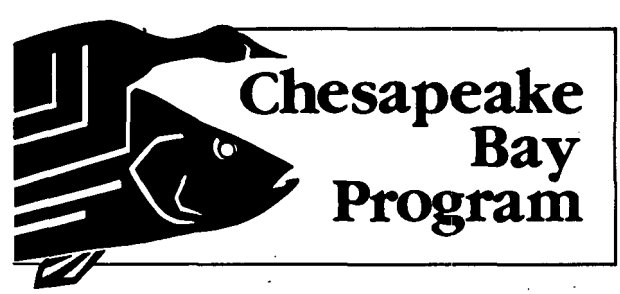
United States Environmental Protection Agency
CBP/TRS 5/87

August 1987

A Comparison of Preservation Techniques for Estuarine Water Samples for Analysis of Organic Carbon Fractions



A COMPARASION OF PRESERVATION TECHNIQUES FOR ORGANIC CARBON ANAYSIS IN ESTUARINE WATER SAMPLES

ROBERT C. SIEGFRIED
CHESAPEAKE BAY PROGRAM
VIRGINIA WATER CONTROL BOARD
2111 NORTH HAMILTON ST.
RICHMOND, VA. 23230

JANUARY, 1987

INTRODUCTION:

In order to determine whether freezing of water quality samples was a acceptable method of preservation, the Chesapeake Bay Program Monitoring Subcommittee funded a comprehensive comparison study. Virginia Institute of Marine Science preformed a comparison of five preservation treatments for eight water quality parameters (TP, TDP, OP, NO2, NO23, TKN, Si, TSS). These results were reported in Salley et al 1984. The State of Maryland conducted a comparison of three preservation treatments for particulate nitrogen, phosphorus and carbon samples. These results were reported in Vaas (1986). Old Dominion University provided the laboratory analyses for total organic carbon (TOC) and dissolved organic carbon (DOC). The statistical analysis and graphical presentation of these data is presented in this report prepared by Chesapeake Bay Program staff of the Virginia Water Control Board.

METHODS:

Four stations, two in the James and two in the York rivers, were sampled to give a range of salinities and nutrient levels. Five liter carboys of water were filled at each station and returned to VIMS. The samples for carbon analysis were transported to ODU. Five different combinations of preservation and holding times where employed. Analyses for the 'Day 0' samples (D0) were conducted 24 hours after collection. Samples where frozen for 7 days (D7F) and 28 days (D28F) to examine freezing as a preservation method. Samples where also preserved with acid and analyzed within 48 hours of collection (D1) and after a 28 day (HT) holding time. See Salley et al (1986) for further discussion of sampling.

The laboratory analyses were conducted following EPA Method Reference Number 415.1. Inorganic carbon was purged from the sample, which was then digested to convert organic carbon to CO2, which was measured with an infrared detector. Five replicates where prepared for analysis for each treatment-station combination, but due to the loss of some samples during the purging process, the number of replicates ranged from three to five. The quality assurance and quality control measurements included running standards, duplicates, and spikes. In general, for each treatment three standards were analyzed during the run, four samples where spiked with known concentrations, and 30-40 duplicates were run. The following are the QA\QC results.

Standard= 1.4 mg/l Average	% Diff.	Duplicates Average % Diff. from mean	Spiked Samples Average % Recovery
HT	112	9.8	129.9
D1	122	6.8	110.2
D0	102	7.0	81.9
D7F	114	5.6	89.0
D28F	108	14.4	126.7

Statistical methods similar to those used by Salley et al (1986) were employed to test whether mean carbon concentrations were different between the five treatments for each of the four stations. As with the other preservation comparison reports, comparisons between the same treatments from different stations were deemed not appropriate. Comparisons between treatments from the same station where the major interest in this study. \the use of four different stations was to obtain a range of sample concentrations and matrixes.

Due to the limited number of replicates (3-5) in each treatment, rigorous tests of the data for normality and homogenity of variance could not be performed. Since these tests of the assumptions of parametric statistics were not performed, both parametric and nonparametric statistics were applied to the data.

For each of the four stations, a one way ANOVA was performed to test the null hypothesis that all treatment means were equal. A parametric Tukey's multiple comparison test was preformed to identify which treatment means, if any, were significantly different. Tukey's comparisons assume equal sample size per treatment which was not always true within this experiment. A Scheffe's comparison was conducted at both alpha levels, since this test does not assume equal sample size, but it is not as a powerful test as Tukey's if sample sizes are equal.

A nonparametric ANOVA similar to the Kruskal-Wallis Test was performed by assigning ranks to the data for each station then performing a one-way ANOVA on the ranked data (Vaas, 1986). Tukey's multiple comparisons were also conducted on the ranked data to identify significantly different treatments.

RESULTS:

A listing of the raw data with treatment means and standard deviations for TOC and DOC are presented in Tables I and II, respectively. Table III and IV contains the results of the statistical analysis of the TOC and DOC data, respectively. Plots of the treatment means for each station for TOC and DOC are depicted in Figures 1 - 4 and Figures 5 - 8, respectively. In the discussion of the results an alpha level of 0.05 is considered significant. Results for both an alpha level of 0.05 and 0.01 are presented in the tables.

