UNITED NATIONS UNIVERSITY
Fisheries Training Programme

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Final Project 2008

ENVIRONMENTAL STUDY AND MONITORING OF UNDESIRABLE CHEMICAL SUBSTANCES IN FISH/SEAFOOD: A PROPOSAL FOR LAKE VICTORIA, KENYA

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ABSTRACT

Without a doubt, pollution in Lake Victoria is an issue of major concern. Various studies have shown the presence of undesirable chemical substances in the water, sediment and fish from the lake. In this paper, the current monitoring programme for the undesirable chemical substances in the lake by the Ministry of Fisheries, Kenya has been reviewed with the aim of ensuring safe fish and fish products to all fish consumers and especially local consumers who could be more vulnerable to exposure to undesirable chemical substances through consumption of fish from the lake. The objective of the on-going programme has been expanded to include monitoring of toxic trace metals, polychlorinated biphenyls (PCBs) and dioxins in addition to pesticide residues. Sampling sites have been selected while taking into consideration areas of the lake that are likely to contain higher concentrations of chemical contaminants. Lake Kanyaboli which is closely linked to Lake Victoria has been included in the monitoring programme due to the increased farming activities at the adjoining Yala swamp. The specific objectives of the programme, rationale for the programme, sampling areas, the fish species to be monitored and the chemical contaminants to be measured have all been described. Also described are the period and frequency of sampling, and sampling and sample preparation methods. While sample analysis will continue to be done at an accredited laboratory, it is proposed that limits of detection (LOD) and limits of quantification (LOQ) be agreed on since the results as currently interpreted (pass/failure) do not provide any useful information concerning trends or levels of pollution in the lake. While the estimated costs for implementation of the programme may be high as calculated in the proposal, it is humbly submitted that the need to implement this monitoring programme cannot be overemphasised.

Key words: Lake Victoria, Monitoring programme, Undesirable chemical substances, Fish

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ABBREVIATIONS

ADI	Acceptable daily intake
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
BDL	Below detection limit
BHC	Benzenehexachloride
DDD	Dichloro-diphenyl-dichloroethane
DDE	Dichloro-diphenyl-dichloroethylene
DDT	Dichloro-diphenyl-trichloroethane
DEFRA	Department of environment, food and rural affairs, United Kingdom
EDTA	Ethylene diamine tetra acetic acid
EEC	European Economic Commission
ERL	Extraneous residue limit
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FTP	Fisheries training programme
GC/ECD	Gas chromatography/electron capture detection
GC/MS	Gas chromatography/mass spectrometry
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
IAEA	International Atomic Energy Agency
ICP-MS	Inductively coupled plasma- mass spectrometry
ILO	International Labour Organization
IOC	Intergovernmental Oceanographic Commission
IUPAC	International union of pure and applied chemistry
JECFA	Joint FAO/WHO expert committee on food additives
JMPR	Joint FAO/WHO meeting on pesticide residues
KEPHIS	Kenya plant health inspectorate service
LOD	Limit of detection
LOQ	Limit of quantification
MDL	Method detection limit
MRL	Maximum residue limit
OECD	Organization for Economic Co-operation and Development
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo *para*-dioxin
PCDF	Polychlorinated dibenzofuran
POP	Persistent organic pollutant
PWTI	Provisional tolerable weekly intake
SCF	Scientific committee on food of the European commission
TCDD	Tetrachlorodibenzo-para-dioxin
TDI	Tolerable daily intake
TEF	Toxic equivalence factor
TEQ	Toxic equivalent quotient
TWI	Tolerable weekly intake
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organization
UNITAR	United Nations Institute for Training and Research
UNU	United Nations University
WHO	World Health Organization

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1. INTRODUCTION

Lake Victoria, the second largest freshwater lake in the world and the largest in Africa has an area of 68,800 km^2 and a water volume of 2,750 km^3. The lake is generally shallow with a maximum depth of 83 m and a mean depth of 40 m. It has an irregular shoreline of about 3,440 km in length and lies in catchments of about 184,000 Km^2. The lake which lies astride the equator between latitude 3°12‘S and 0°30‘N, and longitude 31°37‘E and 34°53‘E is situated at an altitude of 1,134 m above sea level (Kayombo and Jorgensen 2006). Although the lake area is shared by three riparian states (Kenya 6%, Tanzania 49%, and Uganda 45%,), its catchment is constituted by five countries namely Kenya, Tanzania, Uganda, Burundi, and Rwanda. Drainage is by a number of large rivers (Kagera, Nzoia, Yala, Nyando, Sondu-Miriu, Kuja-Migori, Mara, Issanga, and Biharamulo) plus many small rivers and streams. River Nile is the single outlet. Lake Victoria surrounds several groups of large Islands (the Sesse or Kalangala and Buvuma of Uganda; Ukerewe of Tanzania and the Mfangano and Rusinga of Kenya) and many small ones (Twong‘o and Sikoyo 2003).

The Lake Victoria, Kenyan basin (Figure 1) which includes the Kenyan part of the lake and its catchment, constitutes 22% of the whole Lake Victoria basin. With a population of over nine million the basin supports one of the densest rural populations in the world with densities of up to 1,200 persons per km^2 in some parts. The altitude of the basin averages 1,157 m above sea level and annual rainfall ranges between 1,200 and 1,600 mm. Temperatures range between 14 and 34°C and there is a long rain season. The main activities through which people support their livelihoods include fishing, farming, and bee keeping, trading activities, quarrying and mining (Phoon *et al.* 2004). Some of these activities are a source of pollution in the lake which threatens plant and animal life while affecting the quality and safety of fish and fish products produced from the lake.

1.1 Pollution in Lake Victoria

Pollutants entering Lake Victoria are unlikely to be rapidly reduced by dilution or out flow as the lake with only one outlet has a flushing time of 123 years and residence time of 23 years (Kayombo and Jorgensen 2006). Since the 1960s, the lake has experienced a serious decline in water quality due to pollution (Odada *et al.* 2004). Although studies have detected low levels of undesirable chemical substances in the water, sediments, and fish species of the lake (Tole and Shitsama 2001, Madadi *et al. 2005,* Lalah *et al*. 2008, Makokha *et al*. 2008, Ongeri *et al.* 2008), the concentration levels are of concern to the food chain as in some cases they have exceeded the maximum World Health Organization (WHO) limits (Makokha *et al*. 2008).

Sources of pollution into lake Victoria include; untreated sewage sludge from major urban centres along the lake shore (Scheren *et al*. 2000), agrochemicals due to their increasing use in the lake basin with large-scale farms of coffee, tea, cotton, rice, maize, sugar and tobacco, industrial polluters e.g. sugar refineries, soft drink and food processing factories, oil and soap mills, leather tanning factories and mining companies. Pollution has also been reported in Feeder Rivers and streams from pulp and paper processing, tanning, fish processing, and abattoirs (Ntiba *et al*. 2001). As demand for land increases due to population increase, urbanization as well as agriculture, forests and wetlands have been cleared leading to environmental degradation and increased sediment deposition in rivers and lakes (Ntiba *et al*. 2004).

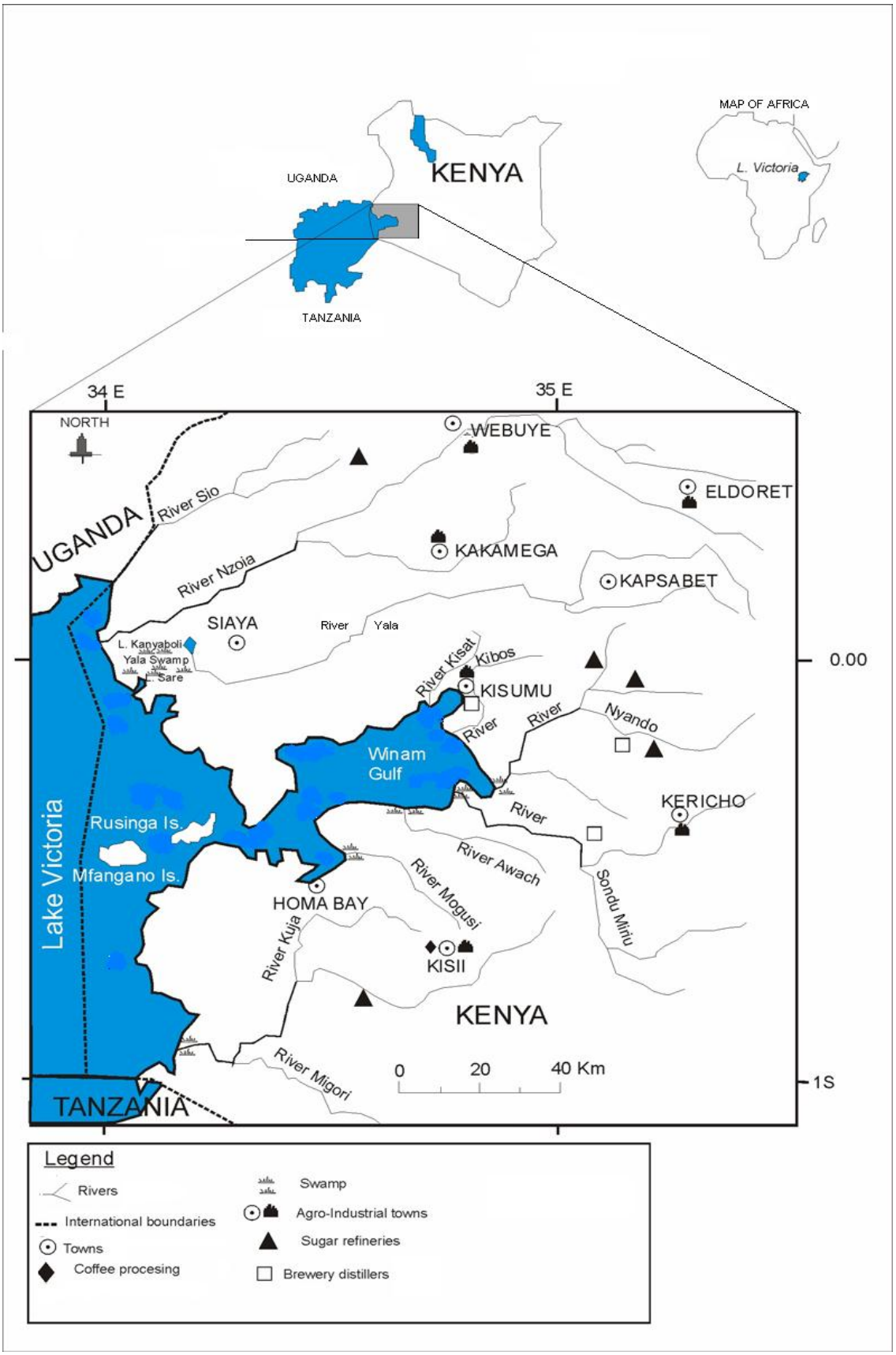

Figure 1: Map of Lake Victoria basin, Kenya showing the drainage area and main towns (Oketch 2004).

There are 87 large towns in the Lake Victoria basin, 51 in Kenya, 30 in Tanzania and 6 in Uganda. Effluent from these towns enters into small rivers which later join up to form the main ones leading to the lake. The eight main rivers in the Kenyan catchment area (Table 1) discharge into the lake an average of 292.1 m^3s^{-1} water (Kayombo and Jorgensen 2006) and hence are the main pollution carriers into the lake.

Table 1: Average discharge (m^3s^{-1}) from river basins in Lake Victoria, Kenya catchment area (Kayombo and Jorgensen 2006)

Basin	Sio	Nzoia	Yala	Nyando	Sondu-Miriu	Awach north	Awach south (Mogusi)	Kuja-Migori	Total
Discharge (m^3s^{-1})	11.4	115.3	37.6	18	42.2	3.7	5.9	58.0	292.1

Although environmental pollution by lead is a worldwide public health problem and many countries have outlawed the use of leaded petrol, African countries have been slow in putting in place regulatory structures on leaded products. In Kenya, the policy and regulatory framework for the control of lead pollution is weak (Makokha *et al*. 2008). An inventory in towns around Lake Victoria indicated that there are high possibilities of oil spillage into Lake Victoria due to the small size and hence inefficient functioning of oil separators/ interceptors of nearly all visited industries. It was also found that many drainage systems from petroleum filling stations were draining oil directly to sewerage systems or to rivers and that bilge oil is regularly discharged into the lake (Kayombo and Jorgensen 2006).

