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The incidence of polychlorinated biphenyl and organochlorine pesticide residues in the eggs of the cormorant (*Phalacrocorax carbo sinensis*): an evaluation of the situation in four Greek wetlands of international importance

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Abstract

This study contributed to identifying the current levels of organochlorine pollutants in four Greek wetlands of international importance (the Evros and Axios Deltas, and Kerkini and Prespa Lakes), using the cormorant *Phalacrocorax carbo sinensis* as a suitable bioindicator in a region where such information is scarce. Residue levels of eight polychlorinated biphenyl (PCB) congeners and 13 organochlorine pesticide (OC) compounds were measured in cormorant eggs. Most PCBs and OCs (except dieldrin and endrin) were found in at least some of the study areas. Median concentrations of five PCBs (IUPAC 8, 20, 52, 138, 180) and of six OCs (α-BHC, β-BHC, lindane, heptachlor, 4,4′-DDE and 4,4′-DDT) differed significantly among the areas. The median totals of the PCBs were highly significant among the areas, being unexpectedly highest in Prespa Lake (68.43 ppb), despite its remoteness, and lowest in Evros Delta samples (12.17 ppb). Aldrin that was found in samples from Evros, Axios and Prespa probably accumulated in wintering grounds. In all of the areas, the relative proportions of α-BHC and 2,4′-DDD were the highest of all OCs. Fingerprint and cluster analyses illustrated overall differences in the PCB patterns, being greatest between the deltas than between the lakes, but, inversely, for OCs the differences were smaller in the deltas. Differences were attributed to large variations in the cormorants' diet between areas and different regimes of pollutant management in the two types of wetland. Correlations of pollutants varied considerably among areas and

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they were more diverse in OCs. The ΣOCs/ΣPCBs ratio indicates agrochemical pollution in all areas. An important finding was that levels of both pollutant groups were too low to have any biological implications on the cormorants and, additionally, suggest that they have a negligible impact on the environment of the wetlands studied.

Keywords: Polychlorobiphenyls; Organochlorine insecticides; *Phalacrocorax carbo sinensis*; Greek wetlands

1. Introduction

Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) were extensively used in the 1960s; however, most have been banned since the 1970s. OCs were used as pesticides and PCBs were used in industry, but both include substances with high toxicity and a persistence in the environment. Studies indicate that some compounds of both groups result in reproduction damage, impairment to wildlife, and/or (particularly in the case of PCBs) various embryonic deformities and mortality (Blus, 1982; Hoffmann et al., 1987; Gilbertson et al., 1991; Yamashita et al., 1993; Dirksen et al., 1995; Ludwig et al., 1995; Custer et al., 1999). They also pose threats to humans, especially at high levels, and/or background exposure to some compounds (Longnecker et al., 1997). Due to their lipophilic structure, both groups tend to accumulate in food chains; therefore they tend to occur at elevated levels, and bioaccumulate in higher organisms (Tanabe et al., 1987; Gagnon et al., 1990; Giesy et al., 1994; Guruge et al., 1997; Harding et al., 1997).

In Greece, OCs and PCBs were banned before the mid-1970s (Albanis, 1997). However, some studies have indicated that both groups persist in the Greek environment (Crivelli et al., 1989; Larsen and Fytianos, 1989; Kilikidis et al., 1992; Albanis et al., 1994, 1995, 1996; Fytianos et al., 1997; Goutner et al., 1997a,b). Systematic monitoring and comparative studies in different areas using higher trophic level receptors are still lacking. Greek wetlands are of particular interest for such studies, as in their vicinity they support considerable human populations where local sources such as water and fish are consumed, and they also contain internationally important populations of wildlife, especially birds.

The levels of organochlorines in seabird eggs reflect the diet of the female, and pollutant body reserves constitute a useful indicator of environmental contamination (Pearce et al., 1989; Furness, 1993). Cormorants (*Phalacrocorax* spp.) are fish feeders, and thus are top predators in aquatic environments; therefore, they are a suitable bioindicator species (Scharenberg, 1991; Ryckman et al., 1998). The aim of this study was to assess and compare the levels of OCs and PCBs in four internationally important Greek wetlands through the analysis of eggs from cormorants of the Eurasian race *Phalacrocorax carbo sinensis* (hereafter referred to as 'cormorant'). We hypothesized that river deltas would bear a much higher pollution loading than lakes because of a continuous transboundary deposition of pollutants.

1.1. Study areas

The wetlands sampled for this study (Fig. 1) contain four (out of a total of five) of the most populated colonies of cormorant in Greece (Handrinos and Akriotis, 1997). The Evros Delta, at the Greek–Turkish border, is the easternmost Greek wetland (40°47′N, 26°05′E), extending over 11 000 ha, and includes a variety of habitats such as temporary and permanent fresh water marshes, salt marshes, lagoons, brackish lakes, coastal sandy islets and beaches with ammophilus and nitrophilous vegetation, bush and forest stripes along the river, and cultivated land (Britton and Hafner, 1978; Babalonas, 1980). The river Evros, originating in Bulgaria, forms the border between Greece and Turkey for approximately 200 km, and it receives considerable amounts of transboundary pollution; of its total drainage area (52 500 km^2) only 6.5% is in Greece. Kerkini

Lake (41°22′N, 23°13′E) is an artificial lake (7300 ha) made as a water reservoir in the 1930s by damming the river Strymon, also originating from Bulgaria. Vegetation includes aquatic species, wet fields and an extensive forest situated at the area where the river enters the lake (Zalidis and Mantzavelas, 1994). The Axios Delta (40°30′N, 22°53′E) is a part of an extensive wetland complex situated at the west coast of the Thermaikos Gulf. Habitats include salt and fresh water marshes, rice fields, lagoons, vegetated coastal islets, limited sandy shores, forested river banks and tamarisk bush land (Kazantzidis et al., 1997). This river originates from the former Yugoslavia and contains considerable pollution, probably being the mostly polluted water body in north-eastern Greece (Fytianos et al., 1986). Lake Mikri Prespa (40°76′N, 21°09′E) extends over 4800 ha and is situated 853 m above sea level. The lake lies at the north-western edge of Greece, bordering Albania and the former Yugoslavia. Habitats include aquatic vegetation, wet meadows, cultivations of reedbeds, and sparse forestland. Cormorants nest on Vidrinetsi island at Mikrolimni, and their population has increased during the 1990s (Catsadorakis, 1997). In these areas, cormorant colonies nest in dense colonies on trees and/or bushes with water beneath them, at Kerkini Lake and the Evros Delta. All four wetlands are wetlands of international importance and are protected by the Ramsar convention. Lake Prespa has been declared a National Park since 1974.

