Journal of Biological Research-Thessaloniki **17**: 154 – 168, 2012
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

Organochlorine pesticide residue concentrations and accumulation patterns in waterbirds and in their prey at Lake Kerkini, a Ramsar wetland, Greece

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Received: 28 March 2011 Accepted after revision: 4 November 2011

The levels of 16 organochlorine pesticides (OCPs) were measured in egg samples of the great cormorant, great-crested grebe, grey heron, little egret, black-crowned night heron and in their prey being bleak, roach, pumpkinseed, goldfish and frogs from Lake Kerkini, NE Greece. Concentrations of most OCPs differed among most bird and prey species. DDTs predominated in all species, contributing to about 80% of the total OCPs in most sample types. Moving up the food chain, the relative contribution of ∑DDTs increased whereas of ∑HCHs and ∑Cycls (cyclodienes) decreased. Regarding trophic transfer, all OCPs exhibited bioconcentration but, in contrast, compounds such as γ-HCH, endrin, heptachlor, heptachlor hepoxide, α-, β-endosulfan and endosulfan sulfate showed low or no biomagnification. No correlations were found between either bioaccumulation (BAF) or biomagnification factors (BMF) and lipid content of biota. Nevertheless, there was a significant relationship between $\log K_{ow}$ (the octanol-water partition coefficient) and BAF of all OCPs. OCP bioaccumulation patterns at Lake Kerkini are probably governed by the chemical properties of compounds and their metabolic ways in biota. The levels of OCPs were too low to have adverse biological effects to the waterbirds. The great cormorant, pumpkinseed and *Rana* frogs are suggested as the most suitable bioindicators for monitoring OCPs at Lake Kerkini due to the highest concentrations of most compounds and showing the greatest bioaccumulative properties.

Key words: bioaccumulation, waterbirds, prey, lake Kerkini, Greece.

INTRODUCTION

Organochlorine pesticides (OCPs) have extensively been used in countries around the Mediterranean basin and in other parts of the world before their ban in the mid-seventies, enforced due to their high toxicity, environmental persistence and bioaccumulation properties (Harris *et al*., 2003; Sethajintanin *et al*., 2004; Corsi *et al*., 2005). The bioaccumulation of OCPs in aquatic wildlife may result from direct water intake and/or prey ingestion and seems related to the octanol-water partition coefficient (K_{ow}) of each compound (Galassi *et al*., 1996; Fisk *et al*., 1998; Mackay & Fraser, 2000), although the relationship between this process and K_{ow} is not straightforward (Swackhamer & Hites, 1988).

Fish-eating waterbirds such as cormorants and herons are top predators in aquatic environments. These birds uptake OCPs through their dietary paths, a fact that established them as suitable bioindicators for monitoring studies (Sanpera *et al*., 2003; Goutner *et al*., 2011). Concentrations of chemicals in eggs tend

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to reflect pollutant uptake by the female foraging close to the colony in the few days prior to egg laying (Muñoz Cifuentes *et al*., 2003; Becker & Muñoz Cifuentes, 2004). The choice of a suitable species is essential because its use as biomonitor in a part of its distribution may not be appropriate in another (Albanis *et al*., 2003). Various marine biota in north Mediterranean have been monitored for OCPs' pollution (Goutner *et al*., 2001; Di Muccio *et al*., 2002; Oliveira Ribeiro *et al*., 2005). Levels dropped gradually due to their banning (Crivelli *et al*., 1999) although the top predators are continuously exposed because of OCPs' environmental persistence (Storelli *et al*., 2005).

Monitoring of OCP residues have been carried out in some European freshwater systems but they are scarce at lakes (i.e. Scharenberg & Ebeling, 1998; Bordajandi *et al*., 2003; Fagotti *et al*., 2005). In Greece, OCP levels have occasionally been investigated in some lakes (Crivelli *et al*., 1999; Konstantinou *et al*., 2000; Golfinopoulos *et al*., 2003) whereas few studies dealing with OCPs' bioaccumulation and biomagnification in marine or lagoon environments were available (Albanis *et al*., 1995, 1996). The initiation of this study was motivated because Lake Kerkini is a wetland of international importance hosting big colonies of colonially nesting waterbirds, an artificial irrigation lake and fisheries for the local societies, and because it is exposed to transboundary pollution. The purposes of this paper are: a) to describe and compare OCP levels in waterbird eggs and in their prey at Lake Kerkini, b) to investigate OCP bioaccumulation patterns from water through prey to waterbird eggs, and c) to suggest the best bioindicator(s) of OCPs at Lake Kerkini.

MATERIALS AND METHODS

Study area

Lake Kerkini in N Greece (41°22′N, 22°13′E), is an artificial lake (with maximum surface 7300 ha and depth 35.5 m) along the Strymon River originating from Bulgaria and flowing into the N Aegean Sea. A forest along the river mouth in the lake (mainly by *Salix alba* and *Salix* hybrids) hosts colonies of pelecaniform and ardeid (herons and relative) species. Lake Kerkini, beside a wetland of international importance under the Ramsar Convention, is a Special Protected Area and Important Bird Area of Greece (Nazirides & Papageorgiou, 1996).

Field sampling

Egg samples were collected between end of March and end of April 2004, depending on the time of laying of each bird species. The levels of organochlorines in bird eggs reflect the diet of the female prior to egg lying and pollutant levels in body reserves, thus constituting a useful indicator of environmental contamination (Pearce *et al*., 1989; Muñoz Cifuentes *et al*., 2003) and residues in one egg in a clutch accurately reflect those in the whole clutch (Custer *et al*., 1990). Under license, one egg was randomly sampled from different nests of great cormorant (*Phalacrocorax carbo*), great-crested grebe (*Podiceps cristatus*), grey heron (*Ardea cinerea*), little egret (*Egretta garzetta*) and black-crowned night heron (*Nycticorax nycticorax*). Soon after collection, egg contents were preserved in chemically cleaned containers. The prey types of some these bird species (except grey heron and great-crested grebe) were mainly bleak (*Alburnus alburnus*), roach (*Rutilus rutilus*), pumpkinseed (*Lepomis gibbosus*), goldfish (*Carassius auratus*) and frogs (*Rana* sp.). Prey types were collected during the birds' egg laying period using an electrofishing device and professional nets (for methodological details, see Antoniadou *et al*., 2007). Water samples were collected during the egg collection period by boat, using a Ruttner sampler (1 L) along 15 stations, 150 m distant from each other in a straight line starting from the Strymon river mouth across a west direction in the lake. In each station, three water samples were taken (surface, column, bottom), homogenized in a container, and 1.5 L of the mix were placed in chemically cleaned glass bottles and transported to the laboratory where they were preserved in a fridge until analysis.

