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INTERACTION OF CLADOPHORA GLOMERATA MACROALGAE AND MICROPLASTIC IN BAHR AL-NAJAF, NAJAF, IRAQ

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Abstract

Bahr al-Najaf is a body of water located on the western side of the city of Najaf in Iraq, behind the Wadi al-Salam cemetery. It is 15 km long and has been subject to drought in recent years. Many species of birds and fish live in it. The Najaf Sea area also contains many sand quarries and brick factories. Many names have been given to the Sea of Najaf since the beginning of its inception. It was known to the Arameans as (Farsha), which means in their language (The Trustworthy), and to the Jews it was known as (Hashir). However, during the Sassanid era, the Sea of Najaf was given the name (Al-Jawf). During the era of Alexander the Great, it was known as (Lake Rumiah) and (the Marshes of Rumiah) and the Arabs in the pre-Islamic era called it (the Sea of Pancia). The idea of drying the Najaf Sea dates back to the time of Alexander the Great, who tried to dry it to revive his lands and invest them in agricultural production, by creating a dam between the Babylon River and the Najaf Sea depression to prevent flood waters from

reaching it. However, it also did not dry completely until the year 1240 AH. As for Now you find it in the form of a body of water, and the dry parts are farms and palm groves.

The changes occurring in the physical and chemical properties of Bahr al-Najaf water body (temperature, pH, conductivity and dissolved oxygen) between December 2024 and December 2025 were due to changes in water levels. The study showed that the Najaf Sea ecosystem was exposed to pollution with plastic particles at a rate of up to 1.9%, especially in the southern and eastern stations of the study (S1, S2, S3, S4). The presence of different types of microplastic (MP) was also detected using FTIR spectroscopy of samples collected from the eight stations by the planktonic network, and transparent polyethylene (PE) was found to be the most abundant plastic particle. The concentrations of MP in the collected samples changed from (1.8×10^3 to 2.1×10^3 units/km²) in December 2024 to (1.6×10^3 to 1.8×10^3 units/km²) in December 2025. The growth and chlorophyll content in the macroalgae *Cladophora glomerata* showed an obvious decrease with the addition of different types of MP. PE has the greatest negative impact on these two parameters. In addition, macroalgae *Cladophora glomerata* showed great potential to absorb and accumulate MP molecules from agricultural media and act as a bioremediation agent.

Keywords: Macroalgae *Cladophora glomerata*, Microplastic, Toxicity, Biosolution, Bahr al-Najaf.

1. Introduction

Microplastic are a prevalent and persistent human risk and serious threatens to the polar regions, coastal waters, lakes, rivers, seas, and oceans (Sun *et al.*, 2026). The accumulation of microplastic waste can affects negatively macroalgae by stimulating inhibition of Growth, oxidation stress, and impaired photosynthesis (Sichen *et al.*, 2026). Recently, the path of microplastic poisoning has confirmed by heteroaggregation and adsorption interaction (Xu *et al.*, 2026) which impairs the absorption of nutrients and light macroalgae (Rasta *et al.*, 2026). Plastic is produced from mineral oil sources such as Polyethylene (LDPE), polypropylene (PP), polyethylene (PE), nylon, polyvinyl chloride (PVC) and polyacrylate and have the fate into our marine and aquatic ecosystem through the tourism activity and synthetic polymers (Salim *et al.*, 2025). The enhanced photochemically oxidation process and mechanical corrosion led to the breakdown of plastic in the ecosystem into microplastic pieces less than 5 mm of size (Rodrigo *et al.*, 2025). The dangerous of microplastic impact on the marine organism food chain has been revealed, and it has affected the ecology and biology of the biota (Shah *et al.*, 2026). Still at present, the macroalgae surface interacted with microplastic not well understood. As fresh water plastic waste accumulates, the macroalgae cell surface is highlighted in scientific research because it is considered a protective shield in the face of microplastic attacks (Luvai and Obiero, 2026). In the same regard, recent works focused on the effects of shading through the absorption of microplastic, as well as the defects that occur on the surfaces of macroalgae cells represents the deformations occurring on the surface of microscopic algae conclusive evidence of the toxicity of microplastic (Campbell *et al.*, 2026). They also represent clear phenomena of injury of macroalgae cell, which in turn helps in explain and assess the obvious microplastic poison impact. Despite the above, it has been found difficult to trace the molecular interactions at the level of microplastic with single cell macroalgae (Chen *et al.*, 2026). A number of recent studies have used scanning electron microscopy and optical microscopy technology (Palka *et al.*, 2026). There is also a scarcity of information about the interaction that occurs between microplastic with different properties and different algae species, which results in different morphological characteristics (Chaudhuri and Balasubramanian, 2026). In recent studies, researchers have documented the interaction between macroalgae species and microplastic particles in the pure aquatic environment and the release of oxidative

substances (ROS) in the algae as well as various changes in photosynthesis and growth (Şentürk and Oskay, 2026).

