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PARAMETRIC AND NON-PARAMETRIC APPROACHES TO FACTORIAL DATA ANALYSIS.

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by

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ABSTRACT:

Factorial design is one of the best ways to screen different factors in a system of study. It allows the study of the main effects and their interactions at the same time. We used the 2^k-factorial design to screen different factors that affect the bacterial sensitivity to antibiotics. Through simulation studies, we examined the sensitivity and specificity of four different approaches to analyze the factorial data under different scenarios. The parametric "ANOVA" showed higher sensitivity and specificity for normally distributed data, but it failed largely for non-normal data. Rank-ANOVA had very high sensitivity and specificity for both normal and non-normal data when replicates were higher. Aligned-rank approach also had higher sensitivity and specificity for normal data, but not for non-normal data. However, it was very sensitive to outliers and thus failed to detect the significant interactions in our settings. Similarly, permutation approach failed to detect the significant interactions largely for non-normal distributions and needed higher number of observations for larger number of factors to run without error. Overall, rank-based ANOVA was the robust approach to analyze the factorial data without losing the statistical power under our simulation settings. We also used the above-mentioned approaches on our real data obtained from 2^k factorial design. Model trimming options are not available in some of the functions that we used and therefore only full models were compared. Based on root mean squared error (RMSE) values all four approaches, except the permutation test, were similar in predicting the response variable. Permutation test was not applicable with all 7 factors and their interactions in the model due to a smaller number of observations, so reduced model without the factor temperature was used for the analysis. All four approaches yielded comparable p-value for the highest

order interaction with 6 factors in the model but when 7 factors were in the model, rank ANOVA had different p-value than other approaches. Since, factorial data with larger number of factors are difficult to run with multiple replicates, assumptions of parametric approach may hold true. Non-parametric approaches like rank-based and aligned-rank-based approaches are more suitable for the purpose.

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Chapter 1: Introduction.

Antibiotics are medications used to treat bacterial infections in humans and animals. With the increased use of antibiotics there is an increase of antibiotic resistance as well. Currently antimicrobial resistance is a global health threat. Recently, U.S. Centers for Disease Control and Prevention (CDC) released a report on antibiotic resistance threats that reported 2.8 million infections and 35,900 deaths a year in U.S. due to antimicrobial resistant pathogen (CDC, 2019). At the same time, the rate of new drug discovery has highly decreased (WHO, 2017). We are in immediate need of new drugs or new approaches to address this public health issue. However, new drug discovery is very expensive, time consuming and complicated. There is always high risk of adverse effects once they are out in the market (Chen et al., 2018). Since we already know pharmacokinetics, risks, and benefits of current drugs, a suitable alternative would be to improve the current drug's efficacy which saves time, money, and effort.

We propose an approach that can improve the efficacy of the current antibiotics by altering bacterial physiology. Bacteria responds to environmental factors differently and this response determines their susceptibility towards the antibiotics. For example, if the environment has magnesium ions in abundance, such magnesium ions will protect and stabilize ribosomes that can increase the resistance against the ribosome-targeting antibiotics like Kanamycin or Erythromycin (Nierhaus, 2014). We can optimize the environmental growth factors of a bacterium for an antibiotic such that the antibiotic demonstrates enhanced killing of that bacterium.

Azotobacter vinelandii, a gram-negative Nitrogen-fixing soil bacterium, is used as the model organism to study this approach. This bacterium is a Nitrogen fixer, effect of

different sugars and ions are already known in this bacterium (Wong, 1988, 1993; Wong, Pei, Bancroft, & Childers, 1995), has the highest rate of respiration and therefore, is constantly at oxidative stress which makes it ideal for this study. The bacterium is also a very close relative of notorious pathogen *Pseudomonas aeruginosa (Ozen & Ussery, 2012)*. Any information obtained from this bacterium will provide valuable information about treating *Pseudomonas* infection as well.

Factorial designs are one of the most efficient designs for optimization studies. They are a series of independent studies conducted at once. Factorial design allows us to study the effect of two or more independent variables on one or more outcome variables simultaneously. The beauty of the factorial design is that it allows us to study the difference within the independent variables, called main effects, and test if those independent variables interact with each other to make a difference. 2^k factorial designs are the simplest ones where k represents the number of independent variables under study and 2 represents the setting of level of independent variables (usually high and low). Broadly, there are three types of factorial design (Tabachnick & Fidell, 2007).

a) Independent factorial design where independent variables are measured using different subjects (between group design). This is the type of the design used in this study.
b) Repeated-measures factorial design where independent variables are measured using same subject.
c) Mixed design where independent variables are measure using both same and different subjects.

There are various parametric and non-parametric approaches under different statistical software to analyze the factorial data. R is a free statistical programming language which is versatile and has dynamic environment. It has many packages that allows us to analyze the factorial data.. However, most of the non-parametric approaches handle only one or two factors only. Therefore, we simulated the real data by varying the number of factors and observations per unique group and different distribution of response variable. We picked few of the widely accepted packages for both parametric and non-parametric approaches to analyze the simulated data under the conditions where basic ANOVA assumptions are either satisfied or violated (Feys, 2016).

Parametric approach: ANOVA.

Analysis of Variance (ANOVA) is a statistical method that is used to analyze the difference in the means between or among two or more groups. . ANOVA is based on the law of total variance according to which the variance observed is separated into "explained" and "unexplained" variances. Explained variances, also called model variance, is the variance explained by the independent variables that are present and not due to error while the unexplained variances are due to error. An F-statistic is used to compute those variances, which is the ratio of the model variance and error variance. ANOVA is considered fairly robust when sample sizes are equal (Tabachnick & Fidell, 2007).

ANOVA is a special case of General Linear Model that makes three major assumptions about the probability distribution of the residual:

1. They are independent.
2. They are normally distributed.

3. Variance of the groups are equal.

ANOVA that model observations meaursed across all combinations of each levels of each factor is called factorial ANOVA. Should any of these assumptions be violated, this parametric approach cannot be used. For this body of work, we will focus only on approaches should the assumptions of normality and/or equal variance within groups be violated.

Non-parametric approaches:

As inferred by their name, non-parametric approaches do not make a distribution assumption about the data. Most are rank based approaches with several published methods for ranking. Whenever there is violation of assumptions (normality or homogeneity of variances) of ANOVA then the choice of method is non-parametric method. However, non-parametric alternatives for the factorial ANOVA are relatively uncommon (Sawilowsky, 1990). Some of the available non-parametric alternatives are reliable for main effects but are subject to increased Type I errors for interactions (Higgins & Tashtoush, 1994).

Popular non-parametric methods are the Kruskal-Wallis and Friedman tests; however, they cannot be used to study interactions because they can handle only one factor. Therefore, we used three popular alternatives: permutation ANOVA, rank-based ANOVA and Aligned-rank-based ANOVA. These approaches are freely available with user-friendly programming language within the statistical software R.

Permutation based non-parametric approach: ezPerm.

Permutation based approaches are simple straight-forward methods for empirically obtaining the distribution of a test statistic. It does this by examining many permutations of the data given the null hypothesis of no effect of exposures on outcome is true. While holding the independent variables (factors) fixed, the overall F-test statistic is calculated for each permutation of the response variable. The number of permutations is set by the researcher. The resulting two-tailed p-value obtained from the permutation iterations is the proportion of obtaining a test statistic more extreme than the observed test statistic using the non-permutated data.

Unlike the traditional parametric statistics and resulting p-values, the permutation p-value is a subset of non-parametric statistics and therefore does not need any statistical assumptions about the population data (Pitman, 1937). However, under null hypothesis, it is assumed that the observations are exchangeable. Permutations are suitable for small data and is not considered ideal for big data.

Due to the iterative nature of the permutation approach, help from statistical software is always appreciated. The ezPerm function from the ez package (Lawrence & Lawrence, 2016) available for R software, performs non-parametric factorial permutation test. It is a randomization test that re-labels the datapoints repeatedly and compares the results with the real data. We will use this package for this body of work.

