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Levels of Heavy Metals and Organochlorine Pesticides of Cyprinid Fish Reared Four Years in a Wastewater Treatment Pond

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Sanitary risks to public health are certainly the most serious bottleneck for wastewater aquaculture extension. Apart from the fact of an eventual transmission to man of pathological organisms such as bacteria, viruses and parasites by fish, the wide occurence in domestic sewage of micro-pollutants like heavy metals and pesticides, generally considered as bio-cumulative, is troublesome. In this respect, short-term analyses cannot give a good idea of potential hazard, as it is widely assumed that accumulation by fish or aquatic animals is related to age, size, feeding habits and retention time in polluted waters (Mitra 1986). We present here the results of a study on a sample of 14 tench (*Tinca tinca*) and 4 rudd (*Scardinius erythrophtalmus*), introduced 4 years before in a wastewater treatment pond of the town of Realmont (France). Both species may be considered as good test for heavy metal and pesticide contamination, according to their feeding behaviour (tench is a bottom feeder, and rudd feeds mainly on plankton and benthos). After the estimation of age, growth and sanitary state of the fish, analytical results of three metals (cadmium, lead and mercury) and six organochlorine pesticides and derived compounds (DDT, heptachloride, aldrin, dieldrin, HCB and HCH) are compared with natural water fish content and discussed in relation to international recommendations concerning food product quality.

MATERIALS AND METHODS

All fish were sampled with an electro-fishing apparatus in the last pond (in a succession of three) of the sewage treatment plant of Realmont. They were measured and weighed immediately, and scales taken for age and growth measurement by the scalimetric method (Daget and Le Guen 1975). The fish were placed in a refrigerator for 48 hours, then liver, kidney and dorsal muscles were taken and frozen.The following analytical methods were employed :
Cadmium and lead : after dissolution of 100 mg of homogenized sample in a mixture of nitric, sulfuric and perchloric acids (24:1:24), the residue was dissolved with acetate buffer (5 ml) and metal content determined with an ESA-2400 voltameter by

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anodic redissolution (detection limits : 0.010 μg/g for Cd and 0.10 μg/g for Pb on a wet basis).
Mercury : mineralization of 500 mg of homogenized sample was carried out by means of a mixture of nitric and sulfuric acids (10:1) in closed tubes. After cooling, urea and distilled water were added to the solution up to 50 mL. Total Hg content was measured by flameless atomic absorption spectrophotometry (stannous chloride as reductor; detection limit : 0.010 μg/g on a wet basis).
The methods used in metal determination were previously tested successfully against standards of NBS freeze-dried beef liver.
Pesticides : after hexane treatment of the homogenized sample, the supernatant was purified by chromatography on florisil column; the pesticide content was determined by gaseous-phase chromatography (detection limit : 1 ng/g on a wet basis).

Cd, Pb and Hg analyses in water and sediment were also carried out to determine the level of these metals in the ponds. The number of samples was : 2 water samples in each pond, 1 mud sample at the sewage input, 2 within the first pond and only one in each of the remaining lagoons. Metals in water were measured by flameless atomic absorption spectrophotometry, directly for cadmium and lead, after cold mineralization in a mixture of nitric and sulfuric acids for mercury. The sediment samples (1 g) were mineralized in a mixture of fluorhydric and perchloric acids (4:1) for cadmium and lead, by nitric acid in closed tubes for mercury. Then, metal content was measured by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

The external appearance of the fish showed a total absence of either parasites or necrosis. Table 1 presents the mean values of the length and weight of the sampled fish at the date of harvest. The age was assessed between 9 and 11 years for tench and 4-5 for rudd.

Table 1. Total length and weight (average values) of the fish sampled for heavy metals and pesticides determination.

	No. of Fish	Total Length (cm)	Weight (g)
Rudd (females)	3	23.6	220
Rudd (male)	1	23.0	190
Tench (females)	5	39.5	1164
Tench (males)	9	34.4	658

Figure 1 shows the average growth curves of the fish, as calculated by scalimetry. The absence of discontinuities in the curves, especially for tench, indicates good environmental and feeding conditions in the pond.

