Assessment of Hexachlorocyclohexane in Soil, Water and Plant Residue Samples from Agricultural Areas of Potohar Region, Pakistan

By

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بسم الله الرحمن الرحيم

In the Name of ALLAH, the Most Gracious, the Most Merciful

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FINAL APPROVAL

It is certificate that we have read the thesis submitted by Ms. (Talat Ara) and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the M.S Degree in Environmental Science

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DEDICATION

To

My Mother

A strong and gentle soul, who taught me patience and faith in ALLAH. Her prayers enabled me to achieve my goals

My Father

Who taught me to trust in ALLAH and believe in hard work. For earning an honest living for us, supporting and guiding during my educational career

"Morality is the judgement to distinguish right and wrong, vision to see the truth, courage to act upon it, dedication to that which is good, integrity to stand by the good at any price"

(Ayn Rand)

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 10/11/17

Talat Ara

CONTENTS

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Best regards,

Talat Ara

LIST OF ABBREVIATIONS

Acronyms	Abbreviations
HCH	hexachlorocyclohexane
GDP	Gross Domestic Product
EPA	Environmental Protection Agency
POPs	Persistent Organic Pollutants
OCPs	Organo-Chlorine Pesticides
WHO	World Health Organization
PTS	Persistent Toxic Substances
GC-ECD	Gas Chromatography Electron Capture Detector
DDT	dichlorodiphenyltrichloroethane
KPK	Khyber Pakhtunkhwa
LRAT	Long Range Atmospheric Transport
PD	Parkinson's Disease
US	United States
DNA	Deoxyribonucleic Acid
AZF	Azoospermic Factor
MOA	Mode of Action
MRL	Maximium Residue Limit
ADI	Acceptable Daily Intake
TMDI	Theoretical Maximum Daily Intake
JMPR	Joint Meeting on Pesticide Residues
FAO	Food and Agriculture Organization

SM	Soil Moisture
SOM	Soil Organic matter
EC	Electrical Conductivity
TOC	Total Organic Carbon
MBC	Microbial Biomass Carbon
MWC	Microwaved Carbon
TDS	Total Dissolved Solids
GC-MS	Gas Chromatography, Mass Spectrometry
SPSS	Statistical Package for Social Sciences
GoP	Government of pakistan
NSDWQ	National Standards for Drinking Water Quality
OM	Organic Matter
nd	Not detected
MAC	Maximium Allowable Concentration
ATSDR	Agency for Toxic Substances and Disease Registry

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ABSTRACT

The present study was conducted to assess residue level of hexacholorocyclohexane in soil, water and plant samples. For this purpose, samples of agricultural soil, water and plant residues were collected from Taxila, Gujar khan, Chakwal, Rawalpindi and Fateh Jang areas of Potohar, Punjab. Results were obtained by Gas chromatography Mass spectrometry analysis which shows higher residue levels in soil, water and plants ranging from 2.43 μg/g – 8.88 μg/g, nd-5.87 μg/g and nd-4.87 μg/g respectively. Residue level of HCH in samples were exceeding the standards of Agency for Toxic Substances and Disease registry (ATSDR) and Maximum Residue Limits of European Union. The results of the present investigation are alarming and alerts the concerned departments for remediation of contamination and proper implementation of Environmental laws.

INTRODUCTION

INTRODUCTION

Pakistan's biggest natural resource is arable land and 25% of total land is under cultivation with world's largest irrigation system. Agriculture accounts 23% of GDP and 44% of labour force (Abrantes *et al.*, 2010). About 17% 0f the agricultural land depends on rain and contributes 10% to the total agricultural production. These rainfed areas are mostly located in the Pothar plateau, the northern mountain and the northeastern plains of the country. The total area of pothar plateau is 2.2million hactors of which cultivated land accounts 1million hactor (Ashraf *et al.*, 2007). Agriculture has a major share in the economy of Pakistan and like other agro based nations it also uses pesticides for higher yields to cope with food shortages. Serious environmental indications arise due to the extensive use of illegal pesticides on the pretext of low cost of production and higher yields due to comprehensive nature (Abrantes *et al.*, 2010). According to the EPA definition a pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mice and other animals, unwanted plants (weeds), or microorganisms) (Morillo and Villaverde, 2017). United Nations Environment Program Governing Council in 1995 identified 12 Persistent organic pollutant posing threat to environment and human health, one of these POPs was a fungicide and eight were insecticides (Morillo and Villaverde, 2017). Persistent organic pollutants (POPs) are capable of bio-magnification and bio-accumulation, long-range transport and non-degradability thus POPs are of international concern due to their negative impacts on the ecosystem (WHO, 2011). Persistent Organic Pollutants (POPs) consist of a highly toxic and hazardous group of chemicals known as Organochlorine pesticides (OCPs) (Park *et al.*, 2011). One of the major sources of OCPs in the environment is the agricultural soil as it serves as an ultimate depository for the present and past residues (Pozo *et al.*, 2011; Weaver *et al.*, 2012). After applying pesticide to a crop field only

0.1% of the pesticides reach to the pest while the remaining 0.99% contaminates soil, water and air (Sultana, et al., 2014; Pimentel, 1995). In 2001 the Stockholm Convention was taken up to minimize and ultimately eliminate the release of POPs into the environment and many commercial products containing POPs were banned (Ahad *et al.*, 2010; Alamdar *et al.*, 2014). Being a signatory to Stockholm convention it is obligatory for Pakistan to enforce basic plan of this convention. However, OCPs are still produced in Pakistan and easily available in the market. Pakistan is among the countries that holds the world's largest reservoirs of out dated pesticides (Syed and Malik, 2011). There are almost 300000 metric tons of outdated pesticides in 93 nations (Food and Agriculture Organization of United Nation, 2014) these pesticides also contain POP's (Jennings & Li, 2015). Pakistan holds one of the world's largest stockpiles of obsolete pesticides, factories involved in OCP production and dumping sites closer to cities have been destroyed (Syed and Malik, 2011).

The worldwide consumption of pesticides is about two million tons per year of which 69% is consumed by Europe and USA and only 31% are used by the rest of the world (Aktar *et al.*, 2009). Pakistan is ranked second among the South Asian countries on the basis of pesticide consumption (Randhawa *et al.*, 2007). The most commonly studied organochlorine pesticide is Hexachlorocyclohexane (HCH) which has been declared as POP in 2009 (Vijgen *et al.*, 2011). HCH has some known isomers of which HCH alpha (α), beta (β), gamma (γ) and delta(δ) are critical for environment (Li *et al.*, 2011). The most toxic isomer of HCH is γ-HCH or lindane, which is a constituent of agro-chemicals used for pest control and applied in medical treatments (Voldner and Li, 1995).

Venier and Hites (2014) studied HCH isomer trends and sources in air samples collected from great lakes of North America. The use of technical HCH was restricted in North America in

1970's when replaced by another HCH isomer named lindane. This study suggested that urban areas might be more important contributors of OCPs than previously considered (Venier and Hites, 2014).

Organochlorine pesticides have serious health impacts on animals and human's due to its toxic nature, it can cause neurological, reproductive and immunological disorder. OCPs can bio-accumulates and incorporates into the food chain posing a great threat to living beings (Kalyoncu *et al.*, 2009). Human exposure to OCPs is mainly through consumption of contaminated food. Therefore, a research was conducted to assess levels of OCPs and potential human health risk associated with OCPs after consumption of edible cattle tissues (Mahmoud *et al.*, 2016).

Development in agricultural and industrial sector has led to irreversible damage to environment and natural resources hence, the number of protected areas worldwide has raised (Cosentino, 2005). It has been proved that these protected areas are vulnerable to chemical contamination specially persistant toxic substances (PTS) by anthropogenic activities (Cabrerizo *et al.*, 2012; Yang *et al.*, 2008).

In late 1960s and early 1970s period of green revolution, Pakistan imported huge amount of pesticides from Europe and USA to eradicate malaria, locust control and for pest control in Pakistan (Ahad *et al.*, 2010). The presence of such deleterious chemicals in the environment together with natural processes such as evaporation and surface runoff ultimately enters the food. The people today are showing concern over the increase in contamination of environment because these hazardous chemicals are identified to alter normal hormones and effect human health and wildlife (Vos *et al.*, 2000).

A research study by Sultana *et al.* (2014) was aimed to assess the contamination status of organochlorine pesticides (OCPs) in agricultural soil and ambient air samples of Indus Basin, Pakistan and probable risks associated with air–soil exchange and health impacts. GC-ECD was used for quantitative analysis of samples. Among the other OCPs detected in the samples the most dominant compounds were ΣDDTs (Soil: 320 ng/g) and ΣHCHs (α-HCH 51%) HCH ranges from 7-27ng/g. Per soil quality guidelines the concentration of DDT and HCHs were exceeding the permissible limits, hence it poses a serious threat to the biodiversity of Indus Basin (Sultana *et al.*, 2014).

Liu *et al.* (2016) investigated the residue level and risk assessment of pesticides, the relationship between fruits and soil, for this purpose persimmons, jujubes and their soils were collected from China. The soils tested for residue levels indicated much higher concentrations of pesticides in persimmon soil (68.2%) and jujube soil (79.2%) than in fruits. Concentration of HCH was 4.5µg/kg – 14.2µg/kg in persimmon soil and 7.9µg/kg – 41.0µg/kg in jujube soil samples. Most frequently occurring pesticides were DDT in jujube soil and HCH in persimmon soil (Liu *et al.*, 2016).

Persistence and long-range transport ability of HCHs when combine with natural processes contaminates vast areas, it is of utmost importance to control HCH contamination in the environmental compartments, for the effective implementation of global monitoring plan. Further research findings are needed to limit formulation and use of banned HCH pesticides and control regional environmental pollution which ultimately results in global climate change.

Pesticides are causing Hexachlorocyclohexane contamination in agricultural soil, water and plant in the Potohar region.

Hexachlorocyclohexane are banned persistent organic pollutants but are still in use in many parts of the world, Pakistan being an agriculture economy uses HCHs in pesticide formulations to obtain higher crop yields. These pesticides entering the food chain, bio-accumulates and becomes one of the causes of many chronic disorders.

The present study will provide a baseline data for further research and analysis on pesticides (HCHs) in Potohar region of Pakistan and the study will help to limit formulation and use of illegal pesticide.

The current study is to investigate the concentration of Hexachlorocyclohexane (HCHs) and allocation profile in the soil, water and plants residues from agricultural areas along Potohar region in Pakistan. The main objectives include:

- To determine HCH in agricultural soils, irrigation water and crop residues of Potohar region
- To identify most contaminated areas among the five selected sites

LITERATURE REVIEW

LITERATURE REVIEW

Pesticides are used in agriculture, forestry, domestic and health sectors to control pest and related benefits (Aktar *et al.*, 2009). Persistent organic pollutants consist of well-known organochlorine pesticides such as hexachlorocyclohexane (HCHs) and dichlorodiphenyltrichloroethane (DDT) (Cofield *et al.*, 2007). They are extremely toxic, stable and non-degradable in natural environment (Li *et al.*, 2016: Yadav *et al.*, 2016). Historically used technical HCH contains approximately: α 60-70%, β 5-12%, γ 10-15% and δ 6-10% isomers (Zhang *et al.*, 2015; Willett *et al.*,1998). Later pure linden was used as Pesticides in many countries which contain more than 90% γ-HCH and replaced technical HCH (Zhang *et al.*, 2015).

2.1 Production and Usage of HCH

After the first half of the twentieth century global population was growing fast, agricultural practices were intensified to meet the rising global food demand (Merrington *et al.*, 2004) and the use of chemicals such as pesticides became wide spread (Ali *et al.*, 2014). During late 60's and early 70's Pakistan imported thousand tons of pesticides from Europe and United States of America for eradication of malaria and control of crop pests (Ahad *et al.*, 2010). The overall consumption of pesticide in Pakistan was 7000 tons per annum in 1960 and reached up to 78,132 tons per annum in 2003 (Khan *et al.*, 2010; Syed and Malik, 2011). In Pakistan, the use of banned toxic chemicals has decreased gradually and significantly over the years (Khan *et al.*, 2010). Even after the implementation of pesticide ordinance and rules in 1971 and 1973, the government lacks a strategy for obsolete and banned pesticides in custody (Ahad *et al.*, 2010). Therefore; Pakistan has the largest stock of outdated pesticides in the world. Reportedly about 5000 tons of obsolete pesticides exist in different provinces of Pakistan of which 128 tons in Balochistan, 2016 tons Sindh, 179 tons in KPK and 3803 tons in Punjab (Khwaja *et al.*, 2006).

