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Organochlorine residual concentrations in cattle egret from the Punjab Province, Pakistan

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Abstract In this study, residual concentration of organochlorine pesticides (OCPs) in the sediments, prey, and eggs of *Bubulcus ibis* were measured from three breeding heronries from the Punjab province of Pakistan. Pattern of contamination in eggs followed the order: DDTs > HCHs > heptachlor > aldrin. Overall, pesticide residual concentrations were greater in eggs of cattle egrets collected from heronry on the River Ravi. Among HCHs, γ-HCH was more prevalent in eggs, whereas DDTs followed the order: DDD > DDE > *p*,*p*′-DDT > *o*,*p*′-DDT. Eggshell thinning was detected which showed negative relationship with residual concentration of DDE. In prey samples, residual concentration of POPs followed the order: DDTs > HCHs > dicofol > heptachlor; however, contamination pattern in sediments followed a slightly different order: DDTs > heptachlor > dicofol > HCHs > dieldrin > aldrin. Concentration of β-HCH was more prevalent in sediments and comparatively greater concentrations of POPs were measured in sediments collected from the River Ravi. Dicofol was found for the very first time in the biological samples from Pakistan, and its concentration was measured as relatively high in eggs from heronry from the River Chenab. Residual concentrations measured in eggs were below the levels that could affect egret populations. Biomagnification of the total OCPs through the food chain was evident in three breeding heronries. The concentration of DDE measured in eggs of the cattle egret suggests the need for monitoring this contaminant in other bird species at different trophic levels.

Keywords Persistent organic pollutants · Biomonitoring · Colonial water birds · Contamination · Biomagnifications · DDTs

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Introduction

Multilateral environmental agreements (MEAs) address the environmental problems being faced by the international community and vouch for common responsibility among nations for the environmental protection. Pakistan is the signatory to several MEAs aiming at sustainable development of natural resources and has ratified all three conventions related to chemicals and hazardous waste such as Basel, Rotterdam, and Stockholm.

Pakistan signed the Stockholm convention on persistent organic pollutants (POPs) on 6th December 2001 which was ratified on 17th April 2008. Being signatory of the Stockholm convention, the use of most of the OCPs were banned in Pakistan; however, these contaminants are still detected in high concentration in various physical and biological environmental compartments (Malik and Zeb 2009). According to Tariq et al. (2003), aldrin, chlorodane, and mirex had never been registered, while dieldrin and endrin were deregistered in the early 1980s, whereas DDT and toxaphene were registered in the early 1990s. Hexachlorocyclobenzene and heptachlor were deregistered during 1996 and 1997. Most of the pesticides were imported from the USA and Europe in large quantities during the 1960s and 1970s to eradicate and control locust, crop pest, and malaria, which were later distributed by the provincial departments of plant protection (Tariq et al. 2003). The use of chemicals in agriculture practices was started in 1954 with the formulation of 254 metric tons of pesticides and consumption reached over 7,000 tons per annum by mid-1960 and 16,226 metric tons in 1976–1977 (Baloch 1985). During the last two decades, pesticide consumption was alarmingly increased along with the number of sprays per crop (Tariq et al. 2003). According to agriculture statistics of Pakistan, the consumption of agricultural pesticides was 13,072 million tons during the late 1980s, which doubled in 1995 and then peaked in 2000 (61,299 million tons), worth hundreds of dollars.

In 1980, pesticide import and distribution was transferred from the public to private sector that boomed from this business. Organochlorine pesticides, viz. DDT and HCH, were produced locally. DDTs and HCHs have been completely banned in developed countries as well as in Pakistan from agricultural use. However, due to their persistence, high levels remained in many parts of the globe. Moreover, OCPs, DDT in particular, are still in use for sanitation campaigns against vector borne diseases in developing countries including Pakistan where its illegal use cannot be ignored.

Certain POPs have been responsible for the reduction and impairment of reproduction (Custer et al. 1998, 1999) and may be associated with adverse effects on the survival of Ardeids (Connell et al. 2003) resulting in population decline (Turusov et al. 2002; Sakellarides et al. 2006) and may cause adverse health effects in human beings (Tanabe and Kunisue (2007) particularly in young babies due to transfer across the placenta and via breast milk (Malarvannan et al. 2009). High levels of POPs such as DDTs can result in breeding failures and a negative effect on reproductive success (Baker and Sepu'lveda 2009; Harris et al. 2003) particularly in Ardeid species (De Luca-Abbott et al. 2001). POPs have a variety of acute and chronic pathological, neurotoxic effects and can disrupt the endocrine and immune systems (Yamashita et al. 1993), causing interference with transport of calcium resulting in eggshell thinning (Connell et al. 2003), and may cause genetic mutations resulting in internal and external malformations (Burger et al. 2007).

POPs are highly prone to atmospheric transportation and are deposited on the earth's surface. Their use is becoming widespread, representing a global contamination problem, and had been measured in high concentration even in remote regions such as the Arctic (Mallory et al. 2005). These chemicals persist in the environment with long half-lives, are highly volatile, extremely stable, and bio-accumulate (Keithmaleesatti et al. 2007). These environmental contaminants are mainly manufactured synthetically, released into the environment as a result of anthropogenic activities, and had been reported worldwide in different physical and biological compartments of terrestrial (Jaspers et al. 2007; Cid et al. 2007; Qadir et al. 2008; Dauwe et al. 2009; Qadir and Malik 2009) as well as aquatic ecosystems including pristine Arctic environments (Mallory et al. 2005) due to their toxicity, non-biodegradability, and biomagnification at higher trophic levels.

Scarce information is available on the rate or total amount of chlorinated pesticides used in Pakistan in different environmental matrices, and relatively sparse are the data on occurrence, concentrations, fate, and possible effects of OCPs on biological organisms. According to Tariq et al. (2007), over 47 studies have been conducted in Pakistan in the last 40 years that are related to the estimation of OCPs to assess their occurrence, geographic distribution, and trends in various environmental compartments. These studies differ

in site selection criteria, sample collection methods, and species and tissue type of biota collected. Pesticides were found in drinking water resources (Ahad et al. 2000, 2006; Tariq et al. 2003) and surface soils and sediments collected from different areas of Pakistan. Jabbar et al. (1993) estimated pesticide residues in cropland soils and shallow groundwater in Punjab, Pakistan. DDT, DDE, and HCH were measured in blood samples and fat tissues samples collected from hospital patients of Quetta City of Baluchistan Province, Pakistan (Krawinkel et al. 1989). Similarly, Parveen and Masud (2001) estimated OCPs, organophosphate, and synthetic pyrethroids in human blood samples collected from cotton-growing areas of Pakistan. Azmi et al. (2006) reported a significant increase in the level of different enzymes such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and alkaline phosphatase in the blood samples collected from farm workers from a rural area of Gadap exposed to pesticide residues such as cypermethrin, deltamethrin, polytrin-C, diazinon, monocrotophos, DDT, and DDE. Saqib et al. (2005) detected pesticide residues in muscles, liver, and fat of fish species captured from Kalri and Haleji lakes. Bhalli et al. (2006) assessed the genotoxic effects of pesticides on workers involved in the pesticide industry in Multan district, Punjab, Pakistan and reported a decrease in the level of SChE in the industrial workers occupationally exposed to pesticide residues. The literature also suggested that little is known about contamination and toxic impacts of OCPs in biological organisms including birds (Tariq et al. 2003). Very few studies have been conducted to assess the accumulation of pesticide contamination in physical and biological matrices.

