

**MICROPELLET PARTICLES: A VECTOR OF HYDROPHOBIC ENDOCRINE
DISRUPTING CHEMICALS IN LAGOS LAGOON**

ABSTRACT

The occurrences of plastic waste materials in the aquatic environment are receiving enormous attention all over the world due to its negative impacts on aquatic organisms. Micropellet litters have been found to adsorb and absorbs persistent organic endocrine disrupting chemicals (EDCs). Endocrine disrupting chemicals are recognized toxic chemicals to humans and organisms. This study quantifies occurrences of micropellet particles in Lagos Lagoon and their EDCs contents. Surface water and sediment sample collection was conducted from 2016 to 2017 at eight sampling locations with three points established in each of the sampling station. The chemical analysis of EDCs was conducted by gas chromatography coupled with electron capture detector and flame ionization detector. Micropellet particles occurrence was highest in surface water (67%) compared to (33%) in sediment during the period of sampling. EDCs contents reflect contamination of PCBs and PAHs in the extracted micropellet particles. Some sampling stations contained relatively higher PAHs concentrations but very low concentrations of PCBs. Since, micropellet particles and EDCs cannot be removed completely from the aquatic environment, reduction of impending hazards ought to rely on curtailing disposal of plastic materials and sensitizes the populace on general disposal methods in order to minimize interaction of plastic particles with EDCs which are likely to pose significant effects on aquatic fauna.

27 **Keywords:** Micropellet particles, endocrine disrupting chemicals (EDCs), PCBs; PAHs, Lagos
28 Lagoon

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31 **I. 0 INTRODUCTION**

32

33 Lagos lagoon in Nigeria supplies a number of essential services for indigenous and non-
34 indigenous people in Lagos (Ajagbe *et al.*, 2012) with high influence of solid waste litters in and
35 around the Lagos lagoon has been reported by several authors, but limited studies on its effects
36 in our water bodies (Ajao, 1996; Amaeze *et al.*, 2012; Abiodun and Oyeleke, 2016, Soneye *et*
37 *al.*, 2018). In Nigeria, plastic litter materials in coastal areas as been reported by (Nubi *et al.*,
38 2010; Amaeze *et al.*, 2012; Soneye *et al.*, 2018) as the most common litters in Lagos lagoon and
39 are likely to be a major sources of hydrophobic endocrine disrupting chemicals contributing to
40 the significant decline in aquatic resources thus threatening their long-term sustainability. Plastic
41 wastes materials is one of the most documented waste in aquatic ecosystem globally (Derraik,
42 2002;UNEP, 2016; Gibb *et al.*, 2017) with greater negative impact on aquatic fauna. In the last
43 few decades, plastic products have developed into one of the most largely used materials for
44 many applications. World total plastic production continues to increase yearly (Duis and Coors,
45 2016) owing to the plasticity and the durability of most of the plastic products(PlasticEurope,
46 2015, 2017). Due to the extensive utilization of plastic in many applications coupled with
47 improper disposal methods of plastic waste materials consistently in waterways leading to
48 breakdown into minute's particles due to weathering action and ultra violet solar radiation (Ryan
49 *et al.*, 2009; Andrady, 2011).

50 Plastic wastes materials was proposed as hazardous materials (Rochman *et al.*, 2013) when
51 found in the aquatic environment. Due to increase in population density and economic growth
52 rate there is a clamour for affordable products which have led to increased plastics production as

53 well indiscriminate increased in plastic waste generation in Nigeria (Olanrewaju and Ilemobade,
54 2009; Babayemi, *et al.*, 2018). Despite the intervention of the government in Nigeria, on proper
55 waste disposal methods, solid waste still find their way into the Lagos lagoon at an alarming rate
56 (Olanrewaju and Ilemobade, 2009; Babayemi, *et al.*, 2018). Unlike other substances, majority of
57 plastic waste materials are not easily biodegradable, but instead photodegradable into smaller
58 fragment and further degraded (Arthur *et al.*, 2009) from macroplastic, >5 mm into
59 microplastics <5mm that has increased conspicuously (Thompson *et al.*, 2004, 2009) in the
60 aquatic environment. Several authors reported the ability of microplastics particles to adsorbed
61 and absorbed hydrophobic endocrine disrupting chemicals (HEDCs) at a several magnitude
62 higher than their surrounding water (Rochman *et al.*, 2013; Velzeboer *et al.*, 2014).

63 Plastic wastes materials and hydrophobic endocrine disruption chemicals has been reported by
64 Vethaak and Leslie (2016) to form multifaceted mixture of contaminants in the aquatic
65 environment that increase the availability HEDCs to be readily bioavailability to wide variety of
66 aquatic faunas and eventually to humans in contrast to other naturally sorbent (Bakir *et al.*, 2016;
67 Koelmans *et al.*, 2016). Hydrophobic endocrine disrupting chemicals like polychlorinated
68 biphenyls (Mato *et al.*, 2001, Bakir *et al.*, 2012, 2014) and polycyclic aromatic hydrocarbons
69 (Rochman *et al.*, 2013) are known to adsorb on microplastic surfaces (Teuten *et al.*, 2009).
70 Presently there is increasing concern that aquatic fauna declines in populations and increasing
71 occurrence of endocrine-related syndrome in aquatic organisms are connected to chemicals
72 compound adsorbed on plastic waste materials (Bergman *et al.*, 2013). These chemicals
73 compounds includes but not limited to polychlorinated biphenyl (PCBs), organochlorine
74 pesticides (OCPs) and polycyclic aromatic hydrocarbon (PAHs) are well known hydrophobic
75 persistent organic pollutants that are constrained in most countries Nigeria inclusive may

