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Human Health Risk Surveillance through the Determination of Organochlorine Pesticides by High–Performance Liquid Chromatography in Water, Sediments, and Fish from the Chenab River, Pakistan

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ABSTRACT

The current study assessed the spatio–temporal variations and human health surveillance associated with organochlorine pesticides (OCPs) contamination in water, sediments and fish from Chenab River, Pakistan. The OCPs determinations were made using high performance liquid chromatography with a reverse phase C18 column. The total OCPs levels ranged from 13.33–274.59 ng/L in water, 4.63–239.11 ng/g in sediments and 23.79–387.12 ng/g in fish species. The overall pattern of mean OCPs concentrations followed the order as ΣDDTs > Σendosulfan > aldrin and OCPs pollution pattern among the headworks were Khanki Barrage > Qadirabad Barrage >

Trimmu Barrage > Marala Barrage in all three environmental matrixes during both seasons. The estimated daily intake (EDI) for $\sum$OCPs was found to be 22.44 ng/kg/day. The hazard ratios calculated to assess the carcinogenic risk indicated that the values for $\sum$DDT and aldrin at the 95^{th} percentile concentrations were greater than one, indicating the probability of carcinogenic risk occurrence of one in million populations due to fish consumption. Therefore, these high levels of OCPs and carcinogenic risk through fish consumption highlights the needs of immediate elimination of OCPs from riverine environment of Chenab River and we recommend long-term monitoring based freshwater ecological studies to be conducted in the study area.

KEYWORDS: carcinogenic health risk, Chenab River, high performance liquid chromatography, organochlorine pesticides

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Introduction

The optimistic effects of synthetic chemicals to the industrial development accelerate their production and usage to bloom the industrial based economy (Arslan et al. 2015). In this context, the agrochemicals, particularly the pesticides production in last few decades have been intensified manifolds, to meet the global food demand in increasing world population scenario (Merrington et al. 2004). However, simultaneously studies have reported the harmful effects of pesticides on living organisms and the environment (Paccagnella et al. 1971; Tariq et al. 2007; Aktar, Sengupta, and Chowdhury 2009). Among pesticides, the organochlorine pesticides (OCPs) are of great global concern, due to adverse eco-toxicological effects associated with them. The non-polar and semi-volatile characteristics of the OCPs along with their persistence and bio-accumulative nature characterize them as persistent organic pollutants (Zheng et al. 2013; Parween et al. 2014).

Pakistan is an agrarian country with approximately 70% of its population directly or indirectly associated with agriculture. Hence, over past few decades, a massive rise in pesticides consumption rate in the agricultural sector of the country has been observed (Tariq and Hussain 2004; Azmi et al. 2006; Sultana et al. 2014). Moreover, the application of OCPs in Pakistan has also been reported to control the insect-borne epidemics diseases, domestic pests and termites (Malik et al. 2011; Eqani, Malik, and Alamdar et al. 2012; Eqani, Malik, and Katsoyiannis et al. 2012). According to World Wide Fund for Nature (2007) - Pakistan, pesticides consumption in Pakistan is increasing with an annual rate of 6%; which is expected to increase manifolds in the upcoming years (Economic Survey of Pakistan 2014–2015). However, despite of the fact that Pakistan is a party to the Stockholm Convention on Persistent Organic Pollutants (2001), the indiscriminate inland use of banned OCPs are still reported due to their low cost, effectiveness and inefficient indigenous regulatory mechanism (Tariq et al. 2007; Eqani, Malik, and Mohamm 2011; Syed and Malik 2011). In Pakistan, mass application of the OCPs in recent decades has resulted in the contamination of various environmental sections, including water, sediments and biota (Eqani, Malik, and Alamdar et al. 2012). The existing scenario likely exerts undesirable impacts to exposed populations, including disturbance in the physiological, biochemical, behavioral and developmental responses at different trophic levels of the food web (Beketova et al. 2013). There is growing evidence on carcinogenic, neurological, endocrinal, natal and neonatal disorders arising from acute to chronic exposure to the OCPs (Williams 2013).

In context to the human dietary exposure to the persistent organic pollutants, fish and its products are considered to be responsible for 90% of human persistent organic pollutants intake (Robinson et al. 2016) because fish has a key status in connecting aquatic food web to human lifecycle (Sun et al. 2016). At the same time, fish have been considered as a key bioindicator to monitor the persistent organic pollutants in aquatic ecosystems (Pacini et al. 2013; Eqani et al. 2015). In Pakistan, several studies have been conducted to access the levels of OCPs in surface water (Iram et al. 2009; Ahad et al. 2010; Eqani, Malik, and Katsoyiannis et al. 2012; Akhtar et al. 2014; Mahmood et al. 2014) and surface sediments (Sanpera et al. 2003; Eqani, Malik, and Mohamm 2011; Malik et al. 2011; Ali et al. 2014; Mahmood et al. 2014; Malik et al. 2014; Syed et al. 2014; Ali et al. 2016). However, to date, only three studies have been conducted to investigate the levels of OCPs in fish media of the country (Eqani et al. 2013; Aamir et al. 2016; Robinson et al. 2016). Therefore, the present study aims to evaluate occurrence, distribution and levels of OCPs in water, sediments and fish muscle tissues from four headworks across the Chenab River. To best of authors' knowledge, the conducted study is a pioneer investigation in the study area that would be significantly valuable in global freshwater studies.

