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Ecological status and sources of anthropogenic contaminants in mangroves of the Wouri River Estuary (Cameroon)

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Abstract

Mangroves are critically threatened by human activities, despite the important ecosystem functions and services they provide. Mangroves in Cameroon represent no exception to the worldwide trend of mangrove destruction, especially around Douala, on the Wouri river estuary. In two sites around Douala, we assessed the presence of sterols, PAHs, PCBs, DEHP, DDT and its metabolite p,p’DDE and potentially toxic metals in sediment samples. As a proxy of ecological quality, we measured the diversity and abundance of macrobenthos assemblages. We detected p,p’DDE contamination, with concentrations higher than 3 µg kg⁻¹ in 16 out of 26 samples which were attributed to recent widespread use of DDT. The detection of sterols revealed faecal contamination. Significant sensitivity of the macrobenthos to contaminants was revealed, with possible implications on the overall mangrove vulnerability to climate change and on the provision of ecosystem services to local populations.

Keywords

Mangrove; Contaminants; Macrobenthos; Cameroon; Wouri Estuary; Sediment.
Highlights

- Contaminant and macrobenthonic survey was carried out in two Cameroonian mangrove forests around Douala city, Wouri estuary.
- Heavy metals, PCBs, PAHs, DDT and metabolites, and sterols were recorded in mangrove sediment.
- Crab and mollusc assemblages were assessed in both forests and were correlated with the presence of contaminants.
- Uncontrolled management of urban wastewater was among the most important factor of mangrove contamination.
- Integration of chemical and ecological data is required for a comprehensive risk assessment of peri-urban impacted mangrove forest.
1. Introduction

Mangroves in Africa cover over 3.2 million ha, corresponding to about 20% of their global coastline coverage, with approximately 1.5 million ha located along the Atlantic coast (Giri et al., 2011; Massó i Alemany et al., 2010; Spalding et al., 2010; UNEP, 2007). As a consequence of enormous anthropogenic pressure and multiple threats, Western African mangroves have declined by more than 25% over the past 25 years (Friess and Webb, 2014; Giri et al., 2011). Cameroon harbours approximately 2000 km² of mangroves, distributed along the coast of the Guinean Gulf (Giri et al., 2011). Although mangroves contribute considerably to the social and economic well-being of the Cameroonian coastal inhabitants, their total surface area has decreased by 30% in 20 years (Spalding et al., 2010), mainly due to rapid and uncontrolled urbanization around Douala (Din et al., 2002; Ellison and Zouh, 2012; Nfotabong-Atheull et al., 2013). With a population of more than 2 million people, Douala is the largest city in Cameroon and exerts a huge pressure on the nearby mangroves, with uncontrolled sewage discharge detrimentally affecting the whole ecosystem (Simon and Raffaelli, 2012).

Douala is also one of the major shipping ports in the Guinea Gulf that serves the entire central Africa and refuels oil tankers to export locally extracted oil, another significant anthropogenic impact on the Wouri River estuary mangroves (Alemagi, 2007; Duke, 2016; Price et al., 2000; Walle, 1989). Due to the lack of policy regulation in the management of Cameroonian coastal ecosystems, sand mining and wood harvesting also play an important role in reducing mangrove biodiversity and provision of ecosystem services (Ellison and Zouh, 2012; Nfotabong-Atheull et al., 2011).

Although these multiple impacts threaten Wouri River estuary forests, the major socio-economic activity associated with mangroves for local people is in fact still artisanal fishing, with landings estimated between 76 and 106 tons per year (Gabche, 1997). Fisheries play a significant role in small-scale commercial activities and they are vital in providing a source of protein and income for coastal communities (Nfotabong-Atheull et al., 2009). Thus, the modification of both abundance and diversity of mangrove species and the deterioration of water quality, due to urban and industrial activities, will surely have detrimental consequences on the well-being of local communities (Alemagi, 2007; Nfotabong-Atheull et al., 2011, 2009). Last but not least, vulnerability to climate change, and especially to sea level rise, proved to be exacerbated by the high level of anthropogenic pressure on the Wouri River estuary mangroves (Ellison and Zouh, 2012). In particular, purported impacts of sea level rise on mangroves are (i) the increase of frequency and duration of the tidal inundation that may cause the death of the mangrove trees (exceeding the species-specific physiological thresholds; Ball, 1988), (ii) the impact on the inland fresh groundwater with saline intrusion and contaminants dispersal in the intertidal systems (Woodroffe et al., 2016), and (iii) the change of the topography and hydrology of the sediment (Lovelock et al., 2015).

