Understanding Two-Way ANOVA: A Step-by-Step Guide Using SPSS

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November 8, 2025

The Power of Two-Way Analysis of Variance (ANOVA)

The Two-Way ANOVA, short for Analysis of Variance, serves as an indispensable tool in inferential statistics. Researchers employ this technique when they need to simultaneously evaluate the influence of two distinct categorical independent variables, commonly referred to as factors, on a single continuous dependent variable. Unlike its simpler counterpart, the One-Way ANOVA, the two-way structure enables a much richer analysis by assessing the individual impact of each factor (known as **main effects**) while also examining their combined influence.

The foremost benefit of utilizing a Two-Way ANOVA is its unique capability to detect and quantify an interaction effect. This interaction reveals whether the effect of one factor on the outcome variable is dependent upon the specific level of the second factor. For example, a new medication might significantly reduce blood pressure only when paired with a strict diet regimen; this dependency indicates a crucial interaction that a simpler analysis would miss.

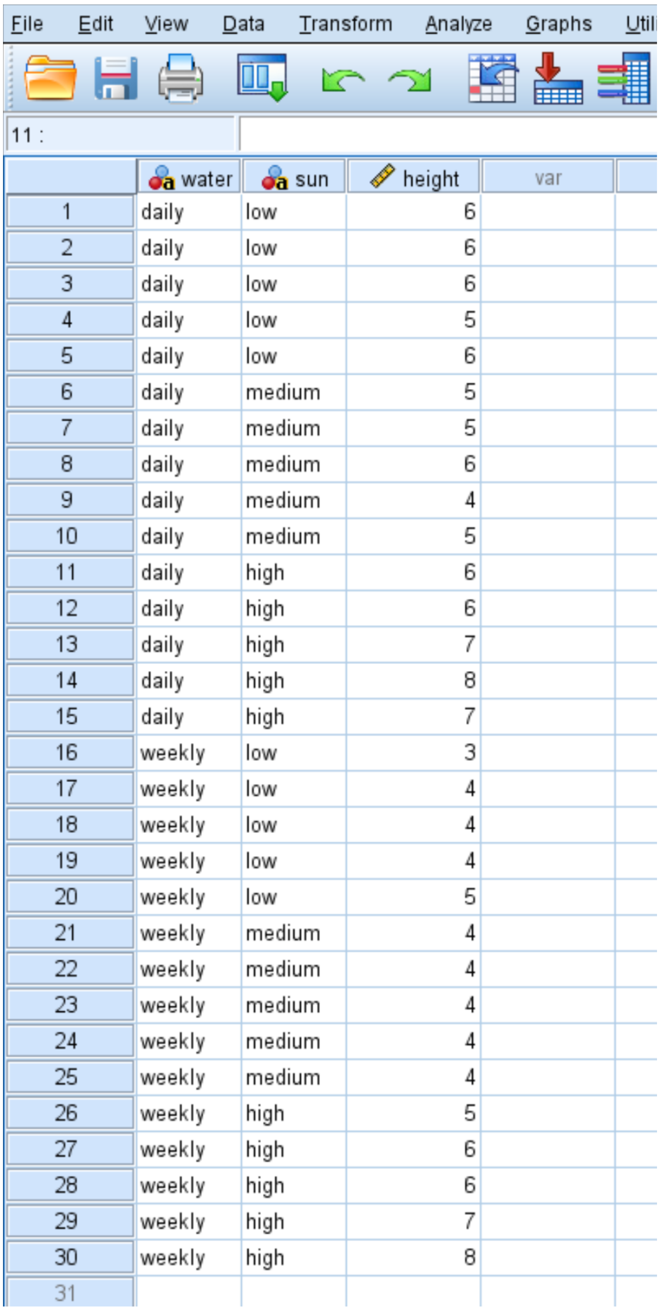
The following guide provides a rigorous, step-by-step methodology for correctly executing and interpreting a Two-Way ANOVA. We will utilize the industry-standard software, IBM SPSS Statistics, ensuring the resulting statistical report is both clear and scientifically accurate.

