Assessment of the Effects of Holding Time on Various Water Quality Parameters

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Newfoundland Labrador

Government of Newfoundland & Labrador
Department of Environment and Conservation
Water Resources Management Division
St. John's, NL, A1B 4J6 Canada

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Assessment of the Effects of Holding Time on Various Water Quality Parameters

ABSTRACT

The Department of Environment and Conservation in Newfoundland and Labrador has identified that many of the water samples that are shipped to the National Laboratory for Environmental Testing in Burlington, Ontario are exceeding parameter holding times as prescribed by NLET's Schedule of Services. There is concern that the integrity of data is being compromised as a result of holding time exceedences. Ten parameters were identified as consistently failing to meet recommended holding times: total nitrogen, nitrate, total phosphorus, dissolved inorganic carbon, dissolved organic carbon, alkalinity, pH, specific conductivity, turbidity and color. This study was conducted to determine if the length of holding time has a significant effect on the concentration or level of any of the identified parameters. Water samples were collected from three water bodies in the province and the samples were analyzed at five different holding times. The study was conducted in two phases; the first phase of sampling was conducted in March 2009, and the second phase was conducted in October 2009, to observe whether or not seasonality had any effect on parameter concentrations at each holding time. The results of the study indicated that although parameter concentrations varied at different levels of holding time, none of the differences were significant at 0.05. This study is of particular relevance to the province of Newfoundland and Labrador because the results indicate that although recommended holding times cannot always be met, the analysis results for the ten parameters of interest are valid and representative of true water quality. Notwithstanding this conclusion, water samples should always be analyzed as soon as possible after collection.

1 INTRODUCTION

Holding times are the length of time a sample can be stored after collection and prior to analysis without significantly affecting the analytical results. Holding times vary with the analyte, sample matrix, and analytical methodology used to quantify the analytes concentration (Keith, 1991).

Water samples from approximately 79 representative freshwater monitoring stations across Newfoundland and Labrador (NL) are collected seasonally and shipped to the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario as part of a federal-provincial water quality monitoring agreement. Due to the remote locations of some monitoring stations and their distance from a courier service, many water samples submitted for analysis are not meeting the maximum recommended holding times prescribed by NLET. Parameter holding times prescribed by NLET are listed in **Appendix I** at the end of this report. Several commonly tested parameters (total nitrogen, nitrate, total phosphorus, dissolved inorganic carbon, dissolved organic carbon, alkalinity, pH, specific conductivity, turbidity and color) have short holding times of 24 to 48 hours. Samples collected in NL typically take two to five days to arrive at NLET, and even though great effort is made to ensure there are enough ice packs in the coolers to keep sample temperatures below $4^{o}C$, the lengthy holding times may be impacting data integrity. The Department of Environment and Conservation (ENVC) in NL must decide if parameter results are valid, despite the fact that holding times are being exceeded, and if other measures need to be explored to ensure the validity of the data.

This study involved the collection of five sets of water samples from each of three water bodies in the province. The samples were shipped to NLET immediately after collection so that analyses could begin within 48 hours. Five holding times were established for analysis: on the first day the samples arrived at the laboratory (T1), and on the 3^{rd}, 7^{th}, 10^{th} and 21^{st} days (T2, T3, T4 and T5 respectively) after the samples arrived. Parameter concentrations at T1 represent the control group to which parameter concentrations at all subsequent holding times were compared. One set of samples from each water body was analyzed at each holding time. The purpose of the study was to determine if concentrations and levels of the parameters of interest changed significantly as holding times increased. The study was conducted in two phases; the first phase of sampling was conducted in March 2009, and the second phase was conducted in October 2009, to see if seasonality had any effect on holding time. The results of this study will guide water sampling protocol for ENVC, particularly in determining whether more resources need to be directed toward sample preservation and enabling shorter holding times to be met.

2 METHODOLOGY

2.1 Data Collection

Five sets of water samples were collected at each of three water bodies in Newfoundland and Labrador on March 2, 2009; Quidi Vidi Lake, a highly developed urban watershed; Corduroy Brook, a moderately developed urban watershed; and Pinchgut Brook, a fairly pristine watershed with low development. A set of water samples consisted of four sample bottles to be analyzed for the following parameters:

Sample Container	Parameters Analyzed
1 x plastic bottle	alkalinity, pH, specific conductance, color, turbidity
1 x glass bottle	nitrates, total nitrogen
1 x glass bottle	DIC/DOC
1 x glass bottle	total phosphorus

The water samples were packed on ice packs in coolers and shipped to NLET immediately after collection. All samples arrived at lab within 48 hours of collection. Sample analysis commenced on the day the samples arrived, which was designated as holding time “day 1.” One set of samples from each water body was analyzed according to the following holding time intervals:

- Same day samples arrived at the laboratory(T1)
- 3 days after samples arrived at the laboratory(T2)
- 7 days after samples arrived at the laboratory(T3)
- 10 days after samples arrived at the laboratory(T4)
- 21 days after samples arrived at the laboratory(T5)

All samples were stored in refrigerators and kept below 4^oC from the time they arrived at the laboratory until they were analyzed. The study was repeated on October 5, 2009 to determine if seasonality had an effect on parameter concentrations at the various holding times. An extra set of five samples was collected at Quidi Vidi Lake in March and October for quality assurance/ quality control comparison.

2.2 Data Analysis

An examination of the analytical results of each water quality parameter indicated that almost all distributions were positively skewed, and the data included outliers, missing values and less than detection results. Lack of normal distribution along with the presence of outliers, missing values and censored data make parametric statistical analysis unsuitable (Helsel and Hirsch, 2002).The data for each parameter were therefore rank-transformed so that parametric analytical methods could be utilized. The data were analyzed using a three-way ANOVA general linear method (GLM). This method was selected because there were three factors (holding time, location and month) and all factors are categorical. The test statistic is the F-test, testing if the means of the groups formed by values of the independent variables are different enough not to have occurred by chance (Wheater and Cook, 2000). If the group means do not differ significantly, then it is inferred that the independent variables did not have an effect on the dependent variable, or in this case, that holding time, location and month did not have a significant affect on parameter concentration. If the F-test shows a relationship, then a multiple comparison test of significance can be used to examine the impact of each independent variable on the relationship (Garson, 2009). In this study, Dunnett's method compared the means of parameter concentration at each holding time T2, T3, T4 and T5 to the mean of the control holding time T1, relative to location and season. All factors are categorical with holding times represented as T1, T2, T3, T4 and T5; sample locations defined as Quidi Vidi Lake, Corduroy Brook and Pinchgut Brook; and months, March and October. This analysis of variance on the ranks of the data tested the null hypothesis that there was no difference between group means, and the alternative hypothesis that at least one group mean was different. The key ANOVA assumptions were upheld by the distributions of the ranked data, that the groups formed by the independent variables were normality distributed and had relative homogeneity of variance. Minitab 15 software was used to compute the GLM analysis with Dunnett's comparison. A large F-value would indicate a high degree of variability between means, and would generate a small p-value. The decision to reject the null hypothesis was established at $p\text{-value} < 0.05$.

The final phase of analysis for this study involved the comparison of Quidi Vidi Lake data with the duplicate set of data collected as part of quality assurance/quality control. For this analysis, a paired t-test was selected because each data point is paired with another. The paired t-test compares two paired groups of data to determine if their differences are significantly different from zero (Helsel and Hirsch, 2002). The null hypothesis was set as: there is no difference in parameter concentrations at each holding time interval between the two groups. The level of significance was established at 0.05.

3 RESULTS AND DISCUSSION

Data for each parameter were arranged by holding time, location and month as shown in the example below.

Total Nitrogen

	T1	**T2**	**T3**	**T4**	**T5**
QVL-M	0.847	0.822	0.800	0.776	0.802
QVL-O	0.715	0.756	0.721	0.696	0.688
QVLQA-M	0.828	0.807	0.813	0.802	0.816
QVLQA-O	0.725	0.796	0.690	0.686	0.683
CB-M	0.901	0.882	0.884	0.862	*
CB-O	0.575	*	0.495	0.475	0.481
PB-M	0.299	0.269	0.291	0.277	0.284
PB-O	0.222	0.229	0.212	0.209	0.196

* = no data available

QVL-M	Quidi Vidi Lake-March
QVL-O	Quidi Vidi Lake-October
QVLQA-M	Quidi Vidi Lake QAQC-March
QVLQA-O	Quidi Vidi Lake QAQC-October
CB-M	Corduroy Brook-March
CB-O	Corduroy Brook-October
PL-M	Pinchgut Brook-March
PL-O	Pinchgut Brook-October

Holding Times:

T1 = analysis conducted on the day the samples arrived at the laboratory

T2 = analysis conducted on the 3rd day after the samples arrived at the laboratory

T3 = analysis conducted on the 7th day after the samples arrived at the laboratory
T4 = analysis conducted on the 10th day after the samples arrived at the laboratory
T5 = analysis conducted on the 21st day after the samples arrived at the laboratory

3.1 Graphs

The data for each parameter were graphed by scatterplot and boxplot to give an initial observation of the effect of holding time on parameter concentration, as well as an indication of data distribution. A scatterplot and boxplot for total nitrogen concentration at each holding time, location and season is shown in **Figures 1 and 2**:

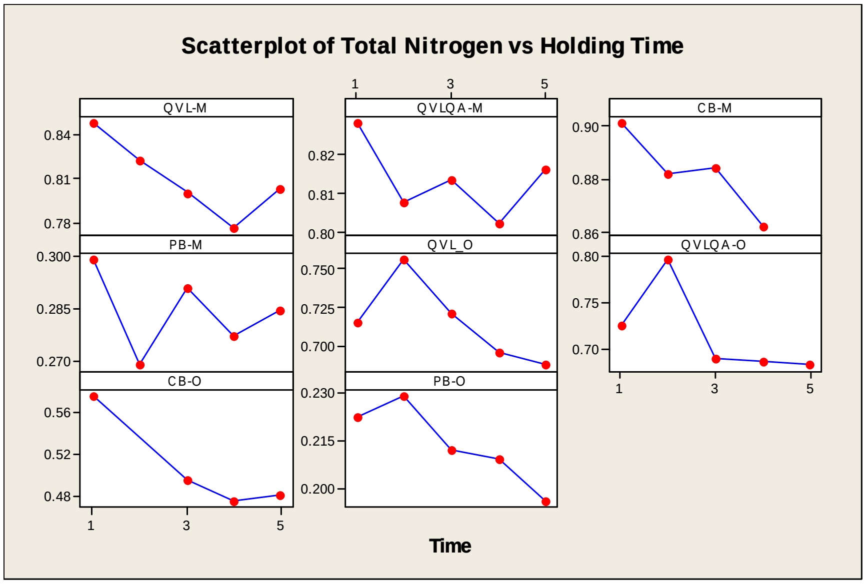


Figure 1: Scatterplot of Total Nitrogen Concentrations

The scatterplots show decreasing total nitrogen concentrations for most data sets.

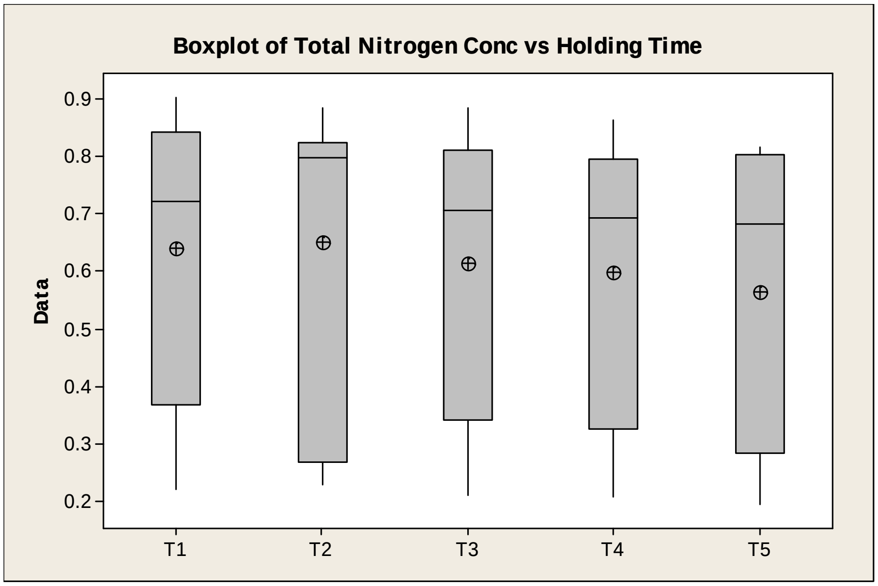


Figure 2: Boxplot of Total Nitrogen Concentrations

The boxplots demonstrate that the means for each holding time are similar in location and show a slight decrease as holding times increase; all distributions are asymmetric.

