2023-09-15

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Boots, B

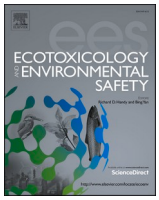
10.1016/j.ecoenv.2023.115291
Ecotoxicology and Environmental Safety
Elsevier BV

Ecotoxicology and Environmental Safety 263 (2023) 115291

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Physical and chemical effects of conventional microplastic glitter versus alternative glitter particles on a freshwater plant (Lemnaceae: *Lemna minor*)

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ARTICLE INFO

Edited by: Professor Bing Yan

Keywords:
Ecotoxicology
Biodegradable plastics
Modified regenerated cellulose
Synthetic mica
Lemna minor

ABSTRACT

Glitters are primary microplastics which are directly littered into the environment, yet the ecological effects have seldom been tested. When microplastics enter the environment, their physical presence and chemical leachate may alter the physiology of primary producers. Glitter can be composed of plastic or natural and/or biodegradable materials, often with additives. Three experiments were run for 14 days to separate chemical and physical effects of different types of glitter: polyethylene terephthalate (PET), biodegradable modified regenerated cellulose (MRC), synthetic mica, and a natural particle control (kaolinite) on several physical characteristics of *Lemna minor* (common duckweed). *L. minor* was exposed to either fresh (chemical and physical effects), leachate from glitter (chemical) or aged glitter (physical). Overall, there was little effect of PET, synthetic mica, kaolinite or of any aged glitter. High concentrations of fresh MRC glitters, however, decreased root length, biomass and chlorophyll content of *L. minor*. Some of these effects were also present when exposed to leachate from MRC glitters, but were less pronounced. Elemental analysis revealed the presence of metals in MRC glitters which may explain these responses. Short-term ecotoxicity of biodegradable glitters can arise due to their physical and chemical properties, but may lessen over time as their surface coating degrades.

1. Introduction

Plastic production is still increasing and doubled from 2000 to 2019 to reach 460 million tonnes (OECD, 2022). Microplastics (plastic particles < 5 mm), either manufactured intentionally ("primary microplastics") or arising from the fragmentation of larger plastic debris ("secondary microplastics") are the most common form of solid waste on Earth (Eriksen et al., 2014; Pabortsava and Lampitt, 2020). In response to concern about the persistence and impacts of microplastics, bans on conventional primary microplastics in products, such as rinse-off cosmetic microbeads, have been implemented around the world (Mitrano and Wohlleben, 2020). As such, alternative primary microplastics made from bio-based, biodegradable (e.g. cellulose, alginate, lignin or starch) or compostable polymers (e.g. polylactic acid) are entering the marketplace instead. There is growing evidence that microplastics made of bio-based and/or biodegradable polymers can exert the same, or stronger, negative effects on biota as conventional microplastics (Green, 2016; Green et al., 2016; Green et al., 2017; Green et al., 2019; Boots et al., 2019; Straub et al., 2017; González-Pleiter et al., 2019). The effects could be due to the physical presence of the particles or due to the release of leachates during biodegradation. Indeed, cellulose- and starch- based products contain more chemical compounds (including plasticizers, additives and coatings) and induce stronger in-vitro toxicity than conventional plastic products (Zimmermann et al., 2020). Weathering will change the physiochemical properties and may alter the toxicity of microplastics (Liu et al., 2020), but very few studies have compared the effect of pristine versus aged microplastics (Olubukola et al., 2022). In addition, the toxicity of microplastics designed to biodegrade quickly may dissipate after aging, but this has not yet been investigated.

Despite playing a vital role in supporting aquatic food webs, there is little research on the effects and impacts of microplastics on aquatic plants (Kalčíková, 2020). To date, research has shown variable effects of microplastics on freshwater plants, for example, microplastics with a

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https://doi.org/10.1016/j.ecoenv.2023.115291
Received 19 December 2022; Received in revised form 20 June 2023; Accepted 20 July 2023
Available online 24 July 2023

smooth surface (microbeads) had no effect on the leaf growth and chlorophyll content of duckweeds *Spirodela polyrhiza* (Dovidat et al., 2020) and *Lemna minor* (Mateos-Cárdenas et al., 2021; Kalčíková et al., 2017). Contrarily, sharp microplastics reduced the viability of root cells (Kalčíková et al., 2017) and decreased root length (Rozman et al., 2021) in *L. minor*. As well as being useful model organisms in laboratory studies, *L. minor* also fulfills an important role in many freshwater ecosystems; serving as food for fish and waterfowl and providing nursery grounds and refuge for invertebrates and fish with their bundles of roots (Fourounjian et al., 2020).

Glitter is a unique type of primary microplastic which has been overlooked but is starting to gain attention from the scientific community (Tagg and Ivar do Sul, 2019; Perosa et al., 2021; Piccardo et al., 2022a; Vargas et al., 2022). Glitters are flat, often sharp edged, particles consisting of several layers; an inner "core" composed of either plastic (usually biaxially-oriented polyethylene terephthalate) or a biodegradable alternative (e.g. plant-based plastic such as modified regenerated cellulose (MRC)), and a reflective layer (e.g. aluminium or mineral pigment) and all held together with a thin outer plastic layer (e.g. styrene acrylate) (Blackledge and Jones, 2007). Natural or synthetic mica (fluorphlogopite) is also used as glitter, especially in cosmetics. Glitters are widely used in clothing, arts and crafts, cosmetics and body paint from where they can enter waterways incidentally (when rinsed off the body or through washing clothing), reaching aquatic environments through wastewater and sewage effluent. For example, glitter was found in half of all tested wastewater samples from Norwegian domestic wastewater treatment plants (Lusher et al., 2017) and were the third most common (behind fibres and fragments) particle types (accounting for 24%) in waste activated sludge from a wastewater treatment plant in Australia with an estimated daily discharge of 2.7–3.0 $\times$ 10^7 in activated sludge and 2.5–2.7 $\times$ 10^6 glitter particles from effluent (Raju et al., 2020). This study concluded that glitter likely constitutes a major source of primary microplastics contaminating terrestrial (e.g. agricultural systems receiving sludge) and aquatic (via effluent) environments (Raju et al., 2020). Indeed, large local direct input occur when glitter is used in great quantities at protests (a.k.a. "glitter bombing") and celebratory events such as festivals (Yurtsever, 2019; Perosa et al., 2021). Glitter has been found in the aquatic environment in freshwater sediments (Ballent et al., 2016; Hurley et al., 2018) and in rivers (estimated 523 and 1403 glitter particles sec^{-1} flow down the Thames and Putney rivers, respectively, during peak ebb tides; Rowley et al., 2020) but is underestimated due to technical issues with common methods of detection used for other microplastics (Yurtsever, 2019) leading to a low (4–11%) recovery rates from samples (Piccardo et al., 2022b).

