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Comparison of Organochlorine Pesticides, PCBs, and Heavy Metal Contamination and of Detoxifying Response in Tissues of *Ameiurus melas* from Corbara, Alviano, and Trasimeno Lakes, Italy

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Abstract Accumulation of cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), hexachlorobenzene, γ-HCH, DDTs, and PCBs has been investigated in the muscle of *Ameiurus melas* sampled during the same period from Lake Corbara, Alviano, and Trasimeno. Glutathione content and the enzymatic activities of glutathione reductase, glutathione peroxidase, catalase, glutathione S-transferase, and glyoxalase I were examined in gills, liver, and kidneys of each specimen. Catfish from Alviano, compared to those of Corbara and Trasimeno, showed the highest contamination of DDTs and PCBs and the lowest levels of biochemical parameters. Most likely, OCPs and PCBs content might be responsible for the compromised antioxidant status in these specimens.

Keywords *Ameiurus melas* · Antioxidant response · Heavy metals · Organic contaminants

Although the open use of many of these pollutants—polychlorinated biphenyl (PCB), DDT, hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB)—has been prohibited in most industrialized countries, considerable residues still remain in the environment. These molecules, such as HCB and p,p′-DDE—the latter being the major metabolite of DDT—exhibit high octanol/water partition coefficients ($LogK_{ow} > 4$) and are selectively partitioned from water into bed and suspended sediments and into the lipid tissue of aquatic biota. Fish are considered as sentinel organisms in the aquatic ecosystem to assess the environmental contamination of water by xenobiotics, because fish can be chronically exposed to different substances, such as heavy metals and pesticides, and they can bioaccumulate by direct exposure or through the food chain. Fish can be considered an excellent early warning system for environmental health problems that could potentially lead to human health concerns. In fact, the interaction of pollutants with living organisms can be a cause of oxidative stress, which might result in a variation of antioxidant systems (Winston and Di Giulio, 1991). Some components of these systems involve reduced glutathione (GSH) and certain antioxidant 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) and glyoxalase I (GI).

In the present study, catfish *Ameiurus melas* Raf., an economically important freshwater fish species in Italy, was chosen as the experimental model to investigate the contents of heavy metals, organochlorine pesticides, and PCB in specimens sampled from Alviano, Corbara, and Trasimeno. These three lakes, located in Central Italy, show vastly different geomorphologic and physical characteristics: Corbara and Alviano are artificial basins, affected by an anthropic charge of the Tiber River with urban and industrial waste; Trasimeno is a natural basin, not affected by industrial activities; but Trasimeno is bordered by farms (mostly hog operations) and agricultural activities. Our previous study, conducted on the muscles of catfish sampled from Corbara and Trasimeno during the winter of 1996–1997, showed evidence of the highest level of Pb, Hg, zinc (Zn), and HCB in specimens from Corbara

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(Galarini et al., 2002). Following that study, organic contaminants, such as PCB and DDT, were analyzed in muscle and antioxidant parameters in gills and liver of catfish sampled seasonally during 1997–1998 from Lake Trasimeno (Elia et al., 2006). The results indicated an increased content of organic contaminants through the sampling months, and the biochemical parameters were also positively correlated with the investigated pollutants. Until this writing, no information is available regarding organochlorine and heavy metal contamination in catfish from the Alviano reservoir. Therefore, the purpose of this study was to identify, among a suite of contaminants, the appropriate bioaccumulation marker that could be used to assess the inland water contamination and to verify the health state of this fish species by evaluating its antioxidant response. Therefore, the levels of copper (Cu), Hg, Pb, Cr, HCHs, HCB, DDTs (o,p-DDE, p,p′-DDE, o,p-DDD, p,p′-DDD, o,p-DDT, p,p′-DDT), and PCBs (AR1242, AR1254, AR1260) were measured in muscle, and the levels of GSH+2GSSG, GST, GI, GPx, GR, CAT were examined in liver, kidney, and gills of each specimen.

Materials and Methods

The three lakes Alviano, Corbara, and Trasimeno differ greatly in geomorphologic and physical characteristics: Trasimeno is the fourth largest lake in Italy, and because of the peculiar morphology of its basin (126 km^2; average depth of 4 m) and shoreline, Trasimeno is defined a laminar or shallow lake. Corbara is a dam reservoir built in 1963 on the Tiber River with a maximum depth of about 40 m and a surface of 15 km^2. Lake Alviano is also an artificial basin, which derives from the Tiber, with an area of 3.49 km^2 and a maximum depth of 9 m. Alviano is situated below the confluence of the Paglia River with the Tiber.

