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Chemical contaminants in fish species from rivers in the North of Luxembourg: Potential impact on the Eurasian otter (*Lutra lutra*)

Aurore Boscher [a,*], Sylvie Gobert [b], Cédric Guignard [a], Johanna Ziebel [a], Lionel L'Hoste [a], Arno C. Gutleb [a], Henry-Michel Cauchie [a], Lucien Hoffmann [a], Gérard Schmidt [a]

[a] EVA-Department, Environment and Agro-biotechnologies, Centre de Recherche Public – Gabriel Lippmann, rue du Brill, 41, L-4422 Belvaux, Luxembourg

[b] MARE Center-Laboratoire d'Océanologie, Université de Liège, Bât. B6c, Sart-Tilman, B-4000 Liège, Belgium

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ABSTRACT

Contamination levels of PCBs, and of the heavy metals cadmium (Cd), lead (Pb) and mercury (Hg) were analyzed in four fish species from seven rivers in the North of Luxembourg. During August and September 2007, 85 samples of fish were collected belonging to four species: the stone loach (*Barbatula barbatula*, $n = 12$ pools), the chub (*Squalius cephalus*, $n = 36$), the barbel (*Barbus barbus*, $n = 23$) and eel (*Anguilla anguilla*, $n = 14$). The concentration of seven indicator PCBs ($\sum_7$PCBs) reached a mean of 39 ng g^{-1} and varied between 4.0 and 346.2 ng g^{-1} (wet wt) depending on the site and species. Fish collected at Wallendorf on the Our River and sites on the Wiltz and the Clerve rivers showed the highest concentrations for PCBs. In comparison with 1994, PCB levels in fish decreased strongly during the last decade in these rivers. Lead was detected at low levels (0–181.4 ng g^{-1} wet wt). Mercury concentrations ranged between 10.3 and 534.5 ng g^{-1} (wet wt) exceeding maximum tolerable levels for human consumption of 500 ng g^{-1} in two fish out of 85. Chubs and eels from the Sûre River were the most contaminated by mercury. Cadmium levels varied between 4.0 and 103.9 ng g^{-1} (wet wt). In addition to mercury in fish, cadmium was the most problematic pollutant on the Our, the Wiltz, the Clerve and the Troine Rivers, because values found in 20% of fish exceeded the threshold of about 10–50 ng g^{-1} (wet wt) recommended for human health.

The total PCB level predicted to accumulate in livers from otter potentially feeding on these fish based on a previously published mathematical model is 37.7 μg g^{-1} (lipid wt), which is between a proposed "safe level" and a "critical level" for otters. Rivers in the North of Luxembourg are thus to some extent polluted, and the establishment of otter populations could be affected by current levels of contamination.

1. Introduction

The omnipresence of hazardous chemicals in the environment is a threat for the biodiversity and human health. Among these contaminants, PCBs (polychlorinated biphenyls) and heavy metals are known for their toxic effects, and are often found in high concentrations in the environment leading to negative impacts on wildlife.

PCBs consist of 209 congeners with varying numbers and positions of the substituted chlorine atoms. They have been produced in large quantities in the form of technical mixtures such as Aroclor 1242, 1254 and 1260, the last two numbers corresponding to the percentage in weight of chlorine. PCBs have been banned since the 1980s, and are listed among the POPs (Persistent Organic Pollutants) in the Stockholm convention. PCBs are endocrine disrupting compounds (Brouwer et al., 1998) and play a role in immunological, neurobehavioral, dermal and ocular alterations, but also reproductive toxicity and cancer (Wren et al., 1987; Arnold et al., 1995; Chu et al., 2003). PCBs accumulate in sediments and bioaccumulate in the aquatic food chain, due to their physico-chemical characteristics, such as their high lipophilicity and resistance to degradation. Birds and mammals, which are at the top of the aquatic food chain accumulate these contaminants and become vulnerable to their effects (Leonards et al., 1994; Cifuentes et al., 2003).

Among heavy metals dispersed in the environment, cadmium (Cd), lead (Pb) and mercury (Hg) are ubiquitous and have potentially high hazardous effects. In humans and animals, cadmium affects a number of organs: kidney, lung, bones, placenta, brain and the central nervous system. Other effects that have been observed include reproductive toxicity, hepatic effects, hematological effects, and immunological effects (ATSDR, 2008). Exposure to high levels of metallic, inorganic, or organic mercury can permanently

* Corresponding author. Tel.: +352 47 02 61 436; fax: +352 47 02 64.
E-mail address: boscher@lippmann.lu (A. Boscher).

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damage the brain, kidneys, and developing foetus. In humans, effects on brain functioning may result in irritability, shyness, tremors, changes in vision or hearing, and memory problems (ATSDR, 1999). Methylmercury (CH_3Hg) is the most toxic form of mercury, which affects the immune system, alters genetic and enzyme systems, and damages the nervous system. Lead is also a poisoning metal, and the organs or systems, which may be affected, are the hematological, nervous, cardiovascular, reproductive and immune systems as well as the kidney (ATSDR, 2007).

Hence, monitoring these aquatic pollutants is an important step in the management of aquatic and semi-aquatic wildlife. The biomagnification of these toxic substances may exert negative effects on top predators such as the Eurasian otter (*Lutra lutra*) (MacDonald and Mason, 1994; Smit et al., 1996a; Smit et al., 1998; Mason, 1998; Roos et al., 2001; Kruuk, 2006).

The Eurasian otter is a semi-aquatic mammal, which can be found along rivers, lakes, ponds, streams and rocky coasts. Its diet consists mainly of fish (50–94%), but can also include birds, insects, frogs, crustaceans and sometimes, small mammals. Generally, otters are opportunists and take a much higher proportion of abundant species, which are easier to catch than scarce species.