Although glitter likely poses a greater risk than microbeads due to its complex chemical composition and greater prevalence as litter (Raju et al., 2020; Kurniawan et al., 2021), to date, there has been very few studies examining the impacts of glitter on the environment. The first study on glitter by Green et al. (2021) found that 60 mg L^{-1} of either plastic (PET) glitter or alternative glitters (MRC, natural mica and synthetic mica) reduced the biomass of primary producers in the water column and reduced the root length of *L. minor*. This study, however, was unable to determine whether the observed effects on duckweed were due to the physical or the chemical (leachate) characteristics of the glitters and whether effects lessen over time as particles age and potentially release surface additives.

Here we assess the effects of plastic glitter (with a core of PET), alternative glitters (synthetic mica or with a core of biodegradable MRC) and natural particles (kaolinite) added either as i) pristine particles, ii) leachate or iii) aged particles on the growth and chlorophyll content of *L. minor*. Furthermore, we present elemental analysis of leachates from each type of glitter to improve our mechanistic understanding of any observed biological effects.

2. Materials and methods

2.1. Characterisation of glitter

2.1.1. Glitter morphology; scanning electron microscopy

Four different types of glitter were chosen because they are available on the market. These included conventional plastic PET glitter (PET), synthetic mica (mica), MRC with an acrylic coating (MRC1), MRC without an acrylic coating (100% plastic-free) (MRC2) were used, of which the latter two were marketed as biodegradable. Morphological analysis of the glitter samples was performed by scanning electron microscopy (SEM) using a JEOL 6610 LV SEM (Tokyo, Japan). Samples were placed on stubs with adhesive tape and sputter-coated with gold prior to analysis. SEM observations were performed in low vacuum mode, with an accelerating voltage of 15 kV. ImageJ (Schneider et al., 2012) was used to measure the mean dimensions based on 25 measurements of each micrograph per sample.

2.1.2. Fourier transform infrared spectroscopy

The composition of the glitter samples was investigated by Fourier transform infrared spectroscopy (FTIR). Spectra were acquired with a Vertex 70 spectrometer (Bruker, Germany) by collecting the signal with an attenuated total reflectance accessory (ATR), using 32 scans and a resolution of 4 cm^{-1}, over the range 4000 − 400 cm^{-1}. The obtained spectra were compared to multiple spectral databases (i.e. Bruker Optics ATR-Polymer Library, KIMW ATR-IR Polymer Library, ATR-FTIR Library Polymer, etc.).

2.1.3. Elemental analyses using ICP-OES and ICP-MS

Analytical reagents-grade chemicals (HNO_3 (70%) and HCl (37%) were purchased from Fisher Scientific, UK) were used in the preparation of all solutions. All the plastic and glassware were cleaned by soaking in 10% v/v HNO_3 subsequently rinsed in deionized and then in ultrapure water prior to use. The presence of different elements in the glitter samples was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a iCAP7000series (ThermoScientific, UK) and inductively coupled plasma mass spectrometry (ICP-MS) with a iCAP RQ ICP-MS (ThermoScientific, UK). The 4 different glitters were separately acid digested in triplicates (n = 3), by adding 0.25 g of to 5 mL of HCl:HNO_3 (1:4), subsequently heated to gentle boiling until all smoke was emitted and the solution became clear. The digests were then cooled down to room temperature, filtered to remove any solid residues using Grade 51 ashless hardened quantitative filter paper, (Fisherbrand, UK), and further diluted to 25 mL with ultrapure water. Procedural blanks of acid digestion and filtration with no glitter material were also prepared in triplicates. For the calibration curves, a series (n = 4) of multielement standard solutions were prepared combining single element standard solutions (K 1000 mg L^{-1}; Si 50 mg L^{-1}; P 10,000 mg L^{-1}; S 10,000 mg L^{-1}; 100 mg L^{-1} for all the other analysed elements; Fisher Scientific, UK).

2.2. Experimental setup and design

2.2.1. Microcosm setup

Common duckweed (*Lemna minor*) was used as a model organism to assess the effects of different types of glitter. In addition, clay mineral kaolinite was included as a natural particle for comparison. It is important to compare the effect of naturally occurring particles to ascertain whether microplastics are indeed more toxic than the former (Gerdes et al., 2019; Schür et al., 2020). Duckweed was grown from stock (Blades Ltd, UK) in natural mineral water (Ca^{2+} = 80 mg L^{-1}, Mg^{2+} = 26 mg L^{-1}, Na^{+} = 6.5 mg L^{-1}, K^{+} = 1 mg L^{-1}, SiO_2 = 15 mg L^{-1}, HCO_3^- = 360 mg L^{-1}, SO_4^{2-} = 14 mg L^{-1}, Cl^- = 10 mg L^{-1}, NO_3^- = 3.8 mg L^{-1}, dry residue at 180 °C: 345 mg L^{-1} and pH = 7.2) with continuous oxygen supply via an air bubbler with an average temperature of 18 °C. Only green healthy-looking plants were used in the experiment,

any that were pale or yellowed were discarded. All experiments were done using this same type of water. We collected continuous PAR data using a S-LIA-M003 Photosynthetic Light (PAR) smart sensor coupled with a H21-USB Hobo Micro Weather Station and the average daytime PAR over the experimental duration was 59.60 ± 1.06 μmol m^{-2} s^{-1}.

