Title Secondary microplastics were prevalent in sediment in a freshwater UK urban river

Authors Reina M. Blair[a*], Susan Waldron[a], Vernon Phoenix[c], Caroline Gauchotte-Lindsay[b]

Affiliations [a]School of Geographical and Earth Sciences, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK

Email**:** r.blair.1@research.gla.ac.uk; Susan.Waldron@glasgow.ac.uk

[b]School of Engineering, University of Glasgow, Glasgow, G12 8LT, Scotland, UK

Email: Caroline.Gauchotte-Lindsay@glasgow.ac.uk

[c]Department of Civil and Environmental Engineering, University of Strathclyde, Glasgow, G1 1XQ, Scotland, UK

Email: vernon.phoenix@strath.ac.uk

***Corresponding Author** Reina M. Blair

School of Geographical and Earth Sciences, Room 211, Main Building, East Quad, University of Glasgow, Glasgow, G12 8QQ, UK

Email: r.blair.1@research.gla.ac.uk

Phone: +44 (0) 141 330 4376

Abstract

Plastic pollution has been documented in terrestrial and aquatic environments worldwide, with growing concern for 'microplastics' (MPs, <5 mm). Understanding of the sources, fate, and impact of MPs remains limited, particularly in freshwater environments. Furthermore, their small sizes and a lack of standardised methodology hinders monitoring and risk assessment of these emerging contaminants. Here, the distribution of microscopic debris in an urban river close to the marine environment in the West of Scotland was investigated to assess the prevalence of MPs. Bank sediment samples were collected twice from the River Kelvin in Glasgow and were size-fractionated and processed for extraction of MPs by density separation. Light microscopy and scanning electron microscopy with energy dispersive spectroscopy, were employed for characterisation and quantification of microdebris of sizes ranging from 2.8 mm to 0.45 μm. Sample MP spiking and use of procedural blanks allowed the influence of processing on field data quality to be considered. The predominant type of MPs were fibres, comprising >88% of total MP counts, but fibre content in blanks suggested potential contributions from background contamination. Final MP abundances were estimated at 161-432 items per kg dry sediment. In addition, metallic and glass pellets were observed in high abundances in settled material and could be easily misidentified by visual inspection. Thus, compositional analysis is needed to avoid analytical errors from MP misidentification and overestimation.

Key words: microplastic, emerging contaminants, freshwater, electron microscopy, fibres

Abbreviations

MP	microplastic(s)
mm	millimetre
kg	kilogram
cm	centimetre
SE	sampling event
°C	degrees Celsius
TS	total solids
DS	density separation
NaCl	sodium chloride
ρ	density
g	grams
mL	millilitre
µm	micrometre
DI	deionised
C	carbon
SEM	scanning electron microscopy
EDS	energy dispersive spectroscopy
BSE	backscatter electron
Ti	titanium
Br	boron
Si	silica
Al	aluminium
nm	nanometre
MSFD	Marine Strategy Framework Directive

1. Introduction

Plastic production and subsequent pollution are global environmental concerns. Global plastic generation has exhibited an upwards trend since the 1950s, reaching 335 million tonnes in 2016, a 10% increase from 2015 levels (Plastics Europe, 2017). Moreover, an estimated 8300 million metric tonnes of plastic have been produced since 1950 to date, with approximately 6300 million metric tonnes of plastic waste created until 2015, of which only 9% was recycled (Geyer et al. 2017). Plastics are persistent materials, so when discarded as waste they can accumulate in landfills and the environment for a long time (Geyer et al., 2017) and pose a threat to biodiversity, ecosystems services and potentially human health (Eerkes-Medrano et al., 2015).

Arising from its aesthetic and environmental impacts, plastic contamination has received increasing attention from the public and scientific communities for several decades (Coe and Rogers, 1997; Derraik, 2002; Blair et al., 2017), especially larger, visible pieces. Of recent concern is microscopic plastic debris commonly referred to as microplastics (MPs), typically less than 5 mm in size GESAMP, 2015), although a formal definition and lower limit have not been established (Blair et al., 2017). They are divided, broadly, into primary or secondary types (GESAMP, 2015), though these definitions are also not standardised. Primary MPs are produced intentionally and are typically small spherical pellets, while secondary MPs, such as fibres, fragments, and flakes are formed indirectly from the breakdown of larger plastic pieces. Primary types have garnered the most media and public attention, namely pre-production pellets and microbeads. The widespread attention given to primary MPs has prompted actions worldwide sometimes leading to country-wide bans on the use of microbeads (e.g., in the Netherlands, Canada, USA, United Kingdom, and New Zealand). Despite the greater focus on primary MPs, secondary types may be of increasing abundance, particularly fibres released into wastewater from washing of synthetic fabrics (Browne et al., 2011). Fragmented secondary MPs may increase in quantity over time, long after primary inputs are reduced since larger pieces may continue to degrade into smaller plastic particles. Currently, the contribution of different

sources to overall MP loadings to the environment and the relative importance of primary and secondary types remains poorly understood (Duis and Coors, 2015; GESAMP, 2015).

Research focussed on understanding the sources, distribution, fate, and impact of MP fractions in the environment is increasing rapidly (Blair et al. 2017; Horton et al., 2017), but knowledge of MP pollution in oceans compared to freshwater environments remains more advanced (Thompson et al., 2009; Wagner et al., 2014; Eerkes-Medrano et al., 2015). Coastal and beach surveys conducted between 1980 and 2001 worldwide, revealed that plastic waste can account for 50-90% of all marine litter and that MP materials have been accumulating rapidly in oceans and shorelines over the past few decades (Derraik, 2002). More recently, interest in MPs in freshwater systems has been rising (Eerkes-Medrano et al., 2015) as these are known to be important transport vectors of land-based contaminants to coastlines and open sea environments. Widespread MP abundances have been observed in river and lake surveys of water and sediment samples collected from North American, Asian, and European locations (Blair et al., 2017) with the highest concentrations in freshwaters to date observed in highly contaminated areas of Lake Taihu, China (Su et al., 2016) and in sediment of the River Tame (Hurley et al., 2018). Nevertheless, the role of fluvial waters as conduits of MPs to the marine environments from terrestrial sources has been largely unknown due to a lack of empirical data, although this is a rapidly growing field. Investigating the abundance and nature of MPs in rivers close to estuarine and marine environments, particularly in urban and industrialised catchments where MP could be higher (Nizzetto et al., 2016; Hurley et al., 2018), can potentially further our understanding of this link.

Globally, there is high variability regarding MP abundances and distribution of primary and secondary types (Blair et al., 2017). This may be because MPs are highly diverse in shape, size, colour, and density, resulting in high variability in their distribution in space and time, even within localised environmental compartments. Thus, it is important to increase spatio-temporal coverage and generate further local and regional datasets to improve our understanding of this variability. Nevertheless, the diverse nature and small sizes of MPs render them difficult to measure and monitor

(Hidalgo-Ruz et al., 2012; Tagg et al., 2015). Consequently, there is a lack of unified research methodology for isolation, identification and quantification of MPs both in oceans and freshwaters, reducing comparability among available surveys. Differences in sampling, density separation and sample digestion techniques, and visual assessment of MPs exist (Hidalgo-Ruz et al., 2012). Recently, analytical techniques have been employed more frequently to determine the chemical composition of the recovered pieces, a step that is important for discriminating MPs from other confounding materials that may be mistaken for plastics, for example cellulose fibres (Wesch et al., 2016). Current methodological limitations can lead to errors in characterisation and quantification of MPs from environmental samples, thus method validation of extraction and identification protocols should be routinely tested to understand where uncertainty can be introduced and improve the ability to characterise confidently.

This study sought to determine the prevalence and distribution (size, type and colour) of MPs in a site representing of sediment accumulation in the River Kelvin in the west end of Glasgow, Scotland, close to its discharge to the Clyde estuary. Combined physico-chemical characterisation approaches based on light microscopy and electron microscopy with energy dispersive spectroscopy (SEM-EDS) were used for identification and enumeration of microscopic debris from riverbank sediment. These were required to explore the viability of visual identification of MP and the need to draw on instrumental analysis in routine testing for source verification. This study contributes to generation of spatio-temporal datasets and understanding of what methods are needed for extraction and characterisation of MPs from freshwater environments globally.

2. Materials and Methods

2.1 Site and sampling

The River Kelvin is a freshwater river approximately 34 km long and discharging into the Clyde Estuary, making it particularly suitable to evaluate the role of fluvial systems in the fate and transport

of MPs from continental to oceanic waters. Bulk sediment samples from the surface to a depth of 8 and 10 cm, respectively, were collected with a spade in December 17, 2015 (sampling event 1, SE1) and February 15, 2016 (sampling event 2, SE2) from the River Kelvin bank (55° 52' 8.742", -4° 17' 19.0278", **Fig. 1**). The sample site was selected to be representative of dense urban areas and as it was geomorphologically favourable for sediment deposition as was in a bend of the river. Samples were collected in aluminium tins and wrapped in aluminium foil for transport to the laboratory, five minutes away.

Fig. 1 Location of the sample collection site in a river bend section in the River Kelvin in the west of Glasgow, Scotland, UK.

2.2 Sample processing

The methodological approach employed for sample processing broadly follows methods discussed in the literature (Hidalgo-Ruz et al., 2012; Blair et al., 2017). Throughout the process, care was taken to minimise sample contamination by avoiding the use of plastic materials and including the use of procedural blank controls to check for background contamination.

First, samples were weighed in aluminium trays before and after oven-drying overnight (~24 hours) at 100°C, and mass of total solids (TS) in grams (g) was calculated as the weight of the dried samples. Using an automatic shaker for a duration of 10 minutes, oven-dried samples were sieved into the following size classes: 2.8 mm, 2.0 mm, 1.4 mm, 1.0 mm, 0.71 mm, 0.5 mm, 0.355 mm, 0.25 mm, 0.18 mm, 0.125 mm, 0.09 mm, and 0.063 mm, producing 13 sub-samples for each sampling event. Size fractionation was employed to assess how different types of MPs are associated with different sediment grain sizes. Each size class fraction was weighed and stored in a glass bottle until further processing.