1.1.1 Chemical contaminants in Lake Victoria water and sediments:

Various studies carried out at different locations of Lake Victoria have indicated the presence of various undesirable chemical substances in the lake water and sediments (Makundi 2001, Tole and Shitsama 2001, Ljung 2002, Madadi *et al* 2005, Lalah *et al* 2008, Ongeri *et al* 2008).

Trace metals

At Mwanza town which is at the shores of Lake Victoria, Tanzania, sediments were found to contain Chromium (Cr) and Cobalt (Co) in addition to metals of terrestrial origin (Potassium (K), Calcium (Ca), Titanium (Ti), Manganese (Mn), Iron (Fe), Rubidium (Rb), Strontium (Sr), Yttrium (Y), and Zirconium (Zr)). Samples from the town centre contained high concentrations of Vanadium (V), Copper (Cu), Zinc (Zn) and Lead (Pb), while samples from the industrial area contained the highest concentration of V, Cu, Zn, Arsenic (As) and Pb. It was concluded that industrial and sewage wastes discharged into the lake were the main sources of heavy metal contamination (Makundi 2001). In the Ugandan cities of Kampala, Jinja and Entebbe, discharges contained high concentrations of Cadmium (Cd), Cu, Pb and Zn in comparison with Swedish environmental guidelines. A reduction of the above metals after the water had passed through areas of wetland was observed. In Kampala the reduction was 99%, while it was found surprising that Sese islands had higher concentrations of Pb and Cr than 55% of the sampling points in the cities (Ljung 2002).

In Winam gulf of Lake Victoria, Kenya, the mean concentration of heavy metals in water was found to be 0.12-0.45 mg Pbl^{-1}, 0.01mg Cdl^{-1} and 0.16-1.82 mg Crl^{-1} while in sediments, the concentrations were 21.2-76.2 mg Pbl^{-1}, 0.4-2.8 mg Cdl^{-1} and 37.6-394 mg Crl^{-1} (Tole and

Shitsama 2001). An analysis of heavy metals in rivers flowing into Winam gulf found the mean sediment concentration of exchangeable cations (in $\mu g g^{-1}$) for Silver (Ag), Cd, Co, Cu, Mn, Nickel (Ni), Pb, Tin (Sn), and Zn ranging from 0.01 to 263 (for Mn at Kuja river). The mean total dissolved metal (0.45 μm filter cut-off) ($\mu g l^{-1}$) and mean total sediment concentrations ($\mu g g^{-1}$ dry weight) were found to be as in the Table 2 below (Lalah *et al.* 2008):

Table 2: Mean total dissolved metal ($\mu g l^{-1}$) and mean sediment concentrations ($\mu g g^{-1}$ dry weight) of heavy metals in water flowing into Winam gulf of Lake Victoria (Lalah *et al.* 2008).

Heavy Metal	Ag	Cd	Co	Cr	Cu	Mn	Ni	Pb	Sn	Zn
Dissolved Metal	Nd* - 16	Nd - 8	Nd – 23.3	Nd - 50	5-157.5	50-3276	Nd-54.1	7 – 93.6		25-219.5
Sediment concentration	Nd-8.34	Nd-1.78	0.48-1.75	2.92-5.36	3.90-150.2	133.5-7237	4.33-42.29	3.09-66.06	23.39-7.83	23.39-350.8

* Not detected

Enrichment of Cd and Pb was found in all the river sediment samples with factors ranging from 2.12 at Kisat river mouth to 4.41 at Awach for Cd and from 1.49 at Kisat river mouth to 2.38 at Nyando river mouth for Pb (Lalah *et al.* 2008).

An investigation on the distribution and sources of trace metals including Cd, Cu, Zn, Pb and Fe in the Winam gulf lake and river waters found concentrations (in $\mu g l^{-1}$) ranging from <1.79 (Cd), <3.83 (Pb), <1.53-3.86 (Cu), 4.37-11.6 (Zn), 11.8-2,440 (Fe). The sediment concentrations (in $\mu g kg^{-1}$ x 10^3) ranged from 0.19 -1.91 (Cd), 6.86-138 (Pb), 18-100(Cu), 36.2-443 (Zn) and 960-73,200 (Fe). It was also confirmed that trace metal concentrations accumulate downstream in the rivers both in water and sediments and that the rivers studied (Sio, Nyamasaria, Nyando and Sondu- Miriu) were major sources of heavy metal load into the Winam gulf (Ongeri *et al.* 2008).

Organochlorines

Organochlorine pesticide residues were found in the Lake Victoria catchment, Kenya at levels ranging from below detection limit (BDL)-0.44 $\mu g l^{-1}$ in river Nzoia water, between BDL-0.34 $\mu g l^{-1}$ in river Sio water, BDL- 0.26 $\mu g l^{-1}$ in water from Sio Port, and between BDL-0.31$\mu g l^{-1}$ in water from Lake Victoria at Marenga Beach. The detection limits for analysed pesticides ranged from 0.001 to 0.004 $\mu g l^{-}$ (Madadi *et al. 2005*).

1.1.2 Chemical contaminants in Lake Victoria fish

A chemical must be in a bioavailable form in order to produce toxic responses in marine organisms or their consumers. A chemical is bioavailable if it is in a form that can move through or bind to the surface coating (e.g. skin, gill epithelium, gut epithelium, cell membrane) of an organism and thereby elicit biological responses. Bioavailable chemicals may accumulate to high, potentially toxic concentrations in the tissues of marine organisms or their consumers if they have a higher affinity for some tissue compartment (e.g. lipids) than for the ambient water or if they bind to tissue components. Naturally occurring bioavailable chemicals are bioaccumulated by marine organisms, often to concentrations much higher than those in the ambient seawater. Increased inputs to the marine environment of these chemicals from man's activities can result in an increase in the concentrations in water and enhanced

bioaccumulation in the tissues of marine organisms to concentrations that are toxic to the organisms themselves or their consumers, including man (Neff 2002).

Studies carried out on the Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) which are fish species of commercial value in Lake Victoria have found different concentrations of undesirable chemical substances in their muscles (Mitema and Gitau 1990, Henry and Kishimba 2006, Kasozi *et al.* 2001, Birungi *et al.* 2007, Ejobi *et al.* 2007, Makokha *et al.* 2008).

Trace metals in fish

Nile perch (*L. niloticus*) samples collected from fish processing factories at the shores of Lake Victoria in Mwanza and Musoma, Tanzania were found to contain low levels of heavy metals. The concentration ranges (μgg-1 wet weight) were: Pb < 0.01- 0.08, Cd < 0.001- 0.04, Cu 0.01- 0.97, and Zn < 0.01- 18.94. The concentration of total mercury ranged between 31.0 and 684.2 ngg^{-1} ww (Machiwa 2005). In Kisumu, Kenya, lead levels in fish (μgg^{-1}) were found to range between 1.0 and 3.3 with the lead levels in all the fish samples being above the WHO maximum limit of 0.2 μgg^{-1} (Makokha *et al*. 2008); such a result for Kenya indicates that there is real risk of lead poisoning from eating fish.

Nile tilapia (*Oreochromis niloticus*) has been used as a bio indicator species in active bio monitoring of trace heavy metals. In the Nakivubo wetland along lake Victoria, Uganda, fish kills were recorded (up to 77.5%) in less than six weeks indicating high levels of pollution. The order of accumulation of metals in tissue after six weeks was in the order Cu >Zn >Cr >Mn and gills >liver >muscle (Birungi *et al.* 2007)

Organochlorines in fish

In Uganda, an analysis for organochlorine residues found significantly higher levels of lindane, α-endosulfan, p, p'-Dichloro-diphenyl-dichloroethylene (DDE), p, p'-Dichloro-diphenyl-trichloroethane (DDT) and dieldrin in Nile perch (*Lates niloticus*) than Nile Tilapia (*Oreochromis niloticus*). No difference was found in the distribution of pesticide residues in the different parts of Nile tilapia (*O.niloticus*), although a difference for p, p'-DDE was observed in the Nile perch (*L.niloticus*) (Kasozi *et al.* 2001). Also in Uganda, 6 organochlorine pesticide residues and 3 polychlorinated biphenyl (PCB) congeners were found in Nile perch (*L.niloticus*) fillet samples in the following proportions: Hexachlorobenzane(6.7%), dieldrin(3,3%), p, p'-DDE(83.3%), o, p'-Dichloro-diphenyl-dichloroethane (DDD)(6.7%), p, p'-DDD(3,3%), p, p'-DDT(20%), PCB-153(13.3%), PCB-138(13.3%) and PCB-180(16.7%). The concentrations of these contaminants in muscle were however low with the mean concentration of total DDT being 0.001 $mgkg^{-1}$ fresh weight and the highest recorded level 0.003 $mgkg^{-1}$ fresh weight. DDE constituted on average 94% of the total DDT in the fillet (Ejobi *et al.* 2007). In liver samples, 9 organochlorine pesticide residues and 4 PCB congeners were found in the following proportions: hexachlorobenzene (HCB)(20%), α-hexachlorocyclohexane (HCH)(13.3%), β-HCH(6.7%), lindane(10%), dieldrin(36.7%), p,p'-DDE(83.3%), o,p'-DDD(3.3%), p,p'-DDD(33.3%), p,p'-DDT(13.3%), PCB-52(3.3%), PCB-101(16.7%), PCB-153(16.7%), and PCB-138(13.3%). The mean total DDT was 0.003 $mgkg^{-1}$ fresh weight, with the highest concentration of 0.01 $mgkg^{-1}$ fresh weight (Ejobi *et al*. 2007).

Samples of Nile tilapia (*Oreochromis niloticus*) and Nile perch (*Lates niloticus*) collected from fish landing stations in Lake Victoria, Tanzania were found to contain pesticide residues of up to 0.003, 0.03 and 0.2 $mgkg^{-1}$ fresh weight (0.7, 3.8 and 42 $mgkg^{-1}$ lipid weight) of fenitrothion, DDT and endosulfan, respectively. Mean levels within sites were up to 0.002, 0.02 and 0.1 $mgkg^{-1}$ fresh weight (0.5, 0.5 and 16 $mgkg^{-1}$ lipid weight), respectively (Henry and Kishimba 2005). The fact that higher levels of p, p'-DDT than the degradation products (p, p'-DDD and p, p'-DDE), and higher levels of endosulfan isomers (α and β) than the sulphate, in fish samples, were detected implied recent exposure of fish to DDT and endosulfan, respectively. Most of the fish samples had residue levels above the average method detection limits (MDLs), even though they were within the calculated allowable daily intake (ADI) (Henry and Kishimba 2006).

In eighty two samples of either Nile perch (*Lates niloticus*) fish muscle or fat analysed for organochlorine residues in Lake Victoria, Kenya, 9 organochlorine residues were detected in the following percentages: α-benzenehexachloride(BHC)/HCB-40%; β-BHC/HCB-40%; γ-BHC/HCB/lindane-4%; aldrin-9%; dieldrin-1%; p, p'-DDE-73%; p, p'-DDD-9%; o, p'-DDT-170; and p, p'-DDT-11%. All levels of organochlorine residues were below the extraneous residue limit (ERL), apart from just one sample of fish fat which had sum of DDT above ERL. Higher levels of organochlorines were found in samples from Mbita as compared to Luanda fishing point (Mitema and Gitau 1990).

