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RESEARCH ARTICLE

Plastic microbeads from cosmetic products: an experimental study of their hydrodynamic behaviour, vertical transport and resuspension in phytoplankton and sediment aggregates

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Hydrodynamic behaviour and the transport pathways of microplastics within the ocean environment are not well known, rendering accurate predictive models for dispersal management of such pollutants difficult to establish. In the natural environment, aggregation between plastic microbeads and phytodetritus or suspended sediments in rivers and oceans further complicate the patterns of dispersal. In this laboratory study, the physical characteristics and hydrodynamic behaviour of a selection of common plastic microbeads, as used in exfoliation skincare cosmetic products, were investigated. Additionally, the potential for aggregation of these microbeads with phytodetritus and suspended sediments, as well as the subsequent sinking and resuspension behaviour of produced aggregates, were investigated with roller tanks, settling columns and erosion chamber. Physical characteristics of the plastic microbeads showed great heterogeneity, with various densities, sizes and shapes of plastic material being utilised in products designed for the same purpose. The majority of the plastics investigated were positively buoyant in both freshwater and seawater. Aggregation between plastic microbeads and phytoplankton was observed to be swift, with even extremely high concentrations of plastics being rapidly scavenged by suspended algal material. Following aggregation to sizes of 300 to 4400 μm diameter, some formerly buoyant plastics were observed to settle through the water column and enter the benthic boundary layer with settling velocities ranging between 32 and 831 m day^{-1}. These aggregates could be resuspended in the laboratory under critical shear velocities of 0.67–1.33 cm s^{-1} (free stream velocities of > 10 cm s^{-1}). This rapid aggregation and subsequent settling indicates a potentially important transport pathway for these waste products, a pathway that should be considered when modelling discharge and transport of plastic microbeads and determining the ecosystems that may be at risk from exposure.

Keywords: Plastic Microbeads; Personal Care Products; Marine Pollution; Microplastic Transport Pathways; Microplastic Sink; Diatom Aggregates

Introduction

The global distribution of plastic pollution in the aquatic environment is a current topic in pollution research and policy making. Though the presence of large-sized plastic wastes in the world oceans has been studied for decades, microplastics and their potential impacts on ecosystem functioning has recently come to prominence as a topic of serious concern (Ivar and Costa, 2014). A common source of primary microplastics are the microbeads found in consumer skincare products, such as facial cleansers, body and shower scrubs and tooth pastes. These can be characterized as synthetic, non-degradable, water insoluble, solid materials comprising a range of polymers and additives (Leslie, 2014) which vary greatly in size (100 μm to more than 1000 μm), shape (from amorphic to spherical) and quantity used in different commercial products (Fendall and Sewell, 2009). After use, products containing microbeads are designed to be washed down the drain with household wastewater (Tanaka and Takada, 2016). Microbeads have been found in the Great Lakes, in rivers, on beaches, and in subtidal sediments, wastewater effluents and the coastal and pelagic ocean worldwide (Eriksen et al., 2013; Leslie, 2014; Castañeda et al., 2014; Eerkes-Medrano et al., 2015; Cheung and Fok, 2016; Isobe, 2016). Various studies have shown that waste water treatment plants are often incapable of capturing microplastics efficiently during the purification process (Chang,

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2015; Rochman et al., 2015; Lechner and Ramler, 2015; Mintenig et al. 2017) and that freshwater systems transfer plastic debris into the ocean (Moore, 2008; Thompson, 2010). Microbeads are now recognised as a significant contaminant of the marine environment (Browne, 2015; Napper et al., 2015; Rochman et al., 2015).

Although numerous studies on identification, quantification and characterization of plastics at sea have been published (e.g., Morét-Ferguson et al., 2010; Hidalgo-Ruz et al., 2012), the transport pathways and deposition sites (particularly for small microplastics) remain poorly understood. A large proportion of produced plastics remain buoyant and are dispersed by winds and currents over long distances, accumulating in areas of confluence such as gyres, eddies and fronts (Moore et al., 2001; Hammer et al., 2012; Eriksen et al., 2013). The density of plastic varies greatly with composition and structure, commonly ranging from 0.83 to 1.38 g cm^{-3} (Andrady, 2011). This range of densities has resulted in debris being observed in many contrasting marine environments, including neustonic (sea surface microlayer), benthic and coastline environments (Barnes et al., 2009; Thomson, 2010; Andrady, 2011; Cole et al., 2011; Bergmann et al., 2012). The vertical distribution of plastic debris concentrations has been documented to drop exponentially with water depth, with smaller material plastic fragments tending to be less buoyant (Reisser et al., 2014). Quantities of microplastics (< 4.75 mm) at the sea surface have been measured to be less than models have predicted, given the estimated release rates of material to the environment, suggesting a removal of plastic fragments of a few mm or less from surface waters on a large scale (Cózar et al., 2014; Eriksen et al., 2014). These removal processes may result from rapid degradation of microplastics into microscale-sized objects not observed in these published studies or by ballasting processes such as biofouling, consumption by organisms, entrainment in settling detritus, downwelling and/or beaching. Although these processes are partially understood, the localisation and quantification of microplastics in the marine environment remains largely unknown (Thompson et al., 2004; Ballent et al., 2013).

The objective of this study was to experimentally investigate microplastic removal via entrainment in settling phytodetritus and subsequent potential for transport within the benthic boundary layer. The hypothesis was that the aggregation of originally near-buoyant microbeads (from commercial skincare products) with organo-mineral aggregates may result in vertical export from upper ocean layers to the seafloor, and subsequent lateral transport following resuspension. The output from the study should improve transport models for these plastic waste materials, following their release from water treatment plants into the riverine and marine environments.

Methods

To improve our understanding of the transport and fate of microbeads in the environment, a number of experimental studies were conducted. These were aimed at gauging the range in physical size of microbeads within and between different cosmetic products for facial cleansing, investigating how these microbeads behave in water and how they may aggregate in the environment with algal detritus or sediments. The behaviour of these aggregates was also examined to determine how microbead transport pathways may be changed by aggregation events. The methodologies employed, and the statistical approaches to analysis of the results, are described below.

Characterization of microbead size, shape and buoyancy

Six water-based facial and body cleansers containing microplastics (hereafter referred to as microbeads) were randomly selected, produced by a range of well-known companies and commonly available in German supermarkets (see Table S1 and Figure S1 for details). The total wet weight of each product was determined. Each product was then mixed with 0.75 L of warm water (40°C) and left to rest for 24 hours, to ensure full solubilisation of the creamy soap content of each product. The suspension was then filtered through a 63 µm filter, to extract the solid plastic material. The extracted microbeads were then washed thoroughly with purified water to remove any soap and dried at 50°C for 24 hours. The percentage weight of the extracted microbeads of the product material as a whole was determined. The microbeads were then examined under a standard dark field microscope with 40× magnification. The maximum lengths and widths of 150 individual microbeads were measured using the ImageJ software application (http://imagej.nih.gov). To determine the buoyancy variation between the microbeads from the different products, 50 mg of the plastic extracted from each product were added to small containers with 250 ml freshwater (16°C) and mixed with a vortex mixer for 30 seconds, to bring all material into suspension. These containers were then left to rest for 24 hours and vertical (upward buoyant or downward settling) motion of the plastic was observed within each of the containers. The full procedure for measuring buoyancy was then repeated with filtered (0.7 µm GF/F) North Sea seawater (salinity of 34; 16°C) for each product.