The current study aims to survey the main types of microplastic found in the study site (Bahr al-Najaf in Najaf, Iraq) and evaluate the physical and chemical properties of Bahr al-Najaf water. The research also addresses the effect of the types of microplastic collected by the planktonic network from Bahr al-Najaf water on the interaction with macroalgae. Last but not least, the research also reviews the measurement and study of the exopolymer secreted by macroalgae. And last but not least to measure changes in chlorophyll content and growth in macroalgae as a result of exposure to different types of microplastic.

2. Materials and Measuring

2.1. Study Site and Sampling

Bahr al-Najaf is a prominent geomorphological phenomenon in the Najaf Governorate and in the alluvial plain area. Structurally, it is a depression located on the border between the stable shelf (the Salman Range) and the unstable shelf (the Alluvial Plain Range). The area is traversed by three groups of faults that are divided according to their directions, such as: The White Fault, the Khar Fault, and the Euphrates Fault, in addition to the folds that characterize the study area, are considered one of the largest geomorphological phenomena present in the study area, as its length reached (40) km and its width (19) km, while its area reached (366 km²). It is 15 km away from the city of Najaf towards the west (Abdullah *et al.*, 2026). It is a depression of tectonic origin that has expanded due to weathering factors. The depression is bordered to the north and east by Tar al-Najaf, which is a rocky cliff, consisting of sand and clay rocks. It is bordered to

the west and south by the desert plateau. The depression is covered with sediments composed of clay, silt, sand, and gravel with salt particles (Al Helaly, Al Rammahi and Muhsin, 2026).

Eight water samples were collected between December 2024 and December 2025 from 8 locations (GPS location, S1: 32.030705, 44.220340, S2: 32.015278, 44.244715, S3: 31.997227, 44.270808, S4: 31.983250, 44.290034, S5: 31.967815, 44.262912, S6: 31.976261, 44.244715, S7: 31.986745, 44.226176, S8: 32.008000, 44.212786) in Bahr al-Najaf water body for evaluation the level of microplastic pollution and physical and chemical properties as shown in Fig. 1.



Fig. 1 Study location and sites sampling.

The process of collecting water samples and measuring the physical and chemical properties of the water (dissolved oxygen, pH, temperature, and conductivity) was done on site using a multi-sensor device (SensoDirect 150, Lovibond, Germany). Samples taken from the various sites were stored in one-liter glass bottles after being washed with filtered water. The plankton net used in the research to collect samples from the water of the Najaf Sea had dimensions (length: 190 cm, mesh size: 350 mm, diameter: 45 cm). The method of using the planktonic network was to place it next to the boat and be completely submerged. The speed of the boat was 1.5 km/hour and the process took 30

minutes (Figure 2). Samples were also collected from a depth of a quarter of a meter, adding methyldehyde to the collected samples at a rate of 5%.



Fig. 2 Planktonic net used in the sampling process.

2.2. Isolation of Microplastic

Eight samples were brought to the laboratory from the eight sites that were chosen in Bahr al-Najaf. The models were then filtered using a 0.45 mm filter, then the models were washed using filtered water, and finally they were closed well to be ready for the subsequent laboratory steps. The next step is the step of removing organic materials from the samples using hydrogen peroxide (30%). The samples are washed well with this solution and then returned to clean glass bottles and closed well. The closed sample bottles are transferred to a water bath at 65 degrees with shaking, and the process continues for three days. As a final step, the samples are filtered again, dried, and stored

in aluminum foil until they are examined later. Fig. 3 displays the types of microplastic samples collected from different locations in the waters of Bahr al-Najaf.



Fig.3 Microplastic items of different types, size and color collected from different sites in Bahr al-Najaf.

