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Impact of PFAS compounds on microalgal growth, biochemical composition, and metabolism in *Chlorella minutissima*

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ABSTRACT

This study systematically evaluated the effects of seven PFAS on *Chlorella minutissima*, revealing compound-specific physiological and biochemical responses. PFHxS maintained relatively higher growth rates and Fv/Fm values, with a slight increase in chlorophyll-a (11.64 ± 0.234) and photosynthetic efficiency (0.685 ± 0.005) compared to the control. PFDA induced the highest lipid accumulation ($38 \pm 1.9\%$) along with the lowest protein content ($22 \pm 1.1\%$). Compared to controls, PFHpA and PFDA treated microalgae showed increases in both saturated and unsaturated fatty acids, indicating minimal lipid disruption. In contrast, PFOS strongly reduced unsaturated fatty acids (notably C18:1 and C16:3), leading to a dominance of saturated fatty acids. Meanwhile, PFOA emerged as one of the most disruptive compounds, impairing growth and PSII efficiency, decreasing lipid ($24 \pm 1.2\%$), protein ($26 \pm 1.3\%$) levels, total phenolic compounds (TPC), total flavonoid compounds (TFC), reactive oxygen species (ROS) scavenging activity ($71.73 \pm 1.64\%$), and elevating carbohydrate content to $33.1 \pm 1.65\%$. The heat map and principal component analysis (PCA) of microalgae metabolites exposed to seven different PFAS compounds revealed distinct biochemical responses in terms of carbohydrate, protein, lipid, and phenolic compound levels. Overall, the findings indicate that PFAS with different chain lengths and functional groups induce differential, structure-dependent toxicological responses and that even environmentally relevant concentrations can disrupt algal metabolism, highlighting potential risks to aquatic ecosystems.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of fluorinated compounds characterized by highly stable carbon-fluorine bond which contribute to their persistence in the environment. Their widespread use in industrial and consumer products like non-stick cookware, textiles, and stain-resistant coatings has led to global distribution and growing concern over their ecological and human health impacts [1,2]. Long-chain PFAS, such as perfluorooctanoic acid (PFOA)

and perfluorooctane sulfonate (PFOS), are widely detected in ecosystems and are particularly known for to bioaccumulative nature and links to cancer, immune dysfunction prompting and endocrine disruption prompting strict regulatory measures to limit their use [3]. Additionally, other PFAS compounds including, PFHxS and PFNA, have been linked to liver and thyroid toxicity as well as immune and developmental disorders [4].

In aquatic ecosystems, PFAS compounds are pose significant ecotoxicological impacts. Their toxicity tends to increase with longer alkyl

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chain lengths, affecting a wide range of aquatic species including invertebrates, plankton, and microorganisms [5]. Even substitutes such as octylphenol ethoxylate sulfonate (OBS) and perfluorohexanoic acid (C6), though considered less harmful, still pose potential toxicity risks. PFAS contamination in sediments further complicates remediation efforts and lead to long-term environmental damage [6].

Microalgae are also vulnerable to PFAS exposure, even at low concentrations (<1000 ng/L), leading to inhibition of algal growth, disrupted photosynthetic efficiency, and impaired DNA replication [7]. PFOS and PFOA in particular have shown dose-dependent reductions in algal biomass and photosynthetic efficiency, particularly in species with simpler cellular structures [8]. PFOS, specifically, has been observed to reduce cell membrane permeability, disrupt photosynthesis, and induce oxidative stress, leading to severe physiological damage in *Chlorella vulgaris* [9]. Sensitivity to PFAS compounds varies among different algal groups. Green algae with their rigid cellulose-based cell walls are generally more resistant, while diatoms and cyanobacteria are more susceptible due to their silica frustules and lipopolysaccharide layers [10]. Additionally, co-contaminants such as TiO₂, naphthalene, and microplastics may alter algal responses to PFAS depending on species and environmental conditions [11].

Despite the ecological importance of green microalgae as primary producers and key bioindicators of water quality, their responses to PFAS remain insufficiently explored in a comparative and compound-specific manner. In particular, systematic evaluations across different PFAS classes and chain lengths, as well as integrated cellular and physiological responses, are still limited. Most existing PFAS toxicity studies focus on aquatic invertebrates, with relatively few addressing aquatic plants and green microalgae. Moreover, many studies primarily investigate PFOS rather than considering PFAS as a broader group, leaving significant gaps in understanding the diverse effects of PFAS on microalgal physiology [12,13]. Thus, this study aims to address this gap by examining the effects seven PFAS compounds viz., PFOS, PFNA, PFOA, PFHpA, PFHxA, PFHxS, and PFDA on the growth, photosynthetic pigments, biochemical composition, ROS scavenging activity and metabolic profile of *Chlorella minutissima*.

2. Methods

2.1. Microalgae cultivation

Chlorella minutissima (MCC-27) was obtained from the IARI, New Delhi. The chemicals for preparing Bold's Basal Medium (BBM) were procured Merck, India. To prepare the inoculum, *C. minutissima* was pre-cultivated in 250-mL conical flasks containing 100 mL of BBM. The culture was maintained for log phase (7–10 days) under controlled conditions, with a photoperiod of 16:8 (light: dark) a light intensity of 300 $\mu\text{molm}^{-2} \text{s}^{-1}$ (LED light) at 25 °C.

2.2. Experimental design

Seven PFAS compounds viz., PFOS, PFNA, PFOA, PFHpA, PFHxA, PFHxS, and PFDA (S Table 1) were selected to evaluate their effects on *C. minutissima*. The inoculum of *C. minutissima* previously cultured in BBM was used in these experiments. Stock solutions of PFAS were prepared in methanol. PFAS treatments were applied at concentrations spanning the ng/L, $\mu\text{g/L}$, and 1 mg/L levels to investigate dose-dependent responses. The lower concentrations (ng/L– $\mu\text{g/L}$) were selected to represent environmentally relevant exposure scenarios, whereas the 1 mg/L level was included to evaluate potential toxicity thresholds. No significant changes in the growth of *C. minutissima* were observed at the ng/L and $\mu\text{g/L}$ concentrations (Fig. S1 and Fig. S2). Therefore, the mg/L concentration was selected for subsequent experiments to further investigate the physiological and metabolic effects of PFAS exposure. Cultures with an OD_{680nm} of 0.2 maintained in 500 mL Erlenmeyer flasks containing working volume of 250 mL. The cultures

were incubated and maintained for 14 days as the pre-cultivation phase under controlled conditions, with a photoperiod of 16:8 (light: dark) a light intensity of 300 $\mu\text{molm}^{-2} \text{s}^{-1}$ at 25 °C. Growth was recorded every 24 h in triplicates using a UV–Vis spectrophotometer (Cary 60, Agilent, USA) at 680 nm. Once the cultures reached the stationary phase, the biomass was collected and centrifuged at 5000 rpm for 10 min, then freeze-dried and weighed to determine the dry biomass (g/L). Biomass yield (%) was calculated using Eq. (1):

$$\text{Biomass yield\%} = \frac{\text{Final dry biomass g/L} - \text{Initial dry biomass g/L}}{\text{Initial dry biomass g/L}} \times 100 \quad (1)$$

2.3. Determination of photosynthetic efficiency

The impact of PFAS on the photosynthetic efficiency of microalgae was analyzed on the 10th day of the experiment. For this purpose, 2 mL sample of the algal culture was collected from the flask and centrifuged at 6000 rpm for 10 min to pellet the algal cells. The contents of the cell pellets were then extracted in 2 mL of 99.99% methanol with an incubation at 45 °C for 30 min. Following incubation, the sample was centrifuged again at 5500 rpm for 5 min to separate the supernatant and the residual cell debris. The absorbance of the supernatant was further measured at three specific wavelengths: 665.2 nm, 652.4 nm, and 470 nm, using a UV–Vis spectrophotometer. Chlorophyll-a, chlorophyll-b, and carotenoids were quantified in $\mu\text{g/mL}$ using the following Eqs. (2)–(4) [14].

$$\text{Chlorophyll} - a = 16.72A_{665.2} - 9.16A_{652.4} \quad (2)$$

$$\text{Chlorophyll} - b = 34.09A_{652.4} - 15.28A_{665.2} \quad (3)$$

$$\text{Carotenoids} = (1000A_{470} - 1.63\text{Chlorophyll} - a - 104.9\text{Chlorophyll} - b) / 221 \quad (4)$$

2.4. Chlorophyll fluorescence assay

Chlorophyll fluorescence measurements were performed to evaluate the photosynthetic performance of microalgal cultures after 10th days of growth under experimental conditions. Fluorescence emission was measured at 25 °C using a pulse-amplitude-modulated fluorometer (PAM 2000 Chlorophyll Fluorometer, Heinz Walz GmbH). Before measurement, samples were dark-adapted to ensure full oxidation of the photosystem II (PSII) reaction centers [15]. The minimum fluorescence yield (F_0) was recorded under weak modulated light, followed by the application of a saturating light pulse to determine the maximum fluorescence yield (F_m). Fluorescence signals were recorded and analyzed using PamWin software (Version 1.17). A Li-Cor photon flux meter was used to calibrate and verify the light intensity inside the PAM fluorometer to ensure consistent irradiance conditions. The maximum quantum yield of PSII photochemistry (F_v/F_m) was calculated using the Eq. (5):

