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# Bioaccumulation of Heavy Metals, Organochlorine Pesticides, and Detoxication Biochemical Indexes in Tissues of *Ictalurus melas* of Lake Trasimeno

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Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and heavy metals are persistent substances ubiquitously present in aquatic ecosystems. Their bioaccumulation in biota is governed by the physico-chemical properties of the compounds, the bioavailability from environmental matrices, the structure of the food chain, the lipid content of the species and the metabolism of the exposed organisms. These environmental contaminants have been identified as oxidative stress inducers in fish, because they enhance the production of active oxygen species (ROS). Antioxidant systems in organisms are able to counterbalance oxidative stress (Winston and Di Giulio 1991; Cnubben et al. 2001). Some components of these systems involve reduced glutathione (GSH) and certain antioxidant enzymes, including free radical scavenging enzymes, such as glutathione peroxidases (GPx Se-dependent, or GPx Se-independent enzyme), glutathione reductase (GR) and catalase (CAT). Other associated enzymes are glutathione S-transferases (GST), glyoxalase I (GI) and glyoxalase II (GII). Therefore, the biochemical changes may serve as biomarkers of oxidative damage caused by pollutants in fish, as well as in other organisms. Consequently, biomarkers are important tools in biomonitoring programs, since they allow us to detect the presence of various contaminants in the environment.

The aim of this study was to investigate the changes in antioxidant response and in accumulation of contaminants in catfish, *Ictalurus melas* Raf., collected from Lake Trasimeno, in order to verify the contamination status of certain pesticides and heavy metals and also to identify, among a suite of biochemical indexes, the useful biomarkers that could be utilized in biomonitoring programs. Little information is reported about the organic and inorganic contamination (Galarini et al. 2002; Elia et al. 2005) and for detoxifying enzymes in fish of Lake Trasimeno (Elia et al. 2005). Therefore, Cu, Hg, Pb, Cr and HCHs, HCB, DDTs, PCBs were evaluated in muscle, while oxyradical scavenger (total glutathione, GSH+2GSSG), antioxidant enzymes (GPx, GR, CAT) and protective enzymes (GST, G I, G II) were examined in liver and gills of the same samples. In this study, the muscle was chosen for chemical analyses of contaminants and liver and gills for biochemical analyses. In fact, as already reported in literature, the muscle is known to better reflect a chronic exposure to pollutant inputs, whereas the liver is recognized as the tissue which reflects short-term contaminant exposure (Albaigés et al. 1987).

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## MATERIALS AND METHODS

A sample of one hundred *Ictalurus melas* (mean total length 20.3 ± 1.3 cm; mean weight 107.1 ± 22.8 g), were collected from Lake Trasimeno in August 1997, February and June 1998. After capture liver and gills were immediately carefully excised and washed with cold sodium chloride solution (9 g/l). The gill filaments of both sides were trimmed from the gill arches and the arches were discarded. The tissues were grouped in 5 pools of 4 specimens in August and 8 pools of 5 specimens for February and June. Each pool was subdivided into portions for biochemical analyses. The pooled muscles were utilized only for chemical analysis. The tissues for biochemical and chemical analyses were immediately stored at –80°C and –20°C, respectively.

The concentrations of OCPs and PCBs were determined in the lipid extracted from muscle using a Dionex ASE 200 Accelerated Solvent Extractor. The fat was weighed, dissolved in isoctane and washed with concentrated sulphuric acid (Tulonen and Vuorinen 1996). A quantitative analysis was performed by Perkin Elmer gaschromatograph (AUTOSYSTEM), equipped with two EC detectors and programmable thermal injectors. Helium was used as carrier and nitrogen as make-up gas. The main analytical and confirmation columns were Rtx-5MS and Rtx-1701, respectively, both 30 m x 0.25 mm id x 0.25 μm film thickness. The oven temperature program was: 60°C, 1 min; 60°C to 220°C, 25°C/min; 220°C to 270°C, 15°C/min, 270°C, 20 min. Aroclor 1242, 1254 and 1260 were used to quantify PCBs reported. The quantitation limits of pesticides and PCB mixtures were 10 ng/g and 100 ng/g lipid weight (LW), respectively.
The sample tissues (4-5 g) for determination of Cd, Pb and Cr were placed in quartz crucibles and heated overnight at 400°C. The ash residue was dissolved in 1 N hydrochloric acid. Metals were analyzed by atomic absorption spectrometry (AAS). Cd, Cr and Pb were analysed using a flameless graphite oven with an autosampler (AS-60). The standard additions were used for matrix effects. For total Hg analysis the muscle (1 g) was wet digested at 50°C with concentrated sulphuric and nitric acids and then analysed by cold vapor atomic absorption spectrometry (MHS-10) (Galarini et al. 2002). The quantitation limits were 10 ng/g for Cd and 50 ng/g wet weight (WW) for Cr, Hg, and Pb.

