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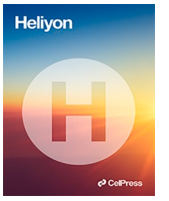

Effects of biodegradable P3HB on the specific growth rate, root length and chlorophyll content of duckweed, *Lemna minor*

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ABSTRACT

The extensive production and use of plastics have led to widespread pollution of the environment. As a result, biodegradable polymers (BDPs) are receiving a great deal of attention because they are expected to degrade entirely in the environment. Therefore, in this work, we tested the effect of two fractions (particles <63 μm and particles from 63 to 125 μm) of biodegradable poly-3-hydroxybutyrate (P3HB) at different concentrations on the specific growth rate, root length, and photosynthetic pigment content of the freshwater plant *Lemna minor*. Microparticles with similar properties made of polyethylene terephthalate (PET) were also tested for comparison. No adverse effects on the studied parameters were observed for either size fraction; the only effect was the root elongation with increasing P3HB concentration. PET caused statistically significant root elongation only in the highest concentration, but the effect was not as extensive as for P3HB. The development of a biofilm on P3HB particles was observed during the experiment, and the nutrient sorption experiment showed that the sorption capacity of P3HB was greater than PET's. Therefore, depleting the nutrients from the solution could force the plant to increase the root surface area by their elongation. The results suggest that biodegradable microplastics may cause secondary nutrient problems in the aquatic environment due to their biodegradability.

1. Introduction

Due to the growing problem of plastic waste and pollution from conventional plastics, there is an increasing interest in producing environmentally friendly products from degradable materials [1,2]. Biodegradable polymers (BDPs) are polymeric materials that can be decomposed under aerobic conditions into carbon dioxide, water, and biomass by the enzymatic action of microorganisms or under anaerobic conditions to methane [3]. Simply put, they become plastics when mixed with additives, which improve their processability and properties. BDPs are being promoted as a viable alternative to conventional plastics in several industrial sectors, some in medicine, others in textile, personal care products, agriculture, and especially as packaging materials [4,5]. BDPs can be made from petrochemicals or renewable materials; they can be polymers produced by extraction from biomass (polysaccharides, polypeptides, lipids), polyesters made by microorganisms and plants or their mixtures [3,6,7].

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Considerable attention is paid to polymers produced from renewable sources, including polyhydroxyalkanoates (PHAs), a group of biodegradable linear polyesters 3, 4, 5, and 6-hydroxy acids, which can be produced under aerobic and anaerobic conditions by various gram-negative and gram-positive bacteria that use them as intracellular reserve materials [8–12]. PHAs were discovered through poly-3-hydroxybutyrate (P3HB) [13,14]. P3HB is a relatively hydrophobic material with a crystallinity greater than 50 %, a high melting point (180 °C), and low elasticity. These properties complicate its processing and limit its possible use on an industrial scale [15,16]. The mechanical properties of P3HB can be improved by various additives, either polymers or plasticizers, or by forming copolymers [17]. However, these additives can have a negative effect on the biodegradation rate of the resulting material [18]. Therefore, substances that facilitate biodegradation, such as starch, are also added to these mixtures. Another disadvantage of P3HB is its high production cost, which can be slightly reduced by using waste materials as feedstock, e.g., used rapeseed frying oil [19]. Biomass, waste products of the sugar industry, or coffee bean hydrolysates can also be used for their production [20].

Similar to conventional plastics, biodegradable plastics can break down into small particles under natural conditions, resulting in large quantities of microplastics [17,21]. Despite their biodegradability, these particles can have a very long life because their degradation is influenced by biotic and abiotic environmental factors such as temperature, humidity, pH, biologically active substances, and the presence and activity of microorganisms [22,23]. Biodegradation processes of BDPs often needs specific environmental conditions, and the biodegradation cannot be completed in many natural environments [24]. BDPs mainly showed high degradability in soil and compost environments. However, this is not always the case of the aquatic environment, for example the degree of biodegradation of polyhydroxybutyrate vary from 30 to 99 % depending on the conditions and the type of water [25].

Microplastics from BDPs have also been found to negatively affect freshwater and marine organisms [26]. In the freshwater amphipod *Gammarus fossarum*, a reduction in biomass gain was observed following exposure to polyhydroxybutyrate compared to the control as a result of their ingestion [27]. The effect of polylactic acid (PLA) microparticles on the marine organism *Arenicola marina* and microalgal biomass on the sediment surface was investigated by Green et al. [29]. There was no adverse effect of PLA on the viability of *A. marina*, but there was a significant decrease in microalgal biomass in PLA-containing sediment compared to the control [28]. However, in general, microplastics from BDPs are largely understudied, so understanding their impacts on the nvironmentt is still limited.

Due to the gradual acceptance of biodegradable replacements for conventional plastics, increased exposure of environmental components by micro-bioplastics can be expected due to their incomplete biodegradation. For this study, we selected P3HB due to its widespread applications and its growing, extensive use in packaging, agriculture, and biomedicine [29]. Further, the price of P3HB production is decreasing [30,31]. Consequently, it can be expected to be widely used in other branches, mainly as a replacement for polypropylene [32]. For those reasons, this study aimed to evaluate the effect of P3HB microplastics on the freshwater floating plant *Lemna minor* and compare the observed results to effects caused by PET microplastics with comparable properties. The effects of microplastics on the specific growth rate, photosynthetic pigments content, and root length are investigated.

2. Materials and methods

2.1. Preparation of microparticles

The P3HB (ENMAT Y3000) microplastics with a spherical shape were used for ecotoxicological tests with *L. minor*. They were obtained as a powder from TianAn Biologic Materials Co., Ltd. (Ningbo City, China). A suspension of P3HB microplastics in MilliQ water was sieved on 63 and 125 μm mesh stainless-steel sieves, and both size fractions (<63 μm and from 63 to 125 μm) were subsequently dried in glass beakers at room temperature in a fume hood.

The PET microplastics were obtained as a powder produced during mechanical recycling from PETKA CZ a.s. (Brno, Czech Republic), repeatedly washed and then prepared similarly to P3HB to obtain the same size fractions.

2.2. Experimental design

The L. minor came from a laboratory culture at the Institute of Chemistry and Technology of Environmental Protection (Faculty of Chemistry, Brno University of Technology, Czech Republic). The plant was cultivated in Steinberg medium [33] under controlled conditions (temperature 23 ± 2 °C, photoperiod 16/8 h).