2.2.2. Addition of glitters and leachate

Fresh (pristine) glitter (new from the packet not yet exposed to the open air or water) was used to assess chemical and physical effects, leachate from glitter for chemical effects and aged glitter (exposed to water and light) for physical effects. The leachate and aged glitter were produced by adding each glitter type to separate Erlenmeyer flasks containing mineral water to a 1 g L^{-1} concentration. The flasks were placed on an orbital shaker for 7 days at room temperature (18 °C). The glitters were separated from the leachate using filter paper (Whatman Cat No 1001–185) and the retained glitter (aged) was dried at room temperature and harvested for subsequent experiments. The collected filtrate was subsequent used to reflect any possible leached chemicals from the glitters. The chemical and physical effects of glitter on *L. minor* were assessed via three separate experiments using 100 mL microcosms. For each experiment, the five types of glitters or their leachate (factor "Glitter") were added at 10, 100 or 1000 mg mL^{-1} (factor "Concentration"). This resulted in an asymmetric design with a dedicated treatment containing no added glitter ("Control") for each separate experiment. Each treatment was replicated five times (n = 5), resulting in total of N = 80 (five levels in Glitter, three levels in Concentration and one control) for each separate experiment, with a grand total of 240 microcosms (Fig. S1). The concentrations (10, 100 and 1000 mg L^{-1}) were chosen to allow comparison of the results to previous research on duckweed and microplastics (Kalčíková et al., 2017; Kalčíková and Kokalj et al., 2020, 2019 used 100 mg L−1) and because the middle concentration (100 mg L^{-1}) is the reference concentration in REACH EU Regulation No. 1907/2006. Each microcosm contained 80 mL of the treatment medium (mineral water with glitter or leachate, representing the different doses). For each microcosm, before introduction, the root length of ten randomly chosen individuals of *L. minor* from healthy stock were carefully cut to 10 mm and plants were weighed to ensure the same starting biomass for each treatment. Duckweed plants with two or three fronds (five of each), to gain a starting total of 25 fronds for each microcosm, were used. During the experimental period (14 days for each experiment), microcosms were kept under fluorescent light tubes, timed corresponding to the British summer daylight for 16 h (5:00–21:00). Any water loss due to evapotranspiration was corrected by compensating with diH_2O. The average temperature during the three experiments was 24 °C.

2.3. Measurements of response variables

During the experimental period, the average number of fronds were counted weekly to assess growth. For this, an image was taken from each microcosm (12 Megapixel, 20 cm high, perpendicular to surface, equal lighting and exposure settings) and the number of fronds counted from the image. At the end of the experiment, after 14 days, distinct individuals of *L. minor* from each microcosm were counted and carefully removed with forceps. Their root length was then measured from ten individuals and averaged for each microcosm. After that, the plants were carefully rinsed, blotted dry and weighed to assess biomass. A random subsample, amounting to half of the recovered *L. minor* biomass, was then weighed, followed by oven-drying at 60 °C for 24 h to gravimetrically determine moisture content. The other half of the biomass was weighed and used to extract chlorophyll. For this, the plants were weighed and added to 5 mL of 90% ethanol, subsequently stored at −20 °C for six days to extract. After that, the chlorophyll content of the fronds was determined with a spectrophotometer by measuring absorption at λ = 645 nm and 663 nm from 1 mL of the extractant. With this, the chlorophyll-a content was calculated with 12.72 $*A_{663}$ – 2.59 $*A_{645}$ and that of chlorophyll-b with 22.9 $*A_{645}$ – 4.76 $*A_{663}$ (Su et al., 2010), summed and expressed as μg g^{-1} dry biomass.

2.4. Statistical data analysis

The three experiments (using pristine, leached or aged glitter) were analysed separately with each dedicated control as reference points using R v4.0.3 (R Core Team, 2020). All response variables were assessed for the assumption of a Gaussian distribution via q-q plots and Shapiro-Wilkinson tests. Homoscedasticity was assessed on residual plots and supported with Levene's tests with the *car* (v3.0–5) package (Fox and Weisberg, 2019). The data were analysed in four steps:

1) The means of the controls from the three experiments were compared using a one-way ANOVA to assess the differences between the experiments at α = 0.05.
2) Changes over time (i.e. root length, biomass and number of fronds) were assessed using t-tests assuming that change equals zero under the null hypothesis for all treatments. To account for inflated familywise error rates with this approach, a Bonferroni correction was applied resulting in an adjusted critical significance level of $\alpha_{adjusted}$ = 0.003 for each response variable under consideration.
3) The effects of the different glitter types and their doses were analysed using an asymmetrical ANOVA because there was a single control group for the controls (n = 5) which was compared to two treatment levels. This was done using the mean squares from two independent ANOVAs and partitioning of the variance. For this a one-way ANOVA with all treatments as separate levels was calculated, followed by a full-factorial, two-way ANOVA without the controls included. To estimate any differences between the levels within the 2nd ANOVA, the residuals of the 1st ANOVA were used. This allowed the variation associated with the controls and that of the other treatments to be distinguished ("Control vs. Others"), contrasted with one degree of freedom (Underwood, 1997). When the main terms were significant (at α = 0.05), pairwise comparisons for the factors in the 2nd ANOVA were computed using Tukey HSD tests.
4) All glitter treatments and concentration were contrasted to the control within an experiment using Dunnett tests with the *multcomp* v1.4-6 (Hothorn et al., 2008) package. To account for inflated familywise error rates, a Bonferroni correction was applied resulting in an adjusted critical significance level of $\alpha_{adjusted}$ = 0.003 for each dependent variable under consideration.

3. Results

3.1. Characterisation of glitter: SEM, FTIR and elemental analyses

3.1.1. Glitter morphology; scanning electron microscopy

SEM micrographs of the 4 types of glitter are presented in Fig. 1. PET glitter particles are smooth and different layers can be seen in the section (Fig. 1a). They are hexagons with an average side length of 146 ± 18 μm, corresponding to an average area of 0.0563 ± 0.0143 mm^2. MCR1 glitter particles are also characterised by a smooth and homogeneous surface (Fig. 1b). They are hexagons with an average side length of 87 ± 5 μm, corresponding to an average area of 0.0197 ± 0.002 mm^2. MCR2 glitter particles are characterised by a fragmented layer of flakes, giving a general roughness to the surface (Fig. 1c). They are hexagons with an irregular shape and surface and an average side length of 86 ± 13 μm and average area of 0.0197 ± 0.0143 mm^2. Finally, MICA glitter particles are characterised by thin flat particles of irregular shape and dimensions (Fig. 1d). Average dimensions were calculated measuring the longer dimension for each particle, obtaining an average value of 101 ± 82 μm.

