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# Comparing the effects of pristine and UV–VIS aged microplastics: Behavioural response of model terrestrial and freshwater crustaceans

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ABSTRACT

Physico-chemical properties of microplastics (MPs) change during weathering in the environment. There is a lack of knowledge about the effects of such environmentally relevant MPs on organisms. We investigated: 1) the physico-chemical changes of MPs due to UV–VIS weathering, and 2) compared the effect of pristine and aged MPs on the behaviour of the water flea *Daphnia magna* and terrestrial crustacean *Porcellio scaber*. Dry powders of MPs were produced from widely used polymer types: disposable three-layer polypropylene (PP) medical masks (inner, middle and outer), polyester textile fibres, car tires and low-density polyethylene (LDPE) bags and were subjected to accelerated ultraviolet–visible (UV–VIS) ageing. Our results show that the extent of transformation depends on the type of polymer, with PP showing the most changes, followed by LDPE, textile fibres and tire particles. Obvious fragmentation was observed in PP and textile fibres. In the case of PP, but not polyester textile fibres, changes in FTIR spectra and surface properties were observed. Tire particles and LDPE did not change in size, but clear changes were observed in their FTIR spectra. Most MPs, aged and pristine, did not affect the swimming of daphnids. The only effect observed was a significant increase in path length and swimming speed for the pristine tire particles when the recording was done with particles remaining in the wells. After transfer to a clean medium, this effect was no longer present, suggesting a physical rather than chemical effect. Similarly, woodlice showed no significant avoidance response to the MPs tested, although there was a noticeable trend to avoid soils contaminated with pristine polyester textile fibers and preference towards the soils contaminated with aged MP of the middle mask layer. Overall, the apparent changes in physico-chemical properties of MPs after accelerated ageing were not reflected in their effects on woodlice and daphnids.

## 1. Introduction

A large amount of plastic waste still ends up in landfills or in the natural environment. In time they degrade into small plastic particles called microplastics (MPs) (1 µm-1 mm) (ISO/TR 21960, 2020), which are ubiquitously present in our surroundings (Castro-Castellon et al., 2022). In recent years, considerable evidence has been gathered to demonstrate their interaction with aquatic (Castro-Castellon et al., 2022) and terrestrial organisms (Selonen et al., 2020). Despite this, their environmental hazard is still not fully clarified. For example, one open question is the impact of environmentally relevant MPs, e.g. particles with a longer residence time in the environment. As reviewed by Rozman and Kalčikova (2022) there is a large discrepancy between MPs used in laboratory research and those found in the environment. Namely, MPs in the environment further undergo various physical, chemical and biological degradation processes cumulatively called weathering or ageing (Jahnke et al., 2017).

The rate of MP degradation in the environment differs mainly due to the time and intensity of exposure to sunlight and the intensity of natural mechanical forces (Ainali et al., 2021). Long-term sunlight radiation including UV light causes the formation of free radicals, oxygen inclusion, hydrogen abstraction, and scission or cross-link of chemical chains

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(Sutkar et al., 2023). Photodegradation combined with thermal degradation chemically ages the surface of MPs, creating oxygenated functional groups such as carbonyl and hydroxyl groups (Liu et al., 2023; Ainali et al., 2021). Physical forces, such as those from waves and wind, can lead to cracks, fractions and holes in the surface (Sutkar et al., 2023) and eventually MPs break down into different sizes and shapes. Besides MP fragmentation, different chemical additives and degradation products can leach from plastic during weathering (Rummel et al., 2017). In terrestrial environments, most of the degradation occurs in the topsoil due to higher mechanical abrasion, UV radiation, increased oxygen availability, and higher temperatures. In aquatic environments, degradation is higher in regions with more intensive sunlight and currents inducing mechanical degradation (Rummel et al., 2017). Taken together, MPs in the environment exhibit various physical and chemical properties not found in pristine plastics (Alimi et al., 2022). These physico-chemical changes of MP properties might significantly affect their hazardous potential (Pflugmacher et al., 2021). Therefore, it is essential to consider the effects of weathering when evaluating the environmental hazard of MPs.

To advance the knowledge of the effects of aged MPs on organisms, we designed a study using two crustacean organisms, water flea *Daphnia magna* (Anomopoda, Daphniidae) (also called daphnid), as a representative toxicity test organism for the freshwater environment, and a woodlouse *Porcellio scaber* (Isopoda, Porcellionidae), as a representative test organism for terrestrial environment. In this study, different behaviour parameters were selected since they were recently suggested to be relevant endpoints to evaluate MP effects (Huang et al., 2023). Daphnia swimming behaviour is a reliable tool for the evaluation of effects and is generally considered more sensitive than acute endpoints, as they integrate systemic responses to toxicants (Bownik, 2017). Further, swimming behaviour is directly related to feeding success and escape from predators. Several different daphnid swimming parameters have been investigated in ecotoxicity studies, including the swimming time, speed, hopping frequency, horizontal and vertical distribution and migration, distance travelled, and swimming trajectory, but swimming speed and distance travelled are by far most applied (Bownik, 2017). Previous studies with MPs and daphnids indicated mostly a reduction in their swimming (Magester et al., 2021, Pan et al., 2022) but also the opposite effect was observed (De Felice et al., 2019). According to our knowledge available studies on the behaviour of daphnids have investigated commercially available primary spherical MPs (polystyrene and polyethylene) while no data are available for environmentally relevant weathered MPs.

The spatial heterogeneity of pollution in the terrestrial environment is generally greater than in the aquatic environment. Because of the differences in pollution between micro-locations, terrestrial animals might avoid pollution to some extent by choosing less polluted soil or food. Woodlice are particularly successful at this as they avoid soil or food polluted with metals (Loureiro et al., 2005; Zidar et al., 2005), veterinary pharmaceuticals (Žižek and Zidar, 2013), and pesticides (Loureiro et al., 2005; Zidar and Fišer, 2022). Currently, there are no reports on whether they can avoid MPs as has been reported for earthworms (Ding et al., 2021). Woodlice avoidance behaviour can be evaluated through several parameters: proportion of time on unpolluted soil compared to the proportion of time on polluted soil, change in speed, the difference in locomotion time and the length of the travelled path on unpolluted and polluted soil. It is assumed that animals that perceive a polluted environment as unfavourable will avoid it, they will spend as little time as possible in a polluted environment and they will move faster in polluted soil to leave this environment as soon as possible (Zidar and Fišer, 2022).

In this study we tested a set of pristine and weathered MPs representing different types of widely used polymeric materials in the environment: three-layer single-use polypropylene (PP) medical masks, polyester textile, car tires, and low-density polyethylene (LDPE) bags. The aim was to investigate: 1) if UV–VIS induced weathering of MP changes their physico-chemical properties, 2) whether MPs affect the behaviour of crustaceans *D. magna* and *P. scaber*, and 3) if UV–VIS induced weathering changes the toxic potential of MPs.