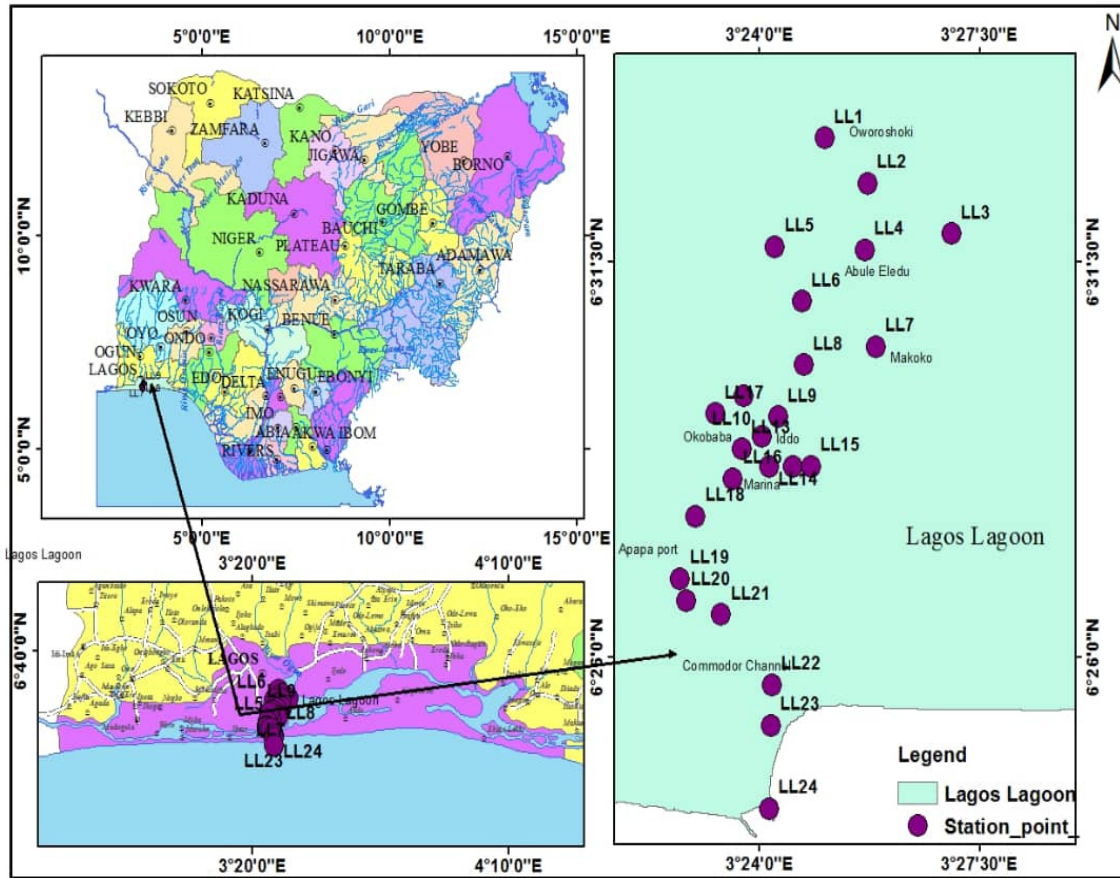
76 interfering with the endocrine system as reported by (Bergman *et al.*, 2013). Some HEDCs are
77 known to cause effects at the present levels found in biota and the environment (Vethaak and
78 Legler, 2012; Bergman *et al.*, 2013). However, the absorption ability of micropellet particles of
79 hydrophobic endocrine disrupting chemicals has not been sufficiently studied. Hence, this study
80 is aimed at evaluating the associated hydrophobic endocrine disrupting chemicals in micropellet
81 particles extracted from surface water and sediment of Lagos lagoon.

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86 **2.0 MATERIALS AND METHODS**

87 **2.1. Sampling area**

88 The study was carried out in one of the biggest estuary in Nigeria Lagos lagoon. The lagoon
89 empties into the Atlantic Ocean through the Lagos harbour, an important channel through the
90 heart of Lagos. Within the Lagos lagoon eight (8) sampling stations were established based on
91 solid waste characteristics of each of the sampling area as reported by past literature (Abiodun
92 and Oyeleke, 2016) (Figure 1 and Table 1). In each of the sampling stations three (3) points
93 were selected to represent the true conditions of the sampling locations (Figure 1) with different
94 wastes littering the surface of the water.



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96 Figure1: Map of the Sampled Study Area

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102 **2.2 Sample Collection Methods**

103 - *Microplastic Samples (355 micron and above)*

104 The surface water was collected by means of e manta trawl net with a circular opening of 15cm
105 by 45cm wide with iron frame, 60 cm length 1.62mm mesh net with 20X 5 collecting bottle was
106 towed behind a speed boat for 30 minutes at each site to sampled microplastic by tow speeds
107 below 3 knots, while speed boat maintains a consistent heading. At each sample site, a target tow
108 length of 500 to 2000 m was established with length based upon the amount of floating debris
109 and waste samples captured at the base of the net end was placed in a clean pre labelled glass

110 sample bottle. To avoid contaminating samples, the manta net and collection vessel were rinsed
 111 methodically (Eriksen *et al.*, 2013). Sediment sampled for the analysis of micropellet particles
 112 were collected with a Van veen grab sampler (0.1m²) in areas of low flow velocity (<0.3m/s) in
 113 each of the stations. Thereafter, sediment was gently stirred and carefully sieved through a
 114 0.5mm mesh sieve. The content of the sieve after washing was transferred into a pre-labelled
 115 container and 10% formalin was added and transfer to the laboratory for further analysis.

116 2.3 Extraction of microplastics

117 The method of extraction employed involved filtration of solids wastes obtained in manta trawl
 118 net surface sampling and sediment sampled while plastic waste materials of appropriate size
 119 were isolated. The sieved plastic waste materials were air dried under the fume hood to
 120 determine the mass in the microplastics sampled. The micropellet particles extracted were
 121 subjected to wet peroxide oxidation (WPO) in the presence of a Fe (II) catalyst to absorbed
 122 organic matter and sediment attached to the plastic waste. In addition the floating plastics were
 123 further isolated from the denser undigested mineral components with a density separator using a
 124 custom 0.45mm filter, air-dried, and plastic material were removed and weighed to determine the
 125 microplastics concentration (Free *et al.*, 2014; Masura *et al.*, 2015).

126 **Table 1: Description of sampling stations and solid waste characterization**

Station Name	Sampling points	Solid waste characterization
Oworonshoki (ST 1)	LL1	Plastic litters, glass, paper, domestic organics, cloths, and human waste.
	LL2	
	LL3	
AbuleEledu (ST 2)	LL4	Plastic litters, glass, paper, domestic organics, human waste, and wood logs.
	LL5	
	LL6	
Makoko(ST 3)	LL7	Plastic litters, glass, paper, domestic organics, human waste, cloths and wood log
	LL8	
	LL9	
Okobaba (ST 4)	LL10	Sawdust waste, wood log, plastic waste, organic wastes and human
	LL11	

	LL12	waste
Iddo (ST 5)	LL13	solid waste dump, cement bag washing, and rubber waste
	LL14	
	LL15	
Marina (ST 6)	LL16	plastics, glass, paper, vegetable waste, human waste
	LL17	
	LL18	
Apapa Port (ST 7)	LL19	Oil and grease, spillages, ship garbage and plastic debris
	LL20	
	LL21	
Commandor channel (ST 8)	LL22	Marine litters
	LL23	
	LL24	

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128 2.4 Test chemicals

129 Analytical grade solvents hexane and dichloromethane were purchased from Sigma Aldrich.
 130 Standards of PCBs, and PAHs were purchased from Accustandard (New Haven, CT, USA). PCB
 131 65 and PAHs mixture Z-014J-0.5X (Naphthalene -d8, acenaphthene-d10, phenanthrene-d10,
 132 chrysene-d12 and perylene-d12) and CLP-LC-SS1 (Nitrobenzene-d5, 1-1'-biphenyl 2-fluorene-
 133 d10 and pterphenyl-d14) were purchased from Accustandard. These standards were diluted with
 134 analytical grade hexane to make calibration, internal, and recovery standards.

