Bull. Environ. Contam. Toxicol. (1991) 46:901–905

Bulletin of Environmental Contamination and Toxicology

Concentration of Metals in Various Larval Stages of Four *Ephemeroptera* Species

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Most of the surface waters in the southern part of Poland have received considerable amounts of wastewaters. Some of the industrial wastewaters contain a large amount of metals (Grodzinski et al. 1984) which can be biologically active, toxic or have a potential for bioconcentration. Field investigations have shown that heavy metals often reduce the abundance and diversity of aquatic insects (Eyres and Pugh-Thomas 1978). Mayfly larvae have been known to be more sensitive to heavy metals than other groups of aquatic insects (Armitage 1980; Winner et al. 1980). Short-term acute toxicity tests have indicated that insects are more tolerant to metals than other invertebrates, i.e., *Daphnia spp.* (Warnick and Bell 1969), in contrast to long-term exposure where some insects have shown to be equally or more sensitive than fish or invertebrates to metals (Spehar et al. 1978; Sodergren 1976). Long-term sublethal exposure using aquatic insects is more ecologically realistic since all stages of their development are exposed. This investigation provided an opportunity to assess the bioconcentration of cadmium, copper, lead and zinc in various stages of the mayflies *Ephemera danica Muller*, *Ephemera vulgata* (L.), *Leptophlebia vespertina (L.)* and *Baetis vernus* Curt. collected from the stream Lane Bloto (South Poland) in which water quality was strongly affected by air pollution.

MATERIALS AND METHODS

Mayfies were sampled from the lowland stream Lane Bloto in the Niepolomice Forest located near Krakow (South Poland). They were collected twice monthly over one year from the sediment and plants using a standard sampler with an inlet surface of 200 cm^2 and 0.3-mm mesh net. Samples were rinsed in the laboratory with distilled water and subsequently fixed with 1.0% formalin. All specimens were removed from the samples, (damaged larvae were discarded), taxonomically identified, measured (body length), weighed and prepared for AA analyses according to the method described by Enk and Mathis (1977). To prepare the samples for AA analysis, mayflies were dried in an oven at 60 °C for 24 hours. Duplicate samples (approximately 100 mg) were digested with a mixture of concentrated nitric (2.4 mL) and perchloric acid (0.6 mL). The samples were heated over medium heat under a hood with watchglass covers on the beakers. Once a sample had been dissolved, the solution was evaporated until the volume had been reduced to approximately 1 mL. The remaining liquid was diluted with distilled deionized water. Metals were analyzed using a Perkin-Elmer Model 503 Atomic Absorption unit equipped with an HGA-74 graphite furnace. Analyses of the QA blanks were performed concurrently with the invertebrate samples. Prior to drying, larvae of each species were separated into different size groups based on body length. Length of each larva was measured from the front margin of the head to the terminal margin of the 10 tergite of the abdomen.

RESULTS AND DISCUSSION

Together with invertebrate samples the water column and bottom sediment samples were collected and analyzed (Jop 1981a). According to these analyses, the water of the Lane Bloto stream is composed mainly of carbonates (60-85%) and calcium (53-76%). The concentration of chlorides, sulfates, sodium or potassium is relatively low. During the summer (June-September), intensive photosynthesis frequently reduces free carbon dioxide to below detection limits, oversaturated stream water up to 130%, and increases pH levels (8.5 to 9.0) causing precipitation of calcium carbonate. The results of metals analyses in the water column and bottom sediments did not show seasonal variations. Slightly higher concentrations of metals were recorded in early spring (March) after the snowmelt. The following mean annual concentrations were recorded in water: 1 μg Cd/L, 17 μg Pb/L, 30 μg Cu, 197 μg Zn/L and sediment: 3 mg Cd/L, 15 mg Pb/L, 26 mg Cu/L, and 193 mg Zn/L.

The life cycle of each mayfly species was different (Jop 1981b). The larvae of *Baetis vernus* formed two clearly separated cohorts within one summer's generation and larvae of both cohorts remained in the stream no longer than 4 months. Larvae of *Leptophlebia vespertina* were present in the water for 10 months as one generation. Both *Ephemera danica* and *E. vulgata* completed one generation in two years and their larvae were always present in the water.