The Eurasian otter strongly declined since 1970 in Western and Central Europe. The otter is considered as very rare in Luxembourg (Schmidt et al., 2008). This decline may be partly due to the increasing contamination of the aquatic ecosystems by PCBs and mercury after the Second World War (Kruuk and Conroy, 1996; Kruuk et al., 1997; Mason, 1998). In a study realized in 1994 by the Otter Group in Luxembourg, the contamination of chub, barbel and eel by PCBs was considered as critical for the otter (Essoe, 1995; Groupe Loutre Luxembourg, 1997; Hugla et al., 1998). The PCB levels measured in fish from all rivers of Luxembourg, varied between 40 and 3500 ng g^{-1} (wet wt) with 50% above the critical limit of 500 ng g^{-1} (wet wt) defined by Weber (1990) for the otter (Hugla et al., 1998).

The aims of the current study were (1) to evaluate changes of contamination in fish from rivers in Luxembourg since 1993–94, (2) to study heavy metal levels in fish and their importance for otter and (3) to calculate from current PCB concentrations in fish expected tissue concentrations in otters in case of recolonization of rivers in Luxembourg and to compare them with postulated effect levels.

2. Animals, materials and methods

2.1. Sample collection

The study area is located in the North of Luxembourg, where ten sites were selected along seven rivers (Fig. 1).

Fish were caught by electro-fishing between August and September 2007. Fish species collected were the stone loach (*Barbatula barbatula*), the chub (*Squalius cephalus*), the barbel (*Barbus barbus*) and the eel (*Anguilla anguilla*). Due to strict restrictions from the Water Management Administration, only three individuals per species were caught, except for stone loach, for which about ten individuals were collected and pooled. The collected fish were then weighed, measured and some scales were taken in order to determine their age by scalimetry.

2.2. Sample preparation

Whole fish were crushed, homogenized and lyophilized. The water content was determined gravimetrically, and the dried samples were kept at −20 °C until analyses.

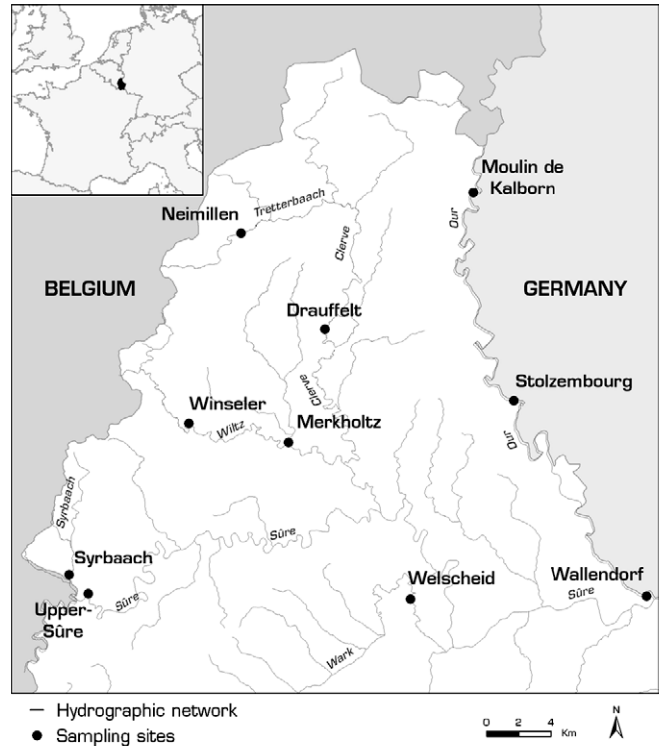

Fig. 1. Map of sampling locations in the North of Luxembourg (the location of Upper-Sûre includes three stations of electrofishing close to each other).

2.3. PCB analyses

Dried samples were extracted by accelerated solvent extraction using an ASE100 from DIONEX (Sunnyvale, USA) equipped with 34 mL extraction cells, which were prepared as follows: at the bottom of the cell a cellulose filter was placed on which a mixture of 0.5–1.5 g of sample spiked with an internal standard (PCB 209), and a pre-washed quartz sand were added until the cell was full, and then a second cellulose filter was placed on top of the cell. The samples were extracted using the following conditions: n-hexane as solvent extraction, 100 °C extraction temperature, 9 min static time, two extraction cycles, the flush volume was 100%, the purge time was set to 100 s by N_2, and the extraction pressure was comprised between 1500 and 1700 psi.

Extracts were collected in 100 mL glass bottles and traces of water were removed by filtration through 4 g of Na_2SO_4 and directly transferred into 100 mL round bottle flasks. The solvent was removed in a rotary evaporator and the fat content was determined gravimetrically. An aliquot was taken up into 1 to 2 mL of hexane and cleaned by adsorption chromatography on a column containing 4 g of florisil, 2 g of activated silica (200 °C, 12 h) impregnated with sulphuric acid (3:2, w/w), a second layer of 2 g of florisil and 2 g of Na_2SO_4. PCBs were eluted with 30 mL of *n*-hexane/diethyl ether (85:15, v/v). Solvent was reduced by a rotary evaporator down to approximatively 800 μL, and then transferred to vials for GC/MS injections.

