Technical Memorandum No. 3:

CHIRONOMID HEAD CAPSULE DEFORMITIES

CHICAGO AREA WATERWAY SYSTEM

HABITAT RESTORATION EVALUATION AND IMPROVEMENT STUDY

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For

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In support of

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Summary and Conclusion

A seven-year macroinvertebrate database was developed by the Metropolitan Water Reclamation District of Greater Chicago (District). The database includes the percent of head capsule deformities of larvae of the Chironomidae family (midges) of Dipera insects. Deformities in midge larvae head capsules have been frequently observed in contaminated sediments. Deformity is generally considered to be a sublethal, teratogenic response to contamination. Herein we summarize the data on chironomid larvae head capsule deformities at sampling stations throughout the Chicago Area Waterway System (CAWS).

Across all 177 samples of midge larvae head capsules that were examined, 10.9% were deformed (±2.8%). Mean rates of head capsule deformities ranged from none at Ambient Water Quality Monitoring (AWQM) Station 55 (Calumet River at 130th Street) to 30.2% at AWQM 100 (Chicago River at Wells Street). In an analysis of variance test, we concluded that there is no significant difference between mean rates of head capsule deformities for those collected on hester-dendy samplers and those collected in ponar dredge samples ($F=2.89$, $p=0.0911$).

We performed correlation analysis to examine the influence of sediment contaminants on head capsule deformities. Based upon Spearman correlation coefficients, the strengths of correlation were significant ($p<0.05$) in the hester-dendy samples for ammonia-N ($r=-0.399$), iron ($r=0.361$), and DDx (DDT + DDE + DDD) ($r=-0.396$). Spearman correlation coefficients were significant for the ponar samples for mercury ($r=0.659$), cadmium ($r=0.339$), copper ($r=0.439$), simultaneously extracted metals (SEM) ($r=0.455$), SEM-acid volatile sulfides ($r=0.454$), total PCB ($r=0.316$) and semi-volatile organic compounds ($r=0.323$). No contaminants displayed strong correlations for both collection methods. This may reflect differences in exposure routes or pathways for macroinvertebrates in ponar samples and hester-dendy samples.

Background

Morphological deformities in midge larvae have been frequently observed in contaminated sediments. Deformity formation is generally considered to be a sublethal, teratogenic response to contamination, and there is a large body of literature on midge head and mouthpart deformities. The results of these studies suggest a relationship between increased incidence of head capsule deformation with toxic stress, but substrate type, season, radioactivity, and genetic factors also contribute to the rate of deformation (Hamilton and Saether 1971; Jeyasingham and Ling 2000; Williams *et al.* 2001). Wiederholm (1984), studying Swedish lakes, found the occurrence of deformed mouth parts in recent and subfossil material of mostly Chironomus, Micropsectra and

Tanytarsus species increased from less than one percent of the larvae at unpolluted sites or time periods to approximately five to 25% at strongly polluted sites. Cushman (1984) studied larval *Chironomus decorus* in experimental ponds and found that head capsule deformations were significantly dose-related to a contaminant, but that the occurrence of deformities appeared to be a less sensitive measure of pollution than changes in abundance, biomass, number of taxa, and species diversity of benthic insects.

Under contract to LimnoTech, Inc., Baetis Environmental Services, Inc. (Baetis) has been retained to analyze macroinvertebrate data collected from the Chicago Area Waterway System (CAWS) between 2001 and 2007. The analysis supports the CAWS Habitat Evaluation and Improvement Study sponsored by the Metropolitan Water Reclamation District of Greater Chicago (MWRDGC). This technical memorandum is intended to:

- Review the data characterizing head capsule deformities in representatives of the dipteran family Chironomidae, a group of non-biting midges
- Examine correlations of the rate of head capsule deformities with sediment contamination in the CAWS.

Methods and Materials

Macroinvertebrates were collected annually each summer from the CAWS from 2001-2007 by MWRDGC, with enumeration, identification and head capsule examination by EA Engineering, Science, and Technology, Inc. (EA) of Deerfield, IL. Figure 1 shows the locations of macroinvertebrate and sediment sampling stations. Macroinvertebrate collection methods included both hester-dendy sampler (artificial substrate) and a ponar (grab) sampler. Most macroinvertebrates were identified to genus; where possible species-level identifications were completed. A detailed description of the methodology is provided by EA in their 2006 report (EA 2006). LimnoTech, Inc. compiled EA's datasets, including head capsule deformities data, into one relational database for this project.

