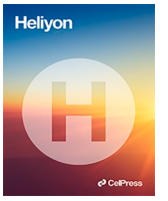
Heliyon 10 (2024) e32881

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

# Recent progress on the toxic effects of microplastics on *Chlorella* sp. in aquatic environments

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ARTICLE INFO

*Keywords:*
Microalgae
*Chlorella* species
Microplastics
Oxidative stress

ABSTRACT

Microplastics (MPs) are emerging contaminants that have harmful effects on ecosystems. Microalgae are important primary producers in aquatic environments, providing nutrients for various organisms. These microorganisms may be affected by MPs. Therefore, it is important to investigate the toxicity aspects of different MPs on *Chlorella* species. It can be seen that the BG-11 culture medium was the most commonly used medium in 40 % of the studies for the growth of *Chlorella* sp. *Chlorella* sp. grows optimally at a temperature of 25 °C and a pH of 7. Most studies show that *Chlorella* sp. can grow in the range of 3000–6000 lux. Moreover, various techniques have been used to analyze the morphological properties of MPs in different studies. These techniques included scanning electron microscopy (SEM), Fourier transform infrared (FTIR), and transmission electron microscopy (TEM), which were used in 65 %, 35 %, and 27 % of the studies, respectively. 53 % of the research has focused on the toxic effects of PS on *Chlorella* sp. Findings show that 41 % of the studies investigated MPs concentrations in the range of 10–100 mg/L, followed by 32 % of the studies in the range of 100–1000 mg/L. The studies found that MPs were used in a spherical shape in 45 % of the cases. The enzymes most affected by MPs were superoxide dismutase (SOD) and Malondialdehyde (MDA), accounting for 48 % of the studies each. Additionally, exposure to MPs increased the activity of enzymes such as SOD and MDA. In general, it can be concluded that MPs had a relatively high negative effect on the growth of *Chlorella* sp.

## 1. Introduction

Plastics are essential materials used in everyday life. In 2019, the global production of plastics was estimated to be 460 million tons [1]. It is predicted that the increase in plastic consumption will lead to a significant rise in the amount of plastic in the ocean by 2050, potentially equaling the quantity of marine organisms [2]. Plastics infiltrate the aquatic ecosystem via water pathways, discharges from industrial enterprises, municipal sewage discharges, as well as wind and tidal dynamics [3]. The widespread use and accumulation of plastics in the environment have raised significant concerns about their impact on various living organisms, including plants, microbiomes, and aquatic species. Several studies have investigated these effects [4,5]. In addition, the degradation of plastics in the environment is slow due to their high molecular weight and hydrophobic properties [6]. However, chemicals and additives used in

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https://doi.org/10.1016/j.heliyon.2024.e32881
Received 14 December 2023; Received in revised form 8 June 2024; Accepted 11 June 2024
Available online 13 June 2024

plastic production can be toxic and harmful to terrestrial and marine organisms [7]. These substances can lead to endocrine problems and cause digestive disorders, stress, growth reduction, and reproductive disorders in living organisms [8,9].

Plastic waste undergoes degradation processes such as weathering, exposure to sunlight, wind, waves, and temperature fluctuations, which lead to the formation of MPs [10]. MPs refer to particles smaller than 5 mm [3], classified into two primary and secondary categories. Primary MPs are microscopically produced and originate from personal care products and synthetic fabrics, while secondary MPs are created when larger plastic materials fragment [11]. MPs and nanoplastics (NPs), due to their small size and their capacity to absorb and transport various pollutants, can travel long distances and accumulate in different environmental compartments, posing a significant threat to ecosystems and human health [12]. These pollutants have a high capacity to adsorb and transport various pollutants, including pesticides [13,14]. MPs are ingested by organisms, penetrate the food chain, and ultimately enter the human body through contaminated food [15]. These pollutants can pose a significant risk to aquatic ecosystems and human health by

