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Organochlorine pesticide residues in feathers of four bird species from western part of Turkey

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Abstract: The use of organochlorine pesticides (OCPs) has decreased considerably worldwide; they have been banned in most countries due to adverse impacts on wildlife. The purpose of the study was to investigate OCP residue levels in wild birds from Aegean, Marmara, Mediterranean, and Central Anatolia geographical regions of Turkey. Feather samples (n = 80) of four bird species in four regions were collected from anthropogenically dead birds such as roadkill and wind turbine collision. Feather samples were analysed using GC/MS and scanned for 23 pesticide congeners. Analysis results showed dichlorodiphenyltrichloroethane (DDT), hexachlorocyclopentadiene (HCCPD), and their derivatives were present in almost all samples at high levels. Regions and birds differed significantly as well as among themselves in terms of total residue levels ($P < 0.05$). The Marmara region (36.257 ng/g) and the common buzzard (*Buteo buteo*) (55.109 ng/g) had the highest residue levels compared with others. The study shows that OCP residues reach a considerable level in birds and other wild threatened species populations. The results also indicate that the feather can be used for terrestrial pesticide monitoring as a nondestructive method.

Key words: Pesticide, residue, organochlorine, bird, feather

1. Introduction

The use of organochlorine pesticides (OCPs) has been banned or restricted by most countries, including Turkey (Kolonkaya, 2006), in the early 1990s (Hagen and Walls, 2005) due to harmful effects on wildlife, environment, and biota (Colborn and Smolen, 1996; Jones and De Voogt, 1999; Lupi et al., 2016). OCPs persist in different environmental components such as soil, sediment, air, or biota (Jones and De Voogt, 1999; Rigét et al., 2019) and bioaccumulate through the lifetime of organisms in the food web (biomagnification) (Daley et al., 2014). The effects of the residues may persist over decades (Batt et al., 2017). OCP residues in wildlife continue to be reported in environmental toxicology studies (Köhler and Triebskorn, 2013; Blévinet al., 2017). The birds, as the top predators of the food web, are the most exposed and affected by OCPs compared to other living organisms (Hoshi et al., 1998; Letcher et al., 2010). Therefore, birds are commonly used as a bioindicator species for monitoring organochlorine and other pesticide pollutants in the environment due to higher exposure (Letcher et al., 2010; Sletten et al., 2016). Higher residues of OCPs in tissues of birds also cause reproductive anomalies (Tiemann, 2008; Goutner et al., 2015), breeding failures (Blévinet al., 2017; Arıkan et al., 2018b), and developmental disorders (Fry, 1995; Tanabe, 2002; Bustnes et al., 2003).

Adipose tissue (Kutz et al., 1991), muscle (Arıkan et al., 2018a), blood, and internal organs such as kidney and liver (Kenntner et al., 2003; Dhananjayan, 2013) were the tissues sampled most for pollutant determination in wild animals. However, using these vital tissues of animals for the determination of pollutants faces practical and ethical issues because of the possible negative impact of such testing on the species (Furness, 1993). Previous research has shown that pesticide accumulation levels are increasing in keratinous tissues as in other tissues of birds contending higher level dwellers of the food web (Hargrave et al., 1992; Corsoliniet al., 2006). Therefore, using keratinous tissue such as mammalian hair (Covaci and Schepens, 2001) and bird feathers (Jaspers et al., 2007; Abbasi et al., 2017; Arıkan et al., 2018b) as nondestructive biomonitoring methods (D'Havé et al., 2006) could be a more effective and widely accepted sampling technique for residue analysis, including OCPs. Besides, studies show

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that nondestructive biomonitoring methods (D'Havé et al., 2006) are a suitable, reliable, cost-effective, and expedient way to investigate and monitor OCPs in wildlife (Schramm, 2008).

There is a limited number of studies on the effects of OCPs on birds from Turkey. Arıkan et al. (2018b) reported OCP residues in feathers as one of the factors associated with the reproductive success of spur-winged lapwing, which is a threatened bird species according to the IUCN Red List for birds of Turkey (Kiziroğlu, 2008). In addition, the northern bald ibis (*Geronticus eremita*), a globally vulnerable bird species, became extinct due to high DDT exposure during the 1960s in Turkey (Kiziroğlu, 1999; Kiliç and Uysal, 2015). Studies show that the detection of OCP residue levels in vertebrates is gaining importance for elaborating environmental protection and conservation action. Nevertheless, data are scarce and gaps exist in data on pollutant levels in birds, to form the basis of conservation action plans in Turkey. Therefore, this study aims to contribute to existing data regarding chemical pollutants in avifauna and determine OCP residue levels for four common bird species from the Aegean, Marmara, Mediterranean, and Central Anatolia geographical regions of Turkey. In addition, results show the bioaccumulation of OCPs and demonstrate the usability of the nondestructive method to monitor pollutants in different geographical regions. Experience concerning pollutants accumulation in birds can be drawn-up for monitoring wildlife.

