Bioaccumulation and the human health risk of *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE in freshwater fish species from the north-eastern Limpopo River valley, Vhembe District, Limpopo Province, South Africa

by

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DISSERTATION

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DECLARATION

I, Sherron Mphephu, student number 18007618, hereby declare that this dissertation for the award of a Master's degree in Zoology at the University of Venda belongs to me. I declare this is my original work and has not been submitted for any degree at any other university or institution. This dissertation does not contain other persons’ writing unless specifically acknowledged and referenced accordingly.

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ABSTRACT

Dichloro-diphenyl-trichloroethane (DDT) has been used globally as an agricultural pesticide since the early 1940s, and both the United States and Canada banned its use in 1972. DDT is still used in the malaria-endemic region of South Africa for vector control through indoor residual spraying (IRS). Studies have reported a link between DDT exposure and prostate cancer risk and/or aggressive disease presentation in Italy, Canada, and the United States. Epidemiological analysis of men with prostate cancer (PCa) in South Africa showed an increased PCa risk in Vhavenda people, the predominant population of Vhembe. The Vhembe District municipality falls in the malaria-endemic region, where DDT is used for vector control. The region of interest is fed by three major tributaries of the Limpopo River, which provide communities with agriculture, washing/bathing and portable water as well food (fish). Therefore, environmental levels of *p,p*-DDT, *p,p*-dichlorodiphenyldichloroethane (DDD) and *p,p*- dichlorodiphenyldichloroethylene (DDE) were measured before and after of the 2022 malaria high-transmission period (HTP), through sampling fish, from the Mutale and Mutshindudi rivers, and Thathe Vondo Dam. Fishes were bought from fishermen at the Thathe Vondo Dam and along the Mutale and Mutshindudi rivers. The fish's edible part (muscle), one piece raw and one piece cooked was tested for DDTs. Fish from the Mutshundudi River had higher DDT residuals than all other sites before the IRS. None of the DDTs were present after the yearly IRS. Daily exposure of DDTs for consumers was estimated by comparing estimated daily intake (EDI) with different criteria. The results revealed that the EDIs in our study were all lower than those criteria. Target hazard quotient (THQ) and risk ratio (R) were used to evaluate non-carcinogenic (toxic) and carcinogenic risks. There was no carcinogenic or toxic risk for humans consuming fish from the Mutale and Mutshindudi rivers and Thathe Vondo Dam. This indicates that DDT, DDD, and DDE in fish are not affecting the PCa burden in the Vhavenda men living in remote communities.

KEYWORDS: Endocrine disrupting chemicals (EDCs); DDTs; malaria vector control; indoor residual spraying (IRS); prostate cancer (PCa); persistent organic pollutants (POPs).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 - GENERAL INTRODUCTION

1.1. Introduction

Chemicals are necessary for everyday living. However, certain chemicals may negatively affect the body's endocrine (hormone) system; such chemicals are referred to as endocrine-disrupting chemicals (EDCs) (Woods et al., 2017). EDCs include some organic chemicals resistant to breakdown by photolytic, biological, and chemical processes and are known as persistent organic pollutants or POPs (Akhtar et al., 2021).

POPs comprise 26 chemical classes consisting of some pesticides, industrial chemicals, and by-products. POPs have exceptionally long half-lives (many years), are distributed over long distances throughout the environment, bioaccumulate in living organisms including humans, and are toxic to both humans and wildlife (https://ipen.org/toxic-priorities/what-are-pops). This group was specified by the Stockholm Convention on POPs (17 May 2004). The Convention, of which South Africa is a party, aimed to guard humans and animals against the possible toxic health effects by controlling the use and production or eliminating or replacing them with alternatives (Bouwman, 2004). Pesticides consist of insecticides, herbicides, and fungicides (Umetsu & Shirai, 2020). Pesticides that are most commonly used worldwide include carbamates, organochlorine (OC), and organophosphorus (OP) (Abubakar et al., 2020).

The OC insecticide dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE) are the two POPs of interest for this study. DDT is used for yearly malaria vector control via indoor residual spraying (IRS) (Bornman et al., 2009). Typical of POPs such as *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE (DDTs) are chemicals that are resistant to chemical, biological, and photolytic breakdown, have long environmental half-lives, are capable of long-range areal transport, and have a strong tendency to bioaccumulate in the lipids of living organisms (Kodavanti et al., 2014). Due to their semi-volatility and resistance to degradation, DDT and metabolites are found ubiquitously in the environment and have been reported in areas where their sources are almost absent (Barnhoorn et al., 2015).

Environmental pollution by any POP compromises goal 6 of the United Nations' sustainable developmental goals where according to Sustainable Development Goal 6: “Clean water and sanitation, besides drinking water, sanitation, and hygiene it also strives to include upholding the quality and sustainability of water resources, for the survival of people and earth (https://www.unep.org/)”.

DDT has been used globally as an agricultural pesticide since the early 1940s, and both the United States and Canada banned its use in 1972. DDT is still used in the malaria-endemic region of South Africa for vector control, through indoor residual spraying (IRS) (Hayes, 2012). Agricultural health studies have reported a link between DDT exposure and prostate cancer (PCa) risk and/or aggressive disease presentation in Italy, Canada, and the United States. Epidemiological analysis of men with prostate cancer in South Africa showed an increased PCa risk in Vhavenda people (OR=1.81 95% CI: 1.01-3.29; P=0.0473), the predominant population of Vhembe (Eeles & Raghallaigh, 2018). Vhembe falls in the malaria-endemic region, where DDT is used for vector control.

1.2. The Rationale of the Study

Bioaccumulation is defined as the ability of a chemical to accumulate in living tissues to levels higher than those in the surrounding environment or is the natural process that progressively concentrates nontoxic levels of pollutants into toxic levels causing adverse side effects (Szynkowska et al., 2018). The bioaccumulation of specific DDT was reported to cause toxic effects in humans (Bornman et al., 2009) and wildlife, including mammals (Bornman et al., 2010). DDT and metabolites bioaccumulate in organismal tissues and different components of the environment such as water, sediment, and other biota (Islam et al., 2018). DDT has been reported from IRS regions in environmental matrices and livestock from homesteads (Barnhoorn et al., 2009; van Dyk et al., 2010; Bouwman et al., 2015), and fish (water sources across South Africa/vicinity of IRS) (Barnhoorn et al., 2009; Gerber et al., 2016; Verhaert et al., 2017 and Volschenk et al., 2019) and humans (Cupul-Uicab et al., 2020; Bornman et al., 2022).

In South Africa, the consumption of wild fish plays an important role in the daily subsistence of communities staying near dams (Tapela et al., 2015). Many studies have indicated that eating DDT-contaminated fish may pose a risk to communities, especially men (Barnhoorn et al., 2009; Verhaert et al., 2017; Volschenk et al., 2019; Cupul-Uicab et al., 2020 and Bornman et al., 2022). Contaminants are not readily metabolized because of their low monooxygenase activity which is reflected by their high bioaccumulative potential for organic chemicals compared to terrestrial organisms (Deribe et al., 2011). Therefore, due to the preferential and high bioaccumulative potential of DDTs, fish should be considered to be the reliable representative of DDT pollution in freshwater systems (Riaz et al., 2021). Major groups depend on the Thathe Vondo Dam, the Mutale and Mutshindudi rivers for water use, and fish as a source of protein. This present work determines the bioaccumulation and the risk of DDTs in the freshwater fish species from the Thathe Vondo Dam, the Mutale and Mutshindudi rivers, and the risk of human intake. This study is also part of a multinational and multiinstitutional study funded by the USA Department of Defence on High-risk PCa in Southern Africa, Unravelling the Genome and Exposome (TARGET Africa). The overarching goal for the TARGET Africa (prosTate cAnceR Genome and Exposome sTudy for Africa) proposal is to reduce lethal PCa in men of African ancestry, by identifying the factors (environmental, lifestyle and molecular) contributing to aggressive PCa in men from southern Africa, with a focus on underserved rural communities. As such, this study focused on fish as possible vectors of human exposure and the associated human health risks.

1.3.1. Aim

This study aims to determine the bioaccumulation and risk of *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE (DDTs) in freshwater fish species from the north-eastern Vhembe district, Limpopo Province, South Africa.

1.3.2. Objectives

The objectives are as follows:

1. Analyze and compare the fish samples from the three different sites: Thathe Vondo Dam and along the Mutale and Mutshindudi rivers.
2. To analyse the before and after IRS fish samples.
3. To Analyse raw and cooked fish muscles.
4. Human Risk Analyses (HRA) of all samples.
5. Compare data with the available international and national literature**.**

1.4. Hypotheses

1. Fish from the site that were reported to have higher DDT residues will also have higher DDT residues.
2. Cooking fish should reduce DDT residues.
3. DDT-contaminated fish may pose a human health risk for people living in IRS areas.
4. DDT from fish is not a link to PCa in Vhavenda Men.

1.5. Study area

Water and fish samples were collected from the northeastern part of the Limpopo River valley, Thathe Vondo Dam, and along the Mutshindudi and the Mutale rivers (Figure 1.1). The Thathe Vondo Dam is one of three dams that are water sources in Thulamela municipality. It is situated near Thohoyandou town, Limpopo province, South Africa, and is close to a 43 m high earth-fill dam with a 286 m crest length (Nemalamangwa, 2020). The Thathe Vondo Dams' capacity is 30.5 million m^3 and has a gross storage capacity of 130 million m^3. This dam is in an upstream forested area of the Soutpansberg Mountains close to the old tea estate and has reduced the impact of agrochemicals as the runoff is limited by vegetation except during peak rainfall (Odiyo et al., 2012).

