Environmental Research 201 (2021) 111495

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Exploring the impacts of microplastics and associated chemicals in the terrestrial environment – Exposure of soil invertebrates to tire particles

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ARTICLE INFO

Keywords:
Tire wear particles
Enchytraeid
Springtail
Isopod
Soil ecotoxicology

ABSTRACT

Abrasion of tire wear is one of the largest sources of microplastics to the environment. Although most tire particles settle into soils, studies on their ecotoxicological impacts on the terrestrial environment are scarce. Here, the effects of tire particles (<180 μm) on three ecologically relevant soil invertebrate species, the enchytraeid worm *Enchytraeus crypticus*, the springtail *Folsomia candida* and the woodlouse *Porcellio scaber*, were studied. These species were exposed to tire particles spiked in soil or in food at concentrations of 0.02%, 0.06%, 0.17%, 0.5% and 1.5% (w/w). Tire particles contained a variety of potentially harmful substances. Zinc (21 900 mg kg^{-1}) was the dominant trace element, whilst the highest concentrations of the measured organic compounds were detected for benzothiazole (89.2 mg kg^{-1}), pyrene (4.85 mg kg^{-1}), chlorpyrifos (0.351 mg kg^{-1}), HCB (0.134 mg kg^{-1}), methoxychlor (0.116 mg kg^{-1}) and BDE 28 (0.100 mg kg^{-1}). At the highest test concentration in soil (1.5%), the tire particles decreased *F. candida* reproduction by 38% and survival by 24%, and acetylcholinesterase (AChE) activity of *P. scaber* by 65%, whilst the slight decrease in the reproduction of *E. crypticus* was not dose-dependent. In food, the highest test concentration of tire particles reduced *F. candida* survival by 38%. These results suggest that micro-sized tire particles can affect soil invertebrates at concentrations found at roadsides, whilst short-term impacts at concentrations found further from the roadsides are unlikely.

1. Introduction

It has been estimated that more than one million tons of tire particles are generated annually both in the European Union and in the United States (Wagner et al., 2018) and almost 6 million tons globally (Boucher and Friot, 2017; Kole et al., 2017). Due to the synthetic nature of the polymers in tire wear, micro-sized particles released from tires are generally considered as microplastics (Eisentraut et al., 2018; Hartmann et al., 2019; Wagner et al., 2018), and abrasion of tire wear is one of the largest sources of micro- and nanoplastics to the environment (Boucher and Friot, 2017; Kole et al., 2017; Sieber et al., 2020; Siegfried et al., 2017; Wagner et al., 2018).

Particles released or generated from tire wear can be classified into different types (Halle et al., 2020). The term ‘tire wear particles’ typically refers to tire tread particles produced in the laboratory or from the simulation of abrasion of tire tread, the outermost surface of the tire attached to the road when driving. Tire and road wear particles are released from tire tread while driving and may also include components released from road surface or other traffic emissions. Crumb rubber, in turn, usually refers to recycled material generated from end-of-life tires e.g. the material used in artificial turf fields. However, the use of this terminology is not always harmonized between different studies. Here,

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https://doi.org/10.1016/j.envres.2021.111495
Received 4 March 2021; Received in revised form 26 May 2021; Accepted 4 June 2021

we use ‘tire particle’ as a general term of all these particles generated or released from tires.

Tire wear consists of elastomers and rubbers (40–60%) (Boucher and Friot, 2017; Wagner et al., 2018; Wik and Dave, 2009), reinforcing agents (20–35%), such as carbon black and silica, process oils (12–15%) and other constituents (5–12%), such as vulcanization agents, preservatives, antioxidants and plasticizers (Wagner et al., 2018; Wik and Dave, 2009). Although efforts have been directed towards decreasing the amount of harmful substances, like polycyclic aromatic hydrocarbons (PAHs), in tire wear (Wagner et al., 2018), they still contain metals and various organic compounds that have the potential to adversely impact both on the environment and human health (Baensch-Baltruschat et al., 2020; Capolupo et al., 2020; Councell et al., 2004; Ginsberg et al., 2011; Halle et al., 2020; Liao et al., 2018; Llompart et al., 2013; Wagner et al., 2018; Watterson, 2017; Wik et al., 2009; Wik and Dave, 2009; Zhang et al., 2018). Most studies on the effects of tire particles have focused on their leachates, assuming that the toxic impacts mainly arise from the dissolving chemicals (Baensch-Baltruschat et al., 2020; Halle et al., 2020; Wagner et al., 2018). However, recently, ecotoxicological studies on particulate material itself have also become more common (Baensch-Baltruschat et al., 2020; Khan et al., 2019; Panko et al., 2013; Redondo-Hasselerharm et al., 2018), bringing out new perspectives such as that the mechanisms of toxicity of particles and leachates may differ (Khan et al., 2019) and that the studies on leachates may overestimate the risks of tire particles (Panko et al., 2013; Redondo-Hasselerharm et al., 2018).

Even though tire wear is considered to be one of the main sources of microplastics in the world’s oceans (Boucher and Friot, 2017; Kole et al., 2017), it has been estimated that only 12% of the particles from tires eventually reach surface waters, whereas 67% end up in soils and the rest in air and in waste water treatment plants (Kole et al., 2017). Despite this, studies on the impacts of tire particles on soil organisms are scarce. To our knowledge, the impacts of tire particles on soil invertebrates have only been investigated in one study on the enchytraeid *Enchytraeus crypticus* (Ding et al., 2020), in two studies on the earthworm *Eisenia fetida* (Pochron et al., 2017, 2018) and in one study on nematode communities (Zhao et al., 2011). Very different outcomes were reported in these few studies. For example, no decrease of *E. fetida* survival at 50% (w/w) (Pochron et al., 2017, 2018), but decreased survival and reproduction of *E. crypticus* already at soil concentrations of 0.024% and 0.12% (w/w), respectively (Ding et al., 2020), were reported. These astonishingly different outcomes may derive from the differences in the test material, such as particle size and chemical composition, or differences in the sensitivity of the test species. This indicates that studies on different species exposed to tire material with relevant particle size distributions are needed to shed light on the susceptibility of soil invertebrates to particle emission from tires.

