

# Journal of Applied Sciences

ISSN 1812-5654





Organochlorine Pesticides and Biomarker Responses in Two Fishes *Oreochromis niloticus* (Linnaeus, 1758) and *Chrysichthys nigrodigitatus* (Lacepède, 1803) and an Invertebrate, *Macrobrachium vollenhovenii* (Herklot, 1857), from the Lake Taabo (Côte d'Ivoire)

<sup>1</sup>Hélène ROCHE, <sup>2</sup>Abiba TIDOU and <sup>1</sup>Ana PERSIC

<sup>1</sup>Ecologie, Systématique and Evolution, UMR8079 CNRS-Université Paris-Sud XI, Bât 362,
F91405 Orsay Cedex, France

<sup>2</sup>Laboratoire des Sciences de l'Environnement, UFRSGE; Université d'Abobo-Adjamé,
02 BP 801 Abidjan 02, Côte d'Ivoire

**Abstract:** The concentrations of organochlorine pesticides (OCPs) were determined in muscle samples of two species of fish, tilapia (*Oreochromis niloticus*) and the catfish (*Chrysichthys nigrodigitatus*) and the prawn (*Macrobrachium vollenhovenii*) found in Lake Taabo (Côte d'Ivoire). Simultaneous measurements of enzymatic biomarkers were made to evaluate the ecotoxicological risk in this hydroelectric reservoir. Lindane and endosulfan were the dominant contaminants, suggesting their current use in neighboring agricultural areas. Other organochlorine (OC) compounds were detected, including some currently banned substances. Ranked in an order of descending concentrations, we found: DDT and its metabolites (17.8-57.2 ng g<sup>-1</sup> dry weight), endrin (7.17-25.0 ng g<sup>-1</sup> dry weight) and heptachlor (7.36-23.6 ng g<sup>-1</sup> dry weight), as well as traces of isomers of chlordane, aldrin and fipronil. The hepatic Glutathione S-Transferase (GST) activity measured in fishes was not correlated with pesticide contamination; whereas the antioxidant biomarkers demonstrated some significant associations, especially hepatic catalase with lindane (R = 0.83) and Glutathione Peroxidase (GPx) with heptachlor epoxide (R = 0.84) and with pp DDT (R = 0.81). In the prawns, acetylcholinesterase (AChE) activity showed significant negative correlations with DDT and its metabolites (R = -0.91). The results of this study emphasize the urgent need for overall environmental risk assessment studies in the region of Taabo and other developing areas.

Key words: Organochlorine pesticides, biomarkers, aquatic biota, Lake Taabo, Côte d'Ivoire

#### INTRODUCTION

In developed countries, the implementation of pollution control programs based on aquatic wildlife, water, sediment and air quality assessments has resulted in the strict regulation of the use of many substances suspected to pose risks to environmental and human health (PAN-Africa, 2003). Nevertheless, the World Health Organization (WHO) estimates that more than 1 million people around the world are affected by exposure to pesticides, causing 200,000 deaths annually (Pimentel et al., 1996; WHO report cited in Touni, 2005). In Africa and other developing regions, stocks of old and outdated pesticides are major sources of toxic risk. According to UN-FAO (2001), over the 50,000 tons of obsolete pesticides stocked in African countries, with 828 tons in Côte d'Ivoire. It is realistic to assume that these amounts are underestimations since the suspected

substances are often leftovers of phytosanitary products stored under uncontrolled conditions and used without proper safeguard, which endangers human population and the environment.

In this context, the Africa Stockpiles Program (ASP) aims to eliminate these stocks and to sensitize the population to the sanitary and environmental risks by recommending the regulation of substances already controlled in developed countries (Curtis and Palmer Olsen, 2004).

In Côte d'Ivoire, the misuse of pesticides in agricultural areas is detrimental to the quality of water and, consequently, could affect the health of aquatic organisms. Pesticide mixtures, used for agricultural treatments, often include some of the most poisonous Persistent Organic Pollutants (POPs) such as aldrin, chlordane, DDT, dieldrin, endrin and heptachlor. In 2002, Houenou (Conway, 2002) stated that the analysis

of fishes caught in the lake of Buyo (Côte d'Ivoire) confirmed the presence of chemical contaminants and clearly showed the biomagnification process along the food chain.

The present study focused on the levels of persistent organochlorine pesticides (OCPs) in three aquatic species (two fishes and an invertebrate) caught in a hydroelectric reservoir, namely Lake Taabo (Côte d'Ivoire).

Any deforestation or clean up of the plantation soils were carried out before the flooding of Taabo's valley; so the nutrients and the pesticides covered by the flooding could contribute to the pollution of the water of Lake Taabo and affect the components of aquatic food web. In general, hydroelectric dams on rivers not only reduce flow, but also cause an enrichment of the sediment with nutrients and pesticides from agricultural areas (Schindler *et al.*, 1995).

To explore this assumption, we quantified persistent organochlorine pollutants in muscle samples from three representative species. Furthermore, we also assessed the responses of environmental stress biomarkers currently used in European biomonitoring parameters have the biomarker programs. Such advantage of providing a quantitative response, as well as valid information reflecting the adverse biological responses towards anthropogenic contaminants. While such studies are mostly conducted in developed countries (Depledge, 1994; Burgeot et al., 1996; Van der Oost et al., 1996; Roche et al., 2002a, b; Buet et al., 2003), they are infrequent in developing countries (Dabrowski et al., 2002).

In this study, the approach consisted of highlighting the link between the concentration of pollutants in tissue and biological parameters, including physiological or biochemical compensatory responses and the processes of pollutant biotransformation. As this investigation constitutes one of the first ecotoxicological studies from Lake Taabo, we expect it to serve as a reference point for further ecological assessments of the chronic adverse effects caused by water pollution in this area. Furthermore, the aim has also been to compare present results with the data reported from other regions in the world, especially from European wetlands, in order to provide additional information to the validation of some biomarkers in natural ecosystems, as previously suggested (Van der Oost *et al.*, 2003).

