APPENDIX A

TOXICITY OF SEDIMENTS COLLECTED FROM MINNESOTA SLIP TO THE MIDGE, *Chironomus tentans*

Toxicity of Sediments Collected from Minnesota Slip to the Midge, *Chironomus tentans*

Author
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Study Period
Start: 12 October 1999
End: 22 October 1999

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Laboratory Project ID
8503-111-007

STATEMENT OF PROCEDURAL COMPLIANCE

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.

David A. Pillard

David A Pillard, Ph.D.
Study Director

16 Dec 1999
Date

STATEMENT OF QUALITY ASSURANCE

The test data were reviewed by the Quality Assurance unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

Anita Rehner

Quality Assurance Unit

December 16, 1999
Date

EXECUTIVE SUMMARY

Six sediment samples, collected from Minnesota Slip in the Duluth Harbor Basin, were received by the Fort Collins Environmental Toxicology Laboratory (FCETL) on October 5, 1999. Ten-day toxicity tests were conducted with these samples using the dipteran midge, *Chironomus tentans*. The test endpoints were survival and growth, as measured by dry weight and ash-free dry weight (AFDW). The results of these tests indicated that the sediments were not toxic to *C. tentans* in the 10-day study when compared to the test control. However, survival in sediment MNS-99-05 was significantly reduced when compared to sediment MNS-99-01.

The following table summarizes the study:

STUDY SUMMARY

Sponsor	Minnesota Pollution Control Agency 520 Lafayette Road N. St. Paul, MN 55155-4194
Project Officer	Judy L. Crane, Ph.D. (651) 297-4068
Study Director	David A. Pillard, Ph.D. (970) 416-0916
Test Facility	ENSR Fort Collins Environmental Toxicology Laboratory (FCETL) 4303 West LaPorte Avenue Fort Collins, Colorado 80521
Location of Data	Data Records and Storage 328 Link Lane #4 Fort Collins, Colorado 80524
Test Substances	Sediments: MNS-99-01, MNS-99-02, MNS-99-03, MNS-99-04, MNS-99-05, MNS-99-06
Test Dates	12 October - 22 October 1999
Length of Tests	10 days
Test Species	*Chironomus tentans*
Source of Organisms	Aquatic Biosystems
Age of Test Organisms	9-10 days (2^{nd} - 3^{rd} instar)
Test Concentrations	Bulk (100%) sediment
Overlying Water	Moderately hard reconstituted water

Results	• No significant reduction in survival, dry weight, or AFDW in any sediment, relative to the control. • Significant reduction in survival in sediment MNS-99-05 relative to sediment MNS-99-01.

1.0 INTRODUCTION

Minnesota Slip is located in the northern section of the Duluth Harbor basin in Duluth, MN. Because of concerns regarding sediment contamination in the St. Louis River Area of Concern, several investigations have been conducted of Minnesota Slip and other nearby contaminated areas. The purpose of this study was to determine if sediments collected from Minnesota Slip were toxic to *Chironomus tentans*. These toxicity tests were part of a sediment remediation scoping project in Minnesota Slip. The study sponsor was the Minnesota Pollution Control Agency (MPCA); the testing laboratory was the ENSR Fort Collins Environmental Toxicology Laboratory (FCETL).

2.0 DESCRIPTION OF TEST AND CONTROL SUBSTANCES

2.1 Test Sediment

Six sediment samples were received on 5 October 1999. Each sample was a composite of the upper 5 cm layer of sediment. Sample containers were 4-liter high-density polyethylene, wide-mouth jars. Sample collection and receipt information is presented in the following table. See Appendix A for chain of custody records.

Sample Name	Collection Date and Time	FCETL Sample # and Date of Receipt	Temp at Arrival (°C)	COC Number	COC Tape Number
MNS-99-01	9/22/99 @ 0950-1030	12809 10/5/99	6	34570	5815
MNS-99-02	9/22/99 @ 1115-1140	12810 10/5/99	6	34570	5815
MNS-99-03	9/22/99 @ 1345-1410	12811 10/5/99	6	34570	5815
MNS-99-04	9/22/99 @ 1505-1535	12812 10/5/99	6	34571	5816
MNS-99-05	9/22/99 @ 1620-1642	12813 10/5/99	6	34571	5816
MNS-99-06	9/22/95 @ 1715-1738	12814 10/5/99	6	34571	5816

Sediment samples were stored at 4°C in the dark until test setup. Sediment was homogenized prior to use. Homogenization consisted of transferring sediment to a clean Nalgene® container and thoroughly mixing the sediment with a stainless steel auger attached to an electric drill for not less than three minutes. The auger and container were thoroughly cleaned and decontaminated (soap, tap water rinse, acetone, tap water rinse, HCl, tap water rinse, DI rinse) between homogenization of each sediment.

2.2 Control Sediment

A control sediment (Florissant reference soil obtained from USGS-Biological Resources Division in Columbia, Missouri) was tested concurrently.

2.3 Overlying Water

The overlying test water was moderately hard reconstituted water (USEPA 1993). Four batches of water were used during the course of the study. Initial characterization of the moderately hard water batches was:

Batch #	Initial Use Date	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (µS/cm)	pH	Ammonia (mg/L N)	TRC[a] (mg/L)
3634	10/11/99	96	66	328	8.1	<1.0	<0.05
3636	10/12/99	96	65	332	8.2	<1.0	<0.05
3641	10/14/99	96	63	328	8.1	<1.0	<0.05
3644	10/17/99	94	62	328	8.2	<1.0	<0.05

[a] total residual chlorine

3.0 TEST CONDITIONS

3.1 Test Methods

The studies were 10-day static-renewal toxicity tests using *Chironomus tentans*. Tests were conducted according to USEPA (1994) and ASTM Method E 1706-95b (ASTM 1997) guidelines. The test was modified to include ash free dry weight (AFDW) as a growth endpoint, per the draft (unpublished) ASTM and USEPA guidelines (personal communication with Drs. David Mount and Chris Ingersoll). The biological responses measured were death (defined as no visible movement or any response to gentle prodding with a blunt probe) and growth (mean dry weight and AFDW per surviving organism). The complete test protocol is included as Appendix B.

3.2 Test Duration

Test duration was 10 days.

3.3 Test Apparatus

Test chambers were 500-ml glass beakers containing 100 ml of test sediment and 175 ml of overlying water. On the day prior to test initiation, sediment was homogenized and placed into the test chambers. Overlying water was added and the resulting mixtures were allowed to settle overnight. At test initiation, ten organisms were to be impartially distributed to each test chamber. Due to a technician error, four chambers received no organisms at test initiation, one chamber probably received 12, and one chamber probably received 20 (see section 6.1 for further details). Eight replicates were tested per treatment for a (target) total of 80 organisms per treatment. All test chambers were held in a temperature controlled water bath under

fluorescent lighting with a photoperiod of 16 hours light:8 hours dark; target test temperature was 23 ± 1°C.

3.4 Feeding

Test organisms were fed 1.5 ml of a 4 g/L Tetramin suspension, in moderately hard water, per test chamber on a daily basis.

3.5 Aeration

On day 1 dissolved oxygen in MNS-99-03 and MNS-99-04 test chambers was at or less than 2.5 mg/L (Appendix C). Therefore, aeration was initiated on day 1 in all test chambers, including the control. The aeration apparatus consisted of a Pasteur pipette (connected to the laboratory air supply), the tip of which was positioned so as not to disturb the sediment. All test chambers were aerated for the remainder of the test.

4.0 TEST ORGANISMS

Chironomus tentans were obtained from Aquatic BioSystems (ABS) in Fort Collins, CO. One lot (99-23) of 9- to 10-day old organisms was received on 10/12/99. At the time of test setup, 20 organisms were subsampled and preserved with 10% osmotically-balanced (sugar) formalin. Head capsule width of these organisms was measured using a calibrated ocular micrometer under an Olympus compound microscope. The mean head capsule width was 0.322 mm (range of 0.28 - 0.525 mm) (Appendix C). USEPA (1994) guidelines indicate that head capsule widths of second instar *C. tentans* range from 0.18 - 0.23 mm and mean head capsule widths of third instar *C. tentans* range from 0.33 - 0.45 mm. These data indicate that most of the test organisms were late second instar to early third instar chironomids, although one organism was probably a late third instar. The test organisms were transferred to moderately hard reconstituted laboratory water at the time of receipt (@1240) and held for approximately 2 hours at test temperature (23°C) until test initiation. No further acclimation was conducted.

5.0 QUALITY ASSURANCE

Acute reference toxicant tests were initiated from the lot of *C. tentans* obtained for this study. Sodium chloride was the reference toxicant with moderately hard water as the dilution water. Tests were 96 hours in length; 24-hour data were also collected, where possible, from the tests for use with ENSR's historical 24-hour reference toxicant database.

6.0 RESULTS

6.1 Biological Data

At test takedown it was discovered that four replicates contained no live organisms. Those replicates were Control B, MNS-99-04 H, MNS-99-05 H, and MNS-99-06 F. Since there were organisms in all other replicates and these four replicates were in a row after test chamber randomization (see data package in Appendix C for randomization chart), it was the study director's judgement that these test chambers were not seeded with test organisms at test initiation. These four test chambers were, therefore, not included in analysis of the data. In addition, 12 organisms were found in MNS-99-01 replicate H and 19 organisms were found in MNS-99-02 replicate E. It is likely that replicate E of MNS-99-02 was double seeded so it was assumed that 20 organisms were put into the test chamber at test initiation. The number of organisms placed into replicate H of MNS-99-01 is not known. However, due to the good survival of all other MNS-99-01 replicates (not less than 90% survival in any replicate) it was assumed by the study director that 12 organisms were placed in this chamber at test initiation. Both of these test chambers were included in the statistical analysis of the data.