135 2.5 Solvent Extraction Procedure

136 Micropellet particles were extracted for determination of contents of PCBs and PAHs.
 137 Approximately 1 g of micropellets was used for extraction, Samples were placed in labelled
 138 amber glass bottle and matrix blanks were used as controls. Matrix blank consisted of virgin
 139 polyethylene pellets that were not exposed to environmental factors outside of production. About
 140 0.3 g of sodium sulphate was added to each amber bottle in order to remove any moisture present
 141 in each sample. Each sample was then spiked with known amounts of internal standards. N-
 142 Hexane was added to each sample in equal amounts of 30ml and shaker for 30 minutes using a
 143 mechanical shaker (Fisher Scientific, Fair Lawn, NJ, USA). The extraction procedure was then

144 repeated a three time (3x) with 15ml of hexane were added to the same amber bottle Extracted
145 samples were then placed in a round bottom flask and attached to rotary evaporator and
146 concentrated to about 2 ml at 40°C.

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148 **2.6 Silica gel- solid Phase Extraction (SPE)**

149 Silica gel based SPE cartridges, Sep-Pak, from (Waters, Milford, MA, USA) and glass syringes
150 were assembled on a Visiprep SPE vacuum (Supelco, Bellefonte, PA, USA). After thorough
151 cleaning and conditioning of SPEs, each extract was loaded into SPE and fractionized with 10 ml
152 of hexane and 10 ml dichloromethane (7:3). Flow rate of solvent through SPEs was carefully
153 monitored at this time. The two fractions were combined and concentrated by the TurboVap,
154 transferred to 2 ml amber vials.

155 **2.7 Identification and Quantification of PCB Congeners and PAHs derivative**

156 Identification and quantification of 28 PCB congeners (congeners (PCB 8, PCB18, PCB 28,
157 PCB 44, PCB 52, PCB 60, PCB 77, PCB 101, PCB 81, PCB 105, PCB 114, PCB 118, PCB 123,
158 PCB 126, PCB 128, PCB 138, PCB 153, PCB 156, PCB 157, PCB 167, PCB 169, PCB 170,
159 PCB 180, PCB 185, PCB 189, PCB 195, PCB 206 and PCB 209) was performed with a gas
160 chromatograph (GC)-electron capture detector (ECD) (Agilent 7890A GC- (ECD Detector)
161 using USEPA Method 608. sixteen(16)PAHs (naphthlaene (NAP), acenaphthylene (ACY),
162 acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene
163 (FLT), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF),
164 benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[ghi]perylene (BghiP),
165 dibenzo[a,h]anthracene (DahA), indeno[1,2,3-cd]pyrene (IND)). Analysis was conducted

166 utilizing Agilent Gas Chromatography (GC-7890A) coupled with Flame ionization Detector
167 (FID) Column: HP5 (30m x 320um x 0.25um) along with internal and recovery standards.

168

169 **2.8 Quality Assurance**

170 All glassware was thoroughly cleaned and baked in the oven at 140°C for thirty minutes
171 (30mins) and glass syringes at 45°C for one hour. During the extraction procedures, samples
172 were all carefully covered with aluminium foil in order to prevent contamination. All glass
173 pipettes, test tubes, and vials were discarded after single usage.

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175 **2.9 DATA ANALYSIS**

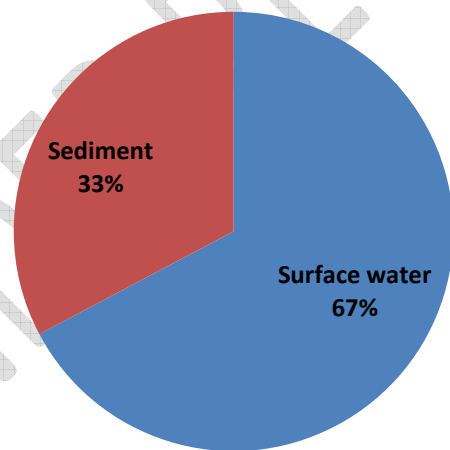
176 Analysis of results was completed by using the sum totals of 28 PCBs and 16 PAHs. One way
177 Analysis of variance (ANOVA) with Pos –Hoc Duncan multiple range test was conducted
178 coupled with descriptive analysis (means and standard deviations) from the sums of each EDCs
179 compound analyzed. Data was sorted micropellets extracted from surface water and sediment
180 according to sampling locations.

181 **3.0 RESULTS**

182 **3.1. Micropellet particles occurrence and distribution among the sampled environmental** 183 **matrices**

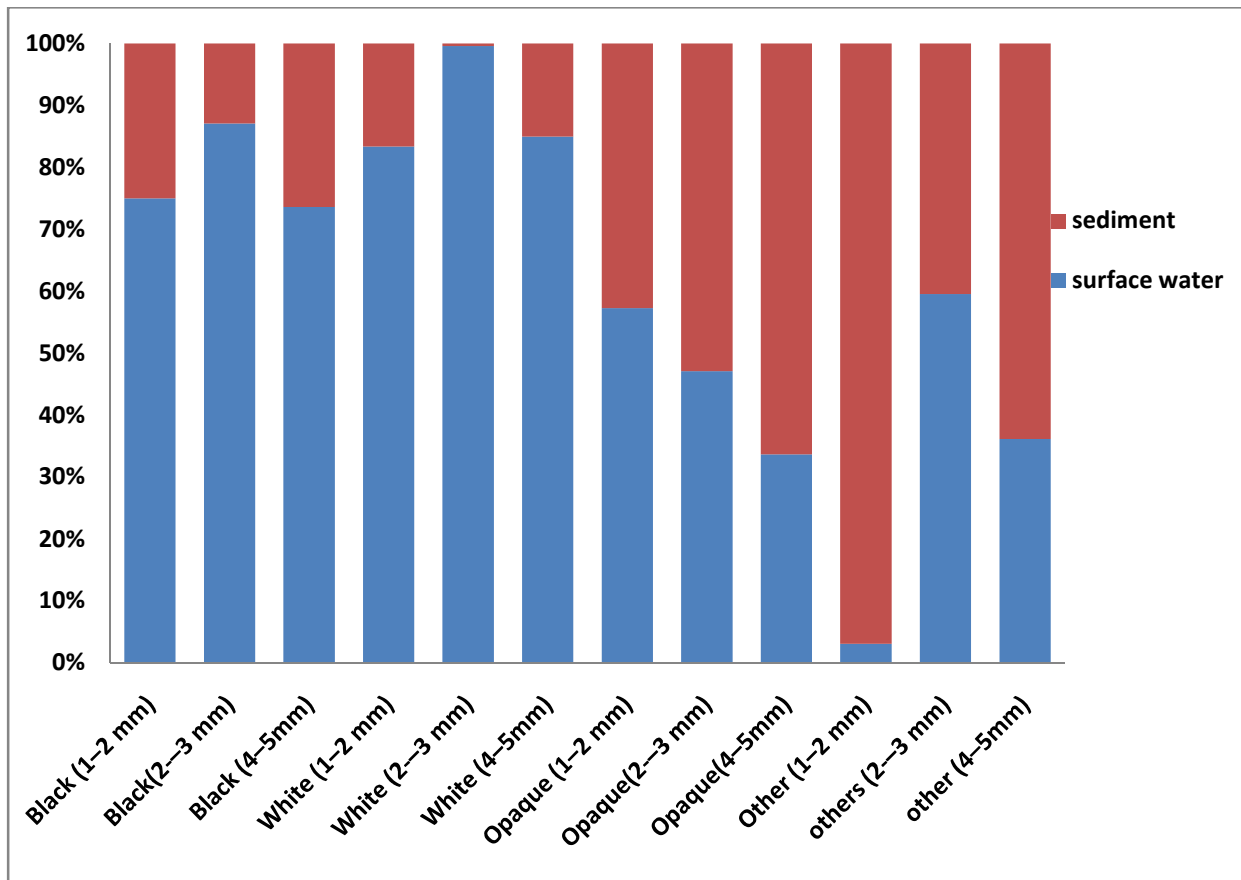
184 The occurrence of micropellets sampled within the environmental matrices indicated that
185 abundance of pellet particles are more in surface water than in sediment. Micropellets particles
186 were present in trawls nets from all sampling stations in surface water. Almost all of the plastic
187 micropellet particles (91.6%) were round in shaped whereas 13.7% were cylindrical with the
188 highest occurrence in surface water (67%) and (33%) in sediment sampled (Figure. 2). Most

