

SEDIMENT TOXICITY AND BIOACCUMULATION OF TOXICANTS  
IN THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*,  
AT TIMES BEACH, BUFFALO, NEW YORK.

by

Jeannie Marie Roper

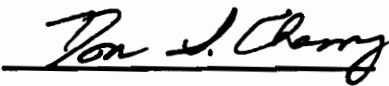
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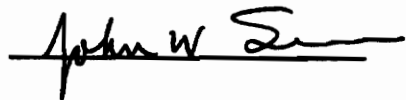
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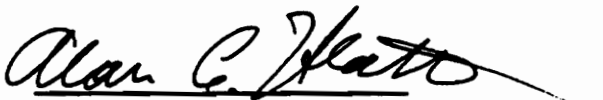
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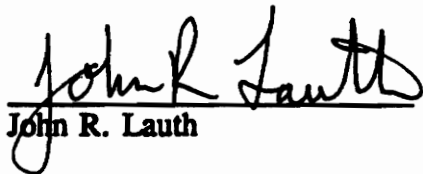
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(ABSTRACT)

This study consisted of a site characterization followed by a biomonitoring study utilizing the zebra mussel, *Dreissena polymorpha*, at the Times Beach Confined Disposal Facility (CDF), located in Buffalo, New York. Concentrations of the selected contaminants (polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and the following metals: arsenic (As), chromium (Cr), barium (Ba), mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se) and silver (Ag), were at or below detection limits in the water column. In the sediment toxicant concentrations were as high as 549 mg/kg for total PAHs, 9 mg/kg for PCB Aroclor 1248, and 54, 99, 6, 355, 637, and 16 mg/kg for the metals: As, Ba, Cd, Cr, Pb, and Hg respectively. To predict contaminant bioavailability, elutriate and whole sediment toxicity tests were performed utilizing the cladoceran,

*Daphnia magna*. The whole sediment tests showed a significant impact. Control survival was 84%, while the sediment treatment survival range was 1-7%. Mean control reproduction was 86.8 neonates, whereas treatment group reproduction ranged from 1.4 to 9.0. Zebra mussels, placed both in the water column (Upper) and at the sediment level (Lower), survived the 34-day exposure. Contaminants which significantly accumulated in zebra mussel tissue during the exposure period (mg/kg) were total PAHs (6.58), fluoranthene (1.23), pyrene (1.08), chrysene (0.98), benzo(a)anthracene (0.60), PCB Aroclor 1248 (1.64), As (0.97), Cr (2.87), and Ba (7.00). The accumulation of benzo(a)anthracene was statistically higher in the Upper mussels; however, this did not occur for any other toxicant. Accumulation of these contaminants in zebra mussel tissue represent a potential hazard to organisms (ie. fish and birds) which feed on them.

I dedicate this thesis to my parents, William and Kathy Roper for their love, support, and encouragement. Thanks Mom and Dad!

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## INTRODUCTION

The introduction of the zebra mussel, *Dreissena polymorpha*, to the United States is believed to have occurred in 1986. The accidental introduction occurred in Lake St. Clair, Michigan when a ship released its ballast water, releasing thousands of veligers it had picked up in a European freshwater port (Miller et al. 1992). Since its original sighting in 1988 (Herbert et al. 1989), the spread of the zebra mussel in North America has raised many potential environmental concerns. Concerns include the control of zebra mussel infestations, possible ecological impacts, and their potential for contaminant uptake. Among these, the potential for contaminant bioaccumulation demands further study. Zebra mussels have a relatively high bioaccumulation potential, that aspect combined with their high filtration rates, could prove to be an environmental hazard for large populations in contaminated areas (Reeders and Bij de Vaate 1992, Fisher et al. 1993, Griffiths 1993).

An area of documented contamination (Marquenie et al. 1990, Stafford et al. 1991), the Times Beach Confined Disposal Facility (CDF) located in Buffalo, New York was the site location. Times Beach lies close to the mouth of the Buffalo River and is separated by a dike from Lake Erie (Figure 1). The 186,000 m<sup>2</sup> site was built to receive dredged material, consisting primarily of contaminants from

industries located along the Buffalo River (an oil refinery, two steel plants, an aniline dye chemical plant and milling facilities). Dredging activities occurred from 1972-76, during this time the CDF was colonized by several plant and animals species, including over 22 bird species. This excited the Buffalo Ornithological Society, and on their request Times Beach was made a designated nature preserve (Stafford et al. 1991). In 1976 all dredging activities were ceased, leaving the site partially filled, and consisting of three ecosystems, upland, wetland and aquatic (Figure 2). This study was limited to the aquatic area, and utilized Times Beach as an area of known contamination.

Since 1981, Times Beach has been the site of government funded studies resulting in 24 reports to the US Army Corp of Engineers (CEWCB) Buffalo District, which have been depicted in journals and conference proceedings, a listing of which can be found in Smith et al. (1992). In 1981 an analysis of the dredged material was performed, which indicated the presence of potentially toxic heavy metal and organic compounds (Marquenie et al 1987). The results of the 1981 study led to a more in depth analysis of the CDF, including a biomonitoring study by Marquenie et al. (1990), where the mussel *Elliptio dilatata* was found to accumulate high concentrations of lower chlorinated PCBs and pesticides. The reports indicate, with respect to the aquatic area, the environmental concerns at this facility include the potential of contaminant leakage into the water column, acute and chronic toxicity to organisms inhabiting the site and the bioaccumulation of contaminants

from the sediment through the food web.

This study is part of a project being conducted by the US Army Corps of Engineers Zebra Mussel Research Program (ZMRP). The ZMRP was developed under the authority of the Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990, Public Law 101-646. The ZMRP is currently researching several zebra mussel issues which are of potential environmental concern. These issues include; biological and chemical control of the mussels, general zebra mussel biology, and environmental effects of zebra mussels colonization in contaminated areas. The issue of interest in this study is whether the zebra mussel can survive in Confined Disposal Facilities. There are multiple CDFs in the Great Lakes (Beyer and Stafford 1993). A concern is that zebra mussels may colonize a CDF as they have industrial plants, locks, and dams along the Great Lakes.

Zebra mussels are effective biomonitors with the ability to rapidly accumulate a variety of toxicants (Neumann and Jenner 1992, de Kock and Bowmer 1993), if they were to populate a CDF, contaminant mobility would be a potential problem. Zebra mussels may bioconcentrate contaminants, resulting in bioaccumulation and/or biomagnification effects through the food web. Another contamination issue is the degree of contamination zebra mussels can accumulate, as they may require special handling in disposal.

Contaminants commonly found in dredged material are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and a group of metals

arsenic (As), chromium (Cr), barium (Ba), cadmium (Cd), mercury (Hg), lead (Pb), selenium (Se), and silver (Ag). The high lipid content in the zebra mussel (Walz 1979, Fisher et al. 1993) is of interest in this study as PAHs have a high lipid solubility and easily incorporate into tissue lipids (Fisher et al. 1993), PCBs and metals are of concern because they bioaccumulate in the tissue of organisms. Often when dredged material is considered unfit for return to the water it is due to high concentrations of either PCBs or Hg (Beyer and Stafford 1993).

This study consisted of a 34-day active biomonitoring study (ABM) as described by Karbe et al. (1975) Foster and Bates (1978), de Kock (1986), and Kraak et al. (1991). Prior to day 0, a preliminary site characterization was performed at Times Beach. In order to characterize the site both chemical evaluations and toxicity tests were performed on samples from Times Beach. Sediment toxicity evaluations consisted of both acute elutriate (water-extractable) and chronic whole sediment (solid phase) tests, utilizing the cladoceran *Daphnia magna*, a recommended organism for sediment toxicity testing due to its sensitivity (Nebeker et al. 1984, Giesey et al. 1989, Burton 1992). The combination of chemical evaluations and toxicity tests were performed to predict ecosystem stress at Times Beach (Burton et al. 1987, Swartz et al. 1990, ASTM 1991b). Toxicity tests were included in the site characterization as a measurement of chemical concentrations alone do not predict contaminant bioavailability. Toxicity tests better predict bioavailability due to simulation of both chemical and environmental

conditions.

Zebra mussels were used as bioindicators to determine accumulation of selected toxicants (PAHs, PCBs, As, Ba, Cd, Cr, Hg, Pb, Se, and Ag) in a location of known toxicant concentrations. Mussels were placed both in the water column (Upper) and on the sediment level (Lower) to determine accumulation with respect to position in the water column (Figure 3). Toxicant concentrations were measured in zebra mussel tissues as previous studies (Van der Velde et al. 1992, Secor et al. 1993, and Kreis et al. 1994) have shown that contaminants are primarily associated with the tissues not the shells.

The objectives of this study were to (1) perform a preliminary site characterization study at Times Beach which included a) chemical analysis of water and sediment samples for the selected contaminants, and b) performance of toxicity tests to predict contaminant bioavailability at the CDF, and (2) to conduct a 34-day biomonitoring study utilizing the zebra mussel, the objectives of which were (a) to determine if zebra mussels can survive in the Times Beach CDF, (b) to compare concentrations of the selected contaminants in the mussel tissues compared to the water and sediment at the CDF, (c) to determine whether mussels placed at Times Beach and a reference site would accumulate the selected contaminants significantly higher than the Day 0 baseline analysis, and (d) to determine if accumulated concentrations of the selected contaminants differed due to mussel position in the water column ie. proximity to the contaminated sediment.



## METHODS

### Water and Sediment Collection

On June 16, 1993 Times Beach was partitioned into 16 sites. Each site was marked with a float tied by rope to a concrete block (Figure 4). At each site both a water sample and sediment sample were taken for chemical analysis (PAHs, PCBs, and metals). Samples were collected in glass "I-Chem" bottles with teflon lined lids. Sediment and water samples were placed in iced coolers, and sent overnight to the US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, where samples were kept at 4°C until preparation for chemical analysis.

Toxicity tests were performed with water and sediment from Times Beach plots. A plot consisted of four sites grouped into a plot, resulting in a total of four plots. Water was collected in the middle of each plot in 20-L plastic containers. Sediment was collected at each site, and later combined into plot sediment composites. All sediment was collected with an Ekman grab sampler. This sampler is a precise sampler, recommended for use in less compacted, fine grained sediments (Downing 1984), such as the dredged material observed at Times Beach. Immediately after collection, sediment for toxicity testing was placed in labeled plastic 3.8-L Ziploc bags. All sediment and water samples were placed on ice, and sent overnight to the laboratory, where samples were kept at 4°C until the toxicity

tests were performed. All sediment was utilized within 6 weeks of sediment collection.

Nearby Grand Island (Figure 5) was the reference site for sediment toxicity tests, it was selected in accordance to requirements set by the USEPA/USACE (1990). The reference point approach was used, as Grand Island sediment was collected from a single reference sediment sampling point. Grand Island sediment is similar in grain size, relatively uncontaminated, and environmentally similar to Times Beach sediment prior to the addition of dredged material (Stafford et al. 1991). These samples were collected and stored following the same procedures used with the Times Beach samples.