Colonial water birds at the upper level of the food chain make them a suitable indicator of persistent organic environmental contamination (Sanpera et al. 2003; Sakellarides et al. 2006; Pol et al. 2009) and have been suggested as useful organisms for monitoring POPs (Jiménez et al. 2007). Studies have reported that populations of various bird species such as cormorants have been declining, which is associated with some OCPs. Studies have reported that these birds have been suffering due to contaminants, thereby affecting their development and reproductive capacity (Blus 1996). Different bird species, especially those belonging to Ardeidae which are at high levels of the food chain, have often been used as bioindicators (Jiménez et al. 2005) to evaluate the presence, persistence, and biomagnifications of POPs. Information on pesticide contamination of Ardeidae in Pakistan is rather limited. Ardeidae are important indicators of environmental degradation caused by toxic chemicals in wetlands, and there are only a few studies in Pakistan that use colonial water birds as an ecological indicator of OCP contamination, e.g., Sanpera et al. (2003) measured POPs in eggs of little egrets from selected wetlands of Pakistan. However, to our knowledge, no such data are available for OCP contamination of Ardeidae species from our three study sites in Punjab district, Pakistan.

In this study, the cattle egret, a medium-sized colonial bird native to Africa and Asia, the most terrestrial heron;elonging to the family Ardeidae that is well adapted to diverse habitats (Telfair 1994), was used as a biomonitor for local environmental pollution and served as a potential indicator of terrestrial pollution on five continents and many islands. Cattle egrets can be used to indicate the organic pollutant profile of both the terrestrial and aquatic environments (Bouwman et al. 2008). The cattle egret has expanded greatly in range from Africa and is now found in Europe and the Americas, while the Asian subspecies (*Bubulcus ibis coromandus*) can be found in the area between India, Japan, Australia, and New Zealand (Bouwman et al. 2008). This species has been found abundant in major rice-growing tracts such as around Sukkur, Larkana, and Hyderabad and cotton belts like Khanewal in Pakistan. Cattle egret feed mostly on grasshoppers, crickets, spiders, flies, frogs, and noctuid moths (Telfair 1994), and their position at the upper level of the food chain make them a suitable indicator of environmental contamination; their responses can provide information regarding environmental changes occurring at lower trophic levels (Malik and Zeb 2009).

The aim of this study is to investigate the accumulation patterns of OCPS in eggs, prey samples of cattle egrets, and surface sediments from three studied heronries to assess if there are

any differences among studied heronries and to compare measured concentration with regional areas studied. The results will provide OCP baseline data for cattle egrets from Punjab province to evaluate the potential risk of OCP contamination for the local avifauna, highlighting the bioavailability, bioaccumulation, magnification, and biotransfer of these pollutants (Swaileh and Sansur 2006). The results will provide data regarding OCP contamination in egret and other bird species from other countries and if the selected species is at risk.

Materials and methods

Three breeding heronries were identified in Punjab province: one at Rawal Lake Reservoir in Islamabad district and two in Khaniwal district along Chenab and Ravi Rivers, Pakistan (Fig. 1). The first heronry was located in the highly urbanized area of Islamabad City, the capital of Pakistan, in close vicinity to the Rawal Lake Reservoir at latitude 33°41′24″N and longitude 73°44′73″E at an elevation of 520 m (asl). The Rawal Lake Reservoir is of ecological significance, protected within an isolated section of the Margalla Hills National Park (Malik and Husain 2006a, b, c, 2007; Bibi et al. 2008). It is a small water reservoir with some associated freshwater marshes, adjacent to a large area of protected woodland on the outskirts of Islamabad, in an area that forms the northeast part of the Potwar Plateau. It is the sole drinking water reservoir for the city of Rawalpindi and serves as an important habitat for wintering waterfowl (mostly *Anas platyrhynchos*). The lake is a partly "Arched Gravity" type reservoir with a discharge capacity of 2,300 m^3/s and covers an area of 8.8 km^2 with a maximum depth of 31 m. The lake has three primary inlets and one outlet. The Kurang stream enters on the northeastern side; Rumli and Quaid-i-Azam University streams enter on the northern side, while the spillway gates are located on the southwestern side. The lake reservoir serves as an important resource for sports and commercial fishery. Fish yields in the lake have declined in recent years. It also is a popular area for outdoor recreation including boating and fishing. The climatic temperature at the Rawal Lake Reservoir varies from an average maximum in winter of 17°C to an average minimum of 3°C. In summer, the temperature varies from 24 to 34°C. The lake reservoir is facing degradation of its water quality

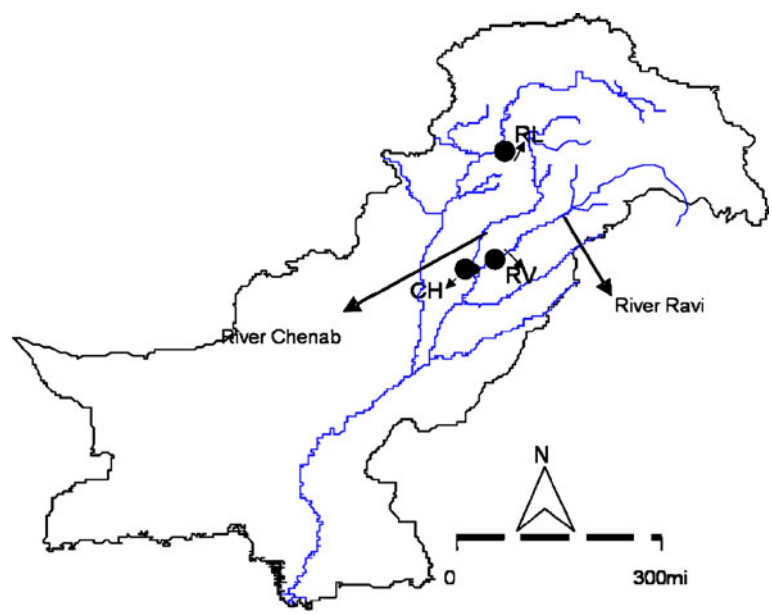

Fig. 1 Location of three breeding heronries of cattle egrets: River Chenab (*CH*), River Ravi (*RV*), Rawal Lake Reservoir (*RL*)

from point and non-point sources of contamination, which include surface runoff from agricultural and urban areas, waste from 360 poultry sheds in its catchments, recreational use of motorboats, car wash activities along the eastern margin of the lake, discharge from the feeding streams (such as Rumli, Shahdra, University stream), shoreline banks which fall into the reservoir, and recreational and human settlements in Bara Kahu, Malpur, Bani Gala, and Noorpurshan villages and the Diplomatic Enclave, Islamabad. There is no treatment plant; raw sewage and municipal waste are fed directly into the natural streams which feed the lake reservoir. The concentration of pollutants in the Rawal Lake Reservoir has increased in recent years (Malik and Zeb 2009).