### MATERIALS AND METHODS

**Study area:** Lake Taabo is located in the Région des lagunes situated within the limits of the dense forest and the savannah near the city of Taabo in the department of Tiassalé (Côte d'Ivoire). It is one of the five biggest

hydroelectric reservoirs built in 1979 by the construction of the Taabo dam on the main tributary of the Bandama River (1,050 km long), in order to ensure the socioeconomic development of Côte d'Ivoire.

The construction of the Taabo dam caused a deceleration of the stream and re-organized aquatic habitats as well as animal and plant populations. Lake Taabo stands as an important halieutic fisheries resource not only for the local populations but also for the inhabitants of Abidjan.

The regional climate is bimodal. The average annual rainfall ranges from 1,000 to 1,300 mm. The Bandama river flows exclusively in the territory of Côte d'Ivoire and its catchment area spreads across 97,500 km². It springs up in the North and stretches to the South where it flows into the Atlantic Ocean in the Gulf of Guinea.

The main agricultural activities are coffee (856 tons per year) and cocoa (6,300 tons per year) plantations. Other crops include yams, plantain bananas and pluvial rice and corn (about 4,300 tons per year). In addition, some farm-produce corporations set up alongside the river or its tributaries.

The aquatic fauna of Lake Taabo consists mainly of Cichlidae, Claroteidae and shellfish and a population of many aquatic insect larvae (Simuliidae, Anopheles etc...). Aquatic vegetation is mainly composed of water lettuce (*Pistia stratiotes*) and the invasive water hyacinth (*Eichhornia crassipes*).

**Information about the specie of interests:** The Nile tilapia (*Oreochromis niloticus*) is primarily an herbivorous Cichlidae with a great economic and ecological role. Its diet is dominated by phytoplankton (*Chlorophycea*, *Cyanophycea*, *Euglenophycea*) or benthic algae, but it becomes omnivorous under aquaculture conditions.

The African catfish (*Chrysichthys nigrodigitatus*) represents the most common species in the aquatic ecosystem of the 'Région des lagunes' and is also reared in aquaculture (Ouattara *et al.*, 1993). It is a generalist feeding on seeds, insects (mainly *Chironomidae*), bivalves and detritus.

The African river prawn (Macrobrachium vollenhovenii) is an omnivorous species, which is intensively exploited, particularly during the spawning period (Lhomme, 1994). These invertebrates generally migrate from estuaries and near shore coastal waters to river systems during the rainy season (July to September) (Goore Bi, 1998).

**Organism sampling:** Ten tilapia, seven catfishes and five prawns were collected by local fishermen in Lake Taabo Côte d'Ivoire (Fig. 1). The fishes and the prawns were

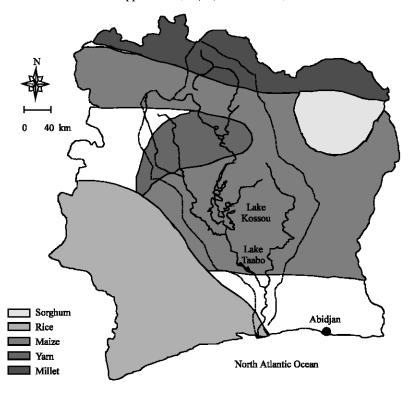


Fig. 1: The Côte d'Ivoire with the catchment area of Bandama River and its two hydroelectric reservoirs, Lake Taabo and Lake Kossou. Major agriculture areas according to the FAO (1996)

stored frozen in dry ice (-80°C) before their use at the laboratory where, the individuals were weighed and measured. Individual specimens were then opened and their organs were carefully dissected. In each animal, muscle samples were collected for pollutant analysis and for AChE activity measurement. AChE activities were also measured on brain samples from the fishes. Other biomarker analyses were performed on the livers of the fishes.

Since the organisms were frozen previously the pesticide and lipid concentrations should be expressed on a dry weight basis. Then, the analyzed dry mass of the muscle tissue was determined by oven-drying at 105°C for 24 h. The muscle lipids were extracted with a chloroform-methanol solution (Folch *et al.*, 1957) and then, were quantified spectrophotometry (Fiske and Subbarow, 1925). All the analyses were carried out on individual samples.

**Organochlorine analyses:** Pesticide grade solvents from VWR international (Strasbourg, France) were used in all tests. The concentrations of OCPs residues were determined in the muscle of fishes and in the total body mass of the prawns, excluding cuticle. Compounds analyzed included isomers of hexachlorocyclohexane

[α-HCH, β-HCH, γ-HCH (lindane), δ-HCH],-heptachlor and heptachlor epoxide-α-chlordane and γ-chlordane-dieldrin, aldrin, endrin and endrin aldehyde-dichloro-diphenyl-trichloroethane (pp'-DDT) and its degradation products, pp'-DDD, pp'-DDE; α-endosulfan, β-endosulfan, endosulfan sulphate and fipronil.

OCPs were purified by Solid Phase Extraction (SPE) on florisil (MgO<sub>3</sub>Si), an extremely polar, magnesia-loaded silica gel, following the EPA method 608 (Bond Elut Florisil, 1 g, 200 μM particle size-Varian). We used the hexane to eliminate polychlorobiphenyls (PCBs) and other similar pesticides. The hexane/diethyl ether (95/5) mixture was used to recover selected OCPs, according to clean-up procedures recommended by Durand *et al.* (1994). The eluates were evaporated to dryness and dissolved in 1 mL of hexane before Gas Chromatography (GC) analysis.

