PERSISTENT ORGANIC POLLUTANTS IN HUMAN MILK FROM WESHA COASTAL COMMUNITY IN PEMBA - TANZANIA

By

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DECEMBER 202

DECLARATION

I hereby declare that this research dissertation is my original work and has not been presented for a degree in any other University or any other award

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We as supervisors confirm that the research dissertation reported was carried out by the candidate under our supervision.

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DEDICATION

This work is special dedication to my mother, my lovely husband (Ame Khamis Adam), and the whole family

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Abbreviation and Acronyms

ADI	-	Acceptable Daily Intake
AMAP	-	Arctic Monitoring and Assessment Programme
bw	-	body weight
CHLs	-	Chlordanes
CV	-	Coefficient variation
DDD	-	Dichlorodiphenyldichroroethane
DDE	-	Dichlodiphenyltrichloroethylene
DDT	-	Dichlorodiphenyltrichroroethane
EDI	-	Exposure Daily Intake
EI	-	Electron ionization
GC-ECD	-	Gas Chromatography- Electron Capture Detector
GC-MS	-	Gas chromatography- Mass spectrometry
HCBs	-	Hexachlorobenzenes
HCHs	-	Hexachlorohexanes
HI	-	Hazard Index
HPLC	-	high performance liquid chromatograph
HQ	-	Hazard Quotient
Kow	-	Octanol-water partition coefficient
LOD	-	Limit of detection
LOQ	-	Limit of quantification
NMS	-	Non-metric multidimessions calling
OCP	-	Organochlorine pesticides

OCPs - Organochlorine pesticides

PBBs - polybrominated-biphenyl

PBDEs - Polybrominated dipenyl ethers

PCA - Pentachloroanniline

PCB - Polychlorinated Biphenyl

PCDDs - polychlorinated dibenzo-dioxins

PCDFs - polychlorinated dibenzo-furans

PCNs - polychlorinated naphthalenes

PHB - polyhalogenated biphenyls

PHDDs - polyhalogenated dibenzo-dioxins

PHDFs - polyhalogenated dibenzo-furans

POPs - Persistent Organic Pollutants

SD - standard Deviation

UNEP - United Nations Environment Program

WHO - World Health Organization

ABSTRACT

Human milk is considered to be vital for health development of infant as it provide infants with well-balanced nutrition and protection against infectious pathogens. The milk also offers psychological and social benefits to breastfed infants. Being very rich in Lipid fat, various Persistent Organic Pollutants (POPs) dissolve and accumulate in the milk to the level that can pose serious health risks to breast-fed infants. The levels of persistent organic pollutants (POPs) in human milk from Wesha coastal community in Pemba were analyzed using GC-ECD and GC-MS. The determined levels were assessed for their composition, variations and the associated health risks to breast-infants. A total of 31 POPs belonging to organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) categories were identified in the analyzed human milk. OCPs were predominated by DDTs while all PCBs were dominated by ortho-congeners. Composition of DDTs revealed highest contribution of the parent p'p-DDT followed closely by p'p-DDE while HCHs had highest contribution of γ–HCH followed by α-HCH indicating combination of both continuous exposures of new and aged sources of pollutants. The levels of DDTs were much higher than the levels reported elsewhere with the exception of those reported in human milk from South Africa. In contrary, PCBs in this study were much lower than many reported levels from other parts of the world. ΣDDTS and ΣPCBs showed positive association and both of them increased significantly with lipid contents indicating that the two groups of POPs are originated from the same sources of exposure and are highly lipophilic in nature. On the other hand ΣHCHs gave strong negative associations with ΣDDTs and ΣPCBs and decreased with lipid content because of its relatively lower lipophilic nature compared to ΣDDTs and ΣPCBs. In contrary, DDTs and PCBs decreased significantly with lactation period, maternal age and parity whereas the HCHs depicted increased trend with lactation period, maternal age and parity proving that the first-born babies are exposed with the highest pollutant load from their mothers. Health risks assessment using both HQ and HI revealed possibility of potential health hazard to breast-fed infants. The follow-up study focusing on assessment of dietary intake of Wesha community is recommended to reveal the potential sources of POPs exposure in human

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Human or breast milk is a complex liquid mixture of high nutritive value produced by the mammary glands located in the breast of human female. It is the first option of infant feeding providing the infant with well-balanced nutrition and protection against potential infectious pathogens while the neonatal immune system completes its development (Sharp *et al*. 2014) Human milk consists of important nutrients such as proteins, amino acids, and carbohydrates, and non-nutritive factors including enzymes, hormones, and immunoglobulin's that are necessary for a proper development (Kishikawa & Kuroda 2009 and Borjand *et al.* 2007). Human milk is considered to be very important key in building immunity system of the infant which help protect infants against several diseases (Chao *et al*. 2005). Following the importance of human milk, World Health Organization (WHO) and several other health organizers have recommended breast feeding duration of two years with the first six months being exclusive breastfeeding (UNEP 2017) despite health benefits, breastfeeding also offers psychological and social development benefits to infants. Human milking has been proved to strengthen bond between mother and child (Boquien 2018). Children who were breastfed for a longer duration were reported to have higher levels of parental attachment and tended to perceive their mothers as more care and less overprotection toward them compared with formula-fed children (Chao *et al*. 2005).

About 87% of milk is composed of water and the remaining part consists of carbohydrates, fats and proteins (Pandey & Voskuil 2011). Lipid fat which represents about 50% calories of human milk includes triglyceride, cholesterol and phospholipids. These chemical contents of fats have both polar and non polar portions which allow various lipophilic chemical compounds likes Persistent Organic Pollutants (POPs) to dissolve in fat layer of milk (Boquien 2018).

POPs are organic compounds characterized by semi- volatility, high environmental persistence, a long half-life, and a high degree of lipophilicity by semi- volatility, high environmental persistence, a long half-life, and a high degree of lipophilicity; the characteristics which enable them to be ubiquitously distributed and accumulated in different environment matrices. Their persistence and lipophilic nature also qualify them with ability to accumulate in fatty foods and therefore biomagnified along the food chain.

Humans are exposed to POPs through environmental and dietary routes but exposure through the food chain remains to be the major exposure route (Papadopoulou *et al*. 2019). Once ingested, POPs accumulate in human adipose tissues and significant portion of them transferred to human milk because the milk is rich in lipid fats. Accumulation of POPs in human milk depends on their physico-chemical proprieties and their metabolic and excretion pathways (Lorenzetti *et al*. 2021) Lipophilic organochlorine compounds such as PCBs, Dioxins and OCPs such as DDT, hexachlorocyclohexanes (HCHs), dieldrin, chlordanes, hexachlorobenzene (HCB) and mirex are known to accumulate in human milk. However, chemical like HCHs which are relatively less lipophilic are normally measured at lower concentrations (Mueller *et al*. 2007).

When women are exposed to POPs through dietary or environment, they may bioaccumulate POPs in their adipose tissue to very large concentrations as both chemical and biological degradations of POPs occur at very slow rates. During breast feeding adipose tissues are activated and large amounts lipophilic contaminants are mobilized and transported with lipids to the mammary glands where they ultimately end up in milk. In this way breast feeding represent the major route of offloading POPs from lactation mother (Gallenberg & Vodicnik 1987). Since nursing infants feed entirely on human milk, they are therefore exposed to particularly high levels of pollutants via breast feeding (Fang *et al*. 2015). Transfer of significant amount of POPs from mother can pose potential and serious health risks to infants. These health effects includ differentiation of cells of the immune system, as well as risk to cognitive development such as spatial learning/memory and motor deficits have been reported elsewhere (Chao *et al*. 2005).

Analysis of the environmental chemical contaminants such as POPs in breast milk has revealed presence of high pollutants load in women from coastal communities and the levels have been associated with the consumption of contaminated seafood (Hu *et al*. 2021and Fång *et al*. 2015). Consumption of marine seafood is associated with POPs pollution because marine environments are widely used as dumping sites of different wastes consequent marine biota receive and accumulate high levels of POPs in their tissues. Presence of even little amounts of harmful contaminants in human milk poses a high risk effect to the infants because their immunity systems are very fragile and therefore more sensitive to toxicity (Kishikawa & Kuroda. 2008). On the other hand human milk has been used as a biological indicator for maternal transfer of environmental contaminants in mammals.

1.2 Statement of the Problem

Breast milk is the first and complete food that provides almost all of the essential nutrients required for infant growth and development. Human milk is also vital for preventing infection and cognitive improvement of babies. Despite these benefits, human milk is vulnerable to accumulation of lipophilic POPs resulting from chronic exposure through both environmental and food chain (dietary) routes. These bio-accumulated lipophilic POPs are transferred to infants through breastfeeding and epidemiological studies have recognized potential health risks to infants exposed to POPs through breastfeeding (Guerranti *et al*. 2011 and Lenters *et al*. 2019). Research have shown that breast feeding mothers from coastal communities consuming seafood as the major components of their diet have elevated levels of POPs in their milk (Hu *et al*. 2021). These elevated levels have been associated with dietary exposure through consumption of contaminated seafood. Zanzibar coastal communities are the regular users of seafood in their daily meal but studies on levels of POPs in human milk in Zanzibar is very scanty despite the existing vulnerability to human exposure in the aisles. This calls for the need of comprehensive investigation on the levels of POPs in human milk and assessing their associated health risk to breast-fed infants.

1.3 Objectives of the study

1.3.1 General objectives

The main objective of the study was to assess the levels of POPs contamination in human breast milk and their associated health risks to infants.

1.3.2 Specific objectives

The specific objectives were:

1. To determine the types and levels of POPs in human milk from Wesha Pemba
2. To assess the composition of the determined contaminants
3. To determine the relationship among the measured POPs
4. To assess variation of POPs with parity and mother age
5. To assess health risk to infants associated with breast feeding

1.4 Research questions

1. What are the types and concentrations of POPs in Human milk from Wesha Pemba
2. What are the composition patterns of the POPs measured in human milk from Wesha Pemba
3. Is there any correlation among the measured POPs in human milk?
4. To what extent the concentrations of the measured POPs in milk vary with maternal age and parity.
5. Is there any health risks associated with the levels of POPs in human milk to breast-fed infants?

1.5 Significance of the study

The present study reveals status of human milk in coastal community of Zanzibar and associated health risks to infants. The finding of this study can be used to package advices to society and will assist health officer to plan strategies to address the situation. Furthermore, the data and information reported in this work provide baseline information for other researchers to refer

CHAPTER TWO

LITERATURE REVIEW

2.1 Persistent organic pollutants and their characteristics

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes (Bernes 1998). Most of these POPs are polyhalogenated hydrocarbons that contain chlorine, bromine, or fluorine. They include pesticides, industrial chemicals, chemicals used in consumer products, and by-products of certain manufacturing and combustion processes (WHO 2010). Despite their high persistence, POPs are also characterized by their semi volatility and lipophic nature. The semi volatility property accord them ability to disperse and move long distance in the atmosphere before being deposited and can be found in region where they have never been used (Ritter *et al*. 1999). Likewise, high persistence and lipohilic properties qualify them with ability to bioaccumulate in many fat-containing tissues and biomagnified along the food chain. Human being at the top of food chain is exposed with these POPs through consumption of fatty rich food contaminated with POPs.

Persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment around the world. In some cases, POPs may undergo degradation to give more persistent and toxic degradation products. Their ability to accumulate along the food chain has raised a worldwide public health. For many years, the World Health Organization (WHO) has collaborated with countries in the development of data on levels of POPs in food as well as human milk. This data has been used to assess the risks to human health posed by exposure to various POPs. In 2004, an

international agreement, the Stockholm Convention on POPs, was adopted by a large majority of the world's countries to reduce the amount of these substances in the environment and in people (UNEP 2017). Under the treaty of Stockholm Convention production and consumption POPs were either banned or restricted and special permit is required to allow the use of restricted POPs. Due to their long term and serious threat to the environment and human health many POPs were banned in industrialized countries as early as the 1960s and 1970s (Skaare *et al*. 2006; WHO 2010).

2.2 Sources of POPs

Persistent organic pollutants (POPs) are both intentionally or unintentionally produced and introduced in the environment. POPs that were intentionally introduced into commercial proved beneficial in pest and disease control, crop production, and industry. These same chemicals, however, have had unforeseen effects on human health and the environment. These chemicals were once used in agriculture, public health, and in industrial processes. Examples of the intentionally POPs are Organochlorine pesticides used in agriculture and public health, PBDEs used as fire retardants and PCBs, which have been useful in a variety of industrial applications such as in electrical transformers and large capacitors, as hydraulic and heat exchange fluids, and as additives to paints and lubricants. Unintentionally POPs are chemicals produced as byproducts in some industrial processes and combustion of medical and municipal waste. These include PCBs, dioxins and furans. In recent researches, some chemicals such as methoxylatedpolybrominated diethyl ethers (MeOPBDEs) with bioaccumulative and persistence effects like OPPs have been shown to occur naturally (WHO 2010).

2.3 Classifications of POPs

Classes of these POPs include the organochlorine pesticides (e.g. DDT, chlordane, toxaphene), the polyhalogenated –biphenyls (PHBs; includes PCBs and PBBs), -dibenzo-p-dioxins (PHDDs; includes PCDDs), - dibenzofurans (PHDFs; includes PCDFs), and the polychlorinated naphthalenes (PCNs) (Muir *et al*. 1992; Ross 2000).

2.3.1 Organochlorine pesticides (OCPs)

Organochlorine pesticides (OCPs) were extensively used to increase the production of agricultural crops by preventing losses due to pest (Heidari 2003). They have also been used to control various vectors, which spread diseases like malaria or plague. OCPs persist in the environment for a long time and as a consequence, they enter the human body through the food chain and may cause serious health problems (John *et al*. 2001). The OCPs include DDTs, Hexachlorohexane (HCHs), cyclodines, Drins, hexachlorobenzene, mirex and toxaphene (Bergonzi *et al*. 2009; Dingemans *et al*. 2011; Ribas-Fito *et al*. 2001).

Dichlorodiphenyltrichloroethane (DDTs) is abroad spectrum insecticides (WHO 1979). DDT is normally found as a mixture of two several related compounds, the major dominated isomers namely 1,1bis-(4- chlorophenyl)-2,2,2-trichloroethane(p,p'-DDT) which contributes about 77% and l-(2- chlorophenyl)-l-(4-chlorophenyl)-2,2,2-trichloro ethane (o,p'-DDT)contribute about 15% (Zarba-Vary 1989). Among the isomer, p,p'-DDT is more stable than o,p'-DDT. In the environment and biological tissue the two isomers can degrade to DDE, DDD and the DDMU. Formation of DDE metabolite is more pronounced under aerobic conditions while DDD is more favored in anaerobic

conditions. The DDE metabolic product is more persistent and toxic than the parent molecule (Noren & Meironyte 2000) and therefore has strong ability to bioaccumulate in tissues with more health concern (Jensen & Slorach 1991). Growing concern about adverse environmental effects especially on wildlife led to severe restrictions and bans in many developed countries in the early 1970s (Ritter *et al*. 1999). As both parent and metabolites are persisted and hydrophobic they have great potential to accumulate in fatty food such as fish and milk. It was the first environmental chemical to be detected in human milk (Laug *et al*. 1951)

Hexachlorocyclohexanes are a mixture of stereoisomer's which differ in the relative position of the chlorine around the hexane ring. The technical commercial insecticide is a mixture of the different isomers which include α -HCH, β-HCH, γ-HCH, δ- HCH and others.The γ- HCH is the only isomer with pesticidal effects while other isomers have no pesticidal effects and thus act as pollutants in the environment. To minimize the effect of pollution another formulation with γ –HCH which known as lindane was developed, consequently use of technical HCH has been widely banned. The β-isomer of HCH is the most persistent with higher ability to accumulate in the fat tissues than the other isomers and is therefore of environmental concern. The β -HCH isomer usually found in highest concentration in the human adipose tissues and milk. An elevated concentration of β–HCH is also attributed by tendency of α- and γ-isomers of HCH to be converted into the β-within biological tissues (Jensen & Slorach. 1991).

Hexachlorobenzene **(**HCB) is a persistent organochlorine chemical that is both a pesticide and an industrial by-product. It is as a fungicide that was first introduced in 1945 for seed treatment (Jensen & Slorach, 1991 and Ritter *et al*. 1999). HCB is formed as an industrial

by-product in chlorination processes, such as wastewater treatment (Courtney 1979). It also forms as a by-product in the manufacturing and production of the wood preservative pentachlorophenol, of chlorinated solvents such as perchloroethylene and carbon tetrachloride, and of various pesticides (Jensen & Slorach 1991, Courtney 1979).

Cyclodiene OCPs are chlorinated derivatives of hexachlorocyclopentadiene. Most of the commercially important cyclodiene pesticides were branded as persistent organic pollutants by the United Nation Environmental Program (UNEP 2017 and Bhatt *et al*. 2009). These pesticides were introduced into the market in the 1950s and were used to control soil insects, cockroaches, termites, grasshoppers, locusts and other pests (Kannan *et al*. 1997). Typical cyclodiene pesticides are aldrin, dieldrin, endosulfan, endrin, heptachlor, heptachlor epoxide, chlordane and many others.

Cyclodienes are very persistent but some of them can degrade/metabolize to give more persistent metabolite. For instance, heptachlorcyclodiene metabolizes to heptachlorepoxide which is extremely lipophilic and persistent in the environment. The metabolite has been found in different tissues and even in soil 14–16 years after application (EXTOXNET 1996)**.** Chlordane is rapidly metabolized in organisms into oxychlordane and γ-chlordane or into impurities such as trans-nonachlor or cis-nonachlor. It is these breakdown products that persist in the tissue of fish, birds, and mammals and that are found in breast milk (Jensen and Slorach1991).

Dieldrin and aldrin are closely related organochlorine insecticides that are extremely persistent in the environment. In the living systems, aldrin breaks down to dieldrin; while in the environment, dieldrin resists bacterial and chemical breakdown processes (Afful *et*

al. 2010). On the other hand endrin is very persistent; however, in the presence of sunlight, it partially decomposes to endrin, ketone and endrin aldehyde (Nollet 2000). Endosulfan decomposes to endosulfansulphate which is equally toxic and bioaccumulative. Both parent endosulfan and metabolites are classified by the World Health Organization as priority pollutants (Weber *et al*. 2010)

2.3.2 Polychlorinated biphenyls (PCBS)

PCBs is a group of POPs which consist of two benzene rings with 1 to 10 chlorine atoms (ATSDR 2000) and general chemical formula of $C_{12}H_{10-n}Cl_n$ (Kmetic *et al*. 2012). The PCBs group has a total of 209 congeners which are named based on the number and position of chlorine atoms in the two benzene rings. These chemical compounds were intentionally manufactured and extensively used in commercial mixtures used in various fields of industry as heat-exchanges fluid in electric transformer and capacitors, additives in paints, carbonless copy paper, and plastics production due to their chemical stability, low flammability, and high dielectric constant (Ross 2004 and Kmetic *et al*. 2012) of industrial applications. Among the congeners six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180) are most often found as environmental contaminants due to their presence in commercial mixtures.

The toxicity of PCB congeners depends on the number and position of chlorine atoms in the molecule (Tesar 2000). The most toxic congeners are non-ortho PCBs, which include PCB-77, PCB-81, PCB126, and PCB-169. Other toxic PCBs are the mono-ortho PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB157, PCB-167, and PCB-189. Toxicity of non-ortho and mono-orho is facilitate by the ability of these congeners to assume a planar or nearly planar conformation similar to that of 2, 3, 7, 8-

tetrachlorodibenzo-*p*-dioxin (TCDD) and have toxic effects qualitatively similar to TCDD. PCBs with two or more ortho chlorines do not assume a planar conformation and are therefore less toxic and depict a different pattern of toxicity to that of TCDD (Metcalfe & Heffner 1995). Determination of resultant mixture of PCBs is done by using Toxic Equivalent Factors (TEFs) which is based on comparison with TCDD. However, the method is not applicable for PCBs with two or more ortho chlorine atoms (Safe 1990).

2.4 Exposures of POPs in the environment

POPs originate mainly from uses in industrial processes, waste incineration and agriculture. Once released into air, water and land, they are subjected to different chemo dynamics depending on physic-chemical characteristics of the particular POPs. They are globally distributed through the air, and ocean currents, travelling long distances via air–water exchange and cycles involving rain, snow and dry particles. As POPs are lipohilic, most of the time they end up absorbed into abiotic environment rich in organic matter or biotic environment rich in fat content. Due to their persistence and lipophilic nature, they concentrate, accumulate and magnify along the food chain (Bernes1998). Fat rich food like fish, milk and others are commonly found with elevated levels of POPs. Consequently, animals and human, especially those that depend on aquatic foods are the most affected (AMAP 1998).

