Chemosphere 226 (2019) 774–781

Contents lists available at ScienceDirect

Chemosphere

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

Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*

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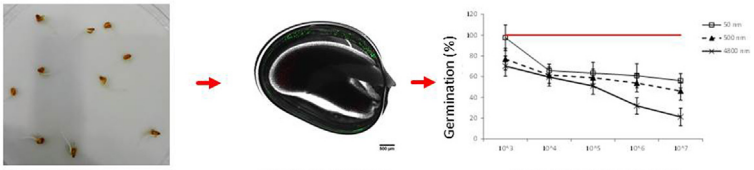
HIGHLIGHTS

- Terrestrial systems accumulate nano- and microplastics but are understudied.
- We exposed a vascular plant to three different sized plastics (50, 500 and 4800 nm).
- Exposure to plastics caused significant impacts on germination and root growth.
- Late germination is likely related to accumulation of microplastics on seed case.
- The observed effects were short-term and transient.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 August 2018
Received in revised form
20 March 2019
Accepted 25 March 2019
Available online 31 March 2019

Handling Editor: Tamara S. Galloway

Keywords:
Nano- and micron-sized plastics
Terrestrial systems
Vascular plant
Cress (*Lepidium sativum*)
Sublethal impacts

ABSTRACT

The impacts of nano- and microplastics (<100 nm and <5 mm, respectively) on terrestrial systems is to the present largely unexplored. Plastic particles are likely to accumulate in these systems primarily by the application of sewage sludge. The aim of the current study was to investigate the effects of three sizes of plastic particles (50, 500, and 4800 nm) on a terrestrial plant (cress; *Lepidium sativum*), using a standardized 72 h bioassay. Cress seeds were exposed to five different concentrations of plastics, ranging from 10^3 to 10^7 particles mL^{-1}. Germination rate was significantly reduced after 8 h of exposure for all three sizes of plastics, with increased adverse effect with increasing plastic sizes. Seeds exposed to 4800 nm microplastics showed a germination rate decline from 78% in control to 17% in the highest exposure. No difference in germination rate occurred after 24 h of exposure, regardless of the size of the plastic used. Significant differences in root growth were observed after 24 h, but not after 48 or 72 h of exposure. Impacts on germination are likely due to physical blockage of the pores in the seed capsule by microplastics as shown by confocal microscopy of fluorescent microplastics. In later stages, the microplastics particularly accumulated on the root hairs. This is the first detailed study on the effect of nano- and microplastics on a vascular, terrestrial plant, and our results indicate short-term and transient adverse effects.

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https://doi.org/10.1016/j.chemosphere.2019.03.163

1. Introduction

Terrestrial emissions of microplastics in industrialized countries predominantly originate from application of sewage sludge to farmlands (Nizzetto et al., 2016). Sewage sludge, also referred to as biosolids, is applied to improve and maintain productive soils and stimulate plant growth in many areas of the world (Singh and Agrawal, 2008). The application of agricultural sewage sludge alone results in a major input of plastic particles into agricultural soils, estimated to be between 63,000 and 430,000 and 44,000–300,000 tons per year of microplastics (<5 mm) in the EU and North-America farmlands, respectively (Nizzetto et al., 2016), and between 2800 and 19,000 tons per year in Australian agro-ecosystems (Ng et al., 2018). In addition, there are also new technological innovations in which plastics are added as a nano-coating to seeds in combination with an active ingredient, such as fertilizers or pesticides. As a result, there is an accumulation of plastic particles in terrestrial systems.

To date, most work on the distribution and toxic potential of nano- and microplastics has focused on aquatic environments, and there is an urgent need for research on potential adverse effects of nanoplastics (<100 nm) and microplastic pollution in terrestrial ecosystems (Bläsing and Amelung, 2018; Horton et al., 2017). In fact, only a few studies have investigated the impact of plastics particles in terrestrial organisms (Chae and An, 2018). For example, in the gut of the earthworm *Eisenia andrei*, microplastics can cause histopathological changes and induce an immune response at 0.1% dry weight (Rodriguez-Seijo et al., 2017). Nanoplastics ingested by the oligochaete *Enchytraeus crypticus* decrease the gut bacterial diversity and body weight at 10% dry weight (Zhu et al., 2018). At microplastic doses of 28, 45, and 60% dry weight, increased mortality and significantly reduced growth rates are reported in the earthworm *Lumbricus terrestris* (Huerta Lwanga et al., 2016).

Fewer studies have investigated the impacts of nano- and microplastics on plants. Most of these studies have been conducted on non-vascular plants, such as phytoplankton. For example, exposure of *Skeletonema costatum*, a marine microalgae, to microplastics resulted in negative effects on growth and photosynthesis (Zhang et al., 2017). Microplastic exposure to freshwater algae *Chlorella pyrenoidosa* resulted in physical damage to the algae and oxidative stress (Mao et al., 2018). To our knowledge, only one study has examined the effects on vascular or so-called higher plants: Kalčíková et al. (2017) exposed duckweed (*Lemna minor;* a freshwater plant species) to microplastics, and a significant reduction in root growth and cell viability was observed, but no effect on leaf growth.