The Thathe Vondo Dam is on the Mutshindudi River and originates in the Soutpansberg mountain range (Sinthumule, 2022). The Mutshindudi River is a tributary of the Luvuvhu River .It is 534 m above sea level. It's roughly 50 km long, with a precipitous drop from a high rainforest area at 1200 meters to a lowland valley at 450 m, where it meets the Luvuvhu River (Fouche et al., 2013). The Mutshindudi River is a small but persistent river that supplies the municipal area of Thohoyandou's domestic needs. There are numerous riffles and rapids that are 20 m broad and 80 m long (Munyai et al., 2023).

The Mutale River is almost entirely contained within Venda's ancestral homeland. The river rises about 870 m above sea level in the Soutpansberg mountain near Lake Fundudzi. It then flows northeast for about 120 kilometres until it meets the Luvuvhu River inside the Kruger National Park (KNP). From this point on, the Luvuvhu flows east for an additional 20 kilometres before emptying into the Limpopo at Crook's Corner, located on the border between South Africa and Mozambique (Martin et al., 2022).

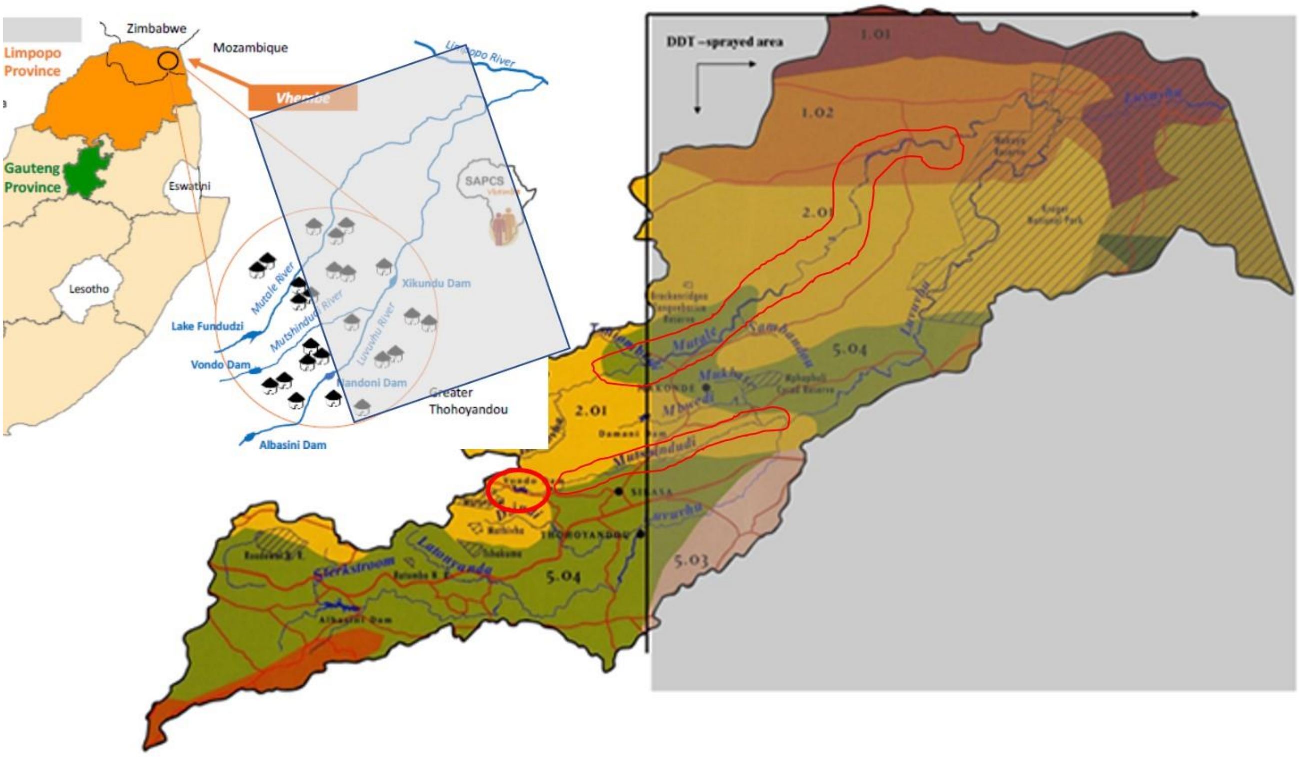

Figure 1: A map borrowed and edited from TARGET Africa indicating the three water sources circled in red including the Mutale and Mutshindudi rivers and the Thathe Vondo Dam. The grey area is showing the area where indoor residual dichlorodiphenyltrichloroethane (DDT) spraying (IRS) is ongoing.

(Note that Vonda Dam on the map is Thathe Vondo Dam)

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CHAPTER 2 - LITERATURE REVIEW

2.1. Endocrine disrupting chemicals

Endocrine disrupting chemicals are substances found in food sources, personal care products, manufactured goods, and the environment (air, soil, or water supply) that interfere with the endocrine system's normal function in a body (Kabir et al., 2015; Monneret, 2017 and Darbre, 2022). Because EDCs are derived from a wide range of sources, people are exposed to EDCs through various channels, such as food, drink, and air we breathe. EDCs may also penetrate the skin to enter the body (Aydemir et al., 2012). According to Kumar et al. (2020), exposure to EDCs can cause a variety of conditions, including altered immune system function, altered sperm quality and fertility, endometriosis, early puberty, altered nervous system function, respiratory problems, metabolic disorders, diabetes, obesity, cardiovascular issues, growth, and neurological and learning disabilities in humans.

EDCs affect the actions of hormones in four different ways in humans and wildlife. In short, as described by Kortenkamp et al. (2011) they:

- imitate hormones by acting like a hormone for instance when an EDC such as DDT perform like oestrogens they are identified as environmental oestrogens.
- interfere with the normal effects of hormones for example, some PCBs may have an anti-estrogenic action.
- by changing hormonal binding to the receptor and changing hormone synthesis, for example, when thyroid hormone production is altered by the flame retardant PBDE-99’
- regulating the levels of hormone receptors available, for example when an EDC will interact with all the estrogen receptors available.

Since EDC affect all hormone systems, it is evident that they therefore affect the development and function of the reproductive organs, adult-onset diabetes and cardiovascular disease (Ashraf & Wilson, 2019).

Among the main categories of EDCs are pesticides, human and animal pharmaceuticals, alkylphenols, phthalates, PFAS, PCBs, PBDEs and selected metals. Pesticides are divided into insecticides, herbicides, and fungicides (Kumar et al., 2023). Again, they are inclusive of organochlorines (OCs), organophosphates (OPs), carbamides, pyrethroids, and neonicotinoids (Kumar et al., 2023). The OCs comprise chlordane, *p,p*'-DDT, *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDD), aldrin, dieldrin and heptachlor epoxide metoxychlor, vinclozolin, atrazine to name a few (Barnhoorn et al., 2015). The OPs include diazinon, malathion and pirimiphos-methyl (Sharma et al., 2010).

Human and animal pharmaceuticals include an array of chemicals. A few groups are antiretroviral drugs (ARVs), antifungals, bacteriostatic antibiotics, anti-epileptics, analgesics (pain relievers), and antipyretics (fever reducers) (Wooding et al., 2017) and personal care products (PCPs) (Archer et al., 2017). Animal pharmaceuticals are veterinary drugs and they usually are the antihelmintics, antibiotics (aminoglycosides, β-lactam antibiotics, macrolides, peptides, sulphonamides and trimetroprin, tetracyclines, quinolones, chloramphenicol, malachite green, coccidiostats (nitroimidazoles, nitrofurans), hormones (anabolic steroids, corticosteroids, tyreostats), β-agonists and tranquillisers (Lau, 2001). The most common carbamides found in SA water include propoxur and carbaryl (Mndeni et al., 2022), while the pyrethroids used in malaria combat are permethrin, alpha-cypermethrin or deltamethrin (Hlongwana et al., 2013; Nardini et al., 2013).

The alkylphenol group is estrogen mimics used as industrial surfactants and in pesticide manufacturing, and wool and metal processing. Alkylphenols acting as EDCs include 4-*t*-octylphenol, 4-nonylphenol, 4-*n*-octylphenol, and 4-*n*-nonylphenol (Ye et al., 2014). Phthalates are found in consumer goods and personal care items used as plasticisers. Many plastics and consumer goods have bisphenol-A (BPA) added to them. PCBs are used as insulators, coolant fluid additives, hydraulic oil additives, and food contact materials while PFAS are used as stain and water repellents, firefighting foams, and other materials (Gore et al., 2014).

Lead (Pb), cadmium (Cd), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), mercury (Hg), and nickel (Ni) belong to the metals which have an important endocrine-disrupting effect on health (Georgescu et al., 2011). The human body is exposed to low levels and long-term metal and element exposures (Lauretta et al., 2009). Pb is an endocrine disruptor, a non-degradable hazardous substance found in the environment that may results in permanent health damage. Changes in thyroid function, such as a decrease in thyroid-stimulating hormone (TSH) and an increase in thyroxine (T4), have been linked to Pb poisoning. Because of its strong attraction for cysteine-rich proteins and metallothionein in the thyroid gland, Cd tends to accumulate there and can be extremely dangerous to the thyroid and alter the hormone’s functions. Thyrotoxicity can result from continuous exposure to Cd (Genchi et al., 2020). Given that As interacts with TRs and ERs and activates genes that are related to thyroid function or oestrogen, endocrine disruption could be a possible way of influencing the association between As exposure and health outcomes like skin, bladder, lung, liver, kidney, prostate, and other cancers. Histone modification, siRNA and DNA damage, methylation, neurotoxicity, and reproductive toxicity are the key areas of developing evidence for Hg-induced toxicity (Shi et al., 2018). Ni has a strong correlation with the prevalence of all breast cancers. exposure to Cu can cause endocrine disturbance and reproductive problems (Liu et al., 2023). Mn caused an endocrine disruption reaction, which was shown by a drop in plasma concentrations (Correia et al., 2021).