Materials and chemicals

All solvents used (hexane, acetone, dichloromethane, methanol, ethyl acetate), were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd, Dublin, Ireland). Alumina, copper, sodium sulphate (pro-analysis) and concentrated sulphuric acid 98%, were from Merck (Darmstadt, Germany). Octadecyl bonded silica (C18, 500 mg) solid-phase extraction (SPE) disks of 47 mm diameter and 0.5 mm thickness, were obtained from Empore™ (Saint Paul, MN, USA). Glassware was soaked, cleaned with chromic solution, thoroughly rinsed with distilled water and acetone and heated at 150°C for 12 hrs. Aluminum foil was rinsed with acetone and dried at ambient temperature prior to use. Cellulose extraction thim-

bles of 35 mm i.d. and 100 mm long were from Whatman (Maidstone, England). Sodium sulphate and thimbles were precleaned by Soxhlet extraction with hexane:dichloromethane (3:1 v/v) for 3 hrs before use.

The organochlorine pesticides analysed in this study were α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, heptachlor, α-endosulfan, β-endosulfan and endosulfan sulphate, γ- chlordane, heptachlor epoxide, aldrin, dieldrin, endrin, *p,p′*-DDT, *p,p′*-DDD and *p,p′*- DDE, purchased from Supelco (Bellefonte, PA, USA).

Analytical Procedures

Extraction of water samples

Extraction of pesticides was performed on unfiltered samples based on (Albanis *et al*., 1998) and, in brief, involved adding methanol modifier (10 ml) to 1 L water samples, percolation through acetone and methanol cleaned C18 disks at a flow-rate of 50 ml min^{-1} under vacuum and collection of pesticides with dichloromethane – ethyl acetate (1:1, v/v). The fractions were evaporated to 0.5 ml under a gentle stream of nitrogen prior to GC injections.

Extraction of egg samples

An aliquot of 5-10 g homogenized egg contents were mixed with anhydrous sodium sulfate and extracted with a hexane:dichloromethane (3:1, v/v) mixture in sonication bath, centrifugation in 50 ml tubes at 4000 rpm for 5 min and evaporation a rotary evaporator to 10 ml. Lipids were then removed by treating the extracts with aliquots of concentrated sulfuric acid until the organic layer remained colourless.

Extraction of fish and frog tissues

Homogenates of 10-15 g from individual fish and frogs or pooled composites of multiple items (of approximately equal tissue mass) were blended with anhydrous sodium sulfate to obtain a fine powder. OCPs from mixtures were then Soxhlet extracted with n-hexane:dichloromethane (3:1, v/v) for 16 hrs. Lipid content was determined gravimetrically using about 20% of the extract. Concentration and lipid removal followed the procedure mentioned above for the eggs.

All extracts were dried, reconstituted in hexane and the clean-up was completed by adsorption chromatography, eluting the colourless layer through short chromatography columns prepared in plastic syringes (10 ml) fitted with glass wool (Singh *et al*., 1998). Solvent blanks analysed for OCPs cross contamination showed the absence of co-eluted organochlorines. The syringes were layered with 5 cm alumina (5% water-deactivated) followed by 1 cm acid-activated copper for the removal of any residual elemental sulphur in the extracts (Wainwright *et al*., 2001) and dried sodium sulphate. The column was washed with 50 ml of the extraction solvent mixture. The purified samples were evaporated in a rotary evaporator to ~5 ml and in gentle N_2 stream at 35°C to ~0.5 ml, then samples were stored in silanized vials in a refrigerator (4°C).

All of the above mentioned extraction methods are described in more detail in Antoniadou *et al.* (2007).

Chromatographic analysis and quality assurance

Samples were analysed with a Shimadzu 2010 gas chromatograph equipped with ^{63}Ni electron capture detector (ECD). Separation was achieved with a DB-5 (30 m × 0.25 mm i.d., J & W Scientific, Folsom, CA) capillary column. The column oven temperature was programmed as follows: 150°C (4 min), 150-260°C (5°C min^{-1}), 260°C (15 min), 260-290°C (10°C min^{-1}), 290°C (10 min). The temperatures were set at 240°C for the injector and 300°C for the detector. Helium was used as carrier at a flow of 1.5 ml min^{-1} and nitrogen was used as make-up gas at a flow of 35 ml min^{-1}. Injections (1 μl) were performed using a Shimadzu AOC – 20i auto injector in splitless mode with the valve open for 30 sec. Quantification of OCPs was performed using internal standard (tetrachloro-m-xylene). Compounds were positively identified if the relative retention time (*versus* the internal standard) differed no more than 0.05 from that of the calibration standards. Procedural blanks were also analysed for every set of 10 samples in order to identify any contamination throughout the analytical procedure. No background interference was found to be introduced by the proposed methodology. Recoveries of spiked OCPs into samples and passed through the analytical procedure were between 92 and 108% (RSD < 10%). In order to calculate recovery efficiencies, peak areas of OCPs from the non-spiked samples were subtracted from the corresponding peak areas in the spiked samples. Both spiked and non-spiked samples were analyzed in triplicate. The mean relative recoveries of the surrogate standard (PCB 209) were 89%, 93% and 87% for fish, frog and waterbird egg samples respectively, with RSD values ($n = 3$) < 8.5%.