All the assay conditions of biochemical parameters have been detailed elsewhere (Elia et al. 2000). Total glutathione (GSH+2GSSG) was determined on the deproteinized supernatant by the GR enzymatic method. Weighed tissue was homogenized in 4 volumes of 1 M $HClO_4$, 2 mM EDTA, maintained for 30 min in ice and centrifuged at 30000 *g* for 20 min.
The study of enzymatic activities was performed on the cytosolic fractions. Weighed samples were homogenized in 10 volumes of 100 mM TRIS buffer, pH 7.8, containing 100 μM phenylmethylsulphonyl fluoride (PMSF). Centrifugation was carried out at 4 °C at 100 000 *g* for 60 min and the supernatant was utilized for the determination of enzymatic activity. Glutathione peroxidase (Se-dependent enzyme) activity was determined with $H_2O_2$ as substrate. The oxidation of NADPH was monitored at 340 nm ($\varepsilon$ = - 6.22 $mM^{-1}$ $cm^{-1}$). Glutathione reductase

activity was determined by following the decrease in absorbance at 340 nm ($\varepsilon$ = -6.22 $mM^{-1}cm^{-1}$) due to the oxidation of NADPH. Catalase activity was measured following the decrease in absorbance at 240 nm due to $H_2O_2$ consumption ($\varepsilon$ = -0.04 $mM^{-1}cm^{-1}$). Glutathione S-transferase activity, with the substrate 1-chloro-2,4-dinitrobenzene (CDNB), was measured by following the formation of the conjugate with GSH at 340 nm ($\varepsilon$ = 9.6 $mM^{-1}$ $cm^{-1}$). Glyoxalase I activity was determined by monitoring at 240 nm ($\varepsilon$ = 3.37 $mM^{-1}cm^{-1}$) the formation of S-D-lactoylglutathione from the hemimercaptal adduct of methylglyoxal (MG) and reduced glutathione (GSH). Glyoxalase II activity was determined at 412 nm ($\varepsilon$ = 13.6 $mM^{-1}$ $cm^{-1}$) by measuring GSH formation in the presence of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and S-D-lactoylglutathione (LSG).

All data were included in the statistical analysis using a single-factor ANOVA with Student-Newman-Keuls'tests (SPSS 9.0 package). The experimental data matrices were submitted to the simple linear Pearson correlation analysis. Significance was tested at $P<0.05$. All variables were log-transformed in order to minimize the impact of outliers.

## RESULTS AND DISCUSSION

The average concentrations of organochlorine compounds (ng/g lipid weight, LW) in catfish sampled from Lake Trasimeno are reported in Table 1. The HCB content in muscle of catfish was 2.5 times higher in June than in February and the value was not detectable in August. As shown in Table 1, γ-HCH was 2 times higher in February than in June, while α-HCH, β-HCH, o,p- DDT and o,p-DDE levels were systematically under the quantitation limit during all the sampling period (data not shown).

The contaminant levels evaluated in muscle of catfish collected from Lake Trasimeno are similar to those generally measured in non-industrialized, temperate areas, although few data are available as yet for organochlorine residues detected in catfish in Italy or in adjacent countries. The amount of organic pollutants is similar to that reported by our previous study of *Ictalurus melas* sampled from the same lake during 1997 (Galarini et al. 2002). Moreover, Roche et al. (2000) found an analogous level of organochlorine contamination in *Ictalurus nebulosus* collected in Camargue (French), except lindane, which was higher than the level reported in our study. According to those authors, the high HCH level might be explained by its systematical use against the rice borer caterpillar over the whole Camargue rice growing area.

Among DDTs, p,p'-DDT was detectable only in June and o,p-DDD was present in August and February (at the same level). The level of p,p'-DDD was about 2 times higher in June than in the other two months, while p,p'-DDE value was similar in all months (Fig. 1). As evidenced in Fig. 1, DDT level was detected only in samples collected in June (p,p'-DDT/DDTs $\approx$ 0.2).