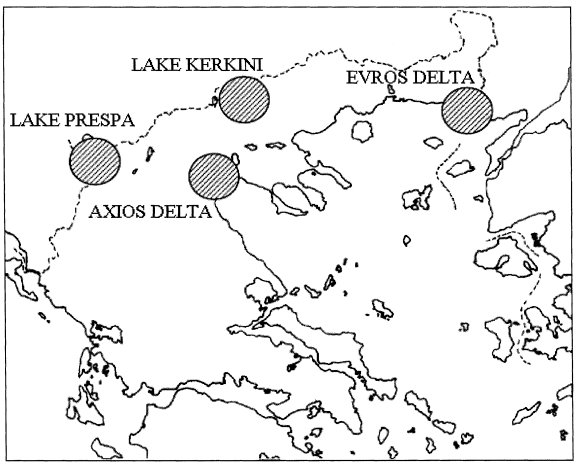

Fig. 1. Map indicating the study areas within the context of Greece.

Table 1
PCBs IUPAC numbers and the corresponding structure

PCB-IUPAC No.	Structure
8	2,4′-Dichlorobiphenyl
20	2,3,3′-Trichlorobiphenyl
28	2,4,4′-Trichlorobiphenyl
52	2,2′,5,5′-Tetrachlorobiphenyl
101	2,2′,4,5,5′-Pentachlorobiphenyl
118	2,2′,4,4′,5′-Pentachlorobiphenyl
138	2,2′,3,4,4′,5′-Hexachlorobiphenyl
180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl

2. Materials and methods

2.1. Sampling procedures

Freshly laid eggs were collected under license from cormorant colonies in April 1997 (Evros Delta, Lake Kerkini and Axios Delta) and 1998 (again Evros Delta, and Lake Prespa). One egg was randomly taken from each clutch sampled. The eggs were opened in the laboratory on the same day, and their contents were preserved, deep frozen, in chemically cleaned jars.

2.2. Materials

The organochlorine pesticides analyzed in this study include: α-BHC; β-BHC; lindane; heptachlor; heptachlor epoxide; aldrin; dieldrin; endrin; 2,4′-DDT; 2,4′-DDD; 4,4′-DDT; 4,4′-DDD; 4,4′-DDE. The following nine PCB congeners, in terms of PCB-IUPAC numbers, were also analyzed: PCB-8, 20, 28, 52, 101, 118, 138, and 180. The corresponding structure of the above PCBs is shown in Table 1.

PCB standards were obtained from the Dr Ehrensdorfer GmbH laboratory in concentrations of 10 mg/ml. A Supelco No. 4-9151 organochlorine pesticide standard mixture in iso-octane was used in concentrations of mg/ml for the chromatographic analysis. All of the solvents used:

hexane, acetone, and petroleum ether were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd, Dublin, Ireland). Florisil (50–100 mesh) and sodium sulfate (pro-analysis) was from Merck (Darmstadt, Germany). Glassware was soaked, cleaned with chromic/sulfuric acid solution, thoroughly rinsed with distilled water and acetone, and heated to 150°C for 12 h.

2.3. Analytical procedures

Whole eggs were homogenized in a blender and an aliquot of 5–10 g was homogenized again with 20–30 g of sodium sulfate in 100-ml glass tubes. The mixture was extracted firstly with 20 ml of a hexane/petroleum ether (1:1) mixture, and then followed twice more with 10 ml of the mixture, using a vortex (2 min), sonication bath (5 min), and manual mixing (5 min) with a glass rod. The extracts were collected in polypropylene centrifugation tubes of 50 ml and centrifuged at 4000 rev./min for 5 min. The supernatant was evaporated in a rotary evaporator to 10 ml, and lipids were then removed by treating the extracts with 0.5–0.8-ml aliquots of concentrated sulfuric acid. The procedure was repeated until the acid layer remained colorless (Pavoni et al., 1991). The clean-up was completed by adsorption chromatography, eluting the colorless layer through a chromatography glass column of 1 cm internal diameter, 20 cm length, provided with a teflon stopcock. The column was packed as follows: 5 cm of Florisil slurry was added first under the gentle tapping of the column and keeping the stopcock open, to avoid bubbles, then 2 cm of dried sodium sulfate was added. The column was washed with 20 ml *n*-hexane. All solvents used for packing the column were degassed in a sonication bath (Fytianos et al., 1997). The purified sample was evaporated in a rotary evaporator to approximately 5 ml, and in a gentle N_2 stream at 35°C to approximately 0.5 ml; then the samples were stored in silanized vials in a refrigerator (−20°C).

2.4. Chromatographic conditions

2.4.1. GC-ECD

A Shimadzu 14B gas chromatograph equipped with a Ni 63 electron capture detector (ECD) was used for the organochlorine residue analysis. The capillary column used was a DB-5, 30 m × 0.32 mm internal diameter, containing (5% phenyl) methyl polysiloxane (J and W Scientific, Folsom, CA) and following the temperature program: 150°C (2 min); 150–200°C (5°C/min); 200°C (45 min); 210–270°C (10°C/min); 270°C (3 min). The temperatures were set at 250°C for the injector and 300°C for the detector. Helium was used as the carrier and nitrogen as the make-up gas. Pure reference standard solutions were used for instrument calibration, recovery, quantification and confirmation. The splitless mode was used for the injection of a 1-μl volume, with the valve opened for 30 s.

2.4.2. GC-MS

Confirmation of organochlorine residues was performed by using a GC-MSD QP 5000 Shimadzu equipped with DB-5 capillary column, 30 m × 0.32 mm internal diameter, containing (5% phenyl) methyl polysiloxane (J and W Scientific, Folsom, CA), used under the following chromatographic conditions: injector temperature 220°C, column program of temperatures: 55°C (2 min); 55–210°C (5°C/min); 210°C (20 min); 210–271°C (20°C/min); 270°C (4 min). Helium was used as the carrier gas at 14 psi. The interface was kept at 270°C. The spectra were obtained at 70 eV. The splitless mode was used for the injection of a 1-μl volume, with the valve opened for 30 s. Two ions (M^+, M^{+2}) were chosen for each pesticide and biphenyl for screening analysis in a selected ion monitoring mode (SIM). Ions were selected after injecting a concentrated solution of compounds and recording the 'total ion chromatogram'. The ion traces were divided into five groups that were recorded sequentially during the injection, on the basis of the retention times of the single substances. For the PCBs, the identification of the selected ions for each degree of chlorination was confirmed by checking the ratios of the intensities of the ions belonging to the same cluster (Raccanelli et al., 1994; Singh et al., 1998). The characteristic ions and their relative intensities, used for the identification of the studied compounds are shown in Table 2.