Scatterplots and boxplots for each parameter are found in **Appendices II and III** respectively. A summary of scatterplot and boxplot observations are found in **Table 1**:

Table 1: Summary of Scatterplot and Boxplot Observations

Parameter	**Scatterplot Observation**	**Boxplot Observation**
Total Nitrogen	Overall decreasing concentration per increasing holding time	Similar location of means for all groups; slight decrease as holding times increase; asymmetric distributions
Nitrate	Slight increasing concentration per holding time in March; no change in October	Similar location of mean; asymmetric distributions
Total Phosphorus	Small variations in concentration per holding time	Similar location of mean; asymmetric distributions
Dissolved Inorganic Carbon	Concentrations increase per holding time in March, decrease in October	Identical location of mean; asymmetric distributions
Dissolved Organic Carbon	Decreasing concentration at 10 days in March, small variations per holding time in October	Similar location of mean; an outlier value at each holding time; asymmetric distributions

Alkalinity	Small variations per holding time	Small variations in mean between holding times; asymmetric distributions
pH	Decreasing levels at first, then increasing	Decrease in mean at 3 days; asymmetric distributions
Specific Conductance	Small variations in levels per holding time	Similar location of mean; asymmetric distributions
Turbidity	General decreasing concentration between 1- 7 days	Small variation in location of means; outlier at 10 days; asymmetric distributions
Color	Decreasing color per holding time in March; small variations per holding time in October	Slight decrease in mean per holding time; asymmetric distributions

3.2 General Linear Method and Dunnett's Comparison

The GLM and Dunnett's comparison, using the F-statistic, generated p-values that would serve as the basis for rejecting the null hypothesis. A p-value < 0.05 was significant evidence to reject the null hypothesis. A summary of p-values generated by the GLM and Dunnett's comparison is shown in **Table 2**:

Table 2: Summary of p-values from GLM Analysis and Dunnett's Comparison

	GLM			**Dunnett's**			
Parameter	**Time**	**Location**	**Month**	**T2**	**T3**	**T4**	**T5**
Total Nitrogen	0.371	0.000	0.000	0.9992	0.9094	0.5005	0.3739
Nitrate	0.552	0.000	0.000	0.4102	0.8882	0.8191	1.0000
Total Phosphorus	0.393	0.000	0.042	0.3493	0.3282	0.9918	0.8214
DIC	0.814	0.000	0.032	1.0000	0.9997	0.7464	0.9993
DOC	0.670	0.000	0.001	0.9976	0.6420	0.9998	0.9849
Alkalinity	0.859	0.000	0.046	1.0000	0.9963	0.7775	0.9999
pH	0.763	0.000	0.000	0.5996	0.9988	0.9584	1.0000
Specific Conductance	0.710	0.000	0.000	0.8733	0.9215	0.9672	0.9999
Turbidity	0.852	0.000	1.000	0.9942	0.8820	0.9414	0.9848
Color	0.289	0.000	0.005	0.9166	0.8903	0.6545	0.3625

The computations for GLM and Dunnett's comparison for each parameter are found in **Appendix IV.** Residual plots were examined for each parameter to verify that there were no violations of the assumptions of normality and equal variance. The residual plots for each parameter are found in **Appendix V.**

3.3 Paired t-Test

The final statistical analysis was computed on the parameter concentrations of the Quidi Vidi Lake water samples compared to the Quidi Vidi Lake duplicate samples, as part of quality assurance/quality control protocol. A p-value < 0.05 was significant evidence to reject the null hypothesis. A summary of p-values generated by the paired t-test is found in **Table 3**:

Table 3: Summary of p-Values from Paired t-Test on QVL versus QAQC Data

Parameter	**Mean Difference**	**P-Value**
Total Nitrogen	0.300	0.757
Nitrate	0.111	0.889
Total Phosphorus	0.300	0.690
DIC	2.700	0.002
DOC	0.300	0.886
Alkalinity	0.310	0.797
pH	0.389	0.542
Specific Conductance	0.670	0.572
Turbidity	2.333	0.026
Color	0.110	0.939

The paired t-test computation for each parameter is found in **Appendix VI.**

4 CONCLUSIONS

The p-values generated from statistical analysis on the data using the GLM and Dunnett's comparison indicated that there was not enough evidence to reject the null hypothesis for any holding time for any parameter monitored. Even though small variations in concentration were evident in the numerical data, scatterplots and boxplots, they were not significant at $\alpha = 0.05$. This study demonstrates that concentrations for the ten parameters identified did not change significantly when analyzed up to 21 days after the samples had arrived at the laboratory.

Parameter concentrations did vary significantly by location, for all parameters at all locations. This is an expected result because Quidi Vidi Lake is the receiving water for a heavily developed urban watershed, while Corduroy Brook is in a moderately developed watershed and Pinchgut Brook is fairly pristine. More importantly, this study concludes that variable parameter concentrations between locations had no impact on holding times in this study.

Seasonality had an impact on turbidity values in this study, as turbidity levels were significantly higher in March than in October. This is reflective of climate conditions at the time of sampling, as rainfall, snowmelt and run-off from road salting operations influenced high turbidity levels in March. The variable turbidity levels had no impact on holding times in this study. No other parameters were significantly influenced by the month of collection, however variability for total phosphorus concentrations and alkalinity levels are approaching significant levels. These seasonal variations do not appear to have any impact on parameter concentrations at each holding time.

The paired t-test on duplicate sets of water samples from Quidi Vidi Lake identified significant differences for two parameters, dissolved inorganic carbon and turbidity. This test, however, holds little power with a very small sample size of two.

In summary, this study concludes that the range of holding times of two to five days that typically elapse between sample collection and laboratory analysis is not affecting data integrity for water samples collected as part of the Canada-Newfoundland and Labrador Water Quality Monitoring Agreement. That being stated, ENVC will continue to strive to reduce holding times to a most practical level, and will continue to follow strict protocols for sample storage and handling.

5 RECOMMENDATIONS

There was no replication in this study. Individual parameters from one sample collected at Quidi Vidi Lake, from one sample collected at Corduroy Brook and from one sample collected at Pinchgut Brook were analyzed at each holding time. The results of this study may have been more reliable if parameters from multiple samples *from each water body* were analyzed at each holding time. Statistical analysis involving replication and interaction could then have been used to interpret the results. ENVC may conduct a second holding times study in the near future, which will include replication.

6 REFERENCES

Keith, Lawrence H.1991. Environmental Sampling and Analysis: a practical guide.

Garson, David G.2009. Univariate GLM, ANOVA, and ANCOVA: Stat notes North Carolina State University http://faculty.chass.ncsu.edu/garson/PA765/anova.htm Copyright 1998, 2008, 2009 by G. David Garson

Helsel, D.R. and Hirsch, R.M.2002. Statistical Methods in Water Resources.

Wheater, C.P. and Cook, P.A. 2000. Using Statistics to Understand the Environment.

Appendices

Appendix I

NLET Parameter Holding Times

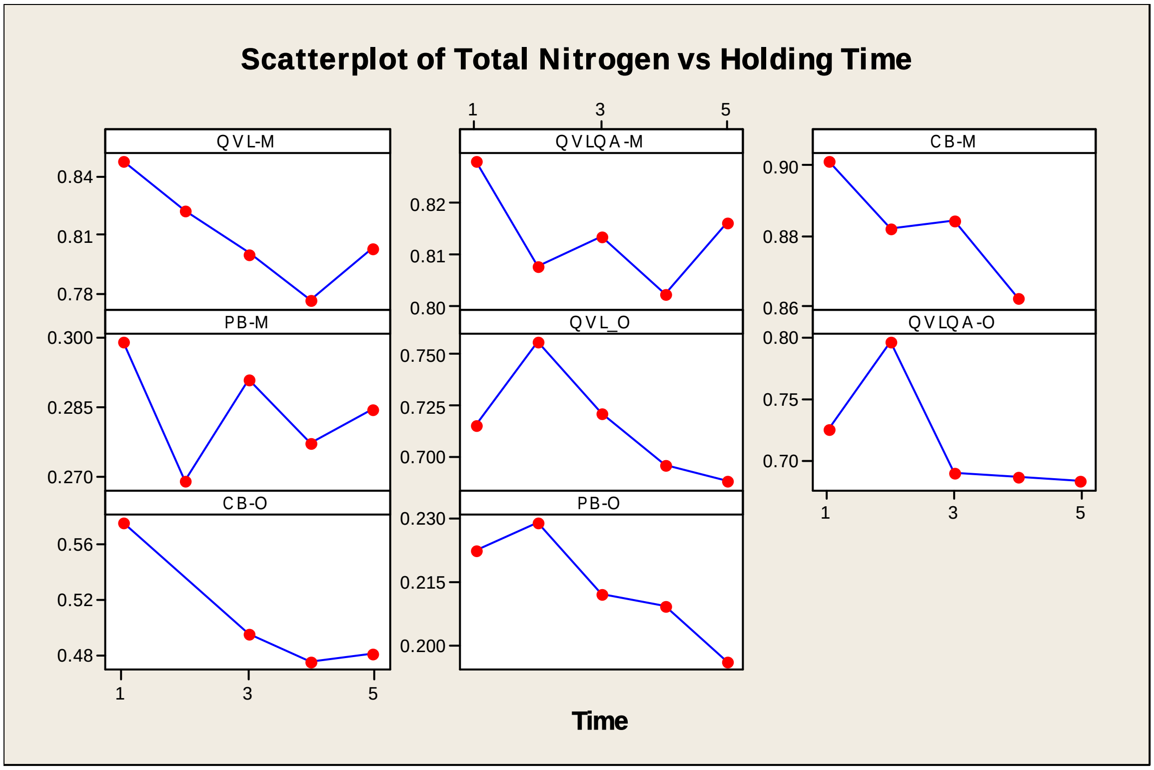
NLET Parameter Holding Times

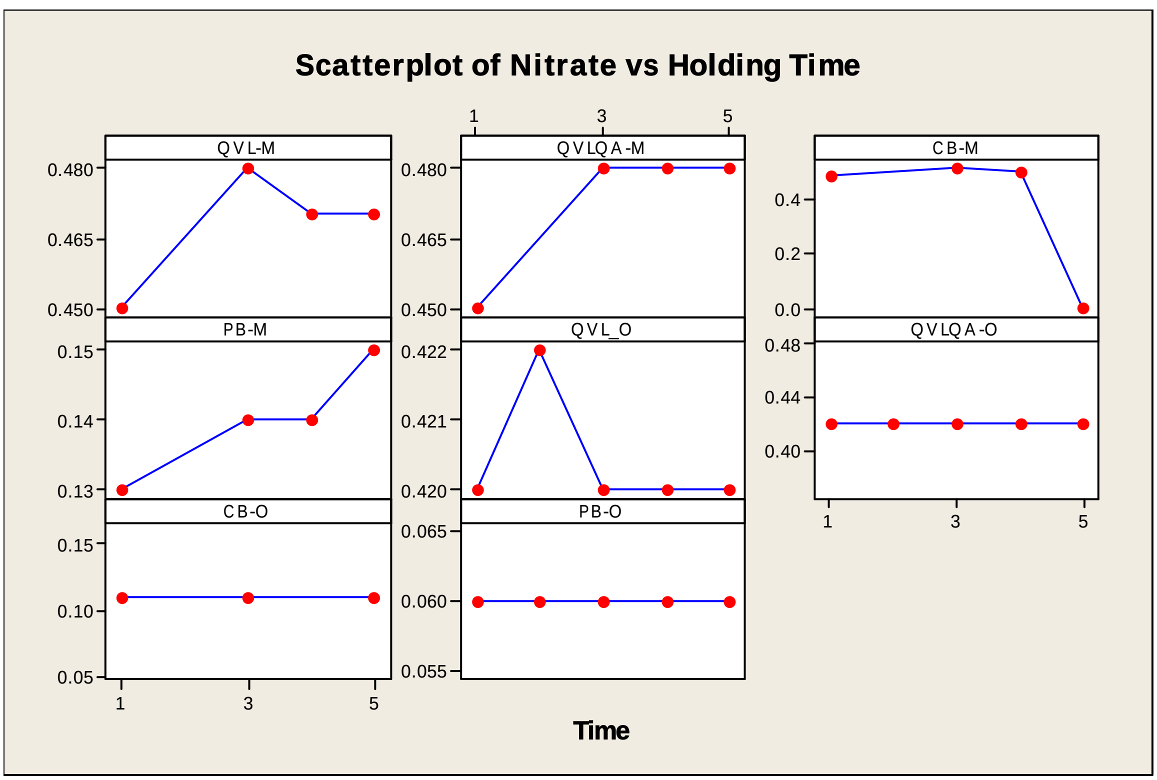
Parameter or Grouping	Bottle Type	Holding Time
Major Ions		
Acidity	500ml polyethylene, round	24 hours
Alkalinity	500ml polyethylene, round	24 hours
Flouride	500ml polyethylene, round	28 days
Chloride	500ml polyethylene, round	28 days
Sulphate	500ml polyethylene, round	28 days
Silica	500ml polyethylene, round	28 days
Calcium	500ml polyethylene, round	8 weeks
Magnesium	500ml polyethylene, round	8 weeks
Sodium	500ml polyethylene, round	8 weeks
Potassium	500ml polyethylene, round	8 weeks
Physicals		
pH	500ml polyethylene, round	24 hours
Conductivity	500ml polyethylene, round	28 days
Colour	500ml polyethylene, round	48 hours
Turbidity	500ml polyethylene, round	24 hours
*Nutrients		
Ammonia	120ml glass, round	24 hours
Nitrate-Nitrite	120ml glass, round	24 hours
Total Khjeldahl Nitrogen	120ml glass, round	24hrs or 7 days if acidified
Total Nitrogen	120ml glass, round	24 hours
Soluble Reactive Phosphorus	120ml glass, round	48 hours
DIC/DOC	120ml glass, round	24 hours
Total Phosphorus	125ml glass, square	1 year
Metals		
Extractable Metals	125ml polyethylene, round	6 months
Total Metals	125ml polyethylene, round	6 months
Total Recoverable Metals	125ml polyethylene, round	6 months
Bacteriological	125ml plastic	24 hours

