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Research Paper

The transport and vertical distribution of microplastics in the Mekong River, SE Asia

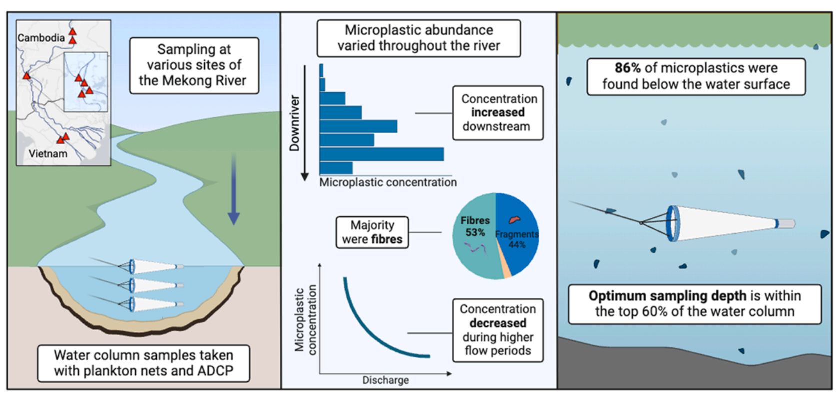
Freija Mendrik [a,*,1,2], Christopher R. Hackney [b], Vivien M. Cumming [c], Catherine Waller [d], Danet Hak [e], Robert Dorrell [a], Nguyen Nghia Hung [f], Daniel R. Parsons [g]

[a] Energy and Environment Institute, University of Hull, UK
[b] School of Geography, Politics and Sociology, Newcastle University, UK
[c] Science Stories Limited, Edinburgh, UK
[d] School of Environmental Sciences, University of Hull, UK
[e] Department of Civil Engineering, Institute of Technology of Cambodia, Cambodia
[f] Southern Institute of Water Resources Research, Viet Nam
[g] International Centre for Informatics and Disaster Resilience, Loughborough University, UK

HIGHLIGHTS

- Microplastics in the Mekong River increased downstream, principally composed of fibres.
- Depth profiles show that 86 % of microplastics were below the water surface.
- An optimum sampling depth is identified to ensure representative sampling.
- Microplastic transport does not follow suspended sediment transport laws.

GRAPHICAL ABSTRACT



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ABSTRACT

Rivers are primary vectors of plastic debris to oceans, but sources, transport mechanisms, and fate of fluvial microplastics (<5 mm) remain poorly understood, impeding accurate predictions of microplastic flux, ecological risk and socio-economic impacts. We report on microplastic concentrations, characteristics and dynamics in the Mekong River, one of the world's largest and polluting rivers, in Cambodia and Vietnam. Sampling throughout the water column at multiple localities detected an average of 24 microplastics m^{-3} (0.073 mg l^{-1}). Concentrations increased downstream from rural Kampi, Cambodia (344 km from river mouth; 2 microplastics m^{-3}, 0.006 mg l^{-1}), to Can Tho, Vietnam (83 km from river mouth; 64 microplastics m^{-3}, 0.182 mg l^{-1}) with most microplastics being fibres (53 %), followed by fragments (44 %) and the most common polymer being

* Corresponding author.
E-mail address: freija.mendrik@plymouth.ac.uk (F. Mendrik).
[1] Current address: International Marine Litter Research Unit, University of Plymouth, Drake Circus, Plymouth, UK.
[2] Current address: School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth, UK.

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polyethylene terephthalate (PET) or polyester. Pathways of microplastic pollution are expected to be from urban wastewater highlighting the need for improved wastewater treatment in this region. On average, 86 % of microplastics are transported within the water column and consequently we identified an optimum sampling depth capturing a representative flux value, highlighting that sampling only the water surface substantially biases microplastic concentration predictions. Additionally, microplastic abundance does not linearly follow discharge changes during annual monsoonal floods or mirror siliciclastic sediment transport, as microplastic concentrations decrease rapidly during higher monsoon flows. The findings reveal complex microplastic transport in large rivers and call for improved sampling methods and predictive models to better assess environmental risk and guide policy.

1. Introduction

Despite the widespread recognition that rivers dominate the global flux and delivery of plastics to the ocean, there is a key knowledge gap regarding the nature of that flux, the behaviour of microplastics (<5 mm) in transport and their pathways from rivers into the coastal ocean [1,2]. Furthermore, rivers are important biodiverse systems that are often overlooked as zones for microplastic accumulation, with ecological risk in fluvial habitats relatively under-examined. Microplastic research has predominantly focussed on marine systems where studies tend to sample either at the water surface or bed sediment [3,4]. Yet, observed estimates of the volume of floating marine plastic debris represents just 2–6 % of the estimated plastic flux entering aquatic systems annually, so it is likely these sampling strategies significantly bias and underestimate plastic loads [1,5]. The lower frequency of riverine plastics studies, coupled with sampling methodologies that have systematically not measured plastics through the entire water column, has prevented progress towards a robust and holistic understanding of microplastic dynamics. Furthermore, this paucity of data and sampling biases impact identification of zones of high microplastic accumulation, as well as curtailing the evolution of effective mitigation and policy measures to reduce ecological, environmental, and societal risk and impact.

The majority of plastic pollution originates from land based pathways through mismanaged waste, urban and storm water runoff, degradation of larger plastics released into the environment, wastewater treatments plants (WWTPs) and industry [6-10]. Microplastic transport in rivers is influenced by both particle properties (including buoyancy, shape and size) and hydrodynamics, such as salinity, turbulence and velocity [11-14]. It has been argued that microplastics can be expected to follow transport behaviours that are comparable to naturally occurring sediment particles of hydraulically equivalent properties [15-19]. As a result, a high proportion of fluvial microplastics have been anticipated to be deposited within bed sediment [20]. However, the majority of microplastics have a number of dissimilar properties to their siliciclastic counterparts, for example microplastics tend to be consisted of elongate particles typically described as fragments, fibres, and films that have been weathered and fragmented. As such their transport behaviour and mechanisms can vary significantly [16,21], introducing uncertainties. Recent microplastics settling experiments have shown that using predictors based on siliciclastic sediment formulae are not always accurate, with microplastics tending to display very different fall rates and variable buoyancy through time [13,14]. Particle fate will also be impacted by biofilm growth and interactions with suspended sediment that may lead to aggregation and floc formation, both of which are likely to increase particle density [22]. Additionally, as plastic moves within a riverine to ocean environment, it will experience turbulence and resuspension in addition to crossing salinity boundaries, which further changes particle buoyancy [13,23]. Therefore, due to their relatively low densities (in comparison to sediment) the majority of microplastics may be in suspension rather than deposited within the bed [16].

Furthermore, there is growing evidence that plastic pollution can harm biota, wider ecosystems and potentially human health in addition to having societal and economic repercussions through damaging shipping, fisheries and tourism [24-28]. Although many of the potential ecotoxicological consequences of plastics are well known, research has only recently begun to explore the detailed transport dynamics of plastics in freshwater aquatic environments [29-31]. Given that rivers are the major source of plastic flux to the ocean, detailed transport dynamics of microplastics will be a first order of control on how various organisms are exposed to microplastics in terms of the distribution and how these pollutants are delivered into coastal seas and the wider marine environment. Therefore, to robustly predict the transport, fate and ecological risk of microplastics in aquatic environments at a global scale, the distribution and abundance of microplastics through the water column of riverine deltas and estuaries must be identified and understood [32,33].

The Mekong River of Southeast Asia has been identified as one of the top contributors to marine plastic pollution, with an estimated plastic load of up to 37,000 tonnes per year [2,34]. The consequences of plastic pollution in the Mekong could be severe due to the high levels of biodiversity of the basin and the millions of people that rely on its productivity for their livelihoods. To our knowledge, few studies have quantified microplastic levels in the Mekong River of Cambodia and Vietnam. Of those that do exist, a study conducted at six sites along the Tien River of the northern Mekong Delta Vietnam found 53.8 ± 140.7 microplastics m^{-3} in surface water and 6.0 ± 2.0 microplastics g^{-1} dried weight in sediment, with the majority being fibres [35]. Microplastics have also been found in peatland areas of the Mekong Delta in Vietnam with average of 192.3 ± 261.3 items kg^{-1} with the majority being fragments (67 %), films (25 %) followed by fibres (7.6 %) [36]. The settling of microplastic fibres from the Mekong River has informed development of plastic transport models for the Mekong directly upstream of Phnom Penh and indicated that large fractions of microplastics move toward the bottom of the water column than previously quantified using conventional models [37]. Haberstroh et al. [38] sampled sites close to Phnom Penh, Cambodia, during the monsoon season and this is also one of the few studies to sample within the water column at various depths (other examples include [39] and [40]). Furthermore, semi-natural field experiments conducted in the Lower Mekong Basin of Cambodia revealed the potential for biofouled plastics to cause hypoxic conditions which may alter wider ecosystem function [41].