Live organisms at the end of the test were all large, dark red, and appeared healthy. A few of the test chambers contained live pupae. These organisms were counted as alive but excluded from measurements of weight. There was an emerged adult in replicate H of sediment 06. This organism was also counted as alive but excluded from weight measurements.

Percent survival and growth of test organisms were determined after 10 days of exposure. Growth was measured both as AFDW and as dry weight. Raw test data are presented in Appendix C. Results are presented in the following table.

Treatment	Mean % Survival (± Standard Deviation)	Mean Dry Wt. Per Surviving Organism (mg) (± Standard Deviation)[a]	Mean AFDW Per Surviving Organism (mg) (± Standard Deviation)[b]
Control	71.4 ± 23.4	2.844 (± 0.503)	1.908 (± 0.402)
MNS-99-01	95.0 ± 5.3	2.525 (± 0.523)	1.925 (± 0.460)
MNS-99-02	89.4 ± 8.6	2.448 (± 0.457)	1.765 (± 0.348)
MNS-99-03	82.5 ± 14.9	2.424 (± 0.240)	1.711 (± 0.161)
MNS-99-04	82.9 ± 13.8	2.970 (± 0.421)	2.208 (± 0.319)
MNS-99-05	81.4 ± 9.0	2.559 (± 0.542)	1.819 (± 0.411)
MNS-99-06	81.4 ± 13.5	2.769 (± 0.423)	2.180 (± 0.360)

[a] Dried at 81°C for > 24 hours.
[b] Ashed at 550 ± 50°C for 2 hours.

6.2 Data Analysis

Significant differences were identified with Toxstat Version 3.4 (West, Inc. and Gulley 1994). Survival data were transformed using arcsine square root. Data normality was evaluated with the Chi-square goodness of fit test (α=0.01); homogeneity of variance was evaluated with Bartlett's test (α=0.01). All data were found to meet the requirements for parametric analysis; where necessary, data were analyzed using a T-test with Bonferroni adjustment (α=0.05).

Comparison of treatment survival to the control was completed by observation since control survival was less than in any of the treatments. Dry weight and AFDW were compared to the control using Toxstat. In addition, per the request of the study sponsor, survival, dry weight, and AFDW from sediments MNS-99-02 through MNS-99-06 were compared to sediment MNS-99-01 using Toxstat (Appendix D). Dry weight and AFDW in the test treatments were not significantly reduced relative to the control. Survival in sediment MNS-99-05 was significantly reduced relative to survival in sediment MNS-99-01. There was no significant reduction in dry weight or AFDW in sediments MNS-99-02, MNS-99-03, MNS-99-04, or MNS-99-06 relative to sediment MNS-99-01.

6.3 Physical and Chemical Data of the Overlying Water

Water quality parameters measured during the test are included in Appendix C. On day 1 dissolved oxygen (DO) concentrations in MNS-99-03 and MNS-99-04 fell to 2.4 and 2.5 mg/L, respectively. Aeration was initiated on day 1 in all test chambers and DO concentrations remained above 5.0 mg/L for the remainder of the study. Test temperature, as measured in the test solutions, remained at 23°C throughout the test. The pH of the test solutions ranged from 7.0 to 8.5. Ammonia was detectable in all treatments on day 0. Ammonia concentrations decreased after aeration was initiated. The following table summarizes physical and chemical data in each test treatment.

Treatment	pH Range (Units)	DO Range (mg/L)	Conductivity Range (µS/cm)	Temperature Range (°C)	Ammonia as N Range (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control	7.0 - 8.2	5.4 - 6.8	342 - 479	23	<1.0 - 1.2	76 - 152	47 - 61
MNS-99-01	7.3 - 8.2	4.5 - 6.6	329 - 341	23	<1.0 - 4.6	88 - 96	62 - 65
MNS-99-02	7.4 - 8.3	4.6 - 6.6	333 - 357	23	<1.0 - 4.9	102 - 104	70 - 76
MNS-99-03	7.3 - 8.4	2.4 - 6.6	391 - 420	23	<1.0 - 9.8	120 - 140	92 - 122
MNS-99-04	7.4 - 8.5	2.5 - 6.6	395 - 436	23	<1.0 - 8.0	116 - 156	106 - 133
MNS-99-05	7.4 - 8.5	3.5 - 6.6	397 - 432	23	<1.0 - 10.8	124 - 154	100 - 126
MNS-99-06	7.7 - 8.4	5.1 - 6.7	373 - 391	23	<1.0 - 1.3	114 - 130	84 - 98

6.4 Reference Toxicant Test Results

Three reference toxicant tests were conducted using *C. tentans* from lot 99-23. Twenty-four hour reference toxicant data from all three tests were included in ENSR's historical reference toxicant database. Two tests also had acceptable 96-hour data. Because previous reference toxicant tests conducted at the FCETL with this species were only 24 hours in duration and only three 96-hour tests have been conducted, insufficient data have been generated to calculate historical 95% control limits for the 96-hour endpoint. The *C. tentans* reference toxicant data sheets and 24-hour reference toxicant control chart are included in Appendix E.

Test Number (8503-109-820-)	Test Period	24-H LC_{50} (mg/L Cl^-)	ENSR FCETL Historical 95% Control Limits for 24-Hour Tests (mg/L Cl^-)		96-H LC_{50} (mg/L Cl^-)
			Low	High	
051	10/12-10/16/99	6,013	5,240	7,094	N/A[a]
054	10/18-10/22/99	5,540	5,107	7,047	3,655
055	10/18-10/22/99	5,169	4,860	7,068	3,914

[a] 96-hour data not acceptable for test 051 due to excessive control mortality at 96 hours.

7.0 PROTOCOL DEVIATIONS

On day 1 the dissolved oxygen level in replicate B of MNS-99-03 was 2.4 mg/L. Aeration was initiated on day 1 in all treatments and dissolved oxygen concentrations remained ≥2.5 mg/L for the remainder of the test.

At test takedown it was discovered that four test chambers contained no test organisms. It was assumed by the Study Director that no organisms were placed in these chambers at test setup. Two other test chambers received more than 10 organisms per chamber.

Ammonia was measured in each treatment on day 6 rather than day 7 as indicated in the test protocol.

Crucibles containing organisms from sediments 05 (replicates E through G) and 06 (replicates A through E) were ashed for 1 hour 55 minutes rather than for at least 2 hours as stated in the test protocol. Organisms from all other treatments/replicates were ashed for a 2- to 3-hour period.

Because of the large number of treatments and replicates, the Chi-square test was used to test for normality rather than Shapiro-Wilk's test.

Because of the failure to seed four of the test chambers at test initiation, there was an uneven number of replicates among treatments. As a result, statistical comparisons were made using a T-test with Bonferroni adjustment rather than analysis of variance with Dunnett's test. However, both of these are parametric tests and this difference in statistical analyses is not likely to have affected data interpretation. It is the Study Director's judgement that the remaining protocol deviations did not impact test outcome.

8.0 REFERENCES

ASTM. 1997. Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, Method E 1706-95b in *1997 Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides.* American Society of Testing and Materials.

USEPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th edition. EPA/600/4-90/027F. United States Environmental Protection Agency, Office of Research and Development.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024. United States Environmental Protection Agency, Office of Research and Development.

West, Inc. and D.D. Gulley. 1994. Toxstat version 3.4. Western EcoSystems Technology, Inc. Cheyenne, WY.

Appendix A

Chain of Custody

(Available upon Request from the MPCA)

Appendix B

Test Protocol

(Available upon Request from the MPCA)

Appendix C

Test Data

(Available upon Request from the MPCA)

Appendix D

Statistical Analysis Comparing Sediments 02 - 06 to Sediment 01

(Available upon Request from the MPCA)

Appendix E

Reference Toxicant Data and Control Chart

(Available upon Request from the MPCA)

APPENDIX B

TOXICITY OF SEDIMENTS COLLECTED FROM MINNESOTA SLIP TO THE AMPHIPOD, *Hyalella azteca*

Toxicity of Sediments Collected from Minnesota Slip to the Amphipod, *Hyalella azteca*

Author
David A. Pillard

Study Period
First Tests Started: 13 October 1999
Last Tests Ended: 8 December 1999

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STATEMENT OF PROCEDURAL COMPLIANCE

I certify that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge, accurate and complete.

D. A. Pillard
David A Pillard, Ph.D.
Study Director

25 Feb 2000
Date

STATEMENT OF QUALITY ASSURANCE

The test data were reviewed by the Quality Assurance unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

Anita Rehner
Quality Assurance Unit

February 25, 2000
Date

EXECUTIVE SUMMARY

Six sediment samples, collected from Minnesota Slip in the Duluth Harbor Basin, were received by the Fort Collins Environmental Toxicology Laboratory (FCETL) on October 5, 1999. Forty-two-day toxicity tests were conducted with these samples using the amphipod, *Hyalella azteca*. The test endpoints were survival, reproduction, and growth (as measured by dry weight per surviving organism). The results of these tests indicated that sediments MNS-99-03 and MNS-99-05 caused significant reductions in amphipod survival. In the sediments that did not cause significant reductions in survival, there were no significant sublethal effects to either reproduction or growth, when compared to the test controls.

