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## **Persistent organic pollutants in sediments of the Wouri Estuary Mangrove, Cameroon: Levels, patterns and ecotoxicological significance**

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### ► To cite this version:

Kevin Mbusnum, Laure Malleret, Pierre Deschamps, Imen Khabouchi, Laurence Asia, et al.. Persistent organic pollutants in sediments of the Wouri Estuary Mangrove, Cameroon: Levels, patterns and ecotoxicological significance. *Marine Pollution Bulletin*, 2020, 160, pp.111542. 10.1016/j.marpolbul.2020.111542 . hal-03152699

**HAL Id: hal-03152699**

**<https://hal.science/hal-03152699>**

Submitted on 2 Mar 2021

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1 **Persistent Organic Pollutants in Sediments of the Wouri Estuary Mangrove,**  
2 **Cameroon: Levels, Patterns and Ecotoxicological Significance.**

3

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12

13 **Abstract**

14 The anthropogenic impact in the Wouri Estuary Mangrove located in the rapidly  
15 developing urban area of Doula, Cameroon, Africa, was studied. A set of 45 Persistent  
16 Organic Pollutant were analysed in surficial mangrove sediments at 21 stations.  
17 Chlorinated Pesticides (CLPs), Polychlorinated Biphenyls (PCBs) and Polycyclic  
18 Aromatic Hydrocarbons (PAHs) have concentrations ranging from 2.2 – 27.4, 1.7 –  
19 31.6 and 83 – 544 ng/g, respectively. The most abundant CLPs were endosulfan,  
20 alachlor, heptachlor, lindane ( $\gamma$ -HCH) and DDT, which metabolites pattern revealed  
21 recent use. Selected PAHs diagnostic ratios show pyrolytic input predominantly. The  
22 sum of 7 carcinogenic PAHs ( $\Sigma$ C-PAHs) represented 30 to 50% of Total PAHs  
23 (TPAHs). According to effect-based sediment quality guidelines, the studied POPs  
24 levels imply low to moderate predictive biological toxicity. This study contributes to  
25 depict how far water resources are shifting within what is now termed the  
26 Anthropocene due to increasing local pressures in African developing countries.

27 **Keywords:** Pesticides, Polycyclic Aromatic Hydrocarbons, Polychlorinated Biphenyls,  
28 **Sediments, Mangrove, Africa.**

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**Introduction**

Alongside the greenhouse gases leading to climate warming, the pollution of the earth's surface does contribute as one of the main components of the global change. The entering into the newly called Anthropocene, characterized by man's predominant influence on the earth system, including the global dispersion of different types of pollutants in all the environmental compartments, represents a major concern for environmental and human health. In particular, the management of coastal areas is becoming a growing issue.

Mangrove forests are one of the most threatened world's coastal ecosystems acting as a fragile connection between marine and fresh water ecosystems (Maiti and Chowdhury, 2013; Duke, 2011). They provide numerous ecosystem services ranging from habitats, food, nursery and breeding areas to marine and arboreal life, firewood, timber and non-timber forest products to local populations. Besides, they play an important role in nutrient cycling, pollution sink, stabilization and protection of coastlines from tsunamis and hurricanes (Ajonina, 2018; Pernot et al., 2015). Anthropogenic pressure on mangroves, such as pollution, deforestation, dam, has risen over the years (Maiti and Chowdhury, 2013) and modify the functioning of mangroves. Beyond the preservation aspect, this may act as an opportunity to use them as biological indicators of anthropogenic pressures, in particular for water quality. In this trend, two compartments of the mangrove are generally examined: the mangrove stand and the sediment. Sediments that accumulate in mangroves are potential sinks of anthropogenic pollution due to high total organic content, anaerobia, rapid turnover and burial (bioturbation) (Raza et al., 2013; Tam and Yao, 2002; Vane et al., 2009). Contaminated sediments can further act as chronic sources of pollution. Actually, contaminants can be remobilized by microorganisms, consumed and retained

1 the benthos and consequently biomagnified through aquatic food chains to higher  
2 trophic levels (Minh et al., 2007; Ross and Birnbaum, 2003). Hence, the assessment  
3 of contaminant levels in mangrove sediments could provide essential information on  
4 the chemical status and ecological risks linked.

5         Since the 2000's, several researchers pointed out the occurrence of diverse  
6 organic and inorganic anthropogenic contaminants including pharmaceutical products,  
7 endocrine disruptors, industrial chemicals and pesticides in mangrove compartments  
8 worldwide (Net et al., 2015; Kouzayha et al., 2013; Maiti and Chowdhury, 2013; Bayen,  
9 2012; Lewis et al., 2011; Bayen et al., 2005; Zheng et al., 2000). But, the lack of data  
10 on contaminant levels in African aquatic ecosystems is often noted (Gioia et al., 2014,  
11 Ssebugere et al. 2019, Fernandez et al. 2007, Brits et al., 2016). African mangrove  
12 covers, however, about 3.2 million hectares (i.e. 20 % of total global mangrove area),  
13 making it the second largest worldwide mangrove ecosystem, following Asia (42 %)  
14 (Giri et al., 2011). Among other anthropogenic pressure and threats, they suffer in the  
15 last decades intense pollution due to large urban and harbour centres rapidly grown in  
16 their vicinity (Lagos and Port Harcourt, Nigeria; Douala, Cameroon; Monbassa, Kenya;  
17 Abidjan, Ivory Coast; Dar Es Salaam and Tanga, Tanzania; Dakar, Senegal; Durban,  
18 South Africa; Djibouti, Djibouti; Suez Canal and Alexandria , Egypt; Tangier, Morocco;  
19 Port Luanda, Angola; Takoradi and Tema Harbour, Ghana). Rapid population growth  
20 and recent expansion of cities with inadequate planning and environmental  
21 management facilities fostered increasing input of various contaminants in the  
22 environment, including the Persistent Organic Pollutants (POPs) through urbanisation,  
23 agriculture and industrialization (Kaiser et al., 2016). POPs are defined as compounds  
24 with toxic properties, a proven resistance to degradation, long-range transport and  
25 bioaccumulation potential in fatty tissues of living organisms (Jones and de Voogt,

1 1999; Lu et al., 2013). Most of the 12 POPs initially listed in 2001 in annex A (for  
2 instance dieldrin, heptachlor, endrin, lindane) were prohibited from the 1970s and  
3 1980s in Europe and North America, but their bans in Africa occurred only recently in  
4 signatory countries of the Stockholm Convention. Other POPs are under restriction of  
5 production and usage (Annex B, for instance DDT, perfluoro compounds) or under  
6 measures to limit their unintentional release (Annex C, for instance PCB,).

7         The present study focuses on the Wouri Estuary Mangrove (WEM) surrounding  
8 the Douala city, Cameroon. It follows a previous study carried out by Fusi et al., (2016)  
9 at two stations in the Wouri estuary (Bois des singes and Wouri Bridge) on  
10 macrobenthos (molluscs and crabs), assessing the ecological status and level of  
11 chemical pollution by anthropogenic contaminants, namely DDT and its metabolites  
12 (DDE, DDD), bis(2-ethylhexyl)phthalate (DEHP), 10 polycyclic aromatic  
13 hydrocarbons (PAHs), 6 polychlorinated biphenyls (PCBs), 16 heavy metals and 4  
14 sterols. Based on statistical analysis, this study revealed significant sensitivity of  
15 macrobenthos to the target compounds and raised the concern that these compounds  
16 could potentially be spread throughout the mangrove zone. The need for further  
17 information on the levels, spatial distribution and toxicity risk of anthropogenic  
18 contaminants throughout the Wouri Estuary Mangrove motivated the present study. It  
19 aims to be a pilot survey of anthropization level of the WEM by Persistent Organic  
20 Pollutants, carried out on 21 stations to ensure an extensive geographical coverage of  
21 the study area. To investigate the level of anthropization, a broad range of compounds  
22 (45) were analysed in the sediment compartment namely: a list of 22 chlorinated  
23 pesticides (CLPs) including 18 Organochlorine Pesticides (OCPs), the 7  
24 Polychlorinated Biphenyls indicator (PCBs) of the International Council for the  
25 Exploration of the Sea (ICES) belonging to the intentionally produced POP, and the 16

1 US EPA Priority Polycyclic Aromatic Hydrocarbons (PAHs) (L. Keith, 2014), which  
2 belongs to the unintentionally produced POP (El-Shahawi et al., 2010). Though not  
3 classified as POPs, among the 22 CLPs targeted, 2 Organophosphorus Pesticides  
4 (OPPs: chlorpyrifos, chlorfenvinphos) and 2 Chloroacetamide Herbicides  
5 (CAHs: alachlor, metolachlor) were investigated because of their toxicity and extensive  
6 use in Cameroon for subsistence farming (Ntiendjui et al., 2009). In addition, alachlor  
7 and metolachlor were one of the most abundant and highly detected pesticides in  
8 surface water from urban and peri-urban areas of Yaounde (capital city, Cameroon)  
9 (Branchet et al., 2018). The sources of the studied compounds were discussed, and  
10 the likelihood of toxicity to aquatic organisms and human health was assessed using  
11 Sediments Quality Guidelines (SQGs) proposed by Long et al. 1998, 1995. Thereafter,  
12 our findings are then compared with those of other studies related to worldwide coastal  
13 environments (estuaries, mangroves, bays and lagoons).

14

## 15 **2 Materials and methods**

16

### 17 **2.1 Study area**

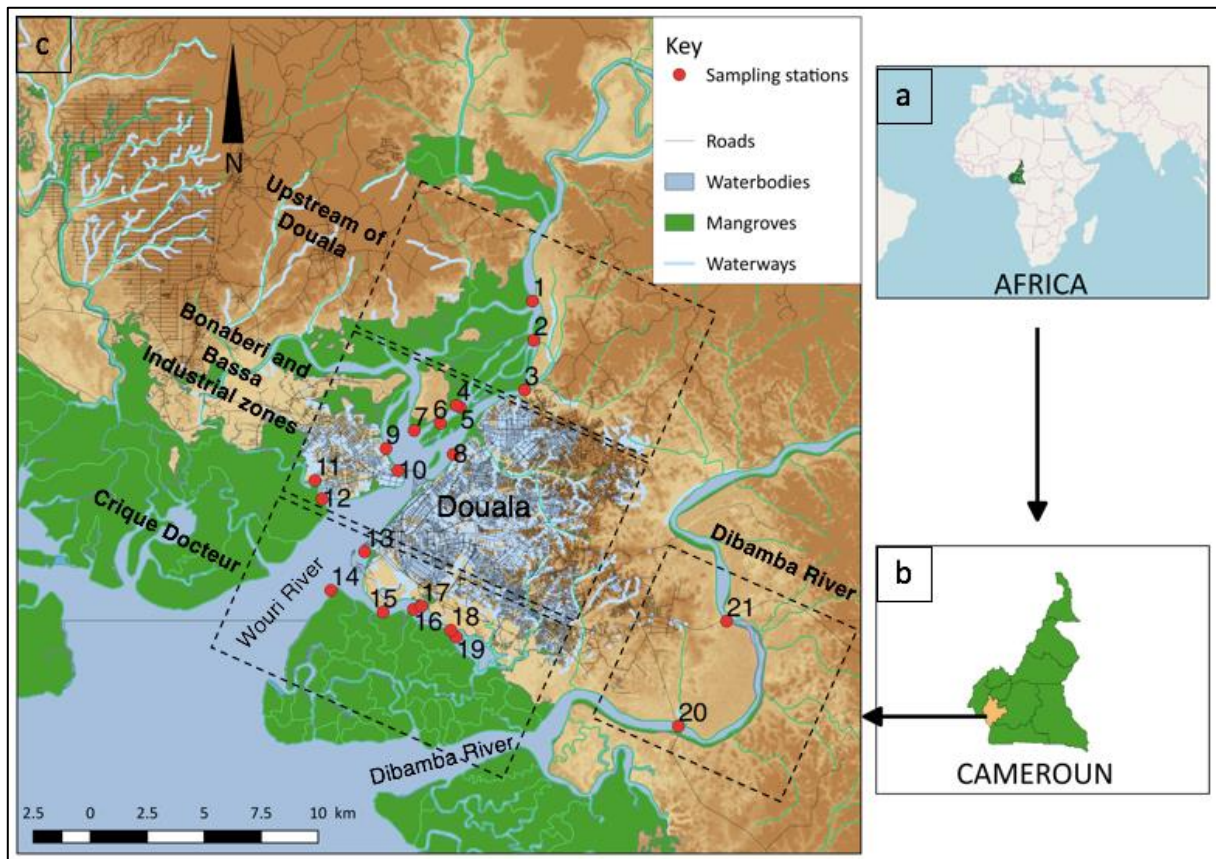
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19 The present study was carried out in the Wouri estuary mangrove (WEM) located  
20 between 4° 03' N and 9° 42' E and covering 650 km<sup>2</sup> along the Cameroonian coastline  
21 in the Gulf of Guinea (Fig. 1). In 2007, the Foundation Working Group International  
22 Wader and Waterfowl Research (WIWO) carried out a water bird census concluding  
23 that WEM qualifies as wetlands of international importance (Ramsar site) according to  
24 criteria of the Ramsar convention (Van der Waarde, 2007). This region has a warm  
25 and humid equatorial climate (mean annual temperature of 27 °C and average annual  
26 precipitation of 4000 mm). The dominant tree species in this mangrove forest are  
27 *Rhizophora racemosa* and *Avicennia africana*. This study mainly focuses on the area

