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First Ecotoxicological Assessment Assay in a Hydroelectric Reservoir: The Lake Taabo (Côte d'Ivoire)

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Abstract Organochlorine pesticides (OCPs) contamination was assessed in marketable species, two fishes (tilapia and catfish) and a prawn from the Lake Taabo (Côte d'Ivoire). Lindane and endosulfan were the main contaminants, suggesting their current use. DDT, endrin, heptachlor plus traces of chlordane, aldrin and fipronil were also detected. In fishes and in prawns, enzymatic biomarkers exhibited significant correlations with OCPs levels, showing the feasibility of a biomonitoring. The transfer of OCPs along the aquatic food web and their immunosuppressive effects in human are discussed. This preliminary study highlights that the pesticide contamination was concomitant with the increase in infectious diseases in the bordering population of this African lake.

Keywords Organochlorine pesticides · Susceptibility to infectious diseases · Lake Taabo · Côte d'Ivoire

In developed countries, the implementation of pollution control programs based on aquatic wildlife, water, sediment and air quality assessments, has resulted in the enforcement of strict regulation of the use of many substances suspected to pose risks to environmental and human health (PAN-Africa 2003). In Africa and other developing regions, stocks of old and outdated pesticides are major sources of toxic risk. According to the UN Food and Agricultural Organization (UN-FAO 2001), over the 50,000 tons of obsolete pesticides stocked in African countries, with 828 tons in Côte d'Ivoire, represent an alarming risk. It is realistic to assume that these amounts are underestimations since the suspected substances are due to a surplus of agricultural inputs, stored under uncontrolled conditions and used without proper safeguard endangering the human populations and the environment. In this context, the aim of the Africa Stockpiles Program (ASP) is to eliminate the pesticide 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 abuse of pesticides in agricultural areas is damaging to the quality of water and, consequently, could affect the health of aquatic organisms and consumers. 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 (cited by Conway 2002) stated that the analysis of fishes caught in the Lake Buyo (Côte d'Ivoire) confirmed the presence of chemical contaminants and clearly showed a biomagnification process in this ecosystem.

In addition to the contamination of the Lake Taabo trophic web, other water-related risk factors are endemic or emerging infectious diseases (malaria or Buruli ulcer) affecting predominantly the children. The immunological effects of POPs are largely documented. We wonder about a potential relationship between the recent development of these diseases and the contamination level of components of the food chain. The construction of hydroelectric dams

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on rivers causes an enrichment of the sediment with nutrients and pesticides from agricultural areas in its catchment. In the case of Lake Taabo, neither deforestation nor cleanups of the plantation soils were carried out before the flooding of the valley. The nutrients and the pesticides covered by the flooding could therefore have contributed to the water pollution and affect the components of the food web. To explore this assumption, the present study focused on the levels of OCPs in three aquatic species (2 fishes and 1 invertebrate) from the Lake Taabo, which is a major food contribution for the bordering populations. While such studies are mostly conducted in developed countries, they are infrequent in developing countries (Berny et al. 2006). Our main objective is to highlight the need for biomonitoring in such a degraded aquatic ecosystem.

Materials and Methods

The Lake Taabo (Department of Tiassalé, Côte d'Ivoire) is located in the 'Région des lagunes' within the limits of the dense forest and the savannah. It stands as an essential halieutic resource for the local populations and the inhabitants of Abidjan. The catchment area of the Bandama river covers 97,500 km^2 and many agricultural activities are located alongside the river or its tributaries.

Ten tilapia, 7 catfishes and 5 prawns were collected in June 2003 by local fishermen in the Lake Taabo. The fishes and the prawns were stored frozen on dry ice (−80°C) before arrival in the laboratory, where the individuals were measured and dissected. The analyzed dry mass of the muscle tissue was determined by oven-drying at 105°C for 24 h. The lipids were extracted with a chloroform–methanol solution and then quantified gravimetrically. All the analyses were carried out on individual samples.

