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Influence of polyethylene microplastic beads on the uptake and localization of silver in zebrafish (*Danio rerio*)

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ABSTRACT

This study aimed to determine whether the uptake and localization of Ag in zebrafish was affected by the presence of polyethylene microplastic beads (PE MPBs). Zebrafish were exposed to 1 μg Ag L^{-1} (radiolabelled with ^{110m}Ag) for 4 and 24 h in the presence or absence of PE MPBs (10, 100 or 1000 MPBs mL^{-1}), and one treatment in which MPBs (1000 MPBs mL^{-1}) were incubated with Ag to promote adsorption. The presence of MPBs, at any of the tested doses, had no effect on the uptake or localization of Ag. However, exposure to the Ag-incubated MPBs (~75% of the Ag bound to MPBs) significantly reduced Ag uptake at both time points and also significantly increased the proportion of intestinal Ag. This study demonstrates that microplastics can alter the bioavailability and uptake route of a metal contaminant in a model fish species.

1. Introduction

The myriad of chemical releases into the aquatic environment necessitates a greater understanding of the possible interactions between pollutants and the effects that they can exert upon each other. In this regard the propensity for microplastics (MPs, defined as < 5 mm in size, Arthur et al., 2009) to sorb other chemicals (organic contaminants and trace metals) from the surrounding environment, affecting both the spatial distribution and the biological interactions of the adhered pollutants (Cole et al., 2011), warrants further investigation. This vector-effect was recently summarised by Syberg et al. (2015), with MPs influencing the behaviour of adsorbed contaminants at three distinct levels; (i) at the 'environmental-vector' level contaminants adhered to MPs are carried to new geographic locations and between environmental compartments (Teuten et al., 2007); (ii) the 'organismal-vector' effect involves the inadvertent ingestion of pollutants by organisms when MPs are mistakenly consumed, with the adhered contaminants subject to dietary uptake (Besseling et al., 2012); and (iii) the 'cellular-vector' effect in which MPs in the micro- or nano-size ranges are taken up into cells (von Moos et al., 2012), possibly by endocytotic or phagogaocytotic processes, allowing adhered contaminants cellular entry.

The ingestion of MPs by aquatic organisms has been widely reported in both laboratory (Browne et al., 2008; Cole et al., 2013) and field (Wright et al., 2013; Lusher et al., 2013; Sanchez et al., 2014) studies. Thus the role of MPs in carrying other contaminants has focussed mainly at the organism level (Besseling et al., 2012; Koelmans et al., 2013; Chua et al., 2014), and such research has prioritized hydrophobic pollutants, including plasticisers (additives in many plastic formulations) and POPs (persistent organic pollutants), such as PCBs (polychlorinated biphenyls) and PAHs (polycyclic aromatic hydrocarbons) (Gouin et al., 2011; Fries and Zarfl, 2012; Koelmans et al., 2013; Oliveira et al., 2013). The potential for metals to interact with MPs has been largely overlooked as plastics surfaces are considered to be relatively inert to aqueous metal cations (Ashton et al., 2010; Holmes et al., 2012). However, the adsorption of metal ions by plastic containers has been reported in ecotoxicological studies (Giusti et al., 1994; Fischer et al., 2007) and silver (Ag, used in the present study as a model metal contaminant), in particular, exhibits strong surface-binding characteristics which requires recognition and mitigation in experimental design (West et al., 1967; Sekine et al., 2015). Commonly used plastic pellets, including polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC), deployed in the San Diego Bay

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area for up to one year accumulated different amounts of nine metals, with some metals (including Ni, Zn and Pb) not reaching saturation within the given timeframe (Rochman et al., 2014). These authors demonstrated that all tested plastic types had the ability to adsorb metals and accumulate increasing concentrations with increasing deployment time. Similarly, plastic resin collected from beaches in South West England were shown to have adsorbed varying concentrations of seven trace metals, in some instances exceeding local sediment concentrations (Holmes et al., 2012). To our knowledge, however, no studies have yet investigated the potential for MPs to affect the bioavailability and flux of metals at the organism level.

The impact of MPs in freshwaters remains relatively unknown as the vast majority of MP research has been conducted in the marine environment and with marine organisms (Wagner et al., 2014). However, the presence of MPs in both freshwater environments (Moore et al., 2011) and biota (Sanchez et al., 2014) suggests that the concern regarding MPs should not be confined to the marine environment. There is also more awareness of the different types of MPs in the environment and particularly the prevalence of microplastic beads (MPBs, microspheres or 'scrubbers') (Zitko and Hanlon, 1991; Gregory, 1996; Fendall and Sewell, 2009). Commonly composed of PE, PP or polystyrene (PS), MPBs are used in skin cleansing and exfoliating products and are small enough in size (<0.5 mm) to evade capture by waste water treatment (Derraik, 2002; Fendall and Sewell, 2009). MPBs were ubiquitously sampled in the Laurentian Great Lakes and were similar in size, shape and composition to beads commonly used in commercial products (Eriksen et al., 2013). Their presence in the North American freshwaters in close proximity to urbanized areas was likely a direct consequence of consumer use (Eriksen et al., 2013). However, precisely determining MBP concentrations in the environment is difficult due to the complexities of plastic sampling at the smallest size range (Cole et al., 2015). Thus in the present study, the MBP concentrations employed (10, 100 and 1000 MBPs mL^{-1}) were similar to those described in the literature (Watts et al., 2014; Cole et al., 2015).