Materials and Methods

Study area and sampling strategy

The Chenab River is a tranboundary river that originates in India and enters into Pakistan where its watershed covers densely populated, industrial cities and agricultural area of the Rachna Doab and Jech Doab, both are the most intensively cultivated areas of Pakistan that follows conventional cultivation pattern, i.e. Kharif (summer season) and Rabi (winter season). The river provides ecological habitat to important aquatic and avian species (Eqani, Malik, and Alamdar et al. 2012; Eqani, Malik, and Katsoyiannis et al. 2012; Altaf et al. 2015). All four major headworks across the Chenab River, i.e., Marala Barrage, Khanki Barrage, Qadirabad Barrage and Trimu Barrage, were selected for the purpose of this study. The selection of these stations was based on their ecological importance and accessibility. The water and surface sediment samples were collected from upstream and downstream points to each headwork site (**Figure 1**), primarily to incorporate the dilution factor due to removal or addition of water flow through different link canals, whereas the fish sampling was conducted at headwork site with the assistance of the Department of Fisheries, Punjab. Details of the sampling sites are provided in **Table 1**. Based on the variation in flow and agricultural practices, the samples were collected from the selected sampling sites in two distinct seasons, i.e., winter and summer between May, 2014 and March, 2015.

Sample collection and preservation

Water sampling

The time proportional composite water samples (n=16), in summer and winter were collected manually in amber glass bottles, pre-rinsed with dichloromethane. The zero headspace was assured, followed by on-site sealing and labeling of samples (American Public Health Association 2005). In order to ensure preservation, the samples were placed in ice box containing wet ice and later transferred to Aquaculture Laboratory at the Sustainable Development Study Centre, Government College University Lahore, where they were stored at –20^{o}C through refrigeration until analysis.

Sediment sampling

Composite surface sediment samples (n=16) were also collected in winter and summer seasons from each sampling site, at depth of 0–10 cm. Each sediment sample was a composite of five sub-samples, collected by a self-designed perforated ~10 cm diameter pipe. The composite samples were transferred to polyethylene bags, followed by labeling using self-administered labeling stickers and on-field preservation in ice box with wet ice. The samples were then transferred to laboratory where they were air dried, sieved (2 mm), frozen, and stored at −20 °C until analysis.

Fish sampling

The fish samples (n= 326) were collected using fishing nets, with the assistance of local fishermen and Department of Fisheries, Punjab from all four headworks across Chenab River. Among the fish species, only five carnivores fish species namely; *Channa marulius* (local name; Saul), *Anguilla rostrata* (local name; Bam), *Channa punctatus* (local name; Daula), *Wallago attu* (local name; Mullee) and *Labeo boga* (local name; Bhangan) were collected from each sampling site. However, the *Wallago attu* from Khanki Barrage and *Anguilla rostrata* from Trimu Barrage were not found in significant quantities to be included in study. The selection of carnivorous species was based on the fact that they exist on higher trophic levels in aquatic ecosystem, thus acting as admirable bioindicators. On field, the collected fish species were identified with the help of fish taxonomist and were screened for sampling on the basis of similar morphological characteristics. The collected samples were then stored in ice box containing dry ice and transported to Aquaculture Laboratory at the Sustainable Development Study Centre, Government College University Lahore; where stored at –20 ^{o}C until analysis.

Sample extraction and clean up

The water samples were passed through Whatman ashless, Grade 42 filter paper, and extracted employing a liquid-liquid method with 25–30ml dichloromethane using a 1 L separatory funnel. The sediment samples (~20 g) and fish samples (~20–30 g) were prepared with solid-liquid extraction with dichloromethane in Soxhlet assembly for 24 h (Eqani et al. 2013; Mahmood et al. 2014). In laboratory, prior to extraction, fish samples were thawed, shucked and homogenized with anhydrous Na_2SO_4 using mortar and pestle (Liu et al. 2010). The surrogate standards of 2, 4, 5, 6–tetrachloro–m–xylene and decachlorobiphenyl (PCB-209) were added to each sample prior to extraction. The extracted samples were then concentrated and the solvent phase was exchanged from dichloromethane to n–hexane using rotary evaporator (Diahan Scientific WEV-1001L). The concentrated extract were then subjected to clean-up process by eluviation through 12 mm diameter alumina/silica columns, packed from top to bottom, with anhydrous Na_2SO_4 (1 cm layer), 50% sulfuric acid-silica (3 cm layer), neutral 3% deactivated silica gel (3 cm layer), and 3% deactivated neutral alumina (3cm layer). The purified segment of sample extract were then concentrated up to 0.2 ml in septa vials using gentle flow of pure nitrogen gas, followed by addition of dodecane (25 μl) as solvent keeper and PCB-141 as an internal standard before analysis through high performance liquid chromatography (HPLC).

HPLC analysis

The selected OCPs, *viz*. α-endosulfan, β-endosulfan, aldrin, *o,p'*-dichlorodiphenyldichloroethylene (*o,p'*-DDE), *o,p'*-dichlorodiphenyltrichloroethane (*o,p'*-DDT), *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), *p,p'*-dichlorodiphenyltrichloroethane (*p,p'*-DDT) were detected from samples using high performance liquid chromatography (Shimadzu, LC 20A Japan) with a reverse phase C18 Column (250 nm ×4.6 mm ID; pore size 5 μm) encapped (Merck Germany) with quaternary pump and photodiode array detector. For organochlorines, acetonitrile: water (75:25 v/v) was used as a mobile phase and 20 µl volume of analyte was injected with 1 mL/min flow rate and temperature was controlled at 30 ˚C.