#### *Daphnia magna* Culturing

All sediment tests were performed with the cladoceran, *Daphnia magna*, which were cultured in aged tap water (Figure 6). The *Daphnia* culture were fed every other day with a 6 mg/L tri-algal mixture of *Ankistrodesmus falcatus*, *Chlamydomonas reinhardi*, and *Chlorella vulgaris*. The tri-algal mixture was kept refrigerated and thawed to room temperature before feeding. On between-feeding days the culture media was stirred. Individuals were placed in fresh media twice a week. The *Daphnia* were maintained at  $25 \pm 1^\circ\text{C}$ , with a 24-hr photoperiod of florescent lights (USEPA 1991). Three weeks prior to the start of a test, individual cultures were started with young from adults less than one week of age who had

produced 3 or more broods (Biesinger et al 1987). Each individual was placed in 150 ml of culture water in a 250 ml beaker. Young picked for the testing had to come from the above organisms, and meet standards set forth by (ASTM 1991a). For all transfers in both culturing and testing the organisms were transferred by a fire polished 6-mm bore pipet.

### Elutriate Toxicity Tests

The sediment and water samples from each plot were stored at 4°C after the June 1993 collection, until the sediment toxicity tests began in July 1993. The elutriate was prepared with a 1: 4 ratio of sediment to site water as described in the (USEPA /USACE 1990; Plumb 1981). The elutriate tests were performed in the controlled environment of a culture room where the temperature stayed at  $20 \pm 1^{\circ}\text{C}$  as recommended (ASTM 1991a; USEPA 1991; Biesinger et al. 1987). Photoperiod for the elutriate tests was 24 hours of light, which followed the recommendation that *D. magna* receive a minimum of 16 hours of light each day (USEPA 1991). At each station 100%, 50%, 10% elutriate concentrations, and a 0% concentration of site water taken at each individual station, were tested. There was also an additional control consisting of the *Daphnia* culture water. Five replicates for each concentration, 10 organisms per replicate, comprising a total of 50 organisms exposed to each concentration (Figure 7). The tests lasted 48 hrs and organisms were checked at 0, 4, 24 and 48 hrs. Temperature, pH and dissolved oxygen were

measured at 0, 24 and 48 hrs. Conductivity, alkalinity and hardness readings were collected at 0 and 48 hrs (Table 1).

#### Whole Sediment Toxicity Tests

Whole sediment toxicity tests were performed using the guidelines set by Nebeker et al. (1984) for the *D. magna* life cycle test. The test consisted of exposing 5-day old *Daphnia* for 10 days, through maturation and the release of three broods. The tests were conducted in 2-L beakers, each containing 200 ml of sediment and 800 ml of site water. The sediment level was determined by volumetric displacement. Five replicates were performed for each site with each replicate containing 20 organisms for a total of 100 organisms exposed per site. Organisms were fed every other day 6 mg/L of the tri-algal mixture used in culturing. The beakers were kept lightly aerated by a glass tipped airline placed approximately 4 cm below the water surface. Temperature and DO were checked twice daily and were maintained at  $25 \pm 1^\circ\text{C}$  and  $>5.9$  ppm respectively. On day 0 and 10, pH, conductivity, alkalinity and hardness were recorded (Table 2). At the end of the tests the original adults and their offspring were counted. Water and fine suspended solids were poured through a 0.5 mm mesh screen, rinsed and placed in clean water to enable counting.

#### Biomonitoring Study

On October 6, 1993, four of the 16 Times Beach sites were selected as sites for mussels placement. The four Times Beach sites were picked for mussel placement based on toxicant concentrations in the sediment. In order to best characterize the system, the highest, lowest and two sites of average contaminant concentrations were chosen. Water and sediment for chemical analysis were taken from each of the four sites for mussel placement as described in the water and sediment collection methods. Mussels for use in the bioaccumulation study were collected from the nearby Black Rock Channel Lock in Buffalo, NY. Black Rock Channel Lock also served as a reference site in the biomonitoring study as the area is relatively uncontaminated. The average mussel size was  $\sim 1.6$  cm. Mussels were collected using a hand-held sampler (Miller and Dye 1992) to scrape clumps of mussels from the walls of the Black Rock Lock. This device consisted of a long metal pole  $\sim 3$  m attached to a screened metal box which held the mussels and allowed water drainage. Immediately after collection, mussels were held between layers of wet paper towels in a 170-L cooler. Mussels were then secured in vented plastic cages, filled halfway, with  $\sim 1300$  grams of mussels. Filled cages were placed in Black Rock Channel Lock water until the 15-min transport by van to the Times Beach CDF.

At one station in the Black Rock Channel Lock and the four Times Beach sites (TB01, TB10, TB11, and TB12) mussels were placed in two positions, both on the sediment (Lower) and in the water column (Upper) (Figure 4). Four additional

samples were randomly placed in Times Beach to allow for quality assurance. quality control (QA/QC) reasons. Sediment level (Lower) mussel cages were placed on a cord that was tied from the float to the sediment with sufficient cord to allow for water level fluctuations. Water column (Upper) mussel cages were tied to the cord ~ 45-cm below the float.

On October 6, 1993, day zero of the bioaccumulation study, four groups of randomly selected mussels were collected for a day 0, baseline analysis. These mussels were placed in iced coolers and sent overnight to the Waterways Experiment Station (WES). There, the mussels were kept in the freezer until preparation for chemical analysis. At Days 19 and 34 mussels were recollected from both Times Beach and the reference station for analysis. The procedures for shipping the recollected mussels to WES were the same as for the baseline mussel analysis. On Days 0, 19, and 34 temperature, dissolved oxygen, pH and conductivity were conducted at the Reference site (Black Rock Channel Lock) and the four Times Beach sites (TB01, TB10, TB11, and TB12). On Day 34 pH and conductivity could not be recorded due to equipment failure (Table 3).

### Chemical Analyses

The water and sediment samples were analyzed for the selected toxicants at the Environmental Chemistry Branch (ECB) at WES. Samples for PAH analysis were extracted, cleaned up and run on a GC mass spectrometer (GC-MS) following

USEPA SW-846 (1986) Method 8270. Samples were reported as Total PAHs which consisted of the sixteen PAHs listed as priority pollutants by the World Health Organization (WHO), the European Economic Community (EEC) and the US Environmental Protection Agency (USEPA)(Keith and Telliard 1979); fluoranthene, pyrene, chrysene, benzo(a)anthracene, phenanthrene, anthracene, naphthalene, acenaphthylene, acenaphthene, fluorene, benzo(a)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene. PCBs were run by USEPA SW-846 (1986) Method 8080. The following PCB Aroclors were analyzed for; 1016,1221, 1232, 1242, 1248, 1254, and 1260. Metals were digested by USEPA SW-846 (1986) Method 3050 for sediment and Method 3010 and 3020 for water. Eight metals were tested for; arsenic, chromium, cadmium, mercury, lead, barium, silver and selenium. Metals were run by USEPA SW-846 (1986) Method 6010 (ICAP).

Tissues were prepared for analysis by manually removing mussel tissue from the shells with forceps (Figure 8). Twenty five grams of wet tissue weight was needed for each analysis and contaminant concentrations were recorded as wet weight. The 16 PAHs analyzed for in the sediments were also analyzed for in the tissues. Tissues used in PAH analysis were extracted using methods in USEPA (1981), cleaned up with silica gel (Warner 1976), and run for PAHs on a GC-MS using Method 8270 in USEPA SW-846 (1986). PCBs were run by USEPA SW-846 (1986) Method 8080. The following PCB Aroclors were analyzed for; 1016, 1221,

1232, 1242, 1248, 1254, and 1260. Tissue samples for metal analysis were extracted, cleaned up and run on a GC mass spectrometer (GC-MS) following the methods in USEPA SW-846 (1986).

### Statistical Analyses

No analyses were performed on the elutriate test data as toxicity was not sufficient to produce an LC50. Data produced from the whole sediment toxicity tests were recorded as survival and production of neonates. Reproduction and adult survival data were used to determine if survival and reproduction were significantly inhibited at the Times Beach Plots (TBP-1 - TBP-4) in comparison to the control. This procedure is based on hypothesis testing. If the data are normally distributed and the variance in number young produced is the same, data are subjected to analysis of variance (ANOVA) to determine if differences exist between treatment means. Steel's Many Rank Test procedure was then used to compare concentration means to control means (Weber et al. 1989).

Analytical laboratory results for contaminant (PAH, PCB, and selected metal) concentrations were submitted as detectable values, J-values (a J-value is reported when a concentration is detected but can not be quantified) and less than detection limit values. For statistical analyses the detectable values and the J-values were recorded as detectable values. The less than detection limit values were divided in half and recorded as detectable values. This was done as recommended by



Rhodes (1981) and Porter et al. (1988) and in accordance with USEPA/USACE (1993-Draft).

The data was tested for normality and equality of variances. Raw data were tested for normality using the Shapiro-Wilks test (Morrison 1976) and for equality of variances by the Levene's test (Snedecor and Cochran 1980). If the data failed one or both of these tests, the data were log transformed then retested for normality and equality of variances. If the data still failed one or both of these tests the data were transformed into Rankits.(USEPA/USACE 1993-Draft)

One tailed T-Tests were performed to determine if the contaminants accumulated in the mussel tissues on Day 34 were significantly greater than those collected on Day 0 (Zar 1984; USEPA/USACE 1993). Anovas (analyses of variance) were performed on a composite of mussel tissues from Day 19 and 34 to determine contaminate concentrations differences due to mussel position in the water column (Upper versus Lower) (Steel and Torrie 1980).

## RESULTS

### Site Characterization

Concentrations of the contaminants were low in the water column. Total PAHs and Total PCBs were found to be below detection limits of 0.01 and 0.00060 mg/kg respectively across the 16 Times Beach Sites (Table 4). Concentrations of metals in the water column were detectable in some samples, but all concentrations were near detection limits (Table 5).

Contaminant concentrations were evident in the Times Beach sediment. Total PAHs were present in the sediment ranging from 11.94 to 549.01 mg/kg. Total PCBs were also detected in the sediment ranging from 2.20 to 8.90 mg/kg (Table 4). The concentration range of metals in mg/kg were as follows: arsenic (27.5-54.0), barium (38.6-99.6), cadmium (1.4-5.9), chromium (170.0-355.0), lead (195.0-637.0), mercury (3.7-16.0). Selenium and silver present in the samples analyzed were below detection limits of 0.50 and 10.00 mg/kg respectively (Table 6).

The acute elutriate toxicity tests had no substantial mortality for *D. magna* exposed to all elutriate concentrations, so no LC50 determinations could be calculated (Table 7). The 10-day whole sediment toxicity tests exhibited significant impairment in survival and reproduction. Percent survival from the reference station

(Grand Island) was high (84%) but was reduced (1-7%) at Plots TBP-1 to TBP-4. Reproductive impairment followed the same overt trend as survivorship. The mean reproduction at the reference site was high (86.8 neonates), with a significant reduction at plots TBP-1 through TBP-4 (1.6 to 9.0 neonates) (Table 8).