The concentrations of cadmium, lead, copper and zinc in whole bodies of the various size groups of the four mayfly species tested in this study are summarized in Table 1. The results showed a relatively wide range of concentrations for each metal analyzed, with cadmium having the smallest and zinc the largest range. The concentrations of each metal in mayfly larvae were proportional to the concentration of metals in the water and sediment, i.e., Cd<Pb<Cu<Zn. The results of this study suggest that the exposure time for mayflies is not critical in bioconcentration of metals, i.e., *Baetis vernus* in 4 months accumulated as much as *Leptophlebia vespertina* in 10 months. Also, colonization seems to be important in relation to the concentration of selective metals. The concentration of copper was similar, while cadmium, lead and zinc were slightly different in two burrowers, *Ephemera danica* and *E. vulgata*. *E. danica* was found primarily in a sandy bottom with low organic matter while *E. vulgata* colonized sediments rich in organic matter.

The bioconcentration potential of metals depends largely on its concentration in water, water hardness, the amount of organic matter present and its ionic stage. During laboratory exposures with the mayfly *Ephemerella grandis*, Nehring (1976) estimated the bioconcentration factors for lead, copper and zinc to be 100.

Table 1. Concentrations of cadmium, lead, copper and zinc in whole bodies of four *Ephemeroptera* species. All values are expressed in $\mu g/g$ dry weight (standard deviation).

Size Groups (mm)	Sample Size n	Dry Weight (mg)	N	Cd	Metal Pb	Cu	Zn
Ephemera danica							
0 - 5	250	165	3	2.1 (0.7)	18.0 (5.8)	44.7 (9.4)	1576 (414)
5 - 10	150	92	3	2.7 (1.1)	17.7 (5.3)	41.6 (16.2)	1342 (378)
10 - 15	100	126	3	4.2 (2.5)	18.0 (6.0)	43.2 (17.1)	846 (212)
15 - 20	100	230	3	3.2 (1.5)	14.7 (3.4)	19.4 (4.0)	456 (65)
20 - 25	10	108	3	3.0 (1.3)	3.5 (1.2)	18.5 (6.1)	241 (51)
Imago	20	169	3	0.4 (0.2)	BDL	18.1 (4.7)	159 (34)
Ephemera vulgata							
0 - 5	250	140	3	8.3 (1.4)	37.7 (7.9)	41.2 (5.0)	821 (62)
5 - 10	200	166	3	7.1 (1.1)	30.5 (8.7)	36.6 (3.8)	756 (49)
10 - 15	100	133	4	4.2 (0.5)	30.2 (9.4)	31.6 (2.7)	621 (57)
15 - 20	50	145	4	3.4 (0.3)	30.0 (9.8)	24.6 (1.7)	368 (26)
20 - 25	10	107	4	3.0 (0.4)	20.0 (3.8)	19.6 (4.8)	357 (44)
Imago	20	139	3	BDL	7.0 (1.2)	12.8 (3.1)	214 (31)
Leptophlebia vespertina							
1 - 2	2000	81	3	6.5 (0.7)	61.7 (7.1)	54.7 (17.1)	3157 (815)
2 - 3	1000	108	3	6.5 (0.5)	57.9 (5.7)	51.6 (13.2)	2834 (754)
3 - 4	600	109	3	6.3 (0.8)	47.9 (6.2)	50.2 (10.5)	2657 (459)
4 - 5	300	97	4	6.2 (0.5)	41.5 (5.9)	46.4 (7.5)	2242 (512)
5 - 6	250	107	4	4.9 (1.0)	25.9 (3.2)	32.4 (8.2)	1822 (502)
6 - 7	150	109	5	4.6 (0.7)	11.6 (3.5)	23.1 (4.9)	1814 (378)
7 - 8	150	124	5	3.6 (0.3)	8.1 (2.1)	8.1 (3.2)	1645 (312)
Imago	150	88	3	1.3 (0.4)	6.6 (2.2)	7.1 (1.8)	1605 (362)
Baetis vernus							
1 - 2	2500	92	3	8.6 (0.9)	66.2 (7.5)	51.5 (15.1)	1521 (395)
2 - 3	1000	96	3	8.3 (1.1)	65.1 (7.3)	46.9 (13.8)	1342 (311)
3 - 4	600	101	3	7.9 (0.8)	54.7 (6.7)	44.6 (13.4)	1266 (287)
4 - 5	300	93	3	7.2 (0.6)	45.7 (5.8)	37.6 (11.2)	1204 (292)
5 - 6	200	109	3	5.1 (0.4)	34.5 (4.1)	37.6 (10.7)	803 (274)
6 - 7	200	160	3	4.5 (1.0)	25.4 (4.7)	31.3 (7.7)	642 (161)
7 - 8	150	145	4	1.9 (0.3)	15.5 (2.3)	27.6 (6.6)	451 (103)
Imago	150	112	3	1.8 (0.3)	8.9 (1.8)	23.6 (5.4)	125 (24)

n - number of specimens/sample, Dry weight in mg is average weight/sample, N - number of samples analyzed, BDL - Below Detection Limit

