Norwegian University
of Life Sciences

Master's Thesis 2021 60 ECTS
Faculty of Biosciences

Determination of organochlorine pesticides in the fish from freshwater lakes of Pakistan

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Master Ecology

Acknowledgement

I humbly offer the gratitude to the Almighty who is the Lord of heavens and earth and what is between them.

This master thesis was written under the supervision of Professor Mr. Ole Martin Eklo of the Faculty of Biosciences NMBU and Dr. Shaukat Ali of the Department of Zoology, Government College University Lahore, Pakistan.

I would cordially thank Mr. Ole for his kind support and supervision throughout my research and thesis writing. I am also grateful to Mr. Shaukat Ali for his support during my field work in Pakistan.

I also acknowledge my family for their continuous financial and moral support during my research.

Fahad Ali Kazmi

Oslo, March 2021.

Abstract

This study is a quantitative analysis of selected organochlorine pesticides in the muscle tissues of fish and water samples from Tarbela (34° N, 72° E) and Sukkur (27°N, 68°E); two major lakes with water from the river Indus as the main source. The study also included a survey to inspect the awareness and attitudes of farmers towards the use of organochlorine pesticides in special areas linked with sampling sites. Extraction and cleanup procedure included QuEChERS which was followed by gas chromatography-mass spectroscopy. Farmers residing in the vicinity of sampling sites were interviewed with a 6-question binary questionnaire. From Tarbela lake 0.019 mg/kg wet weight of dichlorodiphenyldichloroethylene (DDE) was found in the muscle sample of silver carp (*Hypophthalmichthys molitrix*) while 0.015 mg/kg wet weight of endosulfan was detected in the muscle sample of Mahseer (*Tor putitora*). From Sukkur barrage 0.047 mg/kg wet weight of endosulfan was detected in Rohu/Dumbra (*Labeo rohita*). All other samples of fish did not contain the detectable quantities of OCPs. Water from both lakes contained 0.004 mg/L of endosulfan. For the survey part, study found that farmers of age greater than 30, farmers with experience more than 10 years, and those who were literate, used pesticides more frequently and have used dichlorodiphenyltrichloroethane (DDT) more than the farmers of age less than 30, farmers with experience less than 10 years and illiterate farmers. Farmers of age less than 30, literate ones, and those having experience less than 10 years showed greater knowledge about pesticides and their hazards as well as better attitudes towards the environment than the corresponding groups. Type of crop production of the farmers and the pesticide training did not have any significant impact on the facts, knowledge, or attitudes of the farmers. The results from this study however have a degree of uncertainty due to the small number of fish and water samples. This resulted into the insignificance of the correlations. Also, the mistrust between government officials and the farmers can impact the results of the survey.

Sammendrag

Denne studien er en kvantitativ analyse av utvalgte organoklorpesticider i muskelvevet til fisk og vannprøver fra Tarbela (34 ° N, 72 ° Ø) og Sukkur (27 ° N, 68 ° Ø); to store innsjøer med vann fra elven Indus som hovedkilde. Studien inkluderte også en undersøkelse for å inspisere bøndenes bevissthet og holdninger til bruk av organoklorpesticider i spesielle områder knyttet til prøvetakingssteder. Ekstraksjons- og renseprosedyre inkluderte QuEChERS med etterfølgende gasskromatografi-massespektroskopi. Bønder bosatt i nærheten av prøvetakingssteden ble intervjuet med et spørreskjema med 6 spørsmål. Fra Tarbela-sjøen ble 0,019 mg/kg våtvekt av diklordifenyldikloretylen (DDE) funnet i muskelprøven av sølvkarpe (Hypophthalmichthys molitrix) mens 0,015 mg / kg våtvekt av endosulfan ble påvist i muskelprøven til Mahseer (Tor putitora). Fra Sukkur demningen ble 0,047 mg/kg våtvekt av endosulfan påvist i Rohu/Dumbra (Labeo rohita). Alle andre prøver av fisk inneholdt ikke påviselige mengder OCP. Vann fra begge innsjøene inneholdt 0,004 mg/L endosulfan. For undersøkelsesdelen fant studien at bønder over 30 år, bønder med erfaring mer enn 10 år, og de som kunne lese og skrive, brukte plantevernmidler oftere og har brukt mer diklordifenyltrikloretan (DDT) mer enn bøndene under 30 år, bønder med erfaring under 10 år og analfabeter. Bønder i alderen under 30 år, kunne lese og skrive, og de som hadd erfaring under 10 år, viste større kunnskap om plantevernmidler og deres farer, samt bedre holdninger til miljøet enn de tilsvarende gruppene. Hvilke vekster bøndene dyrket og opplæring av plantevernmidler hadde ingen betydelig innvirkning på bøndenes fakta, kunnskap eller holdning. Resultatene fra denne studien har imidlertid en viss usikkerhet på grunn av lite antall fisk og vannprøver. Dette resulterte i redusert sannsynlighet av sammenhengen. Mistilliten mellom myndighetspersoner og bøndene kan også påvirke resultatene av undersøkelsen.

Abbreviations

ADI	Average Daily Intake
AOAC	Association of Official Analytical Chemists
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EPA	Environmental Protection Agency
GC-ECD	Gas Chromatography – Electron Capture Detector
GCU	Government College University
HCH	Hexachlorocyclohexane
HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HQ	Hazard Quotients
KPK	Khyber Pakhtunkhwa
LRAT	Long Range Atmospheric Transport
LW	Lipid Weight
$MgSO_4$	Magnesium Sulfate
MRL	Maximum Residual Limit
N	Total Number of
Na2SO4	Sodium Sulfate
NMBU	The Norwegian University of Life Sciences
OCP	Organochlorine Pesticide
PCSIR	Pakistan Council of Scientific and Industrial Research
POPs	Persistent Organic Pollutants
QuEChERS	Quick Easy Cheap Effective Rugged Safe
SPSS	Statistical Package for The Social Sciences
WHO	World Health Organization
WW	Wet Weight

Glossary

Azad Kashmir	Pakistani administered state of Jammu and Kashmir
Barrage	A man-made dam build on the flow of river to make a reservoir for irrigation
Bioaccumulation	The accumulation of a contaminant by an organism from any of its environment (air, water etc)
Bioconcentration	The accumulation of a water-borne contaminant by an aquatic organism
Bioconcentration factor	The ratio of the concentration of a pollutant in an organism to its concentration in its environment
Biomagnification	The increase in concentration of contaminant in food chain
COVID-19	Coronavirus disease which was discovered at Wuhan, China in 2019 but became pandemic in 2020. It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
Jammu and Kashmir	Indian administered state of Jammu and Kashmir

Key words

Organochlorine pesticide, POPs, fish, farmers, knowledge.

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1. Introduction

1.1. Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) and especially organo-halogenated contaminants are a potential threat to the environment and living organisms due to their semi-volatile characteristics, longer biological half-life, persistence over long distances and lipophilic nature (Burreau et al., 2004; Muir et al., 1990). Organo-halogenated chemicals are proved to have adverse effects on thyroid functions (De Pisarev et al., 1990), carcinogenic effects on soft tissues and liver in different animals (Cabral & Shubik, 1986; Randi et al., 2003) and disorder of mitochondrial function and fatty acid metabolism in humans (Liu et al., 2017). Most of these chemicals are used as pesticides to destroy insects as they are designed in a way that they render many physiological functions of insects thus removing them from the agricultural sites (Jayaraj et al., 2016). Organochlorine pesticides (OCPs) are widely used pesticides across the world with some derivatives banned in developed countries (Jayaraj et al., 2016) and are considered pollutants due to their persistence and bioaccumulative nature (Van Ael et al., 2012). The notorious pesticide dichloro-diphenyl-trichloro-ethane (DDT) which was first explained hazardous by Rachel Carson in her book 'Silent Spring' is banned in developed world but it is still being used in many developing countries which depend mainly on agriculture for their economic development (Gupta, 2004; Malik et al., 2014; Riaz et al., 2018). Only in India 85000 metric ton OCPs are produced and there have been hundreds of mortalities reported due to contamination of pesticides with food which indicates the manhandling of these chemicals (Gupta, 2004).

OCPs used in agricultural fields are mostly dispersed in environment (99%) and only a small amount (1%) is consumed in targeting the pest (Pimentel, 1995). These OCPs become the part of agricultural drainage and are transported to the riverine systems where they either settle down in sediments or keep going to further distances in water (Van Ael et al., 2012). Settling down in sediment saves these OCPs from the direct incident of sunlight and prevents their photochemical destruction (Walczak & Reichert, 2016). In water OCPs are taken up and bioaccumulated in adipose tissues by fish upon exposure to the respiratory, cutaneous and gastrointestinal surfaces of fish (Burreau et al., 2004; Mackay & Fraser, 2000) and then these chemicals keep biomagnifying in trophic levels (Burreau et al., 2004). OCPs affect the epithelium of gills by curling and fusion of primary and secondary lamellae, induce the karyolysis of hepatocytes and increased activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) thus damaging the liver and enhance lipid peroxidation leading to oxidative stress in fish (Karmakar et al., 2016). They also deform the kidney tissues by causing renal tubule swellings and disorganization, induce the necrosis of tubular epithelia and result in to narrowing of the lumen tubes of kidneys (Karmakar et al., 2016; Walczak & Reichert, 2016). Spermatogenesis is affected by OCPs along with the reduced development of gonads leading to decreased sperm count and infertility (Johnson et al., 2013).

In 2001 a global initiative under the UN Environment Program was adopted which came in to force in 2004, this initiative also known as Stockholm Convention aimed at the elimination and restriction on the existence and production/distribution of POPs in the signatory countries (Convention, 2019; Xu et al., 2013). The initial chemicals which were listed as the “dirty dozen” were 12 and most of these were pesticides, however the convention is still adding and proposing new pollutants in their updated lists (Xu et al., 2013). Currently there are 35 POPs in the list of Stockholm Convention until their ninth meeting in 2019 and three chemicals are in the proposal phase (Convention, 2019).

1.2. OCPs in Pakistan

Around 70% of the population of Pakistan depends directly or indirectly on the agricultural sector for their living, thus making it an agrarian country just like its neighbor India. Both countries have increased the use of OCPs during past few decades to maximize their production (Gupta, 2004; Malik et al., 2014; Riaz et al., 2018). Geographically these two countries share a same epitope and some areas divided between them. Major rivers irrigating the plains of these two countries and especially Pakistan, originate from Kashmir and Tibet, and all of those (Indus, Jhelum, Sutlej, Ravi and Chenab) which have to run through Pakistan enter India first. (Zawahri & Michel, 2018). So, the agricultural run-off from India, the monsoon and air current systems along with the agricultural techniques being used in Pakistan make a combined impact on the riverine system of Pakistan. Despite being the signatory member of Stockholm Convention on POPs 2001, there is a reported use of banned OCPs in Pakistan due to their efficacy, availability at low costs and poor law and regulatory measures (Shahid et al., 2016). Due to this immense use of OCPs, these pollutants are found everywhere in soil, sediment, underground water, air and river fish (Alamdar et al., 2014; Malik et al., 2014; Riaz et al., 2018; Tahir et al., 2016), and are increasing in amount (Mehmood et al., 2017) but there are only a few studies which report the bioconcentration in fish at different trophic levels. There is one recent study each for river Kabul and river Chenab which show the elevated concentrations of hexachlorocyclohexane (HCH), aldrin and DDT in different species of fish however there are very few studies reporting the bioconcentration of lake fish which is the prime source of commercial fisheries in Pakistan (Aamir et al., 2016; Riaz et al., 2018).

1.3. Farmers' knowledge and awareness of pesticides

Regarding the general pesticide use in Pakistan by farmers, previous surveys found that there is misuse and mostly overuse of pesticides by the farmers (Khan et al., 2020; Saeed et al., 2017). Farmers are usually told by the dealers about the use and amount of pesticides to be used for the crops or vegetables, sometimes they are unable to read the labels and the directions in national or foreign language due to the lack of education or knowledge of English respectively, so most of the times their training with the pesticides are necessary along with their education to help reduce the negative impacts of mishandling and misuse of the pesticides (Saeed et al., 2017).

1.4. Aims of the study

This study is a quantitative analysis targeting the levels of selected persistent organic pollutants (POPs) analyzed and tested in the fish species of commercially and agriculturally important freshwater lakes of Pakistan to assess the imposed threat by POPs to the biodiversity and human health. This study also served as an advanced continuity of previously conducted studies on the riverine systems (Tahir et al., 2016) and fish species (Riaz et al., 2018). There is reported negligence in the administration of pesticides and control on the sale of banned chemicals (Tahir et al., 2016; University, 2016) so this study also covered the local usage of the pesticides by assessing the knowledge and attitudes of farmers in special areas linked with the sampling sites.