TOTAL ORGANIC CARBON:

For all stations the one-way parametric ANOVA was significant, indicating that not all treatments were equal. The parametric Tukey's multiple comparisons identified the HT treatment to be significant different from the D0 treatment for all stations. For the James River samples, the HT treatment was significantly different from all other treatments, with no other significant differences observed in the other treatment comparisons. The York 1 comparisons identified the HT treatment as significantly different from the D0 and D7F treatments. The D7F treatment was also significantly different from the D28F treatment. The York 2 comparisons identified the HT treatment as significantly different from the D0 and D28F treatments. The D7F treatment was also significantly different from the D28F treatment.

The Scheffe's comparisons identified eleven of the 14 significantly different treatment comparisons identified with the Tukey's comparisons. The James 1 and York 1 comparisons results were the same as with Tukey's test. In the James 2 comparisons, the HT treatment was significantly different from the other treatments except the D28F treatment. In the York 2 comparisons only the HT treatment was significantly different from the D0 treatment, in contrast to the Tukey's test which identified three significant comparisons.

The nonparametric ANOVA (Kruskal-Wallis) procedures also indicated that, at each station, not all treatments were equal. The nonparametric Tukey's multiple comparisons of the ranked data identified a few more significantly different comparisons than the parametric Tukey comparisons. The James 1 comparisons were the same as in the parametric Tukey's, i.e. HT treatment was significantly different from all other treatments. The

James 2 comparisons showed all treatments were significantly different from the D0 treatment except the D1 treatment. The D1 treatment was significanlty different from the HT and D28F treatments. The HT, D7F and D28F treatments were not significantly different from each other. For the York 1 comparisons both the HT and D28F treatments were significantly different from the D0 treatment. The D7F treatment was significantly different from all treatments but the D0 treatment. The York 2 comparisons indicated that both the HT and D28F treatments where significantly different from the D0 treatment and the HT treatment was also significantly different from the D7F treatment.

DISSOLVED ORGANIC CARBON:

The one-way parametric ANOVA was significant for only the York 1 and York 2 stations. The treatment means for the James 1 and James 2 stations were not significantly different. The Tukey's multiple comparisons for the York 1 station identified the D28F treatment as significantly different from the D0, HT and D7F treatments. The York 2 comparisons identified the D28F treatment as significant different from the D0 and HT treatments. The Scheffe's comparisons identified the same significant differences as the Tukey's comparisons.

The nonparametric ANOVA (Kruskal-Wallis) procedure indicated the same results as the parametric ANOVA. The nonparametric Tukey's multiple comparisons of the ranked data identified a few more significantly different comparisons than the parametric comparisons. For the York 1 comparisons the D1 treatment was significantly different from the D0 and HT treatments. As in the parametric comparisons, the D28F treatment was significantly different from all treatments but the D1 treatment. York 2 comparisons indicated that the D28F treatment was significantly different from the D0 and HT treatments, which is the same as in the parametric comparisons.

DISCUSSION:

The basic question of this research is if the preservation methods employed preserve the original organic carbon content of the samples. The D0 treatment was the control against which the other treatments where compared. The HT treatment is the currently EPA approved and accepted method. The freezing treatments were compared to both the control treatment (D0) and the EPA approved treatment (HT) to determine if freezing could be adopted as comparable to the EPA approved method.

Examination of the plots of the TOC results, (Figures 1 - 4), shows a general trend for increasing TOC with increased holding time, whether frozen or acidified. While a decrease in organic carbon could be explained by poorly preserved samples, an increase in not easily explained. Day to day variation in the accuracy of the analytical method may represent as much variation as that introduced with the use of different preservation methods. Based on the Scheffe's comparisons, the HT treatment was significantly different from the control treatment in three out of four stations. The D7F treatment appears to not be significantly different from the D0 treatment. Examination of the plots and raw data shows an increase in variation and mean concentration when comparing the D28F treatment to the control. The D28F treatment is not significanlly different from the D0 treatment in 3 out of 4 stations based on the Scheffe's comparisons.The EPA approved holding time of 28 days with acidification (HT) is significantly different from the control for three out of four stations, and significantly different from the other treatments in six out of the other twelve possible comparisons. Of the five treatments, the EPA approved holding time appears to be the least favorable for the preservation of TOC samples based on comparison with the control treatment. When freezing is compared to HT, especially the short term freezing treatment, this preservation method appears comparable or better. When compared to the control treatment, freezing is somewhat questionable, especially when held for 28 days.

The plots of the DOC results (Figure 5 - 8) do not depict any general pattern to the data. The statistical analyses indicate that there are no significant differences between the treatments from the James 1 and 2 stations. The raw data indicates a larger amount of variance in the D28F treatment than the other treatments. The HT treatment for the James 2 station was also highly variable (range 1.8 to 4.5). The EPA approved 28 day with acidification treatment (D28F) does not appear to be significantly different from the control treatment (D0), in contrast with what was found in the TOC analyses. For the York 1 and 2 stations, the D28F treatments were significantly different from the 'control' treatment (D0), as well as the HT treatment, yet not from the D1 treatment. All seven day freezing treatments (D7F) where not significanly different from the control. Out of 8 possible comparisons between the freezing treatments and the control treatment, only 2 where significantly different. When compared to the control or the HT treatment, freezing appears to be an acceptable method of preserving DOC samples despite a few significant differences. Since these diffenences where detected in the long term freezing treatment, the length of storage time for frozen samples should be as short as possible.

