PCDD/F AND DIOXIN-LIKE PCB ACTIVITIES IN SEDIMENTS FROM THE MTONI ESTUARY (TANZANIA) AS ANALYZED WITH THE CHEMICALLY ACTIVATED LUCIFERASE GENE EXPRESSION (CALUX) BIOASSAY

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Introduction

Polychlorinated dibenzo-*p*-dioxins, -furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) are persistent organic pollutants that are ubiquitous environmental contaminants and still very widespread in this day and age.

Little is known on PCDD/F and dioxin-like PCB sediment levels in the African continent, where the main focus has historically been more oriented toward pesticides as part of the 12 Persistent organic Pollutants (POPs). Few exceptions are South Africa where both chemo- and bio-analytical data are available[1]. For Tanzania as a whole, only selected studies have been carried out mentioning dioxin levels in egg[2] and PCB levels in sediments[3]. Since the Stockholm Convention ratification by the United Republic of Tanzania in 2004, POP inventories have been/are being assembled[4], focusing on dioxin releases and possible source categories (uncontrolled combustion source, medical waste incineration, power generation and heating). Dioxin levels in estuarine sediment have not been reported in Tanzania, to our knowledge, and can provide a valuable indicator for locating point sources. The CALUX bioassay can aid as an important tool (quick and cost-effective) to provide biologically relevant data[5] on PCDD/F and dioxin-like PCB levels.

Materials and methods
Sampling location
Sediment samples were collected in the Mtoni estuary (Figure 1), located 3 km south of Dar es Salaam, during low tide. Samples (E1-E7) were taken from the mixing zone in the Mtoni estuary, including samples from the Mzinga River, the Kizinga River and their confluence in 2 different campaigns (wet and dry seasons). The Kizinga River drains urbanized areas of approx. 700,000 inhabitants whereas the Mzinga River drains more rural areas (approx. 110,000 inhabitants). Both rivers are suspected to carry waste from industrial and residential areas[6]. Important to consider is that a textile factory and a solid waste dumping site are located near the rivers, respectively near the Kizinga river and in between the rivers. For this reason, additional end-member samples (F1-F8) were taken upstream (fresh water) of both rivers and at the mouth of the estuary (marine water).

Sampling was performed according to EPA standards[7], using a hand corer (30 cm height and 6 cm internal diameter). Cores were cut into three intervals (0-3, 3-6 and 6-9 cm deep), stored at -20°C and a total of 50 samples were transported for analysis.

Extraction and clean-up
Lyophilized sediment (5g) was extracted in an Accelerated Solvent Extractor, ASE® (Dionex, USA) with a toluene:methanol (4:1) mixture[8] and 33mL extraction cells (125°C oven temperature, 1500 psi, 10min. static time, 6min. oven heating time, 60s purge at 60% of extraction cell volume, 2 static cycles per sample).

The clean-up and fractionation processes were based on the EPA Method 4435[9] and have been described elsewhere[10]. The sample passes through an acid silica gel column (33% w/w H_2SO_4 on silica gel), an activated copper column and finally through a 1% X-CARB column (all in piggybacked setup). The dl-PCBs and PCDD/F fraction are differentially eluted from the X-CARB column.
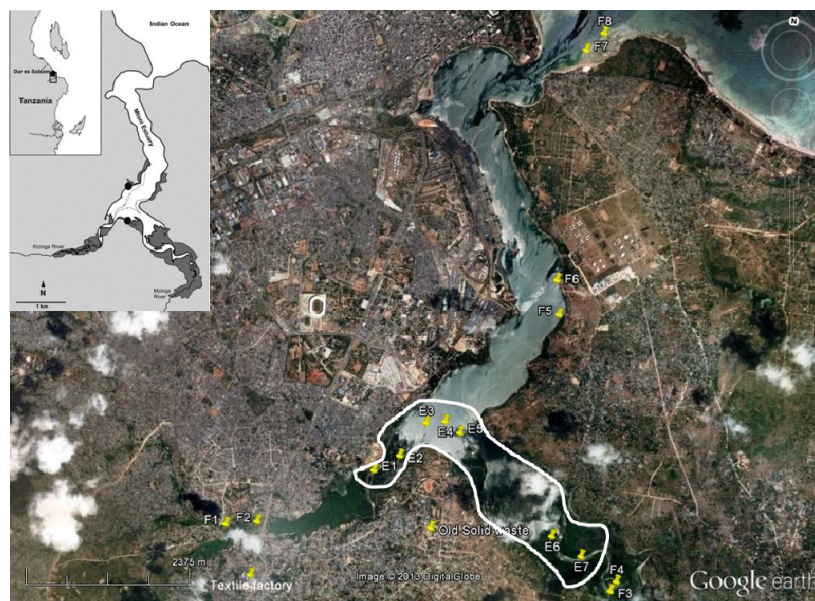
Figure 1: Sampling points in the Mtoni estuary, Dar es Salaam: E1 and E2 in the Kizinga River, E3-5 at the confluence and E6, E7 in the Mzinga River. Stations F1-2 in the Kizinga River, F3-4 in the Mzinga River, F5-6 at the Navy shore and F7-8 at Kigamboni Seaway are additional sampling points. White line delimits the estuarine mixing zone. The solid waste dumping site and textile factory are also indicated.

