ACCSCIENCE PUBLISHING

Asian Journal of Water, Environment and Pollution

ORIGINAL RESEARCH ARTICLE

Inhibitory effects of total flavonoids from *Solidago canadensis* on growth, oxidative stress, and microcystin production in *Microcystis aeruginosa*

Supplementary File

1. Chlorophyll a content

Two-way analysis of variance (Table S1) revealed highly significant main effects of time ($F_{5,72}$ = 135.99, p < 0.0001) and concentration ($F_{5,72}$ = 1,564.83, p < 0.0001), and critically, a significant time × concentration interaction ($F_{25,72}$ = 160.59, p < 0.0001). Simple effects analysis showed that the control group exhibited a continuous increase in chlorophyll a content over time (from ~9 mg/L at day 2 to ~15 mg/L at day 12), while flavonoid-treated groups demonstrated concentration-dependent inhibition that intensified at later stages. High-concentration treatments (80–100 mg/L) caused the most severe suppression, particularly at days 10–12, indicating dynamic concentration–time effects beyond the scope of one-way analysis of variation.

Table S1. Two-way analysis of variance results for the effects of flavonoid concentration and exposure time on chlorophyll a content

Metric	DF	Sum of squares	Mean square	*F*-value	*p*-value
Time	5	16.65167	3.33033	135.98961	<0.0001
Concentration	5	191.61102	38.3222	1,564.8347	<0.0001
Time × concentration	25	98.31744	3.9327	160.58633	<0.0001
Model	35	306.58013	8.75943	357.67942	<0.0001
Error	72	1.76325	0.02449	-	-
Corrected total	107	308.34338	-	-	-

Note: The time × concentration interaction is highly significant, indicating that the effect of flavonoid concentration on chlorophyll a content varies significantly across different exposure times.
Abbreviation: DF: Degrees of freedom.

2. Carotenoid content

Two-way analysis of variance (Table S2) showed highly significant main effects of time ($F_{5,72}$ = 602.96, p < 0.0001) and concentration ($F_{5,72}$ = 544.11, p < 0.0001), with a significant time × concentration interaction ($F_{25,72}$ = 76.18, p < 0.0001). Simple effects analysis revealed that carotenoid content in the control group increased progressively from day 2 to day 12. Moderate concentrations (20–60 mg/L) showed elevated levels at middle-to-late time points (days 6–10), while high concentrations (80–100 mg/L) exhibited suppressed levels throughout the experimental period. This pattern suggests concentration-modulated temporal dynamics in carotenoid accumulation, with moderate doses potentially enhancing carotenoid synthesis as a photoprotective response.

Table S2. Two-way analysis of variance results for the effects of flavonoid concentration and exposure time on carotenoid content

Metric	DF	Sum of squares	Mean square	*F*-value	*p*-value
Time	5	8.52517	1.70503	602.95624	<0.0001
Concentration	5	7.69316	1.53863	544.1108	<0.0001
Time × concentration	25	5.3856	0.21542	76.18097	<0.0001
Model	35	21.60393	0.61726	218.2817	<0.0001
Error	72	0.2036	0.00283	-	-
Corrected total	107	21.80753	-	-	-

Note: The highly significant time × concentration interaction suggests concentration-modulated temporal dynamics in carotenoid accumulation.
Abbreviation: DF: Degrees of freedom.

3. Phycocyanin content

Two-way analysis of variance (Table S3) indicated significant main effects of time ($F_{5,72}$ = 150.21, p < 0.0001) and concentration ($F_{5,72}$ = 276.23, p < 0.0001), and a significant time × concentration interaction ($F_{25,72}$ = 31.63, p < 0.0001). A simple effects analysis showed that phycocyanin (PC) content in the control group increased steadily over time. All flavonoid treatments showed reduced PC levels compared to control, with the inhibitory effect becoming more pronounced at higher concentrations and later time points. Notably, the 100 mg/L group exhibited the lowest PC content at day 12, suggesting cumulative damage to phycobilisome components under prolonged high-dose exposure.

Table S3. Two-way analysis of variance results for the effects of flavonoid concentration and exposure time on phycocyanin content

Metric	DF	Sum of squares	Mean square	*F*-value	*p*-value
Time	5	0.0058	0.00116	150.21315	<0.0001
Concentration	5	0.01067	0.00213	276.22601	<0.0001
Time × concentration	25	0.00611	2.44E−04	31.62955	<0.0001
Model	35	0.02257	6.45E−04	83.51242	<0.0001
Error	72	5.56E−04	7.72E−06		
Corrected total	107	0.02313			

Note: The significant time × concentration interaction indicates cumulative damage to phycobilisome components under prolonged exposure to high doses.
Abbreviation: DF: Degrees of freedom.

4. Allophycocyanin content

Two-way analysis of variance (Table S4) revealed highly significant main effects of time ($F_{5,72}$ = 884.69, p < 0.0001) and concentration ($F_{5,72}$ = 155.52, p < 0.0001), and a significant time × concentration interaction ($F_{25,72}$ = 18.30, p < 0.0001). Simple effects analysis showed a distinct compensatory pattern: allophycocyanin content increased continuously in all treatment groups over time, with flavonoid-treated groups generally higher than the control. Moderate concentrations (40–60 mg/L) exhibited the most pronounced elevation at middle time points (days 6–8), whereas high-concentration groups (80–100 mg/L) showed attenuated increases at later stages. This concentration-dependent temporal shift suggests a

compensatory response of the phycobilisome that becomes saturated under prolonged high-dose exposure, highlighting the need for a multifactorial analysis to capture such complex dynamics.

Table S4. Two-way analysis of variance results for the effects of flavonoid concentration and exposure time on allophycocyanin content

Metric	DF	Sum of squares	Mean square	*F*-value	*p*-value
Time	5	0.02405	0.00481	884.69067	<0.0001
Concentration	5	0.00423	8.46E−04	155.51982	<0.0001
Time × concentration	25	0.00249	9.95E−05	18.29726	<0.0001
Model	35	0.03077	8.79E−04	161.67097	<0.0001
Error	72	3.92E−04	5.44E−06	-	-
Corrected total	107	0.03116	-	-	-

Note: The exceptionally strong time × concentration interaction ($F = 18.30$) indicates that allophycocyanin exhibits a concentration-dependent compensatory response that becomes saturated under prolonged high-dose exposure.
Abbreviation: DF: Degrees of freedom.

Total flavonoids from *S. canadensis* significantly affected the photosynthetic pigment contents of *M. aeruginosa* (Tables S1–S4). Two-way analysis of variance revealed highly significant time × concentration interactions for all four pigments ($p < 0.0001$), indicating that flavonoid effects were strongly dependent on both dose and exposure duration. Collectively, these findings demonstrate that total flavonoids exert differential, time-dependent effects on photosynthetic pigment components: while major pigments (chlorophyll a, carotenoids, and phycocyanin) show concentration-dependent inhibition, allophycocyanin exhibits a concentration-modulated compensatory increase with distinct temporal dynamics. The consistent significance of time × concentration interactions across all four parameters ($p < 0.0001$) highlights the critical necessity of multifactorial statistical analysis for understanding flavonoid-photosynthesis relationships. These differential effects on pigment components ultimately disrupt photosynthetic function, thereby inhibiting algal growth, providing more robust and nuanced insights into the dose–time response characteristics of flavonoid exposure.