In this study, the enchytraeid worm *Enchytraeus crypticus* (Annelida: Oligochaeta), the springtail *Folsomia candida* (Arthropoda: Entognatha) and the isopod *Porcellio scaber* (Arthropoda: Crustacea) were used as ecotoxicological model organisms for studying the effects of micro-sized tire particles on soil invertebrates. The different ecology, size and appearance of these invertebrates (Løkke and Van Gestel, 1998) are likely to result in differences in the rate of ingestion of tire particles and exposure to the various chemicals present in the tire particle material. *P. scaber* is a litter feeder with a size of adults in the range of 10–20 mm, whilst *F. candida* feeds on microbial biomass and is only 3 mm in size. In contrast to these arthropods with chitin-rich cuticles, the soft-bodied *E. crypticus* has a more permeable cuticle allowing greater potential for body surface damage and dermal exposure. In addition, enchytraeids may ingest tire particles and be exposed to tire particle-associated chemicals through the oral route when ingesting soil and feeding on microbial biomass and detritus. In addition to species with differing ecology, different levels of biological hierarchy from the cellular level (electron transfer system (ETS) and acetylcholinesterase (AChE) activity in *P. scaber*) to the population level (survival, reproduction) were studied. In addition, a functional endpoint – the feeding activity of *P. scaber* – was followed. To better understand the contribution of tire particle-associated chemicals to the potential toxicity, a comprehensive list of chemicals was explored including trace elements, metals and a variety of organic compounds (PAHs, pesticides, polychlorinated biphenyls, brominated flame retardants, and benzothiazole). Particles with a size of less than 180 μm were used, corresponding with the size range of emitted tire particles reported in some studies (Grigoratos and Martini, 2014; Kreider et al., 2010).

2. Materials and methods

2.1. Tire particles

The tire particles were produced by Genan (Denmark) from mixed end-of-life passenger car tires by cryo-milling and sieving to the size below 180 μm. The size range was chosen according to the reported size range distribution of emitted tire particles by Kreider et al. (2010) and the studies referred to by Grigoratos and Martini (2014). The powder had a density of 1.16 g cm^{-3} and contained several different synthetic rubbers including SBR, butadiene rubber (BR), and butyl rubber (IIR), 10–35% natural rubbers and 25–35% carbon black. The material was further refined for analysis, as described in the Supplementary material (S1.1.).

A field emission scanning electron microscope (FE-SEM, Zeiss ULTRA plus, Carl Zeiss, Germany) was used to examine the particle size and morphology of tire particles. The particle size distribution was determined using a laser diffraction particle size analyzer Microtrac Bluewave at the University of Ljubljana, as described in the supporting information and in Selonen et al. (2020). The number of particles in the studied tire particle material was highest in the smallest size classes (mean 12.57 μm; Fig. 1A), while in the volumetric distribution, particles between 80 and 110 μm in size dominated (mean 102.9 μm; Fig. 1B). The numerical distribution of the particles shows that about 50% of the particles in our test material were smaller than 10 μm. The FE-SEM confirmed the results of the particle size distribution. The tire particles were semi-angular and irregularly shaped (Fig. 1C and D). Most of the particles were (numerically) in the range from 5 μm to 100 μm. The SEM micrographs revealed that many particles smaller than 10 μm and even reaching the nanoscale were attached to larger particles (Fig. 1C and D). This may indicate that the number of ultrafine particles is easily underestimated in the measurements of tire particles.

2.2. Test soils and experimental setup

The culturing conditions of the test species used are given in the supplementary material (S1.2).

E. crypticus, *F. candida* and *P. scaber* were exposed to tire particles at nominal concentrations of 0, 0.02, 0.06, 0.17, 0.5 and 1.5% w/w (dry weight) in the standard natural soil Lufa 2.2 (Lufa Speyer, Germany; Table S1). The spiking was done one day before starting the exposures by mixing tire particles in with the soil. Five replicates were prepared for each treatment and control, as recommended in standard test guidelines (OECD, 2004, 2009).

In addition to the experiments with spiked soil, experiments with spiked food were established for *E. crypticus* and *F. candida*. These tests were also performed in Lufa 2.2 soil using five replicates for each treatment and control. Nominal concentrations of 0, 0.02, 0.06, 0.17, 0.5 and 1.5% w/w were prepared by mixing tire particles in with a mixture of ground oats and water (1:4), that was used as food for *E. crypticus*, or a mixture of baker’s yeast and water (1:2), that was used as food for *F. candida*. The concentrations were the same as were used in our previous study with polyester textile fibers (Selonen et al., 2020) and represented concentrations that can be found at roadsides and further from the roads (Unice et al., 2012; Wagner et al., 2018; Wik and Dave, 2009).