Quantification of PCBs (IUPAC numbers: 18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 170, 180, 194, 209, standards purchased from LGC Promochem) was carried out on a gas chromatograph Hewlett–Packard HP 6890 series equipped with an autosampler split/splitless injector, and a mass selective detector Agilent 5973 Network, with an ionisation by electronic impact (70 eV). A Restek® Rxi-5ms column (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness) was used. Helium was used as carrier gas with a constant flow of 1.2 mL min^{-1}. A splitless injection volume of 1 μL was used and the injector temperature was maintained at 280 °C. The temperature of the oven was programmed from 80 °C to 280 °C at 5 °C min^{-1} (held for 5 min), to 320 °C at 30 °C min^{-1} (held for 5 min). Analyses were operated in single ion monitoring (SIM) mode on the basis of four target ions and the retention time of the compound to that of a known standard. PCBs were quantified using an internal standard (phenanthrene d10) and the limit of quantification for PCBs was 5 ng g^{-1} (dry weight). The recovery of the method was evaluated by spiking a hake (*Pollachius virens*) sample according to this procedure with the PCB mix at 30 ng g^{-1} and PCB recoveries were between 81% and 100% ($n = 6$). Each sample was analyzed in duplicate.

2.4. Heavy metal analyses

Five hundred milligrams of dried fish were mineralised in Teflon tubes in a microwave digester (Anton Paar Multiwave 3000) (Graz, Austria) with a mixture of HNO_3 (7 mL) and H_2O_2 (3 mL). The power was increased linearly from 0 to 1400 W during 10 min, then held at 1400 W for 5 min, and was finally decreased linearly from 1400 to 0 W during 20 min. After cooling, samples were diluted to 50 mL with deionised water in a volumetric flask. Cd and Pb were quantified by inductively coupled plasma mass spectrometry (ICP/MS) carried out on a Perkin Elmer Elan DRC-e. Total Hg concentration was analyzed by a Direct Mercury Analyser (DMA Milestones). Quality control measurements included analyses of certified reference materials, namely IAEA 359 for Pb, and Cd, and DORM-1 for Hg. Detection limits were 12, 30 and 1 ng g^{-1} in dry weight respectively for Cd, Pb, and Hg. Each sample was analyzed in triplicate for Cd and Pb, and in duplicate for Hg.

2.5. Statistical analysis

Principal component analyses (PCA) were carried out using PRIMER five software (Primer-E Ltd.), and were used to explore relationships between sites or species and contaminants, lipid content and weight.

3. Results and discussion

3.1. PCB contamination

The contribution of the main individual congeners (PCBs: 101, 149, 118, 153, 138 and 180) to the sum of thirteen PCBs is shown in Fig. 2. PCB 153 (hexa-CB) is the main contributor with a

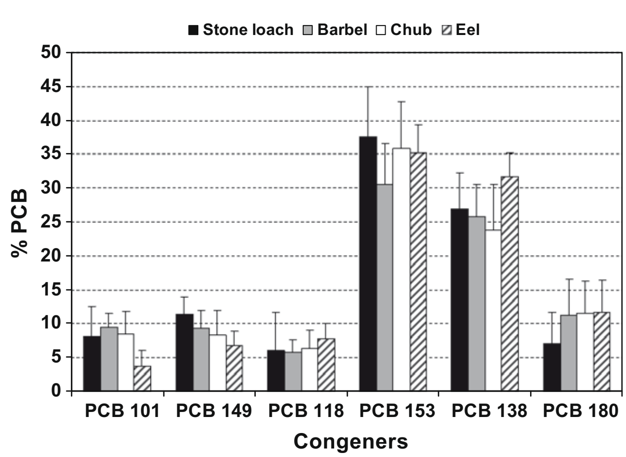

Fig. 2. PCB profiles in fish species from all sites (mean percentage of main PCB congeners within the $\sum_{13}$PCBs, error bars represent standard deviation).

mean ± SD proportion of 35 ± 7% when all fish species are considered together, followed by PCB 138 (hexa-CB) and PCB 180 (hepta-CB) with proportions of 26 ± 6% and 11 ± 4%, respectively. In the literature, it appears that congeners 153, 138 and 180 are the most persistent and are not metabolized by fish (Niimi, 1996; Manirakiza et al., 2002; Bordajandi et al., 2003). PCB patterns indicate that pollution is mainly attributed to the technical mixtures of Aroclor 1260 (85%) and Aroclor 1254 (15%). Hugla et al. (1998) came to similar conclusions in their paper on fish contamination in Luxembourg. Relative portions of different congeners are, however, not exactly the same as the technical mixture because PCB congeners are degraded or metabolized at different rates (Buckman et al., 2006; Baba and Katayama, 2007).

Regarding species and PCA analysis, eel has the highest levels of PCBs. This can be explained mainly by their high lipid content, which was found to be 23.1 ± 5.2% (wet wt) against 5.7 ± 1.3% for barbels, 3.8 ± 1.1% for stone loaches and 3.3 ± 2.0% for chubs (Table 1). $\sum_{13}$PCB concentrations were in the range of 4.7–49.6 ng g^{-1} for stone loaches, 5.3–153 ng g^{-1} for chubs, 8.7–105.5 ng g^{-1} for barbels and 55.4–388.4 ng g^{-1} for eels. Stone loaches were less contaminated than chubs in spite of a similar lipid content, but the fish age and a different diet could be responsible for such a difference (Olsson et al., 2000; Fisk et al., 2001). The Wiltz River, Wallendorf downstream of the Our River and Drauffelt (Clerve River) presented the highest contamination by PCBs among all sites. The origin of pollution on Wiltz River was attributed to the presence of two dumping sites upstream of Winseler and two others situated between Winseler and Merkholtz. It is possible that the origin of the pollution at Wallendorf comes from fish originating from contaminated sites on the Lower Sûre or the Alzette River, which are in connexion with the Our River. The pollution on the Clerve River (Drauffelt) could come from the industrial area of Troisvierges but there is no direct proof for that.

