Chemosphere 366 (2024) 143315

Contents lists available at ScienceDirect

Chemosphere

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

Assessing the ecotoxicological effects of novel cellulose nanocrystalline glitter compared to conventional polyethylene terephthalate glitter: Toxicity to springtails (*Folsomia candida*)

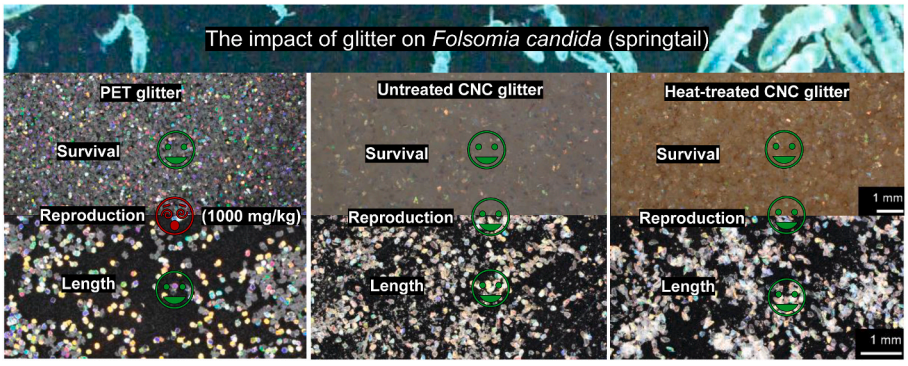
Po-Hao Chen [a,b], Benjamin E. Droguet [d], Ian Lam [b], Dannielle S. Green [e], Silvia Vignolini [d], Zhuyun Gu [b], Shamali De Silva [c], Suzie M. Reichman [b,*]

[a] Office for Environmental Programs, Faculty of Science, University of Melbourne, VIC, 3010, Australia
[b] School of BioSciences, University of Melbourne, Parkville, VIC, 3010, Australia
[c] Environment Protection Authority Victoria, EPA Science, Macleod, VIC, 3085, Australia
[d] Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield road, CB2 1EW, United Kingdom
[e] Applied Ecology Research Group, Faculty of Science and Engineering, Anglia Ruskin University, Cambridge, CB11PT, United Kingdom

HIGHLIGHTS

- Cellulose nanocrystalline (CNC) glitter is a potential glitter alternative.
- Toxicity of polyethylene terephthalate (PET) and CNC glitters to *Folsomia candida*.
- 1000 mg/kg PET glitter resulted in ~61% reproduction inhibition for *F. candida*.
- CNC glitter did not affect the endpoints of *F. candida* tested in this study.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Paolo Pastorino

Keywords:
Collembola
Glitter
Soil invertebrate

ABSTRACT

Glitter is a type of microplastic, and thus there is a need to assess its potential impacts on the environment and to assess the potential for non-plastic cellulose nanocrystal structurally colored glitters as safe and sustainable replacements. The ecotoxicity of glitter has been mostly ignored in the research literature, with only a few published studies focusing on aquatic organisms. Therefore, an exposure experiment was conducted to examine the impact of conventional polyethylene terephthalate (PET) glitter as well as untreated and heat-treated cellulose nanocrystal (CNC) based glitter on the survival, reproduction, and length of *Folsomia candida* (springtail). *Folsomia candida* reproduction was reduced by 61% ($P = 0.013$) after exposure to PET glitter at 1000 mg/kg, while no significant effects were observed on *F. candida* survival and length. In contrast, there were no significant impacts on *F. candida* survival, length, or reproduction when exposed to untreated or heat-treated CNC glitter. These results indicate that exposure to PET glitter may impact soil invertebrates at the population level, and that

* Corresponding author.
E-mail address: suzie.reichman@unimelb.edu.au (S.M. Reichman).

https://doi.org/10.1016/j.chemosphere.2024.143315
Received 15 March 2024; Received in revised form 9 September 2024; Accepted 10 September 2024
Available online 14 September 2024

CNC glitter has potential as a biodegradable non-plastic alternative to PET glitter to decrease detrimental effects on soil ecosystems.

Abbreviations

PET	Polyethylene terephthalate
CNC	Cellulose nanocrystal
MRC	Modified regenerated cellulose
R2R	roll-to-roll process

1. Introduction

Glitter is a type of microplastic produced for its light reflective, sparkling properties with many uses including for art and craft, in clothing and in makeup. Glitter is produced in sizes ranging from 50 to 6250 μm with a uniform shape, such as hexagonal, square, or triangle (Provenza et al., 2022). In general, glitter is composed of three layers: a core polymer film, coated with a colored aluminum layer to create a reflective appearance and then topped with a thin polymer layer (Tagg and Ivar do Sul, 2019). The most commonly used material for the core polymer film is polyethylene terephthalate (PET) (Provenza et al., 2022).

Glitter can be released into the environment, like other microplastics, and so it tends to accumulate and ends up increasing plastic pollution. It has been found in environmental samples including soil and water (Raju et al., 2020; Nithin et al., 2022). Until recently, the amount of PET glitter and other PET microplastics have been underestimated in environmental samples because of the limitations of common microplastics analysis (Yurtsever, 2019; Rowley et al., 2020; Boots et al., 2023). However recent advances in microplastics analysis have seen glitter being identified in sewage sludge and sediment, and glitter were the third most common microplastics in a wastewater treatment plant in Australia (Ballent et al., 2016; Raju et al., 2020). Like other microplastics, glitter has the potential to be toxic to organisms, however, there has been little research on the impact of glitter on organisms. While studies have assessed the impacts of PET glitter on aquatic species such as mussels (*Mytilus galloprovincialis* and *Perna perna*) (Provenza et al., 2022; Abessa et al., 2023), microalga (*Desmodesmus* sp.) (Wang et al., 2023), cyanobacteria (*Microcystis aeruginosa* and *Nodularia spumigena*) (Machado et al., 2023), sea urchins (*Echinometra lucunter* and *Arbacia lixula*) (Abessa et al., 2023), and duckweed (*Lemna minor*) (Green et al., 2021), its toxicity in terrestrial environments remains largely unexplored. Only one study has investigated the impacts of commercial glitters on the earthworm (*Eisenia fetida*) (Trakić et al., 2024)