Numerous studies have indicated the effects these EDCs have on humans and wildlife. Below are a few recent findings of some of the listed EDCs: Recent studies have shown that xenobiotic chemicals' strategies for targeting endocrine systems are complex. It is now known that EDCs target all receptors, including orphan receptors, non-nuclear steroid and nonsteroid hormone receptors (dopamine, norepinephrine, and serotonin receptors), and other biochemical pathways involved in the biosynthesis of steroids (Auriemma et al., 2023; Hajam et al., 2023; Thacharodi et al., 2023). Other nuclear receptors targeted by EDCs include androgen, progesterone, thyroid, and retinoid receptors (Interdonato et al., 2023). Since EDCs are linked to multiple pathogeneses, such as infertility, endocrine dysfunction, altered hormone metabolism, cancer, metabolic syndrome, obesity, diabetes, cardiovascular dysfunction, and neurological and reproductive disorders, they have harmful effects even at low doses (Maniradhan et al., 2023).

However, EDCs have a direct impact on the female reproductive system, leading to issues like accelerated ovarian ageing and decreased follicle development, size, and activity (Ahn et al., 2023). Moreover, negative effects of EDC exposure in females include endometriosis, premature births, polycystic ovarian syndrome, infertility, epigenetic modifications in DNA methylation, genotoxicity, and extended puberty (Robinson, 2023). An increased risk of cardiovascular illnesses, dementia, osteoporosis, mood disorders, sexual dysfunction, and death can result from early or premature menopause caused by dysregulation in the female reproductive system (Aydemir & Ulusu, 2023).

According to studies conducted on animals, pesticides such as vinclozolin, DDT, and DDE increase the risk of developing testicular germ cell cancer (TGCC) (Ahn et al., 2023). Specific wildlife species were essential in proving causal evidence for chemical-induced effects on wildlife. In certain species, impaired development and reproduction that are causally connected to endocrine-disrupting chemicals are well-documented and have resulted in alterations in local or regional populations. Amongst them, probably the most evident example of endocrine disruption brought on by an environmental chemical is the masculinisation (imposex) of female Gastropod snails by tributyltin, a biocide used in antifouling paints (Thacharodi et al., 2023). Because of the extreme vulnerability of Gastropods, imposex has caused local populations all over the world including coastal regions in Europe and the open North Sea to deteriorate or go extinct (Zapata et al, 2023 and Prebensen., 2023)

Many raptor species have experienced dramatic declines in population due to DDE-induced egg-shell thinning in birds (Lesch, 2023). Coral reefs were affected by a UV filter that is frequently found in sunscreen and is thought to be an endocrine disruptor (Fair & Houde, 2023). The immune systems of sea otters, bottlenose dolphins, and polar bears, as well as their reproductive and hormone systems, have all been connected to problems with a group of chemicals referred to as PFAS (Romero et al., 2023).

Some EDCs are referred to as POPs, which are pollutants that can be present in the environment for a prolonged period. POPs is a group of chemicals banned by the Stockholm convention POPs include industrial chemicals (PCBs, furans, PBDEs, and polychlorinated biphenyls, among others), pesticides, and byproducts of industrial processes (dioxins and furans) (Bouwman, 2004; Bornman et al., 2009). These pollutants travel across international borders and have a long life span. These have been reported in locations like the earth's poles, where they have never been used. These bioaccumulate into the food chain, affecting humans and animals and causing several well-known health risks and environmental effects (Alharbi et al., 2018). DDT is considered a POP and will be thoroughly discussed.

According to previous studies, toxic materials are washed into rivers and dams, causing water pollution and leading to a reduction of clean, usable water (Barnhoorn et al., 2015; Verhaert et al., 2017 ;Cupul-Uicab et al., 2020). According to Marchand et al. (2012), freshwater fish species living in rivers and dams face a constant risk of exposure to pollution because their diets directly or indirectly affect the water quality they consume. The three main pollutants in urban and rural areas are pesticides, pharmaceuticals, and metals.

In the agricultural sector, pesticides are very important and positively impact a nation's economy (Haribabu et al., 2018; Gonsioroski et al., 2020). Abubakar (2020) claims lower food production costs result from increased pesticide use. Chemicals called pesticides are applied to crops to keep pests away. Rainwater runoff and atmospheric movements are the main ways pollutants enter aquatic systems (Barnhoorn et al., 2010). According to Tunya et al. (2018) and Bengu et al. (2017), endocrine-disrupting chemicals (EDCs) have contaminated rivers and dams in South Africa. EDCs are environmental contaminants that mimic hormones, disrupt the regular operation of your body's endocrine system, and may consist of a combination of chemicals that affect how hormones are produced and processed.

2.2. Dichlorodiphenyltrichloroethane

The pesticide DDT, an insecticide, despite being banned in the United States for many years, is still a prevalent contaminant at many sites. The para-para (77%) and ortho-para (15%) isomers abbreviated as *o,p*'-DDT, and *p,p*'-DDT combined to form commercially produced DDT for insecticidal purposes, the remaining 8% is made up of various other related compounds (Ricking & Schwarzbauer, 2012). The commercial form of DDT is an amorphous powder with a lower melting point than pure DDT, a white crystalline solid that melts at 80-109°C (Devi, 2020). To be used as a tool to prevent malaria infections, DDT is sprayed on interior walls, ceilings, and eaves via IRS (Gaspar et al., 2015).

Eventually the sprayed DDT accumulates in soil in significant concentrations as a result of repeated sprayings. Its effects on wildlife greatly increased as it became associated with food chains. According to Martínez et al. (2012), there are three types of situations where DDT is found in the world: (1) Locations where it is still being used; (2) locations where it was sprayed years ago; and (3) locations where DDT was transported via air over great distances and found in places where it was never used, such as the Antarctic. Due to DDT's stability, it bioaccumulates in the body tissues of organisms lower in the food chain, which is a food source for other species higher up in the food chain. This had harmful effects on the latter animals. It has been linked to DDT exposure in certain regions where malaria is still a serious public health concern, a drop in raptor populations (Kesic, 2020). DDD and DDE are some of the degradation products that DDT produces in the environment (Hellou et al., 2013).

2.2. Dichlorodiphenyltrichloroethane metabolites

2.2.1. Dichlorodiphenyldichloroethylene

DDE is a chemical molecule produced when DDT undergoes dehydrohalogenation, one of the more common breakdown products (Gohil H and Ogram, 2020). It can also exist in a para-para and orto-para isomer, *p,p'*-DDE and *o,p'*-DDE (Hellou et al,2013).

DDE is commonly found in animal tissue samples because of the high prevalence of DDT in agriculture and society throughout the mid-20th century (Devi, 2020). Because DDE is fat-soluble, like other OCs, it is barely excreted from the body, and its concentrations tend to increase throughout an individual's life, making it potentially harmful. The main exception is the excretion of DDE in breast milk, which passes over a significant amount of the mother's DDE load to the young animal or child (Kuang et al., 2020). This stability causes bioaccumulation in the environment, which intensifies the harmful effects of DDE in addition to accumulation throughout an organism's lifetime (Kesic, 2020). DDE has more potency of causing harm than DDT (Truong et al., 2019). Hu et al. (2021) demonstrated that patients with primary hyperparathyroidism have higher quantities of DDE in their tumours. DDE has been associated with significant changes in the brains of American Robins (*Turdus migratorius*) (Iwaniuk et al., 2006), bioaccumulation in birds' eggs (Carlsson et al., 2011; Vander et al., 2009) and the blubber of mammals (Kesic, 2020).

2.2.2. Dichlorodiphenyldichloroethane (DDD)

Another metabolite is dichlorodiphenyldichloroethane (DDD). DDD toxicity can occur in different potential mechanisms, and they may all operate at once, DDD reduces potassium transport across the membrane and inhibits the inactivation of voltage-gated sodium channels. The channels open normally but are closed slowly, thus interfering with the active transport of sodium out of the nerve axon during repolarisation and resulting in hyperexcitability (Mrema et al., 2013). DDD blocks the action of neuronal adenosine triphosphatases essential for neuronal repolarisation. In addition, DDD prevents calmodulin, a calcium mediator in nerves, from carrying calcium ions, which are necessary for neurotransmitter release. A fully depolarised neuron would not respond to small stimuli, but all of these inhibited functions slow down the rate of depolarisation and make neurons more sensitive to them (Bornman et al., 2007). DDD also negatively affects the reproductive system by binding to the estrogen and androgen receptors and imitating endogenous hormones (Syed et al., 2023)

2.3. DDT and DDE bioaccumulation and biomagnification

Through bioaccumulation, POPs such as DDT may build up over time in animal tissues. As a result, POP levels tend to be higher in older animals than in younger ones. The inability of an organism to eliminate or degrade the chemical and the amount of the chemical entering the organism more quickly than it can be used are the two factors that lead to bioaccumulation (Gerber et al., 2016). Bioaccumulation occurs at the base of a food web, usually within primary producers like phytoplankton. These microscopic photosynthetic organisms absorb POPs directly from the water and accumulate them in their bodies over time. The toxins build up in their tissues because they are absorbed from the water at a rate faster than they can be metabolised or excreted (Wolmarans et al., 2021).

The rise or increase in contaminated substances brought on by polullated environment is known as biomagnification (Uddin et al.,2020). Heavy metals like AS, Hg, and pesticides like DDT and PCBs are contaminants. The organisms absorb these substances from the food they eat. These toxic substances build up in the higher organisms when they prey on the lower food web organisms carrying them (Miller et al., 2020).

Because of its lipophilicity and slow elimination rate, DDT has a high potential for bioaccumulation. Its primary metabolite, DDE, also exhibits this tendency. Due to these features, DDT is biomagnified throughout the foodweb, reaching peak concentrations at the top of the trophic chain, where secondary poisoning episodes may materialise (Tasselli et al., 2023). There are several ways that DDT can enter freshwater ecosystems, including atmospheric deposition, sewage effluent runoff, and runoff from contaminated soils (Bornman et al., 2022). Biomagnification occurs when an animal's concentrations of persistent hydrophobic organic contaminants rise above those of its food (Currier et al., 2020). DDT and its metabolites tend to accumulate in bottom sediments, where they can be stored for decades, after being absorbed into suspended solids and particulate organic matter due to their hydrophobic properties. Sediments can release stored DDT back into the water column and allow it to bioaccumulate in benthic organisms that come into contact with the sediments, potentially leading to biomagnification in the aquatic food chain (Tasselli et al., 2023).