1 located between the Wouri and Dibamba rivers, acting as administrative borders of the  
2 city of Douala, (Fig. 1). This town is the economic capital and the most populated city  
3 of Cameroon. Its present population is about 3 million inhabitants and is expected to  
4 reach 4 million inhabitants by 2030. This rapid urbanisation exerts a strong pressure  
5 on the mangrove ecosystem. The WEM forest is thus highly exploited for its halieutic  
6 resources, firewood, sand extraction and house construction (Longonje N. Simon,  
7 2012). New quarters of the town are now expanding on the mangrove that equally  
8 serves as waste dumps for household and municipal sludge and sewage. Peri-urban  
9 agriculture has led to the conversion of mangroves into agricultural ponds and favours  
10 the cultivation of various crops such as maize, banana, oil palm, green vegetables and  
11 sugar cane. The Douala seaport is central Africa's largest port and serves most  
12 landlocked countries of the African Sub-Saharan region. Douala is characterised by  
13 two major industrial zones known as Bassa and Bonaberi on both sides of the Wouri  
14 River and a dense traffic in the city centre. These activities in conjunction with laxity in  
15 the implementation of laws protecting the environment and mangroves in particular,  
16 lead to continuous huge pressure exerted on the WEM ecosystem.

17

18



1  
 2 Figure 1. Location of the Wouri Estuary mangrove (a) World map, (b) Cameroon map  
 3 and (c) Sampling stations  
 4

## 5 2.2 Sample collection, pre-treatment and contents of moisture and organic 6 carbon

7 Superficial sediments (0 - 5 cm, n=21) were collected by manual coring  
 8 between November and December 2017 (dry season). The study area was divided  
 9 into four mangrove zones (fig. 1 and S.I. 1): i) upstream of Douala (UD) (stations  
 10 1 to 3), ii) Bonaberi and Bassa Industrial Zones (BB) (stations 4 to 12), iii) Crique  
 11 Docteur (CD) (stations 13 to 19) and iv) Dibaba River (DR) (stations 20 to 21).  
 12 During the sampling campaign, field observations revealed the presence of car tyres,  
 13 used oil containers buried into sediments, dock and boating activities at the Douala  
 14 harbor, commercial fishery, waste dumps and effluent discharges (see SI 1). After



1 collection, samples were transferred in aluminium trays of 0.5 L and stored at – 18 °C  
2 until pre-treatment. After thawing, samples were oven dried at 40 °C, ground and  
3 sieved at < 2 mm.

4 Moisture content (Table 1) was determined according to the standard method  
5 ISO 11465 (ISO 11465, 1993). Total Carbon (TC) and Total Inorganic Carbon (TIC)  
6 were determined separately using a TOC solid module HT1300, such that the  
7 difference results in Total Organic Carbon (TOC) (Table 1). For TC measurement,  
8 50 mg of sediments were weighed in a ceramic boat, introduced in a furnace and  
9 combusted at 950 °C under a flux of oxygen. A pre-calibrated (CaCO<sub>3</sub> 12 % Carbon)  
10 non-dispersive infrared detector enabled the quantification of emitted CO<sub>2</sub>. For TIC  
11 measurement, 100 mg of sediments were weighed in a conical flask and reacted with  
12 2 mL of 40 % phosphoric acid, heated at 83 °C and agitated at 350 rpm. The amount  
13 of emitted CO<sub>2</sub> was quantified as above.

14

### 15 **2.3 Reagents and standards**

16 Solvents of suprasolv grade; n-hexane (Hex), acetone (Ace), dichloromethane (Dcm),  
17 methanol (MeOH) and acetonitrile (Acn) were purchased from Merck (Pessac,  
18 France). Hydrochloric acid (35 %), Nitric acid (69 %) and Phosphoric acid (85 %) of  
19 pure grade were from Fisher Scientific (Marseille, France) and VWR (France)  
20 respectively. PAH calibration mix at 10 µg/mL in acetonitrile was supplied by Supelco,  
21 Pesticide 8081 standard mix at 200 µg/mL in hexane: toluene (50:50) were obtained  
22 from Sigma-Aldrich and PCB mix at 10 ng/g in isooctane was provided by Dr.  
23 Ehrenstorfer Laboratories (Augsburg, Germany). The individual pesticides  
24 (Chlorfenvinphos, chlorpyrifos-ethyl, alachlor and metolachlor) at 100 µg/mL in  
25 Hexane were supplied by LGC Standards GmbH D-46485 Wesel. Alumina and copper  
26 powder (>230 mesh) were purchased from VWR International and Merck (Pessac,

1 France), respectively. Certified reference material RTC, CNS391-050, 50 g of PAHs,  
2 PCBs and Pesticides on sediment was obtained from Merck. Agilent Mega Bond Elut  
3 Florisil cartridges, 1 g, 6 ml were supplied by Agilent Technologies.

4

#### 5 **2.4 PAHs, PCBs and CLPs extraction**

6 The extraction of 10 g of sediment was performed by Accelerated Solvent Extraction  
7 (ASE Dionex 350). CLPs and PCBs extraction, adapted from (Villaverde J., 2008),  
8 included a temperature of 100 °C and a static time of 5 min (2 cycles) at a pressure of  
9 1500 psi. The solvent used was a mixture of Hex/Acn (1/1) with a flush volume of 60%  
10 and a purge time of 90 s. PAHs were extracted in the same way, but with a mixture of  
11 Hex/Dcm (1/1) at temperature and pressure of 150°C and 1500 psi, respectively. The  
12 final extract was always in the range of 45-55 mL when using a thimble size of 33 mL.  
13 An in-cell clean-up was performed by mixing the sample with 4 g of activated alumina  
14 for PAHs extraction, to eliminate polar compounds and with 3 g of activated copper for  
15 CLPs and PCBs extraction, to remove sulphur compounds (Kanzari et al., 2012). The  
16 PAH extracts were concentrated to a drop under a gentle flux of nitrogen and  
17 exchanged in 0.5 mL Acn for further analysis. The CLPs and PCB extracts were  
18 concentrated to a drop under a gentle flux of nitrogen, exchanged in 1 mL Hex and  
19 subjected to a clean-up and fractionation procedure using Solid Phase Extraction  
20 (SPE) with Florisil cartridges (1g, 6 mL), according to the US-EPA 3620 C method. The  
21 elution procedure was optimized as follows: the cartridge was washed by 10 mL of  
22 HEX before loading the 1 mL CLPs and PCBs extract. PCBs were eluted in the first  
23 fraction (F1) with 2.5 mL of Hex (100 %). The targeted pesticides were eluted in the  
24 second fraction (F2) with 5mL of Hex/Ace (80/20 v:v), apart from the most  
25 hydrophobics ones (i.e.  $\alpha$  et  $\beta$  HCH, DDD, DDT), which were recovered in both

1 fractions. Fractions F1 and F2 were concentrated to 0.25-0.5 mL before subsequent  
2 instrumental analysis.

3

#### 4 **2.5 PAHs, PCBs and CLPs analyses**

5 The 16 US EPA priority PAHs (listed in S.I. 2) were analysed by high pressure  
6 liquid chromatography with fluorescence programmable detector (HPLC-PFD) using a  
7 PerkinElmer Flexar FX6 LC and a PerkinElmer Altus A-30 FL detector. Separation was  
8 performed on an Agilent Technologies Pursuit 5 PAH column (250 mm x 4.6 mm i.d. x  
9 and 5  $\mu\text{m}$ ). The elution conditions were as follows; a mobile phase with ACN/Water  
10 (50:50), flow rate 1.2 mL/min, injector and column temperature of 10  $^{\circ}\text{C}$  and 30  $^{\circ}\text{C}$   
11 respectively was applied. For the separation, ACN rate was gradually increased to  
12 100 % after 12 min and maintained for the next 12 min and decreased to 50 % for post-  
13 run after 4 min and finally maintained for 3 min for conditioning. The fluorescence  
14 detector was programmed in a 4 channels data mode: 248 - 280 nm (excitation  
15 wavelength,  $\lambda_{\text{ex}}$ ) and 324 - 462 nm (emission wavelength,  $\lambda_{\text{em}}$ ) for selective detection  
16 of each PAH (see S.I. 2). The PerkinElmer Altus A-30 UV detector was set at 229 nm  
17 to enable the quantification of acenaphthene. Subsequently, data were processed by  
18 Empower 3 software. Quantification was based on a twelve-point calibration curve  
19 plotted between 1-1000 ng/mL.

20 Analyses of CLPs and of the 7 ICES PCBs congeners (listed in S.I. 2) was carried out  
21 by Gas Chromatography-Mass Spectrometry (GC-MS) using a Perkin Elmer Clarus  
22 600 GC and Clarus 600 C MS, equipped with a Restek Rxi – XLB; (30 m x 0.25 mm  
23 i.d. x 0.25  $\mu\text{m}$ ) capillary column. Prior to the injection, internal standard of PCB 116 D5  
24 were added at 40 pg/ $\mu\text{L}$ . A volume of 1  $\mu\text{L}$  was injected in splitless mode with helium  
25 as carrier gas at a constant flow rate of 1 mL/min. The temperature of the injector was

1 driven from 50 °C (iso 0.1 min) to 250 °C (200 °C min<sup>-1</sup> - Iso 10 min). The GC oven was  
2 programmed from 70 °C (iso 2 min) to 175 °C (10 °C/min - iso 4 min) to 320 °C (5  
3 °C/min - iso 10 min). Source ionization was performed by electronic impact at an  
4 ionization energy of 70 eV and a temperature of 250°C. The temperature of the transfer  
5 line was set at 280°C. The mass spectrometer was operated in SIM mode (Single Ion  
6 Monitoring) targeted three specific ions (one quantifier and two qualifiers) for each  
7 studied compound (see Table 2). Subsequently, data were processed with TurboMass  
8 Version 5.4.1 software using the NIST MS Search Version 2.0 library. Quantification  
9 was based on the internal standard (PCB 116 D5) calibration curves obtained from the  
10 analysis of standard solutions at seven concentration levels (0.1, 0.5, 1. 5, 10, 20 and  
11 100 pg/μl).