3.1.2. Fourier transform infrared spectroscopy

The FTIR spectrum of the PET glitter particles (Fig. S2a) shows all the

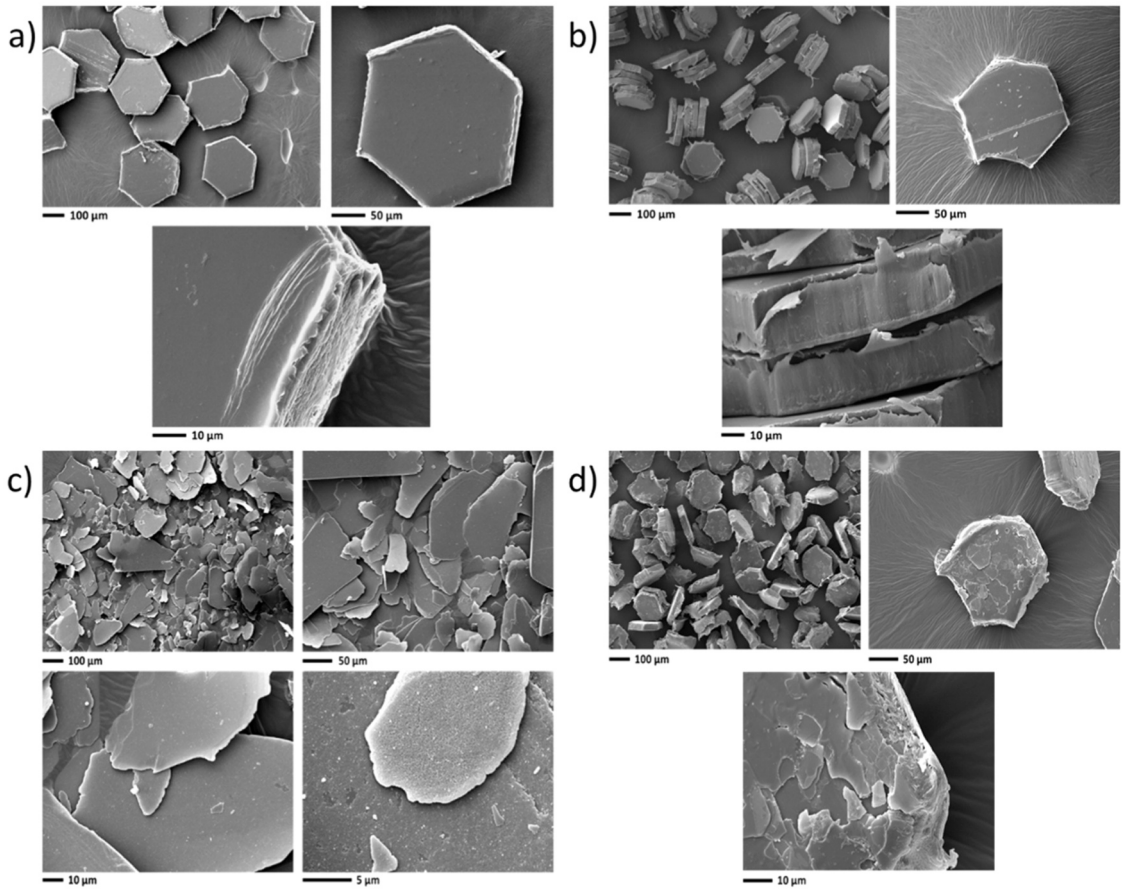

Fig. 1. Scanning electron micrographs of (a) PET, (b) MRC1, (c) Mica and (d) MRC2 glitter particles at different magnifications.

characteristics absorption bands of PET: CH_2 asymmetric stretch (2962 cm^{-1}), C═O stretch (1714 cm^{-1}), C-O stretch (1244 and 1092 cm^{-1}), aromatic ring in-plane CH bend (1017 cm^{-1}), aromatic in-phase CH wag (722 cm^{-1}) (Noda et al., 2007).

A clear identification was not possible through comparison with spectral libraries for the MRC1 glitter particles (Fig. S2b), but according to the composition provided by the manufacturer, the core material of MRC1 glitter is modified regenerated cellulose with an acrylic coating. Indeed, the FTIR spectrum of MRC1 glitter shows a strong absorption band at 1717 cm^{-1}, that corresponds to the carbonyl C═O stretching vibration band of acrylic compounds. The additional bands observable in the region 1665–1021 could be due to the interaction among the C-H, C-O-C and C-O bonds of cellulose with those of the acrylic resin.

The FTIR spectrum of MICA glitter particles (Fig. S2c) was identified as a silicate, confirming the glitter composition provided by the manufacturer. Finally, MRC2 glitter particles (Fig. S2d) were identified as regenerated cellulose (i.e. rayon, viscose, etc.) by comparison with spectral databases. This confirms the composition of the glitter core material (modified regenerate cellulose) stated by the manufacturer. The characteristic broad O-H stretching band around 3336 cm^{-1} is visible along with the CH2 asymmetric (2928 cm^{-1}) and symmetric (2859 cm^{-1}) stretching; HOH bending of absorbed water (1664 cm^{-1}); CH bending (1372 cm^{-1}) and wagging (1314 cm^{-1}); C-O-C asymmetric stretching (1155 cm^{-1}); C-O stretching (1015 cm^{-1}) (Carrillo et al., 2004; Comnea-Stancu et al., 2017).

3.1.3. Elemental analyses using ICP-OES and ICP-MS

Elemental analyses of glitters detected 19 different elements (Table 1) with a wider range and higher quantities of elements detected in Mica and MRC glitters. For example, the highest concentration of Na, V, Mn, Fe, Al, Si, Mg, K, Ti, Cr and Tl were detected in Mica whilst MRC1 glitter had the highest concentration of S and Ni and MRC2 had the highest concentration of Cu, Co and Pb (Table 1). PET glitter had the highest concentration of Ag and Sb.

3.2. Effects of glitter (physical and chemical) on Lemna minor

3.2.1. Effects of glitter on change in the number of fronds

The change in number of fronds under the control conditions for the three separate experiments (glitter, leachate and aged) were not significantly different after two weeks of growth ($F_{2,12}$ = 0.59, p = 0.572) with a mean (± SEM) change in fronds for the controls of 19 ± 1.1 (Fig. 2a-c).