Reported concentrations were not corrected for the recoveries of surrogate standard. Peaks less than three times the noise level were considered below the detection limit. Detection limits ranged from 0.3 to 0.5 ng g^{-1} and from 0.05 to 0.1 ng g^{-1} for egg and fish or frog samples respectively, on a wet weight basis. Secondary confirmation was performed on representative samples using a GC-MS, QP 5000 Shimadzu equipped with DB-5 MS capillary column, 30 m × 0.25 mm i.d. contained (5% phenyl) methyl polysiloxane (J & W Scientific, Folsom, CA), following the previous oven temperature programme. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The injector and interface temperatures were 240°C and 290°C respectively. The spectra were obtained at 70 eV. The splitless mode was used for injection of 1 μl volume, with the valve opened for 30 sec. Two cluster of ions M^+ and $(M+2)^+$ for each pesticide were chosen for screening analysis in selected ion monitoring mode (SIM). Detection limits typically ranged from 0.1 to 1 ng g^{-1} (ww).

Data analysis and statistical procedures

All statistical comparisons refer to lipid-corrected geometric means. All statistical assessments were limited to the chemicals detected in more than 50% of all sample sets. A value one-half the lowest limit of detection was assigned to samples with undetectable contaminant concentrations if detectable quantities were found in at least half of the samples. Due to the small sample sizes and the non normal distribution of the congener concentrations (Shapiro-Wilks tests), the statistical evaluation is based on the use of non-parametric tests (Hario *et al*., 2004). Residue differences among birds and prey species were tested separately using Kruskal-Wallis test. Correlations were tested using non-parametric Spearman Rank Correlation. Cluster analysis (with Euclidean-distances as distance measure and single linkage as a linkage rule) was used to evaluate overall differences in pollution patterns among sample types. Results were considered as significant at p-values < 0.05 throughout the study. Lipid-normalized bioaccumulation factors (BAFs), being useful for comparisons across different species (Kelly *et al*., 2004; Borgå *et al*., 2005) were calculated according to the equation:

$$BAF = (OCPs \text{ in organism})_{\text{lipid adjusted}} / (OCPs \text{ in raw water})$$

Dietary OCP enrichment was calculated using the biomagnification factor (BMF) according to equation:

$$BMF = (OCPs \text{ in waterbird})_{\text{lipid adjusted}} / (OCPs \text{ in prey})_{\text{lipid adjusted}}$$

BMF values higher or lower than 1 indicate biomagnification and elimination of the pollutant respectively.

All statistical analyses were performed with STATISTICA 6.0 (Statsoft Inc.).

RESULTS

OCP levels and patterns in biota

All 16 compounds of OCPs analysed were detected in bird eggs and in their prey (except α-HCH in little egret eggs and heptachlor epoxide in pumpkinseed). Significant differences in geometric mean concentrations among waterbirds were detected in β-HCH, lindane, aldrin, dieldrin, heptachlor, γ-chlordane, α- and β-endosulfan, *p,p′*-DDT and *p,p′*-DDE (Table 1). Great cormorant egg samples had highest (geometric) mean concentrations of eight compounds (α-HCH, β-HCH, lindane, dieldrin, heptachlor epoxide, α-endosulfan, *p,p′*-DDT and *p,p′*-DDE) and also maximum concentrations of nine compounds (α- and β-HCH, lindane, dieldrin, endrin, heptachlor, heptachlor epoxide, *p,p′*-DDD and *p,p′*-DDE). Grey heron eggs had highest concentrations of four compounds (endrin, γ-chlordane, endosulfan sulfate and *p,p′*-DDD). Black-crowned night heron eggs had highest concentrations of two compounds (heptachlor, and β-endosulfan) but none in maximum. Great-crested grebe egg samples had highest concentrations of only one compound (aldrin) and three compounds in maximum levels (aldrin, α-endosulfan and *p,p′*-DDT) whereas little egret eggs had highest mean levels of only δ-HCH and maximum levels of only β-endosulfan (Table 1).

Regarding waterbirds' prey, the highest mean concentrations of six compounds (β- and δ-HCH, heptachlor, γ-chlordane, endosulfan sulfate and *p,p'*-DDD) were found in frog samples [where maximum values of six compounds were also detected (α- and β-HCH, aldrin, endrin, heptachlor epoxide and *p,p′*-DDD] (Table 2). The highest mean concentrations of four compounds, namely lindane, β-endosulfan, *p,p′*-DDT and *p,p′*-DDE were detected in pumpkinseed samples, where maximum concentrations on five compounds were also found (lindane, β-endosulfan, en-

TABLE 1. Geometric mean concentrations (ng g^{-1} lipid weight) of organochlorine pesticides in waterbird eggs from Lake Kerkini. Bold lettering indicates the highest geometric mean concentration of each compound reported among all bird species. Numbers enclosed in squares indicate the maximum compound concentrations measured in bird egg samples. NS: non-significant