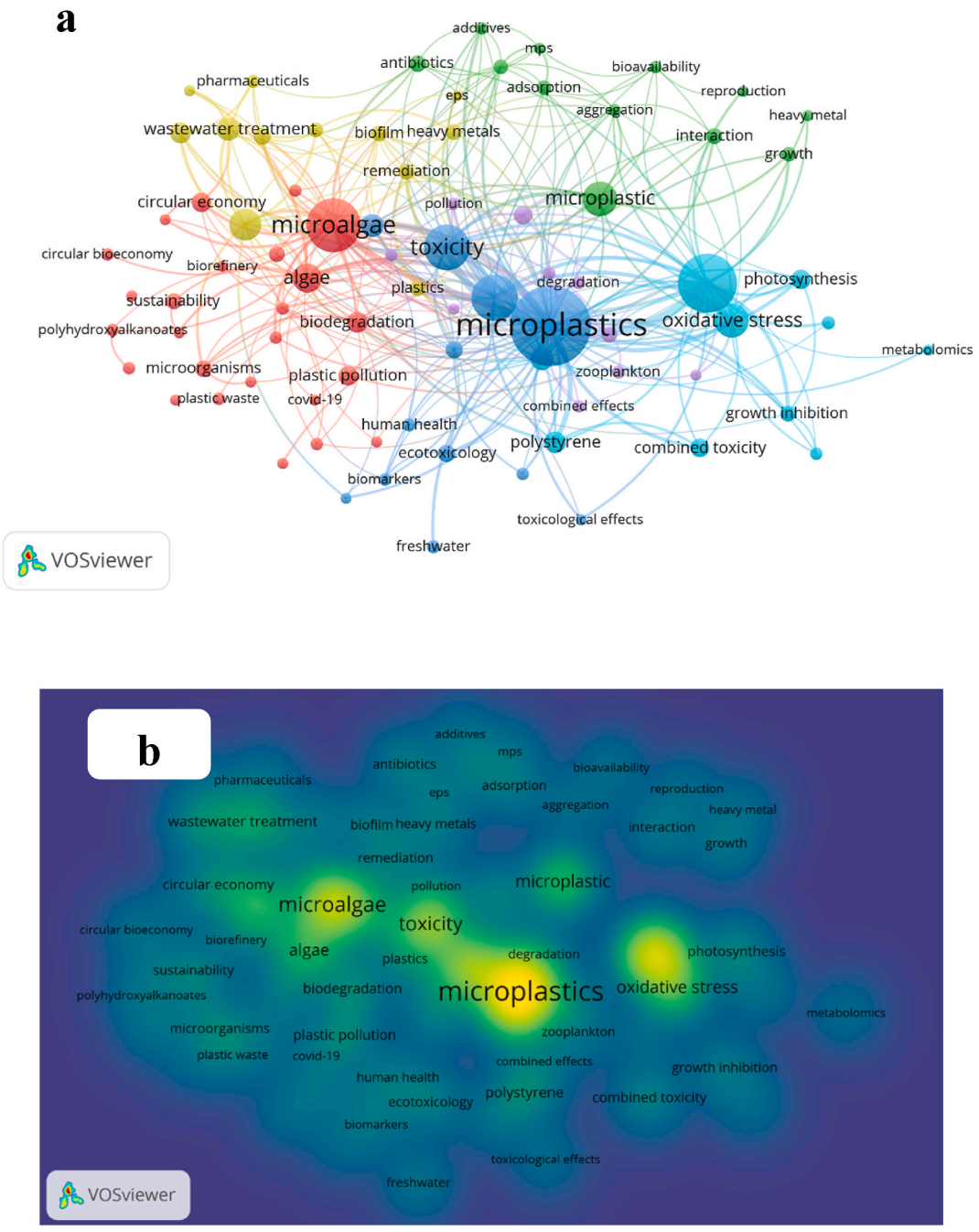


**Fig. 1.** a) Communication network of MPs interacting with algae and visualization of relationships between links and nodes and b) Density map.

absorbing and transporting other pollutants, including heavy metals [16]. Heavy metals, such as copper, cadmium, lead, and chromium, can induce oxidative stress in algae. This stress can lead to reduced growth rates, changes in pigment production, and the development of antioxidant defense mechanisms [17]. These tiny plastic particles adsorb various organic pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), perfluorinated alkyl substances (PFAS), polybrominated diethers (PBDs), pesticides, heavy metals, pharmaceuticals, personal care products (PPs), radionuclides, and nutrients, thereby increasing their toxicity [18,19]. In a study, MPs were found in the tissues of marine organisms in the seawater [20]. The adverse effects of MPs on marine organisms include a reduction in feeding rates, physical damage, induction of oxidative stress, and decreased neurofunctional activity [21]. These pollutants can alter growth conditions and inhibit microorganism metabolic processes [22]. MPs can reduce biodiversity and disrupt different ecosystems, thereby having negative effects on the overall health and functioning of aquatic and terrestrial environments [23,24]. Beneficial microorganisms, such as the yeast Saccharomyces cerevisiae and various species of algae, play a crucial role in preserving a healthy environment and promoting sustainable practices [25–28].

Microalgae are the most abundant microorganisms in aquatic environments, comprising approximately 72,500 species. These microorganisms are responsible for half of all photosynthesis on Earth [29]. Microalgae have a relatively high growth rate and are capable of converting solar energy into chemical energy, fixing atmospheric carbon dioxide, and serving as primary producers. They also provide essential nutrients for terrestrial and aquatic organisms [30]. Small disturbances in microalgae populations may have serious consequences for food webs. Microalgae are also used to monitor environmental changes and assess ecological risks [31]. Some materials, such as zeolites, can effectively stimulate the growth of marine microalgae due to their high surface area and rough texture, providing a favorable environment for algal cultures [32]. However, these microorganisms may be affected by heavy metal pollutants, such as chromium. Therefore, a bioindicator can be used to assess the levels of these pollutants in aquatic environments [33]. MPs can inhibit the growth of microalgae, affect their photosynthesis, and induce oxidative stress in them. Moreover, these small plastic particles can adhere to the surface of microalgae, obstructing the exchange of materials and reducing the microalgae's access to light, compromising their photosynthesis [34,35].

*Chlorella* sp. are the most notable unicellular green microalgae that commonly grow in aquatic environments [36]. These green microalgae are considered important biological indicators in aquatic environments and constitute a significant part of the food chain in aquatic ecosystems [36,37]. These microorganisms have properties such as sensitivity to toxic substances, tolerance to high temperatures, and the ability to grow in small amounts of nutrients [35]. Moreover, *Chlorella* sp. have a simple cell cycle, photosynthetic activity, and metabolic pathways that are similar to those of higher plants. They are also used in various applications, such as biofuel production and water treatment. But this type of algae may be affected by various pollutants, such as microplastics, which pose challenges to their activity in the environment. Therefore, understanding the impact of MPs on *Chlorella* sp. can have implications for ecosystem health, food security, and industrial processes [38]. The objective of the review was to assess the impact of MPs on *Chlorella* sp., aiming to gain insights into the ecological repercussions of MPs contamination on microalgae and devise strategies to mitigate these impacts. In addition, this study investigated the effect of inhibitory factors, such as polymer type, *Chlorella* sp., exposure time, and properties of MPs, including size, shape, and concentration, on the growth of *Chlorella* sp.

## 2. Methodology

### 2.1. Search strategy

A bibliographic survey was conducted to identify the available papers. The research papers were reviewed on August 20, 2023, by searching through the ScienceDirect, PubMed, Google Scholar, and Scopus databases. For the study, keywords such as "microplastics", "algae", and "*Chlorella*" were used, leading to the identification of 655 articles. The search was based on the titles, keywords, and abstracts of papers. Screening was conducted by applying exclusion criteria. In this study, articles that examined the inhibitory impact of microplastics (MPs) on the growth of fungi and zooplankton were excluded. Additionally, articles written in languages other than English, technical reports, conference papers, and short communications were also omitted.

Fig. 1 shows the keyword map representing the global research trend, created using VOSviewer 1.6.19 software. The minimum number of occurrences for a term was 4. According to Fig. 1, the 46 keywords with the highest frequency appeared most frequently in the research papers consulted through their titles, abstracts, and keywords. The term "microplastics" has the strongest semantic association with other words. Fig. 1a shows an overlay visualization map, where circles with labels represent items. The size of the circle and its label indicate the keyword's weight, with larger sizes indicating greater importance. Some lower-weight keywords are not displayed to prevent overlap. The proximity of keywords is significant, with closer words indicating stronger connections. The overlay image includes a color bar representing the publication year of articles. Fig. 1b shows a visualization map of keyword density. Keywords are represented by labels, and each keyword is assigned a color indicating the density of words in that area. The color range goes from blue to green to yellow, with yellow indicating higher density. The density map highlights "microplastics", "microalgae", "oxidative stress," and "toxicity" as hotspots, suggesting potential for further research in these areas.

### 2.2. Review and categorization

Summary information of articles was categorized based on the following criteria: *Chlorella* sp. and the growth conditions required for *Chlorella* sp. (including culture medium, temperature, and pH), the type, size, and shape of the polymer, concentration of MPs, and exposure time.