2.3. Microplastic Characterization

Through what was collected from various sites in the waters of Bahr al-Najaf, it was found that the types of microplastic commonly found in the water in different forms are polystyrene (PS) (blue and white), polyvinyl chloride (PVC), and polyethylene (PE), and they were in sizes ranging from (250-550 nm). Laboratory samples obtained from Chengdu Huaxia Chemical Reagent Co. Ltd., China were used for the purpose of making standard samples used in the current study in the culture media of macroalgae used in the study for bioremediation. First, a suspension of microplastic is made in distilled water, and then the suspension is exposed to ultrasound waves in a special device for this purpose for 40 minutes to obtain a solution with a concentration of 1 g/L of microplastic, which is preserved for later use in experiments.

In the same way mentioned above, samples are prepared with different concentrations of microplastic and different types ranging from (0, 25, 250 mg/L of culture medium) for the macroalgae used in the study. The chemicals used in all stages of the current study are chemicals used for laboratory analysis, and all the prepared samples and cultivation media for the algae were dispersed so that they do not stick to the container containing them or settle. Infrared spectroscopy technology was used to analyze microplastic samples collected from water samples at various stations in Bahr al-Najaf. The device used in the study is FTIR (Bruker, Tensor II, German manufacture), with a spectral accuracy of up to 4 cm^{-1} and works within the range of $4000\text{--}400\text{ cm}^{-1}$ in order to diagnose the type of plastic in different samples. The tests were carried out in the laboratories of the Chemical Engineering Department at the College of Engineering, Al-Qadisiyah University.

2.4. Algae Cultivation

Cladophora glomerata (Green algae) is a macro-algae used in the study and the culture medium suitable for growing this type of algae was Chu's No. (10) under the conditions of : pH 8.5, temperature: $15\text{--}25^{\circ}\text{C}$, light intensity: $4000\text{--}6000\text{ lux}$, wave length of 980 for optimal density and 14-20 days cultivations with continues ventilation (Praba *et al.*, 2016). Solution containing algae in a volume of 250 ml in a conical flask under the culture conditions mentioned above was used for cultivating and propagating algae. Moreover, shaking four times a day to prevent the algae from settling at the bottom of the container.

2.5. Biochemical Factors Analysis

For the purpose of measuring the changes occurring in growth and the amount of chlorophyll in macroalgae as a result of their exposure to the influence of microplastic particles, the optical density was measured after planting the macroalgae in media containing different concentrations of microplastic particles and different types of them. The measurement procedures are summarized in the following stages: First, measuring the optical density of the macroalgae is measured at a wavelength of (680 nm) using ultraviolet and visible spectrometry (UV-Vis) technology (apel PD-303 spectrophotometer, Japan). As for measuring the amount of chlorophyll, it was measured according to the modified method as follows. The method consists of first placing 10 ml of macroalgae for 10 minutes in a centrifuge (number of revolutions 5000 rpm) to obtain algae cells and then washing them several times with distilled water. Steinman method was used for chlorophyll estimation (Steinman *et al.*, 2017). The fresh macroalgae *Cladophora glomerata* samples of 5 to 350 mg were dried at first and placed in vial with 15 mL of methanol then covered with foil. Secondly the vial was placed in refrigerator for a period of 2 h. Aliquot of 2 mL was transferred to cuvette of 3.5 mL in the spectrophotometer instrument for reading the spectra at 750 and 665 nm respectively. Another 0.1 mL acidified sample with HCL of 0.1 Normality was analyzed using spectrophotometer at 750 and 664 nm respectively after 90 sec. Finally, the content of chlorophyll was estimated in the form of ($\mu\text{g/g}$) using the following formula:

$$\text{Chlorophyll } (\mu\text{g}/\text{cm}^2) = 26.7(E_{664b} - E_{665a}) * (Ve / \text{Stratum Area } (\text{cm}^2)) * L (\text{cm}) \dots (1)$$

Following that:

$E_{665a} = (\text{sample absorbance at } 664 \text{ nm} - \text{blank absorbance at } 664 \text{ nm}) - (\text{sample absorbance at } 750 \text{ nm} - \text{blank absorbance at } 750 \text{ nm})$ before acidification

$E_{665a} = (\text{sample absorbance at } 665 \text{ nm} - \text{blank absorbance at } 665 \text{ nm}) - (\text{sample absorbance at } 750 \text{ nm} - \text{absorbance of blank at } 750 \text{ nm})$ after acidification.

