BASELINE LEVELS OF PERSISTENT ORGANIC POLLUTANTS IN SEDIMENT AND TISSUES OF FISH AND INVERTEBRATES FROM THE CONGO RIVER (Democratic Republic of Congo)

Verhaert V[1], Covaci A[1, 2] , Musibono DE[3], Bervoets L[1], Verheyen E[1, 4] and Blust, R[1]

[1] Laboratory of Ecophysiology, Biochemistry and Toxicology, Dep. of Biology, University of Antwerp, B-2020 Antwerp, Belgium; [2] Toxicological Centre, Dep. of Pharmaceutical Sciences, University of Antwerp, B-2610 Wilrijk, Belgium; [3] Laboratory of Ecotoxicology, Dep. of Environmental Sciences, University of Kinshasa, DR Congo; [4] Evolutionary Ecology Group, Dep. of Biology, University of Antwerp, B-2020 Antwerp, Belgium and the Vertebrate Department, Royal Belgian Institute of Natural Sciences, B-1000 Brussels, Belgium

Introduction

The Congo River is one of the largest freshwater systems of the world and considered pristine, although very little information is available concerning the presence of anthropogenic pollution and its impact on biodiversity and human health. Both urban and industrial activities may result in the release and accumulation of organic pollutants in the aquatic system but there is no or very little regulation and control. In addition, also the global transport and atmospheric deposition of pollutants may be a source of contamination[1].

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs), can be transferred across trophic levels of the food chain by the processes of bio-accumulation and bio-magnification where they can be toxic to all organisms of the food web. As a consequence, most of them are banned globally[2]. The DR Congo has ratified the Stockholm Convention in 2005 and POPs are banned from importation, sale and use. Nevertheless, there is evidence of their presence and continued use[3].

The present study aims to evaluate the occurrence of the most important POPs, such as OCPs (DDTs, HCHs and HCB), PCBs and PBDEs in sediments, invertebrates and fish from selected sites in the Basin of the Congo River. To our knowledge, this study is the first to investigate the presence of POPs in the Congo River.

Materials and methods

Study area

The Congo River is situated in the Democratic Republic of Congo, Central Africa. It is the second largest river in Africa and has the second largest watershed of the world (3 680 000 km²), after the Amazon[4]. Samples were taken in May-June 2010, during an expedition on the Congo River, organised by the Royal Belgian Institute of Natural Sciences, the Royal Museum of Central Africa, the National Botanical Garden and the University of Kisangani.

The studied area (Fig. 1) was situated between Kisangani and Bumba and comprised selected sampling locations of three tributaries, i.e. Itimbiri (1), Aruwimi (2), Lomami (3) and the Congo River itself, i.e. near Isangi (4) and Kisangani (5).

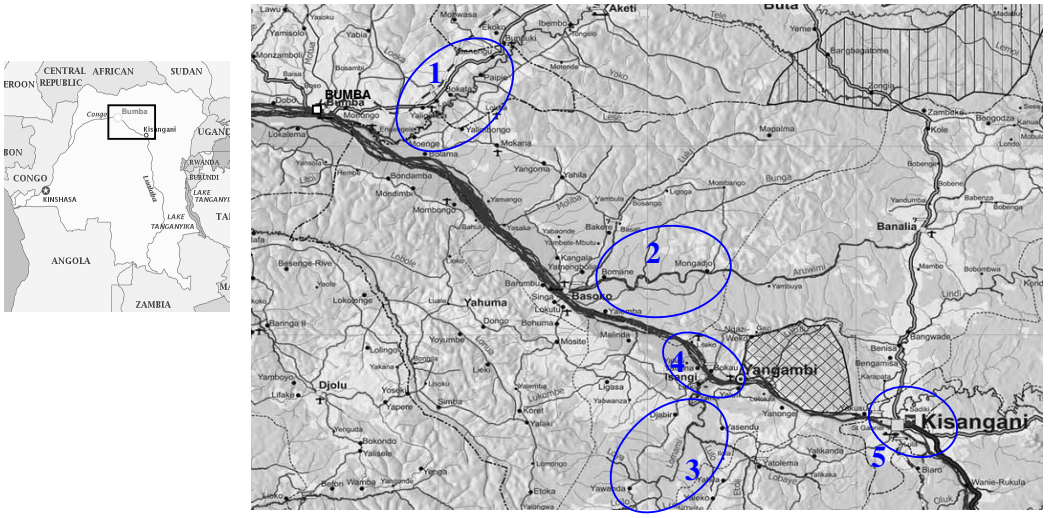

Fig. 1: Sampling locations 1. Itimbiri, 2. Aruwimi, 3. Lomami, 4. Congo River (Isangi), 5. Congo River (Kisangani)