196 *2.3 Extraction by density separation*

197

198 After fractionation, density separation (DS) with a saturated NaCl solution (ρ ~1.2 g cm^{-3}) was used to
199 separate low-density MP pieces. Briefly, ~25 g (or entire volume if less than 25 g) of oven-dry
200 sediment from each size fraction was mixed with 40-68 mL of salt solution to cover the sediment,
201 manually shaken vigorously for 1 minute and left to settle overnight (~24 hours). After 24 hours, the
202 supernatant was filtered through Whatman 0.45-µm cellulose filters to collect suspended debris. The
203 filter paper was rinsed three times with deionised (DI) water to remove excess salt, then transferred to
204 petri dishes to dry at room temperature (18-21ºC). During processing of SE1 samples, re-suspension
205 of some settled sediment (i.e. those deposited after the 24 hour period) was observed during
206 decanting. Thus, a second settling step was introduced for processing of SE2 samples in which the
207 supernatant was transferred into a clean beaker before filtration, covered, and left to settle for two
208 additional hours to allow for further settling of re-suspended solids and reduce their potential transfer
209 to filters.

210

211 The DS extraction method was validated via recovery tests using river bank sediment collected from
212 the same study site, spiked with different types of MP standards. Polyethylene (0.71-0.85 mm
213 diameter, ρ =0.96 g c^{-3}), polypropylene (2.45 mm diameter, ρ =0.866 g c^{-3}), and polystyrene (4.4 mm
214 diameter, ρ =1.048 g c^{-3}) microbeads purchased from Cospheric LLC (Santa Barbara, California) were
215 used to mimic primary MPs. Nylon toothbrush bristles and rope fragments, polypropylene cleaning
216 brush bristles, and polyethylene mesh fruit packaging fragments produced in the lab were used to
217 mimic fibrous secondary MPs. Briefly, approximately 20 g of oven-dried sediment were spiked with
218 10 beads or 15 fibre-like fragments, in triplicates for each polymer type, thoroughly mixed, and
219 processed the same way as field samples (See Sections 2.3 and 2.4). Recovery efficiencies were
220 calculated as [number of pieces extracted/number of pieces spiked] * 100).

221

222 Procedural blanks consisting of NaCl solution were produced with every filtration sequence to
223 account for background contamination.

2.4 Identification and quantification

First, a stereo microscope was used to identify MPs based on physical appearance. Here, samples different from sediment grains (i.e. more rounded, pitted, fibre-like, coloured or transparent) were identified and counted, and pieces in sizes ranging <2.8 mm to 0.7 mm were picked out with metal tweezers into glass vials and photographed with a Leica MC120 HD camera connected to a Leica $MX7_5$ microscope. Pieces smaller than 0.7 mm were not extracted this way as they were too small to manipulate and could be lost during manual transfer; these fractions were counted and saved on the filter paper until further instrumental analysis. Settled solids were also inspected under light microscopy to detect presence of high-density polymers (ρ>1.2 g cm^{-3}).

Representative aliquots of suspected MPs from each category and size fraction were examined using a FEI Quanta 200F scanning electron microscope (SEM) coupled with energy dispersive spectroscopy (EDS), enabling determination of elemental composition. The aliquot was selected from the SE1 samples and comprised suspended and settled pieces. Briefly, samples were prepared by placing individual pieces >0.7 mm on double-sided adhesive carbon discs (9-mm diameter), mounted on 9-mm specimen stubs and imaged by SEM-EDS operating at an accelerating voltage of 20 keV in the secondary electron and backscattered mode. Suspended pieces <0.7 mm that could not be separated manually with tweezers were transferred onto the SEM stub by "pressing" the C adhesive over the filter paper and using a light microscope to verify that the target piece was successfully transferred onto the stub. If it was not possible to transfer a piece after multiple tries, a square of filter paper was cut around it and placed on the stub. The compositional data were used to discriminate plastics from non-polymers since the plastics are carbon-based and other materials are expected to be non-organic. Electron microscopy assessment of the aliquot was used to refine the approach to the visual identification of MPs for the remaining samples under light microscopy.

The sum of pieces counted in all size fractions was used to quantify MP abundance for each sampling event by visual characterisation under light microscopy (stage 1) followed by chemical

252 characterisation by SEM-EDS analysis (stage 2) to compare visual and chemical assignation of MPs.
253 Abundances were calculated as [total number of suspected MPs/mass of TS] and expressed in items
254 per kg of dry sediment.
255
256 **3. Results and Discussion**
257
258 *3.1 Method validation tests and blanks*
259
260 Recovery rates for MP microbead standards were 100 % for all polymer types, sizes, and densities
261 (**Fig. 2**) while average recovery rates for fibre-like secondary MPs were lower than for primary MPs,
262 ranging from 49 (±10.2) to 58 (±7.7) % for mesh packaging fragments and nylon rope pieces,
263 respectively (**Fig. 2**). Lower recovery rates for fibrous MPs may be attributed to a tendency to cluster
264 together and adhere to the inorganic matrix and walls of the container, and may present a challenge
265 for separation and thus accurate quantification of this type of MP.
266
267 Fibres were the only type of materials observed in procedural blanks (**Table 1**). Fibre content in
268 blanks were similar to those observed in other studies (Dris et al., 2015; Horton et al., 2017; Hurley et
269 al., 2018). Only a handful of freshwater studies have included use of blanks as verification, but when
270 reported they were considered negligible compared to those observed in field samples (Dris et al.,
271 2015; Horton et al., 2017) or determined to be non-plastic (Hurley et al., 2018). Thus, the field data
272 were not blank corrected in this study. Nevertheless, their occurrence in blank controls suggests
273 background contamination, meaning that the field samples may contain a non-river contribution of
274 fibres that could result in an overestimate. Conversely, their lower recovery rates could result in an
275 underestimate in both the sample and the blank. As fibres seem to be a predominant MP category in
276 this and many studies, more blank and standard control tests are needed to reduce these uncertainties
277 and improve confidence in results.
278

279 **Fig. 2** Recovery tests for density separation using various types of microplastic standards: purchased
280 microbeads (polyethylene, **PE**; polypropylene, **PP**; and polystyrene, **PS**), and fibre-like fragments
281 produced in the lab (PP bristles from a cleaning brush, nylon bristles from a toothbrush, nylon rope,
282 and PE mesh packaging).
283
284 *3.2 Microplastic categories*
285
286 Suspected MPs were observed in all size fractions and were classified into three broad categories: (1)
287 pellets, (2) fibres, and (3) fragments (**Fig. 3**).
288
289 **Fig. 3** Light microscopy images of suspected microplastics in size-fractionated sediment samples
290 from the River Kelvin in suspended and settled material before chemical characterisation. Items
291 shown are: pellets (a), fibres (b) and fragments (c).
292
293 *3.2.1 Micropellets*
294
295 At stage 1, five micropellets were observed in suspended material in SE1 only (**Table 1**), but these
296 were determined to be non-plastic at SE2. Visually, these pellets were dark-coloured and similar in
297 appearance to those reported in a previous study in the St. Lawrence River (Castañeda et al., 2014).
298 Pellets in the St. Lawrence River were determined to be polyethylene microbeads based on chemical
299 characterisation by differential scanning calorimetry, thus suspended pellets in the River Kelvin were
300 suspected to be also MPs. However, SEM-EDS analysis performed here showed suspended pellets
301 were primarily metallic (**Fig. 4**). The physical similarities but differing elemental compositions
302 between the two studies indicate that non-MP pellets can be easily mistaken for MPs by visual
303 inspection alone. The absence of primary MPs in this study contrasts with reports from earlier
304 freshwater studies in urban catchments that found primary MPs to be more common than secondary
305 forms based on visual and chemical characterisation (Zbyszewski and Corcoran, 2011; Eriksen et al.,
306 2013; Castañeda et al., 2014; Hurley et al., 2018). The high recovery rates for pellets from the

validation tests provided confidence that, although no MP pellets were isolated from the environmental samples for this study, this was likely due to their absence from the site and not due to extraction error.

Visual examination revealed that micropellets were the predominant type of MPs in settled material by count across all size fractions for December and February samples, respectively (**Supplementary Material**). Settled micropellets consisted mostly of dark spheres similar to suspended ones, with a few clear and white- or cream-coloured pieces (**Fig. 3a**). Micropellets were present mainly in the mid-range particle size fractions (0.25-0.7 mm). These were also present in clusters or aggregations of pellets that appeared to have been fused or melted together. Owing to their physical resemblance to micropellets observed in previous studies (Castañeda et al., 2014), an aliquot of settled pellets representing varying colours and sizes, was analysed by SEM-EDS to assess whether they were high-density MPs or non-plastic. The chemical composition was determined to be mostly metallic for dark pieces, while light-coloured pellets were mostly silica (**Fig. 4**). While these micropellets were not MPs and therefore not the focus of this study, their high concentrations might warrant further evaluation to determine source of origin since they do not occur naturally in the aquatic environments and if similar in size, shape, and colour as their MP counterparts, could also be harmful to the aquatic fauna if ingested. It is also important to be aware of their presence as they could be mistaken for MPs by visual inspection, especially if extracted by density separation as here. As metals have higher density, it would be expected that DS would not extract these materials. In this study, the five pellets in SE1 extracted by DS at stage 1 may be explained by the presence of a porous surface that was only evident during examination of structural composition in SEM-EDS images.

Fig. 2 Backscattered electron image and elemental spectra for common micro-pellets observed in River Kelvin sediment. Pellets were determined to be non-plastic based on absence of a strong carbon signal.

334 *3.2.2 Microfibres*

335

336 Fibres were the most abundant type of suspended microdebris (**Table 1**), consisting primarily of
337 coloured pieces (i.e. black or dark blue, light blue, and red). Micro-fibres of similar characteristics
338 were observed in other freshwater ecosystems (Ballent et al., 2016), where fibres <2 mm identified
339 visually with a stereo microscope were found to be the predominant type of MPs, alongside fragments
340 in the same size range. In the River Kelvin sediment, fibres were observed in isolation, in clusters and
341 embedded in sediment grains (**Fig. 3b**). Microfibres were observed mostly in the lower size fractions
342 (<0.090), with the <0.0063 mm size fraction containing nearly 34% and 44% of total fibres in SE1
343 and SE2 samples respectively (**Supplementary Material**). However, their small sizes and tendency
344 to cluster made it challenging to identify and enumerate visually by light microscopy, especially in the
345 <0.06 mm fractions (**Fig. 3b**), potentially leading to their underestimation. No fibres were observed in
346 settled material after DS.