#### Zebra Mussel Survival

The majority of the mussels, placed in both the Upper and Lower positions appeared to be alive after the 34-day *in-situ* study. This was based on observation of the mussels upon collection. The majority of the mussels were not open or gaping and their tissues were intact.

#### PAH Bioaccumulation

The total PAH concentration in the water column at the 4 sites of mussel placement was >0.010 mg/L. Total PAHs in the sediment averaged 164.41 mg/kg, and ranged from 19.27 mg/kg at the low spot TB01 to 340.95 at the hot spot TB10. Total PAH accumulation in the mussel tissue (Upper and Lower combined as they were not statistically different see Table 12) at Times Beach was 6.58. At the individual sites the concentrations ranged from 5.60 to 10.40 (Table 9). Total PAH concentrations in the 34-day exposure tissues were significantly higher than those in the water column or the day 0 analysis, but did not reach the levels seen in the sediment (Figure 9).

No significant PAH accumulation occurred at the Black Rock Channel Lock reference site. At Times Beach, total PAHs, fluoranthene, pyrene, chrysene and benzo(a)anthracene accumulated significantly at Day 34 compared to the Day 0 baseline tissue analysis. Detectable levels of phenanthrene, anthracene, acenaphthene, fluorene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, and benzo(g,h,i)perylene were found in the Day 34 mussel tissues, although the concentration levels were not statistically higher than the baseline analysis (Table 10).

The individual PAHs, which significantly accumulated in the Upper and Lower mussel tissue (fluoranthene, pyrene, chrysene and benzo(a)anthracene), were all found in the sediment but not detectable in the water column. Acenaphthene, fluorene, phenanthrene, anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene) were present in the sediment, detectable but not significant in the Upper and Lower tissues, and undetectable in the water column. Napthalene, acenaphthylene and dibenzo(a,h)anthracene were not detected in water, tissue (Upper and Lower) or sediment samples. There where no cases were a PAH was present in the tissues (Upper and Lower) but not in the sediment (Tables 10 and 11). No significant position differences were observed at the reference station. At the Times Beach sites PAH accumulation between mussels placed in the water column (Upper) and mussels placed at the sediment-water interphase (Lower) followed the general

trend of greater accumulation in the Upper mussels than in the Lower. This apparent difference was not found to be significant statistically, with the exception of benzo(a)anthracene concentrations showed a slight statistical increase between Upper and Lower. Total PAHs, fluoranthene, pyrene, and chrysene had no significant position differences across the four Times Beach sites. This was based on the combined data from mussels collected on Days 19 and 34 for the PAHs which accumulated significantly over the study (Table 12).

#### PCB Bioaccumulation

Seven PCB Aroclors (1016, 1221, 1232, 1242, 1248, 1254 and 1260) were analyzed for in Times Beach water and sediment. In the water column, all Aroclors were below detection limits (0.00060 mg/l). In the sediment the only prevalent Aroclor was Aroclor 1248 (at 3.44 mg/kg). Aroclor 1260 was also detected at 0.40 mg/kg (Table 13).

In the tissues (Upper and Lower combined) from mussels exposed to Times Beach for 34 days, concentrations of Aroclors 1248 and 1260 were detected (1.64, and 0.21 mg/kg respectively). However, only Aroclor 1248 accumulated significantly compared to the Day 0 baseline analyses of Aroclors 1248 and 1260 (0.24 and 0.40 mg/kg respectively). No significant accumulation occurred at the Black Rock Channel Lock reference station (Table 14).

At Times Beach, across the 4 sites of mussel placement, Aroclor 1248

concentrations (Upper and Lower combined) in mussels exposed to the CDF for 34 days (1.64 mg/kg) were significantly higher than water column concentrations (<0.00060) and approximately half that of the sediment (3.44 mg/kg) (Figure 10). This general trend was also seen at each site individually (Table 15).

At both the reference site (Black Rock Channel Lock) and the Times Beach sites (the four sites combined) PCB Aroclor 1248 concentrations in Upper versus Lower mussels were not significantly different. This was based on the combined data from mussels collected on Days 19 and 34 (Table 16).

#### Metal Bioaccumulation

In the water column, As, Cr, Ba, and Pb were detected in low concentrations, near detection limits. Hg, Cd and Ag were not detected. In the sediment, As, Cr, Ba, Hg, Cd, Pb, and Se were present at 47, 285, 98, 7, 7, 492, and 1mg/kg respectively. Ag was not detected in the sediment (Table 17).

In the tissues (Upper and Lower combined) from mussels after the 34-day Times Beach exposure, concentrations of all metals except Ag were detected. However, only As (0.96 mg/kg), Cr (2.87 mg/kg), and Ba (7.00 mg/kg) accumulated significantly compared to the Day 0 baseline analysis (initially 0.82, 1.02, and 2.40 mg/kg respectively). Day 34 concentrations of Cr and Ba showed a two fold concentration increase over Day 0. No significant accumulation occurred at the Black Rock Channel Lock reference station (Table 18).

At Times Beach, across all sites, As, Cr and Ba tissue concentrations (Upper and Lower combined) from mussels exposed for 34 days, were significantly higher than water column concentrations. Tissue concentrations of these metals were only a fraction of the concentrations measured in the sediment (Figure 11). This general trend was evident at each site individually. As in tissues collected from the four sites ranges from 0.88 - 1.03, while As in the sediment ranged from 41.4 - 51.8 mg/kg. Tissue concentrations of Cr and Ba at the four sites ranged from 1.4 - 4.8 and 6.7 - 7.0 mg/kg respectively, sediment concentrations ranged from 260-332 and 88-111 mg/kg (Table 19).

At both the reference site (Black Rock Channel Lock) and the Times Beach sites (the four sites combined), no significant position differences (Upper versus Lower) were seen due to mussel placement. This was based on the combined data from mussels collected on Days 19 and 34, looking at As, Cr and Ba, the only metals to significantly accumulate over the study (Table 20).

## DISCUSSION

### Preliminary Site Characterization

Chemical concentrations of a water or sediment sample do not predict contaminant bioavailability (Luoma and Bryan 1981, Adams et al. 1985, Ditoro et al. 1991). For a given contaminant concentration the percentage that is biologically available can range from 0 to 100% (Burton and Scott 1992). Levels of total PAHs, total PCBs, and the selected metals (As, Ba, Cr, Cd, Hg, Pb, Se, and Ag ) were at or near detection limits in the Times Beach water samples collected from all 16 sites. The acute elutriate (water phase) test performed in this study did not have significant mortalities to permit generation of an LC50. These results indicate that water column contaminants may not be of environmental concern in terms of water column toxicity.

Concentrations, in the mg/kg range, of total PAHs, total PCBs, and several metals (As, Ba, Cd, Cr, and Pb) were present in Times Beach sediment. Whole sediment toxicity tests tested with Times Beach sediment resulted in highly significant mortality and reproductive impairment to the cladoceran, *Daphnia magna*. This indicates that Times Beach sediment may pose potential environmental hazards relative to sediment toxicity and the bioaccumulation of toxicants from the sediment by aquatic organisms.



The chemical evaluations and the combination of elutriate and solid phase toxicity tests demonstrated that the sediment is toxic, but may not be releasing dissolved materials into the overlying water column. This is in accordance with studies showing that *D. magna* are generally more sensitive to whole sediment than elutriate exposures (Burton et al. 1989). The toxicity and impairment measured in the whole sediment toxicity tests indicate environmental concern as bioavailable contaminants can be directly transferred from the sediment (Adams et al. 1992, Black et al. 1991).

This preliminary phase of the study indicates sediment toxicity, along with the potential of toxicant bioaccumulation and biological impairment to organisms exposed to the Times Beach CDF. It was demonstrated that these contaminants are not sediment bound but bioavailable to the cladoceran, *D. magna*.

#### Water Quality at Times Beach

Water column concentrations of As, Cr, and Ba were all below the Federal limits acceptable for drinking water of 0.05, 0.05 and 1.0 mg/l respectively as documented in the Handbook of Toxic and Hazardous Chemicals and Carcinogens (Sittig 1986; Sittig 1991). There are no water quality criteria for PAHs and PCBs in drinking water in the United States; however, the World Health Organization European Standard for drinking water recommends PAH concentrations limit (based on the composite of six PAHs; fluoranthene, benzo(a)pyrene, benzo(ghi)perylene,

benzo(b)fluoranthene, and indeno(1,2,3-cd)pyrene) of 0.2 ug/l (Sittig 1986), which is below the detection limits of this study. Thereby, Times Beach water would not be deemed undrinkable in the United States due to contaminant levels.

The United States Environmental Protection Agency (USEPA) have water quality criteria for permissible concentrations of total PCBs, As, and Cr in water to protect freshwater aquatic life (Sittig 1986, Sittig 1991, Peddicord et al. 1991). For total PCBs the limit is 0.014 µg/l, which was below detection limits of this study. The criteria for metal impacts on the freshwater aquatic life are listed for trivalent Cr (not to exceed  $e^{(1.08 \ln(\text{hardness}) + 3.48)}$  ug/l), hexavalent Cr (18 µg/l as a 24-hr average, not to exceed 21.0 µg/l), and trivalent As (not to exceed 440 µg/l). Unfortunately this study evaluated only total Cr and As. Therefore, this study can state that toxicant levels were low in the water column, but may or may not be above criteria levels harmful to aquatic life.

### Zebra Mussel Survival

The survival of zebra mussels in a CDF is of potential environmental concern as there are multiple CDFs located along the zebra mussel infested Great Lakes (Beyer and Stafford 1993). While these CDFs are not infested with zebra mussels, it is important to recognize their survival ability, thereby making infestation an existing concern. The survival of zebra mussels in the soft sediment of Times Beach (Lower mussels) is supported by recent reports in the United States. Dermott and Munawar

(1993) found large populations of zebra mussels existing in soft sediments throughout the central and eastern basins of Lake Erie. Reports of mussels in muddy sand and silt sediments occurred in Lake St. Clair (Hunter and Bailey 1992) and the western basin of Lake Erie (Nalepa and Schloesser 1993). Originally, zebra mussels were not found in the sediments in North America. This was not due to an inability to survive in sediment, but rather a growth pattern similar to European mussels (Ramcharan et al. 1992), where the mussels slowly form duses on small particles in soft sediments, eventually resulting in mussel aggregations over areas of soft sediment.

#### Position Differences

Concentration differences of the PAH benzo(a)anthracene due to position (Upper versus Lower) were statistically greater for mussels placed in the less contaminated water column than at the highly contaminated sediment level. There were no significant accumulation differences for total PAHs, fluoranthene, pyrene, chrysene, PCB Aroclor 1248, arsenic, chromium, or barium. The reason the majority of the contaminants bioavailable to zebra mussel showed no significant position differences may be due to the water fluctuations at Times Beach. The water at Times Beach is influenced by Lake Erie seiche action. On one sampling date the water was churned to the degree that sediment was encrusted on the floating site markers. Another possibility is that as the general trend was greater

accumulation in the Upper samples, the length of the study may have been a limiting factor, thereby a longer study may provide significant differences.

