Appendix R
USCG Dry Cargo Sweepings Scientific Investigation: Biological Characterization—Nutrient Enrichment

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Introduction

This memorandum provides documentation of the nutrient enrichment activities conducted as part of the Biological Characterization for the U.S. Coast Guard Dry Cargo Sweepings Impact Analysis Project (Volpe et al., 2006). The nutrient enrichment assays were the only activities conducted as part of this task.

Materials and Methods

Dry deck taconite, limestone, eastern coal, and western coal sweepings were collected in October 2006 and sent to Applied Sciences Laboratory (ASL) in Corvallis, OR for the preparation of the simulated slurry tested in the nutrient enrichment study. The collection of sweepings materials is described in the accompanying technical memorandum, "USCG Dry Cargo Sweepings Scientific Investigation: Sweepings Characterization".

Standard algal growth stimulation assays were conducted for each type of cargo to assess the effects of dry cargo sweepings on Great Lakes phytoplankton communities. The basic approach was to create a slurry of dry cargo material and lake water and to test the supernatant water for potential effects on the natural algal community. The supernatant water (elutriate) was tested for nutrient chemical content, and toxicological analyses. The results of the toxicological analyses are presented in an accompanying technical memorandum, "USCG Dry Cargo Sweepings Scientific Investigation: Sweepings Characterization – Toxicological Analyses". Creation and testing of the slurry followed the basic protocols for dredge materials testing for biological-effects as provided in Section 11 of the EPA/ACOE Manual "Evaluation of Dredged Material Proposed for Ocean Disposal" (EPA, 1991) with the exception of the use of higher dilutions as required to meet estimated field conditions. Lake water and fall algal assemblages from Lake Erie and Lake Superior were tested. Lake Erie and Superior were selected because they represent the two ends of the nutrient/primary production gradient present in all the Great Lakes.

Water and a composite of the natural lake phytoplankton community were freshly collected from Lake Erie and Lake Superior at suspected sweepings deposit locations. Side sonar maps of the sample locations are presented in Figures 1 and 2. A description of the side sonar mapping activities is presented in a separate technical memorandum. At each lake sampling location, water column profiles of temperature and dissolved oxygen were measured using calibrated field meters, and the chlorophyll maximum layer was identified using in-situ flourometry. Water for slurry preparation was collected from the chlorophyll maximum.

Water from Lake Erie was collected on Oct. 8, 2006. A Seabird 19plus was deployed from the Rebel Queen research vessel to approximately 35 ft. in a total lake depth of 42 ft. After equipment upcast, a review of the temperature data identified no definitive thermocline, with a constant temperature of approximately 16 degrees Celsius throughout the depth profile. Fluorometric readings were also relatively constant throughout the depth profile. Water quality readings at this location are presented in Table 1. A slight maximum was observed at approximately 28 ft. with a reading of 1.86 CFU. Subsequently, 10-Liter Niskin bottles were deployed to this to depth to retrieve the lake water. Collected lake water was filtered into 5-gallon cubitainers with an aquarium net to remove large particles and organisms that may deplete the phytoplankton assemblage. Water and samples were shipped on ice ASL for overnight delivery.

Water from Lake Superior was collected on Oct. 17, 2006. A Seabird 911plus was deployed from the Research Vessel Blue Heron. A double thermocline, with a maximum chlorophyll reading at approximately 13 ft. was observed in the downcast, and Niskin bottles were deployed at this at this depth. Water was collected as for Lake Erie. After collecting water, a sediment corer was deployed, and taconite pellets were observed in the retrieved upper sediment layer.

Once water was received by ASL, a subsample of several liters of the lake water was mixed with a known weight of dry cargo material, stirred for 30 minutes, and settled for 1 hour. The supernatant from that slurry provided the elutriate water to be used in the algal stimulation bioassays. Dry cargo material was provided from each of 8 ships; 2 ships per each of the 4 cargo types (eastern coal, western coal, taconite, and limestone). Dry material from similar cargo types was combined in equal weights to produce the 4 final elutriates for testing.