Aligned Rank based Non-parametric approach: aligned rank transform.

The Aligned Rank Transform (ART) is another non-parametric approach for factorial designs. This approach by Villacorta (*aligned.rank.transform()* function in art package) allows researchers to examine both main effects and interactions (Wobbrock,

Findlater, Gergle, & Higgins, 2011). It is based on Higgins and Tashtoush formula for completely random designs (Higgins and Tashtoush, 1994, pp. 203-204). Responses are aligned for each main effect or interaction such that every term is sequentially stripped away except the term for which alignment is being done. Thus, aligned responses are then ranked in a new column. For example, in a two-factor experiment with effects A, B, interaction A*B, and response Y, testing for the significance of effect A will first strip estimates of effects B and A*B from Y resulting say YA′, leaving only effect of A. Then YA″ is created which is ranked YA′. A full factorial ANOVA is then run with YA″ as the response with model terms A, B, and A*B. This examines only the effect of A through the ANOVA table and the effects of B and A*B are ignored. This process then repeates for the effects of remaining factors.

In case of ties, ranks are averaged. For N independent variables, the function creates 2N-1 aligned columns and 2N-1 ranked columns. Finally, factorial parametric ANOVA is run on the ranked responses. By using the responses, the normality and variances assumptions are indeed met. Like the permutation approach, we can use the ART package in R to help implement this approach.

Rank based Non-parametric approach: raov.

A slightly modified aligned rank approach is the more general rank-based (R) estimation and inference for linear models (Kloke & McKean, 2012). Just like the typical least squares method used in the parametric ANOVA, rank-based estimation also finds the coefficients of main effects and interaction (beta), however, unlike the least squares method, where minimization of the sum of the squared deviations is done, the rank-based ANOVA uses a reduction in dispersion tests (Jaeckel, 1972) where reduction in

dispersion of residuals are done using some score function. It computes the rank-based analysis for all $2^k - 1$ hypotheses for testing main effects and interaction of all orders. A score function has to be chosen for rank-based fitting according to the distribution of residuals. For assistance, we use the Rfit package, developed by John D. Kloke and Joseph W. McKean, utilizing the *raov()* function that uses an algorithm described in Hocking (1985) and has several score options. The score options and their recommended usage are shown in table 1. The default option is Wilcoxon scores. If the distribution of the errors is known then associated scores can be assigned, however, a user-defined score function can be created to best fit the data.

Table 1 Score functions available in Rfit package.

Score	Keyword	Recommended usage
Wilcoxon scores	wscores	Light to moderate tailed
Normal scores	nscores	Light to moderate tailed
Bent scores	bentscores1, bentscores2, bentscores3, bentscores4	Skewed distribution

For the three non-parametric approaches outlined above, the driving assumption is the independence of the both the independent variables and of subjects. The assumptions of equal variances and normality generally as required by parametric approach like ANOVA are often not met in practice. Specially, for ordinal data where means are not defined, the parametric models are not appropriate. Non-parametric approaches on the other hand are appropriate for both metric and non-metric data. However, parametric tests are robust if the assumptions are met.

Given the uncertainty with real data, our goal is to examine the performance of parametric and non-parametric approaches under varying violations of statistical

assumptions required for ANOVA. We simulated an array of possible real data with various settings to analyze through parametric, non-parametric and robust approaches. Based on sensitivity, specificity, and accuracy of the tests, one can pick the approach that best fits the real factorial data.

Chapter 2: Methods.

Azotobacter vinelandii bacteria was grown in Burk`s Nitrogen free media with 2% glucose or galactose in rotary shaker at 30$^{o}$c and 200 rpm for 24-36 hours. Overnight culture of the bacteria was adjusted to OD 600 of 0.2 to get the log phase cells. Culture plates of Burk`s Nitrogen free media with 2% agar were made with different growth factors. OD adjusted bacteria was mixed with autoclaved 0.5% agar solution and overlayed on top of the plates. Antibiotic discs (erythromycin) were kept on the overlayed plates for the screening purpose and the plates were incubated at 30^{o}c for 24-36 hours. For the factorial design experiment, wells were made on plates and different combination of all the selected factors were kept in each well such that each well would represent one run of the experiment. Finally, the area of zone of inhibition was measured in square millimeters.

Screening of factors:

Culture plates prepared with different factors at various levels were used for the screening purpose. Factors which influenced the antibiotic efficacy were chosen for the further experiments.

Factorial design:

Two level full factorial balanced design was used to analyze effect of the environmental factors on bacterial antibiotic sensitivity. Seven different factors were selected from the screening process. The summary of the variables at different levels is shown in table 2. The experiment was repeated 5 times and the area of zone of inhibition was recorded in square millimeters as the response variable.

Table 2 Environmental growth factors at two levels used in the factorial design.

Factors	Levels	Values
Independent		
1. Sugar	Glucose (2%)	1
	Galactose (2%)	0
2. pH	6	1
	7	0
3. Temperature	$30^{o}c$	1
	$40^{o}c$	0
4. Magnesium	1mM	1
	10mM	0
5. Calcium	1mM	1
	10mM	0
6. Nitrate	0mM	1
	40mM	0
7. Mannose	0%	1
	2%	0
Dependent		
1. Area of zone of inhibition	(mm^2)	Numeric

We applied four different tests viz: parametric (ANOVA), permutation, aligned rank, and rank ANOVAs to the data obtained from the experiment. Full models were built including all possible interaction among all the factors. Based on the performance of the four tests in simulation study (in next chapter), results were interpreted.

Chapter 3: Simulation Studies.

Simulations are controlled experiments that represent simplified versions of a real experiment. It allows us to study the effect of varying certain parameters on other parameters. We can also measure the robustness of the estimators by checking our models if violation of any assumption occurs.

For ease of interpreting, all settings have been listed in the Table 3 and 4 below. In summary, all settings follow the same basic format. We allowed the number of binary independent variables to vary from 2 to 8 so that it encompasses 7 independent variables of our real data. We allowed 5, 10, 30, 50, and 100 different replicates for each unique combination of independent variables. Under each specific setting, 100 simulated datasets were sampled such that the first 50 datasets were done specifying the coefficient of highest order interaction (for example $\beta_{highest}$ of 3 factor setting is β_7 for three-way interaction) as significant and second 50 datasets were done using in-significant highest order interaction. For each specific replicate, sensitivity, specificity, and accuracy are defined as below:

Sensitivity = number of times $\beta_{highest}$ identified as significant among first 50 simulations.

Specificity = number of times $\beta_{highest}$ identified as insignificant among second 50 simulations.

Accuracy = number of times $\beta_{highest}$ identified correctly as significant and insignificant out all 100 simulations.

Table 3. Model specification and settings for simulation study.

Number of independent variables (k)	2	4	6	8
Number of interactions (all level)	1	11	57	247
Number of runs (2^k) for single replicate	4	16	64	256
Number of Replicates	5, 10, 30, 50, 100	5, 10, 30, 50, 100	5, 10, 30, 50, 100	5, 10, 30, 50, 100
Total sample size	20, 40, 120, 200, 400	80, 160, 480, 200, 800, 1600	320, 640, 1920, 3200, 6400	1280, 2560, 7680, 12800, 25600

Table 4. Settings for simulations. X is the design matrix including main effects and interaction term, β is a vector of coefficients of all main effects and interaction terms including the intercept; s1 and s2 are arbitrary standard deviation; and e1 and e2 are vectors of error terms.