### Toxicant Bioaccumulation

Generally due to aqueous solubilities, toxicants in aquatic systems adsorb to organic and inorganic particulate materials, become deposited in bottom sediment (Goldberg 1976, Neff 1979, Wakeham and Farrington 1980, Baker et al. 1991), and accumulate in the tissues of aquatic organisms (Neff 1984, Fisher et al. 1993). Our study supports this pattern, as toxicant concentrations in the water column were near or below detection limits, present themselves in the sediment, and became detectable in mussel tissues. After the 34-day exposure, tissue concentrations of total PAHs, fluoranthene, pyrene, chrysene and benzo(a)anthracene all accumulated significantly over the initial tissue analysis at Day 0. Previous studies evaluating PAH concentrations in Times Beach fish (Marquenie et al. 1987) have indicated limited PAH bioavailability in the aquatic ecosystem. Zebra mussel tissues exposed to Times Beach accumulated total PAHs, fluoranthene, pyrene, chrysene, and benzo(a)anthracene at concentrations higher than the water column and less than the sediment. The accumulation of chrysene and benzo(a)anthracene is of environmental concern as chrysene and benzo(a)anthracene are mutagenic and carcinogenic (Kauss and Hamdy 1991). Zebra mussel accumulation of potentially mutagenic and carcinogenic PAHs is of concern due to the potential of zebra mussel induced

bioaccumulation of contaminants through the food web (Reeders and Bij de Vaate 1992, Fisher et al. 1993).

The remaining 12 PAHs showed no significant accumulation during the 34-day *in-situ* study, including the potentially carcinogenic PAH, benzo(a)pyrene (BAP), known to accumulate well in aquatic animal tissues (Neff 1979, Kauss and Hamdy 1991). BAP concentrations in the sediment were low (~ 5 mg/kg) at Times Beach, possibly explaining the detectable but insignificant increase of this PAH in the study tissues. PAHs can be taken up by filtration of the water column, above the sediment as seen with the mussel *Mytilus* (Pruell et al. 1986, Dame and Dankers 1988, Doherty 1990), or from a food source (Clark and Finley 1975, Fisher et al. 1993) which should also be considered. It is thought that the latter approach is a slower means of uptake. While it could have been a combination of all three, accumulation of PAHs in mussel tissue appears to be primarily from the water column as described in Neff (1984).

PCBs are of particular concern in the Buffalo, New York area, and were present in the material dredged from the Buffalo River to Times Beach (Marquenie et al. 1990). PCBs are man-made contaminants with high environmental persistence and a tendency to accumulate in the food chain, possibly adversely affecting organisms at the top of food webs, including man. Previous site studies have indicated Times Beach PCBs to be bioavailable. Stafford et al. (1986) reports that PCB concentrations in gamefish were of potential concern for public health, and

Marquenie et al. (1990) found several PCB congeners (15, 28, 52, 49, 44, 70, 101, and 87) accumulated in the mussel *Elliptio dilatata* during a 35-day biomonitoring study. In the present study, PCB Aroclor 1248 was determined to be the predominant Aroclor at Times Beach. After the 34-day Times Beach exposure, concentrations of PCB Aroclor 1248 in zebra mussel tissues (Upper and Lower combined) accumulated significantly, ranging from 1.30-2.03 mg/kg at the four Times Beach sites. These concentrations are of environmental concern as they approach the FDA action limit (the highest level of a toxicant acceptable for human consumption) for shellfish of 2 mg/kg (Peddicord et al. 1991). The initial day-0 analyses were low, thereby zebra mussels accumulated levels approximately half of the sediment concentrations over a 34-day period. This ratio of sediment to tissue concentrations is similar to a passive bioaccumulation study by Clarke et al. (1988) utilizing crayfish and several fish species at the Chicago Area CDF, in Chicago, Illinois.

Of the selected eight metals, six (As, Ba, Cd, Cr, Hg, and Pb) were present in the sediment. Copper (Cu) and Zinc (Zn) were not included in this study as previous studies at the CDF (Stafford et al. 1991) have indicated they are not a concern in the Times Beach aquatic ecosystem. Previous fish studies at Times Beach (Marquenie et al. 1987) indicated bioavailability of As, Cd, and Hg. Of the metals relevant to this study, Cr, Pb, Cd, Hg, and Se are known to accumulate in

zebra mussel tissue (Reeders and Bij de Vaate 1992, de Kock and Bowmer 1993, Mersh and Johansson 1993, Secor et al. 1993). During the 34-day Times Beach exposure the mussels in this study significantly accumulated As, Cr, and Ba. There are no FDA action limits for these three metals however, LeBlanc (1980) reported LC50 values for the toxicity of Ba to the cladoceran, *Daphnia magna*, to be >530000 and 410000 µg/l for 24 and 48 hour exposures. Mussel tissue concentrations were a magnitude higher at 7.0 mg/kg, thereby it is evident that toxic levels of Ba are present in the Times Beach aquatic ecosystem. As is present at toxic levels in the system. The Australian National Health and Medical Research Council on Standards for Metals in Food lists the maximum concentrations of wet weight edible portions of As in mollusks to be 1.0 mg/kg (Peddicord et al. 1991), which is at the level accumulated in the exposed zebra mussels (0.97 mg/kg).

Zebra mussels accumulated the potentially harmful contaminants, PCB Aroclor 1248 and As, to concentrations of environmental concern. This presents the potential environmental hazard of bioconcentration and bioaccumulation effects through the food web. This is a concern in the Times Beach CDF as previous studies have indicated movement of contaminants through the food webs of the Times Beach aquatic ecosystem. In 1991 (Stafford et al.) found that the Times Beach mallard ducks had significantly higher concentrations of Cd and Hg than those from a reference site. Toxicant accumulations by zebra mussels also raise

environmental concerns along contaminated portions of the Great Lakes as recent reports state several duck species have included zebra mussels in their diet (Wormington and Leach 1992, Mitchell and Carlson 1993, and Hamilton et al. 1994). Documented zebra mussel consumers common to the Great Lakes include the Greater scaup, Lesser scaup, Bufflehead, and Common Goldeneye (Hamilton et al. 1994). The Lesser scaup has been reported at Times Beach (Stafford et al. 1991). At Times Beach, contaminants were high in the sediment and high in the visceral mass of the zebra mussel. This indicates a potential bioaccumulation problem of the zebra mussel uptaking contaminants resulting in contaminant mobility to birds which feed on the mussels.



## SUMMARY

The preliminary site characterization study of the Times Beach CDF indicated the potential of biological impairment to organisms exposed to the Times Beach CDF. Concentrations of total PAHs, total PCBs, arsenic, barium, cadmium, chromium, lead, and mercury were near or below detection limits in the water column and present in sediment samples as high as 549, 8.9, 54, 91.6, 5.9, 299.0, 637.0, and 16.0 mg/kg, respectively. Toxicity tests demonstrated that the Times Beach sediment was toxic to the cladoceran, *D. magna*, unlike the water which was not bioavailable and relatively nontoxic. The biomonitoring study indicated that zebra mussels can survive in a CDF. At the Times Beach CDF the mussels survived for 34 days both in the water column and at the sediment level. Contaminant bioavailability was confirmed at Times Beach, as during the 34-day biomonitoring study zebra mussels accumulated total PAHs, the individual PAHs; fluoranthene, pyrene, chrysene, and benzo(a)anthracene, PCB Aroclor 1248, arsenic, chromium, and barium above detection limits. This accumulation was significantly greater than the initial Day 0 evaluation, therefore indicating a potential concern that uptake from contaminated sediments by zebra mussels could result in bioaccumulation effects through the food web, in particular to waterfowl feeding on the mussels. At the

Times Beach CDF, zebra mussels accumulated statistically higher concentrations the PAH benzo(a)anthracene when placed in the water column compared to placement at the sediment level. There were no significant accumulation differences due to position for total PAHs, fluoranthene, pyrene, chrysene, PCB Aroclor 1248, arsenic, chromium, or barium.



Figure 1. Times Beach Confined Disposal Facility Near Buffalo, New York.

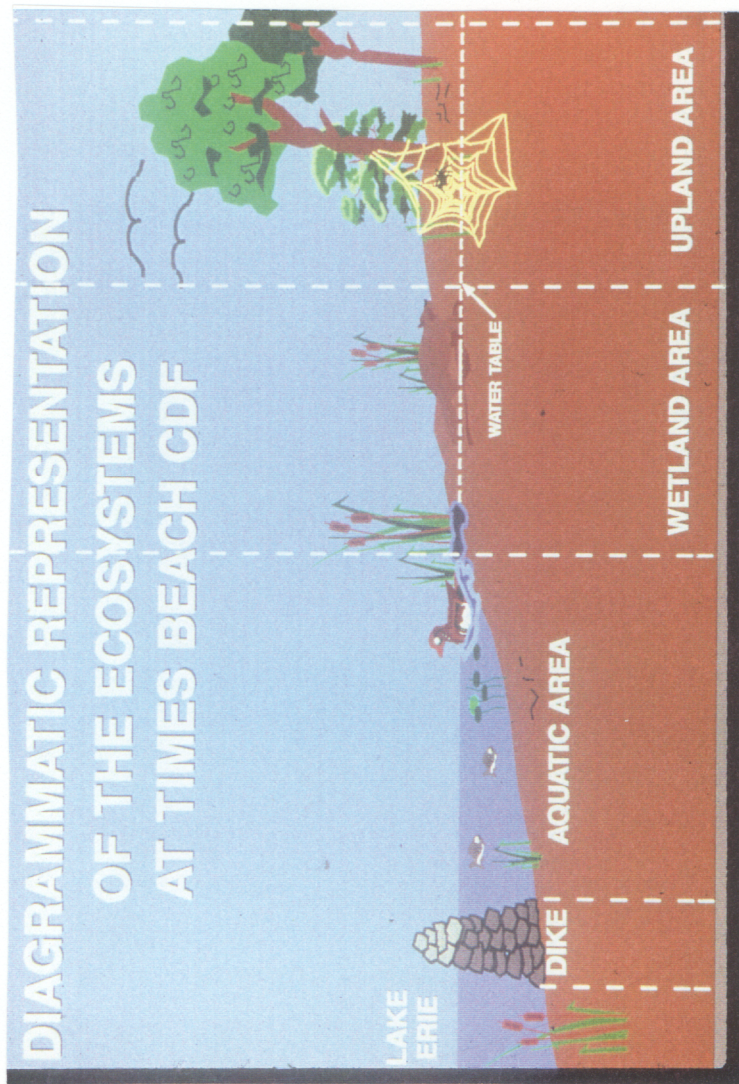


Figure 2. Artist Illustration of the ecosystems at the Times Beach CDF.

