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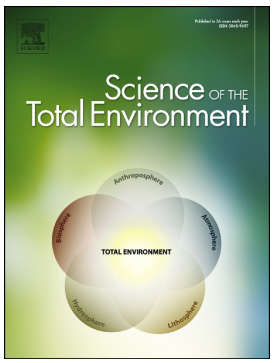

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Colour and size influences plastic microbead underestimation, regardless of sediment grain size

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ABSTRACT

The quantification of microplastics in environmental samples often requires an observer to determine whether a particle is plastic or non-plastic, prior to further verification procedures. This implies that inconspicuous microplastics with a low natural detection may be underestimated. The present study aimed at assessing this underestimation, looking at how colour (white, green and blue), size (large; ~1000 μm and small; < 400 μm) and grain size fraction may affect detection. Sediment treatments varying in grain size were inoculated with known quantities of low-density polyethylene microbeads extracted from commercially bought facial scrubs. These microbeads varied in colour and size. Once extracted using a density separation method microbeads were counted. An overall underestimation of 78.59 % may be a result of observer error and/or technical error. More specifically, the results suggested that microbeads varying in colour and size have a different detection probability and that these microbead features are more important in underestimation likelihoods than grain sizes.

Key words

microplastics, analytical methods, detection probability, observer error

Conflict of interest

There are no conflicts to declare.

1. Introduction

Microbeads, an abrasive media, used in personal care products and air-blasting (Gregory, 1996) were identified as a source of microplastic pollution in the 1990s (Zitko and Hanlon, 1991). In the United States alone, trillions of microbeads are discharged into the aquatic environment every day (Rochman et al., 2015). Eriksen et al. (2013) found on average 43 000 particles km^{-2} in the Laurentian Great Lakes, while Castañeda et al. (2014) estimated microbead contamination in the St Lawrence River sediment to be ~13 759 particles m^{-2}; in both instances, the authors suggested that the primary source of contamination was from commercial products such as facial scrubs.

Unlike microfibers, which are brightly coloured and elongated making them relatively easy to detect, microbeads have a relatively similar shape to natural particles and as a result are thought to be often underestimated (Castañeda et al., 2014; Napper et al., 2015). Furthermore, given that grain size fractionation characteristics vary spatially, and that the potential source microbead size spectrum is considerable (Napper et al., 2015), microbead underestimation levels are highly likely to vary in space. Our understanding of microbead contamination has been supplemented by alternate methods to quantify load within a defined area. One method involved the enumeration of microbeads in popular consumer products; Napper et al. (2015) estimated that between 4 594 and 94 500 microbeads sourced from six brands of facial products alone can potentially pass through the sewerage works. Van Wezel et al. (2016) modelled the potential microbead load entering an aquatic environment using information on product use, sales, population densities, microbead properties and waste management which supported the findings of Van Wezel et al (2016). Similarly, quantification of microplastics in wastewater treatment plant (WWTP) effluent and sludge was used by Rochman et al. (2015) to determine the amount of microbeads entering the surrounding environment.

While the underestimation of microbead densities in sediments is often acknowledged, few studies have assessed the level of this error (but see Conkle et al., 2018). The overarching aim of the present study was to quantify the extent of underestimation using the traditional density separation method. Microbeads varying in colour (white, green, blue) and size (large; ~1000 µm and small; < 400 µm) were inoculated into sediment of different grain size fractions (<63 µm, 63–125 µm, 125–250 µm, 250–500 µm, 500–1000 µm). We hypothesised that there would be a difference in the detectability of microbeads varying in colour and size. We also hypothesised that microbead recovery rates would differ significantly among sediment grain size classes.