The aim of the present study was to determine the influence of PE MPBs (typical of those found in consumer products) on the uptake and localization of Ag in zebrafish (*Danio rerio*). In teleost fish the primary sites of uptake and toxic action associated with waterborne and dietary metal exposure, including Ag, are the gills and the intestine, respectively (Wood et al., 1999; Bury et al., 2003). We hypothesize that if zebrafish are co-exposed to waterborne Ag and MPBs, Ag will adsorb to the MPBs which will be ingested by the zebrafish. This would facilitate a change in uptake route for Ag from water to diet, and change the localization of Ag from gill to intestine, accordingly. Thus, we ask (i) whether the uptake and localization of Ag in zebrafish (during a 24 h exposure to 1 μg Ag L^{-1} using the radiotracer ^{110m}Ag) is affected by the presence of MPBs, as predicted. If so, (ii) is this effect dependant on the dose of MPB (10, 100 or 1000 MPBs mL^{-1}) present in the Ag exposure and (iii) is the effect enhanced if Ag and MPBs are incubated together prior to the zebrafish exposure, to allow increased time for Ag adsorption to the plastic surfaces.

2. Methods

2.1. Preparation and characterisation of microplastic beads

Clear PE MPBs were purchased dry from Cospheric LLC (Lot #: 100929-3-B, Santa Barbara, CA, USA). The manufacturers stated that the MPBs were 100% PE with a corresponding density of 0.96 g/cm^3 and had a size range of 10–106 μm. The MPBs in their pristine state were hydrophobic and in order to be dispersed in water the beads required treatment with a surfactant (polyoxyethylenesorbitan monooleate, purchased as 'Tween80 Biocompatible Surfactant', Cospheric LLC). To minimize the amount of Tween80 carried into the zebrafish exposures, we tested the dispersion of the MPBs in artificial freshwater (OECD 203, (OECD, 1992), used as zebrafish media) after varying the surfactant treatment. The manufacturer's guidelines suggested a surfactant solution of 0.1% was needed to disperse the MPBs in water, but dispersion was equally achieved with a 0.01% solution (data not shown). Moreover, it was possible to filter (1 μm nylon mesh) and rinse the MPBs prior to dispersion in the freshwater medium.

To accurately add the different MPB doses (10, 100 and 1000, MPBs mL^{-1}) to the zebrafish Ag exposures, we related MPB weight to bead numbers. An initial 5 mL stock was prepared at a concentration of 0.1 g mL^{-1} and the MPB numbers in the dispersion were determined with a Thoma cell counting chamber. From this, 0.1 g of the purchased powder was determined to contain $\backsim 7.0 \times 10^5$ individual MPBs ($6.7 \times 10^5 \pm 5.4 \times 10^4$, $n = 3$ replicate solutions with 10 measurements from each solution) and therefore 1.0×10^6 MPBs was calculated to weigh 0.15 g. When dispersed in 1 L (volume used for the zebrafish exposures) one million MPBs would equate to 1000 MPBs mL^{-1} (i.e. 0.15 mg mL^{-1}, used as the top MPB dose). This was verified with a Coulter Counter® Multisizer TM Z3 (Beckman Coulter, Miami, FL, USA) which showed that our estimates were >90% accurate. This weight to number relationship was used a basis for each of the MPB doses.

A drop of the 0.1 g mL^{-1} stock suspension was placed on a glass slide and imaged by a Nikon SMZ18 stereomicroscope equipped with NIS-Elements Basic Research software (Nikon, Tokyo, Japan). The size distribution of the MPBs was determined by measuring the diameters of 100 individual beads on selected microscopy images using imaging software (ImageJ). The composition of the pristine PE MPBs was verified non-destructively using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FT-IR) spectroscopy. The single bounce diamond internal reflectance element (2×2 mm) was covered with the pristine beads (as received from Cospheric LLC) and 20 scans were run at a resolution of 2 cm^{-1} between 4000 and 650 cm^{-1} on a Bruker Alpha FT-IR instrument (Bruker, Billirica, MA, USA). Spectra were processed using Opus software supplied by Bruker. To investigate whether the surfactant coating or incubation with 1 μg Ag (described in the next section) altered the MPBs. ATR-FT-IR spectroscopy was also conducted on beads after each step of the Ag incubation. Thus, surfactant-coated MPBs, MPBs dispersed in artificial freshwater and MPBs dispersed in artificial freshwater containing 1 μg Ag, prepared identically to those used in the zebrafish exposure, with the exception of using non-radiolabeled Ag (Ag as $AgNO_3$ was used instead), were dried at room temperature and then analysed by ATR-FT-IR, as described. A spectrum of $AgNO_3$ (Sigma Alrich) was also analysed as for comparison.

