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ORIGINAL PAPER

Impact of polystyrene microplastics on major marine primary (phytoplankton) and secondary producers (copepod)

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Abstract

The effect of microplastic adsorption on marine microalgae *Tetraselmis suecica*, *Amphora subtropica,* and copepod *Pseudodiaptomus annandalei* was investigated in the present study. Fluorescence microscopic images were used to evaluate MP interactions with algae and copepods. *T. suecica* growth rate decreased with effects of 0.1 µm polystyrene exposure to 75 µl/100 ml (0.899 to 0.601 abs), 50 µl/100 ml (0.996 to 0.632 abs) and 25 µl/100 ml (0.996 to 0.632 abs), respectively. On the other hand, at 10th day of experiment, the control *T. suecica* showed the highest growth rate (0.965 abs), chlorophyll concentration (Chl-'a' = 21.36 µg/L; Chl-'b' = 13.65 µg/L), and cell density (3.3×10^6 cells/ml). A marine diatom *A. subtropica* absorbed 2.0 µm microplastics, and the maximal inhibition rate increased at higher MP concentration until 10th day. The highest MPs (75 µl/100 ml) treatment resulted in decreased growth rate of *A. subtropica* from 0.163 to 0.096 abs. *A. subtropica* (without MPs) had the highest lipid concentration of 27.15%, whereas *T. suecica* had the lowest lipid concentration of 11.2% (without MP). The maximum survival (80%) of *P. annandalei* was found in control on 15th day whereas on 12th day, the microplastics ingested copepod had the lowest survival rate (0%). On 15th day, the maximum Nauplii Production Rate (NPR) (19.33) female^{-1} was observed in control, whereas the minimum (17.33) female^{-1} NPR was observed in copepod ingested with MPs. The maximum lipid production (17.33% without MPs) was reported in control, whereas MPs fed copepods had the lowest lipid production (16%). Long-term exposure to polystyrene microplastics significantly reduced algae growth and chlorophyll concentration and also NPR and lipid concentration rate of copepod. We inferred that microplastic exposure of algae and copepods might results in persistent decreases in ingested carbon biomass over time.

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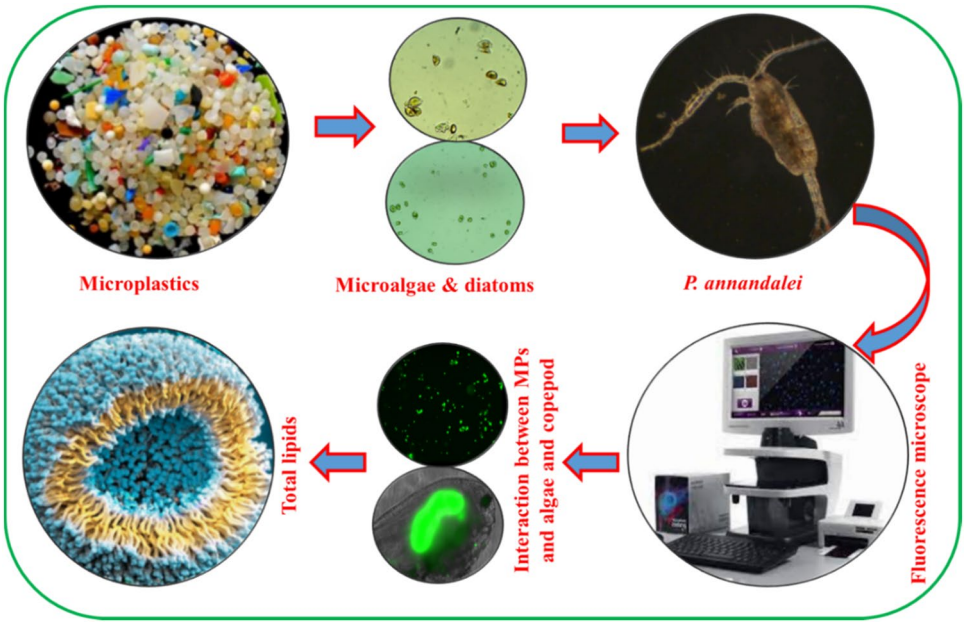
Graphical abstract



Keywords Microalgae · *Pseudodiaptomus annandalei* · Microplastics · Diatom · Total lipids

Introduction

Plastic production has increased significantly since the large-scale industrial manufacturing started in early 1950s. Plastics are used in almost every aspects of daily life (Plastics Europe, 2016) and the majority of plastics are used in automotive industry (8.9%), electrical and electronic devices (5.8%), agriculture (3.3%), packaging (39.9%), building and construction (19.7%), and other sectors (22.4%). The plastic products would gradually break down into fragments over time, especially if they were continuously exposed to ultraviolet radiation (of sunlight) and high temperatures. This fragmentation would reduce the plastic material sizes from macroscopic to microscopic and finally nano-sizes. The increasing awareness of plastic pollution has resulted in the documentation of more scientific and societal concerns regarding the effects of microplastics (MPs) with diameter less than 5 mm (Gregory and Ryan, 1997; Kako et al. 2011, 2014; Yoon et al. 2010; Kubota, 1994; Zarfl and Matthies, 2010; Isobe et al. 2014; Maximenko et al. 2012). Microplastics can be categorized as either primary or secondary MPs based on their manufacturing process. Primary microplastics are tiny particles that are released into the environment directly or indirectly through domestic and industrial effluents, spills and sewage discharge. The most commonly found primary microplastics are fibres, pellets, film, fragments and spheres (Kang et al. 2015; Lusher 2015; Li et al. 2016). Secondary microplastics are produced by photo-oxidation of UV radiation, mechanical transformation and microbial degradation (Andrady and Neal, 2009; Cole et al. 2011). The smaller particles broken down into nanoplastics (1–100 nm) with toxicological characteristics (Koelmans et al. 2015; da Costa et al. 2016). Ingestion of microplastics by marine organisms including plankton, fish, benthic organisms that can be trophically transferred (Browne et al. 2008; Wright et al. 2013). Overall reported the impacts of microplastics on marine organisms such as growth delay, oxidative stress, reduction of feeding activity, genotoxicity, neurotoxicity and reduction of reproductive fitness (Mazurais et al. 2015; Li et al. 2018). Microplastics adsorption by microalgae influence the sinking rate of both microalgae and buoyant microplastics (Ballent et al. 2013). Biofouling of microplastics may also enhance their absorption by biota (Andrady 2011). Under stressful conditions such as a lack of light or nutritional shortage, microalgae produce polysaccharides molecules, known as exopolysaccharides which can subsequently coagulate, allowing algal cells to aggregate (Long et al. 2015; Staats et al. 2000). Copepods are secondary producer and popular model for ecotoxicological studies among zooplankton because they play an important role in aquatic ecosystem and are sensitive to environmental stressors (Ananth and Santhanam, 2011). Polystyrene is a transparent