Table 2
Molecular weight, retention time and mass spectra data of the GC-MS analysis

Organochlorine	Molecular weight	Retention time (DB-5)	Mass spectra data m/z ions, (ratios)
α-BHC	288	13.33	181 (100), 183(94)
PCB-8	222	13.82	222 (84.8), 224(50.8)
β-BHC	288	14.20	181 (87.6), 183(88)
Lindane	288	14.70	181 (100), 183(97.2)
Heptachlor epoxide	386	17.33	353 (94), 355(72)
PCB 28	256	18.01	256 (82), 258(73.6)
PCB 20	256	18.67	256 (85), 258(71.2)
Heptachlor	370	19.18	272 (100), 274(96)
PCB 52	290	20.30	290 (78), 292(100)
Aldrin	362	21.82	66 (100), 265(27)
4,4′-DDE	316	24.87	246 (100), 248(65.6)
PCB 101	324	28.83	324 (63.2), 326(100)
Dieldrin	378	32.19	79 (100), 81(35.2)
2,4′-DDD	318	32.93	235 (100), 237(67.2)
Endrin	378	34.88	263 (42.4), 265(30.4)
PCB 118	324	38.02	324 (58.8), 326(100)
4,4′-DDD	318	38.42	235 (100), 237(64)
2,4′-DDT	352	39.67	235 (100), 237(71)
4,4′-DDT	352	47.06	235 (100), 237(68)
PCB 138	358	48.36	358 (41.6), 360(94)
PCB 180	392	64.73	394 (100), 396(96)

2.5. Statistical procedures

The concentrations of the pollutants were not normally distributed, thus the median of each pollutant and the total median concentrations of PCBs (hereafter ΣPCBs) were compared by Kruskal–Wallis χ^2 tests. We calculated the ratio of total organochlorine concentrations (ΣOCs) and ΣPCBs (ΣOCs/ΣPCBs) in samples as a measure of agrochemical or industrial pollution (Fossi et al., 1984; Pastor et al., 1995b), and we then compared the median values between the four areas. Cluster analysis (with Euclidean distances as a distance measure and single linkage as a linkage rule), was used to evaluate the overall differences in pollution patterns among the areas studied.

3. Results

3.1. Polychlorinated biphenyls

Most of the eight PCB congeners analyzed for were detected in all areas, with the exception of PCB 118, which was not found in eggs from Kerkini Lake or Axios Delta, and PCB 138, which was not found in the Kerkini and Prespa samples. The median concentrations of five of the congeners (IUPAC 8, 20, 52, 138 and 180) differed significantly among the areas (Table 3), and the proportion of the eight individual congeners varied between cormorant eggs from each area, resulting in different fingerprints (Fig. 2). Median concentrations of PCB 8, 20, 52 and 180 were highest in the samples from Lake Prespa; PCB 138 was highest in the Axios Delta samples. Maximum levels of the congeners 8, 20 and 118 were found in the samples from Prespa Lake, congeners 28 and 180 from Kerkini Lake, and congeners 138 and 52 from the Evros and Axios Deltas, respectively. The median totals of the eight PCB congeners were highly significant between the areas (Table 1); the highest were in the Prespa samples (68.43 ppb) and the lowest were in the Evros samples (12.17 ppb), being intermediate in the other areas.

A cluster analysis of percentage levels of each

Table 3
Organochlorine and PCB concentrations (ppb wet wt.) in cormorant eggs from Greek wetlands (means are given for comparisons with the literature)

	Evros Delta ($N = 38$)				Kerkini ($N = 15$)				Axios Delta ($N = 12$)				Prespa ($N = 12$)				Statistics	
	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	K–Wallis χ^2	P
PCBs																		
PCB8	1.16	0.94	0.00	5.08	0.13	0.00	0.00	1.84	0.76	0.00	0.00	8.90	5.60	4.33	0.00	14.14	30.083	< 0.0001
PCB20	0.67	0.64	0.00	1.61	0.39	0.42	0.00	0.94	1.09	0.66	0.00	6.22	3.67	3.45	2.64	6.24	14.973	0.002
PCB28	2.96	2.12	0.00	18.01	11.61	3.34	1.34	124.19	8.37	2.82	0.80	58.20	40.60	32.72	0.00	115.68	2.938	0.401, n.s.
PCB52	1.74	1.29	0.00	9.74	3.98	1.43	0.88	36.74	5.13	1.80	0.65	37.07	4.02	3.37	0.93	9.45	11.020	0.015
PCB101	4.20	2.43	0.00	16.07	5.49	3.41	0.00	21.85	3.03	2.38	0.00	12.29	6.89	1.42	0.00	25.20	0.159	0.984, n.s.
PCB118	0.75	0.00	0.00	10.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.42	0.00	0.00	57.92	4.294	0.231, n.s.
PCB138	2.48	0.00	0.00	65.39	0.00	0.00	0.00	0.00	22.96	24.40	4.95	40.83	0.00	0.00	0.00	0.00	59.384	< 0.0001
PCB180	2.29	1.64	0.40	15.65	14.24	2.83	0.79	114.28	6.65	6.75	0.85	13.52	12.35	9.68	2.72	36.30	24.251	< 0.0001
ΣPCBs	16.24	12.17	3.40	109.00	35.85	15.10	5.36	171.62	47.99	41.13	9.43	148.41	79.56	68.43	11.58	172.52	20.994	0.0001
Organochlorines																		
α-BHC	0.05	0.00	0.00	0.41	0.14	0.09	0.00	0.51	0.65	0.34	0.00	2.81	0.38	0.11	0.00	1.20	22.043	0.0001
β-BHC	26.49	21.27	5.66	67.45	75.05	46.21	14.09	429.76	54.97	39.46	12.24	144.60	142.98	78.52	53.14	538.46	24.517	< 0.0001
Lindane	0.65	0.00	0.00	6.07	0.00	0.00	0.00	0.00	0.42	0.00	0.00	1.94	4.76	2.14	0.00	26.50	11.209	0.011
Heptachlor	0.40	0.30	0.03	2.65	0.40	0.41	0.11	0.78	0.27	0.26	0.00	0.58	0.95	1.00	0.11	1.34	18.081	0.0004
Heptachlor epoxide	8.68	6.15	0.57	29.71	4.21	3.56	1.35	8.72	7.77	5.59	0.81	17.57	3.81	3.24	0.00	7.96	4.672	0.197, n.s.
Aldrin	0.10	0.00	0.00	1.04	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.09	0.26	0.00	0.00	1.51	6.457	0.091
4,4′-DDE	0.42	0.14	0.00	4.02	0.00	0.00	0.00	0.04	1.41	1.02	0.00	4.98	0.00	0.00	0.00	0.00	31.932	< 0.0001
2,4′-DDD	111.16	84.76	8.58	336.11	115.58	93.50	45.18	438.87	148.15	66.28	33.69	825.75	122.59	115.47	24.05	217.40	4.801	0.186, n.s.
2,4′-DDT	0.29	0.00	0.00	5.18	1.00	0.00	0.00	8.18	0.86	0.00	0.00	5.14	0.00	0.00	0.00	0.00	4.536	0.209, n.s.
4,4′-DDD	0.31	0.00	0.00	9.28	0.43	0.00	0.00	5.84	0.58	0.00	0.00	6.97	0.70	0.00	0.00	8.34	0.989	0.804, n.s.
4,4′-DDT	2.98	2.92	0.00	9.05	3.33	3.51	0.37	5.25	3.20	3.67	0.00	5.48	16.55	15.19	11.96	25.79	19.127	0.0003
ΣOCL/ ΣPCBs	12.75	11.31	1.65	34.98	10.91	10.01	1.69	37.10	4.75	3.28	2.41	14.20	6.69	3.46	0.77	23.28	15.712	0.0013

