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Nanoplastic exposure inhibits growth, photosynthetic pigment synthesis and oxidative enzymes in microalgae: A new threat to primary producers in aquatic environment

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ABSTRACT

The acute toxicity of graded concentrations of polysterene nanoplastic (PS NPs) spheres (Size 0.1 μm) was evaluated to ascertain the effects of NPs on growth, vital photosynthetic pigments, protein and oxidative stress enzymes. The findings show that PS NPs inhibited the growth of microalgae (*Chlorella vulgaris* and *Spirulina* (*Arthrospira*) *platensis*) in a dose-dependent manner. The growth inhibition percentage reached 40.12 % for *C. vulgaris* and 42.57 % for *S. platensis*, compared to the control. Additionally, pigment content decreased by 31.62 % to 35.06 %, while protein content dropped by 37.27 % to 48.48 % of both the tested microalgae as the concentration of PS NPs in the medium increased. The oxidative stress created by PS NPs was evident from an increase in catalase and peroxidase activity. The findings conclusively endorse that NPs pollution in the aquatic environment will disrupt the functioning of ecosystems through its detrimental effects on microalgae forming the base of the food chain and supporting the successive trophic levels in the aquatic environment. This research will give a deeper insight into the ecotoxicological impacts of NPs in aquatic environments and the baseline information will be helpful in developing an effective strategy for mitigation of plastic pollution with a greater emphasis on nanoplastics.

1. Introduction

With the increasing population, plastic has become one of the most ubiquitous and persistent environmental pollutants. Since the 1950s, global plastic production has skyrocketed from 1.5 million metric tons to 400.3 million metric tons by 2022 (Statista, 2023). It may reach 33 billion tons by 2050 (Rochman et al., 2013). Larger plastics, under a variety of environmental forces, including UV radiation, wind action, wave action and microbial activity, are broken down into smaller particles such as microplastics (MPs) (< 5 mm) (NOAA, 2008) and nanoplastics (NPs) of particle size < 100 nm (Galloway et al., 2017) or < 1 μm (Gigault et al., 2018). MPs and NPs have been detected in territorial water (Güven et al., 2017), rivers (Wang et al., 2017; Sarkar et al., 2019), tidal creeks (Robin et al., 2020), seawaters (Sunitha et al., 2021), coastal areas (Karthik et al., 2018), snow (Aves et al., 2022) and even in clouds (Xu et al., 2023).

NPs and MPs have garnered significant attention from the scientific community due to their ecotoxicological risks to aquatic organisms. These particles threaten the health of exposed aquatic species, impacting them across various trophic levels, from lower to higher. Ecotoxicological studies have shown that MPs and NPs negatively impact various aquatic organisms, including plants (Xiao et al., 2022), fish (Barría et al., 2020), crustaceans (Li et al., 2022), and aquatic microbes (Hao et al., 2023). It causes genotoxic (Brandts et al., 2022), cytotoxic (Kaur et al., 2022), neurotoxic (Van Tran et al., 2023), immunotoxic (Kim et al., 2021), biochemical (Guerrera et al., 2021), and haematological alterations (Kim et al., 2021) in exposed organisms. These tiny plastics have also been shown to negatively affect aquatic plants by altering their morphological and biochemical parameters, including frond and root size, plant growth, chlorophyll content, and malondialdehyde levels. Additionally, they have been reported to adsorb onto plant surfaces (Ge et al., 2021; Ceschin et al., 2023).

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Among the toxicity studies of NPs studied so far are focused on aquatic feeding animals, with scarce information on microalgae. Microalgae, form the base of aquatic food webs and are crucial for oxygen production, as well as nitrogen and phosphorus biogeochemical cycles (Xiang et al., 2023). Even minor disruptions to these organisms can have significant impacts on the entire ecosystem. Therefore, the effects of nanoplastics on microalgae deserve greater attention. Furthermore, the toxic effects of nanoplastics on algae remain inadequately explored in current research. Few studies have demonstrated that tiny plastics can impede growth, reduce photosynthetic efficiency, and cause other toxic effects on microalgae (Prata et al., 2018; Mao et al., 2018; Li et al., 2020). Gao et al. (2021), in a meta-analysis study, demonstrated that MPs and NPs significantly affect various aspects of aquatic microalgae, including growth inhibition, photosynthesis, pigment production, and metabolism, with the concentration required to induce 50 % growth inhibition (EC_{50}) reported to be above 25 mg L^{-1}. However, some studies have also shown the opposite results. In a study by Sjollema et al. (2016), the marine flagellate *Dunaliella tertiolecta* exposed to MPs at a high concentration of 250 mg L^{-1} showed reduced growth rate, though photosynthetic efficiency remained unaffected. Canniff and Hoang (2018), found that plastic microbeads could benefit microalgae growth by serving as substrates for microalgae. Further research is urgently needed in light of the contradictory findings and the limited number of microalgae species tested. In addition, Yan et al. (2021), demonstrated that NPs are more hazardous than MPs. Though NPs are more toxic than MPs, till now, more focus has been given to MPs. Assessment of the effect of NPs is a relatively underexplored area.

Therefore, such studies can address the critical knowledge gap by evaluating the effects of polystyrene nanoplastics (PS NPs) on two ecologically significant microalgae species, *Chlorella vulgaris* and *Spirulina platensis*. By examining key parameters such as growth, photosynthesis, protein content, and oxidative stress responses, alongside high-resolution surface morphology by Scanning Electron Microscopy (SEM) and biochemical profiling by Fourier Transform Infrared Spectroscopy (FTIR), this research provides impactful insights into the physiological and biochemical disruptions caused by nanoplastics exposure. The findings of these studies could offer valuable insights into the ecological risks posed by NPs, emphasizing their potential to disrupt aquatic ecosystems.

2. Material and methods

In the current study all the media and consumables were procured from HiMedia Laboratories Pvt Ltd, Mumbai, India and Sigma-Aldrich, India. The instruments used included Sedgwick-Rafter cell counter (Partex Products, Mumbai), UV–visible spectrometer (UV-3092, LABINDIA, India), scanning electron microscope (SEM, FEI Quanta 200) and 3000 Hyperion Microscope with Vertex 80 FTIR System (Bruker, Germany).

2.1. Culture conditions

Two microalgal species, *C. vulgaris* and *S. platensis,* were selected for the toxicity study. The algae were received from the Algal Biotechnology Lab, Aquatic Environmental Management Discipline of ICAR–Central Institute of Fisheries Education (ICAR-CIFE), Mumbai, India. *C. vulgaris* was cultured in BG-11 medium and *S. platensis* was cultured in modified CIFE medium. Both the microalgae were cultured separately in a 2 L erlenmeyer flask at 25 ± 2 °C under 3500 ± 200 lux light intensity provided by cool-white fluorescent lamps with a 12:12 hour light/dark cycle and manually shaken three times a day (morning, mid-day and evening) to prevent the settlement of algae. Microalgae were periodically transferred into a new medium to maintain the culture.

