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Organochlorine and Metal Pollution in Aquatic Organisms Sampled in the Doñana National Park During the Period 1983–1986

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The study area, Doñana National Park, is located in the South South-West of Spain, and this is one of most important reservation of Europe.

Samples of aquatic organism were obtained from the principal waterway of Doñana National Park to determine the degree of organochlorine and metal contamination of this environment.

The sampling was carried out during the period 1983-1986 in order to collect six aquatic species in four sites along the "Brazo de la Torre" (Figure 1). An agricultural area in the North-West side of the Park and a working mine at about 40 km from its northern boundary were considered as the likely main polluting sources of organochlorine pesticides, PCBs, and heavy metals respectively.

MATERIALS AND METHODS

The aquatic organism species chosen for analysis were: American crayfish (Procambarus clarckii), carp (Cyprinus carpio), barbel (Barbus barbus), grey mullet (Mugil capito), eel (Anguilla anguilla), and frog (Rana perezi). Specimens were immediately weighed, wrapped in aluminium foil and frozen at -18°C.

The composite aquatic organism samples, each of different species consisted of 1-21 adults.

Extraction, purification and analysis of organochlorine compounds presents in the muscle tissue samples were carried out according to a method previously described (Cromartie et al. 1975; Kaiser et al. 1980). The organochlorine contaminant analysis consisted of hexane extraction of a homogenized fish-sodium sulfate mixture on a Soxhlet extractor, clean-up of the extracts on a partially deactivated Florisil column; the pesticides and PCBs were separated into four fractions. Determination was made by gas chromatography using an electron affinity detector.

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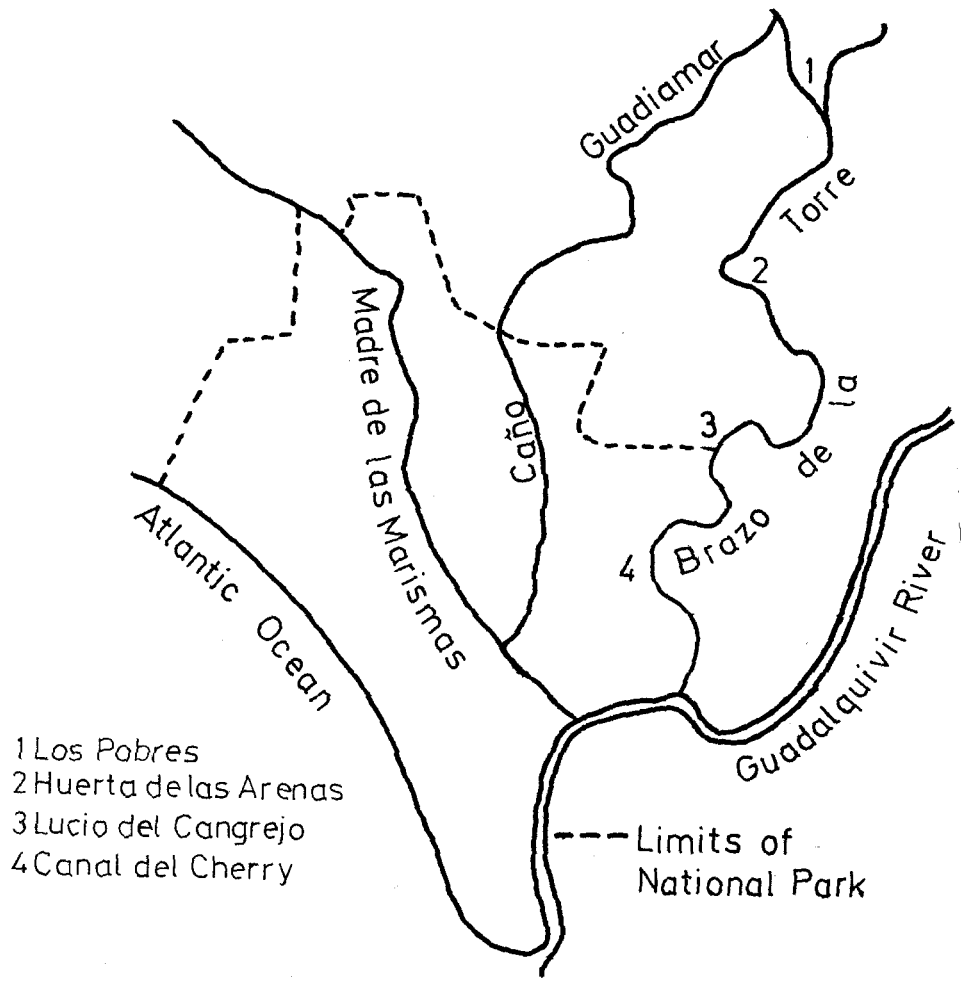

Figure 1.- Stations sampled for determination of organochlorine compounds and heavy metals in freshwater fish of Doñana National Park, 1983-1986.