The bioassays consisted of triplicate samples of three experimental conditions plus controls:

- 100 mL of 100% elutriate water added to 800 mL of lake water.
- 100 mL of 50% elutriate water added to 800 mL of lake water.
- 100 mL of 10% elutriate water added to 800 mL of lake water.
- 100 mL of clean, cargo-less elutriate water with 800 mL of lake water (control)

All conditions contained approximately the same initial inoculate of lake phytoplankton because the lake water solutions were created from the same, homogenized lake water and because the elutriate water had been pre-filtered and is phytoplankton-free. The initial elutriate and dilutions of elutriate were created using pre-filtered EPA water piped from Lake Superior at the Duluth EPA laboratory. The most concentrated of the dilution series mimics the condition of no initial dilution for the plume of slurry water as it might be discharged from a ship (see attachment to the Sweepings Chemical Characterization Technical Memorandum for a description of how the concentration of slurry discharged from a ship was estimated).

Each sample consisted of 900 mL of water incubated in ½ gallon, open, wide-mouth glass jars, lightly bubbled with an air line to gently circulate and aerate the phytoplankton mix. Initial elutriate water and lake water samples were collected at the start of the experiment.

At time 0 of the experiment, the elutriate and lake water mixture was analyzed for nutrients. In addition, the control mixture was filtered for chlorophyll a analysis.

The experimental samples were incubated in a growth chamber at conditions designed to mimic field conditions at the time of collection (mid-epilimnion temperature and midday light) on a 14 hours light: 10 hours dark cycle. The incubation proceeded for 96 hours (4 days). At the end of the incubation, water was collected from each flask for chlorophyll a analysis. The results for chlorophyll were then analyzed using a basic 3 x 4 Analysis of variance (ANOVA) design to test for significance of difference among conditions and for differences between experimental and control conditions.

A total of 8 lake/cargo combinations were tested (2 lakes x 4 cargo types). Bioassay jars, were incubated simultaneously for 96 hours. Nutrients and biological material tested from initial elutriate/lake water mixtures and final test conditions were as follows:

Raw water:

- Total kjeldahl nitrogen (initial mixture and final from all flasks)
- Total recoverable phosphorus (initial mixture and final from all flasks)
- Ammonium-N
- (Nitrate + nitrite)-N

0.45 um filtered water: (all for initial mixture and final from all flasks)

- Dissolved ortho-P, filtered at ASL prior to shipment.

1.2 um filtered (GF/C), algae on filters:

- Initial, raw lake water (duplicated for each lake) and final from all flasks for chlorophyll a determination.
- 200 mL of water was filtered, then the filter was folded in half with the material inside; frozen and kept dark until extraction and fluorometric analysis within 3 weeks of collection.
- Used low vacuum filtration so as not to break algal cells (< 5 atm)

Results

Chlorophyll

As expected, prior to any addition of dry cargo sweepings, Lake Erie water had a significantly greater amount of chlorophyll than Lake Superior. All Lake Erie slurries showed growth over the 4 days. Simulated eastern coal and iron deck sweepings slurries with both Lake Erie and Lake Superior water were significantly stimulatory compared to controls, but western coal and limestone slurries were not (Figures 3 and 4). Analysis of Variance (ANOVA) results are presented in Table 2. The variability between dilution series within any one set of type of cargo and lake was also analyzed. The analyses within series identified iron as being significantly more stimulatory with greater concentrations of elutriate. Eastern coal did not show similar, positively increasing chlorophyll with higher elutriate concentrations. For Lake Erie, the relationship was significant but negative (i.e., more elutriate produced less chlorophyll, not more), suggesting that low concentrations of the coal are stimulatory (thus, higher than controls, as shown before) but higher concentrations are not.

Nutrients

Nitrogen and phosphorus nutrient compounds were measured in the nutrient stimulation experiments to provide a direct measure of nutrient differences among treatment effects. All experimental and control flasks were assessed at the end of the test (i.e. day 4) for concentrations of ammonia (NH_3-N), nitrate (NO_3-N), total kjeldahl nitrogen (TKN), dissolved ortho-phosphate (OP), and total phosphorus (TP) fractions. TKN and NO_3-N results were added to provide an estimate of total nitrogen (TN). In addition, the ratios of TN to TP (N:P) were computed as a measure of the tendency of the ambient concentrations to provide nitrogen or phosphorus-based limitations to algae growth. Increases of TN or TP over control conditions were considered indicative of increases in nutrients as supplied by the slurries. Complete nutrient laboratory reports are provided as a CD Attachment to this memorandum.

