Day 4: One-Way and Two-Way ANOVA using R $\,$

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Topics Covered

- One-Way ANOVA
 - Data Entry and Data Manipulation
 - Hypothesis Testing
 - Checking Assumptions
- Two-Way ANOVA
 - Data Entry and Data Manipulation
 - Hypothesis Testing
 - Checking Assumptions

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Preliminaries

1.1 Setting Up RStudio

In order for us to be on the same page all throughout the discussion, set up RStudio as explained in the following video.

1.2 Installing the needed R packages

install.packages(c("tidyverse", "ggpubr", "rstatix", "markdown", "rmarkdown", "tinytex"))

One-Way ANOVA

Data Entry

Load the necessary packages.

```
library(tidyverse)
## -- Attaching packages -----
## v ggplot2 3.3.5 v purrr
                                  0.3.4
## v tibble 3.1.6 v dplyr 1.0.7
## v tidyr 1.1.4 v stringr 1.4.0
## v readr 2.1.0 v forcats 0.5.1
## -- Conflicts -----
                                          ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()
                      masks stats::lag()
library(ggpubr)
library(multcomp)
## Loading required package: mvtnorm
## Loading required package: survival
## Loading required package: TH.data
## Loading required package: MASS
## Attaching package: 'MASS'
## The following object is masked from 'package:dplyr':
##
##
       select
## Attaching package: 'TH.data'
```

```
## The following object is masked from 'package:MASS':
##
## geyser
```

Load data to R using read_csv() function of the readr package of tidyverse and save it with a variable name oneway_data.

```
# Load and save
oneway_data <- read_csv(file = "data/Tubo-USEP_One-Way Cleaned Data for R.csv")
# Preview
oneway_data</pre>
```

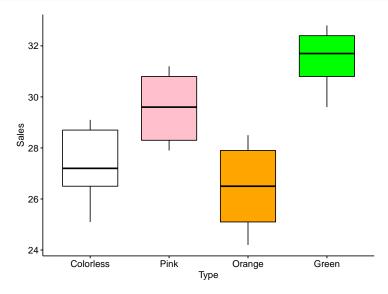
```
## # A tibble: 5 x 5
## Observation Colorless Pink Orange Green
##
    <dbl> <dbl> <dbl> <dbl> <dbl> <dbl> <
## 1
                 26.5 31.2 27.9 30.8
           1
## 2
          2
                 28.7 28.3
                           25.1 29.6
## 3
          3
                 25.1 30.8 28.5 32.4
## 4
          4
                29.1 27.9
                            24.2 31.7
          5 27.2 29.6
## 5
                            26.5 32.8
```