The other two heronries were located in the Khaniwal district. One was located in the close vicinity of Sardarpur village at latitude 30°06′34″N and longitude 71°81′40″. This heronry was located about 1/2 km away from the River Chenab in cotton fields irrigated with water diverted from the river; whereas the other heronry was located in the village of Faqiranwala about 1 km from the River Ravi at latitude 30°62′64″N and longitude 71°84′78″E in cotton fields. The River Ravi is heavily polluted from industrial and municipal waste from the cities of Lahore, Kalashkaku, and Qasur. Major anthropogenic sources of pesticide contamination of the River Chenab include agricultural runoff; industrial and urban effluents from Sialkot, Gujarat, and Cheniot cities; and atmospheric deposition.

The literature suggested that egg sample size varied considerably among different studies, e.g., egg sample sizes of little egret varied between three and five per heronry (Keithmaleesatti et al. 2007), 12 eggs from brown boobies at eight colonies from the northern Gulf of California to southern Mexico (Mellink et al. 2009), five to 15 eggs per heronry in the study of Henny et al. (2003), three to five eggs per colony of great blue herons (*Ardea herodias*) from Indiana (Baker and Sepu'lveda 2009), three to 14 eggs of little egrets from three breeding heronries of three selected wetlands of Pakistan (Sanpera et al. 2003), five to 11 eggs from two cattle egret heronries from south Africa (Bouwman et al. 2008), and 20 eggs of cattle egrets from Tai Lake in China (Dong et al. 2004). Therefore, in this study, from each heronry, a total of 10 nests were sampled, and one egg from each nest was collected.

The eggs, prey, and sediment samples from two heronries in Khanewal district were collected in May and from Rawal Lake Reservoir in June 2007, respectively. Before the collection of eggs and prey samples, nests were marked with numbered plastic tags, and a total of 10 nests were sampled. From each nest, one egg was collected randomly and a total of 10 fresh eggs were collected from each of the three heronries. Eggs were wrapped in foil, placed in the icebox to protect from breakage, and transferred to the Environment Biology Laboratory at Quaid-i-Azam University, Islamabad. Whole egg was refrigerated in the laboratory at −4°C for later pesticide residual analysis.

The prey items were collected with the help of a wooden fork from the feeding ground within 10 km of each heronry and were temporarily stored in polyethylene envelopes. Chick regurgitates were also collected from 3- to 5-day-old chicks. Two samples per week were collected in order to have a good idea of the diet. In case of small prey like insects, the sample included >50 prey items and >10 items in case of larger prey like fishes and toads, were collected from more than 10 different nests. For analysis, small individuals of the same species were pooled to obtain the composite sample. Prey items were thoroughly washed with distilled water, dried, and identified. These samples were ground with mortar and pestle to powdered form and stored in sterilized glass vials before further analysis.

Surface sediment was collected in polypropylene jars from all sites in May 2005 at 3–8 cm depth and stored at −4°C until further analysis. Five sediment samples were taken from each site at different locations within a radius of 10 km which were most frequently used by the cattle egrets for foraging. Foraging sites were identified during repeated field visits. Sediments were collected by using stainless steel spoons and deposited in a glass bowl. Prior to sampling, equipment was washed in a dilute solution of free phosphate detergent, rinsed with deionized water, and finally with methanol. Sediments were allowed to air dry, mixed and sieved through a stainless steel

mesh, and collected in 500-ml pre-cleaned glass container for further analyses.

Chemical analysis was performed for nine selected OCPs, viz. β-HCH, γ-HCH, heptachlor, aldrin, dicofol, DDD, DDE, *o*,*p*′-DDT, and *p*,*p*′-DDT at the Toxicology Laboratory National Agricultural Research Center (NARC), Islamabad, Pakistan, according to methods given in Sanpera et al. (2003). Eggs were opened after thawing, and contents (yolk and albumen) were homogenized using a small blender, taking care to keep the foam. Egg samples were homogenized and mixed with anhydrous sodium sulfate, ground to free-flowing powder, and extracted with *n*-hexane followed by cleanup with acid silica. For prey and sediment samples, a similar method was used for the egg samples. According to Sanpera et al. (2003), cyclodienes are partially degraded by the acid attack; therefore, the values measured should be considered as underestimated.

Pesticide standards with purity higher than 95% were obtained from Ehrenstorfer (Augsburg, Germany). Stock standard solutions of 1,000 ng/μl were prepared by exact weighing and dissolving in *n*-hexane containing 10–15% acetone, while working standard solutions were made by diluting the stock standards and stored in the dark at 4°C. Working standard solutions were freshly prepared by dilution in the same solvent. All the solvents and chemicals were purchased from Merck (Germany). Nitrogen gas of high purity (99.999%) was used during the analysis.

A Perkin Elmer AutoSystem gas chromatograph (GC) equipped with an electron capture detector (ECD-Ni63), capillary column (P.E. no. N931-2414, methyl 10% phenyl-silicone, 17 m, 0.32 i.d., 0.5 mm o.d., 0.5 μm film thickness), and Turbochrom data analysis hardware/software system was used. During analysis, the injector (splitless mode) and detector temperature was kept at 225 and 300°C, respectively. Initial oven temperature was set at 100°C, which was held for 5 min and ramped to 160°C at a rate of 15°C/min to 190°C at a rate of 2°C/min. The backup pressure of carrier gas (N_2) was kept 12 psi. The carrier flow rate was kept at 10 ml/min, whereas the pressure of makeup gas (N_2) was 32 ml/min. Standard/sample measuring 1.0 μl was injected with a 10-μl Hamilton syringe by the solvent flush injection technique. A vortex mixer (vortex TRA 0300-100 Gyromixer) was used for shaking during extraction of samples, and a centrifuge (Hettich Zentrifugen, D-78532 Tuttlingen, Germany) was used for phase separation. A Buchi R-114 rotary evaporator (R-114, Buchi, UK) coupled with Buchi heating bath was used to concentrate sample extracts.