Quality control and assurance

Quality control and assurance procedures were firmly followed throughout the samples preparation and analysis to ensure the quality of results. All organic solvents and inorganic salts used were of HPLC grade/ analytical grade and purchased from Merck KGaA (Germany). The surrogate and internal standards were purchased from CPAchem Ltd., Zagora, Bulgaria. To prevent any contamination to the sample, prior to use, the glassware was thoroughly rinsed with distilled water and baked at 450 °C in muffle furnace. Calibration standards were used on daily basis to ensure instrumental calibrations and follow up standards were run after every 20th sample to obtain precise results. The mean values for surrogate recovery were 85.1% and 91.4% for 2, 4, 5, 6–tetrachloro–m–xylene and decachlorobiphenyl, respectively.

Statistical analysis

Basic descriptive statistics were performed using Statistica 10.2.

Health risk assessment

The human health risk, both non-carcinogenic and carcinogenic risks *via* fish consumption from the contaminated area was assessed through calculating estimated daily intake and hazard ratios as previously followed by Jiang et al. (2005) and Robinson et al. (2016). The hazard ratios for carcinogenic risk were calculated at 50th percentile and 95th percentile exposure concentrations by dividing estimated daily intake with carcinogenic benchmark concentration as given by

$$Hazard\ ratio = \frac{Estimated\ daily\ intake}{Carcinogenic\ benchmark\ concentration} \quad (1)$$

The estimated daily intake and carcinogenic benchmark concentration values were computed using the following, respectively.

$$Estimated\ daily\ intake = \frac{consumption\ rate \times contaminant\ concentration}{body\ weight} \quad (2)$$

$$Carcinogenic\ benchmark\ concentration = \frac{risk\ level \times body\ weight}{consumption\ rate \times oral\ slope\ factor} \quad (3)$$

The average body weight for the adults (60 kg) and fish consumption rate (8 g/person/day) in the studied area were determined through non-structured questionnaire survey. The risk level was set to one in one million populations for all calculations and oral slope factor for OCPs were obtained by values evaluated by the Integrated Risk Information System of the US Environmental Protection Agency (United States Environmental Protection Agency 2007). The non-carcinogenic risk was by making comparison of *estimated daily intake* with the available acceptable daily intake values. The non-carcinogenic risk for also assessed for DDTs through hazard ratios at the 50^{th} and 95^{th} percentile by substituting reference dose with slope factor in Eq. (3) (Robinson et al. 2016).

Results and Discussion

OCPs levels in surface water

Descriptive statistics of selected OCPs in surface water from the four Headworks of Chenab River are presented in **Table 2**. Among the studied OCPs, the pattern of mean concentration of OCPs in water followed the order as: ΣDDTs (sum of *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT) > Σendosulfan (sum of α-endosulfan and β-endosulfan) > aldrin. Among isomers/metabolites of DDTs and endosulfan, similar order of magnitude was observed during both winter and summer seasons; *p,p′*-DDT > *p,p′*-DDE > *o,p′*-DDT > *o,p′*-DDE and β-endosulfan > α-endosulfan, respectively. The ΣOCPs levels in surface water samples from the headworks across Chenab River revealed that the highest contamination was found at Khanki Barrage (142–274.59 ng/L), followed by Qadirabad Barrage (53.78–136.92 ng/L), Trimmu Barrage (41.9–80.89 ng/L), and Marala Barrage (14.53–33.71 ng/L).

A comparison of OCP concentrations in surface water from other studies reported from Pakistan and other developing countries are provided in **Table 3** and **Table 4**, respectively. The nationwide comparison revealed that the mean ΣDDTs concentrations in current study were comparable to those previously reported by Eqani, Malik, and Katsoyiannis et al. (2012) and higher than those by Mahmood et al. (2014) from Chenab River and its tributaries, respectively. However, the concentration was lower than those detected from Rawal Lake and Simly Lake by Iram et al. (2009). While, the aldrin and Σendosulfan concentrations were higher than those reported from study areas of Chenab River (Eqani, Malik, and Katsoyiannis et al. 2012; Mahmood et al. 2014).

For the comparison of results from the current study with previously reported data from developing countries, an inconstant trend was observed. The ΣDDTs levels in surface water from current study were found higher than those from Republic of Macedonia (Veljanoska-Sarafiloska, Jordanoski, and Stafilov 2013), India (Kumarasamy et al. 2012), Iran (Behfar et al. 2013; Kafilzadeh 2015) and China (Tang et al. 2008; Cui et al. 2015). In contrast, the lower ΣDDTs concentrations were determined than those reported from three Indian rivers, i.e. Ganges, Yamuna, and Ghaggar (Singh, Malik, and Sinha 2007; Kaushik et al. 2008, 2010). Similarly, the aldrin concentrations from Chenab River were higher than those from India (Kumarasamy et al. 2012), China (Cui et al. 2015), Ghana (Kuranchie-Mensah et al. 2012) and Ethiopia (Teklu, Adriaanse, and Van den Brink 2016). Nevertheless, the aldrin concentrations were found lower than some previously published data from India, Egypt and Iran (Singh, Malik, and Sinha 2007; Said et al. 2008; Behfar et al. 2013). However, the Σendosulfan levels in present data were found to be lower than all the previously consulted results from different developing countries (Singh, Malik, and Sinha 2007; Behfar et al. 2013; Kafilzadeh 2015; Teklu, Adriaanse, and Van den Brink 2016).

