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Is color a matter of concern during microplastic exposure to *Scenedesmus obliquus* and *Daphnia magna*?

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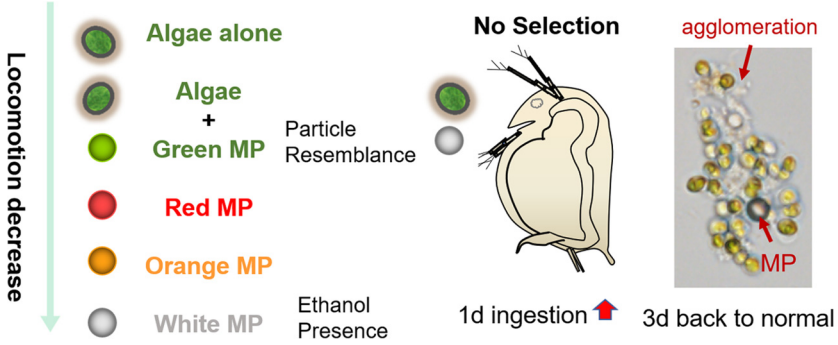
GRAPHICAL ABSTRACT



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ABSTRACT

Toxicities of microplastics (MPs) on aquatic organisms have been widely investigated often by using white or transparent MPs. However, various colored MPs scatter in the real aquatic environment. Here we investigated four colored MPs' effects on *Scenedesmus obliquus* algal growth first. Under the light condition, algal growth increased initially due to hormesis stimulation and then decreased gradually at higher MP concentrations. Green colored MPs exhibited the lowest inhibition effect, probably due to their resemblance to algae; white MPs inhibited the algal growth significantly, which was attributed to the presence of ethanol. Turbulence condition seemed to diminish algal growth differences among groups, but it led to slight oxidative stress. Furthermore, we also tested MP effects on *Daphnia magna* feeding ability. Results indicated that daphnids were probably not able to distinguish colored MPs from algae. But their algae ingestion amounts increased when MPs reached to 40% of algal cells, probably because daphnids could widen their filtering gapes when food quality decreases. However, this phenomenon did not last until the 3rd day, as the agglomeration of MPs and algae made them settle down. Overall, our results highlighted the color may alter some MP effects and is necessary to be considered in (eco) toxicological studies.

1. Introduction

Microplastic (MP) is becoming a contaminant with emerging concern in the environment, especially when the global plastic production amounts are still increasing annually (Lebreton and Andrady, 2019; Halden, 2015). MPs can enter into aquatic environments via direct input or indirect fragmentation of larger plastic debris and consequently lead to negative impacts on organisms in the aquatic

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environments (Chen et al., 2018, 2019; Yin et al., 2018; Deng et al., 2018). Due to different manufacturing purposes, MPs are found with various colors either in environmental matrix or aquatic organisms. Industry adds pigments to polymers, such as different colored alkyd resins used in marine application plastics, and yellow to orange colored toys may contain lead chromate pigments (Greenway and Gerstenberger, 2010; Shtykova et al., 2006). Moreover, environmental weathering can lead to color fading or change the original color to be yellowish (Chen et al., 2019). It has been reported that the colored MPs are the most dominant ones among the collected plastic samples in water, accounting for 50.4–86.9% of the total MPs. The detected colors include white, green, yellow, orange, red, blue, grey, and black in different marine fishes (Wang et al., 2017; Shaw and Day, 1994; Su et al., 2016; Peters et al., 2017; Zhang et al., 2018). In the natural environment, color is one of the most essential evolution features for organisms, either for avoiding predation by their predators or for capturing proper light resources (Croce and Van Amerongen, 2014; Karpestam et al., 2014). Previous studies indicated that predators were prone to ingest MPs with colors resembling their prey, either through preferably selection or mistakenly ingestion (Boerger et al., 2010), suggesting that distinct colored MPs are likely to exert different ecological risks in the natural environment; whereas it is poorly understood yet.

Microalgae are at the basis of energy resources, which can convert inorganic compounds into polysaccharides, proteins, and lipids (Mata et al., 2010). The growing input of MPs and nanoplastics into the aquatic environment may lead to inhibitory rates on algal growth (Mao et al., 2018; Besseling et al., 2014). The dose-dependent adverse effects on algae growth were mainly observed in the first sensitive stage (the lag phase and the earlier logarithmic phase) (Sjollema et al., 2016). Moreover, the algal biomass reduction and photosynthesis toxicity were usually observed at very high exposure concentrations (> 100 mg/L) (Mao et al., 2018; Besseling et al., 2014). Adsorption, aggregation, and oxidative stresses are the main explanations involved in the algal growth inhibition (Mao et al., 2018; Zhang et al., 2017). The shading effect is another important reason for growth inhibition by particulates, such as bulk black carbon or carbon nanoparticles (Long et al., 2012). But the white-colored and transparent MPs may not cause apparent shading effects on algae. A previous study suggested that the shading effect did not exist for white-colored polyvinyl chloride (PVC) particles for microalgae *Skeletonema costatum* (Zhang et al., 2017), but it is still unknown for other colored MPs and needs to be further explored.