The residue levels of OCPs were quantitatively determined by external reference standard method. Mixture of standard solutions was injected into GC followed by a sample injection to qualitatively identify the analytes of interest on the basis of their respective retention times and peak areas, which were used to calculate the concentration of pesticide residues. For every set of 10 samples, a procedural blank and spiked sample consisting of all reagents was run to check for interference and cross contamination. Method performance was assessed by evaluating the quality parameters such as recovery, correlation coefficient, limit of detection (LOD), and limit of quantification (LOQ) given in Table 1.

Table 1 Purity, retention time (*R.T.*), correlation coefficient, LOD, and LOQ of the studied pesticides

Pesticides	Purity (%)	R.T. (min) mean ± SD	Correlation coefficient "*r*"	LOD (ng/g)	LOQ (ng/g)
β−HCH	99.9	4.58 ± 0.01	0.999	6	11
γ−HCH	99.9	5.12 ± 0.02	0.998	2	3
Heptachlor	99.6	6.54 ± 0.02	0.999	5	14
Aldrin	98.5	7.45 ± 0.01	0.989	6	12
Dicofol	96.5	8.18 ± 0.02	0.998	6	12
DDD	98.9	11.93 ± 0.01	0.999	6	12
DDE	97.5	12.45 ± 0.02	0.993	5	10
o,*p*′-DDT	99.5	14.26 ± 0.01	0.995	3	6
p,*p*′-DDT	99.5	16.34 ± 0.01	0.997	5	14

Recoveries were assessed by analyzing uncontaminated matrices ($n = 3$) spiked at the level of 50, 100, and 200 ng/g for each of the studied OCPs on the same day. Mean recovery (%) of OCPs ranged from 70% to 98% while the correlation coefficient "r" ranged from 0.993 to 0.999. LODs and LOQs were calculated on the basis of signal-to-noise ratio (S/N) 3 and 10, respectively. The values of peak area were used in regression template 6.3.1 to calculate the pesticide residual level which is expressed in nanograms per gram (Ambrus and Miller 2003).

Differences in mean concentrations of residual pesticides in eggs, prey, and sediment samples of three breeding heronries were evaluated using one-way analysis of variance.

Results

Concentrations of OCPs in eggs

Basic descriptive statistics of OCP residual concentrations in eggs are presented in Table 2 which indicated that mean concentrations of heptachlor, aldrin, and dicofol were significantly different among three heronries. Pattern of OCP concentration in three heronries followed the order >River Ravi > River Chenab > Rawal Lake Reservoir. Concentration of OCPs in eggs was in the order: DDTs > HCH > dicofol > heptachlor > aldrin. Among studied DDTs in the current study, DDD, DDE, o,p'-DDT, and p,p'-DDT were detected in 90%, 70%, 63%, and 60% egg samples from three heronries. Concentrations of ΣDDTs were greater in eggs collected from heronry located at the River Ravi (35.44%) followed by heronry at the Rawal Lake Reservoir (35.3%) and the River Chenab (29.3%), respectively. Residual concentration of DDTs in eggs from three heronries followed the order: DDD > DDE > p,p'-DDT > o,p'-DDT. Mean concentrations of DDE and DDD in eggs collected from heronry at River Chenab was highest. In contrast, relatively higher concentration of DDT isomers were measured in heronry at Rawal Lake Reservoir and among these, p,p'-DDT were more prevalent. ΣHCHs concentration was higher as compared to those of cyclodienes. Among HCHs, β-HCH was more prevalent. Among cyclodienes, only heptachlor was recorded in all egg samples from heronry at River Ravi and Chenab, whereas aldrin was found only in eggs collected from heronry at River Chenab. Residual

Table 2 Basic descriptive statistics of OCP residual concentration in eggs (ng/g) collected from three heronries, $n = 10$ for each heronry

Compounds	*N*(detected)		CH	RV	RL	*P* value
β−HCH	30 (18)	Mean ± std	17.9 ± 28.6	47.1 ± 51.2	10.1 ± 13.7	0.21
		Min–max	ND–89.1	ND–178.9	ND–35.7	
γ−HCH	30 (22)	Mean ± std	29.9 ± 23.5	21.8 ± 19.2	12.9 ± 17.9	0.45
		Min–max	ND–64.6	ND–64.3	ND–56.2	
Heptachlor	30 (7)	Mean ± std	18.9 ± 21.5	6.6 ± 14.1	ND	0.0
		Min–max	ND–56.3	ND–38.4	ND	
Aldrin	30 (3)	Mean ± std	8.7 ± 18.1	ND	ND	0.0
		Min–max	ND–54.9	ND	ND	
Dicofol	30 (12)	Mean ± std	48.3 ± 53.3	38.4 ± 50.2	10.0 ± 21.3	0.01
		Min–max	ND–129.3	ND–155.0	ND–56.8	
DDD	30 (27)	Mean ± std	126.2 ± 105.9	165.6 ± 114.3	152.9 ± 95.1	0.56
		Min–max	ND–372.0	ND–317.2	56.6–344.7	
DDE	30 (21)	Mean ± std	64.2 ± 60.4	58.2 ± 62.6	53.256.4	0.86
		Min–max	ND–188.2	ND–188.2	ND–154.3	
o,p'-DDT	30 (19)	Mean ± std	36.3 ± 20.0	28.7 ± 2.5	28.8 ± 36.7	0.39
		Min–max	14.1–71.7	ND–95.5	ND–111.3	
p,p'-DDT	30 (18)	Mean ± std	27.0 ± 32.2	41.4 ± 45.4	57.7 ± 86.5	0.08
		Min–max	ND–75.1	ND–152.3	ND–269.4	
∑HCH		Mean ± std	239.1 ± 84.1	344.3 ± 9.0	114.7 ± 19.8	
∑DDTs		Mean ± std	60.7 ± 34.0	73.4 ± 27.4	73.1 ± 29.0	

RV River Ravi, *CH* River Chenab, *RL* Rawal Lake Reservoir

concentration of dicofol was measured greater in eggs from heronry at River Chenab (48.3 ng/g) followed by heronry at River Ravi (38.5 ng/g) and Rawal Lake Reservoir (10 ng/g).

Concentrations of OCPs in prey samples

Mean concentration of DDTs was greater than those of HCHs and cyclodienes (Table 3). Pattern of OCPs in prey of cattle egrets followed the order: >River Ravi > River Chenab > Rawal Lake Reservoir, whereas DDTs followed the order: DDD > DDE > *o*,*p*′-DDT > *p*,*p*′-DDT. Among DDT metabolites, the highest concentration of DDD was found in Rawal Lake Reservoir (86.7 ng/g) followed by River Ravi (85.3 ng/g) and River Chenab (80.8 ng/g). Similarly, higher concentrations of HCHs were also measured from heronry at River Ravi (66.1 ng/g), followed by River Chenab (56.5 ng/g) and Rawal Lake Reservoir (40.6 ng/g). Concentration of β-HCH was measured higher in prey samples collected from heronries at River Chenab and Rawal Lake Reservoir, whereas γ-HCH concentration was higher in prey samples collected from heronry at River Ravi (36.8 ng/g) and lowest in prey samples collected from heronry at Rawal Lake Reservoir (9.9 ng/g). Mean γ-HCH concentration in prey samples was also significantly different between three heronries. Aldrin was recorded only in prey samples collected from Rawal Lake Reservoir, whereas heptachlor was measured only in heronries at River Ravi and Chenab.