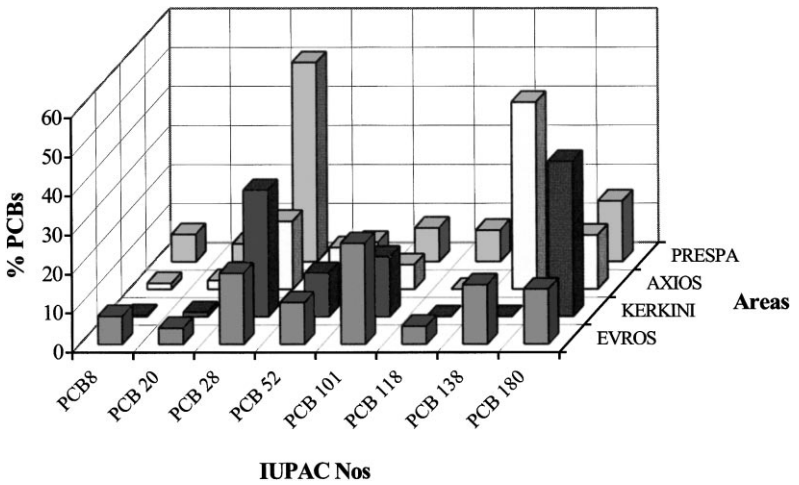

Fig. 2. Fingerprint of PCB congeners detected in cormorant eggs in four wetlands areas in Greece.

congener in the whole sample from each area provided two distinct groups: in one, the Axios Delta was clearly separated from the other areas, and in the other, the two lakes were associated separately from the Evros Delta (Fig. 3). Hence, a great overall dissimilarity was found between the PCB pollution patterns of the river deltas, and a similarity was found between the lakes.

Spearman Rank Correlation Coefficients between congener concentrations 28 and 52 were significant in all areas. For Kerkini Lake samples, it was the only significant correlation found. For Evros Delta samples, significant correlations were also found between congeners 20 and 138; in Axios Delta samples, between 8 and 38, and 138 and 180. In Prespa Lake samples significant correlations were also found between 8 and 20, 20 and 52, 20 and 180, and 52 and 180 (Appendices A and B).

3.2. Organochlorine pesticides

Of the thirteen compounds analyzed in cormorant eggs, two, namely dieldrin and endrin, were below detection limits in all of the study areas. Lindane and aldrin were not detected in samples from Kerkini Lake, and 4,4′-DDE and 2,4′-DDT in samples from Prespa Lake. Out of eleven compounds detected, the median concentrations of organochlorines were significantly different in six (α-BHC, β-BHC, lindane, heptachlor, 4,4′-DDE, and 4,4′-DDT). The highest median values of β-BHC, lindane, heptachlor and 4,4′-DDT were found in samples from Prespa Lake, and of α-BHC and 4,4′-DDE in samples from the Axios Delta. Maximum concentrations of β-BHC, lindane, aldrin and 2,4′-DDT were detected in Prespa samples, α-BHC, 4,4′-DDE and 2,4′-DDD in samples from Axios, heptachlor, heptachlor epoxide and 4,4′-DDD for Evros samples, and only 2,4′-DDT in Kerkini samples.

In all of the wetlands studied, the relative proportions of β-BHC and 2,4′-DDD predominated over all other compounds, resulting in characteristically similar fingerprints of OCs (Fig. 4). Only low levels of lindane and α-BHC were found in the samples, which is the reverse of the trend found among other environmental samples such as sewage sludge, mussels and bream. In all areas, the median concentrations of heptachlor epoxide were higher than those of heptachlor, indicating a gradual transformation of the latter.

A cluster analysis of percentage levels for each organochlorine compound in the whole sample from each area indicated two separate groups: one including only Prespa Lake, and another where the two delta areas were associated between them, and with Kerkini Lake (Fig. 5). In

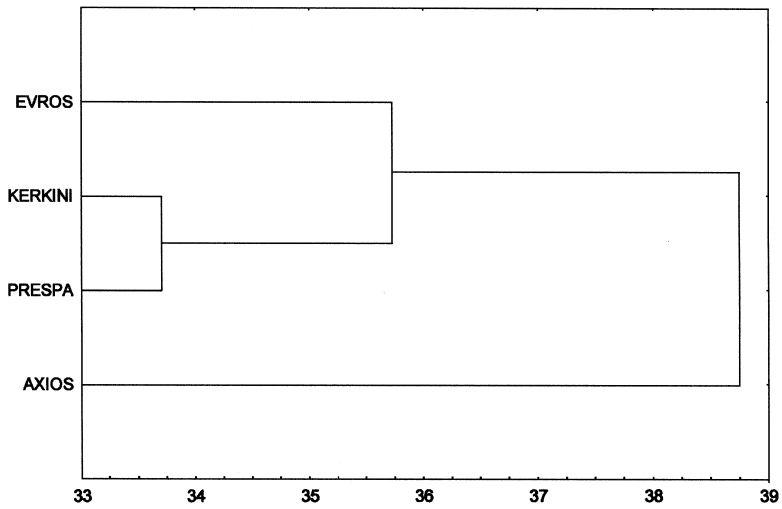

Fig. 3. Cluster indicating the relationship of areas studied in % levels of PCB congener in cormorant eggs. Euclidean distances as distance measure and single linkage as a linkage rule were used.

this analysis pattern, the river deltas were grouped more closely than lakes, which probably reflected differences in the development of agriculture of the studied areas.

Statistically significant Spearman rank correlations between compound concentrations varied greatly among the study area samples, without any uniform pattern occurring. In Evros and Prespa samples, heptachlor showed a significantly positive correlation with heptachlor epoxide. For DDT metabolites, significant correlations were only found between 4,4′-DDE and 2,4′-DDT (Evros, Prespa) and 4,4′-DDE and 4,4′-DDT (Evros). A statistically significant relationship was found between the analyzed compounds, but a different pattern was found for each area.