Microbead aggregation and aggregation behaviour experiments

To investigate how microbeads may behave and aggregate in the marine environment, environmentally relevant concentrations of microbeads and appropriate algal and sediment material were required for the experimental set-ups.

Microbead concentrations

Concentrations of 1,000 to 24,000 microbeads L^{-1} were prepared for the experiments. We considered this range sufficient to experimentally replicate plastic concentrations reported from sewage disposal pipes to offshore North Sea sediments (Leslie et al., 2013; Castañeda et al., 2014; Mintenig et al., 2017). We did not extract microplastics directly from the environment for the experimental studies, as we aimed to conduct reproducible work with materials of a reasonably uniform nature which we could determine in the laboratory.

Algal material

To simulate environmental exposure to algal material, the large pelagic centric diatom (~ 6–20 μm) *Thalassiosira weissflogii* (Bacillariophyceae) was selected as an appropriate phytoplankton species with which to carry out aggregation experiments. The species is abundant in the North Sea and tends to flocculate into marine snow during the decline of the annual spring bloom (Hoppenrath et al., 2007). Cultures of *T. weissflogii* were cultivated in filtered (0.7 μm GF/F) seawater at 16°C under daily illumination of 12 hours in a temperature-controlled laboratory. The algae were diluted in 1.15-L roller tanks to achieve concentrations of 10^6 cells L^{-1} prior to each experimental run (Seebah et al., 2014). Such a concentration is representative of a diatom bloom in coastal waters, where cell concentrations between 10^5 and 10^8 cells L^{-1} have been reported, and therefore is ecologically relevant for aggregation studies (Raymont, 1980; Venrick, 1998).

Sediment material

To simulate exposure to organo-mineral particulate material in the water column, as may occur during periods of marine sediment resuspension or during riverine transport, riverbed samples from the Weser and Ems estuaries were used in experimental runs (Riethmüller et al., 1988; Grabemann et al., 1997; Van de Kreeke et al., 1997).

Aggregation methodology

To determine how microbeads may aggregate with various materials in the environment, a series of experimental aggregation experiments were carried out, in which differing concentrations of plastic microbeads, diatom cells and river sediments were used (**Table 1**). The first experimental set-up (set-up 1) involved a range of environmentally high concentrations of microbeads, with no diatom cells or river sediments added; set-up 2 used the same range of high microbead concentrations but with *T. weissflogii* cells also added. Set-up 3 involved a range of moderate concentrations of microbeads with *T. weissflogii* cells; set-up 4 used the same range of moderate microbead concentrations with *T. weissflogii* cells but with riverine sediments also added. In set-ups 2–4, one treatment was incubated with *T. weissflogii* cells only (no microbeads or sediments) to obtain a standard for natural aggregation. For all aggregation experiments, a standard roller tank setup was utilized, with aggregations monitored over a 72-hour period. Aggregation tanks, housed in a temperature-controlled laboratory at 16°C, were filled with filtered (0.7 μm GF/F) North Sea water (salinity of 34).

To assess how rapidly turbidity changed over time in the experimental runs (**Table 1**), 4.5 ml samples were carefully removed from each roller tank at discrete time intervals (0, 1, 2, 4, 8, 12, 24, 48, 72 hours). Aggregates were allowed to settle for about 5 minutes before sampling, and turbidity (in Nephelometric Turbidity Units, NTU) was measured

Table 1: Design of multifactorial experiments with 15 treatments investigating aggregation rates of various microbead quantities with algal detritus and riverine sediments in seawater. DOI: https://doi.org/10.1525/elementa.317.t1

Set-up[a]	Treatment #	Concentration of algae (*T. weissflogii*), plastic microbeads, and sediments			
		Algal content (total # of cells)	Plastic content		Sediment content (mg L^{-1})
			(mg L^{-1})	(estimated total # of microbeads)	
1	1	0	261	24000	0
	2	0	174	16000	0
	3	0	87	8000	0
2	4	10^6	261	24000	0
	5	10^6	174	16000	0
	6	10^6	87	8000	0
	7	10^6	0	0	0
3	8	10^6	43	4000	0
	9	10^6	22	2000	0
	10	10^6	11	1000	0
	11	10^6	0	0	0
4	12	10^6	43	4000	130
	13	10^6	22	2000	130
	14	10^6	11	1000	130
	15	10^6	0	0	0

[a] In all cases, 1.15-L experimental roller tanks were used, with North Sea water of salinity 34 at 16°C.

with an AquaFluor (Turnerdesigns.com) turbidity meter. Three replicate measurements were taken for each time interval during each experimental run. Results were then averaged and are provided with standard deviations in the supplementary materials (Table S2). As turbidity is a proxy for the concentration of fine particles in suspension, a reduction of turbidity over time was inferred to represent the removal of fine particles from suspension via aggregation. Aggregates were photographed in suspension using a fixed focal length particle camera (Imaging source DFK-41F02) with back illumination at the end of the 72-hour incubation time. The camera took 20+ images of ~ 1 cm area with a shallow focal depth of ~ 1 mm. The maximum lengths of 50 'in-focus' aggregates captured in these images were measured using 'Image J'.

Settling velocity of aggregates

Settling velocities of the 72-hour aggregates formed in each of the experimental aggregation runs were determined following the method of Pabortsava et al. (2011). Aggregates were carefully siphoned with a tube of 1-cm diameter to the top of a 1-m settling column and allowed to settle through filtered (0.7 μm GF/F) North Sea water of salinity 34. Sinking aggregates were photographed with the DFK-41F02 camera with back illumination. Images were taken every 2 seconds for a 60-minute period. A fishing line of 250-μm diameter was used for image size calibration. Subsequent frames were combined into image stacks using 'Image J'. For 'in-focus' aggregates within a stack image, the size and distance travelled by each aggregate through successive frames were measured. From these measurements settling rates (m day^{-1}) were computed.

Critical shear velocity for aggregate resuspension

To determine experimentally the likely resuspension behaviour of the 72-hour aggregates following settlement to the seafloor through the benthic boundary layer, critical shear velocities of aggregates were measured following the method described in Pabortsava et al., (2011). Briefly, the 72-hour aggregates from each aggregation experiment were carefully siphoned into separate erosion chambers (Thomsen and Gust, 2000) in the temperature-controlled laboratory at 16°C and allowed to settle to the chamber floor. Following settlement, flow velocities were slowly increased in each chamber to determine the flow conditions under which the various aggregates would resuspend and laterally advect. The critical shear velocity u^*_{crit} for bedload and suspended transport of these aggregates was determined by raising the u_* by 0.1 cm^{-1} every 5 minutes. Qualitative observations of aggregate behaviour were also documented.

Statistical analysis of aggregate size, turbidity and settling velocity results

Rates of aggregation within each experimental set-up were compared with two-way ANOVA tests. The two independent variables used in each set-up test were 'time' in hours since start of experimental run (9 levels: 0, 1, 2, 4, 8, 12, 24, 48, and 72 hours) and 'treatment' (3–4 levels in each set-up, representing the various roller tank contents, as detailed in **Table 1**). The dependent variable was 'turbidity' (NTU), as a proxy indicator of aggregation. A one-way ANOVA was used to determine whether there was a significant difference in aggregate sizes 72 hours after delivery of the contents to each roller tank used in experimental set-ups 1–4 (the independent variable was 'experimental treatment', and the dependent variable was 'aggregate size' in μm). A further one-way ANOVA was used to determine if particle settling rates differed significantly between treatments (the independent variable was 'experimental run', i.e., settling aggregates from each set-up, with the dependent variable 'settling rate' in cm $hour^{-1}$ as measured for each in-focus aggregate photographed in the experimental runs). Where appropriate, post-hoc tests were used to determine which treatments resulted in statistically significant differences. Levene's test of homogeneity was used to assess whether or not the variability in all comparisons of data was homogeneous or not. In situations where the data distribution differed significantly between treatments by Levene's test, Welch's robust F ANOVA test statistic is reported.

