The Impact of PS Microplastics on Green Algae *Chlorella vulgaris* Growth

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Abstract Even the mostly introductory studies carried out in recent years serve as proof of the diverse threat microplastics may pose for water sources, environment, and biota. That is why microplastics (MPs) pollution is starting to look like a puzzle almost beyond any solution. Microplastics in the aquatic ecosystem could lead to substantial damage on the growth as well as digestion, reproduction, and excretory systems of organisms. The present study investigates the negative impact microplastics cause on the growth of an algae species (*Chlorella vulgaris*) grown in the experiment environment where PS microplastics were introduced alongside the medium. The state of the algae was investigated, with reference to photosynthetic pigment (chlorophyll-a) and optical density (OD) values, and with a view to understanding the impact of microplastics on the growth and development of cellular biovolume of algae. The analyses revealed that MPs inhibited biovolume growth from day one on; the larger the MP dose applied, the higher the level of inhibition. The bioexperiments ran with microplastics of various doses including 1, 2, 4, 6, and 8 mg/L produced an overall increase in the algae biomass values as of the end of the 7th day; yet the biomass figures were found to fall as the experiments were sorted by MP concentration levels, from the lowest to the highest.

Keywords: algal growth, biota, *Chlorella vulgaris*, inhibition, microplastics

1. Introduction

The last 5 years saw increased interest among the scientific community on MP pollution. Various studies referred to the need to assess the complex ecotoxicological impact such pollution may have on biota (Cole *et al*., 2015; Huvet *et al*., 2016; Avio *et al*., 2016; Hartmann *et al*., 2017; Rochman *et al*., 2013; Katsnelson, 2015; 2014; Oliveira *et al*., 2013).

A number of studies try to assess and understand any negative impact microplastics observed in substantial quantities in fresh water sources on the surface, such as lakes and rivers, might have on organisms throughout the food chain from planktons to animals, and even humans (Eriksen vd., 2013). The studies so far focused on invertebrates, zooplanktons, mussels (Wegner vd., 2012; Van Cauwenberghe vd., 2015), worms (Besseling vd., 2012; Wright vd., 2013) and fish (Khan vd., 2015) applying certain experiments to make these organisms ingest microplastics, with a view to monitoring the impact thereof on their digestive and defacation systems as well as on their growth and reproduction. Microplastics' potential negative impact on algae, daphnia, and copepod (Cole vd., 2015) have also drawn substantial research interest, and recently the scientists have been spending more time on these issues. Further studies on mystacoceti, a species of whales using filter feeding to ingest oysters and jellyfish etc. revealed that the species is exposed to excessive volumes of micro-trash, in other words, microplastics (Fossi vd., 2012).

The studies on the impact of micro/nano-plastics on the development (Besseling *et al*., 2014), reproduction, and photosynthesis of algae are quite recent (Bhattacharya *et al*., 2010), and more studies on this matter are needed.

In the same vein, the engineered nanoparticles' impact on algae were also reviewed (Fullerene (C_{60}), Ag, TiO_2, ZnO, Fe_2O_3, CuO, quantum dots, single-walled carbon nanotubes, multiwalled carbon nanotubes etc), yet, their uptake and the toxicity mechanisms remain to be elucidated (Navarro *et al*., 2008).

In this study, bioassays have been conducted to evaluate the impacts of PS microplastics on a green algae *Chlorella vulgaris*.

2. Material and Methods

2.1. Equipments

The climate cabinet (dev/pet) (0-7000 lux) was used during the incubation of the algal culture medium. The devices used during the experiments are analytical balance (±0.0001 g), magnetic stirrer (heating), refrigerated microcentrifuge, spectrophotometer.

2.2. Strain and growth medium

Chlorella vulgaris (Beijerinck) strain was bred on BG11 medium (Rippka ve ark., 1979), under axenic conditions (see Fig.1). The contents of the BG11 medium are specified in Tables 1, 2 and 3. 20 mL of algae culture was inoculated in 180 mL sterile culture medium in 250 mL erlenmayer flasks, followed by 10 days in the air-conditioning cabin at a temperature of 25ºC subject to 5000 lux of light (12 hour light, 12 hour dark) provided by full-spectrum lamps, and shaken 3 times a day.

