrations in Hydropsyche sp. and Test Media

Aquarium Number	*Hydropsyche sp. Log Mean µg Cd/g ± SD	*Water -Log Mean Cd_T ± .01	Mean pCd ± SD	%Cd Free
1	-0.11 ± .34	8.66	8.68 ± .01	95
2	-0.08 ± .15	8.33	8.87 ± .03	29
3	0.69 ± .12	7.94	8.89 ± .08	11
4	1.82 ± .16	5.98	6.86 ± .03	13
5	1.01 ± .07	8.16	8.48 ± .01	48
6	1.92 ± .08	7.20	8.12 ± .03	12
7	1.50 ± .08	7.00	9.15 ± .03	0.7
8	0.61 ± .10	8.74	9.97 ± .03	5.9
9	1.92 ± .08	5.93	9.18 ± .15	0.1
10	0.43 ± .10	8.45	8.47 ± .03	96
11	2.47 ± .05	6.08	7.73 ± .09	2.2
12	1.72 ± .06	7.46	7.49 ± .03	93
13	0.43 ± .05	7.89	10.18 ± .05	0.5
14	0.41 ± .04	8.50	8.52 ± .03	95
15	2.41 ± .07	6.10	7.81 ± .08	1.9

* For day 3 to end of experiment.

TABLE III

Comparison of Cd Concentrations in Living and Dead Organisms

Aquarium Number	Day	Status	Average Weight (g)	µg Cd/ g Larvae	-Log Mean Cd_T	pCd
11	1	Living	.0008	80	6.08	7.73
11	1	Dead	.0026	49	6.08	7.73
11	2	Living	.0023	124	6.08	7.73
11	2	Dead	.0012	87	6.08	7.73
11	4	Living	.0034	209	6.08	7.73
11	4	Dead	.0039	149	6.08	7.73
13	1	Living	.0020	1.01	7.89	10.08
13	1	Dead	.0019	.589	7.89	10.08
13	2	Living	.0021	1.42	7.89	10.08
13	2	Dead	.0048	.467	7.89	10.08
14	5	Living	.0066	1.17	8.50	8.52
14	5	Living	.0041	2.58	8.50	8.52
14	5	Dead	.0044	1.38	8.50	8.52

correlation will inevitably be observed between free ion and organism cadmium concentrations even if the free and complexed cadmium are actually accumulated to the same degree by the organisms. The large difference in the strength of the association between free Cd ion versus organism cadmium and total Cd versus organism cadmium, however, clearly indicates that both free Cd and Cd bound to NTA are accumulated by Hydropsyche sp.. These results contrast with those of DODGE and THEIS (1979) who found that complexation of copper with glycine and other organic ligands greatly reduced its uptake by Chironomus tentans. POLDOSKI (1979) found that uptake of cadmium by Daphnia could be either inhibited, increased or unaffected by complexation of cadmium with various organic ligands. These discrepancies suggest that the relative importance of various modes of uptake probably vary as a function of the aquatic insect species and/or type of metal.

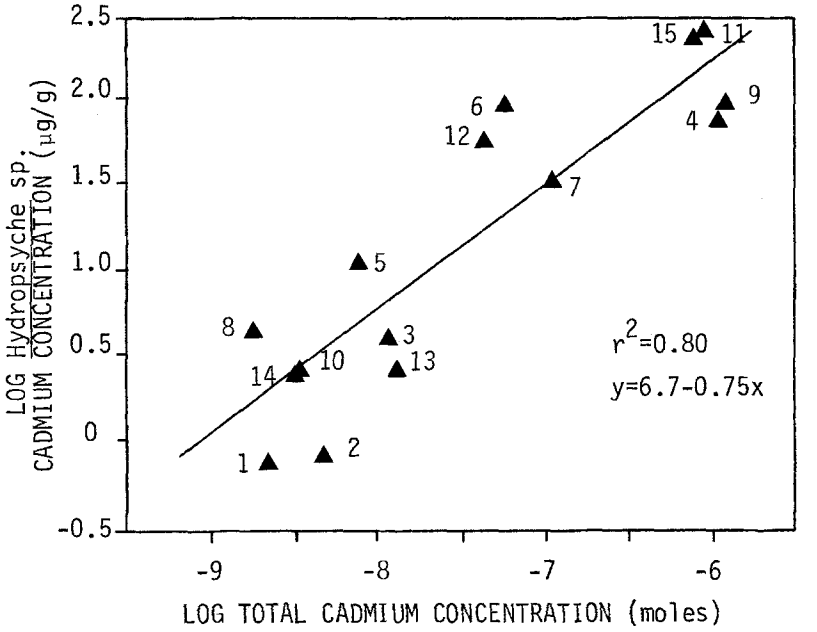

FIGURE 2. Cadmium concentration in Hydropsyche sp. as a function of total cadmium concentration in water. Numbers identifying data points refer to aquaria numbers in Table I.