12

## 13 **2.6 Quality Assurance and Control (QA/QC)**

14 All analytical data were subjected to strict quality assurance and control. Procedural  
15 blanks composed of glass beads and Fontainebleau sand, and certified reference  
16 material (CRM CNS 391 for PAHs, PCBs and pesticides on sediment) were processed  
17 for each batch of 7 extractions in the same procedures used for field samples in this  
18 study. Procedural blanks were evaluated using the criteria that blank levels of target  
19 analytes should be at least three times below the detection limit (signal-to-noise ratio  
20 3:1). The accuracy of the method was tested by analysing native pollutants in certified  
21 sediment. Obtained recoveries were 62-94 % for pesticides, 76-94 % for PCBs and  
22 69-95 % for PAHs (see SI 2). Inter-day replicates (n=7) lead to relative standard  
23 deviations of 3-14 % for pesticides, 3-9 % for PCBs and 3-12 % for PAHs. The reported  
24 limit of detection (SI 2) was set as the concentration level yielding to a signal-to-noise  
25 ratio of 3:1.

1 Prior to each extraction, 4,4-DDE D8 and PCB 156 D3 were added as surrogate  
2 standards at 100 pg/ $\mu$ L to check the analytical procedure for CLPs and PCBs,  
3 respectively. Mean  $\pm$  SD recoveries were 85 $\pm$ 7 % and 91 $\pm$ 5 % for 4,4-DDE D8 and  
4 PCB 156 D3, respectively. If surrogates' recoveries differ from more than  $\pm$ 15 %,   
5 sample extraction was redone.

6 Instrumental QC was performed by regular analyses of solvent blanks and random  
7 injection of standards. Measured values were not deviating more than 15% from the  
8 theoretical values. The following conditions had to be met for an unequivocal  
9 identification and quantification of the analytes: (1) retention time matching that of the  
10 standard compound within  $\pm$ 0.15 min, (2) signal-to-noise ratio greater than 3:1, (3)  
11 detection of the qualifier ions when compounds were analysed by GC/MS. All  
12 concentrations are reported on dry mass.

13

### 14 **3 Results and discussion**

#### 15 **3.1 Spatial distribution and profiles of PAHs, PCBs and CLPs**

##### 16 **3.1.1 Polycyclic Aromatic Hydrocarbons (PAHs)**

17 The concentration of PAHs ranged from 83.35 (station 8) to 544.31 ng/g (station  
18 17) with mean and median of 218.79 and 185.0 ng/g, respectively (Table 1). Lower  
19 concentrations of PAHs close to 100 ng/g were measured upstream of Douala (stations  
20 1-3). This may be attributed to the fact that these stations are located upstream the  
21 anthropogenic pressure and the probable tidal flushing of pollutants adsorbed on  
22 sediment particles. In the Bonaberi and Bassa Industrial zones, stations 9 and 11 had  
23 the highest PAHs concentrations of respectively 299.44 and 251.51 ng/g. At station 9,  
24 PAHs input is probably due to massive burning of mangrove woods with mollusc shells  
25 for basic fertilization by liming (Stogiannidis and Laane, 2015). At station 11, effluent

1 discharge of wood treatment industry may be the source of high PAHs concentrations  
2 through creosote release (Stogiannidis and Laane, 2015).

3 At the creek Doctor, were determined at stations 13, 18 and 19 contained PAHs  
4 concentrations of 279.08 to 376.97, which can be explained by the vicinity of Douala  
5 port and by wastes discharges. The most contaminated stations 15 and 17 with PAHs  
6 levels of 405.83 and 544.31 ng/g can be linked to exhaust emission from boating  
7 activities during commercial fishery and from waste dumps. The Creek Doctor area  
8 receives the discharge of sludge, sewage, municipal wastes. The sales of petroleum  
9 products and automobile repair garage are also located here. In addition, the Mgoua  
10 river known as the “black river” draining part of the Bassa industrial zone discharges  
11 in this area. Solange et al., (2014) reported high PAH levels (480000 – 333000 ng/g)  
12 in surface sediments along the Mgoua river close to a hydrocarbon treatment industry  
13 (Nettoycam) located in the Bassa industrial zone.

14 According to a classification scale proposed by (Baumard et al., 1998) 8 stations  
15 out of the 21 studied are moderately contaminated (100–1000 ng/g dry weight), while  
16 only three stations (1, 6, 8) are slightly contaminated, with a total PAH concentration  
17 of 0–100 ng/g dry weight, and none were corresponding to high contamination levels  
18 (1000–5000 ng/g dry weight).

1 Table 1. PAH concentrations (ng/g of dry weight) in sediments of the WEM with Sediments Quality Guidelines (Long et al., 1995)

2

	NA (2)	ACY (3)	ACE (3)	F (3)	PHE (3)	ANT (3)	FL (4)	PYR (4)	BaA (4)	CHRY (4)	B(b)FL (5)	B(k)FL (5)	BaP (5)	DBA (5)	B(ghi)P (6)	IP (6)	ΣPAHs	LPAHs/ HPAHs	ΣCPAHs (%)	TEQ CPAHs/ TPAHs (%)	TOC (SD) (%)
1	12.0	15.4	1.4	4.5	15.1	2.3	11.4	10.8	6.5	0.6	4.0	0.9	4.0	0.9	0.8	1.1	91.7	1.2	19.6	6.7	3.3 (0.02)
2	14.7	25.0	2.6	2.4	9.1	3.5	6.0	6.3	4.0	2.6	1.8	0.6	2.4	1.7	13.2	18.8	114.7	1.0	27.8	5.8	7.5 (0.13)
3	12.5	17.7	2.0	2.2	10.5	1.4	12.0	3.5	16.8	9.5	14.2	0.7	0.6	1.8	2.8	3.6	111.9	0.7	42.2	5.3	4.4 (0.02)
4	8.8	28.9	1.7	4.1	9.8	3.6	12.5	18.1	6.9	3.5	1.4	0.6	0.8	1.4	13.4	35.9	151.4	0.6	33.4	4.4	6.3 (0.15)
5	12.4	24.7	1.7	3.2	12.1	2.6	90.6	20.4	7.2	1.9	1.2	1.6	0.6	1.5	1.2	2.8	185.5	0.4	9.1	1.8	3.9 (0.04)
6	21.9	14.7	2.5	2.6	2.4	0.9	14.5	2.6	4.2	6.1	10.8	0.6	2.8	4.6	1.8	1.2	94.1	0.9	32.1	9.6	7.1 (0.08)
7	20.2	27.1	1.5	5.3	18.0	1.2	6.4	2.6	13.6	1.3	25.9	0.7	0.9	1.3	0.7	12.5	139.2	1.0	40.4	5.4	6.5 (0.11)
8	6.5	9.1	7.2	2.3	7.4	2.3	3.9	9.8	5.3	3.3	6.5	2.9	2.2	0.9	10.5	3.5	83.4	0.7	29.4	5.6	6.0 (0.08)
9	28.5	28.7	1.9	6.8	25.1	6.2	33.5	40.4	29.9	21.0	26.1	11.7	13.8	3.6	17.0	5.2	299.4	0.5	37.2	7.9	6.4 (0.18)
10	11.6	15.8	1.2	5.2	15.7	3.7	23.4	22.6	14.5	10.1	13.9	7.2	10.2	1.5	3.0	3.6	163.2	0.5	37.3	9.1	6.0 (0.05)
11	25.3	9.9	2.4	6.6	16.3	5.3	25.0	63.2	16.6	18.4	11.5	7.8	14.6	2.6	17.0	9.0	251.6	0.4	32.0	8.3	6.5 (0.15)
12	13.2	20.8	2.4	5.7	29.5	3.8	23.0	29.1	9.1	8.0	16.0	5.0	7.9	1.8	10.7	8.6	194.5	0.6	28.9	6.7	6.9 (0.16)
13	20.2	27.1	1.9	4.8	18.1	5.1	25.3	29.1	18.3	15.5	68.0	6.6	10.2	3.3	11.0	14.8	279.1	0.4	48.9	8.5	5.6 (0.03)
14	10.3	24.3	1.6	4.8	10.5	4.6	4.4	28.6	10.8	5.8	1.8	0.7	1.2	0.9	10.2	23.5	144.0	0.6	31.1	4.0	5.5 (0.12)
15	9.9	10.0	2.4	7.1	29.4	7.9	31.2	68.5	45.9	34.1	87.5	11.4	16.6	3.9	34.4	5.8	405.8	0.2	50.5	8.5	6.7 (0.14)
16	13.5	32.1	2.0	5.8	21.0	6.2	31.7	42.6	28.1	4.9	2.3	4.3	1.1	2.0	17.9	21.7	237.2	0.5	27.2	3.5	6.9 (0.10)
17	64.5	23.1	2.6	11.9	32.5	8.7	47.7	60.7	58.9	47.2	102.5	16.1	28.8	6.3	31.7	1.4	544.3	0.4	48.0	9.5	6.6 (0.46)
18	23.1	21.0	1.6	10.3	26.8	16.8	35.4	73.5	9.6	5.3	6.6	8.8	2.1	2.2	20.8	26.5	290.3	0.5	21.0	3.0	10.1 (0.02)
19	14.8	30.5	2.2	8.8	26.5	8.2	39.5	52.0	39.1	24.9	52.5	15.4	2.4	3.7	26.2	30.5	377.0	0.3	44.7	4.9	8.3 (0.08)
20	10.5	12.3	2.5	4.1	17.8	5.4	45.5	39.2	23.2	17.4	25.0	11.7	18.2	3.6	17.7	8.2	262.2	0.3	40.9	10.5	6.4 (0.14)
21	7.0	23.0	1.7	3.9	10.1	4.3	12.9	59.2	11.7	1.5	1.6	4.0	7.1	1.8	8.8	15.4	173.9	0.4	24.7	6.8	8.1 (0.06)
Min	6.5	9.1	1.2	2.2	2.4	0.9	3.9	2.6	4.0	0.6	1.2	0.6	0.6	0.9	0.8	1.1	83.4	-	9.1	1.8	-
Max	64.5	32.1	2.6	11.9	32.5	16.8	47.7	68.5	58.9	47.2	102.5	16.1	28.8	6.3	34.4	35.9	544.3	-	50.5	9.5	-
Mean	17.2	21.0	2.2	5.4	17.0	5.0	25.5	32.5	18.1	11.6	22.9	5.7	7.1	2.4	13.1	12.1	218.7	-	33.6	6.5	-
Median	13.2	23.0	2.0	4.8	15.7	4.3	23.4	29.1	13.6	6.1	11.5	4.3	2.8	1.8	11.0	8.6	185.5	-	32.1	6.7	-
TPAHs	361.3	440.9	46.8	112.5	363.4	104.0	535.8	682.9	380.3	242.5	480.9	119.1	148.3	51.1	270.7	253.4	-	-	-	-	-
ERL	160	44	16	19	240	85.3	600	665	261	384	-	-	430	63.4	-	-	4022*	-	-	-	-
ERM	2100	640	500	540	1500	1100	5100	2600	1600	2800	-	-	1600	260	-	-	44792*	-	-	-	-