## 2. Materials and methods

### 2.1. Tested microplastics

#### 2.1.1. Production of microplastics

We tested three layers of PP MPs from medical masks (inner, middle and outer layer of the masks) (Jemec Kokalj et al., 2022a,b), tire particles (Selonen et al., 2021), polyester textile fibres (Selonen et al., 2020), and LDPE fragments (Jemec Kokalj et al., 2021) (Table 1). The abbreviations of MP samples are the following: Inner layer of polypropylene medical mask ("inner mask"), middle layer of polypropylene medical mask ("middle mask"), outer layer of polypropylene medical mask ("outer mask"), polyester fibres ("textile fibres"), car tires ("tire particles") and low-density polyethylene particles from bags ("LDPE"). All MPs were produced from their original materials by cryo-milling where the milling bowl was first cooled down in liquid nitrogen for 4 min, subsequently, the milling was carried out with a horizontal homogeniser (Milli Mix 20; Domel, Slovenia). The milling of the samples was performed at a horizontal frequency of 28 Hz, for 2.5 min using milling balls (diameter, 25 mm). After the milling, the samples, except for textile fibres, were sieved through a 250-μm-pore sieve.

#### 2.1.2. Ageing of microplastics

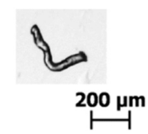
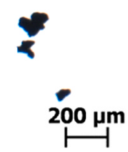
Since natural weathering tests are often time-consuming and difficult due to inability to control weather, accelerated laboratory ageing is often used for material degradation research. In our research, we used a Xenon chamber with controlled temperature, relative humidity, and UV irradiation conditions, according to EN ISO 4892–2 to reproduce the characteristics of direct sun exposure (UV irradiation). Dry powders of MPs were exposed in glass Petri dishes for 1000 hours to UV–VIS radiation under the following conditions in a Xenon chamber: irradiation = 55 W/m$^2$, T = 50 °C, temperature of the black body ($T_{bb}$) = 68 °C, RH = 50 %.

#### 2.1.3. Physico-chemical characterisation of microplastics

The particle size and any changes on the surface of the polymer particles after exposure to accelerated ageing in a Xenon chamber were examined with two microscopes: (a) a field-emission scanning electron microscope (FE-SEM) Zeiss ULTRA plus (Zeiss, Germany) (Fig. 1) and (b) a low-vacuum scanning electron microscope (LV-SEM) JSM-IT500LV (Jeol, Japan) (Figure S2). The accelerating voltage was set to 2 kV and 10 kV in the case of used FE-SEM and LV-SEM, respectively. Images were obtained by secondary electron detection. Before the FE-SEM analysis, samples were coated with a thin 10 nm Au/Pd layer to increase the electron conductivity, while for LV-SEM analysis no extra coating on samples' surface was used, only low vacuum between 40 Pa and 33 Pa. The particle size distributions calculated based on the number distribution were determined with a light microscope Imager Z2m (Zeiss, Germany) using the image processing software AxioVision 4.8.2. Samples of MPs were placed on the holder and at least 10–15 different MP images on different positions where performed. Approximately 200–400 objects were analysed. Based on a statistical analysis performed with the AxioVision software, the mean diameters with standard deviation were determined. In addition, the length of a fibre-like object (*fibre length*) with standard deviation was determined for samples with a flattened fibre shape. The results were expressed as the size (an average diameter and/or fibre length value) with standard deviation (SD) according to the number particle size distribution.

Fourier transform infrared-attenuated total reflectance (FTIR-ATR; diamond crystal) spectrometry with a PerkinElmer FTIR Spectrum spectrometer was used to investigate the structural changes induced by accelerating weathering exposure. Spectra were taken in the range

**Table 1**
The list of microplastics (MPs) tested, their average size (obtained by optical microscope and evaluation software program), shape and carbonyl indices (CI) after ageing. The abbreviations of MPs are the following: Inner layer of polypropylene medical mask (“inner mask”), middle layer of polypropylene medical mask (“middle mask”), outer layer of polypropylene medical mask (“outer mask”), polyester fibres (“textile fibres”), car tires (“tire particles”) and low-density polyethylene particles from bags (“LDPE”).

| MP designation | Pristine MPs Size±SD (μm)[a] | Aged- MPs Size±SD (μm)[a] | Carbonyl index (CI) for aged MPs | Pristine and aged MPs Shape | Appearance |
|---|---|---|---|---|---|
| **Inner mask** | 72.6±49.1<br>Fibre length:173.4±162.3 | 31.3± 25.3<br>Fibre length:58.6±59.4 | 2.24 | flattened fibres |  |
| **Middle mask** | 92.8±82.8 | 37.8±28.8 | 1.62 | fragments |  |
| **Outer mask** | 93.2±55.1<br>Fibre length: 226.9±208.1 | 61.3±41.2<br>Fibre length: 125.1±119.5 | 1.30 | flattened fibres |  |
| **Textile fibres** | 58.3±39.9<br>Fibre length: 183.7±181.8 | 60.9±29.6<br>Fibre length: 125.6±88.5 | / | flattened fibres |  |
| **Tire particles** | 57.8±27.0 | 62.2±48.8 | 0.75 | fragments |  |
| **LDPE** | 176.0±186.9 | 180.9±165.6 | 0.20 | fragments |  |

[a] mean ± standard deviation, numeric distribution.

400–4000 $cm^{-1}$ with an average of 3 scans at a resolution of 4 $cm^{-1}$. The spectra were then normalised to identify changes in the polymer structure and the functional groups indicative of weathering. The FTIR-ATR spectra were compared before and after xenon arc exposure.

FTIR spectroscopy facilitates the observation of changes in the carbonyl band (C═O), giving rise to a method known as the carbonyl index (CI) (Andrady et al., 2015). The CI serves the specific purpose of tracking the absorption band of carbonyl species formed during photo- or thermo-oxidation processes within the 1,850–1,650 $cm^{-1}$ range. This is achieved by measuring the ratio of the carbonyl peak to a reference peak methylene ($CH_2$) scissoring peak from 1,500 to 1,420 $cm^{-1}$. To compare the intensity of carbonyl groups formed in the MP after ageing their bond indices were used. These carbonyl indices were calculated following Eq. 1, where SAUB is specified area under band:

$$\text{Carbonyl index(CI)} = \frac{\text{SAUB}(1,850\text{–}1,650)cm^{-1}}{\text{SAUB}(1,500\text{–}1,420)cm^{-1}} \quad (1)$$

The presence of plastic-associated chemicals (organic chemicals and metals) was characterised in pristine MPs. The complete list of chemicals is available in these publications: textile fibers (Selonen et al., 2020), tire particles (Selonen et al., 2021), LDPE (Jemec Kokalj et al., 2021), and medical mask MPs (Jemec Kokalj et al., 2022a, b). In none of these studies, the effects were attributed to the chemicals leaching from the microplastics, as the concentrations of chemicals were considered relatively low.

## 2.2. *Experiments with Daphnia magna*

### 2.2.1. *Exposure set-up and test concentrations*

Test concentrations of 1, 10 and 100 mg MPs/L were chosen based on our previous MPs research with daphnids (Jemec Kokalj et al., 2018), and 100 mg/L is commonly regarded as the highest relevant concentration of a pollutant to test. All MP test materials were dispersed in ADaM medium (Klüttgen et al., 1994) with Tween 40 (0.0024 %) and vortexed. The highest concentration (100 mg/L) was prepared by weighting, while 10 mg/L and 1 mg/L were prepared by diluting the 100 mg/L solution. ADaM medium with Tween 40 was used as a control (hereafter referred to as control).