The following table summarizes the study:

STUDY SUMMARY

Sponsor	Minnesota Pollution Control Agency 520 Lafayette Road N. St. Paul, MN 55155-4194
Project Officer	Judy L. Crane, Ph.D. (651) 297-4068
Study Director	David A. Pillard, Ph.D. (970) 416-0916
Test Facility	ENSR Fort Collins Environmental Toxicology Laboratory (FCETL) 4303 West LaPorte Avenue Fort Collins, Colorado 80521
Location of Data	Data Records and Storage 328 Link Lane #4 Fort Collins, Colorado 80524
Test Substances	Sediments: MNS-99-01, MNS-99-02, MNS-99-03, MNS-99-04, MNS-99-05, MNS-99-06
Test Dates	13 October - 24 November 1999 (MNS-99-01 to MNS-99-03) 27 October - 8 December 1999 (MNS-99-04 to MNS-99-06)
Length of Tests	42 days
Test Species	*Hyalella azteca*
Source of Organisms	Environmental Consulting and Testing
Age of Test Organisms	7 days (test beginning 13 October) and 8 days (test beginning 27 October)
Test Concentrations	Bulk (100%) sediment

Overlying Water	Moderately hard reconstituted water augmented with ~50mg Cl^-/L
Results	Significant (α=0.05) reduction in survival in sediments MNS-99-03 and MNS-99-05.

1.0 INTRODUCTION

Minnesota Slip is located in the northern section of the Duluth Harbor basin in Duluth, MN. Because of concerns regarding sediment contamination in the St. Louis River Area of Concern, several investigations have been conducted of Minnesota Slip and nearby contaminated areas. The purpose of this study was to determine if sediments collected from Minnesota Slip were toxic to *Hyalella azteca*. These toxicity tests were part of a sediment remediation scoping project in Minnesota Slip. The study sponsor was the Minnesota Pollution Control Agency (MPCA); the testing laboratory was the ENSR Fort Collins Environmental Toxicology Laboratory (FCETL).

2.0 DESCRIPTION OF TEST AND CONTROL SUBSTANCES

2.1 Test Sediment

Six sediment samples were received on 5 October 1999. Each sample was a composite of the upper 5 cm layer of sediment. Sample containers were 4-liter high-density polyethylene, wide-mouth jars. Sample collection and receipt information is presented in the following table. See Appendix A for chain of custody records.

Sample Name	Collection Date and Time	FCETL Sample # and Date of Receipt	Temp at Arrival (°C)	COC Number	COC Tape Number
MNS-99-01	9/22/99 @ 0950-1030	12809 10/5/99	6	34570	5815
MNS-99-02	9/22/99 @ 1115-1140	12810 10/5/99	6	34570	5815
MNS-99-03	9/22/99 @ 1345-1410	12811 10/5/99	6	34570	5815
MNS-99-04	9/22/99 @ 1505-1535	12812 10/5/99	6	34571	5816
MNS-99-05	9/22/99 @ 1620-1642	12813 10/5/99	6	34571	5816
MNS-99-06	9/22/95 @ 1715-1738	12814 10/5/99	6	34571	5816

Sediment samples were stored at 4°C in the dark until test setup. Sediment was homogenized prior to use. Homogenization consisted of transferring sediment to a clean Nalgene® container and thoroughly mixing the sediment with a stainless steel auger attached to an electric drill for not less than three minutes. The auger and container were thoroughly cleaned and decontaminated (soap, tap water rinse, acetone, tap water rinse, HCl, tap water rinse, DI rinse) between homogenization of each sediment.

2.2 Control Sediment

A control sediment (Florissant reference soil obtained from USGS-Biological Resources Division in Columbia, Missouri) was tested concurrently. Because the 42-day reproduction study is new and little historical data exist, the Study Director decided to include 6 control sediment treatments, one with each test sediment.

2.3 Overlying Water

The overlying test water was moderately hard reconstituted water (USEPA 1993) that was augmented with 50 mg/L of Cl^-, added as NaCl. Previous experience with *H. azteca* has shown that addition of chloride to the overlying water can significantly improve organism health and control performance. Because of the long length of the tests, several batches of water were prepared over the duration of these studies. Initial characterization of the Cl^--enhanced moderately hard water batches was:

Batch #	Date Measured	Cl^- (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (µS/cm)	pH	Ammonia (mg/L N)	TRC[a] (mg/L)
3640	10/12/99	48	98	64	492	8.2	NM[b]	NM
3645	10/18/99	48	94	64	496	8.1	<1.0	<0.05
3654	10/24/99	NM	94	65	490	8.4	<1.0	<0.05
3657	10/25/99	49	98	63	497	8.1	<1.0	<0.05
3664	10/28/99	50	98	64	499	8.2	<1.0	<0.05
3665	10/31/99	49	100	66	495	8.1	<1.0	<0.05
3668	11/3/99	48	96	63	485	8.1	<1.0	<0.05
3672	11/5/99	49	96	63	496	8.2	<1.0	<0.05
3674	11/9/99	48	96	65	500	8.2	<1.0	<0.05
3679	11/11/99	49	98	62	505	8.2	<1.0	NM
3680	11/15/99	49	98	65	504	8.1	NM	<0.05
3687	11/18/99	50	98	65	506	8.1	<1.0	<0.05
3691	11/21/99	49	96	66	498	8.2	NM	<0.05
3696	11/26/99	49	98	66	506	8.1	<1.0	<0.05
3703	12/3/99	48	96	63	500	8.1	<1.0	NM

[a] TRC = total residual chlorine
[b] NM = not measured

3.0 TEST CONDITIONS

3.1 Test Methods

The studies were 42-day static-renewal toxicity tests using *Hyalella azteca*. Methods for this test have not yet been published, but are available in the draft USEPA and ASTM guidelines for conducting sediment toxicity tests (personal communication with Drs. David Mount and Chris Ingersoll). The biological responses measured were death (defined as no visible movement or any response to gentle prodding with a blunt probe), reproduction (number of young produced per female) and growth (mean dry weight per surviving organism).

On days 0 through 28 the organisms were exposed to the test sediments with overlying water. On day 28 all organisms were removed from the test chambers. Live organisms were counted and organisms from replicates A through D were removed for determination of dry weight. Organisms from replicates E through L were placed back in test chambers that contained overlying water only (and a small piece of Nitex netting). On day 35 the number of surviving adults and young were counted; young were removed from the test chambers. On day 42 the number of surviving adults and young were counted and the surviving adults were removed for determination of dry weight and identification of males and females. Adult *H. azteca* from each replicate were placed in 10% sugar formalin. Under a compound microscope males were identified by the enlarged second gnathopod (Figure 1). Organisms were then rinsed with deionized water, dried at 60-90°C, and weighed. Reproduction for each replicate was determined by summing the number of young produced on days 35 and 42 and dividing by the number of females in the test chamber at test termination (see Figure 2).

The complete test protocol is included as Appendix B.

3.2 Test Duration

Test duration was 42 days (28 days of sediment exposure and 14 days of water-only exposure).

3.3 Test Apparatus

Test chambers were 300- or 500-ml glass beakers containing 100 ml of test sediment and 175 ml of overlying water. On the day prior to test initiation, sediment was homogenized and placed into the test chambers. Overlying water was added and the resulting mixtures were allowed to settle overnight. At test initiation, ten organisms were impartially distributed to 30-ml plastic cups containing 15 ml of overlying water. The number of amphipods in each cup was verified by laboratory technicians. Amphipods were then introduced into a test chamber by gently submerging the cup and swirling it in the water until all of the amphipods swam out. Twelve replicates were tested per treatment for a total of 120 organisms per treatment. All test chambers were held in a temperature-controlled water bath under fluorescent lighting with a photoperiod of 16 hours light:8 hours dark; target test temperature was 23 ± 1°C.

3.4 Feeding

Test organisms were fed 1.5 ml of a yeast-trout chow-cereal leaf suspension per test chamber on a daily basis.

3.5 Aeration

Previous testing with *Chironomus tentans* under the same test conditions indicated that dissolved oxygen in some of the test sediments might drop below 2.5 mg/L. In addition, dissolved oxygen concentrations in some of the chambers with test sediments was low (<4.5 mg/L) on day 0 (after the overnight settling period) (see Appendix C). Aeration was, therefore, initiated in all test chambers, including the control, on day 0 after organisms had been added to the test chambers. The aeration apparatus consisted of a Pasteur pipette (connected to the laboratory air supply), the tip of which was positioned so as not to disturb the sediment. Aeration was continued through day 28; test chambers were not aerated from days 28 through 42 during the water-only exposure.

4.0 TEST ORGANISMS

Hyalella azteca were obtained from Environmental Consulting and Testing in Superior, WI. Since two batches of tests were set up, two lots were used. Lot 99-22 was received on 10/12/99; organisms were 6 days old at the time of receipt and 7 days old at test set up. Lot 99-27 was received on 10/26/99; organisms were 7 days old at the time of receipt and 8 days old at test set up. The test organisms were transferred to moderately hard reconstituted laboratory water at the time of receipt and held at test temperature (23°C) until test initiation. No further acclimation was conducted.