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (5)$$

2.5. Biochemical composition analysis

To extract lipids from the freeze-dried algal biomass, the modified Bligh and Dyer method [16]. In this process, 20 mg of freeze-dried algal biomass was subjected to ultrasonication at 40 kHz for 10 min to disrupt the cells. The disrupted cells were then treated with chloroform:methanol (2:1; v/v) and stirred at 150 rpm for 3 h on an orbital shaker. Once homogenized, the suspension was centrifuged to pellet the solids, and the liquid supernatant was carefully decanted into a clean screw-cap glass tube. The supernatant was treated with 0.034% MgCl₂ and centrifuged at 5000 rpm for 10 min. The lower phase was then carefully

aspirated. Further, 2 N KCl was added to the collected lower phase, centrifuged at 5000 rpm for 10 min. Carefully aspirated the lower phase, added chloroform: methanol: water (3:47:48; v/v/v), and centrifuged at 5000 rpm for 5 min. Separated the lower phase and dried the remaining contents of the tube to obtain total lipids. The lipids thus obtained were measured gravimetrically. The percentage of lipid content was calculated using the following Eq. (6):

$$\text{Lipid content (\%)} = \left[\frac{\text{Total intracellular lipids (g)}}{\text{Dry cell weight (g)}} \right] \times 100 \quad (6)$$

The lipids were transesterification into fatty acid methyl esters (FAME) as follows: the lipid extract was treated with a 6% methanolic H_2SO_4 . The prepared sample was heated in hot water bath for 1 h at 90 °C. For recovering FAMEs, hexane was added and washed with distilled water by centrifuging the suspension for 5 min at 5000 rpm. The hexane layer comprising FAME was collected. FAME analysis was performed by using gas-chromatography-mass spectrophotometry (GC-FID) [16].

The total carbohydrate content was determined according to Bisht et al. [17]. Absorbance was recorded at 490 nm with a spectrophotometer, using distilled water as the blank. A standard curve was generated from known glucose concentrations.

Crude protein content was quantified by first determining the elemental composition of the microalgae biomass. This was performed using a CHNS elemental analyzer (Elementar vario EL cube), which provided the total nitrogen percentage (% N). Following this, the algae crude protein % (w/w) was derived by multiplying the measured nitrogen content by a specific conversion factor of 4.78 using Eq. (7), a methodology consistent with Laurens et al. [18].

$$\text{Algae crude protein \%} \left(\frac{w}{w} \right) = \%N \times 4.78 \quad (7)$$

2.6. Phytochemical analysis and ROS scavenging activity

2.6.1. Preparation of microalgal extract

To prepare the extract, 10 mg of freeze-dried algal biomass was added to a falcon tube containing 1 mL of distilled water [19]. The tube was placed in an ice and sonicated using a Cole parmer Ultrasonic Processor (Cat. no. 04711-45) Model no. CPX750) using a probe tapered microtip diameter 6 mm at 750 W, 20 Hz at 40% amplitude for 5 min at room temperature. After sonication, the mixture was centrifuged at 5000 rpm for 5 min and the supernatant was collected for further analysis and marked as algal extract.

2.6.2. Determination of TPC

TPC of the algal biomass was determined using the Folin-Ciocalteu method [20]. 20 μL of extract was mixed with 450 μL of distilled water bringing the total volume to 500 μL . Then, 250 μL of Folin-Ciocalteu reagent was added to the mixture forming a blue complex. To neutralize the solution and enhance the reaction, 2 mL of 7.5% sodium carbonate was added. The mixture was thoroughly mixed and incubated at room temperature for 90 min to allow the reaction to develop fully. After incubation, the absorbance of the solution was measured at 765 nm using a UV-Vis spectrophotometer. TPC was expressed as gallic acid equivalents (mg GAE/g DW) using Eq. (8):

$$\text{TPC} \left(\frac{\text{mg GAE}}{\text{g DW}} \right) = \frac{c \times v}{m} \quad (8)$$

where, c is the polyphenol content from the calibration curve (mg GAE/mL), v is the chemical volume utilized (mL), and m is the sample weight (g DW).

2.6.3. Determination of TFC

The aluminum chloride colorimetric method was used to determine the TFC, consistent with the methodology reported by Agbo et al. [21].

In a 5 mL falcon tube, 20 μL of the microalgae extract was mixed with 0.15 mL of 5% sodium nitrite and 0.15 mL of 10% aluminium chloride. After a 5 min interval, 1 M sodium hydroxide was added to the above mixture. The solution was then adjusted to the final volume of 5 mL with distilled water and allowed to react. The absorbance of the resulting solution was measured at 510 nm using a spectrophotometer. A standard quercetin solution was used to generate a calibration curve, and the total flavonoid content was expressed as quercetin equivalents (mg QE/g DW) based on the absorbance values using Eq. (9).

$$\text{TFC} \left(\frac{\text{mg QE}}{\text{g DW}} \right) = \frac{c \times v}{m} \quad (9)$$

where, c is the flavonoid content from the calibration curve (mg QE/mL), v is the chemical volume utilized (mL), and m is the sample weight (g DW).

2.6.4. Estimation of H_2O_2 scavenging activity

The scavenging activity of reactive oxygen species (ROS) was evaluated using a hydrogen peroxide (H_2O_2) scavenging assay [22]. Algal extract 100 $\mu\text{g/mL}$ were incubated with 0.6 mL of 40 mM H_2O_2 in phosphate buffer (pH 7.4). The absorbance of the reaction mixture was measured at 230 nm, and the scavenging activity was expressed using Eq. (10) to evaluate the effectiveness of ROS scavenging activity.

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100 \quad (10)$$

2.7. FTIR analysis

To identify the functional groups, both PFAS-treated and control microalgal biomass samples were analyzed using a Bruker Invenio FTIR spectrometer. The spectra were recorded in the range of 4000–800 cm^{-1} with a resolution of 4 cm^{-1} .

2.8. Extraction of metabolites and non-targeted analysis

For metabolite analysis, 100 mg of lyophilized *Chlorella minutissima* biomass was extracted with 10 mL of 80% methanol and subjected to sonication for 5 min using a probe sonicator. The mixture was then kept at -20°C for 10 min, followed by centrifugation at 10,000 and 4°C for 20 min. The supernatant was collected and dried under vacuum. The resulting extract was re-dissolved in 100 μL of acetonitrile water solution (1:1, v/v). Metabolite separation was performed using an ultra-high-performance liquid chromatography system coupled with a triple quadrupole mass spectrometer (LCMS-8050TM, Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source. The separated metabolites were then eluted into the mass spectrometer using ESI source at positive mode [23]. The drying gas flow was maintained at 6 L/min at 250°C , while the sheath gas flow was set to 9 L/min at 350°C , with a nebulizer pressure of 25 psi. Metabolite analysis (including amino acids, lipids, phenolics and flavonoids, pigments, organic acids, and carbohydrates) were performed using GC-FID (for lipids) and LC-MS (for the other metabolites). Metabolites were identified by comparing their *m/z* values and retention times with published reference data and publicly available databases. The primary sources for metabolite annotation were the Metabolomics Workbench [24] and the FoodB (Food Database) [25]. Metabolite identification was performed at Confidence Level 2 (putative annotation). Specifically, amino acids, pigments, phenolics, and organic acids were putatively annotated by matching their accurate masses and retention times (RT) with those of authentic standards (S Tables 10–15). PFAS were confirmed by matching their precursor ion, product ion(s), and chromatographic retention time with authentic standards analyzed on the same instrument. Amino acids were

identified using standard. FAME were identified using a FAME Standard Mixture (Cat. No. 1269119, United States Pharmacopeia (USP) Reference Standard, Sigma-Aldrich, USA). Identification was based on comparison of retention times and mass spectra with those of the reference standard and verified against the NIST mass spectral library (match score > 95%). All fatty acids were expressed as FAME derivatives. Other metabolites (pigments, phenolics, organic acids) were putatively annotated by matching their accurate mass and retention time with authentic standards (S Tables 10–15).

2.9. Statistical analysis

All experiments were conducted in triplicate ($n = 3$), and results are expressed as mean $\pm$ standard deviation (SD) to ensure accuracy, reproducibility, and consistency across replicates. For metabolite analysis, instrument precision and repeatability were evaluated through five replicate injections of standard mixtures, and the relative standard deviation (RSD) values were calculated to assess analytical stability. Statistical analysis and data visualization were performed using GraphPad Prism (version 8.4.3) and OriginPro 2024 software. One-way analysis of variance (ANOVA) was performed, followed by a post hoc Tukey's multiple comparison test to identify significant differences at $p < 0.05$ among treatments.