CALUX analysis

CALUX analyses were carried out using the new mouse hepatoma cell line (H1L7.5c1) as described elsewhere[10]. Briefly, cells were maintained in alpha minimal essential medium (α-MEM) supplemented with 10% (v/v) foetal bovine serum (FBS) and seeded in 96-well plates. After 24-hour incubation and cells reaching a monolayer, sample extracts were dosed in triplicate along with a TCDD-standard curve. PCDD/F fractions were subjected to a 9-point serial dilution curve, whereas dl-PCBs made up a 4-point dilution curve. Cells were again incubated over a 24-hour period after which lysis and measurement were performed using Luciferase assay substrate and a Glomax 96-well plate reader (both from Promega, The Netherlands). Data analysis was performed in Excel where statistical analysis and BEQ quantification[11] involved fitting the 4-parameter Hill equation or the newer Box-Cox and Slope ratio method[11] (Excel Solver add-in enabled). This last method uses Box-Cox regression[11] to linearize part of the response curve; the equation's slope can then be used, much like the Hill EC_{50}, to generate a BEQ by taking the ratio of the sample slope over that of TCDD.

Results and discussion

The CALUX H1L7.5c1 bioassay

This latest, 3rd generation mouse hepatoma cell line has improved sensitivity[12] towards PCDD/Fs and dioxin-like PCBs and was most suited for the task. The importance of full dose curves is demonstrated (see Figure 2A) as the additivity principle is not upheld despite (1) using sulphuric acid to remove acid labile interferences and (2) the separation of PCDD/Fs and dioxin-like PCBs to counteract known antagonistic effects.

Quality Control

DMSO solvent controls showed no increase in response compared to fitted background values (p= 0.45 for a two-tailed Student T-test), whereas media control (p= 0.06 for a two-tailed Student T-test) was borderline for a significant difference. Procedural blank samples (n=6) spiked with an in-house Quality Control solution (0.250pg TCDD/μL DMSO) ranged in recoveries from 85-120% for the PCB fraction and 91-115% for the PCDD/F fraction.
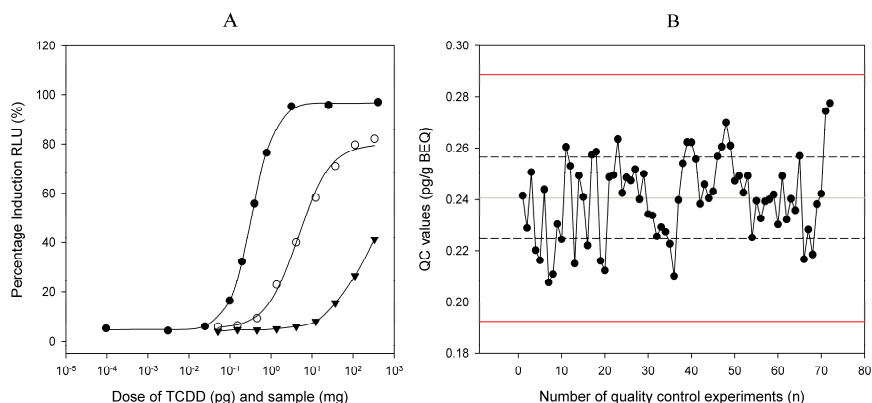
Figure 2: (A) Typical TCDD-standard curve (●), sample with full dose response (○) and sample not attaining an upper plateau (▼) using Hill regression. (B) Scatter chart of QC analyses during the study where dotted lines indicate mean ± 1SD and full (red) lines indicate mean ± 3SD.

