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‘The whole of science is nothing more than a refinement of everyday thinking’

Albert Einstein (1879 – 1955)

To

My

son Elvis

and

daughter Elicia

ABSTRACT

In order to describe and evaluate the past and current status, and predict the future status of mangrove ecosystem in relation to changing human activities in the coastal areas and its environment in Tanzania, mangrove sediments from the Mtoni estuary were analysed for elemental (C, N) and isotopic ($\delta^{13}C$, $\delta^{15}N$) signatures to establish the role of anthropogenic influence on the contribution of organic carbon and nitrogen in the tropical mangrove sediments. Moreover, metals, PCDD/Fs and dioxin-like PCBs were analysed to describe their current sources and fates in response to anthropogenic and natural changes. Lastly, the study intended to determine and compare the variability in the distribution of potential micro-pollutants in the mangrove sediments of Tanzanian coast.

Sandy particles dominated the mangrove sediments, with less than 6% of organic matter (OM) in the estuary and less than 9% in the tributaries. Similarities in the levels of total organic carbon (TOC) and total nitrogen (TN) with depth were observed at all stations, indicating a homogenous, well-mixed top 9-cm sediment layer. A clear gradient of TOC and total nitrogen (TN) levels from the riverine (Kizinga and Mzinga) stations to the estuarine mouth was observed with highest content in most upstream stations and decreasing towards the mouth of the estuary. No clear seasonal trend of C/N ratio was observed except in the confluence region where the ratios were higher in wet than dry season. Wet season $\delta^{15}N$ values were lower while $\delta^{13}C$ values were higher compared to dry season values for all the stations. Mzinga $\delta^{15}N$ values were higher than Kizinga values in both seasons while the opposite was observed for $\delta^{13}C$, but all were lower than the confluence values. Correlations of the geochemical parameters for both wet and dry seasons suggest that OM in Mtoni sediments originated mainly from sources that are ^{15}N enriched and ^{13}C depleted and are linked to OM degradation processes. Combined elemental and isotopic signatures indicated that wet season values were dominated by sewage material while dry season values were dominated by mangrove material. Quantitative estimation of the contribution of the identified sources indicated that sewage material contributed between 60% and 90% of the sedimentary OM in the wet season and mangrove litter between 44% and 98% in the dry season.

Metal levels in the Mtoni sediment layers (0-3, 3-6, and 6-9 cm) showed neither distinct depth gradient nor variation between wet and dry seasons. A clear gradient from the upstream of the Kizinga River to the estuarine mouth was observed for all metals, except As. This was also observed from upstream of the Mzinga River to the estuarine mouth. Pearson correlation matrix and Principal component analysis (PCA) indicated that the selected compounds could be separated in 3 groups: (1) Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb; (2) Cd and TOC, which were strongly anti-correlated and (3) Sr. Enrichment factors (EF) indicated that Sr was most probably derived from natural origin, while other metals in the Mtoni estuary originated from anthropogenic activities. The levels of anthropogenic metals are indicative of human induced environmental change and have repercussions on the future status of the estuary and of the coastal area of Tanzania as a whole.

PCDD/Fs values as analysed by chemically activated luciferase gene expression (CALUX) method and expressed as bioanalytical equivalency (BEQ) values ranged from 5.7 ± 1.4 to 39.9 ± 5.8 pg BEQ/g sediment in wet season and from 14.1 ± 2.0 to 32.8 ± 4.7 pg BEQ/g sediment in the dry season. High levels were observed in Kizinga River and stations close to the mouth of that river. Dioxin-like PCB (dl-PCB) levels ranged from 0.21 ± 0.03 to 0.53 ± 0.03 pg BEQ/g sediment in wet season and from 0.22 ± 0.03 to 0.59 ± 0.04 pg BEQ/g sediment in the dry season. Higher PCDD/F and dl-PCB levels in sediments are probably related to open burning of plastic scraps, household burning of wood or charcoal and traffic related emissions, which all occur in the Dar es Salaam region. The denser population and the more intense industrial activities in the Kizinga River Basin may explain the enhanced PCDD/F and dl-PCB levels observed in the sediments of that River compared to those in the Mzinga River Basin. Enhanced levels in the Kizinga River (up to 400 pg-BEQ/g) observed in the third sampling campaign showed a clear decreasing concentration gradient in the downstream direction. The presence of these pollutants in the sediments poses a threat to the biological community living in the Mtoni estuary.

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ABBREVIATIONS AND ACRONYMS

BEQ	Bioanalytical Equivalency
C/N	Carbon to Nitrogen ratio
CALUX	Chemically Activated Luciferase Gene Expression
CALUX-BEQ	Chemically Activated Luciferase Gene Expression-Bioanalytical Equivalency
CV	Coefficient of Variation
dl-PCBs	Dioxin-like PCBs
DMSO	Dimethylsulfoxide
EC	Effective Concentration
EF	Enrichment Factor
EPA	Environmental Protection Agency (of the United States)
FBS	Foetal Bovine Serum
HTOC	High Temperature Oxidative Combustion
IAEA	International Atomic Energy Agency
MEM	Minimal Essential Medium (α-)
OM/POM	Organic Matter/Particulate Organic Matter
PCA	Principal Component Analysis
PCBs	Polychlorinated Biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDD/Fs	Polychlorinated dibenzo-*p*-dioxins-Polychlorinated dibenzofurans
PCDFs	Polychlorinated dibenzofurans
POPs	Persistent Organic Pollutants
QA	Quality Assurance
QC	Quality Control
RLU	Relative Light Units
TC	Total Carbon
TCDD	Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-)
TEF(s)	Toxicological Equivalent Factor(s)
TEQ	Toxicological Equivalency
TN	Total Nitrogen
TOC	Total Organic Carbon
UNESCO	United Nations Educational, Scientific and Cultural Organisation
XDS	Xenobiotic Diagnostics Systems

GENERAL INTRODUCTION

MANGROVE SEDIMENT AS A BIOGEOCHEMICAL INTEGRATOR OF MICRO-POLLUTANTS

CHAPTER ONE: MANGROVE SEDIMENT AS A BIOGEOCHEMICAL INTEGRATOR OF MICRO-POLLUTANTS

1.1 MANGROVE ECOSYSTEM AS A BIOGEOCHEMICAL ENVIRONMENT

Superimposed by natural variability, the contemporary period is characterised by an ever-increasing utilization of materials, energy and space that emanate from the growing human population, industrial activity and intensive agricultural activities. Anthropogenic (i.e., human) activities due to industrialisation, urbanisation, and population growth have resulted in formation and release of stressors (micro-pollutants), which have the potential to impact the physical (e.g., affecting a temperature change), biological (e.g., altering habitat consumption), and chemical (e.g., resulting in increased risk of cancer) environments of the marine coastal system. These substances are brought into the ecosystem either directly (through anthropogenic activities) or indirectly (through natural processes). Once in the environment they are transformed through physico-chemical, microbial and biological processes, during their transfer from their source into the marine ecosystem. As a result of severe increase of human impacts on aquatic ecosystems there has been an increasing flux of the chemicals in the marine environment.

Mangrove forests have shown to play an important role in the biogeochemistry of contaminants in tropical coastal areas (Tam and Wong, 1996; Tam and Wong, 2000; Janaki-Raman *et al.,* 2007). They can be considered as chemical reactors due to biochemical and physiological processes as well as their role in sediment chemical reactions that significantly affect the pollutant mobilities (Ramos de Silva *et al.,* 2006). The capacity of mangrove mud to accumulate discharged materials has made mangrove areas to become dumping sites for solid wastes and for sewage disposal (Defew *et al.,* 2005; Mtanga & Machiwa, 2007; Kamaruzzaman *et al.,* 2008). The high rates of sediment accretion and stabilised vegetated nature make the mangrove wetlands highly efficient recorders of environmental changes and, therefore, can be used to reconstruct coastal changes (Janaki-Raman *et al.,* 2007; Kamaruzzaman *et al.,* 2008; Raju *et al.,* 2010). Though the record can be altered by sediment mixing effects that eventually affect the preservation of physical sedimentary structures, this will depend on the rate of sediment accumulation as well as the nature, intensity and depth of mixing (Kamaruzzaman *et al.,* 2008). The elevated levels of micro-pollutants recorded in mangrove sediments can thus reflect the long-term pollution caused by human activities (Tam and Wong, 2000).

The micro-pollutants can therefore be considered as inputs (source) to marine sediment and the sediment as an output (sink) to these chemicals. At times, the system can be at equilibrium or rather operates under steady-state conditions, particularly when a mass balance between inputs and outputs has been achieved. However, the system may be perturbed, possibly by anthropogenic activities, and therefore change towards a new equilibrium state. Transportation and transformations processes within the reservoir will affect the temporal and spatial distribution of the micro-pollutants. Whereas the transport effects are dominated by the hydrodynamic regimes, transformations are affected by biological (microbial degradation, bioaccumulation and biomagnification), chemical (dechlorination, reduction and oxidation) and geological (sedimentation) processes. Therefore, the mangrove sediment as an entity can be considered as representing a biogeochemical environment.

1.2 MANGROVE SEDIMENTS AS INTEGRATORS OF POLLUTANTS

Mangrove fine sediments (or mud-clay) can act as repositories for contaminants coming from the atmosphere, water layer or brought by surface run-off. Mangrove sediments can bind to organic pollutants which adsorb to the extensive surfaces provided by the fine particulate sediments. On the other hand, metals can be trapped by forming complexes. Micro-pollutants may later become remobilised when there is change in conditions of the environment (Vicente-Beckett *et al.,* 2006). Consequently, filtered pollutants in water will accumulate in estuarine and mangrove sediments (Kruitwagen *et al.,* 2008). The pollutants integrated in mangrove sediments include, among others, organic matter, metals, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) as detailed below.

1.2.1 Stable Isotopes and Isotopic Composition in Organic Matter

In natural conditions, most elements have more than one stable isotope. Stable isotopes which are useful in biogeochemical research have (i) a large mass difference between the rare and the abundant isotope, (ii) a low atomic mass leading to large mass differences, (iii) the rare isotope that represents a small fraction of the total elemental occurrence (e.g. approximately 98.89% of all carbon is ^{12}C, and only 1% is ^{13}C; Boutton, 1996), and (iv) more than one oxidation state (McSween *et al.,* 2003; Sulzman, 2007; White, 2007). However, the relatively homogeneous distributions of isotopes of naturally occurring elements in the Earth's crust ensure only little variations of the elements isotopic composition (Emerson and Hedges, 2008).

It is of general knowledge that outer shell electrons of an atom control chemical reactions; thus, the chemical behaviour of the various isotopes of an element is qualitatively similar (Sulzman, 2007; Emerson and Hedges, 2008). While possessing the same fundamental chemical properties, the different isotopes of an element differ in the density and rates of diffusion and evaporation which are the direct consequences of their atomic mass (Jardine, *et al.,* 2003; Bickert, 2006). As a result of the difference in atomic mass, there is variation in the rate of reaction as well as isotopic bond strength (Sulzman, 2007; Emerson and Hedges, 2008; Hoefs, 2009). In that way, heavier isotopes of an element typically form slightly stronger bonds to other atoms, and molecules containing heavier isotopes move somewhat more slowly at a given temperature owing to their greater mass (Emerson and Hedges, 2008).

Variations in physical and chemical properties arising from differences in atomic mass of a chemical element are a consequence of unequal stable isotope composition within different materials and are linked to isotope fractionation (Hoefs, 2009). This phenomenon can be explained by the fact that kinetic energy is constant for a given element in fixed environmental surroundings (Sulzman, 2007; Hoefs, 2009). Isotopic composition in organic matter can be expressed using a fractionation factor (α) and a delta value (δ) as explained below.

1.2.1.1 Fractionation Factor (α)

In biogeochemistry, the equilibrium constant, *K,* for isotope exchange reactions is often replaced by the fractionation factor, α, (White, 2007; Hoefs, 2009). The process of isotope fractionation is mathematically described by comparing the isotope ratios of the two compounds in chemical equilibrium (X↔Y) or of the compounds before and after a physical or chemical transition process (X→Y). The isotope fractionation factor, α_{x-y}, is then defined as the ratio of the two isotope ratios (IAEA-UNESCO, 2000):

$$\alpha_{X-Y} = \frac{R_X}{R_Y}$$

where R = isotope ratio = abundance rare isotope/abundance abundant isotope.

If the isotopes are randomly distributed over all possible positions in the compounds X and Y, then α is related to the number of atoms exchanged, n, by:

$$\alpha = K^{1/n}$$

where K is the equilibrium constant.

Recently, it has been a common practice to replace the fractionation factor by the separation factor, ε, (which is defined as $\varepsilon = \alpha - 1$), because $\varepsilon \times 1{,}000$ approximates the fractionation in parts per thousand, similar to the delta, δ, value (Hoefs, 2009) as shown in the next subsection.

1.2.1.2 The Delta (δ) Value and Isotopic Ratio Expression

For a chemical compound, whose isotopic composition has been determined by conventional mass spectrometry, the isotopic composition is expressed in terms of δ values (Sulzman, 2007; Hoefs, 2009). Since the isotopic differences between various materials are actually very small, isotopic compositions are reported as the relative deviation from the isotope ratio of a standard reference material, and the results are expressed in parts per thousand, ‰ (Emerson and Hedges, 2008), with units given in per mil (Sulzman, 2007; White, 2007). In general terms, it is convenient to write:

$$\delta(\mathrm{y}) = \left(\frac{R_{sample}}{R_{std}} - 1 \right) x 10^3$$

where y = heavy isotope of an element (e.g. ^{13}C, ^{15}N), and R = the ratio of the heavy isotope (e.g. ^{13}C, ^{15}N) over the light isotope (e.g. ^{12}C, ^{14}N) in the sample (R_{sample}) and standard (R_{std}).

For expressing the δ^{13}C of carbon compounds, the standard reference material is Pee Dee Belemnite. For the δ^{15}N of nitrogen compounds, the standard reference is atmospheric N_2 gas.

1.2.1.3 Carbon and Nitrogen and their Stable Isotopes as Environmental Tracers

Organic matter in a given marine ecosystem can be supplied from both *in situ* primary producers (autochthonous sources) and external sources of organic material (allochthonous sources) (Graham, *et al.,* 2001; Yu *et al.*, 2010). The autochthonous sources involve primary production by phytoplankton, microphytobenthos and higher plants as well as chemoautotrophic production. On the other hand, allochthonous sources include marine and riverine inputs as well as direct domestic, industrial and runoff inputs from the terrestrial environment (Middlelburg and Nieuwenhuize, 1998).

Intertidal mangrove ecosystems are an important interface for the carbon cycle in tropical coastal environments and are considered to have significant impact on global carbon cycling due to their productivity in the terrestrial ecosystem (Marchand, *et al.,* 2008). They can transfer organic carbon to nearby environments in the form of litter, particulate or dissolved organic matter (Bouillon *et al.,* 2003) and they have the ability to accumulate and store large amount of organic matter (Bouillon *et al.,* 2007) in their sediments.

The amount and origin of organic matter will depend on biological (consumption, removal), chemical (degradation) and physical (tidal amplitude) factors acting on mangrove sediments (Bouillon *et al.,* 2003). In addition to the natural organic matter sources, human inputs, that involve large volumes of poorly defined solid and human wastes which are sometimes discharged directly or after little treatment into the area, significantly contribute to the sediment and water chemistry of the mangrove ecosystem (Grace *et al.,* 2008).

Sources of organic matter to mangrove sediments have been evaluated using their molar organic carbon to nitrogen ratio (C/N) (Hu *et al.,* 2006; Marchand *et al.,* 2008) and stable carbon and nitrogen isotopic signatures. Their use as source indicators relies on the fact that different organic matter sources have different signatures (Marchand *et al.,* 2008). The use of C/N ratio and stable carbon and nitrogen isotopes as environmental tracers is briefly presented below.

1.2.1.3.1 Organic Carbon to Nitrogen Ratio as a Tracer

Changes in the C/N ratio in sediments have been used as a palaeoenvironmental proxy for the source of organic matter within sediments (Ramaswamy *et al.,* 2008; Yu *et al.,* 2010). The use of the ratio relies on the ability to distinguish and account for the varying sources of organic matter being transported into a mixed water system such as a mangrove estuary. Fluctuations in this ratio over time represent a shift in the sediment source at a given location (Woods, 2009). Specifically, C/N ratio has been used to describe the fractional contributions of different end-members contributing the organic matter or organic carbon in the environment (Gonneea *et al.,* 2004; Giani *et al.,* 2009; Barros *et al.,* 2010).

1.2.1.3.2 Stable Carbon and Nitrogen Isotopes as Tracers of Origin and Fate of Organic Matter

Stable isotope analysis is widely used in environmental, biogeochemical and organic geochemical studies to trace the dominant sources and fate of carbon and nitrogen in the changing environment (Zhang *et al.,* 2007) and for identifying the sources of the elements accumulating in sediments (Cloern *et al.,* 2002; Jardine *et al.,* 2003; Emerson and Hedges, 2008). Stable isotopes have been used to determine the presence and/or effects of allochtonous materials and the origin and destination of organic matter in marine environment (Salazar-Hermoso, 2007). This relies on their ability to record both source (equilibration) and process (fractionation) information (Emerson and Hedges, 2008). Since the stable isotopic signatures can persist over geologic time even through severe changes in chemical composition (Emerson and Hedges, 2008), it is possible to study the incorporation of different carbon sources into food webs on the condition that there is a sufficiently large difference in the isotopic composition of the different primary carbon sources such as terrestrial material, phytoplankton and benthic microalgae (Bouillon *et al.,* 2002).

Stable isotopes have been potential discriminators of organic matter source and fate in marine sediment (Ramaswamy *et al.,* 2008; Machiwa, 2010). They are used particularly to: (i) identify origins (e.g., terrestrial or marine pollutant), (ii) infer processes (e.g., heterotrophic nitrification), (iii) estimate rates (e.g., soil carbon turnover) and (iv) constrain, confirm or reject derived models (Sulzman, 2007). Carbon and nitrogen stable isotopes can discriminate terrestrial and marine sources, thereby identifying the origin of organic matter (Middlelburg and Nieuwenhuize, 1998; Ramaswamy *et al.,* 2008; Sampaio *et al.,* 2010b) by considering the differences that exist between naturally abundant isotopes in terrestrial and marine organic matter (Sampaio *et al.,* 2010a). This is because (i) consumers fractionate carbon and nitrogen in predictable ways that allow for the identification of the isotopic composition of their food resource(s), (ii) relative contributions of some primary producers can be differentiated by the variation in the carbon isotopic composition of plants as a result of differences in growth environment and the biochemical pathways of photosynthesis and biosynthesis (Cloern *et al.,* 2002; Machiwa, 2010) and (iii) isotopic ratios are conservative, hence the mixing of different sources will determine origin and distributions of organic matter in natural systems (Cifuentes *et al.,* 1988). Stable isotope compositions analyses are therefore valued as tracers of important biogeochemical processes due to the fact that compositions integrate the cumulative results of various ongoing processes such as the transfer of organic elements in trophic levels, climate change and marine productivity (Zhang *et al.,* 2007; Emerson and Hedges, 2008).

1.2.1.4 Analytical Methods used to determine bulk Elemental and Stable Isotopes in Sedimentary Organic matter

Total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) can be analysed in the sediment solid phase using a CHN elemental analyser. Organic matter in a complex sample is quantitatively converted to CO_2 N_2, SO_2, and H_2O by high temperature oxidative combustion at >900°C in an O_2 atmosphere. The gases are then isolated and purified by cryogenic distillation or gas chromatography. Since all substances carry a unique signature (proportion) of given variable forms, their identification by isotope ratio mass spectrometry (IRMS) in the samples is possible (Jardine *et al.,* 2003).

Isotopic composition of the gases are determined by simultaneous collection of masses in the IRMS (Boutton, 1996; Sulzman, 2007), e.g. for CO_2, 44 ($^{12}C^{16}O^{16}O$), 45 ($^{13}C^{16}O^{16}O$), and 46 ($^{12}C^{18}O^{16}O$). The isotopic composition of the gases derived from a sample is compared to that derived from the standard with known $^{13}C/^{12}C$ ratio for carbon and $^{15}N/^{14}N$ for nitrogen (Boutton, 1996). An automated preparation with low costs per sample and a large sample throughput offers a great advantage of this technique (Hoefs, 2009). Since the heavier isotope has less interaction with the stationary phase in the GC column, it will be eluted first allowing each peak to be integrated well for correct isotopic ratio. For analytical reasons, a working reference standard is analysed roughly after every certain number of samples in the same manner as the samples (Sulzman, 2007).

1.2.2 Metals in Mangrove Sediments

Metal elements in sediments exist in different chemical forms as free ions, dissolved species ans as inorganic and organic complexes in the dissolved phase (Spencer and MacLeod, 2002; Du Laing *et al.,* 2009), and as carbonates, silicates, sulphide, Fe-Mn oxyhydroxides and organic matter in the solid phase. They may enter the estuarine environment via a number of pathways (Spencer and MacLeod, 2002) such as natural processes (including erosion of ore-bearing rocks, wind-blown dust, volcanic activity and forest fires) and processes derived from anthropogenic activities (Dell'Anno *et al.,* 2003; Chatterjee *et al.,* 2007; Tranchina *et al.,* 2008). Anthropogenic sources in mangrove ecosystems arise from industrial effluents and wastes, urban runoff, sewage treatment plants, runoff from agricultural field and domestic garbage dumps (MacFarlane and Bruchett, 1999, 2001; Dell'Anno *et al.,* 2003; Chatterjee *et al.,* 2007; Tranchina *et al.,* 2008). In addition, discarded automobiles and dumping metallic substances have been the common anthropogenic inputs of metals (Kamau, 2002; Praveena *et al.,* 2010) that find their way into the marine ecosystem.

Metals are essentially a permanent addition to the aquatic environment as they cannot be chemically degraded, and are not subject to biological degradation. As a consequence, they get accumulated locally (MacFarlane and Bruchett, 2001; Defew *et al.,* 2005) and/or transported over long distances (Marchand *et al.,* 2006). Metals are trapped by the sediments as a result of sedimentation of suspended particles. Both natural (e.g. bioturbation, tides, erosion, etc) and anthropogenic (e.g. land pollution, dredging, etc) processes and activities tend to act on metals in the sediments (Woods, 2009), but the major procecess controlling the distribution of the metals in the sediments is early diagenesis, mainly oxidation of organic matter. In aerobic environments, oxygen reduction is the main process, but in anaerobic conditions, nitrates, oxides and hydroxides of Mn and Fe and sulphates consecutively become the electron acceptors in the oxidation process. With the exception of sulphate reduction that precipitates the metals as sulphides; other diagenetic reactions release the metals in the sediments. Metals are released from particulate organic matter (POM) after degradation of POM and from Fe and Mn oxy-hydroxides after reduction and dissolution of these minerals.

Marine environment is the ultimate destination of virtually all substances from areas affected by pollution (Raju *et al.,* 2010). Since metals are toxic, persistent, and non-degradable in the environment, the contamination of sediments by the metals represent the greatest ecological risk to coastal marine environment even at very small concentrations (Dell'Anno *et al.,* 2003) especially to the human population that relies on marine resources (Nobi *et al.,* 2010).This has several effects to the biological community, including diseases in plant and animal species, local or complete extinction of some species and loss or modification of habitat (Raju *et al.,* 2010). A rank ordering of the toxicity of metals is: mercury (most toxic) > cadmium > copper > zinc > chromium > nickel > lead and arsenic (least toxic) (McLusky and Elliott, 2004). With such high concentrations of metals expected and detected in sediments, the bioavailability indices assumes considerable importance with respect to bioaccumulation within organisms living in the mangrove environment (Defew *et al.,* 2005).

Although metals are natural constituents of the earth's crust and are present in all ecosystems, their concentrations have been dramatically increased by human activities (Raju *et al.,* 2010). Increased population, urbanisation, industrial activities, urban agriculture as well as exploitation of natural resources have resulted to converting open spaces into residential, industrial and agricultural areas (De Wolf & Rashid, 2008). This has led to the occurrence of pollutants in the mangrove system due to uncontrolled disposal of untreated domestic and industrial wastes (De Wolf & Rashid, 2008) into the streams, valleys, rivers and finally into the marine ecosystem. Most of the anthropogenic pollution of the marine realm has been the outcome of these human activities (Raju *et al.,* 2010).

1.2.2.1 Sediments as Carriers of Metals in the Marine Ecosystem

Sediments are considered a suitable medium to study the contamination of aquatic environments (Sprovieri *et al.,* 2007) because they are metal carriers and the metals partition with the surrounding waters reflecting the quality of an aquatic system. Sediment samples have added advantages over other environmental samples when it comes to analysis of metals. Metals accumulate in sediments, particularly in organically rich sediments, thus are easily measured and much less susceptible to accidental contamination. Furthermore, sediments offer a degree of time integration, as they are much affected by sediment characteristics that vary in particle size and organic carbon content (Rainbow, 1995). However, measurements of metals in sediment provide an assessment of total metal present, not of that portion that is available for uptake and accumulation i.e. the fraction that is of ecotoxicological relevance (Rainbow, 1995).

Sediments can act as a source (MacFarlane & Burchet, 2000) and a long-term store for metals in the marine environment (Spencer and MacLeod, 2002) particularly when triggered by changes in abiotic conditions such as pH, redox potential and salinity. Geochemical sediment characteristics can infer the sources of pollution (Chatterjee *et al.,* 2007). Because of their large adsorption capabilities, sediments are the major arsenal for metals and can document various changes in contamination (Nobi *et al.,* 2010). In this way, profiles of pollutants in cores of marine sediment can record pollution due to pollutant stability and as a result of insignificant mobility after deposition, pollutants will leave imprints in the sediment (Chatterjee *et al.,* 2007). Sediments tend to integrate geochemical processes; hence the information from sediments can establish the long term behaviour of metals in marine environment reflecting the history and anthropogenic impact (Kamau, 2002; Janaki-Raman *et al.,* 2007; Alaoui *et al.,* 2010). Therefore, sediment core studies can be useful for ascertaining the impacts of anthropogenic and natural activities on the mangrove ecosystem (Chatterjee *et al.,* 2007). Hence, metal content found in the sediments may reflect a diversified set of natural processes, from erosion to early diagenesis, and anthropogenic influences (Alaoui *et al.,* 2010).

Mangrove sediments, besides being rich in organic matter and sulphide, they have a large proportion of fine particles, low pH, anaerobic as well as reducing nature (Tam and Yao, 1998; MacFarlane & Burchet, 2000; Tam and Wong, 2000; Defew *et al.,* 2005; Janaki-Raman *et al.,* 2007;

De Wolf & Rashid, 2008). Because of these, they can effectively trap and favour retention of water-borne metals (MacFarlane & Burchet, 2000; Defew *et al.,* 2005; De Wolf & Rashid, 2008). This is only possible through either (i) immobilising the metals in the anaerobic sediments by adsorption on ion exchange sites on sediment particle surfaces, (ii) incorporation into lattice structures of the clay particles, (iii) precipitation as insoluble sulphides (MacFarlane and Bruchett, 2001) or (iv) oxidation of sulphides between tides (Defew *et al.,* 2005), which further mobilises the once trapped metals to increase their availability (De Wolf & Rashid, 2008).

1.2.2.2 Monitoring Metal Pollution in Sediments

The absolute concentration of metals in marine sediments never indicates the degree of contamination coming from either natural or anthropogenic sources because of grain-size distribution and mineralogy (Tam and Yao, 1998). Anthropogenic disturbances can be evaluated without any complexity by comparing the sediment metal content of the study area with the world averages and the values of nearby areas. Various biogeochemical tools have used to compare and monitor metal pollution in location and depth as a way to differentiate between the natural variations from changes due to anthropogenic deposition of elemental concentrations. These tools include, among others, enrichment factor (EF), which is briefly discussed below.

1.2.2.2.1 Enrichment Factor and the Reference Metal

Enrichment factor (EF) is a useful indicator reflecting the status and the degree of environmental contamination (Feng *et al.,* 2004). EF is regarded as a convenient measure of geochemical trends and is calculated as:

$$EF = \frac{\left[X/M \right]_{Sample}}{\left[X/M \right]_{Crust}}$$

where X is the concentration of the metal studied and X/M is the ratio of the amount of studied metal to a reference metal. Whereas, EF>1 indicates magnification, more abundance that the average, absence of enrichment or depletion relative to the Earth’s crust is denoted by unity.

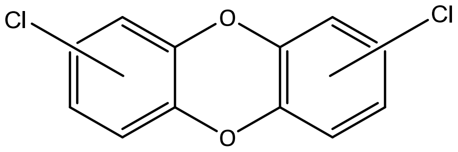
The EF calculation seeks to reduce the metal variability associated with variations in mud/sand ratios, and is a convenient tool for plotting geochemical trends on a large geographic domain with varied sediment particle sizes (Abrahim and Parker, 2008).

Normalisation of the measured amount of metal to a reference metal, such as Al and Fe, is a convenient method to differentiate whether the metal originate either from anthropogenic activities or lithogenic sources (Zhou *et al.,* 2007) as well as for the regional comparison. The normalisation elements tend to co-vary with grain size and as such their use can represent several underlying geochemical relationships (González-Macías *et al.,* 2006). In addition, the elements act as a proxy for the clay content (Abrahim and Parker, 2008). Due a linear relationship that exist between the normaliser concentrations and the fine particle-size fractions (silt+clay) of the samples in most sedimentary environments (Liu *et al.,* 2003), geochemical normalisation can compensate for both granulometric and mineralogical variability of metal concentrations in sediments (Aloupi and Angelidis, 2001). Thus, the use of normaliser as a substitute for the granulometric variability of the sediments is justifiable.

Iron can be used to normalise the metal data because (i) it is associated with fine solid surfaces and its distribution is not related to other metals and (ii) it is available in a relatively high and uniform natural concentration, and is therefore not expected to be substantially enriched from anthropogenic sources in estuarine sediments (Abrahim and Parker, 2008). However, it is a redox sensitive element and therefore behaves quite differently than most of the trace metals that are not redox sensitive. Aluminium can also be used as a normalisation element due to its consistence as being a major constituent of fine grained aluminosilicates in which the metals become associated. In addition, Al is highly refractory and anthropogenic influences on the metal are generally minimal (Rokade, 2009).

1.2.3 Dioxins and Dioxin-Like PCBs in Sediments

Polychlorinated dibenzo-*p*-dioxins (**1**) and polychlorinated dibenzofurans (**1**) are halogenated, tricyclic aromatic compounds, comprising of two benzene rings connected by one or two oxygen atoms (El-Kady *et al.,* 2007; Srogi, 2008; Gevao *et al.,* 2009). Both chemical groups have chlorine atoms that give rise to several isomers or congeners which can be differentiated by the position and total number of chlorine atoms present (Killops and Killops, 2005).



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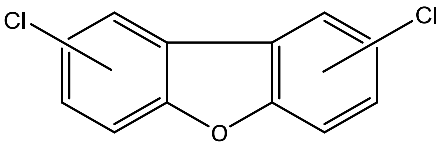


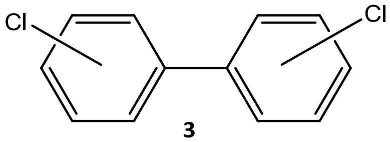
2

Hence, 75 possible PCDD and 135 possible PCDF congeners (Table 1.1) can be formed, where those having the same number of chlorine atoms form a homologue group (Smith and Lopipero, 2001; Srogi, 2008;). As the two chemical families (dibenzo-dioxins and dibenzofurans) are closely related in structure, they are commonly known as dioxins (Smith and Lopipero, 2001; Gevao *et al.,* 2009) and abbreviated as PCDD/Fs.

Table 1.1: Numbers of Possible PCDD and PCDFs Homologues and Congeners

Number of Halogen substitutions (Homologue)	**Number of Congeners**	
	Dibenzo-*p*-dioxins	**Dibenzofurans**
Mono-	2	4
Di-	10	16
Tri-	14	28
Tetra-	22	38
Penta-	14	28
Hexa-	10	16
Hepta-	2	4
Octa-	1	1
Nona-	-	-
Deca-	-	-
Total	**75**	**135**

Polychlorinated biphenyls, PCBs (**3**) are chemically similar in structures to the PCDD/Fs. They, however, lack oxygen atoms and are less structurally rigid (rotation possibilities of the C-C bond depending on the substitution pattern of both benzene rings) compared to PCDD/Fs.



3

Several chlorination patterns of the parent biphenyl exist and give rise to a total of 209 individual PCB congeners (Killops and Killops, 2005), as shown in Table 1.2.

Table 1.2: **Number of possible PCB congeners by degree of chlorination**

Degree of Chlorination (Homologue)	PCB formula	Number of congeners
Mono-	$C_{12}H_9Cl$	3
Di-	$C_{12}H_8Cl_2$	12
Tri-	$C_{12}H_7Cl_3$	24
Tetra-	$C_{12}H_6Cl_4$	42
Penta-	$C_{12}H_5Cl_5$	46
Hexa-	$C_{12}H_4Cl_6$	42
Hepta-	$C_{12}H_3Cl_7$	24
Octa-	$C_{12}H_2Cl_8$	12
Nona-	$C_{12}HCl_9$	3
Deca-	$C_{12}Cl_{10}$	1
Total		**209**

According to World Health Organisation (WHO), twelve PCBs that have 4 to 8 chlorine atoms, including four non-ortho (IUPAC Nos. 77, 81, 126 and 169) and eight mono-ortho (IUPAC Nos. 105, 114, 118, 123, 156, 157, 167 and 189) are conformationally similar to the PCDD/Fs. These PCB congeners elicit similar biochemical and toxic responses to dioxins and are therefore known as dioxin-like PCBs (Smith and Lopipero, 2001; Sanctorum *et al.,* 2007b; Okay *et al.,* 2009; Pan *et al.,* 2010). Due to their chemical stability, lipid solubility, and ubiquitous prevalence in the environment, PCDDs, PCDFs and PCBs are listed in the Stockholm Convention as Persistent Organic Pollutants (POPs) (El-Kady *et al.,* 2007; Shen *et al.,* 2008). The POPs list contains 12 initial compounds: 9 organochlorine pesticides and the PCDDs, PCDFs and PCBs as the remaining 3 compounds (each in fact represents a group of compounds). Within the dioxins (PCDD/Fs) group, 7 PCDDs and 10 PCDFs are considered toxic (Van den Berg *et al*. 2006). The PCB group contains 12 congeners that are in this category.

1.2.3.1 Sources and Use of PCDD/Fs

PCDD/Fs are present in the environment as unintended by-products of various technological processes and, as such, have never been produced commercially (Roots *et al.,* 2004). They may originate from natural combustion processes, such as bushfires and volcanoes (Birch *et al.,* 2007). Anthropogenically, they are produced during incomplete combustion processes (Pan *et al.,* 2010) when wastes containing chlorine and carbon, like polyvinyl chloride plastics, are incinerated (Manahan, 2000; Liu *et al.,* 2006; Birch *et al.,* 2007; De Wolf & Rashid, 2008; Terauchi *et al.,* 2009). The formation of dioxins in such incinerators takes place due to the presence of chlorine, carbon, oxygen as well as catalytic metals (Manahan, 2000).

From an industry point of view, PCDD/Fs can be formed during manufacture of various chlorinated chemicals (Müller *et al.,* 2002; Ryoo *et al.,* 2005; El-Kady *et al.,* 2007). In this specific case, dioxins are produced as by-products of chemical reactions (Pan *et al.,* 2010), e.g. when products such as wood preservatives and pesticides such as pentachlorophenol (PCP) are produced. Thus, elevated PCDD/F concentrations are typically associated with highly urbanised and industrialised areas (Müller *et al.,* 2002). It's these areas where combustion processes from waste incinerators, power plants and automobile exhausts (Zhang *et al.,* 2010), cement manufacture, cigarette smoke (Gevao *et al.,* 2009), municipal wastewater effluents (Moon *et al.,* 2009) as well as industrial processes, such as pulp bleaching and metal refining/melting are found (Bruckmeier, 1997).

1.2.3.2 Sources and Use of PCBs

PCBs were once produced worldwide as commercial chemicals (Koistinen *et al.,* 1997; Srogi, 2008). The first synthesis of PCBs was described in 1881 (Schmidt and Schultz, 1881), and from 1930 their industrial application started (Erickson, 1997). Since then, PCBs have been found in many industrial and consumer products (Liu *et al.,* 2006; Wang *et al.,* 2007) and widely used in electronic appliances and hydraulic fluids (Shen *et al.,* 2008; Yang *et al.,* 2009). Well-known sources of PCBs include the historical use and disposal of industrial PCB products and by-products of waste incineration (Pan *et al.,* 2010). PCBs can also be formed during the operations of electric arc furnaces and can occur in effluents from paper mills and the chloroalkali industry. Non-ortho PCBs, which do not originate solely from commercial PCB mixtures, can be formed during coal combustion and industrial waste incineration particularly when the combustion temperature is not sufficiently high for destruction, that is less than 800 °C (Chi *et al.,* 2007).

PCBs have been used as anti-corrosion materials, coolants and insulators in heat transfer systems such as transformers (Shen *et al.,* 2008; Srogi, 2008), and as capacitors in electrical industries (Pan *et al.,* 2010). Moreover, they have been used as plasticizers, hydraulic and dielectric fluids, fire retardants, lubricating oils (Bruckmeier, 1997) and additives in paints (Nhan *et al.,* 1999), pesticides and inks (Killops and Killops, 2005; Srogi, 2008; Fouial-Djebbar *et al.,* 2010). Commercial PCB mixtures were sold under the names of, among others, Aroclor, Fenclor, Phenochlor, Clophen and Kanechlor (Srogi, 2008). The high chemical stability (low flammability), high thermal stability, high electrical insulating properties (El-Kady *et al.,* 2007), high electrical resistance and low volatilities made the PCBs suitable for their intended commercial use (Killops and Killops, 2005).

During their manufacture and use, PCBs can be released into the atmosphere through smokestacks and weathering of asphalt and other substances as well as burning products containing these PCBs (Zhang *et al.,* 2010). They can also be released from leaking transformers containing PCBs, incineration of wastes, burning domestic garbage as well as improperly and illegally disposed industrial wastes (Wang *et al.,* 2007).

1.2.3.3 Chemodynamics of Dioxins and Dioxin-Like PCBs

When dioxins and dioxin-like pollutants are adsorbed onto soil, their predominant fate is to remain sequestered on the soil surface and be released into the water body through soil erosion. In the water column they primarily undergo sedimentation and burial, thereby providing their ultimate environmental sink in aquatic sediments (Smith and Lopipero, 2001). Dioxins and dioxin-like PCBs can enter the aquatic environment through coastal processes such as riverine discharges and homeland run-off, and can be stored in mangrove sediments (Hu *et al.,* 2005). Moreover, atmospheric transport via dust particles, industrial emissions and long range deposition also contribute to accumulation in sediments (Danis *et al.,* 2006).

1.2.3.3.1 Fate of PCDD/Fs in Sediments

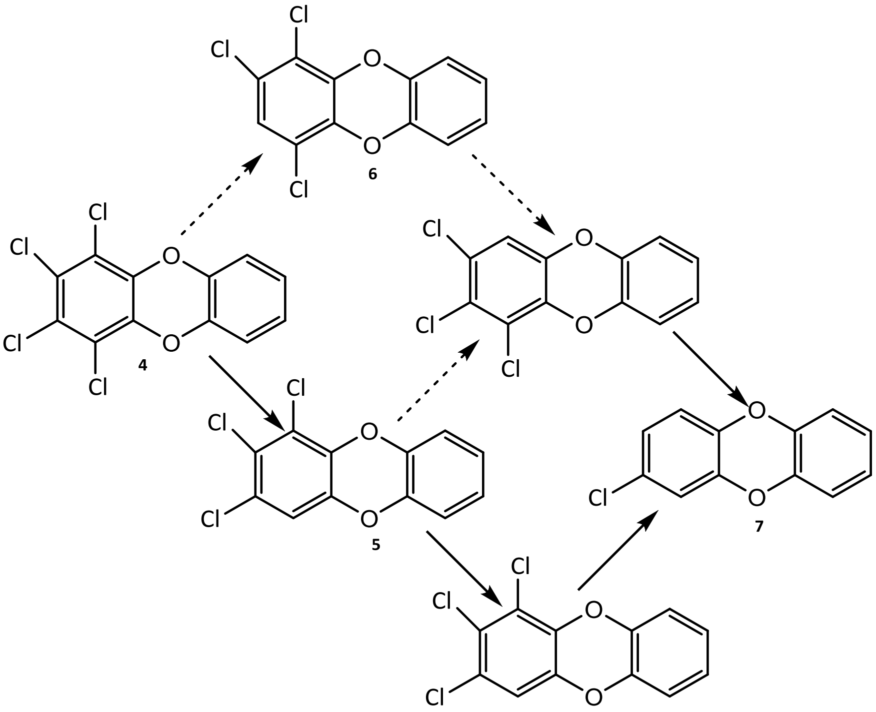
PCDD/Fs are thermally stable and resistant to biodegradation in the environment. The fate of these compounds is therefore governed by their low reactivity (persistence), volatility (potential for vapour-phase transport), low solubility in water and high lipophilicity (Table 1.3). In view of their global distribution, these chloroaromatics appear to photodegrade slowly. Usually, the more chlorine atoms that are present, the more stable is the compound and therefore the greater the persistence (Wright, 2003). As a result, they are ubiquitous in the environment and are detected in approximately all natural aquatic environmental samples, especially in suspended particulate matter within the water column where they become bound (Wright, 2003).

