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RESEARCH ARTICLE

THE STUDY ON THE ACCUMULATION EFFECT OF AGING POLYLACTIC ACID MICROPLASTICS ON CHROMIUM IN THE MARINE MEDAKA (ORYZIAS MELASTIGMA)

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ABSTRACT

The toxic effects of polylactic acid (PLA) microplastics (MPs) on aquatic organisms have garnered widespread attention. However, there is insufficient research on the impact of aged PLA-MPs on the accumulation of heavy metals within organisms. Therefore, this study focused on UV-aged PLA-MPs, utilizing inductively coupled plasma mass spectrometry to determine the accumulation of chromium in marine medaka following joint exposure to original and aged PLA-MPs with chromium. The results indicated that the color of the aged PLA changed from milky white to yellow, the surface of the PLA became rougher with noticeable grooves and protrusions, and there was a significant increase in oxygen-containing functional groups, primarily carbonyl (C=O) and hydroxyl (O-H) groups. Aged PLA significantly increased the Cr content in the marine medaka, with Cr primarily accumulating in the fish's visceral mass. Additionally, after joint exposure to aged PLA and heavy metals, the PLA content within the marine medaka was significantly higher than that of the original PLA. The findings of this study suggest that aged PLA enhances the accumulation effect of chromium in the medaka. Compared to original PLA, the joint exposure to aged PLA and heavy metals may exhibit greater toxicity.

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INTRODUCTION

Microplastics (MPs) are widely present in marine environments, with research indicating that at least 10% of plastic waste ultimately ends up in the ocean[13]. Annually, up to 10 million tons of plastic enter the oceans[2], and studies show that the cumulative amount of plastic waste in marine environments exceeds 150 million tons[6]. Given the increasing severity of plastic pollution, biodegradable plastics, which offer the advantage of complete degradation as an alternative to traditional plastics, are being widely utilized. Among these, polylactic acid (PLA) is a common bio-based biodegradable plastic made from the polymerization of lactic acid produced through the fermentation of plants[11]. As an environmentally friendly material, PLA has become an important alternative to traditional plastics. However, PLA requires specific humidity and temperature conditions for complete degradation, and when discarded in natural environments, it does not fully degrade, leading to the generation of MPs that can have adverse effects on the environment and health. Research has shown that zebrafish prefer to ingest PLA over polyethylene terephthalate (PET), resulting in specific changes in gut microbiota diversity and promoting the proliferation of species closely related to energy metabolism, cellular processes, and fish diseases[1]. However, most current studies focus on the toxicity of PLA-MPs themselves, with limited reports on the carrier effects of PLA-MPs. Therefore, this study uses marine medaka as the research subject to investigate the accumulation effect of aged PLA in combination with the commonly found heavy metal ion chromium in the environment. This aims to provide data support for a comprehensive assessment of the ecological toxicity effects of PLA-MPs in the environment.

MATERIALS AND METHODS

Microplastics and reagents: PLA-MPs (Dongguan Mingyuxing Plastic Raw Materials Co., Ltd.), chromium metal ion standard (National Electronic Materials Analysis and Testing Center), nitric acid (Xilong Chemical Reagent Co., Ltd.), and hydrogen peroxide (Xilong Chemical Reagent Co., Ltd.).

UV aging and characterization of MPs: Accurately weigh 0.5 g of PLA and spread it evenly in a petri dish. Place it in a UV aging chamber using a UV light source of 254 nm, turning the microplastics daily to ensure uniform aging for 20 days. Scanning electron microscopy (SEM) (ZEISS EIGMA, Germany) will be employed to characterize the morphology of PLA before and after UV aging, while Fourier-transform infrared spectroscopy (FTIR) (PerkinElmer FT-IR Microscope Spotlight 400) will be used to determine the changes in the spectra of the microplastics before and after aging, investigating whether there are any alterations in functional groups and chemical properties.

Exposure experiment: In this experiment, uniform-sized adult marine medaka (4 months old) were selected. Prior to the start of the exposure experiment, the fish were fasted for 24 hours. The medaka were randomly assigned to 20 beakers of 2 L each, with 4 fish in each

beaker. Four treatment groups were established: a blank control (artificial seawater), chromium (Cr) alone exposure (100 μg/L), unaged PLA microplastics + Cr co-exposure (MPs 1 mg/L + Cr 100 μg/L), and aged PLA microplastics + Cr co-exposure (aged MPs 1 mg/L + Cr 100 μg/L), with 5 replicates for each treatment group. During the exposure period, a 14-hour light and 10-hour dark cycle was maintained, and the exposure lasted for 7 days. To keep the exposure solutions clean and to maintain stable concentrations of microplastics and heavy metals, the exposure solution was replaced every 48 hours. During the experiment, feeding was conducted twice daily at 9:00 AM and 6:00 PM, ensuring that the fish could consume the food within 15-30 minutes. Any leftover food and fish feces were promptly removed.

