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Review

Soil microplastics – current research trends and challenges: preliminary results of the earthworm *Eisenia fetida* impact on glitters

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This paper shortly introduces the topic of soil microplastics by presenting a comprehensive review of the current state of the art in this field. It focuses mainly on the role of primary microplastics, particularly glitters, due to their large surface-to-volume ratio. From conclusions about current challenges and research directions, after analysis of the crucial "knowledge gaps", one can point out the lack of a detailed description of the influence of microplastics on biota. The earthworms are promising model organisms that may play in soil ecosystems a similar role to the bivalves in waters. Thus, this mainly review paper was enlarged by the preliminary results of studies on glitters and bioglitters naturally aged in milli-Q water and soil with *Eisenia fetida*. Fourier-transform Infrared (FTIR, microscopy in the reflectance mode) and Raman spectroscopy (780 nm) were used to identify the subsequent notable changes in studied materials. The presence of the polymer (PET) core in the standard glitter particles is confirmed. In addition, the leakage of dyes from bioglitters was observed within the 9-week experiment. Tested bioglitters decompose in a slightly different way. Blue and pink pigments had entirely different stability as only one disappeared without a trace under UV light (blu), and the other remained stable in solution, confirmed by UV-Vis spectra. The nephelometry shows sedimentation of glitters after turbulent agents in the environment. This paper presents the natural weathering of primary microplastics in the presence of *Eisenia fetida*.

Keywords: *Eisenia fetida*, glitters, soil microplastics, Raman spectroscopy, primary microplastics

1 Introduction

Microplastics (MPs) (Galloway & Lewis, 2017) and nanoplastics (NPs) (Gonçalves & Bebianno, 2021) in the environment are currently one of the most interdisciplinary and extensively studied research topics (Ali et al., 2021). Although during last years, marine microplastics (Fossi, Akdogan & Guven, 2019) have drawn much more attention due to the global ocean being the final destination of anthropogenic pollutants (Gonçalves & Bebianno, 2021), it is the soil environment to be probably under the highest pressure (Ng et al., 2018). According to recent estimations, only biosolids can be a source of 2.8–63 t·ha^{-1} of nanoplastics. Therefore, one can assume that sludge and water from the wastewater treatment plants carry the following microplastics in a single year: 63,000–430,000 t in Europe, 44,000–300,000 t in North America and 2,800–19,000 t in Australia. Moreover, 79% of ~6, 00 million t of plastic wastes is deposited on landfelds and constitutes the aboundant secondary source. Among many sources of microplastics one should focus on the primary ones (Praveena, Shaifuddin & Akizuki, 2018) due to their large surface to volume ratio and potentially higher mobility, biocompatibility, and ability to form a substrate for biofilm, toxicity, and sorptive capacities. This paper will focus on the impact of the soil environment and *Eisenia fetida* on the natural aging of glitters, one of the most typical primary microplastics. However, for a long time, they were nearly neglected in studies (Yurtsever, 2019). The complete picture of interactions includes the reverse interaction and substantial changes in soil structure when contaminated by plastics, animals' possible response, and their role in the fate of microplastics in soil. This short review will cover all those aspects in the following paragraphs.