OCPs levels in surface sediments

The descriptive statistics of OCPs levels in the surface sediments from Chenab River are summarized in **Table 2**. The overall pattern of OCPs mean concentrations followed the trend as ΣDDTs > Σendosulfan > aldrin during winter season, and ΣDDTs > aldrin > Σendosulfan during summer season, respectively. In consistent with ΣOCPs pattern in surface water, the ΣOCPs concentrations in surface sediments from the headworks across Chenab River followed the same pattern of contamination, i.e. Khanki Barrage (160.1–239.11 ng/g) > Qadirabad Barrage (40.42–49.23 ng/g) > Trimmu Barrage (33–44.11 ng/g) > and Marala Barrage (4.63–8.6 ng/g).

For the OCP levels countrywide comparison, the mean ΣDDTs concentrations in the sediments of the Chenab River were higher than those reported from different water bodies of Punjab, Province (Malik et al. 2011), Mehmood Booti Drain (Ali et al. 2016), Soan River, Korang River, Ling Stream and Lai Nullah (Malik et al. 2014), Haleji Lake (Sanpera et al. 2003) and coastal sediments from Pakistan (Bano and Siddique 1991; Ali et al. 2014). However, findings were comparable or slightly higher than those in sediments from Nullah Aik and Nullah Palkhu (Mahmood et al. 2014), Chenab River (Eqani, Malik, and Mohamm 2011), River Ravi (Syed et al. 2014) and Karachi Ghas Bander (Sanpera et al. 2003). The aldrin concentrations in the current study were comparable to those previously reported from Chenab River and River Ravi (Eqani, Malik, and Mohamm 2011; Malik et al. 2011).

In comparison with developing countries, the ΣDDTs concentrations in sediments from the current study were higher than results from Egypt (Said et al. 2008; Barakat et al. 2013), Congo (Verhaert et al. 2013), China (Yun et al. 2014), Iran (Kafilzadeh 2015) and Republic of Macedonia (Veljanoska-Sarafiloska, Jordanoski, and Stafilov 2013); comparable to those reported from Egypt (Barakat et al. 2012) and India (Kumarasamy et al. 2012) and lower than those from Mexico (Hinojosa-Garro, Burgos Chan, and Rendón-von Osten 2016) and Thailand (Poolpak et al. 2008). The comparison of aldrin concentrations exhibited a comparable pattern with the data from Egypt (Barakat et al. 2012, 2013) and Ghana (Kuranchie-Mensah et al. 2012); however, much lower concentrations were observed than those previously cited from Egypt (Said et al. 2008), India (Kumarasamy et al. 2012), Thailand (Poolpak et al. 2008) and China (Yun et al. 2014). In context to Σendosulfan concentrations in sediments, the current study have shown the comparable or lower pattern than those reported from other developing countries (Poolpak et al. 2008; Barakat et al. 2013; Kafilzadeh 2015; Hinojosa-Garro, Burgos Chan, and Rendón-von Osten 2016).

OCPs levels in fish

The OCPs levels in five carnivorous fish species from headworks across the Chenab River are provided in **Table 5**. The mean ΣOCPs concentrations in fish samples ranged from 23.79 to 387.12 ng/g. In consistent to the ΣOCPs levels trend in surface water and sediments from the current study, the lowest levels of ΣOCPs in fish samples was observed at Marala Barrage (23.79–43.41 ng/g). The mean ΣDDTs concentrations in fish samples varied between 20.45–363.03 ng/g, with highest mean value of 363.03 ± 98.88 ng/g in *Channa marulius* from Khanki Barrage. The

Σendosulfan and aldrin showed relative lower mean concentrations than ΣDDTs, ranging 2.03–30.31 ng/g and not detected–33.02 ng/g, respectively.

The comparison of OCPs levels in fish reported by other studies from Pakistan (**Table 3**) elucidates that the levels of ΣDDTs in current study were found to be comparable to the data reported from Kabul River (Aamir et al. 2016) and Chenab River (Eqani et al. 2013); and much higher than those from Indus River (Robinson et al. 2016). However, the mean concentrations of aldrin were comparable or higher than the findings of Eqani et al. (2013) from Chenab River. The OCPs concentrations comparison with other developing countries (**Table 4**) have shown heterogeneous trends with higher levels of mean ΣDDT concentrations in present data than those reported from China (Cui et al. 2015), Republic of Macedonia (Veljanoska-Sarafiloska, Jordanoski, and Stafilov 2013), India (Malik, Singh, and Ojha 2007; Kaur et al. 2008), Egypt (Said et al. 2008), Ghana (Kuranchie-Mensah et al. 2013) and Iran (Kafilzadeh 2015). However, the mean ΣDDT concentrations were found to be comparable or lower than those in fish samples from Ethiopia (Yohannes, Ikenaka, Nakayama et al. 2014) and Mexico (Hinojosa-Garro, Burgos Chan, and Rendón-von Osten 2016), respectively. The aldrin levels from Chenab River were found comparable or slightly higher than the levels reported in fish from Ethiopia (Yohannes, Ikenaka, Nakayama et al. 2014), China (Cui et al. 2015), India (Malik, Singh, and Ojha 2007), Egypt (Said et al. 2008) and Ghana (Kuranchie-Mensah et al. 2013).

