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# Research for the Presence of Pesticides in Fish from the Sassandra and Bandama Rivers in the Localities of Bouafle, Guessabo and Soubre, Ivory Coast

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study aims to investigate the presence of pesticides in fresh fish caught in the Sassandra and Bandama rivers of the Sassandra-Marahoué district.

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**Study Design:** Fish is an important source of protein, the quality of which depends on many actions carried out directly or indirectly by man during fishing, fish trading, preservation operations (smoking, drying, refrigeration, etc.) the use of pesticides on cultivable areas along the rivers.

**Place and Duration of Study:** Sampling was carried out in three fishing areas of the Sassandra-Marahoué district and the chemical analyzes were carried out at the Agrovalorization Laboratory of the Jean Lorougnon Guédé University in Daloa and at the National Laboratory for Agricultural Development Support (Côte d'Ivoire).

**Methodology:** : A total of 390 samples of fresh fish, including 130 of each of the Tilapia, Chrysichthys and Labéo species, were collected randomly under aseptic conditions directly from various fishermen from the Sassandra River in the towns of Soubré and Guessabo and from the Bandama River in the locality of Bouaflé.

**Results:** Analysis of the fish samples revealed about fifteen active substances from pesticides. Crimidine and Desisopropylatratzine showed the highest levels in Bouaflé Tilapia with 29.55% and 22.73% respectively, Metobromuron, Simazine and Fénuron at 29.55% each in Chrysichthys and Labeo, each substance was found in a single sample with a rate of 2.27%. In the Guéssabo samples, Metoxuron and Monuron are the highest substances in fresh Tilapia with 18.60% each. Certain substances such as Crimidine, Aldicarb, Parathion-ethyl and Parathion-methyl were not found in the Chrysichthys samples as well as Isoproturon, Metobromuron, Metoxuron and Monuron in the *Labeo*. The highest substances in the Soubré samples are Metobromuron at 18.60% in *Tilapia*, Parathion-methyl and Terbutryn at 18.60% in *Chrysichthys* and Crimidine in *Labéo* at 9.30%.

**Conclusion:** This study clearly shows the presence of pesticide residues in fresh fish caught in the Bandama and Sassandra rivers. Precautions should be taken to eliminate or at least reduce the impacts of these on the environment and especially on human health.

*Keywords: Fresh fish; pesticides; Sassandra river; Bandama river.*

## 1. INTRODUCTION

Côte d'Ivoire has, in addition to its opening on the Atlantic Ocean, many water bodies and rivers including lagoons and rivers for the development of fishing and aquaculture. According to N'guessam *et al*., 2017 fish is the main source of animal protein for Ivorian consumers, with per capita consumption in 2014 at 15.90 kg/capita/year [1,2,3]. This demand for fishery products remains strong and sustained due to their relative ease of access. But their quality depends on all the actions carried out directly or indirectly by man during fishing, fish trading, conservation operations (smoking, drying, refrigeration, etc.) and the use of pesticides in cultivable areas along rivers [4,5,6]. Indeed, the search for arable land suitable for market gardening and/or rice growing leads the population to colonize along the rivers. This proximity to rivers makes these environments favorable for pests, diseases and fungi, thus encouraging farmers to use pesticides to control and protect their crops [7,8]. This often abusive and non-recommended use of chemicals affects all regions of Côte d'Ivoire and particularly the localities of the Sassandra-Marahoué district. If along the rivers is an area suitable for crops, on the other hand it represents great risks for the marine and coastal environment in the production areas [9,10]. Through the phenomenon of erosion, pesticides and their residues end up in surface water (watercourses and bodies of water) as well as in groundwater and seawater [11,12]. A large number of insecticides and some herbicides and fungicides can have a toxic effect for aquatic organisms, and can have a harmful effect on the natural environment (Kreuger quoted by Merghid [12]). According to our knowledge, pesticides are chemicals, and any material that contains a chemical composition is dangerous for human health. Thus, the marketing of contaminated fish can also represent a threat to human health [12,13]. It is within this framework that the present study falls, the general objective of which is to search for the presence of pesticides in fresh fish caught in the Sassandra and Bandama rivers of the Sassandra-Marahoué district. Specifically, it is a question of searching by the HPLC method, for traces of pesticides in fish samples from three different localities in the district.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Biological material

Three species of fish among the most consumed and most present in the Bandama and Sassandra rivers were the subject of this study. These are the Tilapia (*Tilapia*), the jawbone (*Chrysichthys*) and the Labéo (*Labéo*). These three species were chosen because they are not only among the most consumed fresh or smoked, but also because they are present in the waters of the three localities studied.