At the time of test setup, 51 organisms from lot 99-22 and 55 organisms from lot 99-27 were subsampled and preserved with 10% osmotically-balanced (sugar) formalin. These organisms were used to determine the initial dry weight of the test organisms. The mean dry weight of *H. azteca* from lot 99-22 was 0.011 mg (Appendix C) and the mean dry weight of *H. azteca* from lot 99-27 was 0.052 mg (Appendix D).

5.0 QUALITY ASSURANCE

Acute reference toxicant tests were initiated using the lots of *H. azteca* obtained for this study. Sodium chloride was the reference toxicant with moderately hard water as the dilution water. Tests were 96 hours in length; 24-hour data were also collected for use with ENSR's historical 24-hour reference toxicant database.

6.0 RESULTS

6.1 Biological Data

Percent survival and growth of test organisms were determined after 28 days of sediment exposure. Survival and number of young produced on day 35 was determined. Survival, growth and reproduction at test termination (day 42) were also measured. Raw test data (except day 42 dry weight) are presented in Appendices C (sediments MNS-99-01 - MNS-99-03) and D (sediments MNS-99-04 - MNS-99-06). Survival results are presented in the following table.

Treatment	28-day Mean % Survival (± Standard Deviation)[a]	35-day Mean % Survival (± Standard Deviation)[a]	42-day Mean % Survival (± Standard Deviation)[a]
Control 1	84.2 (± 15.6)	80.0 (± 20.0)	77.5 (± 18.3)
Control 2	85.0 (± 2.4)	83.7 (± 14.1)	83.7 (± 14.1)
Control 3	88.3 (± 20.4)	93.8 (± 7.4)	91.3 (± 8.3)
Combined Controls 1-3	85.8 (± 16.1)	85.8 (± 15.3)	84.2 (± 14.7)
MNS-99-01	93.3 (± 8.9)	92.5 (± 8.9)	90.0 (± 7.6)
MNS-99-02	85.8 (± 10.0)	80.0 (± 12.0)	80.0 (± 12.0)
MNS-99-03	19.2 (± 18.3)	13.8 (± 13.0)	13.8 (± 13.0)
Control 4	77.5 (± 16.6)	65.0 (± 30.7)	63.8 (± 29.2)
Control 5	70.0 (± 16.5)	66.2 (± 22.6)	65.0 (± 21.4)
Control 6	80.8 (± 17.8)	78.7 (± 18.1)	75.0 (± 17.7)
MNS-99-04	69.2 (± 16.8)	72.5 (± 18.3)	71.3 (± 17.3)
MNS-99-05	24.2 (± 21.5)	25.0 (± 22.7)	25.0 (± 22.7)
MNS-99-06	75.0 (± 15.7)	77.5 (± 7.1)	77.5 (± 7.1)

[a] Day 28 mean survival is based on all 12 replicates; Days 35 and 42 survival are based on the remaining eight replicates.

Data sheets with initial organism weight and day 28 dry weight are included in Appendices C and D. Day 42 dry weight data and statistical analysis are included as Appendix E. Dry weight (per surviving organism) results are presented in the following table.

Treatment	28-day Mean Dry Weight (mg) (± Standard Deviation)	42-day Mean Dry Weight (mg) (± Standard Deviation)
Control 1	0.285 (± 0.062)	0.300 (± 0.048)
Control 2	0.332 (± 0.080)	0.296 (± 0.026)
Control 3	0.318 (± 0.034)	0.312 (± 0.40)
Combined Controls 1-3	0.312 (± 0.060)	0.303 (± 0.038)
MNS-99-01	0.298 (± 0.053)	0.322 (± 0.087)
MNS-99-02	0.456 (± 0.226)	0.365 (± 0.034)
MNS-99-03	0.443 (± 0.097)	0.392 (± 0.131)
Control 4	0.364 (± 0.087)	0.374 (± 0.077)
Control 5	0.440 (± 0.262)	0.320 (± 0.044)
Control 6	0.364 (± 0.071)	0.316 (± 0.087)
MNS-99-04	0.527 (± 0.192)	0.516 (± 0.079)
MNS-99-05	0.572 (± 0.332)	0.537 (± 0.086)
MNS-99-06	0.419 (± 0.187)	0.536 (± 0.030)

Reproduction data are included in Appendices C and D. Reproduction results are presented in the following table:

Treatment	Total Number of Females Alive at Test Termination	Mean Reproduction (young/surviving female) (± Standard Deviation)
Control 1	31	1.16 (± 0.70)
Control 2	40	1.50 (± 1.66)
Control 3	42	2.11 (± 1.43)
Combined Controls 1-3	113	1.59 (± 1.33)
MNS-99-01	41	2.32 (± 1.30)
MNS-99-02	36	2.55 (± 1.11)
MNS-99-03	7	0.42 (± 0.80)
Control 4	19	2.77 (± 2.61)
Control 5	31	1.65 (± 1.65)
Control 6	38	0.80 (± 1.02)
MNS-99-04	28	2.27 (± 1.86)
MNS-99-05	13	1.30 (± 1.99)
MNS-99-06	34	3.73 (± 2.76)

6.2 Data Analysis

Controls 1, 2, and 3 all met the acceptability criterion of 80% survival on day 28. All endpoints (survival, reproduction and dry weight) for these treatments were statistically compared and were not found to be significantly different (Appendix F). Therefore, data from all three of these controls were combined and used as the control for comparison to MNS-99-01, MNS-99-02, and MNS-99-03.

Controls 4 and 5 had less than 80% survival on day 28. These controls were not used for comparison to treatments MNS-99-04, MNS-99-05, and MNS-99-06; only control 6 was used.

Significant differences were identified with Toxstat Version 3.4 (West, Inc. and Gulley 1994). Survival data were transformed using arcsine square root. Normality was evaluated with the Chi-Square or Shapiro-Wilks test (α=0.01); homogeneity of variance was evaluated with Bartlett's test (α=0.01). Where data met the requirements for parametric analysis; data were analyzed using analysis of variance with Dunnett's test or (for unequal replicates) a T-test with Bonferroni adjustment (α=0.05). Where parametric requirements were not met, data were analyzed with Steel's Many-One Rank test.

Survival in MNS-99-03 and MNS-99-05 were significantly lower than in the respective controls. Because survival was significantly lower in these two sediments, they were excluded from analysis of sublethal endpoints.

Neither reproduction nor dry weight of any of the remaining test treatments was significantly less than in the respective controls. Computer printouts from Toxstat 3.4 of the statistical analysis of survival, reproduction, and day 28 dry weight are presented in Appendices G (MNS-99-01 - MNS-99-03) and H (MNS-99-04 - MNS-99-06).

6.3 Physical and Chemical Data of the Overlying Water

Water quality parameters measured during the test are included in Appendices C and D. Aeration was initiated on day 0 in all test chambers. On day 0 (before organisms were added and aeration initiated) dissolved oxygen concentrations in sediments MNS-99-01, MNS-99-02, and MNS-99-03 were ≤4.2 mg/L. All other dissolved oxygen measurements were ≥5.0 in all treatments for the remainder of the test. Aeration was terminated on day 28 at the end of the sediment exposure. Test chambers were not aerated during the water-only exposure phase of the test and dissolved oxygen concentrations remained greater than 5.3 mg/L for the remainder of the test. On day 11, test temperature in Controls 1, 2, and 3 and sediments MNS-99-01, MNS-99-02, and MNS-99-03 was 17°C. This decrease in temperature was due to a malfunctioning refrigeration/heating unit (Remcor™). The unit was repaired and test temperatures remained between 22 and 24°C for the remainder of the test. The pH of the test solutions ranged from 7.3 to 8.6. Ammonia was detectable in all test sediments, except MNS-99-06, on day 0. Ammonia concentrations decreased after aeration was initiated. The highest ammonia concentration (38 mg/L) was measured on day 0 in MNS-99-03. Because the test included a “with-sediment” period (days 0-28) and a “without-sediment” period (days 28-42), water quality data are presented accordingly in the following tables.

Water Quality Data from Days 0-28

Treatment	pH Range (Units)	DO Range (mg/L)	Conductivity Range (µS/cm)	Temperature Range (°C)	Ammonia as N Range (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control 1	7.3 - 8.3	5.6 - 7.5	515 - 655	17 - 23	<1.0	100 - 176	47 - 64
Control 2	7.3 - 8.4	5.5 - 7.5	522 - 675	17 - 24	<1.0	98 - 174	43 - 63
Control 3	7.3 - 8.4	5.3 - 7.6	518 - 664	17 - 23	<1.0	102 - 174	43 - 68
MNS-99-01	7.6 - 8.4	4.2 - 7.2	478 - 540	17 - 23	<1.0 - 1.9	102 - 106	61 - 74
MNS-99-02	7.5 - 8.5	4.2 - 7.4	478 - 565	17 - 23	<1.0 - 1.9	102 - 114	64 - 86
MNS-99-03	7.7 - 8.5	3.4 - 7.4	560 - 631	17 - 23	<1.0 - 38	148 - 154	109 - 120
Control 4	7.6 - 8.3	6.0 - 7.3	486 - 605	22 - 24	<1.0	110 - 138	48 - 64
Control 5	7.5 - 8.4	6.0 - 7.2	517 - 581	22 - 23	<1.0	98 - 130	50 - 66
Control 6	7.5 - 8.4	6.1 - 7.2	509 - 588	22 - 24	<1.0	96 - 146	67 - 105

