

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

ABSTRACT

The occurrence of plastic waste materials in the aquatic environment is receiving enormous attention all over the world due to its negative impacts on aquatic organisms. Micropellet litters have been found to adsorb and absorb persistent organic endocrine disrupting chemicals (EDCs). Endocrine disrupting chemicals are recognized toxic chemicals to humans and organisms. This study quantifies occurrence 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 concentration 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 sensitizing 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.

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

28 I. 0 INTRODUCTION

29

30 Lagos lagoon in Nigeria supplies a number of essential services for indigenous and non-
31 indigenous people in Lagos (Ajagbe *et al.*, 2012). High influence of solid waste litters in and
32 around the Lagos lagoon has been reported by several authors (Ajao, 1996; Amaeze *et al.*, 2012;
33 Abiodun and Oyeleke, 2016, Soneye *et al.*, 2018). In Nigeria, plastic litter materials in coastal
34 areas as been reported by (Amaeze *et al.*, 2012; Abiodun and Oyeleke, 2016, Soneye *et al.*,
35 2018) as one of the most common litters in Lagos lagoon and are likely to be a major sources of
36 hydrophobic endocrine disrupting chemicals contributing to the significant decline in aquatic
37 resources thus threatening their long-term sustainability. Plastic wastes materials is one of the
38 most documented waste in aquatic ecosystem globally (Derraik, 2002; UNEP, 2016) with greater
39 negative impact on aquatic fauna (Gibb *et al.*, 2017). In the last few decades, plastic products
40 have developed into one of the most largely used materials for many applications. World total
41 plastic production continues to increase yearly (Duis and Coors, 2016) owing to the plasticity
42 and the durability of most of the plastic products (PlasticEurope, 2015, 2017). Due to the
43 extensive utilization of plastic in many applications coupled with improper disposal methods of
44 plastic waste materials consistently in waterways leading to fragmentation into microparticles
45 due to weathering action and ultra violet solar radiation (Ryan *et al.*, 2009; Andrady, 2011).
46 Plastic wastes materials was proposed as hazardous materials (Rochman *et al.*, 2013) when
47 found in the aquatic environment (UNEP, 2016). Due to increase in population density and
48 economic growth rate there is a clamour for affordable products which have led to increased
49 plastics production as well as indiscriminate increase in plastic waste generation in Nigeria
50 (Olanrewaju and Ilemobade, 2009). Despite the intervention of the government in Nigeria, on
51 proper waste disposal methods, solid waste still find their way into the Lagos lagoon at an

52 alarming rate (Olanrewaju and Ilemobade, 2009; Babayemi, *et al.*, 2018). Unlike other
53 substances, majority of plastic waste materials are not easily biodegradable, but instead
54 photodegradable into smaller fragment (Arthur *et al.*, 2009) from macroplastic, >5 mm into
55 microplastics <5mm that has increased conspicuously (Thompson *et al.*, 2004, 2009) in the
56 aquatic environment. Several authors reported the ability of microplastics particles to adsorbed
57 and absorbed hydrophobic endocrine disrupting chemicals (HEDCs) at a several magnitude
58 higher than their surrounding water (Rochman *et al.*, 2013; Velzeboer *et al.*, 2014).

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

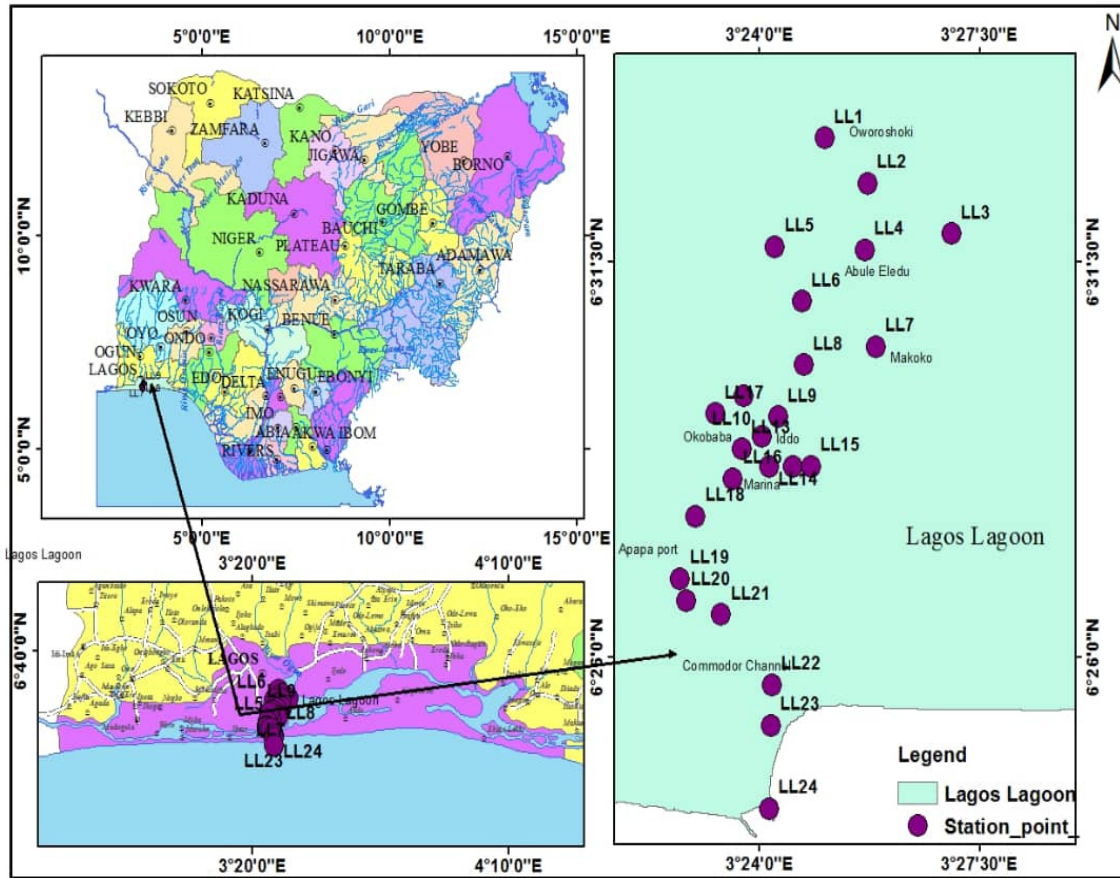
75 of micropellet particles of hydrophobic endocrine disrupting chemicals has not been sufficiently
76 studied. Hence, this study is aimed at evaluating the associated hydrophobic endocrine disrupting
77 chemicals in micropellet particles extracted from surface water and sediment of Lagos lagoon.