Results

Physical and hydrodynamic characteristics of microbeads

The percentage weight of microbeads in the products investigated ranged from 2.08 to 5.86% per milligram wet weight of the retail products. Products 1 and 5 had the highest plastic concentrations of 5.83 and 5.86% by volume, respectively (Table S1).

The shape and size distribution of plastic particles were highly heterogeneous. The majority of microbeads were not uniform and spherical but showed a variety of irregular shapes (**Figure 1**). All products had a groundmass consisting of small, white, amorphous particles that were hardly visible to the human eye within the cleanser but could be sensed on the skin when used. In addition, most products (all but product 2) contained larger, coloured, spherically shaped microbeads that were easily distinguishable and visible to the human eye (**Figure 1**). Product 1 contained many microbeads that were long and thin and, due to their high surface area, easily broken into smaller fragments (**Figure 1a**). The plastic content of product 2 consisted of many (only) small, homogeneously sized particles, with the majority being round or elliptical in shape (**Figure 1b**). Product 3 contained primarily small particles, relatively uniform in shape, with a few larger blue spheres (**Figure 1c**). In product 4, diverse plastic particles were found: white amorphous shaped microbeads, light blue oblate spheroids and large, dark blue spheres (> 0.2 mm; **Figure 1d**). Product 5 contained primarily small particles, relatively uniform in shape, with a few larger green spheres also included in the mix (**Figure 1e**). Similar to products 3 and 4, product 6 consisted of amorphous plastic fragments and threads (**Figure 1f**) intermixed with occasional larger blue spheres. Maximum particle lengths ranged from 22 to 1586 μm across all six products tested. A physical description and size distribution of individual

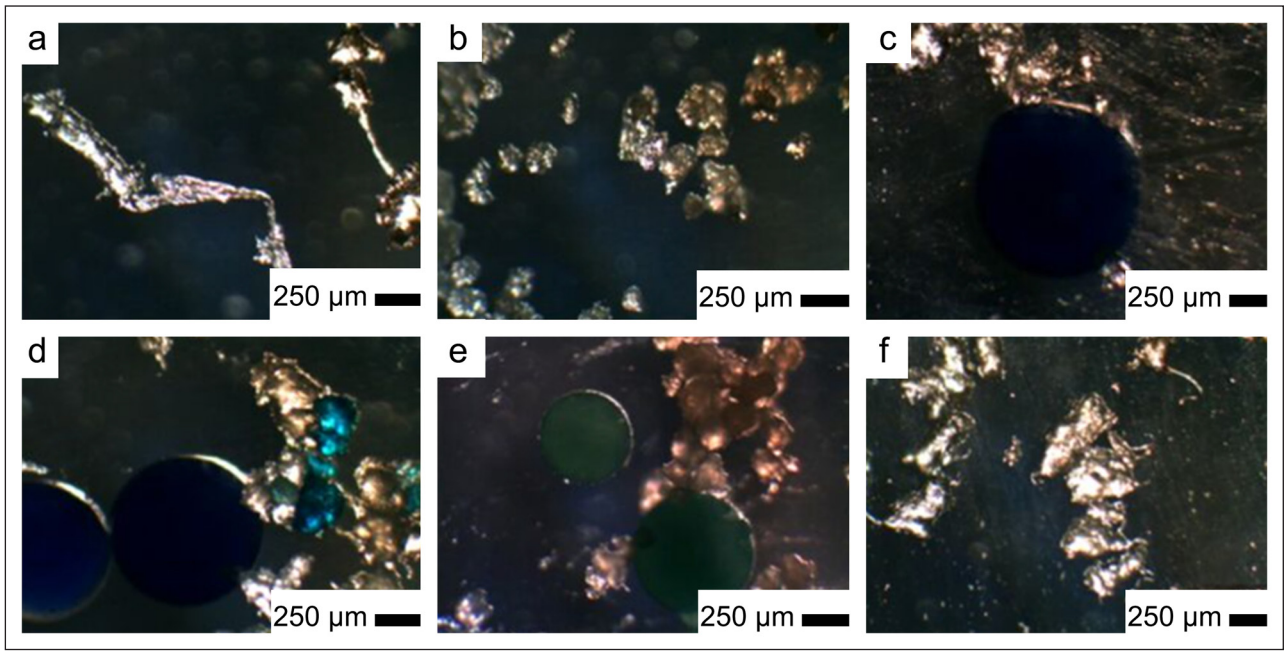

Figure 1: Images of microbeads extracted from personal care products. Microbeads were imaged under a standard dark field microscope with 40x magnification. All products contained small white amorphous particles **(a–f)**, while five (all but product 2) contained large spherical beads (readily visible in c–e; the larger beads present in products 1 and 6 were not captured by respective images in a and f). Product 1 (a) contained large, often long and thin microplastics. Panels a, b, c, d, e and f represent products 1, 2, 3, 4, 5 and 6, respectively. DOI: https://doi.org/10.1525/elementa.317.f1

Table 2: Size, shape and buoyancy characteristics of 150 individual microbeads extracted from products 1–6. DOI: https://doi.org/10.1525/elementa.317.t2

Product #	Particle length (µm)[a]			Buoyancy behavior in small containers		
	Maximum	Minimum	Mean ± STD[b]	Microbead description[a]	In freshwater	In seawater
1	1586	63	473 ± 231	white amorphous, blue spherical	positive	positive
2	636	82	177 ± 84	white amorphous, blue spherical	positive	positive
3	608	22	173 ± 131	white amorphous, blue spherical	positive (white), negative (blue)	positive (both)
4	555	76	242 ± 83	white amorphous, light blue elliptical, dark blue spherical	positive	positive
5	555	49	151 ± 68	white amorphous, green spherical	positive	positive
6	568	27	226 ± 118	white amorphous, blue spherical	positive	positive

[a] Determined by dark field microscopy.
[b] Standard deviation.

microbeads prior to aggregation experiments are provided in **Table 2** (see Dataset S1 for complete details).

Buoyancy observations in seawater showed that microbeads flocculated swiftly following cessation of vortex mixing and accumulated in a matter of seconds at the surface of each container. In general, the large spherical particles (where present in a product) exhibited the strongest positive buoyancy, though a proportion of smaller amorphous particles also accumulated on the surface within the first 30 seconds following mixing. Qualitative observations (Table S3) indicated that smaller particles remained the longest in suspension, rising to the surface within minutes rather than seconds. Across all products, a surface layer of microbeads had developed within all containers, with no particles visible in suspension after 24 hours. Microbeads from each product showed broadly similar

behaviour when placed in freshwater, though with less rapid buoyancy. Only product 3 demonstrated a slightly different behaviour: the large spherical beads in product 3 stayed in suspension for hours, eventually sinking to the bottom, indicating that these product microbeads had a higher density than the surrounding freshwater. After these preliminary investigations of size, shape and buoyancy of the microbead contents of these facial cleansing products, the majority of microbeads were characterized as buoyant, while only a small proportion of product 3 (blue spherical beads, estimated < 5% of product microbeads) demonstrated neutral and negative buoyancy in freshwater only. Product 3 was selected for further experimental studies to determine the aggregation, settling and lateral transport potential for the microbeads within this product in the environment.

