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ABSTRACT

Fish and amphibian egg cultures were used to determine the embryopathic effects of cadmium, mercury, and zinc released from natural and metal-enriched sediments, and to develop egg culture bioassay procedures suitable for monitoring bottom sediments for hazardous contaminants. Eggs of the narrow-mouthed toad (Gastrophryne carolinensis), goldfish (Carassius auratus), and rainbow trout (Salmo gairdneri) were cultured in contaminant-free water added to natural and metal-enriched sediments. Exposure was initiated after fertilization (toad, goldfish) or at 10 days prehatching (trout) and maintained continuously through 4-10 days posthatching. Sediments were enriched with 0.1-100 ppm cadmium and mercury and 1.0-1000 ppm zinc.

Natural control sediments contained average concentrations of 0.052 ppm mercury, 1.0 ppm cadmium, and 108.2 ppm zinc. Substantial frequencies of mortality and teratogenesis occurred for all 3 animal species when eggs were cultured over natural sediments further enriched with as little as 0.1-1.0 ppm cadmium or mercury and 1-10 ppm zinc. Survival of trout embryos and alevins closely paralleled sediment test concentrations. The sediment TL_{50} concentrations for trout stages cultured from 10 days prehatching through 10 days posthatching were approximately 1 ppm for mercury, 2.15 ppm for cadmium, and 210.6 ppm for zinc. Sediment metals were substantially more lethal to eggs and embryos than to free-living larvae or fry.

Only small fractions of sediment-bound metals were released to culture water during the 20-day trout culture period, with approximate release rates of 5% for mercury, 4% for cadmium, and 1% for zinc. However, culture water concentrations ranged from 0.11-6.40, 2.1-7.2, and 17.0-122.8 ppb for sediment concentrations of 0.052-106.7, 1-121, and 108-1157 ppm mercury, cadmium and zinc, respectively. The inverse relationship between sediment release rates and aqueous retention levels likely resulted from differential loss of the 3 metals from culture water by volatilization, adsorption to glass surfaces, accumulation by test organisms, and/or readsorption by sediment.

Trout embryos and alevins concentrated sediment-released mercury to high tissue levels. At mercury sediment concentrations of 1, 12.1 and 106.7 ppm, tissue accumulation levels for the 20-day exposure period were 41, 269, and 902 ppb, respectively.

Trout eggs cultured over natural sediment collected from 4 Inner Bluegrass streams gave hatching frequencies which correlated closely with 1) levels of sediment metal contamination and 2) density and diversity of aquatic fauna. As bioindicator results compared closely with independent ecological indicators used to evaluate water quality, short-term egg hatchability tests possibly may be used to predict long-term ecological effects of aquatic and sediment-bound contaminants.

Descriptors: Sediment Metals, Bioassay, Bioindicator, Eggs and Embryos

INTRODUCTION

It has been well established that many trace metal contaminants which enter natural water resources accumulate to high levels in bottom sediments. Cadmium, mercury, zinc, and various other heavy metals have been reported at concentrations up to 100 ppm or more in bottom sediments of numerous U.S. waterways (1-5). Jernelov (6) showed that fish exposed to bottom sediments spiked to 100 ppm mercury, accumulated tissue mercury to 3 ppm in 15 days. As bottom sediments serve as spawning substrates for many aquatic species, it is essential to determine whether metal release rates are sufficient to reduce egg hatchability or impair embryonic development (e.g., lethality, teratogenesis). This is particularly important as fish embryos are known to exhibit high bioaccumulation potentials for certain metals. For example, trout and catfish embryos treated with 0.1-3.0 ppb mercury for 5 days in a continuous flow system concentrated tissue mercury at 500-2000 times the exposure levels (7).

The objectives of this study were to determine 1) whether cadmium, mercury, and zinc released from natural and spiked sediments affect hatchability and development of fish and amphibian eggs and 2) whether egg culture bioassays may be used to monitor the release of hazardous substances from bottom sediments.

LITERATURE REVIEW

During recent years environmental toxicologists have placed increased emphasis on the distribution and cycling of trace contaminants among the abiotic and biotic compartments of aquatic ecosystems. Such considerations are essential to an understanding of the factors governing the biological availability and assimilation of trace contaminants which enter water resources. Studies in this area have been concentrated largely on a limited number of trace metal contaminants which originate from multiple sources of pollution (e.g.,cadmium, copper, mercury, zinc). Investigations on mercury have provided the greatest insight into mechanisms which regulate the distribution of trace elements within water resources (6, 8-17).

When introduced into aquatic ecosystems, mercury is distributed among the major compartments, including tripton (e.g., suspended solids, organic detritus), bottom sediments, biomass (living organisms) and water. Relative

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uptake by the various compartments will depend on such factors as the oxidation state of the mercury, physical and chemical characteristics of the water (e.g., redox potential, pH), and the availability of mercury binding sites or adsorbing surfaces. In the oxygenated waters supporting living organisms, mercury generally predominates in the +2 state (12), and is rapidly removed from solution as a result of 1) adsorption by suspended solids and bottom sediments (e.g., clay minerals), 2) binding to organic detritus, and/or 3) assimilation by living organisms (e.g., plankton). Prevailing fluxes among these various compartments subsequently result in a progressive accumulation of mercury in bottom sediment and biomass.

Bottom sediments generally constitute the principal "sink" for mercury and numerous other trace metal contaminants. Sediment concentrations of 1-10 ppm Hg generally have been observed in contaminated waters, and levels up to 100 ppm or more have been reported in various instances (13, 15-21). For example, Hancock (2) recorded a maximum level of 187 ppm Hg in sediment collected from the Tennessee River in Western Kentucky. Sediment from contaminated aquatic resources may contain 10,000 times more mercury than found in the water (8, 22). The mercury content of sediment-free water taken from most polluted regions usually does not exceed 1 ppb, and seldom is greater than 5 ppb (12, 16, 23).