347

348 During SEM-EDS analysis at stage 2, fibres exhibited a strong C peak, sometimes accompanied by a
349 smaller O peak (**Fig. 5**). Therefore, fibres could not be dismissed as non-plastic from their density and
350 chemical composition, resulting in equal counts at stages 1 and 2. Fibres comprised approximately
351 88% and 95% of all plastic pieces in SE1 and SE2, respectively, in the final enumeration. However,
352 other non-plastic fibres such as cellulose-based ones can exhibit a similar structure and C signal
353 (Remy et al., 2015), and SEM-EDS does not allow for distinction between them (**Fig. 5**).
354 Spectroscopy analysis via FTIR and Raman has been used successfully for further isolation of MP
355 from non-MP fibres (Remy et al., 2015), highlighting the need for advanced chemical characterisation
356 tools for proper MP quantification, especially in the case of fibres.

357

358 Similarly, others have reported the predominance of fibres (Ballent et al., 2016; Su et al., 2016),
359 especially in systems associated with wastewater treatment as such fibres typically break off synthetic
360 textiles and are released via household sewage (Browne et al., 2011; Magnusson and Nóren, 2014).
361 While the selected site in the River Kelvin is not located near a discharge pipe from a wastewater

362 treatment facility, it has been suggested that fibres can be transported for greater distances (Ballent et
363 al., 2016), thus their presence may be attributed to distant inputs upstream from the study site.
364 Conversely, atmospheric fallout can act as a significant source of these MP types, confirmed from
365 rooftop samples collected in urban Paris (Dris et al., 2015) and further supported by the presence of
366 microfibres in our procedural blanks (See Section 3.1). Thus, fibre content in blanks could be a result
367 of aerial deposition of fibres released during wear and tear of sampling or lab gear. However, fibre
368 content in drinking tap water tested in multiple countries (Kosuth et al., 2018) may suggest potential
369 background contamination of fibres even in water purification systems, but this was not tested here
370 and no other studies on MPs in drinking water are currently available.
371
372 **Fig. 3** Backscattered electron image and elemental spectra for common micro-fibres (top) observed in
373 River Kelvin sediment and a 100% cotton fibre standard (bottom). Fibres exhibited a strong carbon
374 signal, but MP could not be discriminated against cellulose fibres.
375
376 *3.2.3 Microfragments*
377
378 The third category comprises fragmented or flake-like pieces that had uneven edges and appeared to
379 have broken off larger pieces. Suspected MP fragments were observed in suspended and settled
380 material and consisted mainly of coloured pieces (**Fig. 3c**). Counts varied between sampling events
381 and quantification stage and although the highest counts were observed in the 0.71 mm size fraction at
382 stage 1, this was not the case for the final counts, and they did not seem to concentrate around a
383 specific size fraction in a discernible pattern. Because high-density polymers can be present in the
384 environment, all settled fragments that physically resembled plastic materials were counted as
385 suspected MP at stage 1 and analysed for chemical composition. Unlike pellets that consistently had
386 little to no C, and fibres that consistently were mostly C, SEM-EDS signals for fragments were more
387 varied and complex.
388

389 Suspended flake-like fragments with a strong C signal (**Fig. 6a**) became visible only during SEM-
390 EDS imaging. This is likely explained because these pieces were captured on the filter paper after DS,
391 and, while not visible under light microscopy, they were transferred onto the adhesive while
392 attempting to transfer other materials like fibres using the "pressing" method. Furthermore, electron
393 microscopy enables greater resolution than light microscopy, making SEM-EDS a powerful tool for
394 detection of smaller pieces like these that may be overlooked by visual inspection, and highlights the
395 detection limits of visual techniques.
396
397 Other suspended fragments showed a strong C peak, but exhibited additional elemental signals
398 including Ti, Br, and Si (**Fig. 6b**). These pieces were counted as MPs, due to their strong C signal and
399 low densities, but further analysis via spectroscopy tools (e.g. Raman, FT-IR) should be employed in
400 these cases to identify the type and source of these (and similar pieces) to be conclusive. Only one of
401 ten settled MP fragments showed a strong C signal in the SEM-EDS analysis (**Fig. 6c**). This may
402 indicate high-density plastic fragments, for example, polyvinyl chloride from construction
403 applications, or polytetrafluoroethylene and engineering polyesters from industrial applications that
404 would need heavier liquids to be extracted (Hidalgo-Ruz et al., 20120). The remaining settled pieces,
405 while initially expected to be plastic due to their bright colours and shapes, showed no carbon signals
406 at stage 2 (**Fig. 6d**) and therefore were rejected from final counts.
407
408 Fragments comprised 12% and 5% of total MP counts in SE1 and SE2, respectively (**Table 1**). While
409 most studies report either pellets or fibres as the predominant forms of MP debris, and a diversity of
410 fragments generally have been observed across rivers and lakes worldwide, only a few studies have
411 reported fragments as the predominant form of these materials (Vianello et al., 2013; Wagner et al.,
412 2014; Hurley et al., 2018). However, fragments may become more abundant if plastic litter already
413 present in the environment continues to degrade into smaller fractions. Thus, more information on

414 degradation or fragmentation rates of different polymers may play a key role in understanding this
415 category (Hidalgo-Ruz et al., 2012).
416
417 **Fig. 4** Backscattered electron image and elemental spectra for common micro-fragments observed in
418 River Kelvin sediment showing floated microplastics (a) and (b), settled microplastic (c), and settled
419 non-microplastic (d) pieces. Pieces were identified as microplastic on the basis of a strong carbon
420 signal.
421
422 *3.3 Microplastic abundances*
423
424 Suspected MPs abundance at identification stage 1 supported initial estimates of 220 items per kg of
425 dry sediment in SE1 and 448 items per kg of dry sediment in SE2. Final MP abundance at stage 2
426 were 161 and 432 items per kg of dry sediment in SE1 and SE2 samples respectively (**Table 1**).
427 Sediment samples collected from German rivers and inspected visually (Wagner et al. 2014) and
428 chemically (Klein et al., 2015) found 34-64 items per kg dry weight in the Rivers Elbe, Mosel,
429 Neckar, and Rhine, and fragments accounted for 60% of total microplastics, with the remainder being
430 fibres (Wagner et al., 2014). However, abundances can be spatially and temporally variable, with
431 other sediment samples from the Rhine yielding 228-3,763 items per kg, and further 786-1,368 items
432 per kg in the River Main (Klein et al., 2015). At these sites, the relative abundance of spheres and
433 fragments compared to other shapes was highest in the 63−200 μm and 200-5000 μm size fractions,
434 respectively, while fibres were most abundant in size fractions <200 μm compared to their
435 concentration in higher size fractions (Klein at el., 2015). In addition, sediment MP abundances in the
436 River Thames were found to range from 18.5±4.2 to 66±7.7 particles per 100 g (equivalent to 185 and
437 660 particles per kg) of sediment across four sites, with fibres as the main type in three sites and
438 fragments in the fourth, based on visual and chemical characterisation (Horton et al., 2017). High MP
439 contamination was observed in multiple river channels in the Mersey and Irwell catchments in
440 Northwest England, where 517,000 particles per m^2 were observed on the River Tame (Hurley et al.,
441 2018).

442

443 The relative abundance of secondary MP types observed here is also consistent with those from other
444 freshwater studies conducted in Lake Hovsgol (Free et al., 2014), the Raritan River (Estahbanati and
445 Fahrenfeld, 2016), and urban Paris (Dris et al., 2015), although this comparison can only be expressed
446 qualitatively as different measurements and units were used. Methods and measurement units used in
447 reporting results need harmonising for improved risk assessment and to facilitate discussion across
448 studies. Nevertheless, the predominance of secondary MPs in the River Kelvin and other freshwater
449 catchments supports the general assumption that most MPs in the environment originate from the
450 breakdown of larger pieces (Duis and Coors, 2016). Coloured pieces were more frequent than white
451 and translucent pieces (**Fig. 7**), but further data is needed to determine whether this is an accurate
452 reflection of their greater abundance in the environment, or if this is attributed to selection bias.
453 Indeed, it has been suggested that fibre-like and bright-coloured pieces may be easier to find (Hidalgo
454 Ruz et al., 2012; Cole et al., 2014) and could be a source of analytical bias.
455

456 Although only one location was sampled in the River Kelvin, the site is of lower energy and so
457 sediment deposition can occur. Thus, the abundance of MPs here may support previous interpretations
458 that processes affecting deposition of fine sediment similarly influence MPs (Vianello et al., 2014;
459 Nizzetto et al., 2016), and may explain why fibres were more abundant and concentrated in the lower
460 size fractions. Further comparative data from the local catchment is needed to improve our
461 understanding of MP behaviour in these systems. In addition, the distinctly different abundances
462 observed between December and February samples in the River Kelvin suggests that high local
463 variability can be expected, likely because MP contaminants encompass a wide array of highly-
464 diverse particles and thus will not be evenly distributed in space and time. Hence, it is crucial to
465 increase the spatial coverage of surveys through research like this, and the comparability across
466 studies to fully understand this variability (Turra et al., 2014) and improve reliable assessment of their
467 distribution and abundance in aquatic environments.
468

469 This research shows that freshwater river sediments close to marine estuary systems contain MPs,
470 with fibres numerically dominant, and thus it is likely that freshwater systems are a feeder of marine
471 MPs, mobilised for example to the marine environment by large flows (Nizzetto et al., 2016; Hurley
472 et al., 2018). Moreover, the fate of MPs in these systems may be influenced by the association of
473 different MP types and sizes with different sediment grain size fractions and some MPs may be
474 retained (Nizzetto et al., 2016). Thus, consideration of different particle-size fractions and areas
475 where sediment accumulates is needed in river MP studies to improve understanding of MP emissions
476 to oceans.
477
478 **Table 1** Microplastic counts in River Kelvin sediment sampled December 17, 2015 (SE1) and
479 February 15, 2016 (SE2) by category, and total counts and abundance aggregated across all size
480 fractions for stages 1 (visual characterisation) and 2 (chemical characterisation).
481
482 **Fig. 7** Percentages of coloured and non-coloured (i.e. white and translucent) pieces observed in River
483 Kelvin sediment samples at each characterisation stage (data is pooled for both sampling events).
484
485 *3.4 Visual vs chemical characterisation*
486
487 Counts and relative abundance of suspected MP types were used to compare the efficacy of visual and
488 chemical characterisation techniques to discriminate plastics from other non-plastic microdebris and
489 the sediment matrix before and after SEM-EDS analysis. Visually, identification of pieces that were
490 different than sediment grains was possible by light microscopy although this was increasingly
491 difficult in the fractions smaller than 0.125 mm due to decreasing resolution, and it was nearly
492 impossible to distinguish plastic from non-plastic microdebris. As a result, visual characterisation may
493 lead to overestimation of MP pieces due to misidentification, because floatation of non-polymer
494 microdebris can occur and because non-plastic pellets and fragments can be easily confused for MP
495 given their physical similarities. Visual inspection is often used in methodological approaches for
496 initial enumeration and identification (Hidalgo-Ruz et al., 2012; Blair et al., 2017). However, heavy