3 ( ) = number of rings, NA= Naphthalene, ACY= Acenaphthylene, ACE= Acenaphthene, F= Fluorene, PHE= Phenanthrene, ANT= Anthracene, FL= Fluoranthene, PYR= Pyrene,  
4 BaA= Benzo(a)Anthracene, CHRY= Chrysene, B(b)FL= Benzo(b)fluoranthene, B(k)FL= Benzo(k)fluoranthene, BaP= Benzo(a)pyrene, DBA= Dibenzo(a,h)anthracene, B(g,h,i)P = Benzo(g,h,i)pyrene,  
5 IP= Indeno(1,2,3-cd)pyrene, HPAHs= High Molecular Weight PAHs, LPAHs= Low Molecular Weight PAHs, ΣPAHs = Sum of 16 PAHs, TPAHs = Sum of individual PAHs, CPAHs= Carcinogenic  
6 PAHs, LPAHs= Low Molecular Weight PAHs, HPAHs= High Molecular Weight PAHs, TEQ CPAHs= Toxic Equivalents of CPAHs, ERL= Effect range Low, ERM= Effect Range Median, SD = Standard  
7 deviation

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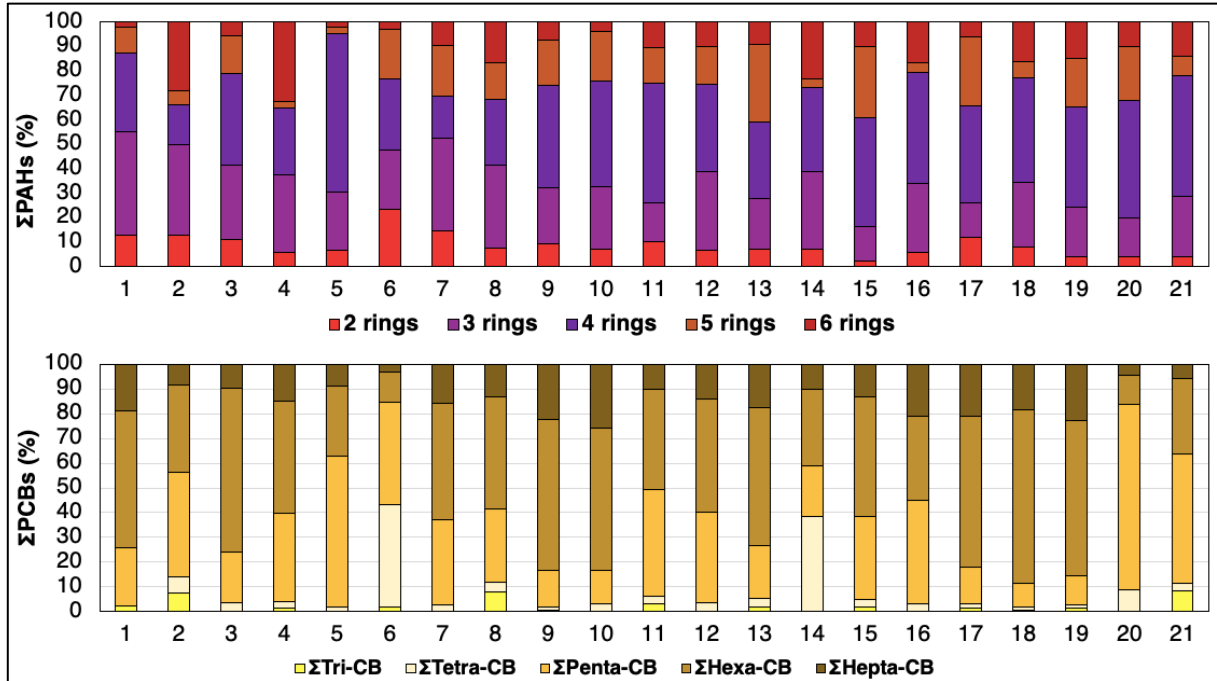
2 TOC content ranged from 3.27 (station 1) to 10.13 % (station 18) with mean and  
3 median very close of 6.43 % and 6.48 % respectively, indicating a symmetrical  
4 distribution of the values (table 1). The rather high organic contents are typical of  
5 mangrove sediments and can originate through three main inputs: mangrove plants  
6 (annual litter fall and underground roots), sediment trapping of suspended matter from  
7 coastal waters and benthic animals (Kristensen et al., 2008; Yong et al., 2011). TOC  
8 is observed to increase from upstream to downstream of the Wouri river. This is due  
9 to the deposit of coarser material upstream where sand extraction is mostly carried out  
10 whereas fine particles with higher TOC content are deposited downstream where the  
11 energy of the rivers is low.

12 In contrast to studies that reported a positive correlation between sediment  
13 organic carbon and concentration of POPs in mangroves (Tam and Yao, 2002; Vane  
14 et al., 2009), sediments of the WEM show poor correlations ( $R^2 = 0.35$ ) for TOC against  
15 PAHs. This therefore suggests that the distribution and concentrations of PAHs in  
16 sediments of the WEM are not controlled or driven by the sediment's OC content.  
17 Similarly, a poor correlation was found between TOC and PAH concentrations in  
18 surface coastal sediments of the Gulf of Mexico (Wang et al., 2014). According to  
19 (Simpson et al., 1996), correlation between PAHs and sediments' OC was only  
20 significant for highly contaminated sites, where the total PAH concentrations were  
21  $> 2000$  ng/g.

22 Concerning the distribution of the different compounds studied, tri-aromatic (15-  
23 35%) and tetra-aromatic (20-60 %) compounds are mostly accumulated in surface  
24 sediments (Fig. 2). The most abundant PAHs in increasing order were Pyrene (PYR),  
25 Fluoranthene (FL), Benzo(b)Fluoranthene (B(b)FL), Acenaphthylene (ACY) and



1 Benzo(a)Anthracene (B(a)A) referred to as High Molecular Weight PAHs (HPAHs) with  
 2 4 to 6 rings, except ACY referred as Low Molecular Weight PAHs (LPAHs) with 3 rings  
 3 (Table 1).



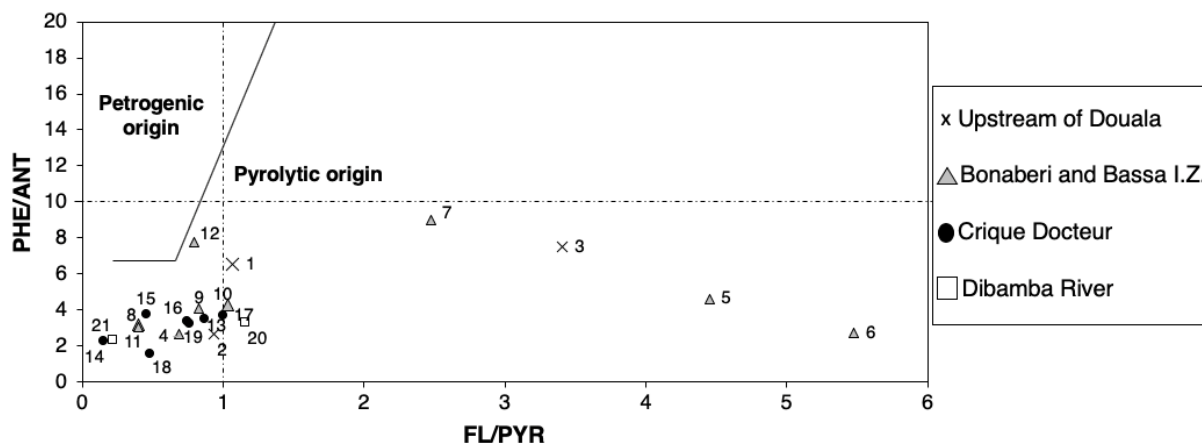
4  
 5  
 6 Figure 2. Percentage distribution of PAHs (top) and PCBs (bottom) in sediments of  
 7 the Wouri Estuary Mangrove

8  
 9 PAH source characterisation was based on source specific diagnostic ratios  
 10 such as PHE/ANT and FL/PYR distinguishing between pyrolytic (<10 and >1,  
 11 respectively) and petrogenic (>10 and <1, respectively) origins in sediments (Budzinski  
 12 et al., 1997a). The same authors stated that, these two ratios must be studied  
 13 simultaneously so as to provide a good estimate of PAH sources.

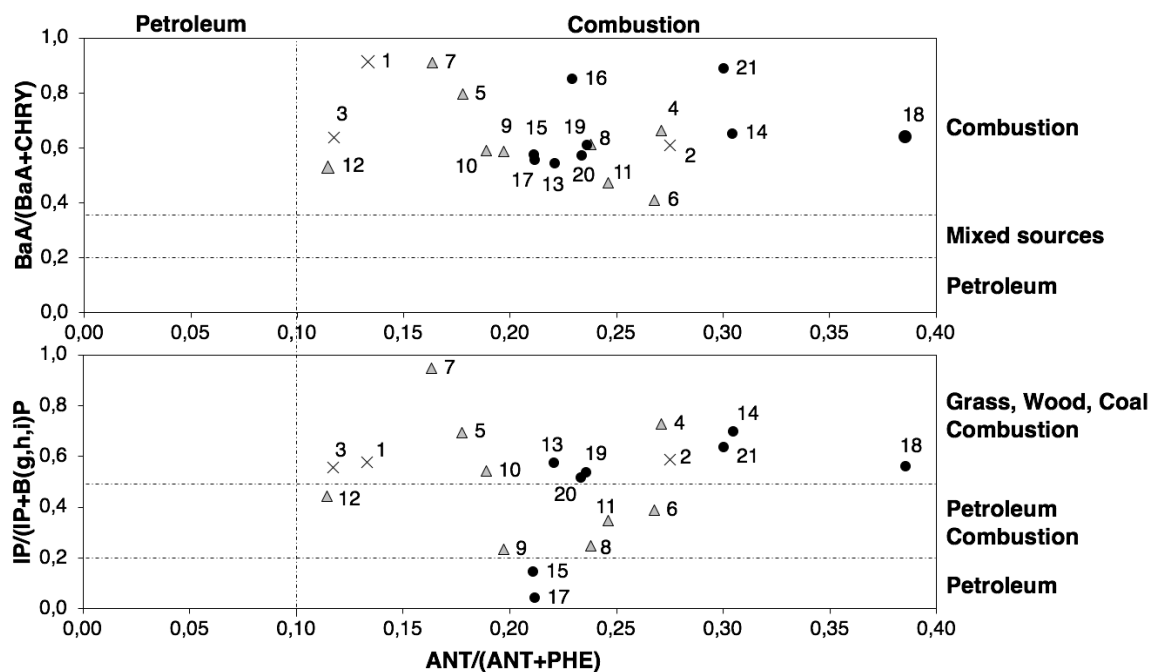
14 The predominance of FL, PYR and to a lesser extent PHE (Table 1) observed  
 15 in WEM sediments indicates the pyrolytic source of the contamination (Page and  
 16 Boehm, 1999). PHE/ANT values lower than 10 were measured in all stations and 33  
 17 % of the stations satisfy FLY/PYR < 1 (Fig. 3) expressing a predominance of pyrolytic  
 18 sources. This is reinforced by the PAH distribution dominated by HPAH

1 ( $\Sigma\text{LPAHs}/\Sigma\text{HPAHs} < 1$  in 85 % of the stations - Table 1). HPAHs from pyrolytic sources  
 2 enter aquatic environments through contaminated soil or direct deposition (Budzinski  
 3 et al., 1997; Morillo et al., 2007; Stogiannidis and Laane, 2015), pointing out that this  
 4 could be the major sources of PAHs in the WEM. Similar results were obtained by Aly  
 5 Salem et al., (2014) for sediments of the Egyptian Red Sea coast (Suez Gulf, Aqaba  
 6 Gulf and Red sea). Other ratios like  $\text{BaA}/\Sigma(\text{BaA}+\text{CHRY})$  and  $\text{ANT}/\Sigma(\text{ANT}+\text{PHE})$  enable  
 7 to distinguish between combustion and petroleum sources while  $\text{IP}/\Sigma(\text{IP}+\text{B}(\text{g,h,i})\text{P})$   
 8 enable the discrimination between various pyrolytic sources (de Almeida et al., 2018;  
 9 Mahdi Ahmed et al., 2017; Barakat et al., 2013, 2011; Asia et al., 2009; Vane et al.,  
 10 2009; Mille et al., 2006; Yunker et al., 2002) (Fig. 3). These ratios  $\text{BaA}/\Sigma(\text{BaA}+\text{CHRY})$   
 11  $> 0.35$ ,  $\text{ANT}/\Sigma(\text{ANT}+\text{PHE}) > 0.10$  and  $\text{IP}/\Sigma(\text{IP}+\text{B}(\text{g,h,i})\text{P}) > 0.5$ , reveal that the  
 12 dominant pyrolytic source of PAHs in sediments of the WEM corresponds to the  
 13 combustion of biomass (grass, wood and coal) and petroleum. This could be supported  
 14 by activities such as dense traffic, harbour, fish smoking, sustained by mangrove wood  
 15 taking place in the study area.