Recently, in response to the demand for "eco-friendly" products, biodegradable glitter has been developed and promoted as an alternative to PET glitter. The predominately used material for biodegradable glitter is regenerated cellulose or modified regenerated cellulose (MRC), mainly from *Eucalyptus* trees, that replace the core PET film (Green et al., 2021). In addition, fluorphlogopite mica, which could be mined or synthesized, is also used as a replacement material in biodegradable glitter for its colour and shine (Becker et al., 2015). These commercially available biodegradable glitters are still coated with a colored aluminum layer and topped with a thin plastic layer (Green et al., 2021). Despite previous assumptions about their lower toxicity, recent research suggests that commercially available biodegradable glitters may exhibit comparable or even greater toxicity to aquatic organisms than PET glitter (Green et al., 2021). For instance, a follow-up study demonstrated that MRC glitters at concentrations of 100–1000 mg L^{-1} reduced the root length, biomass, and chlorophyll content of duckweed, while PET glitter and other controls did not (Boots et al., 2023).

Cellulose nanocrystals (CNC) are a novel bioderived material that can be extracted from many biological sources, from cotton to wood pulp and many agricultural wastes (Vanderfleet and Cranston, 2021). Such material has recently raised considerable interest in academia and industry to produce more sustainable optical materials from pigments to glitter (Klockars et al., 2019; Frka-Petesic and Vignolini, 2019; Parker et al., 2023). Droguet et al. (2022) presented a roll-to-roll (R2R) process to produce large-scale CNC structurally colored films. Their colour arises from the peculiar helicoidal ordering of the CNCs within the film. These metre-long CNC films, once ground, produce water-stable photonic microparticles that could be an alternative to PET glitter and commercial biodegradable glitter, without the need for a core polymer layer, aluminum layer, or thin plastic layer. To prevent the degradation of the CNC film's surface, a thermal treatment was applied to the CNC films to obtain water-stable glitter-like microparticles (Droguet et al., 2022).

As a new glitter material, there is a need to assess the potential toxicity of CNC glitter in aquatic and terrestrial environments, therefore, the aim of the research presented here was to investigate the toxicity of PET and CNC glitters to a soil organism, *Folsomia candida* (springtail).

2. Material and method

2.1. Soil preparation

The soil was collected from the Dookie campus of the University of Melbourne, Victoria, Australia (−36° 19′ 60.00″ S, 145° 41′ 59.99″ E). After collection, the soil was air dried for 14 days, followed by sieving to <2 mm. Then, the sieved soil was frozen in a −20 °C freezer to eliminate other soil organisms. After 14 days, the soil was removed from the freezer and left for ~24 h to return to room temperature. Sieved soil was confirmed as free from microplastics by Nile Red staining method (Li et al., 2019; Prata et al., 2019). In brief, soil was first digested by 30% H_2O_2 for 24 h to remove organic matter, and then potential microplastics were separated from soil by saturated $ZnCl_2$. Once separated from the experimental soil, microplastic particles stained with Nile Red were identified and differentiated from soil particles under light and fluorescence microscopy. No microplastic particles were found in our soil. General soil characteristics are summarized in Table 1.

2.2. Glitter preparation

In this study, three glitters were selected for ecotoxicity testing: polyethylene terephthalate (PET) glitter, untreated cellulose nanocrystal (CNC) structurally colored particles, and heat-treated CNC particles (Table 2). The size, color, and shape of PET glitter were observed under a light microscope (Leica Stereoroom S9i) (Fig. 1). The density of PET glitter was tested, as per Li et al. (2018). In brief, 1–3 mg of the PET glitter was added to a series of density gradient solutions from 0.8 g/cm^3 to 1.5 g/cm^3 made by different compositions of $ZnCl_2$, water, and ethanol. The PET glitter had a density between 1.3 g/cm^3 and 1.4 g/cm^3, which was in agreement with the density of PET microplastics measured by Li et al. (2018). A conversion of particles to mass for PET glitter and CNC film was done by counting the particles in a known mass of glitter under light microscopy (Leica Stereoroom S9i). In brief, about 0.4–0.5 mg of glitter were weighed and recorded, and then photographed under light microscopy. The number of glitter particles were counted using the software ImageJ (Schneider et al., 2012). Three replicates were made. Overall, 0.1 mg of PET glitter was equivalent to 193–198 particles. Conversion from particle to mass-based density for CNC film was harder to determine as the higher size range lead to larger variation in the particles per milligram (Table 2).

Table 1
Physicochemical characteristics and respective methods used for soil ecotoxicity tests.