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79 **2.0 MATERIALS AND METHODS**

80 **2.1. Description of sampling area**

81 The study was carried out in one of the biggest estuary in Nigeria Lagos lagoon is located
82 between longitude 3°23" and 3°53" and latitude 6°26" and 6°37"N. The lagoon empties into the
83 Atlantic Ocean through the Lagos harbour, an important channel through the heart of Lagos.
84 Within the Lagos lagoon eight (8) sampling stations were established based on solid waste
85 characteristics of each of the sampling area as reported by past literature (Abiodun and Oyeleke,
86 2016) (Figure 1 and Table 1). In each of the sampling stations three (3) points were selected to
87 represent the true conditions of the sampling locations (Figure 1) with different wastes littering
88 the surface of the water.



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90 **Figure1: Map of the Sampled Study Area**

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96 **2.2 Sample Collection Methods**

97 **- Microplastic Samples**

98 The surface water was collected by means of manta trawl net with a circular opening of 15cm by
99 45cm wide with iron frame, 60 cm length 1.62mm mesh net with 20X 5 collecting bottle was
100 towed behind a speed boat for 30 minutes at each site to sampled microplastic by tow speeds
101 below 3 knots, while speed boat maintains a consistent heading. At each sample site, a target tow
102 length of 500 to 2000 m was established with length based upon the amount of floating debris
103 and waste samples captured at the base of the net end was placed in a clean pre labelled glass

104 sample bottle. To avoid contaminating samples, the manta net and collection vessel were rinsed
 105 methodically (Eriksen *et al.*, 2013). Sediment sampled for the analysis of micropellet particles
 106 were collected with a Van veen grab sampler (0.1m²) in areas of low flow velocity (<0.3m/s) in
 107 each of the stations. Thereafter, sediment was gently stirred and carefully sieved through a
 108 0.5mm mesh sieve. The content of the sieve after washing was transferred into a pre-labelled
 109 container and 10% formalin was added and transfer to the laboratory for further analysis.

111 2.3 Extraction of microplastics

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

121 **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 waste
	LL11	
	LL12	
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	

122

123 **2.4 Test chemicals**

124 Analytical grade solvents hexane and dichloromethane were purchased from Sigma Aldrich.
 125 Standards of PCBs, and PAHs were purchased from Accustandard (New Haven, CT, USA). PCB
 126 65 and PAHs mixture Z-014J-0.5X (Naphthalene -d8, acenaphthene-d10, phenanthrene-d10,
 127 chrysene-d12 and perylene-d12) and CLP-LC-SS1 (Nitrobenzene-d5, 1-1'-biphenyl 2-fluorene-
 128 d10 and pterphenyl-d14) were purchased from Accustandard. These standards were diluted with
 129 analytical grade hexane to make calibration, internal, and recovery standards.

130 **2.5 Solvent Extraction Procedure**

131 Micropellet particles were extracted for determination of contents of PCBs and PAHs.
 132 Approximately 1 g of micropellets was used for extraction, Samples were placed in labelled
 133 amber glass bottle and matrix blanks were used as controls. Matrix blank consisted of virgin
 134 polyethylene pellets that were not exposed to environmental factors outside of production. About
 135 0.3 g of sodium sulphate was added to each amber bottle in order to remove any moisture present
 136 in each sample. Each sample was then spiked with known amounts of internal standards. N-
 137 Hexane was added to each sample in equal amounts of 30ml and shaker for 30 minutes using a

138 mechanical shaker (Fisher Scientific, Fair Lawn, NJ, USA). The extraction procedure was then
139 repeated a three time (3x) with 15ml of hexane were added to the same amber bottle Extracted
140 samples were then placed in a round bottom flask and attached to rotary evaporator and
141 concentrated to about 2 ml at 40°C.

142

143 **2.6 Silica gel- solid Phase Extraction (SPE)**

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

150 **2.7 Identification and Quantification of PCB Congeners and PAHs derivative**

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

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

163

164 **2.8 Quality Assurance**

165 All glassware was thoroughly cleaned and baked in the oven at 140°C for thirty minutes
166 (30mins) and glass syringes at 45°C for one hour. During the extraction procedures, samples
167 were all carefully covered with aluminium foil in order to prevent contamination. All glass
168 pipettes, test tubes, and vials were discarded after single usage.

169

170 **2.9 DATA ANALYSIS**

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

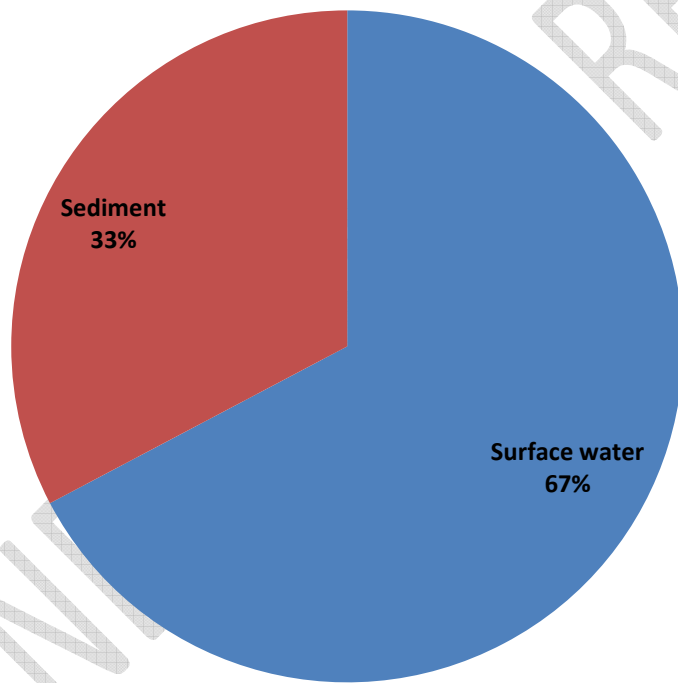
176 **3.0 RESULTS**

177 **3.1. Micropellet particles occurrence and distribution among the sampled environmental** 178 **matrices**

