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Environmental Bromine in Freshwater and Freshwater Organisms: Factors Affecting Bioaccumulation

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In the past, more fish kills have been observed in the West Fork of Stones River, located in Murfreesboro, Tennessee, than in any other city in the state. Although low dissolved oxygen and/or improperly treated sewage have been implicated in these kills, there is evidence to support a combination of causative agents (BURDICK et al. 1973). During this study, significant quantities of bromine were observed in the x-ray fluorescence spectra of fish and crayfish taken from the waters of the area. Closer examination of these samples revealed that the fish and crayfish collected below a chlorinated sewage treatment plant effluent had lower concentrations of bromine in their tissues than those collected in untreated and uncontaminated waters. This prompted a more thorough study of the factors that contribute to bromine levels in water, fish and crayfish (CASTO 1977 and WOODS et al. 1977). These studies take on added significance in light of the recent findings by MINEAR et al. (1978) that Murfreesboro, Tennessee ranks second among 29 cities studied in the state for the concentration of trihalomethanes in the municipal water supply.

MATERIALS AND METHODS

Water. Stream samples were collected in polyethylene bottles and analyzed for bromide by the phenol red bromination method (STANDARD methods 1976). Analysis of water samples collected for bromide determination indicated no loss of bromide within sixty days, provided the sample was refrigerated and stored in a tightly sealed full bottle. Methods for dissolved oxygen and residual chlorine were also performed according to STANDARD METHODS (1976).

Fish and Crayfish. Fish samples were collected using a Dirigo "500" Electrofisher. Crayfish were collected by seining. The fish and crayfish were washed with deionized water and ground with an equal volume of deionized water in a blender. The resultant slurries were lyophilized at -50°C and 50 µm Hg.

The fine powder from the lyophilization was analyzed for bromine by x-ray fluorescence and neutron activation analysis. A 25 millicurie Cd-109 excitation source was used to excite characteristic x-rays. Excitation time was > 1000 seconds. The concentration of bromine in a sample of *Hypentelium nigricans* was determinated by x-ray fluorescence using the method of standard additions.

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This value was compared with that obtained by neutron activation analysis of an identical sample. For neutron activation, a Cf-252 source with a neutron flux of 1.42 x 10^8 neutrons/cm^2/sec was used. Sample irradiation time was 110.9 hours with a waiting time of 32 hours before counting. The sample was then counted for approximately 17 hours to ensure sufficient statistical accuracy.

RESULTS AND DISCUSSION

Water. The map in Figure 1 shows the sampling stations used during the study. Stations 1, 2 and 3 are above the treated sewage effluent.

