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Temporal variation of heavy metal contamination in fish of the river lot in southern France

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ABSTRACT

The present study aims at assessing the current situation of the heavy metal contaminated River Lot (SW France). Several fish species were captured in October 1987 and 2007 at three sampling sites. The concentration of copper, zinc, cadmium and lead were quantified in fish muscle and liver as well as in environmental samples (water, sediment, moss). The decrease in heavy metal concentrations in fish tissue between 1987 and 2007 reflects the decrease of heavy metal concentrations in the environment. Concentrations found in 2007 are comparable to those published by a study conducted in the 1990s. The situation of the River Lot has improved over the last 2 decades, although there is still margin for amelioration according to US EPA criterion to protect freshwater aquatic life. The average concentrations of cadmium in fish muscle in 2007 were above the maximum safe for human consumption defined by the European Commission.

1. Introduction

Used either as a means of transportation, as a source or water, or, worse still, as a sink for waste, the aquatic environment no doubt suffers the consequences of industrial activities. Albeit efforts – to a greater or lesser extent – to contain waste and reduce environmental impacts, a river system will naturally drain all areas surrounding it, washing into the aquatic environment many harmful substances. Many river systems in the industrialized world are thus the focus of regular monitoring of their contaminant load. In the European Union (EU), all member states must comply with water quality standards. When surpassing the maximum permissible levels, efforts must be undertaken to further control emissions. The present water framework directive in force in the EU works towards obtaining environmental qualities that will ensure high protection for aquatic ecosystems against toxic substances (Directive 2000/60/EC of the European Commission, 2000).

The River Lot, an affluent of the Garonne in southwest France, is known for its long history of intense heavy metal pollution and has been the focus of studies dating back to the 1970s (Audry et al., 2004; Blanc et al., 1999; Labat et al., 1977). In an effort to determine a recent Cd (cadmium) budget of the Lot-Garonne River system, Blanc et al. (1999) estimated that approximately 85% of the Cd in the River Lot is derived from anthropogenic origin. As such, the main sources of heavy metal pollution are the now closed mining sites situated along the Riou-Mort River, a tributary of the Lot in the Decazeville area. Apart from significant continuous input of Zn (zinc) and Cd between 1842 and 1987 due to the activity of a Zn ore manufacturing center (14.6 t/year of Cd until 1986, Dauta et al., 1999) there occurred an accidental spillage at this site in 1986. On that occasion, contaminated waste released from the area into the Riou-Mort River caused the mortality of several tonnes of fish inhabiting downstream from the accident site. In response, the Agence de l'Eau Adour-Garonne (AEAG; water agency for the Adour-Garonne River-basin) requested a follow-up on the concentrations of heavy metals in the tissues of native fish species (part of the present study). Almost two decades later, concern about the health of the local consuming population resulted in a survey and report on the risk of consuming the exposed fish (Ricoux and Gasztowtt, 2005). In addition to the historical heavy metal pollution affecting the River Lot, the natural flow of the river has been altered due to the construction of many locks and a few dams. The presence of barriers to the water flow leads to the accumulation of particulate matter to which trace elements are bound (Dauta et al., 1999). When the reservoirs release water, large amounts of heavy metals become water-borne and are transported downstream (Grousset et al., 1999; Lapaquellerie et al., 1995). Such dams are thus an additional source of heavy metal contamination to the watershed. As a result, heavy metals bioaccumulate to considerably higher concentrations in fish species of the Gironde estuary, where the

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Garonne River joins the Atlantic Ocean, than in several other heavily polluted estuaries throughout the world (Durrieu et al., 2005).

Organisms that inhabit an aquatic environment are useful bioindicators of the impact of the presence toxic heavy metals (Arain et al., 2008). However, the presence of such pollutants at high concentrations does not necessarily present a threat to the exposed organisms as they may not be uptaken by the organism at all or, if uptaken, its metabolism can deal with (tolerate and/or excrete) the load (Fernandes et al., 2008). Therefore, coupling the concentration of pollutants in tissues of exposed organisms to the concentrations found in the environment, while taking into consideration relevant abiotic factors (e.g. water hardness, temperature, particulate matter) as well as the tolerance capacity of the organisms, are key processes in environmental risk assessment.

Cu (copper) and Zn are essential elements for growth and development and their uptake from the environment is regulated according to nutritional demand through homeostatic control (Couture and Rajotte, 2003; Watanabe et al., 1997; Wiener and Giesy, 1979). However, Cu and Zn become toxic at concentrations above the limits of homeostatic control (McKim et al., 1978; Sinley et al., 1975). Cd and Pb are non-essential heavy metals that do not have dedicated regulatory mechanisms (Viarengo, 1989)and are therefore more toxic to organisms. As such, Cd and Pb (lead) are classified as priority substances by the European Commission (COM, 2006). The tissues selected for bioaccumulation analysis were of liver and muscle. The liver is the main site of accumulation, biotransformation and excretion of pollutants in fish (Moon et al., 1985; Triebskorn et al., 1997). The axial muscle is the section of fish that is consumed by human beings; therefore it is required to verify whether contaminants like heavy metals are within the recommended limits for human consumption.

Most studies on the impact of heavy metals on exposed organisms focus along a gradient of pollution in one moment in time (Andres et al., 1999; Fabris et al., 2006; Fernandes et al., 2008; Labat et al., 1977; Reynders et al., 2008) or compare two different seasons (Mzimela et al., 2003). Fewer studies have addressed the long-term temporal variation of such impacts (Bietz et al., 1997; Maes et al., 2008; Nakhle et al., 2007). This knowledge is nevertheless important to characterize the evolution of the environmental quality over long periods of time. The situation in the River Lot is continuously monitored for heavy metal loads in sediment and water. However, the contamination of biota is less documented. The objective of the present study was thus to assess how heavy metal levels in fish species from the River Lot have evolved over the last 20 years.

