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THE AMERICAN
UNIVERSITY IN CAIRO

# Analysis of organochlorine pesticides levels in irrigation water from various water sources across Egypt

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

By Omar Tarek Ahmed Khairy

Under the supervision of

Dr. Tamer Shoeib

Fall 2019

## Dedication

To my beloved Father and Mother who always pushed me to become the best version of myself and who always showered me with endless care and love.

To my beloved wife, may our life always be filled with love, peace and laughter.

## Acknowledgments

I would like to express my deepest gratitude and appreciation to my supervisor, **Dr. Tamer Shoeib**, who was very patient with me throughout my whole period of studying for my degree, always understanding of my working hours and coaching me on regular basis. Working with Dr. Tamer was a real pleasure, with his continuous support and guidance, he helped make this journey smooth and worth the effort and long working hours.

Special thanks goes to **Dr. Nermin Salah** who taught me a lot in Mass Spectroscopy and without her help and guidance I probably wouldn't have made it so far. Working with Dr. Nermin felt like working with a close friend and not with a supervisor. Thanks again for your all your continuous assistant, your encouragement and your kind words.

I would also like to thank **Mr. Mahmoud Abdel Moez** from the department of Chemistry, AUC. You were always very keen to help me with anything I needed in terms of lab supplies or equipment. I always appreciated your advice and guidance and really enjoyed our chats during the long nights that I spent working in the instrumental analysis lab.

I can't thank enough my **father and mother** for all their continuous love and support that they provide me. Whenever I felt like giving up, they would always give me the boost I needed to work even harder than before, they always knew the right words to say. I am nothing without you.

Finally, I would like to thank my **wife; Marwa Hamed.** You always believed in me even when I didn't have that faith in myself. I will always remember our discussions of my work over the phone and I will always cherish the level of patience you had back then to listen to my continuous chatter, guide me and push me on. Thank you, my support and number one fan.

# Abbreviations

| | |
|---|---|
| POPs | Persistent Organic Pollutants |
| OC pesticides | Organochlorine Pesticides |
| PCBs | Polychlorinated Biphenyls |
| DDT | Dichlorodiphenyltrichloroethane |
| o,p-DDD | 1,1-(Dichlorodiphenyl)-2,2-dichloroethane (A by-product of DDT) |
| HCH | Hexachlorocyclohexane |
| HCB | Hexachlorobenzene |
| OP | Organophosphates |
| pKa | Acid-base ionization constant |
| EPA | USA Environmental Protection Agency |
| MRL | Minimum Reporting Limit |
| LOQ | Limit of Quantitation |
| LOD | Limit of Detection |
| GC | Gas Chromatography |
| ECD | Electron Capture Detector |
| MS | Mass Spectroscopy |
| SIM | Single Ion Monitoring |
| SPE | Solid Phase Extraction |
| SPME | Solid Phase Microextraction |
| ELISA | Enzyme Linked Immunosorbent Assay |
| EI | Electron Ionization |
| WHO | World Health Organization |
| N.D | Not detected |

## Abstract

Organochlorine pesticides are extensively used in agriculture to cope with the world’s increasing population and demands for nutrition. This class of pesticides poses a serious risk to human health upon chronic exposure to elevated levels. Determination of organochlorine pesticides levels in water has been extensively covered in literature in Egypt and other parts of the world. The need for up-to date data and statistical information regarding the levels of organochlorine pesticides in water is ever increasing. These compounds, although a lot have been prohibited from use decades ago, can still be detected in trace levels in water due to their long half-lives and bioaccumulation. This study contributes to the existing pool of data of pesticide levels in water and expands on it by assessing the levels in new sources of irrigation water and fish farms as well. All samples collected were extracted using Solid-Phase Extraction technique and analyzed using GC-ECD as well as GC-MS. Levels of 7 pesticides was determined and reported for both instruments. Among the 7 reported oc pesticides, heptachlor was found to be the most recurring with a frequency of detection of 52.9% in GC-ECD and 18.18% in GC-MS and mean values of 0.09 μg/l and 0.10 μg/l respectively. Mean values reported were found to be higher than the acceptable limit of WHO of 0.03 μg/l which indicates a significant level of pollution that requires future mitigation. o,p-DDD was not detected in any of the samples, while p,p-DDT was detected in only one sample with a value of 0.06 μg/l. This study indicates that although DDT was heavily used in the past decades in Egypt, its prohibition from the 1980s forward may have contributed to the decrease of its levels in the natural water resources to the point that its almost not detected in this study. Fish Farms (Tanks) marked the highest frequency of pesticides detection with 100% detection with GC-ECD and 33% detection with GC-MS. Mean values of total oc pesticides in GC-ECD for fish farms samples was 0.334 μg/l for GC-ECD and 1.484 μg/l for GC-MS, a possible evidence of bioaccumulation throughout the food chain.

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# 1. Chapter One: Background and Review of Literature

## 1.1. The Stockholm Convention

A Review Committee comprised of 31 government designated experts in chemical assessment and management convened in Stockholm Sweden in 2001and decided to sign an agreement to limit the use and importation of a class of chemicals that are labelled as Persistent Organic Pollutants (POPs)[1].

These may be defined as xenobiotic chemicals that are carbon-based and tend to deposit in living tissues, bioaccumulate throughout the food chain and eventually reach the human body through dietary consumption. These compounds include synthesized chemicals intended for agricultural and industrial usages (such as organochlorine pesticides and polychlorinated biphenyls; PCBs)[2].

The governing council of the United Nations Environment Program UNEP requested ,in May 1995, that a global assessment process be conducted and to result in a list of chemical compounds for which recommendations and international action should be taken to mitigate their effects. The list consisted of 12 POPs that came to be known later as the “Dirty Dozen”. These chemicals included nine pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene), two industrial chemicals (hexachlorobenzene, polychlorinated biphenyls (PCBs)) and two of their by-products (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF))[1].

The Stockholm Convention actions were in effect in May 2004 with the objectives of eliminating production and use of POPs while mandating registration of specific exemptions for their production or use and to limit their importation. The convention also promoted the use of best available techniques to limit the release of POPs into the environment and mandating proper management of available stockpiles and wastes[1].

## 1.2. The Rise and Evolution of Pesticides

The very first civilizations in the world started with the discovery of agriculture. Humans turned from Hunter-Gatherers to Harvesters of agricultural crops and these abundant sources of food gave rise to the very first cities. Naturally, as humans began forming established societies many hundreds of years ago, they began seeking methods to protect their crops. Formulations of toxic compounds of sulfur, arsenic, lead and nicotine were applied throughout history and used as insecticides. [3]

These are what are nowadays referred to as first-generation pesticides and they were largely derived from plants. It was the arrival of DDT that ushered in the so called second generation of pesticides. It even earned entomologist Paul Mueller a Nobel Prize in 1948[3].

After World War II more organochlorine pesticides were in use and the age of OC pesticides started and lasted till the 1970s. The search for safer alternatives gave rise to organophosphate and carbamate insecticides[4].

The contribution of pesticides towards increased agricultural crop yields throughout the past century is well studied and documented. The green revolution of the 1940s was built on the foundations of the development of high-yielding agricultural crops, the development of modern irrigation technologies and the widespread and frequent use of agrochemicals, including synthetic products, for the mitigation of pests and insects [4].

Since the 1940s, pesticides are being used to enhance food production and quality. The increasing world population and demands for nutrition places more stress to boost the agricultural yield without the need to increase the agricultural land. In addition, constraints such as shying away from deforestation, insufficient water resources or the need to maintain the ecological reserves, the world had to make use of what agricultural land was available. Technically, pesticides do not enhance agricultural production as fertilizers do, however, they do prevent crop losses and thus contribute to higher yields [5]

While for requirement of 2900 kcal/day, the daily average caloric intake in developed countries is around 3500 kcal/day which relies heavily on livestock products. In developing countries on the other hand, daily average caloric intake is around 2000 kcal/day relying heavily on cereal grains.

Over the last decades a new class of pesticides called systemic pesticides came to existence. These types of compounds have the ability to move systemically through the xylem of plants and provide some kind of protective coating that repels, through its toxicity, insects and fungi[4].

Currently, there is a huge number of pesticide compounds belonging to more than one hundred chemical classes and they are used worldwide.

### 1.3. Egyptian Pesticides Market

In Egypt there is a total of around 6 million acres of cultivable land distributed between the Nile Delta and the Nile River Valley. These areas represent a mere 5-7% of the total area of Egypt while no other areas are dedicated for agricultural activities in the rest of the lands except for some oases that rely heavily on underground waters or the north western coasts along the Mediterranean that rely on rain water for irrigation[6].

Cairo, a densely populated urban hub acted as the basin of some million metric tons of various pesticides that were being dumped into its waters since the 1950s up till now[6].

Cotton was and still is one of the most important Egyptian crops and one of the important pillars of Egyptian economy. In the 1950s, protecting such a vital resource from pests justified the heavy use of insecticides. Hence, insecticides were applied these days by high pressure ground sprayers to mitigate the cotton leafworm. The first insecticide mixture to be used contained mainly DDT. However, as time progressed and the cotton worm began to show signs of resistance, other types of pesticides had to be used with toxaphene being the most commonly used during the 1950s and 1960s. Protection of crops is not just limited to cotton, corn, rice, sugarcane and many other crops were treated with large quantities of pesticides of various classes[5].

The Egyptian Pesticide Market gradually changed from the 1950s and 1960s, where the use of organochlorine (OC) compounds was common place, to the use of organophosphates (OP) and carbamates as evident in the high usage of chlorpyrifos, an organophosphate from 1969 to 1990 where the usage of around 13,500 total metric tons of the chemical was recorded. Organophosphates and carbamates were thought to cause less environmental damage at the time. The average use of pesticides in Egypt has decreased significantly from around 26,000 metric tons from 1970-1979 to around 12,000 metric tons from 2000-2006[7].

While Africa on the whole is not a huge importer of pesticides in the global market, North Africa shows some signs of intense pesticides usage that can be attributed to the cotton-growing regions of Egypt and Sudan. Many of the large-scale commercial farms that produce coffee for example, as in the case in Kenya, tend also to rely heavily on pesticide usage. Many of the obsolete classes of OC pesticides in Europe and North America are still being sold and used in Africa[3].

A.O. Barakat / Environment International 30 (2004) 309–322

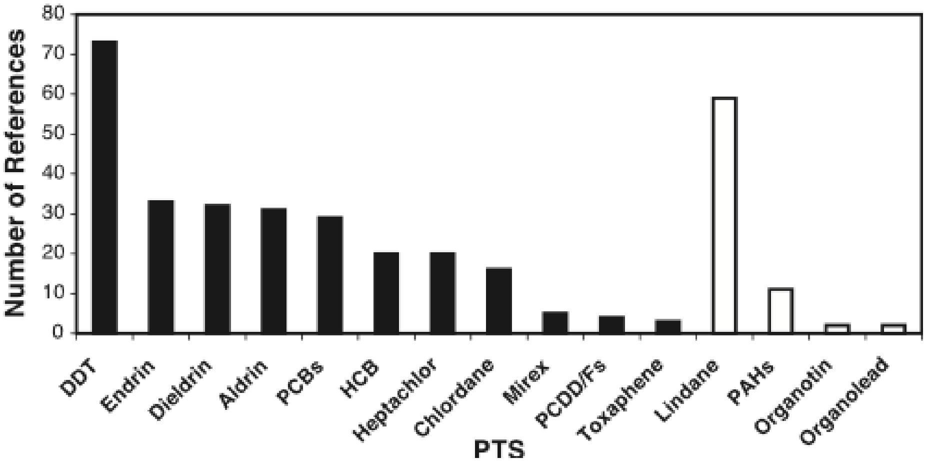

Fig. 1. A literature survey of PTS covering the years 1980–2001.

**Figure 1**: A statistical bar chart summarizing the frequency of oc pesticides reporting in Egyptian literature throughout the period of 1980-2001 [7]

Figure 1 summarizes the number of references to a number of persistent toxic substances (PTS) in Egypt. From this figure we can see that organochlorines were one of the heavily studied classes of pesticides, especially DDT and Lindane. This is followed by Endrin, Dieldrin ,Heptachlor and Hexachlorobenzene. The compounds chosen in this study are; Alpha-HCH, Heptachlor, Aldrin, Heptachlor Epoxide Isomer B, o,p-DDD, Endrin and p,p-DDT. These include some of the compounds classified by the Stockholm Convention as persistent organic pollutants (POPs) and consequently bear high significance[7].