The control flasks and day 0 measurements of raw lake water indicated an overwhelming tendency of the conditions to favor phosphorus limitation. N:P ratios ranged from 10 to 657. Values between 7 and 20 indicate possible mixed N or P limitation, values over 20 indicate phosphorus limitation and values less than 7 indicate nitrogen limitation to algae growth. In addition, only 6 out of 84 experimental flasks indicated co-limitations of algae growth (primarily for western coal in Lake Erie); the rest were indicative of phosphorus limitation (N:P values well over 20).

As summarized below, ANOVA tests of differences among experimental conditions indicated very few significant differences between control and experimental conditions with respect to nutrients. Analysis of Variance (ANOVA) results are presented in Appendix Table 3. The N:P results indicate that phosphorus should have been the most stimulatory nutrient to explain the chlorophyll results. The expectation is that TN and TP would be conservative over the course of the experiment but that dissolved fractions would be dynamic, as related to uptake by growing algae. Dissolved fraction buildup would indicate a lack of uptake by algae and probably growth limitation by the other major nutrient. The following statistically-significant differences among groups at the end of the incubation period were observed:

- NO_3-N: Higher concentrations than controls for limestone and western coal in Lake Superior.
- NH_3-N: No significant differences.
- TKN: Higher concentrations than controls for western coal in Lake Erie. Controls were higher than experimental conditions for eastern and western coal in Lake Superior.
- TN: Higher concentrations than controls for western coal in Lake Erie and higher controls than experimental for eastern and western coal in Lake Superior.
- OP: Uniformly undetected in the samples.
- TP: Higher concentrations than controls for western coal in Lake Erie and iron in Lake Superior.

It is worth noting that the lower N:P ratios associated with western coal additions to Lake Erie were also associated with both N and P buildup in concentrations in the experimental conditions. However, those buildups were not associated with increased algae growth.

Observations of significant algal growth (additions of eastern coal or iron) were associated with some N and P compound buildup and high N:P ratios, indicative of N additions to the flasks from slurries but without dissolved N uptake (eastern coal, only). The iron additions (the most stimulatory of growth) showed no such uptake effects for N or P. However, iron may be stimulatory itself, in addition to the separate effects of N and P nutrients (e.g., Weger et al., 2002).

The results indicate the probability that eastern coal and iron stimulations of algal growth were associated with P additions, but those net additions were relatively small and not measurable as increased concentrations of TP in the growth chambers (except possibly for iron in Lake Superior). Some of the larger N additions were measurable as significant differences between groups but were not associated with stimulations of growth or noticeable uptake of dissolved N fractions. The evidence from TN and TP measurements indicates that western and eastern coal slurries added N whereas western coal and iron added P to the experimental flasks.

References

U.S. Environmental Protection Agency. 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal.* Office of Water. EPA 503/8-91/001. February.

Volpe National Transportation System Center, Parsons Brinckerhoff Quade & Douglas, Inc., and CH2M HILL. 2006. *Scientific Approach for Dry Cargo Sweepings Impact Analysis*. Prepared for the United States Coast Guard, Washington, D.C.

Weger, H. G. Middlemiss, J. K. Petterson, C. D. 2002. Ferric chelate reductase activity as affected by the iron-limited growth rate in four species of unicellular green algae (Chlorophyta), *J. Phycology* Vol 38 (3): 513-519.

Table 1. Water Quality at Lake Water Sample Locations

Parameter	Lake Erie	Lake Superior
Location	41 33.5556 N, 82 31.8256 W	46 52.4466 N, 91 44.7593 W
Lake Depth (ft.)	42 ft.	275 ft
Fluorescence	1.36-1.86 CFU	0.77-3.55 mg/m^3
Depth at Max. Fluorescence (ft.)	28.32	13.01
Temperature (Celcius)	16.3-16.8	4.06-9.51
Conductivity (mS/cm)	0.21-0.22	0.06-0.07
Turbidity (NTU)	2.19-3.24	Not Available
DO (mg/L)	7.94-10.1	10.5-12.3

Appendix Table 2. Analysis of Variance (ANOVA) comparisons, chlorophyll concentrations, Day 4 of stimulation bioassay experiments. Statistically-significant comparisons, only.