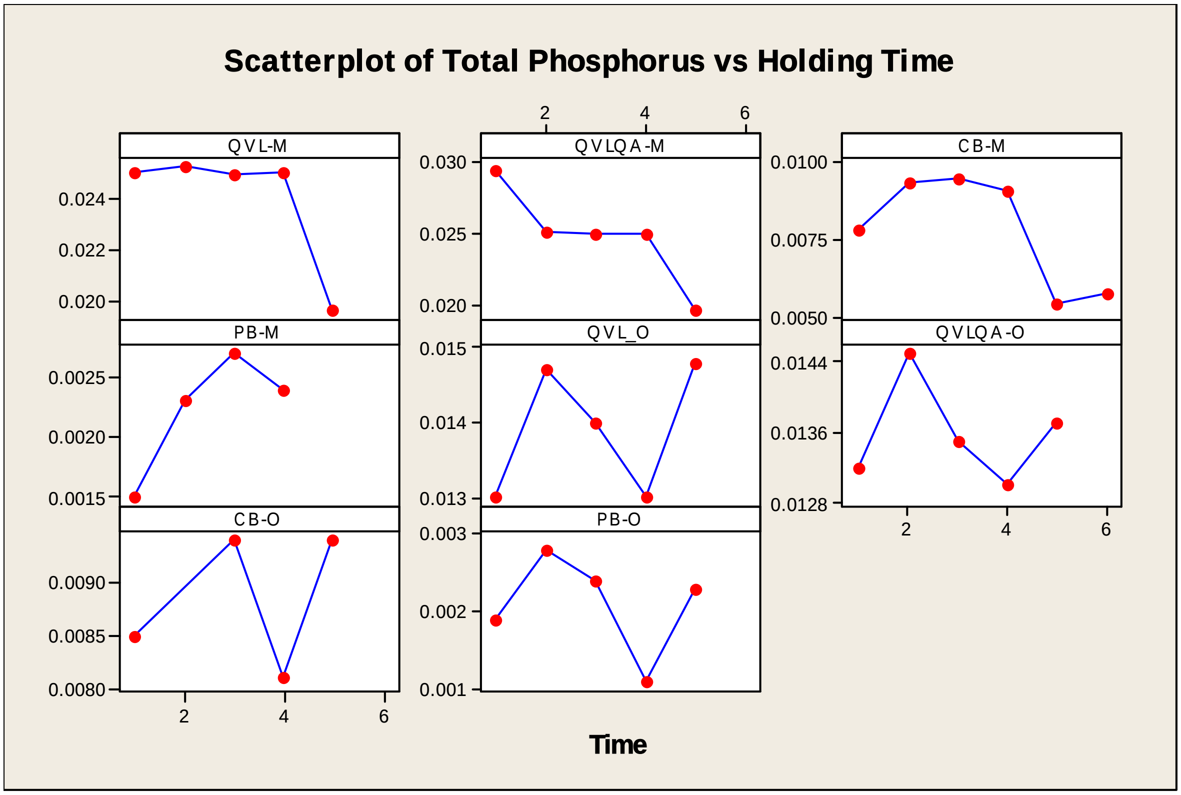
- For nutrient parameters, the bottle should be filled to the rim to minimize sample degradation.

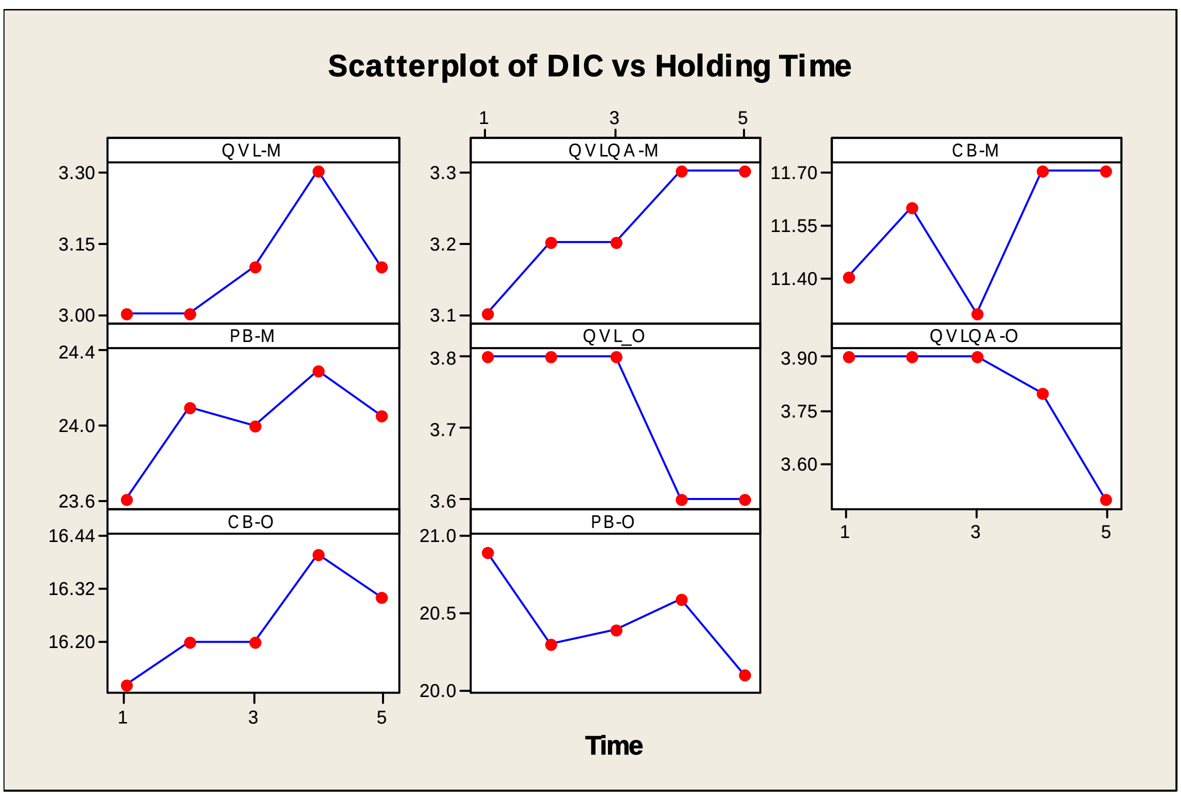
Appendix II

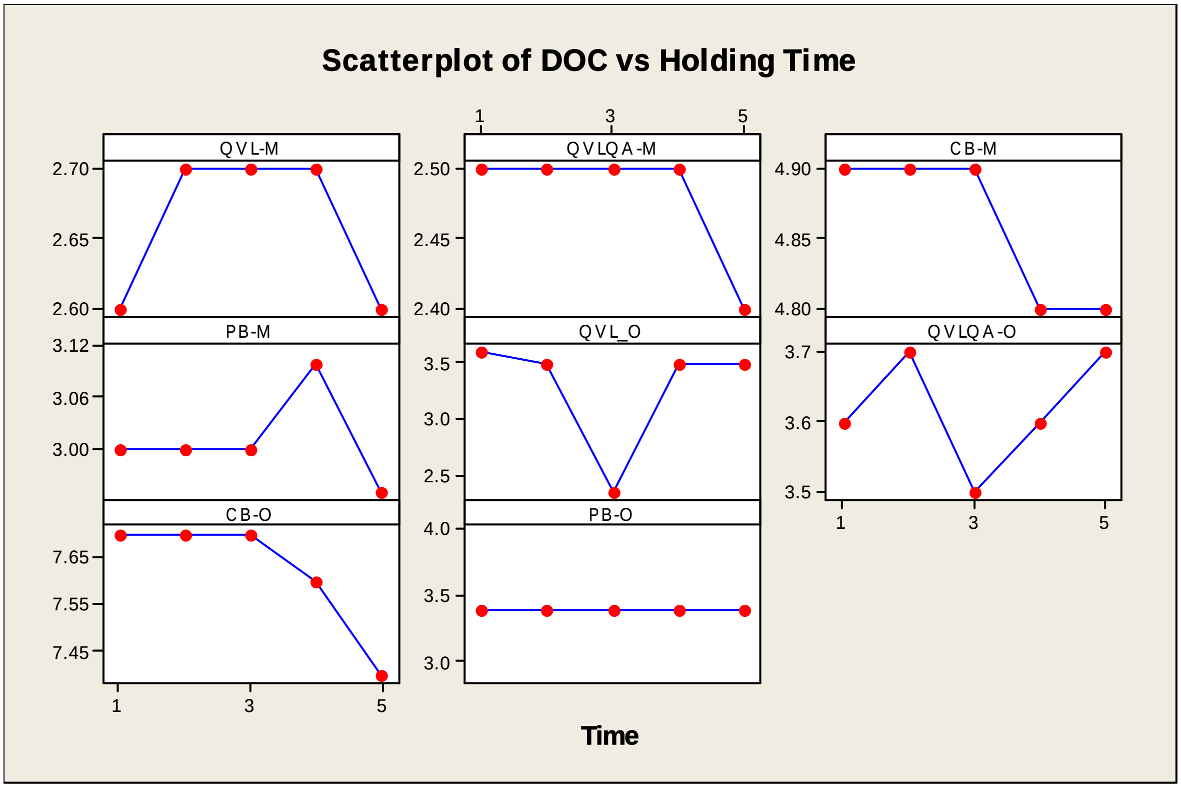
Scatterplots of Parameters versus Holding Time