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1.1 Soil microplastics

Although, in 2018, more than one-third of scientific papers were related to marine microplastics (~38,3%) and only <4% considered the plastics on land (He et al., 2018), this proportion is rapidly changing due to the confirmed effects of MPs and NPs on agrosoils (Tian et al., 2022). For instance, the presence of high-density polyethylene (HDPE), polylactic acid (PLA), and multiwall carbon nanotubes (MWCNTs) can influence the bacterial communities' structure and geochemical properties of soils, altering their pH, carbon content, nitrogen and phosphorus cycle (Wang et al., 2022). Similarly to marine microplastics, soil ones are a challenge in their sampling, monitoring, purification protocols, standarization of methods, identification on a large scale, and estimations of toxicity. They also have not fully understood the mechanisms of MPs transport, degradation and fate in the environment. However, some aspects are unique and worth a more extensive discussion. One can claim that MPs in soils occur 4–23 times more than in the ocean. Firstly, the main sources of MPs in soil ecosystems (Ya et al., 2021) are related to mulching and polymer film leftovers on the fields (especially of the low-density polyethylene, LDPE), sludge, wastewater irrigation, industrial dust and atmospheric deposition with particles from the car tyres abrasion included (Lackmann et al., 2022). The positive effect of aerofoils has made LDPE mulching popular. It makes soil warmer and constraints evaporation. It is estimated that only in China (2011), more than 20 million hectares were mulched and those in North-West China contributed to one of the significant contamination related to MPs. In Qinghai province, 240–3,660 particles per 1 kg were detected (Lang et al., 2022). Mulching is mainly done with LDPE. However, some biopolymers are tested instead, such as the PLA/PHA (polylactic aide, polyhydroxyalkanoate), PBAT (polyadipinian co-terephthalate butyl) or mixtures of starch and polyesters. Among primary sources, one can distinguish fibres from synthetic clothes and abrasive particles (mainly polypropylene, PP) from peelings and scrubs. Secondary sources are derived from macro debris on the land fields and fragmented due to the UV radiation, mechanical interactions, and chemical and biological gradual decomposition during natural aging. An interesting example typical for soils is related to nylon fibre in garden mowers. One can estimate it to be the source of at least 6,000 up to 11,000 particles of MPs per minute (Luo et al., 2022). Recently the attention is drew also to natural fertilization as river sludge carries the load of freshwater contaminants. In fields treated by residues of waste water treatment plants a lot of synthestic fibres were found (Schell et al., 2022). Dominant types are plypropylene, polyesters, polyethylene. The concentration of 16 $mg.kg^{-1}$ of dry soil was reached. Light soils are more prone to vertical particle migration, leading to further distribution, as provided in the preliminary tests.

1.2 Consequences of MPs presence in soils

Contrary to marine microplastics, synthetic particles in soil are directly disposed of in the vicinity of the production chain, particularly integrated into cops from agrosoils (Sajjad et al., 2022). That enhances the probability of impact on humans. Nanoplastics can even be transported directly to tissues and accumulate inside plants. They directly influence the system soil-plant by the impact on the roots system, the velocity of photosynthesis, type of biofilm, carbon cycle, and transport of nitrogen and phosphorous. The observed effect is more visible in the case of smaller than larger particles due to the enlarging surface-to-volume ratio and the crucial role of the hydrophobic interphase. Moreover, all synthetic materials can release added compounds, such as plasticizers, softeners, stabilizers, flame retardants, dyes, catalysts or monomers. Some of those are highly toxic – for instance, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochloride pesticides (DDT, HCH). One more negative effect is the synergy of xenobiotics or higher contamination risk by adsorbed pollutants, including metals (As, Cd, Pb, and Zn). Furthermore, the presence of MP at high concentrations substantially changes the soil structure, water capacity, and grain size distribution (Guo et al., 2020). Thirdly, the presence of diverse bacteria and fungi capable of biodegradation is not to be neglected as one of the crucial features of soils. Their destructive effect has been confirmed on PE (*Arthrobacter* sp., *Streptomyces* sp., *Stenotrophomonas* sp., *Comamonas* sp., *Delftia* sp., *F. falciforme*, *F. oxysporum*, *F. lilacinum*, and *Aspergillus flavus*), LDPE (*Bacillus tropicus*, *Stenotrophomonas* sp., *Achromobacter* sp., *Aspergillus oryzae*, *Streptomyces* sp., *Nocardia* sp., and *Rhodococcus* sp.), HDPE (*Achromobacter xylosoxidans*), PS (*Pseudomonas* sp., *Cephalosporium* sp., *Mucor* sp.). Among factors influencing the biodegradation, one can point out the molecular weight (it is advisable to keep it <1,000 $mol.dm^{-3}$), not branched polymer chain, and functional groups as a starting point of enzymatic reaction (mainly ester, amid, ether), high content of amorphous phase, hydrophilic surface, its roughness, water absorption. MPs can be biomineralized to CO_2 and CH_4 or CO_2 and H_2O in anaerobic or aerobic conditions, respectively. Also, the presence of earthworms influences MPs' stability, fate, transport, and behaviour, which will be further discussed in one of the following paragraphs. Finally, the long-term perspective and threshold where

the system will spontaneously fail to balance are still uknown and represent critical challenges for future studies.