V_e = volume of the extract in 90% acetone (mL) , 26.7 = correction of absorbance

L = length of the light path throw cuvette in cm.

2.6. Exopolymer Substance Determination

The amount of exopolymer (EPS) secreted by macroalgae as a result of its exposure and interaction with microplastic particles was measured using a method modified from Li and Li and Yang (Li and Yang, 2007). The macroalgae cells are first separated according to the method mentioned previously in the analysis of biochemical factors section. In order to obtain a suspension of algae with microplastic particles, a 90% NaCl solution was used and the suspension was kept in a conical flask at a temperature of 60 degrees Celsius with continuous shaking for two hours. To obtain the amount of external polymer (EPS) generated from the action of macroalgae, the macroalgae is extracted by placing the previous suspension in a centrifuge at 9000 rpm for 15 minutes, after which a sample of the external polymer is obtained for examination under the microscope.

2.7. Statistical Analysis

The experimental and control data for the current study were statistically analyzed using the Statistica 12 program. A one-way analysis of variance (ANOVA) was also performed to examine the role of differences between the experimental groups, assuming homogeneity and normality, and below the standard significance level (0.05).

3. Results and discussion

3.1. Water quality of Bahr al-Najaf.

The quality of water body in the the Bahr al-Najaf were tested using the physicochemical properties of pH, Temperature (T), Dissolved oxygen (DO) and Conductivity (C_e) for the period between December 2024 and December 2025 and the date were recorded and presented in Table 1. The acidity of Bahr al-Najaf water body using pH values were slightly acidic in general in all the recorded date in the different sites with slight differences between all sites for the period mentioned for previously. The recorded data of pH values were in the range between (4.28 to 4.45) in December 2024 and between (4.17 to 4.26) in December 2025. Temperature of the water body also were recorded and were found in the average values of 14°C to 18°C in the period of the study having small differences between the different sites. While, the DO data were found to have obvious changes for the period of the study and for the different sites. The DO data were changed between (6.34 to 10.21 mg/L) in December 2024 and (8.32 to 12.41 mg/L) in December 2025. The reduction in the values of DO for the period of the study is an indicator for dangerous changes in the water quality of Bahr al-Najaf and can be related to the decrease of the water level in the this period and due to the high competition

between the different organisms in the water environment. The final parameter was recorded in the present study was C_e and the values for this property were changed between (12.72 to 13.43 ms) and (10.72 to 12.63 ms) in the period of the study with small changes between the different sites. Once again the decrease of the water level in Bahr al-Najaf led to change the quality of the water body to more salty as found from the data recorded for this period.

Table 1 The physicochemical properties of Bahr al-Najaf.

| (a) December 2024 | | | | |
|-------------------|------|-----------------|----------|------------------|
| Station name | pH | Temperature, °C | DO, mg/L | Conductivity, ms |
| S1 | 4.44 | 15 | 8.20 | 13.43 |
| S2 | 4.41 | 15 | 9.19 | 13.34 |
| S3 | 4.39 | 15 | 6.34 | 13.22 |
| S4 | 4.45 | 15 | 6.71 | 13.18 |
| S5 | 4.32 | 15 | 10.18 | 12.88 |
| S6 | 4.31 | 15 | 9.92 | 12.97 |
| S7 | 4.28 | 15 | 10.18 | 13.43 |
| S8 | 4.27 | 15 | 10.21 | 12.72 |
| (b) December 2025 | | | | |
| Station name | pH | Temperature, °C | DO, mg/L | Conductivity, ms |
| S1 | 4.25 | 14 | 8.38 | 12.59 |
| S2 | 4.24 | 14 | 10.42 | 10.72 |
| S3 | 4.21 | 14 | 9.43 | 12.53 |
| S4 | 4.26 | 14 | 8.32 | 11.34 |
| S5 | 4.20 | 14 | 12.38 | 11.21 |
| S6 | 4.18 | 14 | 12.39 | 11.12 |
| S7 | 4.19 | 14 | 12.32 | 12.63 |
| S8 | 4.17 | 14 | 12.41 | 12.37 |