Soil property	Value	Method
pH	6.3 ± 0.1	1:5 soil/water extract (Rayment and Lyons, 2010)
Electrical conductivity (EC) (μS/cm)	136 ± 5	1:5 soil/water extract (Rayment and Lyons, 2010)
Water holding capacity (%)	4	Determination of the maximum WHC of the soil (OECD, 2016)
Total Carbon (%)	0.83	Dry combustion method by LECO CNS
Total Nitrogen (%)	0.09	TruMAC Analyser
Carbon: Nitrogen ratio	9.2	
Soil texture (%)	Sand 19 Silt 11 Clay 70	Particle-size analysis (Gee and Bauder, 1986)
Effective Cation Exchange Capacity (ECEC) (cmol/kg)	21	Exchangeable bases method by 1 M ammonium acetate at pH 7.0 (Rayment and Lyons, 2010)
Calcium (%)	42	
Magnesium (%)	51	
Potassium (%)	3.1	
Sodium (%)	3.8	
Metal(oid) concentration		
Arsenic (mg/kg)	22	1:3 Nitric/HCl digest with APHA 3125
Cadmium (mg/kg)	<0.5	ICPMS (Rayment and Lyons, 2010)
Chromium (mg/kg)	105	
Copper (mg/kg)	118	
Lead (mg/kg)	11	
Manganese (mg/kg)	2893	
Mercury (mg/kg)	<0.1	
Nickel (mg/kg)	58	
Selenium (mg/kg)	0.7	
Silver (mg/kg)	<1	
Zinc (mg/kg)	33	

Table 2
Summary of glitter characteristics.

Glitter type	PET glitter	Untreated CNC glitter	Heat-treated CNC glitter
Main composition	Polyethylene terephthalate	Cellulose nanocrystals	Cellulose nanocrystals
Size	~100 μm	~64–177 μm	~64–177 μm
Color	Silver	Multi-colored	Multi-colored
Shape	Hexagonal film	Irregular particles	Irregular particles
Density	1.3–1.4 g/cm^3	1.5 g/cm^3	1.5 g/cm^3
Particles in 0.1 mg (mean ± standard deviation)	195 ± 3 particles	201 ± 7 particles	201 ± 6 particles

Untreated and treated CNC glitter was produced by researchers at the University of Cambridge (Droguet et al., 2022). Treated CNC glitter shares similar characteristics with untreated CNC glitter, with the only difference being the heat treatment added to the preparation process before grinding to extend survival under environmental conditions. The heat treatment process was that CNC glitters were exposed to 180 °C in an oven for 30 min. The information on composition, size, and color was provided by researchers at the University of Cambridge (Table 2), and the shape was observed under a light microscope (Leica Stereoroom S9i) (Fig. 1).

2.3. Culture and synchronization of Folsomia candida

Established laboratory cultures of *Folsomia candida* were used for this study (De Silva, 2016). *Folsomia candida* were cultured in 125 mL polypropylene vessels with a charcoal base made with a mixture of 18 g plaster of Paris, 2 g activated charcoal, and 12 mL ultrapure water (≥18.2 MΩ cm). The charcoal base was made at a slight gradient so that free water could be provided at the bottom and dried yeast as food at the top. Cultures were placed in a box to provide a completely dark environment, and the temperature was maintained at 18–22 °C. Every 3–4 days, the vessels were opened for aeration and to check the water and food content.

Synchronization was conducted to obtain first instar juveniles (0–3 days since hatching) by transferring 30–50 healthy adult *F. candida* into new vessels with the charcoal substrate. Each vessel contained 7–8 mg of dry bakers' yeast and 2–4 drops of ultrapure water. Synchronization vessels were maintained in the dark with a temperature of 18–22 °C. After three days, adults *F. candida* was removed from the vessels. The life cycle of *F. candida* fit the investigation of de Lima e Silva et al. (2021) in our laboratory environment; thus, juveniles hatched at ~10 days with 1st instar juveniles (0–3 days) utilized for the exposure experiment.

2.4. Exposure experiment for Folsomia candida with polyethylene terephthalate glitter, untreated cellulose nanocrystal glitter, and heat-treated cellulose nanocrystal glitter

The exposure experiment was adapted from Fountain and Hopkin (2005), OECD 232 (OECD, 2016), and de Lima e Silva et al. (2021). A total of eleven treatments were tested, including three types of glitter with three concentrations (10, 100, and 1000 mg/kg) of each, a soil control, and a charcoal control. The selected concentrations were based on environmentally relevant concentrations, while a broad range was chosen considering the small amount of information on glitter concentrations in soil. The base medium for the charcoal control was made from a mixture of charcoal (1 g), plaster of Paris (9 g), and ultrapure water (6 mL). Each treatment was replicated five times. For each

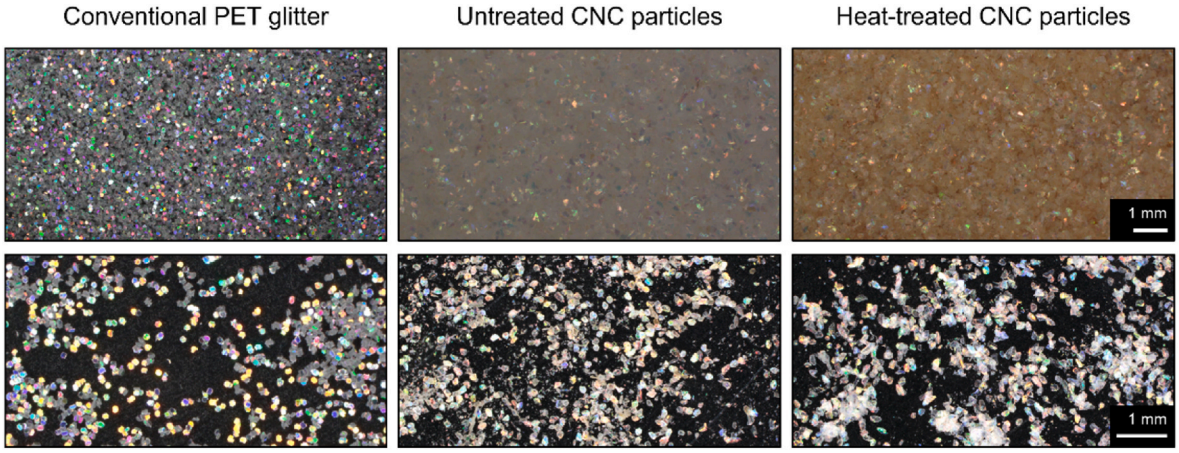


Fig. 1. Images from a light microscope of PET, untreated cellulose nanocrystal and heat-treated nanocrystal glitters.

