ToxGenie Algae & Cyanobacteria, Growth Inhibition Test Analysis Report

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Test Type:	Definitive Test

Study No.:	Test-0123	**Test Material:**	Test Material
Test Species:	Pseudokirchneriella subcapita	**Exposure Time:**	72 hours

Table 1. Biomass (cells/mL) of Pseudokirchneriella subcapita during the 72 hours exposure

(Unit: mg/L)

Dose	Replicate No.	0-hour	24-hours	48-hours	72-hours
Control	1	5000	27800	162000	526000
Control	2	5000	27800	164000	551000
Control	3	5000	29600	198000	581000
Control	4	5000	27400	150000	536000
Control	5	5000	27900	154000	528000
Control	6	5000	30800	181000	584000
	Mean	5000	28550	168167	551000
	SD	0	1344	18115	25954
1	1	5000	30700	187000	594000
1	2	5000	28600	151000	621000
1	3	5000	28600	179000	566000
	Mean	5000	29300	172333	593667
	SD	0	1212	18903	27502
2.2	1	5000	29600	161000	532000
2.2	2	5000	28400	154000	506000
2.2	3	5000	28500	152000	511000
	Mean	5000	28833	155667	516333
	SD	0	666	4726	13796
4.8	1	5000	27300	131000	434000
4.8	2	5000	27800	145000	463000
4.8	3	5000	25300	131000	422000
	Mean	5000	26800	135667	439667
	SD	0	1323	8083	21079
10	1	5000	22400	99800	330000
10	2	5000	21600	96800	339000
10	3	5000	21000	86500	350000
	Mean	5000	21667	94367	339667
	SD	0	702	6976	10017
23	1	5000	13400	27400	49200
23	2	5000	14200	29900	52600
23	3	5000	11300	27800	51800
	Mean	5000	12967	28367	51200
	SD	0	1498	1343	1778
50	1	5000	7440	6680	6930
50	2	5000	5990	6240	6530
50	3	5000	7520	6740	6320
	Mean	5000	6983	6553	6593
	SD	0	861	273	310

0-hour: Nominal initial biomass, SD: Standard Deviation

Table 2. Increase of biomass in the control cultures within the 72 hours test period

Replicate No.	0-day	1-day	2-day	3-day	0-3 day
1	5000	27800	162000	526000	105.2
2	5000	27800	164000	551000	110.2
3	5000	29600	198000	581000	116.2
4	5000	27400	150000	536000	107.2
5	5000	27900	154000	528000	105.6
6	5000	30800	181000	584000	116.8
Mean					110.2

Validity Check: The biomass in control cultures increased by a factor of 110.2. This exceeds the OECD 201 validity criterion (factor >= 16), indicating that the test conditions allowed for sufficient unrestricted exponential growth.

Table 3. Mean CV(%) for section-by-section specific growth rate in the control cultures

Replicate No.	1-2 day	2-3 day	Mean	SD	CV(%)
1	1.7626	1.1777	1.4701	0.4136	28.1305
2	1.7748	1.2119	1.4933	0.3981	26.6565
3	1.9005	1.0765	1.4885	0.5827	39.1446
4	1.7001	1.2735	1.4868	0.3016	20.2884
5	1.7083	1.2321	1.4702	0.3367	22.9019
6	1.7710	1.1714	1.4712	0.4240	28.8178
Mean					27.6566

SD: Standard Deviation

Validity Check: The mean coefficient of variation (CV) for section-by-section specific growth rates was 27.66%. This is within the OECD 201 limit (<= 35%), demonstrating that stable exponential growth was maintained throughout the test duration.