Aggregate size distributions

The average and standard deviation of aggregate sizes formed in all roller tank runs are given in **Table 3**, with photographs of aggregates shown in **Figure 2**. Results are given below for each set-up, 1–4 (using microbeads from product 3) plus the phytoplankton-only control.

Set-up 1. After 72 hours in the roller tanks, experimental runs with an estimated 8000, 16000 and 24000 microbeads L^{-1} formed aggregates of a size range of < 400 to 2100 μm, giving a larger average size (836 ± 365 μm) than that of the individual unaggregated microbeads (173 ± 131 μm). An increase in microbead concentrations correlated positively with an increase in average aggregate size, though the comparably high standard deviations in these measurements rendered this observed increase statistically insignificant (**Table 3**; **Figure 3A**).

Set-up 2. Exposing 10^6 cells L^{-1} of *T. weissflogii* phytoplankton with high densities of microbeads (estimated at 8000, 16000, 24000 L^{-1}) in the roller tanks resulted in the formation of larger aggregates than were formed by microbeads alone, though these were smaller than aggregates formed by placing comparable cell densities of the algae in the roller tanks without any addition of microbeads (3194 ± 1917 μm; **Table 3**; **Figure 3B**). The mean size of these aggregates was 1991 ± 826 μm across all treatments, considerably greater than the average aggregate size produced in the roller tanks containing only microbeads (set-up 1). Aggregates formed in set-up 2 exhibited a greater size heterogeneity than those produced via set-up 1, ranging from < 600 to 4400 μm. The lowest concentration of microbeads used in set-up 2 (8000 L^{-1}) produced the greatest fraction of large aggregates, with the highest average aggregate length (**Table 3**).

Set-up 3. Moderate concentrations of microbeads (estimated at 1000, 2000, 4000 L^{-1}) and phytoplankton (10^6 cells L^{-1}) produced aggregates of smaller average size (1038 ± 342 μm) than those formed in set-up 2 (**Table 3**; **Figure 3C**).

Set-up 4. Moderate concentrations of plastic microbeads (estimated at 1000, 2000, 4000 L^{-1}), and phytoplankton (10^6 cells L^{-1}) additionally enriched with 130 mg L^{-1} of fine sediments, simulating aggregation processes in estuaries and coastal waters, resulted in formation of aggregates ranging from < 300 to 2800 μm diameter, with an average size of 1248 ± 644 μm, (**Table 3**; **Figure 3D**).

Table 3: Aggregate size distribution, turbidity decrease and settling velocity following a 72-hour aggregation period in roller tanks. DOI: https://doi.org/10.1525/elementa.317.t3

Set-up	Treatment	Contents	Estimated total # of microbeads	Resulting aggregates				
				Mean size (μm) ± STD[a]	Size range (μm)	Mean size (μm) ± STD across set-up	Turbidity decrease (%) over 72 hours	Settling velocity (m d^{-1})
1	1	microbeads only	24000	935 ± 446	370–2090	836 ± 365	75	buoyant
	2		16000	891 ± 347	480–2100		64	
	3		8000	682 ± 218	360–1270		72	
2	4	microbeads, phytoplankton	24000	1810 ± 807	520–3890	1991 ± 826	67	buoyant
	5		16000	1915 ± 686	540–3620		57	
	6		8000	2248 ± 920	850–4410		56	
3	8	microbeads, phytoplankton	4000	1144 ± 342	490–2100	1038 ± 342	54	91 ± 27
	9		2000	984 ± 375	380–1980		57	
	10		1000	987 ± 282	310–1810		48	
4	12	microbeads, phytoplankton, sediments	4000	1358 ± 731	270–2670	1248 ± 644	48	559 ± 178
	13		2000	1206 ± 642	350–2610		43	
	14		1000	1180 ± 545	280–2810		51	
2, 3, 4	7, 11, 15	phytoplankton only	0	3194 ± 1917	990–7650	3194 ± 1917	54	53 ± 22

[a] Standard deviation.

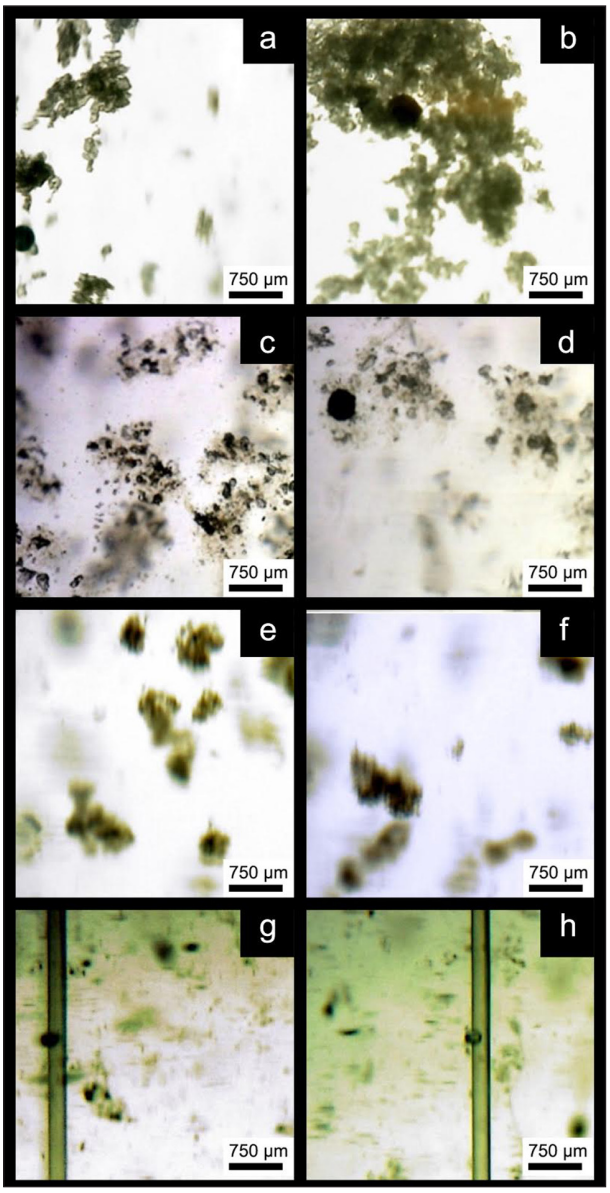

Figure 2: Photographs of aggregates following a 72-hour aggregation period in the roller tanks. Set-up 1 **(a, b)** included high concentrations of microbeads only, estimated at 24000 and 16000 L^{-1} (treatments 1 and 2, respectively); set-up 2 **(c, d)**, same high concentrations of microbeads, aggregated with 10^6 cells L^{-1} of *T. weissflogii* (treatments 4 and 5, respectively); set-up 3 **(e, f)**, moderate concentrations of microbeads, estimated at 4000 and 2000 L^{-1} (treatments 8 and 9, respectively), aggregated with 10^6 cells L^{-1} of *T. weissflogii*; and set-up 4 **(g, h)**, same moderate concentrations of microbeads, aggregated with 10^6 cells L^{-1} of *T. weissflogii*, and 130 mg sediments (treatment 12). Images in panels a–f were taken in aggregation tanks for treatments 1, 2, 4, 5, and 8, 9, respectively (Table 1). Images in panels g and h were taken in the settling columns from treatment 12. A fishing line of 250 µm in diameter (as in panels g and h) was used to calibrate image size. DOI: https://doi.org/10.1525/elementa.317.f2

Aggregation of phytoplankton cells. For comparison, aggregates formed in runs with only phytoplankton (10^6 cells L^{-1}) placed in the roller tanks exhibited a wide size range of 990–7650 µm, with an average aggregate size of 3194 ± 1917 µm (**Table 3**).

