

Non-dioxin-like PCBs in ten different fish species from the Danube river in Serbia

Saša Janković · Marijana Ćurčić · Tatjana Radičević ·
Srđan Stefanović · Mirjana Lenhardt ·
Ksenija Durgo · Biljana Antonijević

Received: 30 April 2010 / Accepted: 22 November 2010
© Springer Science+Business Media B.V. 2010

Abstract This work has been developed to examine the level of non-dioxin-like (ndl) PCBs (28, 52, 101, 138, 153 and 180) in (a) ten different freshwater fish species from the Danube river, (b) two sampling points: up and downstream of the industrial zone of the city of Pancevo (ecological hot spot in Serbia) and (c) two time points i.e., in 2001 and 2006. Obtained results would serve to analyse spatial, temporal and congener profile

characteristics of ndl PCBs cumulated in fish tissues due to environmental pollution. Sixty-four samples of the following species were collected: wels (*Silurus glanus*), pike (*Esox lucius*), bream (*Abramis brama*), crucian carp (*Carassius carassius*), pike perch (*Stizostedion lucioperca*), barbel (*Barbus barbus*), tench (*Tinca tinca*), sterlet (*Acipenser ruthenus* L.), common carp (*Cyprinus carpio*) and bighead carp (*Hypophthalmichthys nobilis*). Gas chromatography coupled with electron capture detector was used for analysis of ndl PCBs. Total ndl PCBs content in upstream samples ranged from 2.7 to 98.1 ng/g and from 4.9 to 68.3 ng/g in 2001 and 2006, respectively. During the 5 years, ndl PCBs content increased significantly in downstream samples i.e., ndl PCBs varied from 13.7 to 46.1 ng/g and from 14.4 to 107.2 ng/g in 2001 and 2006, respectively. PCBs 138 and 180 were predominant congeners in 2001, while in 2006 the most abundant PCB congeners were 138 and 153. In 2006, the presence of PCB 28 and PCB 52 has indicated a recent contamination event. Data on continual monitoring of PCBs in all relevant environmental compartments together with appropriate biomonitoring data are expected to give comprehensive insight into the fate and behaviour profile of these contaminants.

S. Janković (✉) · T. Radičević · S. Stefanović
Institute of Meat Hygiene and Technology,
Kačanskog 13, 11000 Belgrade, Serbia
e-mail: sasa@inmesbgd.com

M. Ćurčić · B. Antonijević
Department of Toxicology “Akademik Danilo
Soldatović”, Faculty of Pharmacy,
University of Belgrade, Vojvode Stepe 450,
11221 Belgrade, Serbia

M. Lenhardt
Institute for Multidisciplinary Research,
University of Belgrade, Kneza Višeslava 1a,
11030 Belgrade, Serbia

K. Durgo
Department for Biochemical Engineering,
Faculty of Food Technology and Biotechnology,
Zagreb University, Kršnjavoga 25,
1000 Zagreb, Croatia

Keywords ndl PCBs · Fish · Danube · Serbia

Introduction

Polychlorinated biphenyls (PCBs) are a family comprising 209 chemically related compounds that were widely used in a variety of industrial applications due to their insulating and fire-retardant properties. Improper use and disposal, as well as industrial accidents, due to the persistence, liposolubility and tendency of PCBs to bioaccumulate in animal tissues and milk resulted in their spread in the environment often situated remotely from the production or disposal site (Smith and Gangolli 2002). Fish and other seafood at all life stages, readily take up organochlorinated compounds, including PCBs, from water with rather high bioconcentration factors making them useful indicator organisms for the evaluation of pollution of the aquatic environment. As a result of this bioconcentration, PCB levels in aquatic organisms can be up to one million times higher than their concentration in the aquatic environment. Accumulation of PCBs in fish depends on their concentration in water, the life span, species and fat content of the fish (Bayarri et al. 2001; Bordajandi et al. 2006). Nowadays, they are of great concern because of their persistence and bioaccumulation through the food chain and their toxic effects on wild life and humans. The main human intake, almost 90% of PCBs, is via food consumption (Liem et al. 2000; Lim et al. 2004). Fish consumption, although with relatively small share in total humans' diet, compared with other food products (meat and milk), can contribute up to 50% of the intake of PCBs (Kiviranta et al. 2004; Antonijevic et al. 2007). Some social groups may be exposed to higher PCBs concentration as a result of their particular diet. Thus, the aquatic environment is the major route of PCBs entry to the food chain.

Different PCB congeners have different biological properties. Dioxin-like (dl) PCBs are able to bind to the aryl hydrocarbon (Ah) receptor and have toxicological properties similar to those of the highly toxic compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Non-dioxin-like (ndl) PCBs have been shown to elicit different types of responses than the dl PCBs, including neurological, neuroendocrine, endocrine, immunological and carcinogenic effects (EFSA

2005). These effects occur via multiple toxicity pathways, not involving the Ah receptor. Therefore, unlike dl PCBs, no health based guidance value for human exposure has been established for ndl PCBs because their toxicity is still insufficiently understood. Since ndl PCBs constitute a major part of the PCBs found in food and human tissues, several regulatory and advisory boards have recommended that more information should be gathered about the toxicity of ndl PCBs (ATSDR 2000).