Set Number	Description
Set 1	Assumes each factorial combination (group) is sampled from a normal distribution and all groups have equal variances. ($Y \sim X\beta + e_1$, $e_1 \sim N(0, s_1^2)$).
Set 2	Assumes each factorial combination is sampled from a normal distribution and at least one group has different variance than other groups. ($Y = (Y_1, Y_2)$: $Y_1 \sim X\beta + e_1$, $Y_2 \sim X\beta + e_2$, $e_1 \sim N(0, s_1^2)$, $e_2 \sim N(0, s_2^2)$).
Set 3	Assumes each factorial combination is sampled from a non-normal distribution but all groups have equal variance. ($Y \sim X\beta + e_1$, $e_1 \sim t(r)$), where r is arbitrary degrees of freedom.
Set 4	Assumes each factorial combination is sampled from a non-normal distribution and at least one group has different variance than other groups. ($Y = (Y_1, Y_2)$: $Y_1 \sim X\beta + e_1$, $Y_2 \sim X\beta + e_2$, $e_1 \sim t(r)$ and $e_2 \sim$ Cauchy (μ, σ), where r is arbitrary degrees of freedom, μ is location parameter and σ is scale parameter for Cauchy distribution.

Simulated model specifications and parameters for each setting were defined as a continuous outcome (Y) as a function of k binary independent variables and all pairwise interactions (also all lowered powered interactions). Using matrix notation, we can specify model as $Y = X\beta + e$, where $Y = [Y_1, Y_2, .. Y_N]^T$ is N×1 vector of responses (dependent variable), X is $N \times 2^k$ design matrix, $\beta = [\beta_0, \beta_1, .. \beta_{(2^k-1)}]^T$ is $2^k \times 1$ vecotor of

regression coefficients, $e = [e_1, e_2 \ldots e_N]T$ is $N \times 1$ vector of error terms, N is number of observations and k is number of factors (independent variables).

For example, the theoretical model for 3 binary independent variables with 5 replicates would be:

$$Y_N=\beta_0+\beta_1X_1+ \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1X_2X_3 + e,$$

where X_1, X_2, and X_3 represent each binary factor, β_w denotes the coefficients of the main effects and interaction term (w= 1,2,..7), β_0 the intercept and N=1,2,3..40 (for 3 factors with 5 replicates N=2^3*5). For each setting we examined four different combinations of normality and group variances. These are summarized in Table 4. For example for normal distribution with equal variance (set 1), error terms were sampled from normal distribution as $e_i \sim N(0,4^2)$, where $i = (1, N)$. Two error terms $e1_i$ *and* $e2_j$ were sampled and combined for normal distribution with unequal variance (set 2) such that $e1_i \sim N(0,4^2)$, and $e2_j \sim N(0,2^2)$, *where* $i = (1, N/2)$ *and* $j = (N/2 +1, N)$. For non-normal distribution with equal variance (set 3), error terms were sampled from Student's t-distribution with 0.5 degrees of freedom (r), as $e_i \sim t(r)$, $i = (1, N)$. For non-normal distribution with unequal variances (set 4), two error terms were combined such that one was sampled from Student's t-distribution with 0.5 degrees of freedom, as $e_i \sim t(r)$, $i = (1, N/2)$; and the other from Cauchy distribution with location parameter (μ) of 0 and scale parameter (σ) of 3 as $e2_j \sim$ Cauchy (μ, σ), $j = (N/2 + 1, N)$. Vector of coefficients would be set as $\beta=(\beta_0, \beta_1, \beta_2, \beta_3 \ldots \beta_7)=(5, 5, 5, 5, 7.5, 7.5, 7.5, 10)$ for the first 50 datasets and $\beta= (5, 5, 5, 5, 7.5, 7.5, 7.5, 10^{-5})$ for second 50 datasets for each sets 1 through 4 mentioned above.

We applied parametric, permutation, aligned rank, and rank ANOVAs to each dataset within each setting so that direct comparisons could be made.

Chapter 4: Results.

Simulation studies were conducted to compare different approaches used in this thesis for factorial data analysis. Sensitivity, specificity, and accuracy of the approaches under different simulation settings were compared. We varied independent variables from 2 to 8 and allowed 5, 10, 30, 50, and 100 different replicates for each unique combination of independent variables. We sampled 100 simulated datasets from each unique combination such that the first 50 had significant highest order interaction and second 50 datasets had in-significant highest order interaction.

ANOVA:

In basic ANOVA, sensitivity was highly affected when the sampled data was from non-normal distribution. The type II error (probability of identifying interaction as insignificant when it is significant) was as high as 100% even when the number of observations were set high for just two independent variables. Table 5 shows the summary output of ANOVA test in the simulated data. Irrespective of the numbers of independent variables and replicates, the ANOVA approach failed to detect significant highest order interaction for non-normal sampled data (sets 3 and 4). The sensitivity of the normally distributed error term increased as the number of replicates increased. However, we did observe sensitivity as low as 6% for 8 factors with 5 replicates under normal distribution with equal variance (set 1). Specificity was consistently high for all factors at all replicates indicating low type I error (probability of identifying interaction as significant when it is insignificant). However, type I error as high as 12% (6 factors with 10 replicates under normal distribution with unequal variance) was observed.

Table 5. Summary table of simulation using ANOVA. NE: Normal and equal variance, NU: Normal and un-equal variance, NNE: Non-normal and equal variance, and NNU: Non-normal and unequal variance.

		Sensitivity				Specificity				Accuracy			
Factors	Replicate	NE	NU	NNE	NNU	NE	NU	NNE	NNU	NE	NU	NNE	NNU
2	5	0.74	0.9	0.06	0.02	0.92	0.92	1	0.98	0.83	0.91	0.53	0.5
	10	0.96	1	0.06	0.04	0.94	0.94	1	1	0.95	0.97	0.53	0.52
	30	1	1	0	0.02	0.94	0.9	0.98	1	0.97	0.95	0.49	0.51
	50	1	1	0	0	0.98	0.96	1	1	0.99	0.98	0.5	0.5
	100	1	1	0	0.02	0.96	0.96	1	0.98	0.98	0.98	0.5	0.5
4	5	0.24	0.42	0	0	0.94	0.94	1	1	0.59	0.68	0.5	0.5
	10	0.54	0.76	0	0	0.9	0.9	1	1	0.72	0.83	0.5	0.5
	30	0.94	1	0.02	0	0.98	0.98	0.98	1	0.96	0.99	0.5	0.5
	50	1	1	0	0.02	0.98	0.96	1	1	0.99	0.98	0.5	0.51
	100	1	1	0	0	0.94	0.94	1	1	0.97	0.97	0.5	0.5
6	5	0.14	0.14	0	0	0.94	0.96	1	1	0.54	0.55	0.5	0.5
	10	0.16	0.22	0.02	0	0.92	0.88	0.98	1	0.54	0.55	0.5	0.5
	30	0.48	0.64	0.02	0	0.92	0.94	0.98	1	0.7	0.79	0.5	0.5
	50	0.56	0.82	0	0	0.98	0.96	1	1	0.77	0.89	0.5	0.5
	100	0.82	0.92	0	0	0.92	0.92	1	1	0.87	0.92	0.5	0.5
8	5	0.06	0.06	0	0	0.94	0.96	1	1	0.5	0.51	0.5	0.5
	10	0.06	0.1	0	0	0.96	0.96	1	1	0.51	0.53	0.5	0.5
	30	0.12	0.18	0	0	1	1	1	1	0.56	0.59	0.5	0.5
	50	0.16	0.28	0	0	0.96	0.96	1	1	0.56	0.62	0.5	0.5
	100	0.38	0.5	0	0	0.98	0.98	1	1	0.68	0.74	0.5	0.5

Permutation:

For the permutation approach, we observed that sensitivity was very low when the sampled data were from non-normal distribution (sets 3 and 4) as compared to when data were from normal distribution (sets 1 and 2). The summary of the permutation on simulated data is given in table 6. When the number of factors were 4 or higher, 5 and 10 replicates did not produce sufficient number of observations to run the *ezPerm()* function. With 8 factors and 100 replicates, permutation was not run due to time limitation. We found type I error as high as 66% (6 factors with 30 replicates under normal and un-equal variance condition) and upto 100% type II error were observed. On the contraty, specificity was above 90% under all simulation settings.