A one-way ANOVA test of aggregate size measurements across treatments indicated that there was a significant difference in aggregate diameters by treatment (at the 95% threshold): ANOVA, $F(9,490) = 44.46$, $p < 0.001$. The Bonferroni test determined that all aggregates formed in set ups 1–4 were significantly smaller than those formed in roller tanks dosed only with algal cells ($p < 0.05$). Aggregate sizes formed in set ups 1–4 were not significantly different from each other, given the large standard deviations associated with the measured aggregates ($n = 50$) and the great variability in aggregate form. This variability led to an occasional considerable overlap in individual measurements across treatments, even in cases where tank-produced aggregate types exhibited very different diameter means, such as between treatment 12 (4000 microbeads L^{-1} plus sediment) with a mean diameter of 1358 ± 731 µm and treatment 4 (24,000 microbeads L^{-1} without sediment) with a mean of 1810 ± 807 µm.

Turbidity changes during aggregation

During all roller tank aggregation experiments, a decrease in turbidity over time was observed over the 72-hour aggregation period (**Table 3**). For complete turbidity data recorded at each measurement point, please see Table S2.

Set-up 1. A significant decrease in turbidity was observed during aggregate formation of microbeads only (ANOVA, $F(8,72) = 14.215$, $p < 0.001$) by 72, 64 and 75%, respectively, for the estimated 8000, 16000, and 24000 microbeads L^{-1} over the full aggregation period of 72 hours.

Set-up 2. With an estimated 8000, 16000, and 24000 microbeads and phytoplankton (10^6 cells L^{-1}), turbidity decreased by 56, 57 and 67%, respectively, over 72 hours. Statistical results indicated that significant factors in determining the measured turbidities were: time in hours (ANOVA, $F(8,72) = 48.082$, $P < 0.001$), treatment (ANOVA, $F(3,72) = 106.316$, $P < 0.001$) and interaction effects between hours and treatment (ANOVA, $F(24,72) = 1.486$, $P < 0.001$). Turbidity reduction over the aggregation period was less rapid and less pronounced within roller tanks containing only phytoplankton (set-up 2, treatment 7; ANOVA, $F(3,72) = 73.398$, $P < 0.001$).

Set-up 3. With an estimated 1000, 2000, and 4000 microbeads L^{-1} and phytoplankton (10^6 cells L^{-1}), turbidity decreased by 48, 57 and 54%, respectively, over 72 hours of aggregation. Even with these reduced concentrations of microbeads available for aggregation, turbidity reduction differed by treatment (ANOVA, $F(3,24) = 1.991$, $P < 0.001$). The Bonferonni post-hoc test indicated that reference values from unenriched roller tank samples differed significantly from the other tanks and by time (ANOVA, $F(8,72) = 13.480$, $p < 0.001$; Table S2).

Set-up 4. With an estimated 1000, 2000, and 4000 microbeads, phytoplankton (10^6 cells L^{-1}) and sediments (130 mg L^{-1}), turbidity decreased over time from the initial elevated turbidity values by 51, 43 and 48%, respectively. As with set-up 1, significant determinants of the observed turbidities were the time of measurement