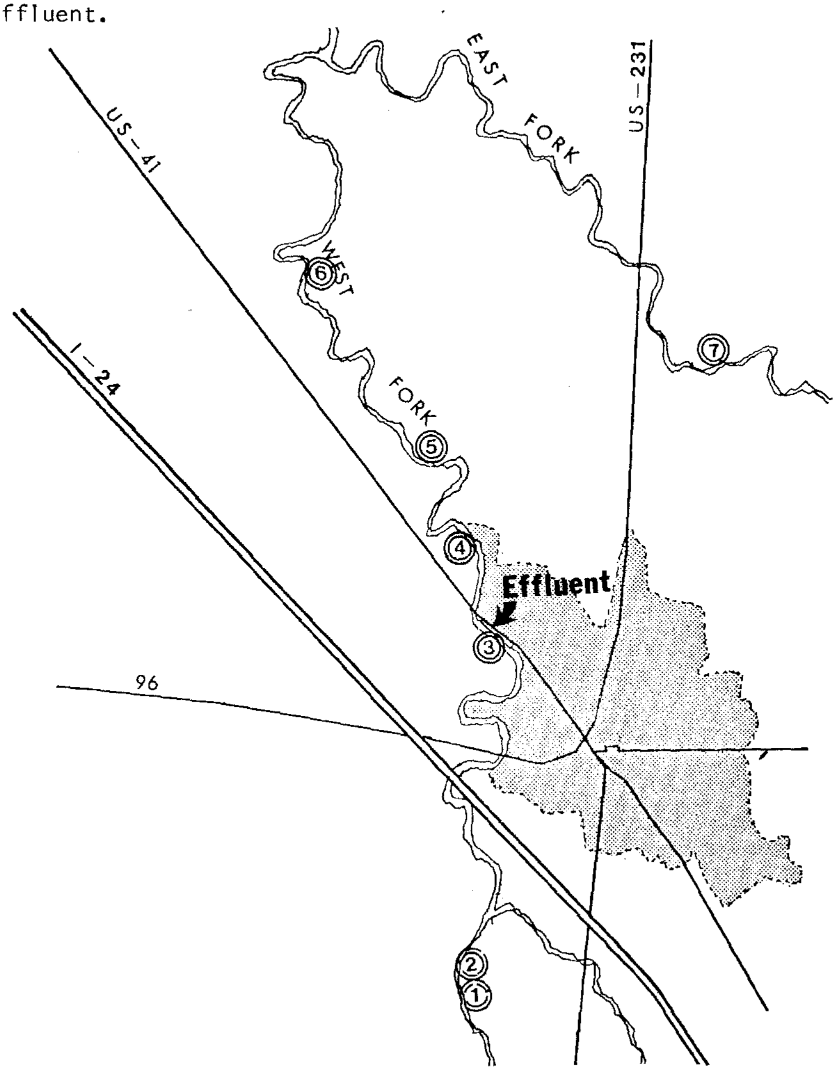

Figure 1. Stones River, Showing the Sampling Stations Established Along the Stream.

The treated sewage effluent enters the stream approximately 100 meters downstream from station 3. Station 4 is immediately below the effluent.

A summary of the bromide levels in the water is presented in Figure 2.

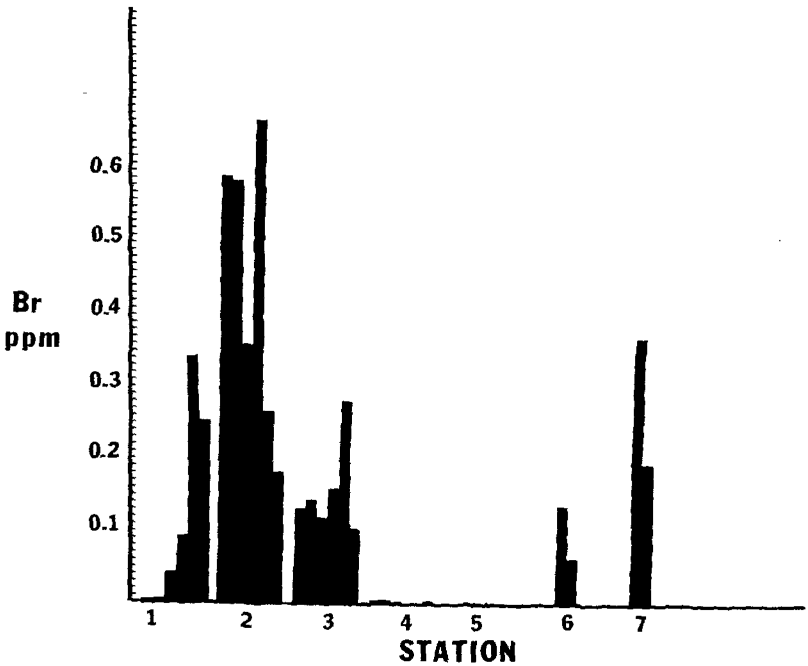

Figure 2. Bromine Concentrations at Various Sampling Stations.

Station 1 was shallow and had a relatively low flow during four of the sampling periods. In addition, the ambient temperature was much higher (near 26°C). Water temperatures for stations 2 and 3 ranged from 18°C to 21°C during the summer months but dropped to 14°C by October.

Generally, the amount of bromide in the water above the wastewater outfall varies inversely with the increased flow due to rainfall. Although levels as high as 0.67 ppm occur at points upstream, the bromide level appears to stabilize at approximately 0.2-0.3 ppm in free-flowing, nonpolluted water. Samples taken in the East Fork of the Stones River (Station 7) have bromide levels similar to those of the West Fork.