Table 6 Summary table of simulation using permutation. NE: Normal and equal variance, NU: Normal and un-equal variance, NNE: Non-normal and equal variance, and NNU: Non-normal and unequal variance.

		Sensitivity				Specificity				Accuracy			
Factors	Replicate	NE	NU	NNE	NNU	NE	NU	NNE	NNU	NE	NU	NNE	NNU
2	5	0.74	0.9	0.06	0.06	0.92	0.92	0.98	0.98	0.83	0.91	0.52	0.52
	10	0.96	1	0.1	0.12	0.94	0.96	1	0.98	0.95	0.98	0.55	0.55
	30	1	1	0.06	0.08	0.94	0.9	0.98	0.94	0.97	0.95	0.52	0.51
	50	1	1	0.02	0.04	0.96	0.96	1	1	0.98	0.98	0.51	0.52
	100	1	1	0	0.04	0.96	0.98	1	0.96	0.98	0.99	0.5	0.5
4	5	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	10	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	30	0.94	1	0.02	0	0.98	0.98	0.98	0.96	0.96	0.99	0.5	0.48
	50	0.96	1	0	0	0.98	1	0.98	0.98	0.97	1	0.49	0.49
	100	1	1	0.02	0.02	0.94	0.94	1	0.98	0.97	0.97	0.51	0.5
6	5	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	10	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	30	0.4	0.66	0.04	0.02	0.94	0.92	1	1	0.67	0.79	0.52	0.51
	50	0.62	0.76	0.02	0	0.98	0.96	0.94	0.98	0.8	0.86	0.48	0.49
	100	0.86	1	0	0.02	0.92	0.92	0.96	0.96	0.89	0.96	0.48	0.49
8	5	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	10	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
	30	0.08	0.12	0.02	0.02	0.96	1	1	1	0.52	0.56	0.51	0.51
	50	0.32	0.44	0.08	0.08	0.9	0.92	0.96	0.98	0.61	0.68	0.52	0.53
	100	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*

Aligned Rank Transform:

We used *aligned.rank.transform()* function from the art package in R for this approach. Summary of the aligned rank transform approach in the simulated data is presented in Table 7. Since, aligned rank transform is a non-parametric approach, we expected it to perform well regardless of sampled data distrbutions.. However, the sensitivity of this approach was very low for the normally distributed data (sets 1 and 2) for higher number of factors. It significantly underperformed when trying to detect the insignificant highest order interactions term for non-normal conditions when four or more

factors were used in the model. For higher number of factors, higher number of replicates (higher number of observations) increased the sensitivity but not the specificity.

Table 7. Summary table of simulation using aligned rank transform. NE: Normal and equal variance, NU: Normal and un-equal variance, NNE: Non-normal and equal variance, and NNU: Non-normal and unequal variance.

		Sensitivity				Specificity				Accuracy			
Factors	Replicate	NE	NU	NNE	NNU	NE	NU	NNE	NNU	NE	NU	NNE	NNU
2	5	1	1	0.38	0.5	0.9	0.92	0.98	0.98	0.95	0.96	0.68	0.74
	10	1	1	0.36	0.5	0.94	0.94	0.96	0.98	0.97	0.97	0.66	0.74
	30	1	1	0.3	0.54	0.94	0.84	1	0.96	0.97	0.92	0.65	0.75
	50	1	1	0.14	0.5	0.98	0.94	0.98	1	0.99	0.97	0.56	0.75
	100	1	1	0.14	0.36	0.98	0.98	1	0.92	0.99	0.99	0.57	0.64
4	5	1	1	1	0.92	0.94	0.92	0.12	0.18	0.97	0.96	0.56	0.55
	10	1	1	0.94	0.98	0.88	0.86	0.04	0.06	0.94	0.93	0.49	0.52
	30	0.94	1	1	0.96	0.96	0.92	0.02	0.04	0.95	0.96	0.51	0.5
	50	1	1	0.98	0.94	0.98	0.96	0.02	0.04	0.99	0.98	0.5	0.49
	100	1	1	1	1	0.94	0.9	0	0.02	0.97	0.95	0.5	0.51
6	5	0.14	0.16	0.96	1	0.94	0.94	0.04	0	0.54	0.55	0.5	0.5
	10	0.18	0.28	0.98	0.98	0.94	0.84	0.02	0	0.56	0.56	0.5	0.49
	30	0.36	0.66	1	1	0.92	0.88	0	0	0.64	0.77	0.5	0.5
	50	0.62	0.88	1	1	0.98	0.96	0	0	0.8	0.92	0.5	0.5
	100	0.8	0.92	1	1	0.92	0.9	0	0	0.86	0.91	0.5	0.5
8	5	0.08	0.12	1	1	0.94	0.92	0	0	0.51	0.52	0.5	0.5
	10	0.04	0.14	1	0.98	0.96	0.96	0	0.02	0.5	0.55	0.5	0.5
	30	0.2	0.18	1	1	1	1	0	0	0.6	0.59	0.5	0.5
	50	0.14	0.38	1	1	0.94	0.94	0	0	0.54	0.66	0.5	0.5
	100	1	1	1	1	0.98	0.96	0	0	0.99	0.98	0.5	0.5

Rank ANOVA:

We used *raov()* function from the Rfit package for this approach with the default option, Wilcoxon scores. Rank based ANOVA was very consistent in identifying the significant and in-significant highest order interactions (Table 8). Both, for normal and non-normal sampled data, it had high accuracy. When there were 8 independent variables in the model, its sensitivity was low but not the specificity which is probably due to less

number of observations. Under all simulation settings, with sufficient number of observations, rank ANOVA had greater accuracy compared to all other approaches.

Table 8. Summary table of simulation using rank ANOVA. NE: Normal and equal variance, NU: Normal and un-equal variance, NNE: Non-normal and equal variance, and NNU: Non-normal and unequal variance.

		Sensitivity				Specificity				Accuracy			
Factors	Replicate	NE	NU	NNE	NNU	NE	NU	NNE	NNU	NE	NU	NNE	NNU
2	5	0.76	0.92	0.42	0.42	0.94	0.9	0.96	0.94	0.85	0.91	0.69	0.68
	10	0.94	1	0.78	0.8	0.92	0.94	0.94	0.94	0.93	0.97	0.86	0.87
	30	1	1	1	1	0.94	0.86	1	0.98	0.97	0.93	1	0.99
	50	1	1	1	1	0.98	0.94	0.98	0.98	0.99	0.97	0.99	0.99
	100	1	1	1	1	0.98	0.98	0.96	0.94	0.99	0.99	0.98	0.97
4	5	0.28	0.52	0.16	0.14	0.94	0.92	0.84	0.88	0.61	0.72	0.5	0.51
	10	0.52	0.78	0.36	0.38	0.88	0.88	0.88	0.94	0.7	0.83	0.62	0.66
	30	0.94	1	1	0.88	0.96	0.94	1	0.96	0.95	0.97	1	0.92
	50	0.98	1	1	0.98	0.96	0.96	0.98	0.94	0.97	0.98	0.99	0.96
	100	1	1	1	1	0.94	0.9	0.9	0.94	0.97	0.95	0.95	0.97
6	5	0.14	0.22	0.3	0.24	0.94	0.94	0.74	0.8	0.54	0.58	0.52	0.52
	10	0.22	0.3	0.18	0.1	0.92	0.8	0.96	0.92	0.57	0.55	0.57	0.51
	30	0.36	0.62	0.58	0.46	0.92	0.86	0.92	0.92	0.64	0.74	0.75	0.69
	50	0.62	0.88	0.86	0.56	0.98	0.96	0.96	0.92	0.8	0.92	0.91	0.74
	100	0.8	0.92	0.98	0.88	0.92	0.88	0.94	0.96	0.86	0.9	0.96	0.92
8	5	0.1	0.18	0.36	0.18	0.92	0.86	0.6	0.82	0.51	0.52	0.48	0.5
	10	0.04	0.16	0.14	0.06	0.96	0.92	0.86	0.96	0.5	0.54	0.5	0.51
	30	0.2	0.18	0.12	0.22	1	1	0.86	0.96	0.6	0.59	0.49	0.59
	50	0.14	0.34	0.36	0.26	0.94	0.94	0.86	0.96	0.54	0.64	0.61	0.61
	100	0.22	0.48	0.44	0.34	0.98	0.96	0.92	0.96	0.6	0.72	0.68	0.65