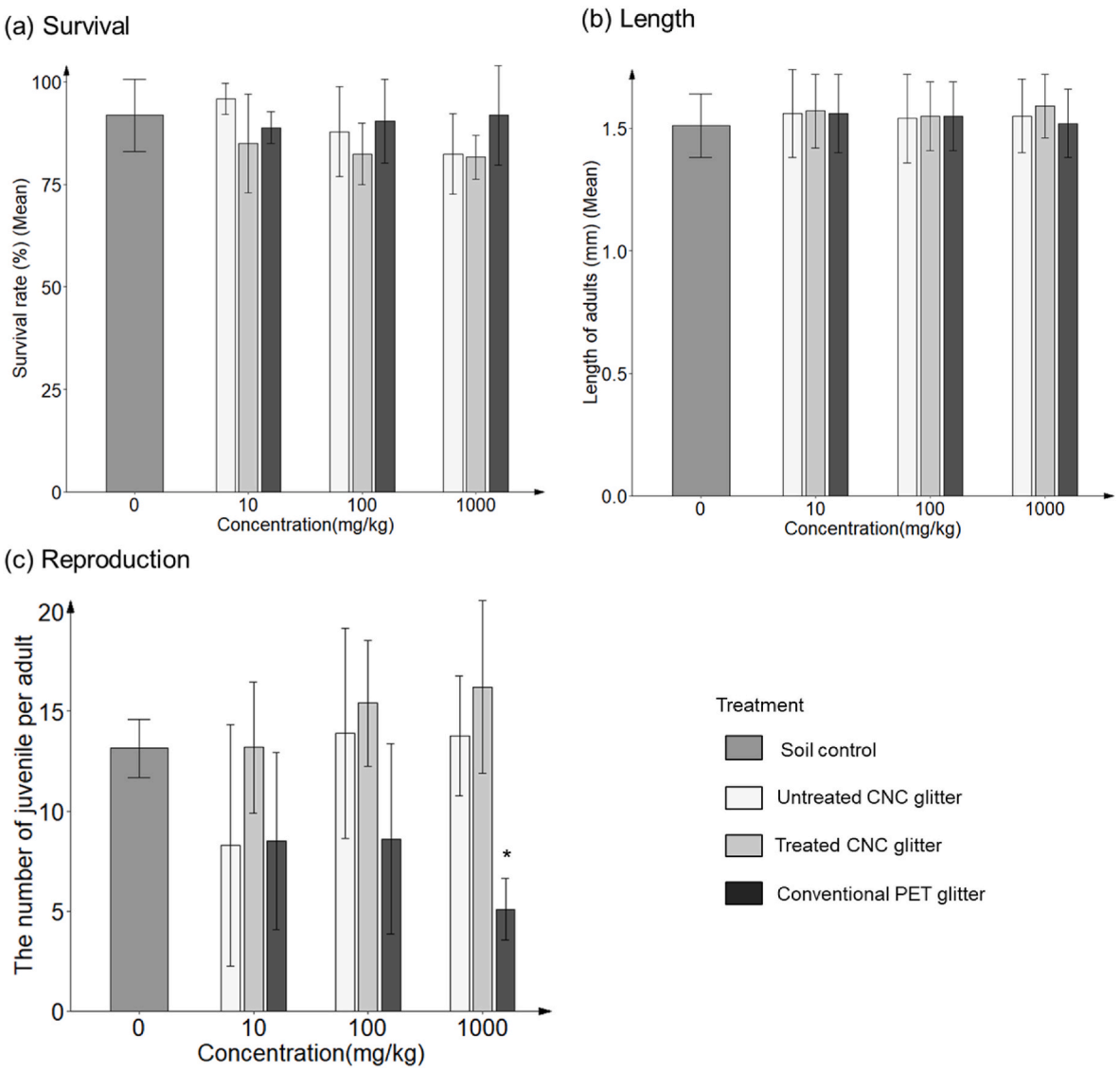


Fig. 2. *Folsomia candida* survival, length, and reproduction after 28 days of exposure to soil spiked with conventional polyethylene terephthalate (PET) glitter, untreated cellulose nanocrystal (CNC) glitter, and heat-treated cellulose nanocrystal (CNC) glitter. Values are mean ± standard deviation (n = 5). Three concentrations were tested (10, 100, and 1000 mg/kg) for three glitter types in soil. * represents $P < 0.05$.

treatment, twelve to sixteen synchronized juveniles were counted and added to a 75 mL polypropylene container containing 10 g soil (with or without glitter) or charcoal medium. At the start of the experiment, the soil was moistened by adding 3–4 mL of ultrapure water, and ~0.1 mg (4–5 pellets) of dried yeast to each vessel. The charcoal medium was flattened to sit at a slight angle to ensure *F. candida* juveniles had access to a range of moisture conditions within each vessel. Vessels were incubated in the dark at 20–22 °C. Every one to two days, the vessels were opened, aerated, water and food replenished as necessary, and any yeast with mold removed.