The influence of MP on algae deserves pressing concern, as any potential toxic effects on them may affect higher trophic leveled organisms, such as zooplankton (Wan et al., 2018). Take *Artemia franciscana* for example; they can accumulate and excrete the nanoplastics that were adsorbed on microalgae *Dunaliella tertiolecta* from prey to predator (Bergami et al., 2017). But for larger particles, many zooplankton (i.e., *Acartia clausi* and *Calanus pacificus*) have a size-selection strategy for prey (Cole et al., 2013). *Centropages typicus* became to gain a discernible ability to avoid eating larger beads (20.6 μm) after MP exposure, but were still unable to discriminate the smaller ones (7.3 μm, which is similar to their prey algae size) (Cole et al., 2013). This ability is probably due to the numerous chemoreceptors and mechanoreceptors on the mouthpart of zooplankton, which can differentiate algae cells from MPs (Cole et al., 2013). Under such circumstances, zooplankton can protect themselves through size-selection strategies for bigger sized plastic particles, but they are still under threat by the smaller sized particles. The abundance of MPs in water increases by orders of magnitude with the size decreases (Shim et al., 2018). Recently, a particular focus has been given to small-sized MPs (< 10 μm). Novotna et al. (2019) reported that the MPs in the outflow of drinking water treatment plant were mainly in size range of 1–10 μm (Novotna et al., 2019). Also, over 93% of the detected MPs were smaller than 10 μm in bottled water, and the maximum quantified MP concentration reached 35 436 MPs/L (Oßmann et al., 2018). Therefore, the understanding of zooplankton responses to small-sized MPs needs to be explored further.

The physical properties of MPs can be changed in the aquatic environment. *Scenedesmus obliquus* is usually negatively charged due to the carboxyl groups on their cellulose surfaces (Bhattacharya et al., 2010). In the natural aquatic environment, normal uncoated MPs are usually slightly anionic with zeta potential values below zero at environmental-related pH, electrolyte concentrations, and natural organic matter contents (Li et al., 2018a). Thus, algae and MPs can exist together relatively stable at the beginning of their interaction. But with longer interaction periods, the extracellular polymeric substances (EPS) excreted by algae can form sticky agglomerates, and the hetero-agglomeration of algae and MPs may occur due to algae biofilm formation or excretion process by organisms (Long et al., 2017; Thornton, 2002; Wright et al., 2013). Thus, MPs will become easy to transport vertically due to their agglomeration and heavier density, and their environmental (eco)toxicological effects will also change accordingly. In the present study, we aimed to understand (1) how colored MPs affect the algae (*Scenedesmus obliquus*) growth; (2) if different environment conditions (such as light and light plus turbulence) will affect algal growth differently (3) if colored MPs could interfere in the zooplankton (*Daphnia magna*) ingestion for green algae.

2. Materials and methods

2.1. Chemicals

The polystyrene MPs were purchased from BaseLine Chromtech Research Centre (Tianjin, China). Four colored MPs were chosen (white, green, orange, and red) with the same particle size of 5 μm according to the manufacturer and these MPs had been washed by Milli-Q water before usage. All other reagents and chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). The zeta potential of each kind of MP and algae was measured by dynamic light scattering on a Zetasizer Nano Series software (Malvern Panalytical Co., UK) and the results can be found in Table S1.

2.2. Culture of Scenedesmus obliquus and Daphnia magna

S. obliquus (FACHB-276) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, China. The algae were cultured in 250 mL flasks containing 100 mL of BG11 medium at 23 ± 1°C (Table S2). The culture was continuously illuminated with fluorescent lamps and shaken twice manually every day. *D. magna* was cultured in M4 medium (a mixed trace element composition medium) (Table S3) (Arl et al., 2019). Each culturing glass tank contains 30 neonates in 500 mL fully aerated M4 medium at 23 ± 1°C. *D. magna* was fed with a constant amount of *S. obliquus* for food, and the M4 medium was renewed twice a week.

2.3. Preparation of microplastic exposure solutions

Exposure groups were separated into five groups, namely control group ([control]), white MP exposure group ([white]), green MP exposure group ([green]), orange MP exposure group ([orange]), and red MP exposure group ([red]). A wide concentration range of MPs were added according to the initial algal cell numbers by 0.01% (C1), 10% (C2), 20% (C3), 40% (C4), 100% (C5), 200% (C6), 400% (C7), 1000% (C8), and 1500% (C9), respectively, which is in accordance with 6 MP/mL (C1), 6 000 MP/mL (C2), 12 000 MP/mL (C3), 24 000 MP/mL (C4), 60 000 MP/mL (C5), 120 000 MP/mL (C6), 240 000 MP/mL (C7), 600 000 MP/mL (C8), and 900 000 MP/mL (C9), respectively. The reported MP concentrations (< 10 μm) in raw water or drinking water was 1.5–35 MP/mL (Oßmann et al., 2018; Pivokonsky et al., 2018). According to our estimation, the MP concentration will increase by 83–150 times, which equals to 122-5 315 MP/mL by 2060 on a business-as-usual scenario (detailed calculation can be found in

Supporting Information-section I). Therefore, the C1 concentration (6 M P/mL) was chosen according to the current small-sized MP pollution status, and the C2 concentration (6 000 M P/mL) was chosen according to the predicted MP concentrations in 2060. The C3-C9 were selected to explore the MP effect on algae growth with even higher MP concentrations.