Comparison	Average of tested values (mg/L)	Overall ANOVA results (1)	Detailed Comparisons in Concentrations (2)
LAKE SUPERIOR			
All elutriates:	Control = 1.035 E Coal = 1.393 W Coal = 1.189 Limestone = 1.182 Iron = 1.808	P < 0.05	Eastern Coal > Control P = 0.0009 Iron > Control P < 0.0001
LAKE ERIE			
All elutriates:	Control = 10.522 E Coal = 12.364 W Coal = 8.765 Limestone = 10.592 Iron = 13.365	P < 0.05	Eastern Coal > Control P = 0.0497 Iron > Control P = 0.0034

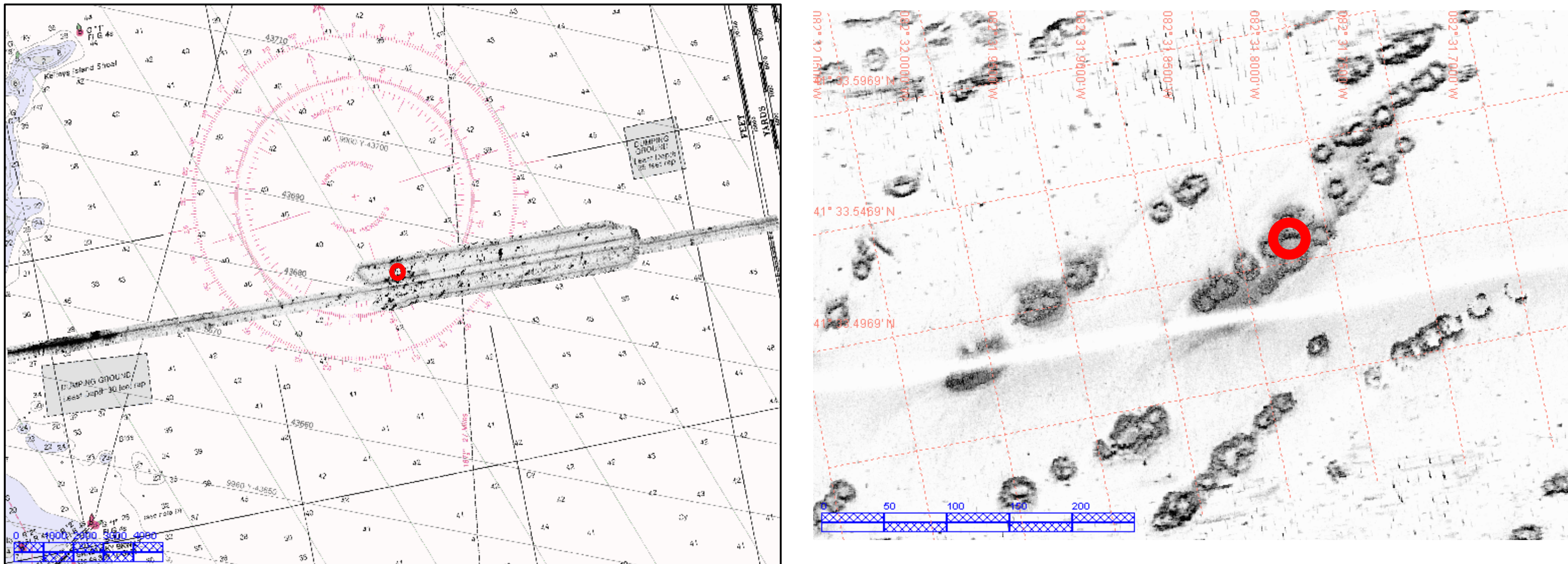
1. Least-squares regression. F-test.
2. Fisher's PLSD test (Projected Least Significant Difference), for post-hoc differences between pairs of ANOVA groups.

Appendix Table 3. Analysis of Variance (ANOVA) comparisons, nutrient concentrations, Day 4 of stimulation bioassay experiments. Statistically-significant comparisons, only.