179 The occurrence of micropellets sampled within the environmental matrices indicates pellet
180 particles occurs more in surface water than sediment. Almost all of the plastic micropellet
181 particles (91.6%) were round in shape, with only 8.4% non-cylindrical in shape; maximum
182 occurrence in surface water (67%) and (33%) in sediment sampled (Figure. 2). Most common
183 colour in all size class of micropellet particles extracted in surface water and sediment in all the

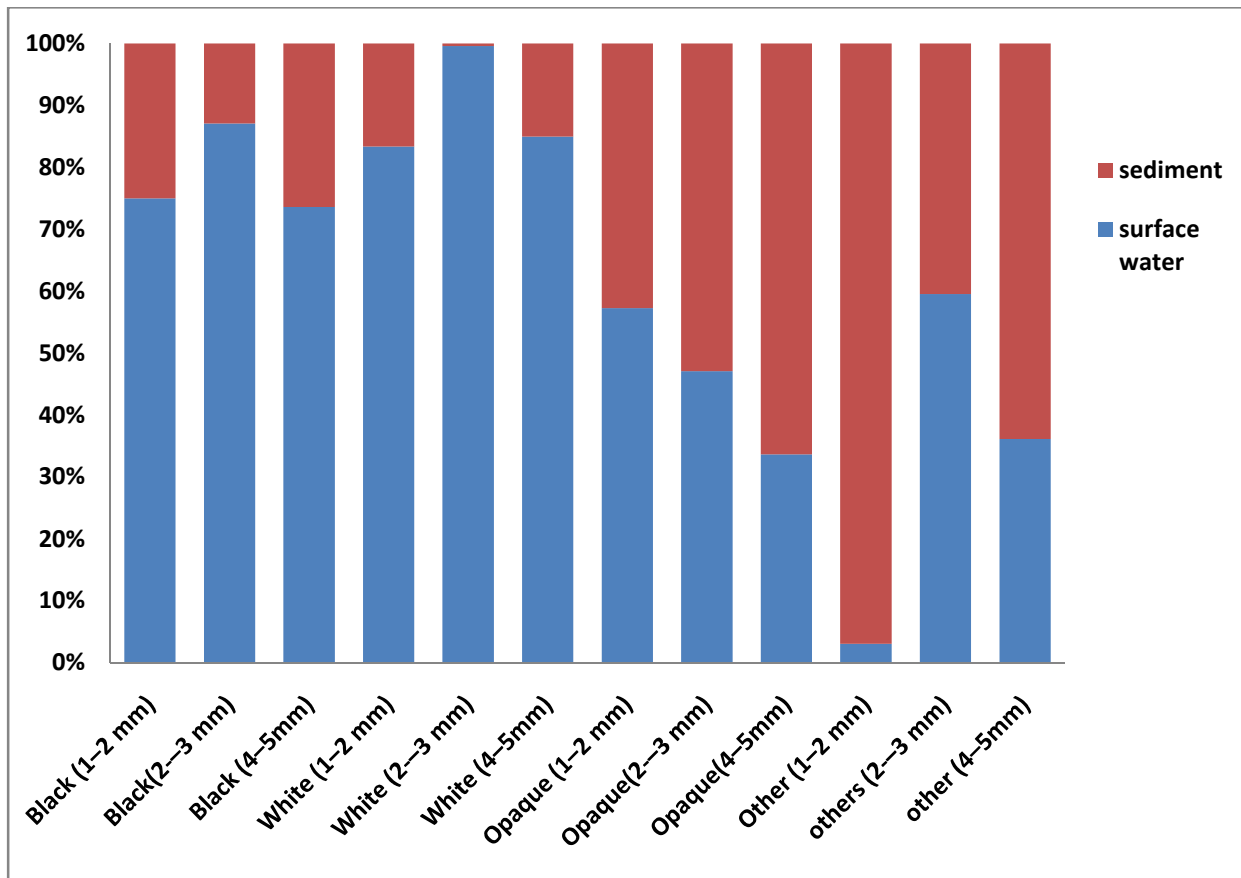
184 sampling stations was white (46.97) and opaque in sediment (53.03) (Figure 3). Majority
185 (63.20%) fell between 2–3mm size classes in surface water while 26.8 % size class in sediment
186 was between 1-2mm. The highest mean occurrence concentration of micropellet particles was
187 observed in surface water samples from the marina axis at sampling point LL16 (4692
188 micropellets particles/L), followed closely by samples collected at Commodore channel at
189 sampling points LL22 (4165 Micropellets particles/L) and the lowest occurrence is observed at
190 Makoko station at sampling point LL7 (figure 4).