Table 4. CV(%) of average specific growth rates in replicate control cultures during the whole test period

Replicate No.	0-3 day	0-1 day	1-2 day	2-3 day
1	1.5520	1.7156	1.7626	1.1777
2	1.5674	1.7156	1.7748	1.2119
3	1.5851	1.7783	1.9005	1.0765
4	1.5582	1.7011	1.7001	1.2735
5	1.5532	1.7192	1.7083	1.2321
6	1.5868	1.8181	1.7710	1.1714
Mean	1.5671	1.7413	1.7695	1.1905
SD	0.0156	0.0462	0.0718	0.0672
CV(%)	0.9943	2.6556	4.0592	5.6482

SD: Standard Deviation

Validity Check: The coefficient of variation (CV) for average specific growth rates in control replicates was 0.99%. This meets the OECD 201 criterion (<= 7% for Pseudokirchneriella subcapita), confirming acceptable experimental precision and reproducibility.

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Table 5. Average Growth Inhibitions (%) of Pseudokirchneriella subcapita

Dose	Replicate No.	Average Growth Rate	Inhibition (%)
Control	1	1.5520	0.0000
Control	2	1.5674	0.0000
Control	3	1.5851	0.0000
Control	4	1.5582	0.0000
Control	5	1.5532	0.0000
Control	6	1.5868	0.0000
	Mean	1.5671	0.0000
	SD	0.0156	0.0000
1	1	1.5925	-1.6178
1	2	1.6073	-2.5633
1	3	1.5764	-0.5908
	Mean	1.5920	-1.5906
	SD	0.0155	0.9866
2.2	1	1.5557	0.7269
2.2	2	1.5390	1.7927
2.2	3	1.5423	1.5836
	Mean	1.5457	1.3678
	SD	0.0089	0.5647
4.8	1	1.4879	5.0576
4.8	2	1.5094	3.6817
4.8	3	1.4785	5.6540
	Mean	1.4919	4.7977
	SD	0.0158	1.0114
10	1	1.3966	10.8846
10	2	1.4055	10.3123
10	3	1.4162	9.6331
	Mean	1.4061	10.2767
	SD	0.0098	0.6265
23	1	0.7622	51.3663
23	2	0.7844	49.9450
23	3	0.7793	50.2710
	Mean	0.7753	50.5274
	SD	0.0117	0.7446
50	1	0.1088	93.0569
50	2	0.0890	94.3215
50	3	0.0781	95.0168
	Mean	0.0920	94.1317
	SD	0.0156	0.9936

SD: Standard Deviation

Table 6. Model Comparison result: Fitted Models to the Dose-Average Growth Inhibition (%)

Model Name	R2	MSE	Res. SD	AIC	Norm. P
Exponential	0.8836	121.78213	10.79000	231.36	0.000
Gompertz	0.9838	16.98272	3.67918	184.08	0.000
Logistic	0.9972	2.87897	1.61606	141.49	0.363
Log-Logistic	-0.0000	1046.15054	32.34425	282.98	0.000
Weibull	0.9970	3.16549	1.74107	143.76	0.035
Linear	0.9842	16.53847	4.06675	183.45	0.241
Logistic with Hormesis	0.9803	20.63340	4.22856	188.75	0.078

Best Model: Logistic (AIC = 141.49, R2 = 0.9972)

MSE: Mean Squared Error, Res. SD: Residual Standard Deviation, AIC: Akaike Information Criterion

Norm. P (Normality Test P-value)): Shapiro-Wilk Test P-value (P > 0.05 indicates normal residuals)

Table 7. Point Estimates: Average Growth Inhibition (%)

(Unit: mg/L)

Level	Estimates	95% Lower Confidence Limit	95% Upper Confidence Limit
ErC10	11.2625	10.2818	12.2431
ErC20	15.2013	14.5102	15.8924
ErC25	16.6513	16.0498	17.2527
ErC30	17.9325	17.3968	18.4682
ErC40	20.2085	19.7425	20.6746
ErC50	22.3143	21.8412	22.7873
ErC60	24.4283	23.8814	24.9752
ErC70	26.7336	26.0521	27.4151
ErC75	28.0427	27.2660	28.8194
ErC80	29.5366	28.6346	30.4387
ErC90	33.6962	32.2884	35.1040

Note: Logistic Model R2 = 0.9972 (>= 0.9). Estimates based on non-linear regression model.