There were significant increases in the number of fronds in the controls, and also when exposed to some of the different concentrations of the glitters, except any of those made of PET (Table S1a). There was a significant difference between the applied doses ($F_{2,64}$ = 4.26, p = 0.018), but not between the glitter types (Table 2a), with ~1.4 more fronds when exposed to 10 mg mL^{-1} and ~1.6 more when exposed to 100 mg mL^{-1} compared to *L. minor* exposed to 1000 mg mL^{-1}. When *L. minor* was exposed to leachate, the number of fronds significantly increased in all treatments except for kaolinite and MCR1 at 10 mg mL^{-1} (Table S2a). The number of fronds was significantly greater with MCR1 at 1000 mg mL^{-1} (Dunnet test p < 0.001) compared to the control, with ~2.2 times more fronds (Fig. 2b). There was a significant interaction between the type of glitter and dose ($F_{8,64}$ =12.1, p < 0.001), where *L. minor* with MRC1 at 1000 mg mL^{-1} had a greater increase in fronds than the other glitters applied at 10 mg mL^{-1}. When exposed to aged glitter, the number of fronds significantly increased for most treatments (Fig. 2c; Table S3a), but none were significantly different from *L. minor* grown under control conditions (Table S3b). This was mirrored by no detectable differences between the types ($F_{4,63}$ =

Table 1
Analytical results of elements (μg/g) detected by ICP-OES or ICP-MS in either PET, MRC1, MRC2 or MICA glitter (mean ± SD, n = 3).

	Analysis using ICP-OES			
Element	**PET**	**MRC1**	**MRC2**	**MICA**
Na	111.5 ± 62.36	778.31 ± 31.07	725.73 ± 18.41	1091.69 ± 48.3
S	11.93 ± 1.42	404.41 ± 14.98	168.43 ± 5.29	49.63 ± 5.1
V	0.34 ± 0.08	0.59 ± 0.15	5.09 ± 0.38	225.39 ± 9.65
Mn	8.32 ± 1.57	0.75 ± 0.06	3.7 ± 0.13	64.58 ± 2.78
Fe	14.49 ± 6.66	6.13 ± 4.98	614.51 ± 20.47	1618.14 ± 41.42
Cu	0.27 ± 0.08	48.65 ± 4.04	87.91 ± 11.88	< LOD*
Zn	1.38 ± 0.53	34.06 ± 2.66	59.53 ± 7.85	6.09 ± 0.67
Al	1372.42 ± 191.17	1960.98 ± 51.53	1255.78 ± 51.53	42,493.49 ± 1879.21
Si	216.53 ± 20.28	99.55 ± 18.61	< LOD	228.54 ± 105.28
Mg	40.06 ± 17.98	0.99 ± 0.38	1920.6 ± 68.39	71,251.16 ± 2264.25
K	< LOD	< LOD	1338.55 ± 48.77	51,275.51 ± 2649.27
Ti	< LOD	1.46 ± 0.16	39.29 ± 3.63	193.4 ± 15.21
	Analysis using ICP-MS			
Cr	251.59 ± 70.84	663.24 ± 931.08	185.59 ± 11.06	1260.58 ± 85.27
Co	196.17 ± 89.1	153.46 ± 144.66	314.11 ± 234.97	90.67 ± 8.71
Ni	54.35 ± 21.15	649.9 ± 551.42	432.78 ± 16.06	443.94 ± 18.27
Ag	110.39 ± 69.61	49.39 ± 20.76	44.49 ± 44.26	< LOD
Sb	82,559.03 ± 10,921.35	1.58 ± 6.91	2.18 ± 15.25	166.45 ± 268.65
Ti	< LOD	< LOD	7.78 ± 3.15	110.24 ± 9.48
Pb	68.33 ± 8.17	2209.09 ± 58.05	4879.7 ± 543.68	585.71 ± 43.76

* LOD = limit of detection

0.87, p = 0.488), nor the applied doses ($F_{2,63}$ = 0.35, p = 0.709) (Table 2c).

3.2.2. Effects of glitter on change in root length

In all three experiments, duckweed roots had a starting length of 10 mm, the final root length after 14 days was measured and used to calculate the change in root length over this duration. The change in root length under control conditions for the three separate experiments (glitter, leachate and aged) were not significantly different ($F_{2,12}$ = 1.49, p = 0.263) with a mean (± SEM) change in root length during the experimental period of 28 ± 1.9 mm. When exposed to fresh glitter (physical and chemical), the roots of *Lemna minor* all significantly increased in length (Table S1c) regardless of the treatment and the status of the glitter (Fig. 3a) except for MRC1 and MRC2 at 1000 mg L^{-1}. In addition, when *L. minor* was exposed to the highest concentration (1000 mg L^{-1}) of both glitters made of MRC the roots grew significantly less than the other treatments (Fig. 3a), with ~2.4 times less growth compared to the controls for MRC1 (Dunnett p = 0.001) and ~9.4 times less for MRC2 (Dunnett p = 0.001). A measurable effect of these glitters was also detected at the 100 mg L^{-1}. This pattern mirrored root growth of *L. minor* when exposed to the leachate from glitter (chemical) (Fig. 3b), where all roots significantly increased in length (Table S2d), but there was a significant effect of the dose and the type of glitter; with *L. minor* having significantly less root growth when exposed to 1000 mg L^{-1} of MRC1 (Dunnett p = 0.001) compared to the control (~1.9 reduction). When exposed to aged glitter (physical), all roots significantly increased in length (Fig. 3c), but there was no significant difference between the glitter type nor their dose.

3.2.3. Effects of glitter on change in biomass

The control conditions for the separate experiments (glitter, leachate and aged) were not significantly different ($F_{2,12}$ = 0.51, p = 0.611) with a mean (± SEM) change in biomass for the controls of 51 ± 2.9 mg (Fig. 4a-c).

Under all treatments of fresh glitter, *L. minor* significantly increased in biomass (Table S1e) except when exposed to 1000 mg mL^{-1} of MRC1 and 100 or 1000 mg mL^{-1} of MRC2. There also was a significant difference between the types of plastic (Table 2a), but with an interaction between the applied dosage ($F_{8,64}$ = 2.27, P = 0.034) and this was only significant when compared to the control for MRC2 at 100 mg mL^{-1} (~1.7 time less, Dunnett p = 0.013) and 1000 mg mL^{-1} (~10.9 times less, Dunnett p < 0.001; Table S1f). When *L. minor* was exposed to leachate from glitter the effects were less pronounced (Fig. 4b), with no significant differences between the controls and treatments at any dose (Table S2e). There was a significant difference between treatments, with PET and MRC2 at 1000 mg mL^{-1} being less than MRC1 at 100 and 1000 mg mL^{-1}. With aged glitter, the biomass of *L. minor* significantly increased (Table S3e), except MRC2 at 10 mg mL^{-1} (t-test p = 0.003). The biomass was significantly different depending on the added dose (Fig. 4c), regardless of the glitter source (10 vs 100 mg mL^{-1} Tukey p = 0.017; and 100 vs 1000 mg mL^{-1} Tukey p = 0.045).