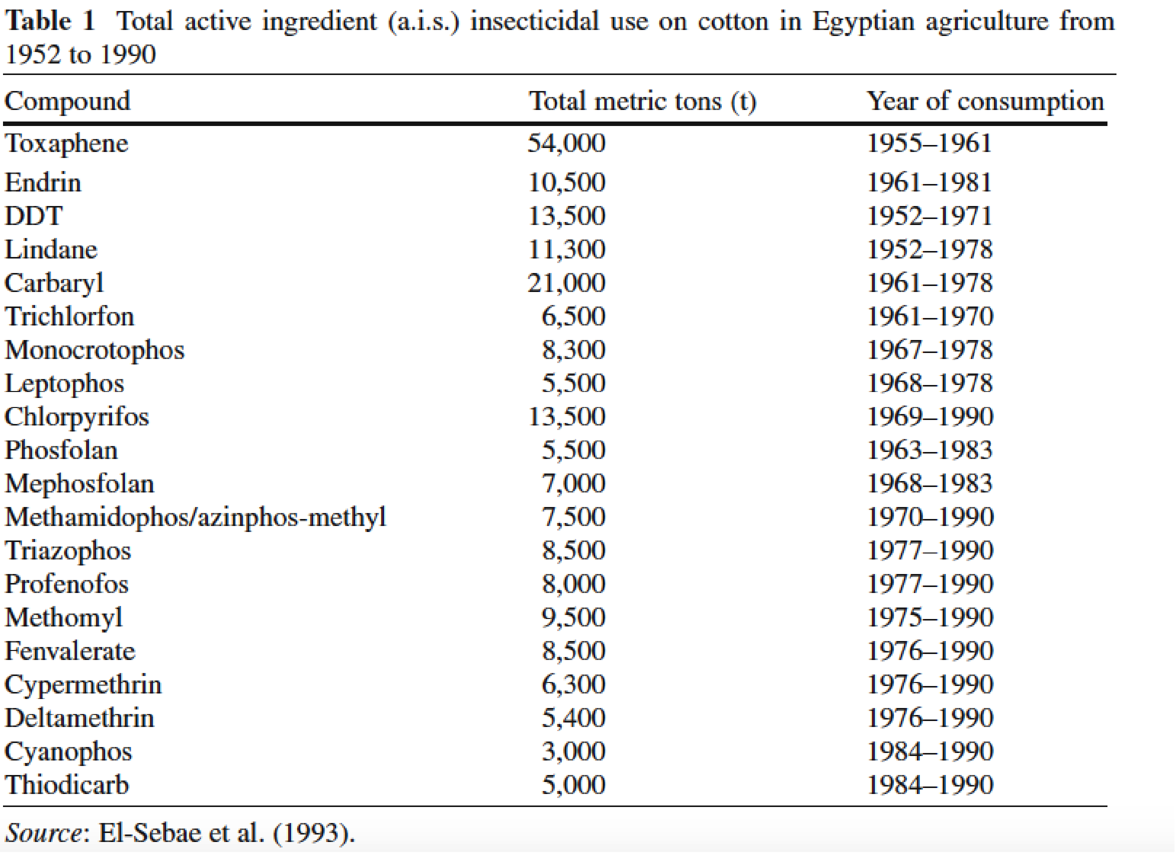

**Table 1** Total active ingredient (a.i.s.) insecticidal use on cotton in Egyptian agriculture from 1952 to 1990

| Compound | Total metric tons (t) | Year of consumption |
|---|---|---|
| Toxaphene | 54,000 | 1955–1961 |
| Endrin | 10,500 | 1961–1981 |
| DDT | 13,500 | 1952–1971 |
| Lindane | 11,300 | 1952–1978 |
| Carbaryl | 21,000 | 1961–1978 |
| Trichlorfon | 6,500 | 1961–1970 |
| Monocrotophos | 8,300 | 1967–1978 |
| Leptophos | 5,500 | 1968–1978 |
| Chlorpyrifos | 13,500 | 1969–1990 |
| Phosfolan | 5,500 | 1963–1983 |
| Mephosfolan | 7,000 | 1968–1983 |
| Methamidophos/azinphos-methyl | 7,500 | 1970–1990 |
| Triazophos | 8,500 | 1977–1990 |
| Profenofos | 8,000 | 1977–1990 |
| Methomyl | 9,500 | 1975–1990 |
| Fenvalerate | 8,500 | 1976–1990 |
| Cypermethrin | 6,300 | 1976–1990 |
| Deltamethrin | 5,400 | 1976–1990 |
| Cyanophos | 3,000 | 1984–1990 |
| Thiodicarb | 5,000 | 1984–1990 |

*Source*: El-Sebae et al. (1993).

**Figure 2** shows the relative large consumption of Endrin, DDT and Lindane with respect to other pesticides. [5]

Egypt is industrializing with a fast rate with intensive use of chemicals in a wide array of industrial sectors as shown in Table 1. The great majority of the chemicals used are imported into the country, with nearly all industrial activities being concentrated in Greater Cairo, 40% in Alexandria and the rest is in the Delta, Upper Egypt and New Cities[3].

## 1.4. Chemical properties of Persistent Organic Pollutants (POPs)

### 1.4.1. Physicochemical Properties of Organochlorine Pesticides

Physicochemical properties determine how a chemical compound will behave in the environment and can also provide researchers with clues regarding the availability and concentration of the pesticides in certain areas and media of the environment[8]. Compounds such as aldrin, endrin and dieldrin are not degraded by naturally known mechanisms and tend to accumulate in the tissues of living organisms as well as in the waters of the rivers that are in proximity to agricultural lands[9]

#### 1.4.1.1. Water Solubility

Water solubility determines the maximum concentration of a pesticide that is dissolved in water at equilibrium. This parameter is usually reported in mg/l at 20°C. Pesticides with high water solubility will be typically prevalent in high concentrations in ground water, and will be carried away easily by irrigation water to reach water sources and are thus critical for interpreting routes of mammalian excretion.

Generally products that are more soluble leach into greater depths, the pH of the medium also governs the ionization of the molecules. The solubility of other salts and minerals in water can result in the decrease of pesticide solubility.

Water solubility changes depending on a number of factors like the chemical structure, the molecular size and weight, the functional groups and their locations, the freedom of rotation, the physical state of the compound, the solvent and the environmental conditions[9].

#### 1.4.1.2. Vapor Pressure

Vapor pressure determines the distribution of a chemical compound between the liquid and gas phase i.e evaporation, or between solid and gas phase i.e sublimation. It measures the partial pressure exerted by the chemical in the gas phase when in equilibrium between the gas phase and one of the two previously mentioned phases.

The higher the vapor pressure the more volatile the chemical compound. Knowing the pesticide volatility can help us draw conclusions regarding its evaporation rate from the soil to the air.

Vapor pressure is dependent, usually in a non-linear manner on the temperature. In tropical regions, vapor pressure plays a key role in the evaporation of pesticide molecules from water and the surface of vegetations and to lesser degrees from soil.

It can also be expected that compounds with high volatility can induce toxicity through inhalation[9].

#### 1.4.1.3. Acid-Base Ionization Constant (pKa)

This term describes the equilibrium between the dissociated and the undissociated form of any acid, in other terms the balance between the ionic and non-ionic forms of the acid. The higher the pKa, the weaker is the acid.

Knowing the pKa of different pesticides can give us clues as to how they distribute between the soil and water, in order to estimate a leaching rate. Moreover, close estimations of pKa values can guide us to proper optimization of the extraction techniques since it is easier to extract the pesticide compound in its non-ionic form, which can be done by simple pH adjustment of the solution[9].

#### 1.4.1.4. Octanol-Water Partition Coefficient

The octanol–water partition coefficient, Kow, is the equilibrium ratio of concentrations of a two-phase system comprised of water and n-octanol. In other words, the partition coefficient is the ratio between the concentration of pesticide in the n octanol, non-aqueous layer to the concentration of the pesticide dissolved in the water layer[9].

This parameter gives an indication of the lipophilicity of the pesticide molecule and therefore can help us draw conclusions regarding its bioaccumulation tendencies and its half-life as will be later discussed. Knowledge of this parameter can also prove useful when working with reversed phase liquid chromatography, as this can drive the choice of the reversed phase stationary phase according to the polarity of the molecule[9].

#### 1.4.1.5. Half Life

The half-life of any chemical compound can be described as the time needed for a compound to reach half of its initial concentration. The half-life is dependent on several factors; the physicochemical properties, the environmental conditions, the pesticide application history and the biomass concentrations.

Better insights of the mechanisms of pesticide persistence in soil can be gained from improved understanding of the environmental parameters that influence the half-life including biodegradation[9].

### 1.4.2. Physicochemical Properties attributed to Bioaccumulation

Bioaccumulation is a key factor that determines the end destination and effects of any xenobiotic compound. The phenomenon can lead to a much higher concentration of pesticides in fatty tissues than in the environment. There are multiple pathways by which pesticides could be uptaken by living organisms. This could be through skin permeation, inhalation or through ingestion. The concentration of pesticides can therefore increase throughout the food chain and this can pose a significant hazard to the ecosystem and to humans. The most important physicochemical property in this regard is the octanol-water Partition coefficient. Generally, chemicals with values higher than 3 can show significant accumulation, while the mean value of organochlorine pesticides is something close to 6[8].

### 1.4.3. Modes of Action of Organochlorine Pesticides

The mechanisms and chemical pathways that the pesticides undertake inside the human body are important to understand their toxicity but are also key to understanding the modes of action and efficacy of pesticides. Pesticides are bioactive compounds and they target certain enzymes and receptors within the pests to activate or inhibit specific physiological actions[10]. Most insecticides disrupt neurological transmission and can kill the insects over a short period of time. On the other hand, herbicides target the weeds mechanisms of survival and are not instantaneous in action. Organochlorine pesticides are generally insecticides. They target the synapses or the axons especially the cholinergic system inhibiting the acetylcholinesterase enzyme[10]. However, this can have unintended toxicological effects on humans. The newest class of insecticides, neonicotinoids target specific binding sites on the acetylcholine esterase enzyme and can cause acetylcholine accumulation in the synapses ,effectively poisoning insects while having lower human toxicity[10].

### 1.4.4. Toxicological Properties of OC Pesticides

#### 1.4.4.1. DDT

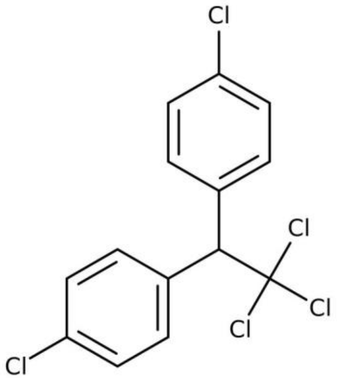

**Figure 3:** DDT

**DDT is also called:** p, p0-dichlorodiphenyltrichloroethane; 2,2-bis( p-chlorophenyl) 1.1.1-trichloroethane; 1,10-(2,2,2-trichloroethylidene) bis(4-chlorobenzene); or Dicophane[10]. DDT is a colorless to white non-polar powder with a faint odor. It has a melting point close to 109°C and decomposes at high temperatures[10].

DDT causes low to moderate acute toxicity, high doses can cause severe headaches, dizziness, disorientation, convulsions and tremors. The high tendency of DDT for bioaccumulation can cause delayed effects that can manifest after years from the initial low-level exposure. Chronic effects can include liver damage and central nervous system degeneration. It was also found to have teratogenic effects affecting fetal development, and estrogenic-like effects. It mostly accumulates in adipose tissue and is very resistant to biodegradation. DDT is still detected in many agricultural environments 30 years after it was banned[10].

Metabolites of DDT include o,p-DDD, p,p-DDE, and p,p-DDD. The isomers of DDT include o,p-DDT and p,p-DDT.

#### 1.4.4.2. Lindane

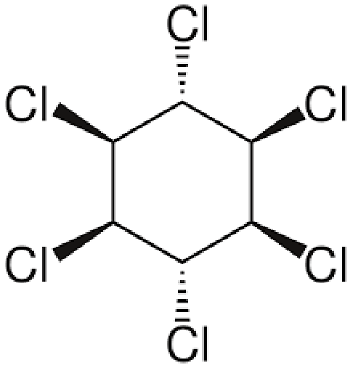

**Figure 4:** Lindane

**Lindane is also called:** γ- 1,2,3,4,5,6-hexachlorocyclohexane; γ-benzene hexachloride; γ-BHC; hexachlorocyclohexane _γ-isomer. Lindane is a white crystalline substance with a melting point of 112.5°C. It has a seven other stereoisomers, is insoluble in water and soluble in non-polar organic solvents[10].

