Analysis of a Non-Replicated Split-Split Plot Experiment

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Analysis of a non-replicated split-split plot experiment

A Master's Project

By

Emily Simmons Sim
§ 1 INTRODUCTION

A major obstacle in the analysis of experimental data, in many situations, is the lack of “true” or “complete” replication. In some disciplines, researchers are very aware of the importance of replication and the methods for correctly replicating an experiment. In other subject areas, however, researchers are less aware of what it means to properly replicate an experiment. Due to this lack of awareness, many non-replicated experiments are carried out every year. For many of these non-replicated experiments, there is no satisfactory statistical analysis.

The subject of this report is the analysis of two non-replicated experiments in environmental engineering. First, we analyze the data sets using two traditional methods that assume true replication. We then present an analysis that uses information from both experiments to analyze the individual experiments. We believe that this last analysis is superior to the traditional alternatives.

The organization of the rest of this report is as follows. In section two we summarize some background material on the analysis of factorial, split plot, and split-split plot experiments. In section three we introduce the data that were analyzed for this report. In section four we describe the three analyses we carried out. Finally, in section five, we compare and discuss the results of the three analyses.

§ 2 THREE TYPES OF DESIGNS

Factorial designs (including randomized complete block designs), split plot designs, and split-split plot designs all involve a factorial structure of treatment factors, meaning that all levels of every factor are “crossed” with all levels of the other factors. Thus, every factor level combination is present in the experiment. These designs differ in the constraints imposed on the randomization of the experimental units to the different factor level combinations. In a factorial design, an equal number of experimental units are randomly assigned to each factor level combination, with no restriction on the randomization. A split plot design also has an equal number of experimental units assigned to each factor level combination, but the randomization of the second factor is carried out within the levels of the first factor. A split-split plot design is an extension of a split plot design. A split-split plot design adds a third factor, the randomization for which is carried out within the levels of the
second factor. These designs are best illustrated by example. Because the split plot and split-split plot designs originated in agriculture, the terminology of these designs, and our following examples, have an agronomic flavor.

**Factorial Designs**

Suppose a farmer wants to increase her yearly corn yield. She is interested in three varieties of corn (A, B, and C) and three levels of fertilizer (I, II, and III). Because there are three levels of each factor, there are nine possible combinations of corn and fertilizer. To create an experiment with a factorial design, the farmer might use two pieces of land, one for each replicate of the experiment. Then, she would divide each piece into nine sections, and randomly assign one of the nine possible treatment combinations to each of the nine sections. In other words, she does the equivalent of writing each possible combination on a piece of paper (resulting in nine pieces of paper), putting them in a hat, and then drawing out one at random for each of her nine sections of land. After doing this process for the first piece of land, she would repeat the process for the second piece. One possible outcome of this type of randomization process is shown in Table A.

<table>
<thead>
<tr>
<th>Table A: Factorial Randomization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rep 1</strong></td>
</tr>
<tr>
<td>Corn Variety A</td>
</tr>
<tr>
<td>Fertilizer Level I</td>
</tr>
<tr>
<td>Corn Variety A</td>
</tr>
<tr>
<td>Fertilizer Level II</td>
</tr>
<tr>
<td>Corn Variety B</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
</tr>
<tr>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level II</td>
</tr>
<tr>
<td><strong>Rep 2</strong></td>
</tr>
<tr>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level II</td>
</tr>
<tr>
<td>Corn Variety A</td>
</tr>
<tr>
<td>Fertilizer Level I</td>
</tr>
<tr>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
</tr>
<tr>
<td>Corn Variety B</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
</tr>
<tr>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
</tr>
</tbody>
</table>

2
To measure the yield of each section of land, she might choose three corn stalks at random from each section and count the number of ears on each plant. The usual model for analyzing the data from the farmer’s experiment is:

\[ Y_{ijkl} = C_i + R_k + (CR)_{ik} + F_j + (CF)_{ij} + (FR)_{jk} + (CFR)_{ijk} + \varepsilon_{ijkl} \]

Where

- \( Y_{ijkl} \) = observed yield for the \( l \)th plant, in the \( k \)th replication, for the section of land that contains the \( i \)th variety of corn and the \( j \)th level of fertilizer.
- \( C_i \) = the effect of the \( i \)th variety of corn, a fixed effect, \( i = 1, 2, 3 \).
- \( R_k \) = the effect of the \( k \)th replication, a random effect, \( l = 1, 2 \).
- \((CR)_{ik}\) = the interaction between the \( i \)th variety of corn and the \( k \)th replication, a random effect.
- \( F_j \) = the effect of the \( j \)th level of fertilizer, a fixed effect, \( j = 1, 2, 3 \).
- \((CF)_{ij}\) = the interaction between the \( i \)th variety of corn and the \( j \)th level of fertilizer, a fixed effect.
- \((FR)_{jk}\) = the interaction between the \( j \)th level of fertilizer and the \( k \)th replication, a random effect.
- \((CFR)_{ijk}\) = the interaction between the \( i \)th variety of corn, the \( j \)th level of fertilizer, and the \( k \)th replication, a random effect.
- \( \varepsilon_{ijkl} \) = the error or residual term, \( l = 1, 2, 3 \).