	P. carbo (n = 11)			*P. cristatus* (n = 13)			*A. cinerea* (n = 11)			*E. garzetta* (n = 11)			*N. nycticorax* (n = 13)			K-Wallis H (n = 59, df = 4)	*p*
	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max		
α-HCH	**3.98**	2.50	92.25	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	8.878	NS
β-HCH	**52.55**	3.10	2529.27	9.04	3.10	26.45	44.74	3.10	275.25	17.32	3.10	49.89	5.69	3.10	53.07	22.314	< 0.001
γ-HCH (lindane)	**18.81**	1.90	119.67	7.26	1.90	38.50	3.02	1.90	19.53	3.69	1.90	39.57	7.70	1.90	142.69	14.013	0.007
δ-HCH	4.52	1.90	582.85	2.45	1.90	20.59	3.94	1.90	861.74	**6.20**	1.90	291.36	2.95	1.90	40.46	1.104	NS
Aldrin	2.26	1.90	12.70	**7.84**	1.90	53.51	2.71	1.90	15.05	2.23	1.90	11.09	2.22	1.90	12.24	18.904	0.001
Dieldrin	**32.33**	1.90	100.03	16.51	1.90	81.44	12.56	1.90	39.97	7.39	1.90	52.14	3.24	1.90	22.67	18.415	0.001
Endrin	11.28	3.10	49.17	11.12	3.10	25.36	**14.26**	3.10	33.96	8.76	3.10	20.76	13.39	3.10	28.80	6.123	NS
Heptachlor	5.39	1.90	62.59	2.86	1.90	60.26	1.90	1.90	1.90	3.20	1.90	10.90	**7.64**	1.90	45.59	16.851	0.002
Heptachlor epoxide	**3.97**	2.50	69.77	2.50	2.50	2.50	2.50	2.50	2.50	2.76	2.50	7.53	2.50	2.50	2.50	6.276	NS
γ-chlordane	8.87	1.90	21.55	2.52	1.90	73.04	**21.07**	7.80	120.00	2.18	1.90	8.79	2.72	1.90	19.42	32.516	< 0.001
α-Endosulfan	**165.30**	53.78	338.69	34.92	9.94	446.02	116.27	57.71	313.08	5.21	1.90	31.43	20.22	5.95	57.23	39.488	< 0.001
β-Endosulfan	15.49	3.10	71.13	13.96	3.10	35.07	10.15	3.10	153.76	32.91	3.10	195.24	**60.45**	13.30	148.31	22.360	< 0.001
Endosulfan sulfate	4.12	3.10	15.95	3.32	3.10	7.68	**6.29**	3.10	119.37	4.82	3.10	23.48	5.88	3.10	33.47	3.249	NS
p,p'-DDT	**98.33**	53.91	197.37	27.74	2.50	280.88	47.88	28.40	97.66	23.31	2.50	45.54	25.52	15.33	49.97	27.930	< 0.001
p,p'-DDD	4.84	3.10	23.27	4.03	3.10	12.32	**5.96**	3.10	21.97	5.22	3.10	13.59	4.30	3.10	15.38	2.524	NS
p,p'-DDE	**5295.93**	4145.35	7439.24	1628.57	1296.62	2953.30	3817.33	2467.45	6053.47	1524.46	537.58	5345.42	2606.86	1108.57	5043.70	36.168	< 0.001
Mean *p,p'*-DDT/ *p,p'*-DDE	53.86	26.24	95.14	58.72	6.07	684.55	79.72	47.89	140.45	65.41	21.90	415.48	105.80	54.62	161.22	13.53	0.01

TABLE 2. Geometric mean concentrations (ng g^{-1} lipid weight) of organochlorine pesticides in waterbirds' prey and in water from Lake Kerkini. Bold lettering indicates the highest geometric mean concentration of each compound reported among all prey species. Values enclosed in squares indicate the maximum compound concentrations measured among prey species. Statistics did not include water data. NS: non-significant

	C. auratus (n = 10)			*A. alburnus* (n = 10)			*R. rutilus* (n = 9)			*L. gibbosus* (n = 11)			*Rana* sp. (n = 10)			Water (n = 15)			K-Wallis H	
	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max	G. mean	Min	Max	(ng l^{-1})	Min	Max	(n = 50, df = 4)	*p*
α-HCH	**7.11**	1.95	24.32	1.83	0.40	3.86	3.61	0.40	10.37	0.81	0.40	11.75	2.36	1.30	46.94	0.32	0.05	1.58	16.625	0.002
β-HCH	4.25	0.50	21.42	1.12	0.50	2.45	3.93	0.50	13.30	5.28	0.50	32.49	**9.97**	1.65	34.04	0.71	0.10	1.60	13.405	0.010
γ-HCH (lindane)	12.02	3.24	26.65	23.33	11.31	32.16	27.24	7.13	73.13	**41.52**	11.80	123.77	28.75	0.65	142.19	0.64	0.05	12.19	14.107	0.007
δ-HCH	2.04	0.20	15.72	1.32	0.20	283.82	11.16	2.40	42.21	10.38	0.20	58.84	**33.56**	0.65	156.82	3.54	0.48	32.76	16.875	0.002
Aldrin	2.22	0.30	49.22	**4.57**	0.30	16.59	0.98	0.30	30.57	1.95	0.30	26.39	4.50	1.00	88.77	0.16	0.10	0.54	5.228	NS
Dieldrin	10.57	0.98	130.77	**12.80**	0.20	60.35	2.57	0.20	13.84	1.76	0.20	108.38	4.43	0.65	24.99	0.48	0.10	2.10	7.992	NS
Endrin	14.05	4.41	56.42	**21.24**	7.57	45.35	11.76	5.21	21.59	13.14	0.50	53.99	11.74	1.65	183.17	0.15	0.10	0.97	3.531	NS
Heptachlor	16.99	2.48	115.00	27.85	10.90	77.96	26.27	8.79	50.83	28.76	5.31	88.93	**41.55**	0.65	273.55	0.76	0.10	2.70	6.043	NS
Heptachlor epoxide	1.71	0.40	12.68	2.99	1.48	5.19	**3.80**	1.47	10.03	0.40	0.40	0.40	2.15	1.30	19.18	0.36	0.05	1.22	27.547	< 0.001
γ-chlordane	1.38	0.30	118.94	2.05	1.44	3.40	4.31	1.66	40.35	3.74	0.30	15.40	**7.29**	1.00	74.40	0.49	0.10	1.13	8.508	NS
α-Endosulfan	49.27	18.05	174.46	31.31	12.19	87.65	**50.52**	18.03	116.17	37.36	6.90	108.63	7.52	0.65	40.93	2.59	0.10	9.12	16.412	0.003
β-Endosulfan	58.92	9.62	346.51	50.16	5.39	282.02	143.35	90.31	250.83	**164.39**	10.08	483.79	12.10	1.65	344.92	0.49	0.10	4.33	15.569	0.004
Endosulfan sulfate	4.10	0.50	35.19	18.44	4.82	62.40	13.04	6.12	33.16	37.32	3.92	131.09	**47.35**	13.31	79.00	0.16	0.10	1.22	21.583	< 0.001
p,p′-DDT	1.57	0.40	29.19	3.97	0.40	17.67	7.97	1.67	43.30	**11.34**	0.40	57.26	4.97	1.30	47.28	0.21	0.20	0.45	8.576	NS
p,p′-DDD	10.24	3.39	47.63	20.28	10.81	45.68	14.05	5.76	29.56	29.00	4.33	72.19	**49.22**	22.54	102.16	0.33	0.03	4.11	21.670	< 0.001
p,p′-DDE	828.19	393.94	2088.91	1098.44	744.86	1517.51	1221.36	360.99	2269.96	**1374.45**	256.53	3150.90	214.20	42.28	1733.62	0.57	0.10	10.91	19.408	< 0.001
Mean *p,p′*-DDT/ *p,p′*-DDE	527.63	28.32	3068.50	276.37	66.72	3423.73	153.18	34.59	493.46	121.25	31.50	4079.25	43.13	0.89	356.98	2.69	0.50	54.54	11.63	0.020