Table 1.3: Physico-chemical Properties of Some PCDD/Fs: after Srogi, (2008)

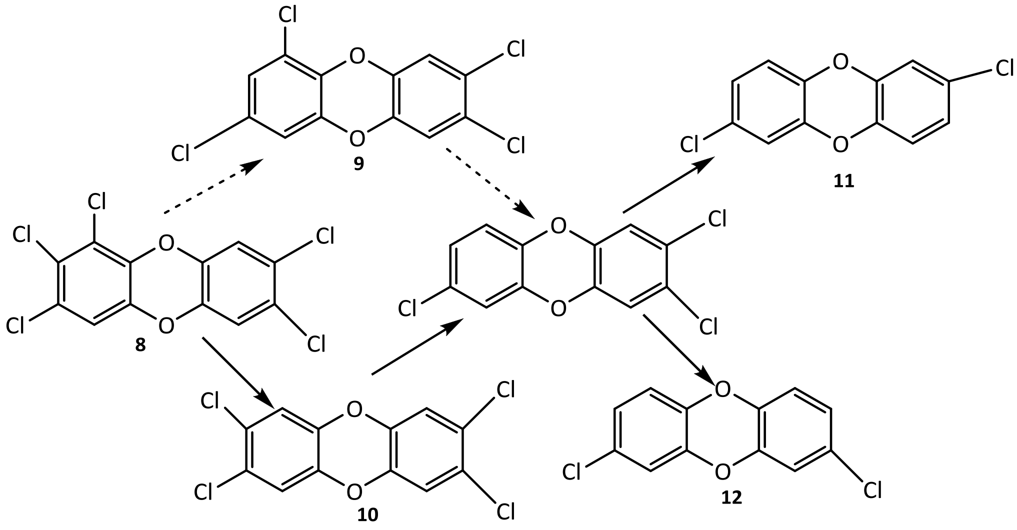
Homologue group	Solubility at 25°C mg/L	Log K_{ow}	Vapour Pressure mm Hg at 25°C	Henry's Law Constant atm-m^3/mol
TetraCDD	7.9 x10^{-6} - 4.7 x 10^{-4}	6.6-8.7	7.4 x 10^{-10}- 7.5 x 10^{-9}	7.01-16.1 x 10^{-6}
PentaCDD	1.18 x 10^{-4}	8.64-9.48	6.6 x 10^{-10}	2.6 x 10^{-6}
HexaCDD	4.42 x 10^{-6}	9.19-10.4	3.8 x10^{-11}	44.6 x 10^{-6}
HeptaCDD	1.9 x 10^{-3} - 2.4 x 10^{-6}	9.69-11.38	5.6 X 10^{-12}- 7.4 x 10^{-8}	1.31 x 10^{-6} - 2.18 x 10^{-5}
OctaCDD	7.4 x 10^{-8} - 2.27 x 10^{-9}	8.78-13.37	8.25 x 10^{-13}	37.9 x 10^{-6}
TetraCDF	4.2 x 10^{-4}	6.2	2.5 x 10^{-8}	6.06 x 10^{-4}
PentaCDF	2.4 x 10^{-4}	6.4	2.7 x 10^{-9}	2.04 x 10^{-4}
HexaCDF	1.3 x 10^{-5}	7.0	2.8 x 10^{-10}	5.87 x 10^{-4}
HeptaCDF	1.4 x 10^{-6}	7.9	9.9 x 10^{-11}	5.76 x 10^{-4}
OctaCDF	1.4 x 10^{-6}	8.8	3.8 x 10^{-12}	4.04 x 10^{-5}

In sediment, PCDD/Fs tend to associate with organic matter owing to high lipophilic and low water solubility capacities (Gevao *et al.,* 2009). Due to low water solubility and volatility, they are deposited into aquatic systems, mostly in sediments which act as a sink and a long-term source in the marine sediment system (Müller *et al.,* 2002; Pan *et al.,* 2010). Once adsorbed to particulate matter, they exhibit little potential for significant leaching or volatilisation and hence, their persistence in the environment is emphasised (Smith and Lopipero, 2001). Moreover, their chemical stability affords significant bioaccumulation and biomagnifications in the marine biota (Ryoo *et al.,* 2005).

The degradation of dioxin congeners is through the dechlorination process that leaves the parent molecule (dibenzo-dioxin or dibenzofuran) intact. For example, 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (**4**), undergoes anaerobic reductive dechlorination to give 1,2,3-trichlorodibenzo-*p*-dioxin (**5**) and 1,2,4-trichlorodibenzo-*p*-dioxin (**6**) that further degrade to 2-chlorodibenzo-*p*-dioxin (**7**). Similarly, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (**8**) gives 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (**9**) and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (**10**) which degrade further to 2,7-dichlorodibenzo-*p*-dioxin (**11**) and 2,8-dibenzo-*p*-dioxin (**12**) (Killops and Killops, 2005). There are various degradation pathways, but those of 1,2,3,4- TCDD and 1,2,3,7,8- PCDD have been shown below to depict the high chemical stability and recalcitrant nature of these compounds.



Scheme 1.1



Scheme 1.2

PCDD/Fs are highly toxic (Ryoo *et al.,* 2005) and can produce a variety of potential health effects in organisms which include dermal toxicity, immunotoxicity, teratogenicity, carcinogenicity and other adverse effects on reproduction, development and endocrine functions (Smith and Lopipero, 2001; Roots *et al.,* 2004). The toxicity associated with PCDD/Fs is induced through the binding of the compounds to the aryl hydrocarbon receptor, AhR (Denison and Heath-Pagliuso, 1998; Hahn 2002). The effect is more pronounced to those isomers presenting the chlorines in the 2,3,7,8 positions (7 PCDDs and 10 PCDFs), which are more toxic (Srogi, 2008; Okay *et al.,* 2009; Pan *et al.,* 2010). However, increasing the substitution causes a decreased potency (Smith and Lopipero, 2001).

Contamination of sediments may pose a risk to marine organisms that tend to bioaccumulate PCDD/Fs and to other organisms through the ingestion of contaminated food and feedstuff (Eljarrat *et al.,* 2001).

1.2.3.3.2 Fate of PCBs in Sediments

PCBs have extremely low solubilities in water (Okay *et al.,* 2009), low vapour pressure (Smith and Lopipero, 2001) and high octanol-water coefficients i.e. large log K_{ow} values (Yang *et al.,* 2009). PCB degradation in the environment occurs only slowly (Zhao *et al.,* 2010). Like the PCDD/Fs, increasing the number of chlorine atoms in a molecule increases the stability and persistence (Wright, 2003). Despite their recalcitrant nature, lower substituted PCBs (up to 4 chlorines) can be aerobically degraded by some bacterial strains through meta-cleavage of the unchlorinated 2,3-carbons. Oxidation may also take place on the chlorinated ring, provided that the 2,3 carbons in the chlorinated ring are not obstructed.

When released from their sources, they travel long distances and are finally deposited (Killops and Killops, 2005; Lee *et al.,* 2006; Wang *et al.,* 2007), where they have the potential to elicit their effects. Partial volatility of PCBs at ambient temperatures will direct their remobilisation and release into the atmosphere from both soil and water. Re-deposition of PCBs is brought about by wet or dry deposition (Killops and Killops, 2005). From what is known on the physico-chemical properties coupled with their widespread occurrence, persistence (Smith and Lopipero, 2001), and bioaccumulation (Kang *et al.,* 1997; Nhan *et al.,* 1999; Birch *et al.,* 2007; Bhavsar *et al.*, 2008), PCBs tend to accumulate and are likely to be associated primarily with sediments (Killops and Killops, 2005).

Sediment transport in water bodies is the dominant transport mechanisms responsible for the widespread occurrence of PCBs in the marine environment (Smith and Lopipero, 2001). Being hydrophobic in nature, they will very quickly bind to organic rich suspended particles in the water column and later transported to the bottom sediments where their incorporation can be delayed due to resuspension and bioturbation (Gevao *et al.,* 2009). Remobilisation of surficial sediment-associated PCBs can occur during natural events, such as high river flows and storms or during human activities (Yang *et al.,* 2009). As a result, they continue to be detected for very long periods (Zhao *et al.,* 2010). The major concern of sediment-associated chemicals is their entry in the food chain and the toxicity and adverse effects (Lee *et al.,* 2006; Gevao *et al.,* 2009; Terauchi *et al.,* 2009).

Like the PCDD/Fs, the biological effects and toxicity of PCBs depend on the number of chlorines and the position of the halogens in the compound (Killops and Killops, 2005; de Souza *et al.,* 2008; Bhavsar *et al.*, 2008). PCBs tend to elicit endocrine disruption (Wang *et al.,* 2007) and are potent inducers of liver microsomal aryl hydrocarbon hydroxylase (Eljarrat *et al.,* 2005). Due to contamination and their toxic effects, these dioxin-like compounds pose a major environmental and human health risk (Bhavsar *et al.,* 2008; Okay *et al.,* 2009). In humans, PCBs are believed to cause reduced male fertility, long-term behavioural and learning difficulties, particularly in children (Wright, 2003).

1.2.3.4 Sediment as Source and Sink of Dioxins and Dioxin-Like Compounds

The occurrence of chlorinated organic compounds in the marine environment is of international concern due to their persistence, toxicity, bioaccumulation and biomagnification (Tyler and Millward, 1996). The concern is aggravated when it comes to mangrove ecosystem because of their importance as growth niches and areas for mammals, birds and reptiles to obtain food. For humans, the use of the ecosystem as a source of materials is linked to various socio-economic activities. The hydrodynamics of mangrove ecosystems favour deposition of sediments and it is in sediments where the various pollutants concentrate (de Souza *et al.,* 2008).

Coastal marine environments are known to receive large amounts of pollutants and have been considered as most susceptible areas for the accumulation of toxic compounds (Kumar *et al.,* 2008). They usually act as temporary or primary long-term sinks (Müller *et al.,* 1999; Chi *et al.,* 2007; Pan *et al.,* 2010) for PCDD/Fs and PCBs and consequently act as the source of these substances to the ocean and biota (Guzzella *et al.,* 2005). Being the principle pool for pollutants, the estuary signifies a source for further release to other environmental compartments (Zhao *et al.,* 2010).

Sediments can be used to evaluate pollutant sources, historical trends, and fate processes (Müller *et al.,* 1999; Lee *et al.,* 2006; Moon *et al.,* 2009) since the amounts of contaminants in sediments reflect their regional or global discharges. The estuary and its surrounding environment can therefore be used as indicator for long-term monitoring of many environmental contaminants (Zhao *et al.,* 2010).

Marine disposal of sewage sludge and contaminated municipal and industrial wastewater can release a large amount of these anthropogenic compounds resulting into contamination of coastal marine environments (Eljarrat *et al.,* 2001; Gevao *et al.,* 2009). Natural and anthropogenic sources continuously add various compounds to the aquatic ecosystem where they pose a serious threat because of their toxicity, long time persistence, bioaccumulation, and biomagnifications in the food chain (Kumar *et al.,* 2008). In addition, contaminated sediments may constitute a particular threat to associated biota and other organisms in the marine environment (Zhao *et al.,* 2010). Increased industrialisation, population and various anthropogenic activities contribute to their elevated levels in the environment (Kumar *et al.,* 2008).

1.2.3.5 Analysis of Dioxin and Dioxin-Like Chemicals in Sediments

Various analytical methods have been used to characterise PCDD/Fs and dioxin-like PCBs in sediments. Gas chromatograph - high resolution mass spectrometry (GC-HRMS) offers a possibility to chemically identify and quantify individual congeners (Schecter *et al.,* 1999; Besselink *et al.,* 2004) in the matrix and enables the assessment of risks associated with the congeners (Long *et al,* 2006). The GC-HRMS technique assumes that (i) the additivity principle of pollutant response or effect holds (ii) there is an absence of agonistic and antagonistic interactions and (iii) the compounds produce a response (toxicity) under the same dioxin-like mechanism. However, this technique has limitations in that PCDD/Fs and dioxin-like PCBs exist as complex mixtures of congeners that elicit synergistic and/ or antagonistic interactions with different toxicity mechanisms (Schroijen *et al.,* 2004; Joung *et al.,* 2007). Chemical analysis of individual congeners particularly in small concentrations can be very expensive and time consuming. More so, the presence of compounds with AhR affinity but not commonly measured and absence of toxicological equivalents (TEQ) for several congeners further limit the use of this analytical method (Long *et al.,* 2006; Joung *et al.,* 2007).

Biological assays utilising either biomolecular techniques (e.g. immunoassays) or living materials (e.g. *in vitro* chemically activated luciferase gene expression, (CALUX) have been developed to overcome the observed drawbacks in the chemoanalysis method. Unlike the chemoanalysis, bioassay techniques screen for chemicals with selective and specific biochemical interactions (Roy *et al.,* 2002). In particular, CALUX bioassay screens for chemicals with AhR potential (Schecter *et al.,* 1999; Song *et al.,* 2006) and produces a single integrated biological equivalency (CALUX-BEQ) of the mixtures instead of congener specific information (Besselink *et al*., 2004). It also measures a response which is a single toxicity end-point produced by AhR active compounds that cannot be measured (Joung *et al.,* 2007) by chemoanalysis or when the target compounds are below the chemo-analytical detection limit. Moreover, the CALUX bioassay provides an account of the non-additivity, violating the assumption in chemoanalysis (Hurst *et al.,* 2004).

CALUX bioassay uses genetically modified cells (hepatoma cells stably transfected with a reporter gene) which respond to chemicals that activate the cytosolar AhR by induction of luciferase (Denison *et al*., 2004 and see Figure 1.1).

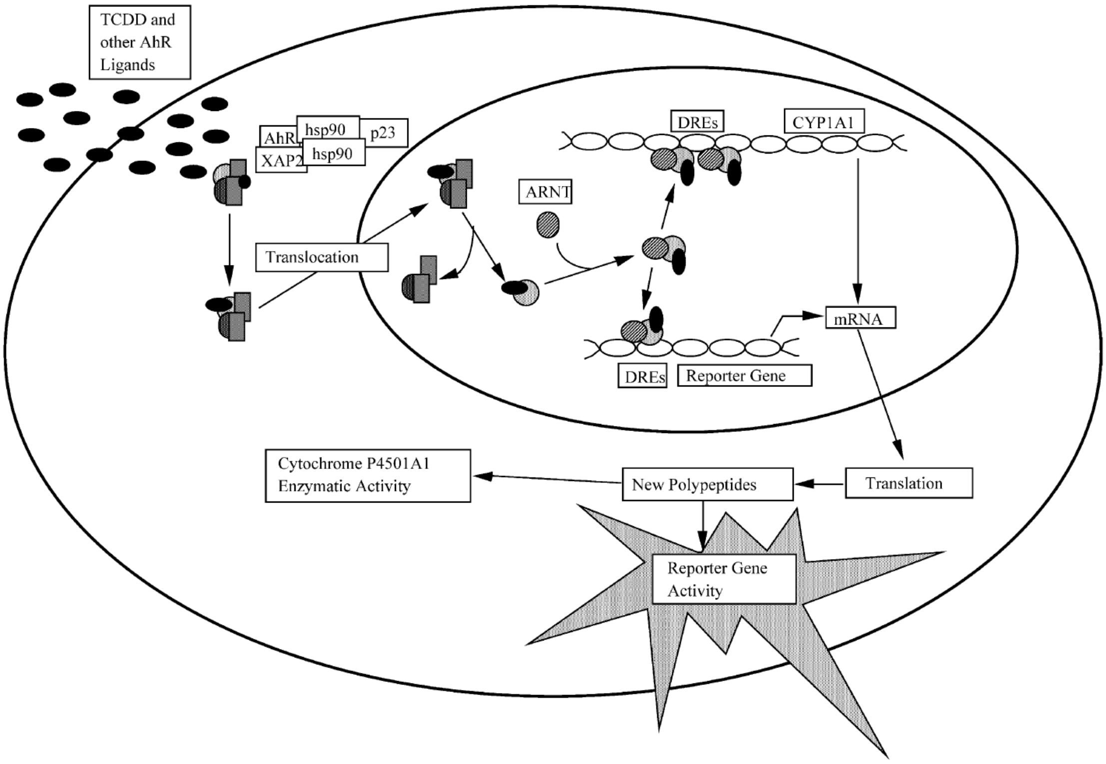


Figure 1.1: Molecular Mechanism of TCDD induced gene expression and related AhR agonists (Denison *et al.,* 2004)

The AhR is a protein complex with great affinity and low capacity (one site per molecule) that binds to various polyhalogenated aromatic hydrocarbons (Denison *et al.,* 2003). As a ligand-dependent transcription factor, the AhR not only binds and is activated by dioxins and related chemicals but is also responsible for mediating the toxicity of these chemicals (Windal *et al.,* 2005). The third generation recombinant mouse hepatoma cell lines, H1L7.5c1, stably transfected with luciferase reporter gene, pGudLuc 7.5 (He *et al*., 2011, Van Langenhove *et al.,* 2011) offers advantages of sensitivity because it contains five dioxin responsive domains (DRDs) each with four dioxin response elements (DREs).

Once dioxins and dioxin like-PCBs interact with the AhR, the formed AhR-ligand complex is translocated to the nucleus (Murk *et al.,* 1996; Schecter *et al.,* 1999; EPA, 2008). In the nucleus, it binds to dioxin responsive elements (DRE) in the dioxin responsive domain (DRD) stimulating the transcription of a luciferase gene (Murk *et al.,* 1996; Joung *et al.,* 2007; Sanctorum *et al.,* 2007a). The toxicity of these pollutants is produced either as a change in gene expression mediated through the AhR or by interference with other pathways (Hurst *et al.,* 2004). Estimation of relative potency and toxic potential can therefore be done by measuring the activation level of AhR gene expression (EPA, 2008).

CALUX can be used for the detection and quantification of dioxins, furans, and dioxin-like PCBs present in biological and environmental samples (Schecter *et al.,* 1999; Song *et al.,* 2006). It has been used to analyse sediment, soil, fly ash, stack gas emissions, food, feed, blood, and water suspected of being contaminated with the chemicals (Song *et al.,* 2006; EPA, 2005). The bioassay is particularly useful for the rapid screening of total concentrations of the compounds in sediments (Song *et al.,* 2006). Results obtained with the CALUX bioassay are given in BEQ as opposed to the TEQ associated with GC-HRMS. The biologically more relevant data is simply given by the ratio of TCDD's EC_{50} (Effective Concentration at 50% induction) over the EC_{50} of the sample and are expressed in pg TCDD BEQ/g sediment (Elskens *et al.,* 2011). The approach is fast and more cost-effective and offers an alternative for the identification and quantification of the AhR agonist chemicals (Schecter *et al.,* 1999; Song *et al.,* 2006).

1.3 CURRENT SITUATION OF MANGROVE ECOSYSTEMS IN TANZANIA

Majority of anthropogenic activities are more and more localised near the coastal areas (Tranchina *et al.,* 2008). The increasing growth of human population along the coastal areas has deteriorated the environmental quality of most rivers and the effects of anthropogenic activities are higher in coastal areas adjacent to urban cities (Tam and Wong, 2000). Mangroves occupy mouths of most large rivers along the coast of Tanzania (Figure 1.2). They stretch along the coastal districts from Tanga to Mtwara with the largest continuous mangrove areas being found on the coasts of Tanga district in the north, the Rufiji River delta in Kilwa and Lindi districts, and in Mtwara, where the Ruvuma River forms an estuary close to the Mozambique border (Mainoya *et al.,* 1986). Despite their importance to humans, wildlife and global carbon balance, mangroves are generally undervalued and overexploited. Coastal and estuarine areas have been the final repositories of persistent pollutants (McLusky and Elliott, 2004; Chatterjee *et al.,* 2007), exposing the various mangroves to high degree of contamination. Due to their position and trapping capacity, the pollutant levels are likely to reflect the use of pollutants in the shores and the surroundings (Kruitwagen *et al.,* 2008).

It is a fact that coastal cities in East Africa are expanding rapidly due to high population growth and constant immigration from inland areas. Like other countries, Tanzania is experiencing increasing impacts of environmental degradation, with rapid population growth and the industrial activities putting tremendous pressure on the local environment. The increasing degradation of coastal resources are fuelled by poverty, rapid population growth along the coasts as well as lack of proper management and awareness (Taylor *et al.,* 2002). The major sources of micro-pollutants in coastal mangrove systems in Tanzania include production and use of chemicals and consumer products, waste incineration, uncontrolled combustion processes, power generation and burning of plastic wastes.

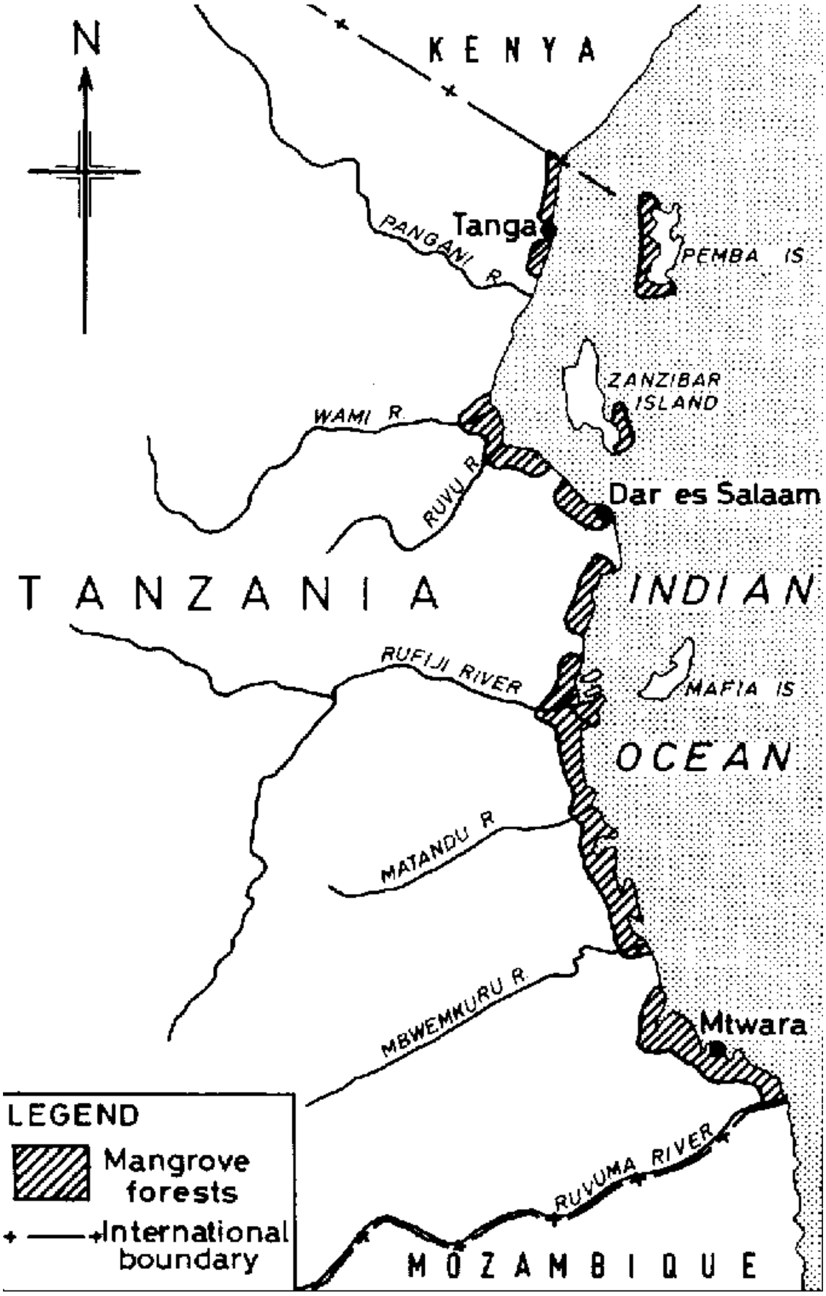


Figure 1.2: Map showing the location of mangrove forests in Tanzania

Tanzania has a few industrial centres, with most of them concentrated in the Dar es Salaam city. Being both the major industrial as well as the largest city, Dar es Salaam's population and level of industrial activities have steadily increased (Machiwa, 1992). This increased development has resulted in an uncontrolled disposal of domestic and industrial wastes (Ak'habuhaya and Lodenius, 1988; Machiwa, 1992). Most domestic wastes generated by the Dar es Salaam residents are discharged directly into the streams untreated and, as such, transported towards the coastal area (De Wolf *et al.,* 2001). Some factories have been built close to the rivers where there are deliberate or accidental releases of industrial effluents into local waters that end up into the mangrove ecosystem. The twelve rivers (including Kizinga and Mzinga) draining different areas of the city constitute the major transport pathways for pollutants from residential and industrial areas into the Indian Ocean (Gaspare *et al.,* 2009). The Msimbazi River, which is among the waterways draining the urban area of Dar es Salaam, has been identified as highly polluted (Ak'habuhaya and Lodenius, 1988; Machiwa, 1992; De Wolf *et al.,* 2001). It is anticipated that the similar situation may exist in the Mtoni estuary where Kizinga and Mzinga streams enter the ocean.

There is an increased contamination of the coastal area by increased urban wastewater production, riverine discharge, dumping and uncontrolled sewage drainage (Machiwa, 1992; PUMPSEA, 2007). The sewage contains various substances such as organic matter, nutrients, pathogenic microorganisms, metals, suspended solids and organic pollutants. Studies indicate that there are significant concentrations of metals (Machiwa, 1992; Machiwa, 2000; Mremi and Machiwa, 2003), pesticide residues (Mwevura *et al.,* 2002) and PAHs (Gaspare *et al.,* 2009) in sediments in the near shore mangrove areas of Dar es Salaam. The various industrial, economical and social activities suggest that metals and the organochlorine compounds (PCDDs, PCDFs and PCBs) might be released in high quantities to the coastal environment. Due to lack of effective urban planning and the rapid population growth resulting from urbanisation and industrial development, undesirable consequences are expected.

1.3.1 Anthropogenic Organic Matter in the Coastal Tanzania

Mangrove environments in coastal areas have been increasingly impacted by various socio-economic activities. Natural and anthropogenic changes including industrial and domestic pollution (oil spills, effluents, wastes and global climate change) are among the unprecedented threats. This is aggravated by the pressures from increased coastal population and the exploitative use of resources. Economic and developmental activities have resulted in increased sedimentation rates in coastal areas and increased inputs of anthropogenic organic carbon to the mangrove ecosystem (Hu *et al.,* 2006). Mangroves in the coastal Tanzania are thus affected by both pollution drain as well as human clearance for firewood, tannin extraction, timber, building poles and to obtain land for salt extraction (Mainoya *et al.,* 1986; De Wolf *et al.,* 2001).

In Tanzania, significant amounts of different agricultural, domestic, and industrial wastes are discharged into the streams, rivers, estuaries and ocean. As estuaries serve as final receptors of natural and anthropogenic organic matter carried by rivers into the ocean (Lü and Zhai, 2006), most of these loads are deposited in the mangrove environments, which continuously receive a presumably high influx of anthropogenic products. As a result, the type, nature and content of organic matter in Tanzanian mangrove sediments and their C/N, $\delta^{13}C$ and $\delta^{15}N$ may reveal different signatures.

1.3.2 Metals in the Contemporary Coastal Ecosystems

Majority of anthropogenic activities are more and more localised near the coastal areas (Tranchina *et al.,* 2008). Industrialisation and urbanisation along the coastal towns and cities have led to a strong risk of metal contamination (Tam and Yao, 1998). Urban and industrial activities have contributed to the input of significant amounts of metal pollutants into the marine environment and directly affect the coastal ecosystems in which they find a final destination. Mangrove areas are close to urban cities and they continuously receive industrial and domestic run-off that contain metals in various forms (Defew *et al.,* 2005; Kamaruzzaman *et al.,* 2008). Direct and indirect disposal of waste products into rivers and estuaries, especially those in industrial and urban areas, has led to a significant increase in metal contamination (Alaoui *et al.,* 2010). Metals from incoming tidal water and fresh water sources are rapidly removed from the water body and deposited onto the mangrove sediments. Hence, the contemporary metal concentrations in coastal marine environment have increased (Tranchina *et al.,* 2008) and mangrove ecosystems are under serious human threat (Tam and Wong, 2000).

At present, the anthropogenic contribution of the metals into the marine environment in Tanzania and the impacts of metal contamination in the coastal ecosystems are alarming. There has been an increase in industrial activities, traffic and traffic related activities, urban agriculture in valleys and near rivers that drain their water into mangrove ecosystems and direct contamination via dumping of metallic substances or wastewater discharges (Ak'habuhaya and Lodenius, 1988; Machiwa, 1992; Machiwa, 2000; De Wolf *et al.,* 2001; Taylor *et al.,* 2002; Mremi and Machiwa, 2003). As the lives of people have higher dependence on the coastal resources for fishing, salt extraction, tourism and other related activities, even a small variation in the ocean surroundings can have severe impact on those lives.

1.3.3 Dioxins and Dioxin-Like PCBs in the Contemporary Tanzania

Although the official use of dioxin-like PCBs in Tanzania is not known, the presence of these substances in transformers, electrical equipments, ship painting and other industrial activities is common. In addition, the involuntary production, especially via incineration, of dioxins is neither known. In the coastal Tanzania, there are a lot of municipal, chemical and even hospital wastes that are directed into the Indian Ocean with incineration and burning being the main treatments. This probably increases the levels of the pollutants. Wood burning is a common source of household emissions as most households use either charcoal or firewood for cooking. In many local households, plastics have become a common substance to light fire on charcoal when burnt. Recently, vehicle emissions resulting from traffics and importation of used and old cars are now common in Tanzania. Public allegations that electrical transformers are vandalised in search of their coolant for unspecified domestic and commercial use in large cities calls for research in different food and environmental matrices in a way to create public awareness on these types of fluids in general.

Because of omnipresence, the dioxins and dioxin-like pollutants co-exist in most environmental matrices. Therefore, simultaneously monitoring their levels in environmental samples, particularly the mangrove ecosystem, is highly desired in order to assess their sources and fate in the marine system (Liu *et al.,* 2006). As estuarine sediments are an important sink for these contaminants in the aquatic environment, the study of sediments is an important step in mapping possible source and transport pathways of the contaminants to the marine ecosystem (Covaci *et al.,* 2005).

1.4 DESCRIPTION OF THE MTONI ESTUARY

The Mtoni estuary is located at approximately 3 km south of Dar es Salaam (Tanzania) and is fed by two rivers: Kizinga and Mzinga. The creeks have mangrove trees such as *Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora mucronata and Sonneratia alba* species growing on both sides (Mlay *et al., unpublished)*.

The average annual precipitation of the Dar es Salaam region is around 1100 mm (Mtoni *et al.,* 2012). December and January have an average precipitation rate of 194 and 89 mm, respectively, while these rates in July and August are much lower with 48 and 47 mm respectively. River flows in the Dar-es-Salaam area are mainly controlled by the precipitation rate in the previous period. The flows of Kizinga and Mzinga rivers are highest in the wet season (the highest discharge rates can go up to 15 m^3/s for the Kizinga River and 7 m^3/s for the Mzinga River). In the dry season, base-line flows of 1 m^3/s in the Kizinga River and even lower in the Mzinga River were observed (Van Camp *et al.,* 2013).

The Kizinga river that drains the urbanised and industrialised (small to medium scale) areas of Keko, Chang'ombe, Kurasini and Temeke (approximately 400,000 inhabitants; NBS, 2003) is suspected to carry a variety of wastes and discharges originating from agricultural, industrial as well as residential sources (Taylor *et al.,* 2002). There is also a textile factory built within the Kizinga river catchment.

The Mzinga river, on the other hand, drains the rural and non-industrialised areas of Vijibweni, Tuangoma and Mji Mwema with a population of around 90,000 (NBS, 2003). Due to rapid growth of settlements along the Mzinga creek resulting from increased human population, the river is suspected to carry agricultural and residential wastes and discharges presumed to be emptied into the creek. The estuary further receives inputs from the Dar es Salaam harbour which is located near the mouth of the estuary during diurnal tides (up to 5 m amplitude) and from the Mtoni solid waste dumping site located in between the two rivers.

Therefore, the Mtoni estuary is highly impacted (PUMPSEA, 2007) by discharges of various origin: (1) the Kizinga and Mzinga rivers draining the mangrove forest (Kruitwagen *et al.,* 2008), (2) the wastewater drainage systems from industrial and residential areas (of a population of around 500,000 inhabitants; NBS, 2003), (3) charcoal and wood burning, (4) mangrove harvesting for residential places, (5) salt mining, (6) tourism and (7) agriculture (Taylor *et al.,* 2002).

1.5 OBJECTIVES

The general aim of this study was to describe and evaluate the past and current status of mangrove ecosystem and to predict the future status by relating it to changing human activities in the coastal areas and its environment in Tanzania. Specifically, the study aimed at (1) establishing the role of anthropogenic influence on the contribution of organic carbon and nitrogen in the mangrove sediments via the use of stable carbon and nitrogen isotopes as proxies and (2) describing the current sources and fate of key pollutants (metals, PCDD/Fs and PCBs,) in the Tanzanian mangrove ecosystems in response to anthropogenic and natural changes. Moreover, the study intended to determine and compare the variability in the distribution of potential micro-pollutants in the mangrove sediments of Tanzanian coast.

1.6 THESIS LAYOUT

The thesis has been organised into 5 chapters. ***Chapter One*** gives an outline of the mangrove sediment as a biogeochemical environment as well as source and sink of micro-pollutants. It also describes the organic matter composition as well as the use of carbon and nitrogen stable isotopes and nitrogen to carbon ratio as environmental tracers in sediment. The chapter also gives a description of sources, fate and effects of metals and dioxins and dioxin-like PCBs in mangrove sediments. It lastly gives the current situation of mangrove ecosystem in coastal Tanzania.

The next 3 chapters, 2 to 4, which form the main body of the thesis, address the specific objectives of the work. Each of the chapters gives a brief introduction, descriptions of the sampling procedures, methods on sample preparation and instrumental analysis, quality control and quality assurance measures, description of the results, detailed discussion and conclusion. ***Chapter Two*** reports the carbon and nitrogen dynamics as indicated by total organic carbon (TOC), total nitrogen (TN), stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta\ ^{15}N$) and carbon to nitrogen (C/N) ratio in the estuarine mangrove sediments. Temporal and spatial variations of the geochemical parameters were also determined. Sources of the sedimentary organic matter were identified by a combination of geochemical parameters. Carbon content contribution of the terrestrial component was quantitatively estimated using the stable carbon and nitrogen isotopes assuming the marine environment is a two end-member system.

Chapter Three reports the distribution of two major, two minor and seven trace elements as quantified in coastal sediments using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Correlation coefficients of the different metals were estimated to determine the factors controlling their behaviour in tropical mangrove sediment. Enrichment factors and principal component analysis (PCA) using varimax rotated factor analysis were also determined to identify the sources of metals in a way to assess the anthropogenic activities as indicators of human induced environmental change.

Chapter Four reports the levels of dioxins, furans and dioxin-like PCBs as detected by chemically activated luciferase gene expression, CALUX assay, a reporter gene bioassay that uses genetically modified cells. The induction by these chemicals in the recombinant cells was measured as luminescence and compared to the response of a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) standard. The amount of pollutants in any given sample was converted to a bioanalytical equivalent (BEQ) and expressed in pg TCDD BEQ/ g sediment. The current levels of these micro-pollutants in different samples enabled to identify the possible pollution sources and determine any historical input and extent of biological exposure in the mangrove ecosystem.

The last chapter, ***Chapter Five,*** gives the general conclusion and recommendations of the study based on the findings obtained in the chapters two to four.

1.7 REFERENCES

Abrahim GMS, Parker RJ (2008). Assessment of heavy metal enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zealand, *Environ Monit Assess,* 136: 227–238.

Ak'habuhaya J, Lodenius M (1988). Metal pollution of River Msimbazi, Tanzania, *Environmental International,* 14: 511-514.

Alaoui AM, Choura M, Maanan M, Zourarah B, Robin M, Conceição MF, Andrade C, Khalid M, Carruesco C (2010). Metal fluxes to the sediments of the Moulay Bousselham lagoon, Morocco, *Environ Earth Sci,* 61: 275–286.

Aloupi M, Angelidis MO, (2001). Normalization to lithium for the assessment of metal contamination in coastal sediment cores from the Aegean Sea, Greece, *Marine Environmental Research*, 52: 1-12.

Besselink HT, Schripper C, Klamer H, Leonards P, Verhaar H, Felzel E, Murk AJ, Thain J, Hosoe K, Schoeters G, Legler J, Brouwer B (2004). Intra- and interlaboratory calibration of the DR Calux bioassay for the analysis of dioxins and dioxin-like chemicals in sediments. *Environ. Toxicol. Chem.* 23: 2781–2789.

Bhavsar SP, Reiner EJ, Hayton A, Fletcher R, MacPherson K (2008). Converting Toxic Equivalents (TEQ) of dioxins and dioxin-like compounds in fish from one Toxic Equivalency Factor (TEF) scheme to another, *Environment International* 34: 915–921.

Bickert T (2006). Influence of Geochemical Processes on Stable Isotope Distribution in Marine Sediments *in* Schulz HD, Zabel M (*Eds*.), Marine Geochemistry, Second Edition, Springer-Verlag, Berlin Heidelberg.

Birch GF, Harrington C, Symons RK, Hunt JW (2007). The source and distribution of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofurans in sediments of Port Jackson, Australia, *Marine Pollution Bulletin* 54: 295–308.

Bouillon S, Middelburg JJ, Dehairs F, Borges AV, Abril G, Flindt MR, Ulomi S, Kristensen E (2007). Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania), *Biogeosciences*, 4: 311–322.

Bouillon SA, Dahdouh-Guebas F, Rao AVVS, Koedam N, Dehairs F (2003). Sources of organic carbon in mangrove sediments: variability, and possible ecological implications, *Hydrobiologia* 495: 33–39.

Bouillon SA, Raman V, Dauby P, Dehairs F (2002). Carbon and Nitrogen Stable Isotope Ratios of Subtidal Benthic Invertebrates in an Estuarine Mangrove Ecosystem (Andhra Pradesh, India), *Estuarine, Coastal and Shelf Science*, 54: 901–913.

Boutton TW (1996). Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change *in* Boutton TW, Yamasaki SI (*Eds.*), Mass Spectrometry of Soils, Marcel Dekker, Inc., New York, pp. 47-82.

Bruckmeier BFA, Jüttner I, Schramm KW, Winkler R, Steinberg CEW, Kettrup A (1997). PCBs and PCDD/Fs in Lake Sediments of Großer Arbersee, Bavarian Forest, South Germany, *Environmental Pollution,* 95 (1): 19-25.

Chatterjee M, Silva Filho EV, Sarkar SK, Sella SM, Bhattacharya A, Satpathy KK, Prasad MVR, Chakraborty S, Bhattacharya BD (2007). Distribution and possible source of trace elements in the sediment cores of a tropical macrotidal estuary and their ecotoxicological significance, *Environment International,* 33 (3): 346-356.

Chi KH, Chang MB, Kao S (2007). Historical trends of PCDD/Fs and dioxin-like PCBs in sediments buried in a reservoir in Northern Taiwan, *Chemosphere* 68: 1733–1740.

Cifuentes LA, Sharp JH, Fogel ML (1988). Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary, *Limnol. Oceanogr.,* 33 (5): 1102-1115

Cloern JE, Canuel EA, Harris D (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system, *Limnol. Oceanogr.,* 47 (3): 713–729.

Covaci A, Gheorghe A, Voorspoels S, Maervoet J, Steen Redeker E, Blust R, Schepens P (2005). Polybrominated diphenyl ethers, polychlorinated biphenyls and organochlorine pesticides in sediment cores from the Western Scheldt river (Belgium): analytical aspects and depth profiles, *Environment International* 31: 367–375.

Danis B, Debacker V, Miranda CT, Dubois P (2006). Levels and effects of PCDD/Fs and co-PCBs in sediments, mussels, and sea stars of the intertidal zone in the southern North Sea and the English Channel, *Ecotoxicology and Environmental Safety* 65: 188–200.

de Souza A, Torres JPM, Meire RO, Neves RC, Couri MS, Serejo CS (2008). Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in sediments and crabs (*Chasmagnathus granulata*, Dana, 1851) from mangroves of Guanabara Bay, Rio de Janeiro State, Brazil, *Chemosphere* 73: S186–S192.