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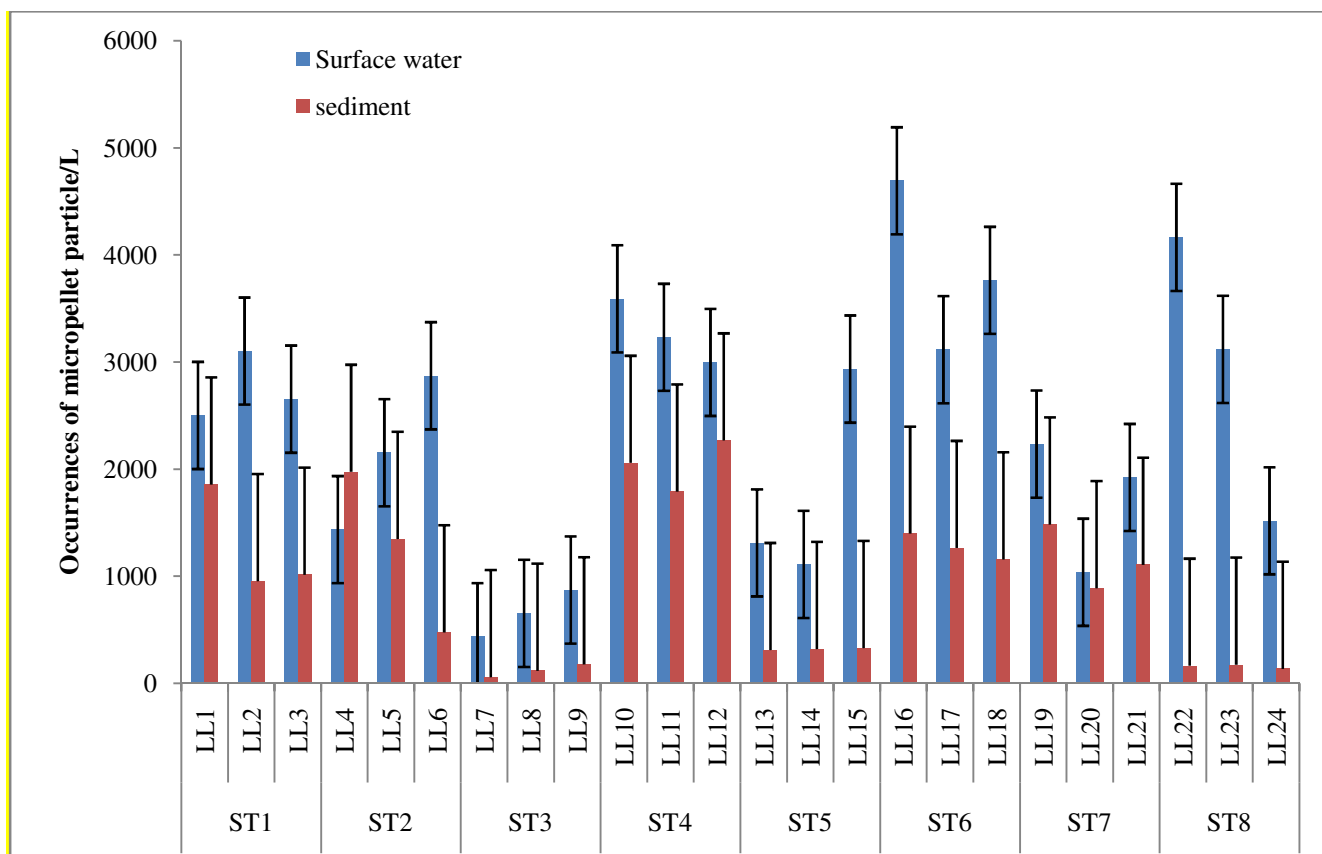
Figure 2: Distribution of micropellet particles in environmental matrices



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

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200

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

202

203 **3.2 EDCs contents in extracted pellet particles from surface water and sediment**

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

207

208 **3.2.1 PCBs in Micropellet particles extracted from surface water and sediments**

209 The Σ PCBs concentration varied between 76 and 1043 ng g⁻¹, which was significantly P (< 0.01
 210 and 0.05) higher in the surface water than in sediment (Figure 5). The maximum Σ PCBs
 211 concentration was found at ST 5 from micropellet particles extracted from surface water while

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

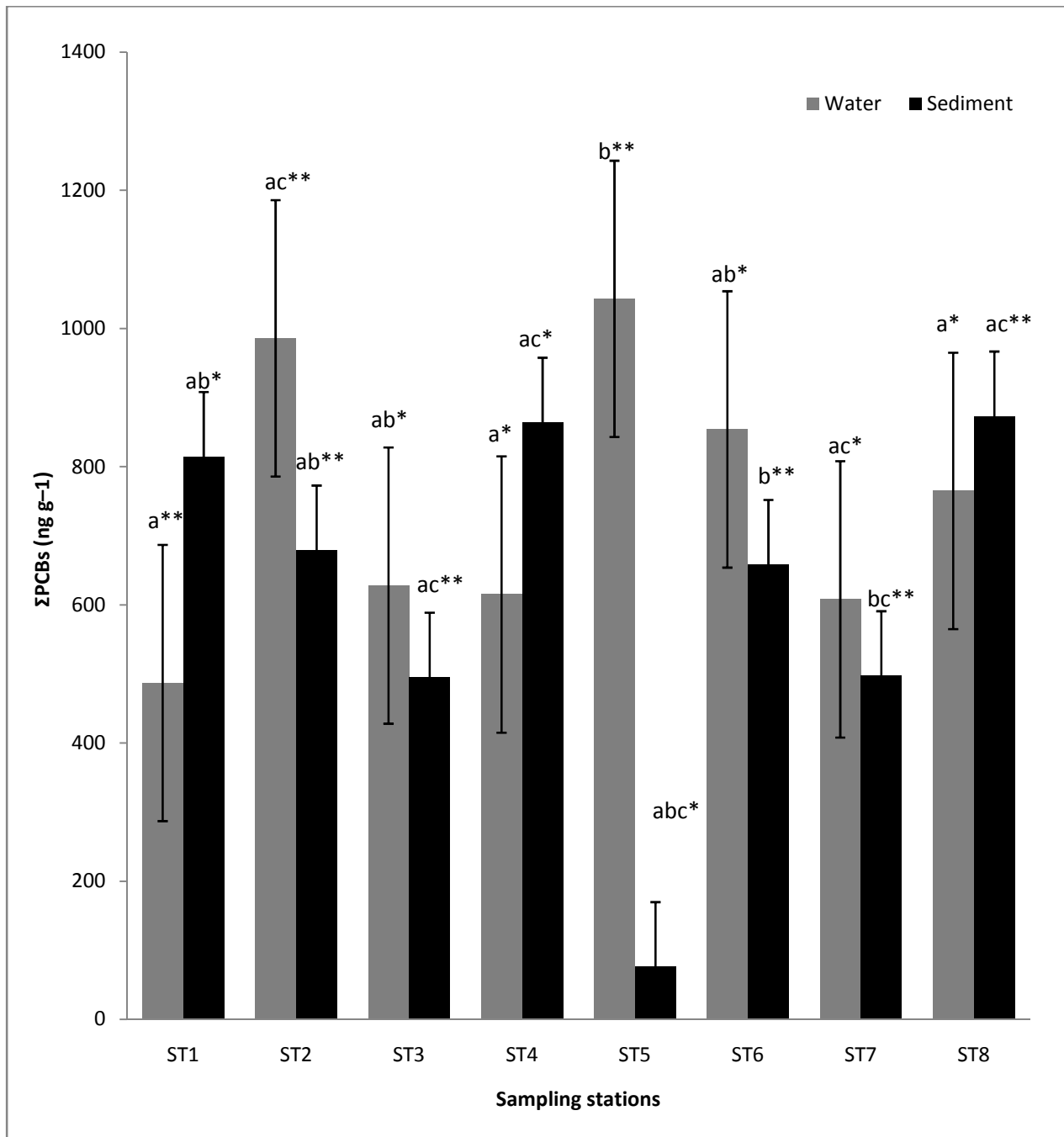
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UNDER PEER REVIEW



