TOXICITY OF SEDIMENTS AND PORE WATER
FROM BRUNSWICK ESTUARY, GA

by

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ABSTRACT

A chlor-alkali plant in Brunswick, Ga, discharged >2 kg mercury/day into a tributary of the Turtle River-Brunswick Estuary from 1966 to 1971. Mercury concentrations in sediments collected in 1989 along the tributary near the chlor-alkali plant ranged from 1 to 25 ug/g (dry weight), with the highest concentrations found in surface (0 to 8 cm) sediments of subtidal zones in the vicinity of the discharge site. Toxicity screening in 1990 using Microtox® bioassays on pore water extracted on site from sediments collected at 6 stations distributed along the tributary indicated that pore water was highly toxic near the plant discharge. Ten-day toxicity tests on pore water from subsequent sediment samples collected near the plant discharge confirmed high toxicity to Hyalella azteca, and feeding activity was significantly reduced in whole-sediment tests. In addition to mercury in the sediments, other metals (chromium, lead, and zinc) exceeded 50 ug/g, and PCB concentrations ranged from 67 to 95 ug/g. On a molar basis, acid volatile sulfide concentrations (20 to 45 umoles/g) in the sediments exceeded the metal concentrations. Because acid volatile sulfides bind with cationic metals and form metal sulfides, which are generally not bioavailable, toxicities shown by these sediments are thought to be due to the high concentrations of PCBs.
INTRODUCTION

A chlor-alkali plant in Brunswick, GA, discharged >2 kg mercury/day into the Turtle River-Brunswick Estuary from 1966 to 1971 (Windom et al. 1976). During the early 1970's, mercury residues in fish and birds associated with this estuary exceeded the U.S. Food and Drug Administration's action level of 1.0 ug/g, causing restrictions to be placed on hunting and fishing (Odom 1974, Gardner et al. 1978). These restrictions were lifted during the later 1970's, after discharges of mercury were reduced to <0.2 kg/day.

The lower Turtle River-Brunswick Estuary system provides important habitat for threatened (brown pelican, Pelecanus occidentalis) and endangered species (Florida manatee, Trichechus manatus), in addition to other important species such as wood stork (Mycteria americana), black duck (Anas rubripes), clapper rail (Rallus longirostrus), least tern (Sterna antillarum), loggerhead turtle (Caretta caretta), and striped bass (Morone saxatilis). The estuary also supports economically important sport and commercial fisheries and crabbing.

To determine the current levels of mercury and other contaminants in the part of the estuarine system that received the high discharges of mercury and to assess potential environmental impacts, sediments were collected for analysis and toxicity testing.

METHODS AND MATERIALS
This study consisted of an initial evaluation of the sediments to determine mercury concentrations after 20 years of reduced discharge, followed by testing of sediments and sediment pore water to determine if there was a toxic threat to biota of the system. Toxicity testing included a reconnaissance screening of pore water extracted on site from sediments collected along the creek channel receiving effluents using the Microtox bioassay. After identifying the most toxic area based on the reconnaissance survey, more intensive toxicity testing was conducted on pore water and sediment collected from stations within that area. The intensive toxicity testing consisted of exposing Hyalella azteca to whole sediment and sediment pore water for a 10-d period, along with an additional Microtox bioassay on the pore water. Sediments tested with H. azteca were analyzed for metal and organic contaminants.

Sample Site Location

The Turtle River-Brunswick Estuary is located in southeastern coastal Georgia adjacent to the city of Brunswick, which supports a number of industries (Fig. 1). The Turtle River lies west of Brunswick and provides a major inflow to the estuary. Effluents from the chlor-alkali plant, located northwest of Brunswick, were discharged into an unnamed tidal creek that empties into the Turtle River.

To avoid confusion on sampling locations and measurements or studies conducted on sediments collected at the various locations
considered in this study, stations are numbered consecutively from the mouth of the tidal creek to the backwater area. The respective analyses and tests conducted on sediments from each station are summarized in Table 1.

Sample Collection and Handling

Sediment samples for mercury analyses were collected in 1989 from 5 sites (Stations 1, 2, 3, 10, 12) distributed above and below the discharge (in the vicinity of Station 6) from the chlor-alkali plant (Fig. 1). At these sites, sediment samples were collected from the *Spartina alterniflora* marsh, intertidal zone, and the subtidal zone. Sediments were collected to a depth of 15 cm with a 5-cm diameter Wildo® core sampler. These cores were fractionated into surface (0 to 8 cm) and subsurface (8 to 15 cm) layers, which were analyzed separately for mercury. Following nitric flux digestion, mercury was analyzed by cold vapor atomic absorption using a Perkin-Elmer Model 403 Atomic Absorption unit, Perkin-Elmer Model 056 recorder, Technicon Sampler I, and Technicon Pump II.