Sample collection

At each sampling location samples of sediment, fish and invertebrates were collected at different sampling points. Sediment samples were taken with a Petite Ponar Grab (Wildco). Fish were collected with nets, and then filleted and skinned to obtain muscle tissue. Six fish species have been selected based upon their distribution throughout the axial length of the study area: *Marcusenius sp. (*Mormiridae*),* Shoulderspot catfish *(Schilbe marmoratus,* Schilbidae*),* another catfish species *(Schilbe grenfelli,* Schilbidae*),* Bigeye squeaker (*Synodontus alberti,* Mochocidae*),* Spot-tail robber (*Brycinus Imberi,* Alestidae*),* Sharktail distichodus (*Dystichodus fasciolatus,* Distochontidae).

Concerning invertebrates, a shrimp species African caridina *(Caridina Africana,* Atyidae) and a prawn species *Macrobrachium sp.* (Palaemonidae) were also collected with nets. Two apple snail species (*Lanistes ovum* and *Pila sp.*, Ampullariidae*)* were bought from the local population. All samples were collected in May and the beginning of June and stored at -20°C until analysis.

Chemicals

The following compounds were included in the analysis: 33 PCB congeners (IUPAC numbers: CB 18, 28, 44, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 151, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, 206, 209), 7 PBDEs (IUPAC numbers: 28, 47, 99, 100, 153, 154, 183), DDXs (o,p'-DDD,o,p'-DDT, o,p'-DDE, p,p'-DDD, p,p'-DDE, p,p'-DDT), chlordanes – CHLs (TC, CC, TN, OxC), HCHs (α-, β-, γ-hexachlorocyclohexane) and HCB. BDE 209 was also targeted in sediment samples. All solvents and chemicals were purchased or prepared as described previously[5, 6].

Sample preparation

The methods used for the determination of POPs in sediment and biota samples have been previously described and validated[7] and are briefly given below. For the biota samples, the whole amount available of freeze-dried fish muscle (0.2-6.2g) and invertebrates (0.1-4.1g) were homogenized with anhydrous Na_2SO_4, spiked with internal standards (CB 143, BDE 77, ε-HCH) and extracted for 2h by hot Soxhlet with 100 ml hexane/acetone (3/1, v/v). After lipid determination, the extract was cleaned-up on 8 g acidified silica. After elution of analytes with 20 ml hexane and 15 ml dichloromethane, the cleaned extract was concentrated. For the sediment, the same procedure was followed, but 5 g of activated copper powder was added and mixed with the sample. The samples are spiked with internal standards (CB 143, BDE 77, ^{13}C-BDE 209, ε-HCH). For the clean-up step, 2 g of copper powder was added on top of the acid silica.

Analysis

PBDEs, HCHs and chlordanes were measured with an Agilent 6890-5973 gas chromatograph coupled with a mass spectrometer (GC-MS) and equipped with a 30 m x 0.25 mm x 0.25 μm DB-5 capillary column. The MS was operated in electron capture negative ionisation (ECNI) mode and was used in the selected ion-monitoring (SIM) mode with ions m/z = 79 and 81 monitored during the entire run and specific ions for OCPs. PCBs, DDXs, and HCB were measured with the same GC-MS system as for the PBDE determination, operated in electron ionisation (EI) mode and equipped with a 25 m x 0.22 mm x 0.25 μm HT-8 capillary column. The MS was used in the SIM mode with 2 ions monitored for each PCB homologue group or OCP.

Quality assurance/quality control (QA/QC)

For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank, which ensures > 99 % certainty that the reported value is originating from the sample. For analytes that were not detected in procedural blanks, LOQs were calculated for a ratio S/N equal to 10. LOQs depended on the sample intake and on the analyte and ranged between 1 and 4 ng/g lipid weight (lw) and 10 and 50 pg/g dry weight (dw). QC was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs and PBDEs in whale blubber) and CRM 536 (PCBs in harbour sediment) was used to test the method accuracy. Obtained values were not deviating more than 10 % from the certified values. The QC scheme is also assessed through regular participation to interlaboratory comparison exercise organised by the US National Institute of Standards and Technology.