2.5 Human exposures to POPs

Humans get exposed to POPs through either active (diet) or passive uptake routes such as occupational accidents and the environment including indoor. (Hedley *et al* 2010). Man being the highest link of the food chain, the main source of exposure to POPs by humans

is diet; primarily from ingestion of fatty foods such as dairy products, meat and fish. Dietary exposure to POPs can also be through consumption of contaminated vegetables and fruits from agricultural soils. Researchers have established that over 90% of POPs exposure to human comes from food, especially fish (Li *et al*. 2008).

Within the human and other mammals POPs distribute in different tissues and bioaccumulate in fat-rich tissues such as milk in females. As human milk is rich in fat, it bioconcentrate a significant amount of different which is the maternally transferred to young ones. Infants are usually exposed to POPs through placental and maternal transfer. Placental transfer refers to pollutants exposure to foetus in uterus via the placenta while maternal transfer is through breast feeding (Al-Othman *et al*. 2014). These maternal transfers have been associated with different accumulation pattern in male and female mammals. In male, levels of POPs normally increase with age but in female levels increase to maturity and the fall down as females are engaged in reproduction (Mwevura *et al*. 2010). Exposure of POPs to humans and other mammals is known to cause adverse health effects on their health; the effects may include cancer, damage to the nervous system, reproductive disorders, or disruption of the immune system (WHO, 2010). It is obvious that POPs exposure to delicate and undeveloped body of the new born will have more pronounced potential health risks.

2.6 POPs in human milk

Several studies have reported the presence of POPs in human milk and other parts of the human body from different parts of the World. The concentrations of pesticide residues, in human milk, vary considerably depending on the type of pesticide, from country to

country and were influenced by factors such as legalization and the culture of society and diet (Sharaf *et al*. 2008).

The most common types of POPs found in human milk are OCPs, PCBs, Dioxins and PBDEs. Others are perfluorinated compounds (PFCs), and chlorinated paraffins (CPs). In general domination of one group of POPs at given area depends on the intensity of pollutants sources of that area. For instance, OCPs are always dominating in human milk from agricultural areas or areas applied with DDT to combat mosquito and PCBs and dioxins are more prevalent in milk from industrial dominating areas (Hassine *et al*. 2012, Bouwman *et al*. 2012, Haraguchi *et al*. 2009, and Hu *et al*. 2021)

Levels of POPs measured in human milk from different parts of the world are also depending on the magnitude of pollution sources and route of exposure to nearby community. In general human milk from coastal communities (Hu *et al*. 2021) and from area sprayed by POPs (Bouwman *et al*. 2012) have been found to carry much higher levels of POPs compared to other communities. Higher levels of POPs in human milk from coastal community have been liked with frequent consumption of fish and other sea food from the areas.

In some human milk studies, measured levels have been compared with set standards for human consumptions from different countries but have been also used to estimate daily uptake doze of POPs and used to determine hazard quotient (HQ) and hazard index (HI). In many studies the levels are lower than the set standards are within a safe HQ and HI values; however, there are cases of higher levels than set standards. Levels of p,p-DDE in human milk from Middle and Upper Egypt (Saleem & Ahmed 2001) ranged from 3.353

to 67.159 µg/l, with some levels exceeding the Extraneous Residue Limits (ERLs) of (50 µg/l) issued by WHO 1996 .

Occurrence of several POPs in human milk and their possible associated health risks have necessitated the establishment of worldwide monitoring of POPs in human milk. In this project by UNEP project human milk is used as a monitoring media (UNEP 2017**)**. Tanzania is one of the project beneficiaries but the project has limited coverage and only few selected community can be selected from the country. Zanzibar being coastal community, consume significant amounts of fish and other seafood that have been pointed to be major source of POPs into human milk. Some of the coastal community like wesha houses hot spots with potential sources POPs and other organic pollutants. For instance Wesha power station drained different waste associated with power generation into the coastal nearby coastal areas.

CHAPTER THREE

METHODOLOGY

3.1 Study area

This study was conducted at Wesha, a small coastal village located at Chake-Chake District, Southern region of Pemba Island in Tanzania (Coordinates: $5^{O}15^{'}$S, $39^{O}7^{'}$E). The village is about 12 km from Karume Airport and 5.2 km from Chake-Chake Town. The coastline of Wesha has semi- enclosed bay with small opening to Pemba channel of Indian Ocean. (Ali 2015)

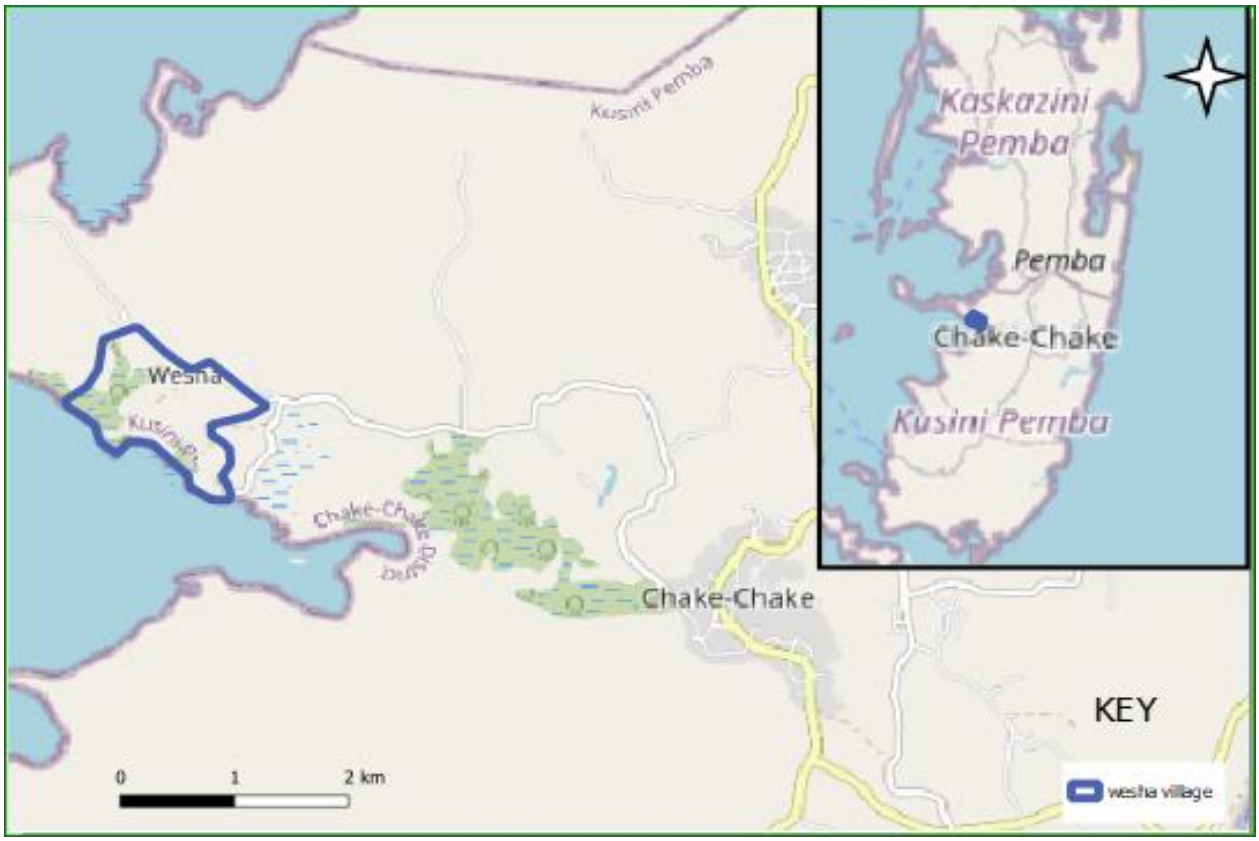

Figure 3.1: Map of the study area

The village is well known for hosting different socioeconomic activities such as fishing

and fish landing, oil deporting and storage and tourism related activities. The area also hosted power plant, which was the only sources of electricity in Pemba. The power plant operated for nearly 30 years but was then abandoned in 2010 following connecting Pemba Island Power grid from Tanga. The plant was consuming thousands of litters of heavy oil per month and a significant amount was in stock when it was abandoned. The plant was located few meters from coastal line and the generated wastes (oil residues) were stored in underground tanks with the power plant premises. The compound within the power plant was also used as a storage and workshop to repair of transformers.

During heavy rains a notable amount of oil residues overflew from the residue storage tank to coastal areas and resulted to detrimental degradation of the coastal environment. About 3 hectors of mangrove cover and their associated flora and fauna disappeared leaving the area like an aquatic desert. Recent studies have revealed elevated concentrations of oil associated chemicals such as PAHs and diesel product in sediment and edible biota from the nearby coastal waters. (Ali 2015 and Abdalla 2019). This pollution status was amplified by new oil leak following the attempt to transfer the unused oil from the oil reservoir tanks (Seif, 2019).

The area was selected for study because was highly exposed and impacted by different sources of bioaccumulative chemicals such as heavy duty oil and transformers associated with the power plant. (Ali 2015). Furthermore, the same species of edible biota that have been confirmed to contain bioaccumulative pollutants form part of regular diet of the villagers.

3.2 MATERIALS

Materials used at different stages of this study include Glassware, Equipments, Chemicals and Reagents. The following glassware were used were glass jar with teflon lined cock for collection of sample, Erlenmeyer flasks, pipettes, filtering funnels, graduated test tubes, glass column and glass vials. All apparatus were pre cleaned before use by washing with general and then successively rinsed with ethanol and acetone before being backed at 150 over night. Chemicals and reagents used at different stages of this study included anhydrous sodium sulphate, florisil, dichloromethane, hexane, cyclohexane and acetone. All chemicals and reagents used were either analytical or HPLC grade.

Various equipments were used in this study. The equipment includes analytical balances, rotary evaporator, overhead shaker, centrifuge, Gas chromatography (Varian 3400, and Hewlett Packard 6890) with electron capture (GC-ECD) and Gas chromatography equipped with mass spectroscopy machine (GC-MS). Other materials that were used in the study are aluminium foil, centrifuge tubes, and glass wool.

3.3 Methods

3.3.1 Ethical clearance and Sample collection.

A total of 35 breastfeeding mothers were invited to attend special informative session on the importance of carrying out this study. They were allowed to seek clarifications before making any decision. Out of 35 mothers, 30 voluntarily agreed to donate sample for the study under specified conditions and were requested to give their written consent by

filling the Informed Consent Form (Appendex 1). The age of mothers ranged from 20 to 40 years. The detailed information of the sample donors are summarized in the Table 3.1.

Table 3.1 show the information of the sample Donors

Sample Code	**Maternal Age**	**Parity**	**Child Age**
S1	40	10	1.8 years
S2	40	09	06 months
S3	25	03	07 months
S4	26	02	1.2 years
S5	35	10	1.5 years
S6	32	06	1.2 years
S7	40	05	09 months
S8	30	09	06 months
S9	32	16	1.5 years
S10	31	05	07 months
S11	27	04	1.5 years
S12	27	03	01 month
S13	30	07	1.3 months
S14	26	03	09 months
S15	26	03	09 months
S16	26	03	03 months
S17	25	05	06 months
S18	22	04	05 months
S19	28	09	03 months
S20	30	03	03 months
S21	26	02	10 months
S22	23	01	06 months
S23	30	06	03 months
S24	23	02	03 months
S25	21	01	1.5 years
S26	23	01	01 year
S27	30	06	1.8 years
S28	37	14	1.9 years
S29	22	01	4.5 months
S30	30	07	1.5 years

The samples from donors were collected according to the GEF/ UNEP 2017 guideline.

The donors were provided with pre-cleaned glass jars labeled with the donor's individual identification code. With a protected screw cap to collect and store the milk samples. The samples were collected manually directly into clean glass jars with a Teflon lined screw cap under the assistance of trained nurses at Wesha dispensary. A minimum of 15 ml of milk was collected from each mother in one breast while infant is nursing on the other breast, to take advantage of the let-down reflex of the mother. The collected milk samples in the capped glass jar were immediately stored into ice box for transportation to laboratory. In the laboratory samples were frozen at -20°C until the sample preparation, extraction and analysis.

3.3.2 Determination of fat content

Fat content will be determined using gravimetric method using mass of unextracted milk and mass of fat obtained after extraction.

Percentage Fat in the milk = $\frac{Mass\ of\ fat\ extracted}{Mass\ of\ unextracted\ milk} \times 100$

3.3.3 Sample extraction

Extraction

Milk samples were defrosted and a mass of 10 g was weighed and thoroughly homogenized with anhydrous sodium sulphate in the conical flask to give dry powder. The powder was then extracted by shaking successively with 60 ml, 30 ml, 30 ml of dichloromethane/cyclohexane (3:1v/v) using overhead shaker for 30 minutes. The extract was then filtered through granular anhydrous sodium sulphate to remove the remained traces of water and rotary concentrated to a volume of 2 ml.

Extraction of samples

Concentrated extracts

3.3.4 Clean-up of Sample Extracts

The concentrated extract was quantitatively transferred to a 1-cm diameter glass column packed with 10 g of 4% deactivated florisil slurry and 2 g sodium sulfate at the top. The column was then eluted with 60 ml hexane and the eluents was collected in the conical flask. The 60 ml hexane was concentrated using rotary evaporator to nearly 2 ml and then further concentrated to exactly 2ml by a gentle stream of N_2 gas. (Hong *et al*. 1992).The concentrated cleaned eluents was then put in the vials and stored at – 20 ^{o}C before being analyzed

3.3.5 GC-MS Analysis of sample

Analysis of OCPs in the sample was done by GC-MS-2010 Shimadzu Gas Chromatography coupled with electron capture detector (GC-ECD) and then confirmed by using Mass Spectrometry (GC-MS). The GC was operated using Restek-5MS column (30m x 0.25mm x 0.25µm) and initial oven temperature of 90^{o}C for 2 minutes. The oven temperature was then increased to 280^{o}C for 4 minutes at the rate of 6^{o}C/minute. The injection temperature was set at 250^{o}C with splitless injection mode. The flow rate of carrier gas helium was 1.2ml min^{-1}. The mass spectrometry was operated in electron

Ionization (EI) mode (MS) at 70eV.The ion source temperature and interface temperature in MS were 230^{o}C and 300^{o}C respectively.

3.3.6 Identification, Confirmation and Quantification

Identification of gas chromatograph (GC) peak of samples was done by comparing gas chromatographic retention times of authentic standards of the GC-ECD analysis. Confirmation of the identified peak was done by comparison of GC-MS scan method which involves the use of Mass Spectral Library & Search Software. The library search giving more than 90% was further confirmed by comparing the GC-MS of the authentic standard and the suspected analytes. On the other hand quantification of OCPs was accomplished by using calibration curve of peak heights of authentic standards.

3.4 DATA ANALYSIS

Analysis of data from milk samples employed different statistical treatments including descriptive statistics, simple linear regression and non-metric multidimessions calling (NMS). The descriptive statistics and simple linear regression analysis were carried out using Graph Pad Prism version 8.1.1.330 and were intended to establish association among the pollutants. The NMS was performed using PCOrd version 7.07. Nonmetric multidimensional scaling which avoids the assumption of linear relationships between variables by using ranked distances was used. Absolute concentrations were used, with Gower-ignore-0 as distance measure and a maximum of 500 iterations were allowed. The tests were done with 500 and 249 runs with real and randomized data, respectively.

3.5 HEALTH RISK ASSESSMENT

Estimated daily intake (EDI in ug/kg bw/day) of a single or group of pollutants depends

on mass of infant and the amount (mass) of milk consumed per day by infant

$$\text{EDI} = \frac{\%\ \text{lipid}}{100} \times \frac{\text{Mass of milk taken by infant per day} \times \text{Concentration of pollutant } (\frac{\mu g}{g\ lm})}{\text{Mass of infant (kg)}}$$

The mean values of EDI and Acceptable daily intake (ADI) of different POPs in milk are normally estimated based on the assumption that 5 kg infants consume an average of 700 g of milk daily (Oostdam *et al*. 1999 and Cok *et al*. (2012). Thus mean values of EDI are calculated using the following formula:

$$EDI = \left(\frac{P}{100}\right) \times 140\ X\ C$$

Where P = Percentage of fat in the milk

$\left(\frac{P}{100}\right)$ = fraction of lipid in the milk

140 = factor (g/kg) obtained by dividing (700g/5kg)

C = concentration (μg/g) of pollutant in milk

The calculated values of EDI and ADI from different standards were used to determine hazard quotient (HQ)

$$\text{HQ} = \frac{\text{EDI}}{\text{ADI}}$$

The health risk associated with individual pollutant or group of pollutants in the milk sample was then assessed by using HQ values. HQ< 1, indicate no obvious health risk while HQ >1 indicate possible health risk associate with the pollutant. USEPA (2000), Hu *et al*. (2021)

Health risk was also assessed using Hazard Index (HI) which is basically consider cumulative health effect of all pollutants measured in the given milk sample. The values of HIs are calculated as the summation of HQs of all pollutants found in the milk sample (Sue *et al*. 2020)

$$\mathrm{HI} = \sum \mathrm{HQs}$$

Health risk using HIs was then estimated using the same criteria that $\mathrm{HI} < 1$, indicate no obvious health risk while $\mathrm{HI} > 1$ indicate possibility of obvious health risk associate with consumption of the milk (Khan *et al*. 2014 and Castro-González *et al*. 2019).

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Quality control and quality assurance

The accuracy of the method and quality of analysis was controlled and assured by the use of procedural blanks as well as determination of both method detection limits (LOD).

The results for limit of detection LOD for all analytes are given in Table 4.1.

Table 4.1: Limits of Detection and Quantification (ng/kg wet mass)

Analyte Group	Analytes	LOD	LOQ
HCHs	α- HCH	0.008	0.04
	β-HCH	0.02	0.1
	γ-HCH	0.004	0.02
	δ-HCH	0.03	0.2
DDTs	p,p'-DDT	0.001	0.05
	o,p'-DDT	0.02	0.1
	p,p'-DDE	0.008	0.04
	o,p'-DDE	0.008	0.04
	p,p'-DDD	0.04	0.2
	o,p'-DDD	0.04	0.2
	p,p'-DDMU	0.02	0.1
HCB	HCB	0.005	0.03
PCA	PCA	0.008	0.04
Cyclodiene	Aldrin	0.02	0.1
	Dieldrin	0.008	0.04
	Endrin	0.02	0.1
	α-chlordane	0.02	0.1
	γ-chlordane	0.02	0.1
	oxy- chlordane	0.02	0.1
	Heptachlorepoxide	0.01	0.05
	trans-nonachlor	0.01	0.05
	α-endosulfan	0.02	0.1
	β-endosulfan	0.02	0.1
	Endosulfansulphate	0.02	0.1
PCBs	PCB-28	0.001	0.05
	PCB-52	0.001	0.05
	PCB-101	0.001	0.05
	PCB-118	0.001	0.05
	PCB-138	0.001	0.05
	PCB-153	0.001	0.05
	PCB-180	0.001	0.05

The GC-MS analysis of the procedural blanks showed that the materials and solvents used at different stages of sample preparation are free of contamination. On the other hand, limits of detection (LOD) and limits of quantification of the measured persistent organic pollutants (POPs) ranged between (0.25 – 0.8 and 0.02 to 0.1) ng/g wet weight (ww), respectively. As there were no contaminations in the blanks the recorded values were not subjected to blank correction.

4.2. Biological parameters

The sampled mother composed of age range of 21 to 40years with mean age of 29 years. All of them are unemployed housewives engaged in fishery and agriculture. Their feeding habit shows that seafoods such as fish, mussels and shrimps form a part of their daily meal. This population had a recorded number of pregnancies reaching gestational age (parity) between 1 to 16 children. During sampling these mothers were lactating children of varying age. The average age of the children was varied from 1to 21 months.