Sediments included in the reconnaissance toxicity testing were collected in September, 1990, with a Ponar grab at Stations 4, 7, 8, 9, 10, 12 (Fig.1). Immediately following sediment collection at each site, pore water was collected for the preliminary toxicity screening. Pore water was extracted using a vacuum operated device consisting of a 60-cc polyethylene syringe, air line tubing, and a fused-glass airstone (Winger and
Lasier 1991) from an essentially undisturbed sediment sample after it was carefully emptied into a polyethylene pan. After extraction of 20-40 ml, the syringes containing the pore-water samples were stored on ice in a cooler until later in the day when toxicity was determined using Microtox®.

The reconnaissance toxicity screening showed that pore water from sediments collected near the mouth of a drainage canal that emptied into the tidal creek was the most toxic (Stations 4 and 7). Sediment samples were collected the next day with the Ponar grab from 3 sites (Stations 5, 6, and 7) within the area showing this high toxicity. Five to 8 Ponar grabs of sediment were collected at each of the 3 sites and placed in polyethylene pans and homogenized. Prior to homogenization, pore water was extracted in the field for testing the same day with Microtox®. The homogenized samples from Stations 6 and 7 were returned to the laboratory where they were maintained in the dark at 20°C until testing, which began about 4 wk after collection. In the laboratory, toxicity tests were conducted on both the whole sediment and sediment pore water from Stations 6 and 7.

Sediments tested with *H. azteca* (Stations 6 and 7) were analyzed for metals, organic contaminants, acid volatile sulfides, and organic carbon. Metals were determined by inductively coupled plasma emission spectrophotometry using a Jarrell-Ash 1100 Mark III. Organic chemicals, including organochlorine pesticides, PAHs, and PCBs, were measured following Soxhlet extraction and clean up by capillary gas
chromatography (CGC) with a flame ionization detector for aliphatic hydrocarbons, a CGC with electron capture detector for pesticides and PCBs, and a mass spectrometer detector in SIM mode for aromatic hydrocarbons (Wade et al. 1988). Acid volatile sulfides of the sediments were determined using the ion-selective electrode method (Allen et al. 1991). Total organic carbon of the sediments was determined using the Coulometrics Carbon Model 5020 Analyzer.

Standards, blanks, spiked blanks and samples, and duplicate samples were included in the analytical method. Procedural blanks were below the limits of analyte detection, which were 0.01 µg/g for mercury, 0.2 µg/g for arsenic and selenium, 0.02 for cadmium, chromium, lead, and zinc, 0.05 for copper, 0.1 for nickel, and 0.01 for pesticides, PCBs, and aromatic hydrocarbons. Percent recovery from spiked blanks and samples averaged 80% for aromatic hydrocarbons, 91% for pesticides and PCBs, and 98% for metals. Differences between duplicate samples were 13.3% for aromatic hydrocarbons, 0% for pesticides and PCBs, 2.9% for metals, 9.4% for mercury, 10% for arsenic, and 0% for selenium. Quality control data for these analyses are available on request from the authors.

Toxicity Testing

Microtox® toxicity tests were conducted according to the standard assay procedure (Microbics Inc. 1991). A serial dilution of pore water with diluent was inoculated with
photoluminescent bacteria (Photobacterium phosphoreum) and incubated for 5 and 15 min before reading the amount of light emitted. Bioluminescence of the bacteria was measured with the Microtox® Model 500 Toxicity Analyzer, which is a temperature controlled (15°C) spectrophotometer. EC50s (effective concentration that reduces bioluminescence by 50%) were calculated using software provided with the instrument (Microbics Inc. 1991). EC50s reported in this paper are from the 5-min readings.

Static, 10-d toxicity tests were conducted on both whole sediment and sediment pore water using juvenile (2-3 mm) Hyalella azteca. Six replicates for whole-sediment tests and 5 replicates for pore-water test, with 5 animals/replicate, were included for each treatment. Tests were conducted in 150-ml beakers at 20°C. In pore water tests, 110 ml of solution were used, and for whole sediment tests, the beakers were prepared using 30 ml of sediment and 110 ml of reconstituted sea water. Reference sediment and pore water extracted from the reference sediment were used as controls for testing H. azteca. The reference sediment, collected from St. Andrews Bay, Bay County, Florida in November 1989, was stored in the dark at 20°C prior to use.