Due to the lack of standardised protocols for microplastic ecotoxicity testing, there is no accepted procedure for how organisms should be exposed in ecotoxicity tests. Accordingly, we used several approaches to expose *L. minor*. The effects of (i) suspension (without replacement), (ii) suspension exchange during the test, which used Li et al. [34] procedure in tests with TiO_2 nanoparticles, (iii) leachate and (iv) direct weighing (due to surface tension, some insufficiently wetted P3HB particles remained floating on the surface). Tests were conducted with both size fractions.

The suspension (i) was prepared by weighing the desired amount of P3HB and transferring it to a volumetric flask; then, the volumetric flask was filled to the mark with Steinberg medium. The suspension thus prepared was ultrasonicated for 10 min to disperse the particles.

In the case of the exchange test medium (ii), the suspension thus prepared was exchanged twice during the test.

Leachate (iii) was prepared as follows: a suspension of P3HB in Steinberg medium was prepared and then incubated for seven days under the same conditions as the *L. minor* test. The medium was then filtered through a 0.8 μm pore size filter.

In direct weighing (iv), P3HB was weighed in the required amount, transferred to a test vessel, and 100 mL of medium was added.

Since the results (Section 3) showed no significant difference between the preparation methods, the test was performed with PET in

suspension only (i). Also, leachate from PET was not tested because our previous study showed no effects of PET leachate on various endpoints of *L. minor* [35].

The experiments were conducted in 150 mL glass beakers, and each was filled with 100 mL of test suspension, leachate, or medium. The concentration of P3HB microparticles was 0, 10, 50, and 100 mg/L, corresponding to 3.20 10^{13} particles in 1 g for the fraction <63 μm and 2.39 10^{13} particles in 1 g for the fraction <125 μm. The number of particles was determined based on their distribution in suspension (figure S1–S4 in Supporting Information). This concentration range was selected based on our initial tests and previous studies on the toxicity of microplastics to *L. minor* [36]. The PET was used in the same concentrations as P3HB.

Each experiment involved three replicates of the same exposure concentrations was repeated three times and was carried out according to the OECD guideline No. 221 [37] and used in many studies when microplastics were tested [36,38–40]. The initial number of leaves was nine, so more than 50 % of the surface area was available for plant growth. Roots were carefully removed from the plants before the start of the test. This experiment was conducted at a temperature of 24 ± 1 °C, and evaporation of the medium was prevented during the test by covering it with polyethylene foil. A photoperiod of 16/8 h (light/dark) was set at a light intensity of 4300 lx at the plant level, and the duration of the experiment was seven days.

Images of P3HB and PET microplastics were taken with an optical microscope and ocular camera, 10 × /0.25 objective before and after the experiment.

At the end of the experiment, the number of leaves was counted (n = 9), and the average specific growth was calculated (details are given in section 2.6, Data analysis).

2.3. Measurement of root length

L. minor (n = 75) were placed on laminated graph paper and photographed with a Nikon D3100 digital camera and an AF-S Micro NIKKOR 40 mm 1:2.8 G lens (Nikon, Japan). Afterward, the root length was evaluated in the ImageJ program.

2.4. Photosynthetic pigment determination

Photosynthetic pigments (n = 9), chlorophyll *a and b*, and total carotenoids were determined by a modified Arnon method described by Radić and Pevalek-Kozlina [41]. Approximately 30 mg of fresh plant material from each treatment was used for the photosynthetic pigment analysis. The samples were homogenized in 80 % ice-cold acetone (w/v), and the absorbance of the supernatant was read at 470, 642.8, and 646.2 nm.

2.5. Sorption experiment

Both P3HB and PET microplastic fractions were incubated in the Steinberg medium for seven days at the same concentrations (0, 10, 50, and 100 mg/L) and under the same conditions as in the *L. minor* assay. Media samples were collected at 0, 24, and 168 h and filtered through a 0.45 μm pore size nylon filter. The nitrate-nitrogen concentration in these samples was determined spectrophotometrically using a commercial kit (Spectroquant®, Millipore, 109713).

To evaluate the effects of nutrient depletion of the medium, an additional test was also prepared in which the medium contained 5 %, 10 %, 20 %, 30 %, and 40 % less nutrients than the original Steinberg medium. The ecotoxicity test with *L. minor* was then performed as described for P3BH.

2.6. Data analysis

The average specific growth rate after seven days of exposure was calculated according to ISO 20079 (2005) as follows:

$$\mu = \frac{\ln(N_j) - \ln(N_i)}{t}$$

where μ (d^{-1}) is the average specific growth rate, N_j (/) is the number of leaves at the end of the experiment, N_i (/) is the number of leaves at the beginning of the experiment, and t (d) is the exposure time (seven days).

$$\%I_r = \frac{(\mu_C - \mu_T)}{\mu_C} \bullet 100$$

where $\%I_r$ is the percentage inhibition of the average growth rate, μ_c is the mean growth rate for control, and μ_T is the mean μ for specific sample treatment.

The content of photosynthetic pigments was calculated according to Lichtenthaler [42]. Results are given in mg of chlorophyll per gram of fresh plant mass.

The statistical significance of the differences between control and individual concentrations was determined using Dunnett's test in the R program, with differences considered significant if $p < 0.05$.