Human health risk assessment

Non-carcinogenic risk characterization

The current levels of aldrin, endosulfan and DDTs in fish from the Chenab River were compared to the tolerance/ maximum residual limits set by different regulatory bodies globally.

The studied levels of DDTs in fish were found to be well within the tolerance limits set by the Punjab Pure Food Rules (Government of Punjab 2011) for aldrin (200 ng/g), endosulfan (200 ng/g) and DDTs (1250 ng/g). However, the comparison of concentrations with the maximum residual limits set by the international and regional regulatory bodies revealed that the DDTs in fish samples were found to be exceeding the limits set by United States Environmental Protection Agency (i.e. 14.4 ng/g) (United States Environmental Protection Agency 2000), the European Union (50 ng/g) (Binelli and Provini 2004) and Codex Alimentarius Commission of the United Nations Food and Agriculture Organization (300 ng/g) (Food and Agriculture Organization 2017). Similarly, the endosulfan concentrations were found in excess to the permissible limit of 10 ng/g established by the European Union. In order to estimate non-carcinogenic risk to human health, the estimated daily intake values were also calculated and compared with the acceptable daily intake through fish consumption. The calculated EDI (**Table 6**) were found within the proposed acceptable daily intake 10000 ng/g (Food and Agriculture Organization 2011). However, the current EDI levels of DDTs were higher than the data previously reported from Pakistan (10.7 ng/g) (Eqani et al. 2015), China (14.7 ng/g), and Ethopia (1.1–2.46 ng/g) (Jiang et al. 2005; Yohannes, Ikenaka, Saengtienchai et al. 2014). Furthermore, the hazard ratios were calculated for DDTs on the basis of available reference dose for DDTs (0.5 µg/kg/day) at the 50^{th} and 95^{th} percentile concentrations that indicates the presence of risk related with the consumption of fish from Chenab River (**Table 7**).

Carcinogenic risk estimation

The carcinogenic risk was calculated on the 50th and 95th percentile concentrations for aldrin and different isomers of DDTs (**Table 7**). The calculated HRs on 50^{th} percentile concentration basis suggested absence of significant carcinogenic risk which is strengthen by similar findings reported from Indus River and Chenab River, Pakistan (Eqani et al. 2015; Robinson et al. 2016). However, lower fish consumption rate (8 g/person/day) in the present study area; as compared to those from China (30 and 105 g/person/day) (Jiang et al. 2005), Ethopia (30 g/person/day) (Yohannes, Ikenaka, Saengtienchai et al. 2014) and India (47 g/person/day) (Muralidharan, Dhananjayan, and Jayanthi 2009) might be a governing factor for lower risk levels calculated for residents in the vicinity of Chenab River, Pakistan.

The calculated hazard ratio values for DDTs and aldrin at 95th percentile concentrations were exceeding one, indicating the carcinogenic risk occurrence among one in million individuals due to contaminated fish consumption. The current status is further strengthened by the previously published reports from the Indus River, Pakistan (Robinson et al. 2016), South Korea (Moon et al. 2009) and China (Jiang et al. 2005); where calculated HRs for the 95^{th} percentile concentration were greater than 1 while for the 50^{th} percentile were observed less than unity. Among the studied OCPs, the highest HR was recorded for aldrin that might be attributed to the higher cancer slope factors for aldrin (17 mg/kg–day^{-1}). Furthermore, the current calculated ratios may not be the actual representation of carcinogenic and non-carcinogenic risk to humans in the study area as some factors such as consumption of vegetables, animal meat and other environmental contaminants were not considered in present study. Therefore, possibly actual risk to humans in the vicinity of Chenab River would be much higher that needs to be assessed through a comprehensive study, incorporating all other factors.

Conclusion

The spatio-temporal variations and human health surveillance associated with OCPs in the water, sediments and fish from four major headworks along Chenab River, Pakistan were assessed.

Among the headworks, the Khanki Barrage and Marala Barrage were found to be most polluted and least polluted sites, respectively. This might be attributed to the fact that Khanki Barrage is the immediate downstream barrage to the most agriculturally productive area of Pakistan, whereas Marala Barrage is the most upstream site on the Chenab River in Pakistan; The upstream region is characterized by low agricultural activities in Jammu and Kashmir and India possibly due to uneven gradient. The overall pattern of mean OCPs concentrations in all three studied environmental matrixes followed the trend as ΣDDTs > Σendosulfan > aldrin during both seasons, i.e., winter and summer. The carcinogenic-human health risk assessments due to consumption of fish showed that the hazard ratio values were exceeding the value of 1 at the 95^{th} percentile concentration for aldrin and ∑DDTs, indicating the probable cancer risk occurrence of one in million individuals. Therefore, a long-term monitoring program for OCPs in fish from the region is needed to precisely evaluate the human health risk associated with riverine fish consumption. We recommend well-founded regulatory measures to be established and implemented in accordance with the Stockholm Convention on Persistent Organic Pollutants (2001) to which Pakistan is also a party.

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Table 1. Description of sampling sites across the Chenab River.