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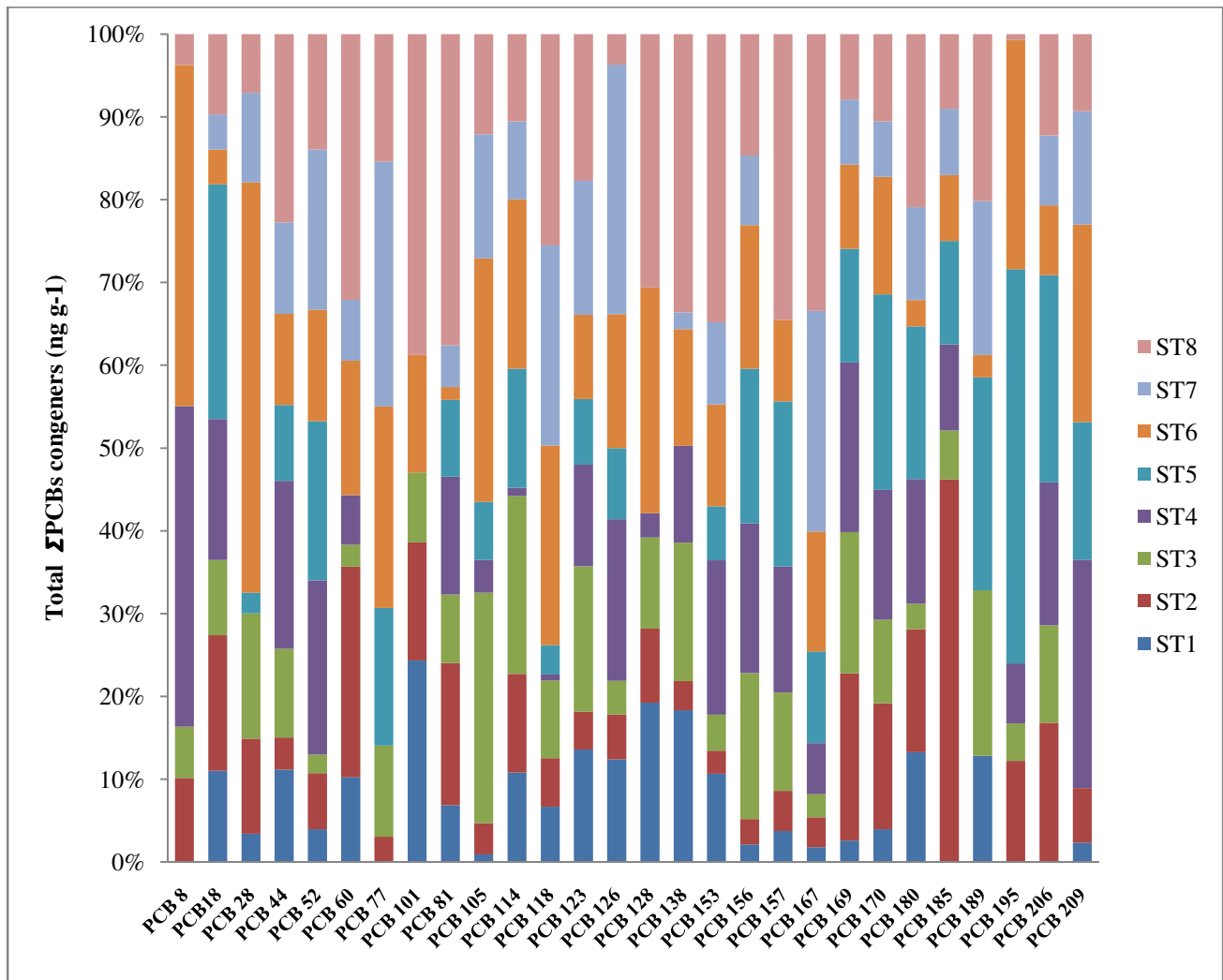
222 Figure 5: Mean concentrations of ΣPCBs (28 congeners) in microplastics (ng g⁻¹) in Surface
 223 water and sediments.

224 Means and standard deviations of three replicates are shown; bars with different letters (a,b,c,d)
 225 indicated mean differences among sampling stations according to one-way ANOVA and post-
 226 hoc Duncan multiple range test; single asterisks (*) indicates p < 0.05 and double asterisks (**)
 227 indicated p < 0.01 significant difference between sampling station and environmental matrices