C. vulgaris and *S. platensis* were selected for this study due to their role as critical biological indicators in aquatic environments. As primary producers, they are essential for nutrient cycling and exhibit traits such as sensitivity to pollutants, high-temperature tolerance, and growth in low-nutrient conditions. Additionally, their simple cell cycles, photosynthetic activity, and metabolic pathways closely resemble those of higher plants (Janssen et al., 2022; Panahi et al., 2016).

2.2. Toxicity experiment

2.2.1. Test chemical and preparation of working solution

This study utilized analytical grade polystyrene nanoplastics (PS NPs) with a size of 0.1 μm, sourced from Sigma-Aldrich, India. Double-distilled ultrapure water was used as the suspension medium to prepare the stock solution. The concentrations used in our study were selected to simulate potential future scenarios based on current trends in plastic production. For the toxicity study, working solution of 1, 5, 10, 50, and 100 mg L^{-1} concentrations were prepared. The selected concentrations simulate potential future scenarios, reflecting the rapid rise in plastic production and NPs release, which could reach levels up to 10^{14} times higher than current microplastics (Besseling et al., 2019). While exceeding present levels, these worst-case scenarios enable robust risk assessment for primary producers like microalgae, aligning with ecotoxicological studies to understand potential environmental impacts.

2.2.2. Experimental setup

The algal growth inhibition test was conducted based on the OECD guidelines 201 (OECD, 2011), with minor modifications when required. For the test, an inoculum of *C. vulgaris* and *S. platensis* was pre-cultured in respective sterilized growth media two days before the actual study to ensure that the microalgae cells were in the exponential growth phase. The initial cell density was maintained at 1.4 × 10^6 cells mL^{-1}. Algal cells were inoculated in their respective media containing five different concentrations of PS NPs, i.e., 1, 5, 10, 50 and 100 mg L^{-1}. Control cultures were kept without the addition of PS NPs. The experiment was conducted under controlled photoautotrophic conditions, with each test concentration performed in triplicate. A 96 h growth cycle was followed to ensure the microalgae were in the exponential growth phase during the experiment. During the study, samples were collected every 24 h to determine the specific growth rate, pigment content, protein content and antioxidant enzymes of both the algae.

2.2.3. Estimation of growth parameters

The cell numbers of both algae were determined using a Sedgwick-Rafter cell counter (Partex Products, Mumbai). The specific growth rate (SGR) (day^{-1}) of the microalgae was then calculated using the formula outlined in the OECD guidelines (OECD, 2011).

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{T_t - T_0}$$

Where, N_0 is the cell numbers at time T_0 and N_t is the cell numbers at time T_t. The following equation was used to determine the doubling time (t_d) (day) of microalgae (Levasseur et al., 1993).

$$t_d = \frac{\ln(2)}{\mu}$$

Where, μ is the SGR of microalgae.

2.2.4. Estimation of growth inhibition rate

The growth inhibition rate (IR%) was calculated based on the estimated SGR of both the microalgae. Treatment SGR was compared with the control SGR on a particular day and IR% was calculated using the following formula (Xi et al., 2019).

$$IR\% = \frac{\mu_c - \mu_t}{\mu_c} \times 100$$

Where, μ_c and μ_t are the SGR of the control and treatment groups, respectively.

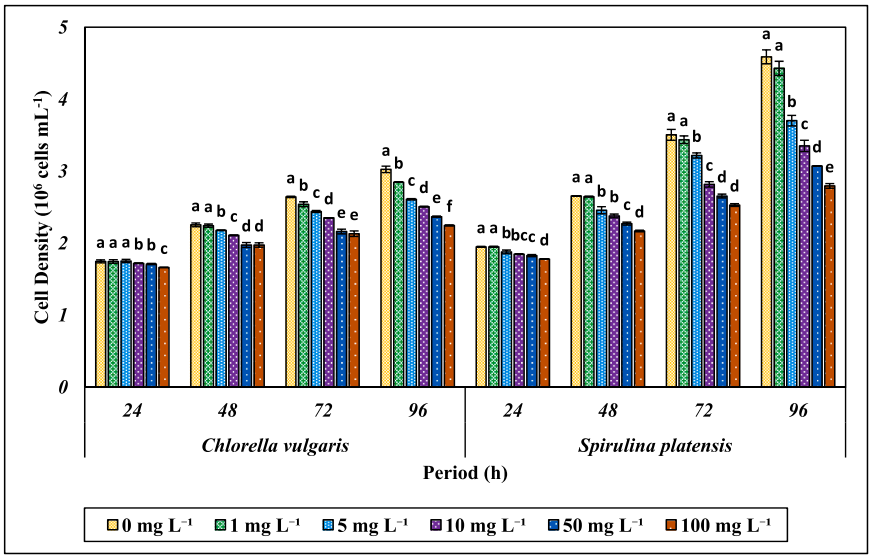

Fig. 1. Effect of PS NPs (1 to 100 mg L^{-1}) on cell density (10^6 cells mL^{-1}) of *C. vulgaris* and *S. platensis* at different times (24, 48, 72 and 96 h). Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

2.2.5. Estimation of pigment content

For pigment estimation, 5 mL algal samples, including control, were drawn from each treatment. Algal samples were centrifuged at 8000 rpm for 10 min at 4 °C (Universal 320R, Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was discarded and 5 mL N,N-dimethylformamide (DMF) was added to the remaining pellets and well mixed. The suspension was incubated overnight at room temperature under dark conditions. Post incubation, the suspension was centrifuged and the collected supernatant was subjected to spectrometry. To determine the chlorophyll and carotenoid content, optical densities were measured at 447 nm, 647 nm and 664 nm wavelengths using a double beam UV-visible spectrometer (UV-3092, LABINDIA, India). The pigments such as chlorophyll a (Chl-a) (Moran, 1982), chlorophyll *b* (Chl-b) (Moran, 1982), total chlorophyll (EPA, 1994) and carotenoids (Chamovitz et al., 1993) were calculated using the following formulas.

$$\text{Chl} - \text{a}\ (\mu\text{g mL}^{-1}) = \text{OD}_{664} \times 11.92$$

$$\text{Chl} - \text{b}\ (\mu\text{g mL}^{-1}) = -5.6 \times \text{OD}_{664} + 23.26 \times \text{OD}_{647}$$

$$\text{Total chlorophyll}\ (\mu\text{g mL}^{-1}) = \text{Chl} - \text{a} + \text{Chl} - \text{b}$$

$$\text{Carotenoid}\ (\mu\text{g mL}^{-1}) = [\text{OD}_{461} - (0.046 \times \text{OD}_{664})] \times 4$$

2.2.6. Estimation of protein content

Protein estimation was done by Lowry et al. (1951), method with Bovine serum albumin (BSA) was used as standard and absorbance of each sample was measured at 660 nm using a double beam UV-visible spectrometer.