Scenedesmus spp. are widely recognized as high-quality food resources for cladocerans (i.e., *D. magna*) (Ahlgren et al., 1990). The carbon content of *S. obliquus* was 2.30×10^{-11} gC/cell with carbon to dry weight ratio of 0.50 (Boersma and Vijverberg, 1996). The initial algae concentration was set at 4 μgC/mL to ensure the food amount for *D. magna* (Stibor and Navarra, 2000), when we accounted for the algae settling effects on the carbon concentration dilution. In the *D. magna* ingestion experiment, 0%, 10% (C2), 20% (C3) and 40% (C4) MPs of algal cell numbers were added together with *S. obliquus* to *D. magna*. The MP percentages were chosen according to the calculated MP environmental concentrations and due to the different growth effects in the algae exposure tests.

2.4. Algal growth measurement

For the algae exposure experiment, the initial algae concentration was set at 60 000 cells/mL. Exponentially growing *S. obliquus* was exposed to different colored MPs for 72 h and 120 h. The MP effects on the algal growth were measured under different exposure concentrations. The experiments were performed with two scenarios: one is under light illumination solely, and the other one is under both light and gentle turbulence together. Natural turbulence can be formed by wind or inflows and outflows of natural water bodies (Zhang et al., 2019), and the turbulence has been reported to be an important factor in nutrients uptake and light availability for algae (Bordet et al., 2017). To facilitate the algal growth quantification process, we established a linear curve of algal cell numbers versus optical density (OD) values (Fig. S1). Meanwhile, the control samples were checked that their growth amounts within 72 h had increased at least 16 times to ensure within the logarithmic phase.

2.5. Algal cell locomotion behavior after exposure to different colored microplastics

The color effects of MPs on algae were also monitored from a microscopic view under an inverted microscope (Nikon Ti-U, Japan). The algal cell locomotion behaviors were traced on a disk-shaped chamber fabricated by silica gel on a gas-liquid interface (Zhu and Liu, 2019). Camera (sCMOS, pco. Edge 5.5, Germany) is utilized to capture the movement of algal cells within 5 min. The video images were processed by Image J's descendant Fiji, and their trajectories and average locomotion speeds were also plotted and calculated by Fiji (Schindelin et al., 2012).

2.6. Oxidative stress biomarkers analyses

Algae samples were harvested by centrifugation at 2 500 G for 10 min at 4 °C, and the exposure medium was removed and washed with fresh BG11 medium. Then, the samples were homogenized on ice for further measurements. The non-enzymatic oxidative stress was tested by using the total antioxidant capacity (TAC) assay. The tests were performed using commercial TAC and protein bicinchoninic acid (BCA) kits (Nanjing Jiancheng Bioengineering Institute, China). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a free radical. The antioxidants can scavenge the DPPH to extents according to the antioxidants in algae samples, and the antioxidant capacity can be calculated as U per mg protein (Janaszewska and Bartosz, 2002). Meanwhile, two representative enzymatic related antioxidants activities (catalase (CAT) and glutathione peroxidase (GPx)) and the level of the reduced form of glutathione (GSH) were also measured according to our previous study (Chen et al., 2017).

2.7. Daphnia ingestion experiment with the presence of microplastics

Medium M4 was aerated before exposure and stirred gently daily to ensure algae were suspending in the solution during the algae concentration quantification. The algae ingestion amounts were measured and calculated by optical density (OD) values. The ingested algae amounts were calculated by deducting the growth amount during a day. The algal growth amount was calculated according to the control by the following equation. The specific growth rate, which means the rate at which algal cell concentration increases per unit time.

$$\mu = (1/x)\cdot(dx/dt)$$

in which, μ is the specific rate of growth (d^{-1}), x is the algal cell concentration (cell number/mL), and t is the time (d).

The survival status of *D. magna* was checked every day, and we did not find any lethal effects on daphnids during the whole experiment.

2.8. Statistics

The data differences among different colored MP exposure groups were firstly tested for normality of variance. Subsequently, they were evaluated by the one-way analysis of variance (ANOVA) followed by Turkey's test when parametric assumptions were met, or assessed by non-parametric multivariate rank test when non-parametric assumptions were met. Different letters on bars represent $p < 0.05$ between each other. All analyses were performed with SPSS (version 20.0, SPSS software Co., USA). Figures were plotted with GraphPad Prism (version 8.0, GraphPad Software Co., USA).