1.3 Glitters as a benchmark primary microplastics

Glitters perfectly fulfil all requirements for typical primary microplastics in the environment: they have linear dimensions <5 mm already in the production stage, are abundant and thrown directly into the environment (with sludge, wastewaters, dust, and litter), are polyphase materials composed from the polymer core (usually PET) and shinning aluminium layer covered with acrylic lattice. Sometimes other copolymers, organic and inorganic compounds, can be found. The chemical composition can be complex, and it is worth characterizing it with an excellent spatial resolution, for instance, using the FTIR with the synchrotron source (Vernoud et al., 2011). Surprisingly, glitters were nearly not studied for such a long time. Although perfectly known and used in forensic science (Najjar & Bridge, 2020), their environmental fate only recently drew attention (Tagg & Ivar do Sul, 2019). Those shiny particles, not to be confused with shimmers based on mica, can vary in shapes and sizes (50–6,500 µm). However, they are usually hexagonal with a diameter of ~200 µm. It is estimated that 1 g can be a source of nearly 50,000 microplastic particles. In their composition, one can find PET, PBT, PCV, SBC, PS, epoxy, aluminium, and Fe_2O_3. Glitters are considered inert for health, not including accidents like ingestion (Dela Cruz, Fukui & Hudgins, 2018), but probably influence the environment, especially when having irregular shapes (Green et al., 2021). That is relevant also to bioglitters that are getting more and more popular and claimed neutral being produced from cellulose, regenerated modified cellulose, mica, synthetic mica or polyhydroxyalkanoate (PHA), poly(lactic acid) (PLA). However, further studies are needed to clarify issues of their potential ecotoxicity. Thus, this paper chose glitters as model microplastics for the soil-worms-MP tests.

1.4 Variety of interactions between Eisenia fetida and microplastics

Considering the problem of mutual interactions between plastics and earthworms, one can point out the three crucial aspects. Firstly, the natural degradation of materials can be further triggered by microbial taxa in the guts of animals. For example, it was already proven that selected bacteria from the guts of *Lumbricus terrestris* contributed to the aging and decomposition of low-density polyethylene (Huerta Lwanga et al., 2018). Gram-positive bacteria *Brevibacillus borstelensis* and *Rhodococcus rubber* can shorten the carbon chain with an effect already visible after 3–9 months. Only 4 weeks were needed for *Microbacterium awajiense*, *Rhodococcus jostii*, *Mycobacterium vanbaalenii*, *Streptomyces fulvissimus*, *Bacillus simplex*, and *Bacillus* sp. to reduce the size of LDPE efficiently. The byproducts were observed: volatile alkanes such as octadecane, docosane, ticosane, and icosane. *Eisenia andrei* decomposed the PE (180–300 µm) into nanoplastic (Kwak & An, 2021).

Secondly, glitters and other microplastics in soil substantially change their water loading capacity, hydrophobicity, and grain structure. Due to that, visible results in plant cultivation can be observed. Moreover, the traces of foils left in soil are an abundant source of microplastics. All in all, microplastics change the environment, which causes at least an effect on animal behaviour. It is primarily seen in their burrows morphology and topography. MPs are transported vertically (being at the same time separated accordingly to their dimensions) by earthworms, similar to seeds (McTavish & Murphy, 2021a), and incorporated into the walls of their burrows (Huerta Lwanga et al., 2017). That makes them more available for the adsorption of other xenobiotics using the same transport paths. Moreover, actively transported MPS are more prone to biodegradation and influence the biofilm in deeper layers. To better understand the transport of MPs, 2D and 2D mapping of burrows are helpful (Dittbrenner et al., 2011), assisted by computer tomography (Rogasik et al., 2014). Biodegradable materials can be also ingested by *Lumbricus terrestris* (Zhang et al., 2018).

Thirdly, the potential ecotoxicological effect is not excluded as the interaction is mutual. One can list the following documented effects on *Eisenia fetida*, *Eisenia andrei*, or *Lumbricus terrestris*: damage to tissues and digestive tracks, immunological stress, higher mortality, and reduced reproduction and growth. For instance, PE harmed spermatogenesis and comeloocyte viability in *Eisenia andrei* (Kwak & An, 2021). In the presence of polystyrene (PS), the accumulation of toxic copper and semimetals increased and induced an immunological response (Xu, Yang & Yu, 2021). Bioaccumulation of synthetic fibres with long retention in digestive tracks was confirmed for *Lumbricus terrestris* (Lahive et al., 2022). In the presence of MPs, the dufulin ($C_{19}H_{22}FN_2O_3PS$) effect was enhanced, causing the altering of metabolic paths in *Eisenia fetida* and provoking oxidative stress (Sun et al., 2021). Interestingly, also green polymers are not inert and can influence behaviour. Strong avoidance of MP in concentration >40 $g \cdot kg^{-1}$ was reported (Ding et al., 2021).

