Arch. Environ. Contam. Toxicol. 44, 360–368 (2003)
DOI: 10.1007/s00244-002-2044-z

ARCHIVES OF Environmental Contamination and Toxicology

Persistent Organic Pollutants in Little Egret Eggs from Selected Wetlands in Pakistan

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Received: 5 March 2002/Accepted: 19 August 2002

Abstract. The main goal of the present work has been to study the use of egret eggs to assess environmental pollution by POPs (HCB, HCHs, cyclodienes, DDTs and PCBs) in three Pakistani wetlands that are presumed to be affected by different types of pollution. Taunsa Barrage, affected mainly by agricultural pollutants; Karachi Harbor because of the supposed exposure to industrial activity-related POPs; and Haleji Lake as a relatively pristine area because of its location in a stony desert. Taunsa Barrage and Haleji Lake are wetlands of international importance according to the Ramsar Convention, while the Karachi Harbor is of interest because of the large human population living there. Eggs of the white (*Egretta garzetta garzetta*) and dark (*Egretta garzetta gularis*) morphs of Little Egrets were used as monitoring tools. Concentrations were also determined in several prey in this species' diet and in the sediments collected in their foraging areas. Differences in egg pollutant content among the three localities were significant for all the compounds. Overall, the eggs from Haleji Lake and Karachi showed, respectively, the lowest and highest percentages of detection and organochlorine concentrations. Biomagnification from sediments to prey and then to eggs has been documented in the three areas studied and is accompanied by higher percentages of detection of different compounds through the compartments. Differences in the biomagnification factor among the areas were small, even when differences in pollutant concentrations were high, suggesting that eggs are reliable indicators of POPs in the environment. The values found were generally lower than those reported for the eggs of large herons from North America or the Mediterranean basin, and are about the same order of magnitude that those of other medium-sized egrets from other parts of the world.

Organochlorine compounds are still used by developing countries, mainly for the treatment of agricultural pests and for the control of malaria vectors (Scholtens *et al.* 1990). Some features of tropical ecosystems, such as high temperatures and heavy rain, affect the environmental fate of these pollutants by increasing their dispersion rate (Iwata *et al.* 1994; Kannan *et al.* 1995; Custer 2000). Despite their widespread use, little information is available on the impact of persistent organic pollutants in tropical areas.

In Pakistan, the use of agricultural pesticides is presently concentrated on cotton fields, located mainly in the Punjab and Sindh provinces and to a lesser extent in Baluchistan. Lesser amounts of pesticides are applied to other crops, such as vegetables and fruits. Most of these pesticides are insecticides against white flies, jassids, aphids, bollworms, and other harmful insects. The estimated use in Pakistan ranged from 13,000 metric tons for 1990–1, to 30,471 metric tons for 1995–6 (STAT-USA on the Internet, United States Department of Commerce). Predatory birds, such as egrets and herons (Family Ardeidae), are at the top of the aquatic food webs, and are therefore particularly susceptible to pollutant bioaccumulation. Since they accumulate organochlorines over time, these waterbirds have often been used as indicators of ecosystem health (De Lucca-Abbot *et al.* 2001). Several authors argued that the eggs of predatory birds are effective materials for monitoring both the exposure to and the effects of the contaminants (Kushlan 1993; Cobb *et al.* 1995; Aurigi *et al.* 2000; Erwin and Custer 2000; De Luca-Abbot *et al.* 2001). Moreover, egg sampling outweighs the shortcomings of experimental studies on contamination (Blus and Henny 1997).

During 1999 and 2000, we conducted a research project aimed to assess the pollution status of selected wetlands in Pakistan and China.

In this paper the use of egret eggs for assessing the general environmental pollution by persistent organochlorine pollutants (POPs) is evaluated and the contamination levels for three Pakistani wetlands that are presumed to be subject to different types of pollution. We collected eggs of the Little Egret (*Egretta garzetta*) as our main study material. In order to estimate the exposure of the birds to pollutants, we also ana-

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lyzed samples of their prey and of the sediments collected within their foraging areas.

Material and Methods

Study Areas

During the egret breeding seasons of 1999 and 2000, fresh eggs, prey, and sediments were sampled at the following three study areas (Figure 1).

- Haleji Lake is a freshwater reservoir with associated marshes and adjacent seepage lagoons, set in a stony desert about 80 km north of Karachi in the Sindh province. The area is owned by the Sindh Government, and designated a Wildlife Sanctuary and Ramsar Site. Since there is no agriculture or industrial activity in the surroundings, pollutants could reach the reservoir mainly by atmospheric deposition.
- Taunsa Barrage, Punjab province, is a storage reservoir behind a barrage on the River Indus. It has been declared a Ramsar Site and a Wildlife Sanctuary. Agriculture (mainly cotton, sugar cane, wheat and fodder crops), livestock grazing, and forestry occur in adjacent areas, but the barrage is used also for power generation, so presumably pesticides and some PCBs are to be found.
- Karachi Harbor, or "Ghas Bunder" is an area of tidal creeks, mangrove swamps, and intertidal mudflats, and was expected to receive considerable urban and industrial pollution. About 45% of Pakistani industries are located near Karachi and all their effluents, plus the domestic sewage from the city (more than 8 million people), find their way, untreated, into the sea (Khan 2000). Karachi Harbor is also exposed to ship wastes and oil spills from the oil terminal. Therefore, a high incidence of PCBs was expected at this study area.