Data Manipulation

```
oneway_data %>%
  gather(key = "Type", value = "Sales", -Observation) %>%
  {\it \# mutate(across(c(Observation, Type), ~as\_factor(.x)))}
  mutate(across(Observation:Type, ~as_factor(.x))) -> clean_oneway_data
clean_oneway_data
## # A tibble: 20 x 3
##
      Observation Type
                           Sales
##
      <fct> <fct>
                          <dbl>
## 1 1
                Colorless 26.5
## 2 2
                 Colorless 28.7
## 3 3
                 Colorless 25.1
## 4 4
                  Colorless 29.1
## 5 5
                  Colorless 27.2
## 6 1
                  Pink
## 7 2
                 Pink
                            28.3
## 8 3
                 Pink
                            30.8
## 9 4
                 Pink
                            27.9
## 10 5
                  Pink
                             29.6
## 11 1
                  Orange
                             27.9
## 12 2
                  Orange
                             25.1
## 13 3
                             28.5
                  Orange
## 14 4
                             24.2
                  Orange
## 15 5
                  Orange
                             26.5
## 16 1
                  Green
                             30.8
## 17 2
                  Green
                             29.6
## 18 3
                  Green
                             32.4
## 19 4
                  Green
                             31.7
## 20 5
                  Green
                            32.8
```

```
str(clean_oneway_data)
## tibble [20 x 3] (S3: tbl_df/tbl/data.frame)
## $ Observation: Factor w/ 5 levels "1","2","3","4",..: 1 2 3 4 5 1 2 3 4 5 ...
             : Factor w/ 4 levels "Colorless", "Pink", ...: 1 1 1 1 1 2 2 2 2 2 ...
                : num [1:20] 26.5 28.7 25.1 29.1 27.2 31.2 28.3 30.8 27.9 29.6 ...
## $ Sales
clean_oneway_data %>%
  sample_n(10)
## # A tibble: 10 x 3
##
     Observation Type
                           Sales
##
     <fct>
                 <fct>
                           <dbl>
##
   1 1
                 Green
                            30.8
## 2 5
                 Green
                            32.8
## 3 4
                 Pink
                            27.9
## 4 5
                 Colorless 27.2
##
   5 1
                 Colorless 26.5
                 Pink
## 6 1
                            31.2
## 7 4
                 Green
                            31.7
## 8 4
                 Colorless 29.1
## 9 5
                 Pink
                            29.6
## 10 3
                 Orange
                            28.5
levels(clean_oneway_data$Type)
## [1] "Colorless" "Pink"
                              "Orange"
                                          "Green"
```

Data Visualization



Hypothesis Testing

The One-Way ANOVA Table in R

```
one_aov <- clean_oneway_data %>%
  aov(formula = Sales ~ Type, data = .)
one_aov %>%
  summary
```

```
## Df Sum Sq Mean Sq F value Pr(>F)
## Type 3 76.85 25.615 10.49 0.000466 ***
## Residuals 16 39.08 2.443
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

In the table,

- \bullet Df degress of freedom
- \bullet Sum Sq sum of squares
- $\bullet\,$ Mean Sq mean sum of squares
- ullet F value value of F statistic
- Pr(>F) p-value

Thus, from the table

$$SSB = 76.85$$
 $MSB = 25.615$ $F = 10.49$ (5.1)

$$SSE = 39.08$$
 $MSE = 2.443$ (5.2)

(5.3)

Similar to when you look up at an F-table, the p-value can be computed using the following R code.

```
pf(q = 10.49, df1 = 3, df2 = 16, lower.tail = F)
## [1] 0.0004652698
```

Checking Assumptions¹

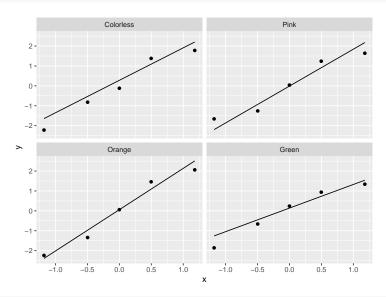
6.1 Checking Normality Assumptions

 $Shapiro-Wilk\ Test$

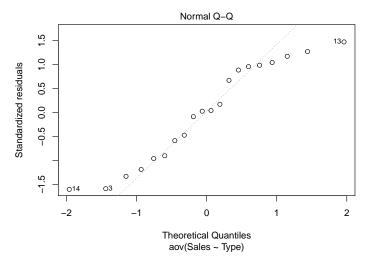
The Shapiro-Wilk test tests the null hypothesis that the samples come from a normal distribution against the alternative hypothesis that the samples do not come from a normal distribution.

¹Except for most of the codes, the contents of this section are obtained from this link