1.2 Case for an improved environmental monitoring programme for Lake Victoria

The first environmental monitoring programme for Lake Victoria, Kenya was initiated by the competent authority on fish and fisheries products in the country (Ministry in charge of Fisheries), in October 1999. This was after the European Union (EU) imposed a ban on fish imports from the three East African states (Kenya, Uganda and Tanzania) due to reported use of chemicals in harvesting fish in Lake Victoria. The aim of the monitoring programme is to demonstrate the absence of pesticides in fish and ensure the quality and safety of fish and fisheries products from the lake (Fisheries Department, Kenya 2003). While this monitoring programme has served its purpose and the EU ban was lifted, it is now considered necessary to review and improve it so as to continue ensuring the safety and quality of fish and fish products to all and especially the local fish consumers. This is supported by the findings of recent studies as described earlier. Local fish consumers may be more vulnerable because much of the fish for local consumption is harvested by artisanal crafts closer to the shores as compared to the fish for export which mainly comes from offshore.

The reasons it is considered that a review of the present monitoring programme is necessary are the following:

- **Objectives:** The main objective of the current monitoring programme is to monitor the levels of pesticide residues to ensure product safety, while specific objectives are to determine the levels of residues in Nile perch (*Lates niloticus*) and tilapia (*Oreochromis niloticus*) fish, water and sediments (Fisheries Department, Kenya 2003).

  The World Health Organization has identified a list of priority contaminants that require monitoring in different products including fish to assess the potential to human exposure. Those identified for monitoring in fish include: aldrin, dieldrin, dichloro-diphenyl-trichloroethane (DDT (*p, p'*- and *p, o'*-)), Dichloro-diphenyl-dichloroethane

(DDD (*p, p'*-)), dichloro-diphenyl-dichloroethylene (DDE (*p, p'*- and *p, o'*-)), endosulfan (α, β and sulfate), endrin, hexachlorocyclohexane (α, β and γ), hexachlorobenzene, heptachlor, heptachlor epoxide, chlordane, polychlorinated biphenyls (PCBs) (congeners (IUPAC numbers) 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 167, 169, 180 and 189), dioxins (polychlorinated dibenzo *para*-dioxins (PCDDs) and Polychlorinated dibenzofurans (PCDFs)), mercury, lead, and cadmium (WHO 2004). While some of these contaminants are being monitored in the current programme, it is considered necessary to monitor all so as to have factual data on their absence/ presence and ensure safety and quality of Lake Victoria fish.

While it is not considered necessary to monitor contaminants in water and sediments (as the main aim of this monitoring programme is to ensure safety and quality of fish and fisheries products), other fish species of commercial value need to be included in the programme and especially those considered to have a higher probability of bio accumulating contaminants.

- **Sampling sites:** The criterion for selection of sampling sites was based on the quantity of fish landed. The four sites selected were estimated to receive 90% of fish landings from Lake Victoria, Kenya. These included, Marenga to the north, Uhanya at the centre, and Karungu and Muhuru bay to the south (Fisheries Department, Kenya 2003).

  While it may have been right to use this criterion in selecting the sampling sites, none of the sampling sites is close to an area that may be considered to have high concentrations of contaminants (hot spot). It is therefore considered necessary to review the criteria and focus on those areas that may be considered as hot spots. Furthermore, the results of the analysis done so far on samples from the current sampling sites have not indicated any risk in the fish from those areas while in a study discussed earlier (Makokha *et al.* 2008) levels of lead were higher than the maximum (WHO) limit in fish from Kisumu. Monitoring of hot spots will also provide information to the competent authority and other relevant institutions to take action to reduce pollution. It is also considered important to include Lake Kanyaboli in the monitoring programme because of the increasing farming activities at Yala swamp which is connected to the lake (Ochieng *et al*. 2008). This is necessary in order to guarantee the safety and quality of fish and fish products from that lake.

- **Sampling plan:** Duplicate samples of fish for each species are collected per sampling site during each sampling exercise (Fisheries Department, Kenya 2003). While the cost of monitoring may have influenced the decision on the number of samples, such few samples may not provide enough data to facilitate result interpretation and compilation of a conclusive report on the levels of undesirable chemical substances in the Lake Victoria fish. More samples are proposed for the monitoring programme per sampling site but pooled to keep the cost of monitoring low.

- **Sample analysis and results interpretation:** While samples are analysed at Kenya plant health inspectorate (KEPHIS) laboratory which is an accredited reference laboratory in the country, there is need to agree on the limits of detection (LOD) and limits of quantification (LOQ). This is because the results as currently interpreted without quantification (pass/failure) based on the AOAC 17th edition do not provide

any useful information concerning trends or levels of pollution. It is also considered necessary to make functional the Fisheries laboratory in Kisumu so that sample preparation can be carried out there without risk of contaminating the samples and cut the costs of monitoring.

2. METHODOLOGY

The methodology employed in this project included a review of the following:

- Published United Nations Environmental Programme (UNEP) reports and guidelines on environmental monitoring of chemical contaminants.
- Other literature related to global efforts on environmental monitoring and undesirable chemical substances of concern in environmental monitoring.
- Literature on Lake Victoria and its basin.
- Literature on pollution affecting Lake Victoria and its sources.
- Literature on studies that have been carried out to determine levels of chemical contaminants in the Lake Victoria.
- Literature on the current monitoring programme by the Ministry of Fisheries, Kenya on undesirable chemical substances in Lake Victoria.

The main tool utilized in literature review was the web of science on the internet.

There was also participation in sample analysis at MATIS laboratories so as to fully appreciate the use of different analysis methodologies in a monitoring programme. The methodologies some of which would be applicable in Kenya included the following:

- Fish sample homogenization and handling for analysis of contaminants.
- Sample freeze drying and digestion prior to trace metal analysis using inductively coupled plasma- mass spectrometry (ICP-MS) instruments. Trace metal analysis using ICP-MS instruments.
- Water and fat content determination in fish.
- Extraction methods for pesticide residue and PCB analysis.
- Pesticide residue analysis using gas chromatography/mass spectrometry (GC/MS) method.
- Indicator-PCB analysis using gas chromatography/electron capture detection (GC/ECD) method.
- Sample preparation for dioxin measurements.
- Interpretation of results of sample analysis.

3. THE PROPOSED MONITORING PROGRAMME

The proposed monitoring programme is titled: Environmental study and monitoring programme for undesirable chemical substances in fish/seafood from Lake Victoria, Kenya. It is designed with the overall aim of protecting public health. It is an improvement of the current monitoring programme for Lake Victoria and has been developed with a focus on protecting all fish consumers while taking into consideration areas of the lake likely to have higher concentrations of undesirable chemical substances (hot spots). It is intended for implementation by or on behalf of the Ministry of Fisheries Development, Kenya which is the country's competent authority on fish quality.

3.1 Objectives of the monitoring programme

As the main aim of this study is to protect public health, contaminant concentrations in edible fish tissues will be compared with international/national regulations to determine compliance. Specific objectives for the monitoring programme include the following:

- To find out contaminant concentrations in edible muscles of the fish and determine if there is need for further surveillance or mitigation.
- To identify areas of the lake that may be sufficiently contaminated to require fisheries controls and action to reduce pollution.
- To detect trends in contaminant concentration levels with time.
- To detect any seasonal variation in contaminant concentrations.
- To determine the need for monitoring the contaminant concentrations in other commercial fish species and other age/size groups in the fish species under study.

3.2 Rationale for the programme

The need to study and monitor the fish/seafood from Lake Victoria in terms of undesirable chemical substances arises out of the following concerns:

- Lake Victoria contributes over 90% of Kenya's total fish production. The lake is also the source of the Nile perch (*Lates niloticus*) which is the main fish species of export value to the country. Other fish species of commercial value in the lake include Nile tilapia (*Oreochromis niloticus*), Dagaa (*Rastrineobola argentea*), and African catfish (*Clarias gariepinus)* (Aloo 2006).
- Most of the towns in the Lake Victoria basin including the three main and industrial ones Kisumu, Kakamega and Homa bay which have been discharging raw or poorly treated sewage into the lake (Scheren *et al*. 2000, Nzomo 2005).
- The Lake Victoria basin is mainly (80%) an agricultural catchment (Majaliwa *et al*. 2003). With a population exceeding 30 million, pressure on forests, wetlands, rangelands and marginal agricultural lands has led to increased sediment load to rivers and the lake (Swallow *et al*. 2002). Sediment loads from such areas are high in nutrients and organic matter (Folliot and Brooks 1986). Moreover, the use of

agrochemicals has been increasing in the lake basin because of large farms of coffee, tea, cotton, rice, maize, sugar and tobacco (Ntiba *et al.* 2001).

- There has been an increase in mechanized farming activities in Yala swamp which is a major feature within the catchment of both Lake Victoria and Lake Kanyaboli (Ochieng *et al.* 2008).
- A paper manufacturing company discharges its waste water into river Nzoia, one of the main rivers serving the lake (Ntiba *et al.* 2004, Nzomo 2005).
- Gold mining activities in the Lake Victoria basin could be a source of mercury (Campbell *et al.* 2003) or other contaminations.

3.3 Sampling areas

The study will be carried out on Lake Victoria, Kenya and Lake Kanyaboli which is linked by a swamp to Lake Victoria. Selection of sampling sites for the study has been done so as to include main river mouths, and areas around the major towns (Figure 2); the assumption is that these areas are likely to have higher concentrations of undesirable chemical substances (hot spots). Sampling will be done around the mouth of rivers; Nzoia, Yala, Nyando, Sondu, Awach, and Kuja. There will be two sampling areas around Kisumu town, one on the southern side at the mouth of river Nyamasaria and another on the northern side at the mouth of river Kisat. There will be a sampling area around Homa bay town, and at Mbita point. The sampling at Mbita point is to target fish from offshore waters which are not expected to have been exposed to high levels of chemical contaminants. This will be done for comparison purposes. There will also be one sampling point at Lake Kanyaboli.

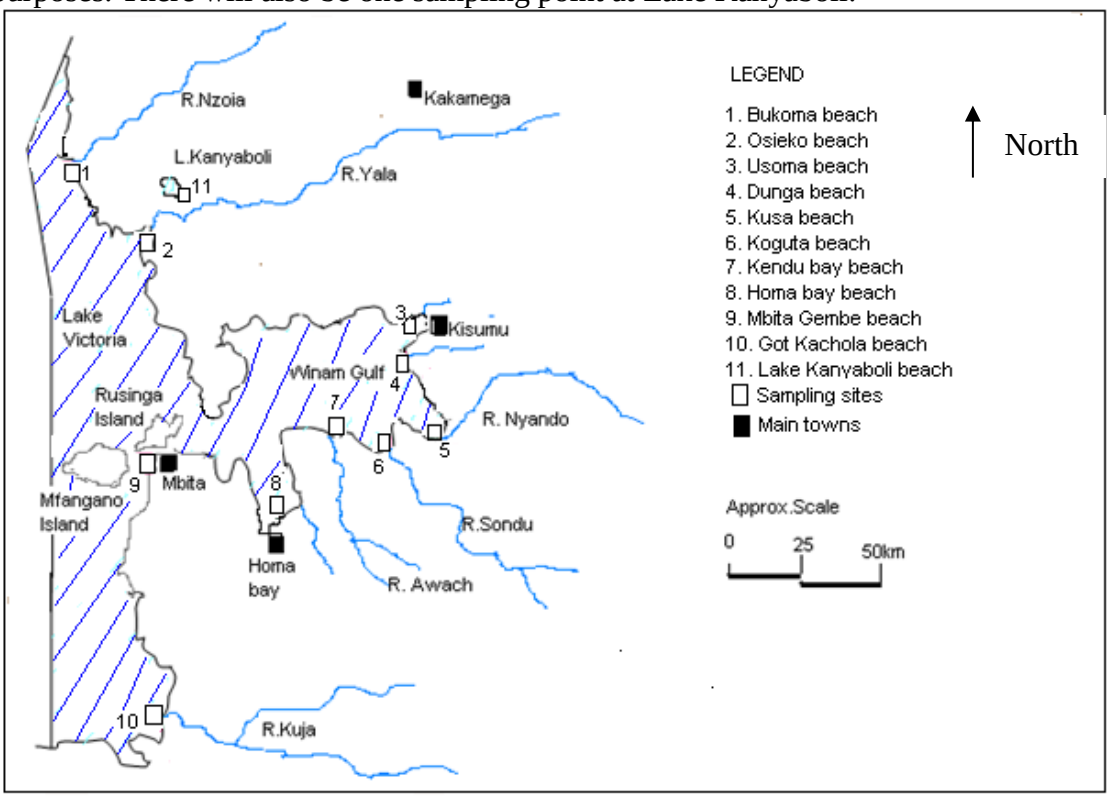

Figure 2: Lake Victoria, Kenya showing proposed sampling sites for a monitoring programme for undesirable chemical substances in fish/seafood

3.4 Fish species to be monitored

Two fish species that are of commercial value have been selected for this study: the Nile perch (*Lates niloticus*) and the African catfish (*Clarias gariepinus*). The two being carnivorous and omnivorous respectively (Njiru *et al.* 2008, Kees *et al.* 1997) hence at the end of food chain are likely to bio-accumulate more undesirable chemical substances when compared to other fish species in the lake. It has been demonstrated for example (Li *et al.* 2008) that carnivorous fish preferring benthic position as their feeding habitat have a higher concentration of total mercury (higher mercury bioaccumulation) than other fish species. For Lake Kanyaboli however, tilapia (*Tilapia nilotica*) which is herbivorous will be used as it is the main fish type in the lake (Ochieng *et al.* 2008). In sampling areas where contaminant concentrations may be found to be high during the initial stages of this study, a decision would be made to include other fish species of commercial value in the monitoring programme.

