ASSESSMENT OF PESTICIDE AND HEAVY METAL RESIDUES IN TILAPIA FISH FROM MACHAKOS AND KIAMBU COUNTIES, KENYA

A thesis submitted in partial fulfillment of requirements for Masters Degree of University of Nairobi (Pharmacology and Toxicology)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this work to my beloved Parents Mr. and Mrs. Samson Omwenga who continually supported me both financially and morally throughout my education.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ATSDR	Agency of Toxic Substances and Disease Registry
CUPs	Current-use Pesticides
P,p’DDD	Dichlorodiphenyldichloroethane
p, p’DDE	Dichlorodiphenyldichloroethene
DDT	Dichlorodiphenyltrichloroethane
EC	European Community
ESP	Economic Stimulus Program
ECD	Electron Capture Detector
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GLC	Gas Liquid Chromatography
GOK	Government of Kenya
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
MFO	Mixed Oxidase Function
OC	Organochlorine
PCB	Polychlorinated Biphenyls
POP	Persistent Organic Pollutants
PNUE	Programme des Nations Unies pour l'environnement (United Nations Programme for the Environment)

WHO	World Health Organization
KBU M	Muscle sample from fish in Kiambu County.
KBU L	Liver sample from fish in Kiambu County.
KBU G	Gonad sample from fish in Kiambu County.
KBU B	Brain sample from fish in Kiambu County.
KAN M	Muscle sample from fish in Machakos County
KAN L	Liver sample from fish in Machakos County
KAN G	Gonad sample from fish in Machakos County
KAN B	Brain sample from fish in Machakos County.

ABSTRACT

Exposure to pesticides, heavy metals and other chemical residues cause harmful effects; they can cause injury to human health as well as to the environment. Humans take up these chemicals through skin absorption, respiration and ingestion of contaminated food. Among all foods, fish is one of the main sources of chemical contaminants although fish products account only for about 10% of diet or less. The present study was conducted in Kiambu and Machakos counties with objectives of determining the concentration levels of pesticides and heavy metals in edible parts of fish from inland fish farms, The dietary intake of pesticide and heavy metal was also estimated and compared with acceptable daily intakes of the World Health Organization (WHO) guidelines so as to assess their potential health hazard.

A total of two hundred and thirty tilapia fish samples (n= 230) were collected from Kiambu and Machakos counties in the months of September and October 2011. Following wet digestion, the levels of lead and cadmium were determined in muscle, liver, gonad and brain of tilapia fish using Atomic Absorption Spectrophotometer (AAS) fitted with cadmium and lead lamps set at their respective wavelengths. To evaluate organochlorine concentration, fish samples were extracted, cleaned and analyzed using Gas Liquid Chromatography (GLC) fitted with Electron Capture Detector (ECD).

The data was subjected to descriptive statistics and Analysis of Variance (ANOVA) to test levels of significance at 95% confidence limit using Statistical Analysis Software (SAS) 9.0 version. Significance was noted at $P< 0.005$.

In Kiambu county, mean lead levels ($x \pm s.d$) in brain, liver, gonad and muscle were 31.31 ± 28.27, 17.33 ± 20.64, 16.62 ± 15.42 and 3.78 ± 2.22 ppm respectively against `the benchmark

value of 0.5ppm.The average cadmium levels (x ± s.d) in the brain, liver, gonad and muscle were 7.25± 6.59, 5.35 ± 6.12, 3.35 ± 4.16 and 1.66 ± 2.48 ppm respectively against the benchmark level of 0.05ppm.However, only positive samples were considered. Out of the total number of samples analyzed, only 40% were positive for lead and cadmium in Kiambu county and 34 % in Machakos County.

In Machakos county, mean lead levels (x ± s.d) in the brain, liver, gonad and muscle were 30.40±20.56, 7.88 ±7.25, 13.29±14.28 and 12.22±22.96 ppm respectively against the benchmark level of 0.5ppm.The average cadmium levels (x ± s.d) were 3.91±5.84, 2.90±3.58, 1.25±1.38 and 1.12±1.13ppm respectively against the benchmark level of 0.05ppm.

The Organochlorines exhibiting the highest concentration (x ± s.d) were *o,p* ‘-DDT with a mean of 2.098±4.097 µg Kg^{-1} followed by p’p-DDD 1.684±3.666 µg Kg^{-1} in the brain tissues. This was followed by p’p-DDD in muscle at 0.916±1.287 µg Kg^{-1} and pp-DDT at 0.916±1.916 µg Kg^{-1}. Machakos County had *p,p’*-DDT at a concentration of 0.158 µg Kg^{-1} ,*p,p*’-DDD had 0.097 µg Kg^{-1} and *p’*-DDT had a concentration of 0.016±0.016 .

Statistically, there was no significant difference in the tissues analyzed for the concentration of Pb in the two counties since pr/t/>0.05. Cadmium concentration in the gonad had a significant difference between Kiambu and Machakos County since pr/t/=0.05.The brain, liver and muscle did not show any significant difference in the concentration of Cadmium between the two counties since pr/t/ >0.05 .

The results of the current study indicate the presence of relatively high levels of lead and cadmium above permissible limits in fish from the study areas and recommend controlling industrial and agriculture effluents into surface water and proper sitting of ponds to minimize the risk of contamination of farmed fish by pesticides and heavy metal. However it was noted that the number of samples that were positive with regard to the presence of all residues was small compared to the total number of samples analyzed.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Inland fish farming is increasingly being seen as an alternative to ease the current pressure on formal fish resources, provision of food and nutritional security and as a vehicle for income and employment generation particularly for the youth and women in Kenya. However, there is little understanding about the constraints facing farmers such as high fish mortality rates which could be caused by fish diseases or poisoning among other causes. Poisoning occurs when pesticides and other environmental contaminants enter the water system through surface runoff, leaching, and/or erosion and are taken up by fish.

Pesticides constitute a major group of potential environmental hazards to man and have been routinely used in most countries of the world to control harmful pests. Some of these pesticides are the persistent organic pollutants (POPs). The contamination of POPs is a significant health problem because they can be accumulated and magnified through the food chain, thus causing adverse effects to human health. Accidental contamination of POPs in the environment has been cited and therefore the risk assessment of POPs in food for human health is important and necessary, (Binelli & Provini, 2004).

Contamination by heavy metals has been associated with discharges from urban centers, waste and sewage leakages into rivers as well as entry of various agricultural chemicals, petroleum products and domestic wastes into rivers and canals, M'onica et al., (2006). This work, therefore, sought to provide baseline information on levels of pesticide and heavy metal contamination in fresh water farmed fish samples collected in selected Counties in Kenya. The results will be used to identify the mitigation measures required to improve and promote the safety of fresh water farmed fish in Kenya for local and export market.

1.2 Justification

Aquaculture is one of the fastest growing food production activities in the world, FAO (1997). It is one of the sectors selected by the government of Kenya to benefit from the Economic Stimulus Program (ESP) in order to contribute to economic recovery and attainment of Vision 2030. . In the years 2009 and 2010, the government committed up to Ksh 1.12 billion for the construction of 200 fish ponds in 140 constituencies at a cost of Ksh. 8 million per constituency, GOK, (2010).

Although inland fish farming is fast becoming established in Kenya, there is little information regarding the safety of the end product and hence potential hazard to humans. Several chemical contaminants from the agricultural fields, comprising of pesticides and other agrochemicals have been reported in the drainage systems and are likely to jeopardize the quality of the water bodies that support the fishery industry. Also, there is little understanding about the constraints facing farmers such as high mortality rates which could be caused by fish diseases or poisoning. During research period, information on pesticide and heavy metal residues in inland fish farming was fragmentary and inadequate. There was need for data on persistent organic pesticides and heavy metal residues in fresh water farmed fish for public health reasons. The information obtained was used to advise farmers on ways of mitigating against contamination and marketing of the fish especially on export to European countries based on the WHO Guidelines. The information was used to advise policy makers on developing satisfactory techniques that can combine optimal agricultural productivity and environmental protection.

1.3 Objectives of the Study

1.3.1 General objective

The general objective of this study was to determine the concentration levels of pesticides and heavy metals in edible parts of tilapia fish from inland fish farms from selected Counties in Kenya.

1.3.2 Specific objectives

1. To determine Organochlorine pesticide and heavy metal concentration in edible fish samples collected from Machakos and Kiambu Counties in Kenya.
2. To compare the relationship between levels of organochlorines and heavy metals to the performance of Tilapia fish from the two counties.
3. To estimate pesticide and heavy metal dietary intakes by citizen and compare them with the WHO guidelines so as to asses potential health hazard.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pesticides and their Harmful Effects

Pesticides refer to any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies,(Food and Agricultural Organization, 1983).

Pesticides have contributed to dramatic increases in crop yields and in the quantity and variety of the diet and have also helped to limit the spread of certain diseases. Despite the benefits accrued, pesticides have harmful effects; they can cause injury to human health as well as to the environment. The range of these adverse health effects includes 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).

In particular Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are two principal environmental pollutants due to their toxicity, mobility and persistence. Humans take up POPs through skin absorption, respiration and ingestion of contaminated food. Skin absorption and respiration are not the main route. Some researchers have confirmed that more than 90% of contaminants come from food, (Furst, *et al*. 1990). Among all foods, fish is one of the main sources of chemical contaminants although fish products account only for about 10% of diet or less, (Alcock *et al*. 1998).

Organochlorine compounds such as dichlorodiphenyltrichloroethane (DDT), lindane and aldrin were used in Kenya between the mid 1940's and late 1970's in agriculture and aerial control of mosquitoes in the Lake Victoria region. The use of persistent organic pesticides was banned or restricted in Kenya in 1986, (Pest Control Records 1986). Examples include endrin, toxaphene, heptorchor, chlordane and aldrin.

The pesticides mainly exert their detrimental effects on non-target organisms through chronic toxicity and sub-lethal exposure as they accumulate in fatty tissue of living organisms. DDT and its metabolites cause microsomal enzyme induction, egg-shell thinning in birds and tumor induction. DDT also reduces the reproductive success of birds and fish. Aldrin and dieldrin are potentially carcinogenic while lindane and other hexachlorohexane (HCH) isomers produce liver tumors in mice, (Mugachia *et al.* 1992).

Earlier studies conducted by Mitema and Gitau (1990) detected low levels of BHC, aldrin, dieldrin, lindane, and p,p'-DDT in Nile perch from Lake Victoria. The p,p'-DDT and its metabolites formed the largest proportion of the organochlorine pesticide residues in the fish samples. The presence of these residues was attributed to the previous use of the pesticides in agriculture and aerial control of mosquitoes in the Lake Victoria region. (Mugachia *et al.,* 1992b) found presence of organochlorine pesticide residues in six species of fish from the Athi River estuarine. They reported presence of p,p'- DDE, p,p'-DDT, p,p'-DDD, γ-HCH, α-HCH, heptachlor and o,p'-DDD in fish samples.

A number of persistent organochlorines and highly toxic organophosphates, which have been banned or severely restricted, are still marketed and used in many developing countries. Although DDT is reported to have been gradually abandoned owing to pest resistance and international concern, it is secretly being used by farmers in Uganda, (NEMA, 2000). In a recent interview in Uganda, 2% of the farmers confessed to using DDT, (Wasswa, 2009). Its residues have also been detected in fish samples from Lake Edward, as well as in soil samples in western Uganda, (Ssebugere *et al*. 2009; Ssebugere *et al*., 2010: Wasswa *et al.* 2011) reports high levels of aldrin and dieldrin in sediments from the Uganda side of Lake Victoria. The misuse of pesticides by concerned individuals, in addition to lack of or weak national policy on pesticides has led to the outbreak of adverse effects especially in developing countries, (Mansour, 2004). Organochlorine pesticides in the environment are characterized by high chemical stability; poor water solubility and low vapor pressures, (Bouwman, 2004) and are Consequently referred to as persistent organic pollutants, (Darko *et al*., 2008). In East Africa, OC pesticides have been in use since the 1940s and have tended to accumulate in soil, and sediment, (Madadi *et al.,* 2006 ; Werimo *et al*. 2009). Owing to ingestion of sediment by fish, OC pesticides and their residues find their way into edible fish, (Licata *et al*., 2003). Health risks associated with OC metabolites are well documented, (Engel *et al*., 2000). As such, out of the 21 persistent organic pollutants (POPs) that have been ear-marked for phase-out and elimination in the World, fourteen are OC pesticides; dichlorodiphenyltrichloroethane (DDT), aldrin, endrin, dieldrin, chlordane, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, chlordecone, lindane and pentachlorobenzene, (Stockholm Convention, 2001, 2009).

The determination of organochlorines and heavy metal residues in fish may give an indication of the extent of aquatic contamination and accumulation characteristics of these compounds in the aquatic biota that will help in understanding the behavior and fate of these persistent chemicals (Kannan *et al.* 1995).

The application of many current-use pesticides (CUPs) in agriculture and public health has increased with the transition from use of older or banned legacy pesticides that are persistent, bioaccumulative or toxic such as the organochlorine insecticides DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane) or chlordane (octachloro-4,7-methanohydroindane). Many of the CUPs are considered to be of lesser environmental concern due to the perception of generally more favorable physico-chemical properties such as shorter half lives or decreased potential for bioaccumulation due to lower octanol–water partitioning coefficients (*K*ow), both of which would be expected to contribute to a lower potential for toxicity in exposed organisms. While, in most cases, the concentrations of CUPs found in the environment are much lower than that expected to cause direct lethality in non-target aquatic species, there is still only limited information regarding their sub-lethal effects. Unfortunately, as a result of their higher use levels, CUPs are being identified more frequently in environmental surface water, sediment and air samples in some parts of the world, e.g., British Columbia, Canada and elsewhere (Harris *et al.* 2008).

2.2 Toxicity of pesticide

Pesticide toxicity depends on the compound family and are responsible for acute poisonings as well as for long term effects, including cancer and adverse effects on reproduction in humans and wildlife, (Maroni *et al.*, 2006; Moline *et al.*, 2000).Toxicity of a pesticide is a measure of the capacity or ability of the pesticide to cause injury or illness and is determined by subjecting test animals to varying dosages of the pesticide active ingredient. Toxicity pathways are normal pathways for maintaining cellular functions but when sufficiently perturbed, will lead to an adverse health outcome and the magnitude of the adverse effect is related to dose at the cellular level, the timing of the perturbation and the susceptibility and life stage of the host, (Linda *et al*. 2009).

Harmful effects due to acute toxicity is injury from a single high exposure and generally of short duration via any route of entry while chronic toxicity and chronic effects occur from exposure to small doses of the active ingredient repeated over a long period of time. Chronic effects from exposure to pesticides include birth defects, severe depression, irritability, confusion, delayed reaction time, drowsiness, insomnia and production of benign or malignant tumors, genetic changes, birth defects, nerve disorders, endocrine disruption and `reproduction effects

2.3 Routes of Exposure to Pesticides

Pesticide exposure can be dermal, oral, through inhalation, or through the eyes. In children dietary ingestion is one of the pathways they are exposed to pesticides, (Akland *et al*., 2000; Berry, 1997; Thomas *et al*., 1997) and more so during normal oral exploration of their environment and via dermal contact with floors and other surfaces.