On day 28, the experiment was harvested and *F. candida* reproduction, survival, and length were recorded. Vessels were flooded by adding ultrapure water and then carefully stirred, such that adults and juveniles floated on the water surface. Each vessel was photographed by smart phone as well as under a light microscope at magnification 1-2 × (Leica Stereoroom S9i). The number of adults and juveniles were counted, and the adult length was measured from the center of the head to the tail of the body using the software ImageJ (Schneider et al., 2012). Adults were recorded as dead if they did not float on the water.

2.5. Statistical analysis

The survival rate was calculated by the count of adults divided by the number of juveniles added at the start of the experiment. The reproduction was calculated by the number of juveniles present at the end of the experiment divided by the count of adults. Statistical analyses were conducted to compare the survival, growth, and reproduction between concentrations. The soil control and charcoal control treatments were compared to ensure the soil quality was suitable for *F. candida*. Survival rate, the number of juveniles, and mean length were analyzed with Kruskal-Wallis test to detect significant differences between treatments ($P < 0.05$), followed by post hoc multiple comparison (Dunn's test) when $P < 0.05$ to identify significant different treatments.

In each soil control vessel, the survival of the adults was >80% and the number of juveniles were >100, which was above the OECD 232 quality control criteria of 80% and 100, respectively (OECD, 2016). *Folsomia candida* survival in the soil control was 14% higher than that in charcoal control ($P = 0.037$), with no significant difference in adult length ($P = 0.80$) between the soil and charcoal controls. The reproduction behavior of *F. candida* also performed better in soil than in

Table 3
Comparison of ecotoxicity studies from the literature and the present study on the impact of microplastics on *Folsomia candida*.

	Zhu et al. (2018)	Ju et al. (2019)	Selonen et al. (2020)	Selonen et al. (2021)	Present study		
	Microplastic tested						
Polymer type	Polyvinyl chloride (PVC)	Polyethylene (PE)	Polyethylene (PE)	Mixed rubber polymers	Polyethylene terephthalate (PET)	Untreated cellulose nanocrystal	Treated cellulose nanocrystal
Particle size	80–250 μm	32% < 50 μm, 25% 50–200 μm, 43% 200–500 μm	12 μm - 2.87 mm	5–100 μm	~100 μm	45–125 μm	45–125 μm
Particle shape	particles	microbead	fibers	irregular particle	hexagonal glitter	irregular glitter	irregular glitter
Experimental design							
Test concentration (s)	1000 mg/kg	50, 200, 1000, 5000, 10000 mg/kg	200, 600, 1700, 5000, 15000 mg/kg	200, 600, 1700, 5000, 15000 mg/kg	10, 100, 1000 mg/kg		
Exposure period	28 and 56 days	28 days	28 days	28 days	28 days		
Endpoints recorded (only significant effects compared to control treatment presented)							
Reproduction	* (28.8% decrease at 1000 mg/kg)	* (29.8% decrease at 10000 mg/kg)	n.s	* (38% decrease at 15000 mg/kg)	* (61% decrease at 1000 mg/kg)	n.s	n.s
Survival	n.s	* (26% decrease at 10000 mg/kg)	n.s	* (24% decrease in soil at 15000 mg/kg)	n.s	n.s	n.s

* = $p < 0.05$; n.s.: not significant; N/A: no test in the study.

charcoal medium with higher juveniles in the soil control compared to in the charcoal control at the end of the experiment (P = 0.016). Therefore, the charcoal control was not reported on further.

All statistical analysis, including descriptive analysis (mean value, standard deviation), Kruskal-Wallis test, Dunn's test, and visualization of data, were conducted in R environments (version 4.0.5) with the "ggpubr" and "FSA" packages (Kassambara, 2020; Ogle et al., 2022).

3. Results

3.1. Survival and length

Folsomia candida survival was high in all treatments, ranging from 82% to 96% (Fig. 2). The mean body length of adults at harvest ranged from 1.5 to 1.6 mm (Fig. 2), which is within the expected length of *F. candida* at sexual maturity (1.5 mm–3.0 mm), (Fountain and Hopkin, 2005). There was no significant difference (Kruskal-Wallis $X^2 = 13.359$; d.f. = 9; P = 0.147) in survival between the soil control and the conventional PET glitter, untreated CNC glitter, and treated CNC glitter treatments at all concentrations tested. In addition, there was no significant difference (Kruskal-Wallis $X^2 = 3.228$; d.f. = 9; P = 0.950) in the body length of *F. candida* adults between the soil control and the PET glitter, untreated CNC glitter, and treated CNC glitter treatments at all concentrations tested.

3.2. Reproduction

Folsomia candida reproduction was significantly reduced by exposure to PET glitter (Kruskal-Wallis $X^2 = 21.277$; d.f. = 9; P = 0.011, figure 3, Supplementary Information, S1). Thus, the PET glitter treatment had ~61% lower number of juveniles in the 1000 mg/kg treatment after 28 days compared to the soil control (P = 0.013). However, there was no significant difference between the number of juveniles at the end of the experiment in the soil control and untreated CNC glitter and treated CNC glitter treatments at any concentration (P > 0.05) (See Supplementary Information S1). In addition, reproduction of *F. candida* exposed to 1000 mg/kg PET glitter was ~62% and 68% lower than for *F. candida* exposed to untreated CNC glitter (P = 0.012) and treated CNC glitter (P = 0.003) at the same concentration.