polymer contains styrene monomers with specific gravity of 1.04–1.07 g cm^{-3} and its soluble in various organic solvents such as esters, aromatic hydrocarbons and ketones. PS is resistant to salts, alkalis, mineral oils, organic acids and alcohols. Less weight PS foam provides many applications such as roofing, refrigerators, building walls and freezers. PS caused the gastrointestinal tract infections and toxic to the cellular level (Cole et al. 2015). According to Cai et al (2018), PS particles from laboratories, might be a source of main plastics particulate pollutants and their particles can create a variety of issues depending on their size, shape and functional groups. Polystyrene is a primary particle caused the delayed growth, feeding capacity and reproductive development in marine organisms (Alimi et al., 2018). The aggregation and attachment of polystyrene to marine organisms from microalgae to copepod were not investigated by earlier studies; hence, the present study was aimed to assess the effects of microplastics adsorption on marine microalgae *Tetraselmis suecica*, *Amphora subtropica* with effects on growth rate, chlorophyll concentration, lipid profile besides survival, nauplii hatching and lipid profile of copepod *P. annandalei.*

Materials and methods

Experimental setup

Pre-cultured *T. suecica* (PSBDU002) and *A. subtropica* (KM099276) at the logarithmic growth phase were taken from microalgae culture collection of marine planktonology and aquaculture laboratory of Department of Marine science, Bharathidasan University, and added into 100 ml of Conway's and TMRL-enriched seawater medium was used as a control, of which 10% inoculum was added. Depending on the size of the microalgae, two distinct Polystyrene microplastics (0.1 µm (Lot# MKCH2797) amine modified fluorescent orange beads for *T. suecica* and 2.0 µm (Lot#MKCJ4402) fluorescent yellow green beads for *A. subtropica*) were obtained from Sigma-Aldrich and individually injected to each algal culture to achieve concentrations of 25, 50 and 75 µl/100 ml, respectively. All the experiments were incubated under controlled laboratory conditions that remained constant throughout the investigation (23 ± 1 °C, 50 µmol photons/(m^2 sec^{-1}) and a 12 h/12 h (light/dark) cycle). The aggregation and attachment of MPs to algae were evaluated over an entire culture cycle, from seeding to stationary growth phase for each algal species. The microalgal physiology was examined through the analysis of their growth rates, cell density and chlorophyll for assessing the photosynthetic ability which is used to identify the potential impacts of micro-PS on phytoplankton community. All groups were maintained as triplicate.

Determination of photosynthetic pigment

Chlorophyll was extracted by taking 0.5 g of algae to which 5 ml 90% acetone was added. It was kept undisturbed overnight and the optical density of the chlorophyll was measured next day with a UV/Vis spectrophotometer (Spectro 20D plus, U.S.A). The amount of chlorophyll was calculated according to the equations of Jeffrey and Humphrey (1975).

Chlorophyll analysis for green algae

Chlorophyll 'a' (µg/ml) = 11.93 E_{664}–1.93 E_{647}.
Chlorophyll 'b' (µg/ml) = 20.36 E_{647}–5.50 E_{664}.

Chlorophyll analysis for diatom

Chlorophyll 'a' (µg/ml) = 11.47 E_{664}–0.40 E_{630}.
Chlorophyll 'c' (µg/ml) = 24.36 E_{630}–3.73 E_{664}.

Determination of microalgae growth rate

Algal growth was measured by taking absorbance. Absorbance was measured every 2 days once for the period of 10 days by recording the changes in optical density at 680 nm with a UV/Vis spectrophotometer according to Lichtenthaler (1987).

Determination of algal cell density

Microalgal cell concentration was determined through cell counting using haemocytometer. The haemocytometer is a slide glass size 30 mm × 70 mm × 4 mm thick. It has 2 chambers and cells counts were performed at the center part. The specific growth rate of microalgae was calculated using Eq. 1 derives formula from Andersen (2005).

$$Cell\ Density = \frac{Average\ number\ of\ cells\ per\ square \times Dilution\ factor}{Volume\ of\ square\ (10^4)}.$$

Extraction of total lipids from microalgae

The total lipids of the algae were extracted by mixing chloroform–methanol (4:2 v/v) by adopting the standard procedure of Folch et al. (1957). A mixture of 2 ml methanol and 1 ml chloroform was made and added to 1 g algal biomass. It was kept for 24 h at room temperature to dissolve the lipids properly. The mixture was centrifuged at 3000 rpm for 10 min. Supernatant was separated, 2 ml of chloroform was again added to the pellets and shaken properly. It was

again centrifuged at 3000 rpm for 5 min and supernatant was separated. After adding 2 ml of 1% KCL to the supernatant, two layers were formed. Lower layer was pipette out and weighed.

$$Lipid\ content\ (\%) = wt.\ of\ lipid\ (g) \times 100 / wt.\ of\ algal\ sample\ (g).$$

Collection and identification of copepods

The zooplankton samples were collected from the Vettar estuary (10°46' N Lat. 79°12' E Lon.) using plankton net with 158 µm mesh. The collected samples were immediately transported to laboratory by providing with vigorous aeration using battery aerator. The zooplankton samples were thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *P. annandalei* was isolated with fine brush, needle and stempel pipette and identified under microscope using the key of Davis (1955); Kasturirangan (1963). Based on the keys provided by the authors, the species was confirmed for their taxonomy and used for culture.