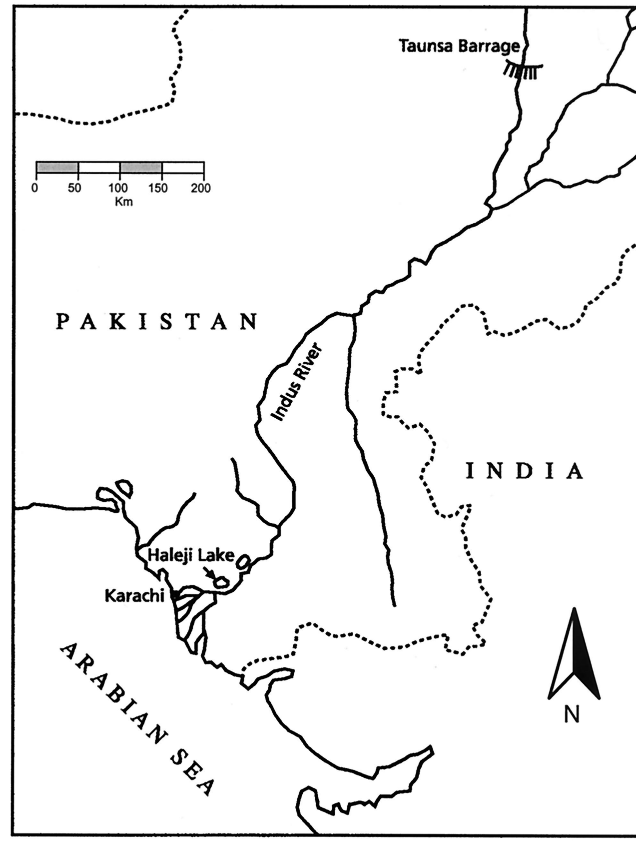

Fig. 1. The study areas in Pakistan

Samples

At each study area, one suitable Little Egret colony was located, sample nests were marked with numbered plastic tags, and eggs were collected and keep frozen until laboratory analysis. Unfortunately, at Haleji Lake and at Taunsa Barrage, Little Egrets were abundant but their nests were unattainable, thus sample size was limited. On the other hand, the nests at Karachi Harbor were easily accessible and two eggs per nest were collected in order to estimate the effect of the female on egg pollutants (Custer *et al.* 1990 and references therein). In order to avoid pseudoreplication, a mean nest value was computed and used for descriptive statistics. The white morph of the Little Egret (*Egretta garzetta garzetta*) is widespread through inland Pakistan, and was present at the first two study areas, while the dark morph (*Egretta garzetta gularis*) is found near the seashore from the Arabian Peninsula to India and was present only at the Karachi study area (Roberts 1991; Perennou *et al.* 2000).

Sediment samples were collected, using a 30-cm depth core, at the foraging areas that were most frequently used by the egrets during breeding. The use of foraging habitats was assessed during repeated surveys of all the available wetlands within 10 km of the study colony, which is the foraging range currently exploited by Little Egrets around their breeding sites (Hafner and Fasola 1992). Sediments were allowed to air-dry under field conditions and then taken to the lab for analysis.

Prey samples were also collected by taking advantage of the spontaneous regurgitates of the Little Egret chicks when handled. These samples were measured, weighed, and preserved in alcohol (60°). Whenever possible, prey items were sorted and identified. Small items of the same species were pooled to obtain a composite sample. At Haleji Lake, prey samples corresponded to four species of fish: *Oreochromis niloticus* (21 fish pooled in five samples), *Puntius phuturio* (38 fish pooled in two samples), *Colisa lalia* (10 fish pooled in three samples), and *Glossogobius giuris* (six fish). At Karachi, Abu mullets (*Liza abu*) were collected, 15 fish being included in each pooled sample. At Taunsa, prey samples corresponded to one pool of nine undetermined fish.

Analysis

Chemical analysis was done at the Laboratory of Toxicology (School of Veterinary Science, Universitat Autònoma de Barcelona) according to Mateo *et al.* (1999) for the following organochlorine compounds: Hexachlorobenzene (HCB); hexachlorocyclohexane α, β, and γ (lindane) isomers (HCHs); heptachlor epoxide and α-endosulfan (cyclodienes); *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, and *o,p′*-DDT (DDTs); and polychlorinated biphenyls (PCBs). Fish and egg samples were homogenized with anhydrous sodium sulphate, followed by extraction with n-hexane and clean-up with sulfuric acid. The same procedure was used for sediment samples, but without anhydrous sodium sulfate.

Organochlorine analyses were carried out by high resolution gas chromatography on a Perkin-Elmer AutoSystem equipped with a 30-m fused silica capillary column of 0.53 mm ID (BPX5 from SGE, Ringwood, Australia), coupled to an electron capture detector (ECD).