Treatment	pH Range (Units)	DO Range (mg/L)	Conductivity Range (µS/cm)	Temperature Range (°C)	Ammonia as N Range (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
MNS-99-04	7.8 - 8.6	5.5 - 7.2	551 - 598	22 - 24	<1.0 - 3.4	128 - 130	107 - 108
MNS-99-05	7.7 - 8.6	5.0 - 7.1	566 - 585	22 - 23	<1.0 - 4.7	128 - 134	102 - 116
MNS-99-06	8.0 - 8.5	5.5 - 7.4	539 - 559	22 - 23	<1.0	114 - 124	80 - 88

Water Quality Data from Days 29-42

Treatment	pH Range (Units)	DO Range (mg/L)	Conductivity Range (µS/cm)	Temperature Range (°C)	Ammonia as N Range (mg/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control 1	7.9 - 8.4	5.5 - 7.0	511 - 532	23	<1.0	92 - 96	65 - 68
Control 2	7.9 - 8.4	5.3 - 6.9	510 - 534	23 - 24	<1.0	96 - 98	63 - 69
Control 3	7.9 - 8.3	5.4 - 6.9	505 - 525	23 - 24	<1.0	92 - 94	64 - 68
MNS-99-01	7.9 - 8.3	5.4 - 6.8	504 - 526	23	<1.0	94	63 - 68
MNS-99-02	7.9 - 8.4	5.3 - 6.9	516 - 530	23	<1.0	94 - 96	63 - 69
MNS-99-03	7.9 - 8.3	5.3 - 6.8	514 - 594	23	<1.0	98	63 - 66
Control 4	7.7 - 8.2	5.8 - 7.0	517 - 535	22 - 23	<1.0	94 - 108	66 - 67
Control 5	7.8 - 8.2	5.5 - 6.8	514 - 533	22 - 23	<1.0	90 - 102	66 - 67
Control 6	7.8 - 8.2	5.3 - 6.8	514 - 525	22 - 23	<1.0	94 - 96	66 - 67
MNS-99-04	7.8 - 8.2	5.3 - 6.8	512 - 527	22 - 23	<1.0	96 - 106	64 - 66
MNS-99-05	7.8 - 8.2	5.5 - 6.8	510 - 516	22 - 23	<1.0	92 - 104	65 - 67
MNS-99-06	7.9 - 8.2	5.6 - 6.8	512 - 518	22 - 23	<1.0	90 - 96	64 - 68

6.4 Reference Toxicant Test Results

Two reference toxicant tests were conducted using *H. azteca* from lot 99-22 and one reference toxicant test was conducted with organisms from lot 99-27. Twenty-four hour reference toxicant data from all three tests were included in ENSR's historical reference toxicant database. All three tests also had acceptable 96-hour data. Because previous reference toxicant tests were conducted at the FCETL with this species were only 24 hours in duration and only three 96-hour tests have been conducted, insufficient data have been generated to calculate reliable historical 95% control limits for the 96-hour endpoint. The *H. azteca* reference toxicant data sheets and 24-hour reference toxicant control chart are included in Appendix I.

Test Number (8503-109-820-)	Lot of *H. azteca*	Test Period	24-H LC_{50} (mg/L Cl^-)	ENSR FCETL Historical 95% Control Limits for 24-Hour Tests (mg/L Cl^-)		96-H LC_{50} (mg/L Cl^-)
				Low	High	
052	99-22	10/13-10/17/99	2,969	1,909	3,781	1,563
053	99-22	10/13-10/17/99	2,770	1,901	3,772	1,459
062	99-27	10/27-10/31/99	2,770	1,893	3,759	1,648

7.0 PROTOCOL DEVIATIONS

On day 11, the temperature of the overlying water in the test chambers (Controls 1, 2 and 3 and sediments MNS-99-01, MNS-99-02, and MNS-99-03) was 17°C due to a malfunctioning heating/cooling unit. The device was repaired and temperatures remained between 22 and 24°C for the remainder of the test. It was the Study Director's judgement that this deviation had no impact on the test.

From days 28-42 the test chambers contained 300 to 350 ml of water rather than 150 to 275 ml of water, as stated in the protocol. The reason for this difference is that since sediment had been removed on day 28, a larger volume of water was needed to raise the water level to the Nitex screen on the side of the beaker through which water drained when the test chambers were renewed. This deviation had no impact on the test.

Day 28 survival of Controls 04 and 05 was less than the acceptable level of 80%. These controls were dropped from the analysis.

8.0 REFERENCES

USEPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 4th edition. EPA/600/4-90/027F. United States Environmental Protection Agency, Office of Research and Development.

West, Inc. and D.D. Gulley. 1994. Toxstat version 3.4. Western EcoSystems Technology, Inc. Cheyenne, WY.

Appendix A

Chain of Custody

(Available upon Request from the MPCA)

Appendix B

Test Protocol

(Available upon Request from the MPCA)

Appendix C

Test Data for Sediments MNS-99-01, MNS-99-02, and MNS-99-03

(Available upon Request from the MPCA)

Appendix D

Test Data for Sediments MNS-99-04, MNS-99-05, and MNS-99-06

(Available upon Request from the MPCA)

Appendix E

Day 42 Dry Weight Data and Statistical Analysis

(Available upon Request from the MPCA)

Appendix F

Statistical Comparison of Controls 01, 02, and 03

(Available upon Request from the MPCA)

Appendix G

Statistics for Sediments MNS-99-01, MNS-99-02, and MNS-99-03

(Available upon Request from the MPCA)

Appendix H

Statistics for Sediments MNS-99-04, MNS-99-05, and MNS-99-06

(Available upon Request from the MPCA)

Appendix I

Reference Toxicant Data and Control Chart

(Available upon Request from the MPCA)

APPENDIX C

HISTORICAL DISTRIBUTION OF TOTAL PAHs, MERCURY, LEAD, AND ZINC AT MNS-99-04R AND MNS-99-13R

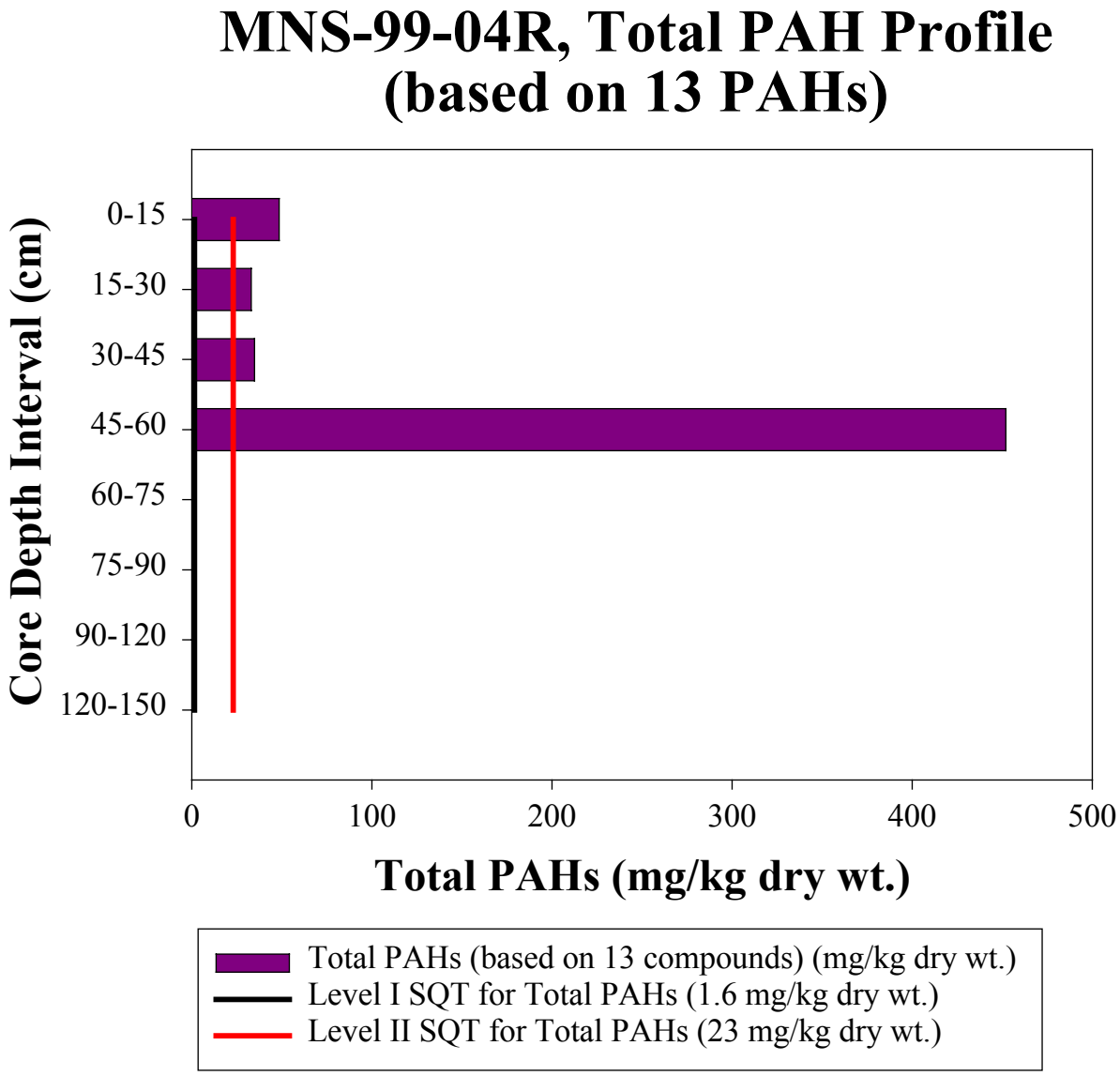

Figure C-1. Historical distribution of total PAHs (mg/kg dry wt.) at site MNS-99-04R.