Descriptive and inferential statistics were derived for the 2001-2007 macroinvertebrate database using SAS software (Vers. 9.1, SAS Institute Inc. Cary, NC). In all cases, data were examined for normality using the Shapiro-Wilks test. Because very little of the macroinvertebrate abundance data are normally distributed, nor could they be transformed to approximate a normal distribution, we generally used nonparametric statistical methods, which are independent of the population distribution. Correlation analyses, for example, relied on Spearman correlation coefficients unless otherwise indicated. For all inference tests, conclusions have been based on a significance level, α, of 0.05.

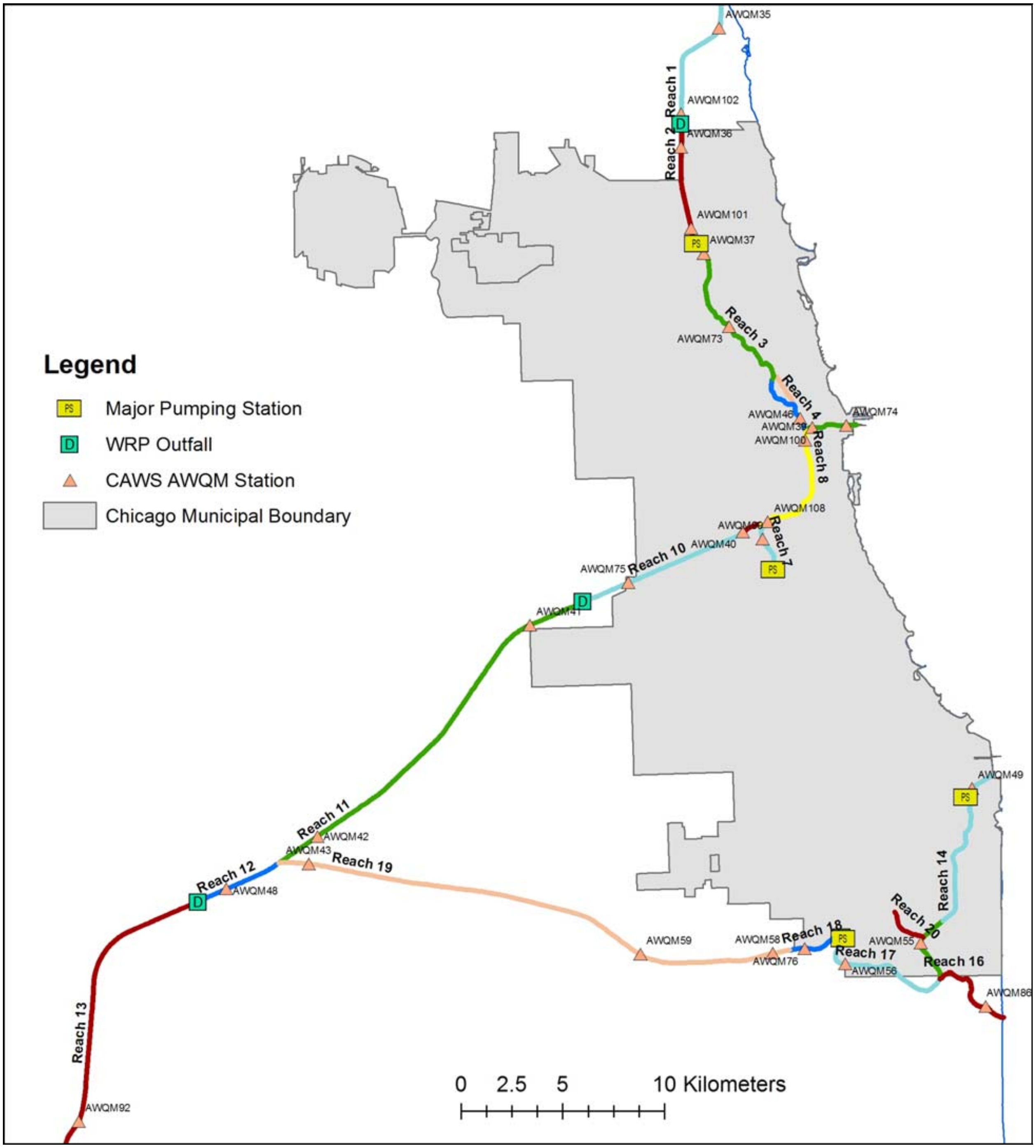

Figure 1. Locations of AWQM Stations in the Chicago Area Waterway System

Results and Discussion

From 2001 through 2007, EA examined 177 CAWS macroinvertebrate samples for chironomid head capsule deformities. Overall, head capsule deformities were observed in 10.9% of chironomid samples (±2.8%). Sampling statistics over the seven-year study period are given in Table 1. Mean rates of head capsule deformities ranged from none at Ambient Water Quality Monitoring (AWQM) Station 55 (Calumet River at 130th Street) to 30.2% at AWQM 100 (Chicago River at Wells Street).

Table 1

HEAD CAPSULE DEFORMITY STATISTICS

Station_ID	N	Mean	Minimum	Maximum
AWQM 100	6	30.2	0	100
AWQM 101	3	7.0	0	13.3
AWQM 102	8	1.7	0	6.9
AWQM 108	6	9.8	0.66	27.3
AWQM 35	7	6.3	0	33.3
AWQM 36	12	3.3	0	16.7
AWQM 37	3	23.7	0	52.4
AWQM 39	4	4.1	0.6	14.3
AWQM 40	6	12.8	1.0	33.3
AWQM 41	15	19.3	0	100
AWQM 43	8	4.3	0.5	12.5
AWQM 46	12	23.3	0	100
AWQM 49	4	7.3	0.7	20.0
AWQM 55	2	0	0	0
AWQM 56	10	19.0	1.7	66.7
AWQM 58	4	7.8	0.4	15.0
AWQM 59	14	4.5	0	11.1
AWQM 73	6	8.0	0	40.0
AWQM 74	10	24.6	0	100
AWQM 75	8	3.0	0	6.9
AWQM 76	10	10.1	0	50.0
AWQM 92	15	4.0	0	16.7