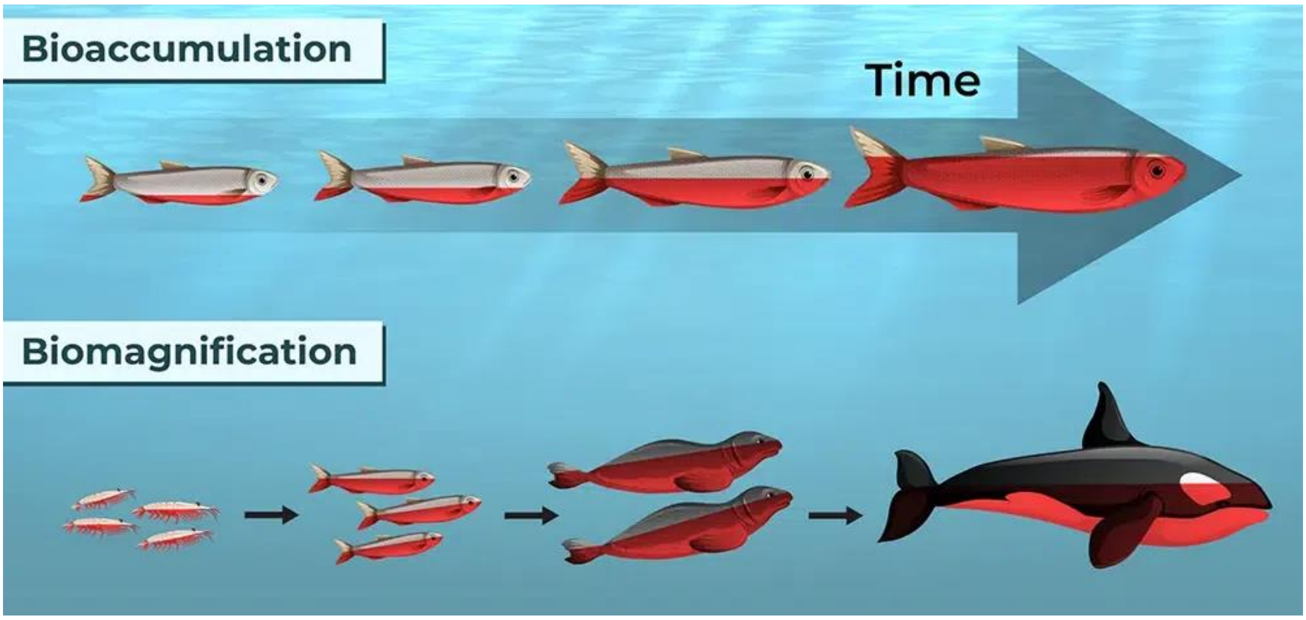

Figure 2: Showing the difference between bioaccumulation and biomagnification in fish species. (Borrowed from https://www.geeksforgeeks.org/difference-between-biomagnification-and-bioaccumulation/).

2.4. DDT and human health risks including PCa

Human health risk is the possibility that something will damage or negatively impact human health (Dippong et al., 2021) Danger does not guarantee bad things will happen. It's only possible. The degree to which you are at risk for health problems depends on several characteristics known as risk factors. Assessing the risk to human health is a procedure used to determine the nature and possibility of harmful health effects in humans who may be exposed to chemicals in contaminated environmental media at some point in the future (Neris et al., 2019). The Environmental Protection Agency (EPA) employs risk assessment to define the type and extent of health hazards to human populations, including locals, tourists, and adults and children. Along with plants, birds, other wildlife, and aquatic life, the EPA also calculates the risks to ecological receptors (U.S. Environmental Protection Agency (EPA), 2012)

Over the past 60 years, DDT has proven to be one of the few accessible and efficient methods for controlling malarial vector mosquitoes, which cause over 300 million disease cases and over 1 million fatalities annually (Padmanaban et al., 2022). DDT exposure damages the nervous system (Morris, 2022). High exposure causes vertigo, trembling, agitation, and convulsions in affected individuals (Kedari, 2020; Richardson et al., 2019) Stated that long-term neurological and cognitive issues are present in workers who have been exposed to DDT. Babies born prematurely or short for gestational age are more common among pregnant women exposed to DDT (Anand et al., 2019). Because of its estrogenic qualities, DDT is regarded as a chemical that disrupts hormones (Amir et al., 2021). According to the EPA, DDT may cause cancer in humans.

Populations dwelling near rivers and dams depend heavily on the everyday consumption of wild fish for protein mostly because of poverty and water from surrounding rivers for drinking, washing and cooking purposes (Loucks et al., 2005; Hamerlynck et al., 2019). In Limpopo province, harvesting fish is a regular activity. Residents of the nearby communities buy any freshwater fish that is for sale from roadside vendors. According to an earlier investigation, DDT concentrations were considerably higher in tap water, indoor air, floor dust, outside soil, portable water, leafy crops and chicken samples and eggs (Barnhoorn et al., 2010; Bornman et al., 2012; Forbes et al., 2021; van Dyk et al., 2012). In the Vhembe district of Limpopo province, DDT is used for yearly malaria vector control via indoor residual spraying (IRS) (Bornman et al., 2009), which can end up in the nearby river through different ways, including washed into the rivers in rainfall and wind. Vhavenda people, who make up the majority of the Vhembe district population, had a higher prostate cancer risk, according to an epidemiological study of men with the disease in South Africa (Maladze, 2020). Prostate cancer is strongly considered to be linked to exposure to environmental EDCs such as DDT (Eeles & Raghallaigh 2018; Marima et al., 2021). Thus, consuming fish and drinking water from nearby dams and rivers could be another way to be exposed to DDT, increasing the risks of PCa.

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CHAPTER 3 - MATERIALS AND METHODS

3.1 Ethical consideration

This study has been cleared by the Univen Animal Environmental and Bioinformatics Research Ethics Committee (AEBREC). Ethical clearance no: FSEA/22/BS/17/1707. (Appendix A)

3.2 Fish and tissue sampling

A total of 120 fishes, 20 per site, per collection were bought from local fishermen in the surrounding area of the Mutale River, the Thathe Vondo Dam and the Mutshindudi River (Figure 3.1). Local fishermen were recommended by local communities for fish at these sites. The collection of pre-IRS fish was done from the 31 of August 2022 to the 19 of September 2022 and post-IRS from 16 to 30 April 2023. Twenty fishes caught from the Thathe Vondo Dam were bought at Thathe village. The ones from the Mutshindudi River were bought at Ngudza village, and fish from the Mutale River was bought at the Mutale Water Affairs and Sambandou village. The species were bought as per availability. Fishes were dead when they were bought. After collection, the fish were taken to the lab and kept in the freezer at -20°C. 2 g Two fish muscle tissue samples were collected per fish and stored in aluminium foil until further laboratory processing. One piece of each sample was cooked for 10 minutes using a hotplate. The raw and cooked samples was stored at -20°C until analyses. The fish muscle tissue was grouped according to the same species, same location, and the same date for further analyses. This data is available in Appendix B.

3.3 Chemical analysis

Ms Mphephu performed the chemical analyses at the FDA laboratories in Pretoria. Standard methods were used as developed and used by the SANAS-accredited FDA laboratories. The procedures were developed according to Bordet et al. (2002) and written in the standard operating procedures (SOPs) of the laboratory.

3.3.1 Sample extraction

Samples were weighed to 1.00g (±0.02g) in 50mL polypropylene tubes, including nine blank samples for fortification, using a kern PCB balance. A known amount of target analyt spiked blank samples by using appropriate secondary standards (0.1 ppm and 10 ppm) to compensate for matrix effects. A matrix match calibration curve was spiked using an electronic pipet (eVol XR) to simulate the matrix effect of the samples analysed. An extraction solvent of 10 mL hexane and acetone (1:1) was added to the samples. Samples were homogenised using an Ultra-turrax for 15 seconds per sample. The samples were then shaken overnight.

3.3.2 Sample preparation

C18 SPE cartridges were conditioned on a SPE vacuum manifold with 10mL hexane petroleum ether (85:15). Samples were centrifuged for 5 minutes at 3000rpm, 10^{o}C using an Allegra X22r centrifuge. The supernatant was transferred to another polypropylene tube and centrifuged again using the same centrifuge conditions. The Supernatant was transferred to glass test tubes and a Pasteur pipette(3mL) was used to remove the aquatic layer. All test tubes were evaporated to dryness at 40°C under the gentle stream air using a Turbovop (from Boitage). The samples were reconstituted with 4mL of acetone to dichloroethane (1:1), sonicated using a digital ultrasonic cleaner (Biobass) for 10 minutes. The extract was loaded into cartridges and collected into another glass test tubes. The SPE cartridges were eluted with 10Ml hexane: petroleum (85:15). The eluate was evaporated to dryness under a gentle stream of air at 40^{o}C. Lastly, the contents of the test tubes were reconstituted with 1mL of methanol and sonicated for 10 minutes. Extracts were filtered through a 0.22 µm ptFE filter into a vial.

3.3.3 Instrument analysis

The filtered extracts were subjected to gas chromatography tandem mass spectrometry (GC-MS/MS) analysis (Shimadzu QC 2010). The chromatographic condition included an injection temperature of250°C, a spitless injection, an injection volume of 1µL, on a Phenomenex column (2B- multi residue-1 Guardian) with dimensions of 300m × .25m (ID) × 0.25 µm (film thickness). The temperature programs was 50^{o}C for 1 minute then 25^{o}C/min to 125^{o}C for 0 min then 10^{o}C to 300^{o}C for 15 minutes. The run time was 36.5 minutes. The mass spectrometry (Shimadzu TQ 8030) conditions were an interface and EI ion source of 250^{o}C. The solvent delay was 1.5 minutes, the start mass was 45µg, and the end mass was 500µg. The quantification of analytes was accomplished using a matrix-assisted calibration curve on Microsoft Excel 2010.