2. Material and methods

2.1. Study area and sampling

The present study focuses on the most downstream section of the River Lot (Fig. 1) in the southwest region of France. The Lot is an affluent of the Garonne, the largest river of the Northern side of the Pyrenees Mountains. Three sampling sites were selected: Cajarc, Luzech and Le Temple. All sites were downstream from the confluence with the heavily polluted Riou-Mort River (Fig. 1) and located immediately upstream from locks/dams, considered as possible reservoirs of contaminated sediment (Dauta et al., 1999).

In all studies, fish were handled in accordance with national and international guidelines for the protection of animal welfare (Directive 86/609/EEC of the European Commission). Fish surveys took place in October 1987 and October 2007. Fishing was performed by Electrofishing (Electro-Pulman, 300–400 V, 1.5 A max. current); progress was made via fishing boat. In the deeper and calmer areas fishing was performed with monofilament nylon gill-nets of different mesh sizes (18–33 mm). Of the fish species captured, only those that were present at all three sites in both surveys are considered here. The selected species are roach (*Rutilus rutilus*), bream (*Abramis brama*) and perch (*Perca fluviatilis*). Sampling of liver and muscle was carried out in the field in 1987 and in the laboratory in 2007. Fish were carefully conserved on ice during transportation and while awaiting dissection the following day. From each fish, liver and muscle samples, $1.1\pm0.36\%$ and $11.2\pm3.62\%$ of their total body weight, respectively, were taken and pooled into composite samples of 1–6 individuals. Fish in each composite sample did not differ in total length by more than 25%. Composite samples were necessary to reduce the final number of samples, and hence reduce the costs for heavy metal quantification. The composite samples were weighed and kept at $-20\,^{\circ}$C in clean 100 ml high-density polyethylene vials until analysis.

Data on environmental variables were provided by the AEAG for each sampling site: conductivity, pH, water temperature and concentration of Cu, Zn, Cd and Pb in water, sediment and aquatic moss (Table 2; Fig. 2). As the AEAG did not monitor water concentrations of heavy metals at those sites in 2007, the environmental data considered in the present publication are from 2006. This did not pose a problem as during the 10 preceding years (1996–2006), fluctuations in average yearly water temperature ($16.79\pm0.93\,^{\circ}$C), pH (7.7 ± 0.12) and conductivity ($222.16\pm22.39\,\mu S\,cm^{-1}$) were minor, and, with the exception of Zn concentrations in the sediment, heavy metal concentrations in the water, sediment and aquatic moss have maintained relatively constant (Fig. 2). Over the period of years between 1982 and 2006, noticeable fluctuations occurred in heavy metal concentrations of Cu_{water} (until 1997), Cu_{moss} and $Zn_{sediment}$. $[Zn]_{water}$ and $[Zn]_{moss}$ decreased considerably until 1989 and then maintained relatively constant thereafter. Apart from these exceptions, there was a general decrease in heavy metal concentrations in all three compartments.

2.2. Heavy metal quantification

2.2.1. Sample preparation and analysis in 1987

Samples were immersed in a solution of NH_4NO_3 (10%), using a ratio of 2 ml/g of sample. They were then dried until no weight loss occurred (110 °C) to determine the water content and calcined (450–550 °C) for 2 h. The ashes were then mixed with 5 ml of water, 10 ml of HCl and 5 ml of HNO_3 (6.3%), boiled for 10 min and dried at 110 °C until no weight loss occurred. The residues were further treated with 20 ml of HNO_3 (6.3%), boiled and filtered with paper filter (0.45 μm). Chemical analysis was performed on the filtrates by the AEAG on an Atomic Absorption Spectrometer (Perkin Elmer 4100ZL).

2.2.2. Sample preparation and analysis in 2007

The frozen samples were freeze-dried for 48–72 h to determine the water content and then homogenized using a mortar and/or knife. Tissue mean water content was $80\pm4\%$. In total 100 mg of dry sample was weighed in pre-weighed polytetrafluoroethylene vials. Liver samples were treated with 0.1 ml H_2O_2 (30% ultrapure, Merck) for 15 min. prior to further digestion. All samples were ultrasonicated for 30 min with 0.5 ml bidistilled concentrated (biΔ conc.) HNO_3 (home-distilled in quartex system). Another 500 μl biΔ conc. HNO_3 was added and the closed vials were heated for 12 h at 90 °C. After cooling down, 0.5 ml H_2O_2 were added and the closed vials were heated for 12 h at 70 °C. Finally, the samples were dried at 60 °C for 3–4 days. The resulting dried matter was dissolved in 2 ml biΔ conc. HNO_3. Aliquots were further diluted in HNO_3 2% and, according to the dilution factor, a certain amount of In/Re (indium/rhenium) was added as internal standard. Blanks with no fish tissue were run with each batch of samples to monitor contamination of used reagents. A fish standard (Standard Reference Material® 1947, Lake Michigan Fish Tissue) for As (arsenic), Cu and Zn was prepared with the same protocol to check the validity of the analytical method. The heavy metal concentrations found in the fish standard analyzed with our protocol were thus compared with the certified values. Heavy metal measurements were performed on an Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7500 ce, Agilent Technologies) at the Laboratoire des Mécanismes et Transferts en Géologie, Toulouse, France. Cu, Zn, Cd and Pb average concentrations in the blanks and the ICP-MS detection limits were, respectively, 0.213/0.031, 1.832/0.021, n.d./0.003 and n.d./0.013 μg/kg (n.d.—not detected).