Though bottom sediments sequester a substantial fraction of the mercury which enters aquatic ecosystems, sediment-accumulated mercury may be remobilized back into overlying water. Jernelov (6) showed that fish exposed to bottom sediment containing 100 ppm inorganic mercury accumulated up to 3 ppm methyl mercury in their tissues after treatment for 15 days. Estimates indicate that the resorption of mercury from bottom sediments may continue for 10-100 years after pollution is curtailed (10, 24). The rate of resorption depends on various factors, including the composition of the sediment (e.g., sulfides, percent organic matter), redox potential, microbial activity, and the depth at which the mercury is stratified (10, 12, 21). Bothner and Carpenter (21) recently reported a relatively short half-life of 1.3 years for sediment-bound mercury. Methylation of inorganic mercury by microbial action reportedly facilitates

remobilization from sediments into water, contributing to the accumulation of methyl mercury by aquatic organisms (6, 9-12, 25-27).

Many other trace metals also are known to accumulate progressively in bottom sediments (e.g., As, Cd, Cr, Cu, Ni, Mn, Pb, Zn). Sediment concentrations up to 100 ppm or more are not uncommon for such metals (2, 4, 5, 19, 28, 29). For example, bottom sediments from the Tennessee River in western Kentucky contained arsenic and zinc up to 46.7 and 167 ppm (2), and cadmium has been reported to reach 50 ppm in sediment from the Holston River in Tennessee, with sediment-bound zinc accumulating to 1000 ppm or more (4). Whether such metals are remobilized from sediment in sufficient quantities to affect living organisms is not clear. However, Sanchez and Lee (30) have shown that sediment binding of copper is affected by changes in carbonate alkalinity, and there is evidence that arsenic, selenium and tellurium may undergo methylation (31-33).

As noted above, mercury which enters aquatic resources is rapidly redistributed from the water to other compartments, accumulating progressively in sediment and biomass. Bioassimilation of mercury involves uptake directly from water or secondarily from bottom sediment (e.g., mercury remobilization) and tripton (e.g., organic detritus consumed as food). The assimilation rate varies with such factors as the form and concentration of mercury, physical and chemical characteristics of the water, and the size and species composition of the biomass (8, 9, 12). Bioaccumulation of mercury by living organisms is a function of assimilation rate/body elimination rate, and may vary according to animal species or individual characteristics (e.g., age, size, metabolic rate). Generally, the bioaccumulation potentials of different forms of mercury (e.g., Hg^{+}, Hg^{++}, CH_3Hg^{+}) increase with chemical or physical properties which facilitate bioassimilation (e.g., lipid solubility) and physiological properties which increase biological half-life (e.g., high tissue affinity, low excretion rate).

Organic forms of mercury exhibit longer biological half-life and accumulate to higher levels in animal tissues (8, 14-16). Depending on temperature, which affects metabolic rate, the biological half-life of methyl mercury may range up to 1 1/2 years in trout and 2 years in pike (34, 35). Mercury accumulation ratios have been reported to be 1000 for macrophytes and freshwater phytoplankton, 10,000-100,000 for certain fresh-

water invertebrates, and 1000-10,000 for fish (8, 9, 17, 34, 36-38). Pike (36) and trout (38) are known to concentrate mercury above levels found in water by factors of 5000 and 10,000, respectively. When exposed for 2 months to water containing 0.05 ppb methyl mercury, brook trout were found to accumulate 0.5 ppm mercury in their tissues (8, 38). In the same series of experiments, methyl mercury administered at 1 ppb was selectively concentrated 30,000 times in certain fish organs.

The progressive buildup of mercury in the biomass of aquatic ecosystems results from both bioaccumulation at the organismal level and biomagnification through the food chain, in which species at higher trophic levels (e.g., fish, fish-eating mammals) feed on other mercury-concentrating organisms (8, 34, 36, 39). Tissue mercury at or above 0.5-1.0 ppm has been reported for numerous species of fish taken from U.S. waters (2, 15-18). The combination of bioaccumulation and biomagnification increases the prospects for chronic effects of mercury on the health and reproduction of exposed animals. Furthermore, a human health problem may result, since edible tissues of fish and other organisms may concentrate mercury in excess of the FDA limit of 0.5 ppm (15-17). In 1970, 18 states within the U.S. reported fish which exceeded the FDA limit for mercury (17). Mercury at 0.5 ppm or more was observed in 73% of fish collected from the Tennessee River in western Kentucky, with an upper range of 10-34 ppm (2, 40). Though other trace elements have received far less attention than mercury, initial data (41-43) indicate that a number of them accumulate to significant levels in aquatic organisms (e.g., Ag, As, Cd, Cu, Ge, Mn, Pb, Se, Sn, Tl, V, W, Zn).

Certain investigators (31) have de-emphasized the environmental risk of coal-derived mercury, largely because of the low concentration found in the majority of coal deposits (e.g., 0.1-0.2 ppb). However, this circumstance is offset, at least in part, by the ability of aquatic organisms to concentrate appreciable quantities of mercury from environmental levels as low as 30 parts per trillion (8, 38).

As inorganic trace elements vary significantly in physical and chemical properties (44), their behavior in aquatic ecosystems may be expected to differ substantially. Pending further definition of their aquatic chemistry, we may assume that biological availability will increase with properties which facilitate bioaccumulation (e.g., methylation, lipid

solubility, long biological half-life) and decrease with characteristics which promote selective accumulation and long environmental half-life in abiotic components of water resources (e.g., sediment binding).

MATERIALS AND METHODS

Test animals. Studies were undertaken to determine the effects of metals remobilized from bottom sediments on hatchability and development of fish and amphibian eggs. The species selected for study included rainbow trout (Salmo gairdneri), goldfish (Carassius auratus), and narrow-mouthed toad (Gastrophryne carolinensis). This choice was based in part on seasonal availability, suitable egg production, and good handling characteristics for in vitro propagation. In addition, this selection of test animals included species with representative patterns of reproduction, involving a number of important developmental variables which may respond differentially to sediment-borne toxicants (e.g., egg type, yolk quantity, hatching time, reproductive habitat).