Some PCB congeners are highly persistent and can, therefore, currently be found in the environmental media, particularly in sediment and fish matrices. Such congeners were considered as PCB tracers, irrespectively of the dl or ndl PCBs types. In this respect, analysis of food matrix contamination profiles allows us to identify six most commonly found congeners (PCB-28, 52, 101, 138, 153 and 180), which account for approximately 50% of all PCB congeners present in human food of animal origin and in human fat, and are called indicator PCBs (Arnich et al. 2009). Among these indicator PCBs, the highly persistent *di-ortho* substituted PCB-138, 153 and 180 have been already covered by specific regulations in some European countries. The less persistent PCB-28, 52 and 101 were included since they were found in significant amounts in some contaminated foodstuffs or were considered as indicators of recent contamination (Arnich et al. 2009).

United Nations Environment Programme (UNEP)/United Nations Centre for Human Settlement (UNCHS) established the Balkan task force, with the aim of carrying out the assessment of impact of the recent conflict on the environment in Serbia (UNEP/UNCHS 1999). The task-force, in the report, declared city of Pancevo, (southern part of Serbian province of Vojvodina, 15 km northeast from Belgrade-44°52' N 20°38' E), as one of the ecological hot spots and proposed immediate implementation of measures to be taken in order to avoid serious consequences on humans' health. Additionally, Pancevo is a city where a major industrial complex is situated (petrochemical plant, fertilizer plant and major oil refinery). These facilities are considered the most important sources of pollutants in the Danube river ecosystem. All these data, including data

on sediment concentrations of PCBs in this area, ranging from 80 to 1,600 ng/g (UNEP/UNCHS 1999) have indicated that the levels of ndl PCBs in fish could reflect environmental contamination. Therefore, this work has been developed to examine the level of ndl PCBs (28, 52, 101, 138, 153 and 180) in (a) ten different freshwater fish species from the Danube river, (b) two sampling points: up and downstream of the industrial zone of the city of Pancevo and (c) two time points i.e., in 2001 and 2006. Obtained results would serve to analyse spatial, temporal and congener profile characteristics of ndl PCBs cumulated in fish tissues due to environmental pollution.

Experimental

During 2001 and 2006, 64 samples of ten different fish species have been collected using random sampling design: wels (*Silurus glanus*), pike (*Esox lucius*), bream (*Abramis brama*), common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), pike perch (*Stizostedion lucioperca*), barbel (*Barbus barbus*), tench (*Tinca tinca*), sterlet

(*Acipenser ruthenus* L.) and bighead carp (*Hypophthalmichthys nobilis*). Two locations were chosen as sampling points: up and downstream from city of Pancevo, Serbia (Fig. 1). According to our knowledge, the upstream sampling site is free of any major industrial complex that could cause pollution in this location. The distance between up and downstream fishing sites is approximately 300 km, allowing the minimization of the influence of fish migration behaviour on results obtained in this study.

After being caught, all fish specimens were kept frozen at -20°C before analysis. The head, tail, backbone and fins were removed from the partially frozen fish. Edible parts were chopped into 2 to 3 cm thick portions and homogenized.

Residues of PCBs in the fat extracted from fish samples were analyzed according to the USDA Analytical Chemistry Laboratory Guidebook (1991). All standards and reagents were purchased from Promochem (Wesel, Germany). PCBs were extracted and separated by elution from fat in small glass columns filled with partially deactivated alumina. The eluate was evaporated to an appropriate volume. An aliquot

Fig. 1 Sampling locations, upstream and downstream from Pancevo, Serbia



Table 1 Freshwater fish species collected from the Danube upstream and downstream from Pancevo

Fish species and number of samples	2001		2006	
	Upstream	Downstream	Upstream	Downstream
Carp	+	–	+	+
Pike	+	–	+	–
Barbel	–	–	+	+
Wels	+	–	+	+
Sterlet	–	+	+	+
Bighead carp	–	–	–	+
Bream	–	+	–	+
Pike perch	+	+	–	+
Tench	–	–	–	+
Crucian carp	+	+	–	+

of 1 μL was injected into a gas chromatograph coupled with electron capture detector.

Gas chromatograph GC Varian Model 3800 equipped with a ^{63}Ni electron capture detector (ECD) and Varian VF 5-ms column (30 m \times 0.25 mm i.d. and 0.25 μm film thickness) were used for analysis of PCBs. Operating conditions

were as follows: injector 250°C; detector 300°C; column oven program: initial 50°C raised to 200°C at 50°C/min, hold 2 min then raised to 215°C at 2.5°C/min, hold 5 min and finally raised to 230°C at 2°C/min, hold 9.5 min. The highly purified nitrogen carrier gas flow was 1 mL/min. Data acquisition was performed by Varian Star software.

