

EVALUATION OF THE RISK OF CONTAMINATION BY PESTICIDES

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ABSTRACT

Pesticides have the particularity of accumulating mainly in aquatic ecosystems. The concentration of pesticides in such contaminated ecosystems appears to be a potential health risk resulting from the human consumption of fish from these ecosystems. The aim of this study is to determine the pesticide residue levels of *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis*. 120 samples of each species, distributed from 96 samples for sector IV and 24 samples for sector V, were collected from February 2014 to January 2015. These were analyzed by gas chromatography coupled to a spectrometer of mass (GC / MS), after extraction and purification respectively by accelerated solvent extraction (ASE) and by adsorption chromatography on Oasis HLB Plus cartridges. Pesticide concentrations detected in fish muscle ranged from 0 to 40.66 µg / kg in the study area. All values found for the health risk index (HI) in *Chrysichthys nigrodigitatus* and *Tilapia* hybrid muscles are well below 1 (HI < 1). Pesticide residues detected in both fish species do not pose a potential risk to human health.

Keywords: Contamination, pesticides, *Chrysichthys nigrodigitatus*, *Tilapia*, risk.

RESUME

EVALUATION DU RISQUE DE CONTAMINATION PAR LES PESTICIDES

Les pesticides ont la particularité de s'accumuler principalement dans les écosystèmes aquatiques. La concentration de pesticides dans de tels écosystèmes contaminés apparaît comme un risque potentiel pour la santé résultant de la consommation humaine de poissons de ces écosystèmes. Cette étude a pour objectif de déterminer la concentration de résidus de pesticides chez *Chrysichthys nigrodigitatus* et *Tilapia zilli* × *Tilapia guineensis*. 120 échantillons de chaque espèce, repartis en raison de 96 échantillons pour le secteur IV et 24 échantillons pour le secteur V, ont été collectés de février 2014 à janvier 2015. Ceux-ci ont été analysés par chromatographie en phase gazeuse couplée à un spectromètre de masse (GC / MS), après Extraction et purification respectivement par Extraction Accélérée par Solvant (ASE) et par chromatographie d'adsorption sur cartouches Oasis HLB Plus. Les concentrations de pesticides quantifiés et non détectés dans les muscles des poissons variaient entre 0 et 40,66 µg/kg dans la zone d'étude. Toutes les valeurs trouvées pour l'indice de risque pour la santé (HI) dans les muscles de *Chrysichthys nigrodigitatus* et de *Tilapia* hybride sont nettement inférieures à 1 (HI < 1). Les résidus de pesticides détectés chez les deux espèces de poisson ne présentent pas de risque potentiel pour la santé humaine.

Mots-clés : Contaminations, pesticides, *Chrysichthys nigrodigitatus*, *Tilapia*, risque.

INTRODUCTION

The beneficial effects of pesticides in increasing agricultural production are far-reaching. In the same time these have indirect and harmful effects on the environment, especially aquatic, and pose a public health problem. Pesticides in food products and drinking water pose an immediate threat to human health, whereas other contaminants gradually build up in the environment and in the human body, causing disease long after first exposure [1]. Apart from their direct toxicity to humans and animals, pesticides have the particularity of accumulating in the mostly aquatic ecosystems of which they are the major pollutants. Epidemiological and other laboratory studies in animals have shown that exposure to different chemical families of pesticides such as organochlorine (OC), organophosphate (OP), carbamate can cause a range of neurological health effects such as loss of coordination and memory, reduced visual ability and reduced motor signalling [2, 3]. Worldwide, an estimated three million cases of pesticide poisoning occur every year, resulting in an excess of 250,000 deaths [4]. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish [5]. Bioaccumulation of pesticides in fish species are capable of reaching toxic levels in the fish even when exposure is low [6]. The toxicity of pesticides to target and non-target organisms generally depends on the amount present in the environment, the proportion available to the biota and ultimately on the amount actually encountered and absorbed by the organism [6]. Moreover, the overuse of pesticides can cause a decline in populations of different fish species [3, 7].