Comparison	Average of tested values (mg/L)	Overall ANOVA results (1)	Detailed Comparisons in Concentrations (2)
LAKE SUPERIOR			
Eastern Coal: TKN	Control = 3.130 100% = 0.802 50% = 0.665 10% = 0.952	NS	Control > 50% P = 0.047
TN	Control = 3.493 100% = 1.158 50% = 1.023 10% = 1.323	NS	Control > 50% P = 0.046
Western Coal: NO3-N	Control = 0.363 100% = 0.370 50% = 0.381 10% = 0.368	P < 0.05	50% > 10% P = 0.032 50% > Control P = 0.003
TKN	Control = 3.130 100% = 0.572 50% = 1.000 10% = 1.000	NS	Control > 100% P = 0.039
TN	Control = 3.493 100% = 0.942 50% = 1.381 10% = 1.368	NS	Control > 100% P = 0.040
Iron: TP	Control = 0.018 100% = 0.025 50% = 0.028 10% = 0.028	NS	50% > Control P = 0.018
Limestone: NO3-N	Control = 0.366 100% = 0.381 50% = 0.369 10% = 0.363	P < 0.05	100% > 50% P = 0.044 100% > 10% P = 0.018 100% > Control P = 0.002
LAKE ERIE			
Western Coal: TKN	Control = 0.846 100% = 1.260 50% = 0.806 10% = 0.725	P < 0.05	100% > 50% P = 0.003 100% > 10% P = 0.001 100% > Control

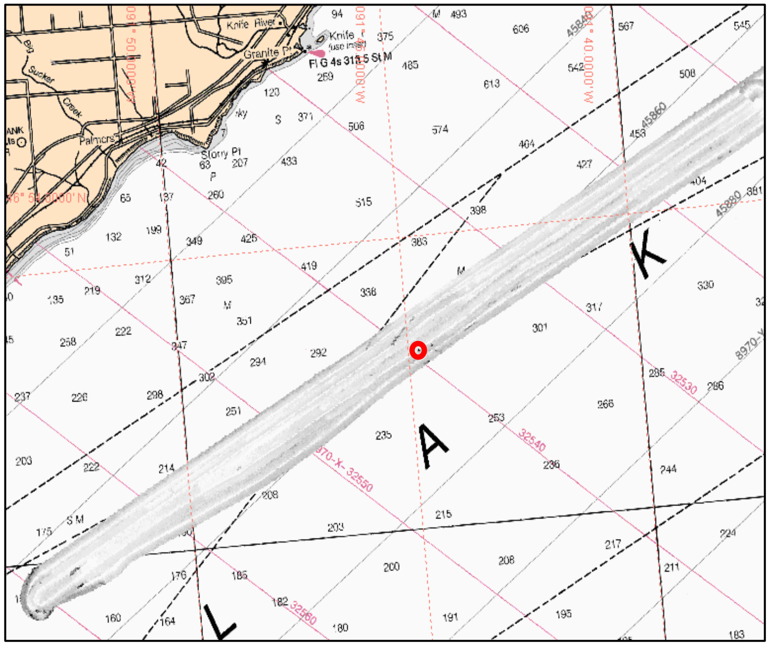
			P = 0.002
TN	Control = 0.993 100% = 1.425 50% = 0.965 10% = 0.867	P < 0.05	100% > 50% P = 0.002 100% > 10% P < 0.001 100% > Control P = 0.001
TP	Control = 0.023 100% = 0.128 50% = 0.056 10% = 0.021	P < 0.05	100% > 50% P < 0.001 100% > 10% P < 0.001 100% > Control P < 0.001 50% > 10 % P = 0.0001 50% > Control P < 0.0001

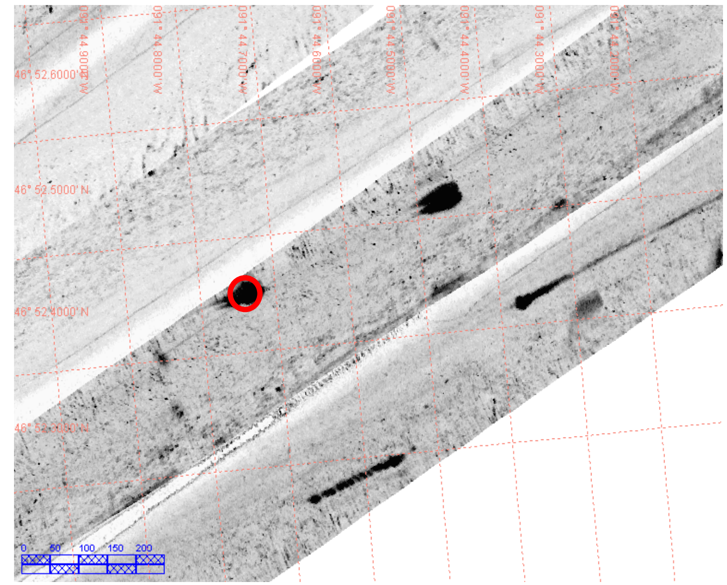
1. Least-squares regression. F-test.
2. Fisher's PLSD test (Projected Least Significant Difference), for post-hoc differences between pairs of ANOVA groups.
3. NS = not significantly different among all groups combined.