3.5 Contaminants to be measured

Undesirable chemical substances to be studied at each sampling area (Table 3) have been selected based on literature review and the author's knowledge of the geographical area.

Table 3: Sampling areas, contaminants to be monitored in the sampling areas and their probable sources in the Lake Victoria basin

Sampling Area	Contaminants to be measured	Probable contaminant source/s
Nzoia river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, sewage/industrial waste from towns upstream
	Dioxins & PCBs	Pulp and paper mill, industrial/sewage waste from towns upstream
Yala river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, sewage/industrial waste from towns upstream
Kisat river mouth, Kisumu north	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Kisumu town
	Dioxins & PCBs	Industrial and sewage waste from Kisumu town, waste incinerators
Nyamasaria river mouth, Kisumu south	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Kisumu town
	Dioxins & PCBs	Industrial and sewage waste from Kisumu town, waste incinerators
Nyando river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage/industrial waste from towns upstream
Sondu river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage/industrial waste from towns upstream
Awach river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage waste from towns upstream, natural

Homa bay town	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Homabay town
	Dioxins & PCBs	Industrial and sewage waste from Homabay town, waste incinerators
Mbita point	Pesticide residues	Water currents
	Trace metals	Water currents, natural
	Dioxins & PCBs	Water currents, atmosphere
Kuja river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, industrial and sewage waste from towns upstream
L.Kanyaboli	Pesticide residues	Agricultural activities
	Trace metals	Sewage waste from towns upstream, natural

3.5.1 Pesticide residues

The pesticide residues to be measured are mainly organochlorine chemicals primarily composed of carbon, hydrogen and chlorine. They are man-made and characterised by their ability to persist in the environment and mostly bio magnify in the food chain. Organochlorine pesticides are associated with many harmful effects in human including; acute and persistent injury to the nervous system, lung damage, injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects, and cancer (Mansour 2004)

Organochlorine pesticides to be measured include the following:

- **Aldrin and dieldrin** – In plants and animals aldrin is transformed to dieldrin, hence, the concentration of aldrin tends to be below limit of detection (LOD) in food samples. Dairy products, meat products, fish, oils and fats, often contain dieldrin. The maximum value in the EU is set for the sum of aldrin and dieldrin. The maximum residue limits (MRLs) recommended by the FAO/WHO Joint Meeting on pesticide residues (JMPR) range from 0.02 to 0.2 $mgkg^{-1}$ product (WHO 1989). EU MRL for the sum of adrin and dieldrin in animal fats is 0.2 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Total DDT (dichloro diphenyl trichloroethane)** – Total DDT is used to refer to the sum of p, p'-DDT, o, p´-DDT, p, p'-DDE, o, p´-DDE, and p, p´-DDD. JMPR/ Codex has set acceptable daily intake (ADI) at 0.02 $mgkg^{-1}$, and the extraneous residue limits (ERLs) for total DDT in animal fat at 5 $mgkg^{-1}$ (FAO/UNEP 1991). In the EU the ERL is 1.0 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Endosulfan** – includes the sum of α, β and metabolite, endosulfan sulfate. FAO/WHO ADI of endosulfan is 0.008 $mgkg^{-1}$ body weight (WHO 1988a). The EU MRL in animal fats for the sum of α, β and endosulfan sulfate (endosulfan) is 0.1$mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Endrin** - FAO/WHO ADI levels for endrin are set at 0.0002 $mgkg^{-1}$ body weight. MRLs for endrin established by codex alimentarius commission range between 0.0008-1 $mgkg^{-1}$ in products of animal origin (WHO 1991a). EU MRL for endrin in animal fat is 0.05 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Hexachlorocyclohexane (HCH)** – Includes α, β and γ (Lindane) isomers. JMPR has established the ADI for lindane which is the active substance in HCH at 0 – 0.008 $mgkg^{-1}$ body weight. FAO/WHO MRLs for lindane range between 0.05 – 3 $mgkg^{-1}$ (WHO 1991b). The EU MRLs in animal fat are 0.2 $mgkg^{-1}$ for the α- HCH isomer, 0.1 $mgkg^{-1}$ for the β- HCH isomer and 0.02 $mgkg^{-1}$ for the γ HCH isomer (lindane) (Council Directive 86/363/EEC 1986).

- **Hexachlorobenzene (HCB) –** HCB exists as a single compound. The total average daily intake of HCB in the general population varies between 0.0004 and 0.003 μg $HCBkg^{-1}$ body weight per day. Intakes of nursing infants are estimated to range from <0.018 to 5.1 μgkg^{-1} body weight per day. A tolerable daily intake has been set at 0.17 μgkg^{-1} body weight per day (WHO 1998). The EU MRL for HCB in animal fat is 0.2 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Heptachlor** – Includes the sum of heptachlor and heptachlor epoxide. JMPR has set the ADI for man at 0 - 0.0005 $mgkg^{-1}$ body weight. FAO/ WHO has also set MRLs for heptachlor in animal fat at 0.15 – 0.2 $mgkg^{-1}$ (WHO 1988b). EU MRL for heptachlor in animal fat is 0.2 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

- **Chlordane** – Includes α, γ chlordane, oxychlordane and trans-nonachlor which are the most common isomers. JMPR has set ADI at 0-0.0005 $mgkg^{-1}$ body weight. Maximum residue tolerance for food ranges from 0.2 to 0.5 $mgkg^{-1}$ for the sum of α and γ isomers of chlordane and oxychlordane (WHO 1988c). EU MRL for chlordane in animal fats is 0.05 $mgkg^{-1}$ (Council Directive 86/363/EEC 1986).

3.5.2 Polychlorinated biphenyls (PCBs)

These are a mixture of 209 chlorinated compounds (congeners) that are classified as persistent organic pollutants (POPs). Most bio accumulate in animals due to their stability and are also toxic depending on the amount of chlorine they contain (WHO 1992). Higher levels of PCBs and related compounds in human are associated with various health effects such as lowering intelligence quotient, disorder of thyroid gland, higher rate of endometriosis in women, declining thyroid hormone levels, higher rate of diabetes in pregnant women, lowering age at menarche, and altering play behaviour in children at school age (Masuda 2003) WHO has recommended the monitoring of congeners (IUPAC) nos. 28, 52, 77, 101, 105, 114, 118, 123, 126, 138, 153, 156, 167, 169, 180 and 189 as priority contaminants in fish (WHO 2004). Seven of these congeners ((IUPAC) nos. 28, 52, 101, 118, 138, 153 and 180, also known as indicator PCBs) are regarded as essential for routine monitoring and have been found to be relatively abundant in fish (Boscolo *et al.* 2006). MRL for PCBs in food of animal and plant origin in many countries ranges between 0.008 and 3.0 $mgkg^{-1}$ (WHO 1992).

3.5.3 Dioxins and dioxin like PCBs

Dioxins and dioxin-like PCBs are a group of structurally related chemicals which persist in the environment, may bio accumulate in food and human tissues and are toxic. They are considered to have similar mechanisms of toxicity and so are grouped together when considering potential risks even though they originate from different sources. Dioxin is used to refer to polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Dioxins are ubiquitous pollutants produced in small quantities in most combustion

processes and as by-products in some industrial processes involving chlorine such as waste incineration, chemical and pesticide manufacturing and pulp and paper bleaching. The most toxic of these compounds is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD and the toxicity of other dioxins and dioxin like PCBs are measured in relation to it (DEFRA 2002).

Dioxins and dioxin-like PCBs are never found as individual congeners but occur as complex mixtures with only some congeners exhibiting 2,3,7,8-TCDD-like toxicity. In order to sum up the toxicity of different congeners of dioxins and Dioxin-like PCBs, the concept of toxic equivalence factors (TEF) are used to facilitate risk assessment and regulatory control and to express analytical results in terms of TCDD toxic equivalent concentration (TEQ) (Bergkvist *et al.* 2008). The toxic equivalent quotient (TEQ) is broadly the amount of 2,3,7,8-TCDD that would give the same overall effect (DEFRA 2002).

There is a wide range of toxic effects of dioxins. The most sensitive are immunosuppression, developmental and reproductive toxicity, as well as neurological behavioural effects. Cancerogenic effects are induced at higher exposure (Nau 2006). Dioxin‘s hormone-like activities include decreased sperm count, immune suppression, increased genital malformation and neurobehavioral effects in the offspring of animals (Masuda 2003)

The scientific committee on food (SCF) of the European commission and the joint FAO/WHO expert committee on food additives (JECFA) have set the tolerable daily intake (TDI) of dioxins and dioxin–like PCBs in food at 2 $pgkg^{-1}$ body weight. In a study on exposure to dioxin–like pollutants via different food commodities in Swedish children and young adults, it was found that individuals most highly exposed were characterised by a high consumption of fish (Bergkvist *et al.* 2008). The current EU maximum levels for sum of dioxins (WHO-PCDD/PCDF-TEQ) and sum of dioxins and dioxin-like PCBs (WHO-PCDD/PCDF-PCB-TEQ) in the muscle meat of fish and fish products excluding eel are 4.0 pgg^{-1} wet weight and 8.0 pgg^{-1} wet weight respectively (Commission regulation (EC) No 1881 2006).

3.5.4 Toxic metals

The contamination of aquatic environment by metals due to natural and anthropogenic sources is a worldwide environmental concern. While heavy metals can be accumulated by marine organisms through a variety of pathways including respiration, absorption and ingestion, exposure to the toxic ones may lead to several effects in aquatic animals including tissue damage, respiratory changes, alteration of biochemical and physiological mechanisms and ultimately death (Oner *et al.* 2008). In this programme, three metals that have been prioritised by WHO for monitoring in fish to assess how the potential human exposure will be measured. These include the following:

- **Mercury:** Mercury (Hg) has been well known as an environmental pollutant hence much attention has been focused on its contamination in aquatic ecosystems. Fish are known to accumulate mercury and the uptake and depuration of mercury in fish is affected by several biological factors such as fish species, size, weight, length and age. In a study carried out on 37 subtropical fish species, total mercury concentrations were found to vary among the species. Higher mercury concentrations were found in carnivorous species demonstrating greater mercury bioaccumulation in species with more predatory feeding habits. While no significant difference was found between fish grouped by habitat preference and feeding habit, carnivorous species preferring

benthic positions had higher concentrations of mercury than others suggesting that mercury accumulation is related to the interaction of feeding habit and habitat preference (Li *et al.* 2008).