497 reliance on the visual and manual components at nearly every step of the process can introduce
498 potential for selection bias (Cole et al., 2014) and is limited by what is reasonably visible with or
499 without the aid of a microscope. While this detection limit will depend on the individual doing the
500 identification, it is recommended that visual characterisation is not used for pieces smaller than 0.5
501 mm (Hidalgo-Ruz et al., 2012), a limit much higher than the lower limit set by sampling (e.g. 0.3 mm
502 for neuston nets) and filtration (e.g. 0.7 micron for glass fibre filters) methods, including those used in
503 this study.
504
505 Here, the chemical composition data from SEM-EDS was useful mainly for separation of non-plastic
506 pellets and fragments in both suspended and settled material, but it was not useful for MP fibre
507 identification. Further analysis by spectroscopy techniques such as Raman and FTIR-ATR (Blair et
508 al., 2017) are likely necessary for proper MP fibre enumeration. While chemical characterisation by
509 SEM-EDS and other complementary techniques like Raman and FTIR spectroscopy can aid to
510 overcome detection limits and misidentification from visual characterisation (Wesch et al., 2016), it is
511 important to note their limitations. First, these techniques can be extremely time-consuming and may
512 be costly. For similar logistical reasons, it was possible only to analyse a microfibre sub-aliquot via
513 SEM-EDS in this study. Care was taken to ensure that the sub-aliquot was representative of all types,
514 colours, and size categories, but extrapolation of SEM-EDS results to the rest of the sample is
515 undertaken visually and could result in some MP items being overlooked or misidentified. Second,
516 chemical characterisation may be also subject to selection bias as MP specimens needed to be isolated
517 from other media and manually transferred to the instrument for analysis, depending on the ability of
518 the researcher to first find these pieces visually. Lastly, instrument aided detection is also subject to
519 size limitations. For Raman and FTIR, this is considered to be in the range of 0.5 and 10 μm,
520 respectively (Hale, 2017), although this may vary according to the equipment employed.
521
522 A combined approach that uses visual and multiple chemical characterisation techniques can address
523 some of these methodological limitations. Combined or stepwise approaches are becoming more
524 common in recent routine testing as a way to optimise extraction and characterisation methods and

525 reduce analytical errors (Hidalgo-Ruz et al., 2012; Horton et al., 2017). Further, new studies are
526 recognising the impact of visual reliance on size limitations and proper MP identification and are
527 using advanced FTIR mapping techniques to develop automated methods (Primpke et al., 2017). This
528 is an important step forward in method development because a lower size limit for MPs is yet to be
529 established. In addition, automated methods will be crucial for emerging nanoplastic (<100 nm)
530 research that may become more abundant in the environment as their use increases in future trends in
531 technological applications and as macro- and microplastic waste continues to degrade (Koelmans et
532 al., 2014).
533
534 **4. Conclusions**
535
536 While MP pollution research is experiencing rapid development, this remains a new area of water
537 research still in its early stages, especially for free-flowing freshwater sources. Inter-comparison of
538 available freshwater surveys is complicated by the differences in environmental compartments
539 examined (e.g. water, sediment, biota), as well as differing methodologies and units used for reporting
540 results. Therefore, it is necessary to expand the spatio-temporal datasets to inform what needs to be
541 measured and monitored and assess the severity of their threat. This study found that secondary MPs
542 were more abundant than primary MP types, but that high variability can be observed in MP counts
543 and distribution. Recent bans and industry and public voluntary actions to phase out the use of
544 microbeads in consumer products may contribute further to the decrease of primary MPs in similar
545 fluvial systems. Conversely, secondary MPs may increase in numbers in these systems, as plastic
546 waste continues to be generated and broken down in the environment, making the secondary types a
547 greater threat and harder to manage. Thus, management strategies will need the development of
548 concerted actions to effectively reduce further secondary inputs.
549
550 Improving confidence in reporting results is key for development of adequate policies and regulations
551 to control the release and spread of these emerging contaminants in the environment. A reliable
552 assessment of MP pollution and predominant MP types and sources is required, but this can only be

achieved by improved qualitative and quantitative assessment and standardisation of methods and units of measure to guide the ongoing research. The results in this study suggest that current protocols can be subject to both under- and overestimation of different types of MPs, potentially leading to inaccurate assessment of the distribution and abundances of primary, secondary, and total MPs in environmental samples. This could result in mitigation efforts that are largely misdirected. This and previous studies have found that fibres are the most abundant type of secondary MPs, especially in urban settings and in association with wastewater treatment, so their accurate assessment is highly relevant in MP research. However, their recovery from environmental samples is low and there is a high probability that a portion of recovered fibres might not be plastic, but their accurate characterisation is challenging as fibres are perhaps the most difficult to examine with FTIR-ATR techniques. Further, it may be difficult to assess confidently if results are an accurate reflection of spatio-temporal patterns, or how much of this is due to selection bias and misidentification errors. Thus, validation of protocols with the use of blank controls and recovery tests should be used routinely when reporting results, but such tests are not commonly used or reported in the literature, presenting a crucial gap in MPs research.

The outcomes of this and similar studies are expected to contribute to generating incisive understanding of the distribution and behaviour of MPs in inland waters, making it relevant for academia, government and industry worldwide, and producing useful information for legislators, manufacturers, and industry to inform mitigation strategies and identify where controls should be implemented. Thus, this study adds to a currently limited, but growing body of work exploring the role of freshwaters in MP transport and storage.

Acknowledgements

We thank Kenneth Roberts for his technical assistance in the field and laboratory, and Peter Chung at the Imaging Spectroscopy and Analysis Centre (ISAAC) at the University of Glasgow for his assistance with SEM-EDS measurements.

Funding

This work is funded by the Scottish Government's Hydro Nation Scholars Programme. The programme is supported by CREW (Scotland's Centre of Expertise for Waters) and administered by the James Hutton Institute

References

Ballent A, Corcoran PL, Madden O, Helm PA, Longstaffe FJ (2016) Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar Pollut Bull 110:383-395

Blair RM, Waldron S, Phoenix V, Gauchotte-Lindsay C (2017) Micro- and nanoplastic pollution of freshwater and wastewater treatment systems. Springer Science Reviews 5:19-30.

Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol 45:9175-9179

Castañeda RA, Avlijas S, Simard, MA, Ricciardi A (2014) Microplastic pollution in St. Lawrence River sediments. Can J Fish Aquat Sci 71:1767-1771

Coe J, Rogers D (1997) Marine Debris: Sources, Impacts and Solutions. Springer, New York.

Cole M, Webb H, Lindeque PK, Fileman ES, Halsband C, Galloway TS (2014) Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci Rep 4

609 Derraik JG (2002) The pollution of the marine environment by plastic debris: a review. Mar Pollut
610 Bull 44:842-852
611
612 Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B (2015) Microplastic contamination in an
613 urban area: a case study in Greater Paris. Environ Chem 12:592-599
614
615 Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a
616 specific focus on personal care products), fate and effects. Environ Sci Eur 28:2
617
618 Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a
619 review of the emerging threats, identification of knowledge gaps and prioritisation of research needs.
620 Water Res 75:63-82
621
622 Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Amato S (2013) Microplastic
623 pollution in the surface waters of the Laurentian Great Lakes. Mar Pollut Bull 77:177-182
624
625 Estahbanati S, Fahrenfeld NL (2016) Influence of wastewater treatment plant discharges on
626 microplastic concentrations in surface water. Chemosphere 162:277-284
627
628 Free CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ, Boldgiv B (2014) High-levels of
629 microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull 85:156-163
630
631 GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection)
632 (2015) Sources, fate and effects of microplastics in the marine environment: a global assessment.
633 Kershaw PJ (ed.). IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Rep. Stud.
634 GESAMP No. 90
635

636 Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made. Sci Adv
637 3:1-5.
638
639 Hale R (2017) Analytical challenges associated with the determination of microplastics in the
640 environment. Anal Methods 9:1326-1327
641
642 Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a
643 review of the methods used for identification and quantification. Environ Sci Technol 46:3060-3075
644
645 Horton A, Svendsen C, Williams RJ, Spurgeon J, Lahive E (2017) Large microplastic particles in
646 sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective
647 quantification. Mar Pollut Bull 114:218-226.
648
649 Horton A, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017) Microplastics in freshwater and
650 terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and
651 future research priorities. Sci Total Environ 586:127-141
652
653 Klein S, Worch E, Knepper TP (2015) Occurrence and spatial distribution of microplastics in river
654 shore sediments of the Rhine-Main area in Germany. Environ Sci Technol 19:6070-6076
655
656 Koelmans AA, Besseling E, Shim WJ (2015) Nanoplastics in the aquatic environment. In: Marine
657 Anthropogenic Litter. Bergmann M, Gutow L, Klages M (eds) Springer, Berlin pp. 325–342
658
659 Kosuth M, Mason SA, Wattenberg EV (2018) Anthopogenic contamination of tap water, beer, and
660 sea salt. PLOS One 13:e0194970
661

662 Magnusson K, Norén F (2014) Screening of microplastic particles in and down-stream a wastewater
663 treatment plant. Technical Report published for IVL Swedish Environmental Research Institute,
664 August 2014; Swedish Environmental Research Institute: Stockholm, Sweden
665
666 Nizzetto L, Bussi G, Futter MN, Butterfield D, Whitehead PG (2016) A theoretical assessment of
667 microplastic transport in river catchments and their retention by soils and river sediments. Environ Sci
668 Process Impact 18:1050-1059
669
670 Plastics Europe (2017) Plastics—The Facts 2017. An Analysis of European Plastics Production,
671 Demand and Waste Data (Plastics Europe, Brussels) Available
672 http://www.plasticseurope.org/application/files/5715/1717/4180/Plastics_the_facts_2017_FINAL_for
673 _website_one_page.pdf (accessed 26 February 2018)
674
675 Primpke S, Lorenz C, Rascher-Friesenhausen, Gerdts G (2017) An automated approach for
676 microplastics analysis using focal plan array (FPA) FTIR microscopy and image analysis. Anal
677 Methods 9:1499-1511
678
679 Remy F, Collard F, Gilbert B, Compere P, Eppe G, Lepoint G (2015) When Microplastic Is Not
680 Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass
681 Macrophytodetritus Environ Sci Technol 49:11158-11166
682 Sanchez W, Bender C, Porcher JM (2014) Wild gudgeons (Gobio gobio) from French rivers are
683 contaminated by microplastics: preliminary study and first evidence. Environ Res 128:98-100
684
685 Su L, Xue Y, Li L, Yang D, Kolandhasamy P, Li D, Shi H (2016) Microplastics in Taihu Lake, China.
686 Environ Pollut 2016:711-719
687
688 Tagg AS, Sapp M, Harrison JP, Ojeda JJ (2015) Identification and quantification of microplastics in
689 wastewater using FPA-based reflectance micro-FT-IR imaging. Anal Chem 87: 6032-6040

