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Concentrations of PCBs, organochlorine pesticides and heavy metals (lead, cadmium, and copper) in fish from the Drôme river: Potential effects on otters (*Lutra lutra*)

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Abstract

In this study samples of ten species of fish were analyzed for concentrations of organochlorine pesticides, PCBs and heavy metals (Pb, Cd, and Cu). Fish were captured using electric fishing on ten sites along the Drôme river (Rhône-Alpes region). Quantitative determination of the organochlorine and PCBs compounds was performed by gas chromatography–electron-capture detection (GC–ECD). The concentrations of heavy metals were determined by atomic absorption spectrophotometry.

Samples contained detectable concentrations of lindane, PCBs, and heavy metals but at concentrations below the maximum residue limit (MRL). Non-parametric statistical analysis was performed to distinguish groups of sites with different levels of contamination. PCBs concentrations increased along the river with four groups of sites significantly different from each other. Cadmium concentrations were below the MRL. Lead contamination showed two groups significantly different and a repartition similar to PCBs. Copper contamination was correlated with the localization of vineyards.

We assessed the potential effects of contamination the otter (*Lutra lutra*). The concentrations of all pollutants analyzed in fish sampled in this study are lower than the threshold values described in literature. The Drôme river is relatively unpolluted river, and the establishment of otter populations should not be affected by pollution.

Keywords: Polychlorinated biphenyls; Organochlorine pesticides; Heavy metals; Pollution; Otter (*Lutra lutra*)

1. Introduction

Aquatic environmental quality currently receives a great deal of attention. Some organochlorine pesticides and polychlorinated biphenyls have been banned for several years (PCBs have not been produced since 1977) but are still found in the environment because of their tendency to persist. These contaminants and some heavy metals accumulate up the food chain and cause various harmful effects on wildlife (Yamaguchi et al., 2003).

In the late of 1950s and 1960s, a steep decline in otter (*Lutra lutra*) took place throughout most of western Europe, and was probably caused by such substances (Mason and Wren, 2001; Mason and Macdonald,

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1994). PCBs can have detrimental effects on the reproduction of otters (foetal toxicity, sterility) at levels as low as 50 μg kg^{-1} (Weber, 1990).

In France, otter population are instinct in the northern and eastern parts of the country but the species is still thriving on the Atlantic coast and in the Massif Central, where a population expansion has been recorded recently (Fonderflick et al., 1995; Rosoux et al., 1995).

In Rhône-Alpes, otters are present in Ardèche, where spraint samples and footsprints have been observed (Bendelé, 2000). In the Drôme (neighbouring department), the population's status is being studied, but otters seem to have disappeared.

The aim of this study was to evaluate PCB, OC, and heavy metal (Cd, Pb, and Cu) concentrations in fish of the Drôme river and to assess whether such burdens would be compatible with the survival of the otter or with the re-colonization of the area with otter. We chose the Drôme river because we think that otters could be back there soon and it is very crucial to monitor the water pollution and fish contamination, in order to maintain this colonizing population.

2. Materials and methods

2.1. Monitoring sites

The study area is located in the Rhône-Alpes region. The Drôme river is 106 km long and drains an area of approximately 1640 km^2. A total of 10 sites were selected along the river Drôme and its affluents Bez and Archianne. The sites were selected according to the localization of principal sources of pollution (upstream and downstream from the main urban and sewage discharge points) (personal communication, Water Agency—Maison des Ramières) (map 1).

2.2. Fish sampling

The fish were captured using electric fishing (powered by a 220 V electric generator) in July 2003, except for the Ramières (site 9) and Archianne (site 1) samples, which were collected in April 2003. Collected fish (12 species) were weighed, measured for total length and classified by species and size. Each batch (same species, same size) was ground and kept frozen (−20 °C) until further analysis.

2.3. Chemical analysis

2.3.1. OC and PCB analyses

A 1.0 g sample was taken from each batch and 30 ml of hexane/acetone 75/25 mix was added. Each sample was blended with an Ultraturrax® (Ika, Werke, Germany). The supernatant was removed and filtered through a phase separator membrane. This extraction procedure was performed twice. The extract was evaporated at 60 °C in a rotary evaporator. The dry extract was dissolved in 10 ml hexane.