2.2.7. Preparation of cell free extract of microalgae for estimation of oxidative enzymes

In every 24 h, 10 mL of algal sample was collected from each treatment and control. Samples were centrifuged at 8000 rpm for 15 min at 4 °C to harvest the algal cells. The cells were washed with 0.1 M potassium phosphate buffer (pH 6.5) and resuspended in the same buffer. The samples were subjected to an ultrasonic homogenizer (Hielscher UP200S ultrasonic processor, Germany) to sonicate the cells (Sabatini et al., 2009). Sonication was performed for 5 min, with 30-second pulses followed by 10-second breaks while maintaining a low temperature throughout the process. After sonication, the samples were centrifuged, and the resulting supernatant was collected as a cell-free enzyme extract.

2.2.8. Estimation of oxidative enzymes

Catalase (CAT) enzyme activity was determined using Aebi et al. (1984), method with slight modifications. The spectrophotometric absorbance decrease, resulting from H_2O_2 decomposition, was measured at 240 nm (A_{240} nm) and CAT activity was then calculated using a molar extinction coefficient (ε) of 45.2 $Mm^{-1}cm^{-1}$. One CAT activity unit (U) is the amount of enzyme required to decompose one mole of H_2O_2 mg^{-1} soluble protein per minute at 30 °C. Peroxidase activity (POD) was determined using Andrade et al. (2006), method. The formation rate of oxidized guaiacol or tetraguaiacol was monitored by measuring the spectrophotometric absorbance at 470 nm (A_{470} nm) and calculated using a molar extinction coefficient (ε) of 26.6 $mM^{-1}cm^{-1}$. The quantity of enzyme needed to produce one mM of tetraguaiacol per minute was defined as one unit of peroxidase. The oxidative enzyme activities were standardized per milligram of protein content.

2.3. Interaction study between PS NPs and microalgae

2.3.1. Scanning electron microscope (SEM) study

After 96 h, control algal cells and 100 mg L^{-1} PS NPs treated algae were selected for the SEM study. About 5 mL of algal culture was collected and centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was discarded and algal pellets were washed two times to remove the media. The resulting pellet was fixed overnight in 2.5 % glutaraldehyde solution at 4 °C. After fixation, the cells were washed twice using 0.5 M potassium phosphate buffer (pH 7.1). The samples were then dehydrated for 20 min at room temperature using ethanol solution (gradient concentrations: 30, 50, 70, 80, 90, 95 and 100 %), then dried using a critical point drier. Algal cells were mounted on copper stubs and sputter-coated with gold-palladium before observing under a scanning electron microscope (SEM, FEI Quanta 200).

2.3.2. Fourier transform infrared (FTIR) spectroscopy study

FTIR analysis was conducted to examine whether exposure to PS NPs causes alterations in the functional groups of microalgae. A lower concentration (10 mg L^{-1}) treatment and a higher concentration (100 mg L^{-1}) treatment were selected for the study. Algal cells were harvested by centrifugation and the collected pellets were washed three times with 0.5 M potassium phosphate buffer (pH 7.1) and dried. The dried cells

Table 1
Effect of PS NPs (1 to 100 mg L^{-1}) on growth rate (day^{-1}) and doubling time (day) of *C. vulgaris* and *S. platensis* at times 96 h.

Concentration (mg L^{-1})	*C. vulgaris*		*S. platensis*	
	Growth rate (μ) (day^{-1})	Doubling time (Tg) (day)	Growth rate (μ) (day^{-1})	Doubling time (day)
0	0.186 ± 0.002[a]	3.735 ± 0.040[a]	0.291 ± 0.009[a]	2.382 ± 0.028[a]
1	0.170 ± 0.000[b]	4.068 ± 0.001[b]	0.282 ± 0.001[a]	2.457 ± 0.024[a]
5	0.149 ± 0.000[c]	4.662 ± 0.026[c]	0.237 ± 0.005[b]	2.922 ± 0.061[b]
10	0.139 ± 0.000[d]	4.998 ± 0.020[d]	0.212 ± 0.005[c]	3.266 ± 0.088[c]
50	0.124 ± 0.000[e]	5.570 ± 0.036[e]	0.191 ± 0.000[d]	3.635 ± 0.023[d]
100	0.111 ± 0.001[f]	6.237 ± 0.046[f]	0.167 ± 0.002[e]	4.149 ± 0.073[e]

Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

were subjected to an FTIR study using a 3000 Hyperion Microscope with Vertex 80 FTIR System (Bruker, Germany). FTIR spectroscopy was performed on spectra ranging from 4000 to 400 cm^{-1} with a data interval of 1 cm^{-1} and a resolution of 4 cm^{-1}.

2.4. Statistical analysis

The data were analysed using SPSS software, version 22.0 for Windows. The obtained data were initially assessed for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results indicated that all datasets followed a normal distribution, allowing for further statistical analysis. A one-way ANOVA followed by Duncan post hoc test was conducted to identify significant differences, with a p value of < 0.05. The experiment was performed in triplicate, and the results are presented as the mean ± standard error of the mean. Pearson correlation and FTIR data were analyzed using Origin Pro software (Electronic Arts Inc., California, United States).

3. Result and discussion

3.1. Effect of PS NPs on the growth of microalgae

After exposure to PS NPs, a significant ($p < 0.05$) decrease in cell density was observed compared to the control (Fig. 1). After 96 h, cell numbers were reduced by 25.76 % and 39.06 % in 100 mg L^{-1} PS NPs treatments, for *C. vulgaris* and *S. platensis* respectively, compared to the control. Similarly, the growth rate was also observed to be decreased significantly ($p < 0.05$) throughout the study for both the tested microalgae (Table 1). As the growth rate declined, the doubling time or generation time showed a significant increase ($p < 0.05$) from the first day of exposure. The doubling time of microalgae itself almost doubled from the control at the highest PS NPs concentration (Table 1). A similar result was recorded in the prior work by Zhang et al. (2017), where they observed decreased cell density of *Skeletonema costatum* at higher concentrations of PVC (Polyvinyl chloride) MPs treatment. Other studies also reported that smaller plastics, i.e., MPs and NPs, reduced microalgae growth (Prata et al., 2018; Mao et al., 2018; Zhao et al., 2019), which agreed with the findings of the current investigation.