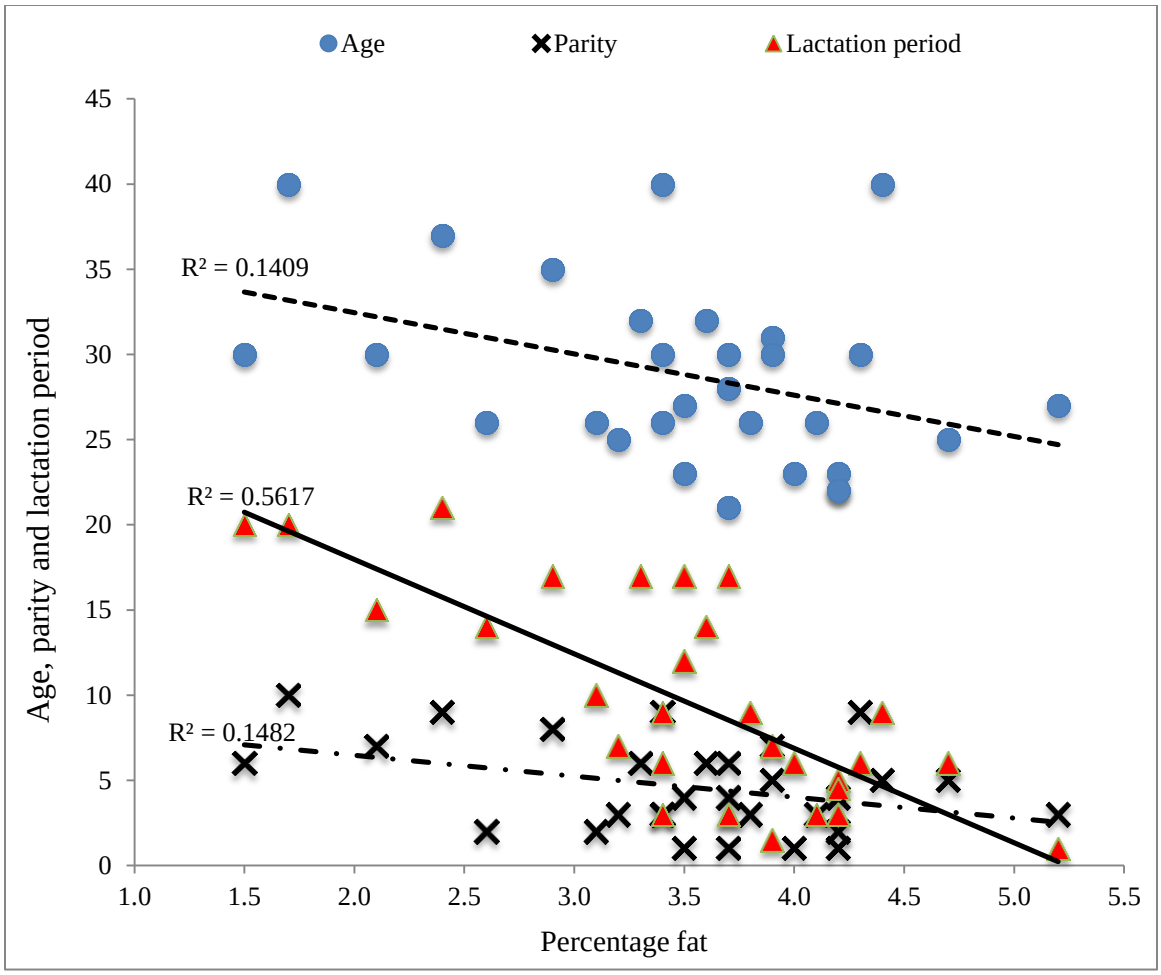

Figure 4.1 Variation of fat content with maternal age, parity and lactation period

Analysis of the sampled milk gave mean fat content of 3.5% varying between 1.5 to 5.2%. A simple linear regression analysis revealed that the fat contents of the milk decrease significantly with mothers ages (p= 0.054), parity (p=0.0322) and lactation period (0.0026). Among the three parameters (Figure 4.1), lactation period showed highest goodness of fit with fat content (R^2= 0.5617) followed with age (R^2 = 0.2532) and then parity (R^2 = 0.1590). The observation suggests that decrease in fat content in milk is more pronounced with prolongation of lactation period. This observation is in agreement with (Paulaviciene *et al*. 2020)_but also contrary to what reported by (Lukacka *et al*. 2018). In general differences in fat variations with prolonged lactation period have been

widely observed and have been reported to depend on mothers feeding behavior and sampling method used to collect milk samples (Puentes *et al*. 2019).

4.3. Persistent organic pollutants

The analysis of persistent organic pollutants revealed the presence of 31 pollutants belonging to two major categories of persistent organic pollutants, namely organochlorine pesticides (OCPs) and polychlorinated biphenyl (PCBs).

4.3.1. Organochlorine pesticides (OCPs)

A total of 24organochlorinepesticides and their metabolites were well measured above the method detection limits. The pesticides fall into three main groups including Hexachlorohexanes (HCHs), Dichlorodiphenyltrichloroethane's (DDTs), and cyclodines. Analysis of samples also revealed presence of other OCPs which do not directly belong to HCHs, DDTs and cyclodienes. These pesticides include Hexachlorobezene (HCB) and pentachloroanisole (PCA). The detailed data is given in Appendix 4.1.

4.3.1.1 HCHs

The measured HCHs included four isomers α, β, γ, and δ. Among the HCHs, α- HCH isomer was most dominant in terms of detection frequency by being presented in all samples. The other isomers; β-HCH, γ-HCH, and δ-HCH were measured in 87%, 97%, and 13% of the samples respectively. The- HCH was measured at concentration ranging from 0.51 - 8.3 µg/kg lipid mass (lm) and mean concentration of 2.82 µg/kglm. The highest concentration was detected in sample S7 and the lowest in sample S18. The β-HCH concentration was up to 19 µg/kglm whereas concentrations of γ-HCH and δ-HCH

were up to 16 and 2.1µg/kg lm respectively. The descriptive statistics of the isomers that were measured at higher frequencies is given in the Table 4.2.

Table 4.2: Descriptive statistics of concentrations (µg/kg lm) of HCHs

Parameter	N	Min	Max	Med	Mean	SD	%CV
α-HCH	30	0.51	8.3	1.7	2.82	2.18	0.88
β-HCH	26	0.36	19	3.15	0.19	5.68	109
γ-HCH	29	0.36	16	3.1	0.16	3.98	98

The measured HCHs isomers gave a total concentration (ΣHCH_4) ranged from 0.55-43.53µg/kg lm (Figure 4.2), with the mean concentration value of 12.53 ± 10.61µg/kg lm. The highest concentration of $\Sigma HCH_4$was measured in sample S7 while the lowest total concentration was in S29.

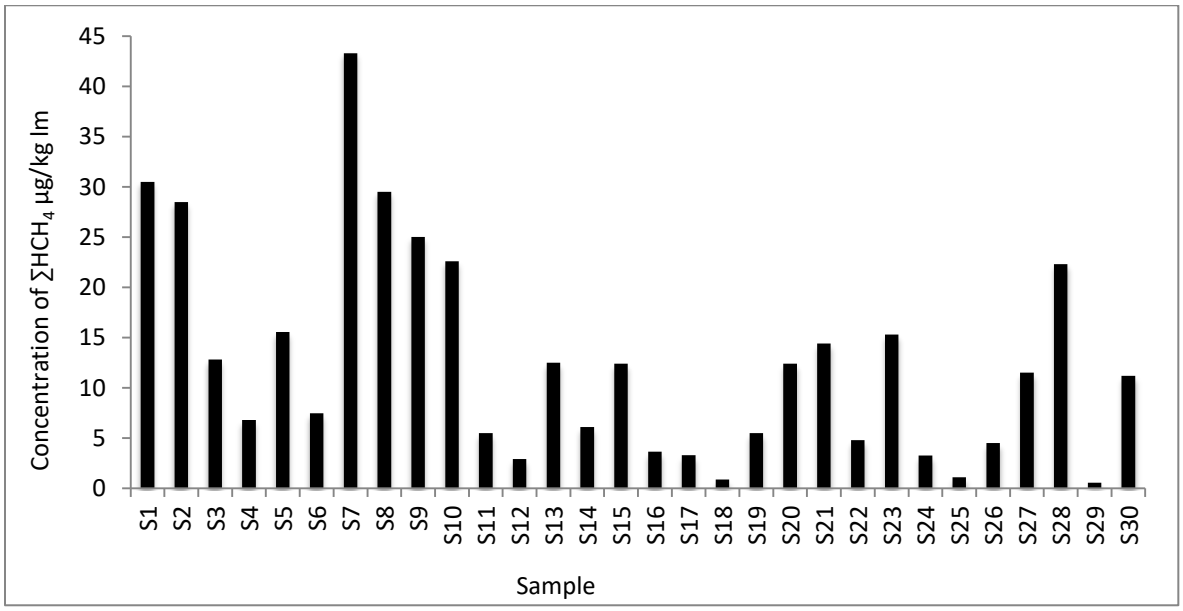

Figure 4.2 Concentrations of Total HCHs (ΣHCH)

4.3.1.2 Composition of HCHs

Analysis of composition gave the following contribution trend to total HCHs; γ-HCH>β-HCH>α-HCH>δ-HCH. The average composition of HCHs isomers are presented Figure 4.3

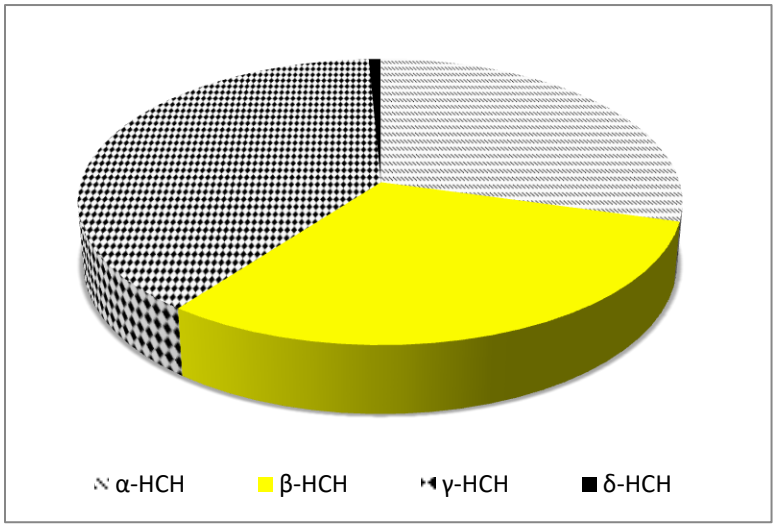

Figure 4.3 Compositions of HCHs

Hexachlorocyclohexane is used in agriculture and public health programs in technical or pure lindane formulation. The technical formulation consists mainly of α- HCH, β-HCH and γ –HCH isomers and lindane formulation consists of almost exclusively of γ-HCH isomer (Saleem & Ahmed 2001). Among the isomers, γ –HCH is less persistent and can be easily biotransformed and eliminated and therefore not expected to stay much longer in biological system compared α- HCH and β-HCH (Kunisue *et al*. 2002).

In this study, γ-HCH dominated the other isomers with average contribution of 39% (range 0 – 66.67%) followed by β-HCH which contributed 30.89% (0 – 83%) of the total HCHs. The isomer β-HCH was followed closely with α-HCH which had a mean contribution of 29.36% (7.1 – 100%) to the total HCHs. The contribution of δ-HCH ranged between 0 – 8.9% of total HCHs with mean value of 0.74%. The ratios of α-/γ-

HCH ranged from 0.02 to 1.91 with mean value of 0.42 indicating low proportion of α-HCH and high proportion of γ-HCH. Therefore, composition and ratio of α-/γ-HCH indicate the dominance of γ –HCH over the other isomers. This relatively high proportion of γ-HCH followed by β-HCH and low proportion of α-HCH implies combination of recent exposure of lindane and past exposure of technical HCH (Tsydenova *et al*. 2007)

Dominance of γ-HCH followed by β-HCH has been also reported in human milk from Arusha Tanzania (Muller *et al*. 2017) and the trend was associated with recent exposure to lindane (Muller *et al*. 2017). The elevated levels of β-HCH in human milk can be explained by the metabolization of γ-HCH to β-HCH (Willett *et al*. 1998) or intensive past exposure of technical HCHs (Minh *et al* 2004 and Tsydenova *et al*. 2007).

4.3.1.3 DDTs

Analysis of DDT pollutants in the human milk samples revealed presence of both fresh and metabolites of ortho-para (o,p) and para-para (p,p) isomers. The fresh p,p′–DDT was measured in all samples and o,p′-DDT was detected in 83 % of all milk samples. The mean concentration of p,p′-DDT was 6100 µg/kg lm ranging between 10000 to 2400µg/kg lm whereas concentration of o,p′-DDT was up to 4100 µg/kg lm. The highest concentrations of both fresh DDT isomers were measured in milk sample S7.

On the other hand, four DDT metabolites including o,p′-DDE, o,p′-DDD, p,p′-DDE, p, p′-DDD, and p,p′-DDMU were detected. The p,p′-DDE was the most abundant metabolite followed by p,p′-DDD and the remained metabolite were rarely detected in milk samples. p,p′-DDE was measured in all samples at mean concentration of 5199 µg/kg lm and at concentration range of 970 - 12000 µg/kg lm. The p,p′-DDD metabolite

was detected in 90 % of the analyzed milk samples and its concentrations were up to 3400 µg/kg lm. The highest concentrations of both p, p′-DDE, p,p′-DDD metabolites were measured in sample S7. On the other hand o, p′-DDE, o, p′-DDD, and p, p′-DDMU were found in only one (3 %) sample. The metabolites o,p′-DDE, o,p′-DDD, and p,p′-DDMU were found in the sample S7 only at concentrations of 80, 6.8 and 1.2 µg/kg lm. The descriptive statistics of the measured concentrations of fresh DDT and the major metabolites are presented in Table 4.3.

Table 4.3: Descriptive statistics of concentrations (µg/kg lm)of DDTs

Analyte	N	Min	Max	Med	Mean	SD	%CV
p,p'-DDE	30	970	12000	5000	5200	1853	48.47
p,p'-DDD	27	510	3400	1000	1220	641	65.28
o,p'-DDT	25	160	4100	760	920	756	96.94
p,p'-DDT	30	2400	10000	6000	6100	1705	40.31

The total concentration of seven members of DDTs group (ΣDDT_7) is presented in Figure 4.4. The concentrations ranged from 6400-30,000µg/kglm, with the mean value of 13,000 ± 4267µg/kg lm. The highest total DDT concentration was measured in sample S7 where the lowest was in S30

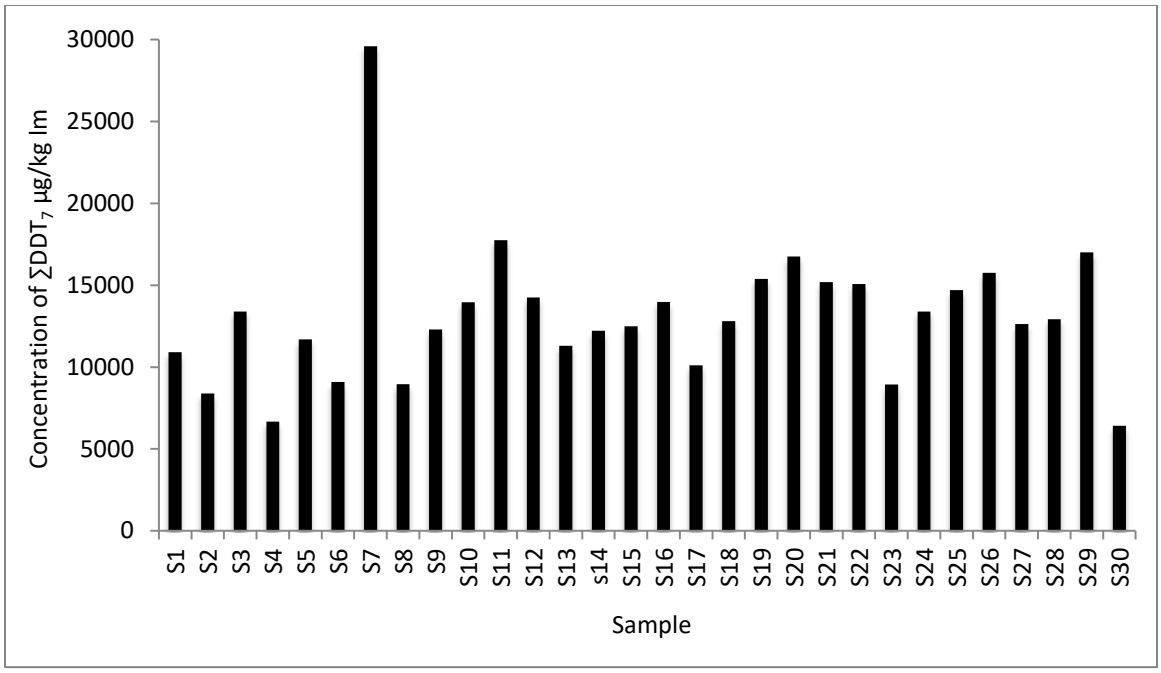

Figure 4.4: Concentrations of Total DDTs (ΣDDT)

4.3.1.4 Composition of DDTs

Different compositions of the measured fresh isomers and metabolites in human milk have been widely used to assess whether the contaminants are associated with fresh exposure or past exposure. Any composition or ratio showing dominance of parent molecules (p,p′-DDT and o,p′-DDT) indicated recent exposure while dominance of metabolite indicate past exposure (Linderholm *et al*. 2010, Muller *et al*. 2017, Jaga & Dharmani 2003 and Solomon & Weiss 2002).

In this study the DDT group was composed of seven members belonging to two parent and five metabolite molecules. The mean percentage contributions trend of the concentration is p,p',-DDT(46.68%)>p,p'-DDE(39.21%)>p,p'-DDD(8.73%)>o,p'-DDT(5.36%)>o,p'-DDE(0.009%)>o,p'-DDD(0.0008)>p,p'-DDMU(negligible). The parent molecule p,p′-DDT dominated the group by contributing between 33.79% and

60.84% of the total concentration of DDTs followed closely by p,p',-DDE which accounted 15.13 to 51.33 % of the total concentration of DDTs group. The average contributions of the individual members of DDTs are presented in Figure 4.5.

This dominance of fresh DDT over the metabolite is also indicated by percentage of the two fresh DDT isomers (p,p',-DDT + o,p',-DDT) in total DDT which ranged from 39.82% to 72.85% (mean = 52.04%).

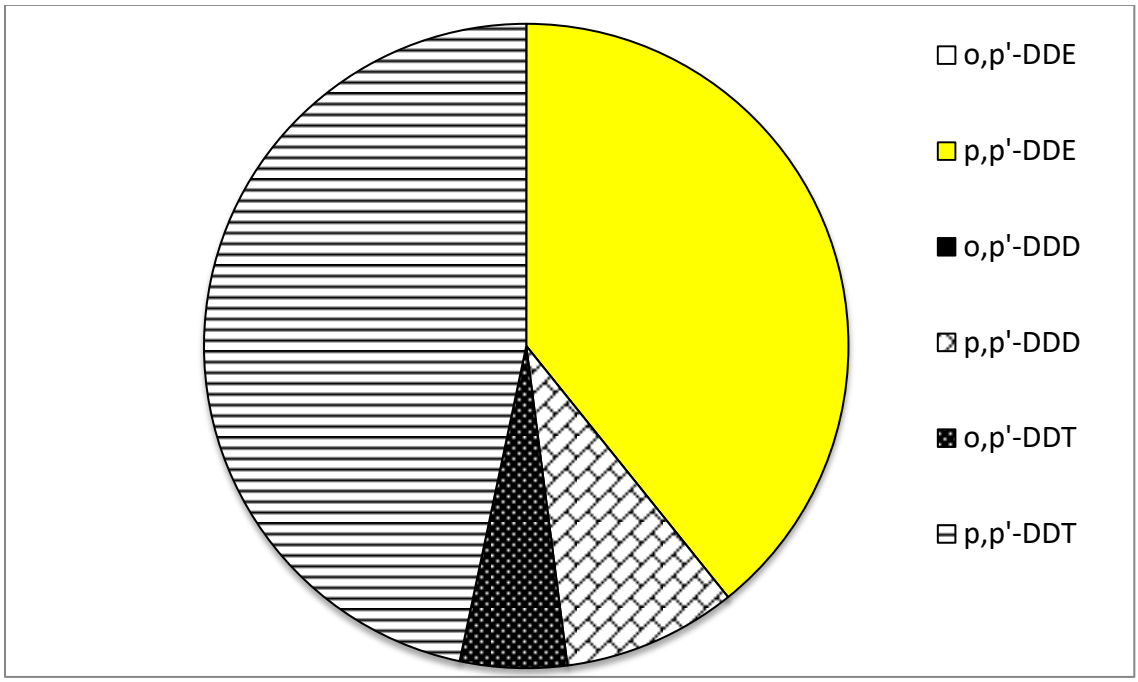

Figure 4.5 Compositions of DDTs

Similarly, the ratio of p,p'-DDE/p,p'-DDT ranged from 0.25 – 1.29 with mean value of 0.86. The mean value less than one shows dominance of parent molecules over the major metabolites (Linderholm *et al*. 2010). In general, the composition pattern and the calculated ratios indicate significant presence of fresh DDT compared to metabolite and therefore suggest recent uptake/exposure of the contaminants in human body. Such prevalence of parent metabolites in human milk has been reported elsewhere (Bouwman

et al. 1990, Saleem & Ahmed 2001 and Muller *et al*. 2017) and has been reported to reflect relatively recent exposure of the mother to DDT from foodstuffs or through direct exposure. On contrary dominance of metabolites has been reported in areas where mothers have affected by past exposure to DDT (Fång *et al*. 2015 and Hu *et al*. 2021).