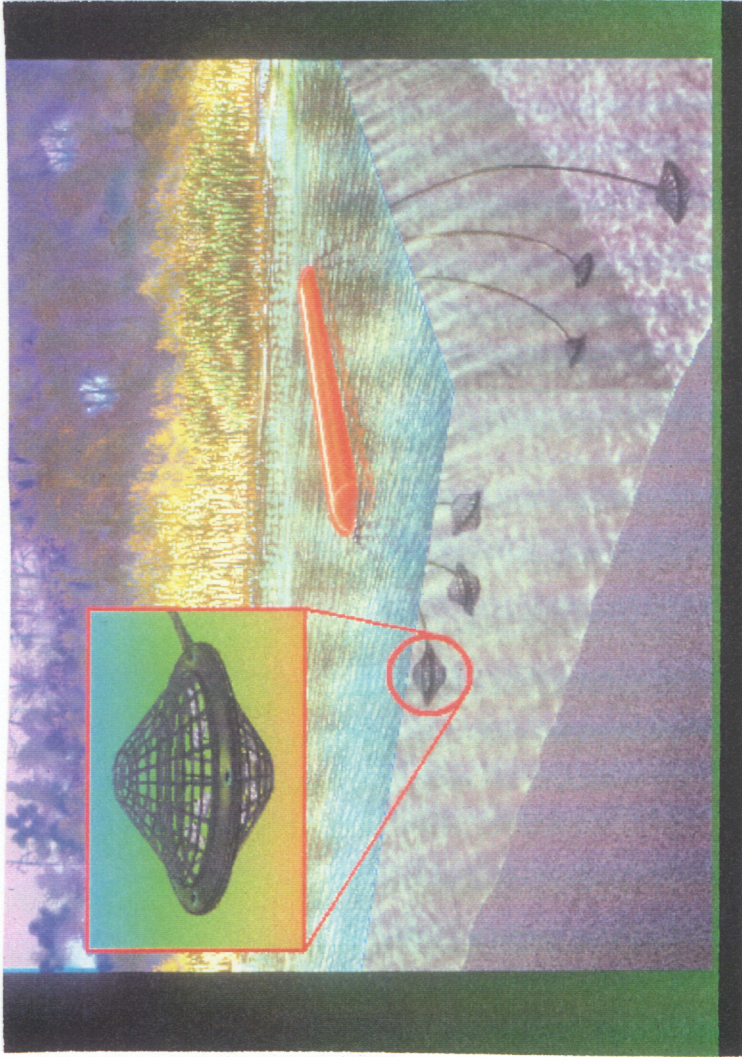


Figure 3. Artist Illustration of Mussel Placement at the Times Beach CDF.

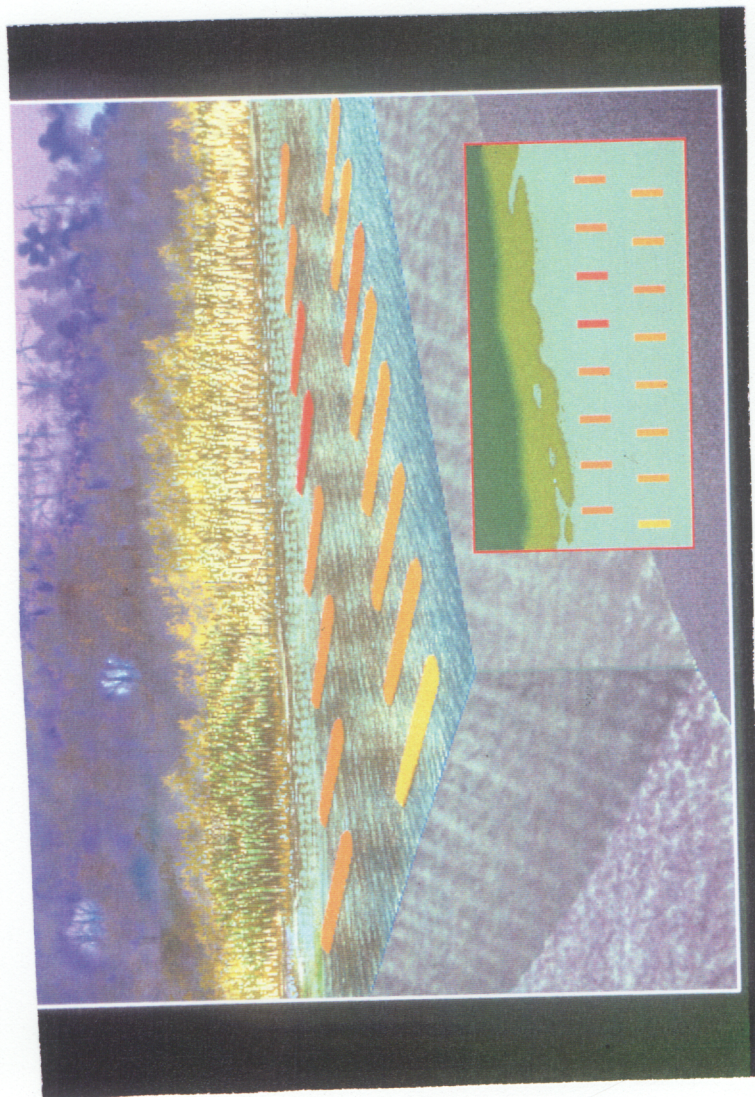


Figure 4. Artist Illustration of the Sixteen Times Beach Sites Used in the Site Characterization Study.

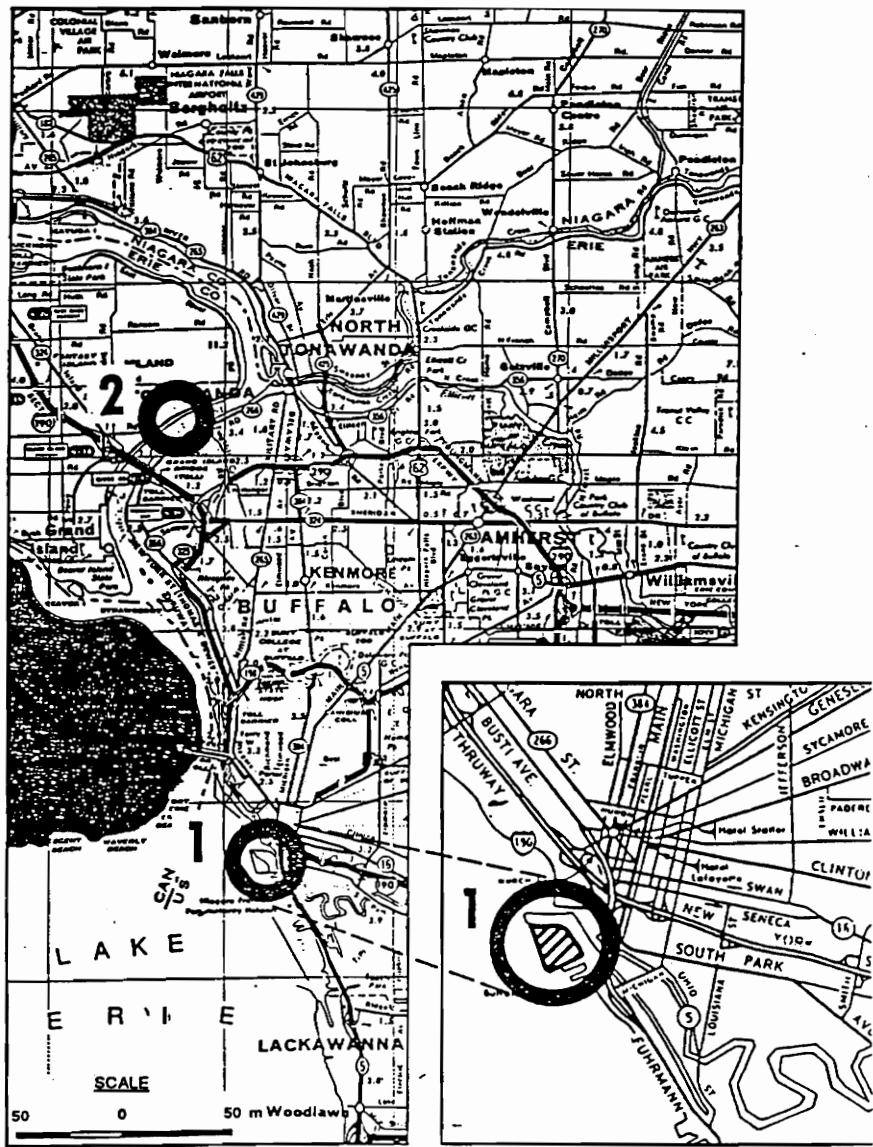


Figure 5. Location of the Times Beach Confined Disposal Facility (1) and the Selected Reference Site for Toxicity Testing, Grand Island (2).



Figure 6. Photograph of the Cladoceran Daphnia magna.





Figure 7. Insertion of the Cladoceran Daphnia magna into the Elutriate Toxicity Test.



Figure 8. Manual Removal of Zebra Mussel Tissues for Chemical Analysis.

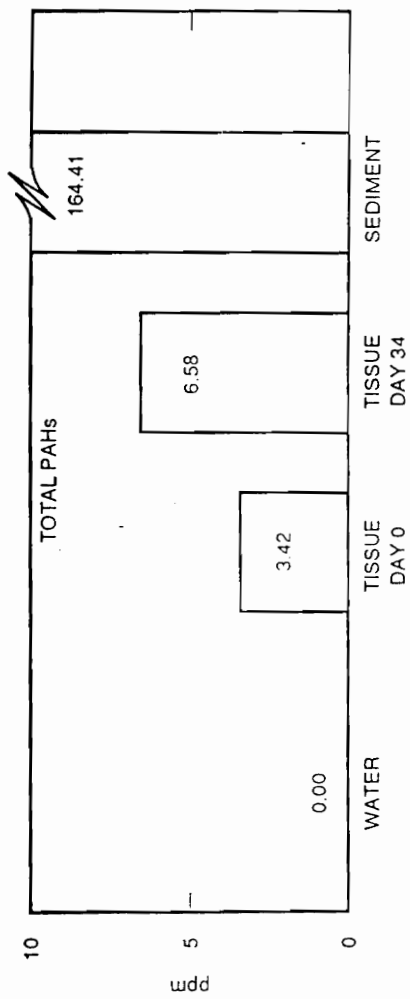


Figure 9. Concentrations of Total PAHs (ppm) in Samples from the Times Beach CDF (all sites combined).