2 Material and methods

2.1 Materials

The following sources of primary microplastics were used: bioglitters Sizzix (pink with number 663881 and blu with number 663879), standard red glitter used for decoration of fugues (distributed by e-chińczyk.pl, PID 1699), polyethene (PE) from Sigma Aldrich (428043-250G). The PE is a pure product, and glitters are not standardized, thus the detailed information about their composition was one of the tasks of this work. Milli-Q water was used for leakage experiments and standard garden soil from AURA (code: 5907735200253). Vermicompost and earthworms *Eisenia fetida* (#5) were provided by EKARGO. For fast checking of pH, K, N, and P, the "Soil test kit "from Worth Gardening by Garland was used, and the Hellings method (chemicals and ceramic plate from RIM KOWALCZYK Sp.J.).

2.2 Methods for the physical and chemical characterization

Spectral and chemical measurements were done at the following research facilities:

- DXR Raman Microscope (Thermo Scientific) with the four lase lines available: 455 nm, 532 nm, 633 nm, 780 nm,
- FTIR microscope Nicolete i10 MX (Thermo Scientific) in a reflectance mode, cooled detector for mapping,
- UV-Vis Lambda 25 (Perkin Elmer) set in the range 200–700 nm without any signal pretreatment,
- AL450T-IR Nephelometer (Acqua Lytic) working in the standard average mode.

The Raman signal was collected with the 10 mm lens at a laser power of 5 mW and 50 µm aperture. The standard green line (532 nm) was substituted in cases of a strong autoluminescence. The FTIR and Raman spectra were described and pre-treated in the OMNIC software using the standard background cut-off. In addition, the atmospheric suppression filter was applied for the FTIR mapping to eliminate the CO_2 and H_2O bands overlapping the diagnostic signal. The author's open-source Python script was used for the final visualisation of data.

2.3 Experiments design

Three tests were carried out and are shortly summarized in Table 1. Firstly, the synthetic red glitter was initially added to soil with *Eisenia fetida* for a two-week exposition. Secondly, two specimens were left for additional 14 weeks, thus aged naturally for 16 weeks. During 14 weeks also, 3 tests with bioglitters were performed. Pink bioglitter was used in two configurations and blue in one. Finally, materials were taken for further studies and will not be described here in detail. At this level of accuracy, the soil parameters check did not show any significant difference with and without the presence of MPs.

Purely physical and chemical weathering was tested with the materials in milli-Q water exposed to UV radiation for 7–9 weeks. The following concentrations of the red glitter and bioglitters (pink and blue) were used: 1 $g.dm^{-3}$, 5 $g.dm^{-3}$, 25 $g.dm^{-3}$, and 50 $g.dm^{-3}$.

3 Results and discussion

Red glitter, pink and blue bioglitters were not labelled with information about their chemical composition. However, the FTIR and Raman spectroscopy allowed identifying the red glitter as a PET polymer (core) coated with several layers (Fig. 1), whereas spectra of both bioglitters looked similar and indicated their cellulose origin (Fig. 2).

The presence of PET in the core is identified by its typical bands (278; 626; 701; 800; 857; 950; 1,000; 1,096; 1,119; 1,295; 1,418; 1,462; 1,615; 1,730; 2,912; 2,968; 3,085 cm^{-1}), especially the strong two at 1,615 and 1,730 cm^{-1} coupled with signal in range 2,900–3,100 cm^{-1}. In the red glitter the following signals were confirmed despite the relatively high background noise: 274; 631; 702; 794; 857; 997; 1,093; 1,116; 1,186; 1,240; 1,290; 1,335; 1,395; 1,416; 1,614; 1,727; 2,965; 3,000; and 3,082 cm^{-1}. They can be assigned to the ester