2. Materials and methods

2.1. Species, sampling, and study area

Eighty feather specimens were collected from four bird species, which were the common buzzard (*Buteo buteo*), common kestrel (*Falco tinnunculus*), common blackbird (*Turdus merula*), and house sparrow (*Passer domesticus*), as the common species for avifauna of Turkey (Kiziroğlu, 2008). Furthermore, these species are targets of human activities. Five specimens per species were taken from each region. The specimens were randomly collected from bird carcasses from roads (Kociolek et al., 2011), collision victims in areas surrounding wind turbines (Smallwood, 2007), buildings (Loss et al., 2014), and tracking nests at four geographical regions across Turkey (Figure 1) over four years between 2016–2020. The locations represent four different habitats, i.e., urban, forest, steppe, and agricultural lands. However, the habitat parameters were not taken into account for statistical analyses because of insignificant comparisons between species and inadequate species sampled from diverse habitats.

2.2. Analytes

Twenty-three OCP congeners were investigated from the groups of pesticides derived from hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), chlordan (CHL), hexachlorocyclopentadiene (HCCPD), and dicofol (dicofol and 2,4'-dicofol). PCB 46 and PCB 143 were used as internal standards and 1,2,3,4-tetrachloronaphthalene (TCN) was used as a recovery standard (Covaci and Schepens, 2001). All standards were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany) at a concentration of 10 ng/μL in isooctane.

2.3. Extraction procedure for feathers

The extraction procedure was based on a method for the determination of persistent organochlorinated pollutants in keratinous tissues (Covaci and Schepens, 2001; Jaspers et al., 2007). Samples were weighed to the nearest 200 mg, spiked with internal standards (PCB 46 and PCB 143; 5 ng of each), and incubated overnight with 4.0 mL of 4 M HCl and 3.0 mL of hexane:dichloromethane. The analytes were extracted from the incubation medium using a liquid–liquid procedure before purification on an Empore™ C18 SPE (3M™, USA) cartridge filled with 500 mg of acidified silica and 250 mg anhydrous sodium sulphate.

2.4. Instrumentation and quality assurance

Chromatography was performed with a Shimadzu QP2010 Ultra GC/MS (Shimadzu Co., Tokyo, Japan) equipped with a 30-m-long, 0.25 mm inner diameter, 0.25-μm-thick film Restek (USA) capillary column. Two 8-point (from 2 to 250 mg/L) calibration curves were generated for quantification by use of OCPs solution in isooctanes; good linearity ($r^2 \geq 0.99$) was observed in the ranges tested. The r^2 (0.98 ± 0.001), percent recovery (97.5 ± 0.15), relative standard deviation (4.27 ± 2.21), limit of detection (0.027 ± 0.01), and limit of quantitation (0.05 ± 0.026) values of the analytical methods were determined from the linear regression of the multilevel calibration. All analyses were conducted at Hacettepe University Pesticide Research and References Laboratory.

2.5. Statistical analysis

The data were tested for normality using the Kolmogorov–Smirnov test. Data did not obey the normality assumption; therefore, nonparametric methods were used. Kruskal–Wallis H test and ANOVA were used to evaluate the level of OCPs concentration in regions and species. The Mann–Whitney U test was used to investigate differences between mean levels of OCPs between birds. The frequency (f) and descriptive statistics of OCPs were specified in an individual sample, region, habitat, and species. SPSS version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. A P-value below 0.05 shows statistical significance.