Real data analysis:

Full factorial design was used to study the effect of environmental factors on bacterial antibiotic sensitivity. Effect of 7 independent variables (factors) viz: sugar, pH, temperature, calcium, magnesium, nitrate and mannose were studied on antibiotic susceptibility of bacterium *Aztobacter vinelandii*. Our data has a total of 640 observations with 5 observations in each unique group. Factors were set to low and high

levels, except for factor “Sugar”, which was assigned either glucose or galactose. Bacterium was grown in a defined medium and area of zone of inhibition of antibiotics under all possible combinations of the 7 factors were measured. Area in square millimeters was used as response variable. We used all four approaches in our real data and used root mean square error (RMSE) to compare between the approaches in our data analysis. RMSE is square root of the variance of the residuals. It indicates the absolute fit of the model to the data. Compared to other measures (like mean absolute error (MAE), mean absolute scaled error (MASE), or mean error (ME)), the RMSE is more sensitive to the occasional large error because squaring the residuals penalizes very large errors heavily. Table 10 shows the comparison between different approaches applied on our real data using RMSE values. We found that all three approaches (ANOVA, aligned rank transform, and rank ANOVA) were equally good in predicting response variable in our real data analysis. Adjusted R^2 could not be used to compare the models because it was not possible to calculate for non-parametric approaches from the functions that has been used in this thesis. In addition, permutation approach could not be used in our real data with all 7 factors due to a smaller number of observations (5 observations per each unique combination of factors). We removed one factor “temperature” and re-ran the model with only 6 factors with all possible interactions through all approaches . Temperature is of least interest among other factors and therefore, model without temperature was used to study the effect of rest of the factors. Calculating RMSE was not possible in permutation approach, so permuation could not be compared with other approaches through RMSE.

When all 7 factors were included in the model, ANOVA and aligned rank transform yielded comparable p-values for the highest order interaction showing it significant as shown in table 9. However, rank ANOVA identified the highest order interaction as insignificant. Since, rank ANOVA was the most accurate one among all the approaches from our simulation study, the highest order interaction may not be significant as indicated by other two approaches (Table 9 and 11). When only 6 factors were considered, all four approaches yielded comparable p-value for the highest order interaction and it was insignificant. Model trimming was not possible in the permutation and rank ANOVA approach (ie., using *ezperm()* function and *raov()* function respectively). Therefore, it is not possible to create best fit model under both approaches.

Table 9. Significance of highest order interaction under different approaches.

Function	p-value	
	7 factors	6 factors
ANOVA	0.026374	0.846363
Rank ANOVA	0.26653	0.87403
Aligned rank transform	0.0216	0.7381
Permutation	*NA*	0.864

Table 10. Root mean square error values of full models created under different approaaches.

	Number of factors used in model	
Approach	7	6
ANOVA	66.59884	81.2854
Rank ANOVA	67.49195	81.5512
Aligned rank transform	66.59884	81.2854
Permutation	*NA*	*NA*

In summary, rank ANOVA and aligned rank transform based ANOVA are better alternatives to parametric tests if the distribution of the data is not known. However, with

many independent variables in the model, aligned rank transform looses its specificity and so, rank ANOVA should be preferred to it for such analysis. Permutation test becomes unmanageable with larger dataset and hence can be used for smaller datasets. There are only few non-parametric alternatives to ANOVA to analyze interactions in factorial data and very few among them are applicable when multiple interactions are present in the data. There should be more study on these tests with larger datasets for better use in real data analysis.

Chapter 5 Discussions.

Factorial designs are commonly used by practitioners in industrial and academic experimentation. The design allows one to investigate the effect of factors and their interaction on response variable and if those effects are significant. Most commonly, the F test is used to test the difference in means of observations grouped by combination of factor level. However, when assumptions of parametric tests like normal distribution of errors and homoscedasticity are violated, non-parametric tests are powerful alternatives for data analysis. Because common non-parametric tests (e.g., Wilcoxon and Friedman) do not examine interaction effects, only a few of the non-parametric tests can be used to handle multi-factorial data with interaction. We used a parametric (ANOVA) and three non-parametric approaches (rank ANOVA, permutation, and aligned rank transform) in factorial data analysis. We first used these approaches in simulated data and evaluated their performance based on sensitivity, specificity and accuracy to detect significant or insignificant highest-order interaction term. We then used the approaches in our real data and analyzed the findings based on the conclusions made from simulated data.

The parametric approach ANOVA performed very well in normally distributed data only. The type II error for ANOVA was very high in non-normal conditions (set 3 and 4) in our simulation study. Since ANOVA assumes that the data is normally distributed and has homogenous variance, it is not surprising to see such high type II errors for non-normal data. It has been reported earlier that violations of several assumptions of ANOVA reduces the power (the probability to avoid the type II error) (Sawilowsky, 1990). Specifically, when the data is heteroscedastic the power of the test is

reduced drastically (Snedecor & Cochran, 1980). Therefore, such condition necessitates non-parametric approach.

Permuation is a non-parametric alternative approach to analyze the factorial data. The *ezPerm()* function from the ez package by Lawrence (Lawrence & Lawrence, 2016) was used in this work for permutation tests because other packages like coin, lmPerm, perm etc., do not include tests for interactions (Jos feys 2016). However, the function *ezPerm()* is a work in progress. Permutation works by resampling the data many times in order to determine a p-value for the resampled data. Therefore a major disadvantage of this approach is that it becomes difficult to manage when multiple factors with interactions are involved. Computation is very time intensive at higher factors with interactions terms involved due to which we were unable to use this approach for 8 factors with 100 replicates. Nonetheless, we observed that lower number of observation results in very high type I and type II errors.

One of the more popular non-parametric techniques is Aligned Rank Transform (ART). It aligns the data and ranks them. Ranks are averaged in case of ties and then common ANOVA procedures are used, making it applicable to any number of factors. The limitation of ART as indicated by authors themselves, is in case of very high proportions of ties in the data, or in case of extremely skewed distribution. Ties are replaced by tied ranks and the skewness is reduced by the rank transformation (Wobbrock et al., 2011). We observed very low sensitivity of ART for the normally distributed data (sets 1 and 2) and very low specificity for non-normal data when four or more factors were used in the model. Others have also reported inflated type I errors in heterosedastic conditions, although not as extreme as ours which is probably due to

increased number of factors in our study (Leys & Schumann, 2010). Since, we used a t-distribution and a combination of a t-distribution and Cauchy distribution to sample non-normal data, we speculated that the outliers from the sampled data could be causing the decrease in specificity. To test this theory, we applied truncated t-distribution and truncated Cauchy distribution to sample our data (to better avoid the outliers). Under such conditions, the aligned rank transform approach had significantly increased specificity (data not shown). Since, this approach aligns the responses for each main effect or interaction by stripping away all effects from it except the one for which alignment is being done using cell means and overall means, outliers can imbalance the aligning and ranking process.