In addition, a total of 72 QC experiments were conducted in this study (see Figure 2B) for reproducibility assessment. A mean of 0.24 ± 0.02 pg TCDD/µL DMSO was found with a CV (coefficient of variation) at 7.2%. The LOD of the H1L7.5c1 bioassay was set at 0.05 pg TCDD/well or 0.22 pg TCDD/g sediment (IUPAC definition of LOD based on fitted background levels and taking into account the starting volume and serial dilution applied to the sample).

Bio Analytical Equivalents (BEQs) in Mtoni Sediment Samples

CALUX-BEQ values of PCDD/Fs in Mtoni estuary sediments were assessed using three methods: the Hill regression equation, Box-Cox transformation (both providing an EC50 in pg/g BEQ) as well as the slope ratio method (providing a single BEQ value). A detailed description of the methods can be obtained elsewhere[11]. The relationships between these methods indicate that they correlate well (r^2 of 0.7909 between Slope ratio and Hill, 0.9452 between Box-Cox and Hill and 0.8710 between Box-Cox and Slope ratio), implying that either of these methods will provide reliable results in this study. Model precision for BEQ determination based on CVs ranged from 6.7-17.4% (Hill model), 5.2-14.3% (Box-Cox transformation) and 5.5-15.5% (Slope ratio method).
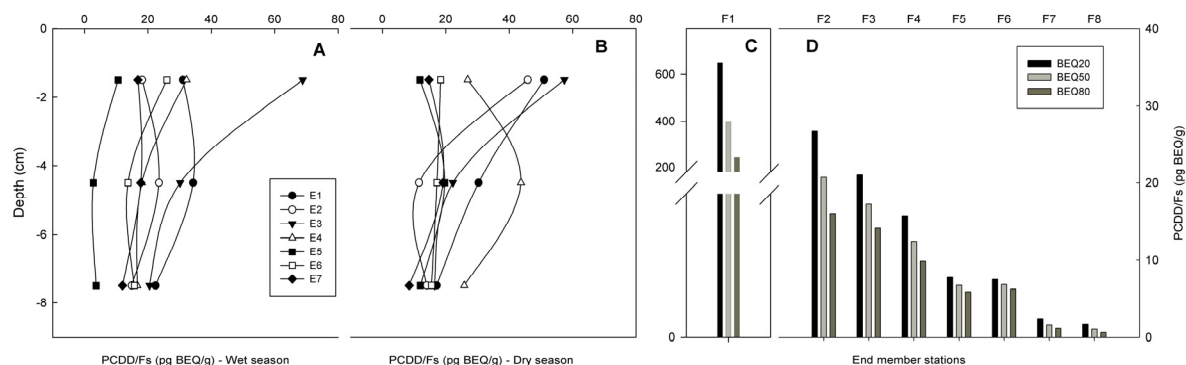

Figure 3: Mtoni BEQ depth-profiles (A, B) and end-member data (C, D) at BEQ 20-50-80.

High levels of PCDD/Fs in both seasons (see Figure 3A and 3B) were found in the Kizinga River (E1-E2) and in confluence stations close to it (E3-E5). Wet season samples contained less PCDD/Fs than dry season samples in the Kizinga River, whereas in the Mzinga stations (E6-E7) it was the opposite. Higher PCDD/F levels at confluence station E3 in both seasons could be due to a local emission source in proximity to the Kizinga River. This is confirmed by the subsequent higher levels at the nearby station E4 in both seasons and lower levels at E5,

a station closer to the Mzinga River. No significant difference (t = 0.55 two tailed; p = 0.59) in PCDD/F levels was observed between the two seasons (n = 42). Dioxin-like PCB levels were generally low (1 to 2 orders of magnitude in BEQ; data not shown) compared to the PCDD/F levels.