3.3.4. Quality assurance and quality control.

An auto-tune was performed before analysis, along with a system degradation check and sensitivity check. A matrix-matched calibration curve was used for the quantification of the analysis. Spiked controls were regulatory injected along with spiked samples to ensure effective extraction of analysis in a sample. The average recoveries of DDTs (o,*p*′-DDD, *p,p’*-DDD, *o,p*′-DDE, *p,p*′-DDE, *o,p*′-DDT, *p,p*′-DDT) in the spiked and matrix-spiked blanks were ranged from 93% to 104%, and from 90% to 105%, respectively.

3.4. Human health risk assessment

A human health risk assessment using a standard protocol described by the United States Environmental Protection Agency (US EPA) in 1991 and 2011 was conducted. The exposure parameters used were stipulated as follows;

a) 70 kg of average body weight,
b) 70 years of life expectancy,
c) and 30 years of exposure duration with weekly fish muscle consumptions averaged at 350g.

3.4.1. Average daily dose and the lifetime average daily dose

To evaluate the risk of contracting cancer and chronic non-cancerous toxic health effects from oral exposure, the average daily dose (ADD) and the lifetime average daily dose (LADD) were used (US EPA, 1991, 2011). The ADD or exposure concentration (mg/kg/d) expected to be ingested during the period of exposure was calculated as follows:

$$\mathrm{ADD} = [\mathrm{C(m)} \times \mathrm{IR} \times \mathrm{ED} \times \mathrm{EF}]/(\mathrm{BW})$$

where C(m) = contaminant concentration (mg);

IR = ingestion rate (kg/d);

ED = exposure duration (years);

EF = exposure frequency (days per year);

BW = body weight (kg).

The LADD or concentration that would yield an equivalent exposure if exposure continued for the entire lifetime was calculated as follows: LADD = ADD/ED.

3.4.2. Carcinogenic risk

The cancer risk estimates represent the risk of developing cancer over and above the background cancer rate. To evaluate the carcinogenic risks from exposures that last less than a lifetime, the ADD was adjusted to LADD or cancer risks were calculated using the formula:

Risk = e/ (oral slope factor ⁄ LADD)

3.4.3. Hazard index (HI) or toxic risk

For toxicants that cause non-cancerous toxic effects, a hazard index (HI) was calculated. The HI compares the expected exposure to a chemical to an exposure that is assumed not to be associated with toxic effects. For oral exposures, the ADD is compared to a reference dose (RfD) (available online at http://www.epa.gov/IRIS/) and was calculated as HI = ADD/RfD. The RfD is a conservative estimate of non-cancer toxic hazards with differences in sensitivity to toxic effects within and between species and differences in toxic effects between chronic and sub-chronic exposures considered. The HI is expressed as milligrams of contaminant per kg body weight per day.

3.6. References

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CHAPTER 4 – RESULTS

4.1.concetration of DDT’s detected in the raw and cooked fish muscle tissue before and after annual IRS.

Muscle samples collected from fish after the annual IRS contained no DDT and metabolites. However, the analyses showed concentration of *p,p*-DDT, *p,p*-DDE and *p,p*-DDD in different fish muscle samples (cooked and raw) from the Mutale and Mutshindudi rivers. The results are presented per site in Tables 1 & 2. DDTs were detected in all fish samples but in most it was below the detection limit of 0.5 µg/kg, reading as zero (FDA Laboratories’ principles). We therefore concluded that no DDTs were detected in those fish samples.

Table 1: DDT and its metabolites were concentrated in muscle samples (µg/kg) wm of fish from the Mutshindudi River before the annual indoor residual spraying (IRS).

Mutshindudi River fish muscle sample	***p,p'*-DDE**		***p,p'*-DDD**		***p,p'*-DDT**	
	Fresh	**Cooked**	**Fresh**	**Cooked**	**Fresh**	**Cooked**
1	5	6	nd	nd	nd	nd
2	29	nd	nd	nd	nd	nd
3	13	24	nd	nd	7	nd
4	nd	10	nd	nd	nd	nd
5	nd	8	nd	nd	nd	nd
6	nd	28	nd	nd	nd	nd
7	nd	17	nd	nd	nd	nd
8	5.2	28	nd	nd	nd	nd
9	83	nd	nd	nd	nd	nd
10	18	27	nd	nd	nd	nd
Mean	15	15				
±SD	26	11				
Median	5	13				

Except for sample 3 that contained 7 µg/kg *p,p*-DDT in the fresh cut, *p,p’*-DDE was the only DDT measured in the fish fresh muscle samples from the Mutshindudi River with a mean concentration 15,37 µg/kg (Table 1). After cooking, it showed to be less at 14.64 µg/kg. From Table 2 it was evident that fishes from the Mutale River, bioaccumulated *p,p’*-DDE, *p,p’*-DDD and *p,p’*-DDT. The mean concentration of all DDTs decreased after cooking. The mean *p,p’*-DDE level decreased from 71 µg/kg to 45 µg/kg after cooking, as did *p,p’*-DDD levels decreasing from 17 µg/kg to 9 µg/kg, *p,p’*-DDT levels from 28 µg/kg to 16 µg/kg and the ∑-DDT levels decreased from 115 µg/kg to 70 µg/kg.

Table 2: DDT and its metabolites concentration in raw and cooked muscles samples of fish species from Mutale River before annual IRS.

Mutale River fish muscle samples	*p,p'*-DDE		*p,p'*-DDD		*p,p'*-DDT		∑-DDT	
	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked	Fresh	Cooked
1	58	78	6	11	17	25	81	114
2	111	16	17		36		163	16
3	72	14	7		18		98	14
4		44		7		12		63
5	35	73		11	11	19	46	103
6	237		37		57		331	0
7	16	44		6		8	16	59
8	15						15	0
Mean	71	45	17	9	28	16	115	70
±SD	72	27	14	2	19	7	105	37
Median	58	44	12	9	18	16	89	69

4.2. Human health risk assessment before annual IRS.

Oral ingestion of fish was assumed making use of default ingestion values from the US EPA (1991). *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT mean concentration were detected from Mutshindudi and Mutale rivers were used for the calculations (Table 3) with the exclusion of Thathe Vondo Dam because no DDTs was detected. The ADD and LADD were calculated making use of the assumptions of body weight, number and size of meals, and exposure duration as described in Section 2.2, with the results shown in Table 3.

4.2.1. Cancer risks before annual IRS

The risk assessment showed a potential risk of developing cancer should contaminated fish from Mutale River be consumed. The total cancer risk calculated was higher for the Mutale River and the lower for Mutshindudi river (Figure 3).

4.2.2. Hazard Index before annual IRS.

The HI was higher for the Mutale River compared to the Mutshindudi River (Figure 4).

Table 3: Mean concentrations of DDT and its metabolites in fresh and cooked fish muscle for human health risk assessments and the average daily dose (ADD) and the lifetime average daily dose (LADD) calculated before annual Indoor Residual Spraying (IRS).

Mutshindudi River	***p,p'*-DDE**		***p,p'*-DDD**		***p,p'*-DDT**		**∑-DDT**	
	Fresh	**Cooked**	**Fresh**	**Cooked**	**Fresh**	**Cooked**	**Fresh**	**Cooked**
Average concetration in muscle(mg/kg)	0.015	0.015	0	0	0.001	0	0.01607	0.01464
ADD(mg/kg/d)	3.29E-05	3.14E-05	0	0	0.0000015	0	**0**	0
LADD(mg/kg/d)	1.41E-05	1.34E-05	0	0	6.43E-07	0	**0.0000148**	0.0000134
RfD	0.0003	0.0003	0.00003	0.00003	0.0005	0.0005	0.0005	0.0005
Hazard Quotient	1.10E-01	1.04571E-01	0.00000E+00	0.000E+00	3.00000E-03	0.00000E+00	0.00000E+00	0.000000E+00
Slope	0.34	0.34	0.24	0.24	0.34	0.34	0.34	0.34
Risk	4.80E-06	4.57127E-06	0.00000E+00	0.00000E+00	2.18571E-07	0.00000E+00	5.01778E-06	4.57127E-06
Mutale River	***p,p'*-DDE**		***p,p'*-DDD**		***p,p'*-DDT**		**∑-DDT**	
Average concetration in muscle(mg/kg)	0.06805	0.0334875	0.008425	0.00445	0.01735	0.0080375	0.0938250	0.0459750
ADD(mg/kg/d)	0.00014582	7.1759E-05	1.80536E-05	9.53571E-06	3.7179E-05	1.72232E-05	**0.0002011**	0.0000985
LADD(mg/kg/d)	6.2495E-05	3.0754E-05	7.73724E-06	4.08673E-06	1.5934E-05	7.38138E-06	**0.0000862**	0.0000422
RfD	0.0003	0.0003	0.00003	0.00003	0.0005	0.0005	0.0005	0.0005
Hazard Quotient	0.48607143	0.23919643	0.601785714	0.317857143	0.07435714	0.034446429	0.402107143	0.197035714
Slope	0.34	0.34	0.24	0.24	0.34	0.34	0.34	0.34
Risk	2.1248E-05	1.0456E-05	1.85694E-06	9.80816E-07	5.4174E-06	2.50967E-06	2.92964E-05	1.43555E-05