Lindane has a high acute toxicity. Its symptoms can range from nausea and vomiting to epileptic convulsions, seizures and cyanosis. Since it is a stimulant to the nervous system, it can lead to very violent convulsions with rapid onset and could lead to death. Ingestion of 2-10 g is probably fatal to humans. Lindane is also carcinogenic leading to liver and lung tumors[10].

#### 1.4.4.3. Endrin

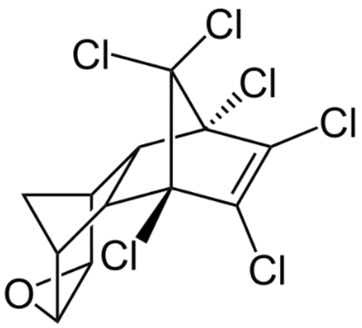

**Figure 5:** Endrin

**Endrin is also called:** 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a ,5,6,8a - octahydroendo, endol, 4:5,8-dimethanonaphth[2,3-b ]oxirene; Hexadrin; Mendrin; Endrex. Endrin is a stereoisomer of Dieldrin. It is a colorless to tan-colored solid with a melting point of around 245°C. It is non-polar, insoluble in water and soluble in organic solvents[11].

Endrin targets the central nervous system where it facilitates nerve conduction leading to myocardial irritability. Other symptoms include nausea, vomiting, respiratory depression and occasional convulsions. It is however, known to be of less severe toxicity than its stereoisomer Dieldrin[11].

### 1.4.4.4. Dieldrin

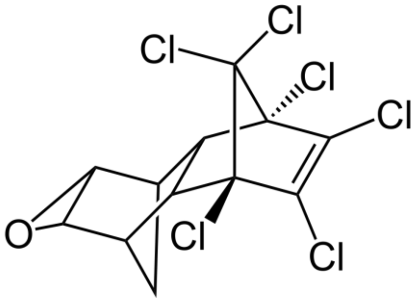

**Figure 6:** Dieldrin

**Dieldrin is also called:** 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a, 5,6,7,8a-octahydroendo, exo-1,4:5,8-dimethanonaphthalene; 3,4,5,6,9,9-hexachloro-1,1a,2,2a,3,6,6a,7,7aoctahydro-2,7,3,6-dimenthanonaphth(2,3-b)oxirene; Octalox; or Dieldrex. Dieldrin, similar to Endrin, is colorless or light tan in color with a melting point of 176°C, is insoluble in water and moderately soluble in organic solvents[10].

Like most organochlorine pesticides, Dieldrin acts on the central nervous system by promoting dopaminergic degeneration. Its neurotoxicity is largely attributed to degeneration of the dopaminergic neurons through mechanisms like oxidative stress, mitochondrial dysfunction, protein clumping and aggregation or programmed cell death. This can lead to an array of neurodegenerative diseases including Parkinson’s disease[10].

### 1.4.4.5. Heptachlor

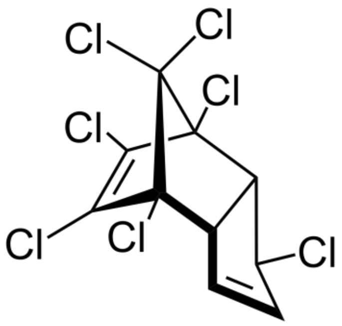

**Figure 7:** Heptachlor

**Heptachlor is also called:** 1,4,5,6,7,8,8-heptachloro-3a, 4,7,7a-tetrahydro-4,7-methanoindene; 3,4,5,6,7,8-heptachlorodicyclopentadiene; 1,4,5,6, 7,10,10-heptachloro-4,7,8,9-tetrahydro-4,7-endomethyleneindene: Heptamul. Heptachlor is a chlorinated dicyclopentadiene insecticide and was historically mainly used for control of termites and ants. Heptachlor is a Persistent Organic Pollutant (POP) that tends to bioaccumulate in the environment upon release [10]. Heptachlor undergoes a photochemical reaction that results in the generation of free hydroxyl radicals that transform heptachlor, in the presence of water, to its congener heptachlor epoxide. This form is more stable than heptachlor and is only slightly water soluble. The persistence of heptachlor can be also attributed to its chlorinated structure, non-planar geometry and the presence of chiral centers[10].

Exposure of animals to heptachlor has been shown to alter the functions of the dopaminergic neurons and contribute to the etiology of Parkinson’s disease in laboratory animals[10].

### 1.4.4.6. Hexachlorobenzene

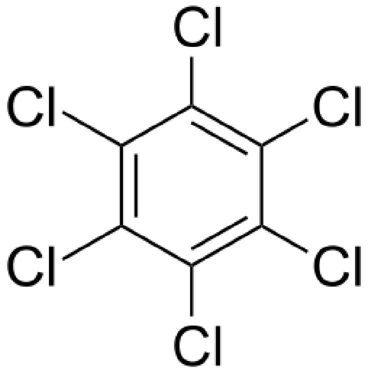

**Figure 8:** Hexachlorobenzene (HCB)

Hexachlorobenzene, also known as Perchlorobenzene, is a dioxin-like compound that is known to have weak interactions with the aryl hydrocarbon receptor and is a disruptor of the body hormones. It is not a naturally occurring compound, but is synthesized by chlorinating benzene[12]. HCB is a white crystalline solid that is insoluble in water, soluble in ethanol and ethyl ether, is very soluble in benzene and has a melting point of 231°C. The compound was used in manufacturing of flammable compounds for military purposes, the manufacture of electrodes, and is used as a fungicide for the treatment of seeds. HCB is now commonly used for laboratory purposes and is produced as a by-product in the manufacturing of some organochlorine solvents and pesticides[13].

According to the Stockholm convention regarding Persistent Organic Pollutants (POPs), the production, marketing and usage of HCB is prohibited. Like most OC Pesticides, HCB tends to bioaccumulate in the tissues of living organisms. The residues of this compound could then be found in animal feed, milk, blood and the adipose tissue of m any birds and mammals. The toxicological mechanisms of HCB involve targeting the liver, thyroid glands, reproductive organs and carcinogenesis.

By activating the aryl hydrocarbon receptor, HCB induces an association of the epithelial growth factor receptor (EGFR) which makes it a potent co-carcinogen[13].

### 1.4.5. Metabolism and Biotransformation

Studies are not only concerned with the analysis and quantitation of pesticides parent molecules, but also with their degradation products[8].

These can be the result of various processes, biological, chemical or physical that result in a vast array of secondary and tertiary products that are known as transformation products (TPs). It has become a practice to include the transformation products in the monitoring studies of food products as identifiers of the parent compound and as part of the minimum reporting limits (MRLs)[8].

The terms biotransformation and metabolism are often used interchangeably. The key pillars of toxicokinetics are absorption, distribution, biotransformation (metabolism) and elimination. Absorption involves the passage of the compounds through membranes and is mainly affected by their physicochemical properties such as that their lipophilicity and acidity along with the properties of the cells. Distribution is the transportation and circulation of the compounds between tissues. It is mainly affected by the affinity of pesticides to bind to plasma proteins and is also dependent on the physicochemical properties of the compounds. Metabolism is the process of biological or chemical change to the structure of the compound within the body of a living organism. Finally elimination is the process by which the cells get rid of the compounds by active cell processes[9]. To have a better understanding of the overall fate of pesticides and their bioaccumulation in the ecosystem, we have to an understanding of the basic phases of metabolism is important.

Phase I metabolism is mainly comprised of basic chemical process such as hydrolysis, oxidation, and reduction. This includes introducing functional groups such as carboxylic, hydroxy or amine groups. This is often followed by a complex series of reactions where phase I metabolites can be further conjugated to form phase II metabolites[9].

Phase II metabolism involves the in-vivo conjugation to endogenous substrates in-vivo. The endogenous substrates can range from peptides like glutathione to sugar conjugates like glucosides or glucoronides[9].

Phase III metabolism refers to the modification and active transport of xenobiotics via transporters and ATP binding, either through covalent bonds or through some way of physical encapsulation within a macromolecular matrix. In animals, this can be evident in xenobiotics such as pesticides being bound to proteins or nucleic acids[9].

## 1.5. Analytical Techniques Used in Quantitation of Organochlorine Pesticides

Analytical method development in the detection and quantitation of POPs has been extensive. This yielded many methods for the extraction, isolation, detection and quantitation of OC pesticides and PCBs[14].

Typically capillary gas chromatography instrument with either electron capture or low-resolution mass spectrometry (MS) detection are used for detecting OC Pesticides[14].

Bioanalytical methods such as Enzyme-linked Immunoabsorbent Assays (ELISA) have been used to quantify most DDT/DDE, HCH isomers, Toxaphene and Cyclodiene OCPs. ELISA methods for OC pesticide analysis are based on the principle of competitive binding between the analyte (OC pesticide molecules) and an excess amount of a labeled analyte/coating antigen (antibody-enzyme conjugate). The reaction is carried out in a well of a polystyrene plate, and the solution is left for equilibration in the first step. The well is subsequently washed and a substrate (chromophore) is added. A change of color will occur due to the reaction between the enzyme and the substrate. The excess of analyte will replace the coating antigen, which will in turn slow the rate of the reaction and the color change which can be detected spectrophotometrically[14].

### 1.5.1. Water Samples Collection

Samples should be collected in glass containers. The bottles must not be rinsed with the sample before collection. All samples must be iced or refrigerated at 4°C and stored for a maximum of 72 hours before extraction. If samples are not going to be analyzed within 72 hours, then the sample pH must be adjusted within 5.0-9.0 by sodium hydroxide or sulfuric acid. All samples should be extracted within 7 days of collection and assayed within 40 days of extraction[15].

### 1.5.2. Sample Extraction Techniques

Sample preparation is generally matrix and analyte dependent and is a major step in the determination of pesticide levels. Liquid samples are generally filtered and/or centrifuged and then undergo extraction procedures[16].

#### 1.5.2.1. Liquid-Liquid Extraction

Liquid-liquid extraction is one of the most commonly used techniques of extraction mainly because of its simplicity and wide range of applicability. This method, however, requires large volume of solvents such as acetonitrile, ethyl acetate, acetone and dichloromethane and typically lacks automation[16].

Two methods are commonly used for liquid-liquid extraction. The first is the so-called Luke Method which basically is extraction by acetone followed by partitioning with a mixture of dichloromethane and light petroleum. The other method is extraction by ethyl acetate in the presence of sodium sulfate. The second method is generally regarded more environmentally friendly since it does not utilize chlorinated solvents such as dichloromethane[16].

#### 1.5.2.2. Solid Phase Extraction (SPE)

Solid phase extraction has been developed as an alternative to liquid-liquid extraction. Today SPE is a widely used technique with versatile applications ranging from extraction of organochlorine pesticides from liquid samples to the purification and cleanup of crude extracts after solvent extraction or liquid-liquid extraction. SPE mainly involves the use of disposable cartridges and discs to trap analytes as the sample solution is passed through the activated stationary phase. The target analytes that have affinity to this stationary phase tend to concentrate on it while other sample components pass through[16].

SPE provides lower solvent consumption than liquid-liquid extraction. SPE of liquid foods generally require further cleanup while water does not. The sorbents or the stationary phases range from C8 to C18 bonded phases on silica or polymeric resins such as polystyrene, Florisil (activated magnesium silicate) or polar solvents such as alumina. These different phases provide a wide range of applicability ranging from polar and non-polar interactions, adsorption, H-bonding and ionic exchange resins. Automated SPE methods have also become dominant in the industry[16].

#### 1.5.2.3. Solid Phase Microextraction (SPME)

Solid Phase Microextraction commonly referred to as SPME is a technique that combines sample extraction, concentration and introduction to the testing instrument in one step. Although SPME is a novel technique that does have various advantages, it tends to sacrifice results’ quality for speed and ease as it does not consider the complexity of a sample matrix (i.e in-situ sample extraction does not always provide satisfactory recovery results).

SPME devices consist of a fused silica fiber with an appropriate stationary phase on which analytes are adsorbed. Analytes are partitioned out of the sample matrix to adsorb on the stationary phase either directly from the liquid sample or from the vapor phase above the liquid sample. Desorption can then take place on the GC instrument itself utilizing elevated temperatures[16].

### 1.5.3. Most Common GC Methods Used

#### 1.5.3.1. GC-ECD

In this technique capillary columns are regularly used. GC-ECD is providing very high selectivity and sensitivity and is routinely and widely used in analysis of OCPs. GC-ECD can detect OCP at levels as low as nanograms per liter.

Electron capture detection technique relies on an isotope, most commonly $^{63}$Ni, to provide a continuous stream of beta particles that give off an electric current. Organochlorine pesticides, being electronegative, tend to uptake these electrons to decrease the electric current which can then be read as a signal. The detector is usually operated at high temperatures ranging from 300 – 350°C[16].