Water fleas *Daphnia magna* were reared at Helmholtz Institute for Environmental Research (UFZ). Neonates of age less than 24 h were used in the experiments. They were exposed individually in 24-well plates (Hund-Rinke et al., 2022, Küster et al., 2023; Kühnel et al., 2023). Each well contained 1 mL of the test medium and one daphnia, altogether 10 animals per experiment were exposed. The exposure took place at 20 ± 1°C in the dark. Each experiment with the same type of MP was performed 2–3 times. After 48 h of exposure, the immobility of animals was checked by microscopic assessment of their mobility (according to OECD TG 202). The animals exposed to 10 mg MPs/L were further used to assess the behavioural endpoints. This concentration was the highest that we were able to record due to the high interferences of particles with the recording system at 100 mg/L.

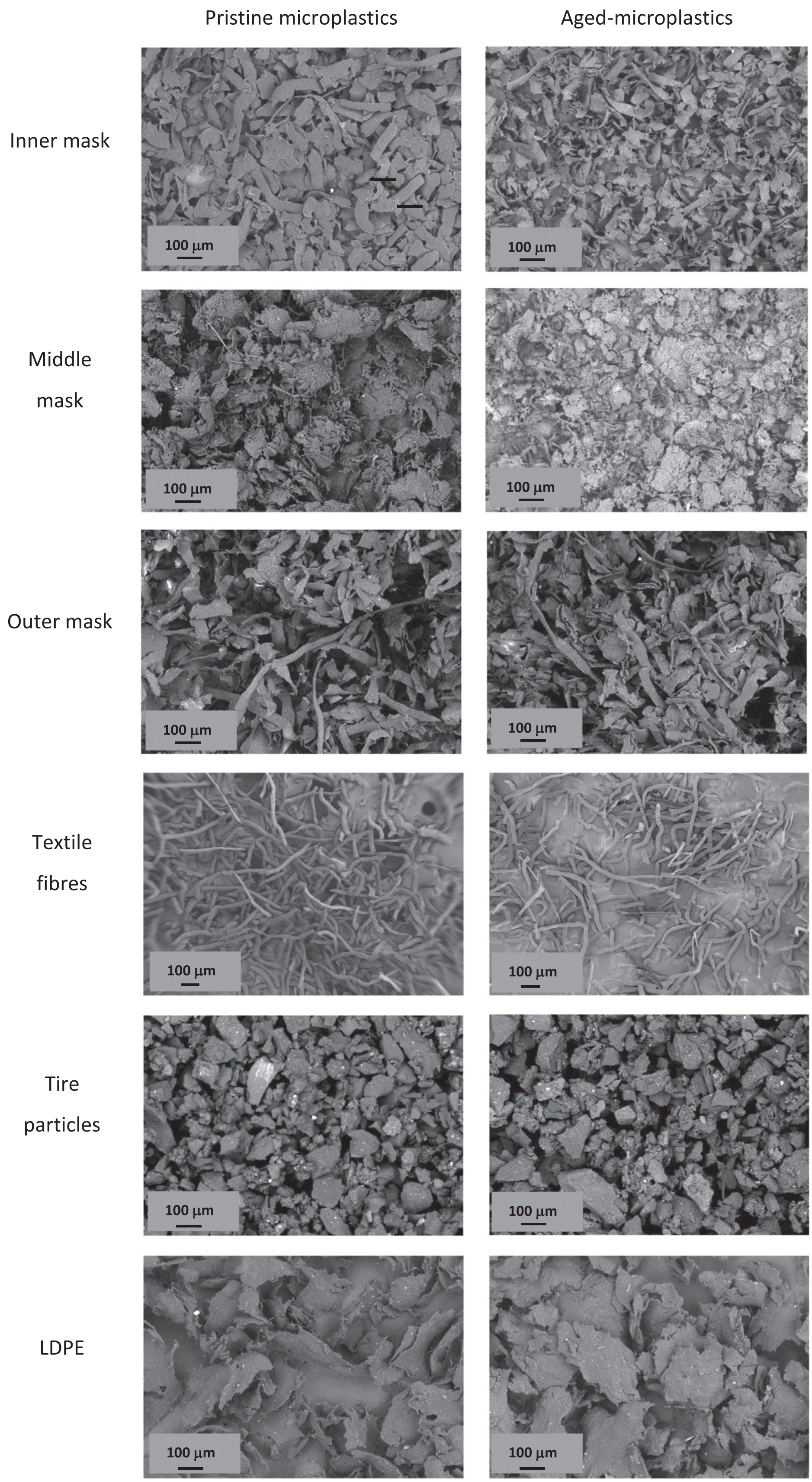



**Fig. 1.** Scanning electron microscopy micrographs of pristine (left column) and aged (right column) microplastics. Low-density polyethylene (LDPE). Images taken by a field-emission scanning electron microscope (FE-SEM) Zeiss ULTRA plus (Zeiss, Germany). Please note a difference in the size of the particles in the case of aged inner, middle and outer layer masks.

### 2.2.2. Behavioural endpoints

Video recording was always done at the same time of the day (morning) to account for different behaviour of daphnids. Before video-recording the exuviae from moulting were removed from the test solutions. Due to the quite low water column in the wells, only the horizontal movement of daphnids was recorded using the ZebraBox equipment (ViewPoint, France). The behaviour tracking protocol was created using the tracking mode of the corresponding software ZebraLab (version 5.13.0.370, Viewpoint Life Sciences, Lyon, France). First, well-spaces for the tracking system were defined manually with a transparent background. The diagonal length of the plate was scaled as a known unit of length within the software to enable the system to mathematically convert pixel changes into mm swimming distance. The detection threshold was adjusted to ensure that software only recognizes the small crustaceans without any background noise. This was usually achieved with a low detection threshold of 35. For the threshold activity levels, large swimming distances were set to 5. Animals were recorded for 10 minutes in the dark followed by 10 minutes of recording under light conditions using the backlight. We used these two illumination regimes because the distance travelled is known to vary between dark and light conditions. Recordings were done in two scenarios: 1) with MPs remaining in the wells, and 2) after the transfer of daphnids to the control medium without MPs. The transfer into a clean medium was done to check for potential physical impairment of daphnid swimming by the presence of the MP. The duration of acclimatization in a clean medium was exactly 30 minutes in the dark according to De Felice et al. (2019). Overall, between 40 and 60 behaviour data points per treatment (type of MPs) were generated.

### 2.2.3. Video and data analysis

Videos were analysed using the accompanying Zebralab v5.15.0.230 software by ViewPoint (France). Data were generated each 30 s within the 10 min period. The data were collected for the length of path travelled and time spent for a certain distance within the 30 s of recording for each sampling well which were used to calculate the speed of swimming. Data were analysed using OriginPro 2023. The difference between controls and different MP exposure groups was calculated using the Kruskal Wallis ANOVA test with Dunnet's test ($p<0.05$). Data were presented as box-plots: 25th ($Q_1$), 50th ($Q_2$, median value) and 75th percentile ($Q_3$) values as boxes, and maximum and minimum values as whiskers excluding outliers. Outliers were designated as data points that were 1.5 times the IQR (interquartile range) greater than the Q3 or 1.5 times the IQR lower than the $Q_1$. Mean values were presented as empty squares.