3. Result and discussion

3.1. Impact of PFAS compounds on growth of *C. minutissima*

Based on the categorization of PFAS concentrations reported in the literature by Wu et al. [26], four ranges were defined: ng/L (<1000 ng/L), $\mu\text{g/L}$ (1–1000 $\mu\text{g/L}$, excluding 1000 $\mu\text{g/L}$), mg/L (1–1000 mg/L, excluding 1000 mg/L), and g/L (>1 g/L). In this study, PFAS exposure at the ng/L and $\mu\text{g/L}$ levels did not cause significant changes in the growth of *C. minutissima*, whereas measurable effects were observed at the mg/L level. Therefore, the growth response of *C. minutissima* was assessed under exposure to various PFAS compounds at a concentration of 1 mg/L over 14 days (Fig. 1). On the 14th day, the cell density of all *C. minutissima* cultures exposed to PFAS compounds was significantly lower than that of the control, except in the PFHxS and PFDA treatments ($p < 0.001$) as depicted in S Table (3). The highest biomass yield (1.025 $\pm$ 0.001 g/L) was recorded in the presence of PFHxS, whereas the lowest biomass yield (0.9023 $\pm$ 0.001 g/L) was observed in cultures exposed to

PFOA. As shown in Fig. 1, exposure to different PFAS compounds led to a significant reduction in the growth of *Chlorella* sp. These findings are consistent with previous reported studies. Zhao et al. [27] demonstrated that *C. sorokiniana* exposed to PFOA at concentrations of 0.05, 0.5, and 5 mg/L for 96 h exhibited a clear concentration-dependent inhibition of growth, primarily associated with oxidative stress (ROS), with approximately 27% inhibition rate at the highest concentration. Similarly, Mojiri et al. [28] reported a progressive decline in cell viability in response to PFOA exposure (1–10 mg/L), with stronger inhibitory effects observed at higher concentrations. In contrast, Marchetto et al. [29] observed no inhibitory effects of PFOS and PFOA on microalgal growth at concentrations below 1 mg/L. However, Hu et al. [30] and Liu et al. [31] documented significant growth inhibition when PFOS concentrations increased up to 20 mg/L. Collectively, these studies highlight that PFAS compounds can exert toxic effects on microalgae in a concentration-dependent manner, emphasizing their persistence and potential for bioaccumulation in aquatic ecosystems.

3.2. Impact of PFAS compounds on photosynthetic pigments of *C. minutissima*

The photosynthetic pigment content reflects the light utilization capacity of algae and conveying electrons, which is directly linked to their growth and serves as a key indicator of physiological status [27,32]. In *Chlorella* sp., chlorophyll-a typically ranges from 0.25 to 9.63 $\mu\text{g/mL}$, accounting for approximately 1–2% of the cell's dry weight [33]. In this study, photosynthetic pigment concentrations showed significant variation ($p < 0.0001$) across the different PFAS treatments (Fig. 2, S Tables 2–6). The maximum concentration of chlorophyll-a (11.64 $\pm$ 0.232 $\mu\text{g/mL}$) was observed in PFHxS-treated cultures, corresponding to an approximately 102.4% increase relative to the control (5.75 $\pm$ 0.075 $\mu\text{g/mL}$), whereas the lowest concentration was recorded in PFOA-treated cultures (2.9 $\pm$ 0.172 $\mu\text{g/mL}$), representing an approximate 49.6% decrease compared to the control. Similarly, the highest chlorophyll-b concentration was detected under PFNA treatment (12.62 $\pm$ 0.405 $\mu\text{g/mL}$), showing an approximate 362.3% increase relative to the control (2.73 $\pm$ 0.035 $\mu\text{g/mL}$), while the lowest value was observed in PFHpA-treated cultures (1.24 $\pm$ 0.616 $\mu\text{g/mL}$), indicating an approximate 54.6% decrease compared to the control. Moreover, carotenoid concentration (3.6 $\pm$ 0.036 $\mu\text{g/mL}$) under PFDA exposure demonstrated a substantial elevation of approximately 106.9% relative to the control (1.74 $\pm$ 0.025 $\mu\text{g/mL}$). On the other hand, the maximum quantum yield of PSII (Fv/Fm), is recognized as a reliable indicator of PSII photochemical efficiency and reflects the intrinsic capacity of microalgal cells to convert absorbed light energy into chemical energy [34]. In the present study, the Fv/Fm ratios recorded in PFHxS (0.685 $\pm$ 0.005) and PFDA (0.667 $\pm$ 0.501) treated microalgal cultures were higher than that of the control (0.645 $\pm$ 0.014), suggesting that PSII efficiency was maintained or slightly enhanced under these treatments. However, statistically significant alterations ($p < 0.0001$) were observed only in PFOS (0.573 $\pm$ 0.003) and PFOA (0.587 $\pm$ 0.008)-treated cultures, indicating a more pronounced impact of these compounds on photosynthetic performance (S Tables 2, 6). These findings are consistent with previous reports demonstrating that Fv/Fm is more sensitive to PFOS exposure than to PFOA, highlighting differential toxicity among PFAS compounds [35].

Notably, the PFHxS induced enhancement in chlorophyll-a was accompanied by increased cell density in *C. minutissima* (Fig. 1), suggesting improved light-harvesting efficiency and photosynthetic capacity. In contrast, PFOS-treated cultures exhibited comparatively lower pigment levels and photosynthetic yield, which corresponded with the lowest growth performance, indicating impaired photosynthetic function and stress-mediated growth inhibition. Recent studies have demonstrated that PFAS exposure can disrupt multiple components of the algal photosynthetic apparatus [36]. Due to the presence of a sulfonic acid functional group and its higher bioaccumulation potential,

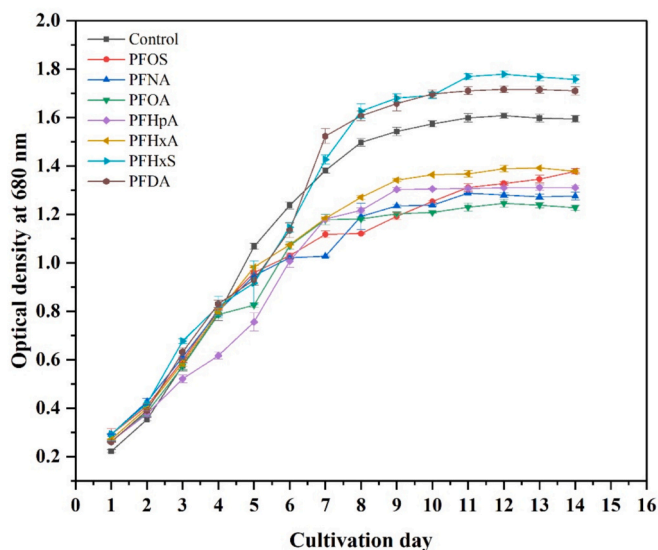


Fig. 1. Growth curve of *Chlorella minutissima* under exposure to different PFAS stress at 1 mg/L concentration.