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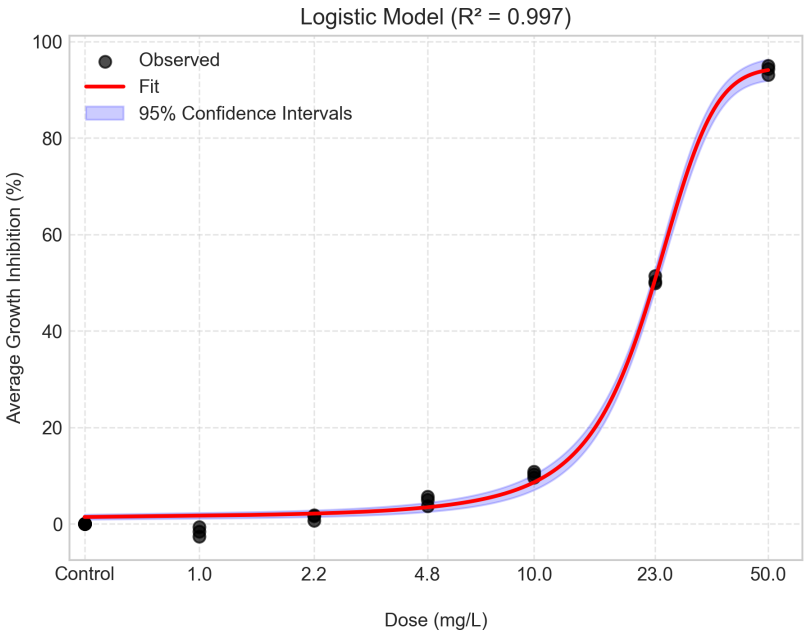

Figure 1. Dose-response curve for Average Growth Inhibition (%).

Table 8. Average Growth Inhibition (%): Quality Assurance (QA/QC) Summary

Parameter	Value / Status
Control Replicates (N)	6
Control CV (%)	0.00
Validity Flag	Pass

Methodology & Interpretation Guide

1. Statistical Parameters:

- *Sample Size (N): 24 (Total number of observed data points).*
- *Degrees of Freedom (DoF): 21 (Calculated as N - Number of Model Parameters [3]).*
- *Higher DoF generally increases the statistical power.*

2. Best Model Selection Criteria:

- *Best Model selected based on AIC (Akaike Information Criterion).*
- *Lowest AIC indicates best balance between goodness-of-fit and complexity.*
- *Secondary criterion: R-squared (R2) >= 0.9.*
- *Consequently, the selected model 'Logistic' was chosen as it showed the best statistical fit (lowest AIC: 141.49) and satisfied accuracy (R2 >= 0.9).*

3. 95% Confidence Interval (CI) Calculation:

- *Method used: Delta Method (Jacobian matrix).*
- *The 95% CI provides a range likely to contain the true parameter value.*

Table 9. Yield Inhibitions (%) of Pseudokirchneriella subcapita

Dose	Replicate No.	Yield	Inhibition (%)
Control	1	521000	0.0000
Control	2	546000	0.0000
Control	3	576000	0.0000
Control	4	531000	0.0000
Control	5	523000	0.0000
Control	6	579000	0.0000
	Mean	546000	0.0000
	SD	25954	0.0000
1	1	589000	-7.8755
1	2	616000	-12.8205
1	3	561000	-2.7473
	Mean	588667	-7.8144
	SD	27502	5.0369
2.2	1	527000	3.4799
2.2	2	501000	8.2418
2.2	3	506000	7.3260
	Mean	511333	6.3492
	SD	13796	2.5268
4.8	1	429000	21.4286
4.8	2	458000	16.1172
4.8	3	417000	23.6264
	Mean	434667	20.3907
	SD	21079	3.8607
10	1	325000	40.4762
10	2	334000	38.8278
10	3	345000	36.8132
	Mean	334667	38.7057
	SD	10017	1.8345
23	1	44200	91.9048
23	2	47600	91.2821
23	3	46800	91.4286
	Mean	46200	91.5385
	SD	1778	0.3256
50	1	1930	99.6465
50	2	1530	99.7198
50	3	1320	99.7582
	Mean	1593	99.7082
	SD	310	0.0568