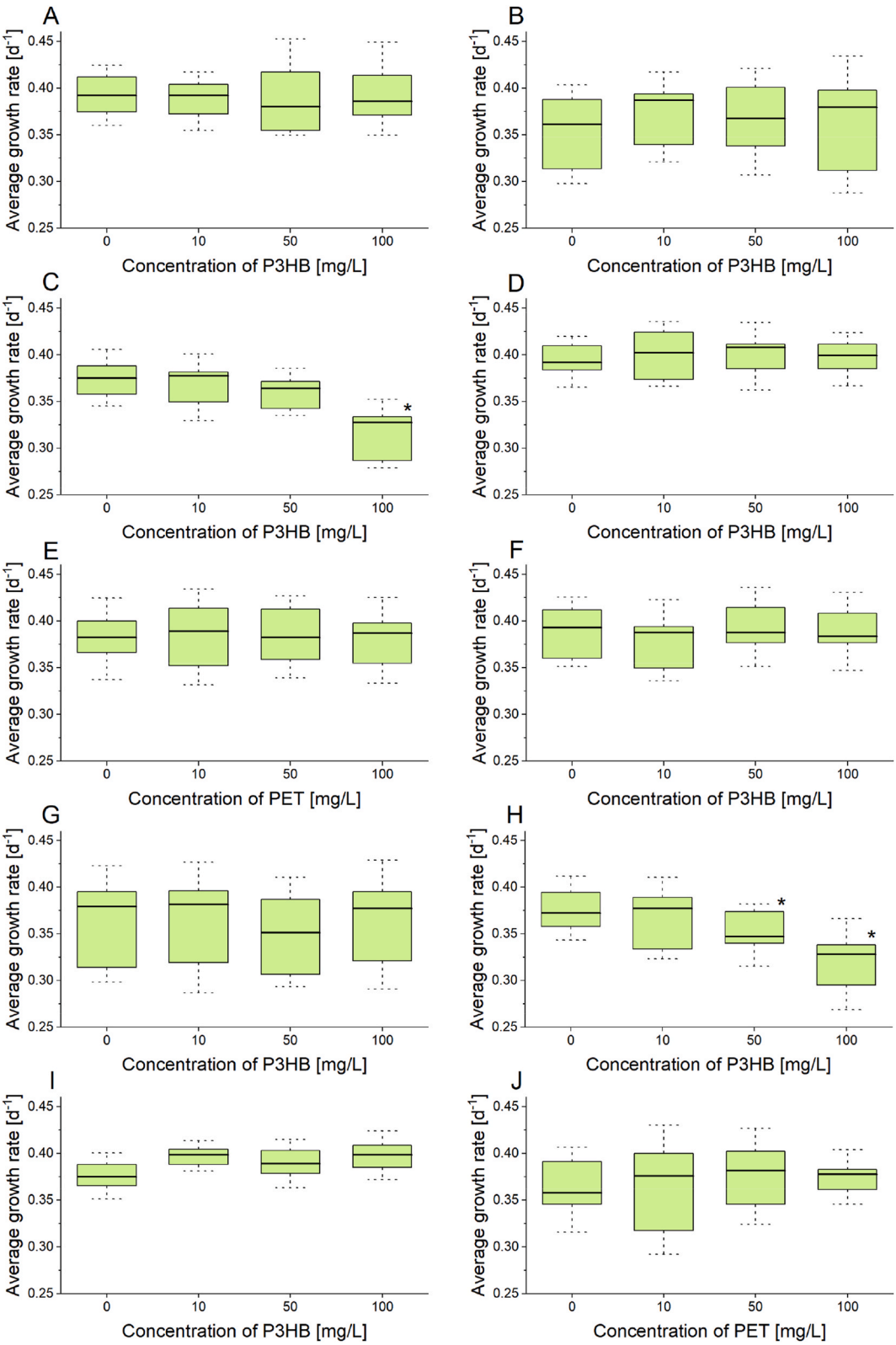

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Fig. 1. Results of specific growth rate: A to E for particles <63 μm (A – suspension (i), B – exchange of suspension (ii), C – leachate (iii), D – direct weighing (iv) of P3HB, and E – suspension of PET) and F to J for particles from 63 to 125 μm (F – suspension (i), G – exchange of suspension (ii), H – leachate (iii), I – direct weighing (iv) of P3HB, and J – suspension of PET). Asterisks denote statistically significant differences compared with the control treatment (p-value <0.05).

3. Results

3.1. Effect of P3HB on the specific growth rate of Lemna minor

The results of this study showed that the specific growth rate of *L. minor* was not affected in the case of suspension test (i), exchange suspension during the test (ii), and direct weighing of P3HB microplastics into the test vessel (iv). Similarly, suspension of PET microplastics did not affect the specific growth rate under those exposure scenarios (Fig. 1).

On the other hand, leachate (iii) from both size fractions of P3HB showed a statistically significant difference at the highest concentrations – 100 mg/L for particles <63 μm, 50 and 100 mg/L for particles from 63 to 125 μm. The specific growth rate inhibition was concentration dependent: leachates from 10, 50, and 100 mg/L caused 4.72 %, 4.01 %, and 15.95 % for particles <63 μm, and 2.79 %, 7.61 %, and 17.69 % for particles from 63 to 125 μm, respectively (Fig. 1).

3.2. Effect of P3HB on chlorophyll content

Both fractions of P3HB microplastics in the different treatments ((i) – (iv)) did not have a negative effect on the content of photosynthetic pigments in *L. minor* leaves (chlorophyll *a and b*, carotenoids). Similarly, PET did not affect photosynthetic pigment content compared to the control (Figs. 2 and 3).

3.3. Effect on root growth

Both P3HB size fractions had statistically significant effects on *L. minor* root growth in all treatments. The effect was concentration-dependent regardless of the preparation of the suspension ([i], [ii], [iv]); the leachates (iii) of P3HB also caused a statistically significant elongation of the roots of *L. minor*.

Similarly, the length of the roots increased with increasing concentration of PET microplastics, but a statistically significant difference from the control was observed only at the highest concentration (100 mg/L, Fig. 4).

3.4. Sorption experiment

To determine the degree to which the presence of microplastics affects the composition of the medium, a series of sorption experiments were conducted on both PET and P3HB. In the presence of microplastic particles <63 μm, there was up to 30 % reduction of the concentration of $N–NO_3^-$ in the case of P3HB, while there was only 12 % for PET (figure S5). For the second size fraction of microplastics (from 63 to 125 μm), the depletion of $N–NO_3^-$ in both cases was slightly higher. Thus, sorption occurs for both types of plastics, but a statistically significant effect on root growth was observed only for P3HB and the highest concentration of PET (Fig. 4). In addition, biofilm growth on the settled P3HB and PET was observed at the bottom of the beaker, where filamentous microorganisms occurred (Fig. 5). However, only in the treatments with P3HB did visual inspection indicate other microbial cells attached to particles (Fig. 5B).