This model assumes the following distributions:

- \( R_k \) are independent and identically distributed as \( N(0, \sigma^2_R) \).
- \((CR)_{ik}\) are independent and identically distributed as \( N(0, \sigma^2_{CR}) \).
- \((FR)_{jk}\) are independent and identically distributed as \( N(0, \sigma^2_{FR}) \).
- \((CFR)_{ijk}\) are independent and identically distributed as \( N(0, \sigma^2_{CFR}) \).
- \( \varepsilon_{ijkl} \) are independent and identically distributed as \( N(0, \sigma^2_e) \).

Letting \( MSS \), with appropriate subscripts, denote the mean sums of squares for the corresponding terms in the model, the appropriate F-ratio for testing whether there is a significant interaction between the variety of corn and the type of fertilizer is \( (MSS_{CF})/(MSS_{CFR}) \). The F-ratio for testing for differences between the fertilizer types is
and the F-ratio for testing for differences between the varieties of corn is 
\( \frac{(MSS_C)}{(MSS_{CR})} \).

**Split Plot Designs**

Logistically, it might be rather difficult for the farmer to divide each piece of land into nine sections and randomly assign each of the nine combinations to one of the sections. It might be much easier for her to plant the corn varieties in strips (or plots), and then randomly assign the fertilizer levels to different sections within those strips. Therefore, the farmer divides each of her two pieces of land into three strips, and randomly assigns one of the three corn varieties to a strip. In split plot design terminology, the corn variety is called the “whole plot factor”, because the land is divided into “whole plots” (the strips) and the varieties of corn are randomly assigned to these whole plots. She then divides each of the strips of land, or whole plots, into three “sub-plots”, and each level of fertilizer is randomly assigned to one of the three sub-plots within each strip. Thus, each strip receives each level of fertilizer. In split plot terminology, fertilizer is called the sub-plot factor. Table B shows one possible result for the two pieces of land.

**Table B: Split Plot Randomization**

<table>
<thead>
<tr>
<th>Rep 1</th>
<th>Rep 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Variety A</td>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level I</td>
<td>Fertilizer Level I</td>
</tr>
<tr>
<td>Corn Variety C</td>
<td>Corn Variety B</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
<td>Fertilizer Level III</td>
</tr>
<tr>
<td>Corn Variety B</td>
<td>Corn Variety C</td>
</tr>
<tr>
<td>Fertilizer Level III</td>
<td>Fertilizer Level III</td>
</tr>
</tbody>
</table>

| Corn Variety A | Corn Variety C |
| Fertilizer Level I | Fertilizer Level I |
| Corn Variety C | Corn Variety B |
| Fertilizer Level III | Fertilizer Level III |
| Corn Variety B | Corn Variety C |
| Fertilizer Level I | Fertilizer Level I |

| Corn Variety B | Corn Variety A |
| Fertilizer Level III | Fertilizer Level III |
| Corn Variety C | Corn Variety A |
| Fertilizer Level III | Fertilizer Level III |
| Corn Variety A | Corn Variety C |
| Fertilizer Level I | Fertilizer Level I |

| Corn Variety B | Corn Variety A |
| Fertilizer Level II | Fertilizer Level II |
| Corn Variety C | Corn Variety A |
| Fertilizer Level III | Fertilizer Level III |
| Corn Variety A | Corn Variety C |
| Fertilizer Level I | Fertilizer Level I |

| Corn Variety B | Corn Variety A |
| Fertilizer Level II | Fertilizer Level II |
| Corn Variety C | Corn Variety A |
| Fertilizer Level III | Fertilizer Level III |
| Corn Variety A | Corn Variety C |
| Fertilizer Level I | Fertilizer Level I |
If the farmer randomly samples three stalks of corn from each section and counts the number of ears on each stalk, then the usual model for analyzing the data from her experiment is:

\[ Y_{ijkl} = C_i + R_k + (CR)_{ik} + F_j + (CF)_{ij} + (CFR)_{ijk} + \varepsilon_{ijkl} \]

Note that the only difference between the split plot model and the factorial design model is that the split plot model does not contain \((FR)_{jk}\), the interaction between the fertilizer and the replication. The split plot model has the same distributional assumptions as the factorial model.

The appropriate F-ratio for testing whether there is a significant interaction between the varieties of corn and the fertilizer types is \((\text{MSS}_{CF})/(\text{MSS}_{CFR})\). The F-ratio for testing for differences between the fertilizer types is \((\text{MSS}_F)/(\text{MSS}_{CFR})\), and the F-ratio for testing for differences between the corn varieties is \((\text{MSS}_C)/(\text{MSS}_{CR})\).