Designing the Study: The Plant Growth Case

To demonstrate the practical application of this statistical model, we will examine an experimental design focused on biological factors influencing plant development. Imagine a botanist investigating the growth of a specific species. Her hypothesis posits that plant height, which is our continuous response variable, is primarily determined by two experimental factors: **sunlight exposure** (Factor 1) and **watering frequency** (Factor 2).

To test this hypothesis rigorously, she cultivates 30 genetically identical plant seeds under strictly controlled conditions for two months. She systematically manipulates the factors, assigning plants to specific conditions: sunlight exposure is set at three levels (Low, Medium, High), and watering frequency is set at two levels (Daily, Weekly). This controlled manipulation allows for the isolation and measurement of the distinct and combined effects of these variables.

The collected data, structured for input into SPSS, includes the final height (in inches) for each of the 30 plants, categorized by their assigned levels of sunlight and water conditions. We aim to determine if watering, sunlight, or their potential interaction effect leads to a statistically significant difference in plant height and, if so, identify exactly which conditions yield superior growth.



	water	sun	height	var
1	daily	low	6	
2	daily	low	6	
3	daily	low	6	
4	daily	low	5	
5	daily	low	6	
6	daily	medium	5	
7	daily	medium	5	
8	daily	medium	6	
9	daily	medium	4	
10	daily	medium	5	
11	daily	high	6	
12	daily	high	6	
13	daily	high	7	
14	daily	high	8	
15	daily	high	7	
16	weekly	low	3	
17	weekly	low	4	
18	weekly	low	4	
19	weekly	low	4	
20	weekly	low	5	
21	weekly	medium	4	
22	weekly	medium	4	
23	weekly	medium	4	
24	weekly	medium	4	
25	weekly	medium	4	
26	weekly	high	5	
27	weekly	high	6	
28	weekly	high	6	
29	weekly	high	7	
30	weekly	high	8	
31				

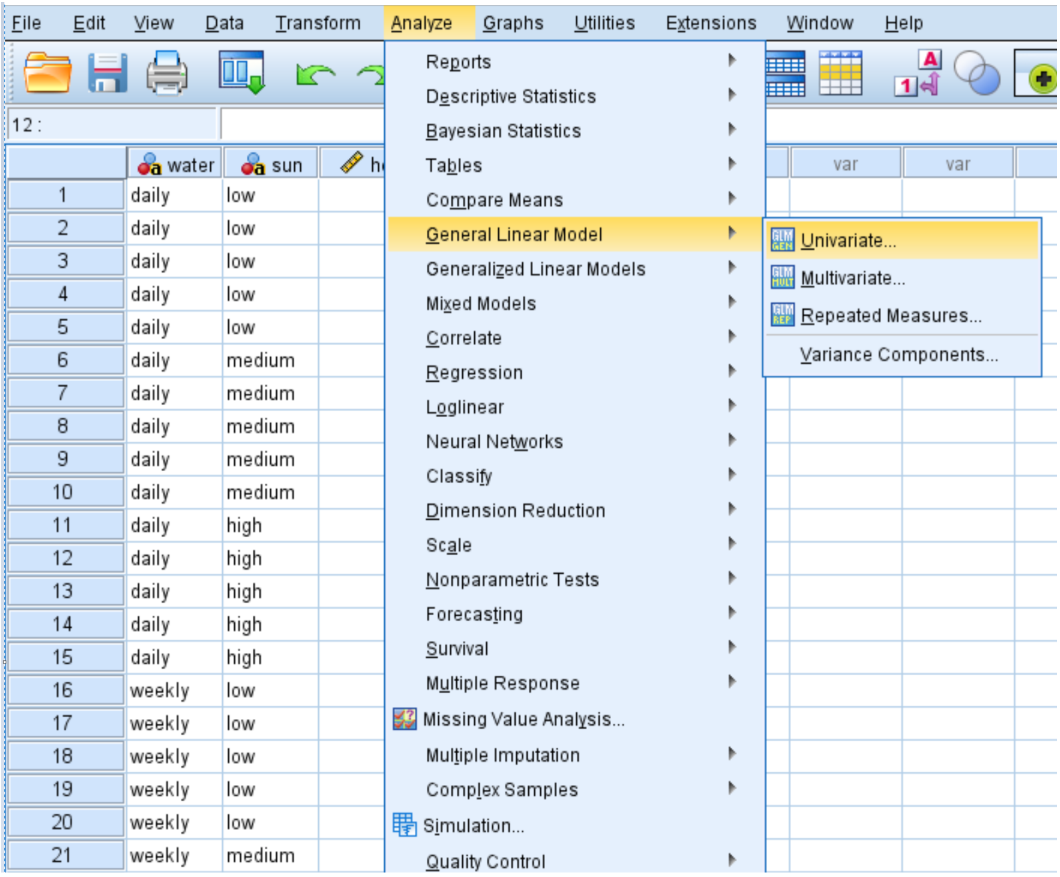
Step 1: Initiating the Analysis in IBM SPSS Statistics