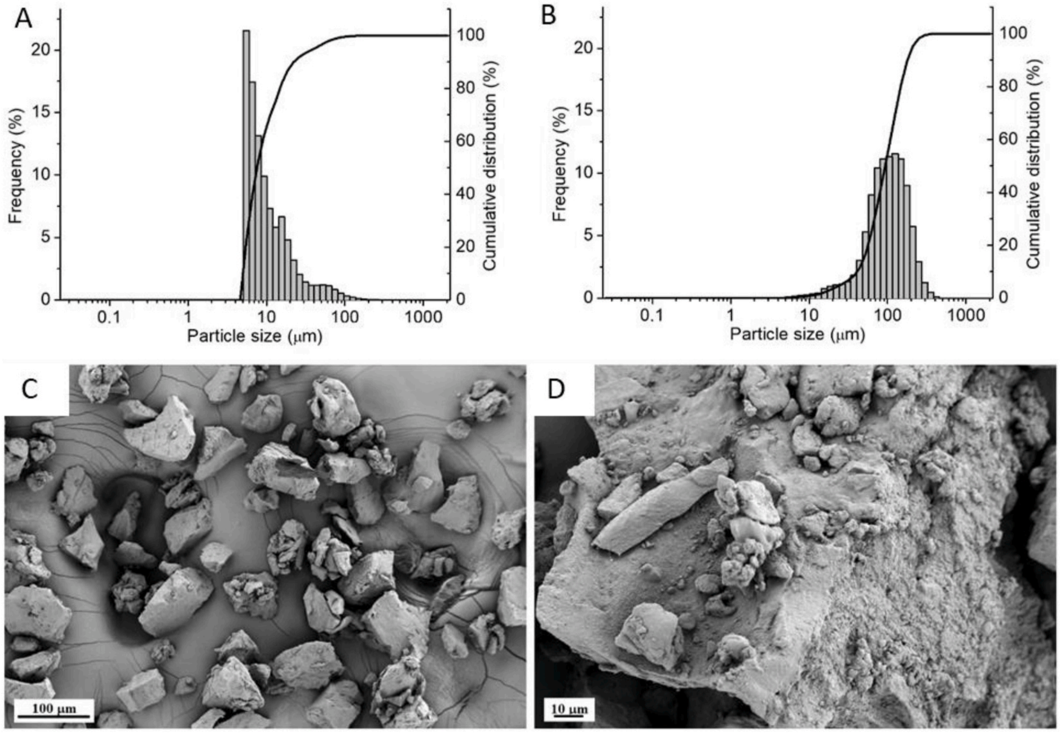

Fig. 1. Size distribution of the tire particles used in the toxicity experiments with soil invertebrates, expressed as number (A) and volume (B) distribution, and characterization of tire particles with field emission scanning electron microscope (FE-SEM) taken at 500X (C) and 2000× (D) magnification.

All test soils were adjusted to a moisture content corresponding to 40% (*P. scaber*) or 50% (*E. crypticus* and *F. candida*) of the water holding capacity (WHC) to reach optimal humidity conditions for the different test species. The tests were performed at 20 ± 2 °C in the dark (*P. scaber*) or under a light:dark cycle of 16:8 h (*E. crypticus*, *F. candida*).

Soil pH was measured at the beginning and at the end of the tests in 0.01 M $CaCl_2$.

2.3. *Enchytraeus crypticus survival and reproduction test*

Effects on enchytraeid reproduction and survival after exposure to tire particles spiked in soil or in food were determined according to OECD guideline 220 (OECD, 2004) with small modifications following Castro-Ferreira et al. (2012). Ten mature *E. crypticus* with visible clitellum and of a similar size were placed into 100 mL glass jars containing 30 g moist soil and 10 mg ground oats mixed in water (1:4). The soil moisture content was adjusted, and additional food added once a week. After 3 weeks, the enchytraeids were fixed with ethanol and stained with Bengal rose (Sigma Aldrich). After 1–3 days of staining, most of the soil particles were removed by washing through a sieve and the enchytraeids were transferred with water into a white tray and photographed (Nikon D5200). The adults and juveniles were counted from the pictures after increasing their visibility by adjusting the color balance of the pictures using Adobe Photoshop CC 2018.

2.4. *Folsomia candida survival and reproduction test*

Springtail tests were conducted following OECD guideline 232 (OECD, 2009). Ten *F. candida* with an age of 10–12 days were introduced into 30 g moist test soil in 100 mL glass jars and fed with 5 mg baker's yeast mixed with water (1:2). The soil moisture content was adjusted, and springtails fed once a week. After four weeks of exposure to tire particles spiked in soil or in food, the content of each test jar with springtails was transferred to a 250 mL beaker. Then 100 mL water was added and the number of *F. candida* adults and juveniles floating to the surface were counted from the picture taken of the water surface (Nikon D5200) using ImageJ®.

2.5. *Porcellio scaber survival and feeding activity test*

To assess the impacts of tire particles on the feeding activity and survival of *P. scaber*, five isopods were placed into each replicate 200 mL glass jar containing 30 g moist test soil and dry leaves of common hazel (*Corylus avellana*) (Selonen et al., 2020). The soil moisture content was checked every three days. Once a week during the 3-week exposure, mortality was assessed by visual inspection of the lack of isopod response to mechanical stimuli. The hazel leaves were also weighed and replaced. Isopod feeding activity was calculated as dry leaf weight mass loss per surviving animal.

2.6. *Acetylcholinesterase (AChE) and electron transfer system (ETS) activity of Porcellio scaber*

To investigate the effects on acetylcholinesterase (AChE) and electron transfer system (ETS) activity, *P. scaber* were exposed to tire particle concentrations of 0, 0.05, 0.5 and 1.5% w/w (dry weight) in soil following the same procedure as described above. After 3 weeks exposure, 10–15 animals per test group were stored at −20 °C for further measurements. Sample processing, measurements and calculations for AChE and ETS activity are described in full in the supplementary material (S1.4.). Briefly, AChE activity was analysed according to Jemec et al. (2008), and ETS activity according to De Coen and Janssen (1997). Protein concentrations were measured using the BCA™ Protein Assay Kit (Pierce, Rockford, IL, USA). All protein and enzyme activity measurements were done in triplicate using a microplate reader: Cytation 3 imaging reader (Biotek, USA).