**Table 1**
Effects of MPs on the growth inhibition of *Chlorella* sp.

| Type of MPs | *Chlorella* sp. | Growth conditions | Analyzes | Rate of inhibition | Ref. |
|---|---|---|---|---|---|
| PET | Chlorella sp. | BG-11 medium + modified f/2, 30 °C, 6000 Lux | SEM, TEM | 21.1 % | [39] |
| | Chlorella sp. | modified bold basal medium, 27 °C, 12/12-h light/dark cycle, Ph = 6.4-6.8 | Spectrophotometer at $\lambda$ of 670 nm, Camera equipped optical, microscope | 3.85 % | [40] |
| | C. vulgaris | OECD medium. | Fluorescence, microscopy, Wraycam microscope camera. | – | [41] |
| | C. pyrenoidosa | 25 °C, 5000-5500 xl, 12/12-h light/dark cycle, pH = 7 | | | |
| | C. vulgaris | Tris-acetate phosphate (TAP) medium, 23 °C, 100 μmol/m2/s 12/12-h light/dark cycle | FTIR, SEM, DLS, FCM, Spectrophotometric | 45 % | [42] |
| PE | C. Pyrenoidosa | BG-11 medium 25 °C, 4800 lux, light-dark ratio of 12:12h. | HPLC, spectrophotometer under 680 nm | 12.7 % | [43] |
| | C. vulgaris | Ningbo No. 3 medium, 27 °C, 100 μmol/m2/s light-dark ratio of 12:12h | FTIR, SEM, EDS laser particle size analyzer, Optical microscope | 47.24 % | [44] |
| | C. vulgaris | BG-11 liquid medium, 25 °C,46 μE m−2 s−1 light-dark ratio of 12:12h, pH = 7.1 | FT-IR, Raman spectrometer, fluorescence, spectrophotometer 785-nm diode laser spectrophotometer under 680 nm | 10.3 % | [45] |
| | C. vulgaris | BG11 medium, 25 °C, light-dark ratio of 12:12h, pH: 7.2–7.6 | FT-IR, SEM under confocal laser scanning microscopy | 40 % | [46] |
| | C. vulgaris | BG-11 medium, 26 °C, 3600 lux, pH: 7.1, light-dark ratio of 12:12h | SEM, FTIR | 85 % | [47] |
| | C.vulgaris Beij | BG-11 medium, 25 °C, 25 μmol photons $s^{-1}m^{-2}$ light-dark ratio of 12:12h | SEM | 3.4 % | [48] |
| | C. vulgaris | Ningbo No. 3 medium, 27 °C, 5000 lux, light-dark ratio of 12:12h | SEM, FESEM | | [49] |
| | C. vulgaris | Ningbo No. 3 medium, 27 °C, 5000 lux, light-dark ratio of 12:12h | SEM, FESEM ultraviolet–visible spectrophotometer (UV1800) | | [50] |
| | C. vulgaris | Ningbo No. 3 medium, 25 °C, light-dark ratio of 12:12h | SEM, ICP-OES, GC-MS, X-ray fluorescence, spectrometer | 33 % | [51] |
| PS | C. pyrenoidosa | BG-11 medium 25 °C, 40 μmol photons/($m^{2\cdot}$ sec),12:12 h light: dark cycle | SEM, TEM A pulse-amplitude-modulated, fluorometer AquaPen-C AP-C 100, microscopy | 29.5 % | [52] |
| | C. vulgaris | f/2 medium 27 °C,5000 lux, 12:12 h light: dark cycle. | SEM, XRD, FTIR hemacytometer optical microscope spectrophotometer microplate reader | 18.06 %, | [53] |
| | C. pyrenoidosa | f/2 medium 22C, 4500 lux 14 h light:10 h dark cycle | FE-SEM, TEM | 10 % | [54] |
| | C. pyrenoidosa | f/2 medium, 25 °C; 14 h light:10 h dark cycle | FE-SEM, TEM | <10 % | [55] |
| | C. vulgaris | Bold Basal Medium (BBM) (25 °C-26 °C) pH = 6.8 and 7.2 | SEM, ICP-OES | 20.33 % | [56] |
| | C. vulgaris | f/2 Medium 18 °C, 100 μmol/m2. sec 12:12 light: dark cycle. | LDH, FTIR, SEM TEM, ImageJ software | 55 % | [57] |
| | C. pyrenoidosa | 24 °C, 3000–4000 lx, 12:12 h light: dark cycle. | TEM, optical microscopy | <10 % | [58] |
| | C. pyrenoidosa | BG-11 medium 25 °C, 4800 lx light-dark ratio of 12:12h | SEM, TEM | 27.73 % | [59] |
| | C. vulgaris | BG11 medium 16 °C, 50 μmol $m^{-2}$ $s^{-1}$, light dark regime of 16:8 h | pulse amplitude modulation, fluorometry, flow cytometry | 51 % | [60] |
| | C. vulgaris | Ningbo No. 3 medium | SEM/ATR-FTIR optical microscope pycnometer | – | [61] |
| | C. marine | in the dark at 4 °C for 24 h. | SEM, TEM | | [62] |
| | C. vulgaris | BG-11 and f/2 medium, 25 °C, 3600 lx, 12:12 h light-dark cycle | integrated biomarker response (IBR), FT-IR, SEM | 18.47 % | [63] |
| | C. vulgaris | 25 °C, 45–50 μmol photon $m^{-2}$ $s^{-1}$, 12:12 h light-dark cycle pH = 7.5 | RT-PCR, Spectroscopy, HPLC-MS/MS | 73.1 % | [64] |
| | C. Sorokiniana | Bold's basal medium, 22 °C, 35.4 μmol/m2/s 12:12 h light dark cycle | TLC CFscan colour digital camera, Spectrophotometer, Confocal laser scanning microscopy | 11–12 % | [65] |
| | C. Sorokiniana | BG-11 medium 25 °C, 2000 lx 12:12 h light-dark cycle | SEM, Dichlorodihydrofluo escein diacetate (DCFH-DA) probe, FTIR | 27.27 % | [66] |

(*continued on next page*)

