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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 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 and the diversity and abundance of macrobenthos assemblages, as a proxy of ecological quality. We detected contamination by p-p'DDE, with concentrations higher than 3 µg kg⁻¹ in 16 out of 26 samples which we attributed to the widespread use of DDT. The detection of sterols revealed faecal contamination. Furthermore, significant relationships between the macrobenthic assemblages and contaminants indicate the first stages of cryptic ecological degradation, which could have severe implications both on mangrove vulnerability to sea level rise and on the provision of ecosystem services to local populations.

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Dear Editor,

Please find enclosed our manuscript **“Ecological status and sources of anthropogenic contaminants in mangroves of the Wouri River Estuary (Cameroon)”** by Fusi, Beone, Suci, Sacchi, Trevisan, Capri, Daffonchio, Din, Dahdouh-Guebas & Cannicci.

Contamination of intertidal ecosystems, especially along tropical coastlines, is an important issue due to a rapid increase in human population size and accessibility to exploit the resources that these ecosystems provide, principally fisheries. Despite the fundamental functions and services provided by mangrove forests, they are among the most anthropogenically affected intertidal environment worldwide.

In this study we focused on the Wouri estuary, an important region in Cameroon, and in particular the mangrove around Douala. This area is heavily affected by mangrove clearance and overexploitation due to uncontrolled population growth.

The investigation was carried out in two mangrove forests northward and south-eastward of the city of Douala respectively. We analysed the levels of heavy metals, PAHs, PCBs, DDT and sterols. Additionally, we surveyed the macrobenthic assemblages, recording environmental parameters such as temperature, porewater pH and conductivity.

Despite detecting a low level of contamination, except for the DDT metabolite, analysis of sterols revealed the first stages of contamination by urban wastewater. Macrobenthic assemblages differed between the two forests. By merging these results, we evaluated that this incipient contamination could affect the macrobenthic species composition despite the chemical concentrations detected being unimportant relative to the international standard.

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This study is the first to carry out heavy metal assessment in a Cameroonian mangrove forest and highlights a possible connection between contaminants and macrobenthonic assemblages even at a low level of contamination. Notably, this study encourages the integration of chemical analysis with ecosystem ecological traits, such as faunal composition, for monitoring purposes. This baseline study provides reliable data for a comprehensive risk assessment of Cameroonian mangrove forests and highlights the necessity to perform similar monitoring studies in anthropogenically affected mangroves worldwide.

I hope you find our manuscript suitable to be considered for publication in Marine Pollution Bulletin.

I look forward to hearing from you.

Yours faithfully,

Marco Fusi
(PhD, KAUST)

A handwritten signature in black ink that reads "Marco Fusi". The signature is written in a cursive, flowing style.

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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 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 and the diversity and abundance of macrobenthos assemblages, as a proxy of ecological quality. We detected contamination by p-p'DDE, with concentrations higher than 3 $\mu\text{g kg}^{-1}$ in 16 out of 26 samples which we attributed to the widespread use of DDT. The detection of sterols revealed faecal contamination. Furthermore, significant relationships between the macrobenthic assemblages and contaminants indicate the first stages of cryptic ecological degradation, which could have severe implications both on mangrove vulnerability to sea level rise and on the provision of ecosystem services to local populations.

Keywords

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

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 Alemán 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 (Spalding et al., 2010). 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, serving the entire central Africa and providing oil tankers to export locally extracted oil, another significant anthropogenic impact on the Wouri River estuary mangroves (Alemagi, 2007; Price et al., 2000; Walle, 1989). In addition, 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 ecosystem services provision (Ellison and Zouh, 2012; Nfotabong-Atheull et al., 2011).

Although these multiple impacts threaten Wouri River estuary forests, the major socio-economic activity within 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 ecosystems 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).

Based on these alarming premises, 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., 2005; Lewis et al., 2011; MacFarlane et al., 2007; Peters et al., 1997; Vane et al., 2009). Compounds such as coprostanol (5 β (H)-Colestan-3 β -ol) can be used in conjunction with other sterols to determine the relative abundance of sewage in sediments. Coprostanol, in particular, is a faecal sterol generated by microbial activity on cholesterol and it 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 recently proved 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 important for the local economy. Crabs and molluscs, in fact, form an important link between primary detritus at the base of the food web and consumers at higher trophic levels (Sousa and Dangremond, 2011). By consuming litter, crabs can promote nutrient mineralization and recycling within the forest.

1 Furthermore, their bioturbation activities undoubtedly alter the physico-chemical characteristics of
2 soil (Kristensen, 2008) and enhance below-ground organic carbon retention (Andreetta et al., 2014).
3 Finally, since mangrove macrobenthos diversity and functioning are known to be strongly impacted
4 by contaminants (see Cannicci et al. 2009, Bartolini et al. 2011, Penha-Lopes et al. 2011 for east
5 African mangrove benthos), their abundance and diversity is useful in assessing the degree of
6 bioavailability of anthropogenic pollutants and the actual impact on the biological components.
7

8 **2. Material and Methods**

9 *2.1 Area description*

10 The study was carried out in two mangrove forests located at different sites along the Wouri
11 estuary: Wouri Bridge forest (4°4'19.10880''N; 9°42'5.81312'' E, hereafter WB) and Bois des
12 Singes forest (4°0'49.67706''N; 9°40' 28.10325''E, hereafter BS), located northwest and southwest
13 of Douala, respectively (fig. 1).
14

15 The climate of the region belongs to the Equatorial regime (Din and Baltzer, 2008), characterised
16 by a long rainy season (March – November) and a short dry season (December – February). Heavy
17 rainfall (approximately 4000 mm per year), stable high temperatures (annual average temperature is
18 26.7°C) and high humidity throughout the year (approaching 100%) are typical for this region. The
19 tidal regime is semi-diurnal with an average amplitude of 2.5 m. Soils are grey or black muds, of
20 silty, sandy or clayey texture, derived from fluvial sediments relatively rich in organic matter with a
21 high C:N ratio due to the reduced biological activity (Campo and Darius, 2004). Annual salinity
22 variation in the region ranges between 0 and 20‰. During the long monsoon season, mangrove
23 water salinity is consistently <10 ‰. During the dry season, salinity varies between 4 and 20 ‰
24 (Din and Baltzer, 2008).
25