3.5. Effect of nutrient depletion

Additional experiments with depletion of nutrients in the Steinberg medium (containing 5 %, 10 %, 20 %, 30 %, and 40 % fewer nutrients than the control under the same conditions as the P3HB and PET tests) confirmed that with decreasing nutrient concentrations in the medium, there was a slight inhibition of growth compared to the control (the highest was 6.5 % in the sample with the least amount of nutrients, i.e., 40 % fewer nutrients than in the control. No changes in photosynthetic pigment content were observed. However, a statistically significant increase in root length was observed at all concentrations and increased with decreasing nutrient concentrations in the medium (Fig. 6).

4. Discussion

Recent research on microplastics has shown that different types of microplastics can affect growth and development of aquatic plants [43,44]. In contrast, natural particles such as wood or cellulose particles showed no effects [40]. Therefore, we aimed to investigate the effect of bio-based and biodegradable P3HB and compare it with that of non-biodegradable PET. PET was selected for its P3HB-like properties mainly density (1.38 g/cm^3) and wettability – the contact angle of PET varies between 66° and 81°, that is, it is also poorly wettable compared to P3HB [45]. In fact, both particles belong to the group of polyesters, but P3HB is, unlike, a biodegradable polymer. Although, recent work [46] suggested that PET hydrolysis can also occur under specific conditions, e.g., about 4.5 years is needed for 50 % PET degradation under neutral pH in marine water, it can be concluded that the lifetime of P3HB and PET

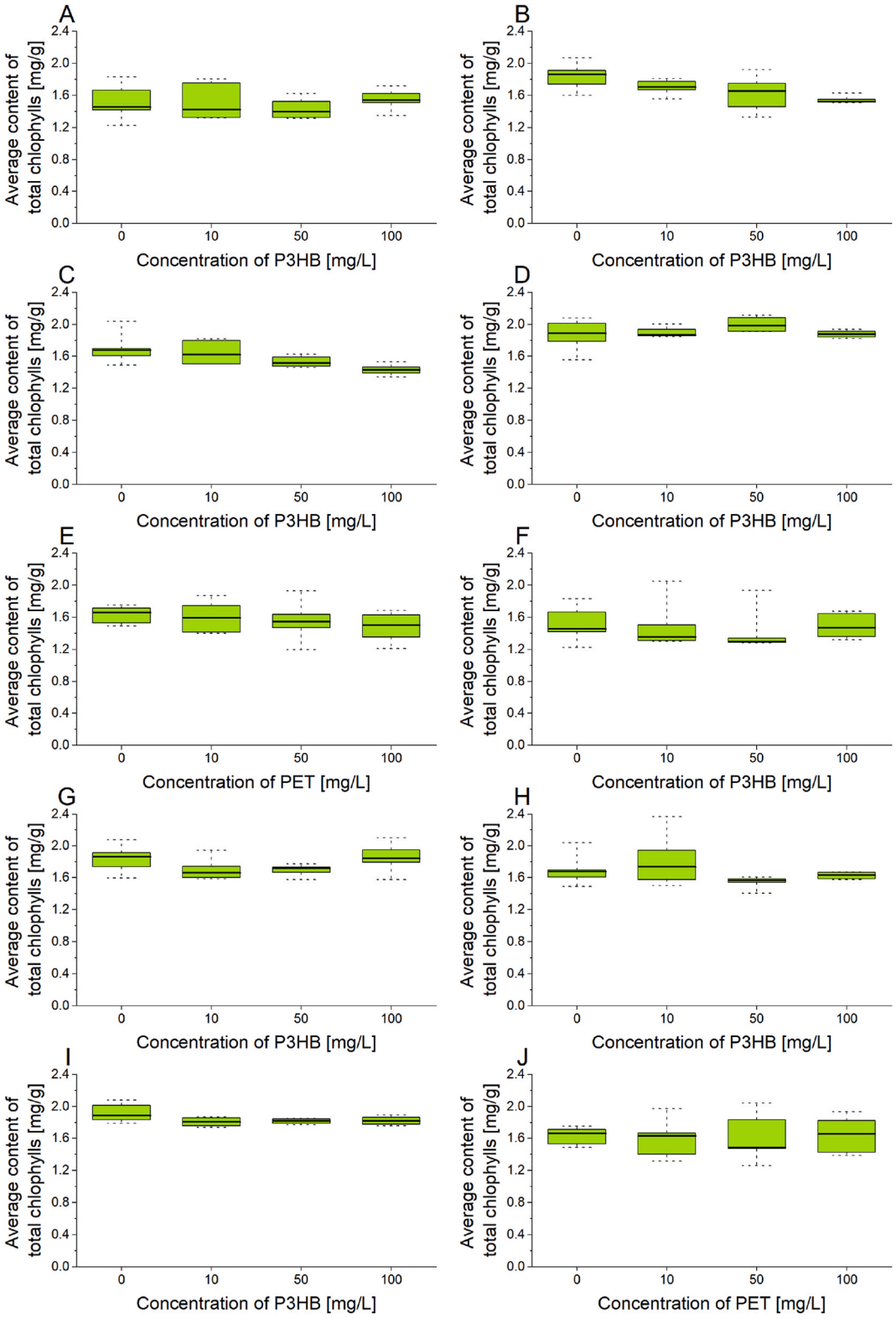

Fig. 2. Results of average content of total chlorophylls: A to E for particles <63 μm (A – suspension (i), B – exchange of suspension (ii), C – leachate (iii), D – direct weighing (iv) of P3HB, and E – suspension of PET) and F to J for particles from 63 to 125 μm (F – suspension (i), G – exchange of suspension (ii), H – leachate (iii), I – direct weighing (iv) of P3HB, and J – suspension of PET).