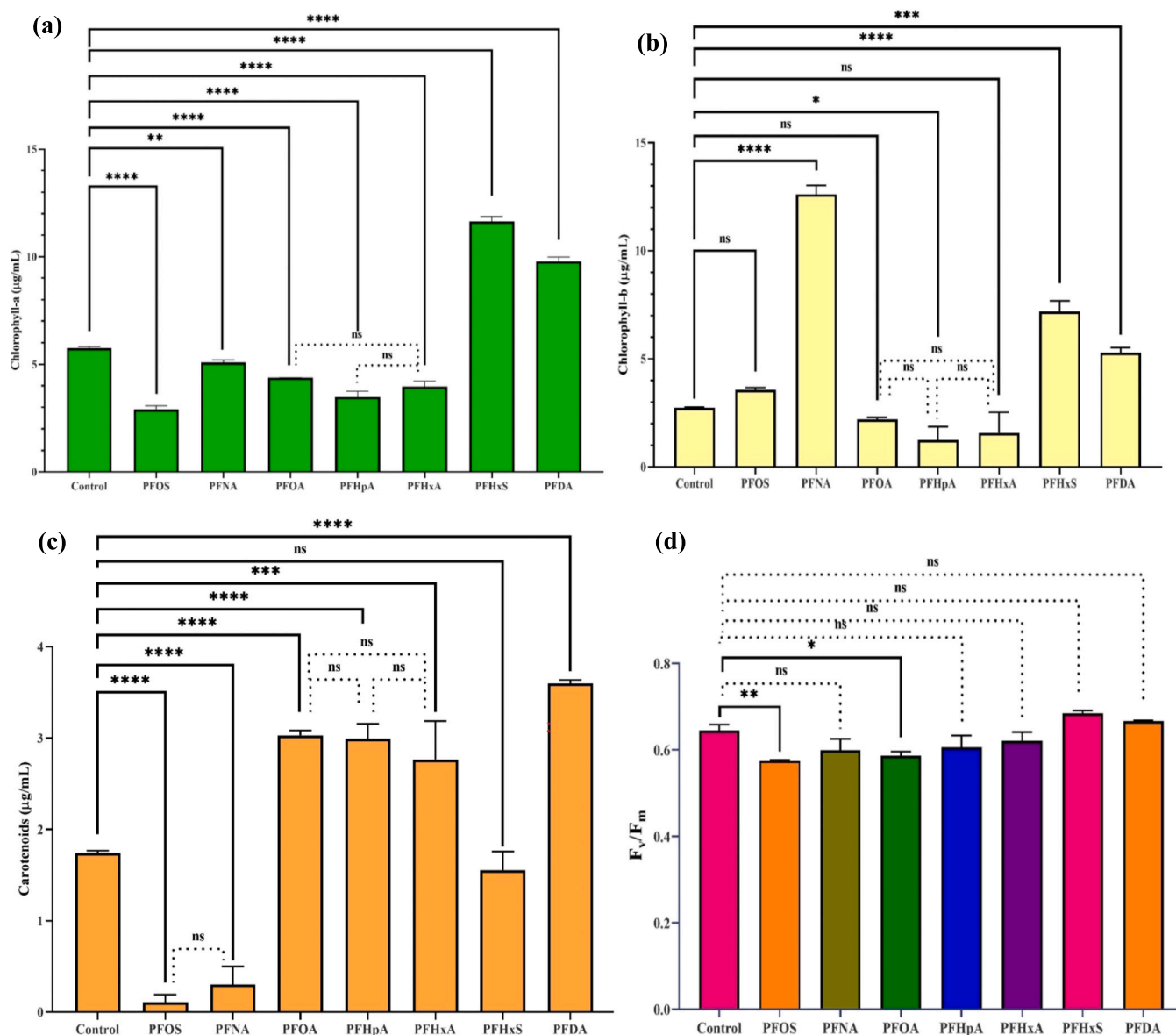


Fig. 2. Influence of PFAS treatment on photosynthetic pigment concentration: (a) Chlorophyll-a, (b) Chlorophyll-b, (c) Carotenoids, and (d) Photosynthetic efficiency (Fv/Fm). Bars represent Mean ± SD. Solid black lines and asterisks indicate statistical significance (*): * = $p < 0.05$ (significant), ** = $p < 0.01$ (more significant), *** = $p < 0.001$ (highly significant), **** = $p < 0.0001$ (extremely significant), while dotted black lines and 'ns' denote non-significant difference.

PFOS exhibits a stronger tendency to accumulate within algal cells, leading to more pronounced damage to photosystems. Compared with PFOA, PFOS has been shown to exert greater inhibitory effects on the synthesis of essential PSII core proteins, including D1 and D2, as well as on genes associated with pigment biosynthesis, such as *psbB* and *chlB* as reported by Mao et al. [35]. Collectively, these structural and molecular disruptions reduce the efficiency of light energy utilization, impair electron transport, and limit ATP and NADPH production, thereby constraining the Calvin cycle and associated dark reactions. This photosynthetic impairment is reflected in reduced photochemical efficiency, as indicated by declines in Fv/Fm values under PFOS stress [36]. Some similar changes are reported by Liao et al. [37] for photosynthetic performance of *Microcystis aeruginosa* at chlorophyll-a levels, promoting cellular proliferation under combined PFAS and heatwave conditions. Consistent with these findings, Mojiri et al. [28] observed that both *C. vulgaris* and *S. obliquus* achieved their maximum total chlorophyll contents at 0.5 mg/L PFAS after 7 days, 16.8 mg/L for *C. vulgaris* and 16.1 mg/L for *S. obliquus* whereas total chlorophyll content declined precipitously at 10 mg/L PFAS after the same exposure duration. Further

analysis of photosynthetic performance, as reported by Wu et al. [38] showed that both long-chain and short-chain PFAS significantly reduced photosynthetic pigment content (−24.29% and −15.86%, respectively) as well as electron transport activity (−9.75% and −4.35%, respectively), with the inhibitory effects being less pronounced for short-chain PFAS. Similarly, carotenoids in microalgae serve as essential protective pigments capable of reacting to lipid peroxidation by-products, thereby mitigating oxidative stress by quenching ROS and preserving the integrity of the photosynthetic process [39] and once the photosynthetic pigments capture light energy, the internal electron transport process begins, ultimately producing carbohydrates and lipids, which facilitates carbon dioxide fixation and oxygen generation [38]. Under PFAS exposure, pigment levels often exhibit concentration and compound-dependent variations, reflecting either stress adaptation mechanisms or pigment degradation.

3.3. Impact of PFAS compounds on biochemical composition of *C. minutissima*

The biochemical composition of *C. minutissima* typically consists of 25–60% protein, 10–30% carbohydrates, and 10–30% lipids, with proportions influenced by species, growth conditions, and environmental stress factors. In the present study, PFAS exposure substantially altered the biochemical composition of *C. minutissima* (Fig. 3). In the control cultures, lipid, protein, and carbohydrate contents were $26 \pm 1.3\%$, $28 \pm 1.4\%$, and $35 \pm 1.75\%$, respectively. Among the PFAS treatments, lipid accumulation generally increased with perfluoroalkyl chain length among perfluorocarboxylic acids. PFDA (C10) exhibited the highest lipid content ($38 \pm 1.9\%$), representing a 46.1% increase compared to the control, followed by PFNA (C9). In contrast, the short-chain PFHxA (C6) resulted in the lowest lipid accumulation. Although PFOA (C8) belongs to the medium-chain group, it showed comparatively reduced lipid levels, suggesting compound-specific metabolic interference despite similar carbon length. Moreover, perfluorosulfonates with PFHxS (C6) and PFOS (C8), both promoting elevated lipid content, correspond to approximately a 30.7% increase relative to the control. This suggests that functional group chemistry, in addition to chain length, influences cellular lipid accumulation. The elevated lipid levels under PFAS exposure represent a well-documented stress-induced adaptive metabolic response in microalgae. Exposure to xenobiotic compounds disrupts the cellular redox balance by enhancing ROS generation while impairing antioxidant defense mechanisms, thereby disturbing the equilibrium between ROS production and elimination [40]. Similar oxidative stress-mediated metabolic perturbations have been reported during chemical exposure, including amoxicillin treatment in *C. minutissima* [39] and PFOA treatment in *S. quadricauda* [41], demonstrating that adverse environmental conditions can significantly alter intracellular metabolic homeostasis. Under such oxidative stress conditions, microalgal cells redirect carbon flux from photosynthetic carbon dioxide fixation away from protein biosynthesis toward neutral lipid accumulation, particularly triacylglycerol formation. This lipogenic phase is typically characterized by reduced cell division, partial downregulation of the photosynthetic apparatus, and enhanced storage lipid biosynthesis [42,43]. Such metabolic reallocation serves both as an energy storage strategy and as a protective mechanism to dissipate excess reducing equivalents generated during impaired photosynthetic electron transport. The key regulatory enzymes involved in this process include acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS). ACCase catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the

committed and rate-limiting step of fatty acid synthesis, while FAS mediates the sequential elongation of acyl chains to produce long-chain fatty acids [44]. Upregulation of these enzymes enhances fatty acid and triacylglycerol biosynthesis, thereby contributing to lipid accumulation and potentially modifying chloroplast energy balance and membrane dynamics [45,46]. However, contrasting findings have been reported. Wu et al. [38] observed increased membrane permeability (58.82%), elevated malondialdehyde levels (33.84%), and a decrease in total lipid content (-12.26%) under PFAS exposure, suggesting that excessive oxidative damage may impair lipid biosynthesis. Therefore, lipid accumulation under PFAS stress appears to be concentration and compound-dependent, reflecting either an adaptive metabolic shift under moderate stress or metabolic suppression under severe toxicity. Collectively, these findings support the interpretation that the observed increase in lipid accumulation, particularly under long-chain PFAS exposure, represents an adaptive metabolic shift driven by oxidative imbalance and carbon reallocation toward storage lipid biosynthesis.

As shown in Fig. 3, the control exhibited the highest carbohydrate content ($35 \pm 1.75\%$), serving as the physiological baseline. On the other hand, medium-chain PFOA-treated microalgae maintained a comparable level ($33.1 \pm 1.65\%$), indicating only a modest reduction ($\sim 5.4\%$), suggesting relatively moderate metabolic disturbance. In contrast, short-chain PFHxA exposure resulted in the lowest carbohydrate content ($28.7 \pm 1.43\%$), corresponding to a $\sim 17.9\%$ decrease, might indicate stress-induced shifts in carbon allocation. Under stress, photosynthetically fixed carbon is often reallocated from protein synthesis toward storage compounds such as carbohydrates or lipids, particularly when growth becomes limited by nutrients such as nitrogen or phosphorus [47]. Mild stress conditions may allow carbohydrates to remain relatively stable, whereas stronger or structurally disruptive PFAS (particularly certain short- or long-chain PFAS) can trigger enhanced lipid accumulation. In addition, PFAS exposure may indirectly influence nutrient homeostasis, particularly nitrogen and phosphorus balance. During the onset of a lipogenic phase, cell division slows, the photosynthetic apparatus may partially decline, and ATP and reducing equivalents are preferentially directed toward the synthesis of neutral lipids such as triacylglycerols. This process often results in reduced carbohydrate pools as carbon skeletons are diverted toward nitrogen-free storage compounds, thereby increasing the cellular C/N ratio [41]. These findings are consistent with the study by Liao et al. [37], who reported significant disturbances in carbon and nitrogen metabolism in *M. aeruginosa* under PFAS exposure and PFAS–heatwave co-treatment, including marked upregulation of C/N metabolic pathways. Furthermore, carbohydrate metabolism is closely linked to intracellular phosphorus availability. Previous studies have shown that carbohydrate accumulation becomes pronounced when intracellular phosphorus levels decline below a critical threshold [48,49]. In such conditions, carbohydrates and overall biomass production may initially increase as carbon assimilation continues while cell division slows. Therefore, the observed carbohydrate fluctuations under PFAS exposure may reflect stress-induced shifts in carbon partitioning, potentially linked to nutrient imbalance.