Sampling and measurement of exposure solutions: During the exposure period, 20 mL samples of the exposure solution were collected daily. After pretreatment according to relevant methods, the concentration of chromium ions was measured using an inductively coupled plasma optical emission spectrometer (ICP-OES) (NexION 1000G, Agilent, USA).

Measurement of Chromium ions: After 7 days of exposure, the medaka were sampled. The fish from each beaker were collected and placed on ice to be euthanized. Before dissection, the medaka were rinsed three times with physiological saline. The fish were then dissected into three parts: the head, body, and internal organs, which were placed in disposable centrifuge tubes for weighing and recorded as wet weight.To digest the tissue samples, 6 mL of HNO_3 was added, and the samples were shaken in a constant temperature incubator (37°C, 180 r/min) for 48 hours. The liquid portion was filtered into a 25 mL colorimetric tube using a disposable syringe filter, and then diluted to 10 mL with distilled water. Next, in a fume hood, the solution was heated on a heating plate to remove the nitric acid, reducing its concentration to below 5%. After cooling to room temperature, the solution was filtered again using a disposable syringe filter and brought to a final volume of 10 mL with distilled water. The concentration of chromium ions was then measured using an ICP-MS. Finally, the concentrations of chromium were calculated based on the weights of the various tissues obtained during dissection, expressed as wet weight (μg/g).

Measurement of MPs: The dissection method for medaka was the same as that used for heavy metal measurement. To each tissue sample, 2 mL of hydrogen peroxide solution was added for digestion. The samples were then shaken in a constant temperature incubator (37°C, 180 r/min) for 48 hours. After digestion, the samples were filtered using a 5 μm filter membrane, and the filtration process was rinsed several times with distilled water filtered through a 0.45 μm membrane. The collected filter membranes were placed in clean glass petri dishes and left to dry at room temperature. The MPs on the filter membranes were then photographed and counted using a stereo microscope.

Data processing: The formula for calculating heavy metal concentration is as follows:

$$C_1 = \frac{C_0 \cdot V}{m_2 - m_1}$$

C_1——Cr ion concentration (μg/g)
C_0—— Cr ion detection concentration (μg/L)
M_2——Centrifuge tube + Tissue sample mass (g)
M_1——Centrifuge tube mass (g)
Data analysis was performed using Excel 2019 and SPSS Statistics 2024, with one-way ANOVA conducted. $p < 0.05$ was considered statistically significant. Graphs were created using Origin 2021.

RESULTS AND DISCUSSION

Physicochemical properties of UV-Aged PLA-MPs: After 20 days of UV aging, the color change of PLA is shown in Fig. 1. The color of PLA changed from milky white to light yellow, which may be due to the presence of a large number of unsaturated chromophores in PLA. Further characterization of the unaged and 20-day aged PLA was conducted using SEM. The surface of the original PLA was relatively smooth and even, with a dense structure and no visible grooves or cracks. However, after 20 days of UV aging, the surface of the PLA became noticeably rough, uneven, and exhibited various holes, grooves, and cracks of different sizes (Fig. 2). These results indicate that UV aging altered the surface morphology of PLA. Such changes may lead to alterations in the physicochemical properties of PLA, thereby providing more adsorption sites for heavy metals (6), which could influence the environmental behavior of heavy metals.