SD: Standard Deviation

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Table 10. Model Comparison result: Fitted Models to the Dose-Yield Inhibition (%)

Model Name	R2	MSE	Res. SD	AIC	Norm. P
Exponential	0.6562	542.62068	23.12983	267.22	0.000
Gompertz	0.9538	72.99425	8.20961	219.08	0.012
Logistic	0.9777	35.18073	5.80793	201.56	0.032
Log-Logistic	-0.0000	1578.40010	39.72908	292.85	0.000
Weibull	0.9756	38.59118	6.20138	203.78	0.441
Linear	0.8421	249.29032	15.78893	248.56	0.005
Logistic with Hormesis	0.9665	52.80522	6.73673	211.31	0.077

Best Model: Logistic (AIC = 201.56, R2 = 0.9777)

MSE: Mean Squared Error, Res. SD: Residual Standard Deviation, AIC: Akaike Information Criterion

Norm. P (Normality Test P-value)): Shapiro-Wilk Test P-value (P > 0.05 indicates normal residuals)

Table 11. Point Estimates: Yield Inhibition (%)

(Unit: mg/L)

Level	Estimates	95% Lower Confidence Limit	95% Upper Confidence Limit
EyC10	4.9584	3.5325	6.3842
EyC20	7.1704	6.1612	8.1796
EyC25	8.0125	7.0785	8.9466
EyC30	8.7654	7.8424	9.6884
EyC40	10.1184	9.0836	11.1532
EyC50	11.3840	10.1339	12.6341
EyC60	12.6651	11.1389	14.1914
EyC70	14.0731	12.2110	15.9353
EyC75	14.8778	12.8149	16.9408
EyC80	15.8015	13.5004	18.1026
EyC90	18.4146	15.3230	21.5062

Note: Logistic Model R2 = 0.9777 (>= 0.9). Estimates based on non-linear regression model. Unit: mg/L

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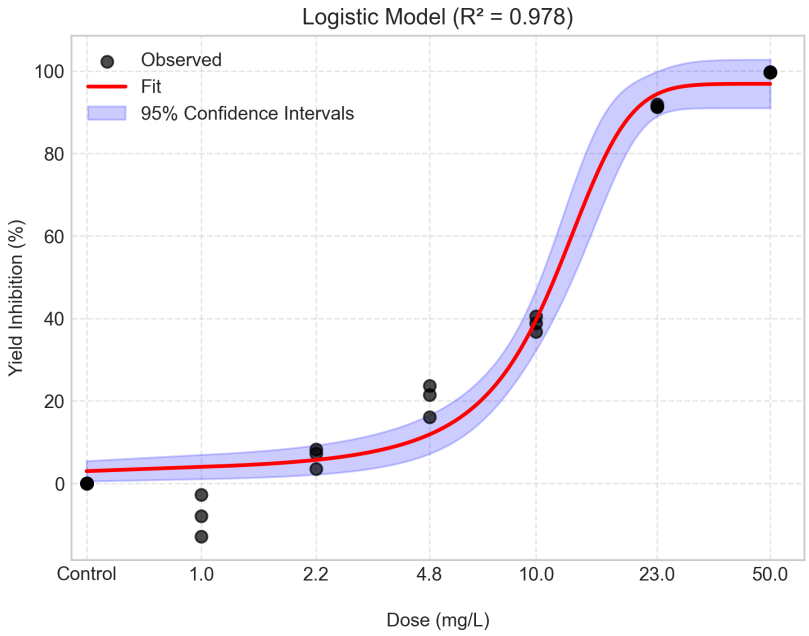

Figure 2. Dose-response curve for Yield (%).