2.7. *Chemical analyses*

The concentrations of 24 trace elements (Zn, Al, Co, Cu, Ti, Pb, Mn, Li, Ba, Ni, Cr, V, Sr, Sn, As, Cd, Bi, Sb, Mo, Ag, Te, Be, Tl, Se) in the tire particle material were determined using inductively coupled plasma mass spectrometry (ICP-MS; iCAP™ Q instrument, Thermo Fisher Scientific). As Zn was the dominant metal found in the tire particle material both in the present and in previous studies (see below), Zn concentrations of the soils were also specifically measured, using High-Resolution Continuum Source Atomic Absorption Spectrometry (HR–CS–AAS; Analytik Jena ContrAA 700).

In addition, polyaromatic hydrocarbons (PAHs), benzothiazoles, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (BDEs), organochlorine pesticides (OCPs) and organophosphorus pesticides in tire particles and test soils were analysed after liquid-liquid extraction (Llompart et al., 2013) and QuEChERS (Acosta-Dacal et al., 2021; Correia-Sá et al., 2012; Fernandes et al., 2013), respectively. For the determination of chlorinated (12 OCPs, 4 PCBs), brominated (7 BDEs), phosphorous (6 OPPs) and sulfuric (benzothiazole) organic compounds in tire particles and test soils, a gas chromatograph (GC-2010) with an electron capture detector (GC-ECD) and photometric flame detector (GC-FDP) from Shimadzu was used. The methods were validated according to the Eurachem Guide (Magnusson and Örnemark, 2014).

Details of the sample preparation, extraction methods, method validations and the chemical analyses are described in the supplementary material (S1.5).

2.8. *Data analyses*

For effects on the AChE activity of *P. scaber*, the median effective concentration (EC50) was determined by fitting the dose-response model of Haanstra et al. (1985) to the data. For other response parameters: survival and reproduction of *E. crypticus* and *F. candida* and survival, feeding activity and ETS activity of *P. scaber*, no median lethal (LC50) or median effective (EC50) concentrations could be calculated due to the slight effects of tire particles. For all response parameters, no-observed effect concentrations (NOECs) were estimated using a one-way ANOVA for testing the differences among treatments in general, followed by a Dunnett's post hoc test. When the data did not meet the assumptions of normal distribution and homoscedasticity for parametric tests, a Kruskal-Wallis test followed by a Mann-Whitney *U* test was applied.

3. Results

3.1. *Chemical analyses*

The following trace elements were detected in the tire particle material, in order of decreasing concentrations: Zn > Al > Co > Cu > Ti > Pb > Mn > Li > Ba > Ni > Cr ≈ V ≈ Sr > Sn ≈ As ≈ Cd > Bi ≈ Sb > Mo ≈ Ag ≈ Te ≈ Be ≈ Tl (Table S2). The most abundant metals were Zn (21 900 mg kg^{-1}), Al (1270 mg kg^{-1}), Co (134 mg kg^{-1}) and Cu (128 mg kg^{-1}). Zinc concentrations in soil increased with increasing concentration of tire particles to reach 188 ± 22 mg kg^{-1} Zn at the highest tire particle concentration (1.5%), which was a bit lower than was expected based on the Zn concentration in the tire particle material (Table S3).

The tire particle material contained the polyaromatic hydrocarbons (PAHs) fluorene, phenanthrene, fluoranthene, pyrene, benzo[b+j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[ghj]perylene at detectable concentrations (Table S4). The highest PAH concentrations were detected for pyrene (4.85 ± 0.04 mg kg^{-1}), fluoranthene (1.35 ± 0.06 mg kg^{-1}), phenanthrene (1.27 ± 0.1 mg kg^{-1}) and benzo[ghi]perylene (0.492 ± 0.1 mg kg^{-1}). All PAHs detected in the tire particle material were also detected in the test soil, showing an increasing trend with increasing concentration of tire particles (Table S4).

Tire particles also contained benzothiazole, four different polybrominated diphenyl ethers (BDE 28, BDE 99, BDE 154, BDE 153), four different polychlorinated biphenyls (PCB 28, PCB 101, PCB 118, PCB 153), chlorpyrifos, hexachlorobenzene (HCB), p,p'-DDE, dieldrin, endosulfan, lindane and methoxychlor at detectable concentrations (Table S5). From these compounds, the highest concentrations in tire particles were detected for benzothiazole (89.2 mg kg^{-1}), chlorpyrifos (0.351 mg kg^{-1}), HCB (0.134 mg kg^{-1}), methoxychlor (0.116 mg kg^{-1}) and BDE 28 (0.100 mg kg^{-1}). From these organic compounds, only benzothiazole and endosulfan were detected in the test soil spiked with tire particles (Table S5). For benzothiazole, the highest concentration was detected at 1.5% tire particles in the soil (0.0195 mg kg^{-1}).

3.2. *Toxicity of tire particles*

Soil pH-$CaCl_2$ after spiking was 5.6–5.7. This decreased slightly during the exposures to 5.4–5.6 but was not affected by the tire particles (Table S6).

Tire particle exposure decreased the reproduction of the enchytraeid *E. crypticus* by 18% and 20% at the lowest (0.02%) and highest (1.5%) concentrations in the soil, respectively (Fig. 2C, Table S7; U = 3, p = 0.047 for both comparisons). Tire particles in soil had no effect on *E. crypticus* survival, and food exposure did not affect survival or reproduction of the enchytraeids (Fig. 2A,B,D).

Survival and reproduction of the springtail *F. candida* were not significantly different between the treatments, due to the high variability between the replicates, but did show a slight dose-related decrease (Fig. 3, Table S7). Compared to the control, the survival of *F. candida* was decreased by 24% and 38% at the highest concentration of tire particles in soil and in food, respectively (Fig. 3A and B). At the highest concentration of tire particles in soil, reproduction of *F. candida* was on average 38% lower than in the control (Fig. 3C).