Mercury may be found in landfills, mine tailings, contaminated industrial sites, soils and sediments. When deposited, mercury changes form primarily by microbial metabolism to methyl-mercury (MeHg). This is the form that collects in organisms (bioaccumulation) and concentrates up food chains (biomagnifications), especially in the aquatic food chains. Methyl mercury is therefore the form of greatest concern as nearly all mercury in fish is methyl-mercury (UNEP CHEMICALS 2002).

Methyl-mercury adversely affects both humans and wildlife and many people are currently exposed to levels that pose risks. The compound readily passes the placental barrier and the blood-brain barrier and is a neurotoxicant, which may in particular cause adverse effects on the developing brain. Exposure to methyl mercury during pregnancy is therefore of highest concern. Small increases of methyl-mercury exposures may also cause adverse effects on the cardiovascular system leading to increased mortality. Moreover, methyl-mercury compounds are considered carcinogenic to humans (UNEP CHEMICALS 2002).

WHO/FAO Maximum allowed/recommended level of methyl-mercury in fish is 0.5 $mgkg^{-1}$, except in predatory fish where the level is 1.0 mg $MeHgkg^{-1}$. The same levels apply in the EU except that for the predatory fish, the level applies to specifically named fish and the levels are given for wet weight. JECFA has established tolerable weekly intake (TWI) of 3.3 μgkg^{-1} body weight for methyl-mercury (UNEP CHEMICALS 2002) while the EU has recommended a provisional tolerable weekly intake (PTWI) of 1.6 μgkg^{-1} body weight (Commission regulation (EC) No 1881 2006).

- **Lead:** Lead is a heavy metal that is toxic at very low exposure levels and has acute and chronic effects on human health. Lead is toxic to multi organ systems with effects ranging from enzyme inhibition and anaemia to disorders of the nervous, immune and reproductive systems, impaired kidney and cardiovascular functions and even death. It is accumulated in bones which may serve as a source of exposure later in life. Organo-lead compounds, such as tri-alkyl-lead and tetra-alkyl-lead compounds, are more toxic than inorganic forms of lead. Due to their rapid growth and maturation, biological characteristics and behaviour, children are more vulnerable to the effects of lead exposure (UNEP CHEMICALS 2008a).

  Natural sources of lead include volcanic activities and weathering of rocks while anthropogenic sources include lead impurities in fossil fuels and other extracted or treated metals, mining and processing activities, manufacturing, use, disposal, recycling and reclamation, incineration of municipal waste, open burning and mobilization of historical lead releases previously deposited in soils, sediments and waste. Emissions from leaded petrol, metal processing including recycling, mining

activity and probably oceans can be considered as the sources of relevance for the long-range transport of lead (UNEP CHEMICALS 2008a).

JECFA has established a provisional tolerable weekly intake (PTWI) for lead of 25 μgkg^{-1} body weight per week. This limit was adopted in the EU regulation 1881/2006 which also set the MRL for lead in fish muscles at 0.3 $mgkg^{-1}$ wet weight (Commission regulation (EC) No 1881 2006).

- **Cadmium:** Cadmium is a non-essential and toxic element for humans mainly affecting the kidneys and the skeleton. It is also a carcinogen by inhalation and its accumulation in bone may serve as a source of exposure in later life (UNEP CHEMICALS 2008b).

  Cadmium is released into the environment from natural sources like volcanic activity and the weathering of rocks. It is also released through anthropogenic means like the mobilization of cadmium impurities in raw materials such as phosphate minerals, fossil fuels and other extracted, treated and recycled metals – particularly zinc and copper. Other sources of cadmium pollution include open burning or incineration of municipal waste and mobilization of historical cadmium releases deposited in soils, sediments, and landfills (UNEP CHEMICALS 2008b).

  JECFA has established a provisional tolerable weekly intake (PTWI) for cadmium of 7 μgkg^{-1} body weight per week which corresponds to 1 μgkg^{-1} of body weight per day. WHO however recognizes that the margin between the PTWI and the actual weekly intake of cadmium by the general population is less than 10-fold and that this margin may even be narrower in smokers (UNEP CHEMICALS 2008b). EU regulation 1881/2006 has set the MRL for cadmium in muscle meat of fish at 0.05 $mgkg^{-1}$ wet weight except in the muscle meat of anchovy (*Engraulis species*), bonito (*Sarda sarda*), common two-banded seabream (*Diplodus vulgaris*), eel (*Anguilla anguilla*), grey mullet (*Mugil labrosus labrosus*), horse mackerel or scad (*Trachurus* species), louvar or luvar (*Luvarus imperialis*), sardine (*Sardina pilchardus*), sardinops (*Sardinops* species), tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*) and wedge sole (*Dicologoglossa cuneata*) where it is 0.1 $mgkg^{-1}$ wet weight and muscle meat of swordfish (*Xiphias gladius*) where it is 0.3 $mgkg^{-1}$ wet weight (Commission regulation (EC) No. 1881 2006).

Below (Table 4) is a summary of the chemical contaminants to be measured and their maximum residue limits in muscle tissue of fish as set by the EU directive 86/363/EEC and regulation (EC) No. 1881/2006.

Table 4: Chemical contaminants to be measured in a monitoring programme for undesirable chemical substances in fish/seafood from Lake Victoria, Kenya and the EU maximum residue limits

Contaminant group	**Criteria for control**	**EU MRLs (Maximum residue limits)**	**Units**
Organochlorine Pesticides residues	Sum of Aldrin/Dieldrin	0.2	$mgkg^{-1}$
	Total DDT	1.0	
	Endosulfan	0.1	
	Endrin	0.05	
	γ-HCH isomer (Lindane)	0.02	
	α- HCH isomer	0.2	
	β- HCH isomer	0.1	
	HCB	0.2	
	Heptachlor	0.2	
	Chlordane	0.05	
Dioxins and dioxin like PCBS	Sum of WHO-PCDD/PCDF-TEQ	0.4	pgg^{-1}
	Sum of WHO-PCDD/PCDF-PCB-TEQ	0.8	
Heavy metals	Mercury (Hg)	0.5	$mgkg^{-1}$ (Wet weight)
	Lead (Pb)	0.3	
	Cadmium Cd)	0.05	

3.6 Period and frequency of monitoring

This study will be carried out for a period of not less than five years so as to gather enough data to facilitate the compilation of a comprehensive report for decision making. Sampling will be done twice every year, and the period for each sampling exercise shall not exceed one month. To ensure that any variation in contaminant concentrations is not due to seasonal environmental changes, the exercise shall be conducted at about the same time every year as possible.

3.7 Methods

The methods to be used in this programme include sampling, sample preparation and sample analysis. The first two of these methods will be carried out by or with the supervision of an authorized fish inspector while the third will be carried out at an accredited laboratory.

3.7.1 Sampling

Sampling will be done by an authorised fish inspector according to the Codex Alimentarius - general guidelines on sampling. Large size fish (>4kg) will be targeted for sampling as they are expected to have accumulated more contaminants. The same size of sample must however be targeted from all sampling sites so as to facilitate determination of spatial variation. The following equipment will be required:

- Cool box and ice for fish storage
- Polyethylene bags
- Aluminium foil
- Labels

Fishing boats with targeted fish from the sampling area will be selected randomly for sampling. Ten samples of fish of the same size for each species will be randomly taken from different selected fishing boats for pooling. The assumption is that the samples taken will be representative of the sample species at the sampling area. Fish of the targeted size will be sorted out first before a random sampling matrix technique is applied without replacement. The technique will be the 5x2 units' matrix outward and downward in the boat.

Sampled fish will be wrapped in aluminium foil and put into polyethylene bags of not less than 200μ gauge. They will be labelled and sealed in such a manner that unauthorized opening is detectable. The samples will then be put in a cool box with ice (ice: fish ratio of 2:1) for transportation to Kisumu Fisheries laboratory for sample preparation. In event that the samples cannot be worked on immediately, they will be frozen and kept at temperature below -18^{o}C. Sample preparation at Kisumu laboratory will be done when arrangements for transportation of the final samples to the analysing laboratories are ready so as to avoid repeated freezing and thawing of samples.

The sample labels shall contain the sample reference code with the following coded information:

- Sample type – NP: Nile perch, AC: African catfish, and TL: Tilapia
- Sampling site – 01-11 as per the legend in Figure 2
- Serial number – Next sequential number for each sampling exercise starting from 001

  Example: NP/01/001 for Nile perch from Bukoma beach, for the first sampling under this programme.

The following information will be recorded in the field book for each of the samples:

- Sample reference code
- Owner of boat sampled/registration number of the boat
- Type of sample
- Sampling site
- Date and time of sampling
- Size of sample- Average weight and length of the 10 samples for each species
- Any additional information likely to be of assistance such as transport time and conditions

3.7.2 Sample preparation:

The fish samples if not fresh will be thawed. The following equipment will be required:

- Filleting knife
- Filleting table
- Homogenizer- Stainless steel
- Sample containers- 200g glass bottles with screw cap
- De-ionized water.
- Sodium citrate/EDTA (Ethylene diamine tetra acetic acid) 2% solution
- Stainless steel spoon

All the 10 fish samples picked at each sampling point for each species will be pooled to make one sample. Each fish will be filleted to produce two fillets which will be de-skinned and all the 20 fillets homogenized together. Each final sample will be handled individually and all fish contact surfaces cleaned thoroughly and rinsed with de-ionized water before another sample is handled to prevent cross contamination. The homogenizer will be run with sodium citrate/EDTA 2% solution (acid) for about 2 minutes after washing and before rinsing with de-ionized water.

With acid-cleaned stainless steel spoon, approximately 200g of the homogenized sample will be taken and put into each of 4 acid-rinsed glass sample bottles. Screw caps will be lined with acid-rinsed aluminium foil or Teflon cap inserts. The bottles will then be affixed with labels and the final samples frozen and kept at temperatures not higher than -18^{o}C pending transportation to the final laboratory for analysis. One of the samples will be retained as a backup sample. The samples will be transported frozen.

The following information will be contained in the final sample label:

- Sample reference code with additional information on sample test group as follows:
  - PS: Pesticide
  - HM: Heavy metals
  - DX/PCB: Dioxins, dioxin like PCBs and indicator PCBs
  - BU: Backup sample

  Example: NP/ 01/PS/001

- Type of sample: Homogenized fish
- Size of sample: Approximately 200g
- Source of sample: Kisumu, Kenya
- Date of preparation: date prepared.
- Any additional information that may be of assistance to the analyst such as mode and condition of transportation.

A filled official sample submission form (Appendix) will accompany each sample to the analysing laboratory

3.7.3 Sample analysis:

Analysis of samples will be carried out at an accredited laboratory to be selected through the normal government procurement procedures. The laboratory will be required to show proof that it participates in international inter-laboratory quality control studies/ proficiency tests. The laboratory will also be required to define limits of detection (LOD) and limits of quantification (LOQ) that facilitate results interpretation.

3.8 Data analysis and results interpretation

The results received from the analysing laboratories will be entered into excel worksheet for ease of analysis and interpretation. The raw data will be entered as in the Tables 5 - 7 below.

Table 5: Proposed data entry table for pesticide residues in fish muscle

Sample code	Adrin/ Dieldrin	Total DDT	Endo sulfan	Endrin	HCH	HCB	Heptachlor	Chlordane
	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}
NP/01/001								

Table 6: Proposed data entry table for dioxins and PCBs in fish muscle

Sample code	PCDD/ PCDF	Dioxin like-PCBs	Sum of Dioxin and dioxin like-PCBs	Sum of Indicator PCBs
	Pgg^{-1} WHO-TEQ	pgg^{-1} WHO-TEQ	pgg^{-1} WHO-TEQ	μgkg^{-1}
NP/01/001				

Table 7: Proposed data entry table for toxic trace metals in fish muscle

Sample code	Mercury	Lead	Cadmium
	$mgkg^{-1}$ wet weight		
NP/01/001			

3.8.1 Determination of spatial variation

From the raw data, contaminant concentrations for each contaminant at different sampling sites will be plotted as shown in Table 8. Bar graphs will be used to show the variation of the contaminant concentrations at the different sampling sites. For each contaminant concentration, analysis of variance (ANOVA) will be carried out to determine if there is any significant difference between the contaminant concentrations in the same fish type at different sites.

