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# Persistent Organochlorine Compounds in Sediment and Biota from the Haleji Lake: A Wildlife Sanctuary in South Pakistan

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The manufacture and use of organochlorine compounds in developed nations has decreased remarkably during the last three decades, but in some developing countries they are still used in the treatment of agricultural pests and as insecticides for vectors of malaria. In tropical ecosystems the environmental fate of these pollutants is affected by high temperatures and heavy rain, which increase the dispersion rate (Iwata et al. 1994, Kannan et al. 1995). However, few data on organochlorine pollutants in biota from tropical ecosystems have been published and, to our knowledge, no data are available from wetlands in Pakistan.

Haleji Lake is a 'clean' freshwater reservoir with associated marshes and adjacent brackish seepage lagoons, covering an area of 10.53 $km^2$, with a maximum depth of 6 m. It is located in a stony desert of limestone and sandstone bedrock, about 80 km north of Karachi, Thatta District (24°48' N 67°44' E, see figure 1). The lake supports abundant aquatic vegetation which forms extensive beds and floating patches and constitutes an important staging and wintering area for waterfowl. The area is owned by the Sindh Government, and designated a Wildlife Sanctuary and Ramsar Site. The lake and marshes provide a breeding area for herons and egrets; the most abundant species are the Night Heron (*Nycticorax nycticorax*) and the Intermediate Egret (*Egretta intermedia*). Also, Indian Pond Herons (*Ardeola grayii*), a few Little Egrets (*E. garzetta*) and Purple Herons (*Ardea purpurea*) are found. The population of Intermediate Egrets has been little studied, though it is the predominant egret species on the lake. This species builds small colonies, both in submerged reed-beds and on islets (Sindh Wildlife Management Board 1986, Roberts 1991).

In 1999, an EU cooperation (EU-INCO) research project was started with the aim of monitoring contaminant levels in wetlands of special interest in both Pakistan and China. The work performed in the Haleji Lake forms part of this project. Eggs of Intermediate Egrets were chosen as monitoring tools to estimate the impact of organochlorine pesticides in this area because of the fish-eating habits of this species, which is at the top of the food chain, and the suitability of birds eggs to reflect the pollution burden of the breeding female (Pastor et al. 1995, Fasola et al. 1998). Also, since the breeding population of Intermediate Egrets inhabiting

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**Figure 1.** The location of Haleji Lake.

Haleji Lake is non-migratory, the results provide information about the pollution status in the area. To obtain an estimate of exposure of birds to pollutants, organochlorine levels were determined in several prey items from the diet of this species and in sediments from its foraging areas.

## MATERIALS AND METHODS

During May 1999 we took 22 fresh eggs from 20 nests of Intermediate Egrets. Whole eggs were frozen for later analysis. In two cases, two eggs were collected from the same nest and a mean nest value was computed and used for statistical purposes.

Ten samples of sediment were taken in the same area using a 30 cm depth core. Sediments were allowed to air-dry under field conditions and then transported to the lab for analysis.

We also collected samples of chicks regurgitates. In the field, prey samples were measured, weighed and preserved in alcohol (60°). In the laboratory, prey items were classified and small individuals of the same species were pooled to obtain a composite sample. Samples corresponding to four species of fish were analysed: *Oreochromis niloticus* (21 fish pooled in 5 samples), *Puntius phuturio* (38 fish pooled in 2 samples), *Colisa lalia* (10 fish pooled in 3 samples), *Glossogobius giuris* (n = 6) and one indeterminate species of crustacean, a caridean shrimp (29 shrimps pooled in 3 samples).

Chemical analysis of organochlorines were done at the Laboratory of Toxicology (School of Veterinary Science, Universitat Autònoma de Barcelona) following Mateo et al. (1999). Organochlorine compounds analysed include: Hexachlorobenzene (HCB), hexachlorocyclohexane isomers (HCHs), heptachlor epoxide, α-endosulfan, DDT compounds (DDTs) and polychlorinated biphenyls (PCBs). Fish and egg samples were homogenised with anhydrous sodium sulphate, followed by extraction with n-hexane and clean-up with sulphuric acid. The same procedure was used for sediment samples, but without anhydrous sodium sulphate. PCBs#1 and #209 were used as internal standards. High resolution chromatographic analyses 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 reported. Recoveries of selected pesticides and PCBs were calculated and considered satisfactory (70%-100%), but no corrections were made based on recoveries. Blanks were processed between samples to check the absence of external contamination.

Eggshell thickness was measured using a DIGIMATIC counter (Mitutoyo). Accuracy of measure was to the nearest 0.01 mm. For each egg, thickness was measured five times at the equator, and the arithmetic mean was considered.