The concentrations of OCPs residues were determined in the lipids of fish muscle and in the lipids from the total body mass of the prawns, excluding cuticle. Compounds analyzed included isomers of hexachlorocyclohexane [α-, β-, γ-(lindane) and δ-HCH], heptachlor, heptachlor epoxide, α- and γ-chlordane, dieldrin, aldrin, endrin and endrin aldehyde, dichloro-diphenyl-trichloroethane (pp'-DDT) and its degradation products, pp'-DDD, pp'-DDE; α- and β-endosulfan, endosulfan sulphate, and fipronil. OCPs were purified by solid phase extraction (SPE) on florisil (MgO_3Si), following the EPA method 608 (Bond Elut Florisil, 1 g, 200 μM particle size, Varian). They were analyzed by gas chromatography with an autosystem XL (Perkin-Elmer), using ECD (electron capture detection) (^{63}Ni). The procedure was adapted from the 8081a EPA Method. (Oliveira-Ribeiro et al. 2005). All reference materials were produced following an ISO9001 certified laboratory. The detection limit ranged from 0.01 to 0.05 ng g^{-1} dry weight (dw).

The fish livers were homogenized in cold buffer (Tris 50 mM pH 7.4) with a Potter Elvehjem homogenizer and centrifuged at 1,000*g* at 4°C for 10 min to obtain the supernatant S1. S1 was re-centrifuged (12,000g) to obtain the post-mitochondrial fraction (S12). Glutathione S-transferase (GST), superoxide dismutase (SOD), catalase, glutathione peroxidases (GPx) activities were assessed in S12 and acetylcholinesterase (AChE) activity was determinated in muscle and brain S1, as described previously (Buet et al. 2006). Inter-species variations were compared using one-way analysis variance (ANOVA) followed by Scheffe's and Bonferroni-Dunnett posthoc tests. Relationships between contaminant concentrations and biological data were tested using the Spearman's rank correlation test. A p-value <0.05 was considered significant.

Results and Discussion

The weight of catfishes, tilapia and prawns were 60 ± 8 g, 45 ± 3 g and 17.5 ± 1.9 g, respectively. The catfishes were the fattiest species ($p < 0.002$) the lipid contents of samples were 127 ± 17 mg g^{-1} dw, 69 ± 8 mg g^{-1} dw and 71 ± 4 mg g^{-1} dw in catfishes, tilapia and prawn, respectively. The analyzed OCPs were divided into two groups: the OCPs 'banned' in Europe (in some cases for more than 30 years) and the 'allowed OCPs' (compounds in current use). In the three species, about 80% of the contamination were due to banned pesticides (Table 1). The tilapia appeared as the more contaminated fish species, although the catfish had the highest lipid content. In such a field study, we hypothesized that sampled tilapia belongs to a lower and potentially less mobile age-group than the catfish. According to Duponchelle and Legendre (2000), they have reached their size at maturity and their longevity is from 4 to 7 years. Therefore, the highest levels of contamination in the least fatty fishes could be results of long-term exposure.

Table 1 OCPs contamination in tilapia, catfishes and prawns from the Lake Taabo

ng g dw^{-1}	Tilapia *O. niloticus* (10)	Catfishes *C. nigrodigitatus* (7)	Prawns *M. vollenhovenii*(5)
ΣAnalyzed OCPs	702 ± 120	597 ± 74	608 ± 166
% Banned OCPs	82.6 ± 1.6	74.7 ± 2.5	81.4 ± 4.0