Our aim was to perform the first baseline study on the ecological status and pollution of the strongly impacted Wouri River estuary mangroves, collecting data on both the presence of anthropogenic pollutants in sediments and the structure and diversity of macrobenthic populations as a proxy for healthy ecosystem functioning (Cannicci et al., 2009, 2008). To assess the level of chemical pollution, we targeted the major anthropogenic compounds usually found in peri-urban impacted mangrove forests world-wide, i.e. organochlorine compounds, such as DDT and its metabolites, phthalates such as bis(2-ethylhexyl)phthalate (DEHP), polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), heavy metals and sterols (Bayen et al., 2016, 2005; Lewis et al., 2011; MacFarlane et al., 2007; Peters et al., 1997; Vane et al., 2009). Compounds such as coprostanol (5β(H)-Colestan-3 β -ol) can be used in conjunction with other steroids to determine the relative abundance of sewage in sediments. Coprostanol, in particular, is a faecal sterol generated by microbial activity on cholesterol and is considered as a chemical marker of faecal contamination, especially from humans (Bull et al., 2002; Fattore et al., 1996; Mudge et al., 1999; Peng et al., 2005; Sherwin et al., 1993).
Diversity and abundance of crab and mollusc populations were recently shown to be key-determinants of the maintenance of mangrove ecosystem function and services (Cannicci et al., 2008; Duke et al., 2007; Lee, 2008), such as the provision of nursery sites for fish stocks which is of great importance for the local economy. Crabs and molluscs form an important link between primary detritus at the base of the food web and consumers at higher trophic levels (Sousa and Dangremont, 2011). By consuming litter, crabs can promote nutrient mineralization and recycling within the forest. Furthermore, their bioturbation activities undoubtedly alter the physico-chemical characteristics of soil (Kristensen, 2008) and enhance below-ground organic carbon retention (Andreetta et al., 2014). Finally, since mangrove macrobenthos diversity and functioning are known to be strongly impacted by contaminants (see Cannicci et al. 2009, Bartolini et al. 2011, Penha-Lopes et al. 2011 for east African mangrove benthos), their abundance and diversity is useful in assessing the degree of bioavailability of anthropogenic pollutants and the actual impact on the biological components.

2. Material and Methods

2.1 Area description

The study was carried out in two peri-urban mangrove forests located at different sites along the Wouri estuary: Wouri Bridge forest (4°41’19.10880”N; 9°42’5.81312” E, hereafter WB) and Bois des Singes forest (4°0’49.67706”N; 9°40’ 28.10325”E, hereafter BS), located north-west and south-east of Douala, respectively (fig. 1), with a distance between them of 11 km. Both of these stands are at about 10 hectares in extension and are largely affected by the uncontrolled expansion of urban areas due to the rapidly increasing population of Douala city (Simon and Raffaelli, 2012). Thus, they are representative sites to assess the possible presence of pollutants in peri-urban mangroves.

The climate of the region belongs to the Equatorial regime (Din and Baltzer, 2008), characterised by a long rainy season (March – November) and a short dry season (December – February). Heavy rainfall (approximately 4000 mm per year), stable high temperatures (annual average temperature is 26.7°C) and high humidity throughout the year (approaching 100%) are typical for this region. The tidal regime is semi-diurnal with an average amplitude of 2.5 m. Soils are grey or black muds, of silty, sandy or clayey texture, derived from fluvial sediments relatively rich in organic matter with a high C:N ratio due to the reduced biological activity (Campo and Darius, 2004). Annual salinity variation in the region ranges between 0 and 20%. During the long monsoon season, mangrove water salinity is consistently <10‰. During the dry season, salinity varies between 4 and 20‰ (Din and Baltzer, 2008).

According to a survey by Saenger & Bellan (1995), the floristic composition of Wouri Bridge forest, a 40 year old stand, is dominated by Avicennia germinans, Rhizophora racemosa, R. mangle and R. harrisonii and the mangrove associate Pandanus sp. Bois des Singes is an older stand and has a different floristic composition, represented only by the three Rhizophora species listed above. Hereafter, the belts studied in the Wouri Bridge forest will be referred to as the Avicennia belt, Pandanus belt and Rhizophora belt.

In these systems the faunal composition includes vertebrates, such as mammals like the sirenian species Trichechus senegalensis and several cetaceans. There are birds, of which particular significance is the high abundance of african skimmers, grey pratincoles, open-billed storks and common green shanks, reptiles such as four species of sea turtles (see Diop et al., 2014), and fishes (many of them of commercial importance), and a wide range of invertebrates, mainly crabs (belonging to the families Sesarmidae and Ocypodidae) and molluscs (extensively described by Ngo-Massou et al. 2012), which constitute the bulk of benthic diversity in the these mangrove ecosystem.

2.2 Sediment Sampling
A total of 20 sediment samples were collected in September 2009 in the two mangrove systems for trace metals and organic compounds analysis, choosing five random replicates in each forest. Five samples from BS and five from WB were taken from the upper layer of superficial sediment (0 – 10 cm) and other five samples from BS and five from WB were taken from the layer underneath (11 – 20 cm). The sediment cores were collected using an Eijkelkamp Multisampler™ piston corer (10 cm diameter). The samples were then placed in glass jars, covered with aluminium foil and immediately transferred to a portable freezer and stored at -20°C until analysis.

2.3 Analytical methods

2.3.1 Solvents, chemicals and standards

The solvents used were acetone, hexane, dichloromethane and isooctane, obtained from Sigma Aldrich and Fluka Co., Steinheim, Germany. Standard reference materials for trace metals analysis were supplied by the Community Bureau of Reference Sample (BCR): Certified Reference Materials CRM 277 and CRM 320 and 142 R. Analytical standards for a mixture of PCBs (IUPAC nr. 28, 52, 101, 118, 138, 153, 180), a mixture of PAHs (anthracene, benzo[a] anthracene, benzo [jbk]fluoranthene, benzo [a] pyrene, benzo [ghi] perylene, chrysene , fluoranthene, indeno[1,2,3-cd]pyrene, phenanthrene, pyrene), bis(2-ethylhexyl)phthalate (DEHP) and the internal standards Anthracene-d$_{10}$ and Perylene-d$_{12}$ were purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Analytical sterol standards, Coprostan-3-ol, 5 α-Cholesterol-3β-ol, Cholesterol and 5 β-Cholesterol-3α-ol, analytical Bis(trimethylsilyl)trifluoracetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), used for sterols derivatisation, and analytical standards for 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (p’DDE) were purchased from Sigma Aldrich and Fluka Co, Steinheim, Germany.