Trout and goldfish were obtained from Federal fish hatcheries in Erwin, Tennessee, and Frankfort, Kentucky, respectively, and the toads were purchased from Charles Sullivan, Nashville, Tennessee.

Trout and toad eggs and sperm were collected for test purposes from gravid animals by artificial spawning procedures previously described (45-47). Fertilization was accomplished by mixing sperm and eggs in spawning trays for fifteen minutes. Freshly fertilized goldfish eggs were collected from hatchery ponds.

Test sediments. Sediments used for test purposes were collected from 2nd and 3rd order Inner Bluegrass streams of the Elkhorn and Hickman divisions of the Kentucky River drainage system. The major source of sediment was Steele's Run, at a site near the junction of Old Frankfort Pike and Redd Road, and 0.7 mile upstream from the mouth of South Elkhorn Creek. Steele's Run, a 2nd order limestone-bed stream of the Elkhorn drainage, supports a healthy aquatic biota and is essentially free of major sources of pollution.

Samples were restricted to the upper 5 cm of sediment and were collected 3 to 5 feet from shore at water depths of 1 to 2 feet. Texture and physico-chemical characteristics of the sediment are given in Table 1.

Texture analysis was conducted by Bouyoucos' hydrometer procedure, as modified by Cox (48). Determination of pH was made on a 1:1 mixture of sediment and distilled water, according to the procedure reported by Jackson (49). Specific gravity values were obtained by the method of Carlisle and Caldwell (50), and percent volatile solids was analyzed by the procedure given in Standard Methods (51). Total iron and calcium were determined by atomic absorption spectrophotometry, after digestion of sediment samples with equal proportions of nitric and sulfuric acid (52). Extractable iron was determined by AAS analysis, following the HCl/H_2SO_4 extraction procedure of Perkins (52, 53). Exchangeable calcium was analyzed by AAS, subsequent to extraction of sediment samples with ammonium acetate (52, 54).

In evaluating the use of egg culture bioassays for monitoring sediment-accumulated toxicants, a limited number of samples were collected from Shelby Branch, Town Branch, and Wolf Run at sites previously described (55). As noted by Birge et al. (55), these streams have suffered significant degrees of trace metal pollution in recent years, resulting in marked reductions in aquatic biota in Town Branch and Wolf Run, and moderate decreases in density and diversity of fish and macroinvertebrates for Shelby Branch (56).

Bioassay procedures. Cultures were established in 500 ml pyrex chambers. Sediment to be tested was layered to a depth of 2 cm and covered with 350 ml of sterile, synthetic culture water. Water was added using a surface-oriented inlet tube to minimize disruption of the bottom sediment. After culture dishes were filled, the inverted inlet was used to provide moderate, continuous aeration, maintaining dissolved oxygen at or near saturation. Synthetic culture water was prepared from distilled, double deionized water, having a conductivity of 0.25 µmhos or less. Routine monitoring was conducted for background contaminants. Calcium, magnesium, sodium and potassium salts were added as given in Table 2. This basic stock was prepared to give a water hardness level of 200 ppm $CaCO_3$ and a pH of 7.5-8.0. Other physicochemical characteristics are shown in Table 2, and a more detailed account of the culture water may be found in earlier publications (46, 57). During the bioassay treatment period, variation in test water parameters generally did not exceed 10%.

Using a sample size of 100, treatment was initiated at fertilization for the narrow-mouthed toad, 2-4 hours post-fertilization for the goldfish, and on the 15th day of development for trout embryos. All cultures were continuous through 4-10 days posthatching. Hatching occurred on developmental days 3, 3.5 and 24 for narrow-mouthed toad, goldfish, and trout embryos, respectively. Cultures were maintained under subdued light in walk-in environmental rooms, with temperatures regulated at 20-21° C for toad and goldfish embryos and 13-14° C for trout. All cultures were monitored at regular daily intervals for temperature, dissolved oxygen, ammonia, water hardness and pH, using a YSI tele-thermometer with thermocouple, YSI oxygen meter (model 51A), Orion ammonia and water hardness electrodes, and a Corning digital pH meter (model 110, with expanded millivolt scale). In addition, cultures were inspected to gauge extent of embryonic development and to remove dead organisms. At 3-4 day intervals culture water also was analyzed for trace metals (e.g., Cd, Hg, Zn) by atomic absorption spectrophotometry.

In Table 3, bioassay data were summarized by giving percent mortality at hatching and 4 days posthatching and by stating parenthetically the percent of teratogenic animals appearing in surviving populations at hatching. Frequencies of mortality and teratogenesis both were control adjusted in Table 3, with values determined as frequency of response in metal-enriched cultures over frequency of response in Steele's Run control cultures. In all other tables, bioassay results were not control adjusted and were expressed at percent embryonic and post-embryonic survival. Control bioassays were performed using non-spiked Steele's Run sediment. Analyses for teratogenesis were performed only at hatching and 4 or 10 days posthatching. In Tables 4 and 8, grossly teratogenic survivors were counted as lethals in computing survival values at hatching and 4-10 days posthatching. Frequencies of teratogenesis were not included in data expressed in Tables 5-7.