26 According to a survey by Saenger & Bellan (1995), the floristic composition of Wouri Bridge forest
27 is dominated by *Avicennia germinans*, *Rhizophora racemosa*, *R. mangle* and *R. harrisonii* and the
28 mangrove associate *Pandanus* sp. Bois des Singes forest has a different floristic composition,
29 represented only by the three *Rhizophora* species listed above. Hereafter, the belts studied in the
30 Wouri Bridge forest will be referred to as the *Avicennia* belt, *Pandanus* belt and *Rhizophora* belt.
31

32 The faunal composition includes vertebrates, such as birds, reptiles and fish, and a wide range of
33 invertebrates, mainly crabs (sesarmids and ocypodids) and molluscs (extensively described by Ngo-
34 Massou et al. 2012), which constitute the bulk of benthic diversity in the region.
35

36 *2.2 Sediment Sampling*

37 Sediment samples were collected in the two mangrove systems. Sediment sampling for trace metals
38 and organic compounds content was performed in September 2009, with five random samples taken
39 from each mangrove. The upper layer of superficial sediment (0 – 10 cm) and the layer underneath
40 (10 – 20 cm) were collected using an Eijkelpamp Multisampler™ piston corer. The samples were
41 then placed in glass jars, covered with aluminium foil and immediately transferred to a portable
42 freezer and stored at -20° C until analysis.
43

44 *2.3 Analytical methods*

45 *2.3.1 Solvents, chemicals and standards*

46 The solvents used were acetone, hexane, dichloromethane and isooctane, obtained from Sigma
47 Aldrich and Fluka Co., Steinheim, Germany. Standard reference materials for trace metals analysis
48 were supplied by the Community Bureau of Reference Sample (BCR): Certified Reference
49 Materials CRM 277 and CRM 320 and 142 R. Analytical standards for a mixture of PCBs (IUPAC
50 nr. 28, 52, 101, 118, 138, 153, 180), a mixture of PAHs (anthracene, benzo[a] anthracene, benzo
51 [j]k]fluoranthene, benzo [a] pyrene, benzo [ghi] perylene, chrysene, fluoranthene, indeno[1,2,3-
52 cd]pyrene, phenanthrene, pyrene), bis(2-ethylhexyl)phthalate (DEHP) and the internal standards
53 Anthracene-d₁₀ and Perylene-d₁₂ were purchased from Dr. Ehrenstorfer GmbH, Augsburg,
54 Germany. Calibration curves, prepared by dilution of stock solution with hexane, for PAHs and
55

1 PCBs and DEHP were obtained at concentrations between 0.01 – 0.2 mg l⁻¹ and 0.1 and 2 mg l⁻¹,
2 respectively, using anthracene d₁₀ (1.14 mg l⁻¹) and perylene d₁₂ (1.05 mg l⁻¹) as internal standards.
3 Analytical sterol standards, Coprostan-3-ol, 5 α-Cholestan-3β-ol, Cholesterol and 5 β-Cholestan-
4 3α-ol, analytical Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane
5 (TMCS), used for sterols derivatisation, and analytical standards for 1,1,1-trichloro-2,2-di(4-
6 chlorophenyl)ethane (DDT) and 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene (p-p'DDE) were
7 purchased from Sigma Aldrich and Fluka Co, Steinheim, Germany. Calibration curves for sterols
8 were obtained at concentrations between 0.05 – 5 mg L⁻¹ by the dilution of stock solution with
9 isooctane. The calibration curves for p-p'DDE and DDT were created at concentrations between
10 0.01 and 1 mg L⁻¹.
11

12 2.3.2 Trace metals analysis

13 All analyses were performed one month after sampling. Trace element concentrations of Mn, Pb,
14 Cr, Cu, Zn, V and Ni were determined by inductively coupled plasma optical emission spectrometry
15 (ICP OES, Perkin Elmer Optima 2100 DV spectrometry, Massachusetts, USA). Concentrations of
16 Co, As, Se, Mo, Cd, Sn, Sb and Tl were determined by inductively coupled plasma mass
17 spectrometry (ICP MS, Agilent Technologies mod. 7700x with Octapole Reaction System ORS,
18 Santa Clara, USA) following aqua regia digestion according to Bettinelli et al. (2000). Mercury was
19 determined by an automatic solid Hg analyzer AMA 254. Certified and experimental values
20 exhibited consistent values, recoveries ranging between 93 to 106% with repeatability better than
21 8% using CRM 277 'Estuarine Sediment' and CRM 320 'River Sediment'.
22

23 2.3.3 Organic compounds analysis

24 For the extraction of PAHs (anthracene, benzo[a] anthracene, benzo [j]fluoranthene, benzo [a]
25 pyrene, benzo [ghi] perylene, chrysene, fluoranthene, indeno[1,2,3- cd]pyrene, phenanthrene,
26 pyrene, PCBs (IUPAC nr. 28, 52, 101, 118, 138, 153, 180), DEHP, DDT and its metabolite,
27 samples were treated according to Zaccone et al. (2009). After extraction with Soxhlet using a
28 hexane (80%) and acetone (20%) mixture, concentration of the extracts using a Buchi B-811
29 Rotavapor, the obtained solutions were divided into two equal parts. A 5 ml aliquot of the extract
30 was evaporated under gentle flow of nitrogen, recovered with 0.5 mL of hexane containing the
31 internal standards anthracene d₁₀ (1.14 mg l⁻¹) and perylene d₁₂ (1.05 mg l⁻¹), centrifuged and
32 analyzed with GC-MS to determine the presence of benzo[j]fluoranthene (m/z 252),
33 benzo[a]pyrene (m/z 252), benzo [ghi]perylene (m/z 276), and indeno[1,2,3- cd]pyrene (m/z 276),
34 DEHP (m/z 149), DDT (m/z 246) and p-p'DDE (m/z 235). The remaining 5 mL of the extracts
35 were cleaned-up through a Florisil column, evaporated under gentle flow of nitrogen, dissolved in
36 0.5 mL of hexane containing the internal standards anthracene d₁₀ (1.14 mg l⁻¹) and perylene d₁₂
37 (1.05 mg l⁻¹), and then analysed to determine the presence of anthracene (m/z 178), chrysene (m/z
38 228), benzo [a]anthracene (m/z 228), phenanthrene (m/z 178), pyrene (m/z 202) and fluoranthene
39 (m/z 202), and of PCBs (m/z 256 for nr.28, m/z 292 for nr. 52, m/z 326 for nr. 101 and nr. 118, m/z
40 360 for nr.138 and nr. 153 and m/z 394 for nr.180).
41

