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Interactive Effects of Nano-Polystyrene and Light Spectra on Growth and Phytohormone (Auxin and Gibberellin) Production in *Chlorella vulgaris*

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ABSTRACT

This study investigated the intricative effects of nanopolystyrene particles (NPS) and varying light spectra on the growth and phytohormones (auxin and Gibberellin) production in *Chlorella vulgaris*, a microalgae crucial to aquatic ecosystems, addressing critical aspects of nanoplastic pollution. The algae's physiological and hormonal response was evaluated under varying light spectra (blue, red, and white) and NPS concentrations (5, 10, 20, and 40ppm) over a 23-day period. Results demonstrated that blue light significantly enhanced algae growth rate and increased auxin production, with the highest auxin levels reaches 61.53 µg/g.w. at 5 ppm under blue light on the 20 day. Conversely, red light provides more effective in stimulating gibberellin synthesis, marginally outperforming the blue spectrum. High concentrations of nanopolystyrene (20-40 ppm) consistently inhibited growth and biomass production (e.g. a 15-20 % growth delay under blue light at 40ppm compared to 5ppm) primarily due to oxidative stress and physical shading. Morphological analysis using scanning electron microscope (SEM) revealed significant cellular damage and the accumulation of nanoparticles biofilms on algal cells at 40ppm after 10 days of exposure, visually conforming the physical impact. These finding underscore the complex interplay between specific lighting conditions and neoplastic contamination, high lighting the potential environmental hazards posed by these particles to primary producers in aquatic food webs. The study provides a more profound understanding of the hormonal and physiological adaptable of microalgae to environmental stresses, contributing valuable data to the ongoing discourse on plastic pollution and its ecological consequence.

INTRODUCTION

The global increase in plastic pollution has become a major environmental concern in recent years due to its detrimental effects on ecosystems **(Rezaei *et al.*, 2020; Widiarti *et al.*, 2025)**. Previous studies have estimated that approximately 300 million tons of plastic waste are generated annually, with about 10% eventually entering the ocean from various sources **(Waring *et al.*, 2018)**. Over time, plastic waste undergoes mechanical and biological degradation, breaking down into smaller fragments known as microplastics (<5 mm). These microplastics further degrade into nanoplastics, defined as particles less than 100nm in size **(Molenaar *et al.*, 2021; Noor *et al.*, 2025)**.

Microplastics and nanoplastics are prevalent in aquatic environments, especially in urban areas where they enter through stormwater systems and direct human activities such as fishing, ship-based dumping, and accidental spills. The release of domestic, agricultural, and industrial wastewater into marine systems has further intensified concerns about nanoplastic contamination **(Andrady, 2011)**.

These microscopic particles can impair photosynthesis and hinder algal growth by obstructing light penetration and gas exchange **(Sjollema *et al.*, 2016)**. As these particles infiltrate aquatic systems from various industrial sources, it becomes essential to investigate their effects on different aquatic organisms—particularly phytoplankton, which form the foundation of the aquatic food web.

Algae, like plants, possess photosynthetic capabilities and contain a variety of pigments, including chlorophylls and carotenoids **(Takaichi, 2025)**. Their growth and pigment production are influenced by light characteristics such as wavelength (color), intensity, and exposure duration, which can modulate photosynthetic efficiency, chlorophyll content, and the activity of plant hormones and enzymes **(Maltsev *et al.*, 2021)**.

Plant hormones, even in trace amounts, play a crucial role in regulating growth and development processes such as cell division, elongation, differentiation, germination, dormancy, and senescence **(Thapa *et al.*, 2024)**. Hormones such as auxins and gibberellins are commonly found in higher plants, algae, and ferns, and have also been detected in mosses and lichens. Auxins are particularly important in promoting plant growth and development **(Fujita & Hasebe, 2009)**. In addition, they have been identified in green algae as well **(Bentley-Mowat & Reid, 1999)**.

Gibberellins are responsible for promoting cell elongation and growth in both higher plants and green algae, even at low concentrations **(Fujioka & Sakurai, 1997; Dumale *et al.*, 2018)**. Although present in smaller amounts within green algal cells, these hormones remain sensitive to environmental influences, resulting in significant physiological changes. *Chlorella vulgaris*, known for its adaptability to both freshwater and marine environments and its rapid growth cycle, serves as an ideal model organism for biochemical and physiological studies.

Several studies have examined the impact of nanopolystyrene on algal physiology. For example, **Li *et al.* (2025)** investigated the effects of micro- and nanoplastics on algal community structure, pigment composition, photosynthetic activity, and oxidative stress markers. Additionally, **Qahtan and Al-Zurfi (2024)** studied the influence of nanopolystyrene on the chlorophyll content of *Chlorella vulgaris*. However, a significant research gap remains regarding the combined effects of nanopolystyrene particles and varying light spectra on algal physiological responses, particularly growth and phytohormone production.

While previous research has explored the individual effects of nanoplastics and light conditions, their potential synergistic or antagonistic interactions under realistic

environmental settings remain poorly understood. The current study aimed to address this gap by comprehensively investigating the interactive effects of nanopolystyrene (NPS) particles and different light spectra (blue, red, and white) on the growth and phytohormone (auxin and gibberellin) production in *Chlorella vulgaris*. Additionally, the study seeked to uncover the physiological and hormonal adaptive responses of *C. vulgaris* to combined environmental stressors, thereby contributing to a deeper understanding of the ecological consequences of plastic pollution.

MATERIALS AND METHODS

Purification of algal cultures

A pure isolate of the green alga *Chlorella vulgaris* used in the present study was obtained from the Advanced Environmental Laboratory, Department of Environmental Sciences, College of Science, University of Kufa. To ensure axenic conditions, a sample of the algal culture was streaked on solid nutrient agar and incubated at 37°C for 24 hours to confirm the absence of bacterial and fungal contaminants **(Anderson, 2005)**. Species identity was verified via light microscopy, observing characteristic morphological features such as spherical cell shape and a cup-shaped chloroplast.