REFERENCES

Salley, B. A., J. G. Bradshaw and B. J. Neilson. 1986. Results of Comparative Studies of Preservation Techniques for Nutrient Analysis on Water Samples. Gloucester Piont, Virginia Institute of Marine Science.

Vaas, P. A. 1986. Freezing of Estuarine Nutrient Samples as a Preservation Technique: The Analysis of Particulate Nitrogen, Carbon, and Phosphorus Fractions. Maryland Office of Environmental Programs, Ecological Modeling and Analysis Division. Technical Report No. xxx.

TABLE I

TOTAL ORGANIC CARBON ANALYSES

JAMES 1	HT	D1	D0	D7F	D28F
	5.8	2.7	2.2	2.9	2.8
	4.9	2.5	2.4	2.7	3.1
	5.6	2.6	2.4	2.5	2.8
	3.7	3.0	2.9	2.6	3.3
			3.3	2.5	3.0
MEAN	5.0	2.7	2.6	2.6	3.0
STD.DEV.	0.8	0.2	0.4	0.1	0.2

JAMES 2	HT	D1	D0	D7F	D28F
	4.9	3.2	3.1	3.3	3.1
	4.6	2.9	3.0	3.3	3.3
	5.1	2.9	2.8	3.2	5.8
	4.8	3.1	2.8	3.4	3.5
	5.0	3.3	3.2		3.6
MEAN	4.9	3.1	3.0	3.3	3.9
STD.DEV.	0.2	0.2	0.2	0.1	1.0

YORK 1	HT	D1	D0	D7F	D28F
	5.4	3.4	3.4	2.8	3.6
	3.9	3.6	3.5	2.8	3.3
	3.8	3.6	3.2	2.9	3.9
	4.3	3.1	3.1	3.2	4.5
		4.2	3.1	3.3	4.7
MEAN	4.4	3.6	3.3	3.0	4.0
STD.DEV.	0.6	0.4	0.2	0.2	0.5

YORK 2	HT	D1	D0	D7F	D28F
	3.6	3.6	2.5	2.8	3.6
	3.6	3.0	2.9	2.6	3.3
	3.7	3.3	2.5	3.1	2.7
		2.8	3.0	2.7	4.3
			2.5	2.9	4.2
MEAN	3.6	3.2	2.7	2.8	3.6
STD.DEV.	0.0	0.3	0.2	0.2	0.6

TABLE II

DISSOLVED ORGANIC CARBON ANALYSES

JAMES 1	HT	D1	D0	D7F	D28F
	2.6	2.3	2.1	2.8	2.4
	2.0	2.2	2.2	2.5	2.6
	2.5	2.2	2.2	2.3	2.2
	2.6	2.5	2.5	2.3	3.9
			2.6	2.1	2.5
MEAN	2.4	2.3	2.3	2.4	2.7
STD.DEV.	0.2	0.1	0.2	0.2	0.6

JAMES 2	HT	D1	D0	D7F	D28F
	2.5	3.5	2.6	2.7	2.2
	1.8	2.9	2.4	2.6	2.5
	4.3	2.8	2.5	2.5	3.4
	2.4	3.0	2.5	2.8	2.4
	2.7	3.6	2.6		3.2
MEAN	2.7	3.2	2.5	2.7	2.7
STD.DEV.	0.8	0.3	0.1	0.1	0.5

YORK 1	HT	D1	D0	D7F	D28F
	2.1	2.5	2.3	2.2	2.8
	2.3	2.5	2.4	2.1	2.3
	2.2	2.7	2.2	2.3	3.3
	1.7	2.6	2.2	2.4	3.8
		3.2	2.2	2.6	4.6
MEAN	2.1	2.7	2.3	2.3	3.4
STD.DEV.	0.2	0.3	0.1	0.2	0.8

YORK 2	HT	D1	D0	D7F	D28F
	2.9	3.1	3.2	2.5	2.6
	3.0	2.7	2.6	2.2	2.4
	3.1	2.9	3.1	3.0	2.0
		2.5	3.0	2.3	2.4
			2.5	2.5	1.9
MEAN	3.0	2.8	2.9	2.5	2.3
STD.DEV.	0.1	0.2	0.3	0.3	0.3

TABLE III.

TOTAL ORGANIC CARBON

	James 1	James 2	York 1	York 2
One-way ANOVA	0.0001	0.0001	0.0013	0.0038

PARAMETRIC

TUKEY'S Mult. Comparisons	James 1	James 2	York 1	York 2
	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F
D1	.	.	.	.
HT	# #	# #	* .	* .
D7F	. . #	. . #	. . #	. . .
D28F	. . # .	. . * .	. . . *	# . . *

Scheffe's Comparisons	James 1	James 2	York 1	York 2
	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F
D1	.	.	.	.
HT	# #	# #	* .	. .
D7F	. . #	. . #	. . #	. . .
D28F	. . # .	. . . .	. . . *	* . . .

	James 1	James 2	York 1	York 2
KRUSKAL-WALLIS NONPARAMETRIC ANOVA	0.0018	0.0001	0.0001	0.0041

NONPARAMETRIC

TUKEY'S Mult. Comparisons	James 1	James 2	York 1	York 2
	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F	D0 D1 HT D7F
D1	.	.	.	.
HT	# *	# #	# .	* .
D7F	. . #	* . .	. * #	. . *
D28F	. . * .	# * . .	* . . #	* . . .