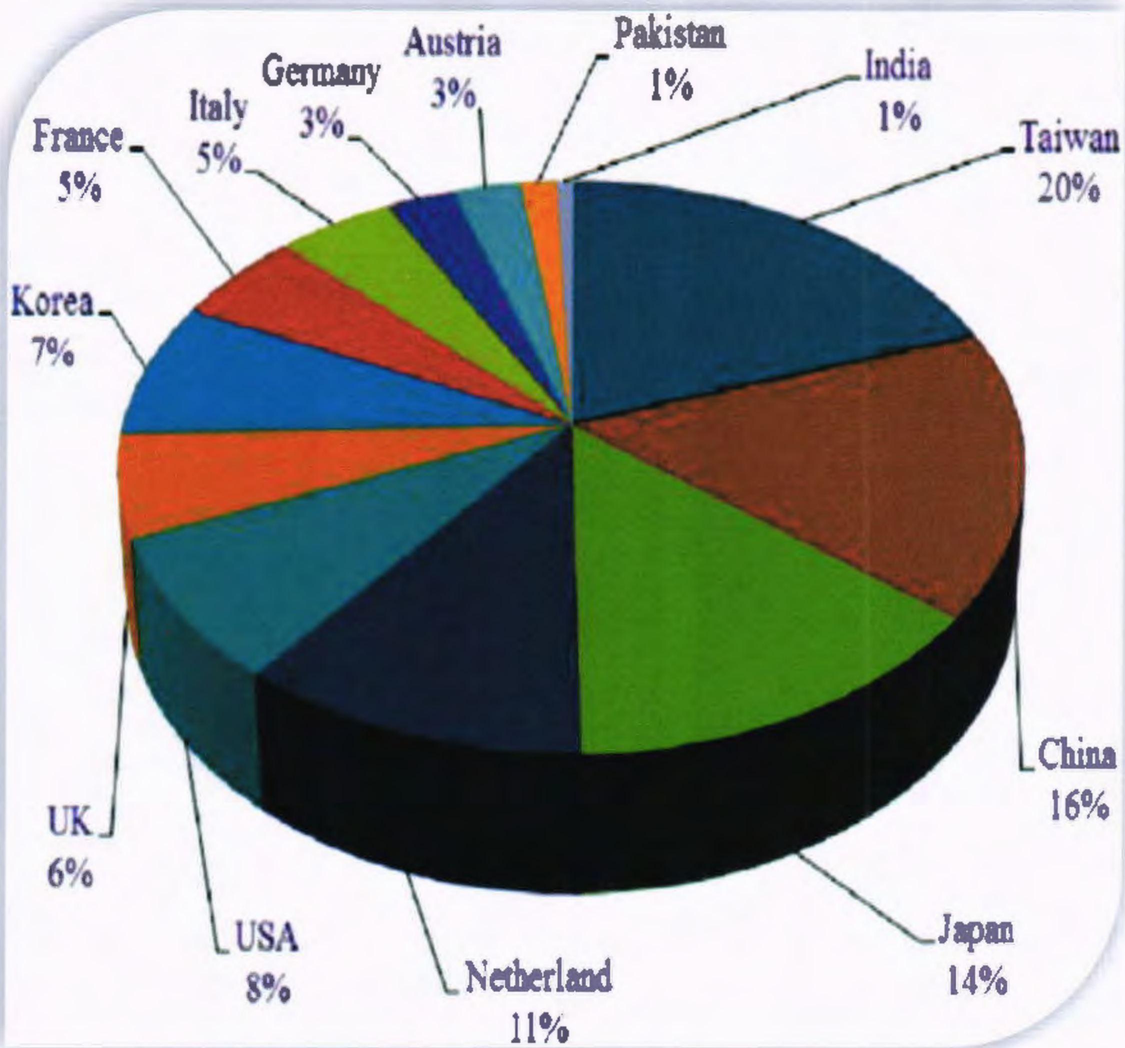

Figure 2.1: Worldwide pesticide consumption (Yadav *et al.*, 2015)

2.2 Properties of HCH's

During the last decades, Persistent Organic Pollutants including HCHs have gained much attention due to its properties which include;

a) Non-degradability

POPs are non-degradable in the environment therefore they are resistant to any means of degradation either chemical, physical, biological or microbiological. (Yadav *et al.*, 2015; Afful *et al.*, 2010). β-HCH are less volatile and resistant to microbial degradation and hydrolysis, while α-HCH and γ-HCH are more volatile isomers of HCH can stay in air and travel over long distance (Wu *et al.*, 2013). By the action of soil microbes or light γ-HCH can be converted to α-HCH (Zhang *et al.*, 2015).

b) Long Range Atmospheric Transport (LRAT)

Pesticides can travel to long distances, away from the source through air and Ocean currents (Nadal *et al.*, 2015; Hung *et al.*, 2013). Antarctica is considered a very isolated continent on Earth even then the presence of POPs in Antarctica has been recognized by scientists (Nash, 2011). A study conducted by Zhang *et al.* (2015) for the assessment of OCPs residue level, their distribution and potential sources in King George Island, West Antarctica. Samples were collected from different environmental matrices and analyzed through High Resolution Gas Chromatography and High-Resolution Mass Spectrometry technique. The results indicate presence of twenty-three OCPs, among these DDT and HCHs were main contaminants in all samples.

c) Bioaccumulation potential

Pesticides can bioaccumulate in fatty tissues, breast milk and blood due to its property of lipidsolubility (William *et al.*, 2008) and enters food chain (Yadav *et al.*, 2015). Human exposure to OCPs is mainly through consumption of contaminated food. Therefore, a research was conducted to assess levels of OCPs and potential human health risk associated with OCPs after consumption of edible cattle tissues (Mahmoud *et al.*, 2016). Total of 135 random samples of tongue, liver and kidney were collected from slaughter houses in Mansoura, Zagazig and Ismailia cities, Egypt. HCHs were highest OCPs and showed highest concentration (448ng/g lipid weight) in tongue samples from Mansoura (Mahmoud *et al.*, 2016).

d) Toxicity

Pesticides can cause toxic effects on health such as, neurotoxicity, carcinogenicity and imunotoxicity (Nadal *et al.*, 2015; Chao *et al.*, 2014). These pesticides can affect not only human health but also dangerous for aquatic life (Yadav *et al.*, 2015). A study conducted in the Faroe Islands also reported a small, but significant relationship between serum levels of β-HCH and increased risk of Parkinson disease (PD) (Richardson *et al.*, 2011; Petersen *et al.*, 2008). Exposure to pesticides was considered as a possible risk factor for developing Parkinson disease. Therefore, a case control study was conducted by Richardson *et al.* (2011) in University of Texas Southwestern Medical Center and Emory University, 283 serum samples of PD patients were investigated to determine association between β-HCH and PD disease. Samples were collected and study in two discrete periods from 2001-2003 and 2006-2008. Although the data obtained was consistent with decrease of β-HCH in environment from 2001-2008, but results shows higher levels of β-HCH in serum is associated with increased risk of PD disease (Richardson *et al.*, 2011).

2.3 Health Impacts of HCHs

Persistent organic pollutants (POPs) are capable of bio-magnification and bio-accumulation, long-range transport and non-degradability thus POPs are of international concern due to their negative impacts on the ecosystem (WHO, 2011). Organochlorine pesticides have serious health impacts on animals and human's due to its toxic nature, it can cause neurological, reproductive and immunological disorder (Kalyoncu *et al.*, 2009). Health impacts of POPs results from chronic, cumulative and long-term exposure to one or more substances, mostly through non-atmospheric pathway. The most common contributing exposure pathway for POPs and semi-volatile contaminants seems to be through dietary uptake (Perelló *et al.*, 2015, Nadal *et al.*, 2015). Alpha-hexachlorocyclohexane (α-HCH) is a structural isomer of HCH that have been used as insecticide globally. Although at present HCH is neither produced nor used in United States but exposure to HCH isomer is of concerning issue, due its historic usage and persistence in environment. The U.S. Environmental Protection Agency (EPA) classifies alpha-HCH as a probable human carcinogen (Bradely *et al.*, 2016). α-HCH causes liver tumors in rat and mice. The mode of action (MOA) of liver tumors involved rapid cell growth or mitogensis.

According to latest studies Y-chromosome microdeletion is related to male infertility. However, certain environmental pollutants are recognized to harm DNA of differentiating and maturing germ cells in the male reproductive tract (Khan *et al.*, 2010). Therefore; a study was conducted by Khan et al. (2010) to determine the seminal HCH and its isomers in relation to semen quality and Y-chromosome microdeletion in azoospermic factor (AZF) region. For analysis 50 fertile and 50 infertile male samples were taken and results showed a higher concentration of HCH and its isomers (α, β and γ) in infertile group similarly, the major microdeletions were also observed in azoospermic patients. This study concluded a susceptibility of male germ line to potential mutagenic activity of HCH which is a risk factor and can lead to spermatogenic failure (Khan *et al.*, 2010).

2.4 Maximum Residue Limit

Residues of persistent pesticides stays for longer periods on the target crops and later enters to human body through food chain (Bhushan *et al.*, 2013). The residue level of these pesticides should not go beyond maximum limits which may be harmful to human health. In order to monitor residue levels of these pesticides in food chain certain limits have been proposed and developed which includes, maximum residue limits (MRLs), acceptable daily intake (ADI) and theoretical maximum daily intake (TMDI) (Bhushan *et al.*, 2013). In areas with good agricultural monitoring the MRL is the maximum residue limit of pesticides considered normal if found in a product treated with them. An ADI is the maximum allowed intake of pesticides from all dietary sources in a day which may not effect health. The TMDI is an estimate of the maximum intake of pesticides with the existing MRLs for a person, resulting from a dietary source. Globally there are no available and acceptable standards for pesticide residues. However, FAO and WHO have recommended a standard acceptable limit for POPs which is zero tolerance (FAO/ WHO, 2011). The standards and limits recommended by FAO and WHO are widely accepted and adopted. These acceptable standards are proposed in a Joint Meeting on Pesticide Residues (JMPR) and the Codex Alimentarius Commission. JMPR recommendations evolves after a thorough review of international data, along with the analysis of presence and cure and effect of pesticides (FAO and WHO, 2011; Fishel, 2010).

2.5 Environmental Legislation regarding HCHs

The most important international conventions which deals with POPs includes the Rotterdam convention which addresses the prior informed consent procedures, certain hazardous chemicals and pesticides in international trade, secondly the Stockholm convention deals specifically POPs and third is the Basel convention which deals with transboundary movement of hazardous wastes and their disposal (Ali *et al.*, 2014). Agricultural Pesticide Ordinance, 1971 and Agriculture Pesticide Rules, 1973 under the guidelines of FAO were first establish rules and regulations for pesticide manufacture, import, sale and monitoring. Later, some changes were made in the ordinance in favor of importers because Pakistan relay on imported pesticides. Pakistan signed the Rotterdam Convention in 1999 at United Nations in New York and became the 67th member country of the Convention. The aim of the Convention was to share trade information about chemicals and pesticides per international rules and regulations (Ali *et al.*, 2014; GoP, 1999). Pakistan also ratified the Stockholm Convention in July 2000 and became party of the convention. Although there is an environmental legislation on POPs in Pakistan, even then there is a huge gap between legislation and implementation (Ali *et al.*, 2014). Malik (2003) and Bhambhro (2004) also reported lack of implementation of Agricultural Pesticide Ordinance and poor coordination among regulatory bodies.

2.6 HCH Assessment in Soil

A study was conducted in Wuhan which is the largest city in central China to investigate 21 OCPs, their distribution and sources in agricultural soils (Zhou *et al.*, 2013). The study reported higher levels of OCPs as compared to some other cities in China and other countries. The total HCH concentrations ranged from nd to 100.58ng g^{-1} in soil and major contaminated regions were Hannan, Huangpi and Xinzhou districts of Wuhan. These higher concentrations of many OCPs were probably attributed to excessive agricultural practices in the area (Zhou *et al.*, 2013).

Yu *et al.* (2013) reported a research conducted in Pearl River Delta, China which is considered as economically prosperous region. This region has well developed agriculture and consumes highest amount of pesticides for the purpose. Total 151 samples were collected from Pearl river delta for assessment of pesticides. Organochlorines were quantitatively analyzed via GC-ECD technique. Hexachlorocyclohexane ranges from no- detectable to 62ng/g. The results show a higher concentration of HCH in Guangzhou which may be due to the fresh input of lindane. Whereas depending on the soil texture and type concentration of HCH was found to be in the following order clay > loamy > sandy (Yu *et al.,* 2013). While pesticides enhance agricultural production on the other hand they bio- accumulates through food chain and poses a serious risk to human health. In recent years public health concern had gained momentum therefore many countries and regions have established maximum residue limits for food stuff, similarly national food monitoring programs for pesticides have been enacted worldwide (Jardim and Caldas, 2012; Lozowicka *et al.*, 2014; Skretteberg *et al.*, 2015).

Alamdar *et al.* (2014) conducted a research on obsolete pesticide dumping grounds of Hyderabad city, Pakistan to assess contamination level of organochlorine pesticides in soil and air samples. The results were astonishing, concentration of DDT (soil: 77–212,200 ng/g; air: 90,700 pg/m^3) and HCH (soil: 43–4090 ng/g; air: 97,400 pg/m^3) were highest in pesticide dumping grounds compared to samples from surrounding industrial and residential areas. According to OCPs diagnostic indicative apart from historic residues, there was fresh DDT in put in the area under study. Elevated levels of OCPs at dumping site and its surroundings pose serious threat to health and safety of biological organisms (Alamdar *et al.*, 2014).

Being an agriculture country the overall organochlorine pesticide consumption in India is nearly three fourth of annual pesticide consumption (Chakraborty *et al.,* 2015). Recently high concentrations of OCPs have been reported in the atmosphere of seven major Indian cities (Chakraborty *et al.*, 2010). OCPs were therefore determined in 81 soil samples from New Delhi, Agra, Kolkata, Mumbai, Goa, Chennai and Bangalore city, which shows highest levels endosulfan followed by DDT and HCHs (Chakraborty *et al.*, 2015). India is ranked third largest country in the world on the basis of technical HCH consumption (Kutz *et al.*, 1991; Iwata *et al.*, 1993). During 1948-2000 about 1057 tonne of HCHs was consumed (Wei *et al.*, 2007) whereas from1990 to 2004 lindane (γ-HCH) consumption in India was 6840 tonne which is 2% of the global usage (Vijgen, 2006) and in 2013 lindane has been banned in India (Chakraborty *et al.*, 2015).