Table 12. Yield Inhibition (%): Quality Assurance (QA/QC) Summary

Parameter	Value / Status
Control Replicates (N)	6
Control CV (%)	0.00
Validity Flag	Pass

Methodology & Interpretation Guide

1. Statistical Parameters:

- *Sample Size (N): 24 (Total number of observed data points).*
- *Degrees of Freedom (DoF): 21 (Calculated as N - Number of Model Parameters [3]).*
- *Higher DoF generally increases the statistical power.*

2. Best Model Selection Criteria:

- *Best Model selected based on AIC (Akaike Information Criterion).*
- *Lowest AIC indicates best balance between goodness-of-fit and complexity.*
- *Secondary criterion: R-squared (R2) >= 0.9.*
- *Consequently, the selected model 'Logistic' was chosen as it showed the best statistical fit (lowest AIC: 201.56) and satisfied accuracy (R2 >= 0.9).*

3. 95% Confidence Interval (CI) Calculation:

- *Method used: Delta Method (Jacobian matrix).*
- *The 95% CI provides a range likely to contain the true parameter value.*

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Table 13. Data Summary for Average Growth Rate

(Unit: mg/L)

Dose	1	2	3	4	5	6	Mean	SD
Control	1.5519	1.5674	1.5851	1.5582	1.5532	1.5868	1.5671	0.0142
1	1.5925	1.6073	1.5764	nan	nan	nan	1.5921	0.0126
2.2	1.5557	1.5390	1.5423	nan	nan	nan	1.5457	0.0072
4.8	1.4879	1.5094	1.4785	nan	nan	nan	1.4919	0.0129
10	1.3965	1.4055	1.4162	nan	nan	nan	1.4061	0.0080
23	0.7621	0.7844	0.7793	nan	nan	nan	0.7753	0.0095
50	0.1088	0.0890	0.0781	nan	nan	nan	0.0920	0.0127

SD: Standard Deviation *(nan: not-a-number)*

Table 14. Shapiro-Wilk's test for normality on the Average Growth Rate

Dose	Statistic	P-value	Result
Control	0.85320	0.16703	Normal Distribution
1	0.99944	0.95470	Normal Distribution
2.2	0.89048	0.35582	Normal Distribution
4.8	0.95056	0.57176	Normal Distribution
10	0.99756	0.90566	Normal Distribution
23	0.91102	0.42151	Normal Distribution
50	0.97266	0.68278	Normal Distribution

P>0.05 indicates Normal Distribution.

Final decision: The Average Growth Rate follows a normal distribution.

Table 15. Levene's test for homogeneity on the Average Growth Rate

Statistic	P-value	Result
0.33572	0.90857	Equal Variances

P>0.05 indicates Equal Variances.

Final decision: The Average Growth Rate shows equal variances.

Statistical Analysis Procedure & Decision Tree

The statistical analysis for 'Average Growth Rate' was conducted based on the following decision steps:

1. Assumption Verification (Normality & Homogeneity):

- *Since the raw data satisfied both the Shapiro-Wilk test (Normality) and Levene's test (Homogeneity of Variance), no data transformation was required.*
- *Consequently, the analysis proceeded directly to Parametric Tests.*

2. Data Transformation Strategy:

- *Not applicable (The raw data already satisfied statistical assumptions).*

3. Final Selected Test Method:

- *Because the data satisfied the required statistical assumptions (Normality and Homogeneity of Variance), the T-Test with Bonferroni Adjustment (Parametric) was selected as the post-hoc test.*
- *Prior to this post-hoc analysis, an Omnibus Test (One-Way ANOVA) was performed to confirm if any statistically significant differences exist among the dose groups ($p < 0.05$).*
- *The selected method is appropriate for comparing multiple treatment groups against a single control group to determine the NOEC/LOEC (No Observed Effect Concentration/Lowest Observed Effect Concentration) or NOAEL/LOAEL (No Observed Adverse Effect Level/Lowest Observed Adverse Effect Level).*

Table 16. One-Way ANOVA on the Average Growth Rate

F-statistic	P-value	Result
5289.6988	0.0000	Significant differences among doses

P<0.05 indicates a statistically significant difference among groups.