= Significant difference between means at alpha=0.01.
* = Significant difference between means at alpha=0.05.
. = No significant difference between means at alpha=0.05.

D0= DAY 0 (CONTROL)
D1 = DAY 1
HT = EPA APPROVED HOLDING TIME, 28 DAYS WITH ACIDIFICATION
D7F = SAMPLE FROZEN FOR SEVEN DAYS
D28F = SAMPLE FROZEN FOR 28 DAYS

TABLE IV:

DISSOLVED ORGANIC CARBON

	James 1	James 2	York 1	York 2
One-way ANOVA	0.4568	0.4136	0.0026	0.0072

PARAMETRIC

TUKEY'S Mult. Comparisons	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F
D1	.				.				.				.			
HT	.	.			.	.			.	.			.	.		
D7F	.	.	.		.	.	.		.	.	.		.	.	.	
D28F	.	.	.	.	.	.	.	.	#	.	#	*	*	.	*	.

Scheffe's Comparisons	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F
D1	.				.				.				.			
HT	.	.			.	.			.	.			.	.		
D7F	.	.	.		.	.	.		.	.	.		.	.	.	
D28F	.	.	.	.	.	.	.	.	*	.	*	*	*	.	*	.

	James 1	James 2	York 1	York 2
KRUSKAL-WALLIS NONPARAMETRIC ANOVA	0.6715	0.1034	0.0002	0.0075

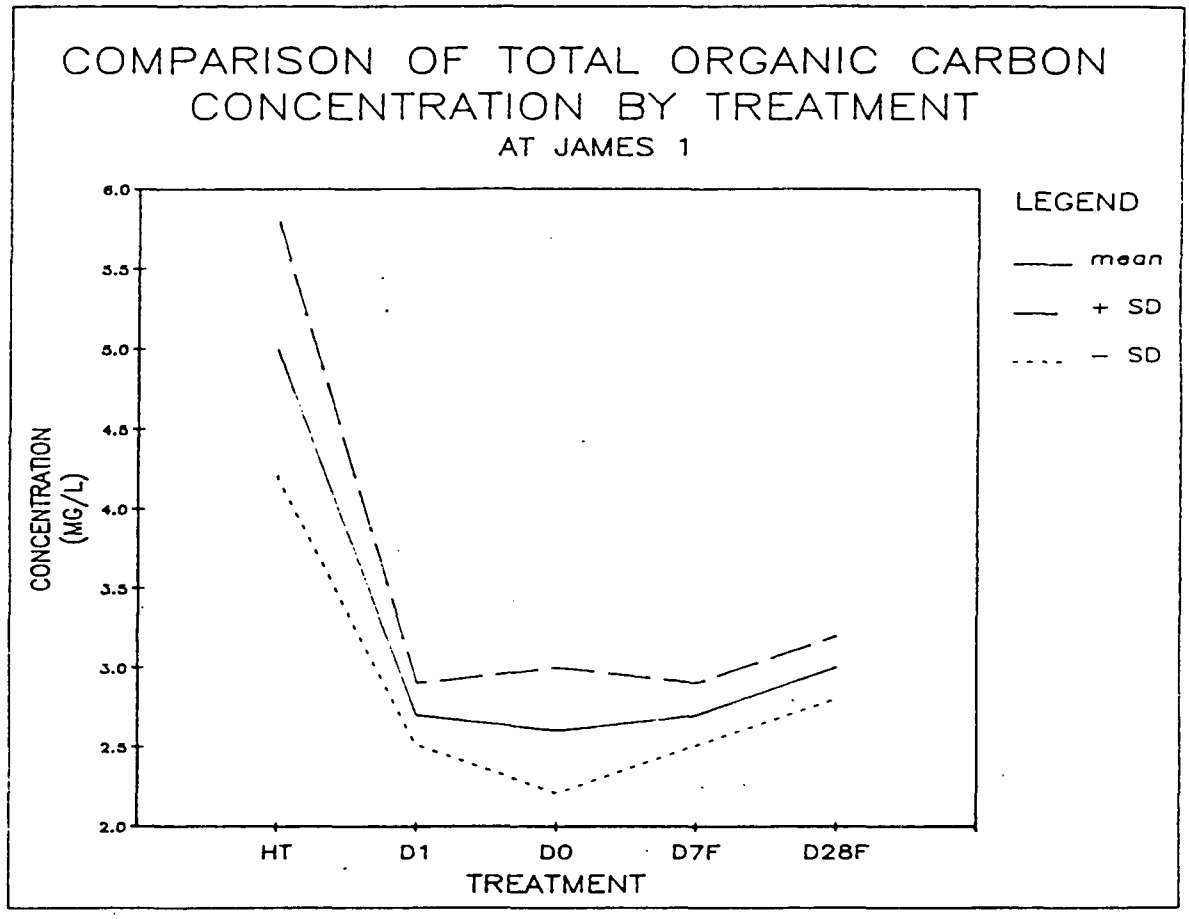
NONPARAMETRIC

TUKEY'S Mult. Comparisons	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F	DO	D1	HT	D7F
D1	.				.				*				.			
HT	.	.			.	.			.	#			.	.		
D7F	.	.	.		.	.	.		.	.	.		.	.	.	
D28F	.	.	.	.	.	.	.	.	#	.	#	*	*	.	*	.