The execution of the Two-Way ANOVA within the SPSS environment begins by accessing the appropriate statistical module. The Two-Way ANOVA is categorized under the framework of the General Linear Model (GLM), which is designed for flexible regression and variance analyses. Follow these menu selections precisely to open the main dialog box:

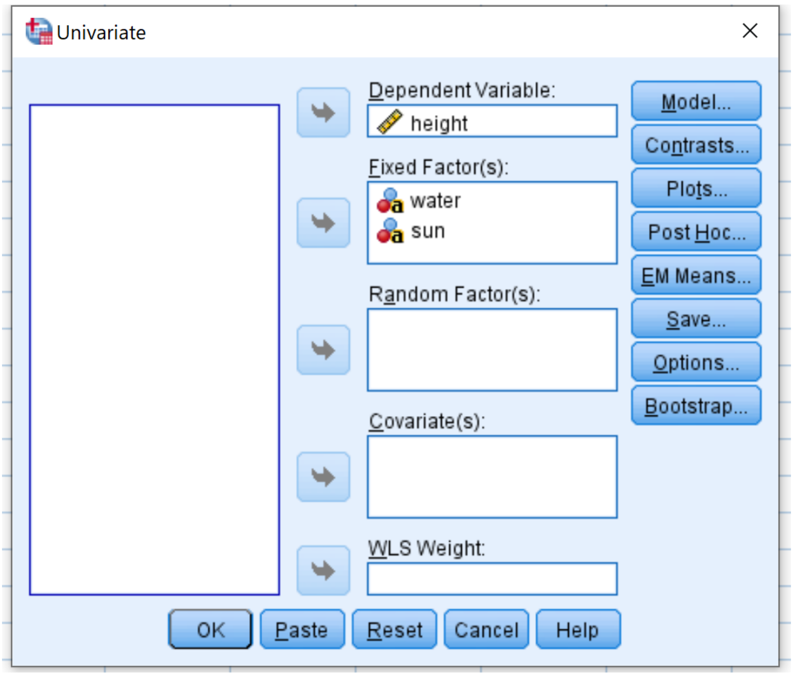
Click the **Analyze** tab found in the main menu bar.
Hover over **General Linear Model** (GLM).

Select the **Univariate** option. This choice is correct because we are analyzing the impact of multiple factors on a single dependent (univariate) outcome variable.

The following image displays the resulting GLM dialog box, where we will define the variables based on our experimental design:



