Australia's National
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Microplastic quantification in wastewater

Wastewater influent and effluent trends over a 10 month period

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Executive summary

Plastics are comprised of polymers made up of a diverse number of repeating chemical units. They are ubiquitous in modern society due to their properties that allow them to be used economically in a broad range of applications. Plastics can be released to wastewater after use in personal care products (microbeads), from fibrous materials such as clothes, or through degradation of materials such as packaging, paints and adhesives. Plastics in wastewater are commonly of a small size and are known as microplastics when they are <5 mm in any dimension. Because of their size and chemical nature, microplastics are generally resistant to physical and biological treatment technologies used in wastewater treatment plants (WWTPs), leading to their discharge into the environment. Although the concentrations of microplastics in wastewater discharges are typically low (a few to hundreds of particles per litre), this can still represent millions to billions of microplastic particles being discharged into freshwater or marine environments each day. There is considerable concern relating to the presence of microplastics in aquatic environments, due to their physical impacts on organisms or through the potential to transfer toxic chemicals associated with the microplastics when aquatic organisms mistake microplastics for a food source.

Because plastics are so integral in our society, mitigation strategies are complex and challenging to implement, although some management options are available that are relatively straightforward to implement. For example, one approach to reducing plastic microbeads in wastewater has been to work with personal care product manufacturers to remove microplastics from their products. Each individual product can contain hundreds of thousands of microplastics. A voluntary industry agreement (VIA) to remove microplastics from personal care products came into effect in Australia in July 2018 when more than ~94% of personal care products that had previously contained plastic microbeads were reformulated without microplastics. One line of evidence to determine whether the VIA was effective in mitigating microbeads entering the wastewater stream is to monitor wastewater for microbeads over time. This is challenging as wastewater is a complex matrix, containing high levels of particulate matter that are not microplastics. Extensive clean-up of wastewater is therefore required for analytical procedures, in combination with visual inspection to quantify and characterise the isolated microplastics.

Visual inspection for quantification relies on an experienced operator to make a representative count of the microplastics and then often taking a sub-set of the microplastics for confirmation of their polymer composition using a technique such as Fourier transform infrared spectroscopy (FTIR). This approach can be highly subjective in the identification and isolation of microplastics. Recent, semi-automated approaches with FTIR can allow identification, quantification and characterisation of microplastics for a relatively rapid and reliable analysis of microplastics in wastewater.

The objectives of this project are to:

(1) Apply semi-quantitative FTIR microscopy for trends in the quantification and characterisation of microplastics in wastewater influent and effluent samples in two WWTPs, with different treatment levels (primary and tertiary), over a 10 month period. This methodology will also be applied to a single collection of

biosolids from seven WWTPs all located in the greater Sydney metropolitan area;

(2) Assess the effectiveness of the VIA in Australia through monitoring trends over 10 months of microbeads in wastewater collected at two of the largest WWTPs in Sydney, based on their respective wastewater treatment levels;

(3) Use the assessment of the number of microplastics quantified in wastewater to determine the effect wastewater treatment levels have on the removal of microplastics from wastewater.; and

(4) Compare the types and quantities of microplastics present in biosolids sourced from a range of WWTPs to assess trends across the greater Sydney region, as well as using this information for a preliminary assessment of the potential implications this can have for terrestrial systems receiving biosolids as a soil amendment.

This study highlights a relatively rapid method of analysing microplastics in wastewater and biosolids that can be incorporated in monitoring programs, as long as adequate quality assurance procedures are also included to account for microplastic contamination and recovery from complex environmental samples.

The presence of microbeads (1–3 particles/L) in wastewater influent at both Cronulla and Malabar WWTPs indicated that products containing plastic microbeads are still used within these catchments. The infrequent detection at Cronulla WWTP (8% of influent samples) and for the two influent streams, S1 (42%) and S2 (25%), at Malabar WWTP, with relatively low counts suggest a low degree of use of microbead containing products. Additional monitoring of wastewater in these catchments and in other Australian cities, alongside ongoing market surveys, would further confirm this.

Wastewater entering Malabar and Cronulla WWTP each day was estimated to contain between 2.4×10^{10} and 6.1×10^{10} (or 24,000 to 61,000 million microplastic particles), based on combined S1 and S2 flows, and 0.087×10^{10} and 1.4×10^{10} (or 870 to 14,000 million microplastic particles), respectively.Wastewater being discharged from Malabar and Cronulla WWTPs each day was estimated to contain between 0.54×10^{10} and 12×10^{10} (or 5,400 to 120,000 million) and 0.86×10^{8}–3.5×10^{8} (or 86 to 350 million) microplastics per day, respectively. This represents a removal rate of up to 79% through primary treatment at Malabar WWTP, although there was occasionally minimal removal apparent,and >98% at Cronulla WWTP through tertiary treatment.

The majority of microplastics are removed through association with sludge within the WWTPs. Collection of biosolids from Malabar and Cronulla WWTP showed microplastics with similar characteristics as those found in the wastewater, with a high proportion of polypropylene (PP) fragments and a lesser extent of polyethylene (PE) and polyethylene terephthalate (PET) also present. Overall, microplastic fibres were much less common than microplastic fragments.

The presence of microplastics at concentrations of 4.5×10^{4}–3.23×10^{5} microplastics/kg (or 45,000 to 323,000/kg) in biosolids collected from Malabar, Cronulla and five other WWTPs (Quakers Hill, Rouse Hill, St Marys, West Camden and Winmalee) in greater Sydney was consistent with other wastewater surveys in Europe, North America and Asia. While there are concerns this can lead to

the transfer of microplastics to the terrestrial environment through biosolid application, it is unlikely that these concentrations will lead to adverse impacts based on a comparatively small number of published terrestrial ecotoxicity studies on microplastics. While there is the need to undertake a more comprehensive analysis of the potential terrestrial impacts of microplastic loads in biosolids, this also needs to be balanced against the many environmental benefits that the reuse of biosolids has in agricultural soil amendment.

This study highlights a relatively rapid method of analysing microplastics in wastewater and biosolids that can be incorporated in monitoring programs, as long as adequate quality assurance procedures are also included to account for microplastic contamination and recovery from complex environmental samples.

Shortened forms

ABS	co-(Acrylonitrile-butadiene-styrene) (polymer)
ATR FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy (for large particles)
BOD	Biological oxygen demand
CBZ	Carbamazepine
DDD	Defined daily dose
EC	Electrical conductivity
ENCP	Estimated number of contributing people
EP	Population equivalent
EVA	co-(Ethylene-vinyl acetate) (polymer)
Fe	Iron (used in Fenton reaction)
FTIR	Fourier transform infrared [spectroscopy or microscopy]
HCl	Hydrochloric acid (used in Fenton reaction)
H_2O_2	Hydrogen peroxide (for organic matter digestion)
HDPE	High density polyethylene (polymer)
HPLC	High performance liquid chromatography (used for pharmaceuticals analysis)
LC-MS/MS	Liquid chromatography tandem mass spectrometry (used for pharmaceuticals analysis)
LDPE	Low density polyethylene (polymer)
MCT	Mercury cadmium telluride (detector used in FTIR)
PA	Polyacrylamide (polymer)
PAN	Polyacrylonitrile (polymer)
PC	Polycarbonate (polymer)
PE	Polyethylene
Penol4RO	Pentaerythritol tetraricinoleate
PET	Poly(ethylene terephthalate)
PMMA	Poly(methyl methacrylate)
POUA	Polyolefin UV absorber
PP	Polypropylene
PU	Polyurethane
PVA	Polyvinyl acetate (polymer)
PVC	Polyvinyl chloride (polymer)
PVS	Poly(vinyl stearate)

SAN	Styrene-acrylonitrile resin
SOT	Sotalol
SPE	Solid phase extraction
SWSOOS	South Western suburbs ocean outfall sewer
TRM	Trimethoprim
TSS	Total suspended solids
VEN	Venlafaxine
VIA	Voluntary industry agreement
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant
$ZnCl_2$	Zinc chloride

1 Introduction

Plastics are widely used in societies throughout the world and their contamination of the environment is correspondingly widespread. Plastics contaminating the environment can be classified as macroplastics (plastics ≥5 mm in any dimension) or microplastics (<5 mm), with the size of plastic important in terms of its transport throughout the environment, the approach taken to their measurement in the environment and their impacts on organisms living in contaminated environments (Botterell et al., 2019; Browne et al., 2013; Burns and Boxall, 2018; Cole et al., 2011; UNEP, 2016).

Plastic pollution is considered to be a 'wicked problem', in that the sources and breadth of contamination is widespread and varied, human attitudes and behaviour are the main drivers of contamination, and the science of sampling and data analysis is still maturing (Belontz et al., 2019). All these factors make source control and mitigation of microplastic pollution extremely challenging, requiring extensive resources and strong, unified leadership to manage the problem. One measure that has been taken to influence consumer behaviour to reduce microplastics inputs into the environment has been to enact legislation to prevent the sale of personal care products (such as cosmetics, toothpastes and cleaning products) that contain microplastics. For example, the USA has passed the *Microbead-Free Waters Act (2015)* and Canada has enacted *Microbeads in Toiletries Regulations (2017)*, which prevent the manufacture and distribution of microbead containing products (Government of Canada, 2017; U.S. Government, 2015). Other countries in Asia, Europe and the Pacific have already enacted bans on microbead-containing products (www.beatthemicrobead.org; accessed January 2020). In Australia, the Federal Government has developed a voluntary industry agreement (VIA) to phase out microplastics in personal care products that will be lead to regulatory measures if there is evidence that the VIA has not been effective in reducing the number of microbeads in products (NSW EPA, 2016). The effectiveness of the VIA can be assessed through analysis of products available on the market in Australia, which has found 94% of products do not contain microplastics as of February 2018 (O'Farrell, 2018). Also, analysis of wastewater flows for microbeads, after microplastic-containing products have been rinsed off, represents another line of evidence that can be used to determine the extent of use of personal care products containing microplastics within Australian communities.

Wastewater treatment plants (WWTPs) have been identified as a significant pathway of microplastics to the marine environment, although higher WWTP treatment levels (e.g. secondary and tertiary) have been shown to effectively remove a large proportion of microplastics from wastewater prior to discharge (Gatidou et al., 2019; Magni et al., 2019; Simon et al., 2018; Talvitie et al., 2017; Ziajahromi et al., 2017). While wastewater treatment, especially higher levels of treatment, can effectively mitigate the contamination of the marine environment with microplastics, there is also increasing concern that this may be shifting the burden of contamination from marine to terrestrial ecosystems (Ng et al., 2018; Nizzetto et al., 2016). This is because the chemical nature of microplastics means that they are highly resistant to chemical and

biological degradation and their main pathway of removal from WWTPs is through hydrophobic association with sludges formed and/or collected during wastewater treatment.

Biosolids, produced from treating wastewater sludge, represent an important stream of waste management and are widely used for beneficial reuse in Australia (and globally) for improving soil structure and fertility. Their use is strictly regulated, and properly assessing the implications of microplastics in biosolids will allow for their safe and beneficial use. Knowledge relating to microplastics entering the terrestrial environment is currently poorly defined and quantifying and characterising microplastics in biosolids represents a preliminary step in informing future management of the potential risks of microplastics in biosolids (Weithmann et al., 2018).

Accurate quantification and characterisation of microplastics in both terrestrial and aquatic systems is necessary to further our understanding of the potential risks they may have for organisms living within these ecosystems. Currently, methodology for quantification and characterisation of microplastics in environmental samples is still developing and standardised approaches to this are required to ensure robust conclusions are drawn from monitoring studies (Hermsen et al., 2018; Koelmans et al., 2019; Löder and Gerdts, 2015; Miller et al., 2017). The main approach for quantification of microplastics has been through visual inspection using microscopes, with characterisation of polymer type also requiring isolated particles to be analysed using an instrumental analytical technique, such as infrared (IR) or Raman spectroscopy or mass spectrometry (Blair et al., 2019; Carr et al., 2016; Fuller and Gautam, 2016; Mason et al., 2016; Murphy et al., 2016; Talvitie et al., 2017; Ziajahromi et al., 2017). More recently, techniques combining the quantification and characterisation of microplastics using an instrument have been developed, which have the advantage of reducing the time of analysis, sample handling and operator judgement and subsequent errors (Löder et al., 2015; Mintenig et al., 2017; Simon et al., 2018; Tagg et al., 2015). This project applied a FTIR methodology combined with microscopy (FTIR microscopy) that allows a semi-automated analysis of an entire wastewater or biosolid sample that has been prepared for analysis. This reduced the time required for analysis and the potential error in characterising and quantifying each microplastic in a sample.

The objectives of this project were to:

(1) Apply semi-quantitative FTIR microscopy for the quantification and characterisation of microplastics in wastewater influent and effluent samples in two WWTPs, with different treatment levels (primary and tertiary), over 10 months. This methodology was also be applied to a single collection of biosolids from seven WWTPs all located in the greater Sydney metropolitan area;

(2) Assess the effectiveness of the VIA in Australia through monitoring trends over 10 months of microbeads in wastewater collected at two of the largest WWTPs in Sydney, based on their respective wastewater treatment levels;

(3) Use the assessment of the number of microplastics quantified in wastewater to determine the effect wastewater treatment levels have on the removal of microplastics from wastewater.; and

(4) Compare the types and quantities of microplastics present in biosolids sourced from a range of WWTPs to assess trends across the greater Sydney region, as well as using this information for a preliminary assessment of the potential implications this can have for terrestrial systems receiving biosolids as a soil amendment.

2 Methods and materials

2.1 Wastewater treatment plants (WWTPs)

Two WWTPs in the greater Sydney region were selected for monitoring of microplastics in their wastewater over 10 months based on their contrasting treatment levels (primary and tertiary), their relatively large catchment populations (approximately 1.5 million and 250,000, respectively) served to be broadly representative of the region, and their discharge into the marine environment.

Sydney Water operates 16 WWTPs in the greater Sydney region and it is not possible to undertake monitoring at all of these WWTPs. The major WWTPs (Bondi, Malabar and North Head), in terms of volumes of wastewater treated, use primary treatment where physical treatment of wastewater, including screening and sedimentation, is employed prior to marine discharge. Other WWTPs, (e.g. Shellharbour and Cronulla), also employ a secondary (biological nutrient removal) and tertiary (disinfection) treatment levels prior to marine discharge. Malabar and Cronulla WWTPs were selected for assessment of microplastics in their wastewater streams, since their different treatment levels would contribute to understand the effect different treatment levels may have on the amount of microplastics being discharged in effluents to the marine environment. Also, by selecting the two WWTPs that service the largest population equivalents in Sydney for their respective treatment levels, ensured the representativeness will be greatest for other coastal WWTPs in Sydney and in other major urban centres of Australia.

Cronulla WWTP treats approximately 50 ML/day to a tertiary level and has a near-shore discharge at Potter Point, while Malabar WWTP treats approximately 10 times this amount (~500 ML/day) to a primary level and discharges its effluent more than 3.5 km offshore at a maximum depth of ~80 m. The wastewater flowing into the WWTPs (influent) and the treated wastewater leaving the WWTPs (effluent) were sampled in a way where (as near as possible) the same packet of wastewater entering and leaving the respective WWTPs had their microplastic loads quantified and characterised. This would give an indication of the extent of removal of microplastics for either primary or tertiary treatment levels prior to marine discharge. While this was a good approximation at the Malabar WWTP due to the 'plug flow' nature of the influent and effluent, this was less accurate for the Cronulla WWTP, where internal recirculation of waste streams meant that the packet of influent was likely to be fragmented during the treatment process (Figure A 1 and A 2). Composite samples of influent at both WWTPs were also sub-sampled for analysis of pharmaceuticals to estimate the number of people contributing to the wastewater stream over the 24 h compositing period. This was to attempt to account for the variability in microplastic loads measured in the influent streams.

Seven WWTPs in the greater Sydney area were selected for analysis of microplastic loads and characteristics in biosolids. Due to the physicochemical properties of microplastics (e.g.

hydrophobic) and the high organic content of sludge generated during the wastewater treatment process, it is likely that microplastics are associating with sludge, or biosolids, during wastewater treatment. A once-off collection of biosolids was undertaken to determine whether microplastic numbers and characteristics in biosolids sourced from other WWTPs in the Sydney region were consistent with the results from Cronulla and Malabar WWTPs. The seven WWTPs, with the exception of Malabar, all had treatment levels of secondary or greater (Table 1).

Summary of selected WWTPs

Wastewater collected over a 10 month period from 2 WWTPs:

- Malabar (Primary treatment); two influent and one effluent location
- Cronulla (Tertiary treatment); one influent, one post-influent and one effluent location
- November, December 2018, February, May, July and September 2019

Biosolids collected in September 2019 from 7 WWTPs:

- Malabar
- Cronulla
- Quakers Hill
- Winmalee
- St Marys
- Rouse Hill
- West Camden

2.2 Sample collection procedure

2.2.1 Wastewater collection

Wastewater samples were collected for microplastics analysis from programmed automated sample collectors positioned at each sampling point, corresponding with licenced EPA sampling points in Malabar and Cronulla WWTPs (Figure A 1 and A 2). At Malabar WWTP, composited samples were collected from SWSOOS1 (S1) and SWSOOS2 (S2) influent streams, as they comprised 30% and 70% of total influent flows, respectively. Along with influent samples from Cronulla WWTP, samples were also collected following settling of the influent sample, prior to activated sludge treatment (C P Inf) that was equivalent to primary treated effluent. Due to limited recirculation at this point, this sampling point was broadly comparable with the primary effluent being discharged from Malabar WWTP, in terms of treatment level.

For each sample collection period, wastewater samples were distributed evenly amongst 4 x 8 L glass collection jars enclosed within a refrigerated compartment maintained at 4°C for the entire duration of compositing. The collection jars had been pre-cleaned with multiple rinses of ultrapure water and wiped out with lint-free paper towel (Kimwipes™) and collection lines were flushed thoroughly between sample collections.

Wastewater samples were transferred to fill clean 10 L HDPE carboys and immediately sealed with an HDPE screw cap lid containing a rubber seal. Ultrapure water (18.2 MΩ.cm; Milli-Q®) transported from the laboratory was also collected in the same manner as the wastewater samples. Carboys had been previously cleaned with Decon 90® detergent, ultrapure water, methanol, acetone and a final rinse of ultrapure water.

Influent samples were also sub-sampled for analysis of various pharmaceuticals, which were immediately transferred to 3 × 500 mL clean, amber glass bottles, acidified with 0.25 mL of concentrated sulfuric acid (H_2SO_4) and stored on ice for transport back to the laboratory for processing within 12 h.

Additional samples were also collected for total and volatile suspended solids (TSS and VSS) analysis at Sydney Water analytical laboratories, while pH and electrical conductivity (EC) were measured *in-situ* for each collected sample.

Table 1 Summary of sample collection dates for wastewater and biosolids from WWTPs

SAMPLE COLLECTED	WWTP	SAMPLING LOCATION
Influent (C Inf)	Cronulla	5th-6th November 2018 (Nov18)
Post-influent settling (C P Inf)		5th-6th December 2018 (Dec18)
Effluent (C Eff)		5th-6th February 2019 (Feb19)
		30th April-1st May 2019 (May19)
SWSOOS 1 (S1)	Malabar	29th-30th July 2019 (Jul19)
SWSOOS 2 (S2)		25th-26th September 2019 (Sep19)
Effluent (M Eff)		
Biosolids	Cronulla Malabar Quakers Hill Rouse Hill St Marys Winmalee West Camden	20th-24th September 2019

2.2.2 Biosolids collection

Biosolids samples were collected directly after centrifugation of sludges by compositing from approximately 10 grab samples, with a total sample mass of ~1 kg per WWTP. Biosolids were collected in amber glass jars, that had been previously cleaned by rinsing with Decon 90® detergent solution, ultrapure water, methanol, acetone and baked at 400^{o}C for 2 h. Biosolids samples were transported on ice to the laboratory for preparation and analysis.

For field blank samples, builders sand was acid-washed with 10% hydrochloric acid (HCl), rinsed multiple times with ultrapure water and acetone then baked at ~600°C in a muffle furnace for 3 h. Approximately 100 g of sand was placed in sample containers (prepared in an identical manner to biosolid collection jars), alongside empty sample containers for biosolids collection, and transported to respective WWTPs. Operators at the WWTP were instructed to open the lid of the field blank containers for as long the biosolids were being collected. Field blanks were transported back to the laboratory with the biosolids and processed in an identical manner as the biosolids.

2.3 Microplastic extraction procedures

For the isolation and analysis of microplastics in wastewater and biosolid samples, it was necessary to remove non-microplastic organic and inorganic matter. The amount material that

required removal is evident based on the relatively high (up to 500 mg/L) TSS and VSS loads in the wastewater, especially influent samples (Table A 3). The presence of organic and inorganic matter interferes with the absorbance of incident IR wavelengths required for effective FTIR microscopy analysis and needed to be removed as much as possible without the concomitant loss of microplastics. Wastewater and biosolid samples were therefore treated with a Fenton reagent to digest organic matter, while inorganic particles were removed from samples using density separation using zinc chloride ($ZnCl_2$). Following this, residual solutions containing microplastics were filtered through a nominal 25 µm Hollander weave mesh (Sefar Pty Ltd; Sydney, Australia) to isolate the microplastics for FTIR microscopy (Figure 1).

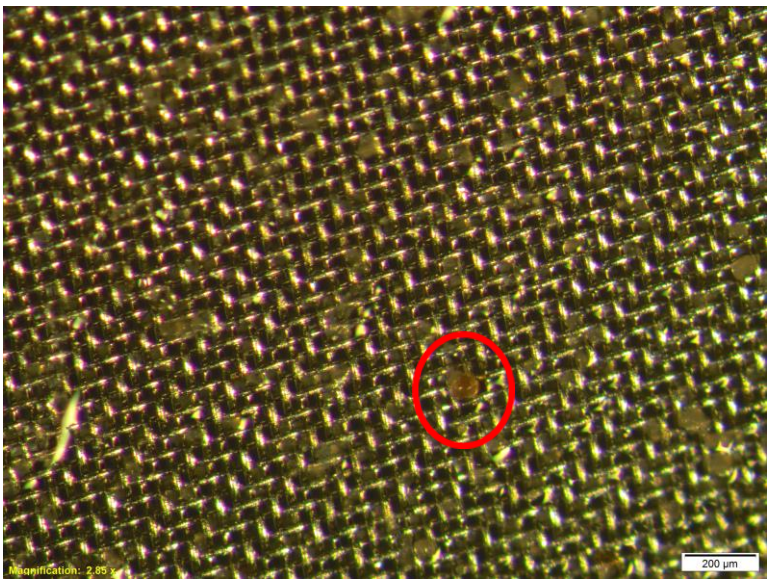


Figure 1 Micrograph of stainless steel mesh used for filtration of wastewater samples.
For reference, an orange PE microbead (~50-60 µm diameter; circled in red) is present in the approximate centre of the image.

2.3.1 Wastewater samples

Fenton reagent and initial reaction with wastewater

The digestion of organic matter in the wastewater samples was undertaken by a 2-stage Fenton process, where wastewater was digested prior to filtration and then the filtrate was subjected to further Fenton digestion. The Fenton process consists of the oxidation of organic matter with hydrogen peroxide (H_2O_2) in the presence of an Fe(II) (or Fe^{2+}) catalyst (Koppenol, 1993). The Fe(II) catalyst is oxidised by H_2O_2, leading to the production of Fe(III) (or Fe^{3+}) and a highly reactive hydroxyl radical (OH·) :

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \quad (1)$$

The Fe(III) can also react with H_2O_2, although at a considerably lower rate due to the comparatively lower water solubility of Fe(III):

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH \cdot + H^+ \quad (2)$$

The hydroxyl radical is a highly reactive species that effectively destroys organic matter and has been used for reducing organic loads in wastewater and, more recently, for assisting the isolation of microplastics from environmental samples (Babuponnusami and Muthukumar, 2014; Hurley et al., 2018; Tagg et al., 2017).

Wastewater samples were quantitatively transferred to a 5 L glass container and initially digested through the addition of Fenton reagent in glass containers and allowed to digest for ~2 h. The Fenton reagents, $FeSO_4.7H_2O$ and H_2O_2 (25%), were obtained from Sigma-Aldrich (Australia). The Fe(II) catalyst was prepared as a 150 g $FeSO_4.7H_2O$/L solution and its pH was adjusted to ~3 using HCl, as the Fenton reaction is considerably more efficient at this pH (Babuponnusami and Muthukumar, 2014). Both the Fe(II) catalyst and H_2O_2 solutions were filtered through a 0.7 µm GF filter (Whatman® GF/F) in a glass Millipore® filtration apparatus and collected in clean amber glass Schott bottles and refrigerated until use (within 24 h). The Fe(II) catalyst was first added at 40 mL/L wastewater and swirled gently to ensure mixing. The H_2O_2 solution was then added at 5 mL/L wastewater and again swirled gently. The samples were allowed to digest for ~2 h, when the production of small bubbles had ceased and an orange/brown Fe(III) precipitate had formed in the bottom of the containers (Figure 2 (b)

). After this, concentrated (12 M) HCl was added at 5 mL/L wastewater to solubilise Fe(III) precipitates and continue the Fenton reaction for a further 12 (Figure 2 (c)

).

It was not necessary to do this initial Fenton step with the Cronulla effluent samples due to the low organic matter content of these solutions. These samples were directly filtered through the stainless steel filters.

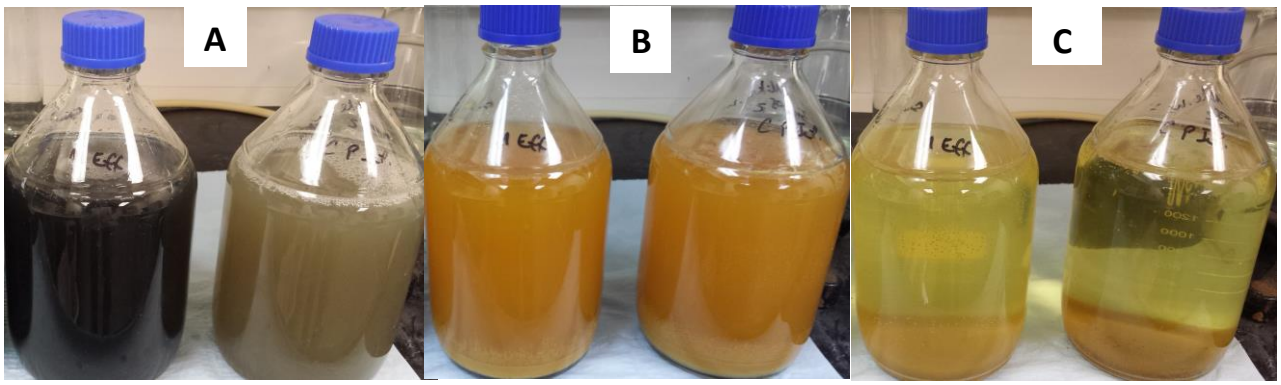


Figure 2 Fenton treatment of wastewater samples showing (a) untreated wastewater, (b) wastewater after Fenton treatment and (c) wastewater following addition of HCl

Filtration of wastewater

After the initial Fenton reaction, samples were then filtered through a 25 µm stainless steel Hollander weave mesh cut to fit a 47 mm vacuum filtration unit. After cutting the stainless steel for filtration, the pieces were washed with a Decon 90® detergent solution, rinsed thoroughly with ultrapure water, methanol, and dichloromethane and then placed in a muffle furnace at 600°C for 1 h. Samples were carefully decanted through the mesh keeping the filtration unit and sample container covered at all times with a fresh piece of aluminium foil. Any solution remaining, due to the presence of undigested inorganic matter, was quantitatively subtracted from the initially measured volume. All glass surfaces, including the sample bottle and filtration apparatus, were rinsed twice with ultrapure water, methanol and dichloromethane, onto the stainless steel filter. After the stainless steel filters were dried under vacuum, they were carefully transferred to a clean glass jar and sealed with a metal enclosure for a further Fenton reaction.

Additional Fenton reaction

All filtered samples were then subjected to an additional Fenton reaction by immersing the filters in a Fenton reagent solution, prepared as above, by adding 5 mL of both $FeSO_4.7H_2O$ solution and H_2O_2 to the filters (total volume 10 mL). The glass jars that the stainless steel filters were collected in served as reaction vessels for the digestion process. The reaction vessels were kept in an ambient temperature water bath to absorb the heat generated by the reaction to prevent it from becoming too vigorous. After ~1 h, the reaction had visibly stopped whereupon 0.5 mL HCl was added to solubilise the Fe(III) precipitate and left for a further 12 h. The solutions were then removed by filtering the stainless steel filters as previously described, with reaction vessels and the filtration apparatus carefully rinsed with ultrapure water, methanol and dichloromethane between each sample.

Density separation with saturated $ZnCl_2$ solution

Microplastics have a lower density than many other solids and this has been previously exploited for separating microplastics from environmental matrices (Browne et al., 2011; Coppock et al., 2017; Imhof et al., 2012; Mahon et al., 2017; Ziajahromi et al., 2017). $ZnCl_2$ was selected for the density separation step due to its relatively high density (1.6 g/mL) when prepared as a saturated solution, which has the potential to ensure that even higher density polymers (e.g. PVC) float (Coppock et al., 2017; Imhof et al., 2012). A 1 kg/L solution of $ZnCl_2$ (Sigma-Aldrich Australia) was therefore prepared for density separation of the filtered particles collected from the Fenton digestion. The $ZnCl_2$ solution was also filtered through a 0.7 µm GF/F filter prior to use. The stainless steel filters were carefully placed in a 50 mL glass culture tube (with the bottom of the filter pressed against the wall of the tube), 20 mL of $ZnCl_2$ solution was added and the culture tubes were vigorously shaken on a vortex mixer for ~1 min, ensuring all the particulates had visibly detached from the stainless steel filter. After this, the culture tubes were centrifuged for 30 min at 1300 rpm (~500 *g*), the stainless steel filter was carefully removed and rinsed with 3 × 1 mL ultrapure water, including the underside of the filter, placed on the filtration apparatus and the $ZnCl_2$ solution was carefully decanted through the filter, leaving ~5 mL in the culture tube. The filtration apparatus was again rinsed through the filter with ultrapure water, methanol and dichloromethane before the dry filter was carefully placed in a clean glass jar in preparation for analysis of the microplastics by FTIR microscopy.

2.3.2 Biosolids samples

Biosolids sample preparation followed a similar procedure to the wastewater samples. Approximately 2 g (wet weight) of biosolid was removed from the collection jar and placed in clean sealed glass jars, frozen at -20°C overnight and freeze-dried (ModulyoD, Thermo Electron Corporation) for 4 days to remove all moisture. Sample containers were covered with two-ply low-linting paper tissues (Kimwipes™) secured in place with elastic bands. Following freeze-drying, the dry biosolid samples were then subjected to Fenton digestion, followed by density separation and centrifugation (following the same methodology as the wastewater filters). Extracted samples were then filtered through a 25 µm stainless steel mesh filter in preparation for sample analysis of the microplastics by FTIR microscopy.

2.3.3 Sample preparation for FTIR microscopy analysis

A purpose-designed filtration apparatus, suitable for use with standard filtration setups (e.g. Merck Millipore™) was constructed for final filtration of wastewater samples before analysis (Figure 3). The design specifically concentrated the filtered particles onto a 13 × 13 mm square area suitable for imaging using the microscope of the FTIR microscope. A 47 mm diameter aluminium oxide filter (Whatman® Anodisc; 0.02 µm pore size) was secured in the filter holder part. This filter holder has an alignment peg and hole on the top and bottom to ensure the orientation of the sample area was maintained between all steps from depositing sample to scanning by FTIR microscopy.

Figure 3 Filtration apparatus for mounting Anodisc filter for final filtration of collected particulates.

Each stainless-steel filter mesh and glass jar were rinsed and sonicated with methanol to disperse the collected material into the methanol. The methanol suspension was quantitatively transferred to the filtration rig and collected onto the Anodisc filter under gentle vacuum. Silicone gaskets were used in between different components of the filtration apparatus to avoid leakage of solvent and particles. The Anodisc filter, while still mounted on the filter holder, was separated from the filtration apparatus and placed in a clean, covered glass petri dish prior to microscopy.

The Anodisc filters containing filtered wastewater residue were examined under a magnifying glass and large particles (~2 mm or greater) were removed. These were placed between two microscope slides, an optical image recorded, and were set aside for separate attenuated total reflectance (ATR) FTIR analysis.

The material collected on the Anodisc filter was imaged with an Olympus BX61 upright optical microscope, 5x objective in reflection mode, using both bright field and dark field configurations (Figure 4). Dark field images were mainly used for visual confirmation due to their greater contrast for translucent particles.

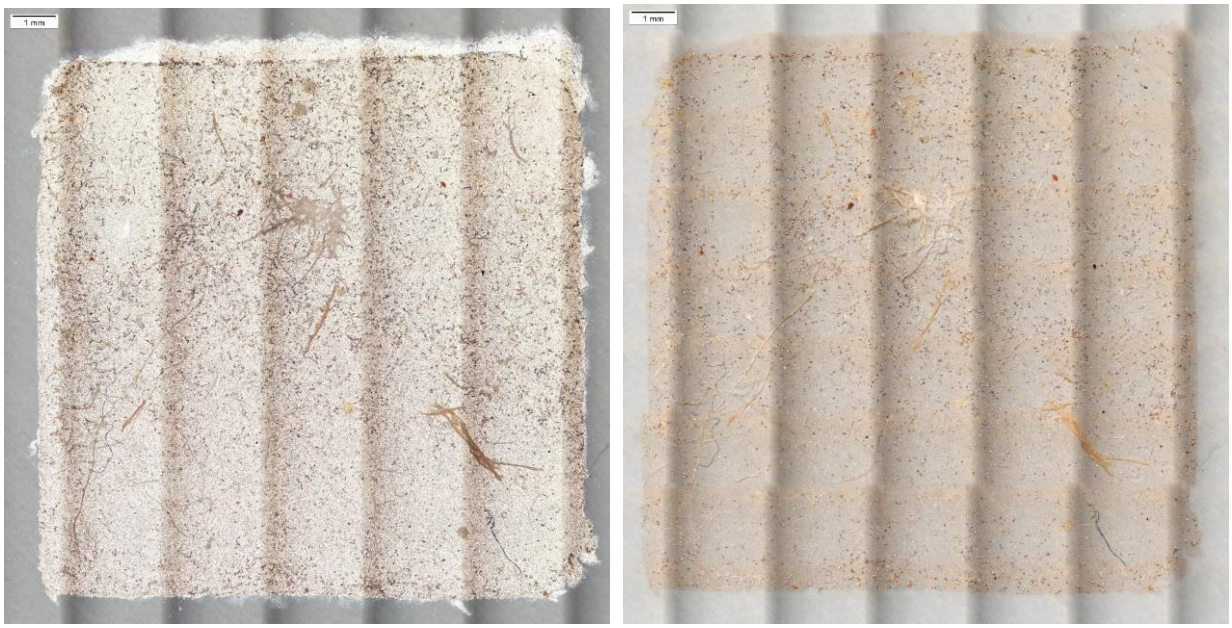


Figure 4 Representative brightfield (L) and dark field (R) optical image of material collected onto an Anodisc filter. The vertical dark lines are artefacts of stitching many images with non-uniformed illumination together.

The optical density of material on the Anodisc filter was assessed and those that were deemed to have too high a density of material to enable accurate FTIR microscope analysis were rinsed and sonicated with methanol and the methanol suspension was divided into two or three portions that were quantitatively transferred to the filtration apparatus and collected onto multiple Anodisc filters.

Analysis of the larger particles that were previously removed was performed using a Thermo Scientific Nicolet 6700 FTIR spectrophotometer with a diamond crystal ATR accessory from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1}. The data were collected and processed with Thermo Scientific OMNIC Specta software. Any larger particles found to be polymers had their optical images calibrated for scale, processed to measure the size and shape of the particle with the results incorporated within the FTIR microscopy analysis.

2.4 Pharmaceutical extraction procedures

Knowledge of population equivalents (EP) within a WWTP catchment is important to understand the treatment capability required for a WWTP to reduce pollution (especially nutrient) loading, rather than knowing the number of people that are serviced by the WWTP. Where the population numbers in a WWTP catchment is required, such as in wastewater epidemiology studies, various chemical methods have been used to get a more accurate estimate of a population. Population sizes have the potential to vary within a WWTP catchment, affecting the conclusions of epidemiological studies in wastewater (Ort et al., 2010; Zuccato et al., 2008). Chemical methods for estimating people can include monitoring ammonia, biological oxygen demand (BOD), nutrient loads, biochemicals (e.g. creatinine and cholesterol) or pharmaceuticals and personal care products in wastewater influent (Been et al., 2014; Chen et al., 2014; Lai et al., 2011). Pharmaceuticals have previously been used for this purpose since they are specific to human activity, have data available relating to their use within a population and their pharmacokinetics that, in combination with wastewater flow, can be used as a reasonable reflection of population numbers within a catchment (Lai et al., 2011). The estimated number of contributing people (ENCP) within a catchment was then used as a comparison with microplastic loads, especially microbeads, in wastewater influent. Ideal pharmaceutical candidates include those that are used consistently throughout the year (i.e. to treat chronic conditions), have a relative high use (either through prescription numbers and/or dose size) or are excreted from the body as a high proportion of the unmetabolised chemical and are relatively stable within wastewater to enable concentrations for reliable quantification (Lai et al., 2011). Four pharmaceuticals were selected based on a preliminary analysis of a range of pharmaceuticals in the influent from the two WWTPs. They were carbamazepine, used to treat epilepsy, bipolar disorder, trigeminal neuralgia; sotalol used to treat hypertension; trimethoprim, used to treat bacterial infections; and venlafaxine, used to treat depression and anxiety. Carbamazepine, sotalol and venlafaxine are used to treat conditions that require long-term therapy so it is expected that their use patterns would be reasonably stable over time. Despite being used to treat short-term infections, trimethoprim is an antibiotic that is commonly detected in wastewater and was present at comparable concentrations with the other pharmaceuticals in the preliminary sampling.

To quantify pharmaceuticals in wastewater, triplicate water samples were transferred, chilled, to a laboratory, filtered through a 0.7 µm glass fibre filter (Whatman® GF/F) and then passed through a pre-conditioned solid phase extraction (SPE) cartridge (Waters HLB; 6 mL, 200 mg) at a rate of ~1 mL/min. Prior to passing through the SPE cartridge, one of each triplicate sample was split into equal volumes and one of the solutions was spiked with 50 µL of a methanol solution containing a 10 mg/L mixture of pharmaceuticals for assessment of recovery. SPE cartridges were processed within 12 h of sample collection and were then stored at -18°C. Immediately prior to analysis, SPE cartridges were eluted with 2 × 3 mL of methanol and 1 × 3 mL of dichloromethane, which was blown down under nitrogen until dry and reconstituted in 1 mL 90% water:10% methanol for analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

2.5 Microplastic analytical procedures

2.5.1 Data collection

The IR spectral data acquisition of microplastics isolated on the Anodisc filter was performed on a Thermo Scientific Nicolet iN10 FTIR microscope with OMNIC Picta v9 (Thermo Scientific). The microscope was set up in transmission mode with a MCT imaging detector having 8 × 2 array of elements. The Anodisc filter was mounted on a holder that was put in a specially constructed adapter plate for the microscope stage. This adapter plate has an alignment peg to keep the filter holder in the correct orientation and location so that every 15 × 15 mm sample analysis area was placed in an exact position once loaded to the microscope stage. The adapter plate also has three grub screws built in so that the operator can manually tilt the filter holder to ensure the entire surface of the filter was in focus and within 100 µm in height relative to the microscope objective lens.

The FTIR background signal was collected with 128 scans at the beginning of an imaging acquisition at a location on the Anodisc filter away from the sample collection region. The background spectrum for all elements of the imaging detector was set at the same point on the sample. Typical parameters for imaging were: spatial resolution of 25 µm and a spectral resolution of 4 cm^{-1}; frequency range, from 4000 to 1300 cm^{-1}; one scan. A typical 15 × 15 mm sample area scan took approximately 2.5 hours and generated a data file of ~2 GB.

The areas immediately surrounding the collection area (Figure 5) were imaged at a lower resolution (50 µm spatially and 8 cm^{-1} spectrally) to check for any microplastics that may have moved outside the main scanning area (15 x 15 mm) during transfer of the Anodisc from the filtration apparatus from the optical microscope to the FTIR microscope. If plastic particles were found outside the main sample area, or a certain region within the sample was out of focus due to unevenness of the Anodisc filter, that particular region would be re-scanned in the FTIR microscope with standard spatial and spectral resolution (i.e. 25 µm spatially and 4 cm^{-1} spectrally). These additional datasets were later overlaid on the main data set, realigned, trimmed and cut as appropriate in the custom-built software IR Map Merger. The resulting dataset was then used for further analysis. All data acquisition for one sample typically took between 3 to 5 hours.

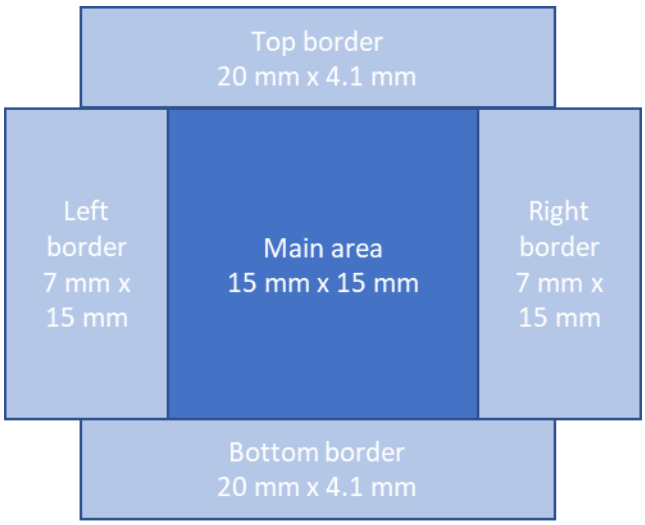


Figure 5 Imaging scheme for FTIR microscope analysis, with the darker shading representing the high resolution (25 µm spatially and 4 cm^{-1} spectrally) image collection and the lighter margins representing the lower resolution (50 µm spatially and 8 cm^{-1} spectrally) image collection areas

2.5.2 Spectral data processing and analytical workflow

The process for analysing the data collected was partially automated via a number of in-house written scripts and programs and was comprised of three steps: (1) bulk data processing to generate correlation profiles against standard materials; (2) polymer identification and assignment; and (3) particle counting, measurement and classification.

Bulk data analysis for correlation profiles

Preliminary work identified microplastics present in wastewater via individual matching of the FTIR spectra with libraries of spectra of polymers using the OMNIC Picta software. From these results, a knowledge of the plastic types found in these specific wastewater samples was gained and this limited library was utilised for the setup of the automated analysis. Some polymer types have more than one reference spectra to reflect different varieties or sources of the polymer. During this step a series of statistical profiles were also created to measure the minimum, average, maximum, and peak-to-peak variation of the absorbance values of the spectrum at the pixel location. Other spectral features were also measured and created as profiles: absorbance values at 2200 cm^{-1} to measure the broad range absorbance, area under the peak between 3000 and 2800 cm^{-1} to measure the strength of the C-H bond stretching peak.

Table 2 Polymers used for automated analysis of microplastics

Polyethylene (PE)	Poly(vinyl chloride) (PVC)	Polyurethane (PU)
Polypropylene (PP)	Polycarbonate (PC)	co-(Ethylene-vinyl acetate) (EVA)
Poly(ethylene terephthalate) (PET)	Styrene-acrylonitrile resin (SAN)	Polyacrylonitrile (PAN)
Polyolefin UV absorber (POUA)	Alkyd resins	co-(Acrylonitrile-butadiene-styrene) (ABS)
Nylon	Silicones	

For the bulk data processing step, OMNIC Picta software was used to create a series of 'correlation profiles' against a list of reference spectra for standard polymers as well as some commonly found non-plastic materials like cellulose (for paper and wood based materials), zein (for protein based materials) and oleates (for esters of fatty acid). Pentaerythritol tetracinoleate (Penol4RO) was commonly found in many of the wastewater and biosolid samples but little information could be found on its function as a polymer or whether it exists as a polymer, i.e. as a molecule comprised of a series of repeating monomer units. It was therefore not included in the analysis of samples. Each of these profiles contains a map of values between zero (no correlation) to one (identical) for the particle spectrum at any given pixel in comparison with the reference spectrum.

A typical sample analysed for correlation profiles against 22 reference spectra takes approximately 3.5 hours (Figure 6).