AWQM 99	4	2.4	1.6	3.4

Macroinvertebrate samples were collected using two methods, the hester-dendy multi-plate sampler (HD) and the ponar dredge (PN). Table 2 displays the head capsule deformity statistics by collection method. There were 107 samples collected by the hester-dendy method that were examined for chironomid head capsule deformities; the mean rate was 8.9%. There were 70 samples collected using the ponar dredge and the mean rate of deformities was 13.9%. In an ANOVA (analysis of variance) test, we concluded that there is no significant difference between mean rates of head capsule deformities for the two collection techniques ($F=2.89$, $p=0.0911$).

Table 2
HEAD CAPSULE DEFORMITIES BY COLLECTION METHOD

Station_ID	Method_Code	N	Mean	Minimum	Maximum
AWQM100	HD	6	30.2167	0	100
AWQM101	PN	3	7.0333	0	13.3
AWQM102	HD	5	0.6600	0	1.8
AWQM102	PN	3	3.3667	0	6.9
AWQM108	HD	4	3.1700	0.7	4.9
AWQM108	PN	2	23.0100	18.8	27.3
AWQM35	HD	4	8.5750	0	33.3
AWQM35	PN	3	3.3667	0	8.7
AWQM36	HD	7	0.8800	0	2.8
AWQM36	PN	5	6.6520	0	16.7
AWQM37	PN	3	23.6667	0	52.4
AWQM39	HD	4	4.1000	0.6	14.3
AWQM40	HD	4	2.5875	1.0	3.9
AWQM40	PN	2	33.3300	33.3	33.3
AWQM41	HD	12	17.4042	0	100
AWQM41	PN	3	26.7833	0	42.8
AWQM43	HD	4	4.6550	0.5	12.5
AWQM43	PN	4	3.8150	1.1	6.7
AWQM46	HD	9	26.9444	0	100
AWQM46	PN	3	12.2200	0	20
AWQM49	HD	2	11.5600	3.1	20
AWQM49	PN	2	2.9750	0.7	5.3
AWQM55	HD	1	0.0000	0	0
AWQM55	PN	1	0.0000	0	0
AWQM56	HD	6	8.3650	1.7	20
AWQM56	PN	4	34.9225	3.0	66.7
AWQM58	HD	2	0.5000	0.4	0.6
AWQM58	PN	2	15.0000	15	15.0
AWQM59	HD	6	1.6000	0	4.8
AWQM59	PN	8	6.7600	0	11.1
AWQM73	HD	3	0.3667	0	0.6
AWQM73	PN	3	15.5667	0	40.0
AWQM74	HD	6	9.9550	0	25.0
AWQM74	PN	4	46.6650	16.7	100
AWQM75	HD	7	3.4143	0	6.9
AWQM75	PN	1	0.0000	0	0
AWQM76	HD	1	0.0000	0	0
AWQM76	PN	9	11.2144	0	50.0
AWQM92	HD	10	4.2570	0	16.7
AWQM92	PN	5	3.5580	0	9.1
AWQM99	HD	4	2.4175	1.6	3.4