2.2. Zebrafish husbandry

Adult zebrafish (0.3–0.7 g, obtained from University of Sheffield, strain AB wildtype) were kept in static glass aquaria containing 125 L of artificial freshwater (OECD 203 (OECD, 1992) with 4:1 Ca:Mg and 10:1 Na:K ion ratios, respectively). Water temperature was maintained at 28 °C and the water was continuously filtered through a biological filter. Twenty litres of water were renewed each day. Fish were fed daily on 3% their body weight with flake food (Aquarian® Tropical Flake food), but were not fed 24 h prior to experimentation. A 12 h light/dark photoperiod was maintained throughout.

2.3. Zebrafish exposure

The present study contained six experimental treatments (A–F) in which zebrafish were exposed to 1 μg Ag L^{-1}, but treatments differed in the presence or absence of MPBs, as follows: (A) 1 μg Ag L^{-1} exposure only, (B) exposure in the presence of Tween80 (i.e. surfactant control), (C) exposure in the presence of 1000 MPBs mL^{-1}, (D) exposure in the presence of 100 MPBs mL^{-1}, (E) exposure in the presence of 10 MPBs mL^{-1}, and (F) exposure where the MPBs (equivalent to 1000 MPBs mL^{-1}) were incubated with the 1 μg Ag for 96 h (termed as 'Ag-incubated MPBs'). In freshwaters (such as OECD 203 medium used in the present study) Ag^{+} would be the dominant species (Hogstrand and Wood, 1998; Ratte, 1999), accounting for up to 90% of the total Ag in some cases (Croteau et al., 2011). Each treatment (A–F) was performed in duplicate beakers (i.e. 12 exposures in all) with 8 fish randomly assigned per beaker. The total Ag concentration of 1 μg L^{-1} consisted of 0.48 μg L^{-1} added as ^{110m}Ag (equivalent to 0.1 MBq, specific activity of 209 MBq mg^{-1} Ag, Institute of Atomic Energy POLATOM Radioisotope Centre, Poland) and 0.52 μg L^{-1} non-radiolabelled Ag (added as $AgNO_3$, Sigma Aldrich). ^{110m}Ag activity was measured with a LKB Wallac 1282 CompuGamma gamma counter (Wallac, Turku, Finland) with a counting window between 198 and 245 keV (counter efficiency of 51%). Blank samples were used alongside samples to determine the background level of radiation.

Based on our previous determination that 0.15 g of MPBs produced a 1 L dispersion of 1000 MPBs mL^{-1}, MPBs were weighed out accordingly for treatments C–F. MPBs were then dispersed in 4 mL of 0.01% Tween80 overnight, after which time MPBs were filtered (1 μm mesh filter) from the surfactant, rinsed and resuspended in 25 mL of artificial freshwater. For Treatment F (Ag-incubated MPBs), the radiolabelled 1 μg Ag was added to the 25 mL suspension in order to allow Ag time to adhere to the plastic surfaces within a restricted volume. Three additional replicates were made of this treatment in order to determine the extent of Ag to MPB binding. All 25 mL suspensions (with and without added Ag) were kept for 96 h under constant agitation (150 rpm, Innova™ 2100, New Brunswick Scientific, CT, USA). To prevent photo-oxidation in the Ag-incubated MPBs suspension, these treatments were wrapped in aluminium foil. All other Ag solutions were also kept in the dark until addition to the exposure tanks. After 96 h, the three additional Ag-incubated MPBs replicates were again filtered and both the MPBs on the nylon filter and the filtrate were assayed for ^{110m}Ag.

Prior to the experiment, the 25 mL MPB suspensions (Treatments C–F) were thoroughly rinsed into 1.5 L plastic beakers lined with plastic bags with artificial freshwater and made up to 1 L. MPBs were vigorously stirred to ensure dispersion through the water column. Plastic bag-lined beakers for Treatments A and B similarly contained 1 L of artificial water and 100 μL of 0.01% Tween80 was added to Treatment B (surfactant control) as an estimate of any potential surfactant that was carried over into the exposure. Treatments A–E were then spiked with 1 μg of radiolabelled Ag. Eight zebrafish fish were randomly placed in each of the 12 beakers commencing the experiment. Temperature and light conditions were the same as previously described for husbandry.