In addition to a correlation between PCB congeners and between OC compounds, significantly positive correlations were found between ΣPCBs and ΣOCs in three out of four areas studied (Evros: $r_s = 0.376$, $P = 0.020$; Kerkini: $r_s = 0.696$, $P = 0.004$; Axios: $r_s = 0.636$, $P = 0.026$) and

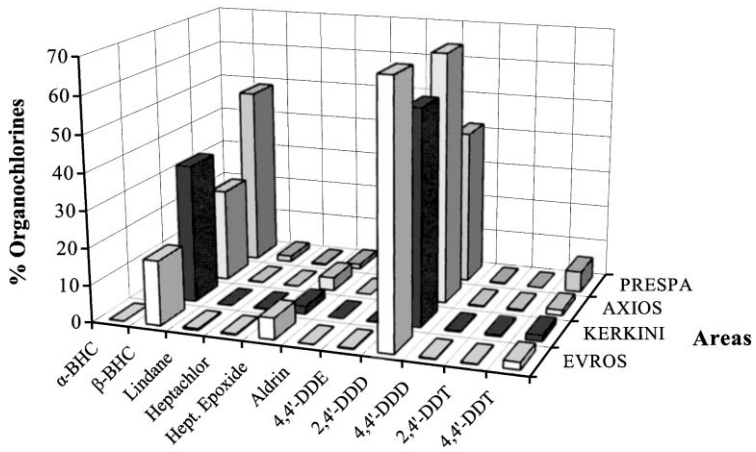

Fig. 4. Fingerprint of organochlorine pesticides detected in cormorant eggs in four wetlands areas in Greece.

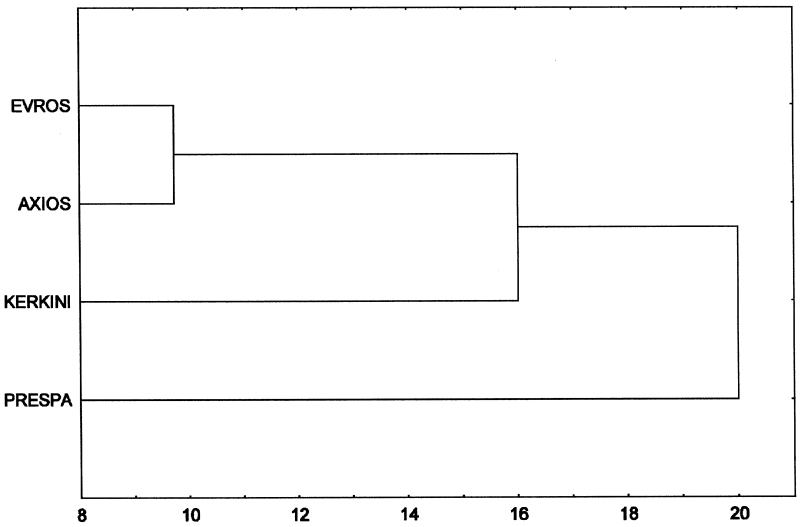

Fig. 5. Cluster indicating the relationship of areas studied in % levels of organochlorine pesticides in cormorant eggs. Euclidean distances as distance measure and single linkage as a linkage rule were used.

between ΣPCB and 4,4′-DDE in the Axios Delta ($r_s = 0.804$, 0.002).

The medians of the ratio ΣOCs/ΣPCBs differed significantly between the areas studied (Table 1), being much higher in samples from Evros and Kerkini than those from Axios and Prespa suggesting: (a) a higher agrochemical pollution in the first two wetlands; and (b) agrochemical dominating over industrial pollution in all areas.

4. Discussion

4.1. Polychlorinated biphenyls

Of the PCBs detected in our samples, congeners 138 and 180 were the most elevated, at least in some areas. These compounds were also found in high concentrations in the eggs of the *Phalacrocorax* species (Van den Berg et al., 1994; Allen and Thompson, 1996; Mason et al., 1997), their livers (Scharenberg, 1991; Platteeuw et al., 1995; Guruge and Tanabe, 1997), and in the eggs of other waterbirds in the Mediterranean, with levels depending on species (Focardi et al., 1988; Pastor et al., 1995a,b). The concentrations of most mono- and di-*ortho* coplanar PCBs (i.e. 28, 118, 138, 180) were higher than non-*ortho*-substituted congeners in different examined specimens (Hong et al., 1998). These congeners have a particular biological interest, exhibiting a considerable bioaccumulation starting at low trophic levels (Gagnon et al., 1990; Pruel et al., 1993; Scrimshaw and Lester, 1995), are persistent in the environment and toxic (Safe, 1990; Hong et al., 1998). The total PCB content of 100 μg/g in lipid was found to produce reproductive impairment in the livers of cormorants (Zimmerman et al., 1997). Significant embryo mortality in cormorants was associated with colony means exceeding 300–350 pg/g TEQs (dioxin equivalents) (Hong et al., 1998; Custer et al., 1999). Their bioaccumulative properties are due to their structure, characterized by the presence of chlorine atoms in the *ortho*, *meta* and *para* positions of at least one biphenyl ring (2,4,5 substitution pattern) (Gagnon et al., 1990; Metcalfe and Metcalfe, 1997).

Elevated concentrations of the median totals of the eight PCB congeners in Prespa samples was a rather surprising finding, due to the remoteness of the area and the lack of industrialization, at least in the Greek part. There has probably been a transboundary pollution from the neighboring countries (as is supposed to has occurred in the case of DDT in the mid-1980s; Crivelli et al.,

1989). Levels of congeners such as 101, 118, 138, 180 that had been measured in the eggs of Dalmatian Pelicans, with similar diets, were much higher in the mid-1980s (Crivelli et al., 1989), indicating that although PCBs still persist, they have diminished in the Prespa area. The pollution by PCBs of the Axios Delta and nearby coasts has been found to be transboundary (through the Axios River from the former Yugoslavia) and of municipal origin (Larsen and Fytianos, 1989).

In each area the concentrations of individual congeners varied between cormorant eggs. The uptake of PCB residues by cormorants, and the consequent differences between geographical areas, have been greatly attributed to feeding habits (Scharenberg, 1991; Dirksen et al., 1995; Platteeuw et al., 1995; Boudewijn and Dirksen, 1997; Guruge et al., 1997; Hoshi et al., 1998). Thus, in examining this assertion, below we take into an account the diet of cormorants in our study areas.

At Prespa, the cormorants' diet consisted mainly of *Rutilus orhidanus prespensis*: (Prespa road) (our unpublished data), an omnivorous fish species. In the egg samples from this area, congener 28 predominated over all other PCBs. This congener, like other low PCB congeners (di- tri- and tetra-chlorinated) was found to predominate in surface water, plankton and fish in non-point source areas (Tanabe et al., 1984; Hoshi et al., 1998). The relative fish species *Rutilus erythrophthalmus*: (red eye roach), was found to bioaccumulate in PCBs at a high rate (approx. 10^6), and tri-PCBs (28 and 31) were among the congeners found in its tissues at a concentration level of 40 μg/kg (Bazzanti et al., 1997). There is evidence of a species-specific bioconcentration factor of individual PCB congeners in fish (Gagnon et al., 1990) and a close correlation of PCB contents and their lipids (Harding et al., 1997). Thus, elevated concentrations of PCB 28 in Prespa were probably due to the particular prey type(s) consumed by cormorants. However, as few cormorants spent the winter in Prespa (Catsadorakis 1997) PCB 28 may have partly been accumulated by females in their wintering grounds and, being a relatively persistent congener, was then excreted via eggs caused by the females' detoxification system (Guruge and Tanabe, 1997). Detoxification mechanisms may also account for the relatively high levels of other congeners of PCB 28 in the other wetlands.