Table 2 Content of ndl PCBs in the Danube fish (ng/g fresh weight) upstream and downstream from Pancevo (2001)

Species	28	52	101	138	153	180	Σ PCBs
Upstream							
Carp	nd	nd	1.7	10	5.9	18.1	35.7
Carp	nd	nd	3.8	14.4	9.4	24.8	52.4
Carp	nd	nd	2	6.6	4.6	8.8	22
Carp	nd	nd	1.4	8.4	4.5	9.2	23.5
Crucian carp	nd	nd	1.2	1.2	0.4	3.2	6
Crucian carp	nd	nd	4.4	11.2	6.7	51.2	73.5
Pike	nd	nd	1.4	2.1	1.5	2.6	7.6
Pike perch	nd	nd	0.4	0.7	0.4	1.2	2.7
Pike perch	nd	nd	0.5	1	0.5	1	3
Wels	nd	nd	4	16.5	9.7	23.7	53.9
Wels	nd	nd	1.2	6.2	3.8	3.7	14.9
Wels	nd	nd	8	27.9	16.6	45.6	98.1
Range	–	–	0.4–8	0.7–27.9	0.4–16.6	1–51.2	2.7–98.1
Mean	–	–	2.5	8.8	5.3	16.1	32.8
Median	–	–	1.6	7.5	4.6	9	22.8
Downstream							
Bream	nd	nd	3	4.4	2.7	5	15.1
Bream	nd	nd	7.4	8.2	4.6	11.6	31.8
Bream	nd	nd	2.5	5.2	2.8	5	15.4
Bream	nd	nd	1.6	5	2.5	4.6	13.7
Crucian carp	nd	nd	6.7	3.3	2	2.1	14.1
Crucian carp	nd	nd	10.5	11.9	6.4	17.3	46.1
Pike perch	nd	nd	2.2	2.8	1.6	14.1	20.7
Sterlet	nd	nd	4.6	6.2	3	4.4	18.4
Sterlet	nd	nd	4.4	9.9	5.3	9.8	29.4
Range	–	–	1.6–10.5	2.8–11.9	1.6–6.4	2.1–17.3	13.7–46.1
Mean	–	–	4.8	6.3	3.4	8.2	22.7
Median	–	–	4.4	5.2	2.8	5	18.2

nd not detected

Table 3 Content of ndl PCBs in the Danube fish (ng/g fresh weight) upstream and downstream from Pancevo (2006)

Species	28	52	101	138	153	180	Σ PCBs
Upstream							
Barbel	2.6	3	10.7	23.9	19.4	8.7	68.3
Carp	0.6	1.1	2.5	7	8.8	4.5	24.5
Pike	0.5	0.5	1.5	0.5	1.4	0.5	4.9
Pike	0.9	0.9	2.4	11	12.2	5.1	32.5
Sterlet	1.7	1.4	8.1	5.8	6.8	2.2	26
Sterlet	1.6	1.4	8.1	5.6	6.6	2.5	25.8
Sterlet	0.8	1	6.8	4	4.1	1.1	17.8
Sterlet	1.2	1.6	6.4	5.8	5.5	2.1	22.6
Sterlet	1.8	1	6.4	5.2	4.5	1.7	20.6
Sterlet	1.6	1.3	5.6	5.9	5.9	3.1	23.4
Sterlet	1.3	1.3	6.5	5.2	4.6	2.3	21.2
Sterlet	1.7	1	6	3.8	3.9	1.2	17.6
Sterlet	1.8	1	7.6	4.8	4.2	1.7	21.1
Sterlet	1.6	1.3	7	9	8.4	3.2	31.5
Sterlet	2	1.5	8.2	7	7	5.5	31.2
Sterlet	2	1.2	8.3	10.8	10.6	7	39.9
Sterlet	1.6	1.3	6.6	7.1	6.6	3	26.2
Sterlet	1	1	5.1	6	5.9	3.1	22.1
Wels	1.3	1.3	4.5	21.2	17.6	9.8	55.7
Range	0.5–2.6	0.5–3	1.5–10.7	0.5–23.9	1.4–19.4	0.5–9.8	4.9–68.3
Mean	1.5	1.3	6.2	7.9	7.6	3.6	28
Median	1.6	1.3	6.5	5.9	6.6	3	24.5
Downstream							
Barbel	1.3	1.9	6.2	9.6	9.9	4.4	33.3
Bighead carp	3.7	4.1	10.4	24.5	22.6	15.7	81
Bighead carp	3.5	4.8	7.4	15.6	14.4	6.3	51.8
Bream	2.3	2.6	6.6	10.7	11.6	6.6	40.4
Bream	2.2	2.4	15.9	7.2	6.1	3	36.8
Carp	5.2	4.4	10.7	40.9	34.3	11.7	107.2
Crucian carp	2.3	2	4.3	6.6	7.4	3.7	26.3
Crucian carp	2.3	1.5	2	6.4	6.5	3.6	22.3
Pike perch	1.5	1.4	2.5	5.7	6.2	3.5	20.8
Pike perch	2	2.2	6.9	9.4	8.7	2.8	32
Tench	1.8	2.5	2.1	2.6	3.7	1.7	14.4
Sterlet	6.5	8.1	13	14.1	14.1	10.2	52
Sterlet	6.4	12.3	18.4	21.5	22.4	13.3	94.3
Sterlet	2.9	6.1	8.5	11.5	11.5	9.5	50
Sterlet	3.9	5.9	7.8	10.8	10.4	6.6	45.4
Sterlet	3.3	5.4	8.9	11.8	11.9	7	48.3
Sterlet	1.1	1.7	7.5	12.8	12.5	3.8	39.4
Sterlet	1.4	1.8	5.4	8.9	9.1	5.2	31.8
Sterlet	2.1	2.4	8.1	9.3	9.1	6	37
Sterlet	5.8	9.1	7.9	12.3	11.6	9.7	56.4
Sterlet	3.5	3.9	3.4	7	7.2	5.6	30.6
Wels	2.8	3.7	11.9	13.7	14.4	10.7	57.2
Wels	2.3	3.1	9.6	35.7	37	12.5	100.2
Wels	2.5	3.1	12.2	28.5	23.6	8.6	78.5
Range	1.1–6.5	1.4–12.3	2–18.4	2.6–40.9	3.7–37	1.7–15.7	14.4–107.2
Mean	3	4	8.2	14	13.6	7.2	49.5
Median	2.4	3.1	7.8	11.2	11.6	6.4	42.9

