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# Ecotoxicity of River and Spring Sediment Along the Hanford Reach

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The Hanford Site was established in 1943 in order to produce plutonium for some of the nuclear weapons tested and used in World War II (Dirkes and Hanf 1997). These historic operations resulted in the production of both radiological and nonradiological wastes. In 1988, the Hanford Site was placed on the National Priorities List for environmental cleanup by the US Environmental Protection Agency. In recent years, efforts at the site have focused on characterizing and remediating contaminants in these wastes.

Because some of the wastes were discharged directly into the Columbia River or have leached into the river via the groundwater pathway, several studies have examined risk to ecological receptors associated with the river (e.g., Friant and Brandt 1994; PNNL 1998). It is apparent that data gaps are extensive and additional data are needed to better characterize potential ecotoxicity of river sediments on the Hanford Site. Assessing sediment toxicity should provide integrated information on contaminant input from multiple sources (Power and Chapman 1992; Ingersoll 1995). The purpose of this study was to evaluate potential ecotoxicity of sediment samples collected primarily from areas where past operations were conducted along the Hanford Reach.

## MATERIAL AND METHODS

Sample locations along the Columbia River on the Hanford Site in southeastern Washington state are shown in Figure 1. Sediment was collected in the fall of 1994 and 1995 from five riverbank spring and four riverbed sites. These included the 100-B Area Riverbank Spring (100-B Spring), 100-N Area Riverbank Spring (100-N Spring), 100-D Area D Island (D Island), 100-F Area Riverbank Spring (100-F Spring), 100-F Slough-upriver (100-F Slough-u), 100-F Slough downriver (100-F Slough-d), Hanford Slough (H Slough), Hanford Townsite Riverbank Spring (HT Spring), and 300 Area Riverbank Spring (300 Spring). The 100 Areas housed nine nuclear reactors that operated from 1944-87 (PNNL 1998). All but N Reactor discharged contaminated cooling water to the river. Wastes were also discharged to the ground, contaminating groundwater which flows into the Columbia River. The 300 Area operations, including nuclear fuel fabrication and research, generated wastes which were discharged to ponds located near the river.

The top two cm of sediment were collected from each location, using either a plastic spoon for riverbank spring sediment or an Ekman grab sampler for river sediment. Samples were quickly transferred into clean polyethylene containers with no head space.

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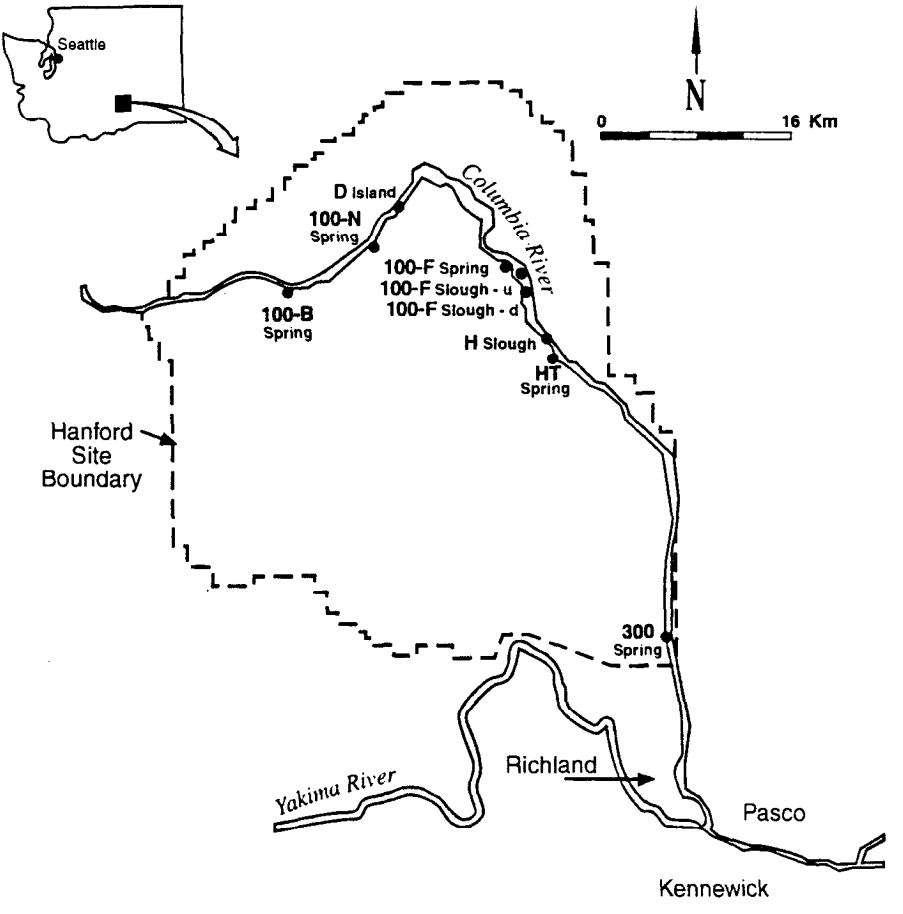