Concentrations of OCPs in sediments

Overall pattern of detected OCP residues in sediments from three heronries was in the order: >Rawal Lake Reservoir > River Ravi > River Chenab. Residual concentrations of DDT metabolites were higher compared to its isomers (Table 4). Greater concentration of DDD, DDE, and *p*,*p*′-DDT was measured in sediments collected from Rawal Lake Reservoir. Among DDT isomers, residual concentration of *p*,*p*′-DDT was more prevalent. HCHs exhibited highest concentration in River Ravi. Residue concentration of β-HCH and γ-HCH was comparatively lowest in sediments collected from River Chenab. Significant differences in residual concentrations of *p*,*p*′-DDT, β-HCH, aldrin, and dicofol in the sediments of three heronries were found.

Table 3 Basic descriptive statistics of OCP residual concentration in prey samples (ng/g) collected from three heronries, $n = 10$ for all heronries

Compounds	*N* (detected)		CH	RV	RL	*P* value
β−HCH	10 (9)	Mean ± std	31.0 ± 14.9	29.2 ± 7.0	30.7 ± 16.6	0.45
		Min–max	20.4–41.6	24.3–34.2	ND–46.6	
γ−HCH	10 (7)	Mean ± std	25.4 ± 19.4	36.7 ± 28.9	9.90 ± 10.9	0.32
		Min–max	11.7–39.2	16.3–57.2	ND–22.3	
Heptachlor	10 (2)	Mean ± std	14.6 ± 20.7	16.0 ± 22.7	ND	0.23
		Min–max	ND–29.3	ND–32.1	ND	
Aldrin	10 (1)	Mean ± std	ND	ND	8.6 ± 21.0	0.74
		Min–max	ND	ND	ND–51.6	
Dicofol	10 (2)	Mean ± std	10.31 ± 14.5	21.6 ± 30.6	ND	0.21
		Min–max	ND–20.6	ND–43.3	ND	
DDD	10 (8)	Mean ± std	85.2 ± 11.2	80.7 ± 7.5	86.7 ± 82.0	0.99
		Min–max	77.3–93.2	75.4–86.1	ND–211.8	
DDE	10 (6)	Mean ± std	28.9 ± 9.3	37.4 ± 29.2	12.5 ± 19.5	0.18
		Min–max	22.3–35.5	16.7–58.1	ND–41.0	
o,*p*′-DDT	10 (7)	Mean ± std	35.2 ± 33.8	31.7 ± 10.4	17.0 ± 21.5	0.32
		Min–max	11.3–59.2	24.3–39.1	ND–52.3	
p,*p*′-DDT	10 (3)	Mean ± std	ND	13.5 ± 19.2	11.6 ± 18.6	0.89
		Min–max	ND	ND–27.1	ND–41.8	
∑HCH		Mean ± std				
∑DDTs		Mean ± std				

RV River Ravi, *CH* River Chenab, *RL* Rawal Lake Reservoir

Table 4 Basic descriptive statistics of OCPs residual concentration in sediments (ng/g) collected from three heronries, $n = 5$ for each location

Compounds	*N* (detected)		CH	RV	RL	*P* value
β−HCH	15 (8)	Mean ± std	3.3 ± 4.6	5.6 ± 3.2	5.9 ± 8.7	0.03
		Min–max	ND–9.4	ND–8.2	ND–19.5	
γ−HCH	15 (11)	Mean ± std	2.2 ± 1.6	4.0 ± 2.8	2.4 ± 2.7	0.46
		Min–max	ND–4.5	ND–7.8	ND–6.4	
Heptachlor	15 (4)	Mean ± std	12.4 ± 18.4	8.6 ± 7.3	3.3 ± 7.3	0.18
		Min–max	ND–41.1	ND–42.9	ND–16.4	
Aldrin	15 (3)	Mean ± std	2.3 ± 5.1	4.6 ± 6.5	ND	0.01
		Min–max	ND–11.5	0–13.9	ND	
Dicofol	15 (4)	Mean ± std	12.5 ± 18.4	11.3 ± 16.2	ND	0.0
		Min–max	ND–40.9	ND–35.0	ND	
DDD	15 (12)	Mean ± std	10.2 ± 6.8	11.1 ± 7.4	21.1 ± 14.3	0.23
		Min–max	ND–19.2	ND–19.3	ND–35.2	
DDE	15 (7)	Mean ± std	4.4 ± 6.4	8.8 ± 12.2	12.1 ± 13.5	0.22
		Min–max	ND–14.2	ND–24.6	ND–32.5	
o,*p*′-DDT	15 (7)	Mean ± std	4.5 ± 7.1	5.2 ± 5.0	4.9 ± 7.0	0.61
		Min–max	ND–16.4	ND–11.2	ND–15.2	
p,*p*′-DDT	15 (11)	Mean ± std	6.5 ± 4.3	8.6 ± 5.4	13.6 ± 13.7	0.03
		Min–max	ND–11.3	ND–13.9	ND–31.2	
∑HCH		Mean ± std	13.9 ± 3.96	20.7 ± 12.3	20.7 ± 12.3	
∑DDTs		Mean ± std	6.4 ± 3.1	8.4 ± 5.9	12.9 ± 7.7	

RV River Ravi, *CH* River Chenab, *RL* Rawal Lake Reservoir

Discussion

The accumulation of OCPs in the cattle egrets is a reflection of contamination of the food chain (Fig. 2) and provides evidence of local environmental pollution. The total OCP concentration in three environmental matrices indicated increasing trend from sediments to prey to eggs. A similar trend of biomagnifications was reported in other studies (Sanpera et al. 2003). Relatively, sediments and prey samples collected from the heronry along the River Ravi were less contaminated, while the eggs collected from the Rawal Lake Reservoir heronry were more contaminated. This indicates biomagnifications and accumulation of studied OCPs along the food chain highlighting the non-biodegradable, accumulative, and lipophilic nature of most of the POPs (Dauwe et al. 2009). Bioaccumulation of POPs has been related with contamination of prey samples, the trophic level to which the organism belongs, the ability to metabolize or eliminate contaminants, and migratory patterns of bird species (Naso et al. 2003). The results indicated that differences in concentration of OCPs between the studied heronries can be mainly due to differences in contamination of foraging habitat of cattle (Malik and Zeb 2009) and the prey items they consume. Three studied areas may receive OCP contamination from different sources including agricultural and urban surface runoff, and untreated municipal and industrial effluents (Qadir and Malik 2009). These birds follow cattle in grasslands, feeding on disturbed insects, follow closely behind fire fronts feeding on burned prey (Bouwman and Hoffman 2007), and even feed on chicks of nesting birds (Bouwman et al. 2008). During the breeding period, cattle egret used freshwater, marshy areas along the Rawal Lake Reservoir and rice fields as foraging habitats (Malik and Zeb 2009). The current study revealed differences in prey items from three studied areas, and these differences could also be related to contaminant residual level between three heronries. Prey samples from River Ravi and Rawal Lake Reservoir were relatively more contaminated. Cattle egret from Rawal Lake Reservoir feed their young mainly with fishes, whereas the prey from River Ravi contained large toads and caterpillars which contain relatively higher amounts of pesticides (Table 5). The bioaccumulation pattern observed in the current study stresses the importance of