The PCBs content was about twice as high in June than in the other months: AR1242 was absent in February, AR1260 increased in June and AR1254 showed an increasing trend all over the sampling period (data not shown). As a general tendency, the organochlorine residues increased in June compared to the other

sampling months. A strongly positive correlation was found between HCB, PCB and DDT concentrations; on the contrary, no relationship was apparent between levels of HCH and these organochlorines (data not shown).

**Table 1**. Organochlorine compounds (ng/g lipid weight) and heavy metals (ng/g wet weight) in catfish of Lake Trasimeno (mean ± SD).

| Substances | August (n=5) | February (n=8) | June (n=8) |
|---|---|---|---|
| HCB | nd c | 11.8 ± 1.3 b [10-14] | 29.5 ± 3.8 a [24-36] |
| γ-HCH | nd c | 31.1 ± 8.0 a [20-41] | 14.3 ± 1.9 b [12-17] |
| DDTs | 115.0 ± 9.9 b [99-123] | 124.6 ± 11.1 b [113-139] | 216.3 ± 9.4 a [200-227] |
| PCBs* | 629.0 ± 71.5 b [533-722] | 621.5 ± 68.6 b [525-715] | 1319.0 ± 173.0 a [1068-1508] |
| OCPs | 115.0 ± 66.4 b [99-123] | 167.5 ± 60.4 b [144-189] | 260.0 ± 112.5 a [241-278] |
| Hg | 99.0 ± 13.0 [86-115] | 73.1 ± 48.0 [nd-115] | 113.0 ± 32.3 [67-160] |
| Pb | 297.8 ± 75.1 a [220-408] | 176.9 ± 97.2 b [103-364] | 125.8 ± 24.9 c [85-163] |
| Cr | 143.4 ± 14.0 a [129-161] | 72.0 ± 10.4 b [54-90] | 36 b [nd-98] |
| Cd | 14.2 ± 4.1 a [11-21] | 5 b [nd-16] | 3 b [nd-13] |

*Semi-quantitative estimation of the total PCB congeners (present in a mixture of Aroclors 1242, 1254 and 1260). Value ranges are indicated in brackets. Different letters (a, b, c) indicate significant differences between the sampling periods in the same row ($P < 0.05$); nd: not detected. n = number of pools.

This trend is probably due to similar mechanism of accumulation of the correlated substances. Biomagnification is the process resulting from bioconcentration and bioaccumulation that increases contaminant concentrations in tissues through the trophic levels. There is little evidence in literature suggesting that γ-HCH and heavy metals such as Pb, Cr, Cd biomagnify in aquatic food webs; on the other hand, DDTs, PCBs and mercury (as organic compound) are well known as having the potential to biomagnify (Suedel et al. 1994). Thus, the highest values of organochlorines in fish collected in June might be attributed to the seasonal changes in some chemical-physical factors or/and food availability. The observed differences in organochlorine contents are referred to levels measured on fat basis, and being the percentage of muscle lipid nearly the same for all analysed pools (≈1.8%), the seasonal trend does not change when the measured concentrations are referred to wet weight.

Heavy metals had the opposite trend (Table 1), showing higher levels in August compared to the other months. These heavy metals became undetectable in

