BASELINE MONITORING OF SELECTED ORGANOCHLORINE PESTICIDES (OCPS) AND ORGANOPHOSPHORUS PESTICIDES (OPPS) IN THE ARABIAN GULF

BY

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A Thesis Presented to the
DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

ENVIRONMENTAL SCIENCE

April 2013

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

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I would like to dedicate my thesis to

My beloved parents, my wife, brothers and sisters

ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Basheer Chanabasha for the continuous support of my MS study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Master study.

Besides my advisor, I would like to thank the rest of my thesis committee: Dr. Assad Al-Thukair and Dr. Bassam Al-Tawabini, for their encouragement, insightful comments, and hard questions.

Last but not the least; I would like to thank my brothers and friends for supporting me spiritually throughout my life.

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LIST OF ABBREVIATIONS

OPPs: Organophosphorus Pesticides

DDE: Dichloro(diphenyl)ethylene

DDD: Dichlorodiphenyldichloroethane

PCBs: Polychlorinated biphenyls

CNS: Central Nervous System

OCPs: Organochlorine Pesticides

POPs: Persistent Organic Pollutants

DDT: Dichlorodiphenyltrichloroethane

UNE: United Nations Environmental Program

EPA: Environmental Protection Agency

AcHE: Acetylcholinesterase

SPM: Suspended Particulate Matter

ECD: Electrical Conductivity Detector

GC: Gas Chromatography

HCH: Hexachlorocyclohexane

GPC: Gel-Permeation Chromatography

KD: Kuderna-Danish

LD: Lethal Dosage

FDA: Food and Drugs Administration

OPI: Organophosphorus Insecticides

ACS: Accredited Standard

MS: Mass Spectrometry

TOC: Total Organic Carbons

SPE: Solid-phase Extraction

UAE: Ultrasound-Assisted Extraction

ABSTRACT

Full Name : Jamal Mohammed Al-Aamri

Thesis Title : Baseline Monitoring of Selected Organochlorine Pesticides (OCPs) and Organophosphorus Pesticides (OPPs) in the Arabian Gulf

Major Field : Environmental Science

Date of Degree : October 2013

In this study, baseline monitoring of Organchlorine Pesticides (OCPs) and Organophosphorus Pesticides (OPPs) were determined in four different sampling locations comprising Tarut Bay, Jubail Coastal Area, Tarut Island and Al-Oyoon agricultural area. Samples included sea water, groundwater, sediment and Biota's (Fish species). Solid samples were extracted using ultrasonication assisted extraction (UAE) and liquid samples were processed using solid-phase extraction (SPE) procedures. After extraction, analyses were performed by Gas chromatography-mass spectrometry (GC). The results apparently demonstrate that DDD, a metabolite of DDT is prevalent in all samples and it has the greatest concentrations among the other pesticides. The average of DDD in all water samples is 0.118 mg/L and the highest concentration is 0.300 mg/L. Quantitatively and collectively, the level of OCPs in Tarut Bay was reckoned at 1.628 mg/L with the average being 0.407 mg/L. OCPs were not determined in Fresh Water samples at both Tarut Bay and Al-Oyoon. Similarly, OPPs remained undetected in Sea Water samples. In Biota, both OCPS and OPPs were quantified, however, the level of OCPs is orders of magnitude elevated than OPPs. Interestingly however, the levels of OCPs detected in Biotas are within acceptable limits according to WHO provisions. The highest concentration of OPPs in Biota was found to in Indian Mackerel and Crab, the lowest value being visible in Shrimp. Invariably, the preponderance of these pesticides in Tarut Bay clearly exceeds that of Jubail coastal area, and this is attributable to run-off from the agricultural activities carried out in Tarut Island.

ملخص الرسالة

الاسم الكامل: جمال محمد علي العامري

عنوان الرسالة: تحديد القياسات الأولية لكمية المبيدات الحشرية العضوية الكلورية والفسفورية في الخليج العربي

التخصص: علوم البيئة

تاريخ الدرجة العلمية: أكتوبر 2013

من خلال هذه الدراسة تمّ قياس تراكيز مجموعة من المبيدات الحشرية العضوية الكلورية والفسفورية في أربع مناطق على ساحل الخليج العربي بالمنطقة الشرقية من المملكة العربية السعودية. الدراسة شملت خليج تاروت، والمنطقة الساحلية بالجبيل وجزيرة تاروت ومنطقة العيون الزراعية بالأحساء. وتنوّعت العينات من عينات من مياه البحر والآبار الجوفية وعينات من التربة البحرية بالإضافة إلى عينات عضوية من أسماك متعددة من السوق المحلي. استخدمت خلال الدراسة طريقتان من طرق الاستخلاص المعروفة والمتداولة وهما الإستخلاص بالتمرير بطبقة صلبة (SPE) والإستخلاص المتسارع باستخدام الموجات فوق الصوتية (UAE). بعد عملية الاستخلاص، تمّ تحليل المستخلصات عن طريق جهاز كروماتوغرافيا الغاز - مطياف الكتلة. أظهرت النتائج أن المبيد الحشري ثنائي كلورو ثنائي فينيل ثنائي كلورو الإيثان (DDD) ،وهو المستقلب من المبيد الحشري ثنائي كلورو ثنائي فينيل ثلاثي كلورو الإيثان (DDT)، كان موجودا في جميع العينات التي تحليلها. وقد كان معدل تركيزه في جميع العينات 0.118 ملجم/ل وأعلى تركيز له هو 0.300 ملج/ل. وكانت المبيدات العضوية الكلورية عند أعلى مستوياتها في خليج تاروت حيث كان أعلى تركيز هو 1.628 وقد كان معدل التركيز في الخليج نفسه حوالي 0.407 ملجم/ل. وخلال الدراسة لم يستطع كشف أي تراكيز للمبيدات العضوية الكلورية في المياه الجوفية في جزيرة تاروت ومنطقة العيون الزراعية. كما أنّه لم يتم كشف أيا من المبيدات العضوية الفسفورية في عينات المياه البحرية. لوحظ أيضا ارتفاع مستويات المبيدات العضوية الكلورية بقدر كبير عن مستويات المبيدات العضوية الفسفورية. بمقارنة مستويات المبيدات العضوية الكلورية في عينات الأسماك مع متطلبات منظمة الصحة العالمية، وجد أن التراكيز في المعدلات الآمنة. كان غير مستغربا أن تكون مستويات المبيدات الحشرية بنوعيها في خليج تاروت أكبر منها في منطقة الجبيل

الساحلية وذلك يعزو إلى وجود قنوات مائية تصل المزارع المنتشرة في جزيرة تاروت بخليجه بعكس منطقة الجبيل الصناعية.

CHAPTER 1

INTRODUCTION

Pesticides have been used thousands of years ago and the main target was to control pests. In ancient time, various types of pesticides were used for example, sulfur had been used 5,000 years ago by Sumerians to control insects and mites. Chinese also tried to control body lice by some arsenic compounds and mercury. Livestock, crops and human being as well were protected by the use of oil, ash and sulfur by the Greeks and Romans. In fact, in many civilities, the concern of preserving food and protecting it was addressed by the use of some material such as smoke and salt. Most of pesticides are being used in the agricultural part. Pesticides and other agrochemicals are used to ensure adequate food supply [1].

Since the introduction of dichlorodiphenyltrichloroethane (DDT) was found as an effective pesticide to kill insect and help improving the agriculture products. However, the toxicity of DDT to other non-target beneficial organisms is a major issue [2]. DDT is belongs to a group of chemical pollutants known as Persistent Organic Pollutants (POPs). The persistence nature of chlorine-carbon bond in POPs makes them difficult to degrade.

Most of the POPs are halogenated hydrocarbons and organochlorines pesticides which are the dominant class of pollutants. As the number of chlorine atoms increase, the persistence of the compound increases. These compounds have low solubility in water and high solubility in lipids (ie they possess high liposolubility), thus explaining why they could bioaccumulate and bioconcentrate in organisms. Cycoloaromatic chlorinated hydrocarbons with higher molecular

weights are also known as bioaccumulative compounds. They can bio-accumulate in animals and humans and could be biomagnified by factors of up to 70,000 fold [3]. They share many characteristics in common for example, lower molecular weight chlorinated hydrocarbons are toxic with shorter half-life.

Due to their toxicity, long-transport and persistency, POPs got an international attention, and rules were set to regulate or prohibit their uses globally. The first call for global action to handle the POPs' issue was by the Governing Council of the United Nations Environment Program (UNEP) in 1995. A global environmental treaty known as Stockholm Convention to control POPs was signed in 2001 and identified twelve POPs known as the dirty dozen. However, the Convention was put into force effective on 17th May 2004. The identified chemicals were categorized into three Annexes (A, B and C) and each Annex has its own chemicals and restrictions. In 2009, nine more chemicals were included in the list. The Convention aims to eliminate or reduce the production and the use of these chemicals [4].

Many Environmental programs to monitor POPs were established. A guidance to monitor POPs was prepared by the UNEP [4]. In fact, as part of the convention, the signed parties should help in studying the effectiveness of the convention by providing national reports on their monitoring programs and non-compliance data. Many countries have shared their experience with such programs and provided sufficient data to the UNEP about the fate and the concentrations of these persistent organics in their environments.

Saudi Arabia signed the Convention in 2001. However, no regualr moniroting program for POPs was estabished. A workshop was completed in July 2010 in Jeddah to prepare for constructing new environmental programs to monitor POPs in the Red Sea.

The famous category of POPs are the organochlorine pesticides (OCPs). They are well known class of pesticides that have brought major hazards to the environment due to their persistence property that allow them to last for longer time. Those pesticides were replaced by another class of chemicals that contain phosphorus-carbon bond which is easily degraded when subjected to the sunlight and water. However, the toxicity of these class of pesticides is greater than the OCPs.

During this study, selected OCPs and OPPs were monitored and detailed descriptions of OCPs and OPPs are summarized in the next pages.

1.1. Organochlorine Pesticides (OCPs)

1.1.1. History and Structure

Oraganochlorine Pesticdes (OCPs) were introduced for the first time in 1940s. Although they were extensively used especially after the Second War to increase the production of different crops, public concerns and research institutes called to stop their uses due to certain visible health hazards and environmental impacts. They have been banned from different countries. Their persistence in the environment makes them still available in different level of concentrations despite their restricted use 30 to 40 years ago. Organochlorine pesticides simply could be defined as any hydrocarbons with chlorine atoms in their structure. Organochlorine pesticides have similar chemical structures, showing chlorine-substituted aliphatic or aromatic cyclic rings. Owing to their structural similarities, these pesticides have certain common physicochemical characteristics such as persistence, toxicity, bioaccumulation, and long-range transport potential. Bioaccumulation of organochlorine pesticides is defined as a log K_{OW} value higher than five or bioaccumulation factor in aquatic species exceeding 5000. These pesticides are nonpolar and semi-volatile, enabling their entry in the atmosphere and transport over long distances globally, predominantly by air mass movements. They predicted to reach polar or high mountainous regions and are ultimately deposited in cold regions by snow via cold condensation and global distillation phenomenon [10]. These pesticides are characterized by their cyclic structure, number of chlorine atoms, and low volatility. They contain four categories: dichlorodiphenylethanes, cyclodienes, chlorinated benzenes, and cyclohexanes. All of them in common share a similar pair of carbon rings, one ring being heavily chlorinated [11]. Heptachlor, whose structural formula is depicted in Fig. 1, is an organochlorine pesticide used for over 40 years in the control of termites

and other soil insects. It's approved use in several countries has however been gradually withdrawn [12]

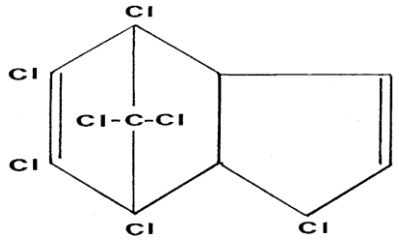


Fig. 1 Schematic structural formula of Heptachlor

Due to their strong chlorine-carbon bond, they are very stable and environmentally non degradable. They undergo very slow decomposition process. DDT easily degrades into dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), which are more persistent than the parent compound. The half-life of p,p′-DDE and β-HCH in humans has been estimated as more than 7 years, whereas γ-HCH has a half-life of only 20 hours [13]. Because of their relative semi-volatility, they can move and circulate in the environment by air, water, soil, vegetation and animals. Treated Food and related products also represent viable conduits by which these pesticides are transported. Given that organochlorine pesticides are essentially non-polar molecules, their tendency to solubilize in hydrocarbon-like environments (hydrophobic), such as the fatty material in living tissues is exceedingly high.

Their solubility in water if at all is only to a small degree. Despite the volatility of Organochlorine pesticides, a property which culminates in their presence in sufficient quantities in the atmosphere, they adsorb appreciably to soils and sediments. Ultimately, their concentration escalates, eventually surpassing that of the surrounding water bodies by certain orders of magnitude. In water and sediments, Organochlorine pesticides, being lipophilic, have the propensity to bioaccumulate and bioconcentrate in tissues of fish and other aquatic organisms.

Plants, birds, terrestrial animals, agricultural livestock, and domestic animals have also evidenced bioaccumulation of Organochlorines. Here, their concentrations rise gradually by orders of magnitude as they move via the food web, especially as they approach higher organisms in the trophic levels. Organochlorine pesticides are known to possess low acute toxicity to humans when inhaled or ingested at low concentrations. Nonetheless, the fundamental fact that they could mimic human hormones such as estrogen, or exhibit other properties that cause long term detrimental health effects is far from being overestimated. Headache, nausea, vomiting, dizziness, convulsions, mood change muscle tremors, liver damage, and death are some of the visible lethal effects of organochlorine pesticides at extreme concentrations [14]. Variable uses of organochlorine pesticides have been banned following their potential hazardous effects to humans and the detectable deleterious consequences on animals and plants in the environment.

1.1.2. Toxicity

The ultimate impact of organochlorine pesticide exposure on health is predicated on the specific pesticide, exposure level, the timing of exposure and the individual, with divergent pesticides bringing in their train a wide spectrum of health symptomatics.On the basis of toxicity and guided by toxicokinetic study, Organchlorines are comprehensively grouped below [15]:

Highly toxic Organochlorines

- Aldrin
- Dieldrin
- Endrin(banned by Agency [USEPA])
- Endosulfan

Moderately toxic Organochlorines

- Chlordane
- DDT (banned by the EPA)
- Heptachlor
- Kepone
- Lindane
- Mirex
- Toxaphene

Organohlorine have neurotoxic effects and are intimately involved in ion channel modification. There are inexhaustible reports about metabolic disorders and hyperglycemia cases. Besides, oxidative stress and diabetes, other metabolic disorders are recorded in acute and chronic exposures to pesticides.

Organochlorines largely affect metabolism of lipids in the adipose tissues and alter glucose pathway in other cells. In this context, there are substantial in vitro and in vivo but few clinical studies about the mechanisms underlying these toxicological effects. Induced cellular oxidative stress through affecting mitochondrial respiration and the explicit disruption of neuronal and hormonal integrity of the body represent an absolute commonality in the mechanism of action of Organochlorines, Carbamates and Organophosphates.

1.1.3. Metabolism

Indeed, economic and public health gains emanating from the application of synthetic pesticides remain incontrovertible. However, contemporary research has proved widespread environmental contamination by OCPs. In fact, the levels have reached unprecedented global proportions driving to a terminal point in the era of their extensive use. As a result of their intrinsic negative health as well as environmental damages due to long half-life and corresponding persistence, these pesticides have been suspended from the Global market.

Metabolism of Organochlorine insecticides is principally mediated by Microorganisms. This notwithstanding, the persistence and bioavailability of several organochlorine insecticides in soils and aquatic habitats for protracted periods has been recorded. This is probably partly attributable either to the resistance of the insecticide to microbial decomposition or to the transformation to active metabolites and complexes with some components of the environment which are primarily resistant to microbial degradation. Microbial degradation of organochlorine insecticides is achieved via reductive dechlorination, dehydrochlorination, oxidation, and isomerization of the parent molecule. The fate of organic pollutants in the environment is influenced by environmental factors such as pH and temperature affecting the activity of microorganisms [16]. Degradation of Lindane by bacteria (*Streptomyces sp*) and fungi (*Pleurotus sajor-caju*) in soils has been successful. However, decomposition rates are affected at high concentrations of the pesticide presumably due to toxicity of active metabolites [12, 16].

1.2 Organophosphorus Pesticides (OPPs)

1.2.1 History and Structure

Organophosphorus pesticides represent a diverse group of agrochemicals with essential insecticidal properties. Emanating from research by Lange and von Krueger (in 1932), esters of monofluorophosphoric acid was found to be highly toxic. Parathion, the first vital insecticide was developed by G. Schrader in 1944. Below illustrates the structure:

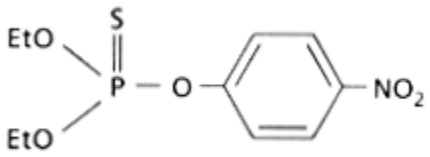


Fig. 2 Schematic structural formula of Parathion

1.2.2 Toxicity

The fundamental mechanism of OPs toxicity has been clearly studied – they function largely as the enzyme acetylcholinesterase(AcHE) inhibitors. Decrease in AcHE activity is function of frequent exposure to Ops, a factor used to assess the levels of human exposure to OPPs. Where OP concentrations entering to body are relatively high, this approach is relevant for professional exposure determination. However, significantly decreased AcHE activity is not usually caused by low OP concentrations which are present continuously. Consequently, exposure of larger populations needs to rely on assessing the levels of OP metabolites, such as alkylphosphate in urine [17].

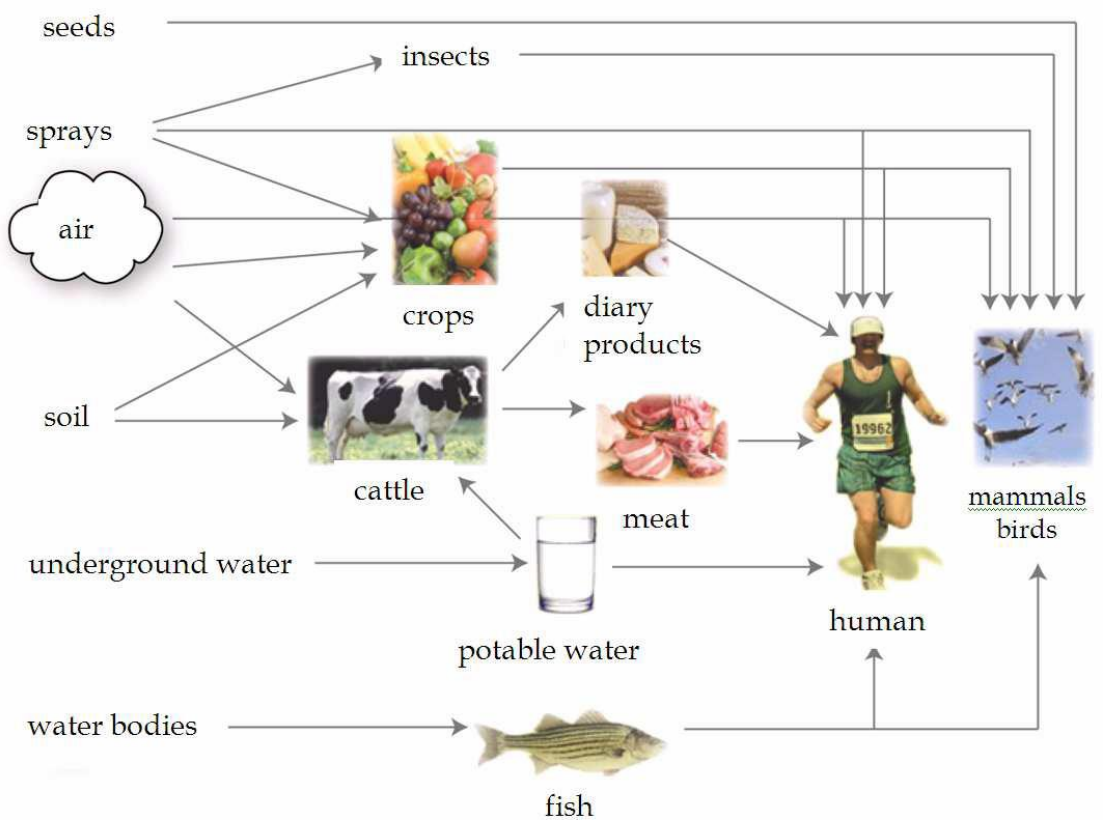


Fig. 3 Schematics of the various exposure routes to Ops [18]

1.2.3 Metabolism

Given that the metabolism of organophosphorus pesticides usually come with rapidity, it is imperative to analyze the metabolite(s) relative to the unchanged organophosphorus pesticide to assess its poisoning correctly. Figure 4 depicts the metabolic pathways of a typical thiono kind organophosphorus pesticide with dialkoxy functional groups (R1 and R2).