Copepod culture

About 50 individuals of *P. annandalei* were isolated and stocked in 1000 ml beaker contained seawater. The marine microalga *Isochrysis galbana* at the concentration of 25,000 to 30,000 cells/ml. was given as feed for copepod *P. annandalei*. The water quality parameters, viz., temperature (23–26° C), salinity (28–30 PSU) and pH (7–8.5) were maintained at on optimized condition. The generation time of *P. annandalei* under optimal conditions was about 10–12 days and having 6 nauplii and 6 copepodite stages including the adult. Finally, the adult gravid female copepods were used to restart the stock culture.

Microplastic beads

The latex beads, carboxylate-modified polystyrene, fluorescent yellow-green were purchased from (Sigma-Aldrich, USA) L4530 (Lot # MKCF0821) aqueous suspension with 2.0 µm mean particle size was used for the present experiment.

Experimental setup

The copepod, *P. annandalei* was cultured in filtered seawater. The 2 µm green fluorescent microplastic beads were used for the experimental study. The control copepods were fed with *I. galbana* with the concentration of 25,000 cells/ml and 2 µl/100 ml MPs was ingested in the experimental copepod. Control and experimental groups were triplicated. Survival rate, nauplii production rate and total lipid was determined in control and experimental copepod.

Effect of MPs ingestion on survival rate of *P. annandalei*

The survival rate of copepods was analyzed for the period of 15 days. 10 numbers of healthy *P. annandalei* were picked up from the stock culture by using fine brush and then transferred to a 100 ml beaker filled with sterile seawater and fed with *I. galbana* and inoculated with 2.0 µm fluorescence yellow green MPs. The number of live copepod specimens remaining in each chamber was recorded daily. Dead copepods were removed from treatments. The survival rates of copepods in respect to experimental and control treatments were measured by subtracting the final density of copepods from initial density of copepods.

Assessment of MP ingestion on nauplii hatching of *P. annandalei*

For determining the nauplii producing capacity of microplastic-ingested *P. annandalei* and control *P. annandalei* five individual female with viable egg sacs were stocked in a glass test tube containing 20 ml of filtered seawater. Copepod *P. annandalei* was stocked in the beaker filled with seawater and 2 µl of MPs. The copepod stocked with only microalgae *I. galbana* was control. The copepod was examined at regular intervals (every 2 h) for the release of nauplii. Once the nauplii were released, the adult female was carefully removed from the test tube and the nauplii were counted under the microscope.

Determination of total lipids from *P. annandalei* fed with MPs

The copepod *P. annandalei* was cultured with control (*I. galbana*) and fed with MPs was harvested using the 48 µm mesh. The harvested copepod sample was first washed with filled seawater followed by distilled water. After removing the excess moisture using blotting paper copepod sample was subjected to drying in an oven. Lipid content of copepod was estimated by the method of Folch et al. (1957). In brief, a known amount of dried copepod sample was homogenized in 10 ml of chloroform–methanol mixture (2/1 v/v). The homogenate was centrifuged at 2000 rpm. The supernatant was washed with 0.9% saline solution (KCl) to remove the non-lipid contaminants and allowed to separate. The upper phase was discarded by siphoning. The lower phase was allowed to dry in a hot air oven and the weight was taken.

$$(\%)\ of\ Lipid = \frac{Amount\ of\ lipid\ in\ the\ sample \times 100}{Weight\ of\ the\ sample\ taken}.$$

Statistical analyses

The statistical analysis of the obtained data was assessed with one-way analysis of variance (ANOVA) with help of SPSS by using Regression Analysis Test (DMRT).

Results

Effect of MPs adsorption on algal photosynthesis

The green algae *T. suecica* (0.1 µm MPs) were subjected to three different concentrations of microplastics (25, 50, and 75 µl/100 ml) and 2.0 µm of green fluorescent MPs were injected in to *A. subtropica* culture flask with three different concentrations (25, 50 and 75 µl/100 ml). The impacts on their photosynthesis and growth rate were monitored every 48 h for 10 days.

Effect of MPs (0.1 µm) on growth rate, cell density and chlorophyll concentration of *T. suecica*

The growth rate, cell density and chlorophyll concentration of *T. suecica* was increased in microplastics suspension culture and no homo-aggregates were formed from day 1 to day 4. In MPs exposure treatment homo-aggregation was observed from 6th day onwards. However, the rate of growth and chlorophyll content decreased from 8th day to 10th day under 75 µl/100 ml (0.899 to 0.601 abs) MPs exposure followed by 50 µl/100 ml (0.996 to 0.632 abs) and 25 µl/100 ml (0.792 to 0.52 abs). The control experiment showed maximum growth rate (0.965 abs), chlorophyll concentration (Chl-'a' = 21.36 µg/L; Chl-'b' = 13.65 µg/L) and cell density (3.3×10^6 cells/ml) on the 10^{th} day of experiment. Only the microalgae (in suspension) were taken into account for the estimation of growth rate and chlorophyll (Fig. 1). *T. suecica* exposed to 0.1 µm polystyrene for different exposure time revealed a positive correlation and significantly affected chlorophyll 'a' ($R^2 = 0.94$; $P < 0.01$) and 'b' ($R^2 = 0.89$; $P < 0.02$) concentrations when compared to control ($R^2 = 0.59$; P0.05). At different exposure periods, the growth rate of *T. suecica* was not significantly different from the control ($R^2 = 0.34$; $P > 0.081$). Cell density was decreased in experimental algae as compared with control showed non-significance and reduced maximum cell density concentration ($R^2 = 0.140$; $P < 0.496$).