#### 2.1.2 Technical material

It is made up of the equipment usually used in a food and industrial microbiology laboratory, in particular consumables and reagents. The main technical equipment for this study is the High Performance Liquid Chromatography (HPLC) chain.

#### 2.1.3 Fish sampling and transport

The sampling of fresh fish was carried out directly as soon as each fisherman landed. Tilapia, Chrysichthys and Labeo are the species taken directly by in situ identification using the identification keys for freshwater fish species made available to us by the services of the Ministry of Animal and Fisheries Resources (MIRAH).

A total of 390 samples of fresh fish, including 130 of each of the *Tilapia*, *Chrysichthys* and *Labéo* species, were randomly collected from various fishermen near the Sassandra River in the towns of Soubré and Guessabo and the Bandama River in the locality of Bouaflé. The collection of the samples took place throughout the year 2022. These samples were taken under aseptic conditions directly from the fishermen, then transported to the laboratory in clean coolers containing ice and kept at 4°C before the analysis time.

### 2.2 Methods

#### 2.2.1 Dosage of pesticides in collected fish samples

High Pressure Liquid Chromatography, now known as High Performance Liquid Chromatography (HPLC), is the chromatographic technique used to identify, quantify, separate and purify individual compounds present in a blend.

#### 2.2.2 Principle of an HPLC chain

The principle of HPLC is, like all other variants of chromatography, to use the differences in physico-chemical properties of different compounds to separate them. The compounds to be separated (solutes) are dissolved in a solvent. The solvents used (acetone, hexane, dichloromethane, acetonitrile) are of quality for the analysis of pesticide residues (purity=99.9%). The mixture is introduced into the liquid mobile phase (eluent). Depending on the nature of the molecules, they interact more or less with the stationary phase in a tube called a chromatographic column [8].

The mobile phase, pushed by a pump under high pressure, travels through the chromatographic system. The mixture to be analyzed is injected and then transported through the chromatographic system. The compounds in solution are then distributed according to their affinity between the mobile phase and the stationary phase. At the column outlet using an appropriate detector, the different solutes are characterized by a peak. The set of recorded peaks is called a chromatogram [8].

**a.** *Tilapia* **b.** *Chrysichthys* **c.** *Labéo*

**Fig. 1. Fish species sampled**

**Table 1. Distribution of fish samples collected**

| Locality | Fresh fish samples | | |
|---|---|---|---|
| | **Tilapia** | **Chrysichthys** | **Labéo** |
| Bouaflé | 44 | 44 | 44 |
| Guessabo | 43 | 43 | 43 |
| Soubré | 43 | 43 | 43 |
| Sous Total | 130 | 130 | 130 |
| Total général | 390 | | |

A Jasco high performance liquid chromatograph (Jasco, Bouguenais, France) was used. It is equipped with a pump (PU 2080 Plus) and coupled to a UV absorption detection system (MD 2015 Plus) with diode array (UV-DAD). The assembly is controlled by a computer equipped with Ezchrom Elite operating and data acquisition software.

#### 2.2.3 Extraction of pesticides

The extraction of the compounds begins with the cutting of the fish into small pieces before grinding them in a mortar. Then, 25 g of the ground material are weighed in a 1000 ml Herlen Meyer in which 100 ml of Acetone then 50 ml of Bidistilled Water are added. Subsequently, the solution is filtered on whatman paper in a separating funnel before adding 2.5 to 5 g of sodium chloride. The whole is vigorously checked so as to obtain a phase separation. An aqueous phase above and an organic phase below. Using a ground-neck flask, the organic phase is collected by filtering it again on whatman paper. Then, dry evaporation using a rotavapor is carried out before adding 10 ml of hexane [14].

#### 2.2.4 Purification of compounds

Purification is carried out by activating the C18 cartridge with 10 ml of methanol and passing the previously concentrated sample through this C18 cartridge. Then, the cartridge is dried and the pesticides retained by it are recovered with 5ml of methanol before being transferred into a Vial for HPLC quantification.

#### 2.2.5 Data processing and statistical analyzes

These data were analyzed using descriptive statistics (frequencies, means, standard deviations). The analysis of variance (ANOVA) was carried out with Past 4.11 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Evaluation of the presence of active substances of pesticides in samples of fresh fish

*3.1.1.1 Rate of active substances from pesticides in fresh Tilapia*

In the Tilapia samples from the Guessabo fishing area, all the pesticide molecules found belong to the family of urea derivatives. These include Metoxuron (0.0019 mg/kg), Monuron (0.0093 mg/kg) and Isoproturon (0.0012 mg/kg). They have a concentration well below the quantitative limit.

