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Increased bio-toxicity of leachates from polyvinyl chloride microplastics during the photo-aging process in the presence of dissolved organic matter

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ABSTRACT

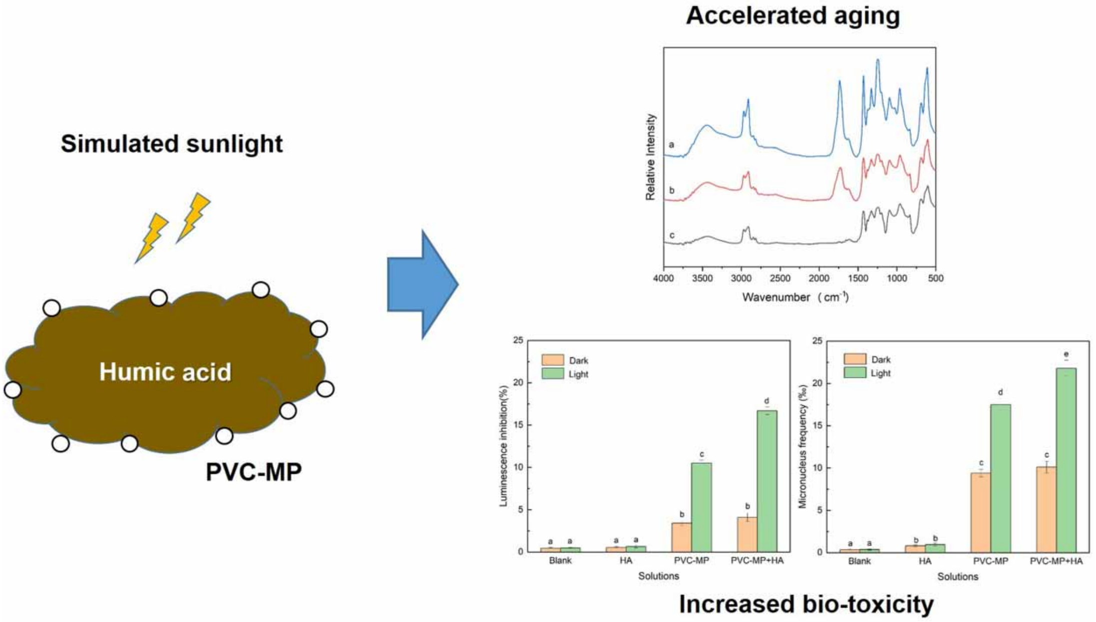
The pollution caused by microplastics (MPs) has gained global attention due to their potential risks to organisms and human health. The process of photo-aging, which plays a crucial role in the transformation of MPs in aquatic environments, has the potential to influence the ecological risk posed by these particles. Dissolved organic matter (DOM) is a prevalent photosensitizer in surface waters that has been shown to facilitate the transformation of various organic compounds by generating reactive oxygen species under light irradiation. The present study investigated the influence of humic acid (HA), a typical component of DOM, on the photo-aging process of polyvinyl chloride MPs (PVC-MPs), using Fourier transform infrared spectroscopy, as well as assessing the resulting ecological risk through bioassays. The results revealed that the presence of HA enhanced the photo-aging of PVC-MP. Moreover, the leachate exhibited higher acute and genetic toxicity under light irradiation when compared to dark conditions. Notably, the presence of HA significantly increased the toxicity of the leachate, emphasizing the need to consider the impact of DOM when assessing the ecological risk of MPs in surface waters. These findings contribute to a more comprehensive understanding of the potential risks associated with microplastic pollution in natural environments.

Key words: bioassay, humic acid, microplastics, photo-aging

HIGHLIGHTS

- PVC-MP leachates exhibited higher acute and genetic toxicity under irradiation in the presence of dissolved organic matter.
- A bioassay provided a cost-effective means to assess the ecological risks posed.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Microplastics (MPs), consisting of plastic particles or fibers with sizes below 5 mm, have emerged as a global concern due to their potential toxicity. A significant proportion of the millions of tons of plastic produced annually enters the environment and undergoes natural weathering processes, resulting in the formation of MPs and nanoplastics (Wu *et al.* 2020). These small plastic particles can persist in the environment for extended periods, potentially reaching hundreds of years (Zhou *et al.* 2021). The harmful effects of MPs on various aquatic organisms, including zooplankton (Botterell *et al.* 2019), marine mammals (Moore *et al.* 2020), microalgae (Gao *et al.* 2023), and benthic foraminifera (Langlet *et al.* 2020), have been reported. These impacts are not solely attributed to the physical presence of MPs but also to the release of chemicals from the particles. Aging processes can alter the properties of MPs, such as particle size, specific surface area, roughness, morphology, functional groups, and the release of chemicals, thereby influencing the ecological risks associated with these particles (Luo *et al.* 2022).

