POLYCHLORINATED BIPHENYLS (PCBs) IN MUSCLE OF THE WILD COMMON CARP *(CYPRINUS CARPIO)* FROM THE LAKE PIEDILUCO

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Introduction

Lake Piediluco is situated in the centre of the Italian peninsula and is, in order of importance, the second lake of Umbria. This is a natural lake (surface: 1.7 km^2, volume: 20,000,000 m^3, max depth: 20 m) regulated for hydroelectric purposes. Its basin has been enlarged (from 74 km^2 to 3204 km^2) through the connection to the Nera and Velino rivers, becoming a reservoir subjected to the charge and discharge processes of its waters. At present, the lake is in an eutrophic condition[1], mostly due to the pollution load produced by the important breeding activity of trout farms[2,3] located along the Nera River, upstream of the derivation channel entering the Lake Piediluco. Moreover, high organic load and marked changes of hydrodynamic conditions cause algal blooms and, under extreme meteorological conditions, water anoxia. The presence of hydrophobic and persistent organic contaminants such as polychlorinated biphenyls (PCBs) in fish of the lake Piediluco has been documented[4], however this lake has not been extensively studied. Since the use and manufacture of PCBs have been banned for more than 30 years now, it is interesting to determine the current levels of PCBs in wild common carp (*Cyprinus carpio*) whose feeding ecology involves direct contact with the sediment and a mostly benthic diet. Levels in these fish would reflect bioaccumulation from exposure to historically PCB-contaminated sediments. In this study, levels of PCBs in common carp were determined to investigate the extent of accumulation in fish (muscle tissue) of different ages and relate the concentrations to the length of exposure. Common carps are bottom feeders that do not migrate extensively, have long life spans (up to 38 yrs.) and reproduce rapidly. Therefore, their attitude to bioaccumulate pollutants from the sediment is high. Juveniles start to bioaccumulate at a very early stage of their life as a result of their feeding habits. Furthermore, common carp is able to survive hypoxia, and even anoxia for short periods, due to the utilization of ethanol pathway rather than lactate pathway[5]. For these reasons, carp are good species for monitoring the bioaccumulation of organic pollutants, particularly in hypoxic/anoxic water conditions when the survival of other fish species is precluded.

Materials and methods

Sampling and samples preparation

The samples were wrapped in aluminum foil, immediately frozen and transported to the laboratory. Overall fifty eight samples of carps were collected during 3 years in the lake Piediluco : sixteen in 2007, twenty one both in 2008 and 2009. Firstly the collected fish were weighted and the sex was identified: 24 were females and 34 males. Than the samples were submitted to chemical analysis for PCBs determination.

The fish was opened and the bones, organs and skin removed; the dorsal muscle tissues were sampled from each carp together with all the subcutaneous fat, and the whole was homogenized and stored at -20 °C.

Analytical method

The described analytical method enables the analysis of eighteen PCB congeners:T_3CB-28, T_4CB-52, P_5CB-95, P_5CB-99, P_5CB-101, P_5CB-105, P_5CB-110, P_5CB-118, H_6CB-138, H_6CB-146, H_6CB-149, H_6CB-151, H_6CB-153, H_7CB-170, H_7CB-177, H_7CB-180,H_7CB-183 and H_7CB-187 (mixture purity >98,5% at 10 ng μL^{-1} in isooctane purchased from Dr. Ehrenstofer, Ausgburg, Germany).

Thirty grams of muscle were weighted on a Petri glass plate, frozen at -80 °C overnight and freeze dried (8 h). The lyophilized samples were extracted with a mixture of n-hexane/acetone (1:1) (pesticide-grade reagents and solvents were from Carlo Erba, Milano, Italy) by means of an accelerated solvent extractor (ASE 200 Dionex

Corporation, Sunnyvale, CA). The solvent was removed under a reduced pressure and the extracts were cleaned-up on a Extrelut NT-3 (Merck, Darmstadt, Germany) column acidified with 3 ml of concentrated sulphuric acid connected on top of a silica cartridge 1 g/6 ml (International Sorbent Technology Mid Glamorgan, UK). After loading, the analytes were directly eluted with 13 ml n-hexane. The solvent was removed from the purified extracts under a nitrogen stream, the samples were dissolved in iso-octane (0.5 ml) and injected in a Agilent-Technologies Gaschromatography (6890 N) equipped with a 7683 series automatic injector, a PTV inlet and a μ-ECD detector. One microlitre of sample was injected in splitless mode and the injector temperature was programmed as follows: 0.08 min at 80 °C, increased to 270 °C at 700 °C/min, kept at 270 °C for 20 min. The chromatographic separation was achieved in temperature programmed mode using a 30 m x 0.25 mm diameter Rtx-5MS/Integra Guard column (5% diphenyl–95% dimethyl polysiloxane, film thickness 0.25μm, Restek Corporation, Bellefonte, PA, USA) as follows: 1 min hold at 60 °C, ramp to 190 °C at 10°C /min, 13 min hold at 190 °C, ramp to 270 °C at 3 °C /min, 6.33 min hold at 270 °C.
In all positive samples the identification of the analytes was confirmed by GC–MS (Agilent-Technologies Gas chromatography 6890 N, mass detector 5973 inert, 7683 series automatic injector) using the same column and the same thermal program used in GC-ECD. Each of the PCB was monitored acquiring at least one target ion and two qualifiers. The H_6CB-155 and O_8CB-198 (Dr. Ehrenstorfer, Reference Materials, Augsburg, Germany) were used as internal standards to obtain a final injected concentration of 50 ng/mL. They were used for calibration and recovery evaluation. Total lipid content of fillets was gravimetrically determined.

