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Effects of Nanopolystyrene on the Chlorophyll pigment content of Chlorella vulgaris

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ABSTRACT

This study used a long-term toxicity test to investigate how the microalga *Chlorella vulgaris* responded to nanopolysteren exposure under different light spectrums. Our experiment involved subjecting algae to polystyrene doses of 0, 0.5, 5, and 10 mg/L. We design an incubator consisting of three sections, each offering a distinct red, blue, and white light spectrum, for the purpose of cultivating algal colonies. Chlorophyll-a and b were estimated at consistent time intervals (1, 4, and 10 days) during the 29-day incubation period, using three replicate samples for each treatment. Experimental results have demonstrated a reduction in both algal cell viability and chlorophyll concentration when exposed to (5 and 10 mg/l) of nanopolysterene. The findings of our study indicate that the growth and chlorophyll content of *C. vulgaris* are significantly affected by the red-light spectrum and with the increase of nanopolyesterne concentrations.

Introduction

There is environmental concern of nanopolysterene (NPS), a kind of polystyrene characterized by its size on the nanoscale scale, arises from its possible effects on aquatic ecosystems [1]. Nanopolystyrene distinguishes itself from bigger polystyrene particles by its higher surface area relative to volume, which benefits in enhanced interaction with biological organisms [2]. These properties can define the interaction between NPS and aquatic microorganisms, such as algae [3]. The unicellular green alga *C.vulgaris* is an essential species in aquatic habitats, serving a vital function in primary production and nutrient cycling. Its responsiveness to environmental changes characterizes it as a valuable bioindicator for evaluating the impacts of contaminants. Recent study show that nanopolystyrene may have substantial physiological effects on *C. vulgaris*, affecting its growth, photosynthetic efficiency, and general well-being [4].

The adverse effects of nanopolystyrene on *C. vulgaris* are influenced by multiple pathways. Foremost, NPS particles have the potential to modify the physical and chemical characteristics of the aquatic environment, therefore changing the availability of nutrients and the quality of water. Furthermore, the direct contact between nanoparticles (NPS) and algal cells can result in damaging the cells or disturbed metabolic functions. Furthermore, the ability of nanopolystyrene to transport and discharge hazardous chemicals introduces an additional level of intricacy to its characteristics.

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An extensively used synthetic polymer, polystyrene, has been a major source of environmental contamination, especially in aquatic habitats [5].

In aquatic environments, the enduring presence of polystyrene particles, especially microplastics, presents a possible hazard to aquatic flora and fauna. One crucial aspect to consider is the influence of polystyrene on chlorophyll pigments, which are vital for the process of photosynthesis in aquatic plants and algae[5].

Persistent solutes (PS) are substances that can persist in the environment for long durations [6,7] and are widely considered to be pollutants in aquatic ecological systems [8]. Biological deterioration, weathering, and UV photo-oxidation of larger PS plastic fragments in aquatic settings lead to the production of smaller particles called micro (<5 mm) and nanoparticles (1–1000 nm%). A wide range of consumer products, including paints, biosensors, nanocomposites, waterproof coatings, nanomedicine, photonics, medical diagnostics, cosmetics, and pharmaceuticals, use PS nanoplastics (PS-NPs) due to their unique properties such as high adsorption capacity, large surface area to volume ratio, and remarkable surface curvature, which enable them to be highly mobile [13]. Researchers have often used engineered PS particles as a model of plastic micro- and nanoparticles to study their toxicity and effects on aquatic organisms[14, 15, 13; 16 ,17,11,18]. Most published data currently focus on the toxicity of PS microplastics in microalgae[7], despite reports of the toxic effects of PS-NPs on microalgae. For instance, PS-NPs lowered chlorophyll levels, stopped growth [19], slowed down photosynthesis, and made more reactive oxygen species (ROS) [20]. Moreover, researchers have observed the adhesion of PS-NPs, particularly positively charged nanoplastics like PS-NH2, onto algal surfaces [21,15].

Green microalgae, which are primary producers at the base of food webs, play an important role in ecosystem productivity and sustainability[22]. Any disturbance to them could have ecosystem-wide consequences [23].