However, data relating to spatial gradients in microplastic fluxes through a large, transboundary river system, such as the Mekong is lacking. The majority of riverine microplastic studies are geographically constrained, focused on China, North America, or Western Europe, despite numerical models, supported by observations, predicting the disproportional contribution of Southeast Asian rivers in plastic emissions to the ocean [2,34,42]. This disparity is driven by factors such as high population densities in coastal regions, inadequate waste management systems and the ongoing plastic waste shipments from the Global North to the Global South for processing, despite existing bans [1,43]. Furthermore, field investigations on microplastics tend to sample only at the water surface or directly from the riverbed, with concentrations being highly variable for several reasons, such as differences in river magnitudes and seasonal influences. The lack of sampling throughout the water column may result in inaccurate concentrations being reported as polymers have varying densities and therefore it is likely that different plastics will be segmented throughout the water column. There is, therefore, an urgent need to capture more robust observations of microplastic transport in rivers that are expected to be significant contributors to the oceanic plastic flux throughout the water column: not only to validate the models being used to predict these fluxes, but to also better enable targeted remediation practises within these basins.

Here, we present field measurements distributed across a much wider range of sites throughout the lower alluvial reaches of the Mekong River and its delta, in Cambodia and Vietnam. We provide insights into the distribution of microplastics and how this varies with depth across eight sites in addition to quantifying the particulate flux and assessing the implications for discharge into the ocean. The overarching aim of this study is to quantify the flux and vertical distribution of microplastics in a large river system of the Mekong River and its tributaries. Specifically, this paper also addresses the following research questions: i) Where in the water column are microplastic concentrations greatest and does this correspond with polymer densities? ii) Where is the most representative point in the water column to sample for microplastics; is surface sampling a representative approach to sampling in large river systems?

2. Materials and methods

2.1. Site Locations

We sampled across the Mekong and its main distributary in the Mekong delta, the Bassac River, at eight locations throughout Cambodia and Vietnam, in July 2019 (Fig. 1). The Mekong River is the longest (4800 km) river in Southeast Asia and has the 10th largest water discharge globally [44,45]. A monsoon-driven flood pulse occurs annually, with the majority of the volume of water carried by the river concentrated into a single wet season [46,47]. This flood pulse sustains ecological productivity by transporting huge amounts of nutrients and sediments, creating diverse habitats – such as the Tonle Sap Lake. Consequently, the Mekong River is one of the most biodiverse freshwater ecosystems globally, containing large and diverse fisheries with the Lower Mekong Basin supporting between 1000–1700 species and having an estimated yield of 4.4 million tonnes per year [48-50]. The ecological productivity is the basis for the livelihoods and food security of the majority of the 70 million people that live in the basin [51]. Other major water-dependent economic sectors are agriculture, and energy such as hydropower production [45]. Yet the resources of the Mekong River are vulnerable to seasonal changes of sediment load, water quality and river flow [50].

The upstream extent of our sampling was the town of Kratie (population ~27,000) and the rural area of Kampi, ~250 km north of Phnom Penh, Cambodia. Kampi is at the bedrock to alluvial transition [52] and the location of a series of deep pools in the Mekong channel which is natural habitat of the Irrawaddy Dolphin [53]. Moving downstream, we sampled close to the Cambodian capital, Phnom Penh (population = 2,014,000). Specific sites were located on the Tonle Sap River, Mekong River (upstream and downstream of the Chaktomuk junction) and Bassac River. The Chaktomuk junction is a major hydrological node in the Mekong system, representing the apex of the Mekong Delta, and the connection between the Mekong River and the Tonle Sap Lake, the largest freshwater lake in Southeast Asia. In the Mekong Delta we sampled at two sites around Can Tho, Vietnam, (population 1,531,000), the largest city on a distributary of the Mekong River in Vietnam, and a busy waterway, Can Tho River.

2.2. Water sample collection

Sampling occurred in July 2019, corresponding with the beginning of an increase in daily discharge of the Mekong River during the rainy season. The majority of the annual discharge (80 %) is seen during the Mekong's flood pulse between June and November and concentrates in a single wet season peak which typically occurs in August/September [46,47]. Five plankton nets, with a mesh size of 250 μm, were attached to a vertical line at 4 m intervals with a 5 kg weight on the end. This allowed samples to be collected at the surface (top layer 0–0.5 m) and at fixed distributions throughout the water column, with the number of depth samples collected dependent on the depth of the river at each location. For example, if the depth of the river was 10 m, water samples were collected at the surface, and at approximately 4 m and 8 m. Pressure sensors were attached to the rope at the position of the nets to measure the depths at which samples were taken. The nets were deployed from the back of a stationary boat for 300 s in the middle of the channel at each location. On retrieval, each net was thoroughly rinsed from the outside to ensure all sample inside the net were collected into the cod end of the net. Each cod end was then removed and rinsed with deionised water and sample was transferred into one glass bottle per net before being sealed for transfer to the laboratory for separation and analysis. The net was then rinsed inside and out before the next sample was collected. In total, 31 water samples were collected. At the same time as the nets were deployed, a 1200 KhZ Teledyne Rio Grande Acoustic Doppler current profiler (ADCP) was used to record instantaneous three-dimensional flow velocity profiles (at 0.25 m bin sizes) at each of the sample locations. These profiles enabled quantification of the flux of water passing through the net at each sample depth and thus estimate concentration levels.

2.3. Separation and filtration

Water samples were first vacuum filtered onto 55 μm-pore size Whatmann GF filter papers, covered, and dried at room temperature. Next, each filter paper was placed in a glass flask and 30 ml of H_2O_2 (30 %) was added. Flasks were put in a shaking incubator at 50 °C, 100 rpm for 24 h to digest any organic material. H_2O_2 (30 %) at 50 °C was chosen as it has been shown to be an efficient reagent for digesting organics while causing minimal damage to any microplastics present [54,55]. Trials were also run on mock samples to test multiple digestion methods and confirmed that this was the most efficient methodology. Finally, 200 ml of deionised water was added to each sample, vacuum filtered, rinsed twice, and dried at room temperature.

2.4. Identification of microplastics

Filter papers were first analysed with an Olympus SZX10 microscope, Olympus UC30 camera, and (Olympus) CellSens software to identify and count suspected microplastics. The particles were then examined for polymer content using Fourier transform infrared (FT-IR) spectroscopy analysis, with a Thermo Fisher Scientific iN10 Nicolet spectrometer equipped with the OMNIC Picta software (Thermo Scientific OMNIC Series). Due to the large number of particles being identified as potential microplastics, 10 % of each type of particle seen at every depth at each site was tested to gain a representation of every type observed, resulting in 719 suspected particles being verified. The spectra were recorded with 12 scans in the region of 800–6000 cm^{-1}. The spectrum of a particle is recorded and compared to well-established polymer libraries in addition to contamination libraries which were identified from control samples. Examples of FT-IR spectra are shown in Supplementary material Fig. 1. Particles were determined plastic if there was a match of at least 70 %. Suspected particles were analysed according to the sample location, depth, and particle type. If one particle in the subcategory was identified as a polymer, all particles of the same types (for example black fibres) were assumed to be the same polymer at that location and depth. Plastics were organised as plastic type (fibre, fragment, film) and polymer type: polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), Low-density polyethylene (LDPE) and “other” which includes non-typical polymers such as polyacrylonitrile (PAN).