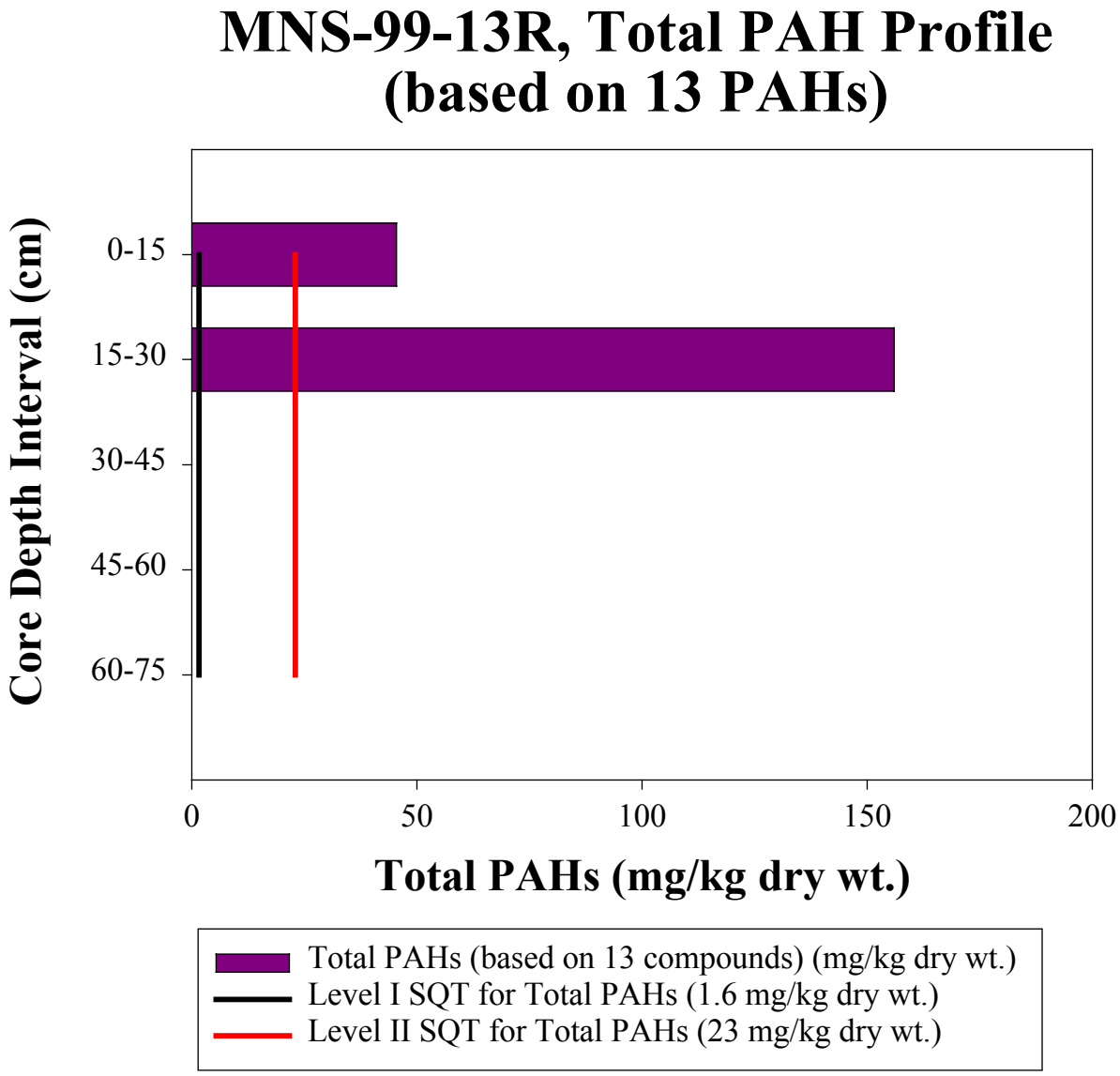

Figure C-2. Historical distribution of total PAHs (mg/kg dry wt.) at site MNS-99-13R.

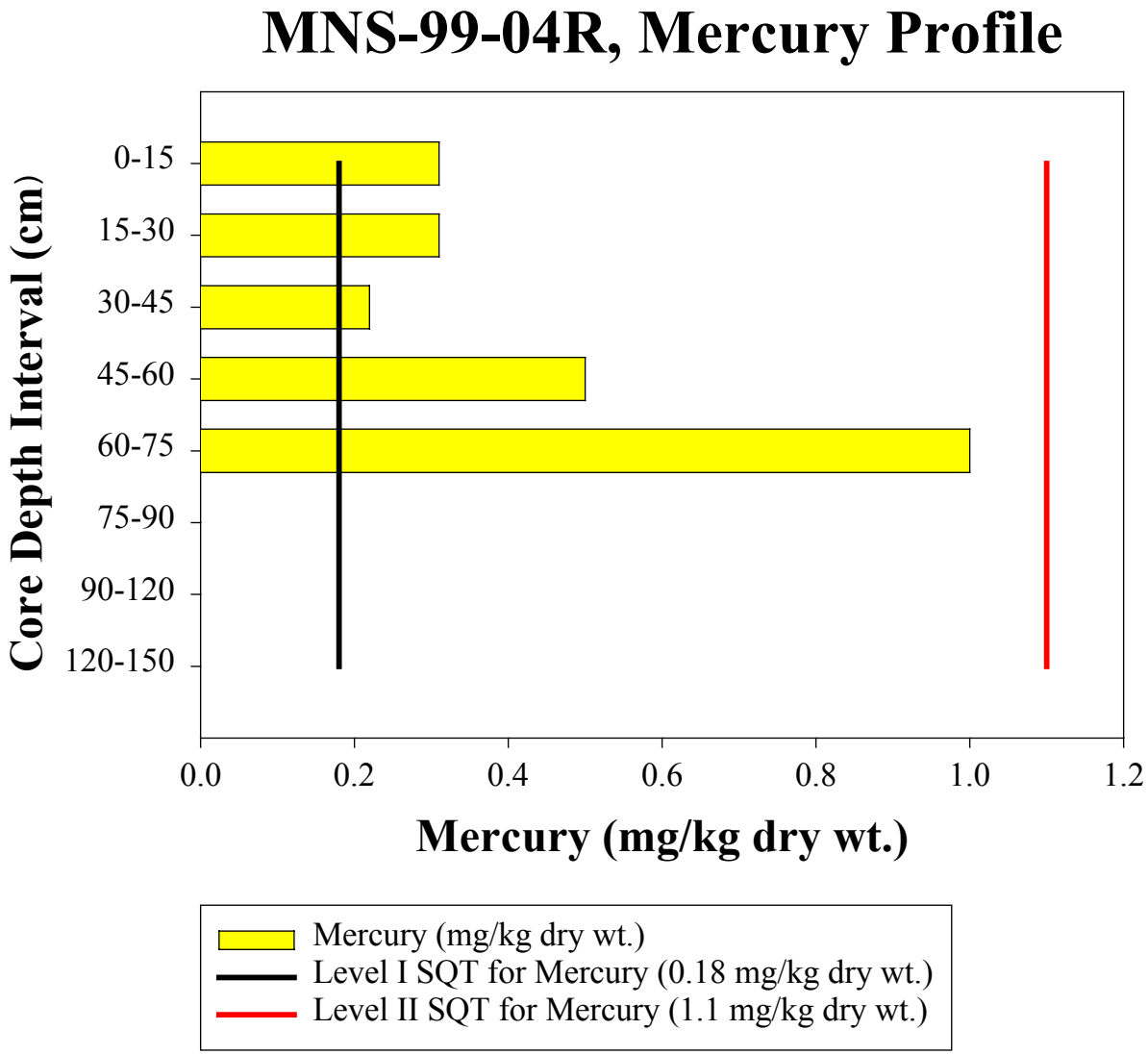

Figure C-3. Historical distribution of mercury (mg/kg dry wt.) at site MNS-99-04R.