Preparation of polystyrene nanoparticles

Polystyrene nanoparticles were prepared following modified protocols **(Hunter, 2021; Rasham, 2024)**. One gram of polystyrene beads was dissolved in 4mL of ethyl acetate in a conical flask equipped with a magnetic stir bar. The mixture was stirred at 40°C and 500rpm for 2 hours until fully dissolved. Using a glass pipette, the solution was slowly added to 50mL of ethanol in a 100mL beaker while stirring at 400rpm. The resulting dispersion was transferred to test tubes and centrifuged at 4,000rpm for 10 minutes.

The supernatant was discarded into a hazardous waste container, and 40mL of distilled water was added to each test tube. Samples were vortexed and recentrifuged; this washing process was repeated twice more. The final concentration of polystyrene nanoparticles was determined by drying the pellet in a pre-weighed vial at 60 °C and measuring the mass difference. The suspension was stored at room temperature in a sealed glass container. Before use, the sample was homogenized with an ultrasonic device to ensure even dispersion without settling.

Culture media preparation

BG-11 medium was prepared in the laboratory with all required components **(Anderson, 2005)**. The medium was sterilized via autoclaving and distributed into forty-five 1-liter glass flasks upon cooling.

Experimental design

Chlorella vulgaris was cultured by inoculating 20mL of pure algal culture into 480mL of BG-11 medium in each flask. Cultures were incubated in a custom-built algal

incubator at 25 ± 2°C. The incubator had three shelves, each equipped with a ventilation fan, a thermostat, and a regulated light source operating on a 16:8 hour light-dark cycle.

Three light treatments were applied:

- White LED (12.59 µmol/m²/s)
- Blue LED (40.79 µmol/m²/s)
- Red LED (12.81 µmol/m²/s)

Light intensities were measured using a Yowexa YW-552 luxmeter (China) and set according to previous studies. Each light treatment included five nanoparticle concentrations: 0 (control), 5, 10, 20, and 40mg/ L of nanopolystyrene (NPS), with three replicates per treatment. The flasks were gently shaken twice daily to prevent cell settling, improve gas exchange, and ensure even exposure to light and nutrients.

Determination of growth rate

Algal growth was monitored over 21 days using optical density (OD) measurements at 680nm **(Fogg, 1975)**. A 1mL sample was withdrawn every 24 hours and measured in a quartz cuvette. Sterile BG-11 medium was used as a blank. OD readings were taken for all samples under red, blue, and white light treatments.

The specific growth rate (K) was calculated using the formula **(Xianghu *et al.*, 2002)**:

$$K = (\log ODt - \log OD_0) \times 3.332 / t$$

Where:

- **K** = growth rate
- **t** = time (days)
- OD_0 = optical density at day 0
- **ODt** = optical density at time **t**

Biomass estimation

To estimate biomass, 25mL of culture was collected on days 4, 10, and 16. Samples were centrifuged at 6,000 rpm for 15 minutes, and residual water was removed through repeated centrifugation. The wet weight of the algal pellet was measured using a sensitive analytical balance **(Tredici, 2004)**.

Phytohormone quantification (Auxin and Gibberellin)

Phytohormone levels were quantified using a spectrophotometric method **(Ünyayar *et al.*, 1996)**. A 2mL algal extract was combined with 6mL methanol, 2.5mL chloroform, and 1.5mL ammonium hydroxide, and then diluted to 12mL with distilled water. The pH was adjusted to 2.5 using 1N HCl or NaOH. Absorbance was measured at 254nm for gibberellin and 280nm for auxin. Concentrations were calculated using calibration curves generated from standard concentrations (0.02– 0.12µg/ g).

Sample preparation for SEM analysis

On day 16, a 10mL algal sample was fixed with 2.5% glutaraldehyde for 24 hours at 4°C. The sample was centrifuged and washed three times with buffer. It was then dehydrated through an ethanol gradient (30, 50, 70, 90, and 100%), with 15 minutes in

each solution and centrifugation between steps. Dehydrated cells were transferred to a critical point dryer.

Dried samples were mounted on aluminum foil and attached to SEM stubs. A 10nm gold coating was applied using a sputter coater. The samples were then imaged using scanning electron microscopy (SEM).

Statistical analysis

All data were analyzed using IBM SPSS Statistics v26.0. A univariate analysis of variance (ANOVA) was performed for a factorial design with three factors: NPS treatment concentration, time, and light spectrum. Tukey's post hoc test was applied to determine significant differences at $P< 0.05$.

RESULTS AND DISCUSSION

1. Effects of nanopolystyrene (NPS) on the growth rate of *Chlorella vulgaris*

The growth rate of *Chlorella vulgaris* was assessed under varying concentrations of nanopolystyrene (NPS) and three different light spectra (blue, red, and white). Growth was closely linked to light intensity, with overall patterns indicating that blue light resulted in the highest growth rates, followed by white, and then red. The growth phase initiated around the fourth day and progressed steadily until reaching a plateau between the 15th and 16th days for all treatments. Afterward, a gradual decline in growth was observed across all spectra, which was attributed to the cumulative stress effects of light exposure and NPS treatment.

In contrast, control groups (no NPS exposure) maintained growth for longer durations—up to day 22 under white and red light, and until day 20 under blue light—before declining due to natural aging or nutrient depletion.

Blue light spectrum

Under blue light, all treatments demonstrated a rapid increase in growth rate during the exponential phase. However, cultures treated with lower concentrations of NPS (5– 10mg/ L) exhibited higher growth rates compared to those treated with higher concentrations (20– 40mg/ L) (Fig/ 1). This aligns with the well-documented role of blue light in enhancing chlorophyll *a* and *b* absorption, photosynthetic efficiency, and ATP production, which support cell division **(Han *et al.*, 2004; Takahashi *et al.*, 2007; Miki *et al.*, 2017; Wang & Praetorius, 2022)**.