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3 Figure 3. Cross-plot of PAH ratios for Wouri Estuary Mangrove sediments: PHE/Ant  
4 versus FL/PYR (top) BaA/(BaA+CHRY) and IP/(IP+B(g,h,i)P) versus  
5 ANT/(ANT+PHE) (bottom)  
6

### 7 3.1.2 Polychlorinated biphenyls

8 The concentrations of  $\Sigma$ PCBs range from 1.7 (station 2) to 31.6 (station 10) ng/g  
9 with mean and median of 16.0 and 17.1 ng/g (Table 2). The highest PCB  
10 concentrations were measured at the Bonaberi and Bassa industrial zones (stations 9,  
11 10) and to a lesser extend at Crique Docteur (stations 16, 17, 18). A recent study by  
12 (Ouabo et al., 2018) showed high PCB levels in soils from three informal recycling sites  
13 in Douala, located in the Bonaberi and Douala I.Z. (fig 1). As aforementioned, Crique  
14 Doctor is also a hub for the disposal of untreated wastes (municipal, household,  
15 sewage and sludge), which can generate PCBs either through leaching or burning.  
16 During the sampling campaign, old car tyres, various plastic wastes, electronic wastes  
17 (e-wastes) were noticed and could spread out PCBs during their decomposition.

1 Station 20 found along the Dibamba river shows also one of the highest PCB levels  
2 (25.8 ng/g). The outfall in this river, of industrial effluents from the Bassa industrial  
3 zone, housing industries using pigments in the manufacturing of plastics, writing  
4 implements, and textiles can explain these findings.

5 The PCB pattern is dominated by hexachlorobiphenyls (PCB 138 and PCB 153)  
6 and pentachlorobiphenyls (PCB 101 and PCB 118) representing a total of 47 % and  
7 30 % of the  $\Sigma$ PCBs (Fig. 2). This could be due to recent and/or chronic PCB  
8 contamination and to the higher persistence of highly chlorinated PCBs in sediments  
9 (Bodin et al., 2011). This can be supported by the fact that despite the ban of PCB  
10 import in Cameroon since 2011, it's use is authorized till 2025 but wastes should be  
11 eliminated before 2028 on the national territory (UNEP-POPS-NIP-CAMEROON-  
12 COP6, 2016). Our results furthermore revealed the use of highly chlorinated PCB  
13 mixtures such as Aroclor 1254 (electrical capacitors and transformers, vacuum pumps,  
14 hydraulic fluids, plasticiser resins) and 1260 (electrical transformers and hydraulic fluid)  
15 (54 and 60 % chlorine by weight respectively), Phenoclor DP6 or Clophen A60. Similar  
16 PCB patterns were identified in sediments of the Hong Kong coastal mangrove (Tam  
17 and Yao, 2002), Senegal Estuary mangroves (Bodin et al., 2011) and Congo River  
18 Basin (Kilunga et al., 2017; Verhaert et al., 2013).

19 Considering the measurement of the 7 ICES PCB only, our discussion can only  
20 be restricted to intentionally produced PCBs coming from commercial mixtures and  
21 single relatively unaltered sources. However, the presence of numerous other  
22 congeners resulting from environmental reactivity or from the formation of inadvertent  
23 PCBs, through wastes burning or pigments for instance, can be suspected in the WEM.  
24 Hence, even if costly, extending the number of targeted congeners would provide

1 better insights in the estimation of PCBs totals and in the apportionment of sources  
2 (Megson et al., 2019).

3

### 4 **3.1.3 Chlorinated Pesticides**

5 In the Wouri mangrove zone, with the exception of endrin, all the pesticides  
6 targeted in our study were detected at least once above method detection limit (MDL)  
7 (see figure 4). The presence of normally prohibited substances, such as aldrin,  
8 heptachlor, 4,4'-DDT, 4,4'-DDD, 4,4'-DDE, lindane, chlordane and dieldrin must be  
9 highlighted. The latter have also been found in sediments of other African countries,  
10 such as Tanzania (Kruitwagen et al., 2008), Nigeria (Ibigbami et al., 2015), Benin  
11 (Yehouenou et al., 2014), Egypt (Barakat et al., 2013), Tunisia (Ben Salem et al.,  
12 2017). This can be explained by the sale and illegal disposal of stocks of pesticides  
13 that are now banned but not destroyed and also by the persistent nature of some of  
14 these compounds. DDT and metabolites, two metabolites of lindane, as well as  
15 alachlor are the most frequently detected pesticides (frequency above 50 %). (M. Fusi  
16 et al., 2016) also mention the presence of DDT and metabolites in sediments in the  
17 same area. It is only since 2002 that DDT has been banned in Cameroon. High  
18 detection frequency of DDT and metabolites (4,4' DDT, 4,4' DDD and 4,4' DDE) was  
19 similarly found in sediments from the Atlantic coast of Morocco (Benbakhta et al., 2014)  
20 and it was attributed to its long half-life ( $T_{1/2} = 2 - 25$  years) (Chattopadhyay and  
21 Chattopadhyay, 2015) and its recent and/or chronic use. Lindane ( $\gamma$ -HCH, >99 %) had  
22 a relatively low detection frequency (detected on 29% of cases), while  $\alpha$  and  $\delta$ -HCH  
23 isomers) were present on more than 50 % of stations with  $\alpha$ -HCH being predominant  
24 in most stations. This may indicate significant historical use of the technical HCHs  
25 mixture, containing 60-70 % of  $\alpha$ -HCH. Early ban in 1989 (UNEP-POPS-NIP-

1 CAMEROON-COP6, 2016) and rapid degradation could also explain dieldrin's low  
2 concentration and detection frequency. 90 % of this molecule was found to degrade  
3 within one month in tropical regions (Bodin et al., 2011).

4 To assess the level of contamination in the area, figure 4 shows for each  
5 pesticide, the distribution over the 21 stations in three classes of pesticide  
6 concentration (<0.1, [0.1-1], >1 ng/g) (fig. 4). For the majority of the stations, it can be  
7 observed that these compounds are detected at very low concentrations, even below  
8 the MDL (<0.1 ng/g), especially trans-chlordane, which is detected at about 40 % of  
9 the stations but always at very low levels. On the other hand, in nearly 50 % of cases,  
10 alachlor and endosulfan were quantified at non-negligible concentrations (> 1 ng/g).  
11 Similarly, the results presented by the Stockholm Convention national implementation  
12 plan report indicated that endosulfan persists in Cameroon and is also present in  
13 several African countries, such as Mali, Senegal and Togo. In addition, in about 30%  
14 of the samples, HCH, aldrin, metolachlor, heptachlor, cis-chlordane, DDT and  
15 metabolites were detected at intermediate levels between 0.1 and 1 ng/g.

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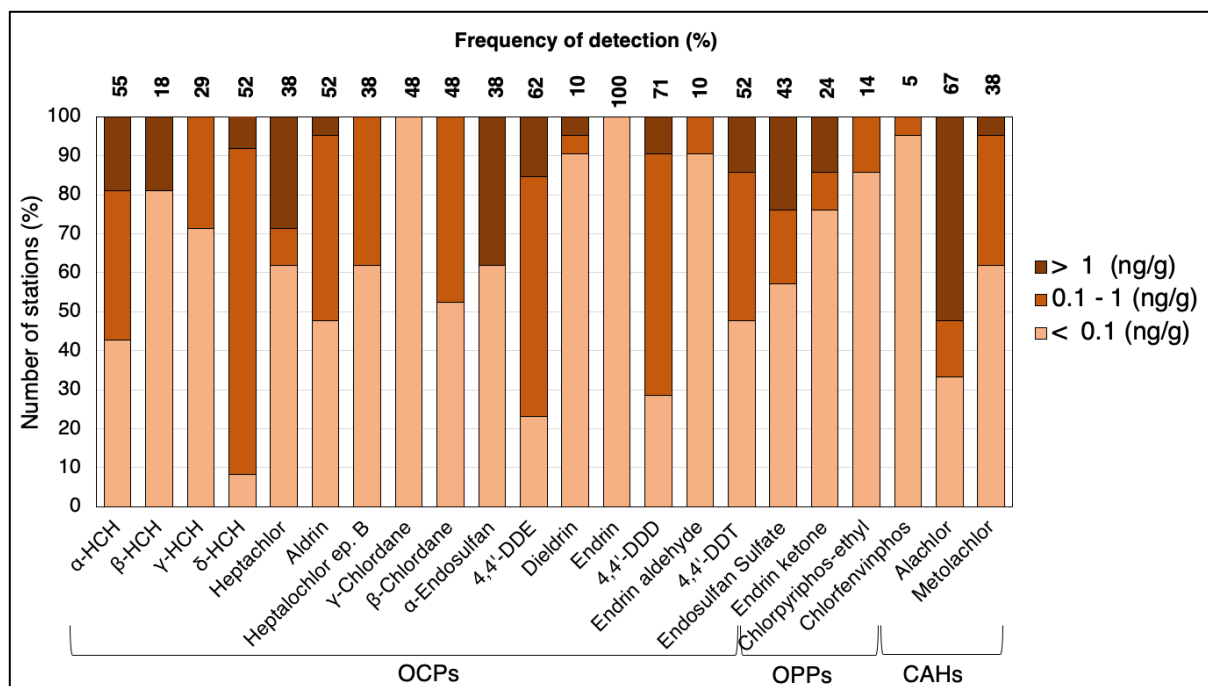


Figure 4. Concentration ranges and Frequency of detection of chlorinated pesticides in the Wouri Estuary Mangrove

CLP concentrations ranged from 2.2 (station 12) to 29.1 ng/g (stations 19 and 21) (mean and median values 10.6 and 10.0 ng/g, respectively) (Table 2). The contamination was mainly in endosulfan (stations 2, 6, 10, 13-17, 19, 21), alachlor (stations 1-4, 9-10, 15, 18-20), heptachlor (stations 1, 3, 5, 16, 21), DDT and metabolites (spread over all stations). Highest levels of the sum of pesticides were encountered in stations 19 (Bois des singes) and 21 (Dibamba river). This is explained by the activities that characterize the latter zone, such as (1) treatment against vectors of diseases such as malaria in the Ndogpassi, Nylon and Village districts, which are close to the Dibamba River which flows into the Wouri estuary, (2) peri-urban agricultural activities practiced in the Yassa area which is in connectivity with Dibamba hydrosystem. At Bois des singes (station 19), the presence of municipal wastewater disposal in basins without treatment could explained the high level measured.