3.2. Acute growth inhibition of PS NPs on microalgae

The growth inhibition rate (IR%) was determined by comparing the growth rate (μ) of the microalgae population across different treatments to that of the control. A significant difference ($p < 0.05$) in IR% was observed for both algal species at varying concentrations of PS NPs (Fig. 2). The findings indicated that *S. platensis* exhibited the highest growth inhibition percentage at 100 mg L^{-1} treatment, reaching 42.57 %, while *C. vulgaris* showed a slightly lower inhibition percentage of 40.12 %. Our findings are in agreement with previous study by Yang et al. (2020), reporting 44.50 % growth inhibition of *C. pyrenoidosa* at 100 mg L^{-1} treatment. To facilitate further understanding and comparison, Table 2 consolidates growth inhibition data from various studies. This highlights the variability in growth inhibition rates across different microalgae species, as demonstrated by available ecotoxicological studies. There is a possibility that the composition and synthesis processes of MPs/NPs plastics may have contributed to their varied effects on microalgae. Furthermore, there is a high possibility that the NPs were unevenly distributed within the testing medium (Bellingeri et al., 2019). The lack of homogeneity of these particles may result from particle behavior itself, i.e., the fact that they do not behave as soluble chemicals under standardized toxicity tests. The behavior of NPs differs

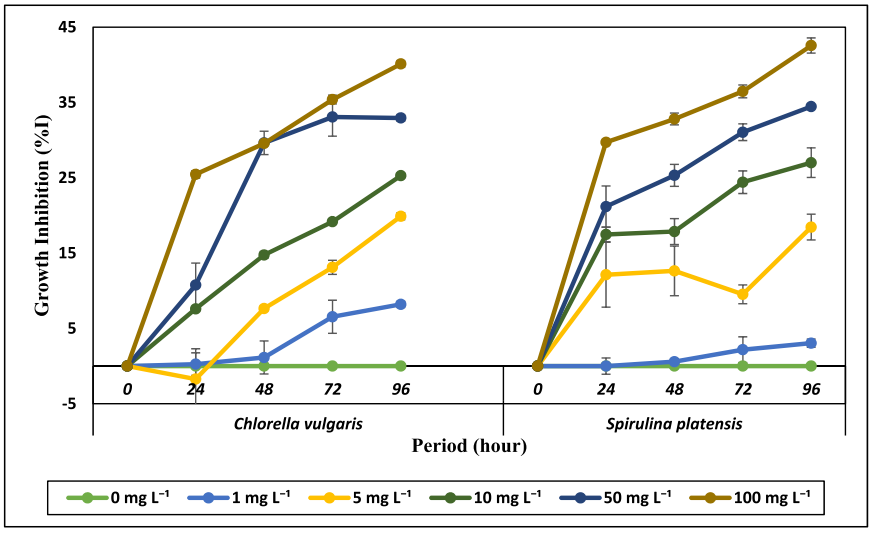

Fig. 2. Effect of PS NPs (1 to 100 mg L^{-1}) on growth inhibition (IR%) of *C. vulgaris* and *S. platensis* at different times (24, 48, 72 and 96 h). Data shown in mean ± SE of three replicate ($n = 3$).

Table 2
Effect of various types MPs and NPs on microalgae growth inhibition percentage (IR%) reported by several authors.

MPs and NPs	Concentration (mg L^{-1})	Microalgae	Growth inhibition (IR %)	References
PS	80	*Chaetoceros meulleri*	37.9	Wang et al., 2021
PVC	100	*Skeletonema costatum*	36.8	Zhu et al., 2019
PS	100	*Microcystis aeruginosa*	60.16	Zheng et al., 2021
PVC	100	*Chlamydomonas reinhardtii*	34	Wang et al., 2020
	200		49.1	
PS	100	*Chlamydomonas reinhardtii*	48.2	Li et al., 2020
PS	100	*Chlorella pyrenoidosa*	38.5	Mao et al., 2018
PP HDPE	250	*Acutodesmus obliquus*	37.7 41.6	Ansari et al., 2021
PS	100	*C. vulgaris*	40.12	Current study
PS	100	*S. platensis*	42.57	Current study

(PS: Polystyrene, PVC: Polyvinyl chloride, PP: Polypropylene, HDPE: High density polyethylene).

from that of chemicals dissolved in a test medium since they undergo many processes and transformations, including agglomeration and settling (Peijnenburg et al., 2015).

3.3. Effect of PS NPs on pigment content of microalgae

Pigment concentrations serve as essential indicators of plant growth, offering a swift and non-invasive approach to assess toxicity in plants. Contaminants interacting with the light-processing complex in microalgae can disrupt the electron transport chain in photosynthesis (Aves et al., 2022). The current study used Chl-a, Chl-b, total chlorophyll and carotenoid content as a growth indicator. A significant difference ($p < 0.05$) in pigment content was observed in all the treatments as compared to the control of *C. vulgaris* and *S. platensis* (Tables 3 and 4). After 96 h of exposure to PS NPs, the highest concentrations of Chl-a and Chl-b were observed in the control groups of both algae, while the treatment groups exhibited a dose- and time-dependent reduction in chlorophyll content. A similar pattern was seen in total chlorophyll content, with the highest level (17.43 μg mL^{-1}) recorded in the control group, which gradually decreased as the concentration of PS NPs increased. Maximum inhibition in carotenoid content was found 31.62 % and 35.06 % in *C. vulgaris* and *S. platensis*, respectively, in 100 mg L^{-1} treatment during the 96 h study.

Findings of various other studies supported our findings, for instance, Wu et al. (2019), found that Chl-a content of *C. pyrenoidosa* was inhibited by 31.08 % at 100 mg L^{-1} PP (polypropylene) treatment. The chlorophyll content of *Skeletonema costatum* was negatively affected by MPs in the 50 mg L^{-1} PVC treatment during acute trials (Zhang et al., 2017). The decrease in Chl-a content could be due to one of the following reasons. First, microalgae and NPs would form hetero-aggregates of different sizes, which prevent microalgae from multiplying (Long et al., 2017; Lagarde et al., 2016). Second, the build-up of intracellular reactive oxygen species (ROS) inside the cells, damages chloroplasts and prevents chlorophyll synthesis (Geoffroy et al., 2003; Wu et al., 2019). Third, NPs may have a shading effect on microalgae cells preventing microalgae from accessing light and hindering their ability to photosynthesis (Long et al., 2015; Garrido et al., 2019). In addition, NPs can adversely affect thylakoids and result in reduced photosynthesis rate. Photosynthesis can be inhibited by reduced electron transport rates and impaired electron donor side of PSII under NPs treatment (Mao et al., 2018).

Table 3
Effect of PS NPs (1 to 100 mg L^{-1}) on pigment content of *C. vulgaris* at different times (24, 48, 72, 96 h).