Specimens of *Ameiurus melas* were sampled from Trasimeno (n = 25; mean ± SE; TL 21.4 ± 0.2 cm; wt 127.06 ± 5.29 g), Corbara (n = 30; mean ± SE; TL 21.2 ± 0.4 cm; wt 122.98 ± 7.85 g) and Alviano (n = 30; mean ± SE; TL 21.0 ± 0.8 cm; wt 124.34 ± 20.48 g). All specimens were collected within 24 hr using fyke nets. The samplings from Trasimeno, Corbara, and Alviano were conducted during the same week in August 2002. Immediately after capture, catfish were sacrificed by cervical dislocation, weighed, and measured. Liver, gills, kidneys, and muscle were removed. The gonads of both sexes were immature. The sex ratio for each sampling site was near 1:1 (Trasimeno 1:1.08, Corbara 1:1.14, Alviano 1:1 in favor of the males). Therefore, the tissues were grouped in 5 pools of 5–6 specimens of both sexes. The tissues for the biochemical and chemical analyses were immediately stored at –80°C and –20°C, respectively. The muscle was chosen for chemical analyses of contaminants, whereas the other tissues were chosen for biochemical analyses. The muscle is known to reflect better a chronic exposure to pollutant inputs, whereas the liver is the tissue that reflects short-term contaminant exposure (Albaigés et al., 1987). Furthermore, liver is the major tissue for detoxification of exogenous and endogenous electrophilic compounds, and gills and kidneys can be also exposed to toxic compounds.

The pesticides investigated in this study were: HCB (hexachlorobenzene), α-HCH, β-HCH, γ-HCH (lindane), o,p-DDE, p,p′-DDE, o,p-DDD, p,p′-DDD, o,p-DDT, and p,p′-DDT. Aroclor 1242, 1254, and 1260 were used to quantify PCBs. The organochlorine compounds (OCPs and PCBs) were determined in the lipid extracted from muscle using a Dionex ASE 200 Accelerated Solvent Extractor, as reported in Elia et al. (2006) . The fat was weighed, dissolved in isooctane, and washed with concentrated sulfuric acid. A quantitative analysis was performed by Perkin Elmer gaschromatograph (AUTOSYSTEM, Perkin Elmer, Waltham, MA, USA), equipped with two EC detectors and programmable thermal injectors. Helium was used as carrier and nitrogen as make-up gas. The main analytic and confirmation columns were Rtx-5MS and Rtx-1701, respectively, both 30 m $\times$ 0.25 mm id $\times$ 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 were 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 analyzed using a flameless graphite oven with an autosampler (AS-60). The standard additions were used for matrix effects. For total Hg analysis, the muscle was wet digested at 50°C with concentrated sulfuric and nitric acids and then analyzed 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.

Total glutathione (GSH+2GSSG) content was determined by the GR recycling assay at 412 nm according to the original assay and under conditions described previously (Elia et al., 2003). Weighed samples were homogenized in 10 vol of 100 mM TRIS buffer, pH 7.8, containing 100 µM PMSF and bacitracin 0.1 mg ml^{-1}. Centrifugation was carried out at 4°C at 100,000 g for 60 min, and the supernatant was used for the determination of enzymatic activities (Elia et al., 2003). Glutathione peroxidase (GPx) activity was determined by using hydrogen peroxide as substrate in 100 mM sodium phosphate pH 7.5 EDTA 1 mM (340 nm, ε mM = –6.22). Glutathione reductase (GR) activity was determined in 100 mM sodium phosphate pH 7.0 by monitoring the reduction in absorbance caused by the oxidation of NADPH (340 nm, ε mM = –6.22). Catalase (CAT) activity was measured in 100 mM sodium phosphate buffer pH 7.0, 12 mM H2O2 (240 nm, ε mM = –0.04). Glutathione S-transferase (GST) was assayed in 0.1 M sodium phosphate, pH 6.5 with the substrate CDNB 1 mM and GSH 1 mM (340 nm, ε mM = 9.6). Glyoxalase I (GI) activity was determined by using 1.5 mM GSH/methylglyoxal hemithioacetal as substrate (240 nm, ε mM = 3.37). Enzyme assays were performed at a constant room temperature of 20°C, and blanks were subtracted from the sample absorbance. Protein concentration supernatant fractions were determined by the method of Lowry et al. (1951).