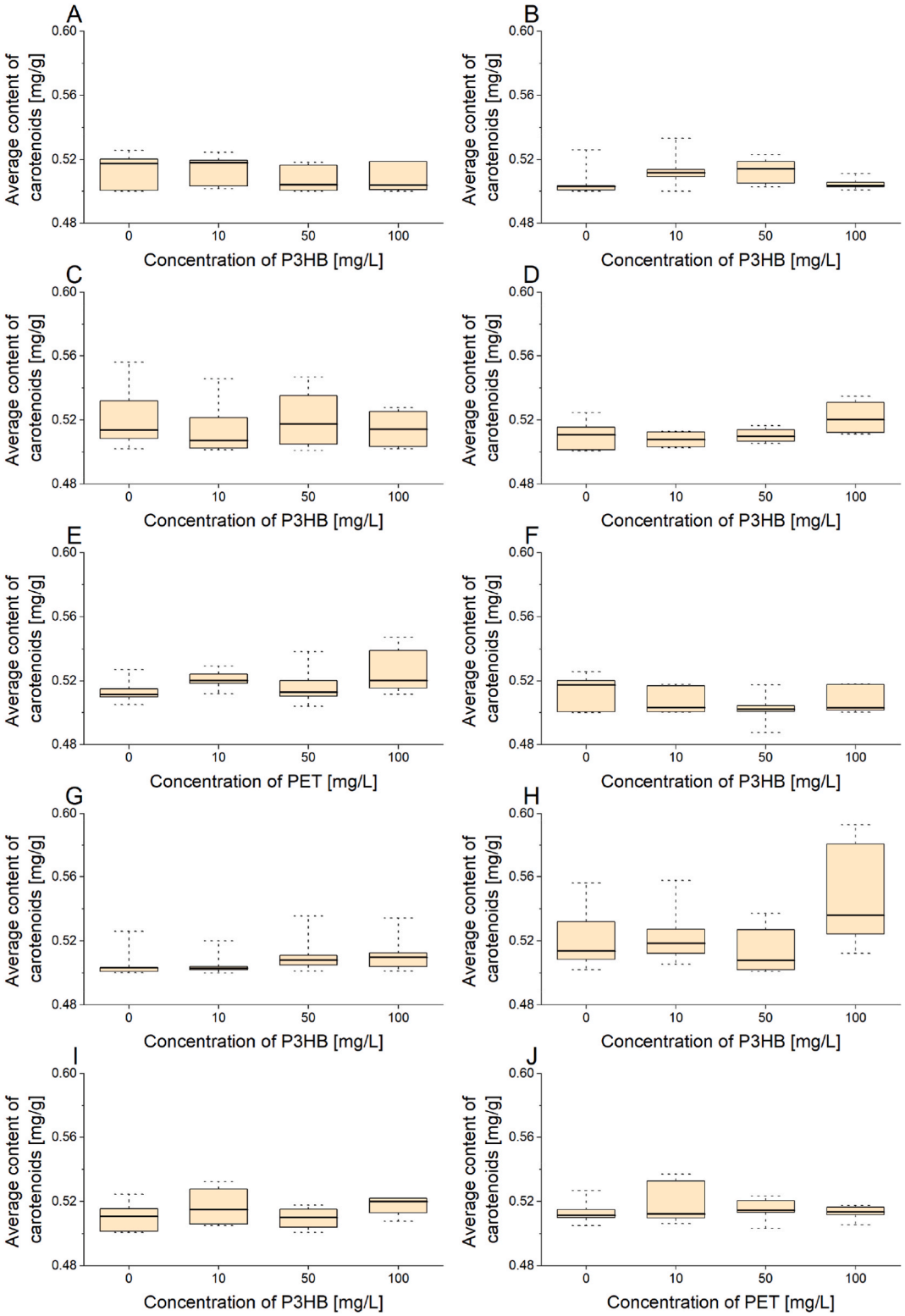

Fig. 3. Results of average content of carotenoids: A to E for particles <63 μm (A – suspension (i), B – exchange of suspension (ii), C – leachate (iii), D – direct weighing (iv) of P3HB, and E – suspension of PET) and F to J for particles from 63 to 125 μm (F – suspension (i), G – exchange of suspension (ii), H – leachate (iii), I – direct weighing (iv) of P3HB, and J – suspension of PET).