42 GC-MS analysis was performed according to Zaccone et al. (2009). Total Ion Monitoring (TIM)
43 and Selected Ion Monitoring mode (SIM) were used for identification and quantification of
44 substances. Overall concentration of PAHs and PCBs in sediment is the sum of the 12 PAHs and 7
45 PCBs, respectively, analysed individually (mean of five samples for each site and season) expressed
46 on a dry weight basis. Recoveries were between 84 and 130% for PAHs and between 70 and 115%
47 for PCBs. The limit of detection (LOD) was 2 µg kg⁻¹ for PAHs, 0.5 µg kg⁻¹ for PCBs, 5 µg kg⁻¹ for
48 DEHP and 0.5 µg kg⁻¹ for p-p'DDE.
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50 The sterols were analysed following the method proposed by Froehner et al. (2009), which includes
51 Soxhlet extraction, clean-up with silica-aluminium column, derivatisation and detection and
52 quantification by GC-MS. The LODs were 0.2 µg kg⁻¹ for coprostan-3-ol and 0.4 µg kg⁻¹ for 5 α-
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1 Cholestan-3 β -ol, Cholesterol and 5 β -Cholestan-3 α -ol. The recoveries of sterols/stanols were
2 between 65 and 80%.

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

5 6 2.4 *Macrobenthos survey*

7 In both forests, the survey was carried out during spring tide in September – October 2009. In each
8 forest, two random transects (100–500 m apart) were established in each vegetation belt following a
9 nested design. Along each transect, three 2 \times 2 m² quadrats were randomly sampled to assess the
10 abundance and density of the brachyuran and molluscan populations. Based on the complexity of
11 the habitat and the diverse behavior of the study species, different sampling techniques were used to
12 assess the abundance of the various groups of macrofauna. Due to their high densities, molluscs
13 were counted in a sub-quadrat of 50 \times 50 cm² placed within the sampling quadrat. Small sesarmids
14 were counted visually throughout the quadrats. Large sesarmids were assessed by counting the
15 number of operational burrows within the quadrats, since previous studies in South Africa and
16 Kenya have clearly shown that these refuges are occupied by single crabs (Berti et al., 2008;
17 Emmerson, 2001; Fratini et al., 2000; Skov et al., 2002). In order to refine the evaluation of crab
18 and mollusc numbers, and due to the accumulation of leaf litter obscuring crabs, after observation
19 for 1 hour in every quadrat we removed fallen leaves and logs to count the hidden specimens.
20 Furthermore we measured temperature, pH and conductivity of the sediment for each plot in each
21 location using an Acorn pH 6 meter probe (Oakton Instruments).
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27 28 2.5 *Statistical analyses*

29 A non-metric multidimensional scaling ordination (nMDS) was performed on the basis of a Bray-
30 Curtis dissimilarity matrix calculated on untransformed data to visualize patterns of macrobenthic
31 composition across sites. Furthermore, a PERMANOVA (Anderson et al., 2008) was used to test
32 the null hypothesis of no differences in macrobenthos assemblages and temperature, pH and
33 conductivity across the factor Site (fixed, orthogonal, two levels: WB – Wouri Bridge and BS –
34 Bois des Singes), Belt (fixed, orthogonal three levels: *Rhizophora*, *Pandanus* and *Avicennia*) and
35 Transect (random, nested in Site, 2 levels). PERMANOVA was also used to test the null hypothesis
36 that there were no differences in contaminants, with the factors Site (fixed, orthogonal, two levels:
37 WB – Wouri Bridge and BS – Bois des Singes), Belt (fixed, orthogonal three levels: *Rhizophora*,
38 *Pandanus* and *Avicennia*) and Sampling Depth (fixed, orthogonal two levels: 10 and 20 cm). In the
39 statistical analysis of contaminants, all values were normalised as performed by Dafforn et al.
40 (2012), and Spearman's correlation was performed in order to eliminate covariate variables. For
41 contaminants, the Euclidean distance was used to calculate the dissimilarity matrix. DistLM
42 Analysis was performed to test the relationship between the ecological and anthropogenic factors on
43 the composition of the macrobenthos assemblages.
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48 3. Results

49 3.1 *Sediment analysis*

50 Ten PAHs and six PCBs congeners concentrations were lower than 0.3 mg kg⁻¹ (fig. 2) and 20 μ g
51 kg⁻¹ (table 1) respectively. No statistical differences were recorded among sites, belts and sampling
52 depths. Similarly, metals (fig. 1) and DEHP (table 2) concentrations were consistently lower than
53 1.5 mg kg⁻¹. DDT was absent, whereas its metabolite p-p'DDE (table 2) was found at
54 concentrations higher than 3 μ g kg⁻¹ in 16 samples. Furthermore, no statistical differences among
55 the samples or between sites, belts and depths were observed. Cholesterol and 5 α -cholestan-3 β -ol
56 concentrations were lower than 1 mg kg⁻¹ (fig. 2), while concentrations of coprostan-3-ol and 5 β -
57 cholestan-3 α -ol were lower than 0.4 mg kg⁻¹ (with the exception of two samples). No statistical
58 differences were detected among sites, belts and sampling depths.
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1 Ratios between different sterols are presented in table 3 and they provide information concerning
2 the source of contamination (according with Froehner et al. 2009), revealing widespread high
3 disturbance in both forests.