Additionally, studies on the effects of engineered nanoparticles (which are in the same size range as nanoplastics) on plants have shown both enhancing and inhibitive effects on plant growth (Ma et al., 2010; Miralles et al., 2012). The absence of studies on the effect of nano- or microplastics on terrestrial plants (Chae and An, 2018; de Souza Machado et al., 2018) results in a major knowledge gap which needs to be urgently addressed, especially in light of global biosolid usage in agricultural fields, and previous work on the effects of engineered nanomaterials on plants. Therefore, the goal of the current study was to increase our understanding of uptake or accumulation and the potential adverse impacts of nano- and microplastics on terrestrial vascular plants. To this end, we used the cress (*Lepidium sativum* L.), a terrestrial plant species frequently used in ecotoxicological assessments, to assess sites of adsorption, uptake and impact on germination, growth, and chlorophyll production.

2. Materials and methods

2.1. Plastic particles

Green fluorescent plastic particles (Fluoro-Max Green Fluorescent Polymer Microspheres) at nominal sizes of 50, 500, and 4800 nm were purchased from Fisher Scientific (Landsmeer, The Netherlands). These particles are internally dyed with a Firefli™ Fluorescent Green (468/508 nm) and are readily brought in suspension. In order to remove potential surfactants, the following cleaning protocol was applied before use of the particle in the assays. First, the solution was vortexed for 30 s and sonicated at 42 kHz for 10 s. Next, the required amount of solution was pipetted in 1.5 mL micro centrifuge tubes (Fisher Scientific) and centrifuged for 5 min at 10,000 rpm. The supernatant was then pipetted off and replaced with distilled water. This was repeated three times. Next, stock solutions of 10^7 particles mL^{-1} were prepared and stored at 4 °C until use.

2.2. Toxicity assessment

The experiment was based on a protocol developed by Hoekstra et al. (2002). Cress seeds (*Lepidium Sativum* L.) were purchased from a local store and kept in a dry location until use. At the start of the experiments, ten seeds were placed on five layers of cellulose grade filter paper (90 mm; Fisher Scientific) in 9 cm Petri dishes. Seeds were exposed to five concentrations for each size of plastic particles: 10^3, 10^4, 10^5, 10^6, and 10^7 particles mL^{-1}, and distilled water was used as a control. At the start of the experiment, 5 mL of each concentration (10^3 -10^7 plastics mL^{-1}) was added to the Petri dishes, with 6 replicate Petri dishes per concentration ($n = 6$) containing 10 cress seed each. The Petri dishes were randomly placed in an incubator (IPP110, Memmert GmbH, Schwabach, Germany), and incubated at 24 °C, at a relative humidity of >80%, and constant 6000 lux top illumination.

2.2.1. Seed germination

At 8 and 24 h after the start of the exposure, the number of germinated seeds were recorded. The visible root emerging from the split seed case was used as the operational definition of seed germination (Hoekstra et al., 2002). To compare the impact of different sized plastic particles, the percentage of relative seed germination (RSG) after 8 and 24 h of exposure was calculated as follows:

$$\text{RSG}\ (\%) = \frac{\text{Number of seeds germinated in exposure concentration}}{\text{Number of seeds germinated in control}} \times 100$$

The RSG of control was used for comparison and was always set at 100% for ease of comparison across treatments and particle sizes.

2.2.2. Root- and shoot growth

At 24, 48, and 72 h after the start of exposure the root length (mm) was recorded (Hoekstra et al., 2002). In addition, at 48 and

72 h after the start of exposure the shoot length (mm) was determined. The root and shoot length were measured manually using a ruler under a dissection microscope and rounded up to the nearest millimeter. For ease of comparison between differently sized plastics the percentage of relative root growth (RRG) was calculated after 24, 48 and 72 h using the following formula:

$$\text{RRG}\ (\%) = \frac{\text{Mean root length in concentration}}{\text{Mean root length in control}} \times 100$$

The percentage of relative shoot growth (RShG) was calculated after 48 and 72 h using the following formula.

$$\text{RShG}\ (\%) = \frac{\text{Mean shoot length in concentration}}{\text{Mean shoot length in control}} \times 100$$

2.2.3. Chlorophyll contents

For experiments in which cress were exposed to 50 and 4800 nm plastic particles total chlorophyll, chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) levels were assessed after 72 h of exposure. Chlorophyll was determined spectrophotometrically based on an established procedure (Farooq et al., 2016; Porra et al., 1989). In brief, topmost fully expanded fresh leaves were extracted for the pigments. Levels were determined in a dark room and the samples were kept on ice to impede chlorophyll degradation. For each concentration, three samples were prepared. A 1.5 mL microcentrifuge tube containing the leaves, 0.04 g of quartz sand and a sterilized metal ball (5 mm in diameter), was filled off with ice-cold 100% methanol and shaken for 1 min at 30 shakes/sec using a Retsch Mill (Retsch Mixer Mill MM200, Retsch, Haan, Germany). Subsequently, the solution was centrifuged for 1 min at 13,200 rpm (Eppendorf MicroCentrifuge 5415 D, Eppendorf) and the supernatant was removed and placed on ice. This procedure was repeated until 5 mL supernatant was collected.