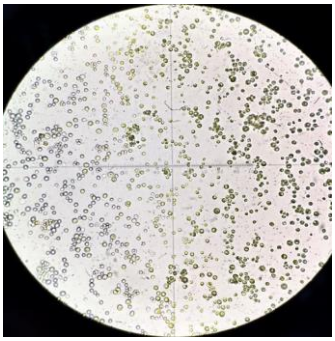

Figure 1. *Chlorella vulgaris*

Table 1. BG-11 liquid medium

Compound	Amount
$MgSO_4$. $7H_2O$	7.5 g L^{-1}
Sitrik asit	0.6 g L^{-1}
EDTA-Na_2	0.1 g L^{-1}
A5 stock solution	100 mL L^{-1}
$CaCl_2.2H_2O$	3.6 g L^{-1}
$(NH_4)_5[Fe(C_6H_4O_7)_2]$	0.6 g L^{-1}
Na_2CO_3	2 g L^{-1}

Table 2. A5 stock solution

Compound	Amount (g L^{-1})
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$Na_2MoO_4.2H_2O$	0.31
$ZnSO_4.7H_2O$	0.22
$Co(NO_3)_2.6H_2O$	0.05
$CuSO_4.5H_2O$	0.08

Table 3. BG-11 medium (Rippka *et al*., 1979)

Compound	Amount
$NaNO_3$	1.5 g L^{-1}
$NaHCO_3$	1 g L^{-1}
K_2HPO_4 (1M)	0.2 mL L^{-1}
BG11(Concentrated Stock)	10 mL L^{-1}

2.3. MP concentrations

In this study, 1µm diameter polystyrene microplastic was used (CAS No:89904). Applied MP doses to algae were calculated taking into account the LC50 value. The study has been studied as two different sets at various concentrations. Applied MP concentration to algae were 1.05 mg L^{-1} , 2.1 mg L^{-1} , 3.2 mg L^{-1} , 4.2 mg L^{-1} , ve 6.3 mg L^{-1} for the 1st set; and 1 mg L^{-1}, 2 mg L^{-1} , 4 mg L^{-1}, 6 mg L^{-1} , 8 mg L^{-1} for 2nd set, respectively.

2.4. The experimental medium

Prior to microplastic application, 200 mL of *Chlorella vulgaris* culture was prepared and they are kept in the conditioning cabinet (at a temperature of 25 ° C) for adaptation under certain conditions for 10 days. OD measurements were made for every day. The cultures were refreshed at the end of 10 days; chlorophyll-a to be 1 µg mL-1 and amount of culture to be 5 mL.

Microplastic solutions are prepared at certain concentrations (for 1st set; 1.05 mg L^{-1}, 2.1 mg L^{-1}, 3.2 mg L^{-1}, 4.2 mg L^{-1}, ve 6.3 mg L^{-1} ; for 2nd set için 1 mg L^{-1} , 2 mg L^{-1} , 4mg L^{-1} 6 mg L^{-1}, 8 mg L^{-1}) , and applied to fresh culture. During the experimental period, changes in the amount of photosynthetic pigment (chlorophyll-a) and optical density (OD) of *Chlorella vulgaris* were planned to be measured and recorded daily. Yet, when the results could not be derived as expected, photosynthetic pigment and OD analyses were replaced by a process to count algae one by one to assess biovolume thereof, in order to be able to assess the growth and development of *C.vulgaris*.

Enumeration of algae were performed with a Palmer-Maloney counting chamber and an Olympus BX51 light microscope.

The following mathematical formula was applied to assess the density of the cells counted in each mL (Wetzel and Likens, 1991).

$$Cell\ Number/mL = \frac{C\ .\ 1000(mm^3)}{A.D.F}$$

C, counted cell number; A, surface of counting area (mm^2); D, depth of counting area (mm); F, unit of counting area. The cellular biovolume was assessed with reference to the formulae to calculate the volume of the form, based on the geometrical formulation of *C.vulgaris* cells' dimensions, followed by the conversion of the values to biomass (Wetzel and Likens, 1991; Edmondson, 1959; Sun and Liu, 2003).

2.5. Measurement of optical density (OD)

The optical density (OD) of Chlorella vulgaris was obtained by absorbance measurement on a spectrophotometer at 750 nm wavelenght. The measurements was performed with 1/10 dilution using BG11 medium (100 µL culture, 900 µL BG11 medium). Ultrapure water was used as a blank solution during the measurements. Each measurement was followed for 7 days.

2.6. Photosynthetic pigment analysis (chlorophyll-a)

Chlorophyll-a measurements was performed with 1/10 dilution using pure methanol (100 µL culture, 900 µL pure methanol). First,1 minute vortexing was performed and then it was analyzed by spectrophotometer at 665 nm after centrifugation for 2 minutes by microcentrifuge at 13,800 rpm at +4 °C. Pure methanol was used as a blank solution during the measurements (Mackinney, 1941).