At 4 h and 24 h, four zebrafish individuals were removed from each beaker (i.e. 8 fish per treatment at each time point). Fish were sacrificed with an overdose of MS222 (Tricaine mesylate, Sigma Aldrich) and gill and intestine were removed from each fish. Dissected tissues and the remaining body were weighed and then assayed for radioactivity. Weights and radioactive counts per minute (CPM) were summed to derive whole fish values. The specific activity of the radiotracer was used to determine the total Ag tissue concentrations which are expressed on whole body wet weight basis (as ng Ag g^{-1} zebrafish (ww)).

2.4. Statistical analysis

No differences were found between zebrafish taken from replicate tanks of each treatment and in the absence of tank-specific effects data from replicate treatments were combined. Levene's test for normal distribution was performed on the combined datasets prior to statistical analysis. Significant differences in the Ag concentrations between treatments groups were determined by one-way analysis of variance (ANOVA) with post hoc Tukey HSD test. Percentage data was arcsine transformed prior to analyse. Differences were considered significant at $p \leq 0.05$. All statistical analysis was performed in SPSS version 20 (SPSS Statistics for Windows, SPSS Inc., Chicago, IL, USA). All data are presented as mean values ± standard deviation (s.d).

3. Results

3.1. Characterisation of the microplastic beads

Imagery of the MPBs used in this study showed that they were spherical in nature (Fig. 1A). A mean size of 59 ± 19 μm and size range of 19–107 μm ($n = 100$), with the size distribution centred around the mean, verified the manufacturer's product information (Figure B). ATR-FT-IR spectroscopy of the pristine beads confirmed the composition as PE and compared favourably with the spectrum for low-density PE from the software's reference library (Fig. 1D).

The 96 h incubation 1×10^6 MPBs with 1 μg Ag, that replicated the exposure scenario in Treatment F, showed that 76.3 ± 2.4% of the recovered radiolabelled Ag was associated to the MPBs and 23.7 ± 2.4% remained within the filtrate (Ag recovery was 92.7 ± 4.2%, $n = 3$, Fig. 1C). The unrecovered Ag was most likely lost via container adsorption. The association of Ag to MBPs did not appear to affect the surfaces of the PE beads as analysed by ATR-FT-IR (Fig. 2). Successive steps of the incubation procedure; 24 h surfactant treatment, 96 h dispersion in freshwater and then spiked with 1 μg Ag, did not affect the PE spectra. In addition there were no detectable peaks that corresponded to the spectrum generated from the analysis of the $AgNO_3$ sample (Fig. 2). However, any Ag adsorbed to the PE MBPs would likely be below the detectable limit for ATR-FT-IR analysis (0.1–1 %).

3.2. Uptake and localization of Ag in zebrafish

Of the 96 zebrafish exposed across all treatments there were 3 treatment unrelated mortalities, and 3 individuals were excluded due to their small size and difficulties in performing dissections. Following 4 and 24 h exposure to only 1 μg L^{-1} Ag (Treatment A), zebrafish accumulated 8.6 ± 2.8 and 19.5 ± 5.3 ng Ag g^{-1} zebrafish (ww), respectively (Fig. 3). Neither the surfactant control (Treatment B) nor the presence of MPBs in varying doses (1000 MPBs mL^{-1} (Treatment C), 100 MPBs mL^{-1} (Treatment D), 10 MPBs mL^{-1} (Treatment E)) significantly affected the uptake of Ag at either time point. However, when zebrafish were exposed to the Ag-incubated MPBs (Treatment F), the uptake of Ag was significantly reduced even though the total exposure concentration remained the same (1 μg L^{-1}); 5.0 ± 0.8 and 10.0 ± 1.7 ng Ag g^{-1} zebrafish (ww) at 4 and 24 h, respectively ($p < 0.05$, Fig. 3).

For all treatments except Treatment F there were similar patterns of Ag localization (Fig. 4). Ag distribution in zebrafish exposed in Treatments A–E and sampled at 4 h, was 15.4–21.2 % in the gills, 0.8–2.1 % in the intestine and 77.0–82.9 % in the remaining body (presented as ng Ag g^{-1} zebrafish (ww) concentrations and percentages, Fig. 4A and C). In comparison, zebrafish sampled at 4 h

