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Reliability of Aquatic Insects Versus Water Samples as Measures of Aquatic Lead Pollution

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During 1971-72, NEHRING (1973, 1976) determined that aquatic insects could serve as biological monitors of heavy metal pollution under certain conditions. Furthermore, concentration of heavy metals by two or three orders of magnitude in the insects made detection of low level metal pollution easier.

While working in Iran (1976-77) the authors conducted a field evaluation on the Chalus River using the methodology set forth by NEHRING (ibid.). Historically, the Chalus River supported large spawning runs of Caspian salmon (*Salmo trutta caspius*); however, in recent times chronic lead pollution from mining activity and silting of the stream by irrigation returns essentially eliminated the salmon run.

The purposes of the study were to: 1) evaluate the accuracy of the concentration factor method (ibid.) in determining the amount of lead pollution in the Chalus River, and 2) determine if several orders of aquatic insects would give repeatable results comparable to water sample analysis by flame atomic absorption spectrophotometry (hereafter AAS).

METHODS AND METERIALS

Test aquatic insects included a single genus or species of the taxonomic groups Ephemeroptera (Heptageniidae), Diptera (Tipulidae), Trichoptera (Hydropsychidae - *Macronemum*), Odonata (Anisoptera), and Plecoptera (Perlidae - *Paragnetina* and Nemouridae - *Nemoura*) and were chosen as test organisms based on abundance in the Chalus River. Insect larvae and naiads were put in a collection jar on ice, transported to the laboratory, rinsed in distilled water, dried to a constant weight, and analyzed according to NEHRING (ibid.). Control insects, taken from a non-polluted tributary of the Chalus River, were used as a comparison against collections from the Chalus River and in aquarium experiments to document the rate of lead uptake when exposed to dissolved lead versus a lead-contaminated substrate of silt, mud, and organic matter from the Chalus River.

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Analysis of water samples was done by AAS with organic extraction-complexation reagents (APDC/MIBK)* according to Standard Methods (1975). In one instance water samples were also analyzed by AAS after concentration through evaporation.

RESULTS AND DISCUSSION

Three groups of control insects were placed in two different aquaria, one containing dissolved lead and the other a lead-contaminated substrate of silt, mud, and organic matter from the Chalus River. Aquatic insect larvae and naiads in both aquaria were cropped off at periodic intervals. Although total lead in the water was 1 mg/l and 0.2 mg/l in the aquaria with dissolved lead and lead-contaminated substrate, respectively, each group of insects concentrated lead at a similar rate and to a similar level. Subsequent removal to lead-free aquariums revealed a more rapid loss of lead in stonefly naiads and caddisfly larvae exposed to dissolved lead than in either species exposed to the lead-contaminated substrate. Mayfly naiads did not survive either exposure; thus no documentation of post-exposure loss was possible in the mayfly. (Figures 1, 2, 3).

These results indicate the mechanism of lead uptake and loss varies with the type of exposure, thereby necessitating the determination of concentration factors under field conditions. During late 1976 and early 1977 field concentration factors for six different groups of aquatic insects were determined for lead as indicated in Table 1. To derive a field concentration factor, the average lead accumulation (ug/g) for each species is divided by the lead level in the stream (mg/l). These concentration factors were used to determine an estimated lead level in the stream during subsequent sampling periods two to six months later.

TABLE 1 Determination of Field Concentration Factors for Lead.

Organism Description	$\bar{X}$ Pb (ug/g) Insects	Pb (mg/l) in Water	Concentration Factor	
Tipulidae (one species)	4037.8	0.50	8076	
Perlidae (Paragnetina)	669.75	0.14	4784	$\bar{X}$= 3636
Perlidae (Paragnetina)	497.62	0.20	2488	
Heptageniidae (one species)	2835.4	0.50	5671	
Nemouridae (Nemoura)	1013.2	0.14	7237	
Hydropsychidae (Macronemum)	1787.4	0.50	3575	
Anisoptera (one species)	980.38	0.26	3771	$\bar{X}$= 3890
Anisoptera (one species)	802.07	0.20	4010	

In Table 2, estimated lead in the stream (determined by the concentration factor method) and lead levels determined by AAS rarely differed by more than 0.1 mg/l. Excessive suspended solids with a high lead content in the effluent negated any attenuation of the lead levels in the Chalus River as tributary streams increased