Table 17. Post Hoc Analytical Method: T-Test with Bonferroni Adjustment on the Average Growth Rate data.

Control	Dose	T-statistic	P-value	Adjusted P-value	Result
Control	1	-2.26797	0.05765	0.34590	No significant difference from control
Control	2.2	2.16602	0.06701	0.40206	No significant difference from control
Control	4.8	6.79026	0.00026	0.00153	Significant difference from control
Control	10	16.06565	0.00000	0.00001	Significant difference from control
Control	23	76.84990	0.00000	0.00000	Significant difference from control
Control	50	133.90980	0.00000	0.00000	Significant difference from control

P<0.05 indicates a statistically significant difference from the control.

Table 18. Average Growth Rate: NOEC and LOEC

(Unit: mg/L)

NOEC	2.2
LOEC	4.8

** NOEC: No Observed Effect Concentration*
** LOEC: Lowest Observed Effect Concentration*

Note: NOEC/LOEC are typically used for Effects on Biotic Systems where exposure is expressed as concentration (e.g., mg/L).
Note: In accordance with OECD TG 201, a statistically significant increase in growth at low concentrations (hormesis) is not considered an adverse effect and is therefore excluded from the LOEC determination.

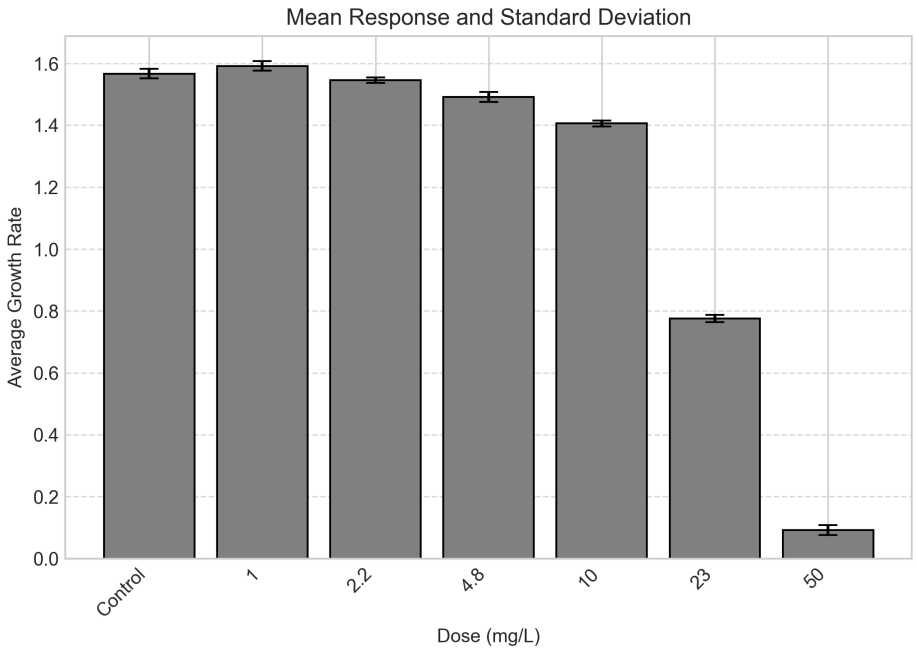

Figure 3. Dose-response plot for Average Growth Rate. Bars represent the mean and Standard Deviation (SD).