Sun *et al.* (2016) revealed the contamination status of Yangtze River Delta, China. Total of 241 topsoil samples were collected from agricultural lands of YRD which is considered economically prosperous region in China. In their research Sun *et al.* (2016) investigated a wide range of endocrine disrupting compounds such as, organochlorine pesticides, Phthalate esters and polybrominated diphenyl ethers. Quantification of OCP was carried out using GC-ECD. Concentration of HCH ranges from 0.37 to 30.3ng/g less then 50ng/g which is a less strict grade II limit for HCH and DDT in agricultural soil by environmental quality standards for soil (China National Environmental Protection Agency, 2008).

Tibetan plateau is the world's largest plateau due to its high elevation and low temperature it is sometimes regarded as "third pole" different from other truly remote poles with small population and few agricultural practices (Wang *et al.*, 2016). A research study on agricultural soil and crop samples collected from Tibetan plateau (TB) showed very low DDT and HCH concentration. The average concentrations of DDTs and HCHs in the agricultural soil, highland barley and rape were 1.36, 0.661, 1.03 ng/g dry weight and 0.349, 0.0364, 0.0225 ng/g dw, respectively (Wang *et al.*, 2016). These concentrations were similar to that of wild plants of TB therefore the presence of HCH and DDT can be attributed to the long-range transport property of these compounds (Wang *et al.*, 2016).

2.7 HCH Assessment in Water

Pesticides such as HCH and DDT residues are commonly found in freshwater bodies (Tao *et al.*, 2007; Zhou *et al.*, 2008). Southeastern lake Chaohu, once known for aesthetic beauty and ample biodiversity is among the five largest lakes in China. (Li *et al.*, 2010; Xu *et al.*, 1999, 2001; Zhang *et al.*, 2007). Xu *et al.* (2001) suggested that the reason behind the deterioration of lake Chaohu is chemical stress by pesticide contamination. The residue level and potential source of HCHs in water from lake Chaohu were analyzed by GC-ECD and results indicate a higher concentration of HCHs (1.58 to 31.66 ng L^{-1}) in water from lake Chaohu which is higher than those in other lakes of China (He *et al.*, 2012).

The rate of metabolism of HCH in fish tissues is very slow (Guo *et al.*, 2008; Masci *et al.*, 2014) therefore, contaminated fish tissues are better indicator of aquatic pollution (Lu *et al.*, 2010; Masci *et al.*, 2014). More than 54 fish species live in the Kabul river most of which are rich source of food for humans (Yousafzai *et al.*, 2008). Nevertheless, industrial waste water, agricultural runoff and sewage waste discharge chemicals into the river (MOE-PAK, 2005). Aamir *et al.* (2016) conducted a study to assess HCH and DDT contamination in river Kabul, Pakistan. Organochlorines were quantitatively analyzed using gas chromatography combined with electron capture detector GC-ECD. The order of distribution of Σ (HCHs +DDTs) in all fish tissues was liver>muscle>stomach>gills. Study showed HCHs contamination in selected fishes were due to its recent use in the area (Aamir *et al.*, 2016).

2.8 HCH Assessment in Plants

Sojinu *et al.* (2012) reported a study conducted in oil exploration areas of Niger Delta in Nigeria, where concentrations and distributions of organochlorine pesticides (HCHs) were examined in higher plants. Among the plants sampled from Olomora study area Newbouldalaevis showed the maximum HCH concentrations (α-HCH 99.1ng/g, γ-HCH 141.1ng/g and δ-HCH 91.1ng/g) whereas Pennisetum purpureum plants sampled from Oginni showed highest concentration for HCH (α-HCH 47.74pg/g, β-HCH 59.87pg/g, γ-HCH 29.14pg/g and δ-HCH 20.30 pg/g). The HCHs detected in the plant samples might have resulted from intense farming practices and pesticide usage for insect control. Soil serves as an ultimate reservoir for a number of pollutants (Zhang *et al.,* 2011) and is a secondary source of contamination for water and air (Tao *et al.*, 2008).

Another study was reported by Mehmood *et al.* (2014) for the assessment and dietary uptake of organochlorine pesticides along the tributaries of river Chenab, Pakistan. Samples of air, soil and crops (wheat and rice) were collected from Gujranwal division of Punjab province, Pakistan. The technique used for the analysis of samples includes gas chromatography, electron ionization combines with mass spectrometry GC-EI-MS. The study reported presence of all OCPs in soil samples except mirex, where HCH (range, 4.54-18.9 ng g^{-1}) was among the dominant pollutants. Similarly analysis of rice and wheat crops also indicated HCH (for rice; range, 1.48–27.6 ng g^{-1}, Wheat; range, 0.35–4.53 ng g^{-1}) as dominant OCP after DDT. These results reveal a serious risk to the residents of the study area by consuming contaminated crops (Mehmood *et al*., 2014).

The pesticide residue levels in fruits and vegetables have been reported many times as compared to nuts. Nuts are a rich source of minerals, fibers, vitamins and fatty acids and highly beneficial for health. Most of the pesticides used for cultivation of nuts are hydrophobic in nature which incorporates in lipid matrix of nuts and ultimately pesticide residue passes on to consumers (Liu *et al*., 2016). Therefore, Liu *et al.* (2016) aimed to study the pesticide residue levels in three types of nuts (chestnut, pinenut and walnut). Samples were collected from seven major nut producing areas in China and analyzed for twenty-nine pesticides (organophosphates, organochlorines, pyrethroid, Triadimefon and buprofezin) by gas chromatography with Electron capture detector (GC-ECD). Among Organochlorines alpha-HCH was found in 1 walnut sample (2.0μgkg−1) and 1 pinenut sample (3.4μgkg−1). But there was no significant health impact associated with consumption of nuts (Liu *et al.*, 2016).

Hence it has become clear from the literature that organochlorine pesticides exist in different environmental compartments. The transboundary and long-range transport property of these hazardous chemicals have serious impacts on both biotic and non-biotic components around the world.

Table 1. review of literature

Author/Year	**Title**	**Instrumentation**	**Results**
Barron *et al.*, 2017	Residues of organochlorine pesticides in surface soil and raw foods from rural areas of the Republic of Tajikistan	GC-ECD Gas chromatography combined with electron capture detector	Pesticides contamination was higher in soil samples and lower in dietary products. Highest conc. of DDT, HCH and endosulfan
Fang *et al.*, 2017	Organochlorine pesticides in soil, air and vegetation at and around a contaminated site in southwestern of China: Concentration, Transmission and Risk Evaluation	Gas chromatography coupled with DFS mass spectrometer	At the contaminated site: ΣHCHs (top soil: 19.1 mg/kg d.w., air: 52.3 ng/m3, vegetation: 0.17 mg/kg d.w). Around the contaminated site: ΣHCHs (top soil: 129 ng/g d.w., air: 5.09 ng/m3, vegetation: 81.8 ng/g d.w.)
Sun *et al.*, 2016	Concentrations, distribution, sources and risk assessment of organohalogenated contaminants in soils from Kenya, Eastern Africa	GC-ECD and MS	Conc. of HCH ranges from nd-7.38µg/kg d.w. DDT was dominant OCP ranges from nd-30.04µg/kg d.w.

Qu *et al.*, 2016	The status of organochlorine pesticide contamination in the soils of the Campanian Plain, southern Italy, and correlations with soil properties and cancer risk		Conc. of DDT and HCH were 0.08-1231 ng/g and 0.03-17.3 ng/g
Qu *et al.*, 2015	Risk assessment and influence factors of organochlorine pesticides (OCPs) in agricultural soils of the hill region: A case study from Ningde, southeast China	GC-ECD	Conc. of HCH was lower than DDT but higher than all other OCPs. Conc. of HCH was 0.45-151.21ng/g d.w.
Min *et al.*, 2015	Assessment, Composition and Possible Source of Organochlorine Pesticides in Surface Soils from Ürümqi, China	GC-ECD	HCH ranges from nd-30μg/kg, Concentration of HCH was highest after DDT.
Da *et al.*, 2014	Analysis of HCHs and DDTs in a sediment core from the Old Yellow River Estuary, China	GC-MS	Based on dry weight ΣHCH 0.001–14.85ng/g and ΣDDT 0.04–1.07ng/g

Kim *et al.*, 2014	Distributions of new Stockholm Convention POPs in soils across South Korea	GC-MS	HCHs were detected in 85% of soil samples ranging from non-detectable (ND) to 0.358 ng/g
Ge *et al.*, 2013	Composition, distribution and risk assessment of organochlorine pesticides in soils from the Midway Atoll, North Pacific Ocean	Gas chromatograph and Ion Trap Mass spectrometer GC/ITMS	Among the six OCPs detected in Midway Atoll two were HCH isomers. ΣHCHs ranges from nd-127ng/g whereas DDT ranges from 1.4-643ng/g.
Mishra *et al.*, 2012	Contamination levels and spatial distribution of organochlorine pesticides in soils from India	GC-ECD	Mean concentrations of total HCH in district Nagaon were 825 ng/g (range: 98–1945 ng/g) and total DDT were 903 ng/g (range: 166–2288 ng/g). Mean concentration of total HCH and total DDT in Dibrugarh were 705 ng/g (range: 178–1701 ng/g and 757 ng/g (range: 75–2296 ng/g)

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Sampling

In the present study different agricultural sites of HCHs application were selected in Potohar region of Pakistan (Figure 3.1). Sampling was done after crop harvest in the selected sampling areas which includes Taxila, Fatehjang, Rawalpindi, Gujar Khan and Chakwal. Soil and plant samples were collected to check the status of HCHs concentration in soil and uptake chemical by plant tissues at the same time water samples were also collected from available water resources at the sampling sites such as canal, hand pump and tube well. All the samples were collected by using contaminants free tools and were stored in zip bags and water samples in sampling bottles, all samples were kept below four degree centigrade durring transfer to lab.

After transporting to the lab all the samples were retaged and soil samples were divided into two parts one for basic analysis was air dried and other for pollutants study was freeze dried. Soil samples were air dried, crushed and seived through 2mm pore size sieve before starting analysis. For the plant samples after transporting to lab, fresh weight was measured later samples were washed with deionized water and stored below 4 degree Centigrade. Map of Potohar region is shown in figure 3.2.

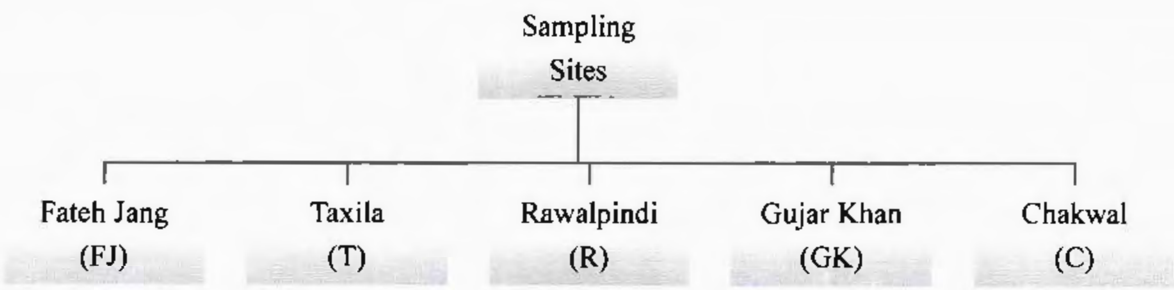

Figure 3.1: sampling areas of Potohar region

3.2 Physico-chemical Analysis of Soil

Analysis of basic physico-chemicals parameters of soil includes soil moisture, soil texture, pH, Electrical conductivity, total organic carbon, microbial biomass carbon and organic matter.

3.2.1 Soil Moisture (SM)

To determine soil moisture content, take 10 gm of soil sample in a petri plate of known weight. Allow it to dry at 105°C for 24 hours in a digital oven with lid unfitted. soil moisture was calculated by using the following formula:

$$\text{Soil Moisture (\%)} = \frac{\text{weight of wet soil} - \text{weight of oven dry soil}}{\text{weight of oven dry soil smple}} \times 100$$

3.2.2 Soil Texture

Soil texture was determined by using hydrometer (Bouyoucos, 1962).

3.2.3 pH

Soil pH was determined by using multi meter model (APHA, 2005). Multi meter was callibarated by using buffer solutions of pH 4, 7 10 before further anylasis of samples. After callibaration soil suspension was prepared and then pH was measured using multi meter model (MM 40 +).

3.2.4 Electrical Conductivity

Electrical conductivity was determined using multi meter model (MM 40 +). Electrical conductivity of clear filtrate was measured in microsiemen (µS) by using EC meter (Muhammad *et al.*, 2008).

3.2.5 Total Oraganic Carbon (TOC)

Total organic carbon was determined by titration method (Walkley, 1947). To measure TOC 1gm of air dry soil was taken in a conical flask, then 10ml of 1N Potassium dichromate and 20ml of concentrated Sulphuric Acid (H_2SO_4) was added and mixed well. After 30minutes 200ml distilled water and 10ml of Phosphoric Acid (H_3PO_4) was added and solution was allowed to cool. Finally, the solution was titrated against iron ammonium sulphate by adding 10-15 drops of diphenylamine as indicator until brilliant green (end-point) is achieved. Same procedure was repeated without any added soil sample to calculated reading of blank solution.

$$\% \text{ Oxidizable Organic Carbon} = \frac{[\text{Volume of } blank - \text{Volume of } sample] \times 0.3 \times Molarity}{Weight\ of\ soil}$$

% Total Organic Carbon = 1.334 × % Oxidizable Organic Carbon

3.2.6 Soil Organic Matter (SOM)

Soil Organic Matter is calculated using titration methof of Walkley (1947).

% Organic Matter =1.724 × % Total Organic Carbon

3.2.7 Microbial Biomass Carbon (MBC)

Microbial biomass carbon was determined by micro-waved irradiation and extraction method (Islam and Weil, 1988).

MBC (mg C kg $soil^{-1}$) = (Micro-Waved C_{ext} - C_{ext}) × 2.64

3.3 Physico-chemical Parameters of Water

Some basic parameters of water samples were determined which include pH, electrical conductivity, total dissolved soilds, alkalinity, temperature and total hardness.

3.3.1 pH

pH of water samples was calculated with the help of multi meter model (MM 40 +) (APHA, 2005)

3.3.2 Electrical Conductivity

Electrical Conductivity was determined using multi meter model (MM 40 +).

3.3.3 Total Dissolved Solids (TDS)

Total dissolved solids were calculated by Richards (1954) as:

TDS mg/l = Electrical conductivity µs/cm × 0.64

3.3.4 Alkalinity

The alkalinity of water sample is determined by titrating it against standard acid solution using indicators like phenolphthalein and methyl orange (Jayalakshmi *et al.*, 2011).

3.3.5 Total Hardness

50 ml of water sample is titrated against 0.01M EDTA (Disodium salt) solution by using Solo Chrome Black T as an indicator (Jayalakshmi *et al.*, 2011).

3.4 Determination of HCHs in Soil, Water and Plant Samples

To determine the concentration of different isomers of HCH in the soil, water and plant samples.

3.4.1 HCH Analysis in Soil

For the assessment of HCHs in soil (figure 3.4.1), 5gm of soil sample was taken in a 100ml teflon tube, then 10ml of dichloromethane was added and mixture was extracted by sonicating for 20min. After sonication mixture was centrifuged for 5min at 4000rpm and the supernant was collected. Then the extract was eluted by adding 1-2ml of a mixture od n-hexane:dichloromethane (v/v 50:50) and supernatant was extracted for HCH and dried by nitrogen sparging. Solid residues were redissolved in 1ml acetonitrile (Li-Hong *et al.*, 2006).

3.4.2 HCH analysis in Water

For the assessment of HCH in water (figure 3.4.2), 100ml water sample was taken in a flask, then 10ml of dichloromethane was added and mixture was extracted by sonicating for 20min. After sonication mixture was centrifuged for 5min at 4000rpm and the supernant was collected. Then the extract was eluted by adding 1-2ml of a mixture od n-hexane:dichloromethane (v/v 50:50) and supernatant was extracted for HCH and dried by nitrogen sparging. Solid residues were redissolved in 1ml acetonitrile (Li-Hong *et al.*, 2006).

3.4.3 HCH analysis in Plants

Assessment of HCH in plants is shown in (figure 3.4.3).Plant residue was subjected to water bath extraction for 20min in ethyl acetate. The extract was passed through silica gel packed column under reduced pressure and then dried by nitrogen sparging. Eluent was re-dissolved in 1ml of acetonitrile for GC- MS analysis (Li-Hong *et al.*, 2006, Rhind *et al.*, 2013).

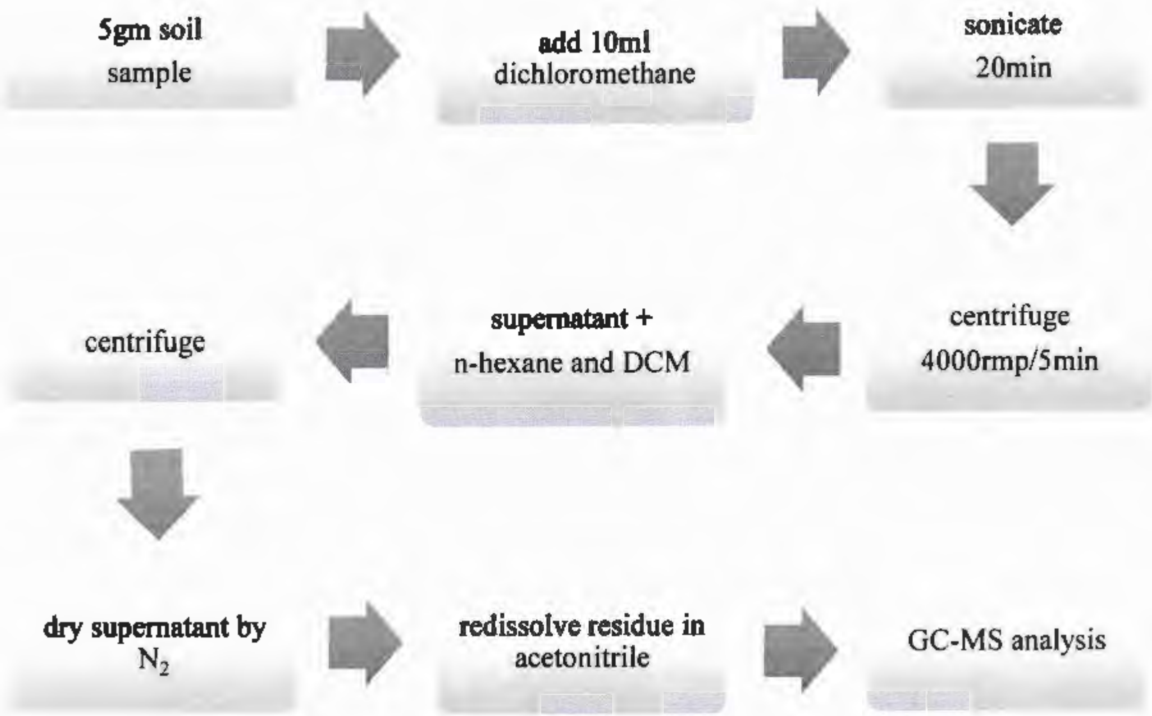

Figure 3.4.1: Flow chart for the assessment of HCH in soil sample

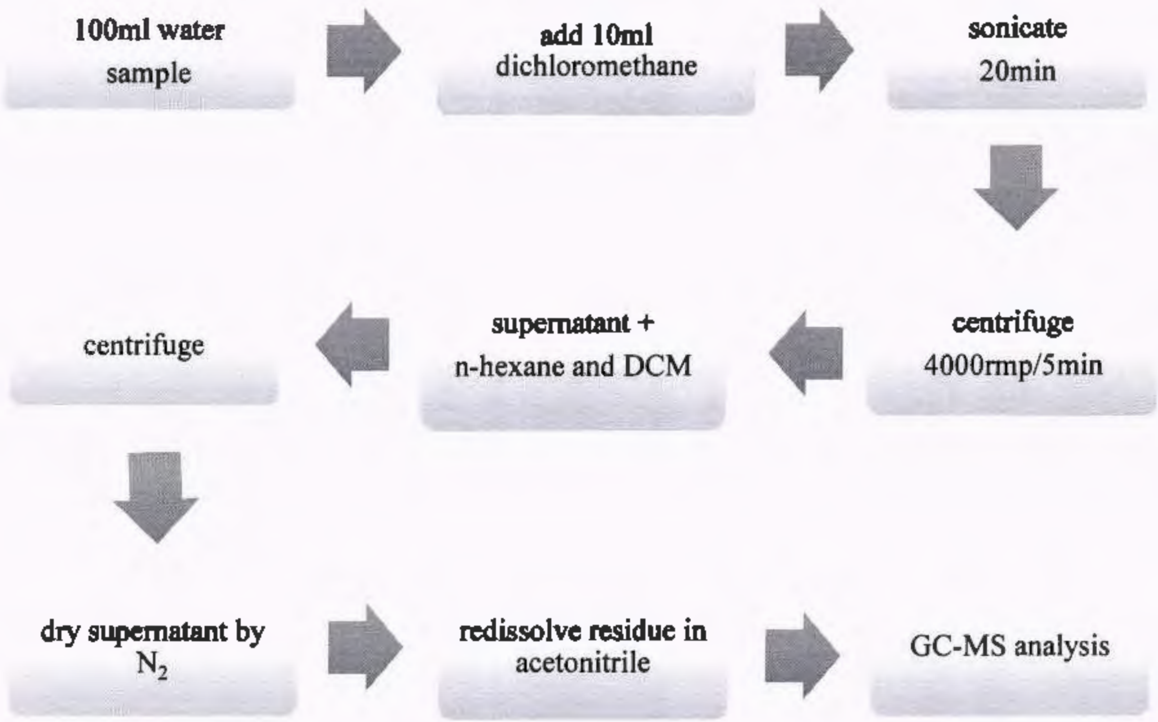

Figure 3.4.2: Flow chart for the assessment of HCH in water sample

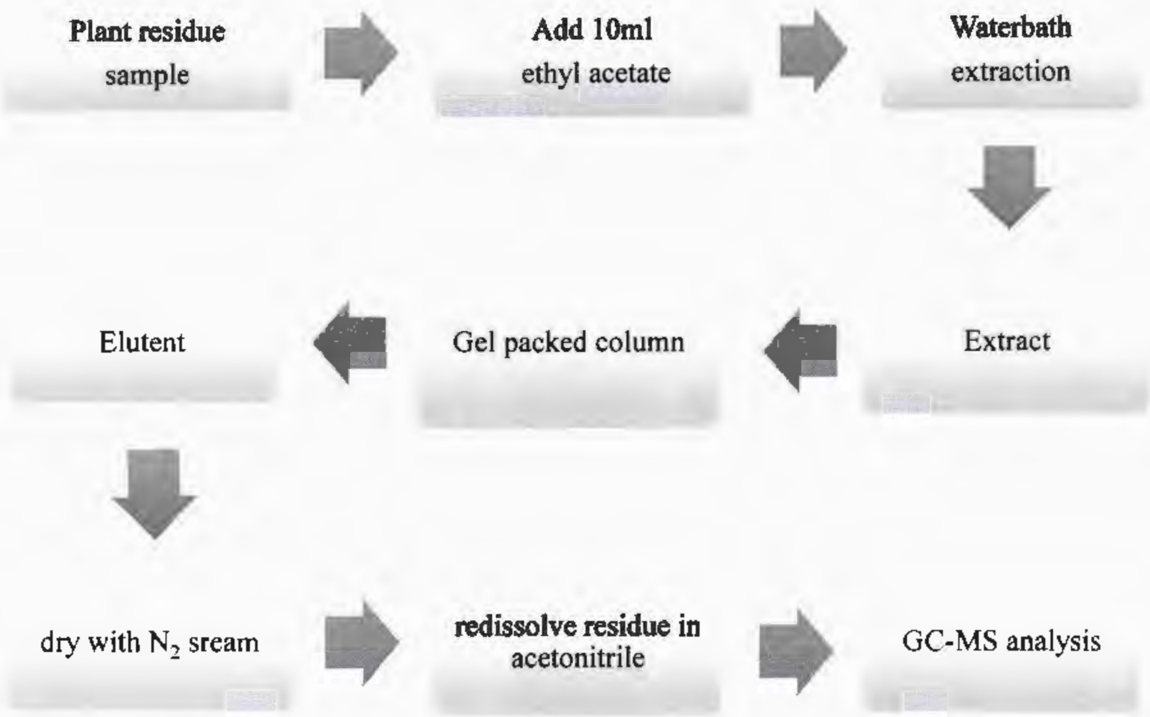

Figure 3.4.3: Flow chart for the assessment of HCH in plant residue

3.5 Instrumental analysis

For the analysis of hexachlorocyclohexane samples were run on Gas chromatography, mass spectrometry combined with Electron capture detector GC-MS-ECD (Malik *et al.*, 2011).

3.6 Quality Assurance

Quality control and quality assurance was ensured by maximizing recovery of standards and samples. 1 standard and 1 duplicate sample was analyzed on GC-MS and the process was repeated after every 10 samples for the validation of results.

3.7 Statistical analysis

Data was tabulated in Excel and SPSS (version 18) software was used for data analysis.

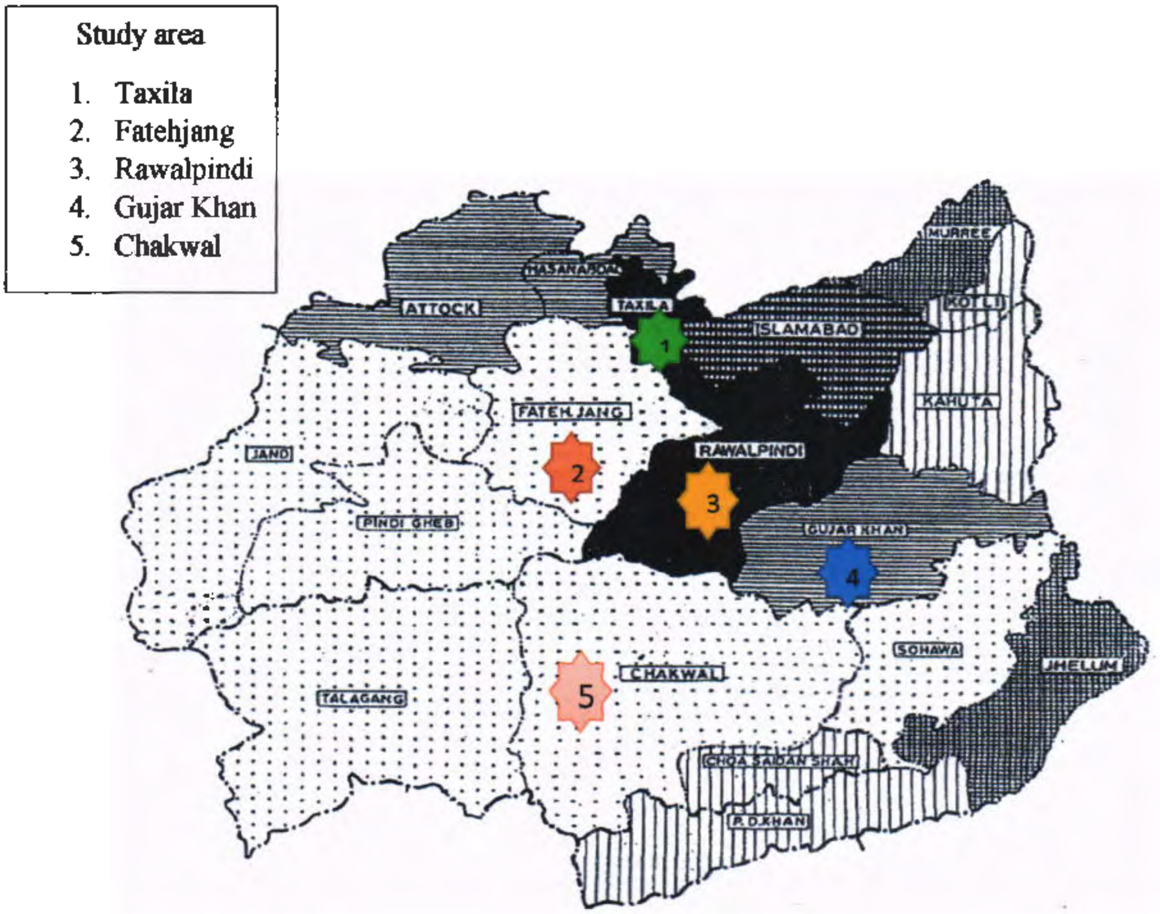

Figure 3.2: Map of Potohar

Source: GoP (1998) Census Report of Pakistan, Population Census Organization, Islamabad

RESULT AND DISCUSSION

RESULT AND DISCUSSION

Results of some basic Physico-chemical parameters of agricultural soil samples and irrigation water samples are discussed as follows:

4.1 Soil Parameters

4.1.1 Soil pH

Mean pH values of soil samples were between 5.8 and 8.4 shown in figure 4.1.1. Mean pH of Gangu, Jandyal and sirkap areas of Taxila were calculated as 6.33, 5.77 and 6.47. Whereas soil of Fatehjang shows slight basic pH, calculated pH values for Ghari hassu khan, Sadiqabad and Sadqal were 8.13, 8.32 and 8.37. In Gujjar Khan slight acidic pH was observed of Ugahoon, Sapiali and Guliana soils were 6.64, 6.97 and 5.76 respectively. Soil pH of Sankalan, Dhudial and Morian areas of Chakwal were 6.61, 6.32 and 7.07. Mean pH values of Bucha, Mandra and Moza arazi moari in District Rawalpindi were 6.13, 6.77 and 7.67.

Soil pH is the measure of acidity or alkalinity of soil. From the previous findings, pH higher than 8.4 or lower than 5.0 is not suitable for optimum plant growth and results in lower crop yield (Rai *et al.*, 2011). In the present study soil pH lies between 5.8-8.4 which is favorable pH for plant growth. Soil of Taxila, Gujjar Khan, Chakwal, Bucha and Mandra areas have a pH < 7.5 (fig.4.1.1) which is considered excellent for agriculture and supports high value crops, fruits and vegetables (Rehman *et al.*, 2010). Mean pH of soil is above 8 in Fateh Jang samples and similar result was reported by (Malik *et al.*, 2013; Shaheen *et al.* 2008). Soil of the semi-arid potohar plateau was derived from sandstone and loess parent material with fine particles, resulting in medium to coarse textured soil (Ali, 1967). Nature of soil is calcareous in pothar plateau (Brinkman, 1967) therefore results in high pH which may increase with soil erosion and exposing calcium carbonate content present in the subsoil (Shaheen *et al.*, 2008). Soil with pH above 8.5 needs suitable amendments (acid, gypsum) per the requirement for the reclamation purpose (Rehman *et al.*,2010). Soil pH of organic fields is higher as compared to conventionally managed fields (Nautiyal *et al.*, 2010; Drinkwater *et al.*, 1995; Reganold *et al.*, 1987)

4.1.2 Electrical Conductivity

Mean Soil electrical conductivity was between 1057- 1946 µs/cm (fig. 4.1.2). In Taxila highest EC 1946 µs/cm was observed in Jandyal samples whereas, EC of Gangu and Sirkap soil was 1331.67 µs/cm and 1072 µs/cm. Soil EC of Chakwal samples were 1447.33 µs/m and 1547 µs/cm in Sankalan and Moorian whereas EC of Dhudial soil was 1474.53 µs/cm. Mean EC values of Bucha and Moza arazi moari areas of dist. Rawalpindi were 1126 µs/cm and 1233 µs/cm whereas EC of Mandra sample was 1058.57 µs/cm which is lowest among all the samples. Highest EC (1942.67 µs/cm) was observed in Sapiali sample of Gujjar Khan whereas EC of Ugahoon and Goliana were 1057 µs/cm and 1291 µs/cm. Mean EC values of Ghari hassu Khan, Sadiqabad and Sadqal villages of Fatehjang were calculated as 1140.33 µs/cm, 1269.33 µs/cm and 1343.33 µs/cm respectively.

Soil EC is closely related to other soil properties such as, soil texture, salinity, cation exchange capacity CEC, organic matter level and soil drainage conditions. Potohar plateau is a rainfed region (Ashraf *et al.*, 2007) and two-third of the annual rainfall occurs during monsoon season (July-August) (Muhammad *et al.*, 2008). Salinity and sodicity is a common inherent problem in aird and semi-arid region of the world due to high temperature and less rainfall. Due to high temperature water moves upward and dissolved salts gradually accumulates at the surface of soil. Hence during dry winter season salts accumulates on the surface of ground and wash down to deeper layers during wet season (monsoon) (Hussain *et al.,* 2002). In Punjab, shallow water table and higher rate of evapotranspiration are major factors contributing to soil salinity i.e EC>4 ds/m as shown in (table.2). High EC values (salinity/sodicity) effects the physical and chemical properties of soil such as soil structure, accumulation of high sodium content in soil effects the plant growth (Muhammad *et al.,* 2008). Subsurface evaporation basin technique is proposed to benefit from monsoon and deep ploughing is suggested to reduce salts by leaching process and enhance productivity (Hussain *et al.*, 2002).

Table 2: Classification of soil into various classes of salinity/sodicity (Malik *et al.,* 1984)

Status	**EC ds/m**	**pH**
Normal	<4.0	<8.5
Saline	>4.0	<8.5
Saline-Sodic	>4.0	>8.5
Sodic	<4.0	>8.5

4.1.3 Total Organic Carbon

Mean values of Percentage of total organic carbon in Potohar samples were between 0.36-1.79% as shown in figure 4.1.3. Highest TOC value (1.37%, 1.63% and 1.79%) was observed in Gangu, Jandyal and Sirkap areas of Taxila. TOC values of Ugahoon, Sapiali and Guliana were calculated as 0.52%, 0.43% and 0.79%. Soil samples from Chakwal showed low values of TOC which were calculated as 0.36%, 0.52% and 0.62% in Sankalan, Dhudhial and Moorian areas. Total organic carbon percentage in Bucha (0.78%) and Moza arazi moari (0.56%) were lower than Mandra (1%) samples from dist. Rawalapindi. Whereas %TOC in Ghari hassu khan, Sadiqabad and Sadqal areas of Fatehjang were 0.74%, 0.64% and 0.77%.

Schumacher, (2002) states that total organic carbon is a chemical component of organic matter which indicates the presence of organic matter in soil. Organic carbon can be in the form of freshly deposited leaves or branches or it can be in a highly-decomposed form such as humus. In Punjab, organic carbon is generally less than 1% (Azam, 1988), Total organic carbon was higher in Taxila soil due to the fresh input of cow manure to enhance soil fertility and growth of cultivated vegetables (spinach, bitter guard, pumpkin and zucchini). Whereas in other sampling areas major crops were cultivated (Maize, peanut and pear millet) under natural soil conditions with low TOC content.

4.1.4 Soil Organic Matter

Mean values of Percentage of organic matter of Potohar samples is shown in figure 4.1.4. Highest SOM (2.36%, 2.81% and 3.08%) was observed in Gangu, Jandyal and Sirkap, Taxila. Minimum value of SOM i.e. 0.62 was observed in Sankalan, Chakwal whereas Dhudial and moorian samples showed 0.90% and 1.07% of SOM. Fatehjang sites were relatively rich in SOM, mean values of SOM obtained in Ghari hassu khan, Sadiqabad and Sadqal samples were (1.27, 1.13 and 1.17) %. Gujjar Khan samples had mean SOM values of 0.88%. 0.67% and 1.29% in Ugahoon, Sipiali and Guliana.

In Pakistan, soil nutrients available for optimium plant growth are mostly insufficient for maximum crop yield (Rafiq, 1996; Khalid *et al.*, 2012). Semi-arid lands have poor microbial activity (Garcı´a *et al.*, 1994a), therefore microbial biomass is low and soil is poor in organic matter (Garcı´a-Gil *et al.*, 2000). Potohar being semi-arid region receives less rainfall, low soil moisture content reduces soil microbial activity and soil becomes poor in organic matter. On the other hand, during wet season (July-September) rate of microbial activity increases and SOM content is enhanced. Some reasons behind the decline of SOM in Potohar includes nutrient mining by repeated cropping, water erosion, insufficient use of fertilizers and crop residues not being recycled (Shaheen *et al.*, 2008). Organic matter content in semi-arid soils is mostly less than 1% (Muhammad *et al.*, 2008). Khalid *et al.* (2008) reported only 10% of soils samples from Chakwal with SOM above 1%. Another study by Sarwar *et al.* (2008) in Pindi Bhatian reports OM of control soil as 0.56%. which is <1%. In the present study SOM in Fateh Jang was observed low, similar results were reported by Shaheen *et al.*, (2008) in a study conducted in Fatehjang. SOM in Taxila sample were high due to the fresh input of cow manure in vegetable fields. Soil fertility can be enhanced by adding compost, crop residues like wheat and rice straws after harvest can be used as compost instead of burning them. Municipal solid waste and cow manure can also be utilized to make composts for soil fertility and crop productivity (Sarwar et al., 2008).

4.1.5 Microbial Biomass Carbon

Mean microbial biomass carbon of all five areas is shown in figure 4.1.5. Soil samples from Taxila have a mean MBC value as 0.53mg/kg, 1.03mg/kg and 1.18mg/kg in Gangu, Jandyal and Sirkap areas. MBC values of Gujjar khan samples were lower than Taxila and Chakwal samples, mean value of microbial biomass carbon in Ugahoon, Sapiali and Goliana were 0.7mg/kg, 0.72mg/kg and 65mg/kg. Samples from Sankalan, Dhudhial and Moorian villages of Chakwal were calculated for mean MBC as 88.mg/kg, 0.73mg/kg and 0.92mg/kg. District Rawalpindi samples showed mean MBC value as 0.77mg/kg, 1.04mg/kg and 0.8mg/kg in the areas of Bucha, Mandra and Moza arazi moari. MBC of Fatehjang samples were as 1.1mg/kg, 0.72mg/kg and 0.89mg/kg in Ghari hassu khan, Sadiqabad and Sadqal villages.

In the present study, the overall soil MBC is very low in all five areas studied. MBC of Taxila is higher compared to other sites because of cow manure present in the soil at the time of sampling, Malik *et al.* (2013) reports increase in microbial biomass carbon by incorporating organic amendments in soil. The soil of Potohar plateau is known to have low OM content and insufficient plant nutrients like nitrogen (N) and Phosphorous (P). Potohar region receives two third of the annual rainfall during monsoon (July-September) and suffers from drought stress afterwards. Due to insufficient soil moisture, fertilizers cannot be used to supplement nutrient deficiencies of soil, under such condition nutrients stored in and released by soil microbes becomes important for crop yield (Srivastava and Singh,1991). In the subtropical dry farming (rain-fed) regions like potohar there is a lack of information regarding the role of soil microbes as a sink and source of plant essential nutrients (Khan & Joergensen, 2006). Incorporating organic amendments in soil can stimulate microbial activity in soil as well as enhances plant growth (Malik *et al.*, 2013).

4.1.6 Soil Texture

Soil of Potohar region is derived from sandstone and loess parent material and contain relatively high proportion of fine particles, therefore soil of the region is medium to coarse textured (Ali, 1967). Soil texture of Gujjar Khan and Fatehjang are (medium textured) loam (Table.3), these soils are capable of cultivation and supports all common crops (Khalid *et al.*, 2012). Soil of Chakwal and Rawalpindi are (light textured) sandy clay loam (Table.3) these soils have low water holding capacity, thus for the improvement of the physical conditions of such soils incorporation of farm yard manure is needed (Khalid *et al.*, 2012). Soil of Taxila is heavy textured, clay loam (Table.3) and is derived from shale, such soils have relatively high EC values because of greater pore size than sandier soil (Grisso *et al.*, 2009).

4.1.7 Soil Moisture

Soil moisture is directly related to soil texture, highly textured soils have low water holding capacity. Clay soil is relatively rich in SM compared to sandy soils due to sufficient water holding capacity, microbial activity is high during wet season and enhances SOM.

Table 3: Soil texture and soil moisture content of five different areas of Potohar region

Area	Sand (%)	Silt (%)	Clay (%)	Soil Texture	Soil moisture
Taxila	40.2	30	29.8	Clay loam	22.5%
Gujjar Khan	39.9	42.3	17.8	Loam	13.5%
Chakwal	50.2	22	27.8	Sandy clay loam	15%
Rawalpindi	50.9	23.3	25.8	Sandy clay loam	11.72%
Fatehjang	42.2	37.9	19.9	Loam	10%

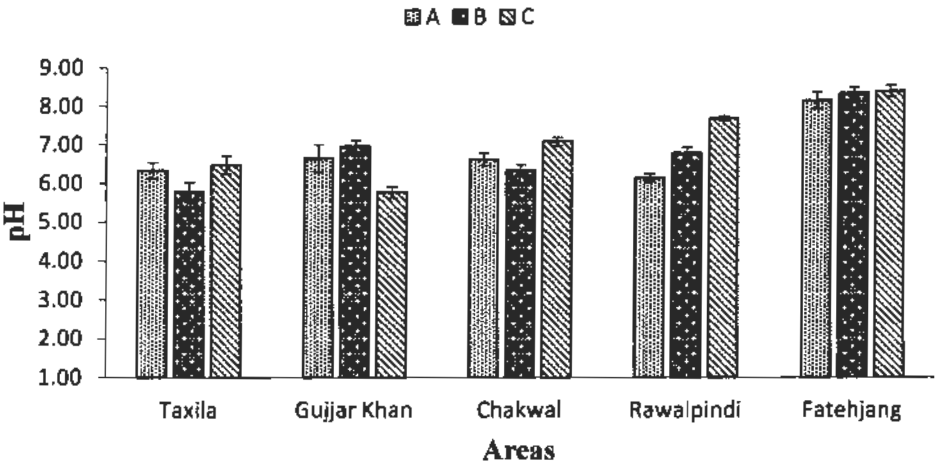

Fig 4.1.1: pH of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliaana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

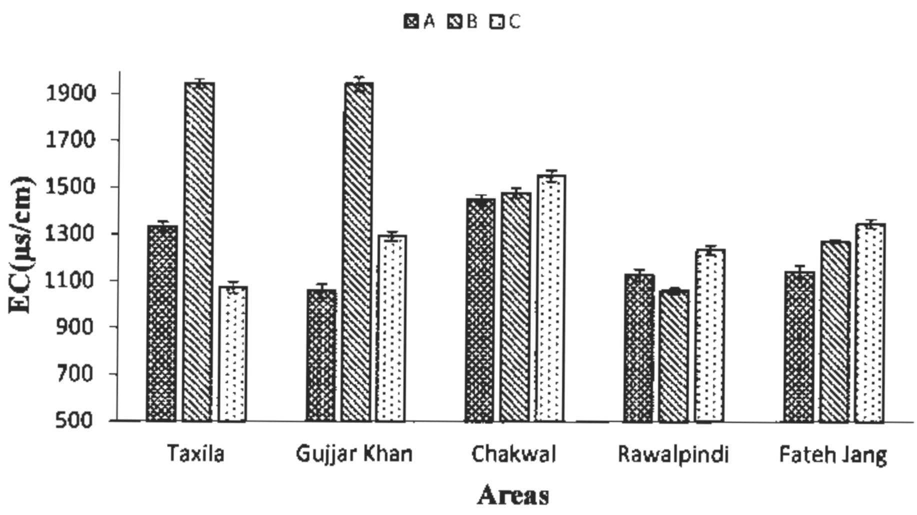

Fig 4.1.2: Electrical conductivity of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Ugahoon, B: Sapiali, C: Guliana, Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

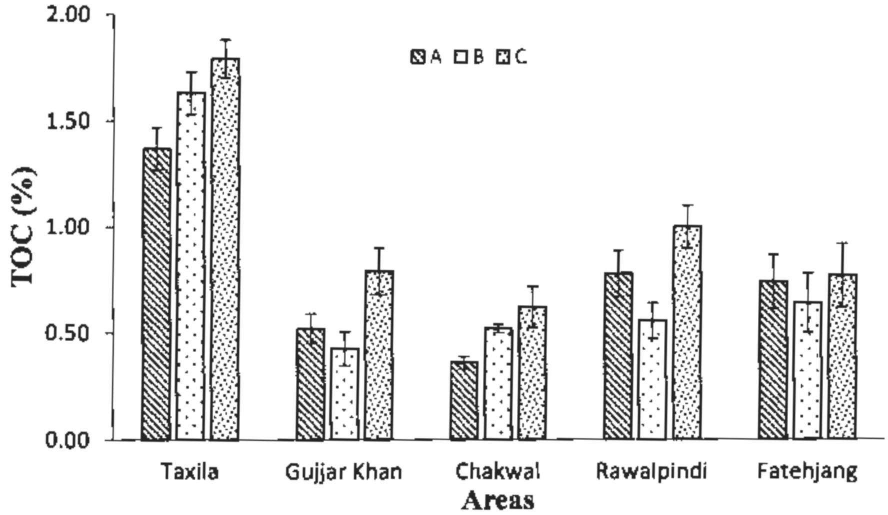

Fig 4.1.3: TOC of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliana) Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

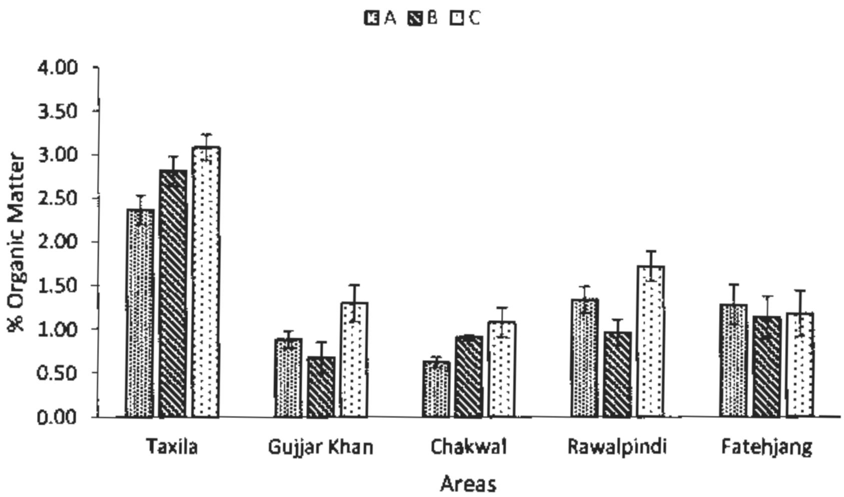

Figure 4.1.4: Organic matter of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

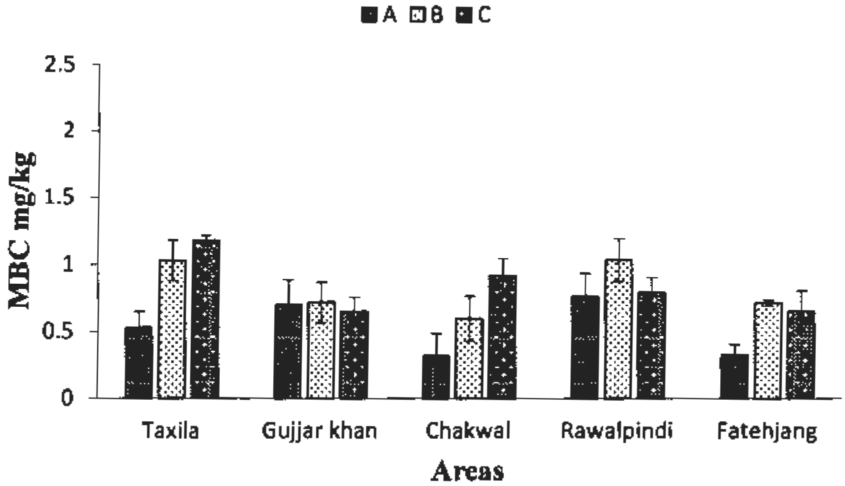

Figure 4.1.5: Microbial biomass carbon of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

4.2 Water Parameter

Different parameters of water analyzed are as follows:

4.2.1 pH

Mean pH of water samples from potohar are shown in figure 4.2.1. Highest mean pH value 8.88 was observed in Morian water sample from Chakwal whereas lowest mean pH 6.95 was observed in Sadiqabad sample from Fatehjang. Mean pH of Taxila samples were 7.2, 7.47 and 7.61 in Gangu, Jandyal and Sirkap villages. Mean pH values of Gujjar Khan water samples were calculated as 7.7, 7.79 and 7.48 at Ugahoon, Sipiali and Gliana areas. In Chakwal mean pH value of Moorian water sample was higher (8.8) than Sankalan (7.71) and Dudhial (7.71) water samples. Mean pH values of Rawalpindi samples were calculated as 7.1, 7.44 and 7.88 in Bucha, Mandra and Moza arazi moari waters. Mean pH of Ghari hassu khan and Sadqal were 7.65 and 7.77 whereas pH of Sadiqabad sample was 6.95 relatively less than other two areas of Fatehjang.

pH is an essential parameter for testing water quality and chemistry (Shahid *et al*., 2015). Higher pH has no direct effect on human health but it has some indirect effects on health by changing water quality parameters such as pathogen survival and solubility of metals. However, water with higher pH values tastes bitter (US EPA, 1977). pH of Water samples in the present study ranges 6.65-8.8 whereas standard limit by World Health Organization (WHO) and National Standards for drinking water quality (NSDWQ) is pH 6.5-8.5 (Table.4). A sample taken from a hand pump from Chakwal district (pH 8.8) is exceeding the set limit, similarly Muhammad *et al*. (2010) reported groundwater pH 7.56-8.5 whereas, all the other samples collect from Potohar region were within the standard limits of WHO and NSDWQ. Similar work was reported by (Jalil & Khan, 2012; Shahid *et al.*, 2015; Muhammad *et al.*, 2010) in their respective researches.

4.2.2 Electrical Conductivity of Water

Electrical conductivity of water samples is shown in figure 4.2.2. Highest EC values (1561.69µs/cm, 1359.01µs/cm and 1860.01µs/cm) were observed in Sankalan, Dhudial and Moorian villages of Chakwal. Mean EC values were relatively low in Rawalpindi samples 591.01µs/cm, 461.02µs/cm and 705.01µs/cm in Bucha, Mandra and Moza arazi moari. Mean EC of water in Gangu, Jandyal and Sirkap were 768.02µs/cm, 373.01µs/cm and 1207.04µs/cm among all the samples least EC (373.01 µs/cm) was of observed in Jandyal water in Taxila. Electrical conductivity of Gujjar Khan water samples was 1208.03µs/cm, 1570.02µs/cm and 693.01µs/cm in Ugahoon, Sapiali and Goliana areas. Mean EC value of Fatehjang water samples were 1035µs/cm, 1145µs/cm and 986µs/cm in Ghari hassu khan, Sadiqabad and Sadqal village.

Electrical conductivity is an easier way of determining concentration of electrolytes in water containing mineral salts. In the present study, EC ranges between 373.01-1860.01 µs/cm whereas drinking water standard by WHO and NDSWQ is <1000 µs/cm (Table.4). Samples collected from underground sources showed the highest EC >1000. Among the 15 water samples 8 samples were exceeding the standard limits, while 7 samples were within the standard limits. Water electrical conductivity values below 0.75 ds/m is considered acceptable for irrigation and will have no adverse effect except salt sensitive crops. However higher EC values of water are not safe and will cause salinization (Waheed *et al.*, 2010).

4.2.3 Total Dissolved Solids of Water (TDS)

Total dissolved solids of water in Potohar region are shown in figure 4.2.3. Highest mean TDS was observed in Chakwal samples with 968.47mg/l, 842.59mg/l and 1153.22mg/l in Sankalan, Dhudial and Moari water. Lowest mean TDS 231.25mg/l was calculated in Jandyal water sample while the rest of two sites in Taxila have mean TDS values as 491.53mg/l in Gangu and 760.34mg/l in Sirkap water sample. Gujar Khan samples had TDS values as 760.96mg/l at Ugahoon, 326.77mg/l in Sipiali and 429.65mg/l in Goliana areas. Water samples from Rawalpindi showed relatively low mean TDS values such as 244.40mg/l in Bucha, 285.78mg/l in Mandra and 437.03mg/l in Moza arazi moari. Mean TDS values of Fatehjang water samples were 641.63mg/l, 709.79mg/l and 611.31mg/l at Ghari hassu khan, Sadiqabad and Sadqal villages.