2.3.2 Trace metals analysis

All analyses were performed one month after sampling. Trace element concentrations of Mn, Pb, Cr, Cu, Zn, V and Ni were determined by inductively coupled plasma optical emission spectrometry (ICP OES, Perkin Elmer Optima 2100 DV spectrometry, Massachusetts, USA). Concentrations of Co, As, Se, Mo, Cd, Sn, Sb and Tl were determined by inductively coupled plasma mass spectrometry (ICP MS, Agilent Technologies mod. 7700x with Octapole Reaction System ORS, Santa Clara, USA) following aqua regia digestion according to Bettinelli et al. (2000). Mercury was determined by an automatic solid Hg analyser AMA 254. Certified and experimental values exhibited consistent values, recoveries ranging between 93 to 106% with repeatability better than 8% using CRM 277 ‘Estuarine Sediment’ and CRM 320 ‘River Sediment’. Certified soils and sediments were supplied by The Community Bureau of Reference Sample (BCR – IRMM, Joint Research Center, Retieseweg, B-2440, Geel, Belgium): CRM 142 ‘Light Sandy Soil’, CRM 277 ‘Estuarine Sediment’ and CRM 320 ‘River Sediment’. Samples were handled according to the supplier’s specifications. The MDL value was also calculated by analysing the blanks prepared on different working days. This parameter was assumed to be three times the blanks’ standard deviation (See table S1 in the supplementary material).

2.3.3 Organic compounds analysis

For the extraction of PAHs (anthracene, benzo[a] anthracene, benzo [jbk]fluoranthene, benzo [a] pyrene, benzo [ghi] perylene, chrysene, fluoranthene, indeno[1,2,3-cd]pyrene, phenanthrene, pyrene, PCBs (IUPAC nr. 28, 52, 101, 118, 138, 153, 180), DEHP, DDT and its metabolite, samples were treated according to Zaccone et al. (2009). After extraction with Soxhlet using a hexane (80%) and acetone (20%) mixture and concentration of the extracts using a Buchi B-811 Rotavapor, the obtained solutions were divided into two equal parts. A 5 ml aliquot of the extract was evaporated under a gentle flow of nitrogen, recovered with 0.5 mL of hexane containing the internal standards anthracene d$_{10}$ (1.14 mg l$^{-1}$) and perylene d$_{12}$ (1.05 mg l$^{-1}$), centrifuged and analyzed with GC-MS to determine the presence of benzo[jbk]fluoranthene (m/z 252),...
The survey assessed ion values. The survey was randomly sampled to assess the abundance and density of the brachyuran and molluscan populations. The surveys were replicated for each spring tide period, at the same time of the day for the same time of observation in agreement with the methods described in Skov et al. (2002).

2.3.4 Quality control

Calibration curves, prepared by dilution of stock solution with hexane, for PAHs and PCBs and DEHP were obtained at concentrations between 0.01 – 0.2 mg L⁻¹ and 0.1 and 2 mg L⁻¹, respectively, using anthracene d₁₀ (1.14 mg L⁻¹) and perylene d₁₂ (1.05 mg L⁻¹) as internal standards. The calibration curves for p-p'DDE and DDT were created at concentrations between 0.01 and 1 mg L⁻¹, using anthracene d₁₀ (1.14 mg L⁻¹) and perylene d₁₂ (1.05 mg L⁻¹) as internal standards. Calibration curves for sterols were obtained at concentrations between 0.05 – 5 mg L⁻¹ by the dilution of stock solution with isooctane. Recovery experiments (in triplicate) for PAH and PCBs were performed on a Certificate Reference Material (CRM) IMEP-21 obtained from the European Commission–JRC–IRM. The LODs were 0.2 µg kg⁻¹ for coprostan-3-ol and 0.4 µg kg⁻¹ for 5α-Cholestan-3β-ol, Cholesterol and 5β-Cholestan-3α-ol. The recoveries of sterols/stanols were between 65 and 80%.

The sediment total organic carbon (TOC) and total nitrogen (TN) in pore water were determined using the standard methods recommended by SSSA (Sparks et al., 1996).

2.4 Macrobenthos survey

In both forests, the surveys were carried out during three consecutive spring tides in September–October 2009. In each forest, two random transects (100–500 m apart) were established in each vegetation belt following a nested design. Along each transect, three 2 × 2 m² quadrats were randomly sampled to assess the abundance and density of the brachyuran and molluscan populations. The surveys were replicated for each spring tide period, at the same time of the day for the same time of observation in agreement with the methods described in Skov et al. (2002).
on the complexity of the habitat and the diverse behaviour of the study species, different sampling techniques were used to assess the abundance of the various groups of macrofauna. Due to their high densities, molluscs were counted in a sub-quadrat of 50 × 50 cm² placed within the sampling quadrat. Small sesarmids were counted visually throughout the quadrats. Large sesarmids were assessed by counting the number of operational burrows within the quadrats, since previous studies in South Africa and Kenya have clearly shown that these refuges are occupied by single crabs (Berti et al., 2008; Emmerson, 2001; Fratini et al., 2000; Skov et al., 2002). In order to refine the evaluation of crab and mollusc numbers, and due to the accumulation of leaf litter obscuring crabs, after observation for 1 hour in every quadrat we removed fallen leaves and logs to count the hidden specimens. Furthermore, we measured temperature, pH and conductivity of the sediment water for each plot in each location using an Acorn pH 6 meter probe (Oakton Instruments).