**Split-Split Plot Designs**

A split-split plot design is similar to a split plot design, except that the sub-plots are divided into sub-sub plots, and a third factor of interest is applied to the sub-sub plots. Using the farmer’s example, suppose she is also interested in two types of herbicide (a and b). After dividing her land into whole plots (and randomly assigning the corn varieties) and then into sub-plots (and randomly assigning the fertilizer levels), she would divide each of the sub-plots into two sections, called “sub-sub-plots”, and randomly allocate herbicide type a to one sub-sub-plot and herbicide type b to the other. Thus, herbicide is called the sub-sub-plot factor. Using the split-plot design from Table B and adding the sub-sub plots, her two pieces of land might look like Table C.
If the farmer measures the yield of each section of land by randomly selecting three stalks of corn and counting the number of ears on the stalk, then the usual model for analyzing the data from the split-split plot experiment is:

\[
Y_{ijklm} = C_i + R_l + (CR)_{il} + F_j + (CF)_{ijl} + H_k + (CH)_{ikl} + (FH)_{jk} + (CFH)_{ijkl} + (CFHR)_{ijklm} + \varepsilon_{ijklm}
\]

Where

- \( Y_{ijklm} \) = observed yield for the \( m^{th} \) stalk in the \( i^{th} \) replication, for the section of land containing the \( i^{th} \) variety of corn, the \( j^{th} \) level of fertilizer and herbicide type \( k \).
- \( H_k \) = the effect of the \( k^{th} \) type of herbicide, a fixed effect, \( k = 1,2 \).
- \( (CH)_{ikl} \) = the interaction between the \( i^{th} \) variety of corn and the \( k^{th} \) type of herbicide, a fixed effect.
- \( (FH)_{jk} \) = the interaction between the \( j^{th} \) level of fertilizer and the \( k^{th} \) type of herbicide, a fixed effect.
\((CFH)_{ijk}\) = the interaction between the \(i^{th}\) variety of corn, the \(j^{th}\) level of fertilizer and the \(k^{th}\) type of herbicide, a fixed effect.

\((CFHR)_{ijkl}\) = the interaction between the \(i^{th}\) variety of corn, the \(j^{th}\) level of fertilizer, the \(k^{th}\) type of herbicide, and the \(l^{th}\) replication, a random effect.

The distributional assumptions for this model are the same as the distributional assumptions for the factorial and split plot models, with one addition. This model assumes:

- \((CFHR)_{ijkl}\) are independent and identically distributed as \(N(0, \sigma^2_{CFHR})\).

The appropriate F-ratio for testing whether or not the interaction between the variety of corn, level of fertilizer, and type of herbicide is significant is \((MSS_{CFH})/(MSS_{CFHR})\). The F-ratio for testing the significance of the interaction between the level of fertilizer and the type of herbicide is \((MSS_{FH})/(MSS_{CFHR})\). The F-ratio for testing the significance of the interaction between the variety of corn and the type of herbicide is \((MSS_{CH})/(MSS_{CFHR})\), and the F-ratio for testing for differences between the types of herbicide is \((MSS_{H})/(MSS_{CFHR})\). F-ratios for terms that are common to the split-split plot model and the split-plot model are the same for the two models.

Recall that in Table B, the experimental units for the whole plot factor are the whole plots. This means that the experimental units for testing for differences between the varieties of corn are the strips in which the varieties are planted. Thus, there are three experimental units for the whole plot factor in Rep 1, and three experimental units for the whole plot factor in Rep 2. If we only had one replicate, then each of the corn varieties would appear in only one experimental unit, and we would not be able to test for differences between the varieties. We have two replicates, therefore, we can test for differences between the corn types (the whole plot factor is testable). This illustrates the major problem with non-replicated split plot designs -- the whole plot factor is not testable. Likewise, split-split plot designs also require the whole plot factor to be replicated in order to be testable.
§ 3 THE DATA

The problem was brought to the USU Statistical Consulting Center in 1999. The client had conducted two experiments on soil beneath the surface of a pond at a contaminated site. The first was a “short-term” experiment involving the times 0, 2, 6, 10, 20, and 32. The other was a “long-term” experiment involving the times 0, 5, 32, 70, 136, and 196. Both experiments were identical in protocol.

For each of the two experiments, the client dug 12 cores into the bottom surface of the pond, thus she had 24 cores. Each core was split into a “top,” “middle” and “bottom” portion (2 cm – 5 cm, 7 cm – 10 cm, and 12 cm – 15 cm, respectively). All 12 “top” portions were thoroughly mixed together, all 12 “middle” portions were mixed together, and all 12 “bottom” portions were mixed together. Thus, there were three “batches” of material corresponding to different levels beneath the bottom surface of the pond. These batches, along with the control, are the levels of the factor “soil” (SOIL); the material used in the control came from some other source.