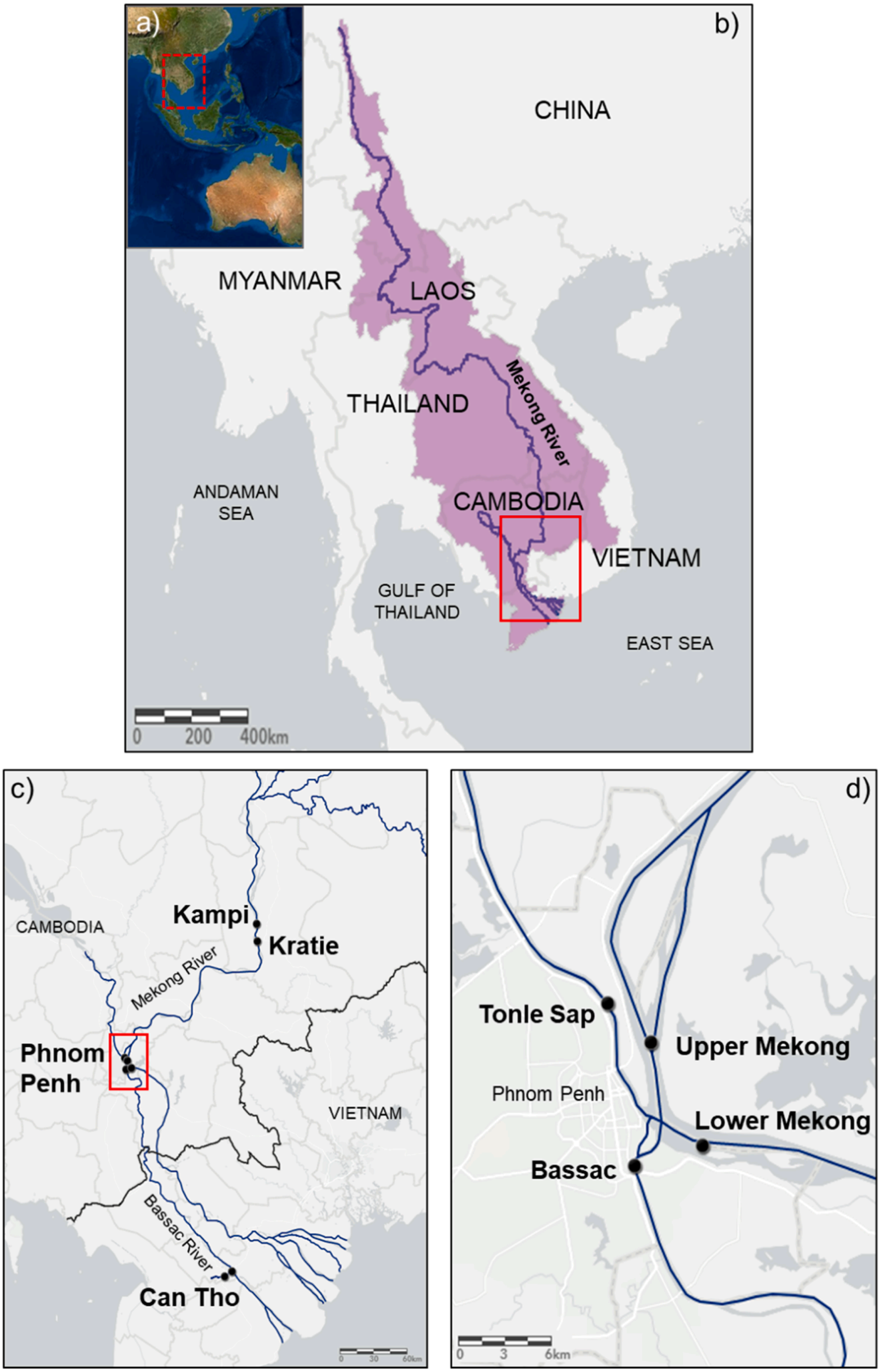

Fig. 1. a) Overview of Mekong River location b) The Mekong River Basin area c) Sampling locations within the Mekong Basin in Cambodia (Kampi, Kratie and Phnom Penh) and Vietnam (Can Tho) d) The sampling locations around Phnom Penh (Tonle Sap, Upper Mekong, Bassac, Lower Mekong). Basemap for a) World Imagery. Basemap for b), c) and d): Light Gray Canvas Map, layers: GMS Major River Basin and Main Rivers, Great Mekong Subregion Secretariat.

2.5. Prevention of contamination

Several measures were taken during the analysis to prevent samples from being contaminated by airborne particles, such as textile fibres. During collection and laboratory analysis, cotton clothing including lab coats were worn instead of synthetic materials in addition to Laboratory Latex gloves. All liquid reagents (deionised water, H_2O_2 and ethanol) used were passed through a filter paper (55 μm-pore size Whatmann GF) using a vacuum pump before being used. Glass equipment was triple rinsed using filtered deionised water and stored sealed to prevent contamination. Work surfaces were cleaned with filtered 100 % ethanol and all processes were performed in a fume hood to prevent airborne contamination. During each step of analysis, a filter paper was placed on the work surface to account for contamination and procedural blanks were also run to determine contamination risks. These filter papers were examined using optical and FT-IR analysis as above. If polymers were identified, they were added to a contamination library which the environmental samples were all compared to. The plankton net mesh polymer (nylon) was also added to a contamination library. Out of the 719 suspected particles tested, only 3 matched the contamination library, which were removed from the analysis.

2.6. Calculation of microplastic concentration and flux

For all flux (rate of flow of microplastics) and concentration (number of microplastic particles per given volume) calculations, the total volume of water, V (m^3), passing through the sampling net was calculated:

$$\boldsymbol{V} = \boldsymbol{vAt} \tag{1}$$

Where v denotes the average downstream flow velocity, m s^{-1}, A denotes cross sectional area, m^2, of the net and t is the length of sampling, s.

Concentration of microplastics $C_{mp\#}$, count per m^3, is calculated as follows:

$$\boldsymbol{C_{mp\#}} = \frac{\boldsymbol{n_{mp}}}{\boldsymbol{V}} \tag{2}$$

Where n_{mp} denotes number of microplastics counted per sample.

We also calculate concentration of microplastics C_{mpd}, g ml^{-1}, based on the density and an assumed hypothetical cylindrical shape characteristics of each polymer type identified within each sample where:

$$C_{mpd} = \sum C_{mpd\,j} = \sum \pi r_j^2 l_j d_j n_{mp\,j} \tag{3}$$

$C_{mpd\ j}$ is the weight-per-volume estimate of concentration for polymer *j*, r_j^2 is the average radius (m) of microplastic polymer *j* as measured from microscope analysis, l_j is the average length (m) of microplastic polymer *j* as measured from microscope analysis, d_j is the density (g/cm^3) of polymer defined from www.matweb.com, *j*, and $n_{mp\ j}$ is the number (count) of microplastics of polymer *j* identified in each sample. Fibres were shown to have an average radius of 29 μm (st dev = 15.8 μm) and fragments were shown to have an average radius of 23 μm (st dev = 4.5 μm).

Microplastic flux can then be calculated as either count per second, $Flux_c$ or as a density-based calculation, $Flux_d$ where:

$$Flux_c = QC_{mp\#} \tag{4}$$

$$Flux_d = QC_{mpd} \tag{5}$$

where Q, m^3/s, is the discharge within the portion of water sampled (acquired from ADCP data).

2.7. Statistical analysis

Regression analysis was carried out using Poisson distribution to assess the spatial (site) differences between total microplastic concentration/flux. Spearman rank test was used to determine correlation between microplastic concentration/flux and depth at a significance level of $p < 0.05$. All analysis was conducted using R Studio [56].

3. Results

All samples were found to contain microplastics. 1444 particles were determined as plastic, with counts varying with location and depth, and a greater number of microplastics particles observed at downstream sample sites. There are significant differences in total microplastic concentration between sites: Kampi (2 microplastics m^{-3}, 0.006 mg l^{-1}, $p < 0.01$) and Kratie (3 microplastics m^{-3}, 0.009 mg l^{-1}, $p < 0.05$) had considerably lower microplastic concentrations while Lower Mekong Phnom Penh (40 microplastics m^{-3}, 0.134 mg l^{-1}, $p < 0.05$) and Can Tho River (64 microplastics m^{-3}, 0.182 mg l^{-1},$p < 0.01$) had considerably higher microplastic concentrations (Fig. 2).

When looking at how microplastic concentration varied with depth at each location, patterns are very varied (Fig. 3). On average, 86 % of microplastics (concentration from count) were observed below the water surface: 67 % at Kratie, 83 % at Tonle Sap, 98 % at Lower Mekong Phnom Penh, 93 % at Bassac Phnom Penh, 94 % at Bassac River Can Tho and 83 % at Can Tho River (Kampi and Upper Mekong Phnom Penh had no surface sampling). Spearman's rank correlation was conducted to determine if there is a relationship between microplastic concentration and depth. No correlation was observed apart from a positive correlation for Bassac River Can Tho ($p < 0.05$) where at the surface the concentration was 1 microplastic m^{-3} increasing to 3 microplastics m^{-3} at 3.3 m and 5.4 m to 5 microplastics m^{-3} at 7.5 m and 11.5 m. When looking at microplastic flux with depth, patterns change slightly (supplementary material, Fig. 2), and no correlation was observed for flux at any site apart from Bassac River Can Tho ($p < 0.05$) with positive correlation with depth.

Of the total microplastics found, 53 % (766) were fibres, 44 % (623) were fragments and 3 % (42) were films (Fig. 4). No plastic pellets or spheres were observed. The majority of the microplastics were classified as PET and "other" with 35 % (501) being PET (density = 1.38 g cm^{-3}), 34 % (493) "other" (density = 1.04 g cm^{-3}), 22 % (313) PP (density = 0.92 g cm^{-3}), 5 % (79) LDPE (density = 0.94 g cm^{-3}) and 4 % (60) PE (density 0.91 g cm^{-3}; Fig. 4). The size distribution of microplastics was mostly in the 1 mm-3 mm range (35 %) with only 5 % being < 0.1 mm and 7 % being 3–4 mm (Fig. 4). Examples of microplastics and their associated FT-IR spectra are shown in Supplementary material, Fig. 1.

No distinct pattern of polymer type with depth was observed across sites (Fig. 5). The distribution of microplastics in the water column differed by location; however, on average, 86 % of microplastics were detected below the surface sample. Across all sites, 50 % of the total microplastic flux was concentrated within the top 40 % of the water column (Fig. 5)."