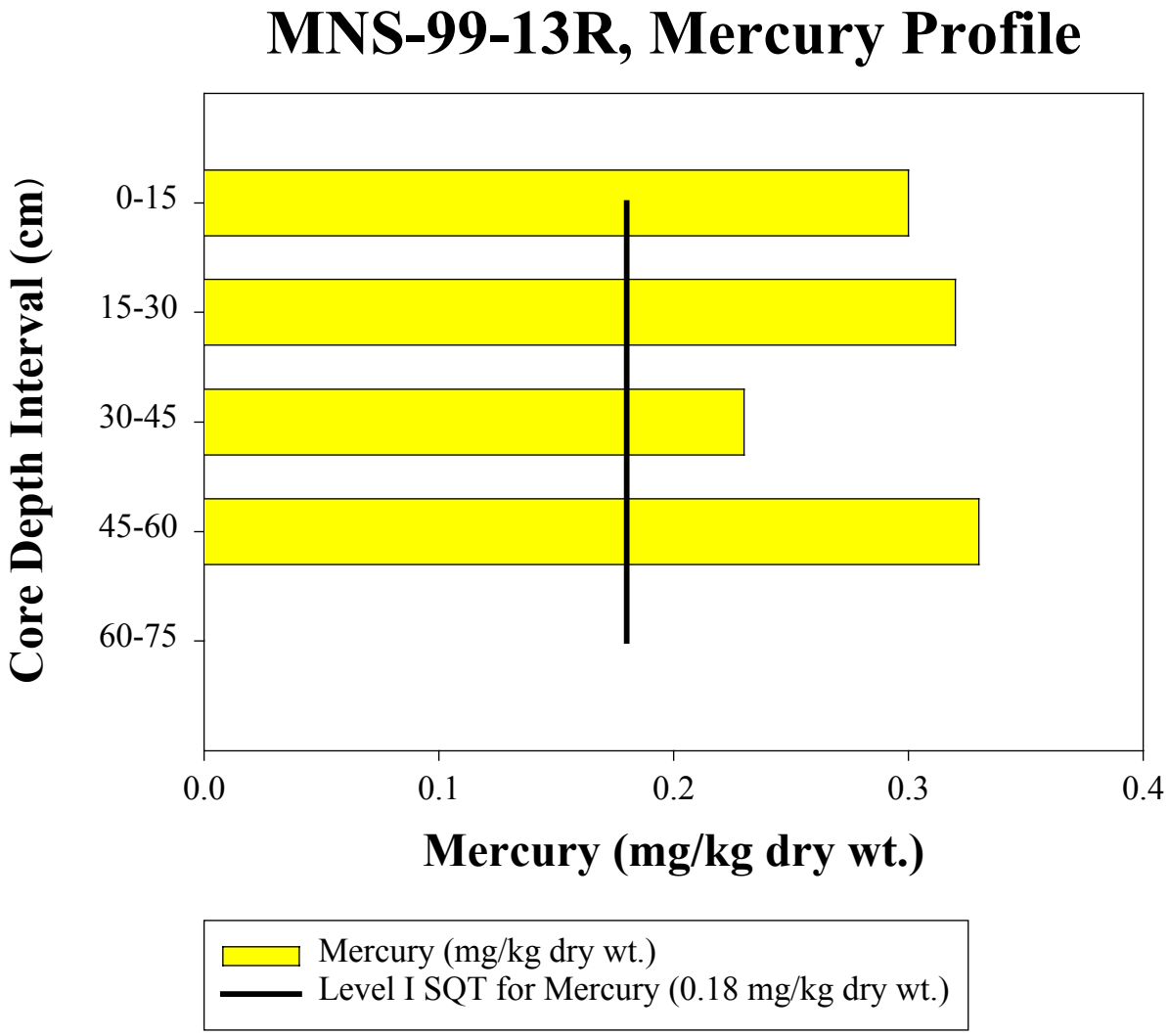

Figure C-4. Historical distribution of mercury (mg/kg dry wt.) at site MNS-99-13R.

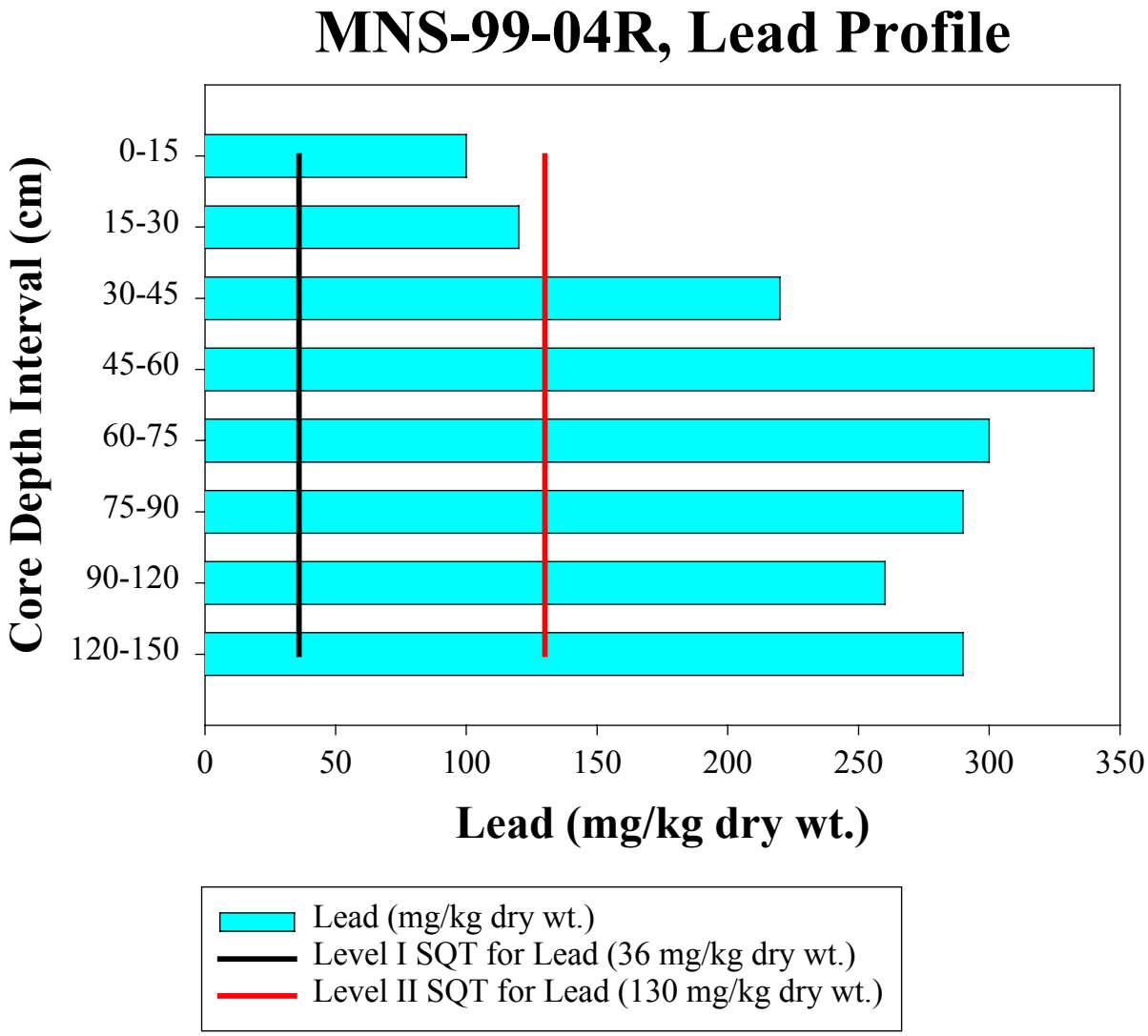

Figure C-5. Historical distribution of lead (mg/kg dry wt.) at site MNS-99-04R.