Consecutively, chl *a,* chl *b*, and total chlorophyll content were determined using a spectrophotometer (Shimadzu UV-1800 UV-VIS Spectrophotometer, Tokyo, Japan), with absorbance set at 665.2 nm and at 652 nm b, as determined after running a full absorbance spectrum. Baseline excitation at 750 nm was subtracted from each value as. The following equations (adjusted according to Lichtenthaler (1987)) were applied to quantify chlorophyll content: chl *a* (mg L^{-1}) = $16.29A_{665.2\text{-}750}$- $8.54A_{652\text{-}750}$ and chl *b* (mg L^{-1}) = $30.66A_{652\text{-}750} - 13.58A_{665.2\text{-}750}$, where A is absorbance.

2.3. Localisation of plastic particles on L. sativum

Seedlings exposed to control and microplastics of 4800 nm in diameter were imaged at 4, 8, 24, 48 and 72 h of exposure. Localisation of smaller microplastics is challenging as single particles might be too small or the particles might be missed due to the low depth of field at high magnifications. Therefore, cress exposed to 4800 nm microplastics were imaged. The seedlings were stained with a 10 μM propidium iodide dye for better orientation in the focal plane. Propidium iodide stains the plasma membrane, yet does not interfere with the microplastics and was therefore appropriate to outline cells (Blancaflor et al., 1998). Only the highest concentration (10^7 microplastics mL^{-1}) was used for imaging as proof of concept where on the cress seedling nano- and microplastics are accumulating. The imaging was specifically directed at the root and shoot of the seedlings as well as the seed case in the early stage (4 and 8 h after the start of exposure).

Overview images of seedlings were taken using a Leica MZ 16FA fluorescence microscope with a GFP filter (excitation at 470/40 nm, barrier at 525/50 nm) and equipped with a digital camera (DFC 420) and image acquisition software of Leica. Images of all seedlings, control and exposed, were pictured using the same settings throughout the experiment. Detailed imaging of seed casing, root, shoot, and root hairs was performed on a Zeiss LSM confocal laser microscope (Zeiss GmbH, Oberkochen, Germany) equipped with a 488 nm argon-ion laser for excitation of green fluorescent microplastics and a 543 nm helium-neon laser for excitation of propidium iodide. The fluorescence emission was collected at 505–530 nm with a bandpass (BP) filter for fluorescent microplastic detection and avoiding endogenous fluorescence of plant tissue (Koo et al., 2015) while propidium iodide emission wavelength was collected at 560 nm with a longpass (LP) filter. All images were taken using an EC Plan-Neofluor 20x/0.50 M27 objective, an EC Plan-Neofluor 10x/0.30M27 objective or a Zeiss EC Plan-Neofluor 2.5c/0.075 objective. Images were acquired with the operating software Zeiss Zen 2010 (Zeiss, Oberkochen, Germany) and subsequently merged and processed with ImageJ (Abràmoff et al., 2004) using the LSM Toolbox.

2.4. Statistical analysis

Statistical analysis was done using the R environment (R v3.3.0; R Core Team). All results are presented as the mean ± SEM. In all cases, Petri-dishes (n = 6/treatment) were used as unit of replication. The data were screened for normality (Shapiro-Wilk test, using the car v2.1–5 package) and homogeneity of variance (Levene's test, using the car v2.1–5 package). Next, the effects of the different concentrations were analysed through separate analyses of variance (ANOVA) for each time interval and the corresponding microplastic size, with α set at 0.05. Despite the robustness of ANOVA for the violation of the aforementioned assumptions, p-values which are close to α need to be cautiously interpreted when a violation of the assumptions does occur. Where applicable, this was noted in the result section. Tukey's post-hoc tests were conducted when significant differences were observed.

3. Results

3.1. Germination rate

Seed germination rate was significantly reduced for all three sized plastics (ANOVA, $p < 0.01$; Table 1). There was a size- and dose-dependent effect on germination at 8 h, with increasing reductions found with increased plastic size. For instance, at an exposure of 10^7 particles mL^{-1}, the mean standardized germination rate, expressed as RSG, was 56%, 46%, and 21%, for 50, 500, and 4800 nm plastics respectively (Fig. 1). Importantly, the effect of nano- and microplastics on seed germination disappeared after 24 h of exposure (ANOVA, $P > 0.05$), and germination reached close to 100% regardless of the size of nano- and microplastics or exposure concentration (Table 1).

3.2. Sub-lethal impacts

A significant difference (ANOVA; $p < 0.01$) was found in root growth after 24 h of exposure for 50 nm and 500 nm, but not for 4800 nm (ANOVA; $p = 0.09$; Table 2). Interestingly, exposure to 50 nm particles resulted in a significant increase in root growth relative to control, while exposure to 500 nm resulted in a significant decrease in root growth (Table 2; Fig. 2). No significant differences (ANOVA; $p > 0.05$) in root growth were observed after 48 and 72 h of exposure, regardless of plastic size (Table 2, Fig. 2).