De Wolf H, Rashid R (2008). Heavy metal accumulation in *Littoraria scabra* along polluted and pristine mangrove areas of Tanzania, *Environmental Pollution* 152: 636-643.

De Wolf H, Ulomi SA, Backeljau T, Pratap HB, Blust R (2001). Heavy metal levels in the sediments of four Dar es Salaam mangroves Accumulation in, and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda), *Environment International* 26: 243-249.

Defew L H, Mair JM, Guzman HM (2005). An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama, *Marine Pollution Bulletin* 50: 547–552.

Dell'Anno A, Mei ML, Ianni C, Danovaro R (2003). Impact of bioavailable heavy metals on bacterial activities in coastal marine sediments, *World Journal of Microbiology & Biotechnology* 19: 93–100.

Denison MS, Heath-Pagliuso S (1998). The Ah receptor: a regulator of the biochemical and toxicological actions of structurally diverse chemicals. Bulletin of environmental contamination and toxicology, 61(5): 557–68.

Denison MS, Nagy SR (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annual review of pharmacology and toxicology, 43: 309–34. doi:10.1146/annurev.pharmtox.43.100901.135828

Denison MS, Zhao B, Baston DS, Clark GC, Murata H, Han D (2004). Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals, *Talanta* 63: 1123–1133

Du Laing G, Rinklebe J, Vandecasteele B, Meers E, Tack FMG (2009). Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review, *Science of the Total Environment* 407: 3972–3985.

Eljarrat E, Caixach J, Rivera J (2001). Evaluation of dioxin contamination in sewage sludge discharges on coastal sediments from Catalonia, Spain, *Wat. Res.* 35 (11): 2799–2803.

Eljarrat E, De La Cal A, Larrazabal D, Fabrellas B, Fernandez-Alba AR, Borrull F, Marce RM, Barceló D (2005). Occurrence of polybrominated diphenylethers, polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in coastal sediments from Spain, *Environmental Pollution*, 136: 493-501.

El-Kady AA, Abdel-Wahhab MA, Henkelmann B, Belal MH, Morsi KS, Galal SM, Schramm KS (2007). Polychlorinated biphenyl, polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran residues in sediments and fish of the River Nile in the Cairo region, *Chemosphere* 68: 1660–1668.

Elskens M, Baston DS, Stumpf C, Haedrich J, Keupers I, Kroes K, Denison MS, Baeyens W, Goeyens L (2011). CALUX measurements: Statistical inferences for the dose–response curve, *Talanta* 85: 1966-1973

Emerson S, Hedges J (2008). Chemical Oceanography and the Marine Carbon Cycle, Cambridge Universtity Press, UK.

EPA (2005). Xenobiotic Detection Systems: CALUX by XDS: Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment, Innovative Technology Verification Report, EPA/540/R-05/001; U.S. Environmental Protection Agency: Washington, DC.

EPA (2008). Method 4435: Method For Toxic Equivalents (TEQs) Determinations for Dioxin-like Chemical Activity With the CALUX® Bioassay, Washington, DC.

Erickson MD (1997). Analytical chemistry of PCBs, Second Edition, CRC Press, Boca Raton FL, 667 *as cited in* Fouial-Djebbar D, Badjah Hadji Ahmed AY, Budzinski H (2010). Determination of organochlorine compounds in coastal marine sediments from the southern west of the Mediterranean Sea. *Int. J. Environ. Sci. Tech.,* 7 (2): 271-280.

Feng H, Han X, Zhang W, Yu L (2004). A preliminary study of heavy metal contamination in Yangtze River intertidal zone due to urbanization. *Marine Pollution Bulletin* 49: 910–915.

Fouial-Djebbar D, Badjah Hadji Ahmed, AY, Budzinski H (2010). Determination of organochlorine compounds in coastal marine sediments from the southern west of the Mediterranean Sea. *Int. J. Environ. Sci. Tech.,* 7 (2): 271-280.

Gaspare L, Machiwa JF, Mdachi SJM, Streck G, Brack W (2009). Polycyclic aromatic hydrocarbon (PAH) contamination of surface sediments and oysters from the inter-tidal areas of Dar es Salaam, Tanzania, *Environmental Pollution*, 157: 24–34.

Gevao B, Jaward FM, Uddin S, Al-Ghadban AN (2009). Occurrence and concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in coastal marine sediments in Kuwait, *Marine Pollution Bulletin* 58: 424–455.

González-Macías C, Schifter I, Lluch-Cota DB, Méndez-Rodríguez L,Hernández-Vázquez S (2006). Distribution, Enrichment and Accumulation of Heavy Metals in Coastal Sediments of Salina Cruz Bay, México, *Environmental Monitoring and Assessment* 118: 211–230.

Grace VB, Mas-Pla J, Novais TO, Sacchi E, Zuppi GM (2008). Hydrological mixing and geochemical processes characterization in an estuarine/mangrove system using environmental tracers in Babitonga Bay (Santa Catarina, Brazil), *Continental Shelf Research* 28: 682–695.

Graham MC, Eaves MA, Farmer JG, Dobson J, Fallick A E (2001). A Study of Carbon and Nitrogen Stable Isotope and Elemental Ratios as Potential Indicators of Source and Fate of Organic Matter in Sediments of the Forth Estuary, Scotland, *Estuarine, Coastal and Shelf Science*, 52: 375–380.

Guzzella L, Roscioli C, Viganò L, Saha M, Sarkar SK, Bhattacharya A (2005). Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in the sediments along the lower stretch of Hugli estuary, West Bengal, northeast India, *Environment International* 31: 523– 534.

Hahn ME (2002). Aryl hydrocarbon receptors: diversity and evolution. Chemico-biological interactions, 141 (1-2): 131–60.

He G, Tsutsumi T, Zhao B, Baston DS, Zhao J, Heath-Pagliuso S, Denison MS, (2011). Third-generation Ah receptor-responsive luciferase reporter plasmids: amplification of dioxin-responsive elements dramatically increases CALUX bioassay sensitivity and responsiveness. Toxicological sciences: an official journal of the Society of Toxicology, 123 (2): 511–22.

Hoefs J (2009). Stable Isotope Geochemistry*,* Sixth Edition, Springer-Verlag Berlin Heidelberg.

Hu J, Peng P, Jia G, Mai B, Zhang G (2006). Distribution and sources of organic carbon, nitrogen and their isotopes in sediments of the subtropical Pearl River estuary and adjacent shelf, Southern China, *Marine Chemistry* 98: 274– 285.

Hu J, Wan Y, Shao B, Jin X, An W, Jin F, Yang M, Wang X, Sugisaki M (2005). Occurrence of trace organic contaminants in Bohai Bay and its adjacent Nanpaiwu River, North China, *Marine Chemistry* 95: 1–13.

Hurst MR, Balaam J, Chan-Man YL, Thain JE, Thomas KV (2004). Determination of dioxin and dioxin-like compounds in from UK estuaries using bio-analytical approach: Chemical-activated luciferaase expression (CALUX) assay, *Marine Pollution Bulletin,* 49: 648-658.

International Atomic Energy Agency and United Nations Educational, Scientific and Cultural Organization (2000) Environmental Isotopes in the Hydrological Cycle. Principles and Applications. Vol 1. introduction — theory, methods, review. http://www-naweb.iaea.org/napc/ih/documents/global_cycle/Environmental%20Isotopes%20in%20the%20Hydrological%20Cycle%20Vol%201.pdf

Janaki-Raman D Jonathan MP, Srinivasalu S, Armstrong-Altrin JS, Mohan SP, Ram-Mohan V (2007). Trace metal enrichments in core sediments in Muthupet mangroves, SE coast of India: Application of acid leachable technique, *Environmental Pollution* 145: 245-257.

Jardine TD, McGeachy SA, Paton CM, Savoie M, Cunjak RA (2003). Stable isotopes in aquatic systems: Sample preparation, analysis, and interpretation, *Can. Manuscr. Rep. Fish. Aquat. Sci.* No. 2656: 39.

Joung KE, Chung YH, Sheen YY (2007). DRE-CALUX bioassay in comparison with HRGC/MS for measurement of toxic equivalence in environmental samples, *Science of the Total Environment* 372: 657–667.

Kamaruzzaman BY, Ong MC, Noor Azhar MS, Shahbudin S, Jalal KCA (2008). Geochemistry of Sediment in the Major Estuarine Mangrove Forest of Terengganu Region, Malaysia, *Am. J. Applied Sci.,* 5 (12): 1707-1712.

Kamau JN (2002). Heavy Metal Distribution and Enrichment at Port-Reitz Creek, Mombasa, *Western Indian Ocean J. Mar. Sci.* (1): 65-70.

Kang YS, Mats M, Kawano M, Wakimoto T, Min BY (1997). Organochlorine Pesticides, Polychlorinated Biphenyls, Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans in Human Adipose Tissue from Western Kyungnam, Korea, *Chemosphere*, 35 (10): 2107-2117.

Killops S, Killops V (2005). Introduction to Organic Geochemistry, 2rd Edition, Backwell Publishing.

Koistinen J, Stenman O, Haahti H, Suonperä M, Paasivirta J (1997). Polychlorinated Diphenyl Ethers, Dibenzo-p-dioxins, Dibenzofurans and Biphenyls in Seals and Sediment from the Gulf of Finland, *Chemosphere* 35 (6): 1249-1269

Kruitwagen G, Pratap HB, Covaci A, Wendelaar Bonga SE (2008). Status of pollution in mangrove ecosystems along the coast of Tanzania, *Marine Pollution Bulletin* 56: 1022–1042.

Kumar KS, Sajwan KS, Richardson JP, Kannan K (2008). Contamination profiles of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons and alkylphenols in sediment and oyster collected from marsh/estuarine Savannah GA, USA, *Marine Pollution Bulletin* 56, 136–162.

Lee S, Kim J, Chang YS, Moon MH (2006). Characterization of polychlorinated dibenzo-p-dioxins and dibenzofurans in different particle size fractions of marine sediments, *Environmental Pollution* 144: 554-561.

Liu H, Zhang Q, Cai Z, Li A, Wang Y, Jiang G (2006). Separation of polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and dibenzo-furans in environmental samples using silica gel and florisil fractionation chromatography, *Analytica Chimica Acta* 557: 314–320.

Liu WX, Li XD, Shen ZG, Wang DC, Wai OWH, Li, YS (2003). Multivariate statistical study of heavy metal enrichment in sediments of the Pearl River Estuary, *Environmental Pollution* 121, 377-388.

Long M, Andersen BS, Lindh CH, Hagmar L, Giwercman A, Manicardi GC, Bizzaro D, Spanò M, Toft G, Pedersen HS, Zvyezday V, Jens Peter Bonde JP, Bonefeld-Jorgensen EC (2006). Dioxin-like activities in serum across European and Inuit populations, *Environmental Health: A Global Access Science Source* 5:14 doi:10.1186/1476-069X-5-14

Lü X, Zhai S (2006). Distributions and sources of organic biomarkers in surface sediments from the Changjiang (Yangtze River), *Estuary, China, Continental Shelf Research* 26: 1–14.

MacFarlane GR, Burchett MD (1999). Zinc distribution and excretion in the leaves of the grey mangrove, *Avicennia marina* (Forsk.) Vierh, *Environmental and Experimental Botany* 41: 167–175.

MacFarlane GR, Burchett MD (2000). Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh, *Aquatic Botany* 68: 45–59.

MacFarlane GR, Burchett MD (2001). Photosynthetic Pigments and Peroxidase Activity as Indicators of Heavy metal Stress in the Grey mangrove, *Avicennia marina* (Forsk.), *Marine Pollution Bulletin* 42 (2): 233-240.

Machiwa JF (1992). Anthropogenic pollution in the Dar es Salaam harbour area, Tanzania. *Marine Pollution Bulletin* 24: 562–567.

Machiwa JF (2000). Heavy metals and organic pollutants in sediments of Dar es Salaam harbour prior to dredging in 1999. *Tanz. J. Sc.* 26: 29–46.

Machiwa JF (2010). Stable carbon and nitrogen isotopic signatures of organic matter sources in near-shore areas of Lake Victoria, East Africa, *Journal of Great Lakes Research* 36: 1–8.

Mainoya JR, Mesaki S, Banyikwa FF (1986). The distribution and socio-economic aspects of mangrove forests in Tanzania *in* Kunstadter P, Bird ECF, and Sabhasri, S.(Eds), Man in the Mangroves: The Socio-economic Situation of Human Settlements in Mangrove Forests, Proceedings of a workshop held at Nong Nuch Village, Pattaya, Thailand, 27-31 May 1985, United Nations University Press.

Manahan SE (2000). Environmental Chemistry, CRC Press LLC, Boca Raton.

Marchand C, Lallier Verges E, Baltzer F, Alberic P, Cossa D, Baillif P (2006). Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana, *Marine Chemistry*, 98 (1): 1-17.

Marchand C, Lallier-Vergès E, Disnar JR, Kéravis D (2008). Organic carbon sources and transformations in mangrove sediments: A Rock-Eval pyrolysis approach. *Organic Geochemistry* 39: 408–421.

McLusky DS, Elliott M (2004). The Estuarine Ecosystem: Ecology, Threats, and Management, 3rd Edition, Oxford University Press.

McSween HY, Richardson SM, Uhle ME (2003). Geochemistry: Pathways and Processes, 2nd Edition, Columbia University Press, New York.

Mlay AP, Wagner GM, Mgaya YD (*unpublished*). A comparative study of the ecology of four sandy/muddy shores in the Dar es Salaam area. Accessed on 23th August 2012 at http://gridnairobi.unep.org/chm/eafdocuments/tanzania/Ecology_of_muddy_shores_in_Tanzania.pdf

Moon HB, Choi M, Choi HG, Ok G, Kannan K (2009). Historical trends of PCDDs, PCDFs, dioxin-like PCBs and nonylphenols in dated sediment cores from a semi-enclosed bay in Korea: Tracking the sources, *Chemosphere* 75: 565–571.

Mremi SD, Machiwa JF, (2003). Heavy metal contamination of mangrove sediments and the associated biota in Dar es Salaam, Tanzania. *Tanz. J. Sc.* 29: 61–76.

Mtanga A, Machiwa J (2007). Assessment of Heavy Metal Pollution in Sediment and Polychaete Worms from the Mzinga Creek and Ras Dege Mangrove Ecosystems, Dar es Salaam, Tanzania, *Western Indian Ocean J. Mar. Sci.* 6 (2): 125–135.

Mtoni Y, Mjemah IC. Msindai K, Van Camp M, Walraevens K (2012). Saltwater intrusion in the quaternary aquifer of the Dar es Salaam region, Tanzania, *Geological Belgica,* 15 (1-2): 16-25

Müller JF, Gaus C, Prange JA, Päpke O, Poon KF, Lam MHW, Lam PKS (2002). Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in sediments from Hong Kong, *Marine Pollution Bulletin* 45: 372–378.

Müller JF, Haynes D, McLachlan M, Böhme F, Will S, Shaw GR, Mortimer M, Sadler R, Connell DW (1999). PCDDs, PCDFs, PCBs and HCB in Marine and Estuarine sediments from Queensland, Australia, *Chemosphere* 39 (10): 1707-1721.

Murk AJ, Legler J, Denison MS, Giesy JP, Van De Guchte C, Brouwer A (1996). Chemical-Activated Luciferase Gene Expression (CALUX): A Novel *in Vitro* Bioassay for Ah Receptor Active Compounds in Sediments and Pore Water, *Fundamental and Applied Toxicology* 33: 149-160.

Mwevura H, Othman OC, Mhehe GL, (2002), Organochlorine pesticide residues in sediments and biota from the coastal area of Dar es Salaam city, Tanzania, *Marine Pollution Bulletin* 45: 262-267.

NBS - National Bureau of Statistics, (2003). Tanzania Populations and Housing Census: General Report, Accessed on 12th October 2012 at http://web.archive.org/web/20040313070101/http://www.tanzania.go.tz/census/census/tables.htm

Nhan DD, Am NM, Carvalho FP, Villeneuve JP, Cattini C (1999). Organochlorine pesticides and PCBs along the coast of North Vietnam, *The Science of the Total Environment* 237/238: 363-371.

Nobi EP, Dilipan E, Thangaradjou T, Sivakumar K, Kannan L (2010). Geochemical and geo-statistical assessment of heavy metal concentration in the sediments of different coastal ecosystems of Andaman Islands, India, *Estuarine, Coastal and Shelf Science* 87: 253–264.

Okay OS, Karacık B, Başak S, Henkelmann B, Bernhöft S, Schramm KW (2009). PCB and PCDD/F in sediments and mussels of the Istanbul strait (Turkey), *Chemosphere* 76: 159–166.

Pan J, Yang Y, Geng C, Yeung LWY, Cao X, Dai T (2010). Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans in marine and lacustrine sediments from the Shandong Peninsula, China, *Journal of Hazardous Materials* 176: 274–279.

Praveena SM, Aris AZ, Radojevic M (2010). Heavy Metals Dyanamics and Source in Intertidal Mangrove Sediment of Sabah, Borneo Island, *EnvironmentAsia* 3: 79-83.

PUMPSEA: Peri-urban mangrove forests as filters and potential phytoremediators, of domestic sewage in East Africa (2007). Distribution and fate of heavy metals and indicator pathogens in sewage exposed mangroves, Specific Targeted Research Project Report for Project No. INCO – CT2004 – 510863.

Rainbow PS (1995). Biomonitoring of Heavy Metal Availability in the Marine Environment, *Marine Pollution Bulletin* 31 (4-12): 183-192.

Raju K, Vijayaraghavan K, Seshachalam S, Muthumanickam J (2010). Impact of anthropogenic input on physicochemical parameters and trace metals in marine surface sediments of Bay of Bengal off Chennai, India, *Environ Monit Assess,* DOI 10.1007/s10661-010-1621-2.

Ramaswamy V, Gaye B, Shirodkar PV, Rao PS, Chivas AR, Wheeler D, Thwin S (2008). Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from the Ayeyarwady (Irrawaddy) continental shelf, northern Andaman Sea, *Marine Chemistry* 111: 137–150.

Ramos de Silva CA, da Silva AP, de Oliveira SR (2006). Concentration, stock and transport rate of heavy metals in a tropical red mangrove, Natal, Brazil, *Marine Chemistry* 99: 2 –11.

Rokade MA (2009). Heavy Metal Burden in Coastal Marine Sediments of North West Coast of India in Relation to Pollution, *PhD Thesis,* National Institute for Oceanography, Mumbai, India.

Roots O, Henkelmann B, Schramm KW (2004). Concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in soil in the vicinity of a landfill, *Chemosphere* 57: 337–342.

Roy S, Mysior P, Brzezinski R (2002). Comparison of dioxin and furan TEQ determination in contaminated soil using chemical, micro-EROD, and immunoassay analysis, *Chemosphere* 48: 833–842

Ryoo KS, Ko SO, Hong YP, Choi JH, Cho S, Kim Y, Bae YJ (2005). Levels of PCDDs and PCDFs in Korean river sediments and their detection by biomarkers, *Chemosphere* 61: 323–331.

Salazar-Hermoso F (2007). Evaluation of Stable Isotopes for the Identification of Aquaculture Waste in the Aquatic Environment, *M.Sc. Thesis,* The University of Guelph, Canada.

Sampaio L, Freitas R, Máguas C, Rodrigues A, Quintino V (2010a). Coastal sediments under the influence of multiple organic enrichment sources: An evaluation using carbon and nitrogen stable isotopes, *Marine Pollution Bulletin* 60: 272–282.

Sampaio L, Rodrigues AM, Quintino V (2010b). Carbon and nitrogen stable isotopes in coastal benthic populations under multiple organic enrichment sources, *Marine Pollution Bulletin* 60: 1790–1802.

Sanctorum H, Elskens M, Baeyens W (2007a). Bioassay (CALUX) measurements of 2,3,7,8-TCDD and PCB 126: Interference effects, *Talanta* 73: 185–188.

Sanctorum, H, Windal I, Hanot V, Goeyens L, Baeyens W (2007b). Dioxin and Dioxin-Like Activity in Sediments of the Belgian Coastal Area (Southern North Sea), *Arch. Environ. Contam. Toxicol.,* 52: 317–325.

Schecter AJ, Sheu SU, Birnbaum LS, DeVito MJ, Denison MS, and Päpke O (1999). A comparison and discussion of two differing methods of measuring dioxin-like compounds: gas chromatography-mass spectrometry and the CALUX bioassay - implications for health studies, *Organohalogen Compounds,* 40: 247-250

Schmidt, H, Schultz G (1881). Einwirkung von Fiinffach-Chlorphosphor auf das y- diphenol. *Ann. Chim*, 207, 338-344 *as cited in* Fouial-Djebbar D, Badjah Hadji Ahmed, AY, Budzinski H (2010). Determination of organochlorine compounds in coastal marine sediments from the southern west of the Mediterranean Sea. *Int. J. Environ. Sci. Tech.* 7 (2): 271-280.

Schroijen C, Windal I, Goeyens L, Baeyens W, (2004). Study of the interference problems of dioxin-like chemicals with the bio-analytical method CALUX, *Talanta* 63: 1261–1268

Shen H, Han J, Tie X, Xu W, Ren Y, Ye C (2008). Polychlorinated dibenzo-*p*-dioxins/furans and polychlorinated biphenyls in human adipose tissue from Zhejiang Province, China, *Chemosphere* 74: 384–388.

Smith AH, Lopipero P (2001). Evaluation of the toxicity of dioxins and dioxin-like PCBs: A health risk appraisal for the New Zealand population. A report to the New Zealand Ministry for the Environment, 17–101.

Song M, Jiang Q, Xu Y, Liu H, Lam PKS, O'Toole DK, Zhang Q, Giesy JP, Jiang G (2006). AhR-active compounds in sediments of the Haihe and Dagu Rivers, China, *Chemosphere* 63: 1222–1230.

Spencer KL, MacLeod CL (2002). Distribution and partitioning of heavy metals in estuarine sediment cores and implications for the use of sediment quality standards, *Hydrology and Earth System Sciences,* 6 (6): 989–998.

Sprovieri M, Feo ML, Prevedello L, Manta DS, Sammartino S, Tamburrino S, Marsella E (2007). Heavy metals, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in surface sediments of the Naples harbour (southern Italy), *Chemosphere* 67: 998–1009.

Srog K (2008). Levels and congener distributions of PCCDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review, *Environ Chem Lett,* 6: 1-28.

Sulzman EW (2007). Stable isotope chemistry and measurement: a primer *in* Michener R, Lajtha K, (*Eds.*), Stable Isotopes in Ecology and Environmental Science, Second Edition, Blackwell Publishing Ltd.

Tam NFY, Wong YS (2000). Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps, *Environmental Pollution* 110: 195-205.

Tam NFY, Wong YS, (1996). Retention and Distribution of Heavy Metals in Mangrove Soils Receiving Wastewater, *Environmental Pollution* 94 (3): 283-291.

Tam NFY, Yao MWY (1998). Normalisation and heavy metal contamination in mangrove sediments, *The Science of the Total Environment* 216: 33-39.

Taylor M, Ravilious C, Green EP (2002). Mangrove of East Africa, Vol. 12, UNEP World Conservation Monitoring Centre, *Biodiversity Series* No. 13, 26 pp.

Terauchi H, Takahashi S, Lam PKS, Min BY, Tanabe S (2009). Polybrominated, polychlorinated and monobromo-polychlorinated dibenzo-p-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls in marine surface sediments from Hong Kong and Korea, *Environmental Pollution* 157: 724–730.

Tranchina L, Basile S, Brai M, Caruso A, Cosentino C, Miccichè S (2008). Distribution of Heavy Metals in Marine Sedimentsof Palermo Gulf (Sicily, Italy), *Water Air Soil Pollut.* 191: 245–256.

Tyler AO, Millward GE (1996). Distribution and Partitioning of Polychlorinated Dibenzo-p-dioxins, Polychlorinated Dibenzofurans and Polychlorinated Biphenyls in the Humber Estuary, UK, *Marine Pollution Bulletin* 32 (5): 397-403.

Van Camp, M., Mjemah, I. C., Al Farrah, N., Walraevens, K. (2013). Modeling approaches and strategies for data-scarce aquifers: example of the Dar es Salaam aquifer in Tanzania, *Hydrogeology Journal* 21: 341–356.

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., *et al*. (2006). The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences: an official journal of the Society of Toxicology* 93 (2): 223–41.

Van Langenhove K, Croes K, Denison MS, Elskens M, Baeyens W (2011). The CALUX bio-assay: Analytical comparison between mouse hepatoma cell lines with a low (H1L6.1c3) and high (H1L7.5c1) number of dioxin response elements, *Talanta* 85: 2039– 2046

Vicente-Beckett V, Shearer D, Munksgaard N, Hancock G, Morrison H (2006). Metal and Polycyclic Aromatic Hydrocarbon contaminants in benthic sediments of Port Curtis, Technical Report 83, Cooperative Research centre for Coastal Zone, Estuary and Waterway Management, Australia.

Wang H, He M, Lin C, Quan X, Guo W, Yang Z (2007). Monitoring and Assessment of Persistent Organochlorine Residues in Sediments from the Daliaohe River Watershed, Northeast of China, *Environ Monit Assess* 133: 231–242.

Windal I, Denison M, Birnbaum L, Vanwouwe N, Baeyens W, Goeyens L (2005). Chemically Activated Luciferase Gene Expression (CALUX) Cell Bioassay Analysis for the Estimation of Dioxin-Like Activity: Critical Parameters of the CALUX Procedure that Impact Assay Results, *Environ. Sci. Technol.* 39: 7357-7364.

Woods A (2009). Tracing the Distribution of Heavy Metals in the Sediments of the Pearl River Estuary. *M.Sc. Thesis*, Durham University.

Wright J (2003). Environmental Chemistry, Routledge, Francis &Taylor Group.

Yang Z, Shen Z, Gao F, Tang Z, Niu J (2009). Occurrence and possible sources of polychlorinated biphenyls in surface sediments from the Wuhan reach of the Yangtze River, China. *Chemosphere* 74 (11): 1522–30.

Yu F, Zong, Y, Lloyd JM, Huang G, Leng MJ., Kendrick C, Lamb AL, Yim WWS (2010). Bulk organic $\delta^{13}C$ and C/N as indicators for sediment sources in the Pearl River delta and estuary, southern China, *Estuarine, Coastal and Shelf Science* 87: 618 -630.

Zhang H, Zhao X, Ni Y, Lu X, Chen J, Su F, Zhao L, Zhang N, Zhang X (2010). PCDD/Fs and PCBs in sediments of the Liaohe River, China: Levels, distribution, and possible sources *Chemosphere*, 79: 754–762.

Zhang J, Wu Y, Jennerjahn TC, Ittekkot V, He Q (2007). Distribution of organic matter in the Changjiang (Yangtze River) Estuary and their stable carbon and nitrogen isotopic ratios: Implications for source discrimination and sedimentary dynamics, *Marine* Chemistry 106: 111–126.

Zhao L, Hou H, Zhou Y, Xue N, Li H, Li F (2010). Distribution and ecological risk of polychlorinated biphenyls, and organochlorine pesticides in surficial sediments from Haihe River and Haihe Estuary Area, China, *Chemosphere* 78: 1285–1293.

Zhou F, Guo H, Hao Z (2007). Spatial distribution of heavy metals in Hong Kong's marine sediments and their human impacts: A GIS-based chemometric approach, *Marine Pollution Bulletin* 54 (9): 1372-1384.

CHAPTER TWO

USING CARBON AND NITROGEN COMPOSITION, STABLE ISOTOPES AND C/N RATIO TO TRACE SOURCES AND FATE OF ORGANIC MATTER IN THE TANZANIAN COASTAL SEDIMENTS

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CHAPTER TWO: CARBON AND NITROGEN DYNAMICS IN TROPICAL ESTUARINE MANGROVE SEDIMENTS OF MTONI, TANZANIA

2.1 Abstract

Elemental (C, N) and isotopic (δ^{13}C, δ^{15}N) signatures were used as proxies to identify changes in the proportions of sedimentary organic matter (OM) in a tropical estuarine environment, Mtoni estuary and tributaries Mzinga and Kizinga (Tanzania). Sandy particles dominated the mangrove sediments, with less than 6% of OM in the estuary and less than 9% in the tributaries. Similarities in the levels of total organic carbon (TOC) and total nitrogen (TN) with depth were observed at all the stations, indicating a homogenous, well-mixed top 9 cm sediment layer. A clear gradient of TOC and TN levels from the Kizinga and Mzinga riverine stations to the estuarine mouth is observed with highest content in the most upstream stations and a decrease towards the mouth of the estuary. No clear seasonal trend of C/N ratio was observed except in the confluence region where the ratios were higher in wet than dry season. Wet season δ^{15}N values were lower while δ^{13}C values were higher compared to dry season values for all the stations. Mzinga δ^{15}N values were higher than Kizinga values in both seasons while the opposite was observed for δ^{13}C, but all were lower than confluence values. Correlations of the geochemical parameters for both wet and dry seasons suggest that OM in Mtoni sediments originated mainly from sources that are ^{15}N enriched (3.48‰ to 8.14‰) and ^{13}C depleted (-30.8‰ to -23.9%) and are linked to OM degradation processes. Combined elemental and isotopic signatures indicated that wet season values were dominated by sewage material while dry season values were dominated by mangrove material. Quantitative estimation of the contribution of the identified sources indicated that sewage material contributed between 60% and 90% of the sedimentary organic matter in the wet season and mangrove litter between 44% and 98% in the dry season.

2.2 Introduction

Mangrove ecosystems represent a crucial component of global ocean carbon and nutrient budgets due to their ability to accumulate and store a large amount of OM (Bouillon *et al.,* 2007). OM from various sources is trapped in the mangrove sediments followed by subsequent mixing by bioturbation and decomposition (Kristensen *et al.,* 2008). The amount and origin of OM will depend on biological (consumption, removal), chemical (oxidation) and physical (tidal dynamics) factors acting on mangrove sediments (Bouillon *et al.,* 2003). Mangrove areas constitute natural reactors which can be affected by heterogeneous processes due to local changes in the sedimentation, biogeochemical processes as well as salinity conditions (Marchand *et al.,* 2008).

Studies to evaluate OM sources in marine sediments can be done using carbon and nitrogen ratio (C/N) and stable isotopic signatures (e.g. Hu *et al.,* 2006; Marchand *et al.,* 2008). The use of C/N ratios relies on the ability to distinguish and account for the varying sources of OM being transported into a mixed water system such as mangrove estuaries. Fluctuations in this ratio over time may represent a shift in the sediment source at a given location (Woods, 2009). Specifically, C/N ratio has been used to describe the fractional contributions of different end-members contributing to the OM of a given area. For example, a low C/N ratio (< 10) indicates the dominance of OM originating from N-rich planktonic processes or bacterial immobilisation of N during degradation (Meyers, 1994; Cifuentes *et al.,* 1996; Ruttenberg & Goñi, 1997; Lü and Zhai, 2006). On the other hand, the presence of high percentages of terrestrial OM is usually reflected by a higher ratio of C/N (>10) in sediments (Thornton and McManus, 1994; Ruttenberg and Goñi, 1997; Andrew *et al.,* 1998; Lü and Zhai, 2006; Fernandes *et al.,* 2011).

Stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) have been used to determine the presence and/or effects of biogeochemical processes in marine environment (Salazar-Hermoso, 2007). OM isotopic composition generally can be used to, among others, identify sources (Hu *et al.,*2006), inferring processes (e.g., heterotrophic nitrification), estimate rates (Sulzman, 2007) and pathways of OM on the basis of the fact that: (i) consumers fractionate carbon and nitrogen in predictable ways that allow for the identification of the isotopic composition of their food resource(s), and (ii) relative contributions of some primary producers can be differentiated by the variation in the carbon isotopic composition of plants as a result of differences in growth environment and the biochemical pathways of photosynthesis and biosynthesis (Cloern *et al.,* 2002; Machiwa, 2010). In aquatic systems, the conservative nature of stable isotopes and the physical mixing of end-member sources determine the isotopic distributions of OM (Cifuentes *et al.,* 1988) and allow discrimination of terrestrial and marine sources (Middelburg and Nieuwenhuize, 1998; Ramaswamy *et al.,* 2008). For example, low $\delta^{13}C$ values in particulate OM are indicative of selective degradation of OM and dominance of the terrestrially derived carbon (Ogrinc *et al.,* 2005; Lü and Zhai, 2006). High $\delta^{13}C$ values indicate dominance of macrophytes or autogenic sources (Lü and Zhai, 2006). High $\delta^{15}N$ values in sediment are an indication of bacterial metabolism or high degree of nutrient utilisation in highly ranked trophic level organisms. Low $\delta^{15}N$ values indicate productivity and nutrient level variations that occur due to selective degradation and fractionation during degradation of OM (Ogrinc *et al.,* 2005).

Mangrove environments in coastal areas have been increasingly impacted by various socio-economic activities but also natural events such as tsunamis. Anthropogenic and natural changes including industrial and domestic pollution (oil spills, effluents, wastes and global climate change) are among the unprecedented threats. This is aggravated by the unsustainable use of coastal resources as well as pressures from the growing coastal population. In Tanzania, significant amounts of different agricultural, domestic and industrial wastes are discharged into streams, rivers and estuaries. With continuously high influx of anthropogenic products from increased urban population, industrial and agricultural activities, sewage input and burning of solid wastes, the magnitude of the problem cannot be underestimated. Hence, the type, nature and content of OM in Tanzanian mangrove sediments and their C/N, $\delta^{13}C$ and $\delta^{15}N$ may reveal different signatures. Knowing that mangrove sediments can provide an important record of anthropogenic impact on the coastal environments, we used carbon and nitrogen composition, C and N stable isotopes and C/N ratios, to determine the sources and to assess the contribution of anthropogenic factors to the deposition and accumulation of OM in the Mtoni estuary.

2.3 Methodology

2.3.1 Study area

The Mtoni estuary (Figure 2.1) is located at approximately 3 km south of Dar es Salaam (Tanzania) and is fed by 2 rivers: Kizinga and Mzinga. The creeks have mangrove trees such as *Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora mucronata and Sonneratia alba* species growing on both sides (Mlay *et al., unpublished).*

The fresh water input from both rivers is low. An average base-line flow rate of 1 m^3/s is observed in Kizinga River with an increase up to $8m^3/s$ in the rainy season while the water-flow rate in the Mzinga River is unstable and lower than in the Kizinga River (Van Camp *et al.,* 2013). Hence, the effect of the river discharges on the hydrodynamics of the Mtoni estuary is very limited. The seven sampling stations (E1 to E7) are located in the mixing zone and their salinities vary from almost fresh to brackish water with somewhat higher salinities in the dry season (Mangion, 2011). Downstream this mixing zone (stations F5 to F8) the water becomes rapidly sea water while the stations F1 to F4 more upstream have fresh water. This estuarine mixing zone was selected because it integrates influences of natural and anthropogenic sources in the riverine and marine systems.

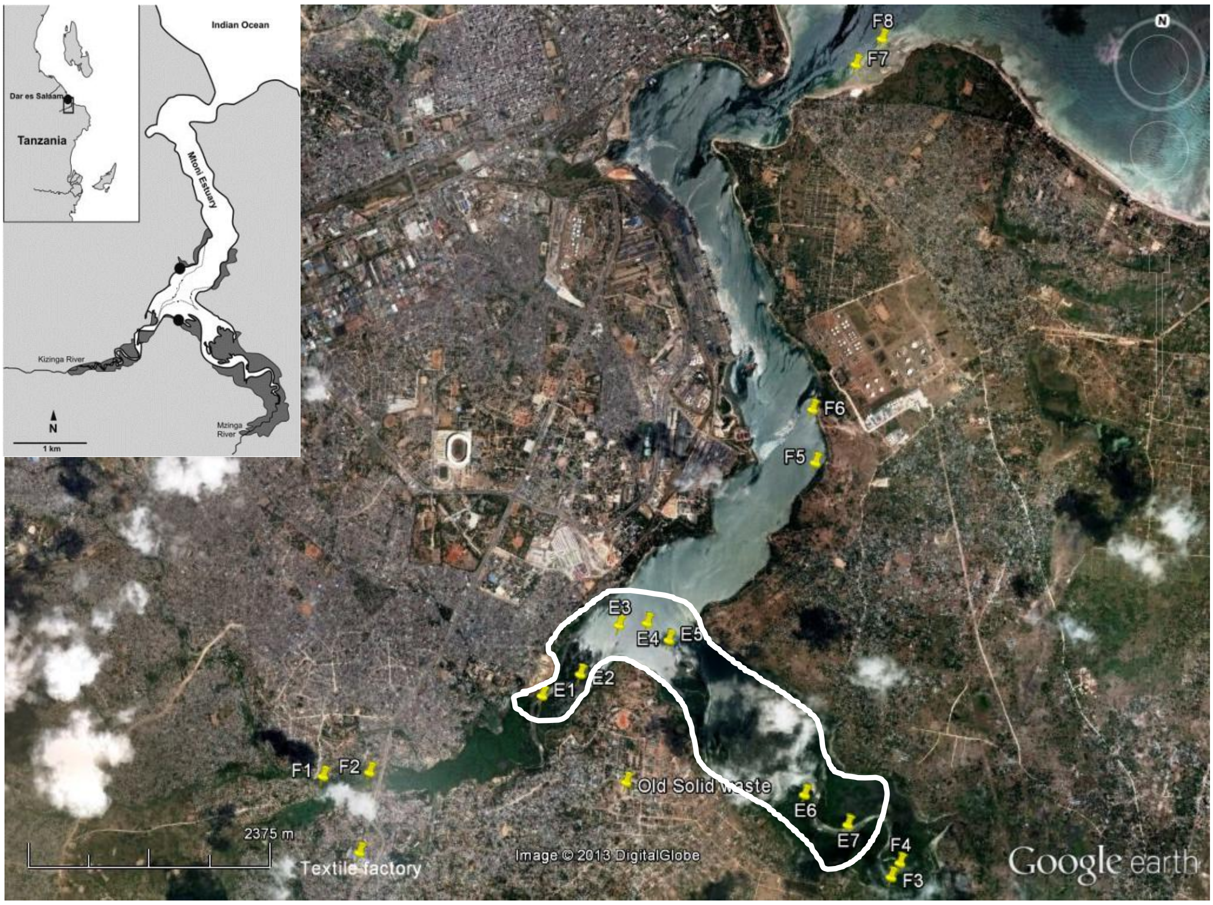

Figure 2.1: **Sampling points in the Mtoni estuary, Dar es Salaam: E1 and E2 in the Kizinga River, E3-E5 at the confluence and E6 and E7 in the Mzinga River. Stations F1-F2 in the Kizinga River, F3-F4 in the Mzinga River, F5-F6 at the Navy shore and F7-F8 at Kigamboni Seaway are additional sampling points. White line delimits the estuarine mixing zone**

The Mtoni estuary is highly impacted (PUMPSEA, 2007) by discharges of various origin: (1) the Kizinga and Mzinga rivers draining the mangrove forest (Kruitwagen *et al.,* 2008), (2) the wastewater drainage systems from industrial and residential areas (of a population of around 500,000 inhabitants; NBS, 2003), (3) charcoal burning, (4) mangrove harvesting for residential places, (5) salt mining, (6) tourism and (7) agriculture (Taylor *et al.,* 2002).

The Kizinga river that drains the urbanised areas of Keko, Chang'ombe, Kurasini and Temeke (approximately 400,000 inhabitants; NBS, 2003) is suspected to carry a variety of wastes and discharges originating from agricultural, industrial as well as residential sources (Taylor *et al.,* 2002). The Mzinga river, on the other hand, drains the rural areas of Vijibweni, Tuangoma and Mji Mwema with a population of around 90,000 (NBS, 2003). Due to rapid growth of settlements along the Mzinga creek resulting from increased human population, the river is suspected to carry agricultural and residential wastes and discharges presumed to be emptied into the creek. The estuary further receives inputs from the Dar es Salaam harbour which is located near the mouth of the estuary during diurnal tides (up to 5 m amplitude) and from the Mtoni solid waste dumping site located in between the two rivers.

2.3.2 Sampling

Sampling of sediments was conducted in the mangrove forests during low tides at Kizinga and Mzinga creeks of the Mtoni estuary (Figure 2.1). Two sampling campaigns were conducted in the estuarine mixing zone (salinity between 20 and 35, Mangion 2011): one during the wet season (19th - 20th January 2011) and a second during the dry season (15th - 16th August 2011). December and January have an average precipitation rate of 194 and 89 mm, respectively, while these rates in July and August are much lower with 48 and 47 mm respectively. River flows in the Dar-es-Salaam area are mainly controlled by the precipitation rate in the previous period. The flows of Kizinga and Mzinga rivers are highest in the wet season (the highest discharge rates can go up to 15 m^3/s for the Kizinga river and 7 m^3/s for the Mzinga river) while in the dry season base-line flows of 1 m^3/s in the Kizinga river and even lower in the Mzinga river were observed (Van Camp *et al.,* 2013). The impact of both rivers on the pollutant levels in the mixing zone can thus best be estimated by sampling in that zone at high (wet season) and at low (dry season) river flow and comparison of the results. Samples were collected from exactly the same locations (seven sampling stations) identified with a hand-held global positioning system (GPS). Two stations were located in the Kizinga River (E1 and E2), two in the Mzinga River (E6 and E7) and three at the confluence of the two rivers (E3, E4 and E5).