2.3. Data analysis

The datasets did not fulfill the assumptions for parametric analysis even when transformed; therefore non-parametric analyses were performed. Differences between heavy metal concentrations in muscle and liver samples between years (species pooled), species (years pooled) and years per species were tested via analysis of variance (GLM; Poisson distribution with χ^2 test or quasipoisson distribution with Fisher's test). In order to assess differences between pairs of species and between-year differences in concentration of the heavy metals in water, sediment and moss, and between-year differences in water temperature, pH and conductivity Mann–Whitney *U*-rank tests were performed. Significance level of 0.05 was adopted in all analysis. To verify whether heavy metal concentrations found in the environment were more related to those found either in fish muscle or in the fish liver, co-inertia analysis was performed with data from both years. Co-inertia analysis performs simultaneous analysis of two datasets. This method maximizes the product of the obtained correlation between the two datasets (in

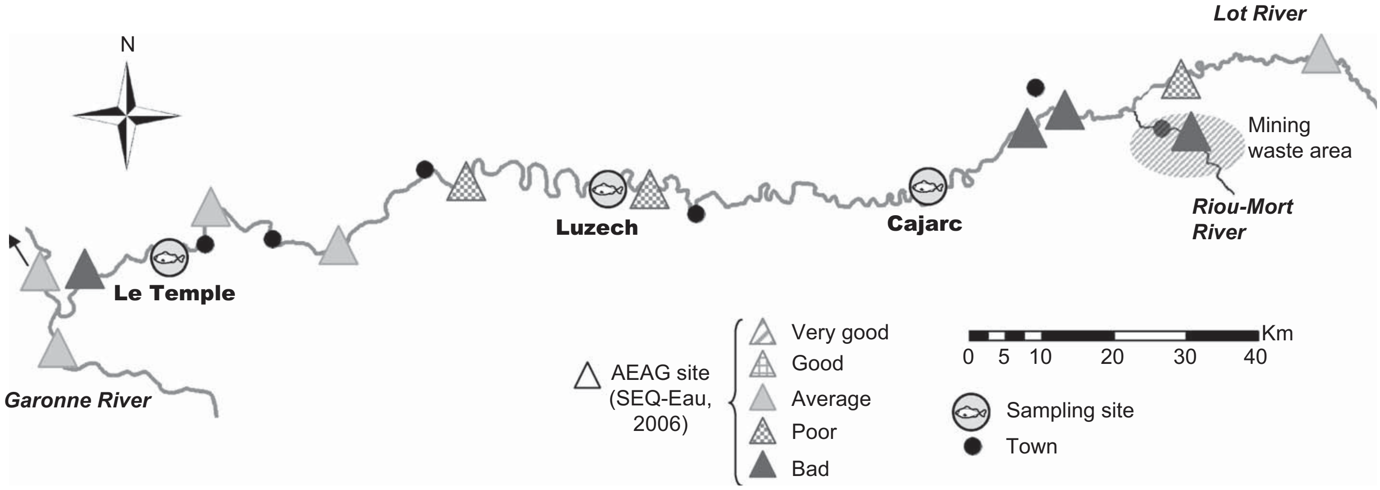

Fig. 1. Map depicting location of the fish sampling sites and the SEQ-Eau classification of the water agency's sampling sites according to heavy metal toxicity to organisms in 2006 (see discussion for further explanations).