In addition to using natural sediments as collected from the designated sites, Steele's Run sediments were enriched by concentrations of 0.1-1000 ppm cadmium, mercury or zinc. Metals used to spike culture sediments were administered as chloride salts using the following procedure. After decanting excess water, 30 gm of wet sediment were dried at 100° C for 24 hours and

TABLE 1

SEDIMENT CHARACTERISTICS

TEXTURE ANALYSIS (%)	
Sand	50.8 ± 1.4[a]
Silt	28.6 ± 1.5
Clay	20.6 ± 1.2
PHYSICOCHEMICAL CHARACTERISTICS	
Volatile Solids (%)	5.82 ± 0.24
pH	7.60 ± 0.03
Specific Gravity (g/ml)	2.55 ± 0.08
Total Iron (ppm)	66,461 ± 1007
Extractable Iron (ppm)	283.0 ± 23.2
Total Calcium (ppm)	44,899 ± 974
Exchangeable Calcium (ppm)	6,747 ± 167

[a]Measurements were made at 22°C. Figures represent mean ± standard error for at least seven measurements.

TABLE 2

SYNTHETIC CULTURE WATER

DISSOLVED SALTS[1] (mg/l)	
$CaCl_2$	150
$MgSO_4 \cdot 7H_2O$	150
$NaHCO_3$	100
KCl	5
CHEMICAL COMPOSITION (mg/l)	
Ca	54.2
Mg	14.8
Na	27.4
K	2.6
Cl	98.2
HCO_3	72.6
SO_4	58.5
Total dissolved solids	328.3
PHYSICOCHEMICAL CHARACTERISTICS[2]	
Hardness (as mg/l $CaCO_3$)	200.0
Total alkalinity (as mg/l $CaCO_3$)	82.0
Conductivity (μmhos/cm)	300.0
Osmolarity (mOsm/Kg H_2O)	12.0
pH	7.9

[1]Prepared in distilled, deionized water (specific conductivity of 0.2 μmhos).

[2]Measurements made at 22° C. Above figures represent mean values for six measurements.

weighed to determine percent moisture content. A sample of drained sediment equivalent to 250 gm dry weight was placed in a 500 ml Erlenmeyer flask. Using a 25 ml aliquot of distilled, deionized water, the selected test metal was added at a sufficient concentration to obtain the desired sediment enrichment level (i.e., 0.1, 1.0, 10.0, 100.0, 1000.0 ppm). Control cultures received a 25 ml aliquot of metal-free distilled, deionized water. Flasks were sealed with parafilm and maintained on a shaker for 5 days, after which contents were filtered under vacuum using no. 1 Whatman paper. During filtering, each sediment sample was rinsed with three 50 ml aliquots of distilled, deionized water. After removal of all excess water, the prepared sediment was placed in the bioassay culture dishes. Subsequent to filtration, the moisture content of the sediment was approximately 15-20%.

In all cases, natural and enriched sediments were analyzed for cadmium, mercury and zinc by atomic absorption spectrophotometry, using a Perkin-Elmer model 503 unit equipped with a mercury cold vapor kit and a graphite furnace (model HGA 2100). Sediment analyses were performed at the initiation and at the completion of all bioassays. Prior to analysis, sediment samples were digested using a mixture of sulfuric and nitric acid (51, 52). Ten-day posthatched trout alevins were analyzed for mercury tissue concentrations by cold vapor atomic absorption spectrophotometry. One-gram tissue samples were digested with a 1:1 mixture of concentrated nitric and perchloric acid, following the procedure by Feldman (58).

RESULTS AND CONCLUSIONS

Bioassays with metal-enriched sediments. Bioassays with metal-enriched cultures are presented in summary form in Table 3. Each of the 3 animal test species was exposed to Steele's Run sediments enriched with each of 3 test metals (i.e., Cd, Hg, Zn). Each test metal was administered at 4 enrichment levels starting at 0.1 ppm and increasing at 10-fold intervals to 100 ppm. The only exception included zinc treatment of rainbow trout embryos, in which sediments were spiked at 1-1000 ppm.

TABLE 3

PERCENT MORTALITY AND TERATOGENESIS AT HATCHING (H) AND 4 DAYS POSTHATCHING (PH)

OF FISH AND AMPHIBIAN EGGS EXPOSED CONTINUOUSLY TO SEDIMENT-BOUND MERCURY, CADMIUM, AND ZINC[1]

TEST SPECIES[2]	METAL ADDED TO SEDIMENT[3] (ppm)	% MORTALITY ON METAL SPIKED SEDIMENT								
		MERCURY			CADMIUM			ZINC		
		CONCENTRATION (ppm)[4]	H	PH	CONCENTRATION (ppm)[4]	H	PH	CONCENTRATION (ppm)[4]	H	PH
NARROW-MOUTHED TOAD	0.1	0.146	45(11)	61	1.34	27(1)	33	104.6	6(3)	14
	1.0	1.188	41(17)	52	2.18	26(9)	33	112.6	3(0)	5
	10.0	12.08	49(16)	64	14.8	31(7)	41	124.5	7(1)	14
	100.0	122.83	47(23)	65	122.8	36(11)	52	222.7	7(2)	8
GOLDFISH	0.1	0.164	50(8)	-	1.38	39(10)	44	100.0	24(1)	27
	1.0	0.810	37(8)	43	2.16	33(9)	42	106.6	18(5)	26
	10.0	10.53	46(12)	53	10.7	41(8)	54	112.5	24(3)	24
	100.0	105.60	52(11)	55	105.5	29(6)	32	227.6	14(1)	14
RAINBOW TROUT	0.1	0.180	11(5)	20	1.45	6(1)	12	-	-	-
	1.0	1.050	29(13)	38	2.15	22(11)	32	115.9	3(0)	5
	10.0	12.10	46(20)	60	12.7	33(16)	44	121.4	11(5)	15
	100.0	106.70	73(31)	85	121.0	56(31)	67	210.6	24(6)	33
	1000.0	-	-	-	-	-	-	1157.0	44(21)	54

[1]Percentages of mortality are expressed as frequency in experimental population/controls. Percentages of survivors bearing gross congenital deformities at hatching are given parenthetically.
[2]Exposure was initiated immediately after fertilization for toad and goldfish, with average hatching times of 3 and 3.5 days, respectively. Treatment of trout embryos was initiated at 15 days of development (eyed stage), with hatching occurring on Day 24.
[3]Enrichment level was obtained by metal innoculation of 250 g sediment.
[4]Actual concentration analyzed for 250 g sediment at initiation of experiment.