2.5 Statistical analyses
A non-metric multidimensional scaling ordination (nMDS) was performed on the basis of a Bray-Curtis dissimilarity matrix calculated on untransformed data to visualize patterns of macrobenthic composition across sites. Furthermore, a PERMANOVA (Anderson et al., 2008) was used to test the null hypothesis of no differences in macrobenthos assemblages and temperature, pH and conductivity across the factor Site (fixed, orthogonal, two levels: WB – Wouri Bridge and BS – Bois des Singes), Belt (fixed, orthogonal three levels: Rhizophora, Pandanus and Avicennia) and Transect (random, nested in Site, 2 levels). PERMANOVA was also used to test the null hypothesis that there were no differences in contaminants, with the factors Site (fixed, orthogonal, two levels: WB – Wouri Bridge and BS – Bois des Singes), Belt (fixed, orthogonal three levels: Rhizophora, Pandanus and Avicennia) and Sampling Depth (fixed, orthogonal two levels: 0-10 and 11-20 cm). In the statistical analysis of contaminants, all values were normalised as performed by Dafforn et al. (2012), and Spearman’s correlation was performed in order to eliminate covariate variables. For contaminants, the Euclidean distance was used to calculate the dissimilarity matrix. DistLM Analysis was performed to test the significant relationship between the ecological and anthropogenic factors and the macrobenthos assemblages.

3. Results
3.1 Sediment analysis
Concentrations of ten PAHs (fig. 2, supplementary table S2) and six PCBs congeners (table 1) were lower than 300 µg kg⁻¹ and 20 µg kg⁻¹ of sediment dry weight respectively. No statistical differences were recorded among sites, belts and sampling depths. Similarly, metals (fig. 2, supplementary table S3) and DEHP (table 2) concentrations were consistently lower than 1500 µg kg⁻¹. DDT was absent, whereas its metabolite p,p'DDE (table 2) was found at concentrations higher than 3 µg kg⁻¹ in 16 samples. Furthermore, no statistical differences among the samples or between sites, belts and depths were observed. Cholesterol and 5 α-cholestan-3β-ol concentrations were lower than 1000 µg kg⁻¹ (fig. 2, supplementary table S4), while concentrations of coprostan-3-ol and 5 β-cholestan-3α-ol were lower than 4000 µg kg⁻¹ (with the exception of two samples). No statistical differences were detected among sites, belts and sampling depths. Interestingly, Coprostanol and 5 β-cholestan-3α-ol were not found in the deepest layers of sediment of any of the belts examined at the Wouri Bridge site.

Ratios between different sterols are presented in table 3 and they provide information concerning the source of contamination (according to Froehner et al. 2009), revealing widespread high disturbance in both forests.

3.2 Macrobenthic assemblages
The two forests were similar in terms of temperature, sediment water pH and sediment water conductivity (PERMANOVA, n=25, F=0.42, df=1.25, p > 0.05; table 4), as well as total N and total OC (PERMANOVA, n=28, F=0.89, df=2.28, p > 0.05; table 4).

In accordance with the species listed by Ngo-Massou et al. (2012) and the reference list edited by Ng & Davie (2008) and Manning & Holthuis (1981), we individuated seven species of sesarmid and two species of mollusc inhabiting the forest. Within Sesarmidae, with an average density of 1.5 individual per square meter, we identified *Perisesarma kamermannii*, *Perisesarma huzardi*, *Metagrapus curvatum* and *Sesarma angolense* as burrowers and *Armases elegans* as a climber. The non-burrowing *Perisesarma alberti* and the potentially phytothelmic *Sesarma buettikoferi* (Fusi et al. unpublished data, fig. 4B), on the other hand, were more dense in the *Avicennia* belt of Wouri Bridge forest (with an average of about 4 individuals per square meter) the former and in the *Pandanus* belt in the same forest (almost 6 individuals per square meter) for the latter. Within molluscs, we recorded the presence of the Thiarid *Pachymelania fusca* in the two forests with a density of at about 400 specimens per square meter and the Potamidid *Tymanotonus radula* (fig. 4A) found only in Bois de Singes forest with a density of at about 40 individuals per square meters.

A significant difference in macrobenthos assemblages between Wouri Bridge and Bois des Singes forest was recorded ($F = 25.655, p = 0.01, \text{fig. 3; fig. 4}$). Specifically, Bois des Singes was characterized by the absence of *S. buettikoferi* and *S. angolense* and a more evenly distributed species density. In Wouri Bridge forest, a dominant species for each belt was observed: *P. alberti* was dominant in the *Avicennia* belt, *S. buettikoferi* in the *Pandanus* belt, while *P. alberti* and *P. kamermannii* were the two most abundant species in the *Rhizophora* belt. There was a notable absence of the gastropod *T. radula* throughout all the Wouri Bridge transects.

### 3.3 Macrobenthos assemblages and contaminants

The DistLM analysis shows a significant relationship between macrobenthos assemblage and sterols, metals, PAHs and C/N data (table 5; fig. 5a) and explains more than 90% of the total variation. In particular, 5 $\beta$-cholestan-3a-ol I, Selenium, Chromium and Zinc explain the highest percentage of variation (20, 15, 8 and 7% respectively, $p < 0.01$). The two species of molluscs, the crabs *P. huzardi* and *P. alberti* appear to be most affected by the variation of the significant environmental variables cited above (fig. 5b).

### 4. Discussion

Mangroves in Cameroon still cover many hectares of estuaries, especially along the Wouri River where a complex system of channels and fens hinder access, and thus direct exploitation, mainly represent by logging for household and coal, and land claim for building new settlements. Nevertheless, the rapid development of Douala together with important commercial and trade activities, due to the presence of the harbour, have contributed to the city being a source of contaminants, which are spreading into nearby mangrove forests. We revealed in this study the presence of contaminants such as PCBs, PAHs, DEHP and heavy metals.