This study used green microalgae C. vulgaris, widely used for toxicity tests due to their easy culture, short growth periods, and high sensitivity to environmental pollution [22,23]. Given the toxicity of noanopolysterene to this species of algae, the study's aim was to determine its effect on the chlorophyll pigment content.

Material and methods:

Algae isolates

Pure axenic cultures of the green alga *C. vulgaris* used in the current study were obtained from the Advanced Environmental Laboratory in the Department of Environmental Sciences, College of Science, University of Kufa. To ensure that the isolates were free of bacteria and fungi, a sample of the algal isolate was planted on the solid culture medium Nutrient agar for testing bacteria and was incubated at 37°C for 72 hours to ensure its purity[24].

PS-NPs preparation and characterization:

A 0.1 g of PS powder and 4 ml of ethyl acetate were combined, weighed, and then added to a conical flask fitted with a magnetic stirrer to create the polystyrene particles [25]. We used scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), and XRD, respectively, to confirm the size, shape, and surface chemical composition of the particles.

Study design:

Twenty ml of pure algae inoculum were taken and added to 480 ml of culture medium BG11 in a special incubator consisting of three sections representing three light spectra in the red spectrum, which has a light intensity of 57.64 micromol/m2/s, the blue spectrum had a light intensity of 256.5 micromol/m2/s, and the white spectrum had a light intensity of 99.16 micromol/m2/s. The samples were incubated in the incubator for 29 days after being exposed to different concentrations of nanopolystyrene (0.5, 5, 10) mg/liter in addition to the control treatment. Three replicates were placed for each treatment in three spectra (red, blue, and white). The photoperiod was (14–10)

light/dark using a timer system under a constant temperature of 25±2 C, and the farm was shaken twice daily to prevent algae accumulation on the walls. The experiment was repeated twice to achieve the goal and accuracy of the results.

Growth rate

The growth rate of Chlorella algae was evaluated by measuring the algae density daily using a spectrophotometer, to extract the optical density at a wavelength of 680 nm[26] . To determine the growth constant (K), the following formula was used [27]:

$$K = \frac{(\mathrm{LnOD1} - \mathrm{LnOD0})}{\mathrm{T}}$$

Determination of Chlorophyll a:

We immediately filtered each chlorophyll foliar into 25 milliliters using 0.45-micron GFC-type filter paper. We filtered the material and then placed it into tight-fitting 10-milliliter test tubes. We firmly sealed ten milliliters of 90% acetone. We stored the tubes at 4°C in the refrigerator for a full day. We then centrifuged the samples at a speed of 3000 revolutions per minute using a centrifuge. The absorbency was determined at 750, 647, 664 and 630nm using a spectrophotometer after gathering the filtrate. We used the following formulas [28] to calculate the concentration of chlorophyll a and b.

$$\textit{Chlorophyll a } (\mu g/L) = (12.7 \times A_{663} - A_{750}) - (2.69 \times A_{645} - A_{750}) \quad (1)$$

$$\textit{Chlorophyll b } (\mu g/L) = (22.9 \times A_{645} - A_{750}) - (4.68 \times A_{663} - A_{750}) \quad (2)$$

Results and discussion:

PS-NPs Description:

The sizes, shapes, and chemical compositions of PS-NPs was Nano sizes as verified by FESEM (Fig. 1) , FTIR (Fig. 4) and XRD (Fig.5). Prior to laboratory exposure, greater characterisation of PS-NPs' physicochemical properties in the exposure medium is required in order to better understand their behaviour and biological impact, as recommended by other investigations[29,30]. The morphology and particle size of PS and NPS were characterized by the FESEM technique. Fig.1 and 2 show monodispersed polystyrene nanoparticles with a particle size distribution of around 34–47 nm. Surfactants are essential to the nanoprecipitation technique. Without surfactant, polystyrene was shown to aggregate rather than produce stable nanoparticles[31]. The morphology of the PS nanoparticles prepared in the prepared in the lab is not spherical, and their average size is about 40 nm.