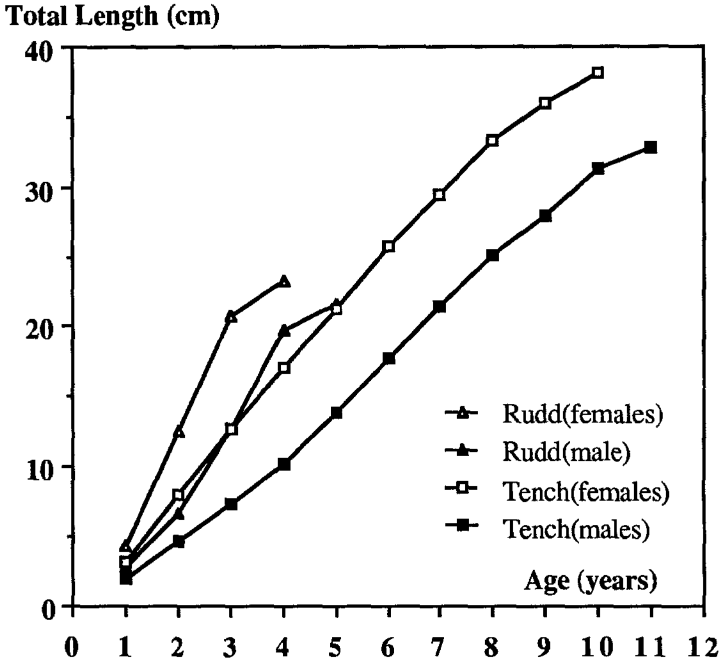

Figure 1. Average growth curves of sampled tench and rudd

Table 2. Cadmium, lead and mercury content of water and sediment of the domestic waste-water treatment ponds of Realmont (results in µg/L for water and µg/g dry weight for sediment).

	Cadmium	Lead	Mercury
Water (range)	n.d.	0.2 - 3.0	n.d.
Sediment :			
at the sewage input	4.0	248.0	1.35
1st pond	4.0	178.0	0.56
2nd pond	≤ 1	48.0	≤ 0.05
3rd pond (fish)	≤ 1	46.0	0.08

Water and sediment concentrations of Cd, Pb and Hg are presented in Table 2. If the water can be considered unpolluted, metal concentrations in sediment are not negligible as they appear similar to the values given by Spiegel *et al.* (1985) for heavy metals in municipal secondary sludge in the US. By comparison, the "natural" cadmium concentration in unpolluted river sediment must not exceed 0.1 to 0.3 μg/g of dry weight (Breder 1988). As pointed out by several authors (Billen 1973; Gardiner 1974; Fitzgerald 1979; Di Giulio and Scanlon 1985), the sediment tends to concentrate heavy metals adsorbed on suspended solids, particularly organic matter. The total suspended solid concentration in these lagoons was very high, ranging between 12 and 128 mg/L. Suspended organic matter (55 to 80 % of total suspended solids), comprised mainly phytoplankton, which can be also responsible for direct metal uptake (Hardy *et al.* 1984).

Table 3. Cadmium, lead and mercury content of fish (results in μg/g wet weight ; n.d. : not detectable i.e. < 0.01 μg/g for Cd and Hg, < 0.10 μg/g for Pb)

	Cadmium average (range)	Lead	Mercury
Rudd :			
liver	0.056 (0.013 - 0.107)	n.d.	n.d.
kidney	0.157 (0.134 - 0.190)	n.d.	n.d.
muscle	n.d.	-	-
Tench :			
liver	0.058 (0.013 - 0.113)	n.d.	n.d.
kidney	0.149 (0.019 - 0.237)	n.d.	n.d
muscle	n.d.	-	-