OCPs were analyzed by gas chromatography with an autosystem XL (Perkin-Elmer), using ECD (electron capture detection) (<sup>63</sup>Ni Source). The procedure was adapted to the EPA Method 8081a. The capillary column used was a PE-5 (Perkin Elmer, Courtaboeuf, France); stationary phase: 5% phenyl 95% methylpolysiloxane, length 30 m, internal diameter 0.25 mm. The carrier gas was nitrogen (12 psi, 20 mL min<sup>-1</sup>), nitrogen was also used as make-up gas (30 mL min<sup>-1</sup>). The oven program of

temperature was: 200°C (12 min), 10°C min<sup>-1</sup> to 210°C (23 min), 40°C min<sup>-1</sup> to 280°C (4 min.). The injector (split) and detector temperatures were 280 and 370°C, respectively. All reference materials (pesticide Mix 1, pesticide Mix 164, fipronil ref. 13645000,  $\gamma$ -chlordane ref. 11230000 and  $\alpha$ -chlordane ref. 11230700) were produced following the ISO9001 certified laboratories of Dr. Ehrenstorfer as part of the Reference Standards Programme provided by the Society CIL Cluzeau (Sainte Foy la Grande, France). The detection limit ranged from 0.01 to 0.05 ng g<sup>-1</sup> dry mass.

Enzymatic biomarkers: In this study, we focused on the biological effects of OCPs on the three organisms from the Lake Taabo ecosystem. The biomarkers were selected in order to investigate several metabolic processes (Cajaraville et al., 2000). These include lipid content, biotransformation process (GST) and antioxidant enzyme activities (catalase, GPX and SOD). When entering the organism, xenobiotics may be detoxified by a biotransformation process that generally leads to the formation of more hydrophilic and more easily excreted compounds than those entering (parent compounds). The Glutathione S-transferase (GST) participates in the conjugation phase (Gundersen et al., 2000; Van der Oost et al., 2003). Numerous biomonitoring programs use the measurement of GST as a biomarker (Cavanagh et al., 2000). Free radicals can be produced during endogenous or exogenous metabolisms and many contaminants have been shown to exert toxic effects related to oxidative stress. Organisms have developed defence systems to control the oxyradical formations. They include superoxide dismutase (SOD), catalase and total and selenium-dependant Glutathione Peroxidases (GPx). Their use as non-specific biomarkers has been recommended in numerous ecotoxicological studies (Choi et al., 2000; Roche and Bogé, 2000). Furthermore, AChE activities were also evaluated. Although AchE is considered as a specific biomarker for neurotoxic contaminants, such as carbamate and organophosphorous insecticides (Andersen et al., 1972; Westlake et al., 1983; Forget et al., 2003; Beliaef and Bocquene 2004), it is increasingly being used to assess the adverse biological effects of complex chemical mixtures (De la Torre et al., 2005).

The fish livers were homogenized in cold buffer (Tris 50 mM pH 7.4) with a Potter Elvehjem homogenizer and centrifuged at 1000 G at 4°C for 10 min to obtain the clarified homogenate. The supernatant was re-centrifuged at 12,000 G, 15 min at 4°C, to obtain the post-mitochondrial fraction. Enzymatic activities were measured in this fraction (supernatant S12). Glutathione S-transferase

(GST) activity was assessed by monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) (Habig et al., 1974). Total superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972) as modified by Buet (2002) based on the auto-oxidation of epinephrine into adrenochrom. Catalase activity was determined by the hydrogen peroxide breakdown method (Aebi 1984). Glutathione peroxidase activities (GPx + Se-GPx) were measured, following the method of Tappel (1978), using cumene hydroperoxide or hydrogen peroxide as substrate. Acetylcholinesterase (AChE) activity was analyzed in clarified homogenates of muscle and brain, according to the method of Ellman et al. (1961). Enzymatic activities were normalized to protein concentration of the extracts measured by the dye-binding assay of Bradford (1976), using bovine serum albumin as standard.

**Data analysis:** Inter-species and inter-site differences were compared using one-way analysis variance (ANOVA) followed by Scheffe's and Bonferroni-Dunnett post hoc tests. Pearson correlations between contamination level and biological data were calculated. All the tests were regarded as statistically significant when p<0.05. Statistical Analysis was performed using Statview software for Macintosh (Abacus Concepts Inc, Berkeley, CA, USA)

#### RESULTS

Information on specimen biometry and lipid contents are shown in Table 1. The two species of fish exhibited similar mass, but the catfishes showed a lipid content two times higher than the others analyzed organisms (vs tilapia, p = 0.0002 and vs prawn, p = 0.0016).

**Pesticide contamination:** The analyzed pesticides were selected according to the persistence of the molecules, to their former excessive use and risks to non-target organisms. The compounds were divided into two groups; the first consisted of the OCPs 'banned' in Europe more than 30 years ago and the second group were represented by compounds currently used, i.e., the allowed OCPs (Table 1).

Although the tilapia seemed to be the most contaminated species, the total OCPs concentration was similar (no significant difference) in the muscle samples from all three components of the food chain of Lake Taabo. More banned insecticides were found in all the species than allowed pesticides (Table 2). The difference in the banned pesticide concentrations was only significant between the tilapia and the catfish (p = 0.015).

Table 1: Biometric parameters, lipid content (mg g<sup>-1</sup> dw) and total impregnation (ng g<sup>-1</sup> dw) by analyzed pesticides of 3 species from Lake Taabo (Côte d'Ivoire)

			Mass	Length	Lipid	$\Sigma$ analyzed	$\Sigma$ banned	$\Sigma$ allowed
Species	Common name	n	(g)	(cm)	content	OC pesticides	OC pesticides	OC pesticides
Oreochromis niloticus	Nile tilapia	10	59.5±7.6	11.5±0.6	$13.8\pm0.9$	702±120	580±990	122±24
Chrysichthys nigrodigitatus	African catfish	7	45.2±2.6	$12.7\pm0.3$	28.8±3.1*	597±740	440±450	157±35
Macrobrachium vollenhovenii	River prawn	5	$17.5\pm1.9$	8.2±0.6	12.5±0.9	608±166	483±129	125±40

<sup>\*:</sup> Significant difference with other species p<0.02

Table 2: Organochlorine pesticides contamination in tilapia (O. niloticus), catfishes (C. nigrodigitatus) and prawns (M. vollenhovenii) from the Lake Taaho