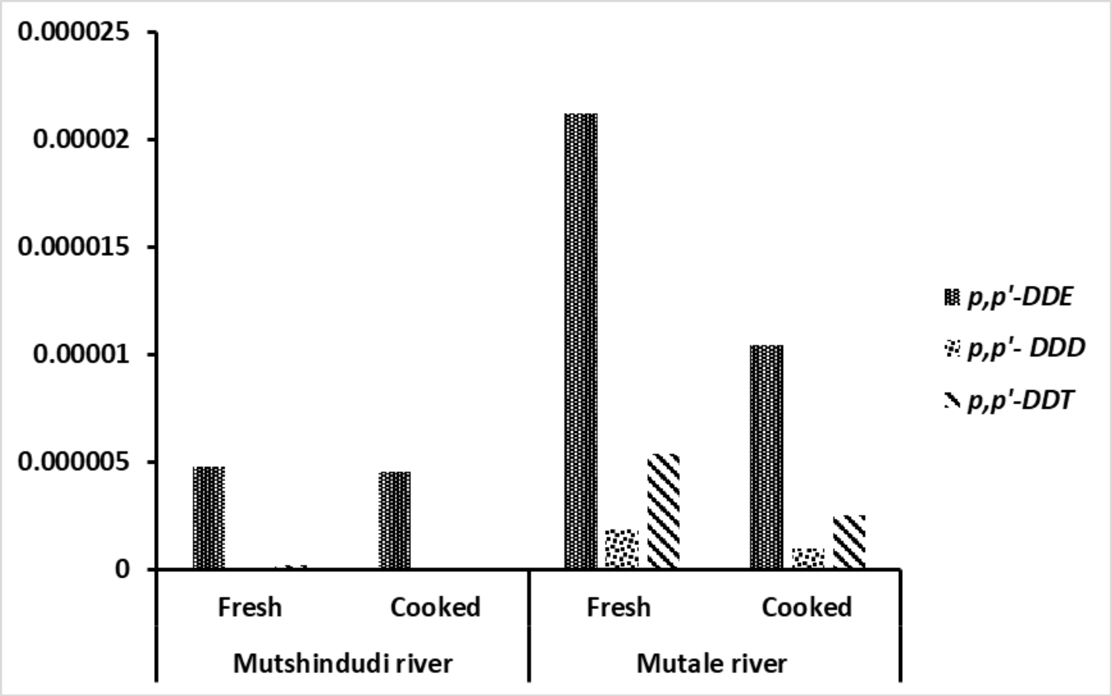

Figure 3: Showing the risk of developing cancer as a result of fish muscle ingestion from the rivers before annual indoor residual spraying (IRS).

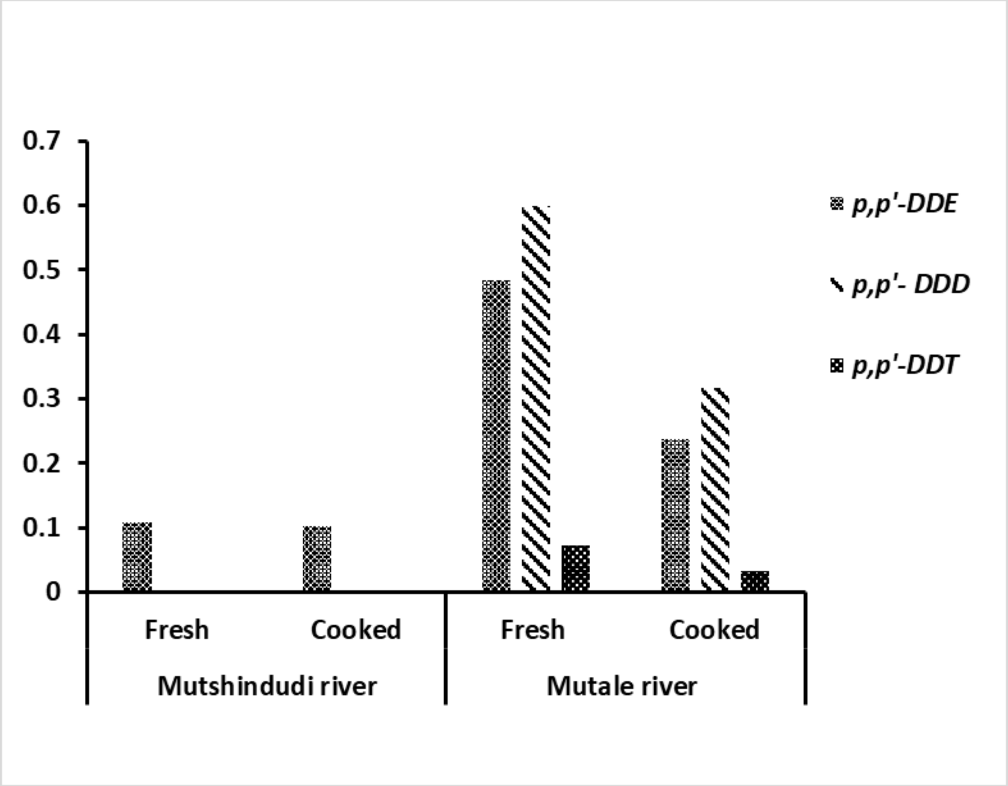

Figure 4: The toxic risks as a result of fish muscle ingestion expressed as a hazard index before annual indoor residual spraying (IRS).

CHAPTER 5 - DISCUSSION AND CONCLUSION

5.1. Discussion

Lately, the Vhembe District has experienced increased anthropogenic activities (Nemalamangwa, 2020). Food security in underdeveloped areas is of concern and pressure on natural ecosystems to ensure food security increases. Rivers are seen as a source of goods and services, primarily protein in the form of fish and freshwater, to the community. To ensure regional and local food security the application of pesticides is also increasing (Fouche, 2015). The solubility of individual compounds and the ability of chemicals to attach to the organic soil matrix are the primary factors influencing POPs, like DDTs, to penetrate water. Chemicals often get to groundwater by leaching and diffuse runoff (Burken, 2014), as well as a variety of other mechanisms such as the physicochemical characteristics of the substance (solubility, degradability), the characteristics of the soil (texture, permeability, amount of organic carbon, and groundwater level depth) in every identified groundwater exposure. Water contamination by DDTs is currently a cause for concern for people worldwide, particularly in nations where the main supply of potable water comes from polluted water sources (Schaider et al., 2014).

The mean DDTs concentrations were higher in the fish from Mutale River compared to the Mutshindudi River. DDTs were found in related studies on different fish species in China (Wang et al., 2013) and the USA (Friese, 2013). The stable isotope results confirmed that cichlids were at the lowest trophic level among the several fish species. Therefore, in various tropical and subtropical regions of Africa, dietary habits did not predict the accumulation patterns discovered by Verhaert et al. (2013, 2017) and Govaerts et al. (2018). According to earlier research on two fish species (*Hydrocynus vittatus* and *Labeo congoro)* from the Olifants River, fish size and lipid content were additional biological factors that have been reported to affect OC pesticide accumulation (Verhaert et al., 2017). Since measuring fish was outside the scope of the study, it was not done, although it might have affected the findings. This work results show the detected level of *p,p'*-DDT was lower than its metabolites, *p,p'*-DDE and *p,p'*-DDD in fish muscle samples from the Mutshindudi and Mutale rivers before IRS (Tables 1 & 2), no detected level of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD was found in the fish muscle of fish from the Thathe Vondo Dam. Among the DDTs detected, *p,p'*-DDE was the most prevalent in all the fish bought and had the highest mean tissue concentrations. The resulting lower concentration of the parent compounds may reflect the current limited use of those persistent OCPs in the Mutale and Mutshindudi rivers. The detected concentration level of DDT and metabolites may be due to a previous historic event with a long half-life (Mitiku et al., 2022).

According to a number of studies carried out in aquatic freshwater systems in Africa and other countries, the sum of DDTs are the most widespread OC pesticides in freshwater fish, while *p,p'*-DDE is the most prevalent and persistent congener (Covaci et al., 2006; Kaur et al., 2008; Barnhoorn et al., 2009; Wepener et al., 2012). Mixed function oxidases' triggered de-chlorination of *p,p'*-DDT to p,p'-DDE is responsible for the high concentrations and excessive accumulation of *p,p'*-DDE in fish species (Schmitt et al., 1990). Wepener et al. (2012) also suggested that the internal biotransformation of DDT to DDE was the cause of the increased levels of *p,p*'-DDE in *H. vittatus* from Lake Pongolapoort. However, for numerous fish, the sun of DDTs were significantly higher than those discovered by Covaci et al. (2006). Based on van Dyk et al. (2010), the use of DDT for malaria vector control in the higher part of the Luvuvhu River is responsible for the high levels of DDT. In South Africa, the application of DDT to control malaria vectors began in the 1940s and continued until 1996. Following this, pyrethroids were used until 2000, at which point DDT was once more used as a result of the discovery of mosquitoes resistant to pyrethroids (Mabaso et al., 2004).

The distribution of the amount of DDTs in different locations might be attributed to several factors. DDTs were found in muscle from fish from the Mutale River in high quantities when compared to the Mutshindudi River. The reason for the high amount of the DDTs in fish from the Mutale River may be due to the slow water current at this point, high malaria vector control activities and migration of DDT-contaminated fishes upstream to downstream from either rivers (Zonkpoedjre et al., 2023). The DDT residues in the muscle tissue of fish from both the rivers were expected as these systems are located in close to settlements where DDT is still used for vector control. Apart from a potential cancer risk following DDT exposure, studies have also shown a risk of developing type 2 diabetes mellitus, altered thyroid hormone levels, affected immune responses in humans and in utero exposure, and delayed neurodevelopment. There is also a greater risk of contracting asthma and a higher chance of contracting breast cancer, especially if the exposure takes place before the age of 14 years, and a risk of both seminoma Tous and non-seminoma Tous testicular carcinoma (Cohen and Jefferies, 2019).