Table 3 presents the analytical results of heavy metals in fish. Since liver and kidney, usually considered as accumulation organs (Fish and Wildlife Service 1985), did not show significant levels of lead and mercury, muscle tissue was not analyzed for those metals. Cadmium was the only metal whose levels in liver and kidney were significantly above the detection limit. Muscle values were below the levels of detection. The average values between males and females were not statistically different ($P < 0.05$), thus average values are shown in Table 3. Kidney accumulates more than liver : the ratio kidney/liver (average of individual ratios) equals 6.04 for rudd and 4.06 for tench. Among the latter, the average value was different between males (2.35) and females (7.14). These values are relatively higher than data published by other authors : Lavoix-Rossigneux (1978) established a 2.4 kidney/liver ratio value in a fish population of a non-polluted river, even though Labat *et al.* (1977) stated a ratio of 6.39 in fish from a river receiving industrial sewage. For fish products of the Atlantic coast of the United States, Frazier (1979) assessed a ratio value in the range 0.18 - 1.35.

If we compare our data with those given by different authors in various natural aquatic ecosystems (Table 4), they are low and similar to those found in non-polluted areas. Even if the muscle content was equal to the detection limit (0.01 µg/g), one could calculate according to the U.S.E.P.A. recommendations for food (Fish and Wildlife Service 1985), that it would be necessary to ingest more than 1.6 kg of fish per individual per day in the best case, 4 in the average case, or 10 in the worst, to exceed the recommended limits (best case : 16 µg/kg diet/day; average case : 40 µg/kg diet/day; worst case : 100 µg/kg diet/day). The current W.H.O. recommendation (1972, cited by Dean and Suess 1985) is less restrictive and would allow a daily intake of 60 - 70 µg/day, which would represent a daily consumption of 6 to 7 kg of fish.

Table 4. Levels of cadmium in fish from different natural waters (results in µg/g wet weight)

Author (s)	Origin	Cadmium	Remarks
Enk and Mathis (1977)	Jubilee Creek (Illinois, USA)	0.08 - 0.15	according to the fish species
Frazier (1979)	Atlantic coast of the U.S.A.	0.01 - 0.24 0.14 - 54 0.19 - 9.8	muscle liver kidney
Labat *et al.* (1977)	Lot River (F) receiving industrial wastes	3.9 11.7 74.8	muscle liver kidney
Lovett (1972)	49 rivers in New York State	0.02 - 0.1	eviscerated fish content
Mathis and Cummings (1973)	Illinois River (U.S.A.)	0.017 - 0.035	muscle
Mathis and Kevern (1975)	Wintergreen Lake (U.S.A.)	0.034 - 0.040	muscle
Stoeppler and Nürnberg (1979)	Baltic Sea, North Sea, and Mediterranean Sea	0.001 - 0.002	muscle

Among pesticides, only HCH and HCB were found in the liver of tench with values above the detection limit (Table 5). The average values for α–HCH and HCB between females and males were significantly ($P < 0.1$) different. Males showed a greater level of accumulation of these compounds than females.

Table 5. Average HCH and HCB levels in the liver of tench (results in µg/g wet weight ; n.d. : not detectable i.e. < 0.001 µg/g)

	Females	Males	Females & Males
Total HCH	0.081	0.134	0.110
α–HCH	0.019	0.045	0.033
β–HCH	n.d.	n.d.	n.d.
γ–HCH (Lindane)	0.061	0.087	0.075
HCB	0.010	0.016	0.014

Compared with concentrations usually occurring in food products from animal origin (fish, meat, fat...), only Lindane appeared to be at a significant level (C.I.E.L. 1976). However, this level was low compared to the Acceptable Daily Intake (A.D.I. : 0.01 mg/kg) recommended by the F.A.O. (1979). It is also below the maximum residue limits for milk products (0.2 mg/kg, fat basis) (F.A.O. 1978).

The levels of cadmium, lead, mercury, and organo-chlorine pesticides found in fish in this study should not be considered as contamination by these compounds. The fish examined were fit for human consumption. The preferential insertion of wastewater-treatment pond aquaculture in rural zones is evidently a factor minimizing the risks on public health. Nevertheless, it is necessary to recommend that careful thought be given to the use of wastewater ponds for aquaculture. It is well established that the concentrations of such micro-pollutants in sewage varies greatly, even in non-industrialized areas.

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