Moreover, the accumulation of OC compounds in the fishes from the same area is not only dependent on the lipid content but also on the habitat, on the dietary intake, on the growth rate and on the metabolism of species. 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 transfer of OCPs, such as endosulfan and endrin, in aquatic food webs, goes mainly from plants to herbivorous–detritivorous compartment. So, the highest concentrations of most OCPs were found in the tilapia, a herbivorous species feeding mainly on benthic algae, as compared to the catfish, a predatory species. More 'banned' than 'allowed' insecticides were found in the three components of Lake Taabo trophic web. The banned pesticide concentrations showed only a significant difference between the tilapia and the catfish ($p = 0.015$). The substances detected at the highest concentrations (ΣHCH, endosulfan and endrin, and to some extent DDT) showed a great variability, both inter- and intra-species (Fig. 1). As we previously mentioned (Roche et al. 2007), lindane and its isomers (ΣHCH) were the dominant substances. At the opposite, the α-chlordane concentrations (data not showed) were below the detection limit in most of the individuals, while the γ-chlordane level could reach a high level in tilapia (up to 87 ng g^{-1} dw). The classification of the pesticides according to their level of detection in the three species were found as follows: HCH > endosulfan > DDT > endrin > heptachlor. Wandan and Zabik (1996a) indicated the dominance of lindane and endosulfan 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. In comparison to other recent studies, the ΣHCH concentration (on a lipid basis) was higher in the fishes from Lake Taabo (ranged from 1.6 ± 0.3 to 4.1 ± 0.7 μg g^{-1} lipids according to the species) than from Lake Tanganyika (>0.1 μg g^{-1}) as described by Manirakiza et al. (2002). Endosulfan exhibited higher concentrations in Taabo organisms than in the fishes from Lake Tanganyika, which values were included between 0.5 and 36.1 ng g^{-1} lipids. The Σ-chlordane concentration was higher in the tilapia than in the prawns and catfishes ($p = 0.002$ and $p = 0.0007$, respectively). 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 its main metabolites were more concentrated in the prawns ($p = 0.041$). The less common OCPs found were aldrin (average from 11.3 ± 4.2 ng g^{-1} dw in tilapia, to 32.4 ± 16.8 ng g^{-1} dw in prawns) and fipronil (average up to 16.5 ng g^{-1} dw in all the species). DDT, endrin and their metabolites were also high. Although these pesticides are officially banned, the farmers still have access to them illegally, in the form of mixture with other OCPs. 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 results 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 time since a DDT application. In the present study, DDE/DDT ratio was lower than 1 suggesting a recent exposure to DDT, direct or by means of food. The bioaccumulation of OCPs through the biomagnification process along the food chains can be considered a secondary poisoning. Wandan and Zabik (1996b) pointed that the water systems of Côte d'Ivoire did not face pollution problems. Although it is likely 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 could be detrimental not only for the contaminated individual but also for the structure of the communities and for the consumers at the top of the food chain.

In the present study, the biomarkers were selected in order to investigate several metabolic processes. These include biotransformation process (GST), antioxidant

Fig. 1 OCPs (sum of isomers and/or metabolites) contamination profile of ■ tilapia (*O. niloticus*), ▩ catfishes (*C. nigrodigitatus*) and □ prawns (*M. vollenhovenii*) from Lake Taabo

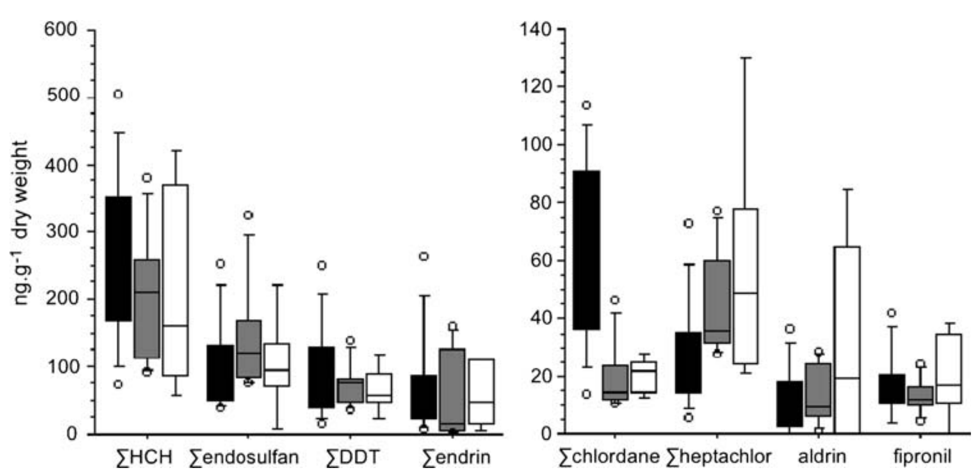

Table 2 Enzymatic biomarkers in tilapia (*O. niloticus*), catfishes (*C. nigrodigitatus*) and prawns (*M. vollenhovenii*) from the Lake Taabo

μM mg^{-1} proteins	Liver				Muscle	Brain
	GST	Catalase	GPx	SOD	AChE	AChE
Tilapia (10)	922 [523–1478]	866 [163–1114]	6794 [2303–13425]	65.5 [3.6–158]	8.40 [3.17–16.7]	0.36 [0.18–0.50]
Catfishes (7)	782 [334–1478]	932 [333–1329]	4048 [2107–6423]	138.5 [17.8–513]	4.80 [1.23–8.04]	0.29 [0.12–0.49]
Prawns (5)	nm	nm	nm	nm	7.05 [4.28–11.6]	nm