Chromatographic conditions were optimized for the separation of compounds under study and to a suitable analysis time: the oven was programmed from the initial temperature of 145°C to 276°C at a rate of 2.5°C/min, with injector (a Jade septum-less injector) temperature of 290°C and detector 310°C, and with carrier gas (He) at a linear velocity of 25 cm/s. Chlorinated compounds were localized using

PCBs #1 and #209 (standards added to the samples from the beginning), and using the Situation Units system for identifying peaks in the chromatograms (Mateo *et al.* 1998). High-resolution chromatographic analysis and quantification of OC residues followed the corrected Ballschmiter and Zell nomenclature system for PCBs (Guitart *et al.* 1993). Aroclor 1254 was used to quantify PCBs. The total PCBs concentration (ΣPCBs) was calculated as the sum of individual congener concentrations. Recoveries of selected pesticides (DDTs and HCHs, 71–100%, $n = 5$) and PCBs (77–100%, $n = 4$) were calculated and considered satisfactory, but no corrections were made based on recoveries. Since cyclodienes are partially degraded by the acid attack their values should be considered as underestimated. Blanks were processed between samples to check the absence of external contamination. Detection limits for the different compounds were as follows: HCBs = 3 ppb; HCHs = 1 ppb; heptachlor-epoxide and α-endosulphan = 1 ppb; *p,p′*-DDE and *o,p′*-DDT = 2 ppb; *p,p′*-DDD and *p,p′*-DDT = 3 ppb; and sum of PCBs = 25 ppb. Eggshell thickness was measured for 32 eggs from the Taunsa and Karachi study areas, using a DIGIMATIC counter (Mitutoyo, accuracy to the nearest 0.01 mm). For each egg, thickness was measured five times at the equator, and the arithmetic mean was calculated.

Statistics

For descriptive statistics on pollutant concentration in eggs, left-censored observations (*i.e.*, values below the limit of detection) were replaced by imputed values obtained by quantile–quantile regression (Gleit 1985; Millard and Neerchal 2001), after having checked the log–normal distribution of data. This procedure let us obtain more unbiased and robust descriptive statistics. Nevertheless, this procedure could not be applied to prey and sediments because of the high percentage of censored observations; thus, descriptive statistics in these samples rely only on detected values.

Rank-order test statistics were used, in order to minimize problems caused by left censored data, for comparisons among localities (Kruskall-Wallis test) and for relationship assessment (Spearman correlation). When *a posteriori* pairwise comparisons between areas were required, we used the Mann–Whitney U test and a sequential Bonferroni procedure to guarantee a tablewise significant level of $\alpha = 0.05$ (Holm 1979). In prey and sediments, for which the number of non-detected values were high, we compared the percentage of left-censored data instead of the average concentrations, by means of permutational exact distribution of the G likelihood statistic.

The egg samples from Karachi Harbor were used to test for a female effect on total organochlorine burden, and on DDTs and PCBs separately, as all the eggs showed values >LOD for these compounds. A random one-way analysis of variance was performed on log-transformed values, once normality and homocedasticity of distributions were verified (Zar 1999).

Results

Most of the contaminants were detected in all the Little Egret egg samples (Table 1). However, the eggs from Haleji Lake had the lowest overall percentages of detected contaminants and showed no trace of cyclodienes. The samples from Haleji Lake showed the lower organochlorine load, whereas those from Karachi had the highest concentrations, although this pattern differs among organochlorine groups.

Tables 2 and 3 describe organochlorine concentrations in prey and in sediments. Many of these samples had values below the standard detection limit, therefore the descriptive statistics were calculated only from determined values. The overall patterns of organochlorine residues at different areas were roughly similar to those of the eggs; the concentrations in the Haleji samples were generally lower, but some compounds, such as HCB and HCHs were found only in this area.

In order to test the female effect, one nest was excluded from the analyses because its variance was very close to the total variance. The residues of total organochlorines, of DDTs, and of PCBs, showed a significant random female effect (Table 4). More than 80% of the total variance can be attributed to differences among females, as can be seen for DDTs in Figure 2. In the eggs, significant differences in pollutant concentration were found among the three localities, for all the compounds (Figure 3). With regard to cyclodienes, only two localities were compared (Taunsa and Karachi M–W U = 24, $p = 0.7$) because these compounds were not detected in the samples from Haleji, suggesting that eggs from this locality had lower concentrations. HCB concentrations differed among areas (K–W $\chi^2 = 12.8$, $p = 0.001$); concentrations at Taunsa were lower than at the other two study areas, that did not differ significantly. HCHs concentrations were significantly higher at Haleji than at Karachi (K–W $\chi^2 = 7.1$, $p = 0.02$), but both areas did not differ significantly from Taunsa, that had intermediate concentrations. DDTs concentrations were significantly lower at Haleji than at the other two areas (K–W $\chi^2 = 6.1$, $p = 0.04$), which in turn were very similar. Moreover, significant differences were found in the ratio DDE/DDTs (K–W $\chi^2 = 15.5$, $p = 0.0004$), because in the Haleji samples almost all the DDTs were in the *p,p′*-DDE form, thus attaining a significantly higher ratio than in the samples from Taunsa and Karachi. PCBs showed the highest difference among localities (K–W $\chi^2 = 20.3$, $p < 0.0001$); lower concentrations were found at Haleji, followed in increasing concentrations, by the samples from Taunsa and from Karachi. The ratio DDTs/PCBs showed the same pattern among areas, but in a reversed order (K–W $\chi^2 = 19.4$, $p < 0.0001$).