* = Significant difference between means at alpha=0.05
. = No significant difference between means at alpha=0.05

DO= DAY 0 (CONTROL)
D1 = DAY 1
HT = EPA APPROVED HOLDING TIME, 28 DAYS WITH ACIDIFICATION
D7F = SAMPLE FROZEN FOR SEVEN DAYS
D28F = SAMPLE FROZEN FOR 28 DAYS

FIGURE 1



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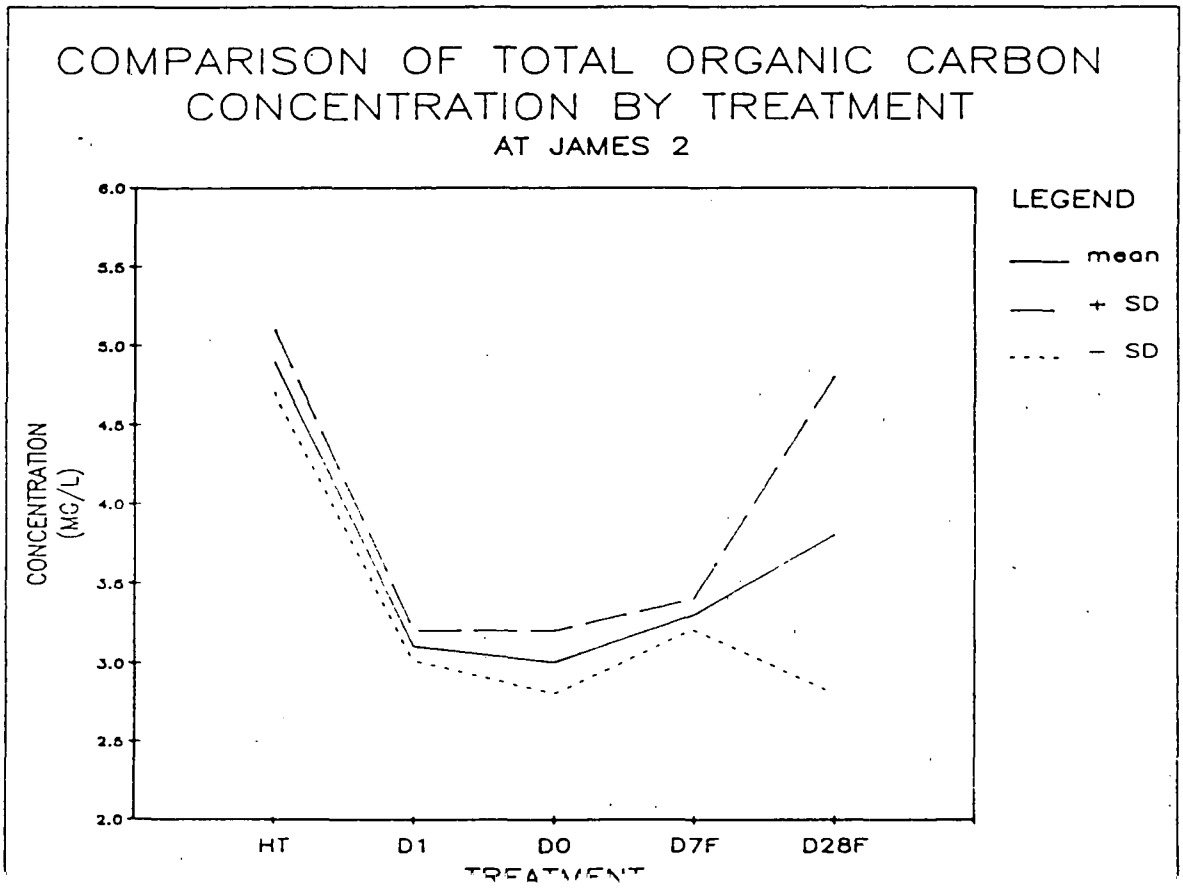