For the isopod *P. scaber*, AChE activity was decreased by 65% at the highest tire particle concentration in soil (Fig. 4D, Table S8), giving an EC50 of 1.2% and a NOEC of 0.5%. No impacts were observed on the survival, growth, feeding activity or electron transfer system (ETS) activity *of P. scaber* (Fig. 4A–C, Tables S7, S8).

4. Discussion

This study provides a view on an emerging environmental challenge – the impacts of tire particles, one of the most abundant microplastic type in the terrestrial environment – by using three invertebrate species as model organisms (enchytraeid *E. crypticus*, springtail *F. candida* and isopod *P. scaber*). A wide variety of compounds were detected in the test material and test soils. The results of this study suggest that tire particles (<180 μm) at low concentrations are not very harmful to soil invertebrates, but at higher concentrations (here, 1.5%) they can induce negative impacts at the macromolecular and population level.

In studies with aquatic organisms, Zn and a variety of organic compounds have commonly been suggested as the main cause for toxic effects of tire particles and their leachates (Camponelli et al., 2009; Panko et al., 2013; Wagner et al., 2018; Wik et al., 2009; Wik and Dave, 2009). The decrease in the AChE activity of *P. scaber* in our study indicates that some of the chemical components of the tire particles were present in a bioavailable form. AChE is predominantly involved in neurotransmission in the cholinergic nervous system and is a known target for specific and non-specific neuroinhibitors including organic substances and metals (Diamantino et al., 2003; Varó et al., 2002). Among the chemicals associated with the tire particles, Zn and benzothiazole were present in high concentrations and could potentially inhibit AChE activity. Zn was previously shown to inhibit AChE activity in the crustacean *Daphnia magna* (Diamantino et al., 2003) and benzothiazole derivatives are known as AChE inhibitors used in medicine (Özkay et al., 2016). Regardless of the inhibition of AChE activity, no changes in survival, feeding activity or ETS activity in *P. scaber* were detected. ETS reflects mitochondrial respiratory electron transfer activity and is therefore commonly used to assess the metabolic activity

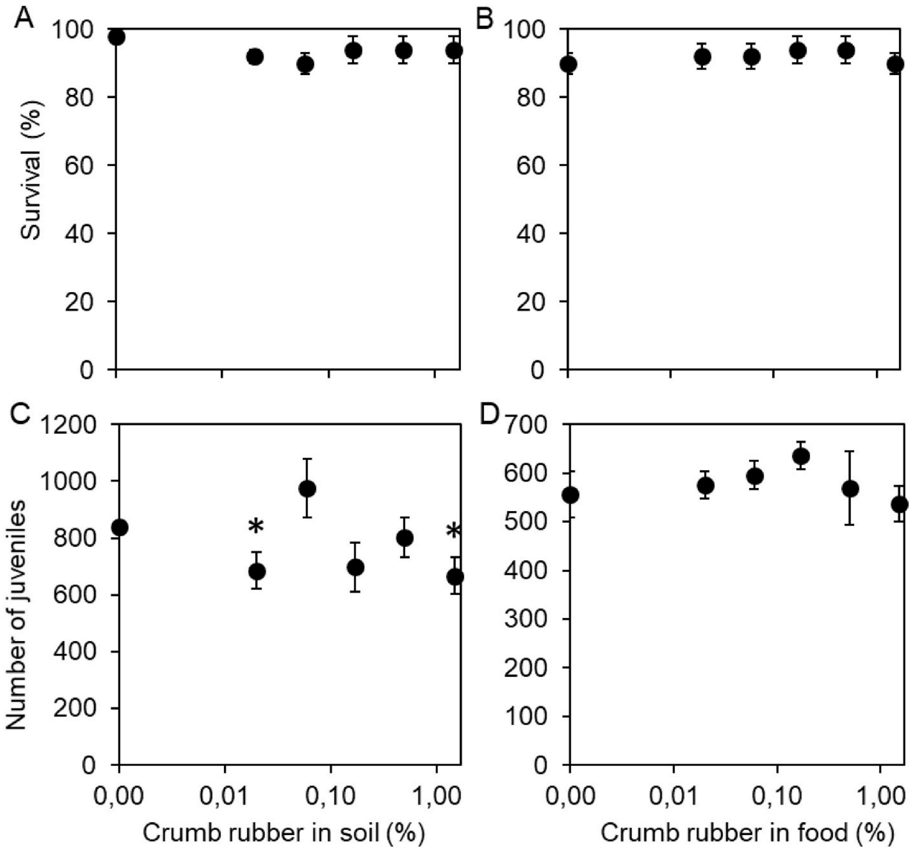

Fig. 2. Survival (A,B) and reproduction (C,D) of *Enchytraeus crypticus* (mean ± SE) exposed for 21 days to tire particles spiked in with Lufa 2.2 soil (A,C) or with food (B,D). Asterisk (*) indicates a significant difference compared to the control ($p < 0.05$).

and energy consumption in organisms exposed to pollutants (Ferreira et al., 2015). The absence of effects on ETS activity in this study is therefore in line with the lack of changes in animal feeding activity. Nevertheless, the observed AChE inhibition indicates that the chemical additives in the tire particles were bioavailable to isopods and induced effects at the biochemical level.