The Kizinga River drains peri-urban environments, which are highly populated and have many small-scale to medium-scale industries. Oppositely, the Mzinga River drains rural environments that are less populated and have very few industries. As a result of various socio-economic activities, various materials (household scraps, plastics, vehicle tires and electronic wastes) are abundantly present in most domestic and industrial wastes. It was observed[13] that waste burning, biomass burning (wood and charcoal) and traffic-related emissions (leaded gasoline exhausts and spills, tire wear) are the major sources of particulate matter in the Dar es Salaam atmosphere. The detected PCDD/Fs and dl-PCBs levels in the estuary can thus reasonably be associated with the open burning of waste, biomass and traffic.

The presence of sources in the vicinity of the sampling stations must be taken into account. For example, upstream station E1 in the Kizinga River is downstream to an untreated wastewater discharge point of a textile factory. A third sampling campaign (for end-members) was organized to verify the higher PCDD/F (Figure 3C and 3D) and dl-PCB levels ($\leq$1 pg BEQ/g) in the Kizinga River and to assess the change of the dioxin levels downstream. Station F1, in the vicinity of a sewage factory pipe and near unauthorized human settlements, shows a high PCDD/F level of 400 pg-BEQ/g. Another upstream Kizinga River station (F2), with 21 pg-BEQ/g PCDD/Fs, is similar to the levels in the upstream Mzinga River stations. A negative concentration gradient of PCDD/Fs and dl-PCBs in sediments is observed in the downstream direction. The levels at the mouth of the Mtoni estuary are very low: 1 to 2 pg-BEQ/g for PCDD/Fs and below LOD for dl-PCBs. These additional results confirm the previous conclusions that the impact of dioxins on the Mtoni estuary is higher in the Kizinga River than in the Mzinga River and that a dilution effect is clearly noticeable in the downstream direction.

References

1. Nieuwoudt C, Quinn LP, Pieters R, Jordaan I, Visser M, Kylin H, Borgen AR, Giesy JP, Bouwman H. (2009); *Chemosphere* 76(6): 774–83.
2. AGENDA, IPEN and Arnika (2005), Contamination of chicken eggs near the Vikuge obsolete pesticides stockpile in Tanzania by dioxins, PCBs and hexachlorobenzene.
3. Kruitwagen G, Pratap HB, Covaci A, Wendelaar Bonga SE. (2008); *Marine pollution bulletin* 56(5): 1022–31.
4. NIP (2005), The United Republic of Tanzania, National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants
5. Goeyens L, Hoogenboom R, Eppe G, Malagocki P, Vanderperren H, Scippo ML, Windal I, Baeyens W, Denison MS, Hädrich J. (2010); *Organohalogen Compounds* 72: 964-967
6. Taylor M, Ravilious C, Green EP. (2003); Mangroves of East Africa - UNEP (p. 28).
7. USEPA (2001); Technical Manual, (EPA-823-B-01-002 October 2001), 208.
8. Baston D.S. and Denison M.S. (2011) *Talanta* 83: 1415-1421
9. USEPA (2008): *http://www.epa.gov/osw/hazard/testmethods/pdfs/4435.pdf*
10. Van Langenhove K, Keupers I, Croes K, Vandermarken T, Denison MS, Baston DS, Elskens M, Baeyens W. (2011); *Organohalogen Compounds* 73: 2132-2135
11. Elskens M, Baston DS, Stumpf C, Haedrich J, Keupers I, Croes K, Denison MS, Baeyens W, Goeyens L. (2011); *Talanta* 85: 1966-1973
12. Van Langenhove K, Croes K, Denison MS, Baston DS, Elskens M, Baeyens W. (2010); *Organohalogen Compounds* 72: 1092-1096
13. Mkoma, S. (2008); Physico-Chemical Characterization of Atmospheric Aerosols in Tanzania, with Emphasis on the Carbonaceous Aerosol Components and on Chemical Mass Closure. Retrieved from https://biblio.ugent.be/publication/470145/file/1880861.pdf