ECD, however, offers the possibility of false positives resulting from interferences of other electronegative molecules like sulfur and phthalate esters. Make-up gas like Nitrogen or Argon/Methane is normally used with ECD detectors and is combined with the flow from the GC column. The gases used must be ultrapure since any traces of water can cause oxidation of the stationary phase of the GC column and can also affect the ECD detector itself[16].

#### 1.5.3.2. GC-MS

Coupling a capillary GC column with an MS detector has shown significant advantages proved in the field of organochlorine pesticides analysis. The MS quadropole detector can quantify pesticides in the range of picograms per liter using Electron Ionization (EI) in selective ion mode. Fragmentation patterns also provide an advantage in identifying eluted compounds with a lower probability of misidentification than in GC-ECD[16].

#### 1.5.3.3. Chiral GC Separation of OCPs

Optically active isomers of Organochlorine Pesticides are very common, for example chlordane and cis/trans-chlordane. Despite the fact that OCPs are manufactured as racemic mixtures, biotransformation in soil and water and the environment as a whole converts these mixtures to optically active compounds. One technique that proves extremely helpful in the separation of chiral isomers is chiral GC. This requires special chiral capillary columns that are basically constituted of cyclodextrins chemically bonded to a polysiloxane. This technique can either be standalone or integrated to any GC technique by redirecting a portion of a sample after elution to this column to provide better separation of chiral compounds[16].

### 1.6. Levels of Organochlorine Pesticides in Egypt According to Literature

Studies of organochlorine pesticides levels of surface water samples in Egypt are extensive in literature[5] however, most studies report on off-shore sea water samples from Alexandria, surface water samples from the Nile Basin or surface water samples from Greater Cairo. Very few studies have listed OC pesticide levels in new urban cities or irrigation sources and water canals other than the Nile Basin. Almost no studies reported oc pesticides levels in water of fish farms.

Hexachlorocyclohexane (HCH) and DDT were the principal oc pesticides in near-shore coastal waters of Alexandria were reported using a combination of GC-ECD and GC-MS methods and the maximum concentration reported 36.97 ngl$^{-1}$ [17]

A total of 25 oc pesticides were determined in samples from 34 locations in Lake Qarun where the total concentrations of oc pesticides ranged 1.01 to 164.8 ng g$^{-1}$ and tended to increase near urban areas reflecting usage trends of these chemicals. This study reported gamma-HCH, heptachlor, aldrin, endrin, p,p-DDE, oxy-chlordane and endosulfan I and II as the most abundant compounds. Through isomer ratio analysis, authors were able to conclude that there were still new inputs of compounds such as lindane, endrin and heptachlor to the environment [18].

A study reported in 2005 to assess pesticide residues in water of the River Nile using Solid Phase Extraction (SPE) and analysis using GC-MS, GC-ECD and GC-NPD. Samples were collected from different sites in Greater Cairo, and on-site solid-phase extraction was applied to a 1 Liter aliquot. In this study samples that gave positive identification of pesticides on GC-ECD and GC-NPD were further tested with GC-MS to ensure accurate reporting. From all samples collected, Aldrin was the most reported pesticide. [19]

The Manzala salt water lake near Alexandria has been gradually transformed from being a preserved Marine environment to a freshwater system. This caused drastic changes in its ecosystem and is a direct consequence of the increased demands for agricultural lands and urban waste disposal at the same time. Pesticides are the main source of pollution in this lake, accumulating in the food chain from shrimp to fish, increasing in concentration by factors of a 1000-fold to 10,000-fold[20]. In a study reporting on this lake samples of water, sediments and fish were collected and tested using the technique of GC-ECD for quantitative determination of pesticides. The maximum level of OCPs reported was 46.253 ng/ml, while the minimum level was

0.289 ng/ml. p,p-DDT ranged from 0.190 ug/l to 4.714 ug/l. Out of four regions analyzed from the Manzala lake, three regions were marked for a mean p,p-DDT level higher than the maximum level allowed by the World Health Organization. These regions were namely the Round Road, Bughas El-Rasoah and Port Said-Damietta Road with mean levels of 4.714, 2.718 and 2.847 ug/l respectively. Among the most frequently reported oc pesticides were gamma-HCH, heptachlor, aldrin and endrin [20].

Another report on Abu Qir Bay from the Eastern Region of Alexandria aimed to quantify PCBs, organochlorine pesticides and chlorpyrifos levels from sediment samples and mussels. In this study which utilized GC-ECD total DDT (Total of DDT by-products; DDE, DDD and DDT) dominated the detected organic pollutants in the sediment samples[21].

One other area covered in Alexandria was the near-shore coastal waters that are in close proximity to swimming areas. In this study water samples were collected from ten sampling stations along the swimming activity areas, extracted in the field and then tested using GC-ECD and GC-MS. Highest concentrations detected were 1.36 ng/l for HCHs and 36.97 ng/l for DDTs during the summer of 2014 and this was attributed to higher sewage activities[22].

In another recent study to investigate OCPs and OPPs along the River Nile, locations were selected throughout a wide region and were chosen according to the location of major agricultural and industrial activities. Samples were collected during the summer of 2013 from 20 sampling sites. It was found that Dieldrin, p,p’-DDE and p,p’-DDT were above the standard guidelines of the World Health Organization with mean values of 1.081, 1.192 and 3.22 ug/l respectively. In this study it was interesting to note the reverse relationship between the mean level of concentration of OCPs and the Region Temperature. OCPs tend to volatilize at warm temperatures and condense at cold ones. A total of 60 water samples were collected from different regions in the Nile Delta. Samples were extracted by liquid-liquid extraction technique and analyzed using GC-ECD detection. The study attributes the high mean levels of OC pesticides detected to the delta agricultural lands wash off. [23]

Lake Burulus, one of the delta lakes connected to the Mediterranean was studied for OCPs levels in 2006. The levels of OCPs varied with seasons and the rate of agricultural and sewage activities. Concentrations of DDT metabolites are the highest reported with p-p’-DDE being the highest with a maximum of 520.13 ng/l.[24]

One study from China determined quantitatively the organochlorine pesticides levels in surface water samples from Baiyangdian Lake in North China. Using GC equipped with a $^{63}$Ni microelectron capture detector, the authors determined the total levels of DDTs and HCHs in the water samples. An average of total HCHs in this study was reported to be 1.59 ng/l while DDTs were not detected in the water samples[25].

Another study from China covered the region of Daya Bay. Total HCHs detected ranged from 35.5 ng/l to 1228.6 ng/l. Total DDTs ranged from 26.8 ng/l to 975.9 ng/l. The study reported that the levels of HCH in several samples exceeded 500 ng/l which is higher than the WHO limit of 20 ng/l[26].

In Kenya, one study determined the levels of 17 OCPs in surface water and sediment samples from Nairubi River. The technique employed was GC-ECD and the mean pesticide residues ranged from below detection to 39.7 ng/l in water samples. These levels are below the maximum permissible limits of WHO in surface water[27].

Mid Black Sea region in Turkey was studied in 2009 (Geyikçi and Büyükgüngor, 2009) to assess the level of contamination of water by organochlorine pesticides. Samples were extracted by SPE technique and analyzed using GC coupled with electron capture detection. The most reported organochlorine pesticides were the isomers of HCH, dieldrin, p,p-DDD and heptachlor epoxide. The study used the EU limit of 0.1 ug/l for all pesticides as a reference and only gamma-HCH was reported to be higher than this limit in two samples with values of 0.2505 ug/l and 0.2046 ug/l[28].

# 2. Chapter Two: Current Study Objectives

The aim of this study is to expand on the already existing pool of data in literature regarding the levels of oc pesticides in the Irrigation and Drainage Water of various locations across Egypt. The study also aimed to provide much needed data regarding the levels of pollution of water from fish tanks and fish farms that is not covered in literature. The organochlorine pesticide compounds chosen for this study are Alpha-HCH, Heptachlor, Aldrin, Endrin and p,p-DDT. The compounds were chosen based on their acceptable recovery values, correlation coefficients from linearity study and their reporting frequency in literature as referenced in figure 1. The obtained results are to be compared with levels established in WHO and to those previously reported in other studies in Egypt evaluating the levels of OC pesticides in coastal waters, the Nile River water and Lake Qarun.

# 3. Chapter Three: Materials and Methods

## 3.1. Samples Collection

Acquisition of representative samples is critical and fundamental to the outcome of the project. From each sampling point 3 samples will be taken to allow for triplicate analysis. 1 L amber-glass containers will be used for sampling with Teflon caps. Around 80 mg of Sodium Thiosulphate can be added to the container prior to extraction to remove free chlorine from the sample. All taps will need to be flushed for 2-3 minutes before sampling to ensure washing of any residues from the sample outlet. Samples should be refrigerated at 4°C till extraction. There is no need for freezing since this is mainly recommended for samples containing fat, where triglycerides can be oxidized to free fatty acids and affect the extraction process[29].

There will be no need to acidify water samples after collection as this is mainly required for the analysis of Dissolved Organic Carbon (DOC) which is out of the scope of this project. The locations of the sampling points will be recorded using a global positioning system (GPS). All samples must be extracted within seven days of collection and analyzed within a maximum of 40 days of extraction[29].

**Figure 9:** El Salam Canal 1

**Figure 10:** El Salam Canal 2

**Figure 11:** 23 meters deep well source

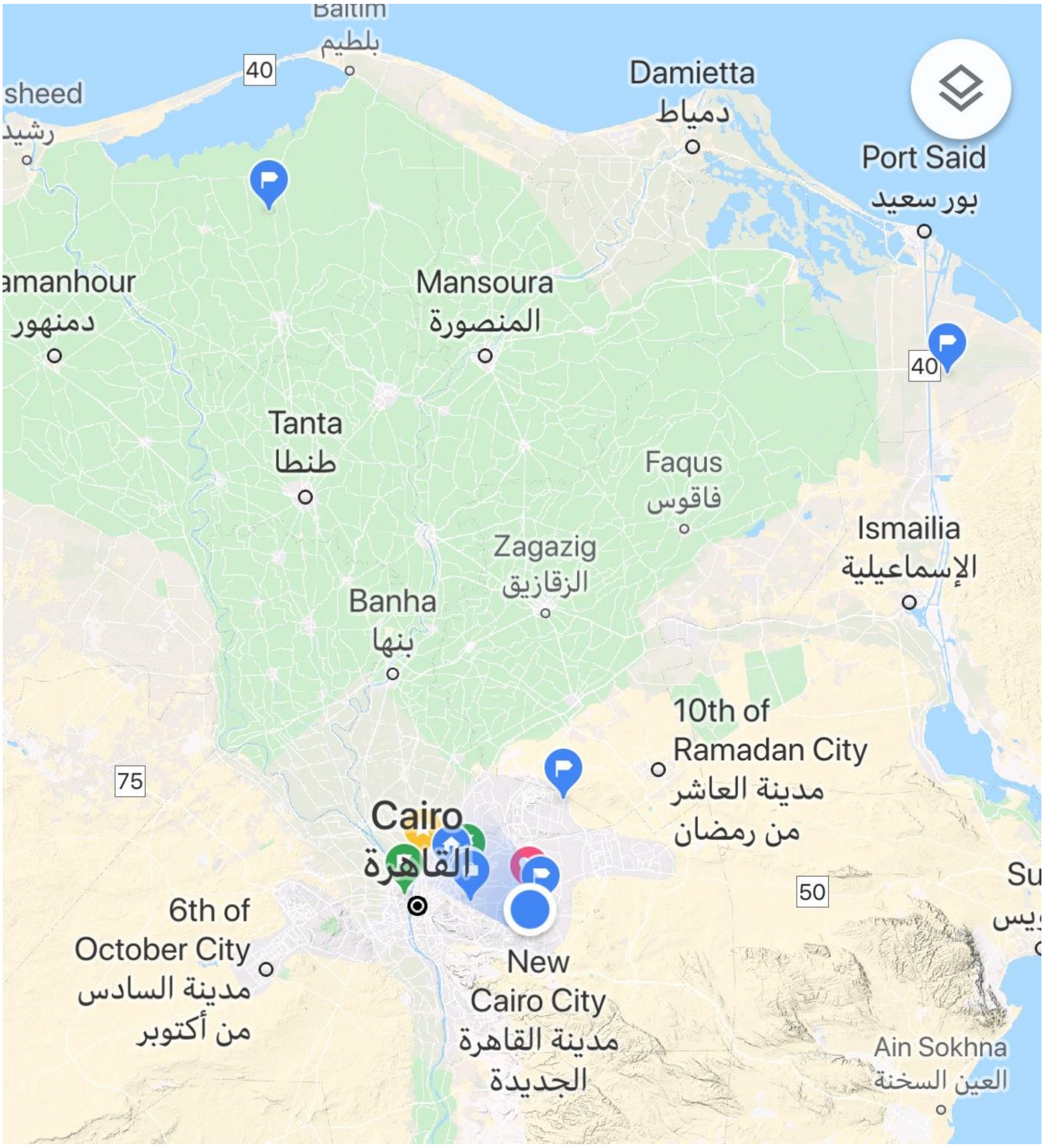

**Figure 12:** Sample locations marked with blue flags

## 3.2. Chemicals and Reagents

A standard Chlorinated Pesticides Mix (Sigma-Aldrich, Catalog No. 49151, Lot No. LC19420V) was used. The mixture contains 13 Organochlorine Pesticides Compounds comprised of Aldrin, Alpha-BHC, Beta-BHC, Dieldrin, Endrin, Gamma-BHC, Heptachlor, Heptachlor Epoxide Isomer B, 2,4’-DDD, 2,4’-DDT, 4,4’-DDD, 4,4’-

DDE, 4,4’-DDT. The minimum concentration of this stock solution is 25.0 µg/ml while the maximum concentration is 249.5 µg/ml.