3.2. The Presence of Microplastic in Bahr al-Najaf

Using the planktonic net in Fig.1, the average contamination percentage was estimated as 1.9 % after testing the microplastic items collected from the different sites in the laboratory. The microplastic species collected from the different sites in the period of the study were changed between (1.8×10^3 to 2.1×10^3 units/km²) and (1.6×10^3 to 1.8×10^3 units/km²) in the period of the study with high changes between site and another as recorded in Table 2. The largest microplastic contamination values in the water body of Bahr al-Najaf were recorded in the sites of south and east of Bahr al-Najaf in the stations of (S1, S2, S3, S4) with nine times higher the other stations, while the lowest values were recorded in the north stations (S5, S6, S7, S8) for the period of the study. The same trend was concluded from the data recorded in the present study compared to the literature (Nie *et al.*, 2026)(Li *et al.*, 2026)(Ghimire, Timalisina and Uprety, 2026). The increase of microplastic contamination percentage led to negative effect on the metabolism energy, immunity and fish reproduction (Siddique *et al.*, 2026)(Khursheed *et al.*, 2026)(Xie *et al.*, 2026). Moreover, a monitoring system should be suggested as the food chain in Bahr al-Najaf water body was effected by the presence of microplastic and finally pass to the human body and affect the human health through eating fish and knowing that the hydrogen peroxide treated microplastic items was non-degradable in the present work.

Table 2 The microplastic items collected in Bahr al-Najaf

| Station name | MPs in October 2019, items/km ² (x10 ⁻³) | MPs in October 2020, items/km ² (x10 ⁻³) |
|--------------|---|---|
| S1 | 2.1 | 1.78 |
| S2 | 2.09 | 1.80 |
| S3 | 2.07 | 1.79 |
| S4 | 2.05 | 1.80 |
| S5 | 1.95 | 1.71 |
| S6 | 1.83 | 1.63 |
| S7 | 1.82 | 1.72 |
| S8 | 1.80 | 1.60 |

3.3. Microplastic Size, Color and Shape in Bahr al-Najaf

The microplastic items were found in the different sites having a variety in type, shape, size and color. However, the most abundant type of microplastic was PP with a ratio in the range of (60 to 70%) in the different sites as analyzed by the FTIR spectroscopy technology along with the presence of another different types like PVC, PE and PS as shown in Fig.6. Moreover, different shapes and sizes were recorded in the different sites with a range of size between 450 to 6200 μm is the most abundant and the wide range were 20 to 6200 μm

3.4. Variation of *Cladophora glomerata* Growth

Fig.4 shows the variation in the optical density of *Cladophora glomerata* with the change in microplastic concentration and time. In the culture media of *Cladophora glomerata*, an adverse effect was concluded in the first stage of growth in the presence of microplastic items with clear differences between the experimental and control samples. Adaptation time of *Cladophora glomerata* with the environment contaminated with microplastic items is different and is more than the time needed for normal environment

without microplastic (Rasta *et al.*, 2026). PVC was found have the higher effect on the growth of *Cladophora glomerata* compared to the other types of microplastic items like PE, PS and PP. special focus should be taken for the effect of microplastic chemical structure on the metabolism of *Cladophora glomerata*.