Photo-aging is a prevalent degradation process that occurs in plastic materials within aquatic environments. Previous studies have documented the changes in the surface structure that accompany the aging of MPs, including increased surface roughness and the formation of specific functional groups, such as vinyl (C–C) and oxygen-containing groups (–OH, –COOH, and –CHO) (Wang *et al.* 2023). Additionally, photo-irradiation can lead to the release of organic substances from MPs (Wang *et al.* 2020; Du *et al.* 2023). The photo-aging process of MPs can be categorized into direct and indirect photo-aging. The former involves the breakage of intermolecular bonds on the surface of MPs upon exposure to ultraviolet light with wavelengths ranging from 290 to 400 nm (Fan *et al.* 2021; Zhang *et al.* 2021). The latter refers to free radical-sensitized photo-aging, which is mediated by chromophores present in the environment or impurities within the polymer (Yang *et al.* 2018; Wang *et al.* 2020). Previous research by Wang *et al.* (2020) indicated that low-molecular-weight organic acids, such as oxalate and citrate, can promote the aging process of polyvinyl chloride MPss (PVC-MPs) and enhance the release of organic chemicals from PVC-MP by generating hydroxyl radicals. Dissolved organic matter (DOM), which is widespread in aquatic environments, often acts as an important photosensitizer. The photochemical degradation of organic pollutants through photolysis sensitized by DOM has been extensively studied in recent years. During this process, reactive oxygen species (ROS) such as hydroxyl radicals ($^{\cdot}$OH), singlet oxygen ($^1O_2$), and triplet states of DOM are formed under sunlight exposure (Chen *et al.* 2016). These ROS also play a crucial role in the degradation and aging of MPss (Duan *et al.* 2022; Liu *et al.* 2022).

Despite the ubiquitous presence of DOM in surface water, limited research has been conducted on its potential influence on the photo-aging behavior of MPs and the resultant ecological risks. The objective of this study was to investigate the impact of humic acid (HA), a common type of DOM, on the photo-aging process of PVC-MPs and the resulting toxicity of leachates.

PVC was selected as the target material due to its widespread usage and presence in water bodies, as well as its greater susceptibility to embrittlement and decomposition compared to other MPs (Browne *et al.* 2010; Wu *et al.* 2019). Considering the complex composition and low concentration of chemicals leaching from PVC-MP, the potential risk of the leachate during the photo-aging process was evaluated by the bioassay. The results of this study will enhance our understanding of the photo-aging process of MPs in natural waters and the associated ecological risks.

2. MATERIALS AND METHODS

2.1. Chemicals

PVC particles with a diameter of 75 μm were obtained from Jiaquan Plastic Co., Ltd (Dongguan, China). HA bought from Sigma-Aldrich (USA) was dissolved in ultrapure water and filtered through a 0.45-μm membrane to prepare a stock solution. The concentration of the HA stock solution, measured in mg C/L, was determined using a carbon analyzer (Shimadzu, Tokyo, Japan). Ultrapure water with a resistivity of 18.2 MΩ·cm was generated using a Milli-Q water purification system (Millipore).

2.2. Aging experiment

The photo-aging experiments were performed using 30 mL quartz glass tubes as reaction vessels. The reaction solution consisted of PVC-MP at a concentration of 100 mg/L, with the option of incorporating either 0 or 5 mg/L HA, along with ultrapure water. The initial pH of each solution was adjusted to 7.0 using hydrochloric acid or sodium hydroxide. Subsequently, the reaction tubes were placed within a photochemical reactor (YM-GHX-V, Yuming Instrument Company, Shanghai, China) equipped with a 500 W central xenon lamp in the center. The reaction solutions were subjected to magnetic stirring, while a constant temperature of 25 °C was maintained throughout the experiment using a recycled cooling water system. Control experiments were also conducted concurrently, employing tubes covered with aluminum foil within the reactor. After an irradiation period of 72 h, the aged PVC-MPs were collected by centrifugation at 6,000 rpm for 10 min and subsequently freeze-dried. Fourier transform infrared spectroscopy (FTIR) analysis was then conducted, utilizing a Nicolet iS 10 instrument (USA), to identify and evaluate the functional groups within the 4,000–500 cm^{-1} range, employing KBr pellet technology. The supernatants obtained from the centrifugation step were collected for subsequent bioassay tests.