Analysis of sample blank showed no interference peaks with the individual PCB congener analysis. The limit of determination for each congener was determined as the mean of 10 times background noise from five reagent blank samples. Methods limits of quantification, which depended on congener type, were in the range 0.2–0.5 ng/g. Analytical quality control was achieved by using certified reference material ERM-BB446 (IRMM, Belgium). Accuracy and intermediate precision were fulfilled according to the specific requirements for determination of ndl PCBs (Community Reference Laboratory for Dioxins and PCBs in Feed and Food, Freiburg, Germany 2008).

The concentrations of target individual congeners (IUPAC numbers 28, 52, 101, 138, 153 and 180) were expressed in ng/g of fresh weight. Values below the limit of detection were assigned to zero.

Descriptive statistics were estimated using ORIGIN program (version 7.1). Mann-Whitney U non-parametric test and principal component analysis (PCA) were performed with Statistica 7.0 software. The differences were considered statistically significant when p value was less than 0.05.

Results and discussion

Non-dioxin-like PCBs levels in fish samples were examined as a measure of freshwater pollution nearby Pancevo. Additionally, the results obtained from upstream and downstream samples in two different time points could also give us the data on both the influence of industry on aquatic ecosystem and current/previous history of pollution.

A summary of freshwater fish species used for ndl PCBs measurement in this study is presented in Table 1.

The content of ndl PCBs in edible tissues of fish samples from the Danube is shown in Tables 2 and 3.

In 2001, the sum of the six indicator PCBs in fish caught upstream from Pancevo was in the range 2.7–98.1 ng/g, with a median value of 22.8 ng/g, whereas in 2006, PCBs content ranged

from 4.9 to 68.3 ng/g with a median value of 24.5 ng/g (Tables 2 and 3). Medians do not differ significantly, pointing out that the upstream levels of ndl PCBs stayed unchanged during the five