In the Bouaflé samples, two molecules from the Triazine family were identified. These include Desisopropylatratzine at 0.00021 mg/kg and Crimidine at 0.00011 mg/kg. These concentrations are well below the limits of quantification thereof.

Those from Soubré showed the obvious presence of Atrazine (0.005 mg/kg), Metobromuron (0.007 mg/kg) and Chlorfenvinphos (0.006 mg/kg). None of these molecules was found with a concentration above the limit of quantification (Table 2).

*3.1.1.2 Rate of active substances from pesticides in Chrysichthys*

No molecule of pesticides was found in the samples of fresh *Chrysichthys* fished either in the locality of Guessabo or in that of Soubré.

Only Simazine (0.0010 mg/kg) and Fenuron (0.014 mg/kg) from the family of Triazines and urea derivatives were found in the samples of *Chrysichthys* from Bouafle. However, their concentrations remain below the authorized quantification limit (Table 3).

*3.1.1.3 Level of active substances from pesticides in the Labéo*

There were no pesticide molecules found in the Labéo samples fished either at Guessabo, Bouaflé or Soubré (Table 4).

### 3.1.2 Appearance rate of active pesticide substances by locality

*3.1.2.1 Appearance of active substances in fish species in the locality of Bouaflé*

The dosage of pesticides in fish revealed about fifteen active substances whose quantities vary from one species to another. Thus Crimidine and Desisopropylatratzine have the highest levels and were found in 13 samples of *Tilapia* with respectively 29.55% and 22.73%. As for *Chrysichthys*, substances such as Metobromuron, Simazine and Fenuron are the most active with a rate of 29.55% each detected in 13 samples. In the *Labeo* each substance was found in a single sample with a rate of 2.27% (Table 5).

*3.1.2.2 Appearance of active substances in fish species in the locality of Guéssabo*

The analysis of fish samples revealed about fifteen active substances from pesticides. These substances were detected at varying rates in the different fish samples. Thus Metoxuron and Monuron are the highest substances in fresh Tilapia with 18.60% each. Certain substances such as Crimidine, Aldicarb, Parathion-ethyl and Parathion-methyl were not found in the Chrysichthys samples. As for Labeo, substances such as Isoproturon, Metobromuron, Metoxuron and Monuron were not detected. As for substances such as Atrazine and Metobromuron, they were detected in 6 samples with a rate of 13.95% each.

*3.1.2.3 Appearance of active substances in fish species in the locality of Soubré*

About fifteen active substances were revealed by dosage of pesticides in fresh fish. The highest substances in the Tilapia samples are Metobromuron at 18.60% in the fresh.

At the level of Chrysichthys, Parathion-methyl and Terbutryn were found to be present in 8 samples analyzed out of 43, i.e. with a rate of 18.60%. On the other hand, Crimidine was present in 4 Labéo samples analyzed, i.e. 9.30%.

## 4. DISCUSSION

The search for the presence of pesticides in fresh fish samples revealed several molecules from different families. This presence of pesticides in the fish samples analyzed demonstrates that the pesticides and their residues are indeed found in surface waters (watercourses and bodies of water) as well as in the animals that live there. In the tropics, the risks of water contamination, linked to erosion and runoff, contributing to “diffuse pollution”, towards surface waters are high [15]. Phenomena of transport by leaching, leaching or runoff lead to the contamination of groundwater,

**Table 2. Content of active substances in *Tilapia***

| Origin | Active substances (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Méto** | **Monu** | **Isop** | **Atra** | **Métob** | **Chlor** | **Dési** | **Crimi** |
| Guessabo | 0,0019 | 0,0093 | 0,0012 | 0 | 0 | 0 | 0 | 0 |
| Bouaflé | 0 | 0 | 0 | 0 | 0 | 0 | 0,00021 | 0,00011 |
| Soubré | 0 | 0 | 0 | 0,005 | 0,007 | 0,006 | 0 | 0 |

***Legend:***
*Atra: Atrazine; Chlor: Chlorfenvinphos; Crimi: Crimidine;*
*Isop: Isoproturon; Métob: Métobromuron; Méto: Métoxuron;*
*Monu: Monuron; Dési: Désisopropylatratzine.*