3. Results and discussion

3.1. Effects of microplastic color on the algal growth under light condition

Under the light condition, the growth stimulation effect was observed for colored MPs at lower concentrations (Fig. 1). The above phenomenon is similar to the hormesis phenomenon which describes processes of organisms exhibit a biphasic response to contaminant exposure; typically, low dose exposure elicits a beneficial response (Mattson and Calabrese, 2010). Previous studies reported that the exposure of copper nanoparticles and copper-carboxylated nanoparticles showed a slight growth stimulation of the green algae *Chlorella vulgaris* and diatom *Phaeodatylum tricornutum*, respectively; whereas the treatment with higher nanoparticle concentrations induced significant inhibition (Zhu et al., 2017; Mykhaylenko and Zolotareva, 2017). Thus, the stimulation effects at the beginning were deemed as a generalized adaptive response to xenobiotic challenges (Iavicoli et al., 2018). After 72 h exposure, the growth-stimulating effect by MPs was slight (mainly occurred in C2-C5), and the highest growth rate increased by 31.3 ± 47.3%, 28.3 ± 32.7%, 21.7 ± 16.7%, and 7.6 ± 14.4% for [white], [green], [orange], and [red] groups if compared with the control, respectively (Fig. S2). At higher MP exposure concentrations (C6-C9), algal growth was gradually decreasing with the increasing MP concentrations, but there were no significant differences from the control, except for C9 in the [orange] group. It indicates that within 72 h, MPs had no significant inhibition effect on algal growth.

The effect of MPs emerged to be obvious after 120 h exposure. At relative lower exposure concentrations (C2-C4), there were significant differences among the different colors (Fig. 1). The growth increment in the [green] group is the highest at MP concentrations of C4 by 95.9 ± 51.8%, following by the [orange] group at C4 by 71.2 ± 41.7%, the [red] group at C3 by 58.3 ± 25.0%, and the [white] group at C2 by 15.3 ± 4.9%. Chlorophyll *a* and chlorophyll b in green algae are the main photosynthetic pigments which absorb blue,

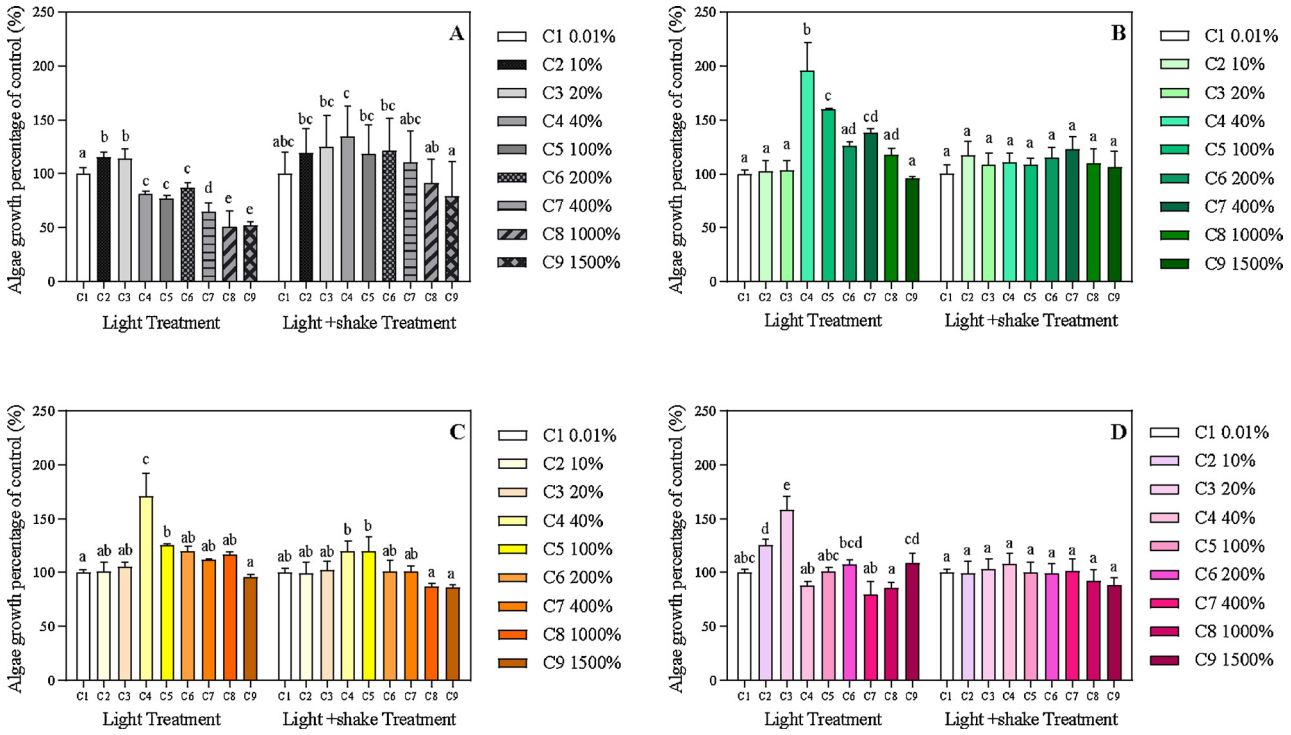

Fig. 1. The algae growth percentages of control in four microplastic exposure groups after 120 h under light condition. (A) white-colored MP; (B) green-colored MP; (C) orange-colored MP; (D) red-colored MP.

red, and orange wavelengths as shown in Fig. S3 (Markager and Vincent, 2001). Due to the suspension status of polystyrene MPs in the algae medium (polystyrene density = 1.0–1.1 g/cm^3) (Li et al., 2018b), the suspended MPs increased the local reflections of the orange or the red lights around algal cells in the [orange] and [red] groups. The microplastic particle numbers can be 15 times higher than the algae cell numbers. As the microplastic particle and algal cells are in the similar sizes, the light reflected by different colored microplastic will exist and we have seen the inhibition effect at high microplastic exposure concentrations. These two kinds of lights can promote the growth of algae, whereas the green-colored MPs may suppress the growth of algae to some extent, relatively. However, we observed an opposite phenomenon in the [green] group.