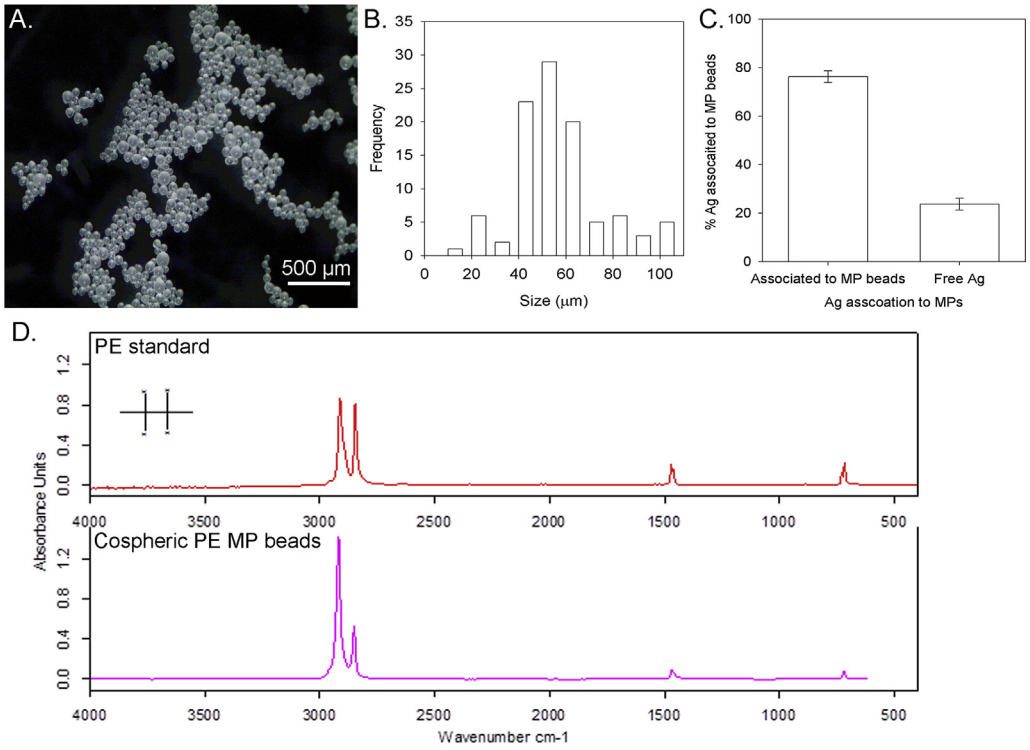

Fig. 1. Light microscopy image of PE MPBs (A) confirmed their spherical nature and were used to determine an average diameter of 59 ± 18 µm (n = 100) with a size distribution close to the mean (B). The composition of the beads was confirmed by ATR-FT-IR spectroscopy with sample spectra comparing well to low density polyethylene standard reference spectrum (D). Following 96 h incubation of MPBs with 1 µg Ag (see text for details), 76.3 ± 2.4% of the recovered Ag was associated to the MPBs with 23.7 ± 2.4% remaining as unbound Ag (n = 3, C).

following exposure to Ag-incubated MPBs (Treatment F) had a significantly greater Ag concentration within their intestine and significantly lower Ag in their body tissue ($p < 0.05$). Proportionally, 27.4 ± 7.0% was found in the intestine, 19.7 ± 6.9% in the gills and 52.9 ± 11.1% in the body. At 24 h there appeared to be a more equal distribution of Ag in gills and intestine in all treatment groups, which meant that the Ag concentration in the intestines of zebrafish exposed to the Ag-incubated MPBs were not significantly different to fish from the other exposure groups, although body concentrations remained significantly lower ($p < 0.05$, Fig. 4B). Nonetheless, fish from Treatment F did continue to exhibit a higher proportion of Ag in the intestine (37.2 ± 6.7%) compared to all other treatments (13.7–24.5 %) ($p < 0.05$, Fig. 4D).

4. Discussion

The aim of the present study was to determine whether the presence of MPBs influenced the uptake and localization of Ag in zebrafish. Our results show that the co-exposure of pristine MPBs and Ag did not alter the uptake and localization of Ag in zebrafish in comparison to Ag-only exposures, irrespective of the tested MPB dose (10, 100 and 1000 MPB mL^{-1}). However, when zebrafish were presented with Ag that was already largely bound to MPBs (Treatment F, where ~75% of the radiolabelled Ag was found to associate to the MPBs), there were two important effects. Firstly, the overall uptake of Ag was lowered and secondly there is a greater proportion of Ag localized to the intestine. Thus, zebrafish presented with Ag in a form in which the aqueous ion was already associated with the plastic beads experienced lower uptake, perhaps due to decreased bioavailability of the metal, but the Ag that was entering the organism was more likely accumulated via the dietary uptake route following ingestion of the MPBs. To our knowledge this is first study to show that MPs can influence the uptake and localization of a metal contaminant, in particular affecting a change in the route of uptake, but only after sufficient time for adsorption to the plastic.