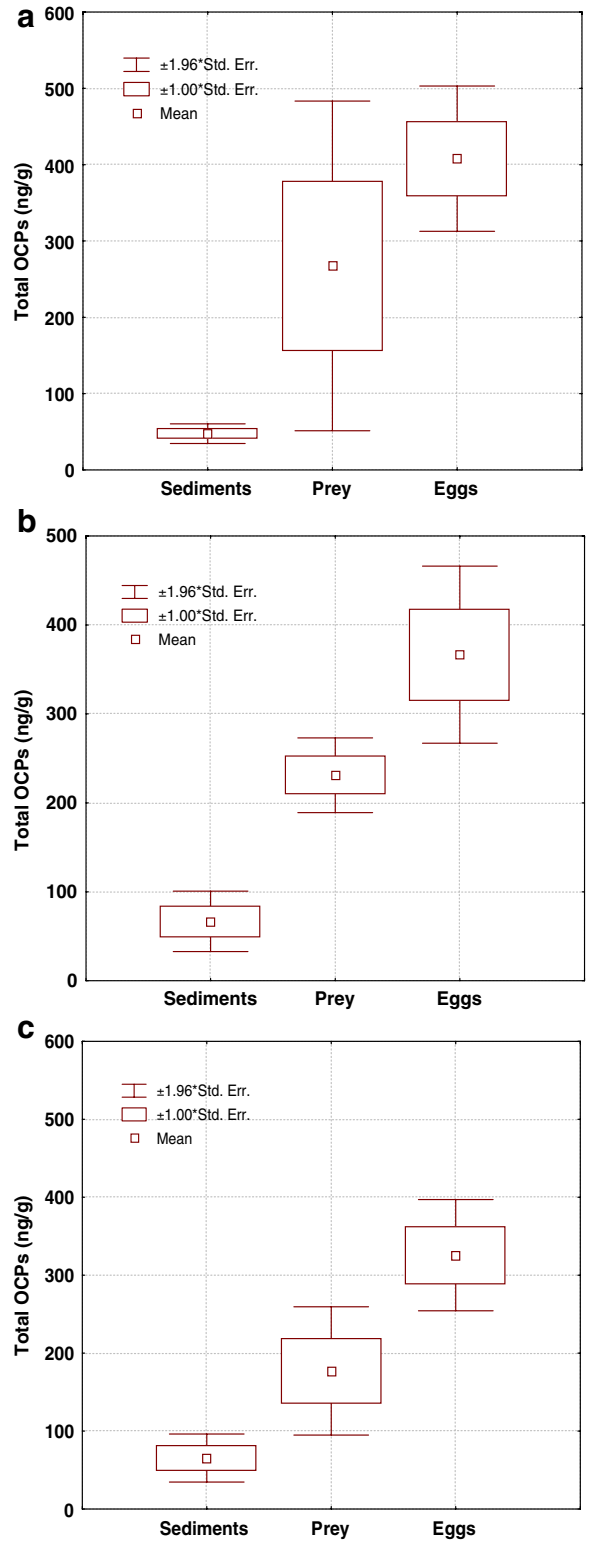

Fig. 2 Biomagnification for total OCP concentrations in egg, prey, and sediments from three breeding heronries: **a** River Ravi, **b** River Chenab, **c** Rawal Lake Reservoir

the use of eggs as an ecological indicator of POP contamination along the food chain.

Among contaminants which were measured in the current study, DDTs were more prevalent in eggs, prey, and sediments. ΣDDT concentrations measured in eggs in the current study were higher than those found in the Audoin's gull (Pastor et al. 1995). However, measured concentrations were lower than those reported in eggs of little egrets (728.3 ng/g) from Pakistan (Sanpera et al. 2003). DDT residues can cause both acute and chronic health disorders including thinning of eggshells and reduction of hormonal level necessary for female birds to lay eggs. DDT may cause direct mortality of birds by directly affecting the nervous system even in birds like robins that feed relatively low on the food chain (Burger et al. 2007). Concentration of ΣDDTs was high in eggs of heronries from River Ravi and Rawal Lake Reservoir as compared to River Chenab. Greater ΣDDTs were also measured in sediments which act as sink for contaminants from Rawal Lake Reservoir (12.9 ng/g) and River Ravi (8.4 ng/g). ΣDDTs in sediment recorded in the current study were lower than those reported in sediments (18–55 ng/g) from Black Sea coast, Turkey (Bakan and Ariman 2004) and higher than those detected in sediments (0.05 ng/g) from the Mediterranean (Iwata et al. 1994). Presence of relatively greater concentration of DDTs in Rawal Lake Reservoir can be due to the use of DDT for malarial control and hygienic purposes or can be related to its use in agricultural activities. Prey samples collected from heronries from River Ravi (40.8 ng/g) and Chenab (37.3 ng/g) were more contaminated compared to those measured in prey collected from Rawal Lake Reservoir (32.0 ng/g). The concentrations measured in prey were greater than those found in the prey of little egrets (10.80 ng/g) from China (Dong et al. 2004) and lower than those found in prey of little egrets (78.4 ng/g) from Pakistan (Sanpera et al. 2003).