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Table 19. Data Summary for Yield

(Unit: mg/L)

Dose	1	2	3	4	5	6	Mean	SD
Control	521000	546000	576000	531000	523000	579000	546000	23692
1	589000	616000	561000	nan	nan	nan	588667	22455
2.2	527000	501000	506000	nan	nan	nan	511333	11264
4.8	429000	458000	417000	nan	nan	nan	434667	17211
10	325000	334000	345000	nan	nan	nan	334667	8179
23	44200	47600	46800	nan	nan	nan	46200	1451
50	1930	1530	1320	nan	nan	nan	1593	253

SD: Standard Deviation *(nan: not-a-number)*

Table 20. Shapiro-Wilk's test for normality on the Yield

Dose	Statistic	P-value	Result
Control	0.84964	0.15636	Normal Distribution
1	0.99989	0.97995	Normal Distribution
2.2	0.88792	0.34801	Normal Distribution
4.8	0.94580	0.55125	Normal Distribution
10	0.99668	0.88986	Normal Distribution
23	0.91456	0.43346	Normal Distribution
50	0.96867	0.66018	Normal Distribution

P>0.05 indicates Normal Distribution.

Final decision: The Yield follows a normal distribution.

Table 21. Levene's test for homogeneity on the Yield

Statistic	P-value	Result
1.83951	0.15084	Equal Variances

P>0.05 indicates Equal Variances.

Final decision: The Yield shows equal variances.

Statistical Analysis Procedure & Decision Tree

The statistical analysis for 'Yield' was conducted based on the following decision steps:

1. Assumption Verification (Normality & Homogeneity):

- *Since the raw data satisfied both the Shapiro-Wilk test (Normality) and Levene's test (Homogeneity of Variance), no data transformation was required.*
- *Consequently, the analysis proceeded directly to Parametric Tests.*

2. Data Transformation Strategy:

- *Not applicable (The raw data already satisfied statistical assumptions).*

3. Final Selected Test Method:

- *Because the data satisfied the required statistical assumptions (Normality and Homogeneity of Variance), the T-Test with Bonferroni Adjustment (Parametric) was selected as the post-hoc test.*
- *Prior to this post-hoc analysis, an Omnibus Test (One-Way ANOVA) was performed to confirm if any statistically significant differences exist among the dose groups ($p < 0.05$).*
- *The selected method is appropriate for comparing multiple treatment groups against a single control group to determine the NOEC/LOEC (No Observed Effect Concentration/Lowest Observed Effect Concentration) or NOAEL/LOAEL (No Observed Adverse Effect Level/Lowest Observed Adverse Effect Level).*

Table 22. One-Way ANOVA on the Yield

F-statistic	P-value	Result
501.9909	0.0000	Significant differences among doses

P<0.05 indicates a statistically significant difference among groups.

Table 23. Post Hoc Analytical Method: T-Test with Bonferroni Adjustment on the Yield data.

Control	Dose	T-statistic	P-value	Adjusted P-value	Result
Control	1	-2.28514	0.05621	0.33725	No significant difference from control
Control	2.2	2.11854	0.07188	0.43125	No significant difference from control
Control	4.8	6.38490	0.00037	0.00224	Significant difference from control
Control	10	13.23668	0.00000	0.00002	Significant difference from control
Control	23	32.19342	0.00000	0.00000	Significant difference from control
Control	50	35.09854	0.00000	0.00000	Significant difference from control

P<0.05 indicates a statistically significant difference from the control.

Table 24. Yield: NOEC and LOEC

(Unit: mg/L)

NOEC	2.2
LOEC	4.8

** NOEC: No Observed Effect Concentration*
** LOEC: Lowest Observed Effect Concentration*

Note: NOEC/LOEC are typically used for Effects on Biotic Systems where exposure is expressed as concentration (e.g., mg/L).
Note: In accordance with OECD TG 201, a statistically significant increase in growth at low concentrations (hormesis) is not considered an adverse effect and is therefore excluded from the LOEC determination.