Ebrie lagoon which is one of the important lagoon in Côte d'Ivoire, fishing agriculture and industrial activities are also carried out on this lagoon. Since October 1999, large mortalities of wild fish have occurred generally at the beginning of the great wet seasons, in the lagoon area of Jacqueline to Dabou including IV and V sectors. The periodicity of 7 to 8 years old mortalities has become more frequent with more important consequences. Mortality peaked in May 2013 with the loss of several hundred tonnes of fish [8]. This now recurrent phenomenon causes from

time to time the closure of fishing and all aquaculture farms dependent on the lagoon. These massive mortalities of fish are detrimental to the many fish farms, to the riverine population highly dependent on fish resources. They also affect all economic activities related to fish. Unfortunately, to date, no study on pesticides has been carry out in IV and V sectors of Ebrie lagoon. So far, the risks of water contamination with pesticides on the population are ignored. Therefore, the study assesses the residual concentrations of pesticides in fish, and potential health risk associated with the pesticide contamination of *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis* fish samples from IV and V sectors of Ebrie lagoon. The choice of *Chrysichthys nigrodigitatus* is motivated by the most affected species during fish mortalities recorded at the Jacqueline (Ahua) nursery station in 2003 and 2013 [8]. As for *Tilapia zillii* × *Tilapia guineensis* (Hybrid *Tilapia*) was noted for its strong presence in samples collected from sectors IV and V of the Ebrie Lagoon, to the detriment of almost non-existent parent species. However, in IV and V sectors covering the department of Dabou and Jacqueline, *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis* are integrated in the population's diet.

MATERIAL AND METHODS

MATERIAL

Study framework

Fish samples were collected from five sampling sites including Layo, Songon, N'djem, Taboth and Ahua in IV and V sectors of Ebrie lagoon according to according to fish mortality rates (Figure). IV and V sectors are located at latitude 05°16'N and longitude 04°15 - 4°30 W. IV and V sectors covering the departments of Jacqueline and Dabou

SAMPLING CAMPAIGN ANALYSIS

Fish samples were caught using a battery of 10 mesh vacuum nets 6, 8, 10, 15, 18, 20, 25, 30, 35 and 40 mm with the fisherman. Each net is approximately 30 m long with a drop height of 1.5 m. samples were collected every month over the period from February 2014 to January 2015 The whole fish samples were removed, frozen and transported for laboratory analysis. sixty

muscle tissues resulting from the dissection of *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis* were analyzed.

CHOICE OF PESTICIDES

Agricultural, industrial and fishing activities taking place in IV and V sectors using pesticides from different biological and chemical families according to Adingra et Kouassi (2011) [9]. Moreover, a study consisting on evaluation of contamination levels for fourteen (14) pesticides in water and sediments in IV and V sectors of the Ebrié Lagoon carried out by Yao *et al.* (2018) from February 2014 to January 2015 [10], showed that the concentrations of simazine, aldicarb, desethylatrazine, metoxuron, crimidine and metolachlor, were the highest.

METHODS

ANALYSIS

The extraction of pesticide residues in muscle tissues was performed by Accelerated Solvent Extraction (ASE) method according to the protocol described by Thierry Polard [11]. Fish samples were previously lyophilized. These were crushed in a mixture 1/3 sample/hydromatrix ratio. Dionex ASE cells of 22 ml were used for sample processing. The bottom of the cell is equipped with 3 cellulose white filters (Whatman, Dionex Corporation) and 2 g of hydromatrix. The cell is then loaded with 10 g of the sample mixture and hydromatrix milled, then supplemented with hydromatrix. Two white cellulose filters are placed at the top of the cell load. The ASE extraction was performed using a mixture of acetonitrile / dichloromethane solvents (75/25, v / v). The mixture is injected into the cell, maintained for 5 minutes at 50 ° C and at a pressure of 100 bar. At the end of the 5 minutes, 60% of the solvent volume is renewed. This sequence is reproduced 3 times at this temperature, then 3 times at 100 ° C [12]. The solvent removed from the cell is loaded with the extracted analytes. The volumes resulting from the extractions at the two temperatures are separated. In order to remove the dichloromethane, each respective volume is filtered through 50 g of anhydrous sodium sulphate and deposited in a funnel filter. The filtrates are evaporated to dryness using an evaporator to reduce the volumes. After the

elimination of the dichloromethane, the purification of the filtrates containing acetonitrile was done by adsorption chromatography on Oasis HLB Plus cartridges. These are washed with 3 solvents: 2 mL of hexane, 4 mL of methanol and then 2 mL of acetonitrile. Each sample then passes on the cartridge and is eluted with 5ml of ACN. The purified extracts are then evaporated and analyzed by Gas Chromatographic coupled with a Mass Spectrometer (GC/MS).