At points where oxidizing conditions exist, either naturally or pollution-induced, bromide levels are at or near the detection limit of the bromide assay method (0.001 ppm). This was found to occur consistently at stations 4 and 5, below the wastewater outfall.

Table 1 summarizes the data for the effects of pH, temperature, dissolved oxygen and residual chlorine on bromide levels in the water above and below the treated effluent. These are representative samples.

Samples taken in February had bromide levels essentially identical to those of other months provided the flow is from ground waters or springs.

The depletion of bromide is obviously due to chlorine addition to the wastewater effluent.

Fish and Crayfish. A typical x-ray fluorescence spectrum of lyophilized *Orconectes diogenes* (crayfish) taken from unpolluted waters is shown in Figure 3. Peaks for K, Ca, Mn, Fe, Cu, Zn, Br and Sr are present. No differentiation can be made between inorganic and organic bromine.

Samples of lyophilized *Hypentelium nigricans* (northern hognose) from stations 2 and 5 were divided and standard additions of 5, 10 and 15 ppm bromide were made by addition of a 1.0 µg/ml KBr solution followed by homogenization and relyophilization as listed in MATERIALS AND METHODS. Extrapolation of the graph gave a bromine level of 29±1.5 ppm (µg bromine/g dry tissue) from the upstream sample and 14 ppm in a sample collected downstream from the wastewater effluent.

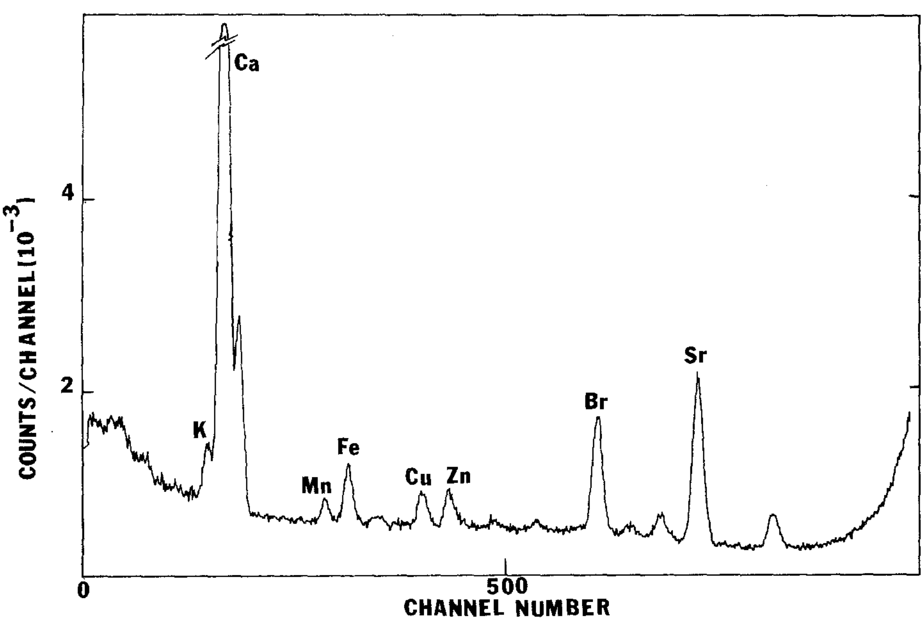

Figure 3. X-ray Fluorescence Spectrum of *Orconectes diogenes* (crayfish).

TABLE 1

Effluent Effects Upon Water Quality Parameters and the Bromide Concentration

Date	Dilution[1] Factor	pH		Temp °C		Dissolved O_2 (ppm)		Chlorine Residual (ppm)		Bromide[2] Level (ppm)
		Above	Below	Above	Below	Above	Below	Effluent	Combined Flow	Above
Aug 26	2.9	5.8	6.1	21	22	6.9	6.7	0.8	0.52	0.18
Sept 25	2.0	7.3	7.2	18	18	5.7	5.5	0.9	0.46	0.10
Sept 27	2.8	7.4	7.3	20	20	5.6	5.5	0.5	0.32	0.18
Oct 16	1.8	7.9	7.8	14	14	6.9	6.4	1.0	0.45	0.28

[1]Dilution Factor = $\frac{\text{Flow of Stream + Effluent}}{\text{Flow of Stream}}$

[2]Bromide levels below the effluent were at or below detection levels (0.001 ppm)

Neutron activation analysis of the same upstream sample gave a bromine content of 21.0±1.0 ppm.