2.4 General signs of Pesticide Poisoning.

Symptoms due to pesticide poisoning can range from mild skin irritation to coma or even death. These vary with classes of chemicals, individuals, duration of exposure and route of entry. It is not surprising therefore that some people may show no reaction to an exposure that may cause severe illness in others. Some of the symptoms which have been reported include central nervous system symptoms like dizziness, headache, confusion and respiratory depression. Muscarinic effects include increased glandular secretions, smooth muscle dysfunction manifested as diarrhea, miosis, blurred vision, involuntary micturation and bradycardia. Nicotinic effects include hypertension, tachycardia, muscle fasciculation, weakness and paralysis. The recovery from acute poisoning depends on the severity of the poisoning and on the availability of treatment, and may last from one day up to a few weeks, (Miranda *et al*., 2004; Lorann and Cheryl, 2002).

2.5 Organochlorine pesticides

Organochlorine compounds are synthetic organic insecticides that contain carbon, hydrogen, chlorine and sometimes oxygen, (Afful *et al*. 2010). This group of synthetic chemicals was introduced for the first time in 1930s and was used extensively in agriculture immediately after the Second World War, (Borrell and Aguilar 2007). The pesticides have been widely used throughout the world since the middle of the 20th century and have been used globally mostly in public health activities in an attempt to combat vector borne diseases in agriculture and animal production, (Bayer and Biziuk , 2007). Consequently, contamination of the environment by organochlorine pesticides is found in many places due to the fact that these compounds are highly lipophilic and do not readily degrade in the environment hence stay in the environment and food web long after being applied, (Tanabe 2002; Heberg *et al.* 2005).They can also be transported by air and water such that trace amounts are all over the world, (Alle *et al.* 2009).

2.5.1 Classification of Organochlorine Pesticides

Organochlorine compounds are divided into three main groups based on their chemical structure.

2.5.2 dichlorodiphenyltrichloroethane group

DDT is one of the most well-known synthetic compound which was widely used to control insects in agriculture and disease vectors. In this group the most important compound is DDT and its metabolites *p,p'*-DDE and *p,p'*-DDD. The metabolites may be observed or recovered in soil or foodstuff as residue many years after use. Exposure to these chemicals can be direct e. g occupational exposure or can be indirect through consumption of foods containing residues of these chemicals. Accumulation along food chain has been investigated and noted that such accumulation can be fatal to humans. DDT was detected in various food commodities e.g. animal products, poultry, fish, human fat and milk, (Kanja *et al*. 1986). DDT has been associated with premature births as well as affecting neurobehavioral functions (Longnecher *et al.* 2001).

In Kenya , there was widespread use of DDT to control pests, especially in the mosquito infested areas,(Kanja *et al.,* 1986). *p,p*-'DDE and p,p DDD enter the environment as contaminants or breakdown products of DDT.

The concern about DDT persistence and negative health effects has had a significant impact on agriculture and vector control. Since DDT is known to induce microsomal enzymes, this may quantitatively alter the response to various drugs and toxic compounds as well as to naturally occurring substances in the body leading to alteration of homeostasis of biochemical processes. In the air DDT is broken down slowly to *p, p*'-DDE and p,p'- DDD by microorganisms, (ATSDR 2002).

2.5.3 Cyclodienes group

This group includes aldrin used as ant killer in construction work, dieldrin, isodrin, heptachlor and endosulfan which are highly insecticidal. Endosulfan is of environmental importance because of its apparent persistence and toxicity to many non target organisms like fish, (Shetty *et al*. 2000).

2.5. 4 Hexachlorocyclohexane Group

Hexachlorocyclohexane (HCH) exists in isomers and most common isomers include α-HCH, ß-HCH, γ-HCH and δ-HCH has been extensively used for the control of insect pest on agriculturally important crops, seeds and vegetables. Lindane (γ-HCH) is commonly used as dust in seed treatment by farmers. Although γ-HCH is persistent and difficult to biodegrade, a few microorganisms have been isolated which can degrade one or more HCH isomers under aerobic conditions. Example of such strain include *Sphingomonas paucimobilis*, (Mrutyunjay *et al.* 2004).

2.5. 5 Metabolism of Organochlorines

Metabolism of the organochlorine pesticides mainly occurs due to the action of the Mixed Function Oxidase (MFO) system located in the ribosome of the smooth endoplasmic reticulum of the hepatocytes, (Janice and James 1976). Fish have low amounts or even deficient of the MFO system and where available, the enzyme system has a lower optimum temperature compared to that of mammal, (Janice and James, 1976). For this reason together with the stability of organochlorine to biodegradation, the rate of metabolism is very slow leading to accumulation of organochlorine in fish.

2.5.6 Mechanism of Action

DDT acts mainly by opening sodium ion channels in neurons, causing them to fire spontaneously, resulting to spasms and eventual death. Insects with certain mutations in their sodium channel gene are resistant to DDT and other similar insecticides, (Denholm, 2006).

2.5.7 Harmful effects of organochlorines

The presence of organochlorine pesticide residues in human adipose tissue have been associated with development of breast cancer due to anti androgenic and estrogenic properties and their effects on sexual activity, (Lopez *et al*. 1996); Ahlborg *et al.* 1995; Hunter *et al.* 1997). After the residues have been metabolized, they are stored in the fat reserves from where they enter in blood circulation and are eliminated through milk, (Cerrkvenik *et al.*, 2000; Nag, 2008).There is uncertainty about the adverse effects that these residues may have after a lengthy human exposure at low doses, (Kodba 2007; Soler and Yolanda 2007).The compounds have been reported to cause a variety of effects including immunologic, teratogenic, carcinogenic, reproductive and neurologic problems in organisms, (Nakata *et al*. 2002).

2.5.8 Immunotoxicological effects of pesticides

Chronic exposure to anthropogenic chemicals has been found to result in sub-lethal effects to the exposed species. For instance, population-level declines in wild salmon stocks as a result of modulation of the fish immune system and resultant increases in mortality,(Arkoosh *et al.* 1998a). A number of studies have found that fish sampled from chemically contaminated environments show changes in phagocytic ability or respiratory burst activity when compared to fish from relatively clean sites, which could predispose these fish to increased susceptibility to infectious diseases, (Lesley *et al*. 2002).

2.6 Heavy Metals

A heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. These metallic chemical elements have a relatively high density and are toxic at low concentrations, (Irwandi and Farida, 2009) and generally do not break down further into less harmful constituents and they accumulate where they are released, (Akan *et al*, 2009).Examples of heavy metals include mercury (Hg), cadmium(Cd), arsenic (As), chromium (Cr), thallium(TI) and lead (Pb) which can be of serious threat to animals and human beings because of their high toxicity and tendency to bioaccumulate in the food chain even at low concentrations, (Suantak *et al*. 2011).

Environmental pollution with toxic heavy metals is mainly due to anthropogenic activities and this has become a global issue,(Gosh and Singh, 2005; Tandy *et al*. 2006).Rapid industrialization has resulted to discharge of potentially toxic trace metals such as mercury, cadmium, copper, chromium and nickel into the marine environment, (Yasar *et al*. 2007).

Heavy metals are regarded as environmental pollutants due to their toxicity, persistency and bioaccumulation problem and their effects on health, (Tam and Wong, 2000).

After entering into aquatic environment they accumulate in tissues and organs of aquatic organisms. Absorption and assimilation depends on ecological, physical, chemical, biological condition, the kind of element and physiology of organisms. The quantification of heavy metals is therefore important for the environment and public health especially in cases of edible products. (Jaffa *et al*., 1998: Temara *et al*., 1996; Warnau *et al*., 1997).

The contamination of food with heavy metals constitutes a serious health hazard depending on their relative levels. Some of these metals, such as cadmium and mercury, affect the kidney and cause symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction. (Luckey and Venugopal, 1977). Moreover, lead causes renal failure and liver damage (Luckey and Venugopal, 1977). Some other metals (e.g., chromium, zinc and copper) cause nephritis, anuria and extensive lesions in the kidney, (Luckey and Venugopal, 1977).

Cadmium is a nonessential heavy metal but it has accumulative polluting effect, and causes toxicity to aquatic organisms even in minute concentrations. Therefore, it is regarded as one of the most toxic elements in the environment. The occurrence of cadmium in considerably toxic amounts was reported by earlier workers in various aquatic ecosystems, (Arno *et al.* 2002; Audrys *et al.* 2004; Chrastny *et al*. 2006). It can act as an endocrine disrupter, interfering with biological functions such as reproduction, growth, development, osmoregulation and the ability to cope up with stress in fish. It causes significant metabolic alterations and injuries of biological system at different levels after entering into the organs of freshwater fishes through the gills, (Pratap and Bonga 1990; Brown *et al.* 1984). Cadmium is reported to cause anemia in a variety of fish species at low as well as high concentrations after entering into the organism of freshwater fishes through the gills, (Larson *et al*. 1976).

Absorption of cadmium compounds is dependent on the solubility of the compounds and accumulates primarily in the kidneys and has a long biological half-life in humans of 10–35 years. There is evidence that cadmium is carcinogenic by the inhalation route. However, there is no evidence of carcinogenicity by the oral route. Cadmium metal is used in the steel industry and in plastics. Its compounds are widely used in batteries and is released to the environment in wastewater, and diffuse pollution is caused by contamination from fertilizers and local air pollution. Contamination in drinking-water may also be caused by cadmium impurities in the zinc of galvanized pipes and solders and some metal fittings. Food is the main source of daily exposure to cadmium, (WHO, 2003).

Lead is another non- essential heavy metal that serves no biological purpose but present in all organisms. It is one of the oldest occupation and environmental diseases in the world. Extensive research indicates that lead may have adverse effects. Lead poisoning is associated with cognitive impairment and also affects the renal, hematologic and neurologic systems, (Needleman *et al*. 1990). It is hazardous as it accumulates in the body and affects the central nervous system. Exposure to lead is a differential diagnosis of microcytic anemia as lead inhibits heme synthesis and increases the rate of erythrocyte destruction, (Schuhmacher *et al*., 1997).Common sources of lead exposure in the environment include lead paint, airborne lead from combustion of petroleum products containing tetraethyl lead, soil or dust near highways or lead painted homes, plumbing leachates from pipes or solder and lead from leaded chips, and batteries, (Committee of Environmental Health , 2005)

2.6.1 Accumulation of heavy metals in fish

Heavy metals can be accumulated by marine organisms through a variety of pathways, including respiration, adsorption and ingestion of water or contaminated food. Some of the common toxic heavy metals that are found in fish include mercury, lead and cadmium. Other heavy metals like calcium, iron, copper, zinc and manganese are essential metals and play important roles in biological system in human and fish,(Irwandi and Farida, 2009). The most non-essential heavy metals of particular concern to fish and surface water are cadmium (Cd), Lead (Pb) and mercury (Hg) which enter into fish mainly via gills,(Ahmed and Hussein 2004).The progressive and irreversible accumulation of these metals in various organs of marine creatures ultimately leads to metal related diseases in the long run because of their toxicity, thereby endangering the aquatic biota and other organisms including humans, (Melville and Burchett, 2002).

2.6.2 Sources of Contamination by pesticides and heavy metals

Wind erosion can carry pesticide residues into the atmosphere that can lead to contamination of surface waters via precipitation, (Dubus *et al*. 2000). Whether they are dissolved in water or carried by sediment, pesticides that are carried off-site can contaminate surface waters, (Willis and McDowell, 1982; Capel *et al.* 2001). Improper cleaning or disposals of containers, as well as mixing and loading pesticides in areas where residues or run-off are likely to threaten surface waters, are other potential sources of contamination. The extent to which a pesticide runs off an agricultural field is determined by the unique combination of climatic, soil, and management factors that characterize each field, crop, and year combination, (Wauchope, 1978; Weber *et al*., 1980; Leonard, 1988;Willis and McDowell, 1982). Studies have reported that only 5% of the sprayed chemicals actually reach the targeted organisms, with the remaining 95% drifting to surrounding areas and eventually becoming an environmental contaminant, (Panagiotis and Weili, 2008).

Lead is found in paint in older houses and in soils near freeways exposed to leaded gasoline in the past. Cadmium is high in cigarette smoke and may also be released from particles of steel belted tires.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area.

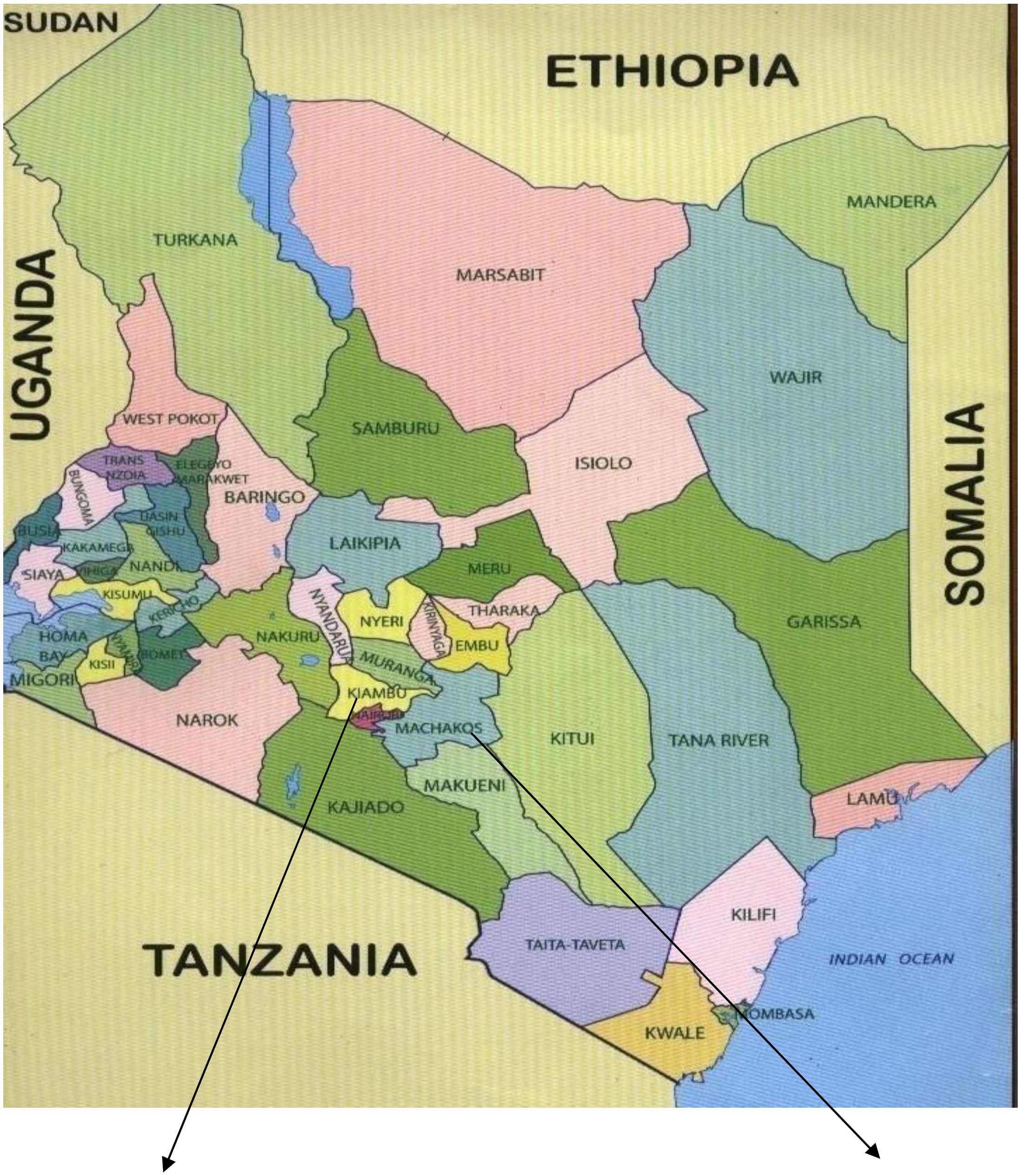
The current study was carried out in Kiambu and Machakos Counties. Kiambu County is in the central province of Kenya, where fish farming and consumption are not traditional practices. The area borders Nairobi city where there is a lucrative market for Fish. The County covers an area of 1,323.9 sq Km². It borders Nairobi City and Kajiado District to the south, Nakuru District to the west, Nyandarua District to the northwest and Thika to the east (figure 3.1). The county lies between latitudes 0°75′ and 1° 20′ south of Equator and longitudes 36° 54′ and 36° 85′ east.