#### Effect of Sterols, p-p-DDE and heavy metals contamination on macrobenthic fauna

In particular, p-p-DDE (DDT metabolite) was found in high concentrations close to the threshold admissible limit (Yang et al., 2007), as previously reported in other mangrove systems by Bodin et al. (2011) and Bhupander and Debapriya (2012). It is likely that this high level is related to recent intensive, and mainly uncontrolled, anti-malaria treatment in the area (Antonio-Nkondjio et al., 2011; Denison, 2013; Etang et al., 2007; Fossog Tene et al., 2013). Unfortunately, these compounds are reported to have a toxic effect on marine organisms (e.g. Bayarri et al. 2001, Mearns et al. 2014) and we strongly suggest that their high levels found in Wouri Bridge might be the reason for the total lack of *Tymanotonus radula* (fig 4, table 2) since the documented endocrine disrupting mechanism of p-p-DDE in molluscs (Matthiessen, 2008). Extremely rapid urbanisation has resulted in a growing urban population that has colonised areas within well-established rainforest and...
mangrove forests. In these areas, settlements consist of rudimentary housing with uncontrolled
discharge of untreated sewage and wastewater into the forests (Nfotabong-Atheull et al., 2011;
Simon and Raffaelli, 2012). This is likely the cause of the presence of sterols detected with a high
ratio of (Coprostanol + epicoprostanol)/Σ Total Stanols that indicates a serious level of sediment
sewage contamination (Froehner et al., 2009; Gern and Lana, 2013). Specifically, we identified high
contamination in 10 of the 26 samples (4 in Wouri Bridge and 6 in Bois des Singes). This
contamination is determined not only by the uncontrolled urbanization taking place in Douala, but
also by the lack of any wastewater treatment management in the city. Indeed, the area nearby Bois
des Singes is highly affected by municipality wastewater discharge, with trucks (personal
observation) releasing tons of untreated wastewater directly into the mangrove, also witnessed
during the survey. This activity is also a good explanation of the fact that we found more highly
contaminated samples in Bois de Singes than Wouri Bridge. Through the macrobenthic survey, we
were able to record highly biodiverse and structured macrobenthic communities in both forests.
However, we recorded two significantly different patterns of macrobenthic assemblage at the two
sites, mainly due to the absence of T. radula (Potamididae) and P. huzardi (Sesarmidae) in Wouri
Bridge forest, while S. buettikoferi was not found in Bois de Singes forest (Fig. 4). Our statistical
analyses found 5 β-cholestan-3α-ol (Gern and Lana, 2013) and four heavy metals, As (Beltman et
al., 1999), Se (Hamilton, 2004), Cr (Lewis et al., 2001) and Zn (Ellis et al., 2004; Schaffelke et al.,
2005), to be the main drivers of differences in crab assemblage (See Fig. 5 and table 5). Indeed,
they are known to be among the more important compounds responsible to disrupt the physiology
of marine species (e.g. Mello and Nayak, 2016 and reference above). If we consider that the main
environmental features, including the tidal regime, were similar between sites and belts, we
hypothesize that these contaminant levels could play a major role shaping the macrobenthos
distribution and density as a result of their differential sensitivity to pollutants and their
concentration. Such a selective ecological effect has been largely described for molluscs in east
African mangrove systems exposed to sewage (Cannicci et al., 2009; Penha-Lopes et al., 2010).

Implications of anthropogenic contamination on mangrove ecosystem

Currently, the interpretation of biological responses as a consequence of contamination remains
complex. A major reason being that organisms in the field are exposed to multiple stressors under
dynamic conditions (e.g. variable micro- and macronutrient loads, changing climatic conditions,
multiple contaminants, tidal cycles and salinity), and potential additive, synergistic or antagonistic
responses to these stressors may occur (Bayen, 2012). Indeed, changes in the diversity/structure of
mangrove ecosystems have been reported as a response to chemical pollution (e.g. Mohamed et al.
2008), which has also been linked to a decline in some populations such as mangrove oysters and
snails (e.g. Roach & Wilson 2009) and molluscs (Cannicci et al., 2009). Kulkarni et al. (2010)
reported low biodiversity indices associated with a low water quality index in mangrove ecosystems
in India. The overall impact of pollution, however, appears to be complex. For example, the patterns
of diversity and species composition recorded in various mangrove forests highly impacted by
humans in Indonesia did not clearly correlate with the impact investigated (Geist et al., 2012).
Moreover, discharge of domestic sewage at low levels caused an increase in crab population size in
east Africa (Cannicci et al., 2009; Penha-Lopes et al., 2011) and did not affect the macrobenthic
communities in Hong Kong (Wong et al., 1997; Yu et al., 1997). Our results indicate that multiple
anthropogenic stressors, and in particular heavy loads of wastewater, although not resulting in the
hypothesised depletion of crab abundance in Wouri River estuary mangroves, can shape their
community composition. The data suggest that contaminant loadings in Wouri river mangroves,
affecting the distribution of macrobenthic species, could lead to the type of cryptic ecological
degradation (sensu Dahdouh-Guebas et al. 2005) shown by Bartolini et al. (2011), who documented
an inverse relationship between the increased biomass of fiddler crabs and their overall engineering
function, thus affecting the whole mangrove ecosystem.
The sterols level recorded in this study strongly indicates a more large contamination by sewage and, in particular, by a first stages of human faecal contamination, as recorded in other studies (i.e. Fernandes et al. 1999, Silva & Madureira 2012). This can have an important effect on the ecology of macrobenthic species (Frena et al., 2016) by changing the chemistry of the sediment where they live dramatically enhancing bacterial activity (Dheenan et al., 2016). The subtle faecal contamination could triggering possible ecological bottom-up effect with relevant ecological consequences on the overall system (Hamilton, 2004), through the modification of secondary consumers primary feeding sources, such as microbenthic (Isobe et al., 2004) or infaunal macrobenthic communities (Moon et al., 2008). Together with the presence of PCBs, PAHs and heavy metals, chromium in particular, raises at least two important concerns. The first is that mangrove sediment receiving wastewater does accumulate a remarkably higher level of long-term contaminants (Tam and Wong, 1995). The second is that they could potentially spread through the whole mangrove area (Agoramoorthy et al., 2008; Yi et al., 2011; Zhou et al., 2007), ultimately affecting, through the trophic chain, the secondary and tertiary consumers which are consumed by the local population. Therefore, they potentially represent a health risk.