3. Results

The results of the analysis from the group of congeners are shown by their mean level in Table 1 as ng/g. Although the Marmara region had the highest total OCP residue levels (36.257 ng/g), no significant differences were found

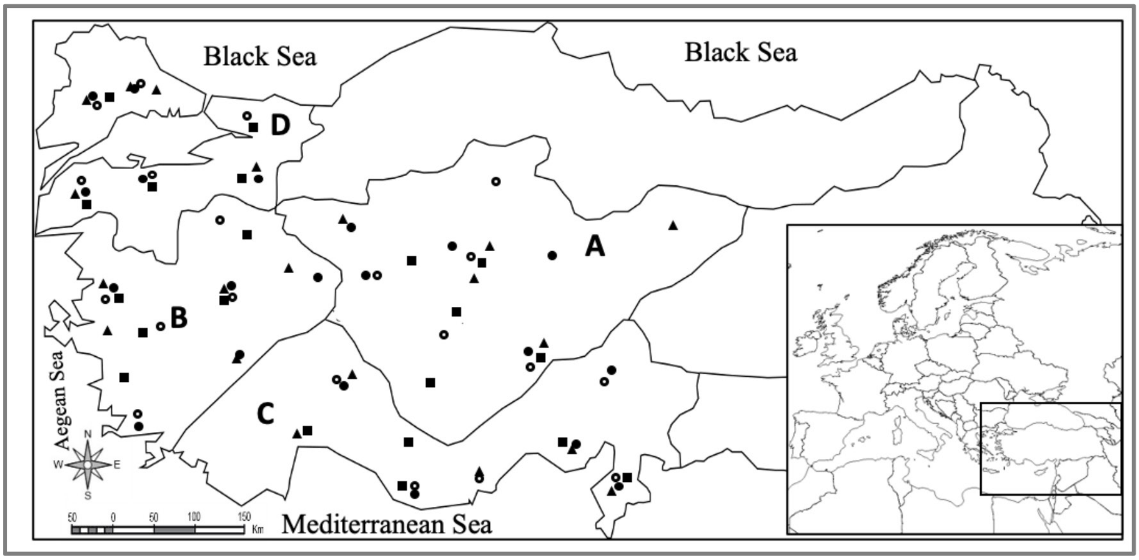

Figure 1. Specimen collecting locations from four geographical regions (A: Central Anatolia; B: Aegean; C: Mediterranean; D: Marmara; triangle: common buzzard, square; common kestrel; circle: common blackbird; ring: house sparrow) across Turkey.

Table 1. The total residue concentrations (ng/g) of OCPs in regions and species.

OCP Group	Species				Region			
	Buteo buteo	*Falco tinnunculus*	*Turdus merula*	*Passer domesticus*	Marmara	Aegean	Mediterranean	Central Anatolia
HCHs	2.812	2.250	1.335	0.338	1.898	1.639	2.053	1.663
DDTs	36.662	26.414	14.590	13.010	26.289	19.909	24.232	23.039
CHLs	5.331	1.691	0.738	0.286	2.465	1.367	2.357	2.160
HCCPDs	8.170	5.390	2.491	0.650	4.257	3.721	6.835	3.731
Dicofols	2.156	1.976	1.097	0.249	1.347	1.255	2.022	1.181

between mean OCP levels (P > 0.05). The Central Anatolia (31.775 ng/g), Mediterranean (31.715 ng/g), and Aegean (27.890 ng/g) regions were lined up with their total OCP levels, respectively. However, beta-endosulfan (0.732 ng/g) and heptachlor (0.543 ng/g) were significantly different in sampled regions (P < 0.05) due to the highest residue in the Mediterranean region. 4,4'-DDT (5.913 ng/g) was the most common and had the highest level of OCP congeners among others in all regions. d-HCH and cis-chlordane were not detected in any region. Furthermore, b-HCH was not determined in feather samples from the Aegean and Mediterranean regions. When the geographical region was not a determinative factor in OCP levels, there was a strong correlation between species and OCP accumulation in feathers (r = 0.829, P = 0.000). Species statistically differed from each other in terms of total OCP levels and all congener groups (P < 0.05), except for those not determined. The common buzzard (55.109 ng/g) had the highest level of OCP residues among the species, which are the common kestrel (37.72 ng/g), common blackbird (20.271 ng/g), and house sparrow (14.532 ng/g). The common buzzard from Marmara had the highest mean levels (66.285 ng/g). However, only the mean level of total DDT (36.66 ng/g) in common buzzard differed significantly by region (P < 0.05). Total HCH level in common kestrel statistically differed by region (P < 0.05). Although Marmara had the highest residue according to the total level of each OCP group, HCH residue in the common kestrel in Central Anatolia (1.673 ng/g) and Marmara (1.909 ng/g) was the lowest among the regions, respectively. Statistically significant differences were found in total OCP (20.271 ng/g), HCH (1.354 ng/g), and CHL (0.738 ng/g) levels in common blackbird according to regions (P < 0.05). Similar to other species, the common blackbird in Marmara had the highest level of pesticide accumulation in feathers (23.853 ng/g). b-, and g-HCH, oxy-, and cis-chlordane were detected in feather specimens of the common blackbird. There were statistically significant differences between HCH (0.338 ng/g), HCCPD (0.649 ng/g), and dicofol (0.249 ng/g) levels in house sparrow by region. HCH, CHL, HCCPD, and dicofol were not determined in feather specimens of house sparrow from the Aegean region. In addition, a-, b-, and g-HCH, oxy-, and cis-chlordan, a-endosulfan were not found in house sparrow feathers.