Additionally, protein content was also strongly influenced by PFAS exposure. PFHxA treatment resulted in the highest protein accumulation ($34 \pm 1.7\%$), showing 21.4% increase over control. Conversely, PFDA exposure led to the lowest protein content ($22 \pm 1.1\%$). This suggests divergent effects of PFAS, where some (PFHxA) enhance nitrogen assimilation or enzyme biosynthesis, while others (PFDA) may impair protein production under elevated oxidative stress. Mojiri et al. [50] similarly reported that low concentrations of emerging contaminants can enhance protein synthesis by stimulating enzyme activity, whereas higher concentrations induce abiotic stress, suppressing protein accumulation.

Overall, these biochemical profiles suggest that different PFAS compounds trigger distinct metabolic profiling in *C. minutissima*, highlighting PFAS-specific impacts on cellular metabolism, likely linked to

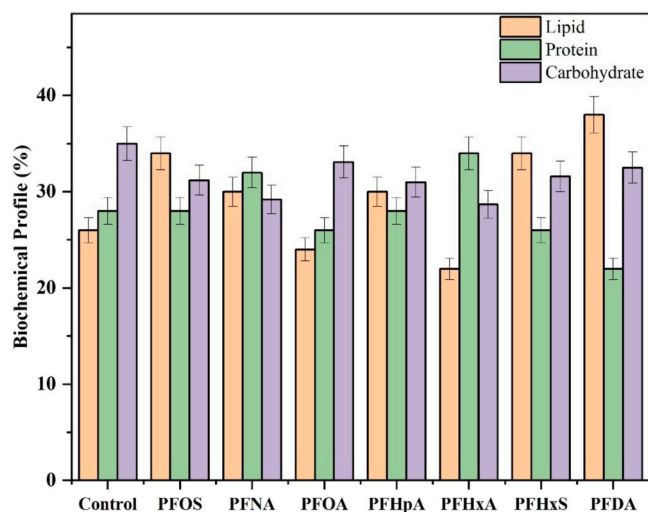


Fig. 3. Influence of PFAS treatment on Biochemical compositions. Bars represent Mean $\pm$ SD.

oxidative stress responses and reallocation of carbon and nitrogen fluxes.

3.4. Impact of PFAS compounds on FAME profile

The analysis of FAME profiles under different PFAS exposures revealed distinct alterations in the distribution of saturated and unsaturated fatty acids compared with the control, indicating disruption of lipid metabolism (Fig. 4). In the control group (total FAME = 95%), saturated fatty acids (53%) and unsaturated fatty acids (42%) were relatively balanced, reflecting normal membrane homeostasis.

However, most PFAS treatments resulted in a reduction in total FAME content, suggesting partial suppression of lipid biosynthesis under xenobiotic stress. Moreover, PFHpA and PFDA exhibited increased total FAME levels relative to the control, along with elevations in both saturated and unsaturated fractions, indicating stimulation of triacylglycerol synthesis, which may reflect activation of de novo fatty acid biosynthesis from CO₂-derived acetyl-CoA within the chloroplast, a pathway centrally regulated under oxidative stress conditions, as an adaptive response to restore redox balance and protect cellular structures [41,42]. Notably, in all PFAS treatments, saturated fatty acids were proportionally higher than unsaturated fatty acids, with PFOS showing marked suppression of unsaturated fatty acids (particularly C18:1 and C16:3). This shift indicates that triacylglycerol accumulation is preferentially enriched in saturated fatty acids, which would be advantageous because less energy is required for the synthesis of polyunsaturated fatty acids from them but more energy is released at oxidation of these compounds, and the accumulation of triacylglycerol comprising long-chain polyunsaturated fatty acids is less beneficial [42,43]. Consistent with these findings, Ha et al. [41] reported that under high PFOA exposure (>12.5 ppm), FAME yield decreased to 67%, likely due to toxic inhibition of lipid biosynthesis. Thus, such alterations may affect the viability and biofuel potential of microalgae, underlining the need for further investigation into PFAS toxicity in algal systems.

3.5. Impact of PFAS compounds on phytochemical composition and ROS inhibition activity of *C. minutissima*

Cellular toxicity in algae is primarily influenced by oxidative stress, which occurs when there is an imbalance between the formation of ROS and the efficacy of endogenous antioxidant mechanisms [51]. Results from the present study revealed that PFAS exposure triggers oxidative stress in *C. minutissima*, leading to changes in the production of antioxidant compounds such as phenolics and flavonoids. TPC, TFC and H₂O₂ (ROS) scavenging activity in the PFAS-exposed microalgae cultures varied significantly ($p < 0.0001$) compared to the control algae biomass (Fig. 5; S Tables 3, 7–9). The control algal biomass exhibited the highest total phenolic content (TPC: 50.39 ± 2.54 mg GAE/g DW) and total flavonoid content (TFC: 40.27 ± 1.11 mg QE/g DW). Among the PFAS-treated groups, PFHxS exposure resulted in the highest TPC (34.66 ± 0.87 mg GAE/g DW) and TFC (24.97 ± 0.69 mg QE/g DW); however, these values were still markedly lower than the control, representing an approximate 31.2% decrease in TPC and 38.0% decrease in TFC relative to untreated cultures. In contrast, PFOS-treated microalgal cultures exhibited the lowest phenolic and flavonoid contents among all treatments, indicating a pronounced suppression of antioxidant-associated secondary metabolites under PFOS stress.

Oxidative activity appeared to be the most sensitive parameter under PFAS exposure, as reflected by alterations in ROS dynamics. ROS typically include singlet oxygen ($^1\text{O}_2$), superoxide ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\text{OH}^{\cdot}$) [52]. Moreover, the antioxidant system plays a critical role in mitigating oxidative damage by scavenging excessive ROS, thereby serving as a primary defense mechanism in algal cells [27]. Among these, $^1\text{O}_2$ is primarily produced in photosystem II, whereas $\text{O}_2^{\cdot-}$ is mainly generated in chloroplasts and mitochondria during oxidative phosphorylation, where it is rapidly converted to H_2O_2 by superoxide dismutase (SOD). $\text{OH}^{\cdot}$ is formed in the chloroplast stroma but, due to its high reactivity and instability, it is quickly transformed into H_2O_2 or H_2O [53]. Notably, the observed increase in ROS levels under PFAS exposure is unlikely to be predominantly driven by $^1\text{O}_2$ or $\text{OH}^{\cdot}$. This is because reduced pigment content and decreased PSII efficiency under PFAS stress likely limited $^1\text{O}_2$ formation, while $\text{OH}^{\cdot}$ is too short-lived to accumulate significantly. Instead,

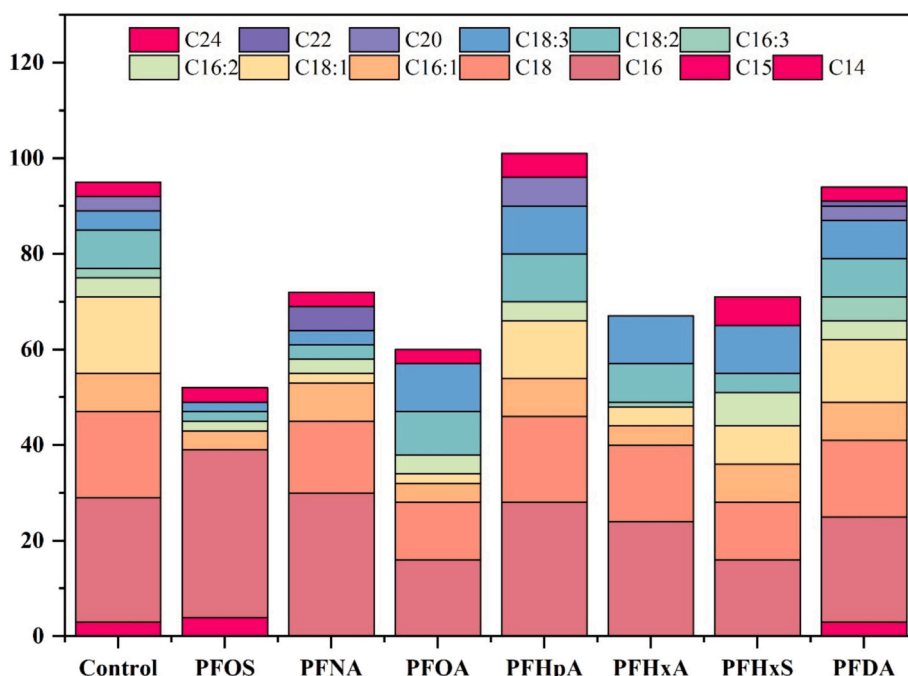


Fig. 4. FAMES profile of microalgae *C. minutissima* under PFAS stress.