Cold vapor atomic absorption was selected as the analytical technique for determining mercury concentration in the fish tissue, in accordance with Uthe et al. (1970); 0.3 to 0.6 g (wet weight) were dissolved with sulfuric and nitric acid mixture, followed by 6% K_2MnO_4.

For the lead, cadmium, copper and zinc analysis each sample was reduced to ashes. The ash was cooled, dissolved and diluted. The residues of metals were determined by the atomic absortion spectrophotometry (Haseltine et al. 1981). Each sample was analyzed for metals at least twice. The lowest limit of reportable residues was 0.01 ppm for pesticides, 0.08 for PCBs, 0.01 for mercury,

0.02 for lead, 0.001 for cadmium, 0.002 for copper and 0.006 for zinc.

RESULTS AND DISCUSSION

The levels of organochlorine insecticides and PCBs found in contaminated fish are detailed in Table 1.

DDE residues were detected in all of the fish, none of these were above the critical level of 1 ppm; this level could represent a potential hazard to carnivores feeding extensively on fish (Hider et al. 1982).

PCBs were detected in all samples, but only two species, from Huerta de las Arenas (Anguilla anguilla, 1985) and Canal del Cherry (Cyprinus carpio, 1983), were above the critical level of 0.5 ppm (Hider et al. 1982).

Residue values detected but not shown in Table 1 were: TDE at 0.01 ppm in carp from Huerta de las Arenas (1983), and Canal del Cherry (1984), and aldrin at 0.01 ppm in American crayfish from Huerta de las Arenas (1983) and Canal del Cherry (1984), grey mullet from Canal del Cherry (1984) and eel from Lucio del Cangrejo (1983), 0.02 ppm in eel from Canal del Cherry (1984), and 0.03 in eel from Huerta de las Arenas (1985). Levels of the other chlorinated pesticides (λ-HCH, heptachlor, heptachlor epoxide and dieldrin) included in the analytical survey were below the limit of detection.

For ease of evolution, the mean concentration of HCHt, DDTt, and PCBs throughout the time of study of the four fish species (Procambarus clarkii, Cyprinus carpio, Mugil capito and Anguilla anguilla) has been plotted in Figure 2.

HCHt concentrations were stationary during the experimental period. DDTt and PCBs levels, after an initial increase, reduced in the final period of the study.

Overall residue appeared greater in eels than those detected in the other fish. The results can be easily justified by the nutritional method of the particular species of fishes. Eel is a fish of prey and in conection with this fact it takes insecticides cumulated in the organisms of its victims together with its food.

The levels of heavy metals (Hg, Cd, Pb, Cu, and Zn) found in the fish are detailed in Table 2. Heavy metals are detected in all of the samples. Levels of metals decreased in order Zn > Cu > Pb > Hg > Cd.

Generally, background levels of mercury in fish are of the order of 0.05-0.2 ppm (Jernelov and Martin 1980), if a background level of 0.2 ppm is assumed, 14 samples (35.8%) exceed this background level. Fish meat from contaminated areas contains

Table 1.- Levels of organochlorine insecticides and PCBs (ppm wet weight) found in aquatic organism from Doñana National Park (Spain)