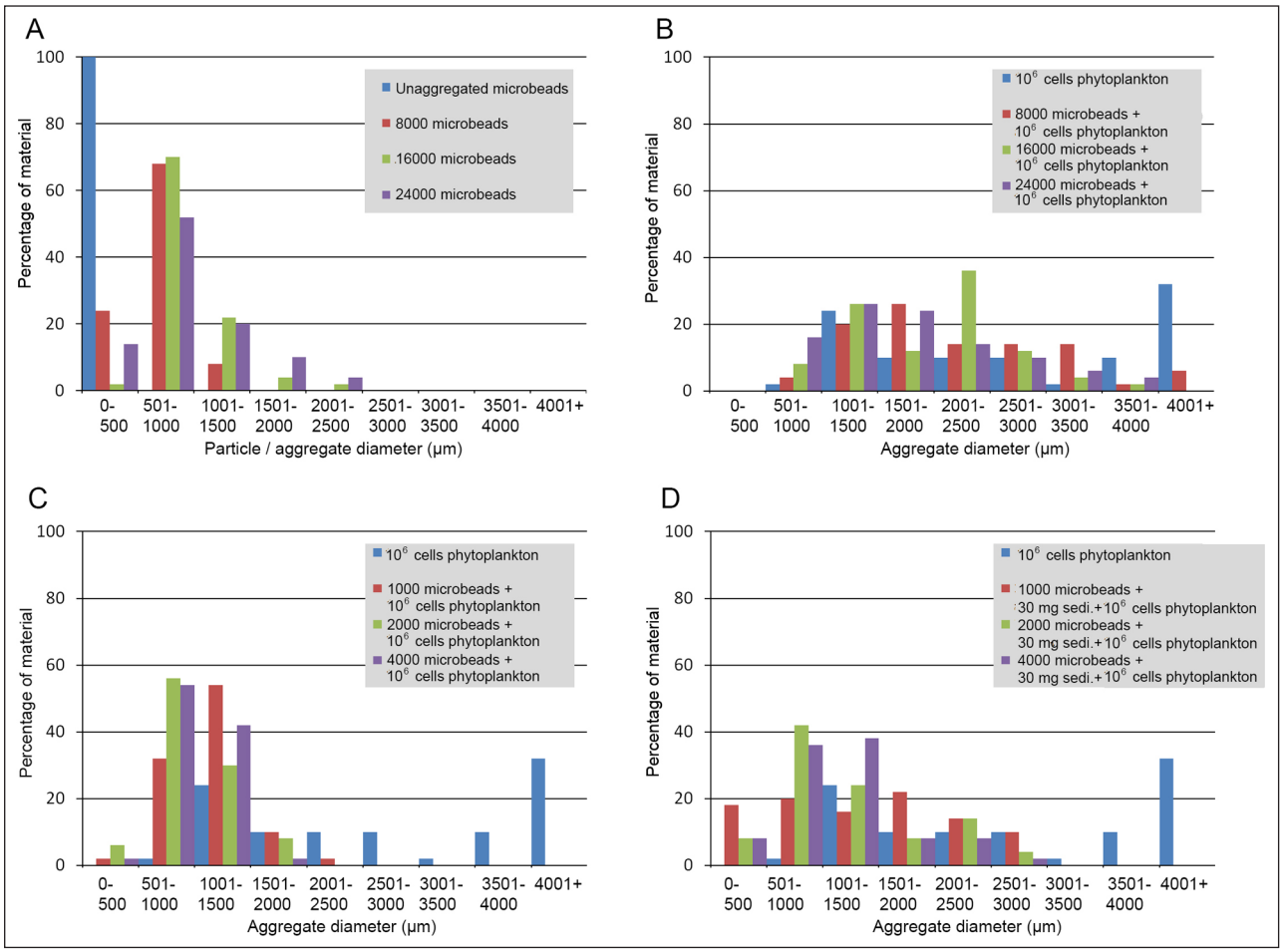

Figure 3: Aggregate size distributions (set-ups 1–4) following a 72-hour period in roller tanks. For experimental aggregations with microplastics, only those extracted from product 3 were used. Set-up 1 **(A)** included high concentrations of microbeads (estimated at 8000, 16000 and 24000 L^{-1}), with unaggregated microbead diameters shown for comparison. Set-up 2 **(B)** used the same high concentrations of microbeads additionally aggregated with 10^6 cells L^{-1} of *T. weissflogii,* with the size distribution of phytoplankton-only aggregates shown for comparison. Set-up 3 **(C)** used moderate concentrations of microbeads (1000–4000 L^{-1}) and 10^6 cells L^{-1} of *T. weissflogii*, with the size distribution of phytoplankton-only aggregates shown for comparison. Set-up 4 **(D)** used the same moderate concentrations of microbeads, 10^6 cells L^{-1} of *T. weissflogii* and 130 mg sediments, with the size distribution of phytoplankton-only aggregates shown for comparison. See Table 3 for details of the treatments (color-coded in A–D). DOI: https://doi.org/10.1525/elementa.317.f3

(ANOVA, $F(8,72) = 229.734$, $p < 0.001$) and interaction between time and treatment effects (ANOVA, $F(24,72) = 22.603$, $p < 0.001$). Bonferroni post-hoc tests indicated that turbidities within roller tanks not enriched with sediment and/or microbeads differed from those enriched with these additives, but that turbidity did not differ significantly between treatments by the microbead concentrations used in set-up 4 (Table S2).

Settling velocity

In set-ups 1 and 2, the aggregates formed in the roller tanks over 72 hours were positively buoyant and returned to the surface of the settling column upon delivery into the first few cm of column water. Therefore, settling rates could only be determined for aggregates formed in roller tanks dosed only with algal cells and those formed in roller tanks containing a moderate microbead concentration (4000 L^{-1}, set-up 3, treatment 8) or moderate microbead concentration and sediments (4000 L^{-1}, set-up 4, treatment 12) (**Table 3**; **Figure 4**). The fastest settling velocities of up to 831 m d^{-1} (mean of 559 ± 178 m d^{-1}) were observed for the organo-mineral and low-plastic concentration aggregates (set-up 4, treatment 12). Microbead-phytoplankton aggregates (set-up 3, treatment 8) exhibited settlement velocities of 32–169 m d^{-1} (mean of 91 ± 27 m d^{-1}). Aggregates formed in the roller tanks by phytoplankton alone exhibited the lowest observed settling velocities with a mean of 53 ± 22 m d^{-1}.

Levene's test indicated that the homogeneity of the settling velocity data was significantly different across treatments, though by using the robust equality of means Welch's F to report the test output, the use of the ANOVA test was considered appropriate given the considerable differences in velocities observed between treatments. The ANOVA test indicated a significant difference in settling velocities across treatments at a 99.9% threshold:

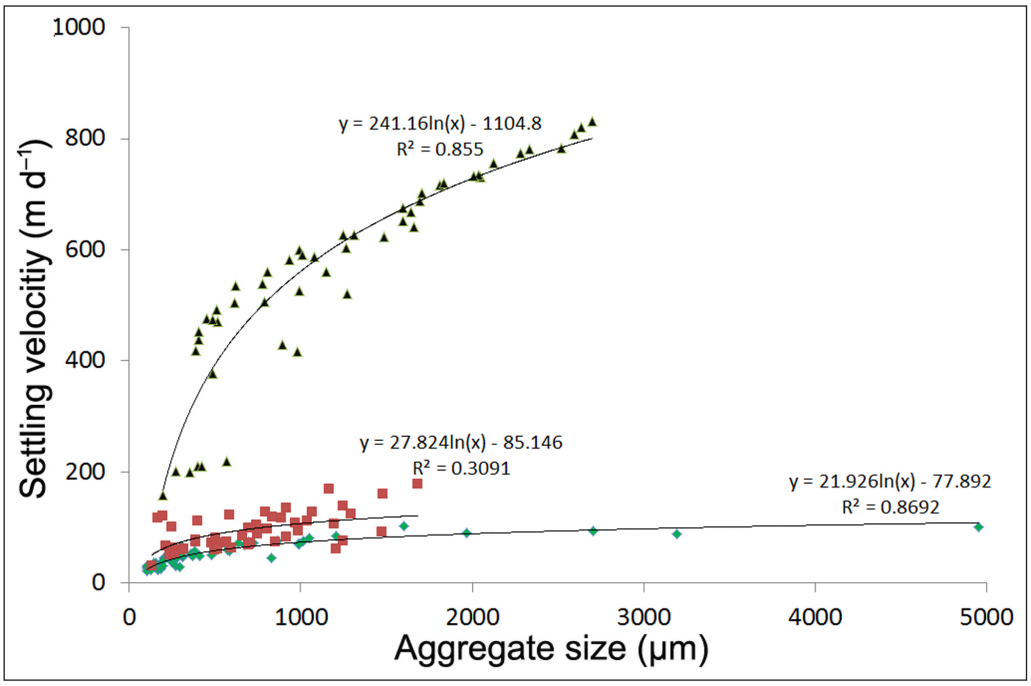

Figure 4: Relationships between aggregate settling velocity and aggregate size. Settling velocity vs. aggregate size for aggregates of phytoplankton-only (*T. weissflogii*) in green, aggregates with phytoplankton and 4000 microbeads L^{-1} (set-up 3, treatment 8) in red and aggregates with phytoplankton, 4000 microbeads L^{-1} and 130 mg L^{-1} sediments (set-up 4, treatment 12) in black. The aggregate sizes presented are the size of the aggregates settling past the camera in the settling tube, with some breakage and further aggregation possible during transport from roller tank to settling tube and within the settling tube prior to passing the camera. Solid lines indicate data fit to a logarithmic regression, with line equations given in the figure. DOI: https://doi.org/10.1525/elementa.317.f4

F (2, 147) = 356.25, $p < 0.001$. The Bonferroni test indicated that this settling rate difference was significant between the aggregates formed from phytoplankton only and those formed from the organo-mineral and plastic additives in the roller tanks of set-up 4 ($p < 0.001$), and between the set-up 3 and set-up 4 aggregate settling velocities ($p < 0.001$). No significant difference in settling velocity was indicated between the phytoplankton-only and low-plastic concentration aggregates formed in set-up 3 (phytoplankton and 4000 microbeads L^{-1}).

Critical shear velocity

Phytoplankton aggregates formed in roller tanks without the addition of microbeads or sediments demonstrated homogeneity in resuspension behaviour. For these aggregates, a low shear velocity of 0.2 cm s^{-1} initiated bedload transport, with most organo-aggregates entering suspended mode at shear velocities of 0.3–0.4 cm s^{-1}. Aggregates with enrichments of 4000 microbeads L^{-1} and sediments (set-up 4) exhibited a markedly different resuspension behaviour, with bedload transport commencing at u^*_{cri} = 0.6–0.7 cm s^{-1}, saltation occurring at u^*_{cri} of 0.8–1 cm s^{-1} and full resuspension observed at u^*_{cri} = 1.3–1.4 cm s^{-1}. An overview of particle behaviour is given in **Table 4**.