Scale in Meters

Figure 1. Location of Lake Erie Sample Location (Red Circle). A Sidescan Sonar Image is Shown on the Right





Scale in Meters

Figure 2. Location of Lake Superior Sample Location (Red Circle). A Sidescan Sonar Image is Shown on the Right

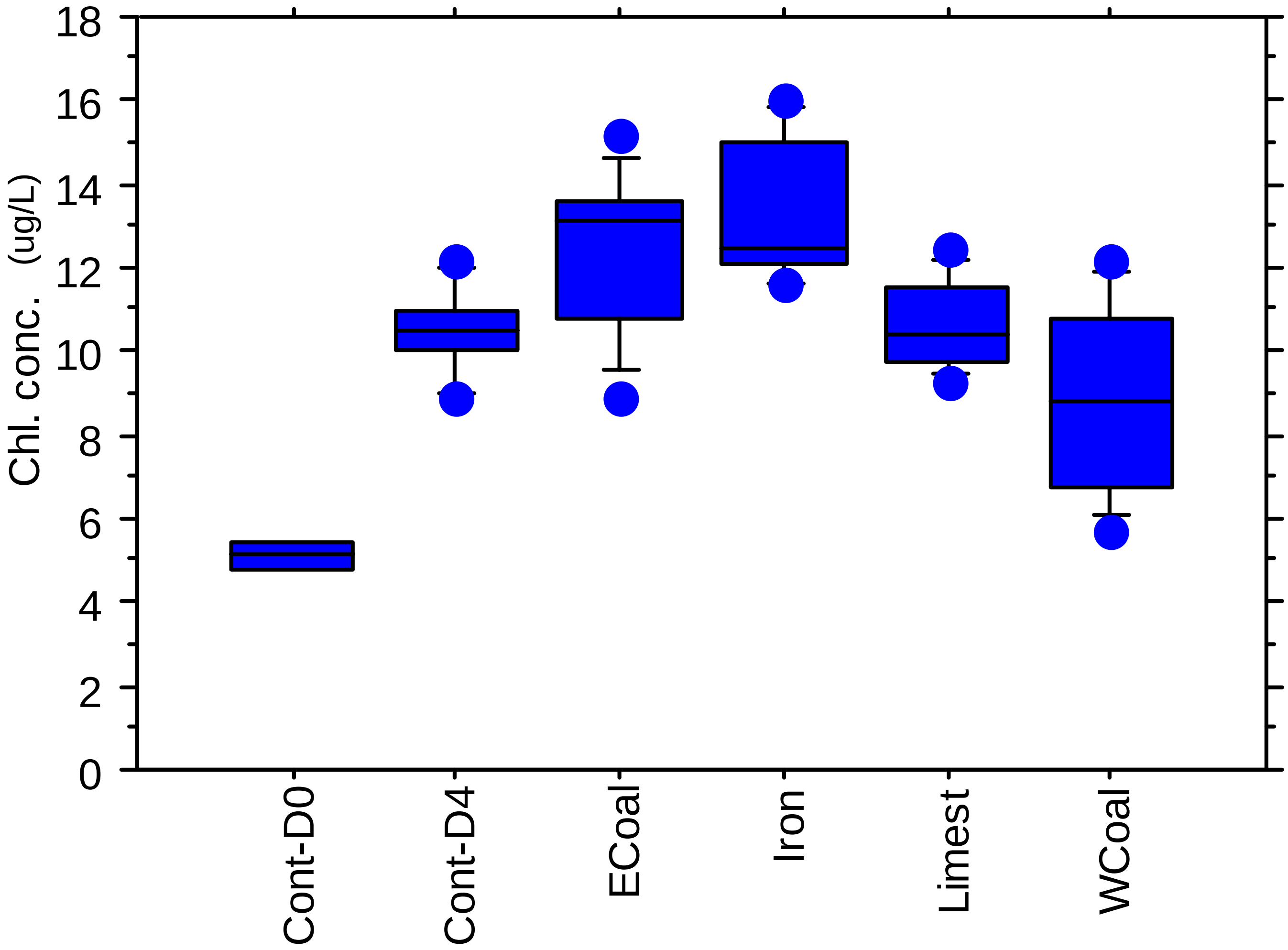


Figure 3. Boxplot of Chlorophyll Concentrations in Simulated Deck Sweepings Slurries with Lake Erie Water and No Dilution