NPs(mg L^{-1})	Chlorophyll a (μg mL^{-1})				Chlorophyll *b* (μg mL^{-1})				Total Chlorophyll (μg mL^{-1})				Carotenoid (μg mL^{-1})			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	5.326 ± 0.100^{a}	8.207 ± 0.007^{a}	9.385 ± 0.100^{a}	14.206 ± 0.032^{a}	1.537 ± 0.020^{a}	2.342 ± 0.140^{a}	2.897 ± 0.093^{a}	3.227 ± 0.107^{a}	6.863 ± 0.113^{a}	10.549 ± 0.150^{a}	12.282 ± 0.005^{a}	17.433 ± 0.090^{a}	1.857 ± 0.019^{a}	2.940 ± 0.031^{a}	3.357 ± 0.001^{a}	4.532 ± 0.008^{a}
1	5.116 ± 0.017^{b}	7.007 ± 0.131^{b}	8.872 ± 0.070^{b}	13.354 ± 0.052^{b}	1.586 ± 0.022^{a}	2.187 ± 0.100^{a}	2.949 ± 0.020^{a}	2.877 ± 0.006^{b}	6.703 ± 0.040^{a}	9.193 ± 0.034^{b}	11.822 ± 0.083^{b}	16.231 ± 0.050^{b}	1.781 ± 0.000^{b}	2.489 ± 0.051^{b}	3.322 ± 0.000^{b}	4.299 ± 0.009^{b}
5	5.084 ± 0.003^{b}	6.773 ± 0.004^{b}	8.682 ± 0.014^{c}	12.377 ± 0.280^{c}	1.327 ± 0.006^{b}	1.482 ± 0.100^{b}	2.702 ± 0.013^{b}	2.689 ± 0.009^{bc}	6.411 ± 0.006^{b}	8.255 ± 0.005^{c}	11.383 ± 0.001^{c}	15.065 ± 0.285^{c}	1.772 ± 0.000^{b}	2.469 ± 0.008^{b}	3.212 ± 0.001^{c}	4.126 ± 0.032^{c}
10	5.051 ± 0.009^{b}	6.529 ± 0.070^{c}	8.374 ± 0.020^{d}	11.403 ± 0.151^{d}	1.294 ± 0.025^{b}	1.471 ± 0.059^{b}	2.425 ± 0.006^{c}	2.499 ± 0.020$_{c}$	6.345 ± 0.026^{b}	8.001 ± 0.048^{d}	10.799 ± 0.023^{d}	13.901 ± 0.156^{d}	1.748 ± 0.001^{c}	2.356 ± 0.001^{c}	2.885 ± 0.021^{d}	3.633 ± 0.125^{d}
50	4.843 ± 0.006^{c}	6.559 ± 0.004^{c}	7.980 ± 0.063^{e}	10.523 ± 0.092^{e}	1.223 ± 0.004^{c}	1.189 ± 0.010^{c}	2.276 ± 0.009^{d}	2.261 ± 0.020^{d}	6.066 ± 0.005^{c}	7.748 ± 0.014^{e}	10.256 ± 0.054^{e}	12.784 ± 0.080^{e}	1.716 ± 0.002^{d}	2.267 ± 0.000^{d}	2.778 ± 0.003^{e}	3.373 ± 0.001^{e}
100	4.810 ± 0.007^{c}	6.276 ± 0.100^{d}	7.235 ± 0.040^{f}	9.621 ± 0.050^{f}	1.076 ± 0.032^{d}	1.055 ± 0.050^{c}	1.948 ± 0.014^{e}	1.873 ± 0.114^{e}	5.886 ± 0.400^{d}	7.331 ± 0.050^{f}	9.183 ± 0.028^{f}	11.494 ± 0.151^{f}	1.676 ± 0.002^{e}	2.154 ± 0.027^{e}	2.720 ± 0.013^{f}	3.099 ± 0.020^{f}

Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

Table 4
Effect of PS NPs (1 to 100 mg L^{-1}) on pigment content of *S. platensis* at different times (24, 48, 72, 96 h).

NPs (mg L^{-1})	Chlorophyll a (μg mL^{-1})				Carotenoid (μg mL^{-1})			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	4.434 ± 0.070^{a}	6.929 ± 0.059^{a}	9.147 ± 0.069^{a}	11.805 ± 0.098^{a}	1.308 ± 0.006^{a}	1.794 ± 0.012^{a}	2.352 ± 0.070^{a}	2.795 ± 0.038^{a}
1	4.244 ± 0.024^{b}	6.469 ± 0.016^{ab}	8.765 ± 0.092^{a}	11.475 ± 0.017^{a}	1.221 ± 0.006^{b}	1.680 ± 0.005^{b}	2.309 ± 0.045^{a}	2.715 ± 0.024^{a}
5	4.148 ± 0.025^{bc}	6.039 ± 0.938^{b}	8.193 ± 0.316^{b}	10.835 ± 0.411^{b}	1.208 ± 0.006^{b}	1.597 ± 0.039^{b}	1.992 ± 0.082^{b}	2.283 ± 0.047^{b}
10	4.049 ± 0.031^{c}	5.201 ± 0.142^{c}	7.057 ± 0.028^{c}	9.488 ± 0.114^{c}	1.170 ± 0.010^{c}	1.417 ± 0.042^{c}	1.768 ± 0.018^{c}	2.086 ± 0.017^{c}
50	3.834 ± 0.048^{d}	4.736 ± 0.076^{cd}	6.663 ± 0.048^{c}	8.495 ± 0.100^{d}	1.145 ± 0.014^{c}	1.295 ± 0.009^{d}	1.598 ± 0.013^{d}	1.964 ± 0.012^{d}
100	3.703 ± 0.078^{d}	4.379 ± 0.158^{d}	6.163 ± 0.036^{d}	7.831 ± 0.028^{e}	1.156 ± 0.019^{c}	1.170 ± 0.039^{e}	1.528 ± 0.009^{d}	1.858 ± 0.019^{e}

Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

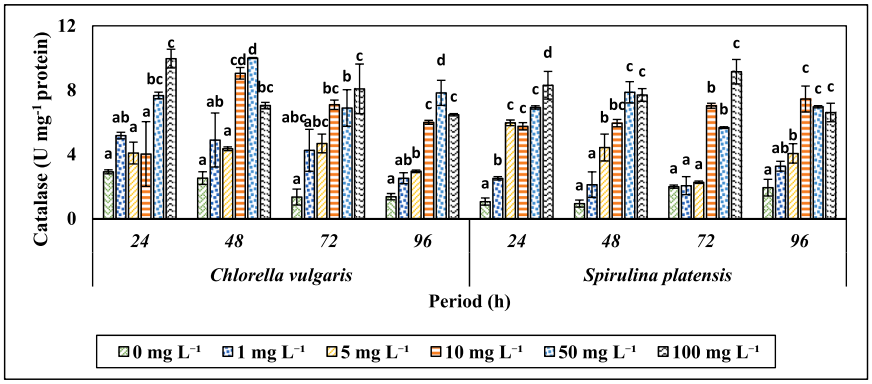

Fig. 3. Effect of PS NPs (1 to 100 mg L^{-1}) on catalase activity (U mg^{-1} protein) of *C. vulgaris* and *S. platensis* at different times (24, 48, 72 and 96 h). Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