The gas chromatography used was Agilent technologies and The capillary column type was HP-5MS (30 m x 0.25 µm x 0.25 mm). The carrier gas helium flow rate was 1 ml/min. One µL of sample is injected in splitless mode. The temperature of the injector was held at 250 °C. The gas chromatography is coupled with a mass spectrometer, oven temperature was at 250 °C and electron capture detector was set at 300 °C. The oven temperature was programmed initially at 60 °C for 1 minute, ramped to 180 °C at 30 C/min and to 220 °C at 2 °C/min, ramped to 250°C at 30°C/min and to 305°C at 3°C/min.

CALCULATION METHOD (EDI AND HI)

Estimated Daily Intake (EDI) was calculated as per international guideline [13, 14]. The spiked samples were allowed to stand for using the equation $EDI = C \times Q/P$. EDI was found by multiplying the average residual pesticide concentration in the muscle tissue of fish species by fish consumption rate in Côte d'Ivoire and divided by the average body weight of adults. In this study, C expresses in $\mu\text{g} \times \text{kg}^{-1}$, Q is Quantity of fish ingested $43.53 \times 10^{-3} \text{ kg / person / day}$ in Côte d'Ivoire. Because according to Ocean World Academy of JAPAN, in Côte d'Ivoire, the average daily per capita fish consumption per year is 15.90 kg [15, 16] and P was 60 kg of adult recommended by WHO. Finally, EDI expresses in $\mu\text{g} \times \text{kg}^{-1} \text{ day}^{-1}$. The estimated Health risk index or Hazard Index (HI) for adults was ($\text{igkg}^{-1} \text{ day}^{-1}$) obtained by estimating the ratios between the EDI by the daily intake (ADI). The equation is $HI = EDI / ADI$.

All statistical treatments: averages, standard deviations, comparisons, and Analysis of Variance (ANOVA) were performed using STATISTICA 7.1. The one-way ANOVA was used. It allowed us to compare seasonal (dry season, wet season and flood season) variances concentrations of each pesticide. When the differences were significant ($p < 0.05$), Tuckey's honestly significant difference (HSD) test appeared necessary :

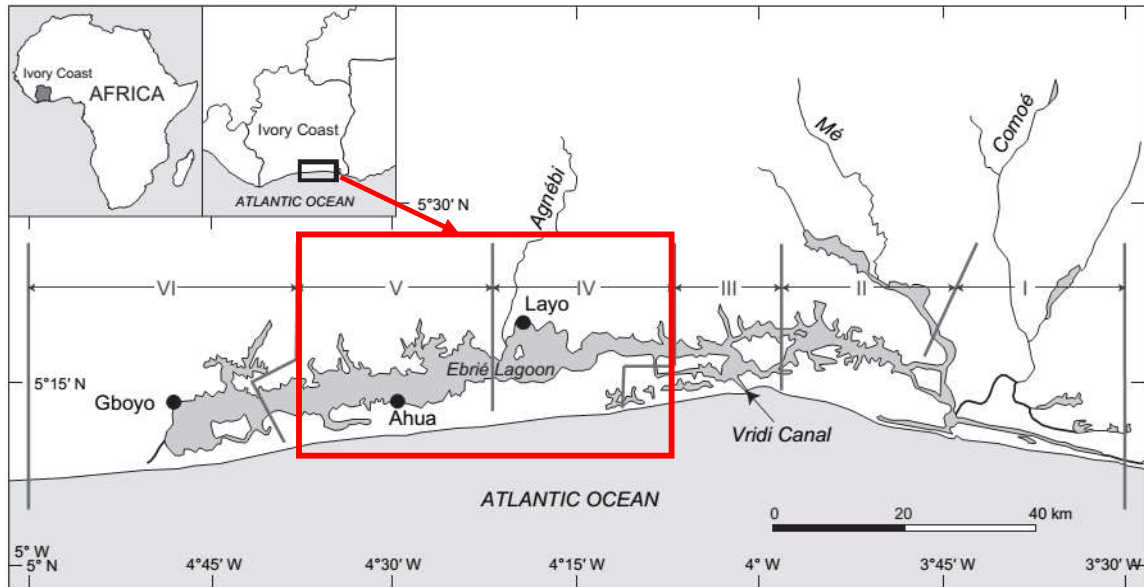


Figure : Location of sampling stations in IV and V sectors of Ebré Lagoon : IV sector : 1-N'Djem, 2-Taboth, 9-Layo, 11-Songon. V sector : 5-Ahua.