5. Conclusions

To our knowledge, this study is the first to investigate the contamination of the Cameroon estuarine and marine environment with Persistent Organic Pollutants (POPS). It contributes to the scarce data and literature on this subject in African countries, which has mainly focused on public health and resistance implications from POPs use. Our data clearly show that the main source of contamination in the mangrove forests surrounding Douala is represented by uncontrolled discharge of urban wastewater and the persistent, illegal and indiscriminate use of DDT. These contaminants, together with four specific heavy metals (As, Cr, Zn, Se) seem to affect the macrobenthonic assemblage of the two study sites, suggesting that Douala peri-urban mangrove is subjected to a complex patchwork of contamination. This documented inflow pollution has serious implications for ecosystem functioning and public health. Therefore we emphasize the necessity to prioritise water quality monitoring and the development of public policies for the wastewater management. Additionally, as highlighted in many studies, human pollution is likely to impair the provision of critical mangrove ecosystem services which are relied upon by local communities. Integrated assessment of macrobenthic assemblages should be considered as a method to detect early contamination patterns, as suggested by our results and confirmed by several other studies. Hence, the present data provide a baseline for further development and environmental management oriented towards anthropogenic pollution by POPs in West Africa, also in the view of monitoring and reducing human impact to mitigate vulnerability of mangroves to the fast climate change.
Acknowledgements

The study was supported by the Cameroon Oil Transportation Company (COTCO), the SP3-People (Marie Curie) IRSES Project CREC (no. 247514), the SC research funds from MIUR (ex 60%) and supported by King Abdullah University of Science and Technology (baseline research funds to DD). We thank Adolphe Nfotabong-Atheull, Vanessa Ngo-Massou and Joseph Bayi for invaluable help during field sampling. We are grateful to Ilaria Marchi for design assistance and Pierluisa Fantini for invaluable laboratory work. Thanks to Jenny Marie Booth for English revision of this manuscript.

References


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Tables

Table 1. Total PCB concentrations in sediment samples. Sample size is shown in brackets. Data are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Forest</th>
<th>Belt</th>
<th>Depth (cm)</th>
<th>PCB tot (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bois des singes</td>
<td>Rhizophora</td>
<td>10 (n=7)</td>
<td>6.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=7)</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>Wouri Bridge</td>
<td>Avicennia</td>
<td>10 (n=3)</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>5.0 ± 3</td>
</tr>
<tr>
<td></td>
<td>Pandanus</td>
<td>10 (n=3)</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rhizophora</td>
<td>10 (n=3)</td>
<td>5.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>4.0 ± 1.2</td>
</tr>
</tbody>
</table>

Table 2. p-p’DDE and DEHP concentrations in sediment samples. Sample size is shown in brackets. Data are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Forest</th>
<th>Belt</th>
<th>Depth (cm)</th>
<th>p-p’DDE (µg kg⁻¹)</th>
<th>DEHP (µg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bois des singes</td>
<td>Rhizophora</td>
<td>10 (n=7)</td>
<td>30 ± 0.2</td>
<td>750 ± 110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=7)</td>
<td>10 ± 0.1</td>
<td>540 ± 90</td>
</tr>
<tr>
<td>Wouri Bridge</td>
<td>Avicennia</td>
<td>10 (n=3)</td>
<td>-</td>
<td>710 ± 370</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>-</td>
<td>530 ± 110</td>
</tr>
<tr>
<td></td>
<td>Pandanus</td>
<td>10 (n=3)</td>
<td>-</td>
<td>380 ± 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>-</td>
<td>1008 ± 390</td>
</tr>
<tr>
<td></td>
<td>Rhizophora</td>
<td>10 (n=3)</td>
<td>40 ± 0.07</td>
<td>750 ± 290</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (n=3)</td>
<td>40 ± 0.07</td>
<td>970 ± 60</td>
</tr>
</tbody>
</table>