690

691 Thompson RC, Swan SH, Moore CJ, vom Saal FS (2009) Our plastic age. Philos Trans R Soc
692 London, Ser B Biol Sci 364:1973-1976

693

694 Turra A, Manzano AB, Dias RJS, Mahiques MM, Barbosa L, Balthazar-Silva D, Moreira FT (2014)
695 Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. Scientific
696 Reports 4:4435

697

698 Vianello A, Boldrin A, Guerriero P, Moschino V, Rella R, Sturaro A, Da Ros L (2013) Microplastic
699 particles in sediments of Lagoon of Venice, Italy: first observations on occurrence, spatial patterns
700 and identification. Estuar Coast Shelf Sci 130:54-61

701

702 Wagner M, Scherer C, Alvarez-Muñoz D, Brennholt N, Bourrain X, Buchinger S, Reifferscheid G
703 (2014) Microplastics in freshwater ecosystems: what we know and what we need to know. Environ
704 Sci Eur 26:1-9

705

706 Wesch C, Barthel AK, Braun U, Klein R, Paulus M (2016) No microplastics in benthic eelpout
707 (Zoarces viviparus): an urgent need for spectroscopic analyses in microplastic detection. Environ Res
708 148:36-38

709

710 Zbyszewski M, Corcoran PL (2011) Distribution and degradation of fresh water plastic particles along
711 the beaches of Lake Huron, Canada. Water Air Soil Pollut 220:365-372

Table 1 Microplastic counts in River Kelvin sediment sampled December 17, 2015 (SE1) and February 15, 2016 (SE2) by category, and total counts and abundance aggregated across all size fractions for stages 1 (visual characterisation) and 2 (chemical characterisation).

Identification Stage	Sampling Event	Sediment Weight, Dry (g)	Microplastics Count (n)					Abundance (items per kg dry sediment)
			Pellets	Fibres	Fragments	Other	Total	
Visual (Stage 1)	SE1	441.49	5	64	23	5	97	220
	SE1 Blanks (n=2)	0	0	3	0	0	3	
	SE2	254.48	0	106	8	0	114	448
	SE2 Blanks (n=4)	0	0	3	0	0	3	
Chemical (Stage 2)	SE1	441.49	0	64	7	0	71	161
	SE2	254.48	0	106	4	0	110	432

Fig. 1 Location of the sample collection site in a river bend section in the River Kelvin in the west of Glasgow, Scotland, UK. Map created using ArcGIS® software by Esri. ArcGIS® and ArcMap™ are the intellectual property of Esri and are used herein under license. Copyright © Esri. All rights reserved.

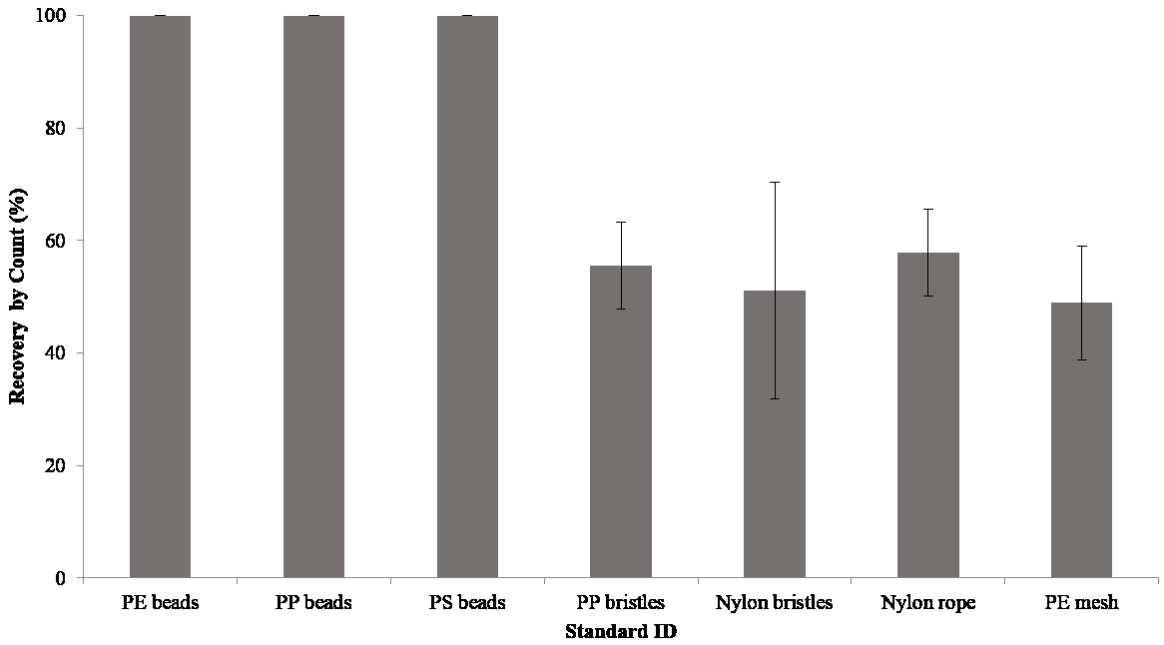

Fig. 2 Recovery tests for density separation using various types of microplastic standards: purchased microbeads (polyethylene, **PE**; polypropylene, **PP**; and polystyrene, **PS**), and fibre-like fragments produced in the lab (PP bristles from a cleaning brush, nylon bristles from a toothbrush, nylon rope, and PE mesh packaging).

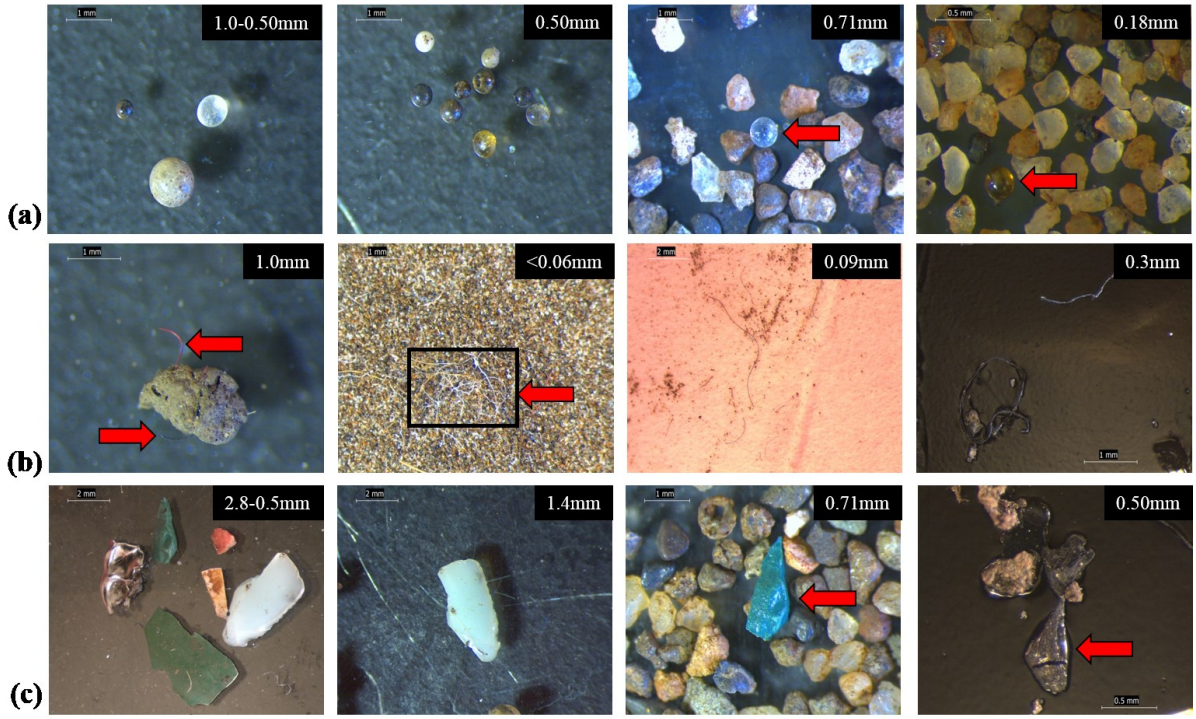

Fig. 3 Light microscopy images of suspected microplastics in size-fractionated sediment samples from the River Kelvin in suspended and settled material before chemical characterisation. Items shown are: pellets (a), fibres (b) and fragments (c).