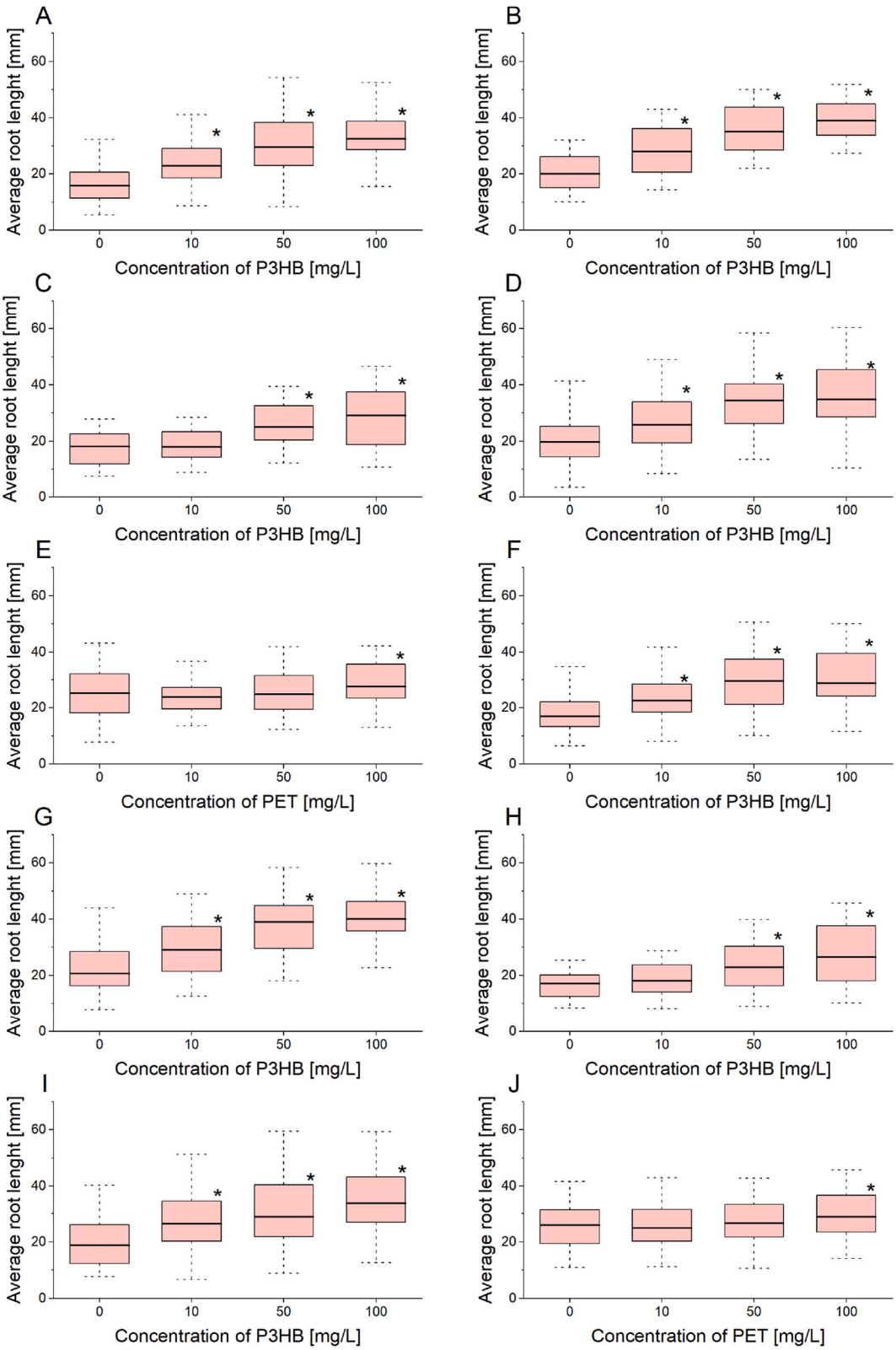

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Fig. 4. Results of measurement of root length: A to E for particles <63 μm (A – suspension (i), B – exchange of suspension (ii), C – leachate (iii), D – direct weighing (iv) of P3HB, and E – suspension of PET) and F to J for particles from 63 to 125 μm (F – suspension (i), G – exchange of suspension (ii), H – leachate (iii), I – direct weighing (iv) of P3HB, and J – suspension of PET). Asterisks denote statistically significant differences compared with the control treatment (p-value <0.05).

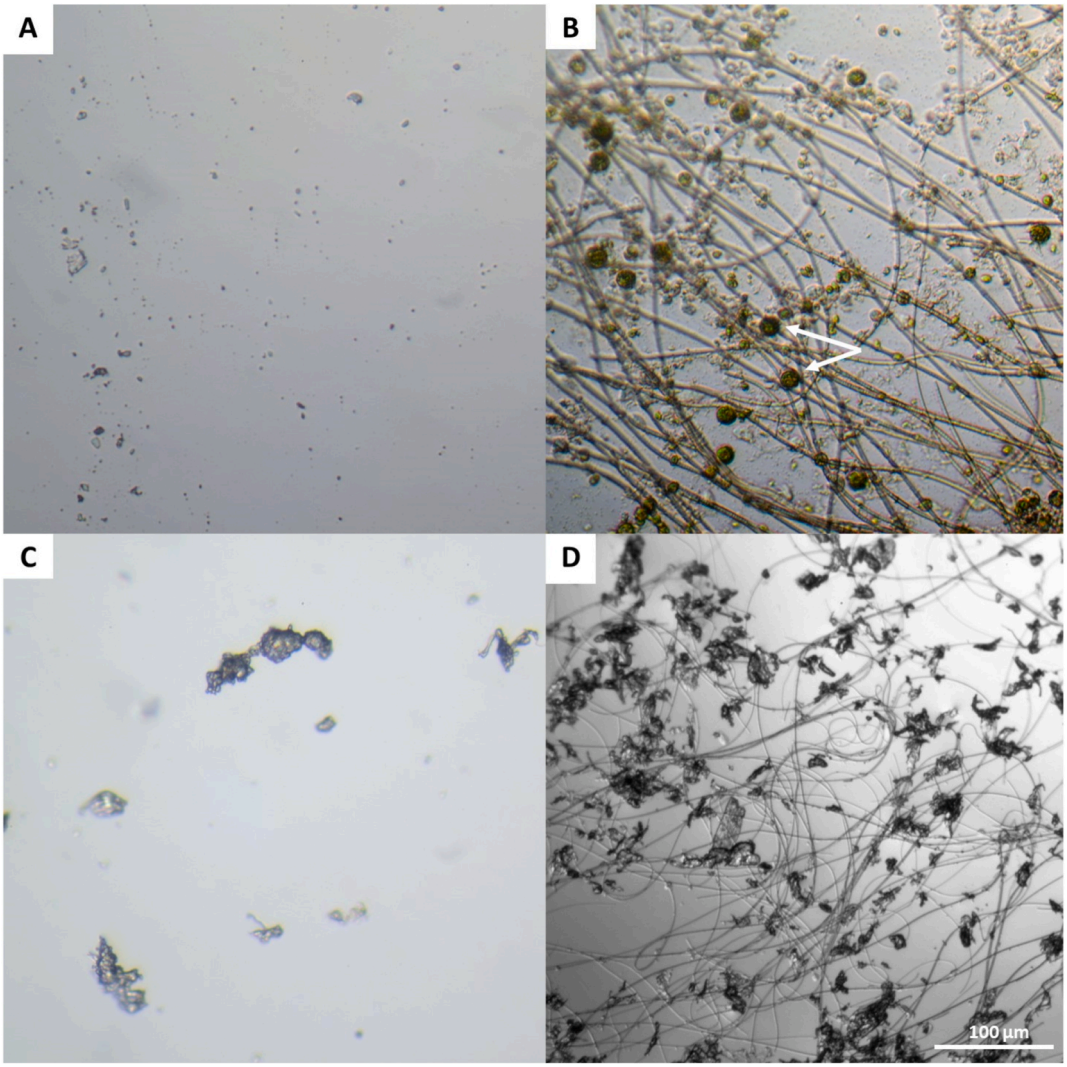

Fig. 5. P3HB particles <63 μm before the test (A) and after seven days of exposure (B), PET particles <63 μm before the test (C) and after seven days of exposure (D). The arrows in Figure B indicate the different types of organisms in the biofilm formed on P3HB microplastics versus PET.

microparticles in natural systems significantly differ.