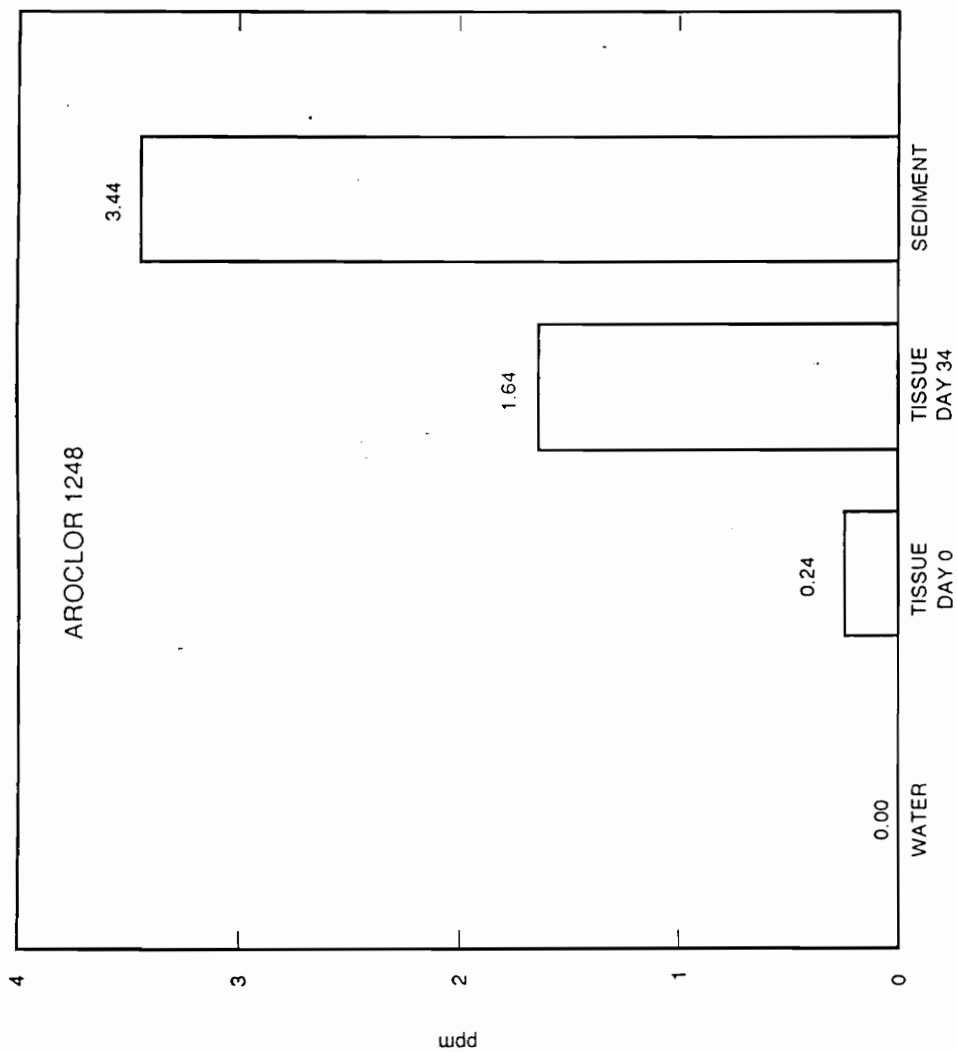


Figure 10. Concentrations of PCB Aroclor 1248 (ppm) in Samples from the Times Beach CDF (all sites combined).

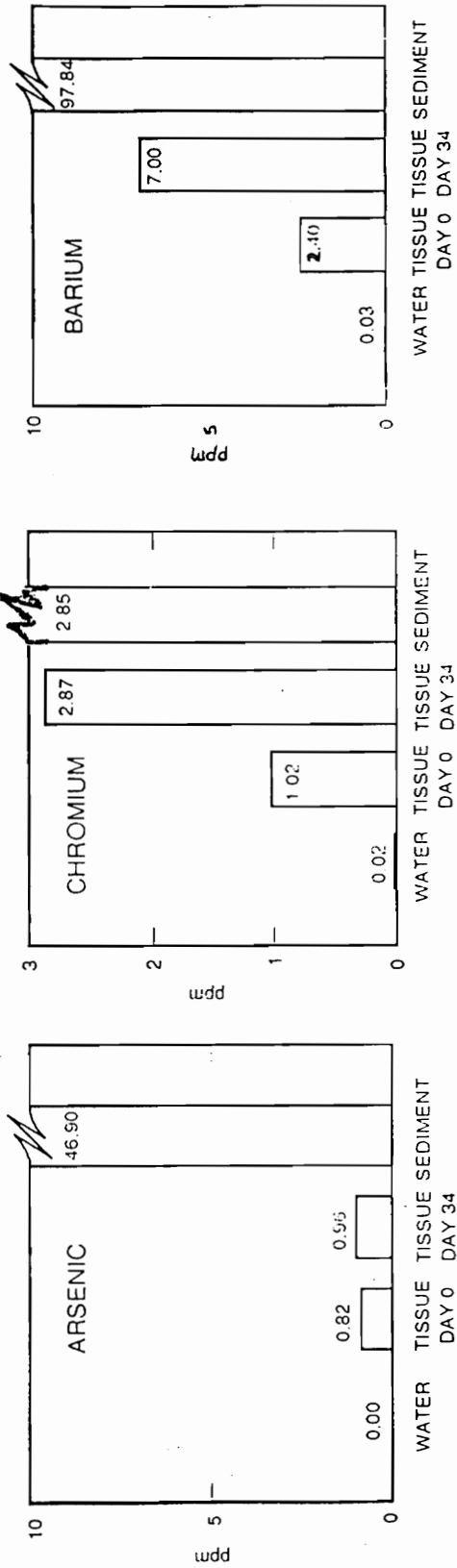


Figure 11. Concentrations of Metals (ppm) in Samples from the Times Beach CDF (all sites combined).

Table 1: Range of Water Chemistry Recorded During the Elutriate Test at Hours 0, 24 and 48.

Sample	Temp °C	DO mg/l	pH	Cond µmhos/cm	Alk	mg/L	Hard mg/L
Culture	21	6.8-7.0	51-53	170	57-61		51-53
GI 100	21	7.1-7.9	114-115	260-300	91-93		114-115
50	21	6.5-7.8	6.4-6.9	260-280	76-81		105-112
10	21	7.0-7.4	6.7-7.0	270-280	76-86		112-133
0	21	6.5-7.0	6.8-7.0	270-290	79-83		111-114
TBP-1 100	21	6.4-7.8	7.0-7.3	330-400	171-175		196-218
50	21	6.7-7.5	7.0-7.3	300-350	97-104		161-167
10	21	6.3-7.5	7.0-7.2	260-280	93-98		136-156
0	21	6.3-7.1	7.3-7.5	250	84-94		115-122
TBP-2 100	21	6.2-6.9	6.4-6.6	340-380	155-169		187-192
50	21	6.0-6.9	6.4-6.6	300-370	99		146-155
10	21	6.3-6.8	6.6-6.8	270-300	80-93		120-147
0	21	6.1-7.1	6.2-6.7	250-270	85-86		109-110
TBP-3 100	21	6.0-6.6	6.1-6.5	400	121-137		157-191
50	21	6.0-6.7	6.5-6.6	350	95-99		155
10	21	6.0-6.9	6.8-7.0	290	80-90		130-139
0	21	6.1-6.9	6.9-7.0	280	80-86		111-121
TBP-4 100	21	7.0-7.4	6.6-6.9	350-390	128		167-168
50	21	6.5-7.1	6.6-7.0	310-330	94-102		141-142
10	21	6.4-6.7	6.9-7.0	290-300	85-93		122-143
0	21	6.5-6.7	6.9-7.0	270-280	80		117-119

Table 2: Water Quality Analysis of the 10-Day Whole Sediment Toxicity Tests with *Daphnia magna*.

Sample	Day	Temp °C	DO mg/l	pH	Cond µmhos/cm	Alk mg/L	Hard mg/L
GI	0	25	>5.9	7.0	580	140	275
	10	25	>5.9	7.4	600	141	283
TBP1	0	25	>5.9	7.2	580	177	300
	10	25	>5.9	7.4	600	168	294
TBP2	0	25	>5.9	7.3	590	145	289
	10	25	>5.9	7.3	600	148	265
TBP3	0	25	>5.9	7.3	500	150	280
	10	25	>5.9	7.5	500	152	248
TBP4	0	25	>5.9	7.2	650	130	390
	10	25	>5.9	7.0	700	97	427

Table 3: Water Chemistry Parameters at Times Beach, New York During the Mussel Biomonitoring Study.

Location	Day	Temp °C	DO mg/l	pH	Cond µmhos/cm
Reference	0	13.7	6.8	6.7	285
	19	12.6	10.0	7.6	200
	34	7.7	9.8		
TB01	0	11.4	9.1	7.1	230
	19	10.4	9.5	8.1	200
	34	2.7	15.0		
TB10	0	12.0	8.7	7.2	250
	19	10.6	11.0	8.9	210
	34	3.6	10.0		
TB11	0	11.4	8.5	7.1	240
	19	10.7	10.2	8.3	200
	34	2.8	15.0		
TB12	0	11.7	8.3	7.2	230
	19	10.5	11.0	9.1	210
	34	4.0	10.0		



Table 4: Concentrations of Total PAHs and Total PCBs found in the Sediment at the Sixteen Times Beach Sites (TB01-TB16).

Site	Total PAHs			Total PCBs		
	n	Water (mg/l)	Sediment (mg/kg)	n	Water (mg/l)	Sediment (mg/kg)
TB01	1	<0.010	14.03	1	<0.00060	3.50
TB02	1	<0.010	48.97	1	<0.00060	5.60
TB03	1	<0.010	16.03	1	<0.00060	3.50
TB04	1	<0.010	23.08	1	<0.00060	3.10
TB05	1	<0.010	19.90	1	<0.00060	3.50
TB06	1	<0.010	25.89	1	<0.00060	4.20
TB07	1	<0.010	17.74	1	<0.00060	3.50
TB08	1	<0.010	52.59	1	<0.00060	8.90
TB09	1	<0.010	11.94	1	<0.00060	4.80
TB10	1	<0.010	549.01	1	<0.00060	5.40
TB11	1	<0.010	27.32	1	<0.00060	4.30
TB12	1	<0.010	230.06	1	<0.00060	3.80
TB13	1	<0.010	13.96	1	<0.00060	3.10
TB14	1	<0.010	31.78	1	<0.00060	5.10
TB15	1	<0.010	15.52	1	<0.00060	3.50
TB16	1	<0.010	45.40	1	<0.00060	2.20

Table 5: Concentrations of Metals (mg/l) found in the Water at the Sixteen Times Beach Sites (TB01-TB16) (n=1).

Site	AS	BA	CD	CR	PB	HG	SE	AG
TB01	0.006	0.033	0.0004	0.010	0.017	0.0002	0.005	<0.001
TB02	0.005	0.064	<0.0001	0.014	0.013	<0.0002	<0.005	<0.001
TB03	<0.005	0.031	<0.0001	0.015	0.011	<0.0002	<0.005	<0.001
TB04	0.006	0.062	<0.0001	0.016	0.012	<0.0002	0.007	<0.001
TB05	0.006	0.030	<0.0001	0.017	0.012	<0.0002	0.006	<0.001
TB06	0.007	0.027	<0.0001	0.018	0.017	<0.0002	<0.005	<0.001
TB07	0.006	0.027	<0.0001	0.018	0.012	<0.0002	<0.005	<0.001
TB08	0.006	0.024	<0.0001	0.020	0.013	<0.0002	0.005	<0.001
TB09	0.006	0.025	<0.0001	0.020	0.012	<0.0002	<0.005	<0.001
TB10	0.005	0.024	<0.0010	0.022	0.012	0.0003	<0.005	<0.001
TB11	<0.005	0.021	<0.0010	0.022	0.014	0.0002	<0.005	<0.001
TB12	<0.005	0.025	<0.0010	0.022	0.014	<0.0002	<0.005	<0.001
TB13	<0.005	<0.020	<0.0010	0.023	0.014	0.0002	<0.005	<0.001
TB14	<0.005	<0.020	<0.0010	0.023	0.015	0.0002	<0.005	<0.001
TB15	<0.005	<0.020	<0.0010	0.023	0.012	<0.0002	0.006	<0.001
TB16	<0.005	<0.020	<0.0010	0.024	0.010	<0.0002	<0.005	<0.001

Table 6 : Concentrations of Metals (mg/kg) found in the Sediment at the Sixteen Times Beach Sites (TB01-TB16) (Dry Weight, n=1).