In addition, in order to better understand the sedimentary organic matter characteristics in the studied estuarine mixing zone (samples E1-E7), a complementary sampling campaign was organised during dry season (2nd October 2012) at 3 locations that are representative of organic matter sources end-members: one site in the freshwaters of Kizinga River (positions F1-F2), one site in the freshwaters of Mzinga River (positions F3-F4) and two sites in the downstream marine waters close to (positions F5-F6) and at the mouth of the estuary (positions F7-F8) (Figure 2.1). All those samples were taken from two sub-sites within a distance of 20 m, except in the Kizinga River. Sample F2 in the Kizinga River was taken at the junction of the river and a textile wastewater stream, while F1 was taken 200 m upstream of F2 very close to unauthorised human settlements.

Sediment sampling during the sampling campaigns was done as described by EPA, (2001) using a hand corer (30 cm height, 6 cm internal diameter). The corer was gently pushed in the mangrove sediments, closed at its upper end with a lid and smoothly removed by twisting and pulling. The sediments were then pushed out of the corer tube using a piston and sectioned into three segments corresponding to depth intervals of 0-3, 3-6 and 6-9 cm. Sampling of complementary sediment samples, however, was done by scooping the top 5 cm sediment layer. All sediment samples were packed in prior labelled and zipped polyethylene bags, stored in iceboxes and later frozen to −20 °C. Sediment samples were then air-transported while frozen to the Laboratory of the Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel (VUB) in Belgium and lyophilised (Leybold Heraus Lyophiliser) prior to the various geochemical analyses (grain size, total and organic carbon and nitrogen, stable carbon and nitrogen isotopes).

2.3.3 Determination of Grain Size Distribution

The grain size distribution was determined at 3 stations with low (station E7), medium (station E2) and high OM content (station E1) in their sediments. Approximately 10 g lyophilised and homogenised sediment sample was prepared by removing salts, OM and carbonates using hydrogen peroxide and hydrochloric acid, respectively. A stable suspension was obtained after rinsing and adding a peptising agent (5 ml). The coarse fraction (above 75 µm) was separated by wet sieving on a 75 µm sieve, then dried at 105°C, and finally dry sieved. The grain-size distribution of the fine fractions 2-75 µm and <2 µm was obtained using the Sedigraph 5100 coupled to a Mastertech 51. The precision for 10 consecutive measurements on aliquots of the same sample was around 1% for every grain-size fraction.

2.3.4 Elemental Carbon and Nitrogen Analyses

Total Carbon (TC) analysis was carried out on untreated sediments placed in tin capsules (about 10 mg) while Total Organic Carbon (TOC) and Total Nitrogen (TN = organic nitrogen) analysis was carried out on pre-treated sediments (acidification of the sample with 5% HCl to remove inorganic components) placed in silver capsules (about 12 mg). For the analyses, a Flash 1112 EA Elemental Analyser (Thermo Finnigan) including a High Temperature Oxidative Combustion (HTOC) unit at 1020°C, was used. The Total Inorganic Carbon (TIC) content was determined from the difference between TC and TOC measurements. Calcium carbonate, $CaCO_3$ content was calculated as a weight percentage from the TIC content assuming that all assessed inorganic carbon was present as $CaCO_3$. A conversion factor of 8.33 was derived from the ratio of molecular mass of carbonate (100.1) to carbon (12.0).

2.3.5 Stable Carbon and Nitrogen Isotopic Analyses

The stable organic carbon isotope ($\delta^{13}C$) composition of the sedimentary OM was determined by analysis of pre-treated sediment sub-samples with 5% HCl, while its stable nitrogen ($\delta^{15}N$) composition was determined on untreated sub-samples. The analyses were accomplished by using a Flash 1112 EA Elemental Analyser (Thermo Finnigan) interfaced to a Delta Plus IRMS (Thermo Electron) operating in a continuous-flow mode according to Bouillon *et al.,* (2004). Data quality control was checked by running a reference standard after every seven samples. The instrument was calibrated using a series of certified standards in quantities (0.1, 0.2, 0.5, 0.7 and 1.0 mg) from the International Atomic Energy Agency (IAEA CH6, sucrose, and IAEA N1, ammonium sulphate), that gave a linear range of isotope ratio values for C and N corresponding to responses within the ranges of the isotopic carbon and nitrogen values in the samples. All stable isotopic data were reported in standard delta notation where the $\delta^{13}C$ and $\delta^{15}N$ are given as the per mil deviation from the C and N-isotope composition of the standards, respectively Pee Dee Belemnite for ^{13}C, and atmospheric N_2 for ^{15}N:

$$\delta(\mathrm{y}) = \left(\frac{R_{sample}}{R_{std}} - 1 \right) x10^3 \qquad (1)$$

where y = ^{13}C or ^{15}N, and R = the ratio of the heavy isotope (^{13}C or ^{15}N) over the light isotope (^{12}C or ^{14}N). Analytical precision ranged from 0.2 to 2.7‰ for carbon and from 0.4 to 2.7‰ for nitrogen.

2.4 Results

2.4.1 Grain size Distribution

Sandy particles dominated the mangrove sediments in the study area, with sand (> 75 µm) contributing for more than 60% of the weight. Highest TOC content in sediments was associated with the finest ganulometry. TOC content correlated well with the finest grain size fraction (< 2 µm, r^2 = 0.92) as well as with the mud+silt fraction (< 75 µm, r^2 = 0.82). It is well-known that fine muddy sediments show higher TOC contents (Baeyens *et al.,* 1991). In the Mtoni estuary, the high sand proportion implies that the mangrove sediments favour abiotic processes such as enhanced diffusion of oxygen in the sediment allowing faster oxidation of organic matter (Holmer, 2003; Davies & Tawari, 2010).

2.4.2 Organic Carbon and Nitrogen in Mtoni estuary Sediments

Only small vertical variations in the C and N contents and the ^{13}C and ^{15}N isotopic compositions of the sediments at the seven sampling stations were observed, indicating a well-mixed top 9 cm layer of these sediments. Results in this paper are therefore further presented as depth averaged values for the top 9 cm of the sediments.

2.4.2.1 TOC and TN levels and C/N ratios

The TOC content in the estuarine mixing zone varied between 0.58 ± 0.07% and 5.66 ± 2.83% during wet season and between 0.65 ± 0.18% and 3.63 ± 0.65% during dry season. The percentage levels of TIC and hence $CaCO_3$ content were generally very low and can thus be ignored. TN content in the estuarine mixing zone varied from 0.06 ± 0.03% to 0.28 ± 0.03% in wet season and from 0.09 ± 0.02% to 0.22 ± 0.1% during the dry season.

There were no obvious seasonal trends in TOC and TN levels between wet and dry seasons (Figures 2.2a and b). For TOC, higher levels were observed during wet season in the Mzinga stations E6 (3.36 ± 0.97%) and E7 (5.66% ± 2.83%) as well as at two of the stations located at the confluence E3 (3.31 ± 0.67%) and E5 (1.56 ± 0.75%). In contrast, Kizinga station E1 (1.95 ± 0.24%), had higher percentages of TOC during dry season while the other stations (E2 in the Kizinga and E4 in the confluence region) showed no seasonal difference. For TN, both Kizinga stations as well as one of the stations in the mixing region (E4) had lower TN in wet season compared to dry season, while the opposite was observed at two of the stations in the mixing zone and one of the stations in Mzinga River.

There was, however, a clear difference between the TOC and TN levels observed in the sediments of the two rivers (stations E1 and E2 versus stations E6 and E7). Percentage levels of TOC and TN were lower in Kizinga stations (TOC: 0.58 ± 0.07% - 1.95 ± 0.24%; TN: 0.06 ± 0.03 – 0.15 ± 0.05%) than in Mzinga stations (TOC: 1.58 ± 0.2% - 5.66 ± 2.83%; TN: 0.13 ± 0.01 – 0.28 ± 0.03%) as well as in the confluence region (TOC: 0.65 ± 0.18% – 3.31 ± 0.67%; TN: 0.09 ± 0.02% - 0.2 ± 0.02%). So, higher values of both TOC and TN were generally restricted to the more rural Mzinga River compared to the other studied stations.

The TOC levels measured in the study area were similar to TOC observed in Indian mangrove sediments (0.6% - 31.7%, Bouillon *et al.,* 2003), Upper Boony estuary, Nigeria (0 – 5.4%, Davies and Tawari, 2010) and Mzinga mangrove sediment (2.2 – 4.8%, Mangion, 2011). However, the values were slightly higher than those observed (0 – 4%) by Barros *et al.,* (2010) in estuarine environments. Observed TN contents in Mtoni estuary were comparable to values observed in similar estuarine environments (0 – 0.35%, Barros *et al.,* 2010) and in similar mangrove sediments (0.17 – 0.32%, Mangion, 2011).

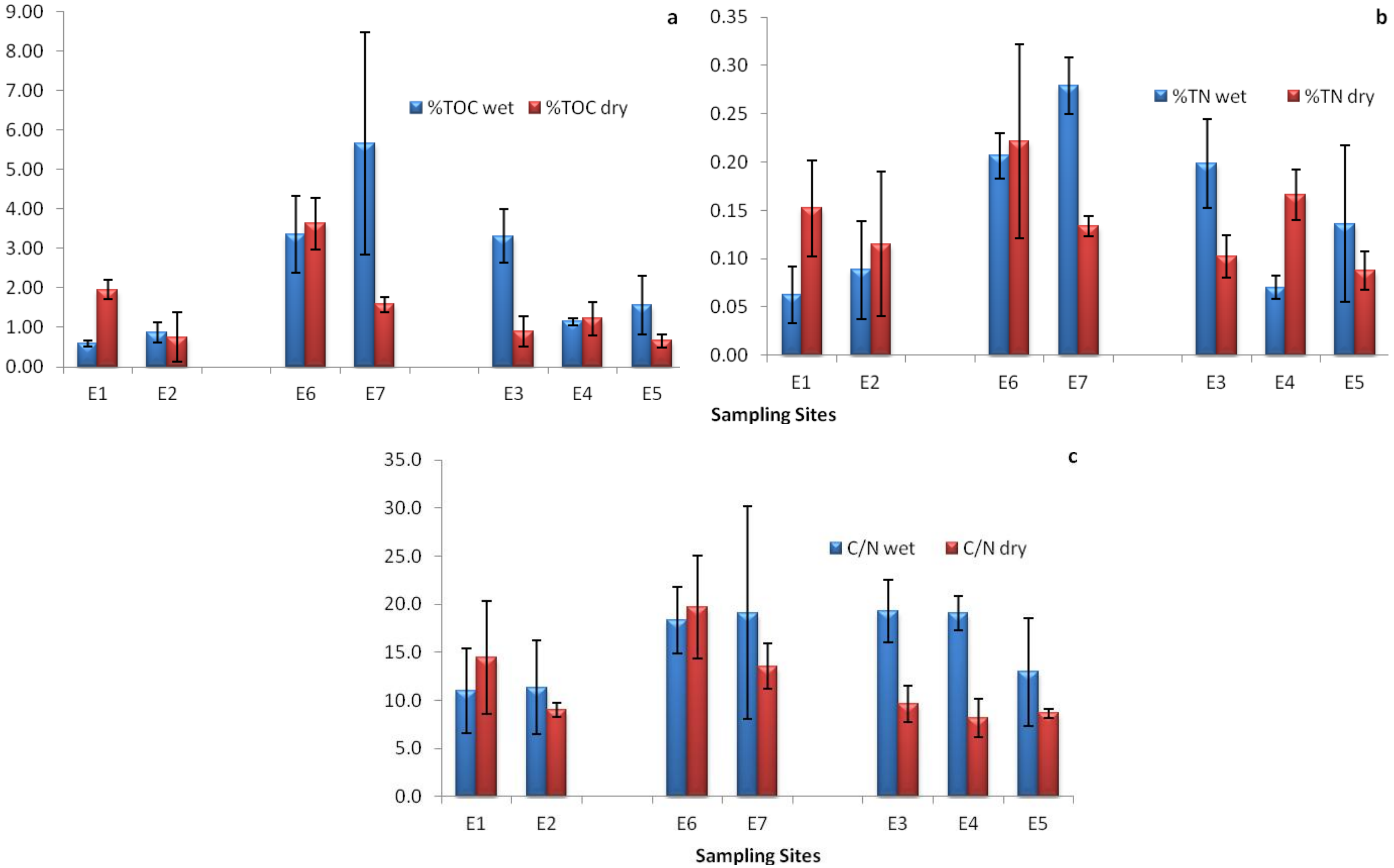

Figure 2.2: **Seasonal variations of percentage TOC (a), percentage TN (b) and C/N ratio (c) in the Mtoni estuary**

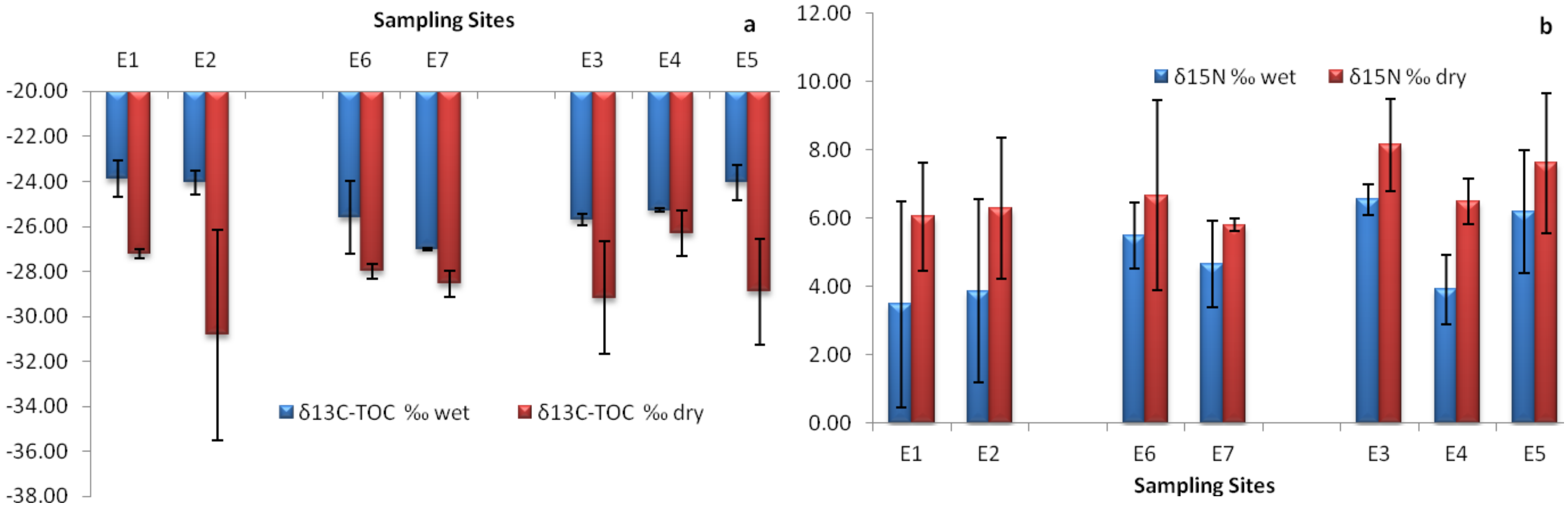

Figure 2.3: Seasonal variation of δ^{13}C (a) and δ^{15}N (b) in the Mtoni estuary

The C/N ratios in the sediments of the estuarine mixing zone varied from 9.4 ± 3.8 to 16.6 ± 2.8 during the wet season and from 7.0 ± 1.7 to 16.9 ± 4.6 during the dry season (Figure 2.2c). There was no clear seasonal trend of the C/N ratio in the upstream river samples. However, in the confluence region, C/N ratios were clearly higher (13.0 ± 6.7 – 16.9 ± 3.0) in the wet season than in the dry season (7.4 ± 0.4 – 8.5 ± 1.8). In addition, higher levels of C/N ratios were observed in more rural Mzinga stations (11.8 ± 1.9 – 19.7 ± 8.6) compared to more urban Kizinga (7.7 ± 0.7 – 14.5 ± 7.7), while the confluence stations displayed an intermediate range (7.3 +1.8 – 16.9 ± 3). The values in the Mzinga stations were similar to those observed (14.8 – 17.1) in an earlier study in that area (Mangion, 2011). The C/N observed in Mtoni sediments were higher (5.5 – 11.3) than those observed in the Gulf of Thailand by Meksumpum *et al.,* (2005), but within the range of values (7.0 – 27.3) observed by Bouillon *et al.,* (2003) for similar mangrove sediments.

2.4.2.2 Isotopic composition

Carbon isotopic values in the sediments of the estuarine mixing zone varied between −27 ± 0.05‰ and −23.9 ± 0.8‰ (mean -25.1 ± 0.1‰) during wet season and between −30.8 ± 4.7‰ and −26.3 ± 1.0‰ (mean -28.4 ± 0.2‰) during dry season (Figure 2.3a). Carbon isotopic values were similar to the values (-29.4‰ to −20.6‰) observed in Indian magrove sediments (Bouillon *et al.,* 2003) and Mzinga creek (-26.1 ± 0.3‰ to -24.9 ± 0.1‰, Mangion, 2011). However, the carbon isotopic values were depleted compared to values (-21.3‰ to -14‰) observed in Dar es Salaam coast by Muzuka (2001) and slightly more enriched than the values (-35‰ to -26‰) for sewage derived OM in creeks of Southern California (Ramírez-Álvarez *et al.,* 2007). Moreover, the observed values were generally lower (cf -21.6‰ to -20.2‰) than those observed in the Gulf of Thailand by Meksumpum *et al.* (2005).

Nitrogen isotopic values in the estuarine mixing zone ranged from 3.5 ± 3.0‰ to 6.6 ± 0.44‰ (mean 4.9 ± 1.2‰) during wet season and from 5.8 ± 0.2‰ to 8.1 ± 1.4‰ (mean 6.7 ± 0.7‰) during the dry season (Figure 2.3b). The observed values were similar to values (1.7‰ to 8.2‰) observed in coastal Dar es Salam by Muzuka, (2001), comparable to values (0.1 – 9‰) observed in sewage environments (Thornton and McManus, 1994; Maksymowska *et al.,* 2000; Ramírez-Álvarez *et al.,* 2007) and more or less similar to values (5.4 – 6.4‰) observed by Barros *et al.,* (2010) in a similar tropical estuary. However, the values were slightly lower than the values (7.4 ± 0.5‰ to 8.0 ± 0.1‰) observed in Mtoni sediments by Mangion (2011) and slightly higher than the range of 0 – 5‰ observed at the Southwestern coast of Thailand by Kuramoto and Minagawa, (2001) and in the Gulf of Thailand by Meksumpum *et al.,* (2005).

A seasonal variation in both C and N isotopic composition of Mtoni estuarine sediments was noticed. δ^{15}N values were lower while δ^{13}C values were higher in the wet season compared to dry season values for all the stations (Figure 2.3a-b). In addition, the isotopic composition also varied spatially during the wet season. The Mzinga δ^{15}N values were higher than Kizinga values in both seasons while the opposite was observed for the δ^{13}C, but all were lower than confluence values. Enhanced variability was observed in δ^{15}N values during the wet season and in δ^{13}C during the dry season (Figures 2.3a-b).

2.4.3 Correlations between geochemical parameters

2.4.3.1 TC and TOC

In order to estimate the contribution of inorganic carbon to the TC level, TC was plotted against TOC (Figure 2.4a) giving $r^2 = 0.98$. From that plot an intercept of 0.16 ± 0.1% was deduced indicating that there is only a very low contribution of inorganic carbon to the sediment carbon reservoir. More than 99% of the carbon pool in the sediments is organic carbon.

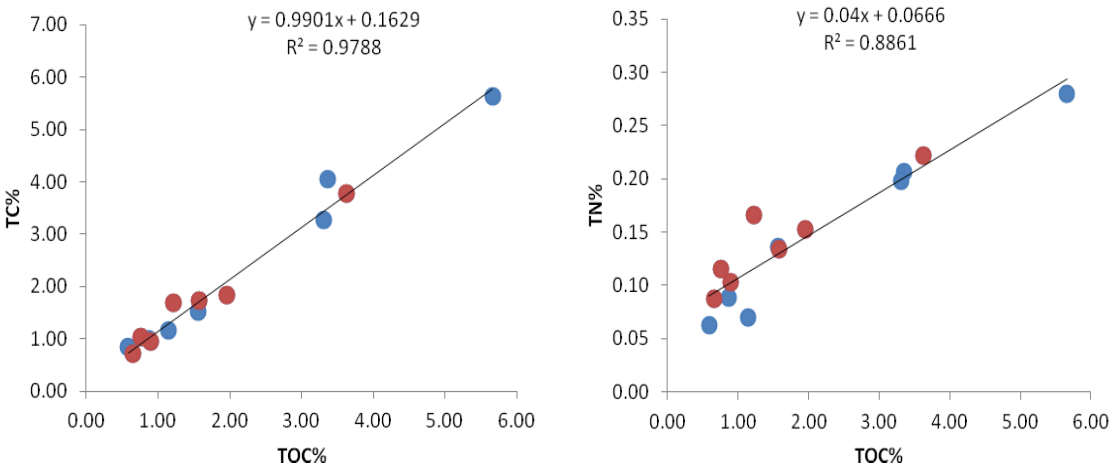

Figure 2.4: **Correlation between %TC and %TOC (a) and %TN and %TOC (b) in the Mtoni sediments. Blue dots indicate wet season and red dots indicate dry season**

2.4.3.2 TN and TOC

The correlation between TOC and TN was used for checking the organic nature of the nitrogen in the sediments. The intercept of that regression (Figure 2.4b) describes the level of diagenetic adsorption of inorganic components (Ku *et al.,* 2007). Indeed, part of the measured TN could be inorganic nutrients (NH_4^+ or NO_3^-) associated with the clay minerals. A good correlation between TOC and TN contents in sediments (slope = 0.04, intercept = 0.07, r^2 = 0.89, Figure 2.4b) was observed which is an indication that they originate from the same OM source (Fernandes *et al.,* 2011). The intercept of this plot (0.07 ± 0.01 %TN), indicates that there could be low amounts of nitrates and ammonium trapped in clay minerals. The slope of 0.04 ± 0.004 is indicative of the presence of OM originating from terrigenous plant vegetation (C/N range 20 – 50), but the contribution of the intercept strongly influences the final C/N ratio: at 1% TOC the C/N ratio amounts to 10 but at 5% TOC that ratio increases to about 20.

2.5 Spatial variation of geochemical parameters in the Mtoni estuary

In order to determine the spatial variation of the geochemical parameters in the estuary, data from all campaigns were used. A clear gradient from the Kizinga upstream stations (F1 and F2) to the estuarine mouth (F7 and F8) in the dry season was observed for the TOC and TN levels (Figure 2.5) with highest content in the most upstream stations and a decrease towards the mouth of the estuary. This is also the case for δ^{15}N values in the Mzinga River and for δ^{13}C values inthe Kizinga River (Figure 2.6).

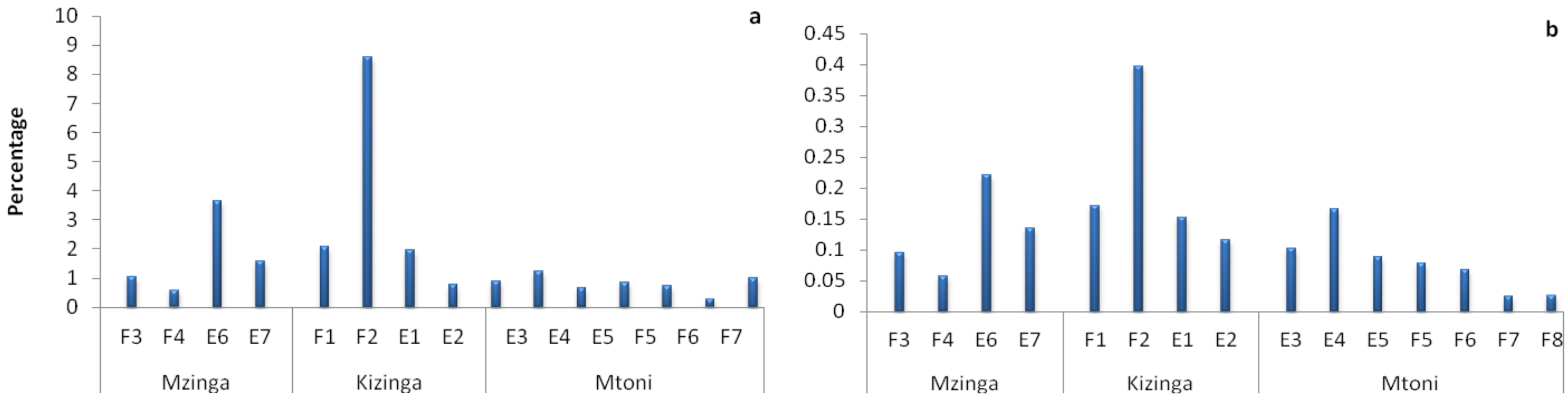

Figure 2.5: Variation of TOC (a) and TN (b) percentages in the Mtoni estuary. Station F2 is the point where the waste water from the textile industry enters the estuary through the Kizinga River

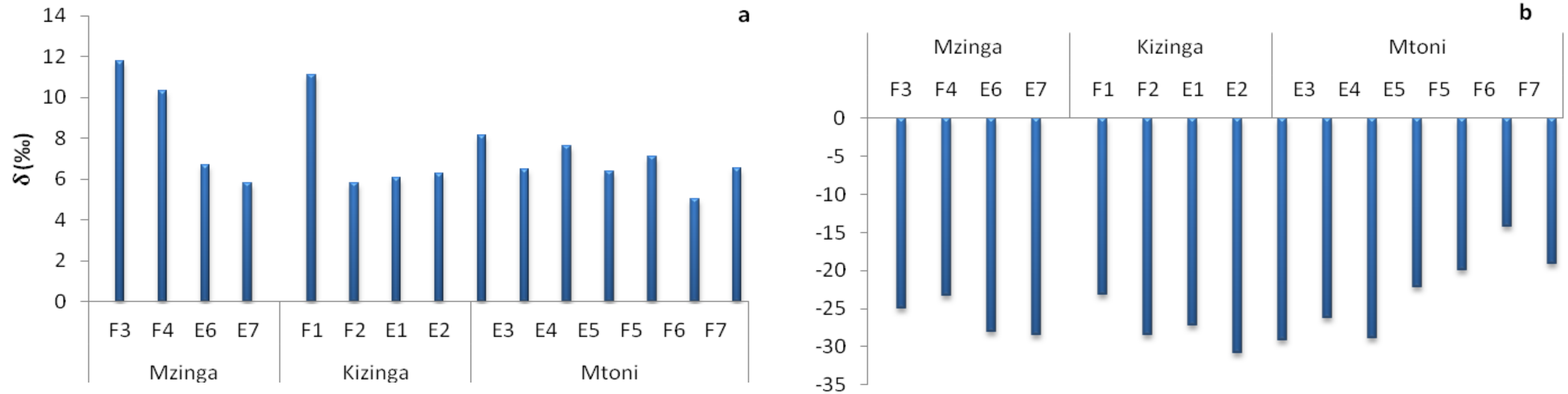

Figure 2.6: Variation of $\delta^{15}N$ (a) and $\delta^{13}C$ (b) in the Mtoni estuary. Station F2 is the point where the waste water from the textile industry enters the estuary through the Kizinga River

2.6 Discussion

2.6.1 Use of C/N ratio and isotopic compositions in sediments to trace the origin of OM in Mtoni estuary

2.6.1.1. General

Human impacted estuarine sediments may contain a large variety of terrigenous, marine and sewage material as potential contributors of OM (Liu *et al.,* 2006). Contribution of each of the sources can be strongly influenced by processes such as transport (tidal enhanced resuspension-settling events, river floods) as well as phytoplanktonic and terrestrial productivities and sewage discharges (Meyers, 1997). In addition, benthic re-mineralisation processes consume sedimentary OM. Both the initial source (Liu *et al.,* 2006; Fernandes *et al.,* 2011) and re-mineralisation processes (Meyers, 1994; Thornton and McManus, 1994) influence the OM characteristics such as the C/N ratio and the isotopic compositions.

The OM content in Mtoni sediments displayed little vertical variation which demonstrates the presence of a rather thick (9 cm) homogenous layer of surface sediments. In addition, there is a significant linear correlation between TN and TOC (Figure 2.4b) contents whatever the season or the sampling station, which is an indication of an invariable source that supplies OM to the estuarine sediments. Moreover, a study performed by Mangion (2011) in Mtoni showed that the carbon isotopic signature of suspended OM was very similar to the one of sediments ($\delta^{13}C$ = -25 to -29 ‰). All these findings suggest that the OM pool in the Mtoni estuarine sediments is submitted to hydrodynamic regimes that constantly homogenises the sediment's upper layer and thus the OM pool (Ruttenberg and Goñi, 1997). In these conditions, benthic OM in the sediment's surface layer is probably permanently mixed with a small amount of freshly deposited OM, while re-mineralisation processes are probably not important enough or anymore (perhaps most of the OM is refractory material) to significantly modify the OM characteristics. Observed variations in the C/N ratios and isotopic composition of the sedimentary OM in this system can thus reasonably be used to trace the origin of OM sources.

2.6.1.2. C/N ratios

OM in estuarine sediments can be originating from different sources which can be traced if their C/N ratios are discriminatory (Graham *et al*., 2001; Perdue and Koprivnjak 2007; Yu *et al*., 2010; Fernandes *et al*, 2011). Changes in C/N ratios usually indicate variations in proportions of OM from different origins (Fernandes *et al.,* 2011). Mtoni sedimentary OM may originate mainly from three main sources: marine OM (basically phytoplankton), mangrove terrestrial plants and urban sewage. Typical ranges of C/N ratios of the three sources are 6.67 for marine OM (= Redfield ratio), 7.7 to 20 for OM from urban sewage (Maksymowska *et al.,* 2000) and 23 to 70 for the organic material from the Mtoni mangrove trees (Mangion, 2011).

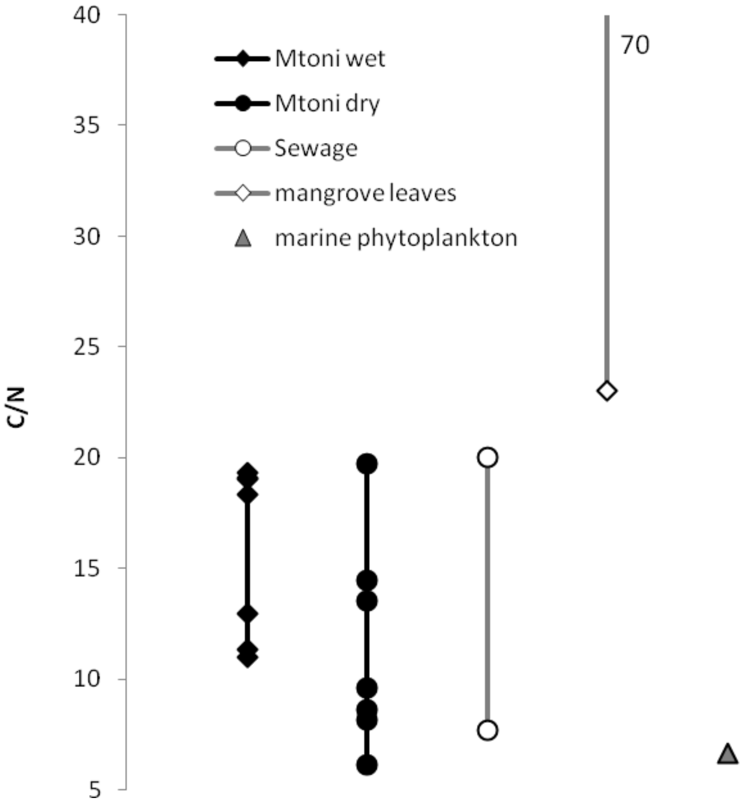

Figure 2.7: **C/N ratios in Sediments describing the different organic matter sources**

All the observed C/N ratios in Mtoni estuary (Figure 2.7) were in the range of sewage OM, above the Redfield ratio, and below the typical range of mangrove tree material. This wide range of C/N ratios (7.3 – 19.7) is an indication that the estuarine sediments incorporate OM from various sources but probably dominated by a sewage source.

However, C/N ratios alone are not a strong reliable indicator of OM sources in sediments because the system can receive OM originating from different sources and each of them can have similar or different C/N ratios. In addition, transport and selective degradation of OM components can alter the original ratios (Meyers, 1997; Ruttenberg and Goñi, 1997; Middlelburg and Herman, 2007). Hence, it is imperative to use the C/N ratios together with other source indicators such as data from stable C and N isotopes to restrain the uncertainties on the origin of the OM (Ruttenberg and Goñi, 1997). Elemental and isotopic values for different OM sources are described in literature (Thornton and McManus, 1994; Andrews *et al.,* 1998; Maksymowska *et al.,* 2000; McSween *et al.,* 2003; Rogers, 2003;, Middlelburg and Nieuwenhuize, 2004; Rwamaswamy *et al.,* 2008; Barros *et al.,* 2010; Sampaio *et al.,* 2010; Ogrinc *et al.,* 2010; Mangion, 2011).

2.6.2 Combined ^{15}N, ^{13}C and C/N ratios

Nitrogen isotopic signatures in sedimentary OM can distinguish various OM sources based on the different signatures between terrestrial and aquatic sources. The δ^{15}N values of the OM content in an estuarine system turns around 8.6‰ for plankton and 0.4‰ for terrigenous C_3 plants (Meyers, 1997). However, in Mtoni estuary which is a peri-urban ecosystem receiving nitrogen rich sewage waters, Mangion (2011) reported a δ^{15}N enrichment of the mangrove biomass with δ^{15}N values ranging between 7 and 12 ‰ (Table 2.1).

Table 2.1: The ^{13}C and ^{15}N ranges for various sources in the Mtoni estuary

Location	^{13}C (‰)	^{15}N (‰)	Reference
Mtoni mangrove sediment (wet)	-27.0 to -23.9	3.5 to 6.5	This study
Mtoni mangrove sediment (dry)	-30.8 to -28.0	6.3 to 6.5	This study
Mzinga mangrove sediment	-24.5	8.1	Mangion, (2011)
Mtoni mangrove forest	-28.9 to -27.3	7.2 to 11.7	Mangion, (2011)
Sewage	-22.6	5.0	Mangion, (2011)

Mangion (2011) further observed that δ^{15}N levels in suspended particulate matter and mangrove sedimentary OM correlated well, indicating that they originate from the same enriched source. There is thus no such agreement with the marine planktonic signature, which excludes it from being a significant source of OM in the estuary. The C/N ratios indicate that sedimentary OM from Mtoni estuary is more comparable to sewage than to mangrove materials. However, the δ^{15}N and δ^{13}C values in the OM of the sediments during the dry season are very similar to those of mangrove plants (Table 2.1).

The elevated δ^{15}N values in Mtoni estuary are attributed to ^{14}N-rich ammonia volatilisation occurring during degradation of wastewater and consequent oxidation of the remaining substrate producing an enriched δ^{15}N nitrate (Mangion, 2011). Highly enriched δ^{15}N values in OM reflect the result of specific biogeochemical processes where the light nitrogen compound leaves the system leaving the heavier one behind (Ramírez-Álvarez *et al.,* 2007). This scenario is common in sewage discharges where the δ^{15}N-depleted urea and ammonia are lost through volatilisation leaving an enriched δ^{15}N waste water (Sampaio *et al.,* 2010). The δ^{15}N signatures observed in the Mtoni estuarine sediments reflect the cumulative effects of sewage and effluent inputs carried by the rivers into the estuary as observed in other ecosystems (Prasad and Ramanathan, 2008).

Seasonal fluctuations observed for the carbon and nitrogen isotopic ratios can be attributed to slightly different sources between seasons. These are well illustrated by the combined δ^{15}N to δ^{13}C signatures (Figure 2.8) where wet season values were closer to sewage sources as compared to dry season values which were closer to mangrove tree values. The observed variations in sources could be accounted by the reduced flow of sewer related materials into the rivers during the dry season, thus increasing the OM contribution from mangrove trees. However, the overlap of δ^{13}C and δ^{15}N values in both seasons is evident, indicative of the presence of an invariably supply of OM from a source with depleted δ^{13}C and enriched δ^{15}N values similar to the observations made by Mangion, (2011) in mangrove sediments impacted with sewage discharges.

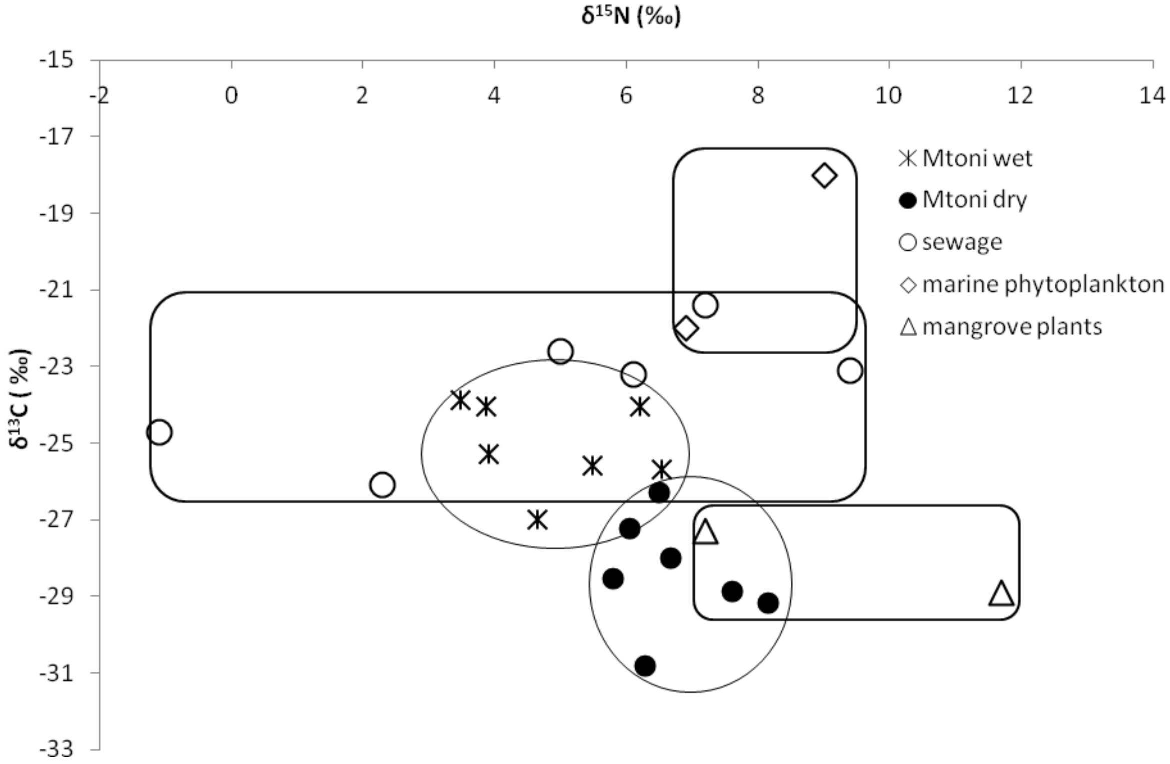

Figure 2.8: **Relationship between $\delta^{13}C$ and $\delta^{15}N$ in Mtoni sediment and different organic matter sources. Values for the sewage and mangrove end-members were adopted from Mangion, (2011).**

The C/N ratios and the plots of $\delta^{13}C$ versus $\delta^{15}N$ clearly identify sewage as a dominant source of OM in the sediments of the Mtoni estuary. Using the signatures, it is evident that spatial patterns and seasonal variability of OM in the Mtoni mangrove sediments are due to changes in the supply of sewage discharges from human and industrial areas. In the wet season, sewage supply is high, while in the dry season, OM from mangrove litter becomes evident due to a decreased riverine volume: this is illustrated by the corresponding $\delta^{13}C$ and $\delta^{15}N$ values (Figure 2.8) whereas the C/N ratios suggests rather sewage supply as the main source.

2.6.3 Use of isotopic ratios in sediments to estimate the contribution of different end-members to the total OM

It has been clearly displayed that despite the contribution from other sources, OM in the Mtoni estuary originates mainly from mangrove as well as sewage materials. In order to estimate the relative proportions of these sources to the total estuarine OM, a traditional isotopic mass balance mixing equation using a two end-member model similar to Schlunz *et al.,* (1999) and Barros *et al.,* (2010), has been applied. The proportions of sewage and mangrove sources were estimated using the equations:

$$f_{Sew} \times \delta^{13}C_{sew} + f_{man} \times \delta^{13}C_{man} = \delta^{13}C_{Sa} \qquad (2)$$

$$f_{sew} \times \delta^{15}N_{sew} + f_{man} \times \delta^{15}N_{man} = \delta^{15}N_{Sa} \qquad (3)$$

$$f_{sew} + f_{man} = 1 \qquad (4)$$

where $\delta^{13}C$ and $\delta^{15}N$ are the stable C and N isotopic composition of the sewage ($_{sew}$), mangrove ($_{man}$) and sample OM ($_{sa}$); and *f* are the fractional contributions of sewage and mangrove OM to the estuarine OM.