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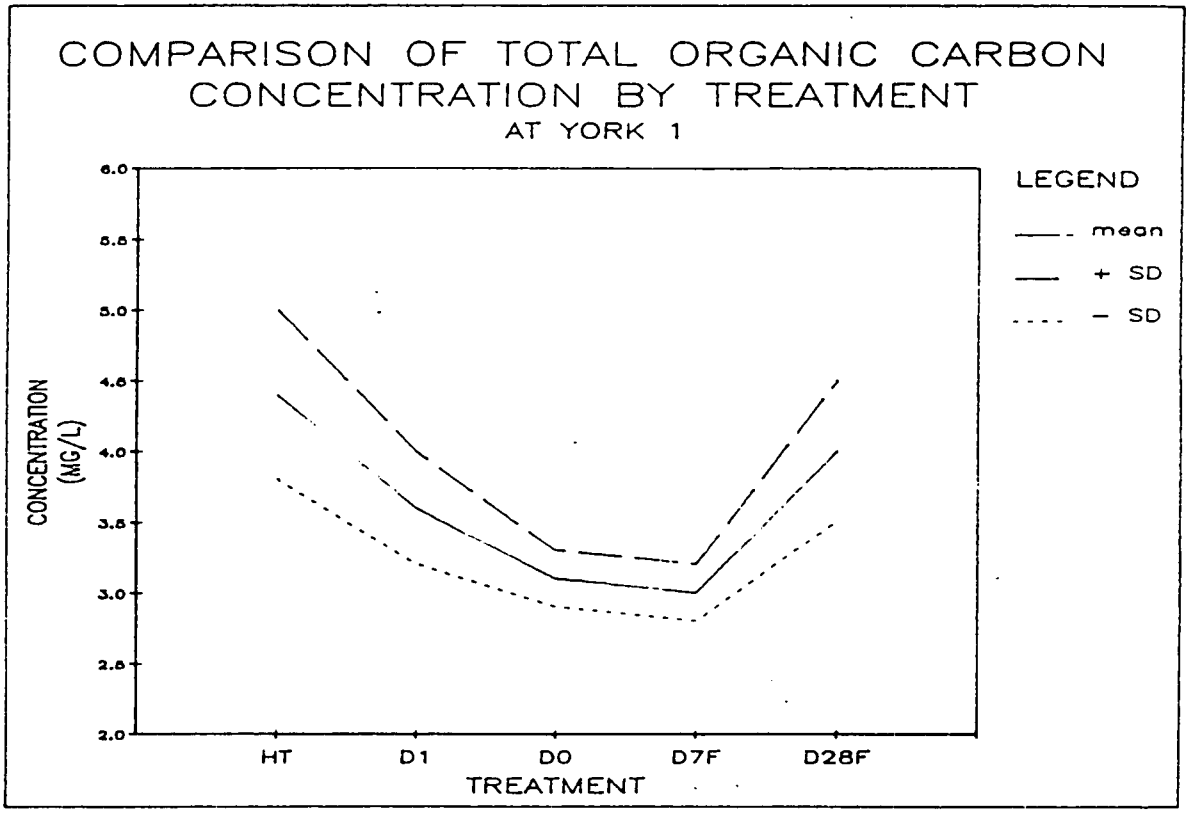

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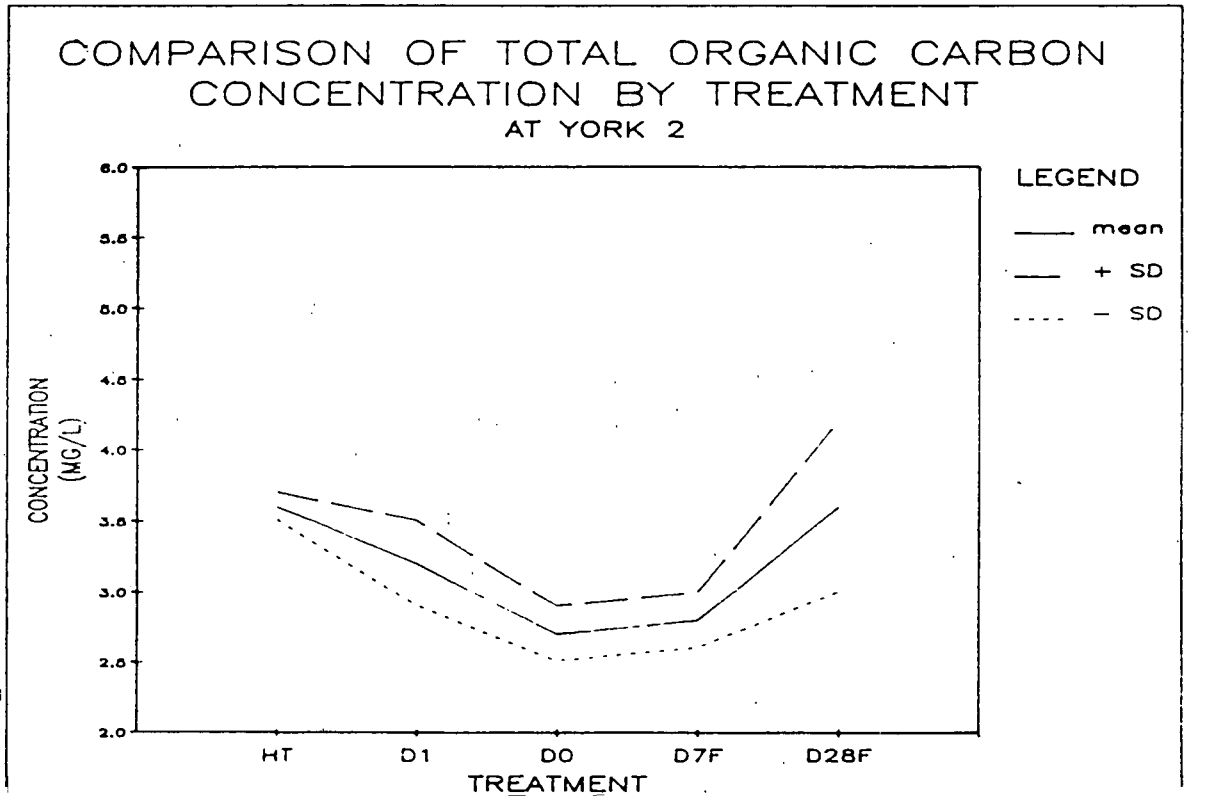