Fig. 3 presents the evolution of PCB concentrations in the North of Luxembourg in the same fish species between 1994 and 2007 (Essoe, 1995; Hugla et al., 1998). Results show a strong decrease of PCB contamination, except for stone loach on Wiltz River indicating that the presence of dumping sites still has a negative impact. This decrease is by a factor of about three during the last thirteen years.

In Luxembourg, Dauberschmidt and Hoffmann (2001) reported total PCBs ((PCB 138 + PCB 153)*3) in fish from Sûre, Our, Syr and Moselle Rivers. The Sûre reservoir was the least contaminated with 3.3 ng g^{-1} (wet wt) in perch ($n = 3$) and 205 ng g^{-1} in eel ($n = 3$), whereas the Moselle River was the most contaminated with 481 ng g^{-1} in perch ($n = 2$) and 4436 ng g^{-1} in eel ($n = 3$). PCB concentrations in gudgeon (*Gobio gobio*, $n = 2$) tissues from Hoesdorf (Our River, close to Wallendorf) amounted to 76.6 ng g^{-1} and 20.1 ng g^{-1} in the skin and the muscle, respectively.

In the same country, Biomonitor (2004) reported total PCB levels ($\sum_7$PCBs*2) in ng g^{-1} (wet wt) in the fillet of gudgeon, roach (*Rutilus rutilus*), brown trout (*Salmo trutta fario*) and eel, caught in 15 rivers between 2000 and 2002. In the latter report, the most polluted rivers were the Moselle, Sûre (Lower-Sûre), Wiltz, and Alzette. In the Moselle River, PCB concentrations were of 1092 and 268 ng g^{-1} in eel ($n = 3$) and roach ($n = 2$), respectively. At Rosport, in the Sûre River (Lower-Sûre), concentrations reached of 278 and 214 ng g^{-1} in eel ($n = 1$) and roach ($n = 2$), respectively. In two sites of the Wiltz River, PCB levels in trout were of 108 and 150 ng g^{-1}. Dauberschmidt and Hoffmann (2001) and Biomonitor (2004) showed that fish from the Moselle were the most contaminated; however, fish from Lower-Sûre and the Wiltz River also presented high contents in PCBs.

Mazet et al. (2005) reported that the mean PCB levels in the whole fish from the Drôme River in France ranged from 7.8 (site 1, $n = 21$) to 56.9 ng g^{-1} wet weight (site 10, $n = 9$) for $\sum_{16}$PCBs,

Table 1
Mean concentrations of $\sum_{13}$PCBs, $\sum_7$PCBs, total PCBs, Cd, Pb and Hg in four fish species from rivers of the North of Luxembourg during the summer 2007 (given in ng g^{-1} wet weight).