We assumed that algae may have a color-recognition function, and it can lead to their different preferences to different colors. For example, the green color is like the color of the algal cells themselves. We recorded the algae moved distances within 5 min, which is a direct reflection of algal cell locomotion ability. From the perspective of MP dynamics, algae had the fastest moving speed in the control group (24.4 ± 12.6 μm/min) (Fig. 2). The detailed algae locomotion trajectories can be observed in Fig. S4. The average locomotion speed of the algal cells in the [green] group was 24.3 ± 16.7 μm/min, which did not show obvious differences in speed values distribution from that of control. But the speed of algae in the [white], [orange], and [red] groups displayed different distribution patterns with most algae swimming speed below 20 μm/min, and the [white] group had a significantly lower speed of 14.5 ± 10.0 μm/min than that of control (p = 0.044). The faster average locomotion speed of algal cells in the [green] group could enable the algae to capture much more light resources. Thus, the more opportunities for the algae to obtain light in the [green] group, the greater biomass of algae produced (Fig. 2).

Compared with the other three colored groups, the slightest hormesis effect was observed in the [white] group at low exposure concentrations (Fig. 1), and its locomotion speeds were the slowest in the meantime (Fig. 2). Although the white MPs reflect almost all wavelengths that they received, the polystyrene particles were suspended in

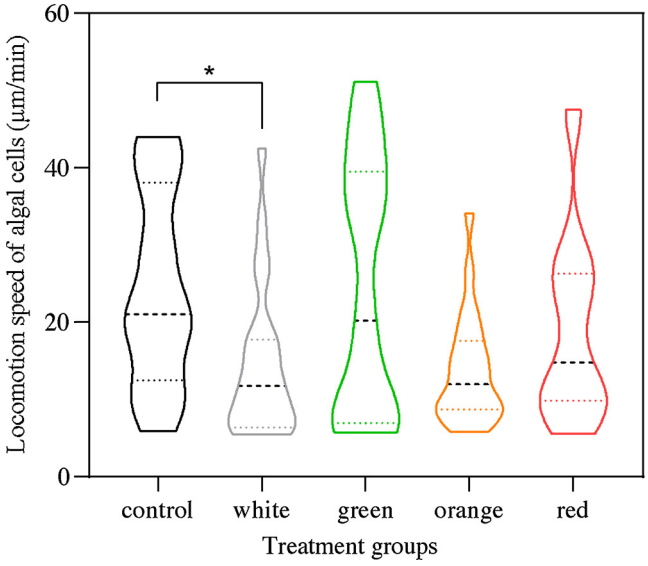

Fig. 2. The swimming speed of algal cells in the [control], [white], [green], [orange], and [red] groups. The three dashed lines represent 25th percentile, median, and 75th percentile values from the top-down order.

the solution, and thus the reflected light will not shade light illumination. Similarly, a previous study suggested that white-colored PVC did not cause any shading effect on algae up to 50 mg/L (the experiment made a special design to make the light transmit the PVC layer first and then to the solution) (Zhang et al., 2017). The growth inhibition in the concentrations of C4-C9 in the [white] group, was probably due to the small amounts of ethanol additive presence in the uniform white polystyrene MPs for better dispersion. With a concentration of 0.05% ethanol in the medium, it will cause around one-third growth biomass reduction for the green algae *Chlorella vulgaris* (El Jay, 1996). The highest ethanol concentration in the algae medium reached 0.036% for