dosulfan sulfate, *p,p′*-DDT and *p,p′*-DDE). In bleak highest mean concentrations of all three drins (aldrin, dieldrin, endrin) were detected and maximum concentration of δ-HCH. Roach samples had highest mean concentrations of two compounds (heptachlor epoxide and α-endosulfan) but none in maximum concentration whereas goldfish samples had highest mean concentration of only α-HCH and maximum concentrations of dieldrin, heptachlor, γ-chlordane and α-endosulfan. In all prey types γ-HCH, endrin, β-endosulfan, and *p,p′*-DDE predominated among compounds of the same group. The differences in geometric mean concentrations were significant for many OCP compounds among prey (except in drins, heptachlor, γ-chlordane and *p,p′*-DDT). Levels of OCPs were generally higher in bird egg samples compared to prey but this was not the rule.

All 16 OCP compounds were detected in the water samples in low concentrations. Maximum concentrations of δ-HCH, lindane, *p,p′*-DDE and α-endosulfan (in the sequence mentioned) were far the highest (Table 2). Among OCPs, α- and/or β-endosulfan were commonly elevated in most biological samples and in water. The mean ratio *p,p′*-DDE/*p,p′*-DDT in bird species' eggs ranged from 53.86 (great cormorant) to 105.80 (black-crowned night heron) and in prey species from 43.13 (frogs) to 527.63 (goldfish). In both bird eggs and in prey, the differences in *p,p′*-DDE/*p,p′*-DDT ratio were significantly different among species (Tables 1 and 2).

The pollution patterns by OCP groups (∑HCHs, ∑DDTs, ∑Cycls (the sum of cyclodiene concentrations analysed) in the different sample types can be distinguished in the fingerprint produced using concentration percentages (Fig. 1). The pattern of ∑HCHs, ∑DDTs, ∑Cycls changed moving up the food chain. In all biological samples, ∑DDTs (especially *p,p′*-DDE) dominated over all other compounds proportionately increasing from frogs to birds. In water, ∑HCHs dominated followed by ∑Cycls and ∑DDTs (Fig. 1). Despite the common occurrence of these OCP groups in the samples, their overall patterns were much different as revealed by a cluster analysis of percent OCP concentrations: water was separated from all samples. Among biological samples, frogs were also placed apart whereas fish prey and birds clustered in distinctly separate sub-groups (Fig. 2).

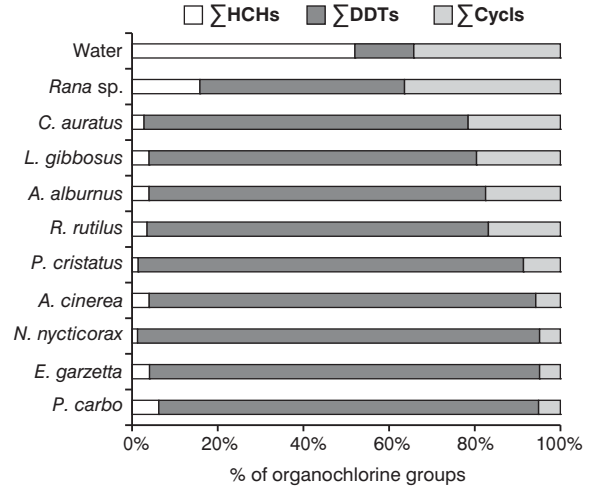

FIG. 1. Concentration percentages of OCP groups (∑HCHs, ∑DDTs, ∑Cycls) indicating pollution patterns in water, prey types and waterbird eggs at Lake Kerkini.

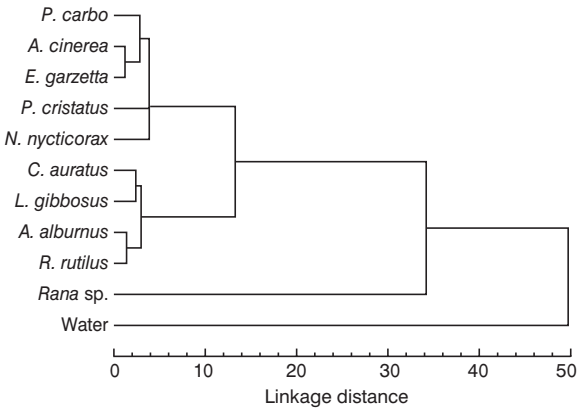

FIG. 2. Cluster analysis of percent OCP levels indicating the relationships in pollution patterns among sample types analysed at Lake Kerkini.

Bioaccumulation and biomagnification

All 16 compounds bioaccumulated in the biota analysed (Table 3). Overall, logBAF values of OCPs varied ranging from 2.57 for δ-HCH in bleak to 6.97 for *p,p′*-DDE in great cormorant. Among all sample types, the maximum BAFs of six OCPs (β-HCH, dieldrin, heptachlor epoxide, α-endosulfan, *p,p′*-DDT and *p,p′*-DDE) were found in great cormorant eggs. Four of maximum BAFs were found in frogs (δ-HCH, heptachlor, endosulfan sulfate and *p,p′*-DDD), two in pumpkinseed (lindane and β-endosulfan), one in great-crested grebe (aldrin), grey heron (γ-chlordane), goldfish (α-HCH), bleak (endrin) and none in little egret, black-crowned night heron and roach. Among prey species, the maximum BAF values of five OCPs were reported in frogs (β-HCH, heptachlor, γ-chlordane, endosulfan sulfate and *p,p′*-DDD), of four in pumpkinseed (lindane, β-endosulfan, *p,p′*-

TABLE 3. Logarithms of bioaccumulation factors (BAF) of organochlorine pesticides in eggs of waterbirds and in their prey. Bold lettering denotes the maximum value of each compound reported among all sample types, and numbers enclosed in squares indicate maximum values reported among prey