Machakos is in a semi-arid agro-ecological zone with no history of fish production. Since water is scarce, its cost is higher than in Kiambu County. The County borders Nairobi city and Thika District to the northwest, Kitui and Mwingi Districts to the east, Kajiado District to the west, Makueni county to the south, Maragwa District to the north and Mbeere District to the northeast (Figure3.3). It stretches from latitudes 0º 45’ south to 1º 31’ south and longitudes 36° 45’east to 37° 45’ east. The two Counties were among the counties that were allocated funds from the economic stimulus package which aimed at construction of 200 fish ponds in each constituency.

Table 3.1 below is a summary of the various sites from where fish was collected.

Table 3. 1: Description of study sites from where fish was collected from in Kiambu and Machakos Counties

Agro-ecological Zone	**Study areas**	**Main activities**	**Fish farming**
Upper Highlands (UH1 – UH2)	Lari and Githunguri districts	Tea and dairy farming	There are about 500 farmers With an estimated total area of37.5 acres currently under fish.
Upper Midland (UM1 – UM2)	Kiambu East, Githunguri and Kikuyu districts	Coffee and dairy farming	There are about 1,000 farmers with an estimated total area of 75 acres currently under fish.
Lower Highlands (LH3 – LH5)	Kikuyu district	Maize and wheat farming	There are about 400 farmers With an estimated total area of 30 acres currently under fish.
Lower Midland (LM4 – LM5) Lower Highland (LH2)	Machakos and Kathiani districts	Extensive livestock rearing, coffee and, drought tolerant cereals and legumes.	There are about 200 farmers with an estimated total area of 15 acres currently under fish.
Upper Midland (UM2 – UM4)	Kangundo and Matungulu districts	Extensive livestock rearing, coffee and drought tolerant cereals and legumes	There are about 150 farmers With an estimated total area of 11.25 acres currently under fish.



Kiambu County **Machakos County**

Figure 3.1: Map of Kenya showing Kiambu and Machakos County (Figure courtesy of Albert K.I., 2011)

Fish were randomly sampled from various sites in Kiambu County (figure 3.2)

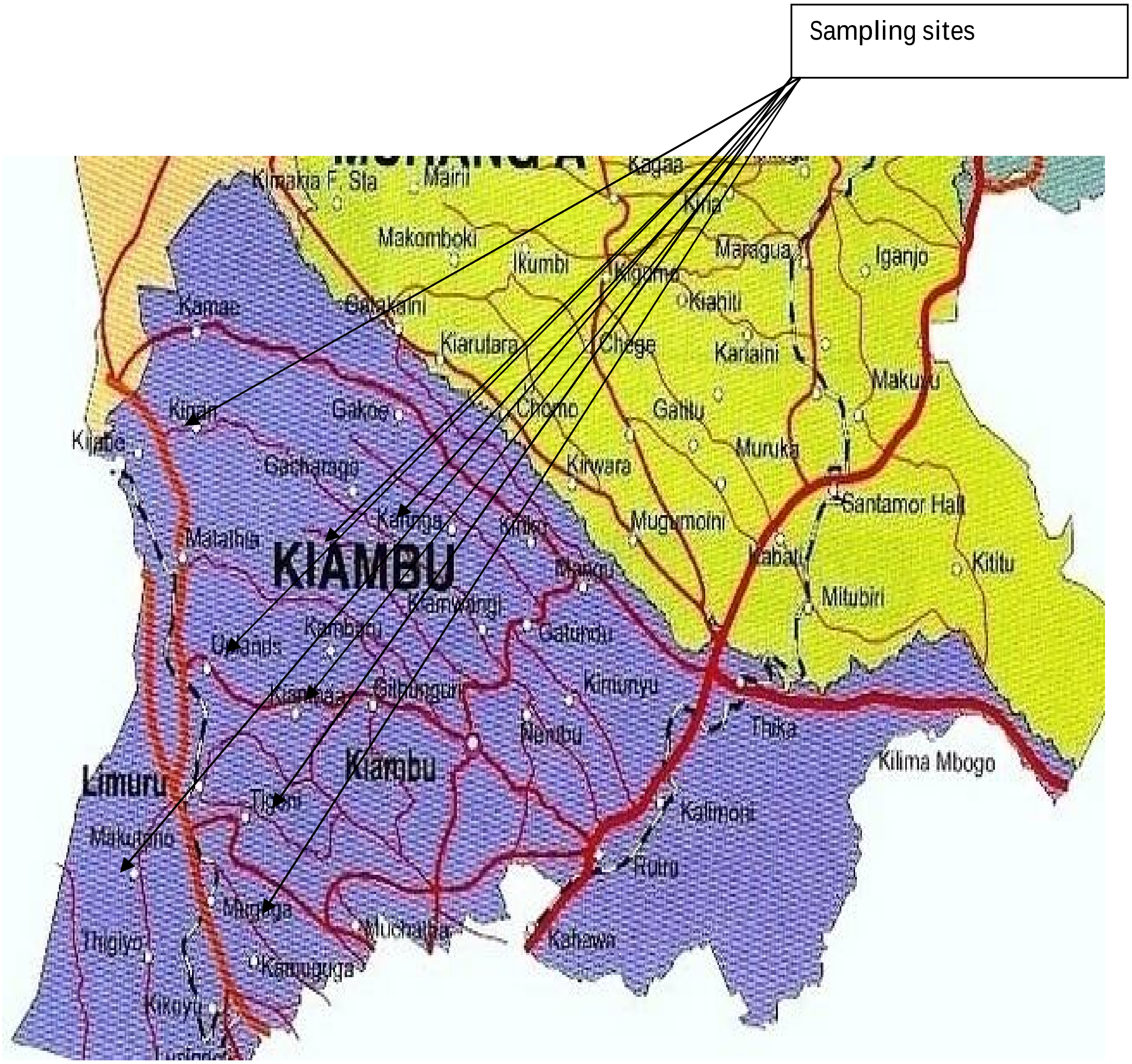


Figure 3. 2: Sampling Sites in Kiambu County

(Figure courtesy of Albert K.I., 2011)

Fish were randomly sampled from Machakos county (figure 3.3)

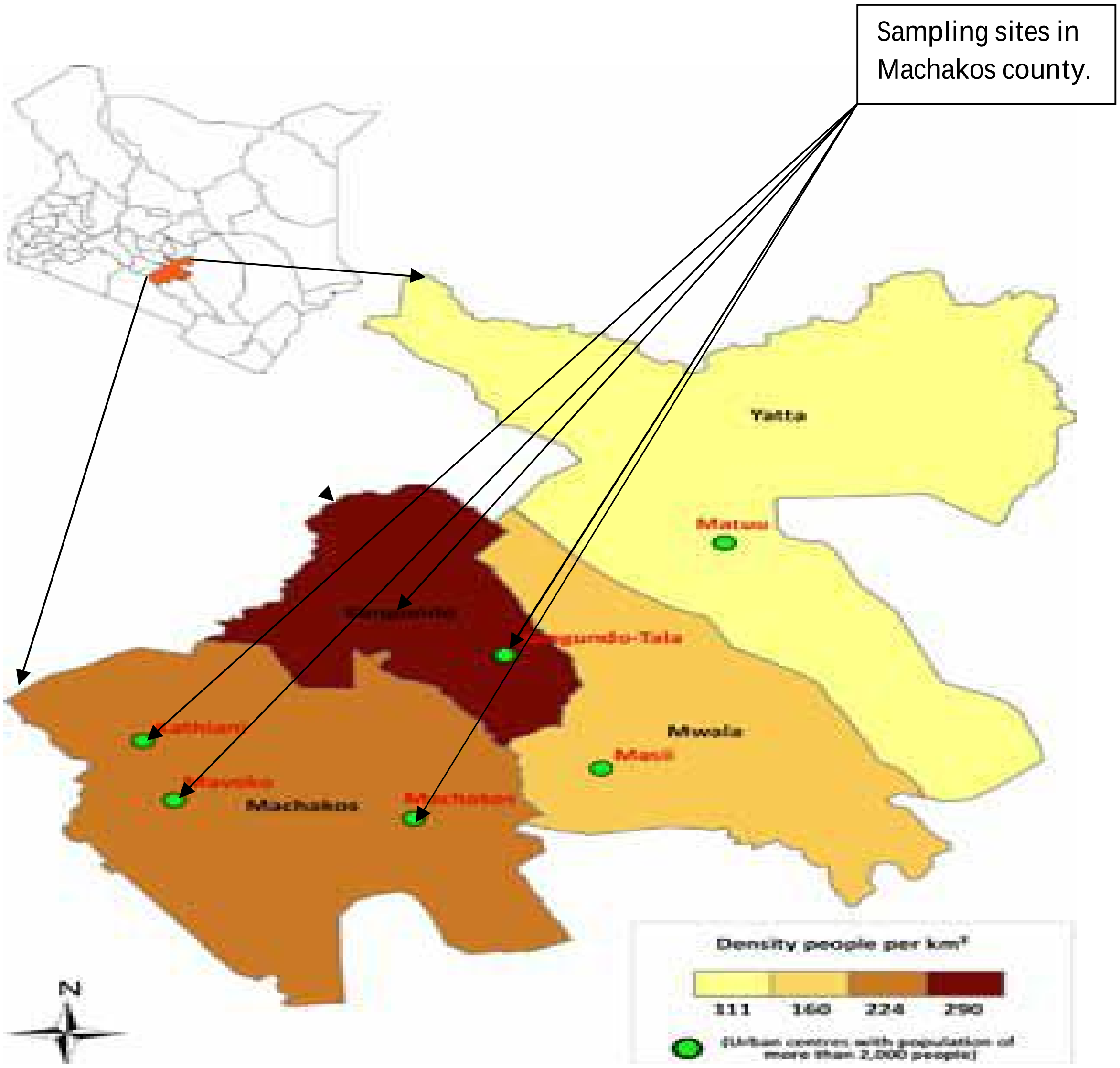


Figure 3. 3: Map of Machakos County showing sampling sites. **(Figure courtesy of Albert K.I., 2011)**

3.2 Sample collection and handling

Individual fish were sampled by net from fish ponds.

Sample size determination was done using the Fisher *et al., (*1991) formula:

$$N = \frac{Z^2pq}{d^2}$$

N-Desired Sample size

p- Proportion in the large population estimated to contain pesticides and heavy metal residues (probability) .

q- Expected contaminated proportion (1-p) (1-0.5) =0.5

z- The standard normal deviation set at 1.96 of the 95% confidence interval.

d- The degree of accuracy desire set at 0.05 significance

Therefore:

$$N = \frac{1.96^2 (0.5 \times 0.5)}{(0.05)^2} = 384$$

Multistage sampling was then conducted whereby two divisions were purposively picked in each county followed by two locations randomly picked in each division. Two sub-locations were picked randomly in which two ponds were randomly picked. A total of 48 ponds were sampled from which 8 fish were randomly picked. However, since there is high correlation in fish in one pond owing to homogeneity of aquatic environment, only 4 fish was randomly picked from each pond.

Following euthanasia, dissection of fish was carried out and livers, brain, muscle and gonads collected. All tissues were packaged using an aluminium foil on ice and immediately transported to the Department of Public Health, Pharmacology and Toxicology. Tissues were then weighed and frozen at - 20^{o} C until analysis for metals and organic compounds was done.

3.3 Analytical procedure.

All glassware were washed in nitric acid solution and rinsed with distilled water. All reagents used during analysis were of analar grade.

3.3.1 Heavy metal Analysis.

3.3.2 Fish sample extraction for heavy metals.

The bench was cleaned with soap and water and rinsed with acetone. Foil paper was spread on the bench. Frozen fish samples were placed on top and thawing allowed to take place. The scales on the fish samples were removed using sterile surgical blades. The sample code was noted and recorded. Following dissection, the muscles, livers, brain and gonads were oven dried at 100^{0}c for 24 hours and then weighed and ground.

The technique used for the separation of and isolation of lead and cadmium was the method from the journal of Association of Analytical Chemists (JAOAC, 1975), which involved the use of nitric acid (70%) and perchloric acid (60%) at the ratio of 3:1. The technique was modified to exclude the following: 10 milliliters of 5% Lanthanum solution during the transfer of the cooled sample to a 50ml volumetric flask; 20 mls of a mixture of nitric acid (70%) and 60 % Perchloric acid in the ratio of 3:1 instead of dissolving the sample in 10 mls of 70% nitric acid until it soaks followed by addition of 3 mls of 60% Perchloric acid; two gram sample were used instead of one gram sample (J AOAC, 1975).

Two grams of each sample were weighed using electronic weighing balance into a 50 ml Pyrex beaker. Twenty milliliters of the mixture containing three parts of nitric acid and one part of perchloric acid was added to the sample. The sample contents in the 50 ml pyrex beaker were left to digest for 12 hours in the fume chamber. The digested sample contents were heated on a sand bath inside a fume chamber. The hot plate heating the sand bath was set at 100^0C at first, and the sample contents heated slowly until frothing.

The sample was heated at 120^0C until white fumes of perchloric acid were produced. At this point, the metals under investigation had separated from the organic matter. The wet oxidized samples were then allowed to cool and then transferred to polypropylene tubes where they were diluted in ultrapure milliQ water to the 50 mls mark. Sample blanks were prepared in the similar manner to the test samples for background correction. Standard solutions for Cd and Pb were prepared from stock solutions (100 ppm). Stock solutions for each metal were prepared by dissolving 1g of pure lead and cadmium metal strip in 1:1 nitric acid and diluted to 1 liter to give a stock solution of 1000 ppm. For each of the stock solutions working standards of 1 ppm, 5 ppm, and 10 ppm working standards were made and calibration curves plotted.

The concentration of lead and cadmium were analyzed using an atomic absorption spectrometer (Spectr AA.10,Varian Techtron Pty Limited) using air-acetylene flame with appropriate hollow cathode tubes using element-specific lamps. Metal concentrations were calculated using a standard curve generated using 1ppm, 5ppm, and 10ppm, and the results were expressed in µg kg^{-1} of dry tissue weight.

3.3.3 Determination of lead and cadmium concentration in fish

The samples were analyzed for lead and cadmium using AAS-Specter AA-10 Varian at the Department of Mines and Geology laboratories.

Aliquots of the filtrates after digestion were used to estimate the concentration of Pb and Cd using Atomic Absorption Spectrophotometer AAS model- Specter AA-10 Varian mounted with Pb and Cd hollow cathode lamps. Calibration of the instrument was carried out using standard solutions that were prepared from commercially available materials. Calibration curves were generated using standards at series of 1ppm, 5ppm and 10 ppm after which the samples were analyzed.

For the analysis of lead detection wavelength was set at 217 nm, the spectral band width at 0.1nm and the fuel acetylene flow rate at 1.5 liters per minute. The lamp current was set at 0.1 amps.

For analysis of Cadmium the detection wavelength was set at 228.8 nm, the spectral band width at 0.5nm and the acetylene flow rate at 1.5 liters per minute. The lamp current was set at 5 amps

3.4 Pesticide Analysis

3.4.1Preparation of Standard Solution

A working stock solution of 1:1000 was prepared using the following procedure.