Concentration of TDS depends on CO_3, HCO_3, Cl, K, NO_3, SO_4, Na, Ca and Mg in water (Muhammad et al., 2010). TDS has secondary impact on human health, TDS is an indicator for high concentration of hazardous substances in water such as., bromide, sulfide and arsenic, which can cause deleterious effects on health (Jalil & Khan, 2012). Highest TDS was observed in Chakwal ground water samples (968.47mg/l, 842.59mg/l and 1153.22mg/l) exceeding the drinking water permissible limits set by WHO and NSDWQ (Table 4). Among the 15 samples collected 8 water samples had a TDS value >500 mg/l which is standard limit by WHO (Table 3). However, per NSDWQ (Table.4) only 1 water sample from Chakwal was found with TDS value >1000mg/l, similar results were reported (Jalil & Khan, 2012; Muhammad *et al.,* 2010). Ground water quality in Lahore was observed by Shahid & Iqbal, (2016). maximum TDS values were exceeding the permissible limit by WHO and NSDWQ for safe drinking water (Table.4).

4.2.4 Alkalinity of Water

Mean Alkalinity of water samples from five major sites in potohar region is shown in figure 4.2.4. Highest alkalinity was recorded in Sankalan (450.83 ppm) Dist. Chakwal and Bucha village (450 ppm) of Rawalpindi. Lowest alkalinity (257.17 ppm) was recorded in Guliana water sample from Gujjar Khan, whereas, alkalinity of other two sites Ugahoon and Sapiali were 275.69 ppm and 350.83 ppm. Alkalinity of Jandyal (270.5 ppm) sample was less than Gangu (350.7 ppm) and Sirkap (360.53 ppm) in Taxila. Alkalinity of water samples from Fatehjang were recorded as 350.15 pm, 350.69 ppm and 400.71 ppm in Ghari hassu khan, Sadiqabad and Sadqal areas respectively. Alkalinity recorded in samples of Mandra and Moza arazi moari were 395 ppm and 420.35 ppm repectively. Similar work was reported by Shahid & Iqbal, (2016) in a ground water case study in Lahore. There is no defined limit for alkalinity of water by WHO and NSDWQ.

4.2.5 Total Hardness of Water

Total hardness of water samples is shown in figure 4.2.5. Highest value of total hardness (448.33 ppm) was observed in Sadqal water sample whereas, in Ghari hassu khan and Sadiqabad it was recorded as 350.92 ppm and 390.5 ppm respectively. Lowest total hardness of water (250.58 ppm) was observed in Morian village of Chakwal whereas, in other two samples collected from Sankalan and Dhudial it was recorded as310.04 ppm and 420.1 ppm respectively. Total hardness of water in Taxila samples were 400.75 ppm, 370.67 ppm and 310.33 ppm in Gangu, Jandyal and Sirkup respectively. Water samples collected from Gujjar Khan had a total hardness calculated as 251.01ppm, 350.83 ppm and 350.15 ppm in Ugahoon, Sapiali and Guliana respectively. Total hardness of water calculated in Bucha and Mandra samples was 290.33 ppm and 251 ppm respectively which is very much less than Moza arazi moari sample (421.01 ppm), Rawalpindi. Calculated total hardness in all the samples were less than 500mg/l, the drinking water limits of NSDWQ, 2008 (Table 4). Total hardness recorded in the present research is less than the maximum hardness (480 and 784ppm) calculated in ground water of Lahore (Shahid & Iqbal, 2016) and very high than that reported by Shahid *et al*., 2015 in drinking water of Punjab.

Table 4: Drinking water Guidelines by WHO and NSDWQ 2008

Parameters	**NSDWQ/EPA**	**WHO**
pH	6.5-8.5	6.5-8.5
EC µs/cm	<1000	<1000
TDS mg/l	<1000	<500
Alkalinity	No standard	No standard
Total hardness ($CaCO_3$)	500mg/l	---------------

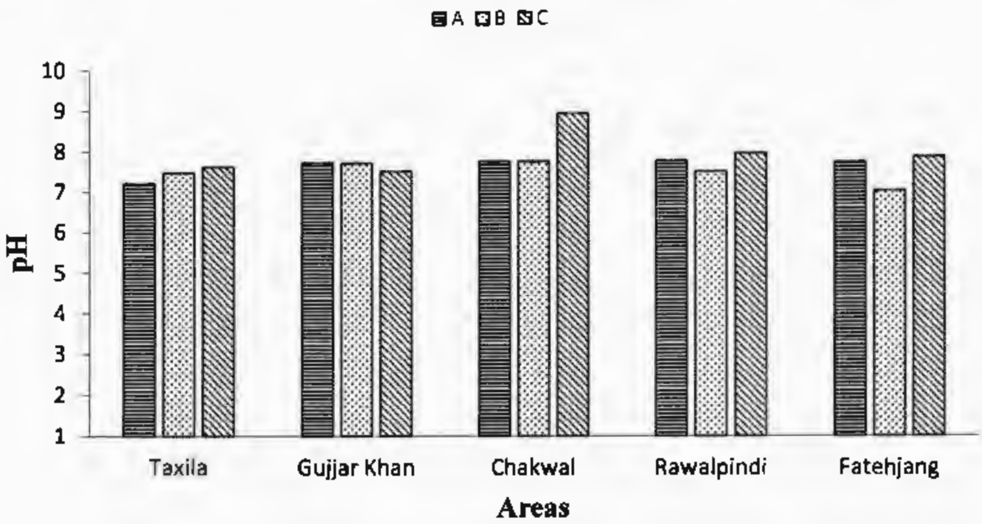

Figure 4.2.1: Water pH of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

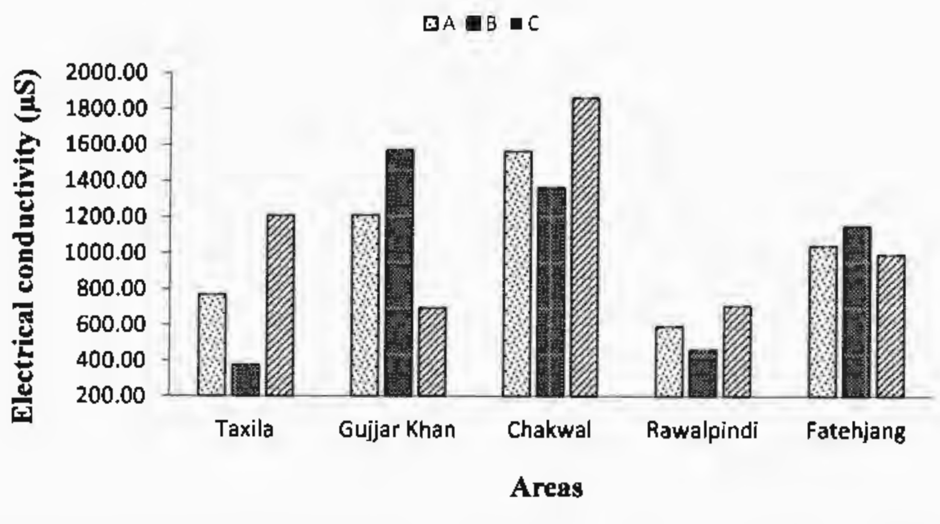

Figure 4.2.2: Water Electrical conductivity of Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Uugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

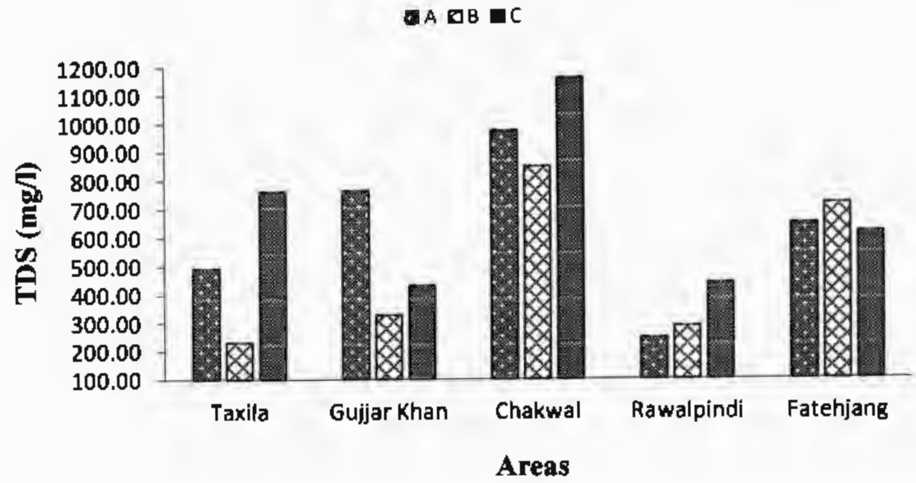

Figure 4.2.3: Total dissolved solids of water in Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Ugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal) samples

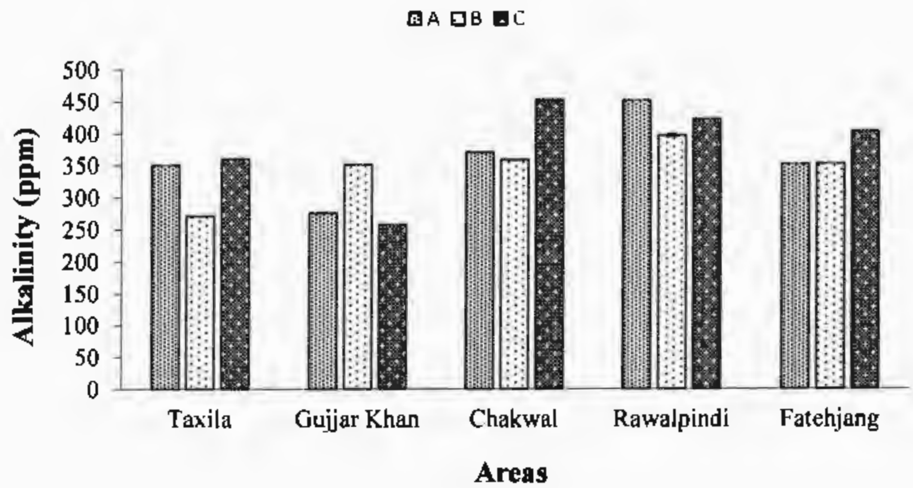

Figure 4.2.4: Alkalinity of water samples from Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Ugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

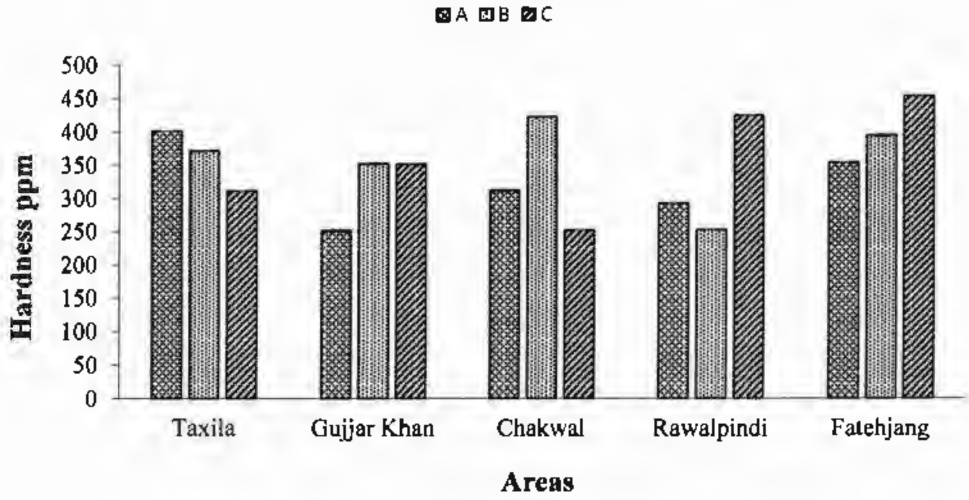

Figure 4.2.5: Total Hardness of water samples from Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Ugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

4.3 HCH Assessment in Soil

The concentration of hexachlorocyclohexane detected in 45 different agricultural soils are shown in figure 4.3. The concentration of HCH in Taxila soil was assessed as 2.43 µg/g in Gangu, 5.74 µg/g in Jandyal and 3.8 µg/g in Sirkap. Whereas concentration of HCH in Gujar khan were (2.99 µg/g, 4.36 µg/g and 5.06 µg/g respectively) and Chakwal samples were assessed as (4.96 µg/g, 5.65 µg/g and4.9 µg/g respectively). Hexachlorocyclohexane concentration detected in Rawalpindi samples (2.74 µg/g, 3.09 µg/g and 4.4 µg/g) were relatively less than other areas. The HCH concentration detected in Fateh Jang soil was 8.8 µg/g in Ghari hassu khan, 4.88 µg/g in Sadiqabad and 7.53 µg/g in Sadqal which were highest recorded values among all samples.