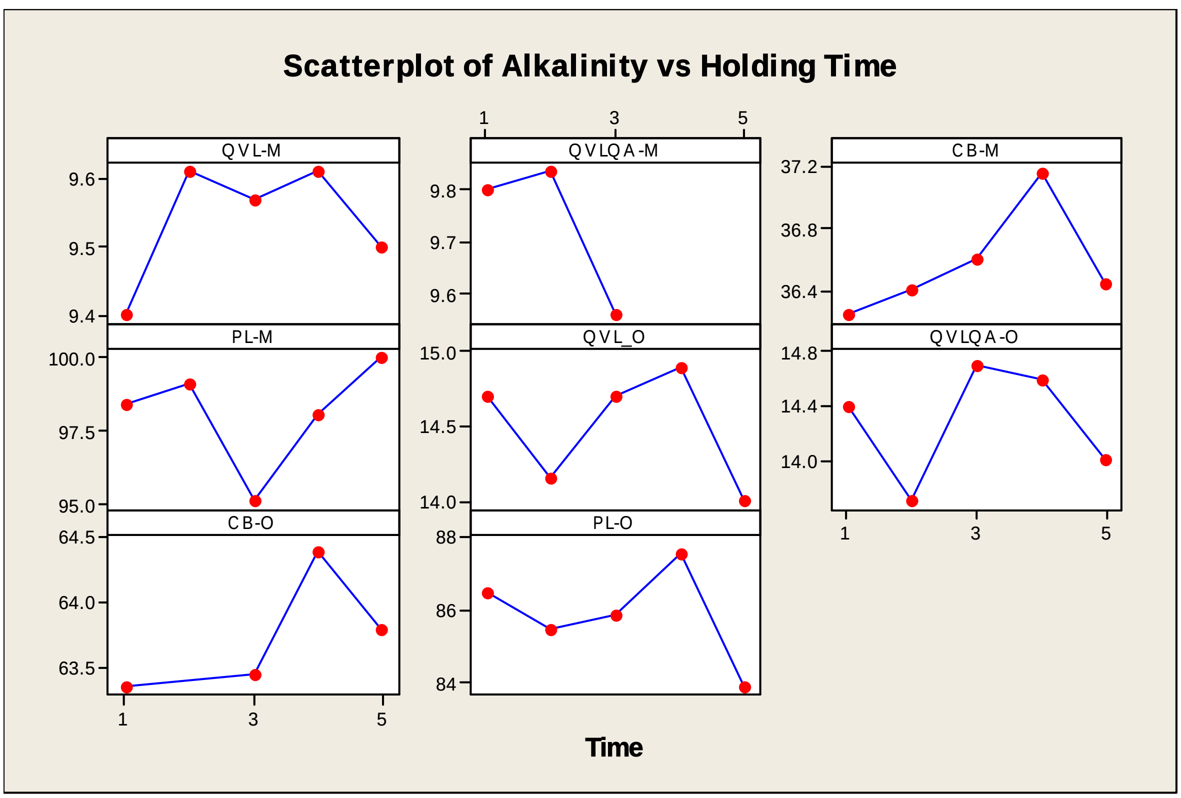

Scatterplot of Total Nitrogen vs Holding Time
QVL-M
QVLQA-M
CB-M
PB-M
QVL_O
QVLQA-O
CB-O
PB-O
Time

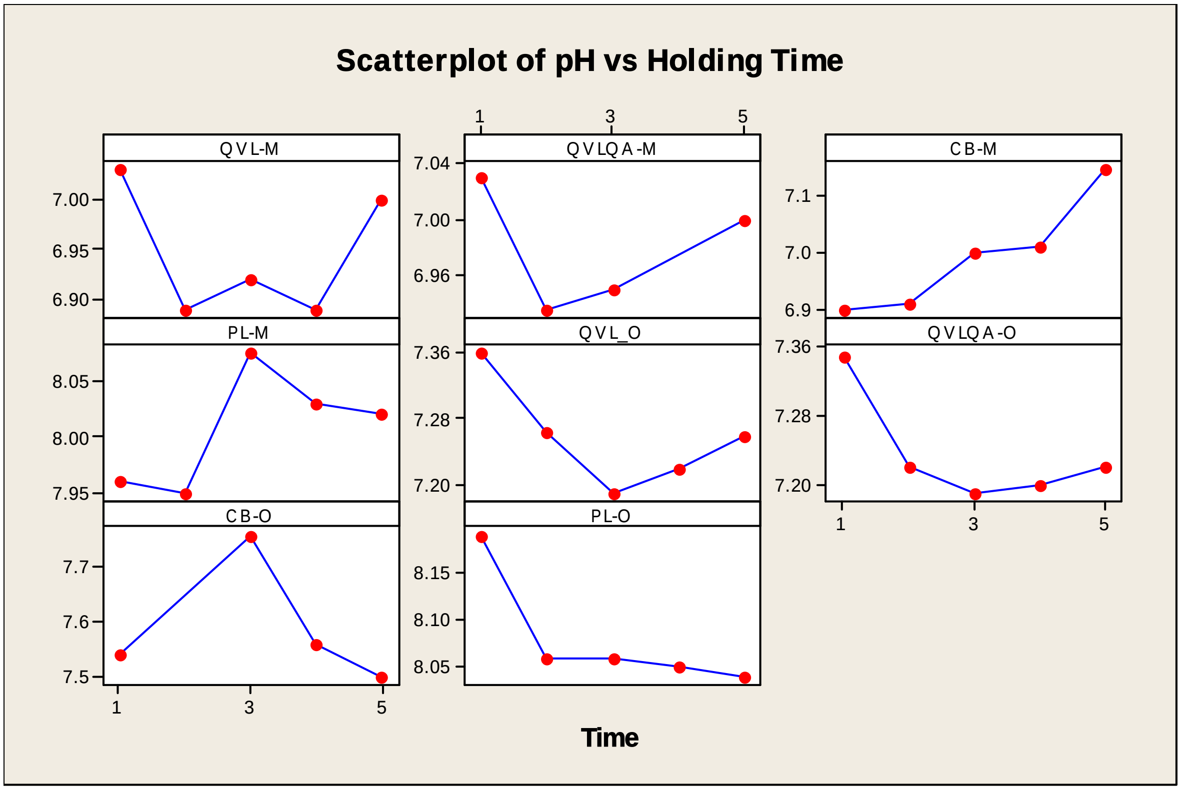

Scatterplot of Nitrate vs Holding Time
QVL-M
QVLQA-M
CB-M
PB-M
QVL_O
QVLQA-O
CB-O
PB-O
Time

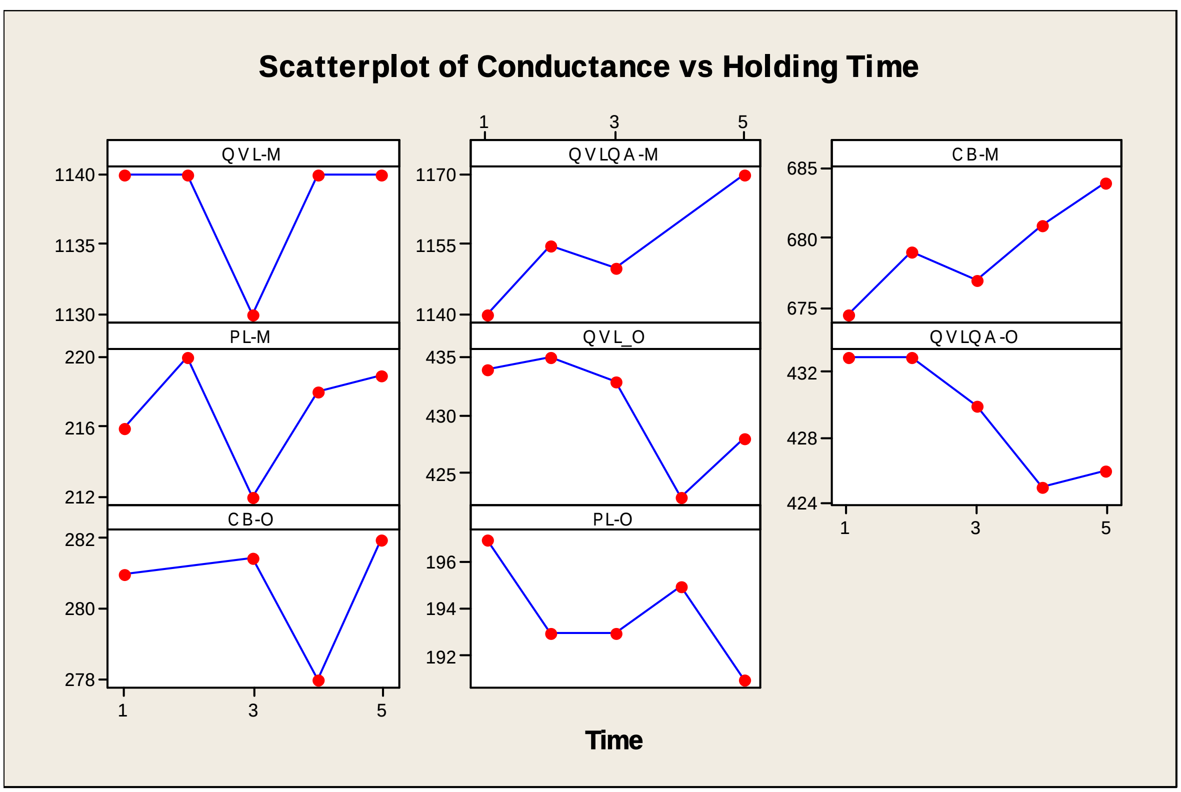

Scatterplot of Total Phosphorus vs Holding Time
QVL-M
QVLQA-M
CB-M
PB-M
QVL_O
QVLQA-O
CB-O
PB-O
Time

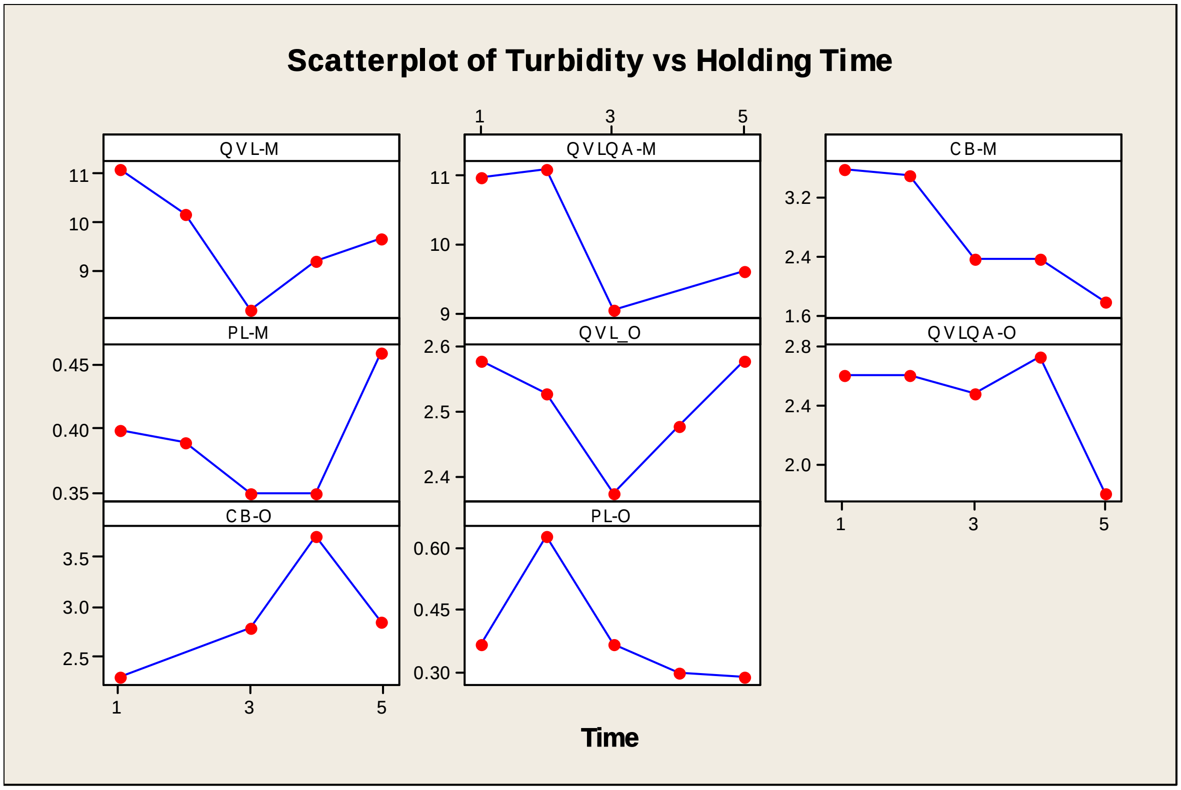

Scatterplot of DIC vs Holding Time
QVL-M
QVLQA-M
CB-M
PB-M
QVL_O
QVLQA-O
CB-O
PB-O
Time