Table 3. Stanol contamination index: percentage of coprostan-3-ol and 5 β-cholestan-3α-ol on total sterols, calculated for the different depths in each belt of the two forests. High levels of contaminants (according with Froehner et al., 2009) are shown in bold.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Site</th>
<th>Depth</th>
<th>Belt</th>
<th>(%) (coprostan-3-ol +5 β-cholestan-3α-ol )/total stanols</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Avicennia</td>
<td>43.9</td>
</tr>
<tr>
<td>A1PW20</td>
<td>Wouri Bridge</td>
<td>20</td>
<td>Avicennia</td>
<td>0.1</td>
</tr>
<tr>
<td>P1PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Pandanus</td>
<td>9.8</td>
</tr>
<tr>
<td>P1PW20</td>
<td>Wouri Bridge</td>
<td>20</td>
<td>Pandanus</td>
<td>0.3</td>
</tr>
<tr>
<td>P2PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Pandanus</td>
<td>47.8</td>
</tr>
<tr>
<td>P2PW20</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Pandanus</td>
<td>5.4</td>
</tr>
<tr>
<td>R1PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Rhizophora</td>
<td>6.3</td>
</tr>
<tr>
<td>R1PW20</td>
<td>Wouri Bridge</td>
<td>20</td>
<td>Rhizophora</td>
<td>7.1</td>
</tr>
<tr>
<td>R2PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Rhizophora</td>
<td>44.5</td>
</tr>
<tr>
<td>R2PW20</td>
<td>Wouri Bridge</td>
<td>20</td>
<td>Rhizophora</td>
<td>3.3</td>
</tr>
<tr>
<td>R3PW10</td>
<td>Wouri Bridge</td>
<td>10</td>
<td>Rhizophora</td>
<td>67.4</td>
</tr>
<tr>
<td>R3PW20</td>
<td>Wouri Bridge</td>
<td>20</td>
<td>Rhizophora</td>
<td>9.0</td>
</tr>
<tr>
<td>R1BS10</td>
<td>Bois des singes</td>
<td>10</td>
<td>Rhizophora</td>
<td>24.0</td>
</tr>
<tr>
<td>R1BS20</td>
<td>Bois des singes</td>
<td>20</td>
<td>Rhizophora</td>
<td>3.7</td>
</tr>
<tr>
<td>R2BS10</td>
<td>Bois des singes</td>
<td>10</td>
<td>Rhizophora</td>
<td>11.9</td>
</tr>
<tr>
<td>R2BS20</td>
<td>Bois des singes</td>
<td>20</td>
<td>Rhizophora</td>
<td>21.6</td>
</tr>
<tr>
<td>R3BS10</td>
<td>Bois des singes</td>
<td>10</td>
<td>Rhizophora</td>
<td>43.1</td>
</tr>
<tr>
<td>R3BS20</td>
<td>Bois des singes</td>
<td>20</td>
<td>Rhizophora</td>
<td>39.1</td>
</tr>
</tbody>
</table>
Table 4. Sediment water temperature, pH and Conductivity and pore water Total Nitrogen (TN) and Organic Carbon (TOC) recorded in each plot during macrobenthos surveys. Data are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Forest</th>
<th>Belt</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Conductivity (mV)</th>
<th>Tot N (%)</th>
<th>Tot OC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bois des singes</td>
<td>Rhizophora</td>
<td>27.35 ± 0.3</td>
<td>6.29 ± 0.06</td>
<td>16.13 ± 2.9</td>
<td>0.24±0.04</td>
<td>4.09±0.49</td>
</tr>
<tr>
<td>Wouri Bridge</td>
<td>Avicennia</td>
<td>26.28 ± 0.4</td>
<td>6.25 ±0.04</td>
<td>20.83 ± 2.3</td>
<td>0.28±0.09</td>
<td>4.55±1.38</td>
</tr>
<tr>
<td></td>
<td>Pandanus</td>
<td>26.9 ± 0.4</td>
<td>6.30 ±0.03</td>
<td>18.08 ± 1.6</td>
<td>0.33±0.2</td>
<td>5.65±0.19</td>
</tr>
<tr>
<td></td>
<td>Rhizophora</td>
<td>26.18 ± 0.06</td>
<td>6.29 ±0.07</td>
<td>19 ± 2</td>
<td>0.38±0.1</td>
<td>6.01±1.25</td>
</tr>
</tbody>
</table>

Ratio values that represent detection of contamination are shown in bold. BS = Bois des Singes; PW=Wouri Bridge.