## 2.3. Experiments with *Porcellio scaber*

### 2.3.1. Experimental set-up

For the experiments with woodlice (Crustacea, Isopoda) laboratory-raised adult male individuals of *P. scaber* in the intermoult phase were used. Animals were recorded in arenas made of transparent polypropylene (PP) pots (diameter: 9.5 cm, height: 6 cm) that were divided into two equally sized chambers with a 3.5 cm high PP barrier with a passage in the middle (Zidar and Fišer, 2022). Pots were filled with a 10 mm thick plaster of Paris darkened with charcoal. The plaster in each chamber was saturated with tap water and covered with a layer of 3 g of Lufa 2.2 soil (Speyer, Germany) previously dried, ground and sifted through a 0.5 mm sieve. One chamber contained unpolluted soil and the other contained soil polluted with selected MPs. The polluted soil contained 3 % (w/dry weight soil) MPs in all cases. In control arenas, both chambers contained unpolluted soil. As a positive control, we used the behavioural response of woodlice to pyrethrin which was tested previously (Zidar and Fišer, 2022). In the case of pyrethrin, the soil was contaminated with 5 mL/kg dry soil of insecticide product Flora Kenyatox Verde Plus (Unichem, Slovenia) which contained 0.2 % of pyrethrin.

### 2.3.2. Video recording

Animals, dorsally marked with an enamel white paint to increase contrast, were placed in test arenas and continuously recorded for three hours. Video recording was performed in a dark environment illuminated with infrared light (850 nm). Four animals were simultaneously recorded with two webcams (Logitech C920) that have been modified for recordings in infrared light. Videos were captured in VirtualDub 1.10.4 at five frames per second and a Full HD resolution ($1920 \times 1080$ pixels).

### 2.3.3. Video analysis

Videos were analysed via video-tracking in Bonsai 2.4.0 (Lopes et al., 2015). The area of each test arena chamber was isolated by cropping. The white spot at the back of the marked animal was extracted from the background by thresholding and the spot's centroid coordinates inside each chamber were determined for each video frame. The behaviour of eight animals was analysed for each type of MP and 12 for the controls. Based on the data thus obtained the proportion of time that woodlice spent on the unpolluted and polluted soil, the time of locomotory activity, path length and average speed on the unpolluted and polluted soil were calculated.

### 2.3.4. Data analysis

The avoidance of polluted soil was determined as significant if animals spent more than half of the recording time on unpolluted soil. Therefore, the percentage of time on unpolluted soil was tested against a fixed value of 50 % with Wilcoxon signed-ranks test. Variables on woodlice locomotor activity (locomotion time, path length, average speed) were given as differences in activity on unpolluted and polluted soil. Positive values mean that activities on polluted soil increased while negative values mean that activities on polluted soil decreased. These values were tested against a fixed value of 0 with Wilcoxon signed-ranks test. All statistical analyses were performed in SPSS 28.0. Data were presented as box-plots as explained previously.

# 3. Results

## 3.1. Effects of ageing on physico-chemical properties of particles

Visual inspection of powders revealed changes in the colour of all three types of MPs from PP medical masks. The MPs from inner and middle layers of the mask changed their colour from white to pale yellow, while the outer blue layer mask MPs changed colour from light blue to turquoise. No evident changes in colour were observed for LDPE, tire particles and textile fibres (Supplementary information, Figure S1). More detailed changes in physico-chemical properties are described below.

### 3.1.1. The size of particles

Table 1 summarises all particle size measurement results and the calculated standard deviations of MP size. We found that three samples (inner mask, outer mask and textile fibres) were flattened fibres shaped and three samples (middle mask, tire particles and LDPE) were in the shape of fragments. The fibres varied greatly in size, with the shortest being microscopically small and the longest being visible without magnification (up to 500 μm). The fragments were irregularly shaped and often gathered into larger agglomerates.

The most significant differences in size before and after ageing were observed in the three PP MPs (inner mask, middle mask and outer mask). Ageing of the inner mask sample, which is in the form of flattened fibres, led to a decrease in their diameter by a factor of 2 from 72.6 μm to 31.3 μm and decrease in their length from 173.4 μm to 58.6 μm, i.e. by a factor of about three. A similar observation was found for the outer mask fibres, where after ageing the average diameter decreased by about one third from 93.2 μm to 61.3 μm and the fibre length decreased by a factor of 2 (from 226.9 μm before to 125.1 μm after ageing). The middle mask

MPs were of different shapes. Their average diameter decreased almost 2.5 times during the ageing process (from 92.8 μm to 37.8 μm). Interestingly, for these PP samples also a narrower range of particle size distribution after ageing was observed, as the value of the standard deviation decreased to about half of the value before ageing. We also observed a decrease of textile fibres length by a third of their original length from 183.7 μm to 125.6 μm after ageing. However, their diameter remained unchanged. The diameter of tire particles and LDPE fragments was also not changed after ageing.

#### *3.1.2. The surface changes and shape of particles*

The shape and surface changes of MPs were investigated by SEM analysis which did not show any significant differences in the shape nor in the surface of aged and pristine MPs of textile fibres, tire particles and LDPE (Fig. 1). However significant changes in the shape and size of particles of all three layers of aged PP MPs from medical masks (inner, middle and outer layer of the masks) were observed, with smaller particles sizes after ageing (Fig. 1). In these PP samples, aged samples consisted of smaller and more dispersed particles than pristine ones. In addition, the SEM analysis revealed that there were no significant differences in the surface morphology of aged and pristine outer mask MPs. In the aged sample of the middle mask, a few cracks were observed on the particles, while no cracks were visible on the pristine ground middle mask sample. On the contrary, in the aged sample of inner mask, a significant number of cracks were observed on the particles compared to the pristine samples, where no visible cracks were detected (Figure S2).

#### *3.1.3. The FTIR spectroscopy results*

Analysis of the FTIR spectra of the aged samples of all three layers of the medical mask made of PP shows most of the bands at similar positions and in similar ratios as the spectra of pristine samples (Figure S3). However, for all aged PP samples, the following additional bands appeared: a weak broad band characteristic of hydroperoxide and alcohol groups in the range of 3600–3050 $cm^{-1}$ (Gardette et. al., 2013), a band in the range of 3090–3000 $cm^{-1}$ indicating CH groups adjacent to C=C double bonds (Smith et. al., 1998), an additional shoulder band at around 1780 $cm^{-1}$ and prominent bands at around 1740 $cm^{-1}$, and 1710 $cm^{-1}$ in the region characteristic of carbonyl groups (Gardette et. al., 2013). Additionally, several weaker bands appeared for all three aged PP MPs between around 1300 $cm^{-1}$ and 1100 $cm^{-1}$, which are characteristic of various oxygen-containing organic compounds (Smith et. al., 1998), and a band at around 965 $cm^{-1}$, which is characteristic of CH groups adjacent to trans double bonds C=C (Smith et. al., 1998) (Figure S3 **a-c**).

The FTIR spectrum of aged tire particles showed weaker bands in similar ratios and at similar positions as the spectrum of pristine of tire particles. In aged samples, an additional weak band appears at around 1710 $cm^{-1}$, which is a characteristic region for carbonyl groups (Smith et. al., 1998). Additionally, a broad weak band appears in the range of approximately 1520 $cm^{-1}$ to 1800 $cm^{-1}$. There is also a change in the ratio between the two bands at 2850 $cm^{-1}$ and 2950 $cm^{-1}$ between pristine and aged samples. The band at around 1100 $cm^{-1}$ for aged tire samples is much larger in comparison to aged tire particles (Figure S3 **d**). In the case of LDPE sample, the FTIR analysis did not show any additional bands in the spectrum of the aged sample compared to the pristine sample. However, there is a slight change in the ratio between the split bands at 1470 $cm^{-1}$and 1460 $cm^{-1}$, as well as 730 $cm^{-1}$ and 720 $cm^{-1}$, which may indicate changes in the interactions of $CH_2$ groups in adjacent chains and the degree of crystallinity (Smith et. al., 1998) (Figure S3 **e**). For textile fibres, no differences in the spectra of aged and pristine samples were observed (Figure S3 **f**).