4 3.2 *Macrobenthic assemblages*

5 The two forests were similar in terms of temperature, pore-water pH and pore-water conductivity
6 (PERMANOVA, $n=25$, $F=0.42$, $df=1,25$, $p>0.05$; table 4), as well as total N and total OC
7 (PERMANOVA, $n=28$, $F=0.89$, $df=2,28$, $p>0.05$; table 4)

8 In accordance with the species listed by Ngo-Massou et al. (2012) and the reference list edited by
9 Ng & Davie (2008) and Manning & Holthuis (1981), we individuated seven species of sesarimid and
10 two species of mollusc inhabiting the forest. Within Sesarimidae, we identified *Perisesarma*
11 *kamermanni*, *Perisesarma huzardi*, *Metagrapsus curvatum* and *Sesarma angolense* as burrowers,
12 *Armases elegans* as a climber, *Perisesarma alberti* as a non-burrower and *Chiromantes buettikoferi*
13 as potentially phytohelmic (Fusi et al. unpublished data). Within molluscs, we recorded the
14 presence of the thiarid *Pachymelania fusca* and the potamidid *Tympanotonus radula*.

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

22 3.3 *Macrobenthos assemblages and contaminants*

23 The DistLM analysis shows a significant relationship between macrobenthos assemblage and
24 environmental data (table 5; fig. 5a) and explains more than 90% of the total variation. In particular,
25 5 β -cholestan-3 α -ol 1, Selenium, Chromo and Zinc explain the highest percentage of variation (20,
26 15, 8 and 7% respectively, $p < 0.01$). The two species of mollusc *P. huzardi* and *P. alberti* appear to
27 be most affected by the variation of the significant environmental variables cited above (fig. 5b).

28 4. Discussion

29 Mangroves in Cameroon still cover many hectares of estuaries, especially along the Wouri River
30 where a complex system of channels and fens hinder access, and thus exploitation, to these forests.
31 Nevertheless, the rapid development of Douala together with important commercial and trade
32 activity due to the presence of the harbour have contributed to the city being a source of
33 contaminants, which are spreading into nearby mangrove forests. We revealed in this study the
34 presence of contaminants such as PCBs, PAHs, DEHP and heavy metals.

35 From the point of view of human health, the levels of contamination cannot be considered harmful,
36 apart from two important exceptions. Firstly, p-p-DDE (DDT metabolite) was found in high
37 concentrations close to the threshold admissible limit (Yang et al., 2007), as previously reported in
38 other mangrove systems by Bodin et al. (2011) and Bhupander and Debapriya (2012). It is likely
39 that this high level is related to intensive anti-malaria treatment in the area (Etang et al., 2007).
40 Unfortunately, these compounds are reported to have a toxic effect on marine organisms (e.g.
41 Bayarri et al. 2001, Mearns et al. 2014). Secondly, extremely rapid urbanisation has resulted in a
42 growing urban population that has colonised areas within well-established rainforest and mangrove
43 forests. In these areas, settlements consist of rudimentary housing with uncontrolled discharge of
44 untreated sewage and wastewater into the forests (Nfotabong-Atheull et al., 2011; Simon and
45 Raffaelli, 2012). This is likely the cause of the presence of sterols detected with a high ratio of
46 (Coprostanol + epicoprostanol) / \sum Total Stanols that indicates a serious level of sediment sewage
47 contamination (Froehner et al., 2009). Specifically, we identified contamination in 10 of the 26

1 samples (4 in Wouri Bridge and 6 in Bois des Singes). Thus, our analysis indicates the first stages
2 of human faecal contamination with a possible important effect on the ecology of macrobenthic
3 species (i.e. Fernandes et al. 1999, Silva & Madureira 2012).

4 We were able to record highly biodiverse and structured macrobenthic communities in both forests,
5 suggesting that they can still be considered as functional ecosystems able to provide crucial
6 ecosystem services (Duke et al., 2007). However, we recorded two significantly different patterns
7 of macrobenthic assemblage at the two sites, mainly due to the absence of *T. radula* (Potamididae)
8 and *P. huzardi* (Sesamidae) in Wouri Bridge forest, while *C. buettikoferi* was not found in Bois de
9 singes forest. The absence of these species could suggest a specific sensitivity to certain
10 contaminants, which has been described in particular for molluscs in east African mangrove
11 systems exposed to sewage (Cannicci et al., 2009; Penha-Lopes et al., 2010), although no
12 significant differences in pollutants were detected between sites.

13 Currently, the interpretation of biological responses as a consequence of contamination remains
14 complex. A major reason being that organisms in the field are exposed to multiple stressors under
15 dynamic conditions (e.g. variable micro- and macronutrient loads, changing climatic conditions,
16 multiple contaminants, tidal cycles and salinity), and potential additive, synergistic or antagonistic
17 responses to these stressors may occur (Bayen, 2012).

18 Indeed, changes in the diversity/structure of mangrove ecosystems have been reported as a response
19 to chemical pollution (e.g. Mohamed et al. 2008), which has also been linked to a decline in some
20 populations such as mangrove oysters and snails (e.g. Roach & Wilson 2009) and molluscs
21 (Cannicci et al., 2009). Kulkarni et al. (2010) reported low biodiversity indices associated with a
22 low water quality index in mangrove ecosystems in India. The overall impact of pollution, however,
23 appears to be complex. For example, the patterns of diversity and species composition recorded in
24 various highly human-affected mangrove forests in Indonesia did not clearly correlate with the
25 impact investigated (Geist et al., 2012). Moreover, discharge of domestic sewage at low levels
26 caused an increase in crab population size in east Africa (Cannicci et al., 2009; Penha-Lopes et al.,
27 2011) and did not affect the macrobenthic communities in Hong Kong (Wong et al., 1997; Yu et al.,
28 1997). Our results confirm that multiple anthropogenic stressors, and in particular heavy loads of
29 human sewage, do not result in predictable depletion of crab population diversity or density in
30 Wouri River estuary mangroves. However, we cannot exclude that sewage loadings could lead to
31 the type of cryptic ecological degradation (*sensu* Dahdouh-Guebas et al. 2005) shown by Bartolini
32 et al. (2011), who documented an inverse relationship between the increased biomass of fiddler
33 crabs and their overall engineering function, thus affecting the whole mangrove ecosystem. In fact,
34 although we did not find differences in contamination between the two sites, we found a relatively
35 strong relationship between macrobenthic diversity and contaminants with a model proposed by
36 DistLM analysis.