**Table 1** (*continued*)

| Type of MPs | *Chlorella* sp. | Growth conditions | Analyzes | Rate of inhibition | Ref. |
|---|---|---|---|---|---|
| | C. pyrenoidosa | BG-11 medium, 28 °C, 3000 Lx, 13/11 h light, pH = 7.2 | SEM, Hemocytometer UV-1100 spectrometer fluorescence electron microscope | 28.96 % | [67] |
| | C. vulgaris | BG11 medium 28 °C, 80 μmol $m^{-2}$ $s^{-1}$, 12/12 h light: dark cycle | automatic algae, counter, fluorometer laser particle size, analyzer video contact angle tester, spectroscopy XRD, SEM, assay kits | 53.63 % | [68] |
| | C. pyrenoidosa | BG 11 medium 25 °C, 4000 lx, 12 h/12 h (light/dark) cycle | SEM, FTIR, flow cytometry, phytoplankton, fluorometer, fluorescence spectrophotometer | 65.13 % | [69] |
| PVC | C. pyrenoidosa | SE medium 25 °C, 50 μmol •photons•$m^{-2}$s-$^{1}$, 12/12 h light: dark cycle | pulse-amplitude-modulated, fluorometer | 44.4 % | [35] |
| | C. GEEL-08 | Bold Basal Medium (BBM), 25 °C, 30–60 min, pH = 6.8 | FTIR thermogravimetric analysis (TGA) | 10.55 % | [70] |
| | C. vulgaris | Ningbo No. 3 medium, 27 °C, 5000 lx, 12/12 h light: dark cycle | SEM, FTIR hemacytometer optical microscope | 31.88 % | [71] |
| | C. pyrenoidosa | BG 11 medium 25 °C, 4000 lx, 12 h/12 h (light/dark) cycle | SEM, FTIR flow cytometry phytoplankton fluorometer fluorescence spectrophotometer | 24.93 % | [69] |
| PP | C. sp. | triple super phosphate (TSP) 25 °C, 3000 lx, pH = 7-8 | SEM, FTIR Spectroquant Pharo 300 M, microscopy | – | [72] |
| PA | C. pyrenoidosa | BG-11 medium 25 °C, 4800 lx 12:12 h light: dark cycle. | spectrophotometer | 16.98 % | [73] |
| PAN | C. Pyrenoidosa | BG11 medium 26 °C, 80 μmol $m^{-2}$ $s^{-1}$, and 12:12 h light: dark cycle. | R software | – | [74] |

## 3. Growth conditions of *Chlorella* sp.

### 3.1. Culture medium

Table 1 shows the effect of MPs on inhibiting the growth of *Chlorella* sp. According to Table 1, *Chlorella* sp. grow in various media, such as BG-11, modified Bold Basal, OECD medium, Tris-acetate-phosphate (TAP), Ningbo No 3, f/2 medium, SE medium, and triple super phosphate (TSP). From Tables 1 and it can be seen that the BG-11 culture medium was the most commonly used medium in 40 % of the studies for the growth of *Chlorella* sp.

### 3.2. Temperature

From Table 1, the growth of *Chlorella* sp. usually occurs in the temperature range of 25 °C–30 °C. Accordingly, 43 % of the studies showed that *Chlorella* sp. grows optimally at a temperature of 25 °C. Although the growth of this type of algae at other temperatures, including 27 °C, 26 °C, and 28 °C has occupied 16.21 %, 10.81 %, and 8.1 % of the studies, respectively. Most studies have identified the optimal temperature for Chlorella sp to be between 25 °C and 35 °C [75,76]. In a study, the optimal temperature found to achieve the highest biomass concentration of C. vulgaris was 25 °C [77].

### 3.3. pH

As shown in Table 1, most *Chlorella* sp. grow in the pH range between 6 and 8. This study showed that *Chlorella* sp. can grow in a wide range of pH values, but the optimum pH for the growth of *Chlorella vulgaris* is found to be 7 [78]. However, in one study, the growth of *C. vulgaris* was dominant at pH 6 to 6.5 [79]. Another study showed that both acidic (pH 3–6) and alkaline (pH 8–9) conditions delayed the growth of *Chlorella vulgaris*. Optimal growth occurred when the pH of the environment was adjusted to 7.5 and 8 [80]. Many studies have reported that adjusting pH and light intensity can increase cell density, which, in turn, influences the photosynthetic activity and growth rate of microalgae [81,82].

### 3.4. Light

According to Table 1, most studies show that *Chlorella* sp. can grow in the range of 3000–6000 lux. The optimal growth of *C. pyrenoidosa* was observed at a light intensity of 8000 lux, resulting in the highest average biomass [83].

### 3.5. Photoperiod

Table 1 shows that most *Chlorella* sp. grow best under a photoperiod of 12 h of light and 12 h of darkness. Of course, some *Chlorella* sp. grew under different photoperiod conditions, including 14 h of light and 10 h of darkness, 16 h of light and 8 h of darkness, and 13 h of light and 11 h of darkness.

## 4. Techniques used in studies

From Table 1, various techniques for analyzing the morphological properties of MPs can be observed. Accordingly, various studies utilized SEM, FTIR, spectrophotometry, and TEM in 65 %, 35 %, 27 %, and 22 % of cases, respectively. Additionally, other methods such as X-ray diffraction (XRD), spectroscopy, hemacytometer, fluorescence microscopy, flow cytometry (FCM), high-performance liquid chromatography (HPLC), optical microscopy, laser particle size analyzer, energy-dispersive X-ray spectroscopy (EDS), Raman spectrometer, ultraviolet–visible spectrophotometer, inductively coupled plasma optical emission spectrometry (ICP-OES), pulse-amplitude-modulated fluorometer, thermogravimetric analysis (TGA), and gas chromatography-mass spectrometry (GC-MS) have been utilized in various studies.

SEM is a microscopic technique used to observe the surface of MPs and detect changes such as cracks, pits, holes, and irregularities [84]. Fig. 2 show the SEM images of the exposure of *C. pyrenoidosa* to different sizes of PS MPs. From Fig. 2, MPs can adhere to the surface of cells. Fig. 2A and B illustrate the exposure of algae to microplastics (MPs) with sizes of 0.1 mm and 1 mm, respectively. Small PS MPs can cause more severe adverse effects on the maximum electron transport rate. The inhibition ratio for MPs under 0.1 mm was almost twice as high as that for 1.0 mm MP.

According to Table 1, FTIR analysis is a widely used method for evaluating changes in polymer functional groups and assessing changes in biomacromolecules on algae surfaces exposed to MPs [85,86]. This technique does not destroy environmental samples, is relatively simple, and has a high screening ability [87]. Xiang et al. (2023) used FTIR to measure changes in biomacromolecules on the surfaces of *C. vulgaris* exposure to polystyrene nanoparticles (PS-NPs) [86]. In another studies, this method was used to investigate the changes in the functional groups of *S. platensis* exposure to MPs [85,88].

Spectrophotometry is a commonly used method to assess the inhibition rate of algal growth in response to MPs. Different studies have utilized the spectrophotometry method to measure algal growth in response to various types of MPs [89–91].

As can be seen from Table 1, the TEM technique has been considered in several studies. This technique provides chemical information and produces high-resolution images with atomic-level spatial resolution. Fig. 3a shows the TEM image of PS microplastic composites with DBP at various concentrations [92,93].