Site Number	Sampling Site Code	Latitude	Longitude	Description
1.	S1	32°41'10.56"N	74°28'31.50"E	Located the upstream to Marala Barrage and serves as most-upstream site in Pakistan, representing the organochlorine pesticides load from India and Jammu and Kashmir
2.	S2	32°38'19.63"N	74°22'48.32"E	Located downstream to the Marala Barrage, after the flow leaves the mainstream through Upper Chenab Canal and Marala-Ravi Link Canal.
3.	S3	32°28'20.40"N	74° 2'15.36"E	Located upstream to Khanki Barrage and represents the pollution load from Sialkot, Gujrat and Wazirabad.
4.	S4	32°23'4.42"N	73°55'34.47"E	Located downstream to Khanki Barrage, until this point the supplementary water from Upper Jehlum Canal (originating from Jehlum River) enters into Chenab River and a portion of Chenab flow

				leaves the mainstream through Lower Chenab Irrigation Canal.
5.	S5	32°19'53.63"N	73°45'40.22"E	Located upstream to Qadirabad Barrage before the confluence of Rasul-Qadirabad Link Canal with Chenab River.
6.	S6	32°18'46.55"N	73°35'33.23"E	Located downstream to Qadirabad Barrage immediately after the Qadirabad-Balloki Link Canal leaves the Chenab River.
7.	S7	31° 9'56.65"N	72°11'43.96"E	Located upstream to Trimu Barrage before the confluence of River Jhelum and Chenab River.
8.	S8	31° 5'2.69"N	72° 8'59.56"E	Located downstream to Trimu Barrage after the water flow from River Jhelum enters the Chenab River.

Table 2. Descriptive statistics of organochlorine pesticides in water and sediments of four major headworks across Chenab River.

Isomers	Water (ng/L)				Sediments (ng/g)			
	Summer		Winter		Summer		Winter	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
α-endosulfan	2.49 ± 3.77	0.3–11.74	4.39 ± 8.1	0.6–24.42	2.55 ± 3.73	0.8–11.75	4.47 ± 8.06	1.1–24.4
β -endosulfan	3.15 ± 4.33	0.72–12.98	4.6 ± 6.02	0.83–16.9	2.48 ± 2.99	0.31–9.15	3.63 ± 4.78	1.15–14.5
Σendosulfan	5.64 ± 8.01	1.5–24.68	8.99 ± 13.52	1.5–41.3	5.03 ± 6.6	1.1–20.81	8.1 ± 12.6	2.2–38.92
Aldrin	4.93 ± 4.76	0.13–13.18	7.69 ± 9.15	0.75–28.22	5.05 ± 6.8	0.1–18.95	5.91 ± 8.12	0.46–21.18
o,p' -DDE	3.76 ± 3.81	ND-11.72	5.91 ± 5.85	ND-18.18	4.42 ± 3.67	0.62–10.55	5.32 ± 5.14	0.3–14.49
p,p' -DDE	18.28 ± 18.18	1.9–56.22	30.48 ± 35.6	3.51–112.17	13.76 ± 22.22	0.82–67.88	17.9 ± 33.44	0.5–98.48

ΣDDE	22.04 ± 21.59	2.3–67.96	36.39 ± 41.21	3.52–130.29	18.18 ± 24.85	1.4–77.21	23.22 ± 37.87	0.8–112.84
o,p' - DDT	4.86 ± 5.25	ND-16.12	6.05 ± 6.55	1.66–21.45	6.8 ± 8.92	ND-23.89	6.97 ± 8.15	1.23–21.44
p,p' - DDT	33.61 ± 30.71	7.55–102.89	48.82 ± 27.38	12.11–95.22	28.92 ± 32.07	1.31–102.2	31.41 ± 28.44	2.6–89.88
ΣDDT	60.51 ± 46.14	11.2–141.6	91.26 ± 62.53	19.8–205.1	53.9 ± 54.47	3.4–140.8	61.6 ± 66.48	5.1–183.7
ΣOCPs	71.08 ± 55.73	13.33-163	107.94 ± 84.44	22-274.6	63.98 ± 66.41	4.6–175.2	75.61 ± 84.46	8-239.11

*ND – Not Detected.

Table 3. Comparison of organochlorine pesticides concentrations in surface water and sediments with previous studies across Pakistan.

Location	Environmental sample	ΣDDT	aldrin	Σendosulfan	Reference
River Ravi	Sediments (ng/g)	8.4 (Mean)	0–13.9	–	Malik et al. (2011)
Chenab River	Sediments (ng/g)	6.4 (Mean)	ND–11.5	–	Malik et al. (2011)
Rawal Lake Reservoir	Sediments (ng/g)	12.9 (Mean)	ND	–	Malik et al. (2011)
Mehmood Booti Drain (Lahore)	Sediments (ng/g)	1.684–10.78	–	–	Ali et al. (2016)
Pakistan	Coastal Sediments (ng/g)	0.07–78	–	–	Ali et al. (2014)
Nullah Aik and Nullah Palkhu,	Sediments (ng/g)	5.84–89.8	–	0.04–1.62	Mahmood et al. (2014)
Soan River	Sediments (ng/g)	4.82–21.71	–	–	Malik et al. (2014)
Korang River	Sediments (ng/g)	2.19–9.87	–	–	Malik et al. (2014)
Ling Stream	Sediments (ng/g)	1.85–8.34	–	–	Malik et al. (2014)