Effect of MPs (2.0 µm) on growth rate, cell density and chlorophyll concentration of *A. subtropica*

Three growth phases (the lag phase, the logarithmic phase and the stationary phase) of *A. subtropica* over a 10 days of incubation period were clearly distinguished in the control groups and algal growth rate, cell density and chlorophyll continuously increased from 0.012 to 0.596 abs, 4.02×10^4 to 2.102×10^5 cells/ml and Chl-a = 3.23 to 15.23 µg/L and Chl-b = 1.56 to 7.15 µg/L from initial day to 10th day, respectively. Then the algae was exposed to 2.0 µm microplastics and the maximum inhibition rate was recorded with increased MPs concentration until 10th day. In the present study, the highest concentration (75 µl/100 ml PS) of microplastic exposure treatment flask inhibit the cell density from 6th day to 10th day. Under 75 µl/100 ml MPs treatments growth rate decreased from 0.163 to 0.096 abs at the end of the study (as compared to the control). Similarly, the chlorophyll concentration was also reduced under highest MP exposure with homo aggregates starting from 6th day to 10th day (Fig. 2). 2.0 µm polystyrene-ingested *A. subtropica* with different exposure days are significantly affected the chlorophyll concentration 'a' & 'b' as compared with control ($R^2 = 0.98$; $P < 0.03$) and ($R^2 = 0.98$; $P < 0.01$). *A. subtropica* growth rate was not significantly different with control at different exposure days ($R^2 = 0.22$; $P < 0.454$). Maximum cell density concentration decreased in ingested one against control ($R^2 = 0.76$; $P < 0.496$).

Effect of MPs on lipid concentration of *T. suecica* and *A. subtropica*

While many microalgal strains were found to naturally have high lipid content. Lipid accumulation refers to increased concentration of lipids within the micro algae cells. In the present investigation the lipid concentration of *T. suecica* and *A. subtropica* was analyzed in relation to control (Without MPs) and different concentrations of MP exposed algae. In the present study, maximum lipid concentration of 27.15% was observed in control *A. subtropica* (without MPs) and the minimum (11.2%) was observed in *T. suecica* (Without MPs). Only minimal changes were observed in the lipid content MPs exposed microalgae (Fig. 3). Lipid concentration was significantly lower in Polystyrene-exposed *T. suecica* compared to control ($R^2 = 0.82$; $P < 0.04$). Similarly, when *A. subtropica* was exposed to 2.0 µm PS, its lipid level reduced in comparison to the control ($R^2 = 0.92$; $P < 003$).

Survival rate of *P. annandalei*

In the present study, the maximum survival rate was observed in copepod fed with *I. galbana* (80%) whereas microplastics ingested copepods were died at the 12th day of