Two ml of fuming sulphuric acid (SO_3 7%) were added and the test tube was shaken immediately. After centrifugation at 3000 rpm for 10 min, a part of supernatant was used for OC chromatography according to Berny et al. (2002) and 1 ml of the supernatant was added to 1 ml of 2% potassium hydroxide in ethanol for PCB analyses. The tubes were placed in a water bath at 50 °C for 30 min. At the end of this period, 2 ml ultrapure water were added, the samples were vortexed and centrifuged once again for 10 min. Samples underwent another acid hydrolysis with 1 ml sulphuric acid. After a final centrifugation, the final supernatant was removed and kept frozen until further analysis.

A gas chromatograph Hewlett–Packard HP5890 series 2 equipped with an electron-capture detector was used. A Restek® Rtx-5 column (Macherey-Nagel, Hoerdt, France) 60 m-long with 0.25 mm internal diameter and 0.25 μm film thickness was used. The temperature program was: 2 min at 75 °C, then 15 °C/min up to 150 °C, from 150 to 265 °C at 1.2 °C/min, then 25 °C/min up to 300 °C. Total duration of analysis was 110 min. Injection was performed automatically with an automatic injector (HP 6890). For each run, 2 μl were injected and each run was followed by a 15 min rinse at 300 °C. Each sample was run in duplicate.

Total PCB concentration was calculated as the sum of 16 individual peaks (IUPAC no. 28, 52, 77, 101, 105, 118, 126, 128, 138, 149, 153, 156, 169, 170, 180, and 187). All standards were purchased from CIL (St Foy la Grande, France) and purity was >99%. Linearity was determined between 5 and 100 ng/g ($r^2 > 0.99$ on standards and spiked samples). Limits of detection were between 0.5 and 1.0 ng/g for individual congeners.

2.3.2. Heavy metal analyses

Concentrations of lead and cadmium were determined for each sample. The grinded samples were dried for 1 h at 110 °C followed by 5 h at 180 °C. After drying, 0.3 g of sample was manually ground in a small dish and diluted in 1 ml of 50% sulfuric acid. The samples were digested for 16 h; digestion temperatures went from 20 °C to 700 °C over the first 10 h, then were held at 700 °C for 6 h. The digests were diluted in 2 ml of 50% nitric acid and gently dried on a hot plate. After cooling, the digests were diluted in 1 ml of 10% nitric acid and transferred to polypropylene tubes where they were diluted in ultrapure milliQ water.

Metal concentrations were analyzed with a Zeeman atomic absorption spectrometer (UNICAM 989 QZ, Thermo Optek, Roissy France) using element-specific lamps. Metal concentrations were calculated using a

standard curve, and the results were expressed in $\mu g\ kg^{-1}$ of dry weight.

2.4. Statistical analysis

Contaminant concentrations between groups (species) were compared using non-parametric multiple comparison tests. The Kruskal–Wallis test and the Mann–Whitney–Wilcoxon test were used to detect significant differences of contamination levels among fish species and monitoring sites using Stat View 5.01 (Sas Institute, Cary North Carolina). Whenever a significant difference was detected among sites by the Kruskal–Wallis test, 2×2 comparisons by means of the Mann–Whitney test were used to separate sites in groups (significant difference between groups, but no significant difference within each group).

3. Results and discussion

3.1. OC and PCB concentrations

The concentrations of organochlorine pesticides were measured in each batch of fish from each site (lindane, endosulfan, DDE, DDD, DDT, heptachlor, heptachlor epoxyde, aldrin, and methoxychlor). Most of the concentrations were below the limit of detection of chemical analyses performed. Only lindane (whose use was restricted in 1975 and banned in 1998 in France) was detectable at each site but the concentrations were small. The highest concentration was $300\ \mu g\ kg^{-1}$. However this concentration does not represent any risk for otters (De la Gorce, 1995).

The whole body concentrations of PCBs ($\mu g\ kg^{-1}$ wet weight) are presented in the Table 1. Among the sites, there were significant differences in total PCB concentration (Kruskal–Wallis, $p < 0.0001$). Fish were increasingly contaminated in downstream samples.

The mean PCB concentration ranged from 7.8 (site 1) to 56.9 (site 10) $\mu g\ kg^{-1}$ (wet weight). There are four groups of sites that were significantly different from one another in terms of PCB concentrations: sites 1, 4, and 5 < site 2 < sites 3 and 6–9 < site 10. ($p < 0.05$). These four groups are represented on Fig. 1.