37 **5. Conclusion**

38 To our knowledge, this study is the first to investigate the contamination of the Cameroon estuarine
39 and marine environment with Persistent Organic Pollutants (POPS). It contributes to the scarce data
40 and literature on this subject in African countries, which has mainly focused on public health and
41 resistance implications from POPs use. Our data clearly show that the main source of contamination
42 in the mangrove forests surrounding Douala is represented by uncontrolled discharge of urban
43 wastewater and the use of DDT. This documented inflow pollution has serious ecosystem
44 functioning and public health implications, indicating the necessity to prioritise water quality
45 monitoring should in the development of public policies and management. Additionally, as
46 highlighted in many studies, human pollution is likely to impair the provision of critical mangrove
47 ecosystem services, which are relied upon by local communities (Yu et al., 2005). Integrated
48 assessment of macrobenthic assemblages should be considered as a method to detect early
49 contamination patterns, as suggested by our results and confirmed by several other studies (e.g.
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Cannicci et al., 2009). 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 a monitoring and a reduction of human impact to reduce vulnerability of mangroves to sea level rise (Ellison and Zouh, 2012).

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Tables

Table 1. p-p'DDE and DEHP concentrations in sediment samples. Sample size is shown in brackets. Data are expressed as mean \pm standard error.

Forest	Belt	Depth (cm)	p-p DDE (mg kg ⁻¹)	DEHP (mg kg ⁻¹)
Bois des singes	<i>Rhizophora</i>	10 (n=7)	0.030 \pm 0.002	0.75 \pm 0.11
		20 (n=7)	0.010 \pm 0.001	0.54 \pm 0.09
Wouri Bridge	<i>Avicennia</i>	10 (n=3)	-	0.71 \pm 0.37
		20 (n=3)	-	0.53 \pm 0.11
	<i>Pandanus</i>	10 (n=3)	-	0.38 \pm 0.04
		20 (n=3)	-	1.08
	<i>Rhizophora</i>	10 (n=3)	0.0400 \pm 0.0007	0.75 \pm 0.29
		20 (n=3)	0.0400 \pm 0.0007	0.97 \pm 0.06

Table 2. Total PCB concentrations in sediment samples. Sample size is shown in brackets. Data are expressed as mean \pm standard error.

Forest	Belt	Depth (cm)	PCB tot (μ g kg ⁻¹)
Bois des singes	<i>Rhizophora</i>	10 (n=7)	6.152 \pm 1.869
		20 (n=7)	6.353 \pm 1.750
Wouri Bridge	<i>Avicennia</i>	10 (n=3)	6.315 \pm 0.411
		20 (n=3)	4.990 \pm 3.033
	<i>Pandanus</i>	10 (n=3)	2.572 \pm 1.167
		20 (n=3)	-
	<i>Rhizophora</i>	10 (n=3)	5.270 \pm 1.503
		20 (n=3)	3.958 \pm 1.199

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

Samples	Site	Depth	Belt	% (coprostan-3-ol + 5 β -cholestan-3 α -ol)/total stanols
A1PW10	PW	10	<i>Avicennia</i>	43.9
A1PW20	PW	20	<i>Avicennia</i>	0.1
P1PW10	PW	10	<i>Pandanus</i>	9.8
P1PW20	PW	20	<i>Pandanus</i>	0.3
P2PW10	PW	10	<i>Pandanus</i>	47.8
P2PW20	PW	10	<i>Pandanus</i>	5.4
R1PW10	PW	10	<i>Rhizophora</i>	6.3
R1PW20	PW	20	<i>Rhizophora</i>	7.1
R2PW10	PW	10	<i>Rhizophora</i>	44.5
R2PW20	PW	20	<i>Rhizophora</i>	3.3
R3PW10	PW	10	<i>Rhizophora</i>	67.4
R3PW20	PW	20	<i>Rhizophora</i>	9.0
R1BS10	BS	10	<i>Rhizophora</i>	24.0
R1BS20	BS	20	<i>Rhizophora</i>	3.7
R2BS10	BS	10	<i>Rhizophora</i>	11.9
R2BS20	BS	20	<i>Rhizophora</i>	21.6
R3BS10	BS	10	<i>Rhizophora</i>	43.1
R3BS20	BS	20	<i>Rhizophora</i>	39.1
R4BS10	BS	10	<i>Rhizophora</i>	23.0

R4BS20	BS	20	<i>Rhizophora</i>	25.6
R5BS10	BS	10	<i>Rhizophora</i>	21.5
R5BS20	BS	20	<i>Rhizophora</i>	35.6
R6BS10	BS	10	<i>Rhizophora</i>	26.0
R6BS20	BS	20	<i>Rhizophora</i>	14.1
R7BS10	BS	10	<i>Rhizophora</i>	68.2
R7BS20	BS	20	<i>Rhizophora</i>	18.8

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

Table 4. Environmental parameters recorded in each plot during macrobenthos surveys. Data are expressed as mean \pm standard error.

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

Table 5. Test for relationships between chemical parameters and 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).