Lai Nullah	Sediments (ng/g)	0.95–4.28	–	–	Malik et al. (2014)
Chenab River	Sediments (ng/g)	4.23–53.60	ND-14.25		Eqani, Malik, and Mohamm (2011)
River Ravi	Sediments (ng/g)	1.5–58	–		Syed et al. (2014)
Haleji Lake	Sediments (ng/g)	1.4–22.2	–	–	Sanpera et al. (2003)
Karachi Ghas Bunder	Sediments (ng/g)	0.4–127.4	–	–	Sanpera et al. (2003)
Karachi	Coastal Sediments (ng/g)	2.7–9.2	–	–	Bano and Siddique (1991)
Degh Nala & Upper Chenab Canal	Sediments (ng/g)	ND-2410	–	–	Tehseen et al. (1994)
Chenab River	Surface water (ng/L)	0.55–590	0.81–16	–	Eqani, Malik, and Katsoyiannis et al. (2012)
Rawal Lake	Surface water (μg/L)	0.23–2.14	–	–	Iram et al. (2009)

Simly Lake	Surface water (µg/L)	0.8–2.87	–	–	Iram et al. (2009)
Nullah Aik and Nullah Palkhu,	Surface water (ng/L)	1.90–20.6	–	0.33–2.01	Mahmood et al. (2014)
Chenab River (Pakistan)	Fish (ng/g)	4.63–308.85	ND-2.15	–	Eqani et al. (2013)
Kabul River (Pakistan)	Fish (ng/g)	16.9–402	–	–	Aamir et al. (2016)
Indus River (Pakistan)	Fish (ng/g)	0.07–6.93	–	–	Robinson et al. (2016)

*ND – Not Detected.

Table 4. Comparison of organochlorines pesticides concentrations in surface water, sediment and fish from Chenab River with previous studies from other developing countries.

Location	Environmental Sample	ΣDDT	aldrin	Σendosulfan	Reference
Campeche (Mexico)	Fish (µg/g)	ND–62.094	0–13.9	ND – 0.9425	Hinojosa-Garro, Burgos Chan, and Rendón-von Osten (2016)
Wuhan (China)	Fish (ng/g)	ND–123.61	ND–9.96	–	Cui et al. (2015)
Lake Prespa (Republic of Macedonia)	Fish (µg/kg)	11.67–13.58	–	–	Veljanoska-Sarafiloska, Jordanoski, and Stafilov (2013)
Punjab (India)	Fish (mg/kg)	0.011–0.014	–	–	Kaur et al. (2008)
Gomti River (India)	Fish (ng/g)	0.68–2.89	0.3–7.84	0.41–2.92	Malik, Singh, and Ojha (2007)
Lake Ziway (Ethiopia)	Fish (µg/g)	0.03–10.6	ND	–	Yohannes, Ikenaka, Nakayama et al. (2014) and Yohannes, Ikenaka,

					Saengtienchai et al. (2014)
Lake Burullus (Egypt)	Fish (ng/g)	5.31–45.13	0.13–4.86	–	Said et al. (2008)
Densu River Basin (Ghana)	Fish (ng/g)	0.06–0.78	0.29–4.26	–	Kuranchie-Mensah et al. (2013)
Lake Tashk (Iran)	Fish (ng/g)	3.751–5.273	–	0.613–0.877	Kafilzadeh (2015)
Lake Qarun (Egypt)	Sediments (ng/g)	ND-5.88	ND-7.98	ND-18.7	Barakat et al. (2013)
Lake Maryut, Alexandria (Egypt)	Sediments (ng/g)	0.07–122.82	ND-9.62	–	Barakat et al. (2012)
Tamiraparani River (India)	Sediments (ng/g)	<0.01–857	<0.02–562	–	Kumarasamy et al. (2012)
Congo River Basin (Congo)	Sediments (ng/g)	0.95–4.28	–	–	Verhaert et al. (2013)
Wuhan (China)	Sediments (ng/g)	2.6–32	3.1–123	–	Yun et al. (2014)
Lake Tashk (Iran)	Sediments (ng/g)	4.182–6.541	–	9.126–15.263	Kafilzadeh (2015)
Campeche (Mexico)	Sediments (μg/g)	ND – 28.912	–	ND – 0.3766	Hinojosa-Garro, Burgos Chan, and

					Rendón-von Osten (2016)
Lake Prespa (Republic of Macedonia)	Sediments (µg/kg)	2.32–4.17	–	–	Veljanoska-Sarafiloska, Jordanoski, and Stafilov (2013)
Mae Klong river (Thailand)	Sediments (µg/g)	<0.001–6.78	0.19–0.94	>0.001–1.34	Poolpak et al. (2008)
Densu river basin (Ghana)	Sediments (µg/kg)	–	2.3–13.54	–	Kuranchie-Mensah et al. (2012)
Lake Burullus (Egypt)	Sediments (ng/g)	1.95–17.39	0.65–30.59	–	Said et al. (2008)
Ganges River (India)	Surface water (µg/L)	ND-1.57	ND-14.25	ND-13.07	Singh et al. (2007)
Yamuna River (India)	Surface water (ng/L)	66.17–722.94	–	–	Kaushik et al. (2008)
Ghaggar River (India)	Surface water (ng/L)	238.59–1005.43	–	–	Kaushik et al. (2010)
Tamiraparani River (India)	Surface water (ng/L)	<0.01–0.72	<0.02–1.5	–	Kumarasamy et al. (2012)
Lake Prespa (Republic of Macedonia)	Surface water (µg/L)	0.036–0.057	–	–	Veljanoska-Sarafiloska,