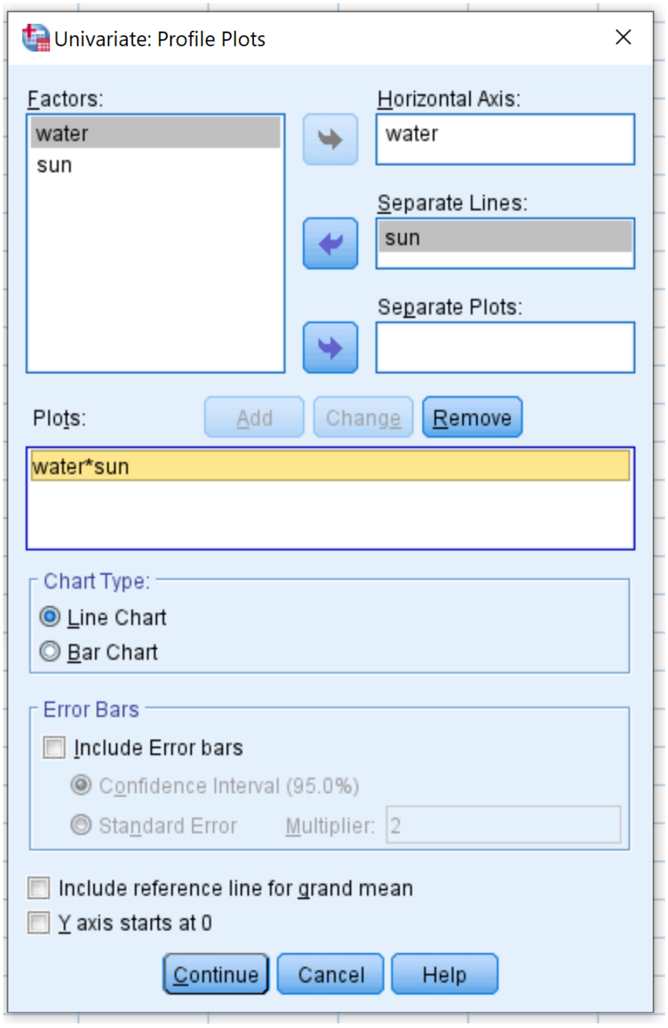
In this dialog box, variables must be correctly assigned to their statistical roles. Our response measure, **height**, is placed into the **Dependent Variable** box. The two factors--**water** and **sun**--which define the groupings we are comparing, must be moved into the **Fixed Factor(s)** box. This configuration clearly informs SPSS of the factorial structure of the plant growth experiment, setting the stage for the calculation of main and interaction effects.



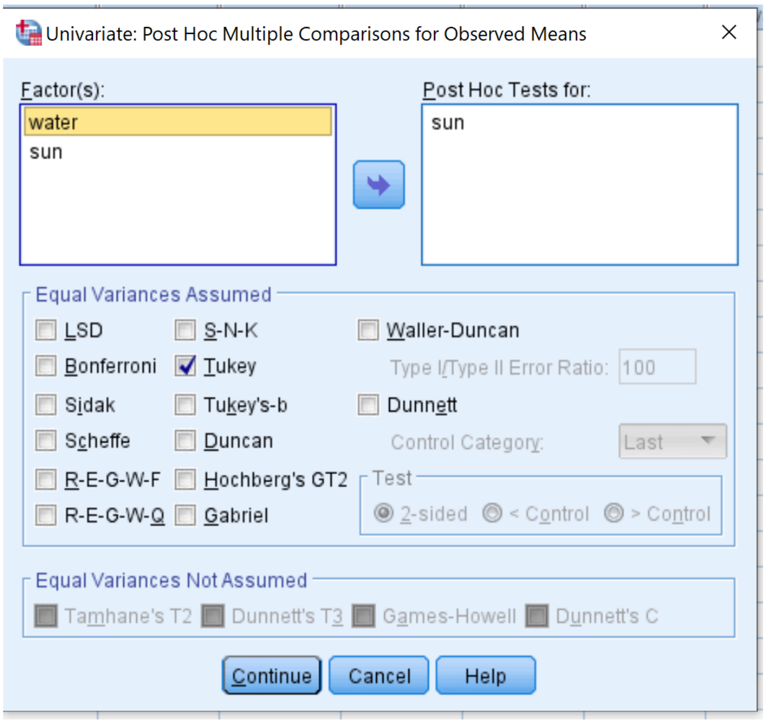
Step 2: Specifying Model Parameters and Visual Outputs

Before running the analysis, several key configuration steps must be performed to generate the necessary outputs for robust interpretation. This involves requesting profile plots for visualization and specifying appropriate Post Hoc Tests to localize significant effects.