3.2.4. Effects of glitter on the total chlorophyll content of the fronds

Once again there were no significant differences between the controls of the three separate experiments ($F_{2,12}$ = 0.05, p = 0.954), with a mean (± SEM) chlorophyll content of 1332 ± 77 μg g^{-1} (Fig. 5a).

When exposed to fresh glitter, the chlorophyll content of *L. minor* exposed to MRC2 at 1000 mg mL^{-1} was significantly different from the

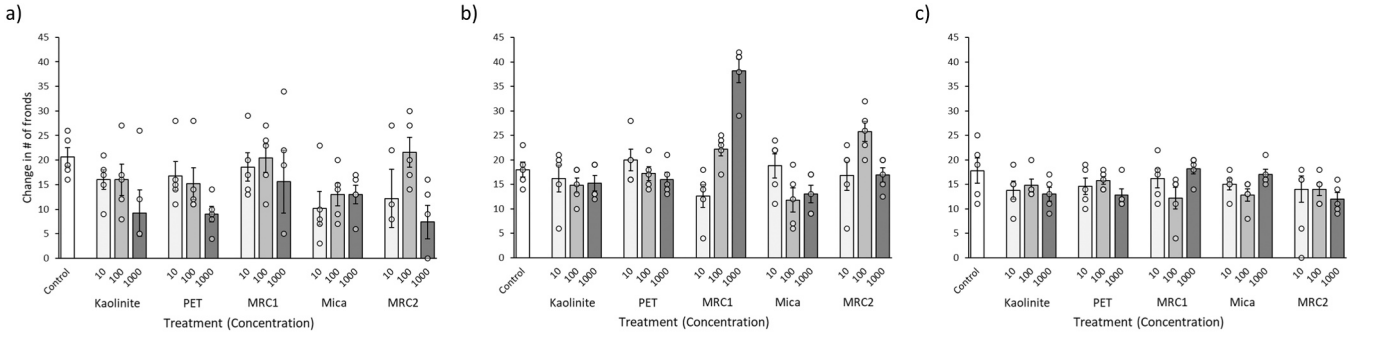

Fig. 2. Change in the number of fronds of *L. minor* after 14 days exposure to different types of glitter at increasing concentrations (mg mL^{-1}) for (a) fresh glitter, (b) leachate from glitter and (c) aged glitter. MRC = modified regenerated cellulose, either with (MRC1) or without (MRC2) an acrylic coating. Mica represents the synthetic mica treatment. Bars represent means (± SEM, n = 5) and superimposed dots are the raw values. Due to loss of a sample, n = 4 for MRC2 at 10 mg mL^{-1} for the aged glitter experiment.

Table 2
Summary of ANOVA results for the raw glitter experiments for change (Δ) in number of fronds, root length (mm), biomass (mg), and chlorophyll content (μg g^{-1} dry biomass) (a) fresh glitter, (b) leachate and (c) aged glitter. Data are degrees of freedom of the numerator (df_1) and denominator (df_2) to calculate F-values, with associated superscript p-values, and significance at α = 0.05 is highlighted in bold.

(a) Fresh glitter Source of variation:	df_1, df_2	Δ fronds	Δ root length	Δ biomass	Chlorophyll
Treatment	15, 64	$1.57^{0.108}$	**$15.63^{<0.001}$**	**$6.71^{<0.001}$**	**$6.09^{<0.001}$**
C†. vs others	1, 64	$3.07^{0.085}$	**$4.29^{0.042}$**	**$5.65^{0.021}$**	$0.09^{0.767}$
Glitter (G)	4, 64	$1.30^{0.278}$	**$29.91^{<0.001}$**	**$15.28^{<0.001}$**	**$8.22^{<0.001}$**
Concentration (Co)	2, 64	**$4.26^{0.018}$**	**$22.88^{<0.001}$**	**$7.85^{0.001}$**	**$6.86^{0.002}$**
G × Co	8, 64	$0.84^{0.527}$	**$8.09^{<0.001}$**	**$2.27^{0.034}$**	**$5.58^{<0.001}$**
(b) Leachate Source of variation:	df_1, df_2	Δ fronds	Δ root length	Δ biomass	Chlorophyll
Treatment	15, 64	**$9.69^{<0.001}$**	**$8.99^{<0.001}$**	**$4.02^{<0.001}$**	**$7.07^{<0.001}$**
C†. vs others	1, 64	$0.03^{0.863}$	$0.38^{0.542}$	$0.42^{0.521}$	$0.06^{0.807}$
Glitter (G)	4, 64	**$11.01^{<0.001}$**	**$7.63^{<0.001}$**	**$6.25^{<0.001}$**	**$17.09^{<0.001}$**
Concentration (Co)	2, 64	$2.64^{0.079}$	**$37.14^{<0.001}$**	**$5.16^{0.084}$**	**$4.76^{0.012}$**
G × Co	8, 64	**$12.01^{<0.001}$**	**$3.71^{0.001}$**	**$3.08^{0.054}$**	**$3.51^{0.002}$**
(c) Aged glitter Source of variation:	df_1, df_2‡	Δ fronds	Δ root length	Δ biomass	Chlorophyll
Treatment	15, 63	$1.44^{0.159}$	$1.34^{0.208}$	$1.82^{0.052}$	**$2.02^{0.028}$**
C†. vs others	1, 63	**$4.12^{0.047}$**	$1.21^{0.275}$	$0.65^{0.422}$	$1.31^{0.257}$
Glitter (G)	4, 63	$0.87^{0.488}$	$1.86^{0.128}$	$0.96^{0.438}$	**$5.27^{0.001}$**
Concentration (Co)	2, 63	$0.35^{0.709}$	$2.15^{0.125}$	**$4.77^{0.012}$**	$0.17^{-0.843}$
G × Co	8, 63	$1.66^{0.127}$	$0.89^{0.533}$	$1.65^{0.129}$	$0.94^{0.487}$

†Contrast of the mean of the Control (C) versus the mean of all other treatments (others). ‡ Residual degrees of freedom was affected for this experiment due to loss of one sample.