Site (river)	*n*	Species	Weight (g) mean (min–max)	Water (%)	Fat (%)[a]	$\sum_{13}$PCBs mean ± SD (min–max)	$\sum_7$PCBs	Total PCBs[b]	mean ± SD (min–max)		
									Cd	Pb	Hg
Syrbaach (Syrbaach)	Pool	Stone loach	4	77.6	4.0	4.7	4.0	8.0	15.9	31.3	46.4
	3	Chub	318 (127–418)	77.9	2.3	13 ± 6 (6.9–18.1)	11.3	22.6	30 ± 35 (8.5–69.6)	26 ± 45 (0.0–77.1)	87 ± 81 (14.6–174.5)
Upper-Sûre (Sûre)	Pools	Stone loach	3	76.6	3.5	8.9 ± 2 (6.9–10.3)	8.1	16.2	24 ± 9 (17.3–33.6)	37 ± 20 (22.7–60.0)	37 ± 22 (13.5–57.6)
	13	Chub	1084 (271–1927)	77.7	2.5	22 ± 12 (7.8–50.3)	18.9	37.7	27 ± 9 (15.8–45.7)	18 ± 9 (0.0–35.0)	298 ± 175 (60.8–534.5)
	8	Barbel	203 (32–530)	73.3	5.3	17 ± 6 (8.7–31.0)	14.8	29.6	28 ± 8 (18.1–39.2)	34 ± 26 (0.0– 71.3)	96 ± 118 (35.1–381.9)
	9	Eel	976 (440–1324)	57.7	21.4	56 ± 21 (36.3–99.9)	52.5	105.2	21 ± 10 (7.4–39.9)	34 ± 10 (18.9–53.5)	317 ± 110 (204.7–508.3)
Moulin de Kalborn (Our)	Pool	Stone loach	4	74.6	5.6	16.7	13.9	27.8	60.2	64.6	77.0
	2	Chub	71 (61–81)	79.4	2.3	13 ± 5 (10.0–16.9)	10.8	21.7	27 ± 14 (17.1–36.3)	0.0	36 ± 14 (25.8–46.3)
	3	Barbel	105 (27–120)	71.1	7.0	26 ± 7 (18.5–33.5)	20.2	40.4	64 ± 15 (46.6–75.2)	61 ± 17 (47.0–79.3)	25 ± 14 (10.3–38.3)
Stolzembourg (Our)	Pool	Stone loach	4	76.2	4.5	12.3	10.7	21.4	44.8	43.2	27.8
	3	Chub	763 (169–1920)	75.5	5.5	20 ± 6 (13.6 26.0)	17.2	34.5	11 ± 4 (7.8–14.6)	3 ± 6 (0.0–10.3)	83 ± 88 (22.9–183.8)
	3	Barbel	44 (31–54)	73.0	6.6	26 ± 6 (21.0–33.0)	22.1	44.3	43 ± 20 (27.8–66.0)	38 ± 29 (11.0–68.9)	26 ± 5 (21.5–31.8)
Wallendorf (Our)	Pool	Stone loach	6	75.6	4.6	49.6	40.9	81.8	20.7	61.0	26.4
	3	Chub	216 (93–286)	70.2	7.0	112 ± 38 (78.1–153.0)	97.6	195.3	12 ± 7 (7.1–20.5)	17 ± 15 (0.0–26.0)	61 ± 25 (34.4–83.4)
	3	Barbel	111 (99–119)	73.2	5.3	94 ± 12 (81.7–105.5)	79.1	158.2	41 ± 23 (18.1–64.7)	30 ± 27 (0.0–49.9)	28.3 ± 4 (24.3–31.2)
	2	Eel	859 (615–1102)	40.2	31.0	387.6 ± 1 (386.9–388.4)	346.2	692.5	34 ± 11 (25.9–41.5)	50.0 ± 0.2 (49.9–50.2)	176 ± 13 (167.2–185.2)
Welscheid (Wark)	Pool	Stone loach	4	78.9	2.9	9.2	7.8	15.6	11.4	46.5	44.7
	3	Chub	630 (81–1636)	78.1	2.8	19 ± 5 (14.9–23.7)	16.5	33.0	6.9 ± 3 (4.0–10.0)	0.0	102 ± 99 (44.4–216.3)
Winseler (Wiltz)	Pool	Stone loach	7	77.7	2.2	38.7	32.0	64.1	60.2	65.9	49.3
	3	chub	94 (64–120)	74.0	3.4	40 ± 14 (29.8–55.5)	33.3	66.6	22 ± 14 (6.7–31.0)	4 ± 8 (0–13.3)	46 ± 18 (28.9–64.0)
Merkholtz (Wiltz)	Pool	Stone loach	4	76.6	4.5	44.3	39.6	79.2	89.0	40.4	33.6
	3	Chub	98 (94–103)	72.2	4.2	52 ± 8 (46.4–61.3)	42.8	85.5	36 ± 11 (23.4–44.3)	67 ± 100 (0–181.4)	62 ± 6 (56.3–68.1)
	3	Barbel	45 (28–80)	71.9	5.1	61 ± 20 (44.3–82.7)	50.8	101.6	58 ± 23 (37.4–82.6)	29 ± 29 (9.3–62.3)	46 ± 33 (25.3–84.2)
Neimillen (Troine)	Pool	Stone loach	5	77.3	3.2	4.3	3.8	7.6	57.5	55.6	37.8
	3	Chub	316 (87–597)	77.4	2.0	11 ± 5 (5.3–15.4)	11.0	22.0	49 ± 9 (42.8–59.5)	40 ± 37 (8.3–80.2)	142 ± 101 (53.1–252.4)
Drauffelt (Clerve)	Pool	Stone loach	5	78.3	3.4	9.9	8.2	16.5	35.8	30.2	165.4
	3	Barbel	1348 (1159–1485)	74.1	5.0	51 ± 5 (46.2–55.0)	47.4	94.7	78 ± 34 (39.1–103.9)	64 ± 76 (15.2–152.2)	127 ± 53 (67.1–170.1)
	3	Eel	721 (502–840)	59.3	22.4	87 ± 8 (79.3–94.9)	78.3	156.6	64 ± 15 (49.7–80.0)	45 ± 11 (32.7–51.6)	159 ± 16 (141.3–171.6)

n = number of individuals analyzed, stone loaches were pooled for analyses.
[a] Percentage of fat in fresh fish.
[b] Total PCBs calculated from seven indicator PCBs ($\sum_7$PCBs $\times$ 2 = $\sum_{total}$PCBs).

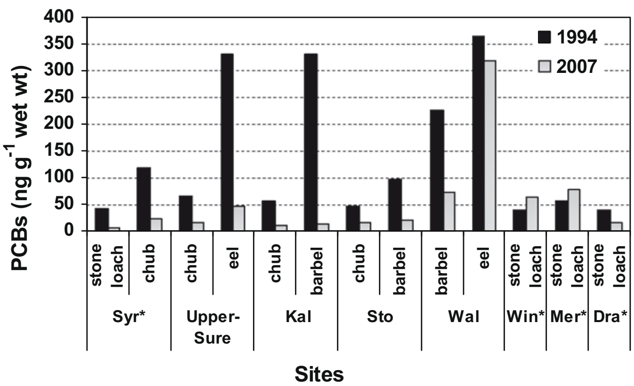

Fig. 3. PCB evolution from 1994 (Essoe, 1995; Hugla et al., 1998) to 2007 (present study). Data are expressed in ng g^{-1} (wet wt), and given for 5 PCBs (IUPAC numbers: 52, 101, 138, 153 and 180), excepted for sites*, where data are given for total PCBs. (Syr = Syrbaach, Kal = Moulin de Kalborn, Sto = Stolzembourg, Wal = Wallendorf, Win = Winseler, Mer = Merkholtz, Dra = Drauffelt).

respectively for the least and the most polluted site. These concentrations are similar to our data, except for Wallendorf. In Belgium, Maes et al. (2008) reported PCB levels (10 congeners) in muscle tissues of 2526 eels from Flanders, and they found PCB concentrations ranging from 3.5 to 12 455 ng g^{-1} with a mean of 605 ng g^{-1} wet weight. In another study in Belgium, Thomé et al. (2005) reported mean PCB concentrations (seven indicator congeners) in the muscle, expressed in ng g^{-1} (wet wt): from 2 to 635 in chub (n = 268), from 57 to 1958 in eel (n = 151) from the Meuse Basin, with two highly contaminated rivers (Vesdre and Sambre Rivers), and from 4 to 16 in chub (n = 26) and 40 in eel (n = 5) from the Rhine basin. Chevreuil et al. (1995) found a ratio of 1.4 for roach and of 2.4 for perch between PCB concentrations in the whole fish and concentrations in the muscle. Therefore, PCB levels can be considered lower in the fillet than for the whole body indicating that PCB concentrations found in Belgium are higher than in the North of Luxembourg.