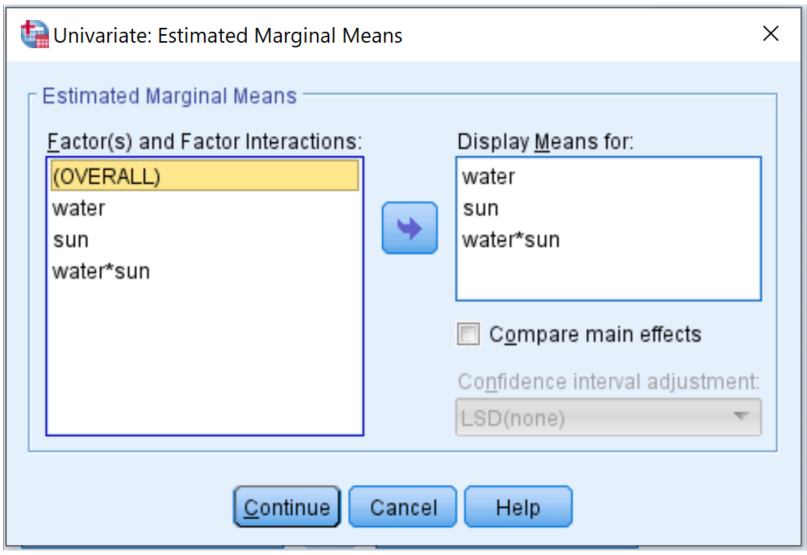
To visually inspect the potential interaction effect, we must generate an interaction profile plot. Click the **Plots** button. Drag the factor **water** to the **Horizontal axis** box, and **sun** to the **Separate lines** box. Crucially, after assigning these variables, click the **Add** button. The interaction term, displayed as **water*sun**, will appear in the Plots box, confirming the request for the interaction visualization. Click **Continue** to exit the Plots submenu.



Next, we address the necessity of pairwise comparisons. Post Hoc Tests are mandatory for any factor found to be significant that possesses three or more levels, as ANOVA only indicates an overall difference exists, but not where it lies. Since our **sun** factor has three levels (Low, Medium, High), click the **Post Hoc** button. Drag **sun** into the **Post Hoc Tests for** box. Select the check box next to Tukey, which is highly recommended for conducting all possible pairwise comparisons while controlling for the family-wise error rate. Click **Continue**.



Finally, click the **EM Means** button (Estimated Marginal Means). Move all factors (water, sun, and the water*sun interaction) into the **Display Means for** box. This ensures that the output includes the statistically adjusted group means, which are essential for accurate interpretation of the main effects. After clicking **Continue**, execute the analysis by clicking **OK** in the main GLM dialog box.



Step 3: Interpreting the Between-Subjects Effects Table

The most critical output generated by the analysis is the **Tests of Between-Subjects Effects** table. This table summarizes the statistical evaluation of the variance attributable to each factor and the interaction term, providing the F-statistic and the corresponding significance value (or p-value) for each source of variation.

The interpretation process must always begin with the interaction effect (water*sun). Only if the interaction is non-significant can we confidently proceed to interpret the main effects independently. We compare the calculated p-value against the predefined alpha level (typically set at 0.05). If the p-value is less than 0.05, the effect is deemed a statistically significant difference.

Tests of Between-Subjects Effects

Dependent Variable: height

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.855[a]	5	7.371	13.960	.000
Intercept	853.333	1	853.333	1616.162	.000
water	9.408	1	9.408	17.818	.000
sun	25.633	2	12.816	24.273	.000
water * sun	1.814	2	.907	1.718	.201
Error	12.672	24	.528		
Total	902.860	30			
Corrected Total	49.527	29			

a. R Squared = .744 (Adjusted R Squared = .691)

Upon reviewing the table, we extract the following key significance values:

Watering Frequency (water): p-value = **.000**
Sunlight Exposure (sun): p-value = **.000**
Interaction Term (water*sun): p-value = **.201**

Since the p-values for both **water** and **sun** are well below 0.05, we confidently conclude that both factors have a highly significant main effect on plant height. Crucially, the p-value for the interaction term (.201) is greater than 0.05. This non-significant interaction confirms that the effect of watering frequency on plant growth does not depend on the level of sunlight exposure; their effects are merely additive.

