Detection of micro plastics in bottled water and sports drinks by fluorescence microscopy and Nile Red dye

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INTRODUCTION

Recent research has reported the presence of micro plastics in tap water and bottled water (BW) but excluded samples of plastic BW. The aim of this study was to determine if micro plastics are present in sports drinks (SD) and BW using fluorescence microscopy and Nile Red dye.

The increasing usage of plastics to support modern lifestyles has drawn concerns about its abundance and the risks to human health. The lightweight and durable nature of plastic makes it ideal to accommodate a wide range of products for food packaging.

Plastics left in the environment as waste form micro plastics upon disintegration into smaller fragments through physical abrasion, weathering and microbial degradation.

Micro plastics are defined by most authors as plastic particles with longest diameter <5mm. There have been some suggestions to redefine the size range for the term micro plastics to be particles which are <1mm, but there is no official lower limit.

Micro plastics are highly mobile after being discarded into the environment while ecosystems heavily contaminated with micro plastics have been identified in convergence areas, such as ocean gyres, or near heavy human activities, such as industrial ports micro plastics have also been identified in remote environments such as Arctic and alpine snow.

Studies have detected the presence of micro plastics in human consumables, such as salt (Yang et al. 2015; Iñiguez et al. 2017), water (Schymanski et al. 2018; Pivokonsky et al. 2018, Zuccarello et al. 2019) and German beers, (Liebezeit and Liebezeit 2014). Inhalation, ingestion and skin perfusions have been suggested to be the main entry route for micro plastics into humans (Wright and Kelly, 2017).

Micro plastic presence can cause harm towards the environment, organisms and human health however, evidence of its impact humans have yet to be identified due to limited evidence available (Schirinzi et al,2017: Wright and Kelly, 2017)

Sports and energy drinks consumption is popular among the young generation (Mintel, 2019). Beside water, sports and energy drinks have grown in consumption with sales increasing from £m 1,800 in 2017 to £m 1,954 in 2019. However the bottled water saw volume sales grow in 2018 by 6% to more than 2.9 billion litres, resulting in the value of the UK market increasing by 5% to £2.15 billion (Mintel, 2019).

Sports and energy drinks are manufactured beverages, packaged in plastic and with lower pH which could potentiate the release of micro plastics. Little is known about the levels of micro plastics in sports drinks.

OBJECTIVE

The objective of this work was to detect and identify micro plastics in sports drinks and in bottled water samples. This study intends to survey the presence of micro plastics in sports drinks using fluorescence microscopy and Nile Red dye and compare the results with concentrations of micro plastics found in water.

MATERIALS AND METHODS

Nile Red, a hydrophobic metachromatic dye, was used to label micro plastics with fluorescence and view the dyed micro plastics under fluorescence microscopy. This relatively novel and inexpensive method uses readily available equipment.

We first tested Nile Red on two commercial polymers dispersed in deionised water to examine the efficacy in staining these with our Nile red solution.

Six different brands of BW were analysed. All water samples were bottled in the EU. The body of all the bottles were made of Polyethylene terephthalate (PET). Eight different brands of SD were analysed. The SD were all manufactured in the EU. The body of all the bottles were made of PET. Considerations were conducted to ensure the open and closing mechanism of the bottles were similar.

Samples were vacuum filtered through polycarbonate track-etched filter membranes, after filtration Nile red solution was added. The membrane was then transferred onto microscopic glass slides and examined under fluorescence microscopy

Images were captured using Micropix Cytocam Software (v 1.4.0.4), to evaluate the morphology of the particles for comparison.

The number of fluorescent particles observed was counted and recorded. The **concentration** of fluorescent particles was calculated based on microscopic observation using the formula as shown:

Concentration (particles/L) = Number of particles per 100 mL x 10

The pH of each sample was determined using a portable pH meter (Jenway, 370 pH meter, EU).

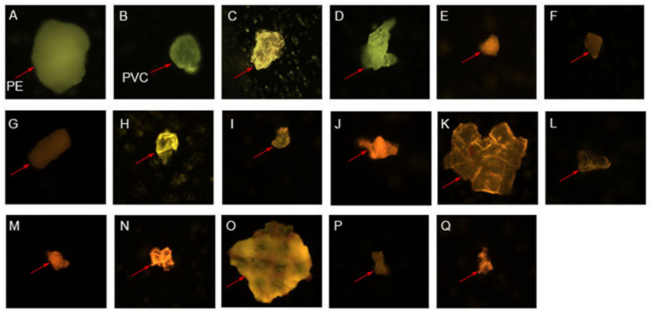

Figure 1. Photos under a fluorescence microscope for Nile Red stained commercial synthetic polymers (A – B), and unknown fragments found in tap water (C), bottled water W1 – W6 (D – I) and sports drinks S1 – S8 (J – Q).