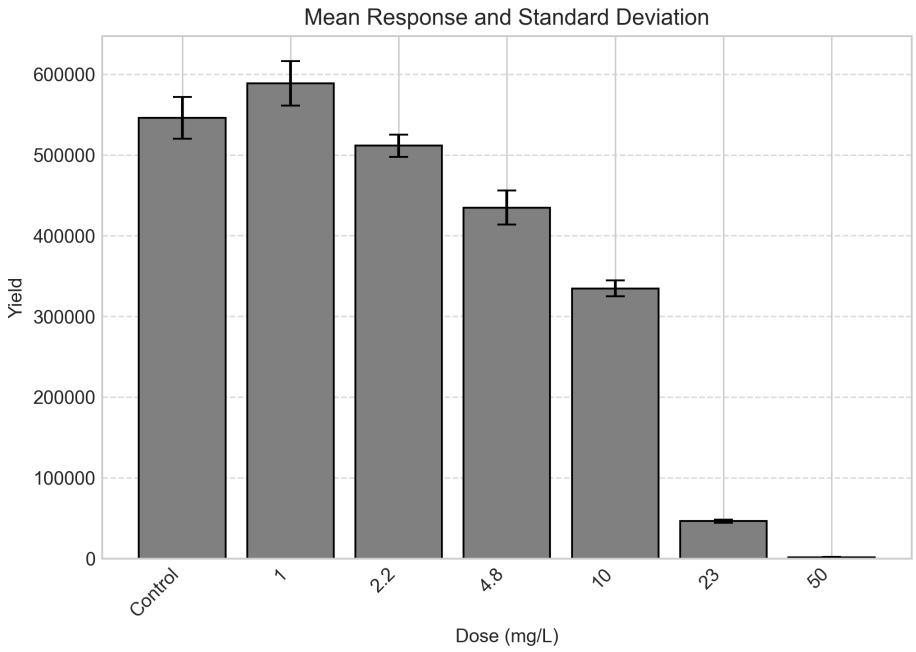

Figure 4. Dose-response plot for Yield. Bars represent the mean and Standard Deviation (SD).

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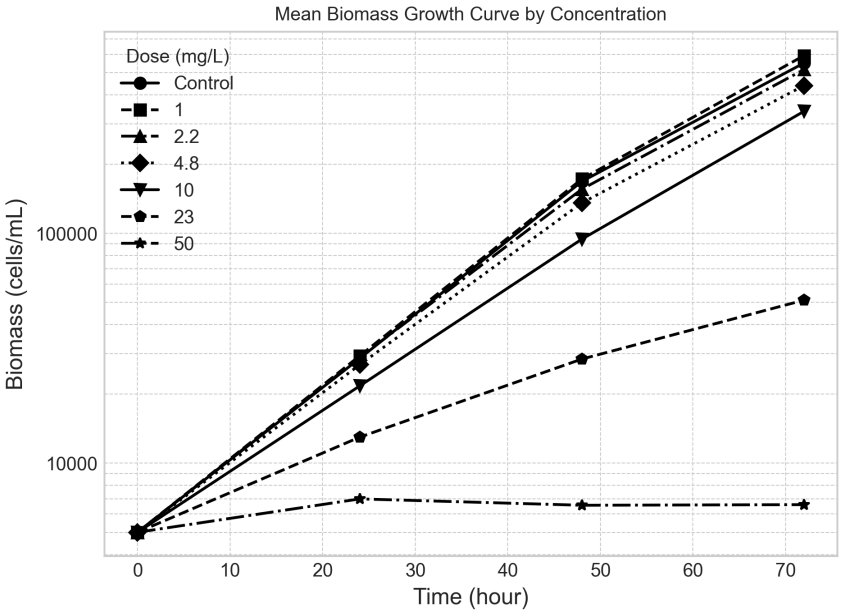

Figure 5. Mean biomass growth curve of Pseudokirchneriella subcapita exposed to different concentrations of the test material over time.

Analyst: ToxGenie