The concentrations of DDTs in fish are highest at the end of the dry season when the flow is at its lowest and no rainfall, according to research by Gerber et al. (2016). As a result, pollutants most likely become concentrated since the water levels are lower than they are at other seasons. This might be the reason why no DDT concentration was detected after the IRS sample, as it was after huge rainfalls and water level and flow were so much higher. DDT itself has been demonstrated to have a variety of harmful impacts on wildlife populations across South Africa (Bouwman et al., 2013) as well as other places, including Ndumo and the surrounding regions (Bouwman et al., 2019), internationally (Fry, 1995). It might possibly be connected to the thinned eggshell, and resultant decreases in bird species' populations, as stated by Gonzalez-Jauregui et al.,(2012).

The US EPA defined human health risk assessment as the process to estimate the nature and probability of adverse health effects in humans exposed to chemicals in contaminated environmental media, now or in the future (USEPA, 2022). Risk assessment for DDTs is estimated using parameters such as estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI). Based on these parameters, the local population health risk from the consumption of fish from the two selected rivers using the DDT concentration from the muscle samples was evaluated. All EDI of DDTs residues exceeded the acceptable daily intake (ADI) recommended by various organizations. This emphasizes the possibility of adverse health effects in humans should fish be consumed over a long-term period (considering the stipulated population parameters in Section 3.4). The results showed that should one consume fish from the Mutale River there is a higher chance of developing cancer while there is a lower chance of developing cancer should one consume fish from the Mutshindudi River. This is considerably higher than the recommended risk value) of 1 in 100 000 as an acceptable risk. However, it is unlikely that individuals will be exposed to fish with similar, high concentrations of DDTs over 30 years unless they are regular fishermen residing in the same area throughout their lifetime and consuming at least 350 g of contaminated fish per week. The US EPA generally considers risks below 1 in 100 000 to be acceptable and above 1 in 10000 as unacceptable.

Steaks of fish (such as S*almo salar*) when baked, boiled, fried, or microwaved, there was no significant difference in POP loss between the cooking methods (Bayen et al., 2005). Moreover, boiling fish muscles did not show any significant DDT concentration reduction pattern (Bhuiyan et al., 2009). Pesticides break down to different degrees whenever food undergoes heating (Keikotlhaile et al., 2010). After cooking the fish muscles some seem to have reduced DDT concentration but some have increased DDT metabolites, meaning that cooking them might increase the breaking down of DDT. Deep frying and also roasting are found to be the best methods of cooking fish to reduce the concentration of DDTs and other pesticides (Alaboudi et al., 2021). concentration of all OCs including DDT in fish was reduced by up to 93%, but not 100% (Islam et al., 2022).

5.2. Conclusion

Thus, the present study recommends the cooking of fish after treating those fishes with different methods such as soaking them in a mixture of sodium bicarbonate solution, vinegar, salt solution and lemon juice, followed by a running tap-water washing to reduce the contamination of the fishes.

It was hypothesized that fish from the site having higher DDT residues, will also have higher DDT residues, it was not clear to say because water from all sit DDTs level was below detection limit reading as zero. Furthermore, it was also hypothesized that cooking fish should reduce DDT residues, DDT did reduce after cooking, but DDE increases in some samples after the cooking process. DDT-contaminated fish may pose a human health risk for people living in IRS areas, in this case, it cannot be said as the result show this fish showing low DDTs levels. Lastly it was hypothesized that DDT from fish is not a link to PCa in Vhavenda Men, the results supporeted the hypothesis as DDTs concentrations were so low.

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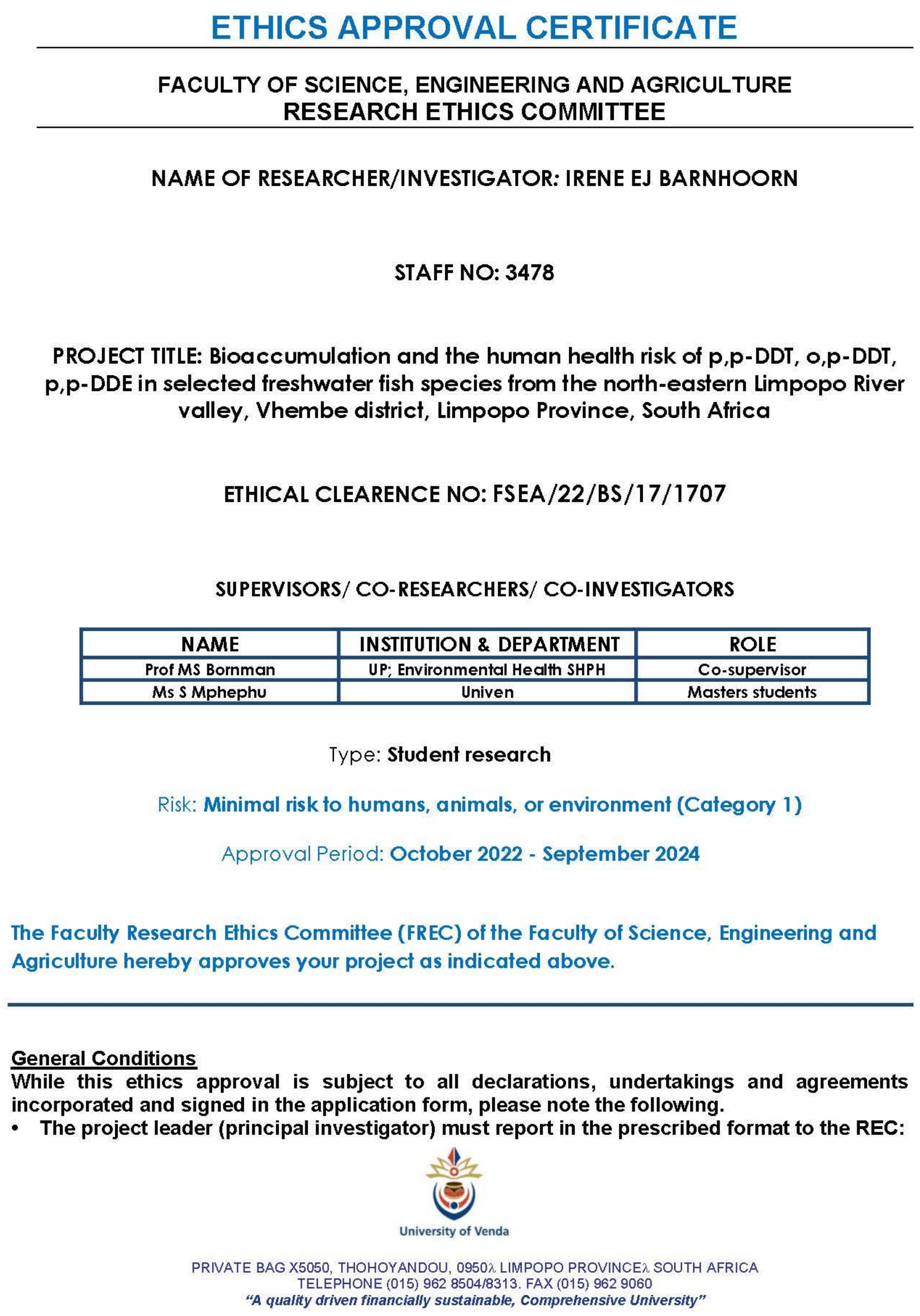
APPENDIX A

ETHICS APPROVAL CERTIFICATE



ETHICS APPROVAL CERTIFICATE

FACULTY OF SCIENCE, ENGINEERING AND AGRICULTURE
RESEARCH ETHICS COMMITTEE

NAME OF RESEARCHER/INVESTIGATOR: IRENE EJ BARNHOORN

STAFF NO: 3478

PROJECT TITLE: Bioaccumulation and the human health risk of p,p-DDT, o,p-DDT, p,p-DDE in selected freshwater fish species from the north-eastern Limpopo River valley, Vhembe district, Limpopo Province, South Africa

ETHICAL CLEARENCE NO: FSEA/22/BS/17/1707

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof MS Bornman	UP; Environmental Health SHPH	Co-supervisor
Ms S Mphephu	Univen	Masters students

Type: **Student research**

Risk: **Minimal risk to humans, animals, or environment (Category 1)**

Approval Period: **October 2022 - September 2024**

The Faculty Research Ethics Committee (FREC) of the Faculty of Science, Engineering and Agriculture hereby approves your project as indicated above.

General Conditions

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following.

- **The project leader (principal investigator) must report in the prescribed format to the REC:**

University of Venda

PRIVATE BAG X5050, THOHOYANDOU, 0950λ LIMPOPO PROVINCEλ SOUTH AFRICA
TELEPHONE (015) 962 8504/8313. FAX (015) 962 9060
"A quality driven financially sustainable, Comprehensive University"

ETHICS APPROVAL CERTIFICATE

  - **Annually (or as otherwise requested) on the progress of the project, and upon completion of the project**
  - **Within 48hrs in case of any adverse event (or any matter that interrupts sound ethical principles) during the project.**
  - **Annually, research projects may be randomly selected for auditing.**
- **The approval applies strictly to the protocol as stipulated in the application form. Should a change to the protocol be deemed necessary during the project, the project leader must apply for approval of these changes before their implementation. Should there be a deviation from the study protocol, without the necessary approval for the change, the ethics approval is automatically forfeited.**
- **The date of approval indicates the earliest date that the project may begin. Should the project have to continue after the expiry date; a new application must be made, and a new approval received before or on the expiry date.**
- **In the interest of ethical responsibility, the FREC retains the right to:**
  - **Request access to any information or data at any time during the course or after completion of the project,**
  - **To ask further questions; Seek additional information; Require further modification or monitor the conduct of your research or the informed consent process.**
  - **withdraw or postpone approval if:**
  - **Any unethical principles or practices of the project are revealed or suspected.**
  - **It becomes apparent that relevant information was withheld from the REC or that information has been false or misrepresented.**
  - **The required annual report and reporting of adverse events was not done timely and accurately,**
  - **New institutional rules, national legislation or international conventions deem it necessary**

ISSUED BY:
FACULTY OF SCIENCE, ENGINEERING AND AGRICULTURE RESEARCH ETHICS COMMITTEE

Date considered: October 2022

Chairperson: Prof. P.O Bessong

University of Venda

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APPENDIX B

Table A: fish species from Mutale River, grouped according to date of collection and same species, before indoor residual spraying.