Fig. 3 shows that the structure of algal cells may be compromised due to exposure to PS MPs. The damage to the structure of the algal cells included plasmolysis, vacuolation, and distortion of membrane structures in the cells after 96 h of exposure to 5 mg/L of PS [95]. Fig. 3b showed that the adsorption of PS onto algal cells altered cell shape and potentially inhibited photosynthetic activity [94].

## 5. Variables affecting the growth inhibition of *Chlorella* sp.

### 5.1. Chlorella sp.

Fig. 4 show that MPs can have a toxic effect on various types of algae, inhibiting their growth. As can be seen from Fig. 4, the majority of research (53 %) has focused on the toxic effects of MPs on *Chlorella* species. Therefore, in this study, *Chlorella* sp. were selected as the target algae. *C. vulgaris* is extensively studied in MPs pollution research due to its suitability as a model organism for toxicity testing, investigating the photosynthetic apparatus, and carbon sequestration. Its ease of cultivation in the laboratory and fast growth rate make it an ideal candidate for testing purposes [96]. Additionally, this microorganism has a short growth cycle, which

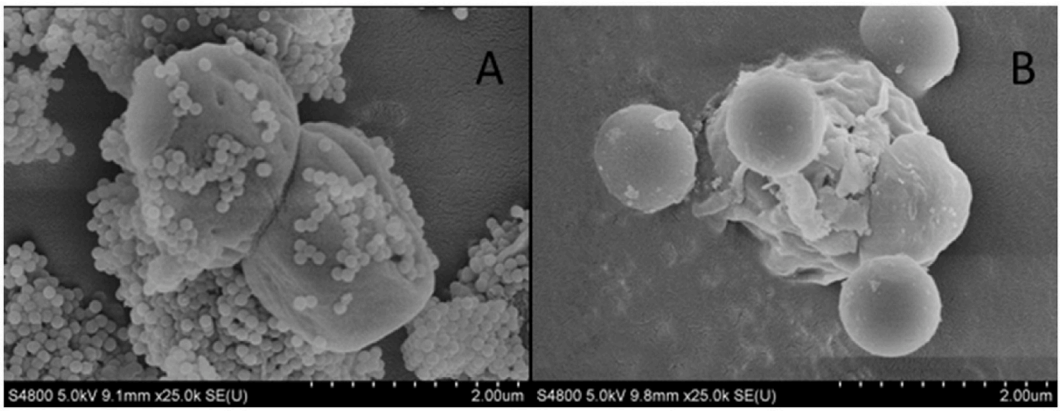


**Fig. 2.** (A) SEM images of *C. pyrenoidosa* exposed to 0.1 mm PS MPs and (B) exposed to 1.0 mm PS MPs [52].

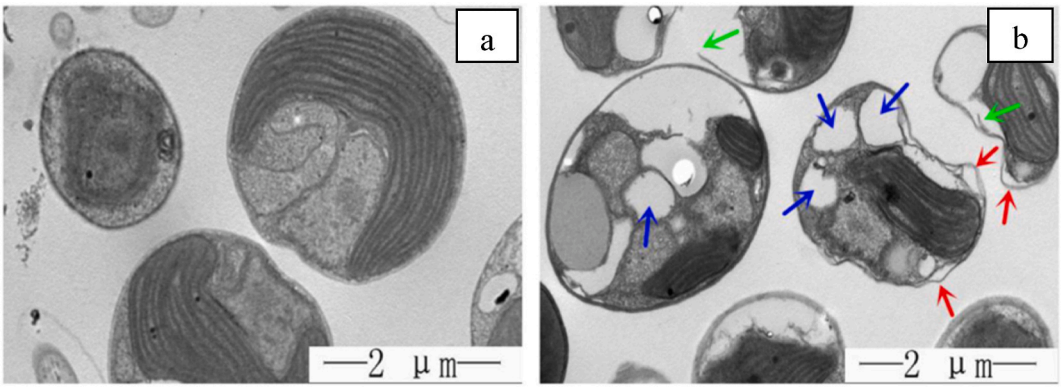


**Fig. 3.** (a) TEM images of the green algae *C. pyrenoidosa* after 96 h exposure to a control (f/2 without silicate), and (b) 0.55 μm PS particles (blue arrows indicated vacuolation; red arrows referred to plasmolysis; and green arrows suggested rupture and damage of cytoderm membranolysis in the algal cells) [94]

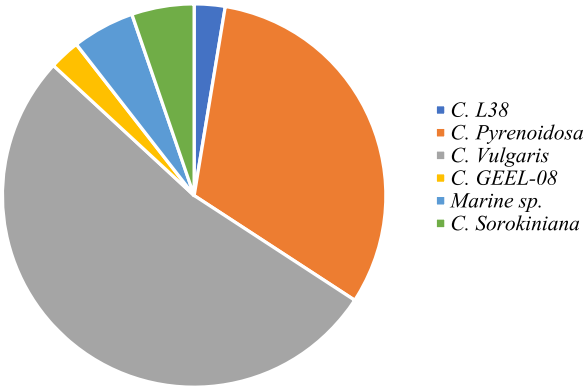


**Fig. 4.** Classification studies based on *Chlorella* sp.

enables a quick assessment of microplastic impacts on growth and other parameters. This species is highly sensitive to MPs, making it a valuable indicator for understanding the effects of MPs on aquatic organisms. *C. vulgaris* is rich in chlorophylls and carotenoids, which serve as excellent biological indicators and play a crucial role in the aquatic food chain. Unfortunately, MPs have been found to have a negative impact on the cell structure of *C. vulgaris*, resulting in various detrimental effects [97]. These effects include a significant decrease in algae growth in exposure to MPs. It has also been shown that the presence of MPs reduces the content of chlorophyll A in *C. vulgaris* and photosynthetic pigments. Furthermore, exposure to MPs leads to cell damage and aggregate formation in *C. vulgaris*. High levels of intracellular superoxide dismutase and malonaldehyde have been observed in the cells of *C. vulgaris* exposed to MPs [98].