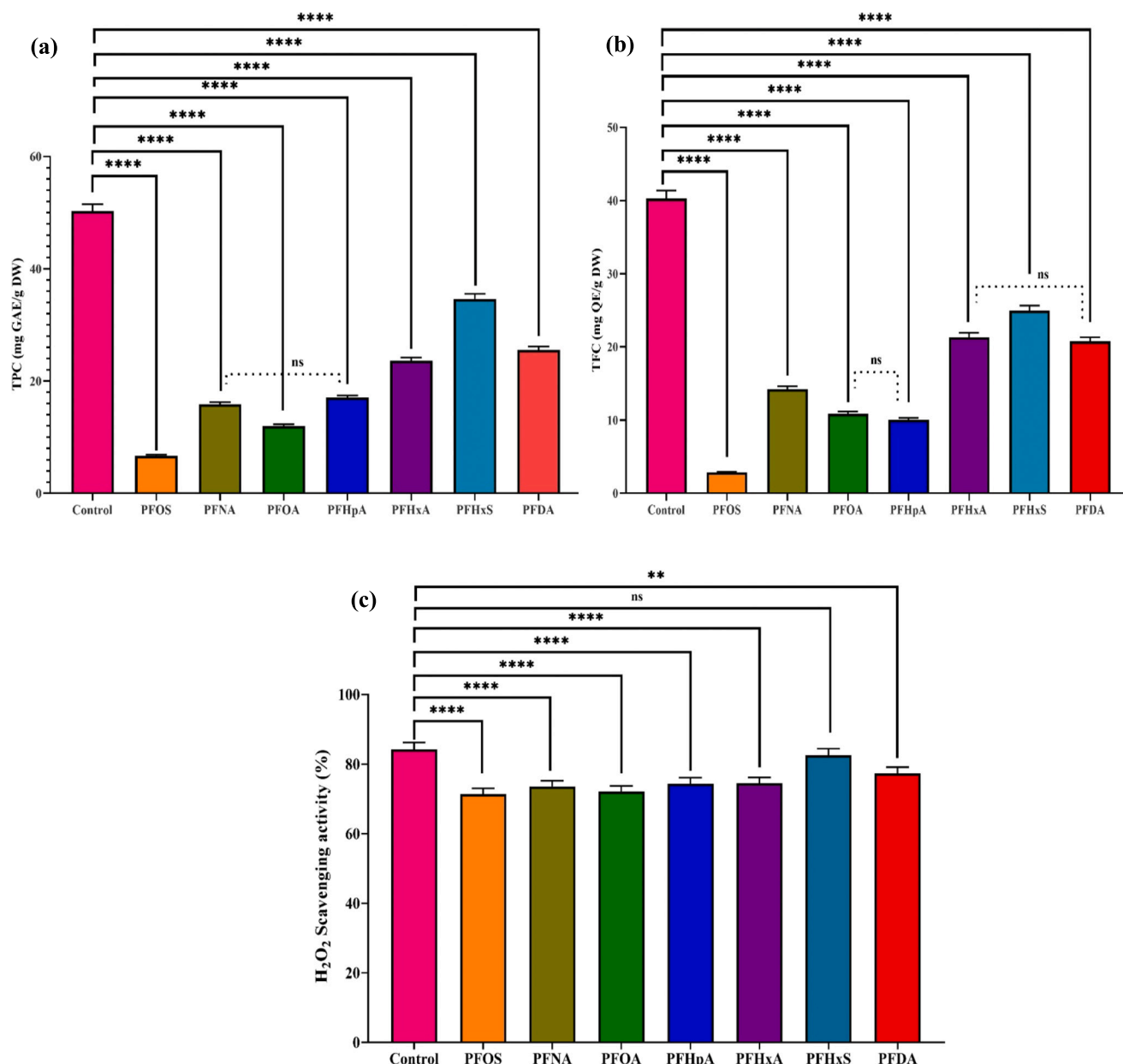


Fig. 5. Effect of PFAS treatment in (a) TPC of microalgae, (b) TFC of microalgae, (c) H₂O₂ scavenging activity (%). Values are denoted as in Mean ± SD. Bars represent Mean ± SD. Solid black lines and asterisks indicate statistical significance (*): ** = $p < 0.01$ (more significant); **** = $p < 0.0001$ (extremely significant), while dotted black lines and 'ns' denote non-significant difference.

impaired electron flow under PFAS stress may promote electron leakage in photosystem I, leading to enhanced O₂[•] production, which subsequently contributes to elevated H₂O₂ levels [54]. Among these, H₂O₂ acts as an essential component of the initial antioxidant barrier, helping to regulate redox balance and protect cellular structures from oxidative stress [55]. In the present study, PFAS exposure altered H₂O₂ scavenging capacity, with the highest activity observed in PFHxS-treated cultures (82.53 ± 0.67%), reflecting a slight reduction of approximately 2.1% compared to the control (84.30 ± 1.94%). Conversely, PFOS treatment resulted in the lowest scavenging activity (71.73 ± 1.64%), corresponding to a pronounced decrease of ~14.9% relative to the control (Fig. 5c). The results demonstrated a significant association ($p < 0.0001$) between H₂O₂ scavenging activity and PFAS exposure in *C. minutissima*, indicating that bioactive compounds play a crucial role in mitigating oxidative stress and preserving cellular integrity under environmental stress conditions. Similar protective responses have been reported with

other pollutants, such as amoxicillin [39]. These findings were also consistent with intracellular ROS levels, suggesting that *C. sorokiniana* employs antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), as part of its defense strategy to counteract oxidative damage [27]. These adaptive responses are critical in managing ROS-induced toxicity and mitigating oxidative damage in algal cells.

3.6. Impact of PFAS compounds on functional groups

FTIR spectra of *C. minutissima* biomass under PFAS exposure exhibited clear biochemical alterations (Fig. 6 and Table 1). Major changes were observed in protein regions (amide I and II bands at ~1500–1650 cm⁻¹), lipid C–H stretching (~2924 cm⁻¹), carbohydrate C–O stretching (~1014–1248 cm⁻¹), and hydroxyl/amino groups (~3278 cm⁻¹). These spectral shifts indicate that PFAS treatments affected the cellular composition of proteins, lipids, carbohydrates, and

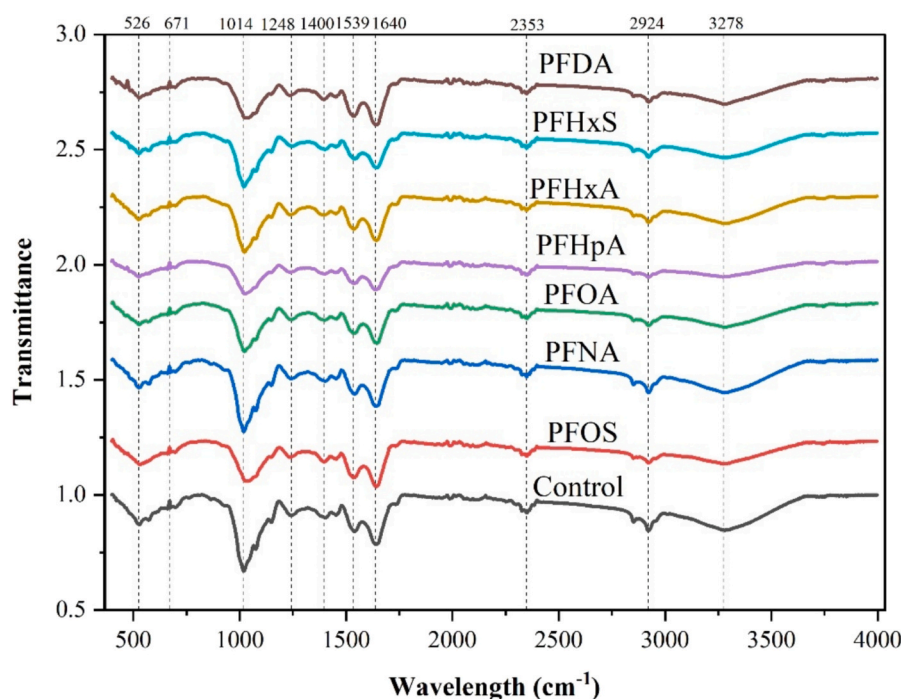


Fig. 6. FTIR spectra of microalgae *C. minutissima* under different PFAS stress.

Table 1

Characteristic FTIR absorption bands of *C. minutissima* biomass under different PFAS treatment.

Wave number (cm ⁻¹)	Vibrations	Functional compounds	Control	PFOS	PFNA	PFOA	PFHpA	PFHxA	PFHxS	PFDA
525–530	C–F stretch	Alkyl halides	–	+	+	+	+	+	+	+
660–680	C–S stretch	Sulfides or thiols	671	+	+	+	+	+	+	+
1000–1200	C=S stretching	Sulfonates	1014	–	+	+	+	+	+	–
1230–1250	P=O stretch	Phospholipids or phosphate groups	1248	–	+	+	+	+	+	–
1400–1450	C–H bending	Methylenes groups	1400	+	+	+	+	+	+	+
1530–1550	N–H bend, C–N stretch	Amides II	1539	+	+	+	+	+	+	+
1640–1650	C=O stretch	Amides I Protein structure	1640	+	+	+	+	+	+	+
2350–2400	C≡N bending	Nitrile	2353	+	+	+	+	–	+	+
2900–2950	C–H stretch	Methylene Chain	2924	+	+	+	–	+	+	+
3200–3300	O–H, N–H stretch	Hydroxyl groups, amines	3278	–	+	–	–	–	–	+

+ sign indicate presence of functional group; – sign indicate absence of functional group.

associated functional groups compared to the control. Taken together, the FTIR spectra highlight that PFAS exposure impacts all major macromolecular pools i.e., proteins, lipids, carbohydrates, and hydroxylated compounds, providing early biochemical evidence of stress responses in *C. minutissima*.