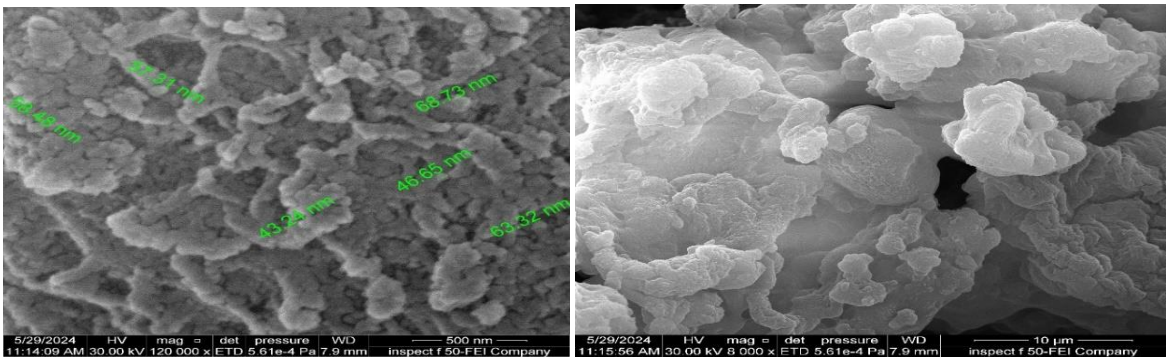


Fig1: FESEM microimage of Nano polystyrene (500nm and 10µm)

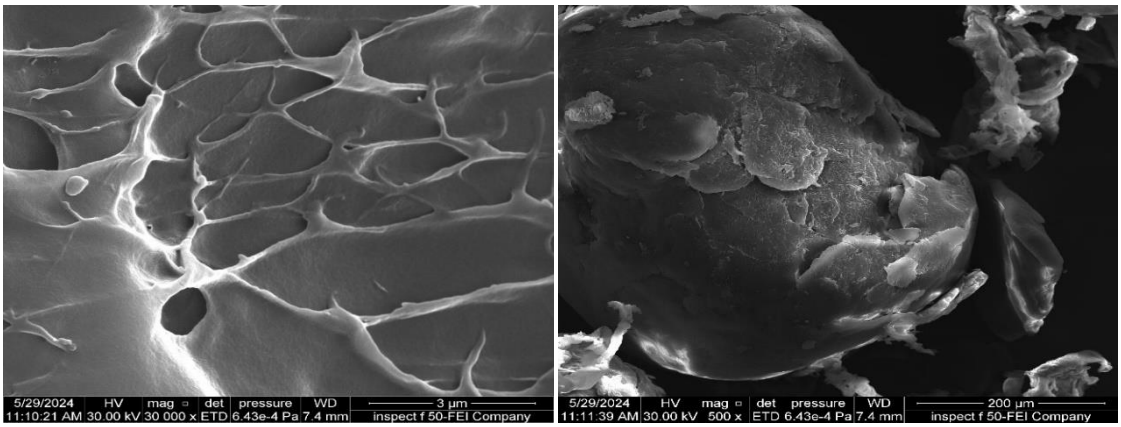


Fig 2: FESEM microimage of polystyrene (3 μm and 200μm)

Fig. (4) illustrates the polystyrene's infrared absorption spectra, Fig. 4 depicts the infrared absorption spectra of polystyrene, with an average particle size of around 40 nm. Multiple absorption peaks are observed within the specified wavenumber range. Alcohol (O-H medium) is represented by absorption peaks at wave numbers 3479 and 3417, carboxylic acid by (O-H stretching) peaks at wave numbers 3049, 2970, and 2884, anhydride by (C=O strong) stretching anhydride by wave number 1776, conjugated alkene by (medium C=C) stretching peaks at wave number 1600, and aromatic C=C stretching vibration absorption by wave number 1504, consistent with previously reported data[32]. These absorption peaks confirm the presence of benzene rings of the compound. The absorption peaks observed at wavenumbers 765 and 711 are characteristic of C-H out-of-plane bending vibration absorption. These peaks suggest the presence of a single substituent within the benzene ring [33].

This FTIR analysis has verified that styrene undergoes a polymerization reaction to form polystyrene. Furthermore, the absorption peaks visible at wave numbers 3479 and 3471 correspond to the stretching vibration absorption of O-H bonds, therefore suggesting the presence of hydroxyl groups. The hydroxyl group can be derived from water or by the process of hydrolyzing a strong acid or a weak alkali salt, such as sodium p-styrene or potassium hydrogen carbonate.