All the apparatus were placed on a foil paper to minimize contamination. Ten ml volumetric flask and forceps were triple rinsed with n-hexane. The 1 ml ampoule with the pesticide mixture was opened after breaking the seal and the content put in a 10 ml volumetric flask. The ampoule with the standard was rinsed five times with n-hexane. This was topped up to 10 mls mark with n-hexane and mixed using a sonicator, capped and then labeled as the 1:10 stock solution.

1ml was pipetted from the 1:10 stock solution using a 500 µl pipette twice and put in a 10 mls volumetric flask and topped up to 10 mls mark with n-hexane, mixed using a sonicator, capped and labeled as the 1:100 stock solution.

1 ml of the 1:100 stock solution was prepared using a 500 µl pipette twice and put in a 10 mls volumetric flask and topped up to 10 mls mark with n-hexane, mixed using a sonicator, capped and labeled 1:1000 working solution.

3.4.2 Cleaning of apparatus

All glassware, mortar and pestles, centrifuge tubes, pipettes and beakers were put in hot water with soap and thoroughly washed with a brush and scotch brite and then rinsed with running tap water to remove all the soap. A further rinsing was done using distilled water followed by final rinse with acetone. The apparatus were then put in the oven to dry at 40^0C.

3.4.3 Fish Sample Extraction

The bench was cleaned with soap and water and rinsed with acetone. Foil paper was spread on the bench. The frozen fish samples were placed on top and thawing allowed to take place. The scales on the fish samples were removed using sterile surgical blades. The sample code was noted and recorded. Dissection was then done to isolate the muscles, livers, brain and gonads.

Extraction of the solid tissues (liver, brain muscle and gonads) was done according to Kanja *et al*, (1986). Three g of each sample was ground together with 4.4 g of acid washed sea sand and 4.5 g of anhydrous sodium sulphate(Na_2So_4).Acid washed sand was used to help crush tough tissues while anhydrous sodium sulphate was used to absorb water.

The pesticide in 4 g of the homogenate was extracted through a glass column with diethyl ether into a weighed centrifuge tube. The eluent was evaporated in a sand bath at 50 ^{0}C. After the tube dried, the sand adhering to the side of the tube was wiped away and the tube weighed. The weight of the fat was obtained by subtracting the weight of the tube.

3.4.4 Clean up

All the glassware apparatus were rinsed with n-hexane for both acid and base clean up. The extract was then dissolved in hexane to make a concentration of 0.05g fat/ml.

3.4.4.1 Acid cleanup

Two hundred millilitres of concentrated sulphuric acid was pre-cleaned with n-hexane. The acid and n-hexane was put in to a separatory funnel, shaken vigorously and then allowed to settle. The acid was removed and washed with another 10 mls of n-hexane. Concentrated sulphuric acid was washed for three rounds then put into an acid dispenser. Two milliters of the cleaned concentrated sulphuric acid was added to 1ml of the sample extract in a 10ml centrifuge tube with a glass stopper and the mixture shaken gently and allowed to stand for one hour in darkness. The mixture was then centrifuged at 3000 revolutions per minute for 10 minutes. The supernatant was drawn using a pre-cleaned Pasteur pipette and transferred in to a 7.5 ml vials with Teflon lined cup and labeled for analysis by Gas Liquid Chromatography.

3.4.4.2 Base clean up

A solution of sodium chloride and orthophosphoric acid was prepared by mixing 11.6 gms of sodium chloride and 6.83 mls of 85% orthophosphoric acid and made up to a liter with distilled water. Two (2) pellets of potassium hydroxide were put into 10 mls centrifuge tube with a glass stopper and 0.1ml of distilled water added. The pellet was allowed to dissolve after which1ml of 99.5% ethanol was added. The remaining 1 ml of the sample extract, after acid clean, was added, shaken gently and placed on a sand bath at 50^0C for 30 minutes then cooled in fridge. Five (5) mls of sodium chloride and orthophosphoric acid solution was added and mixed vigorously. This was then centrifuged at 3000 revolutions per minute for 10 minutes. The supernatant was drawn using a Pasteur pipette and transferred into 7.5 mls vial with lined cup and labeled for analysis by Gas Liquid Chromatography.

3.4.4.3 Identification and quantification of Organochlorine pesticides in fish using Gas Liquid Chromatography.

The type of column that was used is Zebron ZB – 170 IP with the length of 30 meters and internal diameter of 0.25mm X 0.25µm, part no.7HG – G012 -11.The oven program used was 100^0C to 200^0C @ 25^0C / Minute to 240^0C @ 6^0C /minute to 265^0C @ 20^0C/minute for 5min.

The carrier gas was Nitrogen 99.9% pure at a flow rate of 1.6 ml/minute. The detector used was electron capture detector (ECD) at a temperature of 300^0C and injection was splitless at 240^0C and 1 µl of the anylate was injected.

The column, at room temperature was installed in the oven. The carrier gas was then connected and the GC switched on after setting the detector temperature at 300^0C.The Gas chromatograph was left running overnight in order to condition. On the following day 1µl of the Chlorinated Pesticide Mixture (CPM) standard was injected and produced the chromatogram of thirteen OC. Injection of the standard was repeated and reproduced the same chromatogram. 1 µl of the sample extracts was injected and the chromatogram recorded.

3.5 Data recording and Analysis

The data was entered into MS Excel spreadsheet and exported to the statistical package, SAS. The concentration of pesticides and heavy metals were analyzed by descriptive statistics and two way analysis of variance to compare the obtained values and the Minimum residue levels permitted by FAO.

3.6 Toxicological Evaluation

For preliminary assessment of consumer exposure to pesticide residues in inland fish , the estimated daily intake (EDI) was expressed as a percentage of the acceptable daily intake (ADI). The calculations of EDI are described as below:

EDI (ng/kg body weight/day)

= fish consumption(g/kg body weight

= X residue (ng/g).

3.7 Questionnaire

A questionnaire was administered during sampling to obtain more information from the farmers about the general practice in the fishing industry and the level of integration of farming methods and this was later be compared with analytical results from the lab. This helped to understand the use of pesticides and the presence of heavy metal residues and relate to the health and performance of fish.

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CHAPTER FOUR

RESULTS

4.1 Levels of Lead and Cadmium in Fish samples from Kiambu County

4.1.1 Levels of Lead in fish organs from Kiambu County

One hundred and twelve (112) organ samples were analyzed for lead in Kiambu County in which 38(33.93%) were positive for lead. The results are given in table 4:1 below. 42.8 % of the brain samples were positive for lead and had a mean concentration of 31.31 ± 28.27ppm (range 4.07 - 84.3); 25% of the liver sample had a mean concentration of 17.33 ± 20.64.32 ppm (range 2.71 - 54.2); 14% of Gonad had a mean concentration of 16.62 ± 15.42 (range 0.08 - 29.69) and 35.71% muscle had an average mean of and 3.78 ± 2.22 ppm (range 0.21 - 6.58) as shown in table 4.1 below;

Table 4. 1: Mean concentration of lead (mg/Kg) ± SD in different body tissues of fish farmed in Kiambu County

Sample	N	No. of Positive	Percentage positive	Mean ± SD (Positive)	Range (Positives)
Muscle	28	10	35.71	3.78 ± 2.22	0.21 - 6.58
Liver	28	7	25	17.33 ± 20.64	2.71 - 54.27
Gonad	28	9	32.14	16.62 ± 15.42	0.08 - 29.69
Brain	28	12	42.86	31.31 ± 28.27	4.07 - 84.3
Total	112	38	33.93		

In Kiambu County, the concentration of lead in brain was highest followed by the liver, gonad and muscle in that order. The levels of lead in all tissues in Kiambu County ranged from 0.08-84.3 mg/kg. with mean levels (mg/kg) of 31.31, 17.33, 16.62 and 3.78 for brain, liver, gonad and muscle respectively.

4.1.2 Levels of Cadmium in fish organs from Kiambu county

Eighty eight (88) organ samples from Kiambu County were analyzed for cadmium. Out of the brain samples analyzed 31.82% were positive and had a mean concentration of 7.25± 6.59 ppm (range 0.98-16.25) while 40.91% of liver samples were positive with a mean concentration of 5.35 ± 6.12ppm (range0.59-16.53) .Gonad and muscle (31.82% and 40.91%) had a mean concentration of 3.35 ± 4.16 (range 0.41-9.18) and 1.66 ± 2.48 ppm (range 0.29-5.68) respectively as shown in the table 4.2 below.

Table 4. 2: Mean Concentration of cadmium (mg/Kg) ±SD in different body tissues of farmed fish in Kiambu County

Sample	N	No Positive	Percentage Positive	Mean ± SD	Range
Muscle	22	9	40.91	1.66 ± 2.48	0.29-5.68
Liver	22	9	40.91	5.35 ± 6.12	0.59-16.53
Gonad	22	7	31.82	3.35 ± 4.16	0.41-9.18
Brain	22	7	31.82	7.25± 6.59	0.98-16.25
Total	88	32	36.36		

The mean concentration of cadmium was higher in the brain (7.25 mg/Kg) than in other tissues. The lowest levels were found in muscle (1.66 mg/Kg) .

4.2: Levels of lead and cadmium in fish organs from Machakos County

4.2.1 Levels of Lead in fish organs from Machakos County

In Machakos County, 117 organ samples were analyzed for lead out of which 36(30.77%) were positive for lead.38.46% of brain samples and 38.46% of liver samples had an average mean concentration of 30.40± 20.56 and 7.88±7.25 ppm respectively. On the other hand, 26.92% and 23.08% of gonad and muscle organ samples had an average concentration of 13.29±14.28 and 12.22± 22.96 respectively as shown in table 4.3 below.

Table 4. 3: Mean concentration of lead (mg/Kg) ± SD in different body tissues of farmed fish in Machakos County

Samples	N	No. Positive	Percentage Positive	Mean ± Std. Dev (positive)	Range (positive)
Muscle	39	9	23.08	12.22±6.58	1.09- 22.96
Liver	26	10	38.46	7.88±7.25	0.29-21.67
Gonad	26	7	26.92	13.29±14.28	1.57-41.79
Brain	26	10	38.46	30.40±20.56	2.33-65.14
Total	117	36	30.77		

The mean concentration of lead was highest in the brain (30.40 mg/Kg) while the lowest levels were found in the liver (7.88 mg/Kg).

4.2.2 Levels of Cadmium in fish organs from Machakos county

In Machakos county 22.72 and 22.73% of brain and liver samples contained cadmium with an average concentration of 3.91±5.84 and 2.90±3.58 respectively. 22.73% and 36.36% of gonad and muscle samples had a mean concentration of 1.25±1.38 and 1.12±1.13 respectively.

Table 4. 4: Mean concentration of cadmium (mg/Kg) ±SD in different body tissues of farmed fish in Machakos County

Sample	N	No Positive	Percentage Positive	Mean ± Positive (positive)	Range (positive)
Muscle	22	8	36.36	1.12±1.13	0.25-3.67
Liver	22	5	22.73	2.90±3.58	0.58-3.32
Gonad	22	5	22.73	1.25±1.38	0.1-15.5
Brain	26	6	22.72	3.91±5.84	0.21-3.85
Total	92	24	26.09		

The highest concentration of cadmium was observed in the brain followed by the liver, gonad and muscle. Cadmium levels in Machakos County ranged from 0.1 – 15.5 mg/kg with a mean concentration (mg/kg) of 3.91, 2.90, 1.25 and 1.12 in the brain, liver, gonad and muscle respectively.

In general, higher concentration of lead and cadmium were recorded in the brain and liver followed by the gonad and muscle. County wise, Kiambu recorded higher mean concentrations compared to Machakos County.

The highest concentration of lead and cadmium was detected in the brain in both counties as shown in table 4.5 below.

Table 4. 5: Mean Concentration (mg/Kg) ±SD of lead and cadmium in different paired organ samples from Kiambu and Machakos Counties

pairs	Organs	N (positive)	Lead(mg/Kg)	Cadmium(mg/Kg)
Pair 1	[a]Muscle	10	3.78 ± 2.22	1.66 ± 2.48
	[b]Muscle	9	12.22±22.96	1.12±1.13
Pair 2	[a]Liver	7	17.33 ± 20.64	5.35 ± 6.12
	[b]Liver	9	7.88±7.25	2.90±3.58
Pair 3	[a]Gonad	9	16.62 ± 15.42	3.35 ± 4.16
	[b]Gonad	7	13.29±14.28	1.25±1.38
Pair 4	[a]Brain	12	31.31 ± 28.27	7.25± 6.59
	[b]Brain	10	30.40±20.56	3.91±5.84

[a]organs collected from Kiambu County.

[b]Organ collected from Machakos County.

4.3 Organochlorine pesticides

A total of 213 samples were analyzed for organochlorine pesticide residues. Out of this, 125 samples were from Kiambu County while 88 were from Machakos County. In Kiambu County, 30 (24%) were muscle samples, 31 (24.8%) liver samples, 33 (26.4%) gonad samples and 31 (24.8%) brain samples. The samples from Machakos county comprised of 22 (25%) muscle samples, 22 (25%) liver samples, 21 (23.9) gonad samples and 23 (26.1%) brain samples. The results are given in tables 4.6 and 4.7.

4.3.1 Levels of Organochlorine pesticides in fish from Kiambu County

One hundred and twenty five (125) organ samples from Kiambu County were analyzed for organochloride pesticides. 30 (24%) were muscle samples, 31 (24.8%) liver samples, 33 (26.4%)gonad samples and 31 (24.8%) brain samples. The following organochlorine pesticides were identified; BHC group, the cyclodiens aldrin, heptachlor, dieldrin, endrin and the DDT group of compounds.

pp – DDT and its metabolite pp-DDD were the only compounds detected in all the four tissues i.e. muscle, liver, gonad and brain. They ranged between not detectable (limit of detection =0.001 μg Kg^{-1}) to 0.916 μg Kg^{-1} for pp-DDT and not detectable to 1.684 μg Kg^{-1} for pp-DDD . The other compounds were found in one, two or three of the tissues analyzed.

O,p' -DDT had the highest concentration of 2.098±4.097 μg Kg^{-1}in the brain followed by *p' p*-DDD 1.684±3.666 μg Kg^{-1}in the brain tissue. This was followed by *p,p'*-DDD in muscle at 0.916±1.287 μg Kg^{-1} and *p' p*-DDT at 0.916±1.916 μg Kg^{-1} as shown in table 4.6 below.

Table 4. 6: Mean concentration of Organochlorine pesticide levels (µg Kg^{-1}) ±SD in various fish organs in Kiambu County

Organochlorine	Muscle	liver	Gonad	brain
α-BHC	ND	0.236±0.266	0.383±0.15	0.025±0.024
ɤ-BHC	0.072±0.011	0.013±0.033	0.169±0.45	0.022±0.046
ß-BHC	ND	ND	ND	ND
Heptachlor	0.067±0.08	ND	ND	ND
Aldrin	ND	ND	ND	0.172±0.241
Heptachlor epoxide	ND	0.018±0.025	0.003±0.002	0.038±0.068
pp-DDE	0.001	ND	0.017±0.016	0.028±0.034
Dieldrin	0.071±0.075	0.086±0.089	0.012±0.073	0.063±0.083
op-DDD	0.032±0.043	0.015	0.016±0.015	ND
Endrin	0.04±0.05	ND	ND	ND
op-DDT	ND	0.02±0.024	0.116±0.254	2.098±4.097
pp-DDD	0.916±1.287	0.151±0.314	0.116±0.254	1.684±3.666
pp-DDT	0.916±1.916	0.151±0.314	0.274±0.648	0.122±0.220

ND: Not Detected/below detection limit

Lindane had the highest frequency of occurrence and was detected in 36 samples (16.9%) with a mean concentration of 0.0723 ± 0.011, 0.013± 0.033, 0.169±0.45 and 0.022±0.046 in muscle, liver, gonad and brain respectively in Kiambu county.