Site	AS	BA	CD	CR	PB	HG	SE	AG
TB01	46.0	99.6	4.0	261.0	453.0	6.0	<0.50	<1.00
TB02	40.0	89.9	4.6	251.0	451.0	7.4	<0.50	<1.00
TB03	37.0	85.3	4.2	260.0	441.0	8.9	<0.50	<1.00
TB04	34.0	70.7	3.3	196.0	278.0	5.9	<0.50	<1.00
TB05	35.5	80.3	4.4	258.0	407.0	11.8	<0.50	<1.00
TB06	31.0	63.6	4.2	186.0	278.0	5.6	<0.50	<1.00
TB07	43.0	83.3	4.4	261.0	382.0	12.8	<0.50	<1.00
TB08	42.5	69.8	3.6	231.0	296.0	5.9	<0.50	<1.00
TB09	54.0	91.6	5.3	315.0	485.0	14.8	<0.50	<1.00
TB10	52.5	79.3	5.5	336.0	637.0	9.5	<0.50	<1.00
TB11	54.0	87.3	4.3	300.0	451.0	14.2	<0.50	<1.00
TB12	49.0	90.2	5.9	355.0	600.0	10.4	<0.50	<1.00
TB13	51.5	81.6	3.9	299.0	428.0	16.0	<0.50	<0.10
TB14	44.5	54.2	2.2	285.0	233.0	8.0	<0.50	<0.10
TB15	27.5	82.9	3.4	274.0	404.0	8.3	<0.50	<0.10
TB16	29.0	38.6	1.4	170.0	195.0	3.7	<0.50	<0.10

Table 7: Forty-eight Hour *Daphnia magna* Elutriate Test Results taken from the Four Times Beach Plots, the Reference Site (Grand Island) and D. Magna Culture Water.

Sample	# Exposed	Mean Survival*
Culture Water	5 reps (10 organisms/rep)	9.80 ± 0.44
Grand Island 100	5 reps (10 organisms/rep)	10.00 ± 0
50	5 reps (10 organisms/rep)	9.60 ± 0.55
10	5 reps (10 organisms/rep)	10.00 ± 0
0	5 reps (10 organisms/rep)	10.00 ± 0
TB Plot-1 100	5 reps (10 organisms/rep)	8.60 ± 1.67
50	5 reps (10 organisms/rep)	9.80 ± 0.45
10	5 reps (10 organisms/rep)	9.60 ± 0.55
0	5 reps (10 organisms/rep)	9.60 ± 0.89
TB Plot-2 100	5 reps (10 organisms/rep)	8.20 ± 1.10
50	5 reps (10 organisms/rep)	9.60 ± 0.55
10	5 reps (10 organisms/rep)	10.00 ± 0
0	5 reps (10 organisms/rep)	9.80 ± 0.45
TB Plot-3 100	5 reps (10 organisms/rep)	6.60 ± 1.82
50	5 reps (10 organisms/rep)	8.80 ± 1.79
10	5 reps (10 organisms/rep)	9.60 ± 0.89
0	5 reps (10 organisms/rep)	10.00 ± 0
TB Plot-4 100	5 reps (10 organisms/rep)	9.60 ± 0.89
50	5 reps (10 organisms/rep)	10.00 ± 0
10	5 reps (10 organisms/rep)	10.00 ± 0
0	5 reps (10 organisms/rep)	9.80 ± 0.45

\* Not enough toxicity was seen to generate an LC50 in any of the samples

Table 8: Results of 10-Day Whole Sediment Toxicity Test taken from the Reference Site (Grand Island) and the Four Times Beach (TB) Plots.

Site	# Exposed	% Survived	Mean Reproduction
Grand Island	100	84.0 ± 8.94	86.8 ± 15.40
TB Plot-1	100	6.0 ± 6.52*	3.4 ± 4.16*
TB Plot-2	100	7.0 ± 6.71*	9.0 ± 8.57*
TB Plot-3	100	1.0 ± 2.24*	2.8 ± 3.83*
TB Plot-4	100	7.0 ± 8.34*	1.4 ± 1.67*

\* indicates significant difference from the control at the  $\alpha = 0.05$  level.

Table 9: Concentrations of Total PAHs in the Times Beach Water and Sediment Compared to the Study Tissues (Upper and Lower Combined) Collected on Day 34. (Means and Standard Deviations)

Location	Water		Sediment		Day 34 Tissue	
	n	mg/l	n	mg/kg	n	mg/kg
Times Beach (all)	4	<0.010	4	164.41 ± 154.54	26	6.58 ± 1.93
Times Beach 01	1	<0.010	2	19.27 ± 4.82	8	5.60 ± 0.58
Times Beach 10	1	<0.010	2	340.95 ± 30.20	4	10.40 ± 1.53
Times Beach 11	1	<0.010	2	25.59 ± 1.85	6	5.61 ± 1.11
Times Beach 12	1	<0.010	2	271.84 ± 12.73	8	6.37 ± 0.91

Table 10: Accumulation of PAHs in Zebra Mussel Tissues (Upper and Lower Combined) Collected on Day 34 at the Reference and Times Beach Locations Compared to the Day 0 Baseline Tissue Analyses. (Wet Weight, Means and Standard Deviations)

PAH	Baseline n	Baseline mg/kg	Reference n	Reference mg/kg	Times Beach n	Times Beach mg/kg
Total PAHs	4	3.42 ± 0.05	4	3.42 ± 0.12	26	6.58 ± 1.93*
fluoranthene	4	0.10 ± 0.03	4	0.08 ± 0.01	26	1.23 ± 0.48*
pyrene	4	0.16 ± 0.01	4	0.14 ± 0.01	26	1.08 ± 0.45*
chrysene	4	0.26 ± 0.08	4	0.27 ± 0.02	26	0.98 ± 0.20*
benzo(a)anthra	4	0.08 ± 0.04	4	0.10 ± 0.01	26	0.60 ± 0.19*
naphthalene	4	<0.50	4	<0.50	26	<0.50
acenaphthylene	4	<0.50	4	<0.50	26	<0.50
acenaphthene	4	0.25 ± 0	4	0.25 ± 0	26	0.17 ± 0.10
fluorene	4	0.25 ± 0	4	0.25 ± 0	26	0.11 ± 0.10
phenanthrene	4	0.25 ± 0	4	0.25 ± 0	26	0.25 ± 0.33
anthracene	4	0.25 ± 0	4	0.25 ± 0	26	0.13 ± 0.13
benzo(b)fluoranthene	4	0.13 ± 0.05	4	0.25 ± 0	26	0.37 ± 0.07
benzo(k)fluoranthene	4	0.09 ± 0.06	4	0.17 ± 0.06	26	0.23 ± 0.05
benzo(a)pyrene	4	0.16 ± 0.11	4	0.11 ± 0.02	26	0.20 ± 0.05
indeno(1,2,3-c,d)pyrene	4	0.19 ± 0.11	4	0.06 ± 0	26	0.06 ± 0.08
dibenzo(a,h)anthracene	4	<0.50	4	<0.50	26	<0.50
benzo(g,h,i)perylene	4	0.25 ± 0	4	0.25 ± 0	26	0.05 ± 0.09

\* Indicates a significant difference from the Day 0 Baseline analysis.

Table 11: Concentrations of PAHs in the Water and Sediment at the Times Beach CDF.  
(Means and Standard Deviations)

PAH	Water n	mg/l	Sediment n	mg/kg
Total PAHs	4	<0.010	8	164.41 ± 154.54
fluoranthene	4	<0.010	8	25.12 ± 29.30
pyrene	4	<0.010	8	18.22 ± 20.47
chrysene	4	<0.010	8	9.32 ± 10.32
benzo (a) anthracene	4	<0.010	8	8.49 ± 9.61
naphthalene	4	<0.010	8	3.18 ± 3.27
acenaphthylene	4	<0.010	8	1.15 ± 1.07
acenaphthene	4	<0.010	8	10.89 ± 15.00
fluorene	4	<0.010	8	14.90 ± 19.52
phenanthrene	4	<0.010	8	36.06 ± 46.17
anthracene	4	<0.010	8	15.28 ± 18.86
benzo (b) fluoranthene	4	<0.010	8	4.86 ± 4.70
benzo (k) fluoranthene	4	<0.010	8	4.09 ± 3.42
benzo (a) pyrene	4	<0.010	8	5.03 ± 4.84
indeno (1,2,3-c,d)py	4	<0.010	8	3.40 ± 3.18
dibenzo (a,h) anthracen	4	<0.010	8	1.25 ± 1.13
benzo (g,h,i) perylene	4	<0.010	8	3.18 ± 2.83



Table 12: Differences of PAH Concentrations with Respect to Placement in the Water Column (Upper versus Lower) at the Times Beach CDF, Composite of Mussels Collected on Days 19 and 34. (Wet Weight, Means and Standard Deviations) (Sample Number, F-values and P-values included).

PAH	Location	n	F	P	Lower	Upper
Total PAHs	TB-all	22	1.40	0.24	6.64 ± 2.64	7.09 ± 1.86
fluoranthene	TB-all	22	7.20	0.10	1.29 ± 0.75	1.37 ± 0.48
pyrene	TB-all	22	0.29	0.59	1.08 ± 0.57	1.22 ± 0.44
chrysene	TB-all	22	3.81	0.07	0.93 ± 0.24	1.01 ± 0.19
benzo(a)anthracene	TB-all	22	3.89	0.04	0.58 ± 0.22	0.63 ± 0.18 *

\* Indicates a significant difference of contaminant concentration between the two positions (Upper and Lower) at a given location.