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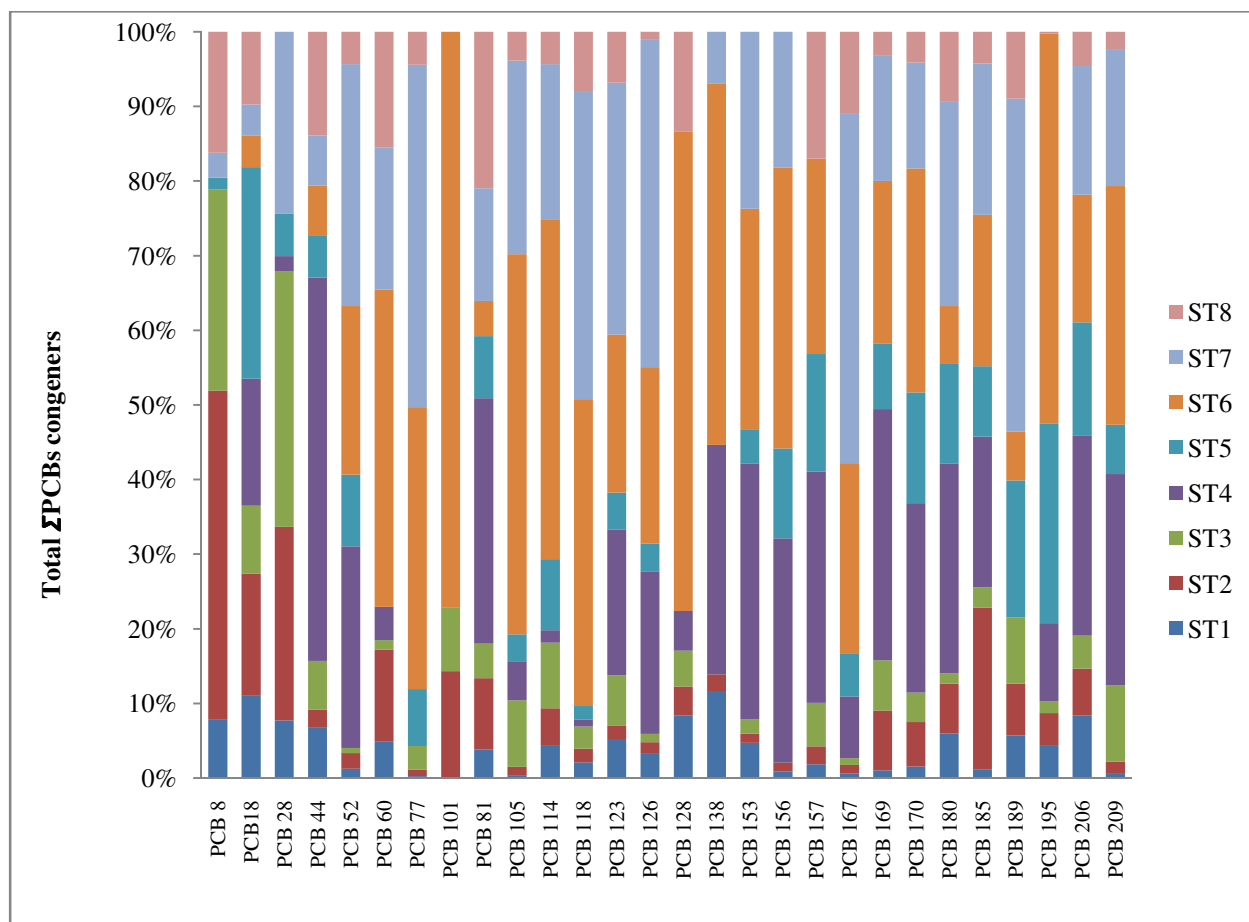
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232 Figure 6: Mean concentrations of ΣPCBs (28 congeners) extracted from micropellets in surface
 233 water

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

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 239 **3.2.2 PAHs in Micropellet particles extracted from Surface water and sediments**

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

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

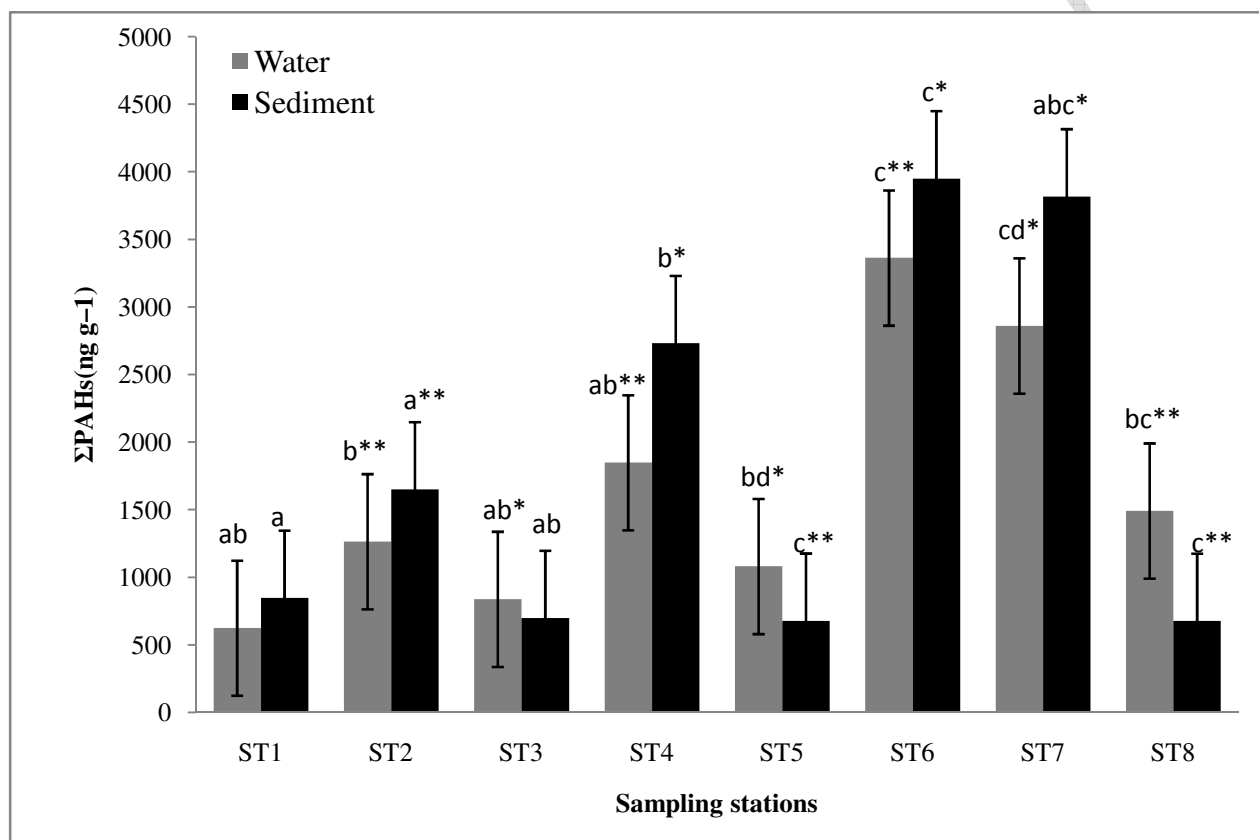
242 station were compared in regards to the environmental matrices, all the stations have PAHs types