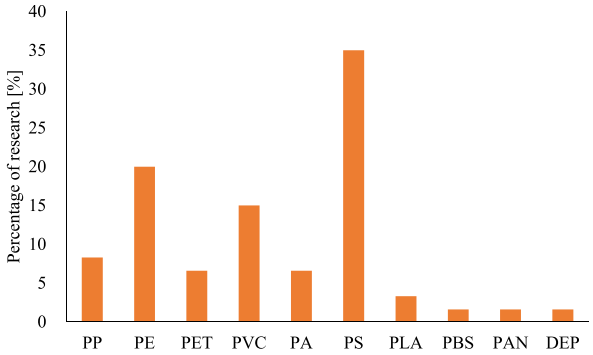


**Fig. 5.** Classification studies based on polymer type.

### 5.2. Polymer type

Based on Fig. 5, the toxic effect of PS on *Chlorella* species was investigated in 35 % of the studies, and it was used more frequently than other types of MPs. PS, as one of the most crucial materials in the modern plastic industry, has gained widespread global usage. This is primarily due to its exceptional physical properties, affordability, abundance, and commercial feasibility [65,99]. This microplastic has toxic effects on *Chlorella* sp., including inhibiting growth and photosynthesis, inducing oxidative damage, and causing morphological changes [55,67,95,97,100,101]. This mechanism of can vary depending on the size and concentration of the polystyrene particles. For example, exposure to smaller PS-NPs can result in greater morphological changes and loss of original shape, leading to a reduction in algal density and size [100]. In contrast, exposure to larger PS-MPs can significantly inhibit the growth of *C. pyrenoidosa* and reduce photosynthesis [67,101]. It has been found that PS causes oxidative damage to algal cells, which leads to a decrease in antioxidant activities and an increase in lipid peroxidation reactions [101]. Additionally, when combined with substances such as dibutyl phthalate or triphenyltin chloride, PS can disrupt cell structure and inhibit photosynthesis [55,95]. A study showed that exposure to PS-MPs significantly reduced colony formation and photosynthetic pigment content compared to both the control and PE-MPs of the same size [48]. PE and PVC can inhibit cell growth in *Chlorella* sp. by increasing oxidative stress [51]. In one study, it was found that PVC had an inhibitory effect on *C. vulgaris* [46]. The study by Song et al. (2020) indicated that PE, PET, and PVC had a negative effect over time, while PP inhibited growth [64]. Su et al. discovered that micro-sized polyethylene (PE), mPA, and polylactic acid (PLA) inhibited the growth of *C. vulgaris* [44].

### 5.3. MPs' size

MP size was known as a crucial factor in ecotoxicological risk assessments for microalgae [102]. The investigation covered six size ranges: 0–0.1 μm, 0.1–1 μm, 1–50 μm, 50–100 μm, 100–1000 μm, and >2000 μm. According to Fig. 6, the majority of studies focused on the 0–100 μm range, accounting for 35 %. Studies show that smaller MPs, due to their more hydrophobic properties, adhere more effectively to microalgae compared to larger MPs. As a result, they have a greater inhibitory effect on the growth of microalgae [75]. A study indicated that smaller MPs have a greater inhibitory effect on microalgae compared to larger plastic debris [83]. Hazeem et al. (2020) found that smaller MPs with a positive surface charge had a greater toxic effect on algae than larger ones, which is consistent with the findings of the Cao (2022) study [83,49]. However, some studies have provided conflicting results.

Mao et al. (2018) observed the highest inhibition rate of *C. pyrenoidosa* when exposed to PS particles with sizes of 0.1 and 1.0 μm [103]. In another study, it was found that the inhibitory effect of PS MPs on the growth of *E. gracilis* increases with an increase in MPs size [104]. Gong et al. (2022) discovered significant growth impacts of MPs of various sizes on *C. vulgaris* [105]. However, another study found that the size of PS MPs had no significant effect on the growth and photosynthesis of microalgae. But oxidative stress and microcystin production increased slightly. These conflicting findings suggest that the relationship between microplastic size and algal growth inhibition may not follow a linear pattern, as intermediate sizes potentially exert different mechanisms on algal growth [75,39–43,83,90,102]. Sjollema et al. (2016) found that smaller polystyrene particles had a greater impact on algal growth compared to larger particles [39]. Also, a study conducted in 2019 found that the toxicity of PS particles to green algae was dependent on their size, with smaller particles showing greater toxicity [40].

### 5.4. Concentration of MPs

Fig. 7 shows that 41 % of the studies investigated MPs concentrations in the range of 10–100 mg/L, followed by 32 % of the studies in the range of 100–1000 mg/L. Additionally, 16 % of the studies examined concentrations greater than 10 mg/L, while 11 % focused on concentrations less than 1000 mg/L. According to Table 1, the lowest concentration tested for marine *Chlorella* sp. was 0.01 mg/L for PS, while the highest concentration of 10,000 mg/L was used for *C. vulgaris* [48,62]. The toxicity of MPs on algae is highly dependent on their concentration and becomes more intense as the concentration increases [1]. Mao et al. (2018) determined that the growth of *C. pyrenoidosa* was inhibited by increasing the concentration of MPs [52]. Similarly, in another study, higher concentrations

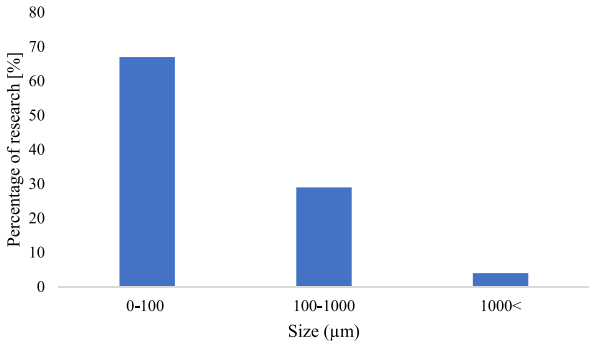


**Fig. 6.** Classification studies based on MPs size.

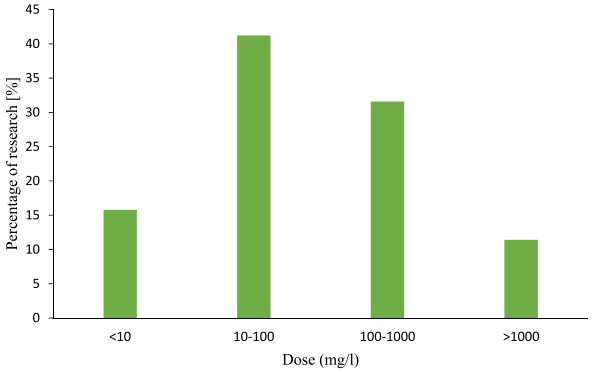


**Fig. 7.** Classification studies based on MPs dose.

of micro-sized mPE and mPA had a greater inhibitory effect on *C. vulgaris* [44]. In some studies, even low concentrations of MPs stimulate the growth of *C. vulgaris*, while higher concentrations can inhibit growth [70] [46,51,71,106,107]. However, a study revealed that lower concentrations of MPs have a greater inhibitory effect on microalgae compared to higher concentrations [53]. Generally, higher concentrations have a greater negative effect on algal growth than lower concentrations [108].