4. Discussion

A wider understanding of the abundance, transport mechanisms and fate of microplastics is necessary to accurately predict riverine microplastic loads into the oceans and understand associated ecological and human risks [57]. The Mekong River is commonly reported to be one of the top polluting rivers globally [2,58]. Despite this, there is limited research on major rivers in Southeast Asia and many approaches to monitoring fluvial microplastic transport have tended to rely only on surface sampling and assumptions to estimate depth concentrations, with vertical distribution at varying flows being essentially unknown [59,40,60].

4.1. Abundance of microplastics in rivers

Microplastics were found in all water samples analysed. The average

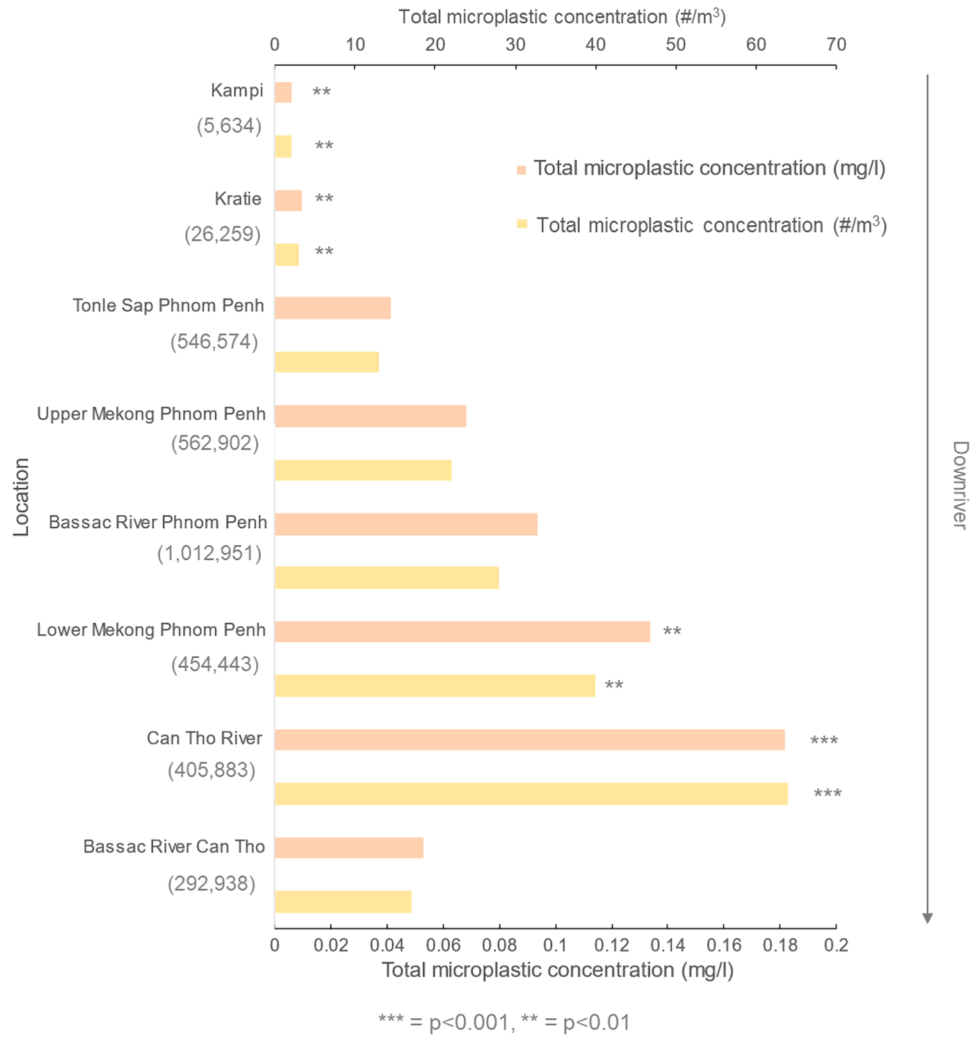

Fig. 2. Total microplastic concentration between all sites and the corresponding location population. Population statistics represent the sum of population within a 5 km radius of the sample location as defined by WorldPop aggregated population count data for 2019 (WorldPop, 2018).

concentration across all sites was 24 microplastics m^{-3} (0.073 mg l^{-1}), yet this was highly variable between sites, with the lowest concentration observed to be 2 microplastics m^{-3} (0.006 mg l^{-1}, Kampi) and the highest 64 microplastics m^{-3} (0.182 mg l^{-1}, Can Tho River). Comparison of results to other studies reporting microplastics in rivers is hampered by different sampling methods and that the majority of studies focus on surface waters. Microplastic levels reported here for the Mekong River and its tributaries are within those reported for surface waters of the North Saskatchewan River, Canada (5 – 88 microplastics m^{-3}; Bujaczek et al. [61]), the Ganges River of India (38 microplastics m^{-3}; Napper et al. [62]), the Marne and Seine rivers of France (38 - 102 microplastics m^{-3}; Dris et al. [39]), and the Amazon River of Brazil (8–39 microplastics m^{-3}; Rico et al. [63]). The range reported here for the Mekong is at the lower end of comparable data from published literature in the region, with a study in various environments of Vietnam (not including the Mekong Delta) reporting microplastic concentration in surface waters of 0.35 to 2522 items m^{-3}, with the highest being in rivers and lowest in bays [64]. Microplastic concentrations in rivers were 2.3, 2.7, 3.9, 93.7, and 2522 items m^{-3} in the Red River, Han River, Dong Nai River, Nhue River, and To Lich River respectively. The Lich River (2522 items m^{-3}) is known to be heavily polluted and receiving large amounts of untreated domestic wastewater, in addition to having low water discharges. Furthermore, a study conducted at six sites along the Tien River of the northern Mekong Delta Vietnam found 53.8 $\pm$ 140.7 items m^{-3} in surface water, with the majority being fibres [35]. However, few studies have directly sampled microplastic concentrations systematically within the water column. This may result in inaccurate concentrations being reported as polymers have varying densities and therefore it is likely that different plastics will be segmented throughout the water column (see Section 4.2). There is, therefore, an urgent need to capture more robust observations of microplastic transport in rivers that are expected to be significant contributors to the oceanic plastic flux: not only to validate the models being used to predict these fluxes, but to also better enable targeted remediation practises within these basins.

Moreover, environmental decision-makers must identify the dominant sources of microplastic pathways to inform management and prevent their entry into the environment, whilst also determining if these sources are consistent across different geographies [65]. The majority of microplastics in this study were classified as fibres (53 %), followed by fragments (44 %) and films (3 %), which was similar to patterns observed in the Amazon River and its tributaries (fibres = 51 %, fragments = 42 % and films = 6 %; [63]). Fibres are often the most common type of plastic found in rivers worldwide, including the Tisza River of Central Europe [66], Sacramento Delta and Mississippi River of North America [65], Nile River, Egypt [67], Guapimirim, Macacu and Maracanã Rivers, Brazil [68], Langat River, Malaysia [57], the Yangtze Estuary, China [69] and the northern areas of the Mekong River Delta [35]. In examining how the dominant microplastic types vary across sampled sites of this study in the Mekong Delta, fibres comprised the majority of total concentrations at Kampi, Kratie, Bassac River Phnom