Table 8: Proposed data entry table for each contaminant in each sample at different sampling sites

Sample code	01	02	03	04	05	06	07	08	09	10	11
Concentration											

3.8.2 Determination of temporal variation

Temporal variation will be determined after data collection for the whole programme period. An evaluation may however be done midway or after three years. For each contaminant at each sampling site, the concentration will be plotted against time as shown in Table 9. Trend lines will be used to show how contaminant concentrations vary with time. ANOVA will also be carried out to determine if there is any significant difference in the contaminant concentrations with time.

Table 9: Proposed data entry table for each contaminant in each sample type at each sampling site at different sampling dates

Serial number	001	002	003	004	005	006	007	008	009	010
Month/ year of sampling										
Concentration										

3.8.3 Determination of seasonal variation

The trend lines plotted to show temporal variation will also show if there is any seasonal variation. However, to determine if there is any significant difference in the contaminant concentrations between seasons, the concentration for each contaminant, in each sample type, at each sampling site, will be determined for each of the two seasons of the year when sampling is done as shown in Table 10. ANOVA will then be carried out to determine if there is any significant difference in the contaminant concentrations between the different seasons.

Table 10: Proposed data entry table for each contaminant in each sample type at each sampling site during different seasons of the year

Year	09	10	11	12	13
Concentration Wet season					
Concentration Dry season					

3.9 Proposed implementation plan

Implementation of this programme will require mobilization of resources initially so as to ensure that once started, the programme does not stall before completion. Such stalling would be a waste of resources if enough data is not collected to facilitate the compilation of a scientific report. This means if the government is to fund the programme, then there should be the necessary commitment to avoid unnecessary delays in availing funds and procuring the necessary equipment and services. Alternatively, the government may wish to identify an inter-governmental organization willing to fund the programme. Implementation will consist of the following stages:

- **Preparatory** - this stage will involve contracting the laboratory to carry out the analysis of samples. The contract should be made for the entire programme as it is important that analysis is done by the same laboratory to reduce variation caused by differences in sample handling. The stage will also involve acquiring all the necessary

tools for implementation and training of the personnel to carry out sampling and sample preparation.

- **Sampling and sample preparation** – these activities will be carried out by the trained personnel supervised by the officer in-charge as per the monitoring programme protocol (Appendix).

- **Analysis of samples** - this will be carried out by the contracted laboratory.

- **Data entry and analysis** - this again will be done by the trained personnel supervised by the officer in-charge of the programme

- **Compilation of reports** – this will be done by the officer in charge of the programme. The reports will include annual, mid-term and final programme reports.

3.9.1 Resources and costs for the programme implementation

The resources and costs for this programme are estimated as follows;

1. **Personnel** – Four authorised fish inspectors including the officer in charge, will collect and prepare the samples (they will require 2 vehicles hence 2 drivers). A three day training on the monitoring programme will be organized before the start of the programme. The cost of their subsistence while carrying out sampling will be calculated as per the government regulations.

   Costing (Kshs):
   Estimated number of days for sample collection - 10
   Current government rates for officers in the field = 2,500 X 10 (days) X 4 (officers) = 100,000
   Current government rates for drivers in the field =1,200 X 10 (days) X 2 (drivers) = 24,000
   Sub-total (Kshs) = 124,000 or €1,181 (based on the current exchange rate of Kshs 105 per Euro).

2. **Laboratory equipments** – these have been listed earlier and it will be assumed that other than the consumables, they are available at the Fisheries department laboratory in Kisumu.

   Costing (Kshs):
   Sample containers – 200(cost per bottle) X 80 (No. of bottles) = 16,000
   Sodium citrate ($Na_3C_6H_5O_7$) (1kg) – 5,000
   Ethylene-diamine-tetra-acetic acid (EDTA) (1kg) –10,000
   Polyethylene bags – 5000
   Aluminium foil – 2000
   Labels – 1000
   Sub-total = 39,000 or €371

3. **Means of transport** – available government vehicles will be used. The cost of running and maintaining them for the period of sampling will however need to be considered. Based on experience, each vehicle will require service at a cost of Kshs 20,000 and 300 litres of petrol.

   Costing (Kshs):
   Service – 20,000 X 2 (Vehicles) = 40, 000
   Petrol – 90 (cost per litre)X 300 X 2 (Vehicles) = 54,000
   Sub-total = 94,000 or €895

4. **Funds for purchase of samples.**
   Twenty fish samples of average weight (5 kg) each will be purchased at every sampling site. The current average price per kg is Kshs 150

   Costing (Kshs)
   Fish samples –150 X 20 (No. of samples) X 5 (Kgs weight) X 11 (No. of sampling sites) = **165,000 or €1571**

5. **Funds for payment to the analysing laboratory**
   This cost will depend on the selected laboratory. For the purpose of this proposal, the cost will be based on the rates submitted in a quotation by Eurofins Analytik Gmbh laboratory in Hamburg, Germany. The rates are for a sample whose initial preparation (homogenization) has been done.

   Costing (Euros):
   Pesticides – 250 (cost per sample) X 22 (No. of samples) = 5,500
   Trace metals –100 (cost per sample) X 22 (No. of samples) =2,200
   Dioxins and PCBs – 710 (cost per sample) X 10 (No. of samples) = 7,100
   Sub-total = €14,800 or Kshs 1,554,000
   Cost of sending 10 kg sample package from Kisumu, Kenya to Hamburg, Germany by DHL (Kshs) **– 23,800 or €227**
   Total cost = **Kshs 1,999,800 or €19,046**
   Contigencies (10%) = **Kshs 199,980 or €1,905**

   Grand total = Kshs 2, 199,780 or €20,950

This grand total is the cost of one seasons sampling exercise and analysis. Assuming the cost remains the same through out the 5 year period of the monitoring programme,

Total Project cost = Kshs 2,199,780 X 10 (No. of exercises) **= Kshs 21,997,800 or €209,500**

3.9.2 Training of implementation personnel

The training described in this text may be extended to as many fish inspectors as may be available so that there can be a pool from where implementation personnel can be identified. This is necessary as monitoring is a long term programme and may also be implemented in other geographical areas in the country. The course outline will be as follows:

- **Course title:** Implementation of a monitoring programme for undesirable chemical substances in fish/ seafood, personnel induction course

- **Course aim:** To provide trainees with the necessary knowledge and skills to be able to implement the monitoring programme successfully.

- **Course objectives:**

  - To link the course with hazard analysis critical control point (HACCP) training on chemical hazards and introduce the trainees to different chemical contaminants, their sources and how they enter into the environment.

  - To introduce the trainees to global efforts and roles played by different institutions in environmental monitoring of undesirable chemical substances.

  - To introduce the trainees to the monitoring programme, its objectives and methodology

  - To describe and discuss in details the monitoring programme protocol (Appendix).

  - To carry out a pilot sampling and practice sample preparation.

- **Course Duration:** The course is designed to take 3 days with lectures and discussions on the first 2 days and practical work on the 3rd day.

- **Course presentation:** the lectures will be delivered through power point presentation and enough time provided for discussion after the lectures.

- **Resources required:** These will include a training venue with the necessary facilities, means of transporting the trainees to a landing site for the pilot sampling and funds to purchase the sampled fish for the sample preparation practical at the Kisumu Fisheries laboratory.

- **Course reference material:**

  - CAC/GL 50-2004. *Codex alimentarius - General guidelines on sampling*
  - Commission regulation (EC) No.1883/2006. Commission regulation (EC) No.1883/2006 of 19 December 2006, *laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs*
  - Ngila M. (2009). *Environmental study and monitoring of undesirable chemical substances in fish/ seafood: A proposal for Lake Victoria, Kenya.* A final project report done in fulfilment of the UNU- Fisheries training programme

  - UNEP CHEMICALS (2004). *Guidance for a global monitoring programme for persistent organic pollutants;* Inter-organization programme for the sound management of chemicals (IOMC) A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD
  - UNEP/FAO/IAEA (1993). *Designing of monitoring programmes and management of data concerning chemical contaminants in marine organisms.* MAP technical reports series No. 77 UNEP, Athens

- UNEP/IOC/IAEA/FAO (1989): *Contaminant monitoring programmes using marine organisms: Quality assurance and good laboratory practice.* Reference methods for marine pollution studies No. 57, UNEP.
- UNEP/FAO/IOC/IAEA (1993): *Guidelines for monitoring chemical contaminants in the sea using marine organisms*. Reference Methods For Marine Pollution Studies No. 6, UNEP

3.9.3 Work plan for the implementation

The annual work plan designed in Table 11 below will be applicable for the whole period of the monitoring programme. The months for the sampling have been selected to coincide with the long rains in March and the dry season in September.

Table 11: Annual work plan for the implementation of the monitoring programme for undesirable chemical substances in fish/seafood from Lake Victoria, Kenya

Activity/Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul.**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Prepare and forward annual budget for the programme												
Requisition of funds for the programme												
Training of implementation team												
Procurement of required equipment												
Sampling and delivery of samples to the laboratory												
Sample analysis and submission of results												
Data entry and analysis												
Compilation and submission of report												

4. CONCLUSION

The fact that pollution is an issue of concern in Lake Victoria has been established. Untreated sewage sludge from major urban centres flows into the lake. All river draining into the lake originate from and/ or pass through rich agricultural areas where agrochemicals are increasingly being used. There are also industrial polluters including sugar refineries, soft drink and food processing factories, oil and soap mills, leather tanning factories, pulp and paper processing, abattoirs and mining companies.

Due to the increase in population and demand for land, forests and wetlands have been cleared leading to environmental degradation and increased sediment deposition in rivers and the lake. Furthermore, pollutants entering into the lake are unlikely to be rapidly reduced by dilution or out flow as the lake with only one outlet has a flushing time of 123 years and residence time of 23 years.

Various studies as described in this document have shown the presence of toxic trace metals, pesticide residues and polychlorinated biphenyls (PCBs) in Lake Victoria waters, sediments

and fish. These undesirable chemical substances negatively affect human and other living organisms hence the need to monitor them to ensure quality and safety of fish and fish products from the lake.

While it has been acknowledged in this document that the Ministry of Fisheries, Kenya already has a monitoring programme for the undesirable chemical substances in Lake Victoria, a case has been built for its improvement. Review of the current monitoring programme has been carried out in the following areas:

- **Objectives:** The objectives have been expanded to include monitoring of toxic trace metals, PCBs and Dioxins in addition to the currently monitored pesticide residues. However, as the main aim of the monitoring programme is to ensure the quality and safety of fish from Lake Victoria, monitoring has been restricted to fish only.

- **Sampling sites:** These have been increased to ten from the current four to include areas of the lake that are considered to contain higher contaminant concentrations. Lake Kanyaboli which is closely linked to Lake Victoria has also been included in the monitoring program due to the increased farming activities at the adjoining Yala swamp.

- **Sampling plan:** The number of samples taken per sampling site has been increased from 4 to 20. The samples will however be pooled for each species so as to keep the cost of monitoring low.

- **Sample analysis and results interpretation:** While sample analysis may continue to be done at the same accredited laboratory, the limits of detection (LOD) and limits of quantification (LOQ) will need to be agreed on so as to give quantifiable results that can provide useful information concerning trends or levels of pollution in the lake.