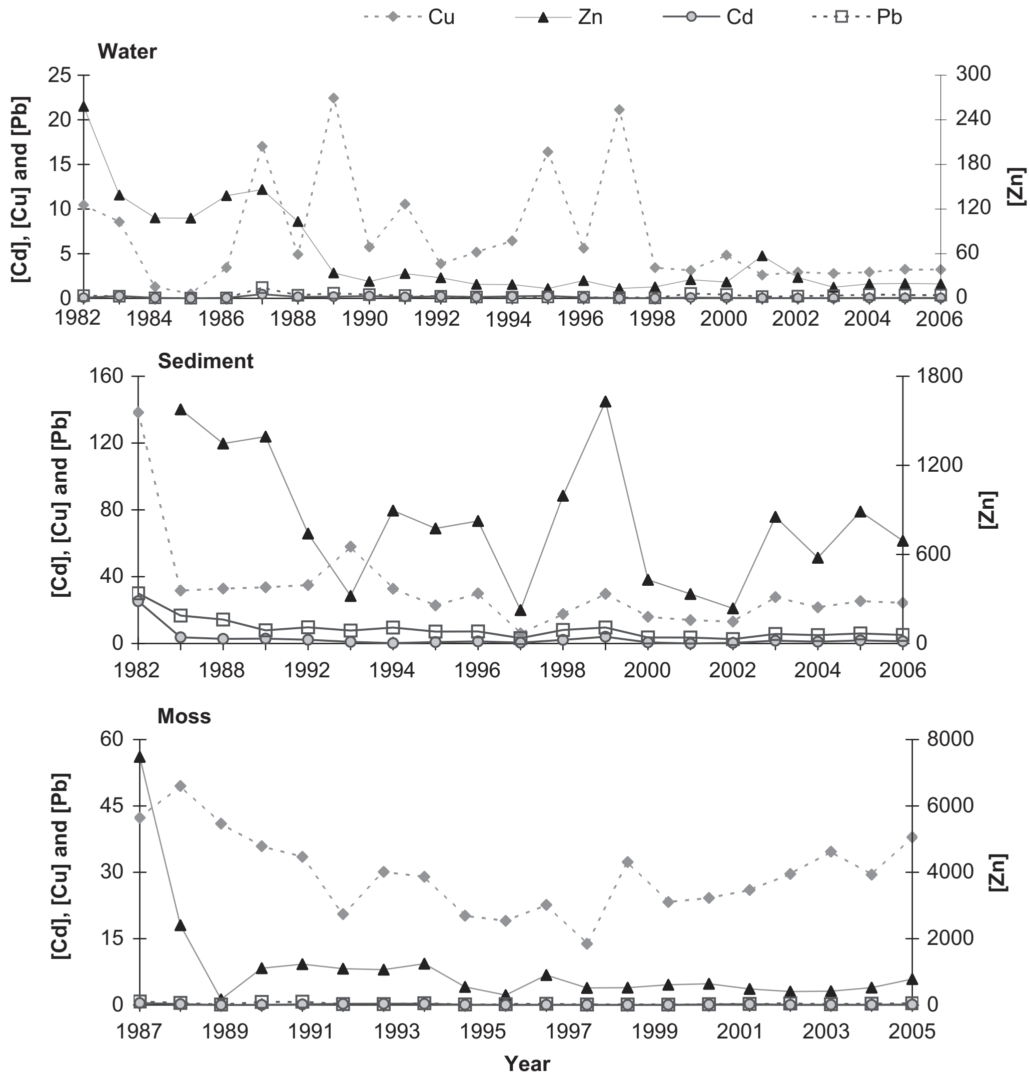

Fig. 2. Average concentrations of Cu, Zn, Cd and Pb in water ($\mu g\,L^{-1}$), sediment ($mg\,kg^{-1}$ wet weight) and moss ($mg\,kg^{-1}$ dry weight) of the River Lot between 1982 and 2006. Concentrations in sediment are missing for 1983–1986, 1990 and 1991, and in the case of Zn for 1982. Concentrations in moss were only available from 1987 onwards.

this case heavy metal concentrations in the environment and in fish), resulting in a coefficient of global similarity (RV coefficient). The closer to 1 the RV coefficients are, the more strongly linked the compared variables are. Monte-Carlo permutation tests indicate whether the co-structure between the two datasets is significant. All statistical analyses were performed using *R* (R Development Core Team, 2008) at a 0.05 significance level.

3. Results

Temperature and pH along the studied segment of the River Lot did not undergo significant changes between registered values in 1987 (17.93±0.92 °C; 7.81±0.04) and in 2006 (17.70±0.87 °C; 7.70±0.20) ($p = 0.2752$ and $p = 0.5127$, respectively). There was a slight increase in conductivity in 2006 (1987: 200.67±29.74 μS cm^{-1}; 2006: 312.33±53.29 μS cm^{-1}) ($p = 0.0495$), although values in both years are within the normal range of the conductivity found in the Adour-Garonne River-basin and favorable to aquatic life (DRI, 2007). Heavy metal concentrations in the water, sediment and moss significantly decreased between the two study periods (Table 2). Exceptions were $[Cd]_{water}$ (concentration of cadmium in the water; $p = 0.5127$), $[Cu]_{moss}$ ($p = 0.8273$) and $[Pb]_{moss}$ (0.5127) that presented no significant differences between the two sampling years.

Analyzed/certified (±uncertainty for certified value, with 95% confidence) As, Cu and Zn concentrations of the fish standard analyzed in 2007 were, respectively, 0.30/0.73 (±0.039), 0.48/0.41 (±0.029) and 3.88/2.66 (±0.08) mg kg^{-1} ww (wet weight). The method therefore provided concentrations that were within the certified ranges for As and Cu, but 46% above for Zn. Higher than standard Zn concentration is more likely a consequence of a contamination that may have occurred outside the clean-room upon ICP-MS analysis. All blanks presented concentrations of heavy metals close to or beneath the detection limit, except for Zn. However, the extent of Zn contamination via reagents and material is insignificant (<0.1%) in comparison to the concentrations found in all fish tissue samples.

Average total lengths of the sampled fish were similar in both the 1987 and the 2007 surveys, validating the comparison between years (Table 1). In general, Cu/Zn/Cd/Pb concentrations in fish liver and muscle decreased between the two study periods (Table 3; Fig. 3). However, $[Cu]_{liver}$ was found to be at similar levels in the liver of the fish captured in both years. Significant differences between species were found only for $[Cu]_{liver}$, bream presenting significantly higher concentrations than perch ($p = 0.0097$; Table 3; Fig. 3). $[Zn]_{liver}$ of bream maintained the same between the two periods (Table 3; interaction with $p<0.05$), whilst of perch and roach the concentrations decreased between 1987 and 2007 (Fig. 3). Heavy metal concentrations were systematically higher in the liver than in the muscle, for both study years and all species ($p<0.05$).

Co-inertia analysis indicated that environmental heavy metal concentrations were clearly related to those found in fish tissues, as co-structure between compared datasets (RV coefficient) was always significant (Table 4). Environmental concentrations were more strongly linked to concentrations in the muscle than in the liver, as the respective RV coefficients were higher.

Table 1
Number of fish sampled, number of fish per composite sample, and average fish length per species and year.

Species	Year	N fish captured	N composite samples	Average length (mm)
Bream	1987	20	3	290.42±76
	2007	17	8	245.19±53
Perch	1987	11	3	273.61±71
	2007	8	5	279.6±45
Roach	1987	52	4	214.54±26
	2007	56	15	206.87±44

Discussion

With the present study, we aimed at assessing the 20-year evolution of heavy metal concentrations in fish from the

Table 2
Average (±SD, for this study) concentrations of Cu, Zn, Cd and Pb in different compartments of the river system, measured along the study area in 1987 and 2006, in other rivers/lakes throughout the world, and the US EPA Criterion for Maximum and Continuous Concentrations, (For further explanations on the criterion refer to Section 4.)

	Water				Sediment			
	Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb
This study, 1987	17.03±3.59*	146.27±98.79*	5.54±5.76	14.47±2.66*	31.67±14.57*	1576.67±406.74*	41.67±20.50*	188±55.24
This study, 2006	3.22±2.03	19.38±1.07	0.5±0.01	4.33±0.58	24.35±7.65	692±313.00	14.75±8.65	57.5±19.50
Lake Boeuf, USA[a]	–	–	–	–	9.08	53.92	–	17.58
Boulder River, USA[b]	11.96	159.88	0.54	1.74	107.63	424.00	3.28	110.33
Asturian rivers, Spain[c]	3.45		0.13	3.65	5.02	–	0.14	9.81
Dipsiz stream, Turkey[d]	0.37	1.05	0.17	0.41	10.83	30.83	0.67	69.67
Orontes River, Turkey[e]	40.30	39.00	11.00	27.00	–	–	–	–
Ontario lakes, Canada[f]	15.67	8.27	0.30	–	–	–	–	–
Lake Beyşehir, Turkey[g]	100.00	160.00	–	–	5.97	–	33.18	–
CMC[h]	13	120	2	65				
CCC[h]	9	120	0.25	2.5				
	Moss							
	Cu	Zn	Cd	Pb				
This study, 1987	42.33±17.01	7477.33±7276.23*	60.47±35.44*	105.00±64.65				
This study, 2006	39.30±10.69	837.67±333.79	17.17±7.74	52.00±9.64				

