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Heavy Metals, HCB and p,p′-DDE in *Ictalurus melas* Raf. from Trasimeno and Corbara Lakes, Italy

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Very small amounts of certain heavy metals are essential for life and it has been stated that they are more important than vitamins since they cannot be synthesized by living matter. Copper, zinc and chromium, although essential at low levels, are very toxic at higher concentrations (Mertz 1981). A quite different situation exists with regard to lead, cadmium and mercury, that, to our knowledge, do not show any essential function for life and are considered only for their negative effects.

The organochlorine pesticides hexachlorobenzene (HCB) and p,p'-DDE, the latter being the major metabolite of DDT, although banned in Italy in the early 1970s, are extensively used in the past. These molecules exhibit high octanol/water partition coefficients ($LogK_{ow}>4$) and are selectively partitioned from water into bed and suspended sediments and into the lipid tissue of aquatic biota. Several studies have reported that organic contaminants with high $LogK_{ow}$ which are present in the water at undetectable levels will bioconcentrate within the lipid tissues of biota (Edwards 1975; Leiker et al. 1991; Phillips 1998).

Aquatic organisms may bioaccumulate and bioconcentrate environmental pollutants to more than 1 000 000 times the concentrations detected in the water column (US EPA, 1992a, b). Fish are considered as sentinel organisms in the aquatic ecosystem to assess the environmental contamination of water by xenobiotics, because they can be chronically exposed to different substances (such as heavy metals and pesticides) and can bioaccumulate by direct exposure or through the food chain (Depledge et al. 1998). *Ictalurus melas*, a bottom-dwelling freshwater teleost, is widely distributed in Italian rivers and lakes and often lives and thrives under extreme conditions of water pollution (Scott and Crosman 1973). The use of catfish as a bioindicator to evaluate the level of aquatic pollution (Leiker et al. 1991; Carpenè et al. 1994) is an approach that allows a more complete comprehension of chronic events and provides a relatively long-term measurement of aquatic contamination, unaffected by short term, intermittent changes in water chemistry.

The few data available concerning the environmental contaminant levels in fish from the lakes in Umbria (Central Italy) are twenty-five years old (Begliomini et al. 1975). Thus, the aim of this study was to obtain quantitative information on the

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concentration of six heavy metals (Cd, Cr, Cu, Hg, Pb and Zn) and of two organochlorine compounds (HCB and p,p'-DDE) in muscle of *Ictalurus melas* collected from Trasimeno and Corbara lakes (Umbria).

MATERIALS AND METHODS

Corbara and Trasimeno lakes show highly different geomorphological and physical characteristics: Corbara is a dam reservoir built in 1963 on the Tiber river with a maximum depth of about 42 meters and a surface of 15 km^2 (Menghini 1975). Trasimeno is the fourth largest lake in Italy and because of the peculiar morphology of its basin, with an ample central zone (81.5 km^2; depth range: 4.50 to 6.30 m) and a very extensive shoreline, it can be defined as a laminar or shallow lake (Carollo 1969). Forty-two specimens of mixed sex of *Ictalurus melas* were caught by professional fishermen during the winter 1996-1997 from different sampling areas of the two lakes. The size, weight, muscular fat and dry matter content of each fish were measured and are given in Table 1.

Table 1. Weight, size, muscular fat and dry matter contents (mean ± SD) of catfish samples from Trasimeno and Corbara lakes

	TRASIMENO	CORBARA
N. of fish	17	25
Weight (g)	139.7 ± 17.8 A	116.1 ± 28.9 B
Length (cm)	22.0 ± 0 .9 a	21.1 ± 1.7 b
Fat (%)	1.8 ± 0.8	2.0 ± 1.3
Dry matter (%)	19.5 ± 0.9 b	21.6 ± 3.3 a

Different letters indicate significant differences among locations for means (A, B: $p \leq 0.01$ and a, b: $p \leq 0.05$)