Linearity
The internal standard multipoint calibration technique (1, 50, 100, 150 and 200 ng/ml in iso-octane) was used to determine the linear response interval of the detector. The regression equations, calculated with the least square method, were obtained by plotting peak area ratios (congener area/internal standard area) versus concentration. Good determination coefficients were obtained for each congener (r^2>0.998). The compliance to linearity was also investigated by calculating the response factors (peak area ratio-intercept/concentration) and verifying that each of the five values obtained for each congener was included in the mean value ±20%.

Limit of detection (LOD) and limit of quantification (LOQ)
The LOD was estimated according to the U.S. EPA guidelines by multiplying the appropriate one-sided 99% t-statistic by the standard deviation. This was obtained from a six repeated analyses of a spiked matrix containing the analytes at a concentration three to five times higher than the estimated LOD. The method detection limits ranged from 0.1 (PCB 105) to 1.4 (PCB 153) ng/g fat, while the limit of quantification was set to 2.5 ng/g fat for all the congeners and verified by analysing spiked samples (n = 6) at 2.5 ng/g fat in order to assess the trueness and the precision. Recoveries were in the range 95–125% and the relative standard deviations were between 1% and 13%.

Trueness and precision
To investigate the precision and trueness of the method, six replicate analyses on a blank muscle spiked with all eighteen congeners at four different concentration levels (2.5, 6, 45, 90 ng/g fat) were performed in order to investigate the performances of the method in all the application field (precision). Recovery rates (±SD) varied between 57% (±5) for PCB 28 and 136% (±11) for PCB 177. Thereafter, a certified reference material (Cod Liver Oil –CRM349 – Community Bureau of Reference, Commission of the European Communities), in which the amounts of six PCB congeners representing all the homologues classes (tri- to epta-chlorinated) were certified, was analysed in six replicates (trueness). The mean measured values for the PCB 28, 52, 101, 153, 138, 180 were not significantly different from the true values certified. Therefore the method is sufficiently accurate.

Results and discussion
The six NDL-PCBs and the eighteen PCBs mean levels found in wild common carp are reported in table 1 giving details on minimum, maximum and mean concentrations per each year of sampling. No significant difference was found, between sexes, for body mass and lipid percent content (Anova, p> 0.05). Male fish captured in 2009 have a body mass significantly higher than fish captured during 2007 and 2008, irrespective to the sex.

A significant positive correlation (Pearson, r= 0.35, p< 0.01; Spearman's Rho, r= 0.40, p< 0.01) was found between body mass and percent lipid content in both sexes, without interaction between sex and body mass (GLM, p> 0.05). In spite of this correlation and of the previously mentioned body mass difference, no significant difference (Anova, p> 0.05) was found among years with regard to percent of lipid content.
Body mass differences were not highlighted between fish sampled during autumn/winter and those sampled during spring/summer (Anova, p> 0.05), though a significant difference was found with regard to the lipid content, that was higher during spring/summer (Anova, p< 0.01). In spite of the absence of significant difference for body mass respect to season, introducing the latter as covariate in the model (GLM), with season as a fixed factor and lipid percent content as dependent variable, resulted in an augmentation of the significance level of the corrected model from p= 0.008 to 0.001.This augmentation of model R^2 from 0.123 (12.3 % of explanation) to 0.224 (22.4 % of explanation), compensates the lipid percent differences related to body mass and unmasks the true role of season as explanatory factor. Accordingly, partial explanatory contribution of season passed from 12.3 % to 9.89 % in the model with body mass as covariate. Effectively, only male fish displayed such seasonal pattern and omitting female from the computation, resulted in a R^2 of 0.30 (30 % of explanation) and a partial explanatory contribution of season of 10.40 %. .
It is to be highlighted that the PCB contamination levels reported as sum of the six indicator congeners take into account of roughly 50% of the total PCB contamination estimated as sum of the eighteen congeners.
Only in 2008 the sum of 18 congeners was positively correlated with body mass of both males and females (female, Pearson, r= 0.91, p< 0.01; Spearman's Rho, r= 0.71, p< 0.01; male, Pearson, r= 0.62, p< 0.05; Spearman's Rho, r= 0.70, p< 0.05), though this probably resulted from the relative lower number of replicates of the years 2007 and 2009 according to sex. No correlation was detected between the sum of 18 congeners and the lipid content (Pearson, p> 0.05; Spearman's Rho, p> 0.05). No significant difference was reported for the sum of 18 congeners, corrected (covariated) for body mass, according to sex and season (Anova, p>0.05). Nevertheless, male fish displayed lower congeners values during 2009 compared to 2008 (Anova, p< 0.05).

Table 1. PCB (ng/g fat weight) levels (range, mean values, standard deviation and median) in wild common carp

6 NDL-PCBs						
Year	n					
		Min	Max	Mean	SD	Median
2007	16	62	1146	525.1	321.1	489.5
2008	21	110.6	5819	902.8	1246.4	477
2009	21	72.7	1159	554.1	372.4	650
18-PCBs						
Year	n					
2007	16	120	2381	1060.8	631.8	972.5
2008	21	229	10322	1706.6	2189.4	1030
2009	21	162.8	2223	1116.6	725.1	1306

Conclusions
Common carp was proved to be a potential good bioindicator due to its feeding and benthic habits, territoriality, long spanning life and hypoxia/anoxia tolerance. Particularly interesting was the correlation between body mass and PCB concentrations, though further investigations are needed to correlate PCB concentrations in sediments with those in fish tissue and to gain information about PCBs partition.

References:

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