The environmental relevance of an exposure scenario in which pollutants interact prior to encountering biota is not abundantly clear, but both MPBs and Ag are released into the environment following consumer use; MPBs from exfoliants and scrubs (Derraik, 2002; Fendall and Sewell, 2009; Eriksen et al., 2013) and Ag from the array of appliances and products that utilize Ag, commonly in the form of silver nanoparticles (Benn and Westerhoff, 2008; Farkas et al., 2011) that have the potential for varying degrees of dissolution (Kittler et al., 2010; Li and Lenhart, 2012). Thus there is the possibility that they would both be transported in urban outflows under conditions that promote adsorption (i.e. relatively restricted volume and constant agitation, much like our preparation of the Ag-incubated MPBs) before introduction into the wider environment and potential interactions with aquatic organisms. Adsorption isotherms of various aqueous metals, not including Ag, to pristine PE pellets show that metal cations are sorbed by the plastic surface (Holmes et al., 2012). However, adsorption was greater in aged pellets where weathering and oxidation may have increased the number of potential binding sites and permeability, and even changed the polarity of the plastic surface to enhance metal accumulation. Moreover, in nature the formation of biofilms and chemical coatings, not forgetting the surfactant that already coats the MPBs, may also increase the adsorptive properties of the plastic surface (Mato et al., 2001; Holmes et al., 2012). Thus, treatments in which zebrafish were co-exposed to Ag and pristine MPBs may have been overly simplified and although the exposure to Ag-incubated MPBs may be the most environmentally relevant scenario, this too may underestimate the extent of adsorption found in

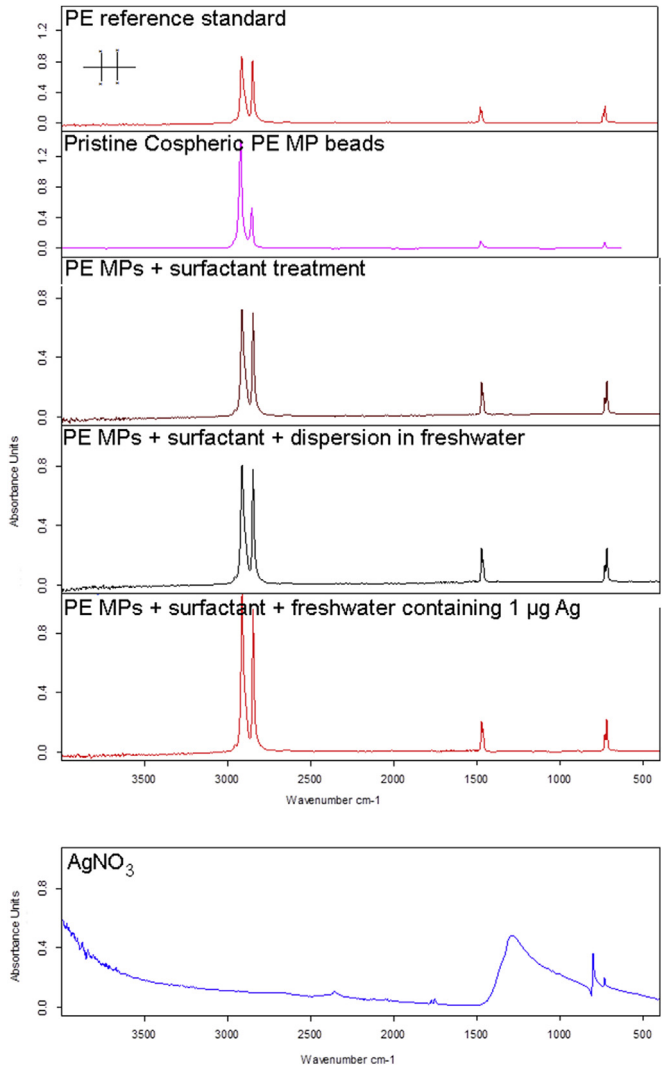

Fig. 2. ATR-FT-IR analysis of the pristine PE MPBs following the different steps of the incubation with 1 µg Ag, namely dispersion in 0.01% surfactant (Tween80), dispersion in artificial freshwater and the addition of Ag. No changes in MPB morphology were apparent after comparison to PE reference spectra. A spectrum for $AgNO_3$ is also shown and presents no overlapping peaks with the PE MBPs.

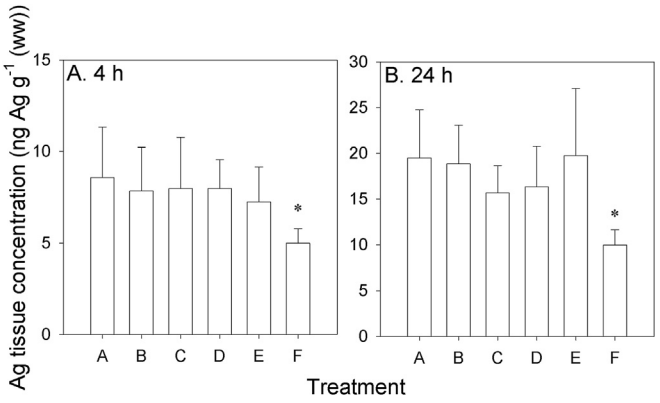

Fig. 3. Mean whole body Ag concentrations in zebrafish (ng Ag g^{-1} zebrafish (ww) ± s.d, $n = 7–8$) exposed to the different 1 µg Ag L^{-1} treatments; 1 µg Ag L^{-1} only (A), in the presence of surfactant (surfactant control, B), addition of 1000 MPBs mL^{-1} (C), addition of 100 MPBs mL^{-1} (D), addition of 10 MPBs mL^{-1} (E), and 'Ag-incubated MPBs' where MPBs (equivalent to 1000 MPBs mL^{-1}) were incubated with Ag for 96 h to promote the adherence of Ag to the surface of the MPBs (F). Results are shown for zebrafish sampled at 4 (panel A) and 24 h (panel B). Results show that fish exposed to the 'Ag-incubated MPBs' accumulated significantly less Ag at both time points ($p < 0.05$, one-way ANOVA with post-hoc Tukey's HSD).

in situ field settings.