Pakistan is ranked second among the South Asian countries on the basis of pesticide consumption (Randhawa *et al.*, 2007). In the present study, value of HCH concentration in soil ranges from 2.43 µg/g – 8.88 µg/g which is higher than Companian Plains of Southern Italy (Qu *et al.*, 2016), higher than 14.2µg/kg – 41.0µg/kg and 0.0364 ng/g reported in agricultural soil of China and Tibetan plateau respectively (Liu *et a*l., 2016 & Wang *et al.*, 2016) and greater than reported in agricultural soil of Wuhan, central China 100.58 ng/g (Zhou *et al.*, 2013). Similarly, residue levels determined in the present study are much higher than those previously reported from Pakistan (Mahmood *et al.*, 2014; Syed *et al.*, 2013; Sultana *et al.*, 2014). However, residue level of HCH in Rawalpindi soil samples is comparable with those of obsolete pesticide dumping site in Hyderabad 43-4090 ng/g (Almadar *et al.*, 2014). The HCH concentration reported in soil and water of obsolete pesticide stores in Pakistan (Ahad *et al.*, 2009) is far greater than those assessed in the present study. Also, the residue levels assessed in contaminated sites of Southwestern of China with mean values of 19.1 mg/kg and 41 mg/kg (Fang *et al.*, 2017) is greater than

HCH concentration detected in the present study. According to China National Environmental Protection Agency the grade II limit for HCH in agricultural soils is 50ng/g which is considered less strict (Sun *et al.*, 2016). If compared to the present study all the soil samples detected for HCH were exceeding the limit assigned by China EPA (2008). Almost all the soils samples were collected from planted soils this might be the reason for higher residue levels of HCH in the study area due to pesticide application.

4.4 Assessment of Hexachlorocyclohexane in Water

The residue levels of Hexachlorocyclohexane were assessed in water samples collected from 15 different water sources in agricultural areas of Potohar. The concentration of HCH detected in all the samples are shown figure 4.4. Highest residue levels were detected in Fateh Jang samples as 3.1 µg/g, 5.87 µg/g and 3.87 µg/g in tap water, hand-pump and tube-well samples respectively. Chakwal samples were detected with higher HCH levels in tube-well (2.79 µg/g) and hand pump (3.98 µg/g & 2.09 µg/g) sources. Whereas HCH residues were not detected in two of samples from Gujar khan while the third sample collected from a hand-pump had a concentration of HCH as 2.98 µg/g. In Rawalpindi, the value of HCH in a tap water sample was 3.68 µg/g Whereas, no HCH residues were detected in the other two samples. Similarly, HCH concentration in Taxila samples were 0.13 µg/g and 2.01 µg/g collected from canal and hand pump respectively.

The residue level of HCH in water samples ranges from nd-5.87 µg/g. According to ATSDR (2005) the concentration of HCH (lindane) consumed by children through water for the lifetime should not exceed 0.0002mg/l. Whereas, the Maximium Allowable Concentration (MAC) by European Union for lindane is 0.1µg/l (Ahaad *et al.*, 2009). However, in the present study none of the samples detected with HCH lies within the limits of ATSDR and EU. Hexachlorocyclohexane residues assessed in the water samples in the present study were higher than those reported in Lake Chaohu, China 1.58-31.66 ng/l (Qin *et al.*, 2012), Biebu Gulf and its tributary rivers in China (Xu *et al.*, 2013), riverine runoff of the Pearl River Delta, China (Guan *et al.*, 2009), Minjiang River Estuary, Southeast China (Zhang *et al.*, 2003) and freshwater sites in and around the city of Amsterdam (Verweij *et al.*, 2004). Whereas, some similar results were reported by Ahad *et al.*, (2009) in Monitoring results for organochlorine pesticides in soil and water from selected obsolete pesticide stores in Pakistan.

4.5 Hexachlorocyclohexane Assessment in Plant Residues

Residue levels of hexachlorocyclohexane were analyzed in 15 plant residue samples collected from Potohar region and the results are shown in figure 4.5. In Taxila the Residue levels of HCH detected in *Lagenaria siceraria, Zea mays* and *Spinacia oleracea* were 0.99 µg/g, 1.68 µg/g and 2.99 µg/g respectively. The residue levels of HCH detected in Chakwal samples were 3.1 µg/g, 2.56 µg/g in *Zea mays* and 3.76 µg/g in *Spinacia oleracea*. The concentration of HCH assessed in Rawalpindi samples were slightly higher than other plants which were 4.87 µg/g in *Citrullus lanatus*, 2.98 µg/g and 2.1 µg/g in *Zea mays* respectively. Whereas, in Gujar khan and Fateh Jang HCH residues were not detected in any of the plant residue.

In the present study residue concentration of HCH in plant residue samples ranges from nd-4.87 µg/g which is higher than 2.0 µg/kg and 3.4 µg/kg reported in walnut and pinenut, China (Liu *et al.*, 2016). Organochlorine pesticide in pine needles in North Xingjang and reported in the Southeast Tibaten Palteau (Lei *et al.*, 2015; yang *et al.*, 2008) were very low in concentration than that of present study. The residue concentration of HCH *Zee* *mays* is 92.86 ng/g in Nigeria (Sojinu *et al.*, 2012) is less than all the detected values for *Zee mays* (1.68, 3.1, 2.5, 2.98 and 2.1) µg/g in the present study. Although the planted soil samples of Gujar khan and Fateh Jang had shown the residues of HCH but no HCH was detected in their plant sample, similar results were reported when the relationship of pesticide contaminated soil and its fruits were analyzed in China (Liu *et al.*, 2016). In the present study residue concentration of HCH detected in all plants samples were exceeding the MRL of European Union (2016) as shown in Table 5.

Table. 5 Maximum residue limit of HCH (mg/kg) in different plants. (EU, 2016)

Scientific name	Common name	MRL of HCH	Annexes
Lagenaria siceraria	Calabash gourd	0.01	II, III B & V
Zea mays	Corn	0.01	II, III B & V
Spinacia oleracea	Spinach	0.01	II, III B & V
Momordica charantia	Bitter gourd	0.01	II, III B & V
Citrullus lanatus	Watermelon	0.01	II, III B & V
Pennisetum glaucum	Pearl millet	0.02	II, III B & V
Arachis hypogaea	Peanut	0.02	II, III B & V

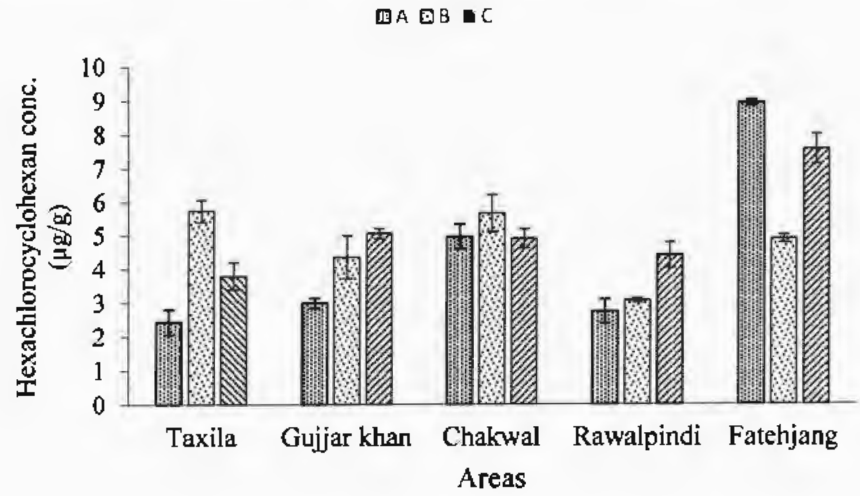

Figure 4.3 Concentration of HCH in agricultural soil samples from Taxila (A: Gangu, B: Jandyal, C: Sirkap), Gujjar Khan (A: Ugahoon, B: Sapiali, C: Guliana), Chakwal, (A: Sankalan, B: Dhudial, C: Morian), Rawalpindi (A: Bucha, B: Mandra, C: Moza arazi moari), Fatehjang (A: Ghari hassu khan, B: Sadiqabad, C: Sadqal)

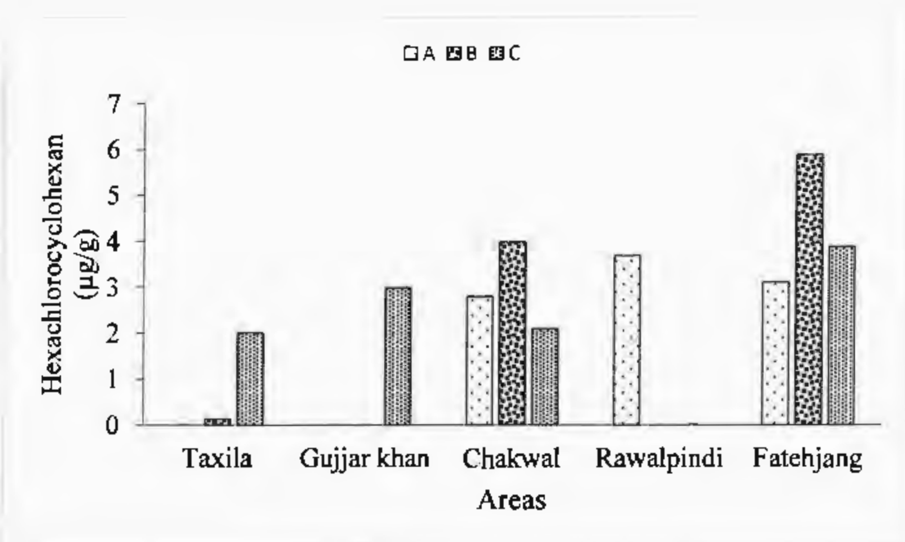

Figure 4.4 Concentration of HCH in water samples from Taxila (A: tube-well, B: canal, C: hand-pump), Gujjar Khan (A: tube-well, B: hand-pump, C: hand-pump), Chakwal, (A: tube-well, B: hand-pump, C: hand-pump), Rawalpindi (A: tap-water, B: tap-water, C: hand-pump), Fatehjang (A: tap-water, B: hand-pump, C: tube-well)

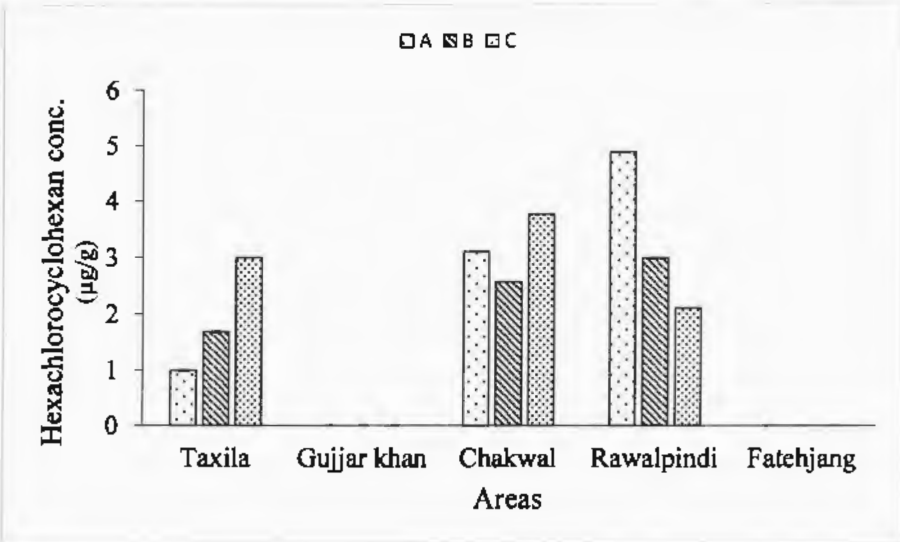

Figure 4.5 Concentration of HCH in plant residue samples from Taxila (A: *Lagenaria siceraria*, B: , *Zea mays*, C: *Spinacia oleracea*), Gujjar Khan (A: *Momordica charantia*, B: *Spinacia oleracea*, C: , *Zea may*), Chakwal, (A: , *Zea mays*, B: , *Zea mays*, C: *Spinacia oleracea*), Rawalpindi (A: *Citrullus lanatus*, B: , *Zea mays*, C: , *Zea mays*), Fatehjang (A: , *Zea mays*, B: *Pennisetum glaucum*, C: *Arachis hypogaea*)

Conclusion

In the current study residue levels of HCH were investigated in soil, water and plant residue samples collected from Potohar region. Hexachlorocyclohexane was used in the formulation of Pesticides and later declared as persistent organic pollutant in 2009 in Stockholm Convention. Unfortunately, all the soil samples investigated in the study were contaminated with HCH, these samples were collected from planted soils therefore there is a huge risk of HCH entering the food chain. Among the 15 water samples analyzed 10 samples were contaminated with HCH and the concentrations were exceeding the standard limits of ATSDR and Maximum Allowable Concentration by European Union. Some of the samples were collected from drinking water sources which poses a serious threat to the local biodiversity. The plant residue samples collected from agricultural soils and assessed for HCH levels were also contaminated, this shows the pesticide uptake capability of certain plant species under favorable conditions. Out of 15 plant samples 9 samples were highly HCH contaminated and exceeded the MRL of EU. However, plants from Gujar khan and Fateh Jang were found un-contaminated which is a good indication for safe food. The present study suggests a poor enforcement of Environmental laws in the area which in addition to aged contamination results in a relatively high level of Hexachlorocyclohexane than in other developing countries.

Recommendation

- Further research and investigation is needed to determine the potential risks associated with Hexachlorocyclohexane present in different environmental compartments of the Potohar region
- Stringent measures should be taken for the implementation of Environmental laws for the protection of environment and biodiversity
- Suitable remediation strategies should be adopted to minimize the impacts contamination on the biodiversity

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