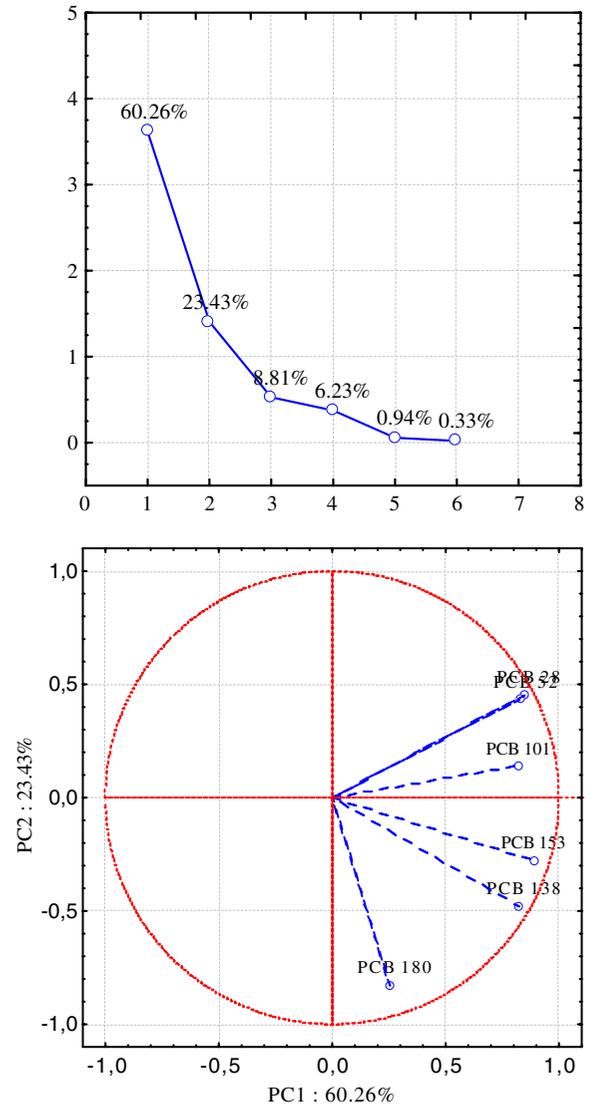


Fig. 2 Eigen values correlation matrix and loadings plot of PC1 (PCB 28) vs. PC2 (PCB 52) from the data set containing information on indicator PCBs (28, 52, 101, 138, 153 and 180) content in ten fish species matrices. *According to Morrison (1967) principal components should account for approximately 75% of the variables. Resulting loading plot has indicated difference in distribution between c and d patterns, what actually presents statistically higher values of indicator PCBs concentration in downstream samples in 2006 comparing with samples from not contaminated location collected in the same time interval

years period. In contrast, ndl PCBs content in fish caught downstream of Pancevo in 2006 was significantly higher than in fish caught at the same locations in 2001 (Table 2). Total ndl PCBs content in fish collected downstream of Pancevo in 2006 was in the range 14.4–107.2 ng/g, with median value of 42.9 ng/g, which was significantly higher than in samples of fish caught upstream during the same year (Table 3). Within the study frame it was not possible to ensure statistically relevant number of individuals of the same fish species, allowing us to avoid variability due to interspecies differences. Intraspecies comparison was only possible to undertake in the case of sterlets (Table 1). Results of statistical analysis of the data on ndl PCBs concentrations in sterlets were in accordance with the results obtained when all species had been included, proving that in 2006 there was significant increase ($p = 0.0013$) in contamination in downstream samples related to upstream ones.

PCA as the multivariate analytical tool is used to reduce a set of original variables and to extract a small number of latent factors (princi-

pal components-PCs) for analyzing relationship among the observed variables. Data submitted for the analysis were arranged in matrices. For analysis of correlation among locations of sampling and collecting time 6×64 correlation matrix was established. Furthermore, for analysis of correlation between sampling locations in 2001, 6×12 and 6×9 matrices were established, respectively, while for analysis of correlation between sampling locations in 2006, 6×19 and 6×24 matrices were established, respectively (Figs. 2 and 3). Finally, for analysis of correlation between the median concentrations of ndl PCBs Mann–Whitney non-parametric test was performed. Using PCA analysis in certain combinations, results have shown statistically significant difference between up and downstream samples collected in 2006 that had been already pointed out in first step of PCA analysis (Figs. 2 and 3).

For the purpose of comparison, levels of ndl PCBs on freshwater fish published in available literature are presented in Table 4. Results similar to ours were published by Vojinovic-Miloradov et al. (2002) for perch, carp and pike, also caught from

Fig. 3 Score plot of the first two principal component from data set containing information on indicator PCBs content in ten different fish species matrices. PC1 = PCB 28; PC2 = PCB52 **a** ndl PCB congeners in upstream samples in 2001; **b** ndl PCB congeners in downstream samples in 2001; **c** ndl PCB congeners in upstream samples in 2006; **d** ndl PCB congeners in upstream samples in 2006