The lipid contents were determined by the Sohxlet extraction method. Dry matter was measured by drying tissues at 105 °C until constant weight. The organochlorine compounds (p,p'-DDE and hexachlorobenzene) were determined in the fat extract following a clean-up with concentrated sulphuric acid (Waliszewski and Szymczynski 1982). The purified samples were analysed by means of a Perkin Elmer gas chromatograph (AUTOSYSTEM) equipped with a ^{63}Ni electron capture detector and a programmable thermal injector. Helium was used as carrier and nitrogen as make-up gas. The analyses were performed using two different capillary columns: DB-5MS (J&W, 30 m; id: 0.25 mm; 0.25 μm) and DB-1701 (J&W, 30 m; i.d.: 0.25 mm; 0.25 μm). The oven temperature program was: 60°C, 1 min; 60°C to 220°C, 25°C/min; 220°C to 270°C, 15°C/min, 270°C, 20 min. The sample tissues (4-5 g) for determination of Cd, Pb, Cd, Zn and Cu were placed in quartz crucibles and heated overnight at 400°C. The ash residue was dissolved in 1 N hydrochloric acid. Metals were analyzed by atomic absorption spectrometry (AAS) using a Perkin Elmer 5100 spectrophotometer. Cu and Zn were determined in an air-acetylene flame. Cd, Cr and Pb were analysed using a flameless graphite oven with an autosampler (AS-60). The standard

additions were used to test for matrix effects. For total mercury the analyses were performed by CVAAS and the detailed procedure is reported elsewhere (Elia et al. 2000). The accuracy of the measurements was checked by calibration with certified materials: BCR (CRM422 - cod muscle) and IAEA (MA-B-3/OC - fish) for heavy metals and organochlorines, respectively. The concentrations found with the method used in this study fell in the confidence interval given by the European Commission and the International Atomic Energy Agency.

Differences among sampling locations were investigated using a single factor One-way Analysis of Variance (ANOVA). Correlation between different parameters were calculated using linear regression. Linear Discriminant Analysis (LDA) is a classification procedure which maximizes the variance between categories and minimizes the variance within categories (Brereton 1990). The method produces a number of orthogonal linear discriminant functions equal to the number of categories minus 1. In this case, from the two groups (lakes), only one function was obtained. All tests were performed by means of statistical software package SPSS 9.0.

RESULTS AND DISCUSSION

The average concentrations of pollutants in catfish sampled from Trasimeno and Corbara lakes were reported in Table 2. No chromium residues were detected. Statistically significant differences between the two sites were observed for lead (206 *vs.* 141 ng/g wet weight), mercury (63 *vs.* 0 ng/g wet weight), zinc (11.34 *vs.* 8.01 μg/g wet weight) and HCB (48 *vs.* 4 ng/g fat weight) although all data showed a generally higher concentration of analites recovered in catfish collected from Corbara.

There are small industries along Trasimeno; the greatest impact upon the lake is therefore very likely exerted by the farms (mostly hog operations) and agricultural activities located mainly on the northern and western sides of the lake. The Corbara basin, on the other hand, is influenced by the very great anthropic charge of the Tiber river with urban and industrial runoff.
Regarding sex effects, no differences in pollutant concentrations were observed between male and female specimens. One of the interesting aspects of this study is that even though DDT and HCB have been banned for about 20 years, detectable levels are still present in aquatic organisms. Since these areas were subjected to massive pesticide treatments for agricultural purposes, this indicates that land can act as a storehouse for environmental pollutants. Mercury concentrations less than 500 ng/g in fish usually are considered natural levels (Winger et al. 1990). In this study mercury contents are lower than those observed by Sangiorgi and Ferretti (1993) in twenty-two *Ictalurus melas* collected in rivers and lakes of an industrial area of Northern Italy.Copper has generally a lower concentration than those for zinc. The Zn/Cu ratios reported in Table 2 are comparable to values measured by Carpené et al. (1994) in white muscle of catfish sampled in rearing ponds of Emilia-Romagna region (Italy). There are few data in the literature about the

Table 2. Average concentrations of pollutants ± SD in catfish sampled from Trasimeno and Corbara Lakes

	TRASIMENO	CORBARA
HCB	4 ± 1 b	48 ± 22 a
(ng/g)	(10-2)	(123-19)
p,p'-DDE	131 ± 48	155 ± 83
(ng/g)	(236-82)	(409-39)
Cu	0.83 ± 0.33	0.90 ± 0.91
(μg/g)	(1.60-0.44)	(5.13-0.48)
Zn	8.01 ± 0.86 B	11.34 ± 2.09 A
(μg/g)	(9.31-6.47)	(15.28-7.29)
Zn/Cu	9.6	12.6
Cd	23 ± 18	23 ±9
(ng/g)	(72-0)	(42-10)
Hg	0 ± 0 B	63 ± 50 A
(ng/g)	(30-0)	(268-20)
Pb	141 ± 42 b	206± 89 a
(ng/g)	(273-99)	(370-69)