Sex is a determinative factor for OCP residues in feathers of common buzzard and common kestrel due to statistically significant differences between genders ($P < 0.05$), where females (59.253 ng/g, 42.541 ng/g) had higher levels than males (50.965 ng/g, 32.899 ng/g). Only the total DDT level among other OCPs statistically differed with gender in both species ($P < 0.05$). On the other hand, there were no statistically significant differences between the genders of common blackbird and house sparrow in terms of total OCP levels ($P > 0.05$).

Twenty-one congeners (except g-HCH and cis-chlordane) among 23 OCPs were observed in birds and also in regions as shown in Table 2. DDT congeners, which are 2,4'- and 4,4'-DDD, and 2,4'- and 4,4'-DDT were determined in all specimens of each species from all regions. 4,4'-DDT (5.913 ng/g) had the highest residue level among other OCP congeners. Mean total DDT (22.669 ng/g) had the highest proportion of total OCPs (31.909 ng/g) compared to other congener groups. Hence, there is a strong correlation between DDT and OCP levels ($r = 0.822$, $P = 0.000$). Dicofols had the lowest residue levels in birds and regions by mean level among OCPs groups. Aldrin is the most frequently detected and highest-level residue (0.901 ng/g) among HCCPD congeners. Similarly, d-HCH (0.887 ng/g) in HCHs, chlorothalonil (0.969 ng/g) in CHLs, and dicofol (0.669 ng/g) are frequently determined congeners in their groups.

4. Discussion

The results show that region is not a factor in the distribution of OCP congener residues in bird feathers from Turkey. In other words, OCP residues in western parts of Turkey are of similar magnitude/level. However, Marmara has the highest level of residues among the regions in terms of the mean residue of congeners. A possible cause could be

Table 2. Concentrations and frequency of organochlorine pesticides (ng/g) in feathers of birds (n = 80).

OCP Group	No	Congeners	Mean	SD	Min.	Max.	Frequency
HCHs	1.	Alpha HCH	0.738	0.799	0.000	3.291	44
	2.	Beta HCH	0.063	0.218	0.000	1.031	6
	3.	Gama HCH	0.000	0.000	0.000	0.000	0
	4.	Delta HCH	0.888	1.170	0.000	2.490	66
DDTs	5.	2,4'-DDE	2.525	1.543	0.932	5.931	80
	6.	4,4'-DDE	1.668	1.183	0.000	5.024	70
	7.	2,4'-DDD	3.309	1.941	1.455	9.251	80
	8.	4,4'-DDD	4.184	2.427	1.784	13.201	80
	9.	2,4'-DDT	5.071	3.030	2.371	18.392	80
	10.	4,4'-DDT	5.913	3.742	1.863	23.519	80
CHLs	11.	Chlorothalonil	0.969	1.284	0.000	6.492	51
	12.	Oxy- chlorodane	0.430	0.715	0.000	2.375	25
	13.	Cis- chlorodane	0.000	0.000	0.000	0.000	0
	14.	Trans- chlorodane	0.607	0.947	0.000	2.094	46
HCCPDs	15.	Alpha- endosulfane	0.476	0.686	0.000	2.281	30
	16.	Beta- endosulfane	0.313	1.040	0.000	1.431	31
	17.	Endosulfan- sulfate	0.803	1.409	0.000	2.471	52
	18.	Heptachlor	0.292	1.512	0.000	2.043	27
	19.	Aldrin	0.901	0.751	0.000	3.021	62
	20.	Eldrin	0.863	1.026	0.000	2.947	63
	21.	Dieldrin	0.527	0.563	0.000	1.994	44
Dicofols	22.	Dicofol	0.699	0.613	0.000	1.953	56
	23.	2,4-Dicofol	0.670	0.807	0.000	1.931	55