There was no consistent pattern in shoot growth for all sizes of plastics tested. We found no significant impacts of 50 and 4800 nm on shoot growth at 48 and 72 h (ANOVA; $p > 0.05$; Table 2 and

Table 1
The effects of three different sized plastic particles (50, 500 and 4800 nm) on seed germination (% of total seeds incubated) of cress *(Lepidium sativum* L.) after 8 and 24 h of exposure. Values are means ± SEM (n = 6). Different letters indicate significant differences (ANOVA, followed by a Tukey post-hoc test, with $\alpha = 0.05$) between treatments at individual time points and plastic sizes.

H after exposure	Concentration (particles mL^{-1})	Percentage of seeds germinated		
		50 nm	500 nm	4800 nm
8	Control	68.3 ± 7.2 a	65.0 ± 4.6 a	78.3 ± 6.4 a
	10^3	66.7 ± 7.7 ab	50.0 ± 6.2 a	55.0 ± 7.0 a
	10^4	45.0 ± 3.9 ab	40.0 ± 4.7 b	46.7 ± 6.1 ab
	10^5	43.3 ± 6.5 ab	38.3 ± 4.9 b	40.0 ± 5.8 ab
	10^6	41.7 ± 7.2 ab	35.0 ± 5.1 b	25.0 ± 5.6 ab
	10^7	38.3 ± 4.4 b	30.0 ± 5.3 b	16.7 ± 6.1 b
24	Control	100 ± 0	100 ± 0	100 ± 0
	10^3	100 ± 0	100 ± 0	98.3 ± 1.7
	10^4	100 ± 0	100 ± 0	100 ± 0
	10^5	98.3 ± 1.7	100 ± 0	98.3 ± 1.7
	10^6	98.3 ± 1.7	100 ± 0	96.7 ± 2.1
	10^7	98.3 ± 1.7	100 ± 0	100 ± 0

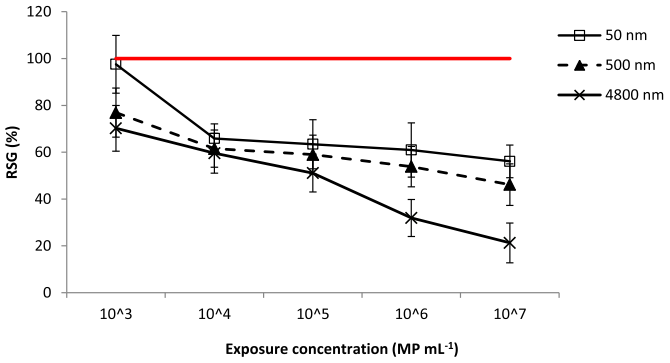

Fig. 1. The effects of three different sized plastic particles (50, 500, and 4800 nm) on Relative Seed Germination (RSG) of cress *(Lepidium sativum* L.) after 8 h of exposure. The red line indicates the control baseline. Values are means ± SEM (n = 6). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3). No significant differences in shoot growth were found for the 500 nm particles after 48 h of exposure (ANOVA; $p = 0.72$). After 72 h of exposure to 500 nm particles, a significant difference in shoot length was observed (ANOVA; $p = 0.008$), although, this was not a dose-dependent response (Table 2; Fig. 3).

Similarly, there was no significant impact of plastics on the Chl *a*, Chl *b* nor on total chlorophyll contents found (ANOVA; $p > 0.05$; Table S1).

3.3. Epifluorescence and confocal microscopy

Images of fluorescent microplastics (4800 nm; 107 particles mL^{-1}) were acquired to determine the sites of accumulation during the first 72 h of cress seedling development. Before germination, at 4 h and 8 h, the plastic particles accumulate in the film coating the seed capsule (testa), particularly in the pores in the surface as shown by a strong green fluorescence indicating accumulation of multiple microplastics (Fig. 4A). Once the radicle ruptures the micropylar endosperm, the microplastics accumulated there continue to adhere to the emerging radicle. This was observed to prompt a gradual spread of microplastic particles on the plant at later growth stages (Fig. 4B).

After 24 h of exposure, the sprouts shoot out through the split seed casing and the root hairs are clearly visible. At this stage, the plastic particles are not only on the seed casing and radicle but also surround the shoot with a tendency of increased accumulation around the root hairs (Fig. S1, Supplementary Material). At 48 h and

Table 2
The effects of three different sized plastic particles (50, 500 and 4800 nm) on root- and shoot length of cress *(Lepidium sativum* L.) after 24, 48 and 72 h of exposure. Values are means ± SEM (n = 6). Different letters indicate significant differences (ANOVA, followed by a Tukey post-hoc test, with $\alpha = 0.05$) between treatments at individual time points and plastic sizes.