Table 13: Concentrations of PCBs in the Water and Sediment at the Times Beach CDF.  
 (Means and Standard Deviations)

PCB	Water		Sediment	
	n	mg/l	n	mg/kg
Aroclor 1016	4	<0.00060	8	<1.53
Aroclor 1221	4	<0.00060	8	<1.53
Aroclor 1232	4	<0.00060	8	<1.53
Aroclor 1242	4	<0.00060	8	<1.53
Aroclor 1248	4	<0.00060	8	3.44 ± 1.58
Aroclor 1254	4	<0.00060	8	<1.53
Aroclor 1260	4	<0.00060	8	0.40 ± 0.18

Table 14: Accumulation of the PCB Aroclors in Zebra Mussel Tissues Collected on Day 34 at the Black Lock (Reference) and Times Beach Locations compared to the Day 0 Baseline Tissue analysis. (Wet Weight, Means and Standard Deviations)

PCB	Baseline n	Baseline mg/kg	Reference n	Reference mg/kg	Times Beach n	Times Beach mg/kg
Aroclor 1016	4	<0.03	4	<0.03	26	<0.03
Aroclor 1221	4	<0.03	4	<0.03	26	<0.03
Aroclor 1232	4	<0.03	4	<0.03	26	<0.03
Aroclor 1242	4	<0.03	4	<0.03	26	<0.03
Aroclor 1248	4	0.24 ± 0.06	4	0.11 ± .01	26	1.64 ± 0.50*
Aroclor 1254	4	<0.03	4	<0.03	26	<0.03
Aroclor 1260	4	0.40 ± 0.18	4	0.20 ± .02	26	0.21 ± 0.14

\* indicates a significant difference from the PCB concentration of the Day 0 baseline analysis.

Table 15: Concentrations of Aroclor 1248 in the Times Beach Water and Sediment Compared to the Study Tissues (Wet Weight, Upper and Lower Combined) Collected on Day 34.  
(Means and Standard Deviations)

Location	Water n	mg/l	Sediment n	mg/kg	Day 34 Tissue n	mg/kg
Times Beach (all)	4	<0.00060	8	3.44 ± 1.58	26	1.64 ± 0.50
Times Beach 01	1	<0.00060	2	2.44 ± 1.51	8	1.30 ± 0.25
Times Beach 10	1	<0.00060	2	5.32 ± 0.12	4	1.71 ± 0.36
Times Beach 11	1	<0.00060	2	2.83 ± 2.08	6	2.03 ± 0.74
Times Beach 12	1	<0.00060	2	3.16 ± 0.91	8	1.64 ± 0.32

Table 16: Differences in Aroclor 1248 Concentrations with Respect to Placement in the Water Column (Upper versus Lower) at the Times Beach CDF, Composite of Mussels Collected on Days 19 and 34. (Wet Weight, Means and Standard Deviations) (Sample Number, F-values and P-values included).

Contaminant	Location	n	F	P	Lower mg/kg	Upper mg/kg
Aroclor 1248	Times Beach-all	21	3.31	0.23	1.22 ± 0.45	1.46 ± 0.65

\* indicates a significant difference between the Upper and Lower placed mussels.

Table 17: Concentrations of Metals in the Water and Sediment at the Times Beach CDF.  
(Means and Standard Deviations)

Metals	Water		Sediment	
	n	mg/l	n	mg/kg
Arsenic	5	0.0020 ± 0.002	10	46.90 ± 6.24
Chromium	5	0.0170 ± 0.006	10	285.00 ± 38.43
Barium	5	0.0270 ± 0.006	10	97.84 ± 18.00
Mercury	5	<0.0002	10	6.96 ± 3.23
Cadmium	5	<0.0010	10	7.00 ± 2.96
Lead	5	0.0510 ± 0.051	10	491.60 ± 97.14
Selenium	5	<0.0010	10	1.08 ± 1.03
Silver	5	<0.0010	10	<0.10

Table 18: Accumulation of Metals in Zebra Mussel Tissues Collected on Day 34 at the Black Rock Channel Lock (Reference) and Times Beach Locations Compared to the Day 0 Baseline Tissue analysis. (Wet Weight, Means and Standard Deviations)

Metal	Baseline n	Baseline mg/kg	Reference n	Reference mg/kg	Times Beach n	Times Beach mg/kg
Arsenic	4	0.82 ± 0.09	4	0.74 ± 0.09	26	0.97 ± 0.23*
Chromium	4	1.02 ± 0.23	4	1.10 ± 0.64	26	2.87 ± 2.13*
Barium	4	2.40 ± 1.62	4	1.25 ± 0	26	7.00 ± 1.62*
Mercury	4	0.30 ± 0.05	4	0.01 ± 0	26	0.03 ± 0.02
Cadmium	4	0.79 ± 0.27	4	0.85 ± 0.06	26	0.47 ± 0.08
Lead	4	2.33 ± 0.25	4	1.04 ± 0.11	26	3.28 ± 1.51
Selenium	4	1.18 ± 0.30	4	0.81 ± 0.05	26	0.90 ± 0.11
Silver	4	<0.04	4	<0.04	26	<0.04

\* indicates a significant increase from the given metal concentration of the Day 0 baseline analysis.

Table 19: Concentrations of Metals in the Times Beach Water and Sediment Compared to the Study Tissues (Wet Weight, Upper and Lower Combined) Collected on Day 34.

Metal	Location	Water n	Water mg/l	Sediment n	Sediment mg/kg	Day 34 Tissue n	Day 34 Tissue mg/kg
Arsenic	Times Beach-all	5	0.002 ± 0.002	10	46.90 ± 6.24	26	0.97 ± 0.23
	TB01	2	0.001 ± 0	4	41.38 ± 4.82	8	1.03 ± 0.13
	TB10	1	0.005	2	51.00 ± 2.12	4	0.93 ± 0.05
	TB11	1	0.003	2	49.00 ± 7.07	6	1.03 ± 0.43
	TB12	1	0.003	2	51.75 ± 3.89	8	0.88 ± 0.12
Chromium	Times Beach-all	5	0.017 ± 0.006	10	285.00 ± 38.43	26	2.87 ± 2.13
	TB01	2	0.011 ± 0.001	4	260.50 ± 5.00	8	4.76 ± 2.74
	TB10	1	0.022	2	302.50 ± 47.38	4	1.52 ± 0.21
	TB11	1	0.022	2	269.00 ± 43.84	6	1.39 ± 0.53
	TB12	1	0.022	2	332.50 ± 31.82	8	2.76 ± 1.19
Barium	Times Beach-all	5	0.027 ± 0.006	10	97.84 ± 18.00	26	7.00 ± 1.62
	TB01	2	0.034 ± 0.001	4	97.38 ± 5.28	8	6.61 ± 1.38
	TB10	1	0.024	2	111.65 ± 45.95	4	8.78 ± 2.93
	TB11	1	0.021	2	95.15 ± 11.10	6	6.77 ± 1.26
	TB12	1	0.025	2	87.65 ± 3.61	8	6.65 ± 0.70



Table 20: Differences in Metal Concentrations with Respect to Placement in the Water Column (Upper versus Lower) at the Times Beach CDF Across All Four Sites, a Composite of Mussels Collected on Days 19 and 34. (Wet Weight, Means and Standard Deviations) (Sample Number, F-values and P-values included).

Contaminant	n	F	P	Lower mg/kg	Upper mg/kg
Arsenic	21	3.53	0.07	0.97 ± 0.17	1.46 ± .17
Chromium	21	1.54	0.22	2.51 ± 1.40	2.64 ± 2.07
Barium	21	2.86	0.10	7.11 ± 1.63	8.10 ± 2.16

\* indicates a significant difference between the Upper and Lower placed mussels.

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# Curriculum Vita

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Waterways Experiment Station  
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## Education:

Master of Science Program in Ecotoxicology, October 1994. Virginia Tech, Blacksburg, Virginia. QCA 3.6

Bachelor of Science, Biology, August 1992. Virginia Tech, Blacksburg, Virginia.

## Relevant Curriculum:

Aquatic Ecotoxicology	Freshwater Ecology
Aquatic Microbiology	General Chemistry
Biochemistry	Hazard Evaluation
Biometry	Microbiology
Conservation Biology	Organic Chemistry
Fish Ecology	Principles of Aquaculture
Fish Environ. Phys.	Principles of Ecology

## Positions Held:

May 1993 - Present  
Graduate Contract Student, US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Fall 1993

Graduate Teaching Assistant, Department of Biology, Virginia Tech,  
Blacksburg, Va.

August 1992 - May 1993

Graduate Research Assistant, University Center for Environmental and  
Hazardous Materials Studies, Virginia Tech, Blacksburg, Va.

Summer 1992

Undergraduate Research Student, Department of Biology, Virginia Tech,  
Blacksburg, Va.

**Related Experience:**

Freshwater acute and chronic toxicity tests

*Ceriodaphnia dubia*

*Daphnia pulex*

*Daphnia magna*

*Moina macrocopa*

*Pimephales promela*

Artificial Stream Systems

Biology Lab Instruction

Certified Scuba Diver

Chemical Extractions

Contaminant Uptake and Mobility Studies

Culturing of Zooplankton

Earthworm Bioassays

Leaf Pack Degradation Studies

Macroinvertebrate Identification

Macroinvertebrate Sampling Techniques

Mussel Biomonitoring Studies

Sediment Toxicity Tests

Statistical Analysis Programs

**Publications and Presentations:**

Roper, J.M., D.S. Cherry and J.W. Simmers. 1994. Bioaccumulation of  
PAHs in the zebra mussel, *Dreissena polymorpha*, Times Beach, New York.  
Platform presentation at the 1994 SETAC Meeting.

Roper, J.M., D.S. Cherry and J.W. Simmers. 1994. Bioaccumulation of metals in the zebra mussel, *Dreissena polymorpha*, Times Beach, New York. Poster presentation at the 1994 Setac Meeting.

Roper, J.M., D.S. Cherry and J.W. Simmers. 1994. Survival and bioaccumulation potential of the zebra mussel, *Dreissena polymorpha*, at Times Beach, NY. (Paper presented by myself at the 1994 meeting of the North American Benthological Society, currently in press for J-NABS)

Roper, J.M., D.S. Cherry and J.W. Simmers. 1994. Bioaccumulation of PAHs by zebra mussels. Poster presentation at the 1994 Corps of Engineers Zebra Mussel Workshop.

Roper, J.M., D.S. Cherry and J.W. Simmers. 1994. Sediment toxicity and the bioaccumulation of toxicants by the zebra mussel *Dreissena polymorpha* at Times Beach, NY. (Paper presented by myself in 4th International Zebra Mussel Conference, paper published in the conference proceedings)

Lynde, S., K. Reubush, J. Roper, W. Van Wart, J.R. Webster and D.S. Cherry. 1992. The use of artificial streams in evaluating leaf degradation: a comparison of breakdown rates based on thermal addition. (Paper presented by S. Lynde at the 1993 meeting of the Association of South Eastern Biologists.)

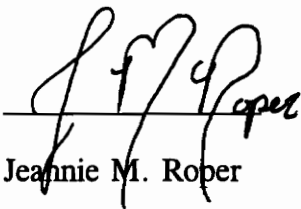
Lynde, S., K. Reubush, J. Roper, W. Van Wart, J.R. Webster and D.S. Cherry. 1992. The effect of temperature and macroinvertebrates on the breakdown of Boxelder leaves in a tributary of the New River, Virginia. (Paper presented by myself at the 1993 meeting of the North American Benthological Society.)

### **Professional Societies:**

North American Benthological Society (NABS)  
Society for Environmental Toxicology and Chemistry (SETAC)  
Phi Sigma (Biological Honor Society)

### **Honors:**

Tuition Scholarship, VPI & SU-- 1993/94  
Phi Sigma induction 1993



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