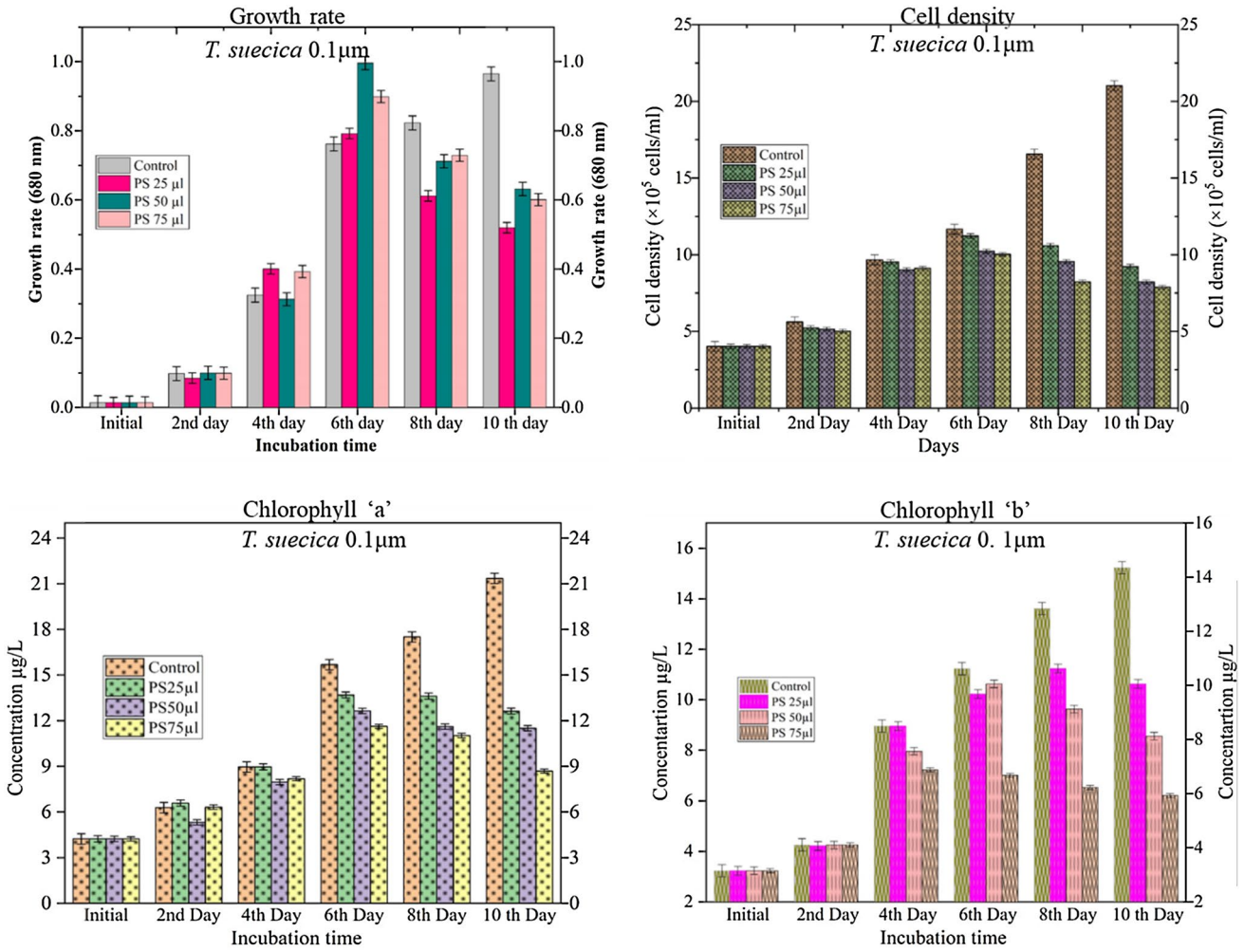


Fig. 1 Chlorophyll 'a' and 'b' concentration showed significance ($P < 0.01$; $P < 0.02$), cell density non-significance ($P < 0.496$) and growth rate not significance ($P > 0.081$). of *T. suecica* in response to MP exposure with size 0.1 µm

experiment and the survival rate of MPs ingested *P. annandalei* was 0% (Fig. 3).

Nauplii hatching rate of *P. annandalei*

The observed results revealed that the microplastic feeding adversely impacts the production of nauplii in copepods. The highest nauplii production rate was observed in the control copepod diet with an average of 19.33 nauplii female^{-1} whereas the lowest nauplii production was observed in MPs ingested *P. annandalei* with 4 nauplii female^{-1} (Fig. 3).

Lipids

In adult *P. annandalei*, approximately 40% of energy derived from their food goes toward the buildup of their lipid store. The presence of lipid concentration in copepod was based on the mass culture production. In the present investigation, micro-algae fed copepod showed highest lipid production (17.3%) whereas the MPs ingested copepods result the lowest lipid profile (Fig. 3). Furthermore, we concluded that a feeding may result in an altered lipid profile. Yet, there was no difference in the total (Plate 1) lipid concentration of copepods at the end of the exposure period (Plate 2).

Discussion

Polystyrene (PS), a high-volume polymer with global market worth more than $30 billion dollars, is one of the plastics often found in oceans (Transparency Market Research, 2014). Because marine microalgae are the major producers at the bottom of the food chain, there is concern about the potentially adverse effects of nano- and micro-sized plastic particles (Kaiser et al., 2011). According to Bhattacharya et al. (2010) the interactions between microplastics and