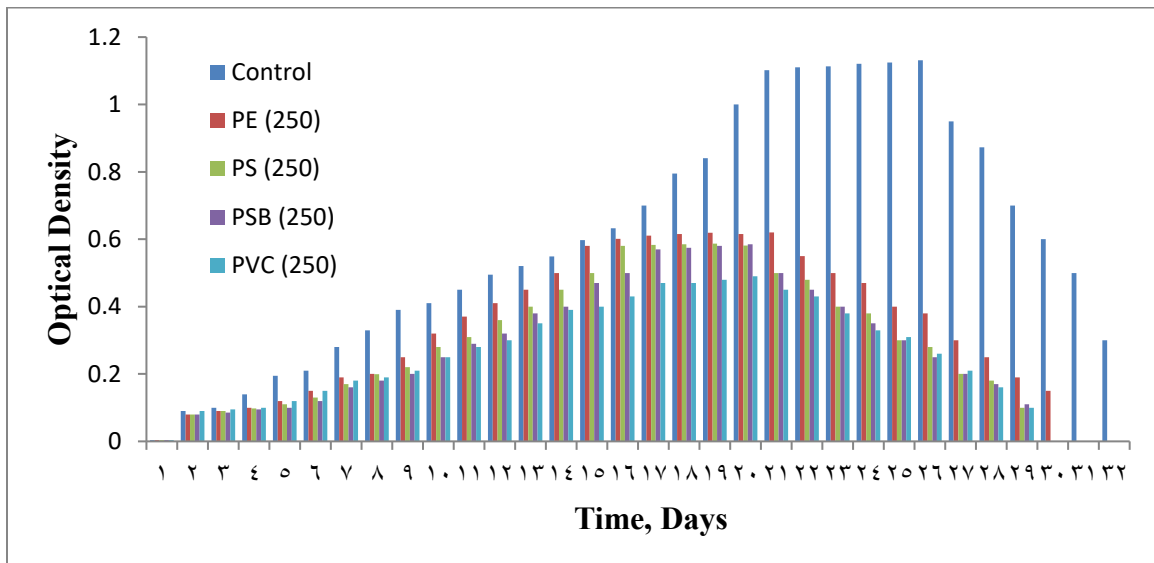
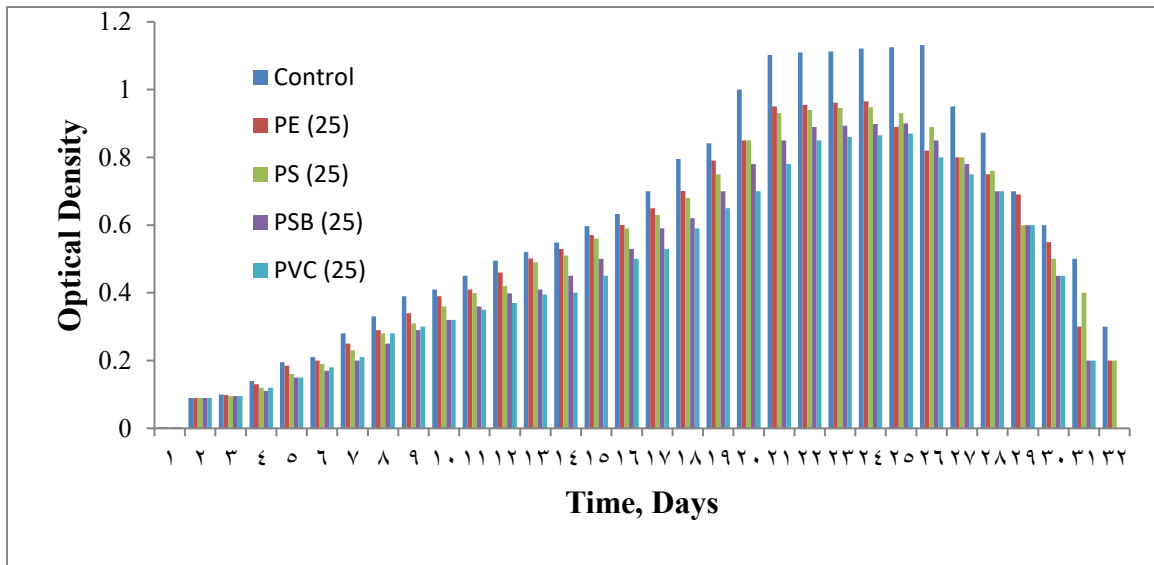


Fig. 4 variation of optical density in *Cladophora glomerata* due to microplastic concentration and time change.

3.5. Effect of Microplastic on Chlorophyll Content

Fig.8 shows the change of Chlorophyll Content with microplastic concentration for *Cladophora glomerata*. The Chlorophyll Content shows increase in the first five days then decrease trend with microplastic concentration with higher Chlorophyll Content in the control sample compared to the experimental samples. Moreover, the next five days showed more decrease in the Chlorophyll Content of experimental samples compared to the control sample. While, the higher change in the Chlorophyll Content was found in the presence of PVC items compared with the lowest change in the presence of PE items. Generally, the higher concentration of microplastic showed a negative effect on the Chlorophyll Content with same trend was concluded for the effect of color. However, the study of the chemical structure for the microplastic items and their effect on the metabolism of *Cladophora glomerata* that led to the changes in the chlorophyll content is recommended.