Next, 36 samples were drawn from each level of SOIL, including the control, and 18 of these samples were randomly assigned to each of two test treatments, which are the levels of the factor “treatment” (TREATMENT). Finally, at each of six time periods, three of the eighteen samples were drawn at random from the treatment in which they were sitting and measurements were taken. These time periods are the levels of the factor “time” (TIME). This form of sampling is termed “destructive”, meaning that at the beginning of the experiment there were 18 samples, after the first sampling there were 15 samples, after the second sampling there were 12 samples, and so forth. Both experiments are split-split plot designs. SOIL is the whole plot factor, TREATMENT is the sub-plot factor, and TIME is the sub-sub-plot factor. The three samples drawn at each time period are not replications, they are “sub-samples” (similar to the three stalks of corn randomly chosen from each section of land in the farmer’s example). Table D is an example of how the randomization in the client’s experiment might look.
<table>
<thead>
<tr>
<th>Top Soil Treatment</th>
<th>Bottom Soil Treatment</th>
<th>Control Treatment</th>
<th>Middle Soil Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Period 2</td>
<td>Time Period 6</td>
<td>Time Period 3</td>
<td>Time Period 5</td>
</tr>
<tr>
<td>Time Period 3</td>
<td>Time Period 2</td>
<td>Time Period 4</td>
<td>Time Period 1</td>
</tr>
<tr>
<td>Time Period 4</td>
<td>Time Period 5</td>
<td>Time Period 5</td>
<td>Time Period 6</td>
</tr>
<tr>
<td>Time Period 5</td>
<td>Time Period 1</td>
<td>Time Period 1</td>
<td>Time Period 2</td>
</tr>
<tr>
<td>Time Period 6</td>
<td>Time Period 4</td>
<td>Time Period 2</td>
<td>Time Period 5</td>
</tr>
<tr>
<td>Time Period 1</td>
<td>Time Period 5</td>
<td>Time Period 5</td>
<td>Time Period 6</td>
</tr>
<tr>
<td>Time Period 2</td>
<td>Time Period 1</td>
<td>Time Period 2</td>
<td>Time Period 5</td>
</tr>
<tr>
<td>Time Period 4</td>
<td>Time Period 6</td>
<td>Time Period 3</td>
<td>Time Period 1</td>
</tr>
<tr>
<td>Time Period 5</td>
<td>Time Period 3</td>
<td>Time Period 6</td>
<td>Time Period 1</td>
</tr>
<tr>
<td>Time Period 6</td>
<td>Time Period 2</td>
<td>Time Period 3</td>
<td>Time Period 5</td>
</tr>
</tbody>
</table>
As shown in Table D, there is no replication of the whole plot factor. Thus, the whole plot factor is not testable. For the client’s experiments to have been replicated, she would have needed more than one batch of “top” soil, more than one batch of “middle” soil, more than one batch of “bottom” soil, and more than one batch of the control. Each batch of “top” soil would have been divided into two parts to receive the treatments, and those parts would have been divided into six portions to be drawn at the different time periods. She would do the same for the batches of “middle”, “bottom”, and control soil.

The client is not to blame for constructing a non-replicated experiment. She consulted a statistician before conducting the experiments, but he misunderstood the designs and told her it was a standard split plot repeated measures analysis, which is incorrect.

§ 4  THREE ANALYSES

Factorial Analysis
Both the “short-term” experiment and the “long-term” experiment have a factorial treatment structure. One might be tempted to use a standard factorial analysis, treating the sub-samples as replicates. Recall, in a factorial design, there is no restriction on the randomization. The client, however, restricted the randomization in her experiments, so they are not factorial designs. Additionally, her sub-samples (the three samples taken at each time period) are not actually replications. Nevertheless, let us see what the results would be if the data were analyzed as if they did come from a replicated factorial design.

We carried out a full factorial ANOVA with SOIL, TREATMENT, and TIME as the crossed factors. Analysis of the residuals (or error terms) discovered that the residuals are non-Normal with non-constant variance (heteroscedasticity). A log transformation solved the problem, and the residuals are now approximately Normal with constant variance. Plots 1.1a, 1.2a, and 1.3a (found in the Appendix) are residual plots for Experiment 1 without the log transformation. We omitted the plot of the residuals by TREATMENT because this plot showed no evidence of heteroscedasticity. Plot 1.4a is a normal quantile plot for Experiment 1 without the log transformation. Plots 1.1b, 1.2b, 1.3b, and 1.4b are the corresponding plots for Experiment 1 after the log transformation. Plots 2.1a, 2.2a, 2.3a, and 2.4a are the residual
plots and normal quantile plot for Experiment 2 without the log transformation. Plots 2.1b, 2.2b, 2.3b, and 2.4b are the residual plots and normal quantile plot for Experiment 2 after the log transformation.