We did not highlight a point source of PCB but there was a progressive increase along the river, compatible with the increase in urbanization and impacts of human activities.

Our results showed that in fish, PCB#153 and 138 were the most abundant (17.7% and 17.0% of the 16 congeners tested, respectively. This result is in accordance with other studies (Chevreuil et al., 1995; Bordajandi et al., 2003; Mazet et al., 2004; Perugini et al., 2004; Storelli et al., 2004) and is not surprising, since congeners 153 and 138 have long half-life (Liem et al., 1994). The bioconcentration of PCBs in aquatic organisms correlates with the degree of chlorination, the stereochemistry, and lipophilicity (Bordajandi et al., 2003).

In spite of the severity of the toxicity and the persistence of organic and metallic micropollutants, data concerning contamination levels in aquatic continental ecosystems, especially in fish, are limited in France. Therefore, it is extremely difficult to demonstrate either temporal or geographical trends of environmental contamination in the country.

Table 2 summarizes reported contamination levels in fresh water fishes in Europe in early 1990s and 2000s. Caution is required in making a direct comparison because of the differences between studies (year, species, methods of analysis, congeners). Table 2 suggests that the levels of PCBs in the fish of the Drôme are not as high as those of many other European rivers.

In 1995, a study (Michelot et al., 1995) in the Rhône-Alpes region compared the PCB contamination level in fish from 10 rivers (Rhône, Isère, Ain, ...). This study showed that the Drôme river was the least polluted with a mean of $73\ \mu g\ kg^{-1}$ (wet weight) whereas in the Rhône PCB concentrations ranged from 184 to $1445\ \mu g\ kg^{-1}$ (wet weight).

In all sites, total PCBs concentrations do not exceed the French Food Standards ($2\ mg\ kg^{-1}$) authorized for

Table 1
Concentrations of PCBs (in $\mu g\ kg^{-1}$ wet weight) in fish collected from the monitoring sites along the river Drôme (France) in 2003

Site	*n*	PCBs (median)	Min–max	Group (no) and median
1	21	**5.2**	(1.3–25.7)	Group 1—6.1
2	6	**16.8**	(10.2–20.9)	Group 2—16.8
3	10	**24.9**	(12.3–49.5)	Group 3—23.9
4	6	**7.9**	(2.7–11.4)	Group 1—6.1
5	10	**6.1**	(3.0–8.1)	Group 1—6.1
6	9	**20.2**	(8.5–37.9)	Group 3—23.9
7	7	**20.4**	(17.4–22.1)	Group 3—23.9
8	10	**23.0**	(14.0–40.1)	Group 3—23.9
9	42	**34.6**	(6.4–98.9)	Group 3—23.9
10	9	**47.4**	(29.0–145.9)	Group 4—47.4

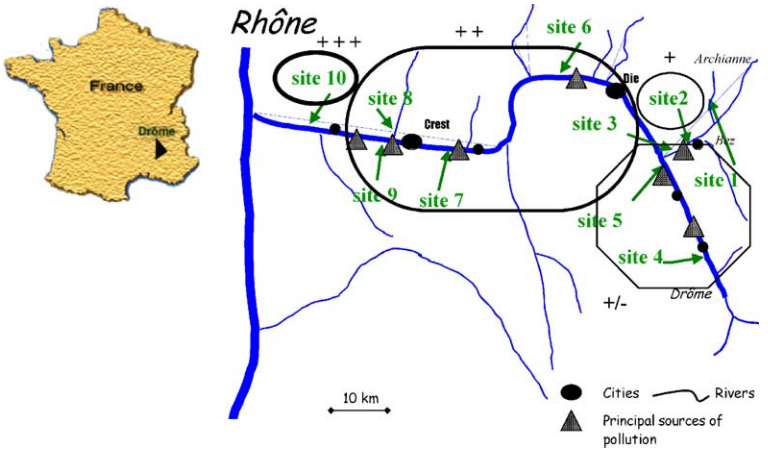

Fig. 1. Different groups of PCBs contamination based on fish samples from the Drôme river (2003) (sites 1, 4, and 5 < site 2 < sites 3 and 6–9 < site 10).