4. Discussion

There was no significant effects of PET glitter, untreated CNC or treated CNC glitters on the survival (P = 0.147) and length (P = 0.950) of *F. candida* over the 28 day experiment (figure 3). However, reproduction of *F. candida* was reduced by the highest PET glitter concentration (1000 mg/kg) compared to the soil control and other glitter treatments (figure 3). These results suggest that CNC glitters have the potential to be an alternative to PET glitter to decrease potential detrimental effects on terrestrial organisms. Besides, heat treatment (30 min at 180 °C) during the R2R process before grinding can enhance the resistance of CNC glitters based on Droguet et al. (2022), and our results further suggest that heat treatment does not cause toxicity from CNC glitters. Therefore, heat-treated CNC glitter has the potential to be a novel non-plastic replacement for PET glitter and its potential ecotoxicity should continue to be explored. There are no other published studies investigating the effects of CNC glitters on soil organisms, thus more research is required to examine the ecotoxicity of CNC glitter compared to PET glitter as well as other "biodegradable" glitters.

There have been few studies on the impact of microplastics on *F. candida*, with research about polyvinyl chloride (PVC), polyethylene (PE), and mixed rubber polymers (Table 3). Given differences in polymer types, particle size and shape, it is difficult to compare these results with the current study. However, it does appear that PET glitter of ~100 μm may be more toxic for reproduction than polyethene and mixed tire rubber particles of similar size (Table 3).

Kim and An (2020) demonstrated that microplastic particle size is important for toxic impacts on *F. candida* with particles smaller than 66 μm being consumed and thus having direct toxic impacts. The PET glitter particles in our study were larger (~100 μm) and thus the mode of toxicity is not likely to be direct toxicity from being consumed.

However, the toxicity of PET glitter to *F. candida* in our study was similar to the toxicity of plain PVC particles (80–250 μm) tested by Zhu et al. (2018) (Table 3). Zhu et al. (2018) hypothesized that a change in feeding *behavior* after exposure to microplastics may lead to an alteration of gut microbiota that may result in reduced reproduction and growth. The toxicity may also have been caused by chemicals in the glitter leaching into the surrounding soil and thus exposing the springtails. Glitter particles have been found to include several potential toxicants including metals, benzene toluene and propylparaben (Abessa

Table 4
Published studies on the identification and quantification of glitter in environmental media.

Location	Sample type	Concentration of microplastics	Particle size of microplastics	Proportion of glitter (%)	Reference
New South Wales, Australia secondary wastewater treatment plant	waste activated sludge	7.91 ± 0.44 particles/L	>1 mm (9.0%) >250 μm (12.7%) >125 μm (39.8%) >38 μm (21.0%), >1.5 μm (9.7%).	24%	Raju et al. (2020)
Norwegian domestic wastewater treatment plants	sludge	6077 particles/kg	261–509 μm	1.70%	Lusher et al. (2017)
Tamil Nadu, India Vellar estuary	sediment	24.8 ± 0.75 to 43.4 ± 0.98 particles/kg	900–2100 μm	21.83%	Nithin et al. (2022)
Pülümür rivers Turkey Remote river	river	28.21 particles/m^3	0.78 ± 0.16 mm	1.18 particles/L	Gündoğdu et al. (2023)

et al., 2023). In addition, the toxic effect of PET glitters on aquatic microalgae was found to be color specific (Wang et al., 2023). Therefore, we suggest that toxicity of PET glitter toxicity to *F. candida* may have been caused by multiple pathways and more research is required to elucidate the mechanisms of PET glitter toxicity in springtails and other soil organisms.

To assess the relevance of our findings in real-world scenarios, we compared the glitter concentrations in the current study to the concentrations of glitter found in environmental samples. Until recently it was thought that glitter was rarely found in environmental samples. However, new analysis methods that can measure low density plastics are now detecting higher concentrations of glitter in environmental samples. Thus, glitter has now been found in water and sludge from wastewater treatment and in sediment (Table 4) but no published study has assessed glitter in soil as yet. The particle size range of glitter in our study (45–125 μm) was comparable with microplastics found in waste activated sludge in New South Wales, Australia (21 % of microplastic particles between 38 and 125 μm) which is the only published study to investigate low density microplastic particles <250 μm (Raju et al., 2020). However, the concentrations of PET glitter that affected the reproduction of springtails in our study (1000 mg/kg, equivalent to ~1, 950,000 particles/kg) was multiple orders of magnitude higher than glitter concentrations that have been detected in environmental samples to date (Table 4). We note though that there are only three published studies that have directly assessed glitter in environmental samples (Table 4) and there are currently no published studies that have measured glitter in soil samples. Thus, we suggest that more research is needed to understand the concentration ranges of glitter, in environmental samples, including soil.

In conclusion, the current study examined the ecotoxicity of PET glitter and CNC films and found that exposure to PET glitter inhibited the reproduction of *F. candida* at a concentration of 1000 mg/kg. In comparison, CNC film had no effects on reproduction, survival, and growth of springtails. Thus, CNC films show potential as a safer, nonplastic replacement for PET glitter, however, we suggest that further tests of CNC ecotoxicity in a wide range of environmental compartments and test species are needed.

CRediT authorship contribution statement

Po-Hao Chen: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benjamin E. Droguet:** Writing – review & editing, Visualization, Resources, Methodology, Conceptualization. **Ian Lam:** Writing – review & editing, Methodology, Investigation. **Dannielle S. Green:** Writing – review & editing, Methodology, Conceptualization. **Silvia Vignolini:** Writing – review & editing, Resources, Conceptualization. **Zhuyun Gu:** Writing – review & editing, Investigation. **Shamali De Silva:** Writing – review & editing, Investigation. **Suzie M. Reichman:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Benjamin Droguet reports financial support was provided by UK Research and Innovation - Engineering and Physical Sciences Research Council. Silvia Vignolini reports financial support was provided by UK Research and Innovation - Engineering and Physical Sciences Research Council. Benjamin Droguet reports a relationship with Sparxell UK Limited that includes: employment and equity or stocks. Silvia Vignolini reports a relationship with Sparxell UK Limited that includes: equity or stocks and funding grants. Benjamin Droguet has patent #WO2023025863A1/GB2610186B pending to Sparxell UK Limited. Silvia Vignolini has patent #WO2023025863A1/GB2610186B pending to Sparxell UK Limited. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank SoilTox Lab members Jordan McCarthy, Stephanie Wallace-Polley, and Antoinette Portelli for technical advice on culturing springtails and experimental design. Thank you to the Environmental Analysis Laboratory for non-microplastic soil analyses. The authors SV and BD would like to acknowledge the EPSRC program grant EP/M025268/1 and the ERC Proof of Principle grant CelloSparx – 101082172.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143315.