The model for Experiment 1 and Experiment 2, separately, is:

\[ \log(Y_{ijkl} + 1) = S_i + T_j + (ST)_{ij} + M + (SM)_{ik} + (TM)_{jk} + (STM)_{ijk} + \varepsilon_{ijkl} \]

Where

- \( Y_{ijkl} \) = concentration of Chloride (Cl) observed for the \( l \)th observation at level \( i \) of SOIL, level \( j \) of TREATMENT and level \( k \) of TIME.
- \( S_i \) = the effect of the \( i \)th level of SOIL, \( i = 1, 2, 3, 4 \).
- \( T_j \) = the effect of the \( j \)th level of TREATMENT, \( j = 1, 2 \).
- \( (ST)_{ij} \) = the interaction between the \( i \)th level of SOIL and the \( j \)th level of TREATMENT.
- \( M_k \) = the effect of the \( k \)th level of TIME, \( k = 1, 2, 3, 4, 5, 6 \).
- \( (SM)_{ik} \) = the interaction between the \( i \)th level of SOIL and the \( k \)th level of TIME.
- \( (TM)_{jk} \) = the interaction between the \( j \)th level of TREATMENT and the \( k \)th level of TIME.
- \( (STM)_{ijk} \) = the interaction between the \( i \)th level of SOIL, the \( j \)th level of TREATMENT, and the \( k \)th level of TIME.
- \( \varepsilon_{ijkl} \) = residual or error term, \( l = 1, 2, 3 \).

This model assumes that the \( \varepsilon_{ijk} \) are independent and identically distributed as \( N(0, \sigma^2_\varepsilon) \).

Table E summarizes the results for both experiments.
As shown in table E, three factors are profoundly significant in both experiments: SOIL, TIME, and SOIL*TIME. Additionally, SOIL*TREATMENT is significant in the second experiment. Using a factorial design, one would conclude that the factors that most effect the level of Chloride in the soil are whether the soil was sampled from the “top”, “middle”, or “bottom” section and how long the soil was in the treatment. This conclusion is erroneous.

### Split-Split Plot Analysis

When the client took three sub-samples at each time period, she believed that these sub-samples were in fact true replications of the experiment. If she had analyzed these data as if they came from replicated split-split plot designs, she would have used a model like the following.

\[
\log(Y_{ijkl} + 1) = S_i + R_l + (SR)_{il} + T_j + (ST)_{ij} + (STR)_{ijl} + M_k + (SM)_{ik} + (TM)_{jk} + (STM)_{ijk} + \varepsilon_{ijkl}
\]

Where

- \( R_l \) = the effect of the \( l \)th replication, a random effect, \( l = 1,2,3 \).
- \( (SR)_{il} \) = the interaction between the \( i \)th level of SOIL and the \( l \)th replication, a random effect.
- \( (STR)_{ijl} \) = the interaction between the \( i \)th level of SOIL, the \( j \)th level of TREATMENT, and the \( l \)th replication, a random effect.

This model has the same distributional assumption as the factorial model for the \( \varepsilon_{ijkl} \)'s. Additionally, this model assumes the following:

- \( R_l \) are independent and identically distributed as \( \text{N}(0, \sigma^2_R) \).
- \( (SR)_{il} \) are independent and identically distributed as \( \text{N}(0, \sigma^2_{SR}) \).
- \( (STR)_{ijl} \) are independent and identically distributed as \( \text{N}(0, \sigma^2_{STR}) \).
Table F summarizes the results of this analysis for both experiments.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>F</td>
<td>DF1</td>
<td>DF2</td>
<td>P-value</td>
<td></td>
<td>F</td>
<td>DF1</td>
<td>DF2</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>18.75</td>
<td>3</td>
<td>6</td>
<td>0.0019</td>
<td></td>
<td>26.93</td>
<td>3</td>
<td>6</td>
<td>0.0007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>3.30</td>
<td>1</td>
<td>8</td>
<td>0.1067</td>
<td></td>
<td>0.83</td>
<td>1</td>
<td>8</td>
<td>0.3878</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil*Treatment</td>
<td>1.34</td>
<td>3</td>
<td>8</td>
<td>0.3289</td>
<td></td>
<td>3.33</td>
<td>3</td>
<td>8</td>
<td>0.0770</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>99.04</td>
<td>5</td>
<td>80</td>
<td>&lt;0.0001</td>
<td></td>
<td>21.02</td>
<td>5</td>
<td>79</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil*Time</td>
<td>3.16</td>
<td>15</td>
<td>80</td>
<td>0.0004</td>
<td></td>
<td>3.77</td>
<td>15</td>
<td>79</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>1.77</td>
<td>5</td>
<td>80</td>
<td>0.1288</td>
<td></td>
<td>1.80</td>
<td>5</td>
<td>79</td>
<td>0.1219</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil<em>Treatment</em>Time</td>
<td>1.37</td>
<td>15</td>
<td>80</td>
<td>0.1813</td>
<td></td>
<td>1.30</td>
<td>15</td>
<td>79</td>
<td>0.2194</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SOIL, TIME and SOIL*TIME are significant factors in both experiments. No other factors are significant in either experiment. Analyzing these data as coming from replicated split-split plot designs, one obtains similar results to the factorial analysis.