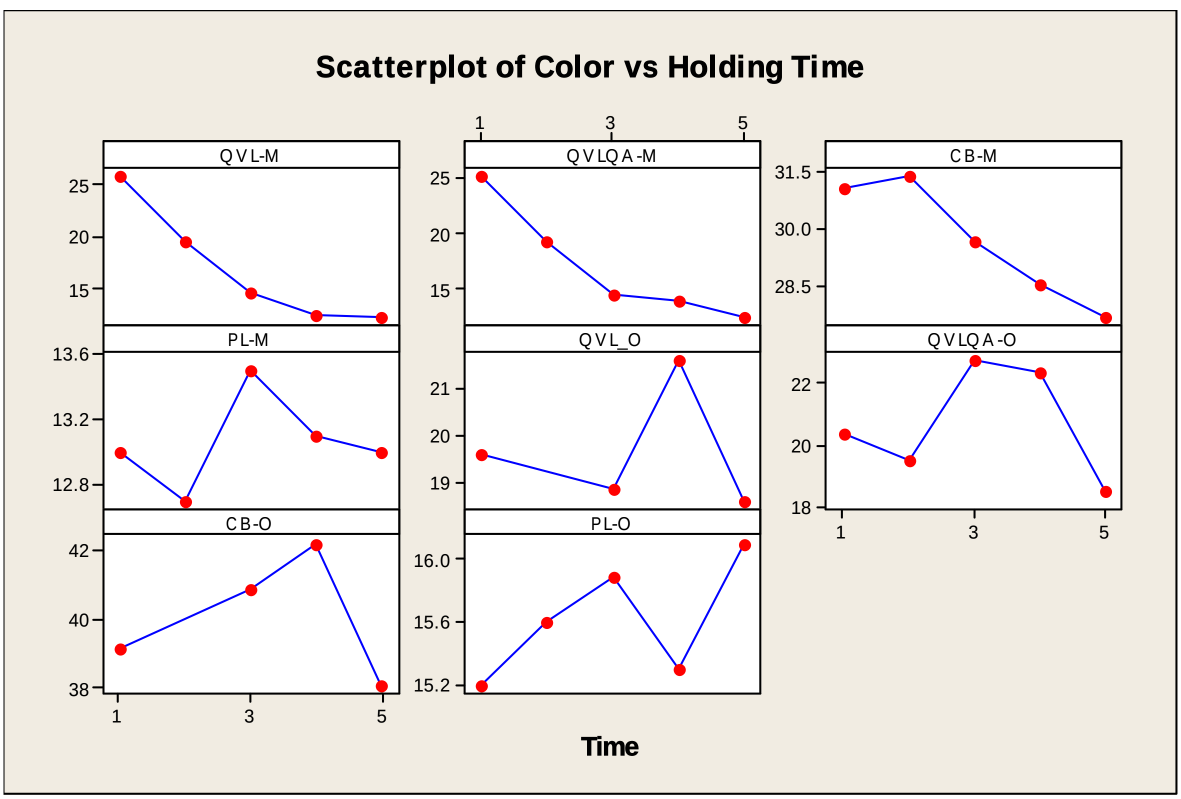

Scatterplot of DOC vs Holding Time
QVL-M
QVLQA-M
CB-M
PB-M
QVL_O
QVLQA-O
CB-O
PB-O
Time


Scatterplot of Alkalinity vs Holding Time
QVL-M
QVLQA-M
CB-M
PL-M
QVL_O
QVLQA-O
CB-O
PL-O
Time


Scatterplot of pH vs Holding Time
QVL-M
QVLQA-M
CB-M
PL-M
QVL_O
QVLQA-O
CB-O
PL-O
Time

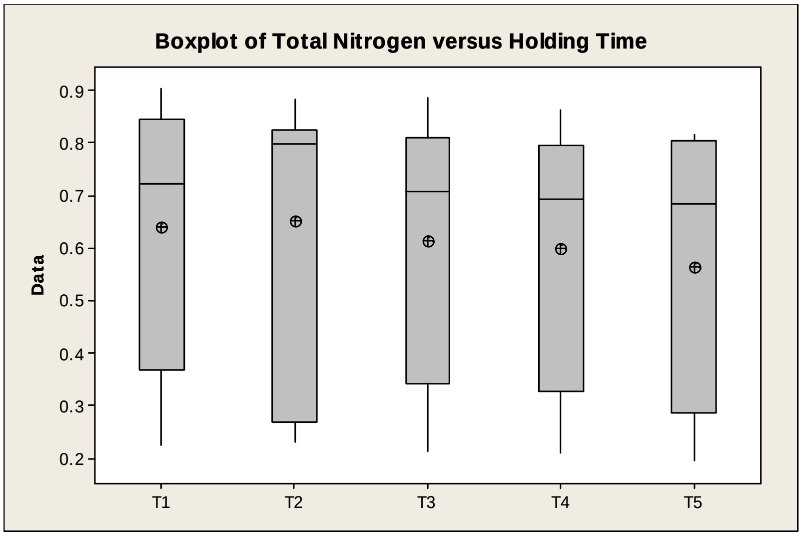

Scatterplot of Conductance vs Holding Time
QVL-M
QVLQA-M
CB-M
PL-M
QVL_O
QVLQA-O
CB-O
PL-O
Time

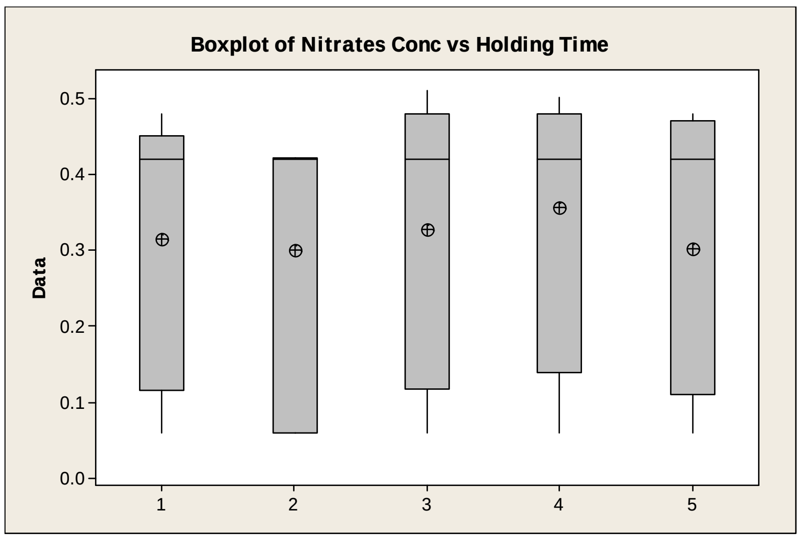

Scatterplot of Turbidity vs Holding Time
QVL-M
QVLQA-M
CB-M
PL-M
QVL_O
QVLQA-O
CB-O
PL-O
Time


Scatterplot of Color vs Holding Time
QVL-M
QVLQA-M
CB-M
PL-M
QVL_O
QVLQA-O
CB-O
PL-O
Time

Appendix III

Boxplots of Parameters versus Holding Time


Boxplot of Total Nitrogen versus Holding Time
Data
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
T1
T2
T3
T4
T5


Boxplot of Nitrates Conc vs Holding Time
Data
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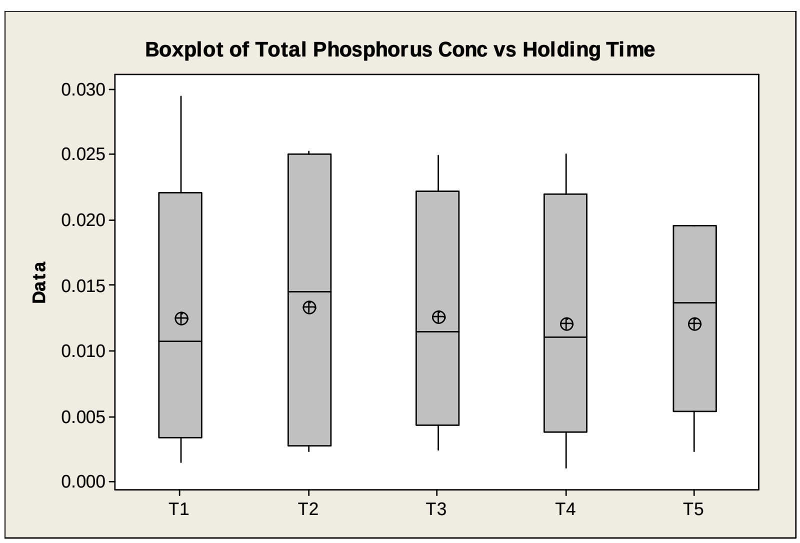
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5


Boxplot of Total Phosphorus Conc vs Holding Time
Data
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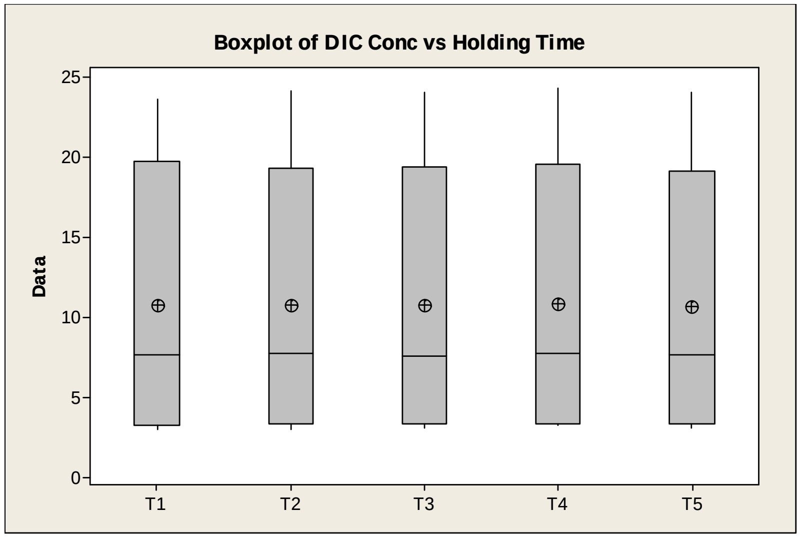
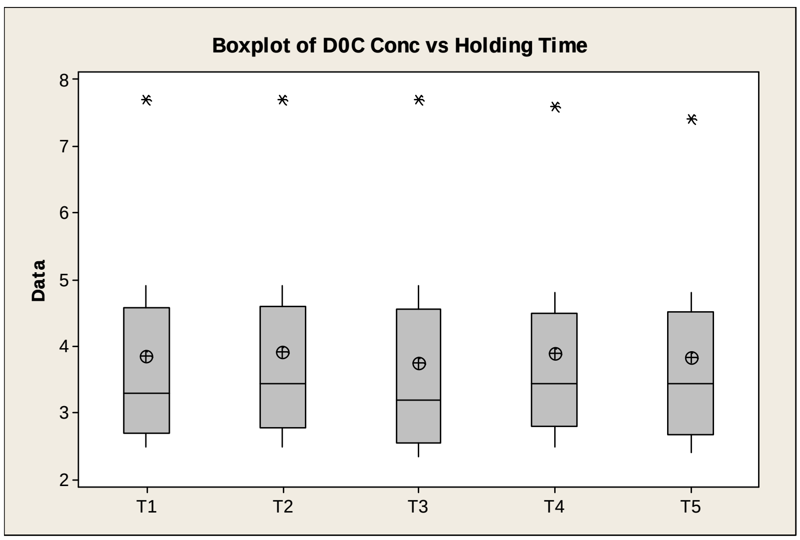
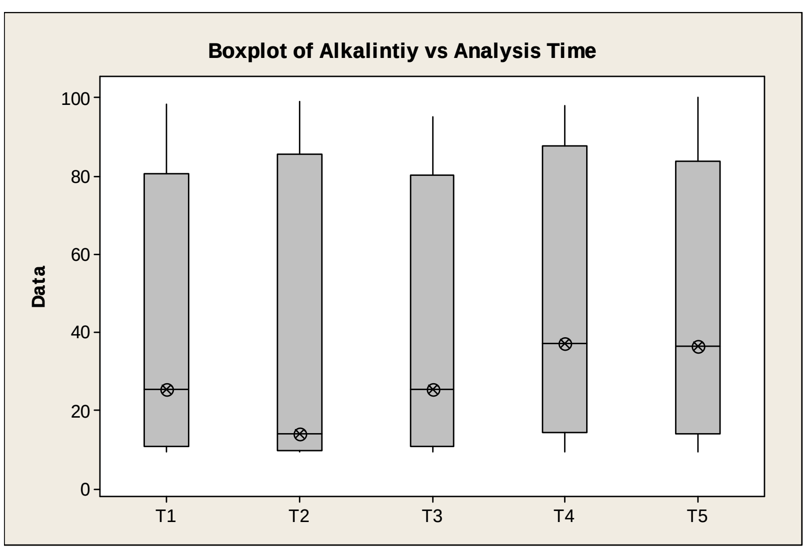
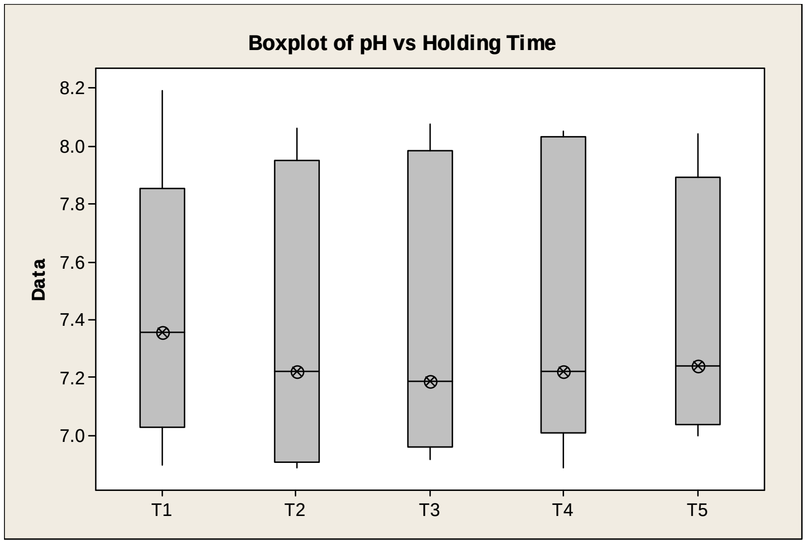
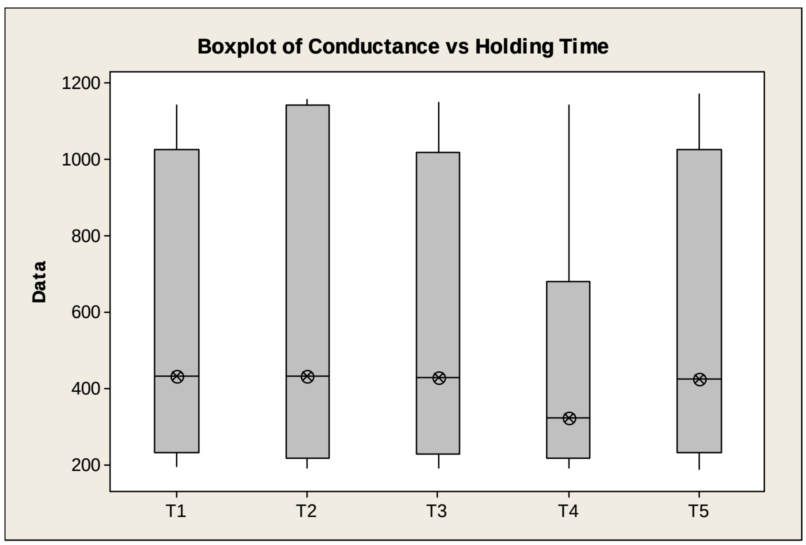
0.025
0.020
0.015
0.010
0.005
0.000
T1
T2
T3
T4
T5