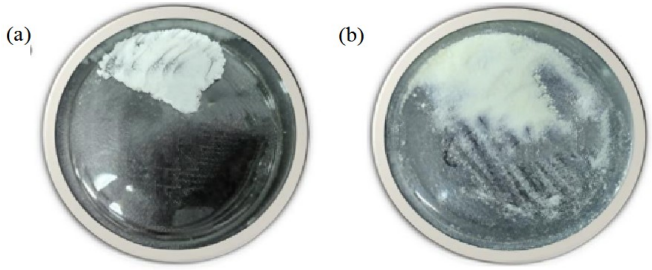

Figure 1. Visual appearance of PLA. (a) Original PLA (b) Aged PLA

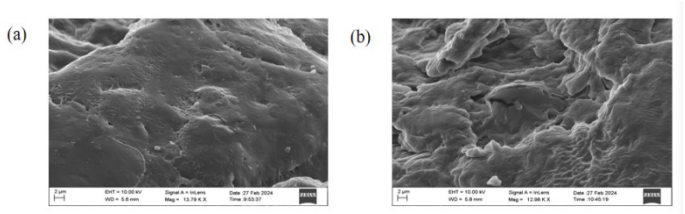

Figure 2. SEM Images of PLA. (a) Original PLA (b) Aged PLA

The FTIR spectra of original and aged PLA are shown in Fig. 3. There were no significant changes in the positions of the absorption peaks before and after UV aging; however, there were notable differences in the absorption peaks of original PLA compared to aged PLA, with a significant increase in functional groups primarily featuring hydroxyl (-OH) and carbonyl (C=O) groups. Significant absorption peaks appeared at 871.3, 1450, 1777.2, 2991, and 3503.1 cm^{-1}, where the peak at 3503.1 cm^{-1} corresponds to the stretching vibration of hydroxyl (-OH), and the vibrational absorption peak at 1777.2 cm^{-1} corresponds to the carbonyl (C=O) absorption peak[7]. After UV aging, the carbonyl absorption peak of PLA microplastics showed significant changes, which is consistent with the findings of Hüffer et al[8]. After UV aging, the increase in surface functional groups such as carbonyl may alter the adsorption capacity of PLA for other pollutants.

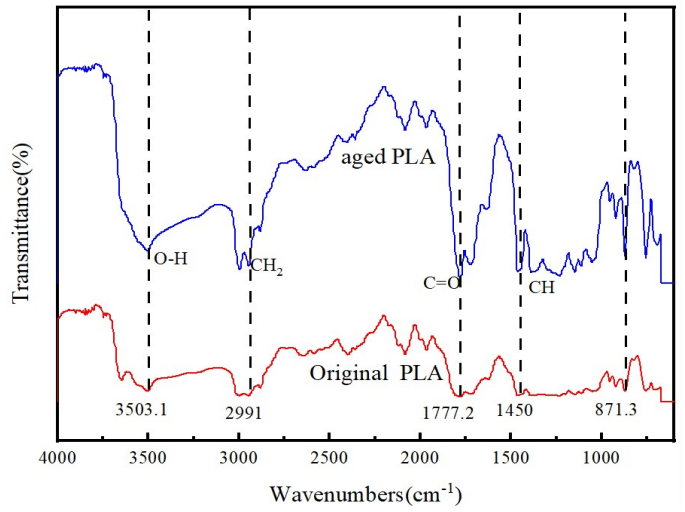

Figure 3. Fourier Transform Infrared Spectra of PLA

Changes in Cr concentration in water samples during exposure: The concentration changes in the exposure solution

over 7 days are shown in Table 1. Since the exposure solution was replaced every two days, there were no significant changes in the concentration, which remained consistently between 80-90 µg/L. There were no significant differences in heavy metal concentrations among the different treatment groups ($p > 0.05$). These results indicate that the addition of PLA did not significantly alter the concentration of Cr ions. A possible reason for this is that the amount of PLA added was relatively small, and the Cr ions adsorbed onto the PLA were insufficient to change the overall concentration of the solution.

Cr Accumulation in Fish: The accumulation of Cr in fish with different PLA treatments is shown in Fig. 4. The addition of PLA resulted in an overall increasing trend of Cr content in the fish, with the increase being most pronounced in the group treated with aged PLA. Specifically, after the addition of aged PLA, the concentration of Cr in the medaka reached its highest level (19.78 µg/g), which was 9.07 and 1.45 times higher than the Cr-only exposure group and the PLA + Cr co-exposure group, respectively (Figure 4(a)).

Table 1. Cr concentration in water samples over 7 days (µg/L)

Treatment	1 day	2 day	3 day	4 day	5 day	6 day	7 day
CK	0.0000b	0.0000b	0.0000b	0.0000b	0.0000b	0.0000b	0.0000b
Cr	86.36±2.8a	85.83±0.87a	85.52±2.11a	85.84±1.35a	85.76±0.82a	84.68±1.77a	85.25±2a
PLA+Cr	88.8±4.06a	85.93±4.67a	88.27±2.86a	85.01±1.75a	87.35±1.37a	84.91±0.76a	88.08±1.6a
Aged PLA+Cr	87.32±2.15a	83.8±2.28a	88.26±1.91a	84.14±1.22a	87.31±1.34a	83.3±2.04a	86.41±1.74a

Note: Letters indicate differences; identical letters denote no significant difference.