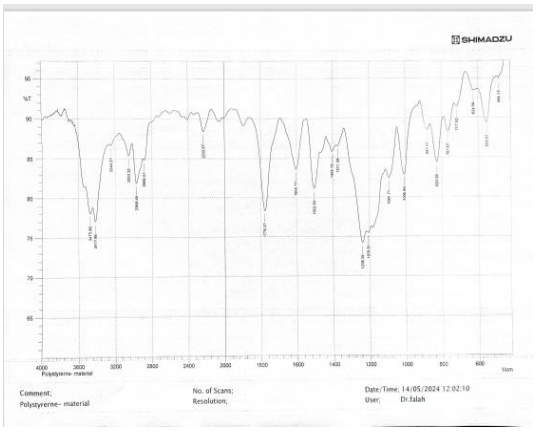


Fig.3: FTIR of polystyrene material

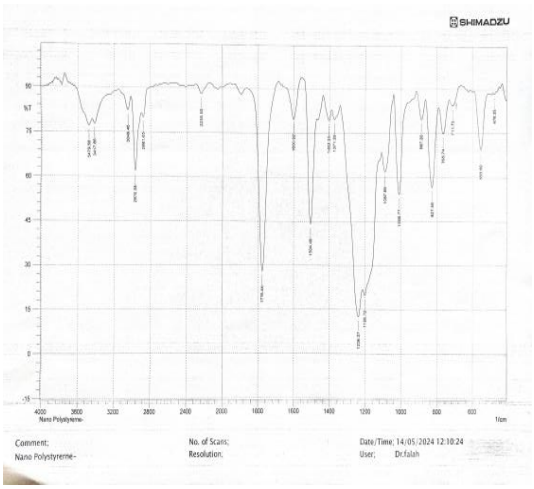


Fig.4: FTIR of Nano polystyrene material

For the purpose of characterizing a solid state sample, X-ray powder diffraction is an effective method. Every crystalline species has a distinct pattern of X-ray diffraction. An investigator can use a diffraction pattern to determine the identity of an unidentified species or to describe the atomic scale structure of a substance that has already been recognized. Fig. 5 displays the polystyrene materials' XRD diffract gram. For polystyrene, the most noticeable peak in the Nano polystyrene is seen at $2\theta = 18.0°$. Crystalline polymer material is indicated by the presence of peaks at $2\theta = 18.0$ and 25.

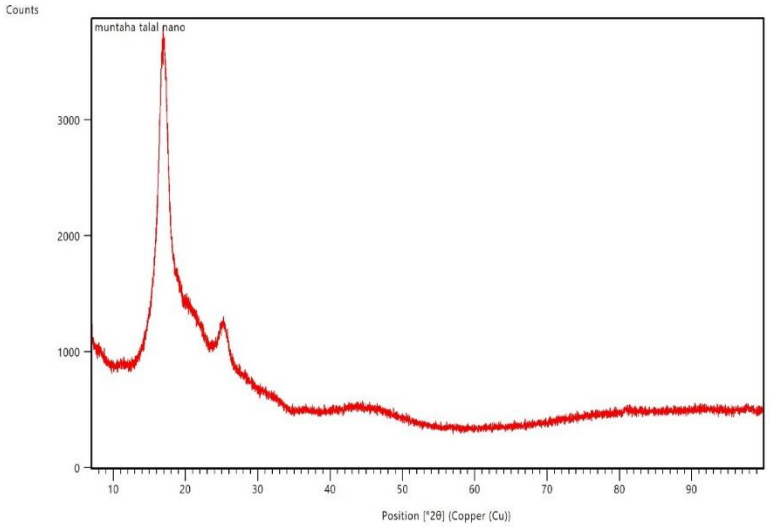


Fig 5: XRD of the Nano-Polystyrene.