Table 5. Test for relationships between sterols, metals, PAHs and C/N with macrobenthos distribution, using permutational multiple regression analysis (DISTLM). AICc: coefficient of regression, SS: sum of squares, F: value of pseudo and its significance p (% Var: percentage of variance explained by each single variable, and % Cumul: cumulative percentage of variance explained, Res. df: residual degrees of freedom). In bold the variable statistically significant related with macrobenthos distribution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AICc</th>
<th>SS(trace)</th>
<th>Pseudo-F</th>
<th>p</th>
<th>% Var.</th>
<th>%Cumul.</th>
<th>Res.df</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ coprostan-3-ol</td>
<td>0.012</td>
<td>113.38</td>
<td>0.29</td>
<td>0.7352</td>
<td>0.001</td>
<td>0.012</td>
<td>24</td>
</tr>
<tr>
<td>+ 5 β-cholestan-3α-ol</td>
<td>0.219</td>
<td>1978.7</td>
<td>6.12</td>
<td>0.0105</td>
<td>0.208</td>
<td>0.219</td>
<td>23</td>
</tr>
<tr>
<td>+ cholesterol</td>
<td>0.291</td>
<td>678.86</td>
<td>2.21</td>
<td>0.1209</td>
<td>0.071</td>
<td>0.291</td>
<td>22</td>
</tr>
<tr>
<td>+5 α-cholestan-3β-ol</td>
<td>0.314</td>
<td>219.05</td>
<td>0.70</td>
<td>0.4644</td>
<td>0.023</td>
<td>0.314</td>
<td>21</td>
</tr>
<tr>
<td>+As</td>
<td>0.434</td>
<td>1151.3</td>
<td>4.27</td>
<td>0.0316</td>
<td>0.121</td>
<td>0.434</td>
<td>20</td>
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<tr>
<td>+Se</td>
<td>0.587</td>
<td>1458.1</td>
<td>7.05</td>
<td>0.0073</td>
<td>0.153</td>
<td>0.587</td>
<td>19</td>
</tr>
<tr>
<td>+Mo</td>
<td>0.604</td>
<td>155.92</td>
<td>0.74</td>
<td>0.471</td>
<td>0.016</td>
<td>0.604</td>
<td>18</td>
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<tr>
<td>+Cd</td>
<td>0.625</td>
<td>197.66</td>
<td>0.94</td>
<td>0.3786</td>
<td>0.021</td>
<td>0.625</td>
<td>17</td>
</tr>
<tr>
<td></td>
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<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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</tr>
<tr>
<td>+Sn</td>
<td>0.637</td>
<td>119.91</td>
<td>0.55</td>
<td>0.5553</td>
<td>0.013</td>
<td>0.637</td>
<td>16</td>
</tr>
<tr>
<td>+Sb</td>
<td>0.669</td>
<td>300.56</td>
<td>1.43</td>
<td>0.241</td>
<td>0.032</td>
<td>0.669</td>
<td>15</td>
</tr>
<tr>
<td>+Cr</td>
<td>0.751</td>
<td>785.68</td>
<td>4.64</td>
<td>0.0322</td>
<td>0.082</td>
<td>0.751</td>
<td>14</td>
</tr>
<tr>
<td>+Cu</td>
<td>0.804</td>
<td>508.87</td>
<td>3.55</td>
<td>0.0613</td>
<td>0.053</td>
<td>0.804</td>
<td>13</td>
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<tr>
<td>+Mn</td>
<td>0.809</td>
<td>45.498</td>
<td>0.30</td>
<td>0.7313</td>
<td>0.005</td>
<td>0.809</td>
<td>12</td>
</tr>
<tr>
<td>+Zn</td>
<td>0.885</td>
<td>717.9</td>
<td>7.18</td>
<td>0.0095</td>
<td>0.075</td>
<td>0.885</td>
<td>11</td>
</tr>
<tr>
<td>+phenanthrene</td>
<td>0.900</td>
<td>149.18</td>
<td>1.57</td>
<td>0.2309</td>
<td>0.016</td>
<td>0.900</td>
<td>10</td>
</tr>
<tr>
<td>+fluoranthene</td>
<td>0.901</td>
<td>6.8011</td>
<td>0.06</td>
<td>0.8834</td>
<td>0.001</td>
<td>0.901</td>
<td>9</td>
</tr>
<tr>
<td>+ benzo[a] anthracene</td>
<td>0.918</td>
<td>166.41</td>
<td>1.71</td>
<td>0.2141</td>
<td>0.017</td>
<td>0.918</td>
<td>8</td>
</tr>
<tr>
<td>+ benzo[ ]fluoranthene</td>
<td>0.922</td>
<td>38.665</td>
<td>0.37</td>
<td>0.71</td>
<td>0.004</td>
<td>0.922</td>
<td>7</td>
</tr>
<tr>
<td>+ benzo [a] pyrene</td>
<td>0.922</td>
<td>-3.598</td>
<td>-0.03</td>
<td>0.9607</td>
<td>0.000</td>
<td>0.922</td>
<td>6</td>
</tr>
<tr>
<td>+DEHP</td>
<td>0.968</td>
<td>85.979</td>
<td>1.13</td>
<td>0.3724</td>
<td>0.009</td>
<td>0.968</td>
<td>4</td>
</tr>
<tr>
<td>+p-p'DDE</td>
<td>0.984</td>
<td>149.9</td>
<td>2.91</td>
<td>0.1425</td>
<td>0.016</td>
<td>0.984</td>
<td>3</td>
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<tr>
<td>+C/N</td>
<td>0.990</td>
<td>56.641</td>
<td>1.16</td>
<td>0.365</td>
<td>0.006</td>
<td>0.990</td>
<td>2</td>
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</table>
Figures and Figure Captions

Figure 1. Study sites. A) Continental overview. B) Woury Estuary where Douala is located and C) Wouri Bridge mangrove forest (WB) and D) Bois de singes mangrove forest (BS). Black squares indicate the exact location where the study was carried on (Image source: Google Earth).
Figure 2. Average concentrations of Sterols (A), PAHs (B) and metals (C), detected in sediment samples. Data are shown according to vegetation belt (Avicennia sp., Pandanus sp., Rhizophora sp.) and sampling depth on the x-axis. Surface sediment upper layer (0-10 cm) and core samples from 11 to 20 cm.
Figure 3. Non-metric multidimensional scaling ordination showing the patterns of distribution of macrobenthic species in the two study forests. ◊ Bois des Singes *Rhizophora*; ● Wouri Bridge *Avicennia*; + Wouri Bridge *Pandanus*; ♦ Wouri Bridge *Rhizophora*. 
Figure 4. Densities of mollusc (A) and crab (B) species in the study sites. Values are expressed as mean ± SE.
Figure 5. Distance-based redundancy analysis plots (dbRDA) of macrobenthos distribution across Wouri Bridge (Avicennia belt (●), Pandanus belt (+) and Rhizophora belt (♦)) and Bois des Singes (◊), in accordance with the contaminants found in the sediment core of each belt. Vectors correspond to environmental variables (A) and species (B). Length and direction of the vectors indicate the strength of the correlation between the variable and ordination axis given the other variables in the model. The radius of the circle denotes a correlation of 1.