We found a negative relationship between eggshell thickness and *p,p′*-DDE concentrations (log-transformed data), as expected, but the relationship was non-significant ($r = -0.25$, $p = 0.09$).

Due to the high proportion of samples below detection limits, we examined organochlorine differences in fish prey between the Haleji and Karachi study areas by comparing percentages of samples above the detection limit (Table 2). Fish from Haleji had lower presence of cyclodienes (G exact test = 8.3, $p = 0.02$) and PCBs than those from Karachi (G exact test = 9.8, $p = 0.01$). On the other hand, HCB was more widespread in fish from Haleji (G exact test = 9.8, $p = 0.01$). No significant differences were found for the other compounds analyzed. The above patterns found for prey are similar to those found for the eggs.

In sediments (Table 3) HCB was present in a significantly higher percentage of samples from the Haleji (G exact test = 15.6, $p = 0.001$), whereas cyclodienes (G exact test = 12.3, $p = 0.005$) and PCBs (G exact test = 17.7, $p = 0.001$) were significantly less frequent at Haleji. DDTs were significantly less frequent in the sediments from Taunsa (G exact test = 11.5, $p = 0.008$). Overall, the percentage of detected contaminants in the sediments (Table 3) agree with the results obtained for eggs and for prey.

The total organochlorine concentrations in the three sample

Table 1. Concentrations and ratios of organochlorine contaminants in the eggs of Little Egrets

		Analyzed (detected)	% detection	Geometric mean (arit.mean)	Q1	Q3	IQR	Max.
Haleji Lake	HCB	8 (6)	75	16.3	12.1	23.5	11.4	25.0
	ΣHCHs	8 (6)	75	170.5	60.7	619.3	558.6	858.0
	Σ Cycs	8 (0)	—					
	Σ DDTs	8 (8)	100	728.3	272.5	2566.5	2294.0	9982.2
	Σ PCBs	8 (3)	37	1.4	0.3	5.8	5.5	22.1
	Σ OCs	8 (8)	100	1138.5	427.3	2827.4	2400.1	10256.9
	DDE/DDTs	8 (8)	100	(1.0)	1.0	1.0	0.0	1.0
	DDTs/PCBs	8 (3)	37	(3826.5)	50.9	8649.4	8598.5	15061.9
Taunsa Barrage	HCB	4 (2)	50	1.7	1.0	2.9	1.9	3.1
	ΣHCHs	4 (4)	100	85.4	44.7	168.3	123.6	185.0
	Σ Cycs	4 (4)	100	129.4	51.2	597.6	546.4	761.3
	Σ DDTs	4 (4)	100	2943.4	926.4	16755.3	15828.8	21192.6
	Σ PCBs	4 (4)	100	100.4	42.3	515.7	473.3	665.0
	Σ OCs	4 (4)	100	3365.3	1159.2	18004.6	16845.4	22739.1
	DDE/DDTs	4 (4)	100	(0.9)	0.8	1.0	0.2	1.0
	DDTs/PCBs	4 (4)	100	(33.3)	17.0	48.9	31.9	53.0
Karachi Ghas Bunder	HCB	14 (13)	93	10.3	7.0	14.8	7.8	31.4
	ΣHCHs	14 (14)	100	44.7	29.6	58.4	28.8	446.8
	Σ Cycs	14 (14)	100	159.9	80.9	354.5	273.6	530.8
	Σ DDTs	14 (14)	100	2665.9	1871.6	3610.4	1738.8	5332.8
	Σ PCBs	14 (14)	100	4203.8	2953.1	5998.0	3044.9	8358.9
	Σ OCs	14 (14)	100	7195.9	4951.7	9279.7	4328.0	13964.8
	DDE/DDTs	14 (14)	100	(0.9)	0.9	0.9	0.1	1.0
	DDTs/PCBs	14 (14)	100	(0.7)	0.6	0.7	0.2	0.9

Q1 and Q3, the first and third quantile; IQR, the interquartile range; max, the maximum determined value. Figures are ng/g dry weight. Eggs mean percentage of water content was estimated as 80.8 (SD = 1.48, n = 40). For ratios, means in brackets correspond to arithmetic means. Samples from Karachi Ghas Bunder correspond to 28 eggs belonging to 14 nests.

materials (Figure 4) show increasing values from sediments to prey to eggs, with average biomagnification factors of 13.0 (CI 95%: 5.4–31.2) for sediments to prey and 72.7 (CI 95%: 35.9–147.1) for prey to eggs. These factors are very similar for the three study areas. Likewise, the percentage of samples below the detection limit decrease from the sediment, the prey, and the egg samples.