P,p'-DDT ranged between not detectable to 0.916 µg Kg^{-1} with a mean concentration of 0.916±1.916, 0.151±0.314, 0.274±0.648 and 0.122±0.220 in muscle, liver, gonad and brain respectively in Kiambu county.

o,p'-DDT that was detected in 25(11.74%) samples and ranged between not detectable to 2.098 µg Kg^{-1} with a mean concentration (µg kg^{-1}) of 0.02±0.024, 0.116±0.254 and 2.098±4.097 in liver, gonad and brain respectively in Kiambu county.

Dieldrin was detected in 24 (11.27%) samples and ranged between not detectable to 0.086 µg Kg^{-1} with a mean concentration (µg kg^{-1}) of 0.071±0.075, 0.086±0.089, 0.012±0.073 and 0.063±0.083 in muscle. liver, gonad and brain respectively.

α-BHC was detected in 19 samples (8.92%) and ranged between not detectable to 0.236 µg Kg^{-1} with a mean concentration(µg kg^{-1}) of 0.236±0.266, 0.383±0.15 and 0.025±0.024 in liver, gonad and brain respectively in Kiambu county. *o,p'*-DDD was detected in 16 samples (7.51%) and ranged between not detectable to 0.032 µg Kg^{-1} with a mean concentration of 0.032±0.043 and 0.016±0.015 in the muscle and gonad.

Heptachlor was in 11 samples (5.16%) with a mean concentration (µg kg^{-1}) of 0.067±0.08 in muscle and a range between not detectable to 0.067 µg Kg^{-1} . Heptachlor epoxide was detected in 11 samples (5.16%) and ranged between not detectable to 0.038 µg Kg^{-1} and a mean concentration(µg kg^{-1}) of 0.018±0.025, 0.003±0.002 and 0.038±0.068 in liver, gonad and brain in Kiambu county.

p,p'-DDE was in 10 samples (4.69%) and ranged between not detectable to 0.028 µg Kg^{-1} and a mean concentration(µg kg^{-1}) of 0.017±0.016 and 0.028±0.034 in the gonad and brain. Aldrin was detected in 3 samples (1.41%) with a mean (µg kg^{-1}) of 0.172±0.241 in the brain in Kiambu county.

4.3.2 Levels of Organochlorine pesticides in fish organs from Machakos County.

Out of the eighty eight (88) samples analyzed from Machakos county, 22 (25%) were muscle samples, 22 (25%) liver samples, 21 (23.9) gonad samples and 23 (26.1%) brain samples.

Lindane, pp-DDE, pp-DDD and pp-DDT were the only pesticides detected in all the four samples i.e. muscle, liver, gonad and brain. The other compounds were found in one, two or three of the tissues analyzed.

pp- DDT had the highest OC concentration and ranged between not detectable (detection limit = 0.001 µg Kg^{-1}) to 0.158 µg Kg^{-1} followed by pp-DDD that ranged between not detectable (detection limit = 0.001 µg Kg^{-1}) to 0.097 µg Kg^{-1} as shown in table 4.7 below.

Table 4. 7: Concentration of Organochlorine pesticide (µg Kg^{-1}) ±SD levels in various fish organs in Machakos County

Organochlorine	Muscle	liver	Gonad	brain
α-BHC	ND	ND	0.013±0.19	0.011
ɣ-BHC	0.013±0.008	0.073±0.01	0.017±0.029	0.029±0.041
β-BHC	ND	ND	ND	ND
Heptachlor	ND	0.014±0.016	ND	ND
Aldrin	ND	ND	ND	0.035
Heptachlor epoxide	ND	0.02±0.005	ND	0.05
pp'-DDE	0.004	0.035±0.018	0.037	0.014±0.15
Dieldrin	0.057±0.08 1	ND	ND	0.009
op-DDD	0.051±0.056	0.132	0.046±0.015	ND
Endrin	ND	ND	ND	ND
op-DDT	ND	0.016±0.016	0.033±0.016	0.029
pp-DDD	0.003±0.004	0.034±0.009	0.032±0.016	0.097±0.012
pp-DDT	0.005	0.158±0.183	0.025±0.019	0.024±0.033

ND: Not Detected/below limit of detection.

.Lindane had the highest frequency of occurrence (16.9%) followed by pp –DDT (13.15%), *p,p'*-DDD (12.68%), *o,p'*-DDT (11.74%), Dieldrin (11.27%), α-BHC (8.92%), Heptachlor epoxide (5.63%), Heptachlor (5.16%), *p,p'*-DDE (4.69%), Aldrin (1.41%) and Endrin (0.94%) in that order.

Lindane residue levels ranged between not detectable to 0.073 µg Kg^{-1} and had a mean concentration (µg kg^{-1}) 0.013±0.008, 0.073±0.01, 0.017±0.029 and 0.029±0.041 in the muscle, liver, gonad and brain respectively in Machakos county.

p,p'-DDT residue levels ranged between not detectable to 0.158 µg Kg^{-1} and was detected in 28(11.74%) of the samples with a mean concentration(µg kg^{-1}) of 0.005, 0.158±0.183 , 0.025± 0.019, 0.024±0.033 in the muscle, liver, gonad and brain respectively.

p,p'-DDD residue levels ranged between not detectable to 0.097 µg Kg^{-1} and had a mean concentration of 0.003±0.004, 0.034±0.009, 0.032±0.016 and 0.097±0.012 in muscle, liver, gonad and brain respectively.

o,p'-DDT residues were detected in the liver and the gonad with a mean concentration of 0.016±0.016 and 0.033±0.016 respectively. The residue levels ranged between not detectable to 0.033 µg Kg^{-1}.Dieldrin residues were detected only in the muscle tissue with a mean concentration of 0.057±0.081.α-BHC residues were present in the gonads with a mean concentration of 0.13±0.19 µg Kg^{-1}.

o,p'-DDD had a mean concentration of 0.051±0.056 and 0.046±0.015 in muscle and gonad in Machakos county and ranged between not detectable to 0.051 µg Kg^{-1} . In Machakos county Heptachlor was present in the liver with a mean concentration of 0.014±0.016 .Heptachlor epoxide had a mean concentration of 0.02±0.005 while *p,p'*-DDE a mean concentration of 0.035±0.018 and 0.014±0.015 µg Kg^{-1} . Endrin residue levels were detected in 2 samples (0.94%) with a mean concentration (µg kg^{-1}) of 0.04±0.05.

4.4 Fish diseases

Majority of farmers 84% reported no massive die offs on the ponds. However, 16% of the farmers noted that they observed die off usually after stocking and immediately after harvesting. The farmers observed gulping of air on the surface of the water as the main symptom observed which may suggest lack of enough oxygen disease or respiratory distress. Only one case in

Kikuyu constituency, Kiambu County was reported to have been due to extensive use of Bestox® (Alphacypermethrin) insecticide on vegetable farm around the ponds.

4.5 Fish production cycle and systems

From the questionairre, average production cycle is 9.36 months in Kiambu and 6.88 months in Machakos. This difference in growth rate could be due to differences in water temperatures or the level of contaminants since harvesting in the two counties is informed by size of the fish. This is also supported by the fact that 31% of the farmers in Kiambu County harvest after 12 months while in Machakos 53% of the farmers harvest at 6 months of age.

The most common fish farming system is semi intensive with Kiambu county having 96% of farmers practicising this type of production system and 80% in Machakos county .

CHAPTER FIVE

DISCUSSION

5.1 Levels of lead in fish samples collected from Kiambu and Machakos Counties

In the current study, lead levels in all tissues in Kiambu county ranged from 0.08- 84.3 mg/kg with mean levels (mg/kg) of 31.31, 17.33, 16.62 and 3.78 for brain, liver, gonad and muscle respectively. In Machakos county, the levels of lead ranged from 0.29 – 65.14 mg/kg with a mean concentration (mg/kg) of 30.40, 13.29, 12.22 and 7.88 for brain, gonad, muscle and liver respectively. These levels exceeded the maximum permissible limits stipulated by Food and Agricultural Organization and World Health Organization (1992) of 0.5 parts per million (ppm) for lead.

The elevated levels of lead observed in the present study are comparable to those reported earlier by Kishe and Machiwa (2003) who reported lead levels of 30.7±5.6 in sediments of Mwanza gulf of lake Victoria, Tanzania. Hounkpatin *et al*., (2012) in a similar study reported slightly lower levels of lead. (26.80 ± 0.57 ppm).

Fish is among the dominant bioindicator species used for acute toxicity assay of pollutants such as heavy metal since much attention has been drawn due to the wide occurrence of metal pollution in aquatic system. The rapid development of industries and agriculture have promoted the increase of environmental pollution although heavy metals in aquatic system can be produced naturally by slow leaching from rocks and soil into water which occurs at low levels. Cadmium (Cd) and lead (Pb) are among the aquatic metal pollutants which usually present at significant levels in water system which pose high toxicities on the aquatic organisms, (Zhou *et al.* 2008).

Different levels of lead were detected in different tissues and this was due to the fact that the concentration of metals depends on species, sex, biological cycle and the portion of the fish analyzed. Moreover, ecological factors such as season, place of development, nutrient availability, and temperature also cause inconsistency of metal concentration in fish organs, (Tuzen, 2003).

The results confirmed the differences of lead accumulation in different tissues.

The current results indicate that the contents of heavy metals are much more in the brain , liver and gonad than in the muscles. Jobling (1995) attributed the high accumulation of heavy metals in brain and liver tissues to the metallothionein proteins which are synthesized in the tissue when fish are exposed to heavy metals to detoxify them. These proteins are thought to play a role in protecting tissues from damage by heavy metals. Moreover, fish liver plays an important role in the metabolism and excretion of xenobiotic compounds, (Rocha and Monterio, 1999).The presence of free protein-thiol group in these tissues also forms strong fixation with heavy metals, (Iwegbue 2008).

The metallothioneins found within the brain tissues are not as inducible as compared to the metal binding proteins found in fish kidney and liver. Moreover, the presence of blood brain barrier in fish brain serves to protect the vulnerable brain tissues from toxic metal perturbations which further prevents fish against neurotoxicity effects. This further supported the presence of Cd and Pb being detected in the fish brain and liver, (Filipovic and Raspor, 2003; Marijic and Raspor, (2007).

Fish flesh muscle is the edible part of fish and frequently employed in assessing human health risks in relation to fish consumption. Karadede & Unlu, (2000) and Khan *et al*., (1989) mentioned that muscle is not an active tissue in accumulating heavy metals. Similar observations were reported by many studies carried out with various fish species (Alam *et al*, 2002).

The presence of mucous layer coating the fish skin surface serves as a barrier which protects the integrity of fish muscle tissues from surrounding contaminants. It serves as the first line of defense against the entrance of heavy metals into fish flesh muscle tissues by forming complexes with the heavy metals. Therefore the muscle tends to bioaccumulate lesser metals compared to the other fish organs, (Schlenk and Benson, 2001; Altindag and Yigit, 2005; Uysal *et al*., 2008)

5.1.2 Levels of Cadmium in fish samples collected from Kiambu and Machakos counties

In the present study, cadmium levels in Kiambu County ranged from 0.29- 16.53 mg/kg with a mean concentration (mg/kg) of 7.25, 5.35, 3.36 and 1.66 for brain, liver, gonad and muscle respectively while in Machakos county Cadmium levels ranged from 0.1 – 15.5 mg/kg with a mean concentration (mg/kg) of 3.91, 2.90, 1.25 and 1.12 in brain, liver gonad and muscle respectively. This levels exceeded the maximum permissible limits stipulated by Food and Agricultural Organization and World Health Organization (1992) of 0.05 parts per million (ppm) for cadmium.

The high cadmium levels observed in the present study are comparable to those reported earlier by Kishe and Machiwa (2000) who reported lead levels of 7.0± 0.2 ppm in sediments of Mwanza gulf of lake Victoria, Tanzania and even higher than the ones reported previously by Hounkpatin *et al*., (2012) (1.79 ± 0.29 ppm for Cd.)

Concentration levels of lead and cadmium in fish are compared to the permissible limits stipulated by Food and Agriculture Organization and World Health Organization.(FAO/WHO, 1992) (table 5.1)

Table 5. 1: Guidelines on heavy metals permissible limits in ppm for food safety

Organization	**permissible limits**	
	Cadmium	Lead
Food and Agriculture Organization/ World Health Organization (1992)	0.05	0.5

Although there were no reported toxic effects by the respondents, the possibility of harmful effects cannot be ruled out after a long period of consumption of farmed fish in the two counties. Accumulation of metal toxicants from the aqueous environment by fish depend upon the availability and persistence of the contaminant in water and food thus, the less available it is the less it will be accumulated, Larsson *et al*., (1985). Heavy metals have a tendency to accumulate in various marine organisms especially fish which in turn may enter into human metabolism through consumption of the fish causing serious health hazards,(Kamaruzzaman *et al*. 2011).

In this study, the levels of Pb and Cd was generally higher in the analyzed organ samples from Kiambu County compared to the Machakos County . However, statistically there was no significant difference in the tissues analyzed for the concentration of Pb in the two counties since pr/t/>0.05. Cadmium concentration in the gonad had a significant difference between Kiambu and Machakos County since pr/t/=0.05.The brain, liver and muscle did not show any significant difference in the concentration of Cadmium between the two counties since pr/t/ >0.05

The higher levels of lead and cadmium detected in organ samples from Kiambu County may be attributed to the high agricultural activities carried out in the county compared to Machakos County that is semi Arid.

Sediments are important sinks for various pollutants including metals and play a considerable role in the remobilization of trace metals in aquatic systems under suitable conditions and in interactions between sediment and water column, (Uzairu et al., 2009). The release of trace metals from sediments into the water body and consequently to fish depend on the chemical fractionation of metals and factors such as sediment pH, and the physical and chemical characteristics of the aquatic system,(Morgan and Stumn 1991).

The most likely sources of lead and cadmium maybe surface runoffs and drainage waters which enter into fish ponds and other water bodies like rivers, surface wells and uncovered bore holes which serve as water sources for fish ponds. Presence of faulty petrol powered water pumps and disposal of empty pesticide containers next to the water sources may have contributed to the load. Lead is not an environmentally mobile metal and is often heavily bound to suspended particulate and sediment material, (Berg et al.,1995). However, according to Bryn and Langston (1992), tilapias are fast growing and short lived fish that are primarily herbivores and feed on water plants especially duckweed.

5.1.3 Organochlorine Pesticides in fish collected from Kiambu and Machakos Counties

The use of chemical pesticide is still indispensable in Kenya due to the hot and humid tropical environmental conditions that are conducive to the development of a myriad of pests, weeds and disease vectors. The public health sector in Kenya also depends heavily on pesticides to control vector – borne diseases such as malaria, sleeping sickness, bilharziasis and filariasis through pesticide spray program aimed at controlling disease vectors such as mosquitoes, tsetse-flies and water snails, Madadi *et al*. (2002). Studies have reported that only 5% of the sprayed chemicals actually reach the targeted organisms, with the remaining 95% drifting to surrounding areas and eventually becoming an environmental contaminant, Panagiotis and Weili (2008).