2.3. Bioassay

The acute toxicity assessment was conducted using the bioluminescence inhibition assay, as described by Villa *et al.* (2014) and Chen & Wang (2018). Ultrapure water served as the blank control in the experiment. Prior to the test, the freeze-dried bacteria *Vibrio qingaiensis* sp. nov. (Q67) was reconstituted. In each quartz tube, 0.1 mL of the test solution was mixed with 0.9 mL of the cultivated bacterial suspension. Subsequently, the luminator (Berthold, Germany) was employed to measure the relative light unit of each test medium and the blank control after a 20-min exposure. The degree of light emission inhibition, which was calculated by comparing the relative light unit (RLU) of each test medium to that of the blank control, was used to determine the acute toxicity of the test solutions.

The genetic toxicity of each test solution was assessed using the *Vicia faba* roots tip micronucleus assay, following the methodology described by Chen & Wang (2018). Dry broad bean (*V. faba*) seeds, which were obtained from the Central China Normal University and harvested from an unpolluted area, were utilized for the assay. The seeds were soaked and germinated at 25 °C until roots measuring 2–3 cm in length emerged. For each test solution and the blank control (ultrapure water), five germinated seeds with uniform root growth were exposed for a duration of 6 h, followed by cultivation at 25 °C for 24 h. Subsequently, the root tips of the beans were excised, fixed using Carnoy liquid (a mixture of acetic acid and ethanol in a ratio of 1:3), stained using the Feulgen staining technique, and finally mounted on slides. The number of micronuclei observed on each slide was counted under a microscope. The genetic toxicity was evaluated based on the frequency of micronuclei, which was represented as the average number of micronuclei per 1,000 cells in each root tip.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (USA) for Windows. To identify significant differences, a one-way analysis of variance (ANOVA) was employed. Statistical significance was considered when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Photo-aging of PVC-MP

The oxidation levels and aging kinetics can be estimated by the changes in the oxygenated groups of MPs during the aging process. As illustrated in Figure 1, compared to the FTIR spectra of pristine PVC-MP, two new absorption bands were observed at 1,730 and 3,400 cm^{-1} after photo-aging, which indicated the presence of $–C=O$ and –OH stretching vibrations, respectively. The presence of $–C=O$ and –OH groups was the result of oxidation reactions during the aging process of MPs, and the intensity of these bands correlates with the extent of aging (Liu *et al.* 2019; Zhu *et al.* 2020). Several published studies have reported the phenomenon of accelerated aging of PVC-MP under light irradiation (Ouyang *et al.* 2022; Tian *et al.* 2023).

Moreover, the intensities of both $C=O$ and O–H were observed to be higher in the presence of HA, suggesting that the presence of HA enhanced the photo-aging process of PVC-MP. Only a few studies have explored the influence of environmental factors such as salinity, low-molecular-weight organic acids, and clay minerals on the photo-aging of MPs (Cai *et al.* 2018; Wang *et al.* 2020; Ding *et al.* 2022). However, the impact of DOM in surface water has received relatively little attention. Wang *et al.* (2020) demonstrated that the presence of low-molecular-weight organic acids could accelerate the photo-aging process of PVC-MP under both simulated and natural sunlight irradiation, primarily due to the formation of •OH radicals. Similarly, HA, being a type of DOM, contains various chromophores that can absorb light and generate reactive oxygen species (ROS), including $^3HA^*$, •OH, and $^1O_2$, which might contribute to the enhanced photo-aging of PVC-MPs.

Photo-aging plays an important role in the degradation process of MPs in the natural environment, which usually involves free radical-mediated reactions initiated by solar irradiation (Zhang *et al.* 2021). Specifically, when exposed to light irradiation, PVC experiences rapid dehydrochlorination, leading to the formation of short sequences of conjugated unsaturation within the polymer. These unsaturated sequences are susceptible to further photodegradation (Ainali *et al.* 2021). In cases where PVC contains chromophores due to impurities or low-molecular-weight organic acids, photosensitized photo-aging may occur, resulting in the formation of polyene structures. These structures can then be oxidized to ketones and alcohols by O_2 and •OH radicals (Wang *et al.* 2020). Given the ubiquitous presence of HA in the natural aquatic environments, it is likely that HA promotes the photo-aging process of PVC-MP by generating ROS, thereby influencing the fate and ecological risk associated with PVC-MP.