**Figure 1.** Sediment sample locations on the Hanford Site

These samples were then stored at 4°C in the dark for a period of three days to eight weeks from the date of sample collection before toxicity testing was initiated.

Toxicity tests included a 28 day survival and growth test with a freshwater amphipod (*Hyalella azteca*) (ASTM 1994a) and a 10 day survival and growth test with a midge (*Chironomus tentans*) (USEPA 1994). For *H. azteca* bioassays, each control and test sample (200 mL sediment and 800 mL overlying water) consisted of four replicates of 20 organisms. Similarly, for *C. tentans* bioassays, each control and test sample (100 mL sediment and 175 mL overlying water) consisted of eight replicates of 10 organisms. Sediment from an uncontaminated site along the Tyger River, South Carolina was used for the control treatment. Overlying water was either obtained from this site on the Tyger River or prepared with 20% diluted mineral water. The *H. azteca* test was conducted under static conditions at 20°C while the *C. tentans* test employed a static renewal design at 23°C with twice daily renewals. Photoperiod was maintained at 16 hr light/8 hr dark in both bioassays. Control mortalities and overlying water chemistry (e.g., temperature, pH, dissolved oxygen) were within acceptable limits (ASTM 1994a; USEPA 1994).

Organism age, feeding regime, and growth assessment followed ASTM (1994a) and USEPA (1994) protocols for *H. azteca* and *C. tentans* tests, respectively. Both bioassays employed juvenile organisms. *H. azteca* were 2-3 mm in length, and *C. tentans* were third instar larvae. *H. azteca* were fed ground rabbit pellets (2 mg/d/replicate), while C. tentans were fed fish flakes (6 mg/d/replicate). Growth was quantified in *H. azteca* by measuring body length from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface, whereas growth was assessed in *C. tentans* by measuring biomass after drying to constant weight at 60°C.

All survival and growth data were normally distributed, as assessed by the Shapiro-Wilk's test. The *F* test was used to evaluate homogeneity of variance between control and test samples. Depending on the outcome of this test, either an equal variance t test or an unequal variance t test was employed to compare control and test sample pairs for survival and growth endpoints (Zar 1984). Two-tailed t tests were applied to detect possible inhibition or stimulation. Alpha was set at 0.05 in all tests.

Radiological and nonradiological contaminant data, corresponding to sample locations and dates (within four months), were obtained from the Surface Environmental Surveillance Project (SESP), Pacific Northwest National Laboratory (*R. Dirkes, personal comm.*). An overall description of these data, including sample collection and analytical methodology, has been published elsewhere (Saldi et al 1995; Saldi and Dirkes 1996). In particular, SESP sediment data included a gamma scan, Sr-90, U-235, U-238, and a suite of metals measured by the inductively coupled plasma (ICP) analysis method. From this data set, three contaminants were selected (i.e., Cr, Sr-90, Cs-137), based on their identification as contaminants of ecological concern in sediment by the Columbia River Comprehensive Impact Assessment study (PNNL 1998). These contaminants had exceeded one or more screening criteria for acute and chronic ecotoxicity in this prior study. These criteria were based on ambient water quality criteria, aquatic biota median lethal concentration (LC50), developmental fish toxicity, and radiation dose to fish.

Toxicological sediment benchmarks for Cr, Sr-90, and Cs-137 were tabulated for comparison to SESP sediment data. Benchmarks for Cr were derived from sediment effect concentrations (USEPA 1996; Jones et al 1997) and from a screening level concentration approach (Persaud et al 1992). Radionuclide benchmarks were selected from Higley and Kuperman (1996), based on the recommendation that chronic dose rates below 100 mrad/d offer protection to plant and animal populations (IAEA 1992).