4.3.1.5 Cyclodienes

The chlorinated cyclodienes detected in this study are composed of drins (aldrin, dieldrin and endrin), Chlordanes (CHLs) {cis(α-)chlordane, trans (γ-) chlordane, oxy- chlordane,, heptachlororepoxide, trans-nonachlor] and endosulfans (α- endosulfan, β –endosulfanand endosulfansulphate).The descriptive statistics on showing their detection frequencies, concentration ranges and samples with highest concentrations are presented in Table 4.4.

The drins group was predominated with by dieldrin interms of both detection of frequency and concentrations followed by far by aldrin. Dieldrin contributed to an average of 98% to total drins. For the samples that were detected with both aldrin and dieldrin the aldrin/dieldrin ratio ranged from 0.02 – 0.06 indicating very low contributins of aldrin. Both aldrin and dieldrin are insecticides that have been extensively used in Tanzania to protect building from termites and in agriculture (Mwevura 2007). Dieldrin is very persistent and hydrophobic (Ritter *et al*. 1999) while aldrin can be rapidly converted into dieldrin in soil, plants and mammals (Bann 1956). The predominance of dieldrin in this study is likely associated with the recent exposure of both aldrin to dieldrin followed with rapid conversion of aldrin to dieldrin and or exposure of the chemicals from aged sources.

Table 4.4: Descriptive statistics of concentrations (µg/kg lm) of cyclodienes

Cyclodiene Group	Individual members	Detection Frequency (%)	Concentration Range (µg/kg lm)	Sample with highest Concentration
Drins	Aldrin	13	<LOQ – 1.4	S23
	Dieldrin	53	<LOQ – 88	S7
	Endrin	3	<LOQ – 0.68	S7
Chlordanes (CHLs)	α-chlordane	30	<LOQ – 4.3	S7
	γ-chlordane	3	<LOQ – 0.46	S7
	oxy- chlordane	53	<LOQ – 9.7	S7
	Heptachlorepoxide	37	<LOQ – 3.3	S2
	trans-nonachlor	17	<LOQ – 5.1	S1
Endosulfans	α-endosulfan	83	<LOQ – 6.1	S11
	β-endosulfan	87	<LOQ – 5.2	S18
	Endosulfansulphate	67	<LOQ – 2.7	S18

Chlordanes were generally measured at low concentrations compared to other cyclodines. Both their detection of frequencies and concentrations depicted the following trend oxy-lk,/lchlordane>heptachlororepoxide>α-chlordane> γ-nonachlor>γ-chlordane.This trend shows predominance of two common metabolites of CHLs (oxy-chlordane and heptachlororepoxide). The metabolites contributed to 68.7% of the total concentration of CHLs. Fresh technical chlordane is composed of trans-chlordane (24%), cis-chlordane (22%), heptachlor (10%), and trans-nonachlor (7%) (Hinckley *et al*. 1990). Formation and predominance of the two metabolites in human milk reflects exposure to aged sources of CHLs. The exposure to aged source is also evidenced by the mean ratio of oxychlordane to trans-nonachlor of 2.1(Fujii *et al*. 2012)

Endosulfans were the cyclodienes that were detected at highest frequencies but at lower concentrations compared to the other groups of cyclodienes. The endosulfans group was

dominated by α-endosulfan followed by β –endosulfan and endosulfansulphate. Endosulfan is the insecticide found in technical form consisting of 70% α-endosulfan and 30% β –endosulfan however, as technical mixture is aging in the environment, α-endosulfan dissipates relatively faster than β–endosulfan (Jia *et al*. 2009). In the human body, both isomers are expected to undergo oxidation to endosulfan sulfate (Casabar *et al*. 2006). Calculation of ratios of α-endosulfan to β–endosulfan in this study ranged between 0.5 – 2.25 (mean value = 1.22) indicating aging as there is a decrease of proportion of α-endosulfan from technical mixture. Presence of endosulfansulfate in more than two-third of the analysed samples (67%) is a clear evidence of the endosulfans in the human body.

Total concentrations of the 11 detected cyclodines ($\Sigma Cyclodine_{11}$) are presented in Figure 4.6. The concentrations of $\Sigma Cyclodine_{11}$ ranged between n.d–116.14 µg/kg lm with mean concentration of 17.22 µg/kg lm. The highest concentration was measured in milk sample S7 and the lowest in samples S14 and S17

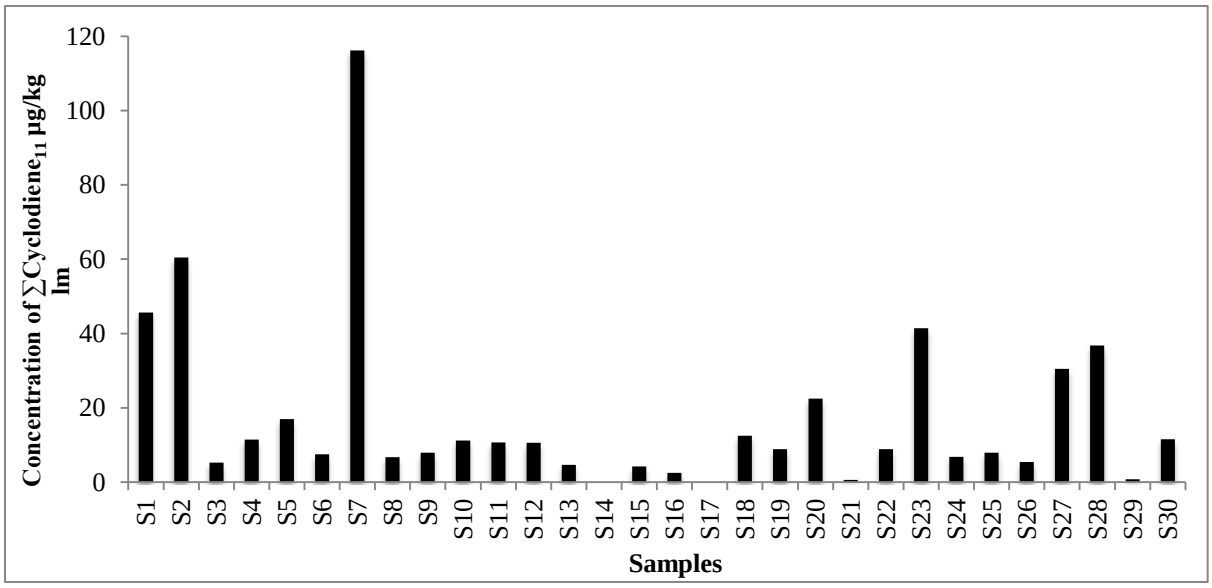

Figure 4.6: Concentrations of total cyclodienes (Σcyclodines)

The contribution trend of cyclodienes groups to the total concentration of cylodines is drines (62.4%)>endosulfans (23.8%)>CHLs (13.8%). The distribution trend is normally governed by their availability within the exposure route and physic-chemical properties such as hydrobhobicity. The majorities of drins are more hydrophobic than the other cyclodines (Ritter *et al*. 1999) and therefore can preferably partition into fat compartment of the body such as human milk.

4.3.1.6 Hexachlorobenzene and Pentachloroaniline

Hexachlorobenzene (HCB) was widely detected in all milk samples at mean concentration of 22.74 µg/kg lm and concentration range of 3.5-56µg/kg lm. The highest concentration of HCB was detected in sample S7 while the lowest was found in sample S26.

Table 4.5: Descriptive statistics of concentrations (µg/kg lm) of HCB and PCA

	N	Min	Max	Med	Mean	SD	%CV
HCB	30	3.5	56	35.5	22.7	11.39	60
PCA	26	0.16	18	4.50	5.01	3.90	91

Pentachloroaniline (PCA) was detected in 87% of the analyzed samples. The concentrations of the metabolite ranged between 0.16-18µg/kglm with its highest concentration detected in sample S7 and the lowest in sample S15. The descriptive statistics of HCB and PCA are presented in Table 4.5 and their concentrations are presented in figure 5

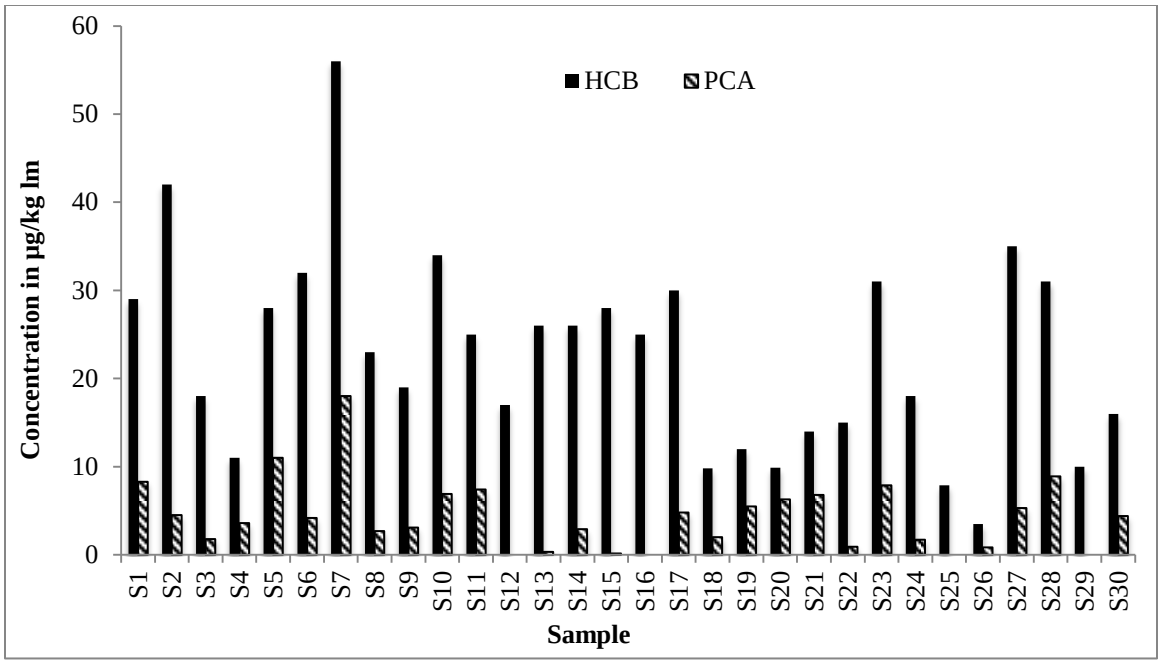

Figure 4.7: Concentrations of HCB and PCA

HCB has high bioaccumulation potential because of its high Kow and high persistence in biota. Presence of HCB in humans is associated with consumption of contaminated of meat and seafood from environmental sources (Sandanger *et al*. 2003) the well known source of HCB in the environment is related its past use as fungicide for seed grains and as a by-product of various manufacturing and combustive process. The other reported source is through biotransformation of HCH isomers to HCB. Use of technical HCH can be more plausible explanation on the presence of HCB in environments that have no industries (Behrooz *et al*. 2008). PCA is degradation product of pentachloronitrobenzene its presence in the environment and then to the food is obviously related to use of pentachloronitrobenzene.

4.3.1.7 Total OCPs

The overall organochlorinepesticide load (ΣOCP_{24}) in the milk is summarized in Figure 4.8. This pesticide load was largely dominated by DDTs which contributed to 99.5% of

the OCP load. The pesticide load in the milk varied from 6453.17 – 29821.44 µg/kg lm with mean concentration value of 13189.77±4283 µg/kg lm.

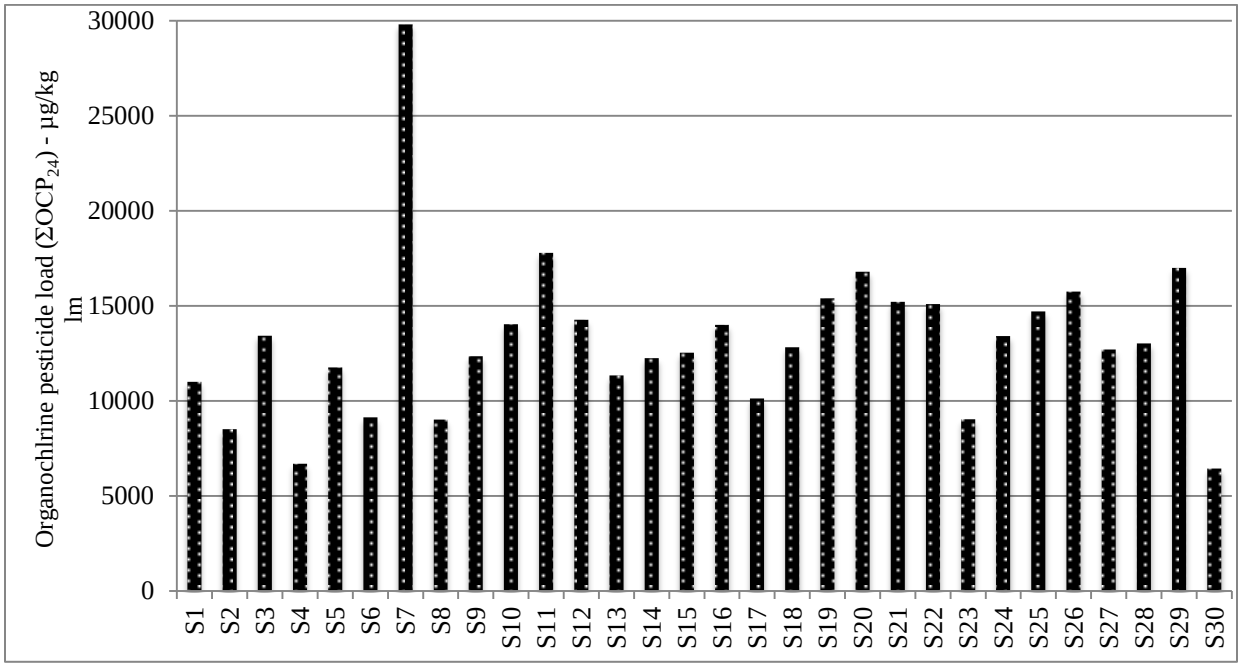

Figure: 4.8: Concentrations of total organochlorine pesticides (ΣOCP)

Sample S7 was found to carry the highest OCP load while the lowest pollutant load was measured in sample S30. All OCP groups including HCHs, DDTs, cyclodines, HCB and PCA were measured at extraordinary high concentrations in S7 compared to other samples. In this regards the concentrations of S7 were regarded as outliers and were not included in statistical analysis.

4.3.1.8 Composition of OCPs

Organochlorine pesticides were highly dominated by DDTs followed by far by HCB, cyclodines, HCHs and PCA. DDT grouped accounted for 98.40 to 99.93% with average contribution of 99.54%. Other groups of OCPs had contribution of less than one percentage. The composition is summarized in Figure 4.9. The extraordinary dominance of DDTs over the other OCP groups is likely due to their extensive use in agriculture and

public health and high persistence. Consequently, they are very ubiquitous in our environment and are very common contaminant in food including sea foods which form a major exposure route of POPs to human. Reviews of on the levels and profiles of POPs from different regions have revealed notable high levels of DDT compared to other OCPs. The prevalence of DDTs in almost all studies was reported to reflect their presence in diet of mothers (Hu, *et al*. 2021, Fang *et al*. 2015, Guerranti *et al*. 2011 and Tanabe & Kunisue 2007). Other OCPs for instances HCHs and PCA are less persistence and they can be degraded relatively easier in both abiotic and biotic environments. Consequent their presence in food and is relatively less than DDTs (Hu *et al*. 2021).

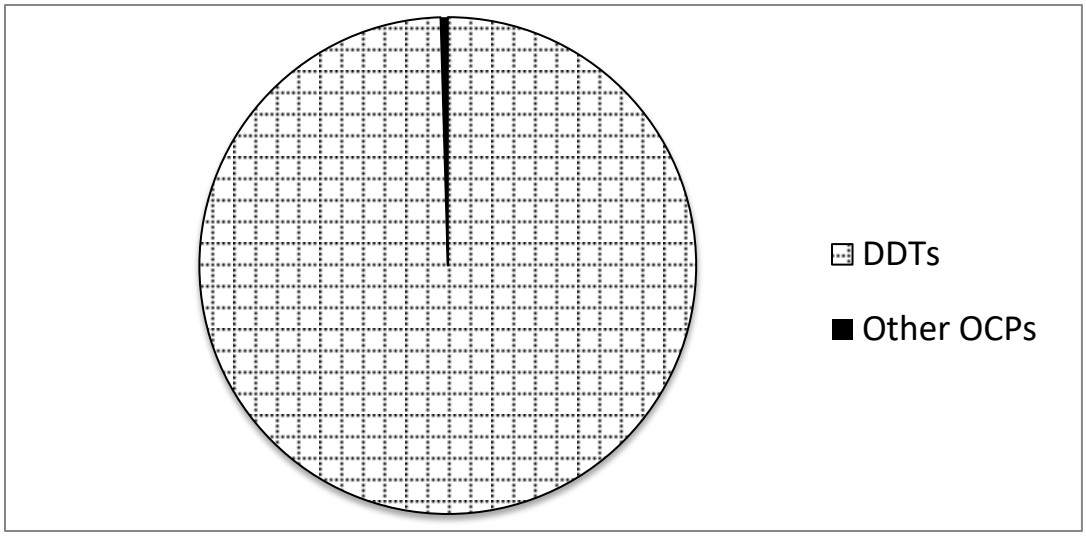

Figure 4.9: Composition of total organochlorine pesticides (OCPs)

4.4. POLYCHLORINATED BIPHENYL (PCBs)

The analysis also revealed presence of PCBs in samples. A total of seven PCB congeners were detected with varying detection frequency, and concentration among the analyzed sample. The detected PCBs in this study included PCBs 28, 52, 101, 118, 138, 153 and 180. Except PCB 118 all detected PCBs are known as indicator PCBs and they are most often determined in the environment due to their presence in commercial mixtures

(Brajenovic *et al*. 2018). Furthermore, PCBs 28, 52, 101,118,153 and 180 were detected at trace levels at different frequencies but the concentration values were below LOQ. Descriptive statistics of the measured congeners covering there detection frequencies, concentration ranges and sample with highest concentration is provided in Table 4.6 and the detailed data is presented in Appendix 3.

Table 4.6: Descriptive statistics of concentrations (µg/kg lm) of PCBs

PCB	Detection Frequency (%)	Concentration Range (µg/kg lm)	Sample with highest Concentration
PCB 28	77	<LOQ – 8.618	S2
PCB 52	53	<LOQ – 2.918	S7
PCB 101	93	<LOQ – 3.618	S7
PCB 118	97	<LOQ – 5118	S5
PCB 138	100	<LOQ – 5218	S3
PCB 153	90	<LOQ – 3618	S29
PCB 180	80	<LOQ – 1818	S2

Distribution of the measures PCBs in the milk samples is given in Figure 4.10. The distribution indicates elevation of PCBs 118, 138 1ne 153 in some of the milk samples.