```
clean_oneway_data %>%
  mutate(Residual = one_aov$residuals) %>%
  ggplot(aes(sample = Residual)) +
  stat_qq() +
  stat_qq_line() +
  facet_wrap(~Type)
```

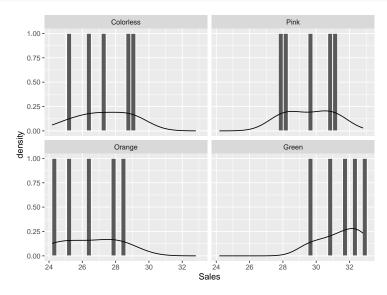


plot(one_aov,2)



Histogram

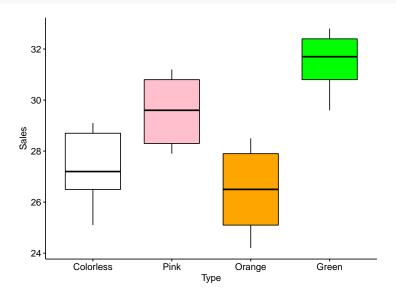
```
clean_oneway_data %>%
  ggplot(aes(x = Sales)) +
  geom_histogram(bins = 30, color = "white") +
  geom_density() +
  facet_wrap(~Type)
```



6.2 Checking Homogeneity of Variance Assumption

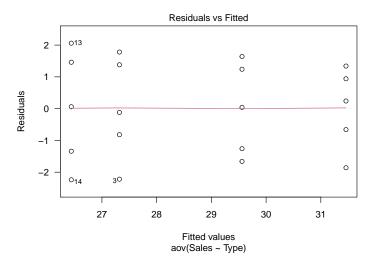
Bartlet's Test

Bartlett's test tests the null hypothesis that the group variances are equal against the alternative hypothesis that the group variances are not equal.



The variability within each group is represented by the vertical size of each box; i.e., the interquartile range (IQR). The boxplot shows that the variability is roughly equal for each group. Let's look at some more ways to test the homogeneity of variance assumption.

Residual vs. Fitted Values Plot

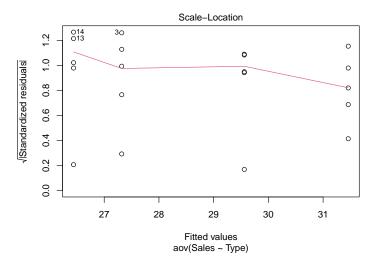


This plot shows the residuals (errors) on the y-axis and the fitted values (pre-

dicted values) on the x-axis. If the variance of each group is equal, the plot should show no pattern; in other words, the points should look like a cloud of random points. The plot shows that the variances are approximately homogenous since the residuals are distributed approximately equally above and below zero.

Standardised Residuals vs Fitted values Plot

plot(one_aov,3)



The more coincident the red line plot to the horizontal line at 1, the lesser possibility the violation of the homogeneity of variance assumption.

Post-hoc

7.1 TukeyHSD (Tukey's Honestly-Significant Difference) post-hoc test in R

There are at least two ways to perform a Tukey's HSD post-hoc in R. One is by using the TukeyHSD function of the pre-installed R package stats. The second is the glht function with "Tukey" option bundled along with the multcomp package.

7.2 Using TukeyHSD of stats package

```
TukeyHSD(one_aov)
```

```
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = Sales ~ Type, data = .)
##
## $Type
##
                     diff
                                 lwr
                                            upr
                                                    p adj
## Pink-Colorless
                     2.24 -0.5880714 5.0680714 0.1479369
## Orange-Colorless -0.88 -3.7080714 1.9480714 0.8099459
## Green-Colorless
                     4.14 1.3119286 6.9680714 0.0034923
## Orange-Pink
                    -3.12 -5.9480714 -0.2919286 0.0281177
## Green-Pink
                    1.90 -0.9280714 4.7280714 0.2580535
                     5.02 2.1919286 7.8480714 0.0005837
## Green-Orange
```

Discussion of Results. Picking up from the significant ANOVA result in our soft drink data, the Tukey's HSD post-hoc analysis result above shows the

following significant comparisons at 0.05:

7.3 Using the multicomp package with "Tukey" option