2. Materials and methods

2.1.Sediment processing

Sediment was collected from an open quarry (33°18'55.82"S, 26°33'33.39"E) on the floodplain of the Bloukrans River near Grahamstown in 2016. Once in the laboratory, all sediment was dried in ovens at a temperature of 60 °C for 48 hours. Dried sediment was then portioned into 500 g subsamples and passed through a series of stacked sieves, agitated by a mechanical shaker, to fractionate the sediment into various grain sizes. Each subsample was shaken on an EMS-8 Electromagnetic Sieve Shaker (set to a power level of 15) for 60 minutes. The sieves used in the fractionation process of each 500 g subsample were 63, 125, 250, 500 and 1000 µm mesh pore resulting in five size classes of sediment for the experiment, namely: size class 1 (<63 µm), size class 2 (63–125 µm), size class 3 (125–250 µm), size class 4 (250–500 µm) and size class 5 (500–1000 µm). The size class 6 (mixed) 500g subsample was comprised of 100g each of size classes 1, 2, 3, 4 and 5, respectively. Once sorted, all sediment was ashed in a furnace at 450 °C for 12 hours to break down any non-geological material. This allowed all

samples to be consistent regarding organic content, thereby removing a potential confound. In addition, the sediment was collected from a field site, which may have been contaminated with microplastics. Exposing the samples to such a high heat made sure no existing plastic was present in the sediment prior to our inoculation. The six sediment size classes were used as sediment treatments.

2.2. Experiment set-up and design

Five replicates (500 g) per sediment treatment were placed in separate 5 L polyethylene containers. Each replicate container for each treatment was then inoculated with the same known quantities of microbeads varying in colour (3; white, green and blue) and size (2; large (*n* = 20 particles), small (*n* = 200/35/20 particles)). All microbeads, identified as polyethylene from individual product ingredients list, were removed from commercially available, off-the-shelf, water-based facial cleansers/body scrubs purchased from a grocery store in Grahamstown, South Africa. Once removed microbeads were dried in an oven at a temperature of 60 °C for 48 hours. Large microbeads ranged in size from 900 to 1100 μm. Small white and green microbeads were too small to individually count, therefore, 0.1 g of each was inoculated in individual containers. Three replicates of 0.1 g of small white and green microbead particles were then counted to determine the average number of microbeads in 0.1 g of material and this equated to ~200 white and ~35 green particles. The small white, green and blue microbeads had sizes ranging from 80 to 280; 200 to 400 and 240 to 400 μm, respectively. Measurements of individual microbeads were made using a ZEISS stereo microscope fitted with eyepiece graticule.

The microplastics were then mixed into the sediment by hand for a period of 30 seconds. Salt water at a concentration of 100 g L^{-1} (density = 1.07 g cm^{-3}) was made using course sea salt

and tap water. The saltwater was then filtered through a 20 µm mesh sieve to remove any potential microplastic contaminants that fell within the size-classes of the microplastics used for inoculating treatments. Two litres of filtered saltwater was then added to each treatment, thereafter the solution was vigorously stirred for 5 minutes and the supernatant sieved through a 63 µm mesh following Claessens et al. (2011) and Thompson et al. (2004). This decanting process was repeated five consecutive times to maximise recovery (Coppock et al., 2017). The entire process was also conducted on control treatments for each grain size whereby sediments were not inoculated with any microbeads. Once samples had been decanted microbeads were counted and recorded using a ZEISS stereo microscope at 40× magnification by a researcher with no prior knowledge of the inoculated samples. The final number of microbeads found was used to determine the percent particle recovery (%) of each microbead type in each sediment treatment. In the controls, 8 flattened microplastic fragments and no spherical microbeads were found over all 30 treatments. As such, all microbeads enumerated in the study were assumed to be from inoculation rather than sediment contamination from the site of collection.

2.3. Statistical analysis

A factorial Analysis of Variance (ANOVA) was performed using colour, size and grain size as the three independent variables with percent particle recovery (%) as the dependent variable. All statistical analyses were conducted in Using SPSS version 16.0 (SPSS Inc., 2007).

3. Results and Discussion

In this study, large white (mean 27 ± 25.5 %) and green (76.5 ± 18.2 %) microbeads had a better recovery than their small sized coloured particles, with small white and green microbeads having recovery rates of 5.5 ± 2.5 % and 8.6 ± 6.0 %, respectively (Fig. 1). However, large blue microbeads (53.2 ± 16.9 %) had a lower recovery than their smaller sized particles (109 ±

28.2 %). This was, however, due to the overestimation of small blue microbeads. The variables "colour" ($F = 200.05$, $df = 2$, $p < 0.001$) and "size" ($F = 18.13$, $df = 1$, $p < 0.001$) had a significant effect on percent particle recovery. There may also be a concern that grain size impairs visual separation of microplastics, especially as fractionation is a well-documented pre-sorting step (Rocha-Santos et al., 2015) and density separation often results in sediments becoming part of the supernatant. However, "grain size" ($F = 0.820$, $df = 5$, $p = 0.537$) did not have a significant effect on percent particle recovery. Overall, the results suggested that white microplastics and microplastics <300 μm may be underestimated in studies. This may be due to their inconspicuous colouring and size, which could be further hindered by biofilm growth and discolouration due to aging although this would need to be investigated with additional experiments.