					Jordanoski, and Stafilov (2013)
Lake Burullus (Egypt)	Surface water (ng/L)	0.07–882.61	0.002–108.42	–	Said et al. (2008)
Densu river basin (Ghana)	Surface water (μg/L)	–	ND-0.02	–	Kuranchie-Mensah et al. (2012)
Debra Zeit (Ethiopia)	Surface water (μg/L)	–	ND	ND-0.031	Teklu, Adriaanse, and Van den Brink (2016)
Wuhan (China)	Surface water (ng/L)	ND-16.71	ND-12.78	–	Tang et al. (2008)
Wuhan (China)	Surface water (ng/L)	0.81–8.28	0.55–6.13	–	Cui et al. (2015)
Lake Tashk (Iran)	Surface water (μg/L)	0.02–0.04	–	0.04–0.08	Kafilzadeh (2015)
Karun River (Iran)	Surface water (μg/L)	0.005–0.09	0.14–1.47	3.85–74.03	Behfar et al. (2013)

*ND – Not Detected.

Table 5. Descriptive statistics of organochlorine pesticides (ng/g) in fish species from four major headworks across Chenab River.

	Speci	Numb	aldri	o,p' -	p,p' -	o,p' -	p,p' -	ΣDD	ΣDD	α-	β-	Σend	ΣOC
Ma rala	*Chan*	7	ND	22.4	10.9	2.71	4.81	7.52	40.86	0.85	1.70	2.55	43.41
	Anaui	12	1.56	19.1	6.99	3.19	3.94	7.13	33.24	0.51	1.88	2.39	37.19
	Chan	27	1.73	15.4	5.78	2.74	4.09	6.83	28.09	0.58	1.74	2.32	32.14
	Walla	13	1.89	10.6	8.77	2.76	3.45	6.21	25.61	0.16	2.00	2.16	29.66
	Labeo	21	1.31	9.81	5.61	1.73	3.30	5.03	20.45	ND	2.03	2.03	23.79
Kh ank	*Chan*	18	ND	56.2	47.8	120.	138.9	258.9	363.0	9.97	14.1	24.0	387.1
	Anaui	21	0.49	66.3	77.6	87.4	94.56	182.0	325.9	13.1	11.7	24.8	351.2
	Chan	15	ND	63.8	55.7	74.6	88.92	163.5	283.2	10.8	15.6	26.4	309.6
	Walla	9	33.0	77.6	34.8	58.1	83.94	142.0	254.6	11.7	18.5	30.3	317.9
	Labeo	19	0.82	54.6	39.0	66.8	70.95	137.7	231.4	7.03	15.0	22.0	254.2
Qa dira	*Chan*	27	4.51	60.9	40.2	56.3	65.85	122.2	223.4	1.56	2.6 +	4.16	232.1
	Anaui	13	ND	41.0	30.4	49.7	69.78	119.4	190.9	2.03	2.45	4.48	195.4
	Chan	17	4.32	70.1	38.3	43.7	51.08	94.83	203.2	2.18	2.43	4.61	212.2
	Walla	21	4.39	35.1	53.9	32.2	58.16	90.42	179.4	1.32	2.0 +	3.32	187.2
	Labeo	13	3.64	31.8	27.8	40.1	55.17	95.36	155.0	1.25	1.82	3.07	161.7
Tr im	*Chan*	25	6.12	25.0	8.61	36.9	33.98	70.91	104.5	2.01	2.82	4.83	115.5
	Anaui	6	ND	21.4	16.9	22.2	34.17	56.43	94.81	1.73	2.78	4.51	99.32
	Chan	16	6.51	24.5	21.5	19.7	25.17	44.89	91.01	1.21	2.90	4.11	101.6
	Walla	14	5.22	38.6	17.7	17.2	27.81	45.07	101.3	2.04	1.78	3.82	110.4
	Labeo	12	4.83	18.8	10.8	17.3	22.81	40.15	69.82	1.19	2.12	3.31	77.96

*ND – Not Detected.

Table 6. Estimated Daily Intake for organochlorines pesticides (ng/kg/day).

Isomers	Mean Concentration (ng/g)	Estimated Daily Intake (ng/kg/day)
aldrin	8.13	1.08
o,p'-DDT	38.18	5.09
p,p'-DDT	27.98	3.73
o,p'-DDE	37.8	5.04
p,p'-DDE	47.04	6.27
ΣDDE	84.84	11.31
ΣDDT	151	20.13
α-endosulfan	3.75	0.50
β-endosulfan	5.4	0.72
Σendosulfan	9.15	1.22
ΣOCPs	168.28	22.44

Table 7. Calculated hazard ratios for organochlorines pesticides using the 50th and 95th percentile concentration bases.

Isomers	*Oral slope factor (mg/kg/day)	Cancer Benchmark Concentration (ng/kg/day)	Hazard Ratio	
			50th Percentile	95th Percentile
aldrin	17	0.441	0.52	**1.33**
o,p'-DDT	0.34	22.069	0.09	0.21
p,p'-DDT	0.34	22.059	0.03	0.33
o,p'-DDE	0.34	22.059	0.02	0.19
p,p'-DDE	0.34	22.059	0.02	0.35
ΣDDE	0.34	22.059	0.04	0.55
ΣDDTs	0.34	22.059	0.17	**1.08**
*Reference dose (μg/kg/day)				

DDTs	0.5	0.2	**1.6**

Oral slope factors and Reference dose were obtained from Dougherty et al. (2000) and United States Environmental Protection Agency (2007).

Figure 1. The study area showing the sampling sites along the Chenab River, Pakistan.