At the highest NPS concentration (40 mg/L), growth was inhibited, likely due to light scattering by nanoparticles that obstructed light penetration. While previous research has examined the independent effects of light spectra and nanoplastic toxicity **(Fu *et al.*,**

2019; Yuan *et al.*, 2020; Wang & Praetorius, 2022; Yang *et al.*, 2024), studies exploring their combined impact are limited. Our findings suggest that blue light may mitigate NPS-induced stress by enhancing photosynthetic capacity, thus reducing toxicity impacts.

Red light spectrum

Growth under red light was slower and more erratic compared to blue light, with a 30– 40% reduction in growth rate. On day 16, the growth rate for the 5mg/ L NPS treatment was 0.171, while that for the 40mg/ L treatment was 0.133 (Fig. 2). A brief decline in growth was observed across all treatments, followed by a temporary surge, and eventually a decrease. In contrast, the control sample exhibited a steady plateau throughout the period.

Statistical analysis revealed significant differences between 5mg/ L and both 10 and 40mg/ L concentrations ($P< 0.05$), but no significant difference was observed between 5mg/ L and the control or 20mg/ L treatments. The decline in growth at higher NPS concentrations may be attributed to oxidative stress caused by prolonged nanoparticle exposure, as nanoparticles have been shown to increase reactive oxygen species (ROS) production, leading to membrane damage **(Fu *et al.*, 2019)**. Additionally, the less efficient photosynthetic response of *C. vulgaris* under red light **(Yuan *et al.*, 2020)** may have compounded the oxidative stress response, rendering the algae more vulnerable to NPS toxicity.

White light spectrum

Under white light, growth showed a moderate increase compared to the red spectrum but remained below that observed under blue light (Fig. 3). Similar to red light, a brief decline in growth rate was observed during the stationary phase, followed by a renewed increase across all NPS treatments except the control. This supports the hypothesis that nanoparticles, rather than light spectrum alone, were responsible for the observed inhibition.

According to **Yuan *et al.* (2020)**, both blue and red light are more effective than white or green in enhancing microalgal cell growth. Blue light promotes higher photosynthetic capacity and photoprotection, leading to greater lipid accumulation, while red light favors carbohydrate synthesis. The mixed spectral properties of white light may offer some adaptability to the algae, but the presence of nanopolystyrene still exerts a strong inhibitory effect on growth.

These findings contribute novel insights into how light quality interacts with nanoparticle pollution in aquatic systems, highlighting the potential for blue light to partially alleviate nanoplastic-induced stress in *Chlorella vulgaris*.

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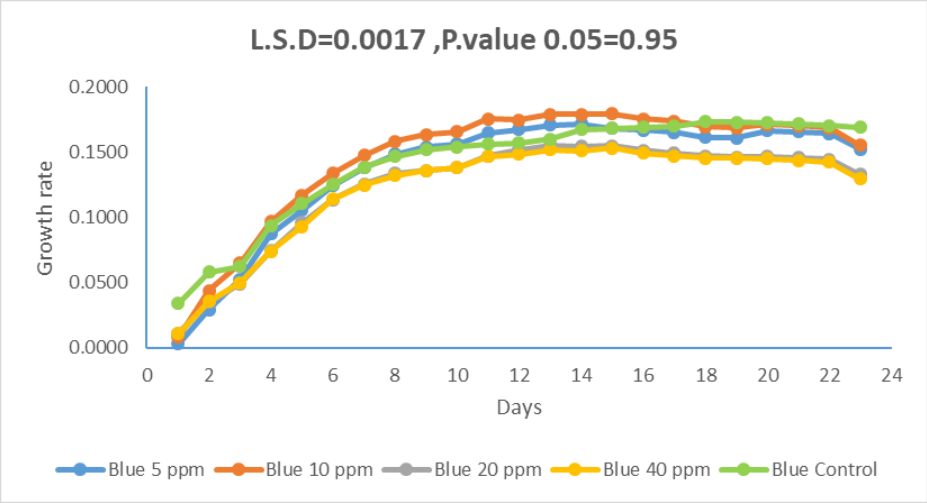

Fig. 1. Effect of nano-polystyrene on growth rate of *C. vulgaris* under blue spectrum during experiment period

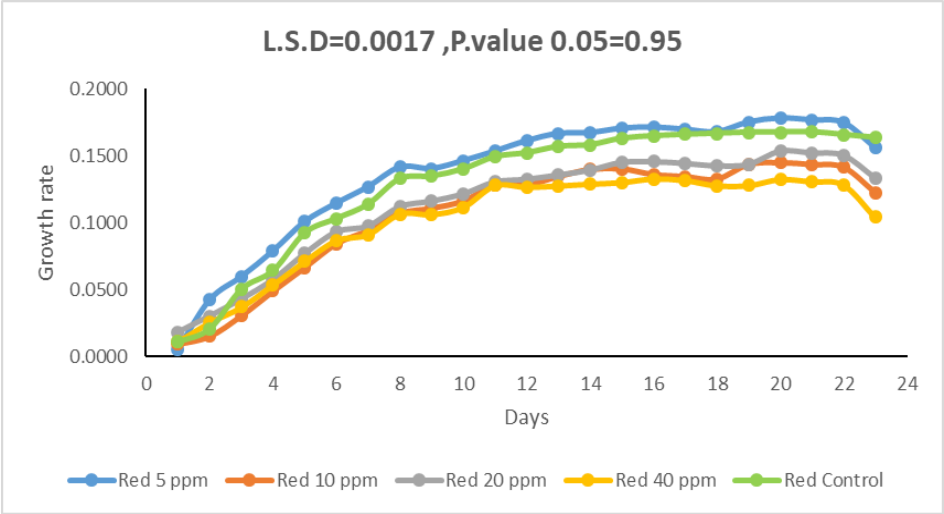

Fig. 2. Effect of nano-polystyrene on growth rate of *C. vulgaris* under red spectrum during experiment period