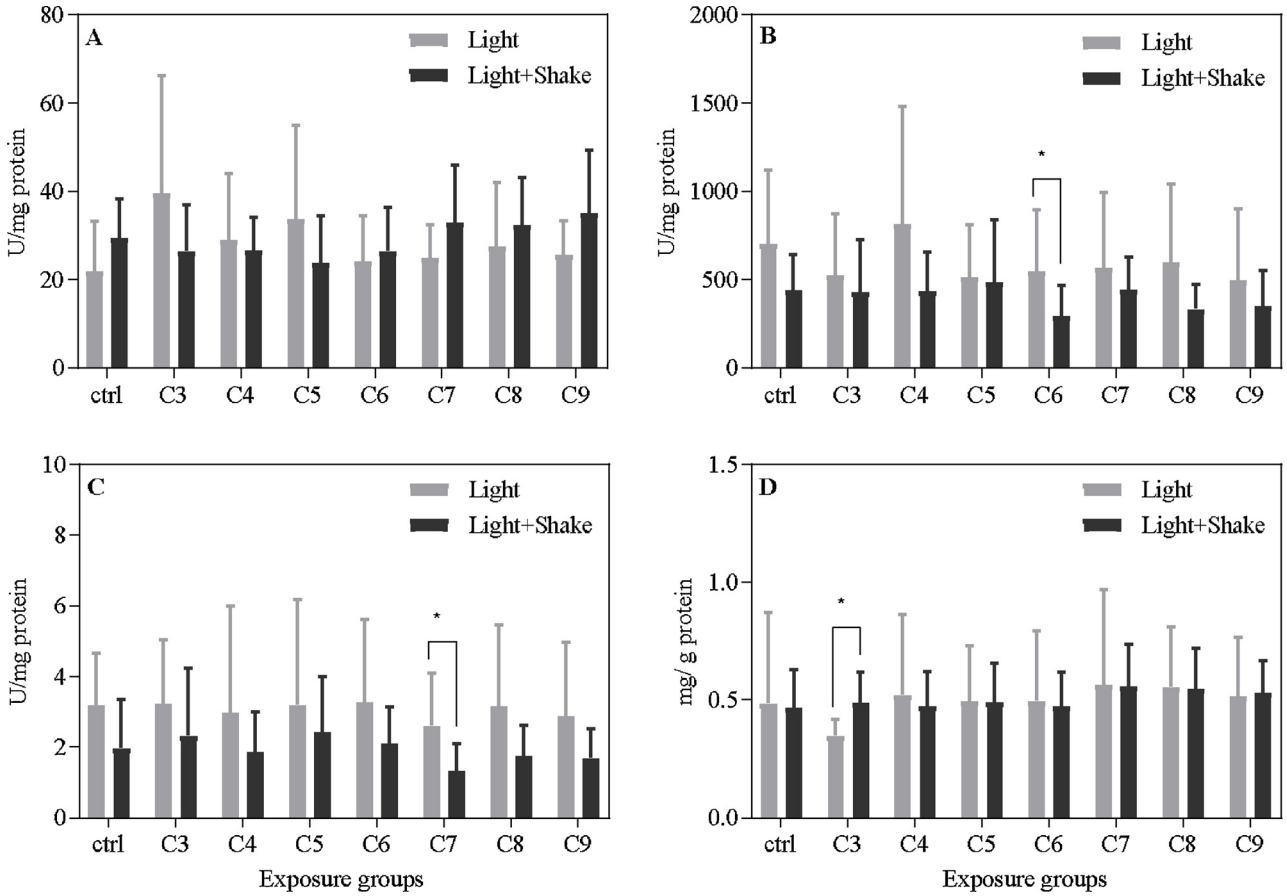

Fig. 3. The oxidative stress biomarkers of algae after exposed to different colored microplastics under either light or light plus turbulence conditions. (A) TAC, (B) CAT, (C) GPx, and (D) GSH. TAC: total antioxidant capacity, CAT: catalase, GPx: glutathione peroxidase, GSH: glutathione.

the C9 group in the present study, and the remaining ethanol in the [white] group could cause an inhibition effect on the algal growth at high exposure concentrations. Thus, it was probably mainly attributed to the presence of ethanol residue but not white-colored MP itself.

Due to the shading effect, the growth rates of algae in the [green], [orange], and [red] groups slightly decreased at exposure concentrations higher than C6, but not significantly different from that of control (except for C7 in the [green] group) (Fig. 1). As there is no severe oxidative damage occurred after MP exposure according to the total antioxidant capacity (TAC) results (Fig. 3A) and no reported toxic additives present in the green- orange- and red-colored MPs according to the manufacturer, the slight inhibition effect induced by high MP exposure concentrations may be attributed to the shading effect. The colorful plastic particles could absorb all wavelengths except for their colored light. Therefore, with increasing colorful MP concentrations, the shading effects would increase accordingly.

3.2. Effects of microplastics on algal growth under light plus turbulence conditions

Unlike the exposure groups only with light illumination, the hormesis effect at lower exposure concentrations did not occur significantly in the light plus turbulence conditions. Turbulence is defined as an eddy-like state of fluid motion where the inertial forces of the eddies are larger than either the viscous of buoyancy forces which tend to damp them out (Zhang et al., 2019). Small-scale turbulence can overcome diffusive transport limitations, so that nutrient uptake is enhanced. For example, the growth rate of *Phaeodactylum* increased by 25–40% at 120 rpm (equals to 0.2 g). But if the turbulence is too large, viscous stresses may mechanically damage the cells or interfere with algal growth processes (Zhang et al., 2019).

There was only slight average increment of algae biomass in C4 (34.5%) for the [white], in C2 (17.3%) for the [green], in C4 (20.1%) for the [orange], and in the C4 (7.9%) for the [red] groups, respectively (Fig. 1). It may be due to the stimulation of the shake condition itself, which masks the hormesis phenomenon by MPs. The turbulence may increase the probability that algae will receive light due to the turbulence effect along with the enhanced nutrients uptake, and therefore, the differences caused by the color effect is no longer obvious. It was surprising that no significant growth inhibition was found in the [white] group at higher exposure concentrations. It may be because that the turbulence led to significant increase of algae growth, which increased the algae growth by 2.4-fold from 7.59E + 6 cells/mL (light only) to 1.83E + 7 cells/mL (light plus turbulence) after 120 h exposure in the control group.