Median values obtained from this study and from literature were used for determining the fractional contributions of the different end-members to the total OM in the estuary. The $\delta^{13}C$ and $\delta^{15}N$ values employed in calculating the OM contributions are given in Table 2.2.

Table 2.2: **Isotopic C and N median values observed in Mtoni and for the identified end-members in the estuary**

Organic matter source	$\delta^{13}C$ value	$\delta^{15}N$ value
Sewage organic matter	-23.5	3.9
Mtoni wet season	-25.2	4.7
Mtoni dry season	-27.8	6.6
Mangrove trees	-28.1	9.5

The model results clearly indicate the dominance of sewage supply in the wet season and that of mangrove supply in the dry season as previously observed from the $\delta^{15}N$ and $\delta^{13}C$ plot (Figure 2.8). The sewage OM contribution in Mtoni sediments ranged from 60 to 90% in wet season and from 2 to 56% in dry season. The remaining OM contribution is from mangrove origin.

2.7 Conclusion

Carbon and nitrogen elements and their stable isotopic ratios in sediments of a tropical estuary, Mtoni estuary, have been determined. Findings have indicated that mangrove sediments from Mtoni estuary are impacted by sewage OM. This sewage OM has a high $\delta^{15}N$ value due to ^{15}N rich ammonia volatilisation occurring during degradation of wastewater. Correlations between sedimentary $\delta^{13}C$ and $\delta^{15}N$ and quantitative estimation of the contribution of each of the various sources have identified two major OM sources for the Mtoni estuary: sewage material in the wet season and mangrove litter during the dry season. Seasonal changes in sewage discharges could explain the spatial patterns and seasonal variability of OM in the Mtoni estuarine sediments. However, when using C/N ratios combined with either $\delta^{13}C$ or $\delta^{15}N$ values only suggest one major source: sewage input. The variability in C/N ratios is, however, less unambiguously related to OM source than it is for the isotope ratios of N and C.

2.8 Acknowledgements

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2.9 References

Andrews JE, Greenaway AM, Dennis PF (1998). Combined Carbon Isotope and C/N Ratios as Indicators of Source and Fate of Organic Matter in a Poorly Flushed, Tropical Estuary: Hunts Bay, Kingston Harbour, Jamaica, *Estuarine, Coastal and Shelf Science* 46: 743–756

Baeyens W, S. Panutrakul, M. Leermakers, J. Navez, M. Elskens (1991). Geochemical processes in sandy and muddy sediments, *Geo-Marine Letters* 11-3/4: 188-193.

Barros GV, Martinelli LA, Oliveira Novais TM, Ometto JPHB, Zuppi GM (2010). Stable isotopes of bulk organic matter to trace carbon and nitrogen dynamicsin an estuarine ecosystem in Babitonga Bay (Santa Catarina, Brazil), *Science of the Total Environment* 408: 2226–2232.

Bouillon SA, Dahdouh-Guebas F, Rao AVVS, Koedam N, Dehairs F (2003). Sources of organic carbon in mangrove sediments: variability, and possible ecological implications, *Hydrobiologia* 495: 33–39.

Bouillon S, Moens Tom, Koedam Nico, Dahdouh-Guebas Farid, Baeyens Willy, Dehairs Frank (2004). Variability in the origin of carbon substrates for bacterial communities in mangrove sediments, FEMS Microbiology Ecology 49: 171-179.

Bouillon S, Middelburg JJ, Dehairs F, Borges AV, Abril G, Flindt MR, Ulomi S, Kristensen E (2007). Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania), *Biogeosciences* 4: 311–322.

Cifuentes LA, Coffin RB, Solorzano L, Cardenas W, Espinoza J, Twilley RR (1996). Isotopic and Elemental Variations of Carbon and Nitrogen in a Mangrove Estuary, *Estuarine, Coastal and Shelf Science* 43: 781–800

Cifuentes LA, Sharp JH, Fogel ML (1988). Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary, *Limnol. Oceanogr.,* 33 (5): 1102-1115.

Cloern JE, Canuel EA, Harris D (2002). Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system, *Limnol. Oceanogr.,* 47 (3): 713–729

Davies OA,Tawari CC (2010), Season and tide effects on sediment characteristics of trans-okpokacreek, upper bonny Estuary, Nigeria, *Agric. Biol. J. N. Am.,* 1 (2): 89-96

EPA (2001). Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual, Office of Water (4305), EPA-823-B-01-002, U.S. Environmental Protection Agency, Washington, DC 20460.

Fernandes L, Nayak GN, Ilangovan D, Borole DV (2011). Accumulation of sediment, organic matter and trace metals with space and time, in a creek along Mumbai coast, India, *Estuarine, Coastal and Shelf Science* 91 (3): 388-399.

Graham MC, Eaves MA, Farmer JG, Dobson J, Fallick AE (2001). A Study of Carbon and Nitrogen Stable Isotope and Elemental Ratios as Potential Indicators of Source and Fate of Organic Matter in Sediments of the Forth Estuary, Scotland, *Estuarine, Coastal and Shelf Science* 52: 375–380.

Holmer M (2003). Mangroves of Southeast Asia *in* Black KD, Shimmield GB (Eds). Biogeochemistry of Marine Systems, Blackwell Publishin, CRC Press.

Hu J, Peng P, Jia G, Mai B, Zhang G (2006). Distribution and sources of organic carbon, nitrogen and their isotopes in sediments of the subtropical Pearl River estuary and adjacent shelf, Southern China, *Marine Chemistry* 98: 274– 285.

Kristensen E, Flindt MR, Ulomi S, Borges AV, Abril G, Bouillon S (2008). Emission of CO_2 and CH_4 to the atmosphere by sediments and open waters in two Tanzanian mangrove forests, *Mar Ecol Prog Ser* 370: 53–67.

Ku HW, Chen YG, Chan OS, Liu HC, Lin CC (2007). Palaeoenvironmental evolution as revealed by analysis of organic carbon and nitrogen: A Case of coastal Taipei Basin in Northern Taiwan, *Geochemical Journal* 41: 111-120.

Kuramoto T, Minagawa M (2001). Stable Carbon and Nitrogen Isotopic Characterization of Organic Matter in a Mangrove Ecosystem on the Southwestern Coast of Thailand, *Journal of Oceanography*, 57: 421-431.

Liu M, Hou LJ, Xu SY, Ou DN, Yang Y, Yu J, Wang Q, (2006), Organic carbon and nitrogen stable isotopes in the intertidal sediments from the Yangtze Estuary, China, *Marine Pollution Bulletin* 52: 1625–1633.

Lü X, Zhai S (2006). Distributions and sources of organic biomarkers in surface sediments from the Changjiang (Yangtze River), *Estuary, China, Continental Shelf Research* 26: 1–14.

Machiwa JF (2010). Stable carbon and nitrogen isotopic signatures of organic matter sources in near-shore areas of Lake Victoria, East Africa, *Journal of Great Lakes Research* 36: 1–8.

Maksymowska D, Richard P, Piekarek-Jankowska H, Riera P (2000). Chemical and Isotopic Composition of the Organic Matter Sources in the Gulf of Gdansk (Southern Baltic Sea), *Estuarine, Coastal and Shelf Science,* 51: 585–598.

Mangion P (2011) Biogeochemical Consequences of Sewage Discharge on Mangrove Environments, *PhD Thesis*, VUB, Belgium

Marchand C, Lallier-Vergès E, Disnar JR, Kéravis D (2008). Organic carbon sources and transformations in mangrove sediments: A Rock-Eval pyrolysis approach. *Organic Geochemistry* 39: 408–421.

Meksumpun S, Meksumpun C, Hoshika A, Mishima Y, Tanimoto T (2005). Stable carbon and nitrogen isotope ratios of sediment in the gulf of Thailand: Evidence for understanding of marine environment, Continental Shelf Research 25: 1905–1915.

Meyers P (1997). Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. *Org. Geochem.*, **27:** 213–250.

Meyers PA (1994). Preservation of elemental and isotopic source identification of sedimentary organic matter, *Chemical Geology* 114: 289-302

Middelburg JJ, Nieuwenhuize J (1998). Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary, *Marine Chemistry* 60: 217–225.

Middelburg JJ, Herman PMJ, (2007), Organic matter processing in tidal estuaries, *Marine Chemistry* 106: 127–147

Mlay AP, Wagner GM, Mgaya YD (*unpublished*). A comparative study of the ecology of four sandy/muddy shores in the Dar es Salaam area. Accessed on 23th August 2012 at http://gridnairobi.unep.org/chm/eafdocuments/tanzania/Ecology_of_muddy_shores_in_Tanzania.pdf

Muzuka ANN, (2001). Sources of organic matter in the Msasani Bay and Dar es Salaam harbour *In*: Richmond, MD, Francis J (*Eds.*) Marine Science Development in Tanzania and Eastern Africa. Proceedings of the 20th Anniversary Conference on Advances in Marine Science in Tanzania, 28th June - 1 July 1999, Zanzibar, Tanzania. IMS/WIOMSA, p. 61-76.

NBS - National Bureau of Statistics, (2003). Tanzania Populations and Housing Census: General Report, Accessed on 12th October 2012 at http://web.archive.org/web/20040313070101/http://www.tanzania.go.tz/census/census/tables.htm

Ogrinc N, Fontolan G, Faganeli J, Covelli S (2005), Carbon and nitrogen isotope compositions of organic matter in coastal marine sediments (the Gulf of Trieste, N Adriatic Sea): indicators of sources and preservation, *Marine Chemistry* 95: 163– 181.

Perdue EM, Koprivnjak JF (2007). Using the C/N to estimate terrigenous inputs of organic matter to aquatic environments, *Estuarine, Coastal and Shelf Science* 73: 65-72.

Prasad MBK, Ramanathan AL (2008). Sedimentary nutrient dynamics in a tropical estuarine mangrove ecosystem, *Estuarine, Coastal and Shelf Science* 80: 60–66.

PUMPSEA: Peri-urban mangrove forests as filters and potential phytoremediators of domestic sewage in East Africa (2007). Distribution and fate of heavy metals and indicator pathogens in sewage exposed mangroves, Specific Targeted Research Project Report for Project No. INCO – CT2004 – 510863.

Ramaswamy V, Gaye B, Shirodkar PV, Rao PS, Chivas AR, Wheeler D, Thwin S (2008). Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from the Ayeyarwady (Irrawaddy) continental shelf, northern Andaman Sea, *Marine Chemistry* 111: 137–150.

Ramírez-Álvarez N, Macías-Zamora J, Burke RA, Rodríguez-Villanueva LV, (2007). use of ^{13}C, ^{15}N and carbon to nitrogen ratios to evaluate theimpact of sewage-derived particulate organic matter on the benthic communities of the Southern California Bight, *Environmental Toxicology and Chemistry,* 26 (11): 2332–2338

Rogers KM, (2003). Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand, *Marine Pollution Bulletin* 46, 821–827

Ruttenberg RC, Goñi, MA (1997). Phosphorus distribution, C:N:P ratios, and δ^{13}C in arctic, temperate and tropical coast sediments: tools for characterising bulk sedimentary organic matter, *Marine Geology*, 139: 123-145

Salazar-Hermoso F (2007). Evaluation of Stable Isotopes for the Identification of Aquaculture Waste in the Aquatic Environment, *M.Sc. Thesis,* The University of Guelph, Canada

Sampaio L, Rodrigues AN, Quintino V, (2010). Carbon and nitrogen stable isotopes in coastal benthic populations under multiple organic enrichment sources, *Marine Pollution Bulletin* 60: 1790–1802

Schlunz B, Schneider RR, Muller PJ, Showers WJ, Wefer G (1999). Terrestrial organic carbon accumulation on the Amazon deep sea fan during the last glacial sea level low stand. *Chem. Geol.* 159**:** 263–281.

Sulzman EW (2007). Stable isotope chemistry and measurement: a primer *in* Michener R, Lajtha K (*Eds.*), Stable Isotopes in Ecology and Environmental Science, Second Edition, Blackwell Publishing Ltd.

Taylor M, Ravilious C, Green EP (2002). Mangrove of East Africa, Vol. 12, UNEP World Conservation Monitoring Centre, *Biodiversity Series,* No. 13, 26 pp.

Thornton SF, McManus J (1994). Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science* **38:** 219–233.

Van Camp, M., Mjemah, I. C., Al Farrah, N., Walraevens, K. (2013). Modeling approaches and strategies for data-scarce aquifers: example of the Dar es Salaam aquifer in Tanzania, *Hydrogeology Journal* 21: 341–356.

Woods A (2009). Tracing the Distribution of Heavy Metals in the Sediments of the Pearl River Estuary, *M.Sc. Thesis*, Durham University.

CHAPTER THREE

METAL DISTRIBUTION AND ENRICHMENT IN MANGROVE SEDIMENT PROFILES AS INDICATORS OF HUMAN INDUCED ENVIRONMENTAL CHANGE IN TANZANIAN COAST

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CHAPTER THREE: METAL DISTRIBUTION AND ENRICHMENT IN MANGROVE SEDIMENTS PROFILES AS INDICATORS OF HUMAN INDUCED ENVIRONMENTAL CHANGE IN COASTAL TANZANIA

3.1 Abstract

Mangrove sediment samples from the Kizinga and Mzinga creeks of Mtoni estuary were analysed for Al, Fe, Mn, Cr Ni, Cu, Zn, As, Sr, Cd and Pb. Metal levels in the sediment layers (0-3, 3-6, and 6-9 cm) showed no distinct depth gradient and there was no variation between wet and dry season. A clear gradient from the upstream of the Kizinga River to the estuarine mouth was observed for all metals, except As. This was also observed from upstream of the Mzinga River to the estuarine mouth. Pearson correlation matrix and Principal component analysis (PCA) indicated that the selected compounds could be separated in 3 groups: (1) Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb; (2) Cd and TOC, which were strongly anti-correlated and (3) Sr. Enrichment factors (EF) indicated that Sr was most probably derived from natural origin, while other metals in the Mtoni estuary originated from anthropogenic activities. The levels of anthropogenic metals are indicative of human induced environmental change and have repercussions on the future status of the estuary and of the coastal area of Tanzania as a whole.

3.2 Introduction

Many environmental pollutants emanating from various sources make the marine system as their final destination. Metal contaminants may enter the coastal environment via a number of pathways such as natural processes (including erosion of ore-bearing rocks, wind-blown dust, volcanic activity and forest fires) and processes derived from anthropogenic activities (Dell'Anno *et al.,* 2003; Chatterjee *et al.,* 2007; Tranchina *et al.,* 2008). Anthropogenic sources in mangrove ecosystems arise from industrial effluents and wastes, urban runoff, sewage treatment plants, runoff from agricultural field and domestic garbage dumps (MacFarlane and Bruchett, 1999, 2001; Dell'Anno *et al.,* 2003; Chatterjee *et al.,* 2007; Tranchina *et al.,* 2008). In addition, discarded automobiles and dumping metallic substances have been the common anthropogenic inputs of metals in the marine ecosystem (Kamau, 2002; Praveena *et al.,* 2010). As metals cannot be chemically degraded and are not subject to biological degradation, they are essentially a permanent addition to the aquatic environment. As a consequence, they get accumulated locally (MacFarlane and Bruchett, 2001; Defew *et al.,* 2005) and/or transported over long distances (Marchand *et al.,* 2006).

Sediments are good transporters of metals, and their partition with the surrounding waters is reflected in the quality of an aquatic system (Rainbow, 1995). Metal content found in the sediments may reflect a diversified set of natural processes, from erosion to early diagenesis and anthropogenic influences (Alaoui *et al.,* 2010). Mangrove sediments act as a source (MacFarlane and Burchet, 2000) as well as a long-term store for metals in the marine environment (Spencer and MacLeod, 2002) particularly when triggered by changes in abiotic conditions such as pH, redox potential and salinity. Mangrove sediments, despite having high organic matter content, sulphides and a large proportion of fine particles, have low pH as well as anaerobic and reducing conditions (Tam and Yao, 1998; Tam and Wong, 2000; Janaki-Raman *et al.,* 2007; De Wolf and Rashid, 2008). In mangroves, metals are therefore trapped by the sediments as a result of sedimentation of suspended particles and physical, chemical and biological processes associated with the surfaces of the sediment organic and inorganic matter (Zheng *et al.,* 1997).

Geochemical characteristics of the sediments can be used to infer the sources of pollution (Chatterjee *et al.,* 2007). Being arsenals for metals, sediments are thus recorders of contamination changes owing to their large adsorption abilities (Nobi *et al.,* 2010). In this way, profiles of pollutant species in sediment cores can be used as pollution records owing to their stability within the sedimentary column and their ability to leave fingerprints in sediments due to lack of or insignificant post-depositional mobility (Chatterjee *et al.,* 2007). Elevated levels of metals recorded in mangrove sediments thus can reflect the long-term pollution caused by human activities (Tam and Wong, 2000).

Increasing human population along the coastal areas has resulted in anthropogenic perturbations of estuarine and coastal environments adjacent to urban areas (Tam and Wong, 2000). In addition, industrialisation, urbanisation and their associated socio-economic activities have contributed to the input of significant amounts of pollutants into the marine environment, directly affecting the coastal systems. Many mangrove ecosystems are close to urban development areas and are obviously impacted by urban and industrial run-off, which contain the metals in the dissolved or particulate form (Defew *et al.,* 2005; Kamaruzzaman *et al.,* 2008). Direct and indirect disposal of waste products into rivers and estuaries has led to a significant increase in pollutant contamination (Alaoui *et al.,* 2010). Metals from incoming tidal water and fresh water sources are rapidly removed from the water body and deposited into mangrove sediments. As a result, the contemporary metal concentrations in coastal marine environment have increased (Tranchina *et al.,* 2008) and mangrove ecosystems are under serious human contamination (Tam and Wong, 2000).

At present, the anthropogenic contribution of the metals into the marine environment in Tanzania and the impacts of metal contamination in the coastal ecosystems are alarming. There has been an increase in industrial activities, street garages, dumping of metallic substances and urban agriculture in valleys and near rivers that drain their water into mangrove ecosystems (Ak'habuhaya and Lodenius, 1988; Machiwa, 1992, 2000; De Wolf *et al.,* 2001; Taylor *et al.,* 2002; Mremi and Machiwa, 2003). Therefore, this study was intended to gain knowledge of the current levels of metal contaminants in mangrove sediments in order to understand the extent of metal pollution, fate and bioavailability of the metals in mangrove ecosystems. The knowledge on the concentration and distribution of metals in the sediments will help to identify the source of pollution in the marine systems of Tanzanian coast.

3.3 Methodology

3.3.1 Study area

The Mtoni estuary (Figure 3.1) is located at approximately 3 km south of Dar es Salaam (Tanzania) and is fed by 2 rivers: Kizinga and Mzinga. The creeks have mangrove trees such as *Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora mucronata and Sonneratia alba* species growing on both sides (Mlay *et al., unpublished)*.

The fresh water input from both rivers is low. An average base-line flow rate of 1 m^3/s is observed in Kizinga River with an increase up to 8 m^3/s in the rainy season while the water-flow rate in the Mzinga River is unstable and lower than in Kizinga River (Van Camp *et al.,* 2013). Hence, the effect of the river discharges on the hydrodynamics of the Mtoni estuary is very limited. The seven sampling stations (E1 to E7) are located in the mixing zone and their salinities vary from almost fresh to brackish water with somewhat higher salinities in the dry season (Mangion, 2011). Downstream this mixing zone (stations F5 to F8) the water becomes rapidly seawater while the stations F1 to F4 more upstream have fresh water. This estuarine mixing zone was selected because it integrates influences of natural and anthropogenic sources in the riverine and marine systems.

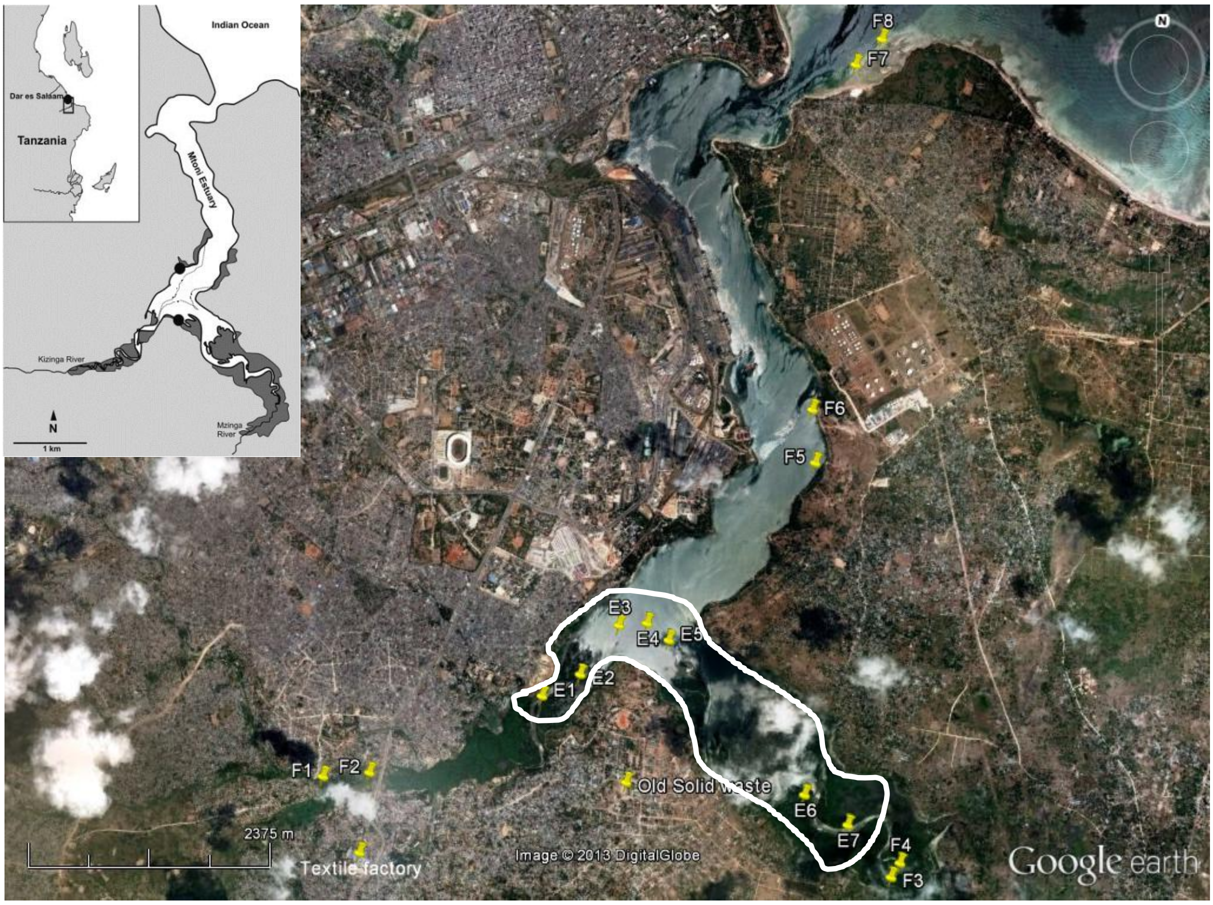

Figure 3.1: **Sampling points in the Mtoni estuary, Dar es Salaam: E1 and E2 in the Kizinga River, E3-E5 at the confluence and E6 and E7 in the Mzinga River. Stations F1-F2 in the Kizinga River, F3-F4 in the Mzinga River, F5-F6 at the Navy shore and F7-F8 at Kigamboni Seaway are additional sampling points. White line delimits the estuarine mixing zone. The solid waste dumping site and textile factory are also indicated.**

The Mtoni estuary is highly impacted (PUMPSEA, 2007) by discharges of various origin: (1) the Kizinga and Mzinga rivers draining the mangrove forest (Kruitwagen *et al.,* 2008), (2) the wastewater drainage systems from industrial and residential areas (of a population of around 500,000 inhabitants; NBS, 2003), (3) charcoal burning, (4) mangrove harvesting for residential places, (5) salt mining, (6) tourism and (7) agriculture (Taylor *et al.,* 2002).

The Kizinga river that drains the urbanised areas of Keko, Chang'ombe, Kurasini and Temeke (approximately 400,000 inhabitants; NBS, 2003) is suspected to carry a variety of wastes and discharges originating from agricultural, industrial as well as residential sources (Taylor *et al.,* 2002). The Mzinga river, on the other hand, drains the rural areas of Vijibweni, Tuangoma and Mji Mwema with a population of around 90,000 (NBS, 2003). Due to rapid growth of settlements along the Mzinga creek resulting from increased human population, the river is suspected to carry agricultural and residential wastes and discharges presumed to be emptied into the creek. The estuary further receives inputs from the Dar es Salaam harbour which is located near the mouth of the estuary during diurnal tides (up to 5 m amplitude) and from the Mtoni solid waste dumping site located in between the two rivers.

3.3.2 Sampling

Sampling of sediments was conducted in the mangrove forests during low tides at Kizinga and Mzinga creeks (Figure 1) of the Mtoni estuary. Two sampling campaigns were conducted: one during the wet season (19th - 20th January 2011) and a second during the dry season (15th - 16th August 2011). December and January have an average precipitation rate of 194 and 89 mm, respectively, while these rates in July and August are much lower with 48 and 47 mm, respectively. River flows in the Dar es Salaam area are mainly controlled by the precipitation rate in the previous period. The flows of Kizinga and Mzinga rivers are highest in the wet season (the highest discharge rates can go up to 15 m^3/s for the Kizinga river and 7 m^3/s for the Mzinga river) while in the dry season, base-line flows of 1 m^3/s in the Kizinga river and even lower in the Mzinga river were observed (Van Camp *et al.,* 2013). The impact of both rivers on the pollutant levels in the mixing zone can thus best be estimated by sampling in that zone at high (wet season) and at low (dry season) river flow and comparison of the results.

Samples were collected from exactly the same locations during both campaigns. Seven sampling stations were identified using a hand-held global positioning system (GPS): two in the Kizinga River (E1 and E2), two in the Mzinga River (E6 and E7) and three at the confluence of the two rivers (E3, E4 and E5).

From the results obtained during the wet and dry seasons in the mixing zone of the estuary, it appeared that the most upstream sampling station E1 in Kizinga River showed higher concentrations for several metals (Cr, Mn, Fe, Ni, Zn and Pb) than the stations in and close to Mzinga River. It was thus interesting to investigate metal levels more upstream in both rivers. In addition, the salinity gradient in the mixing zone is also small and real marine water samples were not included in the previous samplings. We were thus not able to appreciate any evolution of the metal levels from the estuarine mixing zone towards the marine environment. Therefore, an additional sampling campaign was organised at 3 end-members in October 2012: (1) one site in the Kizinga River (fresh water stations F1-F2) much more upstream than stations E1 and E2, (2) one site in Mzinga River (fresh water stations F3-F4) slightly more upstream than stations E6 and E7, but these latter stations were yet compared to the stations F1 and F2 in Kizinga river, much more upstream) and (3) two sites in the marine area, close to and at the mouth of the estuary (respectively marine water stations F5-F6 and F7-F8) (Figure 1).

All those samples were taken from two sub-sites within a distance of 20 m, except in the Kizinga River. The first subsample was taken at the junction of the river and the textile wastewater stream (Figure 1) and the second was taken 200 m upstream of the River very close to unauthorised human settlements.

Sediment sampling was done as described by EPA (United States Environmental Protection Agency [US EPA], 2001) using a hand corer (30 cm height, 6 cm internal diameter). The corer was gently pushed in the mangrove sediments, closed at its upper end with a lid and smoothly removed by twisting and pulling. The sediments were then pushed out of the corer tube using a piston and sectioned into three segments corresponding to depth intervals of 0-3, 3-6 and 6-9 cm. All sediment samples were packed in prior labelled and zipped polyethylene bags, stored in iceboxes and later frozen to -20°C. Sediment samples were then air-transported (frozen) to the Laboratory of the Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel (VUB) in Belgium for metal analyses.

3.3.3 Particle Size and Total Organic Carbon (TOC) Analyses

Effect of grain size on metal concentration in marine sediments is worth noting (Rubio *et al.,* 2000; Trachina *et al.,* 2008) and can be used to compare metal concentrations in sediment profiles from different areas. The grain size distribution was determined at 3 stations with low (station E7), medium (station E2) and high OM content (station E1) in their sediments. Approximately 10 g lyophilised and homogenised sediment sample was prepared by removing salts, OM and carbonates using hydrogen peroxide and hydrochloric acid, respectively. A stable suspension was obtained after rinsing and adding a peptising agent (5 mL). The coarse fraction (>75 µm) was separated by wet sieving on a 75 µm sieve, then dried at 105°C, and finally dry sieved. The grain-size distribution of the fine fractions 2-75 µm and <2 µm was obtained using the Sedigraph 5100 coupled to a Mastertech 51. The precision for 10 consecutive measurements on aliquots of the same sample was around 1% for every grain-size fraction. The amount of Total Organic Carbon (TOC) in the Mtoni sediment samples was determined using a Flash 1112 EA Elemental Analyser (Thermo Finnigan, Italy) by analysing a known weighed amount (about 12 mg) of sediment sub-samples placed in silver capsules and pre-treated by acidification with HCl (5% w/w). TOC was expressed as percentage of the total sediment weight (Mangion, 2011).

3.3.4 Determination of Metal Concentrations in Marine Sediments

The lyophilised sediment samples were pulverised (Fritsch Pulverisette) before chemical treatment. For metal analyses, the samples were then digested using a CEM Microwave Accelerated Reaction System (MARS 5®, Matthews, USA). Prior to digestion, the MARS® HP 500 digestion vessels were cleaned with 2% alkaline Extran (Merck), rinsed with Milli-Q water and then cleaned with Emsure® nitric acid (65% w/w, Merck KGaA, Darmstadt, Germany). For each sample, an analytical amount (0.20 g) of Mtoni sediment was put into the digestion vessel together with Suprapur® Hydrochloric acid, (6 mL, 30% w/w, Merck KGaA, Darmstadt, Germany) and distilled suprapur® nitric acid (2 mL, 65% w/w). The digestion was programmed to operate at 150°C temperature, 1200 W (100%) maximum power, 15 min ramp time, 200 psi maximum pressure and 15 min hold time. After cooling, Milli-Q water (40 mL) was added to each vessel and the contents were transferred into polyethylene bottles ready for analysis. For each digestion session, blank samples as well as certified reference material (LGC 6139, River Clay sediment, Middlesex, UK) were included and treated in the same manner as the samples.

Metal analysis was carried out using a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS, Thermo Finnigan Element II). Samples were diluted tenfold prior to ICP-MS analysis. Metal standard solutions were prepared by serial dilution of stock standard solutions: ICM 224 (Radion), SM 70 (Radion) and XIII (Merck). Prepared working standards (1, 5, 10 and 20 ppm) were run before and after every batch of 10 samples. Eleven metals: two major elements (Al, Fe), two minor elements (Mn, Cr) and seven trace elements (Ni, Cu, Zn, As, Sr, Cd, Pb,) were analysed in each mangrove sediment sample. Indium at a concentration of 1 µg/L was used as the internal standard. The accuracy and precision of the analytical procedures were assessed using the certified reference material (LGC 6139) and procedural blanks.

3.3.5 Statistical Analysis

Statistical methods including Student's t-test, Pearson correlation and Principal Component Analysis (PCA) were used to evaluate differences and elucidate the relationships between various parameters. In all statistical analyses, a value of $p < 0.05$ was set as a criterion to indicate significant differences in the study. Student's t-test was performed using Microsoft spreadsheet whereas Pearson correlation and PCA were performed using Predictive Analytic Software (PASW, v. 16.0) for Windows.

3.4 Results

3.4.1 Quality Control and Quality Assurance

All results were blank corrected using respective mean blank reading prior to determination of the concentrations. Precision of ICP-MS analysis was better than 5% RSD. The percentage recoveries of the measured metals based on the mean values compared to the certified reference materials are given in Table 3.2. Metal recoveries ranged from 93.1% to 117.6% when the certified values for extractable metals were used, while the recoveries ranged from 72.1% to 129.9% when certified values for total metals were used. The results indicated good agreement between the certified and the obtained values except for Cr and Pb when total metal values are used and for Sr because it lacks a certified value for extractable metals.

Table 3.1: Certified and observed mean concentrations (mg/kg) of metals in the certified reference material (LGC 6139) and the percentage recovery (n = 7)

	Extractable metals			**Total metals**		
Metal	**Certified value**	**Obtained value**	**Percent Recovery**	**Certified value**	**Obtained value**	**Percent Recovery**
Al	-	43,300	-	57,000	43,300	76.0
Cr	80.00	94.1	117.6	126.00	94.1	74.7
Mn	-	1,170	-	1,100	1,170	106.4
Fe	-	41,600	-	32,000	41,600	129.9
Ni	38.00	42.1	110.8	44.00	42.1	95.7
Cu	92.00	93.7	101.8	96.00	93.7	97.6
Zn	513.0	580	113.1	530.0	580	109.4
As	27.00	30.7	113.7	-	30.7	-
Sr	-	111	-	154.00	111	72.1
Cd	2.30	2.36	102.6	-	2.36	-
Pb	160.00	149	93.1	176.00	149	84.7

3.4.2 General features of the Mtoni sediments

Sandy particles dominated the mangrove sediments in the study area, with sand (> 75 µm) contributing for more than 60% of the weight. The correlation between TOC and the fine grain size fraction (% < 2 µm) was good (r^2 = 0.92) this was still the case (r^2 = 0.82) between TOC and the mud+silt fraction (% < 75 µm). It is well-known that muddy sediments having a high TOC content but also a high amount of fine grain size fraction (< 2 µm), accumulate by far higher amounts of pollutants than sandy sediments (Baeyens *et al.,* 1991). This means that TOC values can eventually be used to normalise the pollutant concentrations in the sediment versus the mud fraction (% < 2 µm).

3.4.3 Metal Contents in the Mtoni mixing zone

Very limited vertical variations in the metal contents of the sediments layers (0-3 cm, 3-6 cm, 6-9cm) at the seven sampling stations in the mixing zone (E1 to E7) were observed, indicating a well-mixed top 9 cm layer of the sediments in the estuary. This was also the case when the metal contents were normalised to TOC. No significant difference between wet and dry seasons was observed ($p > 0.05$) for all metal concentrations measured in the Mtoni estuarine sediments (E1 to E7). The levels presented further in this paper are therefore average values (over depth and season) of the metal contents in the sediment cores. The spatial distributions of the metals in the estuarine mixing zone are given in Table 3.2 and compared to literature data of trace metals in sediments in tropical estuaries or coastal seas (Table 3.3) especially those related to Mtoni estuary or other Tanzanian coastal waters (Mtanga and Machiwa, 2007; Kruitwagen *et al.,* 2008; Rumisha *et al.,* 2012).

Higher Al content was observed in Kizinga station E1, confluence stations E4 and Mzinga stations E6 and E7. Lowest Al content was observed at confluence station E5. The ranges of Al values observed in the mixing zone were within the range of the values observed by Kruitwagen *et al.,* (2008) and higher than those observed by Rumisha *et al.,* (2012).

The Cr content in the Kizinga station E1 was higher than in other stations in the mixing zone. A more or less similar Cr content was also observed in Mzinga stations E6 and E7 and in confluence stations E3 and E4. Lower Cr content was observed in Kizinga station E2 and confluence station E5. The Cr ranges in the Mtoni were within the range of values observed by Kruitwagen *et al.,* (2008), similar to values observed by Mtanga and Machiwa, (2007) and higher than those observed by Rumisha *et al.,* (2012) in a similar tropical environment (Table 3.3).

The contents of Fe and Mn showed a similar trend. Fe and Mn contents in the mixing zone were higher in the Kizinga station E1 than in both Mzinga stations E6 and E7. However, all were higher than the values in the Kizinga station E2 and in stations at the confluence region. Fe ranges observed in this study were within the range of values observed by Kruitwagen *et al.,* (2008) and higher than those observed by Rumisha *et al.,* (2012). On the other hand, Mn ranges in the Mtoni were higher than those observed by Rumisha *et al.,* (2012).

Ni, Cu and Pb contents in the sediments of the Mtoni mixing zone displayed a similar trend. Their levels were high in Kizinga station E1 and confluence stations E3 and E4. Whereas lower values of the metals were observed at confluence station E5, more or less similar values were observed in Kizinga station E2 and Mzinga stations E6 and E7. The ranges of Ni and Cu values observed in this estuary were within the range of values observed by Kruitwagen *et al.,* (2008), but higher than those observed by Rumisha *et al.,* (2012). Cu ranges in this study were also similar to the values observed by Mtanga and Machiwa, (2007). Pb ranges in this study were lower than those observed by Kruitwagen *et al.,* (2008) and Mtanga and Machiwa, (2007), but higher than the values observed by Rumisha *et al.,* (2012).

Lower values of Zn were observed in the Mzinga stations E6 and E7 than in Kizinga stations E1 and E2 and confluence stations E3 and E4. Confluence station E5 had the lowest values of all. The Zn values in the sediments of the Mtoni mixing zone were higher than the values observed by Rumisha *et al.,* (2012), similar to those observed by Mtanga and Machiwa, (2007) and lower than those observed by Kruitwagen *et al.,* (2008) (Table 3.3).

Table 3.2: **Metal Concentrations (Mean ± Standard deviation (SD) and range) in the Mtoni estuarine mixing zone (n = 6)**

Metals	Concentration (µg/g dw)	Kizinga stations		Confluence			Mzinga stations	
		E1	E2	E3	E4	E5	E6	E7
Al	Mean ± SD (x10^3)	48±21	24.1±7.7	29±16	38±18	18.2±6.6	40.3±8.6	39.1±6.8
	Range (x10^3)	27.6-84.5	13.3-36.8	16.0-59.0	18.4-61.3	9.8-27.9	30.7-56.1	27.7-47.4
Cr	Mean ± SD	35±15	18.9±5.2	26±13	29±12	14.2±4.5	28.3±4.7	27.9±5.2
	Range	21.5-60.2	11.0-26.8	14.3-50.8	14.8-48.0	8.3-20.8	22.6-35.2	19.3-32.8
Mn	Mean ± SD	133±57	68±15	80±26	76±22	56±16	101±44	118±23
	Range	70.0-231.6	41.3-82.2	44.4-113.0	48.2-108.4	35.8-75.0	56.9-164.1	101.1-162.0
Fe	Mean ± SD (x10^3)	25±11	13.2±3.3	16.7±7.6	18.7±7.3	9.5±3.0	20.3±4.3	20.6±3.9
	Range (x10^3)	15.2-45.0	8.0-17.2	9.4-31.0	9.7-30.7	15.5-22.5	15.5-26.7	14.2-24.6
Ni	Mean ± SD	14.7±7.2	6.2±2.0	8.4±4.1	9.7±4.4	4.6±1.6	10.0±2.0	9.7±2.6
	Range	7.7-24.4	3.7-9.2	5.0-16.6	4.6-17.0	2.4-7.2	7.4-13.0	5.8-12.2
Cu	Mean ± SD	14.8±4.5	9.3±2.3	13.2±6.5	13.9±5.0	6.3±1.7	8.8±2.9	10.2±2.3
	Range	11.2-21.5	6.0-10.2	7.9-25.9	6.8-21.9	3.7-8.9	6.9-14.7	6.2-12.6
Zn	Mean ± SD	77±23	49.5±7.6	64±32	61±26	24.8±7.0	44±12	42.9±8.0
	Range	57.3-110.1	42.9-64.0	36.2-125.9	31.9-104.8	16.3-34.7	28.8-66.0	32.6-52.2
As	Mean ± SD	6.6±3.7	2.9±1.0	3.0±1.0	3.5±1.0	1.8±0.8	7.3±1.7	5.4±1.4
	Range	3.8-13.5	1.8-4.6	1.8-4.4	2.2-5.2	0.8-3.1	4.8-8.6	3.6-6.9
Sr	Mean ± SD	28±12	18.9±5.4	30±10	33.8±9.3	21.0±7.3	28.7±8.0	21.4±5.4
	Range	18.2-49.9	11.3-27.6	16.1-42.6	21.4-45.9	12.5-30.1	23.28-44.2	15.3-28.7
Cd	Mean ± SD	0.12±0.02	0.07±0.02	0.12±0.05	0.12±0.04	0.06±0.01	0.06±0.03	0.05±0.02
	Range	0.09-0.14	0.06-0.07	0.07-0.21	0.08-0.17	0.04-0.08	0.04-0.12	0.03-0.07
Pb	Mean ± SD	13.2±4.0	8.0±2.2	11.1±4.6	11.4±4.8	6.9±2.1	10.4±2.1	8.8±1.8
	Range	10.6-19.8	5.1-11.8	7.0-19.6	5.6-18.2	4.1-9.6	8.7-14.3	6.1-10.6

na= not analysed

Table 3.3: Comparative Account of Metal Concentrations (µg/g dw) in Different Tropical Marine Sedimentary Environments

Study site	Mtoni estuary, Tanzania[a]	Mtoni estuary, Tanzania[b]	Mzinga Creek, Tanzania	Mtoni estuary, Tanzania	Msimbazi, Tanzania	S. Buloh & S. Bongsu, Singapore	Hugli estuary, India	Cross River, Nigeria	San Jose lagoon, Puerto Rico	Andaman Islands, India
Al	18,200-48,000	3,300-31,900	-	5,430–61,900	461	-	-	-	-	2,938-4,384
Cr	14.2-35	5.4-460	22.7	14.4-6,240	1.0	16.6–32.1	12.1–84.4	19.2–37.9	-	12.7-20.4
Mn	56-133	32.3-680	-	-	23	-	228.3–716.9	-	-	29.2-134.4
Fe	9,500-25,200	1,600-23,510	-	8,070–59,100	461	-	-	598.2–993.2	1,600–4,600	2,638-4,888
Ni	4.6-14.7	1.8-80	-	7.9–156	0.35	7.44–11.7	6.86–52.5	14.2–35.0	-	7.04-12.0
Cu	6.3-14.8	1.5-400	9.50	3.70–4,050	0.3	7.06–32.0	4.30–45.3	23.0–36.2	29.0–211.0	80.9-87.9
Zn	24.8-77	6.9-260	35.1	43.9–2,450	4.0	51.2–120.2	22.96–205.0	126.4–212.3	266.0–530.0	12.2-23.0
As	1.8-7.3	2.6-8.1	-	-	0.2	-	-	-	4.50–24.0	-
Sr	18.9-34	na	-	-	-	-	-	-	-	-
Cd	0.05-0.10	0.03-0.37	1.05	0.9–28.1	0.01	0.18–0.27			0.20–4.70	0.8-1.52
Pb	6.9-13.2	2.0-32.8	14.6	47.2-385	0.8	12.3-31.0	n.d-44.5	7.2-26.6	16.0-548.0	3.90-5.40
Reference	This Study	This Study	Mtanga & Machiwa, (2007)	Kruitwagen *et al.,* (2008)	Rumisha *et al.,* (2012)	Cuong *et al.,* (2005)	Chatterjee *et al.,* (2007)	Essien *et al.,* (2009)	Acevedo-Figueroa *et al.,* (2006)	Nobi *et al.,* 2010

[a] Values from brackish sediment stations E1 to E7 in the mixing zone; [b] Values from stations F1 to F8 (freshwater to marine sediments); n.d = not detected

A high As content was observed in Kizinga station E1 and Mzinga stations E6 and E7. While lower As values were observed in the confluence station E5, a more or less similar As content was observed in Kizinga station E2 as well as in confluence stations E3 and E4. The range of As observed in the Mtoni estuary was higher than the values observed by Rumisha *et al.,* (2012).