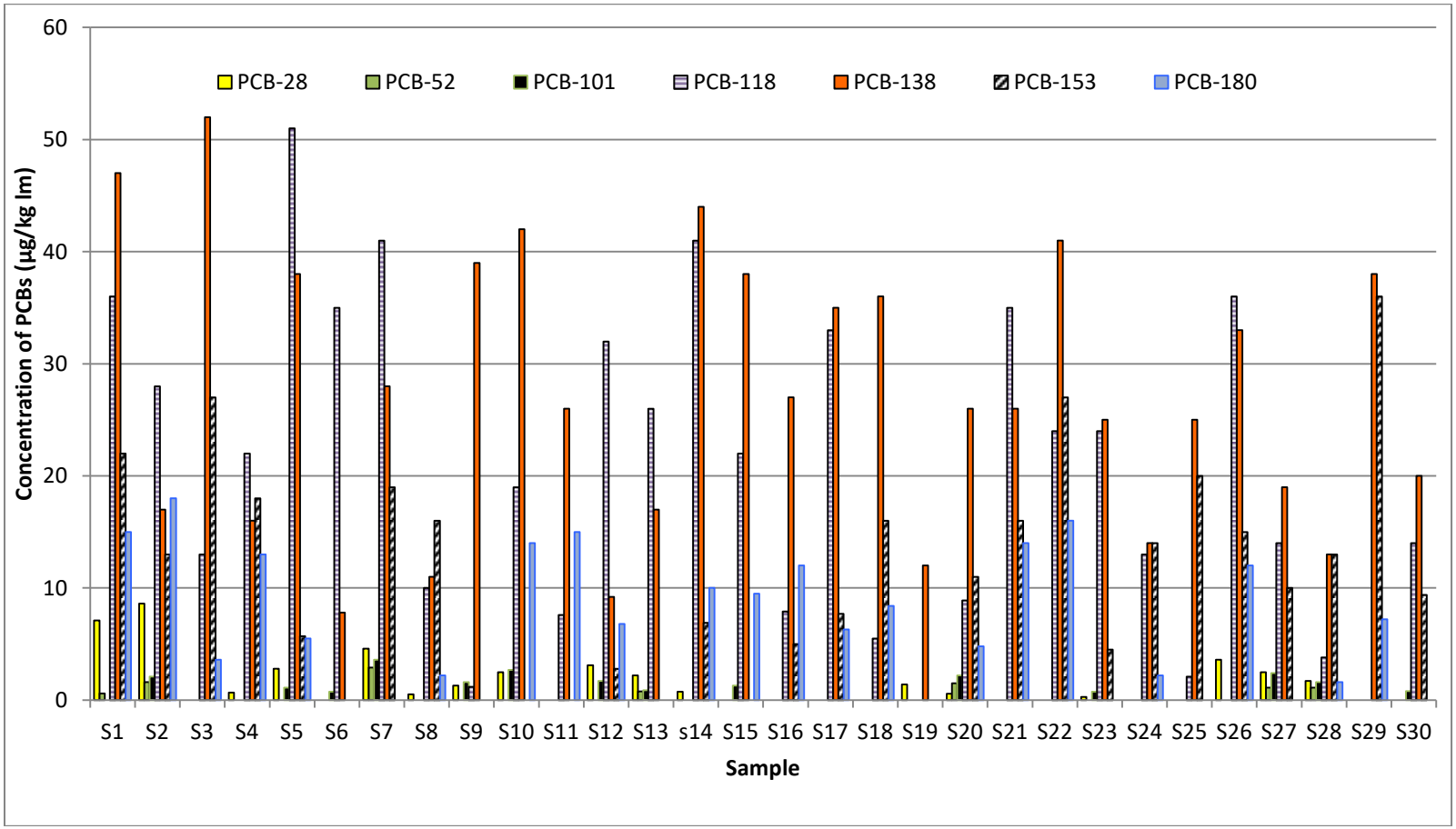

Figure 4.10 Concentrations of PCBs in Milk samples

The measured PCBs resulted to the total PCBs (ΣPCB_7) concentration range of 13 – 128 µg/kg lm with mean concentration of 68±27.2 µg/kg lm. The distribution of ΣPCB_7 is presented in Figure 4.11 the highest value was measured in sample S1 where the lowest was in S19. Other milk samples with more than 100 µg/kg lm are S22, S5 and S14.

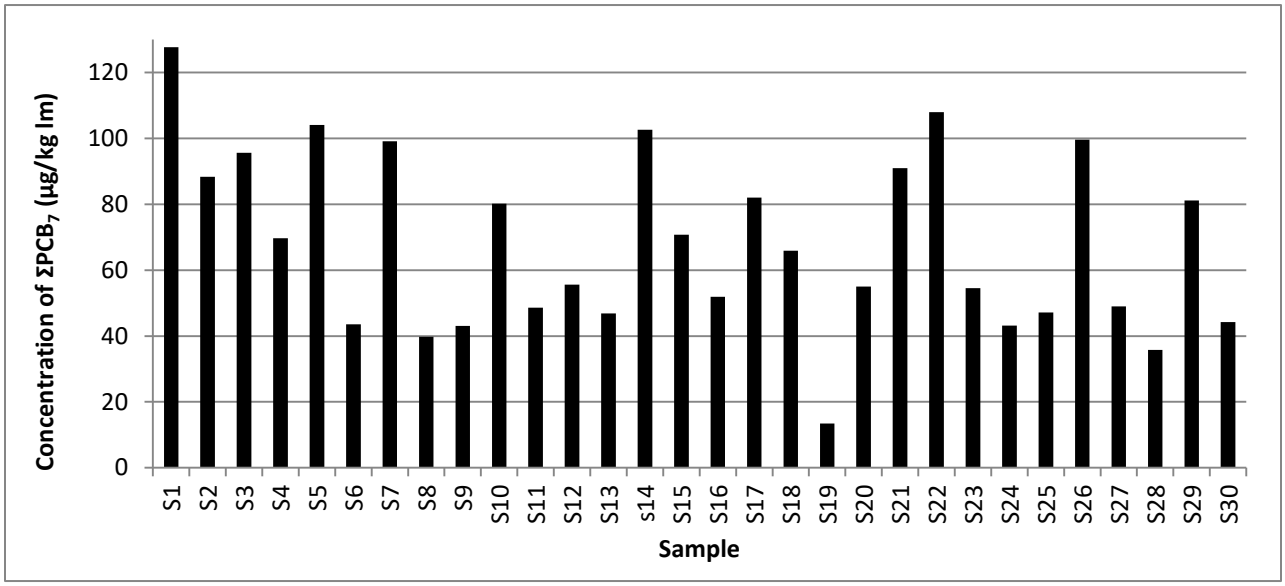

Figure 4.11: Distribution of ΣPCB_7 in Milk Samples

4.4.1 Composition of PCBs

Composition of measure individual PCBs to the concentration of total PCBs is presented in Figure 4.12. The figure shows that, among the measured PCBs, PCB 138 dominated other residues in terms of concentrations followed closely by PCB 118 and PCB 153 whereas the PCB-52 had the lowest contribution of total PCBs. The overall contribution trend of PCBs is PCB-138>PCB-118>PCB-153>PCB-180>PCB-28>PCB-101>PCB-52.

The dominance of PCB-153,180,138 is, due to its high persistence and low environmental degradability, as well as its characteristic of being difficult for organisms to metabolize (McFarland & Clarke 1989).

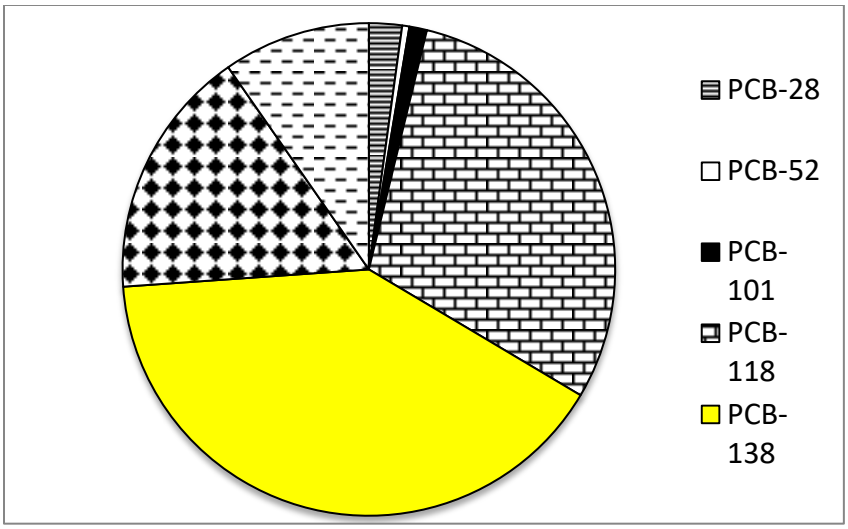

Figure 4.12: Composition of PCBs

4.5 COMPARISON WITH RELATED STUDIES

Types and levels of POPs measured in breast milk in this study were compared with similar study reported elsewhere to reveal the status of human milk contamination at Wesha Pemba. Pollutants (PCBs, DDTs, HCHs, HCBs and cyclodienes) found in this study consist of all common types of POPs reported in human milk worldwide (Fång, *et al*. 2015). These POPs are considered ubiquitous in human milk and their ability to bioaccumualte in fat-rich parts of the body is facilitated by their high persistence and hydrophobic nature (Bernes 1998). The only analyte that was found in this study but not commonly reported in other studies is PCA. Presence of PCA in 80% of the analyzed human milk (0.16-18µg/kg) indicates the exposure of the chemical to mothers and that the chemical can potentially accumulate in human tissues. Tables 4.7 Presents summarized comparison of the concentrations of POPs reported in this study with other related studies from different parts of the world.

Table 4.7: Comparison of concentrations (µg/kg lm) of POPs in breast milk

Country	**ΣDDT**	**ΣHCH**	**HCB**	**ΣCyclodiene**	**ΣPCB**	**References**
Zanzibar	6400 – 30000 13320*	0.55-43.53 12.53*	3.5 –56 22.72*	LOQ – 116.14 17.22*	12 - 128 27.2*	This study
Tanzania	24 - 2400	-	-	937	157	Muller *et al*. 2017
Tunisia	125.8 – 4574.8	63 – 247.6	24.1 – 1470	2 -529	16.4 -1360.2	Ennaceur *et al*. 2007, Hassine *et al*., 2012
Egypt*	517.32	47.97	9.26	-	-	Salem and Ahmed. 2001
Iran*	2554	3780	390	-	1560	Behrooz *et al*. 2008
China	870 – 3600	550 – 1400	56 – 81	6.7 – 16	28 – 42	Haraguchi *et al*. 2009
Japan	97 – 340	49 -210	8.1 – 18	17 – 85	79 - 240	Haraguchi *et al*. 2009
Korea*	180	110	13	14	61	Haraguchi *et al*. 2009
Russia	660 – 1400	410 – 858	77 – 100	19 – 59	240 - 370	Polder *et al*. 1998
Vietnam	1200 -2300	14 – 140	2.5 – 7.4	0.75 – 6.9	74 -84	Minh *et al*. 2004
Taiwan*	333	3.4	NR	10	NR	Chao *et al*. 2005

Indonesia	640 – 1300	14 – 30	1.8 – 2.2	2– 7.7	24 - 33	Haraguchi *et al*. 2009
Australia*	480	190	12	14	NR	Mueller *et al*. 2008
Italy*	1024	NR	38.66	NR	532.87	Guerranti *et al*. 2001
UK*	150	40	17	NR	150	Johnson-Restrepo *et al*. 2007
USA*	64	19	2.3	32	NR	Kalantzi *et al*. 2004

* Levels are presented in mean concentrations

NR = Not reported in the surveyed literature

The concentrations of ΣDDTs measured in this study are much higher than those reported in breast milk from other parts of the world. For instance, the concentration ranges from this study are several folds higher than reported levels in human milk Tunisia, China, Russia, Vietnam and Indonesia. Similarly, the mean value of ΣDDT in this study is 20, 4 and 10 times higher the mean values reported in human milk from Egypt, Iran and Italy respectively (Table 4.7). On the other hand the levels found in this study was lower than those reported in human milk (160 – 140,000 µg/kg lm) from mothers staying in villages where DDT sprayed indoor (Bouwman *et al*. 2012)

The concentrations of ΣHCHs from the current study are much lower than those reported in human milk from many parts of the world (Table 4.7). The differences are likely reflecting the use pattern and intensity of HCHs. Consumption of HCHs in Zanzibar is very small in terms of quantities compared to other parts of developing worlds (Mmochi & Mberek 1998). However, these reported concentrations of ΣHCH are comparable to those reported in milk from Indonesia (Haraguchi *et al*. 2009) but higher than the concentrations recorded in Taiwan (Chao *et al*. 2005)

Comparisons of measured concentrations of HCB reported in this study shows that levels found in human milk from Wesha Pemba are generally lower than those reported from Tunisia, Iran, China, Russia and Italy but comparable with the levels from Egypt, Japan, Korea and UK (Table 4.7). The levels are however higher than the levels from USA, Australia and Vietnam. From Table 4.7 the concentrations of Σcyclodines from this study are comparable and are within the high concentration ranges report in milk from Australia, Taiwan Russia and Japan. But the levels are lower than those reported in milk from Northern Tanzania, Tunisia and USA.

The PCBs reported in this study depicted opposite trend to what was observed for ΣDDT for being within lowest ranges or much lower compared to those reported from other parts of the world (Table 4.7). The differences in levels might be attributed by differences in use and exposure extent to human. The primary route of exposure to PCBs in human body is the consumption of contaminated foods, particularly meat, fish, and poultry. These foods get contaminated from PCBs generated from different sources. The main sources of PCBs are electrical devices such as transformers, capacitors, and incineration of municipal waste. PCBs are also released as a by-product of very high temperature industrial processes. Within the study area the only potential source is electrical devices that were stored within the former Pemba power plant; whereas developed countries have various active sources which contribute to continuous exposure.

4.6 Correlation among POPs

The groups of POPs that were measured at high frequencies include DDTs, HCHs and PCBs. All POPs are hydrophobic and are capable to bioaccumulate in fat-rich tissues however HCH is less hydrophobic compared to DDT and PCBs. Linear regression analysis between POPs revealed positive association of ΣPCBs ($P = 0.0555$) with ΣDDTs and significant negative association of ΣHCHs ($P= 0.0357$) with ΣDDT. The association is presented in log-transformed figure 4.13. This association suggests that both PCBs and DDTs which are very hydrophobic and persistent are originated from the same sources. Many studies have revealed that human milk from coastal communities is contaminated with elevated levels of persistent as the feed on fat-rich sea food. However the load of less persistent and less hydrophobic in human milk is less because mothers can degrade and depurate such chemicals (Hu *et al*. 2021).

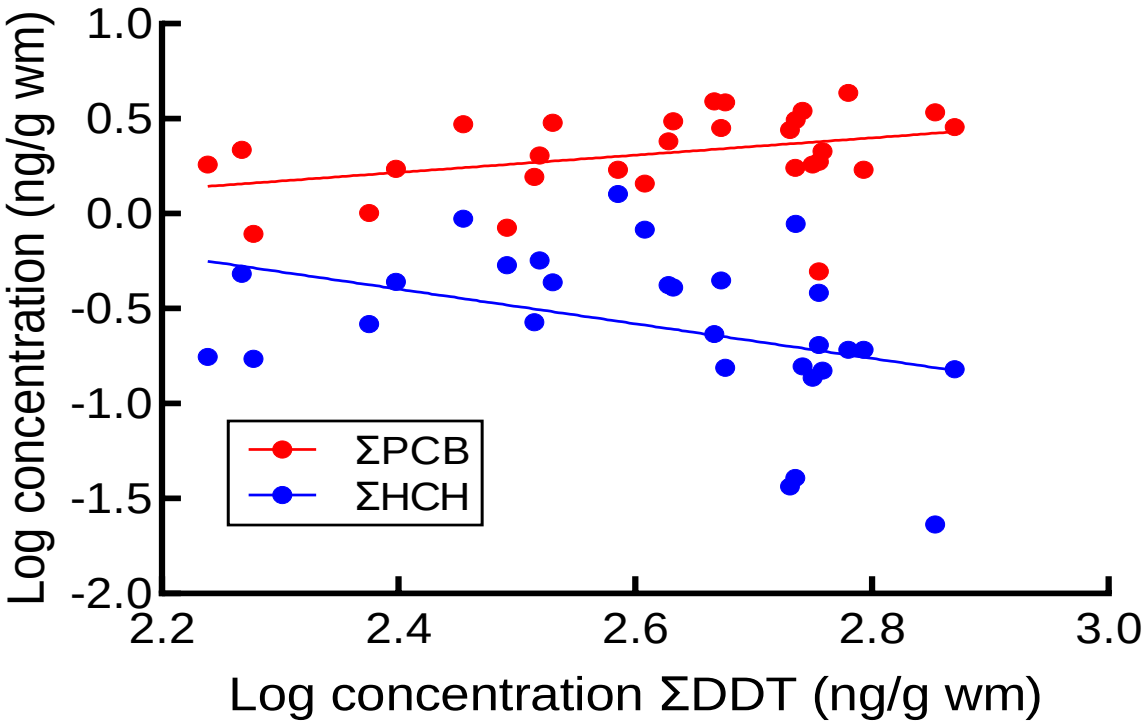

Figure 4.13 Correlation among measured POPs

4.7 Variation of POPs with fat content

The analyzed milk had different fat contents and varying concentrations of the measured POPs. Regression analysis of the three major groups of pollutants showed significant increase of levels of ΣDDTs ($p < 0.0001$) and ΣPCBs ($p= 0.0138$) with the increase of fat content (Figure 4.14) while ΣHCH decreased slightly with fat content. As mentioned earlier that both DDTs and PCBs are very hydrophobic and therefore will preferably partition to the fat. In contrary, HCHs are less hydrophobic and less persistent the properties that reduce their ability to bioaccumulate. In this study ΣHCH was significantly contributed by γ-HCH which is the least persistent and least hydrophobic among the HCHs isomers. This might account for the observed slight negative variation between ΣHCH and fat contents. However increase of both ΣHCH and DDTs has been

reported in human milk from Poland but HCH in Poland study was dominated by β-HCH which is more hydrophilic and fat soluble than γ-HCH (Witczak *et al*. 2021)

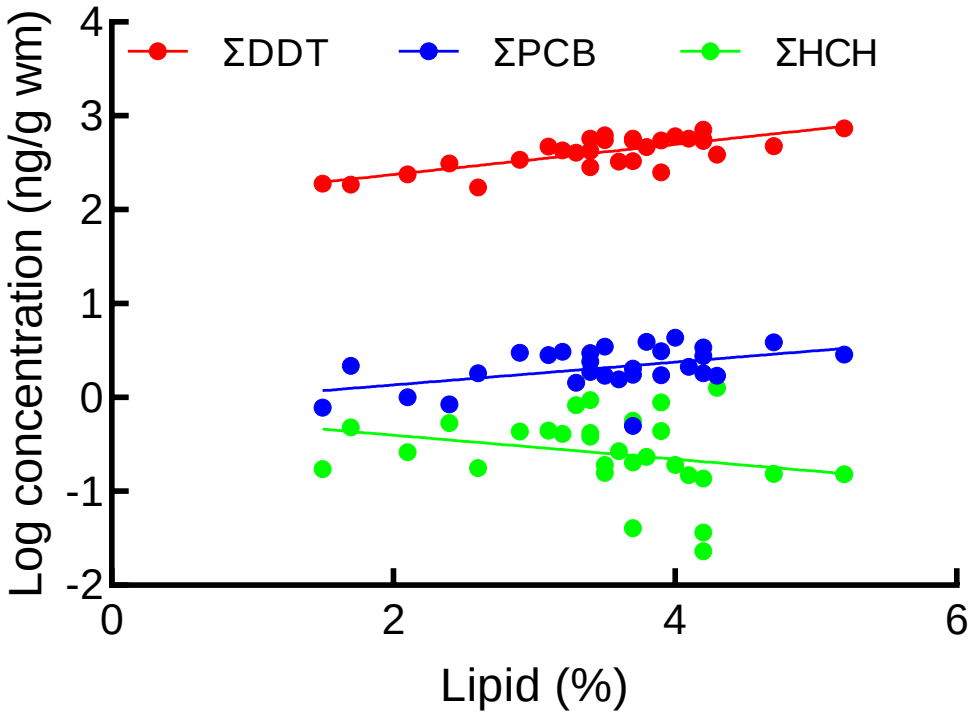

Figure 4.14: Variations of ΣDDT, ΣPCBs and ΣHCH with Fat conten

4.8 Variation with Lactation period

Lactating children of the sampled mothers were of different ages varying from 1 to 21 months. Analysis of pollutants load with lactation period in this study showed minor decrease of pollutant load with lactation period of the children (Figure 4.15, slope = -0.0052). Since mothers are offloading the pollutants through lactation, it is expected that mother's pollutants load will be decreasing with the age. Transfer of significant amount of POPs from mother to offspring has been established to occur during gestation and lactation but the extent is more pronounced during lactation. It has been estimated that

mammals such as dolphin can transfer as much as two third of the total burden of OCPs to their young ones through lactation (Kajiwara *et al*. 2008 and Mwevura *et al*. 2010).