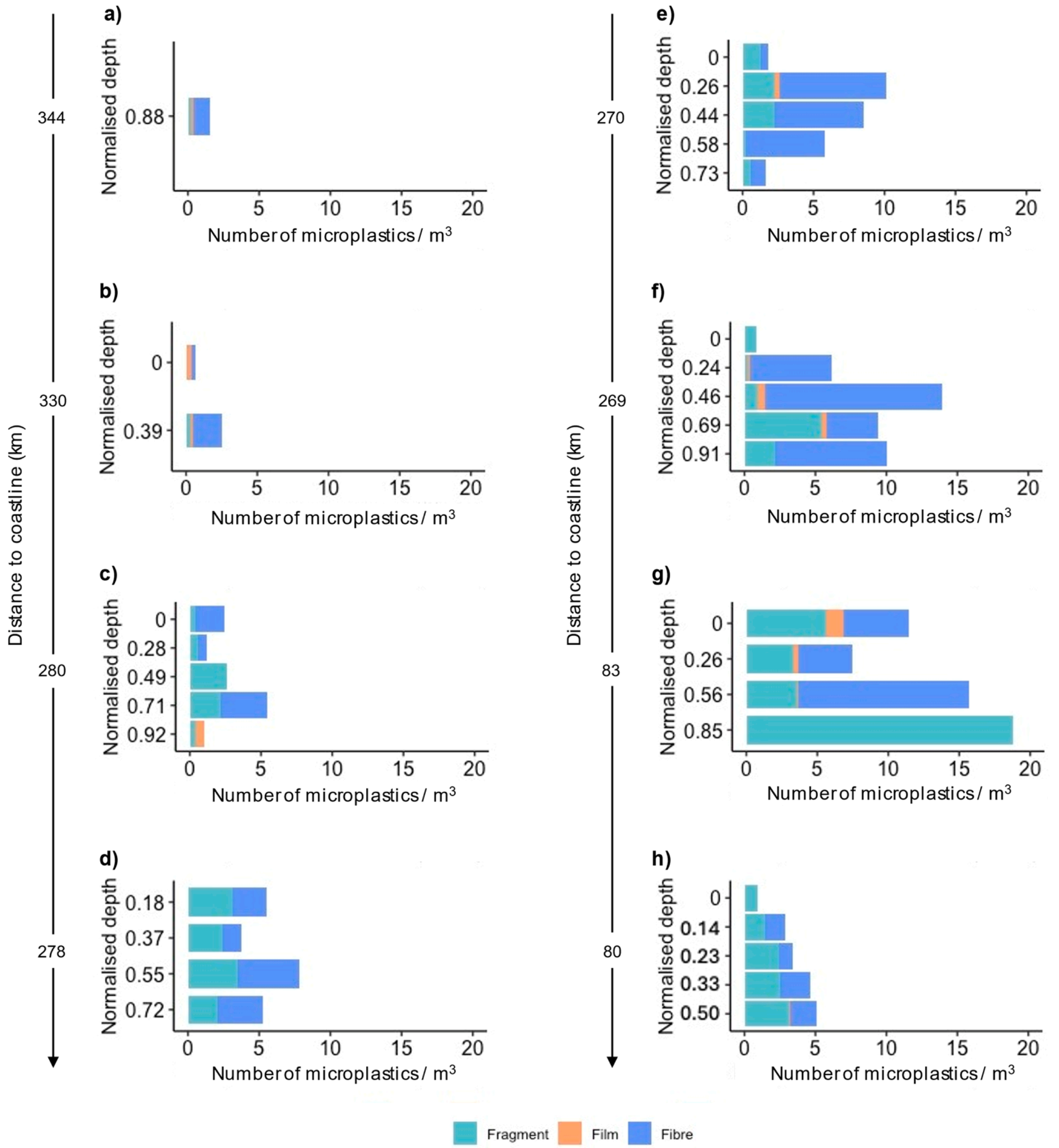

Fig. 3. Microplastic concentration at each location with normalised depth (sample depth divided by max depth) and distance from coastline at a) Kampi, b) Kratie, c) Tonle Sap River Phnom Penh, d) Upper Mekong River Phnom Penh, e) Bassac River Phnom Penh f) Lower Mekong River Phnom Penh, g) Can Tho River and h) Bassac River Can Tho.

Penh and Lower Mekong River Phnom Penh, while both fibres and fragments had similar levels at Tonle Sap River Phnom Penh, Upper Mekong River Phnom Penh, Can Tho River and Bassac River Can Tho (Table 1). In addition, most were determined to be PET (35 %) or "other" (34 %) which included polyacrylonitrile (PAN), or polyacrylates. PET is typically used in packaging for food and drinks and can degrade into fragments, but PET fibres, or polyester, PAN and polyacrylates have several applications such as in clothing and textiles. PET or polyester has also been observed to be the most abundant polymer (32 %) in the Amazon River and its tributaries [63], while others report PP, PE and polystyrene (PS) [70-72].

The variation in the polymers and types of plastics observed highlights the various pathways of plastic pollution in the Mekong and need for mitigation of multiple origin sources. Urban wastewater, such as that from WWTP effluent and stormwater inputs is considered a major pathway for microplastics into aquatic ecosystems, in addition to

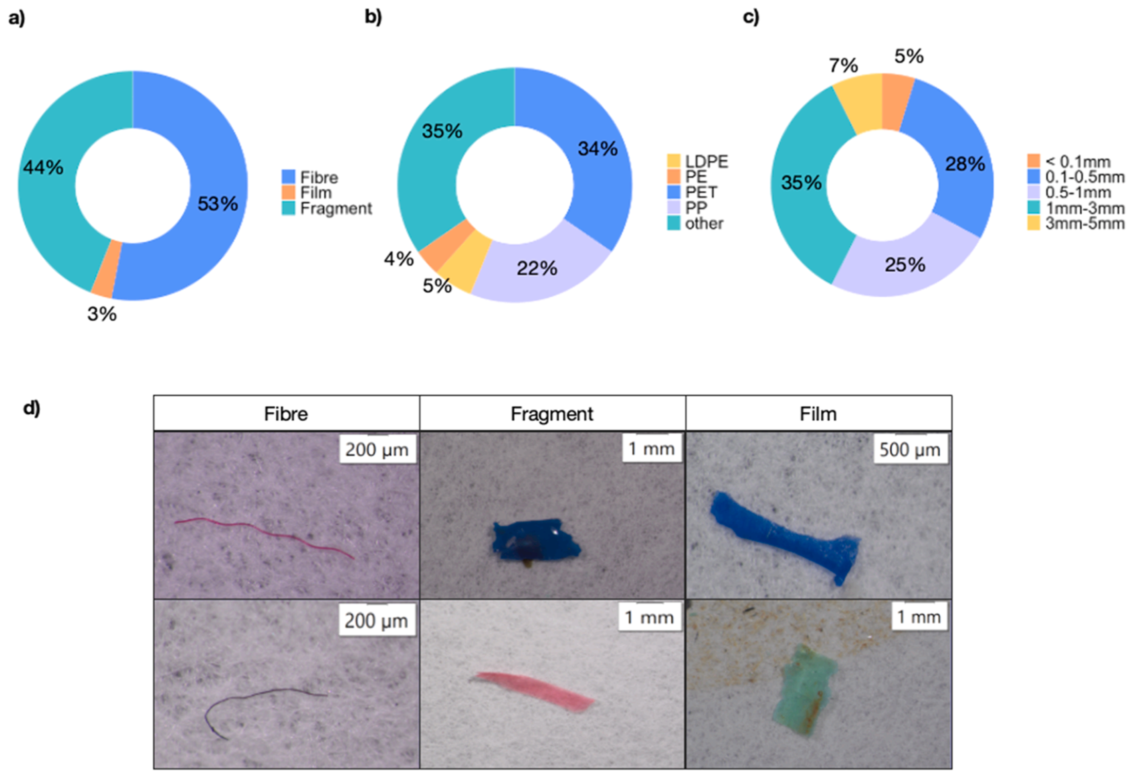

Fig. 4. Characteristics of the total microplastics found across all samples: a) amount of each type of plastic: fibre, film or fragment; b) amount of each polymer type: low-density polyethylene (LDPE), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and "other" which includes non-typical polymers; c) the size range of microplastics: < 0.1 mm, 0.1–0.5 mm, 0.5–1 mm, 1–3 mm and 3–5 mm and d) examples of microplastic types found in the Mekong River a) fibres b) fragments and c) films. Photographed with an Olympus SZX10 microscope, Olympus UC30 camera, and (Olympus) CellSens software.

agricultural drainage, with the majority being fibres and fragments [73-76,65,71,77]. Domestic wastewater often contains large amounts of synthetic fibres such as polyester due to the release from textile washing [78,79]. WWTPs do exist across the Mekong, however they are not widespread and are mostly in urban areas such as Phnom Penh and Can Tho [80,81]. Even when water is cleaned before being discharged into rivers, only a limited number of WWTPs have the ability to filter microplastics [73,77]. As the dominance of fibres was consistent across the area sampled in this study, it highlights the need for improved WWTPs in this region, in addition to more effective waste management to capture fragments.

Furthermore, this study is the first lower Mekong assessment that shows a seaward increasing trend in microplastic concentration and flux, with the most landward rural locations (Kampi and Kratie) having the lowest microplastic concentrations. This rural-urban transition and downstream increase has been observed in other studies due to changes in population density, with densely populated urban environments identified as important sources of microplastics [57,82-86]. In our study, abundance increased dramatically towards Phnom Penh as population increases, with the higher microplastic concentration observed downstream compared to upstream of Phnom Penh, indicating that Cambodia's capital is a key source and pathway for microplastic pollution. Similar results were seen with a study combining visual counts and net sampling of macroplastics, with an increase in plastic concentration and mass transport in the main Mekong branch downstream of Phnom Phen compared to upstream [87]. However, there was a decrease in abundance between the two Bassac River sites, with concentration decreasing from 28 microplastics m^{-3} at Phnom Penh to 17 microplastics m^{-3} at Can Tho, Vietnam. The largest concentration was observed at Can Tho River with 64 microplastics m^{-3}. This suggests a large amount of microplastics travelling within the Bassac river from Cambodia settle in bed sediments or are distributed onto the floodplain through flooding or irrigation practices between Phnom Penh and Can Tho, whilst plastic discharge associated with the city of Can Tho is predominantly routed into the Can Tho River rather than passing downstream of Can Tho in the Bassac River. The high abundance at Can Tho River was expected as it is the largest city on the Mekong delta (population = 1,531,000) with WWTP effluent directly discharging to the Can Tho River. This further highlights the need for more rigorous management of wastewater, particularly in urban areas of the Mekong.