We also calculated the CI indices for aged MPs (Table 1). These were calculated from the specified area under band (SAUB) for C=O and $CH_2$ (Table S1). No CI is given for the textile fibres due to the absence of observable changes during accelerated ageing.

### *3.2. Effects of tested material on Daphnia magna*

No effect on the immobilisation of daphnids according to OECD TG 202 was observed after exposure to 1, 10 and 100 m/L of each MPs for 48 h.

When the daphnids were exposed to MPs for 48 h and afterwards recorded in the dark with MPs remaining in the wells, both the path length and swimming rate were significantly increased only in the case of the pristine tire particles (Fig. 2**,** Fig. 3). No changes were observed for other types of MPs. After turning on the light, the daphnids increased their swimming speed as well as the length of the path in all groups compared to the dark (pay attention to the difference in scale in Figs. 2 and 3). However, as in the dark, significantly changed behaviour (increased swimming speed and path length) in comparison to control was observed only for pristine tire particles. Under light conditions, the variability of data was much higher compared to dark as evident from the distance between minimum and maximum values and interquartile ranges (Fig. 2**,** Fig. 3**).**

In the second scenario when daphnids were transferred to a control medium and recorded in the dark, no changes in swimming speed and path length in the case of aged tire-particles were found anymore compared to controls. In the subsequent recording in the light, there were no changes in the path length, but an increase in swimming speed was noted for pristine middle layer mask (Fig. 2**,** Fig. 3**).**

When comparing the swimming speed and path length in pairs of pristine *vs.* aged microplastics, we only detected a statistically significant difference in the case of tire particles. Pristine tire particles induced an increase in swimming speed and path length, while this was not observed in the case of aged MP. For all other microplastics, no differences between values of swimming rate and path length were observed between aged and pristine materials.

### *3.3. Effects on Porcellio scaber*

In the case of soil contaminated with pyrethrin, woodlice showed a clear avoidance response and changed locomotor activity (Fig. 4**A**). In the case of MPs, both pristine and aged, no statistically significant avoidance response was recorded (Fig. 4**A**). However, we noted two exceptions. The first one was in the case of unaged textile fibres, where all but one animal spent most of the time on uncontaminated soil. The second one was in the case of aged MPs from the middle layer of the mask, where all but one animal spent most of the time on soil contaminated with MPs.

The trend of avoiding soil with pristine textile fibres was evident also in animals' locomotor activity (Fig. 4**B-D**). The time of locomotion and length of the travelled path on soil with pristine textile fibres was significantly shorter while their speed of locomotion evidently but statistically insignificantly increased. On the contrary, in soil with aged MPs from the middle layer of the mask, the animal's locomotion time and path length on contaminated soil statistically significantly increased. The path length was also increased in the case of aged tire particles, but other measured parameters showed no preference for contaminated soil.

## 4. Discussion

We investigated how UV–VIS ageing of microplastics affects their physico-chemical properties and whether these changes are reflected in behaviour of two common ecotoxicity test species, the freshwater crustacean *D. magna* and woodlice *P. scaber*.

### *4.1. Changes in physico-chemical properties of microplastics during ageing*

The most significant changes in physico-chemical properties of MPs after UV–VIS accelerated ageing were found for all three layers of PP

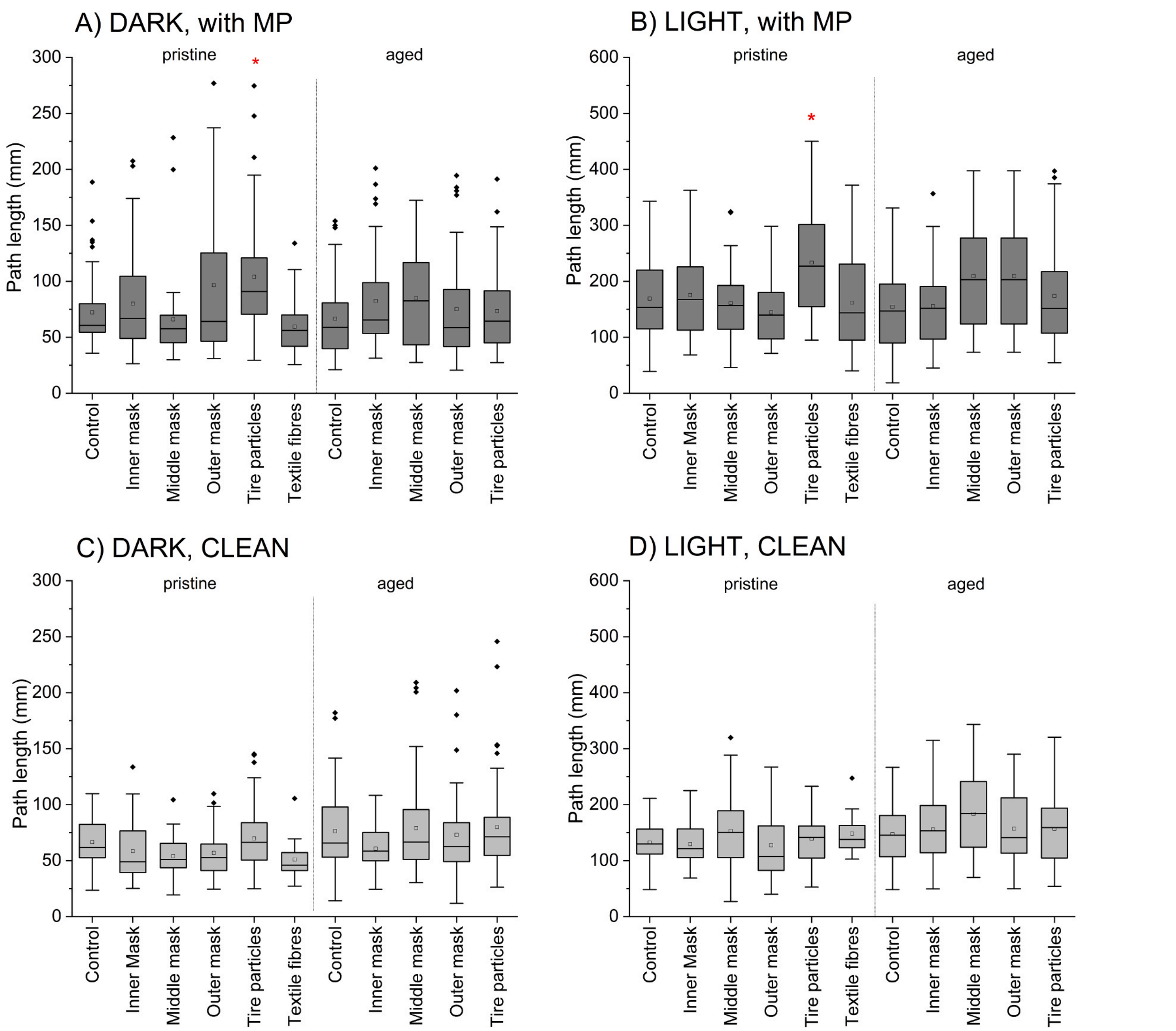



**Fig. 2.** The path length of *Daphnia magna* recorded for 10 min with microplastics (MPs) in the dark (A), and in the light (B), and without MPs (clean) in the dark (C) and light (D). Boxes: 25th, 50th (median values) and 75th percentiles; mean values as empty squares. Kruskal-Wallis ANOVA with Dunnett's test: red *<0.05, n = 40–60 for each box-plot.