189 common colour in all size class of micropellet particles extracted in surface water and sediment
190 across all the sampling stations was white (56.31) and opaque in sediment(62.37 (Figure 3).
191 Majority (73.20%) fell between 2–3mm size classes in surface water while 46% size class in
192 sediment was between 1-2mm. In addition, 87.7% were white whereas 12.2% were black and
193 only 2 were other colour (1 brown). The greatest mean percentage occurrence concentration of
194 micropellet particles was observed in surface water samples from the marina axis at sampling
195 point LL16 (4692 micropellets particles/L), followed closely by samples collected at
196 Commodore channel at sampling points LL22 (4165 Micropellets particles per l) and the lowest
197 occurrence is observed at Makoko station at sampling point LL7 (figure 4).There were no
198 significant differences between any size classes for each colour when percentages based on mass
199 were analyzed.



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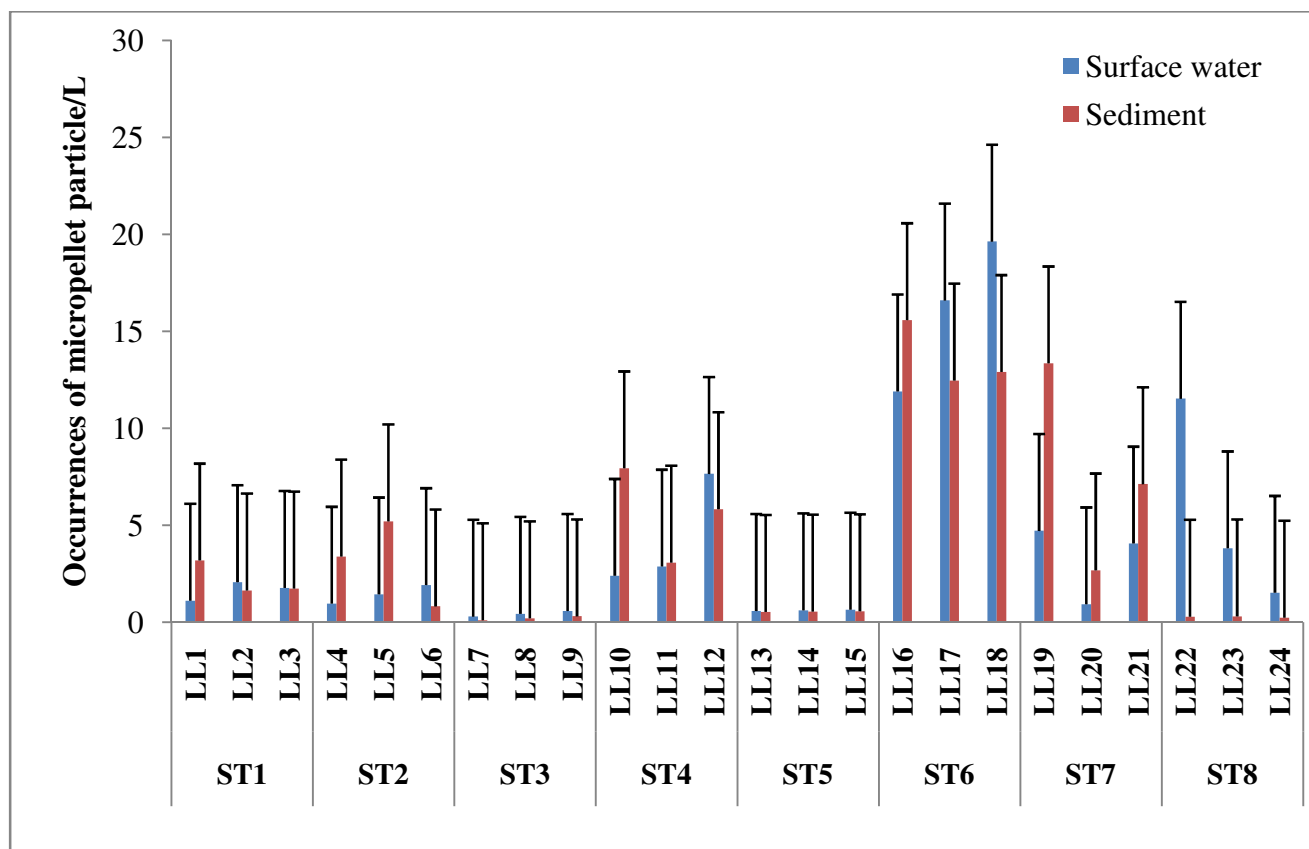
204 Figure 2: Distribution of micropellet particles in environmental matrices



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206 Figure 3: Percentage of plastic micropellet particles with each colour in each size class from each
 207 environmental matrix.

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210 Figure 4: percentage occurrences of micropellet particles in each sampling points

211

212 3.2 EDCs contents in extracted pellet particles from surface water and sediment

213 All micropellets samples contained detectable amount of persistent organic EDCs (figure 5-10),
 214 demonstrating the ubiquitous nature of these contaminants. Inter-stations differences in the
 215 concentrations of individual EDCs were apparent in all the sampling stations.

216

217 3.2.1 PCBs in Micropellet particles extracted from surface water and sediments

218 The Σ PCBs concentration varied between 76 and 1043 ng g⁻¹, which was significantly P (<
 219 0.001) higher in the surface water than in sediment (Figure 5). The maximum Σ PCBs
 220 concentration was found at ST 5 from micropellet particles extracted from surface water while

221 the highest notably concentration of PCBs in micropellet particles extracted from sediment was
222 detected in ST 8 (873 ng g^{-1}), with two to three orders of magnitude higher than that recorded
223 for some of the other stations. In respect of sampled matrices PCB 52 and PCB 77 are the most
224 abundance in surface water and sediment while PCB 195 was relatively low in the pellet
225 particles extracted from surface water and sediment (Figure 6 and 7)