Sr concentrations in the Mtoni mixing zone were all more or less similar. A slightly higher Sr content was observed in confluence station E4 and slightly lower Sr was observed in Kizinga station E2. No data for comparison were available in literature from similar tropical marine environments. The Cd content in the mixing zone was higher in Kizinga station E1 as well as in the confluence stations E3 and E4. In other stations, Cd content was more or less similar. The range of Cd values in the estuary was lower than that observed by Kruitwagen *et al.,* (2008) and Mtanga and Machiwa, (2007), but higher than the values observed by Rumisha *et al.,* (2012) in a similar marine environment.

3.4.4 Metal Contents in additional samples

Metal levels in the additional sampling stations (F1 – F8) are given in Table 3.4. Contents of Cr, Mn, Fe, Ni and Cu were the highest at the riverine station F2 while Zn, Cd and Pb were the highest at riverine station F1 of the Kizinga River. As levels were the highest at stations F3 and F4 in the Mzinga River. A clear gradient from the Kizinga upstream station F1 to the estuarine mouth (F8) is observed in the dry season for all metals except As (Table 3.4). This is also the case for most metals from the Mzinga station F3 to the estuarine mouth (F8) when omitting station F4.

Table 3.4: Metal Concentrations (μg/g dw) in the additional samples from the Mtoni estuary

Metals	Riverine				Marine			
	Kizinga		Mzinga		Navy shore		Kigamboni	
	F1	F2	F3	F4	F5	F6	F7	F8
Al	31,900	7,900	13,900	4,800	17,600	12,700	3,300	4,120
Cr	27.8	460	16.4	6.20	15.5	11.6	5.38	6.46
Mn	384	680	375	163	80.4	80.4	32.3	35.4
Fe	12,400	23,510	9,200	3,600	7,400	5,600	1,600	2,000
Ni	12.1	80	7.23	2.70	5.78	4.33	1.84	2.10
Cu	16.4	400	9.35	4.91	9.12	7.20	1.52	1.94
Zn	260	149	56.4	24.6	38.2	31.4	6.9	8.5
As	3.34	2.61	7.70	8.13	3.39	3.78	4.96	5.10
Sr	na	na	na	na	na	na	na	na
Cd	0.37	0.24	0.10	0.03	0.09	0.07	0.04	0.05
Pb	32.8	10.3	10.4	4.76	10.6	7.92	2.18	2.01

na= not analysed

The ranges of metal concentrations in the additional samples (end members) were, except for Al and Fe, all higher than those in the mixing zone samples. They are now more comparable to those of Kruitwagen *et al.,* (2008) except that the highest levels of Cr, Cu, Cd, Zn and Pb which were still about 10 times higher than our values. However, those high values were all related to one station close to our F2 station in the Kizinga River, which is also the most contaminated in our study. The observed values were all higher than the values reported by Rumisha *et al*. (2012), but the latter studied the coastal area of Tanzania which is a system that is still more diluted than most of the downstream sample stations in our study.

3.4.5 Pearson Correlations and Principal Component Analysis (PCA)

In order to determine the relationship between metals and TOC, Pearson linear correlation matrix was generated and the coefficients are presented in Table 4. PCA was also employed and the results are presented in Table 5. A principal component (PC) is considered significant when its eigenvalue is greater than 1. The measured metal values and %TOC were used as variables (total 12), with the concentrations of the metals in the different sampling stations during wet and dry seasons as objects (total 42). The application of PCA indicated that the 12 variables can be represented by 3 new PCs that accounted for 88.82% of the total variance in the original data sets (Table 5). A two dimensional score plot is given in Figure 3.2. Combining the results of the Pearson correlation matrix (Table 4) and the PCA (Table 5), we can repartition the compounds in 3 groups: (1) Al, Cr, Mn, Fe, Cu, Ni, Zn, As and Pb form PC1, explaining 57.85% of the variance. Correlation coefficients between those elements range from 0.531 to 0.989; (2) Cd and TOC form PC2, explaining 19.5% of the variance. They are strongly anti-correlated with correlation coefficient equal to -0.51; and (3) Sr, which does not correlate with any element forms PC3 explaining 11.47% of the variance.

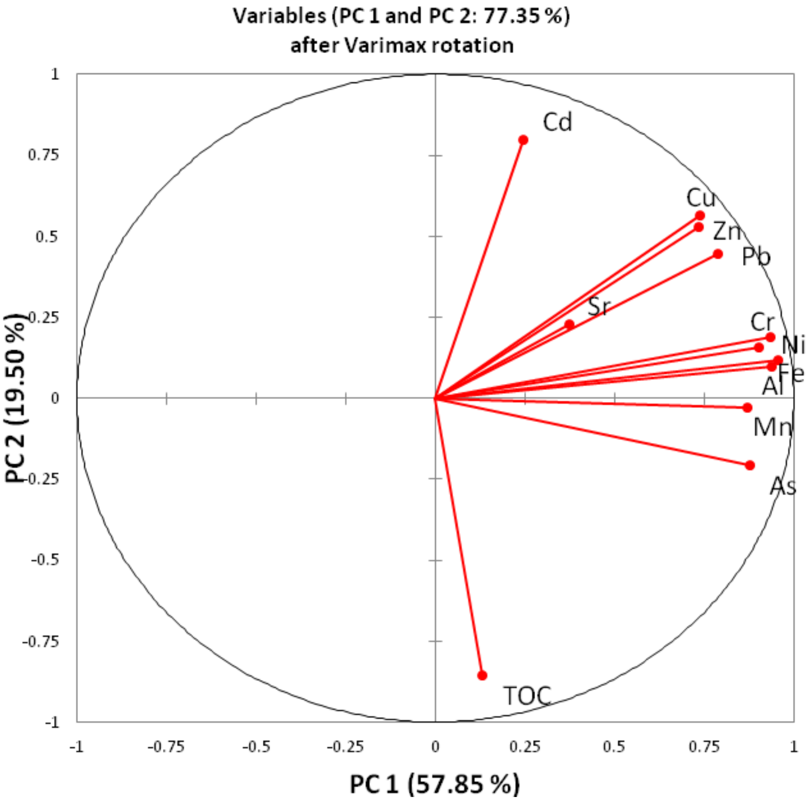

Figure 3.2: **A two-dimensional score plot of metals in relation to %TOC in the Mtoni estuary**

Table 3.5: **Pearson Correlation Coefficients for metals and sediment properties in the Mtoni estuary (n = 42)***

	Al	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**As**	**Sr**	**Cd**	**Pb**	**%TOC**
Al	**1**	**0.979**	**0.816**	**0.977**	**0.906**	**0.764**	**0.743**	**0.845**	**0.579**	**0.372**	**0.852**	-0.003
Cr		**1**	**0.809**	**0.989**	**0.925**	**0.844**	**0.819**	**0.828**	**0.623**	**0.469**	**0.890**	-0.047
Mn			**1**	**0.821**	**0.731**	**0.649**	**0.643**	**0.779**	**0.465**	0.193	**0.661**	0.064
Fe				**1**	**0.926**	**0.808**	**0.786**	**0.879**	**0.571**	**0.417**	**0.863**	0.009
Ni					**1**	**0.793**	**0.777**	**0.801**	**0.548**	**0.467**	**0.847**	0.019
Cu						**1**	**0.893**	**0.531**	**0.567**	**0.734**	**0.877**	-0.295
Zn							**1**	**0.576**	**0.559**	**0.692**	**0.810**	-0.285
As								**1**	**0.486**	0.145	**0.622**	0.202
Sr									**1**	**0.585**	**0.594**	-0.202
Cd										**1**	**0.680**	**-0.510**
Pb											**1**	-0.220
%TOC												**1**

* Significant values at α = 0.05 (two-tailed) are in bold.

Table 3.6: Rotated Principal Component (PC) Matrix

	Principal Components (88.82%)		
	PC1 (57.85%)[a]	**PC2 (19.50%)**	**PC3 (11.47%)**
Al	**0.883**	0.009	0.051
Cr	**0.875**	0.035	0.067
Mn	**0.760**	0.001	0.012
Fe	**0.917**	0.014	0.047
Ni	**0.814**	0.025	0.051
Cu	**0.548**	0.320	0.038
Zn	**0.538**	0.280	0.036
As	**0.768**	0.043	0.056
Sr	0.139	0.052	**0.773**
Cd	0.060	**0.633**	0.183
Pb	**0.623**	0.198	0.064
TOC	0.017	**0.731**	0.000

[a] The percentage in brackets indicates the explained contribution of principal component to the total variance after varimax normalisation.

3.4.6 Enrichment Factors (EF) of the Metals

Enrichment Factors for the metals in the Mtoni estuary were determined relative to the crustal abundance. EF values were normalised to Al (Figure 3.2) after correction of Al levels in the Mtoni sediments based on the percentage recovery. EF values were interpreted as follows: EF <1 = no enrichment, 1<EF<3 = minor enrichment, 3<EF<5 = moderate enrichment, 5<EF<10 = moderate severe enrichment, 10<EF<25 = severe enrichment, 25<EF<50 = very severe enrichment and EF<50 = extremely severe enrichment (Acevedo-Figueroa, 2006; Chen *et al.,* 2007; Essien *et al.,* 2009).

The EF values in the mixing zone samples indicated no enrichment for all metals except Zn, As, Cd and Pb. Whereas minor enrichment (EF <3) was observed for Zn, Cd and Pb, As was enriched in all mixing zone samples (EF values 3-6). Exceptionally high enrichments were observed in additional samples in the riverine stations (F1 and F2 in the Kizinga River and F3 and F4 in the Mzinga River). Kizinga station F2, for example, situated close to where wastewaters from the textile mill enters the Kizinga River, was highly enriched with all metals (EF >4) except Sr. Arsenic (As) was also exceptionally enriched in all the riverine as well as the marine samples (up to EF = 59 in Mzinga station F4, Figure 3.2). Similar As enrichment in all samples was observed in the intertidal sediments of Mbweni, Kunduchi, Msasani, Msimbazi, Mji Mwema and Geza Ulole in the Dar es Salaam coast (Rumisha *et al.,* 2012).

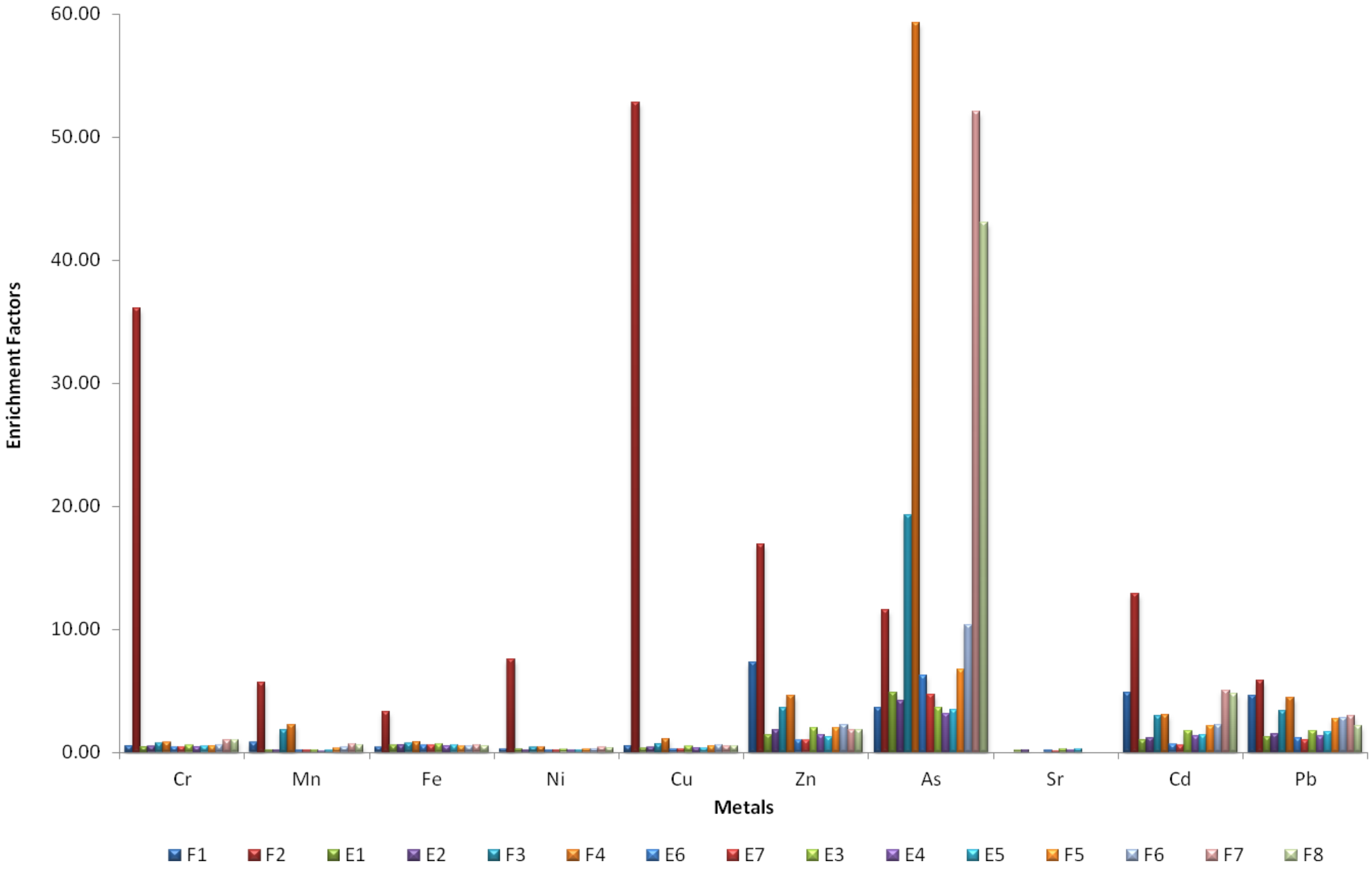

Figure 3.3: Metal Enrichment Factors in samples from the Kizinga River (E1, E2, F1 and F2), Mzinga River (E6, E7, F3 and F4), confluence stations (E3, E4, E5) and downstream area (F5, F6, F7 and F8) of the Mtoni estuary.

3.5 Discussion

3.5.1 Spatial distribution of metal content in sediments

The dominance of the sand fraction in the Mtoni estuarine sediments favours abiotic processes such as enhanced diffusion of oxygen in the sediment allowing faster oxidation of organic matter (Holmer, 2003; Davies & Tawari, 2010). As a result, there is simultaneous release of organic matter-associated pollutants such as metals, which may reduce the metal burden in the sediments.

Sediments from the Mtoni estuary showed varying spatial distributions in metal levels. Higher metal contents were observed in the upstream stations (F1 and F2 in the Kizinga River and F3 and F4 in the Mzinga River) than in the marine and mixing zone samples. This clearly indicates that the main contribution of metals in the estuary comes from the two rivers, which probably accumulate metal pollutants from various natural and human induced processes (see further sources of metals in the Mtoni estuary).

The depth profiles observed indicate that the estuarine sediments are rather fairly well-mixed and the gradient well smoothed out, making the profiles more or less uniform. The sediment mixing caused by tidal currents in the inter-tidal areas (up to 5m) ensures uniform vertical metal patterns and the high salinity (up to <35; Mangion, 2011) may facilitate metal diffusion in the sediments as previously observed by Li *et al.,* (2000). As a result, relatively low variation of metal levels with depth has been observed. Perhaps, a depth of 9-cm is not sufficient enough to observe any difference in the depth profiles. It will be interesting in the future to study the pollutant profiles in the deeper layers.

3.5.2 Sources of metals in the Mtoni estuarine sediments

3.5.2.1 PCA and Correlation coefficient

In aquatic sediments, the metal distribution is governed by many factors and processes: (1) the nature of the sediment (clay/sand/salt fractions, nature and amount of OM), (2) physical processes (mixing, advection, and diffusion), (3) redox processes, and (4) anthropogenic sources. However, it is not always necessary to have all that information to understand the metal distribution in the sediment. Based on the PCA loading and correlation coefficient results, we will try to explain some features observed in the Mtoni estuary sediments as follows:

Principal Component 1: Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb

These correlations suggest a contamination of both ferro and non-ferro metals associated with human activities in electronics and related works (Cu), paints and pigments (Cr), metallurgy and metal construction works (Cr, Mn, Cu, Ni, Al, Fe, Pb), anti corrosion and batteries (Zn), paints, pigments and as additive in petroleum fuel (Pb). Arsenic is extensively used in industry, farming and in agriculture while small amounts are used in, amongst others, the glass and ceramics industry. Arsenic derivates such as arsenates, arsenites as well as arsenic and arsenic acids are used as pesticides and herbicides (De Gieter and Baeyens, 2005). Due to the proximity of the Dar es Salaam harbour, it is likely that these metals are also transported from the harbour. Tides may facilitate the transport of metals upstream into the estuary.

Principal Component 2: Cd and TOC

Cd is a non-ferro metal linked with an anthropogenic source. This is clearly indicated by anthropogenic activities such as paints and pigments, metallurgy and metal constructions and wood preservative. Cd and TOC are negatively correlated. Despite having several anthropogenic activities in common, the source of Cd should be different from those for Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb.

Principal Component 3: Sr

Sr is not correlated with any other metal or TOC. Though the abundance of Sr is lower (about 0.037%) in the earth's crust, it is one of the more abundant elements in sea water (0.0013%). The origin of the Sr levels in the Mtoni sediments should be clearly different from the sources of all other investigated elements.

3.5.2.2 EF data

Concentrations of metals in sediments can be important indicators of toxicological risk, especially when they are substantially above natural levels. To be able to appreciate such increased levels, EF values were calculated. The metal contents from the Mtoni estuary were normalised using Al.

Higher EF values in the Mtoni estuary for several metals at various sites clearly indicate the effect of anthropogenic activities. A similar trend for Cu, Cr, Zn and Pb in the Mtoni estuary (Kruitwagen *et al*., 2008) and for Cr, Zn, As, Cd and Pb in the coastal area of Dar es Salaam (Rumisha *et al*., 2012) was observed. Highly enriched values in the riverine zone as compared to marine and mixing zones could be due to anthropogenic activities in and around the river banks. Agricultural, industrial as well as domestic activities could be the main sources of the metals particularly in this area where (i) very few residents are connected to proper sewage systems, (ii) most industries discharge effluents without proper treatment and (iii) most discharges are directed into the valleys, rivers and ocean implicitly or explicitly (Rumisha *et al*., 2012). For example, stations F1 and F2 in the Kizinga River are close to unauthorised human settlements. In addition, station F2 receives effluents from the nearby households and it is close to a textile factory, which may supply industrial effluents containing various amounts of metals. In the case of Mzinga stations F3 and F4 and estuarine stations F5 and F6, the enrichment could be due to unauthorised human settlements where solid wastes from urban agriculture, households and small industries are trashed away. What is observed in the marine samples (F5-F8) could be due to cumulative effect of pollution from human and industrial sources and the transport of metals from the two rivers. As Figure 3.2 indicates, Al and Fe have background enrichments with no anthropogenic effects. Other analysed metals, however, exceeded the expected natural background levels and therefore have an anthropogenic origin. Metals exceeding background levels must be carefully monitored because they are a potential threat to the ecosystem and humans.

3.5.3 Comparison with Sediment Quality Guidelines

To determine if the metals in the Mtoni estuary pose a threat to marine biota, the metal levels were compared to international sediment quality guidelines because such guidelines specific for the region are not available. Different sediment quality guidelines including the National Oceanic and Atmospheric Administration (NOAA), Canadian Council of the Ministers of the Environment (CCME), Ontario Ministry of Environment Screening level Guidelines and Probable effect levels (PEL) were used for comparison and the values for 7 metals (for Al, Mn, Fe and Sr no guideline values exist) are given in Table 3.7.

Table 3.7: Sediment Quality Guidelines for different metals (µg/g dw)

	NOAA		CCME (TEL)	PEL	ONTARIO (LEL)
	ERL	ERM			
Al	-	-	-	-	-
Cr	81	370	37.3	90	26
Mn	-	-	-	-	-
Fe	-	-	-	-	-
Ni	20.9	51.6	18	36	16
Cu	34	270	35.7	197	16
Zn	150	410	123	315	120
As	8.2	70	5.9	17	6
Sr	-	-	-	-	-
Cd	1.2	9.6	0.6	3.5	0.6
Pb	46.7	218	35	91.3	31

ERL = effect range low, ERM = effect range median, TEL = threshold effect level; PEL = probable effect level; LEL lowest effect level

Comparing the metal levels in the Mtoni estuary and the different sediment quality guidelines, it can be shown that Cr, Ni, Cu, Zn, As, Cd and Pb had lower values compared to CCME, PEL, TEL and NOAA guideline values. Ni, Cu, Zn, Cd and Pb had lower values than the lowest effect levels (LEL) described by the Ontario Ministry of Environment, whereas Cr and As levels were slightly higher that the LEL at the upper range. Using the US EPA toxicity classifications, As was moderately polluted (US EPA range for As is 3-8 ug/g), while other elements were not. This indicates that adverse biological effects related to these metals should be rather limited. However, Kruitwagen *et al.,* (2006) and De Wolf and Rashid (2008) observed some adverse effects on mudskippers and molluscs, respectively.

3.6 Conclusion

Trace metal levels in the Mtoni estuarine sediments do not show a gradient with neither depth nor seasonal variation between the wet and the dry season. A clear decreasing concentration gradient in downstream direction from Kizinga and Mzinga rivers towards the mouth of the estuary is observed for almost all elements except As. Elements from anthropogenic origin can be subdivided in 2 groups: the first consists of Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb, Cd and Sr and the second of Cd anti-correlated with OM.

All the elements from anthropogenic origin also show high enrichment factors. A similar trend for Cu, Cr, Zn and Pb in the Mtoni estuary (Kruitwagen *et al*., 2008) and for Cr, Zn, As, Cd and Pb in the coastal area of Dar es Salaam (Rumisha *et al*., 2012) was observed. Highly enriched areas in the riverine zones as compared to marine and mixing zones could be due to local anthropogenic activities. Agricultural, industrial as well as domestic activities could be the main sources of the metals particularly in this area where (i) very few residents are connected to proper sewage systems, (ii) most industries discharge effluents without proper treatment and (iii) most discharges are directed into the valleys, rivers and ocean implicitly or explicitly (Rumisha *et al*., 2012). For example, stations F1 and F2 in the Kizinga River are close to unauthorised human settlements. In addition, station F2 receives effluents from the nearby households and is close to a textile factory, which could supply industrial effluents containing variable amounts of metals. Between the stations F1 and F2 there is a small metal industry for making local cooking utensils. In the case of Mzinga stations F3 and F4 and estuarine stations F5 and F6, the enrichment could be due to unauthorised human settlements where solid wastes from urban agriculture, households and small industries are trashed away.

Since highly enriched enrichment factors are observed for several toxic metals they should be carefully monitored because they are a potential threat to the ecosystem and humans.

3.7 Acknowledgements

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3.8 References

Acevedo-Figueroa D, Jiménez BD, Rodríguez-Sierra CJ (2006). Trace metals in sediments of two estuarine lagoons from Puerto Rico, *Environmental Pollution* 141 (2): 336-342.

Ak'habuhaya J, Lodenius M (1988). Metal pollution of River Msimbazi, Tanzania, *Environmental International,* 14: 511-514.

Alaoui AM, Choura M, Maanan M, Zourarah B, Robin M, Conceição MF, Andrade C, Khalid M, Carruesco C (2010). Metal fluxes to the sediments of the Moulay Bousselham lagoon, Morocco, *Environ Earth Sci,* 61: 275–286.

Baeyens W, S. Panutrakul, M. Leermakers, J. Navez, M. Elskens (1991). Geochemical processes in sandy and muddy sediments, *Geo-Marine Letters* 11-3/4: 188-193.

Chatterjee M, Silva Filho EV, Sarkar SK, Sella SM, Bhattacharya A, Satpathy KK, Prasad MVR, Chakraborty S, Bhattacharya BD (2007). Distribution and possible source of trace elements in the sediment cores of a tropical macrotidal estuary and their ecotoxicological significance, *Environment International,* 33 (3): 346-356.

Chen CW, Kao CM, Chen CF, Dong CD (2007). Distribution and Accumulation of Heavy Metals in Sediments of Kaoshiung Harbor, Taiwan, *Chemosphere* 66: 1431-1440.

Cuong DT, Bayen S, Wurl O, Subramanian K, Wong KKS, Sivasothi N, Obbard JP (2005). Heavy metal contamination in mangrove habitats of Singapore, *Marine Pollution Bulletin* 50: 1713–1744.

De Gieter M and Baeyens W (2005). Arsenic in fish: implications for human health. Reviews in food and nutrition toxicity, CRC Press, Boca Raton, 4: 57-83.

De Wolf H, Rashid R (2008). Heavy metal accumulation in *Littoraria scabra* along polluted and pristine mangrove areas of Tanzania, *Environmental Pollution*, 152: 636-643.

De Wolf H, Ulomi SA, Backeljau T, Pratap HB, Blust R (2001). Heavy metal levels in the sediments of four Dar es Salaam mangroves Accumulation in, and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda), *Environment International* 26: 243-249.

Defew L H, Mair JM, Guzman HM (2005). An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama, *Marine Pollution Bulletin* 50: 547–552.

Dell'Anno A, Mei ML, Ianni C, Danovaro R (2003). Impact of bioavailable heavy metals on bacterial activities in coastal marine sediments, *World Journal of Microbiology & Biotechnology* 19: 93–100.

Essien JP, Antai SP, Olajire AA (2009). Distribution, Seasonal Variations and Ecotoxicological Significance of Heavy Metals in Sediments of Cross River Estuary Mangrove Swamp, *Water Air Soil Pollut* 197: 91-105.

Janaki-Raman D Jonathan MP, Srinivasalu S, Armstrong-Altrin JS, Mohan SP, Ram-Mohan V (2007). Trace metal enrichments in core sediments in Muthupet mangroves, SE coast of India: Application of acid leachable technique, *Environmental Pollution* 145: 245-257.

Kamaruzzaman BY, Ong MC, Noor Azhar MS, Shahbudin S, Jalal KCA (2008). Geochemistry of Sediment in the Major Estuarine Mangrove Forest of Terengganu Region, Malaysia, *Am. J. Applied Sci.,* 5 (12): 1707-1712.

Kamau JN (2002). Heavy Metal Distribution and Enrichment at Port-Reitz Creek, Mombasa, *Western Indian Ocean J. Mar. Sci.* 1 (1): 65-70.

Kruitwagen G, Pratap HB, Covaci A, Wendelaar Bonga SE (2008). Status of pollution in mangrove ecosystems along the coast of Tanzania, *Marine Pollution Bulletin* 56: 1022–1042.

Li X, Wai OWH, Li YS, Coles BJ, Ramsey MH, Thornton I (2000). Heavy metal distribution in sediment profiles of the Pearl River estuary, South China, *Applied Geochemistry*, 15 (5): 567-581.

MacFarlane GR, Burchett MD (1999). Zinc distribution and excretion in the leaves of the grey mangrove, *Avicennia marina* (Forsk.) Vierh, *Environmental and Experimental Botany* 41: 167–175.

MacFarlane GR, Burchett MD (2000). Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh, *Aquatic Botany* 68: 45–59.

MacFarlane GR, Burchett MD (2001). Photosynthetic Pigments and Peroxidase Activity as Indicators of Heavy metal Stress in the Grey mangrove, *Avicennia marina* (Forsk.), *Marine Pollution Bulletin,* 42 (2): 233-240.

Machiwa JF (1992). Anthropogenic pollution in the Dar es Salaam harbour area, Tanzania. *Marine Pollution Bulletin* 24: 562–567.

Machiwa JF (2000). Heavy metals and organic pollutants in sediments of Dar es Salaam harbour prior to dredging in 1999. *Tanz. J. Sc.* 26: 29–46.

Marchand C, Allenbach M, Lallier-Vergès E (2011). Relationships between heavy metals distribution and organic matter cycling in mangrove sediments (Conception Bay, New Caledonia), *Geoderma*, Vol. 160, 444-456.

Marchand C, Lallier Verges E, Baltzer F, Alberic P, Cossa D, Baillif P (2006). Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana, *Marine Chemistry*, 98 (1): 1-17.

Mlay AP, Wagner GM, Mgaya YD (*unpublished*). A comparative study of the ecology of four sandy/muddy shores in the Dar es Salaam area. Accessed on 23th August 2012 at http://gridnairobi.unep.org/chm/eafdocuments/tanzania/Ecology_of_muddy_shores_in_Tanzania.pdf

Mremi SD, Machiwa JF (2003). Heavy metal contamination of mangrove sediments and the associated biota in Dar es Salaam, Tanzania. *Tanz. J. Sc.* 29: 61–76.

Mtanga A, Machiwa J (2007). Assessment of Heavy Metal Pollution in Sediment and Polychaete Worms from the Mzinga Creek and Ras Dege Mangrove Ecosystems, Dar es Salaam, Tanzania, *Western Indian Ocean J. Mar. Sci.* 6, (2): 125–135.

Nobi EP, Dilipan E, Thangaradjou T, Sivakumar K, Kannan L (2010). Geochemical and geo-statistical assessment of heavy metal concentration in the sediments of different coastal ecosystems of Andaman Islands, India, *Estuarine, Coastal and Shelf Science* 87: 253–264.

Praveena SM, Aris AZ, Radojevic M (2010). Heavy Metals Dyanamics and Source in Intertidal Mangrove Sediment of Sabah, Borneo Island, *EnvironmentAsia* 3: 79-83.

PUMPSEA: Peri-urban mangrove forests as filters and potential phytoremediators, of domestic sewage in East Africa (2007). Distribution and fate of heavy metals and indicator pathogens in sewage exposed mangroves, Specific Targeted Research Project Report for Project No. INCO – CT2004 – 510863.

Rainbow PS (1995). Biomonitoring of Heavy Metal Availability in the Marine Environment, *Marine Pollution Bulletin,* 31 (4-12): 183-192.

Raju K, Vijayaraghavan K, Seshachalam S, Muthumanickam J (2010). Impact of anthropogenic input on physicochemical parameters and trace metals in marine surface sediments of Bay of Bengal off Chennai, India, *Environ Monit Assess,* DOI 10.1007/s10661-010-1621-2.

Rubio B, Nombela MA, Vilas F (2000). Geochemistry of major and trace elements in sediments of the Ria de Vigo (NW Spain): An assessment of metal pollution. *Marine Pollution Bulletin*, 40: 968–980.

Rumisha C, Elskens M, Leermarkers M, Kochzius M, (2012). Trace metal pollution and its influence on the community structure of soft bottom molluscs in intertidal areas of the Dar es Salaam coast, Tanzania *Mar. Pollut. Bull.,* doi: 10.1016/j.marpolbul.2011.12.025

Spencer KL, MacLeod CL (2002). Distribution and partitioning of heavy metals in estuarine sediment cores and implications for the use of sediment quality standards, *Hydrology and Earth System Sciences,* 6 (6): 989–998.

Tam NFY, Wong YS (2000). Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps, *Environmental Pollution* 110: 195-205.

Tam NFY, Yao MWY (1998). Normalisation and heavy metal contamination in mangrove sediments, *The Science of the Total Environment,* 216: 33-39.

Taylor M, Ravilious C, Green E P (2002). Mangrove of East Africa, Vol. 12, UNEP World Conservation Monitoring Centre, *Biodiversity Series* No. 13, pp 26.

Van Camp, M., Mjemah, I. C., Al Farrah, N., Walraevens, K. (2013). Modeling approaches and strategies for data-scarce aquifers: example of the Dar es Salaam aquifer in Tanzania, *Hydrogeology Journal* 21: 341–356

CHAPTER FOUR

DIOXINS AND DIOXIN-LIKE POLLUTION OF MARINE SEDIMENTS AND IMPLICATIONS FOR THE ECOLOGY AND HEALTH OF MANGROVE ECOSYSTEM IN TANZANIA

Based on: Matobola J. Mihale, Kim Croes, Clavery Tungaraza, Willy Baeyens & Kersten Van Langenhove (2013). PCDD/F and Dioxin-like PCB determinations in Mtoni Estuarine Sediments (Tanzania) using the Chemically Activated Luciferase gene expression (CALUX) Bioassay: Accepted for publication in *Environment and Pollution.*

CHAPTER FOUR: PCDD/F AND DIOXIN-LIKE PCB DETERMINATIONS IN MTONI ESTUARINE SEDIMENTS (TANZANIA) USING THE CHEMICALLY ACTIVATED LUCIFERASE GENE EXPRESSION (CALUX) BIOASSAY

4.1 Abstract

Sediments from Mtoni estuary and 2 tributaries, Tanzania, were screened for polychlorinated-p-dibenzodioxins, polychlorinated-dibenzofurans (PCDD/Fs) and dioxin-like PCBs (dl-PCBs) using the chemically activated luciferase gene expression (CALUX) bioassay approach. PCDD/Fs expressed as bioanalytical equivalence (BEQ) values ranged from 5.7 ± 1.4 to 39.9 ± 5.8 pg BEQ/g sediment in the wet season and from 14.1 ± 2.0 to 32.8 ± 4.7 pg BEQ/g sediment in the dry season, with higher levels observed in Kizinga River and stations close to the mouth of that river. Dioxin-like PCB levels ranged from 0.21 ± 0.03 to 0.53 ± 0.03 pg BEQ/g sediment in the wet season and from 0.22 ± 0.03 to 0.59 ± 0.04 pg BEQ/g sediment in the dry season. Higher PCDD/F and dl-PCB levels in sediments are probably related to open burning of plastic scraps, household burning of wood or charcoal and traffic related emissions, all of which occur in the Dar es Salaam region. The denser population and the more intense industrial activities in the Kizinga River basin may explain the enhanced PCDD/F and dl-PCB levels observed in the sediments of that river compared to the levels in the Mzinga River basin. A third sampling campaign, including also stations in the downstream estuary, confirmed the enhanced levels in the Kizinga River (maximum of 400 pg-BEQ/g) and also showed that a clear decreasing concentration gradient in the downstream direction exists. It cannot be excluded that the levels of these pollutants in the sediments of the Mtoni estuary pose a threat to the local biological community.

4.2 Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, which are collectively referred to as PCDD/Fs, have no commercial use and occur in the environment as unintended by-products of technological processes (Roots *et al.,* 2004; Pan *et al*., 2010). PCDD/Fs can be formed from natural combustion processes like bushfires and volcanoes (Birch *et al.*, 2007) or during incomplete anthropogenic combustion processes of chlorinated wastes such as, for example, incineration of polyvinyl chloride plastics (Liu *et al.,* 2006; Birch *et al.*, 2007; De Wolf & Rashid, 2008; Manahan, 2008; Terauchi *et al.*, 2009; Pan *et al.,* 2010). Formation of dioxins in such incinerators occurs due to the presence of both chlorine and catalytic metals (Manahan, 2008). Industrial activities such as metallurgy and manufacture of chlorinated chemicals, like wood preservatives and pesticides, can also produce dioxins (Müller *et al.,* 1999; Ryoo *et al.,* 2005; El-Kady *et al.,* 2007).

Polychlorinated biphenyls (PCBs) were once produced commercially (Koistinen *et al.,* 1997; Srogi, 2007) and used in industrial and consumer products (Liu *et al.,* 2006; Wang *et al.,* 2007), such as anti-corrosion materials, coolants and insulators in heat transfer systems (Shen *et al.*, 2009; Srogi, 2007), electronic appliances and hydraulic fluids (Shen *et al.,* 2009; Yang *et al.*, 2009) and as capacitors in electrical industries (Pan *et al*., 2010). Common PCB sources in the environment include the use as well as disposal of PCB-containing products and the formation of PCBs as by-products of low temperature (less than 800 °C) waste incineration (Chi *et al.*, 2007; Wang *et al.*, 2007; Pan *et al.,* 2010). Twelve of these PCBs have a planar structure and elicit biochemical and toxic responses similar to dioxins and are therefore known as dioxin-like PCBs or dl-PCBs (Smith & Lopipero, 2001; Sanctorum *et al.*, 2007a; Okay *et al.,* 2009; Pan *et al.,* 2010).

Both natural and anthropogenic sources can lead to increased levels of those compounds in estuaries and coastal marine ecosystems (Kumar *et al.,* 2008). Major anthropogenic activities are linked to population growth, urbanisation and industrialisation (Müller *et al.,* 2002; Kumar *et al.,* 2008;) and include effluents from municipal wastewater plants (Moon *et al.*, 2009), combustion processes from waste incinerators or cement manufacturing, power plants and automobile exhausts (Zhang *et al.,* 2010) and industrial processes like pulp bleaching and metallurgy (Bruckmeier *et al.*, 1997).

Mangrove sediments can act as sinks and later as sources of PCDD/F and PCB contaminants to marine environments (Müller *et al.,* 1999; Guzzella *et al.,* 2005; Chi *et al.,* 2007; Pan *et al.,* 2010). Hence, sediments can be used to evaluate pollutant sources, historical trends and fate processes, since the amounts of these compounds in sediments will reflect regional discharges (Lee *et al.,* 2006; Moon *et al.,* 2009; Müller *et al.,* 1999). Contaminated sediments may therefore threaten the lives of organisms in the marine environment due to the toxicity, long time persistence, bioaccumulation and biomagnifications of these lipophilic organic micro-pollutants (Kumar *et al.,* 2008; Zhao *et al.*, 2010).