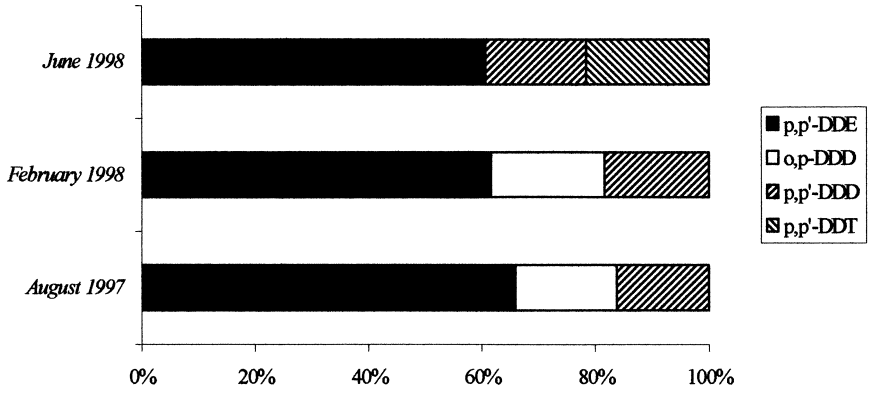


**Figure 1**. Relative distribution of DDT and its metabolites in Ictalurus melas of Lake Trasimeno during 1997-1998.

February (Cd) and June (Cd and Cr), except mercury which did not show statistically significant differences. Regarding heavy metals, European legislation recently fixed the limits for Cd, Hg and Pb in fish products (EC Council Regulation 466/2001). According to this Regulation, the catfish collected in August and two of eight pools sampled in February showed Pb concentrations exceeding the limit of 0.2 mg/kg wet weight. Lead is very widespread in the environment, above all due to its use as gasoline additive in the past. In the 90's, the levels of this element were generally decreasing in italian aquatic ecosystems owing to the gradual introduction of the lead-free petrol (Galarini et al. 1996). Regarding the other two non-essential metals, no samples showed concentrations exceeding the peak value of 0.05 mg/kg and 0.5 mg/kg wet weight in muscle for cadmium and for mercury, respectively.

As reported in Table 2, catfish liver of Lake Trasimeno exhibited higher levels of glutathione in February and June than in August (about 1.5 times), while gills showed the highest significant level in June (about 2.4 times) compared to the other months. There are many reports, from both field and laboratory studies, indicating that heavy metals and organic contaminants in the aquatic environment (chlorothalonil, aromatic hydrocarbons, PAH, PCB and HCB) induce an increase of glutathione level in fish. These results may be due to the scavenging ability of glutathione, which offers a first step protection against oxyradicals (Winston and Di Giulio 1991). In the present study, catfish of Lake Trasimeno revealed that the highest concentration of thiol in examined tissues, mainly in June, was concomitant with the greater level of GR and Se-GPx activity in liver and gills, respectively. In fact, Se-GPx activity in gills was markedly higher in June (about 3 times) than the other two months, while GR hepatic activity increased (about 2.5 times) from August to June. Therefore, the elevated GR catalyzing activity of catfish might indicate an enhancement in total glutathione consumption used as a defense line against organoradicals. Moreover, specimens collected in June exhibited lower CAT activity in both tissues and the activity was strongly

negatively correlated with the organic contaminants in muscle (Table 3). Presumably, the low CAT activity recorded in specimens collected in June could be related to the increase in organochlorine contaminants, which could weaken the tissues antioxidative status. As reported by Ahmad et al. (2000), in catfish exposed to paper mill effluent, a time-dependent increase in GPx and GST and a time-dependent decrease in CAT activities were found. According to the authors the decrease of CAT was related to the presence of some organic and inorganic redox active compounds in the effluent.

**Table 2**. Antioxidant parameters in catfish of Lake Trasimeno (mean ± SD).

| | | August (n=5) | February (n=8) | June (n=8) |
|---|---|---|---|---|
| [1]GSH+2GSSG | Liver | 678.3 ± 68.6 b | 944.3 ± 136.7 a | 1029.1 ±123.4 a |
| | Gills | 69.7 ± 11.6 b | 120.6 ± 64.2 ab | 167.6 ± 31.9 a |
| [2]Se-GPx | Liver | 111.7 ± 15.8 | 112.3 ± 22.6 | 143.2 ± 18.9 |
| | Gills | 20.8 ± 3.7 b | 38.1 ± 15.8 b | 67.6 ± 18.1 a |
| [2]GR | Liver | 14.2 ± 1.5 c | 22.6 ± 3.4 b | 39.2 ± 9.1 a |
| | Gills | 8.3 ± 1.9 | 11.1 ± 4.1 | 15.1 ± 8.6 |
| [3]CAT | Liver | 101.2 ± 23.6 a | 94.9 ± 17.9 a | 55.6 ± 14.1 b |
| | Gills | 33.1 ± 9.2 a | 25.2 ± 7.4 a | 4.1 ± 0.9 b |
| [2]GST | Liver | 266.8 ± 58.5 c | 430.7 ± 80.1 b | 606.8 ± 87.1 a |
| | Gills | 126.8 ± 21.4 b | 352.3 ± 67.2 a | 364.3 ± 88.6 a |
| [2]GI | Liver | 217.7 ± 12.8 b | 215.1 ± 58.7 b | 356.2 ± 98.3 a |
| | Gills | 272.8 ± 50.7 | 252.6 ± 57.2 | 213.8 ± 86.8 |
| [2]GII | Liver | 122.6 ± 19.9 b | 105.8 ± 13.9 b | 169.9 ± 36.1 a |
| | Gills | 49.2 ± 7.4 b | 68.3 ± 10.3 a | 63.8 ± 9.1 a |

[1] nmol/g wet weight; [2] nmol/min/mg protein; [3] μmol/min/mg protein; statistical comparison: different letters (a, b, c) indicate significant differences between sampling periods (in the same row); n = number of pools.