	Birds					Prey				
	P. carbo	*P. cristatus*	*A. cinerea*	*E. garzetta*	*N. nycticorax*	*C. auratus*	*A. alburnus*	*R. rutilus*	*L. gibbosus*	*Rana* sp.
α-HCH	4.09	3.89	3.89	3.89	3.89	**4.34**	3.75	4.05	3.40	3.86
β-HCH	**4.87**	4.10	4.80	4.39	3.90	3.78	3.20	3.74	3.87	4.15
γ-HCH (lindane)	4.47	4.05	3.67	3.76	4.08	4.27	4.56	4.63	**4.81**	4.65
δ-HCH	3.11	2.84	3.05	3.24	2.92	2.76	2.57	3.50	3.47	**3.98**
Aldrin	4.16	**4.70**	4.24	4.16	4.15	4.15	4.47	3.80	4.10	4.46
Dieldrin	**4.83**	4.54	4.42	4.19	3.83	4.35	4.43	3.73	3.57	3.97
Endrin	4.88	4.88	4.98	4.77	4.96	4.98	**5.16**	4.90	4.95	4.90
Heptachlor	3.85	3.57	3.40	3.62	4.00	4.35	4.56	4.54	4.58	**4.74**
Heptachlor epoxide	**4.04**	3.84	3.84	3.88	3.84	3.67	3.92	4.02	3.04	3.77
γ-chlordane	4.26	3.71	**4.64**	3.65	3.75	3.45	3.62	3.95	3.89	4.18
α-Endosulfan	**4.80**	4.13	4.65	3.30	3.89	4.28	4.08	4.29	4.16	3.46
β-Endosulfan	4.50	4.45	4.31	4.82	5.09	5.08	5.01	5.46	**5.52**	4.39
Endosulfan sulfate	4.40	4.31	4.59	4.47	4.56	4.40	5.05	4.90	5.36	**5.46**
p,p′-DDT	**5.67**	5.12	5.36	5.04	5.08	3.87	4.27	4.58	4.73	4.37
p,p′-DDD	4.16	4.08	4.25	4.19	4.11	4.49	4.78	4.62	4.94	**5.17**
p,p′-DDE	**6.97**	6.46	6.83	6.43	6.66	6.16	6.28	6.33	6.38	5.57
% mean lipid content	4.67	7.30	5.24	5.64	6.10	5.10	8.52	5.15	3.45	1.20
SD	0.54	0.67	0.97	0.74	0.62	3.08	1.57	1.94	2.34	0.72

TABLE 4. Biomagnification factors (BMF) of organochlorine pesticides in waterbird eggs for each prey type used. Bold lettering enclosed with squares indicate the maximum BMF value reported for each compound

	C. auratus (n = 10)					*A. alburnus* (n = 10)					*R. rutilus* (n = 9)					*L. gibbosus* (n = 11)					*Rana* sp. (n = 10)		
	PCA[a]	*PCR*[a]	*ACI*[a]	*EGA*[a]	*NYN*[a]	*PCA*	*PCR*	*ACI*	*EGA*	*NYN*	*PCA*	*PCR*	*ACI*	*EGA*	*NYN*	*PCA*	*PCR*	*ACI*	*EGA*	*NYN*	*ACI*	*EGA*	*NYN*
α-HCH	_[b]	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
β-HCH	12.38	2.13	10.54	4.08	_	**46.77**	8.04	39.81	15.41	_	13.36	2.30	11.38	4.40	_	9.95	1.71	8.47	3.28	_	4.49	1.74	_
γ-HCH (lindane)	_	nb[c]	_	_	nb	_	nb	_	_	nb	_	nb	_	_	nb	_	nb	_	_	nb	_	_	nb
δ-HCH	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Aldrin	_	3.54	_	1.01	_	_	1.71	_	nb	_	_	**8.02**	_	2.28	_	_	4.02	_	1.14	_	_	nb	_
Dieldrin	3.06	1.56	1.19	nb	_	2.52	1.29	nb	nb	_	12.57	6.42	4.89	2.87	_	**18.32**	9.36	7.12	4.19	_	2.84	1.67	_
Endrin	nb	nb	1.01	nb	nb	nb	nb	nb	nb	nb	nb	nb	**1.21**	nb	1.14	nb	nb	1.09	nb	1.02	**1.21**	nb	1.14
Heptachlor	_	_	_	_	nb	_	_	_	_	nb	_	_	_	_	nb	_	_	_	_	nb	_	_	nb
Heptachlor epoxide	2.32	_	_	_	_	1.33	_	_	_	_	1.04	_	_	_	_	**9.93**	_	_	_	_	_	_	_
γ-chlordane	6.45	_	**15.32**	_	_	4.33	_	10.28	_	_	2.06	_	4.88	_	_	2.37	_	5.63	_	_	2.89	_	_
α-Endosulfan	3.35	nb	_	nb	nb	**5.28**	1.12	_	nb	nb	3.27	nb	_	nb	nb	4.42	nb	_	nb	nb	_	nb	2.69
β-Endosulfan	nb	nb	_	nb	1.03	nb	nb	_	nb	1.21	nb	nb	_	nb	nb	nb	nb	_	nb	nb	_	2.72	**5.00**
Endosulfan sulfate	_	_	**1.53**	_	_	_	_	nb	_	_	_	_	nb	_	_	_	_	nb	_	_	nb	_	_
p,p′-DDT	**62.64**	17.67	30.50	14.85	16.26	24.74	6.98	12.05	5.86	6.42	12.33	3.48	6.01	2.92	3.20	8.67	2.45	4.22	2.06	2.25	9.64	4.69	5.14
p,p′-DDD	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
p,p′-DDE	6.39	1.97	4.61	1.84	3.15	4.82	1.48	3.48	1.39	2.37	4.34	1.33	3.13	1.25	2.13	3.85	1.18	2.78	1.11	1.90	**17.82**	7.12	12.17

[a] *PCA: P. carbo; PCR: P. cristatus; ACI: A. cinerea; EGA: E. garzetta; NYN: N. nycticorax*

_[b] BMFs were not estimated as congeners occurred in < 50% of samples

[c] nb: no biomagnification

DDT and *p,p′*-DDE), of three in bleak, (drins), of two in roach (heptachlor epoxide and α-endosulfan) and of one in goldfish (α-HCH).

p,p′-DDT and *p,p′*-DDE indicated the highest bioaccumulation tendency in birds, while *p,p′*-DDE and β-endosulfan in prey. δ-HCH showed the lowest bioaccumulation in all biota. Among Cycls, heptachlor had the lowest BAFs in bird species whereas γ-chlordane accumulated mostly in great cormorant, grey heron and frogs. Among drins, endrin had highest bioaccumulation tendency in both birds and prey.