Discussion

One aim of our work was to use eggs of colonial waterbirds, such as the Little Egret, as a bioindicator in pollution assessment studies. The advantages of using eggs have been stated by several authors (Custer *et al.* 1990; Furness and Greenwood 1993; Peakall 1994; Walker *et al.* 2001). One of the most reliable features of eggs is the ease of collecting and handling them, as well as their great homogeneity in composition, which facilitates the interpretation of contamination data in relation to variable biological factors. Moreover, collecting eggs is low risk for the study species population. The selection of a reliable bioindicator was key to our study of contamination in Pakistani wetlands, since no previous information was available on the subject. In order to be a valid bioindicator, the egg contamination must be informative for different areas. The process must also be cost-effective in comparison to the collection of other kind of samples, such as water, sediment, invertebrates, and fish.

Although most organic pollutants pass into eggs, there are few studies documenting the relationship between levels in the eggs and the tissues of the breeding female (Reynolds *et al.* 2001). The outstanding female effect found in egret clutches from Karachi for both DDTs (Figure 2) and PCBs, indicates that the different contamination levels of the females are translated into their eggs, therefore eggs can be used to monitor the status of the adults. Also, a strong female effect validates the strategy of sampling one egg per clutch, as evidentiated by Custer *et al.* (1990) in their study on Black-Crowned Night-Herons, since additional eggs from the same clutch will only provide redundant information. However, a strong laying order effect might be also present in other cases, particularly when female reserves are partially used to form the latter eggs in the clutch, as occurs in some seabird species (Pastor *et al.* 1995 and references therein). Both terms, female or nest effect, have been used to describe the fact that interclutch is larger than intraclutch variability in pollutant concentrations. In our view, since eggs are formed inside the body of the female, the first term is more suitable, because other events can be involved at the nest level. For instance, the extremely high variance observed in one of the Karachi clutches (almost equal to total variance) suggests that, in this case, the two eggs may have been laid by two different females (González-Martín and Ruiz

Table 2. Concentrations and ratios of organochlorine contaminants in the fish prey of the Little Egret

Locality		Analyzed (detected)	% detection	Geometric mean (arit.mean)	Min	Max
Haleji Lake	HCB	16 (14)	88	11.5	1.8	59.7
	ΣHCHs	16 (0)	—			
	Σ Cycs	16 (3)	19	3.9	0.7	10.5
	Σ DDTs	16 (9)	56	23.6	7.6	149.3
	Σ PCBs	16 (2)	13	2.9	1.5	5.7
	Σ OCs	16 (14)	88	33.6	33.5	154.9
	DDE/DDTs	16 (9)	56	(0.7)	0.1	1.0
	DDTs/PCBs	16 (2)	13	(4.4)	3.8	5.1
Taunsa Barrage	HCB	1 (0)	—			
	ΣHCHs	1 (0)	—			
	Σ Cycs	1 (0)	—			
	Σ DDTs	1 (1)	100	78.4		
	Σ PCBs	1 (1)	100	7.4		
	Σ OCs	1 (1)	100	85.8		
	DDE/DDTs	1 (1)	100	(0.9)		
	DDTs/PCBs	1 (1)	100	(10.6)		
Karachi Ghas Bunder	HCB	3 (0)	—			
	ΣHCHs	3 (0)	—			
	Σ Cycs	3 (3)	100	227.7	146.2	349.5
	Σ DDTs	3 (3)	100	32.1	29.6	34.4
	Σ PCBs	3 (3)	100	5.8	4.5	6.6
	Σ OCs	3 (3)	100	267.4	182.2	388.4
	DDE/DDTs	3 (3)	100	(0.3)	0.3	0.4
	DDTs/PCBs	3 (3)	100	(5.7)	4.7	7.6

Min: minimum determined value; max: maximum determined value. Figures are ng/g dry weight. Fish prey mean percentage of water content was estimated as 78.3 (SD = 3.3, n = 20). For ratios, means in brackets correspond to arithmetic means.

1996). Since most organochlorines are lipophyllic and are not easily degraded, they tend to accumulate during the lifespan of an animal and their concentrations are biomagnified through each step in the food web (Hoffman *et al.* 1996; Carey *et al.* 1998). Biomagnification has also been reported for bird eggs (Albanis *et al.* 1996; Hughes *et al.* 1997). For total organochlorines, the increasing concentration from sediments to prey to eggs, was documented for each of our three study areas (Figure 4). This increase is accompanied by higher percentages of detection of different compounds through the compartments (Tables 1, 2, 3) (Tables 1–3) For this reason, sampling egret eggs can solve some difficulties that arise during chemical analysis and result interpretation when many samples have non-detected values. Differences among the three study areas in the biomagnification factor (ratio egg/prey) were small (CV = 16%), even though the differences in pollutant concentrations were high, suggesting that eggs are reliable indicators of POPs contamination in the general environment.

Our study areas, include wetlands that are presumed to receive pollutant inputs from different sources: agricultural run-off, industrial sewage waters, or relatively few pollutants. Because of this variation, it could be expected that the pollutants and their concentrations would differ among the areas. The concentrations found in the samples from Haleji Lake indicate that, on the whole, this can be considered as a relatively pristine area, since the concentrations of total organochlorines, of cyclodienes, of DDTs, and of PCBs were significantly lower than at the other two areas. These results agree with those reported by Sanpera *et al.* (2002) for the Intermediate Egret from the same area. On the other hand, samples from Taunsa Barrage had high concentrations of cyclodienes and of DDTs, and relatively low concentrations of PCBs; the samples from Karachi Harbor had the highest pollutant load among the three study areas. The concentrations of cyclodienes and of DDTs were high at Karachi Harbor, but this area was mainly characterized by the high concentrations of PCBs, a contaminant that originates from industrial activities. The low concentrations of PCBs in the eggs from Haleji, and their absence in fish and in sediments, reflect the low industrial activity around this area. Moreover, this suggests that most of the egrets were sedentary.