References

Abessa, D.M.D.S., Albanit, L.F., Moura, P.H.P.D., Nogueira, V.S., Santana, F.T., Fagundes, K., Ueda, M., Muller, O.P.D.O., Cesar-Ribeiro, C., 2023. A glow before darkness: toxicity of glitter particles to marine invertebrates. Toxics 11 (7), 617. https://doi.org/10.3390/toxics11070617.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., Longstaffe, F.J., 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. 110 (1), 383–395. https://doi.org/10.1016/j.marpolbul.2016.06.037.

Becker, L.C., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., Andersen, F.A., 2015. Safety

assessment of synthetic fluorphlogopite as used in cosmetics. Int. J. Toxicol. 34 (3_Suppl. l), 43S–52S. https://doi.org/10.1177/1091581815613513.
Boots, B., Green, D.S., Olah-Kovacs, B., De Falco, F., Lupo, E., 2023. Physical and chemical effects of conventional microplastic glitter versus alternative glitter particles on a freshwater plant (Lemnaceae: lemna minor). Ecotoxicol. Environ. Saf. 263, 115291. https://doi.org/10.1016/j.ecoenv.2023.115291.
de Lima e Silva, C., van Haren, C., Mainardi, G., de Rooij, W., Ligtelijn, M., van Straalen, N.M., van Gestel, C.A.M., 2021. Bringing ecology into toxicology: life-cycle toxicity of two neonicotinoids to four different species of springtails in LUFA 2.2 natural soil. Chemosphere 263, 128245. https://doi.org/10.1016/j.chemosphere.2020.128245.
De Silva, S., 2016. Effect of Metal Vehicle Emissions on Physiochemical and Biological Properties of a Roadside Soil. RMIT University. PhD Thesis.
Droguet, B.E., Liang, H.-L., Frka-Petesic, B., Parker, R.M., De Volder, M.F.L., Baumberg, J.J., Vignolini, S., 2022. Large-scale fabrication of structurally coloured cellulose nanocrystal films and effect pigments. Nat. Mater. 21 (3). https://doi.org/10.1038/s41563-021-01135-8. Article 3.
Fountain, M.T., Hopkin, S.P., 2005. Folsomia CANDIDA (collembola): a "standard" soil arthropod. Annu. Rev. Entomol. 50 (1), 201–222. https://doi.org/10.1146/annurev.ento.50.071803.130331.
Frka-Petesic, B., Vignolini, S., 2019. So much more than paper. Nat. Photonics 13 (6). https://doi.org/10.1038/s41566-019-0448-9. Article 6.
Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Methods of Soil Analysis. John Wiley & Sons, Ltd, pp. 383–411. https://doi.org/10.2136/sssabookser5.1.2ed.c15.
Green, D.S., Jefferson, M., Boots, B., Stone, L., 2021. All that glitters is litter? Ecological impacts of conventional versus biodegradable glitter in a freshwater habitat. J. Hazard Mater. 402, 124070. https://doi.org/10.1016/j.jhazmat.2020.124070.
Gündoğdu, S., Kutlu, B., Özcan, T., Büyükdeveci, F., Blettler, M.C.M., 2023. Microplastic pollution in two remote rivers of Türkiye. Environ. Monit. Assess. 195 (6), 791. https://doi.org/10.1007/s10661-023-11426-z.
Ju, H., Zhu, D., Qiao, M., 2019. Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, Folsomia candida. Environ. Pollut. 247, 890–897. https://doi.org/10.1016/j.envpol.2019.01.097.
Kassambara, A., 2020. *Ggpubr: "ggplot2" based publication ready plots* (0.4.0). https://CRAN.R-project.org/package=ggpubr.
Kim, S.W., An, Y.-J., 2020. Edible size of polyethylene microplastics and their effects on springtail behavior. Environ. Pollut. 266, 115255. https://doi.org/10.1016/j.envpol.2020.115255.
Klockars, K.W., Yau, N.E., Tardy, B.L., Majoinen, J., Kämäräinen, T., Miettunen, K., Boutonnet, E., Borghei, M., Beidler, J., Rojas, O.J., 2019. Asymmetrical coffee rings from cellulose nanocrystals and prospects in art and design. Cellulose 26 (1), 491–506. https://doi.org/10.1007/s10570-018-2167-7.
Li, L., Li, M., Deng, H., Cai, L., Cai, H., Yan, B., Hu, J., Shi, H., 2018. A straightforward method for measuring the range of apparent density of microplastics. Sci. Total Environ. 639, 367–373. https://doi.org/10.1016/j.scitotenv.2018.05.166.
Li, Q., Wu, J., Zhao, X., Gu, X., Ji, R., 2019. Separation and identification of microplastics from soil and sewage sludge. Environ. Pollut. 254, 113076. https://doi.org/10.1016/j.envpol.2019.113076.
Lusher, A.L., Hurley, R., Vogelsang, C., Nizzetto, L., Olsen, M., 2017. Mapping microplastics in sludge. In: *55*. Norsk Institutt for Vannforskning. https://niva.brage.unit.no/niva-xmlui/handle/11250/2493527.
Machado, M.J., Dextro, R.B., Cruz, R.B., Cotta, S.R., Fiore, M.F., 2023. Response of two cyanobacterial strains to non-biodegradable glitter particles. Aquat. Toxicol. 260, 106590. https://doi.org/10.1016/j.aquatox.2023.106590.