Mean and range [min–max], (n) = number of individuals, nm = not measured

enzyme activities (catalase, GPX and SOD) and AChE which is increasingly used to assess the adverse biological effects of complex chemical mixtures (Table 2). In a recent paper, Fent (2004), in agreement with our previous observations (Oliveira Ribeiro et al. 2005), argued that the assessment of the ecotoxicological potential of contaminated sites should take into account not only easily controllable parameters, such as, interactions of chemicals in complex mixture or their bioavailability, but also adaptive and compensatory processes in the organism of interest. The analysis of the data was made through Spearman's rank correlations. Such relationships were demonstrated in the catfishes between the biotransformation enzyme activity (GST) and the sum of banned or still used OCPs ($p = 0.044$) like for $\sum$OCP and $\sum$endosulfan impregnation ($p = 0.029$ and 0.036, respectively). This observation is consistent with most biomonitoring studies that recommend the use of such an activity as a biomarker of organic chemical exposure in aquatic ecosystems. Because catfishes had higher lipid content, this may influence bioaccumulation of the persistent organic pollutants. In the catfishes, significant correlations were also detected between concentrations of OCPs and hepatic SOD activity. It was the case for endosulfan and fipronil ($p = 0.046$) and more generally, for total, banned or authorized OCPs ($p < 0.05$).

Here, in the tilapia – the 'most contaminated' organism – no correlation was found between hepatic biomarkers and pollutant concentrations. However brain AChE showed significant relationships with heptachlor and fipronil impregnation of muscle ($p < 0.06$).

The most outstanding responses were the negative correlations between muscular AChE activity in the prawn *M. vollenhovenii*, and the level of some OCPs (i.e. ΣOCP, ∴*∴*ΣDDT, ΣHCH, fipronil and α-endosulfan: *p*-values ranked from 0.03 to 0.06). These results are consistent with numerous works listed by Lionetto et al. (2003), which considered that the AChE inhibition, when measured in invertebrates, could be a relevant biomarker in a chemical multi-contamination context.

The present study confirms the contamination of the aquatic species by POPs. As previously mentioned, this contamination is chronic and sufficiently intense to induce answers of some specific biomakers in marketable fishes and prawns (Roche et al. 2007). The US Agency for Toxic Substances and Disease Registry (ATSDR) and the Scientific Committee on Food of the European Commission recommend, as minimal risk levels (MRLs), limits of 1,000 ng g^{-1} for $\sum$DDT, 200 ng g^{-1} for aldrin and heptachlor, 100 ng g^{-1} chlordane and endosulfan and 50 ng g^{-1} for endrin (as concentrations in whole body-tissue). According to this tolerance, the measured levels of OCPs were too high for consumption.

In addition, numerous epidemiologic reports show a dramatic increase in human diseases transmitted by insect vectors, like malaria and Buruli ulcer, an infectious disease due to a mycobacterium (Wansbrough-Jones and Phillip 2006). Such pathologies increase in populations living in humid rural tropical areas, especially near the African hydroelectric reservoirs, notably the Lake Taabo. In Côte d'Ivoire, Brou et al. (2006) demonstrated a relationship between Buruli ulcer prevalence and environmental degradation of wetlands, deforestation, agricultural-water management and increased cultivation. Furthermore, the immunosuppressive effects of OCPs are known. Recently, Cooper et al. (2004) demonstrated evidence of an immunosuppression associated with dietary exposure to DDT, in American farmers. The endemic infectious diseases previously mentioned, affect predominantly children between 5 and 15 years old or immunodeficient individuals. Since the immunotoxicant organochlorines are suspected to induce a decrease of host resistance to infections and to suppress humoral and cell-mediated immune responses, we may assume that the intensity of the pathologies could be correlated with pesticide contamination.

The present ecotoxicological investigations show that all the investigated pesticides, including substances involved in the Stockholm Convention, were found in three representative species of the Lake Taabo ecosystem. High concentrations of compounds such as lindane and endosulfan suggest that these pesticides are still sprayed in the vicinity of the lake or the Bandana River. Because the investigated species constitute a significant lacustrine food resource for the populations neighbouring and inhabiting in the nearest urban area (the city of Abidjan), we pointed out the need for programs about environmental risk assessment, in order to evaluate the quality of African lakes and the actual adverse effects of pesticides in the fish consumers. In addition, it seems essential to investigate the possible links between the dietary pesticide exposure and the prevalence of infectious diseases in Africa.

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