h of exposure	Concentration particles mL^{-1}	50 nm		500 nm		4800 nm	
		Root Length (mm)	Shoot Length (mm)	Root Length (mm)	Shoot Length (mm)	Root Length (mm)	Shoot Length (mm)
24	C	8.87 ± 0.27 a		9.00 ± 0.14 a		10.00 ± 0.31	
	10^3	9.57 ± 0.27 a		9.25 ± 0.28 a		10.27 ± 0.28	
	10^4	8.42 ± 0.13 ab		9.87 ± 0.50 ab		9.05 ± 0.36	
	10^5	8.42 ± 0.44 ab		10.08 ± 0.21 ab		9.30 ± 0.46	
	10^6	8.43 ± 0.33 ab		10.13 ± 0.28 ab		9.28 ± 0.58	
	10^7	7.43 ± 0.25 b		10.93 ± 0.31 b		8.52 ± 0.34	
48	C	21.38 ± 1.27	5.23 ± 0.26	24.50 ± 1.02	5.73 ± 0.28	25.53 ± 1.00	6.47 ± 0.26
	10^3	23.32 ± 1.57	5.25 ± 0.33	24.85 ± 1.02	5.83 ± 0.05	26.52 ± 1.58	5.85 ± 0.23
	10^4	20.81 ± 1.02	5.33 ± 0.32	23.48 ± 0.94	5.55 ± 0.34	24.42 ± 1.05	5.72 ± 0.27
	10^5	20.85 ± 0.61	6.03 ± 0.15	21.72 ± 1.61	5.32 ± 0.32	23.13 ± 0.95	5.37 ± 0.20
	10^6	21.90 ± 1.07	5.50 ± 0.28	23.97 ± 1.01	5.28 ± 0.35	22.78 ± 0.61	5.58 ± 0.25
	10^7	24.12 ± 1.48	5.83 ± 0.40	24.73 ± 1.56	5.73 ± 0.22	23.28 ± 1.12	5.33 ± 0.30
72	C	37.45 ± 2.08	6.62 ± 0.39	38.27 ± 2.04	7.62 ± 0.36 ab	38.73 ± 3.70	7.53 ± 0.62
	10^3	46.83 ± 2.80	6.38 ± 0.25	44.63 ± 2.43	7.90 ± 0.41 ab	38.68 ± 1.09	7.03 ± 0.30
	10^4	43.20 ± 4.05	7.07 ± 0.33	39.70 ± 2.27	8.28 ± 0.36 ab	40.65 ± 2.08	7.00 ± 0.28
	10^5	40.93 ± 2.95	6.32 ± 0.42	40.15 ± 3.04	7.75 ± 0.35 ab	35.47 ± 2.35	7.07 ± 0.28
	10^6	38.80 ± 2.35	6.52 ± 0.28	33.78 ± 1.54	6.18 ± 0.18 b	35.85 ± 2.50	5.88 ± 0.35
	10^7	36.35 ± 2.41	5.90 ± 0.34	37.78 ± 2.41	7.13 ± 0.36 ab	35.15 ± 2.35	6.62 ± 0.35

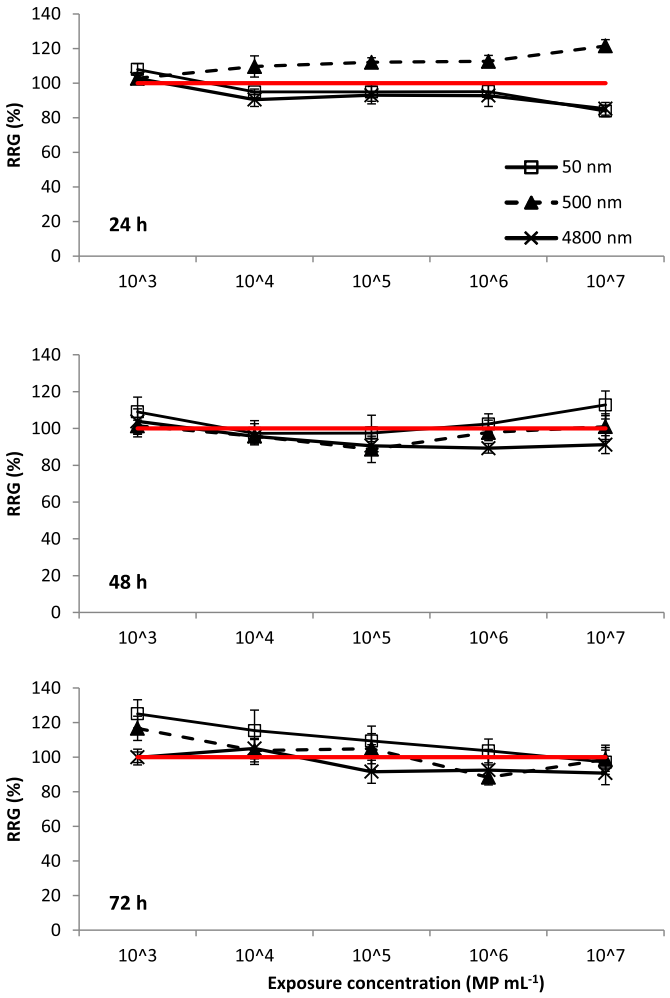

Fig. 2. The effects of three different sized plastic particles (50, 500, and 4800 nm) on Relative Root Growth (RRG) of cress *(Lepidium sativum* L.) after 24, 48 and 72 h of exposure. The red line indicates the control baseline. Values are means ± SEM (n = 6). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