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

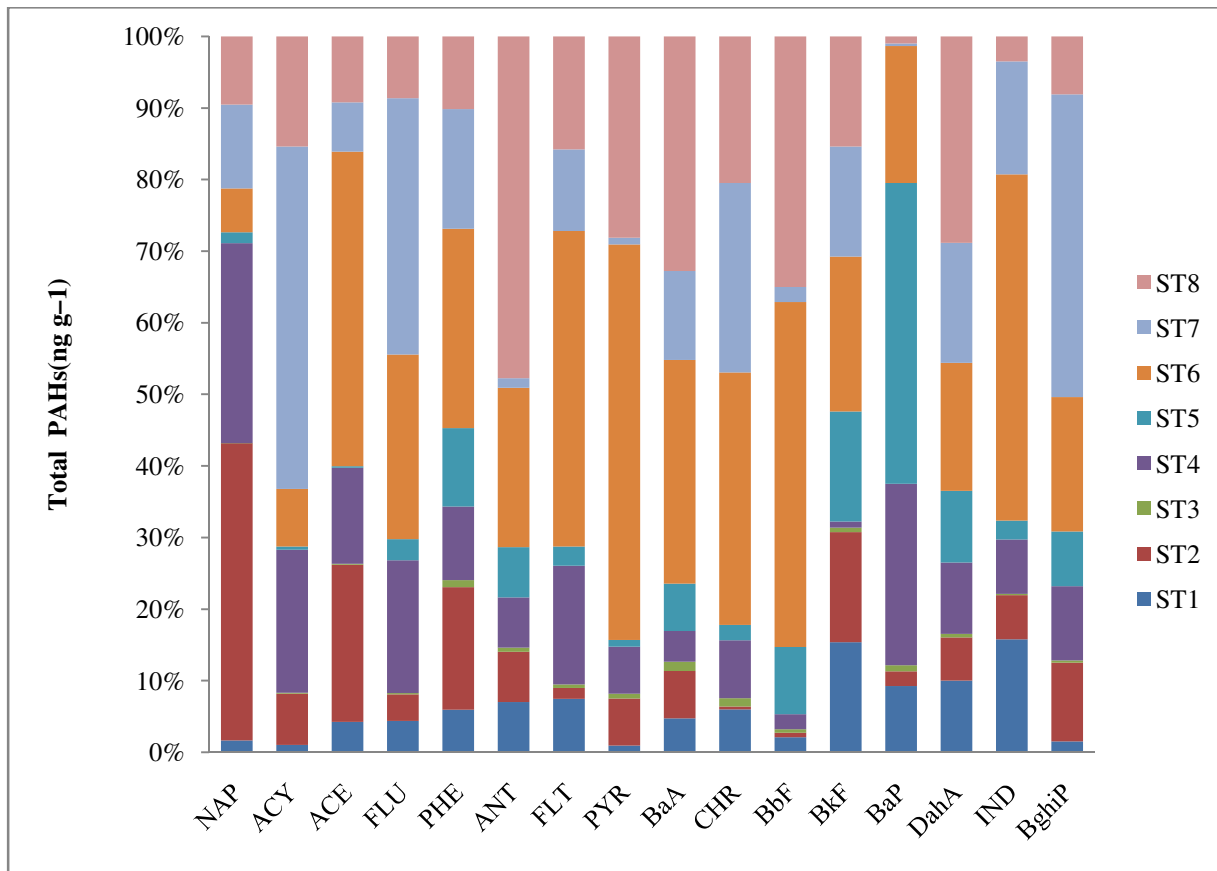
244 PAH. Regardless of stations, 3–4 rings PAH dominated in this study (Figure 9 and 10). The PAH

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

246 environmental matrices). The related distribution pattern for EDCs displayed in ST 7 and ST 6;
 247 contamination of PAHs could be an indication that local contamination sources probably would
 248 have contributed to such difference observed across the sampling station.
 249