* Ammonium pyrolidine dithiocarbamate/ methylisobutylketone

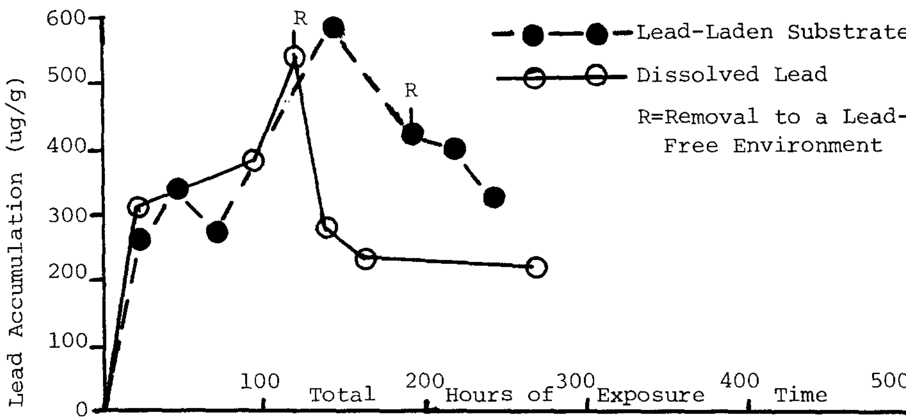

FIGURE 1 Lead Concentration in Trichoptera (Macronemum) Larvae Exposed to Two Lead-Contaminated Environments with Removal to a Lead-Free Environment.

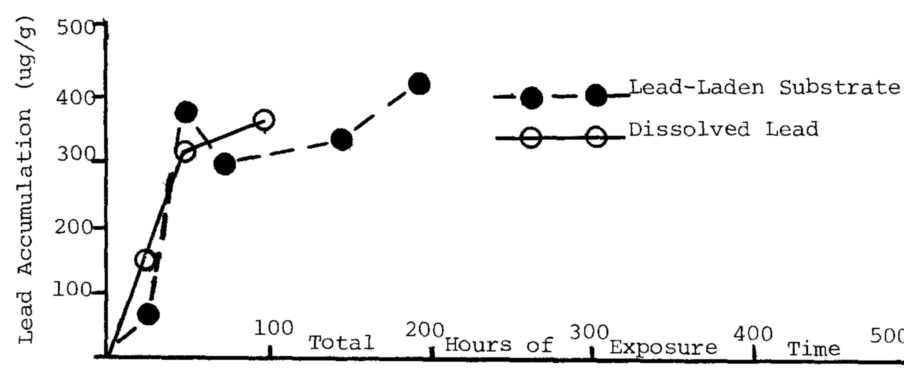

FIGURE 2 Lead Concentration in Ephemeroptera (Heptageniidae) Naiads Exposed to Two Lead-Contaminated Environments.

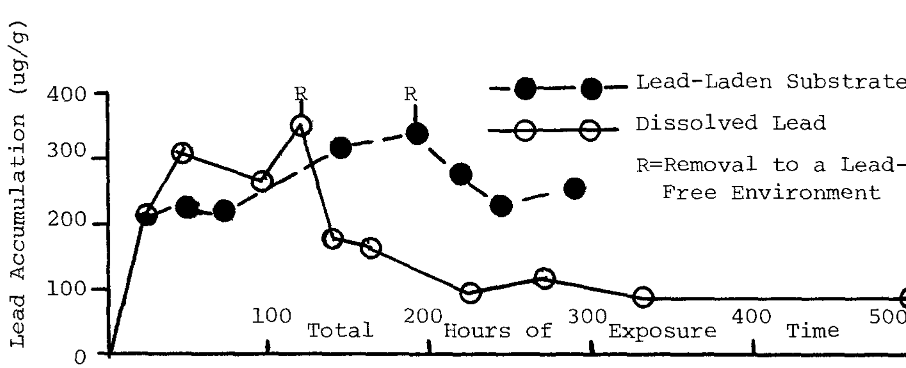

FIGURE 3 Lead Concentrations in Plecoptera (Paragnetina) Naiads Exposed to Two Lead-Contaminated Environments with Subsequent Removal to a Lead-Free Environment.

the total flow. Sampling from April 1976 through May 1977 revealed the Chalus River ranged from 0.14 mg/l to 0.50 mg/l in total lead.