The rank-based approach was very robust in both normal and non-normal conditions. In rank estimation, the Euclidean distance of least squares estimation is replaced with Jaeckel's dispersion function that minimizes the dispersion of residuals and estimates the regression coefficients. It is therefore very similar to traditional least squares method. In summary, rank ANOVA (*raov()*) was the robust method to analyze the factorial data. But even rank based approach failed to detect significant highest order interactions when number of independent factors reached 8 at lower replicates. For 2 independent variables with only one interaction term a total of 3 independent variables are present in the model. However, for 8 independent variables, there are 247 interaction terms making a total of 255 total independent variables in the model. Therefore, 100 replicates might not be sufficient for the model to detect the significant interaction term with 8 factors.

In our real data analysis, we found that all of the approaches used in this study indicate significant interaction between all seven factors, except the rank ANOVA. Although rank ANOVA was the robust approach observed from the simulation studies, we cannot oversee the significance indicated by aligned rank transform and basic ANOVA. All the seven factors can interact with each other and change the antibiotic susceptibility of bacterium to an antibiotic. Even if we consider six-factor interaction when seven factors are included in the model (Table 11), several of them are significant, indicating that these factors change the susceptibility of bacteria to antibiotics differently in presence of other factors.

Appendix.

Supplementary tables

Table 11. Summary of ANOVA, rank ANOVA and aligned rank tests on real data with all seven factors included in the model.

Factors	ANOVA		Rank ANOVA		Aligned rank	
	F value	p-value	F value	p-value	F value	p-value
Ca	16.602	5.34E-05	23.22309	0	17.4144	3.53E-05
KNO3	0.625	0.429586	13.59921	0.00025	0.2001	6.55E-01
Mannose	0.745	0.3885	0.92874	0.33565	0.8070	3.69E-01
Mg	37.018	2.29E-09	14.10916	0.00019	40.6564	4.06E-10
pH	18.133	2.45E-05	21.09639	0.00001	16.8718	4.66E-05
Sugar	51.867	2.13E-12	62.83259	0	51.8660	2.13E-12
Temp	1.213	0.271258	0.81523	0.367	0.6674	4.14E-01
Ca:KNO3	1.185	0.276916	0.02443	0.87585	1.3077	2.53E-01
Ca:Mannose	13.995	0.000204	19.91812	0.00001	14.2042	1.83E-04
Ca:Mg	8.169	0.004435	19.9069	0.00001	7.8865	5.17E-03
Ca:Temp	16.135	6.78E-05	21.46463	0	17.8004	2.90E-05
KNO3:Mannose	1.125	0.289323	22.26553	0	1.3279	2.50E-01
KNO3:Temp	0.03	0.863514	0.01703	0.89621	0.0365	8.49E-01
Mannose:Temp	1.049	0.306213	5.95951	0.01498	1.2226	2.69E-01
Mg:KNO3	0.635	0.425827	3.2685	0.07121	1.7164	1.91E-01
Mg:Mannose	0.366	0.545594	4.72387	0.0302	0.5897	4.43E-01
Mg:Temp	0.485	0.486559	1.65201	0.19927	0.2148	6.43E-01
pH:Ca	15.803	8.04E-05	22.32691	0	18.0843	2.51E-05
pH:KNO3	1.324	0.250365	2.54201	0.11147	1.9766	1.60E-01
pH:Mannose	4.391	0.036611	0.14817	0.70045	3.6475	5.67E-02
pH:Mg	2.064	0.151447	2.66781	0.10301	2.7745	9.64E-02
pH:Temp	11.736	0.000662	11.61487	0.00071	9.9791	1.68E-03
Sugar:Ca	10.909	0.001024	12.62	0.00042	10.0255	1.64E-03
Sugar:KNO3	9.891	0.001757	20.61053	0.00001	9.5159	2.15E-03
Sugar:Mannose	0.25	0.617349	0.27815	0.59814	0.4492	5.03E-01
Sugar:Mg	22.46	2.78E-06	13.86351	0.00022	22.4770	2.76E-06
Sugar:pH	440.223	< 2e-16	480.65815	0	518.7785	7.91E-80
Sugar:Temp	13.232	0.000303	17.51985	0.00003	13.2961	2.93E-04
Ca:KNO3:Mannose	1.856	0.173681	0.02784	0.86754	2.2272	1.36E-01
Ca:KNO3:Temp	0.062	0.803848	1.53068	0.21658	0.0703	7.91E-01
Ca:Mannose:Temp	0.001	0.981049	2.08927	0.14895	0.0191	8.90E-01
Ca:Mg:KNO3	2.811	0.094244	6.18533	0.0132	3.8568	5.01E-02
Ca:Mg:Mannose	4.788	0.029112	2.65673	0.10373	5.9391	1.51E-02
Ca:Mg:Temp	1.961	0.162027	0.03971	0.84213	2.2829	1.31E-01

KNO3:Mannose:Temp	15.727	8.36E-05	7.45135	0.00656	15.4451	9.66E-05
Mg:KNO3:Mannose	0.344	0.557564	0.34424	0.55765	0.4802	4.89E-01
Mg:KNO3:Temp	1.424	0.233306	0.75862	0.38417	1.1072	2.93E-01
Mg:Mannose:Temp	1.902	0.168419	7.70489	0.00571	1.4192	2.34E-01
pH:Ca:KNO3	0.067	0.796281	2.11453	0.14652	0.0006	9.80E-01
pH:Ca:Mannose	0.218	0.640565	2.85168	0.09189	0.3070	5.80E-01
pH:Ca:Mg	1.615	0.20436	0.32546	0.56859	1.3997	2.37E-01
pH:Ca:Temp	3.691	0.055251	3.74732	0.05344	3.5235	6.11E-02
pH:KNO3:Mannose	0.002	0.961112	3.85221	0.05022	0.0191	8.90E-01
pH:KNO3:Temp	3.646	0.056769	3.95522	0.04726	3.2897	7.03E-02
pH:Mannose:Temp	3.019	0.08288	0.02993	0.86271	2.8817	9.02E-02
pH:Mg:KNO3	35.388	5.01E-09	48.76086	0	37.1284	2.18E-09
pH:Mg:Mannose	0.074	0.786009	3.4909	0.06228	0.0637	8.01E-01
pH:Mg:Temp	0.045	0.832992	0.03691	0.84772	0.0003	9.85E-01
Sugar:Ca:KNO3	0.016	0.898033	3.01085	0.08331	0.1892	6.64E-01
Sugar:Ca:Mannose	0.945	0.331457	5.09404	0.02443	1.4214	2.34E-01
Sugar:Ca:Mg	1.151	0.283867	1.35698	0.2446	1.7178	1.91E-01
Sugar:Ca:Temp	7.713	0.005683	12.47028	0.00045	9.3399	2.36E-03
Sugar:KNO3:Mannose	4.182	0.041354	4.06509	0.0443	3.6323	5.72E-02
Sugar:KNO3:Temp	4.588	0.03266	17.14247	0.00004	3.6132	5.79E-02
Sugar:Mannose:Temp	0.074	0.785368	1.83978	0.17557	0.0573	8.11E-01
Sugar:Mg:KNO3	17.334	3.68E-05	25.02918	0	18.7276	1.81E-05
Sugar:Mg:Mannose	0.095	0.75859	0.00046	0.98285	0.0924	7.61E-01
Sugar:Mg:Temp	26.249	4.26E-07	11.10246	0.00092	25.8149	5.27E-07
Sugar:pH:Ca	24.122	1.22E-06	27.82315	0	23.8437	1.40E-06
Sugar:pH:KNO3	9.015	0.002809	3.90628	0.04864	10.5416	1.24E-03
Sugar:pH:Mannose	0.059	0.808688	2.62001	0.10614	0.0363	8.49E-01
Sugar:pH:Mg	0.187	0.665199	7.05401	0.00816	0.2999	5.84E-01
Sugar:pH:Temp	26.466	3.83E-07	34.83018	0	27.5048	2.30E-07
Ca:KNO3:Mannose:Temp	5.291	0.021833	5.59848	0.01835	4.8754	2.77E-02
Ca:Mg:KNO3:Mannose	0.001	0.969416	0.0654	0.79826	0.0000	9.96E-01
Ca:Mg:KNO3:Temp	0.121	0.728028	0.1109	0.73926	0.1653	6.85E-01
Ca:Mg:Mannose:Temp	0.081	0.776731	0.04598	0.8303	0.0110	9.17E-01
Mg:KNO3:Mannose:Temp	0.05	0.823898	0.5218	0.4704	0.0082	9.28E-01
pH:Ca:KNO3:Mannose	1.056	0.304642	2.69195	0.10147	0.7833	3.77E-01
pH:Ca:KNO3:Temp	0.34	0.559945	2.40769	0.12136	0.4314	5.12E-01
pH:Ca:Mannose:Temp	0.11	0.74011	0.62164	0.4308	0.2056	6.50E-01
pH:Ca:Mg:KNO3	2.175	0.140899	3.66538	0.05611	2.2866	1.31E-01
pH:Ca:Mg:Mannose	3.775	0.052569	0.00157	0.96839	4.6651	3.12E-02
pH:Ca:Mg:Temp	0.066	0.797568	0.08875	0.7659	0.0528	8.18E-01
pH:KNO3:Mannose:Temp	0.129	0.719907	1.36039	0.24401	0.1389	7.10E-01
pH:Mg:KNO3:Mannose	1.33	0.249424	0.00305	0.95595	1.4418	2.30E-01
pH:Mg:KNO3:Temp	2.678	0.102348	1.36595	0.24305	1.6795	1.96E-01