Dry weight	abo O. niloticus	C. nigrodigitatus	M vollenhoven
(ng g <sup>-1</sup> )	(10)	(7)	(5)
Banned pesticides	(10)		(5)
α-HCH	3.07±1.32	2.50±0.92	2.90±1.29
w-11C11	[0.02-11.8]	[0.03-6.57]	[0.03-6.79]
β+γ-НСН	222.7±44.7	127.5±26.4	169.4±60.6
p. 1-11011	[0.33-454.8]	[27.8-243.9]	[14.2-324.2]
δ-HCH	49.6±4.61	76.3±16.5	45.5±13.7
Official	[32.9-72.7]	[0.22-138.0]	[15.7-92.2]
heptachlor	7.36±1.19	23.6±4.75*	8.63±4.74
nepatemai	[2.41-13.9]	[0.05-38.1]	[0.06-24.0]
heptachlor epoxide	18.5±6.54	21.2±6.26	48.3±17.1*
neponenioi eponius	[2.80-64.3]	[2.93-43.9]	[10.2-106]
y-chlordane	67.2±10.9*	19.3±4.89	19.9±2.78
,	[13.6-113.3]	[10.2-46.3]	[12.2-27.5]
α-chlordane	0.50±0.48	0.45±0.45	[
	[nd-4.30]	[nd-3.17]	nd
endrin	24.6±8.29	7.17±3.21	25.0±12.8
	[1.51-76.2]	[0.02-22.4]	[4.25-74.2]
endrin aldehyde	51.5±22.7	53.4±26.0	33.6±17.1
•	[<0.01-212]	[<0.01-156]	[<0.01-97.8]
aldrin	11.3±4.15	13.6±4.02	32.4±16.8
	[<0.01-36.6]	[0.47-28.2]	[0.01-84.3]
pp'-DDE	31.5±9.42	17.8±5.01	38.6±10.3
	[4.75-87.7]	[0.05-34.6]	[13.9-71.5]
pp'-DDD	35.4±15.6	19.7±11.1	29.7±14.6
	[0.10-146]	[0.13-79.4]	[0.19-85.6]
pp'-DDT	57.2±17.8	57.1±11.1	28.8±10.0
	[12.1-187]	[25.7-117]	[5.91-56.2]
Allowed pesticides			
fipronil	$17.1\pm4.02$	13.0±2.31	20.5±6.90
	[0.11-41.9]	[4.51-23.8]	[0.13-38.1]
α-endosulfan	17.8±3.92	12.4±2.26	18.6±6.85
	[0.07-35.3]	[2.04-21.3]	[0.06-42.0]
β-endosulfan	34.3±8.64	21.7±4.58	37.7±10.2
	[5.70-81.0]	[8.93-41.8]	[6.77-63.6]
endosulfan sulphate	52.3±17.1	110.0±30.4	48.6±20.5
	[0.24-156]	[52.0-282]	[0.33-118]
Volume one ermanes	1	and none of Feeding marry	(>

Values are expressed as mean $\pm$ SD and range [min-max], (n) = number of individuals, \*: Inter-species significant differences, nd = not detected

The substances detected at the highest concentrations ( $\Sigma$ HCH,  $\beta$ -endosulfan and endrin and to some extent DDT) showed a great variability both within the same species and between the three species tested (Table 2). This range of values indicates random contamination due to the mode of contamination. Consequently, it has proven difficult to fond our significant differences.

The investigated OCPs were detected in all the three species (Fig. 2). Lindane and its isomers (Sigma HCH) were the dominant substances, with a mean concentration ranging from 206±4 ng g<sup>-1</sup> dw in the

catfishes to  $275\pm43$  ng g<sup>-1</sup> dw in the tilapia. Inversely, the  $\alpha$ -chlordane concentrations were very low, below the detection limit in most of the individuals. It was found in only 14% of individuals. The  $\gamma$ -chlordane concentration was higher in the tilapia than in the prawns and catfishes, (respectively p = 0.002 and p = 0.0007). On the other hand, the heptachlor contents were significantly higher in the catfishes than in the tilapia (p = 0.002) or in the prawns (p = 0.012), whereas the concentration of its main metabolite was higher in the prawns (p = 0.041).

The sum of DDT and its metabolites ranged from  $88.7\pm24.7$  to  $74.9\pm12.9$  and  $67.4\pm15.7$  ng g<sup>-1</sup> dw in, respectively the tilapia, the catfishes and the prawns. The less common OCPs found were aldrin and fipronil. Indeed, aldrin concentrations were ranged from  $11.3\pm4.2$  ng g<sup>-1</sup> dw in tilapia, to  $32.4\pm16.8$  ng g<sup>-1</sup> dw in prawns and fipronil concentrations were approximately up to 16.5 ng g<sup>-1</sup>dw in all the species.

The classification of pesticides according to their level of detection in the three species leads to the following scheme: lindane (and isomers) > endosulfan (isomers + metabolite) > endrin+endrin aldehyde > heptachlor + its metabolites (Fig. 2).

**Biomarker assessment:** The metabolic parameters, enzymatic activities and lipid content were provided in Table 3. The catfishes have proven richer in lipid content when compared to the two other investigated species. This lipid content may influence the rate of bioaccumulation of the persistent organic pollutants. The enzymatic activities in fish liver did not show any particular features when compared to the European fishes as we showed previously (Roche et al., 2002a; Buet et al., 2003). The inter-species differences were not very significant; except for the muscle AChE, which was more active in the tilapia than in the catfishes (p = 0.053). Some relationships between the contamination and the lipid contents and/or specific biomarker activities have been highlighted. Generally, OCPs concentrations were correlated with lipid content in the tilapia and in the prawn M. vollenhovenii. In C. nigrodigitatus, only lindane and its isomers, showed a similar significant (R = 0.80).

