Effects of bio-based microplastics on plants and earthworms in the microcosm experiments

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Introduction

Due to the favourable physicochemical properties similar to the common thermoplastics and biodegradability polyhydroxyalkanoates (PHAs) seem to be a potential alternative for petroleum-based plastics (Ganesh Saratale et al., 2021; Chong et al., 2021). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is one of PHAs widely used in agriculture and other applications such as shopping bags, utensils and containers (Chong et al., 2021, Brown et al., 2023). What is more, PHBV is susceptible for the microbiological decomposition and can be synthesized from various waste materials (Brown et al., 2023). Its production is expected to be increasing next years (Chong et al., 2021).

Both conventional as well as bio-based plastics may enter into the soil in a variety of routes such as application of polymer based slow release fertilizers, composts and sludges, plastic mulching, wastewater irrigation and atmospheric deposition (Wu et al., 2021). It was proved that plastic particles (micro- and nanoplastics) might affect the biotic part of soil ecosystems. The majority of the studies that have been carried out so far concerned conventional plastics. However, it was shown that bio-based and biodegradable plastics might also exert negative effects on soil organisms (Qi et al., 2018, Balestri et al., 2019).

Taking into account that microplastics belong to the most ubiquitous pollutants of the soil compartment and that the quantity of bio-based plastics introduced into the market is increasing, it is worth to assess the potential impact of bio-based plastic on soil biota. In this work an innovative PHBV-based plastic material was tested towards its effect on higher plants and earthworms at the community level in the microcosm experiments.

Materials and methods

PHBV-based polymer was selected for testing within the realisation of Bio-plastic Europe Project (Horizon 2020). It contained more than 80% PHBV. According to the nomenclature adopted in the project this was BPE-T-PHBV (Bio-Plastic Europe - Toys - Poly(3-HydroxyButyrate-co-3-hydroxyValerate)) provided by NaturePlast SAS (NP, France) in the form of microparticles.

The microcosm experiments were conducted in the small-terrestrial model ecosystems (STMEs) of total volume 4.3 l. The construction of STME was previously presented by Liwarska-Bizukojc (2022). For 28 days of the experiments each STME was incubated at 20±0.5°C with a 16/8 h light dark regime and the relative humidity ~40% in the acclimatisation chamber FITO 700 (Biogenet, Poland).

A 4 kg of the properly prepared OECD soil containing or not containing (control test) the bio-based plastic tested was gradually added to the STME. The concentration of microplastics in the soil was 2.5% w/w. Then, ten depurated earthworms *Eisenia andrei* were located at the soil surface. After the earthworms had buried themselves in the soil, the seeds of two plants *Sorghum saccharatum* and *Lepidium sativum* provided by Microbiotests (Belgium) were sown. Six seeds of each plant were sown in each STME.

At the end of experiments the percentage of seed germination, mass of fresh shoots and length of shoots, dry matter of shoots, the mortality of earthworms, the fresh mass of depurated earthworms and the earthworm depth distribution in the STME were determined. Moreover, the elemental composition (carbon, hydrogen and nitrogen) of dry matter of plant shoots was determined with the use of an elemental analyzer NA-2500-M (CE Instruments, Hindley Green, UK).

Results and discussion

The percentage of seed germination was lower in the tests with bio-based plastic BPE-T-PHBV in comparison to the control tests. With regard to the dicotyledonous plant *L. sativum* this difference was small (94.4%±9.4 in the tests with bio-based plastic vs. 100%±0 in the control test) and the required level of germination indicated by the manufacturer, i.e. 70%, was significantly exceeded. The seeds of *S. sacharatum* germinated worse than those of *L. sativum* in the performed experiments. In the control run the germination percentage of *S. sacharatum* was at the level 78.8%±2.8, while in the tests with plastic particles reached 66.7%±6.3. At the same time the presence of PHBV-based plastic microparticles did not inhibit the growth either cress or sorghum. The shoot lengths of both plants were higher in the tests with microplastics than in the control tests (Table 1). The same was found with regard to the fresh mass of shoots (Table 1).

Table 1. Effect of BPE-T-PHBV on shoot growth of two higher plants: *Sorghum saccharatum* (SOS) and *Lepidium sativum* (LES).

Tested material	Mean shoot length of individual SOS (cm)	Mean fresh mass of individual SOS shoot (mg)	Mean shoot length of individual LES (cm)	Mean fresh mass of individual LES shoot (mg)
BPE-T-PHBV	18.1±1.2	171.76	5.4±1.1	120.82
Control	12.8±4.4	119.88	5.1±1.1	116.83

The content of dry matter in the plant biomass was higher in the control tests in comparison to the tests with BPE-T-PHBV microparticles. In the case of sorghum the difference between the control tests and bio-based plastic tests was relatively small, i.e. 3.36%, and it was not statistically relevant ($p>0.05$). With regard to cress it reached 9.00% and it was statistically significant ($p=0.00017$). The content of carbon, hydrogen and nitrogen in the plant shoots, sorghum as well as cress, exposed and not exposed to BPE-T-PHBV microparticles was at the similar level. No statistically relevant differences ($p>0.05$) in the elemental composition (CHN) between the control tests and the tests wit bio-based plastic microparticles were found. The value of C:N ratio was at the level of 16.4 and 15.5 for sorghum and 11.8 and 11.3 for cress in the control runs and BPE-T-PHBV runs, respectively. All earthworms survived the microcosm experiments. Their body mass decreased after 28 days because no additional food apart from the organic matter present in the soil was added. The vertical distribution of earthworms in the STMEs differed between the control and bio-based plastic tests. The presence of BPE-T-PHBV microparticles in the soil favoured the downwards movements of earthworms to the bottom soil zone. Thus, about half of earthworms (40-50%) were found in this soil zone in the tests with bio-based plastic particles, while in the control tests it was no more than 10%.

Conclusions

PHBV-based plastic does not affect the germination and growth of terrestrial plants. The elemental composition and C:N ratio of plant biomass are not affected by this microplastic, too. At the same time it contributes to the decrease (9%) in the dry matter content of one of plant species, i.e. *L. sativum*. The presence of PHBV-based plastic does not cause to the mortality of earthworms, whereas it affects the depth distribution of these organisms in the soil. The downward movement of earthworms in the tests with bio-based plastic indicates on the avoidance behaviour under unfavourable living conditions.

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