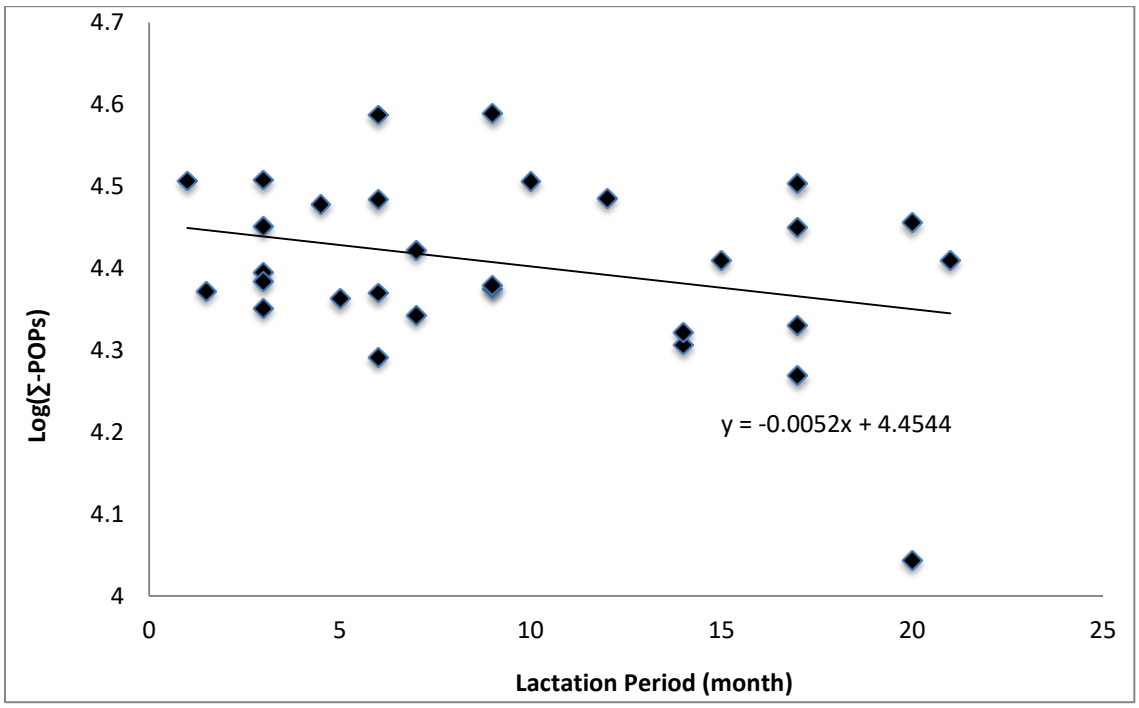

Figure 4.15 Variation of POPs with lactation period

Decrease in concentration is with lactation period is a clear indication of transfer of pollutants from mother to off spring although not significant as estimated in earlier studies. The results might be hampered by the heterogeneity of samples because the background pollutant loads were not the same to all lactating mothers. Similar trend of decrease in concentrations of pollutants with lactation period has been reported in milk from Poland (Witczak, 2021).

4.9 Variations of POP level in Human milk with maternal physiological factors

The human milk samples were donated by mother of different ages and parity, the factors that have been reported elsewhere to influence level of POPs (Mishra & Sharma 2011 and Hassine *et al*. 2012). In this study the total pollutant load in samples were correlated

with maternal ages and parity using individual members of most detected POPs and then overall pollutant load as presented in Figures 4.16 – 4.17.

4.9.1 Variation of pollutants with maternal age

A variation of individual organochlorines with maternal age is presented in Figure 4.16. The figure shows that concentrations of DDTs (parent isomers and metabolites) and PCBs (PCB 138 and PCB 153) decreased with age while HCB and HCHs (α- HCH and β-HCH) increase with age. The differences in observed trend may reflect that decrease in recent exposure of DDTs and PCBs sources, and continuous exposure of HCB and HCHs at different ages.

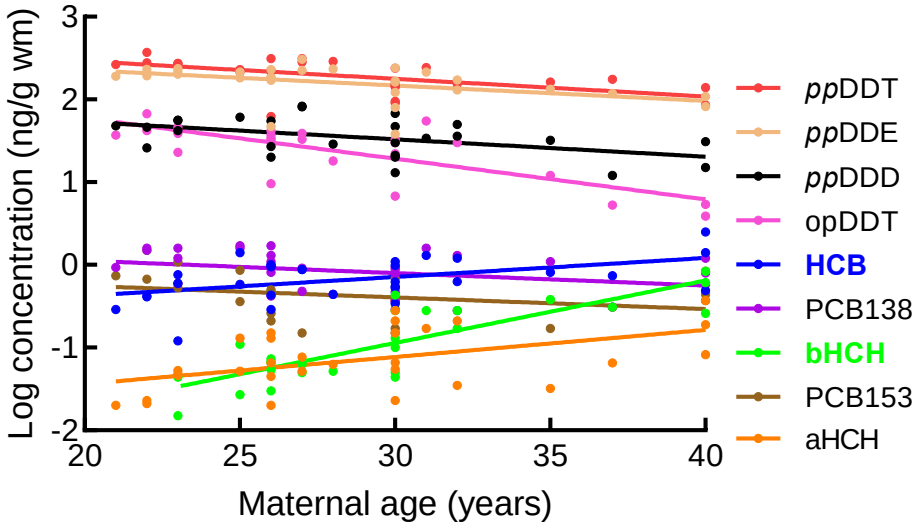

Figure 4.16: Variations of individual POPs with age

A close analysis of the distributions of the measured pollutants within different ages (maternal age groupings) using Non-metric Multidimensional Scaling (NMS) lower maternal ages of 20 -30 years are more rich in DDTs, endosulfans and PCBs while HCHs, HCB, Drins and CHLs are more prevalent at higher ages of 30-40 and above 40

years (Figure17). This confirms that as mothers become older DDTs, PCBs and endosulfans decrease, and the rest increase.

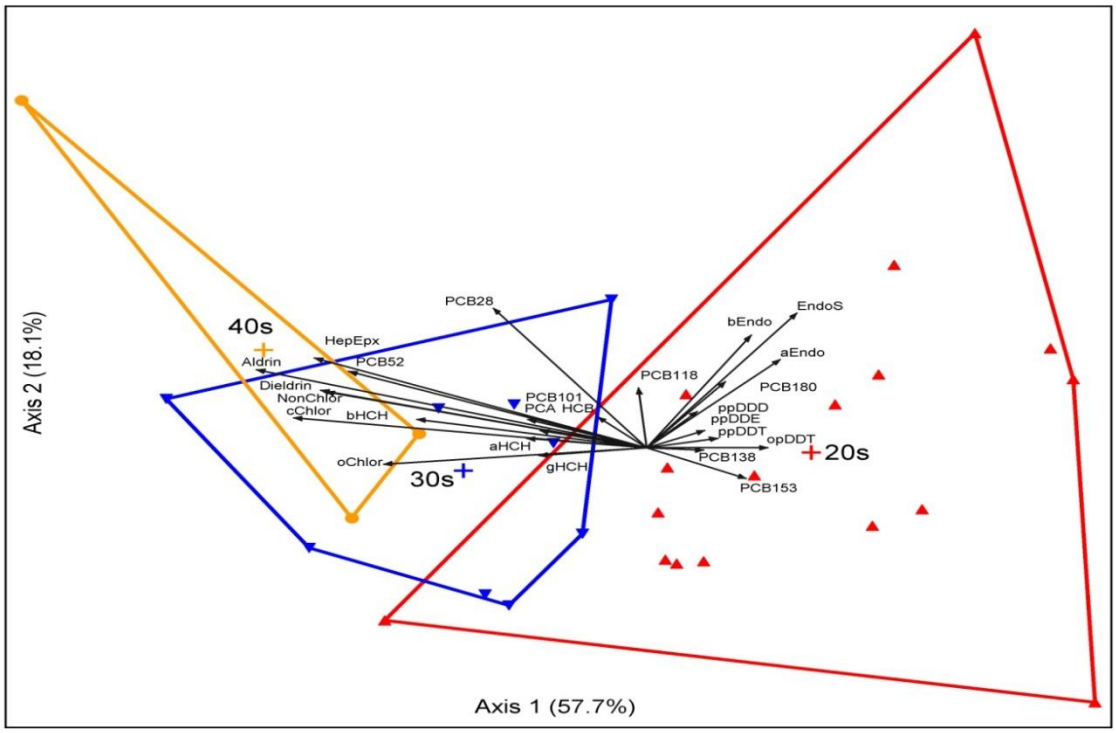

Figure 4.17: Non-metric multidimensional scaled ordination of changes of POPs concentrations with maternal age

Considering variations of overall pollutants with maternal ages, the regressions analysis showed that overall pollutant load (∑-POP) decreased significantly (P = 0.0426) with maternal ages (Fig 4.18A). The overall was likely influenced by decrease of all DDTs which were measured at higher concentrations than the other POPs. These finding indicate that the total POPs body burden of mothers decrease as they become older. Similar trend of decrease in pollutant body burden from young to adult female has been reported in other mammals such as dolphin and the trend has been associated with maternal transfer of pollutants through both placental and lactation transfer (Mwevura *et al*. 2010).

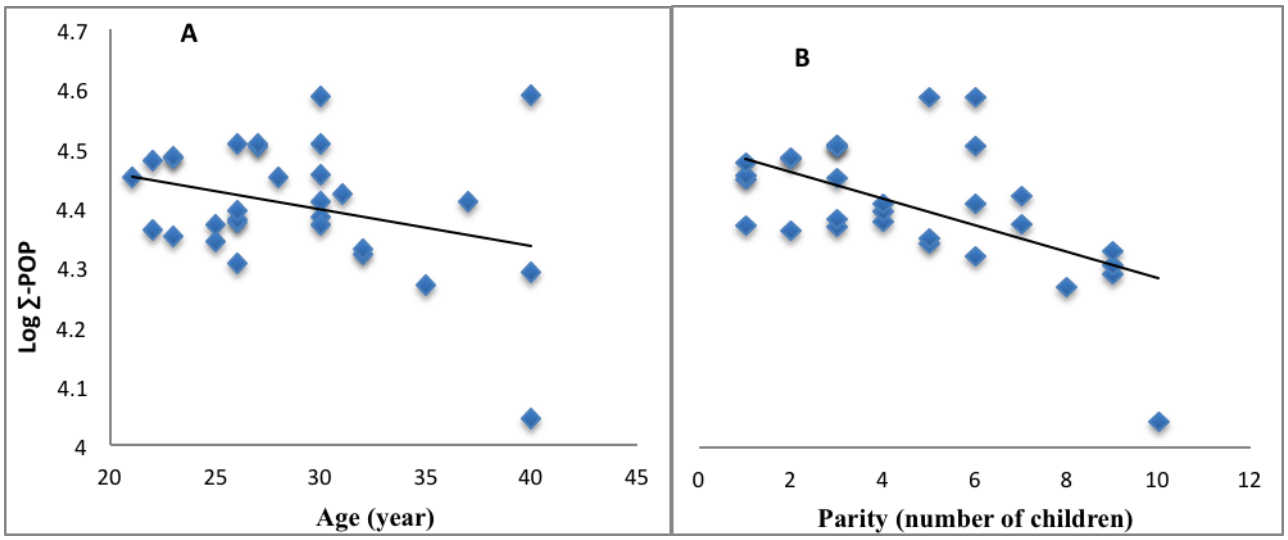

Figure 4.18: Variation of overall pollutant load with age (A) and parity (B)

4.9.2 Variation of pollutants with Parity

Analysis of variation of individual pollutants with number of times that mother has given birth (parity) depicted similar trends to those of age (Figure 4.19). The concentrations of all DDT parent molecules and metabolites as well as PCBs decreased with parity but HCHs and HCB increased with parity. The observed trend can be plausibly explained by span of exposure to contaminates which mainly depend on types of food consumed by mothers (dietary). Increase in pollutant levels with has been liked with continuous exposure by consuming contaminated food while decrease in levels with parity has been associated with absence of exposure and banning of the chemicals (Sudaryanto *et al*. 2006)

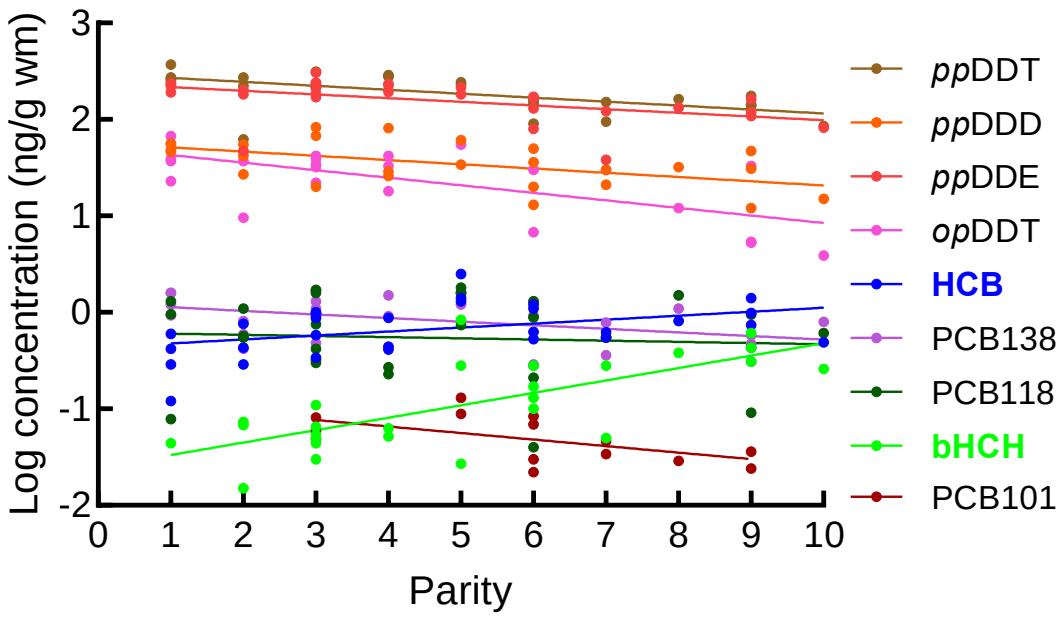

Figure 4:19 variations of individual POPs with maternal parity

A detailed investigation of the distribution of individual pollutants with the parity group (Parity: 1, 2-4, 5-7, and 8-10) using NMS ordination (Figure 4.20) depicted the similar distribution to that shown by age. Lower parities (1 and 2-4) are predominated with DDTs and PCBs while the rest dominated higher parities (5 – 7 and 8 – 10). This distribution indicates that as parity increases from right to left, the proportional contributions of the DDTs, PCBs and endosulfans in milk decreases with an increase in parity towards the left. Thus higher parities have increasing concentrations of chlordanes, dieldrin and aldrin, HCHs, and PCA.

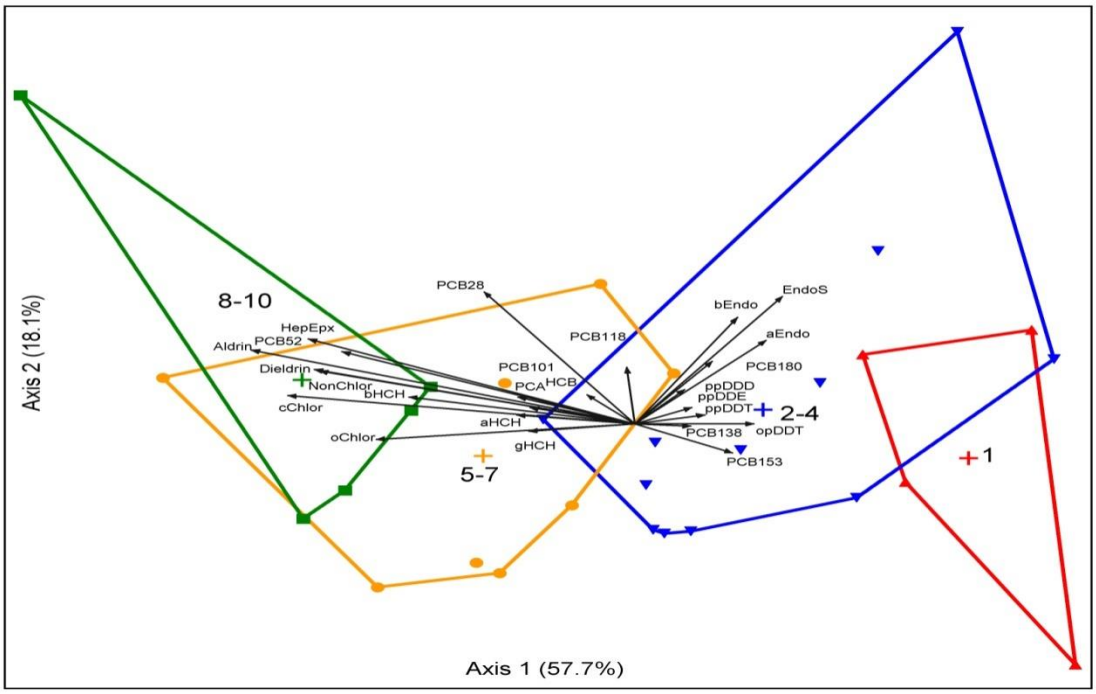

Figure 4.20 Non-metric multidimensional scaled ordinations of changes of POPs concentrations with parity

Assessment of the variation of ∑-POP with parity (Figure 4.19 B) showed significant decrease of the pollutants with parity (P = 0.0286). Like the case of age, the overall decreasing tend was influenced by higher concentrations of DDT than the other POPs. There have been a number of studies that have investigated variations of POPs in human milk with parity worldwide and reported different variation trends. Most of the studies had similar observations of decrease in pollutant burden and thus milk from Primipara mothers have significantly higher levels of POPs than multipara (Malarvannan *et al*. 2009, Bouwman *et al*. 2006, Sudaryanto *et al*. 2006 and Ennaceur *et al*. 2007). Decrease in POPs burden with parity is due to elimination of contaminants via previous periods of lactation and therefore offload a significant portions of their pollutant burden (Haraguchi

et al. 2009 and Mwevura *et al*. 2010). The observations also support the phenomenon that first born infant is likely to receive much higher pollutant load than their sibs (Harris *et al*. 2001 and Bouwman *et al*. 2006). With this regards, lactation is considered as the major route of offloading pollutants burden from the lactating mothers.

Some revealed absence of any strong and direct relations between pollutant concentrations and parity. They either showed weak relation of decrease and/or increase of organochlorines with parity. Increase of concentration with parity has been described to be the effect of continuous intake of organochlorine while decrease as a consequent of maternal transfer coupled with lack or banning of you sources of exposure to human (Devanathan *et al*. 2009, Fång *et al*. 2015, and Hu *et al*. 2021).However, most of the studies that showed no relation with parity have been reported to be hampered by the heterogeneity of the sampled mother.

4.10 Health risks Assessment

Breast milk is considered to be the necessary feeding options for infants as it provides almost all of the essential nutrients and improves infant growth and development (Lorenzetti *et al*. 2021) However accumulation of different kinds of POPS to elevated levels threatens the health of infants at their crucial development stages. Types and levels of toxic chemicals in this study are obviously carry health hazards to breast-fed infants. Concentrations of the pollutants provide ways of analyzing hazards to infants associated with breast-feeding by calculating hazard quotient (HQ) and hazard index (HI). While HQ values indicate health hazard of the single contaminant HIs provide a cumulative health hazard of all contaminants in the human milk. Results on analysis of risk hazard quotient and hazard index from the determined concentrations are presented in Table 4.8.

Table 4.8: Exposure daily Intake (EDI; mg/kg day/bw) Hazard quotient (HQ) of the measured POPs

Parameter	HCB	∑-HCH	DDTs	PCBs
Exposure daily Intake	0.017 - 0.345 (0.111)	0.003 - 0.267 (0.059)	24.28 - 181.72 (965.55)	0.067 - 0.610 (0.333)
Acceptable daily Intake	0.27	0.3	20	1
Hazard quotient	0.064 - 1.278 (0.410)	0.011 - 0.889 (0.196)	1.214 - 9.086 (3.28)	0.067 - 0.610 (0.333)

The calculated exposure daily intake (EDI) of HCHs and PCBs ranged from 0.003 to 0.267 and 0.067 to 0.610 mg/kg day/bw, respectively. All EDI values are below the acceptable daily intake and the HQs ranges is 0.011 - 0.889 for HCHs and 0.067 - 0.610 PCBs. These results suggest no health hazards to infants with respect to HCHs and PCBs contamination in the human milk as the HQ values are less than 1. For the case of HCB the calculate EDI varied between 0.017 - 0.345 mg/kgday/bw and only one out of 30 human milk samples exceeded the ADI. The corresponding HQs for HCB ranged from 0.064 to 1.278 suggest presence of health hazard to infant belong to mother S7. On the other hand, the all calculated EDI values for DDTs (24.28 - 181.72 mg/kgday/bw) exceeded the acceptable (20mg/kgday/bw). Their corresponding HQ values ranged from 1.214 to 9.086 with mean value of 3.28 indicating presence of potential health hazards to all infants with respects to DDTs.