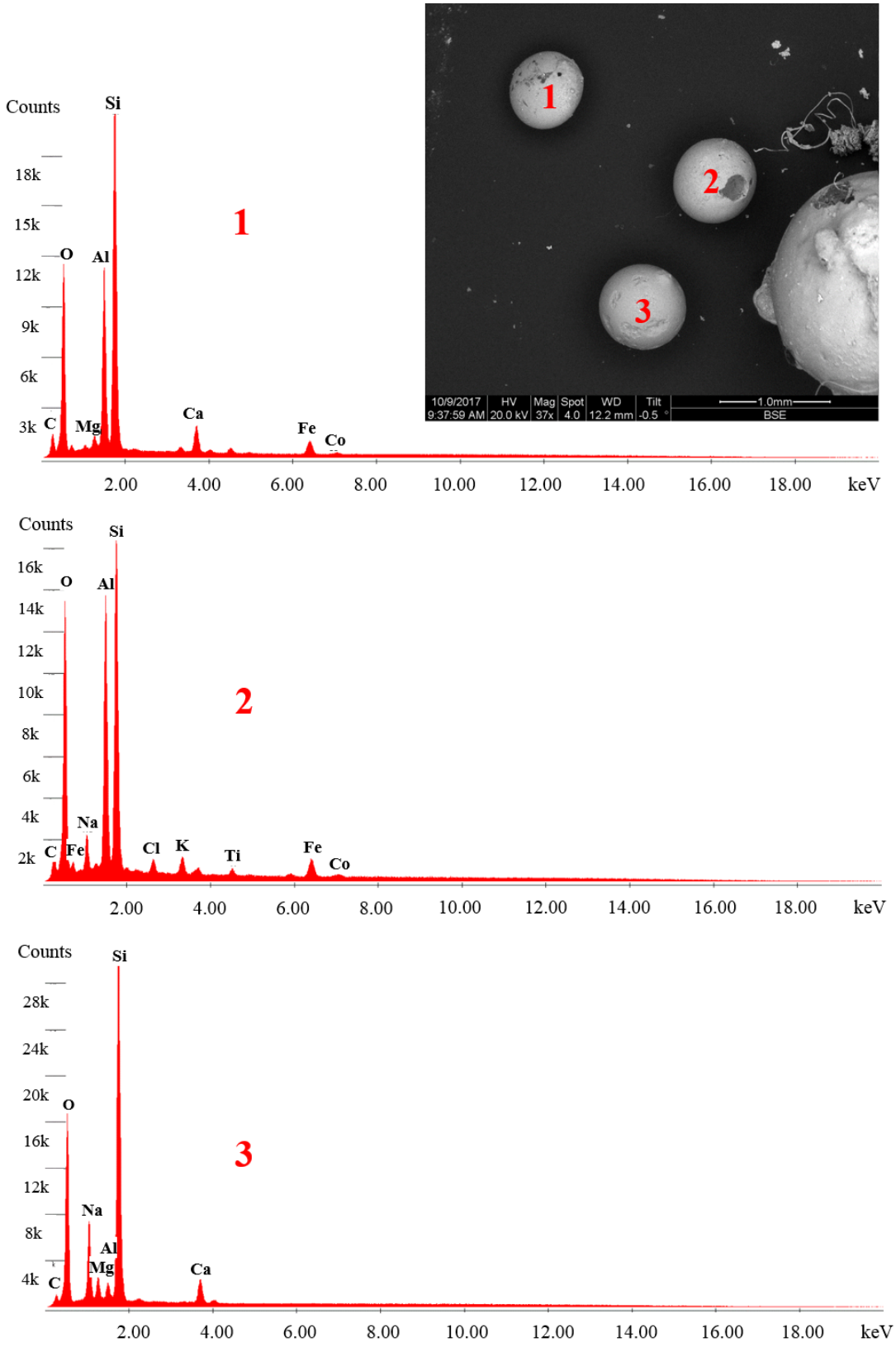

Fig. 4 Backscattered electron image and elemental spectra for common micro-pellets observed in River Kelvin sediment. Pellets were determined to be non-plastic based on absence of a strong carbon signal.

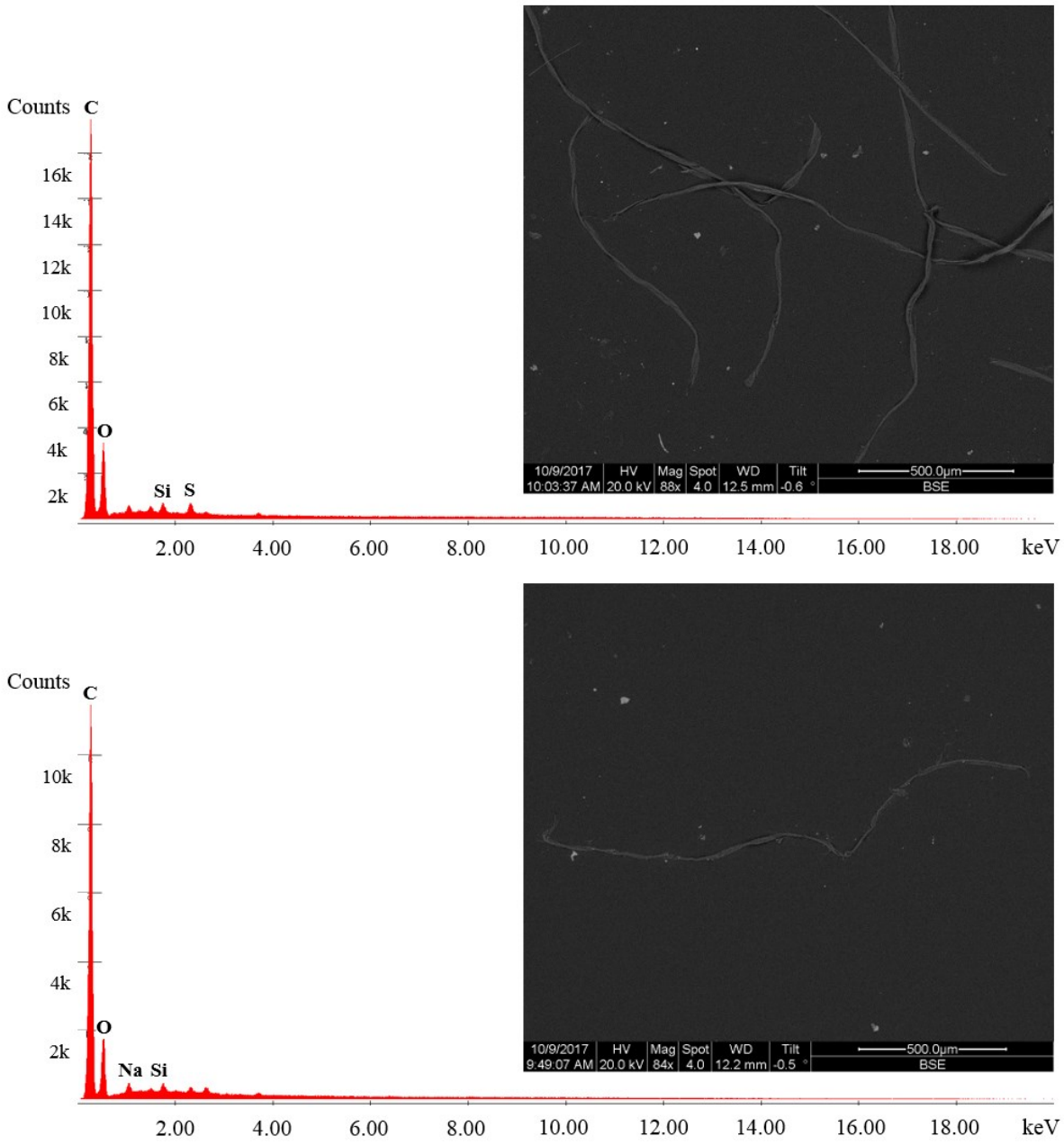

Fig. 5 Backscattered electron image and elemental spectra for common micro-fibres (top) found in River Kelvin sediment and a 100% cotton fibre standard (bottom). Fibres exhibited a strong carbon signal, but MP could not be discriminated against cellulose fibres.

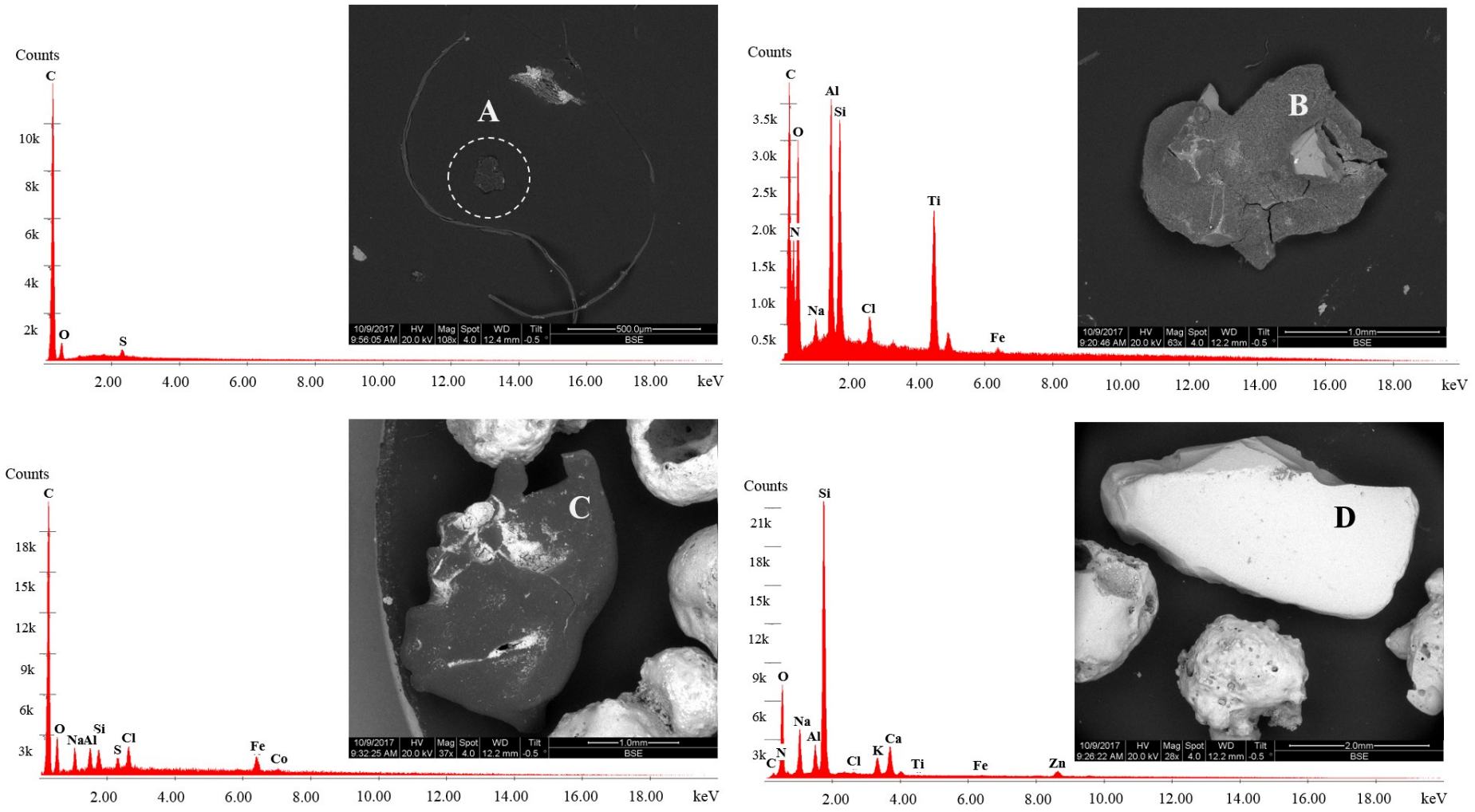

Fig. 6 Backscattered electron image and elemental spectra for common micro-fragments observed in River Kelvin sediment showing floated microplastics (a) and (b), settled microplastic (c), and settled non-microplastic (d) pieces. Pieces were considered microplastic on the basis of a strong carbon signal.