During the examination of chironomids for head capsule deformities, EA recorded the lowest taxa. Unfortunately the taxa identifier was inconsistently recorded, so not all samples have taxa labels. Table 3 summarizes the chironomid taxa, by the method of their collection. Twelve taxa were identified and recorded from the hester-dendy samples. Six taxa were found in the ponar samples. One group, Chironomus sp., was found in sufficient numbers through both sampling methods, and, we found the Chironomus sp. data to be normally distributed. This allows for another ANOVA testing of equal means for the two methods, this test using a lowest taxa group. There were 7 Chironomus sp. samples collected using the hester-dendy technique and the mean rate of deformities was 34.3%. There were 10 Chironomus samples collected using the ponar dredge and the mean rate of deformities was 31.1%. Figure 1 is a box plot of the Chironomus sp. data. An ANOVA test found no significant difference between mean rates of Chironomus head capsule deformities for the two collection techniques (F=0.06, p=0.8055).

Table 3
LOWEST TAXA OF CHIRONOMIDS

Method_Code	Lowest_Taxa	N	Mean	Minimum	Maximum
HD	Chironomus	7	34.3	0	66.6
HD	Dicrotendipes fumidus	2	15.0	10.0	20.0
HD	Dicrotendipes lucifer	2	3.2	2.1	4.2
HD	Dicrotendipes modestus	3	0	0	0
HD	Dicrotendipes neomodestus	3	41.6	4.8	100
HD	Dicrotendipes simpsoni	17	2.4	0	6.6
HD	Glyptotendipes	4	3.7	0	12.5
HD	Nanocladius distinctus	1	9.1	9.1	9.1
HD	Parachironomus	2	23.8	14.3	33.3
HD	Procladius	1	0	0	0
HD	Procladius (Holotanypus)	1	20.0	20.0	20.0
HD	Xenochironomus xenolabis	1	100	100	100
PN	Chironomus	10	31.1	0	75.0
PN	Dicrotendipes lucifer	1	8.3	8.3	8.3
PN	Dicrotendipes modestus	1	11.1	11.1	11.1
PN	Dicrotendipes simpsoni	3	26.0	0.8	50.0
PN	Procladius	13	9.2	0	33.3
PN	Procladius (Holotanypus)	2	5.8	1.1	10.4