In the Axios Delta, the cormorant diet during breeding consisted mainly of small-sized gobiid *Gobius jozo*, living near the bottom and mugilids, encountered in the entire water column (Goutner et al., 1997a). High molecular weight congeners tend to accumulate in river and estuarine sediments (Gagnon et al., 1990; Bazzanti et al., 1997), which probably explains the predominance of PCB 138 in the Axios Delta egg samples, but a relatively elevated percentage (17.4%) of PCB 28 may also reflect the variability of the diet found in this estuarine system (Goutner et al., 1997a).

At Kerkini Lake, the predominant diet is the surface living *Alburnus alburnus*: (bleak) (T. Nazirides, personal communication), which may explain the absence of some higher chlorinated congeners and the occurrence of some low chlorinated congeners. In the Evros Delta, the diet of the cormorants is unknown. The fingerprint of PCBs suggests that the cormorants probably fed on a variety of fish, largely from surface living species. Conclusively, if cormorants accumulated PCBs from sources in their breeding grounds in Greece, differences in the composition and levels of PCBs between the four areas studied were probably due to dietary differences of the populations. The different historical patterns of management regimes of PCBs in the areas studied may have lead to different patterns of pollution of biota and cormorants' prey, reflected in eggs as PCB pollution dissimilarities between river deltas and lakes.

In this study, we measured the levels of eight PCB congeners of which five (Nos 28, 52, 101, 138, 180) are called 'target' or 'indicator' PCBs (Scrimshaw and Lester, 1995; Bachour et al., 1998, respectively). Cogener no. 153 is also included in this list, but we did not analyze it, due to a lack of a standard. There is a difficulty in readily comparing PCB levels in eggs or other biological material between studies, due to the different number of congeners analyzed in each study, as well as different analytical procedures and different presentation of results. Dirksen et al. (1995) multiplied

by two the sum of the six 'target' PCB concentrations, assuming that they accounted for approximately 50% of the total PCB content in cormorant eggs. To compare PCB levels found in this study, we multiplied also by two the sum of the PCB concentrations, according to the above mentioned study (Dirksen et al., 1995). Even if the highest mean (or median) total values are multiplied by two, the concentrations are still low at 159 (136) ppb. Dirksen et al. (1995), summarizing the results of studies on the levels of total PCBs in the eggs of five species of cormorants collected in the 1970s and 1980s, indicated that with the exception of a mean of 0.1 ppm (wet weight) found at St. Martin's Shoal (north-west Lake Huron, Tillitt et al., 1992) the levels in all other 31 sites cited were between 0.5 ppm (SW Ireland) and 71.1 ppm in Naardermeer (The Netherlands). Mean ∑PCBs levels associated with adverse effects to hatching and the breeding success of cormorants were 16–21 ppm (the Netherlands, Dirksen et al., 1995). Mean levels of 23.8 ppm in Great Lakes' double-crested cormorants similarly resulted in reproductive failure (Weseloh et al., 1983). In the same region and in the same species, total PCB means of 3.6–7.3 ppm were associated with live deformities (hard tissue malformations, Yamashita et al., 1993); whereas in Green Bay, USA, total PCB means of 13.6 ppm were not associated to reduced hatching success and the frequency of deformities (Custer et al., 1999). Conclusively, the concentrations of PCBs found in cormorants in our study of the range 3–172 ppb wet wt., are of the lowest ever reported, and they cannot be associated with adverse effects in the biology of this bird (Table 4).

4.2. Organochlorine pesticides

Although organochlorine pesticides (with the exception of lindane) were banned in Greece in 1972 (Albanis et al., 1994), most of them were detected in cormorant egg samples in our areas of study, although at low levels. The occurrence of some of these contaminants in other samples from Greek wetlands varies considerably. Thus, residues of drins (endrin, dieldrin, aldrin) were not found in Dalmatian pelican eggs at Amvrakikos (Albanis et al., 1995), and, as in previous studies, in the Axios Delta, water and sediments (Albanis et al., 1994). In the latter area, drins were also absent from little egret (*Egretta garzetta*) night heron (*Nycticorax nycticorax*) and squacco heron (*Ardeola ralloides*) nestlings and their main food (frogs), but endrin and dieldrin were found in the eggs of little terns (*Sterna albifrons*), and aldrin was found in squacco heron eggs, probably originating from their wintering grounds (Albanis et al., 1996; Goutner et al., 1997b). Pollutants found in cormorant eggs may also accumulate in their wintering grounds (Sommers et al., 1993). The presence of aldrin in some of the cormorant eggs (Evros: in 21.0% of the eggs; Axios: 8.3%; Prespa 33.3%) implies that it may have been accumulated as a part of their breeding population in wintering areas outside of Greece. The relationship of the Greek populations with others is supported by ringing recoveries of cormorants originating from northern and central Europe, but also from other Balkan countries and the Ukraine. In the last mentioned area, the levels of pp′DDE were 57.2 ppm in cormorant eggs, 18.5 ppm in pygmy cormorant (*Phalacrocorax*

Table 4
Comparison of the PCB mean levels of cormorant eggs in this study to those of other studies with known adverse effects to cormorants

Study	Mean total PCBs (ppb)	Species	Area	Effect
This study	32–159	*P. carbo*	Greek wetlands	No effect
Dirksen et al. (1995)	1600–2100	*P. carbo*	Dutch rivers	Reproductive failure
Yamashita et al. (1993)	3600–7300	*P. auritus*	Canadian Great Lakes	Hard tissue malformations
Weseloh et al. (1983)	23 800	*P. auritus*	Canadian Great Lakes	Reproductive failure

pygmeus) eggs, and 15.4 ppm in white pelican (*Pelecanus onocrotalus*) eggs, expressed in dry weight (Fossi et al., 1984).

The higher levels of β-BHC in our egg samples can be explained by the relatively high stability of this compound against metabolism, and points to a chronic rather than an acute contamination (Oxynos et al., 1993). Moreover, the elevated amounts of β-BHC, a lindane metabolite, reflects that lindane is still used as a seed and soil insecticide in various cultivations (Albanis, 1997). Lindane levels were lower than those reported in pelecaniforms in other studies but β-BHC levels (where analyzed) were higher (Fossi et al., 1984; Albanis et al., 1996; Allen and Thompson, 1996). A similar picture occurred with squacco heron eggs in the Axios Delta (Albanis et al., 1996) and little egrets in Italy (Fasola et al., 1998). In these studies the levels of β-BHC were higher that those of cormorant eggs in Greece. The elevated amounts of 2,4′-DDD, in comparison to 4,4′-DDE, was probably due to its presence in zooplankton and in the water column. In zooplankton, the concentrations of 2,4′-DDD were comparable with the concentrations of 4,4′-DDE. Zooplankton possibly acquired DDTs from sediments or from the water column, where the DDD form constitute the major fraction of DDTs (Strandberg et al., 1998).