It must be acknowledged that polymer recognition is influenced by several factors which may cause differences in microplastic identification and quantification. Environmental microplastics will have undergone aging, where abiotic and biotic processes alter their integrity and properties which has implications for accurate analytical assessment and quantification of microplastics [88] The prevalence of different abiotic and biotic degradation processes will depend on the chemical composition of the plastic, and the environment of collection ([89,90]. Furthermore, sample purification and extraction of microplastics often involves a density separation step, but biofouling on aged microplastics can affect buoyancy of particles, complicating separation [91]. Digestion steps used to remove biological material may also cause degradation and fragmentation, affecting polymer identification and size distribution [88].. This fragmentation may also cause particles to fall below the threshold of detection, leading to underestimation of concentrations. Furthermore, analytical methods such as FT-IR spectroscopy are generally validated using virgin, unweathered polymers which differ chemically and physically from aged plastics that have lost spectral information due to increased rugosity and changes in surface groups [92,93]. FT-IR analysis may lead to underestimation of microplastic concentrations, as aged particles, though identified as plastic, might not meet the threshold required to match polymer libraries. This limitation was observed in this study and must be considered when comparing results. To mitigate this, reducing the number of processing steps for

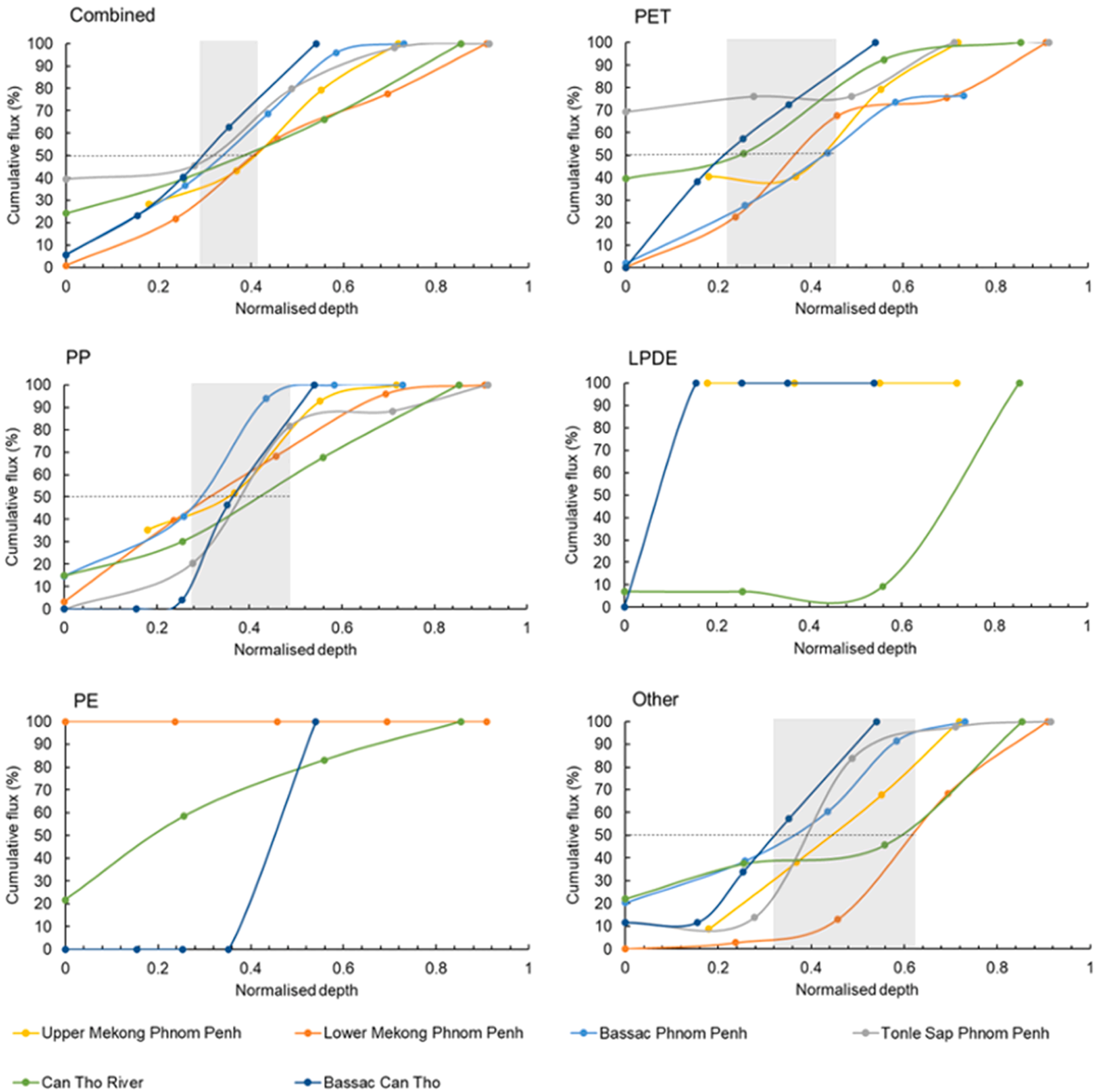

Fig. 5. Cumulative flux of microplastic as a function of normalised depth for each of the microplastic polymers identified at our sampling sites. Dashed lines represent median values (50 %) whilst grey boxes define the region of normalised depths at which 50 % of the flux is observed for each site. Boxes are not present for LPDE and PE due to the small sampling size of these polymers.

extracted microplastics and incorporating appropriate aged reference materials during validation is essential. However, this presents challenges due to the diverse environments and varying exposure times of aged microplastics.

4.2. Plastic distribution in the water column: implications for monitoring and fate

Our data indicates that variations in microplastic abundance with depth at each location must be considered when assessing overall abundance and fate in rivers. Accurately quantifying microplastic abundance in rivers is challenging due to the complex dynamics of fluvial systems, [94], in addition to microplastics becoming biofouled or flocculated, which further influences their density, position in the water column and transport behaviours [95]. Therefore, within the water column microplastics are not uniformly moving or evenly distributed and may be deposited, trapped or remobilised [94]. This is important to recognise in order to determine accurate monitoring techniques and flux estimates. Currently, several different methods exist to quantify and monitor microplastic abundance in rivers, which are primarily used to sample water surface including i) direct sampling using containers such as water samples, buckets or jars; ii) submersible pumps to draw and sample large volumes of water and iii) nets such as manta, neuston and plankton [94].

However, relying solely on surface water concentrations may significantly underestimate the true quantities of microplastics present

Table 1
The total concentration of microplastic types (fibre, fragment and film) across all sampled sites. Numbers in bold indicate which microplastic type was most abundant at that site.

Site	Total concentration / microplastics m^{-3}		
	Fibre	Fragment	Film
Kampi	**1.48**	0.26	0.26
Kratie	**1.84**	0.25	0.49
Tonle Sap River Phnom Penh	5.83	**6.32**	0.60
Upper Mekong River Phnom Penh	10.17	**10.36**	0.00
Bassac River Phnom Penh	**21.05**	6.44	0.37
Lower Mekong River Phnom Penh	**29.82**	8.67	1.07
Can Tho River	29.54	**31.28**	3.02
Bassac River Can Tho	7.31	**10.53**	0.07

(Fig. 3; [40]). The distribution of microplastics in the water column varied by location, but on average, 86 % of microplastics were found below the surface sample. Across our sites, 50 % of the total flux of microplastics was found in the top 40 % of the water column (Fig. 5). We thus identify, similar to [59], that accurately predicting and monitoring microplastic fluxes in riverine systems must include sampling and characterisation of microplastic concentration depth profiles (Fig. 3 and 5).

Interestingly, no distinct patterns of polymer type were observed, suggesting perhaps an influence of turbulent mixing and biofouling on particle settling velocities and thus distribution in the vertical water column. Typically, the most common polymer type detected in rivers are PE and PP [96-100,86,101]; however our study found PET to be the most common type. This could be explained by the sampling strategy, as most studies focus only on surface water, while PET, with a much higher density ($1.38 \ g \ cm^{-3}$) compared to PP ($0.92 \ g \ cm^{-3}$) and PE ($0.91 \ g \ cm^{-3}$) is more likely to sink and may be underrepresented. Indeed, for polymers with a density greater than that of water (PET and "Other"), microplastic flux increased towards the riverbed; thus greater fractions of the total flux are located well below the water surface (Fig. 5). This further underscores that relying solely on surface sampling can introduce bias and will not adequately capture the true nature and magnitude of the flux of these polymers. However, density is also influenced by biofouling, flocculation and aging which impacts settling rates [13,22]. Several studies have identified high density polymers in water samples and low density polymers in sediments [96,102-104] which can be attributed to the effects of turbulence, biofouling and flocculation, leading to particle mixing in the water column. When looking at polymers whose density is lighter than that of water (PP, LDPE and PE) samples taken in the top 50 % of the normalised depth typically capture the greatest percentage of the total flux (Fig. 5), though for LDPE and PE our samples did not capture large enough quantities to provide accurate ranges. Therefore, it appears that different polymer types require sampling across a range of different depths.