## RESULTS AND DISCUSSION

Results of sediment toxicity tests are presented in Table 1. Survival was significantly reduced ($p<0.05$) in 1995 for *H. azteca* at D-Island, 100-F Slough-u, 100-F Slough-d, and H Slough and for *C. tentans* at 100-N Spring and H Slough. Similarly, growth was significantly decreased ($p<0.05$) for *H. azteca* at H Slough in 1995. No toxicity was observed in 1994 tests.

For 1995 bioassays, survival appeared to be a more sensitive endpoint than growth. This may be due to the time course of mortality with early mortality precluding an effect on growth. It may also be possible that specific toxicodynamic factors (i.e., mode of action) or toxicokinetic properties (i.e., absorption, distribution, metabolism, elimination)

**Table 1**. Toxicity tests for sediment samples.

| Bioassay | Year | Location | Survival (%) | | Growth (mm or mg)[a] | |
|---|---|---|---|---|---|---|
| | | | Control | Test | Control | Test |
| *H. azteca* | 1994 | 100-B Spring | 96.3 (4.8)[b] | 86.3 (18.0) | 3.7 (0.1) | 3.6 (0.2) |
| *H. azteca* | 1994 | 100-F Spring | 96.3 (4.8) | 100.0 (0) | 3.7 (0.1) | 3.8 (0.1) |
| *H. azteca* | 1994 | 100-F Slough-u | 96.3 (4.8) | 100.0 (0) | 3.7 (0.1) | 3.5 (0.5) |
| *H. azteca* | 1994 | HT Spring | 96.3 (4.8) | 85.0 (23.8) | 3.7 (0.1) | 3.5 (0.5) |
| *H. azteca* | 1994 | 300 Spring | 96.3 (4.8) | 95.0 (10.0) | 3.7 (0.1) | 4.2 (0.1)*[c] |
| *H. azteca* | 1995 | 100-N Spring | 100.0 (0) | 80.0 (22.7) | 4.1 (0.3) | 4.4 (0.2) |
| *H. azteca* | 1995 | D Island | 90.0 (4.1) | 36.3 (15.5)* | 4.4 (0.1) | 3.7 (0.6) |
| *H. azteca* | 1995 | 100-F Slough-u | 90.0 (4.1) | 16.3 (4.8)* | 4.4 (0.1) | 4.4 (0.5) |
| *H. azteca* | 1995 | 100-F Slough-d | 90.0 (4.1) | 42.5 (19.4)* | 4.4 (0.1) | 3.9 (0.7) |
| *H. azteca* | 1995 | H Slough | 90.0 (4.1) | 32.5 (21.8)* | 4.4 (0.1) | 3.1 (0.4)* |
| *C. tentans* | 1995 | 100-N Spring | 78.6 (9.0) | 34.3 (27.0)* | 1.47 (0.37) | 1.33 (0.42) |
| *C. tentans* | 1995 | D Island | 78.6 (9.0) | 75.0 (17.7) | 1.47 (0.37) | 1.59 (0.34) |
| *C. tentans* | 1995 | 100-F Slough-u | 78.6 (9.0) | 71.3 (19.6) | 1.47 (0.37) | 1.32 (0.17) |
| *C. tentans* | 1995 | 100-F Slough-d | 78.6 (9.0) | 60.0 (21.4) | 1.47 (0.37) | 1.39 (0.35) |
| *C. tentans* | 1995 | H Slough | 78.6 (9.0) | 63.7 (7.4)* | 1.47 (0.37) | 1.57 (0.26) |

*$p<0.05$ for test vs. control.

[a]Body length (mm) for *H. azteca* and dry weight (mg) for *C. tentans* bioassays.

[b]Mean with standard deviation in parentheses.

[c]Test sample produced stimulation (hormesis).

produced lethality without an associated reduction in growth. Finally, the variability observed in growth in several cases may have masked a significant reduction.

*H. azteca* was more sensitive than *C. tentans* for survival at D Island, 100-F Slough-u, and 100-F Slough-d in 1995. This pattern was reversed at 100-N Spring. *H. azteca* also exhibited greater sensitivity for growth at H Slough. Differences in sensitivity are not surprising, considering the two test organisms differ taxonomically and ecologically. Moreover, the range of similarities and differences for survival and growth endpoints reported in the literature for these test organisms (e.g., Phipps et al 1995; Becker et al 1995) demonstrates the need for employing multiple toxicity tests to characterize sediments.