Values for the present study are the annual average of the three sampling sites. Only data for non-reference sites have been considered when reporting other studies. Concentrations in water and sediment are in μg L^{-1} and mg kg^{-1} wet weight (converted from dry weight by dividing concentrations by 1.2), respectively. *Significant differences between present study sampling years ($p<0.05$; Mann–Whitney test). –, data were not available in publication or variable was not studied. CMC, Criterion Maximum Concentration; CCC, Criterion Continuous Concentration.

[a] Aucoin (1999).
[b] Farag et al. (2007).
[c] Linde et al. (1998).
[d] Demirak et al. (2006).
[e] Yilmaz and Dogan (2008).
[f] Rajotte and Couture (2002).
[g] Tekin-Ozan (2008).
[h] US EPA (2006).

Table 3
Result of analysis of variance taking into account the effect of year, species and their interaction.

			Effect					
			Year		Species		Year × species	
Tissue	Element	Test	Resid. dev	*p*-Value	Resid. dev	*p*-Value	Resid. dev	*p*-Value
Liver	Cu	F	92.35	0.759	67.62	**0.006**	66.79	0.598
	Zn	F	147.17	**0.033**	142.17	0.516	140.21	**0.018**
	Cd	F	32.91	**0.001**	31.23	0.497	30.10	0.568
	Pb	Chisq	4.36	**0.004**	1.87	0.288	1.57	0.864
Muscle	Cu	Chisq	7.41	**0.001**	7.31	0.950	7.18	0.939
	Zn	F	37.74	**0.000**	36.42	0.522	33.71	0.257
	Cd	Chisq	4.20	**0.000**	3.46	0.690	3.43	0.989
	Pb	Chisq	2.57	**0.004**	1.04	0.465	0.96	0.962

Given are residual deviances (Resid. dev) and *p*-values from respective tests (F, Fischer's test. Chisq, Chi-square test). *p*-Values$<$0.05 are in bold.

historically polluted River Lot. Data were obtained from fish surveys conducted in 1987 and 2007 at the same river sampling points, and compared. With the exception of $[Cu]_{liver}$, heavy metal concentrations in fish tissues decreased from 1987 to 2007, a trend that is reflected in the decrease of heavy metal concentrations in fish tissue.

$[Cu]_{liver}$ did not vary significantly between the two sampling years, possibly due to the fact that Cu is well regulated by fish below liver concentrations of ca. 10 mg kg^{-1} wet weight (Couture and Rajotte, 2003), which is the case in both surveys of our study. The colloidal fraction of Cu in the River Lot is considerable. In total 30–40% of Cu complexes are hydrophobic, thus with higher affinity to dissolved organic matter (Lemaire et al., 2006), as can be found in most studies reporting the affinity of Cu to organic phases (Mantoura et al., 1978; Benedetti et al., 1996; Lemaire et al., 2006, Santos-Echeandia et al., 2008). This could contribute to an increased bioavailability of Cu to aquatic organisms, including fish. Furthermore, despite a clear decrease between 1987 and 1997 and stabilization since 1998 in $[Cu]_{water}$, and a gradual, albeit small, decrease in $[Cu]_{sediment}$ over the years (Fig. 2), $CuSO_4$ is still heavily applied as a fungicide in vineyards located throughout the surrounding terrestrial basin, contributing to a constant input of Cu into the river basin (Kraepiel et al., 1997). In 2006 River Lot $[Cu]_{water}$ was in general lower than concentrations found in other rivers whilst $[Cu]_{sediment}$ was slightly higher (Table 5). An additional source of Cu could be the erosion of ore deposits in the valley of the River Lot (Schafer and Blanc, 2002). Higher concentrations in the liver than in the muscle in both study years are consistent with studies such as those performed by Andres et al. (2000) and Fernandes et al. (2008).

$[Zn]_{liver}$ in bream was as high in 1987 as it was in 2007, contrary to the decrease observed in perch and roach. Bream is generally a bottom-feeding species whilst perch is carnivorous and roach a water-column dweller. The fact that breams have closer contact to the sediment may explain the nondecrease of the concentration of Zn in their liver. Although $[Zn]_{sediment}$ in 1987 has decreased to less than half in 2006, it has fluctuated considerably. This fact provides an additional explanation to why bream captured in 2007, having been exposed to those fluctuations (given their size, thus age, upon capture), presents as much Zn in their liver as fish captured in 1987.

4.1. Comparison with other studies

A study performed by Andres et al. (2000) on the River Lot reports average concentrations of Cd and Zn in several fish species (including the three in our study) between 1995 and 1997. This study took place 10 years prior to our 2007 survey, and in the same area: from a reference site 41 km upstream of the confluence with the Riou-Mort River, down to Carjarc, the most upstream site in our study.

$[Cd]_{liver}$ and $[Zn]_{liver}$ in the Riou-Mort fish of 1995–1997 are higher than the 1987 averages in the downstream segment of the River Lot (our study). The higher concentrations in the liver are a direct response of higher concentrations of the trace elements in the water at Riou-Mort: ca. 29 and 980 μg L^{-1} of Cd and Zn, respectively, as reported in Andres et al. (2000). With regard to bioaccumulation, the liver, as an organ of continuous detoxification, provides a more immediate assessment of the current environmental levels of pollutants (Bruslé et al., 1996). However, Riou-Mort $[Cd]_{muscle}$ and $[Zn]_{muscle}$ in 95–97 are in general lower than the downstream River Lot averages in our study. The muscle can be considered as an indicator of chronic exposure (Albaigés et al., 1987); the lower concentrations in 95–97 may indicate that the fish were coping better with the high levels of pollution (i.e. eliminating more and storing less) in the Riou-Mort than with the equally high levels of the downstream River Lot in 1987. Fish acclimation to chronic heavy metal contamination has been observed in other fish species, such as mosquitoefish (Klerks and Lentz, 1998), iberian-roach (Lopes et al., 2001) and rainbow trout (Chowdhury et al., 2005).