pH:Mg:Mannose:Temp	1.897	0.169062	0.44571	0.50468	1.4438	2.30E-01
Sugar:Ca:KNO3:Mannose	19.946	9.81E-06	12.49893	0.00044	18.9835	1.59E-05
Sugar:Ca:KNO3:Temp	0.103	0.74799	0.24386	0.62165	0.3229	5.70E-01
Sugar:Ca:Mannose:Temp	0.194	0.65991	4.47668	0.03484	0.2033	6.52E-01
Sugar:Ca:Mg:KNO3	0.075	0.784247	0.19138	0.66196	0.0637	8.01E-01
Sugar:Ca:Mg:Mannose	0.003	0.954803	1.71839	0.19049	0.0254	8.73E-01
Sugar:Ca:Mg:Temp	5.59	0.018437	1.85938	0.1733	5.9619	1.50E-02
Sugar:KNO3:Mannose:Temp	1.3	0.254763	4.60278	0.03239	1.0507	3.06E-01
Sugar:Mg:KNO3:Mannose	0.019	0.891441	3.08126	0.0798	0.0058	9.39E-01
Sugar:Mg:KNO3:Temp	0.013	0.908429	0.39445	0.53025	0.0699	7.92E-01
Sugar:Mg:Mannose:Temp	1.067	0.302003	2.07773	0.15007	1.5091	2.20E-01
Sugar:pH:Ca:KNO3	3.395	0.065953	7.62547	0.00596	4.8226	2.85E-02
Sugar:pH:Ca:Mannose	0.775	0.379067	0.95692	0.32843	0.8478	3.58E-01
Sugar:pH:Ca:Mg	12.348	0.000481	13.95196	0.00021	10.8075	1.08E-03
Sugar:pH:Ca:Temp	2.909	0.088667	4.43314	0.03573	3.3507	6.78E-02
Sugar:pH:KNO3:Mannose	3.125	0.077682	1.29209	0.25619	3.5511	6.01E-02
Sugar:pH:KNO3:Temp	0.131	0.717415	4.16067	0.04189	0.0014	9.70E-01
Sugar:pH:Mannose:Temp	0.364	0.546702	12.15372	0.00053	0.7721	3.80E-01
Sugar:pH:Mg:KNO3	0.008	0.929434	0.19572	0.65838	0.0093	9.23E-01
Sugar:pH:Mg:Mannose	0.191	0.662175	4.56593	0.03309	0.5011	4.79E-01
Sugar:pH:Mg:Temp	18.825	1.73E-05	4.92627	0.02689	18.4504	2.09E-05
Ca:Mg:KNO3:Mannose:Temp	0.99	0.320188	0.85478	0.35564	0.8702	3.51E-01
pH:Ca:KNO3:Mannose:Temp	1.77	0.183956	4.02934	0.04524	1.4362	2.31E-01
pH:Ca:Mg:KNO3:Mannose	1.339	0.247803	3.50914	0.0616	1.1993	2.74E-01
pH:Ca:Mg:KNO3:Temp	10.443	0.001311	18.11382	0.00002	12.6879	4.02E-04
pH:Ca:Mg:Mannose:Temp	1.792	0.181295	1.73197	0.18875	1.9774	1.60E-01
pH:Mg:KNO3:Mannose:Temp	0.001	0.970081	3.12403	0.07774	0.0294	8.64E-01
Sugar:Ca:KNO3:Mannose:Temp	0.901	0.343086	3.68413	0.05549	0.8027	3.71E-01
Sugar:Ca:Mg:KNO3:Mannose	1.186	0.27664	7.3248	0.00703	1.3400	2.48E-01
Sugar:Ca:Mg:KNO3:Temp	0.892	0.345315	2.89446	0.08949	1.3462	2.46E-01
Sugar:Ca:Mg:Mannose:Temp	0.268	0.604935	3.26705	0.07127	0.4544	5.01E-01
Sugar:Mg:KNO3:Mannose:Temp	0.17	0.680555	1.92357	0.16607	0.1628	6.87E-01
Sugar:pH:Ca:KNO3:Mannose	0.015	0.902321	0.02847	0.86609	0.0539	8.17E-01
Sugar:pH:Ca:KNO3:Temp	3.558	0.059826	0.98349	0.32181	4.4385	3.56E-02
Sugar:pH:Ca:Mannose:Temp	2.961	0.085904	17.73596	0.00003	4.2861	3.89E-02
Sugar:pH:Ca:Mg:KNO3	5.603	0.018304	9.32597	0.00238	5.9497	1.51E-02
Sugar:pH:Ca:Mg:Mannose	0.273	0.601306	1.17271	0.27935	0.2894	5.91E-01
Sugar:pH:Ca:Mg:Temp	19.358	1.32E-05	1.39225	0.23857	21.1010	5.49E-06
Sugar:pH:KNO3:Mannose:Temp	6.377	0.011863	2.38977	0.12275	7.0996	7.95E-03
Sugar:pH:Mg:KNO3:Mannose	0.058	0.809495	2.45869	0.11749	0.1185	7.31E-01
Sugar:pH:Mg:KNO3:Temp	5.188	0.023152	9.07202	0.00272	6.1312	1.36E-02
Sugar:pH:Mg:Mannose:Temp	0.638	0.42498	10.52805	0.00125	0.8536	3.56E-01
pH:Ca:Mg:KNO3:Mannose:Temp	1.529	0.216859	0.17648	0.67459	1.7310	1.89E-01

Sugar:Ca:Mg:KNO3:Mannose:Temp	1.995	0.15844	0.04863	0.82555	1.8997	1.69E-01
Sugar:pH:Ca:KNO3:Mannose:Temp	3.643	0.05685	10.30136	0.00141	4.0833	4.38E-02
Sugar:pH:Ca:Mg:KNO3:Mannose	0.05	0.823574	0.2536	0.61477	0.0369	8.48E-01
Sugar:pH:Ca:Mg:KNO3:Temp	0.186	0.666411	0.77968	0.37765	0.3044	5.81E-01
Sugar:pH:Ca:Mg:Mannose:Temp	0.37	0.543382	21.11568	0.00001	0.3502	5.54E-01
Sugar:pH:Mg:KNO3:Mannose:Temp	1.018	0.313449	0.50389	0.47812	1.5202	2.18E-01
Sugar:pH:Ca:Mg:KNO3:Mannose:Temp	4.96	0.026374	1.23724	0.26653	5.3135	2.16E-02

Table 12. Summary of ANOVA, rank ANOVA, aligned rank and permutation tests on real data with six factors included in the model (temperature is excluded).