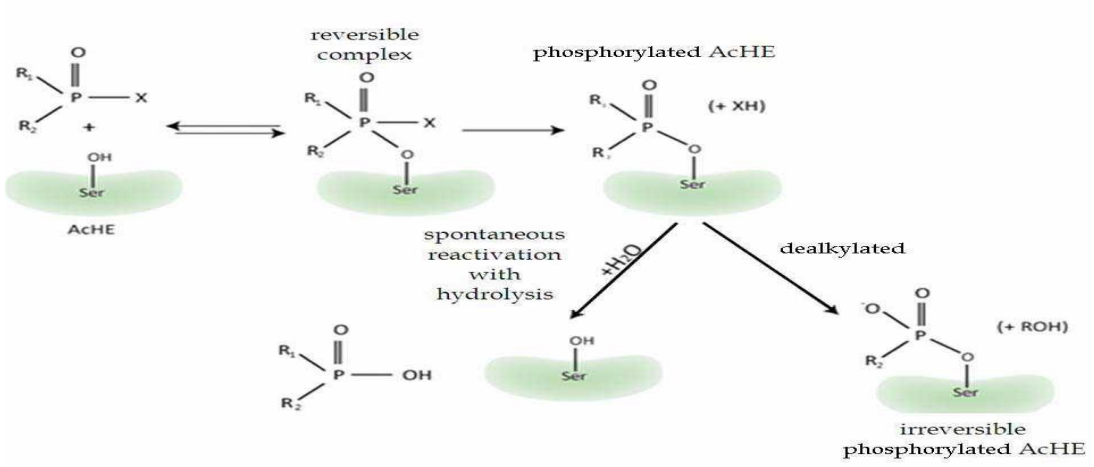


Fig. 4 Metabolic pathways of a typical thiono kind Organophosphorus Pesticide [13]

In mammals, Organophosphorus pesticides undergo both activation of their toxicity and detoxification concurrently. In its unchanged form, the thiono type pesticide (1) elicits nearly no inhibitory effect on cholinesterase, its metabolization by cytochrome P450 (I) into the phosphate type (2), however confers and activates toxicity. The phosphate type pesticide can apparently and potentially inhibit cholinesterase without any further metabolic activation.

Since detoxification and dealkylating reactions by enzyme II are possible, the P450 and glutathion are involved in the enzyme II. The dealkylated form produced, does not inhibit acetylcholinesterase. The esterases III, which hydrolyze organophosphorus pesticides, are generally hydrolyzed by activity of esterases III. “Paraoxonase, A-esterase, phosphatase or arylesterase” represent the diverse groups of enzymes catalyzing such reactions. Related with the metabolism of malathion is carboxyesterase, an Organophosphorus pesticide whose leaving groups have carboxylic acid esters.

Currently, over 900 pesticides are known to be in use with about 600 active substances on the Global market [19]. The calculated theoretical deposit of pesticides is generally less than the observed practical real deposit on target pests. Consequently, less than 5% of the Millions of tons of pesticides applied annually are projected to reach the real target organism. The remaining significant proportion is found deposited on non-target organisms, soil, and transport into water as well as volatilization into the atmosphere [20]. The actual metabolic fate of pesticides is dictated by a multitude of variables acting on the active ingredient. These include; biotic factors (flora and fauna), abiotic factors (temperature, moisture, soil pH, etc.), pesticide physicochemical properties a (hydrophilicity, pKa/b and etc.) and biochemical reactions. Photo degradation, hydrolysis, oxidation, reduction, and rearrangements are the main agents purely accounting for abiotic decomposition owing to chemical and physical transformations of pesticides and their active metabolites. Additionally, pesticides bioavailability may diminish as a consequence of compartmentalization, a phenomenon which transpires as a result of pesticide adsorption and fixation to soil and soil colloids with the chemical structure of the original molecule remaining intact. On the other hand, enzymatic transformation catalyzed by plants and microorganisms via biotic processes is largely the principal detoxification pathway. Three-phase processes may characterize the metabolism of pesticides [21, 22]. Phase I involves transformation of the primary properties of the parent compound through oxidation, reduction, or hydrolysis to less toxic product but more water-soluble than the parent. In phase II, pesticide or pesticide active metabolite undergoes conjugation to glutathione, amino acid or sugar.

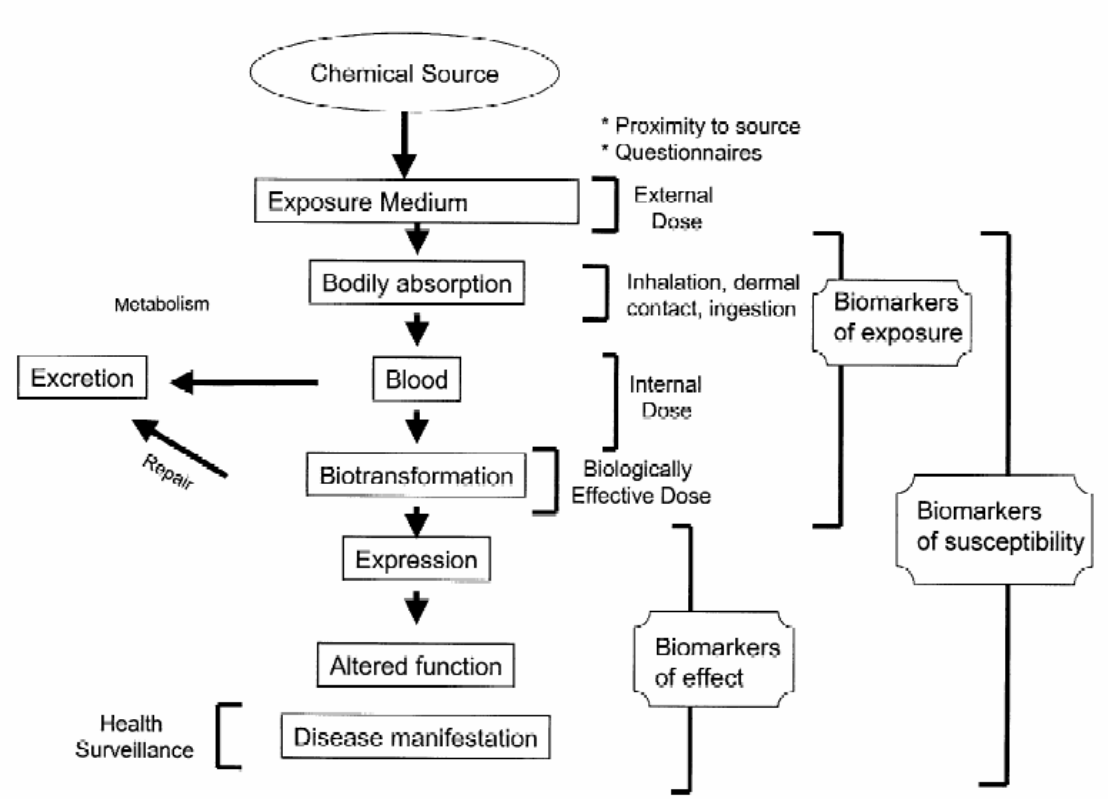


Fig. 5 Metabolism of OPs and effects on organisms

This further raises their polarity and solubility and thus decreases toxicity compared with the original pesticide. As a result of little to no phytotoxicity, Phase II metabolites may be potentially stored in cellular organelles. The third phase is depicted by transformation of Phase II metabolites with the resultant secondary conjugates, which are similarly nontoxic [22]. Phase III metabolism is exemplified in leafy spurge (Euphorbia esula L.) by the conjugation of malonate with the N-glycoside metabolite of picloram and the formation of a gentibioside from the picloram glucose ester metabolite [23].Chlorpyrifos was fed dairy cattle for 2 weeks time span [24].

The original compound and two (oxidized and hydroxylated) metabolites were detected at low concentrations in milk and cream (fat). The levels of all the three compounds however declined dramatically following cessation of administration. Johnson Jr et al., [25]observed concurrence in

the results. Maximum of 0.14% intake through eggs was extracted with dramatic residual depletion from the body [26]. Cook et al., [27] has demonstrated via research that pesticides have series of lethal consequences on rumen fluid found in Ruminants. OPs especially, parathion are actively hydrolyzed by rumen liquor.

Apparently, Cook J.W [28] was probably the first to conceive the enzymatic hydrolysis of Ops. Further visible empirical evidence by Cook J.W [28] highlighted the obvious disappearance of parathion toxicity in Cattle, a phenomenon that is clearly attributable to the metabolic decomposition of parathion by microorganisms associated with the rumen. Williams, Phletus P [29] pointed out that certain class of OP pesticides catalyzes In vitro gas generation through holotrich protozoa resident in the rumen. However, with rumen bacteria used as inoculums source, these compounds had virtually no remarkable impact. At 100, 250, and 500 μcg per ml of media environment (predominantly rumen fluid), Kutches et al., [30] observed that toxaphene were apparently ineffective in inducing decreased In vitro dry matter degradation and eventual disappearance.

The need for monitoring of marine pollution has become a must to ensure food safety and keep a healthy ecosystem. Many nations have established different kinds of monitoring programs for seas under their jurisdiction [4]. Environmental monitoring of sea water quality should help in finding proper strategies to determine the sensitivity of the marine environment to the different sources of pollution it may be subjected to. Environmental monitoring will includes the determination of toxicity levels of compounds at different locations to identify the hot spots or sources of pollution.

Due to the complexity of the seawater sample and the trace concentrations of these toxic compounds, proper sample preparation and analysis should be implemented. International environmental agencies have set very low level of toxic compounds that cannot be determined using conventional analysis methods. Such a low level of concentration requires a pre-concentrate step to enrich the target analytes to be detectable. There are different types of methods to extract the analytes from the environmental water samples and make them ready for analysis.

1.3 PROBLEM STATEMENT

Saudi Arabia has signed the Stockholm Convention in 2001, however, no regualr environmental monitoring programs have been known to monitor the 21 POPs, stated in the Convention. The last data found for some OCPs concentrations was in the Regional Organization for Protection of Marine Environment (ROPME) report in 2005. Therefore, there is urgent need to update the status of the contamination levels of POPs in the Arabian Gulf which is also known of its sensitive ecosystem.

The substitution of the OCPs with OPPs is effective to avoid the persistence of these components. Nonetheless, the health effects of the OPPs made the need to monitor them profoundly important.

1.4 OBJECTIVES

The main objective of this study is to estabilish a base-line monitoring of pesticides in the coastal waters and agriculture areas of Eastern Province of Saudi Arabia.

The specific objectives of the study are:

- To determine the concentrations of selected OCPs and OPPs in aqeous, sediment and biota samples.
- To compare the contaminations levels with the reported concentration values in the literature and WHO acceptable limits.

CHAPTER 2

LITERATURE REVIEW

In this section, some selected papers that represented some investigation studies of organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) levels in different area of the world. These studies are concentrated in their levels in marine water and sediment and with different methods of sampling and extraction techniques. Different concentrations of the contaminants are highlighted in the summary of each paper.

Different samples of sediments and biota were collected from ROPME Sea Area (RSA) from different countries during February to March 2005. These countries are Bahrain, Iran, Kuwait, Oman, Qatar, Saudi Arabia and UAE [31]. The International Sampling Guidelines of IUCN WWF [32] were followed in sampling procedure. The locations of all sampling sites are indicated in the blow map.

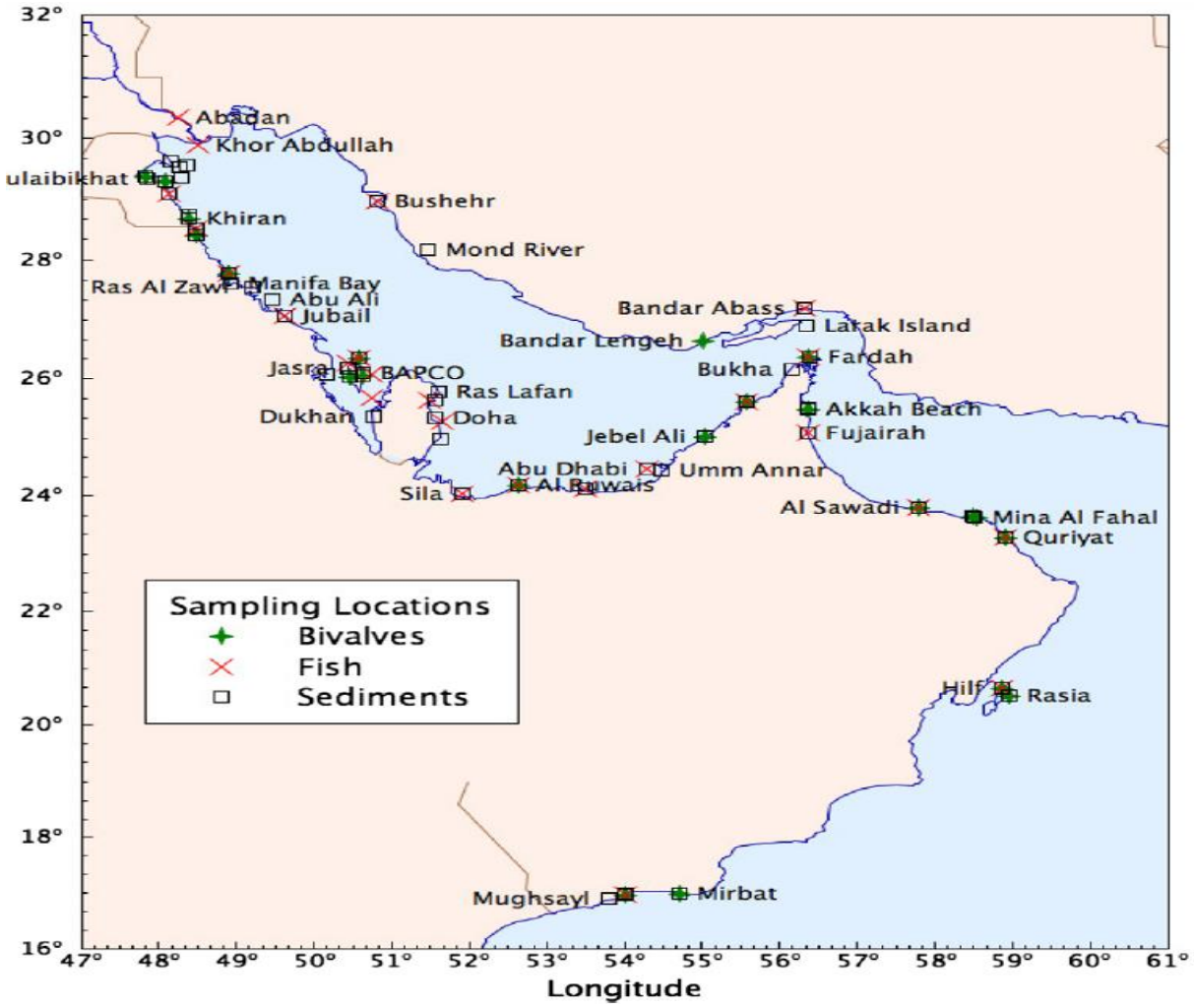


Fig.6 Map of ROPME Sea Area

Sediments samples were prepared and pre-treated before any analysis. Sediments were dried, sieved with mesh sizes from 250 to 1000 um. Then, they were homogenized. TOC and grain size analysis were conducted for each sample. Using a microwave, the extraction of the sediment samples with a mixture of hexane and methylene chloride (1:1) was carried out. Activated copper was used to eliminate sulfur content. The extracts were concentrated by a rotary evaporator. The results and the concentration of the chlorinated hydrocarbon are indicated in the below tables:

Table. 1 Results of the chlorinated hydrocarbon (ROPME)

Country	**Total DDTs (ng/g)**	**Total HCHs (ng/g)**	**Total HCB (ng/g)**
Bahrain	0.003-0.256	0.007-0.022	0.001-0.042
Iran	0.035-0.312	nd-0.0155	0.003-0.092
Kuwait	0.014-3.808	0.006-0.032	0.002-0.022
Oman	0.005-0.079	0.001-0.016	0.003-0.029
Qatar	0.0025-0.093	nd-0.009	0.003-0.053
Saudi Arabia	0.004-0.139	0.001-0.037	0.0015-0.014
UAE	0.0007-0.087	nd-0.012	0.003-0.016

Seventeen sediment samples were collected from the gulf of Aden, Yemen for the determination of pesticides [33]. In this study samples were frozen and dried. 10 g of each sample was put in an accelerated solvent extractor (ASE) with methylene chloride. Then the extracts were fractionated

by a column chromatography (alumina/silica gel). The analytes then were eluted by 1:1 pentane/dichlormethane mixture and concentrated to 1 ml by heating in a water bath at 60 C.

Polychlorinated biphenyl concentrations were found in the range of 0.40 to 4.97 ng/g dry sample weight. The total DDT concentrations were calculated to be from below detection limits to 0.74 ng/g.

Another study was conducted by de Mora et al., [34] in the gulf of Oman for the determination of POPs. Different sediments samples were collected from different location in the Arabian Gulf (Qatar, Bahrain, Oman and the United Arab Emirates). Sediments samples were frozen and sieved with mesh sizes from 250 to 1000 1M and then homogenized. After that, the homogenized samples were extracted by Soxhlet techniques into hexane:methylene chloride mixture. Sulfur interferences were avoided by removing the trace of sulfur by an activated elemental copper. The extracts were then concentrated using a rotary evaporator. The results of the study are tabulated below:

Table. 2 Chlorinated hydrocarbon levels in UAE and Qatrar

Chlorinated hydrocarbon concentrations (pg g^{-1} dry weight) in sediments from Qatar and UAE (Panel A); Bahrain and Oman (Panel B)

Compounds	Qatar					UAE					
	Umm Said	Dukhan	Doha	Ras Laffan	Ras Al Nouf	Jebel Ali	Abu Dhabi	Al Marfa	Al Ruweis	Akkah Head	Akkah Beach
Panel A											
HCB	3.1	3.2	3.0	1.0	5.4	1.3	0.91	5.8	1.4	7.3	4.2
α-HCH	1.8	0.77	1.6	<0.52	2.0	<0.52	<0.52	<0.52	0.59	1.9	<0.52
β-HCH	<1.2	3.2	2.7	<1.2	4.0	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Lindane	3.3	1.5	5.9	21	100	1.2	0.60	<0.53	<0.53	2.6	<0.53
∑HCHs	5.1	5.47	10.2	21	106	1.2	0.6	n.d.	0.59	4.5	n.d.
p,p′-DDT	19	5.6	11	<1.8	<1.8	<1.8	<1.8	5.1	<1.8	<1.8	25
∑DDTs	36.7	23.3	36.2	0.63	17.6	n.d.	1.5	8.96	n.d.	15.4	51.9
cis-Chlordane	<0.68	1.7	<0.68	<0.68	n.a.	3.0	<0.68	3.4	<0.68	<0.68	2.5
trans-Chlordane	<0.66	2.5	<0.66	<0.66	2.1	<0.66	<0.66	<0.66	<0.66	<0.66	<0.66
trans-Nonachlor	<0.43	12	<0.43	0.79	44	<0.43	3.3	0.44	1.4	<0.43	<0.43
Heptachlor	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49	<0.49
Aldrin	3.0	1.3	2.1	<0.44	4.4	3.0	0.55	0.67	1.0	17	1.4
Dieldrin	17	6	4.6	<0.92	5.5	3.7	<0.92	<0.92	<0.92	10	<0.92
Endrin	77	27	22	<2.9	14	<2.9	<2.9	<2.9	<2.9	46	<2.9
α-Endosulfan	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75
β-Endosulfan	28	2.3	1.8	<0.95	<0.95	29	<0.96	<0.96	<0.96	3.4	<0.96
Endosulfan sulfate	<1.3	<1.3	<1.3	<1.3	4.4	<1.3	<1.3	<1.3	2.4	9.7	<1.3
Aroclor 1254	280	160	290	20	230	25	46	13	28	130	22
Aroclor 1260	350	108	500	<10	18	<10	11	<10	14	41	14
∑PCBs	292	81.1	442	5.15	85.1	16.2	32.5	10.1	21.4	58.1	13.3

	Bahrain				Oman					
	Askar	BAPCO	Jasra	North of Meridien Hotel	Al Sawadi	Mina Al Fahal	Ras Al Yei	Hilf	Raysut Port Area	Mughsayl
Panel B										
HCB	6	27	5	6	4.8	10	2.3	2.4	0.94	0.56
α-HCH	<0.45	<0.45	1	1	<0.44	<0.44	<0.44	<0.44	<0.44	<0.44
β-HCH	2	7	3	4	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Lindane	2	6	3	6	3.6	1.1	0.75	2.7	1.2	0.74
∑HCHs	4	13	7	11	3.6	1.1	0.75	2.7	1.2	0.74
p,p′-DDT	22	78	97	19	15	<3	<3	30	<3	<3
∑DDTs	88	430	146	90	53.8	22.5	12.7	85.2	0.92	0.7
cis-Chlordane	4	29	17	6	7.1	<0.93	<0.93	<0.93	<0.93	<0.93
trans-Chlordane	2	4	<1	<1	<0.84	<0.84	<0.84	<0.84	<0.84	<0.84
trans-Nonachlor	13	<0.25	5	7	3.5	2.0	<0.25	<0.25	0.63	<0.25
Heptachlor	2	32	<0.25	2	0.55	2.1	<0.22	<0.22	<0.22	<0.22
Aldrin	<0.25	3	<0.25	19	4.2	2.1	0.91	2.9	1.1	0.47
Dieldrin	22	150	15	7	10	1.4	2.2	5.4	0.70	<0.35
Endrin	15	70	37	26	20	18	<2.4	<2.4	<2.4	<2.4
α-Endosulfan	<0.2	16	10	8	1.7	<0.26	<0.26	2.9	<3.5	<3.5
β-Endosulfan	3	13	5	13	7.3	1.1	2.7	3.0	<1.4	<1.4
Endosulfan sulfate	11	8	6	3	5.5	2.0	<0.37	<0.37	<1.4	<1.4
Aroclor 1254	650	5000	220	550	190	510	12	185	<59	<59
Aroclor 1260	390	7200	81	100	66	3600	13	850	22	<6
∑PCBs	628	7411	175	313	130	1900	14.4	334	17.7	n.d.

n.a. = Not analysed; n.d. = not detected.

Oyugi et al., [35] develop analytical methods for the seawater, sea plants and marine sediments. Samples were sampled at the coastal areas of Kenya-Mombasa and analysed for POPs. Seawater samples were collected in plastic bottles. The sediments samples were collected in a depth of 5 cm of the seabed by using a corer. Then, they were dried, ground and homogenized. Using Soxhlet extraction and with hexane solvent, the pesticides were extracted from the sediments samples and then concentrated.

The sea water samples were also extracted using hexane and dichloromethane, concentrated under reduced pressure and fractionated by a chromatographic column before analysis.

The mean concentration of organochlorine pesticides (OCPs) in sediment samples were found in a range of 0.014 to 2.571 ug/g.