**Table 3. Levels of active substances in fresh *Chrysichthys***

| Locality | active substances (mg/kg) | | |
|---|---|---|---|
| | **Chlorfenvinphos** | **Simazine** | **Fénuron** |
| Guessabo | 0 | 0 | 0 |
| Bouaflé | 0 | 0,0010 | 0,014 |
| Soubré | 0 | 0 | 0 |

**Table 4. Level of active substances in fresh Labéo**

| Locality | Active substances (mg/kg) | | | |
|---|---|---|---|---|
| | Atrazine | Métobromuron | Chlorfenvinphos | Fénuron |
| Guessabo | 0 | 0 | 0 | 0 |
| Bouaflé | 0 | 0 | 0 | 0 |
| Soubré | 0 | 0 | 0 | 0 |

**Table 5. Frequency of appearance of active substances in fish from the locality of Bouaflé**

| Active substances | Number of samples analysed | Number/frequency occurrence of active substances in fish samples (%) | | |
|---|---|---|---|---|
| | | *Tilapia* | *Chrysichthys* | *Labéo* |
| Atrazine | | 5 (11,36) | 3 (6,32) | 1 (2,27) |
| Chlorfenvinphos | | 3 (6,82) | 7 (15,91) | 1 (2,27) |
| Crimidine | | 13 (29,55) | 7 (15,91) | 1 (2,27) |
| Désisopropylatratzine | | 10 (22,73) | 7 (15,91) | 1 (2,27) |
| Isoproturon | | 3 (6,82) | 5 (11,36) | 1 (2,27) |
| Métobromuron | | 3 (6,82) | 13 (29,55) | 1 (2,27) |
| Métoxuron | | 3 (6,82) | 5 (11,36) | 1 (2,27) |
| Monuron | 44 | 3 (6,82) | 3 (6,82) | 1 (2,27) |
| Simazine | | 3 (6,82) | 13 (29,55) | 1 (2,27) |
| Fénuron | | 3 (6,82) | 13 (29,55) | 1 (2,27) |
| Aldicarb | | 3 (6,82) | 3 (6,82) | 1 (2,27) |
| Parathion-éthyl | | 3 (6,82) | 3 (6,82) | 1 (2,27) |
| Parathion-méthyl | | 3 (6,82) | 3 (6,82) | 1 (2,27) |
| Terbutryn | | 3 (6,82) | 3 (6,82) | 1 (2,27) |
| Vinclozolin | | 3 (6,82) | 3 (6,82) | 1 (2,27) |

**Table 6. Frequency of occurrence of active substances in fish from the locality of Guéssabo**

| Active substances | Number of samples analysed | Number/frequency occurrence of active substances in fish samples (%) | | |
|---|---|---|---|---|
| | | *Tilapia* | *Chrysichthys* | *Labéo* |
| Atrazine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Chlorfenvinphos | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Crimidine | | 1 (2,33) | 0 (00) | 1 (2,33) |
| Désisopropylatratzine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Isoproturon | | 5 (11,63) | 1 (2,33) | 0 (00) |
| Métobromuron | | 1 (2,33) | 1 (2,33) | 0 (00) |
| Métoxuron | 43 | 8 (18,60) | 1 (2,33) | 0 (00) |
| Monuron | | 8 (18,60) | 1 (2,33) | 0 (00) |
| Simazine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Fénuron | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Aldicarb | | 0 (00) | 0 (00) | 1 (2,33) |
| Parathion-éthyl | | 0 (00) | 0 (00) | 1 (2,33) |
| Parathion-méthyl | | 0 (00) | 0 (00) | 1 (2,33) |
| Terbutryn | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Vinclozolin | | 1 (2,33) | 1 (2,33) | 1 (2,33) |