Emplacement des stations d'échantillonnage dans les secteurs IV et V de la lagune Ebré
 Secteur IV: 1-N'Djem, 2-Taboth, 9-Layo, 11-Songon. Secteur V: 5-Ahua.

RESULTS

Annual and seasonal concentrations of pesticide residues in muscle tissues in both species with standard deviations in both sectors are presented in I and II tables.

The annual values calculated for the pesticide residues in muscle tissues of *Chrysichthys nigrodigitatus* range between 0 and 12.41 $\mu\text{g}\times\text{kg}^{-1}$ and 0 and 40.66 $\mu\text{g}\times\text{kg}^{-1}$ in IV and V sectors (Table I). The pesticide residue with the highest annual value is desethylatrazine with 12.41 $\mu\text{g}\times\text{kg}^{-1}$ and 40.66 $\mu\text{g}\times\text{kg}^{-1}$ respectively for IV and V sectors. Metoxuron and metolachlor were not detected in IV and V sectors. Only aldicarb,

simazine and desethylatrazine were found for all seasons (Table I) from IV sector. No pesticide residue was identified during the wet and flood seasons except simazine and desethylatrazine detected only during the flood season (Table I) from V sector. No pesticide residues were found in muscle tissue of *Chrysichthys nigrodigitatus* during the wet season in V Sector. Metoxuron and metolachlor were not quantified in *Chrysichthys nigrodigitatus* samples at all seasons in both areas. Statistical analysis using ANOVA showed significant difference in the seasons between concentrations of aldicarb and simazine for *Chrysichthys nigrodigitatus* while a significant difference ($p < 0.05$) was observed between seasons for concentrations of desethylatrazine from IV sector.

Table I : Annual and seasonal concentrations ($\mu\text{g}\text{kg}^{-1}$) pesticide in muscle tissue for *Chrysichthys nigrodigitatus* from IV and V sectors (fresh weight).

*Concentrations annuelles et saisonnières ($\mu\text{g}/\text{kg}$) de pesticides dans les tissus musculaires de *Chrysichthys nigrodigitatus* des secteurs IV et V (poids frais).*

	Annual	Seasons		
		dry Season	wet season	flood season
IV	Metoxuron	0	0	0
	Aldicarb	0.50 ± 0.67	0.75 ± 0.95	0.50 ± 0.57
	Crimidine	0.83 ± 2.58	2.50 ± 4.35	0
	Simazine	1.33 ± 0.88	1.25 ± 1.25	1.25 ± 0.95
	Desethylatrazine	12.41 ± 26.63	1.00 ± 1.41 ^a	12.00 ± 13.85 ^b
	Metolachlor	0	0	0
V	Metoxuron	0	0	0
	Aldicarb	2.00 ± 3.46	6.00 ± 0.00	0
	Crimidine	0.66 ± 1.15	2.00 ± 0.00	0
	Simazine	5.00 ± 7.00	13.00 ± 0.00 ^b	0
	Desethylatrazine	40.66 ± 65.30	6.00 ± 0.00 ^a	0
	Metolachlor	0	0	0

Values with letters a, b, c in superscript show a difference between seasons.

The values of annual concentrations of pesticide residues were relatively low for hybrid *Tilapia*. They range from 0.16 to 4.83 $\mu\text{g}\times\text{kg}^{-1}$ and 0 to 7.66 $\mu\text{g}\times\text{kg}^{-1}$ in sectors IV and V respectively for hybrid *Tilapia*. Desethylatrazine (4.83 $\mu\text{g}\times\text{kg}^{-1}$) from IV sector and simazine (7.66 $\mu\text{g}\times\text{kg}^{-1}$) from V sector were the highest annual

concentrations. From IV sector, only simazine and desethylatrazine have been detected for all seasons (Table II). Further, from V sector, pesticide residues, apart from metolachlor, were regularly present in the dry season (Table II). There is a significant difference ($p < 0.05$) between seasons for aldicarb, simazine and desethylatrazine from IV sector for hybrid *Tilapia*.