Variable	AICc	SS(trace)	Pseudo-F	p	% Var.	%Cumul.	Res.df
+ coprostan-3-ol	0.012	113.38	0.29	0.7352	0.001	0.012	24
+ 5 β-cholestan-3α-ol	0.219	1978.7	6.12	0.0105	0.208	0.219	23
+cholesterol	0.291	678.86	2.21	0.1209	0.071	0.291	22
+5 α -cholestan-3 β -ol	0.314	219.05	0.70	0.4644	0.023	0.314	21
+As	0.434	1151.3	4.27	0.0316	0.121	0.434	20
+Se	0.587	1458.1	7.05	0.0073	0.153	0.587	19
+Mo	0.604	155.92	0.74	0.471	0.016	0.604	18
+Cd	0.625	197.66	0.94	0.3786	0.021	0.625	17
+Sn	0.637	119.91	0.55	0.5553	0.013	0.637	16
+Sb	0.669	300.56	1.43	0.241	0.032	0.669	15
+Cr	0.751	785.68	4.64	0.0322	0.082	0.751	14
+Cu	0.804	508.87	3.55	0.0613	0.053	0.804	13
+Mn	0.809	45.498	0.30	0.7313	0.005	0.809	12
+Zn	0.885	717.9	7.18	0.0095	0.075	0.885	11
+phenanthrene	0.900	149.18	1.57	0.2309	0.016	0.900	10
+fluoranthene	0.901	6.8011	0.06	0.8834	0.001	0.901	9
+ benzo[a] anthracene	0.918	166.41	1.71	0.2141	0.017	0.918	8
+ benzo	0.922	38.665	0.37	0.71	0.004	0.922	7

1	[jbc]fluoranthene							
2	+ benzo [a] pyrene	0.922	-3.598	-0.03	0.9607	0.000	0.922	6
3	+DEHP	0.968	85.979	1.13	0.3724	0.009	0.968	4
4	+p-p'DDE	0.984	149.9	2.91	0.1425	0.016	0.984	3
5	+C/N	0.990	56.641	1.16	0.365	0.006	0.990	2

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Figures and Figure Captions



Figure 1. Study site, showing the location of sediment sampling. A) Bois des Singe mangrove (BS) forest; B) Wouri Bridge mangrove forest (WB).

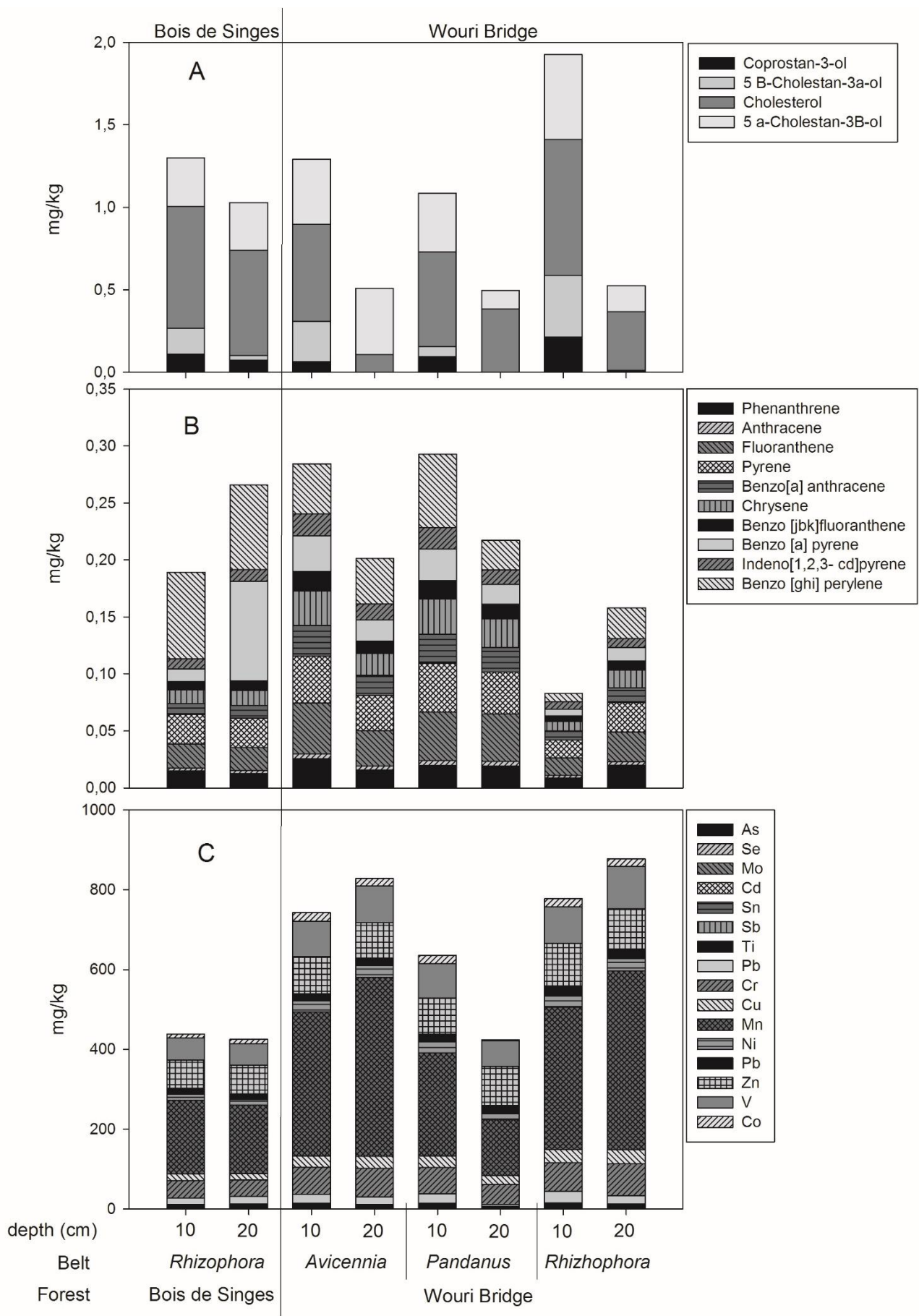


Figure 2. Average concentrations of Sterols (A), PAHs (B) and metals (C), detected in sediment samples. Data are shown according to vegetation belt (*Avicennia* sp., *Pandanus* sp., *Rhizophora* sp.) and sampling depth on the x-axis. Surface sediment upper layer (0-10 cm) and core samples from 10 to 20 cm. Sample size is shown on the x-axis.