medical mask MPs. These MPs became brittle, which is the first indication that some chemical changes have occurred (He et al., 2023). The size (fibre length and diameter) of PP MPs decreased significantly pointing to their fragmentation which was further confirmed by SEM analyses showing several smaller and dispersed particles in the case of aged PP. Evident cracks on the surface of the middle and inner medical mask MPs were discovered. Furthermore, evident changes were detected in FTIR spectra showing additional bands at regions characteristic of carbonyl groups, several weaker bands in regions characteristic of various oxygen-containing organic compounds, and a band characteristic of CH groups adjacent to trans double bonds C=C. For all three PP MPs, higher carbonyl indices were observed compared to other MPs tested in this work. Carbonyl indices were in a similar range as previously reported for PP MPs aged in bio-solids (Alavian Petroody et al., 2023). Intensive photodegradation of PP films (280 nm, 25 °C, 50 %, up to 60 days) was demonstrated by Ainali et al. (2021) who also reported an increase in carbonyl, vinyl and hydroxyl/hydroxyperoxide groups, and the formation of cracks.

The diameters of tire particles and LDPE fragments were not changed after ageing, however, some changes in their FTIR spectra were found. For tire particles, a small additional band characteristic for carbonyl groups appeared, while for LDPE a slight change in the ratio between the split bands at 1470 $cm^{-1}$and 1460 $cm^{-1}$, as well as 730 $cm^{-1}$ and 720 $cm^{-1}$, was noted which may indicate changes in the interactions of $CH_2$ groups in adjacent chains and changed degree of crystallinity (Hamzah et al., 2018; Smith, 2021). Tire material is designed to be resistant to ageing, hence our results are in line with expectations. Polyethylene is generally resistant to photodegradation due to the lack of chromophores, but the presence of impurities, structural defects and carbonyl groups in polymers formed during manufacture or weathering can act as chromophores and this facilitates photodegradation (Fairbrother et al., 2019). It has been previously shown that UV induces the formation of oxidation products, the loss of molecular weight, and the decrease in crystallinity of LDPE (Ainali et al., 2021). A decrease of PET textile fibre length by a third of their original length was shown, but their diameter was not affected. SEM and FTIR analysis did not show any differences in their surface and chemical composition. Fragmentation of PET fibres was previously also demonstrated in artificial freshwater media or natural seawater (Xenon lamp; 1500 W; 65 $W/m^2$, 24 ± 3 °C, 5 months) (Sait et al., 2021).

Our results have shown that the extent of UV–VIS induced ageing is polymer-specific, with PP showing the most evident physico-chemical

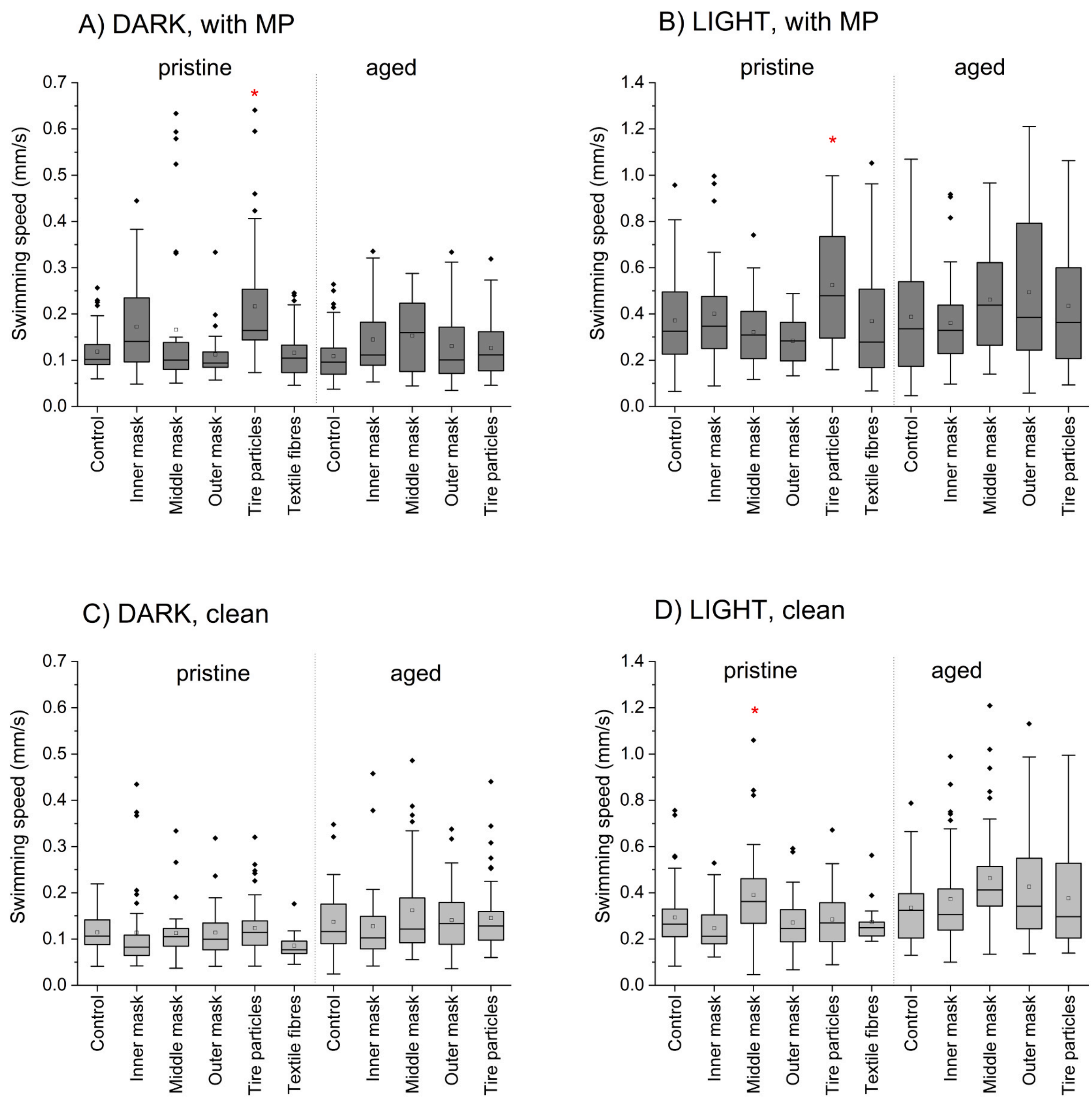



**Fig. 3.** Swimming speed of *Daphina magna* recorded with microplastics (MPs) in the dark (A), and in the light (B), and without MPs (clean) in the dark (C) and light (D). Boxes: 25th, 50th (median values) and 75th percentiles; mean values as empty squares. Kruskal-Wallis ANOVA with Dunnett's test: red *<0.05. n = 40–60 for each box-plot.

changes after ageing, followed by LDPE, polyester fibres and tire particles. This is in line with literature data because, in theory, plastics with a carbon–carbon backbone (PE, PP) have a larger potential to degrade than plastics with heteroatoms in the main chain such as PET (which is the most common representative of polyester) (Zhang et al., 2021). When comparing the degradation of PE and PP, the latter has a lower stability due to the presence of tertiary carbon, which is more susceptible to oxygen attack (Zhang et al., 2021). When PE and PP films were subjected to photodegradation experimentally (280 nm, 25 °C, 50 %, up to 60 days) more intensive changes were found for PP (Ainali et al.; 2021) which is in line with the results of our study.