**Extracted Scale System (ESS) Analysis**

The models usually used to analyze split plot and split-split plot experiments include both fixed and random components. For the client’s experiments, the different levels of soil, the two treatments, and the six time periods are fixed components, as are all of their interactions. The replications and all interactions involving the replication are random. A mixed model may be written as $Y = X\beta + ZU + \varepsilon$, where $Y$ is the vector of values for the response variable, $X$ is the matrix of data for the fixed factors in the model, $\beta$ is the vector of coefficients for the fixed factors, $Z$ is the matrix of coefficients for the random effects in the model, $U$ is the vector of random effects, and $\varepsilon$ is the vector of residual terms. $E[\varepsilon] = 0$ and $\text{Var}[\varepsilon] = R$. $E[U] = 0$ and $\text{Var}[U] = G$. $\text{Cov}[U, \varepsilon] = 0$. Usually, we assume the random components are normally distributed. Often, $R = \sigma^2 I_n$, and in the vast majority of applications, $G$ is a diagonal matrix with variance components as entries of the diagonal.

Note that Experiment 1 and Experiment 2 both have time periods 0 and 32. Also, we deem that time period 6 in Experiment 1 is similar enough to time period 5 in Experiment 2 to treat these two time periods as the same. It is possible, therefore, to combine the two data sets and use the common time periods between the two experiments as true replications, thus allowing one to obtain an estimate of $G$. We combined the data sets for the two experiments and deleted all observations for all time periods except 0, 5, 6, and 32. Then, treating time periods 5 and 6 as though they are the same, we used the following model:
\[ \log(Y_{ijklm} + 1) = S_i + E_i + (SE)_{il} + T_j + (ST)_{ij} + (STE)_{ijl} + M_k + (SM)_{ik} + (TM)_{jk} + (STM)_{ijk} + (STME)_{ijkl} + \varepsilon_{ijkl} \]

Where:

- \( E_i \) = the effect of the \( i^{th} \) experiment, a random effect, \( i = 1,2 \).
- \( (SE)_{il} \) = the interaction between the \( i^{th} \) level of SOIL and the \( i^{th} \) level of experiment, a random effect.
- \( (STE)_{ijl} \) = the interaction between the \( i^{th} \) level of SOIL, the \( j^{th} \) level of TREATMENT, and the \( i^{th} \) experiment, a random effect.
- \( (STME)_{ijkl} \) = the interaction between the \( i^{th} \) level of SOIL, the \( j^{th} \) level of TREATMENT, the \( k^{th} \) level of TIME, and the \( i^{th} \) experiment, a random effect.

This model has the same distributional assumption as the factorial model for the \( \varepsilon_{ijkl} \)'s.

Additionally, this model assumes the following:

- \( E_i \) are independent and identically distributed as \( N(0, \sigma^2_E) \).
- \( (SE)_{il} \) are independent and identically distributed as \( N(0, \sigma^2_{SE}) \).
- \( (STE)_{ijl} \) are independent and identically distributed as \( N(0, \sigma^2_{STE}) \).
- \( (STME)_{ijkl} \) are independent and identically distributed as \( N(0, \sigma^2_{STME}) \).

Table G shows the results of this analysis for the combined data sets.

<table>
<thead>
<tr>
<th>Factor</th>
<th>F</th>
<th>DF1</th>
<th>DF2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>2.19</td>
<td>3</td>
<td>3</td>
<td>0.2676</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.21</td>
<td>1</td>
<td>4</td>
<td>0.6674</td>
</tr>
<tr>
<td>Soil*Treatment</td>
<td>0.12</td>
<td>3</td>
<td>4</td>
<td>0.9442</td>
</tr>
<tr>
<td>Time</td>
<td>3.76</td>
<td>2</td>
<td>16</td>
<td>0.0458</td>
</tr>
<tr>
<td>Soil*Time</td>
<td>0.31</td>
<td>6</td>
<td>16</td>
<td>0.9199</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>0.05</td>
<td>2</td>
<td>16</td>
<td>0.9511</td>
</tr>
<tr>
<td>Soil<em>Treatment</em>Time</td>
<td>0.19</td>
<td>6</td>
<td>16</td>
<td>0.9759</td>
</tr>
</tbody>
</table>
Only one factor is significant, TIME, with a P-value of 0.0458. This result is very different from the results of the factorial and split-split plot analysis, which found SOIL to be profoundly significant.

From the combined data set, we obtained an estimate of $G$, a diagonal matrix. The entries down the diagonal represent the variance estimates for the experiments (EXPT), the SOIL*EXPT interaction, the SOIL*EXPT*TREATMENT interaction, and the SOIL*EXPT*TREATMENT*TIME interaction. The idea of the ESS analysis is to use the common 3 time periods in the two experiments to estimate entries of $G$, and then expand the $G$ matrix to the other time periods. The following table shows the estimates for the different variance components from the $G$ matrix we obtained.