A high Zn concentration is typical for tire wear, since ZnO is often added to tires as an activator of the vulcanization process (Thorpe and Harrison, 2008). The Zn concentration in the tire particle material used in the present study (21 900 mg kg^{-1}) equates to 329 mg kg^{-1} nominal zinc in the highest tire particle treatment in soil. This is close to the EC50s for the effect of Zn in the form of ZnO (461 mg kg^{-1}) and $ZnCl_2$ (348 mg kg^{-1}) on the reproduction of *F. candida* (Lock and Janssen, 2003; Smit and van Gestel, 1996). Although the measured concentration of Zn (188 mg kg^{-1}) was considerably lower than the nominal concentration, it is possible that Zn contributed to the slight toxicity of tire particles to *F. candida* detected at the highest test concentration. Isopods are quite tolerant to Zn exposure with a 14-day EC50 of 2600 mg Zn kg^{-1} for *P. scaber* feeding rate on spiked food (leaves) (Zidar et al., 2003), and a 14-day LC50 for *Porcellionides pruinosus* of 2292 mg Zn kg^{-1} in soil (Tourinho et al., 2013). The concentrations of other major metals, Al (1270 mg kg^{-1}), Co (134 mg kg^{-1}) and Cu (128 mg kg^{-1}), measured in the tire particles are not likely to cause toxic effects on *F. candida* or *P. scaber* alone (Amorim et al., 2005; Bruus Pedersen and van Gestel, 2001; Crouau and Moïa, 2006; Lock et al., 2004; Zidar et al., 2003), but a minor contribution to the overall toxicity of the mixture cannot be totally ruled out.

Although tire particles, along with other traffic-based sources like asphalt and fuel combustion, are an important source of PAHs to the environment, the bioavailability of PAHs in tire particles is generally low (Wagner et al., 2018; Wik and Dave, 2009). The concentrations of PAHs measured in tire particles are not likely to cause impacts on survival or reproduction (Droge et al., 2006). From the analysed organic compounds, benzothiazole was present in the tire particles and test soil in the highest concentrations. Benzothiazole is classified as an emerging organic pollutant, but data on its toxicity to soil invertebrates is still lacking (Herrero et al., 2014; Liao et al., 2018). Benzothiazole and its derivatives induced acute and chronic toxicity to aquatic organisms; the lowest NOEC for effects on daphnids is 11.9 mg L^{-1} (Liao et al., 2018; Nawrocki et al., 2005). Assuming that soil organisms are mainly exposed via pore water and that the sensitivity of soil arthropods equals to that of daphnids, the equilibrium partition concept was applied to estimate a corresponding $NOEC_{soil}$, as described in the supplementary material (S3.1). Doing so, the NOEC for daphnids would correspond with an estimated soil concentration of 70 mg kg^{-1}. This estimation suggests limited risk of benzothiazole for soil arthropods at the concentrations measured in our test soils (0.0125–0.0198 mg kg^{-1}). However, it should be noted that the Predicted No-Effect Concentration (PNEC) for benzothiazole in soil of 0.0172 mg kg^{-1}, reported by EU (2008), is close to the highest test concentration in our test soils (0.0195 mg kg^{-1}).

In the present study, no definite conclusions on the toxicity of tire particles to *E. crypticus* could be drawn, since enchytraeid reproduction was not affected in a dose-related way. In contrast to our results, Ding et al. (2020) found both decreased survival and decreased reproduction of *E. crypticus* at all tested tire tread concentrations above 0.024% and 0.12% (w/w), respectively. The Zn concentration of the tire particles used in our study was about two times higher than that found in the test material used by Ding et al. (2020). The highest tire tread concentration (3%) in the study of Ding et al. – that caused 50.8% reduction in the reproduction – equates to 282 mg kg^{-1} of Zn in soil. This is higher than the highest measured Zn concentration in our study (188 mg kg^{-1}), but below the EC50 of 461 mg Zn kg^{-1} for the effect of ZnO on enchytraeid

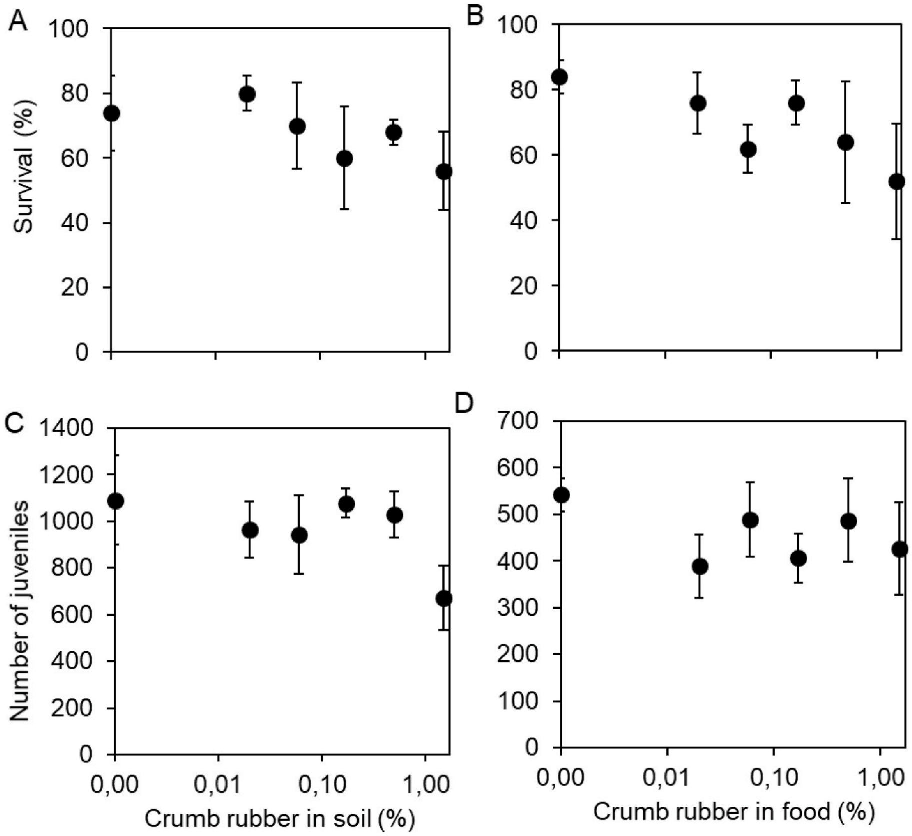

Fig. 3. Survival (A,B) and reproduction (C,D) of the springtail *Folsomia candida* (mean ± SE) exposed for 28 days to tire particles spiked in with Lufa 2.2 soil (A,C) or with food (B,D).