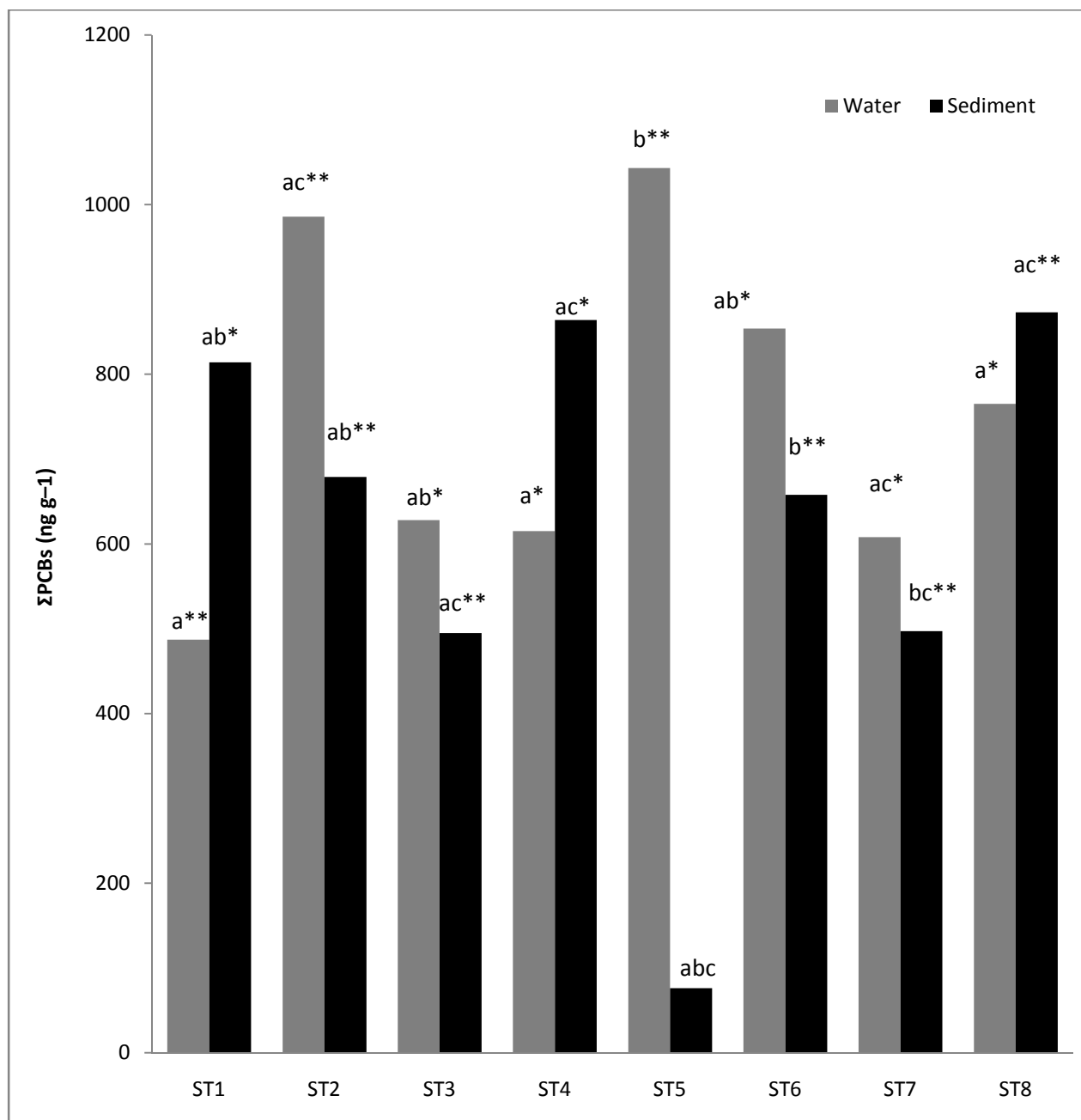
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UNDER PEER REVIEW

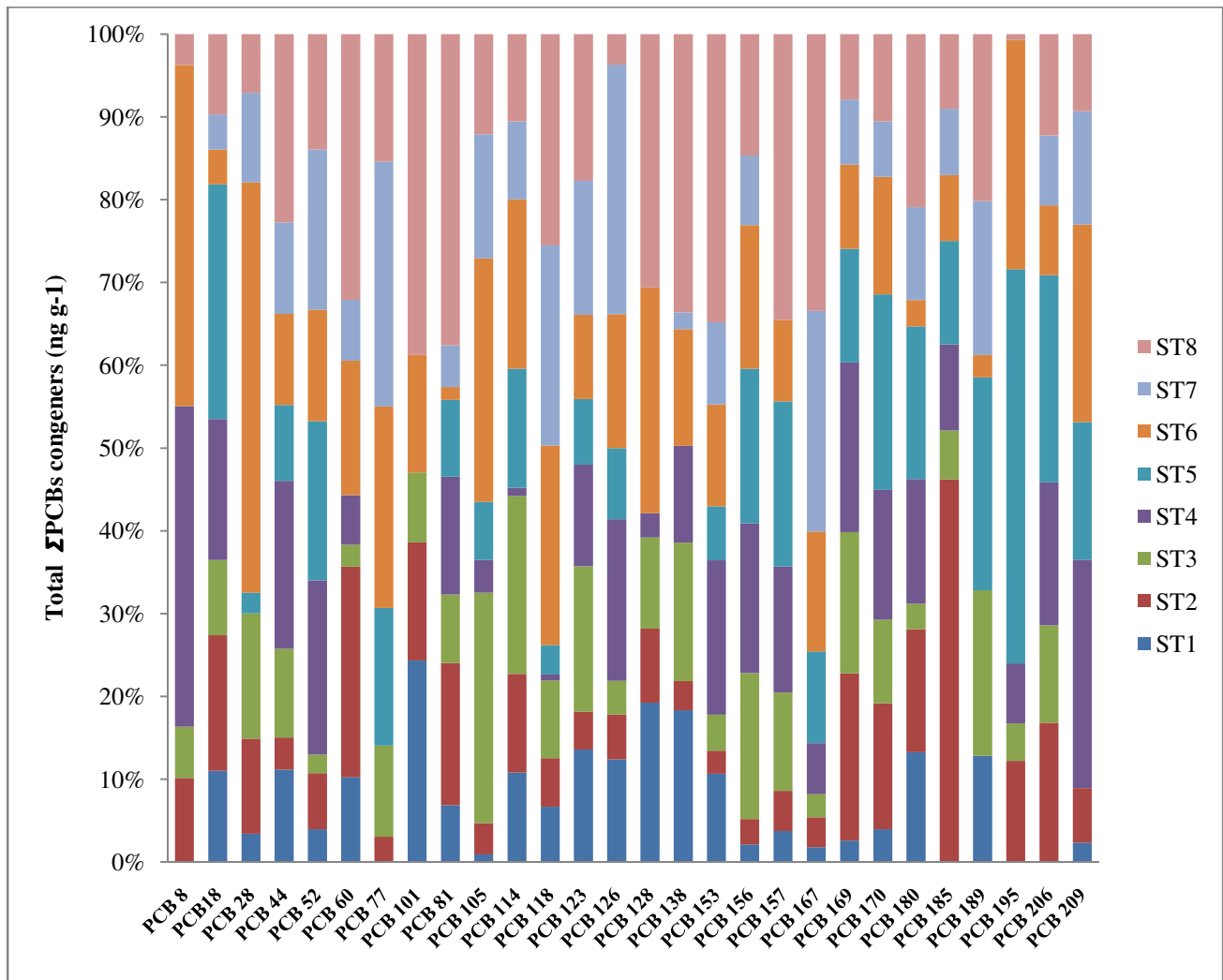


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231 Figure 5: Mean concentrations of ΣPCBs (28 congeners) in microplastics (ng g⁻¹) in Surface
 232 water and sediments.