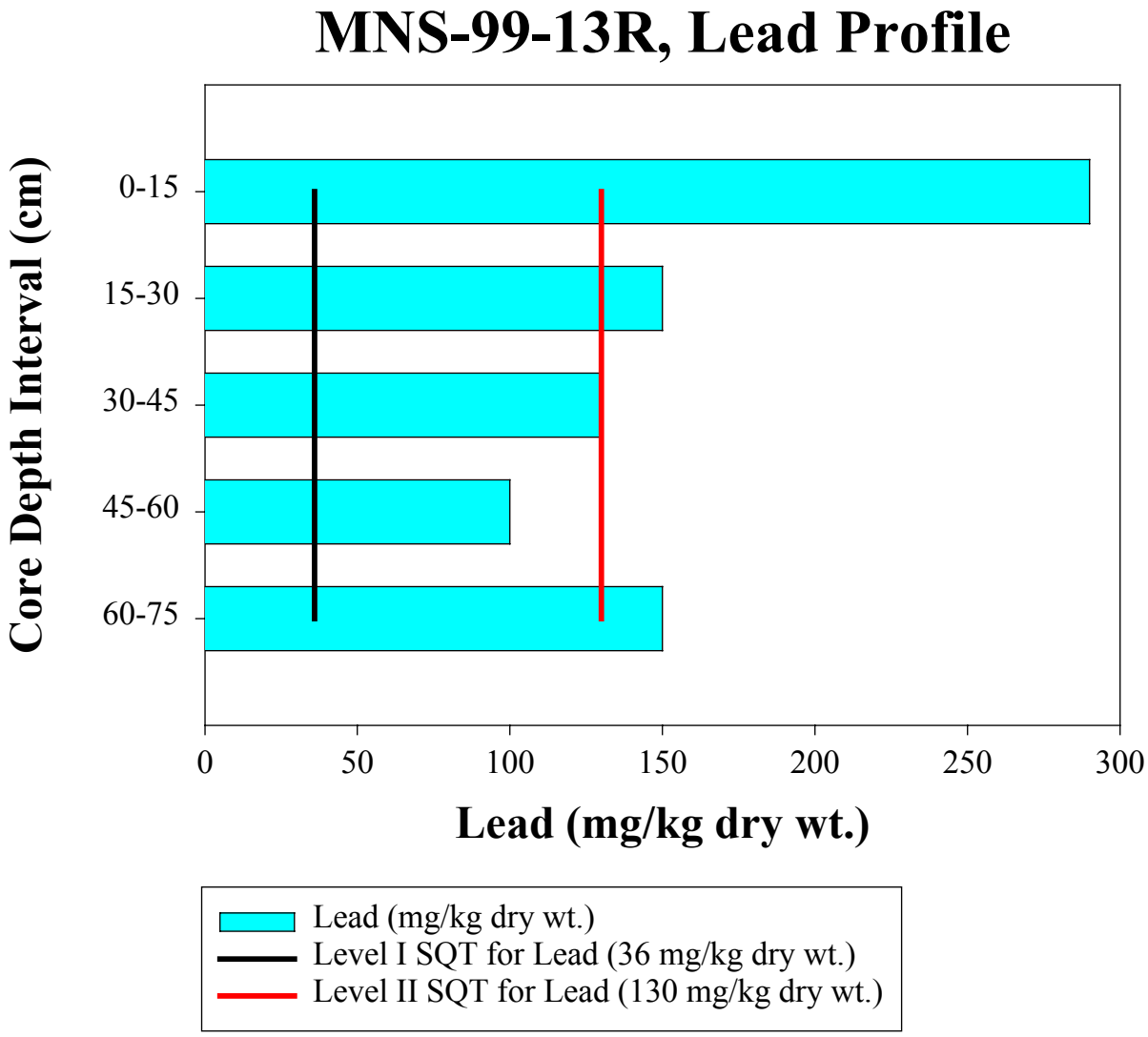

Figure C-6. Historical distribution of lead (mg/kg dry wt.) at site MNS-99-13R.

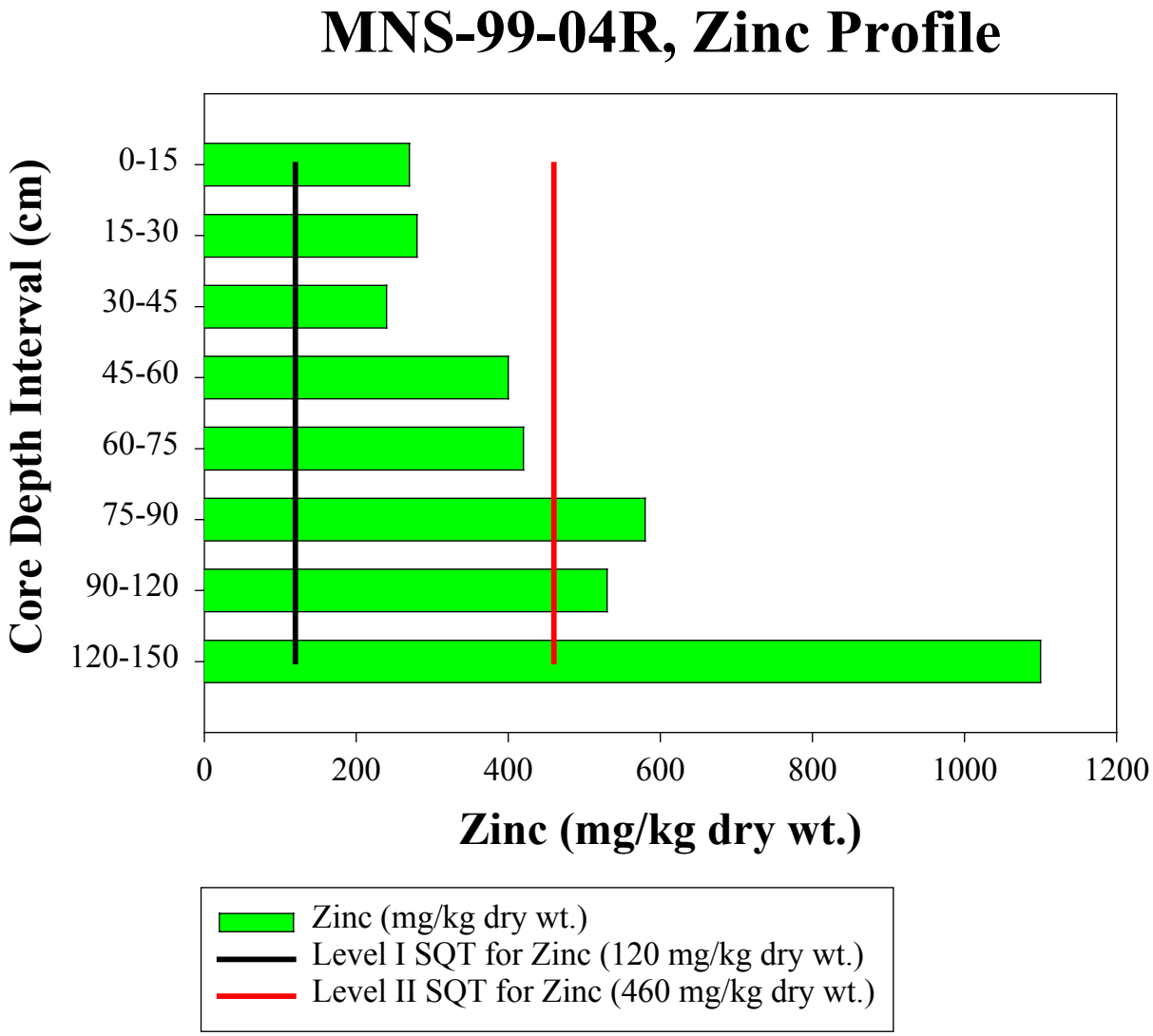

Figure C-7. Historical distribution of zinc (mg/kg dry wt.) at site MNS-99-04R.

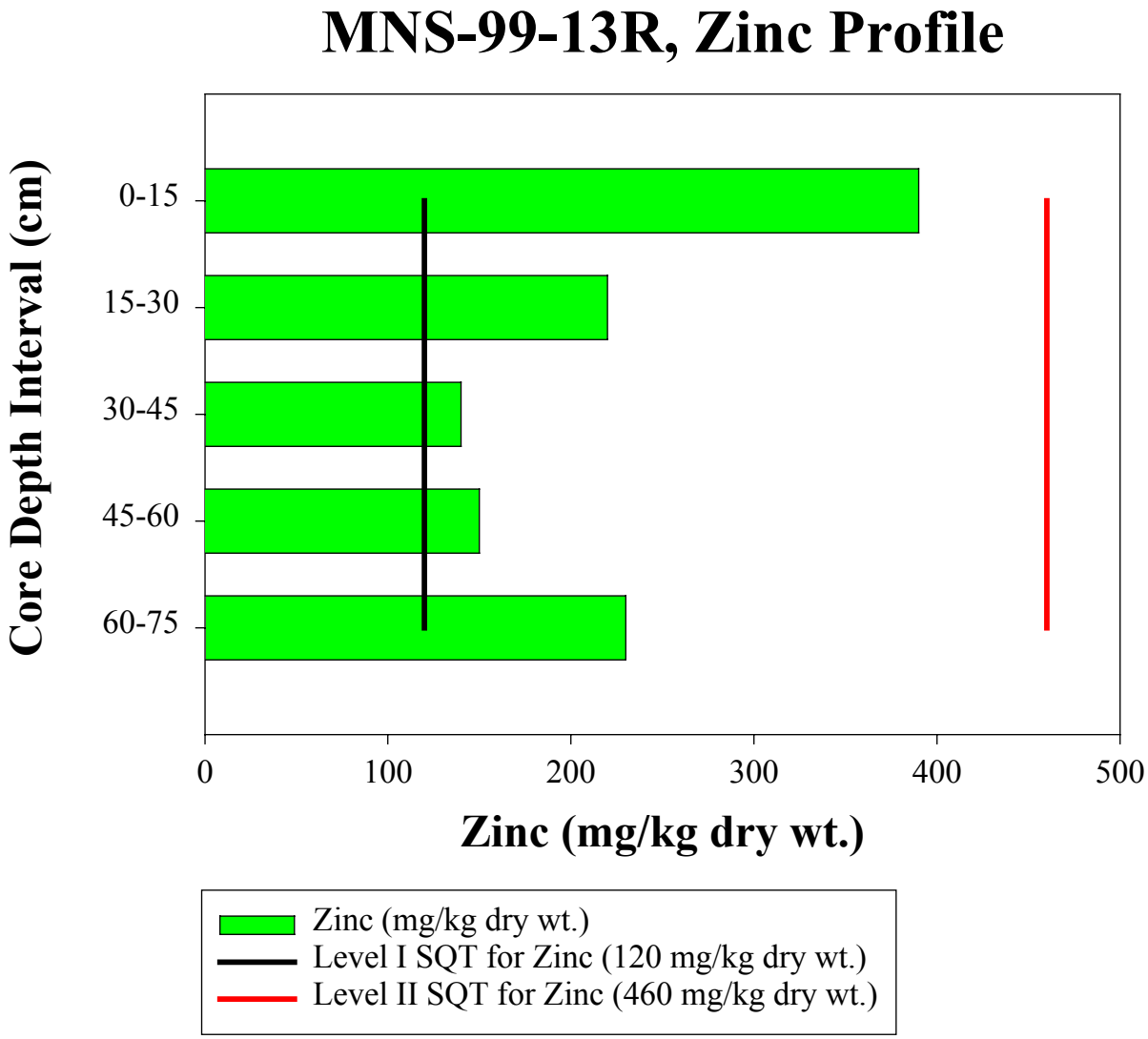

Figure C-8. Historical distribution of zinc (mg/kg dry wt.) at site MNS-99-13R.