3.7. Impact of PFAS compounds on metabolite profiling of *C. minutissima*

The analytical workflow demonstrated excellent precision and reproducibility. The 10 individual phenolic standards yielded RSD values between 0.41 and 6.18%, confirming stable detector response and minimal analytical drift. Similarly, the mixed 20-amino-acid standard solution showed RSD values ranging from 2.27 to 5.82%, validating consistent system performance across all runs (S Table S1). The untargeted analysis revealed significant perturbations in the metabolite profile. The dataset comprises identifications at multiple confidence levels. A subset of key metabolites, including all amino acids and per- and polyfluoroalkyl substances (PFAS), were confirmed using MS/MS fragmentation (S Table 12). The remaining metabolites, including lipids, pigments, and phenolic compounds, were putatively annotated and are presented as hypothesis-generating observations (S Tables 11–14).

The heat map clearly demonstrated that PFAS exposure induced

distinct metabolites in *C. minutissima*, with notable shifts across amino acids, pigments, phenolics/flavonoids, lipids, organic acids, and carbohydrates (Fig. 7, S Tables 11–14). These alterations revealed that each PFAS compound induced distinct clustering patterns, reflecting compound-specific biochemical responses. In particular, most amino acids exhibited either down-regulation or irregular trends across treatments, pointing to disruptions in nitrogen metabolism and protein biosynthesis. For instance, PFOS exposure was associated with elevated levels of certain amino acids, likely reflecting stress-induced protein degradation to support antioxidant defense. Pigments displayed variable regulation. On the other hand, phenolics and flavonoids were largely upregulated, consistent with their role as antioxidants in mitigating PFAS-induced oxidative stress. Organic acids showed mixed responses, reflecting alterations in central carbon metabolism, while carbohydrates generally showed moderate decreases under PFAS stress, indicating a shift of carbon flux toward lipid or protein synthesis. Previous studies reported that long-chain PFAS (C10–C11) predominantly accumulate due to their stronger sorption affinity and lower mobility resulting PFAS accumulation in lower concentration, leading to localized root toxicity in plants, including impaired nutrient uptake, membrane disruption, and oxidative stress [56]. In contrast, short-chain PFAS, owing to their higher water solubility and weaker soil binding, are more readily

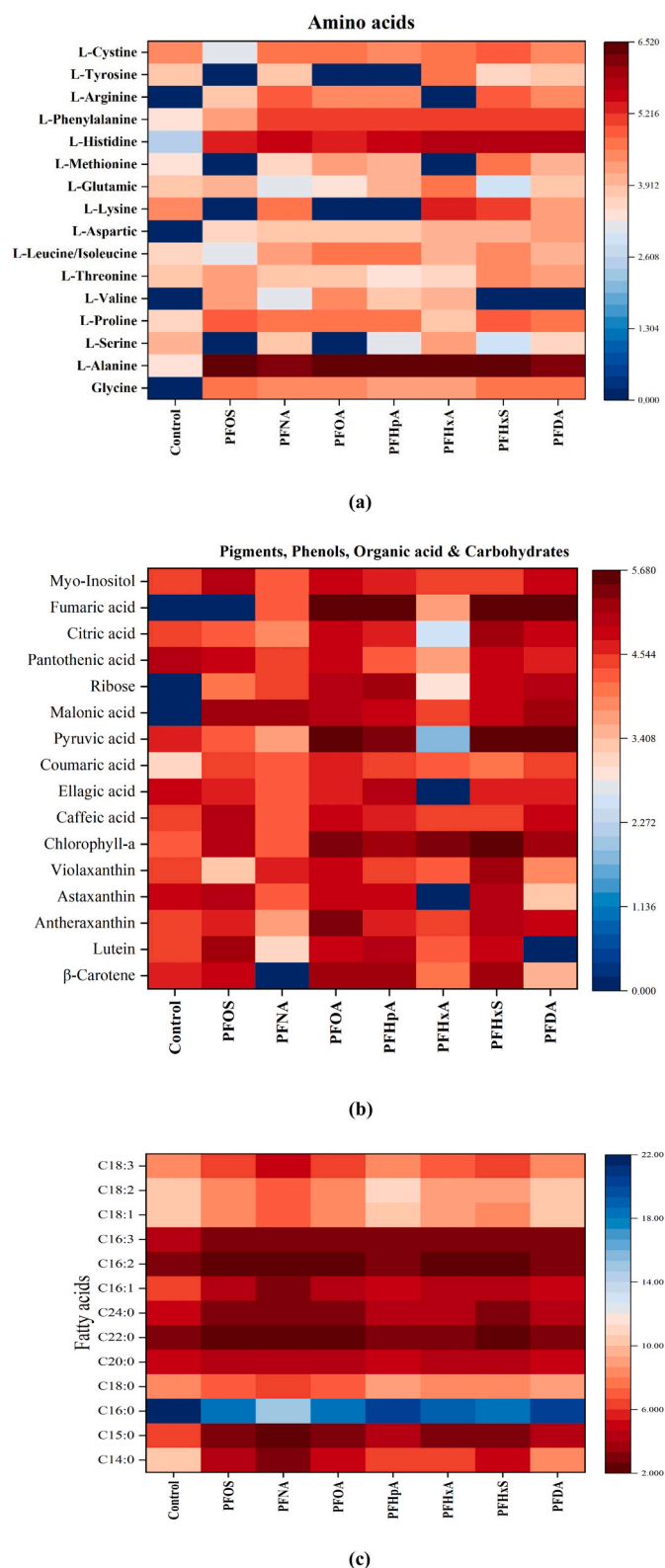


Fig. 7. Heat map of (a) Amino acids, (b) Pigments, Phenolics and Flavonoids, Organic acids and Carbohydrates, (c) Lipids and its derivatives.

translocated to aerial tissues through the transpiration stream [56]. Such differential distribution patterns not only influence plant physiological performance and yield but also elevate toxicological risks along the food chain. Overall, the heat map reinforces that PFAS stress triggers selective up- or down-regulation of key metabolic pathways, reflecting adaptive

strategies of the microalga under chemical stress.

The detection of pyruvic acid, citric acid, and fumaric acid (S Table 13) indicates active glycolytic and TCA cycle flux, providing acetyl-CoA precursors for lipid biosynthesis. The presence of pantothenic acid supports coenzyme A availability, while malonic acid suggests involvement of the acetyl-CoA carboxylase pathway, the rate-limiting step in fatty acid synthesis [43]. Fatty acid synthase (FAS) likely contributes to the observed increase in saturated fatty acids. Ribose detection further implies pentose phosphate pathway activity, supplying NADPH required for reductive lipid biosynthesis [42]. Importantly, identification of a triacylglycerol confirms enhanced neutral lipid accumulation, indicating that PFAS exposure promotes coordinated activation of fatty acid synthesis and storage lipid formation as a stress-adaptive response.

Upon integrated evaluation of the heatmap and PCA, a clear separation among treatments was observed ($PC1 = 83.70\%$, $PC2 = 4.79\%$), indicating that the majority of metabolic variance is explained by $PC1$. The control sample was distinctly separated from all PFAS-treated groups along $PC2$, confirming a unique metabolic profile relative to PFAS exposure. Samples located near the origin exhibited minimal metabolic deviation, whereas those positioned farther along $PC1$ (e.g., metabolite 3) demonstrated strong associations with specific PFAS treatments, reflecting substantial metabolic perturbation. The clustering pattern suggests that metabolic responses are strongly influenced by PFAS carbon chain length and structural characteristics. Long-chain PFAS tended to cluster separately from short-chain analogues, indicating greater metabolic disruption. Furthermore, compounds sharing similar functional groups and comparable chain lengths showed closer spatial proximity in the PCA plot. For instance, PFOS and PFHxS (sulfonates) clustered together, while PFOA, PFNA, and PFDA (carboxylates) formed another cluster, suggesting analogous metabolic perturbation profiles within each functional class. Long-chain PFAS induced more pronounced alterations in key amino acids, including L-alanine, L-histidine, and L-proline, compared to short-chain PFAS, which caused relatively moderate shifts. Likewise, long-chain compounds such as PFDA and PFOS promoted a greater accumulation of saturated fatty acids and a concomitant reduction in certain unsaturated fatty acids. Moreover, carbohydrates and photosynthetic pigments, including ribose, myo-inositol, chlorophyll-a, and carotenoids, also exhibited chain-length-dependent response patterns, with sulfonated PFAS generally exerting stronger effects than their corresponding carboxylates. Collectively, these findings demonstrate coordinated alterations in carbohydrates, lipids, amino acids, and pigments that align with PFAS structural similarity and carbon chain length.