Concentration of HCB and p,p'-DDE are given on a lipid weight basis, while the heavy metals are calculated on a wet weight basis; Maximum and minimum levels recorded are given in parentheses; Different letters denote significant differences among locations for means (A, B: $p \leq 0.01$ and a, b: $p \leq 0.05$).

environmental contaminant levels in fish collected from these two lacustrine ecosystems. The levels of Zn, Pb, Cr and Hg in *Cyprinus carpio, Tinca tinca, Exos lucius* and *Lucioperca* collected during 1972-1973 period were reported by Begliomini et al. (1975). Although a correct comparison was difficult since the previous data were obtained from different fish species of dishomogeneous sizes and ages, certainly, as a consequence of the ban of several pesticides and industrial processes using these heavy metals, the actual levels of mercury, cadmium and chromium are dramatically much lower. Only zinc, which is currently used in the agricultural practices as fungicide and anticryptogamic (Ziram, Metiram, Mancozeb and Propineb) and as fertilizer, did not show a significant decline in tissue of catfish from Trasimeno ($p>0.2$) whereas in Corbara the levels are higher than in the past ($p<0.05$). This is indirectly confirmed by the significant positive relationship between Zn and HCB, indicating a probable common agricultural origin of the two substances (Table 3). Likewise a positive relationship was observed between HCB and p,p'-DDE concentrations, too.

Table 4 shows the results of LDA. The classification function was obtained using the seven variables: Cd, Cu, HCB, Hg, Pb, p,p'-DDE and Zn. The functions at group centroids and standardized coefficients of the discriminant canonical function are reported in Tables 5 and 6. The high percentage (95.1%) of the correctly classified cases demonstrate the utility of the pattern recognition methods to extract useful information for an amount of data. Information was

Table 3. Pearson's moments correlation for biological data and analite contents in catfish from Corbara and Trasimeno lakes

	Weight	Length	HCB	DDE	Cu	Zn	Cd	Hg	Pb
Weight	1.000								
P	-								
Length	.944*	1.000							
P	.000	-							
HCB	-.326	-.285	1.000						
P	.035	.067	-						
DDE	-.059	-.042	.589*	1.000					
P	.712	.792	.000	-					
Cu	.086	.101	.067	-.038	1.000				
P	.592	.531	.677	.813	-				
Zn	-.301	-.217	.521*	.102	-.063	1.000			
P	.056	.172	.000	.526	.696	-			
Cd	-.002	-.126	.104	.119	-.041	-.083	1.000		
P	.991	.434	.518	.457	.801	.604	-		
Hg	-.293	-.223	.535*	.339	-.142	.408*	.076	1.000	
P	.063	.161	.000	.030	.375	.008	.636	-	
Pb	-.309	-.301	.334	.171	.273	.269	.188	.524*	1.000
P	.049	.056	.033	.284	.085	.089	.238	.000	-

The asterisk indicates a correlation at P<0.01.

Table 4. Classification Results of LDA analysis [a,b]

			Predicted Group Membership		
		Lake	Trasimeno	Corbara	Total
Original	Count	Trasimeno	17	0	17
		Corbara	2	22	24
	%	Trasimeno	100.0	0	100.0
		Corbara	8.3	91.7	100.0
Cross-validated	Count	Trasimeno	17	0	17
		Corbara	2	22	24
	%	Trasimeno	100.0	0	100.0
		Corbara	8.3	91.7	100.0

[a] 95.1% of original grouped cases correctly classified

[b] 95.1% of cross-validated grouped cases correctly classified

Table 5. Function at Group Centroids

Lake	Function 1
Trasimeno	-2.638
Corbara	1.869

Table 6. Standardized Coefficients of the discriminant canonical function

	Function 1
Cu	-0.066
Zn	0.488
Pb	0.089
Cd	-0.118
Hg	0.375
p,p'-DDE	-0.977
HCB	1.290

used to relate the pollutant content of catfish with the lake of origin and to classify new cases. The LDA results confirmed what was observed using ANOVA.

Finally, these findings show higher concentrations of all analites in catfish from Corbara, although the values measured can be considered low. These differences are currently being investigated and will be reported at a later date.

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