Some significant correlations were detected between OCPs concentrations and metabolic markers but their

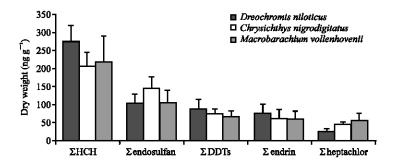


Fig. 2: Major OC pesticides families (sum of isomers and/or metabolites) in tilapia (*Oreochromis niloticus*), catfishes (*Chrysichthys nigrodigitatus*) and prawns (*Macrobrachium. vollenhovenii*) from Lake Taabo

Table 3: Lipid content (mg g<sup>-1</sup> dw) and some enzymatic activities (μM mg<sup>-1</sup> proteins) of biomarkers in tilapia (*O. niloticus*), catfishes (*C. nigrodigitatus*) and prawns (*M. vollenhovenii*) from the Lake Taaho

	O. niloticus	C. nigrodigitatus	M vollenhovenii	
Parameters	(10)	(7)	(5)	
Body lipids	69.4±8.4	127.1±16.7	71.1±3.7	
	[53.7-109.8]	[57.4-178.3]	[60.2-79.4]	
Liver				
GST	922.4±149.5	782.5±137.1		
	[523-1478]	[334-1478]	nd	
Catalase	865.7±126.3	931.9±128.8		
	[163-1114]	[333-1329]	nd	
GPx	6794±1635	4048±741		
	[2303-13425]	[2107-6423]	nd	
SOD	65.5±19.5	138.5±94.1		
	[3.6-158]	[17.8-513]	nd	
Muscle				
AChE	8.40±1.32	4.80±0.91	7.05±1.31	
	[3.17-16.7]	[1.23-8.04]	[4.28-11.6]	
Brain	•	•	•	
AChE	$0.36\pm0.04$	$0.29\pm0.06$		
	[0.18-0.50]	[0.12-0.49]	nd	

Values are expressed as mean±standard deviation and range [min-max], (n) = number of individuals, nd = no detected

values varied according to the species. The highest concentrated pesticides (lindane and its isomers) showed a correlation with liver catalase in O. niloticus (R = 0.83) and liver SOD in C. nigrodigitatus (R = 0.94). Nevertheless, in fishes, no relationship was found between the hepatic GST activity, a biotransformation enzyme and the contaminant levels. Nevertheless, negative correlations revealed, mainly the brain AchE versus fipronil in O. niloticus (R = -0.70) and versus  $\alpha$ -endosulfan in C. nigrodigitatus (R = -0.76). The correlations between the muscular AChE activity and OCPs amount were especially significant in prawns (R = -0.91). The invertebrates showed a synchronized variation of the muscle AChE activity decrease with the increase of DDT (and metabolites), of fipronil and of endosulfan concentrations.

#### DISCUSSION

During their study on surface waters in the Côte d'Ivoire, Wandan and Zabik (1996a, b) observed widespread OCPs contaminated water with a higher rate in rivers and lagoons located in the south of the country. Notably, they pointed out the residues of lindane and of endosulfan. Even if the present concentrations were lower than in other African areas, the researchers stressed that the increase in agricultural activities would require a greater monitoring of the hydrosystems's quality. In such semi-arid countries, the river flow is generally associated with a high evaporation rate. The water bodies are subjected to a continuous input of pollutants due to the use of phytosanitory chemicals, such as pesticides and fertilizers, that are transferred from the surrounding agricultural and industrial activities by atmospheric precipitations (dry or wet), soil leaching and/or by the rivers. The ecological risk relying on ecotoxicological investigations seems obviously a promising approach to appraise the acceptable levels of pollutants for sustainable agriculture development (Babut et al., 2003).

Here the organic pollutants investigated are stable and persistent; their liposolubility implies that they can bioaccumulation and biomagnification (Bremle and Ewald, 1995). All the selected pesticides, whether prohibited or authorized, were found in the tissues of the analyzed fishes and crustaceans. Contrary to common opinion, the tilapia seemed the most contaminated species, although the catfish was the fattest organism. The higher levels of contamination in the fishes could be results of long-term exposure. Besides, the accumulation of OC compounds in the fishes from the same area depends not only on the lipid content but also on habitat, on dietary intake, on the rate of growth and on the metabolism of each species (Pastor et al., 1996). Benthic algae represent an important reservoir of OC pollutants and accordingly, a fundamental link for trophic transfer of these xenobiotics. Pérez-Ruzafa et al. (2000) demonstrated that the input of OCPs, such as endosulfan and endrin, in aquatic food webs takes place mainly through plants to herbivorous-detritivorous compartment. So, higher concentrations of most OCPs were found in the tilapia, an herbivorous species feeding mainly on benthic algae, as compared to the catfish, a predatory species. Nevertheless, in a documented FAO (1996) report on the state of knowledge of the continental fisheries in Côte d'Ivoire, Kassoum (1996) estimates that some fishes consume preys from particular and constant trophic levels in spite of the geographical variations and that, others show a wide feeding spectrum. The plasticity of their food habits confers a capacity to adapt to varied biotopes. The most frequently detected pesticides in the species found in Lake Taabo were lindane and endosulfan. Wandan and Zabik (1996a) showed up the dominance of these two compounds in the aquatic ecosystems in the south of Côte d'Ivoire, since they were still extensively used. Lindane is used for the control of cocoa mirids and other pests and endosulfan as insecticide, acaricide and nematicide widely used for crop protection and for controlling disease vectors. On the other hand, our results were compared to the same analyses performed concomitantly in a catfish (Ictalurus nebulosus) and a crayfish (Orconectes limosus) from a wetland in the South of France (Camargue) (Roche et al., 2000). The species collected in France were less contaminated than the African species from equivalent trophic levels (Table 4), in spite of the French study site represented a rice field area where lindane was intensively used until its prohibition in 1998. This shows that the implementation of regulation is important for wildlife in aquatic ecosystem.