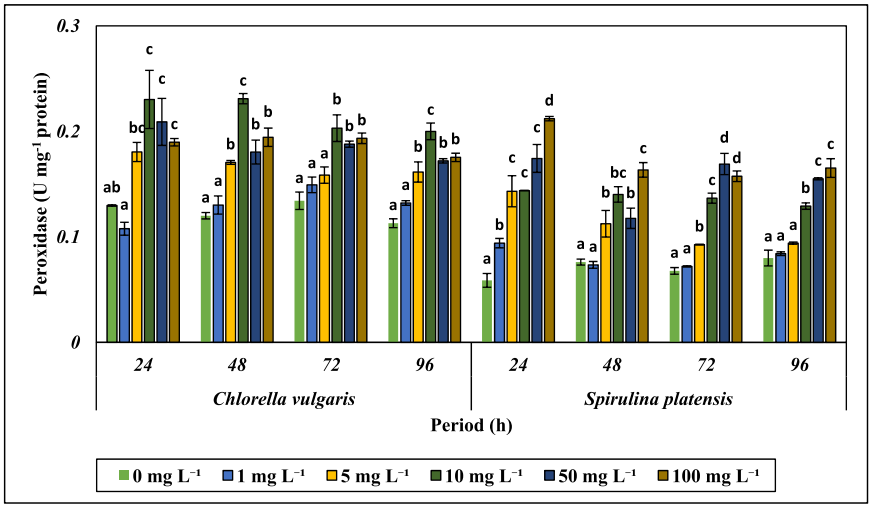

Fig. 4. Effect of PS NPs (1 to 100 mg L^{-1}) on peroxidase activity (U mg^{-1} protein) of *C. vulgaris* and *S. platensis* at different times (24, 48, 72 and 96 h). Data shown in mean ± SE of three replicate ($n = 3$). Different letters indicate significant differences among the treatment groups within the same exposure period at levels of $p < 0.05$.

3.4. Effect of PS NPs on protein content of microalgae

In addition to pigments, protein content was utilized as a biomarker in toxicological studies due to its direct association with the metabolism of organisms (Hu and Wu, 2001). In this research, microalgae exposed to PS NPs exhibited a significant ($p < 0.05$) decrease in protein content after 96 h (Fig. S.1 and S.2). Specifically, after 96 h, protein levels declined by 34.95 % in *C. vulgaris* and by 28.46 % in *S. platensis* at a treatment concentration of 100 mg L^{-1}. This reduction in protein content suggests that normal metabolic pathways may have been disrupted by the concentration of nanoplastics (Xu et al., 2024). Similarly, studies by Sendra et al. (2019) and Yang et al. (2021) reported that exposure to tiny plastics inhibited protein content across all treatments compared to controls. Furthermore, our study confirmed the interaction between PS NPs and microalgal proteins through FTIR spectral analysis, thereby reinforcing our findings.

3.5. Effect of PS NPs on antioxidant enzymes of microalgae

In this study, exposure to PS NPs led to increased catalase (CAT) and

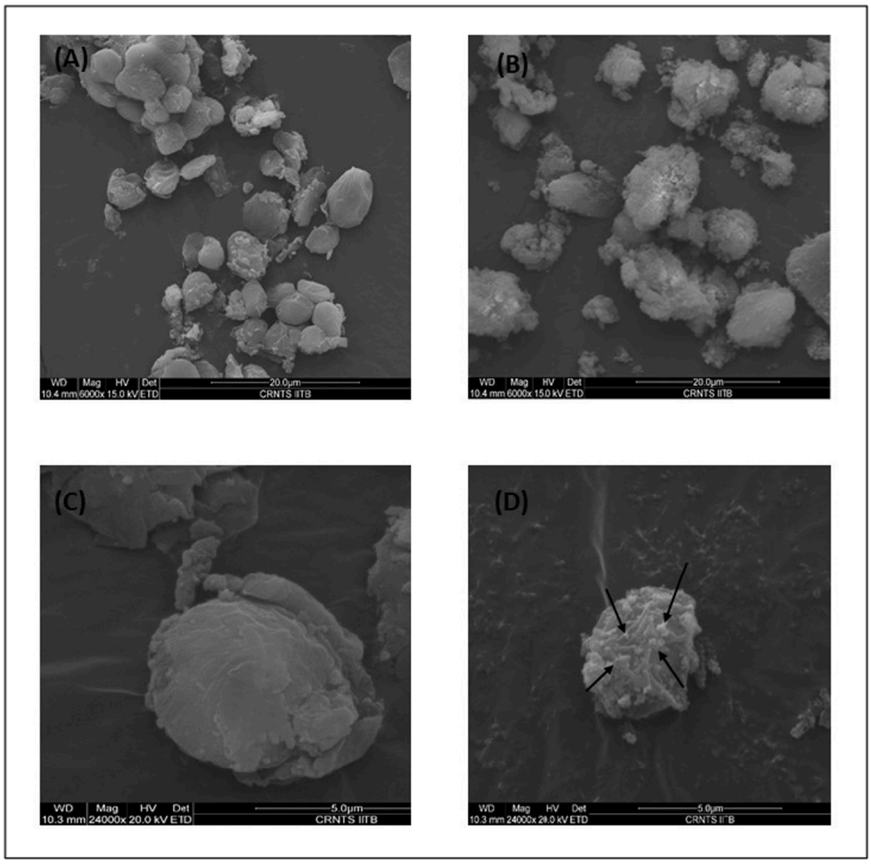

Fig. 5. Scanning electron microscopy analysis of *C. vulgaris* cells after 96 h. (A and C): Control cells. (B and D): 100 mg L^{-1} PS NPs treated cells.

peroxidase (POD) activity in both algae compared to the control. The data revealed that antioxidant enzyme activity increased by up to 4.74 fold compared to the control after 96 h of PS NPs exposure (Figs. 3 and 4). Several other studies have also reported similar findings, where microalgae exhibited increased levels of antioxidant enzymes in response to exposure to MPs and NPs (Yan et al., 2021; Li et al., 2023). Bhattacharya et al. (2010), reported that NPs, induced stress disrupts electron transport, resulting in electron accumulation and elevated ROS production. Excessive ROS can cause lipid peroxidation, overwhelming the intrinsic antioxidant defences and resulting in severe organelle damage. Various antioxidant enzymes such as CAT and POD help mitigate ROS toxicity by scavenging excess ROS (Li et al., 2020; Cao et al., 2015). When algae are in stressed conditions, CAT and POD enzyme is actively elevated and reduce the ROS level to maintain the natural state of algae (Qian et al., 2008; Pandey et al., 2017).

Our investigation also revealed that oxidative enzymatic activity slightly decreased over time. The findings align with Zheng et al. (2021), who observed a decline in SOD levels with prolonged exposure periods. This suggests that NPs might only cause temporary damage to the algae. Alternatively, the higher concentrations of NPs could have disrupted the enzyme synthesis pathway, contributing to this observed trend (Wang et al., 2019).