HIs which give indication of cumulative health hazards of all existing toxicants in human milk were calculated at mean value of 4.22 ranging between 1.70 and 11.86. These values indicated that all infants from sampled mothers are exposed to potential health hazards

associated with the levels of POPs. More than half (56.7%) of infants from sampled mother are exposed to more four times the acceptable levels of toxicants. The exposure to health hazards might be more than what is reported as some of the POPs (cyclodines and PCA) were not included in calculations of HQ and HIs. The situation is worst to infant belong to mother S7 who is exposed to nearly 12 times the tolerable levels of POPs.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMENDATIONS

5.1. SUMMARY

This study investigated human milk from Wesha Pemba coastal community. The milk samples were analyzed for lipid content and levels of different types of POPs. The obtained data were assessed for their composition, correlations, and variations and compared with levels measured in similar studies to reveal their source and status of contamination. The data were also assessed for their associated health risks to breast-fed infants. The investigations of the samples revealed the following major findings:

The lipid contents measured in the human milk of the sampled population varied from 1.5 to 5.2 and found to decrease significantly with maternal age, parity and lactation period. This indicates that as the maternal age and lactation cycles increase and breastfeeding prolonged, the milk becomes less in fat content.

A total of 31 persistent organic pollutants belong to OCPs and PCBs categories were identified in the analyzed human milk. OCPs were extraordinary predominated by DDT groups while all PCBs were ortho-congeners. Among the DDTs, the parent p,p'-DDT had highest contribution followed by p,p'-DDE metabolite indicating recent exposure of DDT (continuous exposure). The ratios of α/ γ HCHs (0.02- 1. 91) and their Percentage composition indicate the dominance of γ–HCH which imply recent exposure to lindane rather than technical mixture. Cyclodines were dominated with dieldrin suggesting past exposure of aldrin which is converted to dieldrin or recent exposure of aged source of dieldrin. Other cyclodine members had minimum contributions to the total concentration

of the group showing that the exposure sources have low concentrations of these cylodiene.

Comparison with related studies showed that, the levels OCPs measured in this study, particularly DDTs were much higher than the levels reported elsewhere. However, the DDT levels were found to be lower than the levels in human milk reported from South Africa. PCBs reported in this study showed opposite trend of being much lower than many reported levels from other parts of the World

Linear regression analysis between POPs revealed positive association of ΣPCBs with ΣDDTs and significant negative association with ΣHCHs with ΣDDT. This implies that ΣPCBs and ΣDDTs are likely shearing sources and degradation pattern in human body but their sources and metabolization properties differ with that of HCHs. Similarly, both ΣDDTs and ΣPCBs were significantly increased with lipid content while ΣHCH showed decreased trends with lipid content. The variations are likely attributed by higher lipophilic nature of DDTs and PCBs which makes these two pollutants to be favorably deposited into lipid compared to HCHs. In contrary, DDTs and PCBs were found to decrease significantly with lactation period, maternal age and parity whereas the HCHs depicted increased trend with lactation period, maternal age and parity. Decrease in trend of DDTs and PCBs is obviously linked with decrease in lipid content with lactation period, maternal age and parity. The findings also indicate that the first-born babies are exposed with the highest pollutant load from their mothers.

Exposure to POPs may obviously result to health problem to infants as they are delicate but the nature and extent of the problem depends on the toxic nature and amount of the

pollutants. Health risks assessment showed that HQ for DDTs and HIs for total pollutants load in all samples exceeded the acceptable level confirming possibility of potential health hazard to breast-fed infants. For instance, the HIs values were 1.70 to 11.86 times higher than the acceptable value.

5.2. CONCLUSIONS

From the summary of the findings the following conclusions have been drawn:-

I. Fat content of the human milk decreases with maternal age, parity and lactation period.

II. Human milk from Wesha is contaminated with several POPs including (OCPs and PCBs) and the levels were highly predominated by DDTs.

III. The measured pollutants are originated from continuous exposure of fresh DDT and γ–HCH pesticides

IV. Levels of OCPs are within the highest ranges of the POPs levels measured in other parts of the world while PCBs are within the lowest ranges.

V. DDTs and PCBs are originated in same sources and share similar degradation patterns in human milk.

VI. Accumulation of DDTs and PCBs was governed by lipid content and they decreased with lactation period, maternal age and parity.

VII. The first born child is likely to receive highest load of lipophilic pollutants from their mothers through breast feeding.

VIII. The measured levels of POPs carry potential risk hazards to breast-fed infants.

5.3. RECOMMENDATIONS

To the best of my knowledge, this is the first study to investigate levels of POPs in Zanzibar. Based on the finding of research the following recommendations are given:

- To extend this types of studies to other coastal communities of Zanzibar to reveal the status of POPs in Human milk in Zanzibar.
- The new studies should follow-up the diet of mothers to give clear understanding of the potential sources of these chemicals.
- The Ministry responsible for health should establish monitoring program on pollution of POPs in human milk to be able to give relevant advices to breastfeeding mothers.
- Following the nutritive and non-nutritive importance of breastfeeding, the feeding is still recommended, despite the presence of these harmful contaminants in breast milk. However, care should be taken and new feeding alternative should be adopted if there is a proven evidence of potential health hazards associated with breastfeeding.

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Appendex 1: Certificate of Consent

Certificate of Consent

I have been invited to take part in the research on Assessment of Persistent Organic Pollutants (POPs) in Human Milk at Wesha. I have been told the purpose and procedures of this assessment. In summary:

Rational and Purpose of the Research

Persistent organic pollutants (often called POPs) are organic compound that resist environmental degradation and stay longer in the environment without losing their toxic effect. These chemicals don't change very much over time and they often are found in fat-containing foods, such as fish, meat, eggs and milk. These chemical find their way into human through feeding. In pregnant and breast feeding female, significant portion of accumulated POPs are mobilized and transported with lipid to the mammary gland where they ultimately end up accumulated in the milk. These contaminants are then passed to the infant during breast feeding. While concerns about POPs have been raised, the evidence for the health advantages of breastfeeding has continued to increase. On a population basis, exclusive breastfeeding for six months is the recommended feeding mode for the vast majority of infants, followed by continued breastfeeding with appropriate complementary foods for up to two years or beyond.

Aim of this study is to assess levels of POPs in human milk and their associated health risks to infants. This kind of assessment reveals actual situation of the problem to and assist responsible authority to develop strategies of reducing the problem and advise the community. The assessment will also support and strengthen national capabilities for the monitoring and sound management of POPs in food.

Procedures

We are asking you to give one 15 ml sample of your milk. The milk can be collected using either manual expression or a breast pump. The sample will be collected at the most convenient way in your home Your sample will be analysed for selected POPs.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected from the survey will not have your name on it, but a number assigned to it instead. During sample collection you will be provided a

glass container marked at 15 ml and a form both will be assigned the same code. You will the hand in thecontainer with sample by pacing on the provided table on your form.

Regarding inadvertent disclosure during publication of results, the consequences are not expected to be significant because your results will not include your name, but will be identified by a code. In addition, only average (mean) results will be reported and not

Alternatives to participation

You do not have to take part in this research if you do not wish to do so, and refusing to participate will not be followed by any consequences. You may stop participating in the research at any time that you wish until your sample has been analyzed; if you choose to end your participation, you will not lose any of your rights as a community member.

Contact information

If you have any questions you may ask them now or later. If you wish to ask questions later, you may contact the following person:

1. Prof. Haji Mwevura, mobile: 0777 844 350, Email: haji.mwevura@suza.ac.tz
2. Nahija Haji Adam, mobile: 0773 520 746, Email: mamaamunta@gmail.com

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study until my sample has analysed. If I choose to withdraw from the study, I understand that I can do so without in any way affecting my right.

Name of Participant: _____ Signature: _____

Date: ___/___/___ (*dd/mm/yy*)

Name of Researcher: Nahija Haji Adam **Signature: _____**

Date ___/___/___ (dd/mm/yy)

Name of supervisor: Prof. Haji Mwevura **Signature _____**

Date ___/___/___ (dd/mm/yy)

FOMU YA RIDHAA YA KUSHIRIKI KATIKA UTAFITI

Hii ni kuthibitisha kuwa, nimealikwa kushiriki kwenye utafiti wa kutathmini kemikali zenye kudumu kwa muda mrefu kwenye maziwa ya binadam utakaofanyika Wesha. Nimeelezewa lengo na utaratibu utakaotumika kufanya utafiti huo. Kwa ufupi nimeelezwa yafuatayo:

Hoja na Lengo la Utafiti:

Kemikali zenye kudumu kwa muda mrefu ni kundi la kemikali ambazo hubakia kwenye mazingira kwa muda mrefu bila kubadilika maumbile yake na uwezo wake wa kudhuru. Mara nyingi kemikali hizo hupendelea kukaa katika vyakula vyenye mafuta kama vile samaki, nyama, mayai, maziwa n.k. Kemikali hizo huingia katika mwili wa mwanadamu kwa njia ya vyakula. Ndani ya mwili wa mama mjamzito, sehemu kubwa ya kemikali hizo hukimbilia kwenye maziwa kwa sababu ya wingi wa mafuta yaliyomo na hatimaye husafirishwa kwa mtoto wakati wa kunyonyesha. Wakati mjadala juu ya athari za kemikali kama hizo kwa mtoto ukiendelea, kumekuwa na ushahidi mzito juu ya faida ya kiafya ya maziwa ya mama kwa mtoto. Hii imepelekea ushauri wa kumnyonyesha mtoto kwa kipindi cha miezi sita mfululizo bila kutumia chakula chochote na kisha kuendelea kumnyonyesha mpaka miaka miwili huku akitumia na vyakula vyengine.

Lengo la utafiti huu ni kutathmini viwango vya kemikali katika maziwa ya mama na athari zake kwa mtoto anaenyonya. Utafiti wa aina hii katika jamii huibua hali halisi ya tatizo hilo na athari zake kwa mtoto. Hii husaidia kuweka mikakati ya kuishauri jamii husika ili kupunguza athari hizo.

Utaratibu

Mshiriki anaombwa kuchangia kiasi cha mililita 15 za maziwa yake. Maziwa hayo yatakamuliwa kwa kutumia mashine au kwa mkono na wewe uko huru kuchagua utaratibu utakaopendelea. Maziwa hayo yatatumika kutafuta aina na viwango vya kemikali zilizomo. Zoezi hili la ukamuaji maziwa litasimamiwa na mhudumu wa afya atakaepewa maelekezo.

Usiri

Taarifa zitakazokusanywa kwenye utafiti huu zitakuwa ni siri. Maelezo utakayotoa hayatowekwa jina lako na badala yake yatapewa namba ya utambulisho. Wakati wa kukusanya maziwa utapewa chombo cha kigae chenye alama ya mililita 15 pamoja na fomu itayokuwa na namba yako ya utambulisho. Baada ya kuweka kiasi kinachohitajika utafunga mfuniko wa chombo ulichopewa na kuweka juu ya fomu yenye namba yako ya utambulisho. Kuhusu usiri wakati wa kutoa machapisho, taarifa itayotolewa itaendelea

kutumia namba za utambulisho na hivyo hakutarajiwi athari yoyote kujitokeza.

Hiari ya kushiriki katika utafiti

Kushiriki katika utafiti huu ni jambo la hiari na unaweza kujitoa wakati wowote ule kuazia hivi sasa mpaka hapo maziwa yako yatakapofanyiwa uchunguzi. Maamuzi ya kujitoa yataheshimiwa na hayatofuatiliwa kwa namna yoyote ile. Aidha ukiamua kujitoa hutopoteza haki yako yoyote ya kijamii na kiraia.

Mawasiliano ya Msimamizi na mtafiti mwanafunzi

Ukiwa na swali lolote unaweza kuuliza hivi sasa au wakati wowote. Ukitaka kuuliza swali baadaye, tafadhali wasiliana na:

1. Prof. Haji Mwevura, simu: 0777 844 350, Barua pepe: haji.mwevura@suza.ac.tz

2. Nahija Haji Adam, : 0773 520 746, Barua pepe: mamaamumta@gmail.com

Nimesoma/Nimesomewa taarifa na nimepewa fursa ya kuuliza swali lolote nilokuwa naol. Na maswali yote niliyouliza yamejibiwa katika kiwango cha kuniridhisha. hivyo nathibitisha kuwa nimeridhia kushiri katika utafiti nikielewa kuwa ninahaki ya kujitoa katika utafiti huu kuanzia sasa hadi hapo sampuli yangu itakapokuwa imefanyiwa uchunguzi. Aidha naelewa kuwa pindipo nikiamua kujitoa maamuzi yangu yataheshimiwa na hayatofuatiliwa kwa namna yoyote ile na wala sitopoteza haki yangu yoyote ya kijamii na kiraia.

Jina la Mshiriki: _____ Saini: _____

Tarehe: ___/___/___

Jina la Mtafiti: Nahija Haji Adam Saini:_____

Tarehe: ___/___/___

Jina la Msimamizi: Prof. Haji Mwevura Saini:_____

Tarehe: ___/___/___

Appendix 2: Questionnaire

Questionnaire for Potential Human Milk Donors

Survey of Human Milk for Persistent Organic Pollutants

CONFIDENTIAL!

Individual Identification Code …… Age of mother ……..

1. Are you prepared to sign the consent form? Yes ☐ No ☐

If yes, attach signed consent form and if no, mother is not eligible to participate in survey.

2. Was your mother born in this country?

Yes ☐ No ☐

3. Were you breastfed? If you know, for how long? _____

Yes ☐ No ☐ Do not know ☐

4. Were you engaged in work other than housework before pregnancy? Yes ☐
No ☐

If yes, please state the duration and describe type of work :
…….

5. Where have you been residing during last 5 years:

urban (city) ☐ rural (countryside) ☐

6. Has the inside of your house been sprayed with DDT in order to prevent mosquitoes?
Yes ☐ No ☐ Do not know ☐

If yes, when? _____

7. How would you describe your dietary habits before pregnancy? Mixed diet ☐
Vegetarian but with milk and eggs ☐

Strictly vegetarian ☐ Other ☐

8. How often, on average, did you eat following foods before pregnancy?

	Fish and fish products (e.g. tuna salad)	Marine mammals (e.g. whales, dolphins	Seafood other than fish and marine mammals (e.g. shrimps, mussels)	Milk and milk products (e.g. cheese, butter, cream, yogurt)	Meat and poultry and derived products (e.g. sausage)	Eggs
Never						
Less than						
Once a week						
Twice a week						
More than twice a week but not every day						
Every						

9. What types of fish do you consume most often?

Fish from the sea ☐ Freshwater fish Both ☐

Please state the species if known: ……

10. How old is your infant?

less than 3 weeks* ☐ 3-4 weeks ☐ 5-8 weeks ☐ more than 8 weeks** ☐

11. Sex of the infant : Boy ☐ Girl ☐

Thank you for your partcicipation

MUONGOZO WA MAHOJIANO NA MAMA ALIYEKUBALI KUSHIRIKI KATIKA UTAFITI

Utafiti wa kemikali zenye kudumu kwa muda mrefu kwenye mazigira katika maziwa ya binadam

Siri!

Nambari ya utambulisho ya Mshiriki …… Umri wa mshiriki ……

1. Je umeshajaza fomu ya kuridhia kushiriki? Ndiyo ☐ Hapana ☐

Kama Ndiyo, aendelee na mahojiano haya, kama hapana asiendelee na mahojiano.

2. Je umezaliwa katika kijiji hichi cha Wesha?

Ndiyo ☐ Hapana ☐

Kama Hapana, taja kijiji ulichozaliwa? _____

3. Je umejihusisha na kazi nyengine yoyote mbali na kazi za nyumbani kabla na baada ya ujauzito?

Ndiyo ☐ Hapana ☐

Ikiwa Ndiyo, Tafadhali elezea aina ya kazi na muda ulitumia kwenye kazi hiyo:

…….....
.....

4. Wapi ulikaa katika kipindi cha miaka mitano (5) iliyopita?

Wesha ☐ Kijiji chengine ☐

Ikiwa kijiji chengine/vyengine, Taja kijiji hicho na muda uliokaa

Kijiji ..... Muda .....

Kijiji ..... Muda .....

5. Je nyumba uliyokaa katika miaka mitano hiyo ya mwisho iliwahi kupuliziwa dawa ya kuulia mbu?

Ndio ☐ Hapana ☐ Sijui ☐

Ikiwa Ndiyo, Taja mwaka wa kupuliziwa dawa _____

6. Vyakula vya aina gani ulipendelea zaidi kutumia kabla na baada ya ujauzito?

Chakula Mchanganyiko ☐ Mboga mboga, maziwa na mayai ☐

Mboga mboga tu ☐ Vyakula vingine ☐

Kama vyakula vyengine, taja vyakula hivyo .....

7. Taja wastani wa kula vyakula vifuatavyo ndani ya miaka mitano iliyopita?

	Samaki	Jamii ya Pomboo na nyangumi	Vyakula vya baharini (kamba, kaa, chaza)	Maziwa na bidhaa zake	Nyama/Kuku na bidhaa zake	Mayai
Sijatumia kabisa						
Mara moja kwa mwezi						
Mara moja kwa wiki						
Mara mbili kwa wiki						
Zaidi ya mara mbili kwa wiki lakini sio kila siku						
Kila siku						

8. Mtoto wako unayemnyinyesha ana umri gani? Umri: .....

9. Huyu ni mtoto wa ngapi kumnyonyesha? .....

10. Taja jinsia ya Mtoto unayemnyonesha? Mume ☐ Mke ☐

Ahsante kwa ushirikiano wako

Appendix 3: Analysed raw data

Sample No	HCH										
	α-		β-		γ-		δ-		∑-HCH		α-/γ-HCH
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	
	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	
S1	0.082	4.8	0.26	15	0.15	8.6	0.036	2.1	0.52	31	0.6
S2	0.19	5.6	0.61	18	0.14	4.1	0.027	0.78	0.97	28	1.4
S3	0.13	4.1	0.11	3.5	0.17	5.2	n.d.	n.d.	0.41	13	0.8
S4	0.020	0.78	0.073	2.8	0.08	3.2	n.d.	n.d.	0.18	6.8	0.2
S5	0.032	1.1	0.38	13	0.03	0.89	0.016	0.56	0.45	16	1.2
S6	0.035	0.97	0.17	4.7	0.06	1.8	n.d.	n.d.	0.27	7.5	0.5
S7	0.37	8.3	0.84	19	0.70	16	n.d.	n.d.	1.9	43	0.5
S8	0.28	6.5	0.43	10	0.56	13	n.d.	n.d.	1.3	30	0.5
S9	0.21	6.5	0.28	8.6	0.33	9.9	n.d.	n.d.	0.83	25	0.7
S10	0.17	4.4	0.28	7.2	0.43	11	n.d.	n.d.	0.88	23	0.4
S11	0.077	2.2	0.063	1.8	0.053	1.5	n.d.	n.d.	0.19	5.5	1.5
S12	0.052	1.0	0.050	0.96	0.050	0.96	n.d.	n.d.	0.15	2.9	1.0
S13	0.055	2.6	0.050	2.4	0.16	7.5	n.d.	n.d.	0.26	13	0.3
S14	0.065	1.7	0.065	1.7	0.10	2.7	n.d.	n.d.	0.23	6.1	0.6
S15	0.13	3.7	0.054	1.6	0.24	7.1	n.d.	n.d.	0.42	12	0.5
S16	0.045	1.1	0.030	0.74	0.074	1.8	n.d.	n.d.	0.15	3.6	0.6
S17	0.052	1.1	0.027	0.58	0.075	1.6	n.d.	n.d.	0.15	3.3	0.7
S18	0.021	0.51	n.d.	n.d.	0.015	0.36	n.d.	n.d.	0.037	0.87	1.4
S19	0.063	1.7	0.052	1.4	0.089	2.4	n.d.	n.d.	0.20	5.5	0.7
S20	0.15	4.4	0.044	1.3	0.19	5.6	0.037	1.1	0.42	12	0.8
S21	0.15	4.9	0.068	2.2	0.23	7.3	n.d.	n.d.	0.45	14	0.7
S22	0.048	1.2	0.044	1.1	0.10	2.5	n.d.	n.d.	0.19	4.8	0.5
S23	0.21	5.7	0.13	3.5	0.23	6.1	n.d.	n.d.	0.57	15	0.9
S24	0.046	1.1	0.015	0.36	0.076	1.8	n.d.	n.d.	0.14	3.3	0.6
S25	0.020	0.55	n.d.	n.d.	0.020	0.55	n.d.	n.d.	0.041	1.1	1.0
S26	0.053	1.5	n.d.	n.d.	0.11	3.0	n.d.	n.d.	0.16	4.5	0.5
S27	0.023	1.5	0.10	6.9	0.047	3.1	n.d.	n.d.	0.17	12	0.5
S28	0.065	2.7	0.31	13	0.16	6.6	n.d.	n.d.	0.54	22	0.4
S29	0.023	0.55	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.023	0.55	n.d.
S30	0.066	1.7	0.28	7.3	0.09	2.2	n.d.	n.d.	0.44	11	0.8
	0.020	0.510	0.015	0.360	0.015	0.360	0.016	0.560	0.023	0.550	0.244
% LOD	**100**	**100**	**87**	**87**	**97**	**97**	**13**	**13**			