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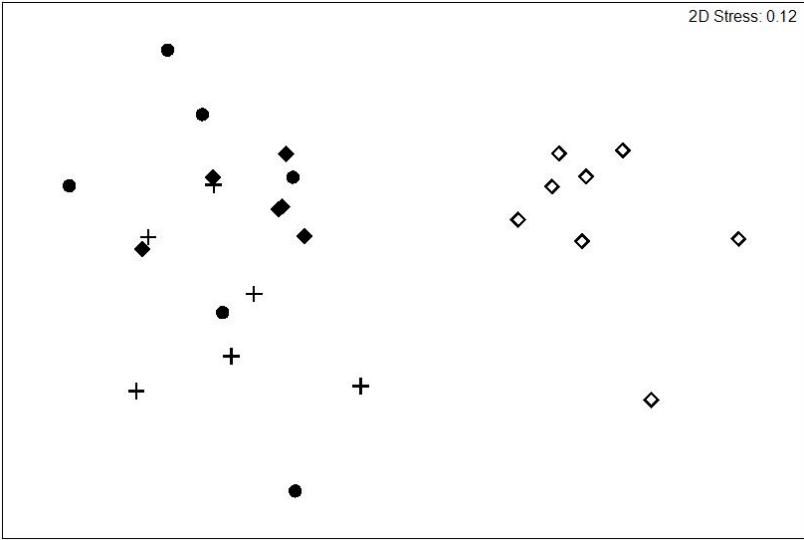


Figure 3. Non-metric multidimensional scaling ordination showing the patterns of distribution of macrobenthic species in the two study forests. \diamond Bois des Singes *Rhizophora*; \bullet Wouri Bridge *Avicennia*; + Wouri Bridge *Pandanus*; \blacklozenge Wouri Bridge *Rhizophora*.

