Aquatic risk calibration of tire wear particle in interaction with leaching time, high temperature and UV radiation based on reproductive toxicity to zooplankton

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Abstract

Toxicity leached from tire wear particle (TWP) has been emerging and getting concern in aquatic ecosystem as a major sink of TWP. Before into water, TWP generated in road will interact with natural factors (e.g., UV, temperature). It is not clear how the interaction with physical parameters influences ecotoxicity of leachate once TWP into aquatic environment. Meanwhile, it is little known how the characteristics of toxicity leached from TWP changes along with time series. Those will be crucial to get more precise risk assessment for TWP. In this study, the dynamics of toxicity of TWP leachate were characterized under different extraction times, in addition, high temperature and UV radiation were regarded as pretreatment of TWP leaching to simulate in-situ road environment. The reproduction inhibition of a typical zooplankton, *Brachionus calyciflorus* was considered as toxicity indicator. The results showed that the dose of toxic effect substance released from TWP was fitted with Chi square density function over time, by which toxicity was estimated to reached apex on 10th day. The UVB radiation dose for a day in summer imposed on TWP reduce the toxicity leached by 28%. The high temperature of 70 °C before TWP into water strengthened the release of toxicity by 280%. High temperature and UV radiation had antagonism on toxicity leaching. This study will provide correction parameters for the environmental risk assessment and management decisions of TWP.

Keywords: TWP; Toxicity leaching; Physical parameter; Time series; UVB radiation; Rotifer

1. Introduction

It has been undoubtable that tire wear particle (TWP) from road does pose threats to organism, no matter in toxicology experiments of laboratory or poisoning incidents observed in the field (Capolupo et al., 2021; Khan et al., 2019; Marwood et al., 2011; Panko et al., 2013; Tian et al., 2021; Turner and Rice, 2010; Wik and Dave, 2006). In addition, as a huge-emission non-point pollutant without effective control measure, TWP deserves indispensability and urgency (Knight et al., 2020; Kole et al., 2017; Wagner et al., 2018). Aquatic ecosystem is a major sink of TWP through road runoff and atmospheric sedimentation (Wik et al., 2008). Once into water, the leaching substances from TWP are a main pathway of toxicity effects, as tire contains much toxic additives (Wik, 2007). Thus, ecological effects and risk assessment of TWP in aquatic ecosystem become nonnegligible.

The leachate has been the research object to evaluate ecological effects of TWP in aquatic environment. As the experiment material, the leachate always was obtained by directly soaked TWP in liquid for a period of time. However, this operation could misestimate environment risk of TWP in water due to neglecting in-situ conditions. On the one hand, before into water, TWP generally undergo the migration process after generated on road surface. During this migration process, TWP is inevitable to interact with environment factors, like high temperature and UV radiation. Especially, in summer day, the temperature of road surface could readily approach to 70°C with strong UV radiation. In some studies, some physical parameters indeed could change the characteristics and organism toxicity of microplastics. It is not clear if the prior

interaction with these physical parameters influences leaching behavior and leachate toxicity after TWP were transferred into water.

On the other hand, in previous studies, the extraction times were set to a fixed period from several hours to a dozen of days in the preparation of TWP leachate. And the period of time was chosen unfoundedly without standard. But it is obvious that the leachate toxicity would be fluctuating over time series as there are releasing and decomposing processes for leachate toxicity from TWP. The time characteristic of leachate toxicity is still unknown after TWP into water, which will influence the determination of its biological effects and environmental risk.

In this study, we exposed the TWP to high temperature and UVB radiation before extraction in water to stimulate environmental physical parameters during migration process into aquatic ecosystem. In addition, the different extraction time series were set to obtain time characteristic of toxicity releasing once TWP in water. The freshwater rotifer, *Brachionus calyciflorus*, was used as the indicator of leachate toxicity by reproduction inhibition.

2. Materials and Methods

2.1 TWP and rotifer

TWP were purchased from a factory that recycles and smashes scrap tires into particles as artificial turf material. These TWP were sieved through 100 μm nylon net, then sealed and stored at 4°C.