A reduction in contaminant uptake was also found following the exposure of the marine amphipod, *Allorchetes compressa*, to POPs (specifically, polybrominated diphenyl ethers (PBDEs)) in the presence of PE MPBs extracted from a commercial facial cleanser (Chua et al., 2014). The authors attributed the reduced uptake of PBDEs to the decreased bioavailability that resulted from the high binding affinity of the organic pollutants to plastic surfaces. In the present study, the bioavailability of the Ag adhered to the MPBs may have been reduced because PE MPBs, with a density of 0.96 g/cm^3, float on the water surface (Eriksen et al., 2013). Despite our efforts to homogenously disperse the MPBs through the water column, they tended to migrate to the water surface over time which was particularly noticeable after 24 h. Thus the reduced uptake of Ag in fish exposed to Ag-incubated MPBs may have occurred, in part, because the MPBs were increasingly aggregating on the surface thereby limiting interactions between Ag and the fish. Similarly, in addition to insufficient adherence time, the buoyancy of the MPBs may also help explain the negligible impact that merely adding MPBs had on the uptake of Ag (Treatments C–E) if the rate of migration from the water column to the surface exceeded the rate at which Ag was able to adhere to the plastic beads. MPB density may therefore be an important factor when considering the role of plastics as a vector for other contaminants and potentially the use of PE MPBs with a greater density or heavier polymer types, such as polystyrene (1.05 g/cm^3) or acrylic (1.19 g/cm^3), may have resulted in changes to uptake and localization of Ag in exposed zebrafish. Greater research into how plastic density may moderate the biological and chemical interactions of MPs is required.

When zebrafish were exposed to Ag-incubated MPBs there was a significant change in the proportion of Ag localized in the intestine. This was most marked at 4 h when 27.4% of accumulated Ag was found in the intestines of fish in exposure group F, compared to 2.1% (maximum proportion) in the intestines of fish in the other groups. Although the difference was less dramatic after 24 h, a higher proportion of Ag in the intestine was still determined for fish exposed to the Ag-incubated MPBs (Fig. 4). The simplest explanation for this is that Ag was incidentally ingested by fish consuming the MPBs, and whilst this may be in keeping with the MP vector effect (Syberg et al., 2015), we cannot claim with certainty that the Ag measured in intestinal tissue was still adhered to MPBs or that the MPBs were also located in the intestine. Ingested microplastics have been sampled from the gastrointestinal tracts of fish species (e.g. Lusher et al., 2013; Sanchez et al., 2014), but the compartments of the gastrointestinal tract are rarely separated. Furthermore, the desorption of adhered contaminants (POPs) from PE and PVC MPs under physiologically relevant gut conditions has been described (Bakir et al., 2014). Generally, PE MPs released POPs at a faster rate than PVC MPs and release was promoted when tests were performed at 38 °C, mimicking conditions found in warm blooded animals (e.g. seabirds), compared to conditions that replicated the guts of cold-blooded animals (Bakir et al., 2014). Such desorption studies have yet to be conducted with metals adhered to MPs, but it must be considered that Ag that entered the fish adhered to the MPBs was desorbed from the plastic's surface before interacting with uptake sites in the intestine.

Knowing the fate of the ingested contaminant is vital if we are to fully understand the implications of MPs transporting adhered contaminants. Research into the dietary bioavailability of metals to