HCB concentrations were generally low at all three study areas. However, they reached the highest concentrations at Haleji Lake, where they were detected in all the compartments (egg, prey, and sediment). The presence of HCB suggests that this fungicide has been dumped into the lake and, because of its great persistence in the aquatic environment, pervaded to all the compartments.

Total DDT concentrations were somewhat higher in egret eggs from Taunsa, and the proportion of p,p'-DDE was similar to that in the eggs from Karachi. Nevertheless, the DDTs at Taunsa corresponded to p,p'-DDD and to p,p'-DDT, while we found a relatively higher proportion of p,p'-DDD over p,p'-DDT at Karachi. DDD and DDT congeners in the eggs from the Haleji were negligible. These differences can be related to the past and present use of this insecticide. While Taunsa is mainly an agricultural area that probably received regular discharges of DDTs, at Haleji, the input of these compounds occurred previously, as shown by the fact that almost all DDTs

Table 3. Concentrations and ratios of organochlorine contaminants in sediments collected within the foraging areas of the Little Egrets

Locality		Analyzed (detected)	% detection	Geometric mean (arit. mean)	Min	Max
Haleji Lake	HCB	10 (6)	60	1.1	0.4	1.7
	ΣHCHs	10 (1)	10	1.0		
	Σ Cycs	10 (0)	—			
	Σ DDTs	10 (3)	30	7.0	1.4	22.2
	Σ PCBs	10 (0)	—			
	Σ OCs	10 (8)	80	2.8	1.2	22.2
	DDE/DDTs	10 (3)	30	(0.8)	0.5	1.0
	DDTs/PCBs	10 (0)	—	(1.0)	1.0	1.0
Taunsa Barrage	HCB	9 (0)	—			
	ΣHCHs	9 (0)	—			
	Σ Cycs	9 (6)	66	5.5	0.7	67.7
	Σ DDTs	9 (0)	—			
	Σ PCBs	9 (7)	78	0.6	0.3	0.9
	Σ OCs	9 (9)	100	2.8	0.3	68.3
	DDE/DDTs	9 (0)	—			
	DDTs/PCBs	9 (0)	—			
Karachi Ghas Bunder	HCB	9 (0)	—			
	ΣHCHs	9 (0)	—			
	Σ Cycs	9 (3)	33	1.3	1.1	1.4
	Σ DDTs	9 (6)	67	7.8	0.4	127.4
	Σ PCBs	9 (6)	67	2.2	0.5	63.4
	Σ OCs	9 (7)	78	8.2	0.8	191.8
	DDE/DDTs	9 (6)	67	(0.4)	0.2	1.0
	DDTs/PCBs	9 (5)	56	(5.7)	0.2	13.4

Min: minimum determined value; max: maximum determined value. Figures are ng/g dry weight. For ratios, means in brackets correspond to arithmetic means.

Table 4. Results of random one-way ANOVA analysis to assess the female effect on eggs from nests sampled in Karachi Ghas Bunder

	Estimated variance	Female effect (% of var)	$F_{12,13}$	Sig.
log ΣDDT	0.0304	86.6%	13.91	<0.001
log ΣPCB	0.0454	82.7%	10.57	<0.001
log ΣOCs	0.0366	83.0%	10.77	<0.001

were in the form of *p,p′*-DDE. On the other hand, Karachi lies at the end of the surface water discharge of the River Indus, whose water has been used for municipal, agricultural, and industrial purposes, without any treatment (Rahman *et al.* 1997). Thus, lower concentrations of *p,p′*-DDT were expected, since agricultural pesticides reach Karachi indirectly, through water supply.

With regard to HCHs, no significant differences were found between the three areas. HCHs concentrations were somewhat higher in the egret eggs from Haleji. However, α- and γ-isomers predominated in the eggs from Taunsa and from Karachi, whereas only β-HCH, the most stable isomer to enzymatic degradation (Tanabe *et al.* 1998; Walker 2000) was found in the Haleji. This suggests that HCHs had been used more recently at Taunsa and Karachi, than at Haleji. The available data on global HCH usage for 1980 and 1990, indicate that its use in Pakistan was lower than in neighboring countries like India or China (Kannan *et al.* 1992; Iwata *et al.* 1994; Li *et al.* 1998), although these compounds can undergo long-range atmospheric transport (Carey *et al.* 1998).