In all 36 bioassays using metal-spiked sediments, embryonic mortality was appreciably higher than for controls cultured over non-spiked sediments. In addition, frequencies of teratogenic animals appearing in surviving populations ranged from 5-31% in 26 of the bioassays performed on metal-enriched sediment, compared to teratogenic frequencies of less than 1% for controls. As seen in Table 3, embryonic mortality and teratogenesis produced by sediment metals decreased sharply in the following order: mercury, cadmium, zinc.

Comparing values at hatching and 4 days posthatching, it is evident that embryonic stages suffered higher rates of mortality. During the 4-day posthatching exposure period, further increases in mortality generally were under 10% and did not exceed 18%. Therefore, we may conclude that sediment metals are more lethal to eggs and embryos than to free-living larvae and fry.

With respect to the trout, frequencies of mortality and teratogenesis increased proportionately with sediment concentrations of mercury, cadmium, and zinc. This correlation was not evident for the toad and goldfish which exhibit considerably shorter, sensitive embryonic periods (i.e., 3-3.5 days). As seen in Table 3, developmental stages of these species generally were more sensitive than trout embryos to sediment enrichment levels of 0.1-1 ppm Hg, Cd, and Zn, but mortality usually did not increase substantially for the sediment enrichment levels of 10-100 ppm. In comparing sensitivity of the three test species, it should be noted that treatment of trout eggs was not initiated until 15 days of development. The fact that early trout stages were omitted from treatment likely resulted in lower frequencies of mortality and teratogenesis. Even so, trout stages were equally or more sensitive than other species at sediment metal enrichment levels of 10 ppm or more.

Results of continuous bioassay treatment of rainbow trout embryos and alevins from 10 days prehatching through 10 days posthatching are given in Table 4, and survival frequencies for this 20-day exposure period are compared with metal concentrations of sediment and test water. Background metal concentrations for Steele's Run control sediment averaged 0.052, 1.0, and 108.2 ppm for mercury, cadmium, and zinc, respectively. Experimental

TABLE 4

TOXIC EFFECTS OF SEDIMENT-BOUND METAL ON EMBRYOS AND ALEVINS

OF RAINBOW TROUT (SALMO GAIRDNERI) AT HATCHING (H) AND 10 DAYS POSTHATCHING (PH)

METAL	METAL ADDED TO SEDIMENT[1] (ppm)	SEDIMENT METAL CONCENTRATION (ppm)[2] MEAN ± S.E.		WATER METAL CONCENTRATION (ppb)		PERCENT SURVIVAL[3]	
		INITIAL	FINAL	MEAN ± S.E.	RANGE	H	PH
MERCURY	0.0 (Control)	0.052 ± 0.001	0.051 ± 0.001	0.11 ± 0.04	0.0-0.3	95	94
	0.1	0.180 ± 0.012	0.161 ± 0.006	0.25 ± 0.09	0.0-0.5	81	70
	1.0	1.050 ± 0.025	1.047 ± 0.029	0.15 ± 0.06	0.0-0.3	58	45
	10.0	12.10 ± 0.85	10.64 ± 0.18	1.83 ± 1.49	0.2-6.3	41	23
	100.0	106.70 ± 5.70	104.13 ± 5.42	6.40 ± 3.66	0.4-15.7	18	0
CADMIUM	0.0 (Control)	1.00 ± 0.03	0.95 ± 0.03	2.1 ± 0.6	0.0-5.0	95	94
	0.1	1.45 ± 0.06	1.40 ± 0.07	4.7 ± 2.4	0.0-14.0	88	80
	1.0	2.15 ± 0.06	2.00 ± 0.03	6.8 ± 1.7	2.0-12.0	66	53
	10.0	12.7 ± 0.2	12.7 ± 0.3	7.5 ± 1.9	2.0-14.0	54	37
	100.0	121.0 ± 1.1	113.0 ± 2.6	7.2 ± 1.5	2.0-13.0	29	11
ZINC	0.0 (Control)	108.2 ± 1.2	106.6 ± 0.4	19.4 ± 1.9	6.0-32.0	95	94
	1.0	115.9 ± 0.6	115.2 ± 0.5	17.0 ± 4.6	5.0-38.0	92	88
	10.0	121.4 ± 1.2	119.8 ± 1.0	21.2 ± 4.0	9.0-36.0	81	75
	100.0	210.6 ± 1.1	211.5 ± 4.3	32.3 ± 7.2	17.0-65.0	68	56
	1000.0	1157.0 ± 2.9	1133.0 ±34.6	122.8 ±15.3	80.0-173.0	42	28

[1]Concentration of metal added to 250 g of sediment.

[2]Sediment metal was analyzed at initiation and termination of bioassay, for an exposure period of 20 days.

[3]Survival frequency of normal animals was determined at hatching (H) and 10 days posthatching (PH). Animals bearing gross congenital deformities were counted as lethals.

cultures were performed using Steele's Run sediment which was further enriched with 0.1-100 ppm mercury and cadmium, and 1-1000 ppm zinc.

Comparing initial sediment metal concentrations with final values determined upon completion of the bioassays, it is apparent that only small quantities of sediment-bound metals were released into the culture water (Table 4). Of the 12 trout bioassays performed with metal-enriched sediments, decreases in sediment metal during the 20-day exposure period were generally less than 5 percent, and exceeded 7% in only 2 instances. The greatest decrease observed was 11% for sediment spiked with 10 ppm Hg. No substantial differences were observed in the release rates for mercury, cadmium and zinc. Averaging data for all enrichment levels, percent metal retained in the sediment after 20 days was 94.8 for mercury, 95.6 for cadmium, and 98.9 for zinc.