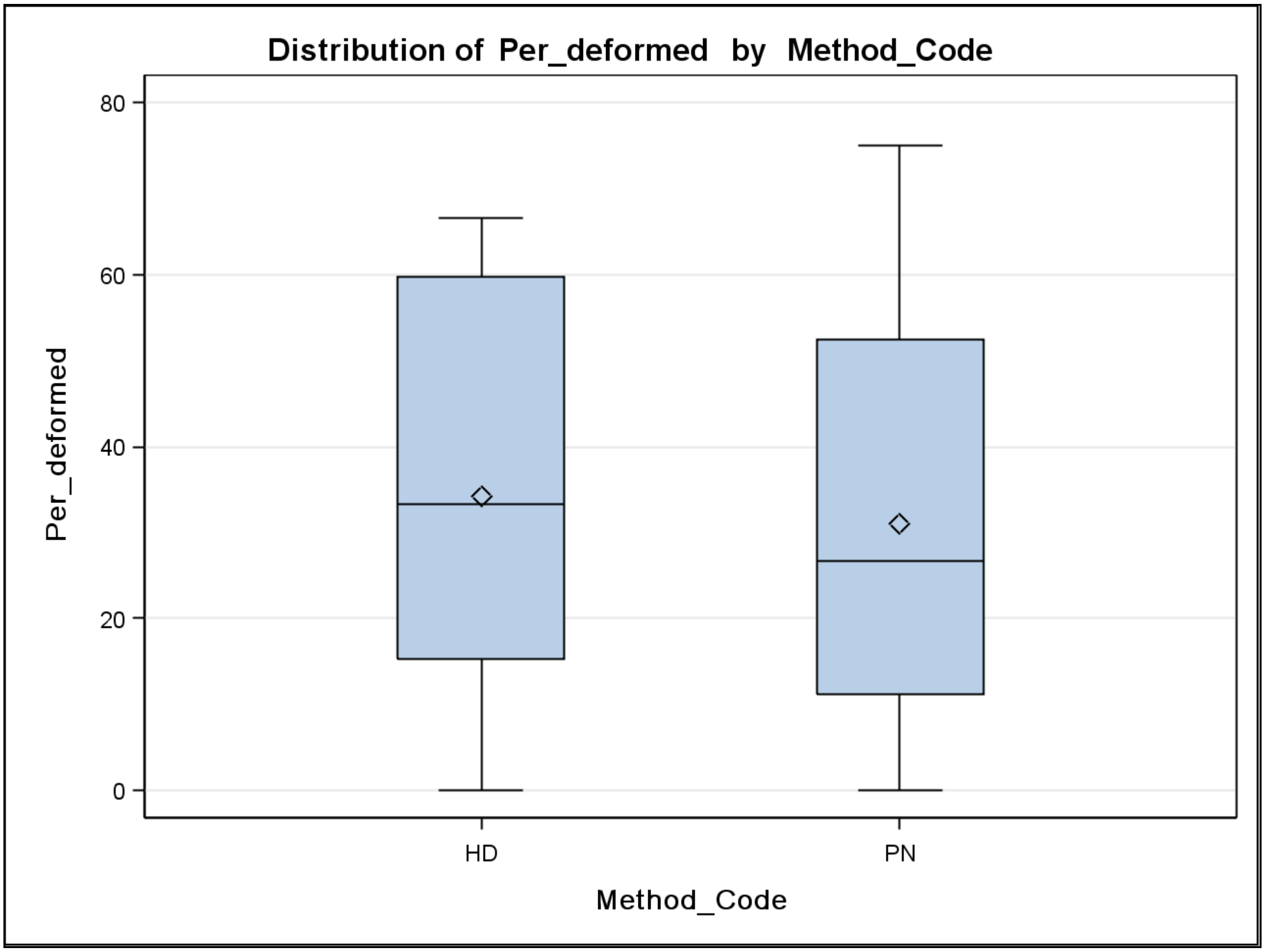

Figure 2. Box Plot of Chironomus sp. Head Capsule Deformities Rates, Grouped by Collection Method.

The Appendix is SAS output from the proc corr procedure and includes a correlation matrix between sediment contamination and the percent head capsule deformities in hester-dendy and ponar samples ($26 \leq N \leq 53$). Based upon Spearman correlation coefficients, the strengths of correlation were significant ($p<0.05$) in the hester-dendy samples for ammonia-N (r=-0.399), iron (r=0.361), and DDx (DDT + DDE + DDD) (r=-0.396). Spearman correlation coefficients were significant for the ponar samples for mercury (r=0.659), cadmium (r=0.339), copper (r=0.439), simultaneously extracted metals (SEM) (r=0.455), SEM-acid volatile sulfides (r=0.454), total PCB (r=0.316) and semi-volatile organic compounds (r=0.323). No contaminants displayed strong correlations for both collection methods. This may reflect differences in exposure routes or pathways for macroinvertebrates in ponar samples and hester-dendy samples.