Concentration of DDD in eggs from three heronries varied between 126 and 166 ng/g which

Table 5 Prey samples of cattle egrets collected from three breeding heronries

Sites	Scientific name	Family	Order	Common name	Total
CH	*Mantid* spp.	Mantidae	Dictyoptera	Caterpillar	23
	Perta spp.	Pertadae	Neuroptera	Lace wing	2
	Lycosa spp.	Lycosidae	Araneae	Spider	1
	Chrotogonus trac	Acrididae	Orthoptera	Toka	1
	Gryllus spp.	Gryllidae	Orthoptera	Field cricket	2
	Aiolopus tamulus	Acrididae	Orthoptera	Grasshopper	2
	Bufo stomaticus	Bufonidae	Anura	Toad	10
RV	*Mantid* spp.	Mantidae	Dictyoptera	Caterpillar	10
	Perta spp.	Pertadae	Neuroptera	Lace wing	3
	Aiolopus tamulus	Acrididae	Orthoptera	Grasshopper	1
	Forficula spp.	Dermaptera	Lapedoptera	Earwig	1
	Bufo stomaticus	Bufonidae	Anura	Toad	19
RL	*Puntius sophore*	Cyprinidae	Cypriniformes	Pool barb	6
	Salmostoma bacaila	Cyprinidae	Cypriniformes	Large razorbelly minnow	40
	Acanthocobitis botia	Balitoridae	Cypriniformes	Mottled loach	1
	Aristichithys nobilis	Cyprinidae	Cypriniformes	Bighead carp	5
	Aspidoparia morar	Cyprinidae	Cypriniformes	None	3
	Channa punctata	Chanridae	Perciformes	Spotted snake head	2

RV River Ravi, *CH* River Chenab, *RL* Rawal Lake Reservoir

were relatively lower than those measured in eggs of *Egretta garzetta* (5–357 ng/g) and *Nycticorex nycticorex* (6–1,203 ng/g) from China (Dong et al. 2004)a and in the eggs of yellow-legged gulls (564.86 ppb ww) from the Northeastern Mediterranean (Albanis et al. 2003). However, measured concentrations were higher than those found in eggs of cattle egrets (0.017 ppm ww) from northwest Mexico (Mora and Anderson 1991) and *E. garzetta* (22–146 ng/g) from Northern Italy (Fasola et al. 1998). The sum of o,p'-DDT and p,p'-DDT was low as compared to the sum of DDD and DDE in eggs collected from heronries from River Chenab and Ravi, highlighting the fact that these highly stable metabolites have the capability to accumulate at higher trophic levels. In the eggs of cattle egrets from heronry at River Ravi, DDD concentration was recorded as higher compared to those measured in heronry at River Chenab. Among DDTs, a relatively greater concentration of DDD was also measured in prey and sediment collected from each heronry. Greater concentrations of DDD were measured in sediments of Rawal Lake Reservoir (21.1 ng/g) followed by River Ravi (11.1 ng/g) and River Chenab (10.2 ng/g). Its higher residual concentrations can be related to metabolic transformation of p,p'-DDT to DDD under anaerobic conditions and to DDE under aerobic conditions (Ozkoc et al. 2007). The sum of o,p'-DDT and p,p'-DDT was high compared to the sum of DDD and DDE in sediments of River Chenab, indicating its recent use in agricultural areas. The low cost of DDT and its use for vector control is an argument for its continued use.

The concentration of DDE reported in the egg samples of cattle egret is lower as compared to those concentrations measured in other countries such as from the USA in eggs of *Nycticorax nycticorax* (Matz and Parsons 2004) and *Pelecanus erythrorynchos* (Blus et al. 1998), Italy in eggs of *E. garzetta* (Fasola et al. 1998), and China in eggs of *N. nycticorax* and *E. garzetta* (Dong et al. 2004). Widespread occurrence of DDT, mainly its metabolite DDE, in the eggs of fish-eating birds such as falcons can cause considerable thinning of eggshells, and consequent difficulties in hatching resulted in a severe population decline (Turusov et al. 2002). Eggshell thinning has been used as a bioindicator of DDE and DDT contamination (Kushlan 1993). DDE, being a highly stable metabolite of DDT, has been detected commonly in bird species. Concentration of DDE in eggs collected from heronry from River Chenab (64.1 ng/g) was greater as compared to eggs collected from River Ravi (58.2 ng/g) and Rawal Lake Reservoir (53.1 ng/g). The concentrations measured were less than those reported

in grey heron (470 ng/g) from France (De Cruz et al. 1997), white-faced ibis (2,100 ng/g) from the USA (King et al. 2003), and peregrine falcon (3,900 ng/g) from Zimbabwe (Hartley et al. 1995) and were within the range reported in the eggs of little egret from the Wat Tan-en non-hunting area Thailand (Keithmaleesatti et al. 2007); however, the residual level recorded in eggs was higher than those in eggs of cattle egret (24 ng/g) from Barberspan and Vaal River (Bouwman et al. 2008).

Birds at higher trophic levels tend to have higher exposure to DDE but not necessarily its effects (Keithmaleesatti et al. 2007). The effect of DDE on shell quality is very species specific, e.g., fish-eating bird species are generally most sensitive towards DDE residue burden and eggshell thinning (Burger et al. 2007). DDE is negatively correlated with eggshell thickness (Mora and Anderson 1991). Jiménez et al. (2007) proposed that the concentration of DDE that affects hatching success ranged between 6 and 10 μg/g. Elliot et al. (2001) found that the highest concentration of DDE in osprey eggs that hatched was 9.2 μg/g ww; in the current study, DDE concentrations measured in cattle egret eggs were below the levels that affect hatching success as proposed by Jiménez et al. (2007) and Elliot et al. (2001). It has also been reported that nests of birds such as great black-backed gulls (*Larus marinus*) with higher blood DDE levels are more prone to be predated by ravens and crows when compared with birds that had lower levels of DDE (Helberg et al. 2005). Threshold level of 1,000 ng/g (ww) of DDE measured in Ardeid eggs can cause a significant reduction in the survival of young birds (Connell et al. 2003). The results of the current study indicated that residual concentration of DDE detected in the eggs from three heronries is well below the level which reduces the survivorship of young birds. The concentration of p,p'-DDE in eggs was higher than 500–6,000 ng/g ww, which causes a reduction in the reproduction of several bird species (Cifuentes et al. 2003). The concentration of p,p'-DDE measured in eggs of a cattle egret were well below those found to generate adverse effects in birds. According to Blus (1996), a concentration of 3,000 ng/g, ww of p,p'-DDE in *A. herodias* eggs can cause reduced hatching. The concentration of DDE measured in eggs of cattle egret was well below this level.

Like other OCPs, HCHs are lipophilic compounds, stored in fatty components of the body (Cid et al. 2007). Residual concentration of ΣHCH followed ΣDDT. Highest ΣHCH concentrations were detected from heronry at the River Ravi (344.3 ng/g) followed by heronries at the Chenab (239.1 ng/g) and the Rawal Lake Reservoir (114.7 ng/g). Concentrations measured in eggs collected from heronries at the River Chenab and the River Ravi were lower than those reported in black-crowned night heron from China (Dong et al. 2004) and far greater to those found in eggs (0.014 ppm) of cattle egrets from northwest Mexico (Mora and Anderson 1991) and *E. garzetta* from the Haleji Lake (170.6 ng/g), Taunsa Barrage (129.4 ng/g), Karachi Ghas Bander (159.5 ng/g) from Pakistan (Sanpera et al. 2003). Jaspers et al. (2005) also measured lower concentration of ΣHCH (41 ng/g) in eggs of *Athene noctua* from Belgium. Comparatively, greater concentrations of HCHs was recorded in prey items collected from heronry at the River Ravi (53.3 ng/g) followed by the River Chenab (40.6 ng/g) and the Rawal Lake Reservoir (22.3 ng/g). These concentrations were lower than those detected in the prey of water birds (80.95 ng/g) from Tai Lake, China (Dong et al. 2004). Concentration of ΣHCHs in sediments was detected as low compared to ΣDDTs. These concentrations were lower than those found in sediments of the north coast of the Vietnam (Nhan et al. 2001) and higher than those found in sediments (9.4 ng/g) of Victoria Harbor (Hong et al. 1995). HCH compounds can undergo long-range transport through the atmosphere, and presence of HCHs in prey, sediment, and eggs can be related to recent exposure and contamination of local ecosystem as well as long-range atmospheric transport.