This proposed monitoring programme has been developed with a focus on protecting all fish consumers while taking into consideration areas of the lake likely to have higher concentrations of undesirable chemical substances. Specific objectives of the programme, rationale for the programme, sampling areas, the fish species to be monitored and the contaminants to be measured have been described. The period and frequency of sampling has been defined while the sampling methods, sample preparation and sample analysis for the programme has also been described. Data analysis and results interpretation will be done so as to be able to determine if there is spatial, temporal, and seasonal variation in contaminant concentrations in the fish species to be studied. The various stages in the implementation of this monitoring programme, training of implementation personnel and the work plan have been described. While the estimated costs for implementation of the programme may be high as calculated in the proposal (€210,000), the need to implement this monitoring programme in order to ensure the quality and safety of fish and fish products from Lake Victoria cannot be overemphasised.

Costs for environmental monitoring may be high, but the cost of not monitoring will be too high to comprehend.

ACKNOWLEDGEMENTS

I wish to thank the United Nations University (UNU) for granting me the fellowship that made my participation in this programme possible. I pay gratitude to the board of directors of the Fisheries Training Programme (FTP) and those who contributed to the success of the programme. Special thanks to the Deputy Director of the Programme Mr. Thor Asgeirsson, for his guidance and Ms Sigridur Kr. Ingvarsdottir for the timely updates and efficient facilitation in most activities during the programme.

I also wish to extend sincere thanks to my supervisors Helga Gunnlaugsdottir and Sasan Rabieh for successfully guiding me through the project. To the staff of Matis at the chemical laboratories both at Skulagata 4 and at Akureyri who I worked with during my project, I feel indebted to all of you for the invaluable information that you were always ready to give.

Lastly and not the least, my thanks to all fellows of the UNU-FTP programme (2008), who not only offered their assistance in one way or the other during the programme but also friendship which becomes so important when one is away from loved ones. My fond memories of the late Li Ge from whom I learnt a lot on computer applications. May her soul rest in peace.

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APPENDIX

Monitoring programme protocol

Monitoring programme for undesirable chemical substances in fish/ seafood from Lake Victoria, Kenya

PERSONNEL GUIDANCE MANUAL

1. INTRODUCTION

The first environmental monitoring programme for Lake Victoria, Kenya was initiated by the competent authority in October 1999 after the European Union (EU) imposed a ban on fish imports from the three East African states. The ban was due to reported use of chemicals in harvesting fish in Lake Victoria hence the competent authority to ensure quality and safety of fish and fishery products initiated the programme which involved the monitoring of pesticide residues in fish and environmental samples from the lake. The aim of the programme was to demonstrate the absence of pesticides in fish and to gain insight on the presence of pesticides in the aquatic environment of Lake Victoria.

The programme has been reviewed in this manual so as to continue ensuring the safety and quality of fish and fish products from Lake Victoria and especially for the local fish consumers who may be more vulnerable (Fish for local consumption is caught more from inshore areas than from offshore while the opposite applies for export fish). Consideration has been given to areas of the lake likely to contain higher concentrations of chemical contaminants while selecting the sampling sites. Two fish species of commercial value that are carnivorous and omnivorous hence at the end of the food chain have been selected for this study, while consideration will be given to other fish species of commercial value where contaminant concentrations may be found to be high. Undesirable chemical substances to be monitored include pesticide residues, dioxins, PCBs, and toxic trace metals. The frequency of sampling has been reduced to twice a year for the recommended period of five years before a review can be carried out.

1.1 Objectives

The main aim of this study is to protect public health. Contaminant concentrations in edible fish tissues will therefore be compared with international/national regulations to determine compliance. Specific objectives for the monitoring programme include the following:

- To find out contaminant concentrations in edible muscles of the fish and determine if there is need for further surveillance
- To identify areas of the lake that may be sufficiently contaminated to require fishery controls and action to reduce pollution.
- To detect trends in contaminant concentration levels with time
- To detect any seasonal variation in contaminant concentrations

1.2 Sampling areas

Selection of sampling sites (Figure 1) for the study has been done so as to include main river mouths, and areas around the major towns; the assumption is that these areas are likely to have higher concentrations of undesirable chemical substances. Sampling will be done around the mouth of rivers; Nzoia, Yala, Nyando, Sondu, Awach, and Kuja. There will be two sampling areas around Kisumu town, one on the southern side at the mouth of river Nyamasaria and another on the northern side at the mouth of river Kisat. There will be a sampling area around Homa bay town, and at Mbita point. The sampling at Mbita point is to target fish from offshore waters which are not expected to have been exposed to high levels of chemical contaminants. This will be done for comparison purposes. There will also be one sampling point at Lake Kanyaboli. The specific sampling sites, include, Bukoma landing beach for Nzoia river mouth sampling area, Osieko landing beach for Yala river mouth, Usoma landing beach for Kisumu northern area, Dunga landing beach for Kisumu southern area, Kusa landing beach for Nyando river mouth, Koguta landing beach for Sondu river mouth, Kendu bay landing beach for Awach river mouth, Homa bay landing beach for Homa bay town area, Mbita gembe landing beach for Mbita area and Got Kachola landing beach for Kuja river mouth area. Lake Kanyaboli main landing beach will be used for the Lake Kanyaboli.

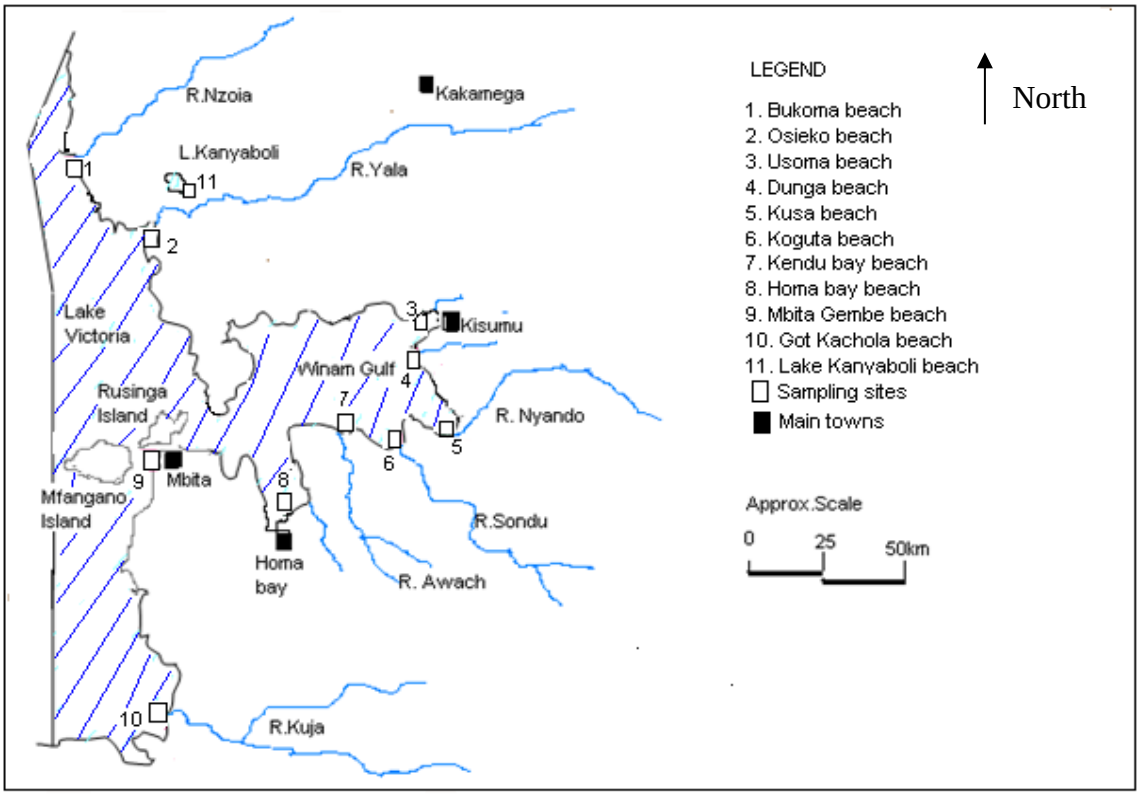

Figure 1: Map of Lake Victoria, Kenya showing sampling sites for monitoring of undesirable chemical substances in fish

1.3 Fish species to monitor

Two fish species that are of commercial value have been selected for this study: the Nile perch (*Lates niloticus*) and the African catfish (*Clarias gariepinus*). The two being carnivorous and omnivorous respectively hence at the end of food chain are likely to bio

accumulate more undesirable chemical substances when compared to other fish species in the lake. For Lake Kanyaboli however, tilapia (*Tilapia nilotica*) will be used as it is the main fish type in the lake. In sampling areas where contaminant concentrations may be found to be high during the initial stages of this study, a decision should be made to include other fish species of commercial value in the monitoring programme.

1.4 Period and frequency of sampling:

This study will be carried out for a period not less than five years so as to gather enough data to facilitate compilation of a comprehensive report for decision making. Sampling will be done twice every year, and the period for each sampling exercise shall not exceed one month. To ensure that any variation in contaminant concentrations is not due to seasonal environmental changes, the exercise shall be conducted at about the same time every year as possible.

1.5 Contaminants to be measured:

Undesirable chemical substances to be measured at each sampling area (Table 1) have been selected based on literature review and the author's knowledge of the geographical area. These include:

- **Pesticides**: Aldrin/ dieldrin, Total DDT (including DDT (*p, p'*- and *o, p'*-), DDE (*p, p'*- and *p, o'*-), and TDE (*p, p'*-)), endosulfan (α, β and sulfate), endrin, hexachlorocyclohexane (α, β and γ), hexachlorobenzene, heptachlor (including heptachlor epoxide), and chlordane.
- **Dioxins:** Polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)
- **Dioxin like PCBs:** Congeners (IUPAC Nos.)77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189
- **Indicator PCBs:** Congeners (IUPAC Nos.) 28, 52, 101, 118, 138, 153, and 180
- **Trace metals:** Mercury, lead and cadmium

Table 1. Sampling areas, contaminants to be studied and their probable sources.

Sampling Area	Contaminants of concern	Probable contaminant source/s
Nzoia river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, sewage/industrial waste from towns upstream
	Dioxins & PCBs	Pulp and paper mill, industrial/sewage waste from towns upstream
Yala river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, sewage/industrial waste from towns upstream
Kisat river mouth, Kisumu north	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Kisumu town
	Dioxins & PCBs	Industrial and sewage waste from Kisumu town, waste incinerators
Nyamasaria river mouth, Kisumu south	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Kisumu town
	Dioxins & PCBs	Industrial and sewage waste from Kisumu town, waste incinerators
Nyando river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage/industrial waste from towns upstream
Sondu river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage/industrial waste from towns upstream
Awach river mouth	Pesticide residues	Agricultural activities
	Trace metals	Sewage waste from towns upstream, natural
Homa bay town	Pesticide residues	Agricultural activities
	Trace metals	Industrial and sewage waste from Homabay town
	Dioxins & PCBs	Industrial and sewage waste from Homabay town, waste incinerators
Mbita point	Pesticide residues	Water currents
	Trace metals	Water currents, natural
	Dioxins & PCBs	Water currents, atmosphere
Kuja river mouth	Pesticide residues	Agricultural activities
	Trace metals	Gold mining activities, industrial and sewage waste from towns upstream
L.Kanyaboli	Pesticide residues	Agricultural activities
	Trace metals	Sewage waste from towns upstream, natural

Below (Table 2) is a summary of the chemical contaminants to be measured and their maximum residue limits in muscle tissue of fish as set by the EU directive 86/363/EEC and regulation (EC) No. 1881/2006.