High Purity solvents were used in standards’ preparation and samples’ extraction. Isooctane was purchased from Sigma-Aldrich (Lot# SZBA2150V), Ethyl Acetate was purchased from Sigma-Aldrich (HPLC Grade, Lot# 921) and Methanol was purchased from Fisher Scientific UK (HPLC Grade, Lot# 1693650). Anhydrous Sodium Sulfate was acquired from Merck, Darmstadt, Germany (Lot# AM1023649 634). Distilled water was used for washing glassware.

**Table 1:** Concentrations of target oc pesticide components in the stock standard solution

| **Analyte** | **CAS-Number** | **Analytical Concentration in µg/ml** |
|---|---|---|
| Aldrin | 309-00-2 | 49.09 |
| Alpha-HCH | 319-84-6 | 24.97 |
| Endrin | 72-20-8 | 198.2 |
| Heptachlor | 76-44-8 | 24.35 |
| p,p-DDT | 50-29-3 | 249.5 |

### 3.3. Preparation of standard solutions

A stock solution was prepared from the Chlorinated Pesticides Mix by diluting 1 ml (1 ampoule) to 100 ml with isooctane. Calibration standard solutions were prepared from this stock by diluting in isooctane. In the table below, the dilution factors for each calibration solution is given along with the theoretical concentrations for each analyte in µg/l

**Table 2:** Preparation solutions for multiple point calibration curves

| **Solution Number** | **Dilution Factor** | **Alpha-HCH** | **Heptachlor** | **Aldrin** | **Endrin** | **p,p-DDT** |
|---|---|---|---|---|---|---|
| **1** | 1 ml in 25 ml | 10.00 μg/l | 9.74 μg/l | 19.64 μg/l | 79.28 μg/l | 99.80 μg/l |
| **2** | 5 ml in 100 ml | 12.50 μg/l | 12.18 μg/l | 24.55 μg/l | 99.10 μg/l | 124.75 μg/l |
| **3** | 2 ml in 25 ml | 20.00 μg/l | 19.48 μg/l | 39.27 μg/l | 158.56 μg/l | 199.60 μg/l |
| **4** | 10 ml in 100 ml | 25.00 μg/l | 24.35 μg/l | 49.09 μg/l | 198.20 μg/l | 249.50 μg/l |
| **5** | 2 ml in 5 ml | 100.00 μg/l | 97.40 μg/l | 196.36 μg/l | 792.80 μg/l | 998.00 μg/l |

According to EPA method 608, the above calibration standards were used to construct calibration curves for each compound and to validate linearity as well. On each day of analysis one calibration solution is injected to ensure that the average response does not differ from the predicted response by more than 15%.

### 3.4. Choice of Sample Volume

In a study published in 2013 aiming to assess Graphene as an efficient sorbent for Solid Phase Extraction of pesticides from water, sample volume was one of the variables studied. The effect of sample volume was studied across 4 different volumes; 50, 100, 200 and 300 ml. Acceptable recoveries (≥86%) were found when the sample volume was less than 200 ml, but strangely enough the recoveries of HCHs decreased when the sample volume was 300 ml. This could be attributed to the weak interaction between HCHs and Graphene, but for the sake of our study a 100 ml sample volume was used. This volume offered satisfactory recoveries (≥70%) for most of the target compounds and saved a lot of time in sample extraction thus offering a simple, efficient and quick method of analysis[30].

### 3.5. Samples Extraction and Preparation

For sample extraction Solid Phase Extraction technique was employed. 100 ml aliquots of the samples were first filtered using Whatman Filter Papers (125 mm Diameter) and were extracted under vacuum pump through a Vacuum Manifold (Waters P/N WAT200677) using SPE C18 cartridges (Strata Phenomenex).

Strata C18 cartridges were first conditioned using 5 ml ethyl acetate, then 5 ml of methanol, followed by 2x5 ml of deionized water for equilibration. 100 ml of water samples were then passed through the cartridge using vacuum. This way the cartridges are loaded with the compounds of concern, and this was followed by an elution step using 10 ml of ethyl acetate. The extracts were dehydrated using 20 mg of anhydrous $Na_2SO_4$ to ensure complete absence of water.

**Figure 13:** Water samples filtration on whatman filters

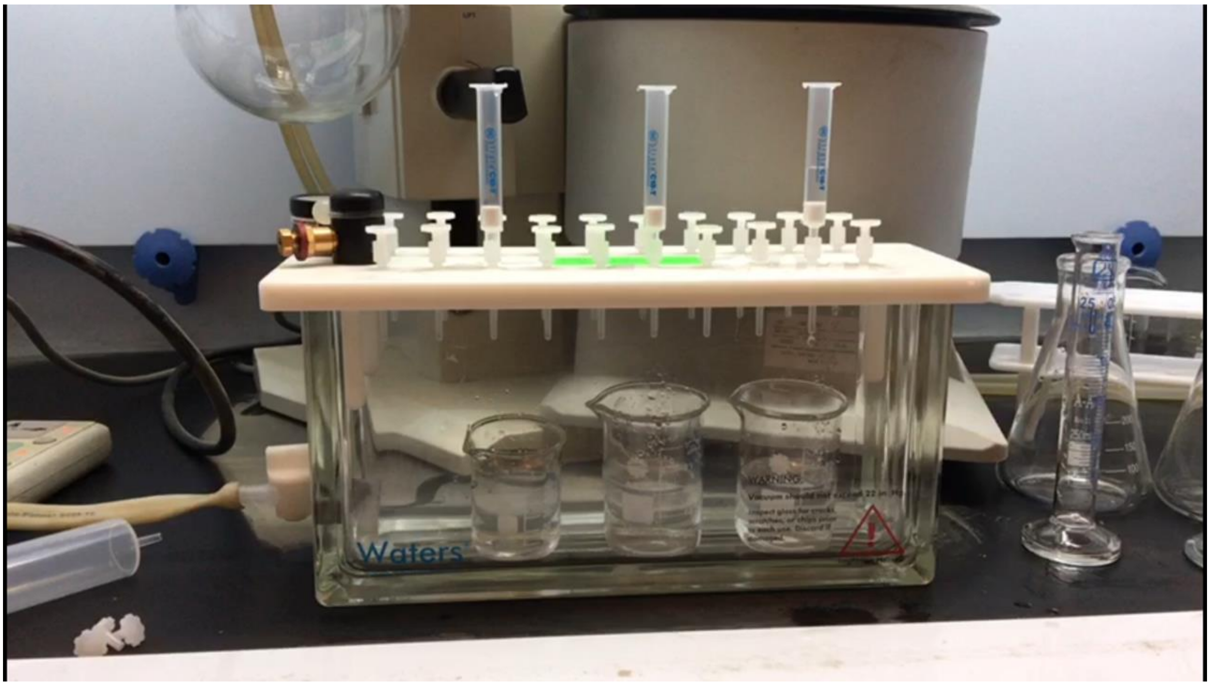

**Figure 14:** Extraction of water samples by Solid Phase Extraction technique (SPE)

## 3.6. Instrumentation and Method

### 3.6.1. GC-ECD

A gas chromatograph equipped with a split/splitless injector (Thermo, Trace 1310) coupled with an electron-capture detector was employed for the analysis. A 30 m TR-5MS capillary column with a 0.25 µm film thickness and 0.25 mm internal diameter (Thermo Scientific P/N 260F142P) was used for compound separation. Helium gas of 99.999% purity was used as a carrier gas while Nitrogen gas of 99.999% purity was used as the make-up gas. Carrier gas flow rate was a constant flow of 1ml/min, while the make-up gas flow was supplied at a rate of 15 ml/min. Injection volume was 1 µl in splitless mode while maintaining an injector temperature of 250°C.

The column oven temperature programming was as follows; initial temperature 100°C, held for 1.0 min. Ramp at 20.0°Cmin was then applied till 180°C. Another Ramp was applied at 5.0°C/min to 270°C. The last Ramp was set at 20.0°C/min to 320°C and held for 2.0 min. The Electron Capture Detector was set at 320°C[31].

### 3.6.2. GC-MS

A gas chromatograph equipped with a split/splitless injector (Thermo, PolarisQ) coupled with an MS detector was employed for the analysis. A 30 m TR-5MS capillary column with a 0.25 µm film thickness and 0.25 mm internal diameter (Thermo Scientific P/N 260F142P) was used for compound separation. Helium gas of 99.999% purity was used as a carrier gas, no make-up gas was used. Carrier gas flow rate was a constant flow of 1 ml/min. Injection volume was 1 µl in splitless mode while maintaining an injector temperature of 250°C.

The column oven temperature programming was as follows; initial temperature 100°C, held for 1.0 min. Ramp at 20.0°Cmin was then applied till 180°C. Another Ramp was applied at 5.0°C/min to 270°C. The last Ramp was set at 20.0°C/min to 320°C and held for 2.0 min. MS transfer line temperature was set at 260°C. Ion source temperature was set at 220°C. Electron energy was 70 eV. Single Ion Monitoring (SIM) was applied for 10 molecular weights to enhance sensitivity; 180, 216, 234, 236, 260, 262, 272, 280, 315, and 352[32].

## 3.7. Method Validation

Before conducting analysis of collected water samples, method optimization and validation had to be made. Method optimization consisted of several trials in which oven program and carrier gas flow were tweaked to obtain the best separation and resolution between analytes. Carrier gas serves to ensure a flat and non-elevated baseline through reducing false signals coming from the electron capture detector.

Method Validation serves to ensure optimum performance of the extraction method and the analytical method used to quantify the organochlorine pesticides in the water samples. Recovery aims to ensure complete extraction of the analytes from the water sample matrix and complete transference to the GC column. Values ranging from 60-130% were considered satisfactory (As stated in EPA method 608 for 60% recovery of chlordane). Repeatability was to be evaluated through injecting sample triplicates. Linearity serves to ensure a linear response of the detector to increasing concentrations of the analytes and determine the working range. Correlation coefficients R higher than 0.998 were considered satisfactory.

Correlation Coefficient = $\sqrt{R^2}$

### 3.7.1. Recovery Study

1 ml of the stock solution was added to triplicate samples of 100 ml distilled water and were extracted according to the procedure described in section 3.5. Recovery was then assessed by calculating expected peak areas versus actual peak areas.

### 3.7.2. Linearity GC-ECD

Linearity was determined by injecting selected concentrations of the calibration solutions in table 2 and performing a linear regression analysis to calculate a correlation coefficient for each compound separately.