1

Table 2. Concentration of CLPs and PCBs (ng/g of dry weight) in sediments of the Wouri Estuary Mangrove

Station	CAHs			OPPs			OCPs								PCBs		
	Alach	Metol	∑CAHs	Chlorp	Chlorf	∑OPPs	∑Endos	∑Chlord	∑Endrin	∑Hepta	Aldrin	Dield	∑HCHs	∑DDTs	∑OCPs	∑PCBs	∑Penta-Hexa CBs (%)
1	3.0	ND	3.0	ND	ND	ND	1.0	0.2	ND	3.1	0.3	ND	0.4	ND	8.1	4.1	79
2	4.2	1.0	5.2	ND	ND	ND	4.0	ND	ND	0.3	ND	ND	1.0	0.3	10.7	1.7	78
3	2.0	ND	2.0	0.8	ND	0.8	ND	ND	ND	6.0	1.0	ND	ND	1.8	11.5	13.1	87
4	1.0	ND	1.0	ND	ND	ND	ND	0.2	ND	0.3	0.6	ND	3.1	0.7	5.9	13.3	81
5	ND	0.9	0.9	ND	ND	ND	ND	0.3	ND	3.5	0.5	ND	ND	5.0	10.0	17.1	89
6	ND	0.2	0.2	ND	ND	ND	10.2	0.2	0.6	0.3	ND	ND	1.8	0.5	13.7	4.4	53
7	ND	ND	0.0	ND	ND	ND	1.6	0.2	ND	0.8	1.0	ND	1.2	1.1	5.9	23.7	82
8	0.5	0.1	0.7	ND	ND	ND	ND	ND	ND	1.0	ND	ND	0.5	0.6	2.8	10.8	75
9	1.6	ND	1.6	ND	ND	ND	ND	ND	ND	0.3	0.5	ND	1.6	0.1	4.0	30.4	76
10	2.0	ND	2.0	ND	ND	ND	4.3	ND	2.2	0.3	0.5	1.1	1.0	0.7	11.9	31.6	71
11	ND	1.7	1.7	ND	ND	ND	ND	0.2	ND	ND	0.7	ND	1.8	1.2	5.5	17.1	84
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.4	ND	1.1	0.4	2.2	6.6	82
13	ND	0.4	0.4	ND	0.3	0.3	11.3	0.2	ND	ND	ND	ND	3.1	0.5	15.8	19.9	77
14	0.6	0.2	0.8	ND	ND	ND	11.1	ND	ND	ND	ND	0.1	0.5	0.4	13.0	5.3	51
15	3.5	ND	3.5	ND	ND	ND	4.6	ND	0.2	ND	ND	ND	1.7	0.4	10.4	12.8	82
16	1.1	ND	1.1	0.9	ND	0.9	4.4	0.2	ND	4.3	1.9	ND	0.6	1.2	14.6	30.1	76
17	0.6	0.2	0.8	ND	ND	ND	11.1	ND	ND	ND	ND	0.1	0.5	0.4	13.0	20.0	76
18	4.9	ND	4.9	ND	ND	ND	2.6	ND	ND	ND	ND	ND	2.1	0.4	10.0	22.7	80
19	4.0	0.2	4.2	ND	ND	ND	18.5	ND	4.5	ND	ND	ND	1.6	0.4	29.1	18.3	74
20	2.3	ND	2.3	ND	ND	ND	ND	ND	0.6	1.2	ND	ND	ND	1.7	5.8	25.8	87
21	ND	ND	ND	0.4	ND	0.4	15.1	0.2	1.7	5.5	ND	ND	2.3	3.9	29.1	7.8	83
Min	ND	ND	-	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	-	1.7	79
Max	4.9	1.7	5.2	0.9	0.3	0.9	18.5	0.2	4.5	4.3	1.9	1.1	3.1	5.0	29.1	31.6	78
Mean	2.2	0.5	1.7	0.7	0.3	0.1	7.7	0.2	1.6	1.9	0.7	0.4	1.4	1.1	11.1	0.2	87
Median	2.0	0.2	1.1	0.8	0.3	0.0	4.6	0.2	1.2	0.9	0.5	0.1	1.4	0.6	10.4	0.1	81

ND = Not determined, CAHs = Chloroacetinamide Herbicides, OPPs = Organophosphate Pesticides, OCPs = Organochlorine Pesticides, Alach = Alachlor, Metol = Metolachlor, Chlorp = Chlorpyrifos-ethyl, Chlorf = Chlorfenvimphos, ∑Endo = Endosulfan I + Endosulfan sulphate, ∑Chlord = Trans + Cis Chlordane, ∑Hepta = Heptachlor + Heptachlor epoxide, Dield = Dieldrin, ∑HCHs = α-HCH, δ-HCH, β-HCH, γ-HCH, ∑DDTs = 4,4' DDT + 4,4' DDE + 4,4' DDD, ∑PCBs = sum of 7 indicator PCBs (CB28, CB52, CB101, CB118, CB138, CB153 and CB180), ∑Penta-Hexa CBs = Sum of Penta CBs (CB101, CB118) and CBs (CB138, CB153)

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The use of pesticides in peri-urban agricultural activities and disease vector control such as malaria was reported by (Antonio-Nkondjio et al., 2011). Moreover, Nfotabong-Atheull et al., (2013) reported that mangrove stands around Douala have been converted into agricultural areas for the cultivation of maize, bean, green vegetables, sugar cane and banana. Furthermore, the development of agriculture upstream of the estuarine zone (UD area) could likely be due to low level of salinity due to high rate of sea water dilution. This can contribute to concentrations of pesticides accounting to around 10 ng/g measured at stations 1, 2 and 3.

For OCPs, the concentration of  $\Sigma$ DDTs (4.4' DDT, 4.4' DDD, 4.4' DDE) ranged from 0.1 to 5.0 ng/g (mean and median of 1.0 to 0.5 ng/g respectively) whereas concentrations of  $\Sigma$ HCHs ( $\alpha$ ,  $\delta$ ,  $\beta$  and  $\gamma$  isomers) ranged from 0.4 to 3.1 ng/g (mean and median of 1.2 and 1.1 ng/g respectively). Endosulfan ( $\alpha$ -Endosulfan + Endosulfan sulphate) was the most abundant pesticide found in WEM sediments (concentrations from 0.95 to 18.5 ng/g and mean and median values of 4.7 and 2.6 ng/g respectively). High levels could be explained by its broad spectrum as insecticide and its large atmospheric pattern transport (Weber et al., 2010). Our results suggest its recent use, which is congruent with its relatively recent prohibition since 2008 in Cameroon.  $\alpha$ -Endosulfan concentrations were higher than those of its toxic metabolite, endosulfan sulfate (ratio between 3 to 75), indicating a slow rate of endosulfan degradation (Kuranchie-Mensah et al., 2012) and relatively recent use. Mean concentration of endosulfan sulfate was higher in WEM (0.97 ng/g) than values previously reported in Ghana (Kuranchie-Mensah et al., 2012). Similarly  $\alpha$ -Endosulfan exhibited higher concentrations in WEM sediments (~ 3.2 ng/g) than in the Atlantic coast of Morocco sediments (Benbakhta et al., 2014) and the Weija Lake in the Densu river basin in

1 Ghana (Kuranchie-Mensah et al., 2012), but lower than those in the Ogbese river  
2 sediments in Nigeria (Ibigbami et al., 2015).

3 OPP concentrations were rather low, ranging from 0.4 – 0.9 for chlorpyrifos-  
4 ethyl and <LOD – 0.3 ng/g for chlorfenvinphos, despite both still in use in Cameroon.  
5 Chlorfenvinphos was introduced in 1963 as an insecticide and acaricide and was used  
6 for controlling ticks in cattle in North-West region of Cameroon (P. Merlin, 1987). In  
7 Cameroon, chlorpyrifos-ethyl is an insecticide frequently used by farmers in the  
8 cultivation of maize (Antonio-Nkondjio et al., 2011; Nwane et al., 2009), its low  
9 concentration and detection frequency in WEM sediments are consistent with a  
10 relatively high water solubility and very short half-life (24 days) in seawater-sediment  
11 system as described previously (Howard, 1991).

12 Levels of CAHs ranged from ND-4.9 ng/g for alachlor and ND – 1.7 ng/g for  
13 metolachlor. Both pesticides are used nowadays in Cameroon. In Cameroon, it has  
14 been specifically used as herbicide for weed control in various crops such as cocoa,  
15 coffee, palm oil, cotton, beans and peanuts and levels above the European Maximum  
16 Residue Limit in 22 out of 72 food samples from the western highlands of Cameroon  
17 were previously detected (Galani et al., 2018).

18 Although DDE is generally the most widely detected and most resistant to  
19 biotransformation compound in the environment (Chattopadhyay and Chattopadhyay,  
20 2015), the abundance pattern of DDTs in the WEM showed that 4,4' DDT > 4,4' DDE  
21 > 4,4' DDD, indicating a recent use of DDT according to Nozar, (2013) and prevailing  
22 anoxic conditions of sediments that favour the formation of DDD. In addition, this  
23 pattern could be observed due to higher solubility of DDT metabolites (DDE and DDD)  
24 (Chattopadhyay and Chattopadhyay, 2015). To assess the recent DDT use, diagnostic  
25 ratios  $(4.4' \text{ DDE} + 4.4' \text{ DDD}) / 4.4' \text{ DDT}$  were computed for stations where all the

1 three compounds (DDT, DDE and DDD) were present (i.e. stations 5, 8, 11, 12, 14, 20  
2 and 21 having calculated ratios of 0.08, 0.35, 2.00, 1.18, 0.40, 0.09 and 0.06,  
3 respectively). Except for stations 11 and 12 located on the western bank of the Wouri  
4 river, the ratios lower than one sign a recent use of DDT (Zhang et al., 2011). Similar  
5 results were found in the mangrove of South Iran Nozar (2013), in line with the recent  
6 recommendation by the World Health Organization (WHO/HTM/GMP, 2011) for the  
7 reintroduction of DDT for vector control in some malaria endemic settings, such as  
8 Africa (Coleman et al., 2008).