Kruskal Wallis ANOVA test and the pairwise differences were tested using a Mann–Whitney *U* test to discriminate differences for each sampling site. All data were run by triplicate, and results are expressed as mean ± ES. The relationship between biochemical parameters and contaminants was evaluated also by Spearman correlation analysis. Significance was tested at 5% ($P < 0.05$).

Results and Discussion

As shown in Table 1, the content of HCB was about 2 times higher in catfish from Alviano than in those of Corbara, and it was not detectable in specimens from Trasimeno. The concentrations of α-HCH and β-HCH were below the limit of detection of the method in all specimens from the three lakes, except for γ-HCH, which was detectable only in fish from Alviano. Our previous report indicated that the higher level of p,p′DDE and the higher level of mainly HCB was found in the muscle of catfish collected from Corbara as compared with that of the same fish species from Trasimeno, both sampled during the same period (winter 1996–1997) (Galarini et al., 2002). The DDTs levels were higher in specimens of Alviano (10 times) and Corbara (5 times) than in those of Trasimeno; p,p′DDE, p,p′DDD, and o,p-DDD were found in highest concentrations in Alviano, o,p,DDE was present only in fish from Corbara, whereas p,p′-DDT and o,p, DDT were not detectable in those fish from Trasimeno. Among the DDT isomers, p,p′-DDE was present in a large amount in specimens from Alviano. p,p′-DDE is the main metabolic product, and it is more persistent than DDT, and thus it is the molecule that is more easily accumulated from organisms. Aroclor (AR1242, AR1254, and AR1260) concentrations were much higher in catfish of Alviano than in those of the other two lakes, and, as a general tendency, the PCBs content in Alviano was much higher than in Trasimeno (about 23 times) and Corbara (about 7.5 times). Regarding the analysis of PCBs content, as the determination is performed on the Aroclor mixtures instead on individual congeners, the results indicate only an overall level of PCBs contamination. Among the total PCB content, AR1254, which is the more predominant congener, was also recorded at the highest concentration in specimens from Alviano. The PCBs values measured for catfish of Lake Alviano appear much higher than those reported for some Italian freshwater fish, such as carp, allice, tench, perch, and pike from Lake Iseo; those values ranged from

Table 1 Organochlorine compounds (ng/g lipid weight) in muscle of catfish collected from Trasimeno, Corbara, and Alviano*

	Trasimeno	Corbara	Alviano
HCB	nd	45.01 ± 2.12 [b]	94.25 ± 3.57 [a]
γ-HCH	nd	nd	12.50 ± 0.87
o,p-DDE	nd	11.75 ± 0.48	nd
p,p′-DDE	78.01 ± 1.10 [c]	111.50 ± 4.81 [b]	899.75 ± 41.55 [a]
o,p-DDD	21.03 ± 0.89 [b]	15.02 ± 0.71 [c]	41.75 ± 3.07 [a]
p,p′-DDD	20.80 ± 0.92 [b]	28.01 ± 2.68 [b]	65.25 ± 4.89 [a]
o,p-DDT	nd	12.75 ± 0.48 [b]	53.75 ± 4.11 [a]
p,p′-DDT	nd	27.25 ± 2.06	23.75 ± 2.66
DDTs	119.80 ± 2.08 [c]	206.25 ± 9.34 [b]	1084.25 ± 45.52 [a]
AR1242	169.20 ± 14.76 [c]	505.25 ± 9.67 [b]	3408.04 ± 145.75 [a]
AR1254	311.60 ± 10.58 [c]	1078.01 ± 42.18 [b]	8285.25 ± 298.60 [a]
AR1260	186.02 ± 18.47 [b]	285.03 ± 15.44 [b]	2259.25 ± 90.81 [a]
PCBs	666.80 ± 25.25 [c]	1868.22 ± 57.92 [b]	13952.50 ± 350.91 [a]
Hg	94.01 ± 5.51 [b]	59.01 ± 5.67 [c]	182.25 ± 14.74 [a]
Pb	319.40 ± 27.48 [a]	168.25 ± 21.12 [b]	260.02 ± 35.21 [a]
Cr	146.60 ± 5.52 [a]	98.75 ± 12.26 [b]	151.25 ± 15.65 [ab]
Cd	16.20 ± 2.06	nd	7.75 ± 1.60

n = 5; mean ± SE

*Semiquantitative estimation of the total PCB congeners (present in a mixture of Aroclor 1242, 1254, and 1260). Different letters (a, b, c) indicate significant differences between sampling site at $P < 0.05$

n, number of pools; nd, not detected (below the limit of detection: OCPs, 10 ng/g LW; PCBs, 100 ng/g LW; Cr, Pb, Hg, 50 ng/g; Cd, 10 ng/g)

1513 to 4915 ng/g lipids (Binelli and Provini, 2004). In spite of this, Bressa et al. (1997) reported a higher value for PCBs (274.36 µg/kg wet weight) in eels' muscle collected from the Po Delta, probably because of the high fat content of the fish species. No data are reported for PCB and DDT levels in sediments or water from Trasimeno, Corbara, and Alviano. However, the low levels of OCP in catfish of Trasimeno can be explained by the complete absence of industrial plants in this basin. On the contrary, Corbara and Alviano are mainly affected by the River Tiber with great urban and industrial waste.