intensive agricultural and industrial activities in a relatively small geographical area when compared with other regions. Our results indicate that the accumulation of OCPs in birds increased from house sparrow to common buzzard or from the bottom to the top of the food web. There is a strong correlation between the trophic level of species in the food web and OCP residues. The accumulation and biomagnification of OCP congeners in bird feathers are directly associated with dietary and ecological niches of the species. The common buzzard and common kestrel have similar feeding behaviour and are at the same trophic level (Cooke et al., 1981; Colborn and Short, 1999). Therefore, despite significant differences, the accumulation of OCPs in feathers shows similarity in terms of residue levels and congener types. The common blackbird and house sparrow are also close to each other in points of OCP residue levels. The order of magnitude for OCP residue in species overlaps the species relations in the food web (Best, 1973; Hargrave et al., 1992; Alleva et al., 2006). Furthermore, the total residue level revealed in species is similar to studies such as the common buzzard (13.9 ng/g) and common kestrel (113.3 ng/g) by Jaspers et al. (2007), and the house sparrow (3.1 ng/g) by Dhananjayan et al. (2011). No data related to OCP residue in feathers of the common blackbird was found in the literature. The species-specific natural history characteristics and behavioural traits play a significant role in the likelihood of contaminant exposure, as expected (Smith et al., 2007). However, the accumulation in species such as common blackbird and house sparrow similarly overlap with their trophic levels (Jiménez et al., 2005). In other words, the species order by means of OCP accumulation is in strong agreement with their ecological relations (Coat et al., 2011) and trophic levels (Alleva et al., 2006). The studies related to OCP residues in the keratinous tissue of mammals (Arıkan et al., 2018a) and birds (Arıkan et al., 2017; Arıkan et al., 2018b) in Turkey show that DDTs are at the highest levels when compared with other congeners. Studies on OCP residues in bird feathers from different countries support our findings that DDTs and congeners have the highest residue among other OCP congeners similar to the case of Turkey (Dauwe et al., 2005; Behrooz et al., 2009; Eulaers et al., 2013). The results of the study revealed that DDT residue levels are highest in the feathers of four species, similar to other studies from Turkey and other countries cited above. Arıkan et al. (2018) stated that 4,4'-DDT had the highest residue in the feathers of Spur-winged Lapwing (*Vanellus spinosus* L.) among other DDT congeners, as shown in the present study. Although there is no conclusive toxicity data on the avian biology of OCP residues in feathers (Espínet al., 2012) until recently, it is known that they have the potential to cause some anomalies, including reproductive failure and declining breeding success (Scharenberg and Looft, 2004; Toschik et al., 2005; Corsolini et al., 2006; Hernández et al., 2008). Arıkan et al. (2018) reported declined fledgling success associated with higher OCP residues in feathers of Spur-winged Lapwing from Turkey. We can conclude that there are potential negative impacts of OCPs on bird species.

Similar studies are needed to elucidate the effects of pesticides on avian biology. If a feather is compared to other tissues such as adipose or muscle, it is clear that other tissues have higher residue levels, as reported in numerous studies (Alomar et al., 2016; Blévin et al., 2017). Bird species change or renew their feathers (Chatt and Katz, 1998; Zuberogoitiaet al., 2005) regularly, depending on age, year, or sex. Therefore, our results indicate that the accumulation in feathers represents only a single period, presumably for one or two years, whereas adipose and muscle tissues provide longer duration accumulation results. Feather accumulation could be an indicator of annual or periodic exposure of birds to OCPs. The species studied show critical exposure to the congeners, although they were banned and not used since the 1990s in Turkey (Kolonkaya, 2006). The level of pesticides may be hazardous for species like the common buzzard and common kestrel in higher trophic levels in the food web. Periodic exposure indicates the magnitude of misused pesticides in the wildlife of Turkey. The specimens in the study could provide reliable data for OCP residue risks countrywide. Another important result of the study is using bird feathers for a nondestructive method as a suitable matrix for biomonitoring of OCP residues in birds and also across the country, as in most studies (D'Havé et al., 2006; Schramm, 2008; Arıkan et al., 2018b). A more comprehensive study using the nondestructive method is required in order to further develop this study and to easily determine the accumulation of OCPs across the country.

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