In a variety of cormorant species, or in the eggs of other waterbirds where DDT metabolites were analyzed, the dominant metabolite was DDE (i.e. Blus et al., 1974; Ohlendorf et al., 1979; Yamashita et al., 1993). In other studies, DDT metabolites are frequently grouped together (as ∑DDTs), making comparisons on each metabolite level with this study impossible. The special interest in detecting DDE in cormorant eggs is associated to the well-established inverse relationship between the DDE content and the shell thickness having consequences on the breeding productivity of populations (Dirksen et al., 1995; Ludwig et al., 1995; Custer et al., 1999). In the cormorant, levels measured in the Netherlands were high (means 2.2–8.3 ppm), with 5% eggshell thinning occurring at levels of 4 ppm (Dirksen et al., 1995). Mason et al. (1997) found a significantly ($P < 0.05$) negative relationship between DDE and shell thickness with mean levels of only 0.183 ppm. A long-term study on the double-crested cormorant indicated that a shell thickness between 0 and 3.9% was associated with DDE levels of 2.4–2.8 ppm, posing no threat to reproduction in birds (Ryckman et al., 1998). The levels of DDE found in Dalmatian pelican eggs in Prespa in the mid-1980s (means of 14.183–21.653 ppb) were too high, resulting in an egg thinning of −12 to −20% (Crivelli et al., 1989). In the well studied double-crested cormorant, means of 3.9 ppm were found at Green bay, Lake Michigan, WI, USA (Custer et al., 1999), and a mean of 14.5 ppm was measured at Lake Huron colonies, Canada (Weseloh et al., 1983). These were associated with eggshell thinning, whereas means as low as 0.59–1.6 ppm had no effects (Henny et al., 1982, 1989). No related effects were found associated with means of 0.48 ppm in *P. pelagicus* (Henny et al., 1982) or with means of 0.67–1.73 ppm in *P. olivaceus* (King and Krynitsky, 1986). Concentrations higher than those found in cormorants in this study, were detected in Dalmatian pelican eggs in the Amvrakikos wetlands (mean of 5.42 ppb; Albanis et al., 1995) and in squacco heron and little tern eggs in the Axios Delta (3.8 and 2.79 ppb, respectively) without affecting the eggshell thickness (Albanis et al., 1996; Goutner et al., 1997a,b). Thus, despite the persistence of DDT metabolites (in particular 2,4′-DDD) in the Greek wetlands we studied, the very low concentrations of DDE, and its absence in two out of four wetlands, seems to be a consequence of the ban of DDT in Greece in the 1970s and poses no threat to cormorants in terms of eggshell thickness.

The levels of heptachlor epoxide were much lower than those found in the olivaceous cormorant (King and Krynitsky, 1986) and in double crested cormorant eggs (Vermeer and Reynolds, 1970; Weseloh et al., 1983; Custer et al., 1999).

Cluster analyses indicates different pollution patterns in deltaic areas and lakes with regard to OCs; probably reflecting in part, as in the case of PCBs, the past use of these pollutants in Greece, but the interpretation of this picture is far from being simple due to the fact that all the wetlands studied are partly polluted by transboundary pol-

lution. These pollution patterns may also reflect the differences in the prey use of cormorants in these two wetland types.

Correlations between organochlorines have also been found in other studies, and frequently are the reason of obscuring the biological effects of each compound or congener (i.e. Blus et al., 1974; Mason et al., 1997; Custer et al., 1999).

Our results indicate that agrochemical sources dominated over industrial pollution in all areas and are in agreement with other studies, indicating that, in contrast to the western Mediterranean, the eastern Mediterranean–Black Sea areas are mostly agrochemically, rather than industrially, polluted (Fossi et al., 1984; Focardi et al., 1988; Crivelli et al., 1989; Pastor et al., 1995b).

5. Conclusions

The findings of this study show that although a considerable number of organochlorines still persist in Greek deltaic areas and lakes, their levels are low. This has important implications. The pollutant levels are not expected to have any adverse effect on the breeding biology of cormorants. No eggshell thinning or deformities are expected. These are important for the future long-term conservation of the populations of the Eurasian race of cormorant. In our study areas, the low pollutant levels detected do not seem to pose a potential hazard to other fish-eating birds or other biota, such as fish. For organochlorines, the environment of these wetlands seems to be safe for humans.

Against our expectations, Prespa Lake was the area most polluted by PCBs and some OCs. It would be worth identifying the sources of this pollution, due to the international importance of this area. Agrochemical pollution and the use of lindane should be controlled in all of the wetlands.

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Appendix A Spearman Rank Correlation Coefficients (lower part of tables) and statistically significant values (higher part of tables) of PCB congener concentrations in areas studied

Evros	PCB8	PCB20	PBB28	PCB52	PCB101	PCB118	PCB138	PCB180
PCB8		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PCB20	0.157		n.s.	n.s.	n.s.	n.s.	0.055	n.s.
PBB28	0.160	−0.092		0.001	n.s.	n.s.	n.s.	n.s.
PCB52	0.205	0.011	0.502		n.s.	n.s.	n.s.	n.s.
PCB101	−0.088	−0.044	0.131	0.052		n.s.	n.s.	n.s.
PCB118	0.045	−0.269	−0.118	−0.068	−0.018		n.s.	n.s.
PCB138	−0.105	0.314	0.036	−0.064	0.170	0.221		n.s.
PCB180	0.016	0.255	0.218	0.099	−0.174	0.039	0.093	

Kerkini	PCB8	PCB20	PBB28	PCB52	PCB101	PCB180
PCB8		n.s.	n.s.	n.s.	n.s.	n.s.
PCB20	0.206		n.s.	n.s.	n.s.	n.s.
PBB28	−0.006	−0.068		0.050	n.s.	n.s.
PCB52	0.199	−0.168	0.514		n.s.	n.s.
PCB101	−0.127	−0.115	−0.459	−0.375		n.s.
PCB180	−0.157	−0.048	0.361	0.214	0.286	

Appendix A (*Continued*)

Axios

	PCB8	PCB20	PBB28	PCB52	PCB101	PCB138	PCB180
PCB8		n.s.	n.s.	n.s.	n.s.	0.011	n.s.
PCB20	−0.252		n.s.	n.s.	n.s.	n.s.	n.s.
PBB28	0.055	0.046		< 0.0001	n.s.	n.s.	n.s.
PCB52	−0.021	−0.042	0.700		n.s.	n.s.	n.s.
PCB101	−0.346	0.012	0.037	−0.146			n.s.
PCB138	−0.501	0.233	0.051	0.193	0.087		< 0.0001
PCB180	−0.124	0.186	0.122	0.191	−0.267	0.683	