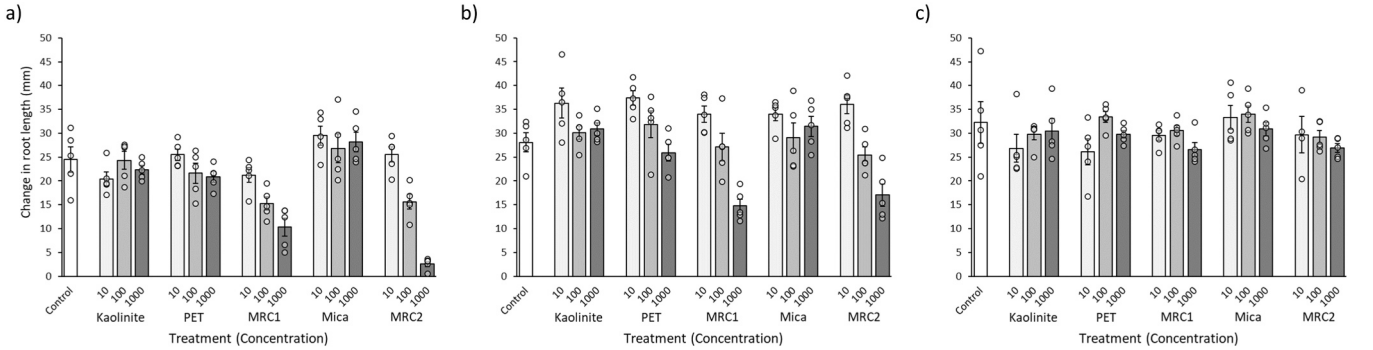

Fig. 3. Change in root length (mm) of *L. minor* after 14 days exposure to different types of glitter at increasing concentrations (mg mL^{-1}) for (a) fresh glitter, (b) leachate from glitter and (c) aged glitter. PET = polyethylene terephthalate, MRC = modified regenerated cellulose and Mica represents the synthetic mica treatment. Bars represent means (± SEM, n = 5) and superimposed dots are the raw values. Due to loss of a sample, n = 4 for MRC2 at 10 mg mL^{-1} for the aged glitter experiment.

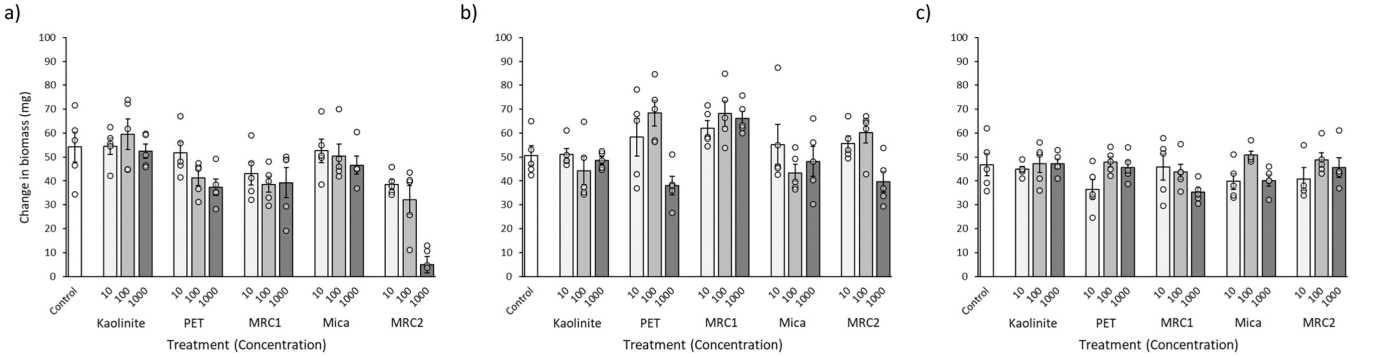

Fig. 4. Change in biomass (mg) of *L. minor* after 14 days exposure to different types of glitter at increasing concentrations (mg mL^{-1}) for (a) fresh glitter, (b) leachate from glitter and (c) aged glitter. MRC = modified regenerated cellulose, Mica represents the synthetic mica treatment. Bars represent means (± SEM, n = 5) and superimposed dots are the raw values. Due to loss of a sample, n = 4 for MRC2 at 10 mg mL^{-1} for the aged glitter experiment.

control (Dunnett p = 0.001; Table S1g) with ~2.8 times less chlorophyll in the fronds compared to the control. When compared to the other treatments, chlorophyll in fronds exposed to MRC2 at 1000 mg mL^{-1} was also significantly ($F_{4,64} = 8.22$, $p < 0.001$; Table 2a) less than that in every other treatment (Tukey $p < 0.01$). *L. minor* exposed to leachate from glitter showed a different pattern, with chlorophyll in fronds exposed to MRC2 at 100 mg mL^{-1} ~1.5 times significantly greater than the control (Dunnett p = 0.002; Table S2g). Chlorophyll content was also significantly different amongst glitter types and doses ($F_{8,64} = 3.51$, p = 0.002); Table 2b), with MRC2 at 100 mg mL^{-1} having the strongest

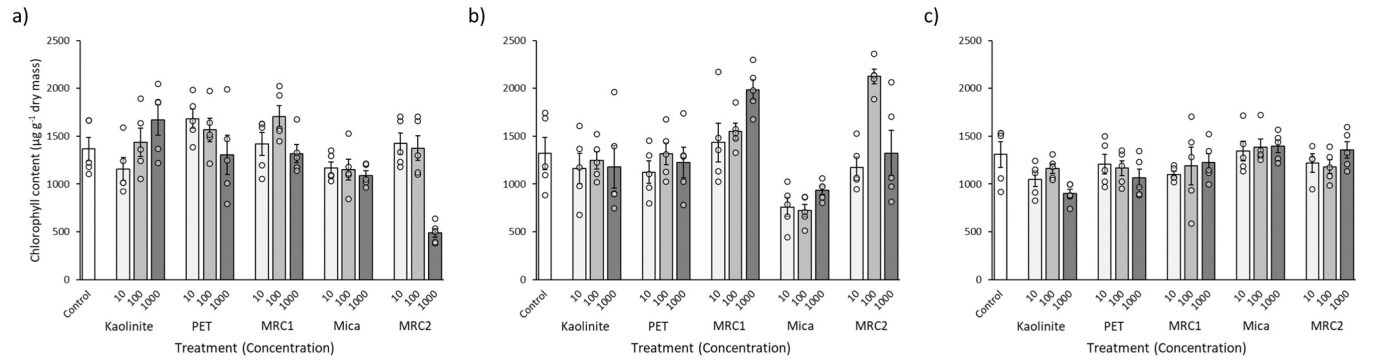

Fig. 5. Total chlorophyll (a and b) content (µg g^{-1} dry biomass) of *L. minor* after 14 days exposure to different types of glitter at increasing concentrations for (a) fresh glitter, (b) leachate from glitter and (c) aged glitter. MRC = modified regenerated cellulose, Mica represents the synthetic mica treatment. Bars represent means (± SEM, n = 5) and superimposed dots are the raw values. Due to loss of a sample, n = 4 for MRC2 at 10 mg mL^{-1} for the aged glitter experiment.

significant difference with PET at 1000 mg mL^{-1} (Tukey p = 0.002). When exposed to aged glitter, the chlorophyll content of *L. minor* was not significantly different from the controls (Table S3g; Fig. 5c). There was a significant difference between the glitter treatments ($F_{8,63} = 5.27$, p = 0.001; Table 2c) with *L. minor* exposed to Mica having significantly more chlorophyll than when exposed to kaolinite (Tukey p < 0.001).