To determine the optimal sampling depth for accurately monitoring microplastics in rivers, the combined flux of microplastics (representing the sum of all polymer types) was analysed and displays an increasing contribution to total flux with depth to approximately 60 % of depth. We estimate that sampling of a few points down to 0.4 of the normalised depth may be sufficient to capture at least 50 % of the cumulative total flux of the combined microplastic load. As such, single point sampling at the surface will not capture the true volume of microplastic fluxes in large river systems like the Mekong, capturing at most 40 % of the cumulative flux, but on average ~10 % of the cumulative flux. To ensure a complete and representative sampling of microplastics transported in the water column, samples should be taken across the range of possible depths rather than focussing on the surface (Figs. 3 and 5). Our data suggests that sampling at depth across at least the top 60 % of the water column will provide a representative sample of polymer type, and capture > 50 % of the cumulative microplastic flux. Thus, if sampling across the entire water column is not feasible due to depths of sampling limitations, efforts should be focussed on sampling the top 60 % of the water column.

The settling, storage and entrainment dynamics of fine material within rivers is complex due to the inputs from multiple sources throughout watercourses and relation to fluvial dynamics [57]. Dispersal in the water column is impacted by local stream velocities, channel depth, particle type and polymer density [40]. Microplastic settling experiments have shown, too, the need to consider biological influence on particle density which increases settling [17,22,13]. All of this highlights that the common practice of sampling only the water surface can cause substantial bias in predicting microplastic concentrations [40]. Depth-integrated sampling is strongly recommended following the results of this study and has been advised by others [59, 94]. However, to provide a comprehensive understanding of microplastic transport in rivers, bed samples should also be taken to determine the quantity of particles settling out.

4.3. *Variation in microplastic fluxes in a monsoonal river system*

To determine if there are changes in microplastic abundance across the hydrograph, this study was compared to Haberstroh et al. [38] who also sampled the Tonle Sap River, Lower and Upper Mekong River and Bassac River at Phnom Penh in August and September 2019 using similar methods, while our study took place in July 2019. During these months, which are within the wet season, the daily discharge will be increasing, which may impact microplastic concentrations. Total microplastic concentrations (surface and all samples within the water column) were compared at each site between our study (July 2019) and Haberstroh et al. [38] (centre of river channel) to determine any trends and relationships between discharge and microplastic concentration. Discharge during July at Phnom Penh was 450 m^3/s at Tonle Sap River, 7609 m^3/s at Upper Mekong River, 6802 m^3/s at Lower Mekong River and 1024 m^3/s at Bassac River. Haberstroh et al. [38] sampled twice at each location, the first during August and the beginning of September (discharge Tonle Sap River = - 6742 m^3/s, due to flow reversal, Upper Mekong River = 25,040 m^3/s, Lower Mekong River = 16,392 m^3/s, Bassac River = 2990 m^3/s) and the second in September (discharge Tonle Sap River = - 9394 m^3/s (Upper Mekong River = 40,799 m^3/s, Lower Mekong River = 26,556 m^3/s, Bassac River = 3715 m^3/s). Average discharge data for all locations and time periods was acquired from a fully validated Mike11 model of the Phnom Penh region forced with observed discharge data developed by the Southern Institute of Water Resources Research (SIWRR), Vietnam [105,106].

Our study (July 2019) reported considerably higher microplastic concentration at the Upper Mekong, Lower Mekong and Bassac River of Phnom Penh compared to August and September 2019 as described by Haberstroh et al. (Fig. 6). Total microplastic concentration decreased from July to September at those locations. For example, the total concentration for the Lower Mekong was 40 microplastics m^{-3} in July, decreasing to 9.23 microplastics m^{-3} in August to 8.72 microplastics m^{-3} in September (reported in [38]). However, concentrations were similar for each month in the Tonle Sap River. In addition, Haberstroh et al., [38] reported the majority of microplastics at the surface and declining with depth overall at Phnom Penh [38].

This may be due to several reasons. First, peak monsoon was during August and September when discharge levels would have been considerably higher compared to July and several flood events occurred. Fig. 7 highlights how lower microplastic concentrations occurred during high flow. Dilution of microplastic concentration due to high flow has been reported elsewhere, and may have occurred in Phnom Penh [57,107]. For example, Fan et al., [108] reported lower microplastic levels during the wet season of the Pearl River, China, attributing this to dilution from increased discharge, a pattern also observed in the Gallatin River, USA [109] and Yangtze Estuary, China [100]. Significantly higher macroplastic (> 5 mm) distribution at the riverbed was reported during low flow periods in shallow waters of the Mekong, Vietnam, compared to

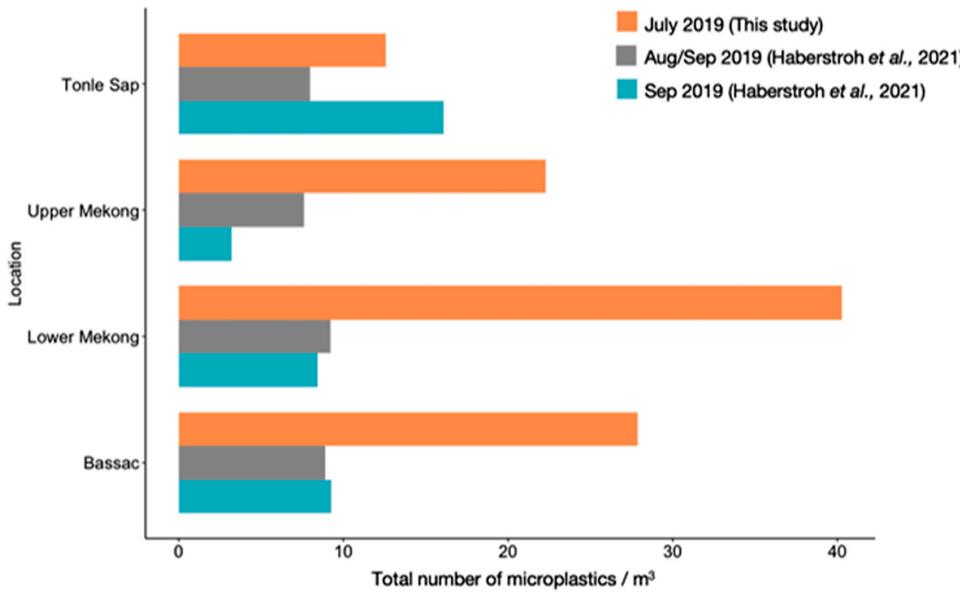

Fig. 6. A comparison of the total microplastic concentration at the Upper Mekong River, Tonle Sap River, Bassac River and Lower Mekong River of Phnom Penh, Cambodia. Microplastic concentration data from July 2019 is from this study while data from August and September 2019 are from [38].

flooding periods [110]. On the other hand, 70–80% of the annual microplastic load to the ocean occurred during the wet season of the Nakdong River, South Korea [96] and similarly in the Brisbane River, Australia, microplastic concentrations was also higher in the wet season [111], likely due to increased connectivity between land and rivers via precipitation. The impacts of increasing discharge are location specific due to factors that influence runoff such as land cover and use, and amount of rainfall, where tropical rivers tend to have higher runoff and sediment yield per unit area compared to other climates [57,112].

Empirical and modelling studies highlight the importance of hydrological regimes in controlling the fate of microplastics, with those carried downstream in suspension expected to be deposited in low flow periods where they accumulate on the riverbed until high flow causes entrainment [113,73,114,13,115]. Furthermore, flooding flushes resuspended microplastics downstream or overbank to be deposited onto floodplains while also delivering plastics from terrestrial sources into the river flow [20]. Post-flooding events have shown significant decreases in microplastics compared to pre-flood, and may be the main supplier of microplastics to the oceans within river systems [114]. Results from the Ganges River report higher numbers of microplastics found pre-monsoon compared to post-monsoon, again highlighting the need for sampling across all seasons [62]. However high flow events also have the potential to drive microplastics in sediment further into the riverbed to less-mobile regions resulting in long-term burial due to hyporheic exchange flow [20,116].

Likewise, it is widely thought that research on natural sediments can provide insights into understanding microplastic transport and fate in aquatic environments [19,117]. In particular, finer grain size fractions have been related to microplastics, suggesting that their distribution is governed by similar mechanisms in river systems [15]. The basic relationship between suspended sediment and discharge is well known with increasing discharge typically resulting in increasing suspended sediment [118]. As our results highlighted differences in microplastic concentrations at various discharges between studies, the relationship was analysed to determine if it follows similar patterns to sediment transport. Fig. 7 demonstrates that microplastic concentration follows the opposite pattern to siliciclastic suspended sediment where an increase in discharge is associated with a decrease in microplastic concentration at all sites around Phnom Penh apart from Tonle Sap River which may be explained by the annual flow reversal during the monsoon. As discussed previously, dilution of microplastic loads appears to have occurred at increased discharge. Furthermore, these results suggest that microplastic concentrations could be supply limited (dependant on the availability of microplastic inputs from surrounding sources) rather than capacity limited (restricted by the river's capability to carry more material) on the flood pulse of the Mekong in Cambodia, as concentrations do not increase with discharge. This highlights the need to sample throughout the year to fully understand and predict changes in microplastic levels in rivers and indicates that we should not rely on sediment dynamics to explain microplastic transport dynamics.