3.2. Heavy metal contamination

Cadmium, lead and mercury levels in fish species from different sites are reported in Table 1 and are expressed in ng g^{-1} (wet wt).

For cadmium and lead the most contaminated species were stone loach and barbel. This observation can be explained by the behaviour of these species in the river. Stone loaches live most of the time on sediments, being therefore always in direct contact with pollutants. Barbels are feeding in the deeper area of the sediment layer, being also in direct contact with the pollutant. The bioconcentration factors (BCF) for fish species concerning cadmium and lead are 3–2213 and 300, respectively (ATSDR, 1993; IAEA, 1994). BCFs of lead and cadmium in fish are low, and there is not really a high bioaccumulation through the trophic chain, but rather a transfer between sediments and fish.

Concerning mercury, chubs and eels from the Upper-Sûre were highly contaminated. Two fish even exceeded values for human consumption. Furthermore, chubs containing high mercury levels were the largest and the oldest ones as shown by PCA analysis (see Section 3.3), as well as by correlation analysis (Figs. 4a and 4b). Rask and Metsälä (1991) and Burger et al. (2002) have already reported the relation between weight, length and age of the fish with mercury levels. Besides, it was often reported that methyl mercury is the major form of mercury (60–85%) present in fish (Houserová et al., 2007; Magalhães et al., 2007), and this form is more toxic than inorganic mercuric form. The BCF of organic mercury ranges from 10 000 to 85 700 (ATSDR, 1999), which is seven times higher than inorganic mercury. The bioaccumulation of mercury in fish is more important than for cadmium and lead.

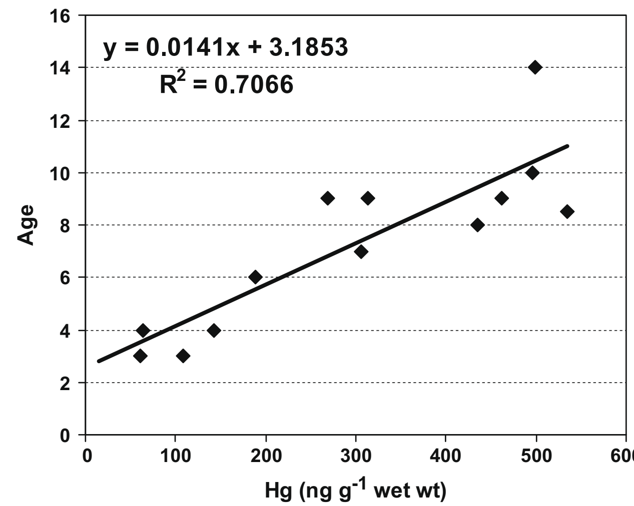

Fig. 4a. Correlation between age (year) and Hg concentration (ng g^{-1} wet wt) for 16 chubs from the Sûre River.

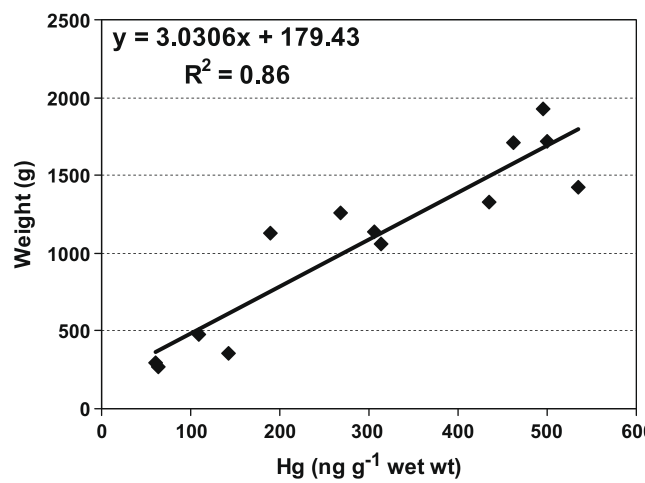

Fig. 4b. Correlation between weight (g) and Hg concentrations (ng g^{-1} wet wt) for the chubs from the Sûre River.

Our data have been compared with those of the previous study (1994) (Groupe Loutre Luxembourg, 1997). In fish from Syrbaach, Upper-Sûre, Stolzembourg, Winseler, Merkholtz and Drauffelt, a general decreasing trend of lead concentration was noted, whereas for cadmium and mercury such a trend is not obvious. For lead, this trend was also observed in the atmosphere and in the seagrass from the Mediterranean, due to the progressive reduction in the use of Pb additives in automobile fuel (Ridame et al., 1999; Gosselin et al., 2006).

In Luxembourg, Biomonitor (2004) reported mercury concentrations between 7 and 250 ng g^{-1} wet weight. The highest levels were found in trouts (n = 3) from Merkholtz (Wiltz River) and Upper-Sûre (Sûre River), with respectively 250 and 240 ng g^{-1}. In Flanders (Belgium), Maes et al. (2008) reported heavy metal concentrations in eels (n = 2526), with a mean of 116.6, 15.8, and 81.2 ng g^{-1} (wet wt) found for Hg, Cd and Pb, respectively. Compared to Flanders, fish from Luxembourg were more polluted by heavy metals.