Statistical analysis

Statistical analysis were conducted using GraphPad Prism 5 (GraphPad Software, Inc) and the SPSS 15.0 statistical package. The level of statistical significance was defined at $p < 0.05$. For concentrations below the LOQ, a value of f*LOQ (with f, detection frequency) was used. After testing the normality of the data (Kolmogorov Smirnov test), data were LOG transformed. Differences between locations were detected using One Way ANOVA with Tukey test. Spearman rank correlation coefficients were calculated between pollution levels in sediment and biota tissues and between invertebrates and fish.

Results and discussion

POP levels in sediment and biota

Table 1 shows mean levels of ∑PCBs, ∑PBDEs, ∑DDXs, ∑HCHs, ∑chlordanes and HCB for sediment (ng/g dw), fish and invertebrate species (ng/g lw). Lipid content of biota(%) is also given. In Fig. 2 an overview of ∑PCBs, ∑PBDEs, ∑DDXs, ∑HCHs concentrations in sediment, fish and invertebrate species for each location is presented.

The overall detection frequency and detected concentrations of POPs in the sediment samples were low. ∑CHLs were below LOQ (0.02 ng/g dry weight) in all collected sediments samples. HCB was only analysed at very low concentrations in 2 out of 26 sediment samples.

∑PCB concentrations in fish from the Itimbiri River (location 1) were significantly higher than concentrations measured at the other sampling locations ($F_{4,65}$=7.003; p=0.000). ∑HCH concentrations in fish from the Aruwimi River (location 2) were significantly higher than concentrations measured in fish collected in the Lomami (location 3) and in the Congo River at Kisangani (location 5) ($F_{4,65}$=3.539; p=0.011). The reason for these differences is not yet clear and will be further investigated.

No significant relations were observed in pollutant concentrations between sediment and biota and between fish and invertebrates. These findings indicate that measurements in sediment are not always reliable to estimate potential bioaccumulation in species.

Overall, concentrations of all compounds were the highest in fish, followed by invertebrates and sediment. This could be indicative for biomagnification of POPs in the Congo River Basin. Hence, further investigation and quantification of biomagnification factors will be conducted.

Table 1. Mean concentrations of ∑PCBs, ∑PBDEs, ∑DDXs, ∑HCHs, ∑chlordanes and HCB in sediment (ng/g dry weight), fish and invertebrate samples (ng/g lipid weight) at the five sampling locations.

Sample	Location	N	Lipid %	∑PCBs	∑PBDEs	∑DDXs	∑HCH	∑CHL	HCB
Sediment	1	2		0.14	0.46	0.05	0.03		
(ng/g dry weight)	2_{fish}	1		0.5	0.7	0.05	0.05		
	2_{inv}	2		0.4	0.7	0.25	0.09		
	3	1		0.18	0.15	0.09	0.03		
	4	1		0.8	0.24	0.12	0.04		
	5	1		0.9	0.5	0.05	0.06		
Fish	1	9	1.4	1457	8	11	11	3	0.5
(ng/g lipid weight)	2	6	3	220	13	14	29	4	3
	3	25	1.6	129	6	11	6	1.2	1.1
	4	20	2.0	189	15	13	10	0.5	2.5
	5	8	1.7	94	10	16	6	1.5	1.2
Invertebrates	1	4	1.7	70	3	9	12	0.6	0.9
(ng/g lipid weight)	2	7	2.1	89	0.9	5	7	0.18	0.18
	3	5	1.8	89	3	10	19	0.18	0.18
	4	5	1.3	99	4	19	18	0.18	0.18

POP levels and human health

Via the consumption of freshwater fish also human health can be impacted by the presence of POPs. Table 3 gives the Minimum Risk Level (MRL) for PCBs, PBDEs, DDXs and γ-HCH defined by the Agency for Toxic Substances and Disease Registry[8]. A person of 70 kg, who eats more than 180g of *Marcusenius sp*. a day (Itimbiri River), exceeds the Minimum Risk Level for PCBs.

Table 2: MRLs (ATSDR) for PCBs, PBDEs, DDXs and γ-HCH

	∑PCBs	∑PBDEs	∑DDXs	γ-HCH
MRL (µg/kg/day)	0.03	7	0.50	0.01
MRL (µg/day) for a person of 70kg	2.10	490	35.00	0.70
Mean concentration in *Marcusenius sp.* (ng/g ww) of Itimbiri River	11.68	0.40	0.27	0.15
Maximum amount (kg ww/day)	0.18	1225	128	5