### 5.5. Shape of MPs

There was limited research on the effect of MPs shape on the growth inhibition of *C. vulgaris*. According to Fig. 8, the majority of the studies found that MPs had a spherical shape (45 %), followed by particles (31 %), fibrous (7 %), fragmented (7 %), asymmetric (7 %), and granular (3 %). The references do not clearly mention the specific mechanisms by which the shape of MPs affects the growth and physiological response of *C vulgaris*. It is likely that the shape of MPs affects these results through various mechanisms, such as altering the physical and chemical environment of algae, inducing stress responses, or interacting with algal metabolic pathways [109]. Overall, the results of the observed studies suggest that the size and type of MPs are likely to influence the growth inhibition of algae.

### 5.6. Contact time

Fig. 9 shows the classification studies based on exposure time. Based on Figs. 9 and 42 % of the studies were conducted within the 0–5 day period. This was followed by 17 % of the studies conducted within the 5–10 day period, 22 % within the 10–20 day period, 5 % within the 20–30 day period, and 14 % within a period of more than 30 days. Exposure to MPs can impact the growth of *Chlorella* sp. However, the magnitude of this effect may vary depending on factors such as the type and concentration of MPs. Moreover, with an increase in exposure time, the algae were able to recover through self-regulation during prolonged exposure to the hazardous environment, leading to enhanced resistance and adaptability [1]. Wenchao et al. (2022) illustrated that the maximum effect of DEP on the growth inhibition of *C. pyrenoidosa* was observed during the 0–2 day period [110]. In a study, the inhibitory effect on the growth of *Chlorella* algae gradually decreases with increasing exposure time to MPs [111]. Also, some studies have shown that a high concentration and small size of PS have a significant inhibitory effect on algal growth [112–114].

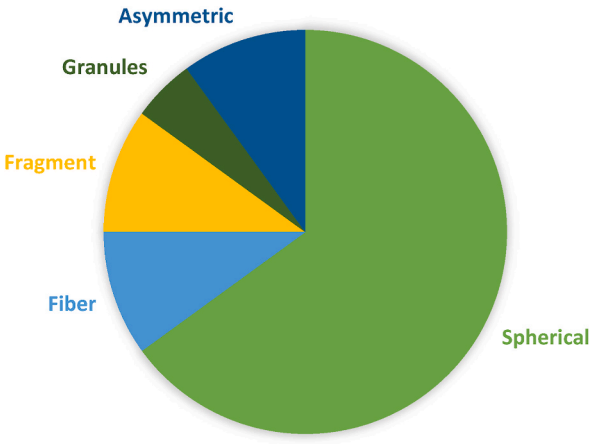


**Fig. 8.** Classification studies based on the shape of MPs.

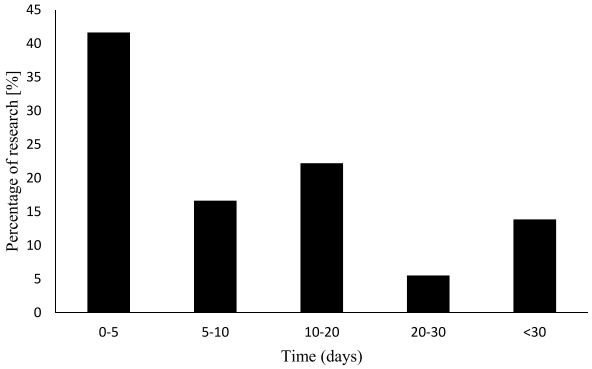


**Fig. 9.** Classification studies based on exposure time.

## 6. Effects of MPs on *Chlorella* sp. enzymatic activity

According to Table 2, the enzymes most affected by MPs were SOD and MDA, accounting for 48 % of the studies each. This is followed by the catalase (CAT) enzyme, which accounts for 40 % of the studies. The effect of MPs on the activity of SOD enzymes in *Chlorella* sp. has been extensively studied. Based on studies, the activity of the SOD enzyme in *Chlorella* sp. increased significantly after being exposed to MPs [115]. This enzyme converts the radical superoxide ($O^{2-}$) into $H_2O_2$ in algal cells and is used to protect against pollutants. Pollutants often have the ability to induce microalga cells to produce antioxidant enzymes, such as SOD, that are involved in the antioxidant protection process to eliminate reactive oxygen species (ROS) [43]. Chen et al. (2020) reported that exposure to MPs increases $O^{2-}$ signal transduction, which subsequently enhances SOD activity [116]. In a study, it was observed that *C. reinhardtii* increases SOD activities as a defense mechanism to combat oxidative stress when exposed to MPs [117]. In another study, exposure of *C. pyrenoidosa* to PS-MPs significantly enhanced SOD activity [118]. In another study, it was reported that MPs increased the SOD content in microalgae cells [43].

According to Table 2, MDA enzyme is another enzyme of interest in studies. This enzyme indicates the level of lipid oxidation in microalgae and the damage caused by MPs to microalgae [121]. This enzyme is a byproduct of membrane lipid peroxidation [122]. In fact, MPs inhibit photosynthesis by attaching to *Chlorella* sp. and reducing the rate of electron transfer. A decrease in the rate of electron transfer leads to the accumulation of electrons, which, in turn, increases the level of ROS. Excessive ROS can lead to lipid peroxidation of the cell membrane, causing an elevation in the content of MDA [123]. A study showed that the MDA activity content of *Chlorella* sp. exposed to PE MPs is higher than PA and PS types. With the increase in the ROS and MDA contents, damage to the cell membrane can occur through lipid peroxidation [124].

As can be seen from Table 2, the CAT enzyme has been considered in 40 % of the studies. As a potent antioxidant, this enzyme catalyzes the conversion of harmful $H_2O_2$ into $H_2O$, similar to other algal enzymes such as SOD, CAT, ascorbate peroxidase, and glutathione reductase. This enzyme protects cells against oxidative damage [125,126]. In one study, the activity of the CAT enzyme increased up to 2.5-fold after exposure to MPs, compared to the control treatment [109]. A significant increase in CAT activity indicates that MPs in the aquatic environment have elevated levels of ROS, which may lead to damage in the algal oxidative system [127]. In addition to reducing the chlorophyll content, photosynthetic efficiency, and photosynthesis rate of *Chlorella* sp., MPs can also inhibit the activity of certain enzymes. For example, they can significantly reduce the activity of enzymes such as peroxidase (POD) and glutathione reductase (GR), which are part of the antioxidant system in algae. Additionally, they can increase the activity of SOD, leading to oxidative stress in algae. This stress can prevent the further growth of algae [106]. In most cases, studies have shown that MPs increased antioxidant enzyme activity [74,128,129]. In one study, the concentration of the lactate dehydrogenase (LDH) enzyme increased after exposure of *C. vulgaris* to PS. This increase was attributed to the damage of cell membrane caused by PS [57].