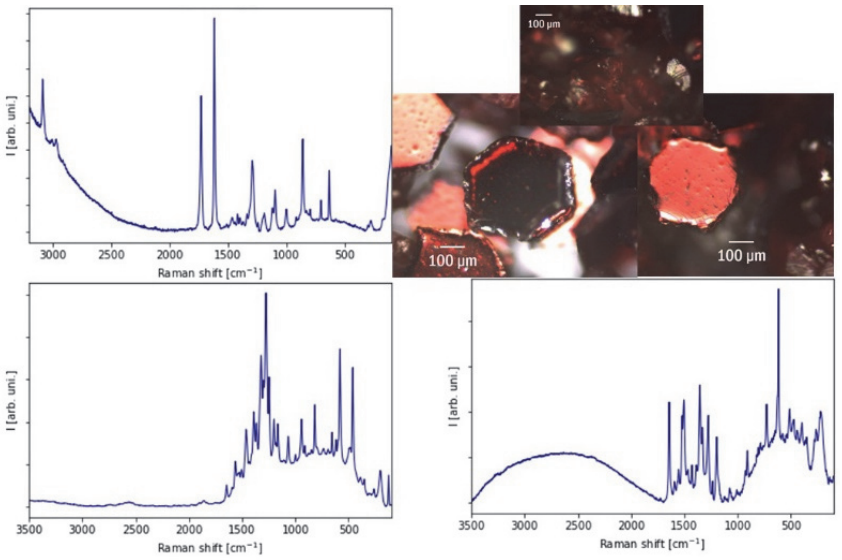
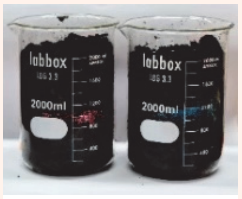


Figure 1 Raman spectroscopy confirmation of a PET core of red glitter and a variety of spectra related to its coating layers

Table 1 Soil burrial test in the presence of earthworms *Eisenia fetida*

Number of experiment	Specimen	Duration time	Description	Other
Test 1 	1.	2 weeks	2 l Becker with one *Eisenia fetida* as a control experiment	
	2.	2 weeks	200 ml of red glitter in the middle; *Eisenia fetida* in a lower layer	
	3.	2 weeks	200 ml of red glitter in the middle; *Eisenia fetida* in an upper layer	
	4.	18 weeks	10% of red glitter homogenouly mixed with soil; one *Eisenia fetida*	
	5.	18 weeks	1,400 ml soil (s) + 600 ml of red glitter (g); *Eisenia fetida* in an upper layer	layers counted from the bottom: 400 (s), 200 (g), 400 (s), 200 (g), 400 (s), 200 (g), 200 (s)
Test 2   	1.	16 weeks	2 × 12 g pink bioglitter in the middle; two *Eisenia fetida* (in a bottom layer)	
	2.	16 weeks	2 × 12 g pink bioglitter in the middle; two *Eisenia fetida* (in an upper layer)	
	3.	16 weeks	2 × 12 g blue bioglitter in the middle; four *Eisenia fetida* (two in the bottom and two in an upper layer)	