Below were the results of Linearity Parameter Validation:

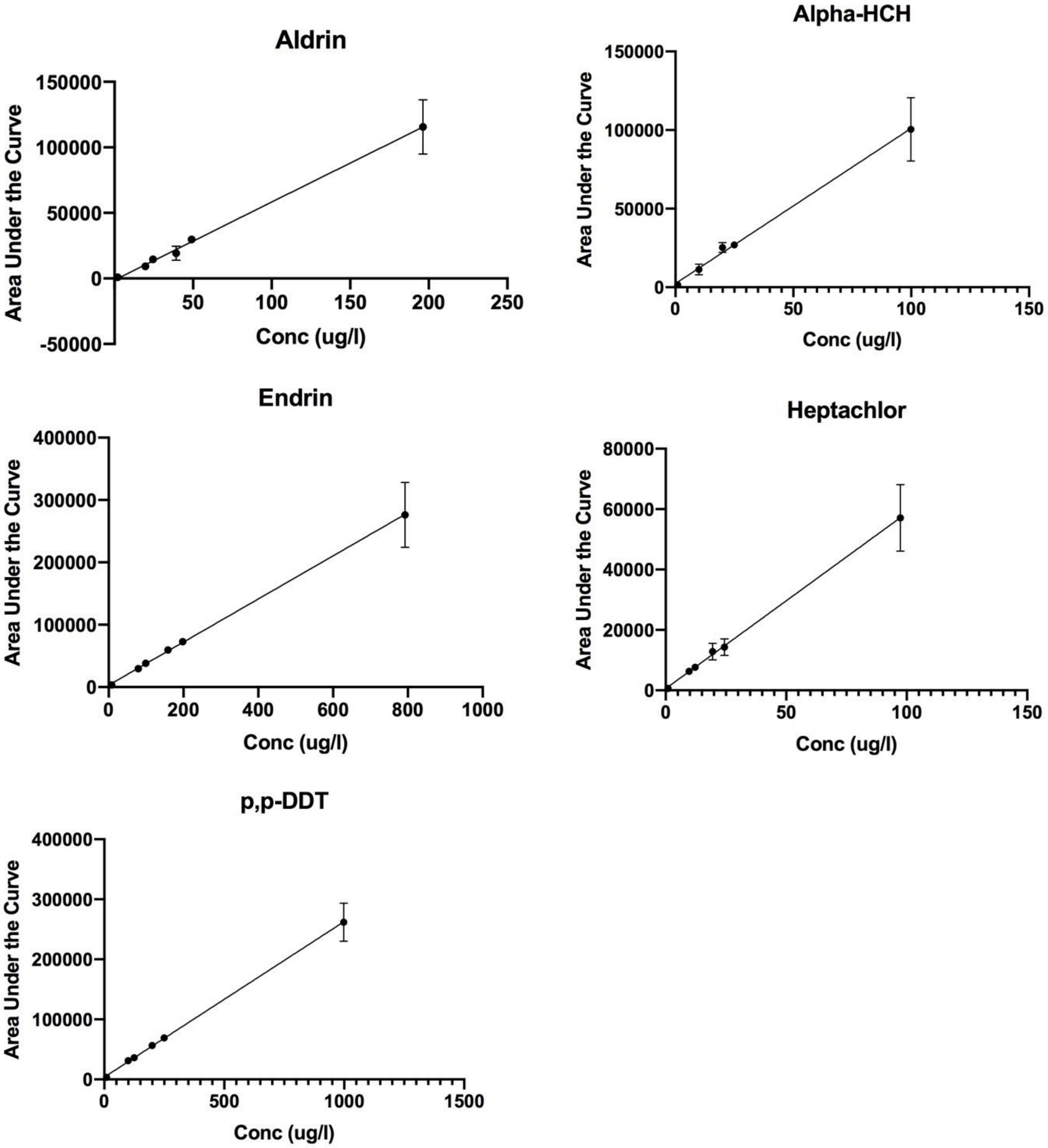

**Figure 15:** Calibration Curves GC-ECD

### 3.7.3. Linearity GC-MS

As in GC-ECD, multipoint calibration curves were prepared for each compound individually with GC-MS. The curves are listed below:

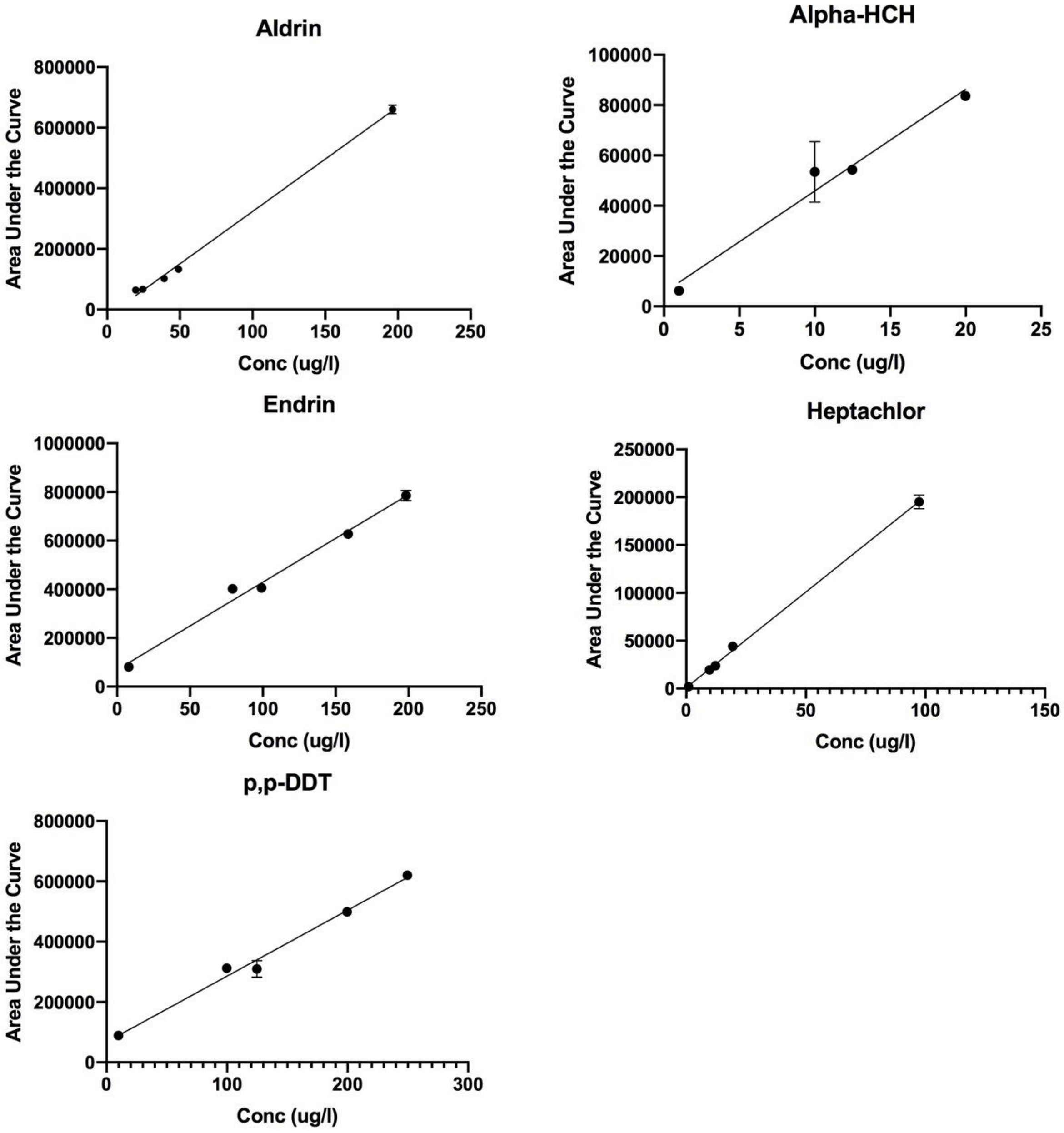

**Figure 16:** Calibration Curves GC-MS

### 3.7.4. Method Validation Summary

**Table 3:** Method Validation Summary

| **Analyte** | **Average Recovery (triplicate samples) %** | **Correlation Coefficient R (GC-ECD)** |
|---|---|---|
| Heptachlor | 87.60 | 0.998 |
| Aldrin | 62.30 | 0.998 |
| Heptachlor Epoxide | 106.00 | 1.000 |
| Endrin | 88.10 | 0.999 |
| p,p-DDT | 110.10 | 0.999 |

## 3.8. LOQ

LOQ was calculated based on the linear regression analysis for each compound. The equation used was:

$$\text{LOQ} = \frac{10 \times \textit{Standard Error of Regression}}{\textit{Slope}}$$

**Table 4:** LOQ reported values for each analyte in µg/l

| **Analyte** | **LOQ (µg/l)** |
|---|---|
| Alpha-HCH | 1.72 |
| Heptachlor | 0.49 |
| Aldrin | 1.03 |
| Endrin | 3.19 |
| p,p-DDT | 3.99 |

## 3.9. Calculation methodology for results reporting

Quantitative results for the detected levels of individual organochlorine pesticides were calculated based on a multi-point calibration curve prepared for each pesticide and considering sample volume extracted as well. Throughout the study results reported are in µg/l.

$$\text{Concentration of pesticide in µg/l} = \frac{(Area\ under\ peak - Intercept)}{\frac{Slope\ of\ linear\ curve}{Dilution\ Factor}}$$

$$\text{Dilution Factor} = \frac{1}{10\ ml\ (Volume\ of\ final\ ethyl\ acetate\ extract)}$$

# 4. Chapter Four: Results and Discussion

One of the main purposes of this project was to provide much needed data regarding organochlorine pesticides levels in drinking and irrigation water sources of new urban cities and other water sources that have not been covered extensively in literature. Samples were collected from various regions around Egypt that have been rarely studied and were filtered, extracted and injected in triplicates using GC-ECD and confirmatory single runs were performed using GC-MS. Samples extracted using Solid Phase Extraction technique. A total of 13 samples were collected from different areas across the country and were tested for organochlorine pesticides levels.

## 4.1. Overview for the concentrations of OC pesticides in the water samples

Using the previously mentioned chromatographic conditions for GC-ECD and GC-MS, the order of peak elution was as follows; Alpha-HCH, Beta-HCH, Gamma-HCH, Heptachlor, Aldrin, Heptachlor Epoxide, p,p-DDE, Dieldrin, o,p-DDD, Endrin, p,p-DDD, o,p-DDT, p,p-DDT.

Incomplete peak resolution was observed between the following pairs of OC pesticides; Beta-HCH and Gamma-HCH; p,p-DDE and Dieldrin as well as p,p-DDD and o,p-DDT. For this reason, these compounds were neither quantified nor reported.

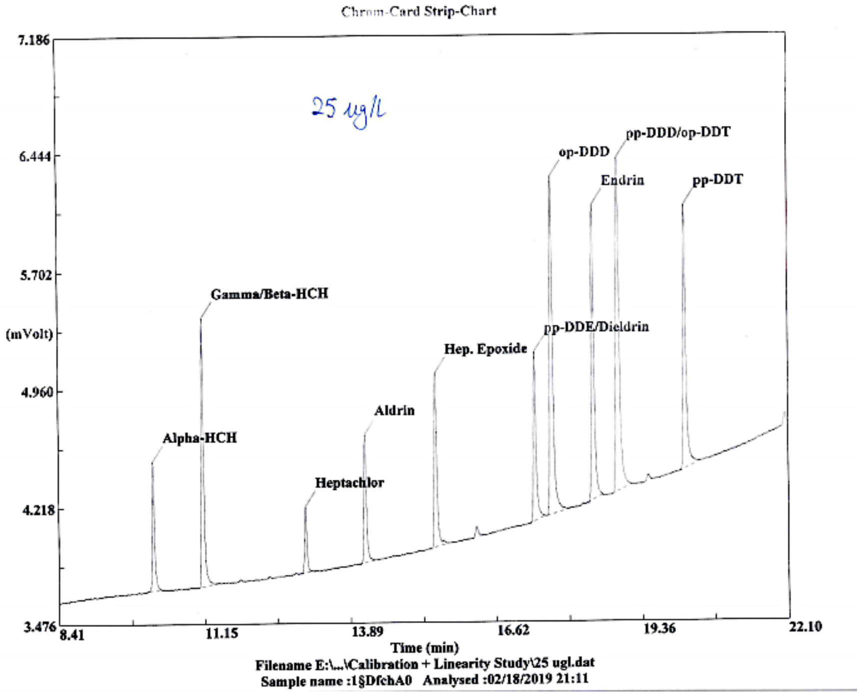

**Figure 17:** GC-ECD Calibration Standard Chromatogram

Heptachlor was the most frequently observed organochlorine pesticide with a detection frequency of 43% ranging from 0.007 μg/l to 0.313 μg/l. The second most frequently observed compound was the Alpha-HCH with detection frequency of 36% with concentrations ranging from 0.153 μg/l to 1.11 μg/l. Other compounds were detected at lower frequencies and some of the investigated compounds were not detected. All results were compared with the WHO guidelines for Drinking-water Quality stated in table 4.

### 4.1.1. Results of samples analyzed using GC-ECD

Table 5 below shows the detailed results for the 13 water samples collected and tested. Results are reported as the mean values of triplicate injections in µg/l. Sample locations are stated in the table alongside the sample source types added in the second column.