reproduction (Lock and Janssen, 2003). Thus, Zn is not likely to explain the different outcomes of the studies. The concentrations of PAHs and several trace metals, like As, Cr, Ni and Pb, in tire particles were considerably higher in the study of Ding et al. than in our study (Table S5), but still at levels not likely to cause lethal or reproductive toxicity to enchytraeids (Droge et al., 2006; Li et al., 2021; Lock and Janssen, 2002a,b; Zhang and van Gestel, 2017). In addition, the smaller size of the particles in our study (<180 μm) compared to the study of Ding et al. (<1400 μm) should increase, rather than decrease, the bioavailability of the toxicants (Wagner et al., 2018; Wik and Dave, 2009). On the other hand, the smaller size of the particles in our study may have induced less physical harm in the digestive tract of the organism. Ding et al. (2020) also found changes in the gut microbiota due to tire particle exposure that can contribute to the decreased fitness of the exposed animals. In any case, the diverging results of the studies imply that rather than from a single chemical component, the toxicity of the tire particles arises from the mixture of different additives that tire material contains (Halle et al., 2020), and that other properties of the particles, such as the size and shape, may also play a significant role. This agrees with the observations on nanoparticles, where the effects on organisms are commonly explained as an interplay of different physicochemical properties of the material (Novak et al., 2019). This also shows the challenge in the field of microplastic research: how to assess the risks of microplastics with varying chemical and physical characteristics, and how to evaluate the contribution of the chemicals and particle themselves.

Although studies on the impacts of different kinds of microplastics on soil invertebrates have been conducted in recent years (Huerta Lwanga et al., 2016; Jemec Kokalj et al., 2018; Ju et al., 2019; Selonen et al., 2020), studies on the impacts of tire particles in the soil remain scarce. Besides our study and that of Ding et al. (2020) on *E. crypticus*, only a few studies exist on the effects of tire wear-based material on soil invertebrates (Pochron et al, 2017, 2018, 2017; Zhao et al., 2011). However, in these studies the size and chemical composition of the particles were not fully described, and only a low number of treatments and replicates were used. Pochron et al. (2017, 2018) reported no effect on the survival of the earthworm *Eisenia fetida* after 33 days of exposure (50% w/w), whilst Zhao et al. (2011) found decreased nematode abundance and changed community structure in tire particle treatments (10% and 15%). A few studies on plants and soil nitrification were also conducted more than 20 years ago. Both Bowman et al. (1994) and Newman et al. (1997) investigated the potential of mixing tire particles with the growth medium for chrysanthemum and geraniums, respectively. In both studies, decreased growth and a reduced number of flowers as well as bioaccumulation of Zn in the shoots due to tire material amendment was found. However, the concentrations in these studies were extremely high, ranging from 22% to 66%. Smolders and Degryse (2002), in turn, found that 10–40% of the Zn in tire particles, that was mixed in with the soil at a concentration of 2.5%, was in exchangeable form after one year of incubation, but that the increased soil pH due to tire particle treatment restricted the mobilization of Zn and increased the nitrification potential of the soil. Such an increase in soil pH was not detected in the present study (Table S6). The divergent outcomes of different studies, as well as the overall lack of knowledge in this field shows the need for more research on the potential impacts of particles emitting from car tires to the terrestrial environment using a variety of terrestrial species from different trophic levels and environmentally relevant particles sizes and concentration ranges.

The test concentrations of tire particles in the present study fall in the range of the gradient from a roadside to further from the road. Tire rubber concentrations of 2300–117 000 mg kg^{-1} (0.2–12%) have been measured or estimated at roadsides, whilst 30 m from the road the concentrations fall to 50–100 mg kg^{-1} (0.005–0.01%) (Unice et al., 2012; Wagner et al., 2018; Wik and Dave, 2009). Furthermore, the Zn