Discussion

The results from this laboratory investigation suggest that microbeads extracted from a number of products aimed at the same cosmetic purpose, namely facial cleansing, may well exhibit distinct and contrasting transport behaviours within the environment. All except one fraction of one facial cleanser were buoyant. Nevertheless, the degree of buoyancy differed by product and appeared to be largely dependent on both particle size and density. These experimental observations support the pervasive view that the majority of microplastics are buoyant, with much research to date focused on the monitoring of their presence by sampling surface waters and beach shore sediments (Hidalgo-Ruz et al., 2012; Cózar et al., 2014; Lusher et al., 2014). However, studies have also reported microplastics in deep-sea sediments (Van Cauwenberghe et al., 2013; Woodall et al., 2014), suggesting that removal of millimeter-sized plastic fragments from the sea surface is occurring on a large scale (Kukulka et al., 2012; Eriksen et al., 2014) and that the sea surface and beaches are not the ultimate or exclusive sinks for plastic pollution.

One focus of our study was to investigate entrainment of selected plastic microbeads into aggregates with algal and coastal lithogenic material, and whether such entrainment would lead to increasingly rapid settlement of microplastic particles. Our results indicate that plastic microbeads can indeed aggregate with algal biomass rapidly, with even high concentrations of microplastics being removed from suspension swiftly by phytoplankton concentrations typically found in coastal waters. When microbeads were exposed to both phytoplankton and riverine sediments, aggregations also took place rapidly. The settling rate observations indicated that a ballasting mechanism, such as the incorporation of microbeads into organic (phytoplankton) or organo-mineral (sediment) aggregates, is

Table 4: Resuspension behaviour of aggregates generated from moderate concentrations of microbeads with phytoplankton and sediments and those from phytoplankton only. DOI: https://doi.org/10.1525/elementa.317.t4

Resuspension stage	Replicate #	Set-up 4, treatment 12[a]		Phytoplankton only	
		u^*_{cri} [b] (cm s^{-1})	Average u^*_{cri} (cm s^{-1})	u^*_{cri} (cm s^{-1})	Average u^*_{cri} (cm s^{-1})
Beginning of resuspension	1	0.6	0.67	0.2	0.20
	2	0.7		0.2	
	3	0.7		0.2	
Bedload transport	1	0.9	0.90	0.4	0.33
	2	0.8		0.3	
	3	1.0		0.3	
Suspended load transport	1	1.4	1.33	0.5	0.50
	2	1.3		0.5	
	3	1.3		0.5	

[a] Roller tanks contained ~ 4000 microbeads L^{-1}, 10^6 cells phytoplankton (*T. weissflogii)* L^{-1} and 130 mg L^{-1} sediments.
[b] Critical shear velocity.

necessary to initiate sinking of (near) buoyant microbeads (Thomsen, 2005).

The volumes of microplastic pollution in the environment reported in the literature differ greatly by study and research area: a recent study examined treated wastewater, sewage sludge and separated light solids from 12 sewage plants all over Germany. The authors detected considerable amounts of microplastics in wastewater effluents, of which many items could be classified as microbeads (Mintenig et al., 2017). The authors estimated an annual discharge of 9×10^7 to 4×10^9 microplastic particles and fibres per wastewater treatment plant. Castañeda et al. (2014) found microbeads in varying abundances in the sediments of the St. Lawrence River, with the highest density of 10^3 microbeads L^{-1}, a figure of comparable magnitude to microplastic concentrations reported from contaminated marine sediments. In a microplastics survey of the Dutch environment, between 9000 and 91000 microplastic particles per m^3 were found in treated wastewater samples, whereas estuarine and North Sea sediments contained 3300 ± 420 to 440 ± 160 microplastic particles per kg dry weight sediment (Leslie et al., 2013). These plastic particles can resuspend easily under elevated coastal and tidal current velocity conditions and re-enter the benthic boundary layer (Leslie et al., 2013). The plastic concentrations selected for set-ups 3 and 4 in the current study were from the higher spectrum of concentrations that have thus far been reported from the environment (10^3 microbeads L^{-1}). Lower concentrations would have been difficult to detect in the roller tanks during experimental runs, and therefore aspects of microbead behaviour would remain obscured. Higher concentrations used in experimental set-ups 1 and 2 were used to study the interaction of microbeads between each other (set-up 1) and with phytoplankton at point sources in coastal waters (set-up 2). Moderate concentrations of microbeads L^{-1} when exposed to phytoplankton in the roller tanks (set-up 3) resulted in swifter aggregation than in experimental runs with higher concentrations of microbeads, possibly as a result of mechanical disassociation of aggregates increasing with higher microbead concentration, or as a result of a greater entrapment of particles by larger fluffy aggregates than can be achieved by the mutual entanglement of smaller microbeads with each other. The morphology of *T. weissflogii* aggregates that incorporated microbeads differed greatly from those formed in roller tanks not dosed with plastics. Organic-only aggregates of *T. weissflogii* were fluffy, fragile with a high porosity and translucent form. In contrast, aggregates that incorporated plastic microbeads (1000–4000 L^{-1}) were generally more compact and lower in porosity. These aggregates were also more rounded, reasonably homogeneous in size, less translucent and green-yellow in colour.

Laboratory measurements of settling velocities of aggregates were conducted in still water conditions. Therefore, potential environmental hydrodynamic effects (e.g., mixing by wave action, tides, seasonal stratification, etc.) on settling rates were not taken into account. Despite this, our results clearly indicate that the observed rapid incorporation of plastic and inorganic material into phytoplankton aggregates alters the settling velocities of these natural aggregates, implying further influence on both the rate of phytoplankton detritus supply and the volume of plastic reaching the seafloor. In our study we observed that on average microplastic-free organic aggregates were sinking at the lowest observed velocities (**Figure 4**). Sinking velocities for marine snow documented in the literature vary by location, season and study. Measured sinking speeds at the decline of the plankton bloom in the Porcupine Seabight in the Atlantic have been reported to be between 100 and 150 m day^{-1} (Lampitt, 1985); in Monterey Bay, California, Pilskaln et al. (1998) reported 16–25 m day^{-1}. For marine snow collected by Thomsen and Van Weering (1998), sinking velocities

of 432 m day^{-1} were reported for the North East Atlantic. Our results for phytoplankton-only aggregates (23–103 m day^{-1}, mean = 53 ± 22 m day^{-1}) are comparable with these ranges of settling velocities. Plastic-only aggregates with no algal content (set-up 1) did not sink, most likely due to the lack of a sufficient ballasting mechanism. Addition of high microplastic concentrations (set-up 2) inhibited the sinking of organic matter, likely due to the high-buoyant plastic content lowering the inherent density of the aggregates. These results indicate that high concentrations of plastics in the natural environment may increase the buoyancy of any naturally occurring aggregates that may incorporate the microbeads. Addition of moderate microbead concentrations (set-up 3) resulted in the produced aggregates sinking at rates of 32–169 m day^{-1} with an average settling velocity of 91 ± 27 m day^{-1} (**Table 3**; **Figure 4**). Further investigations are required to accurately define the degree of change in settling velocity associated with microplastic enrichment.