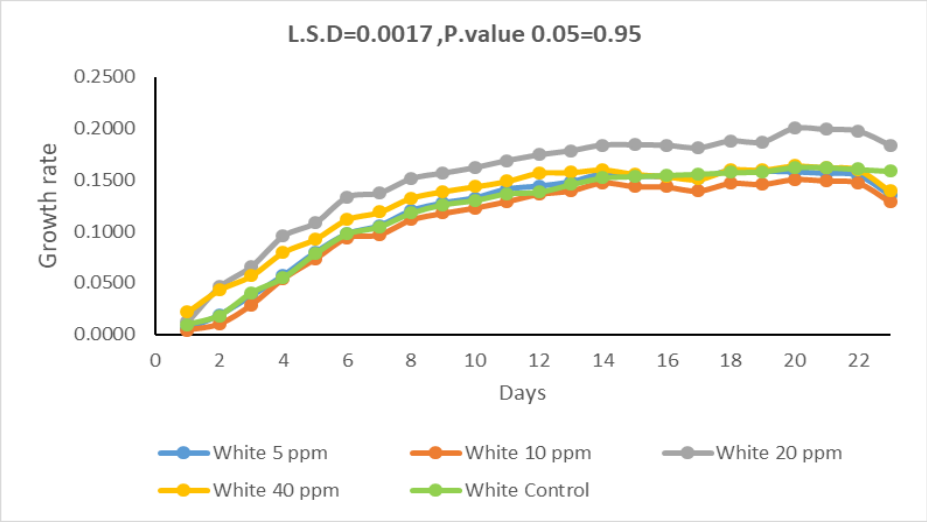

Fig. 3. Effect of nano-polystyrene on growth rate of *C. vulgaris* under white spectrum during experiment period

1. Effects of Ps_NPs on biomass production

The results indicated that the biomass of *Chlorella vulgaris* varied significantly depending on both light spectrum and nanopolystyrene (NPS) concentration. Overall, algae grown under the blue light spectrum consistently produced more biomass than those grown under red or white light conditions.

The highest biomass recorded under blue light was 20.52mg/ L, observed on the fourth day at a concentration of 40ppm NPS. Conversely, the lowest biomass under blue light occurred on the sixteenth day at 10ppm, with a value of 8.56mg/ L. In comparison, the blue light control group (no NPS) achieved a peak biomass of 36mg/ L on the tenth day, while the lowest value was 12.8mg/ L on the sixteenth day (Fig. 4).

Under red light, the maximum biomass was recorded on the tenth day at 20ppm (18.2mg/ L), while the lowest value occurred on the sixteenth day at the same concentration (7.46mg/ L). The red light control group reached its peak on day 10 (31.2mg/ L), and the lowest on day 16 (7.46mg/ L) (Fig. 5).

For cultures exposed to white light, the highest biomass was observed on day 10 at 40ppm (22.66mg/ L), while the lowest was 6.18mg/ L on day 16 at 20ppm. The control group under white light peaked at 33.6mg/ L on day 10, with the lowest biomass recorded on day 1 (13.6mg/ L) (Fig. 6).

Statistical analysis showed significant differences between NPS concentrations and control samples, between light spectra, and across sampling days ($P< 0.05$). These

findings confirm that blue light is more effective in promoting biomass accumulation compared to red and white light. This supports previous studies demonstrating the role of blue light in enhancing photosynthesis and biomass productivity in microalgae **(Vadiveloo *et al.*, 2015)**. That study showed that large-scale cultivation of *Nannochloropsis* sp. under blue-selective photovoltaic filters significantly improved biomass yields while generating electrical energy.

Furthermore, other studies have also confirmed that microalgae grown under monochromatic blue or red light can exhibit equal or even superior growth and productivity compared to those grown under multi-chromatic white light **(Aidar *et al.*, 1994; Chang *et al.*, 2022)**.

When comparing NPS-treated samples to their respective controls, a significant reduction in algal biomass was observed. This decline is likely due to the accumulation of nanopolystyrene particles on algal cell surfaces, which hinders light absorption and reduces photosynthetic efficiency **(Fu *et al.*, 2019)**. Additionally, oxidative stress resulting from prolonged NPS exposure appears to accelerate cellular degradation and advance the onset of the decline phase in treated cultures.

These results align with observations from **Mendonça *et al.* (2023)**, who reported biomass reductions in microalgae exposed to nanoplastics over extended periods. Interestingly, biomass peaked as early as day 4 in several NPS-treated cultures, suggesting either an early-stage stress response or initial adherence of NPS particles to algal cells. In contrast, control groups (without NPS) peaked later in the growth cycle, reflecting a more typical algal development pattern.

Collectively, these findings underscore the dual importance of light spectrum and nanoparticle exposure in shaping algal biomass production and offer further evidence of the ecological risks posed by nanoplastics in aquatic environments.

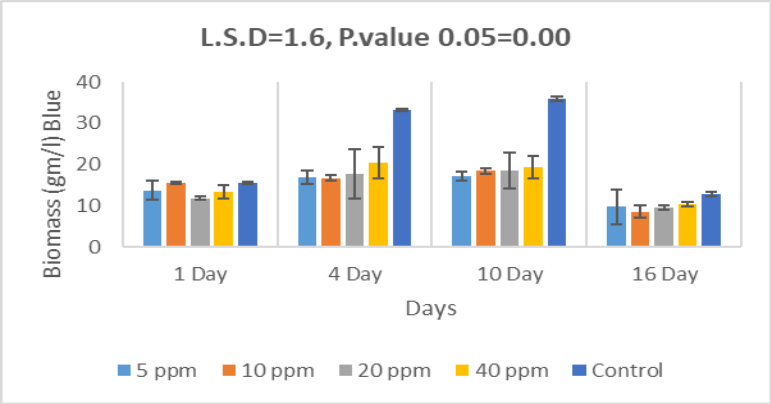

Fig. 4. Effect of nano-polystyrene on biomass of *C. vulgaris* under blue spectrum during experiment period