Effects of PS-NPs on chlorophyll pigments of *Chlorella vulgaris*:

Fig. (6) illustrates the concentration of chlorophyll a pigment in *C. vulgaris* algae exposed to varying NPS concentrations under varying light spectra. The highest concentration of chlorophyll a recorded on the 4th day in the red spectrum at 10 mg/l treatment was 0.203 µg/l, while the lowest value of chlorophyll a recorded during the first day at 0.5 mg/l of NPS was 0.078 µg/l. In the blue spectrum, the highest concentration of chlorophyll a was recorded on the 10th day at a 0.5 mg/l treatment, and the lowest value was recorded on the first day at a 0.5 mg/l of NPS. As at the white

spectrum, the highest concentration of chlorophyll a recorded on the 10th day at control treatment was 0.82 µg/l, while the lowest value of chlorophyll -a was recorded during the first day at 0.5 mg/l of NPS was 0.098 µg/l. The statistical analysis shows that there are significant differences at the level of $P < 0.05$.

Fig. 7 showed the concentration of chlorophyll-b pigment when *C. vulgaris* algae was exposed to different concentrations of NPS under different light spectra. The highest concentration of chlorophyll- a recorded on the 4th day in the red spectrum at control treatment was 1.1 µg/l, while the lowest value of chlorophyll -a recorded during the first day at 5 mg/l of NPS was 0.139 µg/l. While at the blue spectrum the highest concentration of chlorophyll- a recorded on the 10th day at control treatment was 2.27 µg/l, and the lowest value of chlorophyll- a recorded during the first day at 0.5 mg/l of NPS, was 0.107 µg/l. As at the white spectrum, the highest concentration of chlorophyll- a recorded on the 10th day at control treatment was 2.99 µg/l, while the lowest value of chlorophyll -a was recorded during the first day at control was 0.111 µg/l. The statistical analysis shows that there are significant differences at the level of $P < 0.05$.
Moreover, the study a decrease in chlorophyll- a content compared to control. In the current study, we measured the maximum values of chlorophyll- a and chlorophyll- b when we treated *C. vulgaris* algae with LED waves in white, blue, and red at varying concentrations of NPS (0.5, 5, and 10 mg/l). An algae's growth directly correlates with its chlorophyll content, as chlorophyll is essential for light absorption and energy transformation [34]. With an increasing concentration of PS-NPs, the relative contents of Chl-a showed a wobbling trend.

Specifically, 5 mg/l of PS-NPs showed a stronger influence on Chl-a and b blue light, whereas 10 mg/l of PS-NPs showed a greater effect on Chl-a and b with red and white light. With the exception of red light, which decreased at tenth day, the effect of another factor—the light spectrum—on chlorophyll- a, and b however, increased progressively over time. These results were consistent with the previously noted reduction in algal growth, indicating that PS-NPs may impact algae development by interfering with photosynthesis, as the decrease in Chl-a may impede photochemical processes' energy transfer and metabolism [35].
Hazeem *et al*. [16] and [36] revealed that PS-NPs could reduce chlorophyll-a concentration during the exponential growth phase, demonstrating a direct effect on algal development and photosynthesis, which is consistent with our findings. Similar negative effects of PS-NPs were found on chlorophyll- a by [28]. This may affect algae photosynthesis by blocking the transport of light [20]. A recent study found that unstable PS-MPs (1 µm) had a bigger effect on algal photosynthesis than less stable PS-NPs (100 nm), which may explain what happened in this study [37].

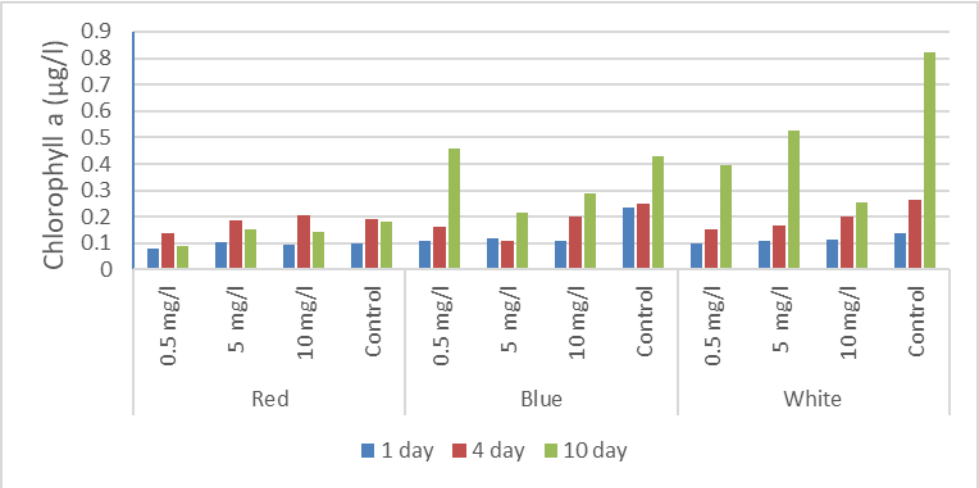


Fig.6: Effect of different concertations of NPS on chlorophyll- a of *C.vulgaris* at different spectra.