Long et al. (2015) studied the aggregation of microplastics with two different algae species: the diatom *Chaetoceros neogracile* and the cryptophyte *Rhodomonas salina*. Interestingly, they determined that the sinking rates of the diatom aggregates strongly decreased following aggregation with microplastics, while the sinking rates of cryptophyte aggregates increased after microplastic incorporation. Cole et al. (2016) examined the effects of microplastics on faecal pellet properties and found that the microplastics-fed copepod *Calanus helgolandicus* egested faecal pellets with reduced densities, a 2.25-fold reduction in sinking rates, and a higher propensity for fragmentation. These various results indicate that microplastic incorporation into natural aggregates of phytoplankton and detritus will affect the transport of organic matter within the marine environment, which may have impacts on the functioning of the marine food web and ecosystem, potentially resulting in a change in faunal distributions as ecosystem niches are altered by the change in particle flux and food supply.

Addition of inorganic material in the current study resulted in strongly increased settling velocities ranging from 158 to 831 m day^{-1}, with a mean velocity of 559 ± 178 m day^{-1}. These results are comparable to the settling velocities of phytoplankton aggregating with drill cuttings (100–175 mg L^{-1} dry weight) measured by Pabortsava et al. (2011). The entrainment of the inorganic material increases the overall density of aggregates and results in elevated settling, with a linear relationship of settling velocity and size. The model of Armstrong et al. (2002) on mineral 'ballast' attached to organic aggregates also explains generally elevated values of settling velocities of aggregates exposed to inorganic material. Studies from continental margins (Thomsen, 2005) showed that once organo-plastic aggregates enter river plumes or reach the seafloor, they most likely incorporate lithogenic material and increase settling velocities. Our results show that plastic microbeads are rapidly scavenged from suspension by aggregates. This rapid incorporation of low density buoyant plastics into aggregates therefore offers a transport route downwards through the water column which cannot be achieved by plastic microbeads without aggregation with settling algal detritus, with incorporation of suspended sediments further increasing the settlement rates. Our results therefore support the hypothesis that vertical transport of microbeads to the benthic environment following phytoplankton aggregation represents a sink for microplastics from the upper waters, explaining the low concentrations that have been measured in the ocean surface (Thompson et al., 2004; Eriksen et al., 2014; Long et al., 2015). Modelling transport pathways for microplastics using buoyancy alone, without the inclusion of the potential aggregation factor, is likely to underestimate the potential rate of downward transport.

Several factors such as size, density, porosity, shape, and stickiness of aggregates play roles in determining the resuspension behaviour of aggregates following settling (Beaulieu, 2003). Our results show that aggregates composed only of *T. weissflogii* resuspended at lower shear velocities than aggregates with entrained plastics and sediments. Our results were within the range observed in literature: Beaulieu (2003) determined values for critical shear velocity, which ranged from 0.4 to 0.6 cm s^{-1} for *Chaetoceros*-derived detritus and from 0.5–0.8 cm s^{-1} for *Skeletonema*-derived detritus. Jago et al. (1993) measured values of 0.45–0.55 cm s^{-1} for organic-rich aggregates deriving mainly from *Skeletonema costatum*. Similar values were also determined by Thomsen and Gust (2000) and by Pabortsava et al. (2011) for the resuspension behaviour of organic aggregates treated with 175 mg L^{-1} of drill cuttings. The results of these resuspension experiments suggest that phytodetrital aggregates with entrained plastics and lithogenic material are less mobile following deposition than those not containing such inclusions and that the sediment content is a particularly prominent factor in determining subsequent transport in the benthic boundary layer.

Resuspension of benthic detritus, bacteria, and settled phytoplankton presents a potentially high-quality food source for suspension feeders (Grant et al., 1997). Many suspension and deposit feeders may ingest resuspended organo-plastic and organo-plastic-lithogenic aggregates as readily as plastic-free organo-aggregates (Moore, 2008; Graham and Thompson, 2009). Ingestion of microplastics has been documented for various intertidal invertebrates, including filter-feeding polychaetes, blue mussels, echinoderms, bryozoans, bivalves and barnacles, as well as deposit-feeding lugworms (Thompson et al., 2004; Ward and Shumway, 2004; Moos et al., 2012; Setälä et al., 2016) and fish, phytoplankton and zooplankton (Boerger et al., 2010; Cole et al., 2013; Hämer et al., 2014; de Sá et al., 2014). Adverse effects on overall fitness, tissue and cells, feeding efficiency, reproduction, photosynthesis and mortality have been documented repeatedly (Browne et al., 2008; Burkhardt-holm, 2012; Moos et al., 2012; Besseling et al., 2014; Cole et al., 2015). Given that plastics have a high affinity to adsorb PCBs and other organic pollutants in aquatic environments, ingestion of plastic particles may also expose benthic marine invertebrates to toxins (Graham and Thompson, 2009) that can be transferred

to higher trophic levels and bioaccumule in the food web (Moore et al., 2001; Eriksson and Burton, 2003; Farrell and Nelson, 2013). Our study reveals that plastics do not readily disassociate from aggregates during the resuspension process (though aggregates may fragment to a minor degree), and therefore the incorporated plastics are rendered available to various fauna for direct ingestion.

Conclusion

Discharge of plastic microbead wastes from cosmetic products following use has been identified as a potentially important primary source of microplastics into the marine environment. These products are washed down the sink after usage and consequently end up in our waterways, where they present various hazards in estuarine and marine environments. Our laboratory experimental study indicates that even microbead-utilizing products with a similar cosmetic application contain plastic material with great diversity of form, density, size, coloration, aggregation potential and hydrodynamic behaviour. To better quantify the likely transport pathways of such material into and within the world oceans, further work is needed. A better description of the contents of these products made available by the manufacturers may assist policy makers in regulating discharge of materials into rivers and water bodies. Our results indicate that for the products investigated, the contained plastics were generally positively buoyant and scavenged quickly by algal material in the experimental water column which acts as a ballasting mechanism to the intrinsic buoyancy of the microbeads. When incorporated into marine aggregates, the microplastic particles used in this study may have an impact on sinking rates by changing aggregate density and morphology. That vertical transport of microbeads following aggregation with phytoplankton material is likely a key transport mechanism for this waste product should be taken into account when establishing predictive transport models for this pollutant, as well as by legislative bodies that determine the best approaches to pollution minimisation.

Data Accessibility Statement

The full data set is accessible in the supplementary files.

Supplemental files

The supplemental files for this article can be found as follows:

- **Dataset S1.** Full .xlsx data set with product information, microbead size distribution, turbidity measurements, aggregate size distribution, settling velocities and resuspension behaviour in multiple worksheets. DOI: https://doi.org/10.1525/elementa.317.s1
- **Table S1.** Product company details and plastic percentage per product volume. DOI: https://doi.org/10.1525/elementa.317.s2
- **Table S2.** Turbidity reduction observed at each time period during roller tank aggregation. DOI: https://doi.org/10.1525/elementa.317.s3
- **Table S3.** Buoyancy behavior of plastic particles in freshwater and seawater. DOI: https://doi.org/10.1525/elementa.317.s4
- **Figure S1.** Pictures of products used in the current study. DOI: https://doi.org/10.1525/elementa.317.s5

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Competing Interests

The authors have no competing interests to declare.

Author Contributions

- Substantial contributions to conception and design: LT, PM
- Acquisition of data: PM
- Analysis and interpretation of data: PM, AP, LT
- Drafting the article or revising it critically for important intellectual content: PM, AP, LT
- Final approval of the version to be published: PM, AP, LT

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