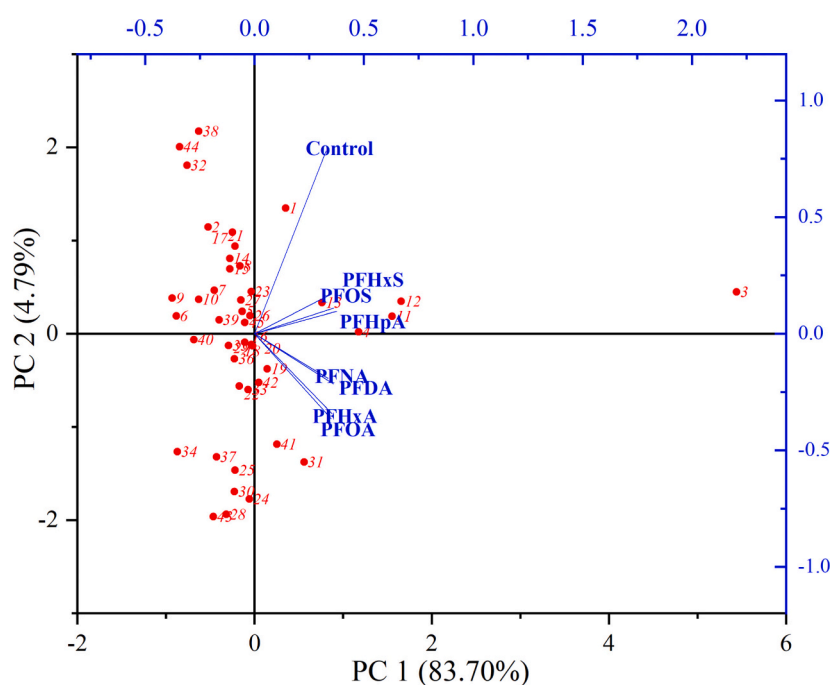
The hierarchical cluster dendrogram (Fig. 8b) further supports the PCA results by grouping PFAS treatments according to similarity in metabolic response profiles using Euclidean distance and complete linkage. PFHxA and PFOA formed a distinct cluster, followed by PFNA and PFDA, while PFHxS and PFOS clustered together, reflecting their shared sulfonate functionality and structural resemblance. The control formed an independent branch, underscoring its clear metabolic distinction from all PFAS-exposed groups.

As this study was designed as an exploratory metabolomic investigation, metabolite identification is reported at the putative level based on accurate m/z and fragment ion matching. Future targeted work will include MS/MS confirmation, isotopically labelled internal standards, and spike-recovery validation to further enhance analytical confidence. Overall, the combined analysis demonstrates that PFAS exposure significantly altered the metabolic profile of *C. minutissima*.

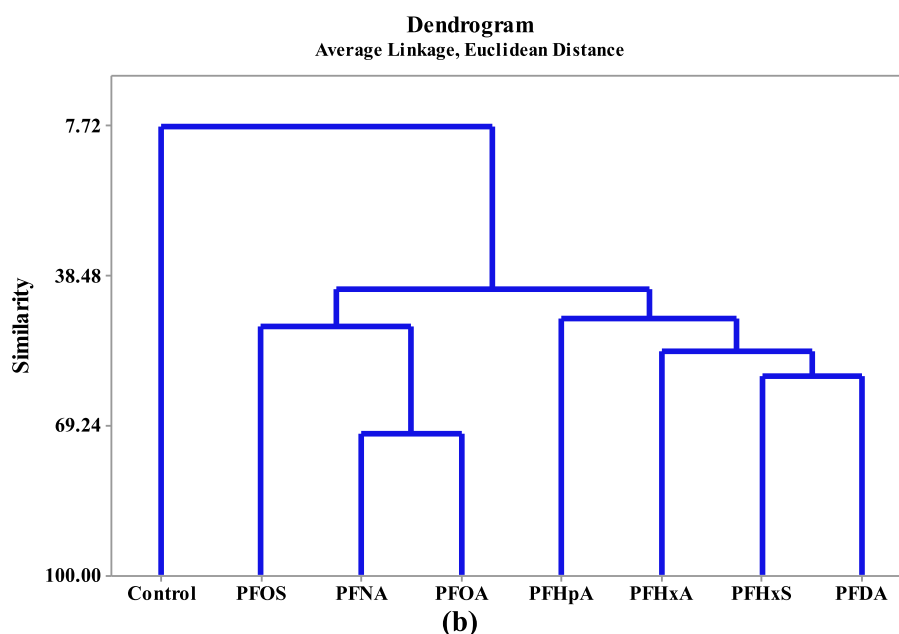
3.8. Structure-toxicity relationships of PFAS compounds

3.8.1. Chain length-dependent toxicity

The higher toxicity of long-chain PFAS is mainly due to their low water solubility, high fat solubility, and strong ability to bind with proteins. These properties allow them to accumulate in fatty tissues and



(a)



(b)

Fig. 8. (a) PCA biplot. Red dots and numbers (1–45) represent individual metabolites included in the PCA analysis; (b) Dendrogram of metabolites of microalgae under different PFAS stress.

form stable complexes in the body, even though they enter cells less easily than short-chain PFAS. In contrast, short-chain PFAS are more water-soluble, have lower molecular weight, and are more easily metabolized and excreted from the body [57,58,59]. The present findings demonstrate that medium- to long-chain PFAS exhibit stronger inhibitory effects on growth compared to short-chain microalgae-treated groups. This trend is consistent with previous reports showing that increased carbon chain length enhances hydrophobicity, membrane

affinity, and bioaccumulation potential, thereby intensifying cellular disruption [56]. Similarly, lipid accumulation generally increased with perfluoroalkyl chain length among perfluorocarboxylic acids. PFDA exhibited the highest lipid content compared to the short-chain PFHxA (C6) resulted in the lowest lipid accumulation. Furthermore, long-chain PFAS induced more pronounced effects and thus tend to cluster separately from short-chain analogues, indicating greater metabolic disruption. Numerous studies have reported in literature that long-chain PFAS

induce pronounced physiological stress across a wide range of aquatic organisms, including fish, daphnids, and aquatic plants [38].

3.8.2. Influence of functional groups

PFAS toxicity also varies depending on their functional groups. For example, perfluoroalkane sulfonic acids (PFSA) generally exhibit higher toxicity than perfluoroalkyl carboxylic acids (PFCAs) with the same carbon chain length. The sulfonic acid group ($-\text{SO}_3\text{H}$) is more polar and chemically stable, making PFSA more resistant to metabolic degradation and excretion. As a result, they tend to accumulate more readily in organisms and cause stronger toxic effects [60]. For instance, PFOS shows significantly higher toxicity to *Chlorella* than PFOA [35]. Previous studies further suggest that functional groups with greater lipophilicity and stronger protein-binding affinity are associated with increased biotoxicity [61,62]. Similarly, PFBS has been reported to exert higher toxicity toward *Chlorella* compared to FBFA [63].

In the present study, the sulfonated compound PFOS exhibited greater toxicity, reflected by the lowest growth ranking, a pronounced decline in photosynthetic efficiency (F_v/F_m), and a significant reduction in ROS scavenging capacity, indicating elevated oxidative stress. Furthermore, perfluorosulfonates, including PFHxS (C6) and PFOS (C8), promoted markedly higher lipid accumulation, showing an approximate 30.7% increase relative to the control. These observations are in agreement with previous findings that F_v/F_m is more sensitive to PFOS than to PFOA exposure highlighting differential toxicity among PFAS compounds [35].

4. Conclusion

The present findings clearly demonstrate that PFAS cannot be considered a uniform class of contaminants; rather, they exert compound-specific biological effects driven by variations in molecular structure, particularly carbon chain length and functional groups.

Among the tested PFAS compounds on green microalgae such as *Chlorella minutissima*, PFHxS and PFDA maintained comparatively higher F_v/F_m values and growth rates, underscoring differential toxicity patterns. Notably, the short-chain PFHxS treatment exhibited the most favourable growth performance, along with a slight enhancement in chlorophyll-a concentration (11.64 ± 0.234) and photosynthetic efficiency (F_v/F_m : 0.685 ± 0.005) relative to the control. Conversely, the medium-chain PFOS treatment ranked lowest in growth performance and showed substantial reductions in protective pigments and antioxidant capacity. PFOS also induced the most pronounced inhibitory effects on cellular growth and PSII photochemical efficiency. This was accompanied by marked antenna expansion and carotenoid accumulation, reflecting severe photophysiological stress. Biochemical composition shifted markedly under PFAS stress. While control microalgae cultures maintained a balanced macromolecular profile, long-chain PFAS such as PFDA promoted the highest lipid accumulation, accompanied by reduced protein content. The consistent lipid buildup across treatments suggests a stress-induced metabolic change, potentially mediated by oxidative imbalance and impaired photosynthetic function. Multivariate analysis further confirmed distinct metabolic fingerprints associated with PFAS chain length and functional group characteristics. By integrating oxidative stress with biochemical and metabolomic endpoints, this study provides evidence that even environmentally relevant concentrations of PFAS can disrupt algal metabolic homeostasis, thereby posing potential risks to aquatic ecosystem stability.

CRediT authorship contribution statement

Jerin James: Methodology, Data curation, Writing – original draft. **Bhawna Bisht:** Data curation, Writing – review & editing. **Alexey A. Dmitriev:** Data curation, Writing – review & editing. **Manisha Nanda:** Writing – review & editing. **Afzal Hussain:** Writing – review & editing. **Mikhail S. Vlaskin:** Writing – review & editing. **Monu Verma:** Data

curation, Writing – review & editing. **Hyunook Kim:** Data curation, Writing – review & editing. **P.K. Chauhan:** Writing – review & editing, Analysis. **Vinod Kumar:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cej.2026.175970>.

Data availability

Data will be made available on request.

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