Additional studies using different organisms, metals and ligands, especially naturally occurring organic ligands, are needed to further evaluate the effect of metal speciation on the accumulation of metals by organisms with the potential for biological monitoring of heavy metals.

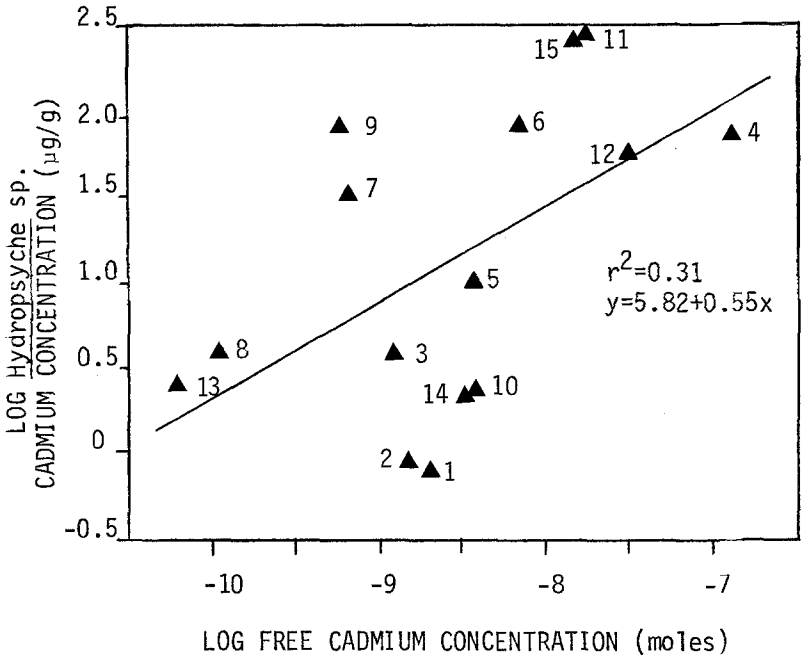

FIGURE 3. Cadmium concentration in Hydropsyche sp. as a function of free cadmium ion concentration in water. Numbers identifying data points refer to aquaria numbers in Table I.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Office of Water Research and Technology, U.S. Dept. of the Interior, Washington, D.C..

REFERENCES

ANDELMAN, J.B.: In: Singer, P.C.: Trace Metal and Metal-Organic Interactions in Natural Waters. Ann Arbor, MI: Ann Arbor Science 1973.
BARICA, J., STAINTON, M.P. AND A.L. HAMILTON: Water Research 7, 1791 (1973).
DEAN, J.M.: Hydrobiologia 45, 33(1974).
DODGE, E.E. AND T.L. THEIS: Environ. Sci. Technol. 13, 1287 (1979).
ERICKSON, S.F., MALONEY, T.E. AND J.H. GENTILE: J. Water Poll. Cont. Fed. 42, R329 (1970).
FIRESTONE, M.K. AND J.M. TIEDJE: Applied Microbio. 29, 758 (1975).
JACKSON, G.A. AND J.J. MORGAN: Limno. Oceanog. 23, 268 (1978).
KORMANDY, E.J.: Limno. Oceanog. 10, 427 (1965).
LIEBER, M. AND W.F. WELSCH: J. Am. Water Works Assoc. 46, 541 (1954).
MATHIS, B.J. AND T.F. CUMMINGS: J. Water Pol. Con. Fed. 45, 1573 (1973).
MCCAULL, J.: Environment 13, 2 (1971).
MINOGUE, J.E.: Masters Tech. Report, Chapel Hill: Univ. NC 1972.
MOREL, F. AND J. MORGAN: Environ. Sci. Technol. 6, 58 (1972).

NEHRING, R.B.: Bull. Env. Contam. and Toxic. 15, 147 (1976).
NEHRING, R.B., NISSON, R. AND G. MINASIAN: Bull. Env. Contam. and Toxic. 22, 103 (1979).
POLDOSKI, J.E.: Environ. Sci. Technol. 13, 701 (1979).
SHUMAN,M.S., SMOCK, L.A. AND C.L. HAYNIE: Water Resources Res. Inst. Report No. 124: Univ. NC 1977.
SMOCK, L.A.: Ph.D. Dissert., Chapel Hill: Univ. NC 1979.
SPRAGUE, J.B.: Nature 220, 1345 (1968).
SWISHER, R.D., CRUTCHFIELD, M.M. AND D.W. CALDWELL: Environ. Sci. Technol. 1, 820 (1967).
SWISHER, R.D., TAULLI, T.A. AND E.J. MALEC: In: SINGER, P.C.: Trace Metal and Metal-Organic Interactions in Natural Waters. Ann Arbor, MI: Ann Arbor Science 1973.
THOM, N.S.: Water Research 5, 391 (1971).
THOMPSON, J.E. AND J.R. DUTHIE: J. Water Poll. Cont. Fed. 40, 306 (1968).
U.S.E.P.A.: Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020 1979.
WILHM, J.L.: Health Physics 18, 277 (1970).