In this study, the organochlorine pesticide residues were generally higher in Kiambu county compared to Machakos County. This could be due to the fact that Kiambu county is a high potential area in agriculture and there is a possibility of OCs use in the past. Another reason could be leaching and surface run-off since 68% of the ponds in Kiambu County were earth ponds as compared to 45% in Machakos County. Further, 89.3% of land on which ponds were constructed in Kiambu County was previously used for growing crops compared to 86% in Machakos county.

High residue levels were detected in brain tissues in both counties followed by the gonads, liver and muscle. This could be attributed to the lipid content of the organs since organochlorine pesticides are lipophilic and tend to accumulate in tissues with high fat content that experience low turn-over rates, Borrell and Aguilar (2007).

Lindane and DDT and its metabolites had the highest frequency of detection in all samples analyzed. This is an indication that some farmers might be illegally using lindane. Lindane was initially used for seed dressing to protect crops against termites. However its agricultural use has been banned in the country due to persistence and toxicity to the untargeted organisms.

DDT and its metabolites were the second highest in frequency though no recent input from anthropogenic sources could be attributed to since *p ,p'*–DDD was much higher than the original compound *p, p'*-DDT. With a half life of 10-20 years in temperate regions, Sericano *et al*., (1990), DDT undergoes degradation to DDE and DDD. The DDE accounts for 50-70% of the DDT burden in the environment, Newsome and Andrews (1993).

Dieldrin was detected in 24 (11.27%) samples while Aldrin was detected in 3 samples (1.41%). Aldrin(1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8 dimethanonaphthalene) is metabolized to dieldrin .Based on the detected residues of dieldrin, which were higher than those for aldrin, the levels detected may be attributed to the previous use of aldrin and dieldrin in the two regions.

The residue levels reported in this study were lower than those reported in marine environment at the Kenyan coast by Wandiga *et al*., (2002), and Getenga *et al*., (2004) in Lake Victoria basin. The difference could be attributed to variations in geographical locations, time differences in terms of the period of study and the extent of previous use of these pesticides. DDT has been banned for agricultural use in Kenya but restricted for public health use.

In comparison to studies carried out in other countries, Mwevura *et al*., (2000) reported lower frequencies of organochlorine pesticide residues of *p,p'*-DDT(25%); *p,p'*-DDE (37%) during dry season, and higher frequencies of *p,p'*- DDT(81%); *p,p'*-DDE (100%); dieldrin (100%) and ΄Y-HCH (6%) in samples from the coastal area of Dar es Salaam, Tanzania. The concentrations of dieldrin and *p,p'*-DDD were notably higher than aldrin and *p,p'*-DDT, respectively, in most of the samples. Since the latter are their degradation products, this indicated possible transformation process taking place on *p,p'*-DDT and aldrin previously in the region. Organochlorines pose potential health hazard and therefore maximum residual limit has been recommended for human consumption by various agencies. FAO recommends a level of 300 µg kg^{-1} or 300 ng g^{-1} as maximum acceptable limit for DDT while Canadian limit is 500 µg kg^{-1}, Mwevura *et al*. (2002). (Table 5.2)

Table 5. 2: Permissible limits (ppm) for OCPs by various organizations

Pesticide	EC, (2001)	FDA, (2000)	FAO, (1983)
Heptachlor	-	0.3	0.3
Lindane	-	0.3	0.3
B-BHC	-	0.3	0.3
Endrin	-	0.3	0.3
Aldrin	-	0.3	0.3
pp-DDT	-	5.0	0.3

The FDA (2001) limits are 5000 µg g^{-1} for DDT and 300 µg g^{-1} for Aldrin, Dieldrin, chlordane, benzene hexachloride, heptachlor, heptachlor epoxide in edible portion of fish while National Academy of Sciences and National Academy of Engineering (NAS/NAE, 1972) recommends a limit of 1000 µg g^{-1} for dieldrin, endrin, heptachlor and chlordane. From a public health point of view, residue levels of organochlorines in all fish samples analyzed in this study are considerably lower than these recommended levels and therefore do not pose a health risk to the populace consuming fish from Machakos and Kiambu counties.

5.1.4 Effects of contaminants on health and performance of fish

In the current study, 16% of the farmers observed mortality after stocking and immediately after harvesting. The farmers observed gulping of air on the surface of the water as the main symptom observed which is an indication respiratory distress. This symptom is consistent with Ammonia Poisoning, Chilodonelloza, a disease that affects the gills and skin of fresh water fish caused by *Chilodonella uncinata* and Ich ,is a protozoal disease of freshwater fish caused by *Ichthyophthirius multifiliis*. Only one case of mass die-offs in Kikuyu constituency, Kiambu County was reported to have been due to extensive use of Bestox® (Alphacypermethrin) insecticide on vegetable farm around the ponds.

Disease occurrence in fish and in fish populations is thought to depend on the interaction of three variables. These variables, controlled by abiotic, biotic, and genetic factors, include the quality of the environment, differential susceptibility of individuals to the pathogen as a result of genetic predisposition or the physiological health of individual members of the host population, and the presence and virulence of the pathogen, (Snieszko 1973).

Chronic exposure to anthropogenic chemicals has been found to result in sub-lethal effects to the exposed species. Arkoosh *et al*. (1998) observed a population-level decline in wild salmon stocks as a result of modulation of the fish immune system and resultant increases in mortality. A number of studies have found that fish sampled from chemically contaminated environments show changes in phagocytic ability or respiratory burst activity when compared to fish from relatively clean sites, which could predispose these fish to increased susceptibility to infectious diseases (Lesley et al, 2002).

In the present study, the level of contaminants was relatively higher in Kiambu county compared to Machakos County. This could explain the fact that the average production cycle is 9.36 months in Kiambu and 6.88 months in Machakos. This difference in growth rate could be due to differences in water temperatures or the level of contaminants among other factors since harvesting in the two counties was informed by size of the fish. This is also supported by the fact that 31% of the farmers in Kiambu County harvest their crop after 12 months while in Machakos 53% of the farmers harvest at 6 months of age.

5.1.5 Calculation of estimated daily intake

Estimated daily intake is calculated as follows;

$$\text{Estimated daily intake} = \frac{\text{portion in Kg consumed /day X Residue level}}{\text{Bwt}}$$

$$\text{Lead in Kiambu county} = \frac{0.2\text{kgX}3.78\text{mg/Kg}}{60}$$

$$=0.0126\text{m/Kg bwt.}$$

The estimated daily intake (EDI) for cadmium in Kiambu county is 0.0055mg/Kg body weigh while that for lead and cadmium in Machakos County is 0.041 and 0.0037 mg/Kg body weight respectively.

On the other hand, the estimated dietary intake for organochlorine is 0.0031µg/kg body weight in Kiambu county and 0.00019 µg/Kg body weight in Machakos County.

The estimated daily intake for Lead and cadmium for Kiambu and Machakos counties calculated above are below the acceptable daily intake of 0.2 and 0.05 mg/kg (Official Journal of European Community, 2001)

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The levels of Pb and Cd in this study was generally high in the analyzed organ samples from Kiambu County compared to the Machakos County .However, there was no significant difference in the tissues analyzed for the concentration of Pb in the two counties since pr/t/>0.05. Cadmium concentration in the gonad was significantly different between Kiambu and Machakos County since pr/t/=0.05.The brain, liver and muscle did not show any significant difference in the concentration of Cadmium between the two counties since pr/t/ >0.05 .

The higher levels of lead and cadmium detected in organ samples from Kiambu County may be attributed to the high agricultural activities carried out in the county compared to Machakos County which is basically a semi arid region.

Lead levels in the liver and the brain were above the international permissible limits while cadmium levels were above the permissible limits in all tissue studied. It is however important to note that lead and cadmium residues were detected in only 40% of all samples analyzed. The residues were below the detection limit in 60% of the samples analyzed. More work needs to be done in this area to identify the actual source of these contaminants. Rearing fish in uncontaminated water, controlling industrial and agriculture effluents into surface water and proper sitting of ponds could minimize the risk of heavy metal contamination of farmed fish for improved human health.

In this study, the organochlorine pesticide residues were generally higher in Kiambu County compared to Machakos County. The OC contamination pathways into water bodies are likely to be nonpoint sources via runoff, atmospheric deposition and leaching due to past agricultural applications and vector control. The levels in this study could be due to the fact that Kiambu

County is a high potential area and there is a possibility of OCs use in the past. Another reason could be leaching and surface run-off since 68% of the ponds in Kiambu County were earth ponds as compared to 45% in Machakos County (Appendix 7). Further, 89.3% of land on which ponds were constructed in Kiambu County was previously used for growing crops compared to 86% in Machakos County.

High residue levels were detected in brain tissues in both counties followed by the gonads, liver and muscle. This could be attributed to the lipid content of the organs since organochlorine pesticides are lipophilic and tend to accumulate in tissues with high fat content that experience low turn-over rates.

Considering the previous and present use of some chlorinated hydrocarbons in agriculture and for vector control in East Africa in general, the levels of pesticide residues found in this study were very low. These low levels suggest a high degradation rate of OCs in the tropical environment.

It is important to note that the concentration levels recorded were those of the positive samples which formed only 40% of all the fish sampled. Sixty percent were negative and were not included in determination of the mean levels.

6.2 Recommendations

1. Establishment of monitoring systems by relevant authorities like NEMA, on handling and disposal of pesticides and pesticide containers at farm level with a view of minimizing contamination of surface waters.

2. Determination of levels of heavy metals, organochlorines residues and other pesticides in fish, soil and foodstuff should be carried out regularly in order to evaluate the possible risk to human health

3. Introduction of programmes aimed at training institutions in areas such as pesticide laws and regulations, handling chemical spills, storage of pesticides, pesticides in the environment, pesticide toxicity and health effects.

4.Educate people on safe surface water management to ensure people do not dispose empty pesticide container in the river, ponds or any other water bodies, draw water directly from surface water using apparatus or wash equipment used for pesticide applications in rivers or ponds in order to reduce contamination by pesticides and heavy metals,

5. Controlling industrial and agriculture effluents into surface water and proper sitting of ponds to minimize the risk of contamination of farmed fish by pesticides and heavy metals.

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APPENDICES

Appendix 1: Data acquisition Questionnaire

A questionnaire was administered to obtain more information from the farmers about the general practice in the fishing industry and the level of integration of farming methods and this was later be compared with analytical results from the lab. This will help understand the effect of pesticides and heavy metal residues on the health and performance of fish

UNIVERSITY 0F NAIROBI
PESTICIDES AND HEAVY METAL ANALYSIS QUESTIONNAIRE
SECTION A: QUESTIONNAIRE IDENTIFICATION *(To be completed before the start of the interview)*

NO.	QUESTIONS	ANSWER CATEGORIES
A.01	Serial number of the questionnaire	
A.02	Name of the enumerator	
A.03	County	
A.04	District	
A.05	Division	
A.06	Sub-Location	
A.07	Village	
A.08	Date of Interview	[ ]/[ ]/[2011]

SECTION B: HOUSEHOLD CHARACTERISTIC

S/ No.	Name	Relation to HH head (a)	Marital status (b)	Sex (c)	Age	Highest level of Education (d)	Main occupation current year (e)
B.01							

- **a)** 1-household head (hh), 2-spouse, 3-son or daughter, 4-father or mother. 5-grandchild, 6-grandparents, 7-other relative, 8-non-relative
- **b)** 1-single, 2-married, with spouse permanently present in the hh, 3-married with spouse migrant, 4-widow or widower, 5-divorced or separated
- **c)** 1-male, 2-female
- **d)** 1-in primary, 2-never completed primary, 3-completed primary, 4-technical school/polytechnic, 5-in secondary, 6-never completed secondary, 7-completed secondary, 7-in college/university, 8-completed college/university.

e) 1-self employed in agriculture, 2-self employed in non-farm enterprise, 3-student, 4-casual worker, 5-salaried worker, 6-domestic worker, 7-unemployed, looking for job, 8-unwilling to work or retired, 9-not able to work (handicapped)

SECTION C: RESOURCE UTILIZATION AND PRODUCTION

C.01	Approximate size of land (in acres)	Number of acres
C.02	In which year did you establish your first fish pond?	
C.03	Indicate the type of holding unit by inserting the total number per each category in the space provided.	Earth pond ☐ Raceway culture ☐ Tank culture ☐ Liner pond ☐ Concrete pond ☐ Others (Specify………………………………)

(C.04)	Date Established (C.05)	Size (C.06)	Species (C.07)	Source of water (C.8)	Ownership (C.9)
Pond 1					
Pond 2					
Pond 3					
Pond 4					

Species Codes	**Water source**	**Ownership Codes**
1=Tilapia monoculture 2=Cat/mud fish mono-culture 3=Tilapia and catfish poly-culture 4=Others (specify) *To accept local names.*	1=River 2=Well 3=Borehole 4=Dam 5=Others (specify)	1=Own 2=Leasehold 3=Freehold

C.10	How does water flow into your pond(s)?	Gravity ☐ Pumping ☐

		Others (specify)
C.11	How often do you top-up pond water?	Once in a week Once in a month When water falls below a certain mark Other (specify)

Feeds: <u>Natural feeds/homemade</u> (*Indicate type e.g. poultry feathers, kitchen wastes, sweet plants, blood-meal others)* <u>Supplementary feeds</u> *(Grain by-products e.g. cereal bran* <u>Complete feeds</u> (*Pellets)* Others (specify) *In case of manufactured feeds, to include the preferred choice of manufacturing company.*			

C.12	Please indicate other major existing enterprises in your farm.
C.13	Do you spray your crops or livestock? If yes, which type of chemical do you use? If no, leave blank.
C.14	Approximately how long have you been using the chemicals?
C.15	Have you ever witnessed massive die-offs in fish in your pond(s)? If yes, at what age of the fish?
C.16	Were the fish showing any abnormal signs before death? If yes, what were the signs?

C.17	Who are the MAIN buyers of your produce of the species you rear in your farm? 1=Consumers (neighbors/passersby) 2=Taking the nearby markets/vending 3=Local small scale traders 4=Middlemen/brokers from large towns, such as	

Species	Main significant buyer	2nd significant buyer

	Nkr, Nrb 5=Exporters 6=Processors 7=Large scale traders 8=Others (Specify ……………………………………………..) 9=None	

Appendix 2: Administration of the Questionnaires

The response rate was 100% since all farmers and agreed to be interviewed. However, in a few cases some farmers did not answer some questions

Appendix 3: Distribution of respondents by age, sex, and education level

Majority of the fish farmers were males (80% for Kiambu County and 74% for Machakos County). There was no significant relationship between the age of the household head and category of farmer in both counties (Table 4-1). The results indicate that fish is kept by a relatively large proportion of farmers who are below fifty years of age (61.5% in Kiambu and 69%) in Machakos counties respectively. Majority of the respondents (69.2 %) were self-employed in agriculture, while 8.0%, 12.8%, 4.4%, 2.8% and 2.8% were self-employed in non-farm enterprise, salaried worker, domestic worker, casual worker and unemployed respectively. This indicates the employment status of the respondents where only around 12.8% had formal employment and 86.8% as not. Both Kiambu and machakos counties had 6% of the farmers having not completed primary level of education. In both kiambu and machakos counties there was significant relationship between the category of farmer and level of education at $p < 0.05$ level.

Appendix 4: Total farm size and land utilization

The average land size was 1.8 hectares in kiambu and 2.5 hectares in Machakos county. Land use comprised of mainly crops and livestock production. The major crops grown were maize, beans, vegetables and fruits. Both counties had cash crops which included coffee and tea. In kiambu county 5.3% of the farmers grew nappier grass mainly as cattle feed for the zero grazed dairy cows. Vegetables are grown mainly for commercial purposes in Kiambu county and to a small extent in machakos county. Fruits are also grown in both counties but they form a bigger percentage 7% in Machakos county due to the production of grafted fruits especially mangoes. This shows intergration of fish farming into crop farming where water from the ponds is used for watering vegetable plots which are usually located near the ponds.