It was predicted that fish from low altitudinal barrage/lake would contain more concentrations of OCPs. The mode of nutrition and weight of the fish was also predicted to have a positive correlation with the bioconcentration of OCPs. Also, the use of banned OCPs was suspected to exist along with the mishandling of pesticides across the different age, education, and experience groups in the farmers. Due to the lack of knowledge and education, it was also predicted that farmers will show least eco-friendly attitudes.

2. Materials and Methodology

2.1. Sampling sites

Although there are numerous small and large lakes in Pakistan, but samples were taken from those freshwater lakes which are major providers of commercial fish and are located on the important rivers. Following freshwater lakes were considered in the sampling.

1. Tarbela lake, (34° N, 72° E) is situated in Khyber Pakhtunkhwa (Northwestern province of Pakistan) on the Indus River (Figure 2.1). It is the first big lake as Indus River comes out of Himalayan and Karakorum ranges which means there should be very little agriculture behind this spot. Tarbela Lake is located on the hilly plain area of elevation 520m with an annual mean temperature of 17˚C, highest 32˚C during summers while lowest of 1˚C during winters. The average amount of precipitation is 1267.5 mm for the year. Fish from this lake is supplied to the different cities of Khyber Pakhtunkhwa (KPK), the federal capital Islamabad and the state of Azad Kashmir. Despite the long-range atmospheric transport of POPs this site was considered a comparative reference point for the values of site 2.

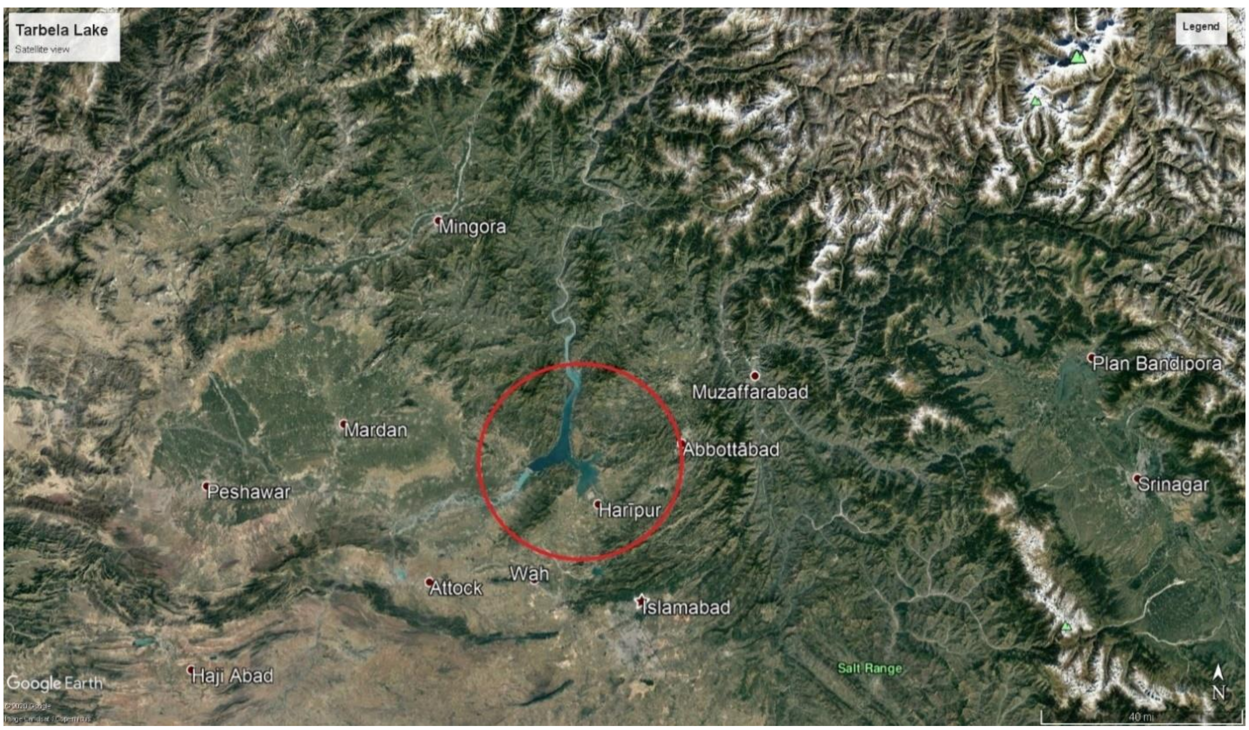

Figure 2.1 The satellite map of Tarbela lake and its geographical location along with the world's highest mountain ranges in its immediate north. Encircled are the lake and the city of sampling. (Source, Google Earth).

2. Sukkur Barrage or formerly called Lloyd Barrage, (27° N, 68° E) is in the Sindh province of Pakistan (Figure 2.2). This place has an annual mean temperature of 28˚C, with the highest of 45˚C during summers while the lowest of 9˚C during winters and an annual precipitation of 13.7mm. Sukkur barrage has the agriculture runoff from whole Punjab. This was considered as the second site for the sampling. The reason for this site was to record the usage of OCPs in the whole Punjab in the form of increased amounts of OCPs in fish tissues. Since all the rivers which flow through Punjab are finally merged in the Indus river behind this spot.

Figure 2.2 The satellite image showing the location of site 2 i.e. Sukkur Barrage along with its geographical situation. (Source, Google Earth)

Embedded between the deserts of Thar and Cholistan, Indus valley is the home to South Asia's hottest cities such as Jacobabad which is famous for its consistent temperature as high as 52˚C during summers. Sukkur barrage was included for sampling due to its location on Indus river when it has merged with every river running through Punjab, the agricultural hub of Pakistan. The detailed map of rivers along with the sampling sites is shown in Figure 2.3.

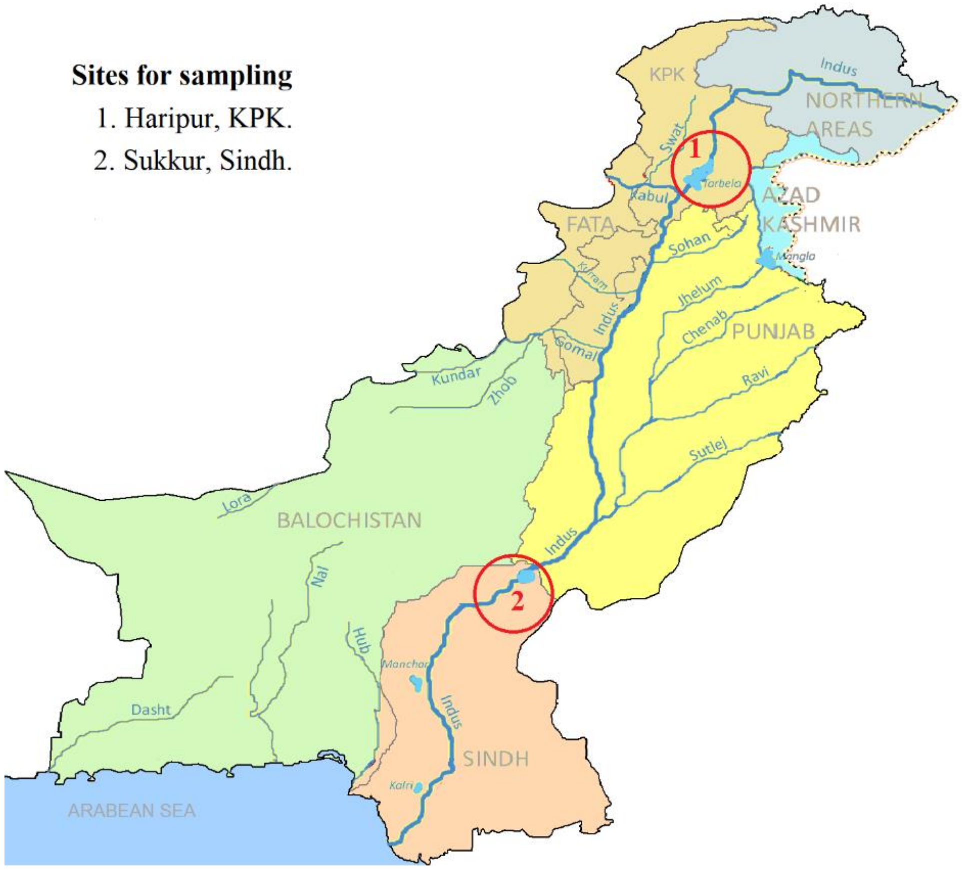

Figure 2.3 Sampling sites: 1 is Tarbela lake in Haripur while 2 is Sukkur barrage in Sukkur, along with the riverine system of Pakistan.

2.2. Targeted species

Five different species of herbivorous, carnivorous, and omnivorous fish were found feasible for the sampling (Table 2.1). Among them *Labeo rohita* was found in both places; Tarbela and Sukkur. Mahseer fish (*Tor putitora*) once found in all five rivers of Pakistan is now limited to the Himalayan rivers and lakes in the Northern Pakistan and Kashmir due to the limitation caused by the construction of dams and barrages (Shafi et al., 2016). So, this was included in the study and its samples were collected only from the Tarbela Lake. Silver carp (*Hypophthalmichthys molitrix*) and Mori fish (*Cirrhinus mrigala)* both being herbivorous were collected from Tarbela and

Sukkur, respectively. However, Catfish *Rita rita* also known as Khagga or Khaggo locally (Soofi et al., 2016) was only found in Sukkur.

Table 2.1 Details of targeted species and their sampling sites.

No.	Local name	Scientific name	Mode of nutrition	Location
1.	Rohu	*Labeo rohita*	Omnivorous	Tarbela Lake
2.	Mahseer	*Tor putitora*	Omnivorous	Tarbela Lake
3.	Silver carp	*Hypophthalmichthys molitrix*	Herbivorous	Tarbela Lake
4.	Khagga	*Rita rita*	Carnivorous	Sukkur Barrage
5.	Mori	*Cirrhinus mrigala*	Herbivorous	Sukkur Barrage
6.	Dumra	*Labeo rohita*	Omnivorous	Sukkur Barrage

2.3. Sampling

Since fishing is prohibited by local government on both sampling sites therefore acquiring fish was depended on the contractors' catch; a contractor is an authorized person who can fish with nets and is responsible for the distribution of fish in the cities around the lakes. Also, there was a risk of fraud in the originality of the fish as many sellers sell farmed fish in the name of wild catch, therefore at site 1 (Tarbela Lake), a local person with sufficient knowledge of fish and its originality was accompanied during the sampling, while at site 2 (Sukkur Barrage) a person from the Sindh Fisheries Department was provided especially for the guidance and acquiring of fish. All the fish for sampling was acquired from the shops situated in the proximity of the lakes and some of them on the lakeshore even.

All fish were identified with their local names and their taxonomy was later confirmed with a taxonomist, FishBase and Fish-Pak dataset (Shah et al., 2019). Fish was weighed on the spot and the initial identity was confirmed.

Muscle samples of three individuals for each species (3x6) were taken during the January 2020. Enough chunk of muscle was cut with sharp blades and stored in Tarsons (made in India) SPINWIN^{TM} centrifuge tubes with conical bottom and sterile bulk, tightly lid and wrapped in aluminum foil immediately. After each sampling was over, samples were frozen overnight and later taken to the freezer of Government College University's (GCU) Toxicology lab where they were stored at -20^{o}C.

Two water samples (100 ml each) were also taken from both sites (1+1) in plastic vials while keeping the zero-space between surface and lid. These samples were stored at 3C at GCU's toxicology lab.

All samples were supposed to get tested through the high performance liquid chromatography (HPLC) methodology used by (Riaz et al., 2018) but the photodiode detector of HPLC at GCU Lahore malfunctioned, so help from other universities was sought, which ended up into a deal with the Pakistan Council of Scientific and Industrial Research (PCSIR) in which they accepted 12 fish samples (2x6) along with the 2 water samples (n14). analytical standard of DDT PESTANAL®, (Sigma-Aldrich USA) was also provided to the PCSIR. However, they confirmed the availability of dichloro-diphenyl-dichloro-ethylene (DDE) and Endosulfan standards with them.

On March 2, 2020, all samples were delivered to the Lahore branch of PCSIR for tests and analysis.

Due to COVID-19 outbreak and the lockdowns following the initial pandemic emergency, all laboratories were closed until the 15th of July 2020.

2.4. Laboratory work

All frozen fish samples were sliced, crushed, and ground using a knife, mortar and grinder and then extracted and cleaned through the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method validated AOAC 2007.01. 15g of grounded sample was added into the 50ml centrifuge tubes and was made even by adding 15ml acetonitrile, vortexed and centrifuged at 4000rpm for 4 minutes then pre-weighed salt packets ($MgSO_4$ 6g and Sodium Acetate 1.5g) were added, vortexed for 30 seconds, mixed vigorously and centrifuged for 5 minutes at 4000 rpm. Extraction was followed by the cleaning step which involved the extraction of acetonitrile extract from the separated organic layer into the dispersive tube containing 50 mg PSA (primary secondary amine), 150 mg C18, 900 mg Na_2SO_4 to clean away all the biological matrix i.e. lipids and proteins from the samples. This was also vortexed and then centrifuged for 10 minutes at 2000 rpm. Supernatant was collected using micropipette into glass vials.

Water samples were passed through 42 grade Whatman ashless filter paper to remove any solid residue. HCl was added to stop any organismic activity and pH was neutralized. Then extraction step was performed adopting liquid-liquid extraction technique described in EPA method 8081 (EPA, 2007).

The final supernatant and concentrated water samples were tested through gas chromatographer equipped with a ^{63}Ni electron capture detector GC-ECD (Shimadzu Japan). Helium was used in the GC step. Tests were performed in the temperature range of 21.3-25.5 degree Celsius with the humidity of 60-65 rh%.

2.5. Quality Assurance

Samples were subjected to quality control to make results reliable and correct. Recovery was assessed by running samples spiked with pure standards and blank samples in the same way real samples were run. This was repeated in between the actual tests approximately after every 5 tests standards were run and observed. Recoveries were ranged between 70 – 110 % and limit of detection was set to be 0.010 mg/kg for fish samples while 0.001 mg/L for water samples. Any amount below these were considered as not detected.

All chemicals, standards and substances were bought from Sigma-Aldrich and Merck, (Germany).

2.6. Farmers' knowledge assessment

To assess the use of banned pesticides in agricultural areas a survey was planned and a 6-point binary questionnaire was designed to check the basic knowledge and attitudes of local farmers in the vicinity of rivers and lakes especially near the sampling sites.

Following questions were asked to farmers after getting consent and basic data about their age, experience, education status, type of crop they produce and acquired pesticide training.

1. Do you use pesticides frequently?	Yes/No	FACT
2. Do you/have you used DDT?	Yes/No	FACT
3. Do you know what an OCP is?	Yes/No	KNW
4. Is OCPs harmful for health?	Yes/No	KNW
5. Do you think OCPs can end up in rivers/lakes?	Yes/No	ATT
6. Do you consider ban on DDT right?	Yes/No	ATT

The first two questions were grouped as to check the fact of pesticide usage, without asking the time of usage to avoid the confusion of asking for a confession of a wrong act (the usage of banned pesticide). The latter two questions were meant to assess the basic knowledge of the farmers about OCPs (during interviews OCPs were named and question was asked orally and simplified to the knowledge level of farmer). The last two questions observed the attitude of the farmer towards ecosystem and sustainable agriculture. Initially more than 50 farmers were approached for interviews but only 40 agreed to take part and their responses were recorded on printed questionnaire. 15 more farmers were approached during COVID-19 break and their responses were also recorded. Questions were asked in their local language which in Khyber Pakhtunkhwa and State of Azad Kashmir was Hindko, while Punjabi in Punjab province. No difficulty was faced in establishing the communication with the respondents.

All 55 interviews were conducted between January-June 2020.

2.7. Human health risk assessment

Human health risk for the consumption of fish from both sites was estimated by following the methodology by (Taiwo, 2019). An average daily intake (ADI) of OCPs was obtained by the following formula.

$$\text{ADI}=\frac{\text{Concentration} \times \text{Ingestion rate} \times \text{exposure duration} \times \text{exposure frequency}}{\text{body weight} \times \text{average time}}$$

Non-carcinogenic risk index was calculated by summing up the hazard quotients (HQ). Which is the ratio of average daily intake (ADI) of OCPs and the reference dose R_fD (µg/kg/day) suggested by United States Environmental Protection Agency and obtained from (Heath et al., 2003). A ratio greater than 1 was considered potential concentration for adverse health effects.

2.8. Statistical analysis:

For the statistical analysis of obtained data, IBM SPSS V22 was used in addition to the GraphPad Prism 8.4.3. Both software applications were used to draw the graphs, Tables, and tests. Before processing the data in these software applications, data was changed into binary form as per requirement. In fish data, herbivore fish was coded as 0 while carnivore 1 and omnivore fish was coded as 2 in the category of mode of nutrition. The questionnaire data was conveniently made binary based on the categories and answers from the respondents as shown in Table 2.2.

Table 2.2 The organization of data into binary form based on different categories.

Factors	Category	Binary code assigned
Age	<30 years	0
	>30 years	1
Education	Illiterate	0
	Literate	1
Produce	Vegetables	0
	Crops (other than vegetables)	1
Pesticide training	No	0
	Yes	1
Experience	<10 years	0
	>10 years	1

All positive responses (yes) in questionnaire were coded as 1 whereas all negative responses (no) were coded as zero.

3. Results

3.1. Sampling results

Five different species were identified and their weights along with the mode of nutrition are presented in the Figure 3.1. The weight of the fish was positively correlated with their level in food chain (Figure 3.2).

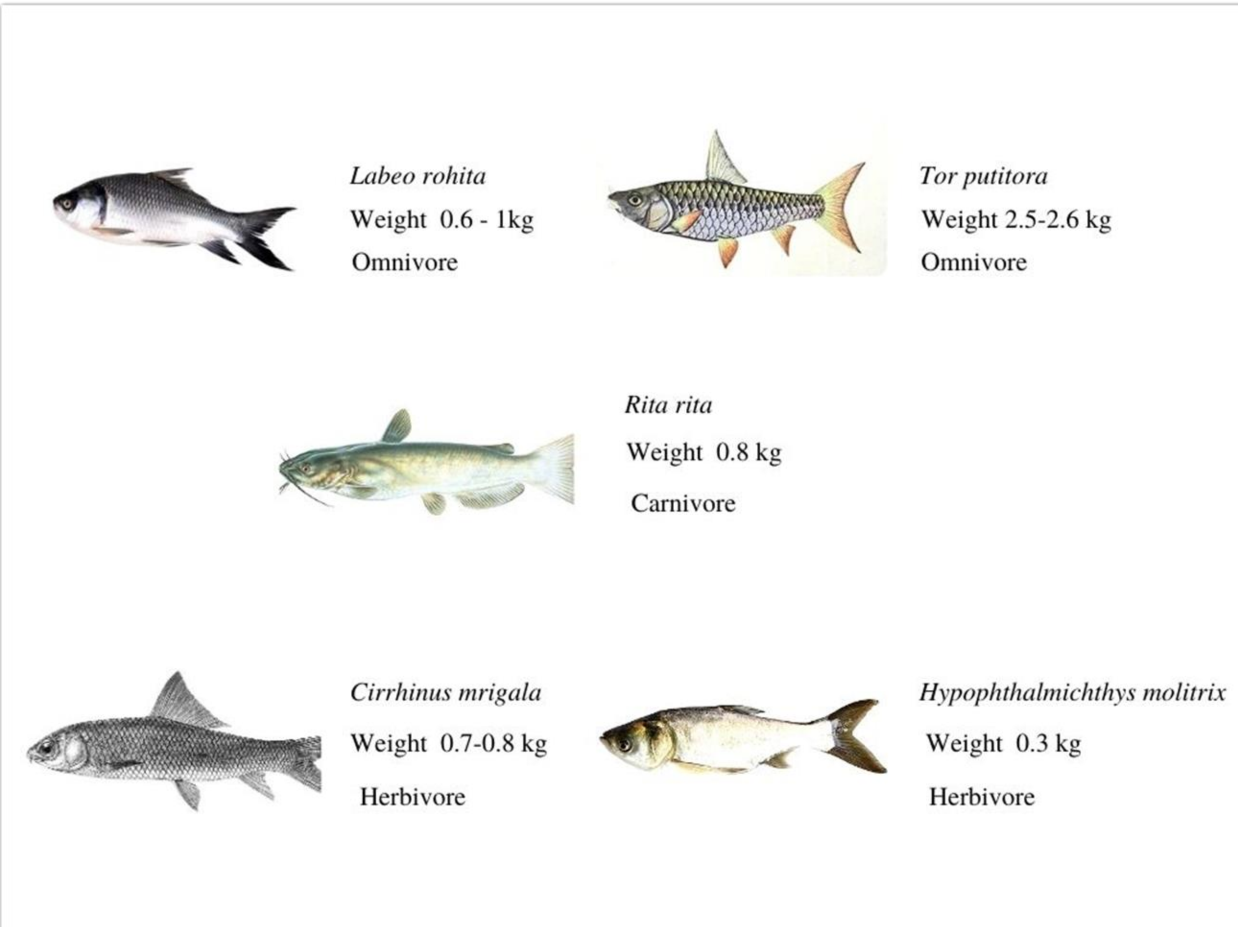

Figure 3.1 Different fish species along with their recorded weight and mode of nutrition.

3.2. Concentration of OCPs in fish and water samples

Only 8 out of 14 samples were detected with organochlorine pesticides. The average concentrations were shared for each sample group of same species. Endosulfan was found in both water samples from each site. No sample had detectable DDT however the highest concentration detected in any sample was for endosulfan in *Labeo rohita* from Sukkur barrage. Detailed results are shown in Table 3.1.

Table 3.1 The concentrations of OCPs found in different samples of fish (mg/kg) *and water (mg/L) after performing GC-ECD along with the bioconcentration factors (BCF in L/kg) and weight of the fish (kg).*

	Sample identity	No. of samples (n14)	Weight	Σ Endosulfan	DDT	DDE	BCF
Samples from Tarbela (site 1)							
1	*Hypophthalmichthys molitrix*	2	0.3	--	--	0.019	--
2	*Tor putitora*	2	2.5-2.6	0.015	--	--	3.75
3	*Labeo rohita*	2	0.6	--	--	--	--
4	Water sample	1		0.004	--	--	
Samples from Sukkur (site 2)							
1	*Rita rita*	2	0.8	--	--	--	--
2	*Cirrhinus mrigala*	2	0.7-0.8	--	--	--	--
3	*Labeo rohita*	2	1	0.047	--	--	11.75
4	Water sample	1		0.004	--	--	

The concentration of endosulfan was found same in each water samples from both sites. However, no other pesticide was detected in water samples.

Overall, the weight of fish and their position in food chain had positive association with the amount of bioconcentration of pesticides ($p>0.01$). Shown in Table 3.2 and Figure 3.2.

Table 3.2 The correlation of weight of fish with the pesticide residue

		Weight of the fish	Pesticide residue
Weight of the fish	Pearson Correlation	1	.124
	P value	--	.815
	N	12	12
Pesticide residue	Pearson Correlation	.124	1
	P value	.815	--
	N	12	12

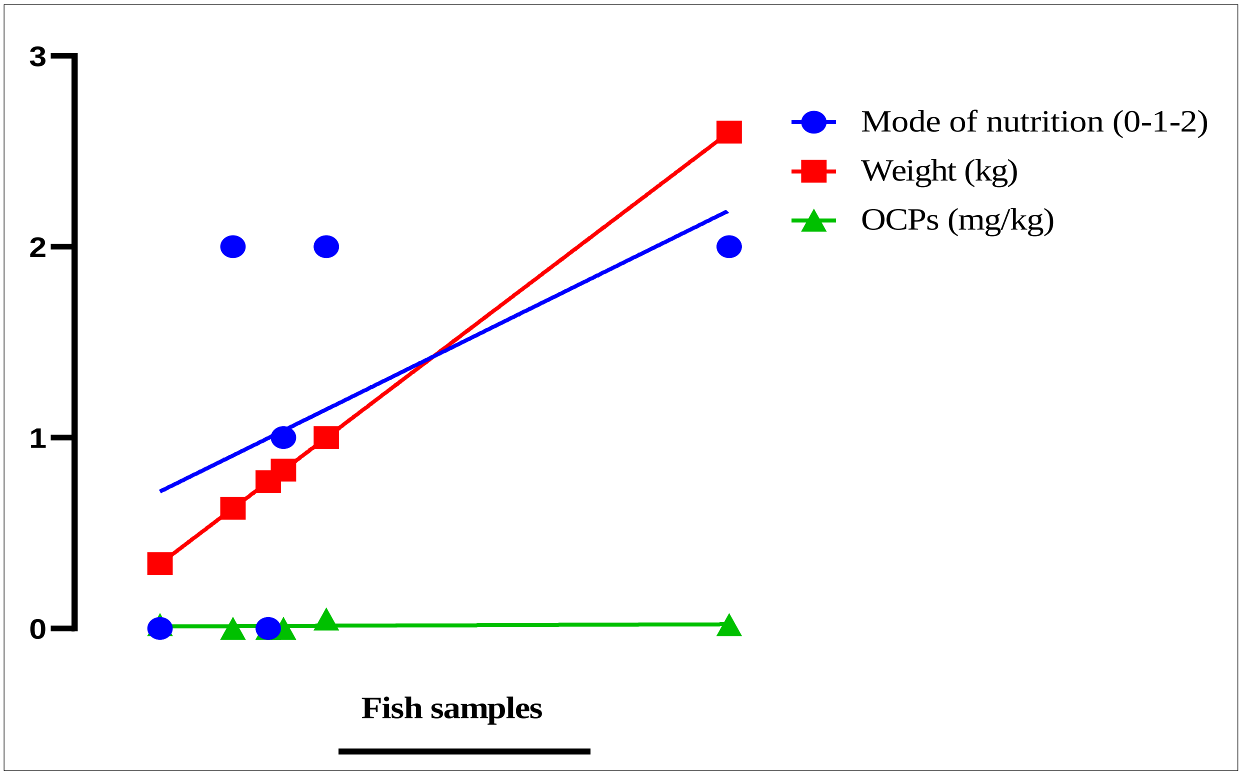

Figure 3.2 Graph explaining the non-linear correlation between weight, mode of nutrition of the fish and the OCP concentration values.

Although the correlation between weight of the fish and the concentration of OCPs is positive but this correlation is weak (P value is 0.7). However, it is a bit stronger in case of mode of nutrition and the bioconcentration of OCPs value as we can see in the Figure 3.2. A simple linear regression

(Figure 3.3) shows a weak yet positive correlation between weight of the fish and the amount of OCPs found in different fish samples.

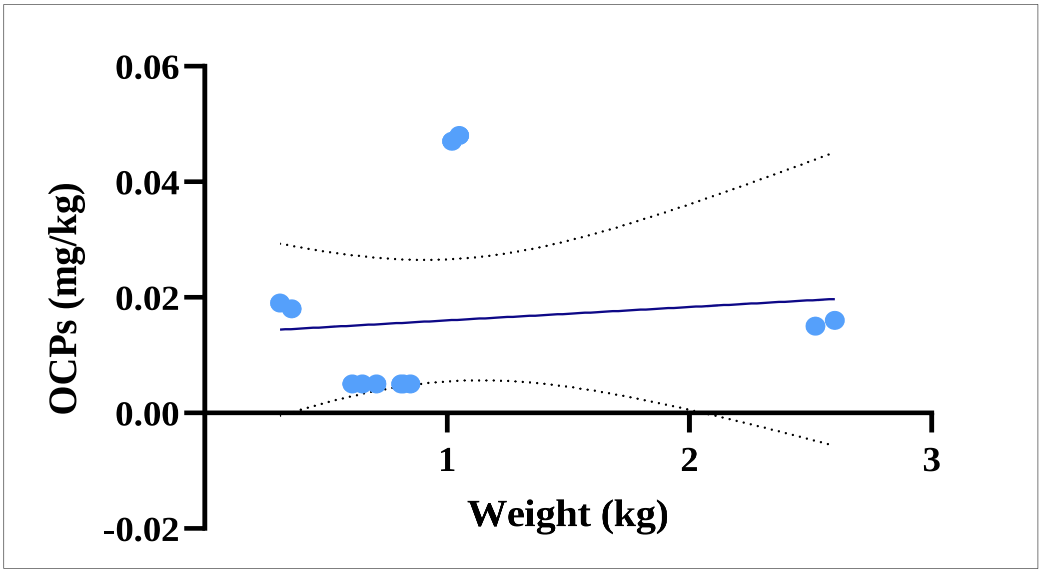

Figure 3.3 Simple linear regression showing a weak yet positive correlation between weight of the fish and the amount of OCPs found in them.

3.3. Farmers' knowledge assessment

The basic information about the farmers was found to be as shown in Table 3.3.

Table 3.3 Basic information collected from the respondents and their interrelation.

Factors	Categories	Percentage within group	Correlations with			
			Education	Produce	Experience	Training
Age	<30	36%	Negative	Negative	Positive	Positive
	>30	64%				
Education	Illiterate	36%	--	Positive	Negative	Negative
	Literate	64%				
Produce	Vegetable	54.5%	--	--	Negative	Negative
	Crops	45.5%				
Experience	<10 years	23.6%	--	--	--	Negative
	>10 years	76.4%				
Training	No	47.3%	--	--	--	--
	Yes	52.7%				

Apparently, majority of respondents were more than 30 years old, and a similar majority was educated too. Most of the farmers had experience of more than 10 years. The correlation between their basic information and factors is discussed in detail (Figure 3.4 and Table 3.4).

Figure 3.4 Correlation between the basic information of farmers (n 55). The scale from red to green represents the intensity of correlation with red (-1.0) being strongly negative while green (1.0) being the strong positive correlation.

Table 3.4 The values of correlation and their significance across the basic data of the farmers.

		Age	Education	Produce	Experience	Training
Age	Pearson Correlation	1	-.414**	-.069	.469**	.117
	Sig. (2-tailed)		.002	.617	.000	.395
	N	55	55	55	55	55
Education	Pearson Correlation	-.414**	1	.159	-.332*	-.034
	Sig. (2-tailed)	.002		.247	.013	.803
	N	55	55	55	55	55
Produce	Pearson Correlation	-.069	.159	1	-.008	-.306*
	Sig. (2-tailed)	.617	.247		.955	.023
	N	55	55	55	55	55
Experience	Pearson Correlation	.469**	-.332*	-.008	1	-.012
	Sig. (2-tailed)	.000	.013	.955		.928
	N	55	55	55	55	55
Training	Pearson Correlation	.117	-.034	-.306*	-.012	1
	Sig. (2-tailed)	.395	.803	.023	.928	
	N	55	55	55	55	55

***. Correlation is significant at the 0.01 level (2-tailed) *. Correlation is significant at the 0.05 level (2-tailed).*

A simple linear regression (see Appendix 1) for multiple factors' comparison also suggested the strength of a negative association of farmers' age and their education level which means older farmers (>30 years) were less educated or not educated as compared to the young farmers (<30 years) who are educated. Also, experience was strongly associated with the age of the farmers in a way that older farmers (>30 years old) had more experience than younger farmers (<30 years old). However, the regressions showed that pesticide training status and produce type did not have any significant association with basic independent factors, such as age of farmers or the co-factors like experience or education. While experience had a negative association with the levels of education which means those farmers who acquired the education have lesser experience in farming than those who quit the school or did not go at all.

The general response from the farmers (N55) shown in Figure 3.5 elaborates the situation which contrasts in every category except for the attitudes towards the pesticide use.

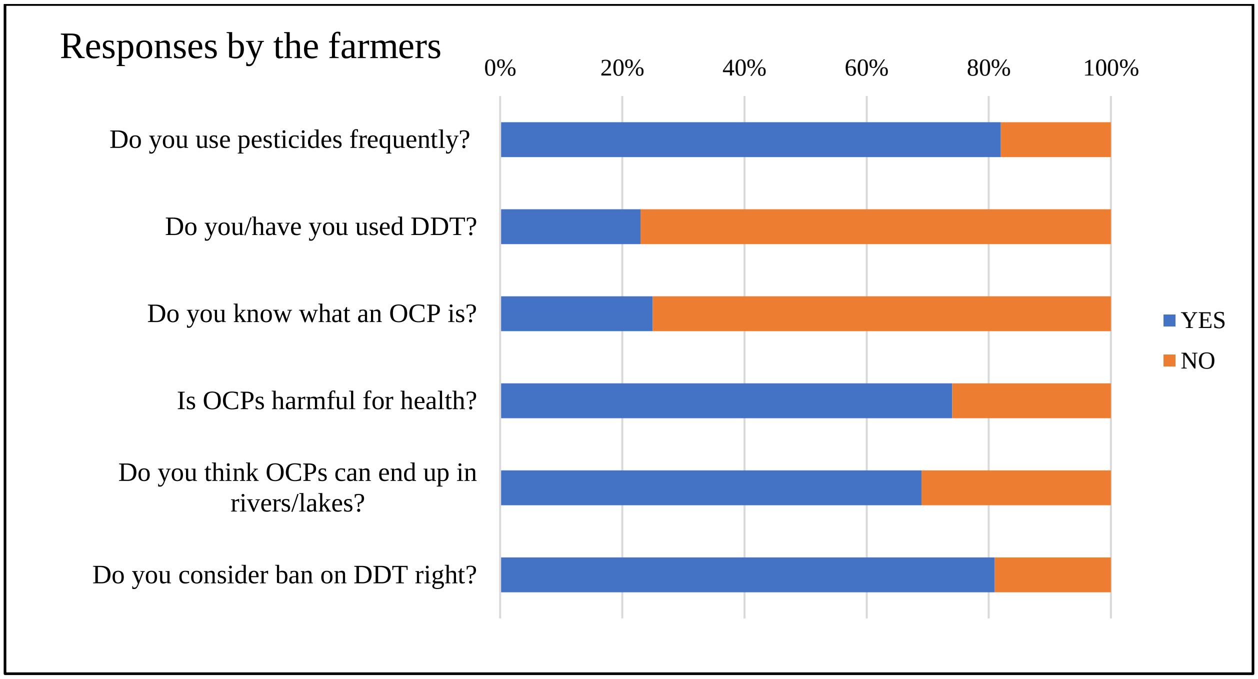

Figure 3.5 Responses by the farmers (overall) to the questions 1 to 6 from top (N55).

When the answers by the farmers were compared within the age groups (Appendix 2 and Figure 3.6), it showed that about 80% and 82% of young (<30 years old) and old (>30 years old) farmers respectively consumed pesticides very often. Only 5% from the farmers of age less than 30 consumed DDT as compared to the farmers of other age group where this percentage was 34%. This indicates the reduction in the use of DDT over time and the new farmers are very less familiar with the use of DDT. Despite knowing very less about what an OCP is, 90% of the young farmers and 65% of the old farmers knew that an OCP is harmful for the human health.

Attitudes of the farmers when compared within the age groups, it showed that 75% of the young while 65% of the old farmers believed that an OCP can end up in rivers or lakes. 90% of young farmers believed that ban on DDT is right. This percentage was lower (77%) for the other age group of the farmers.

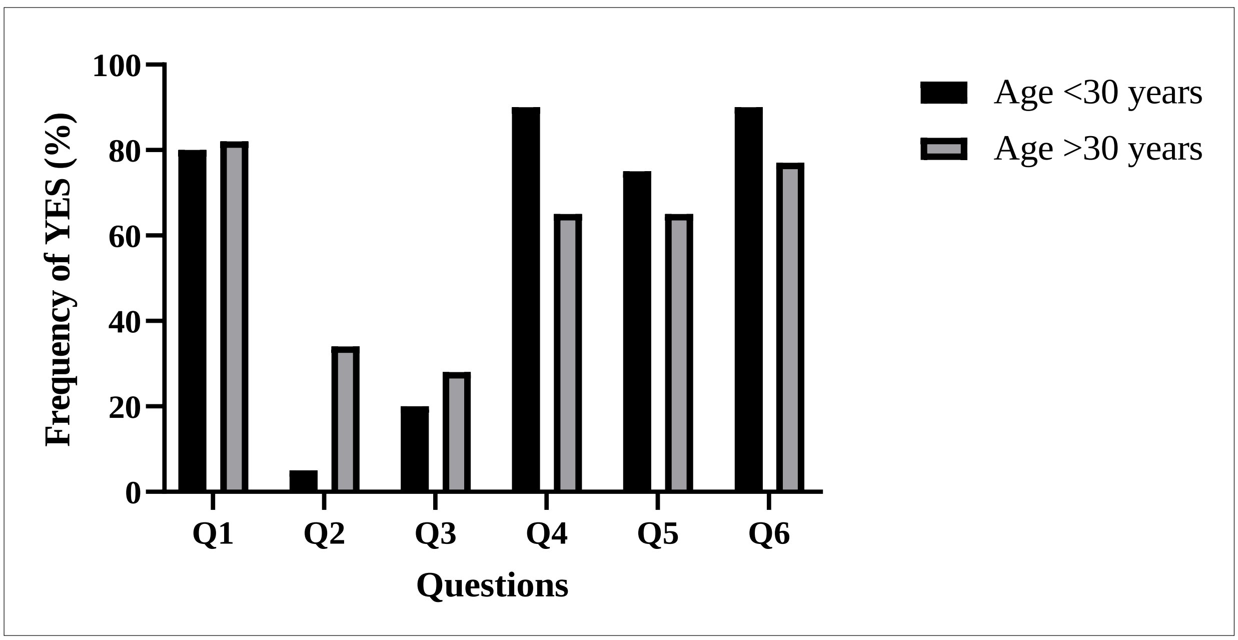

Figure 3.6 Graphical comparison of responses within the age groups of farmers.

When the means of responses were compared within the groups based on education level (Appendix 2 and Figure 3.7) it showed that 75% of illiterate and 85% of literate people confessed the frequent usage of pesticides, while more people (28%) from literate group confessed the use of DDT as compared to the 15% of illiterate farmers. Regarding the knowledge questions, both groups showed very little knowledge about the OCPs 20% and 28% of illiterate and literate farmers respectively, however both groups knew the hazardous impacts of pesticides (illiterate, 65% & illiterate 80%).

For the attitude inspection both groups differed as 55% of illiterate farmers thought that pesticides end up in rivers and lakes while 77% of literate farmers said so. However, majority of both groups agreed that ban on DDT is right (80 and 83% respectively).

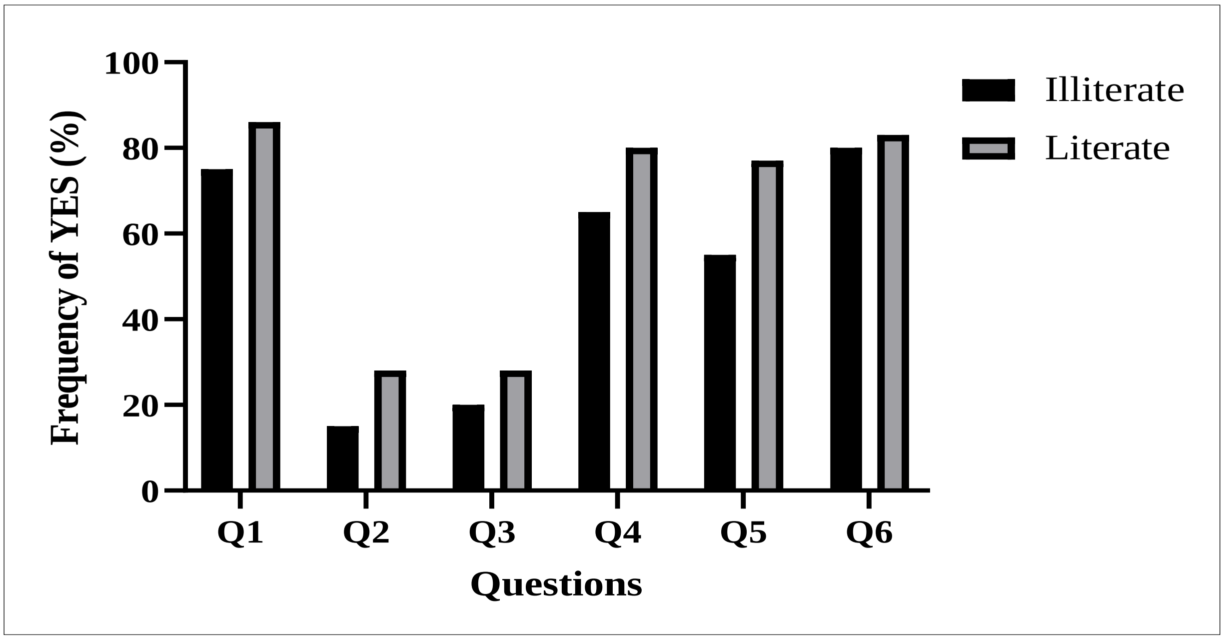

Figure 3.7 Graphical comparison of the responses by the farmers within the education level groups.

When the answers were compared within the groups based on the produce of the farmers, more farmers farming vegetables confirmed the use of pesticides (86%) as compared to the other crop farmers (76%). However, in the case of DDT usage only 13% of vegetable farmers while 36% of crop farmers confessed. (Appendix 2 and Figure 3.8)

For the knowledge assessment, majority of both groups did not know what an OCP is, however almost same majority of farmers in both groups knew the hazardous impacts of pesticides (73-76%).

Similarly, 73% of vegetable farmers and 64% of other crop farmers thought that pesticides can end up in rivers and lakes, and 86% of vegetable farmers and 76% of crop farmers thought the ban on DDT was right.

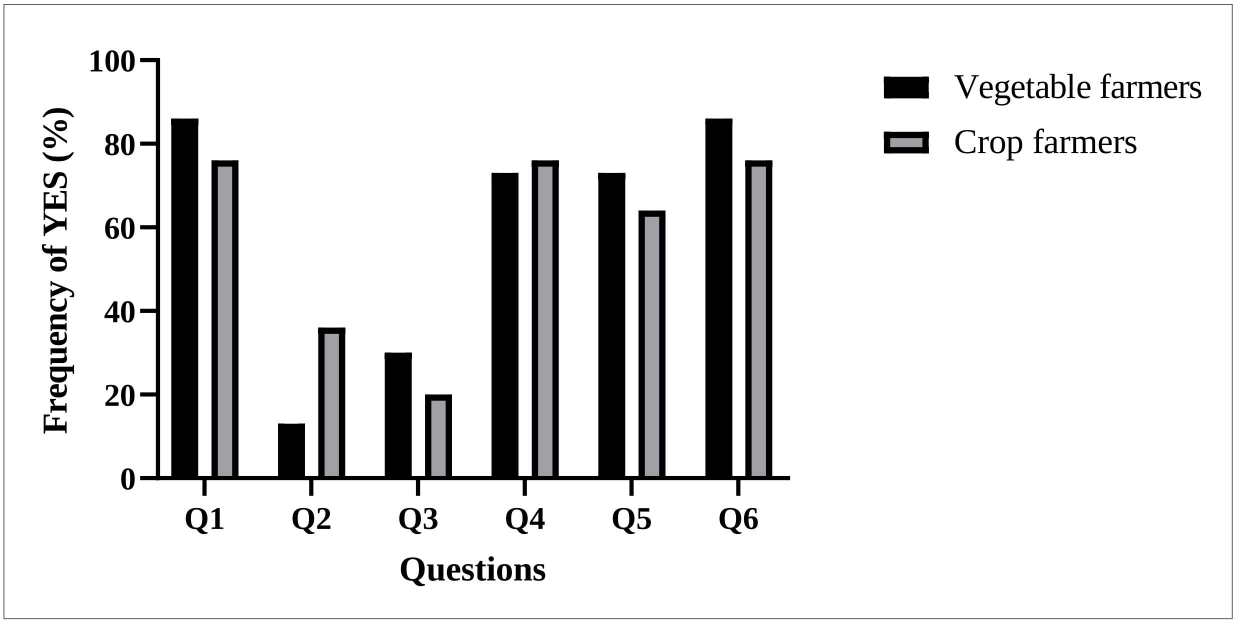

Figure 3.8 Graphical comparison of the answers within the groups of farmers based on their produce.

When the experience of farmers was compared with the responses they gave (Appendix 2 and Figure 3.9), for knowledge part, majority of both groups (85% of new while 80% of experienced farmers) confirmed the frequent usage of pesticides. However, very few (7%) of new farmers and 28% of experienced farmers confirmed the use of DDT.

For knowledge assessment part majority of both new and experienced farmers did not know what an OCP is (only 15 % and 28% knew respectively), however 100% of new farmers knew the hazardous impacts of pesticide usage on human health, this knowledge was with 66% of old farmers on the other hand.

A majority 84% of new farmers believed OCPs can end up in rivers and lakes as compared to the 64% of experienced farmers. 92% of new and 78% of experienced farmers believed the ban on DDT was right.

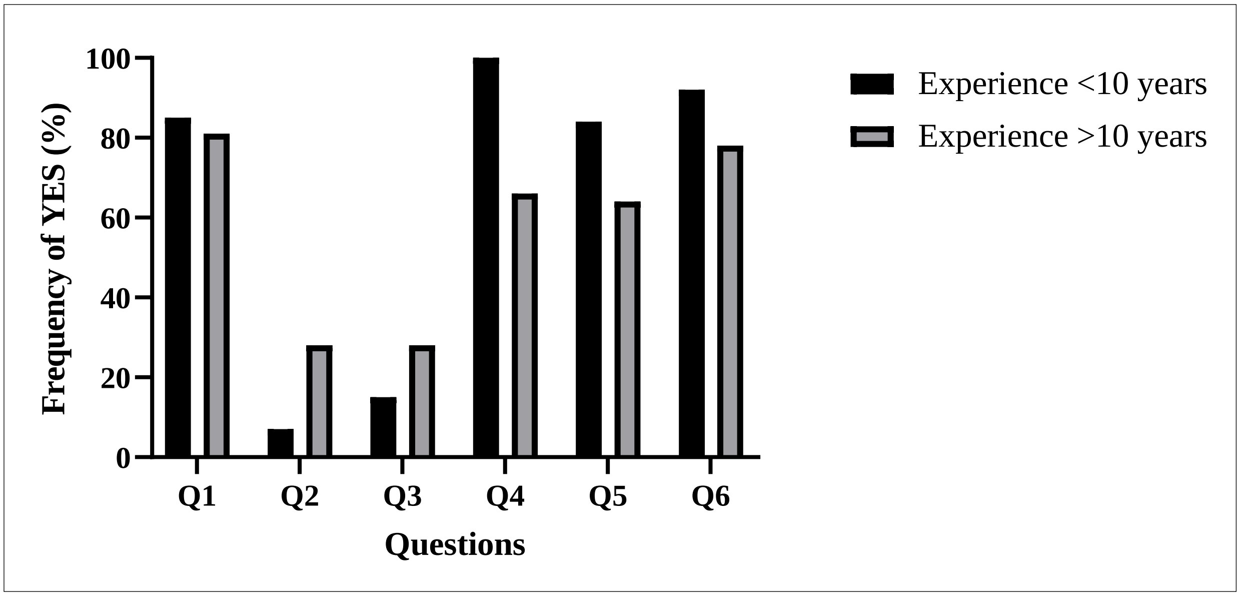

Figure 3.9 Graphical comparison of the experience groups and their responses.

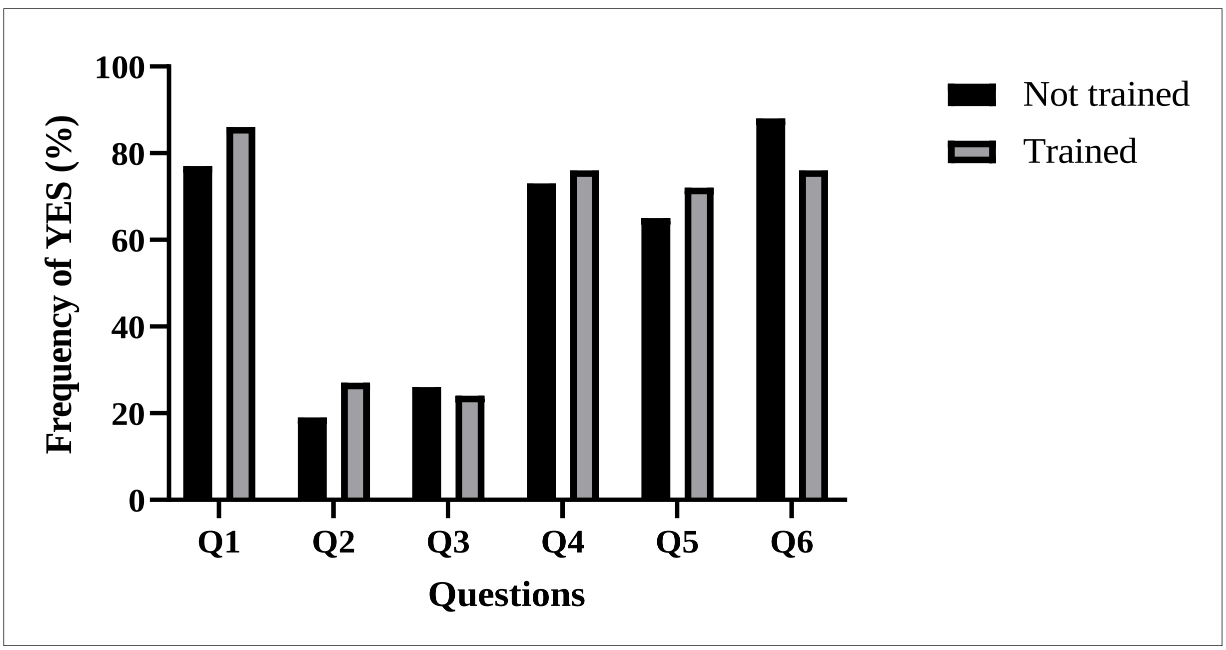

Figure 3.10 Comparison of answers within groups based on the pesticide training status in farmers.

When pesticide training status was compared with the responses of farmers then those who did not get any training confessed lesser use of pesticides (77%) especially DDT (17%) as compared to those who got training (86% and 27%) (Appendix 2 and Figure 3.10). Both groups showed same level of knowledge about the pesticides and their impact on health. Also, there was no significant difference between the attitudes of farmers from both groups. Majority in both groups believed ban on DDT is right.

3.4. Human health risk assessment

Ingestion rate and average body weight was obtained from (Riaz et al., 2018) to be 0.008 kg/day and 60kg respectively for Pakistan. Exposure duration was set to 30 years for adults and exposure frequency to be 350 days a year as adopted from (Taiwo, 2019). The average time for non-carcinogenic risk was kept as the average life expectancy in Pakistan.

Table 3.6 Average daily intake (ADI) for different OCPs in both locations (µg/kg/day) along with the reference dose (µg/kg/day) and the hazard index (HI).

OCP	Location	ADI	RfD	HI
Endosulfan	Tarbela	0.70	6	0.11
	Sukkur	2.19		0.36
ΣDDT	Tarbela	0.80	0.5	1.60
	Sukkur	--		--

Hazard Index (HI) greater than 1 has a potential to cause adverse health effects. The concentration of DDE in fish from Tarbela lake is found to have such impacts.

Consumption of water from both sites is unknown so that was omitted in health risk assessment.

4. Discussion

Working abroad limits one’s options regarding the study design and execution. The choice of methodology and lab work is also affected when there is no back up plan. This happened in the case when proposed strategy was given up and an alternate option was taken for the analysis of samples. Also, while making communication with the farmers it was important to have the knowledge of the very local language to obtain a clear answer. The process of survey went as expected, however due to COVID19 lockdowns and summer holidays, the lab work was delayed for 5 whole months.

4.1. Quality control

While working on the biological samples, it is very important to have a certain protocol to follow because any error can not only ruin the sample but also can lead to results’ uncertainty. That is why the lab was provided with more samples than required in case if something goes wrong, they must have the extra samples to analyze. However, the delay in lab work or contamination can occur which can have impact on results.

4.2. Random errors

During sampling, although it was well taken care of the originality of fish but as stated earlier there was a great risk of fraud in market. This happened once when the retailer told the fish was from Tarbela lake (site 1) and when he was briefed upon why fish was required then the retailer confessed that the fish was from some local farm. This is a single instance, however, there could be places where retailers just hide it. The local farms use the same water from the lake but the fish there is exposed to a different environment and food which can change the results. Despite being accompanied by a local resident while sampling and an official from the fisheries department, some things cannot be trusted entirely. The sample of *Labeo rohita* from Tarbela lake was the most doubted sample in whole field work. The reason is, only this specie is widely cultivated in farms in northern Pakistan. While all other species included in study are not being farmed currently and their genuineness cannot be doubted.

Similarly, for questionnaire survey, even though the communication with farmers was easily build due to the knowledge of local languages, but still there is a mistrust found in farmers regarding the government officials. Most farmers do commit small mistakes in irrigation distribution (since Pakistan’s agriculture majorly depends on irrigation canal system and there is a certain government department which looks after its proper distribution), and whenever these farmers are approached by some person from outside they get suspicious and are reluctant to talk to them. Many people just simply refused to be the part of study, but even those who took part in it, kept asking the questions regarding our association with the government and other stuff which elaborates their fear. It was made sure that farmers feel very relaxed while answering, but the whole satisfaction regarding the fear or reluctance cannot be guaranteed entirely. This can affect the response of farmers.

4.3. Bioconcentration of OCPs in lake fish from Pakistan

POPs and especially OCPs have found to be present in all types of biota samples from extreme southern plains of Pakistan (Ali et al., 2014) to extreme northern glacial lakes in Himalayas (Nawab et al., 2020). This dispersal is attributed to the long-range transport and especially a South-Asia's unique weather phenomenon known as monsoon. In monsoon due to the high temperatures in Indian sub-continent as compared to the Indian ocean, a low pressure zone is formed over land which leads to a counter clock-wise air current system whirling and bringing the series of precipitation in whole Indian sub-continent ending in the Himalayas. The high and cold mountain ranges in north serve as the limiting wall for this seasonal air current system (Kusky, 2014). These monsoon winds take POPs from the agricultural plains of India and Pakistan as a process of long-range atmospheric transport (LRAT) to the climatically arctic-resembling Himalayan region which serves as a cold condenser of these POPs and keeps them in its glaciers and lakes (Ali et al., 2014; Guzzella et al., 2011; Nawab et al., 2020). These glaciers are the major source of most of the rivers running through the plains of India and Pakistan and irrigate the agricultural lands. These rivers not only provide drinking water to the people but also serve as fisheries hotspot as well as migratory routes to the birds. The ecological services of these rivers are numerous and the reason behind the name of India is also traced in the name of river Indus which is the longest of all rivers in Indian subcontinent. The current study also targeted the man-made lakes on river Indus. However, there are also other lakes which are rain-filled or glacier-filled and studies confirm the presence of OCPs in the water, sediment and biota of these lakes (Nawab et al., 2020; Riaz et al., 2020).

Table 4.1 The amount of DDT and OCPs in fish from different barrages on different rivers in Pakistan.

Name of barrage	ΣDDT (ng/g WW)	ΣOCP (ng/g WW)	Elevation (m)	References
Sukkur	14	23	57	(Robinson et al., 2016)
Sukkur	--	47	57	This study
Guddu	12	19	74	(Robinson et al., 2016)
Taunsa	18.5	33.7	131	(Robinson et al., 2016)
Trimmu	70-104	78-115	149	(Riaz et al., 2018)
Qadirababd	155-203	161-232	204	(Riaz et al., 2018)
Khanki	231-363	254-387	213	(Riaz et al., 2018)
Marrala	25.6-40	30-43	264	(Riaz et al., 2018)

In the Table 4.1 the first three barrages discussed are made on river Indus while the later 4 are built on river Chenab. The bioconcentration found in this study can be compared with a previous study at Sukkur barrage. This study has found the higher concentration of OCPs as compared to past study however for DDT it did not find the detectable values. Similarly, the value was higher than the latter two barrages on Indus river; Guddu and Taunsa which lie up-stream from this point. However the value was lesser than the three barrages on Chenab river (Riaz et al., 2018). This could be because of the proximity of these barrages with the agricultural areas. However, in Marala barrage the values for OCPs were far lesser than the other barrages on Chenab as well as the Sukkur. The reason for that could be the location of barrage, since it is built on the Chenab river as close as it enters the Pakistan from the state of Jammu and Kashmir (5km from the border) and there is not much agriculture behind this spot as compared to Punjab (see Figures 4.1 and 4.2).

The values for ΣOCPs are obtained based on the values for HCH, Endosulfan and ΣDDT values as these values were found in most studies and could be compared. The other pesticides such as aldrin, dieldrin, endrin aldehyde etc are omitted due to insufficient data for comparison across the studies.

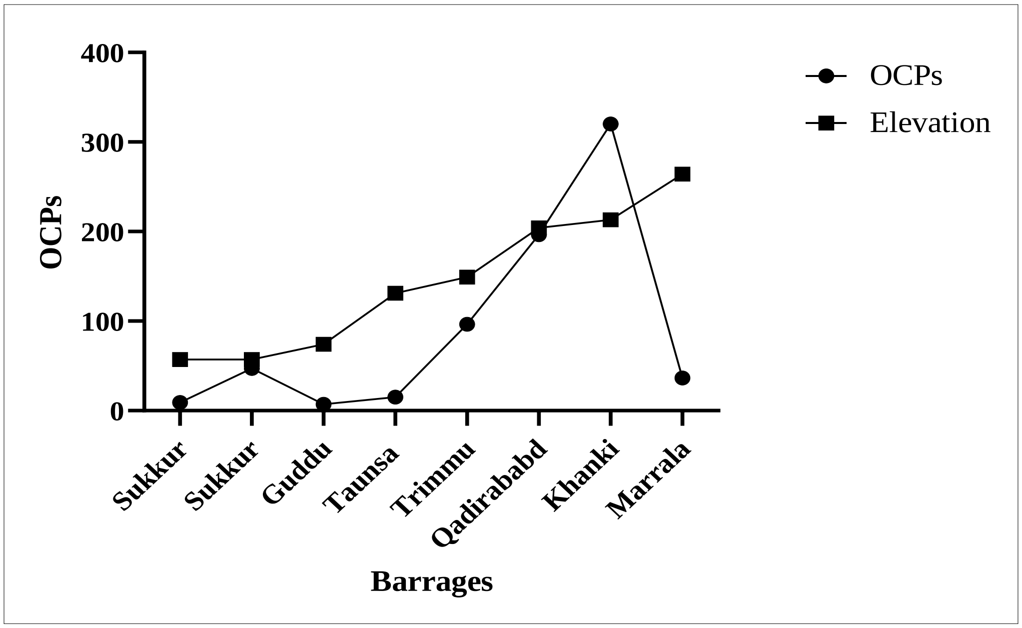

Figure 4.1 The trends of OCPs(ng/g) in fish from barrages at different altitudes.

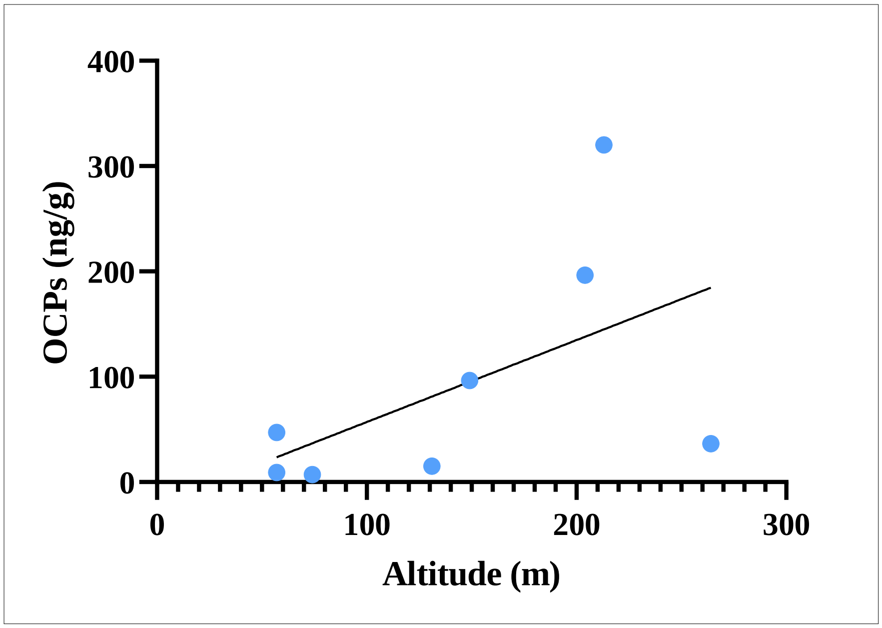

Figure 4.2 Simple linear regression showing a positive correlation of altitude and the bioconcentration of OCPs in barrages made on Indus and Chenab rivers.

Table 4.2 The bioconcentrations of OCPs in different lakes arranged by their elevations from sea level.

Lake name	ΣDDT (ng/g)	ΣOCP (ng/g)	Elevation (m)	Reference
Tarbela	19	34	430	This study
Mangla	351-586 (lw)	355-600 (lw)	332	(Riaz et al., 2020)
Subri	440-587 (lw)	447-593 (lw)	714	(Riaz et al., 2020)
Banjosa	153-254 (lw)	155-256 (lw)	1981	(Riaz et al., 2020)
Shounter	24-39 (lw)	26-46 (lw)	3100	(Riaz et al., 2020)
Ratti Gali	26-34 (lw)	28-38 (lw)	3700	(Riaz et al., 2020)
Upper Kachura	0.20	0.30	2282	(Nawab et al., 2020)
Mahodand	0.45	0.50	2866	(Nawab et al., 2020)
Saif ul Malook	0.30	0.45	3216	(Nawab et al., 2020)

There is a clear trend in this comparison since the increase in the elevation has a negative impact on the concentration of OCPs. Though (Riaz et al., 2020) have used the different standard for measuring the OCPs but even in those we can see a trend of decreasing concentration with an increase in elevation of lakes.

4.4. OCPs in water samples from Pakistan

Similarly, all the water bodies and rivers in Pakistan do contain amounts of OCPs, which varies as varied the bioconcentration of values across the altitude and proximity with agricultural plains. Those lakes/barrages located close to Punjab were found to have higher concentration of OCPs while others located at higher altitude and in other parts of Pakistan were showing the same trend shown by bioconcentration i.e increase in elevation decreased the concentration of OCPs in their water. However, this study found concentration of endosulfan same at both sites while no other OCP was found at detectable limit.

Table 4.3 Comparison of the concentrations of OCPs in different lakes and barrages along the elevation from sea level.

Lake name	ΣOCPs (ng/L)	Elevation (m)	Location	Reference
Sukkur	94.8	57	Indus, Sindh	(Ali et al., 2016)
Guddu	114	74	Indus, Sindh	(Ali et al., 2016)
Taunsa	213	131	Indus, Sindh	(Ali et al., 2016)
Rawal	3000-4000	527	Islamabad city	(Iram et al., 2009)
Simly	3000-11000	695	Islamabad city	(Iram et al., 2009)
Upper Kachura	0.31	2282	Karakorum ranges	(Nawab et al., 2020)
Mahodand	0.29	2866	Alpine Himalaya	(Nawab et al., 2020)
Saif ul Malook	0.23	3216	Alpine Himalaya	(Nawab et al., 2020)
Trimmu	42-81	149	Chenab, Punjab	(Riaz et al., 2018)
Qadirabad	53.7-137	204	River Chenab	(Riaz et al., 2018)
Khanki	142-274	213	River Chenab	(Riaz et al., 2018)
Marala	14.5-33.7	264	Pak-Kashmir border	(Riaz et al., 2018)
Subri	0.004-0.013	332	River Jhelum, Kashmir	(Ullah et al., 2019)
Sukkur	0.004 mg/L	57	Indus, Sindh	This study
Tarbela	0.004 mg/L	430	Lesser Himalaya	This study

4.5. Bioconcentration of OCPs worldwide

Table 4.4 Bioconcentrations of selected OCPs (ng/g) across different climatically tropical and subtropical countries.

Country	ΣDDT	Σ Endosulfan	ΣOCP	References
Kenya (Tana river)	140	--	287	(Wandiga et al., 2002)
Cote d'Ivore (Taabo lake)	109	167.35	319	(Roche et al., 2007)
Nigeria (Lake Geriyo & Chad)	43-5375	342-2918	385-9801	(Shinggu et al., 2015) (Akan et al., 2013)
Ethiopia (Lake Tekezedan & Ziway)	4.15-8.97	0.82	5.07-5.25	(Teklit, 2016) (Yohannes et al., 2014)
Ghana (Lake Volta)	6.50-8.97	4.65-38.50	37-71	(Gbeddy et al., 2015)
Egypt (Lake Manzala & Qarun)	1.89-38	4.75	2.77-39	(Barakat et al., 2017; El-Kady et al., 2017; Kamel et al., 2015)
India (Cauvery river Tamil Nadu, Ludhiana Punjab, Ganges river)	30-1100	--	40-1100	(Bhuvaneshwari & Rajendran, 2012; Kaur et al., 2008; Senthilkumar et al., 1999)
Mexico	16-19	0.3-0.4	17-18µg/kg	(Hinojosa-Garro et al., 2016)
DR Congo	0 - 11	--	11.5	(Verhaert et al., 2013)

The values of OCPs in this study are found to be greater than Ethiopia, Mexico, and Egypt. However, found less than Ghana, India and Nigeria, all populous and agrarian countries along with Kenya and Cote d'Ivore.

Table 4.5 Bioconcentrations of selected OCPs (ng/g) in fish from different countries worldwide.

Country	ΣDDT	Σ Endosulfan	ΣOCP	References
China (Anhui, Nansi, Poyang, Taihu & lakes in Tibetan platue)	0.03 - 5	Not detected – 0.05	3.1 - 350	(Yang et al., 2010; Yin et al., 2020; Zhang et al., 2014; Zhao et al., 2013; Zhao et al., 2014)
Turkey (Kahramanmaras, Konya, Euphrates, Van lakes)	0.01 - 45	--	4 - 47	(Aksoy et al., 2011; Erdogrul et al., 2005; Kalyoncu et al., 2009; Varol & Sünbül, 2017)
South Africa	77 - 351	141 - 516	185 - 978	(Barnhoorn et al., 2015)
Norway (Årungen lake & Etnefjord, fish liver samples)	168 - 444	--	170 - 447	(Sharma et al., 2009; Yang et al., 2010)
Iran (Lake Tashk & Parishan)	8.6 – 8.9	0.7 – 0.8	9 - 10	(Kafilzadeh et al., 2012; Kafilzadeh, 2015)
United States (Virginia, South Carolina, Mississippi, Ohio, Missouri rivers)	8 - 30	--	10 - 37	(Blocksom et al., 2010; Fair et al., 2018; Verhaert et al., 2013)

According to Table 4.5. only fish from South Africa, China and Norway have more amount of OCPs than current study in two big lakes in Pakistan. However, if averaged all the values from current and past studies done in Pakistan a different scenario can be presented. Figure 4.3 shows a graphical elaboration of bioconcentration of OCPs in lake/freshwater fish worldwide.

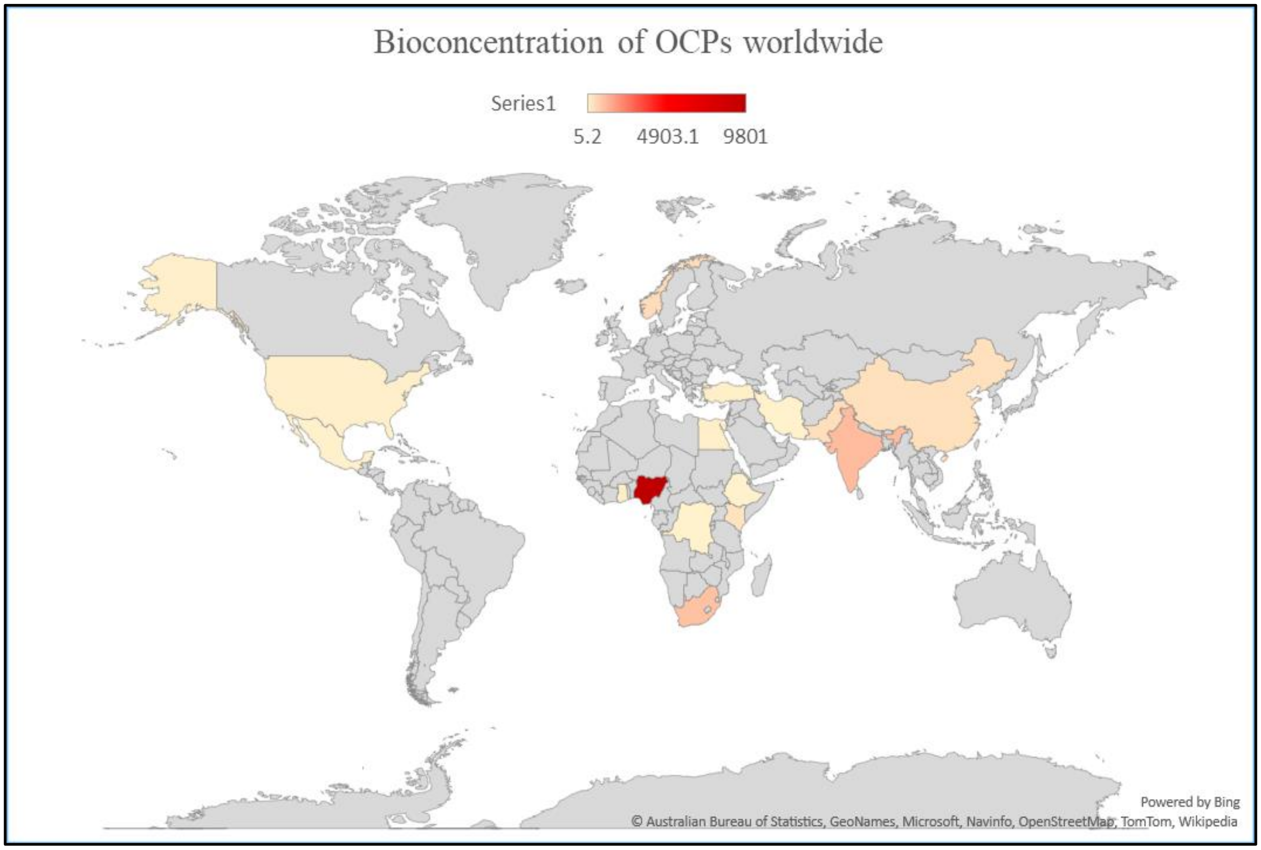

Figure 4.3 Bioconcentration of OCPs (highest recorded values) in freshwater fish worldwide. (map powered by Bing)

4.6. Organochlorine pesticides in freshwater worldwide

According to the comparison of organochlorine pesticide concentration in the freshwater bodies worldwide (Table 4.6), the concentration values found in the present study are greater than those from Brazil, Chile and South Africa while lesser than the agriculturally important and populous countries like China, India, Egypt and Nigeria. Nigeria follows the same trend as shown in table 4.4 in concentration of OCPs in water too.

Table 4.6 Concentration of OCPs (ng/L) in freshwater bodies worldwide

Country name	ΣOCPs	References
China		
Poyang Lake	5.5 – 266	(Zhi et al., 2015)
Tai hu Lake	69 – 223	(Zhao et al., 2015)
Shanghai rivers	43 – 342.7	(Chen et al., 2020; Yang et al., 2019)
Bosten Lake	30 – 90	(Shen et al., 2017)
Dongting Lake	90	(Cao et al., 2021)
India		
Ganga river	46	(Khuman & Chakraborty, 2019)
Yamuna river	78 – 2144	(Agarwal et al., 2015)
Gomti & Cauvery	42	(Agarwal et al., 2015)
Iran		
Shahid Rajaei Lake	120	(Shakeri et al., 2015)
Karun Lake	40	(Jorfi et al., 2021)
Egypt		
Manzala Lake & Nile River	1128 – 7000	(Dahshan et al., 2016; Kamel et al., 2015; Yamashita et al., 2000)
Kenya		
Nairobi River & city	0.2 – 39.7	(Lisouza et al., 2020; Ndunda et al., 2018)
Sabaki & Tana River	110 - 140	(Lalah et al., 2003)
Nigeria		
Niger River	560 – 1629	(Unyimadu et al., 2018)
Ethiope River	1100 – 1400	(Edjerea et al., 2020)
Ogba River	2900	(Ize-Iyamu et al., 2007)
Ikoro River	1100	--
Ovia River	2600	--
Owan River	Nd - 430	(Ogbeide et al., 2015)
Brazil		
Sao Paulo	0.02 – 0.6	(Rissato et al., 2006)

Table 4.6 continued.

Country name	ΣOCPs	References
Chile		
Central Valley	0.1 – 26.2	(Montory et al., 2017)
South Africa		
Johannesburg	0.2 – 36.9	(Amdany et al., 2014)
Vietnam		
Dong Nai River	230 – 590	(Nguyen et al., 2019)
Kazakhstan		
Balkhash Lake	4.02 – 122.8	(Shen et al., 2021)

4.7. Farmers' handling of pesticides

Farmers of Pakistan show a discrete behavior over the uses of pesticides within groups based on their education levels. This study also showed the same pattern and supported that the young and educated farmers show relatively better levels of awareness and attitudes towards the use of pesticides. They are known to the concept of possibly caused aquatic pollution by the excessive pesticidal use. (Khan & Damalas, 2015) have also reported the same pattern where educated farmers have shown better handling of pesticides in Pakistan and (Akter et al., 2018) reported same thing in Bangladesh. This study however did not find any sharp difference between the attitudes of farmers who undergo pesticide training and those who did not, both groups showed the same levels of awareness and attitudes, in contrast to a study conducted in central Punjab which suggested otherwise (Aldosari et al., 2018). This unusual behavior was also reported in Nepal where pesticide training courses did not help developing the attitudes of farmers (Rijal et al., 2018). Farmers' knowledge about the hazardous nature of pesticides and especially OCPs in this study was found better than the farmers of India (Bhanti et al., 2004), where in this study 74% of farmers knew that pesticides and especially the OCPs are dangerous to life, while in North India only 10-20% showed this knowledge. The knowledge was consistently higher in South Indian farmers where 70% farmers knew that pesticides can be dangerous (Mohanty et al., 2013) which roughly equals the knowledge level of farmers in current study.

5. Conclusion

During the past few decades due to intense agriculture and LRAT the problem of OCPs has become worldwide. The results in present study have showed a greater quantities of OCPs in fish from the Sukkur barrage of Indus valley as compared to the fish from the same area and elevation, yet less than the fish from reservoirs existing in the agricultural parts of the Punjab province, Pakistan. Similarly, the fish from Tarbela lake has also shown a greater concentration of OCPs as compared to the other alpine and lesser Himalayan lakes yet less than the urban lakes on the same elevation. The values for ΣDDT in fish exceeded the maximum residual limits (MRL) set by US EPA i.e. 1-1.4 ng/g however less than MRL set by European Union (50 ng/g). Endosulfan concentrations were found exceeding the MRL set by the European Union (10 ng/g). However these values are under the set values by the Government of Punjab for DDT (1250 ng/g) and Endosulfan (200 ng/g) (Riaz et al., 2018). Due to small size of sample in this study, there is a need of continuous monitoring of OCPs in these lakes which are the major source of fisheries and municipal water in Pakistan. This study also showed that very small number of farmers are familiar with the use of DDT. Also, the attitudes of the farmers towards the environment, especially the aquatic life are encouraging. Despite having less knowledge about pesticides and education level, farmers knew the hazardous effects of pesticides on human health. Endosulfan in water and other samples shows its presence in the market with either illegal black marketing or under some false brand. This problem should be addressed by the agriculture departments and the EPAs in all over Pakistan.

6. References

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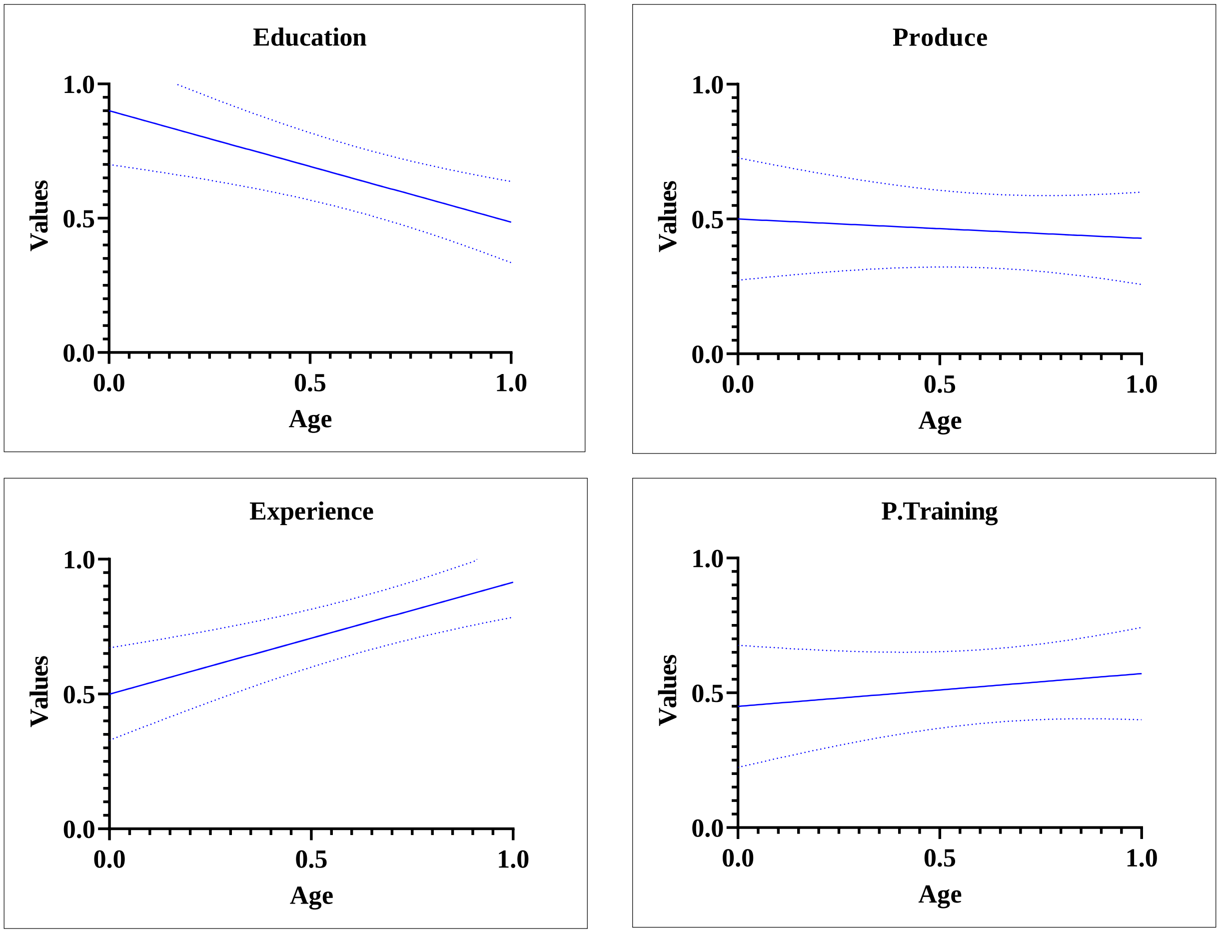
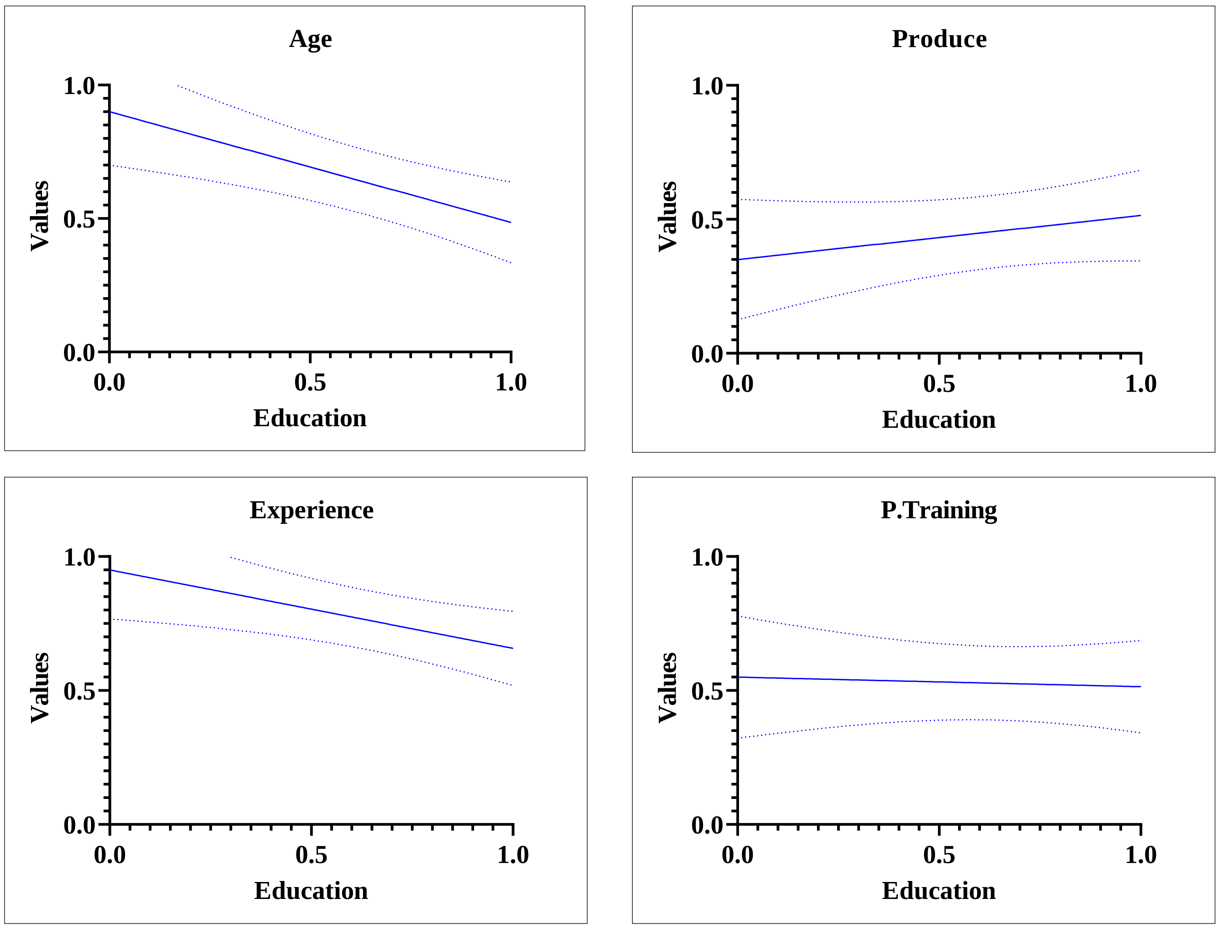
Appendix 1 Simple linear regressions

1.1 Simple linear regression of different basic information factors compared within age groups (<30, >30) on X-axis.



1.2 Simple linear regression of different basic information factors compared within the groups based on education status on X-axis.



Appendix 2 Comparison of answers within groups

2.1 Comparison of the means of responses with the age groups. Data treated as 0 and 1. Negative answers were considered 0 while ‘yes’ was considered 1.

Age		**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**
<30	Mean	.80	.05	.20	.90	.75	.90
	N	20	20	20	20	20	20
	Std. Deviation	.41	.22	.41	.30	.44	.30
>30	Mean	.82	.34	.28	.65	.65	.77
	N	35	35	35	35	35	35
	Std. Deviation	.38	.48	.45	.48	.48	.42
Total	Mean	.82	.23	.25	.74	.69	.81
	N	55	55	55	55	55	55
	Std. Deviation	.38	.42	.43	.43	.46	.38

2.2 The mean comparison of the answers by respondents across the two sub-groups in education category.

Education		Q1	Q2	Q3	Q4	Q5	Q6
Illiterate	Mean	.75	.15	.20	.65	.55	.80
	N	20	20	20	20	20	20
	Std. Deviation	.44	.36	.41	.48	.51	.41
Literate	Mean	.86	.28	.28	.80	.77	.83
	N	35	35	35	35	35	35
	Std. Deviation	.35	.45	.45	.40	.42	.38
Total	Mean	.82	.23	.25	.74	.69	.81
	N	55	55	55	55	55	55
	Std. Deviation	.38	.42	.43	.43	.46	.38

2.3 Comparison of the means of responses within the groups based on the produce of the farmers.

Produce		**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**
Vegetable	Mean	.86	.13	.30	.73	.73	.86
	N	30	30	30	30	30	30
	Std. Deviation	.34	.34	.46	.44	.44	.34
Crops	Mean	.76	.36	.20	.76	.64	.76
	N	25	25	25	25	25	25
	Std. Deviation	.43	.48	.40	.43	.48	.43
Total	Mean	.82	.23	.25	.74	.69	.82
	N	55	55	55	55	55	55
	Std. Deviation	.38	.42	.43	.43	.466	.38

2.4 Comparison of the means of answers across experience groups in farmers.

Experience		Q1	Q2	Q3	Q4	Q5	Q6
<10 years	Mean	.84	.076	.15	1.00	.85	.92
	N	13	13	13	13	13	13
	Std. Deviation	.37	.27	.37	.00	.37	.27
>10 years	Mean	.80	.28	.28	.66	.64	.78
	N	42	42	42	42	42	42
	Std. Deviation	.39	.45	.45	.477	.48	.41
Total	Mean	.81	.23	.25	.74	.69	.81
	N	55	55	55	55	55	55
	Std. Deviation	.38	.42	.43	.43	.46	.38

2.5 comparison of the means of responses and the pesticide training status of the farmers.

Pesticide Training		Q1	Q2	Q3	Q4	Q5	Q6
No	Mean	.77	.19	.26	.73	.65	.88
	N	26	26	26	26	26	26
	Std. Deviation	.42	.401	.45	.45	.48	.32
Yes	Mean	.86	.27	.24	.76	.72	.76
	N	29	29	29	29	29	29
	Std. Deviation	.35	.45	.43	.43	.45	.43
Total	Mean	.82	.23	.25	.74	.69	.81
	N	55	55	55	55	55	55
	Std. Deviation	.38925	.42	.43	.43	.46	.38

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