Step 4: Examining Group Means and Post Hoc Comparisons

With the significant main effects established, we must now delve into the Estimated Marginal Means and the Tukey's test results to characterize the nature and direction of the differences observed.

Analyzing Estimated Marginal Means

The **Estimated Marginal Means** tables present the average plant height calculated for each level of the factors. These means are statistically adjusted, providing the most reliable estimate of the population mean for each factor level, independent of the influence of the other factor. Reviewing these means allows us to identify the superior conditions:

Estimated Marginal Means

1. water

Dependent Variable: height

water	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
daily	5.893	.188	5.506	6.281
weekly	4.773	.188	4.386	5.161

2. sun

Dependent Variable: height

sun	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
high	6.620	.230	6.146	7.094
low	4.890	.230	4.416	5.364
medium	4.490	.230	4.016	4.964

3. water * sun

Dependent Variable: height

water	sun	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
daily	high	6.920	.325	6.249	7.591
	low	5.780	.325	5.109	6.451
	medium	4.980	.325	4.309	5.651
weekly	high	6.320	.325	5.649	6.991
	low	4.000	.325	3.329	4.671
	medium	4.000	.325	3.329	4.671

The direction of the significant effects is clear from the means:

The average height for plants watered daily was **5.893** inches, substantially exceeding the mean for weekly watering.
Among the sunlight conditions, the highest mean height was observed in the high sunlight group, measuring **6.620** inches.
For descriptive purposes, the specific cell mean for plants that received both daily watering and

high sunlight exposure was **6.320** inches.

Interpreting Tukey's Post Hoc Tests

Because the main effect of **sunlight** was significant and involved three levels, the results of the Tukey's test are essential for pinpointing the exact location of the differences among the three exposure levels. This output table provides the adjusted p-values for all possible pairwise comparisons:

Post Hoc Tests

sun

Multiple Comparisons

Dependent Variable: height
Tukey HSD

(I) sun	(J) sun	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
high	low	1.73*	.325	.000	.92	2.54
	medium	2.13*	.325	.000	1.32	2.94
low	high	-1.73*	.325	.000	-2.54	-.92
	medium	.40	.325	.447	-.41	1.21
medium	high	-2.13*	.325	.000	-2.94	-1.32
	low	-.40	.325	.447	-1.21	.41

Based on observed means.
The error term is Mean Square(Error) = .528.
*. The mean difference is significant at the 0.05 level.

Based on the pairwise comparisons, we draw the following conclusions:

High Sunlight vs. Low Sunlight: p-value = **0.000** (Significantly different)
High Sunlight vs. Medium Sunlight: p-value = **0.000** (Significantly different)
Low Sunlight vs. Medium Sunlight: p-value = **0.447** (No reliable difference)

The Tukey's test conclusively demonstrates that high sunlight exposure yields significantly greater plant growth than both medium and low sunlight conditions. However, the data does not support a reliable difference in growth between plants receiving low versus medium sunlight exposure.

Step 5: Formulating the Conclusive Report

The final step in any statistical analysis is generating a clear, standardized summary of the findings, ensuring all calculated evidence from the two-way analysis is included and presented formally:

A Two-Way ANOVA was conducted to examine the effects of watering frequency (daily vs. weekly) and sunlight exposure (low, medium, high) on plant growth (height) among 30 tested plants.

The analysis yielded a **statistically significant effect** for both watering frequency ($F(1, 26) = 28.52$, $p < .001$) and sunlight exposure ($F(2, 26) = 17.58$, $p < .001$). Plants that were watered daily achieved significantly greater growth than those watered weekly.

Subsequent multiple comparisons using Tukey's HSD test indicated that plants receiving high sunlight exposure grew significantly taller than those exposed to medium and low sunlight. Importantly, no significant difference was found between the medium and low sunlight exposure groups.

Furthermore, there was no statistically significant interaction effect detected between watering frequency and sunlight exposure ($F(2, 26) = 1.71$, $p = .201$), confirming that the effects of water and sun on plant height operate independently.