3.6. Interaction study between PS NPs and microalgae

3.6.1. Morphological changes in the microalgae cell due to PS NPs exposure

To investigate morphological alterations induced by PS NPs, cells of *C. vulgaris* exposed to 100 mg L^{-1} and control treatments were selected for SEM analysis after 96 h. The obtained results are depicted in Fig. 5(A-D). Control algae cells (Fig. 5A) were in normal spherical shape and remained round and plump, which is the characteristic feature of healthy *C. vulgaris*. In contrast, after 96 h, 100 mg L^{-1} treated algae (Fig. 5B) showed uneven, grainy surfaces. Deformation in the algal cells was also observed in the treated cells. Deformation of the cell surface indicates that microalgae likely experienced stress under PS NPs treatment, resulting in excessive ROS production (Yue et al., 2018), which subsequently increased oxidative enzyme activity levels. PS NPs can block the transmission channel between microalgae and external substances, hindering the exchange of substances and energy between inside and outside the cell (Yan et al., 2021). Moreover, in higher magnification (24,000 X), SEM analysis revealed that the control (Fig. 5C) cell surfaces were smooth and clear, whereas PS NPs were observed to be deposited at the cell surfaces of 100 mg L^{-1} treated algal cells (Fig. 5D). This reveals the adherence of PS NPs on the surface of microalgae even after repeated washing during sample preparation. This scenario is analogous to the natural ecosystem, where the adhesion of NPs to algal cells may render disintegration challenging, even in the presence of robust wave and wind actions. This circumstance enhances the likelihood of NPs ascending in the food chain. There has been previous evidence for MPs and NPs adhesion to algal surfaces (Bellingeri et al., 2019; Wang et al., 2021; Khoshnamvand et al., 2021). The extracellular polymeric substances (EPS) produced by microalgae cells might be responsible for this adhesion (Cunha et al., 2019; Song et al., 2020). This phenomenon can result in the accumulation of harmful metabolites within algal cells, inhibition of photosynthesis due to reduced light access or hindered light penetration, decreased oxygen transfer to the cells, ultimately causing adverse effects on algal growth (Bhattacharya et al., 2010; Oukarroum et al., 2012).

3.6.2. Alteration in functional group of microalgae due to PS NPs exposure

The impact of PS NPs on the functional groups of microalgae were analysed using FTIR spectra (Figs. 6 to 8). When purchased PS NPs were subjected to FTIR analysis the characteristics peak was observed at wavelength 698 cm^{-1} (Fig. 8). After exposure to PS NPs for 96 h both the

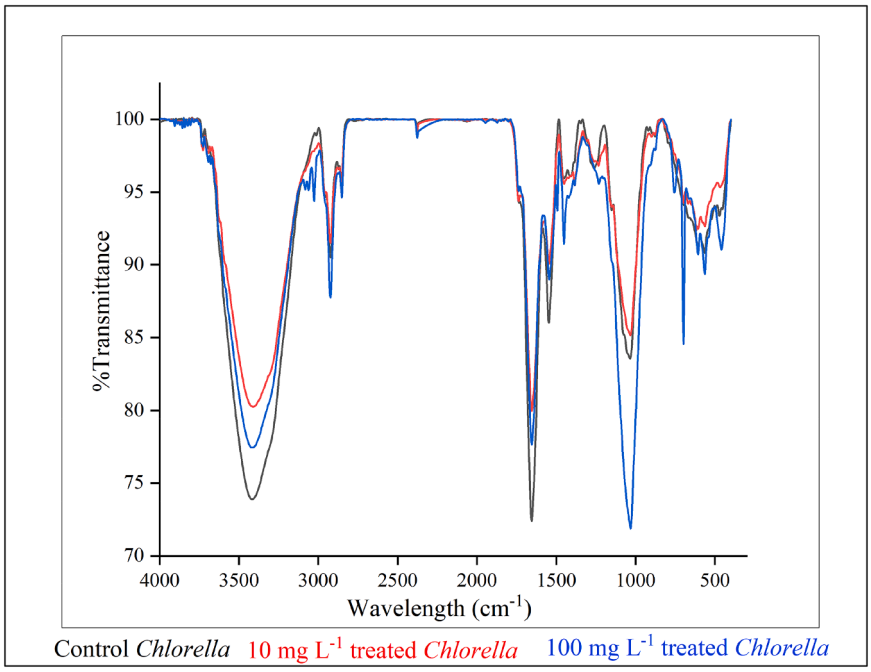

Fig. 6. FTIR spectra of *C. vulgaris* cells after 96 h PS NPs treatment. Black color indicated control cells, red color indicated 10 mg L^{-1} PS NPs treated cells and blue color indicated 100 mg L^{-1} PS NPs treated cells.

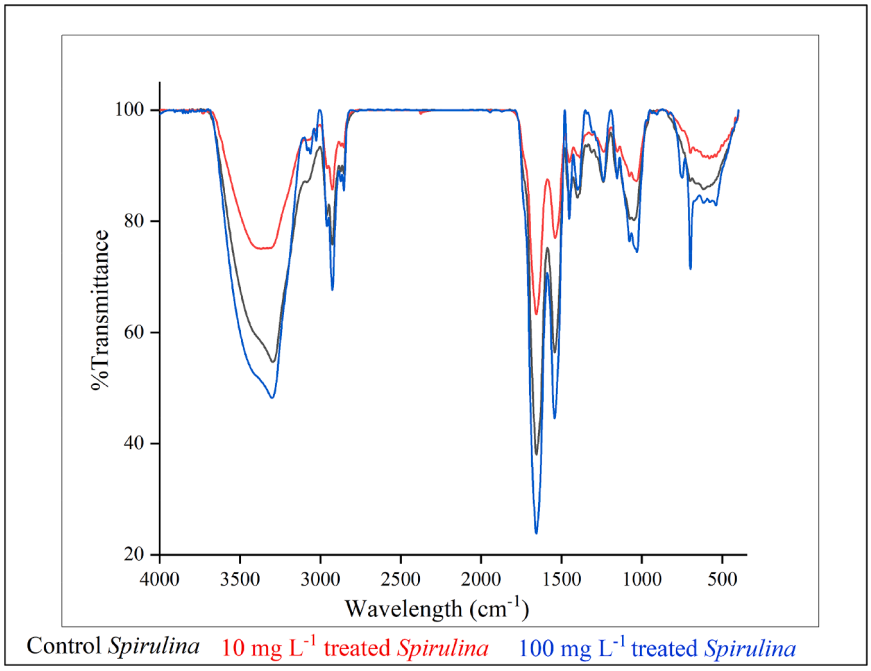

Fig. 7. FTIR spectra of *S. platensis* cells after 96 h PS NPs treatment. Black color indicated control cells, red color indicated 10 mg L^{-1} PS NPs treated cells and blue color indicated 100 mg L^{-1} PS NPs treated cells.

tested microalgal cells were analysed using FTIR and the obtained spectra showed a peak at wavelength 698 cm^{-1}, which was absent in control cells (Figs. 6 and 7). This indicated that PS NPs has deposited onto the surface of microalgae which further supporting the SEM analysis of the study. In addition, FTIR spectra also revealed that nanoplastics treatment altered functional groups of microalgae. For instance, the broad peak between 3100 and 3600 cm^{-1} which corresponds to the –NH and N—H groups of secondary amide II. In the PS NPs treated microalgae, we observed narrowing and stretching of these peaks. The peak at 1655 cm^{-1} signifies amide I of β-sheet structures, are related to proteins. Alteration in these regions which indicates a clear interaction between the NPs and the protein molecules of the tested microalgae (Hollóczki and Gehrke, 2019; Ansari et al., 2021; Hazeem et al., 2020). The bands at 2850–2930 cm^{-1}, representing CH_3 and CH_2 vibrations in aliphatic compounds, indicate hydrocarbons and lipids. The changes in these bands for PS NP treated microalgae suggest that lipid content may be affected under nanoplastics stress (Wang et al., 2018). Both microalgae show distinct FTIR bands at 1030–1035 cm^{-1}, corresponding to