<table>
<thead>
<tr>
<th>Variance Component</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma^2_{\text{EXPT}}$</td>
<td>0.000000</td>
</tr>
<tr>
<td>$\sigma^2_{\text{SOIL*EXPT}}$</td>
<td>0.000000</td>
</tr>
<tr>
<td>$\sigma^2_{\text{SOIL<em>EXPT</em>TREATMENT}}$</td>
<td>0.000000</td>
</tr>
<tr>
<td>$\sigma^2_{\text{SOIL<em>EXPT</em>TREATMENT*TIME}}$</td>
<td>0.503443</td>
</tr>
</tbody>
</table>

To create the estimated $G$ matrix for Experiment 1, we deleted all rows and columns of the $G$ matrix from the combined data set that corresponded to Experiment 2. Then we added in rows and columns for the time periods that were not a part of the combined data set, i.e., times 2, 10, and 20. We used 0.503443 as the estimate for the variance components associated with these times. To obtain the $G$ matrix for Experiment 2, we deleted all rows and columns of the $G$ matrix from the combined data that corresponded to Experiment 1, and then proceeded in the same manner.

The model for the individual experiments is:

$$
\log(Y_{ijklm} + 1) = S_i + E_j + (SE)_{ij} + T_j + (ST)_{ij} + (STE)_{ijkl} + M_k + (SM)_{ik} + (TM)_{jk} + (STM)_{ijk} + (STME)_{ijkl} + \epsilon_{ijklm}
$$
We ran this model in SAS, using the $G$ matrix I created as the dispersion matrix for the random components. The denominator degrees of freedom for the F-tests created by SAS for both experiments were incorrect because SAS assumes the $G$ matrix is known and fixed. We calculated the correct degrees of freedom and used Splus to find the appropriate P-values.

Table I shows the F-values produced by SAS, the degrees of freedom we calculated, and the associated P-values for both experiments.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$DF_1$</td>
<td>$DF_2$</td>
<td>$P$-value</td>
<td></td>
<td>$F$</td>
<td>$DF_1$</td>
<td>$DF_2$</td>
<td>$P$-value</td>
</tr>
<tr>
<td>Soil</td>
<td>2.00</td>
<td>3</td>
<td>3</td>
<td>0.2918</td>
<td></td>
<td>6.06</td>
<td>3</td>
<td>3</td>
<td>0.0865</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.30</td>
<td>1</td>
<td>3</td>
<td>0.6220</td>
<td></td>
<td>0.19</td>
<td>1</td>
<td>3</td>
<td>0.6924</td>
</tr>
<tr>
<td>Soil*Treatment</td>
<td>0.12</td>
<td>3</td>
<td>3</td>
<td>0.9424</td>
<td></td>
<td>0.77</td>
<td>3</td>
<td>3</td>
<td>0.5825</td>
</tr>
<tr>
<td>Time</td>
<td>9.07</td>
<td>5</td>
<td>15</td>
<td>0.0004</td>
<td></td>
<td>4.84</td>
<td>5</td>
<td>15</td>
<td>0.0078</td>
</tr>
<tr>
<td>Soil*Time</td>
<td>0.29</td>
<td>15</td>
<td>15</td>
<td>0.9890</td>
<td></td>
<td>0.86</td>
<td>15</td>
<td>15</td>
<td>0.6130</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>0.16</td>
<td>5</td>
<td>15</td>
<td>0.9734</td>
<td></td>
<td>0.41</td>
<td>5</td>
<td>15</td>
<td>0.8345</td>
</tr>
<tr>
<td>Soil<em>Treatment</em>Time</td>
<td>0.13</td>
<td>15</td>
<td>15</td>
<td>0.9998</td>
<td></td>
<td>0.29</td>
<td>15</td>
<td>15</td>
<td>0.9890</td>
</tr>
</tbody>
</table>

TIME is the only significant factor in both experiments. All other factors are profoundly non-significant, except for SOIL in Experiment 2 with a P-value of 0.0865.

§ 4 CONCLUSION

Clearly, a factorial analysis is not appropriate for the client’s data because the client restricted the randomization process. A split-split plot analysis is also incorrect because the sub-samples taken at each time period are not true replications. The results of the factorial and the split-split plot analyses are similar, but the results of the ESS analysis are very different. We think the results of the ESS are better because ESS allows us to conduct a split-split plot analysis in a non-replicated situation.

Plots 3.1 – 3.4 and 4.1 – 4.4 (found in the Appendix) show the relationship between TIME and TREATMENT for each of the soil types. The mean values of Chloride are along the vertical axis, the values of TIME are along the horizontal axis, and the symbols within the plot represent the TREATMENT (1 for the first treatment, 2 for the second). For Experiment 1, the plot for SOIL = control is Plot 3.1, the plot of SOIL = “top” is Plot 3.2, the plot for SOIL = “middle” is Plot 3.3, and the plot for SOIL = “bottom” is Plot 3.4. For Experiment 2, the plot for SOIL = control is Plot 4.1, and so forth. It appears that the significance of the
factor TIME in Experiment 1 is driven by the first two time periods when SOIL = “middle” and when SOIL = “bottom”, the first two time periods show significantly higher values of Chloride (see Plot 3.3 and 3.4). TIME is almost significant in Experiment 2. It appears that the low P-value for TIME in Experiment 2 is driven by the high value of Chloride for TIME = 196, TREATMENT = 2, and SOIL = “middle” (see Plot 4.3).

Table J shows the results of all three analyses, for both experiments, side by side.