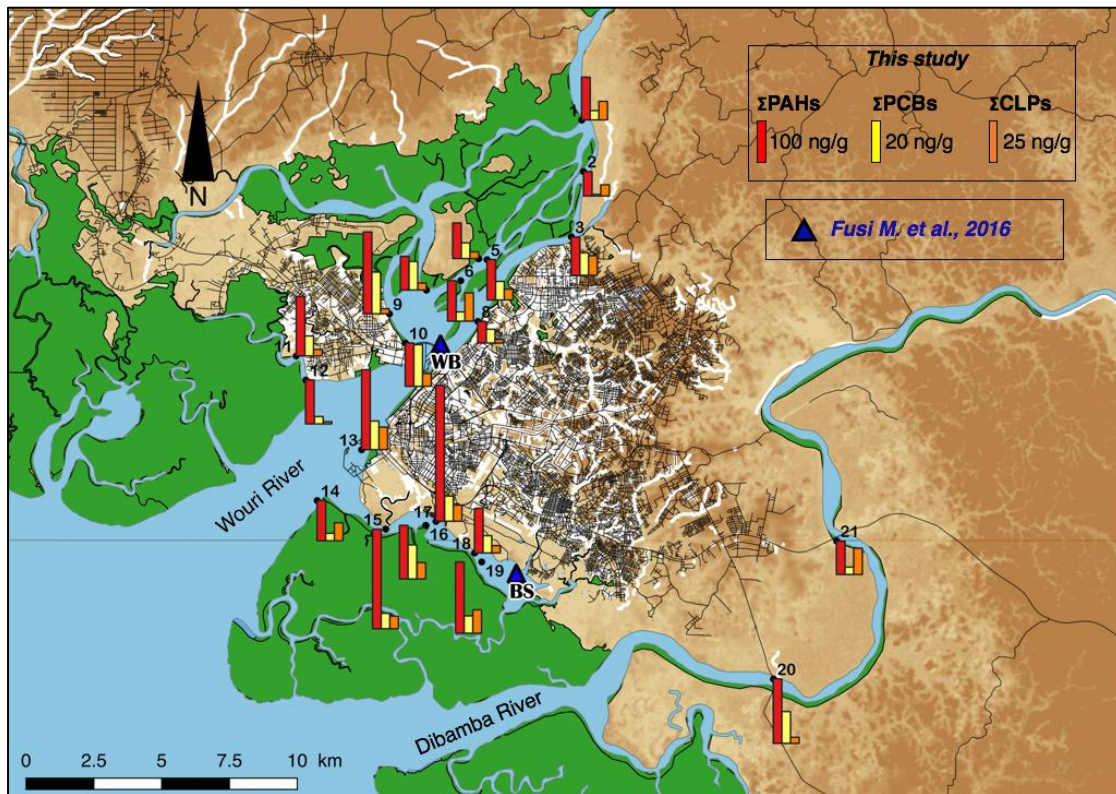
9 The diagnostic ratio ( $\alpha/\gamma$ -HCH) between 3 and 7 indicates the use of technical  
10 HCH (Zhang et al., 2011) while values lower than 3 indicates lindane use (Tao et al.,  
11 2005). Furthermore, low or high  $\alpha/\gamma$ -HCH values were found to reflect current lindane  
12 or historical HCHs uses, respectively (Tao et al., 2005). Due to absence of  $\alpha$  and  $\gamma$   
13 isomers in most samples of the WEM, computed  $\alpha/\gamma$ -HCH ratios were  $<3$  for three  
14 stations (4, 8 and 21). The pattern  $\alpha > \delta > \beta > \gamma$  of lindane isomers suggested an  
15 extensive degradation of lindane into its metabolites, reflected by lindane low half-life  
16 (90 days, (Sang and Petrovic, 1999)) and its degradation speeded up by anaerobic  
17 conditions (Fiedler et al., 1990). However, despite an absence of homologation and a  
18 ban, a stock of lindane still remained in 2005 (UNEP-POPS-NIP-CAMEROON-COP6,  
19 2016).

20 Similar to the Mumbai mangroves, this study shows that despite the restriction  
21 on the use of DDT and lindane in Cameroon, they are found to be persistent in  
22 mangrove sediments. Furthermore, even if prohibited, they are still in usage to sell off  
23 stock or for specific exemptions for use, especially DDT allowed by WHO for malaria  
24 vector control (WHO/HTM/GMP, 2011).

25

## 26 **3.2 Comparison to Worldwide estuary and mangrove areas**

1 Figure 5 summarizes the spatial distribution of PAHs, PCBs and CLPs in the WEM at  
2 the 21 stations investigated. Despite the ban of some of these compounds over the  
3 last 20 years in Cameroon, their presence in mangrove sediments evidenced their  
4 recent and/or chronic uses and also pressures from local as well as global scales.  
5 However, WEM sediments generally show low pollution levels and toxicity risks to  
6 aquatic organisms. The contamination hotspots identified were Crique Docteur and the  
7 Bonaberi and Bassa Industrial area related to the different anthropogenic activities  
8 carried out in these areas.



9  
10  
11 Figure 5. Spatial distribution of PAHs, PCBs and CLPs in sediments of the Wouri  
12 Estuary Mangrove

13 Compared to the previous study carried out in this area (Fusi et al., 2016,  
14 samples collected in 2009) the concentrations of 10 PAHs and 6 PCBs congeners in  
15 2 stations of sediments (BS and WB) were below 300 ng/g and 20 ng/g of sediment  
16 dry weight, respectively. Main conclusions indicated no statistical differences among

1 sites (Wouri Bridge and Bois des singes), belts (*Avicenia sp*, *Pandanus sp*, *Rhizophora*  
2 *sp*) and sampling depths (0 - 10 and 10 - 20 cm). Out of the 21 stations examined in  
3 the present study, we showed also that the distribution of the contamination is relatively  
4 homogenous in the area. Some spots of pollution were nevertheless noticed in the  
5 industrial zones of Bonaberi and Bassa and Crique Doctor.

6       Levels of PAHs contamination have not really changed between 2009  
7 (<300 ng/g) and 2017 (83-544 ng/g). Conversely, higher PCBs levels were measured  
8 increasing from about 5 ng/g in 2009 to 20-30 ng/g in 2017. These compounds were  
9 banned very recently (August 2011) in Cameroon. Moreover, PCBs can enter the  
10 environment through wastes disposal and can be linked to the rapid growth of  
11 urbanization and industrialization in Douala during the past decade. The opposite trend  
12 is noticed for DDE, which has dropped down from 10-40 ng/g in 2009 to level below  
13 1.72 ng/g in 2017. In Fusi et al. 2016, DDT was not detected while 4,4' DDE was the  
14 only metabolite detected at concentrations higher than 3 ng/g. The same author found  
15 4,4' DDE at mean concentration of 30 ng/g (n = 7, depth of 0 - 10 cm) while this study  
16 presents very low mean concentrations of 0.28 ng/g (n = 21, depth of 0 - 5 cm) for  
17 DDE. This large discrepancy could be associated to sand extraction activities highly  
18 carried in this area, which remobilize these contaminants in the water column.  
19 Furthermore, a return to DDT is on the horizon in some African countries to combat  
20 malaria and the WHO has declared itself in favour of a resumption of its use by  
21 spraying in homes. The recent usage of DDT for malaria vector control corroborates  
22 our data showing the prevalence of the parent compound (DDT) over its metabolites  
23 (DDD and DDE).

24       On a global scale, the level of POPs in sediments of mangrove and estuaries  
25 areas across the world are presented in table 3.

1 On a worldwide scale, PAH contamination in the WEM was higher than  
2 mangrove sediments of the Malaysian Peninsular (Raza et al., 2013), Imo river estuary  
3 and mangrove (Nigeria) (Oyo-Ita et al., 2016), the Minjiang river estuary (China) and  
4 the Rembau–Linggi estuary (Malaysia) and the Douro River estuary (Portugal). But  
5 concentrations of PAHs in sediments of the WEM were lower than those of other  
6 mangroves located in Djibouti, Nigeria and Martinique. The highest PAH contamination  
7 was found in sediments of the Iko River estuary mangrove (6100 - 35270 ng/g),  
8 mangroves of Bay of Fort-de -France (18000 - 112000 ng/g), Gironde Estuary (1000 -  
9 2000 ng/g).

10 Levels of HCHs in the WEM are within the range of concentrations found in  
11 sediments of other studies worldwide and higher than those in sediments of the South  
12 China mangrove reserves (Qiu et al., 2019), Falia and Fadiouth estuary mangroves in  
13 Senegal (Bodin et al., 2011). For DDTs, concentrations are generally lower than those  
14 found in sediments of studies worldwide. But it is worth noting that DDT is set to  
15 increase due to current use for malaria vector control. Levels of PCBs in sediments of  
16 the WEM are higher than most mangrove areas such as, mangrove and coastal  
17 sediments in the Persian Gulf (de Mora et al., 2010, Nozar, 2013), mangrove  
18 sediments of the Tanzanian coast (Kruitwagen et al., 2008), Hong Kong mangrove  
19 sediments (Tam and Yao, 2002), S. Buloh and S. Khatib Bongsu Mangroves in  
20 Singapore (Bayen et al., 2005), Sunderban mangrove in India (Binelli et al., 2009). The  
21 results of this study can serve as an early warning of current PCBs contamination in  
22 the WEM. Many entry points for these compounds have been mentioned in our work,  
23 such as improper e-wastes disposal, wastewaters outfall, and growing industrial  
24 activities. The management of this area should be adjusted in the face of these  
25 particular pressures.

1 Table 3. Summary of POP levels (ng/g) in sediments (dry weight) of coastal environments (estuaries and mangroves) worldwide

	Country	Study area and Environment	Main activities	Sampling Stations	Depth (cm)	Concentration range (ng/g) of dry weight (dw)					References
						ΣDDTs	ΣHCHs	ΣOCPs	Σ7PCBs	Σ16PAHs	
MANGROVES	Cameroon	Wouri Estuary Mangrove	Industrial, harbour	21	0 - 5	0.1-0.5	0.4 – 3.1	2.21- 27.4 <sup>E</sup>	1.7- 31.6	83 - 544	This study
	Djibouti	Djibouti-city Mangrove	Oil spills, Industrial	11	0-10	NA	NA	NA	NA	15.7 - 3760.1	Madhi Ahmed 2017
	Cameroon	Wouri Estuary Mangrove	Industrial, harbour	2	0 - 20	10 - 40 <sup>A</sup>	NA	-	2.6 – 6.3 <sup>G</sup>	85 – 250 <sup>B</sup>	Fusi M. et al. 2016
	Nigeria	Imo River Estuary	Industrial, Petroleum	5	0 - 3	NA	NA	NA	NA	28 - 64	Oyo-lta et al 2016
	Senegal	Fadiouth and Falia Mangrove <sup>R</sup>	Agricultural	2	0 - 5	0.3-15.9	0.1–1.9 <sup>N</sup>	1.2-18.2 <sup>O</sup>	0.3 – 19.1	NA	Bodin et al., 2011
	Nigeria	Iko River Estuary Mangrove	Oil spillage, Agricultural		0-10	NA	NA	NA	NA	6100 - 35270	Essien et al 2011
	Tanzania	Tanzanian coast mangrove	Industrial, agricultural	7	0 - 5	0.1-8.6	0.2 – 5.3 <sup>D</sup>	ND	2.3-3.7 <sup>C</sup>	NA	Kruitwagen et al., 2008
	Brazil	Mangroves os Southern Brazil	Industrial, Mariculture	9	0-10	NA	NA	NA	NA	6.8 - 437.3	Assunção et al. 2017
	Puerto Rico	Mangroves of Jobos Bay	Oil refinery, solid waste landfill, pharmaceutical	18	0 - 1	NA	NA	NA	0.42 - 1232	NA	Alegria et al 2016
	Martinique	Mangroves of Bay of Fort-de-France	Agricultural, industrial, nautical, transport	16	0 - 10	NA	NA	NA	NA	18000 - 112000	Mille et al., 2006
	Brazil	Guanabara bay Mangroves	Industrial, petrochemical plants	5	0 - 3	10.6 - 37.4 <sup>K</sup>	0.7 – 2.0 <sup>N</sup>	28.4 – 216.6 <sup>O</sup>	17.8 – 184.2	NA	Santos de souza et al 2008
	China	Mangroves of Nanliu Estuary	Industrial, agricultural	2 <sup>*</sup>	0 - 40	0.2-41 <sup>V</sup>	NA	-	<DL - 0.22 <sup>G</sup>	29-438 <sup>Y</sup>	Kaiser et al 2016
	China	South China Mangroves reserves	Industrial, Agricultural, Aquaculture, Harbour	12	0 - 1	1.14 - 6.82	0.01 - 0.16	-	0.08 - 0.23	NA	Qiu et al 2019
India	Sunderban mangrove	Industrial, agricultural, aquaculture, harbour	7 <sup>*</sup>	0 - 30	NA	NA	NA	0.5 – 26.8	NA	Binelli et al 2009	
Singapore	S. Buloh and S. Khatib Bongsu Mangroves	Aquaculture	2	2 - 7	<0.1 - 0.9 <sup>W</sup>	1.2 – 6.0	-	0.6 – 1.9 <sup>T</sup>	NA	Bayen et al. 2005	
ESTUARIES	Nigeria	Imo river coastal mangrove	Industrial, Petroleum refineries	5	0 - 5	NA	NA	NA	NA	51 – 90 <sup>X</sup>	Oyo-lta et al 2016
	Kenya	Indian Coast of Kenya Estuary	Agricultural, industrial	4	0 - 5	2.3 – 42.0	16.1-1445 <sup>N</sup>	-	NA	NA	Barasa et al., 2007
	Portugal	Douro River estuary	Wastewater, oil refinery	4	50	NA	NA	NA	NA	59 - 156.45	Rocha et al 2011
	UK	Mersey Estuary	Industrial	14 <sup>*</sup>	0 - 100	NA	NA	NA	36 - 1406	626 - 3766	Vane et al. 2007
	Malaysia	Rembau–Linggi estuary	Agriculture, ubanization	21		NA	NA	NA	NA	20 - 112	Raza et al. 2013
	France	Gironde estuary	Industrial	24	0 - 2	NA	NA	NA	NA	1000 - 2000 <sup>U</sup>	Budzinski et al. 1997
	China	Beibu Gulf	Industrial, agricultural	1 <sup>*</sup>	0 - 45	ND-0.5 <sup>V</sup>	NA	-	ND	38 - 74	Kaiser et al 2016
	China	Northern Bohai and Yellow seas	Agricultural, Industrial	35	0 - 10	NA	NA	NA	NA	52.3-1870.6	Jiao W. et al. 2011
	China	Dalliao River Estuary	Industrial, oil, steel factory	35	0 - 5	NA	NA	NA	NA	272 - 1607	Men et al 2009
	China	Minjiang river estuary	Industrial, agricultural	9	1	1.6–13.1	3.0–16.2	28.8–52.1 <sup>O</sup>	15.14–57.9 <sup>F</sup>	NA	Zhang et al. 2003
India	Hugli Estuary, Sunderban	Industrial, agricultural	1	Surface	0.003 - 0.1 <sup>S</sup>	0.003 – 0.3	NA	NA	NA	Bhattachcharya et el 2003	