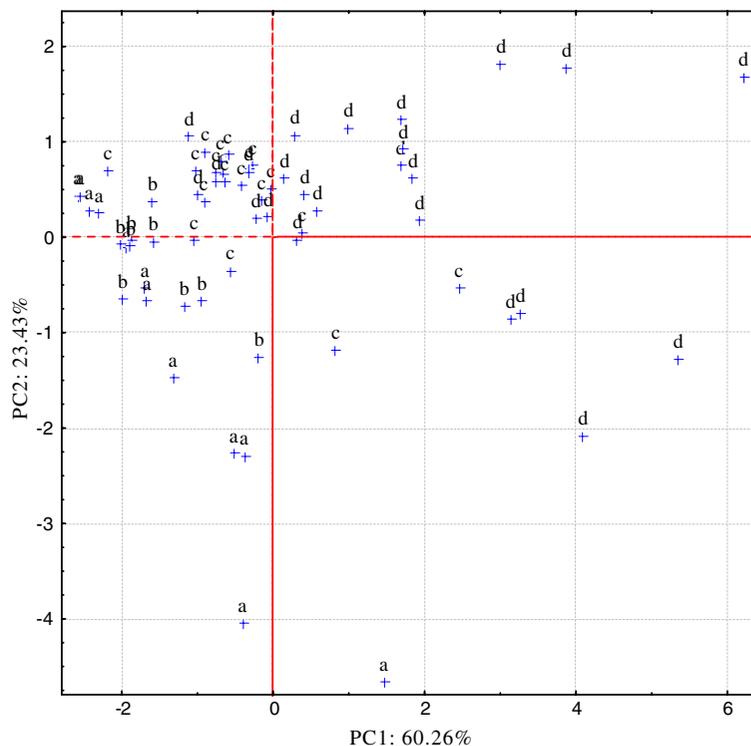


Table 4 Literature data on the levels of ndl PCBs in freshwater fish

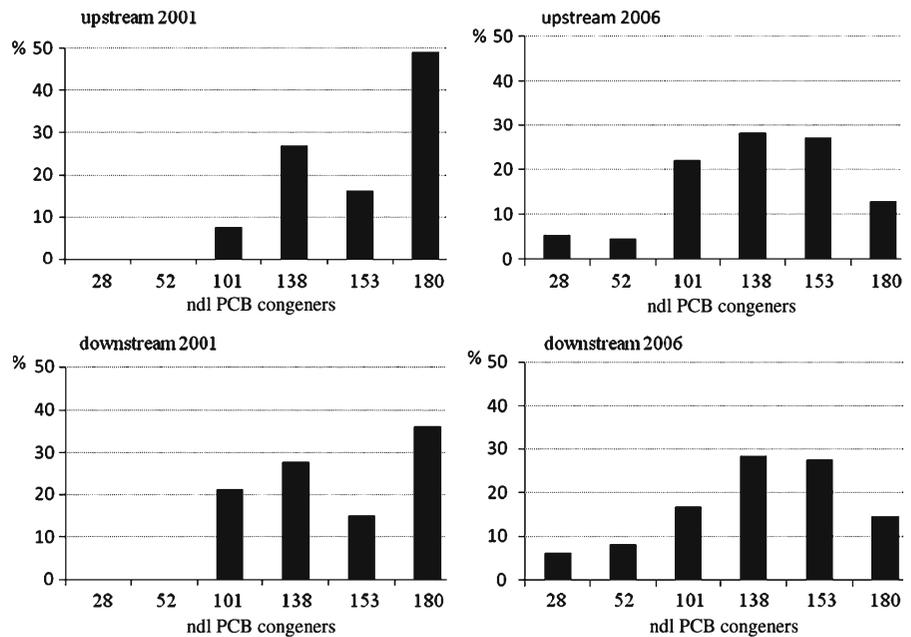
Σ ndl PCBs	Congener					Fish species	Source	Reference
	28	52	101	138	153			
ng/g fresh weight (*ng/g lipids)								
1,037.8	11.5	15.8	63	135	262.5	550	Roach	Bazzanti (1997)
6–22.5	–	–	–	–	–	–	Perch, Carp, Pike	Vojnovic-Miloradov et al. (2002)
370–1,100*	–	–	–	–	–	–	Perch	Falandysz et al. (2004)
8–177	1–5.6	3.3–5.2	0.9–0.65	0.4–4.5	1–1.8	0–2.4	different	Bošnjir et al. (2005)
7.8–56.9	–	–	–	–	–	–	different	Mazet et al. (2005)
7.48	0.14	0.3	0.94	2.2	2.4	1.5	Brown trout	Vives et al. (2005)
37–87	–	–	–	–	–	–	Pike perch	van Leeuwen et al. (2007)
1,451	7.6–233.1	0.5–124.5	3–159.7	14.8–509.8	10.3–226.7	10.3–200	Crucian carp	Zhao et al. (2007)
26.8	–	–	–	–	–	–	different	Arnich et al. (2009)
1.3–6.1	–	–	–	–	–	–	Pike perch, Perch	Waszak and Dabrowska (2009)

the Danube; Bošnjir et al. (2005) for different fish species from the Sava river which is the biggest tributary of the Danube in Serbia; Mazet et al. (2005) for fish from the Drome river (France); van Leeuwen et al. (2007) for pike perch from the Lek river (Netherlands) and Arnich et al. (2009) for freshwater fish from France. However, in some other studies, PCBs concentrations were either higher or lower than the levels found in our study. For example, in the area for the disassembly of obsolete transformers and electrical waste in China, Zhao et al. (2007) as well as Bazzanti (1997) in river Arrone (Italy) after the major contamination episode, determined 10–20 times higher levels of PCBs. In fish from freshwater reservoir in Poland (Waszak and Dabrowska 2009), or from high mountain lake Redo (Spain) (Vives et al. 2005) reasonably lower concentrations of PCBs were found due to low pollution input.

Concerning the congeners’ profile, the presence of congeners 101, 138, 153 and 180 is evident in all the samples collected in 2001 (Fig. 4). PCBs’ profile also showed that congeners 138 and 180 were predominant accounting for 27% and 49%, respectively, in fish caught upstream, and 28% and 36%, respectively, in fish caught downstream of Pancevo. Congeners 28 and 52, known as the indicators of recent exposure were not detected in 2001, pointing out that there was no additional contamination of the Danube river by PCBs usually attributed to accidental release of industrial sludge to the environment. In 2006, the measurements indicated the presence of all indicator congeners. Most abundant PCBs congener was 138 accounting for 28% in both locations (Fig. 4). The presence of PCB 28 and PCB 52 congeners has indicated a recent contamination event. Congeners’ profile in our study are similar to those published in several recent studies (Bazzanti 1997; Zhao et al. 2007; Vives et al. 2005; Carubelli et al. 2007; Zuccato et al. 2008), confirming the fact that highly persistent *di-ortho* substituted PCB-138, 153 and 180 are the prevailing congeners in biological samples.

