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RESEARCH ARTICLE

TOXICOLOGICAL ANALYSIS OF HEADS AND HEADLESS PARTS OF JAWFISH, TUNA, TILAPIA AND MACKERELS CONSUMED IN THE ABIDJAN CITY (CÔTE D'IVOIRE)

OULAI Tokpa Louya[1,*], AKPRO Lathro Anselme[2] and GBOGOURI Grodji Abarin[2]

[1]Laboratory of Biotechnology and Bioprocesses, UFR Engineering Agronomy Forestry and Environment, University of Man (Côte d'Ivoire); [2]Laboratory of Nutrition and Food Security, Department of Food Science and Technology, Nangui Abrogoua University (Côte d'Ivoire).

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**Corresponding Author:*
OULAI Tokpa Louya

ABSTRACT

In this study, toxicological analysis of heads and headless parts of fish the Hydrocarbon Aromatic Polycyclic (PAH) such as pyrene and Benzo (a) pyrene (0.058 and 0.067 µg/kg), Benz (g, h, i) pyrilene, Benzo (a) anthracene. Other compounds such as fluoranthenes and indeno pyrilene have also been detected. The results show the pesticide presence of desethylatrazine (0.021-0.033 µg/g), fenuron (0.023 - 0.042 µg/g), metoxuron (0.622 - 0.623 µg/g), monuron (0.030 - 0.037 µg/g), methabenzthiazuron (0.023 - 0.024 µg /g), simazine (0.089 - 0.090 µg/g), isoproturon (0.062- 0.069 µg/g), cyanazine (0.022- 0.200 µg/g), metamitron (0.042 - 0.042 µg/g) (0.023± 0.0 mg/kg) ofmackerel followed by headless tuna (0.011 mg/kg). The lowest levels are found in the head and headless part of tilapia, as well as in the heads of the jawfish. The heavy metals such as lead and mercury were also detected at low concentrations in the headless parts and heads.

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INTRODUCTION

In a contaminated environment, fish absorb more contaminants than they can eliminate naturally. This results in a concentration toxic substance in of the animal's viscera, flesh and skeleton. The substance bioaccumulation varies according to the species, the age of the fish and the level of contamination of its habitat and food(Corsolini *et al.,* 2005). The phenomenon amplifies from the base to the food chain top: the predatory fish that feed on prey that had already accumulated toxic substances thus concentrate more contaminants(Doganoc, 1995; Andreji *et al.,* 2006).The contaminants of health concern that are most likely to accumulate in of fish flesh and then find their way into the bodies of people who eat them fall into two categories. Heavy metals and organochlorines.Heavy metals are toxic in certain doses but not carcinogenic except for cadmium which are contained in rocks. The latter is slowly released into the environment by geological processes, and also by human and especially industrial activities. Among the heavy metals, four elements deserve special attention because of their accumulation in fish living in a contaminated aquatic environment. These are mercury, lead, cadmium and arsenic(Has-Shön *et al.,* 2006; Mendill and Dogan, 2007).The Mercury [Hg], specifically methylmercury [CH_3Hg^+], a particularly toxic assimilable form of mercury, produced mainly by the action of certain bacteria in the presence of inorganic mercury accumulated in the organic sediments at the bottom or in suspension in the water.The Lead [Pb], and more specifically tetraethyl lead [$Pb(C_2H_5)_4$], a particularly toxic form of lead once used extensively as an anti-knock additive in gasoline.

The Cadmium [Cd], classified as a carcinogen, mainly used in batteries, paints, alloys for engine and welding parts and plastic or synthetic materials.The Arsenic [As], more specifically inorganic compounds (arsenites, arsenoxides and arsenates) generally more toxic than organic compounds, formerly used in the manufacture of pesticides, today mainly used in metallurgy and in the wood's preservation. Organochlorines are reputed to be carcinogenic and relate to all laboratory products used in the manufacture of pesticides and high-performance synthetic oils. These products are dispersed in the environment by human, industrial and domestic activities. The objective of this study was to determine the toxicological dose in the heads and headless parts of fish. Especially, it holds to analyse the hydrocarbon aromatic polycyclic, the pesticides and heavy metals in different parts of fish.

MATERIALS AND METHODS

Materials

The biological material for this study is made up of fish, more specifically heads and headless parts of jawfish (*chrysichthys nigrodigitatus*), mackerel (*Scomber scombrus*), tilapia (*Oreochromis niloticus*) and tuna (*Thunnus albacares*) which were collected in the ''Pêche et Froid'' company of Abidjan (Côte d'Ivoire)