Out of 9 450 inoculated microbeads varying in colour and size, only 2 023 were recovered, with a shortfall of 7 427 microbeads over 30 treatments (Fig. 1). Averaged over the 30 samples, this would equate to a shortfall of 495 microbeads per kg or 78.59 %. Every microbead category, with the exception of small blue microbeads, were underestimated. This study serves as a starting point to begin quantifying and subsequently improving the artefact of observer error.

Observer error is a major concern in microplastic studies as most laboratories still rely on manual sorting and visual identification (Coppock et al., 2017). Even in conjunction with other techniques, such as Fourier-transform infrared spectroscopy (FTIR), visual sorting remains a mandatory step (Hidalgo-Ruz et al., 2012; Coppock et al., 2017). New techniques to minimise observer error are being developed, such as the use of Nile Red (Erni-Cassola et al., 2017; Maes et al., 2017) and Simultaneous Thermal Analysis - Mass Spectrometry (STA-MS;

Dümichen et al., 2015). These studies are still in their infancy and as such, the utilisation of conventional visual identification procedures will likely persist for years to come. The majority of microplastic studies will, therefore, still require an observer to initially decide whether a particle is plastic or non-plastic, prior to further verification procedures.

The low recovery rates could in part be attributed to the density separation method which inadequately removed microbeads from the sediment (technical error). Currently, density separation is the most popular method of extracting microplastics from sediment (Hidalgo-Ruz et al., 2012; Miller et al., 2017), with the web of knowledge article search revealing that in 2018, 16 out of the 19 studies reported using sodium chloride for quantifying microplastics in sediments. Two used sodium iodide (Di and Wang, 2018; Zhang and Liu, 2018), one lithium metatungstate (Eo et al., 2018), one zinc chloride (Lo et al., 2018) and one potassium formate solution (Xiong et al., 2018). Although the solute used varies between studies (see Miller et al., 2017 and Coppock et al., 2017), sodium chloride (NaCl) is still the most commonly used method and hence the one used in this study. While Quinn et al. (2017) estimated a recovery rate of between 85 % and 95 % for sodium chloride, they noted that high density plastics (i.e. PET) were likely missed using this solute. Coppock et al. (2017) on the other hand, stated that NaCl media has a low recovery rate (between 35 and 40 %), which can be compensated for by repeating the decanting method 3 to 5 times, making the method more time consuming. Since the majority of microbeads are made from low-density polyethylene (Cheung and Fok, 2017; Kalčíková et al., 2017), with density values ranging from 0.915 to 0.925 g cm^{-3}(Quinn et al., 2017), compared to sodium chloride (1.07−1.2 g cm^{-3}), this extraction technique should be effective. However, it cannot be ruled out that these results are in part due to the extraction technique. Additionally, it is apparent that although studies have reported the ineffectiveness of sodium chloride in extracting high-density plastics many studies are still using this method

in hopes that most low-density plastics are recovered; this study suggests that this may not the case.

4. Conclusions

Our study suggests that each type and colour of microbead has a different detection probability. The next step would be to consider the issue of microplastic observer error in water samples, which may remove the technical caveat introduced by using the density separation technique and the sorption of microplastics to the polyethylene containers employed in extractions. Future studies would benefit from more replication (>5) as the standard deviation in this study was high. However, it is clear that research into microplastic contamination of the aquatic and terrestrial environments are on the rise. In order for these results to be comparable we should not only focus on standardising sampling and extraction techniques but also enumeration techniques. This will be important when setting up monitoring programmes that are reliant on visual identification. A potential solution may be to employ a global accreditation service. Similar services are used for the identification and enumeration of macroinvertebrates and phytoplankton in both freshwater and marine environments. Although this does not remove observer error, it may reduce it given that it will presumably be conducted by specialists, while also making results more comparable over space and time. Another solution, would be for researchers to quantify observer bias alongside published results allowing readers to better interpret the data.