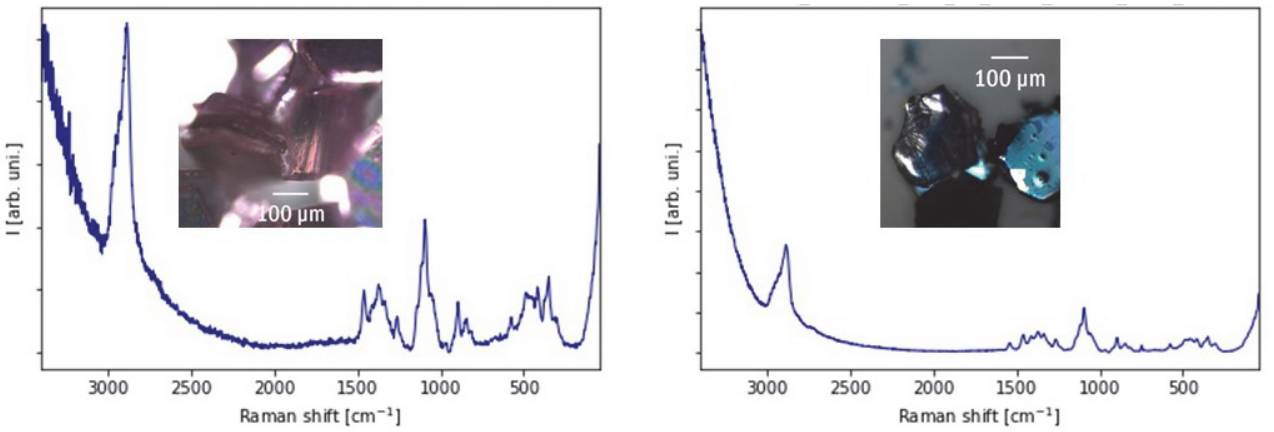


Figure 2 Raman spectra of bioglitters

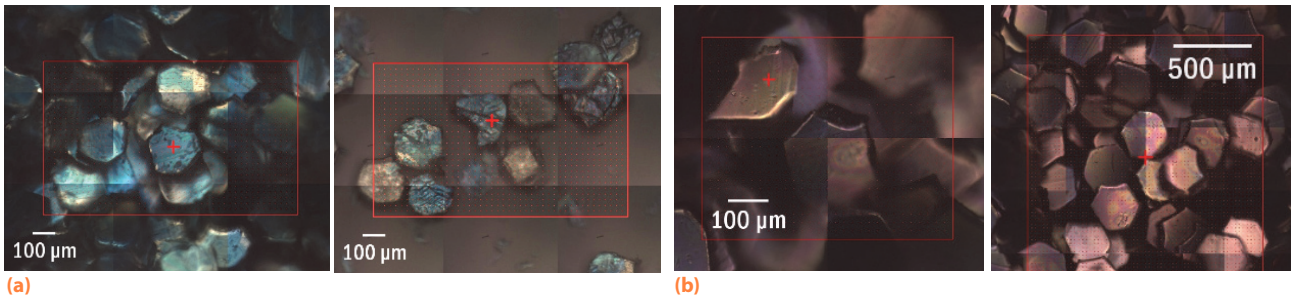


Figure 3 Signs of blue bioglitters aging: a) new and old blue material, b) new pink

C–O and ethylene glycol C–C bonds (1,116 cm^{-1}), the ring in-plane C–H bond and C–C stretching (1,186 cm^{-1}), C–O stretching (1,290 cm^{-1}), CCH and OCH bending (1,416 cm^{-1}), the ring mode 8a (1,615 cm^{-1}), C = O stretching (1,727 cm^{-1}), methylene groups adjacent to the oxygen (2,965 cm^{-1}). The PET signal remained stable during ageing, which was not the case for bioglitters.

During aging in milli-Q water exposed to direct UV radiation, several changes in the structure of bioglitters were observed, such as blanching, gradual decomposition, soaking, weight reduction, and pigment release. The FTIR mapping enabled the more detailed visualization of those effects and their spectral confirmation by peaks broadening (Fig. 3 and Fig. 4).

In all spectral ranges, the characteristic broadening of peaks is observed. Thus, all functional groups are affected, oxidation increases, and the structure exhibits signs of gradual deterioration. Contrary to bioglitters, the red synthetic glitter was not affected, and its PET core was confirmed without substantial changes (Figure 5). The PET spectrum of aged glitter has even lower background noise due to the lack of luminescent coating that fades and becomes opaque.

Despite the signs of visible degradation, many results indicate that biopolymers are not a solution to the plastic problem (Wang et al., 2021). Moreover, some research indicates their direct impact on biota (Ding et al., 2021) and soil structure. Finally, to properly access the "bio" features of the product, not only the polymer type should be controlled, but mainly added compounds, such as: dyes, pigments, plasticizers, traces of catalysts, compatibilizers, and surface coatings. Furthermore, due to numerous properties modifications, those materials often include phthalates, organic pollutants, and brominated or chlorinated derivatives. In addition, the problem of compound leakage during the degradation of materials must be addressed. For example, in the case of both bioglitters, dyes were released into the solution, the blue one decomposed and disappeared before the 7th week of the experiment, but the pink one remained stable and confirmed on UV-Vis spectra (Figure 6 left). Its potential toxic effect is not confirmed, but it would be premature to call it an inert compound. Any leakage changes the boundary conditions for the environmental equilibria, which is particularly dangerous with the unknown tolerance threshold. Another difference was related to the different sedimentation behaviour between the glitter and bioglitter. The nephelometric curves