In general, there are very few studies on the impact of biodegradable microplastics on aquatic organisms; their effects on aquatic ecosystems were not studied until 2015 when Green et al. [28] found that, in some cases, they can negatively affect organisms in the same way as conventional microplastics. Their work compared the effect of PLA, polyethylene (PE), and polyvinyl chloride microplastics on the health and biological activity of lugworms *A. marina* and the nitrogen cycling and primary productivity of the sediment they inhabit. All three types of microplastics, including biodegradable PLA, significantly reduced the biomass of microalgae in the sediment [28]. Su et al. [47] compared the effect of PLA, polybutylene succinate, PE, and polyamide (PA) microplastics (the sizes of each type of microplastic were 57.4, 53.3, 77.8, and 59.9 μm) on the marine algae *Chlorella vulgaris*. PLA showed the most substantial inhibitory effect on the growth of *C. vulgaris*. Interestingly, in contrast to our study, the microplastics appeared to stimulate the contents of photosynthetic pigments (e.g., chlorophyll *a*, chlorophyll *b*, and carotenoids), which was attributed to their ability to act as cellular defences against stress induced by microplastics.

The ecotoxicological research on P3BH is limited, but, for example, tests with secondary P3HB nanoparticles by González-Pleiter et al. [48] demonstrated that with the presence of P3HB nanoparticles (spherical particles with a diameter of around 200 nm, the

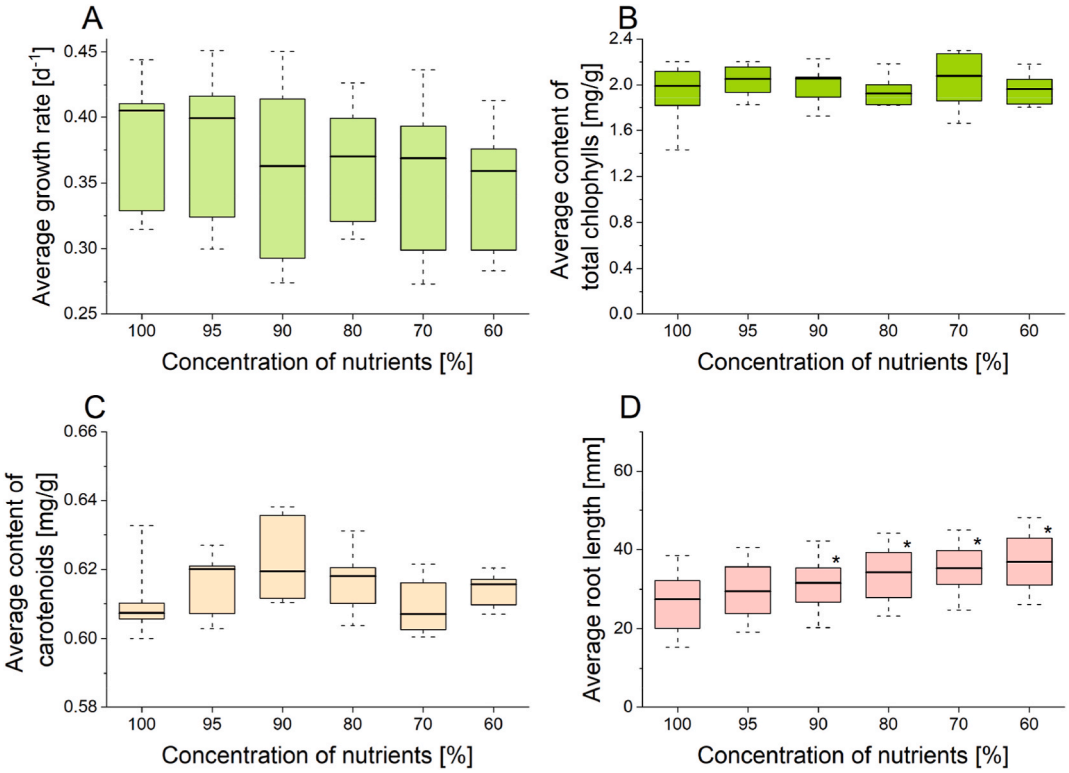

Fig. 6. Results of test with nutrient deficiency: A – average growth rate, B – average content of total chlorophylls, C – average content of carotenoids, D – average root length. Asterisks denote statistically significant differences compared with the control treatment (p-value <0.05).

highest concentration was 100 mg/L) in the medium, there was a significant reduction in the growth of bacteria *Anabaena* (90 %) and algae *Chlorella reinhardtii* (95 %); there was 85 % immobilization of *Daphnia magna* after 48 h. This contradicts our results, which showed no significant acute effect on *L. minor*. This confirms that chemical composition, concentration, and particle properties (and related sorption properties, e.g., towards micropollutants or nutrients) are important ecotoxicological factors in the ecotoxicity testing of micro/nanoparticles.

The results of this study showed limited effects of P3HB microplastics on various endpoints of growth and development of *L. minor*. The minimal effect of P3HB on the specific growth rate of *L. minor* may be caused by the limited contact of the microplastics with the plant biomass. As mentioned earlier, P3HB microplastic particles settled due to their higher density than water (approximately 1.23 g/cm^3) [49], thus leaving enough space on the surface for normal plant growth. Similarly, results of the photosynthetic content analysis suggest a low impact of P3HB microplastics on *L. minor*, as photosynthetic pigments were also not affected by P3HB microplastics. A similar conclusion was reached by Kalčíková et al. [36] when investigating the effect of PE microbeads on *L. minor*. However, P3HB microplastics can be temporarily resuspended in the water column because of turbulence, water flow, or during thunderstorms [50]. The contact angle of P3HB is reported to be between 70° and ~81°, making it slightly hydrophobic [45]. Hydrophobic material naturally tends to separate from water molecules [51]. Accordingly, before the surface of P3HB microparticles is wetted and begins settling, they can float on the water surface and be adsorbed to the leaves and roots of floating plants.

Furthermore, no differences were found between the exposure scenarios (the ways that *L. minor* was exposed to microplastics), so we concluded that using microplastic suspension (i), microplastic suspension exchange (ii), and direct weighing (vi) are appropriate methods for microplastic testing.

Although there was no effect on growth rate and photosynthetic pigment content, P3HB microplastics affected root growth and caused root elongation. A lack of nutrients can cause root length elongation, and consequently, the ability of the root system to nourish increases – the foraging capacity [52]. For example, Cedergreed and Madsen [53] reported that high concentrations of nutrients lead to the shortening or the complete absence of *L. minor* roots, while low levels of nutrients and trace elements lead to elongation, as shown in Fig. 6. As the L. *minor* is a plant with no lateral roots but only one main root, extending the roots is the only way to increase the root area to absorb nutrients from the medium. No other changes (thinning of roots, reduction of root cell viability) were observed during this research. Based on the results of this study, it is plausible that root elongation could be a result of nutrients depletion either 1) due to nutrient sorption on P3HB microplastics as reported by He et al. [54] or 2) the effect of biofilm formed on the surface of the biodegradable polymer.