It is noteworthy that turbulence may lead to slight oxidative damage in the algae. There were no significant antioxidant biomarker differences among different colored MP exposure groups, but some differences observed between light and light plus turbulence groups. The activities of catalase (CAT) and glutathione peroxidase (GPx) were both downregulated in the presence of turbulence, with CAT at C6 and GPx at C7 significantly ($p < 0.05$). Moreover, the reduced form of glutathione (GSH) level increased in the turbulence groups, and GSH at C3 also showed significant differences than that with light treatment only ($p < 0.05$). Therefore, it indicates that the gentle turbulence in the environment may diminish the MP color effects on algal growth, but they may lead to oxidative stress at high MP concentrations.

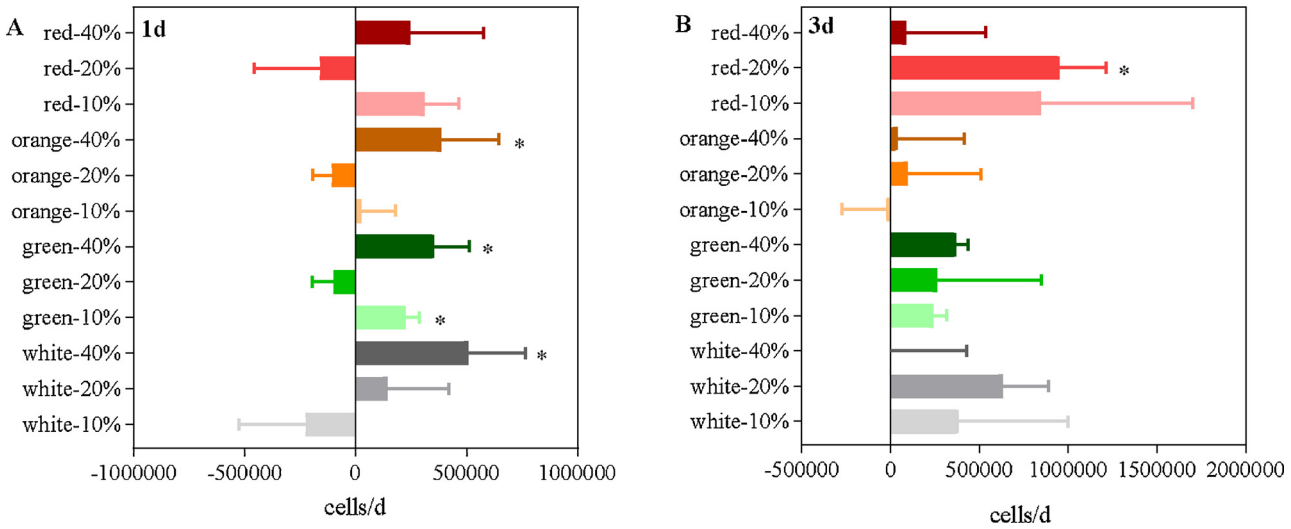

Fig. 4. The ingested algal cell numbers within one day after (A) 1 d or (B) 3 d exposure to different colored microplastics. The percentages of 10%, 20%, and 40% represent the microplastic number percentages of the algal cell numbers.

3.3. Microplastic effects on Daphnia magna prey on algae

We further explored if the color will also affect the algae ingestion ability of *D. magna*. As shown in the previous study, there was no physical damage observed in daphnids after exposure to 5, 10, and 15 μm polystyrene MPs even up to 50 mg/L, because most of the MPs accumulated in the intestinal tract and could be excreted out through anus without sticking on the body (Ma et al., 2016). Therefore, in the present study, we did not aim to quantify the MP uptake and depuration amounts in daphnids but to examine whether the interaction between MPs and algae would affect daphnid's feeding behavior on algae.

Within lower MP exposure concentration groups (10% and 20%), there seems to be no significant changes for the *Daphnia* feeding behavior with ingested algae amounts around the control level (Fig. 4). However, the numbers of ingested algae in all 40% exposed groups ([white], [green], [orange], and [red]) increased when compared with the control, and the first three groups showed significant differences ($p < 0.05$). It suggested that at the beginning of the exposure, 40% MPs of algal cells exhibited a positive effect on *Daphnia* feeding behavior. One explanation is that the feeding behavior of *Daphnia* can be responsive to shifts in food quality (Mandal et al., 2018; Sterner, 1993). Natural food particles range from the highly (termed as "edible") nutritious to very poor (termed as "inedible") food sources (Kretzschmar et al., 1993). When facing a low concentration of inedible algae food source, *Daphnia* will widen its gape. The widened gape is probably due to the lack of edible algae, which induced the *Daphnia* to obtain more food sources. Moreover, the decline in filtering and ingestion rate may often co-occur, which was mainly due to mechanical effects of sticky particles on the filter limbs, rather than a neutrally controlled selection mechanism (Young et al., 1997). But the polystyrene MPs in the present study are round-shaped microbeads which did not contain sticky mucus as the "inedible" algae; therefore, the declined filtering speed was not observed. Thus, the total ingestion amount could increase with widened gape. Another reason is that unlike copepods, *Daphnia* does not obtain a selection strategy between food and larger sized MPs (Cole et al., 2013), not to mention similar sized MPs to their prey algae. Therefore, the above results demonstrated that *Daphnia* did not have a color-selection ability between similar-sized algae and MP particles. If their filter limbs are not stuck, they could ingest more algae with widened gapes at 40% MP concentration.