```
summary(glht(one_aov, linfct = mcp(Type = "Tukey")))
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: aov(formula = Sales ~ Type, data = .)
## Linear Hypotheses:
##
                         Estimate Std. Error t value Pr(>|t|)
## Pink - Colorless == 0
                        2.2400 0.9885 2.266 0.14815
## Orange - Colorless == 0 -0.8800 0.9885 -0.890 0.80991
## Green - Colorless == 0 4.1400 0.9885 4.188 0.00339 **
                        -3.1200
1.9000
## Orange - Pink == 0
                                     0.9885 -3.156 0.02817 *
## Green - Pink == 0
                                     0.9885 1.922 0.25800
                                     0.9885 5.078 < 0.001 ***
## Green - Orange == 0
                           5.0200
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

Two-Way ANOVA

Data $Entry^1$

Load data to R using read_csv() function of the readr package of tidyverse and save it with a variable name twoway_data.

```
# Load and save
twoway_data <- read_csv(file = "data/Tubo-USEP_Two-Way Cleaned Data for R.csv")
# Preview
twoway_data
## # A tibble: 4 x 7
    Fertilizer Manure
                          P1
                                P2
                                      РЗ
                                            P4
                                                  Р5
     <chr>
                <chr>
                       <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>
## 1 High
                High
                        13.7
                              15.8
                                    13.9
                                          16.6
## 2 High
                Low
                        16.4 12.5
                                   14.1
                                          14.4 12.2
## 3 Low
                High
                        15
                              15.1 12
                                          15.7
                                               12.2
## 4 Low
                Low
                        12.4 10.6 13.7
                                           8.7 10.9
```

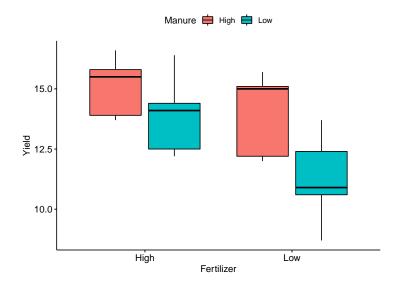
 $^{^{1}\}mathrm{The}$ contents of the succeeding sections are obtained from this link

Data Manipulation

```
twoway_data%>%
 gather(key = Plot, value = Yield, -c(Fertilizer, Manure)) %>%
 mutate(across(Fertilizer:Plot, ~ as.factor(.x))) -> clean_twoway_data
# Structure preview
str(clean_twoway_data)
## tibble [20 x 4] (S3: tbl_df/tbl/data.frame)
## $ Fertilizer: Factor w/ 2 levels "High", "Low": 1 1 2 2 1 1 2 2 1 1 ...
              : Factor w/ 2 levels "High", "Low": 1 2 1 2 1 2 1 2 1 2 ...
## $ Plot
               : Factor w/ 5 levels "P1", "P2", "P3", ...: 1 1 1 1 2 2 2 2 3 3 ...
## $ Yield
               : num [1:20] 13.7 16.4 15 12.4 15.8 12.5 15.1 10.6 13.9 14.1 ...
# Sample preview
clean_twoway_data %>%
 sample_n(10)
## # A tibble: 10 x 4
     Fertilizer Manure Plot Yield
##
     <fct>
                <fct> <fct> <dbl>
## 1 High
                High
                       РЗ
                              13.9
## 2 Low
                Low
                       P5
                              10.9
## 3 High
                High P2
                              15.8
## 4 High
                       P4
                              14.4
                Low
## 5 High
                High P5
                              15.5
## 6 High
                Low
                       P2
                            12.5
## 7 Low
                High
                       P3
                             12
## 8 High
                High
                       P1
                              13.7
## 9 High
                       P3
                              14.1
                Low
## 10 Low
                Low
                       P3
                              13.7
```

Data Visualization

```
ggboxplot(clean_twoway_data,
    x = "Fertilizer",
    y = "Yield",
    fill = "Manure")
```