In comparison to other recent studies, the  $\Sigma$ HCH concentration was higher in the fishes from Lake Taabo than from Lake Tanganyika as described by Manirakiza et al. (2002), or from Karachi Coast (Munshi et al., 2004). Expressed in lipid contamination, the  $\Sigma$ HCH level reached 4.1 $\pm$ 0.7  $\mu$ g g<sup>-1</sup> lipids in the tilapia and 1.6±0.3 µg g<sup>-1</sup> lipids in the catfishes from Lake Taabo, in comparison with less than 0.1 µg g<sup>-1</sup> lipids in the same species from Lake Tanganyika and with a maximum of 3 µg g<sup>-1</sup> lipids in the fishes from the Pakistan coast. However, the contamination level did not differ from the one detected in the catfishes from the Camargue Wetland (France), i.e., 4.4±1.1 µg g<sup>-1</sup> lipids (Persic, 2004). Endosulfan exhibited the same level in the organisms from Lake Taabo and in the fishes collected from Wetland with the concentrations the Camargue ranging from 0.5 to 2.9 ng mg<sup>-1</sup> lipids. However, its concentrations in Taabo organisms were significantly

Table 4: Muscle contamination of some OC pesticides (ng g<sup>-1</sup> dry weight) in a catfish and a crayfish from Camargue wetland (France) (Persic. 2004)

		Orconectes	Ictalurus
Organic	European	limosus	nebulosus
pesticides	regulation	(17)	(12)
γ-HCH (lindane)	Prohibited since 1998	$2.65\pm0.90$	$2.49\pm0.94$
fipronil	Suspended since 2004	41.4±7.89	24.6±4.24
α-endosulfan	Allowed-restricted use	$14.0\pm3.34$	$10.7 \pm 3.31$
aldrin	Prohibited since the 70s	$5.80\pm2.05$	$3.99\pm0.82$
dieldrin	Prohibited since the 70s	12.2±4.16	11.4±5.85
pp'-DDE	Prohibited since the 70s	6.90±2.26	$7.69\pm2.72$

higher than in the fishes from Lake Tanganyika of which values were included between 0.5 and 36.1 ng g<sup>-1</sup> fat (Manirakiza *et al.*, 2002).

DDT, endrin and their metabolite levels were also high. At the same trophic levels the concentration of ΣDDT in fishes from Lake Taabo was three times higher than those reported in the species from Lake Tanganyika. These concentration of  $\Sigma$ DDT suggest that DDT application is probably still in use in Côte d'Ivoire. The ratio of DDE/DDT can be taken as an empirical indicator of the length of time since a DDT application (Fillmann et al., 2002). In the present study, DDE/DDT ratio was low in O. niloticus and C. nigrodigitatus samples (0.63±0.16; 0.36±0.13, respectively) as in prawns (1.17±0.29) denoting recent exposure to DDT and the consumption of contaminated food with DDT. We may assume that DDT inputs have occurred recently. The bioaccumulation of DDT, like other OCPs, through the biomagnification process along the food chains is considered a secondary poisoning. Such pesticides are highly persistent but they exhibit low water solubility. Therefore, it is not surprising that the present results oppose to the conclusion of Wandan and Zabik (1996b). These authors considered that the water systems of Côte d'Ivoire did not face pollution problems. Although it is probable that there is no risk with drinking water, the persistence of the contaminants, their trophic transfer and their accumulation at the highest levels of the trophic web can cause serious damage to the contaminated individual, to the structure of the communities and to consumers at the top of the food chain.

In the tilapia and the prawns, OCPs, especially endosulfan and DDT, were closely associated with lipid content and  $\Sigma$ HCH was correlated to lipid content in both investigated fish species.

Any aquatic ecosystem can be considered as non-exposed due to the permanent and inconsistent input of pollutants. Therefore, because the control data are missing the use of comparative tests in the evaluation of biomarkers is limited. Consequently, the interpretation of the data was made through a correlation analysis. In this

study, no relationship was demonstrated between the biotransformation enzyme activity (GST) and organism contamination. This observation is in contradiction with most biomonitoring studies that recommend the use of such an activity as a biomarker of chemical exposure in aquatic ecosystems (Gavila et al., 2001; Barra et al., 2001). In his study, Fent (2003), in agreement with our previous observations (Roche et al., 2002a, b; Oliveira Ribeiro et al., 2005), argues that the evaluation of the ecotoxicological potential of contaminated sites should take into account not only parameters easily controllable, such as, interactions of chemicals in complex mixture or their bioavailability, but also adaptive and compensatory processes in the considered organism.

Here, some biomarker responses reflect the chronicity of the contamination and the contaminant diversity. In the tilapia-the 'most contaminated' organism-the activity of the hepatic GPX was correlated with OCP contents (namely with heptachlor: R=0.80, aldrin: R=0.76 and  $\Sigma$ DDT concentrations). In addition, the level of the  $\Sigma$ HCH content is statistically linked with other antioxidative activities-catalase in the tilapia (R=0.83) and SOD in the catfish (R=0.94).

The most outstanding response concerns the muscular AChE activity, showing very significant inverse correlations with the rate of OC contamination (R = -0.91), the concentration in  $\Sigma$ DDT and those of authorized pesticides, fipronil and  $\alpha$ -endosulfan in the prawn M. vollenhovenii. These two last insecticides also showed an inverse correlation with the brain AChE activity in analyzed fishes (fipronil: R = -0.70 for tilapia and-endosulfan: R = -0.76 pour catfish). With our results, in accordance with numerous works listed by Lionetto et al. (2003), we can consider that the AChE inhibition in invertebrates could be a good biomarker in a chemical multi-contamination context.

### ACKNOWLEDGMENTS

We thank very much the anonymous referees for their critical reading of the first manuscript. We would also like to thank Professor François Ramade for his substantive and constructive commentary.

## REFERENCES

- Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
- Andersen, R.A., K. Laake and F. Fonnum, 1972. Reactions between alkyl phosphates and acetylcholinesterase from different species. Comp. Biochem. Physiol., 42B: 429-437.

