Activity report on the project "*Evaluating photodegradation products of plastic nurdles and their toxicity in Matagorda Bay*"

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During the last quarter from June to August 2023, we worked mainly on preparation of experimental materials and fish embryos.

Preparation of plastic nurdles and lab photodegradation of two types of nurdles

Polyethylene (PE), polypropylene (PP), polystyrene (PS), polycarbonate (PC) and polyethylene terephthalate (PET) plastic nurdles were purchased from online vendors, including Amazon and eBay. The composition of the plastic nurdles was verified using Fourier Transform Infrared Spectroscopy (FTIR). Subsequently, the nurdles were cleaned using ultrasonication in a 1% H_2O_2 solution for 30 minutes before being subjected to photodegradation experiments.

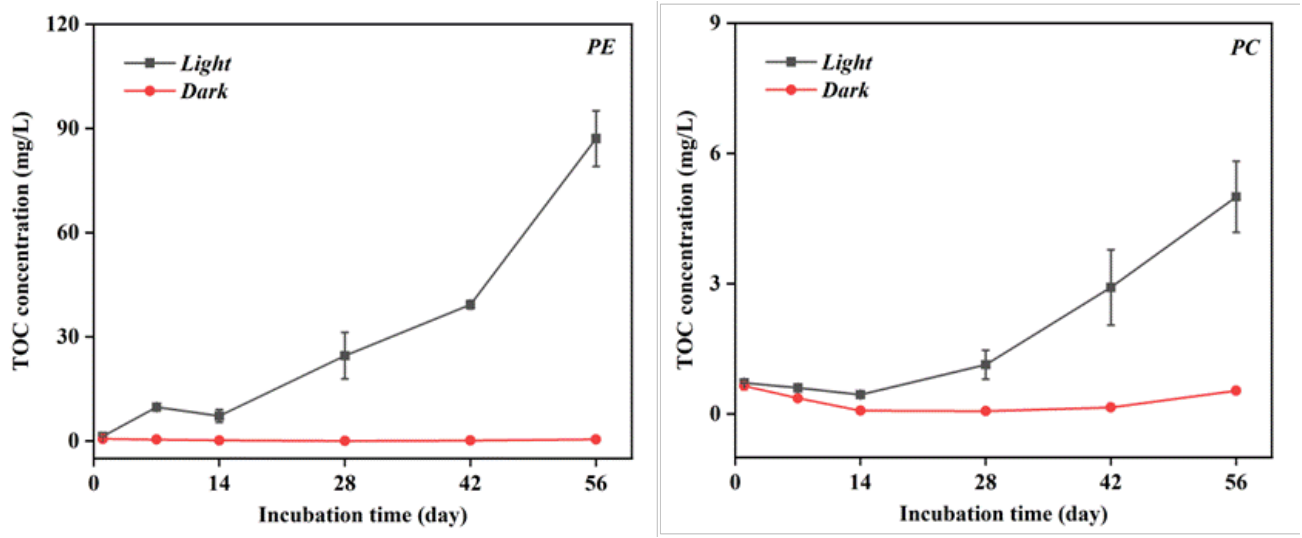
Due to limitations in both solar simulator space and laboratory equipment, the initial photodegradation experiment focused on two specific types of nurdles, PE and PC nurdles, due to their distinct densities. PE nurdles exhibit buoyancy, floating on the surface of seawater, while PC nurdles sink in the water column. This selection aimed to explore the varying behaviors of these plastics under photodegradation conditions.

The photodegradation experiment was conducted in 130 mL quartz bottles. One gram of cleaned nurdles was carefully transferred to each quartz bottle, followed by the addition of 120 mL of artificial seawater with a salinity of 33 ppt. The artificial seawater was prepared by dissolving 33g of pre-combusted artificial sea salt in 10 liters of pure water. The photodegradation process was carried out under a radiation dose of 2 W/m^2, approximately 4-5 times higher than natural sunlight at 0°N, without light/dark cycle. The incubation temperature for the photodegradation experiment was maintained at 35°C. To provide a comparative analysis, a dark weathering treatment was also performed. In this case, 1 g of cleaned nurdles (about 30–40 pieces) was placed in 250 mL pre-combusted amber glass bottles along with 120 mL of artificial seawater with a salinity of 33 ppt. The dark weathering treatment bottles were covered with aluminum foil and positioned within a sealed amber box at a constant temperature of 35°C. This parallel treatment allowed for a comprehensive evaluation of the effects of photodegradation in contrast to dark weathering conditions.