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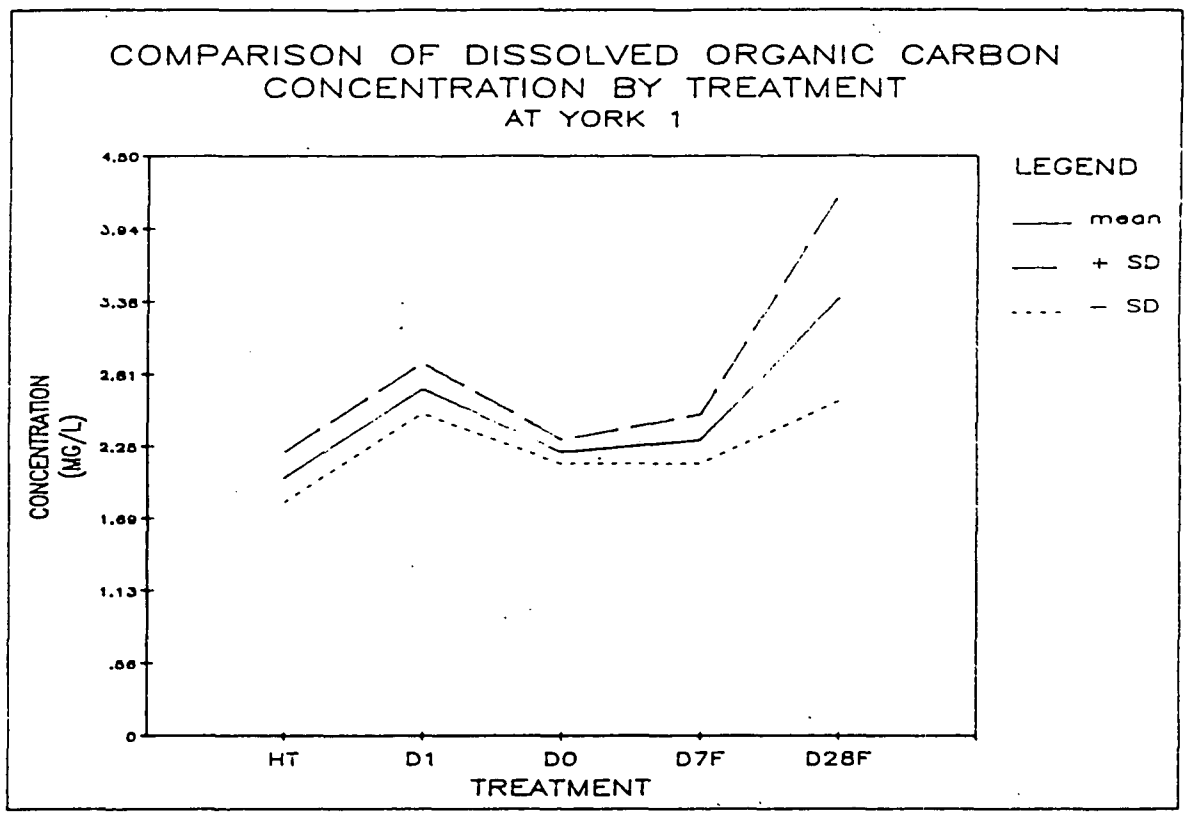

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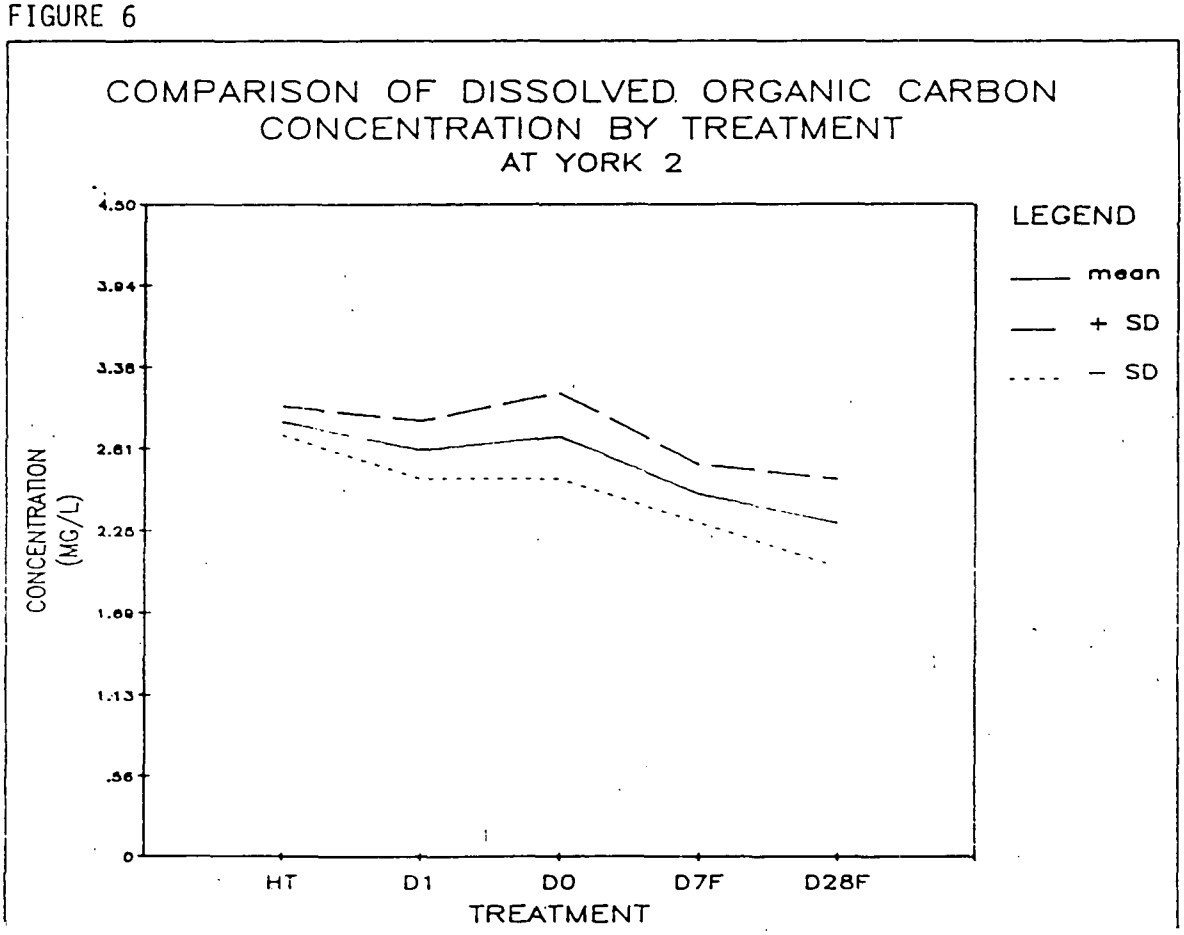