### 4.2. *Effects on Daphnia magna*

We observed no effect of tested MPs, neither pristine nor aged, on the survival of daphnids. Furthermore, contrary to our expectations most of the MPs also did not affect the swimming behaviour of daphnids. The only effect observed was a clear path length and swimming rate increase of daphnids in the case of pristine tire particles when they were recorded with particles present in the wells. When comparing physico-chemical properties of these particles, they did not significantly deviate from other particles in size or shape (Table 1). Tire particles were also the only ones that showed different effects on daphnids after ageing. Interestingly, no evident physico-chemical changes were recorded between aged and pristine tire particles, except for slight changes in FTIR spectra.

When the daphnids were transferred to a medium without tire particles, this effect was no longer present. This indicates that tire MPs impaired daphnids directly during swimming, and they recovered within 30 minutes of acclimation in a clean medium. If the effect had been due to leached chemicals, the animals would not have recovered so fast. We did not assess the attachment of MPs onto the body surface of daphnids to avoid handling animals before video recording, but previous work has shown that MPs can attach to *D. magna* body surface and in particular antennae (De Felice et al., 2019; Magester et al., 2021; Jemec Kokalj et al., 2018). This has been suggested as one of the potential reasons for the effects on daphnids' swimming (Magester et al., 2021). De Felice et al. (2019) suggested that increased swimming might be a

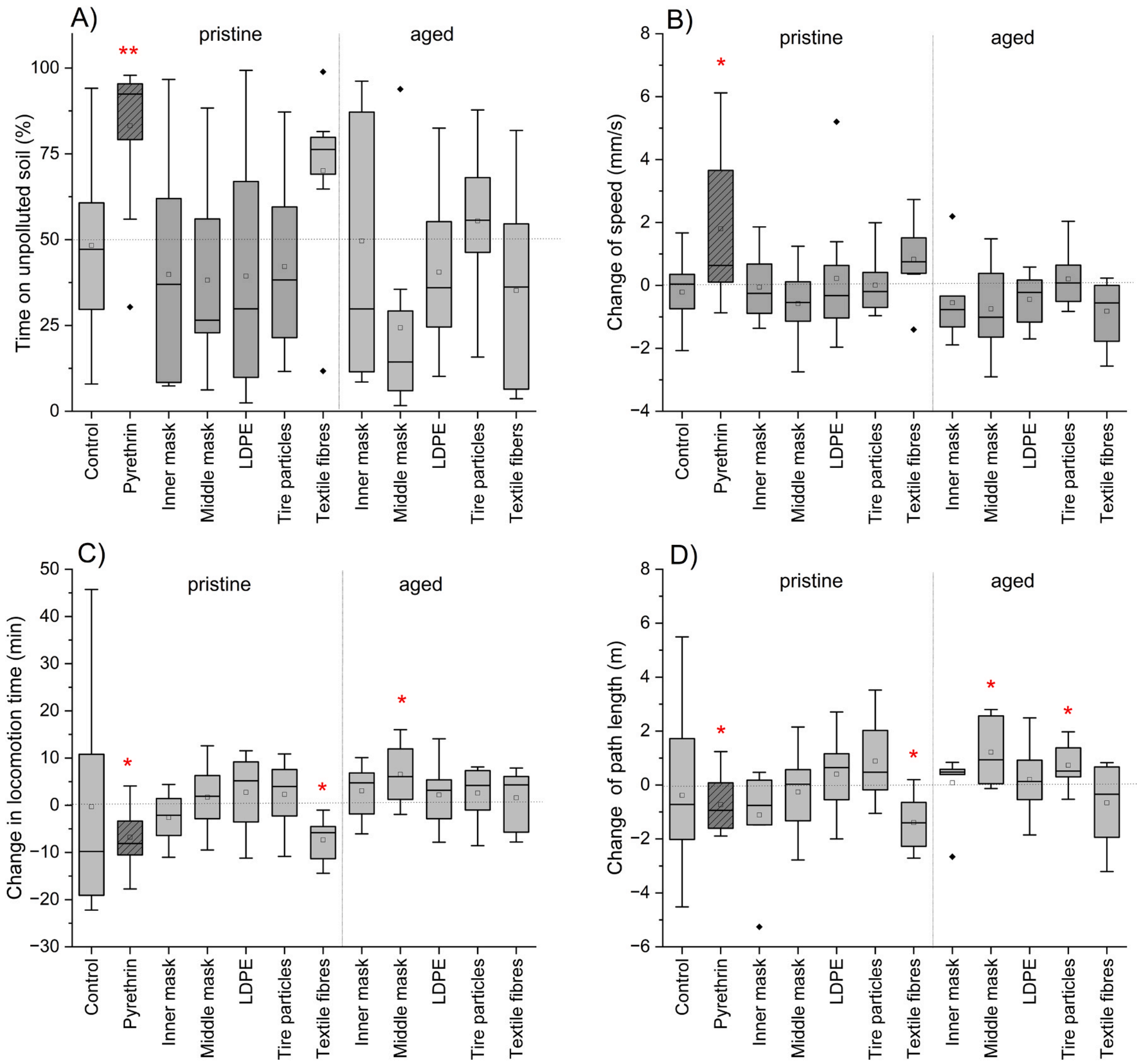



**Fig. 4.** Avoidance response (A) and locomotor activity of animals (B-D) in a free-choice experiment with *Porcellio scaber*. A) The percentage of time on unpolluted soil within the 3 h of observation; B-D) differences between the activity on uncontaminated soil and activity on soil contaminated with microplastic (positive values - activity on polluted soil increase, negative values - activity on polluted soil decrease). Pyrethrin was used as a positive control. Boxes: 25th, 50th (median values) and 75th percentiles; mean values as empty squares. One sample Wilcoxon Signed Rank Test: red *<0.05, red **<0.01, n = 8 for each box-plot with MP, and n= 12 for pyrethrin.

response of daphnids to search for an environment with fewer particles. This is not supported by the results of this study as most of the MPs particles did not induce increased swimming. As daphnids are non-selective filter feeders they are not expected to be able to choose between different types of particles.

Previous studies demonstrated the changed swimming behaviour of daphnids when exposed to MPs. Significant increase in the swimming activity of *D. magna* in terms of distance moved and increased swimming velocity were found after 21-day exposure to 1.25 mg/L and 12.5 mg/L of polystyrene (PS) microparticles (1 μm and 10 μm) under light conditions. Phototactic behaviour was also changed which was evidenced as increased time to reach the light source at 1 μm-exposure, and decreased time in the case of 10 μm (De Felice et al., 2019). On the contrary, Magester et al. (2021) found that 3-day exposure to PS MPs (15.65 μm) slowed down the swimming speed of *D. magna* and change the swimming mode under light conditions which was evidenced as a transition from the horizontal cruising mode to the sinking and jumping mode and prolonged resting time of *D. magna* at the bottom of the water body. Pan et al. (2022) demonstrated decreased hopping frequency of *D. magna* after 1-h exposure to spheric PE particles exposure (160 mg/L, 32–38 μm). All these studies were done with primary spheric microparticles synthesized in the laboratory. The limitation of this selection is that they do not represent realistic scenarios in the environment, where MPs of various sizes (usually larger than those tested previously) and shapes are present (Rozman and Kalčikova, 2022). Furthermore, none of the studies considered reporting the presence of additives in the test suspensions. This can be a serious issue as it has been shown previously that PS nanoplastic suspensions often contain sodium azide which induces toxic effects on daphnids (Pikuda et al., 2018).