Ten pre-weighed leaf disks from conditioned maple leaves were placed in each beaker at the start of the test. Leaves were conditioned by soaking in freshwater for a 4-wk period at 25°C. Prior to use, the leaves were rinsed in fresh water. Leaf disks were made using a paper punch and blotted between paper towels
before they were weighed. At the end of the test, the leaf disks were blotted and reweighed. The amount of leaf material consumed per animal per day was calculated using the following formula:

\[ C = \frac{W_1 - W_2}{AD} \]

where \( C \) is rate of leaf consumption in mg/animal/day, \( W_1 \) is the initial weight of the leaf disks at the start of the test, \( W_2 \) is the weight of the leaf disks at the end of the test, \( AD \) is animal days determined by summing the number of days each animal is alive during the test.

Statistical differences among and between treatments were determined using ANOVA and Dunnett's multiple comparison procedures included in SAS (SAS Institute Inc. 1985). Proportional data were normalized using the arcsine transformation. Significant differences referred to are at the \( p = 0.05 \) level.

RESULTS AND DISCUSSION

Sediment Residue Concentrations

Mercury concentrations in sediments collected from the tidal creek in 1989 ranged from 1 to 26 ug/g (dry weight), with the highest concentrations near the chlor-alkali plant (Fig. 2). The lowest concentrations were measured in sediments closer to the mouth of the creek. Lower concentrations of mercury near the mouth of the creek were attributed to increased tidal flushing.
and greater flows, in comparison with the upstream sites. McLaren and Little (1987) also found higher concentrations of metals at the head of an estuary in Wales compared to the mouth. They attributed this to fine-grained deposition of sediment at the head and dilution at the mouth of the estuary.

The highest concentrations of mercury in sediments collected from this study were found in the subtidal areas, followed by marsh and intertidal zones. In their study of mercury distribution in this salt marsh, Gardner et al. (1978) found low concentrations of mercury in salt-marsh plants and sediments compared to invertebrates and fish, and in the sediments, the highest concentrations were in the upper 5 cm. Similarly, the highest concentrations of mercury measured at each of the sites in this study were in the upper 8-cm fraction.

Mercury levels in sediments from this tidal creek are still elevated, particularly in the vicinity of the plant site, even 20 years after high discharges to the system were stopped. The environmental impacts of these concentrations are not known, and current residue data for biota from this system are lacking. Nevertheless, the potential for environmental degradation is high. Callister and Winfrey (1986) demonstrated that sediment bound mercury is available for microbial methylation and significant amounts of toxic methylmercury can be produced and released from contaminated sediment. Although specific concentrations of mercury in sediments that cause environmental degradation or harmful biotic accumulation are not known, some
relationships are apparent. For example, sediment concentrations (0.4 - 2.2 mg/kg) considerably lower than levels measured in this study were associated with residue levels of mercury in fish >7 mg/kg in an Australian estuary contaminated with fertilizer plant effluents (Talbot 1990). Hildebrand et al. (1980) found a logarithmic relationship of mercury residues in fish with sediment concentrations in the Holston River, Virginia and Tennessee. They reported that as mercury concentrations in sediment approached 3 ug/g, residue levels in fish approached the U.S. Food and Drug Administration's action level of 1 ug/g.

Toxicity Tests

Microtox® toxicity measured during the reconnaissance screening in 1990 on sediment pore water was highest at Stations 4 and 7, an area near the mouth of a tributary to the unnamed tidal creek (Fig. 3). The toxicities shown in this preliminary screening suggest that the chlor-alkali plant discharged effluents into this tributary of the unnamed creek. Based on the high toxicity shown at Station 7 during the reconnaissance survey, sediment samples were collected the following day near the mouth of this tributary at Stations 5, 6 and 7. Microtox® toxicity on fresh pore water extracted on site from these 3 sites showed that Stations 6 and 7 were highly toxic and pore water from Station 5 was only mildly toxic (Fig. 4). Since toxicity at Station 5 was only marginally toxic, it was dropped from further consideration, and efforts were concentrated on Stations 6 and 7,
where whole sediment and pore water were tested with *H. azteca* in 10-d static tests.

Differences in toxicities shown for the 3 sites (Stations 5, 6, and 7) at the mouth of the tributary demonstrate the high variability of contaminant deposition in tidal and fluvial systems (Fig. 4). The distance between Stations 5, 6, and 7 was <10 m. The variability shown in the toxicities among these 3 sites was probably due to nonuniform depositional patterns associated with the channel morphology. Others have also noted significant spatial variation in sediment residues in estuaries (McLaren and Little 1987, Luoma and Phillips 1988). This variability reflects, for the most part, particle size fraction deposition, and contaminants are associated with the finer particles, which generally have higher organic content (Mudroch and Duncan 1986).