Various analytical methods have been used to characterise PCDD/Fs and dioxin-like PCBs in a sediment matrix. Gas chromatography - high resolution mass spectrometry (GC-HRMS) offers a possibility to chemically identify and quantify individual congeners (Schecter *et al.*, 1999; Denison *et al.*, 2002; Besselink *et al.,* 2004; Denison *et al.,* 2004) in the matrix and to enable the assessment of risks associated with the congeners (Long *et al.,* 2006). To estimate the risks from the GC-HRMS results it is assumed that the additivity principle of a pollutant's response or effect is valid, which means the absence of agonistic and antagonistic interactions, and that these effects are produced through the same mechanism of toxicity. However, it has been shown that complex mixtures of PCDD/F and dioxin-like PCB congeners elicit synergistic and/or antagonistic interactions (Joung *et al.,* 2007; Schroijen *et al.*, 2004). In addition, chemical analysis of individual congeners, particularly in small concentrations, can be very expensive and time consuming. Presence of compounds with aryl hydrocarbon receptor (AhR) affinity, but not commonly measured, and the absence of toxicological equivalencies (TEQs) for several congeners further limit the use of this analytical method (Long *et al.,* 2006; Joung *et al.,* 2007). To overcome some of these drawbacks, biological assays utilising either biomolecular techniques (e.g. immunoassays) or living materials (e.g. *in vitro* chemically activated luciferase gene expression, CALUX) have been used as rapid and cost-effective screening methods for chemicals with selective and specific biochemical interactions (Roy *et al.,* 2002). For example, CALUX bioassay screens for chemicals with AhR potential *(*Schecter *et al.,* 1999; Denison *et al.,* 2002, 2004; Song *et al.,* 2006) and produces a single integrated biological equivalency (CALUX-BEQ) of the mixtures (Besselink *et al.,* 2004). CALUX also measures a response which is a single toxicity end-point produced by AhR active compounds that cannot be measured and/or are below the detection level of chemoanalysis (Joung *et al.,* 2007). The major drawback of a bioassay such as CALUX is that there is no information about the congener pattern.

The CALUX method has been explained by various authors (Murk, 1996; Denison *et al.,* 2002, 2004; Croes *et al.,* 2011; Van Langenhove*et al.,* 2011). It uses genetically modified cells (hepatoma cells stably transfected with a reporter gene) which respond to chemicals that activate the cytosolar aryl hydrocarbon receptor (AhR) by induction of luciferase (Denison *et al.,* 2004; Croes *et al.,* 2011). By this method the toxicity of these pollutants such as PCDD/Fs and dl-PCBs is produced either as a change in gene expression mediated through the AhR or by interference with other pathways (Hurst *et al.,* 2004). Estimation of relative potency and toxic potential can therefore be done by measuring the activation level of AhR gene expression (United States Environmental protection Agency, 2008). However, even when a rigorous clean-up and separation procedure of the sample extract is performed, interferences by PCDD/Fs on dl-PCBs and vice versa or by other AhR ligands are still possible (Sanctorum *et al.,* 2007a).

The literature regarding CALUX analyses in marine sediments is very limited. Most of the PCDD/F analyses in the world have been performed with GC-HRMS. In those studies where the CALUX technique was used, the focus was more on method development, on the comparison with the GC-HRMS method and on the screening of food and feed (Van Overmeire *et al.,* 2004; Hoogenboom *et al.,* 2006). In addition, very little data on dl-PCBs are available in literature. The fact is, PCDD/F BEQ levels are in general by far higher than those of the dl-PCBs. Both chemo-analysis and CALUX analysis research on marine sediments in Africa are scarcely documented (Pieters, 2007; Nieuwoudt *et al*., 2009). Regarding Tanzania, only total PCBs in sediment (Machiwa, 1992) and PCDD/Fs and dl-PCBs in free range chicken have been reported (International POPs Elimination Network [IPEN], 2005).

Although there exist no data about the presence of PCDD/Fs and dl-PCBs in the environment (more specifically in the sediment) of Tanzania, the applications of PCBs in electrical transformers and in other equipment is known (Loomis *et al.,* 1997). In coastal Tanzania, there are a lot of municipal, chemical and even hospital wastes that are discharged into the Indian Ocean after incineration and open burning of mixed wastes (Machiwa, 1992). Wood burning is a common source of fuel as most households use either charcoal or firewood for cooking. In many local households, there is uncontrolled burning of plastics. Vehicle emissions are abundant due to increased traffic and importation of old, second-hand cars (Mbuligwe & Kassenga, 1997). The current major outcry of the country has been on the vandalism of electrical transformers (Maleko, 2005) in search of their coolant for unspecified domestic or commercial use. Since Dar es Salaam is by far the largest city in Tanzania, the Mtoni estuary, being the main aquatic system in that area, was selected to study PCDD/F and dl-like PCB levels in the aquatic environment for the first time. The first objective was to assess PCDD/F and dl-PCB levels in sediments of the mixing zone of the Mtoni estuary, including the Kizinga and Mzinga River mouths, during wet and dry seasons. A second objective was to eventually link the observed levels to local sources.

4.3 Methodology

4.3.1 Study area

The Mtoni estuary (Figure 4.1) is located at approximately 3 km south of Dar es Salaam (Tanzania) and receives fresh water input from the Kizinga and Mzinga rivers. The creeks have mangrove trees such as *Avicennia marina, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora mucronata and Sonneratia alba* species growing on both sides (Mlay *et al., unpublished)*. The fresh water input from both rivers is low. An average base-line flow rate of 1 m^3/s is observed in Kizinga River with an increase to 8 m^3/s in the rainy season while the water-flow rate in the Mzinga River is unstable and lower than in the Kizinga River (Van Camp *et al.,* 2013). Hence, the effect of the river discharges on the hydrodynamics of the Mtoni estuary is very limited. The seven sampling stations (E1 to E7) were located in the mixing zone and their salinities vary from almost fresh to brackish water with somewhat higher salinities in the dry season (Mangion, 2011). Downstream this mixing zone (stations F5 to F8), the water becomes rapidly sea water while the stations F1 to F4 more upstream have fresh water. This estuarine mixing zone was selected because it integrates influences of natural and anthropogenic sources in the riverine and marine systems.

The Mtoni estuary is highly impacted (PUMPSEA, 2007) by discharges of various origin: (1) the Kizinga and Mzinga rivers draining the mangrove forest (Kruitwagen *et al.,* 2008), (2) the wastewater drainage systems from industrial and residential areas (of a population of around 500,000 inhabitants; NBS, 2003), (3) charcoal burning, (4) mangrove harvesting for residential places, (5) salt mining, (6) tourism and (7) agriculture (Taylor *et al.,* 2002).

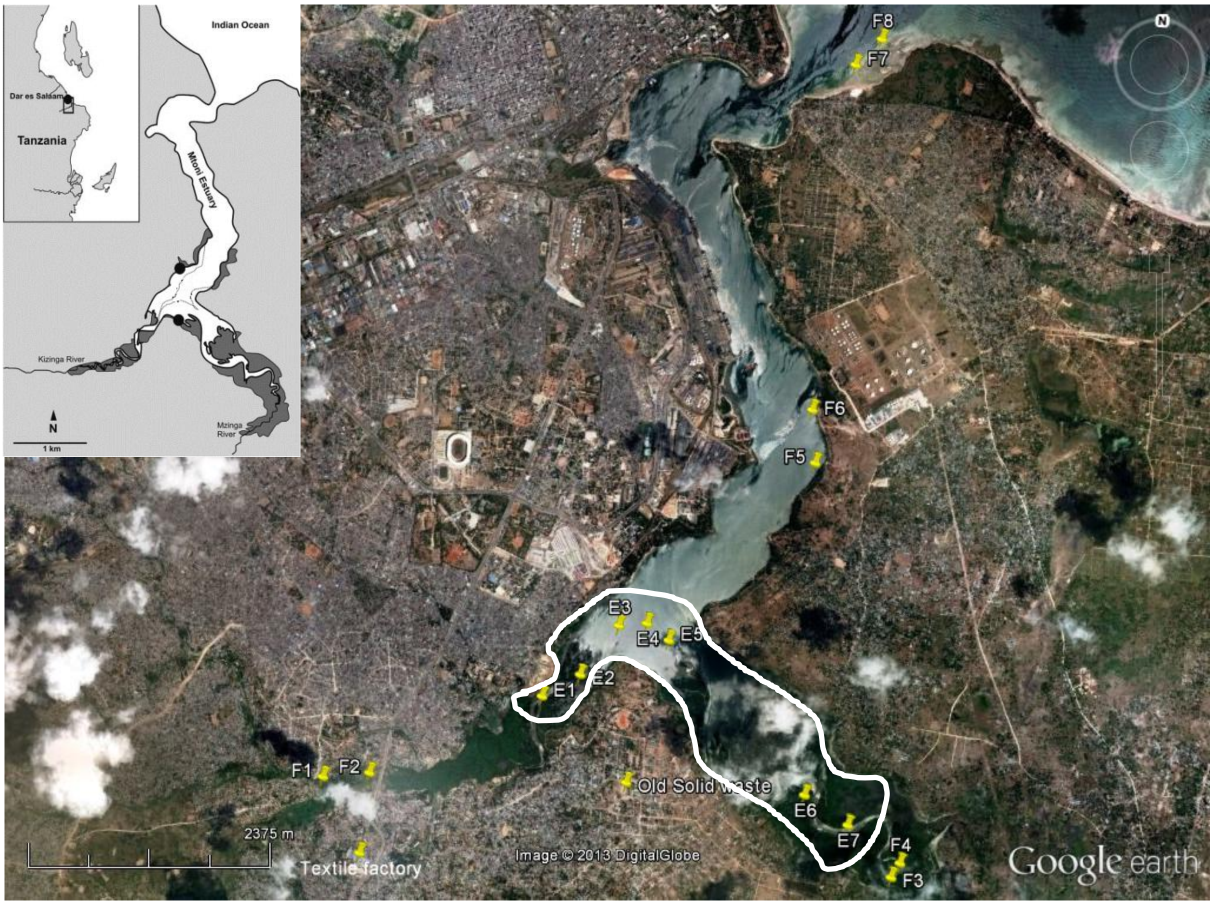

Figure 4.1: **Sampling points in the Mtoni estuary, Dar es Salaam: E1 and E2 in the Kizinga River, E3-E5 at the confluence and E6 and E7 in the Mzinga River. Stations F1-F2 in the Kizinga River, F3-F4 in the Mzinga River, F5-F6 at the Navy shore and F7-F8 at Kigamboni Seaway are additional sampling points. White line delimits the estuarine mixing zone. The solid waste dumping site and textile factory are also indicated.**

The Kizinga river that drains the urbanised areas of Keko, Chang'ombe, Kurasini and Temeke (approximately 400,000 inhabitants; NBS, 2003) is suspected to carry a variety of wastes and discharges originating from agricultural, industrial as well as residential sources (Taylor *et al.,* 2002). The Mzinga river, on the other hand, drains the rural areas of Vijibweni, Tuangoma and Mji Mwema with a population of around 90,000 (NBS, 2003). Due to rapid growth of settlements along the Mzinga creek resulting from increased human population, the river is suspected to carry agricultural and residential wastes and discharges presumed to be emptied into the creek. The estuary further receives inputs from the Dar es Salaam harbour which is located near the mouth of the estuary during diurnal tides (up to 5 m amplitude) and from the Mtoni solid waste dumping site located in between the two rivers.

4.3.2 Sampling

Sampling of sediments was conducted in the mangrove forests during low tides at Kizinga and Mzinga creeks (Figure 4.1) of the Mtoni estuary. Two sampling campaigns were conducted: one during the wet season (19th - 20th January 2011) and a second during the dry season (15th - 16th August 2011). December and January have an average precipitation rate of 194 and 89 mm respectively, while these rates in July and August are much lower with 48 and 47 mm respectively. River flows in the Dar-es-Salaam area are mainly controlled by the precipitation rate in the previous period. The flows of Kizinga and Mzinga rivers are highest in the wet season (the highest discharge rates can go up to 15 m^3/s for the Kizinga river and 7 m^3/s for the Mzinga river) while in the dry season, base-line flows of 1 m^3/s in the Kizinga river and even lower in the Mzinga river were observed (Van *Camp et al.,* 2013). The impact of both rivers on the pollutant levels in the mixing zone can thus best be estimated by sampling in that zone at high (wet season) and at low (dry season) river flow and comparison of the results.

Samples were collected from exactly the same locations during both campaigns. Seven sampling stations were identified using a hand-held global positioning system (GPS): two in the Kizinga River (E1 and E2), two in the Mzinga River (E6 and E7) and three at the confluence of the two rivers (E3, E4 and E5).

From the results obtained during the wet and dry seasons in the mixing zone of the estuary, it appeared that the sampling stations in Kizinga River and close to its mouth showed higher PCDD/F values than the stations in and close to Mzinga River. It was thus interesting to investigate PCDD/F levels more upstream in both rivers. In addition, the salinity gradient in the mixing zone is also small and real marine water samples were not included in the previous samplings. We were thus not able to appreciate any evolution of the PCDD/F levels from the estuarine mixing zone towards the marine environment. Therefore, an additional sampling campaign was organised at 3 end-members in October 2012: one site in the Kizinga River (fresh water stations F1-F2) much more upstream than stations E1 and E2, one site in Mzinga River (fresh water stations F3-F4) slightly more upstream than stations E6 and E7, but these latter stations were yet, compared to the stations E1 and E2 in Kizinga river, much more upstream and two sites in the marine area, close to and at the mouth of the estuary (respectively marine water stations F5-F6 and F7-F8) (Figure 4.1).

All those samples were taken from two sub-sites within a distance of 20 m, except in the Kizinga River. The first subsample was taken at the junction of the river and the textile wastewater stream and the second was taken 200 m upstream of the River very close to unauthorised human settlements.

Sediment sampling was done as described by EPA (United States Environmental protection Agency, 2001) using a hand corer (30 cm height, 6 cm internal diameter). The corer was gently pushed in the mangrove sediments, closed at its upper end with a lid and smoothly removed by twisting and pulling. The sediments were then pushed out of the corer tube using a piston and sectioned into three segments corresponding to depth intervals of 0-3, 3-6 and 6-9 cm. All sediment samples were packed in prior labelled and zipped polyethylene bags, stored in iceboxes and later frozen to -20°C. Sediment samples were then air-transported while frozen to the Laboratory of the Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel (VUB) in Belgium and lyophilised (Leybold Heraus Lyophiliser) prior to the dioxins and dioxin-like compounds analyses.

4.3.3 Chemical reagents and standards

Acetone (Pesti-S grade, minimum 99.9%), n-hexane (minimum 96% assay) and toluene (minimum 99.8% assay) both dioxins and PCB grade, were purchased from Biosolve (The Netherlands). Ethyl acetate (Pestanal, 99.8% assay) was purchased from Sigma–Aldrich (Germany). Sulphuric acid (95 97% w/w, ACS reagent) and Dimethylsulfoxide (DMSO) were obtained from Merck (Germany). Glass fibre filters were purchased from Whatman (UK). Alpha-minimal essential medium (α-MEM), foetal bovine serum (FBS) and trypsin (0.25%) were obtained from Gibco, UK. Phosphate buffered saline (PBS) was obtained from Ambion (UK). Luciferase assay substrate and buffer were purchased from Promega (The Netherlands). Anhydrous sodium sulphate was purchased from Boom (The Netherlands). The X-CARB was purchased from Xenobiotic Diagnostics Systems, XDS Inc, USA and the solution of 2,3,7,8-TCDD standard (50 ng/mL, purity 99%) was purchased from Campro Scientific (The Netherlands).

4.3.4 Determination of Particle Size and Total Organic Carbon (TOC)

The concentration of TOC was determined in all 50 sediment samples with a CHN analyser (Carlo Erba) on a known amount (about 12 mg) of sediment sub-sample placed in a silver capsule and pre-treated by acidification with 5% HCl. The grain size distribution was determined by an external laboratory at 3 stations with low, medium and high organic matter (OM) content in their sediments (stations E7, E2 and E1, respectively) to test the relation between both variables. Approximately 10 g lyophilised and homogenised sediment sample was prepared by removing salts, OM and carbonates using hydrogen peroxide and hydrochloric acid respectively. A stable suspension was obtained after rinsing and adding 5 ml of a peptizing agent. The coarse fraction (> 75 µm) was separated by wet sieving on a 75 µm sieve, then dried at 105°C, and finally dry sieved. The grain-size distribution of the fine fractions 2-75 µm and <2 µm was obtained using the Sedigraph 5100 coupled to a Mastertech 51. The precision for 10 consecutive measurements on aliquots of the same sample was around 1% for each grain-size fraction.

4.3.5 Analysis of dioxin and dioxin-like compounds in Sediments

4.3.5.1 Sample Preparation

In all 50 sediment samples PCDD/Fs and dl-PCBs were analysed. Lyophilised sediment (5 g) was extracted using pressurised liquid extraction in an Accelerated Solvent Extractor, ASE®, (Dionex, USA) with a toluene:methanol (4:1 v/v) solvent system (Baston & Denison, 2011) and 33 mL extraction cells. The ASE extraction conditions were: 125 °C oven temperature; 1500 psi (100 MPa) pressure; 10 min static time; 6 min oven heating time; 60 s purge time; 60% of extraction cell volume as flush volume and 2 static cycles. The extracts were then concentrated in a vacuum centrifuge to near dryness and later re-suspended in n-hexane (5 mL).

4.3.5.2 Column Preparations for Clean up

The clean up and fractionation is based on the EPA Method 4435 (United States Environmental protection Agency, 2008) from which we use the same piggybacked setup of columns. A sequential setup of columns is used to remove Polychlorinated Aromatic Hydrocarbons (PAHs) and break down undesired compounds (acid silica gel) and differentially elute PCDD/Fs and dl-PCBs (X-CARB affinity chromatography column). An additional, third, column was added for sulphur removal. Column preparation is described below and all columns are prepared daily.

An activated copper column (for elemental sulphur removal) was prepared by filling a Pasteur pipette from bottom to top with glass wool and 1 cm of activated (with a 20% hydrochloric acid solution) copper. The activated copper column was first rinsed with Milli-Q de-ionised water (3 x 1 mL) and then with acetone, toluene and n-hexane (each 3 x1 mL) in that order. The activated copper columns were stored submerged in n-hexane to avoid oxidation.

An acidified silica column was prepared by filling a 10-mL disposable column (ID 0.8 mm), from bottom to top, with glass wool, sodium sulphate (0.5 cm^3), sulphuric acid-silica gel (33% H_2SO_4 on silica gel w/w; 4.3 cm^3) and sodium sulphate (0.5 cm^3). The acid silica column was then rinsed with n-hexane (3 x 10 mL).

Similarly, an X-CARB column was prepared by using an open ended tube (ID 0.8 mm), but this was filled (bottom to top) with glass wool, sodium sulphate (0.5 cm^3), 1% X-CARB (1 cm^3 packed), sodium sulphate (0.5 cm^3) and glass wool. The column was inverted and rinsed sequentially with acetone (5 mL), toluene (20 mL) and n-hexane (10 mL).

The acid silica gel column is placed on top, the copper column in the middle and the X-CARB column at the bottom end prior to sample loading. The individual columns are connected to each other and rinsed with n-hexane ensuring that the columns do not run dry.

4.3.5.3 PCDD/Fs and Dl-PCBs Clean Up and Fractionation

The sediment extract in n-hexane was first sonicated for 5 minutes followed by vigorous vortexing. An aliquot (2 mL from the original 5 mL) was quantitatively loaded on the acid silica gel column, followed by elution of the column with n-hexane (total 21 mL). The acid silica gel and activated copper columns were removed once the solvent had passed through. The remaining X-CARB column was further rinsed with extra n-hexane (5 mL), followed by elution with a mixture of 8:1:1 of n-hexane:toluene:ethylacetate (3 x 5 mL) to collect the fraction containing coplanar PCBs (i.e. PCB fraction). The fraction containing the PCDD/Fs (dioxin fraction) was afterwards eluted (back-flushed) with toluene (3 x 5 mL) after inverting the X-CARB column. The PCB and dioxin fractions were later concentrated to dryness in a vacuum centrifuge and re-suspended in n-hexane (4 mL) for CALUX analysis.

4.3.5.4 CALUX analysis

CALUX analysis was performed as described by Van Langenhove *et al.,* (2011). Briefly, mouse hepatoma cells (H1L7.5c1 cell line) were cultured in α-MEM with 10% FBS at 37 °C, 80% relative humidity and 5% CO_2. The cells were seeded (at an approximate density of 7.5 x10^5 cells/mL in a clear bottom 96-well plate (Greiner Bio-One, Germany) and incubated for 24 hrs to reach a monolayer of cells.

TCDD standard solutions (from 125 nM down to 30 pM) were made in DMSO and 4 µL of this solution was transferred to 2 mL of n-hexane. Samples, in 4 mL n-hexane solvent, were serially diluted in n-hexane and 4 µL of DMSO was added to each vial as a carrier solvent. For both the TCDD- and sample solutions, the remaining n-hexane was evaporated using a vacuum centrifuge, leaving behind either 4 µL DMSO with TCDD standard or 4 µL DMSO containing either PCDD/Fs or dl-PCBs from the sample extract.

Prior to dosing, these standards and sample dilutions were diluted 100 times with cell culture media (4 µL standard solution or sample in 396 µL α-MEM with 10% FBS). Final solutions were vortexed vigorously and dosed in triplicate (100 µL per well).

After 24 hrs of incubation, cells were rinsed and visually inspected for abnormalities. Then, cells were lysed and shaken for 5min. Luciferin treatment was performed in the Glomax 96-well microplate luminometer (Promega, USA), where the light output in relative light units (RLUs) was measured. Sample responses were expressed as percentage maximum induction to 2,3,7,8-TCDD (%$TCDD_{max}$).

4.3.5.5 Statistical Analysis of Data

TCDD standards were used to generate the calibration curve. A four-variable Hill equation fitting the calibration curve (Elskens *et al.*, 2011) was used to produce a sigmoid curve of the standard solutions. The calibration equation was then used to convert the measured RLU values of the samples into CALUX-BEQ value (Goeyens *et al.*, 2010) by comparing the sample response curve with the sigmoid dose-response curve (Elskens *et al.*, 2011). Further data treatment was done using slope ratio and Box-Cox transformation methods by linearisation of the non-linear Hill regression equation as described by Elskens *et al.,* 2011). Effective concentrations at 50% $TCDD_{max}$ (EC_{50}) of standard and samples were used to determine the potency or bioanalytical equivalency (BEQ) of the samples. Statistical analysis was performed using Microsoft Spreadsheet for Windows 2007 and graphical representations were performed using Spreadsheet and Sigmaplot programmes (SigmaPlot 10.0). Pearson Correlation and Principal component analysis (PCA) were performed using Predictive Analytic Software (PASW, version 16.0 for Windows) with the PCDD/F and dl-PCB values and the geochemical properties (% total nitrogen (TN), % total carbon (TC) and %TOC) as variables (total 5), and using the concentrations of pollutants in both seasons.

4.3.5.6 Quality Control (QC) and Quality Assurance (QA)

For each batch of samples, a blank sample was introduced through the complete treatment procedure (procedural blank) to monitor the activity contributed by solvents and column matrices used in the sample treatment. Moreover, DMSO and media blanks were added during dosing to detect contamination and to determine the experimental background level of the cell culture. All blank samples were measured in triplicate and were treated in a similar way as real samples. The results with $p < 0.05$ (Student T-test) were considered statistically significant. The limit of detection (LOD) was calculated according to the IUPAC definition. The blank value was taken as the average background of the model fit represented in %RLU relative to the maximum TCDD-induced RLUs (Elskens *et al.,* 2011).

QC experiments were conducted on each 96-well plate, using an in-house QC solution (0.250 pg TCDD/µL), to assess precision of the CALUX method and detect bias. Procedural blank fractions were also spiked with the same QC solution prior to dosing to detect agonistic and antagonistic ligands in that blank solution.

4.4 Results and Discussion

4.4.1 Grain size Distribution

Sandy particles dominated the mangrove sediments in the study area, with sand (> 75 µm) contributing for more than 60% of the weight. The correlation between TOC and the fine grain size fraction (% < 2 µm) was good (r^2 = 0.92) this was still the case (r^2 = 0.82) between TOC and the mud+silt fraction (% < 75 µm). It is well-known that muddy sediments having a high TOC content but also a high amount of fine grain size fraction (< 2 µm), accumulate by far higher amounts of pollutants than sandy sediments (Baeyens *et al.*, 1991). This means that TOC values can eventually be used to normalise the pollutant concentrations in the sediment versus the mud fraction (% < 2 µm).

In the Mtoni estuary, the high sand proportion implies that the capacity of the mangrove sediments to adsorb the dioxins and dl-PCBs is medium to low. On the other hand, a high sand fraction favours abiotic processes such as enhanced diffusion of oxygen in the sediment making pollutants more bioavailable due to faster oxidation of organic matter and simultaneous release of associated persistent organic micro-pollutants such as dioxins (Davies & Tawari, 2010; Holmer, 2003).

4.4.2 The CALUX H1L7.5c1 Assay

The CALUX bioassay integrates the responses of every AhR ligand available in the analysed sample and because of this; it provides only an indication of the possible overall toxicity *(*Van Langenhove *et al.,* 2011). The results presented in this study show the importance of full-dose curves for environmental samples because the additivity principle is not uphold, despite (1) the use of a sulphuric acid silica gel column in the sample clean up step aimed at eliminating interferences caused by PAHs and (2) separation of PCDD/Fs and dl-PCBs avoiding known antagonistic effects between these two compound groups (Van Langenhove *et al.*, 2011).

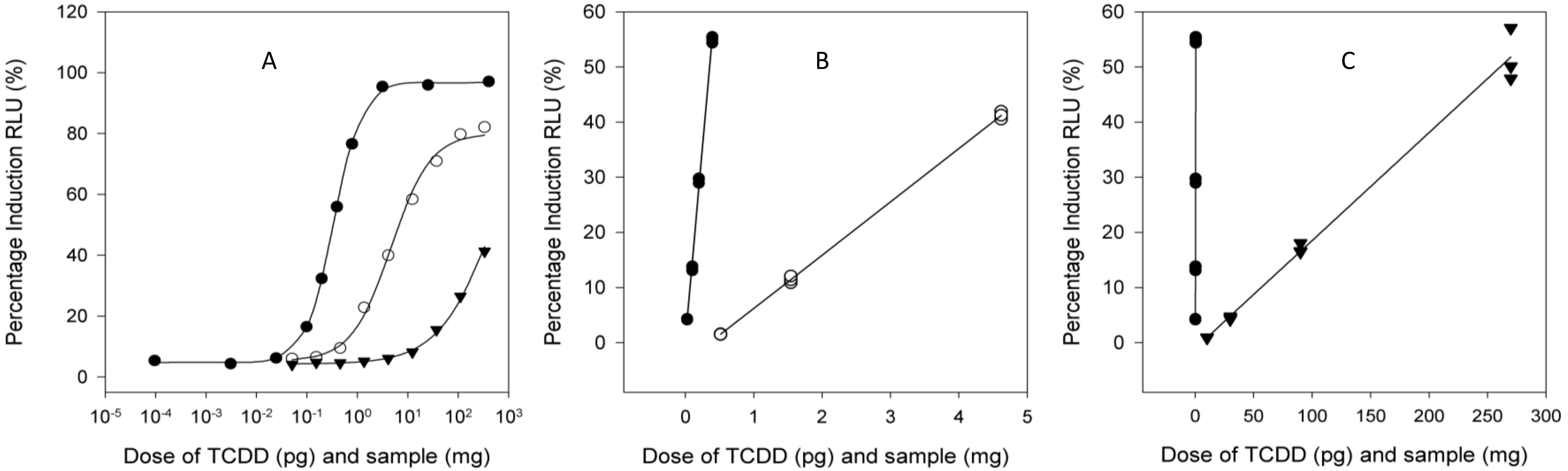

Figure 4.2: **(A): Typical TCDD-standard curve (●), sample with full dose response (○) and sample not attaining an upper plateau (▼) using Hill regression. B: Same data portrayed using the slope ratio method for TCDD (●) and full dose sample (○). C: Slope ratio for TCDD (●) and sample without upper plateau (▼).**

Luciferase induction was reproducible with coefficients of variation (CV) less than 15% for a given standard or sample extract measured in triplicate. Mean values of luciferase response measured in three replicate wells were used to generate the dose–response curves. The dose–response curve of TCDD standards was sigmoidal in appearance as shown in Figure 4.2.

4.4.3 Quality Control

Blank samples (n = 6) spiked with the in-house QC solution (0.250 pg TCDD/µL) ranged in recoveries from 85 to 120% for the PCB fraction and from 91 to 115% for the PCDD fraction, well in accordance with an acceptable relative standard deviation of 20% (80-120% recovery of the TCDD spike). DMSO controls showed no marked difference in response to fitted background values (p = 0.45 for a two-tailed Student T-test). Media controls were generally lower in response, but borderline not statistically significantly different (p = 0.06 for a two-tailed Student T-test).

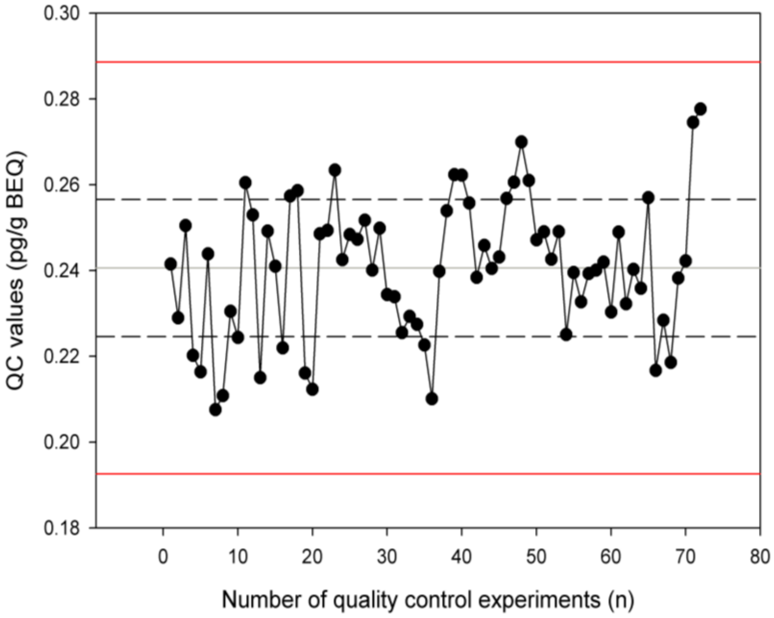

Figure 4.3: **Quality Control scatter chart of analyses during the study. The dotted line above and below the mean (0.24) indicate the mean ± 1SD values. The red lines indicate the mean ± 3SD values.**

A total of 72 QC experiments were performed in this study. When these experiments are plotted on a control chart (Figure 4.3), a mean of 0.24 ± 0.02 pg/g indicated that the analysis procedures were reliable with a coefficient of variation (CV) at 7.2%. The LOD based on the averaged fitted background value and on the DMSO blank was 0.05 pg TCDD/well. Recalculating with the starting volume used in the serial dilution, an LOD of about 0.22 pg TCDD/g sediment was found.

4.4.4 Bioanalytical Equivalencies (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 EC_{50} 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 from Elskens *et al.*, (2011). The relationships between these methods indicate that they correlate well ($r^2 > 0.8$, Figure 4.4), 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).

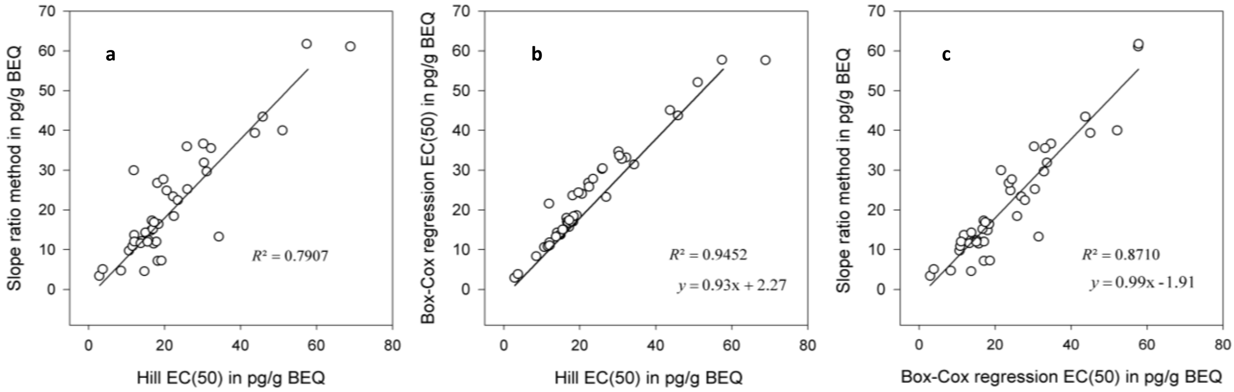

Figure 4.4: **Relationship between PCDD/Fs concentrations (EC_{50}) in Mtoni estuary sediment samples; (a) estimated by Hill equation and slope ratio method, (b) Hill regression and Box – Cox transformation and (c).Box – Cox transformation and Slope ratio**

Since the Hill equation is the most used method and for simplicity of comparison, Hill regression BEQs will be continuously employed in the study and are presented in Table 4.1. To account for non-parallelism that usually exists between dose–response curves of the reference standards and the sample, the BEQ PCDD/F values in a sample were determined based on the EC_{20}, EC_{50} and EC_{80} of the maximum TCDD result ($TCDD_{max}$). Dl-PCBs responses are lower than those of PCDD/Fs owing to their lower toxicity equivalent factors (TEFs). Their potency was therefore determined using inverse prediction, assuming a sample behaves like a diluted TCDD standard solution (Elskens *et al.,* 2011).

In order to eliminate concentration differences emanating from variations in the sand fraction of the sediments, pollutant concentrations in the Mtoni estuary sediments were normalised to TOC. Despite the normalisation, PCDD/F and dioxin-like PCB profiles showed no obvious vertical trends. The rather steady depth profiles indicate that the estuarine sediments are fairly well-mixed making the profiles more or less uniform. It is possible that the hydrodynamics of the estuary (tidal amplitude of up to 5 m at the mouth) provide frequent sediment mixing smoothing out possible vertical concentration gradients. Perhaps, a depth of 9-cm is not sufficient enough to observe any difference and therefore It may be interesting in the future to study the pollutant profiles in deeper sediment layers.

Table 4.1: Mtoni estuary sediment properties and the CALUX-BEQ values for PCDD/Fs and PCBs

				CALUX-BEQ (pg/g sediment)	
Code and Location	**Section depth**	**Season**	**TOC (%)**	**PCDD/Fs EC_{20}-EC_{80} range**	**Dioxin-like PCBs***
E1: S 06°52.443 E 039°17.014	0–3 cm	wet	0.62	44.6 – 21.6	0.35
		dry	1.86	63.3 – 41.1	0.47
	3–6 cm	wet	0.63	45.4– 25.9	0.33
		dry	1.77	53.0 – 17.4	1.03
	6–9 cm	wet	0.50	28.0 – 18.0	0.34
		dry	2.23	23.0 – 12.2	0.26
E2: S 06°52.357 E 039°17.099	0–3 cm	wet	1.02	22.3 – 14.9	0.38
		dry	1.46	55.7 – 37.7	0.63
	3–6 cm	wet	1.02	31.4 – 17.6	0.34
		dry	0.56	15.4 – 8.7	0.26
	6–9 cm	wet	0.56	21.5 – 10.4	0.33
		dry	0.24	20.0 – 10.0	0.24
E3: S06°52.058 E 39°17.355	0–3 cm	wet	2.55	85. 7 – 55.4	0.47
		dry	1.35	72.6 – 45.4	0.41
	3–6 cm	wet	3.57	38.2 – 23.9	0.15
		dry	0.68	43.6 – 11.4	0.40
	6–9 cm	wet	3.83	32.3 – 13.1	0.14
		dry	0.66	19.4 – 14.0	0.11
E4: S 06°52.090 E 039°17.501	0–3 cm	wet	1.04	42.7 – 24.3	0.48
		dry	0.98	81.2 – 8.90	0.27
	3–6 cm	wet	1.15	31.5 - 10.4	0.28
		dry	1.71	49.9 – 38.4	0.55
	6–9 cm	wet	1.23	20.0 – 13.9	0.39
		dry	0.96	42.8 – 15.6	0.10
E5: S 06°52.164 E 039°17.658	0–3 cm	wet	2.38	12.9 – 8.75	0.25
		dry	0.70	15.9 – 8.89	0.55
	3–6 cm	wet	0.90	3.73 – 2.31	0.18
		dry	0.80	35.3 - 11.0	0.47
	6–9 cm	wet	1.40	5.29 – 2.59	0.23
		dry	0.46	16.0 – 9.00	0.36
E6: S 06°52.882 E 039°18.391	0–3 cm	wet	3.12	36.9 – 18.3	0.28
		dry	3.57	24.1 – 14.0	0.39
	3–6 cm	wet	4.42	19.2 – 9.8	0.19
		dry	3.01	25.4 – 11.7	0.26
	6–9 cm	wet	2.54	22.9 – 10.8	0.18
		dry	4.30	22.7 – 10.6	0.24
E7: S 06°52.952 E 039°18.454	0–3 cm	wet	7.50	24.5 – 11.7	0.54
		dry	1.35	19.4 – 11.7	0.19
	3–6 cm	wet	2.41	25.6 – 12.4	0.44
		dry	1.72	25.4 – 14.4	0.23
	6–9 cm	wet	7.07	17.1 – 8. 45	0.60
		dry	1.66	12.4 – 5.85	0.24

*Results were determined by the inverse prediction method

The PCDD/F results from the CALUX screening for the Mtoni estuary sediment extracts are shown in Figure 4.5a. The PCDD/F concentrations in this Figure were based on the Hill BEQ$_{50}$ and ranged from 5.7 ± 1.4 to 39.9 ± 5.8 pg BEQ/g sediment in wet season and between 14.1 ± 2.0 and 32.8 ± 4.7 pg BEQ/g sediment in dry season. Higher levels of PCDD/Fs in both seasons were found in the Kizinga River and in confluence stations close to it. Wet season samples contained less PCDD/Fs than dry season samples in the Kizinga River, however at the Mzinga site 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 ($n = 42$).

Response levels of dl-PCBs (Figure 4.5b) ranged from 0.21 ± 0.03 to 0.53 ± 0.03 pg BEQ/g sediment in wet season and from 0.22 ± 0.03 to 0.59 ± 0.04 pg BEQ/g sediment in dry season. These BEQ values were much lower (about 2 orders of magnitude) than those of the PCDD/Fs. At the 2 Kizinga stations, confluence station E5 and Mzinga station E6, lower PCBs values were observed in wet compared to dry season, while the opposite was true for confluence stations E3 and E4 as well as Mzinga station E7. High dl-PCB levels were generally correlated to high PCDD/s values. The PCB levels detected in the estuary showed no significant variations ($t = 0.27$ two tailed; $p = 0.79$) between the seasons ($n=42$).

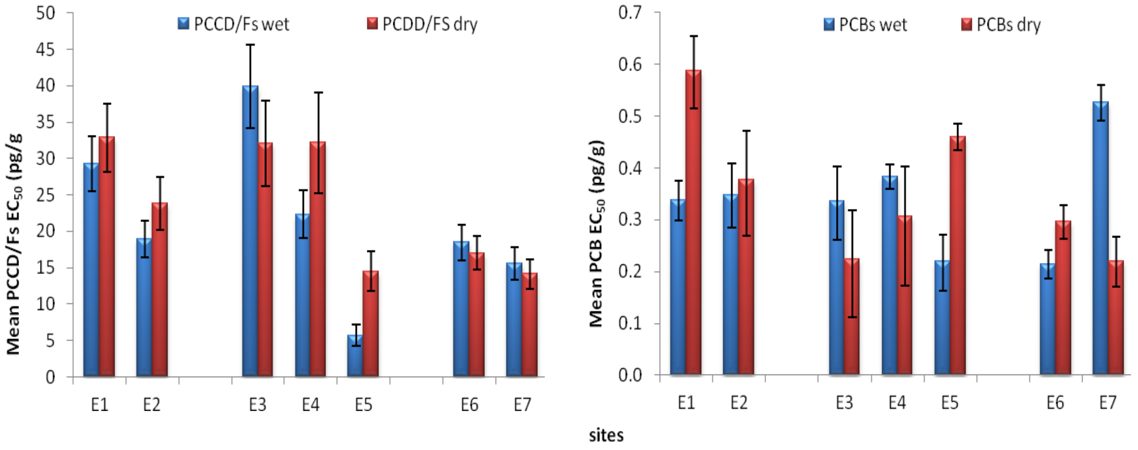

Figure 4.5: **BEQ (with uncertainty error bars) for PCDD/Fs (a) and PCBs (b) in the Mtoni sediments presented as averaged values of the three depth layers in dry and wet seasons.**

The Kizinga River drains the peri-urban environments of Temeke, Mbagala, Ukonga, Charambe, Kijichi, Vituka, Keko, Kitunda, Mtoni and Kurasini which are highly populated and have many small-scale to medium-scale industries. On the other hand, the Mzinga stream drains the rural environments of Mji Mwema, Vijibweni and Tuangoma that are less populated and have very few industries. As a result of various socio-economic activities, polymeric materials (household scraps, plastics, vehicle tires and electronic wastes) are abundantly present in most domestic and industrial wastes. Due to lack of appropriate infrastructures, most of these wastes are collected in open dumping sites and then burnt. Polymeric materials in wastes, when subjected to open burning, may lead to formation of PCDD/Fs and dl-PCBs (Estrellan & Iino, 2010). In addition, 2 other sources of PCDD/Fs and dl-PCBs have to be considered in the study area: (1) charcoal and/ or wood burning is the main source of energy for domestic purposes; (2) privately owned second hand commuter buses that need frequent services are the main means of transport for most residents. Mkoma, (2008) observed that biomass burning (wood and charcoal burning) 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 polymeric waste, domestic wood/charcoal burning and with traffic-related emissions.