Rotifer *B. calyciflorus* clone strain was established from resting egg that was presented by Professor T.W. Snell of Georgia Institute of Technology, USA. The above hard synthetic freshwater was used as culture medium. The culture condition was kept in illumination incubator with 16L:8D light schedule with light intensity of 4000 lux at 25°C. Rotifers were fed with 5×10^6 cell/ml *C. pyrenoidesa*, and the medium was replaced every day. Before experiment, some rotifer with amictic egg were chosen from clone strain and synchronized (Kaneko et al., 2016).

2.2 Preparation of TWP leachate over time series

TWP were soaked with 2 L hard synthetic freshwater (96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in 1 L distilled water) (USEPA, 1985) at 2500 mg/L in 3-L glass bottle for one treatment and mixed uniformly. The mixture was aerated continuously in dark condition at 25°C. The time series endpoints were set at 3, 7, 15, 30, 60 days. Once the time is up, TWP leachate mother liquor was obtained by sieving the mixture through 38 μm nylon net, and frozen at -20°C for temporary storage.

2.3 Pretreatment of TWP exposed to high temperature and UVB radiation

The glass Petri dish with TWP was placed in thermostat water bath with 70°C for 12 hours to stimulate high temperature of road surface in summer. TWP in Petri dish

was exposed to UVB radiation for 11 hours with the intensity 2 mw/cm^2, which is equal to one-day radiation dosage in summer, 81300 mJ/cm^2. The TWP sample was stirred every two hours to ensure uniform exposure. Four treatments were designed with control (CK), high temperature (Heat), UVB radiation (UV) and high temperature followed by UVB radiation (Heat+UV). After exposure pretreatment, TWP sample was soaked to obtain leachate mother liquor according to Part 2.1 for next toxicity test.

2.4 Toxicity test of TWP leachate on rotifer

The TWP leachate mother liquor from every treatment was diluted by rotifer culture medium with concentration gradient (0, 250, 500, 750, 1000 mg/L).

After synchronization of parent, the rotifer individuals with amictic eggs were pick out. Then, neonate born within 4 hours were put into 24-well plate. There was one individual in one well with 1 ml the specific concentration TWP leachate, in which harvested algae exposed to TWP leachate were added at the density of 5×10^6 cell/ml. The plates were placed in illumination incubator with 16L:8D light schedule with light intensity of 4000 lux at 25°C. Survival and offspring number were checked every 12 hours. Each treatment in every generation included 48 individuals.

2.5 Statistics

The average individual reproduction of rotifer at treatment were calculated by dividing the total offspring number by individual numbers (picking out mictic individuals). To eliminate intergenerational difference and display treatment effects more directly, within same generation, relative value to control group (%) of lifespan and reproduction in different treatments was utilized (Formula 1). Positive value means

promotion effect, otherwise inhibition.

$$RV = \frac{V_T - V_C}{V_C} \times 100\% \quad \text{(Formula 1)}$$

Where RV, V_T and V_C was relative value, treatment group value and control group value of average offspring number.

3. Results and Discussion

3.1 Timing characteristic of leachate toxicity after TWP in water

The TWP leachate toxicity initially increased with extraction time then decreased, which reached to the max at 7th day with the most intense reproduction inhibition (Fig. 1). This dynamic showed that the toxicity in leachate from TWP was fit to a two-way model, including releasing and decomposing.

It had been verified in author's previous work that TWP leachate toxicity exhibited hormesis, a typical response model in toxicology. Hormesis is a central concept of toxicology to account for mild stress-induced beneficial effect, which has been observe in many toxic substances(Sun et al., 2021). The lower dose of TWP leachate strengthened the reproduction while high dose caused adverse response, like an inverse bell. The quadratic equation with downward opening could describe the relationship of reproduction capacity to toxicity concentration unit.