In general, GC-ECD could provide lower limits of detection in this study than GC-MS. This could be attributed to the fact that the $^{63}$Ni isotope used in the electron capture detection technique is very specific to halogenated compounds detection. While, on the other hand, in this study we used full scan MS detection at first to qualitatively identify single compounds in the spectrum and then proceeded with using single ion monitoring (SIM) to mark specific ions of interest thereby increasing the specificity of the instrument, lowering detection limits and eliminating interferences.

Most of the samples were analyzed in triplicates with the GC-ECD instrument and summary results are reported in the table 4 as mean values. Heptachlor was detected with the highest frequency of 53% while the highest concentration of all pesticides was Alpha-HCH with a value of 1.11 µg/l in the El Rayah Canal sample. Beta-HCH, o,p-DDD and Endrin were below detection limits in all samples analyzed here.

The levels of Alpha-HCH determined in the samples analyzed here (Table 5) are above the acceptable WHO limit of 0.02 µg/l. It is interesting to note that samples obtained from drainage canals showed the highest frequency of oc pesticides occurrence and the highest mean values of total oc pesticides. This can be attributed to the pesticides' usage for crop protection.

The WHO has set specific guidelines to regulate pesticides usage either for direct addition to water for public health purposes e.g combating insect vectors, or for agricultural purposes. The guideline is called "Guidelines for Drinking Water Quality". The limits provided by this guideline were incorporated in table 4 for comparison, although it has to be noted that these limits apply mainly for drinking water and could be a bit strict for irrigation water used for agricultural purposes but nevertheless they provide good indication for water contamination levels.

Results were also categorized per analyte in table 6. Frequency of detecting any oc pesticide in samples was reported along with the minimum and maximum values. Since Endrin was not detected in any of the samples it was reported to be of 0% detection frequency.

Table 7 categorizes the results as per sample source types. In this table the results are segregated into 3 categories referring to the three sample source types which are; fish tanks, irrigation canal sources and drainage. The lowest frequency of detection for any oc pesticide was reported in the "Irrigation Canal Source" samples while the other two sample source types reported 100% detection frequencies. The mean values of total oc pesticides in µg/l were also reported in this table.

**Table 4:** WHO permissible limits for oc pesticides in drinking water[33]

| **OC pesticide** | **Maximum permissible level µg/l** |
|---|---|
| Alpha-HCH | 0.02 |
| Heptachlor | 0.03 |
| Aldrin | 0.03 |
| Endrin | 0.03 |
| p,p-DDT | 1.00 |

**Table 5:** Mean results in µg/l of oc pesticides in water samples tested by GC-ECD

| Serial Number | Sample Source Type | Sample ID | Mean results from GC-ECD in µg/l<br>Alpha-HCH | Heptachlor | Aldrin | Endrin | p,p-DDT |
|---|---|---|---|---|---|---|---|
| 1 | Fish Tank | **Number 3 Left Fish Tank** | N.D | **0.313** | N.D | N.D | **0.020** |
| 2 | Fish Tank | **Number 4 Right Fish Tank** | N.D | **0.150** | **0.300** | N.D | N.D |
| 3 | Irrigation Source | **Number 7 El Ismailia Canal** | N.D | N.D | N.D | N.D | N.D |
| 4 | Drainage | **Number 1 Fish Farm Drain** | N.D | **0.263** | N.D | N.D | N.D |
| 5 | Irrigation Source | **Number 5 Irrigation Pump Aly Abu Gomaa Farm** | N.D | **0.007** | N.D | N.D | N.D |
| 6 | Fish Tank | **Number 12 Fish Tank Oraby Farm** | **0.153** | **0.067** | N.D | N.D | N.D |
| 7 | Irrigation Source | **Number 1 Irrigation Source El Salam Canal** | **0.470** | N.D | N.D | N.D | N.D |
| 8 | Drainage | **Number 2 North of El Ismailia Canal + Suez Canal** | **0.750** | N.D | N.D | N.D | N.D |
| 9 | Irrigation Source | **Number 3 El Rayah Canal Irrigation Source** | **1.110** | N.D | N.D | N.D | N.D |
| 10 | Irrigation Source | **Number 4 23m deep well** | **0.700** | N.D | N.D | N.D | N.D |
| 11 | Irrigation Source | **Number 9 El Sudan Farm** | N.D | **0.053** | N.D | N.D | N.D |
| 12 | Irrigation Source | **Number 7 Irrigation Pump Hamed Abu Zakaria Farm** | **0.060** | **0.060** | **0.353** | N.D | N.D |
| 13 | Irrigation Source | **Nile River Irrigation Source** | **0.200** | N.D | N.D | N.D | N.D |

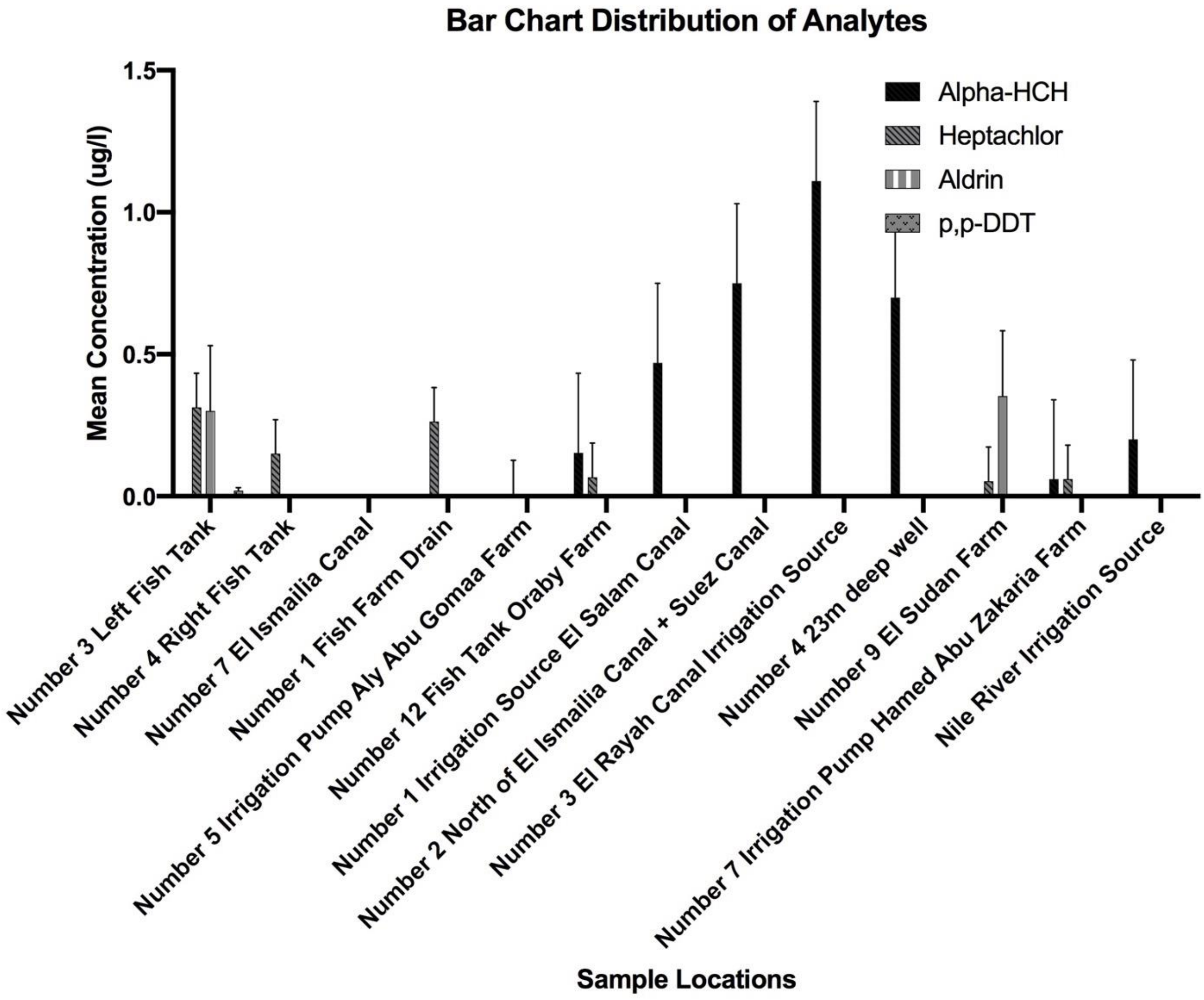

**Figure 18:** Bar Chart distribution for mean pesticides levels (μg/l) across samples

The above figure shows the distribution of the different oc pesticides across the 13 sample locations tested. From this figure it is visible that “El Ismailia Canal”, “El Rayah Canal” and the “Nile River” irrigation sources are of the lowest mean concentrations of detected oc pesticides, while other samples from fish farm sources and drainage sources report higher mean pesticide values.

**Table 6:** Statistical analysis for results reported from GC-ECD

| | **Alpha-HCH** | **Heptachlor** | **Aldrin** | **Endrin** | **p,p-DDT** |
|---|---|---|---|---|---|
| Mean | 0.12 | 0.09 | 0.06 | N.D | 0 |
| Minimum | N.D | N.D | N.D | N.D | N.D |
| Maximum | 1.11 | 0.34 | 1.06 | N.D | 0.06 |
| SD | 0.28 | 0.12 | 0.23 | N.D | 0.01 |
| Detection Frequency | 20.5% | 52.9% | 5.88% | 0% | 2.9% |

**Table 7:** Categorization of water samples results by source type (GC-ECD)

| | **Water Source Category** | | |
|---|---|---|---|
| | **Fish Tanks** | **Irrigation Canal Sources** | **Drainage** |
| Frequency of oc pesticides detection | 100% | 87.5% | 100% |
| Mean value of total oc pesticides in µg/l | 0.334 | 0.377 | 0.507 |

Egypt's recent increasing reliability on fish farms and aquaculture for the country's fish supply has grown to over 1 million ton per year in 2016 (Dickson et.al, 2016). This fact coupled with the fact that the fish farmers have received little to no formal training on the practice raises lots of concerns. 85% of Egypt’s marine production comes from ponds that are concentrated in separate zones in close proximity to the Nile River, supplied directly by water from drainage canals coming from agricultural irrigation systems. Normally most of the aquaculture fish production is either Tilapia or Mullet fish. One study that investigated pesticide residues in Nile Tilapia and Catfish from Assiut City reported levels for oc pesticides in both kinds of fish in the range of µg/Kg. Although the levels did not exceed the recommended permissible levels, they were detected nonetheless. Water is the primary source for this noticeable bioaccumulation and the results of oc pesticides reported in the current study from fish tanks helps highlight a looming problem that poses a high health risk to the Egyptian population[34]. It has to be noted from Table 6 that the irrigation source samples marked the lowest frequency of pesticides detection, while drainage canal samples and consequently fish tank samples marked higher frequencies of pesticides detection[35].

It has to be noted as well that this is one of the first studies in Egypt to cover water samples collected from fish tanks of various fish farms. Given the increasing reliability of Egypt on fish farms as a source of fish, the study provides much needed data regarding the pollution levels of the water used for filling these fish tanks.

**Table 8:** Comparison of pesticide levels reported in current study with literature

| | | | Mean levels of organochlorine pesticides detected (ug/l) | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Study Serial Number** | **Reference** | **Year** | **Total HCHs** | **Heptachlor** | **Aldrin** | **Heptachlor Epoxide Isomer B** | **Endrin** | **DDTs** |
| 1 | Current Study | 2019 | 0.095 | 0.058 | 0.142 | NA | 0.036 | 0.0009 |
| 2 | Detection of persistent OCPs and PCBs congeners in the near-shore coastal waters of Alexandria, Egypt[22] | 2014 | 0.00016-0.00063 | NA | ~0 | NA | ~0 | 0.00022-0.01686 |
| 3 | Monitoring of pesticides water pollution-The Egyptian River Nile[36] | 2016 | ND | ND | ND | ND | 0.403 | 3.22 |
| 4 | Residue levels of organochlorine pesticides in some ecosystem components of Manzala Lake[20] | 2013 | 9.400 | 0.008 | 2.290 | 2.290 | 0.149 | 2.617 |
| 5 | Organochlorine Contaminants in Water, Sediment and Fish of Lake Burullus, Egyptian Mediterranean Sea[25] | 2007 | NA | NA | NA | NA | NA | 0.00007-0.8826 |

Table 8 compares the mean levels of oc pesticides reported in this study with the ones reported in a number of different studies from Egypt as well . Pesticide levels reported in this study don't seem to be remarkably higher than the levels reported in other studies except for Heptachlor which, as discussed before, shows significant occurrence in water samples tested.