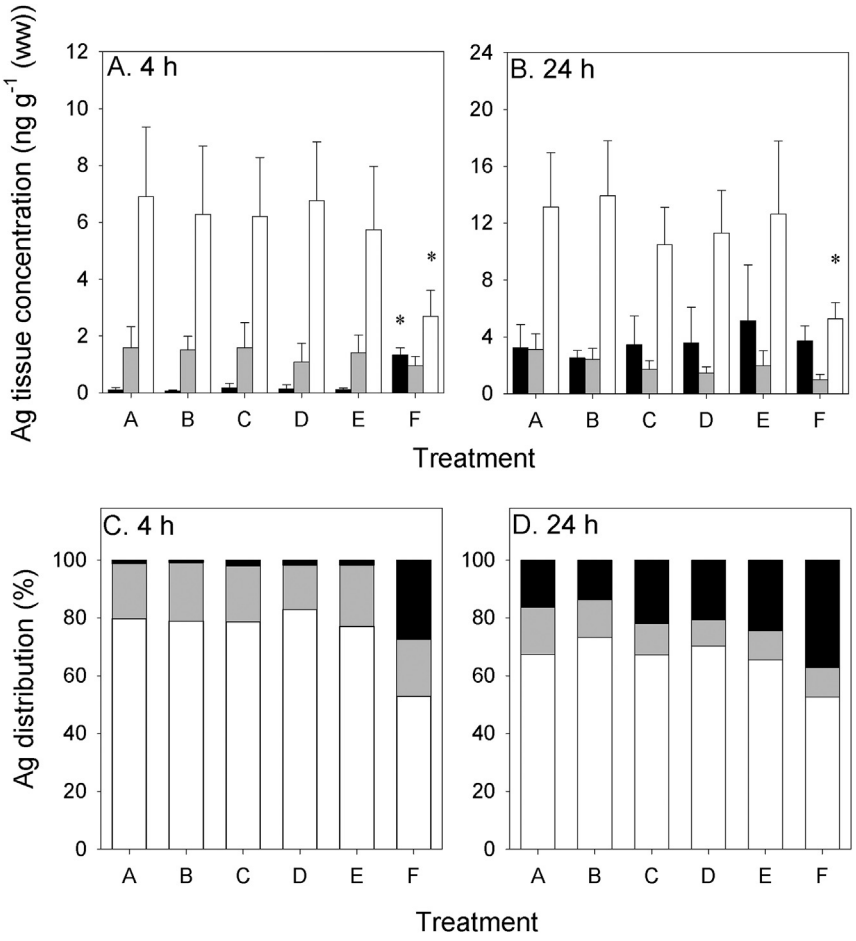

Fig. 4. Localization of Ag in zebrafish tissue. Mean tissue concentrations (ng Ag g^{-1} zebrafish (ww) ± s.d, $n = 7–8$) in the intestine (black bars), gills (grey bars) and body (white bars) are shown for the different 1 μg Ag L^{-1} treatments; 1 μg Ag L^{-1} only (A), in the presence of surfactant (surfactant control, B), addition of 1000 MPBs mL^{-1} (C), addition of 100 MPBs mL^{-1} (D), addition of 10 MPBs mL^{-1} (E), and 'Ag-incubated MPBs' where MPBs (equivalent to 1000 MPBs mL^{-1}) were incubated in Ag solution for 96 h to allow Ag to adhere to the surface of the MPBs (F). Results are shown for zebrafish sampled at 4 (panel A) and 24 (panel B) h post-exposure with asterisk denoting significant differences in tissue concentration ($p < 0.05$, one-way ANOVA with post-hoc Tukey's HSD). Panels C (4 h) and D (24 h) show the tissue concentration data expressed as a % of the total Ag accumulated following each exposure treatment.

fish has shown that ingested metals can cause cytotoxic damage (Khan et al., 2010a, 2010b), physiological changes to the gut environment (Glover and Hogstrand, 2002; Khan and McGeer, 2013) and reproductive perturbation (Boyle et al., 2008). However, in the case of Ag, despite the increase in hepatic Ag concentrations, no significant deleterious effects have been attributed to dietary intake (Galvez and Wood, 1999). In general terms, the fate of the ingested adhered contaminants could, potentially, be summarized into four *in vivo* outcomes (i) contaminants are released from MPs and undergo the same fate as the ingested contaminant in labile form; (ii) contaminants remain adsorbed to the plastic and pass through the organism without effect; (iii) MPs remain in the digestive system, potentially causing blockages and a false sense of satiation (Ryan, 1988), and release adhered contaminants over time; and (iv) a combination of these possible eventualities.

The toxicological importance of these outcomes likely differs and therefore further research is required to understand of these possibilities.

The role of MPs in facilitating the uptake of other contaminants to aquatic organisms has been suggested (Mato et al., 2001; Cole et al., 2011; Syberg et al., 2015), but similar to our study, delineating such affects are not straightforward. In one of the first controlled laboratory studies to look at the effects of MPs on the transport of pollutants, Besseling et al. (2012) exposed the lugworm (*Arenicola marina*) to nineteen different PCBs in the presence of varying concentrations of PS MPs. These authors found that at most there was increase in the accumulation of PCBs by a factor of just 1.1–1.5. Thus some studies suggest that the MP 'organismal-vector' effects may be of limited importance, particularly in the context of risk assessment (Gouin et al., 2011; Koelmans et al., 2013). However, our study adds to the growing body of work that indicates that plastics do have the potential to alter the contaminant–organism interactions (Chua et al., 2014, Oliveira et al., 2013) given the right circumstances. Moreover, it is the first study to show such an effect with a metal contaminant.

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