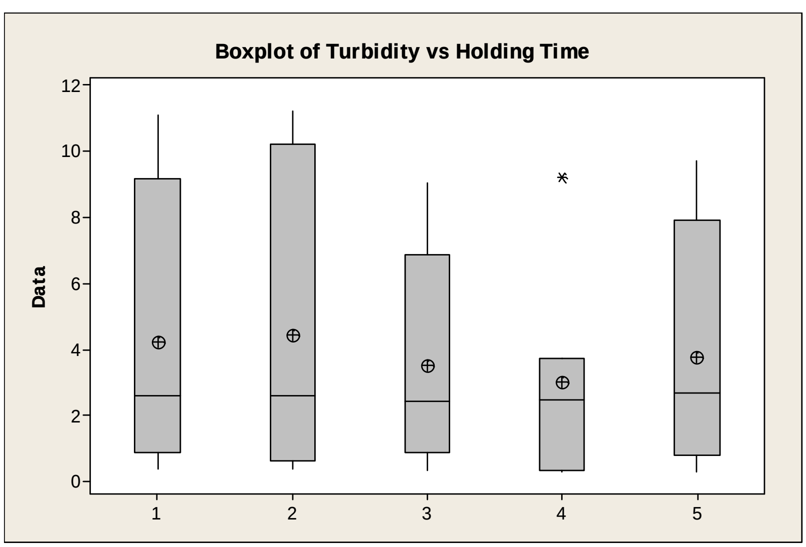

Boxplot of DIC Conc vs Holding Time
Data
25
20
15
10
5
0
T1
T2
T3
T4
T5

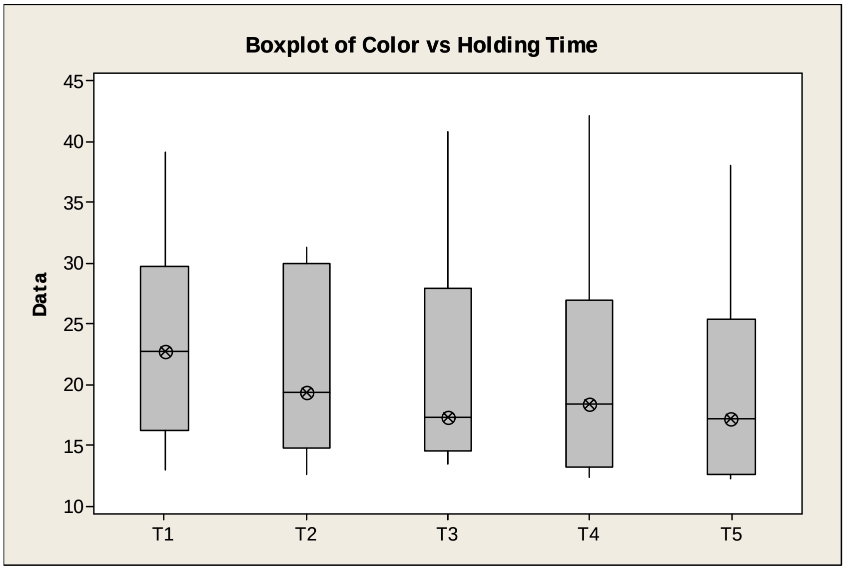

Boxplot of D0C Conc vs Holding Time
Data
8
7
6
5
4
3
2
T1
T2
T3
T4
T5


Boxplot of Alkalintiy vs Analysis Time
Data
100
80
60
40
20
0
T1
T2
T3
T4
T5


Boxplot of pH vs Holding Time
Data
8.2
8.0
7.8
7.6
7.4
7.2
7.0
T1
T2
T3
T4
T5


Boxplot of Conductance vs Holding Time
Data
1200
1000
800
600
400
200
T1
T2
T3
T4
T5


Boxplot of Turbidity vs Holding Time
Data
12
10
8
6
4
2
0
1
2
3
4
5


Boxplot of Color vs Holding Time
Data
45
40
35
30
25
20
15
10
T1
T2
T3
T4
T5

Appendix IV

GLM Computations by Parameter

General Linear Model: TN versus TIME, LOCATION, MONTH

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	1, 2, 3
MONTH	fixed	2	M, O

Analysis of Variance for TN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	186.46	102.61	25.65	1.13	0.371
LOCATION	2	2287.73	2287.73	1143.86	50.34	0.000
MONTH	1	895.36	895.36	895.36	39.40	0.000
Error	20	454.48	454.48	22.72		
Total	27	3824.03				

S = 4.76698   R-Sq = 88.12%   R-Sq(adj) = 83.96%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable TN
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center  Upper      +---------+---------+---------+------
2      -7.19   0.535  8.257              (------------*------------)
3      -9.15  -1.833  5.483           (-----------*-----------)
4     -10.98  -3.667  3.650        (-----------*-----------)
5     -12.21  -4.487  3.235      (------------*-----------)
                                 +---------+---------+---------+------
                             -12.0      -6.0       0.0       6.0
```

Dunnett Simultaneous Tests
Response Variable TN
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.535	2.905	0.184	0.9992
3	-1.833	2.752	-0.666	0.9094
4	-3.667	2.752	-1.332	0.5005
5	-4.487	2.905	-1.545	0.3739

General Linear Model: Nitrates versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for Nitrates, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	82.51	58.94	14.74	0.78	0.552
LOCATION	2	1146.85	1146.85	573.43	30.50	0.000
SEASON	1	828.82	828.82	828.82	44.08	0.000
Error	16	300.82	300.82	18.80		
Total	23	2358.99				

S = 4.33601   R-Sq = 87.25%   R-Sq(adj) = 81.67%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable Nitrates
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower   Center   Upper     +---------+---------+---------+------
2     -4.542  5.65017  15.842         (-------------*--------------)
3     -5.012  1.83333   8.678        (---------*--------)
4     -4.944  2.29340   9.531        (---------*----------)
5     -7.167  0.07118   7.309     (---------*---------)
                                  +---------+---------+---------+------
                               -7.0       0.0       7.0      14.0
```

Dunnett Simultaneous Tests
Response Variable Nitrates
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	5.65017	3.727	1.51582	0.4102
3	1.83333	2.503	0.73234	0.8882
4	2.29340	2.647	0.86640	0.8191
5	0.07118	2.647	0.02689	1.0000

General Linear Model: TP versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for TP, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	92.35	65.36	16.34	1.08	0.393
LOCATION	2	2764.99	2725.69	1362.85	90.10	0.000
SEASON	1	71.50	71.50	71.50	4.73	0.042
Error	20	302.51	302.51	15.13		
Total	27	3231.36				

S = 3.88918   R-Sq = 90.64%   R-Sq(adj) = 87.36%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable TP
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center   Upper     +---------+---------+---------+------
2     -2.530  3.7733  10.077          (------------*-----------)
3     -2.304  3.6667   9.638          (-----------*-----------)
4     -5.221  0.7500   6.721     (-----------*----------)
5     -4.351  1.9524   8.256      (------------*------------)
                                 +---------+---------+---------+------
                              -5.0       0.0       5.0      10.0
```

Dunnett Simultaneous Tests
Response Variable TP
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	3.7733	2.371	1.5917	0.3493
3	3.6667	2.245	1.6330	0.3282
4	0.7500	2.245	0.3340	0.9918
5	1.9524	2.371	0.8236	0.8314

General Linear Model: DIC versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for DIC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	20.22	20.22	5.05	0.30	0.874
LOCATION	2	3538.82	3538.82	1769.41	105.52	0.000
SEASON	1	88.41	88.41	88.41	5.27	0.032
Error	22	368.90	368.90	16.77		
Total	29	4016.34				

S = 4.09490   R-Sq = 90.82%   R-Sq(adj) = 87.89%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable DIC
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center  Upper  -----+---------+---------+---------+-
2     -6.053  0.1667  6.387  (---------------*---------------)
3     -5.887  0.3333  6.553  (---------------*---------------)
4     -3.970  2.2500  8.470       (---------------*---------------)
5     -5.803  0.4167  6.637  (---------------*---------------)
                             -----+---------+---------+---------+-
                               -4.0       0.0       4.0       8.0
```

Dunnett Simultaneous Tests
Response Variable DIC
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.1667	2.364	0.07050	1.0000
3	0.3333	2.364	0.14099	0.9997
4	2.2500	2.364	0.95170	0.7464
5	0.4167	2.364	0.17624	0.9993

General Linear Model: DOC versus TIME, LOCATION, SEASON

```
Factor    Type   Levels  Values
TIME      fixed       5  1, 2, 3, 4, 5
LOCATION  fixed       3  C, P, Q
SEASON    fixed       2  M, O


Analysis of Variance for DOC, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS   Adj MS      F      P
TIME       4    55.38    55.38    13.85   0.60  0.670
LOCATION   2  2709.82  2709.82  1354.91  58.25  0.000
SEASON     1   323.41   323.41   323.41  13.90  0.001
Error     22   511.73   511.73    23.26
Total     29  3600.34


S = 4.82293   R-Sq = 85.79%   R-Sq(adj) = 81.26%


Unusual Observations for DOC

Obs      DOC      Fit  SE Fit  Residual  St Resid
  2  27.0000  18.2667  2.4905    8.7333      2.11 R
 14   1.0000  15.1833  2.4905  -14.1833     -3.43 R

R denotes an observation with a large standardized residual.


Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable DOC
Comparisons with Control Level
TIME = 1  subtracted from:

TIME   Lower  Center  Upper     -+---------+---------+---------+-----
2      -6.66   0.667  7.992             (-------------*--------------)
3     -10.41  -3.083  4.242     (--------------*-------------)
4      -6.99   0.333  7.659            (--------------*-------------)
5      -8.41  -1.083  6.242        (--------------*-------------)
                                -+---------+---------+---------+-----
                             -10.0      -5.0       0.0       5.0


Dunnett Simultaneous Tests
Response Variable DOC
Comparisons with Control Level
TIME = 1  subtracted from:

      Difference       SE of           Adjusted
TIME    of Means  Difference  T-Value   P-Value
2          0.667       2.785    0.239    0.9976
3         -3.083       2.785   -1.107    0.6420
4          0.333       2.785    0.120    0.9998
5         -1.083       2.785   -0.389    0.9849
```