References

Cushman, R.M. 1984. Chironomid Deformities as Indicators of Pollution from a Synthetic, Coal-derived Oil. Freshwater Biology 14(2): 179-182.

EA Engineering, Science and Technology, Inc. (EA). 2006. A Study of the Benthic Macroinvertebrate Community in Selected Chicago Metropolitan Area Waterways during 2003 and 2004. Metropolitan Water Reclamation District Report #07-47.

Hamilton, A.L. and O.A. Saether. 1971. The Occurrence of Characteristic Deformities in the Chironomid Larvae of Several Canadian Lakes. Can. Ent. 103:363-368.

Jeyasingham, K. and N. Ling 2000. Seasonal Influence on Head Capsule Deformities in Chironomus zealandicus (Hudson) (Diptera: Chironomidae). Hydrobiologia 427: 75-82.

Wiederholm, T. 1984. Incidence of Deformed Chironomid Larvae (Diptera: Chironomidae) in Swedish Lakes. Hydrobiologia 109: 243-249.

Williams, D.D., A.I. Nesterovitch, A.F. Tavares, and E.G. Muzzatti. 2001. Morphological Deformities Occurring in Belarusian Chironomids (Diptera: Chironomidae) Subsequent to the Chernobyl Nuclear Disaster. Freshwater Biology 46(4): 503-512.

Appendix

SIMPLE STATISTICS AND CORRELATION ANALYSES FOR SEDIMENT CONTAMINANT CONCENTRATIONS AND CHIRONOMIDAE HEAD CAPSULE DEFORMATION

The CORR Procedure

26 With Variables:	NH3_N Tot_Phos CN Hg Cd Cr Cu Fe Ni Pb Zn Hv_Mtls Ag As AVS SEM SEM_AVS gravel sand silt clay Heptachlor_epoxide Total_PCB DDx SVOC VOC
2 Variables:	HD_Per_deformed PN_Per_deformed

Simple Statistics

Variable	N	Mean	Std Dev	Median	Minimum	Maximum
NH3_N	80	96.16916	176.16207	43.34971	1.29326	1400
Tot_Phos	81	2495	2841	1750	3.70000	19994
CN	82	1.95096	2.77954	0.87532	0	15.58542
Hg	82	0.85720	1.17186	0.48665	0	6.39700
Cd	82	6.65126	13.99237	3.49000	0.20000	121.87000
Cr	82	86.92561	77.91650	63.95000	12.80000	580.85000
Cu	82	150.05890	136.72495	101.55000	8.70000	825.40000
Fe	79	22919	9309	21727	3921	51809
Ni	82	39.14512	28.57443	30.24500	6.60000	204.60000
Pb	82	256.71061	230.46992	181.70000	21.36000	1255
Zn	82	563.46110	426.26106	484.26500	64.00000	2427
Hv_Mtls	82	1104	775.57662	951.36725	171.04300	4628
Ag	79	2.55354	5.08267	0.74500	0	34.80000
As	81	1.51358	2.15770	0.50000	0	10.30000
AVS	63	26.30032	42.10495	8.66000	0.24000	273.40000
SEM	65	54.19267	169.83660	10.20000	0.18000	1030
SEM_AVS	59	4.87216	12.43565	0.80679	0.01363	88.79310
gravel	64	3.95313	6.67713	1.00000	0	35.80000
sand	64	64.06875	23.43388	70.00000	7.40000	97.80000
silt	64	22.55312	17.21450	20.70000	0	63.00000
clay	64	9.41094	10.19695	4.95000	0.80000	48.00000
Heptachlor_epoxide	82	6.93639	5.41567	5.36776	2.00000	36.00000
Total_PCB	82	1763	2664	749.00000	5.37866	13722
DDx	82	143.26389	166.20820	103.67282	9.52744	1095
SVOC	78	159341	497970	53291	2868	3652353
VOC	81	150.84256	886.52013	39.96004	21.51463	8020
HD_Per_deformed	74	7.61331	10.96338	3.90000	0	56.53333
PN_Per_deformed	55	14.58059	15.44069	7.03333	0	60.00000