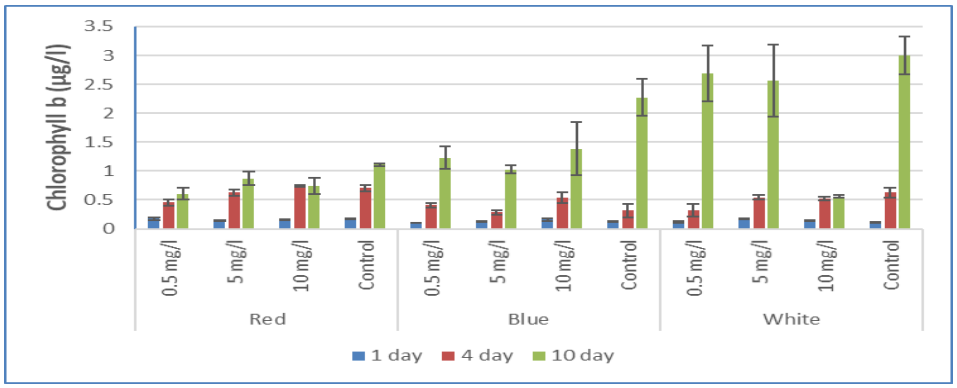
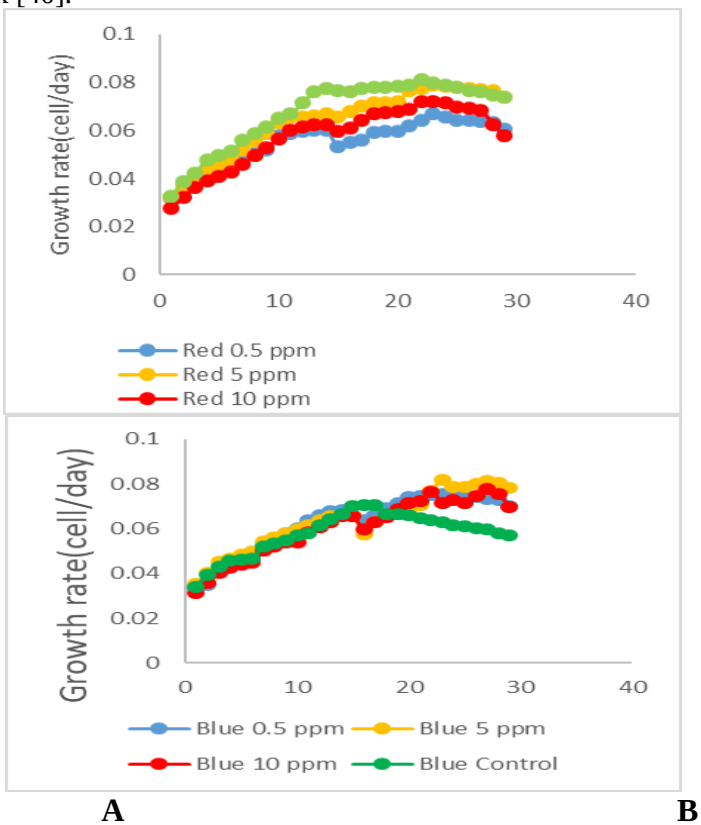