drainage water or surface water, also leading to the contamination of fishery resources [12]. In this study, the fish analyzed contained only low concentrations of pesticides, nothing seems to indicate that these concentrations could present a significant danger to health. This situation is understandable insofar as all the fishermen interviewed during the study claim not to use any pesticides for catching fish. Nevertheless, the presence of the few pesticides obtained in this work can be justified by the types of speculation cultivated around the different fishing areas studied; speculations on which greater or lesser quantities of pesticides were probably applied. Indeed, during the period of reconnaissance of the study areas and the various sampling campaigns, we were able to observe a greater crop rotation at the Bouaflé and Guessabo sites. The cultures of these sites alternated between rice cultivation and market gardening. Unlike these two sites, that of Soubré crossing the city is subject to urban contamination (water from households, sewers and drainage channels, etc.). Contamination of surface water and wells has been noted at sites close to agricultural land around the world [16,17]. In general, the compounds frequently found were atrazine, simazine, alachlor, metolachlor, trifluralin, diazinon, parathion methyl, lindane, endosulfan and aldrin. Surface water was generally more polluted than groundwater, especially near agricultural or urban sites. In France, pesticides were detected in 91% of watercourse quality monitoring points, 75% of water body monitoring points and 70% of groundwater monitoring points between 2007 and 2009. The level of contamination was higher in rivers than in groundwater. The total pesticide concentration was greater than 0.5 µg/L at 18% of the measurement points in rivers and at 3.8% of the points in groundwater. The substances most frequently encountered in waterways were, in almost all cases, herbicides. Most of the exceedances of the standards were due to atrazine desethyl, the main metabolite of atrazine and, to a lesser extent, of atrazine itself. Glyphosate and its metabolite AMPA are the third cause of downgrading [18]. High levels of chlordecone have also been detected in the coasts, rivers, sediments and groundwater of Martinique due to its massive application in banana plantations [19]. Atrazine and endosulfan have been found very often in surface waters in Australia due to high use. Other pesticides found included profenofos, dimethoate, chlordane, diuron, prometryne and fluometuron (Cooper cited by Diop [8]).

Wildlife poisoning depends on the toxicity of the pesticide, the amount applied, the frequency, timing and method of spraying. Many pesticides are toxic to fish. They can be intoxicated by contact during aerial treatment, or by ingestion by eating contaminated food or water, by breathing pesticide vapors or by absorption of pesticides through their skin. Fish, amphibians and aquatic insects are especially susceptible to water contamination by pesticides. Copper fungicides are highly toxic to aquatic organisms, as the risk of copper accumulation in fish and some other aquatic organisms can be high. The effects of pesticides on fish and amphibians have been highlighted. For example, carbaryl has been found toxic to several species of amphibians [20]. Several studies have shown an increased risk of Parkinson's disease associated with exposure to insecticides or herbicides and this risk would be high in the event of exposure to organochlorines, organophosphates or carbamates [8,21]. A study carried out in France showed that occupational exposure to a pesticide was associated with twice the risk of developing Alzheimer's disease. It has also been associated with mild cognitive dysfunction and risk of

**Table 7. Frequency of appearance of active substances in fish from the locality of Soubré**

| Active substances | Number of samples analysed | Number/frequency occurrence of active substances in fish samples (%) | | |
|---|---|---|---|---|
| | | ***Tilapia*** | ***Chrysichthys*** | ***Labéo*** |
| Atrazine | 43 | 5 (11,63) | 1 (2,33) | 1 (2,33) |
| Chlorfenvinphos | | 5 (11,63) | 1 (2,33) | 1 (2,33) |
| Crimidine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Désisopropylatratzine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Isoproturon | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Métobromuron | | 8 (18,60) | 1 (2,33) | 1 (2,33) |
| Métoxuron | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Monuron | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Simazine | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Fénuron | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Aldicarb | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Parathion-éthyl | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Parathion-méthyl | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Terbutryn | | 1 (2,33) | 1 (2,33) | 1 (2,33) |
| Vinclozolin | | 1 (2,33) | 1 (2,33) | 1 (2,33) |

dementia in people with Parkinson's [22]. A series of epidemiological studies carried out since 2000 have concluded that exposure to pesticides can affect spermatogenesis reducing male fertility [23,24,25,26]. Organochlorines are known to be persistent in the environment (high resistance to biodegradation) and for their bioavailability. Thus, they can concentrate in the trophic chain and lead to significant biomagnification in its last link [27,28].

## 5. CONCLUSION

It appears from the present study carried out in Bouaflé, Guessabo and Soubré that pesticides are indeed present in fish products, particularly in the three different species of fish analyzed. Their presence in the sampled fish confirms that human health is in danger. The resulting toxicological risks for humans are very significant (death, endocrine disturbances, congenital malformations, cancers, neurological disorders, immune disorders, etc.). Thus, it is imperative to ensure an ecologically rational use of pesticides in order to maintain the environmental balance, but also, because the health of consumers depends on it. Precautions must be taken to eliminate or at least reduce the impact of these on the environment and especially on human health. In addition, it would be wise to carry out monitoring of the quality of the environment and foodstuffs to verify their compliance with established standards.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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