General Linear Model: ALKALINITY versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for ALKALINITY, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	28.38	23.81	5.95	0.32	0.859
LOCATION	2	2777.78	2783.79	1391.89	75.61	0.000
SEASON	1	82.71	82.71	82.71	4.49	0.046
Error	21	386.60	386.60	18.41		
Total	28	3275.47				

S = 4.29061   R-Sq = 88.20%   R-Sq(adj) = 84.26%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable ALKALINITY
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower   Center  Upper  ----+---------+---------+---------+--
2     -6.757   0.1553  7.068  (-------------*-------------)
3     -5.884   0.6667  7.217    (------------*------------)
4     -4.300   2.2500  8.800       (------------*-------------)
5     -6.800  -0.2500  6.300  (------------*-------------)
                              ----+---------+---------+---------+--
                               -5.0       0.0       5.0      10.0
```

Dunnett Simultaneous Tests
Response Variable ALKALINITY
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.1553	2.614	0.0594	1.0000
3	0.6667	2.477	0.2691	0.9963
4	2.2500	2.477	0.9083	0.7775
5	-0.2500	2.477	-0.	

General Linear Model: ph versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for ph, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	53.83	45.74	11.44	0.46	0.763
LOCATION	2	2411.94	2346.27	1173.13	47.30	0.000
SEASON	1	966.33	966.33	966.33	38.96	0.000
Error	21	520.84	520.84	24.80		
Total	28	3952.95				

S = 4.98017   R-Sq = 86.82%   R-Sq(adj) = 82.43%

Unusual Observations for ph

Obs	ph	Fit	SE Fit	Residual	St Resid
3	3.0000	12.0530	2.5790	-9.0530	-2.12 R
17	37.0000	28.2606	2.6008	8.7394	2.06 R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable ph
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center  Upper  ---------+---------+---------+-------
2     -11.59  -3.568  4.455  (------------*------------)
3      -8.19  -0.583  7.020       (------------*------------)
4      -9.10  -1.500  6.103      (------------*-----------)
5      -7.69  -0.083  7.520        (------------*------------)
                             ---------+---------+---------+-------
                                   -6.0       0.0       6.0
```

Dunnett Simultaneous Tests
Response Variable ph
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-3.568	3.034	-1.176	0.5996
3	-0.583	2.875	-0.203	0.9988
4	-1.500	2.875	-0.522	0.9584
5	-0.083	2.875	-0.029	1.0000

General Linear Model: SpC versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for SpC, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	27.15	18.88	4.72	0.54	0.710
LOCATION	2	2329.49	2291.97	1145.98	130.33	0.000
SEASON	1	751.04	751.04	751.04	85.41	0.000
Error	21	184.65	184.65	8.79		
Total	28	3292.33				

S = 2.96527   R-Sq = 94.39%   R-Sq(adj) = 92.52%

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable SpC
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center  Upper  ------+---------+---------+---------+
2     -3.440   1.337  6.114        (-------------*------------)
3     -5.610  -1.083  3.444  (------------*------------)
4     -5.360  -0.833  3.694   (------------*------------)
5     -4.694  -0.167  4.360     (------------*-----------)
                             ------+---------+---------+---------+
                                -3.5       0.0       3.5       7.0
```

Dunnett Simultaneous Tests
Response Variable SpC
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	1.337	1.807	0.7401	0.8733
3	-1.083	1.712	-0.6328	0.9215
4	-0.833	1.712	-0.4868	0.9672
5	-0.167	1.712	-0.0974	0.9999

General Linear Model: TURBIDITY versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for TURBIDITY, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	60.57	65.17	16.29	0.33	0.852
LOCATION	2	2193.33	2174.22	1087.11	22.24	0.000
SEASON	1	144.30	144.30	144.30	2.95	0.100
Error	21	1026.50	1026.50	48.88		
Total	28	3424.69				

S = 6.99148   R-Sq = 70.03%   R-Sq(adj) = 60.04%

Unusual Observations for TURBIDITY

Obs	TURBIDITY	Fit	SE Fit	Residual	St Resid
22	29.0000	17.1061	3.7015	11.8939	2.01 R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable TURBIDITY
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center   Upper  -------+---------+---------+---------
2      -9.98   1.288  12.552      (-------------*-------------)
3     -13.59  -2.917   7.757  (------------*-------------)
4     -13.01  -2.333   8.340   (------------*------------)
5     -12.26  -1.583   9.090    (------------*------------)
                              -------+---------+---------+---------
                                  -8.0       0.0       8.0
```

Dunnett Simultaneous Tests
Response Variable TURBIDITY
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	1.288	4.260	0.3023	0.9942
3	-2.917	4.037	-0.7226	0.8820
4	-2.333	4.037	-0.5781	0.9414
5	-1.583	4.037	-0.3923	0.9848

General Linear Model: COLOR versus TIME, LOCATION, SEASON

Factor	Type	Levels	Values
TIME	fixed	5	1, 2, 3, 4, 5
LOCATION	fixed	3	C, P, Q
SEASON	fixed	2	M, O

Analysis of Variance for COLOR, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TIME	4	94.33	186.52	46.63	1.34	0.289
LOCATION	2	2811.67	2916.42	1458.21	41.98	0.000
SEASON	1	338.84	338.84	338.84	9.76	0.005
Error	20	694.66	694.66	34.73		
Total	27	3939.50				

S = 5.89348   R-Sq = 82.37%   R-Sq(adj) = 76.20%

Unusual Observations for COLOR

Obs	COLOR	Fit	SE Fit	Residual	St Resid	
1	28.0000	16.4513	3.0805	11.5487	2.30	R
7	31.0000	18.9660	3.4948	12.0340	2.54	R
19	2.0000	12.7013	3.0805	-10.7013	-2.13	R
25	1.0000	11.1180	3.0805	-10.1180	-2.01	R

R denotes an observation with a large standardized residual.

Dunnett 95.0% Simultaneous Confidence Intervals
Response Variable COLOR
Comparisons with Control Level
TIME = 1 subtracted from:

```
TIME   Lower  Center   Upper  --------+---------+---------+--------
2      -7.78   2.515  12.809          (------------*------------)
3     -11.47  -2.417   6.633      (----------*----------)
4     -12.80  -3.750   5.299    (----------*-----------)
5     -14.38  -5.333   3.716  (----------*-----------)
                              --------+---------+---------+--------
                                   -8.0       0.0       8.0
```

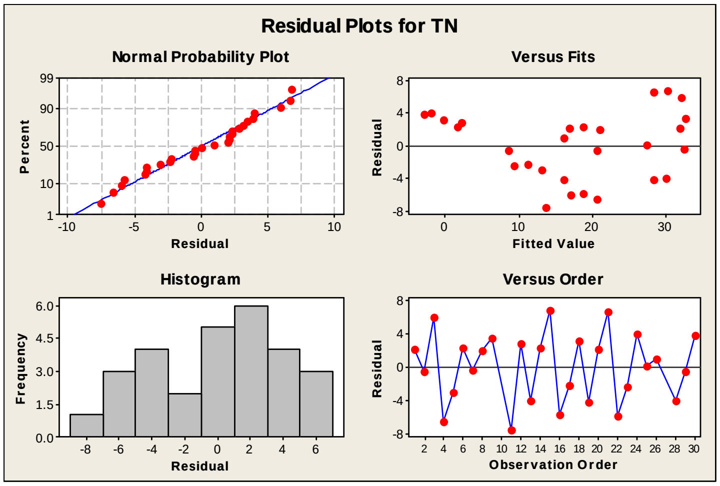
Dunnett Simultaneous Tests
Response Variable COLOR
Comparisons with Control Level
TIME = 1 subtracted from:

TIME	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	2.515	3.871	0.650	0.9166
3	-2.417	3.403	-0.710	0.8903
4	-3.750	3.403	-1.102	0.6545
5	-5.333	3.403	-1.567	0.3625