He concluded that aquatic insects accumulate metals in relative proportion to their concentration in water and the accumulation can be predictable. These conclusions can only apply to short-term exposures where the maximum of two larval stages are exposed and the bioconcentration factor calculated from these experiments can only be applied to that particular larval size and stage.

In the present study, the concentration of all measured metals decreased in consecutive larval stages of four *Ephemeroptera* species, particularly prior to emergence (Table 1). This phenomenon is well documented for aquatic invertebrates (Anderson and Brower 1978; Wright 1980; Jop and Wojtan 1982). Also, the adult form of each species contained significantly lower amounts of each metal. Growth of ephemerids occur by molting subsequent exuviae and during this process partial elimination of metals takes place. Therefore, each of the groups represents at least one instar, more likely two, assuming that the smallest and fastest growing *B. vernus* during the 4 months of larval growth undergo at least 10 instar growth stages. Experimental studies have shown that mayflies from the same geographical region completed larval development with a constant number of instars (Humpesch 1979).

The results obtained from this study have important implications for biological monitoring programs like those outlined by EPA's "Assessment and control of bioconcentrable contaminants in surface waters." Because the concentration of metals in whole body is regulated by physiological processes and varies substantially during the life-cycle, mayflies are of no value for biological monitoring of metals unless the monitoring program includes their complete life cycle to account for differences in the relative metal concentration of instar stages.

Acknowledgments. I gratefully acknowledge K. Wojtan of the Polish Academy of Sciences for his laboratory assistance throughout the study.

REFERENCES

Anderson RV, Brower J (1978) Patterns of trace metal accumulation in crayfish populations. Bull Environ Contam Toxicol 20:120-127

Armitage PD (1980) The effects of mine drainage and organic enrichment on benthos in the River Nent system, Northern Pennines. Hydrobiologia 74:119-128

Enk MD, Mathis BJ (1977) Distribution of cadmium and lead in a stream ecosystem. Hydrobiologia 52:153-158

Eyres JP, Pugh-Thomas M (1978) Heavy metal pollution of the river Irwell (Lancashire, UK) demonstrated by analysis of substrate materials and macroinvertebrate tissue. Environ Poll 16:129-136

Grodzinski W, Weiner J, Maycock PF (1984) Forest ecosystems in industrial regions. Springer-Verlag Berlin, Heidelberg, New York, Tokyo

Humpesch UH (1979) Life cycles and growth rates of *Baetis spp.* (*Ephemeroptera:Baetidae*) in the laboratory and in two stony streams in Austria. Freshw Biol 9:467-479

Jop KM (1981a) Ecology of the forest stream Lane Bloto in the Niepolomice Forest: 1. Chemism of water and bottom sediments and its changes under the influence of industrial pollution. Acta Hydrobiol 23:107-123

Jop KM (1981b) Ecology of the forest stream Lane Bloto in the Niepolomice Forest: 2. Community structure, life cycles, and production of *Ephemeroptera*. Acta Hydrobiol 23:125-141

Jop KM, Wojtan K (1982) Concentration of cadmium and lead in the body of some macrobenthos species from five streams of Southern Poland. Acta Hydrobiol 24:197-210

Nehring RB (1976) Aquatic insects as biological monitors of heavy metal pollution. Bull Environ Contam Toxicol 15:147-154

Sodergren S (1976) Ecological effects of heavy metal discharge in salmon river. Rep Inst Freshw Res Drottningholm 55:91-131
Spehar RL, Anderson RL, Fiandt JT (1978) Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ Poll 15:195-208
Warnick SL, Bell HL (1969) The acute toxicity of some heavy metals to different species of aquatic insects. J Wat Poll Contr Fed 41:280-284
Winner RW, Boesel MW, Farrell (1980) Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can J of Fish Aquatic Sci 37:647-655
Wright DR (1980) Cadmium and calcium interactions in the freshwater amphipod *Gammarus pulex*. Freshw Biol 10:123-133

Received January 28, 1991; accepted February 19, 1991.