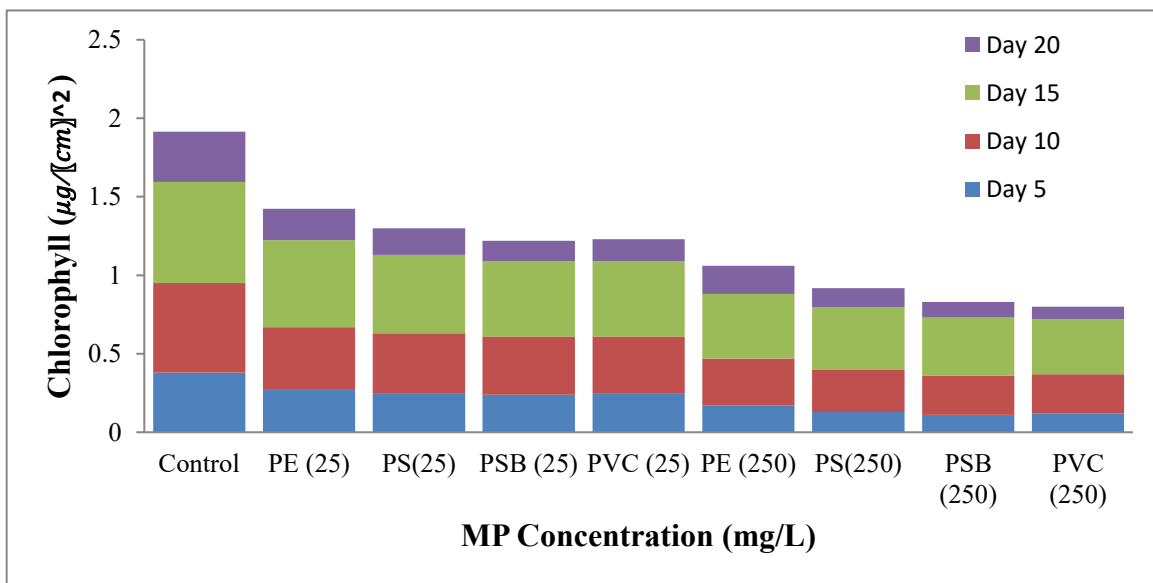


Fig. 5 Change of Chlorophyll content with microplastic concentration for *Cladophora glomerata*

3.6. Variation of Exo-polymeric substances (EPS)

Exo-polymer substance (EPS) can be defined as the excreted polymer from the *Cladophora glomerata* algae in the metabolic process and consist from lipids, DNA, proteins and polysaccharides (Gyte, 2026). *Cladophora glomerata* excreted more two times Exo-polymer with microplastic aggregate in the presence of microplastic compared to the control sample. The concentration and type of microplastic items effect the excretion of Exo-polymer substance from *Cladophora glomerata* which produced a lot of Exo-polymer substance in the culture media with the same results was concluded from other studies (Singh and Qidwai, 2025)(Kanzly *et al.*, 2026). A microscopic photo were shown in Fig. 4 for the different types of microplastic items and their interaction with the *Cladophora glomerata* species. The factors effecting on the efficiency of Exo-polymer substance excretion need to be studied on different macroalgae species and used for bioremediation.

4. Conclusion

Concluded remarks in the present work as follows:

1. the physicochemical properties of pH, Temperature (T), Dissolved oxygen (DO) and Conductivity (C_e) for the period between December 2024 and December 2025 in Bahr al-Najaf were changed generally due to the water level decrease.
2. Microplastic items showed increase by 2.1% between December 2024 and December 2025 in Bahr al-Najaf and a real monitoring system should be used on the disposal of plastic items in Bahr al-Najaf water body.

3. Food chain in Bahr al-Najaf was found to be effected by the increase the concentration of microplastic which led to human risk after eating fish contaminated with microplastic
4. PE items were found the most abundant microplastic in Bahr al-Najaf after checking by the FTIR spectroscopy technology.
5. chlorophyll content and growth of *Cladophora glomerata* were affected adversely in the presence of microplastic in Bahr al-Najaf.
6. Excretion of Exo-polymer substance due to the adsorption of microplastic by *Cladophora glomerata* was found a good bioremediation process in Bahr al-Najaf.
7. The highest microplastic effect was concluded from the PVC items on the chlorophyll content and growth of *Cladophora glomerata*

5. Recommendations

Recommended remarks for the future work as follows:

1. Study of the chemical structure for the microplastic items and their effect on the metabolism of *Cladophora glomerata* gene.
2. The factors effecting on the efficiency of Exo-polymer substance excretion need to be studied on different macroalgae species and used for bioremediation.
3. Study the effect of microplastic chemical structure on the chlorophyll content.

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