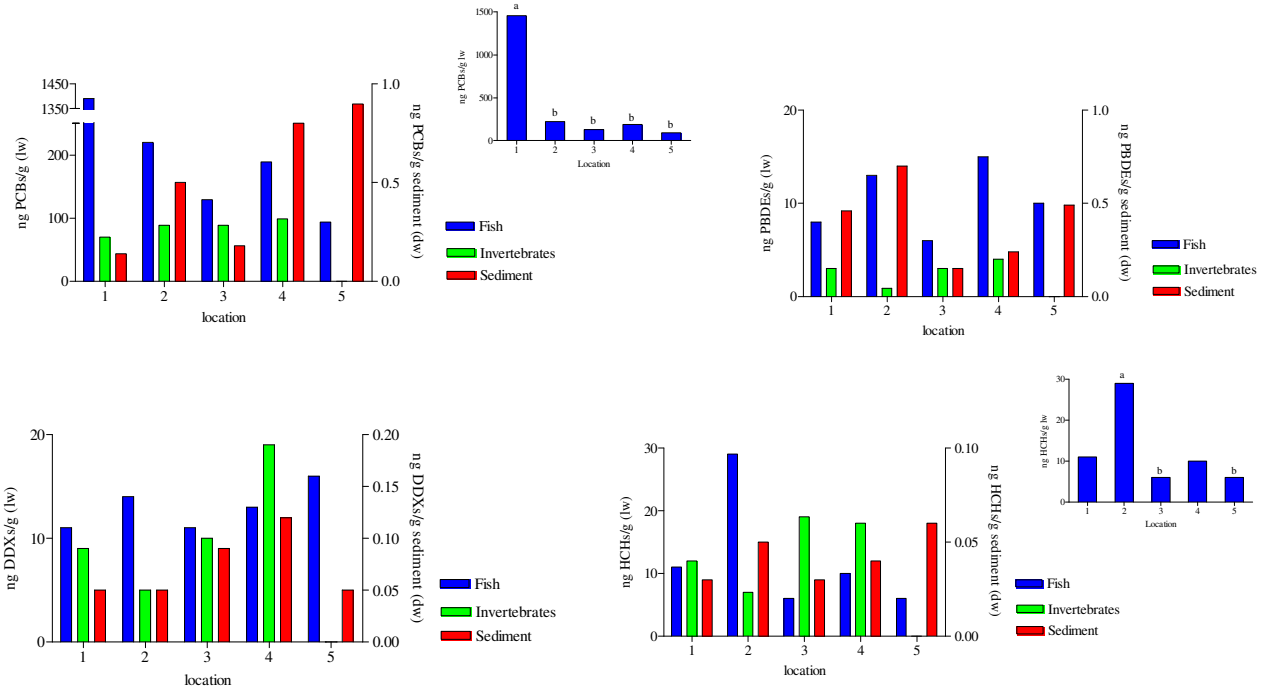

Fig. 2: Mean ∑PCBs, ∑PBDEs, ∑DDXs, ∑HCHs concentrations in sediment, fish and invertebrate species for each location. On the left Y-axis, concentrations in fish and invertebrates are plotted (ng/g lw), the right Y-axis represents sediment concentrations (ng/g dw). Significant differences between locations are presented.

Acknowledgements:

The expedition was organised by a consortium made up of the Royal Museum for Central Africa (coordinator), the University of Kisangani, the Royal Belgian Institute of Natural Sciences and the National Botanic Garden of Belgium with the financial support of the Belgian Development Cooperation, the Belgian Science Policy and the National Lottery. Adrian Covaci is financially supported by a postdoctoral fellowship from the Research Scientific Foundation – Flanders (FWO).

References:

1. Sanusi A, Millet M, Mirabel P, Worthham H. (2000); *Science of the Total Environ.* 263: 263-77
2. Weijs L, Van Elk C, Das K, Covaci A, Blust R. (2010); *Science of the Total Environ.* 409: 228-37
3. UNDP, Ministère de l'Environnement Conservation de la Nature et Tourisme. (2009); *Report*. 279 pp.
4. Dupré B, Gaillardet J, Rousseau D, Allègre C. J. (1996); *Geochimica et Cosmochimica Acta*. 60(8): 1301-12
5. Chu SG, Covaci A, Haraguchi K, Schepens P. (2002); *Analyst*. 127: 1621-26
6. Covaci A, Ryan JJ, *Schepens* P. (2002). *Chemosphere*. 47: 207-17
7. Covaci A, Losada S, Roosens L, Vetter W, Santos FJ, Neels H, Storelli A, Storelli MM. (2008); *Environ Sci Technol*. 42: 8657-60
8. ATSDR. (2010)