Table II : Annual and seasonal pesticide concentrations ($\mu\text{g kg}^{-1}$) in muscle tissue for hybrid *Tilapia* from IV and V sectors (wet weight).

*Concentrations annuelles et saisonnières ($\mu\text{g/kg}$) de pesticides dans les muscles de *Chrysichthys nigrodigitatus* des secteurs IV et V (poids frais).*

		Annual	Seasons		
			dry Season	wet season	flood season
IV	Metoxuron	0.16 ± 0.38	0.25 ± 0.50	0	0.25 ± 0.50
	Aldicarb	1.66 ± 3.11	4.00 ± 4.69	1,00 ± 1,41	0
	Crimidine	0.75 ± 2.00	2.25 ± 3.20	0	0
	Simazine	2.08 ± 1.50	3.50 ± 1.73 ^{ab}	1.25 ± 0.50 ^a	1.50 ± 1.00 ^b
	Desethylatrazine	4.83 ± 9.51	1.00 ± 1.15 ^a	11.00 ± 15.09 ^c	2.50 ± 5.00 ^b
	Metolachlor	0.08 ± 0.28	0.25 ± 0.50	0	0
V	Metoxuron	1.00 ± 1.00	2.00 ± 0.00	1.00 ± 0.00	0
	Aldicarb	1.33 ± 1.52	3.00 ± 0.00	1.00 ± 0.00	0
	Crimidine	1.33 ± 2.30	4.00 ± 0.00	0	0
	Simazine	7.66 ± 12.42	22.00 ± 0.00	0	1.00 ± 0.00
	Desethylatrazine	5.66 ± 8.14	15.00 ± 0.00	0	2.00 ± 0.00
	Metolachlor	0	0	0	0

Estimated Daily Intake (EDI) and Hazard Index (HI) values are presented in Table III EDI values in muscle tissues for *Chrysichthys nigrodigitatus* calculated range from 0 to $9.00 \times 10^{-3} \mu\text{g} \times \text{kg}^{-1} \text{day}^{-1}$ in IV sector and from 0 to $2.95 \times 10^{-2} \mu\text{g} \times \text{kg}^{-1} \text{day}^{-1}$ from V sector. Those of hybrid *Tilapia* range from 3.5×10^{-3} to $5.56 \times 10^{-3} \mu\text{g} \times \text{kg}^{-1} \text{day}^{-1}$. For *Chrysichthys nigrodigitatus*, 9.00×10^{-3} (IV sector) and 2.95×10^{-2} (V sector) were the highest EDI values obtained for desethylatrazine in both sectors. For *Tilapia* hybrid, the highest EDI,

3.5×10^{-3} , was obtained for desethylatrazine in IV sector and 5.56×10^{-3} for simazine in V sector. The results showed the HI values of pesticide residues were ranged between 0 and 1.8×10^{-2} and 1.09×10^{-7} and 7×10^{-3} for IV sector respectively in *Chrysichthys nigrodigitatus* and *Tilapia* hybrid. In V sector, they oscillated from 0 to 5.90×10^{-2} (*Chrysichthys nigrodigitatus*) and from 0 to 8.22×10^{-4} (*Tilapia* hybrid). HI values found in fish muscle are much less than 1

Table III: Values of Estimated Daily Intakes (μgkg^{-1} / day) and Risk Index for Pesticide Health in muscle tissue from IV and V Sectors.

Valeurs des apports quotidiens estimés ($\mu\text{g/kg/jour}$) et de l'indice de risque pour la santé des pesticides dans les muscles de poisson des secteurs IV et V.