3. Results

A glance at the results reveal that, even though chlorophyll-a and OD values were expected to fall by the end of 7 days with reference to the increase in microplastics doses, spectrophotometrical assessment produced a picture of increase in these variables, due to the shade effect caused by microplastics. Hence, the unreliable nature of the results led to a 2nd run of the experiment, with algae being count at this occasion.

The amounts of photosynthetic pigment (chlorophyll-a) and optical density (OD) of the 1st set are given in Tables 4 and 5.

Conc. mg/L	0.day	1. day	2. day	3. day	4. day	5. day	6. day	7. day
Control	0.099	0.032	0.067	0.069	0.083	0.070	0.065	0.075
1.05		0.030	0.052	0.061	0.058	0.052	0.049	0.038
2.1		0.028	0.054	0.072	0.060	0.052	0.045	0.046
3.2		0.032	0.061	0.071	0.078	0.062	0.061	0.068
4.2		0.035	0.081	0.073	0.069	0.061	0.056	0.049
6.3		0.035	0.075	0.079	0.083	0.070	0.064	0.067

Table 4. Chlorophyll-a results for 1.set

Table 5. Optical density (OD) results for 1.set

Conc. mg/L	0.day	1.day	2.day	3.day	4. day	5. day	6. day	7. day
Control	0.016	0.059	0.171	0.253	0.144	0.294	0.314	0.340
1.05		0.145	0.185	0.304	0.330	0.338	0.407	0.420
2.1		0.247	0.329	0.409	0.493	0.465	0.553	0.534
3.2		0.361	0.407	0.498	0.602	0.602	0.704	0.682
4.2		0.433	0.594	0.599	0.688	0.700	0.727	0.783
6.3		0.601	1.169	0.816	0.859	0.887	0.996	0.974

The biomass counting results are given in Figure 2 for the 1st set and in Figure 3 for the 2nd set.

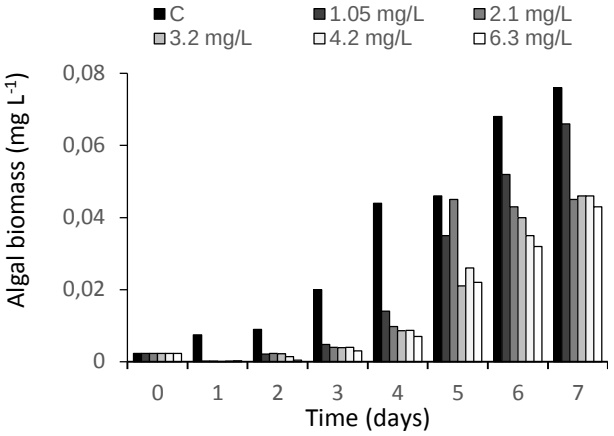

Figure 2. Effect of MP concentrations on daily growth of *C.vulgaris* biomass (1.set)

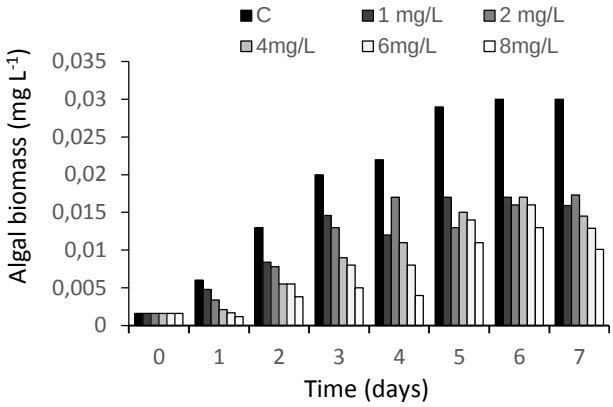

Figure 3. Effect of MP concentrations on daily growth of *C.vulgaris* biomass (2.set)

4. Conclusion

The change various concentrations of microplastics caused on algal biomass over time was reviewed. In general, at every MP concentration level biomass values were observed to exhibit continuous increase through 7 days (see Fig.1 and Fig. 2). However, the daily changes occurring with the biomass values, contrasted against the microplastic dosage applied, revealed that the higher the microplastics concentration, the lower would be the biomass increase. The results received for 2 distinct sets at different concentration levels utilized in the study run parallel to each other. In conclusion, regardless of the dosage applied, µm-size plastics caused shading in the environment and had an impact on photosynthesis by the algae. Furthermore, nm-sized microplastics, in turn, have now been brought into lime light for a review of their impact on the growth, development, and enzyme activities of algae cells.

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