Nithin, A., Sundaramanickam, A., Sathish, M., 2022. Seasonal distribution of microplastics in the surface water and sediments of the Vellar estuary, Parangipettai, southeast coast of India. Mar. Pollut. Bull. 174, 113248. https://doi.org/10.1016/j.marpolbul.2021.113248.
OECD, 2016. Test No. 232: Collembolan Reproduction Test in Soil. Organisation for Economic Co-operation and Development. https://www.oecd-ilibrary.org/environment/test-no-232-collembolan-reproduction-test-in-soil_9789264264601-en.
Ogle, D., Doll, J., Wheeler, P., 2022. dunnTest(.)), A. D. (Provided base functionality of. FSA: Simple Fisheries Stock Assessment Methods (0.9.3). https://CRAN.R-project.org/package=FSA.
Parker, R.M., Parton, T.G., Chan, C.L.C., Bay, M.M., Frka-Petesic, B., Vignolini, S., 2023. Bioinspired photonic materials from cellulose: fabrication, optical analysis, and applications. Acc. Mater. Res. 4 (6), 522–535. https://doi.org/10.1021/accountsmr.3c00019.
Prata, J.C., Reis, V., Matos, J.T.V., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2019. A new approach for routine quantification of microplastics using Nile Red and automated software (MP-VAT). Sci. Total Environ. 690, 1277–1283. https://doi.org/10.1016/j.scitotenv.2019.07.060.
Provenza, F., Anselmi, S., Specchiulli, A., Piccardo, M., Barceló, D., Prearo, M., Pastorino, P., Renzi, M., 2022. Sparkling plastic: effects of exposure to glitter on the Mediterranean mussel Mytilus galloprovincialis. Environ. Toxicol. Pharmacol. 96, 103994. https://doi.org/10.1016/j.etap.2022.103994.
Raju, S., Carbery, M., Kuttykattil, A., Senthirajah, K., Lundmark, A., Rogers, Z., Scb, S., Evans, G., Palanisami, T., 2020. Improved methodology to determine the fate and transport of microplastics in a secondary wastewater treatment plant. Water Res. 173, 115549. https://doi.org/10.1016/j.watres.2020.115549.
Rayment, G.E., Lyons, D.J., 2010. Soil Chemical Methods—Australasia. CSIRO Publishing. https://doi.org/10.1071/9780643101364.
Rowley, K.H., Cucknell, A.-C., Smith, B.D., Clark, P.F., Morritt, D., 2020. London's river of plastic: high levels of microplastics in the Thames water column. Sci. Total Environ. 740, 140018. https://doi.org/10.1016/j.scitotenv.2020.140018.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 7. https://doi.org/10.1038/nmeth.2089. Article.
Selonen, S., Dolar, A., Jemec Kokalj, A., Sackey, L.N.A., Skalar, T., Cruz Fernandes, V., Rede, D., Delerue-Matos, C., Hurley, R., Nizzetto, L., van Gestel, C.A.M., 2021. Exploring the impacts of microplastics and associated chemicals in the terrestrial environment – exposure of soil invertebrates to tire particles. Environ. Res. 201, 111495. https://doi.org/10.1016/j.envres.2021.111495.
Selonen, S., Dolar, A., Jemec Kokalj, A., Skalar, T., Parramon Dolcet, L., Hurley, R., van Gestel, C.A.M., 2020. Exploring the impacts of plastics in soil – the effects of polyester textile fibers on soil invertebrates. Sci. Total Environ. 700, 134451. https://doi.org/10.1016/j.scitotenv.2019.134451.
Tagg, A.S., Ivar do Sul, J.A., 2019. Is this your glitter? An overlooked but potentially environmentally-valuable microplastic. Mar. Pollut. Bull. 146, 50–53. https://doi.org/10.1016/j.marpolbul.2019.05.068.
Trakić, T., Popović, F., Sekulić, J., Hackenberger, D.K., 2024. Ecotoxicological effects of commercial microplastics on earthworm Eisenia fetida (savigny, 1826) (Clitellata; lumbricidae). Agriculture 14 (2), 267. https://doi.org/10.3390/agriculture14020267.
Vanderfleet, O.M., Cranston, E.D., 2021. Production routes to tailor the performance of cellulose nanocrystals. Nat. Rev. Mater. 6, 124–144. https://doi.org/10.1038/s41578-020-00239-y.
Wang, C., Jiang, L., Zhang, Y., Wang, C., He, M., 2023. Stealth microplastics pollutants: toxicological evaluation of polyethylene terephthalate-based glitters on the microalga Desmodesmus sp. and its color effect. Environ. Sci. Pollut. Control Ser. 30 (42), 95975–95987. https://doi.org/10.1007/s11356-023-29147-8.
Yurtsever, M., 2019. Tiny, shiny, and colorful microplastics: are regular glitters a significant source of microplastics? Mar. Pollut. Bull. 146, 678–682. https://doi.org/10.1016/j.marpolbul.2019.07.009.
Zhu, D., Chen, Q.-L., An, X.-L., Yang, X.-R., Christie, P., Ke, X., Wu, L.-H., Zhu, Y.-G., 2018. Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. Soil Biol. Biochem. 116, 302–310. https://doi.org/10.1016/j.soilbio.2017.10.027.