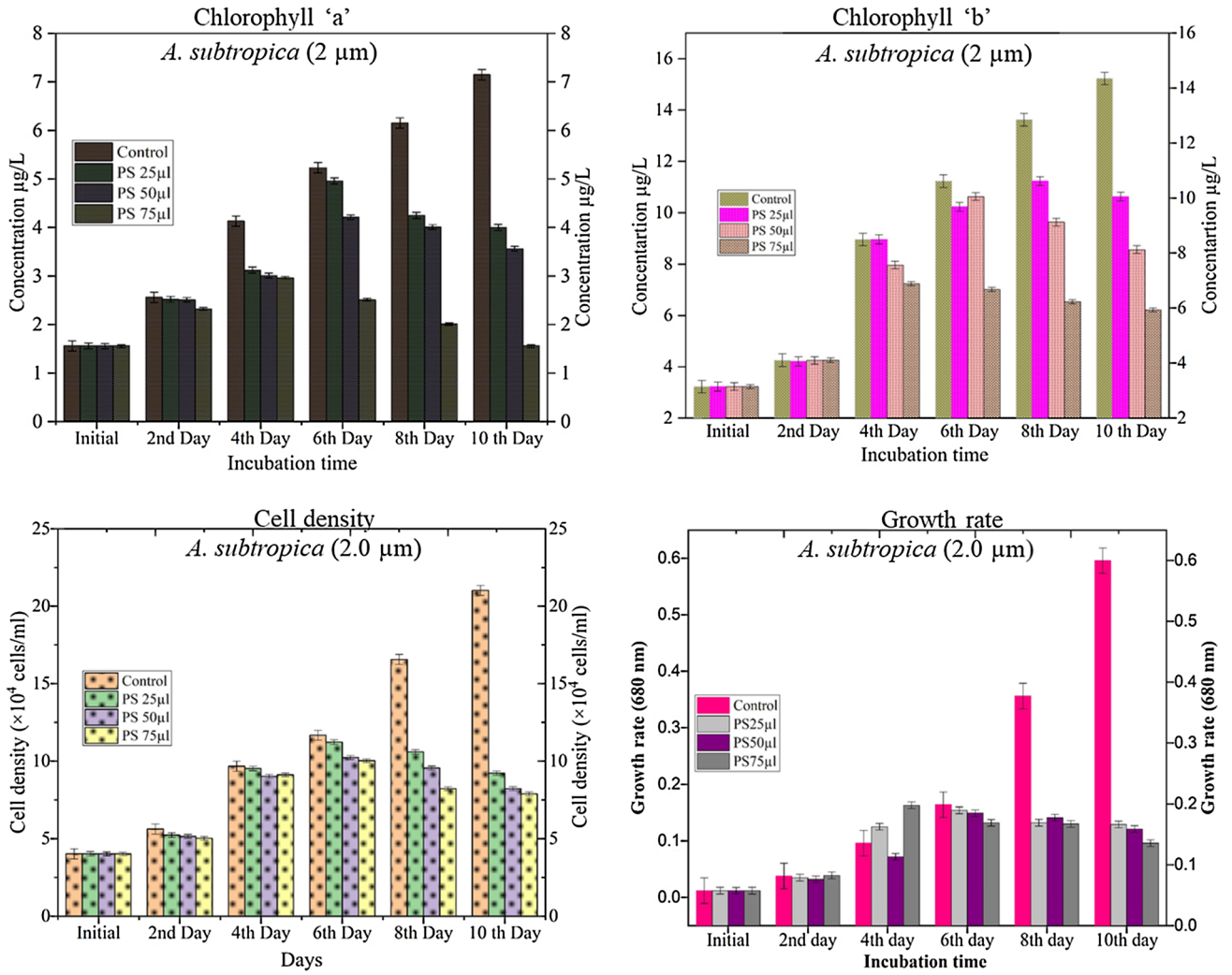


Fig. 2 Chlorophyll 'a' and 'b' concentration showed significance ($P<0.03$; $P<0.01$), cell density non-significance ($P<0.496$) and growth rate not significantly ($P<0.454$) of *A. subtropica* in response to MP exposure with size 2 µm

microalgae are expected to alter cell properties such as size and shape. Additionally, algal cell walls act as barriers to particle penetration and the cell wall characteristics may consequently influence particle sorption. Therefore, the present study was conducted in marine diatom, *A. subtropica* (with a silicate cell wall) and the marine alga *T. suecica* without a cell wall.

Microplastics debris, as an emerging environmental pollutant, is prevalent globally now. Therefore, it is crucial to have a basic understanding of the interactions between microplastics and the organisms. In the present study, the effects of microplastics on microalgae (*T. suecica*) was exposed to 0.1 µm size MPs and then the effects on their photosynthesis and growth were determined every 48 h for the period of 10 days. All the tests were performed under identical temperature, illumination and