The heavy metals (Hg, Pb, and Cd) displayed a lower level in fish of Corbara than in those of the other two lakes, and Cd was present only in specimens of Trasimeno and Alviano (in low amount). Catfish from Alviano and Trasimeno showed lead concentrations exceeding the limit of 0.2 mg/kg wet weight (EC Council Regulation 466/2001). Catfish did not show concentrations of Cd and Hg exceeding the peak value of 0.05 mg/kg and 0.5 mg/kg wet weight in muscle, respectively.

The results of the antioxidant enzymes and total glutathione level of all tissues of catfish of Alviano, Corbara, and Trasimeno are shown in Table 2. GPx activity was different only in the gills of catfish, being the highest in specimens of Corbara and mainly in those of Alviano. It seems that this activity, which was strongly correlated with organic and inorganic contaminants (data not shown), displayed more efficiency to neutralize the ROS induced by contaminants, which were at the highest concentrations in specimens of Alviano. The evaluation of reduced glutathione and antioxidant enzymes in gills, liver, and kidneys of *Anguilla anguilla* exposed to bleached kraft pulp mill effluent, showed that liver was more resistant to oxidative damage than the other two tissues (Santos et al., 2004). Other authors suggested that some contaminants from the petrochemical industry can cause oxidation in fish muscle by impairing the antioxidant system. No effect was observed in the livers of exposed fish, and according to those authors, the liver has a stronger antioxidant capacity than muscle (Avci et al., 2005). In a field study, it was reported by other authors that sculpin (*Myoxocephalus scorpius*) caught from contaminated PAH harbors, displayed higher enzymes activities of GR, CAT, and GPx, as compared with those of the reference site (Stephensen et al., 2000). However, Hamed et al. (2003) found a decrease of GST, GPx, and GR activities in fish exposed to heavy metal polluted sites as compared with controls. The different response of glutathione and glutathione-dependent enzymes in those fish species suggest a dissimilar susceptibility to contaminants. CAT activity in gills was the lowest in fish from Corbara and Alviano, whereas in liver it was the lowest in specimens from Corbara. In kidneys, this enzyme activity was the same for all specimens. It seems that the marked lowering in both tissues of Alviano could be the consequence of the increased $O_2^{-}\cdot$production caused by the marked accumulation of OCPs and Hg in these samples, which may induce a worsen oxidative stress status in these specimens. In a species of fish (*Channa punctatus*) exposed to paper mill effluent, a time-dependent decrease in CAT activity was found, which was related to the presence of some organic and inorganic redox active compounds in the effluent (Ahmad et al., 2000). Among

Table 2 Antioxidant parameters in tissues of catfish collected from Trasimeno, Corbara, and Alviano*

		Trasimeno	Corbara	Alviano
GPx	Gills	22.11 ± 0.77 [c]	31.34 ± 1.99 [b]	56.56 ± 2.70 [a]
	Liver	116.76 ± 7.86	95.32 ± 4.03	117.50 ± 7.84
	Kidneys	62.22 ± 5.91	60.57 ± 4.53	70.15 ± 6.51
GR	Gills	9.48 ± 0.82 [a]	9.28 ± 0.84 [a]	4.87 ± 0.74 [b]
	Liver	14.84 ± 0.72 [ab]	12.24 ± 0.78 [a]	7.83 ± 0.91[b]
	Kidneys	15.05 ± 1.40 [a]	8.16 ± 0.98 [b]	5.76 ± 1.47 [b]
CAT	Gills	35.68 ± 3.98 [a]	16.16 ± 2.17 [b]	18.15 ± 0.46 [b]
	Liver	99.58 ± 8.89 [ab]	72.01 ± 2.37 [a]	113.56 ± 8.15 [b]
	Kidneys	18.50 ± 1.66	15.04 ± 2.02	17.25 ± 1.79
GSH+2GSSG	Gills	74.71 ± 6.21 [a]	55.15 ± 3.56 [b]	34.47 ± 2.01 [c]
	Liver	689.97 ± 5.49 [a]	440.03 ± 25.43[b]	310.47 ± 14.82 [c]
	Kidneys	141.35 ± 8.08 [a]	114.66 ± 4.81 [a]	47.35 ± 3.41 [b]
GST	Gills	130.72 ± 6.76 [a]	114.15 ± 7.80 [a]	88.44 ± 4.72 [b]
	Liver	253.86 ± 26.10 [a]	238.16 ± 23.11 [a]	112.57 ± 13.07 [b]
	Kidneys	146.43 ± 4.48 [a]	109.34 ± 11.41 [a,b]	111.28 ± 3.59[b]
GI	Gills	270.20 ± 24.98 [a]	281.56 ± 10.09 [a]	182.04 ± 12.18 [b]
	Liver	278.90 ± 15.97 [a]	251.20 ± 12.13 [a]	108.82 ± 3.05 [b]
	Kidneys	175.44 ± 7.41 [b]	220.19 ± 5.51 [a]	107.91 ± 3.16 [c]