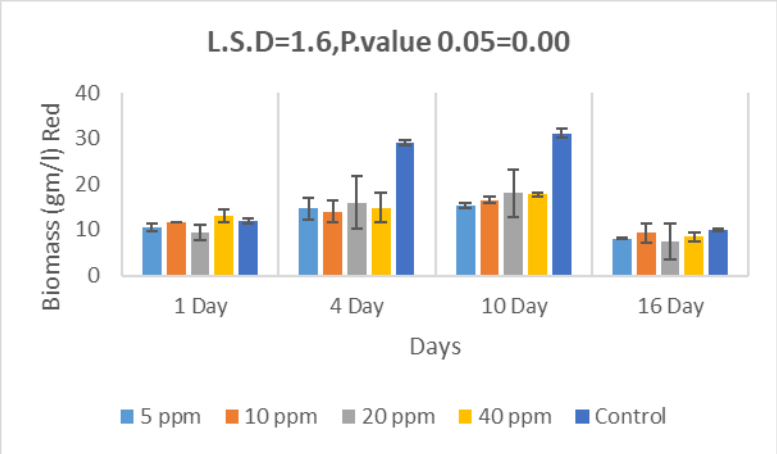

Fig. 5. Effect of nano-polystyrene on biomass of *C. vulgaris* under red spectrum during experiment period

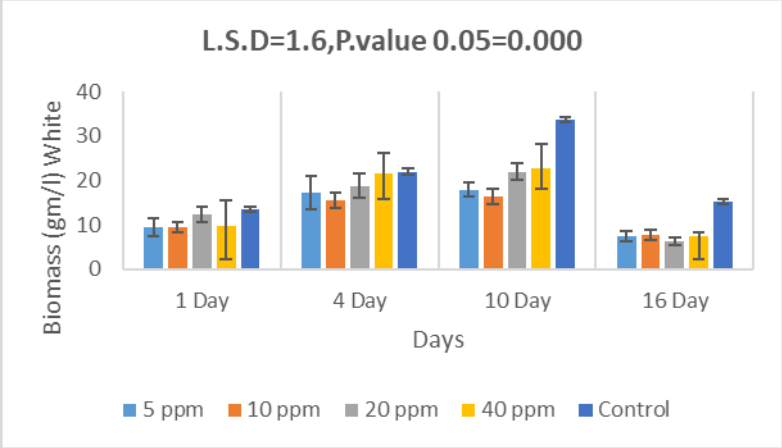

Fig. 6. Effect of nano-polystyrene on biomass of *C. vulgaris* under white spectrum during experiment period

3. Effects of Ps_NPs on auxin production

The current study revealed that light spectrum plays a critical role in modulating the physiological response of *Chlorella vulgaris* to nanopolystyrene (NPS) exposure, particularly in relation to auxin production. A complex interaction was observed between NPS concentration and light type, influencing auxin levels during different stages of the algal life cycle.

The highest auxin concentration was recorded under blue light on the final day of the experiment, reaching 61.537µg/ g.w in the sample treated with 5ppm NPS (Fig. 7). This suggests that lower NPS concentrations may exert a mild stress that triggers a cellular adaptation response, leading to increased auxin production. As supported by recent findings, plant hormones such as auxin function through an intricate network of

signaling pathways that can be activated under environmental stress **(Vanstraelen & Benková, 2012)**. Blue light, with its higher photon energy, enhances photosynthesis and activates metabolic pathways—including those responsible for phytohormone biosynthesis.

Red light exposure resulted in a less pronounced and more variable auxin response (Fig. 8). Notably, the highest auxin concentration under red light (61.185µg/ g.w) was recorded on the final day in samples treated with 20 ppm NPS. Although close in value to the peak recorded under blue light, the overall auxin trends under red light were less consistent. Statistical analysis revealed significant differences between treatments and the control, as well as between light spectra and across sampling days ($P< 0.05$). Red light's limited efficiency in promoting early-stage photosynthesis—due to low absorption by chlorophylls—and the relatively low light intensity used in this study (12.18 µmol/s/m²) likely contributed to reduced auxin synthesis.

Furthermore, NPS-induced stress may have compromised chloroplast function and auxin biosynthesis. This aligns with the findings of **Bhattacharya (2010)**, who reported that plastic nanoparticles can impair photosynthesis through oxidative stress and chloroplast damage, ultimately affecting hormone production and growth.

Under white light, auxin levels showed a mixed initial response but gradually increased over time across most treatments (Fig. 9). The highest value (59.907µg/ g.w) was observed on the final day in samples treated with 20ppm NPS. The lowest auxin concentration among all treatments and light types was 8.685µg/ g.w, also under white light. These results indicate that the broad spectrum of white light—containing blue, red, and green wavelengths—enables *C. vulgaris* to better adapt to environmental stressors, including NPS.

Simultaneous exposure to multiple light wavelengths likely triggered diverse photoreceptors and metabolic processes, enhancing the alga's ability to withstand NPS toxicity. This finding is consistent with **Wang *et al.* (2020)**, who reported that white light allows microalgae to modulate their metabolism in response to nanopollutants, improving stress tolerance and physiological adjustment.

Overall, the findings demonstrate that:

- **Blue light** had the most pronounced effect on auxin production, especially at low NPS concentrations, likely by enhancing photosynthetic efficiency and activating hormone biosynthetic pathways.
- **Red light** was less effective in stimulating auxin production and was associated with greater variability and potential vulnerability to NPS-induced oxidative stress.

- **White light** provided an intermediate response, allowing for gradual adaptation and balanced photoacclimation over time.

These results indicate that nanopolystyrene does not directly inhibit hormone synthesis but exerts its influence indirectly through oxidative damage and cellular stress. The type and intensity of light were the primary determinants of auxin biosynthesis under stress, with blue light enhancing resistance, red light reducing physiological efficiency, and white light enabling moderate growth and long-term adaptation.