3.2. Bio-toxicity

The acute toxicity of the samples expressed as luminescence inhibition is shown in Figure 2. Both the blank control and the HA samples exhibited minimal luminescence inhibition percentages in both dark and light conditions. Compared to the blank control, the leachates of PVC-MP demonstrated significant acute toxicity, with luminescence inhibition percentages

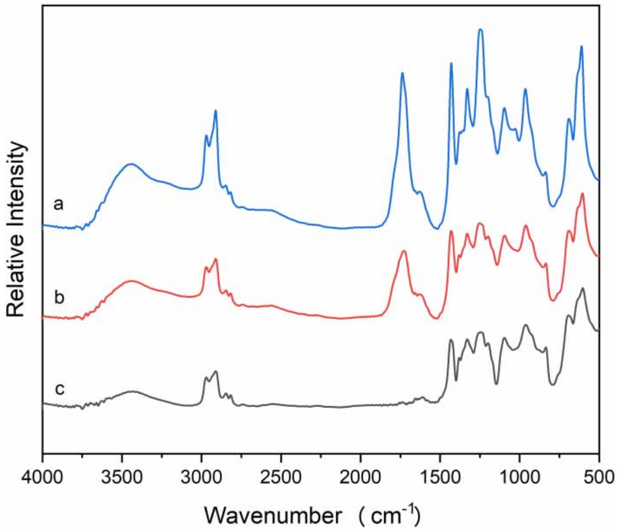


Figure 1 | FTIR spectra of aged PVC-MP in the presence of HA (a), aged PVC-MP in the absence of HA (b), and pristine PVC-MP (c).

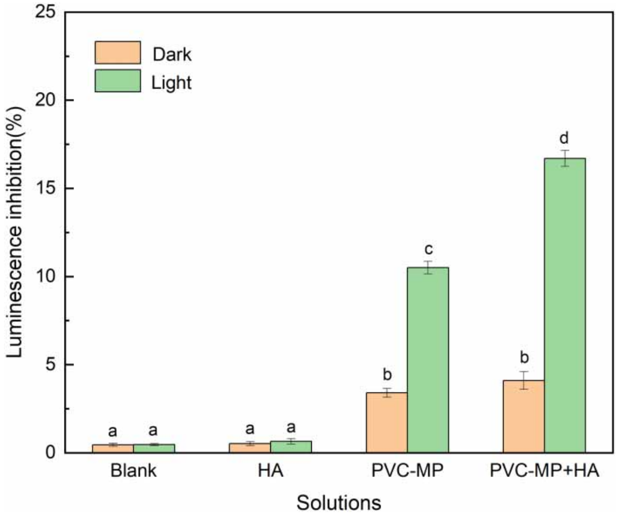


Figure 2 | Acute toxicity of the leachate under various conditions (expressed as luminescence inhibition). Error bars represent the standard deviation calculated from three independent samples. Columns denoted by different lowercase letters (a, b, c, and d) indicate significant differences ($p < 0.05$).

of 3.40 and 10.5% in the dark and under light irradiation, respectively. Furthermore, the acute toxicity of the PVC-MP leachate exhibited a significant increase when exposed to simulated sunlight irradiation. In the presence of HA, the acute toxicity of the leachate remained relatively stable in the dark but exhibited a significant increase under light irradiation, with a luminescence inhibition percentage of 16.7%.

The results for genetic toxicity, which are assessed by micronucleus frequency in root tips, are depicted in Figure 3. The leachates displayed higher genetic toxicity compared to the blank control and the HA solution, both in the absence and presence of light irradiation. Specifically, the micronucleus frequency in root tips was measured at 9.41 and 17.5‰ for the leachates under dark conditions and light irradiation, respectively. Notably, the photo-aging process, especially in the presence of HA, resulted in a substantial increase in genetic toxicity, as evidenced by the micronucleus frequency in the PVC-MP leachates reaching 21.8‰.