During the 5-year period, there was an increase in total ndl PCBs. Additionally, in 2006, significantly higher concentrations were measured in downstream samples, implying that the industry

Fig. 4 Congeners' profile for the six indicator ndl PCBs



situated in the vicinity of Pancevo could be potential source of environmental contamination. Complementary to this are the measurements of PCBs in sediment revealing the concentrations from 80 to 1,600 ng/g (UNEP/UNCHS 1999). Obtained results of our study can be of public concern since there is a global trend of lowering the PCBs levels in different media due to significant reduction of their usage in industrial purposes (Bábek et al. 2008; Linderholm et al. 2010; El-Shaarawi et al. 2010). Besides, another issue related to the consumption of fish and consequent human health risk seems to be appropriate to address. Namely, obtained ndl PCBs concentrations are below the maximum residue level (MRL) of 3,000 ng/g fresh weight, established by Serbian Anonymous (1992), and are also below the limit of 2,000 ng/g proposed by Food and Drug Administration, USA (FDA 2001). For the sake of completeness, it should be mentioned that no maximum levels for ndl PCB in feed and food have been set in the European Union so far. According to Commission Regulation (EC) No. 199/2006 (2006), MRLs based on cumulative risk assessment and relative toxicity regarding 2,3,7,8-TCDD, have been given only for dl PCBs. The maximum level of 100 ng/g fresh weight for the sum of 6 ndl PCBs in fish has been proposed by the European Commission

(EC) draft regulation (AFSSA 2007). It can be seen from the Tables 2 and 3 that, except two samples collected downstream of Pancevo in 2006, all the other values of ndl PCBs fall below the European draft maximum level.

Conclusions

Results obtained in this work have shown temporal increase in ndl PCBs levels in freshwater fish caught from the Danube probably due to certain environmental pollution. Even more, the presence of PCB 28 and PCB 52 has indicated a recent contamination event. Data on continual monitoring of PCBs in all relevant environmental compartments together with appropriate biomonitoring data are expected to give comprehensive insight into the fate and behavior profile of these contaminants. Further studies are also expected to offer more precise assessment of biological response i.e., inter- and intraspecies variability in relation to bioaccumulation capacity and congener profile.

Acknowledgements This study is a part of the Project No.TR20212A granted by Ministry of Science and Technological Development of the R. Serbia.