Synoptically collected contaminant data are presented in Table 2. None of these concentrations exceeded the toxicological benchmarks listed. Cr concentrations were within an order of magnitude of their applicable benchmarks, while Sr-90 and Cs-137 activities were far below their corresponding benchmarks. Where survival was reduced in *C. tentans* and contaminant data were available at H Slough, Cr concentration was roughly 20-42% of the Cr benchmarks. Similarly, where toxicity was observed to *H. azteca* and contaminant data were available at 100-F Slough-u, 100-F Slough-d, and H Slough, Cr concentrations ranged from 15-42% of the Cr benchmarks.

**Table 2**. Selected contaminant concentrations for sediment samples with corresponding toxicological benchmarks.

| Year | Location | Cr (μg/kg) | Sr-90 (pCi/g) | Cs-137 (pCi/g) |
|---|---|---|---|---|
| 1994 | 100-B Spring | NA[a] | NA | NA |
| 1994 | 100-F Spring | NA | NA | NA |
| 1994 | 100-F Slough-u | 21000 | 0.00367 (0.00420)[b]U[c] | 0.204 (0.020) |
| 1994 | HT Spring | 10000 | 0.00682 (0.00445) | 0.25 (0.03) |
| 1994 | 300 Spring | 11000 | 0.0124 (0.0053) | 0.056 (0.058)U |
| 1995 | 100-N Spring[d] | NA | NA | NA |
| 1995 | D Island[e] | NA | NA | NA |
| 1995 | 100-F Slough-u[e] | 8200 | 0.0022 (0.0051)U | 0.486 (0.025) |
| 1995 | 100-F Slough-d[e] | 8200 | 0.0022 (0.0051)U | 0.486 (0.025) |
| 1995 | H Slough[de] | 11000 | 0.0059 (0.0030) | 0.572 (0.035) |
| Toxicological Benchmark | | 56000[f] | 1700[h] | 3400[h] |

[a]NA=Not Available.

[b]Two sigma counting error in parentheses, indicating that a recount would fall within this error approximately 95% of the time.

[c]U=analyte not detected at or above the reported result.

[d]Toxicity observed with *C. tentans.*

[e]Toxicity observed with *H. azteca.*

[f]Benchmark is the lower 10[th] percentile for ranked concentrations associated with effects observed in an *H. azteca* 14-day test (Jones et al 1997).

[g]Benchmark is the lower 5th percentile of the screening level concentration, based on the co-occurrence of sediment Cr concentrations and benthic infaunal species (Persaud et al 1992).

[h]Benchmark results in a dose rate of 100 mrad/d, based on a tissue/sediment concentration ratio equal to one (Higley and Kuperman 1996).

Based on these toxicological benchmarks, it is likely that contaminants other than those identified by PNNL (1998) with available synoptic data (Table 2) contributed to the observed toxicity either independently or interactively. For example, other metals (e.g., As, Cd, Cu, Pb, Ni, Zn) and radionuclides (e.g., Co-60, Eu-155, Pu-239,240, U-235, U-238) have been detected in sediments (Saldi and Dirkes 1996). In addition, diesel as xylene was identified by PNNL (1998) as a contaminant of ecological concern in river sediments, although sediment organics were not monitored by SESP for relevant sample locations and dates. Ancillary sediment chemical and physical data (e.g., redox potential, acid volatile sulfide, total organic carbon, sediment grain size) comprise another data gap, limiting interpretation of toxicity results. These sediment properties are useful in predicting contaminant bioavailability (Hamelink et al 1994; Blanton et al 1995). Temporal variation in these properties may have contributed to differences in toxicity observed between 1994 and 1995 bioassays.

Recommended sediment holding time ranges from less than two weeks (ASTM 1994b; Becker and Ginn 1995) to less than eight weeks (USEPA-USCOE 1998). Although our sample holding times fell within this range (i.e., three days to eight weeks), it should be acknowledged that length and variability of sediment holding time may have influenced toxicity test results. Limits to storage time before testing appear to be a function of both sediment and contaminant characteristics (ASTM 1994b). While it is prudent to initiate testing within two weeks of sample collection, it has been shown in some cases that sediments can be stored at 4°C for up to 12 months without significant alterations in toxicity (Tatem 1988).

It is noteworthy that growth was stimulated ($p<0.05$) for *H. azteca* at 300 Spring in 1994 (Table 1). Hormesis appears to be a general biological phenomenon that may represent an overcompensation to an alteration in homeostasis (Stebbing 1997). Hermetic responses have been reported in a broad range of species, biological endpoints, and chemicals (Delistraty 1986; Calabrese and Baldwin 1997). The stimulatory effect on growth observed in this study may have resulted from exposure to either chemicals or ionizing radiation at low doses (e.g., Luckey 1982).

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