Prespa

	PCB8	PCB20	PBB28	PCB52	PCB101	PCB118	PCB138	PCB180
PCB8		0.016	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PCB20	0.477		n.s.	0.017	n.s.	n.s.	n.s.	< 0.0001
PBB28	0.197	0.111		0.019	n.s.	n.s.	n.s.	n.s.
PCB52	0.373	0.473	0.465		n.s.	n.s.	n.s.	0.019
PCB101	0.034	−0.018	−0.201	−0.069		n.s.	n.s.	n.s.
PCB118	0.025	0.302	0.026	0.126	−0.023		n.s.	n.s.
PCB138	−0.353	−0.062	−0.188	−0.304	0.088	−0.087		n.s.
PCB180	0.361	0.797	0.113	0.467	−0.097	0.384	−0.309	

Appendix B Spearman Rank Correlation Coefficients (lower part of tables) and statistically significant values (upper part of tables) of organochlorine pesticide concentrations in areas studied

Evros	α-BHC	β-BHC	Lindane	Heptachlor	Aldrin	Heptachlor epoxide	4,4′-DDE	2,4′-DDD	2,4′-DDT	4,4′-DDD	4,4′-DDT
α-BHC		n.s.	n.s.	n.s.	n.s.	n.s.	< 0.0001	n.s.	0.001	n.s.	n.s.
β-BHC	0.216		0.008	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lindane	−0.150	−0.423		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.012
Heptachlor	0.081	0.196	0.128		n.s.	0.0001	n.s.	n.s.	n.s.	n.s.	n.s.
Aldrin	0.006	−0.109	0.095	−0.170		n.s.	n.s.	n.s.	n.s.	0.002	n.s.
Heptachlor epoxide	0.023	−0.001	0.163	0.577	0.025		n.s.	n.s.	n.s.	n.s.	n.s.
4,4′-DDE	0.530	−0.138	0.070	0.114	0.139	0.145		n.s.	0.005	n.s.	n.s.
2,4′-DDD	0.240	0.227	0.009	0.306	−0.168	0.072	0.020		n.s.	n.s.	n.s.
2,4′-DDT	0.506	−0.065	−0.216	−0.130	0.081	−0.154	0.447	0.217		n.s.	n.s.
4,4′-DDD	0.165	0.124	0.151	0.195	0.484	0.301	0.181	0.004	−0.069		n.s.
4,4′-DDT	−0.187	0.100	0.384	0.109	0.011	0.078	−0.164	0.201	−0.096	0.188	

Kerkini	α-BHC	β-BHC	Heptachlor	Heptachlor expoxide	4,4′-DDE	2,4′-DDD	2,4′-DDT	4,4′-DDD	4,4′-DDT
α-BHC		n.s.	n.s.	0.028	n.s.	n.s.	n.s.	n.s.	n.s.
β-BHC	−0.020		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.039
Heptachlor	−0.120	−0.186		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Heptachlor epoxide	0.564	0.196	0.466		n.s.	n.s.	n.s.	0.040	n.s.
4,4′-DDE	0.372	−0.124	0.031	0.247		n.s.	n.s.	n.s.	n.s.
2,4′-DDD	−0.030	0.350	−0.090	0.025	−0.309		n.s.	n.s.	n.s.
2,4′-DDT	0.390	0.163	−0.088	0.380	−0.105	0.048		n.s.	n.s.
4,4′-DDD	−0.351	0.193	−0.442	−0.537	−0.105	−0.006	−0.153		n.s.
4,4′-DDT	0.045	0.536	0.261	0.436	−0.247	0.482	0.459	0.042	

Axios	α-BHC	β-BHC	Lindane	Heptachlor	Aldrin	Heptachlor epoxide	4,4′-DDE	2,4′-DDD	2,4′-DDT	4,4′-DDD	4,4′-DDT
α-BHC		0.002	n.s.	n.s.	n.s.	n.s.	< 0.0001	n.s.	n.s.	n.s.	n.s.
β-BHC	0.592		n.s.	n.s.	n.s.	0.011	0.055	n.s.	n.s.	n.s.	0.007
Lindane	−0.152	−0.147		n.s.	n.s.	0.045	n.s.	n.s.	n.s.	n.s.	n.s.
Heptachlor	−0.011	0.158	−0.252		n.s.	0.003	n.s.	n.s.	n.s.	n.s.	0.044
Aldrin	0.003	−0.108	−0.197	−0.092		n.s.	n.s.	n.s.	n.s.	0.022	n.s.
Heptachlor epoxide	0.212	0.502	−0.404	0.562	0.038	n.s.	n.s.	n.s.	n.s.	0.014	
4,4′-DDE	0.790	0.388	0.052	−0.140	−0.084	0.069		n.s.	n.s.	n.s.	n.s.
2,4′-DDD	−0.004	−0.089	−0.071	0.272	−0.008	0.076	0.199		n.s.	n.s.	n.s.
2,4′-DDT	0.161	−0.259	−0.332	−0.246	0.148	−0.147	0.272	0.015		n.s.	n.s.
4,4′-DDD	0.126	0.181	0.110	−0.070	0.455	−0.039	0.134	0.136	−0.146		n.s.
4,4′-DDT	0.385	0.526	−0.056	0.406	−0.302	0.485	0.207	−0.016	−0.030	−0.342	

Appendix B (*Continued*)

Prespa	α-BHC	β-BHC	Lindane	Heptachlor	Aldrin	Heptachlor epoxide	4,4′-DDE	2,4′-DDD	2,4′-DDT	4,4′-DDD	4,4′-DDT
α-BHC		n.s.	n.s.	n.s.	0.029	0.053	n.s.	n.s.	n.s.	n.s.	n.s.
β-BHC	0.208		n.s.	0.004	n.s.	n.s.	0.021	n.s.	n.s.	n.s.	< 0.0001
Lindane	−0.106	0.041		n.s.	n.s.	0.023	n.s.	n.s.	n.s.	n.s.	n.s.
Heptachlor	−0.225	0.560	0.139		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.003
Aldrin	0.436	0.340	−0.164	−0.034		n.s.	n.s.	n.s.	n.s.	0.004	n.s.
Heptachlor epoxide	0.391	−0.226	−0.452	−0.299	0.158		n.s.	n.s.	n.s.	n.s.	n.s.
4,4′-DDE	0.265	−0.457	−0.207	−0.308	−0.120	0.276		n.s.	0.005	n.s.	0.005
2,4′-DDD	−0.116	0.266	−0.276	0.252	0.149	−0.031	−0.039		n.s.	n.s.	n.s.
2,4′-DDT	0.146	−0.387	−0.247	−0.262	−0.146	0.114	0.541	0.065		n.s.	n.s
4,4′-DDD	0.356	0.046	−0.025	−0.016	0.557	0.194	0.104	−0.049	−0.087		n.s.
4,4′-DDT	0.158	0.861	0.244	0.577	0.226	−0.301	−0.544	0.294	−0.201	−0.114	

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