- Babut, M., C. Bonnet, M. Bray, P. Flammarion, J. Garric and G. Golaszewski, 2003. Developing environmental quality standards for various pesticides and priority pollutants for French freshwaters. J. Environ. Manage., 69: 139-147.
- Barra, R., V. Notarianni and G. Gentili, 2001. Biochemical biomarker responses and chlorinated compounds in the fish *Leusciscus cephalus* along a contaminant gradient in a polluted river. Bull. Environ. Contam. Toxicol., 66: 582-590.
- Beliaef, B. and G. Bocquené, 2004. Exploratory data analysis of the Mediterranean component of the BEEP programme. Mar. Environ. Res., 58: 239-244.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
- Bremle, G. and G. Ewald, 1995. Bioconcentration of polychlorinated biphenyls (PCBs) in chironomid larvae, oligochaete worms and fish from contaminated lake sediment. Mar. Freshwater Res., 46: 267-273.
- Buet, A., 2002. Impact biologique des HAP chez l'anguille européenne. Définition et validation de biomarqueurs *in situ*. Ph.D Thesis, Paris 11 University, pp. 194.
- Buet, A., D. Banas, Y. Vollaire, E. Coulet and H. Roche, 2003. Biomarker responses in European eel (Anguilla anguilla) exposed to persistent organic pollutants. A field study in the Vaccarès lagoon (Camargue, France). Chemosphere, 65: 1846-1858.
- Burgeot, T., G. Bocquené, C. Porte and J. Dimeet, R.M. Santella, L.M. Garcia de la Parra, A. Pfhol-Leszkowicz, C. Raoux and F. Galgani, 1996. Bioindicators of pollutant exposure in the Northwestern Mediterranean sea. Mar. Ecol. Prog. Ser., 131: 125-141.
- Cajaraville, M.P., M.J. Bebianno, J. Blasco, C. Porte, C. Sarasquete and A. Viarengo, 2000. The use of biomarkers to assess the impact of pollution in Coastal environments of the Iberian Peninsula: A practical approach. Sci. Total Environ., 247: 295-311.
- Cavanagh, J.E., K.A. Burns, G.J. Brunskill, D.A.J. Ryan and J.T. Ahokas, 2000. Induction of hepatic cytochrome P-450 1A in pikey bream (*Acanthopagrus berda*) collected from agricultural and urban catchments in far North Queensland. Mar. Pollut. Bull., 41: 7-12.
- Choi, J., H. Roche and T. Caquet, 2000. Effects of physical (hypoxia, hyperoxia) and chemical (potassium dichromate, fenitrothion) stress on antioxidant enzyme activities in Chironomus riparius Mg. (Diptera, Chironomidae) larvae: Potential biomarkers. Environ. Toxicol. Chem., 19: 495-500.

- Conway, K., 2002. Improve the environment, improve health in Côte d'Ivoire. In: Division of the International Development Research Centre (IDRC) IDRC Reports magazine, Ottawa, Canada.: http://web.idrc.ca/en/ev-12006-201-1-DO TOPIC.html.
- Curtis, C. and C. Palmer Olsen, 2004. The Africa Stockpiles Programme: Cleaning up obsolete pesticides; contributing to a healthier future. UNEP Ind. Environ. Rev., 27: 37-38.
- Dabrowski, J.M., S.K.C. Peall, A. Van Niekerk, A.J. Reinecke, J.A. Day and R. Schulz, 2002. Predicting runoff-induced pesticide input in agricultural sub-catchment surface waters: Linking catchment variables and contamination. Water Res., 36: 4975-4984.
- De la Torre, F.R., L. Ferrari and A. Salibian, 2005. Biomarkers of a native fish species (*Cnesterodon decemmaculatus*) application to the water toxicity assessment of a peri-urban polluted river of Argentina. Chemosphere, 59: 577-583.
- Depledge, M.H., 1994. The Rational Basis for the Use of Biomarkers as Ecotoxicological Tools. In: Nondestructive Biomarkers in Vertebrates, Fossi, C. and C. Leonzio (Eds.). Lewis Publishers, London, pp: 271-295.
- Durand, J.L., P.A. Monchamp, A.L. Lafleur and H.F. Hermond, 1994. Combined filtration solid phase extraction method for recovering organic substances from natural waters in preparation for mutagenicity testing. Environ. Sci. Technol., 28: 1819-1828.
- Ellman, G.L., D. Courtneyk, V. Andres and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
- FAO, 1996. Agriculture et alimentation en Côte d'Ivoire: http://www.fao.org/giews/french/basedocs/ivc/ivccullf.stm.
- Fent, K., 2003. Ecotoxicological effects at contaminated sites. Toxicology, 205: 223-240.
- Fillmann, G., J.W. Readman, I. Tolosa, J. Bartocci, J.P. Villeneuve, C. Cattini and L.D. Mee, 2002. Persistent organochlorine residues in sediments from the Black Sea. Mar. Pollut. Bull., 44: 122-133.
- Fiske, C. and Y. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.
- Folch, J., M. Less and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
- Forget, J., B. Beliaeff and G. Bocquené, 2003. Acetylcholinesterase activity in copepods (*Tigriopus brevicornis*) from the Vilaine River estuary, France, as a biomarker of neurotoxic contaminants. Aquacul. Toxicol., 62: 195-204.