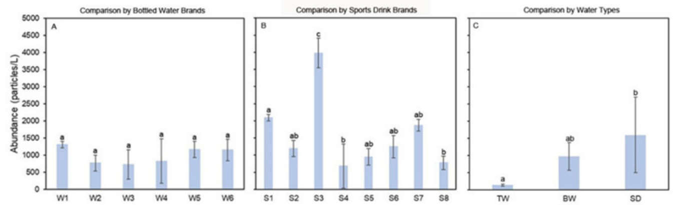

Figure 2. Comparison of the concentration of microplastics (particles /L) in the samples (A−C). Each value represents the mean ± standard deviation. The letters above the bars indicates significant differences ($p < 0.05$). Similar letters indicate that values are not significantly different. The abbreviations on the X-axis are as follows: TW, tap water; BW, bottled water; SD, sports drinks

RESULTS

The commercial synthetic polymer PE, fluoresced in orange (but appeared yellow upon image capturing) while the PVC fluoresced in yellow after staining in Nile red (Figure 1A – B), which provides evidence of the ability of Nile red to stain micro plastics. This finding was consistent with published literature on the use of NR to identify micro plastics.

Numerous particles were identified with fluorescence in both BW and SD. These were of multiple shapes and fluoresced in yellow and orange, hence visual quantification was possible using a light microscope.

The number of particles identified was 350 – 1560 particles/L in BW samples, and 500 – 4330 particles/L in SD samples.

Different water brands showed no significant difference ($p = 0.097$).

There is a statistically significant difference for the mean concentration of particles between SD samples (S1 – S8) ($p = 0.05$) (Figure 2).

The mean concentration of particles was higher in SD (1599 ± 1104 particles/L) compared to BW (1019 ± 352 particles/L). This was not statistically significant ($p= 0.05$).

There is a statistically significant difference for mean pH between BW and SD ($p = 0.001$). The mean pH values were higher in BW (7.03 ± 0.55) compared to SD (3.36 ± 0.24).

There was a statistically significant inverse relationship between the concentration of particles and pH value ($r = –0.355$, $p = 0.039$). The coefficient of determination suggests that pH level explains 12.6% of the variation in abundance of particles in the samples ($R2 = 0.126$).

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Discussion

While a limitation of analysis of micro plastics with Nile Red is co-staining of natural organic material all samples used in our study were processed BW or SD made to EU standards. We felt that the likelihood of significant organic material was small compared to environmental samples. Therefore the dyed particles we identified most likely represent micro plastics.

This research provides evidence on the presence of micro plastics in sports drinks and bottled water. All samples were found to contain micro plastics. To date, this study is the first to suggest the presence of micro plastics in SD.

In this research, it was observed that the particles emitted different colours (e.g. yellow and orange) (Figure 1). This is due to the hydrophobicity of the plastics, which affects the binding of Nile red to the surface, producing colour from yellow to deep red (Maes *et al*. 2017; Karakolis *et al*. 2019).

Synthetic commercial polymers samples such as PE and PVC were used to prove the efficacy in staining these with the Nile Red solution, knowing the approximate size and concentration.

Quantification in this study was done by microscopic observation, as used by Yang et al. (2015) on table salt. This method has been presented as a sensitive and a cost-effective method of quantifying plastic particles as reported by Maes *et a*l. (2017) and Erni-Cassola *et al*. (2017), which is the reason for its use in this research.

Our results demonstrated an inverse correlation between number of micro particles and pH. The low pH value of the SD contained in the bottles may be an influencing factor on the concentration of micro plastics being detected.

The micro plastics detected were >10 µm due to the size of the PCTE filter membrane pores. The pore size of the filter may therefore result in underestimation on the concentrations of particles <10 µm.

Previous reports on micro plastic concentration in BW samples has varied from 14±1.4 particles/L to 325 particles/L. This variation may represent the difficulty in accurately assessing concentration or variation in manufacture and water source.

Tracing the source of micro plastics in the beverages is deemed to be an almost impossible task due to the multiple entry points for contamination to occur. The abundance of micro plastics observed in BW and SD could be a potential public health issue. Until a robust method for determining and detecting the risk of micro plastics, its impact towards human wellbeing being remains uncertain.

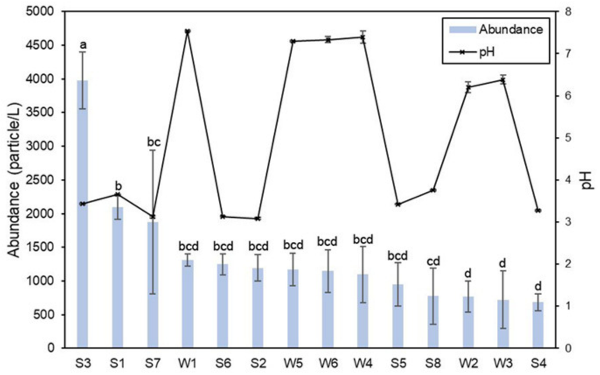

Figure 3. Comparison of abundance of micro plastics in descending order accompanied by individual pH levels for each sample. Each value represents the mean ± standard deviation. The letters above the bars indicates significant differences ($p < 0.05$). Similar letters represent that they are not significantly different.

CONCLUSION

This research has confirmed the feasibility of using Nile red to detect micro plastics in sports drinks and bottled water. Eight different brands of sports drinks and six different brands of bottled water plus tap water were found to contain micro plastics.

To date, this study could be the first to report the presence of micro plastics in sports drinks. This research adds evidence of the extent of micro plastics presence in foods. The results show that higher levels of micro plastics are present in sports drinks and single use plastic bottled water than in tap water. The source and entry of micro plastics into the liquids remains unknown. However it is likely that most beverages and liquid foods packed in plastic packaging will contain micro plastics.

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