Overall, average 2007 concentrations of Cd and Zn in the muscle and liver of all three species are lower than those found in 1995–1997 at the Cajarc site, and 1987 concentrations are clearly higher. More specifically, [Cd] and [Zn] in breams and roaches captured at Cajarc in 2007 (data not discriminated in the present study) are in general lower than in fish captured at the same site in 1995–1997. Such differences may be due to the fact that the results presented by Andres et al. (2000) are an average of successive surveys covering different seasons (autumn and spring) whilst our surveys took place only in autumn. The average water temperature of the River Lot in spring and autumn differs substantially (average from 7.9 to 17.7 °C) and it is known that temperature influences the uptake and metabolism of heavy metal elements by fish (Cogun et al., 2006; Foster et al., 2000; Regoli and Orlando, 1994). Furthermore, $[Zn]_{water}$ at the reference site in 95–97 is higher, and $[Cd]_{water}$ lower, than the averages found further downstream in 2007.

Albeit higher concentrations found in fish tissue in 1995–1997 (Andres et al., 2000) than in 2007 these differences are not substantial with respect to the marked decrease observed from 1987. Remediation efforts after the 1986 accident have resulted in an important decrease of heavy metal concentrations in water, sediment and moss. However, since 1997, Cu, Zn, Cd and Pb concentrations in the sediment have stabilized, being still 1.2, 7.0, 37.2 and 1.7 times higher, respectively, than the pre-industrial background concentrations of the River Lot (Audry et al., 2004). This stabilization is reflected in the similar concentrations of heavy metal elements found in fish liver and muscle in 1995–1997 and 2007.

When assessing the contamination of biota from an impacted area it is often interesting to compare the extent of the contamination with studies performed in other polluted areas in the world (Tables 2 and 5). Heavy metal concentrations found in the three species in our study in 2007 are lower than those found in muscle of brook trout from the Boulder River watershed by Farag et al. (2007) and than in liver of brown trout from rivers in Asturia, Northern Spain (Linde et al., 1998). However, water concentrations in Boulder River watershed are in general much higher (except reference sites) than those found in the River Lot and the Northern Spain Rivers, which in turn have comparable concentrations between them. The fact that brown trout from the