	ADI (μgkg^{-1}) WHO	EDI (μgkg^{-1}) C. nigrodigitatus	HI	EDI hybrid Tilapia	HI
Metoxuron	5	0	0	1.16×10^{-4}	2.32×10^{-5}
Aldicarb	3	3.63×10^{-4}	1.21×10^{-4}	1.20×10^{-3}	3×10^{-4}
Crimidine	5×10^3	6.02×10^{-4}	1.2×10^{-7}	5.44×10^{-4}	1.09×10^{-7}
Simazine	0.52	9.65×10^{-4}	1.85×10^{-4}	1.51×10^{-3}	2.90×10^{-3}
Desethylatrazine	0.5	9.00×10^{-3}	1.8×10^{-2}	3.5×10^{-3}	7×10^{-3}
Metolachlor	3.5	0	0	5.80×10^{-5}	1.66×10^{-5}
Metoxuron	5	0	0	7.25×10^{-4}	1.45×10^{-4}
Aldicarb	3	1.45×10^{-3}	4.83×10^{-4}	9.65×10^{-4}	3.21×10^{-4}
Crimidine	5×10^3	4.79×10^{-4}	9.58×10^{-8}	9.65×10^{-4}	3.21×10^{-7}
Simazine	0.52	3.63×10^{-3}	6.98×10^{-3}	5.56×10^{-3}	1.07×10^{-4}
Desethylatrazine	0.5	2.95×10^{-2}	5.9×10^{-2}	4.11×10^{-3}	8.22×10^{-4}
Metolachlor	3.5	0	0	0	0

DISCUSSION

Mean annual concentrations pesticide residues in muscle tissue of *Chrysichthys nigrodigitatus* are higher than *Tilapia zilli* × *Tilapia guineensis* in both sectors. The only organochlorine (metolachlor), was obtained with concentrations ranging from 0 to $0.25 \mu\text{g} \times \text{kg}^{-1}$ in the muscle tissue of both fishes. Our values are smaller than those found by Ibigbami *et al.*, (2015) in the Ero River in Nigeria for DDT (0.625 to $2.97 \mu\text{g} \times \text{kg}^{-1}$) in *Chrysichthys nigrodigitatus* and *Oreochromis niloticus* [6]. further, they are below the $6 \mu\text{g} \times \text{kg}^{-1}$ reported by Meng *et al.* (2007), in Guangdong Province [17] and at $18.75 \mu\text{g} \times \text{kg}^{-1}$ obtained by Liu *et al.* (2010), from Liaoning Province in China on fish species for same organochlorine [18]. Aldicarb, simazine and desethylatrazine are more present in muscle tissues than other pesticide residues. The accumulation of pesticide residues in muscle tissue varies seasonally for both species. Accumulations of *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis* are higher during the dry season than the wet and floods season. This result could be attributed to their diet. Food availability is low during the dry season compared to the wet season according to Konan *et al.* (2010) [19]. Under these conditions, both species find their food in sediments contaminated with pesticides [20, 21]. Further,

the wet and flood seasons correspond to the nesting and reproduction period in *Chrysichthys nigrodigitatus*. According to Hem *et al.* (1994) [22], the period from August to November (end of the rainy season and the flood season) is the breeding season. This is the period when pairs (females and males) of *Chrysichthys nigrodigitatus* prepare a nest or search for a natural cavity (rocks, trunks and tree roots, etc.) for oviposition. During this period, according to the same authors, the parents remain on an empty stomach, which could reduce the amount of pesticide residues in their organs. Concerning *Tilapia zilli* × *Tilapia guineensis*, during the dry season, cichlids (family *Tilapia zilli* × *Tilapia guineensis*), due to lack of food in the water column they seem to take advantage of the resources contained in the mud [19].

The EDI value of desethylatrazine in the muscle of *Chrysichthys nigrodigitatus* is the highest in this study. This value was comparatively greater than the EDI of pesticides found in fish samples by Ibigbami *et al.*, (2016) in the Rivers of Ogbese, Ero and Elemi in Nigeria [23]. The exposure of human being to pesticides by ingestion passes by the knowledge of the potential risk on the health. The results of EDI and HI calculated in *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis*, showed that the pesticide residues accumulated in muscle tissues of fishes do not present any danger for the consumer.

We can conclude that levels of pesticide accumulation in muscle tissue of *Chrysichthys nigrodigitatus* and *Tilapia zilli* × *Tilapia guineensis* are relatively low in both sectors.

CONCLUSION

The study showed that the accumulation of pesticide residues in muscle tissue was more important in *Chrysichthys nigrodigitatus* than *Tilapia zilli* × *Tilapia guineensis* in both sectors. The accumulation was stronger in the dry season than in the wet and flood seasons from sampled fishes. Pesticide residues intake by people consuming the fishes from IV and V sectors of Ebrie lagoon are low and pose no potential health risk to people in the area.

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