Table 2
PCB content (μg kg^{-1} of wet weight and dry weight) in the whole body of fish from European waters

		Species	PCB (wet weight) (min/max)	PCB (dry weight) (min/max)
Our study[a]	Drôme (France)	12	**7.8–56.9** (1.3/145.9)	**31.2–227.6** (5.2/583.6)
Chevreuil et al. (1995)	Seine (France)	2		**1300–16000**
Teil et al. (1996)	Seine (France)	3		**1792–1847**
Mazet et al. (2004)	Ardèche (France)	10	**135** (35/ 524)	
Lopez-Martin et al. (1995)	Network Catalonia (Spain)	12	**181** (9/2212)	
Bordajandi et al. (2003)	Turia (Spain)	14	**5.14–126**	
Bressa et al. (1997)	Po Delta (Italy)	1	**211–265**	
Binelli and Provini (2003)	Lake Iseo (Italy)	7	**105.9–786.4**	

[a] Our values in wet weight and converted to dry weight (wet wt. = dry wt. × 4) for comparison purposes.

Table 3
Concentrations of lead, cadmium, and copper (μg kg^{-1} dry weight) in fish collected from the monitoring sites along the Drôme river (2003)

Site	*n*	Lead	Cadmium	Copper
1	21	**14.3** (9.9–90.1)	**36.6** (13.1–64.4)	**9536.0** (2813.7–22291.0)
2	6	**10.0** (10.0–22.5)	**11.5** (8.4–18.2)	**13998.3** (10627.6–18911.1)
3	10	**13.0** (10.0–167.1)	**20.3** (10.3–38.0)	**13292.0** (6676.7–19977.5)
4	6	**10.0** (10.0–10.0)	**4.2** (2.5–11.5)	**7065.4** (4251.2–8253.1)
5	10	**10.0** (10.0–88.1)	**20.4** (9.8–96.3)	**8475.6** (5641.2–10124.1)
6	9	**62.3** (11.9–111.9)	**13.4** (7.7–41.6)	**4962.1** (3237.1–10509.2)
7	7	**10.0** (10.0–41.6)	**78.1** (22.1–118.7)	**4324.6** (2783.4–9364.1)
8	10	**67.0** (10.0–146.8)	**15.2** (10.1–36.2)	**4948.5** (2366.9–8224.8)
9	42	**18.3** (10.0–249.5)	**21.1** (6.7–43.5)	**12689.6** (8583.5–35528.5)
10	9	**92.0** (10.0–178.9)	**34.8** (15.6–70.2)	**3156.3** (1535.8–5684.8)

All values [median (minimum–maximum)]; *n* = number of individuals analyzed.

PCB in fish muscle since 16/02/1988 (French Food Safety Agency).

In our study, almost all the sites (site 1–8) have values lower than 50 μg kg^{-1}, which is considered as the "maximum allowable concentration" for reproduction in otters (Weber, 1990). Only two sites (9 and 10) have some values greater than 50 μg kg^{-1}, which is a concentration considered as "level as concern" for otter populations.

Leonards et al. (1994) proposed less severe thresholds. Total PCB concentrations in diet less than 145 μg kg^{-1} (wet weight) is a "concentration without

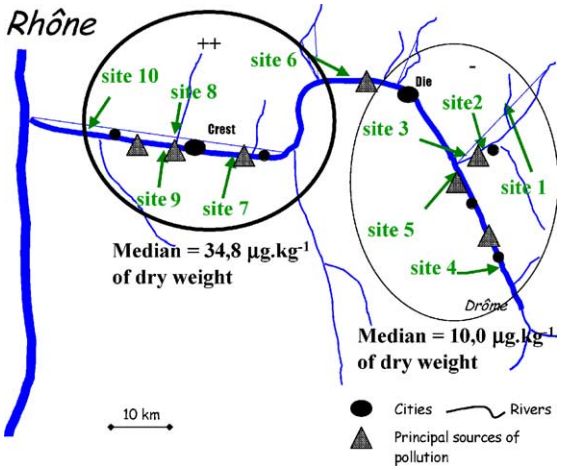

Fig. 2. Different groups of lead contamination based on fish samples from the Drôme river (2003) sites 1–5 < sites 5–10.