It was reported that GST activity was elevated in channel catfish exposed to sediment contaminated with PAH and other xenobiotics (Di Giulio et al. 1993) and in fish from a heavy metal-contaminated site (Lenártová et al. 1997). In the present investigation, elevated GST activity was detected in February and June in both tissues (2.5 times), compared to that measured in August.
This highest enzyme activity could providing evidence of a good capability to counterbalance the oxidative damage which might be induced by the pollutants recorded in this accumulative species. According to our result this enzyme may be designated as useful biomarker for monitoring organic contamination. GI activity in gills of catfish of Trasimeno remained approximately the same, while in liver it reached the highest value in June. GII activity changed in liver and gills raising the highest value in June and in February, respectively. These enzyme activities in catfish liver showed a similar correlation of thiol, GST and Se-GPx enzymes with organic contaminants (Table 3). In literature there are not much information about

the variations of GI and GII enzymes in *Ictalurus melas* exposed to contaminants, except the few reports concern the glyoxalases activities in these fish under mercury laboratory exposure (Elia et al. 2000; Elia et al. 2003).

**Table 3**. Pearson's moment correlation for biological data and analite contents in catfish of Lake Trasimeno. *: $P < 0.05$.

| | HCB | γ-HCH | DDTs | PCBs | Hg | Pb | Cr | Cd |
|---|---|---|---|---|---|---|---|---|
| | | | | LIVER | | | | |
| GSH+2GSSG | 0.7 * | 0.6* | 0.5* | 0.3 | 0.1 | -0.4 | -0.2 | -0.3 |
| Se-GPx | 0.6* | -0.1 | 0.7* | 0.8* | 0.5* | -0.4 | -0.4 | 0.1 |
| GR | 0.8* | 0.3 | 0.8* | 0.7* | -0.1 | -0.7* | -0.7* | -0.6* |
| CAT | -0.7* | 0.1 | -0.7* | -0.7* | -0.3 | 0.5 | 0.7* | 0.3 |
| GST | 0.9* | 0.4 | 0.7* | 0.7* | 0.1 | -0.7* | -0.5* | -0.6* |
| GI | 0.6 | -0.2 | 0.7* | 0.7* | 0.1 | -0.3 | -0.4 | -0.1 |
| GII | 0.5* | -0.3 | 0.7* | 0.6* | 0.1 | -0.3 | -0.4 | -0.4 |
| | | | | GILLS | | | | |
| GSH+2GSSG | 0.7* | 0.3 | 0.5* | 0.5* | 0.2 | -0.5 | -0.5 | -0.5 |
| Se-GPx | 0.8* | 0.3 | 0.8* | 0.7* | 0.2 | -0.6* | -0.6* | -0.5* |
| GR | 0.4 | 0.2 | 0.4 | 0.3 | 0.1 | -0.3 | -0.3 | -0.3 |
| CAT | -0.9* | -0.1 | -0.9* | -0.9* | -0.3 | 0.5 | 0.6* | 0.4 |
| GST | 0.7* | 0.7* | 0.4 | 0.3 | -0.1 | -0.5* | -0.6* | -0.5 |
| GI | -0.4 | -0.1 | -0.3 | -0.3 | -0.2 | 0.00 | 0.1 | 0.1 |
| GII | 0.4 | 0.6* | 0.3 | 0.1 | -0.4 | -0.4 | -0.5* | -0.4 |

Thus, the increase of glyoxalases activities represents an induction of these metabolic detoxification pathways, that might be enhanced during peroxidative processes in living organisms, decreasing α-ketoaldehydes toxicity.
In conclusion, catfish of Lake Trasimeno can be considered as a valuable accumulative species for these pollutants and the biochemical changes recorded in this fish might provide important background information for the implementation of biomarker-based monitoring programs using this fish species.

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