In general, daphnids travelled a smaller path length and had a lower swimming speed in the dark as under light. Their light-induced swimming reaction is well documented (Van Gool and Ringelberg, 2003). We noticed that the difference between minimum and maximum values shown as box-plot whiskers and the interquartile range of data for control exposures as well as for MP treatments is much higher under light conditions compared to dark (Figs. 2 and 3). This has practical implications for further experimental set-ups because it will be more difficult to detect the significant differences in daphnid behaviour due to pollutants under light conditions of recording compared to dark. In this study, we applied a testing system that allowed measuring only horizontal movement. We are aware of the criticism that this approach has received because it does not resemble the environmental conditions and several daphnid-specific behaviours cannot be assessed (Bownik et al., 2017). However, the advantage of this automated system is that it allows the recording of many animals (24) simultaneously (Küster et al., 2023). It is hence well suited for screening of MP effects as was the case in our study. More sophisticated systems also allowing 3D monitoring should then be further applied to study swimming behaviour in detail.

### 4.3. Effects on *Porcellio scaber*

Woodlice showed no significant avoidance response to either of the tested MPs but there was a noticeable trend of avoiding soil polluted with pristine polyester textile fibres and a trend of preferring soil polluted with aged MPs from the middle layer of masks. We cannot simply link the avoidance response of *P. scaber* to certain physico-chemical properties as textile fibres had comparable dimensions and shapes as some other tested MPs. However, we did observe that among MPs only pristine textile fibres clumped together in aggregates, thus these MPs were unevenly distributed in soil and most probably mechanically interfered with animals' locomotion. Another MPs that changed the pattern of woodlice behaviour were aged MPs from the middle layer of the mask. The disintegration of these MPs during the ageing process was the most obvious, as only small fragments with cracks on the surface were left after ageing. In addition, FTIR spectra for aged PP MPs showed additional band at regions characteristic of carbonyl groups. It is known that photodegradation of PP plastic releases different volatile organic compounds, including ketones (Lomonaco et al., 2020). In woodlice, and many other invertebrates, ketones are signal molecules important for aggregation and mating (Fortin et al., 2018). Therefore, in the case of aged PP MPs, chemical signals released from fragments might act as an attractant. In woodlice, behavioural responses are guided and influenced by both chemical and mechanical stimuli. Namely, their two pairs of antennae as well as the rest of the body are equipped with numerous chemo- and mechano-sensory sensilla (Schmalfuss, 1998).

The majority of studies on the behaviour of terrestrial invertebrates upon MP exposure have been performed with earthworms, but to our knowledge, no study has yet been done with woodlice. Ding et al. (2021) tested the avoidance behaviour of earthworm *Eisenia fetida* in a 48-h exposure set-up where they were able to choose between contaminated and non-contaminated soils. Earthworms avoided the soil contaminated with PE, polylactic acid, and polypropylene carbonate MPs (all average size 120 μm,) at concentrations 12 – 50 % w/w. Huerta Lwanga et al. (2016) reported that earthworms *Lumbricus terrestris* migrated to deeper soil layers of mesocosm when exposed to large PE particles (size: < 400 μm) and concentrations (60 % w/w). On the other hand, *L. terrestris* did not avoid soil contaminated with a mixture of MPs (PP, PS, PET and LDPE, size: 250 μm) at concentrations up to 7 % w/w in 48-h (Baeza et al., 2020) nor the soil contaminated with polyester fibres at concentrations up to 1 % w/w (length: 361.6 +/- 387.0 μm, diameter: 40.7 +/- 3.8 μm) after 24-h exposure (Prendergast-Miller et al., 2019). Springtails *Folsomia candida* avoided soil contaminated with 0.5 % and 1 % w/w of PE microplastics (size: <500 μm) after longer exposure (7 days), but not after 48 h (Ju et al., 2019). All these data indicate that environmentally relevant concentrations (up to a few % w/w) most probably do not affect the behaviour of terrestrial invertebrates upon short-term exposure (up to a few days). Ding et al. (2021) showed that the effect of MPs on earthworms depends predominantly on the concentration and not on the type of MPs. Thus, it was suggested that earthworms avoid heavily polluted soil with MPs due to changes of soil physico-chemical properties (Ding et al., 2021). Namely, MPs affect several soil properties, like water holding capacity, bulk density, soil aggregation and porosity, and of chemical properties like pH, organic matter content, nutrient levels, and migration of pollutants (Qui et al., 2022). This might lead to the conclusion that MPs do not necessarily represent a direct chemical trigger for avoidance behaviour of soil invertebrates. This is supported also by our results, as the effect induced by different MPs, pristine and aged, deviated from those that were chemically induced, for example by pyrethrins.

The results of this woodlice study show that these organisms in general do not avoid MPs in acute exposures. This means that they can be potentially chronically exposed to MPs in the environment, as they do not recognize MP-contaminated soils as harmful, or even prefer them, as demonstrated in this study. Other studies with woodlice indeed showed that longer exposures (up to 3 weeks) to MPs induce immune response, and change their metabolic activity (Selonen et al., 2020; Selonen et al., 2021). Furthermore, prolonged multiple-generation exposure might also reveal some other effects, as has been demonstrated for other terrestrial invertebrates and other types of pollutants (Selonen et al., 2020; Selonen et al., 2021; Guimarães et al., 2023).

## 5. Conclusion and outlook

This study provided extensive knowledge on the physico-chemical transformations of PP, LDPE, polyester fibres and tire MPs after UV–VIS induced ageing. The extent of transformation depended on the polymer type, with PP showing the most alterations, followed by LDPE, polyester and tire particles. Evident fragmentation of PP and polyester fibres was recorded, accompanied further by chemical changes reflected in FTIR spectra and also surface properties in the case of PP. Tire particles and LDPE did not change their size, but some chemical changes occurred during ageing. Despite these evident changes in MP properties, this was generally not reflected in the behaviour of daphnids or woodlice. In fact, very few significant changes in their behaviour were observed in the test conditions applied (short duration, worse-case exposure concentrations). There was a trend of woodlice avoiding soil polluted with pristine polyester textile fibres and a trend of preferring soil polluted with aged MPs from the middle layer of masks, which might be due to mechanical and chemical triggers, respectively. Most MPs, aged and pristine, did not affect the swimming of daphnids. The only effect observed was a significant increase in path length and swimming speed for the pristine tire particles when the recording was done with particles remaining in the wells suggesting a physical effect. We would suggest that further tests focus on longer exposure periods, and different set-ups where indirect effects of MPs on physico-chemical properties of test medium could be revealed as previously proposed (Jemec Kokalj et al., 2024).

## CRediT authorship contribution statement

**Primož Zidar:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Dana Kühnel:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Anita Jemec Kokalj:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Data curation, Conceptualization. **Luka Škrlep:** Writing – review & editing, Visualization, Methodology. **Branka Mušič:** Writing – review & editing. **Damjana Drobne:** Writing – review & editing, Funding acquisition. **Andrijana Sever Škapin:** Writing – review & editing, Funding acquisition. **Tina Skalar:** Writing – review & editing, Writing – original draft, Visualization, Methodology.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117020.

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