photoperiod conditions with the exponentially grown cultures at a start density of (5×10^5 cells/ml) (allowing for exponential growth during the experiment). In contrast to photosynthesis, a clear effect of the MPs beads on the growth of *T. suecica* was observed. The highest MP concentration of 75 µl/100 ml showed the lowest microalgal photosynthesis, growth rate and cell density. The growth rate of *T. suecica* was higher on the 6th of the experiment, which might be owing to the homo-aggregation of microalgal cells. The reduction in cell density, algal photosynthesis and growth rate were exposed to 0.1 µm size with concentration of 25 µl/100 ml MPs beads was clearly reduced from 8 to 10^{th} day when compared to the control.

When compared to other species, diatoms like *A. subtropica* expelled large amounts of sticky transparent exopolymeric particles, and these diatoms possess biogenic silica (Passow, 2002). *A. subtropica* is known to easily aggregate for these reasons. The influence of microplastics on the marine diatom *A. subtropica* was studied from the

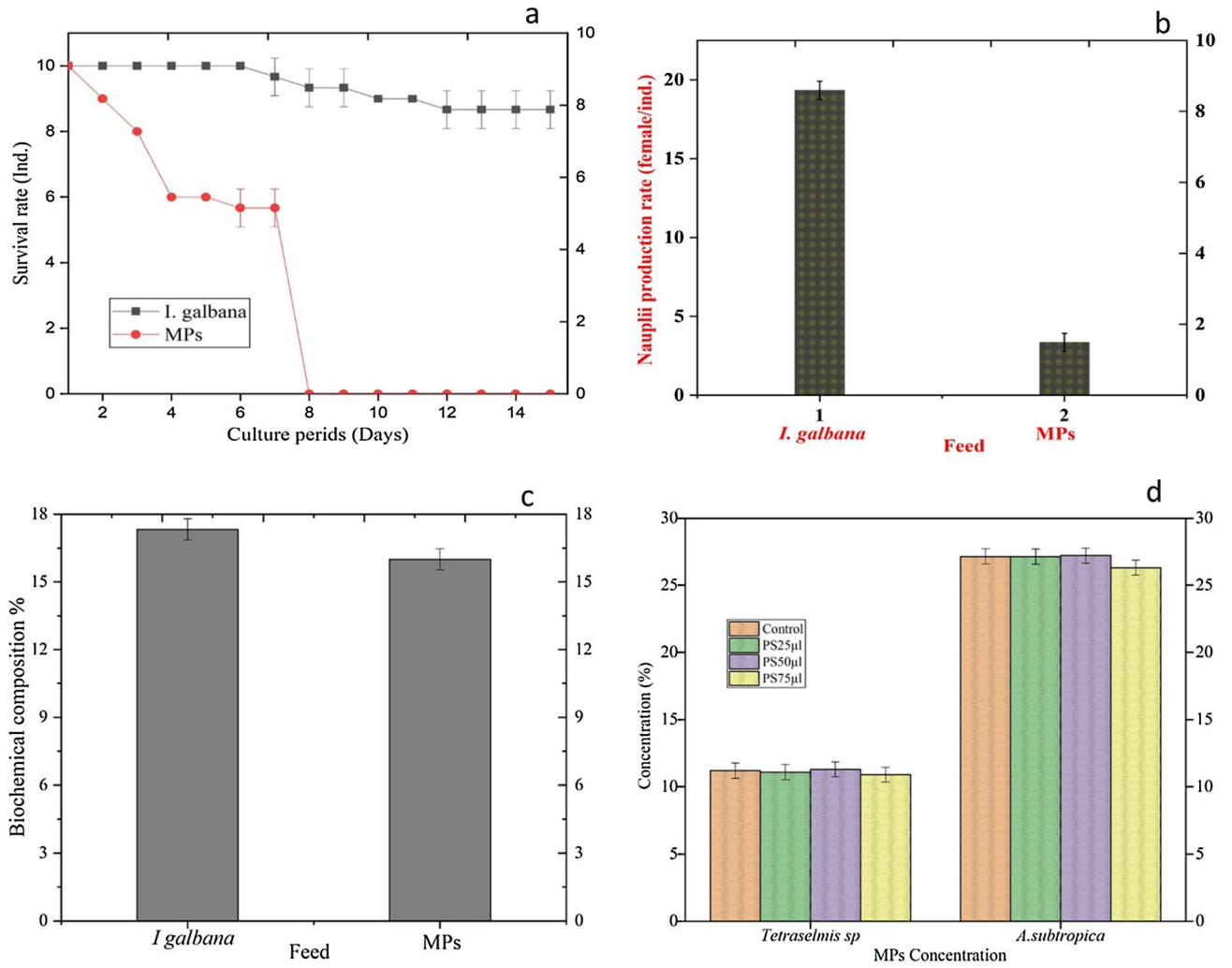
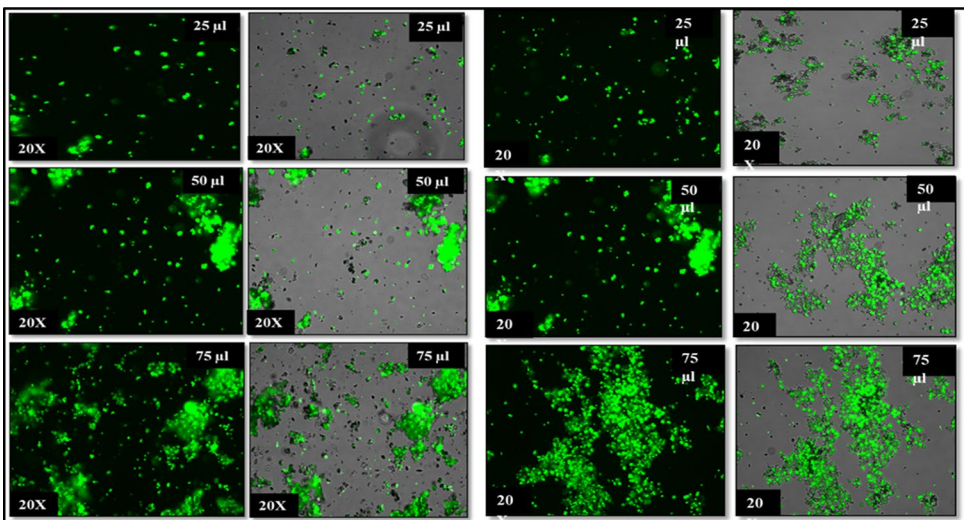