Factors	ANOVA		Rank ANOVA		Aligned rank		Permutation
	F value	p-value	F value	p-value	F value	p-value	p-value
Ca	12.538	0.00043	15.6114	0.00009	13.874	2.15E-04	0
KNO3	0.472	0.49237	8.22037	0.00429	0.271	6.03E-01	0.481
Mannose	0.563	0.45354	0.16274	0.6868	0.618	4.32E-01	0.452
Mg	27.956	1.76E-07	0.28815	0.59161	28.289	1.50E-07	0
pH	13.694	0.00024	15.8777	0.00008	14.416	1.62E-04	0
Sugar	39.17	7.59E-10	43.7415	0	40.960	3.22E-10	0
Ca:KNO3	0.895	0.34461	20.8016	0.00001	1.101	2.94E-01	0.334
Ca:Mannose	10.569	0.00122	3.73236	0.05386	9.897	1.74E-03	0
Ca:Mg	6.169	0.01328	1.00586	0.31632	7.261	7.25E-03	0.017
KNO3:Mannose	0.85	0.35703	10.5412	0.00124	0.751	3.87E-01	0.371
Mg:KNO3	0.48	0.48884	1.34704	0.24628	1.449	2.29E-01	0.488
Mg:Mannose	0.276	0.59939	0.1652	0.68457	0.242	6.23E-01	0.58
pH:Ca	11.935	0.00059	10.9863	0.00098	10.435	1.31E-03	0
pH:KNO3	1	0.31771	0.74958	0.38697	1.021	3.13E-01	0.321
pH:Mannose	3.316	0.06911	2.13171	0.14482	2.867	9.10E-02	0.08
pH:Mg	1.559	0.21239	0.84571	0.35815	2.248	1.34E-01	0.221
Sugar:Ca	8.238	0.00425	10.727	0.00112	9.553	2.09E-03	0.002
Sugar:KNO3	7.47	0.00647	0.38743	0.5339	7.585	6.07E-03	0.004
Sugar:Mannose	0.189	0.66413	1.06278	0.30301	0.505	4.77E-01	0.664
Sugar:Mg	16.962	4.37E-05	8.74276	0.00324	15.055	1.16E-04	0
Sugar:pH	332.455	< 2e-16	342.935	0	374.207	1.30E-64	0
Ca:KNO3:Mannose	1.402	0.23693	2.98162	0.08475	1.747	1.87E-01	0.219
Ca:Mg:KNO3	2.123	0.14568	0.0119	0.91315	2.492	1.15E-01	0.138
Ca:Mg:Mannose	3.616	0.05773	0.00581	0.93928	3.956	4.72E-02	0.059
Mg:KNO3:Mannose	0.26	0.61026	10.9505	0.00099	0.145	7.03E-01	0.611
pH:Ca:KNO3	0.05	0.82248	1.05623	0.30451	0.022	8.81E-01	0.809
pH:Ca:Mannose	0.165	0.6849	1.14961	0.28408	0.063	8.03E-01	0.703
pH:Ca:Mg	1.22	0.26988	0.02355	0.87808	0.570	4.51E-01	0.28
pH:KNO3:Mannose	0.002	0.9662	9.57113	0.00207	0.175	6.76E-01	0.97
pH:Mg:KNO3	26.725	3.24E-07	10.3208	0.00139	29.578	7.95E-08	0
pH:Mg:Mannose	0.056	0.81347	0.00441	0.94709	0.110	7.40E-01	0.794
Sugar:Ca:KNO3	0.012	0.91132	3.14046	0.0769	0.070	7.91E-01	0.909
Sugar:Ca:Mannose	0.714	0.39858	8.46313	0.00376	0.891	3.46E-01	0.425
Sugar:Ca:Mg	0.869	0.35158	0.09932	0.75276	0.591	4.42E-01	0.373
Sugar:KNO3:Mannose	3.159	0.07606	14.9043	0.00013	3.145	7.67E-02	0.089
Sugar:Mg:KNO3	13.091	0.00032	0.23182	0.63036	13.215	3.03E-04	0
Sugar:Mg:Mannose	0.071	0.7894	2.44268	0.11862	0.098	7.54E-01	0.769
Sugar:pH:Ca	18.217	2.30E-05	18.8914	0.00002	17.795	2.86E-05	0
Sugar:pH:KNO3	6.808	0.00931	2.42164	0.12022	8.088	4.61E-03	0.004

Sugar:pH:Mannose	0.044	0.83334	0.36871	0.54395	0.094	7.60E-01	0.831
Sugar:pH:Mg	0.142	0.70685	8.99853	0.00282	0.835	3.61E-01	0.698
Ca:Mg:KNO3:Mannose	0.001	0.97342	0.28329	0.59476	0.023	8.79E-01	0.981
pH:Ca:KNO3:Mannose	0.797	0.37225	0.60244	0.43797	0.576	4.48E-01	0.375
pH:Ca:Mg:KNO3	1.642	0.20051	0.00377	0.95104	2.391	1.23E-01	0.205
pH:Ca:Mg:Mannose	2.851	0.09186	2.78523	0.09568	3.204	7.40E-02	0.097
pH:Mg:KNO3:Mannose	1.004	0.31675	16.5174	0.00005	1.413	2.35E-01	0.305
Sugar:Ca:KNO3:Mannose	15.063	0.00012	1.53376	0.21605	15.493	9.29E-05	0
Sugar:Ca:Mg:KNO3	0.057	0.81193	1.97166	0.16081	0.080	7.77E-01	0.798
Sugar:Ca:Mg:Mannose	0.002	0.96072	4.40897	0.03618	0.097	7.55E-01	0.961
Sugar:Mg:KNO3:Mannose	0.014	0.90558	14.6809	0.00014	0.040	8.42E-01	0.926
Sugar:pH:Ca:KNO3	2.564	0.10985	7.44169	0.00657	3.881	4.93E-02	0.124
Sugar:pH:Ca:Mannose	0.585	0.44455	0.20201	0.65327	0.299	5.84E-01	0.441
Sugar:pH:Ca:Mg	9.325	0.00236	4.04785	0.04469	9.487	2.17E-03	0
Sugar:pH:KNO3:Mannose	2.36	0.12502	0.29074	0.58996	2.886	8.99E-02	0.155
Sugar:pH:Mg:KNO3	0.006	0.93865	0.19383	0.65991	0.011	9.17E-01	0.94
Sugar:pH:Mg:Mannose	0.144	0.70416	0.71698	0.39749	0.108	7.42E-01	0.694
pH:Ca:Mg:KNO3:Mannose	1.011	0.31509	2.6458	0.10437	0.781	3.77E-01	0.305
Sugar:Ca:Mg:KNO3:Mannose	0.896	0.34433	0.04419	0.83357	1.222	2.69E-01	0.333
Sugar:pH:Ca:KNO3:Mannose	0.011	0.91506	0.89147	0.34547	0.136	7.13E-01	0.913
Sugar:pH:Ca:Mg:KNO3	4.231	0.04014	6.78513	0.00943	4.457	3.52E-02	0.037
Sugar:pH:Ca:Mg:Mannose	0.206	0.64974	0.317	0.57364	0.164	6.86E-01	0.646
Sugar:pH:Mg:KNO3:Mannose	0.044	0.83405	0.1109	0.73925	0.007	9.33E-01	0.825
Sugar:pH:Ca:Mg:KNO3:Mannose	0.038	0.84636	0.02516	0.87403	0.112	7.38E-01	0.864

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