Species	(N)	L	Year	γ-HCH	DDE	DDT	PCBs
A. crayfish	(5)	2	1983	0.01	0.03	0.08	0.07
Carp	(4)	2	1983	N.D.	0.05	0.02	0.04
	(5)	4	1983	N.D.	0.10	0.22	0.70
Grey mullet	(3)	2	1983	N.D.	0.10	0.02	0.02
	(2)	4	1983	N.D.	0.02	0.04	0.03
Eel	(2)	3	1983	0.02	0.05	0.08	0.09
	(2)	4	1983	0.02	0.16	0.50	0.17
A. crayfish	(4)	1	1984	N.D.	0.01	0.08	0.08
	(3)	4	1984	N.D.	0.03	0.10	0.10
Carp	(4)	1	1984	N.D.	0.09	0.08	0.11
	(7)	4	1984	0.02	0.08	0.13	0.24
Barbel	(10)	1	1984	N.D.	0.22	0.03	0.07
Grey mullet	(6)	4	1984	N.D.	0.08	0.11	0.07
Eel	(2)	1	1984	0.02	0.60	0.05	0.13
	(8)	4	1984	0.01	0.08	0.11	0.09
Frog	(7)	1	1984	N.D.	N.D.	0.05	0.05
A. crayfish	(6)	1	1985	N.D.	0.04	0.08	0.37
	(6)	2	1985	N.D.	0.02	0.14	0.30
Carp	(5)	1	1985	N.D.	0.08	0.05	0.15
	(5)	2	1985	N.D.	0.06	0.10	0.24
	(3)	3	1985	N.D.	0.02	0.05	0.34
	(5)	4	1985	N.D.	0.11	0.06	0.27
Barbel	(3)	1	1985	N.D.	0.06	0.05	0.19
Grey mullet	(3)	2	1985	N.D.	0.02	0.11	0.32
	(3)	4	1985	N.D.	0.14	0.07	0.29
Eel	(2)	1	1985	0.01	0.26	0.08	0.20
	(3)	2	1985	0.01	0.21	0.23	0.94
	(1)	4	1985	N.D.	0.06	0.05	0.28
Frog	(4)	1	1985	N.D.	0.02	0.04	0.49
A. crayfish	(5)	1	1986	N.D.	0.02	0.06	0.29
Carp	(11)	1	1986	N.D.	0.04	0.07	0.19
	(21)	2	1986	N.D.	0.05	0.06	0.20
	(3)	3	1986	0.01	0.04	0.06	0.35
	(2)	4	1986	N.D.	0.02	0.09	0.39
Grey mullet	(2)	3	1986	N.D.	0.07	0.08	0.19
	(3)	4	1986	0.01	0.05	0.06	0.22
Eel	(1)	1	1986	0.02	0.22	0.07	0.22
	(1)	4	1986	N.D.	0.08	0.06	0.38
Frog	(2)	1	1986	0.01	0.19	0.55	1.08

(N) = Number of aquatic organisms in each composite sample.
L = Location (1 : Los Pobres, 2 : Huerta de las Arenas, 3 : Lucio del Cangrejo, 4 : Canal del Cherry).
N. D. = Not Detected.