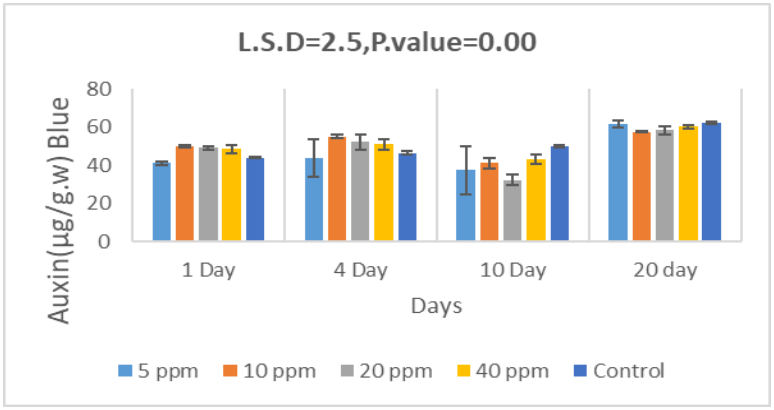

Fig. 7. Effect of nano-polystyrene on Auxin hormone of *C. vulgaris* under blue spectrum during experiment period

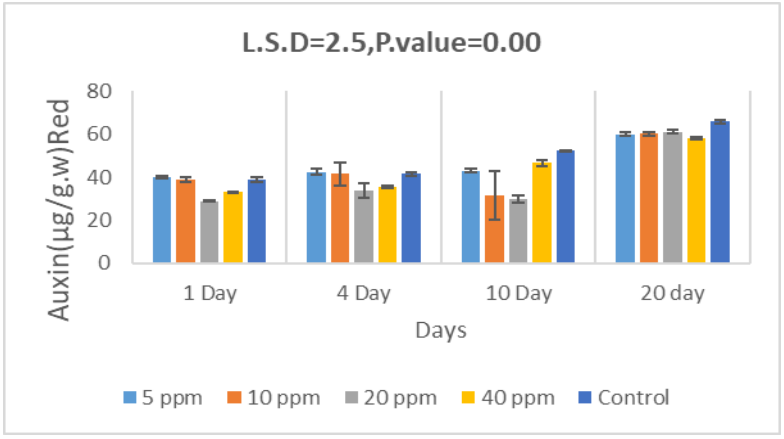

Fig. 8. Effect of nano-polystyrene on auxin hormone of *C. vulgaris* under red spectrum during experiment period

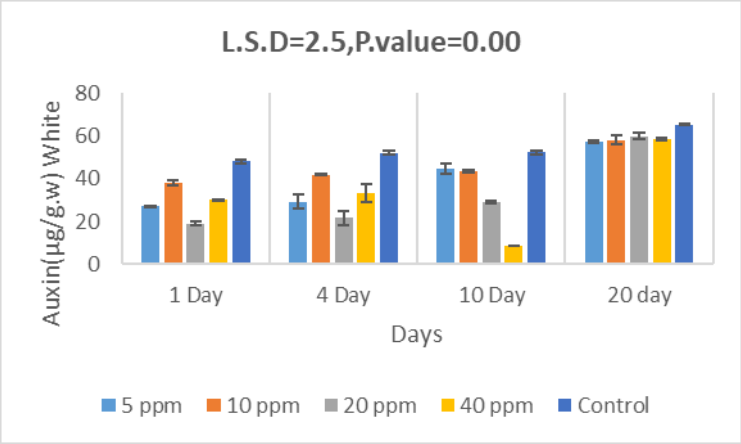

Fig. 9. Effect of nano-polystyrene on auxin hormone of *C. vulgaris* under white spectrum during experiment period

4. Effects of Ps_NPs on gibberellin production

The results showed that gibberellin production in *Chlorella vulgaris* varied according to both the concentration of nanopolystyrene (NPS) and the type of light spectrum used for exposure. Light quality, particularly spectral composition, plays a crucial role in regulating physiological and hormonal processes in microalgae.

Among the three light spectra tested, red light stimulated the highest levels of gibberellin production in both NPS-treated and control groups, slightly outperforming blue light across all treatment levels (Figs. 10, 11, and 12). Statistical analysis revealed significant differences between treatments and the control, as well as between spectra and over time ($P< 0.05$).

The enhanced gibberellin synthesis under red light may be attributed to its greater effectiveness in activating genetic pathways and upregulating enzymes involved in hormone biosynthesis. This is supported by previous research indicating that specific wavelengths can selectively stimulate intracellular signaling cascades and hormonal pathways **(Toman *et al.*, 2024)**.

In contrast, white light exhibited a more subdued effect on gibberellin levels. Although white light includes a range of wavelengths (including red and blue), its broader spectral nature may result in diluted stimulation of specific photoreceptors. Additionally, the relatively low light intensity used in this study may have reduced the activation of gibberellin-related pathways, particularly at higher NPS concentrations (e.g., 40 ppm), where the inhibitory effects of nanoplastics were more pronounced.

Interestingly, across all light types, gibberellin levels increased notably during the later stages of algal growth. This trend may indicate two possible responses: (1) Partial degradation or reduced toxicity of NPS over time, or (2) physiological acclimation of

algal cells to prolonged exposure and stress. The delayed hormone peak suggests that *C. vulgaris* may adapt its internal regulatory mechanisms to reinitiate hormone synthesis once equilibrium is restored.

These findings reinforce the concept that hormone production in algae is governed by a complex interplay between environmental stimuli and internal regulatory systems. Specifically:

- **Red light** was the most effective in promoting gibberellin biosynthesis, indicating strong spectral specificity in hormonal regulation.
- **Blue light** showed moderate stimulation, with production levels consistently below those observed under red light.
- **White light**, despite its broad wavelength range, was the least effective in supporting gibberellin synthesis under NPS-induced stress.

This spectral specificity may be due to the differential activation of distinct photoreceptors within algal cells that are sensitive to certain wavelengths. These photoreceptors likely initiate downstream signaling pathways that regulate the biosynthesis of specific hormones, such as gibberellins and auxins. The dichotomy observed between red and blue light responses supports the hypothesis that hormone regulation in microalgae is wavelength-dependent and closely tied to light intensity and environmental stressors.

In conclusion, the results demonstrate that light spectrum significantly influences the hormonal response of *Chlorella vulgaris* to nanopolystyrene exposure. Red light, in particular, appears to enhance gibberellin production, suggesting a potential adaptive mechanism that supports growth and development even under nanoparticle-induced stress.