Pesticides used in the study area belonged to the chemical groups of organophosphates, Carbamates, pyrethroids , Ivermectin, Amitraz and neonicotinoid. The chemical groups most frequently used were the organophosphates(dimethoate and Steladone), carbamates (lenate) and Synthetic pyrethroids (Bestox, TataALpha,Alphatox and Karate).

Generally Kiambu county had higher pesticide usage than Machakos County as shown in the table below.

Table 4.8 : Chemicals used in the study areas.

		Kiambu county frequecy	Percent	Machakos county frequency	
Valid	foliar feed	6	4.0	17	17
	pesticides	26	17.3	21	21
	insecticides	45	30.0	25	25
	fungicides	18	12.0	37	37
	herbicides	9	6.0		
	n/a	46	30.7		
	Total	150	100.0	100	100

Appendix 5: Livestock enterprises

The main livestock species kept were cattle mainly dairy cows in Kiambu County,goats,poultry,pigs and rabbits. Cattle and goats formed 66.6% and 83% in Kiambu and Machakos counties respectively. This shows a higher degree of integration between fish farming and livestock keeping where manure from the livestock is used as manure in the ponds. Pigs and rabbits were only reported in Kiambu counties which is close to the city of Nairobi which provide ready market for pig and rabbit meat.

Farmers used acaricides to control ticks and other external parasites.Kiambu had higher incidences of acaricde usage at 94% compared Machakos 67%.The acaricides were mostly orgaanophosphates and carbamates.

Table 4.9: Acaricides used in the Study areas.

		Kiambu Frequency	Percent	Machakos Frequency	Percent
Valid	acaricides	141	94	67	67.0
	n/a	9	6	33	33.0
	Total	150	100.0	100	100.0

Appendix 6: Fish farming

Of the total fish farmers interviewed, 85.3% and 75% in Kiambu and Machakos districts respectively were Economic Stimulus Programme fish farmers meaning they were recruited by the government and labour, fingerlings and fish feeds were supplied by the government. The rest of the farmers 14.7% and 25% started fish farming on their own or have been doing it for a long time. This study also established that 89.3% and 80% of the fish pond in Kiambu and Machakos respectively were established in 2009 and 2010 and this the period when the Economic Stimulus Program had been rolled out.

Appendix 7: Fish holding structures

The most common type of pond is the earth pond where Kiambu district has 68% and Machakos district has 45% earth ponds. Machakos district has the highest number of liner ponds having 51% against 30% in Kiambu district . The liner pond is more common in Machakos district because of the scarcity of water in this region, low amounts of rainfall, high rates of evaporation and the poor water holding capacities of soils in these regions and therefore, liners are used as a strategy of conserving the pond water. Earth ponds lose a lot of water through seepage and the problem of water scarcity makes refilling of the pond difficult hence the trend of moving towards investment in liner ponds. The soils in Machakos are also poor compared to Kiambu in terms of water holding capacity and there is a high rate of evaporation. However, on average earth ponds are the most common pond types compared to liner, concrete ponds and tanks.

Table 4.10 Fish holding structures

	Kiambu	Machakos
Pond type	% Respondents(n=150)	% Respondents (n=100)
Liner pond	30	51
Earth pond	68	45
Concrete pond	2	3
Tank culture	-	1
Total	100	100

Appendix 8: Sources of Pond water

Most of the ponds in these two counties were located in the vallies due to closeness to the water source mainly rivers, wells and boreholes. Most of the ponds 52% were filled by gravity were furrows are dug from the water source mainly a river and water is directed to the pond. The remaining 48% of the farmers used pumping to fill their ponds and this increased the cost of management due to the cost of fuel used by the pumps. Farmers mainly in Machakos County who used other methods like ferrying of water using donkey were also grouped under pumping. Rivers form the greater percentage of water sources in Kiambu County because there are more permanent rivers and streams in Kiambu than in Machakos because the region receives higher amounts of rainfall. Fish farmers in Machakos County use different sources of water which include shallow wells 35%, borehole 30% and rivers 27%. It is therefore expensive and tedious to fill the ponds in Machakos County than in Kiambu due to the cost of fuel for pumping and labor for transporting water to the pond. This study also revealed that over 15 % of ponds in Machakos County were dry which further shows that this region lacks water for fish production. Results also indicate that 89.6% of farmers in both counties did not experience any conflict in accessing water for filling the ponds while 10.4% experienced conflict such as complaints of using a lot of water by neighbors located downstream in Kiambu County and complaints of drawing a lot of water from the community boreholes in Machakos County.

Table 4-11: Sources of pond water

Source of water	Kiambu county % Respondents(n=150)	Machakos county % Respondents(n=100)
Borehole	20.7	30
Well	7.3	35
River	62	27
Dam	2	4
Rain	6.7	4
Tapped	1.3	-
Total	**100**	**100**

Appendix 9: Fish feeds and feeding management

Results show that all the farmers in Kiambu and Machakos Counties used complete or formulated commercial feeds for feeding the fish. Most the feeds were supplied by the government through the ESP. In Kiambu County farmers were supplied with feeds from Unga Feeds LTD. and in Machakos County the feeds were from Sigma Feeds LTD. Some Farmers in both counties also used homemade formulations like shrimp meal and omena. Other farmers also used supplementary feed such as wheat and maize bran to feed the fish. Kitchen left overs and vegetable residues were also used as feed. However, these acted as organic fertilizers other than feed due to their raw nature. The average amount feed fed to 1000 fish per day was found to be 1.74kg. Majority of the farmers 62.8% fed the fish twice per day in the morning and in the afternoon. The remaining 35.6% and 1.6% fed the fish once and thrice per day respectively. This results show a gap in the information needs of the farmers because the amount of feed fed to the fish should be determined from their weights at various stages of development. The frequency of feeding should also be determined by the rate of intake of feed by the fish because of fed in excess the decayed feed remains will add the concentration of ammonia gas in the pond which is toxic to fish. Generally it is believed that the contaminants taken in by aquatic organisms are from water, rather than from their food, and may vary with seasonal variation in contaminant availability within the water column.

Table 4.12: Source of formulated fish feeds.

Source of feed	Kiambu	Machakos
	% Respondents(n=150)	% Respondents(n=100)
Supplied by government	82	70
Purchased	18	30
Total	100	100

Appendix 10: Previous land use

Results indicate that 89.3% and 86% of farmers in Kiambu and Machakos counties respectively (Table 4-21) previously used the area with the pond for growing crops before the introduction of the fish farming enterprise.

4.13: Previous land use

	Kiambu	**Machakos**
Previous land use	**% Respondents(n=150)**	**% Respondents(n=100)**
Crop farming	89.3	86
Grazing	0.7	7
Bare land	10	7
Total	**100**	**100**

Appendix 11: Consumption and marketing of fish

Of the total fish farmers interviewed 42% harvested fish for home consumption with 58% harvesting fish only for sale. Majority of the farmers 54% harvested their fish after attaining 8 months maturity age and 88.8% used partial harvesting strategy where only the numbers required are harvested with the rest left to continue growing

Appendix 12: Levels of Lead and Cadmium on Brain, Liver, Gonad and Muscle from fish sampled from Kiambu and Machakos County, Kiambu

	SAMPLE CODE	ORGAN	COUNTY	Pb (ppm)	Cd (ppm)
	KAN05B	Brain	Machakos	65.14	ND
1	KAN35G	Gonad	Machakos	20.49	ND
2	KAN35B	Brain	Machakos	ND	ND
3	KAN39M	Muscle	Machakos	ND	1.25
4	KBU06M	Muscle	Kiambu	ND	ND
5	KBU18G	Gonad	Kiambu	1.04	ND
6	KBU25B	Brain	Kiambu	4.07	ND
7	KAN39L	Liver	Machakos	ND	ND
8	KAN39B	Brain	Machakos	ND	ND
9	KATH04G	Gonad	Machakos	ND	ND
10	KAN33M	Muscle	Machakos	ND	ND
11	KATH04B	Brain	Machakos	ND	ND
12	KBU04G	Gonad	Kiambu	ND	ND
13	KBU04B	Brain	Kiambu	2.71	1.26
14	MACH011G	Gonad	Machakos	ND	ND
15	KAN38B	Brain	Machakos	ND	ND
16	KAN39G	Gonad	Machakos	3.04	ND
17	MACH011B	Brain	Machakos	28.57	ND
18	KBU012B	Brain	Kiambu	ND	ND
19	KBU06B	Brain	Kiambu	27.27	ND
20	KAN38G	Gonad	Machakos	ND	ND
21	KAN05M	Muscle	Machakos	ND	ND
22	KBU12M	Muscle	Kiambu	0.25	ND
23	MACH011L	Liver	Machakos	ND	ND
24	KAN37L	Liver	Machakos	ND	ND
25	KAN39L	Liver	Machakos	ND	ND
26	KBU24L	Liver	Kiambu	ND	ND
27	KAN37B	Brain	Machakos	14.6	ND
28	KBU12L	Liver	Kiambu	ND	ND
29	KBU18B	Brain	Kiambu	ND	ND
30	KAN05L	Liver	Machakos	ND	ND
31	KAN05G	Gonad	Machakos	ND	ND
32	KBU12G	Gonad	Kiambu	ND	ND
33	KBU18M	Muscle	Kiambu	ND	ND
34	KAN33L	Liver	Machakos	ND	1.08
35	KBU18L	Liver	Kiambu	ND	ND
36	KAN03M	Muscle	Machakos	ND	ND

37	KBU25G	Gonad	Kiambu	ND	ND
38	KBU124B	Brain	Kiambu	59.23	ND
39	KBU16M	Muscle	Kiambu	6.0	ND
40	KAN34G	Gonad	Machakos	7.68	ND
41	KBU127B	Brain	Kiambu	190	ND
42	KBU124L	Liver	Kiambu	113.2	11.33
43	KBU26G	Gonad	Kiambu	7.51	ND
44	KAN02B	Brain	Machakos	91.59	62.45
45	KAN89B	Brain	Machakos	36.87	19.07
46	KAN34B	Brain	Machakos	28.99	18.76
47	KAN89M	Muscle	Machakos	8.22	7.77
48	KAN34L	Liver	Machakos	6.85	2.16
49	KBU127L	Liver	Kiambu	157.8	73.68
50	KBU26B	Brain	Kiambu	17.28	12.96
51	KBU13M	Muscle	Kiambu	4.71	8.0
52	KBU13B	Brain	Kiambu	84.3	77.72
53	KAN36G	Gonad	Machakos	ND	0.20
54	KAN34M	Muscle	Machakos	1.42	ND
55	KBU25L	Liver	Kiambu	7.31	1.22
56	KBU25M	Muscle	Kiambu	3.42	0.69
57	KBU169		Kiambu	3.01	0.86
58	KBU02L	Liver	Kiambu	39.21	7.84
59	KAN03B	Brain	Machakos	42.3	3.85
60	KBU26M	Muscle	Kiambu	4.7	ND
61	KBU02M	Muscle	Kiambu	2.84	ND
62	KAN03L	Liver	Machakos	6.13	ND
63	KBU127M	muscle	Kiambu	6.58	ND
64	KAN02G	Gonad	Machakos	73.19	ND
65	KATH06L	Liver	Machakos	21.67	ND
66	KBU16B	Brain	Kiambu	23.98	ND
67	KATH06M	Muscle	Machakos	5.82	ND
68	KBU16L	Liver	Kiambu	3.35	ND
69	KAN36B	Brain	Machakos	12.44	ND
70	KAN02M	Muscle	Machakos	1.55	ND
71	KATH06B	Brain	Machakos	29.27	ND
72	KATH06G	Gonad	Machakos	13.76	ND
73	KBU89G	Gonad	Kiambu	41.79	1.67
74	KBU02B	Brain	Kiambu	48.29	ND
75	KBU124M	Muscle	Kiambu	5.64	5.68
76	KAN36M	Muscle	Machakos	1.09	ND
77	KBU02G	gonad	Kiambu	26.23	ND

78	KBU124G	Gonad	Kiambu	10.26	ND
79	KBU127G	Gonad	Kiambu	13.14	ND
80	KAN09L	liver	Machakos	ND	ND
81	KAN07M	Muscle	Machakos	ND	ND
82	KAN101L	Liver	Machakos	ND	ND
83	KAN07B	Brain	Machakos	60.73	ND
84	KBU07L	Liver	Kiambu	16.24	ND
85	KBU07G	Gonad	Kiambu	4.19	ND
86	KBU00M	Muscle	Kiambu	6.58	ND
87	KAN79B	Brain	Machakos	31.37	ND
88	KAN06G	Gonad	Machakos	ND	ND
89	KAN06M	Muscle	Machakos	ND	ND
90	KAN09M	Muscle	Machakos	ND	0.61
91	KAN79G	Gonad	Machakos	ND	ND
92	KAN79M	Muscle	Machakos	ND	ND
93	KAN09G	Gonad	Machakos	8.93	8.99
94	KAN101M	Muscle	Machakos	3.01	0.90
95	KAN101B	Brain	Machakos	17.20	3.44
96	KAN79L	Liver	Machakos	14.8	2.5
97	KAN101G	Gonad	Machakos	ND	0.17
98	KAN83G	Gonad	Machakos	2.33	ND
100	KATH08G	Gonad	Machakos	ND	ND
101	KATH17M	Muscle	Machakos	6.31	1.70
102	KATH08L	Liver	Machakos	5.70	0.81
103	KATH09M	Muscle	Machakos	ND	0.62
104	KATH04M	Muscle	Machakos	ND	0.25
105	KATH03G	Gonad	Machakos	ND	0.1
106	KATH03L	Liver	Machakos	ND	ND
107	KATH08M	Muscle	Machakos	ND	0.31
108	KATH03M	Muscle	Machakos	ND	0.87
109	KATH08B	Brain	Machakos	0.31	0.41
110	KATH09L	Liver	Machakos	ND	0.6
111	KBU09G	Gonad	Kiambu	ND	0.83
112	KBU14L	Liver	Kiambu	ND	1.94
113	KBU25B	Brain	Kiambu	ND	ND
114	KBU08M	Muscle	Kiambu	ND	0.29
115	KBU14G	Gonad	Kiambu	46.19	11.55
116	KBU14M	Muscle	Kiambu	ND	0.44
117	KBU15M	Muscle	Kiambu	ND	0.39
118	KBU15B	Brain	Kiambu	ND	0.67