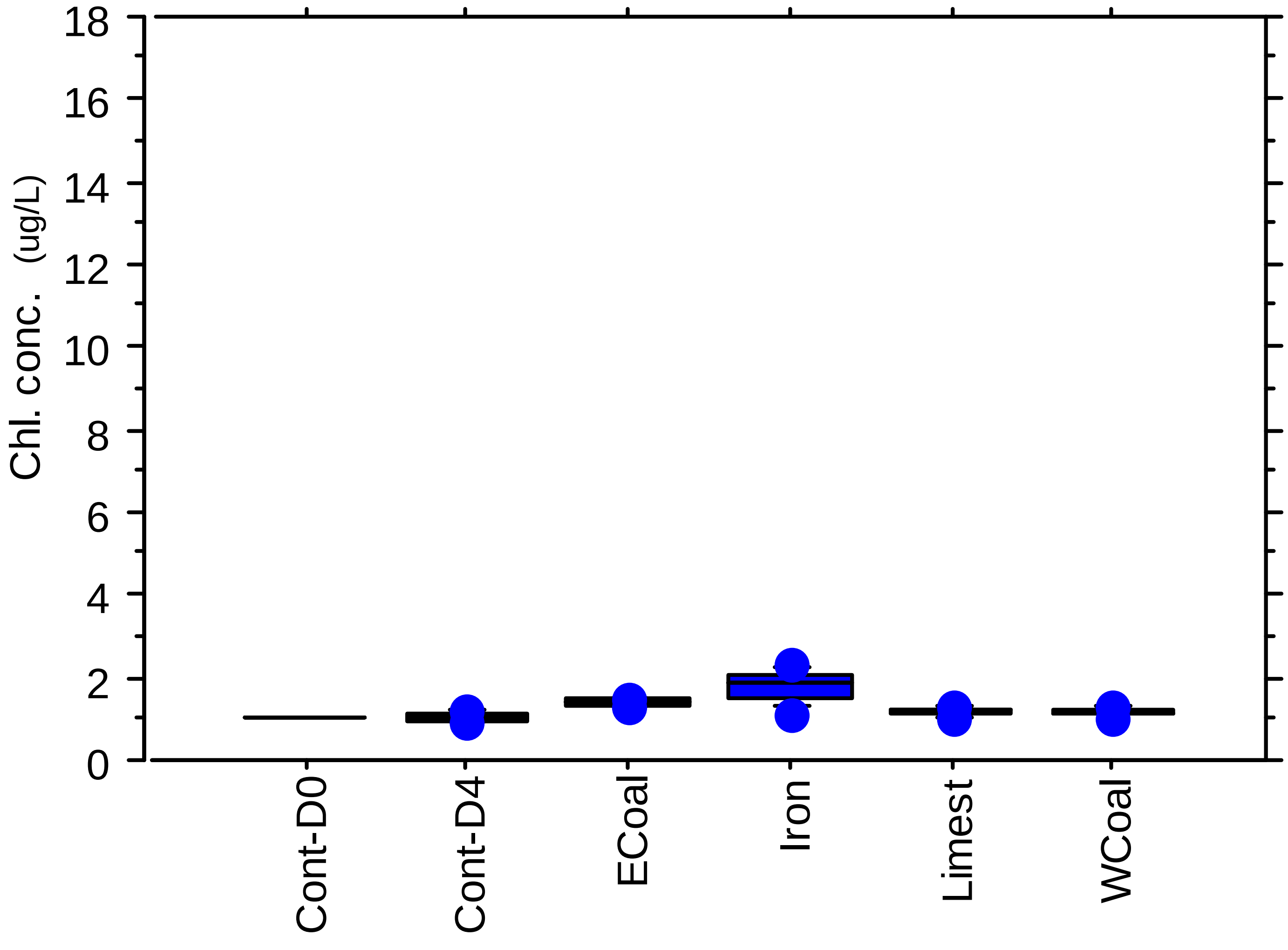


Figure 4. Boxplot of Chlorophyll Concentrations in Simulated Deck Sweepings Slurries with Lake Superior Water and No Dilution