3.3. Principal component analysis (PCA)

An ordination analysis based on data from 85 individuals was performed in order to identify potential relationships between species, sites and the contamination level in fish as well as the weight and lipid content of the fish. The first (PC1) and the second principal components (PC2) explained 36.2% and 26.9%, respectively. Fig. 5 shows the score plot of individuals and variables of the PC1 and PC2, additionally. Distribution of variables in Fig. 5 shows a correlation between Hg and weight on one hand, and between lipid content and PCBs on the other hand.

Regarding sample distribution, eels and chubs appear to be quite well discriminated. Eels from Wallendorf (Our River) and Drauffelt (Clerve River) are isolated by PCB contamination. Chubs from Upper-Sûre (Sûre River) were mainly influenced by the mercury contamination. Barbels and stone loaches from Merkholtz (Wiltz River), Wallendorf and Kalborn (Our River) were mainly influenced by the cadmium and lead contamination.

3.4. Potential risk for the Eurasian otter

3.4.1. PCBs

For the otter, the toxic effects of PCBs are probably particularly mediated by their effect on Vitamin A metabolism and the disruption of thyroid hormones (Simpson et al., 2000). Smit et al. (1996b) proposed two levels for the evaluation of PCBs in fish: a safe level corresponding to a 1% reduction in hepatic retinoid level (EC_1) for $\sum_7$PCBs indicators at 6 ng g^{-1} in wet weight, and a critical level (EC_{90}) for $\sum_7$PCBs at 14 ng g^{-1} in wet weight, which corresponds to 28 ng g^{-1} for total PCBs. In addition, other thresholds based on less sophisticated limits, were presented in the literature for the concentration of total PCBs in fish. The Dutch government recommended that average total PCBs in fish should not exceed 25 ng g^{-1} in wet weight for the conservation of otters (Ministerie van Landbouw en Visserij, 1989). MacDonald and Mason (1994), based on a calculation with prey species and spraints, proposed a safe threshold at 26 ng g^{-1} and a threshold at 50 ng g^{-1} with reproductive failure for otter. Leonards et al. (1994), according to extrapolations from data obtained in American mink (*Mustela vison*), proposed a safe threshold at 145 ng g^{-1} and a critical threshold at 371 ng g^{-1} wet weight. However, Lafontaine and De Alencastro (1999) presented the otter status in Europe with data collected from different others studies and it appeared that otter disappeared when total PCB concentrations in fish were above 145 ng g^{-1} (excepted for gudgeons in Allier (France)), whereas otter was always present when PCB concentrations were below 145 ng g^{-1} (excepted for trouts from Switzerland and for soufies (*Leuciscus souffia*) from

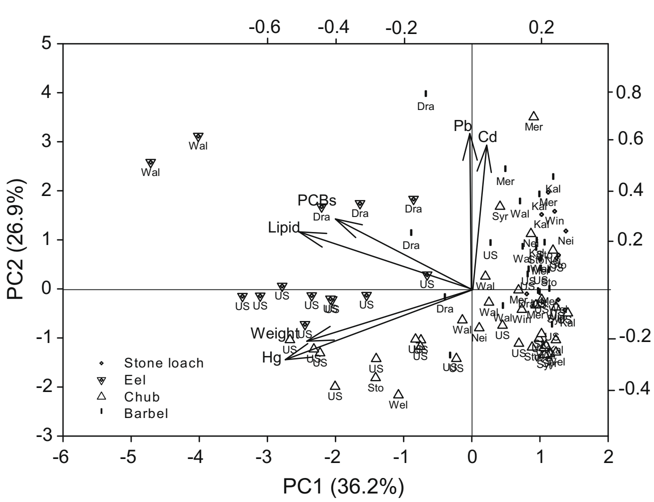

Fig. 5. Biplot PCA of 85 individuals and six variables, PC1 versus PC2. (Syr = Syrbaach, US = Upper-Sûre, Kal = Moulin de Kalborn, Sto = Stolzembourg, Wal = Wallendorf, Win = Winseler, Mer = Merkholtz, Wel = Welscheid, Nei = Neimillen, Dra = Drauffelt).

Buëch, where otters have disappeared). According to these observations, a level of 50 ng g^{-1} in fish (wet wt) was chosen in the present study as the threshold with reproductive effects for the otter, and a second level at 145 ng g^{-1} in fish (wet wt) was chosen as the critical threshold for the survival of otters.

In our study, at six sites out of ten, values of total PCBs in fish (Table 1) were below the limit, which is potentially harmful for the reproduction in otters (50 ng g^{-1}). Eels, for which concentrations of PCBs are often above this limit, are an exception while it has to be considered that eels represented only a minor part of the biomass for these rivers (2–14%, data provided by the "Administration des Eaux et Forêts du Luxembourg: Service Chasse et Pêche" in 1988, and the "Administration de la Gestion de l'Eau du Luxembourg: Service Pêche" in 2005). Thus, in these sites PCB levels should not be a real threat for a future otter population. At other sites, PCB levels in fish represent a potential problem for the reproduction of otters, and PCB concentrations found in chub, barbel and eel from Wallendorf (Our River) were high, exceeding the critical limit for the otter survival.