Three replicates were conducted for each sampling point in both treatment groups. Sampling occurred at designated intervals of 0, 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks. For each sampling point, three replicates were obtained for both the photodegradation and dark weathering treatments. Nurdles from the incubation solution were separated using a vacuum filtration device equipped with a pre-combusted 0.7 μm glass fiber filter paper. Subsequently, 20 mL of the filtrates were collected for total dissolved organic carbon (TOC) analysis (TOC-5000, Shimadzu). And 5 mL of filtrates from each replicate were collected and pooled together for each sampling point and sent to Xu lab for further toxicity analysis. The remaining filtrates,

approximately 90 mL, were carefully transferred into pre-combusted 120 mL amber glass bottles for subsequent leachate characterization analysis.

TOC concentrations in the bottles with nurdles generally increased during the photodegradation process. The TOC concentration curves for both PE and PC nurdles conformed to exponential patterns, indicating a substantial production of TOC without reaching a plateau. Notably, PE demonstrated a substantial increase in TOC concentrations, reaching approximately 90 mg/L (or ppm) after 8 weeks of incubation—equivalent to 1% of the original PE nurdles. In contrast, PC nurdles exhibited a more modest TOC production, with approximately 5 mg/L after the same incubation period. This disparity suggests potential differences in the environmental behavior of PE and PC nurdles under photodegradation conditions. In addition, light treatment generated more TOC than the dark treatment for both nurdles, aligning with findings from relevant studies that highlight the role of light exposure in accelerating the weathering process of plastics. Furthermore, both PE and PC nurdles leached some TOC after 8 weeks of incubation in the dark treatment, approximately 0.5 mg/L for both types of nurdles. This observation raises the possibility of the presence of additives and/or polymers with low molecular weight within the nurdles.



Preparation of marine medaka (Oryzias melastigma) embryos and treatments

To obtain eggs, several pairs of adult medaka were combined in breeding tanks. Medaka fish spawn 10-30 eggs per day per female. The collected eggs were cultured in artificial seawater (35 salinity) in a glass petri dish with methylene blue immediately after collection and kept in an incubator at 28 °C throughout the study. Saltwater medaka embryos were collected just after fertilization, examined for viability under an optical microscope, and randomized into groups in 96-well plates. Two types of plastic particles, polyethylene (PE) and polycarbonate (PC) were photodegraded for 8 weeks under UV and the degraded products in water were collected (see section 1 for detail). PE and PC leachates will be diluted with filtered artificial seawater which was used for medaka culture (35 ppt). Three concentrations of PE (5, 0.5, and 0.1 ppm) and two concentrations of PC (0.5 and 0.1 ppm) will be used for the treatment of medaka fish. The group of medaka embryos without treatment of any plastic particles will be used as a negative control. Each group will include 15 embryos. Water changes will be performed every three days with freshly diluted PE or PC leachates, or control seawater.

Experimental plan for next step

1. Characterization of weathered nurdles and leachates

In the next quarter, nurdles from both treatments will undergo comprehensive analyses from various perspectives. Parameters such as weight loss and color changes will be documented. Additionally, the spectra of the nurdles will be captured through FTIR scans, enabling a detailed examination of potential photochemical reactions during weathering on the nurdles. TOC in the filtrates will be separated with solid phase extraction and analyzed by LC/MS and Pyrolysis-GC/MS, to identify and characterize compound changes in the plastic leachate following the weathering process.

2. Toxicity assessment on PC and PE leachates in medaka embryos

Embryos aforementioned will be examined daily under an optical microscope and checked for mortalities, developmental stages, and maldevelopment of the embryos. For staging, we are going to compare developmental stages to published standards. The abnormalities, fungi, or bacterial infection, and developmental stages, will be all logged. In addition, the hatched embryos will also be recorded, and the hatching rate of each treatment group will be calculated by the end. We will use parameters for maldevelopment include the size of the embryo, body morphology, and dislocation of organs in the embryonic cavities.