## 7. Conclusions and prospect

In conclusion, MPs pose a significant threat to the growth and ecological well-being of *Chlorella.*sp. in aquatic environments. This analysis has provided insights into the harmful effects of MPs on *Chlorella* sp., highlighting their inhibitory impact on growth, photosynthesis, and levels of oxidative stress. The literature reviewed has indicated that *Chlorella* species are typically cultivated using the BG-11 culture medium at a temperature of 25 °C and a pH of 7, with optimal growth occurring within the light intensity range of 3000–6000 lux. Various analytical methods, such as SEM, FTIR, and TEM, have been employed to investigate the morphological characteristics of MPs. The results have shown that PS is the most extensively studied type of microplastic, with concentrations typically ranging from 10 to 100 mg/L. The enzymes most affected by MPs were SOD and MDA, accounting for 48 % of the studies. This is followed by the CAT enzyme, which accounts for 40 % of the studies. Based on the findings regarding the impact of microplastics on *Chlorella* species, several recommendations can be made to mitigate their effects: 1. Reduce Microplastic Pollution: One key recommendation is to reduce microplastic pollution by developing plastics that are easier to recycle and reuse. This approach can help reduce the amount of plastic waste generated. This can be achieved by using materials that are more durable, less toxic, and easier to separate from other waste streams. 2. Regulatory Measures: Governments can play a crucial role in reducing microplastic pollution by

**Table 2**
Effect of MPs on the enzymes of *Chlorella* sp.

| Enzymes | Aspergillus spp. | Polymer | Percentage of studies | References |
|---|---|---|---|---|
| SOD | C. vulgaris | – | 48 | [109] |
| | Chlorella. sp. | PS | | [62] |
| | C. pyrenoidosa | polyamide (PA6) | | [73] |
| | C. vulgaris | PS-PVC | | [53] |
| | C. vulgaris | PVC | | [71] |
| | C. pyrenoidosa | PS | | [58,59] |
| | C. vulgaris | PE, PA, PLA, PBS | | [44] |
| | Chlorella sp. | PS | | [119] |
| | Chlorella. sp. | PP, PE, PET, PVC | | [39] |
| | C. vulgaris | PS | | [63] |
| | C. vulgaris | PE, PVC | | [47] |
| | Chlorella sp. | PVC | | [70] |
| | C. vulgaris | PE | | [50] |
| | C. pyrenoidosa | DBP | | [120] |
| | C. vulgaris | PS | | [66] |
| | C. sorokiniana | PS | | [43] |
| | C. pyrenoidosa | PE, PA, PS | | |
| POD | C. vulgaris | – | 3 | [109] |
| GR | C. vulgaris | – | 7 | [109] |
| | Chlorella sp. | PS | | |
| LDH | C. vulgaris | PS | 3 | [57] |
| Esterase | C. vulgaris | PS | | [86] |
| CAT | C. vulgaris | – | 40 | [109] |
| | C. pyrenoidosa | PAN | | [74] |
| | C. pyrenoidosa | polyamide (PA6) | | [73] |
| | C. vulgaris | PS | | [86] |
| | Chlorella sp. L38 | PP, PE, PET, PVC | | [39] |
| | C. marined and C. vulgaris | PS | | [63] |
| | C. vulgaris | PE, PVC | | [51,63,119] |
| | C. vulgaris | PE | | [47] |
| | C. pyrenoidosa | PS | | [50] |
| | C. vulgaris | PE, PVC | | [58,59] |
| | C. sorokiniana | PS | | [66] |
| | C. pyrenoidosa | PE, PA, PS | | [43] |
| MDA | C. vulgaris | PS-PVC | 48 | [53] |
| | C. vulgaris | PVC | | [71] |
| | C. vulgaris | PE, PA, PLA, PBS | | [44] |
| | Chlorella sp. | PS | | [119] |
| | Chlorella sp. | PP, PE, PET, PVC | | [39] |
| | C. vulgaris | PS | | [63] |
| | Chlorella sp. | PVC | | [70] |
| | C. vulgaris | PE | | [63] |
| | C. pyrenoidosa | DEP | | [110] |
| | C. pyrenoidosa | PS | | [59] |
| | C. vulgaris | PE, PVC | | [47,51] |
| | C. vulgaris | PS | | [86,120] |
| | C. pyrenoidosa | PE, PA, PS | | [43] |
| | Chlorella sp. | PS | | [62] |
| | C. sorokiniana | PS | | [66] |
| | C. pyrenoidosa | polyamide (PA6) | | [73] |
| Alkaline phosphatase (AKP) | C. vulgaris | – | 3 | [68] |
| glutathione peroxidase (GSH-Px) | C. vulgaris | PE | 8 | [50] |
| | C. vulgaris | PS | | [63] |
| | C. sorokiniana | PS | | [66] |

implementing and enforcing regulations that limit the use of microplastics in products and encourage the development of more sustainable alternatives. This includes restrictions on single-use plastics and microbeads in rinse-off cosmetics, as well as improved waste management practices. 3. Research and Monitoring: Continuous research on the ecotoxicological effects of microplastics on algae and other aquatic organisms is essential to understand the gravity of the issue and develop effective strategies to mitigate the impact of microplastics on the environment. Research in this area focuses on the impacts of different types, shapes, and concentrations of microplastics on marine organisms. These impacts include growth delay, oxidative stress, reduced feeding activity, genotoxicity, hindrances to nutrient intake, physical damage, shortened lifespan, decreased fertility, and risks to human food safety. 4. Public Awareness: Public awareness plays a crucial role in reducing the overall burden of microplastics. By raising public awareness about the sources of microplastic pollution, individuals can make informed decisions about their consumption habits and lifestyle choices, leading to a reduction in the amount of microplastics released into the environment. 5. Innovative Solutions: Exploring innovative solutions, such as biodegradable materials and efficient waste management systems, can help address the root causes of microplastic

pollution. Biodegradable materials, such as bio-based materials and compostable polymers, have the potential to reduce microplastic pollution in soils and promote plant growth. These materials can naturally degrade, reducing the amount of microplastics released into the environment. Improving waste management practices, such as implementing advanced filtration technologies and tertiary treatment procedures in wastewater treatment facilities, can effectively intercept microplastics before their release into the environment. This can help reduce microplastic pollution in aquatic ecosystems and protect human health.

## Funding statement

No applicable.

## Data availability statement

Data will be availible upon request.

## CRediT authorship contribution statement

**Fateme Barari:** Writing – original draft, Software. **Mohaddeseh Eydi Gabrabad:** Methodology, Investigation, Formal analysis. **Ziaeddin Bonyadi:** Writing – review & editing, Writing – original draft, Supervision, Resources, Data curation, 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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