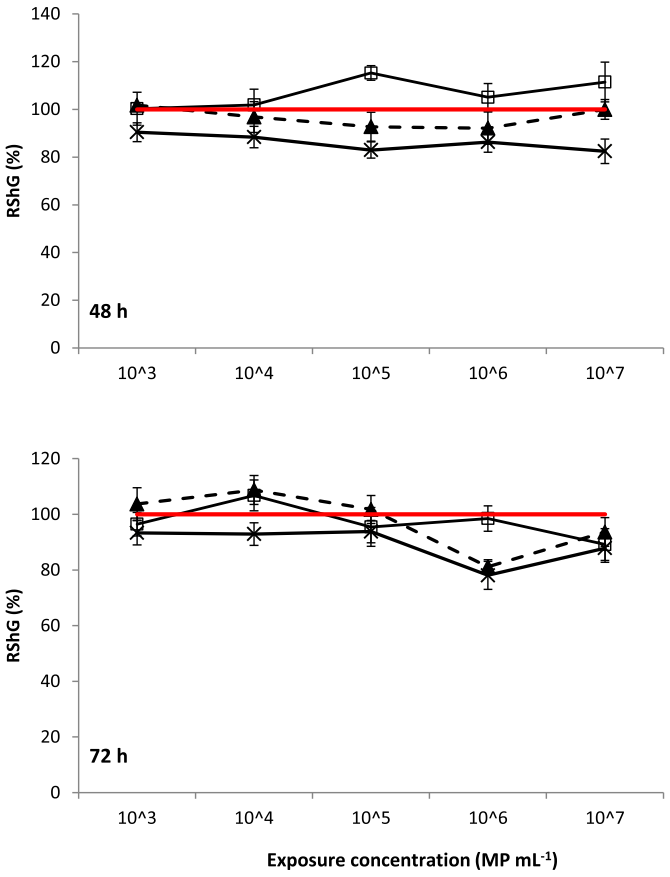

Fig. 3. The effects of three different sized plastic particles (50, 500, and 4800 nm) on Relative Shoot Growth (RShG) of cress *(Lepidium sativum* L.) after 48 and 72 h of exposure. The red line indicates the control baseline. Values are means ± SEM (n = 6). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

72 h, dark green leaves have developed and the root hairs are forming a dense web. Clusters of plastic particles were especially accumulated on the root hairs but also found on the leaves and epidermis (Fig. 5).

4. Discussion

Microplastics enter the terrestrial systems by the application of sewage sludge to agricultural systems and the use in new technologies among other sources, yet effects and potential ecological consequences remain largely unexplored. Here we present the first detailed study on the impact of nano- and microplastics on a higher terrestrial plant species. The seed capsule (testa) of angiosperm seeds protects against adverse environmental conditions and controls germination (Debeaujon et al., 2000). We found that plastic particles particularly accumulate in the pores of the testa of *L. sativum*. Deposits adhering to the surface of the pores can slow down water uptake, as shown in soybean (*Glycine max*) seeds (Calero et al., 1981). This suggests that clogging of the pores with plastic particles might inhibit water uptake and thus delay germination. Given we observed increasingly pronounced effects with the increased size of the plastic, the delay in germination might be caused by physical blocking.

We also observed a change in root growth after 24 h of exposure, with a decrease in root growth when exposed to 50 nm particles, and an increase when exposed to 500 nm plastic particles. A reduction in root growth was also observed by Kalčíková et al. (2017), who found inhibited root growth for the aquatic duckweed species *Lemna minor* exposed to microplastics. While Kalčíková et al. (2017) hypothesized the reduction in growth might be due to physical blocking of the root by the particles, we add a new insight on blocking of pores before germination.

Imaging of fluorescent microplastics indicates the continuous presence, during all growth stages, of the particles on the surfaces of the plants. It is beyond the scope of this study to determine whether actual uptake of the plastic particles across the cell wall of the seedlings took place. However, intracellular uptake of nanoplastics (20 and 40 nm) has been demonstrated in tobacco BY-2 plant cell culture (Bandmann et al., 2012). Whether adsorbed on the epidermis or absorbed intracellularly, microplastics are likely to be ingested by herbivores along with the plant material. Reaching the gut of higher organisms, nano- and microplastics can decrease bacterial diversity and elicit an immune response already at low concentrations (Rodriguez-Seijo et al., 2017; Zhu et al., 2018).