Among HCH isomers, β-HCH isomer is the most prevalent in eggs due to its greater stability to enzymatic degradation, lipophilicity, and its bioaccumulation potential (Tanabe et al. 1998; Walker et al. 2001; ATSDR 2005). β-HCH was more prevalent in eggs from heronry at the River

Ravi than γ-HCH. Residual level of β-HCH measured in eggs from three heronries varied between 10 and 47 ng/g which was comparatively lower than those reported in eggs of *Ardea cinerea* (11–125 ng/g) from France (De Cruz et al. 1997); however, concentrations recorded in eggs from heronry at the Rawal Lake Reservoir were relatively higher than those reported in eggs of *P. erythrorynchos* (>40 ng/g) from Washington (Blus et al. 1998). Greater concentration of β-HCH was also recorded in prey items collected from the Rawal Lake Reservoir than γ-HCH. Greater concentration of γ-HCH was detected in eggs collected from heronry at the River Chenab as compared to β-HCH. γ-HCH is used as an insecticide and fumigant for a variety of insects and is used in the treatment of seeds such as canola and corn, on crops, in warehouses, on domestic and agricultural animals, and for pest control of scabies and lice on humans (ATSDR 2005). Presence of higher levels of γ-HCH in most of the egg samples were probably due to the relatively high stability of this compound against metabolism (Poolpak et al. 2008), and it is not harmful to birds, in contrast to heptachlor and especially its metabolite heptachlor epoxide, which is highly toxic to birds at higher concentrations (ATSDR 2005). Mean concentration of γ-HCH recorded in eggs is far greater than those of other bird species such as the African darter (1.2 ng/g), reed cormorant (0.47 ng/g), African sacred ibis (0.06 ng/g), and crowned plover (0.15 ng/g) from South Africa (Bouwman et al. 2008). Among HCHs measured in prey, γ-HCH was detected in greater concentration than β-HCH in prey items collected from the River Chenab and the River Ravi indicating its use for agricultural practices.

Among cyclodienes, aldrin, dieldrin, and heptachlor were detected in low concentrations in eggs of three heronries as compared to those found in eggs of little egret from different wetlands of Pakistan (Sanpera et al. 2003). Heptachlor and heptachlor epoxide are lethal for birds in concentrations of about 9 ppm (Blus et al. 1985). The concentrations recorded in the current study are too low to have adverse effects on cattle egret population. The concentration of heptachlor measured in eggs collected from heronry from the River Ravi (n.d.–56 ng/g) and the Chenab (n.d.–38.4 ng/g) were higher than those measured in eggs of *N. nycticorax* (2–21 ng/g) from northern Italy (Fasola et al. 1998). Aldrin was only detected in eggs collected from heronry at the River Chenab and measured concentrations (n.d.–54.9 ng/g) were lower than those reported in *E. garzetta* and *N. nycticorax* (4–184 and 7–231 ng/g) from northern Italy (Fasola et al. 1998). Contamination pattern of cyclodienes in sediments of three sites was: >heptachlor > dieldrin > aldrin > endosulfan II. Aldrin, dieldrin, and dicofol were not detected in sediments of the River Ravi and the Rawal Lake Reservoir. The presence of chlorinated cyclodiene pesticides in the food indicated their use in large quantities mainly to control agricultural pests, insect-borne diseases, and for termite control in three study sites. Heptachlor and aldrin were also measured in prey items.

Technical grade dicofol contains <0.1% DDT and causes hyperstimulation of nerve transmissions along nerve axons which inhibit ATPases in the central nervous system (Hurt 1991). To our knowledge, this is the first study from Pakistan in which dicofol was found in biological samples and was measured in greater concentrations in eggs collected from the River Chenab indicating its use as an organochlorine miticide to control mites on agricultural crops and ornamental plants in urban areas. Use of dicofol as an insecticide in gardening and citrus, rice, and cotton fields may contain *o*,*p*′-DDT as byproducts, another pollution source of DDTs and may be one of the reasons for contaminant loads in the local ecosystem. Residual concentration of dicofol in eggs was in the order: River Chenab > River Ravi > Rawal Lake Reservoir. Dicofol was only detected in prey items collected from heronry at River Ravi and Chenab. Catchment of the river Ravi and River Chenab is known for fruit, such as citrus and mangoes, production and agricultural crops, viz. rice, cotton, sugarcane, and wheat crops, and known for their irrigated cropping system. Presence of relatively higher concentration of dicofol in eggs collected from heronries at the River Ravi and the River Chenab can be attributed to its use as a pesticide in agricultural applications. Presence of dicofol in

eggs is a major concern which needs closer attention. Residual concentrations of dicofol measured in the current study was lower than those reported in eggs of captive female American kestrels fed diets containing Kelthane (Wiemeyer et al. 2001).

The results of our study stress the dire need for continuous monitoring of OCP contamination using cattle egrets as an ecological indicator and to perform species-specific toxicity studies to properly evaluate the potential risk of OCPs for birds of this region. There is a dire need for further work to assess the contamination of persistent as well as industrial POPs, etc., their exposure, and potential for toxicity in fish-eating egrets and other top predatory bird species.

Conclusions

Concentration of total OCPs showed an increasing order from sediments to prey and to egret eggs indicating bioaccumulation and biomagnification along the food chain. Comparatively, higher residual level of POPs was measured in heronries from the River Chenab and River Ravi as compared to the Rawal Lake Reservoir. The residual concentrations reported provide a baseline to illustrate and compare the concentration of POPs in other wild bird species exposed to environmental contamination through the food chain. Detailed studies should be conducted to study the fate and ecotoxicological consequences of POPs in bird species especially top predators in Pakistan. Presence and persistence of DDT and its metabolites in eggs of cattle egret are problems of great relevance to cattle egret population. This study concluded that cattle egrets which are at the high levels of the food web can be used as an effective bioindicator to evaluate the presence of persistent contaminants in local environment, and there is a need to monitor long-term trends and POPs on a temporal basis to assess the contamination level.

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