119	KBU14M	Muscle	Kiambu	ND	0.81
120	KBU14B	Brain	Kiambu	74.63	6.81
121	KBU15L	Liver	Kiambu	54.27	ND
122	KBU220M	Muscle	Kiambu	ND	ND
123	KBU2oG	Gonad	Kiambu	ND	ND
124	KBU20L	Liver	Kiambu	ND	ND
125	KBU20B	Brain	Kiambu	5.17	ND
126	KBU30M	Muscle	Kiambu	ND	ND
127	KAN30L	Liver	Machakos	ND	ND
128	KBU40M	Muscle	Kiambu	ND	ND
129	KBU40L	Liver	Kiambu	ND	ND
130	KBU40B	Brain	Kiambu	7.0	ND
131	KBU40G	Gonad	Kiambu	0.08	ND
132	KBU128M	Muscle	Kiambu	ND	ND
133	KBU128G	Gonad	Kiambu	ND	ND
134	KAN39B	Brain	Machakos	3.15	1.68
135	KBU41B	Brain	Kiambu	2.71	1.26
136	KBU28L	Liver	Kiambu	ND	1.25
137	KBU125B	Brain	Kiambu	1.57	1.17
138	KBU41L	Liver	Kiambu	ND	0.85
139	KBU125L	Liver	Kiambu	ND	1.08
140	KBU10G	Gonad	Kiambu	ND	ND
141	KBU10M	Muscle	Kiambu	ND	ND
142	KBU10L	Liver	Kiambu	ND	ND
143	KBU03G	Gonad	Kiambu	ND	ND
144	KAN991G	Gonad	Machakos	ND	ND
145	KBU11G	Gonad	Kiambu	ND	ND
146	KBU03B	Brain	Kiambu	ND	ND
147	KBU03L	Liver	Kiambu	ND	ND
148	KBU10B	Brain	Kiambu	ND	ND
149	KBU09G	Gonad	Kiambu	ND	ND
150	KAN991L	Liver	Machakos	ND	3.32
151	KBU11B	Brain	Kiambu	16.25	16.25
152	KBU150B	Brain	Kiambu	ND	7.17
153	KBU06L	Liver	Kiambu	ND	16.53
154	KBU03M	Muscle	Kiambu	ND	0.91
155	KAN991M	Muscle	Machakos	ND	3.67
156	KBU15G	Gonad	Kiambu	ND	0.41
157	KBU07L	Liver	Kiambu	ND	16.20
158	KBU06M	Muscle	Kiambu	0.21	0.41
159	KBU150G	Gonad	Kiambu	ND	0.44

160	KBU15M	Muscle	Kiambu	ND	0.17
161	KBU07B	Brain	Kiambu	ND	17.40
162	KBU09L	Liver	Kiambu	ND	0.59
163	KATH07G	Gonad	Machakos	ND	0.84
164	KBU11M	Muscle	Kiambu	ND	0.61
165	KBU09B	Brain	Kiambu	ND	2.17
166	KBU150L	Liver	Kiambu	ND	2.33
167	KATH07L	Liver	Machakos	ND	2.25
168	KBU15B	Brain	Kiambu	ND	5.33
169	KBU06B	Brain	Kiambu	ND	2.13
170	KBU15L	Liver	Kiambu	ND	ND
171	KBU09M	Muscle	Kiambu	ND	ND
172	KBU07M	Muscle	Kiambu	ND	ND
173	KBU11L	Liver	Kiambu	ND	4.21
174	KBU06G	Gonad	Kiambu	ND	9.18
175	KAN991M	Muscle	Machakos	ND	ND
176	KATH07G	Gonad	Machakos	ND	ND
177	KBU07G	Gonad	Kiambu	29.69	3.96
178	KATH07M	Muscle	Machakos	ND	ND
179	KBU150M	Muscle	Kiambu	ND	ND
180	KBU24B	Brain	Kiambu	ND	ND
181	KAN33G	Gonad	Machakos	ND	ND
182	KAN05G	Gonad	Machakos	ND	ND
183	KBU06L	Liver	Kiambu	ND	ND
184	KBU24M	Muscle	Kiambu	ND	ND
185	KATH04M	Muscle	Machakos	ND	ND
186	KAN37G	Gonad	Machakos	ND	ND
187	KAN33B	Brain	Machakos	ND	ND
188	KAN57M	Muscle	Machakos	ND	ND
189	KAN03G	Gonad	Machakos	ND	ND
190	MACH011M	Muscle	Machakos	ND	ND
191	KBU04L	Liver	Kiambu	ND	ND
193	KAN38M	muscle	Machakos	ND	ND
194	KAN35L	Liver	Machakos	ND	ND
195	KAN38L	Liver	Machakos	ND	ND
196	KAN35M	Muscle	Machakos	ND	ND
197	KATH04L	Liver	Machakos	ND	ND
198	KAN05B	Brain	Machakos	ND	ND
199	KAN05L	Liver	Machakos	ND	ND
200	KBU15G	Gonad	Kiambu	ND	ND
201	KBU25M	Muscle	Kiambu	2.34	ND

202	MACH63L	Liver	Machakos	7.33	ND
203	MACH63G	Gonad	Machakos	1.57	15.5
204	MACH63M	Muscle	Machakos	2.76	ND
205	KAN89L	Liver	Machakos	0.29	0.58
206	KAN36L	Liver	Machakos	3.63	0.81
207	KAN03M	Muscle	Machakos	3.71	0.62
208	KBU13G	Gonad	Kiambu	19.92	1.65
210	KBU21B	Brain	Kiambu	8.29	0.98
211	KAN06L	Liver	Machakos	2.33	1.43
212	KBU21G	Gonad	Kiambu	7.16	0.81
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Appendix 13: Levels of Organochlorines on Brain, Liver, Gonad and Muscle from fish sampled from Kiambu and Machakos Counties, Kenya

SAMPLE	α-BHC	LINDANE	β-BHC	HEPTACHLOR	ALDRIN	HEPTACHLOR EPOXIDE	4,4' -DDE	DIELDRIN	2,4' -DDD	ENDRIN	2,4' - DDT	4,4' -DDD	4,4 -DDT
M KBU24	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	ND	ND	ND
G KBU124	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND
L KBU124	ND	ND	ND	0.000	ND	ND	ND	ND	ND	ND	0.002	ND	ND
M KBU06	ND	ND	0.101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU124 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU18 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU18	ND	ND	0.386	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU18	ND	ND	0.525	ND	ND	ND	ND	0.012	ND	ND	ND	ND	ND
BLANK 2A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLANK 2B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN07	ND	ND	ND	ND	0.002	ND	ND	0.006	ND	ND	ND	ND	ND
L KAN07	ND	ND	ND	0.000	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN07	ND	ND	ND	ND	ND	ND	ND	0.010	ND	ND	ND	ND	ND
G KAN07	ND	ND	0.013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU25	ND	ND	ND	ND	ND	ND	ND	0.006	ND	ND	ND	ND	ND
G KBU25	ND	ND	ND	ND	ND	0.004	ND	ND	ND	ND	ND	ND	ND
B KBU25	0.000	ND	ND	ND	ND	0.001	ND	0.004	ND	ND	ND	ND	ND
M KBU19	0.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU19	ND	0.000	ND	0.000	ND	0.004	0.035	ND	ND	ND	ND	0.002	ND
G KBU19	ND	ND	ND	ND	ND	ND	ND	0.005	ND	ND	ND	ND	ND
L KBU19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.023	ND	ND
L KBU40	ND	0.000	0.036	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.010	ND	ND
B KBU40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.098	ND	ND
M KAN83	ND	ND	ND	ND	ND	ND	ND	0.002	ND	ND	ND	ND	ND
B KAN83	ND	ND	0.011	ND	ND	ND	ND	ND	ND	0.016	ND	0.001	ND

M KBU27	ND	ND	0.066	ND	ND	ND	ND	0.002	ND	ND	ND	ND	ND
G KBU27	ND	0.000	ND	ND	ND	ND	ND	0.012	ND	ND	0.015	0.006	ND
L KBU27	ND	0.015	0.185	0.000	ND	ND	ND	0.016	ND	ND	ND	0.007	ND
B KBU27	0.009	0.065	ND	0.002	ND	0.141	ND	ND	0.003	ND	ND	0.016	ND
M KBU27	ND	0.013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU26	ND	0.042	ND	ND	0.000	ND	ND	ND	ND	ND	ND	ND	ND
G KBU26	ND	ND	ND	ND	ND	ND	ND	0.019	ND	ND	ND	ND	ND
M KBU29 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU29	ND	ND	ND	0.007	0.002	ND	ND	ND	ND	ND	ND	ND	ND
G KBU29	ND	ND	ND	ND	0.118	ND	ND	ND	ND	0.075	ND	ND	ND
L KBU29	ND	ND	ND	ND	ND	ND	ND	ND	0.013	0.287	ND	ND	ND
M KBU30	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.006	ND	ND	ND
G KBU30	ND	0.016	ND	ND	ND	ND	ND	ND	ND	0.015	ND	ND	ND
B KBU30 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU30	ND	ND	ND	ND	ND	0.369	ND	ND	ND	0.006	ND	0.013	ND
BLANK1A 22/5/12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLANK 1B 22/5/12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN33	ND	ND	ND	ND	ND	ND	ND	0.018	ND	ND	0.062	ND	ND
L KAN33	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.018	ND	ND	ND
B KAN33	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN33	ND	ND	0.023	ND	ND	ND	ND	ND	0.032	ND	ND	ND	ND
M KBU150	ND	ND	ND	ND	ND	ND	ND	0.009	ND	0.008	ND	ND	ND
G KBU150	ND	0.018	ND	0.003	ND	ND	0.142	ND	ND	ND	ND	0.003	ND
B KBU150	ND	0.131	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.054	ND
L KBU150	ND	0.018	ND	ND	ND	ND	0.290	ND	ND	0.016	ND	0.052	ND
M KBU03	ND	ND	ND	ND	ND	ND	0.053	0.004	ND	ND	ND	ND	ND
L KBU03	0.003	0.026	ND	ND	ND	ND	ND	ND	ND	0.012	ND	0.032	ND
B KBU03	ND	0.028	ND	ND	ND	ND	0.519	ND	ND	0.557	ND	0.060	ND
G KBU03	ND	0.023	ND	ND	ND	ND	0.353	ND	ND	0.013	ND	0.057	ND
M KBU04	ND	ND	ND	ND	ND	ND	ND	0.002	0.062	0.116	ND	ND	ND
B KBU04	ND	0.027	ND	ND	ND	ND	ND	ND	ND	0.002	ND	0.057	
G KBU04 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU04	ND	0.027	ND	ND	ND	ND	0.097	ND	ND	0.078	ND	0.079	ND
M KBU04	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.050	ND	0.002	ND
M KBU12ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU12	0.340	ND	ND	ND	ND	ND	ND	ND	ND	0.017	ND	0.000	ND
B KBU12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.011	ND
M KAN991	0.042	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.006	ND
B KAN991	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN991	ND	0.011	ND	0.032	ND	0.162	ND	ND	ND	0.204	0.017	0.000	ND

G KAN991	ND	0.011	ND	ND	ND	0.006	0.004	ND	ND	ND	ND	ND	ND
M KBU106	ND	0.012	ND	ND	ND	ND	ND	ND	ND	0.296	ND	0.006	ND
B KBU106	0.005	ND	0.016	ND	ND	0.006	ND	ND	ND	0.011	ND	0.032	ND
L KBU106	ND	0.010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU106	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.011	ND	ND	ND
M KAN139	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.008	ND
B KAN139	0.004	0.021	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN139	ND	ND	0.037	0.043	ND	ND	ND	ND	ND	ND	0.039	ND	ND
L KAN139	ND	ND	ND	0.011	ND	ND	ND	ND	ND	ND	ND	0.028	ND
M KAN38	ND	0.041	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN38	0.005	0.018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU06	ND	0.025	ND	ND	ND	0.001	ND	ND	ND	ND	ND	ND	ND
L KBU02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU02	ND	ND	0.496	ND	ND	ND	ND	ND	ND	0.040	ND	0.002	ND
G KBU13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU13	0.032	0.242	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU13	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.024	ND	ND	ND
B KBU13	ND	ND	ND	ND	0.034	ND	ND	ND	ND	ND	ND	ND	ND
M KBU34	ND	0.034	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU34	ND	1.159	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU34	ND	1.014	0.425	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.083
G KBU34 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN20 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN20	ND	ND	0.041	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN02	ND	ND	ND	ND	ND	ND	ND	ND	0.002	ND	ND	ND	0.107
L KAN02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU26	ND	ND	0.361	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU26	ND	ND	0.439	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU26	ND	ND	0.212	ND	ND	ND	ND	ND	ND	0.044	ND	ND	ND
B KBU127 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU127	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	ND	ND	ND
L KBU127	ND	ND	0.743	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU127 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU11 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU11 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU11	ND	ND	0.000	0.011	0.023	ND	0.005	ND	ND	ND	2.381	ND	0.042
G KBU11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.072	ND
B KBU08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU08	ND	ND	ND	ND	ND	ND	ND	0.020	ND	ND	ND	ND	ND

G KBU08 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU08 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN05 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN05 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN89 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN89 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN89 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU10 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU 10 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU10 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU10 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU 89	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU89 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KATH03 ND	ND	ND	ND	ND	ND	ND	ND	0.003	ND	ND	ND	ND	ND
L KATH03	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.002	0.003	ND	ND
G KATH03 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH03 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KATH03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN35	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN35 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN35 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN37 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000
G KAN37 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KAN09	ND	ND	ND	ND	ND	ND	0.002	ND	ND	ND	ND	0.000	ND
L KAN09 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN09 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN09ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KATH08 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH08	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	ND	ND	ND
L KATH08 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU07 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU08 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU26	ND	ND	ND	ND	ND	ND	ND	ND	0.002	ND	ND	ND	ND
L KBU26 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU26 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU26	0.041	ND	0.131	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KATH04 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

G KATH04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH04 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KATH04 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU14 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU14 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU14 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KATH09	ND	0.022	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KATH09	ND	ND	ND	ND	ND	0.241	ND	ND	ND	ND	ND	ND	ND
B KATH09	ND	0.099	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KATH09	0.358	ND	ND	ND	ND	ND	ND	ND	0.062	ND	ND	ND	ND
M KAN 63	ND	0.130	ND	ND	ND	ND	ND	ND	0.091	ND	ND	ND	ND
B KAN63	ND	ND	ND	ND	0.351	0.501	ND	ND	0.011	ND	ND	ND	ND
L KAN63	ND	0.019	ND	ND	ND	ND	ND	ND	0.002	ND	ND	ND	ND
G KAN63	ND	0.051	ND	ND	ND	ND	ND	ND	0.005	ND	ND	ND	ND
M KAN63	ND	ND	ND	ND	ND	ND	ND	ND	0.011	ND	ND	ND	ND
L KAN79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KAN79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN79	ND	0.911	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	ND
G KAN101	ND	0.006	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KAN101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KAN101	ND	0.136	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000
M KAN101 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU20 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU20 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU20	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND
G KBU20 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU25 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KATH04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU15 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU150	ND	ND	0.013	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU15	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND
B KBU15	ND	ND	0.007	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KATH05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.064	ND	ND
G KATH05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH05 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KATH06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.080	ND
L KATH06ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH06	3.146	ND	ND	ND	ND	ND	ND	ND	ND	0.019	ND	ND	ND

M KAN00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KBU22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KBU22	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.037	0.008	ND	ND
M KBU22	1.004	ND	ND	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND
L KBU21	ND	ND	ND	ND	ND	ND	ND	0.000	ND	ND	ND	ND	ND
G KBU21	ND	ND	ND	ND	ND	0.001	ND	ND	ND	ND	ND	ND	ND
B KBU21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.000	ND	ND
B KBU28	ND	ND	ND	ND	0.010	ND	ND	ND	ND	ND	ND	ND	ND
G KBU28	ND	ND	ND	0.001	ND	ND	0.002	ND	ND	ND	ND	ND	ND
L KBU28	0.022	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M KBU28	ND	ND	ND	ND	0.156	ND	0.002	ND	ND	ND	ND	ND	0.000
M KATH04	ND	ND	0.008	ND	ND	0.024	ND	ND	ND	0.002	ND	0.001	ND
G KBU24	ND	ND	ND	ND	ND	ND	ND	ND	0.003	ND	ND	0.003	ND
M KBU24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KBU24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
L KATH04 ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B KATH04	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G KATH04	ND	ND	0.016	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND