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Original Article

Presence of selected organochlorine pesticides (OCPs) in sediments and biota of River Satluj, Pakistan: first report

Presença de pesticidas organoclorados selecionados (OCPs) em sedimentos e biota do Rio Satluj, Paquistão: primeiro relatório

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Abstract

In the developed countries, the use of OCPs (organochlorine pesticides) has been banned. However, in South Asia several of them are still in use. In Pakistan and India a constant addition of OCPs into the atmosphere has been indicated by various researchers. In this study levels of selected organochlorine pesticide residues were assessed in sediment and biota collected from four (4) water reservoirs (3 Barrages & 1 Siphon) on the River Satluj Pakistan, along 231 miles (372 km) of River stretch which was further divided into 12 sampling sites. It was aimed to find out the levels of organochlorine pesticide (OCPs) residues in sediments and from selected fish species (*Labeo rohita, Wallagu attu, Cyprinus carpio*) of the River Satluj Pakistan. The Organochlorine residues (seven pesticides) present in samples of sediments and biota were investigated through multi residue method, using Gas Chromatograph (GC-ECD).In the current study, the concentration of DDT, was investigated in *Wallago attu* (0.786-3.987 ppb), *Labeo rohita* (0.779-4.355 ppb) and *Cyprinus carpio* (1.234-5.654 ppb). DDE was also found in *Cyprinus carpio* (1.244-6.322 ppb), *Wallag attu* (0.877-4.221 ppb) and *Labeo rohita* (2.112-5.897 ppb). Aldrin was not observed in *Labeo rohita* and *Wallago attu.* Currently, lindane and DDE was found predominately high in the sediments of study area at SZ-4 (Panjnad Barrage) ranging (2.238-8.226 ppb) and (4.234-6.876 ppb), respectively. Heaptachlor was found only at SZ-4 (Panjnad Barrage) from the sediments with concentration ranging (0.032-234 ppb). In short all other residues investigated were found below the MCL (maximum concentration level) in all the compartments of the study area set by various agencies like WHO/FAO- Codex Alimenterious.

Keywords: water pollution, pesticides, sediments, biota, freshwater management, River Satluj.

Resumo

Nos países desenvolvidos, o uso de OCPs (pesticidas organoclorados) foi proibido. No entanto, no sul da Ásia, vários deles ainda estão em uso. No Paquistão e na Índia, uma adição constante de OCPs na atmosfera foi indicada por vários pesquisadores. Neste estudo, os níveis de resíduos de pesticidas organoclorados selecionados foram avaliados em sedimentos e biota coletados de 4 reservatórios de água (3 barragens e 1 sifão) no rio Satluj, Paquistão, ao longo de 231 milhas (372 km) do trecho do rio, que foi posteriormente dividido em 12 locais de amostragem. O objetivo foi descobrir os níveis de resíduos de pesticidas organoclorados (OCPs) em sedimentos e de espécies de peixes selecionadas (*Labeo rohita*, *Wallagu attu*, *Cyprinus carpio*) do rio Satluj, Paquistão. Os resíduos de organoclorados (7 pesticidas) presentes em amostras de sedimentos e biota foram investigados através do método multirresíduos, utilizando cromatógrafo a gás (GC-ECD). No presente estudo, a concentração de DDT foi investigada em *Wallago attu*

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(0,786-3,987 ppb), *Labeo rohita* (0,779-4,355 ppb) e *Cyprinus carpio* (1,234-5,654 ppb). DDE também foi encontrado em *Cyprinus carpio* (1.244-6.322 ppb), *Wallag attu* (0.877-4.221 ppb) e *Labeo rohita* (2.112-5.897 ppb). Aldrin não foi observada em *Labeo rohita* e *Wallago attu*. Atualmente, lindano e DDE foram encontrados predominantemente altos nos sedimentos da área de estudo em SZ-4 (Panjnad Barrage) variando (2.238-8.226 ppb) e (4.234-6.876 ppb), respectivamente. Heaptacloro foi encontrado apenas em SZ-4 (Panjnad Barrage) dos sedimentos com concentração variando (0,032-234 ppb). Em suma, todos os outros resíduos investigados foram encontrados abaixo do MCL (nível de concentração máxima) em todos os compartimentos da área de estudo definidos por várias agências como a OMS / FAO-Codex Alimenterious.

Palavras-chave: poluição da água, pesticidas, sedimentos, biota, manejo de água doce, Rio Satluj.

1. Introduction

Deposits are discharged into the environment through Riverine sediments that are primary sink of organic contaminants and the best way of lasting observation of numerous pollutants. From various parts of the world, presence of Persistent Organic Pollutants (POPs) in freshwater riverine sediments has formerly been studied (Eqani et al., 2011). According to the past discoveries, POPs are identified in most estuary regions, particularly in the zones near urbanized and industrialized ones. They get and keep substantial amounts of organic contaminants. They also have hostile effects on the adjacent ecological surroundings in addition to physical health of the human beings (Guzzella et al., 2011; Ali et al., 2016a).

Most notable Organochlorines Pesticides (OCPs) like DDT and its metabolite has a dramatic impact on the populations of raptors. Pesticides toxicity extent showed lethal or sub lethal effects on vertebrates, as killing to have impacts on growth and reproduction. Since OCPs era has helped in ensuring that formed compounds are less persistent and capable of time delayed bioaccumulation in food webs (Sanchez-Bayo, 2011).

The use of pesticides in the recent decades has been increased significantly throughout the globe. The basic aim in utilization of these is to protect crops from insect invasion to achieve higher product yields with better results. The use of pesticides in the world is estimated to be 2.5 million tons per annum with constant increase. In 1954, pesticides were introduced in Pakistan, initially with formulation of 254 metric tons. In the end of 1960s and start of 1970s, tons and tons of these pesticides were purchased from Western Countries and America, while few of them, among organochlorines like DDT and benzene hexachloride (BHC) were made indigenously (Ahad et al., 2010; Eqani, 2011, Akhtar et al., 2014a).

Population expansion had not only extended its feet in socio-economic sector, but also impart its effects on agriculture sector. The prime objective of the farmers is to attain more productive yield by any conventional ways like using agro-chemicals like insecticides, pesticides and fertilizers that ultimately give the desired results (Chopra et al., 2011; Eqani et al., 2012; Asghar et al., 2016).

2. Materials and Methods

2.1. Study area

Indus river system flows through Punjab region of South Asia located in India and Pakistan (Punjab word is derived from two words Punj and Ab means five waters, respectively) Figures 1 & 2. The Indus river system is consisting of five tributaries Jhelum, Chenab, Ravi, Bias and Sutlej (Satluj), out of these five Satluj River is the longest one which is known as Zaradros in ancient Greek or Shatadru in Sanskrit. Its origin is from Lake Langa situated at 15000 feet height from sea level in the North Slope of Himalayas mountain range which is in Southwestern Tibet region of China. From its origin, it flows toward Northwest from different valleys of Himalayan range and enter into Himachal Pradesh an Indian state. After crossing Himachal, it enters the Punjab plains near Nangal point

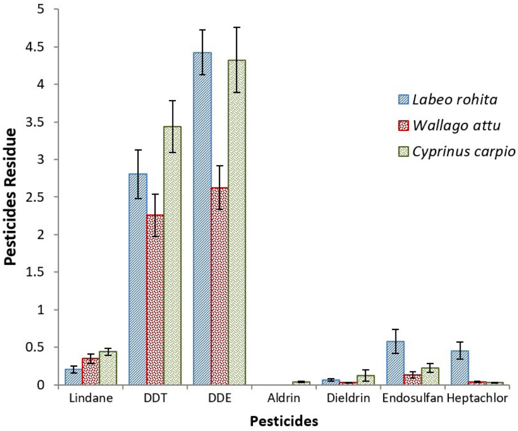

Figure 1. Mean pesticide residues (ng/g) in three fish species.

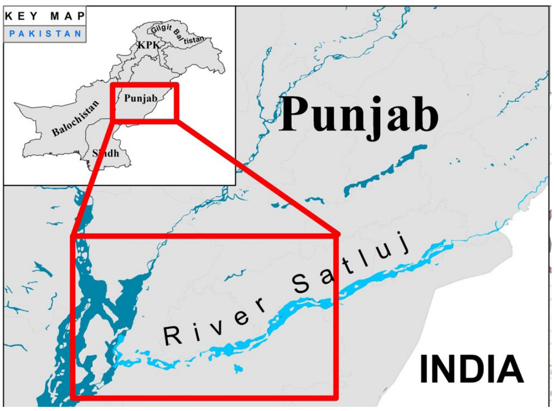

Figure 2. Study Area Map.

and continues to flow southwestward and receive Beas River waters before entering Pakistan's Punjab region and then flows further 220 miles and end up in Chenab River west of former princely state of Bahawalpur. Chenab along with other two tributaries Jhelum and Ravi also merge into the River Indus at the Punjnad located in Southern Punjab (Haider, 2011; Encyclopedia Britiannica Online, 2017).

The length of the Satluj River is approximately 964 miles, one third of which 329 miles flows in Pakistan. For current research, 231 miles (372 km) of River stretch of Satluj was selected for the study purpose, which was further divided into 4 major water reservoirs (Wetlands). It was aimed to find out the levels of organochlorine pesticide (OCPs) residues in sediments and from selected fish species (*Labeo rohita, Wallagu attu, Cyprinus carpio*) of the River Satluj Pakistan.

2.2. Collection of sediment samples

Sediment samples were collected seasonally (Summer: April to September; Winter: October to March) between 9:00 AM and 10:30 AM. Each sampling area was further divided into three sub-sampling points within range of 100 meters. Sediment samples were collected with the help of steel pipe (2 inch diameter), inserted with force to the water column, obtaining 1' sediment layer. A composite sample of sediment was obtained from three sub-sampling points with homogenized way at each sampling site (Akhtar, 2013; Eqani, 2011).

After collection, sediment samples were kept in plastic bags, labelled and brought to the research laboratory of the Department of Zoology, Government College University, Faisalabad, Pakistan.

2.3. Fish sampling and pesticide residual analysis

Detailed preliminary survey was done in selection of sampling sites. It was made possible that every sampling area was marked with a Global Positioning System (GPS, Garmin Nuvi 2597) for re-identification and record that helped for the time of next sampling. Fish sampling was carried out on seasonal basis (already mentioned) keeping the local weather condition in knowledge. The whole sampling activities were started early in the morning with known three sites per day and overall sampling schedule spanned for 1 week. The pesticide residues were studied in selected fish species rohu (*Labeo rohita*), malli (*Wallago attu*), gulfam (*Cyprinus carpio*) collected from upstream and down-stream. The fishes were selected based upon commonly caught and sold in the study area.

2.4. Extraction from fish

For fish samples the extraction method was followed as described in EMP manual (Akerblom, 1995). Breifly; the fish sample of known weight 100 mg was taken, converted into small pieces and homogenized. A mixture of sand, sample and sodium sulphate quantity 10 gm, 10 gm and 30 gm was grinded in a mortar to level when sample float freely. The sample was transferred into conical flask by adding 50 ml ethyl acetate by shaking vigorously up to three minutes. The extract was filtered by passing through the glass wool. The final filtrate was evaporated to two ml by rotary vacuum evaporator at maximum 40 degree Celsius. The solvent was exchanged from ethyl acetate to cyclohexane by evaporation while the volume of the solution was re-adjusted up to two ml.

A mixture of cyclohexane and concentrated sulfuric acid was prepared. The two chemicals in ratios 1:4 were mixed; the upper layer of organic part was withdrawn and discarded. By using a screw cap test tube in which 2 ml extract and 1 ml acid solution and was inverted approximately (40 Sec) for 20 times. After that the mixture was centrifuged for three min until two separate layers appeared. A fresh vial was used to transfer the upper organic layer and was subjected to further pesticide residue analysis by chromatographic techniques.

2.5. Extraction from sediments

The method described by Jabeen et al. (2015) was followed to extract the residue from sediments. For this known quantity (50 g) of dried sample was taken and mixed with sodium chloride (2.5 g), anhydrous sodium sulphate (10 g) and 70 ml of ethyl acetate in conical flask at 60 rpm for one hour. The solvent layer was used in centrifugation of 2500 rpm for 5 minutes and supernatant was dried by using gentle stream of nitrogen. The dried sample was mixed in 0.5 ml acetonitrile before the further chromatographic analysis.

2.6. Instrumental analysis

The collected water samples were employed through high performance liquid chromatography (HPLC) UV-Visible detector while the sediments and fish samples through Gas Chromatograph equipped with Electron Capature Detector (GC-ECD).

2.7. Detection and quantification of pesticides through GC-ECD (sediments and biota)

The Organochlorine residues (seven pesticides) present in samples of sediments and biota were investigated through multi residue method, using Gas Chromatograph (Anwar, 2008; Ullah et al., 2010; Eqani et al., 2011; Gbeddy et al., 2014; Nuapia et al., 2016). The Gas Chromatograph equipped with Electron Capture Detector (GC-ECD) was run under the following limits: The nitrogen gas was used as a carrier gas at a flow rate of 30-32 ml /m with variable temperature settlements (injector temperature: 220°C; oven temperature 150°C held for 4 minutes, then at a rate of 8°C/minute was raised to 290°C, and then hold for four minutes; Detector Temperature: 300°C).

2.8. Quality control and safety

The samples were analyzed after extraction and solvent evaporation, according to the methods proposed by the followings with slight modifications (Anwar, 2008; Ullah et al., 2010; Eqani et al., 2011; Gbeddy et al., 2014; Nuapia et al., 2016). Firstly, 1 µl of standard solution under this study (Lindane, DDT, DDE, Aldrin, Dieldrin, Endosulfan and Heptachlor) was injected and their retention times were noted.

Calibration curves of all the standard pesticides were prepared by using computer software Turbochrome® and then limit of detection was calculated by software Super Cal-5 (Perkin Elmer, Inc., USA). The recoveries of the matrix spiked were in the control limits. After performing solutions of pesticides (standard), one µl aliquot of conc. elute of sample was injected. The retention time was noted and it was within ±2 percent of these standard solutions of pesticides.

Height of these residue peaks was calculated, and residue amount of test solution was found by comparison with the height area obtained from the already known values of relative reference/standard solution in the chromatograms. Detection limits of the method were found by determining the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the Gas Chromatograph. Blank analyses were also performed in order to check interference from the sample. All analyses were carried out in triplicates and the mean concentrations were calculated based on the total number of each sample.

2.9. Data handling

Statistical analyses were carried out by applying Analysis of Variance ANOVA (one-way) by using SPSS-17 software, with significance level ($P< 0.05$); and by MS-Excel -2010 to residues of pesticides were calculated through SPSS and MS-Excel- 2010.

3. Results

Lindane was also detected in sediments of the study area with an average concentration ofSZ-3 (2.20 ppb)< SZ-1 (4.44ppb)< SZ-2 (5.07 ppb) <SZ-4 (6.33 ppb). In fish samples it was observed as *Cyprinus carpio* (0.44ppb)>*Wallago attau* (0.34 ppb)>*Labeo rohita* (0.20 ppb) (Tables 1, 2). It was observed that lindane was more prevalent and persistent in the sediments > fish > water (Tables 1, 2).The Lindane values obtained from the current study were found below in all the compartments of the study area to the Maximum Concentartion Limits (MCL) set by various agencies like WHO/FAO- Codex Alimenterious (Tables 1, 2 and Figures 1, 3).

DDT was found highest in samples of the sediments SZ-2 while lowest in SZ-3 with average concentration of 4.03(ppb) and 2.19 (ppb) respectively. In fish muscles, it was found in *Labeo rohita*(4.42ppb) and *Wallago attu* (2.62 ppb) (Tables 1, 2). The concentration of DDE was observed in the decreasing trend of *Wallago attu* <*Cyprinus carpio* <*Labeo rohita*. In fish tissues aldrin was found only in *Cyprinus carpio* (0.037 ppb) while it was not reported from the other fish species (*Labeo rohita* &*Wallago attu*)

Table 1. Mean±SE of different pesticide residues in selected fish species at study area (ng/g).

Pesticides	Fish		
	Labeo rohita	*Wallago attu*	*Cyprinus carpio*
Lindane	0.204 ± 0.046^{cB}	0.347 ± 0.065^{bAB}	0.442 ± 0.048^{cA}
DDT	2.805 ± 0.325^{bAB}	2.256 ± 0.281^{aB}	3.438 ± 0.343^{bA}
DDE	4.425 ± 0.298^{aA}	2.624 ± 0.289^{aB}	4.324 ± 0.430^{aA}
Aldrin	BDL	BDL	0.037 ± 0.007^{c}
Dieldrin	0.063 ± 0.017^{cA}	0.028 ± 0.005^{bA}	0.123 ± 0.074^{cA}
Endosulfan	0.580 ± 0.159^{cA}	0.135 ± 0.041^{bB}	0.223 ± 0.058^{cB}
Heptachlor	0.455 ± 0.113^{cA}	0.037 ± 0.008^{bB}	0.028 ± 0.004^{cB}

Means sharing similar letter in a row (upper case) or in a column (lower case) are statistically non-significant ($P>0.05$). Upper case letters are used for comparison among fish means and lower case letters are for comparison among different pesticides.

Table 2. Mean±SE of pesticide residues in sediments of study area (ng/g).

Pesticides	Zone			
	SZ-1	SZ-2	SZ-3	SZ-4
Lindane	4.44 ± 0.33^{aB}	5.07 ± 0.39^{aB}	2.20 ± 0.25^{bC}	6.33 ± 0.60^{aA}
DDT	3.39 ± 0.27^{bA}	4.03 ± 0.23^{bA}	2.19 ± 0.31^{bB}	3.39 ± 0.40^{cA}
DDE	2.56 ± 0.21^{cC}	3.78 ± 0.33^{bB}	3.00 ± 0.24^{aBC}	5.29 ± 0.30^{bA}
Aldrin	0.89 ± 0.07^{dA}	0.78 ± 0.06^{cA}	1.11 ± 0.21^{cA}	1.27 ± 0.29^{dA}
Dielderin	0.67 ± 0.28^{dA}	0.67 ± 0.28^{cA}	BDL	1.34 ± 0.19^{dA}
Endosulfan	4.39 ± 0.08^{aA}	BDL	3.14 ± 0.26^{aB}	4.30 ± 0.38^{bcA}
Heptachlor	BDL	BDL	BDL	0.10 ± 0.02^{e}

Means sharing similar letter in a row (upper case) or in a column (lower case) are statistically non-significant ($P>0.05$). Upper case letters are used for comparison among fish means and lower case letters are for comparison among different pesticides. BDL: Below Detection Limit

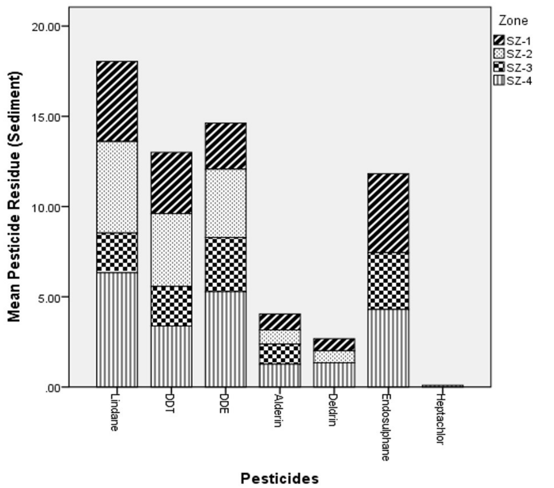

Figure 3. Mean pesticide residues (ng/g) in sediments of study area.

(Tables 1, 2).The findings revealed that in sediments aldrin remained high at SZ- 4 (0.0149 ppb) and low in SZ-1(0.0129 ppb) Tables 1, 2.

The highest concentration of Dieldrin (1.34 ppb) was detected in the sediment samples of SZ-4. The lowest level of Dieldrin in the sediments of SZ-2 remained with average of (0.78 ppb, Tables 1, 2). It was found highest in the fish species of *Cyprinus carpio* (0.123 ppb) >*Labeo rohita*(0.063 ppb) >*Wallagu atto*(0.028 ppb) Tables 1, 2. Heptachlor was detected only in sediments of one study zone of study area with average concentration (SZ-4, 0.010 ppb). Heptachlor was observed more prevalent in the fish than sediments.

4. Discussion

A great quantity of agrochemicals are in the use of farmers. These chemicals can move into the wetlands via running waters as well as subterranean canals. Garbage and wasted waters are added into the wetland by the residents. It is estimated that approximately 0.1% of agrochemicals used affect the target ones while leftover 99.9% scatter through different ways including air, sediments and through other means like water, thus bringing about in contamination of natural resources and disturbing human being's health in addition to other biotic life (Wei et al., 2007; Carvalho et al., 2009; Chopra et al., 2011; Antwi and Reddy, 2015; Zaman et al., 2016; Migheli, 2017; Le et al., 2017).

There are various routes and processes via which OCPs can move into the freshwater's of South Asia. Some of the vital sources and routes of OCPs into the fresh waters include the process of exchange of air and water and deposition (Guzzella et al., 2011), the process of leaching and pond irrigation, disposing of pesticide containers, run-off from agriculture and cleaning of equipments in incautious way (Ahad et al., 2010), its usage as pesticide for the crops as well as in control of malaria. The research showed that the contamination of ground water with POPs in Pakistan is basically the result of various factors that include shallow water, soil characteristics and rigorous use of pesticides (Eqani, 2011; Ali et al., 2016b).

Besides field applications, pesticides are being subjected to the environment by other means like manufacturing, management and transportation. In third world countries, like in Pakistan, the issue is further provoked by inappropriate storing, casual discarding of containers (holding pesticides) in addition to practice of outdated pesticide chemicals (Ahad et al., 2010; Eqani et al., 2011; Akhtar, 2013; Ali et al., 2016a). It occurs normally, because of people associated in agricultural practices lack adequate knowledge of the detrimental causes of pesticides. Resultantly, residues of agrochemicals, contribute to pollution of soil, ground and surface water including drinking water, in addition to shocking yet commercially beverages and so called mineral waters (Minh et al., 2007; Haddaoui et al., 2016).

In the developed countries, the use of OCPs has been banned (Byer et al., 2013; Buah-Kwofie & Humphries, 2017). However, in South Asia several of them are in use even now. For example in Pakistan and India a constant addition of DDTs into the atmosphere has been indicated (Syed et al., 2014). The use of POPs in the developing nations of South Asia may result in the contamination in other regions of the world too, as they have the potential of reaching the distant places. This contamination may occur even in the pristine regions like Arctic and Antarctic. Moreover, the process of POPs recycling from contaminated soils to the atmosphere is another source to environmental pollution round the world (Mendes et al., 2016). The discussed facts support the supposition that in the developing nations of south Asia, POPS contamination is still a great environmental issue (Eqani, 2011; Ali et al., 2016a).

The chemical elements (thought to dangerous for the ecosystem), if discharged into the environment, add in the soil and sediments of aquatic ecosystem. Fish species together with the lower aquatic organisms absorb and transfer them through the food chain to higher trophic levels. Hence, concentrations of POPs (pesticides) in the tissues of fish are determined mainly by the intensity of pollution of the water and food under certain conditions, chemical elements accumulated in the silt and bottom sediments of water bodies can migrate back into the water (Teklet, 2016).

Organochlorine pesticides have constantly proved their importance in agriculture. However, a number of studies have demonstrated that they have negative effects on human's health. The organochlorine pesticides are more accumulated in the fat. They tend to stay until the fat is broken down for energy. The chlorinated organic pesticides can pass through the mother placenta to the unborn child. They lead to many harmful effects such as abnormal development of the immune system, birth defects and fetal death. This is why organochlorine pesticides are considered as one of the main environmental and human health problems in the world (Akhtar, 2014b).

DDT, DDE was found in maximum concentrations from the samples of *Labeo rohita, Wallagu atto* and *Cyprinus carpio*. In the current study, the concentration of DDT, was investigated in decreasing order *Wallago attu* <*Labeo rohita*

<*Cyprinus carpio*. While DDE was remained high in *Cyprinus carpio* (1.244-6.322 ppb) >*Wallago attu* (0.877-4.221 ppb) >*Labeo rohita* (2.112-5.897 ppb). Aldrin was not observed in *Labeo rohita* and *Wallago attu*. The results were in line with the findings of Kafilzadeh (2015) and Teklet (2016). In another study, which was conducted in the Indian Punjab, the amount of OC pesticides in the fish species of freshwater of Punjab (India) was measured and showed the predominance of DDT, while other organochlorine pesticides such as lindane, alderine, dielderine, chlordane, endosulfan and heptachlor were found at lower levels (Kaur et al., 2008).

The current study showed that in the fish samples at the polluted sites, the levels of pesticides' residue of DDT and DDE elevated the higher concentrations which can be a vital source of the transfer of pesticide from fish to human through fish consumption. In fact, preliminary health risk assessment showed daily intake of found pesticides by the fish consuming persons around the River Satluj, was low and did not posing an immediate risk, which were in consistence with the studies of Jabeen et al. (2015).

Moreover, frequent presence of pesticides and their high levels of toxicity together with substantial bioaccumulation in the freshwater ichthyofauna build those toxicants and it is considered they should be required proper consideration in the field of aquatic toxicology. Normally, the aquatic organisms like fish, gather xenobiotic chemicals, particularly those having poor water holding capacity; because of their close contact with medium which bring chemicals in solution form or suspension in addition as fish has to take out oxygen from the same medium by passing vast quantity of water over the surface of gills. Fish death or injury due to the pesticides accumulation is thought to be the primary reason of reducing ichthyofaunal diversity and other organisms as well as humans through the trophics of food chain. Behavioral escaping of contaminants may be the additional factor of reducing the population of fishes Akan et al. (2013).

Unluckily, increased population rate, soil erosion, management issues in water and serious deforestation have caused main reductions in the quality of aquatic ecosystems. The issue is more pronounced in the cities due to careless management in the disposal of domestic and industrial effluents and also the ineffective drainage system that has severely degraded the river water quality (Qadir, 2009; Khan, 2011; Nowell et al., 2014; Azmat et al., 2016).

5. Conclusion

The use of pesticides in the recent decades has been increased significantly throughout the globe. As for current results showed that although the organochlorines are banned for agricultural use in many countries including Pakistan, their level in sediments and fish samples might be due to illegal use of these POPs in the study area and its neighboring regions.

- Present studies revealed that residues of OCPs were present in all the compartments of study area. But their levels were below the Maximum Concentration Limits (MCLs) set by various international bodies.
- Overall, the increasing trend of pesticides accumulation was noticed from upstream to downstream of the river.
- The concentrations of selected pesticide residues were found in increasing trend as fish > sediments.
- A huge amount of agrochemicals are being practiced by farmers which can enter these fresh water wetlands; through running waters and subterranean canals. It was observed during this study, garbage and wastewaters are poured in the wetland by inhabitants.

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