n = 5; mean ± SE

*GPx, GR, GST, GI are reported as nmol/min/mg prot, CAT as µmol/min/mg prot, and GSH + 2GSSG as nmol/g wet weight. Different letters (a, b, c) indicate significant differences between sampling site at $P < 0.05$

n, number of pools

biochemical parameters of catfish from all three lakes, GPx showed a positive correlation with total OCPs and PCBs in gills, whereas the other biochemical parameters were almost all negatively correlated with these contaminants (data not shown). GR activity measured in liver and gills of catfish from Alviano was markedly lower (about 50%) as compared with that of specimens from the other two lakes, whereas the enzyme activity of kidneys was statistically different only from that of catfish from Trasimeno, which was lower by about 65%. Therefore, the depleted GR catalyzing activity of catfish might cause the decrease of reduced glutathione content, which is used as a defense line against organoradicals. Gills and liver of catfish sampled from Corbara and Alviano showed evidence of the lowest GSH+2GSSG level as compared with that of specimens from Trasimeno; the difference was minor for those from Corbara (about 30%) and major for those from Alviano (about 50– 60%). On the contrary, kidneys exhibited the same thiol level for specimens from Trasimeno and Corbara and the lowest content for those from Alviano (about 60%). Our current data showed that the lower glutathione level recorded in all tissues of Alviano and in liver of specimens from Corbara may facilitate the onset of oxidative damage as a result of a decreased defense capability. The steady-state level of oxidative stress depends on both prooxidant forces and antioxidant defense. The rate of ROS generation increased as a result of contaminant exposure either to heavy metals or to organic contaminants. Thus, the depleted thiol level indicates a shifting toward a prooxidant status in catfish from Corbara and mainly in those from Alviano, compared to that of specimens from Trasimeno. Support for this conclusion stems from GST activity in the liver and gills of catfish from Alviano, which was lower than that measured in specimens of Corbara and Trasimeno of about 50% and 30%, respectively. The activity in kidneys was statistically different for fish from Alviano and Trasimeno. The contaminant level in catfish from Alviano was high, probably causing the enzyme inhibition. It is also possible that toxic intermediates produced in the liver may inactivate GST, resulting in reduced hepatic enzyme activity. Further investigations will be required to validate this supposition. As reported in the literature, specimens of *Ameiurus nebulosus* from contaminated sites of PCB, showed a lower thiol level as well as antioxidant enzymes, in various tissues than those of the control sites (Otto and Moon, 1996). In addition, other fish species, *Gobio gobio* and *Rutilus arcasii*, caught downstream and upstream of the waste sites of chemical industries exhibited a reduced thiol level and a decline of GST activity (Almar et al., 1998). GI activity was marked lower in all tissues, mainly in the liver (about 60%) of Alviano in respect to that of Trasimeno and Corbara, probably because of the highest OCPs accumulation causing a compromised detoxifying role against α-ketoaldehydes, usually formed during oxidative processes.

The evaluation of contaminants level in *Ameiurus melas* indicates the highest contamination of organic pollutants, which may result in a marked risk from these contaminants for specimens from Alviano. In addition, the depleted levels of thiol and of certain antioxidant enzymes in almost all tissues of specimens, mainly from Alviano, compared to those from Trasimeno, might reflect the highest levels of the investigated organic contaminants in muscle. To assess better the health state of these ecosystems, further studies should be extended on catfish and other aquatic species from biotops with low contamination levels, in order to allow an early intervention and to prevent any possible toxic effect on the aquatic community.

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