- Gavila, J.F., R. Barra, M.C. Fossi, S. Casini, G. Salinas, O. Parra and S. Focardi, 2001. Biochemical biomarkers in fish from different river systems reflect exposure to a variety of anthropogenic stressors. Bull. Environ. Contamin. Toxicol., 66: 476-483.
- Goore Bi, G., 1998. Contribution à l'étude des crevettes d'eaux douces de côte d'Ivoire. Systématique, Biologie et Analyse socio-économique de la pêche de *Macrobrachium vollenhovenii* (Herklots, 1857) et de *Macrobrachium macrobrachium* (Herklots, 1851) (Crustacea: Decapoda, Palaemonidae) du bassin de la Bia. Ph.D Thesis, Abidjan University, pp. 143.
- Gundersen, D.T., R. Miller, A. Mischler, K. Elpers, S.D. Mims, J.G. Millar and V. Blazer, 2000. Biomarker response and health of polychlorinated biphenyl-and chlordane-contaminated paddlefish from the Ohio River Basin, USA. Environ. Toxicol. Chem., 19: 2275-2285.
- Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione s-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
- Kassoum, T., 1996. Etat de connaissances sur les pêcheries continentales ivoiriennes. PROJET FAO TCP/IVC/4553. In: FAO Project reports AG188/F: pp: 135. http://www.fao. org/docrep/field/009/ag188f/ AG188F00.HTM.
- Lhomme, F., 1994. Les Crustacés Exploitables. In:
  Environnement et Resources Aquatiques De Côte
  D'ivoire. Tome II. Les Milieux Lagunaires,
  Durand, J.R., P. Dufour, D. Guiral and S.G.F. Zabi
  (Eds.). Orstom, Paris, pp. 229-238.
- Lionetto, M.G., R. Caricato, M.E. Giordano, M.F. Pascariello, L. Marinosci and T. Schettino, 2003. Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. Mar. Pollut. Bull., 46: 324-330.
- Manirakiza, P., A. Covaci, L. Nizigiymana, G. Ntakimazi and P. Schepens, 2002. Persistent chlorinated pesticides and polychlorinated biphenyls in selected fish species from Lake Tanganyika, Burundi, Africa. Environ. Pollut., 117: 447-455.
- Misra, H.P. and I. Fridovich, 1972. The generation of superoxide radical during the autoxidation of hemoglobin. J. Biol. Chem., 247: 6960-6962.
- Munshi, A.B., D. Schulz-Bull, R. Schneider and R. Zuberi, 2004. Organochlorine concentrations in various fish from different locations at Karachi Coast. Mar. Pollut. Bull., 49: 597-601.
- Oliveira Ribeiro, C.A., Y. Vollaire, A. Sanchez-Chardi and H. Roche, 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquacul. Toxicol., 74: 53-69.

- Ouattara, M., K. Kouakou and G. Gourène, 1993. Une approche technique peu onéreuse de production de larves et d'alevins de *Chrysischthys nigrodigitatus* (Pisces: *Bagridae*). Agron. Afr., 5: 33-38.
- Pan Africa, 2003. Pesticide Action Network Africa. Annual report.: http://www.pan-afrique.org/english/publication/pg Reports.html.
- Pastor, D., J. Boix, V. Fernandez and J. Albaiges, 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus*, *Mugil cephalus* and *Dicentrarcus labrax*). Mar. Pollut. Bull., 32: 257-262.
- Pérez-Ruzafa, V., S. Navarro, A. Barba, C. Marcos, M.A. Camara, F. Salas and J.M. Gutierrez, 2000. Presence of pesticides throughout trophic compartments of the food web in the Mar Menor Lagoon (SE Spain). Mar. Pollut. Bull., 40: 140-151.
- Persic, A., 2004. Modalités de contamination par les polluants organiques persistants des réseaux trophiques lagunaires. Application de la méthode des isotopes stables. Ph.D Thesis, Paris 11 University, pp: 160.
- Pimentel, D., T.W. Culliney and T. Bashore, 1996. Public Health Risks Associated with Pesticides and Natural Toxins in Foods. In Pesticides: Chemistries/Pesticide Resistance, Radcliffe, E.B. and W.D. Hutchison (Eds.). Radcliffe's IPM World Textbook, University of Minnesota, St. Paul, MN: http://ipmworld.umn.edu.
- Roche, H. and G. Bogé, 2000. In vivo effects of phenolic compounds on blood parameters of a marine fish (*Dicentrarchus labrax*). Comp. Biochem. Physiol., 125C: 345-353.
- Roche, H., A. Buet, O. Jonot and F. Ramade, 2000. Organochlorine residues in european eel (Anguilla anguilla), crusian carp (Carassius carassius) and catfish (Ictalurus nebolosus) from Vaccares lagoon (French National Reserve of Camargue). Effects on some physiological parameters. Aquacul. Toxicol., 48: 443-459.
- Roche, H., A. Buet and F. Ramade, 2002a. Accumulation of lipophilic micro-contaminants and biochemical responses in eels from the Biosphere Reserve of Camargue. Ecotoxicology, 11: 9-18.

- Roche, H., A. Buet and F. Ramade, 2002b. Relationship between persistent organic chemicals residues and biochemical constituents in fishes from a protected area, the French National Nature Reserve of Camargue. Comp. Biochem. Physiol., 133C: 393-410.
- Schindler, D.W., K.A. Kidd, D.C.G. Muir and W.L. Lockhart, 1995. The effects of ecosystem characteristics on contaminant distribution in Northern freshwater lakes. Sci. Total. Environ., 160-161: 1-17.
- Tappel, A.L., 1978. Glutathione peroxidase and hydroperoxides. Meth. Enzymol., 52: 506-513.
- Touni, E., 2005. Pesticides et pauvreté. In: Pesticides et alternatives. Bull. Pesticide Action Network (PAN) Afr., 24: 16.
- UN Food and Agricultural Organization (FAO), 2001. Africa Recovery, 15: 42.
- Van de Oost, R., A. Goksoyr, M. Celander, H. Heida and N.P.E. Vermeulen, 1996. Biomonitoring of aquatic pollution with feral eel (*Anguilla anguilla*): II. Biomarkers: pollution-induced biochemical responses. Aquacul. Toxicol., 36: 189-222.
- Van der Oost, R., J. Beyer and N.P.E. Vermeulen, 2003.
  Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol., 13: 57-149.
- Wandan, E.N. and M.J. Zabik, 1996a. Assessment of surface water quality in Côte d'Ivoire. Bull. Environ. Contamination Toxicol., 56: 73-79.
- Wandan, E.N. and M.J. Zabik, 1996b. Assessment of the contamination of surface water and fish from Côte d'Ivoire. J. Environ. Sci. Health, Part B Pesticides, 31: 225-240.
- Westlake, G.E., A.D. Martin, P.I. Stanley and C.H. Walker, 1983. Control enzyme level in the plasma, brain and liver from wild birds and mammals in Britain. Comp. Biochem. Physiol., 76C: 15-24.
- World Health Organization (WHO) regional office for Africa: http://www.afro.who.int/.