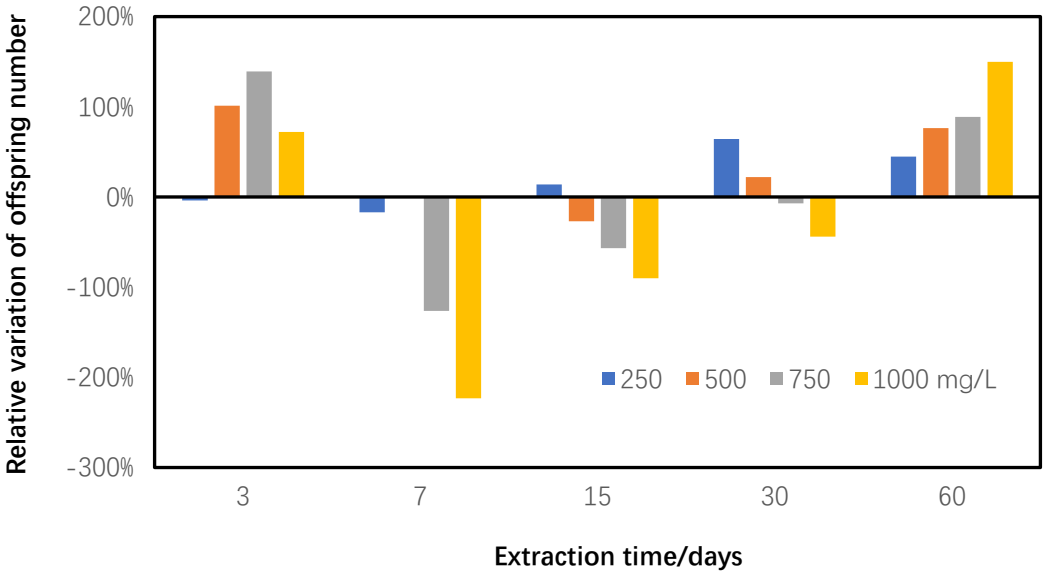

Figure 2. Relative value to control group for average offspring number of rotifer exposed to different-concentration TWP leachate from different extraction time endpoints. Positive value

means promotion effect, otherwise inhibition. Different color represents different concentration (mg/l), respectively.

For every extraction time endpoint, the curve of reproduction capacity to the nominal toxicity concentration gradient could be fit with the same quadratic equation. Assuming the reproduction capacity of rotifer exposed to 1000 mg/L leachate from 60th day as standard, the nominal toxicity concentration was calculated at the same reproduction capacity in other extraction time groups. These nominal toxicity concentration with same biological effect on reproduction should be in reverse ratio to the actual toxicity concentration from different extraction groups. Based on above, the curve of relative actual toxicity concentration of TWP leachate to extraction time was obtained. The curve could be fit with skew distribution, and the max toxicity concentration was predicted to occur at around 10th day after TWP into water (Fig. 2).

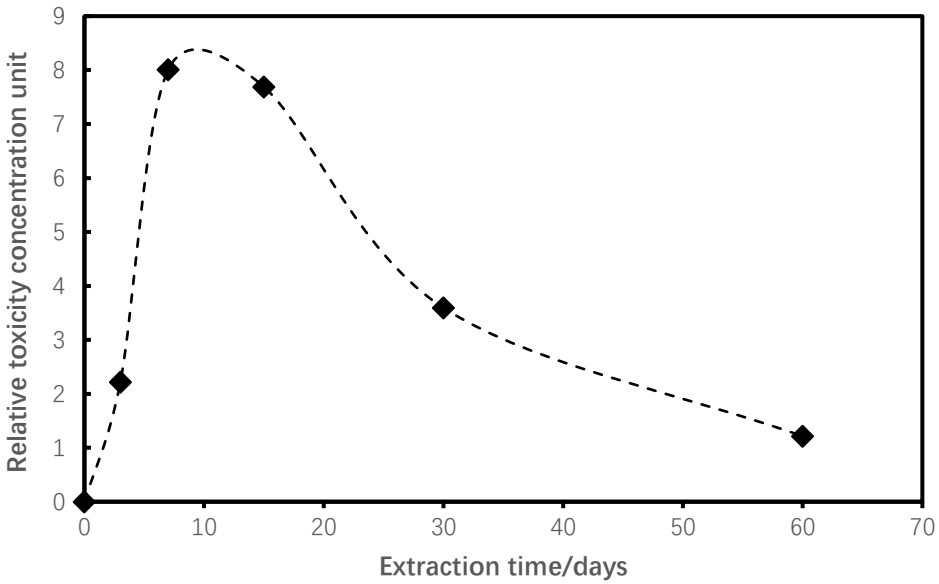

Figure 2. The curve of relative toxicity concentration of TWP leachate to extraction time