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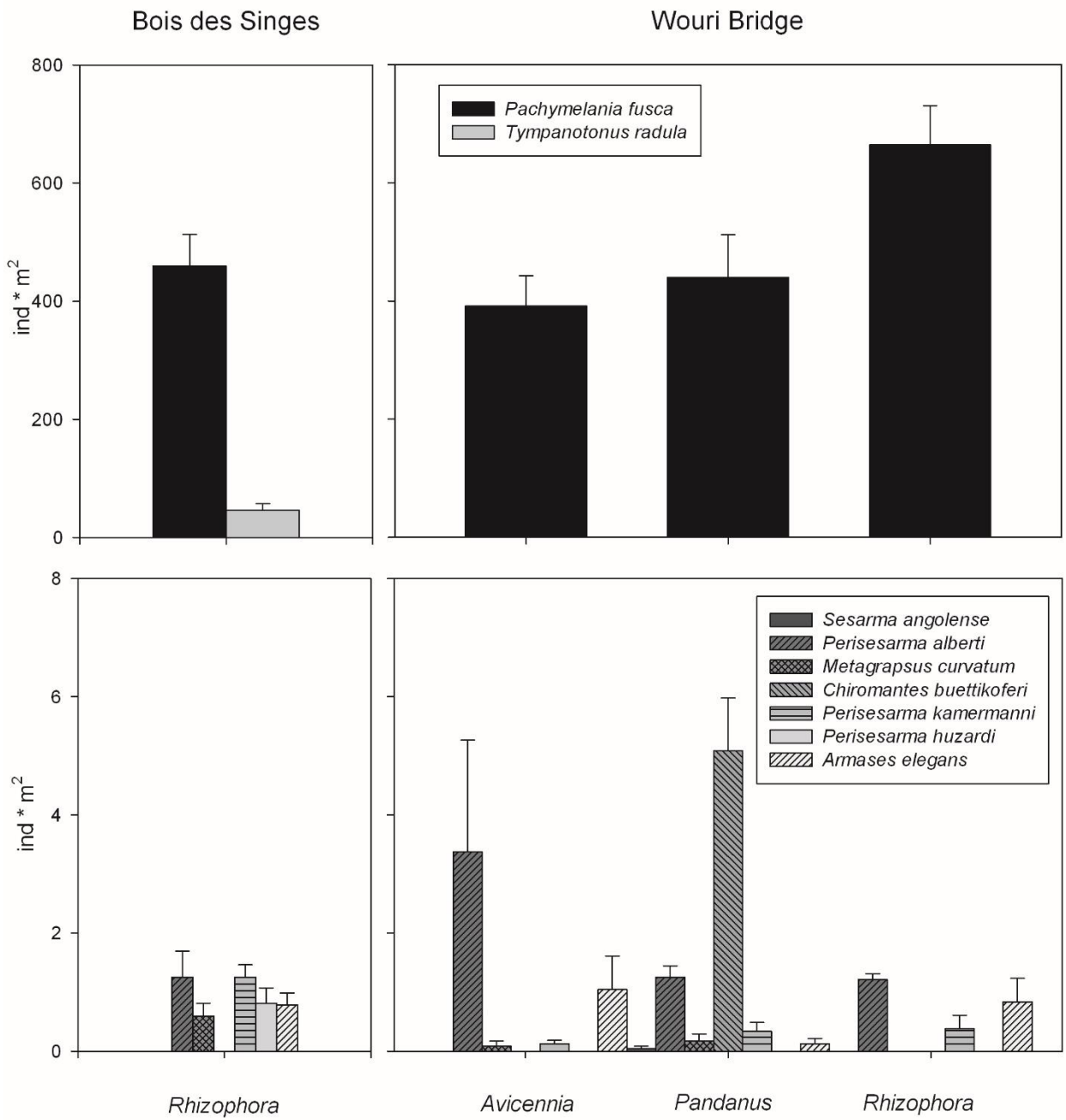
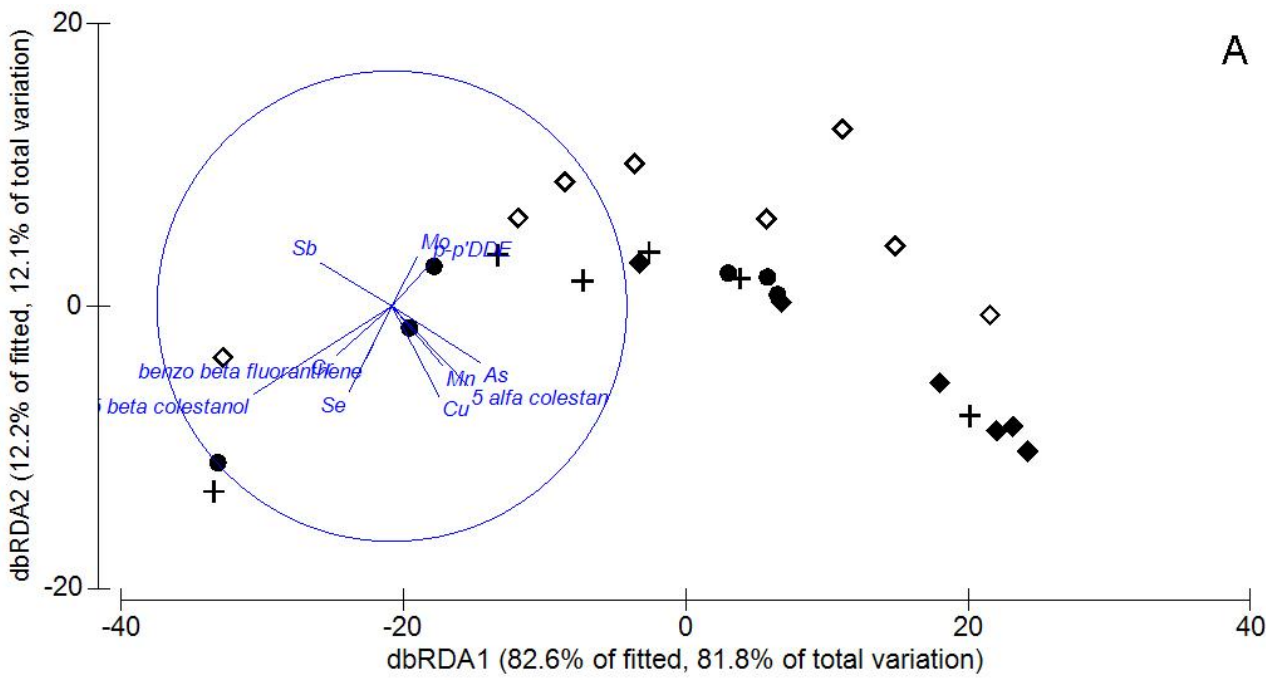
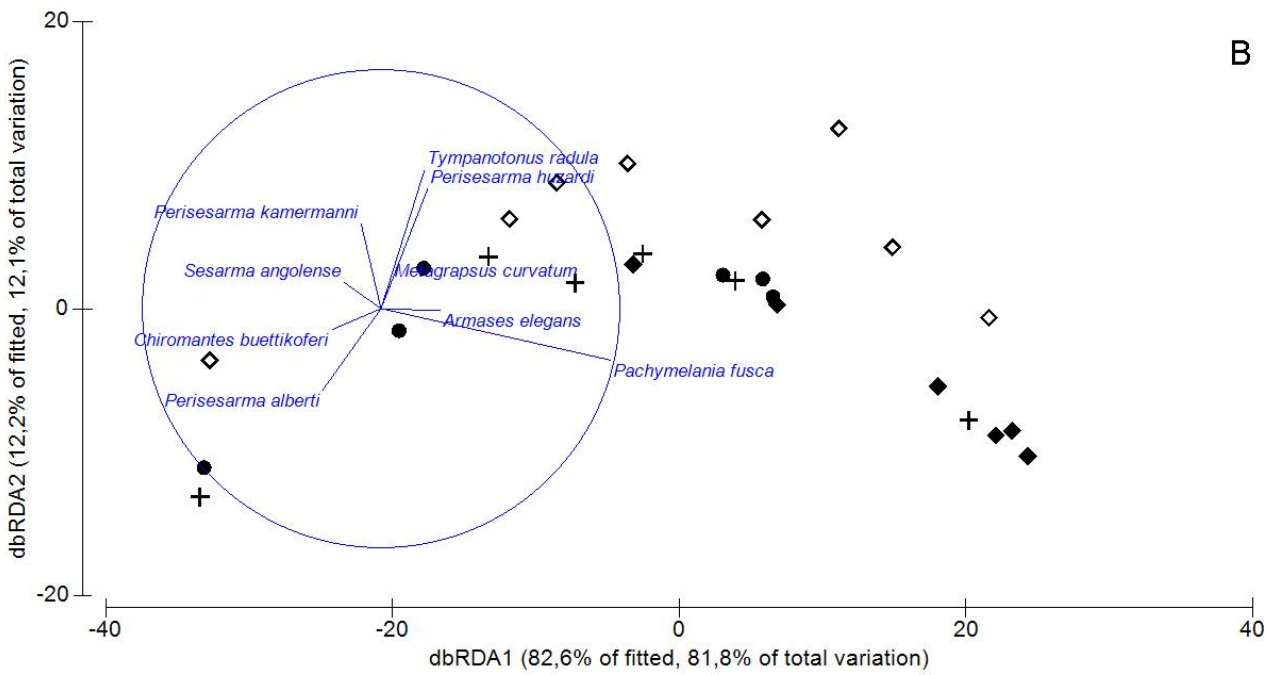


Figure 4. Densities of mollusc (A) and crab (B) species in the study sites. Values are expressed as mean \pm SE.

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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.