In all instances, a distinct inverse correlation existed between sediment metal concentration and percent survival of trout stages. For example, in trout bioassays on mercury-spiked sediment, survival at 10 days posthatching was 94% for control cultures (Steele's Run sediment), and 70%, 45%, and 0% for enrichment levels of 0.1, 1.0, and 100 ppm mercury, respectively (Table 4). Trout survival frequencies for cadmium-spiked sediment were 80%, 53%, and 11% at enrichment levels of 0.1, 1.0 and 100 ppm. Zinc was less toxic to trout embryos and alevins, with survival frequencies of 88%, 56%, and 28% for sediment spiked with 1, 100 and 1000 ppm zinc, respectively.

Though the metal content of bioassay test water increased with sediment metal content, a close linear correlation generally was not obtained. Steele's Run control sediment which initially contained 0.052 ppm mercury gave a mean test water mercury level of 0.11 ppb, amounting to 0.21% of the sediment concentration. As sediment mercury was enriched to 0.18-1.05 ppm, average test water content increased to approximately 0.2 ppb. Thus, 3.5-20-fold increases in sediment mercury produced an approximate 2-fold increase in aqueous mercury. However, when sediment mercury was raised from 1.05 to 12.10 ppm, the culture water concentration increased proportionately to 1.83 ppb. With a further increase in sediment mercury from 12.1 to 106.7 ppm,

the mean culture water level rose to 6.4 ppb. This represented 8.8- and 3.5-fold increases in the mercury content of sediment and culture water, respectively, and the aqueous mercury level of 6.4 ppb was only 0.006% of the sediment concentration.

Sediment cadmium concentrations of 1.00 (control), 1.45, and 2.15 ppm gave test water cadmium levels averaging 2.1, 4.7 and 6.8 ppb. Within this range, increases in aqueous cadmium were approximately proportional to those for sediment cadmium, with aqueous levels averaging 0.21-0.32% of the corresponding sediment cadmium concentrations. However, when sediment cadmium was further increased to 12.7 ppm, the concentration in culture water rose only slightly to 7.5 ppb, and no further elevation of aqueous cadmium was noted when the sediment level was enriched to 121 ppm.

The closest correlation between increases in sediment and test water metal was noted for zinc. Steele's Run control sediment which contained 108.2 ppm zinc gave an average aqueous concentration of 19.4 ppb for the 20-day culture period. When sediment zinc was increased to 121.4, 210.6, and 1157 ppm, average test water concentrations increased nearly proportionately to 21.2, 32.3, and 122.8 ppb. Over the wide range of enrichment levels tested, zinc culture water values averaged 0.011-0.020% of corresponding, initial sediment concentrations.

As presented in Table 4, aqueous metal concentrations increased in the following order: mercury, cadmium, zinc. For example, at a sediment level of 1 ppm, aqueous mercury and cadmium averaged 0.15 and 2.1 ppb, and sediment concentrations of 106.7 ppm mercury, 121 ppm cadmium, and 108.2 ppm zinc gave mean culture water levels of 6.4, 7.2, and 19.4 ppb, respectively. As noted above, a comparison of initial and final sediment metal concentrations showed no great differences in release rates for the three metals. However, averaging data for all sediment concentrations, reductions in sediment metal during the 20-day bioassay period were approximately 5% for mercury, 4% for cadmium, and 1% for zinc. It is evident from these data that release rates for the three metals varied inversely with their aqueous retention levels. This likely resulted from differential loss of the three metals from bioassay culture water, probably through 1) volatilization, 2) adsorption to glass surfaces, 3) bioaccumulation in test organisms, and/or 4) readsorption by sediment. It also should be

noted that for metals such as mercury and cadmium, water retention levels do not provide a reliable index from which to estimate the high concentrations which may accumulate in sediment or the magnitude of embryopathic effects of sediment-borne metals (Table 4).

In Table 5, tissue mercury levels for trout alevins are compared with initial sediment and average test water mercury values. The data clearly showed that trout embryos and alevins accumulated high levels of mercury within the 20-day exposure period, and that tissue accumulation levels correlated closely with sediment mercury concentrations. As sediment mercury was increased from 0.052 to 1.050 ppm, tissue mercury for 10-day trout alevins rose from 0.024 to 0.041 µg/g (ppm). When sediment mercury concentrations were further increased to 12.10 and 106.70 ppm, tissue accumulation levels rose sharply to 0.269 and 0.902 µg/g.

It also is evident that both aqueous and tissue mercury levels increased substantially and proportionately when sediment mercury concentrations were elevated above 1 ppm (Table 5). However, below a level of 1 ppm, fluctuations in sediment mercury content produced less profound effects on aqueous and tissue mercury levels. As also seen in Table 5, tissue mercury levels ranged from 140-270 times aqueous mercury concentrations. For example, trout embryos and alevins exposed to culture water containing 6.4 ppb mercury concentrated tissue mercury to 902 ppb in 20 days.

We should note further that tissue mercury levels for trout alevins correlated closely with survival (Table 5). Exposure for 20 days to sediment containing 1 ppm mercury reduced survival of trout alevins to the TL_{50} range, and gave tissue accumulation levels averaging 0.041 µg/g. When sediment mercury was increased to 12.1 and 106.7 ppm, survival dropped to 33% and 7%, correlating with average tissue mercury levels of 0.269 and 0.902 µg/g.

To provide a more accurate comparison of embryonic and post-embryonic sensitivity to sediment-borne metals, percent survival for populations of trout embryos and alevins was determined at 1-2 day intervals during the 20-day exposure period. The latter was initiated

on the 15th day of development, with hatching occurring on day 24, and these bioassays provided a basis for comparing test responses during the last 10 days of embryonic development to those for the first 10 days posthatching. As seen in Table 5, sediment mercury produced a near linear decrease in survival during the 10-day embryonic period, with substantially less mortality recorded after hatching on day 24. A similar response also was noted for sediment-bound cadmium and zinc (Tables 6,7). This further supports the precept that eggs and embryos supported on a sediment substrate are more subject to the toxic actions of sediment-borne metals than are free-living juvenile stages.