TABLE 2 Comparison of Estimated Lead Levels (mg/l) Determined by Concentration Factors and Water Sample Analysis. (Numbers in Parentheses Indicate Distance from the Pollution Source.)

Method of Analysis	Station C (Control)	Station 1 (8 km)	Station 2 (15 km)	Station 3 (40 km)
Heptageniidae	0.000	0.188	-----	0.156
Tipulidae	0.003	0.250	0.180	0.155
Macronemum	0.028	0.208	0.187	0.131
Nemoura	0.003	0.140	0.056	0.034
Paragnetina	0.017	0.184	0.137	-----
APDC/MIBK	0.030	0.140	0.200	0.260
Evaporation (1)	0.000	0.300	0.170	0.160
Evaporation (2)	0.000	0.300	0.230	0.210

Despite the loss of the critical water samples on two occasions the concentration factor method allowed the authors to estimate the lead pollution level in the Chalus River, as indicated in Table 3.

TABLE 3 Chalus River Lead Levels by Concentration Factor Analysis

Organism	Date	Accumulation/ Conc. Factor	=	Estimated Lead (mg/l)
Nemoura	2/19/77	1045.6/7237	=	0.144
Macronemum	2/19/77	917.06/3575	=	0.257
Heptageniidae	2/19/77	1617.4/5671	=	0.285
Tipulidae	2/19/77	2887.1/8076	=	0.357
Paragnetina	2/19/77	846.04/3636	=	0.233
Paragnetina	4/05/77	1153.2/3636	=	0.317
Heptagennidae	4/05/77	2633.9/5671	=	0.464

On June 11, 1977, lead levels in the Chalus River dropped to the lowest level observed during the study. A bottom-scouring flood preceded our collection by two weeks and carried away the lead-containing sediments in the river. Despite AAS analysis with APDC/MIBK (Standard Methods, ibid.) the lead levels were below detectable limits (0.1 mg/l). Analysis by the concentration factor method revealed an estimated lead level ranging from 0.04 to 0.07 mg/l over the three stations as compared to 0.038 mg/l for the control station. A t-test analysis (DIXON & MASSEY, 1969) of the data revealed no significant difference between the previously polluted stations and the control.

However, a t-test analysis of the data in Table 2 (taken on 3/1/77) revealed a statistically significant difference between the polluted stations and the control. Polluted stations (1,2, and 3) were statistically significantly higher than the control (C) for analysis by the concentration factor method and AAS analysis. When lead levels for a given station were analyzed by the two methods no statistical difference occurred, indicating the concentration factor method produced results as reliable as AAS analysis. Table 4).

TABLE 4 T-Test Evaluation of Lead Levels Determined By AAS Analysis and the Concentration Factor Method.

Comparison (Between Stations)	D.F.	T Value	Significance
Insects vs. Insects			
Station 1 vs. Station C (Control)	7	8.652	$t < 0.005$
Station 2 vs. Station C (Control)	6	4.145	$t < 0.005$
Station 3 vs. Station C (Control)	6	3.597	$0.01 > t > 0.005$
AAS vs. AAS (Between Stations)			
Station 1 vs. Station C (Control)	2	2.032	$0.10 > t > 0.05$
Station 2 vs. Station C (Control)	2	4.914	$0.025 > t > 0.01$
Station 3 vs. Station C (Control)	2	3.122	$0.05 > t > 0.025$
Insects vs. AAS (Within Stations)			$t = 0.95$
Station 1	5	0.502	2.015
Station 2	5	1.565	2.015
Station 3	4	2.084	2.132
Station C (Control)	3	1.241	2.353

Advantages of the concentration factor method are: 1) easier detection of trace levels of pollution not detectable by direct AAS analysis, 2) dried insect samples do not deteriorate with age, thereby eliminating the necessity of immediate analysis for reliable results, and 3) aquatic insects provide a more realistic analysis of the total lead in the stream, including the lead-laden substrate, not accounted for by water samples. Field concentration factors for each genus of insect produced results as reliable as water samples up to six months after initial determination of the concentration factor, indicating normal fluctuations in physical and chemical water parameters do not reduce reliability or accuracy.

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