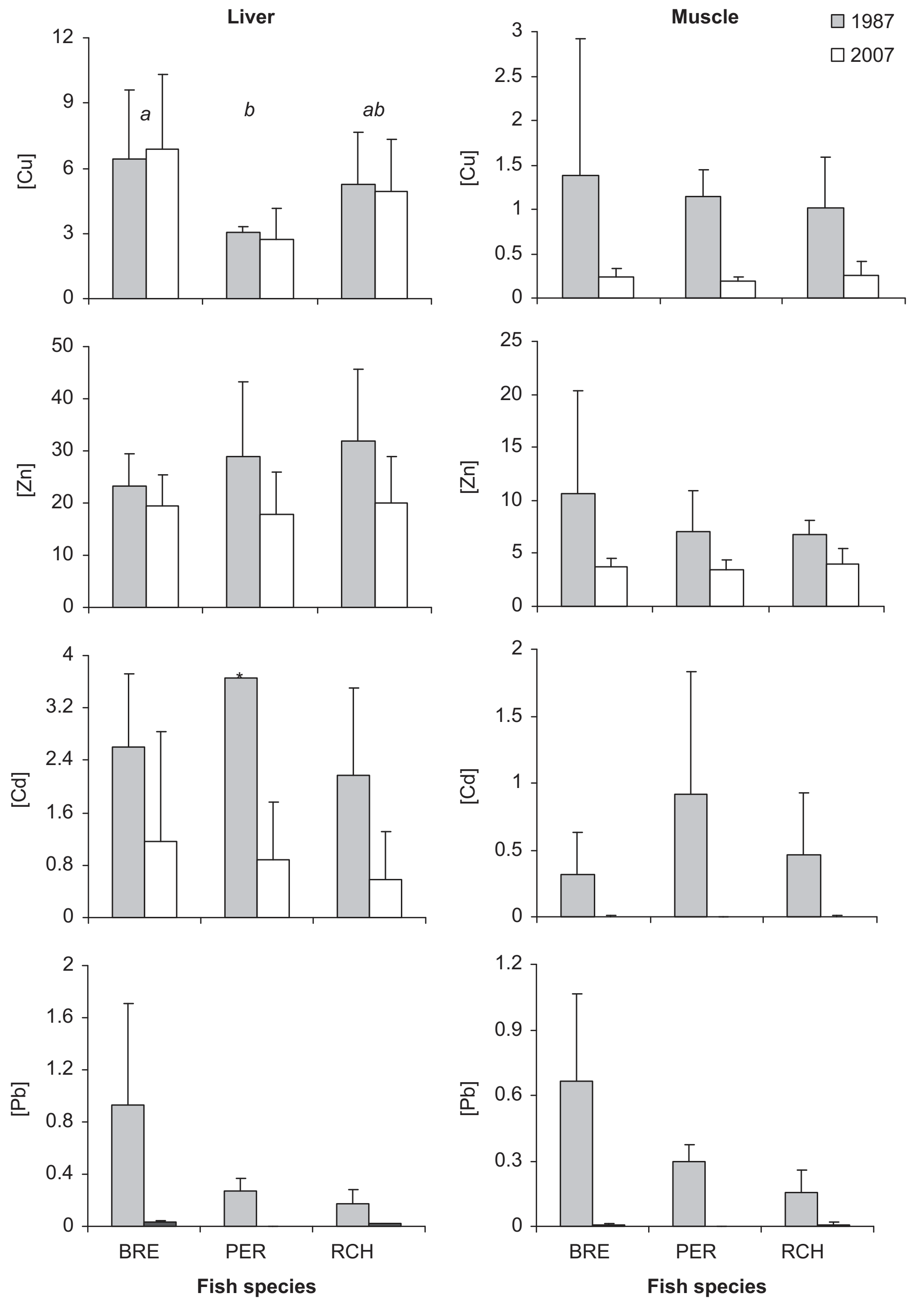

Fig. 3. Average concentrations (mg kg^{-1} ww; ±SD) of Cu, Zn, Cd and Pb in muscle (right) and liver (left) of the three fish species sampled along the River Lot in the 1987 and 2007 surveys. BRE: Bream; PER: Perch; RCH: Roach. * sample size = 1. A different letter indicates presence of significant differences between species (Mann–Whitney test).

Table 4
RV coefficients of co-inertia analysis comparing concentration of heavy metals in the environment (sediment, water and moss) and fish tissues.

	Sediment	Water	Moss
Liver	0.31***	0.4***	0.24*
Muscle	0.64***	0.63***	0.35***

* $p<0.05$.
*** $p<0.001$.

Asturian rivers presented higher heavy metal content than the species in our study, despite water concentrations being similar, maybe due to the different physiology and feeding habits of these species. Another factor could be physical–chemical processes taking place in the Asturian rivers, processes that increase the bioavailability of heavy metals to aquatic organisms. Indeed, the authors state that in these rivers there occur periodic floods in spring and summer, events that often lead to the resuspension of trace elements accumulated in the sediment and consequent increase of bioavailable levels. A study conducted by Rajotte and

Table 5
Other studies' heavy metal concentrations in the liver and muscle of fish and the maximum permissible levels in fish muscle in Europe.

Species	Location	Liver				Muscle			
		Cu	Zn	Cd	Pb	Cu	Zn	Cd	Pb
Bluegill sunfish	Lake Boeuf, USA[a]	–	–	–	–	0.93	14.49	–	2.44
Bream	This study-1987	6.45	23.24	2.60	0.93	1.38	10.68	0.38	0.67
Bream	This study-2007	8.67	21.56	1.17	0.03	0.24	3.64	0.00	0.00
Brook trout	Boulder River, USA[b]	–	–	–	–	118.25	38.30	6.38	0.06
Brown trout	Asturian rivers, Spain[c]	153.90	–	2.23	2.46	–	–	–	–
Chub	Dipsiz stream, Turkey[d]	–	–	–	–	0.79	11.06	0.01	0.23
Crucian carp	Dniester River, Moldova[e]	–	–			3.66	8.43	0.03	1.24
Himri	Orontes River, Turkey[f]	45.72	89.59	2.80	1.55	4.55	9.89	0.12	0.12
Perch	This study-1987	2.82	18.82	3.66	0.20	1.14	7.00	0.90	0.30
Perch	This study-2007	2.72	17.88	0.87	0.00	0.19	3.44	0.00	0.00
Perch	Dniester River, Moldova[e]	–	–	–	–	2.66	9.85	0.03	2.16
Yellow perch	Ontario lakes, Canada[g]	7.64	27.33	1.98	–	1.06	5.91	1.20	–
Roach	This study, 1987	5.03	34.78	2.46	0.13	1.05	6.81	0.83	0.11
Roach	This study, 2007	4.91	20.06	0.58	0.02	0.25	4.04	0.00	0.00
Siberian roach	Dniester River, Moldova[e]	–	–	–	–	2.47	7.66	0.04	2.51
Tench	Lake Beyşehir, Turkey[h]	85.67	27.33	–	–	–	7.40	–	–
Maximum permissible levels for human consumption in Europe[i,j]		–	–	–	–	0.10	100 <	0.05	0.30

Only data for non-reference sites have been considered when reporting other studies.
All tissue concentrations are in mg kg^{-1} wet weight. Tissue concentrations found in dry weight were converted to wet weight by multiplying by a factor of 0.2 (considering an average water content in fish tissues of 80%). –, data were not available in publication or variable was not studied.

[a] Aucoin (1999).
[b] Farag et al. (2007).
[c] Linde et al. (1998).
[d] Demirak et al. (2006).
[e] Sapozhnikova (2005).
[f] Yilmaz and Dogan (2008).
[g] Rajotte and Couture (2002).
[h] Tekin-Ozan (2008).
[i] Commission Regulation No. 1881/2006 (2006).
[j] Ricoux and Gasztowtt (2005).

Couture (2002) reveals heavy metal concentrations in muscle and liver of yellow perch from three polluted lakes in Ontario, Canada, that are in average slightly above those in perch analyzed in the present study in 2007. The heavy metal concentrations that were found in the contaminated lakes were comparable to those in the River Lot in 2006, with Cu slightly higher and Zn slightly lower.

4.2. Aquatic community effects

Concentrations of Cu, Zn and Cd in the river water in 1987 were higher than US EPA Criterion Maximum Concentration (CMC) reflecting the state of high pollution levels (US EPA, 2006; Table 2). The CMC is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect (US EPA, 2006). This situation no longer prevailed in 2006 where the concentrations had unanimously dropped below their respective CMC. In general, concentrations in 1987 were lower than a previous study conducted in 1975 on the River Lot (Labat et al., 1977), indicating that the community had been exposed to concentrations well above the CMC already for several years. Concentrations of all four heavy metals in 1987 and of Cd and Pb in 2006, in the water of the studied area, exceeded the recommended Criterion Continuous Concentrations (CCC). The CCC is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect (US EPA, 2006; Table 2). Therefore, it can be concluded that, according to the US EPA recommendation criteria, the studied segment of the River Lot in 2006 was still contaminated by Cd and Pb to an extent that is harming the aquatic community.