Comparisons of bromine levels in fish and crayfish collected above and below the wastewater effluent are given in Table 2.

TABLE 2

Bromine Concentrations in Fish and Crayfish

	Above Effluent		Below Effluent	
Species	Station	Concentration (ppm)	Station	Concentration (ppm)
Fish:				
Lepomis Microhirus	2	54	4	28
Campostoma anomalum	2	54	4	18
Notropis cornutus	2	33	4	21
Etheostoma blennioides	2	34	4	23
Micropterus dolomieui	2	35	4	25
Micropterus dolomieui	2	34	5	17
Hypentelium nigricans	2	29	4	12
Hypentelium nigricans	-	--	5	14
Hybopsis (nocomis) effusa	1	35	-	--
	Average	38.5	Average	19.8
Crayfish:				
Orconectes diogenes	1	165	4	86
Orconectes diogenes	2	134		

Bromine enrichment is more pronounced in crayfish than in fish. Predator fish species show more enrichment than others.

For the region of the stream directly below the wastewater effluent, bromine exhaustion resulted in a fifty percent reduction in bromine concentration in both fish and crayfish. Fish and crayfish movement in the area of sampling is restricted by a dam located a short distance above the effluent. Migration of species from regions downstream, where bromide levels have somewhat recovered by augmented flow from groundwater, is restricted only by riffles.

The alteration of natural bromine distribution at fractional parts per million levels has not yet been proven to exert harmful effects upon aquatic life or the wholesomeness of the water. However, a recent study of MINEAR et al. (1978) showed that the total trihalomethane concentrations (expressed as ppb) were 82, 108 and 251 for winter, spring and summer, prespectively, for the drinking water supply of the area. Murfreesboro's water supply contained 8 ppb and 77 ppb $CHCl_2Br$ in winter and summer, respectively, while $CHClBr_2$ was found to be 1.5 ppb in the winter and spring and 6.1 ppb in the summer. The bromo species represented a major fraction in several samples and in some cases the majority of the total trihalomethanes. Our data is supported by their observation that trihalomethanes form more readily in higher water temperatures. The higher water temperature would promote depletion of natural bromine by forming free Br_2 from Br^-. We found that bromide levels are lower in warmer waters.

We are presently studying the mechanism(s) for bromine enrichment in fish and crayfish. Brominated tyrosines have been detected in hydrolyzed tissue samples. Additionally, we are investigating the consequences of a large spill of liquid bromine as the result of a highway accident. The accident took place in an isolated area allowing a definitive study of bromine accumulation in plants, soils and other tissues.

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REFERENCES

BURDICK, J.C., DREWERY, W.A., SHELTON, S.P., WELLS, M.R., WOODS, A.E., CHANDLER, C.M. and BROWN, J.W.: A Planning Study of the West Fork-Stones River: Evaluation of Current and Future Water Quality Conditions. A Report Funded by the Environmental Protection Agency under Grant WRC-Tenn-244-Murfreesboro, TN, July 1973.

CASTO, E.: Effects of Wastewater Effluent on Indigenous Bromine in the West Fork-Stones River. M.S. Thesis, Middle Tennessee State University, May 1977.

MINEAR, R.A., BIRD, J.C., and JOHNSON, D.E.: Trihalomethanes in Tennessee Drinking Waters. Presented at Annual Joint Conference of the Kentucky-Tennessee Sections of the American Water Works Association and the Water Pollution Control Association, November 1978.

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 14th Edition American Public Health Association, New York (1976).

WOODS, A.E., CASTO, E., CARTON, R.F. and GLEASON, G.: Indigenous Bromine in Freshwater and Freshwater Organisms. Paper Number 106. Presented at American Chemical Society, Southeast Regional Meeting, Tampa, FLA., November 1977.