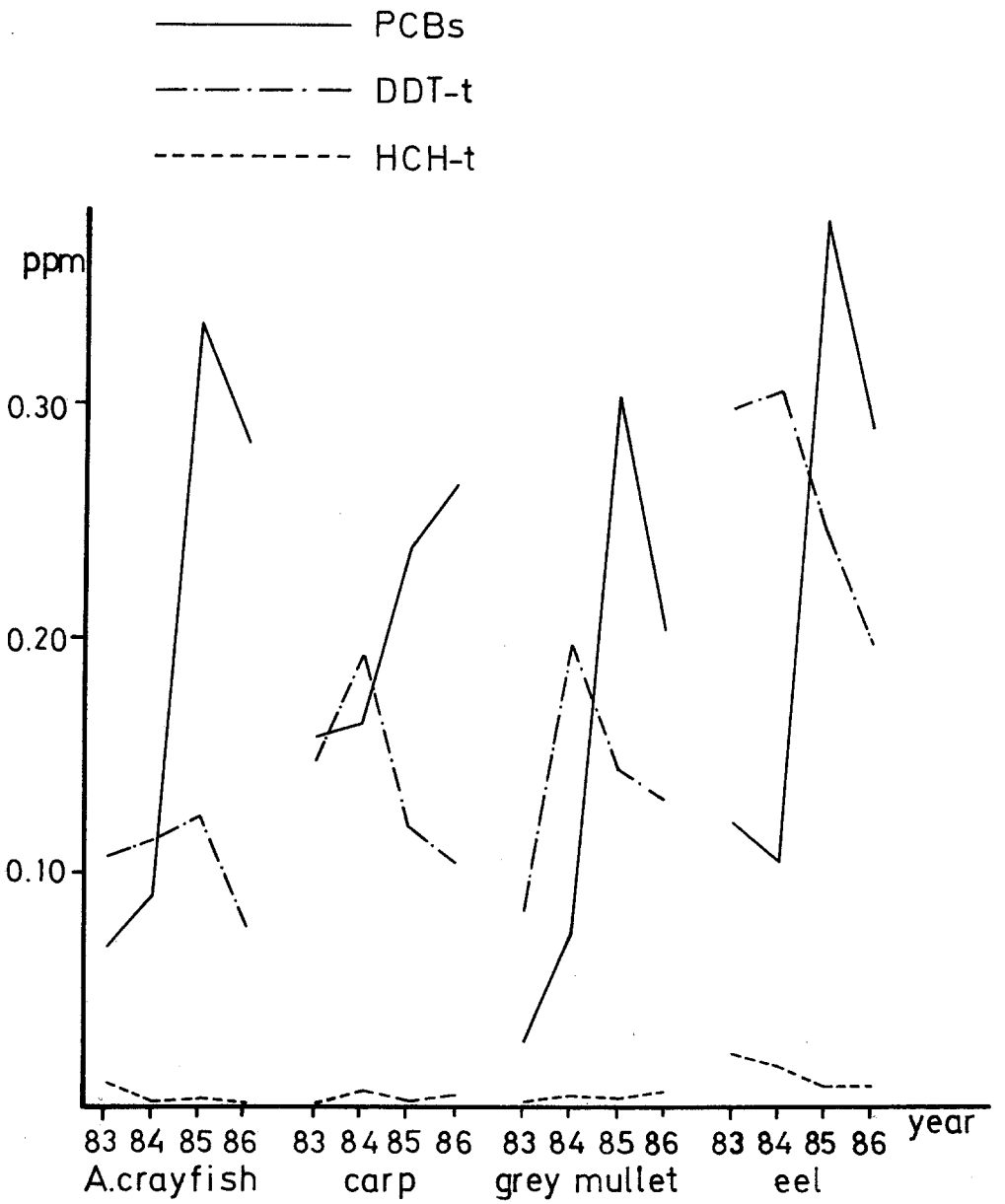

Figure 2.- Variation in HCHt, DDT, and PCB levels along the time of study.

0.01-0.2 ppm of cadmium, mean levels greater than 0.2 ppm occurred in 6 samples (15.3%). Mean levels of lead greater than 1 ppm were recorded in 19 samples (48.7%), indicating a rather high level of contamination. All the samples has levels of zinc lower than 10 ppm. In fish muscle, the levels of copper are reported to be generally less than 1 ppm, these levels of copper in fish muscle are not likely to be of danger to predatory animals

Table 2.- Levels of heavy metals (ppm wet weight) found in aquatic organism from Doñana National Park (Spain).