Trout egg bioassays on natural sediments. In order to evaluate the use of fish eggs as bioindicators for detecting sediment contamination, trout egg cultures were maintained from 10 days prehatching to 10 days posthatching over sediments collected from 4 Inner Bluegrass streams which have suffered various levels of trace metal pollution during recent years (Table 8). As noted above, Steele's Run supports a healthy aquatic ecosystem, and Shelby Branch shows moderate reductions in diversity and density of macroinvertebrate and piscine species. By comparison, Wolf Run supports only a limited piscine fauna and the sediment collecting site on Town Branch was nearly abiotic. In a previous study by Birge et al. (55), fish and amphibian egg cultures were used to perform bioindicator studies on water samples from these and other Bluegrass streams (Table 9). Combining fish and amphibian data, hatchability frequencies for water samples taken from Town Branch, Wolf Run, Shelby Branch, and Steele's Run were 0%, 23%, 87%, and 94%, respectively. Decreases in hatchability correlated closely with reductions in the diversity of fish fauna which have occurred over the past two decades.

In the present study, bioassay cultures were established by adding contaminant-free water to natural, non-spiked sediments. Trout eggs cultured over sediment samples collected from Town Branch, Wolf Run, Shelby Branch, and Steele's Run gave posthatching survival values of 1%, 30%, 81%, and 94%, respectively (Table 8). Generally, sediment and aqueous metal concentrations were higher for cultures which displayed lower survival

frequencies. The survival frequencies obtained for sediment cultures compared closely with those previously reported for egg hatchability tests on water samples collected from these same streams. In addition, results of the sediment bioassays correlated closely with independent ecological indicators used to evaluate water quality (Table 9; ref. 55), providing further evidence that short-term egg hatchability tests may be used to predict long-term ecological effects of aquatic and sediment-accumulated contaminants.

TABLE 5

CORRELATION OF PERCENT SURVIVAL WITH EXPOSURE TIME

FOR TROUT EMBRYOS AND ALEVINS EXPOSED TO SEDIMENT-BOUND MERCURY

ETAL ADDED O SEDIMENT[1] (ppm)	SEDIMENT METAL CONCENTRATION (ppm)[2] MEAN ± S.E.	WATER METAL CONCENTRATION (ppb)[3] MEAN ± S.E.	PERCENT SURVIVAL WITH DEVELOPMENTAL AGE IN DAYS[4]														TISSUE CONCENTRATION (μg/g)
			16	17	18	19	20	21	22	23	24	26	28	30	32	34	
0.0	0.052 ± 0.001	0.11 ± 0.04	100	99	99	98	98	98	96	96	95	95	94	94	94	94	0.024
0.1	0.180 ± 0.012	0.25 ± 0.09	97	97	97	97	95	93	89	87	85	80	75	75	74	74	0.036
1.0	1.050 ± 0.025	0.15 ± 0.06	97	94	91	88	85	79	77	72	67	63	58	56	54	54	0.041
10.0	12.10 ± 0.85	1.83 ± 1.49	97	94	89	84	79	74	68	59	51	43	38	34	33	33	0.269
100.0	106.70 ± 5.70	6.40 ± 3.66	97	84	76	67	60	53	41	33	26	21	14	11	8	7	0.902

[1]Enrichment level was obtained by metal innoculation of 250 g sediment.

[2]Actual concentration analyzed for 250 g sediment at initiation of experiment.

[3]Water analyses were performed in triplicate at approximately 4 day intervals.

[4]Treatment was initiated at 15 days of development (eyed stage). Hatching occurred at day 24 with exposure continuous through 10 days posthatching (day 34).

TABLE 6

CORRELATION OF PERCENT SURVIVAL WITH EXPOSURE TIME

FOR TROUT EMBRYOS AND ALEVINS EXPOSED TO SEDIMENT-BOUND CADMIUM

METAL ADDED TO SEDIMENT[1] (ppm)	SEDIMENT METAL CONCENTRATION (ppm)[2] MEAN ± S.E.	WATER METAL CONCENTRATION (ppb)[3] MEAN ± S.E.	PERCENT SURVIVAL WITH DEVELOPMENTAL AGE IN DAYS[4]													
			16	17	18	19	20	21	22	23	24	26	28	30	32	34
0.0	1.00 ± 0.03	2.1 ± 0.6	100	99	99	98	98	98	96	96	95	95	94	94	94	94
0.1	1.45 ± 0.06	4.7 ± 2.4	98	97	97	96	95	94	91	90	89	86	82	82	81	81
1.0	2.15 ± 0.06	6.8 ± 1.7	98	92	88	86	84	83	79	77	74	67	63	62	61	61
10.0	12.7 ± 0.2	7.5 ± 1.9	98	94	91	87	81	77	74	69	64	57	52	49	47	47
100.0	121.0 ± 1.1	7.2 ± 1.5	98	93	85	78	73	65	56	48	42	37	31	28	25	24

[1]Enrichment level was obtained by metal innoculation of 250 g sediment.

[2]Actual concentration analyzed for 250 g sediment at initiation of experiment.

[3]Water analyses were performed in triplicate at approximately 3 day intervals.

[4]Treatment was initiated at 15 days of development (eyed stage). Hatching occurred at day 24 with exposure continuous through 10 days posthatching (day 34).