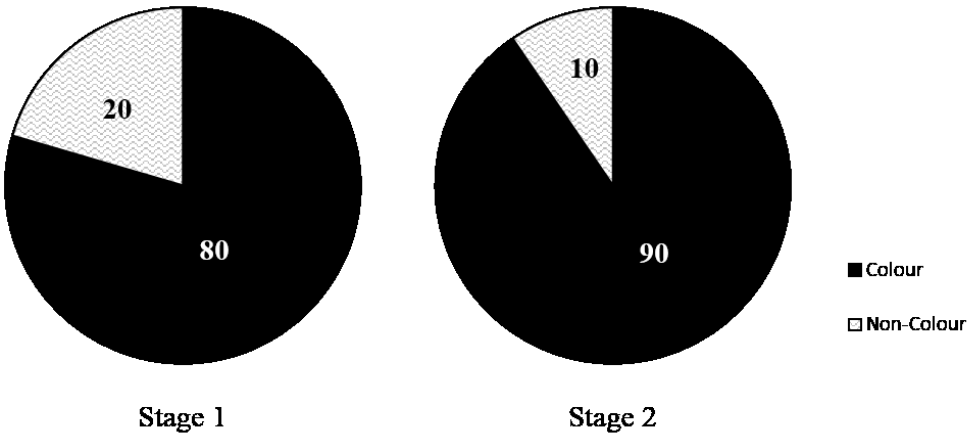

Fig. 7 Percentages of coloured and non-coloured (i.e. white and translucent) pieces observed in River Kelvin sediment samples at each characterisation stage (data is pooled for both sampling events).

Electronic Supplementary Material

Article Title:	Secondary microplastics were prevalent in sediment in a freshwater UK urban river
Authors:	Reina M. Blair*, Susan Waldron, Vernon Phoenix, Caroline Gauchotte-Lindsay
***Corresponding author:**	School of Geographical and Earth Sciences
	University of Glasgow, Glasgow G12 8QQ
	email: r.blair.1@research.gla.ac.uk

Visual counts, suspended

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0	1			1	1		1		2	
1	17/12/2015	0-5	2.00	24.77		2		2				0	3		3		5	
1	17/12/2015	0-5	1.40	24.99				0				0	2		2		2	
1	17/12/2015	0-5	1.00	24.97				0	1			1	2		2		3	
1	17/12/2015	0-5	0.71	24.97				0	5	1		6	1		1		7	
1	17/12/2015	0-5	0.50	25.00				0	2	4		6		1	1		7	
1	17/12/2015	0-5	0.36	25.03				0				0	3		3		3	
1	17/12/2015	0-5	0.25	24.97				0				0			0		0	
1	17/12/2015	0-5	0.18	14.31				0	1	4		5			0		5	
1	17/12/2015	0-5	0.13	3.74				0	1	3		4			0		4	
1	17/12/2015	0-5	0.09	1.01				0	1	3		4			0		4	
1	17/12/2015	0-5	0.06	0.56				0		2		2			0		2	
1	17/12/2015	0-5	<0.063	0.48				0		15		15			0		15	
1	17/12/2015	0-5	blk					0		1		1			0		1	
TOTAL					0	2	0	2	12	32	0	44	12	1	13	0	59	
1	17/12/2015	5-10	2.80	24.96				0				0	2		2		2	
1	17/12/2015	5-10	2.00	25.02		2		2				0	3		3	1	6	
1	17/12/2015	5-10	1.40	25.06		1		1				0	4		4	2	7	
1	17/12/2015	5-10	1.00	24.98				0		2		2			0		2	
1	17/12/2015	5-10	0.71	25.02				0		5		5	1		1		6	
1	17/12/2015	5-10	0.50	25.05				0				0			0		0	
1	17/12/2015	5-10	0.36	24.99				0				0			0		0	
1	17/12/2015	5-10	0.25	25.05				0				0			0	2	2	
1	17/12/2015	5-10	0.18	15.47				0		3		3			0		3	
1	17/12/2015	5-10	0.13	4.22				0				0			0		0	
1	17/12/2015	5-10	0.09	0.95				0		2		2			0		2	
1	17/12/2015	5-10	0.06	0.49				0		1		1			0		1	
1	17/12/2015	5-10	<0.063	0.48				0	5	2		7			0		7	
1	17/12/2015		blk					0		2		2			0		2	
TOTAL					0	3	0	3	5	15	0	20	10	0	10	5	38	
TOTAL BLANKS					0	0	0	0	0	3	0	3	0	0	0	0	3	
TOTAL SE1				441.49				5				64			23	5	97	220

Visual counts, suspended (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0		1		1			0		1	
2	15/02/2016	0-2	2.00	0.11				0		5		5			0		5	
2	15/02/2016	0-2	1.40	0.52				0				0	1		1		1	
2	15/02/2016	0-2	1.00	2.22				0		1		1	1		1		2	
2	15/02/2016	0-2	0.71	7.04				0		3		3	2		2		5	
2	15/02/2016	0-2	0.50	24.71				0		1		1			0		1	
2	15/02/2016	0-2	0.36	28.94				0		1		1			0		1	
2	15/02/2016	0-2	0.25	9.36				0		6		6			0		6	
2	15/02/2016	0-2	0.18	1.53				0		3		3			0		3	
2	15/02/2016	0-2	0.13	0.36				0		3		3			0		3	
2	15/02/2016	0-2	0.09	0.08				0		4		4			0		4	
2	15/02/2016	0-2	0.06	0.05				0		2		2			0		2	
2	15/02/2016	0-2	<0.063	0.05				0		37		37			0		37	
2	15/02/2016	0-2	blk					0		1		1			0		1	
TOTAL					0	0	0	0	0	67	0	67	4	0	4	0	71	
2	15/02/2016	2-4	2.80	2.27				0				0			0		0	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03				0		1		1			0		1	
2	15/02/2016	2-4	0.71	16.43				0		1		1		1	1		2	
2	15/02/2016	2-4	0.50	17.74				0				0	1		1		1	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0		1		1			0		1	
2	15/02/2016	2-4	0.13	0.16				0		1		1			0		1	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0		6		6			0		6	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					0	0	0	0	0	10	0	10	1	1	2	0	12	

Visual counts, suspended (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0			1	1			0		1	
2	15/02/2016	4-6	2.00	1.44				0		3		3			0		3	
2	15/02/2016	4-6	1.40	2.26				0				0			0		0	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82				0				0			0		0	
2	15/02/2016	4-6	0.50	17.65				0		3		3			0		3	
2	15/02/2016	4-6	0.36	11.90				0				0			0		0	
2	15/02/2016	4-6	0.25	3.25				0		3		3		1	1		4	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0		1		1			0		1	
2	15/02/2016	4-6	0.09	0.04				0		1		1			0		1	
2	15/02/2016	4-6	0.06	0.03				0		5		5			0		5	
2	15/02/2016	4-6	<0.063	0.01				0		3		3			0		3	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					0	0	0	0	0	19	1	20	0	1	1	0	21	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0		1		1			0		1	
2	15/02/2016	6-8	1.00	6.49				0		1		1			0		1	
2	15/02/2016	6-8	0.71	13.50				0		1		1			0		1	
2	15/02/2016	6-8	0.50	18.80				0			1	1			0		1	
2	15/02/2016	6-8	0.36	12.49				0				0			0		0	
2	15/02/2016	6-8	0.25	3.99				0		1		1			0		1	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0	1		1		1	
2	15/02/2016	6-8	0.09	0.04				0		2		2			0		2	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0		2		2			0		2	
2	15/02/2016	6-8	blk					0		2		2			0		2	
TOTAL					0	0	0	0	0	8	1	9	1	0	1	0	10	
TOTAL BLANKS					0	0	0	0	0	3	0	3	0	0	0	0	3	
TOTAL SE2				254.48				0				106			8	0	114	448

Visual counts, settled

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight (dry)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0				0			0		0	
1	17/12/2015	0-5	2.00	24.77	2	1	1	4				0	2	1	3		7	
1	17/12/2015	0-5	1.40	24.99		22		22				0	1		1		23	
1	17/12/2015	0-5	1.00	24.97	13	19		32				0	5	2	7		39	
1	17/12/2015	0-5	0.71	24.97	22	38		60				0	4	3	7		67	
1	17/12/2015	0-5	0.50	25.00	5	9		14				0	1	1	2		16	
1	17/12/2015	0-5	0.36	25.03	6	17		23				0		1	1		24	
1	17/12/2015	0-5	0.25	24.97	2	5		7				0			0		7	
1	17/12/2015	0-5	0.18	14.31		1		1				0		1	1		2	
1	17/12/2015	0-5	0.13	3.74				0				0			0		0	
1	17/12/2015	0-5	0.09	1.01				0				0			0		0	
1	17/12/2015	0-5	0.06	0.56				0				0			0		0	
1	17/12/2015	0-5	<0.063	0.48				0				0			0		0	
1	17/12/2015	0-5	blk					0				0			0		0	
TOTAL					50	112	1	163	0	0	0	0	13	9	22	0	185	
1	17/12/2015	5-10	2.80	24.96		1		1				0	1		1		2	
1	17/12/2015	5-10	2.00	25.02	2	5		7				0	2	1	3		10	
1	17/12/2015	5-10	1.40	25.06	5	3	2	10				0		1	1		11	
1	17/12/2015	5-10	1.00	24.98	8	17		25				0	3	3	6		31	
1	17/12/2015	5-10	0.71	25.02	29	25	4	58				0	6	6	12	1	71	
1	17/12/2015	5-10	0.50	25.05	10	16		26				0			0		26	
1	17/12/2015	5-10	0.36	24.99	11	8		19				0		2	2		21	
1	17/12/2015	5-10	0.25	25.05		1		1				0		2	2		3	
1	17/12/2015	5-10	0.18	15.47	1	1		2				0			0		2	
1	17/12/2015	5-10	0.13	4.22	1			1				0			0		1	
1	17/12/2015	5-10	0.09	0.95				0				0			0		0	
1	17/12/2015	5-10	0.06	0.49				0				0			0		0	
1	17/12/2015	5-10	<0.063	0.48				0				0			0		0	
1	17/12/2015							0				0			0		0	
TOTAL					67	77	6	150	0	0	0	0	12	15	27	1	178	
TOTAL SE1				441.49				313				0			49		363	822