Species	(N)	L	Year	Hg	Cd	Pb	Cu	Zn
A. crayfish	(5)	2	1983	0.22	0.42	4.73	12.53	20.11
Carp	(4)	2	1983	0.28	0.06	6.66	1.39	24.38
	(5)	4	1983	0.67	0.07	0.60	0.69	17.18
Grey mullet	(3)	2	1983	0.15	0.05	0.59	1.25	7.84
	(2)	4	1983	0.04	0.09	0.63	1.01	15.72
Eel	(2)	3	1983	0.27	0.19	1.70	2.14	33.00
	(2)	4	1983	0.31	0.05	0.58	0.70	24.72
A. crayfish	(4)	1	1984	0.08	0.53	5.20	32.70	31.37
	(3)	4	1984	0.08	1.01	10.84	17.45	38.46
Carp	(4)	1	1984	0.20	0.08	0.65	0.80	17.84
	(7)	4	1984	0.24	0.07	0.65	1.13	13.02
Barbel	(10)	1	1984	0.13	0.10	0.69	0.85	15.83
Grey mullet	(6)	4	1984	0.39	0.08	0.71	0.97	16.62
Eel	(2)	1	1984	0.12	0.16	0.84	0.84	18.03
	(8)	4	1984	0.22	0.10	0.78	1.50	23.51
Frog	(7)	1	1984	0.08	0.08	1.19	0.80	9.46
A. crayfish	(6)	1	1985	0.10	0.53	3.69	21.23	35.70
	(6)	2	1985	0.15	0.42	4.20	18.96	20.83
Carp	(5)	1	1985	0.14	0.06	0.69	0.33	15.58
	(5)	2	1985	0.26	0.08	0.95	0.40	15.06
	(3)	3	1985	0.08	0.09	0.98	0.77	19.58
	(5)	4	1985	0.21	0.06	0.53	0.29	14.56
Barbel	(3)	1	1985	0.08	0.06	1.33	0.74	10.61
Grey mullet	(3)	2	1985	0.15	0.08	2.03	1.20	11.72
	(3)	4	1985	0.09	0.07	1.85	0.39	9.79
Eel	(2)	1	1985	0.19	0.08	0.58	1.10	16.23
	(3)	2	1985	0.49	0.12	1.72	1.24	30.44
	(1)	4	1985	0.18	0.06	1.68	0.40	17.04
Frog	(4)	1	1985	0.09	0.07	0.48	0.28	7.65
A. crayfish	(5)	1	1986	0.12	0.62	3.97	21.23	31.46
Carp	(11)	1	1986	0.09	0.09	1.15	1.14	17.31
	(21)	2	1986	0.17	0.08	1.00	1.10	16.50
	(3)	3	1986	0.10	0.09	1.05	0.86	11.13
	(2)	4	1986	0.18	0.10	1.03	0.93	13.42
Grey mullet	(2)	3	1986	0.12	0.08	0.96	0.67	10.53
	(3)	4	1986	0.09	0.08	0.94	0.82	9.16
Eel	(1)	1	1986	0.20	0.13	0.98	0.73	17.22
	(1)	4	1986	0.35	0.07	0.84	0.93	17.07
Frog	(2)	1	1986	0.15	0.19	1.82	2.61	31.07

(N) = Number of aquatic organisms in each composite sample. L = Location (1:Los Pobres, 2:Huerta de las Arenas, 3:Lucio del Cangrejo, 4:Canal del Cherry).

Table 3.- Results of two-way analysis of variance on concentrations of organochlorine compounds and heavy metals in freshwater fish, Doñana National Park (Spain).

	F	
	Years	Stations
HCHt	0.896	1.396
DDTt	0.511	1.048
PCBs	10.870**	0.383
Hg	1.624	0.567
Cd	1.000	1.361
Pb	1.152	2.390
Cu	3.575	4.425
Zn	1.695	0.795

F = 6.94 (α=0.05, df1 = 2, df2 = 4).

(Förnstner and Wittman 1981).

From the data on individual species there is no evidence of an increase in metals (Cd, Pb, Cu, and Zn) along the food chain, but American crayfish are more contaminated than other species. Levels of mercury are more elevated in carnivore species.

A two-way analysis of variance (ANOVA) with stations and years as main effects was used to test two null hypotheses: (1) There was no significant difference in pollutant residue concentrations due to location, and (2) there was no effect due to time. The two-way analysis of variance data set consisted of 3 matching stations (Huerta de las Arenas, Canal del Cherry and Lucio del Cangrejo) and 3 time periods (1983, 1985 and 1986). The results of two-way analysis of variance are detailed in Table 3.

No significant difference could be observed in pollutant residue concentrations due to location, and only significant differences in PCBs concentrations were observed from the three years; the major accumulation of these pollutants was observed in 1985, in general.

Correlation among residues of HCHt, DDTt, PCBs, mercury, cadmium, lead, copper, and zinc were calculated and are presented in Table 4.

There are highly significant positive correlations between HCHt/DDTt. PCBs/DDTt, Cd/Pb, Cd/Cu, Cd/Zn, Pb/Cu, Pb/Zn, and Cu/Zn; and there are significant positive correlations between Hg/DDTt and Hg/PCBs; this may be the result of similar storage characterics or similar distribution patterns in ecosystems.

Table 4.- Correlation among residues of organochlorine compounds and heavy metals detected in aquatic organisms from Doñana National Park (Spain).

	HCHt	DDTt	PCBs	Hg	Cd	Pb	Cu
DDTt	0.518**						
PCBs	0.006	0.438**					
Hg	0.179	0.320*	0.371*				
Cd	0.116	0.120	0.061	0.231			
Pb	0.193	0.188	0.129	0.155	0.844**		
Cu	0.206	0.190	0.081	0.229	0.855**	0.690**	
Zn	0.159	0.234	0.189	0.135	0.711**	0.629**	0.629**

** $P<0.001$; * $P<0.005$

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