Fig. 3 Effect of MPs on survival rate (**a**), nauplii hatching rate (**b**), lipids of *P. annandalei* (**c**). The means values shown of three triplicates ± standard deviation and standard deviations were calculated from replications and lipids of *T. suecica* and *A. subtropica* showed significantly ($P < 0.04$; $P < 003$) (**d**)

Plate 1 Fluorescence microscopic images of *T. suecica* and *A. subtropica* exposed to (0.1 and 2 µm) MPs



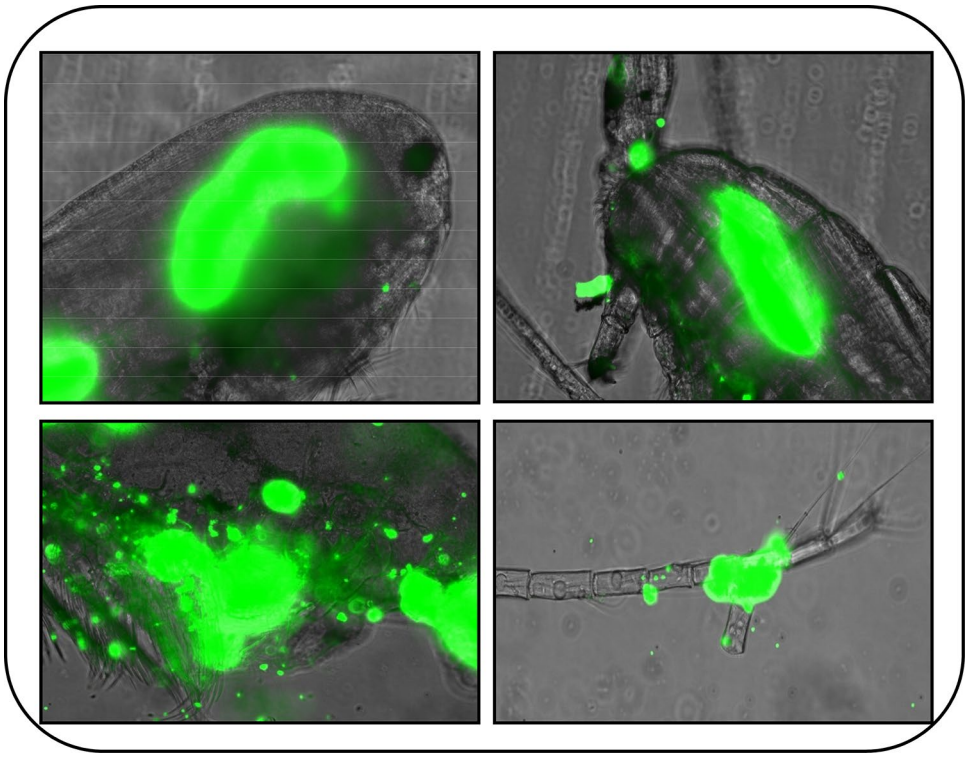


Plate 2 Microplastics accumulation in marine copepod *P. annandalei*

logarithmic to stationary phases in this study. Diatom (*A. subtropica*) was exposed to 2.0 µm size of MPs beads and their effects on photosynthesis and growth were determined every 48 h once for the period of 10 days. All tests were performed under controlled conditions with a start density (4.02×10^4 cells/ml) allowing for exponential growth during the experiment. Among these four different treatments, the highest MPs concentration of 75 µl/100 ml showed the lowest microalgal photosynthesis, growth rate and cell density followed by 50 and 25 µl/100 ml microplastics exposure treatments at the mid stationary phase. The growth rate, cell density and chlorophyll concentration were found to be increased from day 2 to day 6 after it was declined. The minimum of cell density, algal photosynthesis and growth rate were observed with the MP concentration of 25 µl /100 ml. Besseling et al. (2014) demonstrated that PS particles of a similar size (0.07 µm) inhibited the growth of the microalga *Scenedesmus obliquus*. The adsorption of the MPs beads on algae could block light from reaching the photosynthesis, similar studies were earlier carried out by Yufeng Mao et al. (2018) and Marc long et al. (2015). Sjollema et al. (2016) suggested that the nanoscale, particles (0.1 µm) become more easily undergo aggregation (Gigault et al. 2018) and (Galloway et al. 2017), which would cause more interaction between microplastics and algae. These results confirmed that the effect on microalgal growth decreased with increasing beads concentration and size. During the present investigation period, *T. suecica* showed the highest homo aggregation compared to *A. subtropica.* The role of MPs in the fate of aggregates may vary depending on the MPs size, density and composition (Cózar et al. 2014).

Microalgae generate a wide range of lipids with nutritional value. As a result, microalgae play an important role in the aquaculture food chain, primarily as live feeds for larval culture (Brown 1997; Martínez-Fernández et al. 2006). Lipid composition and productivity depend on growth conditions such as growth phase (Xu et al. 2008), medium composition (Valenzuela-Espinoza et al. 2002), irradiance rate (Thompson et al. 1993), and temperature conditions (Renaud et al. 2002). In the present investigation, the total lipid was analyzed in *T. suecica* and *A. subtropica* (with and without MPs). The maximum concentration of lipid was observed in *A. subtropica* without MPs (Control) which may be due to the nitrogen depletion (Valenzuela 2013). The MPs containing microalgae not showed any variation in total lipid concentration. Similar findings were earlier reported in mussels by Smolders and Degryse (2002). Furthermore, we hypothesized that the aggregated microalgae may alter the lipid profile. However, there was a substantial change in total lipid mass of microalgae at end of the exposure period, but the lipid profiles of the control were significantly altered.

Microplastics have been found to be present in many parts of the marine food web, especially in the water column with various impacts on the zooplankton (Cole et al. 2015). This study investigated the effects of microplastic beads of 2 µm that might have on the filtration ability in

copepod *P. annandalei.* The present results showed that the copepod can ingest microplastics (2.0 µm diameter) in the absence of natural food. Microplastics were unknowingly ingested via filter-feeding and later egested in faecal pellets, typically within a matter of hours. Microplastics were found to be trapped between the external appendages of copepods owing to its accumulation on the external surface. The microscopic images of copepod clearly showed that 2.0 µm polystyrene beads clustered within the alimentary canal and aggregated between the setae and joints of external appendages. The presence of 2.0 µm polystyrene beads reduced the algal ingestion rate, nauplii production and survival rates of copepod *P. annandalei*. Similarly, Cole et al. (2013) reported *Temora longicornis* and *Centropages typicus* ingested microplastics with different sizes 7.3, 20.6 and 30.6 µm beads and *Acartia clausi*, *Calanus helgolandicus* ingested 7.3 µm beads affected the feeding and survival rates of the animals. The present study demonstrated that the minimum survival and nauplii production rate was found in microplastic ingested copepod. Prolonged exposure to the microplastics resulted in less egg production with reduced hatching success and survival rate was also reduced within 4–5 days in *P. annandalei*. The present findings shows that microplastics can impede copepod feeding and reduce the nauplii production rate and survival. These effects were most noticeable at 3–4 days after the ingestion of microplastics into the organisms. Lee et al. (2013) found that when exposed to 0.5 and 6 µm microplastics, the number of nauplii which hatched from eggs produced by the benthic copepod *Tigriopus japonicus* was reduced. Similar results have been observed by Bonnet et al. (2005), Cole et al. (2013, 2015). In adult *P. annandalei*, approximately 40% of energy derived from their food goes towards the buildup of their lipid store. (Marshall and Orr, 1955). The present study results concluded that the maximum concentration of lipid was shown in algae fed copepod (19%) and lipid contents were observed in the MPs exposure copepod (18%). Furthermore, there were no differences found in the total lipid concentration of copepods at end of the exposure period.

Conclusion

In the present study, two different microplastic beads were induced in a two-microalga, viz., *T. suecica* and *A. subtropica* over the 10 days experimental period. The significant PS beads aggregation was observed in *T. suecica* (than in the *A. subtropica*). The findings on the effect of MP beads absorption in total lipid production of *T. suecica* and *A. subtropica* showed not much variations observed in lipid production of control and MPs ingested microalgae. The microplastic ingested *P. annandalei*, showed less survival and nauplii production further, microplastic adsorption and consumed copepods showed minimal changes in the lipid production. Higher trophic species that rely on the high lipid content of microalgae and copepods for their own sustenance may be affected by energetic deficits and decreased lifespan of microplastic exposed marine microalgae and copepods. The results concluded that better understand the density of bioavailable microplastics in biota-rich waters, as well as to test whether environmentally relevant concentrations of plastic litter can impact keystone species such as *T. suecica, A. subtropica* and *P. annandalei,* as well as the consequences for commercially important predators.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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