Methods

Dosage of pollutants

Dosage of pesticides : The pesticides that have been analyzed are: Desethylatrazine, Fenuron, Desisopropylatratzine, Metoxuron, Methabenzthiazuron, Linuron, Chlortoluron, Monolinuron, Isoproturon, Propazine, Crimidine, Metamitron. Thus, quantities of 2 g of heads and headless parts of each type of fish were sampled. Thirty (30) mL of a hexane/acetone mixture (75/25, V/V) was then added to the sample. The whole was then ground using an Ultraturrax® grinder (Ika, Werke, Germany), then filtered using a phase separation membrane (Whatman 1 PS). This grinding and extraction step was performed twice. The extract was then evaporated at 60°C using a rotary evaporator (Rotavapor), then taken up in 10 mL of hexane. Two (2) mL of fuming sulfuric acid (SO3, 7%) was then added and centrifuged at 4000 rpm for 10 min. One (1) mL of the supernatant was added to 1 mL of 2% potassium hydroxide (KOH) in ethanol. The tubes were then placed in a shaking water bath at 50°C for 30 min. After cooling, 2 mL of ultrapure water was added and the mixture was centrifuged at 2000 x g for 10 min before undergoing a second hydrolysis with fuming sulfuric acid, as described above. The gas chromatography protocol, combined with an electron capture detector with a 5973N mass spectrometer coupled to a gas chromatograph 6890 GC (Agilent®, 0.30 m column HP5-MS 0.25 mm ID, fineness 0.25µm) was used. For each sample and each standard, 5 µL were injected. The temperature program starts at 85°C maintained for 1 min, then a rise of 6°C/min up to 170°C (maintained for 15 min), then a second rise of 20°C/min up to 280° C, held for 4.33 min. The total analysis time is 50 min. The injector temperature was 250°C and was in splitless mode (Lemarchand *et al*., 2007, 2010, 2011a, b, 2012). The experiment was carried out 3 times.

Dosage of PAHs: Two (2) g of heads and headedless parts of the fish were homogenized for 5 min in 8 mL of acetone then centrifuged at 2000 x g. The supernatant was then placed in separate tubes, and this extraction procedure is performed twice. The samples were then evaporated under nitrogen, the dry residue then being taken up in a solution of acetone and methanol (50:50). The extract was then purified using a 500 mg SPE C18 column, conditioned with 2 mL of acetone and 2 mL of methanol. The column was vacuum dried and the purified samples were taken up in 3 mL of acetone. After evaporation under nitrogen, the samples were again taken up in 1 mL of methanol. The concentrations of the herbicides (Simazine, Terbuthylazine, Cyanazine) dosed were determined by Gas Chromatography coupled with Mass Spectrometry (GC/MS). A 5973N mass spectrometer coupled to a 6890 GC gas chromatograph (Agilent®, 0.30 m column HP5-MS 0.25 mm ID, fineness 0.25 µm) was used. For each sample and each standard, 2 µL were injected. The temperature program starts at 85°C maintained for 1 min, then a rise of 6°C/min up to 170°C (maintained for 12 min), then a second rise of 20°C/min up to 280° C, held for 4.33 min. The total analysis time is 37 min. The injector temperature was 250°C and was in splitless mode. Each sample was identified according to the retention times and 3 to 4 ions depending on the compounds, with predefined contents and 20% variability for each ion. Linearity is confirmed between 100 and 500 ng/g with 5-point calibration curves (r^2> 0.99).The recovery level was determined between 67 and 98% for all the samples and the reproducibility was considered acceptable when the variation coefficients remained below 15%. After a final centrifugation at 2000 x g, the supernatant was used for the detection of PAHs by gas chromatography. The PAHs concentrations were identified from standards available on the market (CIL, St Foy la Grande, France, purity > 99%; level of recovery on the standards greater than 92%; linearity determined between 5 and 100 ng/g (r^2 > 0.99 on standards, 5-point calibration curves) Detection limits vary between 0.5 and 1.0 ng/g for each element Certified samples of fish liver (cod) (cod liver oil BCR349) were used as a quality control reference element.

Dosage of ETMs (heavy metals): Lead (Pb) and cadmium (Cd) were analyzed using the conventional method (Mazet *et al.,* 2005; Lemarchand *et al.,* 2010).

Samples of crushed heads and headless parts (1 to 2 g) were dried for 1 hour at 110°C, then for 5 hours at 180°C. After this drying, 0.3 g of sample was ground in a small container and diluted in 1 mL of 50% sulfuric acid. The whole was then digested for 16 h, the temperature increasing from 20°C to 700°C during the first 10 hours, then being maintained at 700°C for the remaining 6 h. The digested samples were then diluted in 2 mL of nitric acid and dried slowly in a container kept warm. After cooling, the samples were taken up in 1 mL of 10% nitric acid and transferred to polypropylene tubes where they were diluted in ultrapure Milli-Q water. Metal concentrations were measured by atomic absorption spectrometry (UNICAM 989 QZ, Thermo Optek, France) using lamps specific to each element. One (1) g of liver was used for mercury analyses. Next, 1 mL of milli-Q ultrapure water is added, followed by 1.5 mL of hydrogen peroxide (H_2O_2) and 6.5 mL of nitric acid. The mixture was then mineralized in a microwave oven (ETHOS) as follows: temperature increase from 20 to 180°C in 10 min, maintained at 180°C for 15 min. After cooling, the samples were transferred to polypropylene tubes, a 6.4% KMnO4 solution was added. The sample was mixed in 3 mL of a 100 mL solution of 4.0 g MgO and 40 g Mg $(NO_3)_2$ in a small beaker. Furnace temperatures ranged from 20°C to 700°C reached in 8 h, then were maintained at 700°C for 8 hours. After cooling to 100°C, the sample was resuspended using milli-Q ultrapure water and then dissolved in a 50% hydrochloric acid solution. Milli-Q ultrapure water and 2.5 mL of a solution of potassium iodide and ascorbic acid were then added. The mixture was then boiled at 80°C for one hour. Mercury was measured using an atomic absorption spectrometer (Perkin Elmer Analyst), using element-specific lamps after hybrid generation. Each metal analysis sample was conducted in triplicate, and certified reference samples were used for quality control (CRM185R certified values for Pb, Cd, Hg with 95% confidence interval). For each run of analysis, certified samples were used to test recovery (>90%) and a blank run is performed to test for any possible contamination. All the concentrations of metallic and metalloid elements are calculated according to a 5-point calibration curve (r^2> 0.99), and the results are expressed in mg/kg of dry matter. Detection limits are 20 µg/kg for lead, cadmium or mercury.