Hypothesis Testing

11.1 The Two-Way ANOVA Table with Main Effects Only

```
two_aov <- clean_twoway_data %>%
  aov(formula = Yield ~ Fertilizer + Manure, data = .)
two_aov %>%
  summary
```

```
## Df Sum Sq Mean Sq F value Pr(>F)
## Fertilizer 1 17.67 17.672 6.332 0.0222 *
## Manure 1 19.21 19.208 6.883 0.0178 *
## Residuals 17 47.44 2.791
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Similar to the One-Way ANOVA table,

- Df degress of freedom
- Sum Sq sum of squares
- $\bullet\,$ Mean Sq mean sum of squares
- ullet F value value of F statistic
- Pr(>F) *p*-value

Thus, from the table

$$SSR = 17.67$$
 $MSR = 17.672$ $F_C = 6.332$ (11.1)
 $SSC = 19.21$ $MSC = 19.208$ $F_R = 6.883$ (11.2)
 $SSE = 47.44$ $MSE = 2.791$ (11.3)

Similar to when you look up at an F-table, the p-values can be computed using the following R code.

```
pf(q = 6.332, df1 = 1, df2 = 17, lower.tail = F)
## [1] 0.02219209
pf(q = 6.883, df1 = 1, df2 = 17, lower.tail = F)
## [1] 0.01779112
```

11.2 The Two-Way ANOVA Table with Interactions

```
two_aov2 <- clean_twoway_data %>%
 aov(formula = Yield ~ Fertilizer*Manure, data = .)
two_aov2 %>%
  summary
##
                    Df Sum Sq Mean Sq F value Pr(>F)
## Fertilizer
                     1 17.67 17.672
                                       6.368 0.0226 *
## Manure
                     1 19.21 19.208
                                       6.922 0.0182 *
## Fertilizer:Manure 1
                         3.04
                                3.042
                                       1.096 0.3107
## Residuals
                    16 44.40
                                2.775
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

The interaction is not significant so we proceed on using the additive model (i.e., main-effects only).

Checking Assumptions

12.1 Checking Normality Assumptions

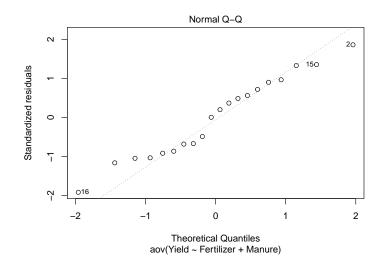
 $Shapiro\text{-}Wilk\ Test$

The Shapiro-Wilk test tests the null hypothesis that the samples come from a normal distribution against the alternative hypothesis that the samples do not come from a normal distribution.

```
shapiro.test(residuals(two_aov))
```

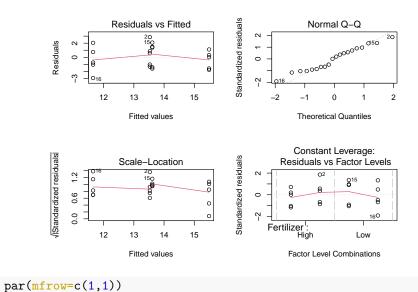
```
##
## Shapiro-Wilk normality test
##
## data: residuals(two_aov)
## W = 0.9634, p-value = 0.6138

QQ Plots
plot(two_aov, 2)
```



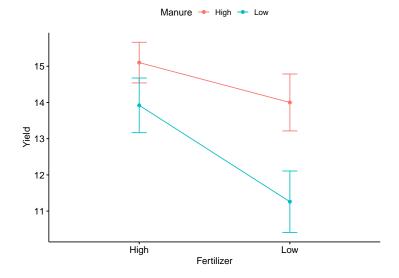
12.2 Checking Homogeneity of Variance Assumption

```
par(mfrow=c(2,2))
plot(two_aov)
```



Mean Line or Interaction Plots

```
ggline(clean_twoway_data,
    x = "Fertilizer",
    y = "Yield",
    color = "Manure",
    add = c("mean_se"))
```



The above figure supports the result before that there is no significant interaction between the two factors.