233 Means and standard deviations of three replicates are shown; bars with different letters indicated
 234 significant differences among sampling stations at the level of $p < 0.05$ according to one-way
 235 ANOVA and post-hoc Duncan multiple range test; single asterisks (*) and double asterisks (**)
 236 indicated a significant difference between environmental matrices at the level of $p < 0.01$.

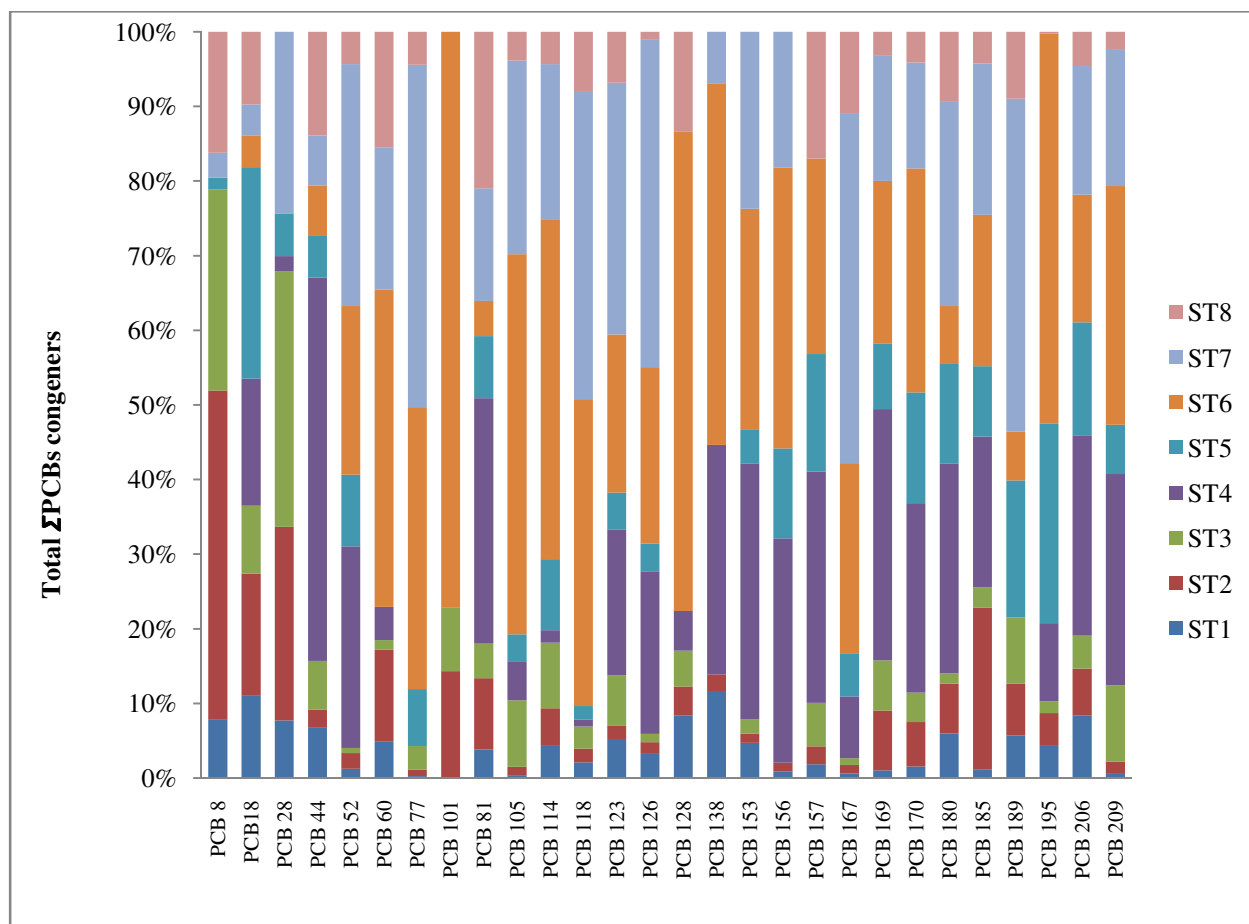
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239 Figure 6: Mean concentrations of ΣPCBs (28 congeners) extracted from micropellets in surface
 240 water

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242
 243 Figure 7: Mean concentrations of Σ PCBs (28 congeners) extracted from micropellets in sediment

244
 245
 246 **3.2.2 PAHs in Micropellet particles extracted from Surface water and sediments**

247 The total PAHs concentration ranged between 46.05 ng g^{-1} (ST1) and $3984.04 \text{ ng g}^{-1}$ (ST 6)

248 within micropellet particles extracted in the environmental matrices (Figure 8). When individual

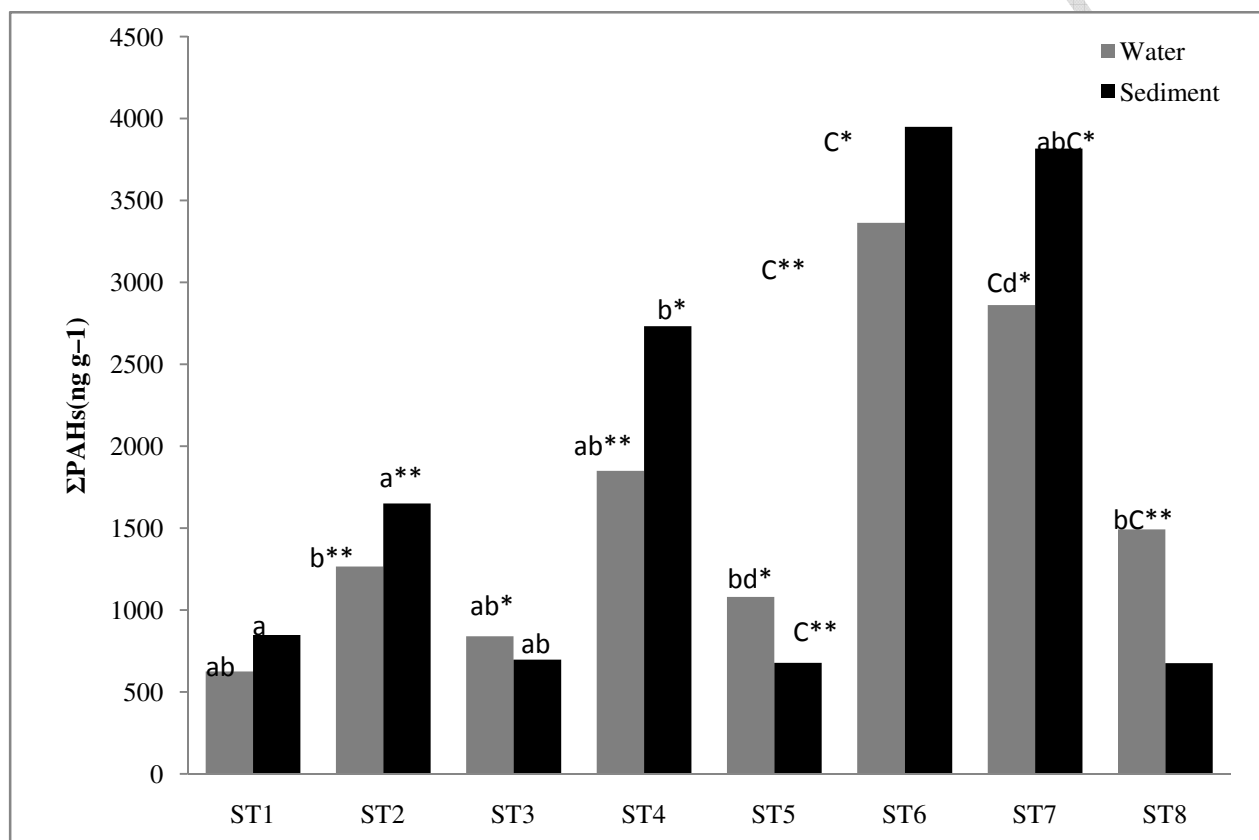
249 station were compared in regards to the environmental matrices, all the stations have PAHs types