The water quality of the Adour-Garonne River-basin has been monitored by the AEAG for several decades. In order to assess the occurrence of contaminants in the aquatic environment, the AEAG has developed a five-level (ranging from 1: good, to 5: bad) index of heavy metal perturbation, the SEQ-Eau, which pools data on contaminant concentrations in water, sediment and water moss (MEDD, 2003). The SEQ-Eau for heavy metals incorporates a classification of the sites according to the water's appropriateness for drinking-water, aquatic sports and aptitude for aquatic life, with regard to 14 different trace elements (including the four elements studied here). This classification is performed for each environmental compartment (water, sediment and aquatic moss) and each heavy metal, and the worst classification is retained. For the most downstream section of River Lot in 2006 (this study) the SEQ-Eau is average to bad (3–5; data provided by the AEAG; Fig. 1). No sites are indexed as 1 (very good) or 2 (good). Downstream from the mining area of Decazeville, the Riou-Mort River has a bad SEQ-Eau. Up to ca. 42 km upstream from the confluence with the Riou-Mort, the Lot has a SEQ-Eau of average to poor (3–4). Some authors adopt points in this section of river as a reference to sites downstream from the Riou-Mort River (Andres et al., 1999; Audry et al., 2004). However, the adequacy of such practice should be questioned, as it possibly affects the conclusions of those studies. Despite all these uncertainties, local fishing authorities are still promoting fishing activities and the attribution of personal fishing permits along the River Lot (Fédération du Lot-et-Garonne pour la Pêche et la Protection du Milieu Aquatique, personal communication).

4.3. Sediment quality

In average, the section of the River Lot studied here was, in 2006, according to Müller's geoaccumulation index (Audry et al., 2004; hereafter referred to as I_{geo}) unpolluted, except in the case of Zn for which it is strongly polluted. I_{geo} is a qualitative indicator to classify sediments according to their pollution intensity. In this classification, the authors consider the sediment heavy metal concentrations in the reservoir immediately upstream from the confluence with the Riou-Mort River as representative of the background concentrations of the River Lot (Audry et al., 2004). However, as proposed by Andres et al. (1999), there may also be inputs of heavy metals via diffuse spills from cities located in the upstream watershed of the River Lot. This is, in fact, reflected in the concentration of Cd in breams (Andres et al., 2000) from a reservoir upstream from the Riou-Mort confluence and only 41 km downstream from the reference site of Audry et al. (2004). However, sediment concentrations of heavy metals in that same section of the River Lot in 2006 (data not discriminated in the present study) were, according to the I_{geo}, unpolluted or unpolluted-to-moderately polluted. On the other hand, sediment concentrations of Zn and Cd in both study years were higher than the probable effect concentrations (PEC; MacDonald et al., 2000), above which adverse effects on the benthic community are expected to occur. Incidentally, these two trace elements were the ones most intensively explored until the closure of the metallurgical activities. Sediment-associated metals may constitute a long-term source of contamination, and thus risk, to higher tropic levels (Eimers et al., 2001; Farag et al., 2007).

Depending on the type of classification applied, and thus which factors are taken into account (toxicity to aquatic or benthic organisms, levels in sediment, water and/or moss, scaling of pollution intensity), the same river location can be classified differently. This indicates that not only one type of classification is sufficient to adequately assess the impact of a contamination on the environment. When possible, environmental risk assessment should include a combination of indexes that cover all environmental compartments concerned and the different biological groups involved. The scaling of such indexes would facilitate inter-index comparisons over time at the same location, and between locations.

4.4. Human health risk

In the 1987 campaign only the average concentration of Zn in the muscle did not exceed the established European Commission regulatory concentration for human consumption (Table 5). Average concentrations of Cu, Zn and Pb in fish muscle in 2007 were below the maximum established European regulatory concentrations. The average $[Cd]_{2007}$ was above the EC maximum, although the concentration in the most consumed species out of the three studied here (perch) was not.

In a humanitarian risk study conducted in 2004 by several official entities of the Adour-Garonne River-basin (of which the River Lot is part of), concentrations of Cd and Pb in muscle of carnivorous fish and eels were in average comparable to those found in the three species sampled in the 2007 campaign (Ricoux and Gasztowtt, 2005). The authors conclude that care must be taken upon consuming certain fish species captured in the Adour-Garonne River-basin, especially in the case of infants that have a diet of high consumption of river fish. The recommendation of the French agency for sanitary security of foodstuffs (Agence Française de Sécurité Sanitaire des Aliments) is to diversify the origin of consumed fish, not preferring too often fish captured in the Adour-Garonne Rivers and avoiding consumption of fish that originate from heavily contaminated areas (Ricoux and Gasztowtt, 2005). These recommendations are based on prevailing concentrations of Cd, Pb and Hg (mercury) in the edible portion of carnivorous fish and eels from the Adour-Garonne River-basin.

5. Conclusion and outlook

As can be seen in the present study, the heavy metal contamination in the River Lot aquatic system has improved over the last 20 years. However, the situation is still not complying with established ecological and chemical requirements of the European Union. As such, the main source of heavy metals to both surface and ground waters – the upstream Riou-Mort River basin – is currently being assessed and measures to control the situation are being taken. The Adour-Garonne Water Agency and the society responsible for the disposal of industrial wastes (UMICORE) are aiming at reducing heavy metal loads, especially Cd, to reach the 2015 limits established by European Union's Water Framework Directive (Prefecture of Aveyron, 2008). Contaminated soils stored within the industrial area are being removed and properly disposed of and/or treated. The local population is being informed of the contamination and a health study has been performed in 2008 to evaluate current accumulation in and exposure of babies, children and adults to arsenic, Cd and Pb. Depending on the results of the study the local health authorities will inform the population on procedures to reduce human exposure. It will be interesting to see how the clean-up of the industrial area will affect the fish populations and the concentration of heavy metals in their tissues.

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