Sample No	HCB		PCA	
	µg/kg	µg/kg	µg/kg	µg/kg
	WM	LM	WM	LM
S1	0.49	29	0.14	8.3
S2	1.4	42	0.15	4.5
S3	0.58	18	0.058	1.8
S4	0.29	11	0.094	3.6
S5	0.81	28	0.32	11
S6	1.2	32	0.15	4.2
S7	2.5	56	0.79	18
S8	0.99	23	0.12	2.7
S9	0.63	19	0.10	3.1
S10	1.3	34	0.27	6.9
S11	0.88	25	0.26	7.4
S12	0.88	17	n.d.	n.d.
S13	0.55	26	0.007	0.35
S14	0.99	26	0.11	2.9
S15	0.95	28	0.005	0.16
S16	1.0	25	n.d.	n.d.
S17	1.41	30	0.23	4.8
S18	0.41	9.8	0.084	2.0
S19	0.44	12	0.20	5.5
S20	0.34	9.9	0.21	6.3
S21	0.43	14	0.21	6.8
S22	0.60	15	0.037	0.93
S23	1.1	31	0.29	7.9
S24	0.76	18	0.071	1.7
S25	0.29	7.9	n.d.	n.d.
S26	0.12	3.5	0.030	0.86
S27	0.53	35	0.080	5.3
S28	0.74	31	0.21	8.9
S29	0.42	10	n.d.	n.d.
S30	0.62	16	0.17	4.4
% LOD	**100**	**100**	**87**	**87**

Sample No	DDE				DDD				DDT							DDMU			
	o,p'-		p,p'-		o,p'-		p,p'-		o,p'-		p,p'-		∑-DDT*		DDE/DDT	o,p'-		p,p'-	
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg		µg/kg	µg/kg	µg/kg	µg/k
	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM		WM	LM	WM	LM
S1	n.d.	n.d.	82	4800	n.d.	n.d.	15	870	3.9	230	85	5000	185	10900	0.96	n.d.	n.d.	n.d.	n.d.
S2	n.d.	n.d.	109	3200	n.d.	n.d.	31	920	5.4	160	139	4100	285	8380	0.78	n.d.	n.d.	n.d.	n.d.
S3	n.d.	n.d.	214	6700	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	214	6700	429	13400	1.00	n.d.	n.d.	n.d.	n.d.
S4	n.d.	n.d.	47	1800	n.d.	n.d.	55	2100	9.6	370	62	2400	173	6670	0.75	n.d.	n.d.	n.d.	n.d.
S5	n.d.	n.d.	133	4600	n.d.	n.d.	32	1100	12	400	162	5600	339	11700	0.82	n.d.	n.d.	n.d.	n.d.
S6	n.d.	n.d.	130	3600	n.d.	n.d.	36	990	n.d.	n.d.	162	4500	327	9090	0.80	n.d.	n.d.	n.d.	n.d.
S7	3.5	80	528	12000	0.30	6.8	150	3400	180	4100	440	10000	1298	29500	1.20	n.d.	n.d.	0.053	1.2
S8	n.d.	n.d.	163	3800	n.d.	n.d.	47	1100	33	760	142	3300	385	8960	1.15	n.d.	n.d.	n.d.	n.d.
S9	n.d.	n.d.	172	5200	n.d.	n.d.	50	1500	30	900	155	4700	406	12300	1.11	n.d.	n.d.	n.d.	n.d.
S10	n.d.	n.d.	215	5500	n.d.	n.d.	34	860	55	1400	242	6200	544	13960	0.89	n.d.	n.d.	n.d.	n.d.
S11	n.d.	n.d.	224	6400	n.d.	n.d.	81	2300	33	950	284	8100	621	17750	0.79	n.d.	n.d.	n.d.	n.d.
S12	n.d.	n.d.	307	5900	n.d.	n.d.	83	1600	39	750	312	6000	741	14250	0.98	n.d.	n.d.	n.d.	n.d.
S13	n.d.	n.d.	122	5800	n.d.	n.d.	21	1000	n.d.	n.d.	95	4500	237	11300	1.29	n.d.	n.d.	n.d.	n.d.
S14	n.d.	n.d.	186	4900	n.d.	n.d.	20	520	42	1100	217	5700	464	12220	0.86	n.d.	n.d.	n.d.	n.d.
S15	n.d.	n.d.	170	5000	n.d.	n.d.	n.d.	n.d.	34	1000	221	6500	391	11500	0.77	n.d.	n.d.	n.d.	n.d.
S16	n.d.	n.d.	230	5600	n.d.	n.d.	n.d.	n.d.	32	780	312	7600	541	13200	0.74	n.d.	n.d.	n.d.	n.d.
S17	n.d.	n.d.	183	3900	n.d.	n.d.	61	1300	n.d.	n.d.	230	4900	475	10100	0.80	n.d.	n.d.	n.d.	n.d.
S18	n.d.	n.d.	193	4600	n.d.	n.d.	26	620	42	990	277	6600	538	12810	0.70	n.d.	n.d.	n.d.	n.d.
S19	n.d.	n.d.	233	6300	n.d.	n.d.	29	790	18	490	289	7800	569	15380	0.81	n.d.	n.d.	n.d.	n.d.
S20	n.d.	n.d.	241	7100	n.d.	n.d.	68	2000	22	650	238	7000	570	16750	1.01	n.d.	n.d.	n.d.	n.d.

S21	n.d.	n.d.	183	5900	n.d.	n.d.	27	880	37	1200	223	7200	471	15180	0.82	n.d.	n.d.	n.d.	n.d.
S22	n.d.	n.d.	236	5900	n.d.	n.d.	56	1400	39	970	272	6800	603	15070	0.87	n.d.	n.d.	n.d.	n.d.
S23	n.d.	n.d.	167	4500	n.d.	n.d.	20	540	n.d.	n.d.	144	3900	331	8940	1.15	n.d.	n.d.	n.d.	n.d.
S24	n.d.	n.d.	202	4800	n.d.	n.d.	42	990	46	1100	273	6500	562	13390	0.74	n.d.	n.d.	n.d.	n.d.
S25	n.d.	n.d.	192	5200	n.d.	n.d.	48	1300	37	1000	266	7200	544	14700	0.72	n.d.	n.d.	n.d.	n.d.
S26	n.d.	n.d.	221	6300	n.d.	n.d.	56	1600	23	650	252	7200	551	15750	0.88	n.d.	n.d.	n.d.	n.d.
S27	n.d.	n.d.	80	5300	n.d.	n.d.	13	880	6.8	450	90	6000	189	12630	0.88	n.d.	n.d.	n.d.	n.d.
S28	n.d.	n.d.	118	4900	n.d.	n.d.	12	510	5.3	220	175	7300	310	12930	0.67	n.d.	n.d.	n.d.	n.d.
S29	n.d.	n.d.	231	5500	n.d.	n.d.	46	1100	67	1600	370	8800	714	17000	0.63	n.d.	n.d.	n.d.	n.d.
S30	n.d.	n.d.	38	970	n.d.	n.d.	30	770	30	770	152	3900	250	6410	0.25	n.d.	n.d.	n.d.	n.d.
% LOD	3	3	100	100	3	3	90	90	83	83	100	100				0	0	3	3

Sample No	Aldrin		Dieldrin		Aldrin/Dieldrin	Endrin	
	µg/kg	µg/kg	µg/kg	µg/kg		µg/kg	µg/kg
	WM	LM	WM	LM		WM	LM
S1	0.022	1.3	0.39	23	0.06	n.d.	n.d.
S2	0.033	0.97	1.5	45	0.02	n.d.	n.d.
S3	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S4	n.d.	n.d.	0.24	9.4		n.d.	n.d.
S5	n.d.	n.d.	0.29	10		n.d.	n.d.
S6	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S7	n.d.	n.d.	3.9	88		0.030	0.68
S8	0.023	0.53	0.12	2.9	0.18	n.d.	n.d.
S9	n.d.	n.d.	0.18	5.5		n.d.	n.d.
S10	n.d.	n.d.	0.24	6.1		n.d.	n.d.
S11	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S12	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S13	n.d.	n.d.	0.006	0.29		n.d.	n.d.
S14	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S15	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S16	n.d.	n.d.	0.078	1.9		n.d.	n.d.
S17	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S18	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S19	n.d.	n.d.	0.24	6.5		n.d.	n.d.
S20	0.026	0.76	0.58	17	0.04	n.d.	n.d.
S21	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S22	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S23	0.052	1.4	1.2	33	0.04	n.d.	n.d.
S24	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S25	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S26	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S27	n.d.	n.d.	0.41	27		n.d.	n.d.
S28	n.d.	n.d.	0.74	31		n.d.	n.d.
S29	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.
S30	n.d.	n.d.	0.28	7.3		n.d.	n.d.
% LOD	13	13	53	53		3	3

Sample No	Chlordane						Chlordene			
	cis- (α-)		trans- (γ-)		oxy-		α-		γ-	
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM
S1	0.009	0.54	n.d.	n.d.	0.061	3.6	n.d.	n.d.	n.d.	n.d.
S2	0.075	2.2	n.d.	n.d.	0.061	1.8	n.d.	n.d.	n.d.	n.d.
S3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S4	n.d.	n.d.	n.d.	n.d.	0.018	0.69	n.d.	n.d.	n.d.	n.d.
S5	0.010	0.34	n.d.	n.d.	0.075	2.6	n.d.	n.d.	n.d.	n.d.
S6	n.d.	n.d.	n.d.	n.d.	0.032	0.88	n.d.	n.d.	n.d.	n.d.
S7	0.19	4.3	0.020	0.46	0.43	9.7	n.d.	n.d.	n.d.	n.d.
S8	0.065	1.5	n.d.	n.d.	0.043	1.0	n.d.	n.d.	n.d.	n.d.
S9	0.040	1.2	n.d.	n.d.	0.018	0.54	n.d.	n.d.	n.d.	n.d.
S10	n.d.	n.d.	n.d.	n.d.	0.015	0.38	n.d.	n.d.	n.d.	n.d.
S11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S13	0.010	0.46	n.d.	n.d.	0.023	1.1	n.d.	n.d.	n.d.	n.d.
S14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S19	n.d.	n.d.	n.d.	n.d.	0.059	1.6	n.d.	n.d.	n.d.	n.d.
S20	n.d.	n.d.	n.d.	n.d.	0.030	0.88	n.d.	n.d.	n.d.	n.d.
S21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S23	n.d.	n.d.	n.d.	n.d.	0.11	2.9	n.d.	n.d.	n.d.	n.d.
S24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S27	0.021	1.4	n.d.	n.d.	0.020	1.3	n.d.	n.d.	n.d.	n.d.
S28	0.031	1.3	n.d.	n.d.	0.019	0.79	n.d.	n.d.	n.d.	n.d.
S29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S30	n.d.	n.d.	n.d.	n.d.	0.14	3.6	n.d.	n.d.	n.d.	n.d.
% LOD	30	30	3	3	53	53	0	0	0	0

Sample No						Nonachlor			∑-Chlordane	
	Heptachlor		Heptachlorepoxide		cis-		trans-			
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM
S1	n.d.	n.d.	0.036	2.1	n.d.	n.d.	0.087	5.1	0.19	11
S2	n.d.	n.d.	0.11	3.3	n.d.	n.d.	0.037	1.1	0.29	8.4
S3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S4	n.d.	n.d.	0.012	0.46	n.d.	n.d.	n.d.	n.d.	0.030	1.2
S5	n.d.	n.d.	0.041	1.4	n.d.	n.d.	0.015	0.5	0.14	4.8
S6	n.d.	n.d.	0.025	0.69	n.d.	n.d.	n.d.	n.d.		
S7	n.d.	n.d.	0.12	2.8	n.d.	n.d.	0.18	4.1	0.92	21
S8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S9	n.d.	n.d.	0.024	0.73	n.d.	n.d.	n.d.	n.d.	0.082	2.5
S10	n.d.	n.d.	0.015	0.38	n.d.	n.d.	n.d.	n.d.	0.030	0.76
S11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.033	1.6
S14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.059	1.6
S20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.030	0.88
S21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11	2.9
S24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S27	n.d.	n.d.	0.012	0.79	n.d.	n.d.	n.d.	n.d.	0.052	3.5
S28	n.d.	n.d.	0.034	1.4	n.d.	n.d.	0.055	2.3	0.14	5.8
S29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
S30	n.d.	n.d.	0.026	0.67	n.d.	n.d.	n.d.	n.d.	0.17	4.3
% LOD	0	0	37	37	0	0	17	17		

Sample No	α-Endosulfan		β-Endosulfan		α-/β-	Endosulfan sulfate		∑-Endosulfan	
	µg/kg	µg/kg	µg/kg	µg/kg		µg/kg	µg/kg	µg/kg	µg/kg
	WM	LM	WM	LM		WM	LM	WM	LM
S1	0.088	5.2	0.063	3.7	1.4	0.019	1.1	0.17	10
S2	0.054	1.6	0.109	3.2	0.50	0.044	1.3	0.21	6.1
S3	0.077	2.4	0.064	2.0	1.2	0.028	0.88	0.17	5.3
S4	n.d.	n.d.	0.024	0.93		n.d.	n.d.	0.024	0.93
S5	tr	tr	0.061	2.1		tr	tr	0.061	2.1
S6	0.13	3.6	0.058	1.6	2.3	0.027	0.76	0.21	6.0
S7	0.097	2.2	0.097	2.2	1.0	0.075	1.7	0.27	6.1
S8	0.034	0.78	n.d.	n.d.		n.d.	n.d.	0.034	0.78
S9	tr	tr	n.d.	n.d.		tr	tr	tr	tr
S10	0.051	1.3	0.037	0.95	1.4	0.082	2.1	0.17	4.4
S11	0.21	6.1	0.10	2.9	2.1	0.060	1.7	0.37	11
S12	0.22	4.2	0.20	3.8	1.1	0.14	2.6	0.55	11
S13	0.059	2.8	tr	tr		n.d.	n.d.	0.059	2.8
S14	n.d.	n.d.	tr	tr		n.d.	n.d.	tr	tr
S15	0.037	1.1	0.0714	2.1	0.52	0.034	1.0	0.14	4.2
S16	0.023	0.57	n.d.	n.d.		tr	tr	0.023	0.57
S17	n.d.	n.d.	tr	tr		tr	tr	tr	tr
S18	0.19	4.6	0.22	5.2	0.88	0.11	2.7	0.53	13
S19	tr	tr	0.028	0.77		n.d.	n.d.	0.028	0.77
S20	0.058	1.7	0.051	1.5	1.1	0.023	0.68	0.13	3.9
S21	0.019	0.62	tr	tr		n.d.	n.d.	0.019	0.62
S22	0.12	3.1	0.13	3.3	0.94	0.1	2.5	0.36	8.9
S23	0.056	1.5	0.063	1.7	0.88	0.036	0.97	0.15	4.2
S24	0.12	2.8	0.092	2.2	1.3	0.076	1.8	0.29	6.8
S25	0.17	4.7	0.078	2.1	2.2	0.041	1.1	0.29	7.9
S26	0.060	1.7	0.074	2.1	0.81	0.056	1.6	0.19	5.4
S27	tr	tr	tr	tr		n.d.	n.d.	tr	tr
S28	n.d.	n.d.	tr	tr		n.d.	n.d.	tr	tr
S29	0.034	0.80	tr	tr		n.d.	n.d.	tr	tr
S30	tr	tr	n.d.	n.d.		n.d.	n.d.	tr	tr
% LOD	83	83	87	87		67	67		

Sample No	PCB															
	28		52		101		118		138		153		180		∑-PCB	
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM	WM	LM
S1	0.12	7.1	0.010	0.59	tr	tr	0.61	36	0.80	47	0.37	22	0.26	15	2.2	128
S2	0.29	8.6	0.054	1.6	0.036	2.1	0.95	28	0.58	17	0.44	13	0.61	18	3.0	88
S3	tr	tr	n.d.	n.d.	n.d.	n.d.	0.42	13	1.7	52	0.86	27	0.12	3.6	3.1	96
S4	0.017	0.67	tr	tr	tr	tr	0.57	22	0.42	16	0.47	18	0.34	13	1.8	70
S5	0.081	2.8	tr	tr	0.029	1.1	1.5	51	1.1	38	0.17	5.7	0.16	5.5	3.0	104
S6	n.d.	n.d.	n.d.	n.d.	0.022	0.75	1.3	35	0.28	7.8	tr	tr	n.d.	n.d.	1.6	44
S7	0.20	4.6	0.13	2.9	0.130	3.6	1.8	41	1.2	28	0.84	19	tr	tr	4.3	99
S8	0.022	0.52	tr	tr	tr	tr	0.43	10	0.47	11	0.69	16	0.095	2.2	1.7	40
S9	0.043	1.3	n.d.	n.d.	0.069	1.6	0.040	1.2	1.3	39	tr	tr	tr	tr	1.4	43
S10	0.098	2.5	tr	tr	0.089	2.7	0.74	19	1.6	42	n.d.	n.d.	0.55	14	3.1	80
S11	n.d.	n.d.	n.d.	n.d.	tr	tr	0.27	7.6	0.91	26	tr	tr	0.53	15	1.7	49
S12	0.16	3.1	n.d.	n.d.	0.060	1.7	1.7	32	0.48	9.2	0.15	2.8	0.35	6.8	2.6	56
S13	0.046	2.2	0.016	0.77	0.046	0.88	0.55	26	0.36	17	tr	tr	n.d.	n.d.	1.0	47
S14	0.029	0.76	tr	tr	tr	tr	1.6	41	1.7	44	0.26	6.9	0.38	10	3.9	103
S15	tr	tr	n.d.	n.d.	0.049	1.3	0.75	22	1.3	38	n.d.	n.d.	0.32	9.5	2.4	71
S16	tr	tr	tr	tr	n.d.	n.d.	0.32	7.9	1.1	27	0.21	5.0	0.49	12	2.1	52
S17	n.d.	n.d.	n.d.	n.d.	tr	tr	1.6	33	1.6	35	0.36	7.7	0.30	6.3	3.9	82
S18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.23	5.5	1.5	36	0.67	16	0.35	8.4	2.8	66
S19	0.052	1.4	n.d.	n.d.	tr	tr	n.d.	n.d.	0.44	12	n.d.	n.d.	n.d.	n.d.	0.50	13
S20	0.019	0.57	0.051	1.5	0.081	2.2	0.30	8.9	0.88	26	0.37	11	0.16	4.8	1.9	55
S21	tr	tr	tr	tr	tr	tr	1.1	35	0.81	26	0.50	16	0.43	14	2.8	91

S22	n.d.	n.d.	n.d.	n.d.	tr	tr	0.96	24	1.6	41	1.08	27	0.64	16	4.3	108
S23	0.010	0.28	tr	tr	0.030	0.76	0.89	24	0.93	25	0.17	4.5	n.d.	n.d.	2.0	55
S24	tr	tr	n.d.	n.d.	tr	tr	0.55	13	0.59	14	0.59	14	0.092	2.2	1.8	43
S25	n.d.	n.d.	n.d.	n.d.	tr	tr	0.078	2.1	0.93	25	0.74	20	tr	tr	1.7	47
S26	0.13	3.6	n.d.	n.d.	tr	tr	1.3	36	1.2	33	0.53	15	0.42	12	3.5	100
S27	0.038	2.5	0.017	1.1	0.084	2.4	0.21	14	0.29	19	0.15	10	n.d.	n.d.	0.78	49
S28	0.041	1.7	0.026	1.1	0.024	1.6	0.091	3.8	0.31	13	0.31	13	0.038	1.6	0.84	36
S29	n.d.	n.d.	n.d.	n.d.	tr	tr	tr	tr	1.6	38	1.5	36	0.30	7.2	3.4	81
S30	tr	tr	tr	tr	0.034	0.8	0.55	14	0.78	20	0.37	9.4	n.d.	n.d.	1.7	44
% LOD	77	77	53	53	93	93	97	97	100	100	90	90	80	80		