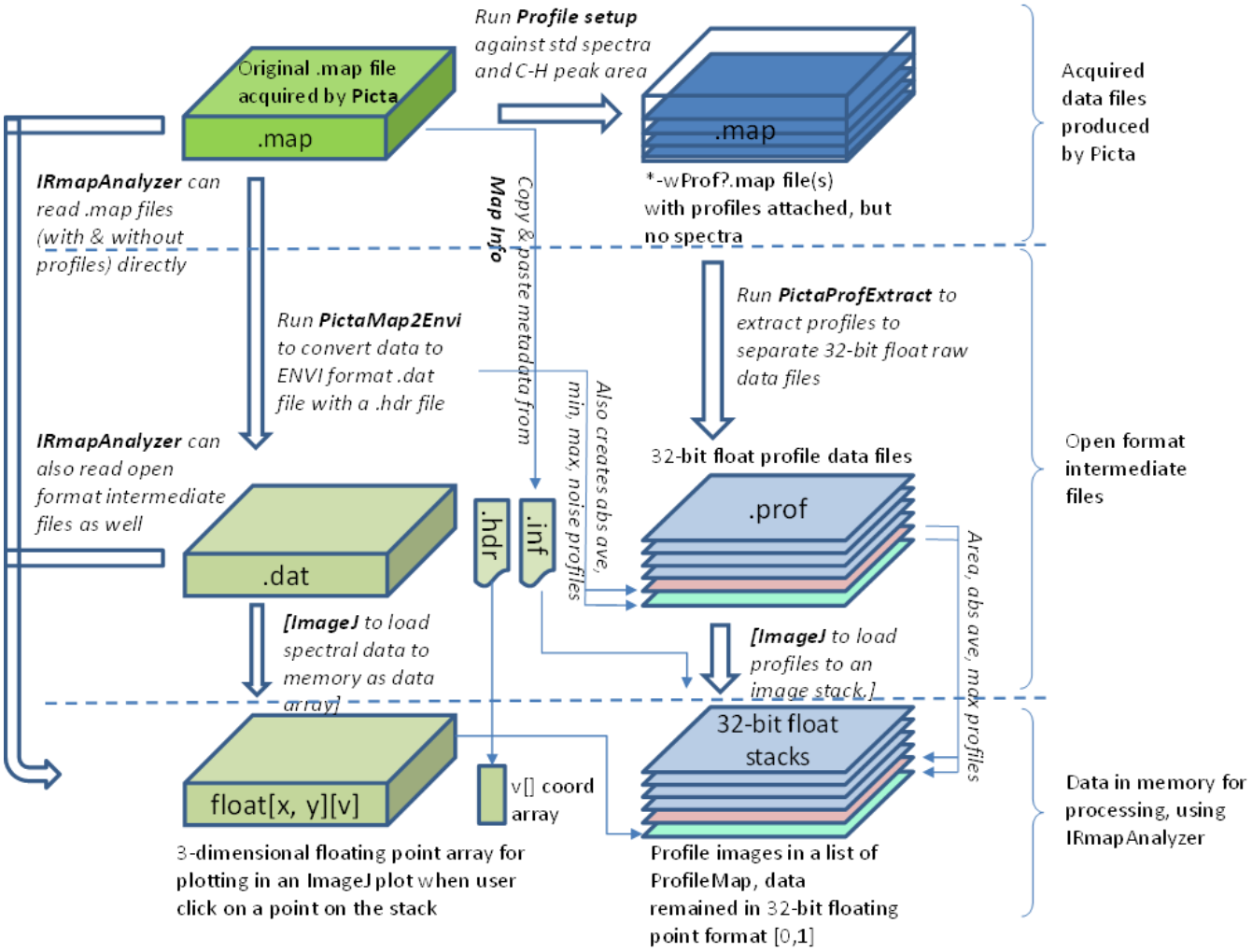


Figure 6 Overview of data flow during the bulk data processing step. Spectral data map acquired during data acquisition step at the top left corner was processed via different pathways and finally loaded to IR Map Analyzer software (bottom) for further analysis.

Material identification and assignment

In this step, the material at each pixel in the sample map was identified and assigned appropriately such that no area is unaccounted for and the same pixel was not assigned to more than one material (Figure 7). Each correlation profile map (Figure 8) had a threshold applied to select only pixels that had a correlation value typically larger than 0.75 to be considered as a candidate for the corresponding reference material. However, different reference materials have different correlation statistics and the threshold value was changed slightly between different profiles to ensure correct identification was mostly achieved in the first steps. For example, PE and PP thresholds were set at 0.75, while the PET threshold was set at 0.6, which was maintained between different samples.

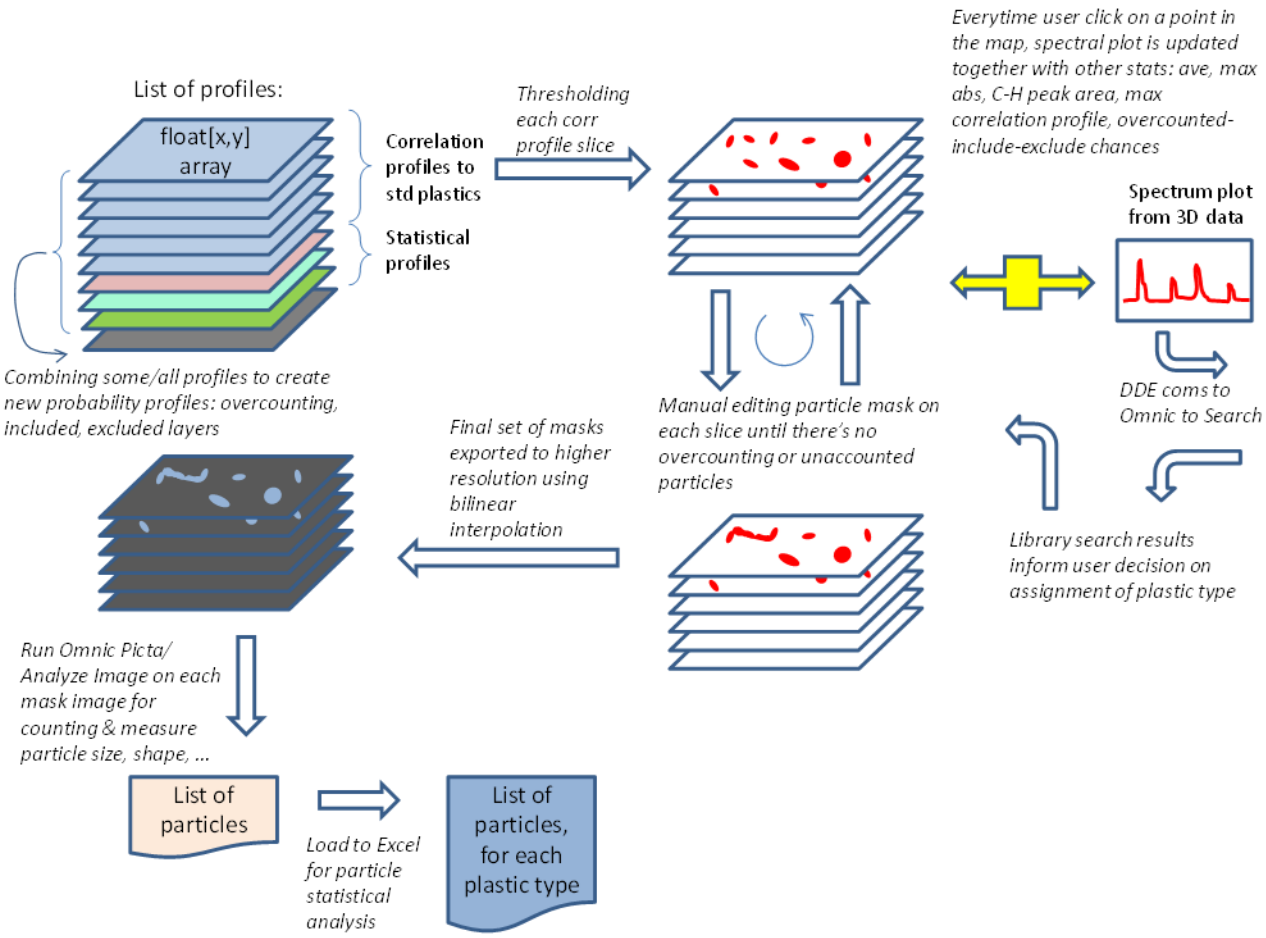


Figure 7 Overview of particle analysis and identification steps within IR Map Analyzer

A binary image of all pixels after thresholding a profile constitutes a mask for that profile. A user can manually change the mask at a pixel to reflect their examination of the spectrum to decide what type of material that pixel should be. A set of working profiles were computed based on the existing profiles to indicate if a pixel is a polymer, substrate, an inorganic/non-polymeric material, an organic/non-polymeric material, unaccounted for (not included in any material) or duplicated (included in more than one material). The set of thresholds for different materials was optimised from expert knowledge gained from the preliminary study such that the vast majority (typically 95 – 99%) of the map area was identified correctly by the automated process. The remaining pixels would be flagged in the working profiles as either unaccounted or duplicated. The infrared spectrum at each of these pixels was manually examined and compared against a set of full commercial libraries of materials, containing more than 4000 spectra of polymers and additives. The user would then decide the most appropriate material assignment for that pixel, then manually set and/or clear the appropriate profile masks. The set of working profiles were then re-calculated until all pixels are accounted for and no double counting existed. Samples with well separated particles and few overlapping spectra only required 30 minutes of manual checking in this step. However, the most difficult samples with many unknown or overlapping material could take an additional few hours.

The binary image of the masks for different plastic profiles were used to count the number of particles, where each particle is defined as a cluster of neighbouring pixels (8-connected neighbour with either common sides or vertex). The size and shape of those particles were also measured. This step could be performed in many common graphic processing software packages. In this study, we imported the mask images to OMNIC Picta for particle counting and measurement. These measurement data were aggregated into an Excel workbook for each sample and statistics for each plastic type was calculated (Figure 7).

2.5.3 Software development

While each step of the process through data processing, analysis, particle identification, polymer identification, determination of size and morphology, and reporting can be performed in the proprietary software which is supplied with a FTIR microscope, there are a number of limitations when analysing environmental samples with a large size and degree of complexity, such as wastewater. The data analysis workflow performed by OMNIC Picta (or any other software we are aware of) is such that the identification of each plastic type must be done separately from acquired data through to a final list of particles. This means any non-identification, misidentification or double counting is not identified until analysis on all particles is complete and compared against each other. Furthermore, any manual correction means re-analysing and cross-checking all particles across each polymer type. Given the large size of each sample and large number of different types of materials present, it is an extremely manual, time consuming, and laborious task.

To streamline the integration of microplastic data analysis and allow it to be more interactive a software named IR Map Analyzer was developed in Visual C# and run on Microsoft .NET platform that automated many of the routine tasks associated with the data processing and interpretation. The goal was to produce software which did not require significant user intervention in order to process multiple samples in a short timeframe, such that an experienced operator could devote the majority of their time to quality control assessment of the results.

The majority of the steps outlined in Figure 6 and Figure 7 have been automated, thereby reducing analytical time considerably per sample but, more importantly, increasing consistency between sample analysis and reducing the potential for ‘human error’.

2.5.4 Microbead and fibre characterisation

While the shape of a microbead and fibre are relatively easy to identify through visual inspection, their parameters need to be defined for automated analysis for microplastics isolated from wastewater samples.

Microbead characterisation

In the case of microbeads, these parameters were set to correspond with objects of a spherical shape. This is despite the majority of microbeads present in consumer products not being of a spherical shape, either because of their manufacture as irregular shapes or due to spherical microbeads being broken up after their release into the sewerage system (Fendall and Sewell, 2009; Napper et al., 2015). The spherically shaped microbead was therefore used as a representative of microbeads found in consumer products due to their more easily identifiable shape (NYSO, 2015).

The circularity (*C*) of a microplastic particle as calculated by OMNIC Picta software is

$$C = \frac{P^2}{4\pi A} \qquad (3)$$

where *A* is the area and *P* is the perimeter of the particles, calculated as total length of joining lines between centres of adjacent pixels. This inherently leaves some area (accounted for in calculation of area) outside the perimeter. When the object is not large enough, this pixelated effect leads to a circularity less than the true value, could even less than one. The example given in Figure 8 (b) shows the perimeter (black line) is 9.66, and the area of the particle (light grey) is 12. It follows that *C* = 0.6, less than the theoretical minimum of one. When such situation occurs, the circularity is automatically corrected to become one (perfect circle) to reflect the fact that as far as the spatial resolution allows, this particle is too small to be detected as anything but a circle.

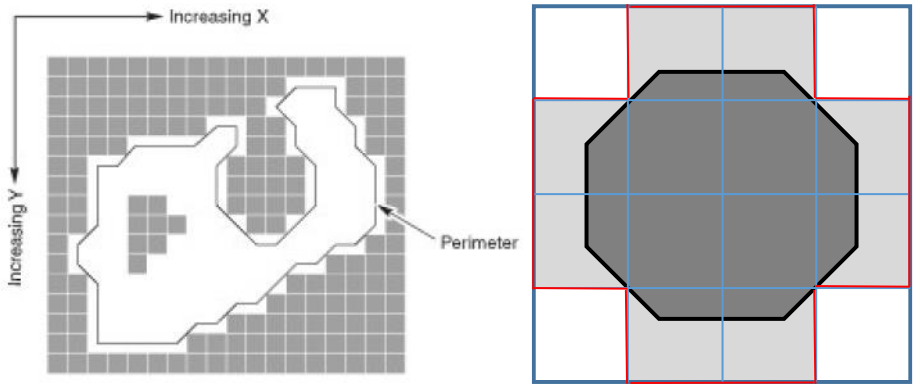


Figure 8 An example of (left) a particle identified from the software as white pixels (equivalent to 25 x 25 μm^2) and (right) a small particle close to the size of pixels (25 x 25 μm^2), where pixelated effects are significant.

Fibre characterisation

OMNIC Picta software calculates two estimates of a feature linear size: fibre length and max projection. Fibre length is a derived length of feature or fibre, after it is straightened into a rectangle of equal area and perimeter. For small features (on any dimension), pixelated effect as seen in the circularity calculation, will underestimate the perimeter, hence underestimate fibre length. If we defined width as area divided by length, it could be greater than length and give a wrong estimate of size.

Max projection is the maximum feature projection (maximum calliper dimension), as the largest separation between points on the feature convex perimeter. This will underestimate the length of a fibre curled up in S or spiral shape, sometimes excessively so if the fibre is very curly. The determination of feature size is that the length is the larger of fibre length and maximum projection, and width is the area divided by length.

The pixelated effects can be reduced by increasing the spatial resolution of the image. Where resolution cannot be increased due to hardware or limits of instrumentation, a software approximation process can be employed. The masked black and white image resolution is increased by the software using bilinear interpolation. This will 'soften' the edges of any particles, introducing increasing grey scale pixels at the edges and round out the sharp corners. Once a new mask is applied with half the greyscale value, the selected area will still closely present the original selection and underestimation of the circumference will be reduced.

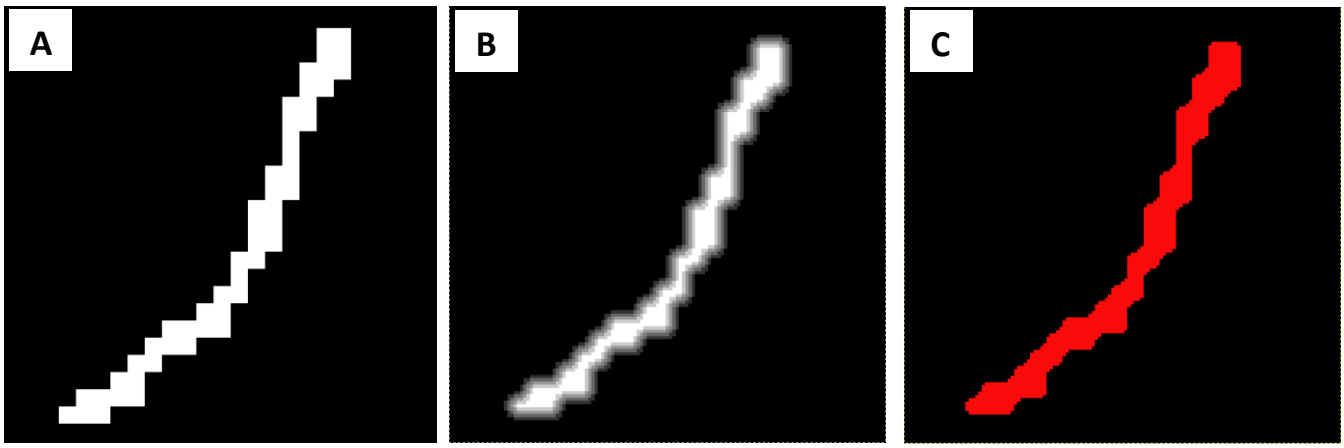


Figure 9 From left to right (a) original black and white mask; (b) mask resolution increased by 5 times using bilinear interpolation producing a greyscale image; (c) new mask of high resolution image with threshold set at the mid-level grey value.

2.6 Pharmaceutical analysis

Following elution of samples from SPE cartridges, pharmaceuticals were quantified by LC-MS/MS using a ThermoFinnigan TSQ Quantum Discovery Max (Thermo Scientific), with HPLC separation performed with a Kinetex C18 100 x 2.1 mm (2.6 µm particle size) column (Phenomenex, USA). MS/MS analysis was undertaken using atmospheric pressure electrospray ionisation (ESI) in positive ionisation mode (Appendix A3). Data was collected and analysed using XCalibur software (Thermo Scientific).

A standard addition methodology was applied following the methodology of Watkinson et al. (2007) to determine the extent of recovery using the relationship:

$$Matrix\ effect = \frac{Concentration\ spiked\ sample - Concentration\ in\ sample}{Concentration\ of\ spike\ solution} \quad (4)$$

with the calculated matrix effect factor used to adjust the concentration determined from the calibration solutions.

The calculated concentrations of the pharmaceuticals were then compared with their prescription data as a means of estimating the number of people that may have been contributing to the wastewater stream for each sampling time period. To calculate the estimated number of contributing people (*ENCP*), the consumption of the pharmaceuticals in a population, the percentage of the dosed pharmaceutical excreted after therapy and the flow rate into the WWTP needs to be included in the following equation:

$$ENCP = C_{ww} \times F_{ww} \times \frac{P_T}{A/365} \quad (5)$$

Where C_{ww} is the concentration of the pharmaceutical in wastewater (influent), F_{ww} is the flow rate of wastewater during the 24 h compositing period, P_T is the total population of Australia and A is the amount of the pharmaceutical consumed in Australia over one year (Lai et al., 2011).

The value for A is obtained from prescription data obtained by the Pharmaceutical Benefits Scheme (PBS), which conducts yearly surveys of pharmaceuticals use in Australia (Department of Health, 2016). The number of prescriptions over a year for each pharmaceutical is compiled and this is converted to the number of defined daily doses (DDDs) per 1000 population per day (Table 3). DDD is a value for each pharmaceutical assigned by the World Health Organisation, which relates to the amount of the pharmaceutical required each day for its intended therapeutic purpose (Department of Health, 2016).

Table 3 Summary of parameters for each pharmaceutical used to calculate ENCP to wastewater flows in the catchment of each WWTP.

PHARMACEUTICAL	%EXCRETION	DDD (GRAMS)	PRESCRIPTION RATE (DDD/1000/DAY)
Carbamazepine	1	1	1.5
Sotalol	75	0.16	2.2
Sulfamethoxazole	25	1.92*	0.06
Trimethoprim	60	0.4	2.2
Venlafaxine	5	0.1	12.1

Source: Department of Health, 2016; Sansom, 2015

2.7 Quality assurance and quality control

Microplastics analysis requires a slightly different methodological approach to extraction and analysis compared with traditional aquatic contaminants (e.g. organic chemicals and metals) and the relatively recent nature of the research means that a consensus on a standardised methodology for their quantification has not yet been reached (Koelmans et al., 2019). Due to the developing nature of the science there is still ongoing debate about robust approaches to the quantification. Plastic (and microplastics) are a ubiquitous material with many applications and their presence has been reported in water (including drinking water), soil, food and air (Koelmans et al., 2019). The challenge with microplastics is therefore ensuring that quantitative methods can demonstrate the values obtained in a particular environmental matrix are as close to the actual

value as possible (accuracy) and are reproducible (precise). Ongoing measures that were taken for sampling and preparation of equipment included continuous replacement of containers for storage of solutions in the laboratory and field, rinsing all containers with only filtered solvents and ultrapure water from the source, filtering all solutions through 0.7 µm GF/F filters prior to every use and rinsing and baking (~600°C) of stainless steel equipment (e.g. tweezers, spatulas). Also, air flow was minimised in the laboratory, where air-conditioners and fume cupboards were not used and personnel handling samples wore only natural materials.

Along with these operational measure, negative controls, or samples reasonably assumed to contain no microplastics, were included during sampling to give an understanding of whether the environment for sample collection, extraction and preparation for analysis had the potential to introduce false positives into quantification, where microplastics from the environment were introduced into samples. Positive controls, or samples where microplastics are quantitatively added, are also an important means of assessing the accuracy and precision of a quantitative methodology.

2.7.1 Negative controls

Negative controls used for the wastewater samples included a laboratory blank and a field blank. A laboratory blank consisted of ultrapure water collected in the laboratory and processed in an identical manner to the wastewater samples. A field blank consisted of ultrapure water being transported to the field in a sampling container and subject to the same environmental conditions as the wastewater samples. In this case, sampling containers with ultrapure water were taken to each sample collection site within the WWTP and the lid of the container kept open for as long as the sample containers. The field blanks were then transported back to the laboratory and processed in an identical manner to the wastewater samples. Both laboratory and field blanks were included for each sample collection period. Clean sand samples were also used as additional negative controls for biosolids samples, as previously discussed.

2.7.2 Positive controls

For the positive controls, fluorescent orange HDPE microbeads (50-60 µm size range; Cospheric LLC, USA) and yellow PET fibres (CSIRO Manufacturing; Clayton, Australia) were added to 4 × 1 L samples of wastewater collected from the Cronulla influent stream in September 2018. These microplastics were used as they were considered likely to be distinct from microplastics already present in wastewater. For this reason, fragments of plastic were not included, as preliminary analysis of wastewater indicated fragments to be a very common microplastic, making added fragments potentially more difficult to distinguish. A total of 10 microbeads and 10 fibres were

counted under a light microscope into clean glass vials and added to 1 L of wastewater using multiple rinses of ultrapure water and methanol. Wastewater samples were used instead of ultrapure water as this would give an indication of the potential for naturally occurring particulate matter to interfere with the extraction and analysis of the microplastics. The vials were examined afterwards for evidence of microplastics that had not transferred into the wastewater. The wastewater samples were then prepared in an identical manner to the other wastewater samples.

2.7.3 Comparison of analytical methodologies – visual counting and FTIR microscopy

Additional samples were prepared identically to the samples for FTIR microscopy analysis for the purpose of visual quantification and characterisation of microplastic particles. Visual quantification and characterisation of microplastics has been the most common approach to environmental samples (Table 5) and this was also undertaken as a cross-validation with the FTIR microscopy methodology. For this comparison, 1 L of Cronulla influent (C Inf) was prepared identically to those samples collected for FTIR microscope analysis for each of the sampling dates from November 2018 to September 2019.

Following preparation of the samples through Fenton digestion and density separation, stainless steel mesh filters were placed directly under a stereomicroscope (Olympus SZX) with a camera attachment (Olympus SC180) and images processed using cellSens software (Olympus Corporation; Tokyo, Japan). The entire filter was screened for microplastic particles, with shape (bead, fibre or fragment) and colour of each particle noted. The operator for this analysis had more than one year's experience in intensive quantification and characterisation of microplastic particles in environmental samples.

Microplastic isolation and analysis for wastewater and biosolids

Removal of organic and inorganic interferences essential for FTIR microscope technique

- Overnight digestion of organic matter (Fenton reagent)
- Density separation of plastics from heavier inorganic material (saturated $ZnCl_2$ solution)

FTIR microscopy analysis of microplastics

- Allows visualisation, quantification and characterisation (size/morphology/polymer) of microplastics isolated from samples
- User input only required for cross-checking data generated from software analysis
- Run time for a 15 × 15 mm sample approximately 3-5 hours

Pharmaceuticals analysis of influent

- Link microplastic loads with estimated number of people within WWTP catchments

3 Microplastics in wastewater

3.1 Quantification and temporal trends of microplastics in wastewater

The FTIR microscope methodology gave information relating to the number of microplastics, their polymer identification and morphology of an entire water (or biosolid) sample that had been pre-treated and filtered onto an Anodisc filter. A summary of the microplastic quantification and characterisation over the six sample collection periods at a tertiary (Cronulla) and primary (Malabar) WWTP is given in Table 4. In general, the numbers of microplastics quantified in all water samples was comparable with other studies monitoring microplastics in wastewater, with particle counts ranging from 19–236 microplastics/L in influent samples to 11–436/L (primary) or 1.9–6.6/L (tertiary) in effluent samples (Table 4 and 5). In both WWTPs, PP dominated the polymer type of identified microplastics, with PE and PET the next most commonly detected. Other polymers that were occasionally detected included all of those in the automated spectral correlation as well as a number of others that were not. These included penol 4RO, polyisobutylene, poly(styrene:acrylate ester), polyacetyl, polybutene and zinc stearate. As previously discussed with penol 4RO, it is likely that zinc stearate identified as fragments were particles that contained high loads of zinc stearate, as opposed to being a polymer. Hence, zinc stearate was not included in the analysis. The other polymers were classified as 'Other' in the quantification of microplastics.

It is unclear why PP was the most common polymer type present in wastewater, as the global production (as well as waste generation) of PP is around half that of PE, which includes low, medium and high density PE (Geyer et al., 2017). Plastics are used for a range of purposes and it may be that materials containing PP are more likely to be used for applications where waste residues end up in wastewater. Other wastewater studies show no clear trends in polymer types predominating as in the present study (Table 4) and, aside from its potentially greater use in various applications vulnerable to entering wastewater, PP may be more susceptible to fragmentation (leading to an increase in particles) within sewerage networks. This aspect requires further investigation.

Fragments were the most common morphology in all water samples (Table 4). This contrasts with other surveys of microplastics in WWTPs, that tend to find fibres are more common than fragments (Table 5). It is notable that other studies typically use a visual sorting step, sometimes prior to chemical analysis such as FTIR. An exception for this trend is noted by only two studies that use this approach (Magni et al., 2019; Talvitie et al., 2015). A follow up study by Talvitie et al. (2017) similarly showed fragments were the most common morphology in effluent, although fibres were predominant in influent. The only study that followed a similar approach to the present study, where treated water samples were directly analysed by FTIR microscopy, found fragments comprised ~99% of influent and effluent samples.

Based on the sample collection design, where it was attempted to sample the same packet of water from the influent through to the effluent stream, the removal rates are apparently greater in the tertiary treated effluent (~98%) compared with the primary effluent (0–79%; Table 4). This also reflects what has been previously reported (Table 5) and discussed later in more detail (*see* Microplastic removal – comparison between influent and effluent loads).

Table 4 Overview of microplastics in Cronulla and Malabar WWTP influent and effluent samples, collected six times over a 10 month period (November 2018-September 2019)

WWTP	SAMPLE LOCATION	NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY
Cronulla	Influent (C Inf)	19-236/L	PP (68%) PE (10%) PET (19%)	Fragment (79%) fibre (21%) bead (<1%)
	Post-influent (C P Inf)	36-597/L	PP (87%) PE (7%) PET (5%)	Fragment (90%) fibre (10%) bead (<1%)
	Effluent (C Eff)	1.87-6.6/L	PP (80%) PE (11%) PET (6%) Alkyd (5%)	Fragment (91%) fibre (9%)
Malabar	SWSOOS 1 (S1)	22-205/L	PP (68%) PE (18%) PET (10%)	Fragment (88%) fibre (11%) bead (1%)
	SWSOOS 2 (S2)	51-101/L	PP (66%) PE (15%) PET (12%)	Fragment (90%) fibre (9%) bead (1%)
	Effluent	11-436/L	PP (80%) PE (7%) PET (9%) Silicone (2%)	Fragment (85%) fibre (13%) bead (2%)

Table 5 Summary of literature for wastewater monitoring of microplastics.

NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY	SIZE RANGE	SAMPLING AND ANALYTICAL TECHNIQUE	LOCATION	REFERENCE
Influent: ~30/L ($8x10^8$/day) Effluent (secondary): ~9/L Effluent (tertiary): ~4/L ($2.2x10^7$/day)	PP (23%) PVS (7%) PE (4%) Other (12%)	Fibre (~53%), fragments/films (~47%), beads (~3%) Fibre (~50%), fragments/films (~50%)	60-2000^{o}μm	Grab Visual sorting + FTIR	UK (1 WWTP)	1
Influent: 610/L Effluent (secondary): 14/L	Undefined	Fragments (~70%) Fibres (~30%) Fragments (~70%) Fibres (~30%)	>20 μm	Pump/composite + mesh stack Visual	Finland (1 WWTP)	2
Influent: 380-687/L Effluent (secondary): 0.7-3.5/L	PET (33%) PP PE PS	Fibres (~70%), fragments (~10%), beads (~5%) Fibres (~30%), fragments (~60%)	>20 μm	Pump/composite + mesh stack Visual sorting + FTIR	Finland (1 WWTP)	3
Influent: 86-243/L (up to $20,000x10^6$/day) Effluent (tertiary): 2-28/L (100-600 $x10^6$/day)	Undefined	Fibres (48-81%) Fragments (<50%) Fibres (>75%%) Fragments (<25%)	>60 μm	Composite (24h) + mesh stack Visual sorting + FTIR	USA (3 WWTPs)	4
Influent: 1-14/L Effluent:0.2-1.7/L (secondary)	PE (20-42%), PP (8-10%), PET (10%), PS (10%) PP (43%), PE (27%), PET (10%), PS (10%)	Fibres (18%), fragments (30%), beads (3%) Fibres (30%), fragments (28%), beads (6%)	43-5000^{o}μm	Pump + mesh stack Visual sorting + Raman microscope	China (7 WWTPs)	5
Influent: 15.7±5/L ($4.1x10^9$/day) Effluent (secondary): 0.25±0.04/L ($6.5x10^7$/day)	PE (5%), PET (11%), PP (3%), acrylic (8%), alkyd (29%), PU (9%), PS acrylic (19%), PVA (3%), PVC (1%) PE (4%), PET (28%), PP (12%), acrylic (12%), alkyd (8%), PVA (4%)	Fibres (19%), fragments (67%), beads (3%)	>11 μm	Grab Visual sorting + FTIR	UK (1 WWTP)	6
Influent: 2,223-18,285/L Effluent (secondary): 19-447/L	PE (10%), PP (12%), PE-PP (13%), PET (14%), Acrylate (27%), PS (2%), PU (6%), SAN (6%) PE (7%), PP (39%), PE-PP (7%), PET (8%), Acrylate (12%), PU (6%), PVC (11%)	Fragments (99%), fibres (1%)	10-500^{o}μm	Composite (24h) FTIR microscope	Denmark (10 WWTPs)	7

NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY	SIZE RANGE	SAMPLING AND ANALYTICAL TECHNIQUE	LOCATION	REFERENCE
Influent: 31±7/L Effluent:2.6±1.4/L (primary) Effluent:0.5-0.2/L (secondary)	PP, PS, PET, Nylon, Polyamide PET	Fibres (70%), fragments (27%), beads (1%) Fibres (60%), fragments (20%), beads (8%)	>63 μm	Grab Visual sorting + FTIR	Canada (1 WWTP)	8
Effluent (0.08-9/L <500 μm) Effluent (0.01-5/L fibre >500 μm)	PE (40% <500 μm) PVA (16% <500 μm) PA, PS (8% <500 μm) PET (74% fibre) PP (9% fibre)	Undefined	>10 μm	Pump + filter Visual sorting + FTIR microscope (20% of sample)	Germany (12 WWTPs)	9
Effluent (secondary): ND	Acrylic, PP, PE, polystyrene-isoprene, PAN	Fragment (~50%) Fibre/film (~40%) Bead (<10%)	125-5000^{o}μm	Flow + mesh stack Visual sorting + FTIR	USA (1 WWTP)	10
Influent: 1-14/L Effluent (primary): 1.5 L ($4.6x10^8$/day) Effluent (secondary):0.48/L ($8.2x10^6$/day) Effluent (tertiary):0.28/L ($3.6x10^6$/day)	PP (8-10%), PE (20-42%), PET (35-80%), PS (4-15%), Nylon (28%)	Fibres (~50->90%) Fragments (~ <10-50%)	>25 μm	Pump + mesh stack Visual sorting + FTIR	Australia (3 WWTPs)	11
Influent: ~90-130/L Effluent (secondary): 2.6/L Effluent (tertiary): 6/L	Undefined	Fragments (23-26%), fibres (55-62%), beads (11-16%) Fragments (13-33%), fibres (61-85%)	>20 μm	Grab + mesh stack Visual	USA (2 WWTPs)	12
Influent: 1/L Effluent (secondary): 0.001/L	Undefined	Undefined	>20 μm	Flow + mesh stack Visual + FTIR	USA (8 WWTPs)	13
Effluent (secondary):	Undefined	Fragments (0-77%), fibres 8-100%), beads (0-6%)	>125 μm	Flow + mesh stack Visual	USA (17 WWTPs)	14
Influent: 15±1/L Effluent (secondary): 0.008±0.0009/L	PE, PP, polyester resin	Fragments (29%), fibres (71%) Fragments (51%), fibres (49%)	>300 μm	Pump + mesh stack Visual	Sweden (1 WWTP)	15

NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY	SIZE RANGE	SAMPLING AND ANALYTICAL TECHNIQUE	LOCATION	REFERENCE
Influent: 20-910/L	Undefined	Fragments (0-25%), fibres (75-100%)	>0.7 μm	Grab	Netherlands (6 WWTPs)	16
Effluent (secondary): 9-142/L		Fragments (0-25%), fibres (25-100%), beads (0-50%)		Visual sorting + FTIR		
Influent: 58±12/L	PET (~90%), PE (~5%), PA (~2%)	Fragments (~9%), fibres (~91%)	>250 μm	Grab + mesh stack	Finland (1 WWTP)	17
Effluent (secondary): 1±0.4/L	PET (~50%), PE (~35%), PA (~1%)	Fragments (~50%), fibres (~50%)		Visual sorting + FTIR/Raman microscope		
Influent: 2.5/L	ABS (40%), PE-PP (14%), PE (17%), PP (4%), PET (4%)	Fragments (80%), fibres (20%)	>8 μm	Grab	Italy (1 WWTP)	18
Effluent (tertiary): 0.4/L	ABS (3%), PE (10%), PET (35%), PA (17%), PU (7%), Acrylates (7%)	Fragments (75%), fibres (25%)		Visual sorting + FTIR microscope		

Source: [1](Blair et al., 2019); [2](Talvitie et al., 2015); [3](Talvitie et al., 2017); [4](Conley et al., 2019); [5](Long et al., 2019); [6](Murphy et al., 2016); [7](Simon et al., 2018); [8](Gies et al., 2018); [9](Mintenig et al., 2017); [10](Dyachenko et al., 2017); [11](Ziajahromi et al., 2017); [12](Michielssen et al., 2016); [13](Carr et al., 2016); [14](Mason et al., 2016); [15](Magnusson et al., 2014); [16](Leslie et al., 2017); [17](Lares et al., 2018); [18](Magni et al., 2019)

ND = not done

The flow rates measured over the 24 h composite sample collection period were around ten times higher in Malabar WWTP compared with Cronulla WWTP (Table 6). While the concentrations, or particles per litre, were similar between the primary effluents of the WWTPs, the total amount of microplastics entering (and being released) from Malabar WWTP would therefore have been expected to be 10 times greater than Cronulla WWTP influent. With the median flow rates at Malabar being consistently around 450 ML/day (Table 6), an estimate of microplastics entering the WWTP would be around 450 million times the reported concentrations or between $2.4x10^{10}$ and 6.1×10^{10} (or 24,000 to 61,000 million particles) microplastics per day. With around 70% of flow contribution coming from the S2 influent stream, the majority of microplastics entering Malabar are coming from S2 due to the similar microplastic concentrations measured in S1 and S2 (Table 5 Figure 13).

Based on comparable median flow rates, the total amount of microplastics entering Cronulla WWTP could therefore be estimated to be around 10% of microplastics in Malabar since the microplastic concentrations were similar (Table 6). Microplastic counts in Cronulla WWTP influent were estimated to be more variable than 10% of Malabar WWTP counts, however, and ranged between 53-400% of S1 and 25-250% of S2 microplastic concentrations over the sampling campaign (Figures 10 and 13). This equates to between 0.87×10^{8} and 1.4×10^{10} (or 87 to 36,000 million particles) microplastics per day estimated to enter Cronulla WWTP, while particle count

estimates for Cronulla WWTP effluent ranged from 0.86×10^8 to 3.5×10^8/day.. The highest degree of variability in microplastic numbers occurred in the Malabar effluent with a coefficient of variation (%CV) of 153%, with much lower variability in Malabar S1 (86%) and S2 (27%). A similarly high degree of variability was noted in the two Cronulla effluent samples (188%), while Cronulla influent (78%) was in the same range as Malabar S1. Whether the sources of microplastics in influent samples and the effect of treatment processes on this variability was more important than the uncertainty associated with sample collection and analysis would require further assessment. Additional replication of samples would be highly beneficial to address this in future, while a further discussion on the reported numbers of microplastics in the present study can be found in Section 3.4 Quality assurance and quality controls.

Table 6 Flow rates of Cronulla and Malabar WWTPs for the sample collection dates.
Flow rates are in megalitres (ML) per day, with the mean ±standard deviation and median (in brackets) for values measured every 15 minutes over the 24 h composite collection period

DATE	CRONULLA INFLUENT	CRONULLA EFFLUENT	MALABAR EFFLUENT
	(ML/day)	(ML/day)	(ML/day)
5th-6th November 2018	47.7±14.3 (45.8)	51.9±18 (59.3)	443±83 (493)
5th-6th December 2018	48±12.9 (45.7)	44.9±14 (45)	458±48 (453)
5th-6th February 2019	46.6±13.7 (49.6)	47±14.8 (47)	458±45 (453)
30th April-1st May 2019	45.3±21.6 (50)	46.4±14.7 (47.4)	457±46 (468)
29th-30th July 2019	47.5±16 (47.5)	46.5±16.7 (45.9)	458±60 (434)
25th-26th September 2019	54.6±30.1 (60.9)	52.9±17 (53.6)	477±61 (482)

3.1.1 Cronulla WWTP

The influent samples at Cronulla WWTP had microplastic particle numbers ranging from 19/L (December 2018) to 236/L (September 2019) (Figure 10). These values are similar to the post-influent samples (C P Inf), which had between 36/L (November 2018) and 597/L (September 2019), while the effluent samples had considerably fewer microplastics present (1.87/L in July and6.6/L in September) (Figure 10). As previously discussed, the mixing processes within Cronulla WWTP make it difficult to assess the removal rate by comparing the influent and effluent samples but these comparative numbers are consistent with differences between microplastics numbers in influent and effluent samples reported in other studies (Gies et al., 2018; Murphy et al., 2016; Simon et al., 2018; Talvitie et al., 2017).

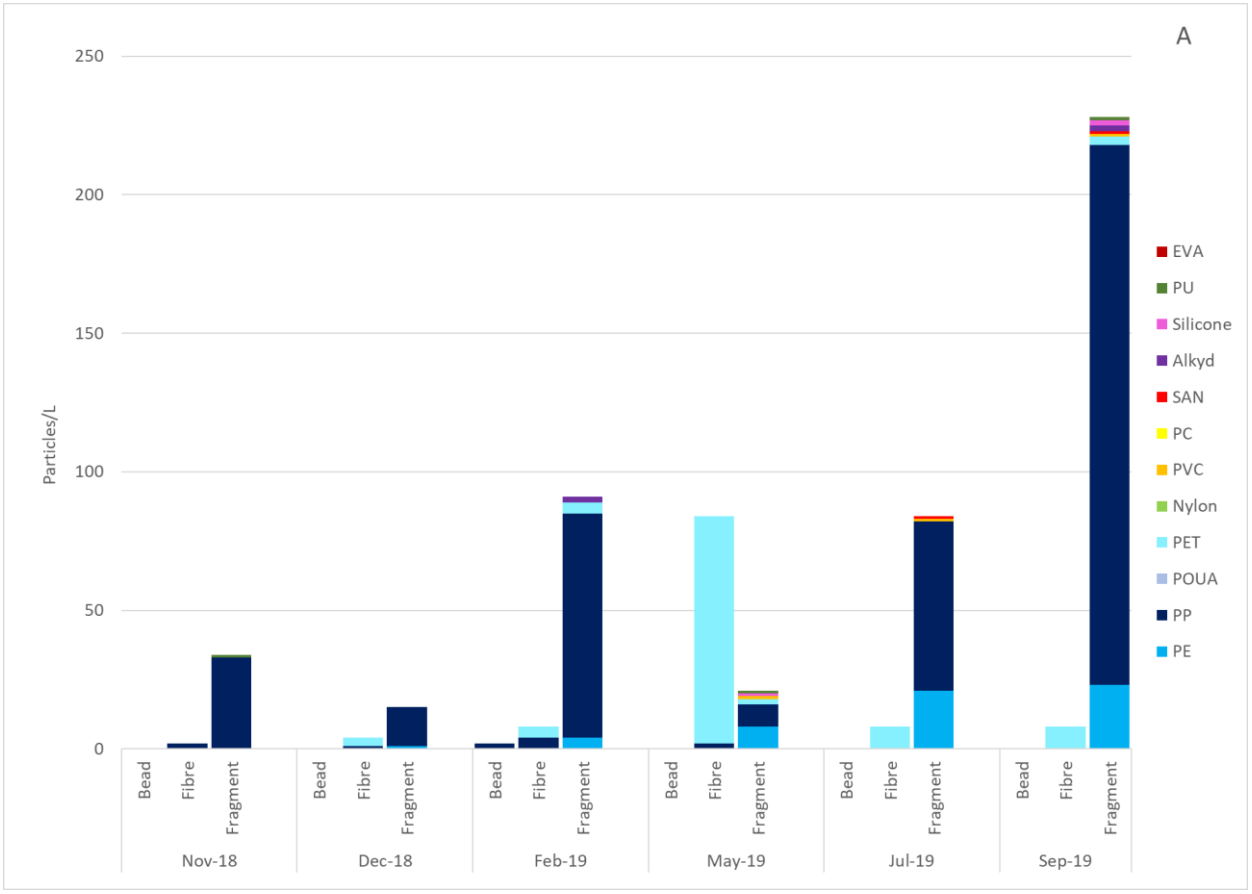
In the majority of samples, PP was the predominant polymer type (68–87%) identified with the exception to this being found for C Inf collected in May 2019 where PET was identified as the predominant polymer (80%; Figure 10). Furthermore, where PP was identified as the predominant

polymer also corresponded with fragments being the predominant polymer morphology (20–99%). Where PET was found to be the predominant polymer corresponded with fibres being the predominant polymer morphology (80%; Figure 10). PET was also found to be the main polymer type associated with fibres in the majority of the other samples collected from C Inf, C P Inf and C Eff (Figure 10).

The majority of quantified PP microplastics were <50 µm at all sampling points of Cronulla WWTP, with >90% of PP microplastics <100 µm (Figure 11). As PET was principally related to fibres, the size distribution of PET tended to be greater than PP (and PE) although the majority of PET particles were still <100 µm (Figure A 17).

Other studies have found microplastic size to be variable, where some studies have found the that the majority of microplastics in wastewater are either <100 µm (Mintenig et al., 2017; Simon et al., 2018; Talvitie et al., 2017) >100 µm (Magni et al., 2019) or a mixture of the two (Leslie et al., 2017; Ziajahromi et al., 2017). A study by Long et al. (2019) found microplastic particles between 63–355 µm were the most common size range in influent and effluent, while Carr et al. (2016) found microplastics <125 µm were the most common size range.

As is further discussed in Section 3.4.1, there was a high degree of contamination noted in the ultrapure water field blanks up until May 2019 that meant there was a low degree of confidence in the all values of microplastics in wastewater. While there was enough wastewater for the influent and primary effluent samples to be re-run, there was insufficient wastewater to quantify the effluent samples and, therefore, only microplastic concentrations were reported for July and September 2019 (Figure 10).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200
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Silicone
Alkyd
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PC
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Nylon
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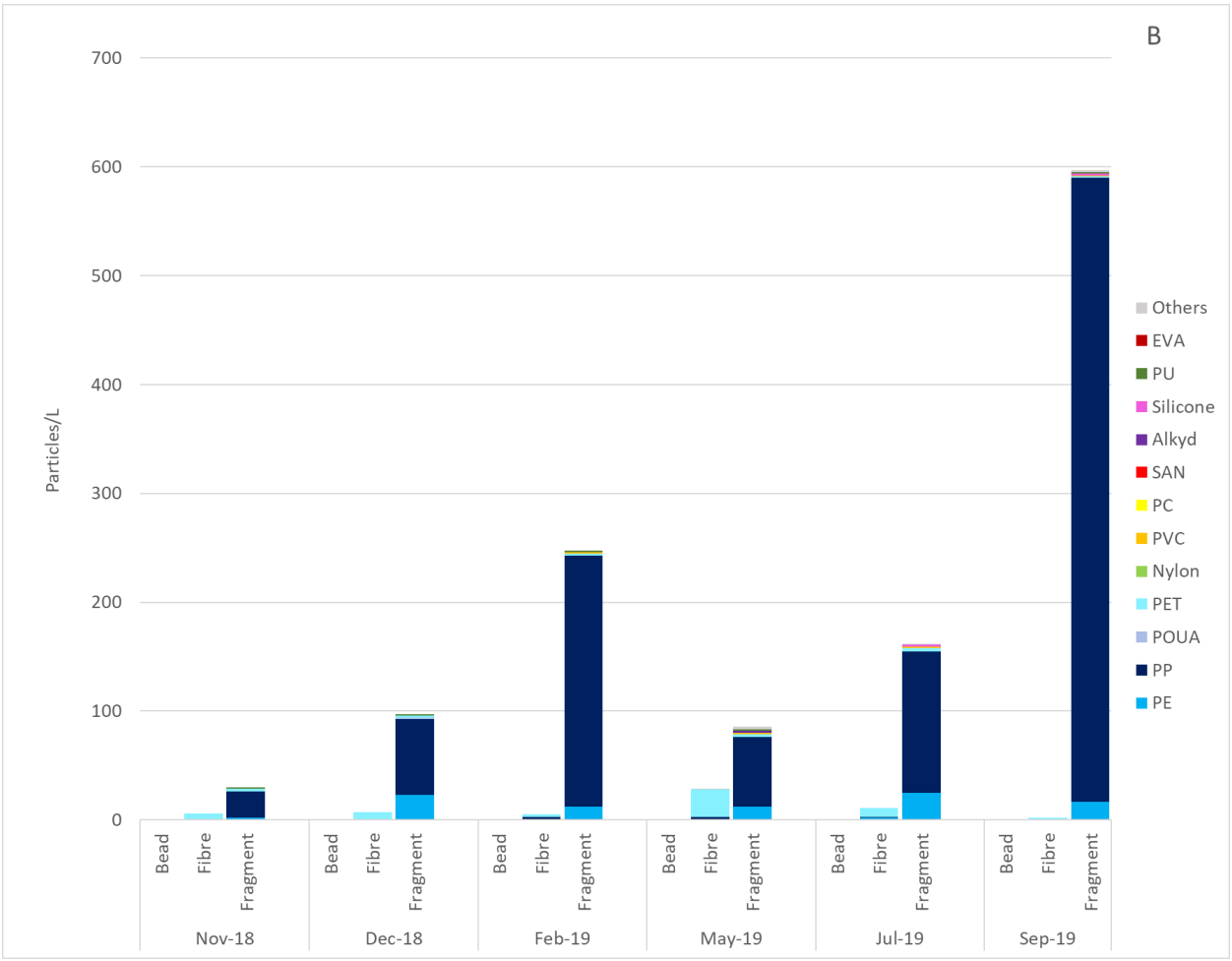
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Others
EVA
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PC
PVC
Nylon
PET
POUA
PP
PE

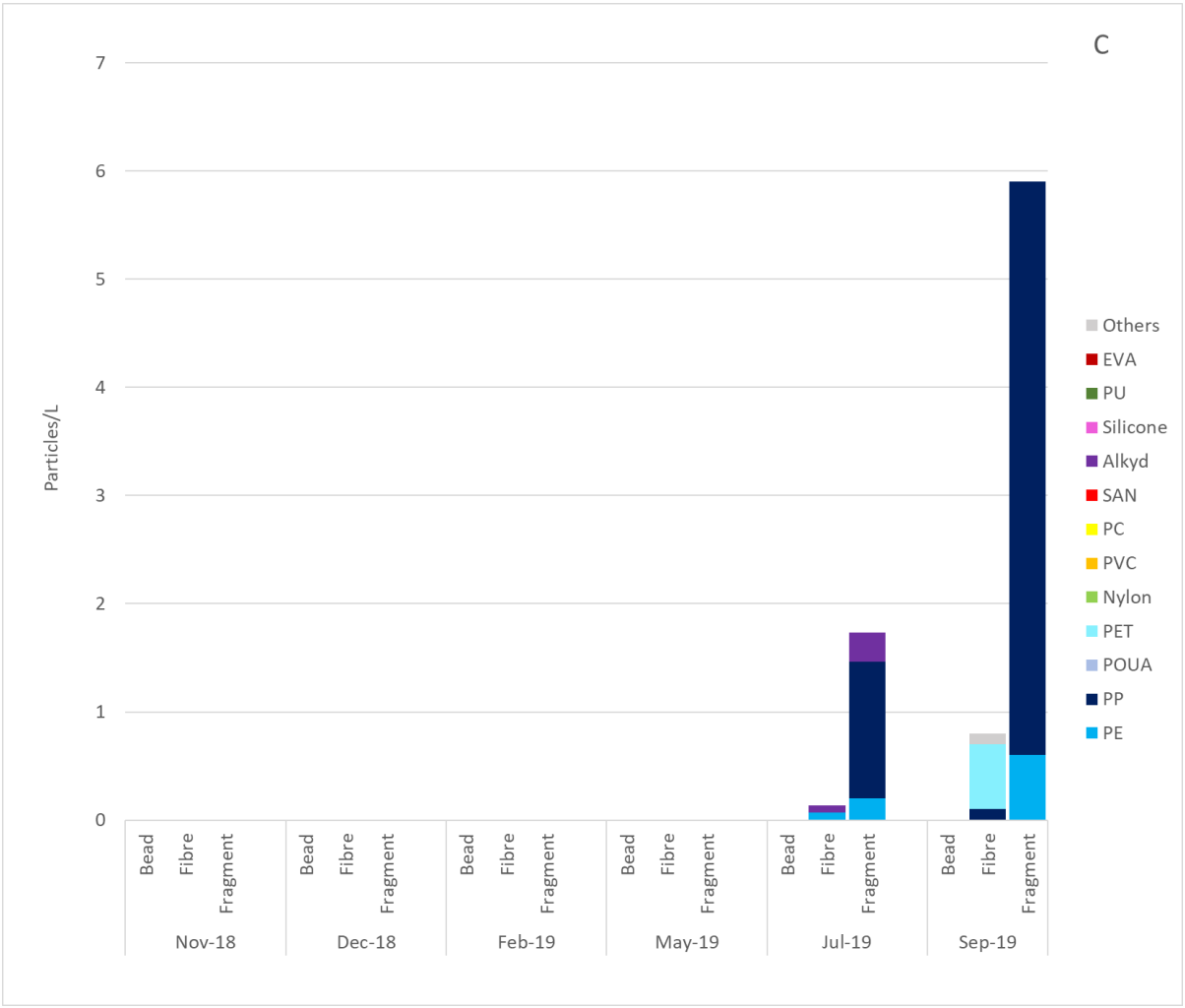


Figure 10 Quantification (number/L) of microplastics in (a) influent, (b) post-influent and (c) effluent samples collected from Cronulla WWTP over the 6 sample collection dates

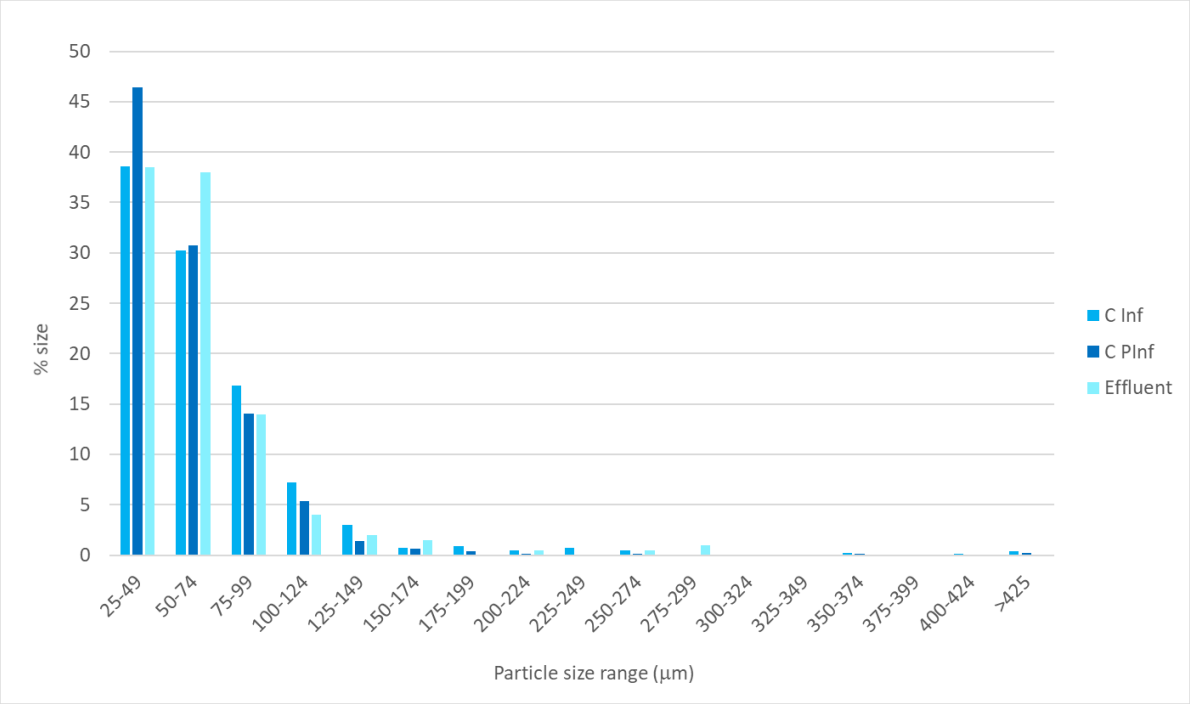


Figure 11 Particle size fraction as a percentage of total PP particles detected in Cronulla WWTP influent, post-influent and effluent, respectively

Comparison of FTIR microscopy with visual counting and characterisation

The Cronulla influent (C Inf) samples over the sampling period were visually inspected for the quantification and characterisation of microplastics as a means of comparison with the FTIR microscopy method. Microfibres were found to be the most common form of microplastics present in the visually counted samples, with microbeads and fragments only occasionally detected (Figure 12). In contrast, quantification of microplastics using the FTIR microscope technique generally led to higher overall numbers of microplastics being counted but, more notably, also a considerably higher proportion of fragments relative to microbeads or fibres (Figure 10). For example, with the Nov 18 samples there was a close agreement in the number of microplastics counted per litre, yet these were characterised as being primarily composed of fragments (*n*=26) by FTIR microscope while visual counting only characterised fibres (*n*=34). There was also a comparable number of microplastics counted in the Dec18 samples between the two methods but for all other samples considerably more microplastics were counted in the samples analysed by FTIR microscopy (Figure 12).

Quantification and characterisation of microplastics in environmental samples, including wastewater, has typically been undertaken using visual assessment of samples (prepared in a similar manner to the present study) and isolating particles for further analysis, usually by IR spectroscopy (Hermsen et al., 2018; Koelmans et al., 2019). Visual identification, isolation and additional instrumental analysis of each particle has the potential to be extremely laborious, especially where particle numbers are >100/sample, which can lead to biases in counting

(Koelmans et al., 2019). This is because long-term analysis using microscopes, especially stereomicroscopes, can enhance variability between operators due to fatigue and its effects on perception of an image (Dieter et al., 2017; Söderberg et al., 1983). The present study has the advantage of using software to scan for all polymers isolated from a sample. The only exception to this was when larger plastics (>2 mm) were identified and removed and for analysis by ATR FTIR. Furthermore, the identification of small (<50 µm) particles by FTIR microscope in the correlation map was often difficult to find a corresponding particle when assessing the visual image. This was especially the case for colourless film, with no clear contrast due to its flat, two-dimensional nature. Using a semi-automated approach to identify all microplastic particles isolated from a sample therefore has the advantage of reducing variability due to operator error and manipulation of samples, as well as identifying particles that may be more difficult to perceive visually. The reason for a greater tendency towards fibre identification through visual analysis, however, may require additional investigation for ongoing development of semi-automated FTIR microscope analysis.

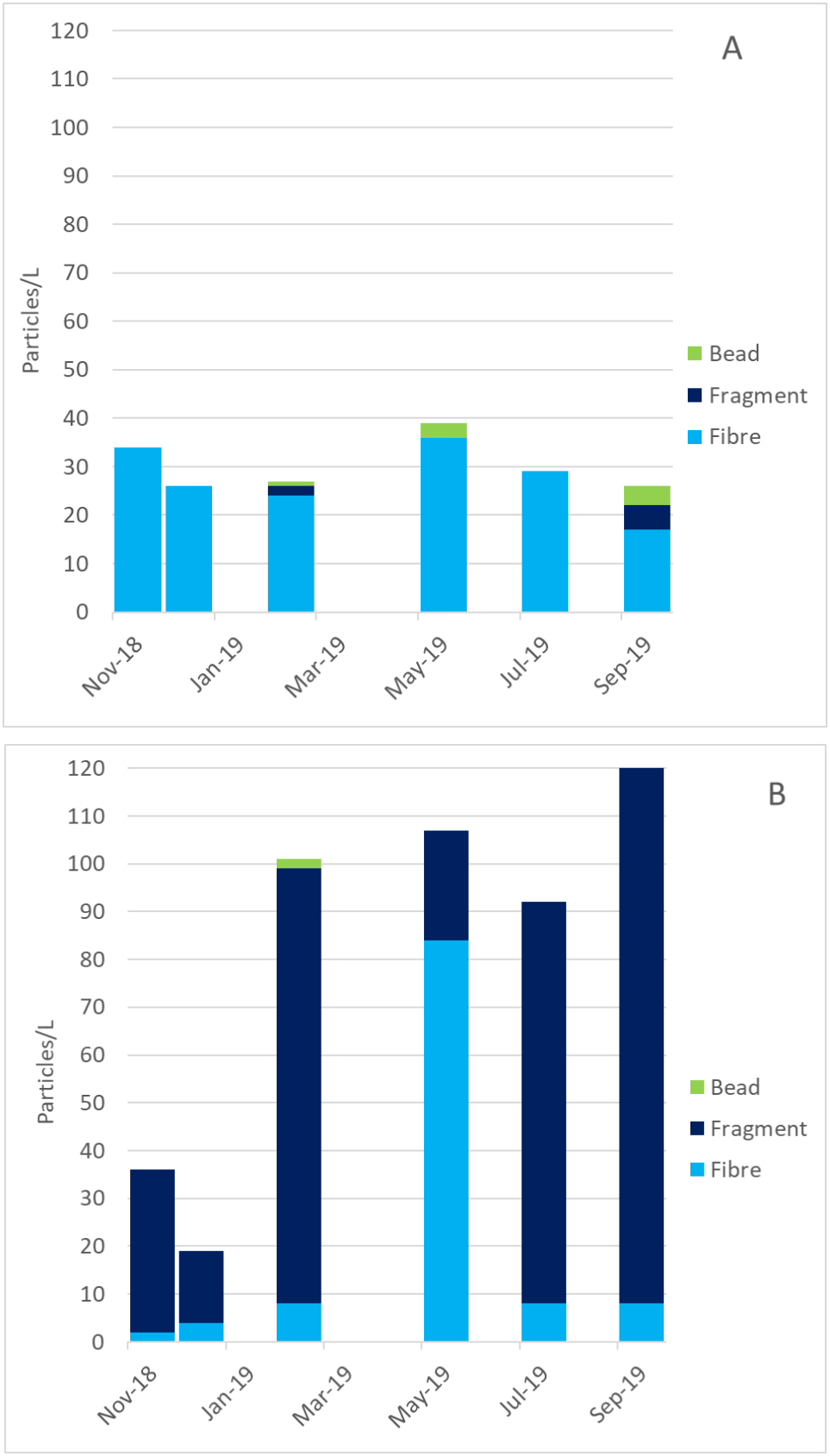
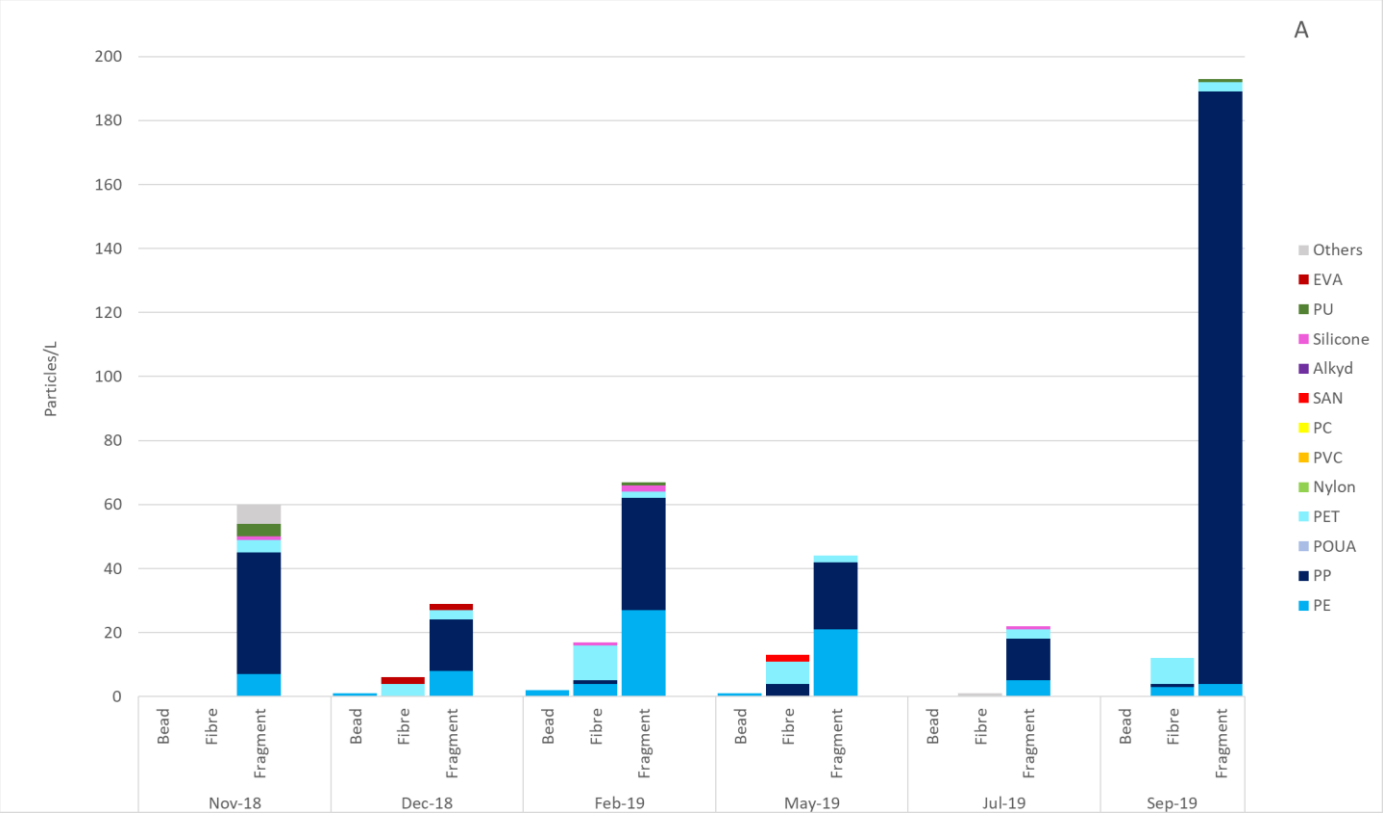


Figure 12 Comparison between microplastics counted using (A) a light microscope and (B) using FTIR microscopy in Cronulla influent samples.
The scale has been changed in (B) for comparative purposes and the total value is 319 microplastics/L for the Sep19 sample.

3.1.2 Malabar WWTP

Microplastic numbers in wastewater collected from Malabar WWTP were similar to those from Cronulla WWTP, although the numbers in Malabar effluent samples were comparatively high (Table 4). This was expected since Malabar WWTP only employs screening and sedimentation (primary) treatment of wastewater, compared with the additional activated sludge, clarification and disinfection (tertiary) treatment at Cronulla WWTP (Figure A 1). There was, however, a reasonable degree of removal of microplastics with the primary treatment when direct comparisons between influent and effluent were made at each sampling period (*see* 3.2 Microplastic removal – comparison between influent and effluent loads).

As with samples collected from Cronulla WWTP, PP predominated as the identified polymer type (63–80%), while fragments were the most common morphology (64–100%) (Figure 13). Similarly, PET was the main polymer associated with the fibre morphology, although a number of other polymers, such as PP, PE, SAN, alkyd resin, silicone, and EVA, were detected as fibres within the S1 and S2 samples (Figure 13).



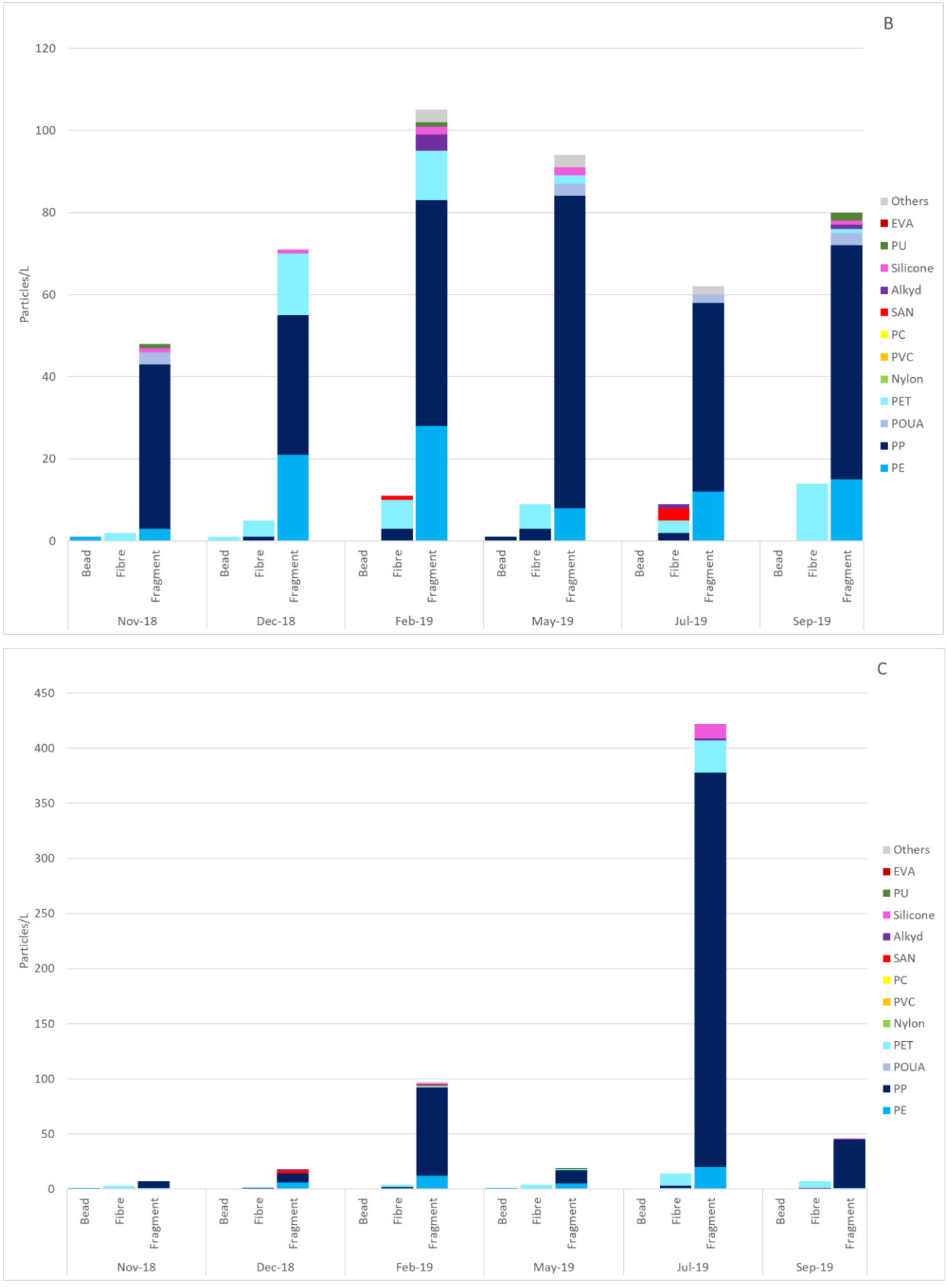


Figure 13 Quantification (number/L) of microplastics in (a) S1 influent, (b) S2-influent and (c) effluent samples collected from Malabar WWTP over the 6 sample collection dates

Quantification and characterisation of microplastics in wastewater

Overview of trends of microplastics in wastewater

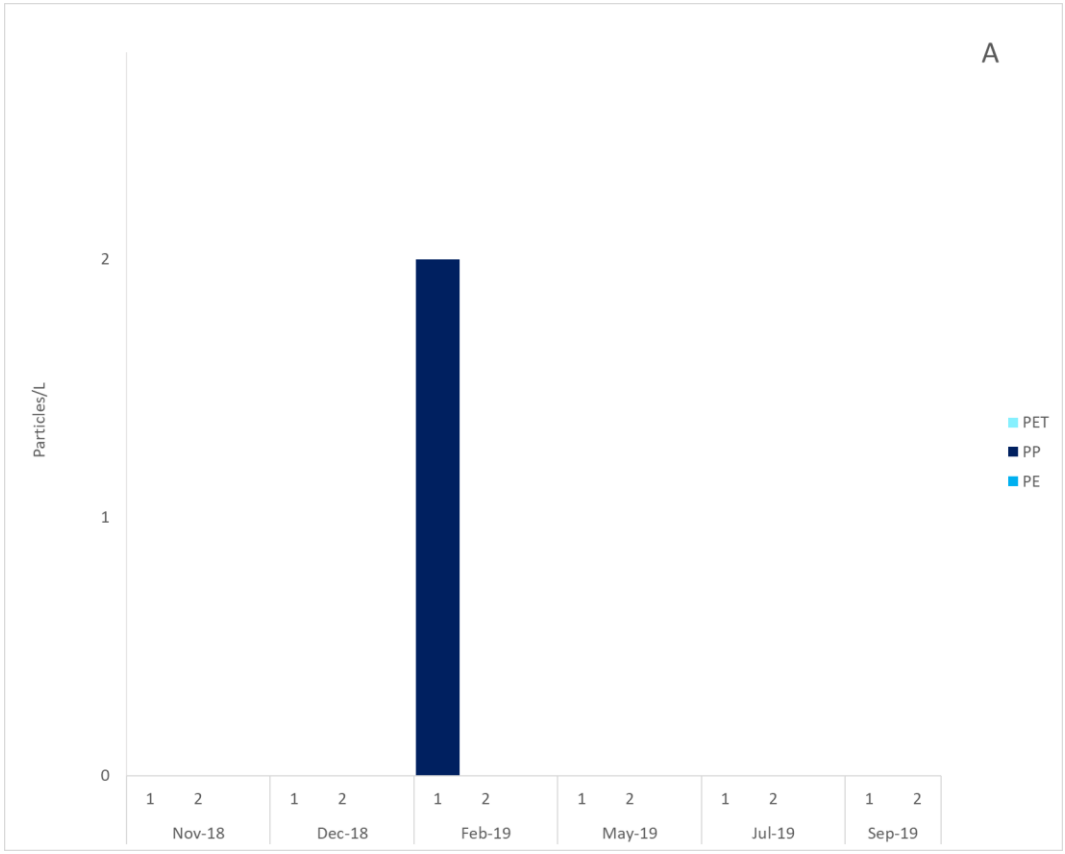
- 19–236 microplastics/L (influent), 11–597 microplastics/L (primary effluent) and 1.9–6.6 microplastics/L (tertiary effluent) during the sampling campaign
- This is equivalent to 8.7×10^{8}–1.4×10^{10} microplastic particles entering Cronulla WWTP and 2.4×10^{10}–6.1×10^{10} microplastic particles entering Malabar WWTP each day
- PP particles predominated the polymer type while microplastics were typically fragments
- PE and PET were also frequently detected at lower numbers; silicone, PC, PU, alkyd, SAN and EVA were all infrequently detected at very low numbers

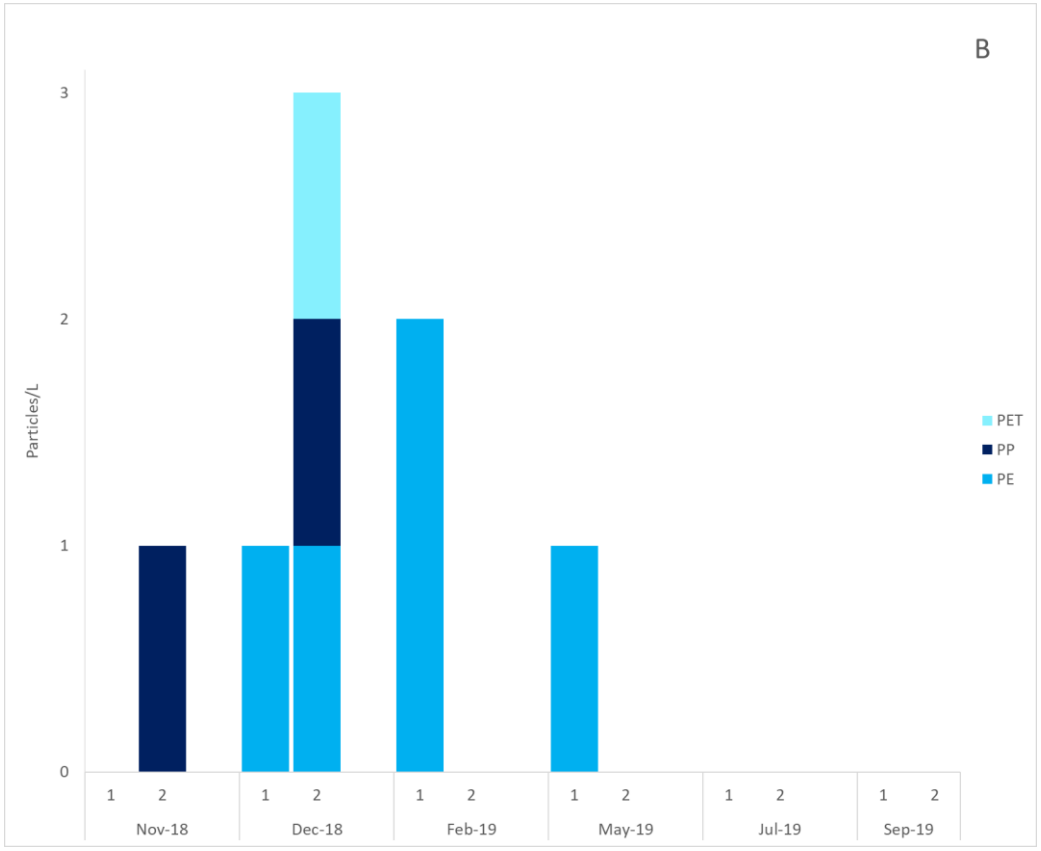
The use of FTIR microscopy compared with visual counting showed differences in numbers and morphology

- Higher numbers of microplastics were counted using FTIR microscopy
- A greater proportion of fragments were detected with FTIR microscopy, while visual counting found a greater proportion of fibres
- Further assessment is required for standardisation between quantification and characterisation methodologies for microplastics in environmental samples

3.1.3 Microbeads

Spherical microbeads were detected in Cronulla WWTP C Inf and C P Inf samples, as well as Malabar WWTP influent (S1 and S2) and effluent samples (Figure 14). Compared with the other microplastic morphologies detected in the wastewater samples, spherical microbeads were very infrequently detected (<1% of morphologies; Figures 10 and 13) and where microbeads were detected, 85% of microbeads were detected in Malabar samples.





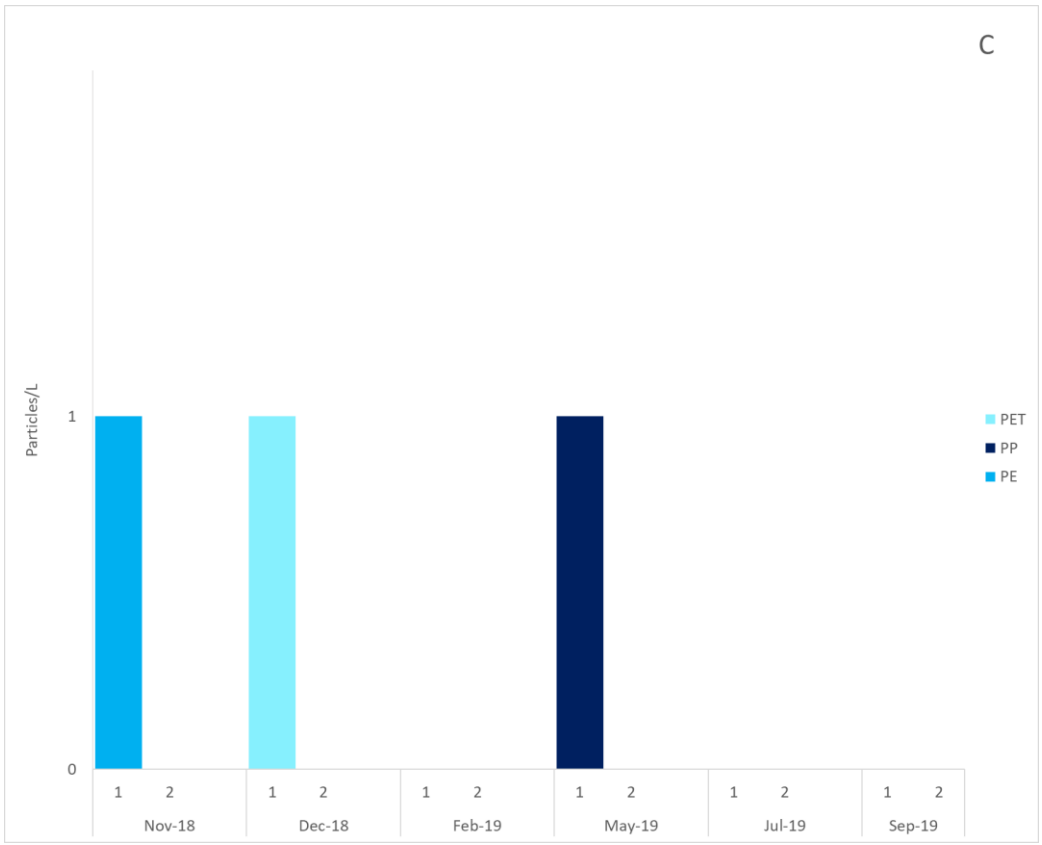


Figure 14 Spherical microbeads quantified in WWTP influent from (a) Cronulla (b) Malabar S1 and (c) Malabar S2 over the 6 sampling periods, in replicate samples. The microbeads identified are also characterised according to the polymer type identified.

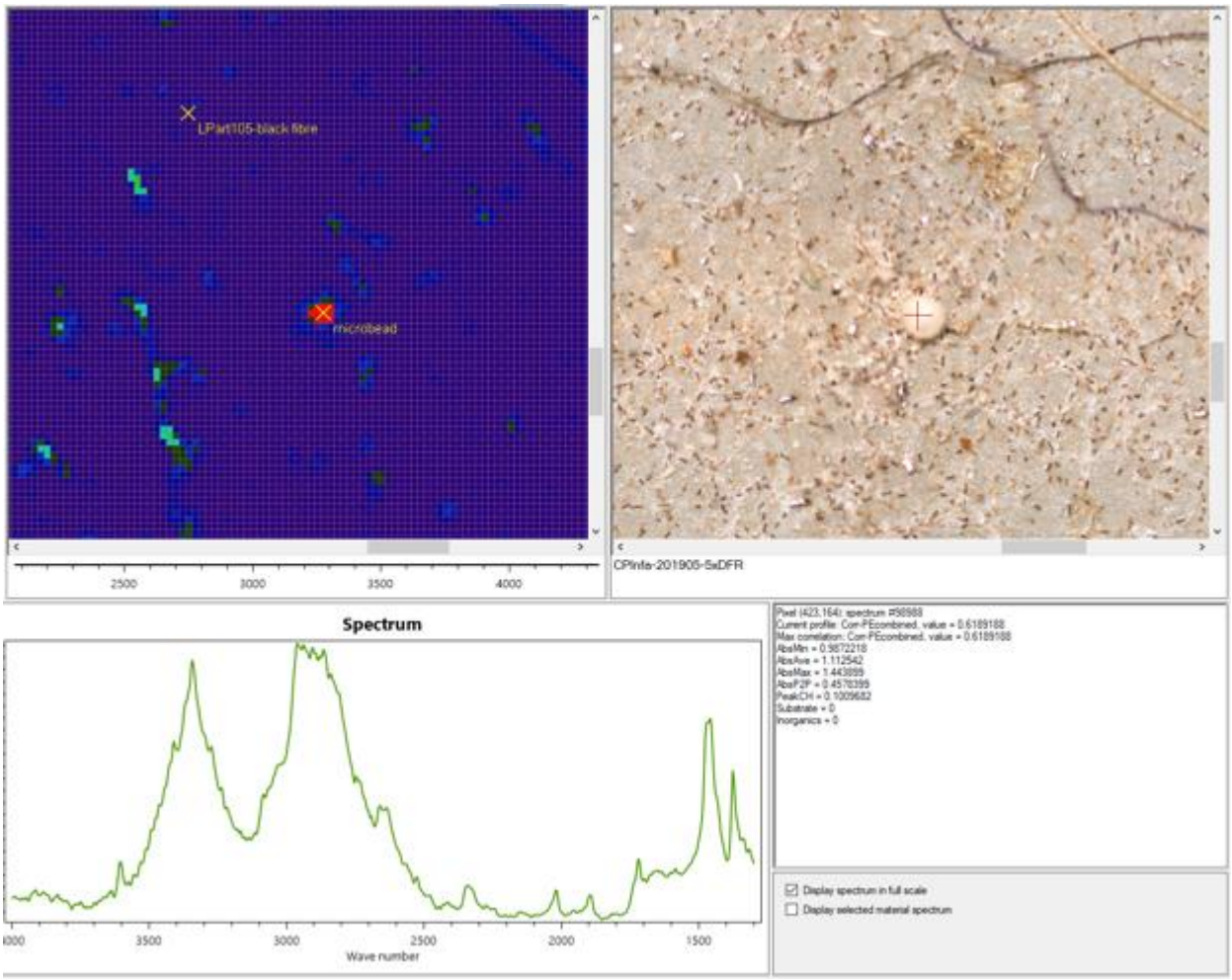


Figure 15 Example of a PE microbead detected in a C P Inf sample during the May 2019 (May19) sample collection period at Cronulla WWTP.
A correlation heat map for PE is in the image to the left of the visual image and the IR spectrum of the detected microbead is given below the images, with the correlation between the particle and PE is given as 0.62. Each square in the correlation heat map is 25 μm^2, which also corresponds to the scale of the visual image.

The majority (55%) of identified microbeads were PE, while 33% were PP and 12% PET (Figure 14). A previous analysis of consumer products sold in Australia suggested that the majority of plastic microbeads are found in personal care products (e.g. face scrubs, make-up, moisturisers) and the majority of the microbeads in these products would be composed of PE (43%), nylon (28%), poly(methyl methacrylate) (PMMA) (10%), PET (5%) and PP (1%) (DoEE, 2018). The high proportion of microbeads in consumer products being composed of PE is consistent with other published literature, which also found randomly sampled consumer products (Fendall and Sewell, 2009; Napper et al., 2015) and those collected in wastewater samples (Murphy et al., 2016; Ziajahromi et al., 2017) are typically PE. A previous study in three Australian WWTPs found spherical beads that were identified as PE as well as nylon, although the relative proportion was not reported (Ziajahromi et al., 2017). For this study microbeads were classified as granular particles and the majority (total of ~0.6–1/L) of PE particles in two of the WWTPs were suspected of having been derived from personal care products. This study was undertaken in October 2015, which occurred prior to the VIA to remove microplastics from personal care products, although it

is difficult to draw comparisons with this study due to differences in methodology. Microbead counts in the present study, when detected, were between 1 and 3/L (1–4/L in visual counting; Figure 12), which are similar with the values found in the study by Ziajahromi et al. (2017). It has been previously estimated that around 6% of the microbeads in consumer products are identifiable as spherical objects (NYSO, 2015), although this is likely to be highly dependent on the product used with some products containing a high proportion of spherical microbeads (Fendall and Sewell, 2009; Habib et al., 2020; Renner Kofi Omare, 2018). Where many secondary (weathered or degraded) microplastics from unknown sources are present in wastewater, they could be mistaken for primary (manufactured), non-spherical microplastics sourced from consumer products and the presence of spherical microbeads therefore offers the best surrogate to assess microbead use.

Based on the flows of wastewater recorded on the day of sampling for Cronulla and Malabar (Table 6) and measured concentrations of spherical microbeads (Figure 14), the number of spherical microbeads quantified in the wastewater is equivalent to 4.5×10^8–9.7×10^8 and 1×10^8 entering Malabar and Cronulla WWTPs, respectively. Estimates of total numbers of microbeads (spherical or not) in facial scrub products are in the range of around 1000 to nearly 20,000 particles/mL, or between 5,000 and 100,000 microbeads per 5 mL daily use (Habib et al., 2020; Napper et al., 2015). At each WWTP, this is equivalent to ~4,500–190,000 (in the Malabar WWTP catchment) and ~1,000–20,000 (Cronulla WWTP catchment) uses of microplastic containing products. These estimates would be considerably (~16 times) greater if the assumption of spherical microbeads making up 6% of microplastics added to consumer products was taken into account. Applying this additional factor is likely to be highly unrealistic, since the upper bounds of the estimated uses per day of microplastic-containing products would be equivalent to or greater than the entire population being serviced by the Cronulla (~2.5×10^5 people) and Malabar (~1.5×10^6 people) WWTPs. These estimates are based on extrapolations of quantifying 1–3 spherical microbeads/L per sampling campaign and the presence of a spherical microbead in the samples could, in theory, be due to the single use of a product containing microplastics.This scenario is also highly unlikely, however, due to the compositing of wastewater samples collecting ~30 L, from respective total flows of approximately 50 and 450 ML, at Cronulla and Malabar WWTPs. That is, a single use of a microbead containing product is unlikely to be picked up when sub-sampling < 0.00006% of total daily flows. The number of uses for microplastic-containing products should therefore exist between the upper and lower bounds of use presented here. A replicate sample from Malabar S1 (Dec18) showed that one PE microbead was found in one sample, while 3 (one each of PE, PP and PET) microbeads /L were found in the other (Figure 14). Also, one sample (Feb19) of C Inf visually counted 1 spherical microbead/L, compared with 2/L detected using FTIR microscopy (Figure 12).

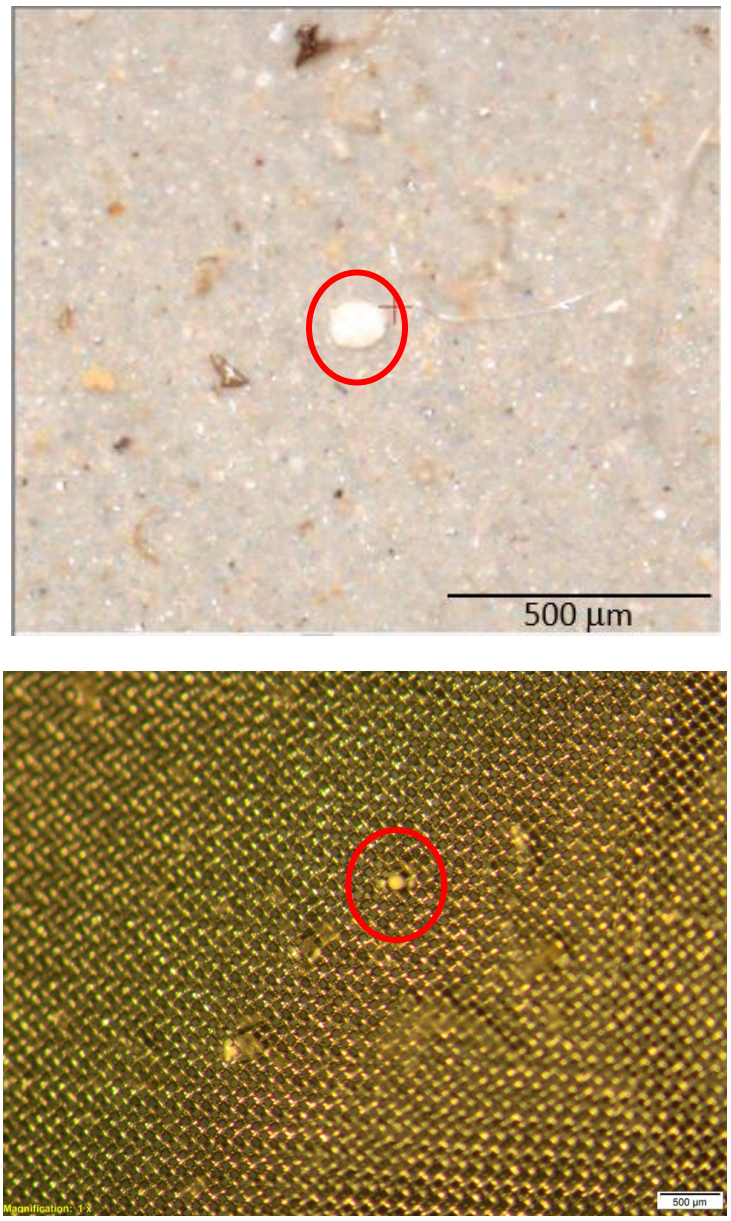


Figure 16 Microbead (circled in red) detected in 1 L of Cronulla influent (CInf) from 24 h sample collection on 5th-6th February 2019 (Feb19) using FTIR microscope (top image) and visual counting with a light microscope (bottom image).
The two methods used separate 1 L samples, prepared identically for analysis. The scale of the micrographs is given in the bottom right corner. The microbead detected using FTIR microscopy was identified as PP, while the identity of the polymer using visual inspection was not done.

All other samples, including replicates, did not contain microbeads, suggesting a lower use of microplastic containing products is currently likely.

The present study commenced ~1 year (as of February 2018) after it was shown ~94% of products (or 4133 of 4400 surveyed products) no longer contained microplastics (or were committed to being removed from the products) due to the VIA (DoEE, 2018; O'Farrell, 2018). Products contributing to spherical microbead loads in wastewater may either be part of the 6% of the products still on the market or that were purchased prior to the substantial phasing out of microplastics in products. This would suggest that as less microplastic-containing products are

available, either through regulation or consumer preference, the presence of microbeads in wastewater will continue to occur less frequently. The latest detection of spherical microbeads in Cronulla WWTP samples using FTIR microscopy was for the Jul19 sample collection for C P Inf (Figure 10) and May19 for all Malabar WWTP samples (Figure 13). The latest (and highest) microbead count in the visual analysis of C Inf samples, however, was for the Sep19 collection and the 3 microbeads counted in May19 samples also did not correspond with any detections in the same sample by FTIR microscopy (Figure 12). These inconsistencies in counts suggests that the lower estimates of use are more likely, such that microbeads are likely to be present at lower concentrations than the estimated 1–4 particles/L where they were detected.

Recoveries of spiked microbeads were 55±25% (*see* 3.4 Quality assurance and quality controls), which would increase the chance of the low quantities of microbeads measured in wastewater being lost during sample preparation. Greater volumes of wastewater may therefore be desirable to give more confidence in the quantification of microbeads, considering the low numbers measured per volume of wastewater. Doing so, however, would need to balance out the extra handling required for sample clean-up, as well as greater quantities of interfering materials in the sample matrix obscuring the FTIR microscope analysis.

The presence of spherical microbeads in wastewater samples demonstrated that products containing microplastics are still being used within the WWTPs catchments. The present study was initiated after the VIA for microbeads was enacted, so that ongoing monitoring would be beneficial to determine whether microbeads are still being released from the diminishing stock of microbead containing products.

Collections of higher volumes over an extended period, when it would be reasonably expected that microbead containing products were exhausted, may prove useful to determine whether a trend of non-detection continues. This should also be done periodically at metropolitan WWTPs around Australia to confirm the trends noted in the Sydney catchment area.

Microbeads

Spherical microbeads were detected infrequently in Cronulla and Malabar WWTP wastewater

- Microbeads (1-3 L) were detected in Cronulla influent (1 sample) and Malabar influent (8 samples)
- Microbeads were PE (55%), PP (33%) and PET (12%)

Microbead containing products likely to still be in use

- Infrequent detection suggests this is not common
- Additional sampling of wastewater in the future could confirm a decline in detections with decreased community use

3.1.4 Pharmaceuticals analysis

The estimated number of contributing people (ENCP) in the Cronulla and Malabar WWTPs showed a high degree of variability for the four pharmaceuticals selected to derive the ENCP value (Table 7 and 8). This includes variability in both the ENCP between the different pharmaceuticals, as well as the ENCP for respective pharmaceuticals. Carbamazepine consistently gave the highest estimate of ENCP at both Malabar and Cronulla WWTPs. It was also the only pharmaceutical quantified in all influent samples, with the other pharmaceuticals quantifiable in only 5 of the 6 sampling periods (Figure A 10). The difference between the maximum ENCP estimates from carbamazepine with the lower estimates of other pharmaceuticals was similar between the WWTPs and varied by a factor of 30–40 (Table 7 and 8).

Carbamazepine and venlafaxine showed the least amount of variability based on the coefficient of variation (%CV), although concentrations measured in wastewater were not consistent during the microplastics sampling for all the pharmaceuticals (Figure A 10). The ENCP values for Cronulla are also considerably lower than the estimates of people in the catchments for Cronulla (~2.5×10^5 people) and Malabar (~1.5×10^6 people) WWTPs, with carbamazepine being closest to this value. The ENCP based on carbamazepine for Malabar WWTP was also approximately 10 times greater than Cronulla WWTP, which is consistent with their relative daily flow rates (Table 6). One assumption related to using pharmaceuticals to determine ENCP is that the prescription data relates to Australia-wide use and that this is reflected in smaller sub-population within a catchment (Lai et al., 2011). If this assumption is not correct then the under- or over-estimation of ENCP could occur.

Carbamazepine (epilepsy, bipolar disorder, trigeminal neuralgia), sotalol (hypertension) and venlafaxine (depression, anxiety) were selected since they are used to treat conditions that require long-term therapy and it was expected that their use patterns would be reasonably stable over time. Trimethoprim (antibiotic) was selected due to relatively high concentrations in the preliminary study but its concentrations varied considerably and were particularly high in Jul19 and Sep19 (Figure A 10). The concentrations of the other three pharmaceuticals were also found to vary to between sampling periods (Figure A 10) and to a much greater extent than the wastewater flows into the respective WWTPs (Table 6). For example, mean carbamazepine concentrations ranged from 3–12±6 ng/L (S1), 12±6–61±10 (S2) and 10±1–33±7 (C Inf) over the microplastics sampling period (Figure A 10). Wastewater collection was undertaken during the week under dry flow conditions, which typically corresponds with a much lower degree of variability in estimated contributing people (Been et al., 2014), while another study deriving ENCP values for five pharmaceuticals over 12 days found %CV for ENCP ranging from 19–31% (Lai et al., 2011). This could either indicate there was a degree of variability in the therapeutic use of these pharmaceuticals in both WWTP catchments or a change in the number of people contributing to the collected wastewater flows (or both).

Furthermore, a detailed analysis of wastewater sampling for the presence of pharmaceuticals has previously shown that variability in the flow of the system and sampling procedure can introduce a substantial degree of variability in overall results and conclusions (Ort et al., 2010). In this study, it

was suggested that continuous (or high frequency) flow-proportional sampling is likely to account for a high degree of uncertainty, especially where there is little information on the system being sampled (Ort et al., 2010). Sydney Water, however, commissioned an analysis on the flow characteristics within both Malabar and Cronulla WWTPs to design a suitable time-proportional sampling program to overcome variability in flow (Table A 1 and A 2). It can also be seen that flow characteristics within the larger Malabar WWTP were consistent throughout all microplastic sampling periods, making these assumptions valid (Table 6; Figure A 4). There was more variability associated with Cronulla WWTP influent, particularly for the Sep19 sample collection (Table 6; Figure A 3), which may have been associated with the 'start-stop' nature of the influent pumping station (Sydney Water, personal communication).

Assuming that the amount of uncertainty within wastewater flows, pharmaceutical consumption and metabolism, wastewater sampling and sample analysis did not cause undue variability from real values, it is evident that the ENCP value did not correspond with microbead or total microplastic loads in wastewater (Figure A 11 – A 16). For the microbeads, the low and intermittent counts in influent would make their association with pharmaceutical loads difficult, due to the inherent variability associated with their analysis. In terms of the total microplastics, however, the greater numbers would give a greater degree of confidence in linking their quantities with pharmaceutical loads. The apparent lack of association between microplastic and pharmaceutical loads could therefore suggest that inputs of these microplastics from domestic sources may have been less important than industrial sources, where a pharmaceutical signal would be expected to be negligible. A previous assessment also found no relationship between the population served by seven WWTPs and the microplastic loads of biosolids collected from the WWTPs (Mahon et al., 2017). The authors concluded that sources such as industrial, stormwater and landfill, may explain this discrepancy.

Based on the observed variability associated with pharmaceuticals analysis, however, it would be desirable to consider the use of additional human biomarkers (such as ammonia, creatinine etc.) collected at a high degree of temporal resolution (e.g. using an *in situ* data logger) (Been et al., 2014; Chiaia et al., 2008; Choi et al., 2018; Lai et al., 2011) to confirm this observation.

Table 7 Summary of estimated number of contributing people in wastewater influent at Cronulla WWTP based on the measured concentrations of four pharmaceuticals (carbamazepine, sotalol, trimethoprim and venlafaxine), their respective pharmacokinetics and wastewater flows for all sampling periods (*n*=6).

PHARMACEUTICAL	MEAN ±SD	MEDIAN	RANGE	%CV
	(x1000 people)	(x1000 people)	(x1000 people)	
Carbamazepine	67±36	59	28.9-156	53
Sotalol	11±8	8	3.6-26	74
Trimethoprim	30±20	24	7.9-64	67
Venlafaxine	27±13	25	10-52	48

Table 8 Summary of estimated number of contributing people in wastewater influent at Malabar WWTP based on the measured concentrations of four pharmaceuticals (carbamazepine, sotalol, trimethoprim and venlafaxine), their respective pharmacokinetics and wastewater flows for all sampling periods (*n*=6).
The value for Malabar represents a combination of S1 and S2 wastewater values.

PHARMACEUTICAL	MEAN ±SD	MEDIAN	RANGE	%CV
	(x1000 people)	(x1000 people)	(x1000 people)	
Carbamazepine	689±458	506	157-1660	67
Sotalol	345±246	275	59-797	71
Trimethoprim	144±143	753	75-424	99
Venlafaxine	667±383	471	267-1342	57

Pharmaceuticals analysis

ENCP did not coincide with microbead or total microplastic loads in wastewater

- Industrial inputs of microplastics may be more important than domestic in both WWTP catchments
- Additional biomarkers in wastewater should be used to confirm this

3.2 Microplastic removal – comparison between influent and effluent loads

The two WWTPs monitored in this study had primary (Malabar) and tertiary (Cronulla) levels of wastewater treatment. The treatment levels of WWTPs are a means to remove solids (primary), reduce nutrient (secondary) and pathogen loads (tertiary) but the treatment processes can also remove other wastewater contaminants, including microplastics. Primary treatment, which uses screens to remove large particulates and skimming and settling to remove smaller particulates, can be effective in reducing microplastic loads. For example, solids removal through primary clarification can remove the majority of microplastics prior to secondary treatment processes (Gies et al., 2018; Murphy et al., 2016; Talvitie et al., 2017). This was generally true for most of the sampling dates, where up to 79% removal of microplastics occurred prior to the effluent stream, although there was no removal measured in the Feb19 samples and a substantial increase in the Jul19 samples (Figure 13). The increase in Malabar effluent load was due to a considerable increase in the number of PP fragments counted and it could also be seen that the proportion of PP particles in the fraction below 50 µm was even greater in the Jul19 sample, relative to influent samples, compared with the overall trends of the other sample dates (Figure 17). This may suggest that during this particular sampling period there was additional mechanical degradation of PP particles during treatment, leading to a higher count of smaller particles.

The similarity, or often higher number, of microplastics in the post-influent (C P Inf) relative to influent (C Inf) samples at Cronulla WWTP, however, indicates that there was very little removal due to the primary treatment process at Cronulla (Figure 10). The treatment process at Cronulla WWTP varies from Malabar, where activated sludge and aerobic zone solutions are continually recycled to a point after the C P Inf collection point, means that it is more difficult to ascertain whether numbers quantified can be related to estimates of removal (Figure A 1). Bearing this in mind, comparisons between the microplastic particles in influent, primary effluent and final effluent at Cronulla WWTP suggest that high removal rates (~98%) are likely to occur within the WWTP, following secondary treatment of the wastewater. This is consistent with other studies that have determined where removal of microplastics within a WWTP is likely to occur (Carr et al., 2016; Gies et al., 2018; Michielssen et al., 2016; Murphy et al., 2016; Talvitie et al., 2017; Ziajahromi et al., 2017). For example, a once off microplastic sampling in three Australian WWTPs that employed primary, secondary and tertiary treatment processes suggested that >90% of microplastics were removed through advanced treatment, although their study did not specifically attempt to collect the same packet of water from influent to effluent.

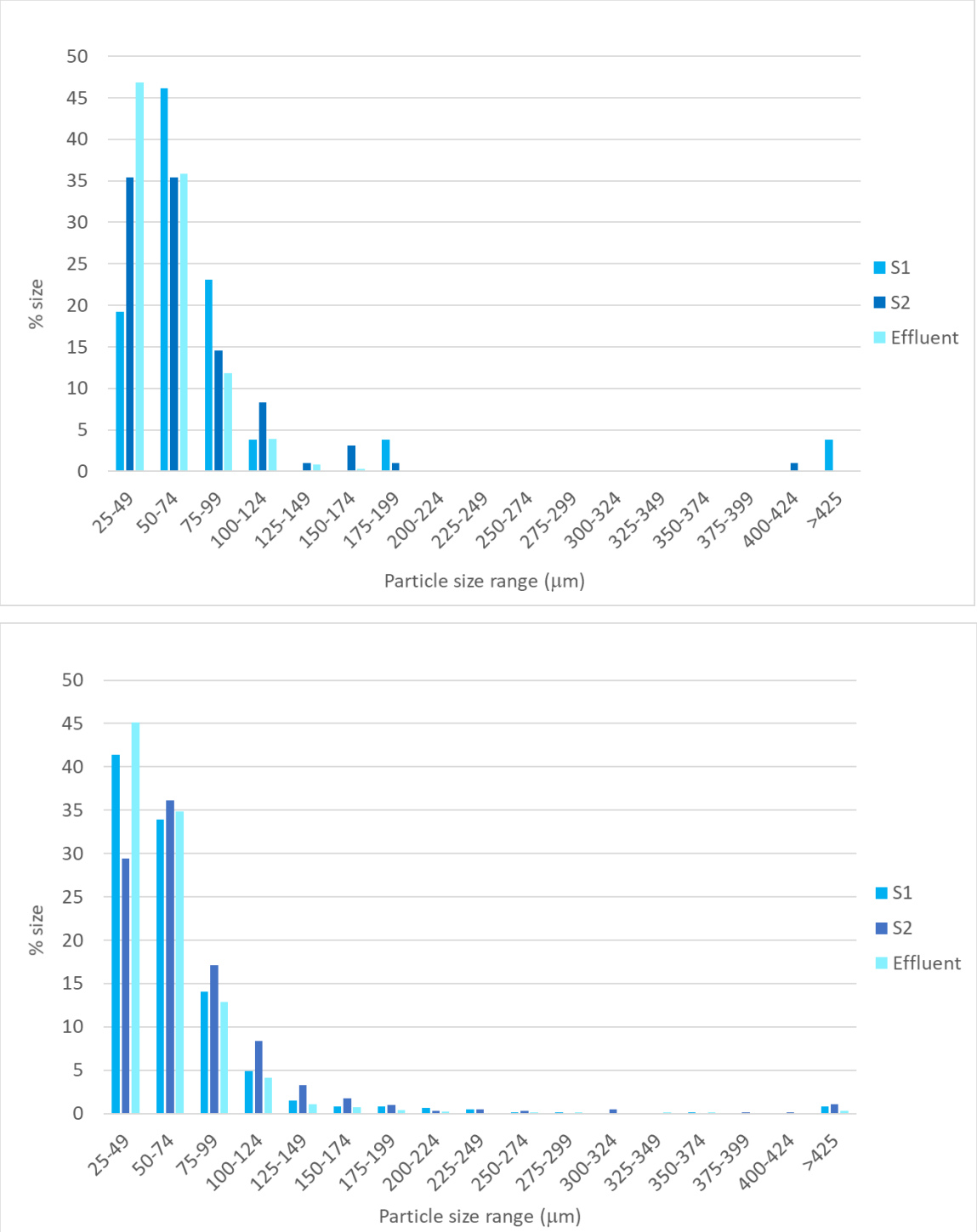


Figure 17 Particle size fraction as a percentage of total PP particles detected in Malabar WWTP influent (S1 and S2) and effluent, respectively, collected on Jun19 (top) and for all samples (bottom)

Similarly high removal rates, however, have also been suggested in other studies from the USA, UK and Europe (Blair et al., 2019; Carr et al., 2016; Michielssen et al., 2016; Murphy et al., 2016; Wisniowska et al., 2018). Although wastewater treatment can drastically reduce the numbers of microplastics being discharged to water bodies, the volumes of effluent discharged each day into receiving waters is still substantial as was the case in the present study. Furthermore, due to the

resistance of PP, PE and PET to biological degradation, the main removal process of microplastics within a WWTP will be through association with particulate matter, which is accumulated in sludges (Carr et al., 2016; Lares et al., 2018; Li et al., 2018; Mahon et al., 2017; Talvitie et al., 2017). With sludges often stabilised to form biosolids that are used in terrestrial reuse applications, microplastics can potentially be transferred to terrestrial systems.

Removal of microplastics from wastewater

Microplastics were removed from the wastewater stream during treatment

- Malabar WWTP (primary treatment) had between 0 and 79% removal
- Cronulla WWTP (tertiary treatment) had >98% removal but no overall removal following primary treatment (C Inf to C P Inf)
- Fragmentation of particles may increase apparent number in effluent

Removal of microplastics is principally through association with sludges

3.3 Microplastics in biosolids

Numbers of microplastics per 1 g of biosolid ranged from 45/g biosolid (Rouse Hill WWTP) up to 323/g biosolid (Winmalee WWTP) (Figure 18). As with the wastewater influent samples at Cronulla and Malabar (Figure 10 and 13), PP was the most commonly detected polymer in all biosolids, followed by PE and PET (Figure 18). PP, PE and PET were commonly identified in biosolids analysed in other studies, as well as a number of other polymers, including alkyd, PS and ABS. The latter polymers made up a substantial proportion of the identified polymers in biosolids in these studies (Magni et al., 2019; Murphy et al., 2016) although, as with wastewater sampling, polymer identification relied on manual manipulation that may have overlooked polymers that were difficult to visually identify. For the WWTPs in the present study, the majority of microplastics were in the smallest (25-50 µm) quantifiable size range and the majority of these were PP (Figure A 19). Although PET followed this trend, it also had a relatively high proportion of particles at higher size distributions due to its fibre morphology.

Microplastic fragments were the most common morphology in Cronulla and Malabar WWTP biosolids, which is consistent with the wastewater samples collected from Cronulla and Malabar WWTPs. This was also the case with the biosolids collected from the other WWTPs with fragments comprising between 90 and 100% of representative morphologies (Figure 18). Other studies generally find fibres to be the predominant morphology in biosolids (Gies et al., 2018; Lares et al., 2018; Leslie et al., 2017; Magnusson et al., 2014) although Magni et al. (2019) found fragments and film to be the most common microplastic morphology, relative to fibres. As previously discussed, the predominance of fibres may relate to analytical methodology employed by the various studies.

Attempts to estimate mass balance flows of microplastics in WWTPs suggest that removal of microplastics through association with sludge is the predominant means of removing microplastics from wastewater. For example, microplastics removal rates of >98% have been observed within WWTPs when comparing influent and effluent loads, with the majority of this attributed to pre-treatment of water through settling or filtering of solids and through activated sludge, where a substantial amount of solids are formed through microbial activity (Murphy et al., 2016; Simon et al., 2018; Talvitie et al., 2017). This aligns with the estimated removal rates of up to 79% at Malabar WWTP and >98% at Cronulla WWTP.

The estimated numbers of microplastics in the biosolids collected from the seven WWTPs (4.5×10^4–3.23×10^5 microplastics/kg) is also of the same magnitude as other studies that estimated numbers of microplastics in wastewater solids, which ranged from hundreds up to 2×10^5 microplastics/kg solid (Table 10). Stabilised biosolids are high in organic matter, nutrients and contain many essential elements and have an important role as a soil amendment in agriculture and land remediation. Beneficial reuse of biosolids is extensively practised in Australia, with 91% of the approximately 370,000 tonnes (dry weight) produced per year being used for this purpose in 2019 (ANZBP, 2020). Of this amount diverted to beneficial reuse, nearly 70% is applied to land used for agricultural purposes. Around one quarter of Australian biosolids production occurs in NSW and ACT, which represents ~90,000 tonnes. Using the above estimates of microplastic

content of dry weight biosolids samples collected from the seven WWTPs, the 4.5×10^4–3.23×10^5 microplastics/kg is equivalent to 4.5×10^7–3.23×10^8 microplastics/tonne. Simon et al. (2018) estimated the average mass of microplastics in wastewater, based on their respective morphology and polymer densities, to be 57 ng/microplastic particle (median 35 ng/particle) in raw wastewater. Applying the worst-case mean value of 57 ng/particle, these numbers of microplastics within the biosolids equates to 0.003-0.02 kg microplastics/tonne biosolid or 230–1,660 kg microplastics in the 90,000 t biosolids produced in NSW (or 950–6,800 kg microplastics in the 370,000 tonnes biosolids produced Australia-wide). This is compared with a previous estimate of 9–63 kg microplastics/tonne biosolid, which equates to 2,800–19,000 tonne microplastics in Australian biosolids each year (Ng et al., 2018). Another assessment of biosolid applications in Europe and North America estimated the amount of microplastics applied to land through biosolid applications would be between 44,000 and 430,000 tonnes of microplastics per year, based on biosolids application rates of up to 70 tonne/ha (Nizzetto et al., 2016). Both of these estimates were based on the potential inputs to wastewaters, rather than through direct measurement of biosolids, which may account for the significant discrepancies of these studies with the present study.

Application rates of biosolids are dependent on the variables such as contaminant and nitrogen concentrations of the biosolids (NSW EPA, 1997) but a conservative approach would be for application rates of 20 dry t/ha every 5 years (Darvodelsky and Hopewell, 2017). Furthermore, this application rate would coincide with incorporation depths of 100 mm to soils with an average bulk density of 1400 kg/m^3 (Darvodelsky and Hopewell, 2017). In this case, each hectare would have 20 dry tonnes of biosolids added to 1,000 m^3 soil, which has a mass of 1.4×10^6 kg or 1400 tonnes soil. If the biosolids contain between 4.5×10^7–3.23×10^8 microplastics/tonne, then between 9×10^8 and 6.5×10^9 microplastics could be applied to the top 100 mm of soil. Using the above assumption relating to the mass of microplastic particles (Simon et al., 2018), this equates to around 0.5–3.7 kg microplastic/ha soil, which may be increased by this amount every five years with repeat applications, assuming this microplastic remains in the top 100 mm of soil. Some extent of dispersal within the soil environment is likely, however, based on the activity of soil invertebrates (Machado et al., 2018).

There have been relatively few terrestrial toxicity studies relating to microplastics. Of the studies that have been undertaken, the amount of microplastics used in the assessments were either at the upper end of ranges detected in the environment or considerably higher (Cao et al., 2017; Huerta Lwanga et al., 2016; Judy et al., 2019; Rodriguez-Seijo et al., 2017; Shang et al., 2020; Zhu et al., 2018). Where effects were noted in these studies, they occurred at concentrations of microplastics much greater than were estimated to be present in biosolids prior to application to agricultural soils. A study by Rodriguez-Seijo et al. (2017) found inflammation of the gut of an earthworm, *Eisenia fetida*, at PE concentrations of 0.0125% w/w (equivalent to 0.125 kg/tonne) in dry soil, which is slightly higher than the worst-case estimate of the biosolids collected in the present study (0.02 kg/tonne). This did not lead to any effects, however, on survival, weight or reproductive ability of the worms (Rodriguez-Seijo et al., 2017). Other studies on earthworms found either no effect or effects at much higher concentrations (>1% w/w) (Cao et al., 2017; Huerta Lwanga et al., 2016; Judy et al., 2019; Lahive et al., 2019). Exposures of greater than

0.1% w/w microplastics were necessary to elicit an effect in other soil invertebrates and microorganisms (Judy et al., 2019; Shang et al., 2020; Zhu et al., 2018). Aside from considerations of terrestrial toxicity, there is limited evidence suggesting the presence of microplastics can also affect soil structure (Lehman et al., 2019; Zhang and Zhang 2020). As with the toxicity assays, these studies on soil structure effects were also done at comparatively high concentrations (~0.1% w/w).

Where repeat applications of biosolids containing microplastics occurs, the accumulation of microplastics may lead to soil concentrations approaching those where effects on soil organisms or structure can occur, although using the 5 yearly reapplication assumption this could take a number of decades. The presence of microplastics in biosolids should be further assessed to ensure that adverse levels do not occur in terrestrial systems, especially over the longer term where microplastics may accumulate from ongoing applications of biosolids. This needs to be carefully balanced, however, against the current practice of beneficial reuse of biosolids. Current reuse practices are considered the most sustainable option for biosolids, in terms of economics, reduction in carbon emissions, reduced reliance on non-renewable soil amendments (e.g. synthetic fertilisers) and the environmental benefits that are associated with this (DSEWPaC, 2012; LeBlanc et al., 2009).

The above assessment is highly dependent on the accurate quantification of microplastics in environmental samples and this can only be assessed through the use of appropriate quality assurance (QA) and quality control (QC). The following section highlights how information relating to concentrations of microplastics should be considered alongside negative and positive controls for samples.

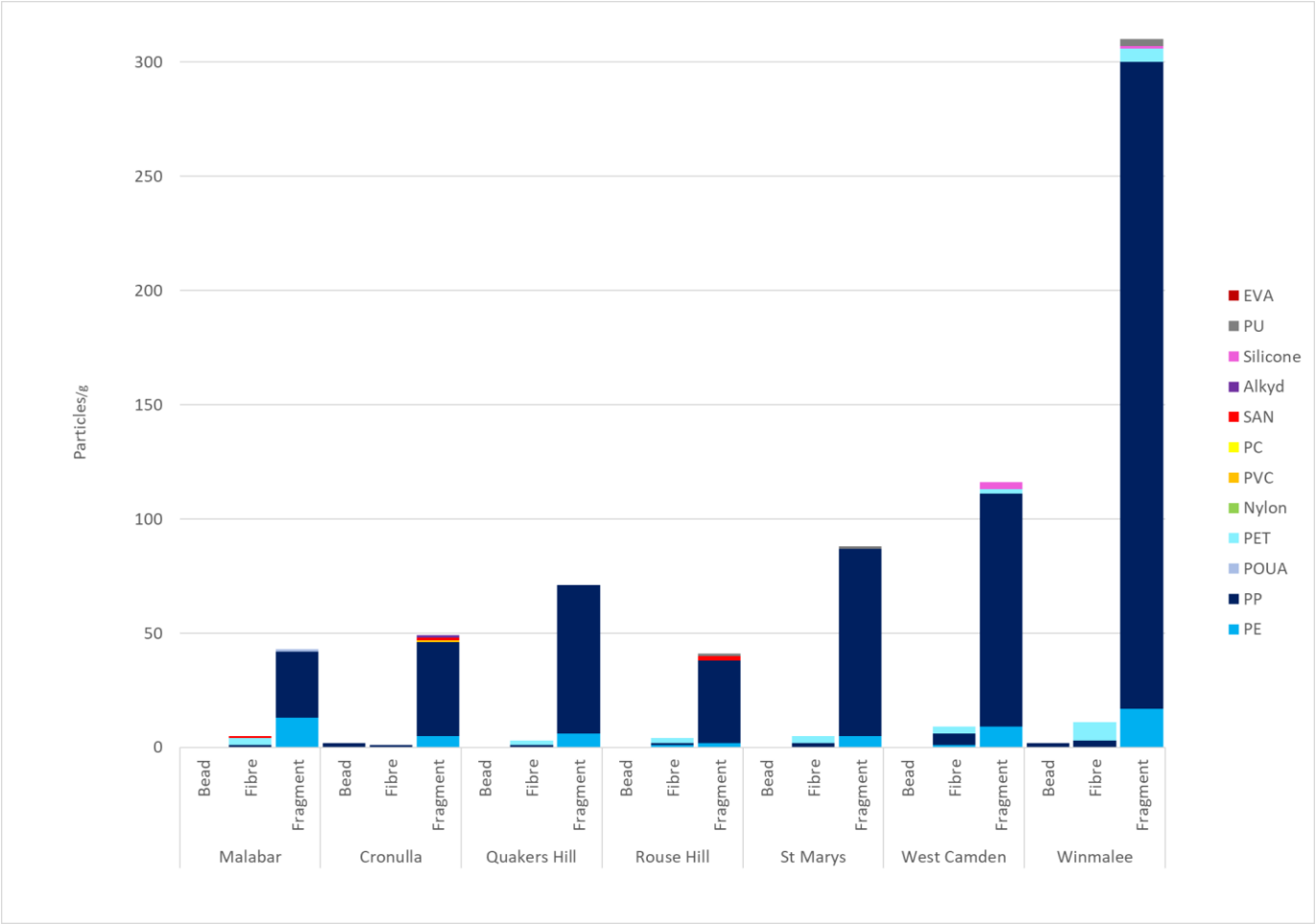


Figure 18 Quantification of microbeads in biosolid samples from the seven WWTPs collected in September 2019.

Table 9 Overview of numbers, polymer type and morphology of microplastics in biosolids collected from seven WWTPs in the Sydney region during September 2019. The numbers of microplastics per kg biosolid are estimated based on the number quantified in 1 g of biosolid from each WWTP.

WWTP	NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY
Malabar	$4.8x10^4$/kg	PP (63%) PE (27%) PET (6%)	Fibres (10%) Fragments (90%)
Cronulla	$5.2x10^4$/kg	PP (85%) PE (10%)	Bead (4%) Fibres (2%) Fragments (94%)
Quakers Hill	$7.4x10^4$/kg	PP (89%) PE (8%) PET (3%)	Fibres (4%) Fragments (96%)
Rouse Hill	$4.5x10^4$/kg	PP (82%) PE (7%) PET (4%)	Fibres (9%) Fragments (91%)
St Marys	$8.8x10^4$/kg	PP (93%) PE (6%)	Fragments (100%)
Winmalee	$3.23x10^5$/kg	PP (89%) PE (5%) PET (4%)	Bead (1%) Fibres (3%) Fragments (96%)
West Camden	$1.25x10^5$/kg	PP (86%) PE (8%) PET (4%)	Fibres (7%) Fragments (93%)

Table 10 Summary of other studies that have quantified and characterised microplastics in biosolids and sludges collected from WWTPs

NUMBER OF PARTICLES	MICROPLASTIC POLYMER	MORPHOLOGY	SAMPLING AND ANALYTICAL TECHNIQUE	LOCATION	REFERENCE
$1x10^3$/kg	Undefined	Undefined	Grab Visual + FTIR	USA (8 WWTPs)	1
720±112/kg	Undefined	Fibres (72%), fragments (20%)	Grab Visual	Sweden (1 WWTP)	2
370-950/kg	Undefined	Fibres (1-2%), beads (0.1%)	Grab Visual	Netherlands (3 WWTPs)	3
1.6-56$x10^3$/kg	Undefined	Fibre (63%) Fragment/film (36%) Microbead (1.3%)	Grab Visual	China (28 WWTPs)	4
2.3-17.1$x10^4$/kg	PET (~>80%), PA (~5%), PE (~10%)	Fibres (88-94%), Fragments (6-12%)	Grab Visual sorting + FTIR microscope	Finland (1 WWTP)	5
1.92-3.16$x10^4$/kg	Polyester (11-36%), alkyd (5-33%), PP (12-22%), PS (10-38%) acrylic (13-33%), PE (5-33%)	Undefined	Grab Visual sorting + FTIR microscope	UK (1 WWTP) Visual	6
1-24$x10^3$/kg	PP (~0-80%) PE (~30-100%) PS (~0-30%) PA (~0-20%)	Undefined (no fibres)	Grab Visual sorting + FTIR microscope	Germany (6 WWTPs)	7
4.4-14.9$x10^3$/kg	PP, PS, PET, Nylon, Polyamide	Fibres (65-82%), Fragments (18-35%)	Grab Visual sorting + FTIR	Canada (1 WWTP)	8
1.87$x10^5$/kg	Undefined	Undefined	Grab Visual sorting + FTIR	Finland (1 WWTP)	9
5.33$x10^4$/kg	PET (15%), PE (18%), ABS (27%), PP (9%), PA (6%), PS (5%)	Fibres (15%) Fragments (34%), Film (51%)	Grab Visual sorting + FTIR microscope	Italy (1 WWTP)	10
2.7-15.4$x10^3$/kg	PE, Acrylic, PET, PP, PA, PEst	Fibres (78.5%) Fragment/film (20.3%) Microbead (0.3%)	Grab Visual sorting + FTIR	Ireland (7 WWTPs)	11

Source: [1]Carr et al., 2016, [2]Magnusson et al., 2014, [3]Leslie et al., 2017, [4]Li et al., 2018, [5]Lares et al., 2018, [6]Murphy et al., 2016, [7]Mintenig et al., 2017, [8] Gies et al., 2018, [9]Talvitie et al., 2017, [10]Magni et al., 2019, [11]Mahon et al., 2017

Microplastics in biosolids

Microplastics accumulated in biosolids collected from 7 WWTPs in greater Sydney

- Estimated loads ranged from 4.5×10^4–3.23×10^5 (or 45,000 to 323,000) microplastics/kg
- Majority of particles were PP fragments <50 µm in size, consistent with wastewater collected from Malabar and Cronulla WWTPs
- Consistent with international studies of microplastics in biosolids that range from 370–1.87×10^5 (or 373 to 187,000 microplastics/kg)

Biosolids are generally used in Australia for beneficial reuse

- Current evidence of ectoxicity suggests loads of microplastics and biosolid application rates are likely to represent a low risk to terrestrial organisms

3.4 Quality assurance and quality controls

3.4.1 Ultrapure water blank samples

The ultrapure water laboratory blanks collected and processed alongside the wastewater samples indicated there was a high degree of microplastic contamination (Figure 19). This became increasingly apparent after the initial sample collection (Nov18) where various contaminant sources may have contributed at various stages of collection and sample preparation. Each time that contamination was noted in the ultrapure water samples, wastewater samples were re-processed from the homogenised (vigorously shaken) archived samples that had been stored in a cool room (<4°C, dark). It was not until the Jul19 sample collection where it was considered that contaminant levels were of an acceptable level for a final re-processing and analysis of the wastewater samples for reporting (Figure 19). The Jul19 ultrapure water samples from the laboratory are used for comparative purposes with the microplastic numbers in wastewaters collected prior to July 2019 in the following discussion. The ultrapure water field blanks transported to Cronulla or Malabar WWTPs is used similarly as a comparison with microplastics characterised in wastewater samples collected from July 2019, at the respective WWTPs.

It has been recommended that laminar flow cabinets may be an effective means to reduce airborne contamination (Koelmans et al., 2019) but this was not utilised in the present study due to concerns of losing microplastic material from samples due to considerable airflow. Parafilm (Bemis Company, Inc, USA) was initially used for sealing wastewater containers and filtration apparatus but it was suspected of contributing to the PP signal and was discontinued. Despite these and other operational measures (outlined in 2.7 Quality assurance and quality controls), the ultrapure water laboratory blanks collected directly within the laboratory prior to processing for the Jul19 and Sep19 samples still contained a number of microplastic contaminants, including PE, PP, PET, SAN and EVA (Figure 19). In contrast with the wastewater samples, the proportion of PP relative to other polymers in the laboratory blanks was generally lower (35-67%) compared with the wastewater samples (63–87%). The number of microplastic particles in the laboratory blanks were also comparatively low with respect to numbers of PP and PE in the wastewater samples, especially for the number of fragments detected. As with the wastewater samples, fragments were the morphology most frequently found (68–100%) microplastic contaminant, while no microbeads were detected (Figure 19).

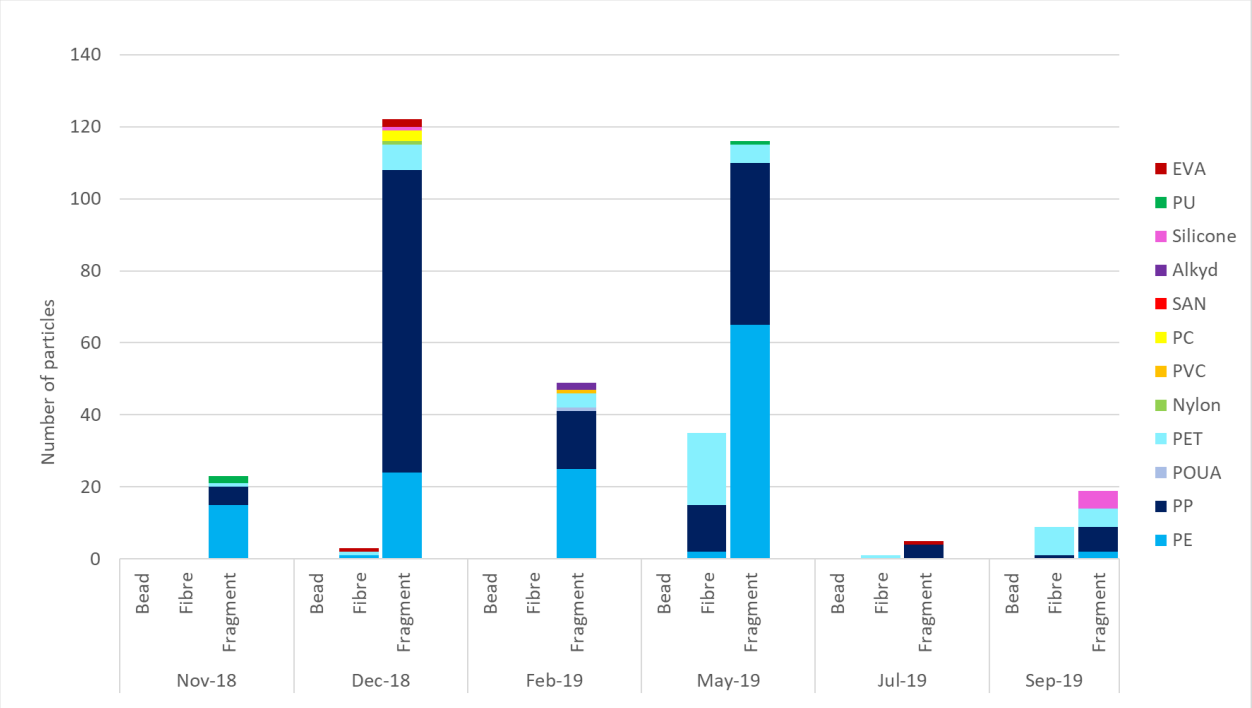


Figure 19 Summary of microplastic numbers, polymer type and morphology measured in ultrapure (MQ) water collected during the monitoring campaign in the laboratory and processed in an identical manner to the wastewater and biosolids samples.

All wastewater samples for final reported quantification values were re-processed and re-analysed after July 2019

The majority of fibres detected were PET, with some PP fibres also present. The amount of PET fibres in a number of wastewater samples was similar to that found in the Sep19 laboratory blanks (8 PET fibres), although more than 8 PET fibres were detected in a number of samples, including Cronulla influent and post-influent and Malabar S2 and effluent (Figures 10 and 13). For the Jul19 laboratory blank, only 1 PET fibre was found (Figure 19) and it is worth noting wastewater samples collected prior to July 2019 were re-processed for analysis in conjunction with the Jul19 sample. A similar level of contamination was also found in field blanks transported to Cronulla and Malabar WWTPs, where no more than 2 PET fibres were detected in the Jul19 and Sep19 samples. Other field blanks processed during the sample collections prior to Jul19 were similarly contaminated as the laboratory blanks collected in the laboratory prior to July 2019 (Figure 19).

This would indicate that the potential exists for the PET fibres detected in the wastewater samples to have been sourced through sample collection and preparation. It should be noted the relatively low PET fibre counts (<2) in the other ultrapure water could also suggest the 8 PET fibres found in the Sep19 ultrapure water laboratory blank were due to a specific contamination event. Low counts (<2) of PET fibres in field blanks (transported to Cronulla and Malabar WWTPs), processed at the same time as the Sep19 laboratory blank. The FTIR microscope image of the 8 PET fibres suggest a similar colour and dimensions (Figure 20), which gives further weight to this being a single contamination source.

Despite this, the number of PET fibres in this Sep19 laboratory blank ranged from 1% (C Inf, May19) to 100% (C P Inf, Sep19; C Eff Jul19) of the number quantified in the wastewater samples.

Although this should be considered against the numbers of PET fibres quantified in Sep19 wastewater and biosolid samples, which were processed alongside this blank, the Sep19 laboratory blank likely represents a worst-case scenario for PET contamination. For fragments, the relative numbers counted in wastewater were typically much higher than the laboratory blanks. For example, numbers of fragments present in laboratory blanks were as low as <1% (M Eff, Jul19) of the wastewater samples and typically <30%, although relative numbers of fragments were as high as 56% (C Eff, Sep19) and 70% (M Eff, Nov18) in limited cases. As previously noted, the proportion of PP fragments in the ultrapure water was less than that of samples, so a higher degree of confidence can be applied to the fragments (the majority of which were PP) detected in the wastewater samples.

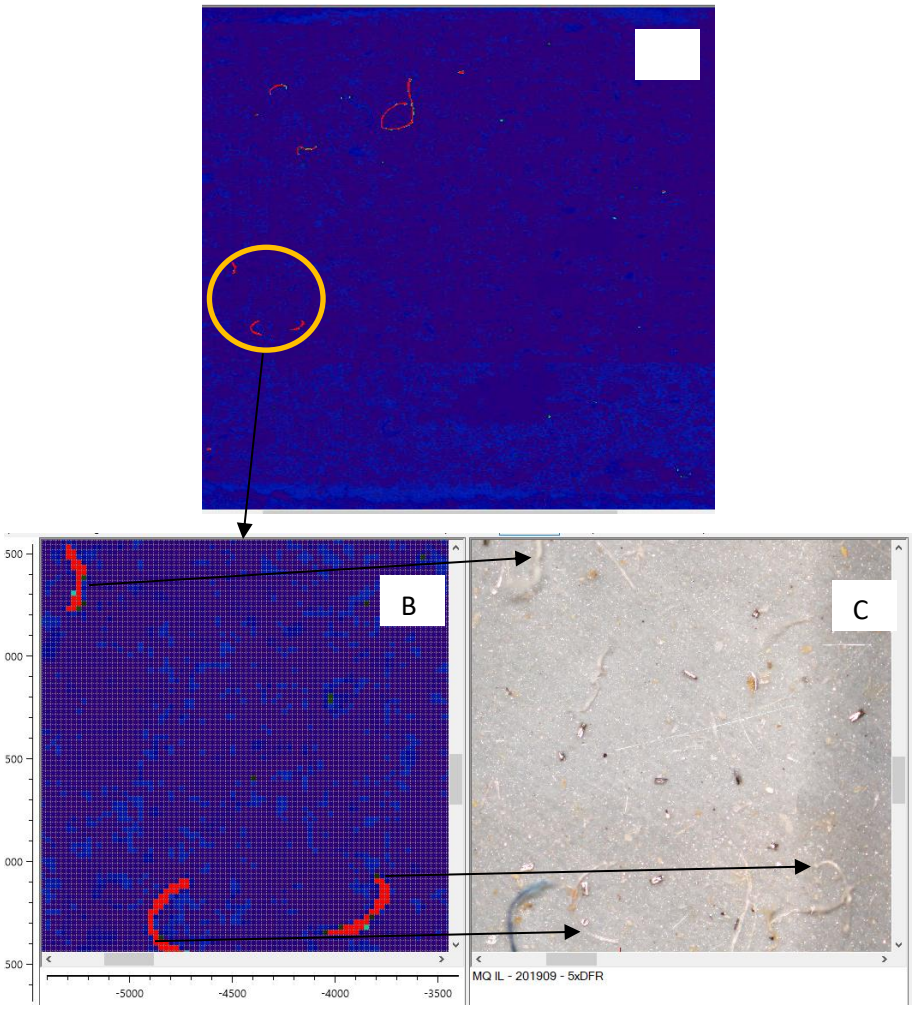


Figure 20 Correlation map of PET fibres (A) in laboratory ultrapure (MQ) water blank sample collected and processed in September 2019. The lower image (B) shows greater detail of the region circled in yellow for three of the eight PET fibres found in the sample.
The adjacent visual image (C) highlights the similarity of the PET fibres (in terms of thickness and colour), which was also the case with the other fibres suggesting a common source of contamination. The size of the upper image is 15 × 15 mm, while the squares in the lower image are 25 × 25 µm. In the correlation maps (upper and lower), red highlights indicate particles with the highest FTIR spectrum match with PET.

It should also be noted that previous studies analysing microplastics in wastewater also found a similar degree of contamination in their water blank samples. For example, Simon et al. (2018) found a median value of 1881 microplastic particles in triplicate 1 L samples of demineralised water, which represented 16.3% of microplastics counted in wastewater samples. PE and PP were equally the most common polymer type in the blank samples for this study as was found in the present study. Another study by Talvitie et al. (2017) found tap water blanks used for their wastewater sampling campaign had contamination levels around 30% of their wastewater samples, primarily in the form of fibres. Tap water controls were also used by Mintenig et al. (2017), where 150 L was extracted in the same manner as wastewater and 130 fibres and 21 particles were counted. The microplastic concentration of these blanks were lower than in the 1 L

of ultrapure water used in the present study, although the absolute number was similar to what was found in the present study (Figure 19). The absolute number of microplastic contaminants is a more relevant measure since the water blank is unlikely to contain any microplastics and is acting as a vehicle for any microplastics present in the air, on extraction equipment or in other solutions. The most common polymer (81%) was PP (Mintenig et al., 2017).

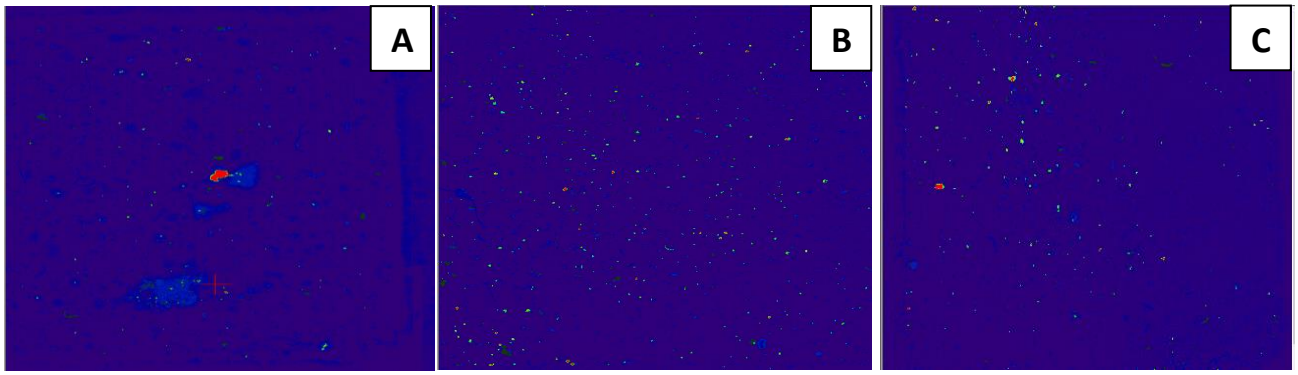


Figure 21 Correlation map of PP fragments in ultrapure (MQ) water blank sample collected and processed in September 2019 from (a) the laboratory, (b) Cronulla (field blank) and (c) Malabar (field blank).

Image sizes are 15 × 15 mm, with red highlights indicating particles with the highest FTIR spectrum match with PP.

3.4.2 Biosolid blank samples

In comparison with the wastewater samples, the number of microplastic particles detected in the biosolids was considerably greater (Figure 22). The number of microplastics quantified in the sand blanks, however, were similar to those in the biosolid samples, and, in the case of Malabar, greater. Since the biosolids samples were prepared for analysis at the same time and using the same methodology as the Sep19 wastewater samples, it is not clear why the sand blanks showed such a high degree of contamination. As with the wastewater samples, the sand blanks had a high proportion of PP (70–94%), the majority of which were fragments (95–98%) (Figure 22). As with the ultrapure water samples, no microbeads were detected in the sand blanks.

According to the protocol, sand blanks were transported to the biosolid collection point and the lid was removed while the biosolid samples were collected. After this point, there was no difference between the water, biosolid and sand samples processing, which occurred alongside the Sep19 water samples. The contamination in the sand blanks, which was reasonably consistent at Cronulla, Quakers Hill, Rouse Hill and St Marys or Malabar, West Camden and Winmalee in terms of number and composition, therefore may have derived from the site of collection (Figure 22). Alternatively, a more likely explanation would be that the source of the contamination came from the sand itself, indicating that it was not adequately prepared prior to transportation. This is considered more likely because of previous studies where similarly high numbers (10^3–10^5

particles/kg) of microplastics were also quantified in biosolids (Table 10). Furthermore, previous studies have also quantified similar numbers of microplastics in wastewaters relative to biosolid loads, which demonstrate similar rates (60% to ~100%) of microplastics being transferred to biosolids during wastewater treatment as in the present study (Gies et al., 2018; Murphy et al., 2016; Talvitie et al., 2017).

In the former case, where contamination is derived from the site of collection, the similar (or in the case of Malabar, higher) numbers of microplastic particles in the sand blanks relative to the biosolids means that the numbers of microplastics counted in the biosolid samples is likely to be considerably lower than estimated. This was the case in separate studies by Magnusson et al. (2014) and Leslie et al. (2017), where hundreds of microplastics per kg were estimated to be present in biosolids. Mintenig et al. (2017) also estimated hundreds of microplastics per kg of sludge at one WWTP, although sludge from five other WWTPs surveyed found concentrations in the 10^3–10^4 particles/kg.

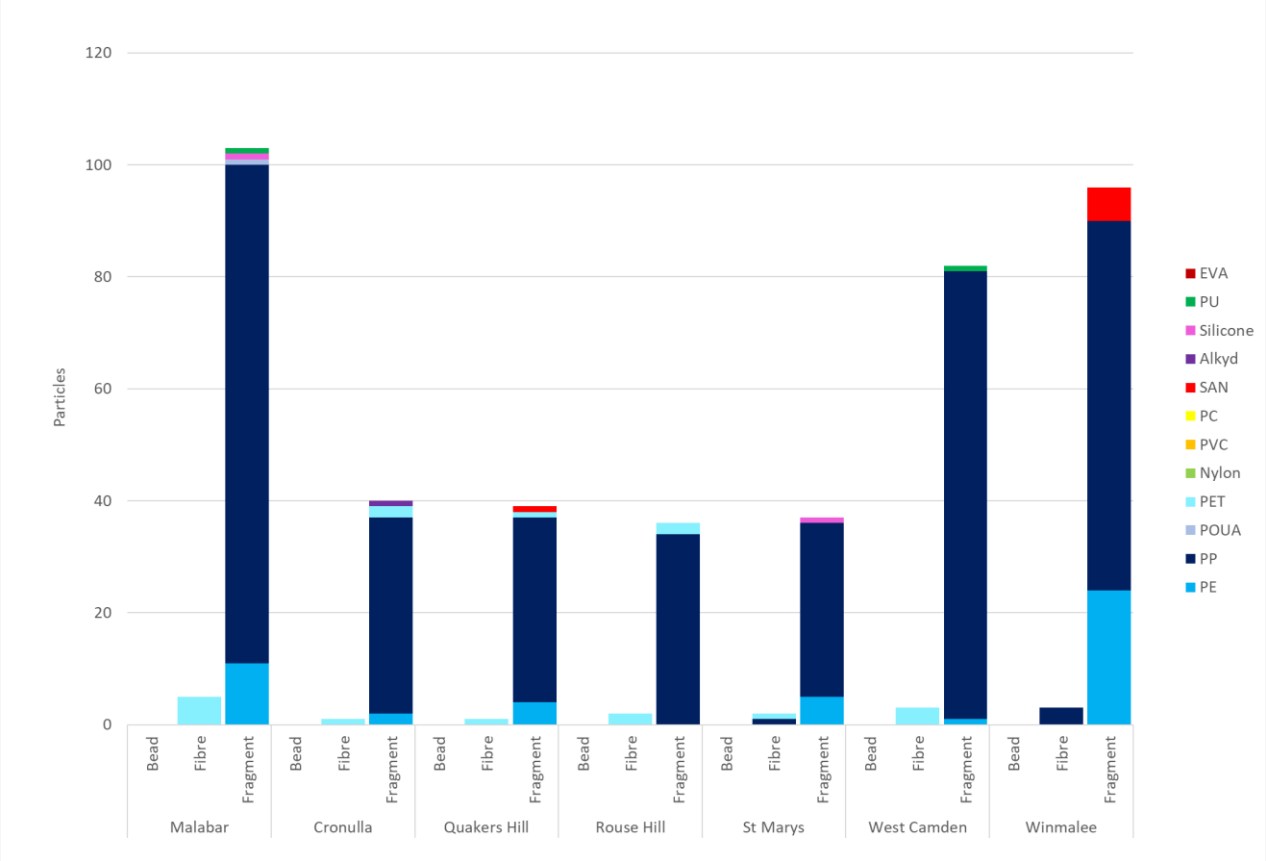


Figure 22 Summary of microplastic numbers, polymer type and morphology measured in sand blanks collected during the biosolid monitoring campaign in September 2019 and processed in an identical manner to the biosolids samples.

In this study, negative controls specific to sludge samples were not used, in that only tap water negative controls were utilised (Mintenig et al., 2017). Based on the average number of polymer counts in three negative control samples, final counts of microplastics in wastewater had these average values subtracted from them (Mintenig et al., 2017). In the case of Simon et al. (2018), a limit of detection was assigned to wastewater samples (3093 particles/L) based on the number microplastic particles detected in the three blank samples. Only one type of blank water (laboratory, Cronulla or Malabar) was used for each time point and this was only reliable in the final two collection periods in the present study. Preferably seven to ten replicate samples are used to generate a limit of detection in analytical chemistry (Winslow et al., 2006) and so it would not have been suitable to use this limited number of blanks to adjust the quantification of microplastics in wastewater samples.

3.4.3 Spike recoveries of microbeads and fibres

The four Cronulla influent samples spiked showed a reasonable recovery for the PE microbeads (55±25%) and PET microfibres (70±43%), although the variability associated with the recoveries were also high (Table 11). In one spiked sample, only 20% of the spiked microbeads (*n*=10) were detected, otherwise their recovery was consistent and >60 %. The PE microbeads were highly visible and interference of microbeads present in the wastewater was unlikely (Figure 23). There was greater variability for the PET fibres due to a low (30%) and high recovery (130%). While a distinctive colour was selected for the PET fibre, the >100% recovery may have been due to the presence of microfibres within the wastewater. Despite this, the results indicated there was a reasonable degree of confidence in the adequate recovery of these two microplastic morphologies when spiked in real wastewater samples.

Table 11 Summary of recoveries of microbeads (*n*=10) and microfibres (*n*=10) spiked in four Cronulla influent samples

SAMPLE	PE MICROBEAD RECOVERY	PET MICROFIBRE RECOVERY
Spike 1	20%	50%
Spike 2	80%	130%
Spike 3	60%	70%
Spike 4	60%	30%
Average (± standard deviation)	55±25%	70±43%

These recovery values are similar to those previously reported by Simon et al. (2018), who undertook recovery assessments for PE fragments (78±12%) and PS microbeads (58±25%).

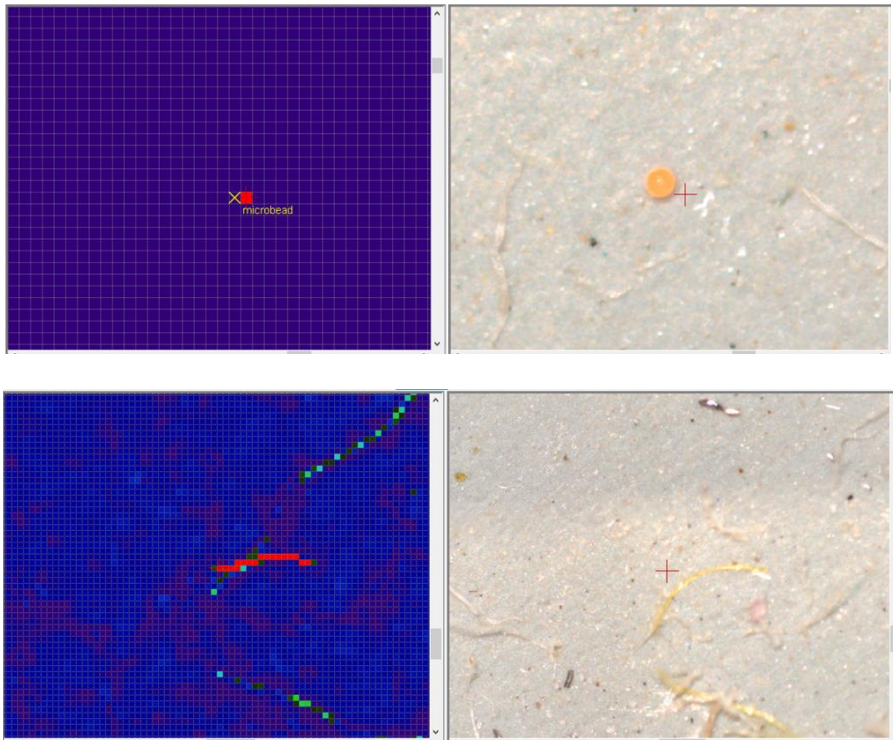


Figure 23 An example of the correlation map (left) and corresponding visual image (right) of a PE microbead (top) and PET fibre (bottom) spiked in Cronulla influent samples for spike recovery assessment.
Each square in the correlation map relates to a size of 25x25 μm.

The accurate quantification of microplastics in environmental samples analysis is critical for making assessments relating to the potential impacts that microplastics may have in the environment and confidence in quantification requires the use of appropriate quality controls. Negative (blank samples) and positive (microplastic spiked samples) controls were used for the present study and revealed a high degree of contamination of negative controls can occur. The absolute numbers of microplastics, especially fragments and fibres, reported here in wastewater and biosolids therefore need to take these controls into account. There is no universally agreed approach to accounting for controls within environmental samples for microplastics and no adjustment of values were made here based on the controls, due to inconsistencies related to control samples and the uncertainty this would further introduce to microplastic numbers in environmental samples. The presence of microplastic contaminants in the negative controls used in the present study, as well as throughout literature, highlights the need for maintaining effective controls to reduce contamination and, where this has been demonstrated to be not entirely effective, used to qualify the final numbers counted. Furthermore, inclusion of recovery

information is important to assess the effectiveness of the technique for isolation of microplastics and their subsequent analysis. This includes not only the numbers of microplastics recovered from spiked samples but also the representativeness of the environmental sample collected for analysis (Hermsen et al., 2018; Koelmans et al., 2019).

Microplastic contamination and recovery

Microplastics were present in blank samples (ultrapure water and sand) throughout the duration of the sampling campaign

- Fragments most common morphology and PP most common polymer but higher proportion of other polymers compared with wastewater and biosolids
- Reflects contamination noted in other studies

Microplastic bead (55±25%) and fibre (70±43%) recoveries indicate some loss and variability of recovery occurred during sample preparation

Inclusion of blanks and demonstration of spike recovery is essential for microplastic quantification studies

4 Summary and Conclusions

4.1 Microplastic quantification and characterisation methodology

The collection of wastewater over 10 months from two (Malabar and Cronulla) WWTPs and a single collection of biosolids from seven (Malabar, Cronulla, Quakers Hill, Winmalee, St Marys, Rouse Hill and West Camden) WWTPs for quantification of microplastic loads required extensive sample clean-up to remove organic and inorganic contaminants for quantification and characterisation (size/morphology/polymer identification) using semi-automated FTIR microscopy. Sample preparation, which included a Fenton digestion of organic matter and $ZnCl_2$ density separation of inorganic particles, took approximately 24 h. The analytical technique after filtration and mounting of samples on Anodisc filters took between 3 and 5 h. This offers a relatively rapid approach to quantifying and characterising microplastics in environmental samples and also has the advantage of characterising and quantifying microplastics in the entire sample. Furthermore, the use of FTIR microscopy and the semi-automated methods developed here substantially reduces the need for user intervention, which can reduce the extent of subjectivity required for counting and identification of polymers, especially for samples containing large numbers (e.g. >100) of particles with difficult contrast or colour properties. The apparent preference in identifying certain morphologies (i.e. fibres or fragments) by visual inspection or FTIR microscopy should be further examined as the field of microplastic analysis continues to mature.

Along with this, the presence of microplastic contamination in blank samples, including ultrapure water and sand, throughout the duration of this sampling campaign highlights the widespread nature of microplastics not only in wastewater but also within the general environment. Literature relating to microplastic quantification in environmental samples also shows that contamination is a widespread issue. Controlling the extent of microplastic contamination within a laboratory can include a number of precautions to limit the degree of contamination, although the inclusion of blank samples at all stages of sample collection, preparation and analysis is equally critical. Also, inclusion of spiked samples to assess the accuracy and precision of the selected sample preparation and analytical methods for microplastics is necessary. Microplastic recoveries using the present methodology was generally good but <100% and there was variability of microplastic recovery.

Microplastic quantification in environmental samples is a developing area of science and many researchers are working towards standardisation of a consistent and robust methodology that gives accurate and repeatable results.

4.2 Microplastics in wastewater and biosolids

Microplastic fragments (90–96% overall) were the most common type of particle found in wastewater samples, with fibres (5–19% overall) less common. Spherical microbeads, used as a surrogate for microbeads sourced from personal care products, were only found infrequently, with only 1–3 microbeads found in analysed samples. The VIA to phase-out microbeads had been in effect for approximately 2 months (since July 2018), with the majority (94%) of companies having already phased-out (or committed to phasing out) 12 months prior to the VIA coming into effect. The presence of relatively few spherical microbeads in wastewater would suggest that personal care products are still being used that contain microplastics, although the infrequent nature of their detection also suggests that widespread use within the WWTP catchments is not likely. The success of the VIA, alongside ongoing market surveys of available consumer products, would be further confirmed through additional future wastewater sampling both in Sydney and other Australian metropolitan centres.

The overall trends of polymer types identified in wastewater found PP (68–87% overall) to be the most common, while PE (7–18% overall) and PET (6–19%) were also detected to a lesser degree. Other polymers, including alkyd, silicone, PU, PC, EVA, SAN and nylon were infrequently detected at even lower proportions. Microplastic numbers at both WWTPs were higher in influent compared with effluent, with removal rates of up to 80% (Malabar WWTP) and >98% (Cronulla WWTP) estimated to occur prior to marine discharge. Although this represents a substantial removal of microplastics from the wastewater stream, it was still estimated that between 5.4×10^{9} and 1.2×10^{11} (or 5,400 million to 120,000 million) and $0.86–3.5\times10^{8}$ (or 86 to 350 million) microplastics per day were discharged from Malabar and Cronulla WWTP, respectively, each day based on their relative flow rates. These loads of microplastics in wastewater is in alignment with other studies that have measured concentrations of microplastics in wastewater collected in Australia, North America and Europe. Current evidence for microplastic particle toxicity would suggest that even in the absence of dilution in the marine environment, these concentrations are still many orders of magnitude less than where toxicity to aquatic organisms has been observed. There is still a paucity of information, however, related to standardised approaches to monitoring microplastics and realistic exposure scenarios, for example, and addressing such knowledge gaps is required to conclusively support this current evidence (Burns and Boxall, 2018).

The predominant removal process of microplastics within a WWTP is through association with biosolids. The collection and analysis of biosolids from seven WWTPs in the greater Sydney area found the estimated load of microplastics in biosolids (4.5×10^{4}–3.23×10^{5} microplastics/kg) which is at the upper end of what has been previously measured in biosolids in Europe, North America and Asia. The polymer characteristics (shape and type) were consistent with what was found in the wastewater collected at Malabar and Cronulla WWTPs, in that they were mainly PP fragments, with a lesser degree of PE and PET also present. Biosolids are used globally for beneficial reuse in agriculture and this is also the case in Australia (and NSW) and this could potentially transfer large quantities of microplastics from the aquatic environment to the terrestrial environment. Based on conservative assumptions of current biosolids application rates, the amount of microplastics

added to soils would take many decades to approach concentrations that have been observed to cause adverse effects in terrestrial organisms. Any changes to reuse regulations for biosolids based on microplastics content should therefore consider the potential for terrestrial toxicity from long-term accumulation of microplastics in terrestrial environments balanced against the considerable environmental and economic benefits in the reduction of landfill, carbon emissions and use of synthetic soil amendments from the reuse of biosolids.

In summary:

- The use of FTIR microscopy for analysis of microplastics in wastewater and biosolids represents a relatively rapid technique that greatly reduces the need for operator input.
- Microplastics were found in WWTP influent at concentrations ranging from 10s to 100s of particles per litre.
- Spherical microbeads were detected infrequently in wastewater, indicating personal care products containing microplastics are still in use within WWTP catchments. Future monitoring of wastewater could confirm whether a declining trend continues.
- Removal of microplastics was up to 79% through primary (screening and settling) treatment and >98% for tertiary (biological treatment and disinfection) treatment. This still represents 10s of millions to 100s of billions of microplastics are being released to the marine environment each day from these two WWTPs.
- Association with biosolids is the main removal pathway for microplastics and concentrations of microplastics in biosolids collected from seven WWTPs in the greater Sydney area were similar to other surveys in Europe, North America and Asia. Based on current evidence, these concentrations are unlikely to lead to adverse impacts on terrestrial organisms. Realistic exposure assessments are required to confirm this and should be considered alongside the many benefits of biosolids reuse for soil amendment.

5 Appendices

A.1 WWTP overview; processes, time proportional sampling and wastewater characteristics

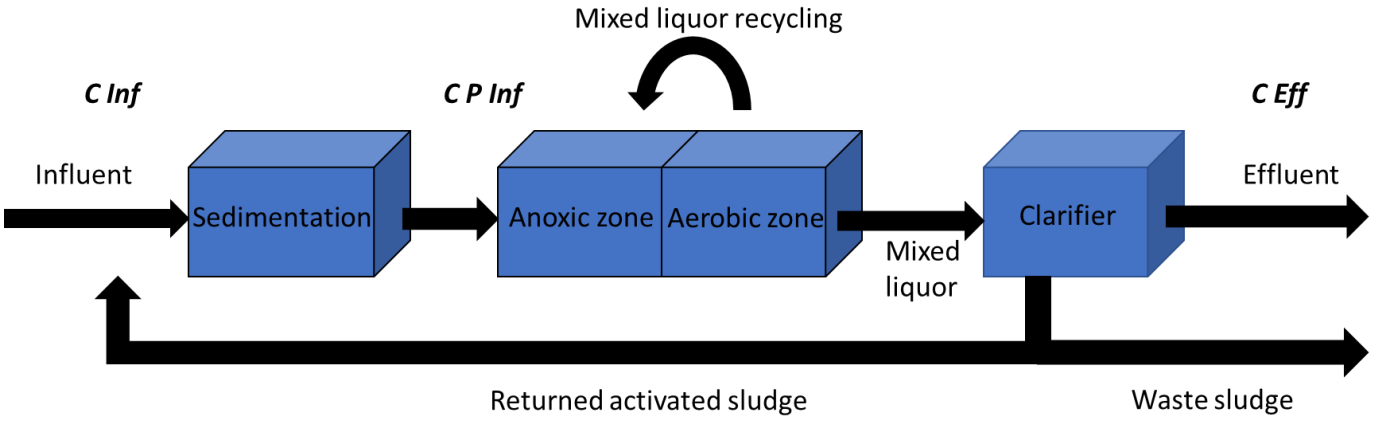


Figure A 1 Representation of wastewater treatment process at Cronulla WWTP.

Bold italic text shows stage of process where wastewater sampling occurred.

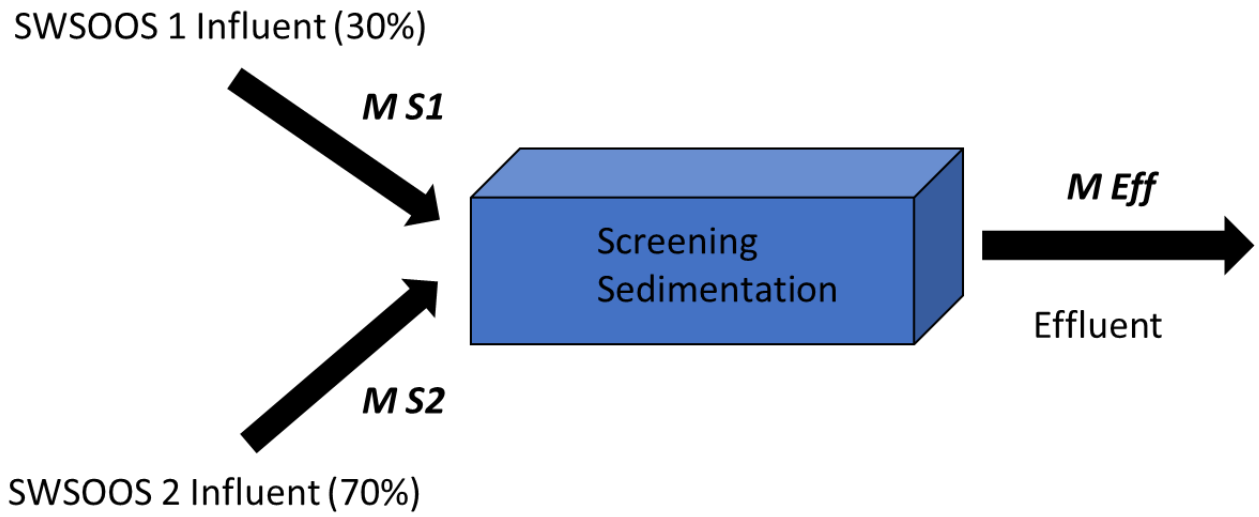


Figure A 2 Representation of wastewater treatment process at Malabar WWTP.

Bold, italic text shows stage of process where wastewater sampling occurred.

Table A 1 Summary of sampling program for time proportional wastewater sampling at Cronulla WWTP.

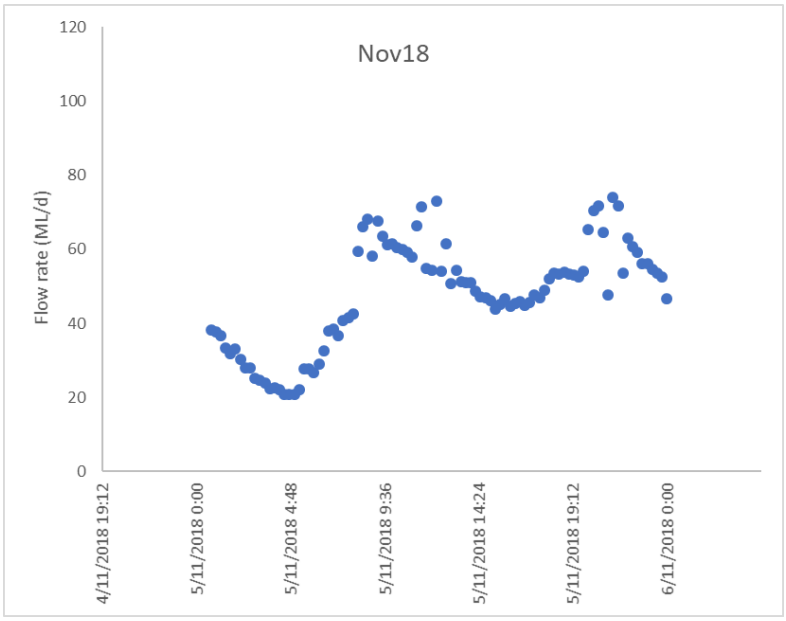
SAMPLE NUMBER	INFLUENT SAMPLE TIME	PRIMARY EFFLUENT SAMPLE TIME	OUTLET SAMPLE TIME
1	0:56	6:11	12:32
2	2:05	7:54	13:24
3	3:37	9:04	14:19
4	5:46	9:55	15:19
5	7:39	10:40	16:19
6	8:54	11:23	17:20
7	9:47	12:07	18:19
8	10:32	12:52	19:15
9	11:16	13:40	20:04
10	11:59	14:31	20:49
11	12:44	15:28	21:32
12	13:31	16:29	22:16
13	14:22	17:32	23:01
14	15:18	18:32	23:47
15	16:18	19:26	0:39
16	17:21	20:16	1:38
17	18:22	21:03	2:52
18	19:17	21:48	4:35
19	20:07	22:34	6:41
20	20:55	23:19	8:16
21	21:40	0:12	9:22
22	22:26	1:11	10:12
23	23:11	2:24	10:58
24	0:00	4:01	11:44

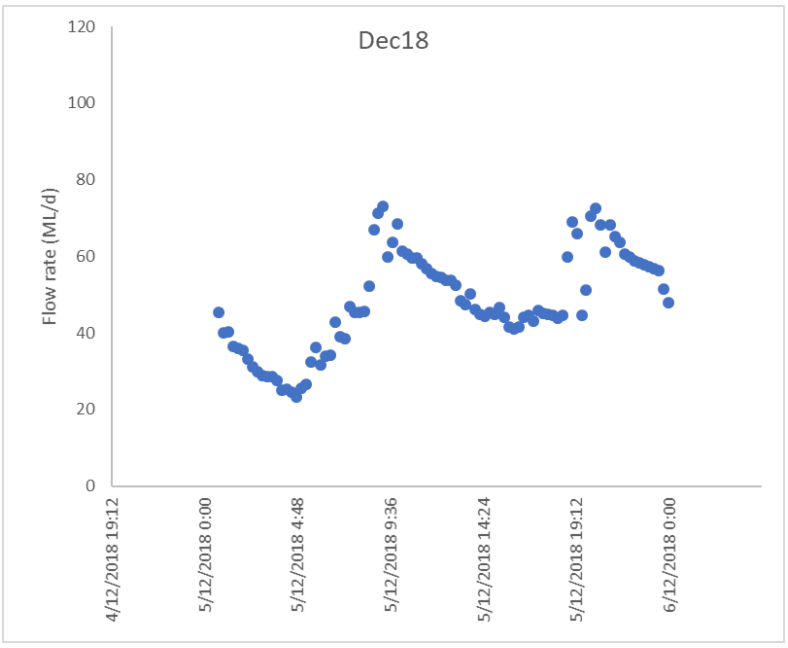
Table A 2 Summary of sampling program for time proportional wastewater sampling at Malabar WWTP

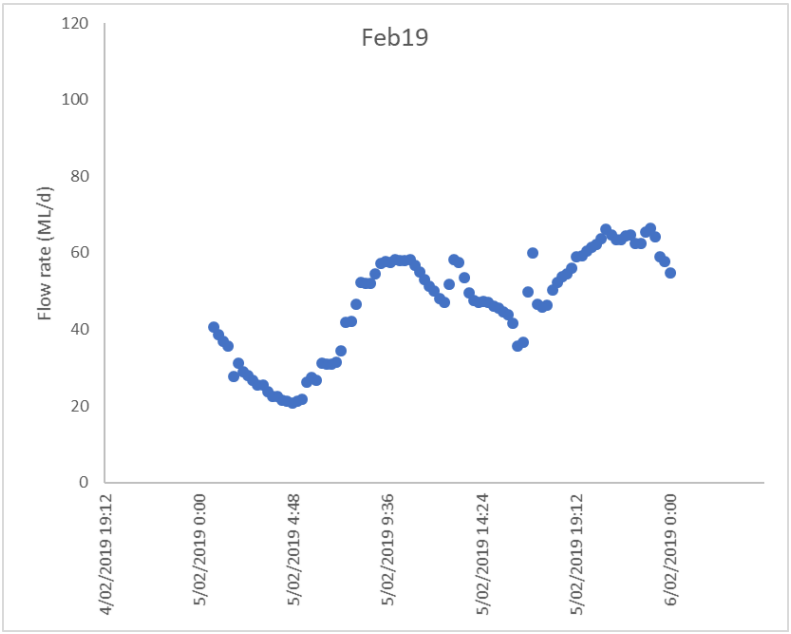
SAMPLE NUMBER	INFLUENT SAMPLE TIME	OUTLET SAMPLE TIME
1	0:51	1:54
2	1:41	2:46
3	2:33	3:39
4	3:25	4:35
5	4:21	5:35
6	5:20	6:40
7	6:24	7:51
8	7:33	9:03
9	8:45	10:13
10	9:56	11:18
11	11:02	12:19
12	12:04	13:18
13	13:03	14:17
14	14:02	15:19
15	15:03	16:22
16	16:06	17:25
17	17:09	18:26
18	18:11	19:27
19	19:12	20:28
20	20:13	21:28
21	21:13	22:25
22	22:11	23:20
23	23:06	0:13
24	0:00	1:03

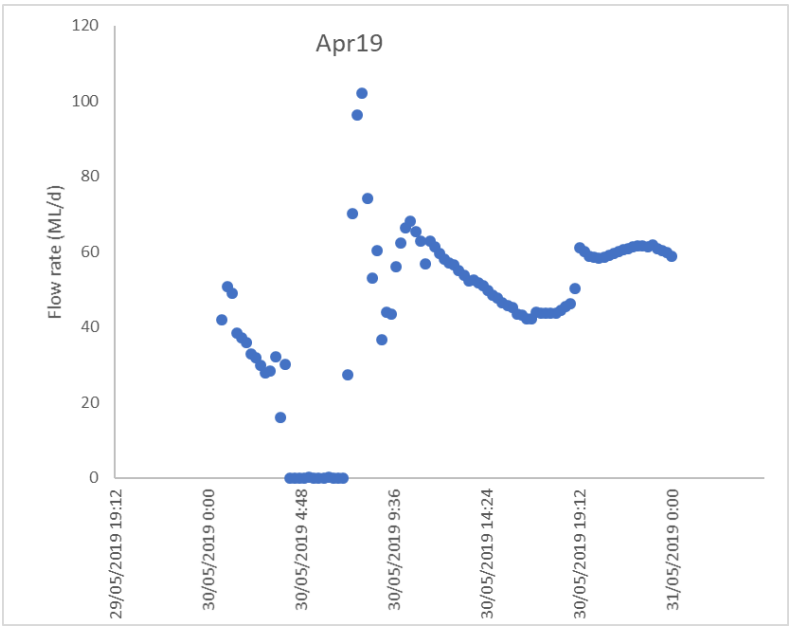
Table A 3 Water quality parameters for sample collection

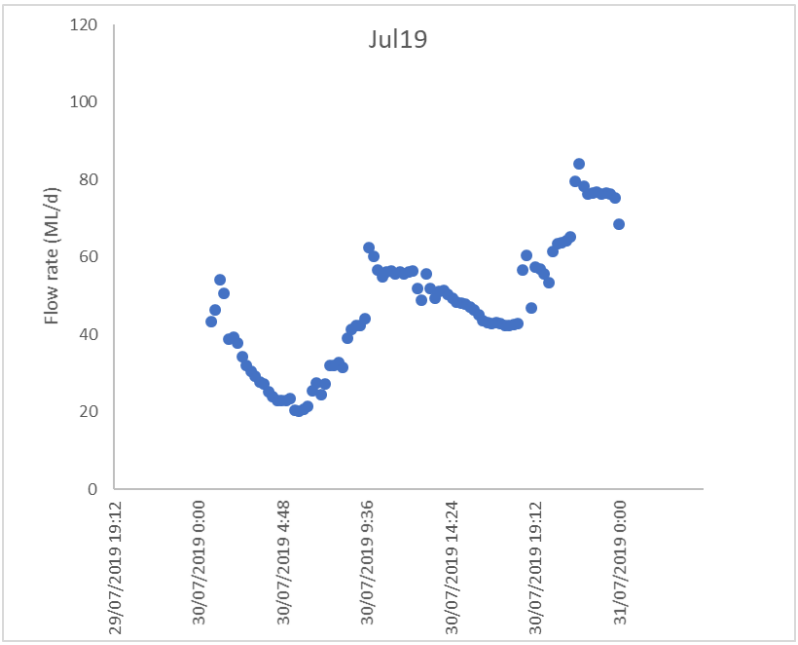
DATE	WWTP	COLLECTION POINT	TIME	PH	EC	TSS	VSS
					(μS/cm)	(mg/L)	(mg/L)
Nov18	Cronulla	C Inf	11:12:08 AM	7.38	1768	290	280
		C P Inf	12:06:15 PM	7.89	2000	110	98
		C Eff	11:41:04 AM	7.38	1603	<2	<2
	Malabar	M S1	9:47:13 AM	7.44	1562	320	290
		M S2	9:20:36 AM	7.45	1374	300	240
		M Eff	8:13:41 AM	7.52	1451	180	150
Dec18	Cronulla	C Inf	11:06:09 AM	7.5	1707	260	240
		C P Inf	11:57:46 AM	8.06	2700	500	410
		C Eff	11:34:21 AM	7.3	1442	<2	<2
	Malabar	M S1	9:46:54 AM	7.48	1777	330	280
		M S2	8:59:20 AM	7.41	1388	350	280
		M Eff	8:21:42 AM	7.42	1468	160	130
Feb19	Cronulla	C Inf	10:58:01 AM	7.42	1596	110	98
		C P Inf	11:37:44 AM	8.01	2000	280	230
		C Eff	11:52:38 AM	6.95	1300	<2	<2
	Malabar	M S1	9:17:17 AM	7.44	1449	330	290
		M S2	8:53:22 AM	7.32	1285	170	150
		M Eff	7:57:47 AM	7.37	1345	160	140
May19	Cronulla	C Inf	10:23:40 AM	7.51	1645	300	270
		C P Inf	10:55:43 AM	7.92	1786	430	360
		C Eff	11:19:04 AM	7.21	1320	2	<2
	Malabar	M S1	8:07:17 AM	7.35	1438	320	270
		M S2	8:25:49 AM	7.25	1414	420	340
		M Eff	8:58:05 AM	7.3	1400	210	160
Jul19	Cronulla	C Inf	10:14:24 AM	7.95	1561	300	270
		C P Inf	10:38:42 AM	7.96	1998	410	360
		C Eff	11:37:26 AM	7.49	1379	4	3
	Malabar	M S1	7:54:02 AM	7.49	1580	320	280
		M S2	8:06:28 AM	7.16	1218	280	240
		M Eff	8:59:35 AM	7.39	1329	180	150
Sep19	Cronulla	C Inf	10:08:38 AM	7.92	1313	270	250
		C P Inf	10:31:15 AM	7.68	1489	80	66
		C Eff	11:35:59 AM	7.21	1151	<2	<2
	Malabar	M S1	7:51:46 AM	7.44	1183	280	250
		M S2	8:06:26 AM	7.23	1180	260	220
		M Eff	8:28:19 AM	7.41	1178	120	100


Nov18
Flow rate (ML/d)
120
100
80
60
40
20
0
4/11/2018 19:12
5/11/2018 0:00
5/11/2018 4:48
5/11/2018 9:36
5/11/2018 14:24
5/11/2018 19:12
6/11/2018 0:00


Dec18
Flow rate (ML/d)
120
100
80
60
40
20
0
4/12/2018 19:12
5/12/2018 0:00
5/12/2018 4:48
5/12/2018 9:36
5/12/2018 14:24
5/12/2018 19:12
6/12/2018 0:00


Feb19
Flow rate (ML/d)
120
100
80
60
40
20
0
4/02/2019 19:12
5/02/2019 0:00
5/02/2019 4:48
5/02/2019 9:36
5/02/2019 14:24
5/02/2019 19:12
6/02/2019 0:00


Apr19
Flow rate (ML/d)
120
100
80
60
40
20
0
29/05/2019 19:12
30/05/2019 0:00
30/05/2019 4:48
30/05/2019 9:36
30/05/2019 14:24
30/05/2019 19:12
31/05/2019 0:00


Jul19
Flow rate (ML/d)
120
100
80
60
40
20
0
29/07/2019 19:12
30/07/2019 0:00
30/07/2019 4:48
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30/07/2019 14:24
30/07/2019 19:12
31/07/2019 0:00

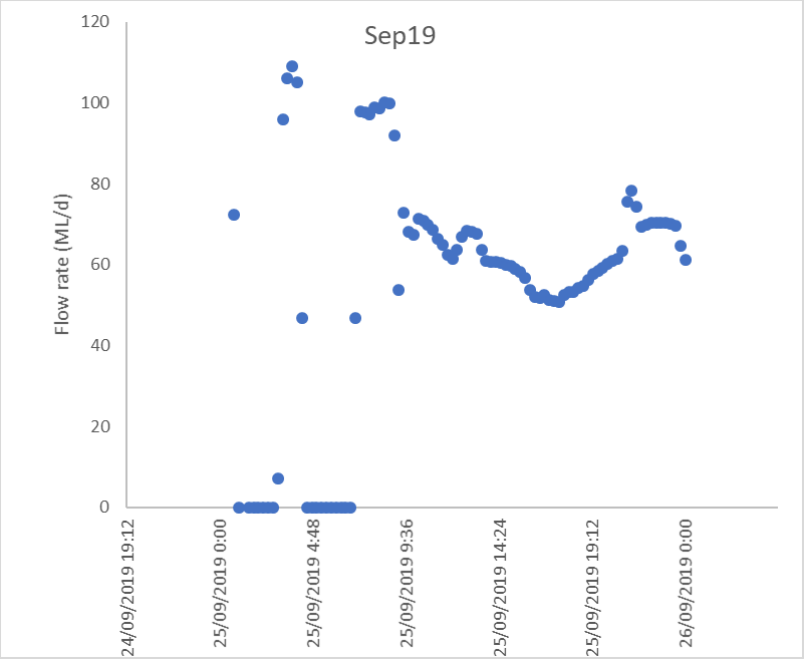
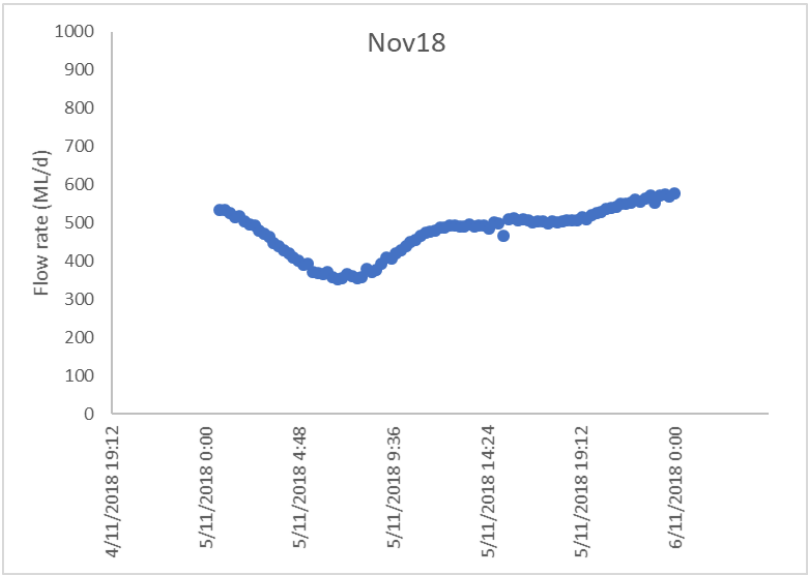
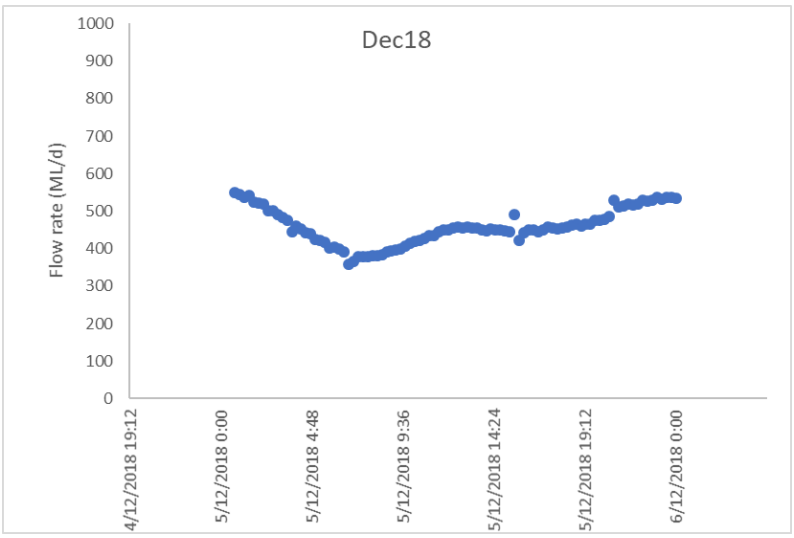
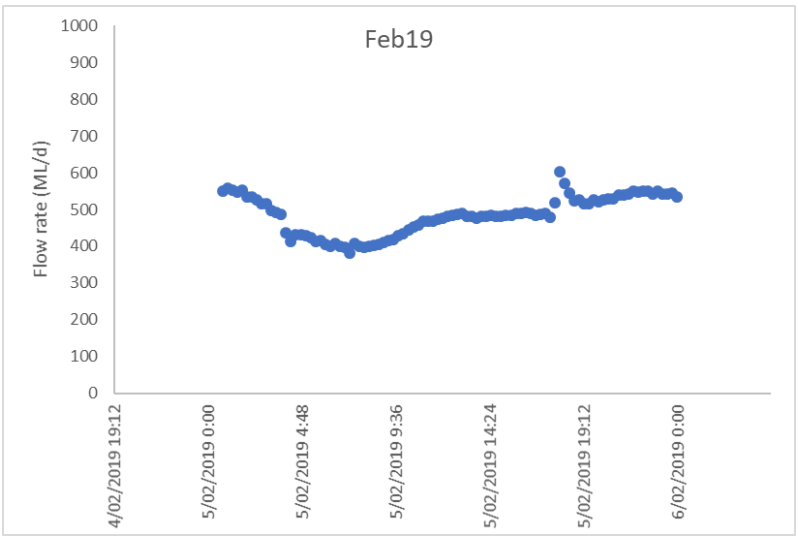


Figure A 3 Flow rate (ML/d) over the 24 h period of wastewater compositing of influent at Cronulla WWTP for each sampling period.

Periods of zero flow reflects the operation of the main influent pumping station in the Cronulla WWTP catchment.


Nov18
Flow rate (ML/d)
1000
900
800
700
600
500
400
300
200
100
0
4/11/2018 19:12
5/11/2018 0:00
5/11/2018 4:48
5/11/2018 9:36
5/11/2018 14:24
5/11/2018 19:12
6/11/2018 0:00


Dec18
Flow rate (ML/d)
1000
900
800
700
600
500
400
300
200
100
0
4/12/2018 19:12
5/12/2018 0:00
5/12/2018 4:48
5/12/2018 9:36
5/12/2018 14:24
5/12/2018 19:12
6/12/2018 0:00


Feb19
Flow rate (ML/d)
1000
900
800
700
600
500
400
300
200
100
0
4/02/2019 19:12
5/02/2019 0:00
5/02/2019 4:48
5/02/2019 9:36
5/02/2019 14:24
5/02/2019 19:12
6/02/2019 0:00

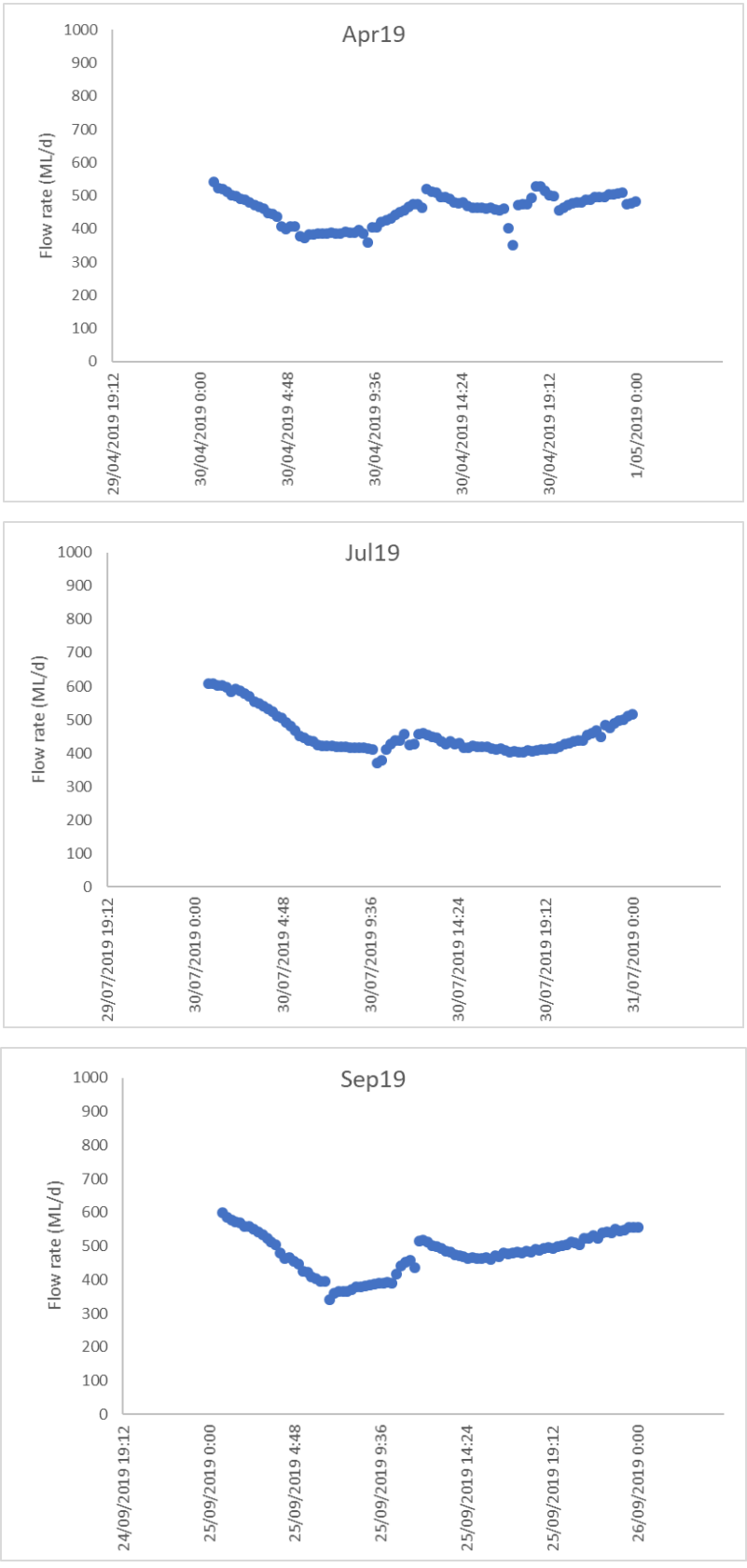


Figure A 4 Flow rate (ML/d) over the 24 h period of wastewater compositing of influent at Malabar WWTP for each sampling period.

A.2 Examples of software workflow for microplastics analysis

Figure A 5 gives an overview of a typical summary of a sample analysed using IR Map software. All the correlation, statistical and working profiles are listed on the right hand side of the image. The polymer profile map pane (top left) shows the currently selected polymer profile in heat map colour scale from dark blue (lowest correlation) to bright yellow (highest) alongside a spatially identical visible image to its right. Any selected pixels included in the profile's mask is highlighted in red. A crosshair can be placed on any pixel in the profile map and the matching crosshair will be placed on the visible image (at top of profile map and visible image). The IR spectrum at the crosshair location is displayed below the profile map along with summary information about the pixel and its spectrum statistics on the point info summary box. The user can freely navigate between different profiles, locations on the map, pan and zoom the map, perform manual search of spectral libraries, set and clear masks using an array of command buttons and keyboard shortcuts. Working profiles (defined by a mathematical formula of existing values on other profiles) can be recalculated quickly in seconds with a single key command.

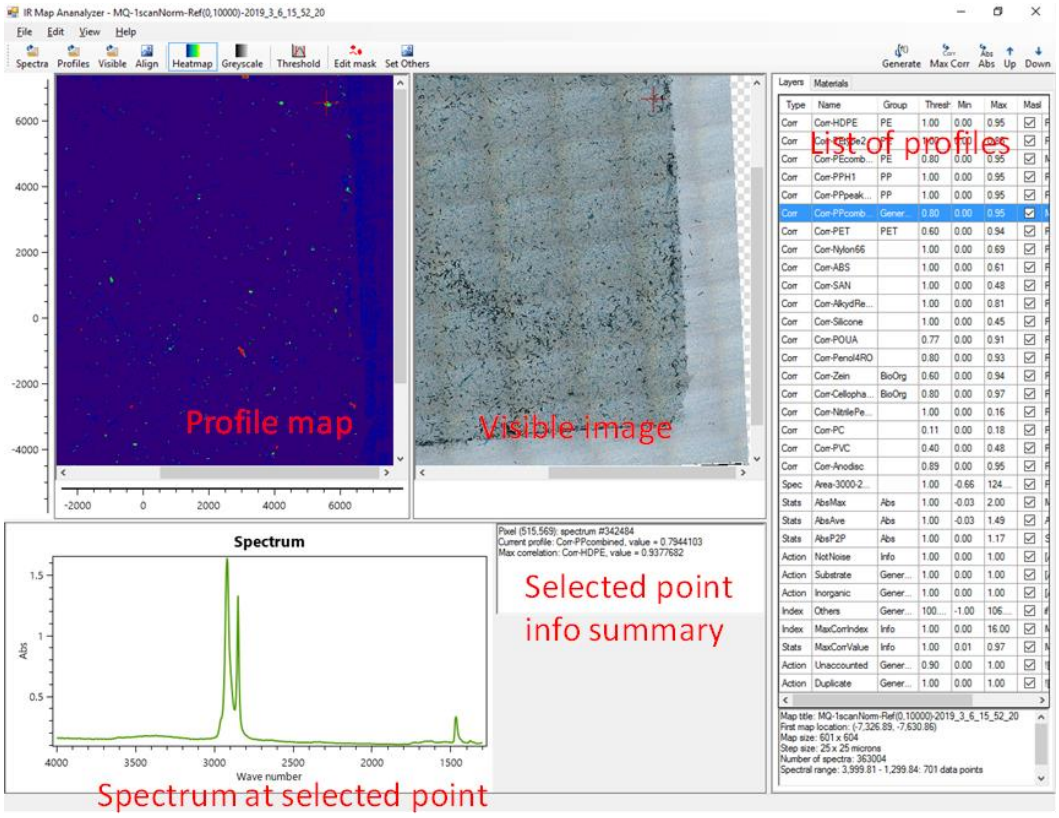


Figure A 5 Example of FTIR microscopy analysis processing screen using IR Map Analyzer software.

Figures A 6-8 give examples of zoomed-in visual images and corresponding correlation profiles of both polymers and non-polymers for various fragments (Figures A 6 and 7) and fibres (Figure A 8) detected in wastewater samples. The presence of the oleate surfactant Penol 4RO (pentaerythritol

tetracinoleate) was common in the wastewater samples and its identification as a polymer fragment was likely to be due to it drying as a film on the filter (Figure A 7). In Figure A 8, the profile of the PAN fibre is interrupted in the correlation profile because part of the fibre had lifted off the filter and was out of focus. In both cases, user intervention was required to not include the Penol 4RO fragment and ensure the PAN fibre was only counted as 1 (rather than 2) fibres. It is also notable that an almost invisible PET fibre was easily accounted for with its IR spectrum included in the correlation profile (Figure A 8). Following analysis using IR Map Analyzer, data was exported to an Excel format that include a full characterisation profile of the identified material (Figure A 9).

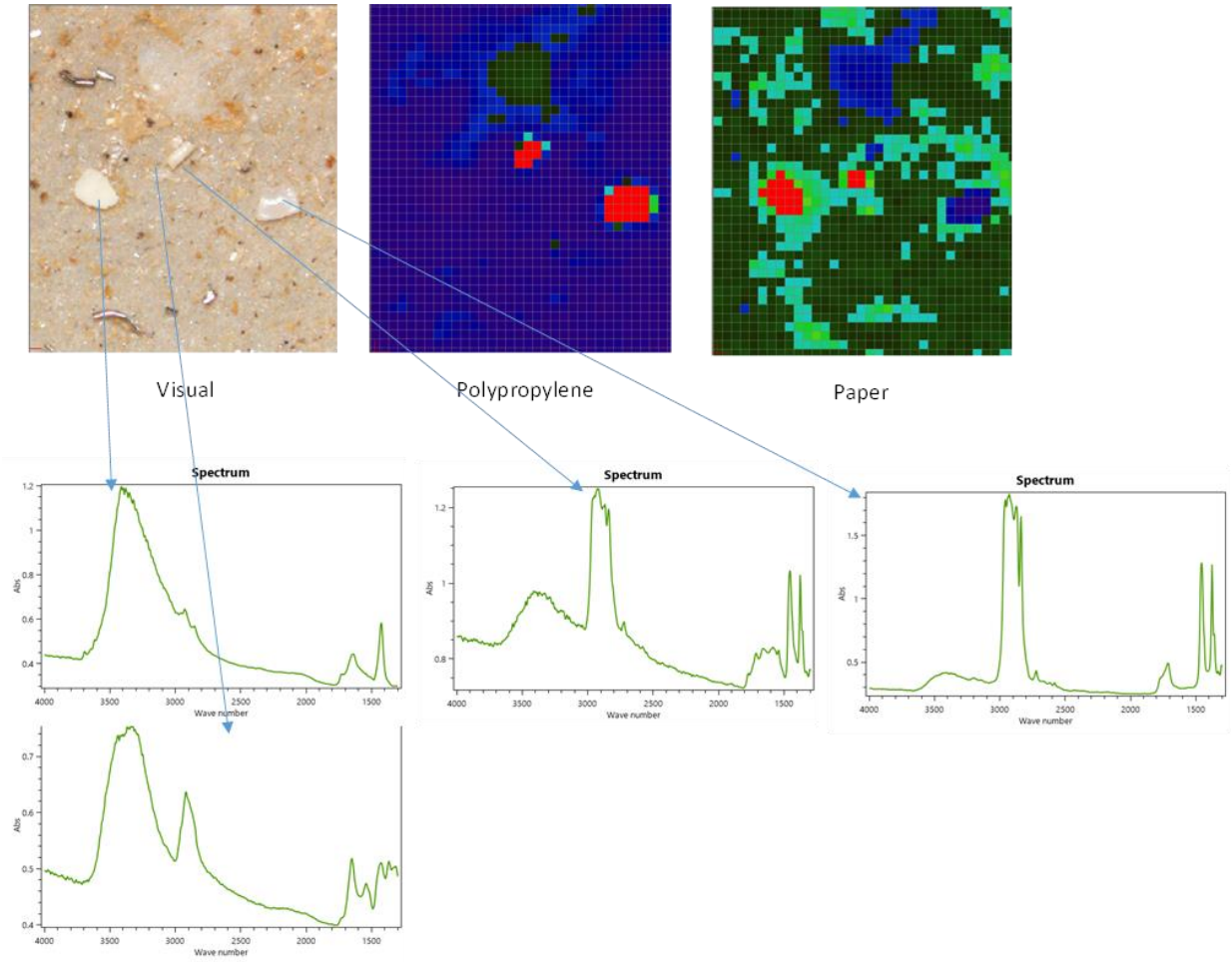


Figure A 6 An example of a typical visual image (top left) and correlation profiles against PP (top middle) and paper (top right) fragments for the same section of sample for Malabar S2 (Dec18). The red pixels are identified as particles of the corresponding material, with spectra corresponding at different locations for the identified particles, shown below the images. Each pixel in the profile map is 25 μm x 25 μm in size.

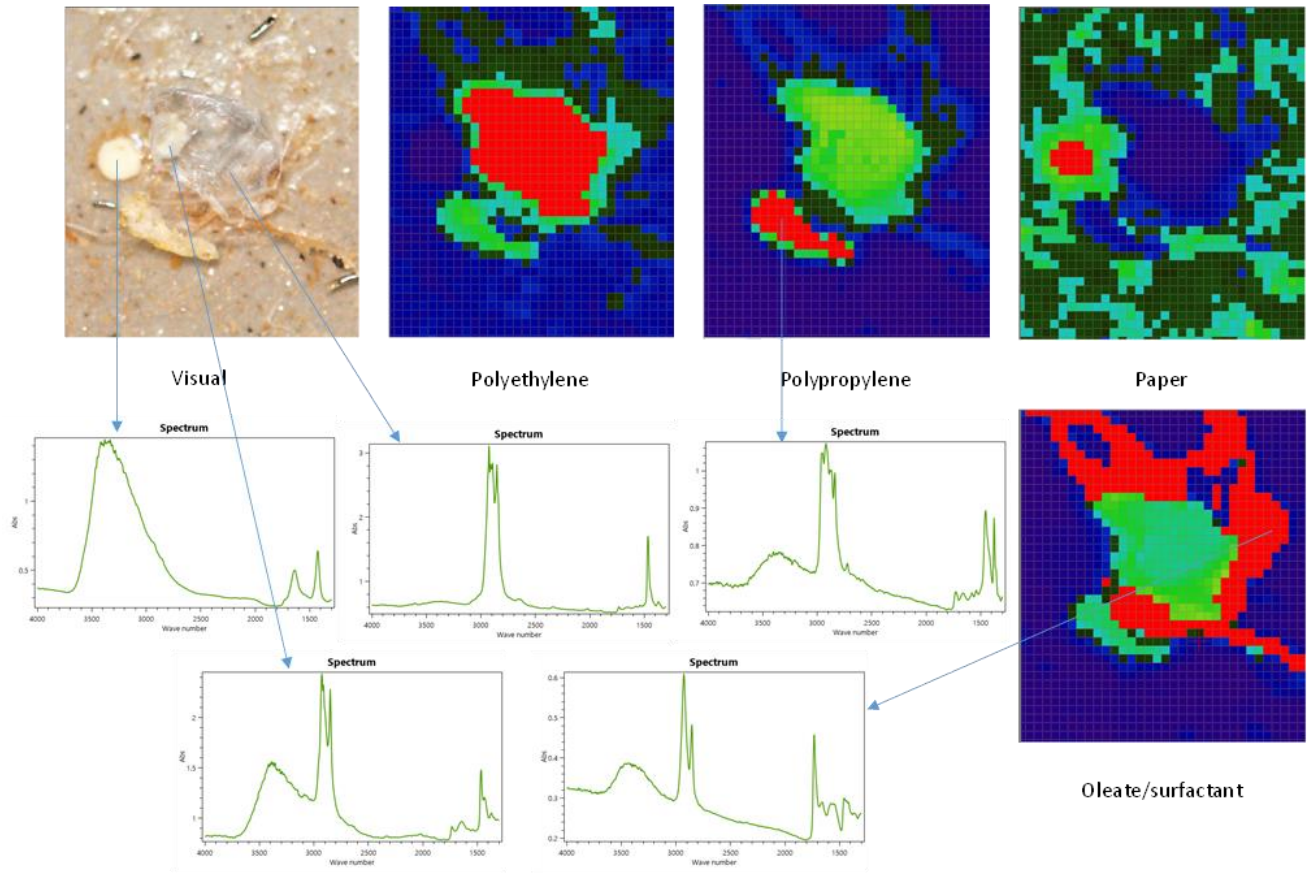


Figure A 7 An example of a visual image (top left) and correlation profiles against (from left to right) PE, PP, paper and Penol4RO fragments for the same section of sample for Malabar S2 (Dec18). The red pixels are identified as particles of the corresponding material, with spectra corresponding at different locations for the identified particles, shown below the images. Each pixel in the profile map is 25 μm x 25 μm in size.

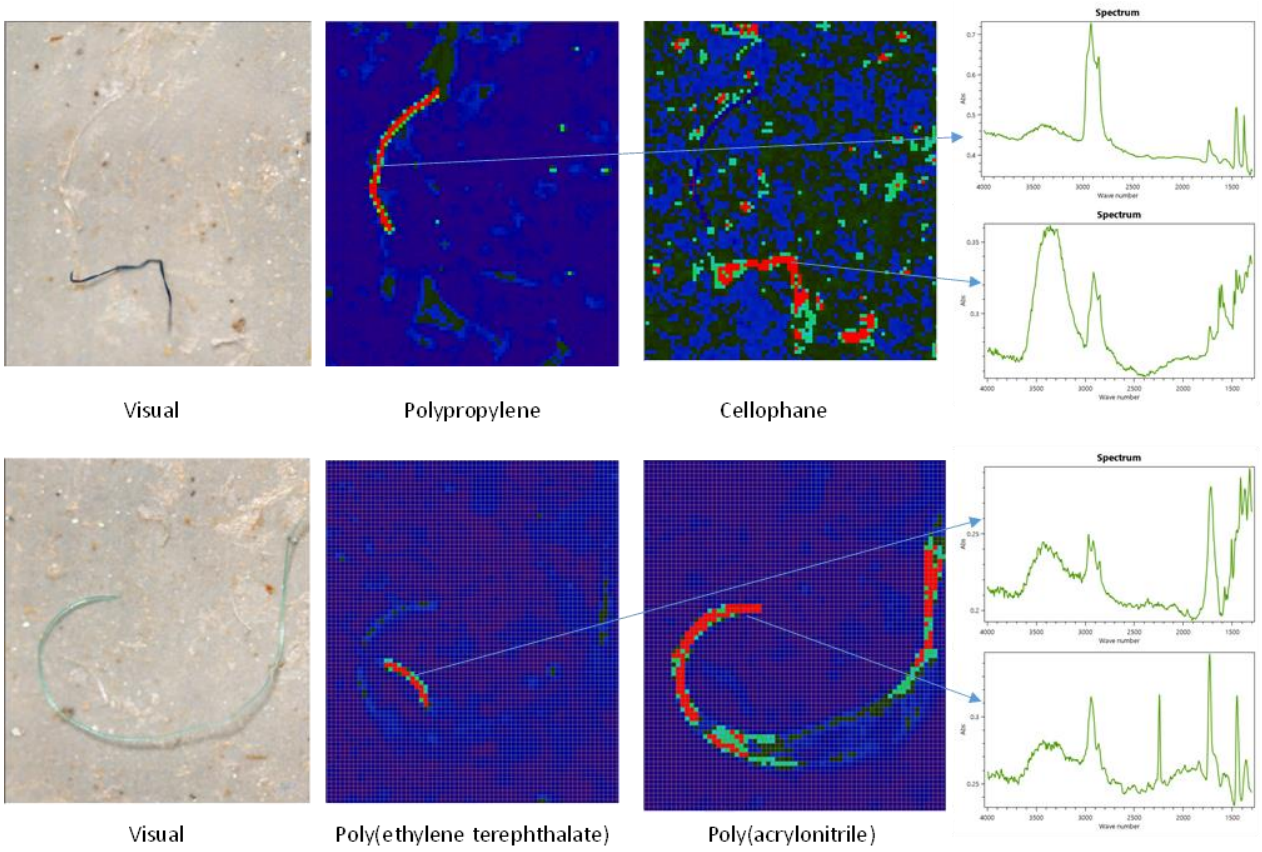
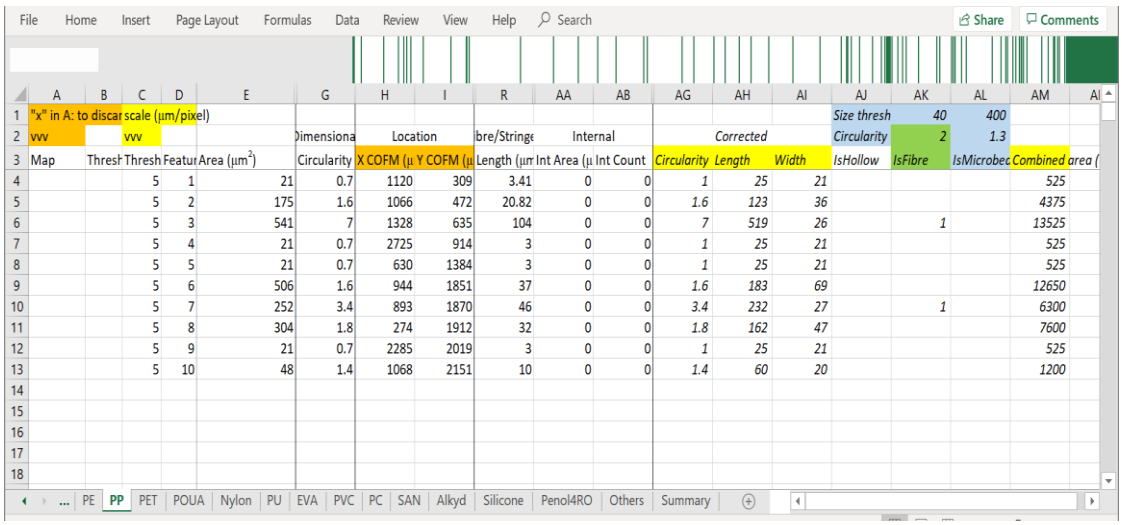
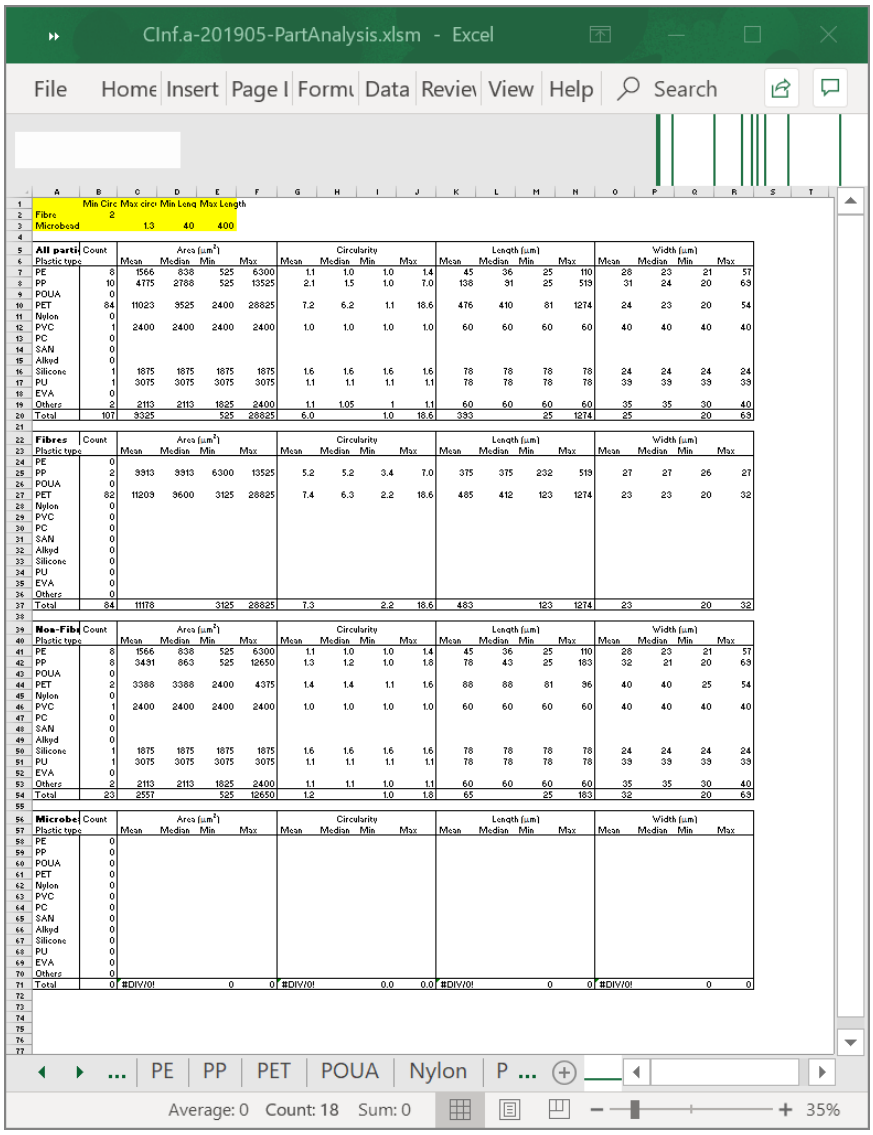


Figure A 8 An example of a visual image (top and bottom left) and correlation profiles against PE and cellophane (corresponding with top visual image) and PET and PAN (bottom visual image) fibres detected in Malabar S1 (May19) sample. The red pixels are identified as particles of the corresponding material, with spectra corresponding at different locations for the identified particles, shown below the images. Each pixel in the profile map is 25 μm x 25 μm in size.



	A	B	C	D	E	G	H	I	R	AA	AB	AG	AH	AI	AJ	AK	AL	AM
1	"x" in A: to discar		scale (µm/pixel)												Size thresh	40	400	
2	vvv		vvv			Dimensiona	Location		bre/Stringe	Internal		Corrected			Circularity	2	1.3	
3	Map	Thresh	Thresh	Featur	Area (µm²)	Circularity	X COFM (µ	Y COFM (µ	Length (µm	Int Area (µ	Int Count	Circularity	Length	Width	IsHollow	IsFibre	IsMicrobea	Combined area (
4			5	1	21	0.7	1120	309	3.41	0	0	1	25	21				525
5			5	2	175	1.6	1066	472	20.82	0	0	1.6	123	36				4375
6			5	3	541	7	1328	635	104	0	0	7	519	26		1		13525
7			5	4	21	0.7	2725	914	3	0	0	1	25	21				525
8			5	5	21	0.7	630	1384	3	0	0	1	25	21				525
9			5	6	506	1.6	944	1851	37	0	0	1.6	183	69				12650
10			5	7	252	3.4	893	1870	46	0	0	3.4	232	27		1		6300
11			5	8	304	1.8	274	1912	32	0	0	1.8	162	47				7600
12			5	9	21	0.7	2285	2019	3	0	0	1	25	21				525
13			5	10	48	1.4	1068	2151	10	0	0	1.4	60	20				1200

PE | PP | PET | POUA | Nylon | PU | EVA | PVC | PC | SAN | Alkyd | Silicone | Penol4RO | Others | Summary



CInf.a-201905-PartAnalysis.xlsm - Excel

	A	B	C	D	E	F
1			Min Circ	Max circ	Min Leng	Max Length
2	Fibre	2				
3	Microbead		1.3	40	400	

	All partic	Count	Area (µm²)				Circularity				Length (µm)				Width (µm)			
6	Plastic type		Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
7	PE	8	1566	838	525	6300	1.1	1.0	1.0	1.4	45	36	25	110	28	23	21	57
8	PP	10	4715	2788	525	13525	2.1	1.5	1.0	7.0	138	91	25	519	31	24	20	69
9	POUA	0																
10	PET	64	11023	9525	2400	28825	7.2	6.2	1.1	18.6	476	410	81	1274	24	23	20	54
11	Nylon	0																
12	PVC	1	2400	2400	2400	2400	1.0	1.0	1.0	1.0	60	60	60	60	40	40	40	40
13	PC	0																
14	SAN	0																
15	Alkyd	0																
16	Silicone	1	1875	1875	1875	1875	1.6	1.6	1.6	1.6	78	78	78	78	24	24	24	24
17	PU	1	3075	3075	3075	3075	1.1	1.1	1.1	1.1	78	78	78	78	39	39	39	39
18	EVA	0																
19	Others	2	2113	2113	1825	2400	1.1	1.05	1	1.1	60	60	60	60	35	35	30	40
20	Total	107	3325		525	28825	6.0		1.0	18.6	393		25	1274	25		20	69

	Fibres	Count	Area (µm²)				Circularity				Length (µm)				Width (µm)			
23	Plastic type		Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
24	PE	0																
25	PP	2	9913	9913	6300	13525	5.2	5.2	3.4	7.0	375	375	232	519	27	27	26	27
26	POUA	0																
27	PET	82	11209	9600	3125	28825	7.4	6.3	2.2	18.6	485	412	123	1274	23	23	20	32
28	Nylon	0																
29	PVC	0																
30	PC	0																
31	SAN	0																
32	Alkyd	0																
33	Silicone	0																
34	PU	0																
35	EVA	0																
36	Others	0																
37	Total	84	11178		3125	28825	7.3		2.2	18.6	483		123	1274	23		20	32

	Non-Fibr	Count	Area (µm²)				Circularity				Length (µm)				Width (µm)			
40	Plastic type		Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
41	PE	8	1566	838	525	6300	1.1	1.0	1.0	1.4	45	36	25	110	28	23	21	57
42	PP	8	3491	863	525	12650	1.3	1.2	1.0	1.8	78	43	25	183	32	21	20	69
43	POUA	0																
44	PET	2	3388	3388	2400	4375	1.4	1.4	1.1	1.6	88	88	81	96	40	40	25	54
45	Nylon	0																
46	PVC	1	2400	2400	2400	2400	1.0	1.0	1.0	1.0	60	60	60	60	40	40	40	40
47	PC	0																
48	SAN	0																
49	Alkyd	0																
50	Silicone	1	1875	1875	1875	1875	1.6	1.6	1.6	1.6	78	78	78	78	24	24	24	24
51	PU	1	3075	3075	3075	3075	1.1	1.1	1.1	1.1	78	78	78	78	39	39	39	39
52	EVA	0																
53	Others	2	2113	2113	1825	2400	1.1	1.1	1.0	1.1	60	60	60	60	35	35	30	40
54	Total	23	2557		525	12650	1.2		1.0	1.8	65		25	183	32		20	69

	Microbe	Count	Area (µm²)				Circularity				Length (µm)				Width (µm)			
57	Plastic type		Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
58	PE	0																
59	PP	0																
60	POUA	0																
61	PET	0																
62	Nylon	0																
63	PVC	0																
64	PC	0																
65	SAN	0																
66	Alkyd	0																
67	Silicone	0																
68	PU	0																
69	EVA	0																
70	Others	0																
71	Total	0	#DIV/0!		0	0	#DIV/0!		0.0	0.0	#DIV/0!		0	0	#DIV/0!		0	0

PE | PP | PET | POUA | Nylon | P ...

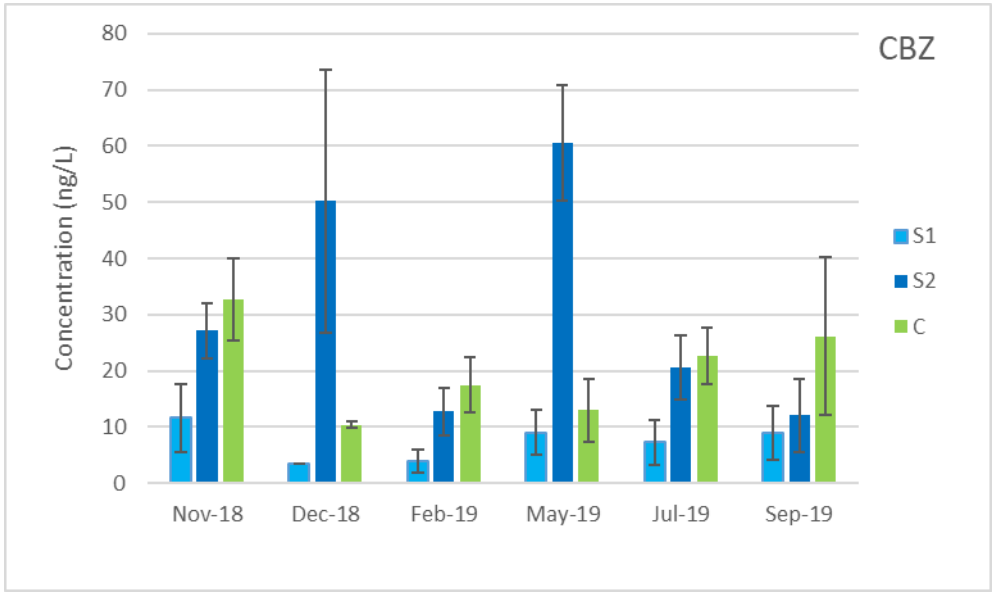
Average: 0 Count: 18 Sum: 0 35%

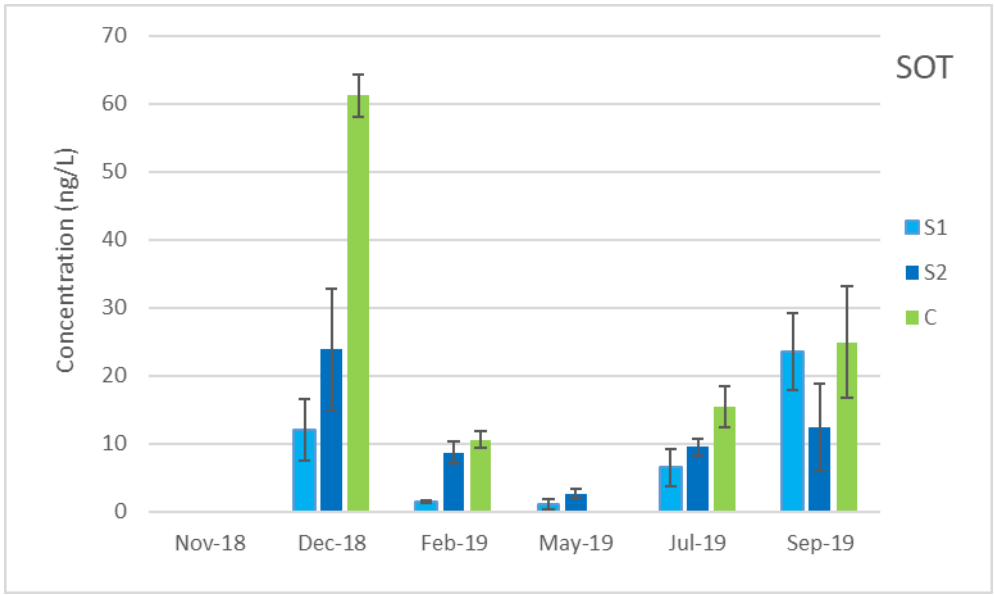
Figure A 9 Example of IR Map Analyzer output to Excel, summarising the overall particle characteristics detected for a polymer type in a wastewater or biosolid sample (top) and a summary of all polymer types detected in a wastewater or biosolid sample for overall quantification of fragments beads and fibres (bottom).

A.3 Summary of quantification of pharmaceuticals

Table A 4 MS/MS parameters for the quantification of pharmaceuticals

COMPOUND	SRM 1	COLLISION ENERGY (V)	SRM 2	COLLISION ENERGY (V)	LOQ (NG/L)
Carbamazepine	237.1 (M+H)→193.1	33	237.1 (M+H)→194.1	18	1
Sotalol	273.1 (M+H)→213.1	17	273.1 (M+H)→255	10	1
Trimethoprim	291 (M+H)→230.1	23	291 (M+H)→261.2	25	2
Venlafaxine	278.2 (M+H)→58.1	19	278.2 (M+H)→260	10	7





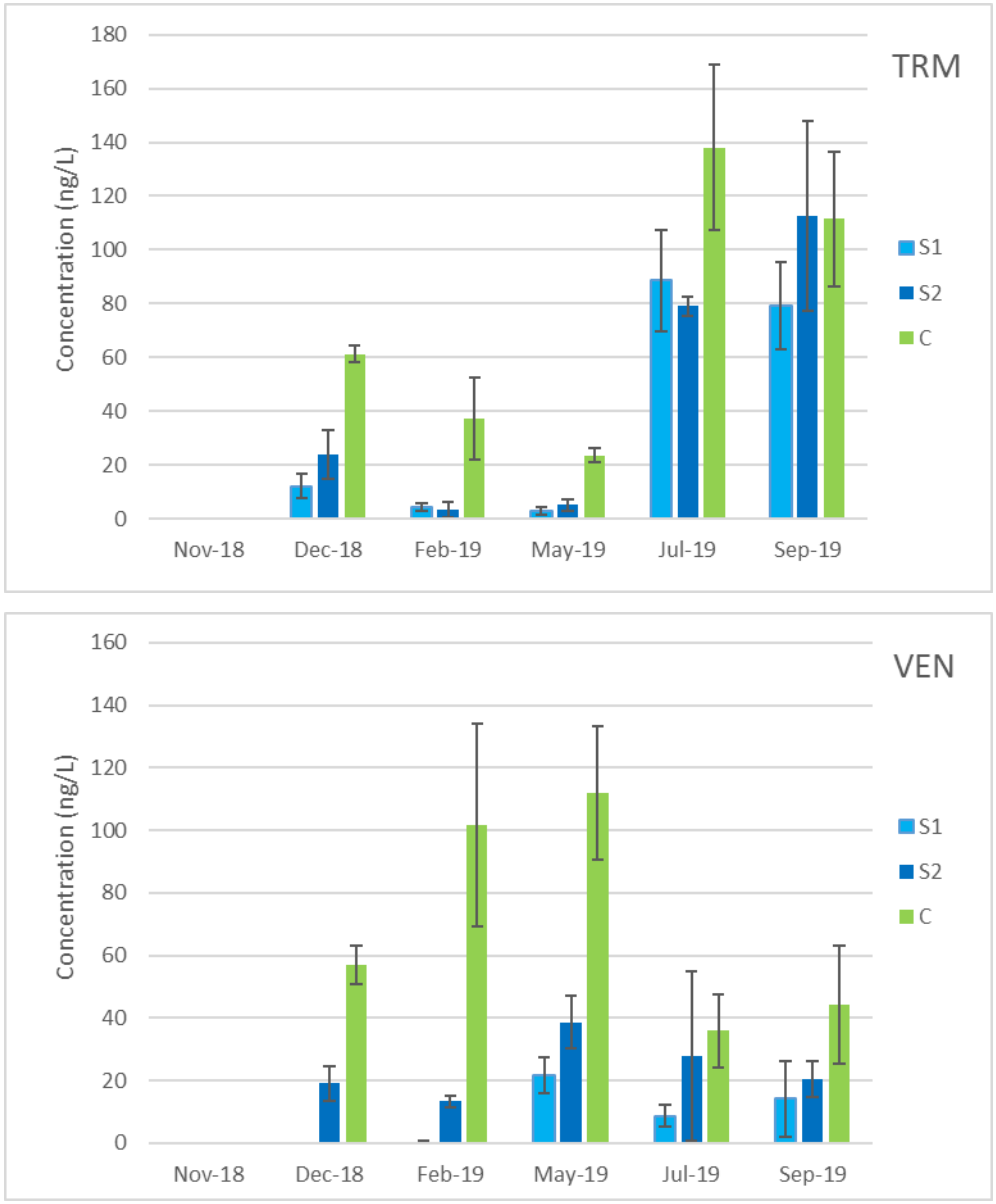
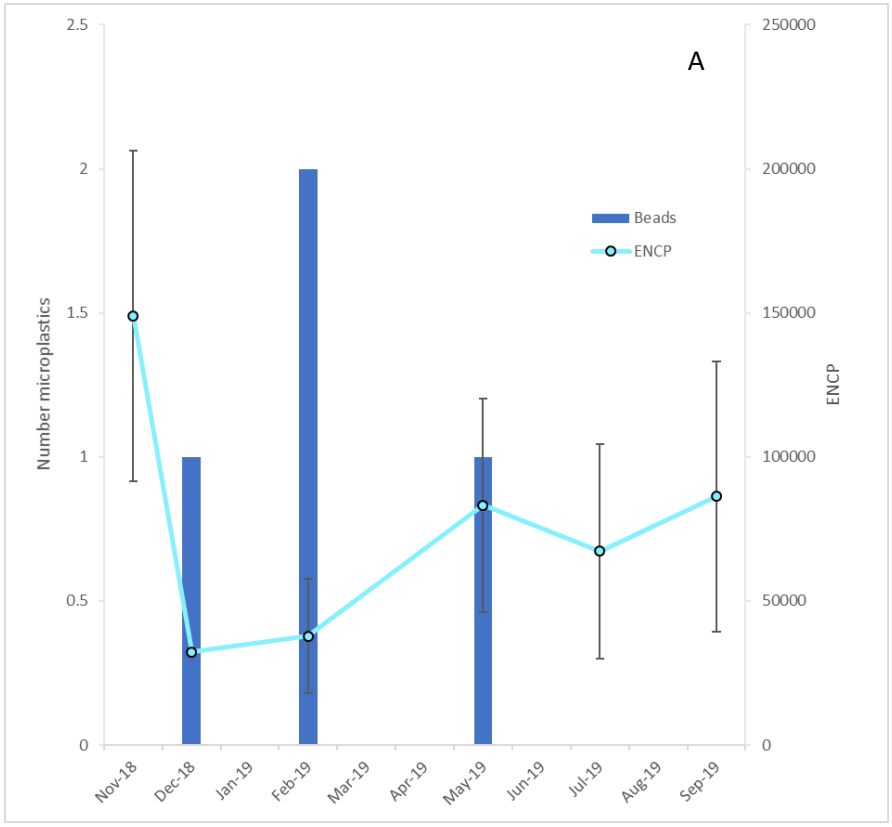
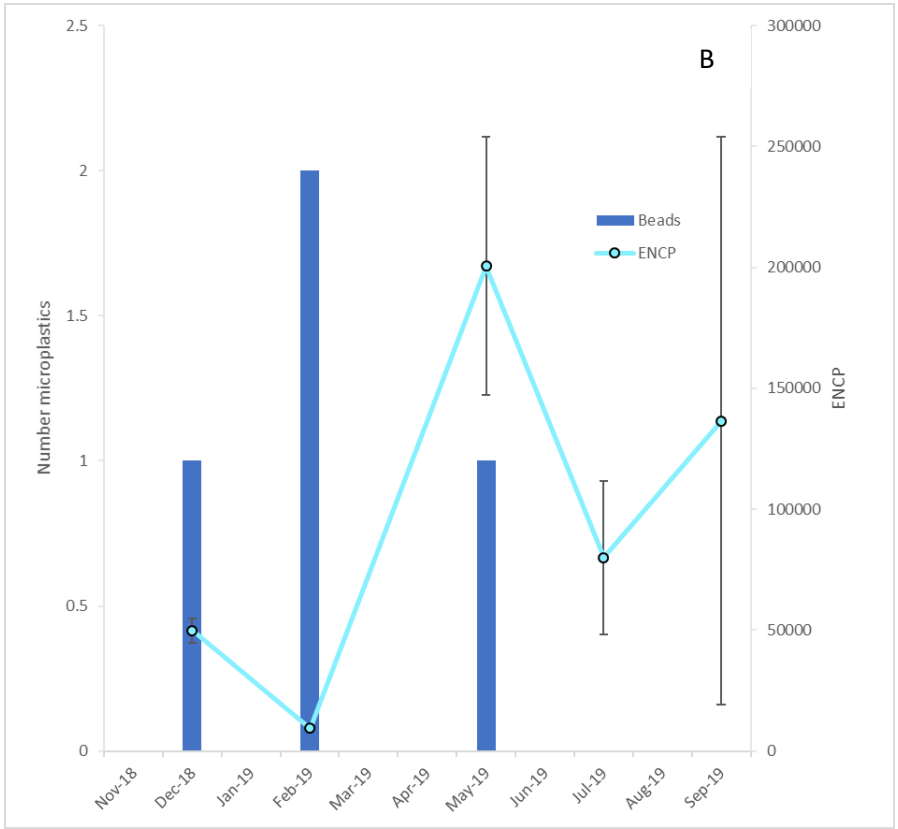


Figure A 10 Measured concentrations of pharmaceuticals carbamazepine (CBZ), sotalol (SOT), trimethoprim (TRM) and venlafaxine (VEN) in the three influent streams at Malabar (S1 and S2) and Cronulla (C).

Table A 5 Pharmaceutical concentrations in wastewater influent and parameters used for estimating contributing population equivalents.
Wastewater flow rates are based on average of values recorded every 15 min over the 24 h composite sampling period; wastewater was collected from 00:56-0:00 at Cronulla and 0:51-0:00 at Malabar. The two influent streams at Malabar (S1 and S2) make up approximately 30% (S1) and 70% (S2) of total flows.

PHARMACEUTICAL	DATE	CRONULLA		MALABAR	
		Flow (ML/day)	Concentration (ng/L)	Flow (ML/day)	Concentration (ng/L)
Carbamazepine	5th-6th Nov 2018	48±14	33±7	475±63	12±6 (S1), 27±5 (S2)
	5th-6th Dec 2018	48±13	10±1	458±48	3 (S1), 50±23 (S2)
	5th-6th Feb 2019	47±14	17±5	485±53	4±2 (S1), 13±4 (S2)
	30th April – 1st May 2019	45±22	13±6	457±46	9±4 (S1), 61±10 (S2)
	30th-31st Jul 2019	47±16	23±5	458±60	7±4 (S1), 21±6 (S2)
	25th-26th Sep 2019	55±31	26±14	477±61	9±5 (S1), 12±6 (S2)
Sotalol	5th-6th Nov 2018	48±14	ND	475±63	ND
	5th-6th Dec 2018	48±13	61±3	458±48	12±5 (S1), 24±9 (S2)
	5th-6th Feb 2019	47±14	11±1	485±53	1.5±0.2 (S1), 9±2 (S2)
	30th April – 1st May 2019	45±22	ND	457±46	1.1±0.7 (S1), 2.7±0.6 (S2)
	30th-31st Jul 2019	47±16	15±3	458±60	6.5±2.7 (S1), 9.5±1 (S2)
	25th-26th Sep 2019	55±31	25±8	477±61	24±6 (S1), 12±6 (S2)
Trimethoprim	5th-6th Nov 2018	48±14	ND	475±63	ND
	5th-6th Dec 2018	48±13	61±3	458±48	12±5 (S1), 24±9 (S2)
	5th-6th Feb 2019	47±14	37±15	485±53	4±1.4 (S1), 3.4±2.8 (S2)
	30th April – 1st May 2019	45±22	24±3	457±46	3±1.4 (S1), 5±2.3 (S2)
	30th-31st Jul 2019	47±16	138±31	458±60	89±19 (S1), 79±4 (S2)
	25th-26th Sep 2019	55±31	111±25	477±61	79±16 (S1), 113±35 (S2)
Venlafaxine	5th-6th Nov 2018	48±14	ND	475±63	ND
	5th-6th Dec 2018	48±13	57±6	458±48	5.4±0.5 (S1), 19±6 (S2)
	5th-6th Feb 2019	47±14	102±32	485±53	1 (S1), 13±2 (S2)
	30th April – 1st May 2019	45±22	112±31	457±46	22±6 (S1), 39±8 (S2)
	30th-31st Jul 2019	47±16	36±12	458±60	9±3 (S1), 28±27 (S2)
	25th-26th Sep 2019	55±31	44±19	477±61	14±12 (S1), 20±6 (S2)


A
Beads
ENCP
Number microplastics
ENCP
2.5
2
1.5
1
0.5
0
250000
200000
150000
100000
50000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
Beads
ENCP
Number microplastics
ENCP
2.5
2
1.5
1
0.5
0
300000
250000
200000
150000
100000
50000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19

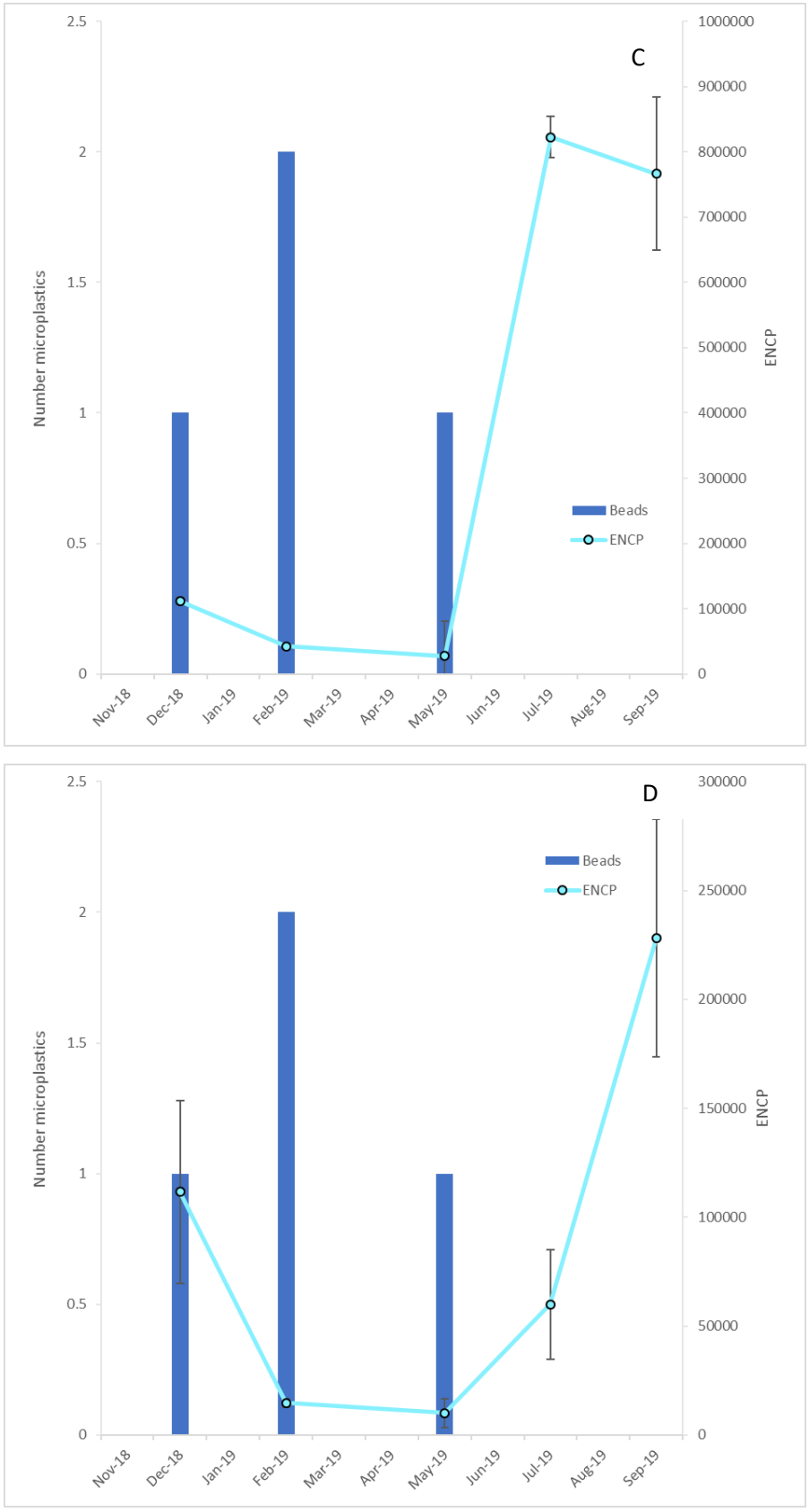
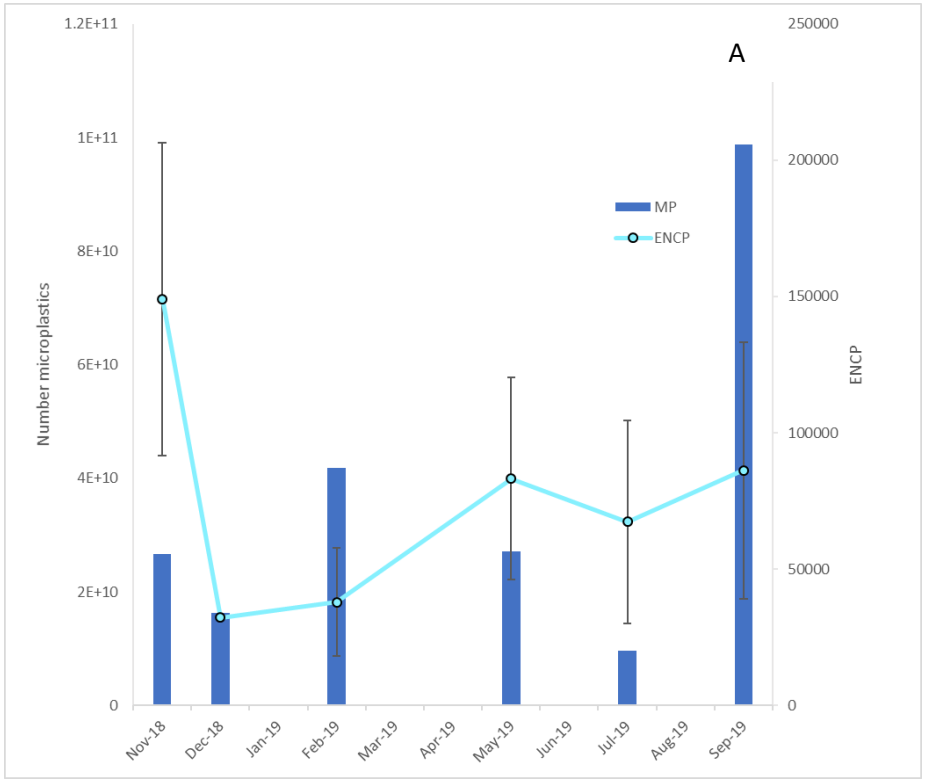
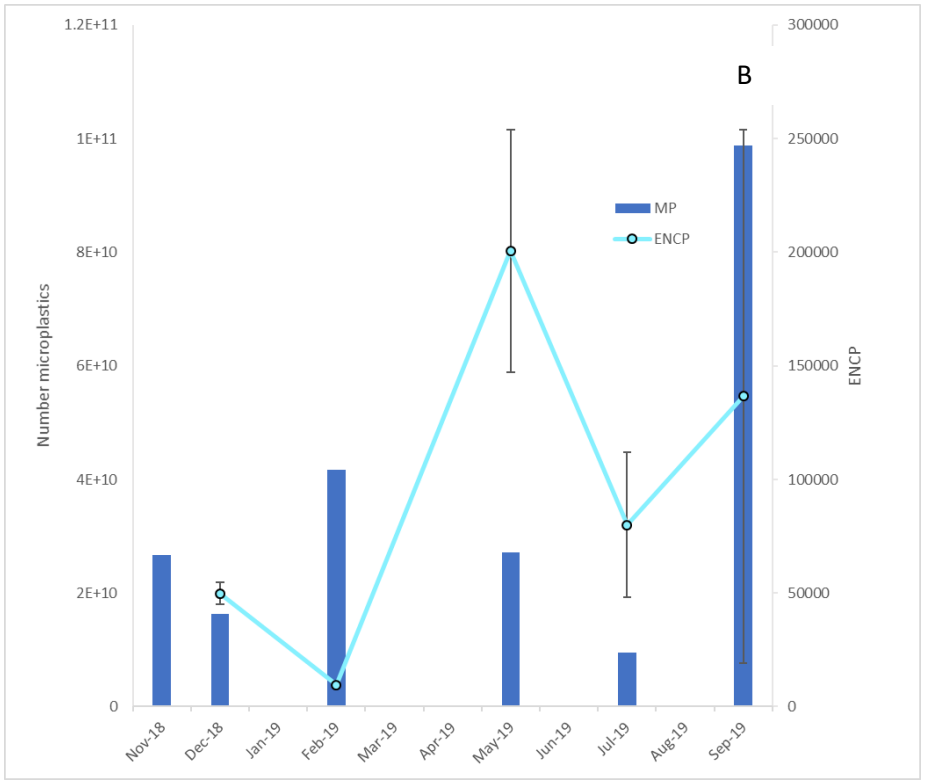


Figure A 11 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with microbead counts for each sampling period in Malabar S1 wastewater.


A
MP
ENCP
Number microplastics
ENCP
1.2E+11
1E+11
8E+10
6E+10
4E+10
2E+10
0
250000
200000
150000
100000
50000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
MP
ENCP
Number microplastics
ENCP
1.2E+11
1E+11
8E+10
6E+10
4E+10
2E+10
0
300000
250000
200000
150000
100000
50000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19

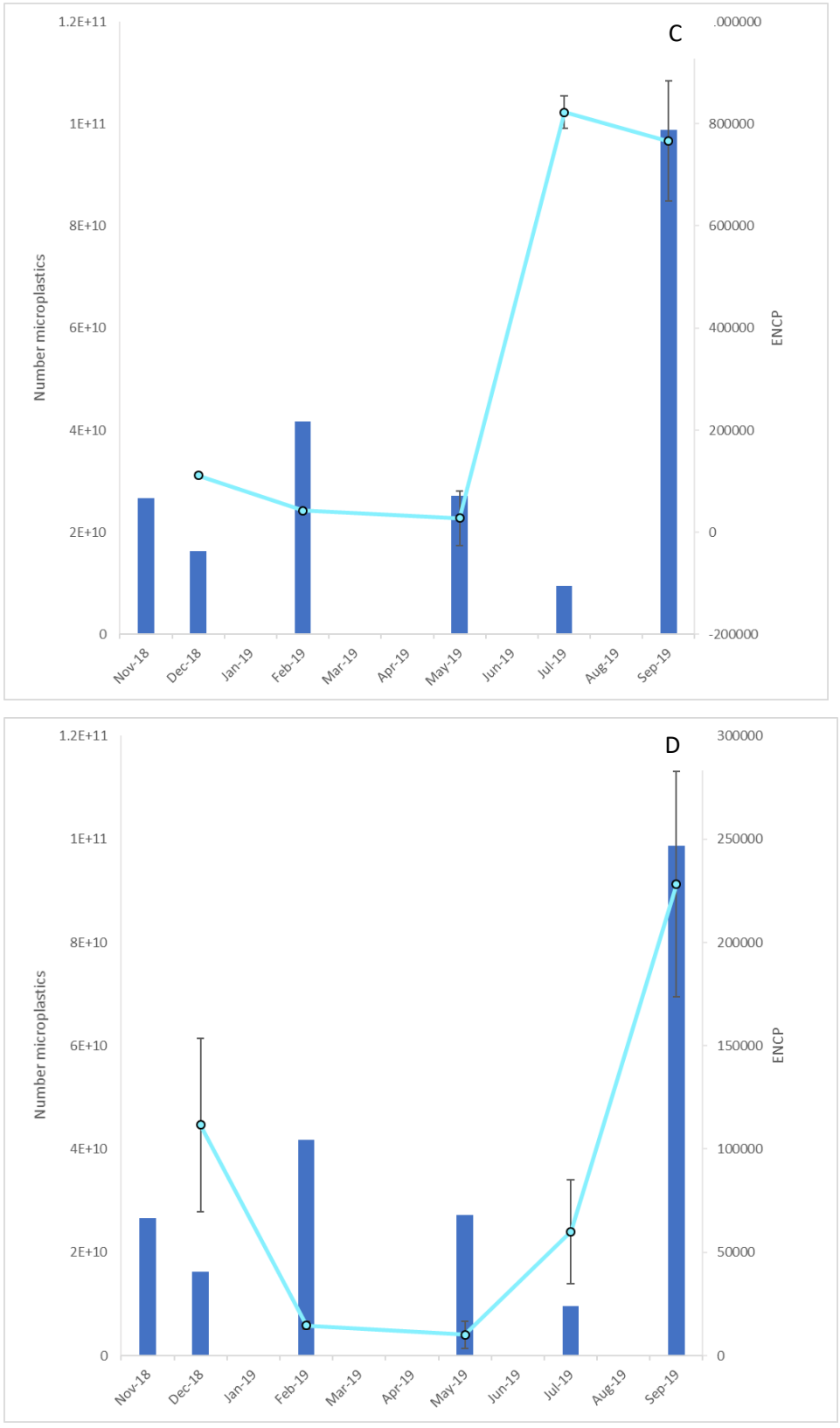
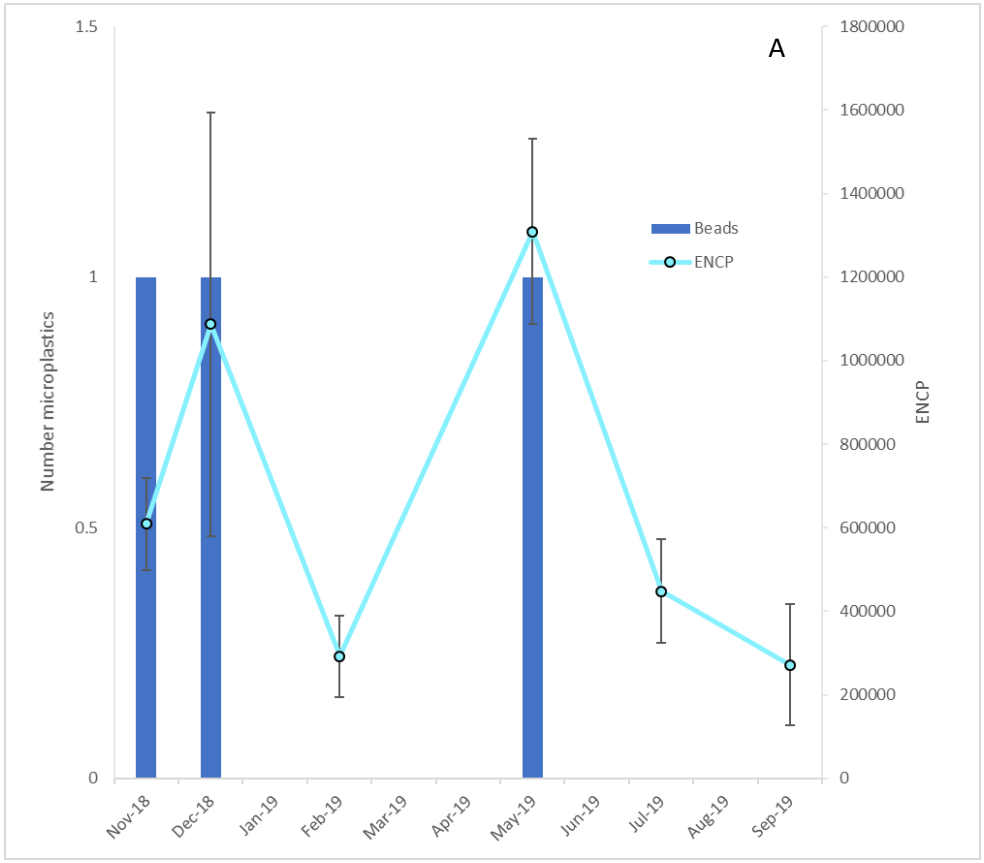
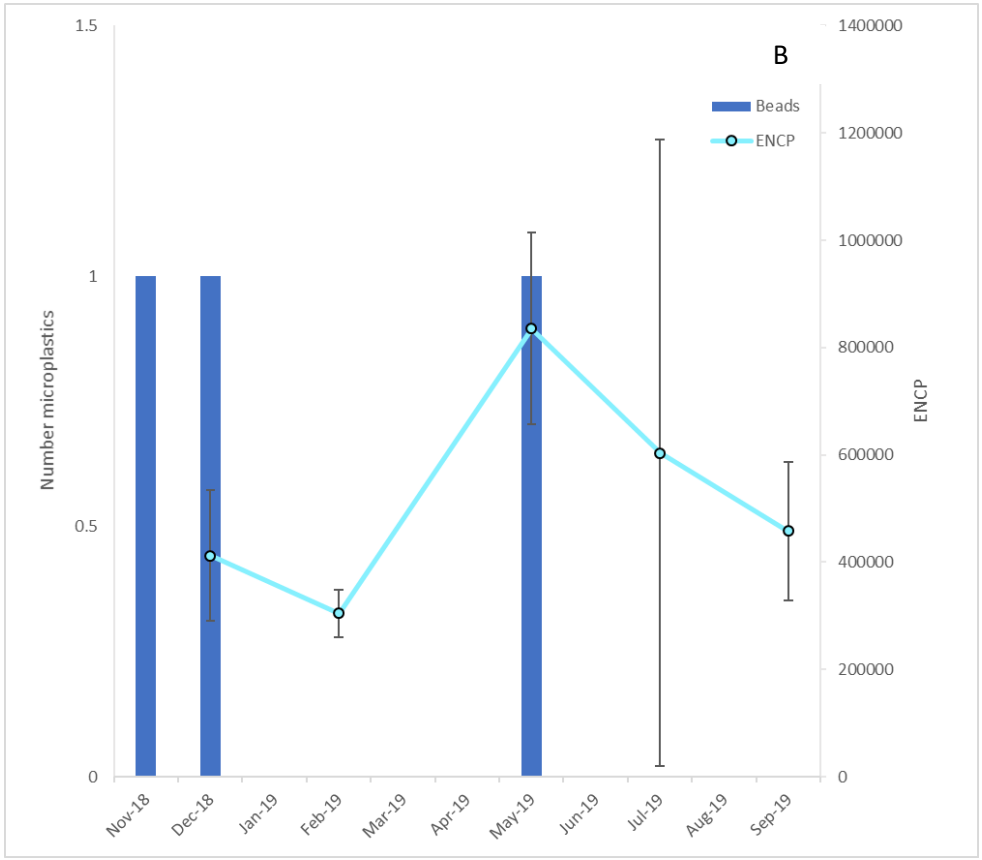


Figure A 12 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with total estimated microplastic counts entering Malabar WWTP through S1 wastewater for each sampling period.


A
Beads
ENCP
Number microplastics
ENCP
1.5
1
0.5
0
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
Beads
ENCP
Number microplastics
ENCP
1.5
1
0.5
0
1400000
1200000
1000000
800000
600000
400000
200000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19

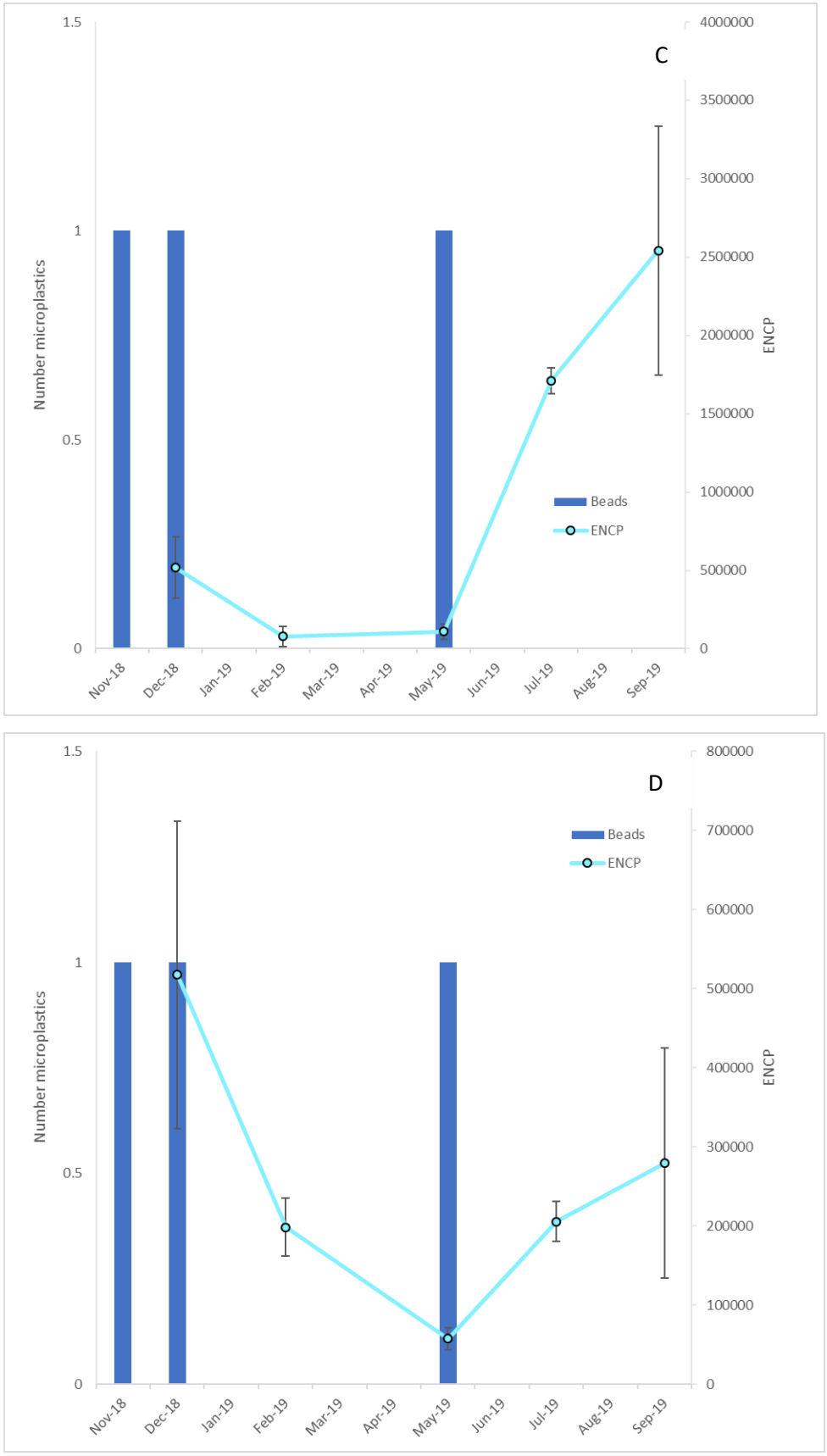
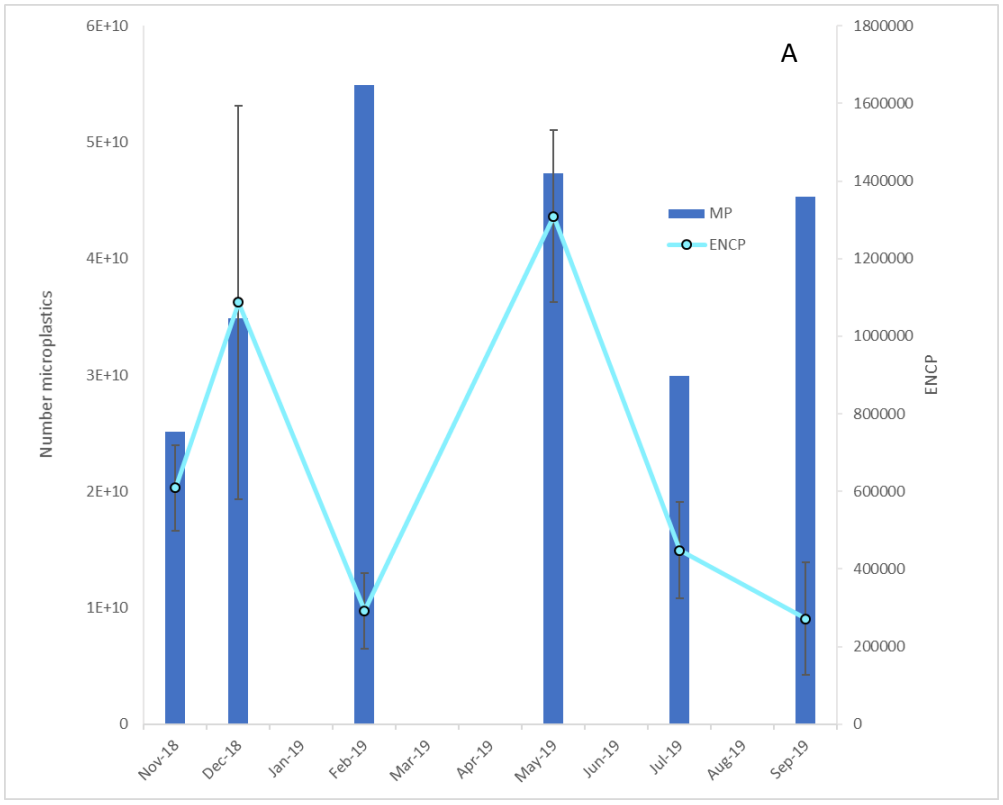
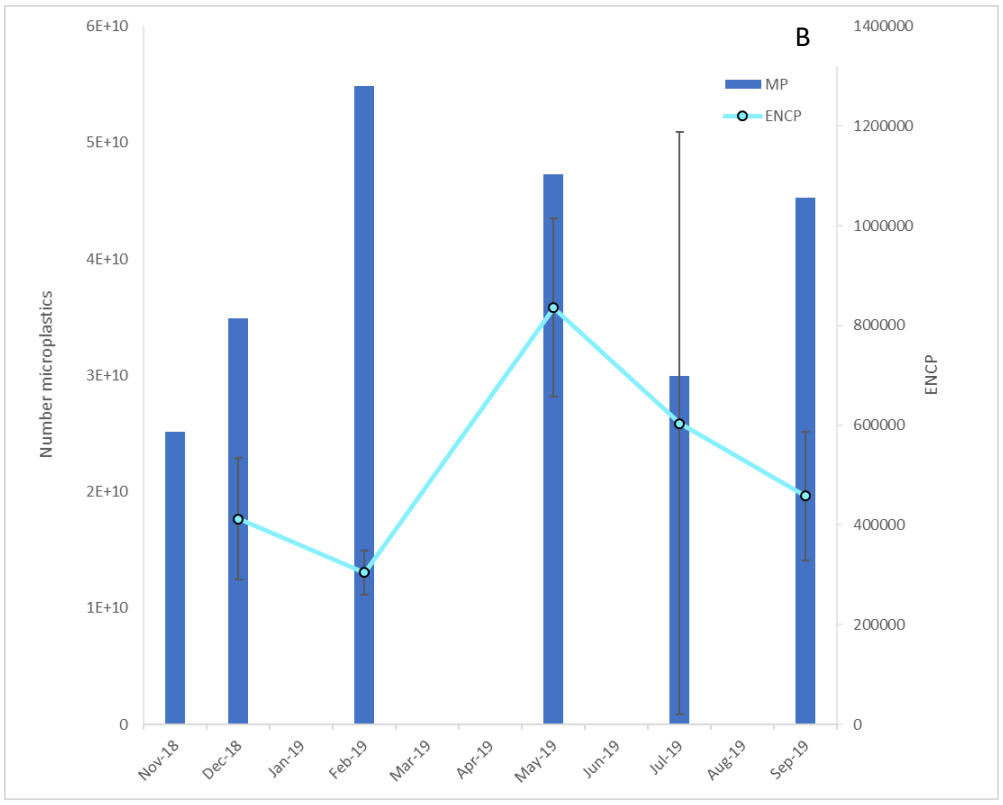
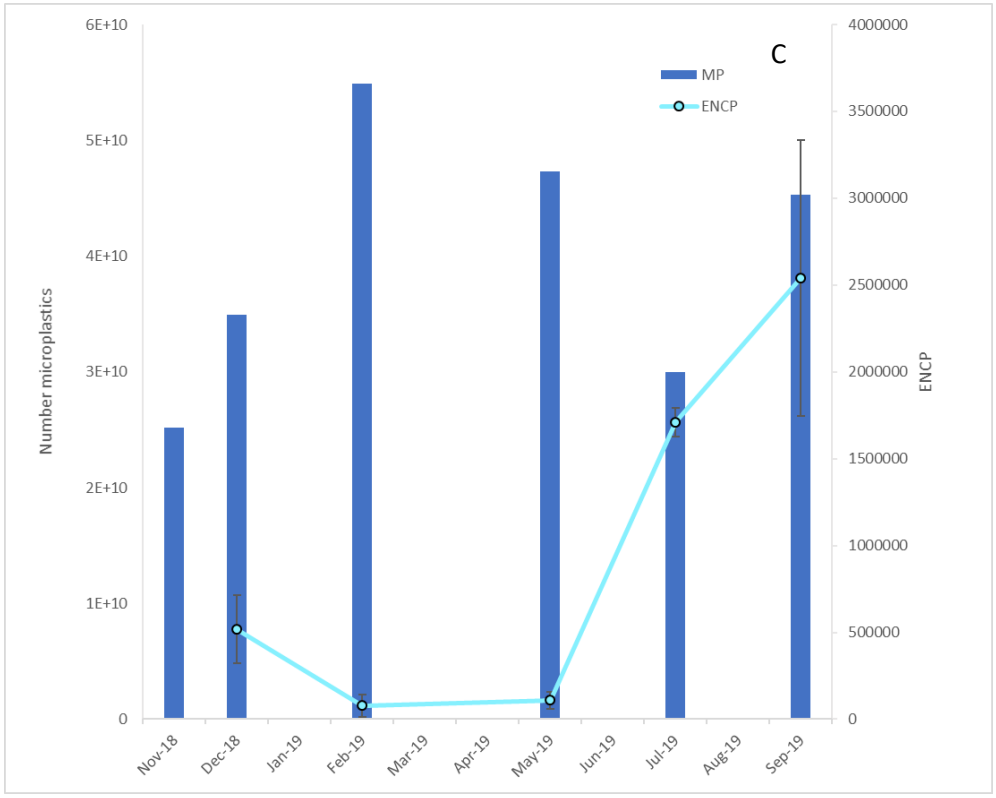


Figure A 13 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with microbead counts for each sampling period in Malabar S2 wastewater.


A
Number microplastics
ENCP
MP
ENCP
6E+10
5E+10
4E+10
3E+10
2E+10
1E+10
0
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
Number microplastics
ENCP
MP
ENCP
6E+10
5E+10
4E+10
3E+10
2E+10
1E+10
0
1400000
1200000
1000000
800000
600000
400000
200000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19



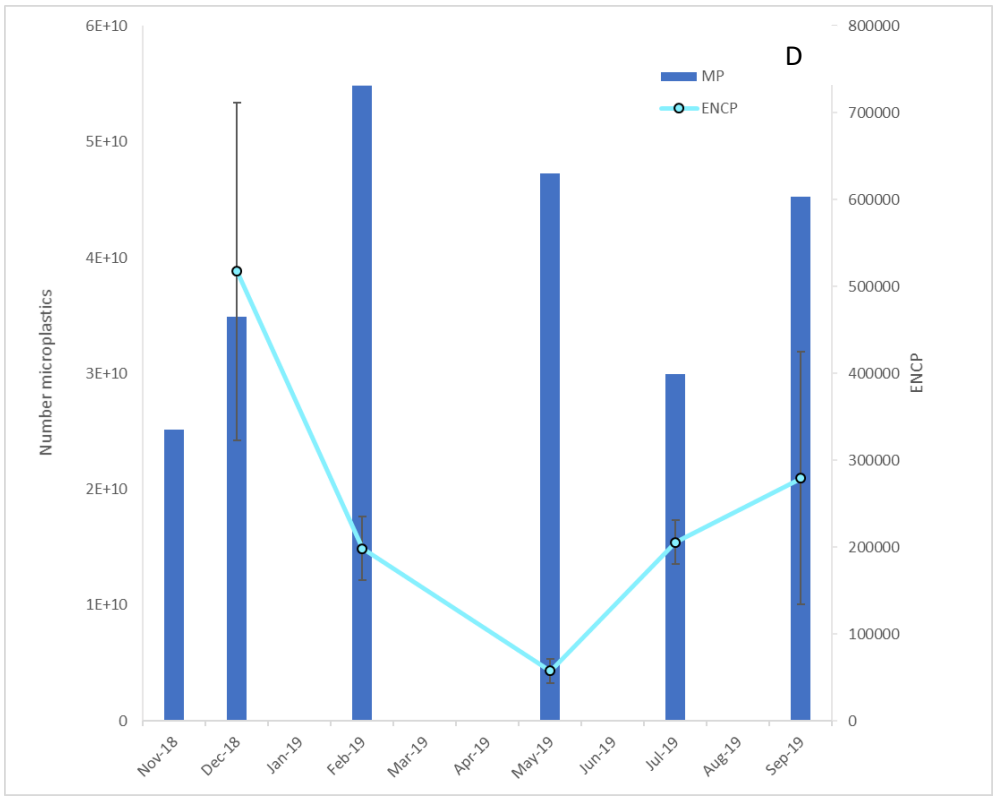
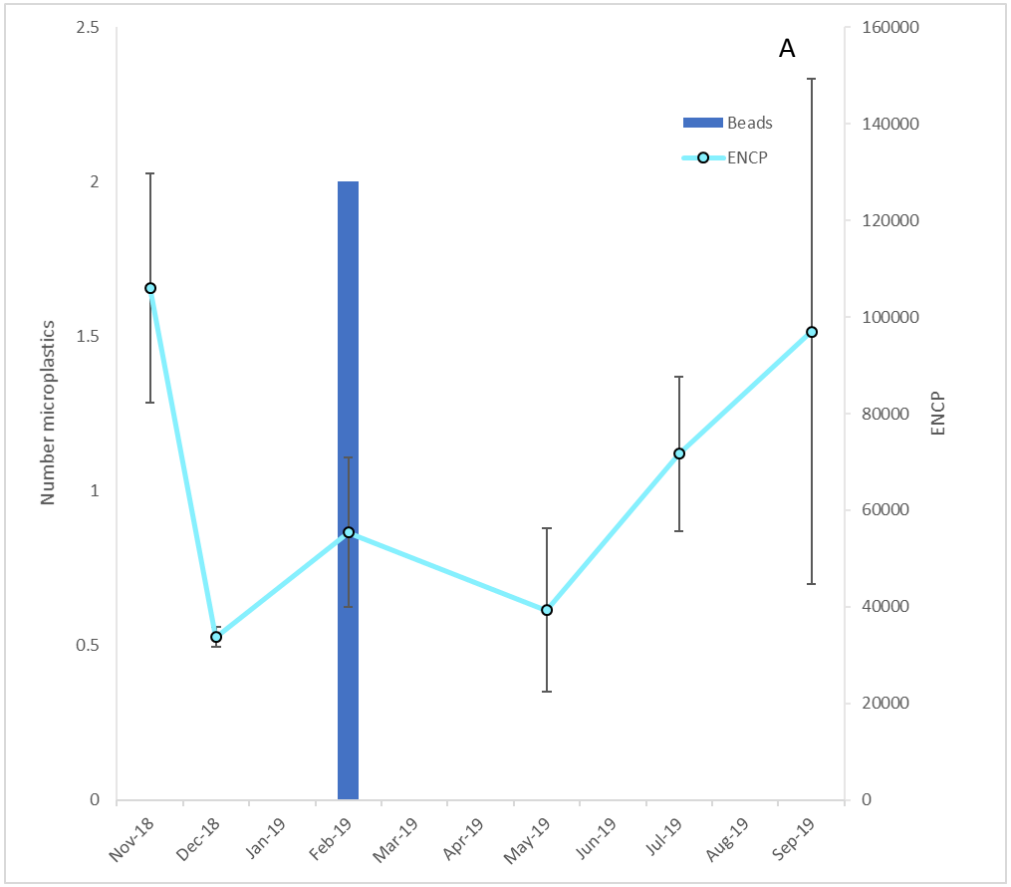
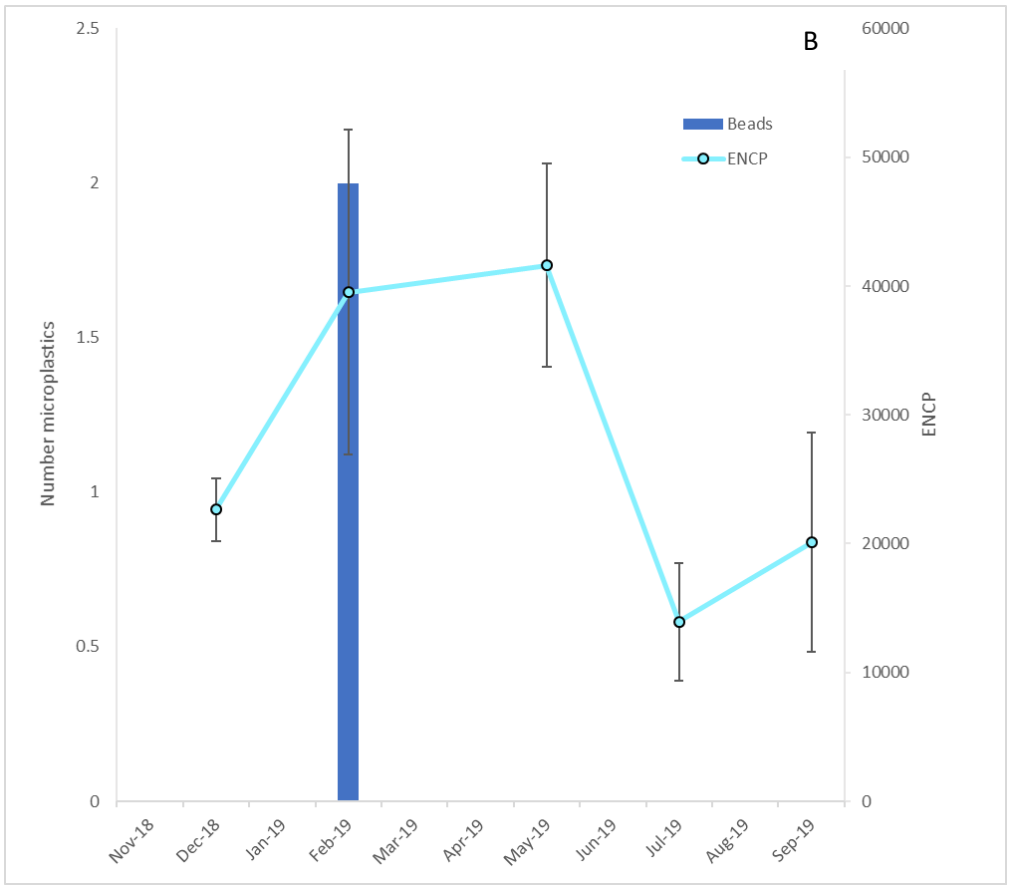
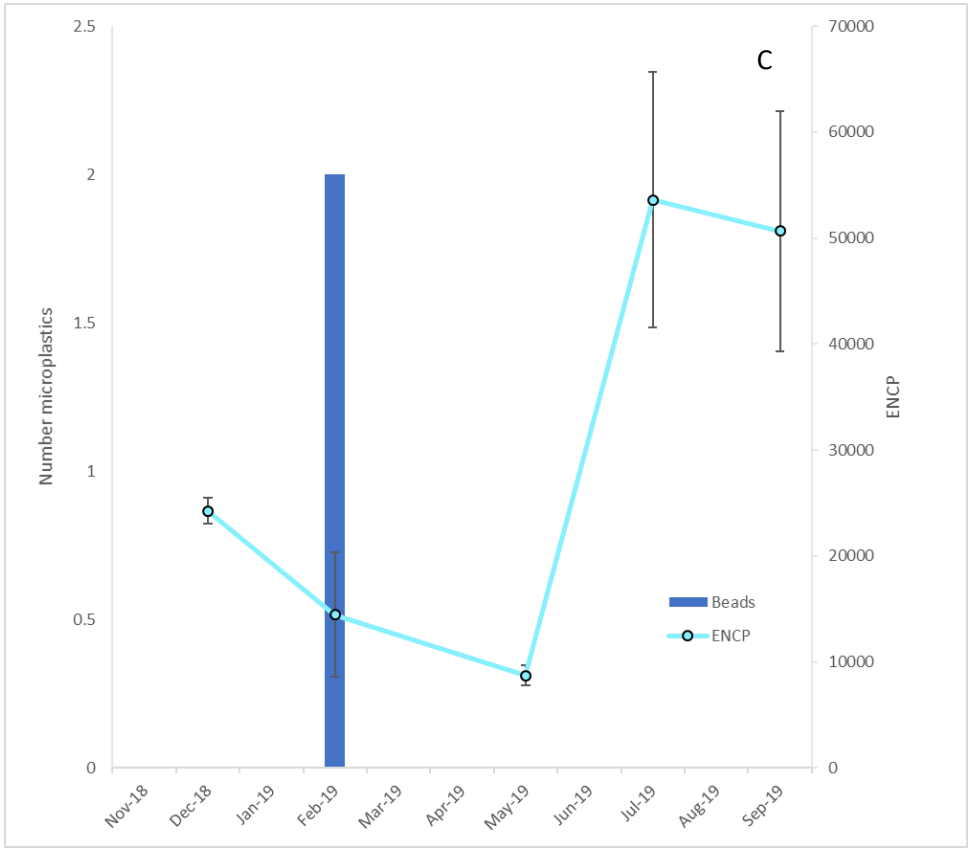


Figure A 14 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with total estimated microplastic counts entering Malabar WWTP through S2 wastewater for each sampling period.


A
Beads
ENCP
Number microplastics
ENCP
2.5
2
1.5
1
0.5
0
160000
140000
120000
100000
80000
60000
40000
20000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
Beads
ENCP
Number microplastics
ENCP
2.5
2
1.5
1
0.5
0
60000
50000
40000
30000
20000
10000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19



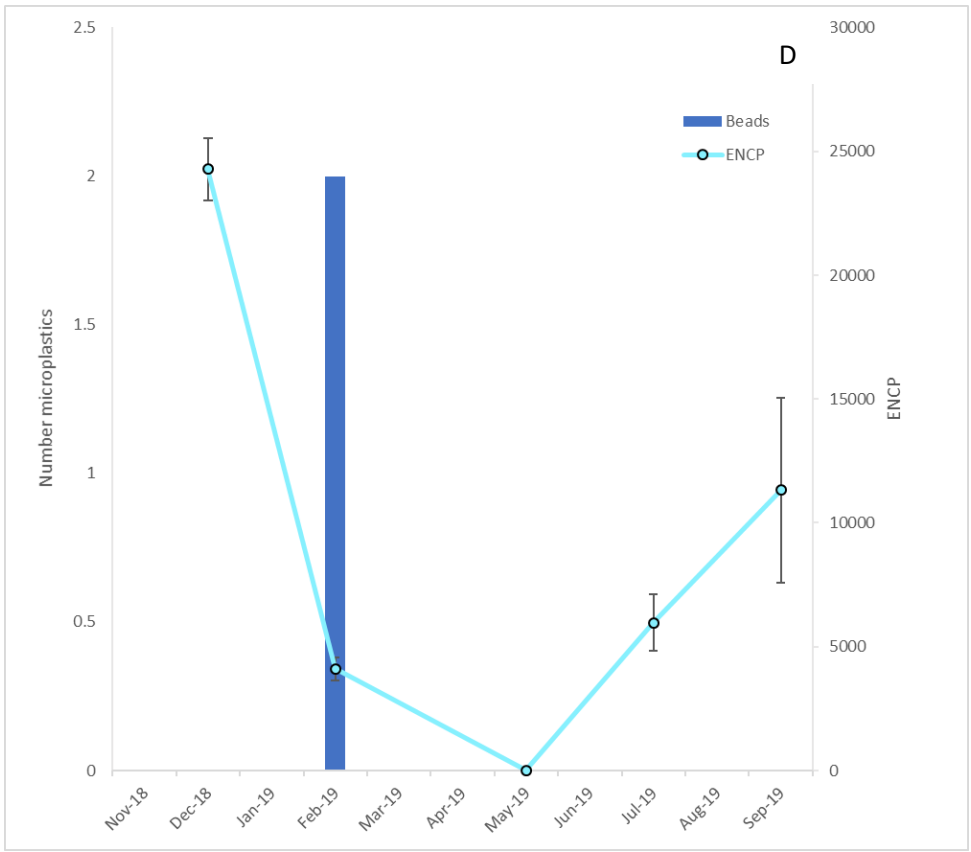
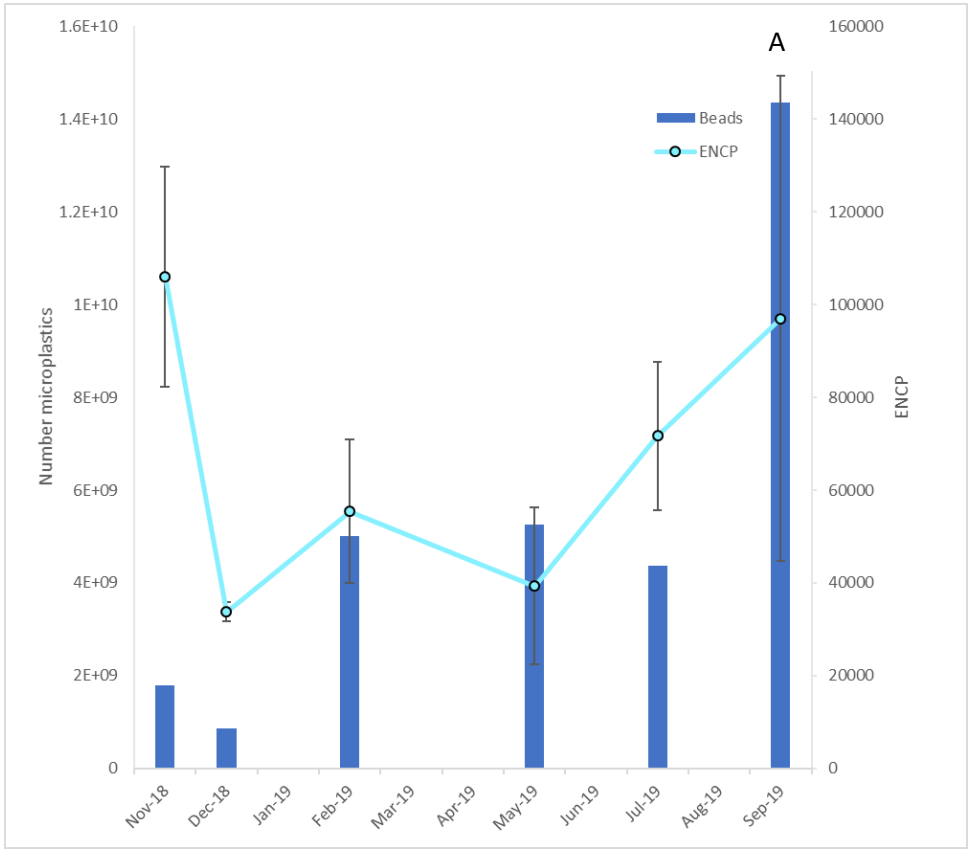
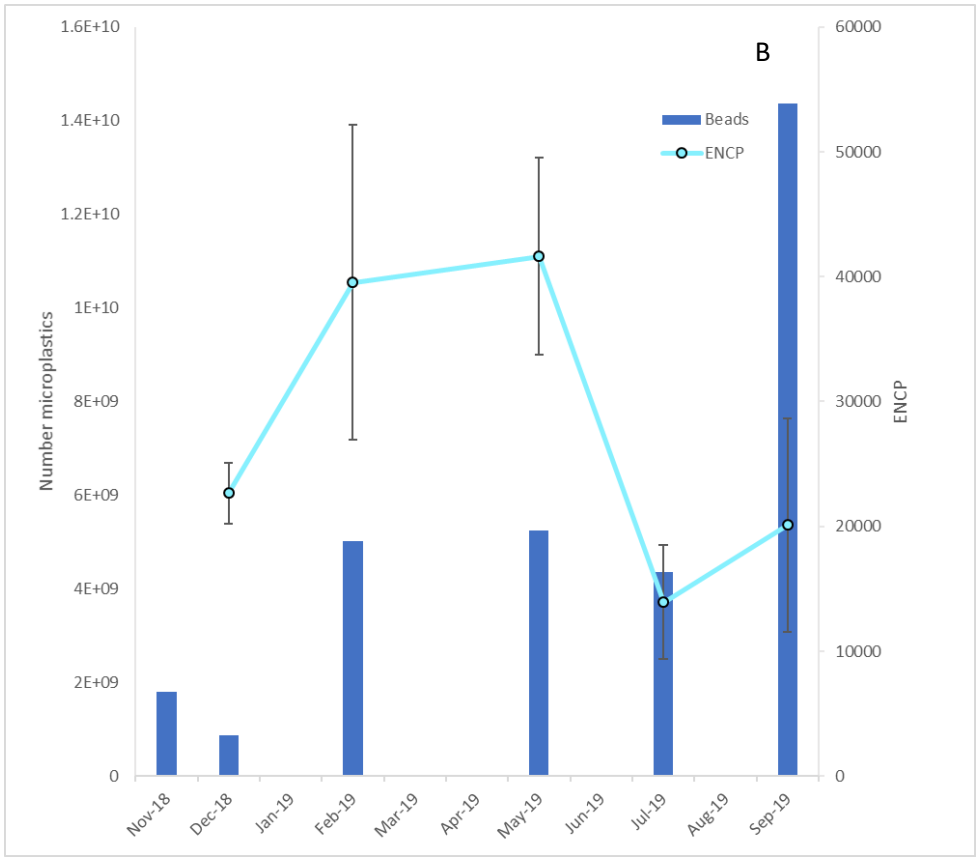


Figure A 15 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with microbead counts for each sampling period in Cronulla influent wastewater.