3.2 Exposure of high temperature and UVB radiation to TWP influenced the toxicity released in water

High temperature treatment before TWP in water intensified leachate toxicity on reproduction, while UVB radiation relieved the toxicity. And these two treatments had antagonistic effect (Fig. 3). High temperature might promote the toxicity release by increasing porosity of TWP and decreasing the bond strength of toxic chemicals to TWP skeleton. It has been verified that the UV radiation could reduce the toxicity of organic pollutants by changing chemical structure.

The high temperature over 70 ℃ and strong UVB radiation are common on road surface where TWP was generated. Before into water, TWP basically goes through these two parameters. However, these two physical processes were neglected in current research about TWP leachate biological toxicity, which would cause miscalculation in the aquatic ecological risk assessment for TWP.

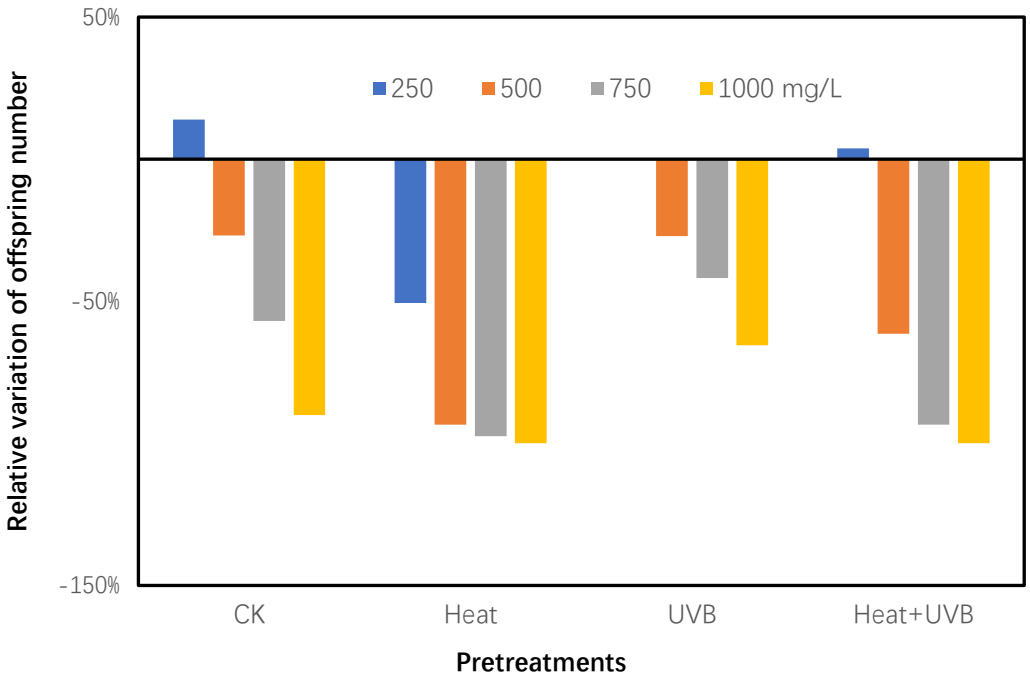

Figure 3. **Relative value to control group for average offspring number of rotifer exposed to leachate from different TWP pretreatment groups.** Positive value means promotion effect, otherwise inhibition. Different color represents different concentration (mg/l), respectively.

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Author contributions

Yanchao Chai: Conceptualization, Methodology, Writing - original draft, Funding acquisition. **Jiaxin Yang:** Supervision, Writing - review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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