Although predicting microplastic transport and fate based on sedimentary laws provides basic insights into potential distribution, it does not take into consideration the complex behaviour of microplastics [19] or geographical differences in microplastic behaviour and patterns in relation to discharge observed. For example, microplastics are present in a range of densities, approximately 0.5–2.65 g m^{-3}, while sediment is often assumed to be 2.65 g cm^{-3} (quartz sand). Furthermore, the density of microplastics may change over time due to biofouling, flocculation and fragmentation, yet exact levels of change are yet to be quantified [95]. Settling experiments have revealed that theoretical approaches from sediment transport are inaccurate for predicting microplastic fate [13,14]. Shape has also been highlighted as more significant in determining fate than for natural sediments as microplastics tend to have more variation in type and form [14,119]. These differences imply that microplastics may not follow predictable sediment dynamics and require distinct monitoring strategies across varying discharge conditions to better predict their fate. Without accounting for these differences, traditional sediment-focused approaches may underestimate the role of microplastics in riverine and oceanic systems. The results provide several implications for predicting microplastic loads and their fate in rivers. Spatial and temporal variation in microplastic concentrations must be accounted for, as they fluctuate with changing seasonal discharge [57]. Concentrations and fluxes of microplastic will change depending on seasonal discharge, with sampling campaigns needing to be conducted throughout the year to form accurate predictions on microplastic loads from rivers into oceans. This includes sampling at lower flows where it is expected that microplastic concentration will be higher, due to decreasing discharge and constant microplastic input. However, this may vary between rivers, with further monitoring needed to determine differences in microplastic concentration patterns worldwide. Understanding these dynamics is essential for forecasting microplastic transport and eventual deposition or export.

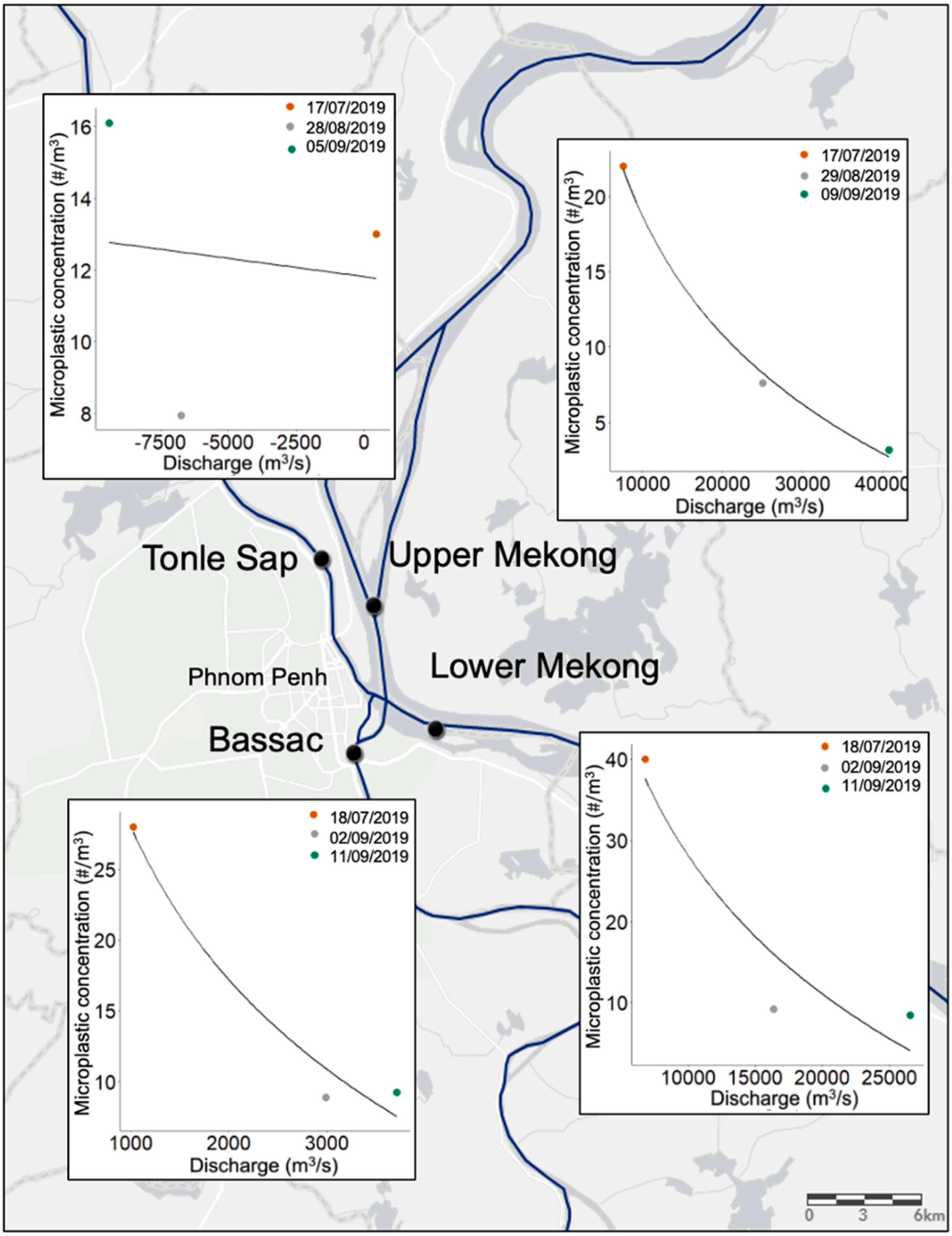

Fig. 7. Microplastic concentration in relation to river discharge at the Upper Mekong River, Tonle Sap River, Bassac River and Lower Mekong River of Phnom Penh, Cambodia. Microplastic concentration data from July 2019 (orange) is from this study while data from August and September 2019 (grey/green) are from [38]. Discharge data from a fully validated Mike11 model of the Phnom Penh region forces with observed discharge data from the river gauge at Kratie. Note the negative discharge at Tonle Sap is negative during August and September, driven by the flow reversal during the wet season.

5. Conclusion

This study investigated microplastic abundance throughout the water column at multiple sites along the Mekong River, a significant contributor to marine plastic waste, and its tributaries of Cambodia and Vietnam. Microplastic concentrations increased downstream and in urban areas, predominantly consisting of fibres and fragments, likely from textiles and packaging. This was attributed to inadequate waste management, especially of wastewater treatment. However, microplastic identification can be hindered by aging and sample processing, potentially causing underestimation. Efforts to collect aged reference materials are essential for improved analysis. We also highlight the importance of sampling throughout the water column, with on average 86% of microplastics seen below the water surface. This demonstrates that riverine microplastic flux predictions may greatly underestimate discharge into the ocean if only using surface water data. Where possible, sampling must occur throughout the water column, to gain representative assessment of microplastic transport. However, if resources do not allow full water column assessment, efforts should be focussed across the top 60% of the flow depth. Furthermore, microplastic concentrations vary with hydrodynamical flows and do not align with suspended sediment transport laws in the Mekong. Flooding may flush microplastics towards the ocean, with higher microplastic concentrations seen before peak flow. However, the impacts of increasing discharge are location specific. Without accounting for these differences, traditional sediment-focused approaches may underestimate the role of microplastics in riverine and oceanic systems. More rigorous monitoring of microplastic transport patterns across multiple sites, both vertically within the water column and seasonally, is essential for accurately predicting their fate and enhancing environmental protection. Microplastics pose significant ecological and socio-economic risks to the Mekong River, including detrimental impacts on various species and associated fisheries. Addressing microplastics at their sources is crucial for preventing contamination. Since most microplastics were fibres from textiles and fragments from packaging, efforts should focus on improving WWTPs and enhancing waste management practices.

Environmental Implication

Microplastics are hazardous due to their persistence, widespread contamination and toxicity to organisms, with rivers being the primary pathway for terrestrial microplastics to oceans. This study reports on microplastic concentrations, characteristics and dynamics in one of the world's longest and polluting rivers. The majority of microplastics are found below the water surface. Therefore, we recommend the need for vertical sampling throughout the water column to determine true microplastic concentrations in major rivers and advise on optimum sampling depths. This will enable more accurate environmental risk predictions and improve strategies to monitor, mitigate and protect aquatic systems for microplastics.

CRediT authorship contribution statement

Danet Hak: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Catherine Waller:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Daniel R. Parsons:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Nguyen Nghia Hung:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Robert Dorrell:** Writing – review & editing, Validation, Methodology, Funding acquisition, Conceptualization. **Christopher R. Hackney:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Freija Mendrik:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vivien M. Cumming:** Writing – review & editing, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2024.136762.

Data availability

Data will be made available on request.

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