Mohammed et al., [36] demonstrated the methodology for the analysis f OPPs from sediment and seawater samples from Port-of-Spain, Trinidad and Tobago**.** Different sediments samples were collected as cores of 5 cm depth. They were dried and homogenized. Using a Soxhlet apparatus and by the use of dichloromethane solvent, the samples were extracted. Elemental sulfur was removed with activated copper to avoid any interference. The volumes of the extracts were reduced by a rotary evaporator.

The total polychlorinated biphenyls concentrations were found from 62 to 601 ng/g (dry weight) and the total of Organochlorine pesticides were from 44.5 to 145 ng/g dw.

In another study from Singapore Wurl et al., [37] reported for POPs in sediment samples, thirteen samples from Singapore's coastal sediments were collected by a grab sample. Samples were dried and homogenized prior to the extraction step. Then, they were extracted in a microwave oven in a mixture of hexane and acetone. The volume of each extract was reduced to 5 ml under the vacuum evaporator. Further reduction was done by using Nitrogen gas. Total concentration of PCB and DDT were from 1.4 to 329.6 ng/g and 2.2 to 11.9 ng/g, respectively. The total HCH concentrations were measured in a range of 3.3 to 46.2 ng/g.

Along the coastal areas of Bohai Sea, different sediment samples were collected using a shovel at a depth of 0-20 cm [38]. Solid-phase extraction cartridges were used to extract the OCPs from the sediment samples with the help of ultrasonication and hexane:dichloromethane mixture (1:1). The

extracts were concentrated by rotary evaporation to 2 ml. The mean average Concentrations of DDT and HCH in the soil samples were found to 1.7 x 10 ng/g dw and 3.5 ng/g dw, respectively.

Sediment samples from 14 different location along the coast of mid-Black sea were collected by [39] using a grab sampler in a depth of 5 cm. The samples were frozen, dried and homogenized. Sediments were Soxhlet extracted in a mixture of hexane and dichlomethane (1:1) the extract then concentrated to few milliliters. Aldrin concentration in the soil sample were found from 19.3 to 87.3 ng/g and DDT ranged from 18.6 to 31.0 ng/g

In another study on OPPs by Abdelhalim., K Salama et al., [40] thirty six samples of water and sediment, or fish were collected from different locations in different seasons. The water samples were collected 50 cm below the level of water. The water samples were then fractionated [41] by a mixture of methylene chloride and sodium chloride. The organic layers were collected and dried and then evaporated to dryness. The residue was dissolved in a mixture of hexane and methylene chloride (1:1).

The sediment samples were collected by an auger at a depth of 5 cm. The sediments samples then were dried with anhydrous sodium sulfate. Liquid-liquid phase extraction was carried out for each sample of sediment. Briefly, the samples were mixed with acetone:n-hexane mixture (1:1) and acidified by concentrated HCl. Distilled water was added and the aqueous layer was collected and fractionated with n-hexane. The organic layer of hexane and the previous organic layer were combined and concentrated to 1 ml at 40 C. The method recoveries for water and sediments samples were found 77.26% and 77.80% respectively.

The organophosphours pesticides were analyzed for all of the collected samples after all preparation steps. The analysis results of the different water samples are summarized as follow:

- Chlorpyrifos levels were found 139, 135.5, 24.5, 303.8 and 89.6 ppb in the water samples that were collected at spring and summer 1999, winter and autumn 2000 and winter 2001, respectively.
- Chlorpyrifosmethyl, Pirimiphos-methyl and profenfos insecticides were found only at winter 2001. (21.8, 23.3 and 41.0 ppb, respectively).
- Malathion results of spring 1999 and winter 2001 were 446.0 and 71.9 ppb, respectively.
- Dizinon was found also in the water samples collected at spring 1999 and winter 2001. (70.5 and 24.6 ppb, respectively).

The residues of OPPs in the sediments samples were also detected. The results are varied from one season to other. Chlorphyrifos was detected in samples collected at winter, spring and autumn 2000 and winter 2001 and their values were 59.5, 133.5, 303.8 and 0.9 ppb, respectively. Chlorphyrifos-methyl was only detected of winter 2001 samples at a concentration of 61.3 ppb. Malathions at autumn 2000 and winter 2001 samples were 5.21 ppb and 2.0 ppb, respectively. Diazinon was found in two seasonal samples of spring 2000 and winter 2001 (279 and 0.9 ppb, respectively).

In the summer of 1993, an investigation study for the contamination of organophosphurs pesticides was carried out in the Bering and Chukchi Seas [42]. The concentrations of chlorpyrifos were 170 ng/g in marine ice and 19-67 ng/g in sea water samples. Laboratories recoveries were found more than 90% of all pesticides. Liquid-liquid phase extraction was used to prepare the samples for analysis. Octachloronaphthalene and 1,3,5-tribromo benzene were mixed with all samples before the extraction. During the extraction methylene chloride solvent was used and the extracts' volumes, then, were reduced to 1.0 ml under N_2 gas.

On March 1998, in the western Mediterranean coast of Valencia, a female specimen of common whale ± 20 Tm weight and 17 m size ± (Balaenoptera physalus) was found dead. One intriguing feature about this animal was its potential to accumulate high concentrations of contaminants, which can possibly give valuable information on the level of pollution in its habitat, stretching from Mauritanian coast to Ligurian Sea (Western Mediterranean, Geneve Gulf). Samples from the liver, kidney and blubber after being collected in triplicate were frozen immediately at 20°C. In an attempt to obtain a homogeneous laboratory sample for each tissue, triplicate samples were cut into small cubes and mixed after thawing.

Samples (15 g, fresh weight) were sliced to form cubes and transferred to a glass funnel. On top of an Erlenmeyerflask, the glass funnel was placed and the temperature increased in a heating cabinet to 65^{o}C for 8hrs. Using n-hexane, glass funnel and flask were rinsed and fused fat was diluted eventually to 30 ml with n-hexane. Before LC clean-up was done, Sodium sulphate was mixed with the hexanic extract. Hexane extracts of 500 mg fatty sample per ml were yielded through this step.

30 g of tissues made into cubes of approximately 10 by10 mm were added to 30 g sodium sulphate and 5 g Celite. The resulting mixture was placed in an erlenmeyer flask. Extraction was achieved under reflux in n- hexane (200 ml) for 1 h. Using filter paper, the extract was carefully filtered and concentrated in a Kuderna Danish evaporator to 10 ml. Ultimately, hexanic extracts of 3 g sample per ml were realized through this step.

Table.3 concentrations of DDT and PCB detected in Whale tissues

Concentrations (mean and relative standard deviation, $n = 4$) of DDT and derivatives and PCBs detected in selected whale tissues.[a]

Compounds	Pesticide concentration ($\mu g \cdot g^{-1}$)		
	Blubber	Liver	Kidney
op′DDE	0.09 (11)	–	0.24 (9)
pp′DDE	7.3 (8)	0.11 (8)	0.23 (10)
op′DDD	0.43 (9)	–	0.08 (8)
pp′DDD	0.64 (11)	–	–
op′DDT	0.71 (10)	–	–
pp′DDT	0.59 (8)	–	–
PCB IUPAC n° 52	1.86 (2)	–	–
PCB IUPAC n° 118	4.28 (7)	–	–
PCB IUPAC n° 138	7.11 (8)	–	–
PCB IUPAC n° 153	7.23 (5)	–	–
PCB IUPAC n° 180	3.78 (10)	–	–
Arochlor 1260	5.13 (6)	–	–

[a] – non detected.

The table above displays the concentrations of DDTs and PCBs detected in the selected whale tissues. Other organochlorine and OP pesticides were simply not observed (limits of detection are between 1 and 10 ng/g [43]. It is observable that the metabolite pp′DDE was the predominant compound within the DDT complex present in the blubber with concentrations reaching 7.3 ppm. This metabolite was also detected in liver and kidney but within lower concentrations. Being the other components minority in the blubber, pp′DDE accounted for about 75%. Residues of pp′DDT, technically referred to as DDT, were detected only in blubber at low concentrations. This concurs with Roots, Ott and Anne Talvari [44] who reported an identical pattern of DDT complex in sea lions and other marine mammals. It is proposed by Anguilar, Alex [45] that the ratio of DDE/DDT is predicated on the length of time since release of the technical DDT is indicative of the chronology of input of DDT. An increasing ratio of DDE/ DDT in Cetaceans was noticed over 20 years, which can be probably ascribed to the decomposition of DDTs to DDE in the environment. According to Aguilar's suggestions, the high ratio of DDE/DDT observed in the whale specimen predicts the low possibility of new DDT input in the area and surrounding regions. However, it should be remembered that metabolic transformation potentials could possibly influence the ratio noticed.

For sampling sake, the eight riverine runoff outlets are strategically split into eastern and western outlets. The eastern outlet is divided into four, comprising Humen, Jiaomen, Honqilimen, and Hengmen while the western outlets split into four as Modaomen, Jitimen, Hutiaomen, and Yamen. The detailed procedures of sample collection are not presented here but can be found elsewhere [46]. In brief encapsulation, sampling was strategically executed monthly, from March 2005 to February 2006, approximately 1 h before the intra-day lower tide. Each sample had a

volume of 40 L and was a composite from subsamples collected at varying points along a cross section of the river. The composites were then stored into 10L brown glass bottles. A vermicular system (pre-cleaned with acetone)were used to filter water samples and suspended particulate matter (SPM) collected with 0.7 mm GF/F glass fiber filters (142 mm diameter, Whatman International, Maidstone, England). These were heated at 450 ^{o}C for 4 hrs before use. SPM-loaded filters were wrapped in pre-cleaned aluminum foil and quickly stored at 20 ^{o}C pending analysis. Immediately following filtration, filtrates were processed. Chromatographic separation and the procedures of extraction are described in detail elsewhere [47, 48] and are presented in the Supporting information.

Results:

The concentrations of P21OCPs and P20PCBs were detected to be 2.57–41.2 ng/L with a mean value of 11.0ng/L and 0.12–1.47 ng/L with a mean value of 0.77 ng/L, respectively. That of DDTs and HCHs were found to read1.08–19.6 ng/L (mean: 3.89 ng/L) and 0.50–14.8 ng/L (mean: 3.69 ng/L), respectively. Essentially, the levels of DDTs and HCHs were detected at extreme end of the global range. The concentrations of PCBs were noticed at levels representing lower global values. It is demonstrable that the concentrations of DDTs and HCHs basically portrayed a seaward decreasing trend from Guangzhou Channel of the Pearl River to the SCS [48, 49, 50]. This leads to a possible plausible conclusion that riverine runoff is probably the important means transporting these contaminants from terrestrial sources to the coastal ocean.

In September 2003 and May 2004 Sediments were sampled from the Hau River – the biggest branch of the Mekong River, which empties into the East Sea after crossing South Vietnam. Stretching from Chau Doc town to Can Tho city and Tranh De estuary, sampling points were

accordingly chosen along the Hau River. Sediments identified as CC and NKSE were obtained close to Can Tho city and those tagged as Hau were sampled at other points along Hau River. Using Ekman dredge at each point, a grab of 5 cm surface sediment was collected. The sediment was homogenized in an aluminum tray and about 200–300 g portion was put in a clean polyethylene bag, placed in boxes packed with gel ice and transported to the laboratory.

Sediments were dried at room temperature in the laboratory, ground and sieved to obtain a particle fraction of less than 2 mm size for the chemical analysis. In a conical flask with 15 ml of water, precisely 15 g of air-dried sediment sample were added. 100 ml acetone was then placed and the flask vigorously shaken for 60 min with the aid of an electric shaker (SR-2W model, Taitec Co. Ltd.). With the aid of a separating funnel containing 100 ml hexane and 600 ml hexane-washed water, the soil solution was filtered. In order to separate entirely the aqueous and the hexane layers, the funnel was vigorously agitated for 15 min and left for about 8hrs. After having discarded the aqueous layer, the hexane layer was washed thrice with 100 ml water. The final solution containing a certain volume of hexane was measured to determine the recovery from initial 100 ml (the recovery value served as a correction factor in calculation process).

With Kuderna–Danish (KD) apparatus, the solution was concentrated to approximately 10 ml and ultimately to 5 ml using gentle nitrogen stream. In a bid to achieve the removal of pigment, humic acids and other organic interferences, equal volume of concentrated H_2SO_4 was added to this solution. This step was done repetitively until the hexane layer reached transparency. Hexane-washed water was used to additionally wash the resulting solution three times. 4 ml of the resulting solution was used for GPC cleanup. Florisil column chromatography was then carried

out as described earlier [51]. Sulfur-containing substances were removed from the final solution after being treated with activated copper. This step was successful with the use of variable strings of copper wires activated by HCl, placed into the solution and maintained for an hour until no black sulfur soot was visible on the copper strings.

The final solution was concentrated to approximately 20 times the initial volume, if necessary, before quantification using GC/ECD. Recovery rates (85%–110%) were obtained for nearly all compounds in the solution. The results were maintained for recovery rates. Highly variable residue levels for DDTs were observed among sampling sites from less than 0.01 to 110 ng/g dry wt. Similar to the distribution, concentrations of PCBs were noticed to be high at sampling sites near urban areas (e.g. NK-SE, CC-1, CC-4, CC-7) but declined downstream. At sampling sites near Long Xuyen town and Can Tho city, DDTs levels were one to two orders of magnitude higher than those from their corresponding downstream sites, the sediment at Hau-1 site in 2004 were exclusive. In the rainy season of 2004, sediment collected at this site showed very high DDTs compared to those in the dry season of 2003.Furthermore, this sample possessed particularly extreme proportion of pp′-DDT that probably implies recent addition of DDT to the river. DDTs might have been transported by storm water from diverse sources including agricultural lands or municipal areas which are disinfected for hygiene purposes and vector control into the river.

The main urban center of the Lower Amazon is Located at the confluent of the Tapajós and the Amazon Rivers in the State of Pará, Santarém (2° 25’ S, 54° 43’ W). The region was chosen for a multitude of reasons. First and foremost, important agricultural activities are predominantly

concentrated around Santarém [52]. Secondly, the region consists of one of the highest concentrations of várzeas of the Brazilian Amazon. In finality, this region harbours the largest fishing port of the Lower Amazon; hence Santarém receives the catches from 14 municipalities along the Amazon River [51, 53].This therefore lends it practically feasibly to collect specimens at Santarém's fish markets which are fairly representative of the entire region under consideration.

The FDA methods are fairly modified to suitably fit our needs. Here, we briefly illustrate the few modifications made. In the extraction step, a mixture of 60 g anhydrous sodium sulfate (Na_2SO_4) and 30 g (wet weight) of fish filet were grounded. The quantity of fish was determined in line with fat content [54]. This was intended to reduce load on the cleanup column retention capacity. Kuderna-Danish concentrator recommended by the FDA was not employed in the concentration step as recommended by FDA but rather we used the rotary evaporation system as it gave better results than the former device. The eluants "2" and "3" (50% methylene chloride, 0,35% acetonitrile, 49,65% hexane (v/v/v) and 50% methylene chloride, 1,5% acetonitrile, 48,5% hexane (v/v/v) respectively were used however, the methyl chloride elution system was strictly followed in the cleanup step. The eluant "1" proposed by FDA was virtually useless in our case as the analytes were collected via these two eluants. All reagents were tested for contamination beforehand and consisted essentially of ACS grade. Prior to analysis, cleaned extracts were accordingly concentrated to 2 ml. OPI analyses were carried out with a Varian

Table 4. Statistical parameters for whole sample and for each species

Group	Size n	Insecticide	Range (ppb)	Median (ppb)	Mean (ppb)	Std dev. (± ppb)	Occurrence[a] (%)
whole sample	120	chlorpyrifos	0,1 - 1,4	0,1	0,3	0,3	59,2
		malathion	0,0 - 1,0	0,1	0,1	0,1	25,8
		methyl-parathion	0,1 - 2,1	0,2	0,3	0,3	68,3
C. monoculus	15	chlorpyrifos	0,1 - 1,0	0,1	0,2	0,3	60,0
		malathion	0,0 - 0,2	0,1	0,1	0,1	13,3
		methyl-parathion	0,1 - 0,4	0,2	0,2	0,1	53,3
C. macropomum	15	chlorpyrifos	0,1 - 1,1	0,2	0,3	0,3	66,7
		malathion	0,0 - 0,7	0,1	0,1	0,2	40,0
		methyl-parathion	0,1 - 0,5	0,3	0,3	0,1	86,7
L. pardalis	15	chlorpyrifos	0,1 - 0,9	0,1	0,2	0,2	46,7
		malathion	< DL - 0,4	< DL	< DL	0,1	13,3
		methyl-parathion	0,1 - 0,7	0,2	0,2	0,2	80,0
M. duriventre	15	chlorpyrifos	0,1 - 0,6	0,1	0,2	0,2	60,0
		malathion	< DL - 0,2	0,1	0,1	0,1	6,7
		methyl-parathion	0,1 - 0,7	0,3	0,3	0,2	60,0
P. flavipinnis	15	chlorpyrifos	0,2 - 1,4	0,4	0,5	0,3	80,0
		malathion	0,1 - 1,0	0,1	0,2	0,2	40,0
		methyl-parathion	0,4 - 2,1	0,8	0,9	0,5	100,0
P. squamosissimus	15	chlorpyrifos	0,1 - 1,4	0,2	0,3	0,3	66,7
		malathion	< DL - 0,2	0,1	0,1	0,1	33,7
		methyl-parathion	0,1 - 0,4	0,2	0,2	0,1	53,3
P. nattereri	15	chlorpyrifos	0,1 - 0,3	0,1	0,1	0,1	46,7
		malathion	< DL - 0,3	0,1	0,1	0,1	6,7
		methyl-parathion	0,1 - 0,5	0,2	0,2	0,1	60,0
S. fasciatum	15	chlorpyrifos	0,1 - 1,1	0,1	0,2	0,3	53,3
		malathion	< DL - 0,3	0,1	0,1	0,1	53,3
		methyl-parathion	0,1 - 0,4	0,2	0,2	0,1	53,3

a: Determines the ratio of specimens in which the compound has been positively detected.
< DL : Smaller than the detection limit

The annual rainfall at the time of the study between 1994 and 1995 was 890.3 and 936.7mm in respectively. This is not controversial as Reconquista river is located in a subtropical region. It is situated in the NW of the Great Buenos Aires (surrounding area of Buenos Aires city) (348410S; 598240W) and is basically a lowland watercourse as shown in Fig. 1. It takes its confluence in Durazno and La Choza streams and ultimately unites with the Luja´n river which flows into the river Plate. It receives 82 small tributaries after flowing for about 55km. Low flow rates (69-103 to 17-105m^3 day^{-1}) are characteristic of Reconquista river. It possesses a variable width between 4 to 14m in S1 and up to 25m in S3. At S1, its depth is 0.5–1.0m and approximately 2.5m in S3. Its basin (1670km^2), is recognized to limit with Matanza river's basin at South and West and with Luja´n river's basin at North and West. The study site harbours over 10 000 industries with a population of about 3.5 million people. It stretches from the upstream boundary (S1) which is primarily agricultural based (72 000 ha), down to Reconquista river mouth in an urban and industrial area (S2–S3) (95 000 ha). The Moro´ n creek (16km long; mean flow of 78-103m^3 day^{-1}) represents its most contaminated tributary stream. Wastewater discharges from residences, cold-storage plants, textile and chemical factories are received by Moro'n creek thus simply adding pollutants to the river [55].

In the extraction process, water samples were sequentially obtained thrice with 25ml hexane each time. Anhydrous sodium sulfate together the extract was dried and concentrated down to 10mL with the aid of Kuderna Danish concentrators. Chromatographic column packed with florisil was used as cleanup system. The column was previously activated for 3 h in an oven at 1308^oC, and anhydrous sodium sulfate (all rinsed with petroleum ether). The extract was injected into the

column. After elution with 6, 15, 50% ethyl ether in petroleum ether, three separate fractions were realized. Maximal flux rate of elution was $5mlmin^{-1}$. Pesticide grade solvents were used entirely throughout. Prior to injection of extracts into the gas chromatographic system for identification and quantification of the pesticides, each eluate was evaporated to yield the corresponding extracts.

Table 5. Concentration ranges of detected pesticides and maximum permitted quantities (MPQ) for protection of aquatic life in superficial fresh water (Agentine Dangerous Law No. 24051/93)

Pesticide	Concentration range ($\mu g\ L^{-1}$)	MPQ ($\mu g\ L^{-1}$)
DDT (sum of isomers)	ND[a]-0.4	0.001
op' DDE	ND[a]-0.1	—
α-HCH	ND[a]-1.1	—
β-HCH	ND[a]-1.3	—
γ-HCH	ND-4.9	0.01
Heptachlor	ND-0.4	0.01[b]
Chlordane (sum of isomers)	ND-0.3	0.006
Endosulphan-II	ND	0.02
Aldrin	ND	0.004
Dieldrin	ND	0.004
Endrin	ND	0.0023

[a] ND: below the detection limit.
[b] Heptachlor + heptachlor epoxide.

CHAPTER 3

MATERIALS AND METHODS

3.1 Methodology for OCPs and OPPs Determination

3.1.1 Sampling

3.1.1.1 Sampling Area

There four sampling areas that were identified to carry out the determination of OCPs and OPPs. These study areas include coastal areas and agricultural areas to assess the difference concentrations of theses pollutants in different sources. Sampling has included as well sea water, sediment and groundwater. Biotas were also collected from different market to be included in the study. The coastal areas are situated in the Eastern province of Saudi Arabia, with Jubail at the northern end and Tarut Bay at the southern end of the study area. The area is home to commercial fishing activities with small to medium, as well as agricultural activities. Household runoff is also collected in these areas. Nine stations were sampled from different locations within these two areas. The samples included eight sea water samples and seven marine sediment samples. The other two sampling areas are located in Tarut Bay and Al-Oyoon agricultural areas. Twelve(12) freshwater samples from different wells and farms have been collected. The maps of these two sampling areas are provided below:

Fig. 7 Sampling Area of OCPs and OPPs in Jubail Coastal Area

Fig. 8 Sampling Area of OCPs and OPPs in Tarut Bay

Fig.9 Sampling Area of Underground water in Tarut Island

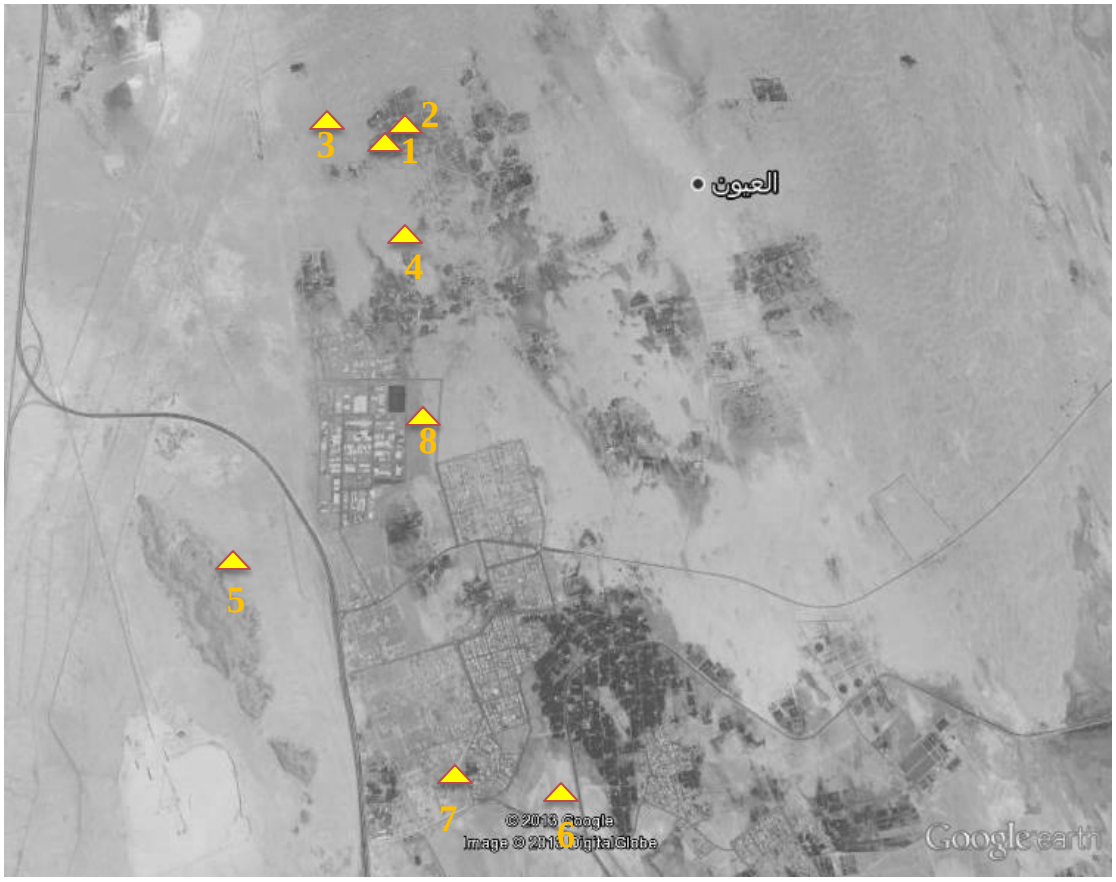


Fig.10 Sampling Area of Subsurface water in Al-Oyoon Agricultural Area

Table 6. Sampling areas for Underground water samples

Sample Name	Coordinates	pH	TDS (mg/L)
TGW1	**26°35'12.96"N, 50° 3'19.57"E**	**7.30**	**3990**
TGW2	**26°35'2.39"N, 50° 3'44.76"E**	**7.50**	**2640**
TGW3	**26°34'8.08"N, 50° 5'10.61"E**	**7.00**	**7850**
TGW4	**26°34'24.09"N, 50° 3'17.71"E**	**6.90**	**6860**
OGW1	**26° 39' 35.25"N, 49° 33' 45.032"E**	**7.80**	**1930**
OGW2	**25° 39' 33.71 "N , 49° 33' 44.88"E**	**7.73**	**1921**
OGW3	**25° 39' 37.55"N, 49° 33' 14.39 "E**	**7.86**	**2440**
OGW4	**25° 38' 53.51"N, 49° 33' 42.66"E**	**7.88**	**1994**
OGW5	**25° 36' 37.50 "N, 49° 32' 38.22"E**	**7.85**	**2180**
OGW6	**25° 35' 18.85 "N, 49° 34' 37.64"E**	**7.93**	**3370**
OGW7	**25° 35' 26.63 "N, 49° 33' 52.79 "E**	**7.93**	**2760**
OGW8	**25° 37' 37.53"N, 49° 33' 53.49"E**	**7.64**	**3310**

Table 7 Sampling areas for marine samples

Area	Coordinates
TSW3	26° 31' 38.139", 50° 11' 51.846"
TS7	26° 28' 50.5698", 50° 14' 0.078"
TSW16	26° 32' 1.086", 50° 5' 22.5162"
TSW17 & TS17	26° 32' 5.2332", 50° 3' 23.8644"
TSW18 & TS18	26° 32' 41.1678", 50° 1' 59.8182"
JSW23	27° 3' 25.6962", 49° 37' 23.4624"
JSW24	27° 4' 0.3684", 49° 38' 23.4054"
JSW25	27° 4' 21.2808", 49° 39' 44.3628"
JSW26	27° 4' 21.2808", 49° 41' 33.7446"

3.1.1.2 Sampling Procedure

Water samples were collected in 1 L Teflon jars in accordance with USEPA surface water sampling SOP (EPA, 1991). Similarly, sediments were sampled following the USEPA procedure for soil, water and solid waste sampling (EPA, 2004). 300 g grab sediment samples were collected in Teflon bottles from each sampled location.

Undergournd water samples were collected after 10 minutes of flushing to ensure fresh and representiative samples. Samples were kept away of sun in a temperature of about 10 ^{o}C.

Biota samples were collected form AlKhobar Fish Market from different species and kept frozen until the time of pretreatment step.

3.1.2 Sample Pretreatment

Because of the low concentrations of the toxic compounds in the environmental samples and the complexity of the matrix, direct analysis of them will show no result. Samples have to be pretreated first to pre-concentrate the components of interest. There are different methods for extracting pollutants from different environmental samples.

Solid-phase Extraction (SPE)

Solid phase extraction was known for the first time in mid-1970. Solid phase extraction is the most common used technique in the sample treatment. It has some advantages among the others such as high recovery, high enrichment factor and short extraction time [56]. The main component of the SPE method is the sorbent material where the environmental sample is passed by the cartridge of the SPE and the target components are selectivity adsorbed. The most commonly used sorbents are C18 silica and graphite carbon.

Ultrasound-assisted Extraction (UAE)

The use of ultrasound energy in the extraction techniques was found to be very helpful. It allows more contact between the solvent and the sample and accelerates the extraction of the target components. More than 233 papers were published in the field of Ultrasound assisted extraction between 2004 and 2007 [57].This technique is commonly used to extract organic compounds from solids. The solid sample is extracted by a suitable extraction solvent and ultrasound energy is applied to it. The bubbles generated by the energy has positive chemical and mechanical effects and allow efficient contact between the solid and the solvent, more extraction and less time [58].

3.1.2.1 Extraction of Water Samples

Solid-phase Extraction (SPE) Method for Sea Water Samples:

1- C18 SPE discs with 47mm Nu-phase fibers (CPI International, USA) were conditioned with deionized water and deployed for the extraction of water samples.

2- One liter of each water sample was washed through the discs via an Ultra ware glass cup.

3- The set was powered by Edwards High Vacuum (B.O.C. Ltd., Crawley, UK).

4- Adsorbed compounds were then eluted by immersing the SPE discs into a beaker with 15 ml of each cyclohexane and acetone and put into the sonication bath. The SPE disc is used only once and then discarded.

5- The mixture was agitated for 30 min at 150 rpm on a Lab Companion Shaker (model SK-600, GEOL Tech, Korea) to accelerate the extraction.

6- After the extraction, traces of moisture were removed by addition of pinches of anhydrous sodium sulfate.

7- Extracts were further pre-concentrated to 1 mL by allowing the solvent for dryness overnight and finally adding 1 ml of hexane. The preconcentrated mixture was transferred into 1 mL plastic vial and 2 μL of the sample was injected into the GC-MS for analysis

3.1.2.2 Extraction of Marine Sediment

Ultra-sound Assisted Extraction (UAE) Method for Sediment Samples:

1- 5 g of each sediment sample was taken in Erlenmeyer flask and 15 mL of cyclohexane and 15 ml of acetone were added and stoppered.

2- The mixture was agitated for 30 min at 150 rpm on a Lab Companion Shaker (model SK-600, GEOL Tech, Korea) to accelerate the extraction.

3- After the extraction, traces of moisture were removed by addition of pinches of anhydrous sodium sulfate.

4- Extracts were further pre-concentrated to 1 mL by allowing the solvent for dryness overnight and finally adding 1 ml of hexane. The preconcentrated mixture was transferred into 1 mL plastic vial and 2 μL of the sample was injected into the GC-MS for analysis.

3.1.2.3 Extraction of Biota

Sample Preparation and Extraction Process for Biota Samples

Biota samples used for analysis in the proposed research was selected from AlKhobar Local Fish Market. The samples were frozen until dissection. A portion of the body part (5 g) was cut and taken to the pretreatment step.

25 mL of acetone and 25 ml cyclohexane was added to the weighed body part in a beaker and it was blended for 10 minutes using an electronic hand blender. The mixture samples were filtered and collected in a beaker. 15 ml of each acetone and cyclohexane were added again to the beaker. The beaker was put into the sonication bath for 30 min at 150 rpm on a Lab Companion Shaker (model SK-600, GEOL Tech, Korea).

The mixture was set aside overnight to allow the evaporation of the solvents. 1 ml of hexane was added to the dried beaker and then transferred into a 1 mL plastic vial and 2 μL of the sample was injected into the GC-MS for analysis

.

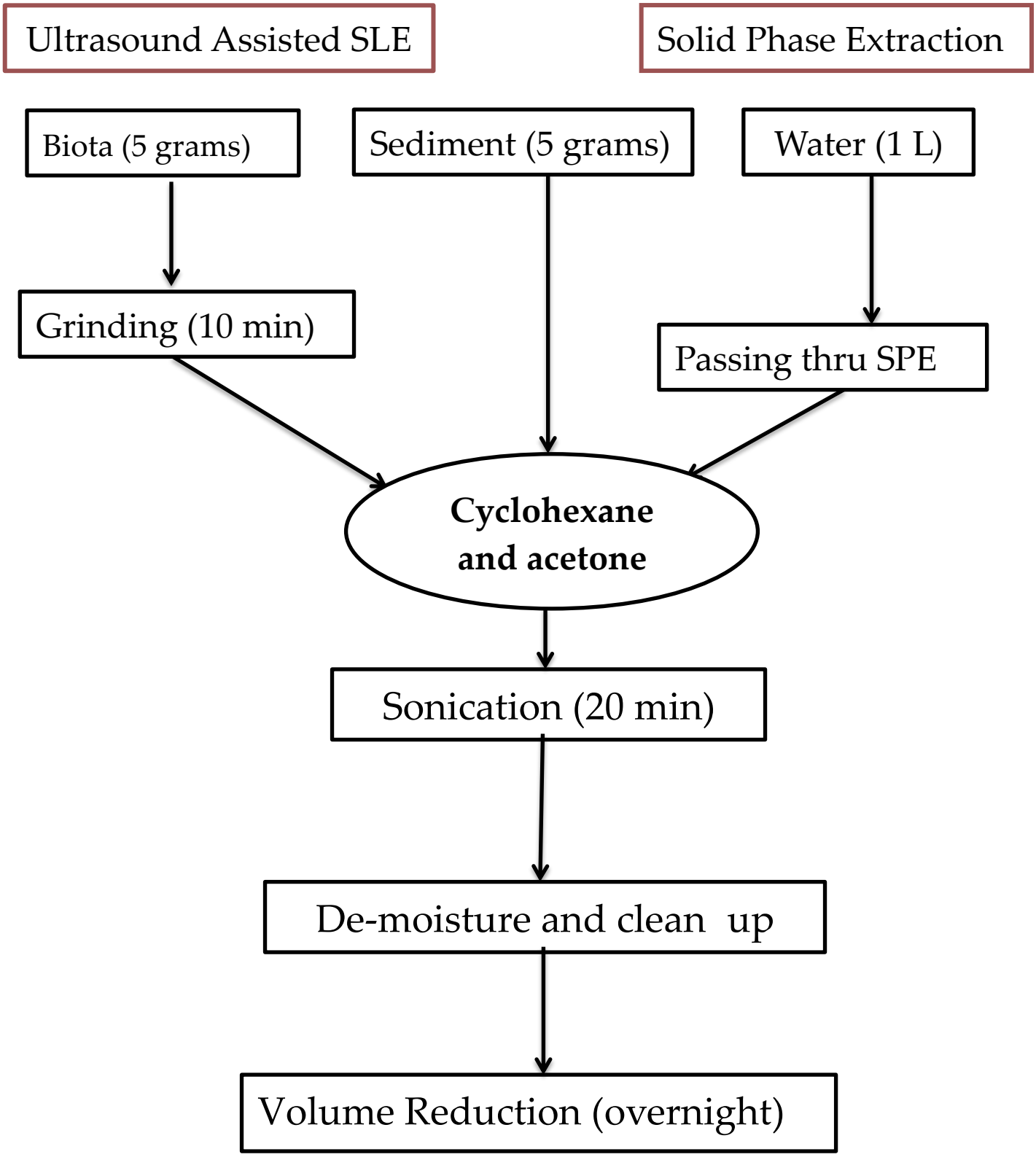
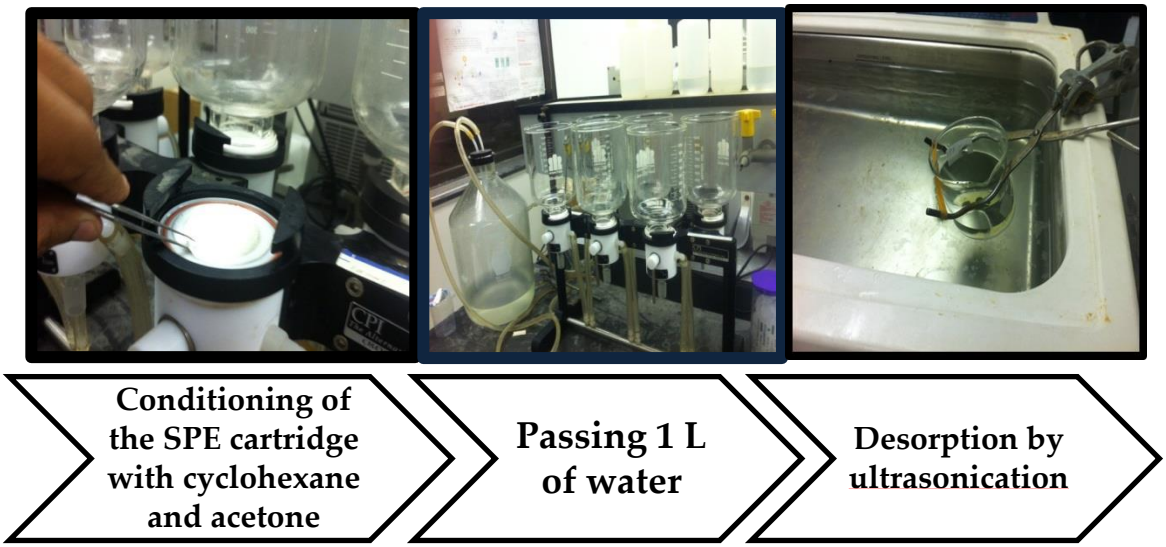


Fig 11. Flow chart of sample extraction and preparation for Biota, Water and Sediments



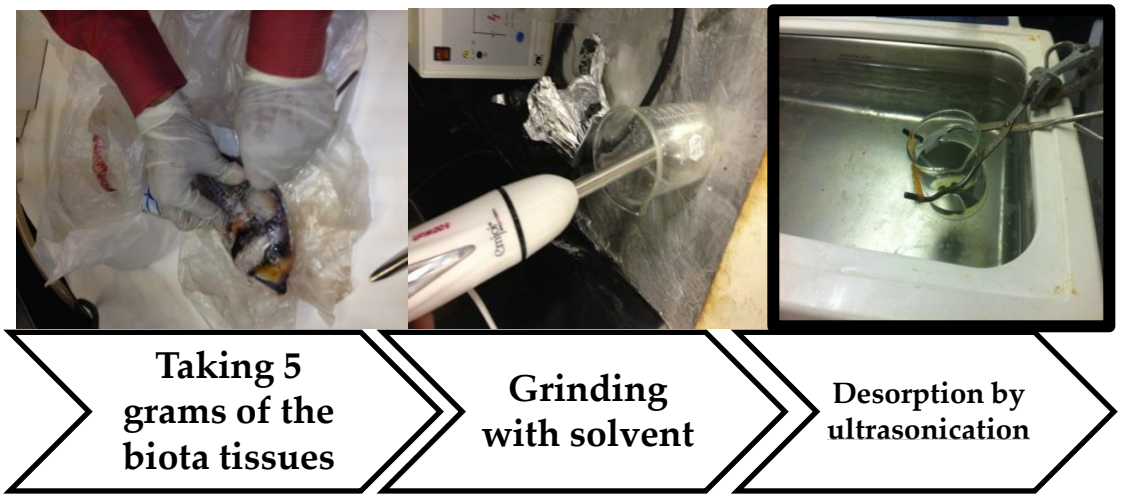


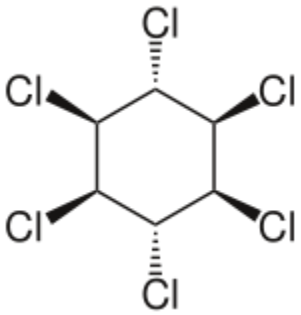
Fig 12. Systematic demonstration of sample extraction processes for Biota

3.1.3 Chromatographic Determination of OPPs and OCPs

3.1.3.1 Target Analytes

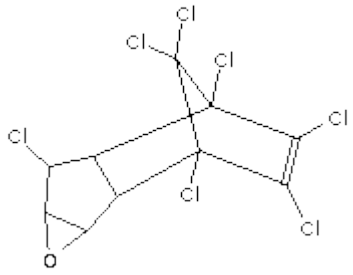
<u>Oragnochlorine Pesticides(OCPs)</u>

1- Alpha-BHC



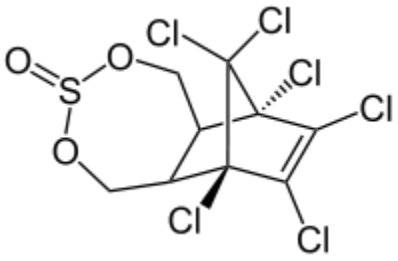
"moderately" acutely toxic. It has an oral LD_{50} of 88 mg/kg in rats and a dermal LD_{50} of 1000 mg/kg

2- Heptachlor Epoxide



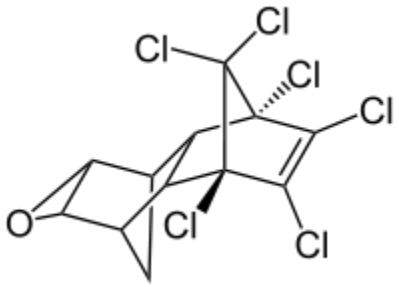
The oral LD_{50} values ranging from 46.5 to 60 mg/kg

3- Endosulfan



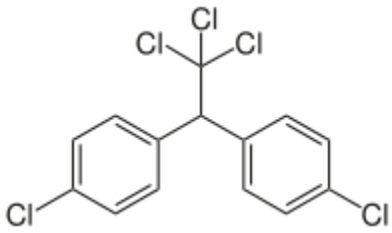
The US EPA classifies it as Category I: "Highly Acutely Toxic" based on a LD_{50} value of 30 mg/kg for female rats, while the World Health Organization classifies it as Class II "Moderately Hazardous" based on a rat LD_{50} of 80 mg/kg.(EPA & WHO)

4- Dieldrin



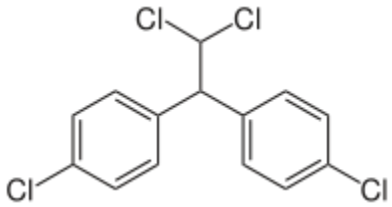
The Acute oral LD50 values in the range of 37 mg/kg body weight in rats to 330 mg/kg in hamsters have been found for dieldrin. (Reported by United Nations Environment Program)

5- DDT



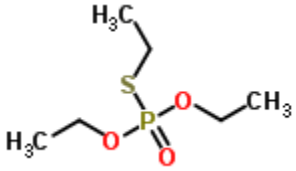
DDT is classified as "moderately toxic" by the United States National Toxicology Program (NTP) and "moderately hazardous" by the World Health Organization (WHO), based on the rat oral LD_{50} of 113 mg/kg

6- DDD



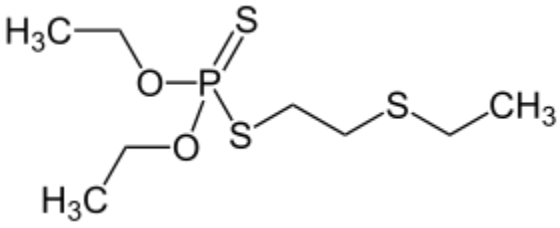
<u>Oraqnophosphorus Pesticides(OCPs)</u>

1- O,O,O-Triethyl Thiophosphate



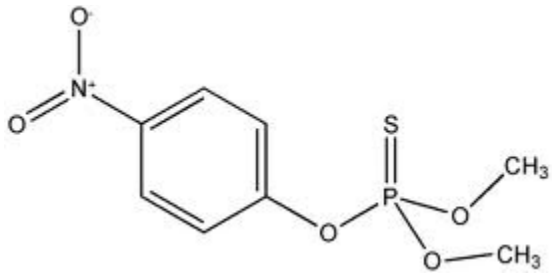
The oral LD_{50} value is 170 mg/kg (Developments in Toxicology and Environtal Science. Vol. 8, Pg. 631, 1980.)

2- Disulfoton



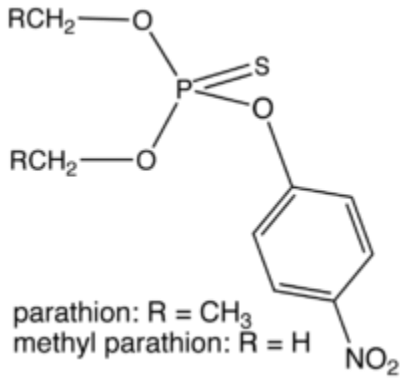
LD_{50} values ranged from 1.9 to 3.2 mg/kg in female rats, 6.2-12.5 mg/kg in male rats (Bombinski and DuBois 1958; Crawford and Anderson 1974; Gaines 1969; Mihail 1978; Pawar and Fawade 1978), 2.7-8.2 mg/kg in female mice, and 5.8-19.3 mg/kg in male mice (Mihail 1978; Pawar and Fawade 1978; Stevens et al. 1972a).

3- Methyl Parathion



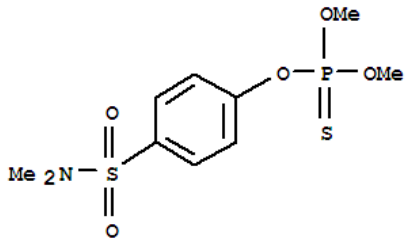
Methyl parathion is highly toxic via the oral route, with reported oral LD_{50} values of 6 to 50 mg/kg in rats, 14.5 to 19.5 mg/kg in mice, 420 mg/kg in rabbits, 1270 mg/kg in guinea pigs and 90 mg/kg in dogs

4- Parathion



The oral LD_{50} for parathion is 2 to 30 mg/kg

5- Famphur



LD_{50} in mammals were determined to be 500 mg/Kg (Organophosphorus Pesticides: Organic and Biological Chemistry," Eto, M., Cleveland, OH, CRC Press, Inc., 1974Vol. -, Pg. 299, 1974)

3.1.3.2 GC-MS Methods

Table 8. GC-MS Methods deployed

• Oven Program 40 °C (5 min), 12 °C/min, 300 °C (10 min)
• Equilibration Time 0.5 min
• Injector Temperature: 250 °C
• Split ratio: 10.0
• Sampling Time: 1.00 m
• Column Flow Helium, 1.49 mL/min (constant flow)
• Pressure: 82.7 kPa
• Transfer Line Temperature 300 °C
• MS Source Temperature 225 °C

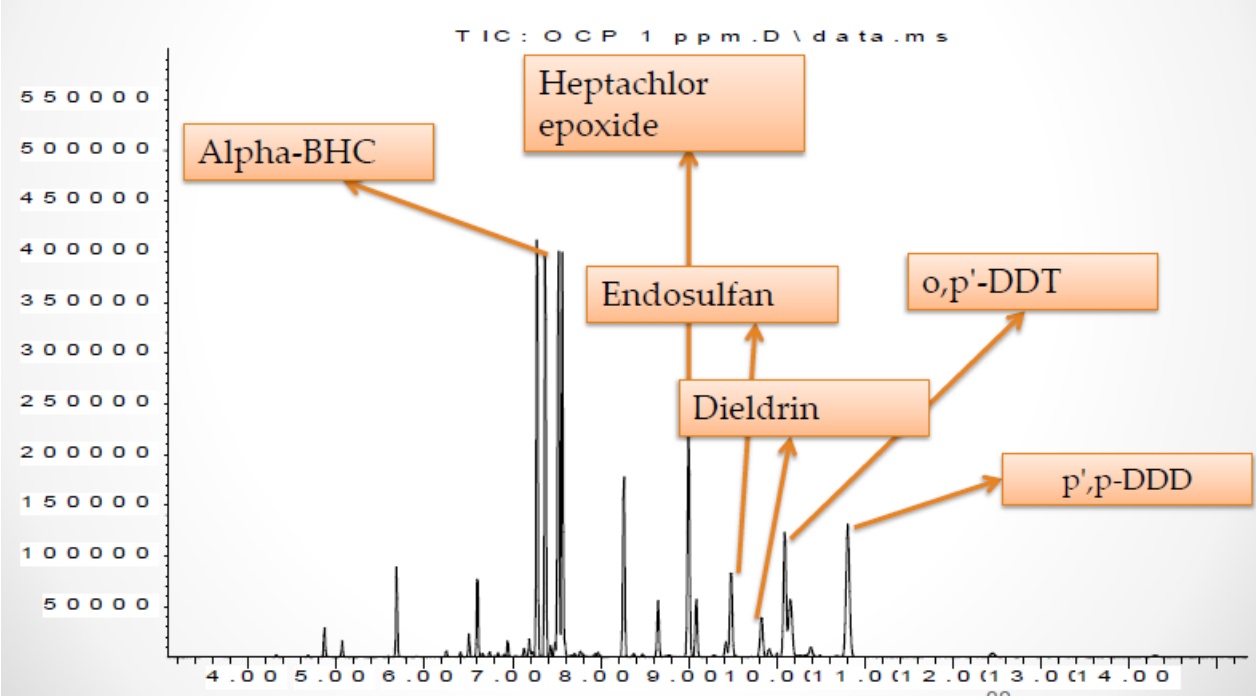


Fig 13. Chromatograms of diverse OCPs detected

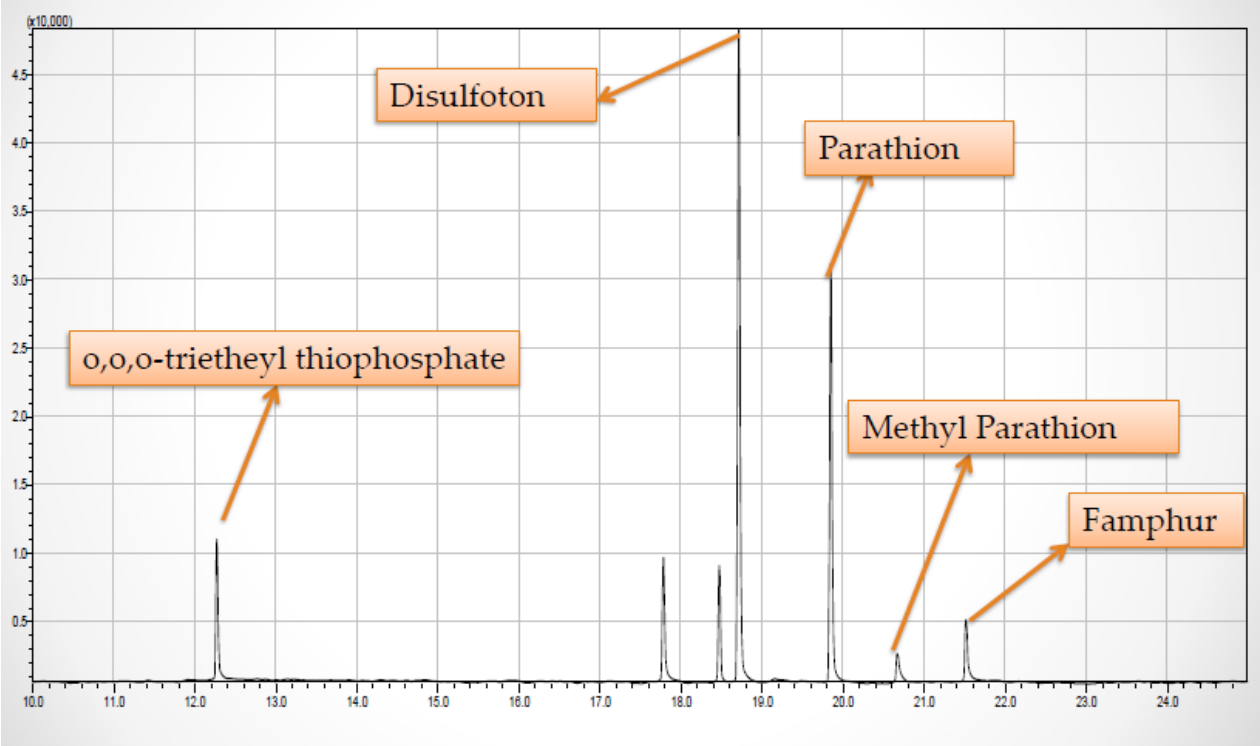


Fig. 14 Chromatograms of various OPPs determined

3.1.3.3 Calibration Curves

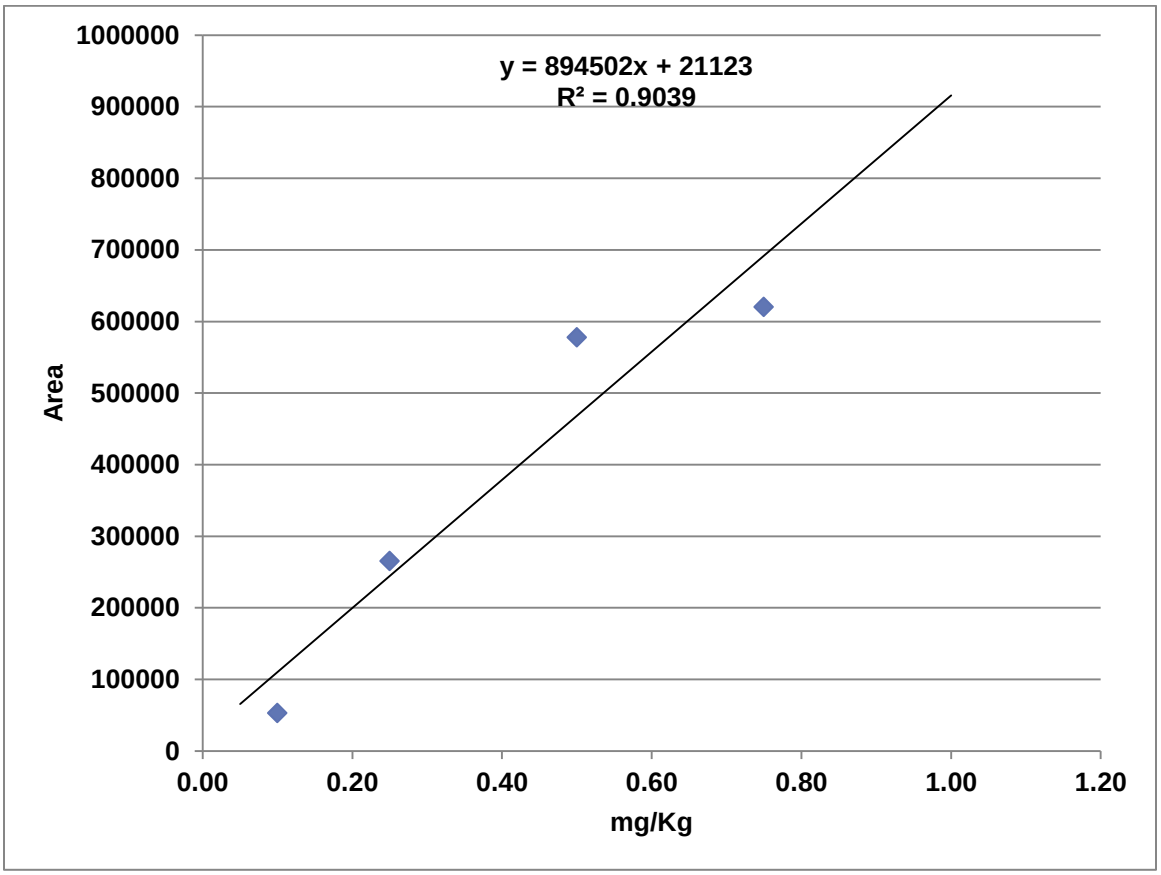


Fig. 15 Heptachlor Epoxide Calibration curve

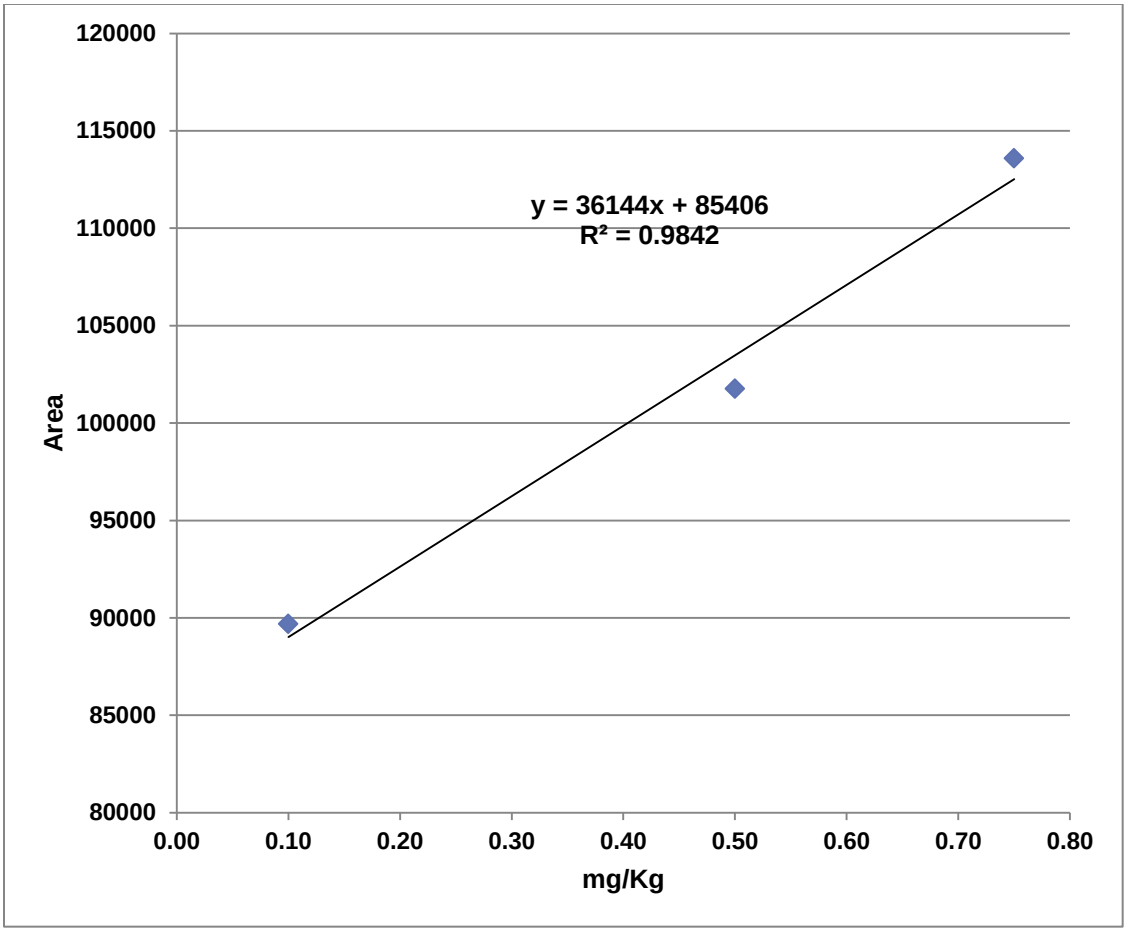


Fig. 16 Dieldrin Calibration curve

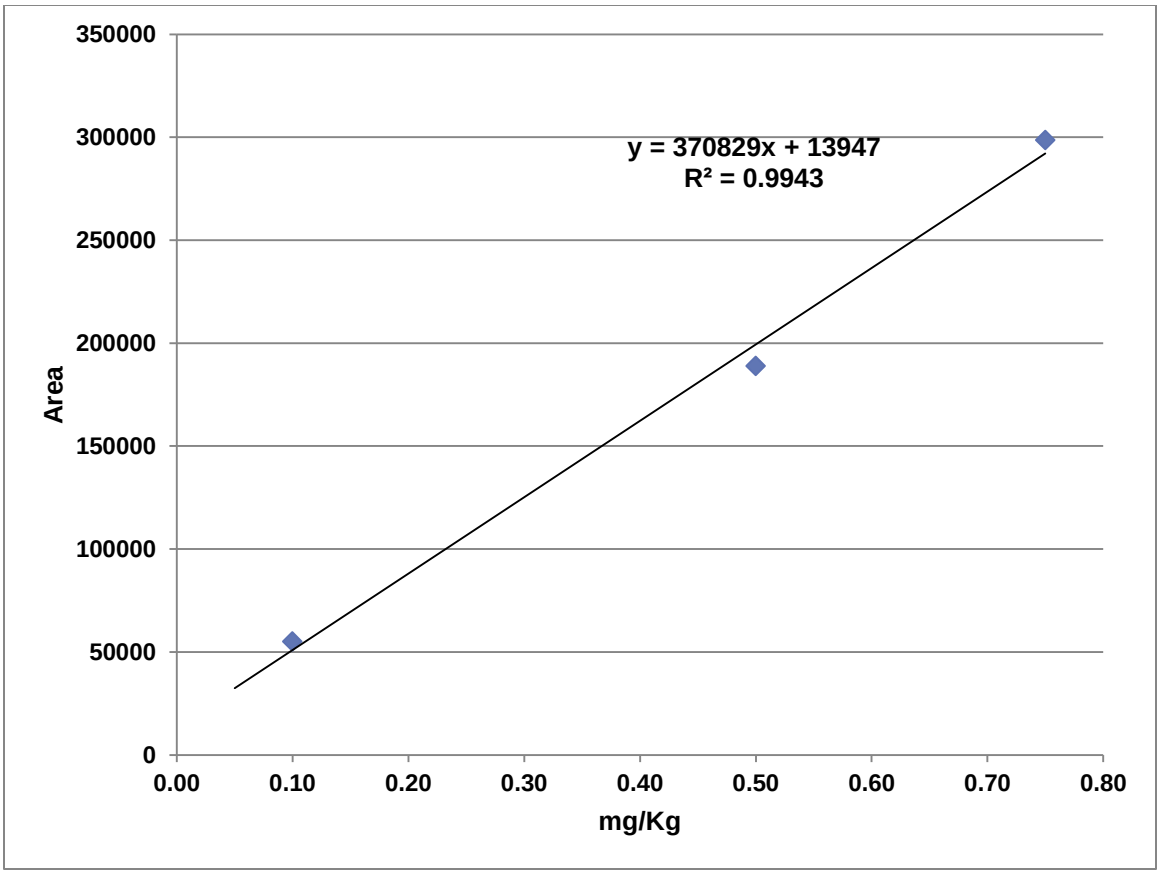


Fig.17 Endosulfan Calibration curve

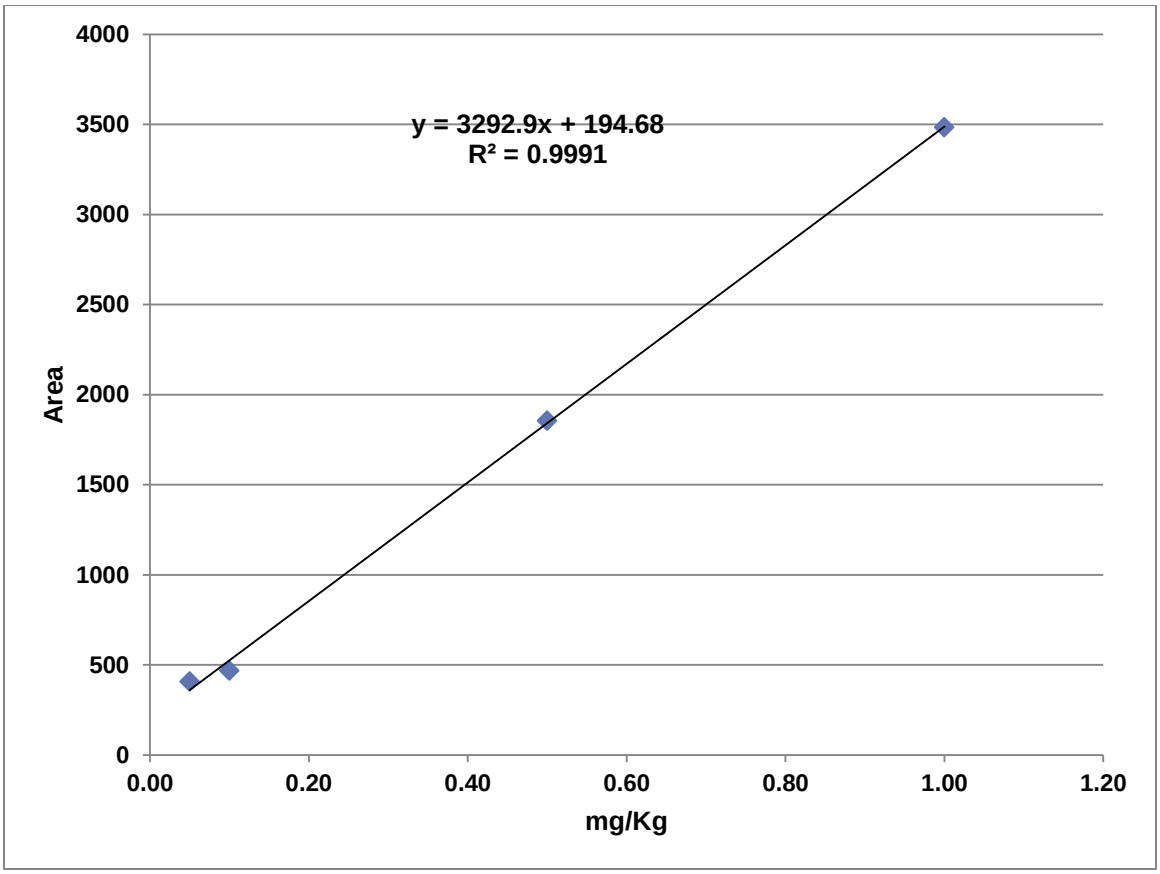


Fig. 18 alpha BHC Calibration curve

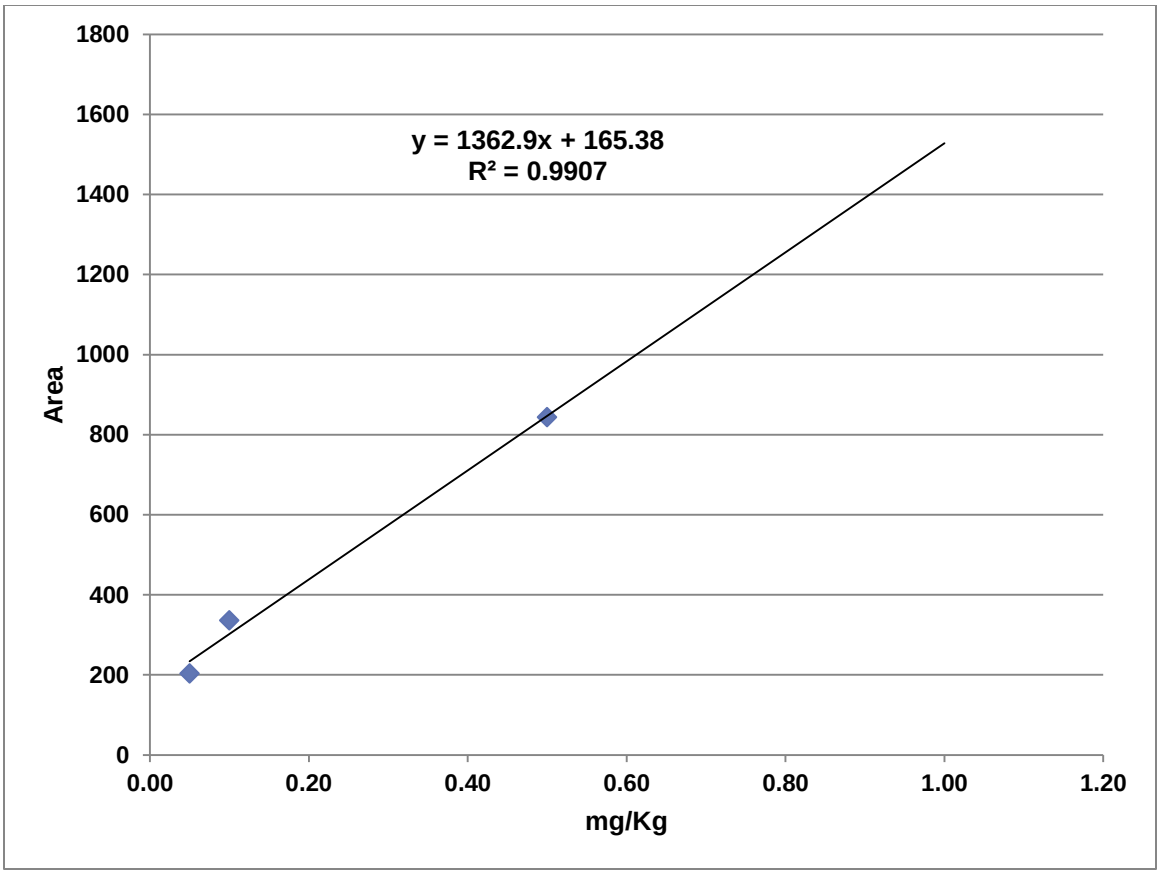


Fig. 19 o,p'- DDT Calibration curve

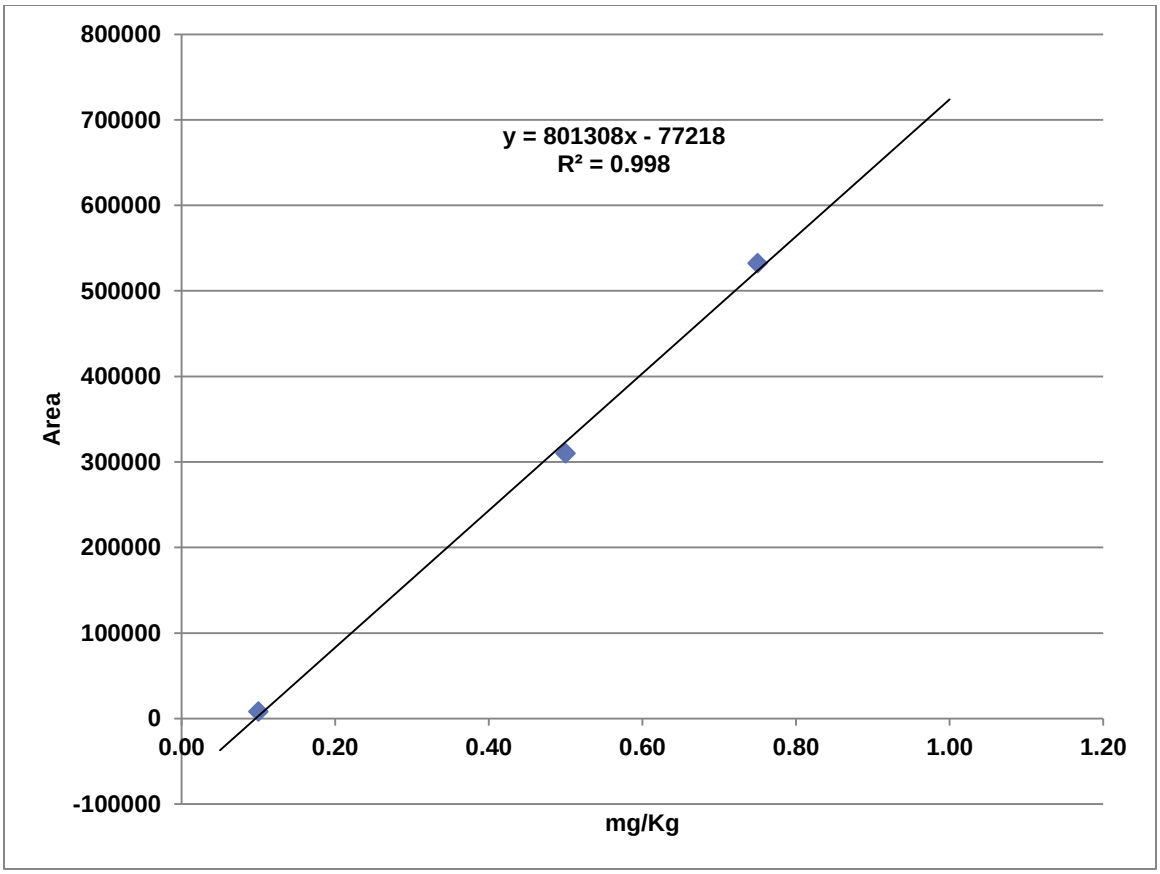


Fig.20 p',p-DDD Calibration curve

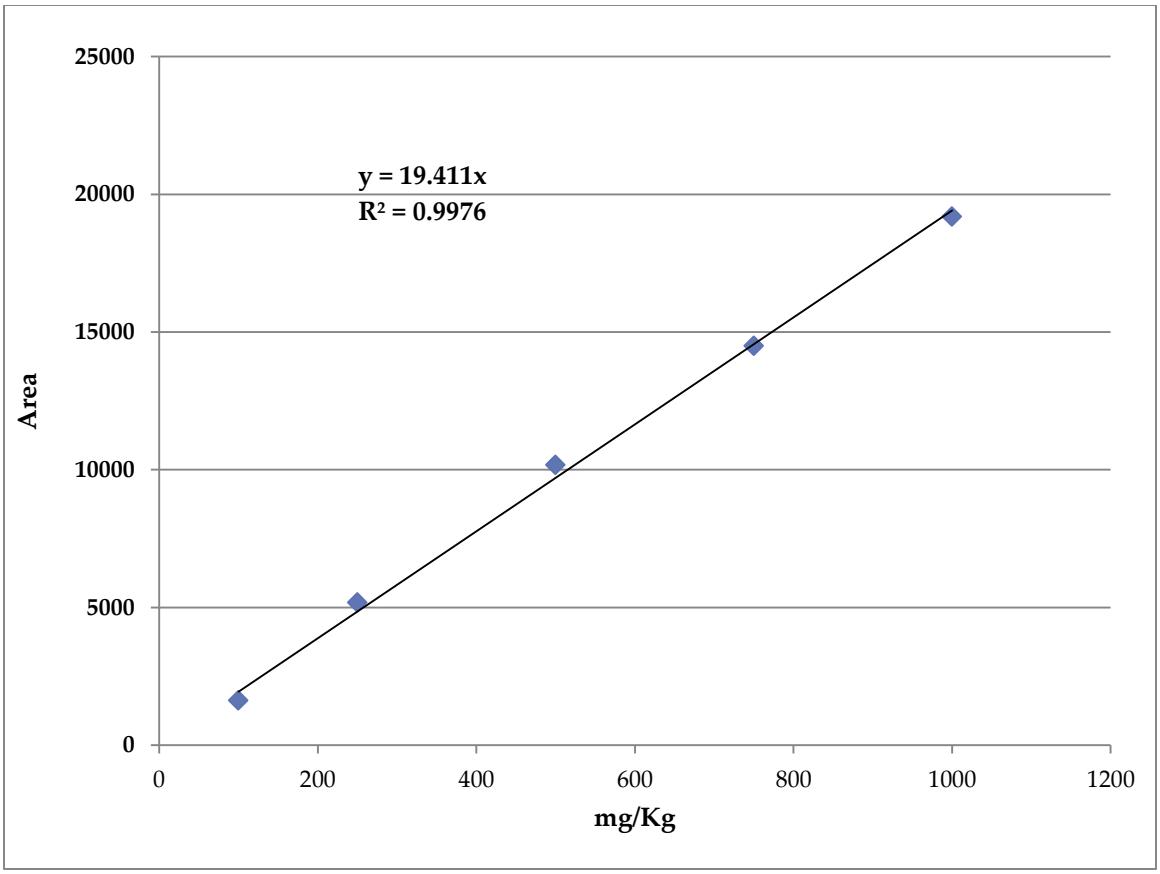


Fig.21 O,O,O-Triethyl thiophosphate Calibration curve

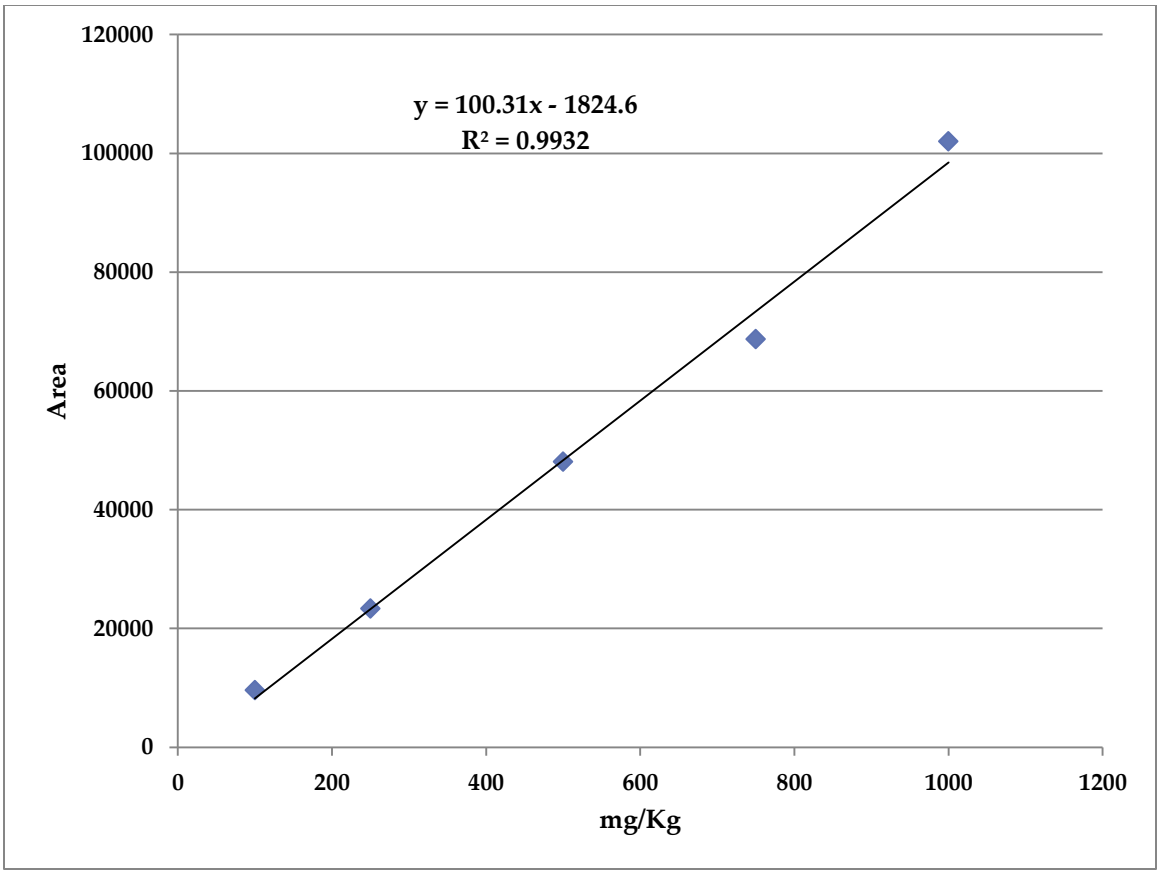


Fig.22 Disulfoton Calibration curve

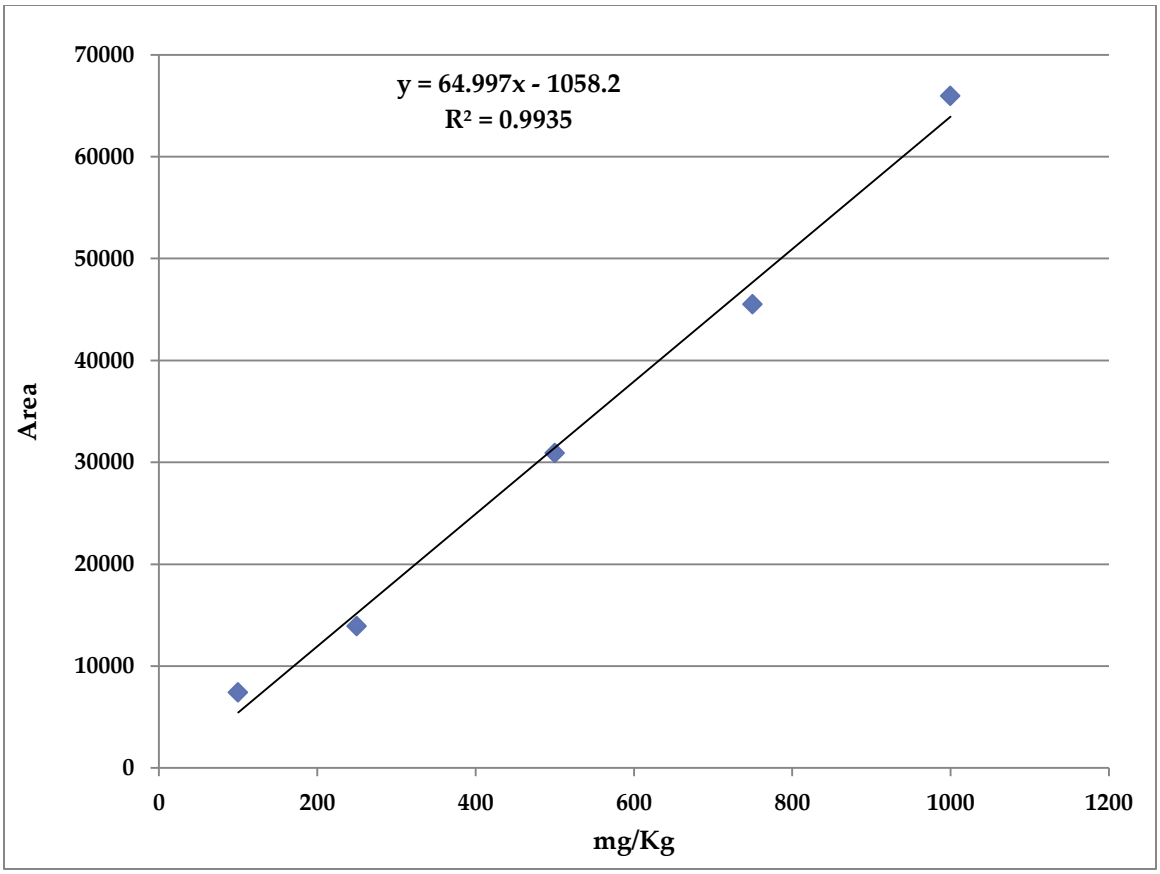


Fig.23 Methyl Parathion Calibration curve

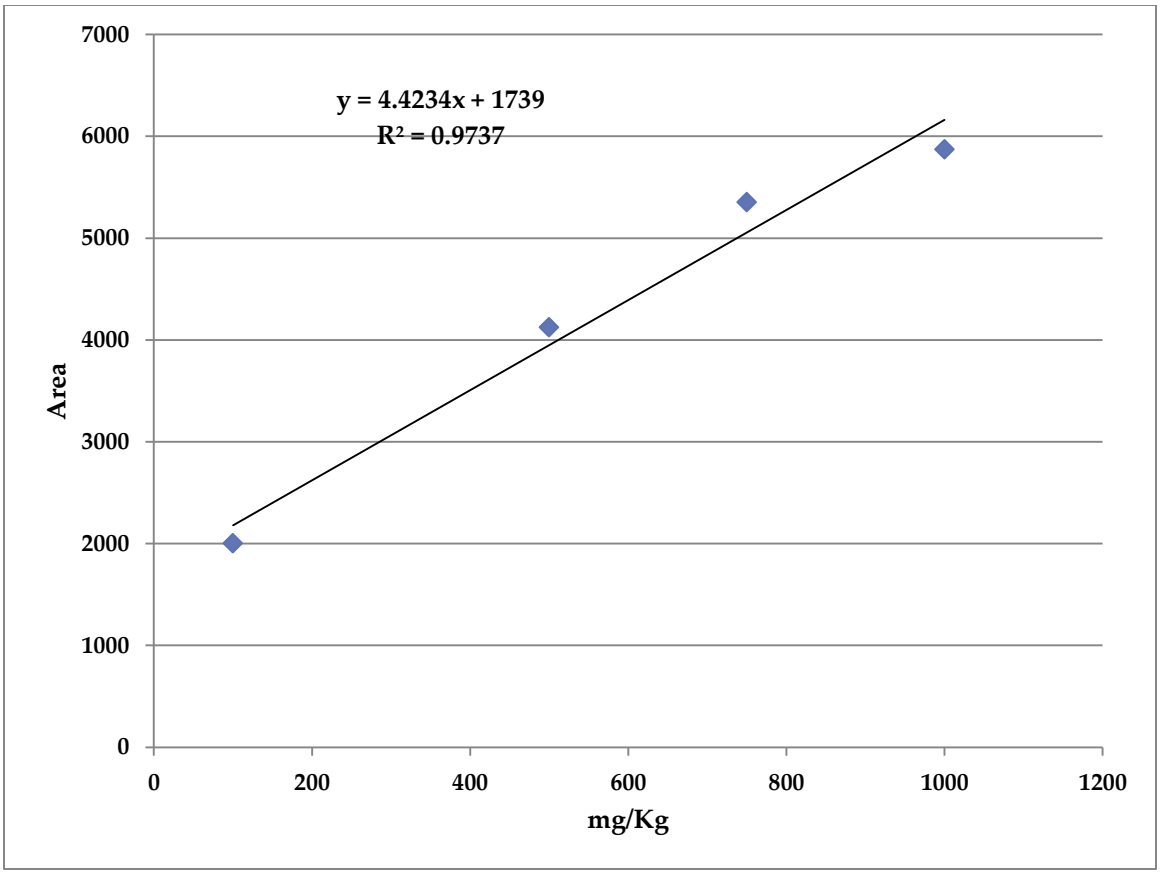


Fig.24 Parathion Calibration curve

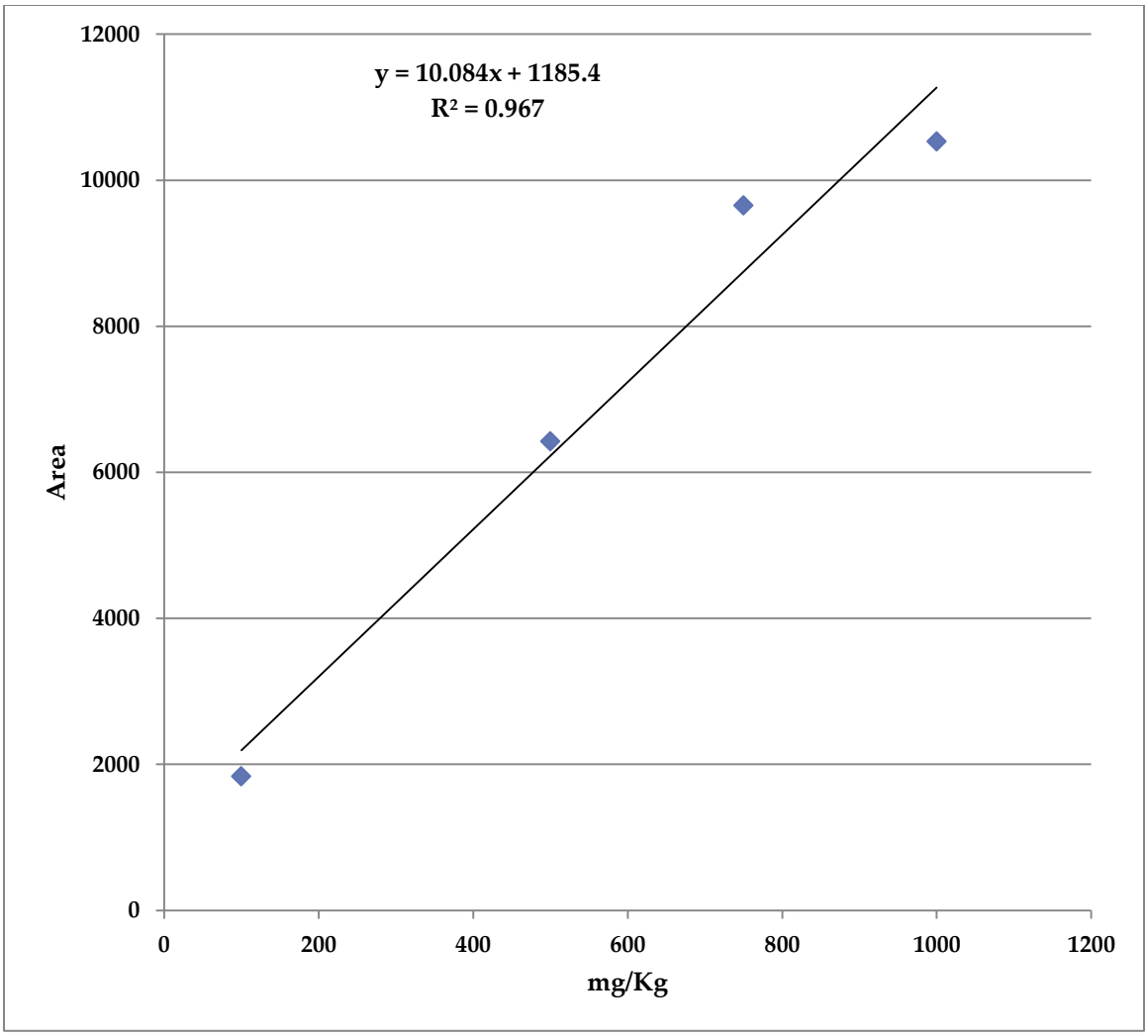


Fig.25 Famphur Calibration curve

3.1.3.4 Method Validation and Recovery

Table 9 Method Validation

Analyte	Linearity (mg L^{-1})	RSD (%, n=3)	LOD (ng L^{-1}, n=3)	LOQ (ng L^{-1}, n=3)	Correlation Cofficient (r^2)
Alpha-BHC	0.1-1.0	15.3	0.010	0.030	0.982
Heptachlor epoxide	0.1-1.0	10.0	0.014	0.043	0.973
Endosulfan	0.1-1.0	14.3	0.028	0.085	0.998
Dieldrin	0.1-1.0	20.7	0.439	1.316	0.995
o,p'-DDT	0.1-1.0	11.0	0.032	0.097	0.987
p,p'-DDT	0.1-1.0	20.3	1.144	3.432	0.998
Triethyl Thiophosphate	0.1-1.0	18.7	0.3926	1.1778	0.998
Disulfoton	0.1-1.0	9.6	0.0664	0.1991	0.993
Methyl Parathion	0.1-1.0	6.3	0.0858	0.2575	0.994
Parathion	0.1-1.0	5.2	0.3175	0.9526	0.974
Famphur	0.1-1.0	2.1	0.3466	1.0398	0.967

Table 10 Method Recovery

Analyte	Recovery (%, n=3)		
	Water	Sediment	Biota
Alpha-BHC	63.49	67.27	79.20
Heptachlor epoxide	79.99	80.29	97.50
Endosulfan	94.26	84.93	93.50
Dieldrin	79.94	86.80	89.10
o,p'-DDT	85.08	73.86	85.60
p,p'-DDT	72.87	68.98	91.80
Triethyl Thiophosphate	82.34	72.34	79.30
Disulfoton	79.30	89.30	69.30
Methyl Parathion	88.30	85.30	84.20
Parathion	77.40	72.80	78.40
Famphur	66.12	62.12	61.40

CHAPTER 4

RESULTS AND DISCUSSION

Table 11 Lethal dosage levels for OCPs and OPPs

Pesticides	LD50 (mg/Kg)
Alpha-BHC	88
Heptachlor Epoxide	46.5-60
Endosulfan	30-80
Dieldrin	37
p,p'-DDT o,p'-DDD	113
Triethyl Thiophosphate	170
Disulfoton	6.5-12.5
Methyl Parathion	6-50
Parathion	2-30
Famphur	500

4.1 Levels of OCPs

4.1.1 Sea Water

Table 12 OCPs levels in Sea Water (mg/l)

#	Name	alpha-BHC	Heptachlor epoxide	Endosulfan	Dieldrin	o,p'-DDD	p,p'-DDT
1	**TSW3**	0.245	0.0191	0.160	0.0145	0.458	0.0853
2	**TSW16**	0.0479	0.0224	0.059	0.0136	0.0424	0.0181
3	**TSW17**	0.0229	0.0167	0.0601	0.0094	0.139	0.0310
4	**TSW18**	0.0179	0.0151	0.0455	0.0066	0.0677	0.00917
5	**JSW23**	0.00932	0.00728	0.0474	0.0091	0.0171	0.0247
6	**JSW24**	0.00374	0.0153	0.0235	0.0225	0.158	0.00315
7	**JSW25**	0.0233	0.00619	0.0644	0.0123	0.0753	0.0285
8	**JSW26**	0.0273	0.0128	0.0444	0.0344	0.0443	0.0116

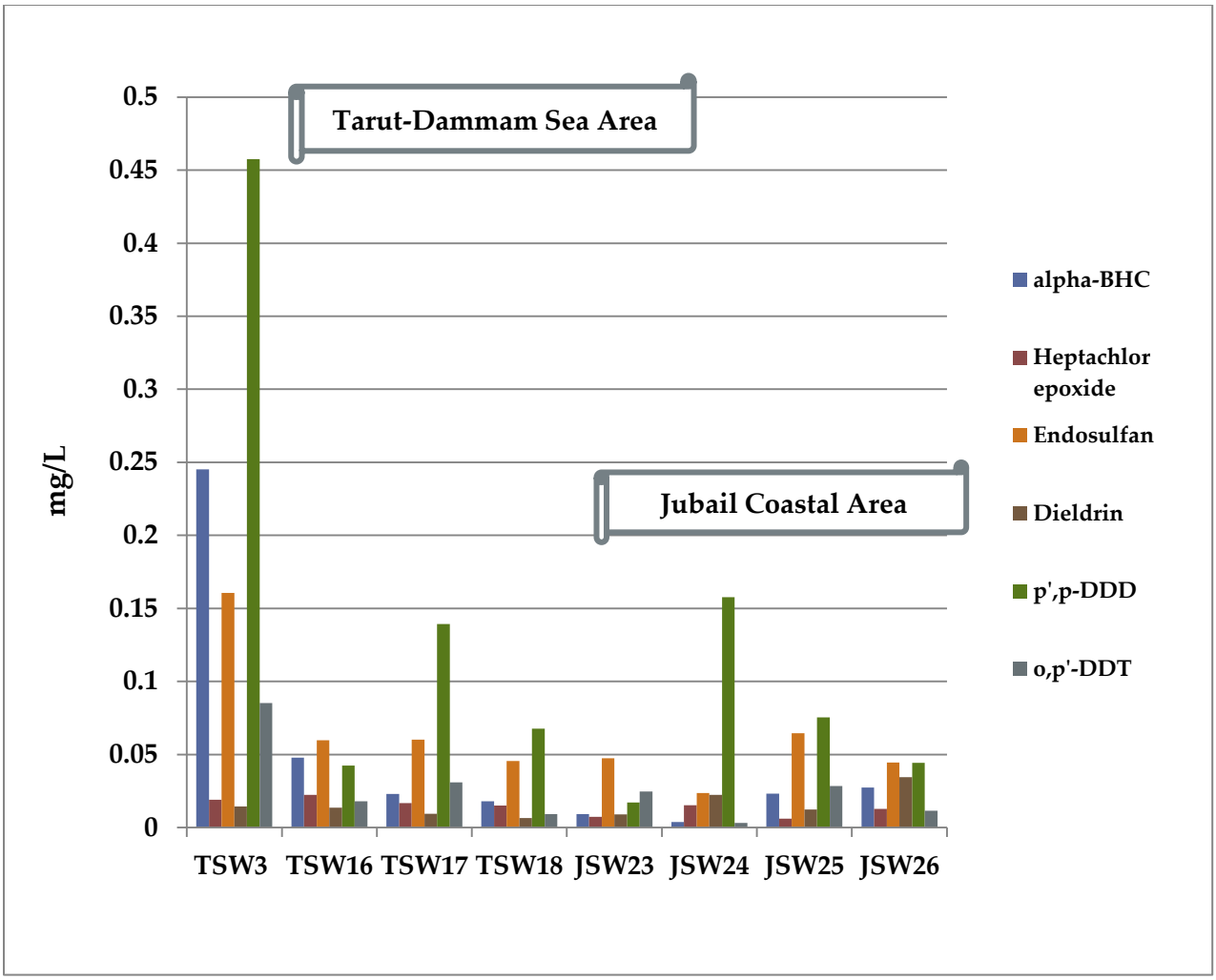


Fig. 26 levels of OCPs in Sea Water samples (mg/L)

Both Fig.26 and Table 12 depicts the concentrations of six Organochlorine Pesticides determined in Sea matrix. The highest concentration of the six Organochlorine pesticides is found in the Tarut Bay samples and particularly in the location TSW3, which is a proximal point to the port. Quantitatively speaking, point TWS3 recorded the highest total concentration of pesticides corresponding to 0.982 mg/l, with the maximum and minimum values being 0.458 mg/l and 0.0145 mg/l respectively. Point JSW23 in Jubail elicited the lowest total concentration of OCPs equivalent to 0.11486 mg/l, with maximum and minimum levels being 0.0474 mg/l and 0.00728 mg/l respectively. In the Jubail Coastal area, the highest total amount of OCPs was determined at point JSW24 corresponding to 0.226 mg/l. Invariably, all the six pesticides were detected in all the sea water samples in Tarut Bay and Jubail areas. Quantitatively and collectively, the level of OCPs in Tarut Bay was reckoned at 1.62797 mg/l with the average being 0.407 mg/l. Jubail coastal area recorded the least level with total and average values reading 0.726 mg/l and 0.181 mg/l respectively. In terms of segregated individual pesticide detections, the highest concentration of 0.458 mg/l, was observed for o,p'-DDD in TSW3 sample. Contrarily, the smallest concentration of 0.04241 mg/l o-p'-DDD was found in Tarut Bay at point TWS16. Similarly, the greater concentration of o,p-DDT is noticed at TWS3 to be 0.0853 mg/l, with 0.00917 mg/l being the minimum level. DDD which is a metabolite of DDT was detected in all samples and had the highest average and concentrations among the other pesticides. The average of DDD in all samples is 0.125 mg/l and the highest concentration is 0.458 mg/l. p,p'-DDT was quantified in all samples with lower average than DDD. Comparatively, at each point, the levels of o,p'-DDD appear to be several orders of magnitude greater than levels of p-p'-DDT. The observed trend is however not paradoxical apparently because p,p'-DDT presumably undergoes decomposition and transformation ultimately precipitating the metabolite o,p'-DDD in the absence of influx of new

p,p’-DDT into the environment. Heptachlor epoxide was identified in all the samples with an average of 0.0144mg/l. The highest concentration was found in TSW16 in Tarut Bay, lowest level of 0.00619 mg/l was determined at JSW25. Endosulfan and dieldrin were also quantified in all samples and the highest concentrations were 0.161 and 0.0344 mg/l, respectively.The average of both of them being 0.0632 and 0.0163 mg/l. One striking observation is that OCPs were not determined in Fresh Water samples at both Tarut Bay and Al-Oyoon Agricultural Areas.

4.1.2 Marine Sediment

Table 13 OCPs concentration in marine sediments (mg/kg)

#	Name	alpha-BHC	Heptachlor epoxide	Endosulfan	Dieldrin	o,p'-DDD	p,p'DDT
1	**TS7**	0.0172	0.0147	0.111	0.0182	0.132	0.0432
2	**TS17**	0.0218	0.0117	0.0772	0.0167	0.0532	0.0244
3	**TS18**	0.221	0.0232	0.0826	0.0199	0.185	0.0350
4	**JS23**	0.0126	0.0171	0.160	0.0127	0.0481	0.0337
5	**JS24**	0.0123	0.0104	0.122	0.0186	0.301	0.0530
6	**JS25**	0.0243	0.00319	0.0850	0.0325	0.0386	0.0119
7	**JS26**	0.00676	0.00255	0.0506	0.0039	0.0715	0.0295

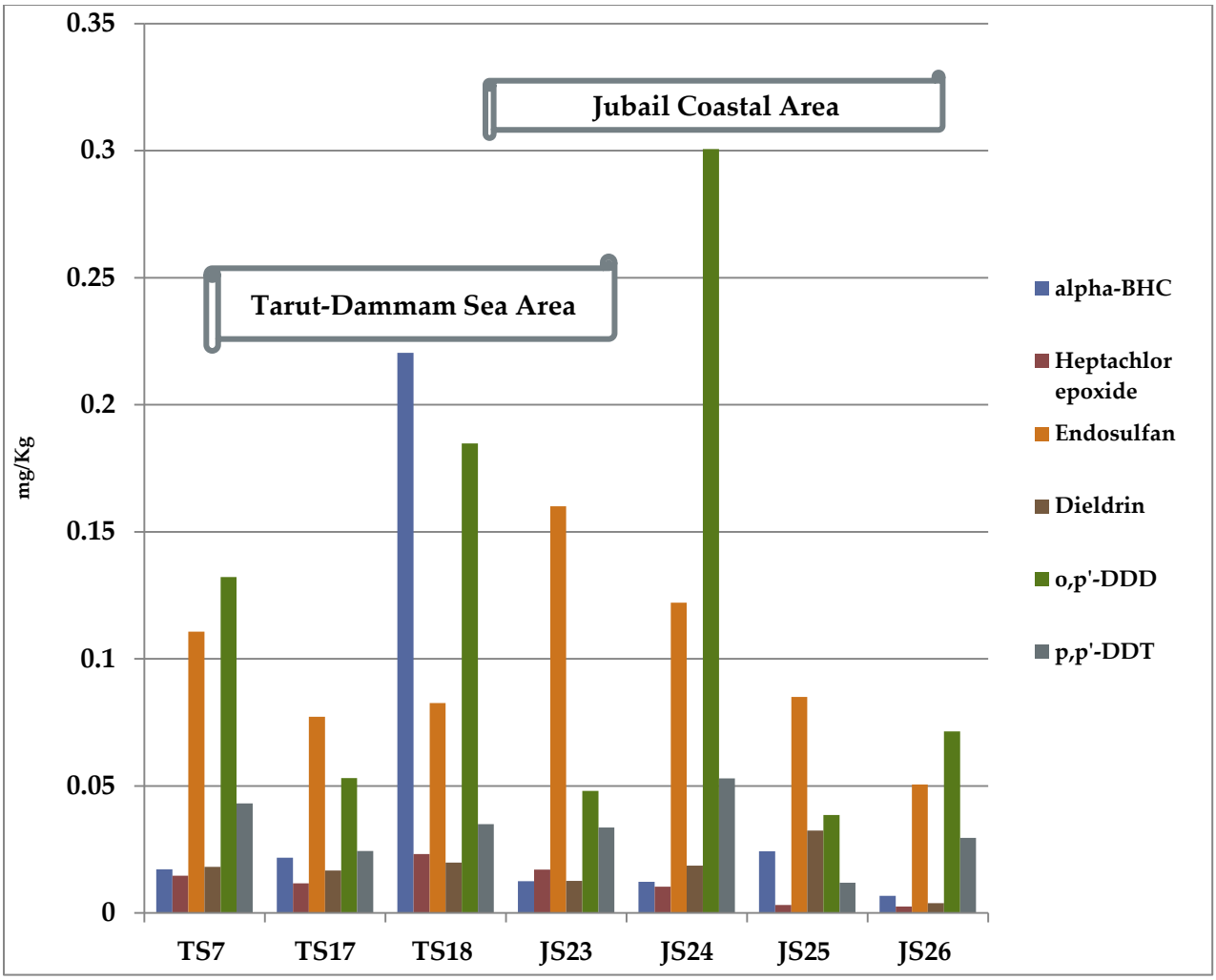


Fig.27 OCPs levels in Marine sediment samples (mg/kg).

The levels of OCPs in Marine Sediments is observable from Fig. 27 and Table 13. In marine sediment, the highest total concentrations of the six different organochlorine pesticides are detected in the Tarut Bay sample and specifically in the location TS18, which is the nearest point to the port. The total concentration of Organochlorine pesticides in Tarut Bay sediment samples is 1.108 mg/kg and the average is 0.369 mg/kg. In the Jubail coastal area sediment samples, the total concentration of the OCPs is 1.162 mg/kg and the average is 0.290 mg/kg.

The six pesticides were detected in all the sea water samples in the two areas. The highest concentration of individual pesticide determined is 0.301 mg/kg, which corresponds to o,p'-DDD in JS24 sample. Point TS18 recorded the highest total levels of OCPs of 0.566 mg/kg with maximum and minimum being 0.221 and 0.0199 mg/kg respectively. Conversely, the least total amounts of OCPs were observed at point JS26 with 0.165 as the greatest value whilst the lowest being 0.00255 mg/kg. Heptachlor epoxide was identified in all of the samples and with an average of 0.0118 mg/kg. The highest concentration was found in TS18 in Tarut Bay. Endosulfan and dieldrin were also quantified in all samples and the highest concentrations were 0.160 and 0.0325mg/kg, respectivelly. The averages of both of them are 0.0984 and 0.0175 mg/kg. Again, DDD was detected in all samples and it has the highest average and concentrations relative to the other pesticides. The average of DDD in all samples is 0.118 mg/kg and the highest concentration is 0.301 mg/kg. p,p’-DDT was identified in all samples with lower average compared to DDD.

As it is anticipated, the presence of these pesticides in the Tarut Bay is higher than Jubail coastal area, and this scenario again, cannot be viewed as being paradoxical. Evidently, agricultural activities are known to be carried out in Tarut Island. The run off of all agricultural farms in Tarut Island is injected into the bay through some artificial channels, hence escalating the influx and levels of pesticides in Tarut Bay. Given that DDD is a metabolite of DDT, the ratio of DDD/DDT

will be a fundamental indicator that there are still some uses of the banned DDT in the areas. However, the ratio of DDD/DDT in the previous data indicates that no more DDT is being injected into the surrounding environment.

4.1.3 Biota

Table 14 OCPs levels in Biota Samples (mg/kg)

Sample	alpha-BHC	Heptachlor epoxide	Endosulfan	Dieldrin	o,p'DDD	p,p'DDT
OYSTER 1	0.049	ND	ND	0.099	0.035	0.074
SHRIMP2	0.057	0.019	0.038	0.107	ND	0.084
SQUID3	0.011	ND	ND	ND	ND	ND
BIYADH4	0.029	ND	ND	0.043	ND	ND
QEED 5	0.011	ND	0.074	0.106	0.031	ND
EMPERIOR6	0.029	ND	ND	ND	ND	ND
CRAB 7	0.026	ND	0.070	0.035	0.027	ND
SOLEA 8	0.083	ND	0.100	0.133	0.023	ND
IBRAHIM 9	0.057	ND	0.069	0.096	0.024	ND
INDIAN MACTERERL 10	0.077	ND	ND	ND	ND	ND

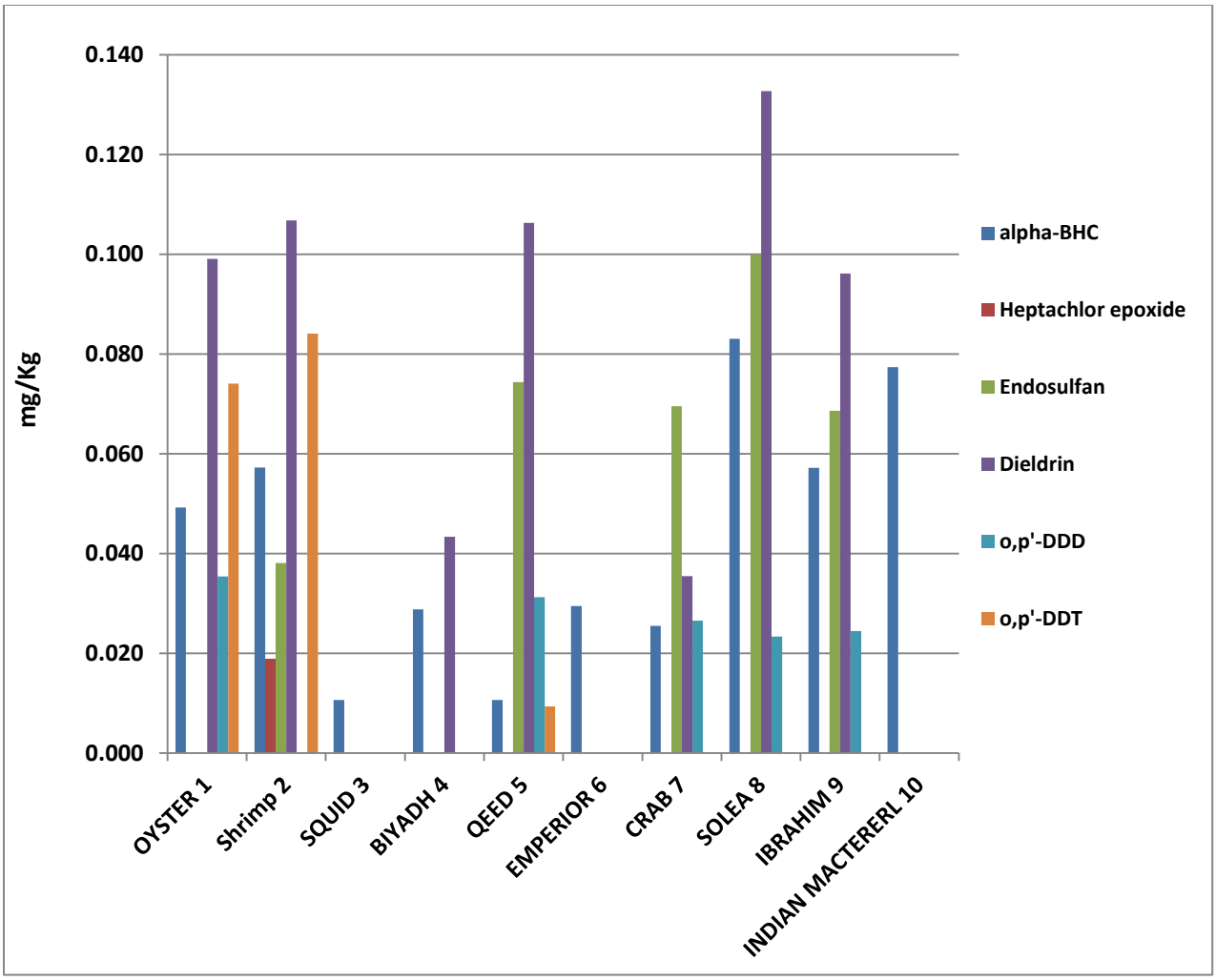


Fig.28 OCPs levels in Biota (mg/kg)

The concentration of OCPs in various Biota samples is shown in table 14. Quantitatively, the amount of Pesticides detected in selected Biotas is relatively low. Dieldrin represents the highest OCPs observed in Biota at maximum and minimum levels of 0.133 and 0.107 mg/kg, corresponding to SOLEA8 and SHRIMP2 respectively. Averagely, the concentration of Dieldrin in Biotas amounts approximately to 0.090 mg/kg. Heptachlor epoxide was determined exclusively in SHRIMP2 at a concentration equivalence of 0.019 mg/kg. With exemption of alpha-BHC which was quantified in almost all the selected Biotas, not all the six categories of OCPs were detectable in selected Biotas. The peak levels of o,p'-DDD reckoned in Biota was 0.035 mg/kg, in OYSTER.The average levels of p,p'-DDT in Biota can be approximated to 0.056 mg/kg. Speaking in terms of relative accumulation of OCPs, SOLEA recorded the highest amount of total biologically accumulated OCPs, amounting to about 0.340 mg/kg. Comparable to the levels of OCPs in sediments and sea water, the levels of p,p'-DDT and o, p'-DDD in Biota appears to follow a common trend demonstrating an obvious transformation of one to the other p,p'-DDT to o,p'DDD. Interestingly, the concentrations of all the determined OCPs in the selected Biotas are well within the levels recommended by WHO for consumer safety and health protection.

4.2 Levels of OPPs

4.2.1 Sea Water and Fresh Water

OPPs were simply not quantified in Sea Water at both Tarut Bay and Jubail Coastal Area. Presumably the open and dynamic Sea environment favours the degradation and rapid disappearance of OPPs residues in sea water. Conversely, OPPs were detected in significant amounts in Fresh Water samples at Tarut Island and Jubail Coasta.Parathion represents highest levels of individual pesticide quantified, occurring at location OGW5 with concentration of about 0.38566mg/L. The least level of single pesticide corresponds to O,O,O Triethyl thiophosphate at 0.015mg/L in site OGW5. In Tarut Island, Parathion again surfaces in high levels and O,O,O-Triethyl thiophosphate being the least, both concentrations measuring approximately 0.306 mg/L(TGW1) and 0.00414 mg/L(TGW2) respectively. Strikingly, the trend in levels of individual pesticide concentration at both Tarut Island and Al-Oyoon Agricultural area appears similar with Parathion being predominant while O,O,O-Triethyl thiophosphate occurring in low abundance. All the targeted pesticides were observed at both locations. Quantitatively, Parathion has the greatest abundance as it is found in sufficient proportions in all sites except OGW3, OGW7 and OGW8.See, Fig. 29 below.

Table 15 Levels of OPPs in Sea Water and Fresh water

Location	o,o,o-Triethyl thiophosphate	Disulfoton	Methyl Parathion	Parathion	Famphur
TGW1	ND	0.00508	ND	0.306	ND
TGW2	0.00414	ND	ND	0.0513	ND
TGW3	ND	ND	ND	0.192	ND
TGW4	ND	ND	ND	0.0333	ND
OGW1	ND	0.0960	0.3291	0.0763	ND
OGW2	ND	ND	ND	0.226	ND
OGW3	ND	ND	ND	ND	ND
OGW4	ND	0.096	0.0104	0.152	ND
OGW5	0.00750	ND	ND	0.386	ND
OGW6	ND	ND	ND	0.0350	ND
OGW7	ND	ND	ND	ND	ND
OGW8	ND	ND	0.0120	ND	ND

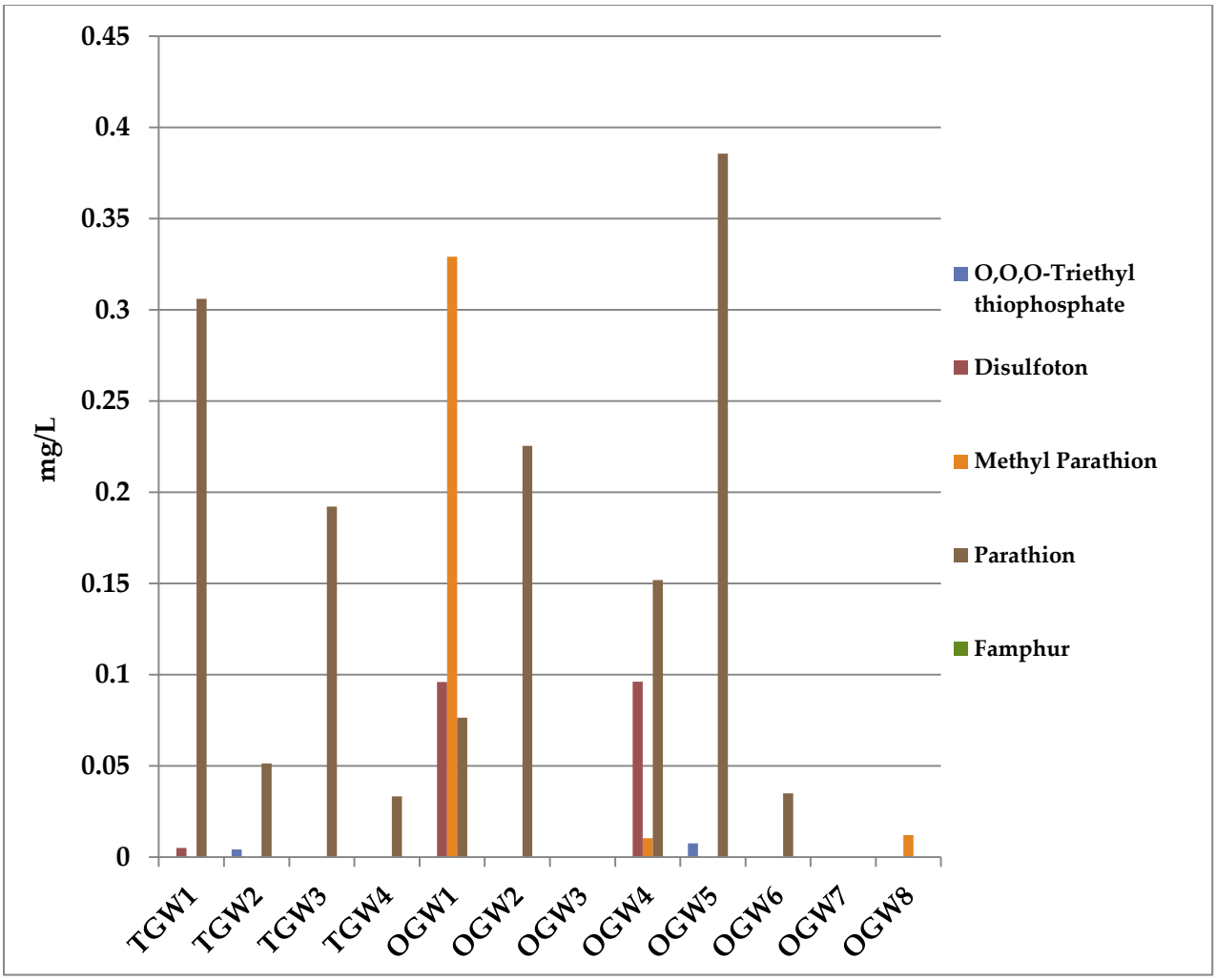


Fig.29 levels of OPPs in Tarut Island and Al-Oyoon Agricultural area (mg/L)

4.2.2 Marine Sediment

Table 16 Levels of OPPs in Marine Sediments (ug/kg)

#	Sample	O,O,O-Triethyl thiophosphate	Disulfoton	Methyl Parathion	Parathion	Famphur
1	**TS7**	ND	1.690	0.00445	0.00605	0.01448
2	**TS17**	0. 660	1.240	0.01108	0.00277	0.00714
3	**TS18**	0.570	4.310	0.00177	0.00223	0.00389
4	**JS23**	0.550	0.600	0.00306	0.0019	0.00523
5	**JS24**	0.470	2.540	0.00339	0.00425	0.00288
6	**JS25**	0.190	0.930	0.00198	0.00587	0.01139
7	**JS26**	0.140	2.900	0.00499	0.00434	0.0041

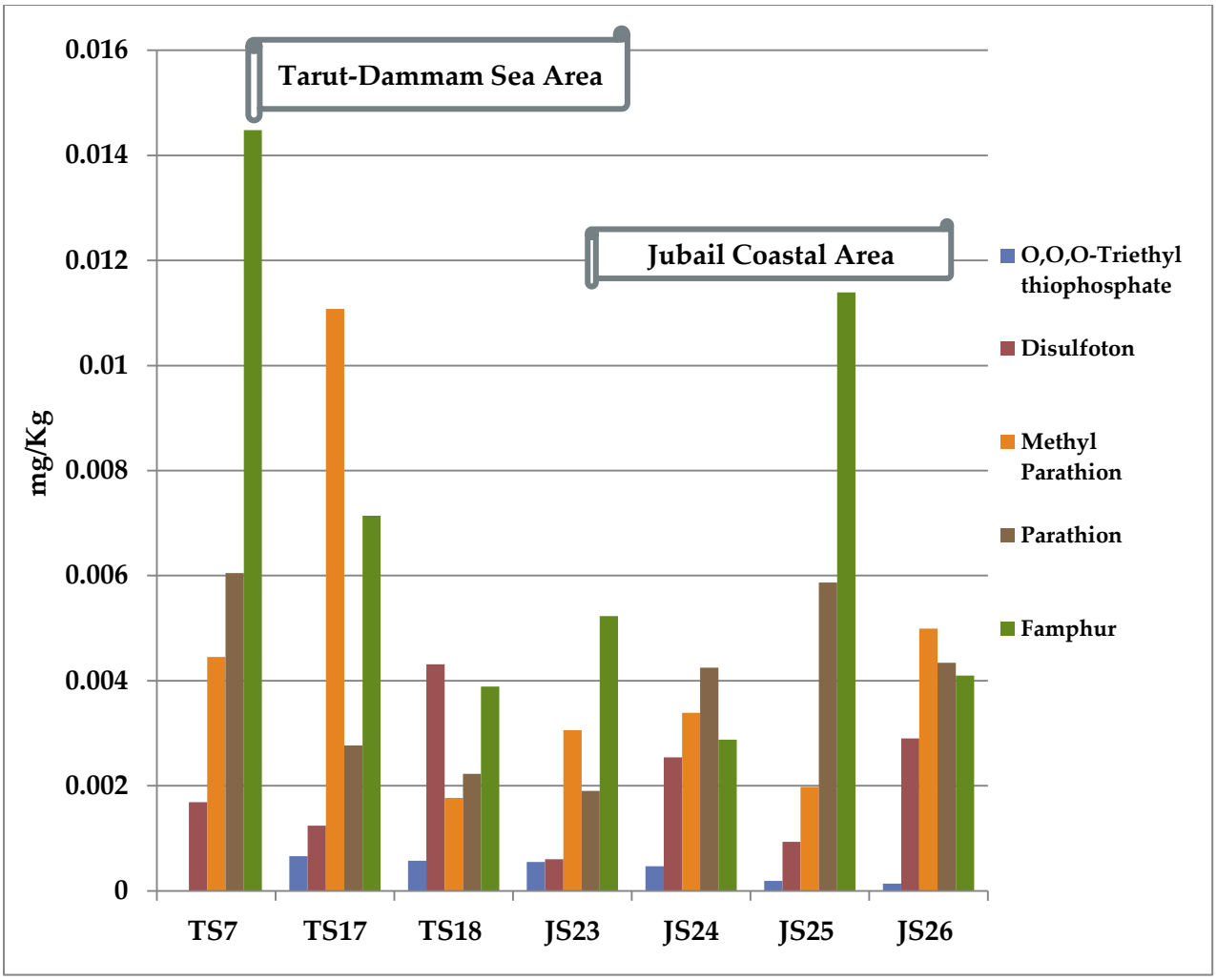


Fig.30 OPPs in marine sediments for Tarut Bay and Jubail Coastal Area (mg/kg)

The level of OPPs in Marine Sediments is vividly demonstrated in table 16. In terms of single pesticide, the peak and lowest levels are both quantified in Tarut Bay at point TS7 with values of 14.48 and 0.570 ug/kg representing Fampur and O,O,O-Triethylthiophosphate(point TS18) respectively. The greatest levels of individual OPPs in Jubail Coastal area were observed at point TS25 with concentration of 11.39 ug/kg (for Famphur). Sampling point TS26 had the least level of OPPs (for O,O,O-Triethyl thiophosphate) at about 0. 14 ug/kg. In totality, Tarut Bay possesses tremendous levels of OPPs compared to Jubail Coastal area with average for both locations being about 20.6 and 15.4 ug/kg respectively. Contrary to levels of OPPs in Sea sample, all the targeted OPPs were quantified in tangible amounts in Marine Sediments. This observation highlights the fundamental fact that marine sediments have the propensity to adsorb and accumulate significant levels of OPPs and probably inhibiting the rapidity of their degradation and ultimate disappearance.

4.2.3 Biota

Table 17 Levels of OPPs in Biota Samples (mg/kg)

Sample	O,O,O-Triethyl thiophosphate	Disulfoton	Methyl Parathion	Parathion	Famphur
OYSTER	ND	ND	ND	ND	ND
Shrimp	0.0469	ND	1.212	ND	ND
SQUID	0.0101	ND	0.00970	ND	ND
BIYADH	ND	0.00819	0.0639	ND	ND
QEED	ND	0.00527	0.00495	ND	ND
EMPERIOR	ND	0.00615	0.0667	0.123	ND
CRAB	ND	0.00525	0.0790	0.158	0.000158
SOLEA	ND	0.0223	0.0964	0.329	ND
IBRAHIM	ND	0.00738	0.0497	ND	ND
INDIAN MACTERERL	ND	0.0109	0.0697	0.312	ND

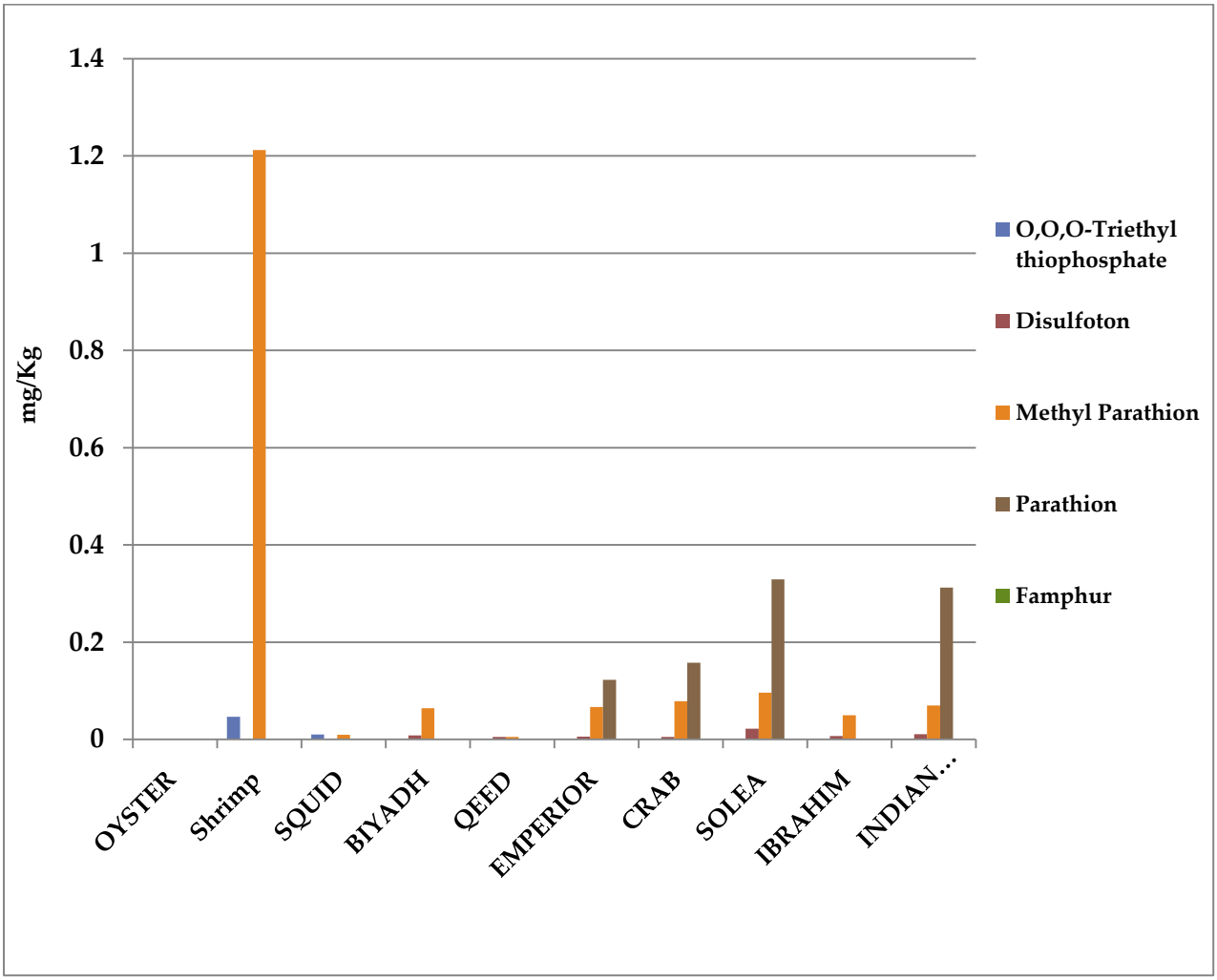


Fig.31 OPPs levels in Biota (mg/kg)

Comparatively, the level of OPPs accumulated in Biota, as shown in table 17 and Fig 31, is found to be several orders of magnitude greater than OCPs. Famphur was quantified exclusively in Crabs with concentration of 0. 158 ug/kg, but remaind virtually undetected in all other Biotas.In a similar vein, O,O,O-Triethyl thiophosphate levels were visible in only in Shrimp and Squid, however, no detection was observed in the remaining Biota samples.Interestingly, non of the pesticides were measured in Oyester Biotas.Methyl parathion reperesents a typical individual pesticide detected in high amounts amongst all the pesticides under consideration.Contrarily, Famphur levels surface as the lowest compared to any single pesticide quantified. Arguably, the greater the intensity of application or use of a particular pesticide, all other factors being equall, leads to a corresponding high preponderance and bioavailability in the environment. Averagely, it could be argued that Shrimp and Squid bioaccumulate the least amounts of pesticides while Crab and Indian Mackerel bioaccumulate susbtantial levels of pesticides, reaching orders of magnitude as high as 0.242 and 0.392mg/kg respectively for all the pesticides combined.

CHAPTER 5

CONCLUSION

Conclusively, it could be asserted that this research represents the first attempt to determine the baseline of several OCPs and OPPs in the Arabian Gulf and other surrounding Agricultural Areas. Amongst the OCPs, o,p'-DDD is highly prevalent in all observed samples except fresh water samples in Tarut Bay and Al-Oyoon. Although it is arguable that no new influx of p,p'-DDT occurs in the environment, o,p'-DDD being a metabolite of p,p'-DDT, forms as a consequence of the decomposition of the latter. Both OCPs and OPPs were determined in Biotas as one would anticipate. Comparatively, the levels of OCPs in Biotas were observed to be elevated than OPPs by certain orders of magnitude. Interestingly however, the levels of OCPs detected in Biotas are within acceptable limits according to WHO provisions. OPPs were quantified in tangible amounts in Marine Sediments. Contrarily, OPPs were unobservable and untractable in Sea Water samples. Quantitatively speaking, Tarut Bay contains the extreme preponderance of Pesticides, a scenario attributable to the intensive Agriculture activities in the Tarut Island.

CHAPTER 6

RECOMMENDATION

Our investigations are not immuned from limitations, hence the levels of OPPs and OCPs in certain samples could not be established. In order to track and decipher the levels of OCPs in Fresh Water samples in Tarut Bay and Al-Oyoon, further research is essential. Similarly, additional investigation is vital to understand the levels of OPPs in Sea Water.

Structered program to monior the levels of organic polloutants should be estabilished in order to assess their risk on human health and environment. New pesticides are introduced to the market and puplic use and effective control should be alaways there.

New methods for detection trace organic pollutatns are required to ensure relaible and continueos determination at different time and level.

Public awareness of the use of pestices should also be increased through the engaging them with the current levels of pesticdes in the environmetn and their dagners.

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