Visual counts, settled (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0				0			0		0	
2	15/02/2016	0-2	2.00	0.11				0				0			0		0	
2	15/02/2016	0-2	1.40	0.52				0				0			0		0	
2	15/02/2016	0-2	1.00	2.22				0				0			0		0	
2	15/02/2016	0-2	0.71	7.04	2			2				0	1		1		3	
2	15/02/2016	0-2	0.50	24.71	3	3		6				0			0		6	
2	15/02/2016	0-2	0.36	28.94		2		2				0			0		2	
2	15/02/2016	0-2	0.25	9.36		2		2				0			0		2	
2	15/02/2016	0-2	0.18	1.53				0				0			0		0	
2	15/02/2016	0-2	0.13	0.36				0				0			0		0	
2	15/02/2016	0-2	0.09	0.08				0				0			0		0	
2	15/02/2016	0-2	0.06	0.05				0				0			0		0	
2	15/02/2016	0-2	<0.063	0.05				0				0			0		0	
2	15/02/2016	0-2	blk					0				0			0		0	
TOTAL					5	7	0	12	0	0	0	0	1	0	1	0	13	
2	15/02/2016	2-4	2.80	2.27		1		1				0			0		1	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03	1			1				0			0		1	
2	15/02/2016	2-4	0.71	16.43		2		2				0			0		2	
2	15/02/2016	2-4	0.50	17.74	2	1		3				0			0		3	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0				0			0		0	
2	15/02/2016	2-4	0.13	0.16				0				0			0		0	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0				0			0		0	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					3	4	0	7	0	0	0	0	0	0	0	0	7	

Visual counts, settled (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0				0			0		0	
2	15/02/2016	4-6	2.00	1.44				0				0			0		0	
2	15/02/2016	4-6	1.40	2.26		1		1				0			0		1	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82	1	2		3				0			0		3	
2	15/02/2016	4-6	0.50	17.65	1			1				0			0		1	
2	15/02/2016	4-6	0.36	11.90	1	3		4				0			0		4	
2	15/02/2016	4-6	0.25	3.25		2		2				0			0		2	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0				0			0		0	
2	15/02/2016	4-6	0.09	0.04				0				0			0		0	
2	15/02/2016	4-6	0.06	0.03				0				0			0		0	
2	15/02/2016	4-6	<0.063	0.01				0				0			0		0	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					3	8	0	11	0	0	0	0	0	0	0	0	11	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0				0			0		0	
2	15/02/2016	6-8	1.00	6.49				0				0			0		0	
2	15/02/2016	6-8	0.71	13.50		1		1				0			0		1	
2	15/02/2016	6-8	0.50	18.80	1	2		3				0			0		3	
2	15/02/2016	6-8	0.36	12.49		1		1				0			0		1	
2	15/02/2016	6-8	0.25	3.99		3		3				0			0		3	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0			0		0	
2	15/02/2016	6-8	0.09	0.04				0				0			0		0	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0				0		1	1		1	
2	15/02/2016	6-8	blk					0				0			0		0	
TOTAL					1	7	0	8	0	0	0	0	0	1	1	0	9	
TOTAL SE2				254.48				38				0			2		40	157

Chemical counts, SEM-EDS

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight (dry)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
1	17/12/2015	0-5	2.80	24.95				0	1			1			0		1	
1	17/12/2015	0-5	2.00	24.77				0				0		1	1		1	
1	17/12/2015	0-5	1.40	24.99				0				0		1	1		1	
1	17/12/2015	0-5	1.00	24.97				0	1			1			0		1	
1	17/12/2015	0-5	0.71	24.97				0	5	1		6			0		6	
1	17/12/2015	0-5	0.50	25.00				0	2	4		6		2	2		8	
1	17/12/2015	0-5	0.36	25.03				0				0			0		0	
1	17/12/2015	0-5	0.25	24.97				0				0			0		0	
1	17/12/2015	0-5	0.18	14.31				0	1	4		5			0		5	
1	17/12/2015	0-5	0.13	3.74				0	1	3		4			0		4	
1	17/12/2015	0-5	0.09	1.01				0	1	3		4			0		4	
1	17/12/2015	0-5	0.06	0.56				0		2		2			0		2	
1	17/12/2015	0-5	<0.063	0.48				0		15		15			0		15	
1	17/12/2015	0-5	blk					0		1		1			0		1	
TOTAL					0	0	0	0	12	32	0	44	0	4	4	0	48	
1	17/12/2015	5-10	2.80	24.96				0				0			0		0	
1	17/12/2015	5-10	2.00	25.02				0				0			0		0	
1	17/12/2015	5-10	1.40	25.06				0				0			0		0	
1	17/12/2015	5-10	1.00	24.98				0		2		2			0		2	
1	17/12/2015	5-10	0.71	25.02				0		5		5	1		1		6	
1	17/12/2015	5-10	0.50	25.05				0				0			0		0	
1	17/12/2015	5-10	0.36	24.99				0				0			0		0	
1	17/12/2015	5-10	0.25	25.05				0				0		2	2		2	
1	17/12/2015	5-10	0.18	15.47				0		3		3			0		3	
1	17/12/2015	5-10	0.13	4.22				0				0			0		0	
1	17/12/2015	5-10	0.09	0.95				0		2		2			0		2	
1	17/12/2015	5-10	0.06	0.49				0		1		1			0		1	
1	17/12/2015	5-10	<0.063	0.48				0	5	2		7			0		7	
1	17/12/2015							0		2		2			0		2	
TOTAL					0	0	0	0	5	15	0	20	1	2	3	0	23	
TOTAL SE1				441.49				0				64			7	0	71	161

Chemical counts, SEM-EDS (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	0-2	2.80	0.23				0		1		1			0		1	
2	15/02/2016	0-2	2.00	0.11				0		5		5			0		5	
2	15/02/2016	0-2	1.40	0.52				0				0			0		0	
2	15/02/2016	0-2	1.00	2.22				0		1		1			0		1	
2	15/02/2016	0-2	0.71	7.04				0		3		3			0		3	
2	15/02/2016	0-2	0.50	24.71				0		1		1			0		1	
2	15/02/2016	0-2	0.36	28.94				0		1		1			0		1	
2	15/02/2016	0-2	0.25	9.36				0		6		6			0		6	
2	15/02/2016	0-2	0.18	1.53				0		3		3			0		3	
2	15/02/2016	0-2	0.13	0.36				0		3		3			0		3	
2	15/02/2016	0-2	0.09	0.08				0		4		4			0		4	
2	15/02/2016	0-2	0.06	0.05				0		2		2			0		2	
2	15/02/2016	0-2	<0.063	0.05				0		37		37			0		37	
2	15/02/2016	0-2	blk					0		1		1			0		1	
TOTAL					0	0	0	0	0	67	0	67	0	0	0	0	67	
2	15/02/2016	2-4	2.80	2.27				0				0			0		0	
2	15/02/2016	2-4	2.00	1.28				0				0			0		0	
2	15/02/2016	2-4	1.40	3.28				0				0			0		0	
2	15/02/2016	2-4	1.00	9.03				0		1		1			0		1	
2	15/02/2016	2-4	0.71	16.43				0		1		1			0		1	
2	15/02/2016	2-4	0.50	17.74				0				0			0		0	
2	15/02/2016	2-4	0.36	9.56				0				0			0		0	
2	15/02/2016	2-4	0.25	2.75				0				0			0		0	
2	15/02/2016	2-4	0.18	0.59				0		1		1			0		1	
2	15/02/2016	2-4	0.13	0.16				0		1		1			0		1	
2	15/02/2016	2-4	0.09	0.04				0				0			0		0	
2	15/02/2016	2-4	0.06	0.02				0				0			0		0	
2	15/02/2016	2-4	<0.063	0.01				0		6		6			0		6	
2	15/02/2016	2-4	blk					0				0			0		0	
TOTAL					0	0	0	0	0	10	0	10	0	0	0	0	10	

Chemical counts, SEM-EDS (continued)

Sampling Event	Sampling Date	Sampling Depth (cm)	Size Fraction (mm)	Sample Weight, Dry (g)	Counts (n)													ABUNDANCE
					Pellets				Fibres				Fragments			Other	TOTAL	
					Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	Clusters	ALL	Non-coloured	Coloured	ALL			
2	15/02/2016	4-6	2.80	3.26				0			1	1			0		1	
2	15/02/2016	4-6	2.00	1.44				0		3		3			0		3	
2	15/02/2016	4-6	1.40	2.26				0				0		1	1		1	
2	15/02/2016	4-6	1.00	4.92				0				0			0		0	
2	15/02/2016	4-6	0.71	10.82				0				0			0		0	
2	15/02/2016	4-6	0.50	17.65				0		3		3			0		3	
2	15/02/2016	4-6	0.36	11.90				0				0			0		0	
2	15/02/2016	4-6	0.25	3.25				0		3		3		1	1		4	
2	15/02/2016	4-6	0.18	0.43				0				0			0		0	
2	15/02/2016	4-6	0.13	0.09				0		1		1			0		1	
2	15/02/2016	4-6	0.09	0.04				0		1		1			0		1	
2	15/02/2016	4-6	0.06	0.03				0		5		5			0		5	
2	15/02/2016	4-6	<0.063	0.01				0		3		3			0		3	
2	15/02/2016	4-6	blk					0				0			0		0	
TOTAL					0	0	0	0	0	19	1	20	0	2	2	0	22	
2	15/02/2016	6-8	2.80	1.51				0				0			0		0	
2	15/02/2016	6-8	2.00	0.47				0				0			0		0	
2	15/02/2016	6-8	1.40	1.91				0		1		1			0		1	
2	15/02/2016	6-8	1.00	6.49				0		1		1			0		1	
2	15/02/2016	6-8	0.71	13.50				0		1		1			0		1	
2	15/02/2016	6-8	0.50	18.80				0			1	1			0		1	
2	15/02/2016	6-8	0.36	12.49				0				0			0		0	
2	15/02/2016	6-8	0.25	3.99				0		1		1			0		1	
2	15/02/2016	6-8	0.18	0.66				0				0			0		0	
2	15/02/2016	6-8	0.13	0.13				0				0		2	2		2	
2	15/02/2016	6-8	0.09	0.04				0		2		2			0		2	
2	15/02/2016	6-8	0.06	0.02				0				0			0		0	
2	15/02/2016	6-8	<0.063	0.01				0		2		2			0		2	
2	15/02/2016	6-8	blk					0		2		2			0		2	
TOTAL					0	0	0	0	0	8	1	9	0	2	2	0	11	
TOTAL SE2				254.48				0				106			4	0	110	432