For only δ-HCH the bioaccumulation factors showed a significant relationship with the % lipid content of samples ($Rs = -0.697$, $p = 0.025$, Spearman Rank Correlations).

OCPs' biomagnification patterns varied greatly. BMF values ranged from 1.01 (aldrin in little egret) to 62.64 (*p,p′*-DDT in great cormorant) both through goldfish (Table 4). β-HCH, *p,p′*-DDT and *p,p′*-DDE were biomagnified through all prey types in all bird egg samples where they were detected (Table 4). Five maximum BMF values were found in great cormorant two of which originated from bleak (α-HCH and α-endosulfan), two from pumpkinseed (dieldrin and heptachlor hepoxide) and one from goldfish (*p,p′*-DDT). Five maximum BMFs were also found in grey heron two of which originated from goldfish (γ-chlordane and endosulfan sulfate), one from roach and one from frogs (*p,p′*-DDE). One maximum value was reported in great-crested grebe (aldrin through roach).

Heptachlor epoxide and α-endosulfan were biomagnified only in great cormorant through all fish prey. γ-chlordane was biomagnified in both great cormorant and grey heron through all fish species and in grey heron also through frogs. β-endosulfan did not show tendencies of biomagnification except in black-crowned night heron through goldfish, bleak and especially frogs. Endosulfan sulfate, being scarce in the samples, was only biomagnified in grey heron through goldfish and roach. Biomagnification of drins followed a very variable pattern in all waterbirds through most prey types with endrin showing the lowest biomagnification tendencies.

Where calculations were possible, BMF of any compound through any prey type showed a significant relationship with the % lipid content of bird samples (Spearman Rank Correlations).

DISCUSSION

Organochlorine pattern in an organism depends on both uptake and elimination processes. As observed in this study, the pattern of ∑HCHs, ∑DDTs, ∑Cycls changed moving up the food chain. Fish and amphibians, in contrast to waterbirds, may partition contaminants directly with water. The relative importance of uptake from water decreases with increasing lipid solubility of a contaminant such as DDTs and trophic position of an organism (Fisk *et al*., 1998). In addition, the higher contribution of compounds from HCHs and Cycls in fish and frogs is consistent with limited metabolic capacities of these organisms. Low elimination rates of PCBs in frog species have also been reported (Leney *et al*., 2006). In contrast, lower relative contributions of HCHs and Cycls in waterbirds are consistent with their higher ability to metabolise and excrete the less persistent compounds. Similar changes of OCs pattern along the food chain, moving from crustaceans and fish to waterbirds, with decreasing contribution of HCHs and Cycls and increasing contribution of DDTs were also previously reported (Borgå *et al*., 2001). Thus, the pattern change reflects the different biomagnification degree of OCs which depends upon the physicochemical properties of the contaminants and the organisms' physiology with respect to contaminant elimination.

The technical endosulfan composition of 70% α-endosulfan and 30% β-endosulfan (Vorkamp *et al*., 2004) was reflected in their relative concentrations reported in water (and biota) in this study. Mean concentrations of HCH isomers in Lake Kerkini water samples had the trend δ-HCH > β-HCH > γ-HCH > α-HCH. In the Strymon River water, some HCHs have also been reported (β-HCH, nd-59 ng l^{-1}; δ-HCH, 12-29 ng l^{-1} and absence of γ-HCH, Golfinopoulos *et al*., 2003; Konstantinou *et al*., 2006). These findings from water analyses with lower levels of γ-HCH and α-HCH could be explained by their easier biodegradation compared to β-HCH and δ-HCH (Willett *et al*., 1998; Badea *et al*., 2009). In addition, the levels of δ- and β- HCH may reflect dissolved and particulate organic matter associated concentrations since they have higher K_{ow} values compared to the other HCH isomers. Most OCPs detected in water in this study have also been detected between 1996 and 2000 in the Strymon River (Golfinopoulos *et al*., 2003; Konstantinou *et al*., 2006) and these rather suggest recent transboundary pollution, though OCPs' presence might also be partly due to wet and dry deposition by atmospheric long-range transport (Cleemann *et al*., 1995). The predominance of β-HCH in bird eggs at Lake Kerkini indicated its high persistence and lipophilic properties (Walker, 1990; Willett *et al*., 1998)

and could indicate technical HCH contamination in some earlier time because α- and γ-HCH isomers can be more readily metabolized (Willett *et al*., 1998). The occurrence and predominance of α-HCH and particularly β-HCH was also documented in an earlier study in great cormorant eggs collected in 1997 (Konstantinou *et al*., 2000) where lindane was absent. These probably suggest either recent use of lindane in the area or transboundary pollution, as suggested above. Anyway, evidence of direct applications of lindane in earlier times at Lake Kerkini is lacking.

In all biota studied, *p,p′*-DDE predominated among DDTs, being the major OCP group. *p,p′*-DDE is known to occur in elevated concentrations in waterbirds (for great cormorant: Kellner & Lage, 2009), fish and frogs (Vives *et al*., 2005) as a result of their dietary intake and/or DDT metabolism (Patil *et al*., 1972). The elevated *p,p′*-DDE levels in prey explain its high concentrations in the waterbird eggs transmitted through the food chain. Increasing proportion of *p,p′*-DDE combined with decreasing proportion of the DDT parent compounds while moving up the food chain have also been found in marine food chains (Borgå *et al*., 2001). The geometric mean *p,p′*-DDE/*p,p′*-DDT sample ratios were elevated at Lake Kerkini denoting a considerably reduced use of DDT in recent years (Gabrielsen *et al*., 1995), photochemical conversion of DDTs in the environment (Brown *et al*., 1986) and, particularly for great cormorant, slow rates of metabolisation and/or a high dietary input of OCPs leading to bioaccumulation (Walker, 1990; Fossi *et al*., 1995).