*H. azteca* showed no mortality in the 10-d toxicity tests on whole sediment from Stations 6 or 7 (Fig 5). There was, however, a significant decrease in feeding activity at the 2 sites compared to the control, with the greatest decrease occurring at Station 6. Behavioral activities of test animals have been shown to serve as biomarkers of stress (Beitinger 1990). Little et al. (1985) summarized information demonstrating that feeding behavior is a sensitive indicator of toxicity, and Crane and Maltby (1991) found high potential for feeding rate of *Gammarus pulex* to reflect water quality. Although there is some evidence that adding food to test chambers may reduce the toxicity of some
contaminants (Cripe et al. 1989), feeding behavior appears to be a biomarker sensitive enough to detect contamination or reduced sediment quality under sublethal conditions. The decrease in feeding behavior measured in this study suggests reduced sediment quality at Stations 6 and 7 compared to the reference sediment.

Sediment pore waters from Stations 6 and 7 were highly toxic to *H. azteca*, with mortalities significantly higher than that shown for the control reference (Fig. 5). As was shown in the Microtox® tests (Fig. 4) and feeding activity on whole sediment, the greatest toxicity of pore water was shown for Station 6.

Water chemistry of pore water and overlying water from the whole-sediment tests were similar to the reference water, except for a minor difference in elevated ammonia concentrations in the pore water from the 2 sites compared to the reference (Table 2). These levels of ammonia, however, were not considered significant in contributing to the toxicity, given the percent unionized ammonia available (Emerson 1975). Concentrations of unionized ammonia (based on temperature and pH of the test water) were calculated to be \( \leq 0.13 \text{ mg/l} \), concentrations considerably lower than those (96-h LC50 = 1.6 - 5.6 mg/l) found by others to be toxic to crustaceans (Williams et al. 1986, Arthur et al. 1987).

Analyses of organic and inorganic contaminants in sediments from Stations 6 and 7 indicated that in addition to mercury, other metals (Cr, Pb, Ni, and Zn) were also high, and DDT, PAHs, and PCBs were also found (Table 2). Acid volatile sulfides in these sediments ranged from 20 to 45 umoles/g. Because acid
volatile sulfides bind with some cationic metals forming insoluble precipitates (DiToro et al. 1990), it is unlikely that metals were responsible for the toxicity shown in these tests.

The high concentrations of PCBs (Table 2) are suspected of being responsible for the toxicity shown, although a Toxicity Identification Evaluation (Mount et al. 1988) was not conducted to verify this. PCBs can be released from sediments to water with the rates dependent upon the concentration in the sediments and specific molecular characteristics of the PCB congeners (Fisher et al. 1983). Toxicity of PCB varies markedly among the various congeners, with LC50 concentrations ranging from 1 to 2,400 μg/l for 7 to 10-d exposures of invertebrates to aqueous PCBs (Stalling and Mayer 1972). LC50s for crustaceans generally range from 5 to 50 μg/l (Eisler 1986). PCB concentrations in pore water from sediments collected at Stations 6 and 7 were estimated (Gschwend and Wu 1985) to be 53 and 26 μg/l, respectively, based on a sediment partition coefficient (Koc) of 42,500 for Aroclor 1254 (Kenega 1980) and 4.2 % organic carbon at Station 6 and 5.9 % at Station 7. These estimates of PCB in the pore water are consistent with the pattern of toxicity for both Microtox® and H. azteca, further corroborating that PCB may be responsible for the sediment toxicity.

SUMMARY AND CONCLUSIONS

High concentrations of mercury were measured in sediments from a tidal creek receiving effluent from a chlor-alkali plant.
The highest concentrations were measured in the surface layers (0 - 8 cm) of the subtidal zone, with elevated levels found near the discharge area and upstream.

Reconnaissance testing of pore-water toxicity from sediments collected along the tidal creek was effective in identifying contaminated areas for further evaluations. Preliminary screening of pore water with Microtox® bioassays indicated the highest toxicity near the mouth of a tributary to the tidal creek near the plant site. Microtox® testing of pore water from additional sediments collected from 3 stations at the mouth of this tributary also demonstrated high toxicity.

Mortality of *H. azteca* was significantly higher in sediment pore water collected from the 2 stations at the mouth of this tributary than in the reference pore water. Feeding activity of *H. azteca* was reduced in whole-sediment tests in comparison with the reference, also indicating reduced sediment quality, although there was no mortality in the whole-sediment tests.