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conclusions or recommendations expressed in this material are those of the authors, and the NRF and Claude Leon Foundation do not accept any liability in this regard.

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Addendum

Figure captions

Fig. 1. Microbead recovery levels (%) in different sediment grain sizes: (a) white, (b) green and (c) blue microbeads.

Highlights:

- Microbeads, found in cosmetics, vary in colour and size.
- Detection probabilities were significantly different between microbeads.
- Microbead characteristics may explain underestimation in *in situ* sampling.

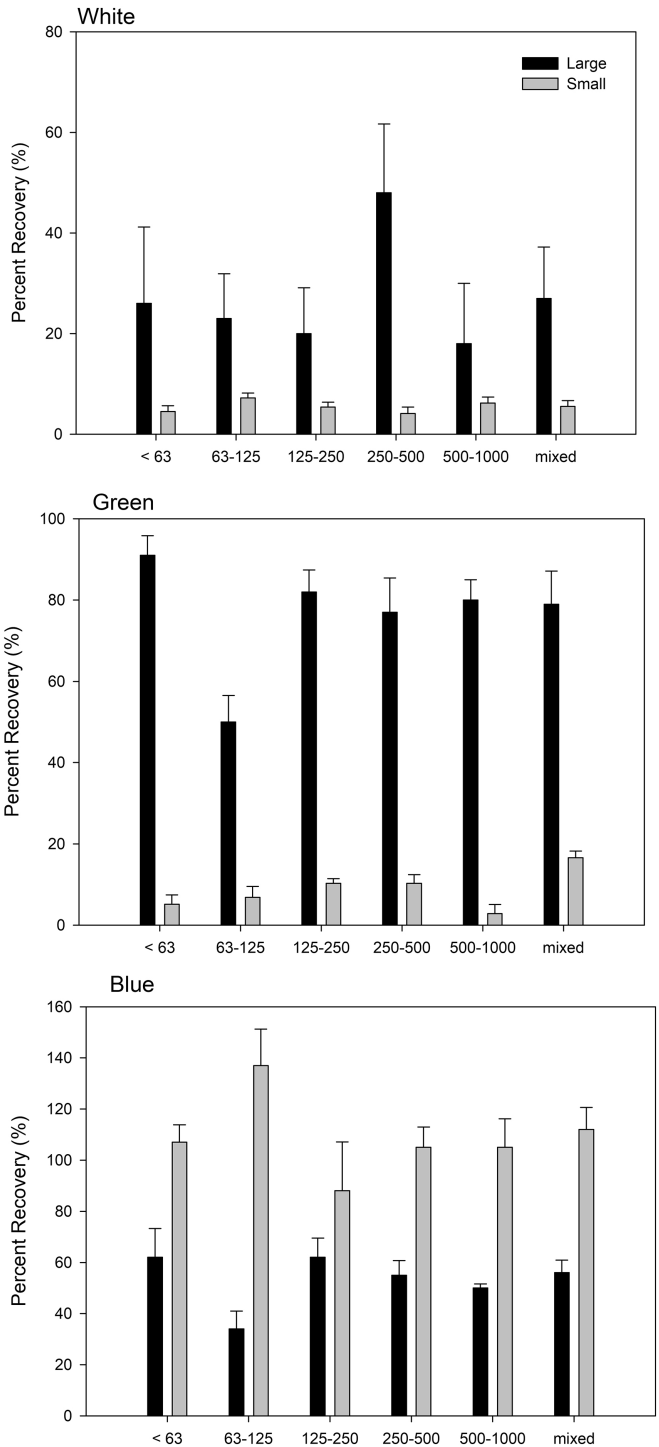
White
Large
Small
Percent Recovery (%)
80
60
40
20
0
< 63
63-125
125-250
250-500
500-1000
mixed
Green
Percent Recovery (%)
100
80
60
40
20
0
< 63
63-125
125-250
250-500
500-1000
mixed
Blue
Percent Recovery (%)
160
140
120
100
80
60
40
20
0
< 63
63-125
125-250
250-500
500-1000
mixed

Figure 1