The CORR Procedure

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations	HD_Per_deformed	PN_Per_deformed
NH3_N	-0.24142 0.0816 53	0.03515 0.8340 38
Tot_Phos	-0.15525 0.2718 52	-0.02509 0.8811 38
CN	-0.13891 0.3212 53	-0.07480 0.6554 38
Hg	0.06281 0.6550 53	0.39290 0.0147 38
Cd	0.10557 0.4519 53	0.16073 0.3350 38
Cr	0.16002 0.2524 53	0.12053 0.4710 38
Cu	0.20516 0.1406 53	0.51139 0.0010 38
Fe	0.22335 0.1079 53	-0.25561 0.1214 38
Ni	0.27805 0.0438 53	0.31248 0.0561 38
Pb	0.32453 0.0177 53	0.25282 0.1257 38
Zn	-0.02060 0.8836 53	-0.01367 0.9351 38
Hv_Mtls	0.19018 0.1726 53	0.14445 0.3869 38
Ag	-0.05779 0.6810 53	0.09043 0.5892 38
As	0.00589 0.9666 53	-0.09038 0.5894 38
AVS	-0.18526 0.2177 46	-0.24213 0.2237 27

The CORR Procedure

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations	HD_Per_deformed	PN_Per_deformed
SEM	0.03160 0.8330 47	0.25169 0.1964 28
SEM_AVS	-0.02316 0.8828 43	0.34413 0.0852 26
gravel	-0.06253 0.6763 47	-0.10294 0.6022 28
sand	0.03897 0.7948 47	0.05337 0.7874 28
silt	-0.12267 0.4114 47	-0.08985 0.6493 28
clay	0.15072 0.3119 47	0.08766 0.6574 28
Heptachlor_epoxide	-0.04874 0.7289 53	0.05794 0.7225 40
Total_PCB	0.16859 0.2275 53	0.11309 0.4872 40
DDx	-0.19253 0.1672 53	-0.00505 0.9753 40
SVOC	0.26487 0.0553 53	-0.01296 0.9385 38
VOC	-0.17874 0.2003 53	-0.07384 0.6507 40

The CORR Procedure

Spearman Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations		
	HD_Per_deformed	**PN_Per_deformed**
NH3_N	-0.39857 0.0031 53	0.19067 0.2515 38
Tot_Phos	-0.20595 0.1430 52	0.22574 0.1730 38
CN	-0.15316 0.2736 53	0.06071 0.7173 38
Hg	0.19060 0.1716 53	0.65907 <.0001 38
Cd	0.02178 0.8770 53	0.33892 0.0374 38
Cr	0.11819 0.3993 53	0.12077 0.4701 38
Cu	0.08475 0.5463 53	0.42869 0.0072 38
Fe	0.36146 0.0078 53	-0.26475 0.1082 38
Ni	0.13759 0.3259 53	0.27725 0.0920 38
Pb	0.06337 0.6521 53	0.25314 0.1252 38
Zn	-0.05897 0.6749 53	0.13720 0.4114 38
Hv_Mtls	0.07587 0.5892 53	0.20887 0.2082 38
Ag	-0.25105 0.0698 53	0.18532 0.2653 38
As	-0.05396 0.7012 53	0.01897 0.9100 38
AVS	-0.00426 0.9776 46	-0.27754 0.1610 27

The CORR Procedure

Spearman Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations		
	HD_Per_deformed	**PN_Per_deformed**
SEM	0.21117 0.1542 47	0.45512 0.0150 28
SEM_AVS	0.22775 0.1419 43	0.45416 0.0198 26
gravel	0.17874 0.2293 47	0.15412 0.4336 28
sand	0.08875 0.5530 47	0.19327 0.3244 28
silt	-0.13372 0.3702 47	-0.11862 0.5477 28
clay	0.16272 0.2745 47	-0.06595 0.7388 28
Heptachlor_epoxide	-0.21028 0.1307 53	0.09130 0.5753 40
Total_PCB	0.17543 0.2090 53	0.31599 0.0470 40
DDx	-0.39639 0.0033 53	0.09506 0.5596 40
SVOC	-0.18769 0.1784 53	0.32305 0.0479 38
VOC	-0.18967 0.1738 53	-0.03607 0.8251 40

The CORR Procedure