In addition to mercury, other metals were measured in the whole sediment, along with high concentrations of PCBs. High acid volatile sulfides (20 - 45 umoles/g) measured in the sediments were thought to form insoluble metal sulfides, making the metals biologically unavailable. The high concentrations of PCBs are suspected of being responsible for the toxicity shown by these sediments. The source of PCBs to this system is not presently known and will require further investigation.
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Table 1. Listing of stations in the Turtle River-Brunswick Estuary where sediments were collected, and a review of the studies conducted on the sediments from the respective stations.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Station</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment collected for mercury analysis</td>
<td>X X X</td>
</tr>
<tr>
<td>Reconnaissance sampling of sediment, toxicity of pore water with Microtox bioassay</td>
<td>X</td>
</tr>
<tr>
<td>Sediment collected from highly toxic area, toxicity of pore water with Microtox bioassay</td>
<td>X X X</td>
</tr>
<tr>
<td>Sediment from highly toxic area taken to lab, toxicity of pore water and solid-phase sediment with <em>H</em>yalella azteca, chemical analysis of sediments</td>
<td>X X</td>
</tr>
</tbody>
</table>
Table 2. Contaminant residues in sediments collected from the Turtle River-Brunswick Estuary and chemistry of sediment pore water and water overlying sediment used in toxicity tests with *Hyalella azteca*.

<table>
<thead>
<tr>
<th>Measurements with Units</th>
<th>Station 6</th>
<th>Station 7</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pore water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkalinity (mg/l)</td>
<td>1,400</td>
<td>1,600</td>
<td>350</td>
</tr>
<tr>
<td>Conductivity (umhos/cm)</td>
<td>22,500</td>
<td>25,500</td>
<td>24,000</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>15</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
<td>7.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>7.5</td>
<td>7.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Total ammonia (mg/l)</td>
<td>12</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Unionized ammonia (mg/l)</td>
<td>0.10</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Overlying water</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Conductivity (umhos/cm)</td>
<td>6,500</td>
<td>8,500</td>
<td>11,000</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>pH</td>
<td>8.2</td>
<td>8.2</td>
<td>8.1</td>
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<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>6.2</td>
<td>6.9</td>
<td>7.5</td>
</tr>
<tr>
<td>Total ammonia (mg/l)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Residues in sediments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic content (%)</td>
<td>4.2</td>
<td>5.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>29.7</td>
<td>25.7</td>
<td>74.6</td>
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<tr>
<td>Total PAHs (ug/g)</td>
<td>1.45</td>
<td>3.02</td>
<td>ND</td>
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<tr>
<td>Total DDT (ug/g)</td>
<td>0.16</td>
<td>0.19</td>
<td>ND</td>
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<tr>
<td>PCBs (ug/g)</td>
<td>95.14</td>
<td>67.33</td>
<td>0.04</td>
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<tr>
<td>AVS-Sulfide (umoles/g)</td>
<td>21</td>
<td>45</td>
<td>NA</td>
</tr>
<tr>
<td>Cadmium (ug/g)</td>
<td>0.5</td>
<td>0.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Chromium (ug/g)</td>
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<td>118.0</td>
<td>42.0</td>
</tr>
<tr>
<td>Copper (ug/g)</td>
<td>14.0</td>
<td>18.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Lead (ug/g)</td>
<td>45.0</td>
<td>63.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Mercury (ug/g)</td>
<td>17.8</td>
<td>24.7</td>
<td>0.16</td>
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<td>Nickel (ug/g)</td>
<td>13.0</td>
<td>17.0</td>
<td>8.7</td>
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<tr>
<td>Zinc (ug/g)</td>
<td>63.0</td>
<td>78.6</td>
<td>54.0</td>
</tr>
</tbody>
</table>
Caption For Figures

Figure 1. Map of Turtle River-Brunswick Estuary showing stations where sediment samples were collected for residue analyses and toxicity testing.

Figure 2. Mercury concentrations in sediments collected from marsh, intertidal, and subtidal zones at five stations in the unnamed tidal creek that discharges into the Turtle River.

Figure 3. Microtox® toxicity of pore water extracted on site from sediments collected during the reconnaissance survey.

Figure 4. Microtox® toxicity of pore water extracted on site from sediments collected in an area identified as highly toxic during the reconnaissance survey.

Figure 5. Percent mortality and feeding rate of Hyalella azteca in 10-d toxicity tests of sediment and sediment pore water from two sites that were identified as toxic during the reconnaissance survey. (* = significantly different than reference, p = 0.05).