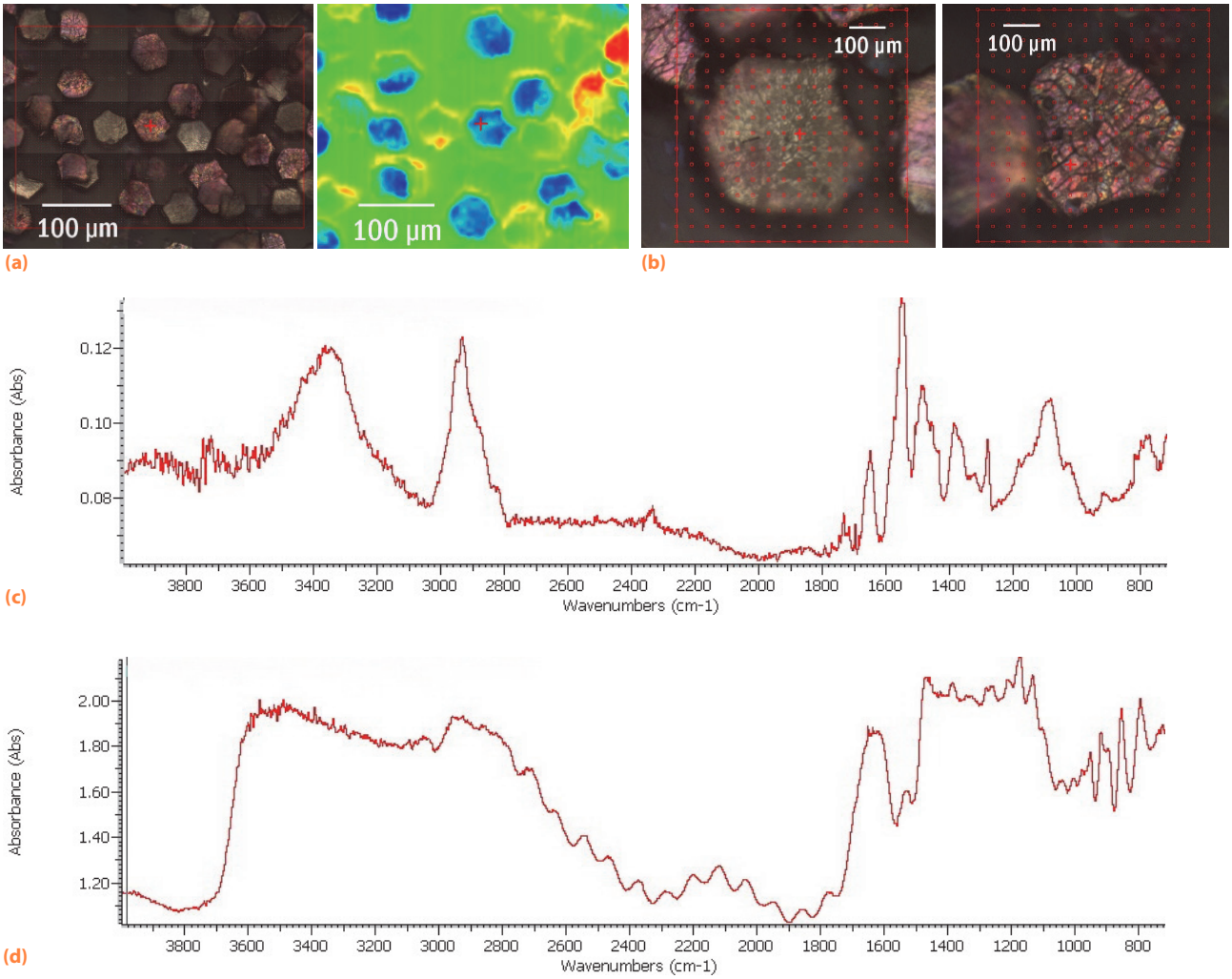


Figure 4 The FTIR confirmation of pink bioglitters ageing: a) mapped aged at b) different levels of decomposition and spectra of c) the pristine and d) old single glitter particle

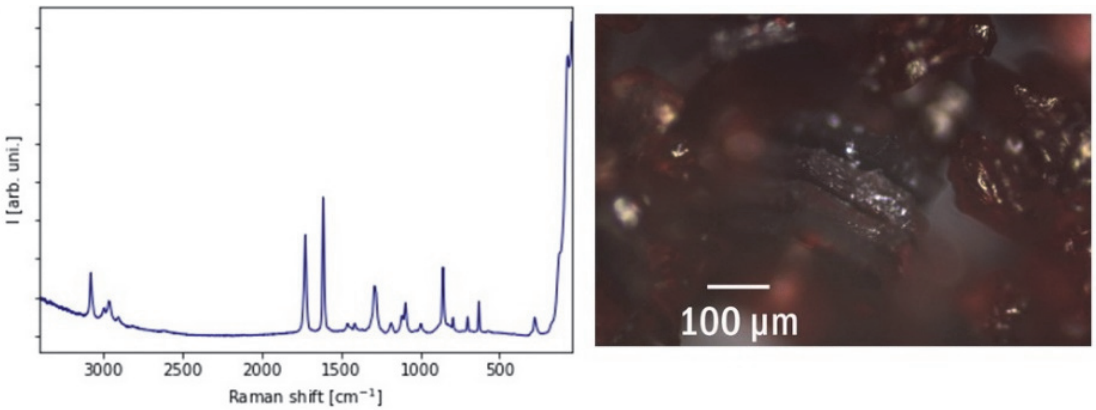


Figure 5 The Raman spectroscopy evidence of lack of aging and chemical changes in PET core of particles