		MUTALE RIVER		
FISH NO	SPECIES NAMES	DATE	LOCATION	COMBINED
1	*O. mossambicus*	1/9/2022	Water Affairs	MT1 F&C
2	*O. mossambicus*	1/9/2022	Water Affairs	
3	*O. mossambicus*	1/9/2022	Water Affairs	
4	*O. mossambicus*	8/9/2022	Water Affairs	MT2 F&C
5	*C. garipienus*	12/9/2022	Water Affairs	MT3 F&C
6	*C. gariepinus*	12/9/2022	Water Affairs	
7	*C. gariepinus*	12/9/2022	Water Affairs	
8	*C. gariepinus*	12/9/2022	Water Affairs	MT4 F&C
9	*C. gariepinus*	12/9/2022	Water Affairs	
10	*C. gariepinus*	12/9/2022	Water Affairs	MT5 F&C
11	*C. gariepinus*	12/9/2022	Water Affairs	
12	*C. gariepinus*	13/9/2022	Water Affairs	MT6 F&C
13	*C. gariepinus*	13/9/2022	Water Affairs	
14	*C. gariepinus*	13/9/2022	Water Affairs	
15	*C. gariepinus*	15/9/2022	Water Affairs	MT7 F&C
16	*C. gariepinus*	15/9/2022	Water Affairs	
17	*C. gariepinus*	15/9/2022	Water Affairs	
18	*C. gariepinus*	15/9/2022	Water Affairs	MT8 F&C
19	*C. gariepinus*	15/9/2022	Water Affairs	
20	*C. gariepinus*	15/9/2022	Water Affairs	

Table B: fish species from Mutshindudi River, grouped according to date of collection and same species, before indoor residual spraying**.**

MUTSHUNDUDI RIVER				
FISH NO	SPECIES NAMES	DATE	LOCATION	COMBINED
1	*M. punctulatus*	11/9/2022	Ngudza	MUT1 F&C
2	*M. punctulatus*	11/9/2022	Ngudza	
3	*M. punctulatus*	11/9/2022	Ngudza	
4	*M. punctulatus*	11/9/2022	Ngudza	MUT2 F&C
5	*M. punctulatus*	11/9/2022	Ngudza	
6	*M. punctulatus*	11/9/2022	Ngudza	
7	*R.moddervis*	11/9/2022	Ngudza	MUT3 F&C
8	*M. punctulatus*	12/9/2022	Ngudza	MUT4 F&C
9	*M. punctulatus*	12/9/2022	Ngudza	
10	*M. punctulatus*	13/9/2022	Ngudza	MUT5 F&C
11	*C. gariepinus*	13/9/2022	Ngudza	MUT6 F&C
12	*M. punctulatus*	19/9/2022	Ngudza	MUT7 F&C
13	*M. punctulatus*	19/9/2022	Ngudza	
14	*M. punctulatus*	19/9/2022	Ngudza	
15	*M. punctulatus*	19/9/2022	Ngudza	
16	*M. punctulatus*	19/9/2022	Ngudza	MUT8 F&C
17	*M. punctulatus*	19/9/2022	Ngudza	
18	*M. punctulatus*	19/9/2022	Ngudza	
19	*O. mossambicus*	19/9/2022	Ngudza	MUT9 F&C
20	*M. capensis*	19/9/2022	Ngudza	MUT10 F&C

Table C: fish species from Thathe Vondo Dam, grouped according to date of collection and same species, before indoor residual spraying.

		THATHE VONDO DAM		
FISH NO	SPECIES NAMES	DATE	LOCATION	COMBINED
1	*O. mossambicus*	31/8/2022	Thathe	TV1 F&C
2	*O. mossambicus*	31/8/2022	Thathe	
3	*O. mossambicus*	15/09/2022	Thathe	TV2 F&C
4	*O. mossambicus*	15/09/2022	Thathe	
5	*O. mossambicus*	15/09/2022	Thathe	
6	*O. mossambicus*	15/09/2022	Thathe	
7	*M. punctulatus*	15/09/2022	Thathe	TV3 F&C
8	*M. punctulatus*	16/9/2022	Thathe	TV4 F&C
9	*O. mossambicus*	16/9/2022	Thathe	TV5 F&C
10	*O. mossambicus*	16/9/2022	Thathe	
11	*M. punctulatus*	16/9/2022	Thathe	TV6 F&C
12	*O. mossambicus*	16/9/2022	Thathe	TV7 F&C
13	*O. mossambicus*	16/9/2022	Thathe	
14	*O. mossambicus*	16/9/2022	Thathe	
15	*O. mossambicus*	16/9/2022	Thathe	TV8 F&C
16	*O. mossambicus*	16/9/2022	Thathe	
17	*O. mossambicus*	16/9/2022	Thathe	
18	*O. mossambicus*	16/9/2022	Thathe	TV9 F&C
19	*O. mossambicus*	16/9/2022	Thathe	
20	*O. mossambicus*	16/9/2022	Thathe	

Table A: fish species from Mutale River, grouped according to date of collection and same species, after indoor residual spraying.

MUTALE RIVER				
FISH NO	SPECIES NAMES	DATE	LOCATION	COMBINED
1	*O.mossambicus*	27/04/23	water affairs	MT1 F & C
2	*O.mossambicus*	27/04/23	water affairs	
3	*O.mossambicus*	27/04/23	water affairs	
4	*S. pilchardus*	27/04/23	water affairs	MT2 F & C
5	*S. pilchardus*	27/04/23	water affairs	
6	*O.mossambicus*	27/04/23	water affairs	MT3 F & C
7	*O.mossambicus*	27/04/23	water affairs	
8	*O.mossambicus*	27/04/23	water affairs	
9	*O.mossambicus*	27/04/23	water affairs	MT4 F & C
10	*O.mossambicus*	27/04/23	water affairs	
11	*O.mossambicus*	27/04/23	water affairs	
12	*O.mossambicus*	27/04/23	water affairs	MT5 F & C
13	*O.mossambicus*	27/04/23	water affairs	
14	*O.mossambicus*	27/04/23	water affairs	MT6 F & C
15	*O.mossambicus*	27/04/23	water affairs	
16	*O.mossambicus*	27/04/23	water affairs	MT7 F & C
17	*O.mossambicus*	27/04/23	water affairs	
18	*O.mossambicus*	27/04/23	water affairs	
19	*O.mossambicus*	27/04/23	water affairs	
20	*O.mossambicus*	27/04/23	water affairs	

Table B: fish species from Mutshindudi River, grouped according to date of collection and same species, after indoor residual spraying.

MUTSHINDUDI RIVER				
FISH NO	SPECIES NAMES	DATE	LOCATION	COMBINED
1	*O.mossambicus*	16/04/23	Ngudza	MUT1 F &C
2	*O.mossambicus*	16/04/23	Ngudza	
3	*O.mossambicus*	16/04/23	Ngudza	MUT2 F &C
4	*O.mossambicus*	16/04/23	Ngudza	
5	*O.mossambicus*	16/04/23	Ngudza	MUT3 F &C
6	*O.mossambicus*	16/04/23	Ngudza	
7	*O.mossambicus*	16/04/23	Ngudza	
8	*M. punctulatus*	16/04/23	Ngudza	MUT4 F &C
9	*M. punctulatus*	16/04/23	Ngudza	MUT5 F &C
10	*M. punctulatus*	16/04/23	Ngudza	MUT6 F &C
11	*O.mossambicus*	30/04/23	Ngudza	MUT7 F &C
12	*O.mossambicus*	30/04/23	Ngudza	
13	*O.mossambicus*	30/04/23	Ngudza	
14	*O.mossambicus*	30/04/23	Ngudza	MUT8 F &C
15	*O.mossambicus*	30/04/23	Ngudza	
16	*O.mossambicus*	30/04/23	Ngudza	
17	*O.mossambicus*	30/04/23	Ngudza	MUT9 F &C
18	*O.mossambicus*	30/04/23	Ngudza	
19	*O.mossambicus*	30/04/23	Ngudza	MUT10 F &C
20	*O.mossambicus*	30/04/23	Ngudza	

Table C: fish species from Thathe Vondo Dam, grouped according to date of collection and same species, after indoor residual spraying.

THATHE VONDO DAM				
FISH NO	SPECIES NAME	DATE	LOCATION	COMBINED
1	*O.mossambicus*	16/04/22	Thathe	TV1 F&C
2	*O.mossambicus*	16/04/22	Thathe	
3	*O.mossambicus*	16/04/22	Thathe	TV2 F&C
4	*M.punctulatus*	16/04/22	Thathe	
5	*O.mossambicus*	16/04/22	Thathe	TV3 F&C
6	*O.mossambicus*	16/04/22	Thathe	
7	*O.mossambicus*	16/04/22	Thathe	TV4 F& C
8	*O.mossambicus*	16/04/22	Thathe	
9	*O.mossambicus*	16/04/22	Thathe	TV5 F & C
10	*O.mossambicus*	16/04/22	Thathe	
11	*O.mossambicus*	16/04/22	Thathe	TV6 F& C
12	*O.mossambicus*	16/04/22	Thathe	
13	*O.mossambicus*	16/04/22	Thathe	TV7 F&C
14	*O.mossambicus*	16/04/22	Thathe	
15	*O.mossambicus*	16/04/22	Thathe	TV8 F&C
16	*O.mossambicus*	16/04/22	Thathe	
17	*O.mossambicus*	16/04/22	Thathe	TV9 F& C
18	*O.mossambicus*	16/04/22	Thathe	
19	*O.mossambicus*	16/04/22	Thathe	TV10 F & C
20	*O.mossambicus*	16/04/22	Thathe	