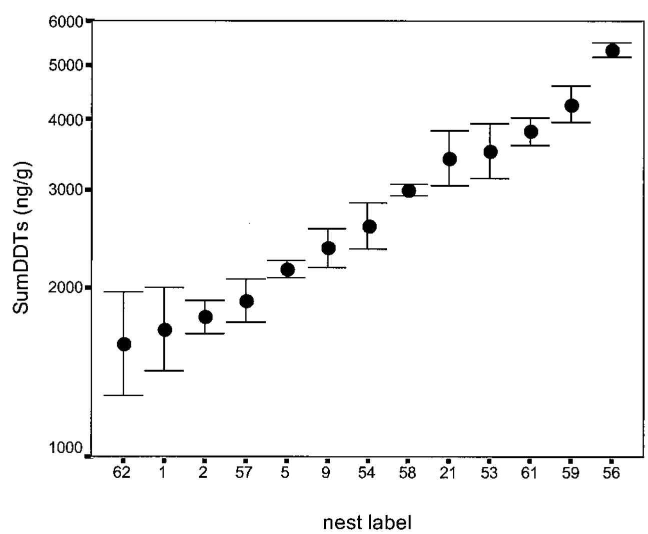

Fig. 2. Nest geometric mean (black dots) and individual egg values (horizontal thick marks) for ΣDDTs concentrations in 13 nests of *E. garzetta* sampled at the Karachi Ghas Bunder. A conspicuous female effect is apparent

Overall, DDTs (*p,p′*-DDE) were the organochlorine contam-

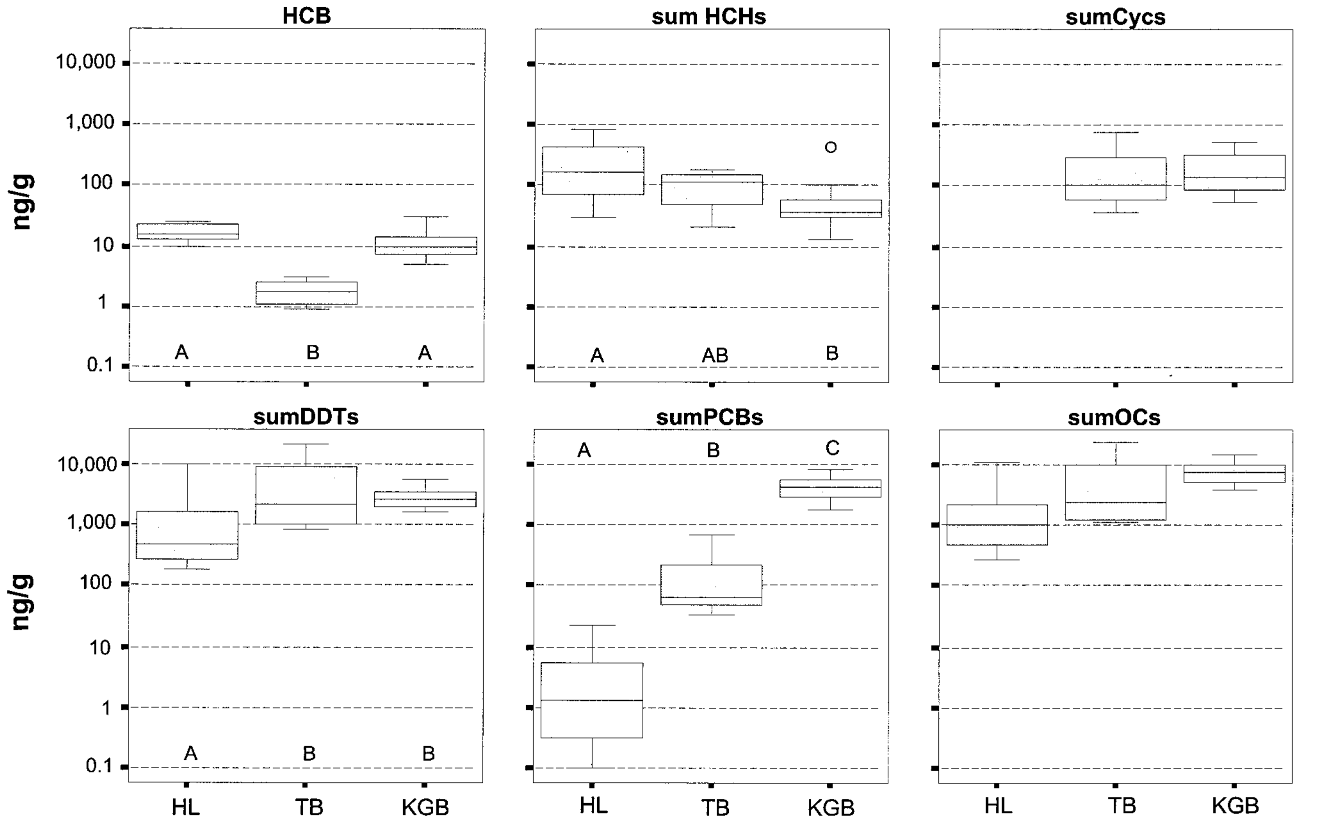

Fig. 3. Box-plot diagrams for different organochlorine contaminants in Little Egret eggs from the three study areas (ng/g, dw, log scale). Horizontal line shows median values, box is interquartilic range, and whiskers correspond to non-outlier range. HL, Haleji Lake; TB, Taunsa Barrage; KGB, Karachi Ghas Bunder. Localities sharing the same letter are not significantly different. This analysis has not been performed for cyclodienes because of lack of data for Haleji Lake and the small number of samples with detectable levels in the other two localities, and for sum of OCs