Despite the presence of the microplastics on the epidermis and root hairs, we observed no impact on leaf growth nor on chlorophyll content. This is in line with previous work with the vascular aquatic plant species *Lemna minor* (Kalčíková et al., 2017). Another study on a marine non-vascular macroalgae (*Dunaliella tertiolecta*)

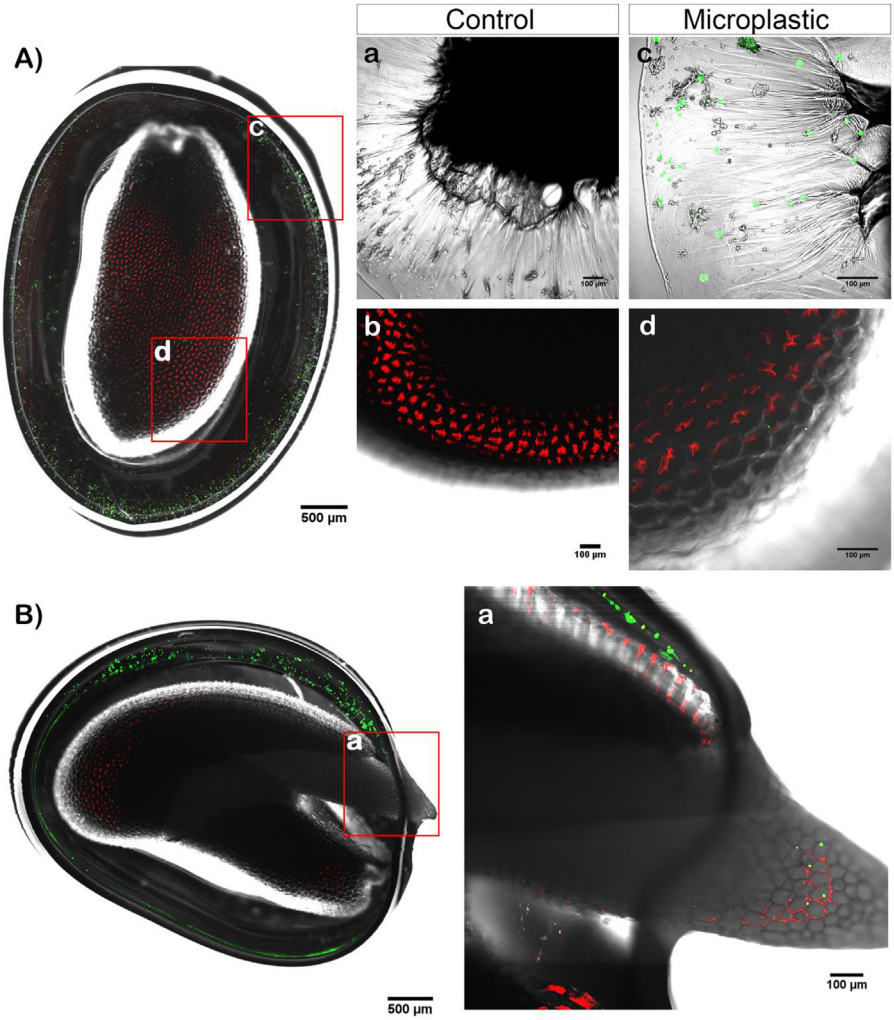

Fig. 4. Accumulation of microplastics (4800 nm) on *L. sativum* seedling within the first 8 h of development. After 4 h of exposure and development, the majority of microplastics (green) accumulate on the pores of the seed coat (A.c) and fewer particles are close to the endosperm of the embryo (A.d). After 8 h or just after germination, microplastics adhere to the emerging radicle (B.a). Propidium iodide (red) was used to stain the plasma membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

exposed for 72 h to three different sizes (50, 500, and 6000 nm) of polystyrene beads found no impact on photosynthesis capacity, however, at very high concentrations (250 mg L^{-1}) growth was negatively affected (Sjollema et al., 2016). In contrast, results on algae (*Scenedesmus obliquus*) exposed to approximately 70 nm polystyrene nanoplastics did observe a reduction in chlorophyll concentration after 72 h exposure (Besseling et al., 2014). Similarly, a study on the algae *Chlorella* and *Scenedesmus* found that exposure to 20 nm polystyrene nanoparticles hindered photosynthesis, possibly by the limitation of light due to shading effects (Bhattacharya et al., 2010).

Direct effects of plastic particles on terrestrial systems and vascular plants in particular are clearly understudied (Chae and An, 2018; Horton et al., 2017; Ng et al., 2018). Reviewing the current literature, Ng et al. (2018) anticipate uptake, potential toxicity, and interaction with other biochemicals of nanoplastics in plants based on the responses of plants to engineered carbon nanoparticles. Still, they emphasize the uncertainty of their conclusions. Thus, the results of the present study are novel in displaying effects of microplastics on cress seedlings, which are nearly incomparable to outcomes of other studies.