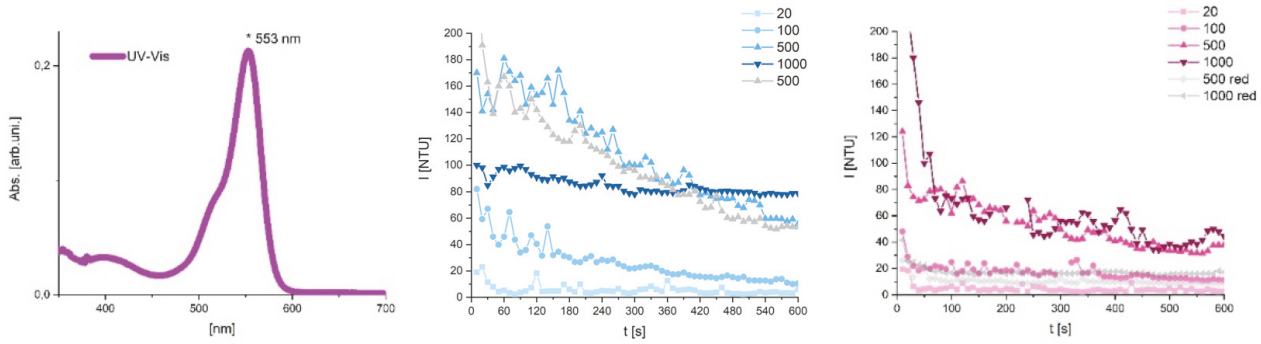


Figure 6 The UV-Vis evidence of leakage (pink dye released from bioglitter and stable despite UV radiation) and the nephelometric curves for glitter (red) and bioglitters (blue and pink, respectively) with different concentrations (numbers indicating the mg for 20 ml of milli-Q water)

(Figure 6) indicated the higher wettability and lower weight of bioglitters compared to glitters. In addition, the gradual sedimentation was observed to have a different course during measurements. Thus, enhanced mobility and transport are postulated for bioglitters.

Finally, the experiments with glitters placed in the soil with the *Eisenia fetida* can be regarded as a modified burrial test of aging. The red glitter remains stable and only slightly affected during all 18 weeks. The only observed change in shining is due to the abrasion of the coating layer. PET core remained unaffected. Contrary, both bioglitters exhibited clear signs of decomposition after 16 weeks. Their total amount decreased. They were also integrated with the plant root system. The majority of those still present were transparent or substantially bleached. All three materials were vertically transported in earthworms' burrials and highly integrated with soil-forming hard grains. They might be used as a source of cellulose indispensable for reproducing those animals. The initial alternate soil-glitter layers changed to randomly spread red material in soil that started to accumulate at the bottom of the backer. All that indicates the interaction between MPs and *Eisenia fetida* with the so far unknown consequences that would be important for evaluation in a longer time-scale of an experiment.

4 Conclusions

The preliminary results in this paper confirm the need for further studies in this direction, mainly to better understand the behaviour of primary microplastics derived from glitters in soil ecosystems. So far, most material studies on glitters have been related to their role in forensic science (Vernoud et al., 2011), and their role as MPs was neglected. Earthworms' presence is crucial for agrosoils and primary microplastics – their fate, transport, behaviour, and overall impact (Manzoor et al., 2021). It is macroscopically visible that *Eisenia fetida* facilitates the vertical migration of glitters and conduces to their high mobility towards lower layers of sediments. Glitters are also incorporated into burrials. Their presence influences the granular structure of soil and inhibits water accumulation. Some traces of agglomerated material were observed. The natural degradation of bioglitters is notable after a few weeks of UV exposition, milli-Q presence or burrial tests. However, their presence in the environment is not neutral, and the leakage of dyes should be further studied. Changes in composition and structure are efficiently detected by FTIR and Raman spectroscopy (Araujo et al., 2018). Future perspectives for this experiment consider monitoring animals' responses, for instance, by measuring the stress level (catalase) and mapping the structure and topography of their burrials (McTavish & Murphy, 2021b). The long-perspective experiment is also planned, and more types of primary microplastics (PE from scrubs) are to be included. Finally, a detailed description of ageing effects noted by Raman spectroscopy is planned. The measurements of soil parameters will be recommended. Additional experiments are currently in progress.

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