TABLE 7

CORRELATION OF PERCENT SURVIVAL WITH EXPOSURE TIME

FOR TROUT EMBRYOS AND ALEVINS EXPOSED TO SEDIMENT-BOUND ZINC

METAL ADDED TO SEDIMENT[1] (ppm)	SEDIMENT METAL CONCENTRATION (ppm)[2] MEAN ± S.E.	WATER METAL CONCENTRATION (ppb)[3] MEAN ± S.E.	PERCENT SURVIVAL WITH DEVELOPMENTAL AGE IN DAYS[4]														
			16	17	18	19	20	21	22	23	24	26	28	30	32	34	
0.0	108.2 ± 1.2	19.4 ± 1.9	100	99	99	98	98	98	96	96	95	95	94	94	94	94	
1.0	115.9 ± 0.6	17.0 ± 4.6	99	97	97	96	96	95	92	92	92	89	88	88	88	88	
10.0	121.4 ± 1.2	21.2 ± 4.0	99	95	93	92	90	89	87	86	85	81	79	79	79	79	
100.0	210.6 ± 1.1	32.3 ± 7.2	99	93	89	85	82	81	78	75	72	66	62	61	60	60	
1000.0	1157.0 ± 2.9	122.8 ±15.3	99	92	83	80	76	75	67	61	53	47	43	41	40	39	

[1]Enrichment level was obtained by metal innoculation of 250 g sediment.

[2]Actual concentration analyzed for 250 g sediment at initiation of experiment.

[3]Water analyses were performed in triplicate at approximately 3 day intervals.

[4]Treatment was initiated at 15 days of development (eyed stage). Hatching occurred at day 24 with exposure continuous through 10 days posthatching (day 34).

TABLE 8

SURVIVAL AT HATCHING (H) AND 10 DAYS POSTHATCHING (PH) OF TROUT EGGS CULTURED OVER NATURAL BOTTOM SEDIMENTS FROM STREAMS OF VARYING DEGREES OF WATER QUALITY[1]

STREAM SELECTION SITE[2]	PERCENT SURVIVAL[3]		METAL ANALYZED	SEDIMENT METAL CONCENTRATION (ppm)[4] MEAN ± S.E.		WATER METAL CONCENTRATION (ppb)[5]	
	H	PH		INITIAL	FINAL	MEAN ± S.E.	RANGE
TOWN BRANCH	14	1	MERCURY	0.270 ± 0.002	0.290 ± 0.002	0.18 ± 0.06	0.0-0.4
			CADMIUM	7.07 ± 0.12	6.65 ± 0.08	3.2 ± 0.7	0.0-6.0
			ZINC	299.0 ± 1.5	297.0 ± 6.1	19.9 ± 2.4	13.0-26.0
WOLF RUN	46	30	MERCURY	0.070 ± 0.004	0.062 ± 0.005	0.17 ± 0.13	0.0-0.8
			CADMIUM	1.40 ± 0.05	1.10 ± 0.06	5.3 ± 1.3	1.0-13.0
			ZINC	147.4 ± 0.5	120.2 ± 0.4	10.3 ± 1.7	3.0-17.0
SHELBY BRANCH	84	81	MERCURY	0.054 ± 0.008	0.044 ± 0.005	0.22 ± 0.12	0.0-0.6
			CADMIUM	1.45 ± 0.03	1.31 ± 0.02	3.1 ± 0.9	0.0-8.0
			ZINC	80.0 ± 1.6	77.0 ± 1.0	10.6 ± 3.2	0.0-26.0
STEELE'S RUN	95	94	MERCURY	0.052 ± 0.001	0.051 ± 0.001	0.11 ± 0.04	0.0-0.3
			CADMIUM	1.00 ± 0.03	0.95 ± 0.03	2.1 ± 0.6	0.0-5.0
			ZINC	108.2 ± 1.2	106.6 ± 0.4	19.4 ± 1.9	6.0-32.0

[1]Treatment was initiated at 15 days of development (eyed stage). Hatching occurred at day 24 with exposure continuous through 10 days posthatching.

[2]Streams selected to represent different levels of water pollution resulting from recent increases in urbanization and industrialization in the Kentucky Bluegrass region. Based on independent ecological indicators, the order of increasing water quality was Town Branch, Wolf Run, Shelby Branch, Steele's Run.

[3]Survival frequency of normal animals was determined at hatching (H) and 10 days posthatching (PH). Animals bearing gross congenital deformities were counted as lethals.

[4]Sediment metal was analyzed at initiation and termination of bioassay, for an exposure period of 20 days.

[5]Water analyses were performed at 3-4 day intervals on synthetic culture water added to natural sediment at onset of the bioassay.

TABLE 9

CORRELATION OF PERCENT EGG HATCHABILITY WITH
POLLUTION-INDUCED REDUCTION IN DIVERSITY OF PISCINE FAUNA

MONITORING SITE[1]	PERCENT EGG HATCHABILITY[2]			DIVERSITY OF FISH FAUNA[3]	
	FISH	AMPHIBIANS	FISH & AMPHIBIANS	# SPECIES/EXPECTED[4]	% SPECIES REMAINING
Cane Run	0	0	0	0/15	0
Town Branch	0	0	0	0/15	0
Wolf Run	40	18	24	2/15	13
East Hickman Creek	56	45	48	8/17	47
West Hickman Creek	63	58	60	11/16	69
Gainesway Branch	70	74	73	7/10	70
Hickman Creek	74	82	80	15/22	68
Shelby Branch	81	90	87	14/17	82
Kentucky River	83	89	87	21/25	84
Elkhorn Creek	86	92	89	43/48	90
Steele's Run	91	97	94	15/15	100

[1]Streams and rivers selected to represent various levels of water pollution resulting from recent increases in urbanization and industrialization in the Kentucky Bluegrass region. Taken from Birge et al., 1975 (55).
[2]Egg hatchability data combined and averaged for four species of fish (rainbow trout, largemouth bass, goldfish, channel catfish) and five species of amphibians (squirrel treefrog, gray treefrog, narrow-mouthed toad, pig frog, red-spotted toad).
[3]Data on fish fauna for past 22 years taken from Carter ('54), Jones ('73), Kuehne ('75), Laflin ('70), MacGregor and Andre ('75), Westerman and Westerman ('75), and Small ('75).
[4]Number species persisting at monitoring sites/number of species reported in earlier censuses.

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