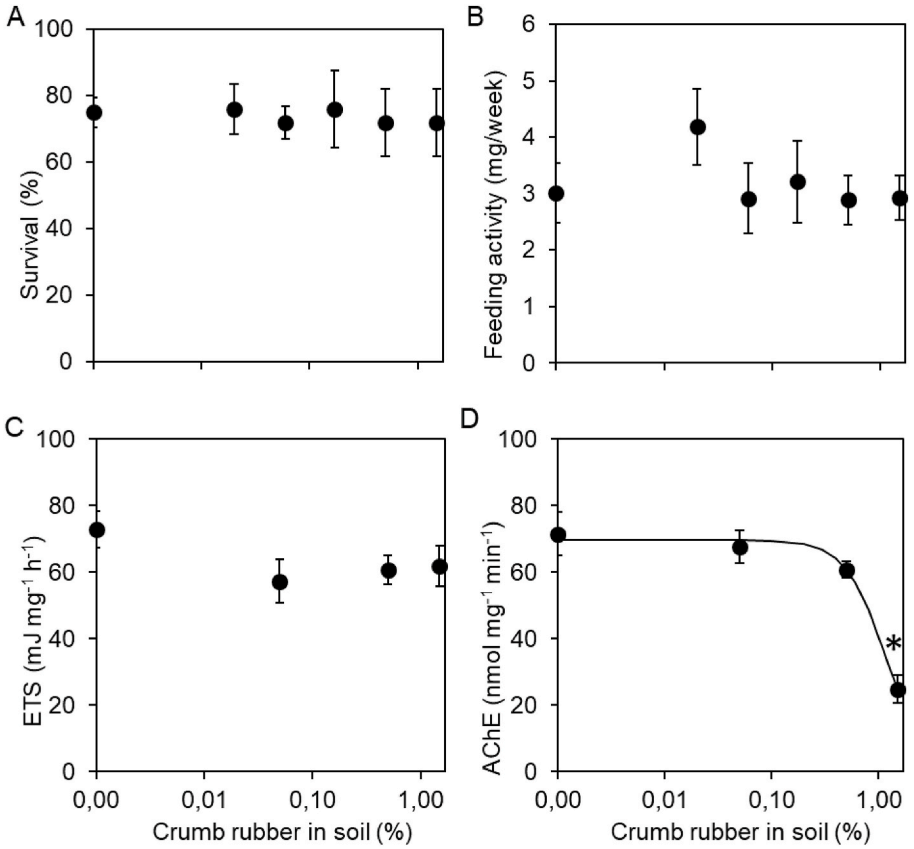

Fig. 4. Survival (A), feeding activity (B), electron transfer system (ETS) activity (C) and acetylcholinesterase (AChE) activity (D) of *Porcellio scaber* (mean ± SE) exposed for 21 days to tire particles spiked in with Lufa 2.2 soil. Asterisk (*) indicates a significant difference compared to the control ($p < 0.05$) and the line shows a fit to the data of a dose-response model by Haanstra et al. (1985).

concentration in the second highest tire particle concentration in the present study equates to the estimated average concentration at roadsides (118 ± 16 mg Zn kg^{-1}), and several studies report concentrations considerably higher than that (de Silva et al., 2021). In addition, the benzothiazole concentration at the highest tire particle concentrations in the test soils is close to the PNEC for benzothiazole in soil reported by EU (2008). Consequently, the slight negative impacts of the tire particles as well as the concentration levels of Zn and benzothiazole at the highest test concentrations in soil in this study indicate that negative changes in soil invertebrate populations are possible at roadsides. Furthermore, as considerably higher concentrations than the 1.5% tested here can be found at roadsides, the impacts of tire particles on soil invertebrates near busy roads may thus be more evident than recorded in this study. In addition, harmful substances from other traffic-based sources, such as fuel, engine oil, brake wear, vehicular exhaust catalysts and particles from road wear contribute to the potential negative impacts on soil organisms at roadsides (de Silva et al., 2021). However, these potential impacts on soil biota at road sides are still poorly known and scattered, with some studies from 1980s showing decreased abundance of soil invertebrates nearby roads, and some showing increased abundances of certain taxa, including isopods and springtails (see review by de Silva et al., 2021).

Our results suggest that negative effects on soil invertebrates in the short term are not probable further from the roadsides. However, because of the challenges in analytical methodology, knowledge on the true tire particle concentrations in different environmental compartments, including soils, is still limited (Eisentraut et al., 2018). Furthermore, long-term studies on soil invertebrates as well as soil community studies on the impacts of tire particles and other emissions from vehicles are also needed (de Silva et al., 2021). As the exposures in the present study lasted only one generation or less, the possibility of longer-term impacts of tire rubber particles on soil invertebrate communities also further from roadsides cannot be entirely excluded.

5. Conclusions

The results of this study suggest that microplastics released from the abrasion of tires can have negative impacts on soil invertebrate populations near to roads, whereas short-term exposure to concentrations currently reported further from the roads did not induce negative impacts. However, the possibility of longer-term impacts of tire particles on soil invertebrate communities further from roadsides cannot be excluded and should be further investigated. The strongest effect of tire particles (<180 μm) was detected for acetylcholinesterase (AChE) activity in the isopod *Porcellio scaber*, which indicates that tire particle-associated chemicals may act as neuro-inhibitors. Benzothiazole was the most abundant organic compound with lower concentrations of PAHs, pesticides and BDE also observed, whilst Zn was the dominant trace element in the test material. As tire wear can contain a variety of different chemicals, the role of different harmful substances in the ecological impacts of tire particles should be investigated more carefully to support the development of more sustainable and eco-friendly tires. In addition, the complexity of its composition as well as the behavior and dispersion of the compounds should be explored simultaneously with the development of analytical methodologies. Finally, the lack of terrestrial impact studies on tire particles, as well as the divergent outcomes of different studies emphasize the need for more research on the potential impacts of particles emitting from tires to a variety of terrestrial species representing different levels of food web.

Credit author statement

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Funding sources

The authors are thankful for the financial support from the Netherlands Organisation for Scientific Research (NWO) [grant number ALWWW.2016.2] and the Research Council of Norway [grant number 271825/E50], in the frame of the collaborative international Consortium IMPASSE; the Maj and Tor Nessling Foundation (Finland); the Fundação para a Ciência e a Tecnologia (FCT)/the Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds (Portugal) [grant number LAQV - UIDB/QUI/50006/2020 and UIDP/50006/2020], PhD grant [Diana Rede and, grant number SFRH/BD/147 404/2019] and Post Doc grant [Virgínia Cruz Fernandes, grant number SFRH/BPD/109153/2015]; and the Slovenian Research Agency (ARRS), through research programme "Integrative zoology and speleobiology" [grant number P1-0184] and research project J1-2482, the infrastructural center Microscopy of biological samples and the ARRS PhD grant (Andraz Dolar). The authors declare no competing financial interest.

Declaration of competing interest

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.

Acknowledgements

We thank Rudo Verweij (VU Amsterdam) and Valentina Perc (University of Ljubljana) for their technical assistance. This study has been conducted within the framework of the international project IMPASSE – Impacts of MicroPlastics on AgrosyStems and Stream Environments, which is financed under the ERA-NET waterworks2015 co-funded call. This ERA-NET is an integral part of the 2016 joint activities developed by the water challenges for a changing world joint programme initiative (water JPI).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111495.

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