Table 2: Chemical contaminants to be measured in a monitoring programme for undesirable chemical substances in fish/seafood from Lake Victoria, Kenya and the EU maximum residue limits

Contaminant group	**Criteria for control**	**EU MRLs (Maximum residue limits)**	**Units**
Organochlorine Pesticides residues	Sum of Aldrin/Dieldrin	0.2	$mgkg^{-1}$
	Total DDT	1.0	
	Endosulfan	0.1	
	Endrin	0.05	
	γ-HCH isomer (Lindane)	0.02	
	α- HCH isomer	0.2	
	β- HCH isomer	0.1	
	HCB	0.2	
	Heptachlor	0.2	
	Chlordane	0.05	
Dioxins and dioxin like PCBS	Sum of WHO-PCDD/PCDF-TEQ	0.4	pgg^{-1}
	Sum of WHO-PCDD/PCDF-PCB-TEQ	0.8	
Heavy metals	Mercury (Hg)	0.5	$mgkg^{-1}$ (Wet weight)
	Lead (Pb)	0.3	
	Cadmium Cd)	0.05	

2. METHODOLOGY

The methods to be used in this programme include sampling, sample preparation and sample analysis. The first two of these methods will be carried out by or with the supervision of an authorized fish inspector while the third will be carried out at an accredited laboratory.

2.1 Sampling

Sampling will be done by an authorised fish inspector according to the Codex Alimentarius - general guidelines on sampling. Large size fish (>4kg) will be targeted for sampling as it is expected to have accumulated more contaminants. The same size must however be targeted from all sampling sites so as to facilitate determination of spatial variation. To make this practical, it will be necessary to start sampling from those areas that have less fish so that the size to be sampled can be set from there. The following equipment will be required:

- Cool box and ice for fish storage
- Polyethylene bags- these should be unused and clean
- Aluminium foil
- Labels
- Means of transport
- Money for purchase of fish
- Measuring ruler- stainless steel
- Weighing scale

Fishing boats with targeted fish from the sampling area will be selected randomly for sampling. Ten samples of fish of the same size for each species will be randomly taken from different selected fishing boats for pooling. The assumption is that the samples taken will be representative of the sample species at the sampling area. Fish of the targeted size will be

sorted out first before a random sampling matrix technique is applied without replacement. The technique will be the 5x2 units' matrix outward and downward in the boat.

For small sized fish e.g. Dagaa, an equivalent of 1kg weight of fish taken from a selected boat will be considered as one sample. It will also be necessary to ascertain the source of fish for the selected boats to be sure the fish is from the targeted fishing area.

Sampled fish will be wrapped in aluminium foil and put individually into polyethylene bags of not less than 200μ gauge. They will be labelled and sealed in such a manner that unauthorized opening is detectable. The samples will then be put in a cool box with ice (Ice: fish ratio of 2:1) for transportation to Kisumu Fisheries laboratory where the initial sample preparation will be done. In event that the samples cannot be worked on immediately, they shall be kept at temperature below -18^{o}C. To avoid repeated freezing and thawing of samples the sample preparation at Kisumu laboratory will be done when arrangements for transportation of the final samples to the analysing laboratories are ready. (Repeated freezing and thawing of samples can lead to loss of body fluids and water content which not only affects the form and concentration of contaminants in the soft tissue but also makes the determination of wet to dry weight conversion factors very difficult.

The sample label shall contain the sample reference code with the following coded information:

- Sample type - NP: Nile perch, AC: African catfish, TL: Tilapia, and RA: Dagaa
- Sampling site – 01-11 as per the legend in figure 3
- Serial number – Next sequential number for each sampling starting from 001

  Example: NP/01/001 for Nile perch from Bukoma beach, the first sampling under this programme.

The following information will be recorded in the field book for each of the samples:

- Sample reference code
- Owner of boat sampled/registration number of the boat.
- Type of sample
- Size of sample –Average weight/ length of the 10 fish samples taken for each species; for Dagaa, the size of sample may be the number of individual fish making up a kilo.
- Sampling site
- Date and time of sampling
- Any additional information likely to be of assistance such as transport time and conditions.

2.2 Sample preparation:

The fish sample if not fresh will be thawed. The following equipment will be required:

- Weighing scale –sensitive for small weight
- Filleting knife
- Filleting table
- Homogenizer- stainless steel

- Sterile sample containers- 200g glass bottles with screw cap
- De-ionized water- purified by filtration.
- Sodium citrate/ Ethylene diamine tetra acetic acid (EDTA), 2% solution
- Pre written labels
- Laboratory gloves

All the 10 fish samples picked at each sampling point for each species will be pooled to make one sample. Each fish will be filleted to produce two fillets which will be de-skinned and all the 20 fillets homogenized together. Each final sample will be handled individually and all fish contact surfaces cleaned thoroughly and rinsed with de-ionized water before another sample is handled to prevent cross contamination. The homogenizer will be run with sodium citrate/EDTA 2% solution (acid) for about 2 minutes after washing and before rinsing with de-ionized water.

With acid-cleaned stainless steel spoon, approximately 200g of homogenized sample will be taken and put into each of 4 acid-rinsed glass sample bottles. Screw caps will be lined with acid-rinsed aluminium foil or Teflon cap inserts. The bottles will then be affixed with labels and the final samples frozen and kept at temperatures not higher than -18^{o}C pending transportation to the final laboratory for analysis. One of the samples will be retained as a backup sample. The samples will be transported frozen.

For dagaa (Rastrineobola argentea), the sample will be homogenized whole as it is consumed.

Care must be taken to ensure samples are not contaminated; all sample contact with plastics must be avoided including use of plastic gloves. Glass sample bottles must also be strictly used.

The following information will be contained in the final sample label:

- Sample reference code with additional information on sample test group as follows:
  - PS: Pesticide
  - HM: Heavy metals
  - DX/PCB: Dioxins, dioxin like PCBs and indicator PCBs
  - BU: Backup sample

  Example: NP/ 01 /PS/001

- Type of sample: Homogenized fish
- Size of sample: Approximately 200g
- Source of sample: Kisumu, Kenya
- Date of preparation: date prepared.
- Any additional information that may be of assistance to the analyst such as mode and condition of transportation.

A filled official sample submission form (*Annex*), will accompany each sample to the analysing laboratory

2.3 Sample analysis:

Analysis of samples will be carried out at an accredited laboratory to be selected through the normal government procurement procedures. The laboratory will be required to show proof that it participates in international inter-laboratory quality control studies/ proficiency tests. The laboratory will also be required to define limits of detection (LOD) and limits of quantification (LOQ) that facilitate results interpretation.

3. DATA ANALYSIS AND RESULTS INTERPRETATION

The results received from the analysing laboratories will be entered into excel worksheet for ease of analysis and interpretation. The raw data will be entered as in the Tables 3- 5 below.

Table 3. Data entry table for pesticide residues in fish muscle

Sample code	Adrin/ Dieldrin	Total DDT	Endo sulfan	Endrin	HCH	HCB	Heptachlor	Chlordane
	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}	μgkg^{-1}
NP/01/001								

Table 4. Data entry table for dioxins and PCBs in fish muscle

Sample code	PCDD/ PCDF	DL-PCB	Sum of Dioxin and DL-PCB	Sum of Marker PCBs
	pg/g WHO-TEQ	pg/g WHO-TEQ	pg/g WHO-TEQ	µg/kg
NP/01/001				

Table 5. Data entry table for toxic trace metals in fish muscle

Sample code	Mercury	Lead	Cadmium
	mg/kg wet weight		
NP/01/001			

3.1 Determination of spatial variation

From the raw data, contaminant concentrations for each contaminant at different sampling sites will be plotted as shown in Table 6. Bar graphs will be used to show the variation of the contaminant concentrations at the different sampling sites. For each contaminant concentration, analysis of variance (ANOVA) will be carried out to determine if there is any significant difference between the contaminant concentrations in the same fish type at different sites. The assumption is that other factors that may affect contaminant concentrations remain constant.

Table 6. Data entry table for contaminant (e.g. Adrin/ Dieldrin) concentration in samples of sample type (e.g. Nile perch) collected at different sites.

Sample code	01	02	03	04	05	06	07	08	09	10	11
Concentration											

3.2 Determination of temporal variation

Temporal variation will be determined after data collection for the whole programme period. An evaluation may however be done midway or after three years. For each contaminant at each sampling site, the mean concentration will be plotted against time as shown in Table 7. Trend lines will be used to show how contaminant concentrations vary with time. ANOVA will also be carried out to determine if there is any significant difference in the mean contaminant concentrations with time.

Table 7. Data entry table for contaminant (e.g. Adrin/ Dieldrin) concentration in sample type (e.g. in N/perch) at sampling site (e.g. Bukoma beach) at different sampling dates.

Serial number	001	002	003	004	005	006	007	008	009	010
Month/ year of sampling										
Concentration										

3.3 Determination of seasonal variation

The trend lines plotted to show temporal variation will also show if there is any seasonal variation. However, to determine if there is any significant difference in the contaminant concentrations between seasons, the mean concentration for each contaminant, at each sampling site, will be determined for each of the two seasons of the year when sampling is done as shown in Table 8. ANOVA will then be carried out to determine if there is any significant difference in the mean contaminant concentrations between the different seasons.

Table 8. Data entry table for contaminant (e.g. Adrin/ Dieldrin) concentration in sample type (e.g. N/perch) at sampling site (e.g. Bukoma beach) during different periods of the year.

Year	09	10	11	12	13
Concentration Wet season					
Concentration Dry season					

4. ANNUAL PROGRAMME WORK PLAN

The annual work plan designed in Table 9 below will be applicable for the whole period of the monitoring programme. The months for the sampling have been selected to coincide with the long rains in March and the dry season in September.

Table 9. Annual work plan for the implementation of the monitoring programme

Activity/Month	Jan	Feb	Mar	Apr	May	Jun	Jul.	Aug	Sep	Oct	Nov	Dec
Prepare and forward annual budget for the programme												
Requisition of funds for the programme												
Training of implementation team												
Procurement of required equipment												
Sampling and delivery of samples to the laboratory												
Sample analysis and submission of results												
Data entry and analysis												
Compilation and submission of report												

REFERENCE MATERIAL

- CAC/GL 50-2004. *Codex alimentarius - General guidelines on sampling*
- Commission regulation (EC) No. 1883/2006. *Commission regulation(EC) No.1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs*
- Ngila M. (2009). *Environmental study and monitoring of undesirable chemical substances in fish/ seafood: A proposal for Lake Victoria, Kenya*. A final project report done in fulfilment of the UNU- Fisheries training programme
- UNEP CHEMICALS (2004). *Guidance for a global monitoring programme for persistent organic pollutants;* Inter-organization programme for the sound management of chemicals (IOMC) A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD
- UNEP/FAO/IAEA (1993). *Designing of monitoring programmes and management of data concerning chemical contaminants in marine organisms.* MAP technical reports series No. 77 UNEP, Athens
- UNEP/IOC/IAEA/FAO (1989): *Contaminant monitoring programmes using marine organisms: Quality assurance and good laboratory practice.* Reference methods for marine pollution studies No. 57, UNEP.
- UNEP/FAO/IOC/IAEA (1993): *Guidelines for monitoring chemical contaminants in the sea using marine organisms*. Reference Methods For Marine Pollution Studies No. 6, UNEP

ANNEX

Sample submission form

REPUBLIC OF KENYA

SHERIES DEVELOPMENT
RTMENT

P.O. BOX 58187
NAIROBI

Sample Delivery/Receipt Form

1. PRE – ANALYSIS DETAILS

1.1 Type of Product			
1.2 Audit ID		1.3 Date of Audit	
1.4 Size of Sample		1.5 Size of Ref. Sample	
1.6 Sample ID		1.7 Sample ID	
1.8 File Reference No.			
1.9 Packaging			
1.10 Condition			

2. ASSESSMENT INFORMATION

2.1 Reference Standard	
2.2 Laboratory Test	

3. DELIVERY OF SAMPLES TO LABORATORY

3.1 Sender's Name		Signed:
3.2 Designation		
3.3 Address		
3.4 Date of Receipt		

4. LABORATORY DETAILS

4.1 Receiver's Name		Signed:
4.2 Laboratory Reference		
4.3 Analyst in charge		
4.4 Institute		

Note: The samples were collected, prepared and transported as per the monitoring programme protocol version 1.0 of 12th March 2009.