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A=DDE, B=10 PAHs, C=10 PCBs, D= 2HCHs, E=18 OCPs, F=21 PCBs, G=6 PCBs, \*=sediment core, H=40 PCBs, i=11 PCBs, J=13PCBs, K= 6DDTs, L= 3HCHs, M= , N= γ-HCH, O= 14 OCPs, P= 28 PCBs, Q= 11OCPs, , R=Ramsar site, S= 5DDTs , T = 40PCBs, , U= 17 PAHs V= 4DDTs, W= 2DDTs, X = 13 PAHs, ΣDDTs = (DDT+DDE+DDD), ΣHCHs = (α-HCH+ δ-HCH+β-HCH+γ-HCH), NA= Not analyzed, ND = Not detected, Y = 15 PAHs, DL = Detection Limit

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**3.3 Ecotoxicological significance**

**3.3.1 Implication for aquatic organisms**

The appraisal of sediment quality and ecological risk assessment from the WEM was carried out following sediment quality guidelines (SQGs) developed for marine and estuarine sediments (Long et al., 1998, 1995). These SQGs express the incidence of adverse biological effects to be observed and or predicted in aquatic organisms closely associated with sediments. Two approaches were applied: the Effect Range Low (ERL) and Effect Range Median (ERM) values as well as the Threshold Effect Level (TEL) and Probable Effect Level (PEL). These values delimit three concentration ranges: (1) concentrations below ERL and/or TEL representing minimal effect ranges below which adverse biological effects would be rarely observed, (2) concentrations equivalent to and above ERL and TEL representing possible-effect ranges within which adverse effects will occur and (3) concentrations equivalent to and above ERM and PEL representing probable-effect ranges within which biological effects are frequently observed. (Long et al., 1998, 1995).

For ΣPAHs, all stations fall to very low levels below the ERL concentrations (4000 ng/g) indicating minimal probability of effects. At these concentrations, adverse biological effects would be rarely observed. In 71 % of stations, ΣPCB concentrations were lower than the ERL and TEL threshold values (Fig. 6), expressing concentrations corresponding to minimal probability for toxic effects to occur. However, 29 % of the sites located in the Bonaberi and Bassa Industrial zones (station 7,9 and 10) and Crique Docteur (stations 16, 18 and 20) exhibited concentrations associated with an occasional probability of toxic effects to occur. For ΣDDTs, up to 80 % of stations have concentrations below ERL and TEL (Fig. 6) thus associated with a minimal probability



1 for toxic effects to occur, whereas 20 % of stations fall between ERL and ERM and  
2 10 % between TEL and PEL indicating concentrations that were occasionally  
3 associated to adverse biological effects. Following the aforementioned results,  
4 sediments throughout the study area predominantly represent concentrations within  
5 which adverse biological effects would be rarely observed. To a lesser extent,  
6 sediments mostly found in the Crique Docteur, Bonaberi and Bassa Industrial zones  
7 represent possible-effect ranges within which adverse biological effects will occur.  
8 None of the analysed stations have concentrations equivalent to ERM or PEL for all  
9 studied compounds (Fig. 6). This is consistent with Fusi et al. 2009 findings, who  
10 observed that the relationship between macrobenthos assemblages and POPs (PAHs  
11 and 4,4' DDE) was not significant accounting for a total variation below 10 %.

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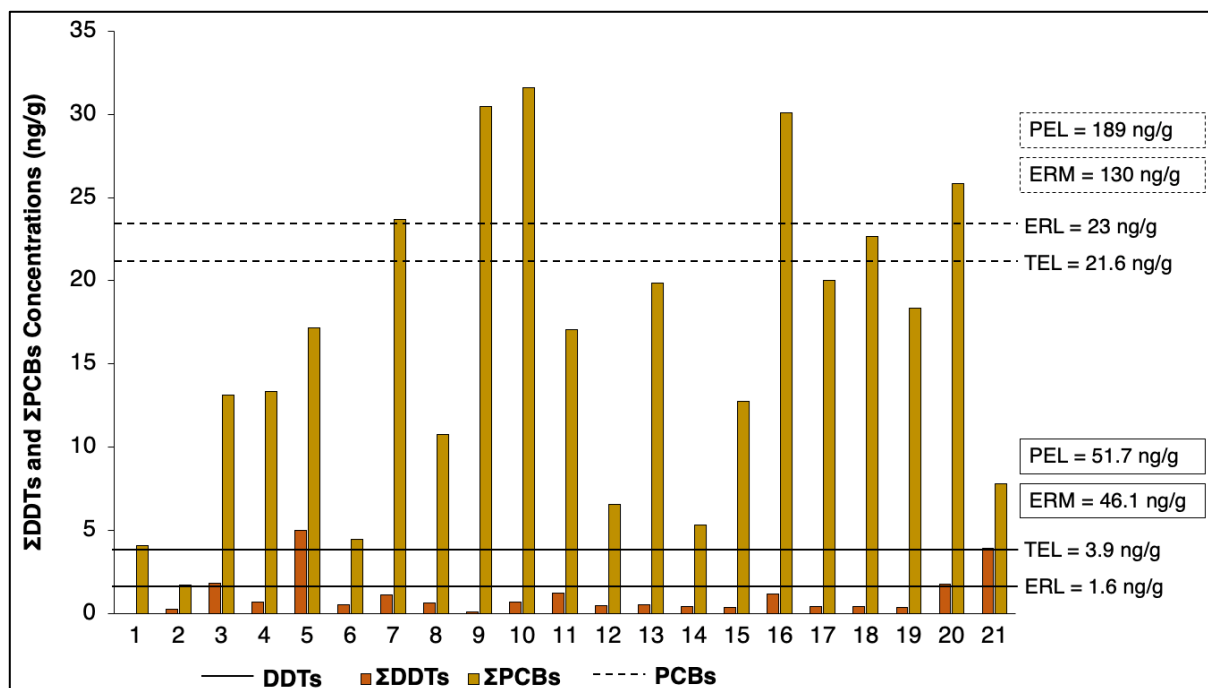


Figure 6. Comparison of DDT and PCB concentrations in sediments of the WEM with Effect-based Sediment Quality Guidelines

### 3.3.2 Implication for Human Health

The pattern of PAHs in the WEM sediments was dominated by (HPAHs) (see Table 1), indicating the presence of strongly carcinogenic and mutagenic PAHs (Karlsson and Viklander, 2008; Laane et al., 2005), apportioning for approximately 25 - 50 % of the total PAH.

Carcinogenicity of WEM sediments was assessed using Toxic Equivalent Concentrations (TEQs) of carcinogenic PAHs (C-PAHs) (Raeisi et al., 2016; Aly Salem et al., 2014; Nasher et al., 2013; Chen and Chen, 2011; Qiao et al., 2006). Among the 7 compounds, B(a)P is the only PAH for which toxicological data are sufficient to derive a carcinogenic potency factor (Aly Salem et al., 2014; Nasher et al., 2013; Hu et al., 2011; Peters et al., 1999). Hence, the carcinogenicity of other PAHs was expressed relative to that of B(a)P. For the 7 C-PAHs, the Toxic Equivalent Factors (TEF) according to the US EPA is given in bracket: Chr (0.001), B(a)A (0.1), B(b)F (0.1),

1 B(k)F (0.1), B(a)P (1), IP (0.1) and DB(a,h)A (1). The total TEQs for C-PAHs was  
2 defined by:

$$\Sigma \text{TEQ}_{\text{C-PAHs}} = \Sigma (C_i \times \text{TEF}_i) \quad (1)$$

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4  
5 where,  $C_i$  is the concentration and  $\text{TEF}_i$  the toxic equivalent factor of  $\text{PAH}_i$  relative to  
6 B(a)P. The  $\Sigma \text{TEQ}_{\text{C-PAHs}}$  ranged from 3.2 – 51.5 ng/g with mean and median of 14.9 and  
7 9.0 ng/g respectively. Higher TEQs were found in station 17, followed by the stations  
8 15, 20 and 13 located in the Crique Docteur (CD) area. This could be attributed to  
9 industrial, harbour and airport activities coupled with the dense traffic in the city of  
10 Douala. The highest contributors of PAHs carcinogenicity include Benzo(a)Pyrene  
11 (47 %), Dibenz(a,h)Anthracene (16 %), Benzo(b)Fluoranthene (15 %),  
12 Benzo(a)Anthracene (12 %). According to Dickhut et al., (2000), motor vehicles are  
13 major sources of these C-PAHs especially in highly populated areas.

14

#### 15 **4. Conclusion**

16 The present study provides comprehensive information on the level of Wouri  
17 Estuary Mangrove anthropization relative to POPs and associated toxicity risks.

18 The concentrations of PAHs and CLPs measured are in the same order of magnitude  
19 of those determined in mangroves of tropical developing countries and corroborate  
20 data published on soil or aquatic organisms in the vicinity of the studied stations. Levels  
21 of PCBs are higher than those reported in similar environment and could be associated  
22 with an occasional probability of toxic effects.

23 The outcome of this work could help reinforce the action plan set up in the  
24 framework of the National implementation plan of the Stockholm Convention in  
25 Cameroon launched since 2012. It contributes in raising awareness for the

1 enforcement of laws protecting the environment and specifically mangrove  
2 ecosystems considering the increasing urbanization of African coastal cities.

### 3 4 **Acknowledgements**

5 We wish to express our gratitude to the French Ministry of Foreign Affairs for supporting  
6 this project. We are equally grateful to the research federation ECCOREV and Labex  
7 OT-Med for supporting this work. We thank IRD Cameroon for the logistic support and  
8 JEA1 RELIFORME team for assistance throughout the sampling campaign. Special  
9 thanks to Pr. Olivier D'HONT (SCAC, Cameroon), Dr. Jean Jacques Braun (IRD,  
10 Cameroon), Dr. Daniel Borschneck (CEREGE), Laurent Vassalo (LCE, AMU), Wulfran  
11 Barthelemy (CEREGE) for their contribution to the realization of this study.

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