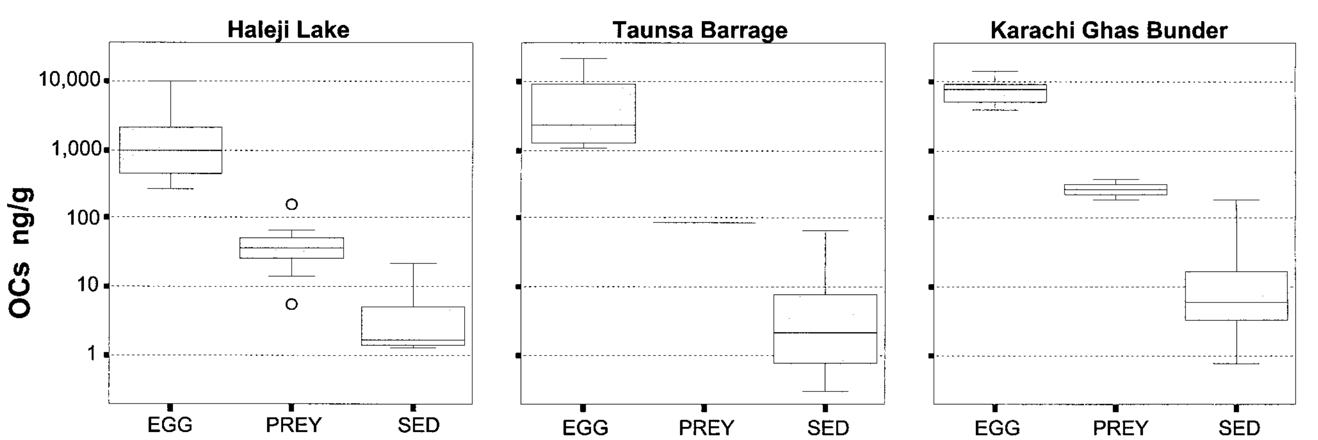

Fig. 4. Box-plot diagrams showing the biomagnification for the sum of organochlorine concentrations at the tree study areas (ng/g, dw, log scale)

inants that reached the highest concentrations, with the exception of PCBs in the samples from Karachi. The values of DDTs we found, were generally lower than those reported for eggs of large herons from North America (Fitzner *et al.* 1988; Custer *et al.* 1997, 1998) and from the Mediterranean basin (Alberto and Peña 1981; Ruiz *et al.* 1991), and were about the same order of magnitude as those of other medium-sized egrets from other parts of the world (Ruiz *et al.* 1991; Mora 1996; Rodgers 1997; Fasola *et al.* 1998). Mean DDE concentrations were, in all the cases, below the 2 μg/g reported by Blus *et al.* (1980) as having biological effects on birds. Only one egg from Taunsa Barrage had DDE concentrations above this level (3.17 μg/g wt/wt), but even in this case, the concentration was below the level associated with reproductive impairment in birds (>10 μg/g wt/wt; Custer *et al.* 1998 and references herein). However, it is worth mentioning that this egg had a thin shell (0.21 mm), about 0.1 mm less than any other eggs, suggesting that *p,p′*-DDE concentrations could have detrimental effects on some individuals of the study population.

With regard to PCBs, our results highlight the ubiquity of these compounds in the Karachi area, with a maximum concentration of 854 ng/g in eggs. The sensitivity of birds to PCBs, and the effects on their reproduction, are highly variable among species (Hoffman *et al.* 1996). Bosveld and Van den Berg (1994) derived LOEL values for PCBs exposure in eggs at 565 ng/g^{-1}, depending on the species and the endpoint involved. Concentrations as low as 100 ng/g in chicken (*Gallus gallus*) eggs were reported to decrease gluconeogenic enzyme activity in experimental conditions (Srebocan *et al.* 1977).

Concentrations of cyclodiene insecticides were moderately high in the eggs from Taunsa and also in the eggs and prey from Karachi. Nevertheless, nothing can be alleged about the effects on birds and on their eggs, as their effects remain largely unknown.

Overall, the results obtained for inland and coastal morphs of egrets are in agreement with the findings of Tanabe *et al.* (1998) for birds of South India, in which inland piscivores and scavengers were found to accumulate greater concentrations of HCHs and DDTs, while coastal piscivores contained comparable or greater amounts of PCBs.

Acknowledgments. This study was funded by the European Union INCO-DC (IC18-CT98-0294) research project. Authors are grateful to Sindh and Punjab Wildlife Departments for logistic support during the field work and for providing sample exportation licenses. We are particularly indebted with Mr. Rasheed Ahmed Khan, Game Officer of Haleji Lake. Also, we are grateful to the staff of WWF-Karachi office and, particularly to Mr. U Khan Baloch from the PARC (Islamabad and Multan) and to G. Gaudenzi (U. Pavia) for their contributions to this research. Dr. R. Guitart and Dr. R. Mateo from the Laboratory of Toxicology (UAB, Barcelona) performed chemical analyses. Comments by E.J. Scollon and an anonymous referee were of great help for improving the manuscript.

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