250
 251 Figure 8: The concentrations of HEDCs in micropellet (ng g⁻¹) of ΣPAHs (16 congeners).
 252 Means and standard deviations of three replicates are shown; bars with different letters (a,b,c,d)
 253 indicated mean differences among sampling stations according to one-way ANOVA and post-
 254 hoc Duncan multiple range test; single asterisks (*) indicates p < 0.05 and double asterisks (**)
 255 indicated p < 0.01 significant difference between sampling station and environmental matrices
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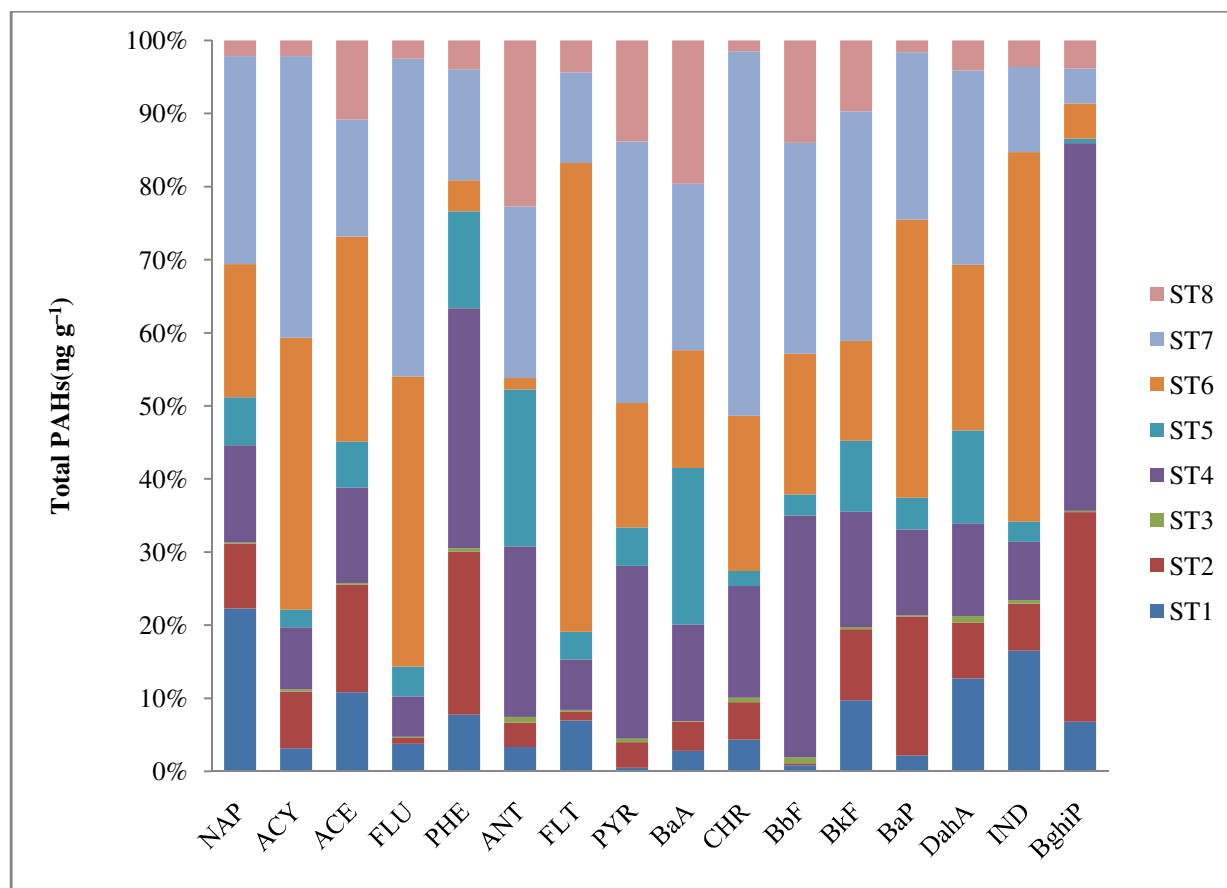


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259 Figure 9: The concentrations of Σ PAHs (16 congeners) extracted from micropellets in surface

260 water

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

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265 4. Discussion

266

267 This study evaluates micropellet particle occurrence and distribution in (surface water and
 268 sediment) and their associated hydrophobic endocrine disrupting chemicals in the extracted
 269 micropellet sampled. In the present study, the micropellet particles collected were higher in
 270 surface water than sediment were mostly opaque and white, a finding in agreement with other
 271 studies that have reported that most micropellet found in environmental matrices are often white
 272 or opaque (Heo *et al.*, 2013; Corcoran, 2015; Veerasingam *et al.*, 2016). This finding is not
 273 surprising because white micropellet particles are the most common colour manufactured
 274 (Redford *et al.*, 1997) worldwide. However, one author have recorded frequency of yellow

275 micropellet (Karapanagioti and Klontza, 2007) and according Veerasingam *et al.* (2016) they
276 are the second most frequently observed micropellet particles often associated with micropellet
277 particles in environmental matrices, a results that was not established in this present study.
278 Although, the difference observed probably may be due to difficulties in colour definition by
279 those researchers counting opaque micropellet particles as “yellow” whereas in the present study
280 they were counted as opaque colour. According to Wright *et al.* (2013), white color composition
281 of micropellet particles are similar in colour to most plankton organisms, a primary food source
282 for most aquatic organisms dwelling in the pelagic zone. The occurrence of micropellets
283 particles within the marine environment is currently well recognized in the water column, at the
284 sea surface and sediments (Law and Thompson, 2014). It has been documented that micropellet
285 particles also accounted for about 10% of all reports of ingestion of aquatic debris, highlighting
286 their importance as a component of aquatic debris (Gall and Thompson, 2015). **The size of**
287 **micropellet particles** makes them accessible to organisms with a range of feeding methods,
288 including: filter feeders (mussels, barnacles), deposit feeders (lugworms) and detritivores
289 (amphipods, sea cucumbers) and zooplankton (Wright, *et al.*, 2013). Due to diversities in their
290 size, a substantial proportion could possibly be discharged into creek, river, estuaries and the
291 oceans. **Micropellet particles have been reported to generally concentrated in the areas of nutrient**
292 **upwelling (Doyle *et al.*, 2011), which possibly accounted for the significant numbers detected**
293 **around solid waste litter and probably influenced by local weather condition systems (Moore *et***
294 ***al.*, 2002; Lattin *et al.*, 2004; Reisser *et al.* 2013).** PCBs were the most frequently encountered
295 organic contaminant, and total PCBs on micropellet particles were higher and varied according
296 to geographical location and frequency of pellets occurrence (Rochman, 2015; Fotopoulou and
297 Karapanagioti, 2017), nevertheless, PAHs concentrations in micropellet particles obtained in

298 this study were generally lower than the values reported elsewhere. Some authors reported high
299 concentrations of priority PAHs contamination in micropellet particles collected in coastal region
300 (Ziccardi *et al.*, 2016; Ivleva, *et al.*, 2017;Mendoza, *et al.*, 2016). Sixteen priority PAHs
301 concentration was detected in the floating marine plastic debris collected from Northern Pacific
302 Gyre varied between undetected and 14459 ng g⁻¹ (Rios *et al.*, 2010). When compared with the
303 concentrations found in marine environment in previous studies, much higher concentrations of
304 PAHs were obtained in micropellet in this study.

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

319 **Conclusion**

320 The present study showed that micropellet particles are present in environmental matrices with
321 differential affinities for sorption of endocrine disrupting chemicals that may alters the hormonal
322 behavioural and physiology of aquatic fauna if injected are likely to threat aquatic resource. This
323 calls for urgent monitoring of Lagos lagoon (where plastics products are abundantly
324 manufactured and used with reckless abandon) and other coastal region in Nigeria in order to
325 mitigate the danger of plastic waste materials in our coastal bodies.

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327 **5.0 Reference**

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