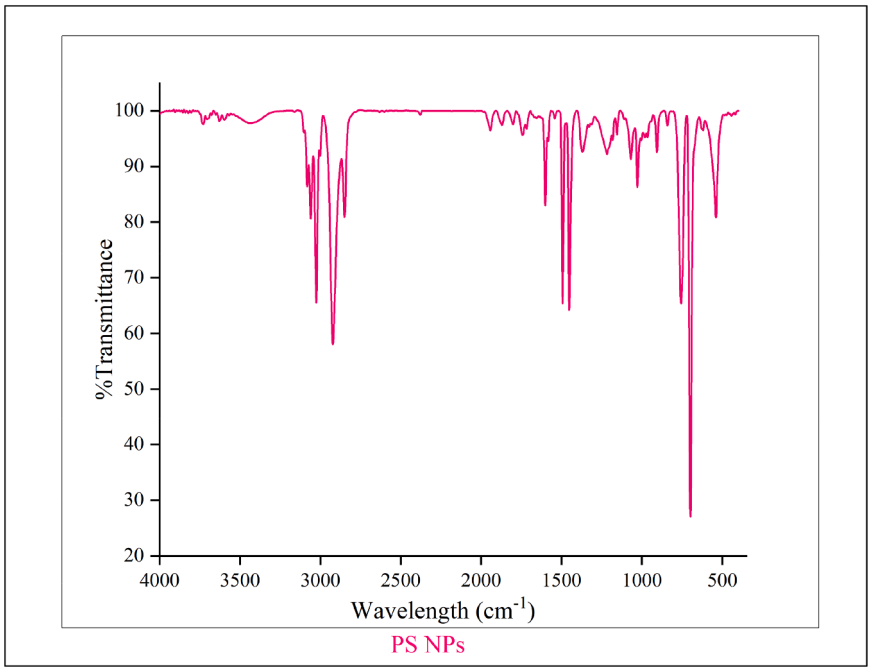

Fig. 8. FTIR spectra of PS NPs.

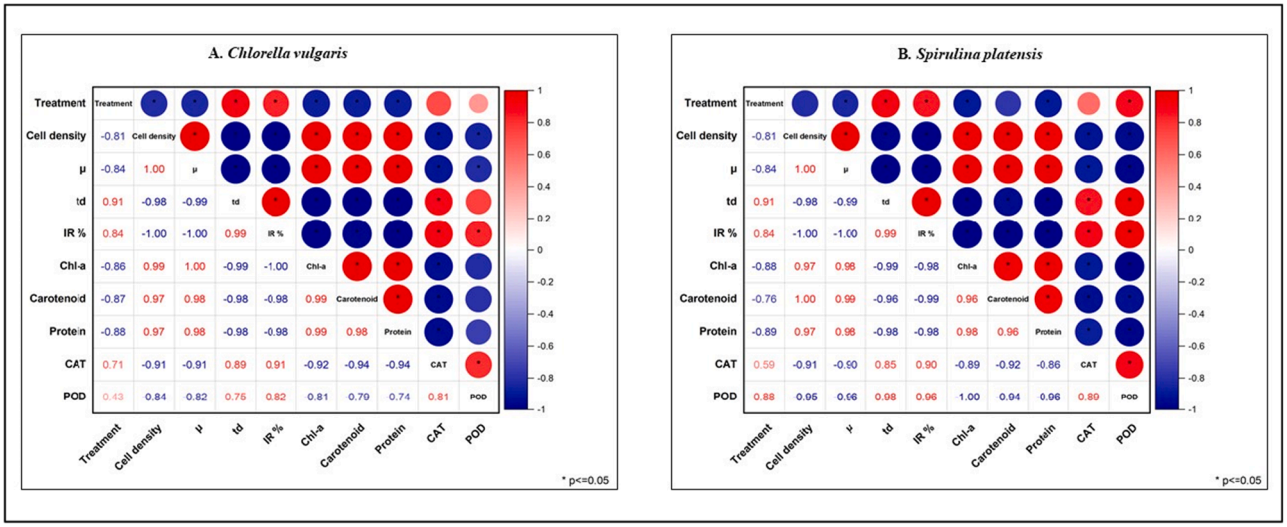

Fig. 9. Pearson's correlation analysis between PS NPs concentration and physiological index of *C. vulgaris* and *S. platensis* after the 96 h ($p < 0.05$).

C–O, C–H, and CH–OH stretches. These bands are more disrupted in treatments with 100 mg L^{-1} PS NPs, indicating changes in carbohydrate content (Wang et al., 2018; Hazeem et al., 2020; Déniel et al., 2020; Ansari et al., 2021). Based on the FTIR results and subsequent data interpretation, it can be inferred that NPs can effectively modify the principal functional groups within aquatic microalgae.

3.7. Pearson correlation analysis between PS NPs concentration and physiological index of microalgae

To understand the impact of PS NPs concentration on various parameters of *C. vulgaris* and *S. platensis*, Pearson correlation analysis was performed (Fig. 9). The results indicated that doubling time, growth inhibition rate, and enzymatic parameters were positively correlated with PS NPs concentration. Conversely, all other parameters exhibited a negative correlation. Pigment levels showed a positive correlation with protein content, growth rate, and cell density. Notably, CAT and POD demonstrated a significant negative correlation with most parameters, except for growth inhibition rate and treatments. Similar findings were reported by Kumar et al. (2024) and Debroy et al. (2024) when plant cells were exposed to emerging pollutants.

4. Conclusion

The study revealed that exposure to PS NPs influenced the growth, pigments, protein content, and oxidative enzyme activity of microalgae in a dose-dependent manner. Elevation in oxidative enzyme activity signifies that the microalgae were under immense stress. SEM and FTIR analyses confirmed that PS NPs disrupted the ultrastructure and altered functional groups of aquatic microalgae. The main purpose of this study was to develop an understanding of the real significance of nanoplastics to aquatic primary producers. Based on our current study, we

recommend that future toxicity research should focus on the transfer of NPs through the food chain, from lower to higher trophic levels, to better understand their potential impact on higher organisms. While our study used pristine PS NPs, it is important to consider that in natural environments, NPs may interact with other pollutants, potentially increasing their toxicity. Therefore, further investigations are needed to assess the combined effects of NPs and other environmental contaminants on microalgae and other aquatic organisms.

CRediT authorship contribution statement

Pritam Sarkar: Writing – original draft, Investigation, Formal analysis. **K.A. Martin Xavier:** Methodology. **Satya Prakash Shukla:** Writing – review & editing, Supervision, Conceptualization. **Govindarajan Rathi Bhuvaneswari:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2025.100613.

Data availability

Data will be made available on request.

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