However, the increased feeding ability alteration at 40% did not last long. On the 3rd day of exposure, the results suggested that the ingested algal cells were almost the same among different exposure groups in comparison with the control, except for the red-2% group (Fig. 3). It is common knowledge that low-density MPs can float or suspend on/in the surface of the water, whereas high-density MPs sink or when low-density MPs become denser (U.S.E.P. Agency, 2006). Initially the density of polystyrene is between 1.0 and 1.1 g/cm^3 (Li et al., 2018b), which can assure its suspension state in the freshwater. However, with the algae inhabiting, the hydrophobicity of the MPs will decrease, and the buoyancy will gradually reduce, thereby accelerating its vertical distribution in the water (Lobelle and Cunliffe, 2011). Moreover, algae could excrete extracellular polysaccharides, which can form sticky particles that can be encapsulated into these algal agglomerates and transported vertically (Wright et al., 2013; Long et al., 2015). It was evidenced by our observation (Fig. 5). Algae and MPs were better separated initially on day 1, whereas the MPs were prone to be wrapped in algae agglomerates after three days and sticky EPS-like stuff can be observed around or within these agglomerates. Therefore, it is possible that most polystyrene MPs were vertically settled at the bottom of tanks, and thus the MP concentration in the water column decreased to concentrations that had no significant effect on daphnids feeding.

4. Conclusions

In conclusion, there seems to be no significant effect on algal growth under the current reported small-sized (5 μm) MP concentrations in the aquatic environment. However, with the MP concentrations increase in the future, different colored MPs could lead to varying effects on algal growth. Among which, green-colored MPs exhibits the lowest inhibition effects, which is probably due to its assembly to algae themselves; whereas the white-colored MPs may lead to apparent inhibition on algae growth, which may be attributed to the presence of additives. As for *D. magna*, they did not appear to have a selection strategy for differentiating similar sized MPs from algae. Moreover, the suspended MPs at 40% of algal cell numbers could increase daphnids' ingestion of algae. However, MPs that coexist with algae for a long time may be wrapped by algae agglomerates and settle down, and then the daphnid's algae ingestion can return to normal state. Therefore, it is necessary to pay attention to the toxic effects of MPs of different colors on aquatic organisms. Furthermore, the occurrence status of MPs (such as suspension and agglomeration) is also essential to be considered when evaluating their potential risks to aquatic environment.

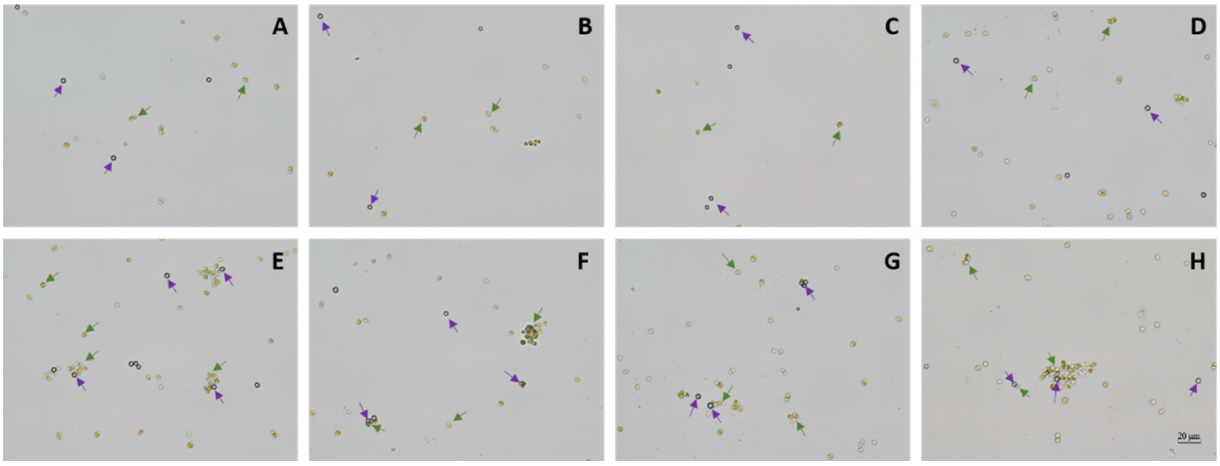

Fig. 5. The interactions between microplastics and algal cells. **A-D**: the initial states of microplastics and algae (day 1); **E-H**: the agglomerations states between microplastics and algae (day 3). The algal cells are in green color, and the microplastics with light colors of white (**A**, **E**), green (**B**, **F**), orange (**C**, **G**), and red (**D**, **H**). The purple arrows point to microplastics, and the green arrows indicate algal cells or algal agglomerates.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2019.121224.

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