The presence of sources in the vicinity of the sampling stations must also be taken into account to explain the detected levels of PCDD/Fs and dl-PCBs. For example, upstream station E1 in the Kizinga River is downstream to an untreated wastewater discharge point of a textile factory. Higher levels of both PCDD/F and dioxin-like PCBs in Kizinga stations compared to Mzinga stations may reflect the combined effects of the denser population and the more industrial activities in the Kizinga river Basin because both are directly related to the various sources of PCDD/Fs and dl-PCBs mentioned here-above.

A third sampling campaign was organised to verify the higher PCDD/F and dl-PCB levels in the Kizinga River and to assess the change of the dioxin levels downstream the previous sampling stations in the estuary (E1 to E5). The most upstream sampling station in the Kizinga River (F1) which is 200 m upstream of a sewage factory pipe and near unauthorised human settlements, is very rich in organic matter (Table 4.2), showed high PCDD/F (400 pg-BEQ/g sediment) and dl-PCB levels (0.91 pg-BEQ/g sediment). The second upstream Kizinga River sample (F2) is also high but not exceptional with 21 pg-BEQ/g PCDD/Fs and 0.63 pg-BEQ/g dl-PCBs. The levels in the upstream Mzinga River samples were close to those of the Kizinga River sample F2 (Table 4.2). A negative concentration gradient of PCDD/Fs and dl-PCBs in sediments was observed in the downstream direction. The levels at the mouth of the Mtoni estuary were very low: 1-2 pg-BEQ/g for PCDD/Fs and below LOD for dl-PCBs. These additional results confirmed the previous conclusions that the impact of dioxins on the Kizinga River Basin is higher than that of the Mzinga River and that a dilution effect is clearly noticeable in the downstream direction.

Table 4.2: CALUX-BEQ values for PCDD/Fs and dl-PCBs in the end-member samples

Code and Location	TOC (%)	CALUX-BEQ (pg/g sediment)		
		PCDD/Fs BEQ50	PCDD/Fs BEQ_{20}-BEQ_{80} range	Dioxin-like PCBs*
F1: S 06°53’01.29’’ E 039°15’48.33’’	8.58	397	652-242	0.91
F2: S 06°52’58.07’’ E 039°16’06.81’’	2.08	20.7	26.7-16.0	0.68
F3: S 06°53’17.67’’ E 039°18’52.06’’	1.05	17.2	21.0-14.2	0.60
F4: S 06°53’13.60’’ E 039°18’52.69’’	0.56	12.5	15.8-9.86	0.74
F5: S 06°51’06.95’’ E 039°18’22.82’’	0.85	6.71	7.70-5.85	0.25
F6: S 06°50'57.46’’ E 039°18'19.16"	0.72	6.82	7.47-6.23	0.23
F7: S 06°49'03.71’’ E 039°18'24.11"	0.28	1.66	2.43-1.14	< LOD
F8: S 06°48'57.62’’ E 039°18'34.60"	0.99	1.03	1.74-0.61	< LOD

*Results were determined by the inverse prediction method

4.4.5 Correlation between pollutants and sediment geochemical characteristics

Calculations of the Pearson correlation coefficients were performed on pollutant levels as well as sediment geochemical characteristics of all samples (n = 42) in the mixing zone in both seasons (Table 4.3). A significant correlation was observed between PCDD/Fs and dioxin-like PCB levels ($r^2 = 0.40$, $p << 0.05$), however, no significant correlations ($p > 0.05$) were found with % TOC although it is well-known that lipophilic compounds such as PCDD/Fs and dl-PCBs tend to preferably associate with organic fractions (Koh *et al.*, 2004; 2006; Pieters, 2007).

Table 4.3: Correlation coefficients between pollutants and various geochemical parameters

	PCDD/Fs	PCBs	%TOC	%TN	%TC
PCDDs	1.000				
PCBs	**0.395**	1.000			
%TOC	0.070	0.208	1.000		
%TN	-0.049	0.120	**0.913**	1.000	
%TC	-0.065	0.172	**0.823**	**0.893**	1.000

Significant values at α = 0.05 (two-tailed) are in bold

The weak correlation between the pollutants and %TOC is contrary to the theoretical expectation similar to Hilscherova *et al.,* (2003), but the sources of TOC and PCDD/Fs or dl-PCBs are quite different in the Mtoni study area. TOC in the estuary and rivers is mainly originating from 2 sources: (1) mangrove degradation compounds and (2) untreated domestic sewage water (Chapter Two), while PCDD/Fs and dl-PCBs originate from open burning processes and traffic related emissions. A mixing of the PCDD/Fs or dl-PCBs with the organic matter in the estuary can occur in all kind of ratios. It is thus not surprising that there exists no correlation between both variables. Good and significant correlations ($p < 0.05$) were observed between % TOC and % TC ($r^2 = 0.82$), % TN and % TC ($r^2 = 0.89$) and between % TOC and % TN ($r^2 = 0.91$).

4.4.6 Pollutant Source analysis by PCA

Multivariate analysis can be used to identify similarities and differences between pollutants in samples as a means to detect possible sources. As we could expect, the PCA results indicated that the variables can be represented by two principal components that accounted for 83.6 % of the total variance in the original data sets (Figure 4.6). Based on the loading distribution of the variables, % TC, % TN and % TOC constituted one related group (PC1), while the pollutants PCDD/Fs and dl-PCBs formed the other group (PC2). This clearly indicates that the sources of the PCDD/Fs and dl-PCBs in the Mtoni estuary differ from that of organic matter.

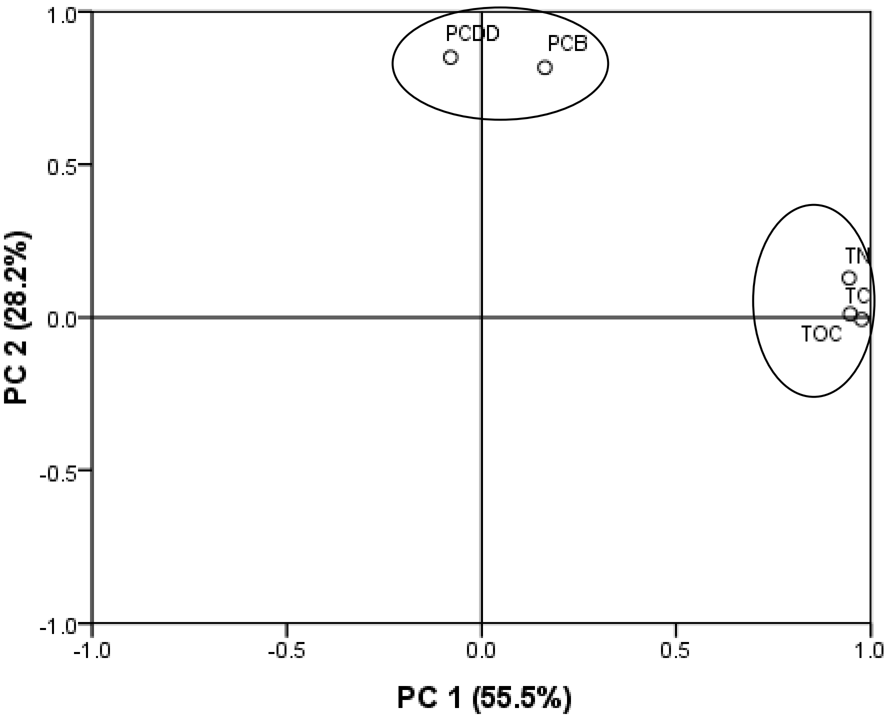

Figure 4.6: **A two-dimensional score plot of the PCDD/Fs and dl-PCBs in relation to geochemical parameters in the Mtoni estuary**

4.4.7 Comparison with Other Studies and with Sediment Quality Guidelines

Little information is available in literature regarding CALUX analysis of PCDD/Fs and dl-PCBs in marine, estuarine and river sediments. No such research has been conducted in Tanzania and Africa as a whole. Research on dioxin-like compounds in such sediments in the world used chemo-analysis or GC-HRMS, because this method is considered as the golden standard (Baeyens *et al.,* 2004). Furthermore, most research focused on PCDD/Fs while no data on dl-PCBs were available for comparison. Comparison with literature data indicated that the observed PCDD/F levels were covering the ranges observed in the literature (see Table 4.4). Only at the Belgian coast where lower values were observed (Sanctorum *et al.,* 2007).

An attempt was made to compare the PCDD/F and dioxin-like PCB levels with the sediment quality guidelines. Since Tanzania lacks these guidelines, National Oceanic and Atmospheric Administration (NOAA), USA and Canadian Sediment quality guidelines were applied to assess the toxicity and risk of the dioxin-like pollutants in the Mtoni estuary ecosystem. Mangrove sediments from Mtoni estuary have higher PCDD/F levels than the NOAA apparent effects threshold (AET) of 3.6 pg-TEQ/g. The levels were also higher than both the threshold effect level (TEL) for Canadian sediment quality guideline (0.85 pg-TEQ/g) and the probable effect level (PEL) of 21.5 pg-TEQ/g. There were no specific guidelines for dl-PCB levels.

Table 4.4: Comparison of PCDD/F and PCB levels (pg-BEQ/g dw) determined by CALUX bioassay in different marine sedimentary environments

Study site	PCDD/Fs[1]	PCBs[2]	Reference
Mtoni estuary, Tanzania[3]	1.0 – 397	<LOD – 0.91	This study
UK estuaries	1.0 - 88	-	Hurst *et al.,* 2004
Hong Kong mudflats	3 – 68	-	Wong *et al.,* 2005
North coast of Bohai Sea, China	3.4 - 28	-	Hong *et al.,* 2012
Masan Bay, Korea	17 - 275	-	Yoo *et al.,* 2006
Belgian coast	0.08 - 42.4	-	Sanctorum *et al.,* 2007b)
West coast, South Korea	3.4 - 11	-	Hong *et al.,* 2012

[1] Values presented are BEQ_{50}; [2] values are inversely predicted; [3] Values indicate the range of mean BEQ of both seasons (n = 6)

The comparisons have shown that there could currently be a risk regarding PCDD/Fs, while the risk associated with dl-PCBs is much lower. Because the toxicity of these chemicals is assumed additive, increasing levels in line with the increasing anthropogenic activities can be alarming to the biological community (Kruitwagen *et al.,* (2006) such as the barred mudskippers and soft bottom molluscs that inhabit the muddy areas. Presence of these pollutants in mangrove sediments may cause impairments, like abnormal growth and malformations. The effects can reach other organisms higher in the trophic level due to their bio-accumulation, bio-concentration and persistent properties. The levels of pollutants observed in the frequently exchanged upper (0-3 cm) layer can have impacts on the distribution and fate of pollutants to mangrove ecosystems and to higher organisms that use mangrove sediment organisms as their food.

4.5 Conclusion

This study is the first of its kind that used *in vitro* bioassay analysis (CALUX) to determine dioxin and dioxin-like compounds in the environments of East Africa. Sediment samples were collected in the Mtoni estuary and its tributaries, the Kizinga and the Mzinga Rivers, in the vicinity of Dar es Salaam. While it is well known that anthropogenic activities as open burning of plastic scraps, household burning of wood or charcoal and traffic related emissions which all frequently occur in the Dar es Salaam region can lead to PCDD/F and dl-PCB production, it was totally unknown if some or all of those sources resulted in the contamination of the nearby aquatic systems. Our CALUX analyses of the sediments in the Mtoni estuary and its 2 tributaries demonstrated that the range of PCDD/F values (1.0-400 pg-BEQ/g-sediment) covers the ranges observed in Western Europe and Eastern Asia sediments. Neither significant seasonal variations nor vertical gradients in the sediments could be observed. For dl-PCBs, we could not find sediment results obtained by CALUX in the literature. Sediments in the Kizinga River, which flows through a denser populated and more industrialised area than the Mzinga River, showed also higher PCDD/F and dioxin-like PCB levels. Finally, the overall BEQ values of PCDD/Fs and dl-PCB levels observed in sediments of the Dar es Salaam region indicate possible ecological and human risks that may emanate from these contaminants.

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4.7 References

Baeyens, W, Panutrakul, I. S., Elskens, M., Navez, M. L. J., & Monteny, F. (1991). Geochemical Processes in Muddy and Sandy Tidal Flat Sediments. *Geo-Marine Letters*, 188–193. http://dx.doi.org/doi:10.1007/BF02431011

Baeyens, Willy, Verstraete, F., & Goeyens, L. (2004). Elucidation of sources, pathways and fate of dioxins, furans and PCBs requires performant analysis techniques. *Talanta*, *63*(5), 1095–100. http://dx.doi.org/doi:10.1016/j.talanta.2004.06.010

Baston, D. S., & Denison, M. S. (2011). Considerations for potency equivalent calculations in the Ah receptor-based CALUX bioassay: normalization of superinduction results for improved sample potency estimation. *Talanta*, *83*(5), 1415–21. http://dx.doi.org/doi:10.1016/j.talanta.2010.11.035

Besselink, H. T., Schipper, C., Klamer, H., Leonards, P., Verhaar, H., Felzel, E., Murk, A. J., *et al*. (2004). Intra- and interlaboratory calibration of the DR CALUX bioassay for the analysis of dioxins and dioxin-like chemicals in sediments. *Environmental toxicology and chemistry / SETAC*, *23*(12), 2781–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15648750

Birch, G. F., Harrington, C., Symons, R. K., & Hunt, J. W. (2007). The source and distribution of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofurans in sediments of Port Jackson, Australia. *Marine pollution bulletin*, *54*(3), 295–308. http://dx.doi.org/doi:10.1016/j.marpolbul.2006.10.009

Bruckmeier, B. F. A., Jüttner, I., Schramm, K.-W., Winkler, R., Steinberg, C. E. W., & Kettrup, A. (1997). PCBs and PCDD/Fs in lake sediments of Großer Arbersee, Bavarian Forest, South Germany. *Environmental Pollution*, *95*(1), 19–25. http://dx.doi.org/doi:10.1016/S0269-7491(96)00118-2

Chi, K. H., Chang, M. B., & Kao, S. J. (2007). Historical trends of PCDD/Fs and dioxin-like PCBs in sediments buried in a reservoir in Northern Taiwan. *Chemosphere*, *68*(9), 1733–40. http://dx.doi.org/doi:10.1016/j.chemosphere.2007.03.043

Croes, K., Van Langenhove, K., Elskens, M., Desmedt, M., Roekens, E., Kotz, A., Denison, M. S., *et al*. (2011). Analysis of PCDD/Fs and dioxin-like PCBs in atmospheric deposition samples from the Flemish measurement network: Optimization and validation of a new CALUX bioassay method. *Chemosphere*, *82*(5), 718–24. http://dx.doi.org/doi:10.1016/j.chemosphere.2010.10.092

Davies, O. A., & Tawari, C. C. (2010). Season and tide effects on sediment characteristics of trans-okpoka creek , upper bonny Estuary , Nigeria. *Agriculture and Biology Journal of North America*, *1*(2), 89–96.

De Wolf, H., & Rashid, R. (2008). Heavy metal accumulation in Littoraria scabra along polluted and pristine mangrove areas of Tanzania. *Environmental pollution (Barking, Essex : 1987)*, *152*(3), 636–43. http://dx.doi.org/doi:10.1016/j.envpol.2007.06.064

Denison, M. S., Pandini, A., Nagy, S. R., Baldwin, E. P., & Bonati, L. (2002). Ligand binding and activation of the Ah receptor. *Chemico-Biological Interactions*, *141*(1-2), 3–24. http://dx.doi.org/doi:10.1016/S0009-2797(02)00063-7

Denison, M. S., Zhao, B., Baston, D. S., Clark, G. C., Murata, H., & Han, D. (2004). Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. *Talanta*, *63*(5), 1123–33. http://dx.doi.org/doi:10.1016/j.talanta.2004.05.032

El-Kady, A. A., Abdel-Wahhab, M. A., Henkelmann, B., Belal, M. H., Morsi, M. K. S., Galal, S. M., & Schramm, K.-W. (2007). Polychlorinated biphenyl, polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran residues in sediments and fish of the River Nile in the Cairo region. *Chemosphere*, *68*(9), 1660–8. http://dx.doi.org/doi:10.1016/j.chemosphere.2007.03.066

Elskens, M., Baston, D. S., Stumpf, C., Haedrich, J., Keupers, I., Croes, K., Denison, M. S., *et al*. (2011). CALUX measurements: statistical inferences for the dose-response curve. *Talanta*, *85*(4), 1966–73. http://dx.doi.org/doi:10.1016/j.talanta.2011.07.014

Estrellan, C. R., & Iino, F. (2010). Toxic emissions from open burning. *Chemosphere*, *80*(3), 193–207. http://dx.doi.org/doi:10.1016/j.chemosphere.2010.03.057

Goeyens, L., Hoogenboom, R., Eppe, G., Malagocki, P., Vanderperren, H., M-l, S., Windal, I., *et al*. (2010). Discrepancies Between Bio-Analytical and Chemo-Analytical Results have a non-Negligible Message. *Organohalogen Compounds*, *72*, 964–967.

Guzzella, L., Roscioli, C., Viganò, L., Saha, M., Sarkar, S. K., & Bhattacharya, A. (2005). Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in the sediments along the lower stretch of Hugli estuary, West Bengal, northeast India. *Environment international*, *31*(4), 523–34. http://dx.doi.org/doi:10.1016/j.envint.2004.10.014

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P. W., Mccabe, J. M., *et al*. (2003). Polychlorinated Dibenzo- p -dioxin and Dibenzofuran Concentration Profiles in Sediments and Flood-Plain Soils of the Tittabawassee River, Michigan. *Environmental Science & Technology*, *37*(3), 468–474. http://dx.doi.org/doi:10.1021/es020920c

Holmer, M. (2003). *Biogeochemistry of Marine Systems*. (K. D. Black & G. B. Shimmield, Eds.)*Journal of Marine Systems* (Vol. 50, pp. 1–34). Blackwell Publishing Ltd. http://dx.doi.org/doi:10.1016/j.jmarsys.2004.05.004

Hong, S., Khim, J. S., Naile, J. E., Park, J., Kwon, B.-O., Wang, T., Lu, Y., *et al*. (2012). AhR-mediated potency of sediments and soils in estuarine and coastal areas of the Yellow Sea region: a comparison between Korea and China. *Environmental pollution (Barking, Essex : 1987)*, *171*(null), 216–25. http://dx.doi.org/doi:10.1016/j.envpol.2012.08.001

Hoogenboom, L., Traag, W., Bovee, T., Goeyens, L., Carbonnelle, S., Vanloco, J., Beernaert, H., *et al*. (2006). The CALUX bioassay: Current status of its application to screening food and feed. *TrAC Trends in Analytical Chemistry*, *25*(4), 410–420. doi:10.1016/j.trac.2006.02.012

Hurst, M. R., Balaam, J., Chan-Man, Y. L., Thain, J. E., & Thomas, K. V. (2004). Determination of dioxin and dioxin-like compounds in sediments from UK estuaries using a bio-analytical approach: chemical-activated luciferase expression (CALUX) assay. *Marine pollution bulletin*, *49*(7-8), 648–58. http://dx.doi.org/doi:10.1016/j.marpolbul.2004.04.012

Joung, K. E., Chung, Y. H., & Sheen, Y. Y. (2007). DRE-CALUX bioassay in comparison with HRGC/MS for measurement of toxic equivalence in environmental samples. *The Science of the total environment*, *372*(2-3), 657–67. http://dx.doi.org/doi:10.1016/j.scitotenv.2006.10.036

Koh, C-H, Khim, J. S., Kannan, K., Villeneuve, D. L., Senthilkumar, K., & Giesy, J. P. (2004). Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea. *Environmental pollution (Barking, Essex : 1987)*, *132*(3), 489–501. http://dx.doi.org/doi:10.1016/j.envpol.2004.05.001

Koh, Chul-Hwan, Khim, J. S., Villeneuve, D. L., Kannan, K., & Giesy, J. P. (2006). Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 2. Dioxin-like and estrogenic activities. *Environmental pollution (Barking, Essex : 1987)*, *142*(1), 48–57. http://dx.doi.org/doi:10.1016/j.envpol.2005.09.006

Koistinen, J., Stenman, O., Haahti, H., Suonperä, M., & Paasivirta, J. (1997). Polychlorinated diphenyl ethers, dibenzo-p-dioxins, dibenzofurans and biphenyls in seals and sediment from the gulf of finland. *Chemosphere*, *35*(6), 1249–1269. http://dx.doi.org/doi:10.1016/S0045-6535(97)00212-9

Kruitwagen, G., Hecht, T., Pratap, H.B., Wendelaar Bonga, S.E., (2006). Changes in morphology and growth of the mudskipper (*Periophthalmus argentilineatus*) associated with coastal pollution. *Marine Biology* 149, 201–211. DOI 10.1007/s00227-005-0178-z

Kruitwagen, G., Pratap, H. B., Covaci, A., & Wendelaar Bonga, S. E. (2008). Status of pollution in mangrove ecosystems along the coast of Tanzania. *Marine pollution bulletin*, *56*(5), 1022–31. http://dx.doi.org/doi:10.1016/j.marpolbul.2008.02.018

Kumar, K. S., Sajwan, K. S., Richardson, J. P., & Kannan, K. (2008). Contamination profiles of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons and alkylphenols in sediment and oyster collected from marsh/estuarine Savannah GA, USA. *Marine pollution bulletin*, *56*(1), 136–49. http://dx.doi.org/doi:10.1016/j.marpolbul.2007.08.011

Lee, S.-J., Kim, J.-H., Chang, Y.-S., & Moon, M. H. (2006). Characterization of polychlorinated dibenzo-p-dioxins and dibenzofurans in different particle size fractions of marine sediments. *Environmental pollution (Barking, Essex : 1987)*, *144*(2), 554–61. http://dx.doi.org/doi:10.1016/j.envpol.2006.01.040

Liu, H., Zhang, Q., Cai, Z., Li, A., Wang, Y., & Jiang, G. (2006). Separation of polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzo-furans in environmental samples using silica gel and florisil fractionation chromatography. *Analytica Chimica Acta*, *557*(1-2), 314 – 320. http://dx.doi.org/doi:10.1016/j.aca.2005.10.001

Long, M., Andersen, B. S., Lindh, C. H., Hagmar, L., Giwercman, A., Manicardi, G.-C., Bizzaro, D., *et al*. (2006). Dioxin-like activities in serum across European and Inuit populations. *Environmental health : a global access science source*, *5*(1), 14. http://dx.doi.org/doi:10.1186/1476-069X-5-14

International POPs Elimination Network. (2005). *Contamination of chicken eggs near the Vikuge obsolete pesticides stockpile in Tanzania by dioxins , PCBs and hexachlorobenzene Contamination of chicken eggs near the Vikuge obsolete pesticides stockpile in Tanzania by dioxins ,* (pp. 0–22).

Loomis, D., Browning, S. R., Schenck, A. P., Gregory, E., & Savitz, D. A. (1997). Cancer mortality among electric utility workers exposed to polychlorinated biphenyls. *Occupational and Environmental Medicine*, *54*(10), 720–728. doi:10.1136/oem.54.10.720

Machiwa, J. F. (1992). Anthropogenic pollution in the Dar es Salaam harbour area, Tanzania. *Marine Pollution Bulletin*, *24*(11), 562–567. doi:10.1016/0025-326X(92)90709-F

Maleko, G. C. (2005). "Impact of Electricity Services on Microenterprise on Rural Areas on Tanzania Development University Of Twente , Enschede .

Manahan, S. A. (2008). *Fundamentals of Environmental Chemistry* (Third Edit., p. 1264). CRC Press.

Mbuligwe, S. E., & Kassenga, G. R. (1997). Automobile air pollution in Dar es Salaam City, Tanzania. *Science of The Total Environment*, *199*(3), 227–235. doi:10.1016/S0048-9697(97)05461-2

Mihale, M. J., Kishimba, M. A., Baeyens, W., & Brion, N. (n.d.). Carbon and Nitrogen dynamics in tropical estuarine mangrove sediments of Mtoni, Tanzania.

Mkoma, S. (2008). *Physico-Chemical Characterisation 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

Mlay, A. P., Wagner, G. M., & Mgaya, Y. D. (n.d.). A comparative study of the ecology of four sandy/muddy shores in the Dar es Salaam area. Retrieved from http://gridnairobi.unep.org/chm/eafdocuments/Tanzania/Ecology_of_muddy_shores_in_Tanzania.pdf

Moon, H.-B., Choi, M., Choi, H.-G., Ok, G., & Kannan, K. (2009). Historical trends of PCDDs, PCDFs, dioxin-like PCBs and nonylphenols in dated sediment cores from a semi-enclosed bay in Korea: tracking the sources. *Chemosphere*, *75*(5), 565–71. http://dx.doi.org/doi:10.1016/j.chemosphere.2009.01.064

Murk, A. (1996). Chemical-Activated Luciferase Gene Expression (CALUX): A Novelin VitroBioassay for Ah Receptor Active Compounds in Sediments and Pore Water. *Fundamental and Applied Toxicology*, *33*(1), 149–160. http://dx.doi.org/doi:10.1006/faat.1996.0152

Müller, J F, Haynes, D., McLachlan, M., Böhme, F., Will, S., Shaw, G. R., Mortimer, M., *et al*. (1999). PCDDs, PCDFs, PCBs and HCB in marine and estuarine sediments from Queensland, Australia. *Chemosphere*, *39*(10), 1707–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10520488

Müller, Jochen F, Gaus, C., Prange, J. A., Päpke, O., Poon, K. F., Lam, M. H. W., & Lam, P. K. (2002). Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in sediments from Hong Kong. *Marine pollution bulletin*, *45*(1-12), 372–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12398408

National Bureau of Statistics, N. B. S. (2003). Tanzania populations and housing census - General report. Retrieved from http://web.archive.org/web/20040313070101/http://www.tanzania.go.tz/census/census/tables.htm

Nieuwoudt, C., Quinn, L. P., Pieters, R., Jordaan, I., Visser, M., Kylin, H., Borgen, A. R., *et al*. (2009). Dioxin-like chemicals in soil and sediment from residential and industrial areas in central South Africa. *Chemosphere*, *76*(6), 774–83. doi:10.1016/j.chemosphere.2009.04.064

Okay, O. S., Karacik, B., Başak, S., Henkelmann, B., Bernhöft, S., & Schramm, K.-W. (2009). PCB and PCDD/F in sediments and mussels of the Istanbul strait (Turkey). *Chemosphere*, *76*(2), 159–66. http://dx.doi.org/doi:10.1016/j.chemosphere.2009.03.051

Pan, J., Yang, Y., Geng, C., Yeung, L. W. Y., Cao, X., & Dai, T. (2010). Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans in marine and lacustrine sediments from the Shandong Peninsula, China. *Journal of hazardous materials*, *176*(1-3), 274–9. http://dx.doi.org/doi:10.1016/j.jhazmat.2009.11.024

Pieters, R. (2007). *An assessment of dioxins, dibenzofurans and PCBs in the sediments of selected freshwater bodies and estuaries in South Africa*. North-West University, Potchefstrrom Campus.

PUMPSEA. (2007). *Peri-urban mangrove forests as filters and potential phytoremediators of domestic sewage in East Africa (2007). Distribution and fate of heavy metals and indicator pathogens in sewage exposed mangroves, Project No. INCO – CT2004 – 510863*.

Roots, O., Henkelmann, B., & Schramm, K.-W. (2004). Concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in soil in the vicinity of a landfill. *Chemosphere*, *57*(5), 337–42. http://dx.doi.org/doi:10.1016/j.chemosphere.2004.06.012

Roy, S., Mysior, P., & Brzezinski, R. (2002). Comparison of dioxin and furan TEQ determination in contaminated soil using chemical, micro-EROD, and immunoassay analysis. *Chemosphere*, *48*(8), 833–842. http://dx.doi.org/doi:10.1016/S0045-6535(02)00129-7

Ryoo, K. S., Ko, S.-O., Hong, Y. P., Choi, J.-H., Cho, S., Kim, Y., & Bae, Y. J. (2005). Levels of PCDDs and PCDFs in Korean river sediments and their detection by biomarkers. *Chemosphere*, *61*(3), 323–31. http://dx.doi.org/doi:10.1016/j.chemosphere.2005.02.093

Sanctorum, H., Elskens, M., & Baeyens, W. (2007). Bioassay (CALUX) measurements of 2,3,7,8-TCDD and PCB 126: interference effects. *Talanta*, *73*(1), 185–8. http://dx.doi.org/doi:10.1016/j.talanta.2007.03.009

Sanctorum, H., Windal, I., Hanot, V., Goeyens, L., & Baeyens, W. (2007b). Dioxin and dioxin-like activity in sediments of the Belgian coastal area (Southern North Sea). *Archives of environmental contamination and toxicology*, *52*(3), 317–25. http://dx.doi.org/doi:10.1007/s00244-006-0063-x

Schecter, A. J., Sheu, S. U., Birnbaum, L. S., Devito, M. J., Michael, S., Health, N., Effects, E., *et al*. (1999). A comparison and discussion of two differing methods of measuring dioxin-like compounds: gas chromatography-mass spectrometry and the CALUX bioassay - implications for health studies. *Organohalogen Compounds*, *40*, 247–250.

Schroijen, C., Windal, I., Goeyens, L., & Baeyens, W. (2004). Study of the interference problems of dioxin-like chemicals with the bio-analytical method CALUX. *Talanta*, *63*(5), 1261–8. http://dx.doi.org/doi:10.1016/j.talanta.2004.05.036

Shen, H., Han, J., Tie, X., Xu, W., Ren, Y., & Ye, C. (2009). Polychlorinated dibenzo-p-dioxins/furans and polychlorinated biphenyls in human adipose tissue from Zhejiang Province, China. *Chemosphere*, *74*(3), 384–8. http://dx.doi.org/doi:10.1016/j.chemosphere.2008.09.094

Smith, A. H., & Lopipero, P. (2001). *Evaluation of the toxicity of dioxins and dioxin-like PCBs: A health risk appraisal for the New Zealand population: A report to the New Zealand Ministry for the Environment* (pp. 17–101).

Song, M., Jiang, Q., Xu, Y., Liu, H., Lam, P. K. S., O'Toole, D. K., Zhang, Q., *et al*. (2006). AhR-active compounds in sediments of the Haihe and Dagu Rivers, China. *Chemosphere*, *63*(7), 1222–30. http://dx.doi.org/doi:10.1016/j.chemosphere.2005.08.065

Srogi, K. (2007). Levels and congener distributions of PCDDs, PCDFs and dioxin-like PCBs in environmental and human samples: a review. *Environmental Chemistry Letters*, *6*(1), 1–28. http://dx.doi.org/doi:10.1007/s10311-007-0105-2

Taylor, M., Ravilious, C., & Green, E. P. (2003). *Mangroves of East Africa - UNEP* (p. 28).

Terauchi, H., Takahashi, S., Lam, P. K. S., Min, B.-Y., & Tanabe, S. (2009). Polybrominated, polychlorinated and monobromo-polychlorinated dibenzo-p-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls in marine surface sediments from Hong Kong and Korea. *Environmental pollution (Barking, Essex : 1987)*, *157*(3), 724–30. http://dx.doi.org/doi:10.1016/j.envpol.2008.11.028

United States Environmental protection Agency. (2001). Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses - Technical Manual, (EPA-823-B-01-002 October 2001), 208.

United States Environmental protection Agency. (2008). Method 4435 for Toxic Equivalents (TEQs) determinations for dioxin-like chemical activity with the CALUX bioassay. Retrieved from http://www.epa.gov/osw/hazard/testmethods/pdfs/4435.pdf

Van Camp, M., Mjemah, I. C., Al Farrah, N., Walraevens, K. (2013). Modeling approaches and strategies for data-scarce aquifers: example of the Dar es Salaam aquifer in Tanzania, *Hydrogeology Journal* 21: 341–356. DOI 10.1007/s10040-012-0908-5

Van Langenhove, K., Croes, K., Denison, M. S., Elskens, M., & Baeyens, W. (2011). The CALUX bio-assay: analytical comparison between mouse hepatoma cell lines with a low (H1L6.1c3) and high (H1L7.5c1) number of dioxin response elements. *Talanta*, *85*(4), 2039–46. http://dx.doi.org/doi:10.1016/j.talanta.2011.07.042

Van Overmeire, I., Van Loco, J., Roos, P., Carbonnelle, S., & Goeyens, L. (2004). Interpretation of CALUX results in view of the EU maximal TEQ level in milk. *Talanta*, *63*(5), 1241–7. doi:10.1016/j.talanta.2004.05.034Wang, H., He, M., Lin, C., Quan, X., Guo, W., & Yang, Z. (2007). Monitoring and assessment of persistent organochlorine residues in sediments from the Daliaohe River watershed, northeast of China. *Environmental monitoring and assessment*, *133*(1-3), 231–42. http://dx.doi.org/doi:10.1007/s10661-006-9576-z

Wong, H. L., Giesy, J. P., Siu, W. H. L., & Lam, P. K. S. (2005). Estrogenic and dioxin-like activities and cytotoxicity of sediments and biota from Hong Kong mudflats. *Archives of environmental contamination and toxicology*, *48*(4), 575–86. http://dx.doi.org/doi:10.1007/s00244-004-0166-1

Yang, Z., Shen, Z., Gao, F., Tang, Z., & Niu, J. (2009). Occurrence and possible sources of polychlorinated biphenyls in surface sediments from the Wuhan reach of the Yangtze River, China. *Chemosphere*, *74*(11), 1522–30. http://dx.doi.org/doi:10.1016/j.chemosphere.2008.11.024

Yoo, H., Khim, J. S., & Giesy, J. P. (2006). Receptor-mediated in vitro bioassay for characterization of Ah-R-active compounds and activities in sediment from Korea. *Chemosphere*, *62*(8), 1261–71. http://dx.doi.org/doi:10.1016/j.chemosphere.2005.07.007

Zhang, H., Zhao, X., Ni, Y., Lu, X., Chen, J., Su, F., Zhao, L., *et al*. (2010). PCDD/Fs and PCBs in sediments of the Liaohe River, China: levels, distribution, and possible sources. *Chemosphere*, *79*(7), 754–62. http://dx.doi.org/doi:10.1016/j.chemosphere.2010.02.039

Zhao, L., Hou, H., Zhou, Y., Xue, N., Li, H., & Li, F. (2010). Distribution and ecological risk of polychlorinated biphenyls and organochlorine pesticides in surficial sediments from Haihe River and Haihe Estuary Area, China. *Chemosphere*, *78*(10), 1285–93. http://dx.doi.org/doi:10.1016/j.chemosphere.2009.12.007

CHAPTER FIVE

GENERAL CONCLUSIONS

CHAPTER FIVE: GENERAL CONCLUSIONS

5.1 Conclusion and Recommendations

Developing countries such as Tanzania experience major societal, economic, industrial and agricultural changes with a severe impact on the environment. A major harbour such as Dar es Salaam is an ideal site for implantation of industrial activities. Increasing labour demand by those industries leads to population migration from rural areas to Dar es Salaam which is rapidly expanding. The infrastructure of the town is not adapted to this population increase, neither is its road infrastructure to the enhanced traffic. Environmental stress is resulting from the unauthorised settlements, the lack of domestic and industrial waste water treatment plants, the open burning of plastics, wood and charcoal, the illegal waste dumping sites, the destruction of mangroves for wood needs, etc.

Since only a very limited number of environmental studies on the Dar es Salaam area exist, we took the opportunity to contribute to that lack of knowledge by studying the impact of the pollution from various sources on the Mtoni estuary, Dar es Salaam.

Sediments integrate during a certain period of time the amounts of pollutants discharged into the aquatic system. They are also easy to sample, to store, to transport and to analyse. Therefore, we selected a number of sediment sampling stations in the Mtoni mixing zone of the estuary. The mixing zone integrates the effects of the riverine system (in this case the Kizinga and Mzinga rivers), local direct sources and the marine system. It appeared that the sediments were well mixed, so that vertical gradients were absent, and that the differences in pollutant concentrations between the wet and dry season were not significant. The results of the two field campaigns in the wet and the dry season clearly demonstrated that in the mixing zone, where the salinity gradient is relatively small, no large concentration differences between stations were found. Therefore a third sampling campaign was organised with sampling further upstream in the rivers and further downstream up to the mouth of the estuary. These results clearly showed a decreasing gradient from the fresh water end-members to the marine end-member for almost all selected pollutants (arsenic was the exception).

Specifically for each of the groups of compounds we investigated, the following specific conclusions can be drawn:

Findings from carbon and nitrogen elements and their stable isotopic ratios have indicated that mangrove sediments from Mtoni estuary are impacted by sewage OM. This sewage OM has a high $\delta^{15}N$ value due to ^{15}N rich ammonia volatilisation occurring during degradation of wastewater. Correlations between sedimentary $\delta^{15}C$ and $\delta^{15}N$ and quantitative estimation of the contribution of each of the various sources have identified two major OM sources for the Mtoni estuary: sewage material in the wet season and mangrove litter during the dry season. Seasonal changes in sewage discharges could explain the spatial patterns and seasonal variability of OM in the Mtoni estuarine sediments. However, when using C/N ratios combined with either $\delta^{13}C$ or $\delta^{15}N$ values only suggest one major source: sewage input. The variability in C/N ratios is, however, less unambiguously related to OM source than it is for the isotope ratios of N and C.

All the elements from anthropogenic origin showed high enrichment factors similar to Cu, Cr, Zn and Pb in the Mtoni estuary (Kruitwagen *et al.*, 2008) and Cr, Zn, As, Cd and Pb in the coastal area of Dar es Salaam (Rumisha *et al.*, 2012). Highly enriched areas in the riverine zones as compared to marine and mixing zones could be due to local anthropogenic activities. Agricultural, industrial as well as domestic activities could be the main sources of the metals particularly in this area where (i) very few residents are connected to proper sewage systems, (ii) most industries discharge effluents without proper treatment and (iii) most discharges are directed into the valleys, rivers and ocean implicitly or explicitly (Rumisha *et al*., 2012). For example, upstream stations in the Kizinga River are close to unauthorised human settlements and to a textile factory, which could supply industrial effluents containing variable amounts of metals. In the case of Mzinga upstream stations, the enrichment could be due to unauthorised human settlements where solid wastes from urban agriculture, households and small industries are trashed away.

A clear decreasing concentration gradient for trace metals in downstream direction from Kizinga and Mzinga rivers towards the mouth of the estuary is observed for almost all elements except As. Elements from anthropogenic origin can be subdivided in 3 groups: the first consisting of Cu, Ni, Cr, Mn, Zn, Al, Fe, As, Pb; the second consists of Cd, which ahs negative correlarion with OM, and the third consisting of Sr having no correlation with either OM or any other metal.

While it is well known that anthropogenic activities as open burning of plastic scraps, household burning of wood or charcoal and traffic related emissions which all frequently occur in the Dar es Salaam region can lead to PCDD/F and dioxin-like PCB production, it was totally unknown if some or all of those sources resulted in the contamination of the nearby aquatic systems. Our CALUX analyses of the sediments in the Mtoni estuary and its 2 tributaries demonstrated that the range of PCDD/F values (1.0-400 pg-BEQ/g-sediment) covers the ranges observed in Western Europe and Eastern Asia sediments. Sediments in the Kizinga River, which flows through a denser populated and more industrialised area than the Mzinga River, showed higher PCDD/F and dioxin-like PCB levels.

Finally, the high overall BEQ values of PCDD/Fs and dl-PCBs observed in sediments of the Dar es Salaam region and the highly enriched EFs for some toxic metals especially As, necessitate a carefully monitoring, because those pollutants are a potential threat to the ecosystem and humans. This is because these pollutants can bioaccumulate. For example, high bioaccumulation factors of As, Cd, Cu, Pb, Zn, Ni and Mn were observed in tissues of soft bottom molluscs (De Wolf *et al.,* 2001*;* De Wolf & Rashid, 2008; Rumisha *et al.,* 2012). Furthermore, the abnormal growth and malformations in mudskippers (*Periophthalmus argentilineatus* Valenciennes) observed by Kruitwagen *et al.,* (2006) in the Mtoni and reduced weight and size observed by Dewolf and Rashid, (2008) are correlated to environmental pollution. Because the toxicity is assumed additive and increasing levels in line with the increasing anthropogenic activities can be alarming because many of these chemicals show bio-accumulation, bio-concentration and persistent properties. The pollutants can finally reach other organisms, higher in the trophic level, that use the mangrove sediment organisms as their food.