Irrespective of the high exposure concentrations used in the current study, the data indicates an intimate link between accumulation of microplastics and delay in germination. To date, very limited data on the occurrence of plastic particles in terrestrial systems is available (de Souza Machado et al., 2018; SAPEA, 2019). Several studies have detected plastic particles in soils (reviewed in SAPEA, 2019), but different approaches were used, and often the focus is relative large particles due to insensitivities of detection techniques (de Souza Machado et al., 2018). Therefore, environmental concentrations of small plastic particles <100 µm are not well known (even in aquatic systems which are much more intensively studies in comparison to terrestrial systems), because standardized procedures for collection, fractionation, characterisation, and quantification are lacking, which results in underestimation especially for smaller particles sizes (Huvet et al., 2016). Concentrations are expected to increase with decreases in particle size, and predicted concentrations of 1 µm particles in aquatic systems range between ~10^2-~10^7 particles·mL^{-1} (Lenz et al., 2016). Accelerating production, deposition and the bioinert character of plastics contribute to further growing environmental concentrations (Horton et al., 2017; Huvet et al., 2016; SAPEA, 2019). It remains to be investigated how soil organisms adapted to an environment consisting of colloids, respond to foreign

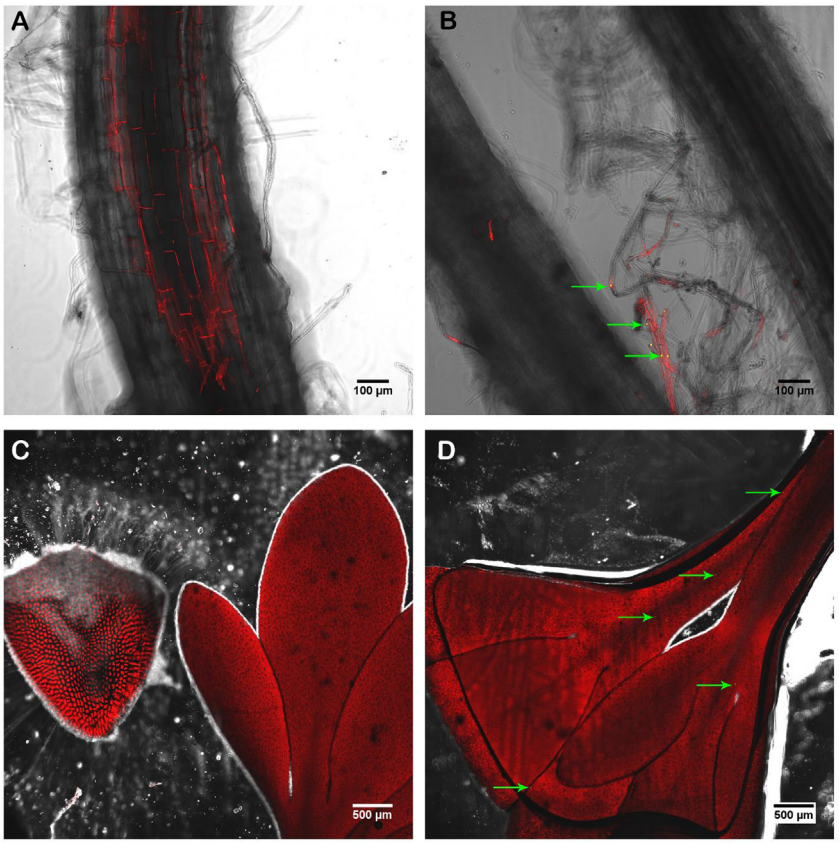

Fig. 5. Accumulation of microplastics (4800 nm) on *L. sativum* seedling after 72 h of development. Image of a stem of a control seedling (A). In exposed seedlings, microplastics (yellow dots at green arrow heads) adhere particularly on the root hairs (B). Image of a leaf of a control seedling (C). A few microplastics are detected as yellow dots (green arrow head) on the leaf of exposed seedlings (D). Propidium iodide (red) was used to stain the plasma membrane. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

particles (such as microplastics) in their natural environment.

In addition to the quantities of particles, the physiochemical properties of different types of polymers and interactions between plastic pollution along with the components of this system remain largely unknown. To this end, there is an urgent need for the further development of standardized sampling-, quantification- and identification methods for the study of plastic particles in soil and terrestrial ecosystems (Bläsing and Amelung, 2018; Costa et al., 2018). This standardization was proven to be very useful when determining levels of microplastics in beach sediment allowing for monitoring pollution levels by non-trained volunteers (Besley et al., 2017; Lots et al., 2017), an approach which could also be used by farmers and governmental institutions that deal with the biosolid applications.

To conclude, we present the first study on adsorption, uptake and the phytotoxicity of nano- and microplastics to a vascular terrestrial plant. The results from the present study demonstrate that plastics particles adsorb particularly on the root hairs. Exposure to plastic particles results in short-term and transient effects on germination rate and root growth. Given our limited understanding of the impact of nano- and microplastics in terrestrial systems, it is key to conduct studies on nano- and microplastics loads, uptake, and effects on terrestrial systems.

Acknowledgements

We gratefully thank Mila Vlottes, Angelo Legrand, Drew Allen, and Lieselot Meelker for advice and suggestions, Eefje de Goede for advice in chlorophyll extraction, Hye Kyong Kim for offering the use of the spectrometer, and Gerda Lamers for assistance in confocal imaging. This research received funding from the Gratama Foundation of the Leiden University Fund (project number 2015–08; provided funds to T.B and M.G.V) and NWO (VIDI 864.13.010; provided to M.G.V and N.R.B). Additionally, we would like to thank the Dopper Foundation Changemaker Challenge for giving additional feedback on the research topic.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.03.163.

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