References

- AFSSA—Agence française de sécurité sanitaire des aliments (2007). *Opinion of the 23rd October 2007 on the establishment of relevant maximum levels for non dioxin-like polychlorobiphenyls (NDL-PCB) in some foodstuffs*. Available from: www.afssa.fr.
- Anonymous (1992). Rolebook on quantities of pesticides, metals and metaloids and other toxic substances, chemiotherapeutics, anabolics and other substances that can be found in foodstuffs. *Off. Journal of FRY* No 5/92.
- Antonišević, B., Matthys, C., Sioen, I., Bilau, M., Van Camp, J., Willems, J. L., et al. (2007). Simulated impact of a fish based shift in the population on 3 fatty acids intake on exposure to dioxins and dioxin-like compounds. *Food and Chemical Toxicology*, 45(11), 2279–2286.
- Arnich, N., Tard, A., Leblanc, J., Le Bizec, B., Narbonne, J., et al. (2009). Dietary intake of non-dioxin-like PCBs (NDL-PCBs) in France, impact of maximum levels in some foodstuffs. *Regulatory Toxicology and Pharmacology*, 54(3), 287–293.
- Bábek, O., Hilscherová, K., Nehyba, S., Zeman, J., Famera, M., et al. (2008). Contamination history of suspended river sediments accumulated in oxbow lakes over the last 25 years Morava River (Danube catchment area), Czech Republic. *Journal of Soils and Sediments*, 8(3), 165–176.
- Bayarri, S., Baldassarri, L. T., Iacovella, N., Ferrara, F., & di Domenico, A. (2001). PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. *Chemosphere*, 43(4–7), 601–610.
- Bazzanti, M. (1997). Distribution of PCB congeners in aquatic ecosystems: A case study. *Environment International*, 23(6), 799–813.
- Bordajandi, L. R., Martín, I., Abad, E., Rivera, J., & Gonzáles, M. J. (2006). Organochlorine compounds (PCBs, PCDDs and PCDFs) in seafish and seafood from the Spanish Atlantic Southwest Coast. *Chemosphere*, 64(9), 1450–1457.
- Bošnić, J., Puntarić, D., Klarić, M., & Šmit, Z. (2005). Polychlorinated biphenyls in freshwater fish from the Zagreb area. *Archives of Industrial Hygiene and Toxicology*, 56(4), 303–309.
- Carubelli, G., Fanelli, R., Mariani, G., Nichetti, S., Crosa, et al. (2007). PCB contamination in farmed and wild sea bass (*Dicentrarchus labrax* L.) from a coastal wetland area in central Italy. *Chemosphere*, 68(9), 1630–1635.
- Commission Regulation (2006). No. 199/2006 on 3 February 2006 amending Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs.
- EFSA—European Food Safety Authority (2005). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the presence of NDL polychlorinated biphenyls (PCB) in feed and food. *The EFSA Journal* 284.
- El-Shaarawi, A. H., Backus, S., Zhu, R., & Chen, Y. (2010). Modelling temporal and spatial changes of PCBs in fish tissue from lake Huron. *Environmental Monitoring and Assessment*. doi:10.1007/s10661-010-1408-5.
- Falandysz, J., Wyrzykowska, B., Warzocha, J., Barska, I., Garbaciak-Wesołowska, A., et al. (2004). Organochlorine pesticides and PCBs in perch *Perca fluviatilis* from the Odra/Oder river estuary, Baltic Sea. *Food Chemistry*, 87(1), 17–23.
- Kiviranta, H., Ovaskainen, M. A. L., & Vartiainen, T. (2004). Market basket study on dietary intake of PCDD/Fs, PCBs, and PBDEs in Finland. *Environment International*, 30(7), 923–932.
- Liem, A. K. D., Fürst, P., & Rappe, C. (2000). Exposure of populations to dioxins and related compounds. *Food Additives and Contaminants*, 17(4), 241–259.
- Lim, Y., Yang, J., Kim, Y., Chang, Y., & Shin, D. (2004). Assessment of human health risk of dioxin in Korea. *Environmental Monitoring and Assessment*, 92(1–3), 211–228.
- Linderholm, L., Biague, A., Mansson, F., Norrgren, H., Bergman, A., & Jakobson, K. (2010). Human exposure to persistent organic pollutants in West Africa—A temporal trend study from Guinea-Bissau. *Environment International*, 36(7), 675–682.
- Mazet, A., Keck, G., & Berny, P. (2005). Concentrations of PCBs, organochlorine pesticides and heavy metals (lead, cadmium, and copper) in fish from the Drome river: Potential effects on ottes (*Lutra lutra*). *Chemosphere*, 61(6), 810–816.
- Morrison, D. (1967). *Multivariate statistical methods*. New York: McGraw-Hill.
- Smith, A. G., & Gangolli, S. D. (2002). Organochlorine in seafood: Occurrence and health concerns. *Food and Chemical Toxicology*, 40(6), 767–779.
- State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany, Community Reference Laboratory for Dioxins and PCBs in Feed and Food (2008). *Specific requirements for determination of NDL PCBs (PCB # 28, 52, 101, 138, 153, 180)*.
- UNEP/UNCHS Balkan Task Force (1999). *The Kosovo conflict consequences for the environment & human settlements*. ISBN 92-807-1801-1, Switzerland.
- US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry-ATSDR (2000). *Toxicological profile for polychlorinated biphenyls (PCBs)*.
- US FDA—Food and Drug Administration (2001). *Fish and fisheries products hazards and controls guidance* (3rd ed.). Rockville: Center for Food Safety and Applied Nutrition.
- USDA, Food Safety and Inspection Service (1991). *Analytical Chemistry Laboratory Guidebook*. Washington DC.
- van Leeuwen, S. P. J., Leonards, P. E. G., Traag, W. A., Hoogenboom, L. A. P., & de Boer, J. (2007). Polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in fish from the Netherlands: concentrations, profiles and comparison with DR CALUX® bioassay results. *Analytical and Bioanalytical Chemistry*, 389(1), 321–333.

- Vives, I., Grimalt, J. O., Ventura, M., Catalan, J., & Rosseland, B. O. (2005). Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta*) from a remote high mountain lake (Redo, Pyrenees). *Environmental Pollution*, 133(2), 343–350.
- Vojinovic-Miloradov, M., Adamov, J., Sekulic, P., Buzarov, D., & Jovetic, S. (2002). *Levels of POPs in Yugoslavia—Case study*. Paper presented at the 1st UNEP Regional Workshop on Assessment of PTS sources and concentrations in the environment, February 4–6 2002, Athens (Greece).
- Waszak, I., & Dabrowska, H. (2009). Persistent organic pollutants in two fish species of Percidae and sediment from the Sulejowski Reservoir in central Poland. *Chemosphere*, 75(9), 1135–1143.
- Zhao, G., Xu, Y., Li, W., Han, G., & Ling, B. (2007). PCBs and OCPs in human milk and selected foods from Luqiao and Pingqiao in Zhejiang, China. *Science of the Total Environment*, 378(3), 281–292.
- Zuccato, E., Grassi, P., Davoli, E., Valdicelli, L., Wood, D., et al. (2008). PCB concentrations in some foods from four European countries. *Food and Chemical Toxicology*, 46(3), 1062–1067.