For descriptive statistics, we computed geometric mean and ranges because the distributions were skewed and those samples in which the compound was not detected were not included. The relationship between organochlorines was assessed by non-parametric Spearman rho statistic; in this analysis non-detected data were replaced by a value equal to half the quantification limit. To compare mean eggshell thickness between egg groups we used the non-parametric U-Mann-Whitney test.

## RESULTS AND DISCUSSION

Descriptive statistics for organochlorine concentrations (ng/g wet weight) in the egg contents and in the egret's prey are provided in Tables 1 and 2, respectively. In Table 1, only those compounds detected in more than 50% of the eggs have been included.

Among HCHs in the eggs, the isomer β-HCH was detected in 50% of the samples, α- and γ-HCH being present only in 25% of the samples at very low concentrations. With regard to DDTs, *p,p'*-DDE was found in all the samples collected, *p,p'*-DDT was detected in 55% of the samples, *p,p'*-DDD only in 4%, while *o,p'*- compounds were not found in any of the eggs. PCBs have been

**Table 1**. Concentration of organochlorine compounds (ng/g wet weight) in the egg contents of Intermediate Egret. To avoid overreplication, in two cases values are the average of the two eggs sampled from the same nest.

| | HCB | β-HCH | *pp'*-DDE | *pp'*-DDT | *ΣPCBs* |
|---|---|---|---|---|---|
| N | 20 | 20 | 20 | 20 | 20 |
| % detected | 90 | 50 | 100 | 55 | 75 |
| Geom. Mean | 4.23 | 29.52 | 165.5 | 8.52 | 2.56 |
| Median | 3.88 | 16.84 | 121.3 | 4.78 | 1.23 |
| Min | 2.21 | 8.87 | 30.00 | 3.36 | 0.95 |
| Max | 62.71 | 1,212 | 18,840 | 86.47 | 54.57 |

**Table 2**. Levels of organochlorine compounds (ng/g wet weight) in the prey consumed by nestlings of Intermediate Egrets.

| SPECIES | | HCB | HEPTA-EPOX | *p,p'*- DDE | *p,p'*-DDT |
|---|---|---|---|---|---|
| *O. niloticus* | *G.M.* | 1.11 | 1.92 | 8.62 | |
| (n=5) | *Min* | 0.34 | | 3.98 | |
| | *Max* | 6.75 | | 25.34 | |
| | *N* | 4 | 1 | 3 | |
| *P. phuturio* | *G.M.* | 2.89 | 0.17 | 1.08 | 3.47 |
| (n=2) | *Min* | 0.95 | | 0.50 | |
| | *Max* | 8.83 | | 2.32 | |
| | *N* | 2 | 1 | 2 | 1 |
| *C. lalia* | *G.M.* | 1.55 | | 1.93 | 3.65 |
| (n=3) | *Min* | 1.17 | | 1.23 | 3.13 |
| | *Max* | 2.44 | | 3.10 | 4.24 |
| | *N* | 3 | | 3 | 2 |
| *G. giuris* | *G.M.* | 5.98 | 1.77 | 3.39 | 2.55 |
| (n=6) | *Min* | 5.41 | | | |
| | *Max* | 59.66 | | | |
| | *N* | 5 | 1 | 1 | 1 |
| Shrimp | *G.M.* | 0.47 | | | 0.85 |
| (n=3) | *Min* | 0.28 | | | |
| | *Max* | 0.78 | | | |
| | *N* | 2 | | | 1 |

referred as the sum of congeners; amongst them only PCB 180 was widely distributed.

In the fish species and in the caridean shrimps from chicks regurgitates, no HCHs and no PCBs were detected, but concentrations of heptachlor epoxide were above the quantification limit in some samples.

In the sediments (N=10), HCB was above quatification limit in 6 samples (Geom. mean = 1.12 ng/g d.w., median = 1.30 ng/g d.w., range = 0.4 - 1.7 ng/g d.w.). The *p,p'*-DDE was above quantification limit in three samples (Geom. mean = 5.13 ng/g d.w., median = 9.40 ng/g d.w., range = 1.4 – 10.30 ng/g d.w.). HCH isomers and other DDT compounds have been quantified only in one sediment sample. PCBs were below quantification limits in all the sediment samples. Therefore only rough data of HCB and DDT bioaccumulation process from sediments to biota can be provided (figure 2).

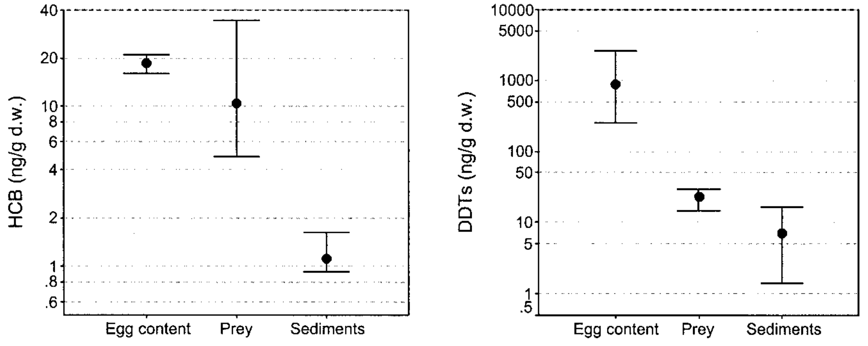


**Figure 2**. Geometric means (black dots) and interquartile ranges for HCB and DDTs in different types of samples analysed, showing bioaccumulation.