Lipid content has been shown to explain a major part of the variation in OCPs accumulation by aquatic organisms (Larson *et al*., 1996; Kucklick & Baker, 1998; Olsson *et al*., 2000) although food web structure and trophic level may affect OCP concentrations (Bentzen *et al*., 1996; Kidd *et al*., 1998). When OCP concentrations increase with trophic level, differences occur in lipid content (Bentzen *et al*., 1996; Kucklick & Baker, 1998), depuration rates (LeBlanc, 1995; Sijm & Van der Linde, 1995), animal size (Bergner, 1985; Olsson, 2000) and exposure duration (Harding *et al*., 1997). Thus, differences in OCs' accumulation among species of the same trophic level may be explained by the uptake degree which is dependent on the physicochemical properties of the compounds (mainly water solubility and lipophilicity), the feeding strategy of the organism and its ability to metabolize OCs. Their metabolization depends on the compounds' structure and the presence and/or efficiency of enzymatic systems for biotransformation. The data of this study suggest that bioaccumulation at lower trophic levels (fish, frogs) seems to depend mainly on the compound of physicochemical properties while at higher trophic levels (waterbirds) biochemical factors play the major role.

Compared to this study, lower BAFs of α-endosulfan, heptachlor and γ-HCH in frogs (*Rana esculenta*) were found in Italy (Fagotti *et al*., 2005). In the Axios Delta, a northern Greek coastal wetland, lower BAFs of HCHs and higher of *p,p′*-DDE were found in black-crowned night heron, little egret and frogs (Albanis *et al*., 1996), probably denoting different food chain pathways. Significant correlation between $\log K_{ow}$ and BAF of all OCPs was found ($BAF = 0.478 \log K_{ow} + 2.15$; $p < 0.001$; $r^2 = 0.25$). The low value of correlation coefficient is due to the variability of BAF data and $\log K_{ow}$ data used and the species considered. Similarly, low correlation coefficients (with $r^2 = 0.34$) have been found also elsewhere (Hoekstra *et al*., 2003). High quality BAF data (with regard to validity and reliability) have been found to present variabilities of > 0.5 log units while $\log K_{ow}$ for many OCs fall within a range of more than 2 orders of magnitude (Nendza *et al*., 2010).

Accumulation of OCPs via food rather than water enhances biomagnification. Differences in physical-chemical properties of OCPs, feeding strategies, and possible biotransformation are usually reflected in the variability of biomagnification between fish and birds (Hoekstra *et al*., 2003). β-HCH was also found to be the principal HCH isomer biomagnified in Barents Sea seabirds (Borgå *et al*., 2001). None of the other HCH isomers was biomagnified at Lake Kerkini consistent with that waterbirds are capable to metabolize and excrete them. Aldrin is readily metabolised to dieldrin, being more resistant to biodegradation (WHO, 1992) explaining its higher BMFs. Endrin, exhibiting a low lipid accumulation profile, can be metabolised in animals (WHO, 1992) and was not biomagnified. Biomagnification of endosulfan in food chains is less likely to occur compared to other organochlorine pesticides (WHO, 1984). In agreement with the present study, limited biomagnification of endosulfan was also found in biota of a Greenland food chain (Vorkamp *et al*., 2004). Heptachlor and heptachlor epoxide, chemically related to endosulfan, exhibited none and low biomagnification, respectively, in birds.

The levels of OCPs detected in bird eggs from Lake Kerkini were lower than those detected for the

same species in other parts of Mediterranean-Black Sea regions and Europe (Fossi *et al*., 1984; Fasola *et al*., 1987; Ruiz *et al*., 1992; Ayas *et al*., 1997; Scharenberg & Ebeling, 1998; Berny *et al*., 2002; Galassi *et al*., 2002). A comparison with them, suggests that the detected concentrations seem too low to have any adverse biological effects. Even regarding *p,p′*-DDE, the dominant OCP in this study, its geometric mean concentrations transformed in wet weight, ranged from 85 ng g^{-1} in little egret to 145 ng g^{-1} in great cormorant eggs. The threshold level associated with adverse effects for little egret and black-crowned night heron causing reduction of young survival is 1000 ng g^{-1} ww (Connell *et al*., 2003); in great cormorant 5% eggshell thinning is caused by concentration of about 4000 ng g^{-1} ww (Dirksen *et al*., 1995); and in great blue heron (*Ardea herodias*) concentration of 3000 ng g^{-1}, ww resulted in reduced egg hatching (Blus, 1996).

Among birds and all other biota, great cormorant eggs had the highest concentrations and BAF- and BMF values through most prey types for a number of OCPs, mainly those of the most persistent. Among prey, pumpkinseed and frogs had the highest concentrations of most compounds and greatest bioaccumulative properties. Additionally, in these two prey types some compounds of the most water-soluble indicated higher levels and bioaccumulation than these found in great cormorant. Thus, great cormorant is suggested as the most useful for OCP biomonitoring at Lake Kerkini whereas pumpkinseed may be useful as alternative biomonitor. *Rana* frogs can provide complementary information in both cases. Extensive use of frogs is not suggested due to the declining trends of their populations.

In conclusion, OCP concentrations detected in water and biota of Lake Kerkini can be considered low. Their bioaccumulation through trophic chain was considerable but their biomagnification generally limited. Prey preferences may have influenced the patterns of pollutants' accumulation in all species studied. The pollution patterns at Lake Kerkini are affected by transboundary pollution, which for some compounds may be more readily detected in water whereas biota pollution patterns suggest a long-term reduction in the earlier use of OCPs. Bioaccumulation patterns at Lake Kerkini are probably affected primarily by biochemical factors such as the metabolic capacity of the organisms, though at lower trophic levels bioaccumulation mechanisms may also depend on physicochemical factors such as water solubility of the pollutants.

ACKNOWLEDGMENTS

We are grateful to the Kerkini Information Center for hospitality and especially to Dr. T. Nazirides and Mr. P. Hatziyiannidis for aid in sample collection. Special thanks to B. Antoniadou and E. Bintoudi for aid in the field and laboratory work. Prof. A. Sinis and Dr. D. Bobori (Laboratory of Ichthyology, School of Biology) provided water-sampling equipment. Thanks to the Ministry of Rural Development and Food for the license of collecting the biological material analysed.

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