Otters are opportunistic hunters and take a much higher proportion of abundant species that are easier to catch (Mason and MacDonald, 1986). As seen before, eels are the most contaminated species, but they are not the most abundant. Therefore the mean concentration of PCBs for all fish species will give an overestimation of the risk for otters. Nevertheless, we have to take eels into account, because this fish will be included in the diet of otters. Under these assumptions, sites representing a risk for otters are in the Sûre River, at Winseler and Merkholtz (Wiltz River), at Drauffelt (Clerve River) and at Wallendorf (Our River) where the average of total PCBs reached 52, 66, 92, 110 and 281 ng g^{-1} (wet wt), respectively.

Projection of PCB levels in otters based on known concentrations in fish could be used in feasibility studies for recolonization. A mathematical model was established to calculate the PCB levels in otter livers from food levels (Gutleb and Kranz, 1998). This model was adapted for the otter using specific parameters, like the assimilation efficiency of otter for PCBs, the food ratio, and the excretion constant. The total PCB level predicted to accumulate in otter livers with this model in case the otter would recolonize Luxembourg was 37.7 µg g^{-1} (lipid weight). This was calculated for a PCB exposure of 730 d (2 years), and the mean of 78.1 ng g^{-1} (wet wt) in total PCBs in all fish, because otter is susceptible to travel along rivers or to migrate from one river to another. Our level predicted in otter livers is between the limit of 10 µg g^{-1} (lipid weight) corresponding to a safe level for otter proposed by the Dutch recommendation (Ministerie van Landbouw en Visserij, 1989), and the limit of 50 µg g^{-1} (lipid weight) corresponding to a critical level for the reproduction of the otter, based on experiments on American mink (Jensen et al., 1977), a species more susceptible to PCBs than otters (Wren et al., 1987). Otter recolonization in the North of Luxembourg may therefore be undermined by the significant contamination of fish by PCBs.

3.4.2. Heavy metals

Data about toxicity thresholds for cadmium and lead for the Eurasian otter are poor. Only Weber (1987) proposed a threshold for entire fish of 500 ng g^{-1} wet weight for cadmium and 2000 ng g^{-1} wet weight for lead. The limit values permitted for the human consumption in seafood, proposed by the European Communities Commission (2001) for cadmium, lead and mercury in fish are respectively of 50, 200 and 500 ng g^{-1} wet weight. Values suggested by Weber (1987) may be too high, as otters eat more fish than humans. As a precaution, our results about the contamination with heavy metals have been compared with the limits for human consumption. For lead, concentrations have been found below the limit value for human health, whereas cadmium was found above the limit values for barbels and stone loaches at the Moulin de Kalborn (Our River), Merkholtz (Wiltz River), but also for stone loaches at Winseler (Wiltz River), barbels and eels at Drauffelt (Clerve River). It is important to note that these concentrations for lead and cadmium may overestimate the risk for human health, because we analyzed the whole fish, whereas humans eat only fillets of fish, and some heavy metals, like cadmium and lead are concentrated in liver and kidney (Burger et al., 2007), whereas otters eat the entire fish.

For mercury some individuals exceeded the limit for human consumption of 500 ng g^{-1} wet weight, namely one chub and one eel from Upper-Sûre. In some studies (Svobodová et al., 1999; Burger et al., 2007; Drevnick et al., 2008), it was found that mercury has a more homogeneous distribution in the fish body. Therefore, it can be assumed that the values derived from whole fish may not have the same intrinsic potential for errors as those for cadmium or lead. However, Peterson et al. (2007) found an excellent regression of Hg concentration in fillet against Hg concentration in whole fish, based on 208 fish analyses. According to their equation, the value of 500 ng g^{-1} Hg in fillet corresponds to 300 ng g^{-1} Hg in whole fish. In this case, 33% of the collected fish from the Upper-Sûre could represent a risk for human health. For the Eurasian otter, Weber (1987) proposed a critical level of mercury at 500 ng g^{-1} (wet wt). Mason (1982, in Chanin, 2003) suggested a safe level for mercury in fish of 300 ng g^{-1} (wet wt). Hovens (1992) calculated that a mean level of 100 ng g^{-1} (wet wt) should be tolerable for otters based on the diet of the thriving population in Shetland. In some countries (Austria, Greece, Shetland) where the population of otters is increasing, Hg levels in fish were found to be below 100 ng g^{-1} (wet wt) (Hovens, 1992; Gutleb, 1995; Christoforidis et al., 2008), whereas in Czech Republic arithmetic means of 175, 410 and 510 ng g^{-1} (wet wt) were found in fish (Dušek et al., 2005; Houserová et al., 2007). Rask and Mestälä (1991) estimated that Hg concentration varied from 150 to 1360 ng g^{-1} (wet wt) in pike (*Esox lucius*) from 17 Finnish lakes. As for PCBs, the mean concentration of mercury for all species could be taken into account to determine the contamination of otters in Luxembourg. So, considering the limit of 100 ng g^{-1} proposed by Hovens (1992), Drauffelt (Clerve River), Neimillen (Troine River) and Upper-Sûre (Sûre River) have an average of Hg concentrations above the limit of 100 ng g^{-1} in wet weight considered as a tolerable limit for otter.

4. Conclusion

In the North of Luxembourg, the contamination by PCB has been significantly decreased between 1993 and 2007. Most fish from the northern part of Luxembourg were characterized by moderate PCB contamination based on threshold levels for the Eurasian otter, excepted at Wallendorf on the Our River, where PCB levels have been found to exceed the critical level for otters. These levels in fish would result in otter tissues coming close to proposed critical levels. In 40% of total fish, mercury concentrations were above 100 ng g^{-1}, the recommended value for the Eurasian otter.

In general, the contamination of fish by PCBs and heavy metals may be considered as problematic and potentially critical for the otter survival if natural recolonization starts to occur.

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