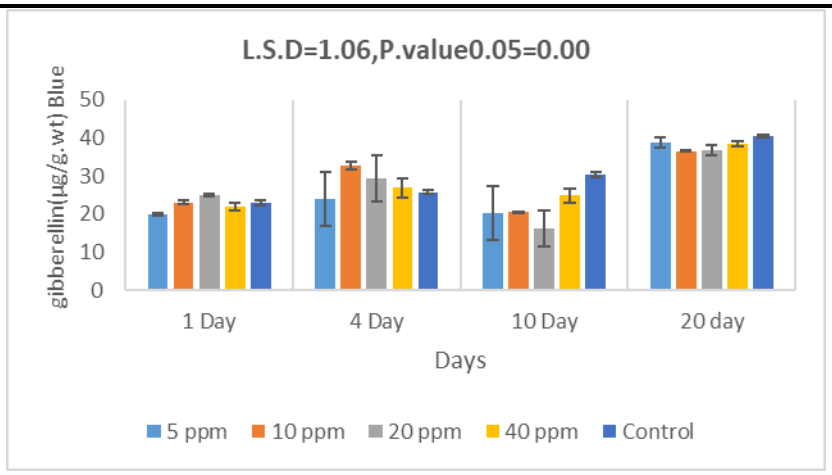

Fig. 10. Effect of nano-polystyrene on gibberellin hormone of *C. vulgaris* under blue spectrum during experiment period

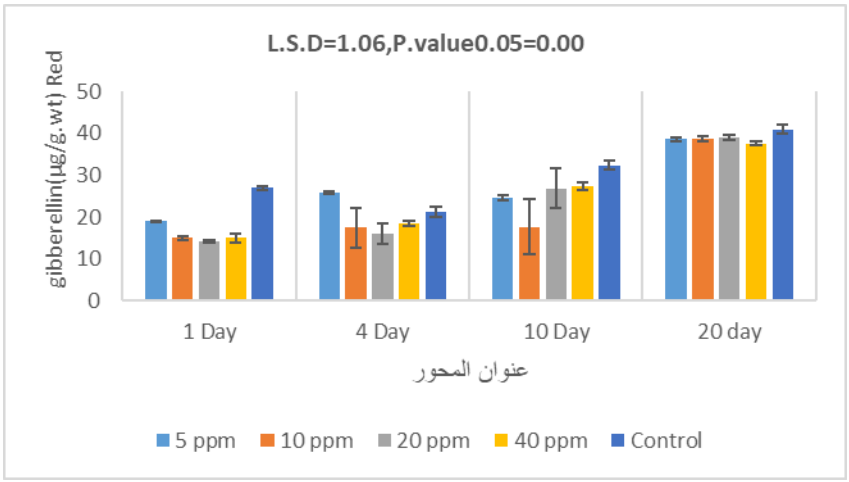

Fig. 11. Effect of Nano-polystyrene on gibberellin hormone of *C. vulgaris* under red spectrum during experiment period

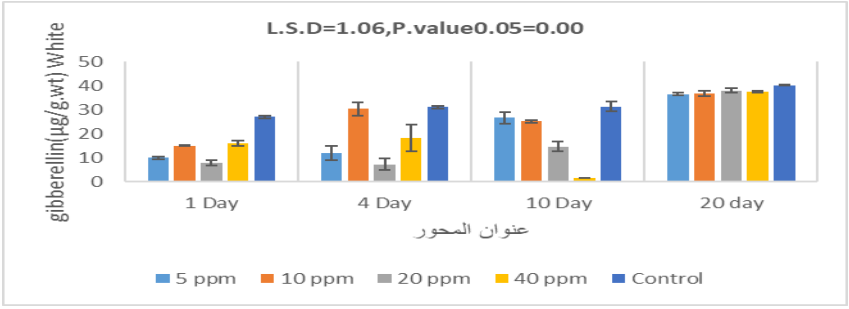

Fig. 12. Effect of nano-polystyrene on gibberellin hormone of *C. vulgaris* under white spectrum during experiment period

5. Morphological changes in microalgae under SEM

A scanning electron microscope (SEM) was used to observe morphological changes in *Chlorella vulgaris* cells following exposure to nanoplastic stress. The natural spherical morphology of *C. vulgaris* serves as an important indicator of cell health. Alterations in this shape provide visible evidence of cellular stress and toxicity induced by nanopolystyrene (NPS) exposure **(Wang *et al.*, 2020)**.

After 10 days of exposure to 40ppm NPS under blue, red, and white light spectra, SEM imaging revealed notable morphological deformations in the algae (Images 1, 2, and 3). The observed changes included thin, irregular film-like structures on the cell surface, nanoparticle accumulation, and biofilm formation. The highly reactive and hydrophobic nature of NPS allows the particles to adhere strongly to algal cells, forming a coating that impairs gas exchange and disrupts normal cell-environment interactions.

These morphological alterations provide strong visual evidence supporting earlier findings of reduced growth rates and biomass production. The SEM images clearly demonstrate the physical impact of NPS on cell integrity, highlighting deformation of the cell wall and loss of the regular spherical shape. This is consistent with prior studies reporting similar detrimental effects of micro- and nanoplastics on algal cells. For example, **Nigam *et al.* (2022)** showed that polystyrene microplastics reduced chlorophyll content and cell count in *Chlorella pyrenoidosa*, attributing the damage to the physical and oxidative stress caused by microplastic accumulation.

The adherence of NPS particles to algal surfaces not only causes shading—thereby reducing light absorption—but also results in mechanical damage, especially to the cell wall, which serves as the first line of defense for microalgae. Such damage impairs critical cellular processes, including nutrient uptake, ion transport, and gas exchange **(Spain & Funk, 2022)**. The SEM analysis in the current study confirmed direct cellular damage, including visible micro-tears in the cell wall, deformation of cell shape, and aggregation of NPS particles on the surface, forming a distinct biofilm layer.