**Table J: All Three Analyses**

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Factor</th>
<th>Factorial</th>
<th>Split-Plot</th>
<th>ESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>DF1</td>
<td>DF2</td>
</tr>
<tr>
<td>Soil</td>
<td></td>
<td>21.53</td>
<td>3</td>
<td>96</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>3.26</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>Soil*Treatment</td>
<td></td>
<td>1.32</td>
<td>3</td>
<td>96</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td>97.70</td>
<td>5</td>
<td>96</td>
</tr>
<tr>
<td>Soil*Time</td>
<td></td>
<td>3.11</td>
<td>15</td>
<td>96</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td></td>
<td>1.74</td>
<td>5</td>
<td>96</td>
</tr>
<tr>
<td>Soil<em>Treatment</em>Time</td>
<td></td>
<td>1.35</td>
<td>15</td>
<td>96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Factor</th>
<th>Factorial</th>
<th>Split-Plot</th>
<th>ESS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>DF1</td>
<td>DF2</td>
</tr>
<tr>
<td>Soil</td>
<td></td>
<td>26.93</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>0.83</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>Soil*Treatment</td>
<td></td>
<td>3.33</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td>21.02</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Soil*Time</td>
<td></td>
<td>3.77</td>
<td>15</td>
<td>95</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td></td>
<td>1.80</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Soil<em>Treatment</em>Time</td>
<td></td>
<td>1.30</td>
<td>15</td>
<td>95</td>
</tr>
</tbody>
</table>
APPENDIX
Plot 1.1a

Resid,

15

10

5

0

-5

-10

-15

Pred
Plot 1.2a

Resid,

15

10

5

0

-5

-10

-15

control soil1 soil2 soil3

SOIL
Plot 1.3a

Resid

15 1
,
,
A
10 2
,
B
5 3
,
,,
A
B
A
A
A
A
0 4
,
D
E
C
A
A
A
A
A
-5 5
,
B
A
A
A
-10 6
,
A
-15 7
,

TIME

0 5 10 15 20 25 30
Plot 1.4a

NORMAL QUANTILE PLOT

Rank for Variable Resid
Plot 1.1b

Resid,
1.0

Pred
Plot 1.2b

Resid.

1.0

1

A

A

0.5

B

B

C

A

A

A

0.0

E

F

C

D

A

0

-0.5

B

A

A

A

-1.0

A

A

-1.5

SOIL
Plot 1.3b

Resid,

\[ \begin{align*}
1.0 & \\
0.5 & \\
0.0 & \\
-0.5 & \\
-1.0 & \\
-1.5 & \\
\end{align*} \]

TIME

\[ \begin{align*}
0 & \quad 5 & \quad 10 & \quad 15 & \quad 20 & \quad 25 & \quad 30 \\
\end{align*} \]
Plot 1.4b

NORMAL QUANTILE PLOT

Rank for Variable Resid
Plot 2.1a

Resid,

200

150

100

50

0

-50

-100

-150

Pred

NOTE: 1 obs had missing values. 33 obs hidden.
Plot 2.2a

Resid

200

150

100

50

0

-50

-100

-150

A

A

A

A

A

A

A

A

A

A

A

SOIL

control

soil1

soil2

soil3

NOTE: 1 obs had missing values. 2 obs hidden.
NOTE: 1 obs had missing values.
Plot 2.4a

NORMAL QUANTILE PLOT

Resid

-150
-100
-50
0
50
100
150
200

Rank for variable Resid
NOTE: 1 obs had missing values.
Plot 2.2b

Resid

2.0

1.5

1.0

0.5

0.0

-0.5

-1.0

-1.5

control soil1 soil2 soil3

SOIL

NOTE: 1 obs had missing values.
NOTE: 1 obs had missing values.
Plot 2.4b

NORMAL QUANTILE PLOT

Rank for Variable Resid
Plot 3.1

-------------------------- SOIL=control --------------------------

Plot of mean*TIME. Symbol is value of TREATMENT.

NOTE: 1 obs hidden.
Plot 3.2

--- SOIL=soill ---

Plot of mean*TIME. Symbol is value of TREATMENT.

NOTE: 3 obs hidden.
Plot 3.3

------------------------------------- SOIL=soil2 -------------------------------------

Plot of mean^TIME. Symbol is value of treat.

NOTE: 3 obs hidden.
Plot 3.4

Plot of mean*TIME. Symbol is value of treat.

NOTE: 3 obs hidden.
Plot 4.1

SOIL=control

Plot of mean*TIME. Symbol is value of treat.

NOTE: 3 obs hidden.
Plot 4.2

--------------------------------------------- SOIL=soill ---------------------------------------------

Plot of mean\*TIME. Symbol is value of treat.

NOTE: 4 obs hidden.
Plot 4.3

Plot of mean\*TIME. Symbol is value of treat.

NOTE: 1 obs hidden.
Plot 4.4

SOIL=soil3

Plot of mean*TIME. Symbol is value of treat.

NOTE: 2 obs hidden.