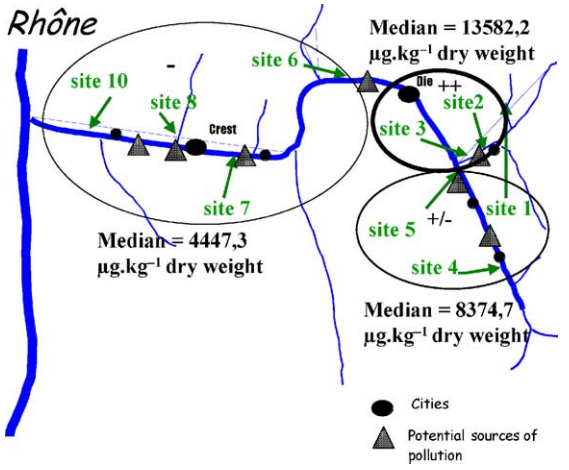

Fig. 3. Different groups of copper contamination based on fish samples from the Drôme river (2003) sites 6–8 and 10 < sites 1, 4, and 5 < sites 2 and 3.

effect'' whereas concentrations in the diet greater than 371 µg kg^{-1} (wet weight) are a ''critical level''.

Those thresholds are extrapolations from toxicological data obtained in American mink. It is not known whether otters are equally susceptible as or less susceptible than minks. In Drôme the concentration is less than the threshold so PCBs should not be a problem for the reproduction or the development of the population.

3.2. Heavy metal contaminations

Table 3 presents lead, cadmium, and copper concentrations in fish in the different sites.

The concentration levels of each heavy metal was significantly different from one another among sites. For lead, there are two groups of sites significantly different: one group upstream (sites 1–5) and one group downstream (sites 6–10) ($p < 0.0001$) (Fig. 2). For cadmium, there were significant differences among sites, but no group could be found. For copper, there are three groups that are significantly different from one another (sites 6–8 and 10 < sites 1, 4, and 5 < sites 2 and 3; Fig. 3).

Lead pollution has a distribution similar to the PCB pollution (Figs. 1 and 2). This is correlated with urbanization and density of population. On the other hand, copper distribution is very different. The concentration is higher in the upstream area and could be associated with the localization of vineyards (local production of

Table 4
Heavy metals content (µg kg^{-1} of dry weight) in fish from European waters

		Species	Pb	Cd	Cu
Our study		*12*	*10–92*	*4.2–78.1*	*3156–13998*
Chevreuil et al. (1995)	Seine (France)	2	600–8900	30–600	4100–6900
Teil et al. (1996)	Seine (France)	Bream	5700	700	5400
		Roach	1200	300	3400
		Perch	100	400	2200
Miramand et al. (1998)	Seine (France)		600–1600	13–110	1900–6500
Henry et al. (2004)	North Sea (France)	4	170 and 40 Pb µg kg^{-1} wet weight	230 and 10 Cd µg kg^{-1} wet weight	14800 and 1200 Cu µg kg^{-1} wet weight
Our study[a]		*12*	*2.5–23.0*	*1.1–19.5*	*789.0–3499.5*
Licata et al. (2003)	Messina (Italy)	1			
		Muscle	280.2	30.2	–
		Gills	200.2	20.0	–
Bordajandi et al. (2003)	Turia (Spain)	3			
		Trout	27.3	1.4	446.0
		Ell	101.8	4.9	977.0
		Barbel	62.0	1.8	793.0

[a] Our values in references converted to wet weight (wet wt. = dry wt./4).

Clairette de Die, sparkling wine), where the utilization of copper is common (fungicidal treatment of vineyards).

Considering the results in Table 4, it seems that the lead and cadmium contamination levels in fish are lower than those found in fish in different studies and the copper contamination is higher, due to the viticultural tradition.

Therefore the concentration of lead and cadmium for each sample of each site was compared with the MRL (EU Food Standard) in the muscle. No sample analyzed had values exceeding the set limits (lead 200 g kg^{-1} wet weight; cadmium 50 μg kg^{-1} (wet weight), European Regulation R466/2001 of 16/03/2001).

There are a small number of analyses for cadmium, lead, and copper in tissues of otters from several parts of Europe (Mason and Stephenson, 2001). Most of them give concentrations of cadmium, lead, and copper in liver and kidney (Gutleb et al., 1998; Mason and Macdonald, 1986; Mason and Stephenson, 2001). In general, all these concentrations are considered to be of no concern for otters, compared with other mammal species. But nothing is known specifically on toxicological effects of low tissue levels of others heavy metals such as cadmium, lead and copper on otters particularly in combination with other contaminants (Gutleb et al., 1998).

It is assumed, therefore based on MRL limits and our data, that the current concentrations of pollutants in the Drôme river should not pose a serious threat to the otters, and that chemical contamination should not be a limiting factor for re-colonization.

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