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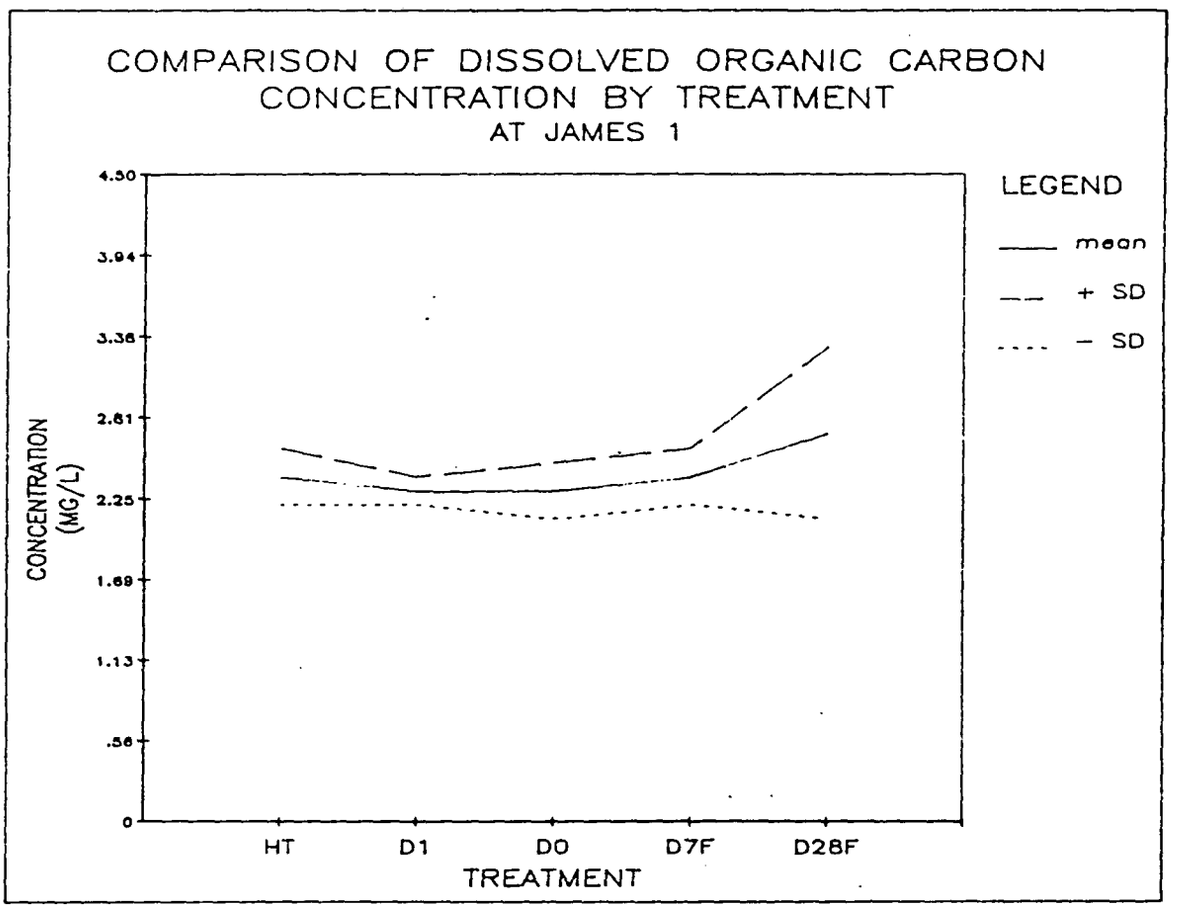

FIGURE 8