4. Discussion

Overall, there were minimal effects of PET glitter, synthetic mica glitter, kaolinite or aged glitters of any kind on the measured variables from *L. minor*. The high concentrations (100 – 1000 mg L^{-1}) of fresh, biodegradable glitters manufactured of modified regenerated cellulose (MRC1 and MRC2) elicited the strongest effects, with decreasing root length, biomass and chlorophyll content (MRC2 only). At a wider scale, a decrease in these variables could compromise the ecosystem services that duckweed provides. For example, duckweeds provide habitat (a.k.a. the "phyllosphere") for microbial communities such as bacteria (O'Brien et al., 2020) and diatoms (Goldsborough, 1993), are a nursery ground for other species and are an important food source for birds and fish (Van Hoeck et al., 2015).

The leachate from the MRC type glitters (representing chemical effects) also caused a reduction in root length, but this was less pronounced than when exposed to fresh glitters (representing physical and chemical effects). The observed responses of *L. minor* when exposed to MRC glitters are, therefore, due to a combination of physical and chemical characteristics of the glitter. Many plastic additives can be easily released into the aquatic environment, becoming available to organisms (Larue et al., 2021). The release of additives from microplastics increases with time and pH, but leaching rate depends on the type of water (Luo et al., 2019). Leachate, isolated from the microplastics particles, can be toxic to a range of aquatic primary producers (Larue et al., 2021). Given that the aged MRC glitters had minimal effects in our study, it is possible that biodegradable glitters could lose toxicity as they age. The toxicity of aged microplastics is uncertain, with some studies finding aging to increase toxicity (e.g. on microalgae; Wang et al., 2020) and others, similar to the current study, finding aging to decrease toxicity (e.g. on an aquatic plant; Pflugmacher et al., 2021). Regarding duckweed, Kokali et al. (2019) found that whilst 100 mg L^{-1} of fresh polyethylene microplastic beads caused a reduction in root length of *L. minor*, when microbeads were aged in low organic-load waters this effect did not occur. However, when aged in high organic-load waters the reduction in root length also occurred (Kokalj et al., 2019). Moreover, 10 and 100 mg L^{-1} of polyethylene microplastics with absorbed silver caused a greater reduction of duckweed root length than pristine microplastics (Kalčíková et al., 2020). This suggests that the effects of aging of microplastics are dependent on the type of water and on the presence of additional chemical compounds, including organic matter.

The current study suggests that the effects on *L. minor* were at least partly due to the chemical characteristics of the modified regenerated cellulose glitters. It is likely that the leaching of degradation by-products on the surface coatings accounts for the observed effects, albeit the exact mechanism remains unknown. MRC glitters caused the strongest effect and contained high concentrations of lead, copper and zinc, it is possible that the leaching of these elements into the water caused the observed responses of *L. minor* as each of these is known to have inhibitory effects on the growth of duckweed (lead: Sobrino et al., 2010; copper and zinc: Dirilgen and İnel, 1994).

In the present study we mostly chemically analysed metals, but there are likely other compounds present in and on the glitters. For instance, extracts from cellulose- and starch-based plastics contained more additional chemicals (many of which were indicative of surface coatings and plasticisers) than conventional plastics tested and triggered stronger in vitro toxicity (Zimmermann et al., 2020). We concur with Zimmermann et al. (2020) that we need to focus more on aspects of chemical safety when designing "eco-friendly" plastic alternatives, especially for items designed for single use such as glitters. Indeed, perhaps in the future avoiding the addition of surface coatings could help reduce toxicity, while finding ways to maintain the shimmer characteristics. Recently, biodegradable glitter made from structurally coloured cellulose nanocrystal films (Droguet et al., 2021) have been developed, which avoids the need for a surface coating. Independent ecotoxicity testing of any new glitter should be done to ensure it does not pose similar risks as other (cellulose-based) glitters. There is evidence that a majority of consumers are willing to pay more for materials perceived to be sustainable (i.e. food packaging; Herrmann et al., 2022) and, as such, alternative materials, including those which are biodegradable, are likely to become more dominant on the marketplace. However, before their widespread adoption, we need to assess what their biological and ecological effects are and how they differ from those posed by conventional plastics.

The ecotoxicological effects of glitter are still very under-represented in the literature and require further attention. Green et al. (2021) found that after 36 days the root length of *L. minor* was decreased by high concentrations (60 mg L^{-1}) of PET and mica glitters as well as by MRC glitter. The lack of effects from PET glitter in the current study could be due to the shorter duration (14 versus 36 days) of the experiments. It is possible that by-products of biodegradable plastics leach faster than those of conventional plastics because they are designed to break down, therefore, effects from more persistent PET glitters may only arise after a longer period. Future research should test the effects and impacts of different glitter types over a longer period. This duration should be at least long enough to allow for the full biodegradation of the MRC glitters (i.e. purportedly <56 days for 90% biodegradation of MRC2 according to TÜV Austria OK Biodegradable WATER certification).

CRediT authorship contribution statement

Dannielle Green: Conceptualisation, Methodology, Resources, Writing – original draft preparation, Supervision. **Bas Boots**: Conceptualisation, Resources, Formal analysis, Visualisation, Writing – original draft preparation. **Brigitta Kovacs**: Investigation, Writing – review & editing. **Francesca De Falco**: Investigation, Writing – review & editing. **Emanuele Lupo**: Investigation, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors like to thank all technical staff at Anglia Ruskin University for their assistance in the laboratory, in particular Leon Stone and Julia MacKenzie. This research was funded by Research England Quality-Related (QR) funding awarded to D.S.G. and B.B. by the Faculty of Science and Engineering, Anglia Ruskin University.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115291.

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