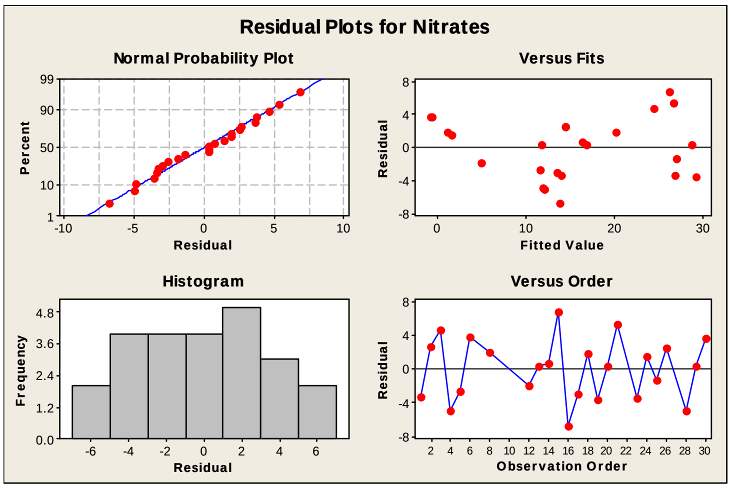
Appendix V

Residual Plots by Parameter

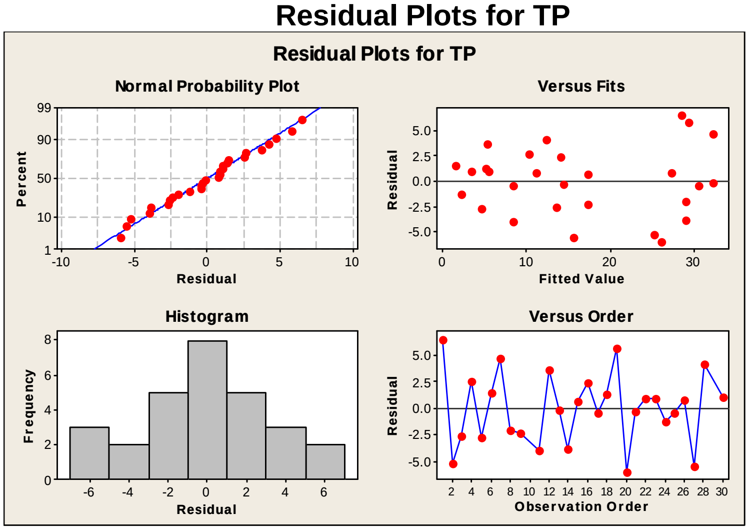
Residual Plots for TN



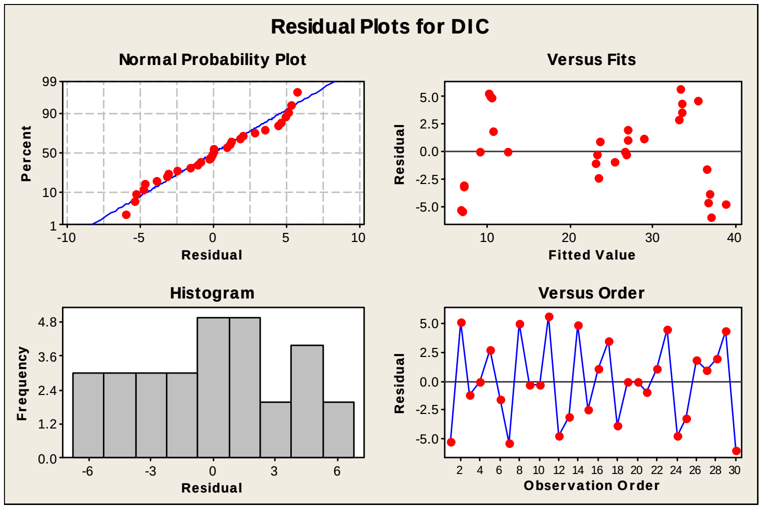
Residual Plots for Nitrates



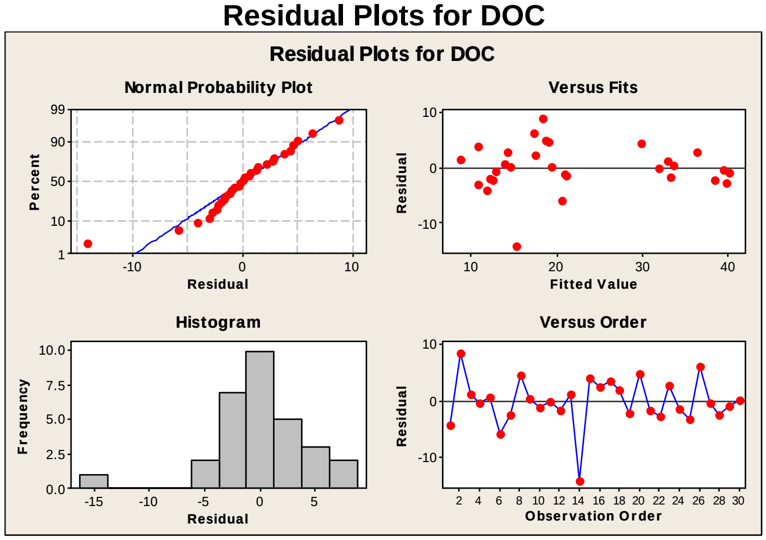
Residual Plots for TP



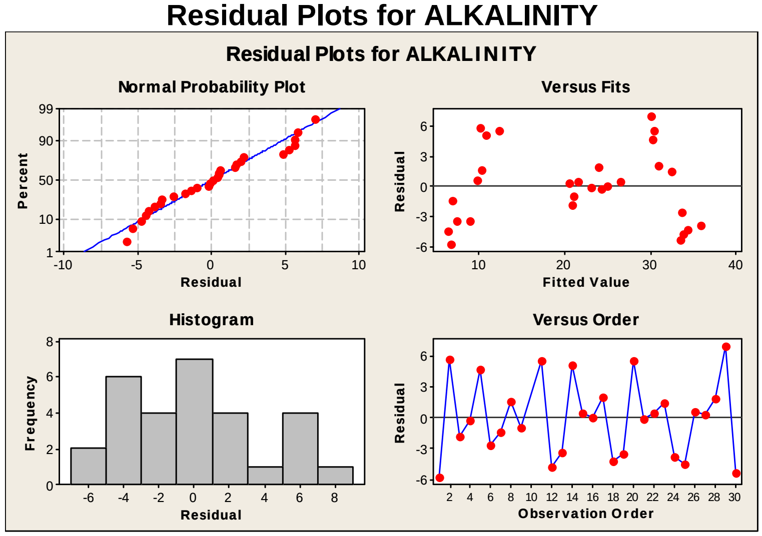
Residual Plots for DIC



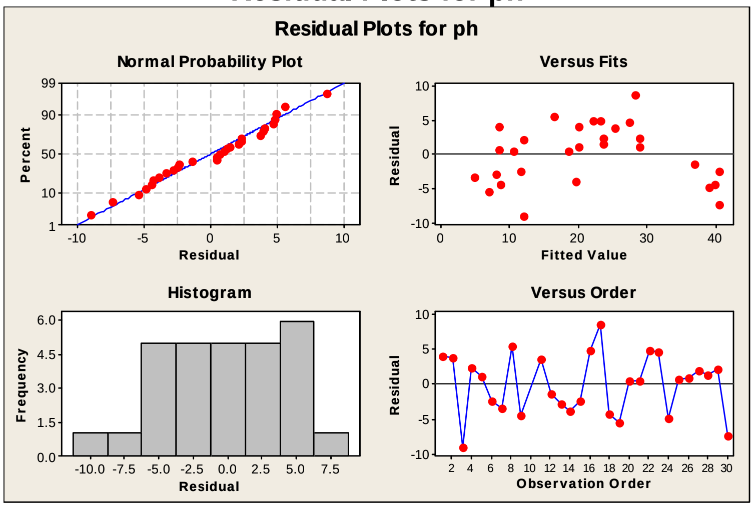
Residual Plots for DOC



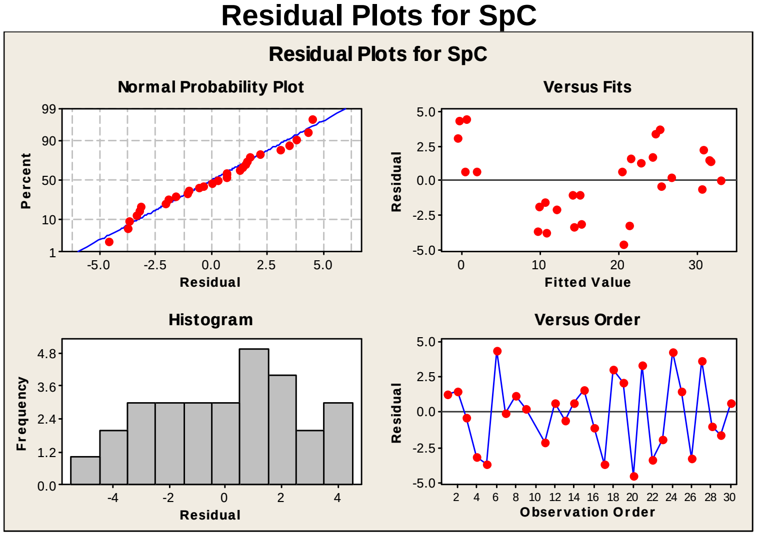
Residual Plots for ALKALINITY



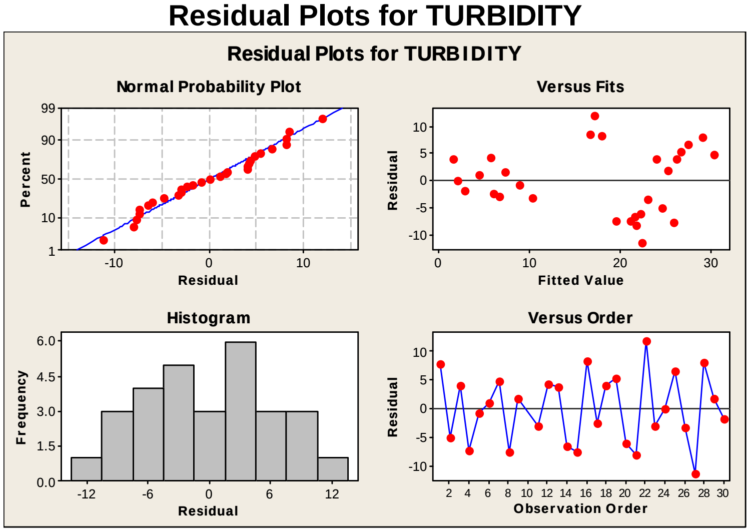
Residual Plots for ph



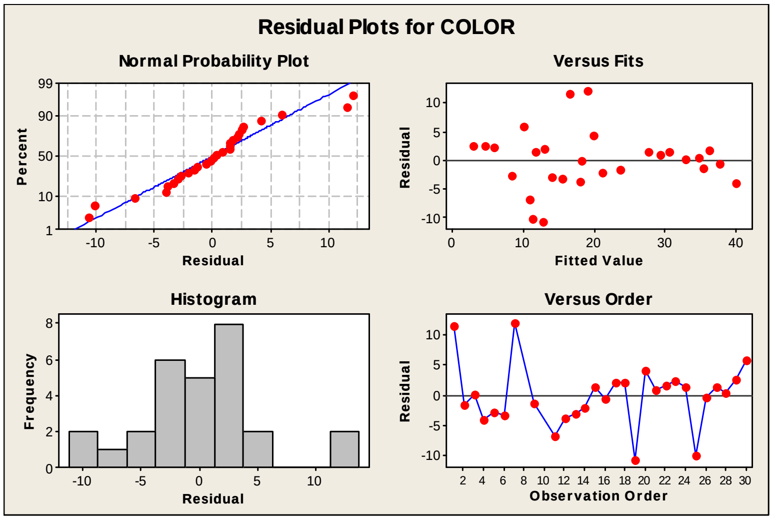
Residual Plots for SpC



Residual Plots for TURBIDITY



Residual Plots for COLOR



Appendix VI

Paired t-Test Computations by Parameter

Paired t-Test and CI: QVL-TN, QVLQA-TN

Paired T for QVL-TN - QVLQA-TN

	N	Mean	StDev	SE Mean
QVL-TN	10	10.35	5.57	1.76
QVLQA-TN	10	10.65	6.54	2.07
Difference	10	-0.300	2.974	0.940

95% CI for mean difference: (-2.427, 1.827)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.32 P-Value = 0.757

Paired t-Test and CI: QVL-NO3, QVLQA-NO3

Paired T for QVL-NO3 - QVLQA-NO3

	N	Mean	StDev	SE Mean
QVL-NO3	9	9.44	4.56	1.52
QVLQA-NO3	9	9.56	5.61	1.87
Difference	9	-0.111	2.315	0.772

95% CI for mean difference: (-1.891, 1.669)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.14 P-Value = 0.889

Paired t-Test and CI: QVL-TP, QVLQA-TP

Paired T for QVL-TP - QVLQA-TP

	N	Mean	StDev	SE Mean
QVL-TP	10	10.35	6.11	1.93
QVLQA-TP	10	10.65	5.95	1.88
Difference	10	-0.300	2.300	0.727

95% CI for mean difference: (-1.945, 1.345)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.41 P-Value = 0.690

Paired t-Test and CI: QVL-DIC, QVLQA-DIC

Paired T for QVL-DIC - QVLQA-DIC

	N	Mean	StDev	SE Mean
QVL-DIC	10	9.15	5.90	1.87
QVLQA-DIC	10	11.85	5.80	1.83
Difference	10	-2.700	2.044	0.646

95% CI for mean difference: (-4.162, -1.238)
T-Test of mean difference = 0 (vs not = 0): T-Value = -4.18 P-Value = 0.002

Paired t-Test and CI: QVL-DOC, QVLQA-DOC

Paired T for QVL-DOC - QVLQA-DOC

	N	Mean	StDev	SE Mean
QVL-DOC	10	10.35	4.45	1.41
QVLQA-DOC	10	10.65	7.24	2.29
Difference	10	-0.30	6.41	2.03

95% CI for mean difference: (-4.88, 4.28)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.15 P-Value = 0.886

Paired t-Test and CI: QVL-ALK, QVLQA-ALK

Paired T for QVL-ALK - QVLQA-ALK

	N	Mean	StDev	SE Mean
QVL-DOC	8	10.38	6.28	2.22
QVLQA-DOC	8	10.06	4.21	1.49
Difference	8	0.31	3.31	1.17

95% CI for mean difference: (-2.45, 3.08)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.27 P-Value = 0.797

Paired t-Test and CI: QVL-ph, QVLQA-pH

Paired T for QVL-ph - QVLQA-pH

	N	Mean	StDev	SE Mean
QVL-ph	9	10.67	6.26	2.09
QVLQA-pH	9	10.28	4.68	1.56
Difference	9	0.389	1.833	0.611

95% CI for mean difference: (-1.020, 1.798)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.64 P-Value = 0.542

Paired t-Test and CI: QVL-SpC, QVLQA-SpC

Paired T for QVL-SpC - QVLQA-SpC

	N	Mean	StDev	SE Mean
QVL-SpC	9	9.44	4.42	1.47
QVLQA-SpC	9	10.11	6.92	2.31
Difference	9	-0.67	3.39	1.13

95% CI for mean difference: (-3.27, 1.94)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.59 P-Value = 0.572

Paired t-Test and CI: QVL-TURB, QVLQA-TURB

Paired T for QVL-TURB - QVLQA-TURB

	N	Mean	StDev	SE Mean
QVL-TURB	9	8.67	6.44	2.15
QVLQA-TURB	9	11.00	5.04	1.68
Difference	9	-2.333	2.562	0.854

95% CI for mean difference: (-4.302, -0.364)
T-Test of mean difference = 0 (vs not = 0): T-Value = -2.73 P-Value = 0.026

Paired t-Test and CI: QVL-COLOR, QVLQA-COLOR

Paired T for QVL-COLOR - QVLQA-COLOR

	N	Mean	StDev	SE Mean
QVL-COLOR	9	9.89	6.43	2.14
QVLQA-COLOR	9	10.00	5.45	1.82
Difference	9	-0.11	4.23	1.41

95% CI for mean difference: (-3.36, 3.14)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.08 P-Value = 0.939