DDT levels is significantly lower than reported in other studies which, considering the fact that it was one of the most reported pesticides in literature and one of the most abundantly used during the last decade in Egypt, is a very positive result that could be highlighting the efforts taken to mitigate the prevalence of this pesticides and the actions that were taken following the Stockholm convention.

When compared to the WHO permissible limits for drinking water, we will find that the mean concentration of Alpha-HCH is above limit (>0.02 μg/l), Heptachlor also exceeds the permissible limit (>0.03 μg/l), Aldrin is higher as well (>0.03 μg/l), while Endrin and p,p-DDT are within the permissible limits of the WHO.

### 4.1.2. Results of samples analyzed using GC-MS

Results obtained from GC-MS analysis in general agreed with the results obtained from GC-ECD. Mass Spectrometry provided additional confirmation for peak identities by observing precursor ions and product ions resulting from fragmentation. Samples were injected once on the GC-MS for confirmatory analysis and assessed against a multi-point calibration curve. The results were then compared against the concentration levels reported from GC-ECD. The same extracted samples that were used for GC-ECD analysis were refrigerated and injected on the GC-MS.

In agreement with the results obtained by GC-ECD, Heptachlor was detected with the highest frequency with a maximum concentration determined of 0.151 µg/l, while Aldrin showed the highest reported concentration for any pesticide at 2.826 µg/l.

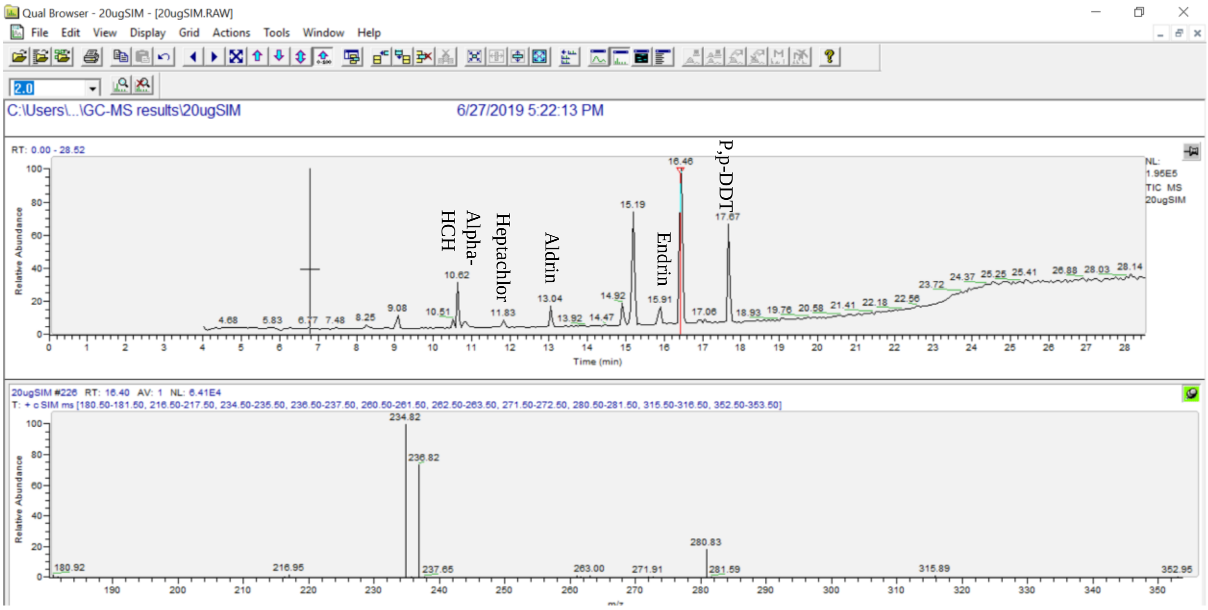

**Figure 19:** GC-MS Calibration Standard Chromatogram

# 5. Chapter Five: Conclusion

In this study samples were collected from some subsiding irrigation canals of the Nile River, from fish farms and from drainage canals as well. This study aimed at expanding the existing pool of data of oc pesticides levels in water from various irrigation sources in Egypt. The aim was to also expand on the existing data with providing much needed data regarding Fish Farms and Tanks to investigate the significance of bioaccumulation. The most occurring pesticide in water samples was Heptachlor with a frequency of occurrence of 53% and mean value of 0.09 µg/l. This is contrary to what was found in literature where most studies marked DDTs with the highest detection frequencies and mean values. This could be a possible indicator that DDT values are starting to regress in the Egyptian environment. Heptachlor levels however may require future mitigation. We can also conclude that Fish Farms (Tanks) are marking the highest detection frequency and mean values of total oc pesticides with 100% detection. Mean values of total oc pesticides for fish farms samples was 0.334 µg/l providing some concern about possible bioaccumulation throughout the food chain.

Opportunities for future work include expanding the pool of samples for higher statistical significance and using an autosampler for overnight injections. This will eliminate any variability that may occur due to human error during injections. Limits of quantitation will improve as well since we will be able to run replicates of the calibration curves and calculating LOQ will have more statistical significance. For future work and since Fish Tanks samples seem to indicate prevalence of pesticides in the fish themselves, we can study the levels of oc pesticides in fish tissues from fish farms as well as fresh fish to gain a perspective on the level of pollution of fish and the levels of human exposure to oc pesticides from this type of food.

# 6. References

1. Stockholm Convention on Persistent Organic Pollutants (POPs). *Encyclopedia of Corporate Social Responsibility* 2336–2336 (2013) doi:10.1007/978-3-642-28036-8_101506.
2. Assessment of persistent toxic substances in the environment of Egypt - ScienceDirect.
3. Mansour, S. A. Persistent organic pollutants (POPs) in Africa: Egyptian scenario. *Human & Experimental Toxicology* **28**, 531–566 (2009).
4. Deadman, M. L. Sources of Pesticide Residues in Food: Toxicity, Exposure, and Risk Associated with Use at the Farm Level. in *Pesticide Residue in Foods: Sources, Management, and Control* (eds. Khan, M. S. & Rahman, M. S.) 7–35 (Springer International Publishing, 2017). doi:10.1007/978-3-319-52683-6_2.
5. Mansour, S. A. Environmental Impact of Pesticides in Egypt. in *Reviews of Environmental Contamination and Toxicology Vol 196* (ed. Whitacre, D. M.) 1–51 (Springer US, 2008). doi:10.1007/978-0-387-78444-1_1.
6. Mansour, S. A. Pesticide exposure—Egyptian scene. *Toxicology* **198**, 91–115 (2004).
7. Barakat, A. O. Assessment of persistent toxic substances in the environment of Egypt. *Environment International* **30**, 309–322 (2004).
8. Pereira, V. J., Arantes Rodrigues da Cunha, J. P., de Morais, T. P., Ribeiro-Oliveira, J. P. & de Morais, J. B. Physical-Chemical Properties of Pesticides: Concepts, Applications, and Interactions with the Environment. *Biosci. J.* **32**, 627–641 (2016).
9. Tsipi, Despina, Botitsi, H. & Economou, A. Pesticide Chemistry and Risk Assessment. *Mass Spectrometry for the Analysis of Pesticide Residues and Their Metabolites* (2015) doi:10.1002/9781119070771.ch1.

10. A Comprehensive Guide to the Hazardous Properties of Chemical Substances (2nd Edition): Pradyot Patnaik; John Wiley and Sons, Inc., New York, 1999, xxiii +984 pages, £122.00, ISBN 0-471-29175-7. *Carbohydrate Polymers* **55**, 455 (2004).

11. Kintz, P. *et al.* A high endrin concentration in a fatal case. *Forensic Sci. Int.* **54**, 177–180 (1992).

12. Hexachlorobenzene as a persistent organic pollutant: Toxicity and molecular mechanism of action.- ClinicalKey. http://0810fsdq2.1103.y.https.www.clinicalkey.com.mplbci.ekb.eg/#!/content/2-s2.0-29128804.

13. Mrema, E. J. *et al.* Persistent organochlorinated pesticides and mechanisms of their toxicity. *Toxicology* **307**, 74–88 (2013).

14. Muir, D. & Sverko, E. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. *Anal Bioanal Chem* **386**, 769–789 (2006).

15. Method 608 Organochlorine Pesticides and PCBs.pdf.

16. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal.- ClinicalKey. http://0810fsdqr.1103.y.https.www.clinicalkey.com.mplbci.ekb.eg/#!/content/2-s2.0-17047943.

17. Detection of persistent OCPs and PCBs congeners in the near-shore coastal waters of Alexandria, Egypt | SpringerLink. http://08102sdsq.1103.y.https.link.springer.com.mplbci.ekb.eg/article/10.1007/s10661-015-4537-z.

18. Persistent organochlorine pesticide and PCB residues in surface sediments of Lake Qarun, a protected area of Egypt.- ClinicalKey.

http://0810fsdsy.1103.y.https.www.clinicalkey.com.mplbci.ekb.eg/#!/content/2-s2.0-23237301.

19. Ali, H., Potter, T., Osman, M. & Mohamed, M. MONITORING OF PESTICIDE RESIDUES IN RIVER NILE WATER FROM EGYPT BY SOLID-PHASE EXTRACTION FOLLOWED BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROSCOPY. doi:http://dx.doi.org/10.21608/ajs.2005.15335.

20. Azab, M. M., Darwish, A. A., Mahmoud, H. A. & Sdeek, F. A. Residue levels of organochlorine pesticides in some ecosystem components of Manzala Lake. *Environ Monit Assess* **185**, 10257–10268 (2013).

21. Khairy, M. A. E.-H., Kolb, M., Mostafa, A. R., EL-Fiky, A. & Bahadir, M. Risk posed by chlorinated organic compounds in Abu Qir Bay, East Alexandria, Egypt. *Environ Sci Pollut Res* **19**, 794–811 (2012).

22. Said, T. O., Okbah, M. A., Mohamed, L. A. & Othman, I. M. Detection of persistent OCPs and PCBs congeners in the near-shore coastal waters of Alexandria, Egypt. *Environmental Monitoring and Assessment* **187**, 353 (2015).

23. Monitoring of pesticides water pollution-The Egyptian River Nile | Journal of Environmental Health Science and Engineering | Full Text. https://jehse.biomedcentral.com/articles/10.1186/s40201-016-0259-6.

24. Said, T. O., El Moselhy, K. M., Rashad, A. A. M. & Shreadah, M. A. Organochlorine Contaminants in Water, Sediment and Fish of Lake Burullus, Egyptian Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology* **81**, 136–146 (2008).

25. Said, T. O., El Moselhy, K. M., Rashad, A. A. M. & Shreadah, M. A. Organochlorine Contaminants in Water, Sediment and Fish of Lake Burullus, Egyptian Mediterranean Sea. *Bull Environ Contam Toxicol* **81**, 136–146 (2008).

26. Wang, Y. *et al.* Residues and ecological risks of organochlorine pesticides in Lake Small Baiyangdian, North China. *Environmental Monitoring and Assessment* **185**, 917–929 (2013).

27. Organochlorine pesticide residues in sediment and water from Nairobi River, Kenya: levels, distribution, and ecological risk assessment | SpringerLink. https://link.springer.com/article/10.1007%2Fs11356-018-3398-8.

28. Geyikçi, F. & Büyükgüngör, H. Monitoring of organochlorine pesticides in the surface waters from Mid-Black Sea Region, Turkey. *Environmental Monitoring and Assessment* **173**, 127–137 (2011).

29. Method 608: Organochlorine Pesticides and PCBs. 33.

30. Han, Q. *et al.* Graphene as an efficient sorbent for the SPE of organochlorine pesticides in water samples coupled with GC–MS. *Journal of Separation Science* **36**, 3586–3591 (2013).

31. Canbay, H., Öğüt, S. & Yılmazer, M. Pesticide residues analysis in lake water and sediment samples from the Golcuk National Park. *Water Resources* **41**, 97–104 (2014).

32. Canbay, H., Öğüt, S. & Yılmazer, M. Pesticide residues analysis in lake water and sediment samples from the Golcuk National Park. *Water Resources* **41**, 97–104 (2014).

33. World Health Organization. *Guidelines for drinking-water quality, Volume 1: Recommendations*. (World Health Organization, 2003).

34. Yahia, D. & Elsharkawy, E. E. Multi pesticide and PCB residues in Nile tilapia and catfish in Assiut city, Egypt. *Science of the Total Environment* **466–467**, 306–314 (2014).

35. Henriksson et al. - 2017 - Benchmarking the environmental performance of best.pdf.

36. Dahshan, H. *et al.* Monitoring of pesticides water pollution-The Egyptian River Nile. *J Environ Health Sci Engineer* **14**, 15 (2016).