Both adsorption of nutrients and biofilm development proceeded differently on P3HB and PET microplastics, although they have equal plastic wettability [45] and comparable sizes. P3HB adsorbed more $N–NO_3^-$ than PET (figure S5), and a significant effect on roots

(elongation) was observed regardless of the P3HB concentration tested. In contrast, the PET microplastics only caused statistically significant root elongation at the highest concentrations. This difference may be due to the fact that P3HB is biodegradable and thus more suitable for biofilm growth than PET (Fig. 5). This supports the uptake of nutrients on P3HB particles compared to PET (figure S5). This is significant mainly for those particles below 63 μm. As can be seen in figure S5, particles between 63 and 125 μm showed a similar trend. We speculate that this may be caused by a change in the specific surface area caused by more extensive aggregation of P3HB particles. In principle, biofilm formation modifies the surface wettability, thereby promoting the rate and extension of the aggregation. In other words, in the presence of denser biofilm on P3HB (Fig. 5), the aggregation is caused by charge-charge interactions. In contrast, in the case of PET, it is caused mainly by the aforementioned separation from water. As a result, the sorption of nutrients by P3HB decreased. Further, it also seems that the biofilm characteristics on PET and P3HB were different (Fig. 5), which influenced the aggregation behaviour of both plastic particles.

The reason for the development of the biofilm on P3HB can be attributed to their biodegradability, as they represent a carbon source for microorganisms and thus are degraded in the aquatic environment [55]. The formation of biofilm on microplastics can significantly alter their properties and, in some cases, even increase environmental risks and ecotoxicity. Microbial attachment to the surface of plastics and biofilm formation can occur within a few days [56]. It can affect properties such as hydrophobicity, density, functional groups, size, surface area, the roughness of microplastics, and their physical and chemical behaviour [57]. For example, the attached biofilm could reduce the hydrophobicity of PE [58,59] and increase the specific surface area and, thus, the adsorption of other pollutants [57]. In addition, the surface of microplastics can also concentrate nutrients dispersed in the surrounding environment to provide sufficient nutrients for biofilm formation [60]. This aligns with the results of Chen et al. [61], who reported that microplastic biofilms could potentially affect the N and P cycles – the N cycle by enhancing denitrification capacity and the P cycle by sorption and microbially mediated P transformation. N and P can be assimilated into and released to the surrounding waters via biofilms, depending on their developmental stage and environmental conditions.

In addition, the specific growth rate of *L. minor* was statistically significantly affected in the test with leachate for both size fractions. This might be caused by chemicals that may be leached from the microplastics during the incubation period (e.g., chloroform for the extraction of P3HB (lysis of cells during P3HB production) [11]. Still, it is more plausible that the effect was also related to nutrient depletion, as it is credible that nutrients were adsorbed to P3HB and utilized by the biofilm during the preparation of the leachate, so the leachate used for the ecotoxicity test had a much lower concentration of nutrients that affected the root growth and induced root elongation.

A fundamentally similar, though not identical, problem with the availability of nutrients for plants in soil is also mentioned in the work of Brtnicky et al. [62]. The authors found that adding P3HB to the soil increased microbial activity due to the preferential use of P3HB as a carbon source. This leads to soil nitrogen depletion around the plant roots, strongly inhibiting plant growth. However, soils are a more microbially active environment than water due to the significantly higher number of microorganisms present. Therefore, abiotic factors such as sorption can be involved in the competition for nutrients between plants and microorganisms in the aquatic system. Even in terrestrial plants, the root: shoot ratio increases due to nitrogen deficiency, and lateral roots shorten, while high concentrations of N–NO_3^- in soil solution inhibit root growth [63]. Kul et al. [64] also state that nitrogen deficiency reduces chlorophyll in the plant leaves and reduces flowering, fruiting, protein, and starch content. In our case, changes in the content of photosynthetic pigments in leaves were not observed; in most cases, there were no changes in the growth of *L. minor*. Thus, it seems that the plant adapted to the reduced content of nutrients in the medium by root elongation, and no acute effect was observed. Accordingly, focusing on the long-term effect of P3HB microparticles on *L. minor* is important.

In submerged rooted plants, which can take up nutrients through both roots and shoots, nutrient uptake by roots from sediment depends on nutrient availability in the water. In contrast, floating plants are unique because roots and leaves receive nutrients from the same source [53]. Root length measurements may be more sensitive than other endpoints (growth and biomass), such as exposure to submerged macrophytes to pesticides [65]. Roots are more critical in nutrient uptake when availability is low, and leaves contribute more at high nutrient concentrations [53]. From an ecological perspective, the increase in the length of the *L. minor* root in the presence of P3HB microparticles is not a large problem for the plant in the short term. Importantly, however, this effect indicates a reduction in nutrient concentration in the water. Nutrients are essential for the growth of algae and aquatic plants, which are a food source for many small invertebrates and fish.

5. Conclusion

This research showed that the presence of P3HB microplastics had no adverse effects on growth or the photosynthetic pigment content in leaves. The only effect observed was an increase in root length with increasing concentration of P3HB, which was shown to be related to the depletion of nutrients from water, but this effect may be transient especially during the initial rapid biodegradation. Furthermore, we speculate that in the short term, *L. minor* can compensate for the shortage by root extension. Even so, *L. minor* serves as a food source and habitat for other organisms, and it is difficult to predict how the lack of nutrients would affect it in the long term. Finally, if P3HB or other rapidly biodegradable plastics are used in marine applications, it is also important to understand that rapid biodegradation could negatively affect aquatic ecosystems and lead to nutrient depletion especially if used in high concentration. For those reasons, the ecological implications of biodegradable plastics should be further investigated.

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Data availability statement

Data will be available on request, for more information contact corresponding author.

CRediT authorship contribution statement

Petra Procházková: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sabina Mácová:** Investigation. **Seçil Aydın:** Investigation. **Helena Zlámalová Gargošová:** Writing – original draft, Conceptualization. **Gabriela Kalčíková:** Writing – review & editing, Writing – original draft, Conceptualization. **Jiří Kučerík:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

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