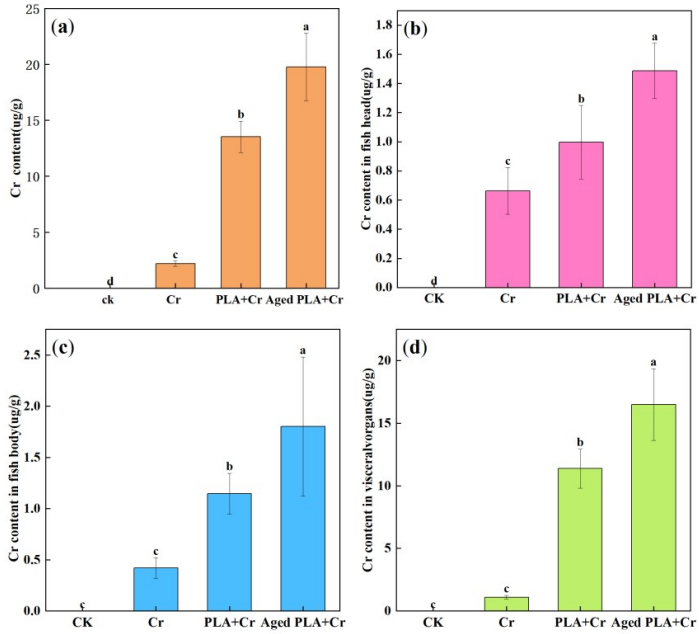

Figure 4. Accumulation of Cr in different parts of medaka. (a) Whole medaka; (b) Fish Head; (c) Fish Body; (d) Viscera. (Different letters indicate significant differences, $p < 0.05$.)

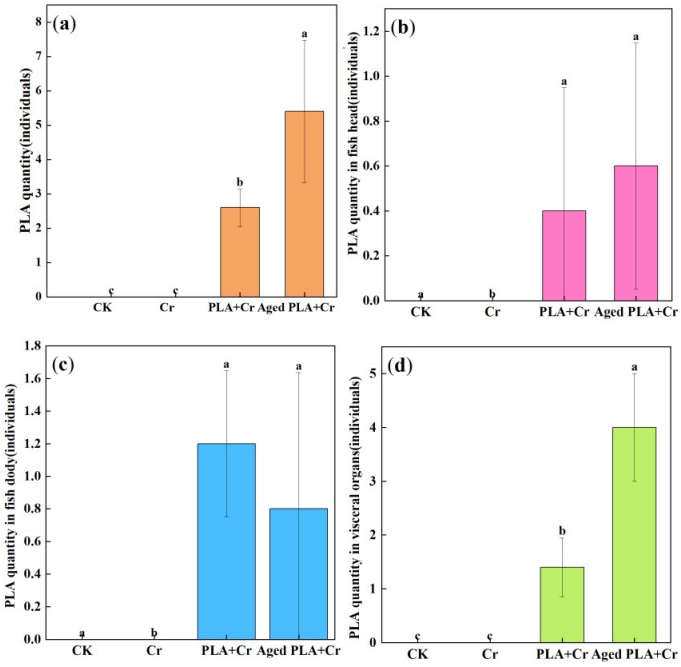

Figure 5. Accumulation of PLA in different parts of medaka. (a) Whole medaka; (b) Fish Head; (c) Fish Body; (d) Viscera. (Different letters indicate significant differences, $p < 0.05$.)

A possible reason for this is that aged PLA can adsorb more heavy metal ions, and when medaka consume MPs that have adsorbed heavy metals, the metals are released into the fish's body, leading to an increase in heavy metal content. On the other hand, there were differences in Cr concentrations in different parts of the medaka after exposure. In the original PLA and Cr co-exposure group, the highest accumulation of Cr in the viscera was 11.37 μg/g, which was 9.97 times and 11.48 times higher than in the fish head and fish body, respectively. In the aged PLA treatment group, the highest Cr concentration in the viscera was 16.49 μg/g, which was 9.16 times and 11.14 times higher than in the fish head and fish body, respectively (Figure 4(b)-(d)). This indicates that Cr accumulates the most in the viscera, possibly because the food consumed by the medaka is primarily released in the gastrointestinal tract for digestion[8].

Distribution of PLA in Fish: The distribution of PLA in fish after co-exposure to different PLA and Cr is shown in Fig. 5. The highest amount of PLA was found in the medaka exposed to aged PLA and heavy metals (Fig. 5(a)). Aged MPs are more likely to accumulate in medaka, possibly because PLA fragments into smaller pieces after aging, leading to greater ingestion by the fish[4]. Research by Fan et al. suggests that aged MPs are more prone to fragmentation, making them easier for organisms to consume[3].The accumulation of MPs in different parts of the medaka is shown in Figure 5(b)-(d). MPs can accumulate in various parts of the fish, with the highest concentration found in the viscera. These results are consistent with findings by Lu et al[9]. This study replaced the exposure solution every 48 hours, maintaining a dynamic balance between PLA accumulation and excretion. Ory et al. propose that this may be due to the phenomenon of "taste trapping," where medaka inadvertently ingest MPs while feeding, leading to their accumulation in the gastrointestinal tract[10].

CONCLUSION

This study focused on the most common biodegradable (PLA) to investigate its accumulation in marine medaka after UV aging and co-exposure to the heavy metal Cr. The results indicate that the physicochemical properties of PLA change after aging, with an increase in oxygen-containing functional groups that provide more attachment sites for Cr. Consequently, this enhances the accumulation of Cr in marine medaka, with the accumulation effect being significantly higher than that observed with original PLA.

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