250 three 5–6 rings PAHs .On the other hand, sites ST1 and ST6 were greatly affected by 2–5rings

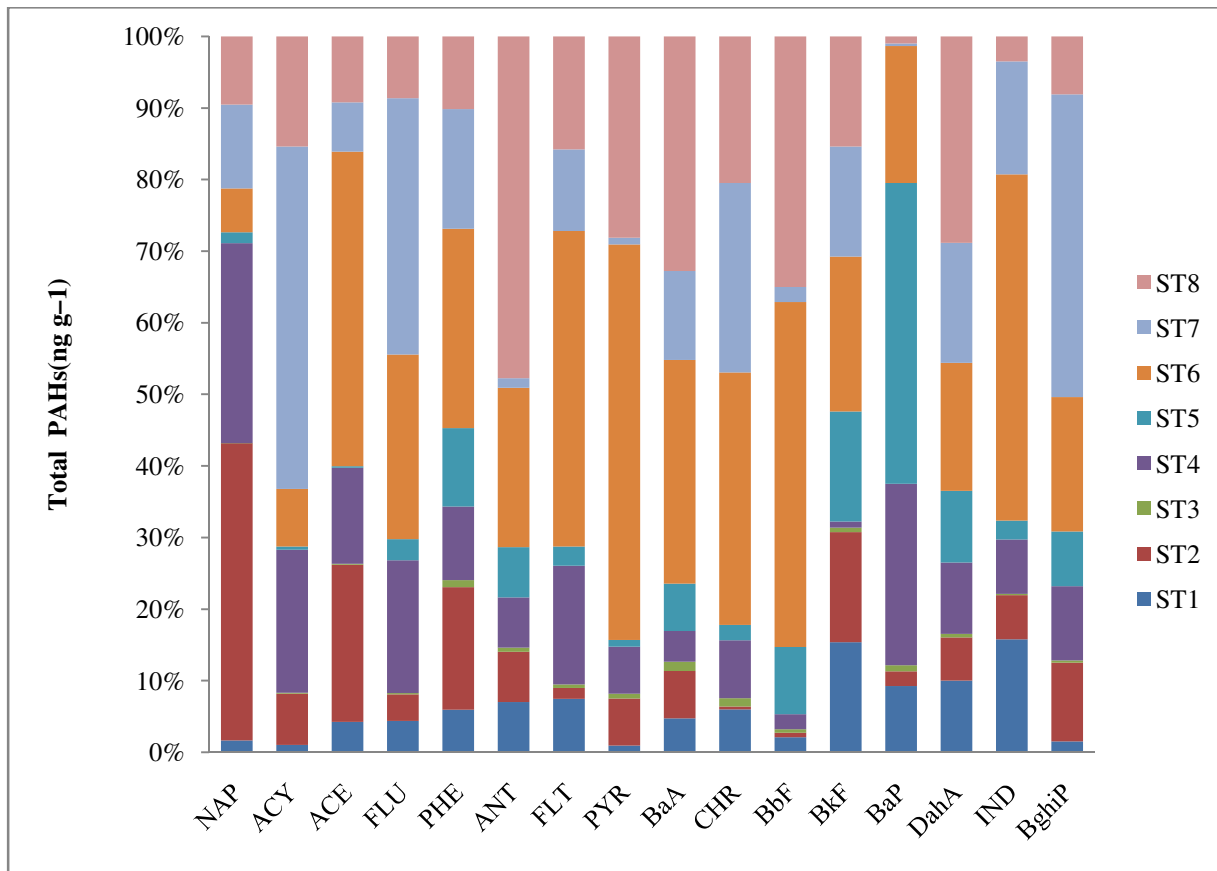
251 PAH. Regardless of stations, 3–4 rings PAH dominated in this study (Figure 9 and 10). The PAH

252 diagnostic ratios (Figure8) indicated PAHs that varied among Stations as well as within the

253 environmental matrices). The related distribution pattern for EDCs displayed in ST 7 and ST 6;
 254 contamination of PAHs could be an indication that local contamination sources probably would
 255 have contributed to such difference observed across the sampling station.
 256