The fact that DDTs , and particularly DDE were detected in every egg analysed points to the ubiquity of DDTs in the lake and in the egret's foraging areas. DDE concentrations of Intermediate Egrets were lower than those reported elsewhere for eggs of large herons, such as the Great Blue Heron (*Ardea herodias*) (Fitzner et al. 1988, Elliott et al. 1989, Custer et al. 1998), and its values were of the same order of magnitude as those reported for other medium-sized herons from the Mediterranean Basin (Ruiz et al. 1991, Albanis et al. 1996, Fasola et al. 1987) and the United States (Mora 1996a). It must be pointed out that mean DDE levels found were much lower than those reported by Blus et al. (1980) to have significant biological effects on birds (2 µg/g w.w.) or by Custer et al. (1997) to impair avian reproduction in herons (10 µg/g w.w).

Nevertheless, the levels of DDE in three eggs (2.27,11. 93 and 29.76 µg/g w.w ), at concentrations which could be detrimental show that some birds are exposed to high DDTs concentrations, probably while feeding in agricultural fields. The two eggs with the highest concentrations came from the same nest (Geom. mean= 18,840 ng/g w.w.), supporting previous findings about the female effect on egg pollutant burdens (Pastor et al. 1995). Moreover, eggshell thickness in these eggs was significantly lower (U Mann-Whitney= 3.00, p= 0.013) than the others we collected. It is well known that high DDE concentrations may induce eggshell thinning in birds (Furness 1993, Blus et al. 1980, Blus 1996), and this has been recognized as one of the main causes of reproductive failure in bird populations.

The fact that DDE was the main metabolite in sediment samples and the high ratio DDE/DDT in the eggs (19.4), suggest that exposure to DDT was not recent. The predominance of DDE would indicate that DDT has been metabolised by the birds or that DDE is the main chemical form reaching the birds through their diet. The analysis of prey indicates that more DDE (in relation to DDT) reaches the birds, because *Oreochromys niloticus*, the main item in the egret's diet (*Ruiz X. &*

*Llorente G., Personal Communication*) contains only DDE. The ratio of DDTs in the eggs in relation to DDTs in *O. niloticus* is high, which shows that these pollutants are biomagnified in the food chain.

With regard to other organochlorines analysed in the eggs, HCHs, HCB, and PCBs followed in abundance to DDTs, their mean concentrations being low. The available data on global HCH usage (for 1980 and 1990) indicate that consumption of this pesticide in Pakistan is lower than in neighbouring 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. HCHs in the eggs corresponded entirely to the isomer β-HCH, the most stable to enzymatic degradation (Tanabe et al. 1998).

In the analysis of correlation between OC compounds, two eggs belonging to the same nest, that exhibited the highest concentration of both β-HCH and *p,p'*-DDE were not considered because if included they should have an extreme effect on correlation estimate . Nevertheless, the extreme organochlorine concentration found in these eggs strongly indicates that the breeding female had been feeding in polluted areas, probably paddy fields, and suggests that although most of the Intermediate Egrets forage in the Haleji Lake or in the seepage lagoons around it, some of them may travel longer distances to exploit other food sources. Even though these data were excluded in the correlation analysis, a positive relationship was found between β-HCH and *p,p'*-DDE (Spearman $r = 0.8$, $n = 19$, $p < 0.01$) and between β-HCH and *p,p'*-DDT (Spearman $r = 0.543$, $n=19$, $p < 0.05$).

No HCH was detected in fish samples or sediment samples. This agrees with the results of several authors (Iwata et al. 1994, Kannan et al. 1995), who reported a low level of accumulation for HCHs in aquatic organisms and sediments, pointing to the high temperatures of the tropics as the cause for the low levels of semivolatile contaminants in these compartments when compared to the higher levels found in air and water.

Heptachlor epoxide was detected in some fish at low levels, but not in the eggs. Similar results were obtained by Albanis et al. (1996) for the eggs and prey from the Squacco Heron (*Ardeola ralloides)* from Greece.

The low levels of PCBs in the eggs and the absence of these pollutants in fish or sediments samples from the Haleji, is indicative of the generally sedentary habits of the Intermediate Egret population and the low industrial activity around the area. The most abundant PCB congeners in the eggs were PCB-153 and PCB-180, in agreement with previous findings in bird tissues and eggs (Mora 1996b, Hoshi et al. 1998).

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