A
Beads
ENCP
Number microplastics
ENCP
1.6E+10
1.4E+10
1.2E+10
1E+10
8E+09
6E+09
4E+09
2E+09
0
160000
140000
120000
100000
80000
60000
40000
20000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19


B
Beads
ENCP
Number microplastics
ENCP
1.6E+10
1.4E+10
1.2E+10
1E+10
8E+09
6E+09
4E+09
2E+09
0
60000
50000
40000
30000
20000
10000
0
Nov-18
Dec-18
Jan-19
Feb-19
Mar-19
Apr-19
May-19
Jun-19
Jul-19
Aug-19
Sep-19

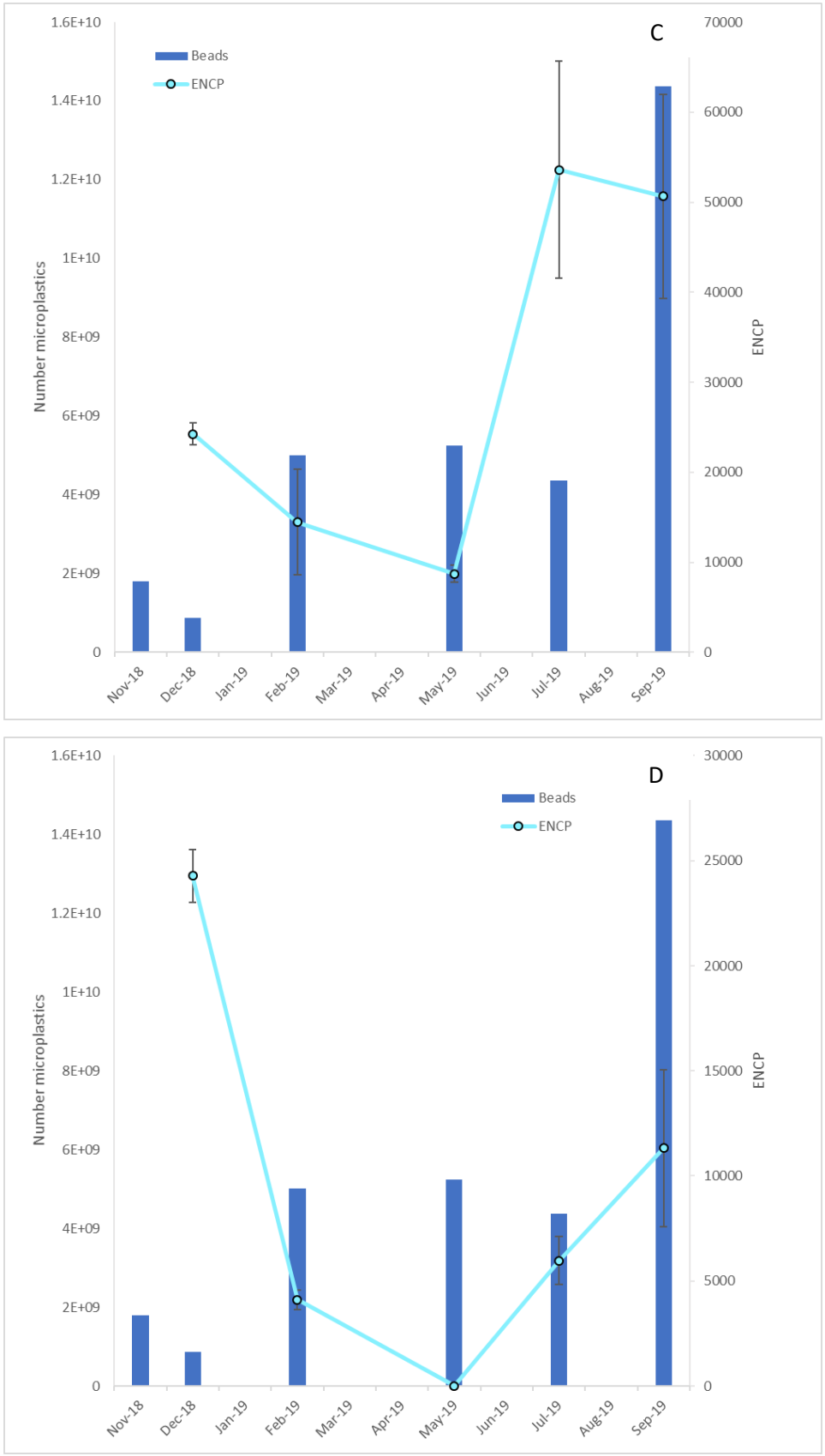
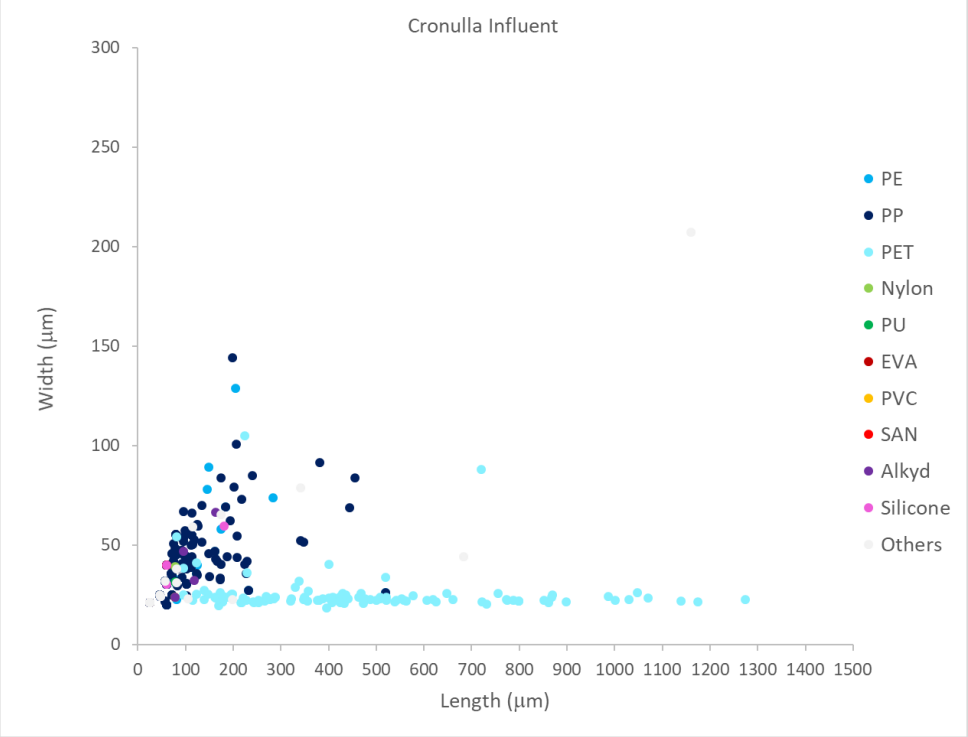
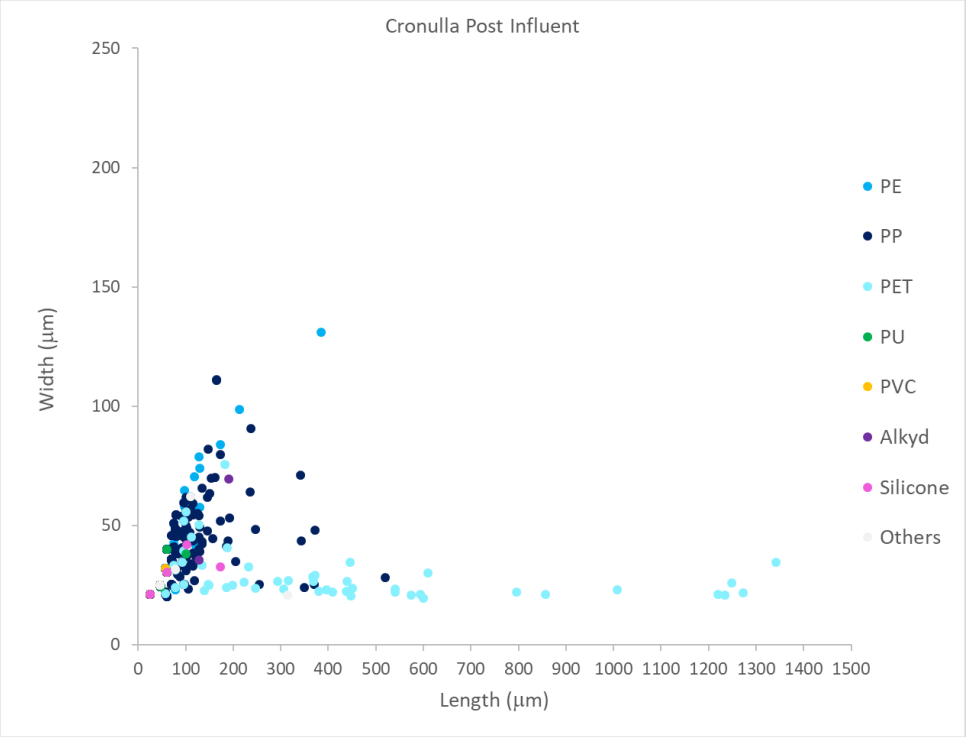


Figure A 16 Estimated number of contributing people (ENCP) based on (a) carbamazepine (CBZ) (b) venlafaxine (VEN) (c) trimethoprim (TRM) and (d) sotalol (SOT) concentration, their respective pharmacokinetics and wastewater flows compared with total estimated microplastic counts entering Cronulla WWTP through wastewater for each sampling period.

A.4 Size distribution of microplastics in wastewater





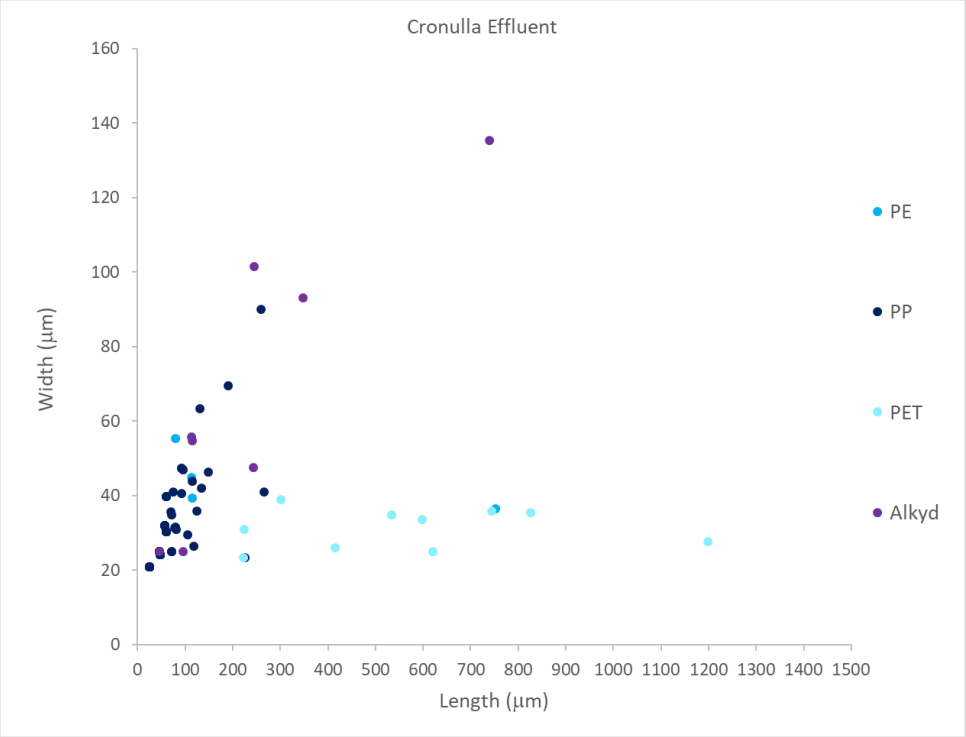
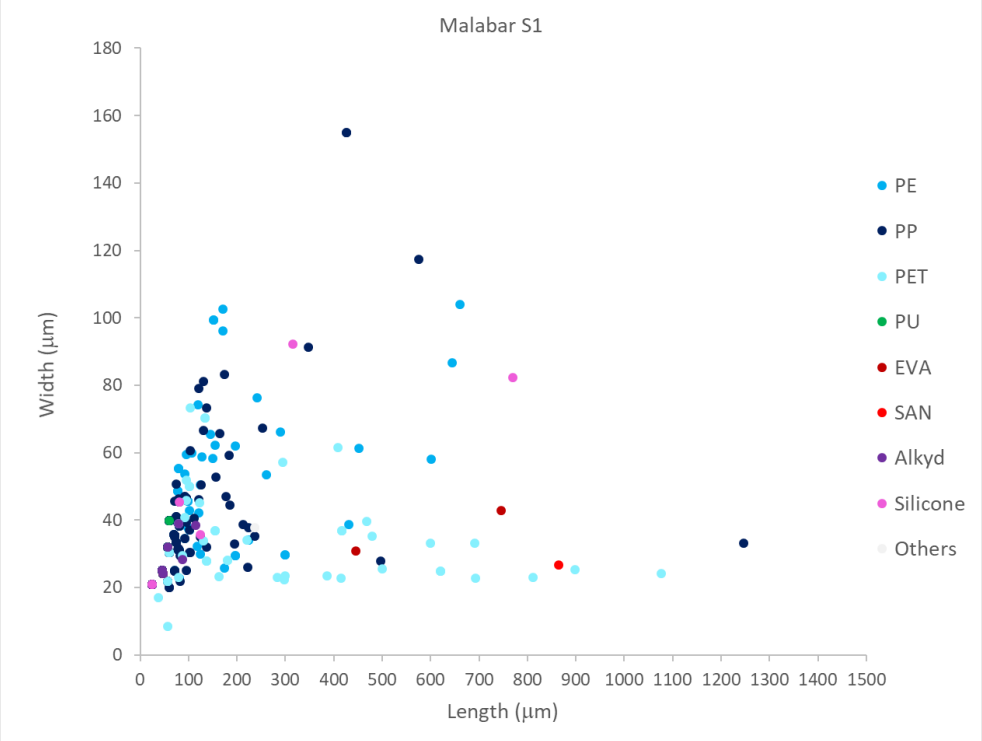
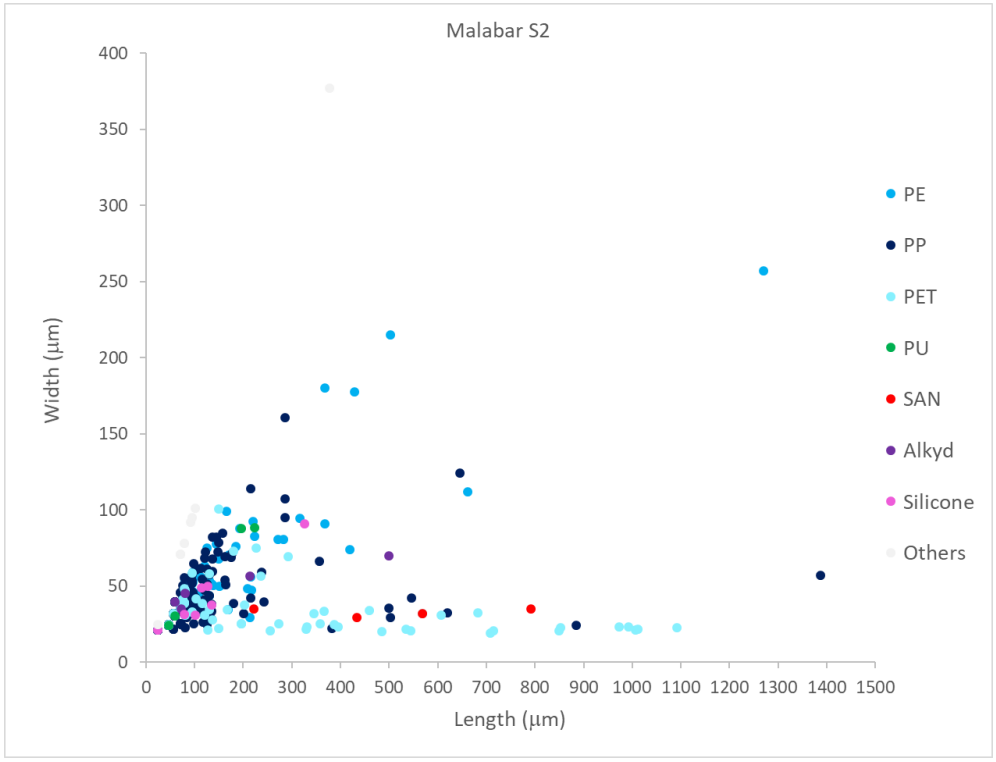


Figure A 17 Size distribution of all polymers detected in Cronulla WWTP influent, primary effluent and effluent for the six sampling events.
The particle size range indicates the lower value in a 25 µm bracket as a percentage of the total particle numbers.





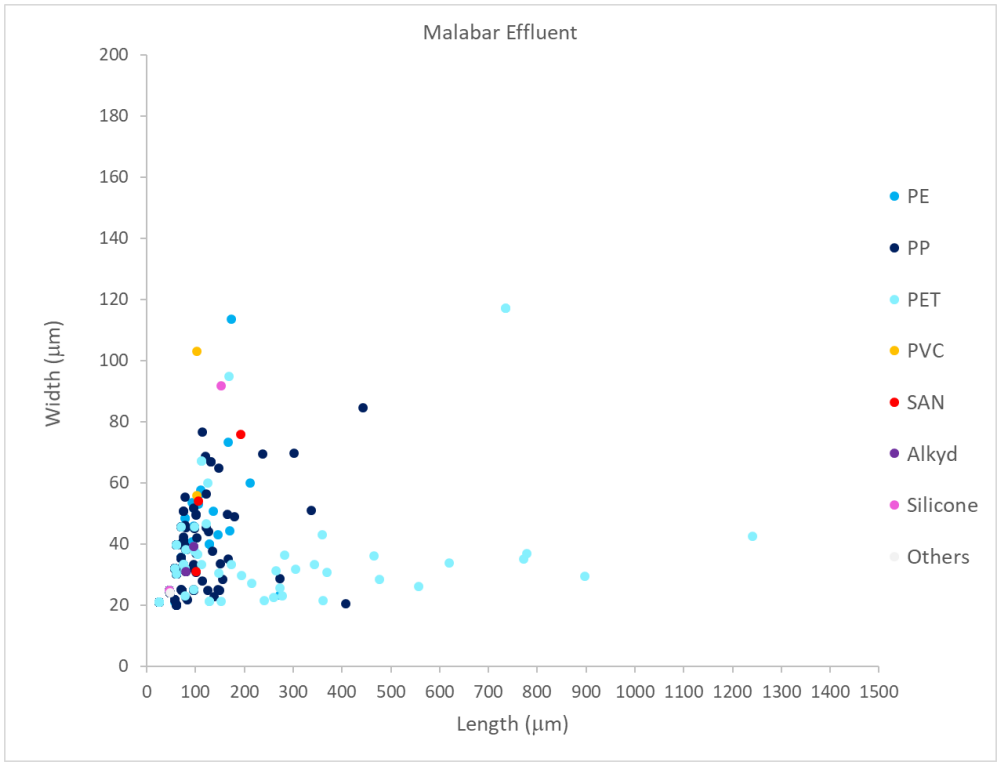
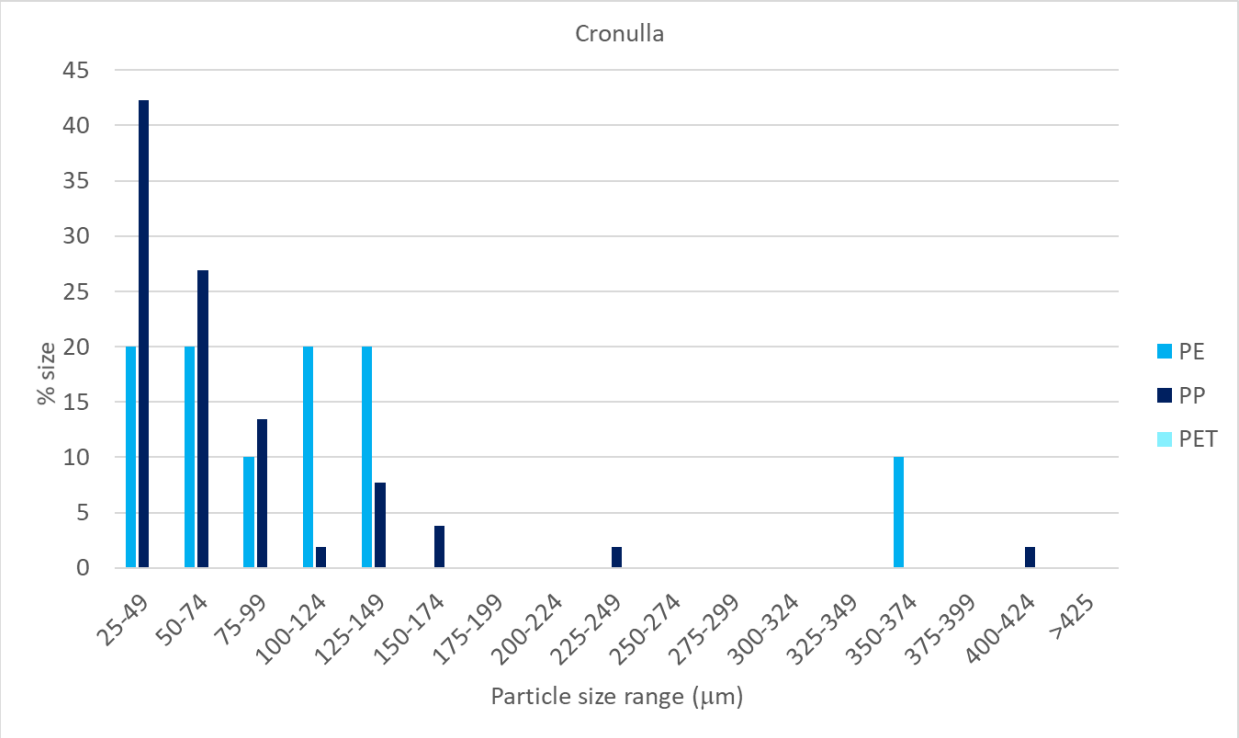
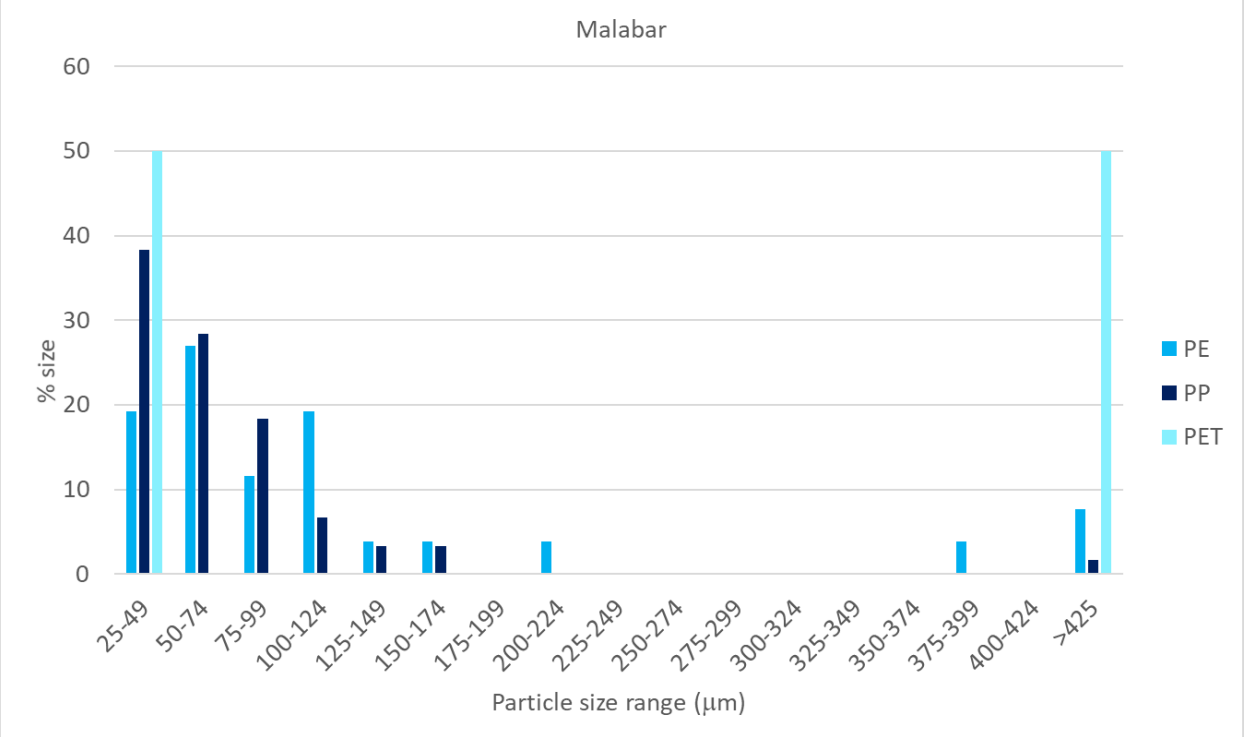
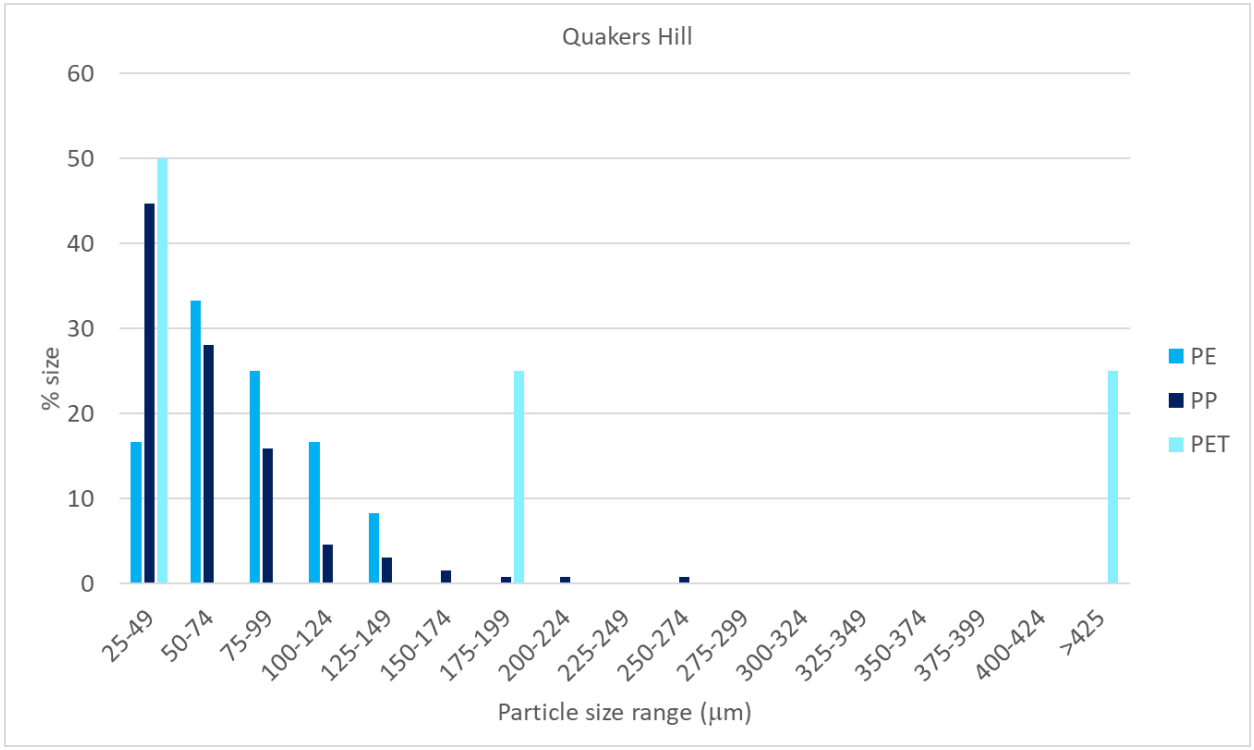


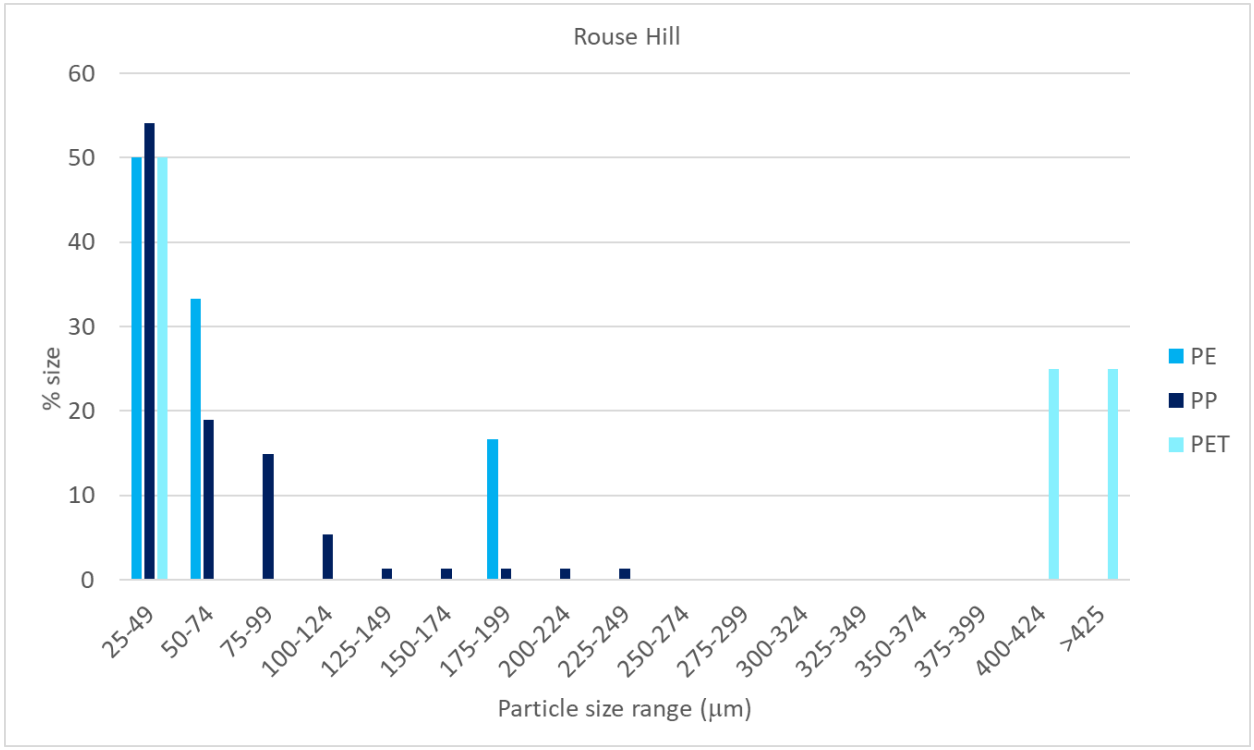
Figure A 18 Size distribution of all polymers detected in Malabar WWTP influent (S1 and S2) and effluent streams for the six sampling events.

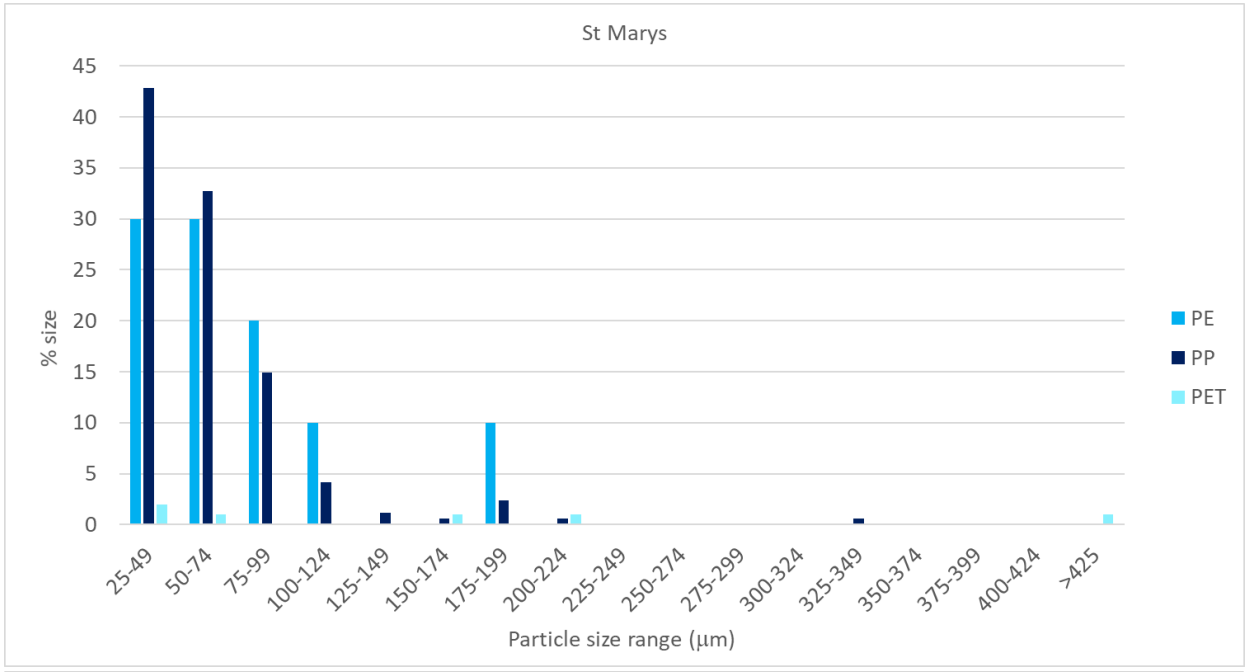
A.5 Size distribution of microplastics in biosolids

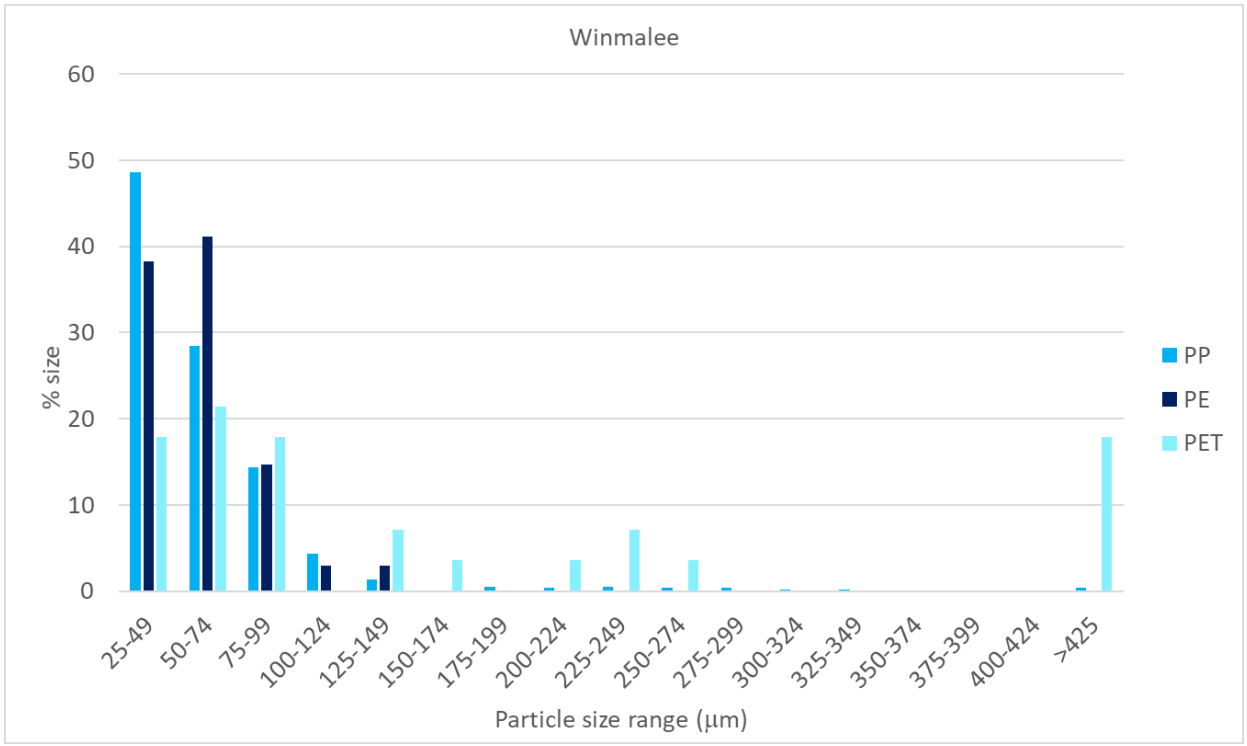





Quakers Hill
60
50
40
30
20
10
0
% size
25-49
50-74
75-99
100-124
125-149
150-174
175-199
200-224
225-249
250-274
275-299
300-324
325-349
350-374
375-399
400-424
>425
Particle size range (µm)
PE
PP
PET


Rouse Hill
60
50
40
30
20
10
0
% size
25-49
50-74
75-99
100-124
125-149
150-174
175-199
200-224
225-249
250-274
275-299
300-324
325-349
350-374
375-399
400-424
>425
Particle size range (µm)
PE
PP
PET


St Marys
45
40
35
30
25
20
15
10
5
0
% size
25-49
50-74
75-99
100-124
125-149
150-174
175-199
200-224
225-249
250-274
275-299
300-324
325-349
350-374
375-399
400-424
>425
Particle size range (µm)
PE
PP
PET


Winmalee
60
50
40
30
20
10
0
% size
25-49
50-74
75-99
100-124
125-149
150-174
175-199
200-224
225-249
250-274
275-299
300-324
325-349
350-374
375-399
400-424
>425
Particle size range (µm)
PP
PE
PET

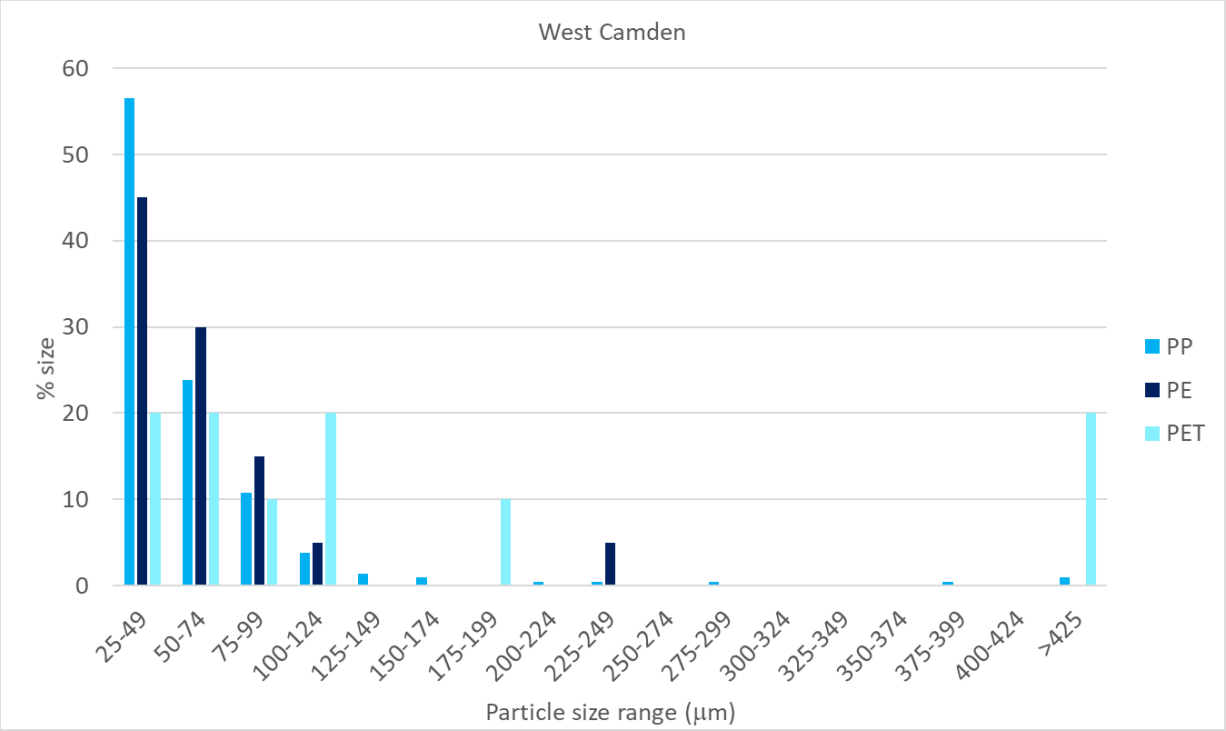


Figure A 19 Size distribution of PP, PE and PET polymers (which represent the majority of biosolids detected) in biosolids collected from 7 WWTPs in September 2019.
The particle size range indicates the lower value in a 25 µm bracket as a percentage of the total particle numbers.

A.6 Summary of field blank samples collected from Cronulla and Malabar WWTPs

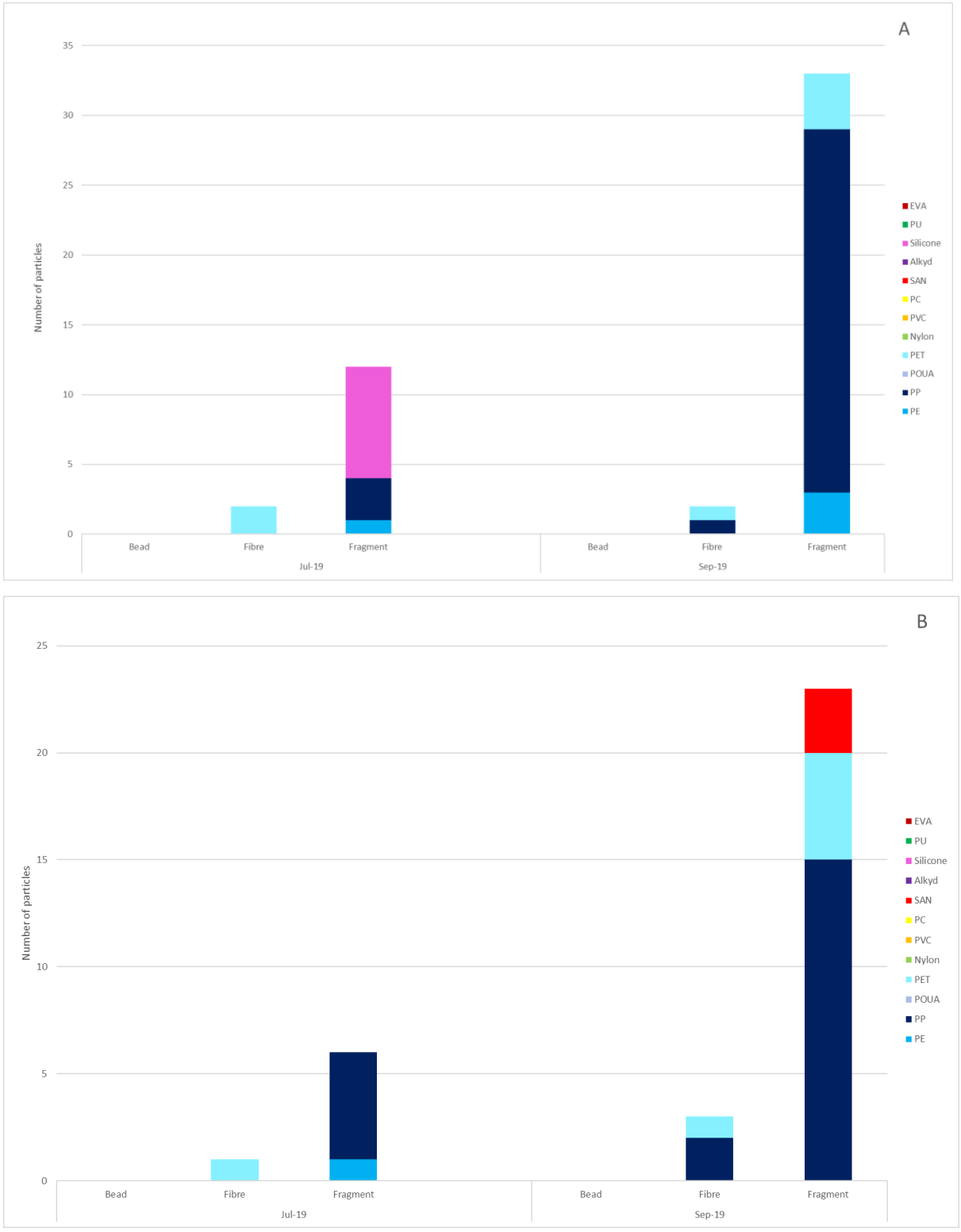


Figure A20 Summary of microplastics present in ultrapure water transported to the field and decanted in conjunction with wastewater collected from (A) Cronulla and (B) Malabar WWTPs

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