This biofilm significantly reduces the surface area available for nutrient and light absorption and imposes mechanical stress on the cell structure. Moreover, the obstruction of gas exchange further compromises photosynthesis and respiration, exacerbating physiological stress **(Behzadnia *et al.*, 2024)**.

These morphological observations offer concrete insight into the physical mechanisms by which nanoplastics inhibit algal growth and productivity. The SEM findings not only corroborate biochemical and physiological data but also highlight the severity of nanoplastic pollution as a multifaceted stressor in aquatic ecosystems, affecting primary producers at both structural and functional levels.

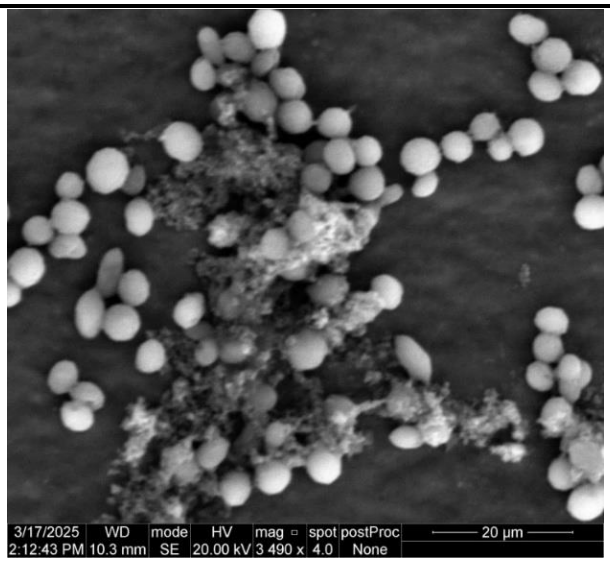

Image 1. SEM showing effect of nano-polystyrene on cell morphology of *C. vulgaris* under blue spectrum during 16 day

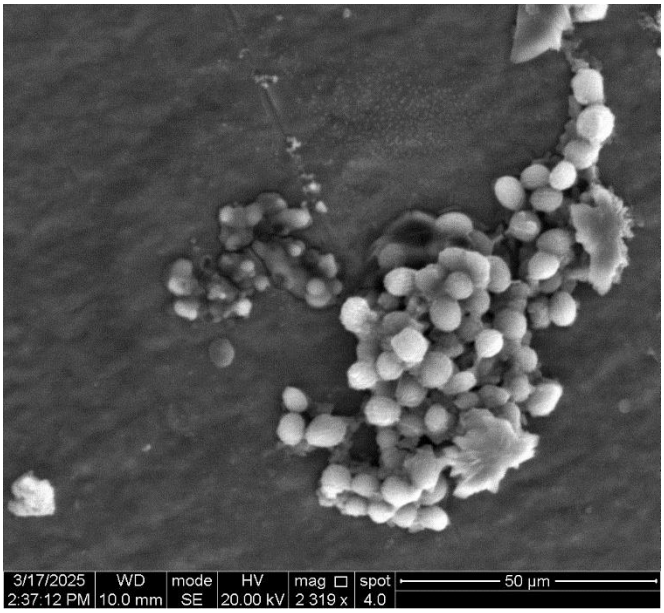

Image 2. SEM showing effect of nano-polystyrene on cell morphology of *C. vulgaris* under red spectrum during 16 day

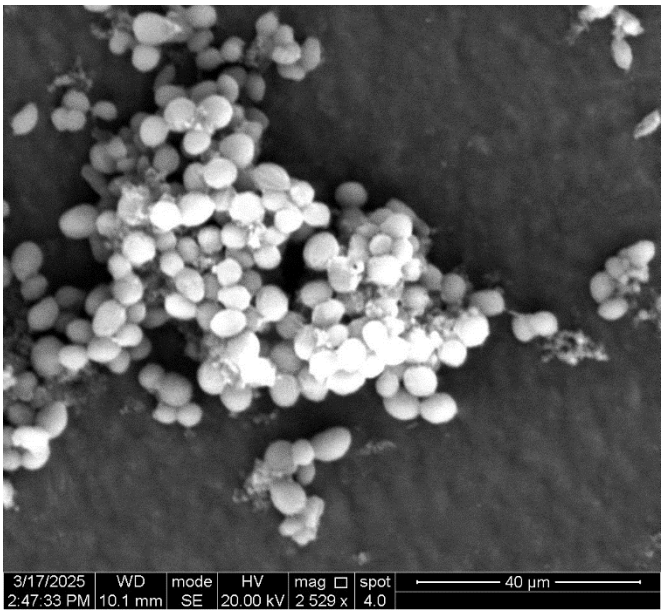

Image 3. SEM showing effect of nano-polystyrene on cell morphology of *C. vulgaris* under white spectrum during 16 day

CONCLUSION

This study unequivocally confirms that nanopolystyrene (NPS) pollution poses a serious and tangible threat to aquatic ecosystems, particularly to primary producers such as *Chlorella vulgaris*. Our findings offer valuable insights into how varying concentrations of NPS particles not only impair algal growth and biomass production but also disrupt internal hormonal regulation, including auxin and gibberellin synthesis. Such disruptions may trigger cascading effects throughout the aquatic food web, including reduced food availability for herbivores and alterations in nutrient cycling dynamics.

The interaction between NPS and *C. vulgaris* extends beyond physical shading; our results indicate direct cytotoxic mechanisms that impair essential biological processes at the cellular level. Importantly, this study highlights the critical role of light spectrum in modulating these impacts. Blue light consistently mitigated NPS-induced growth inhibition and enhanced phytohormone production—particularly auxin—suggesting a protective role under pollutant stress. Conversely, red light exposure often intensified the adverse effects of higher NPS concentrations, resulting in reduced growth and hormonal imbalance. White light provided an intermediate response, offering a broader range of wavelengths that supported partial adaptation.

Overall, these findings underscore that the combined influence of nanopolystyrene and light quality governs the physiological and biochemical responses of *C. vulgaris*. This integrated understanding is essential for evaluating ecological risks associated with nanoplastic pollution in diverse aquatic environments, especially those subject to variable light conditions.

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