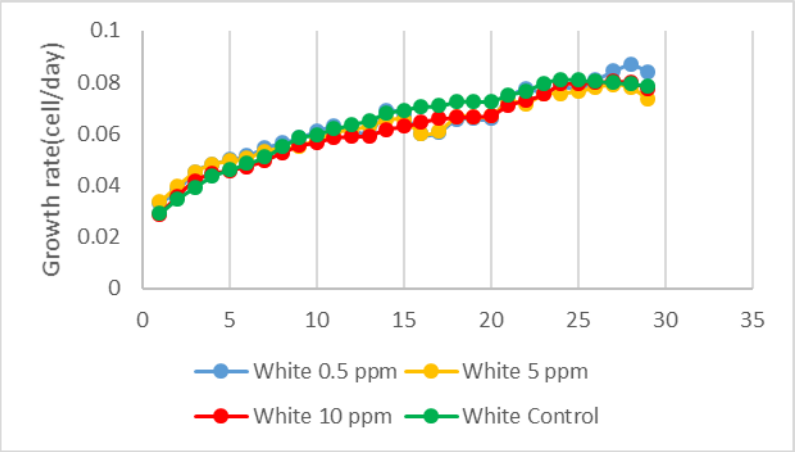
Fig. 7: Effect of different concertations of NPS on chlorophyll- b of *C vulgaris* at different spectra.

Effects of PS-NPs on growth rate:

As shown in Fig. 8, the effects of PS-NPs on the growth rate of C. vulgaris were investigated at different doses ranging from 0 to 10 mg/L. After 29 days of exposure, PS-NPs suppressed the development of C. vulgaris, with all treatments showing a decrease in growth when compared to the control at red light, while under blue light, all treatments decreased on the 16th day and then increased on the 19th day, except control and The increase continued until the 28th day (Fig. 8-B). As for the white color, all coefficients were close to each other and slightly lower than the control (Fig. 8-C), and [38.39] have observed that PS-NPs, at concentrations ranging from 10 to 100 mg/L, have a similar dose-responsive negative effect on the development of freshwater microalgae in the logarithmic phase [39] . Regarding varying PS-NP concentrations, C. vulgaris appeared to be more susceptible to 0.5 mg/l of PS-NPs, exhibiting a 16-day half-life (Fig. 8A). In contrast, there was a clear ($p < 0.05$) reduction in another batch of algae treated with PS-NPs at concentrations of 5 and 10 mg/L, respectively. According to certain research, PS-NPs' impact on algal development rose as their size shrank [40].



A **B**



C

Fig.8: Effect of different concertation's of NPS on growth rate of *C. vulgaris* at A) red light B) blue light C) white light

Conclusion

Nanopolystyrene had an effect on the growth rate, and as polystyrene affected the bio-pigments of the green alga under study, *C. vulgaris*, the concentrations of photosynthetic pigments represented by chlorophyll -a and b decreased with increasing concentration. The light spectrum had a joint effect with nanopolystyrene, especially the red spectrum.

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تأثير النانوبوليستيرين على محتوى صبغة الكلوروفيل لطحلب *Chlorella vulgaris*

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الملخص

تدخل المواد البلاستيكية الدقيقة إلى المسطح المائي من مياه الصرف الصناعي والمنزلي ومحطات معالجة مياه الصرف الصحي والجريان السطحي. ومع ذلك، هناك نقص في الأبحاث حول تأثيرها على الكائنات المائية. استخدمت هذه الدراسة لاختبار سمية واستجابة الطحلب Chlorella vulgaris للتعرض طويل الأمد للبوليسترين النانوي تحت أطياف ضوئية مختلفة. تضمنت التجربة إخضاع الطحلب لجرعات من البوليسترين النانوي (0 و0.5 و5 و10) ملغم / لتر. لقد صممنا حاضنة تتكون من ثلاثة أقسام، كل منها يوفر طيفًا مميزًا من الضوء الأحمر والأزرق والأبيض، لغرض زراعة مستعمرات الطحالب. قمنا بقياس مستويات الكلوروفيل أ وب على فترات زمنية ثابتة في اليوم (1 و4 و10) خلال فترة التجربة التي استمرت 29 يومًا، باستخدام ثلاث عينات مكررة لكل معاملة. وقد أظهرت النتائج التجريبية انخفاضًا في قابلية خلايا الطحالب للبقاء على قيد الحياة وتركيز الكلوروفيل عند تعرضها لجرعات مختلفة من النانوبوليسترين وخصوصا (5 و10 ملغم / لتر). تشير نتائج دراستنا إلى أن نمو وتخليق الكلوروفيل لطحلب C. vulgaris يتأثر بشكل كبير بطيف الضوء الأحمر والتراكيز العالية من النانوبوليسترين.

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