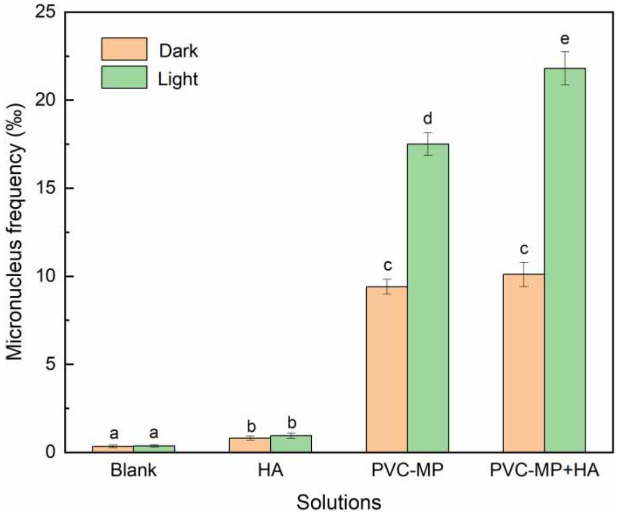


Figure 3 | Genetic toxicity of the leachate under various conditions (expressed as micronucleus frequency). Error bars represent the standard deviation calculated from three independent samples. Columns denoted by different lowercase letters (a, b, c, d, and e) indicate significant differences ($p < 0.05$).

Previous studies have demonstrated the toxic effects of leachates from MPs on various organisms, including the sea urchin *Lytechinus variegatus*, blue mussel *Mytilus edulis*, benthic foraminifera *Haynesina germanica*, and so on (Nobre *et al.* 2015; Langlet *et al.* 2020; Uguen *et al.* 2022). The observed toxicity of these leachates was primarily attributed to the presence of chemical additives in the plastic debris. These additives are compounds added to enhance the performance and functionality of plastic products, and they can readily leach from the polymer matrix into aquatic environments when plastic waste enters such ecosystems (Oliviero *et al.* 2019). Certain organic additives, including plasticizers, flame retardants, stabilizers, antioxidants, and pigments, are known to have carcinogenic, mutagenic, or endocrine-disrupting properties toward aquatic organisms (Groh *et al.* 2019; Do *et al.* 2022). Therefore, it can be inferred that the observed toxicity of PVC-MP leachates in the absence of light is likely caused by the release of the additives present in the plastic particles.

The bio-toxicity of leachate derived from MPs exhibited a significant increase after exposure to light irradiation, in comparison to the dark condition. Previous research has consistently reported an escalation in toxicity for leachates derived from aged MPs. Rummel *et al.* (2019) conducted cell-based bioassays and observed an increased induction of oxidative stress response when exposed to leachates derived from UV-weathered MPs. However, Bejgarn *et al.* (2015) reported different findings that leachate from four plastics became more toxic after light irradiation, while two became less toxic and two did not change significantly, depending on the types of plastic products. The increased toxicity was mainly attributed to the enhanced release of plastic additives under irradiation (Bejgarn *et al.* 2015; Luo *et al.* 2020). Besides, during the photo-aging process of PVC-MP, the degradation of the polymer can result in the release of organic substances. Recent studies have demonstrated the generation of microplastic-derived DOM during the weathering processes of MPs, including photo-aging and biodegradation (Miri *et al.* 2022; Ouyang *et al.* 2023). The MP-derived DOM primarily consists of monomers, oligomers, and oxygenated intermediates derived from MPs, which have been identified as emerging environmental hazards and toxic to organisms (Abolghasemi-Fakhri *et al.* 2019; Xu *et al.* 2022).

In the presence of HA, the toxic effects of the leachates were found to be significantly intensified. Light irradiation can lead to the production of ROS such as ^{3}HA*, •OH, and $^1O_2$. It has been established that ROS could promote the aging of MPs and facilitate the release of microplastic-derived DOM (Lee *et al.* 2020; Xu *et al.* 2022), thereby increasing the bio-toxicity of the leachate. However, to date, no research has specifically investigated the impact of DOM on the ecological risk associated with MPs.

4. CONCLUSION

The process of photo-aging plays a crucial role in the aging of MPs within aquatic environments; thus, it is necessary to address the ecological risks associated with this phenomenon. Given the intricate composition and low concentrations of leachates, bioassays offer a cost-effective approach for evaluating the ecological risks involved. In comparison to the blank controls, PVC-MP leachates exhibited statistically significant acute and genetic toxicity under both dark and light irradiation conditions. Notably, the toxicity of the leachates was considerably higher under light irradiation, suggesting a greater release of toxic chemicals during the photo-aging process. Furthermore, the presence of HA was found to enhance the aging process and intensify the ecological risk associated with the leachates. Considering the ubiquitous presence of DOM in surface water, it is imperative to account for its potential to promote microplastic photo-aging and the resulting increase in bio-toxicity.

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AUTHOR CONTRIBUTION

L.C. conceptualized the work, wrote and reviewed the edited manuscript, and supervised the work. H.Q. wrote the preparation of original draft and visualized the work. K.Y. performed data curation and did software techniques. B.G. investigated the work, analyzed the methodology, and validated the work.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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