257
 258 Figure 8: The concentrations of HEDCs in microplastics (ng g⁻¹) of ΣPAHs (16 congeners).
 259
 260 Means and standard deviations of three replicates are shown; bars with different letters indicate
 261 significant differences among sampling stations at the level of $p < 0.05$ according to one-way
 262 ANOVA and post-hoc Duncan multiple range test; single asterisks (*) and double asterisks (**)
 263 indicate a significant difference between season at the level of $p < 0.01$
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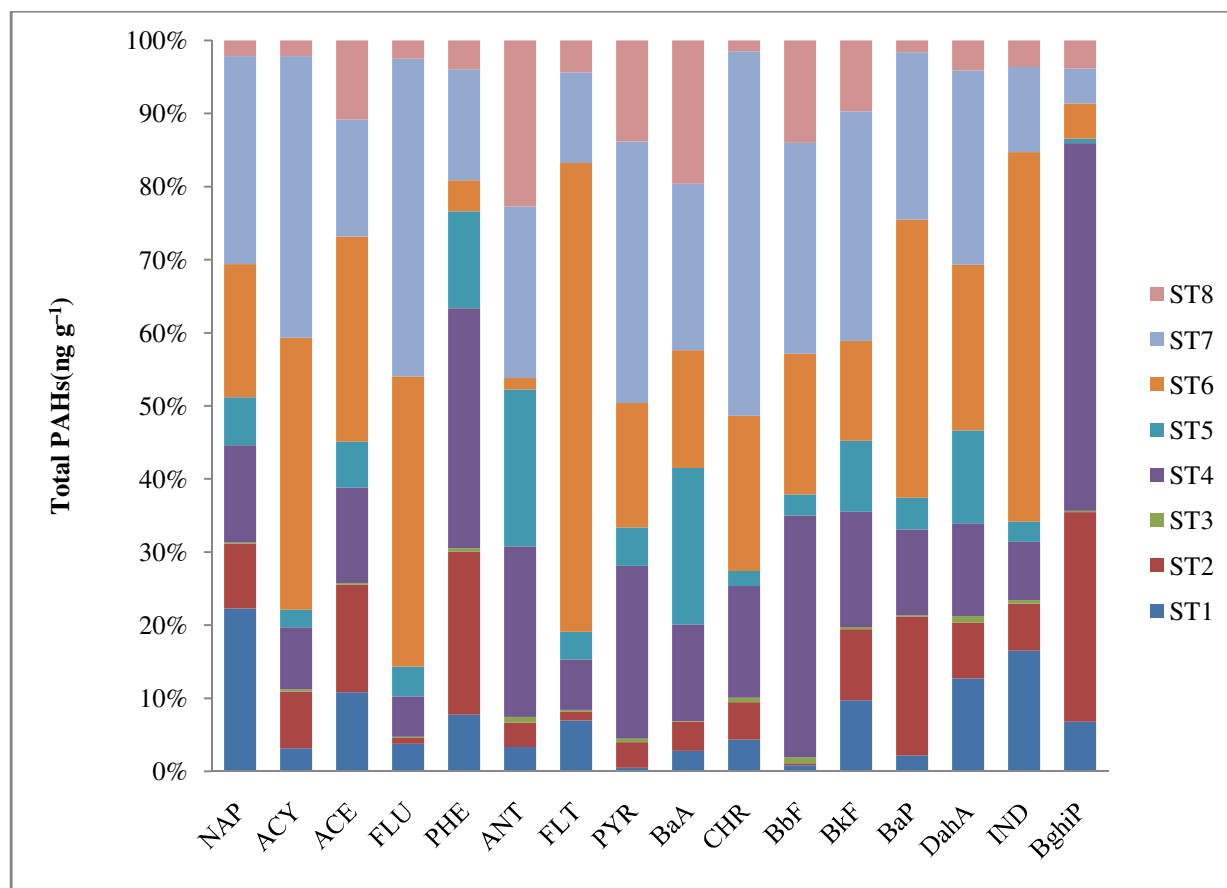


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266 Figure 9: The concentrations of Σ PAHs (16 congeners) extracted from micropellets in surface

267 water

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270 Figure 10: The concentrations of Σ PAHs (16 congeners) extracted from micropellets in sediment

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272 **4. Discussion**

273

274 This study evaluated micropellet particles occurrences and distribution in environmental matrices

275 (surface water and sediment) and their associated hydrophobic endocrine disrupting chemicals in

276 the extracted micropellet sampled. In the present study, the micropellet particles collected which

277 were higher in surface water than in sediment were mostly opaque and white, a finding in

278 agreement with other studies that have reported that most micropellet found in environmental

279 matrices are often white or opaque (Heo *et al.*, 2013; Corcoran, 2015; Veerasingam *et al.*, 2016).

280 This finding is not surprising because white micropellet particles are the most common colour

281 manufactured (Redford *et al.*, 1997) worldwide. However, one author have recorded frequency

282 of yellow micropellet (Karapanagioti and Klontza, 2007) and according Veerasingam *et al.*
283 (2016) they are the second most frequently observed micropellet particles often associated with
284 micropellet particles in environmental matrices, a results that was not established in this present
285 study. Although, the difference observed may probably be due to difficulties in colour definition
286 by those researchers counting opaque micropellet particles as “yellow” whereas in the present
287 study they were counted as opaque colour. According to (Wright, *et al.*, 2013), this white
288 composition of micropellet particles are similar in colour to most plankton organisms, a primary
289 food source for most aquatic organisms dwelling in the pelagic zone. Due to range in
290 micropellet size, a substantial proportion is probably discharged into creek, river, estuaries and
291 the oceans. The occurrence of micropellets particles within the marine environment is currently
292 well recognized in the water column, at the sea surface and sediments (Law and Thompson,
293 2014). It has been documented that micropellet particles also accounted for about 10% of all
294 reports of ingestion of aquatic debris, highlighting their importance as a component of aquatic
295 debris (Gall and Thompson, 2015). Their size makes them accessible to organisms with a range
296 of feeding methods, including: filter feeders (mussels, barnacles), deposit feeders (lugworms)
297 and detritivores (amphipods, sea cucumbers) and zooplankton (Wright, *et al.*, 2013). Due to
298 diversities in their size, a substantial proportion is may possibly be discharged into creek, river,
299 estuaries and the oceans. PCBs were the most frequently encountered organic contaminant, and
300 total PCBs on micropellet particles were higher and varied according to geographical location
301 and frequency of pellets occurrence (Rochman, 2015; Fotopoulou and Karapanagioti, 2017;).
302 The PAHs concentrations in micropellet particles obtained in this study were generally lower
303 than the values reported elsewhere. Some authors reported high concentrations of priority PAHs
304 contamination in micropellet particles collected in coastal region (Ziccardi *et al.*, 2016; Ivleva, *et*

305 *al.*, 2017;Mendoza, *et al.*, 2016). Sixteen priority PAHs concentration was detected in the
306 floating marine plastic debris collected from Northern Pacific Gyre varied between undetected
307 and 14459 ng g⁻¹ (Rios *et al.*, 2010). When compared with the concentrations found in marine
308 environment in previous studies, much higher concentrations of PAHs were obtained in
309 microplastics in this study.

310 Differences in PAHs level across the sampling stations were apparent, even for stations very
311 close to each other. This probably indicated that there is possibility of input PAHs at
312 preproduction of plastic pellets. The presence of EDCs in the environment may have ecological
313 and health consequences not only for aquatic fauna but also for humans, as EDCs can enter the
314 food chain and bioaccumulates. The range of values of polycyclic aromatic hydrocarbons and
315 polychlorinated biphenyl studied confirmed large fluctuations within the period of study possibly
316 influenced by anthropogenic activities. This study corroborated with the reports of previous
317 authors (Nubi, *et al.*, 2010; Amaeze, and Abel-Obi, 2015; Abiodun and Oyeleke, 2016; Soneye,
318 *et al.*, 2018) in South-West Nigeria that plastic waste materials litter the Lagos Lagoon
319 environment. Furthermore, a potential problem associated with micropellet particles
320 contamination is the likelihood of transport of endocrine disrupting chemicals contaminants by
321 plastic waste particles which have been established in this study to adsorbs onto surface of
322 plastic waste materials and may transfer to biota upon ingestion as reported by many authors
323 (Teuten *et al.*, 2009;Devriese *et al.*, 2015; Koelmans *et al.*, 2016;Suaria *et al.*, 2016).

324 **Conclusion**

325 The present study showed that micropellet particles are present in environmental matrices can be
326 of varying colour, size and shape with differential affinities for sorption of persistent
327 hydrophobic organic chemicals that may alters the hormonal behavioural and physiology of

328 aquatic fauna threatening aquatic resource. This calls for urgent monitoring in Lagos lagoon and
329 other coastal region in Nigeria (where plastics products are abundantly manufactured and used
330 with reckless abandon) in order to mitigate the danger of plastic waste materials in our coastal
331 bodies.

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333 **5.0 Reference**

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