Statistical analysis : EXCEL and STATISCA version 8.0 software were the statistical tools used for the analysis of the results. The various results obtained were the mean of three repetitions and were expressed as mean ± standard deviation. The analysis of variance and the Duncan test at the 5% threshold were used to compare the means.

Content of Polycyclic Aromatic Hydrocarbons (PAH): Table 1 presents the main families of PAH molecules detected in the heads and headless parts of jawfish, mackerel, tilapia and tuna. The PAHs detected in the jawfish are pyrene and Benzo (a) pyrene with the respective contents of 0.058 and 0.067 µg/kg in the head parts (head jawfish) then 0.060 and 0.066 µg/g in the heads (head of jawfish). Benz (g, h, i) pyrilene was only quantified in mackerel with levels of 0.074 µg/g in the head part (head mackerel) and 0.072 µg/g in the head (mackerel head). Benzo (a) anthracene was quantified mainly in tilapia with relatively high values of 0.147 µg/g in the head (tilapia head) and 0.147 µg/g in the headed parts (head tilapia). Other compounds such as fluoranthenes and indeno pyrilene have been detected, but at trace levels.

Pesticide content of heads and headedless parts of fish: The results of the contamination of heads and headless parts of fish by organophosphorus pesticides, herbicides, carbamates and triazines are illustrated in Table 3 below. The observed values of desethylatrazine (0.021-0.033 µg/g), fenuron (0.023 - 0.042 µg/g), metoxuron (0.622 - 0.623 µg/g), monuron (0.030 - 0.037 µg/g), methabenzthiazuron (0.023 - 0.024 µg /g), simazine (0.089 - 0.090 µg/g), isoproturon (0.062- 0.069 µg/g), cyanazine (0.022- 0.200 µg/g), metamitron (0.042 - 0.042 µg/g) are all relatively low and below pesticide standard (4 µg/g wet weight). Among the 9 molecules detected, 6 were found in tilapia against 4 in the jawfish, 2 in mackerel and 1 in tuna.

Table 1. Content of Polycyclic Aromatic Hydrocarbons (PAH)

PAH	Concentration (µg/g)								LD (µg/g)	LQ (µg/g)
	Mackerel headless	Tunaheadless	Tilapia headless	Jawfish headless	Mackerel head	Tuna head	Tilapia head	Jawfish head		
fluoranthene	Nd	0,0002	0,003	0,001	Nd	0,0002	0,003	0,0008		
Pyrene	0,014	0,006	0,029	0,058	0,014	0,004	0,028	0,060		
Benzo (k) fluoranthene	Nd	0,0003	Nd	0,001	Nd	0,006	Nd	0,0005		
Benzo (a) pyrene	Nd	0,021		Nd	Nd	0,02	Nd	0,066		
Indeno (1, 2,3-cd) pyrilene	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd		
Benzo(g,h,i) pyrilene	0,74	0,007	Nd	0,009	0,072	0,006	Nd	0,009		
Benzo (a) anthracene	Nd	Nd	0,147	Nd	Nd	Nd	0,147	Nd		
Benzo (b) fluoranthene	0,013	0,004	0,003	0,004	0,013	0,003	0,003	0,005	0,017	0,051

N.B :all values below the limit of quantification are considered traces

Table 2: Pesticide content of heads and headless parts of fish

Family of Pesticides found	Concentration (µg/g)									LD (µg/g)	LQ (µg/g)
		Mackerel headless	Tunaheadless	Tilapia headless	Jawfish headless	Mackerel head	Tuna head	Tilapia head	Jawfish head		
Herbicides derived from urea and nitrogenous heterocycles + Carbamates + Triazines	Desethylatrazine	-	-	0.033	0.021	-	-	0.022	0.021	0.006	0.018
	Fenuron	-	0.033	0.042	-	-	0.023	0.042	-		
	Desisopropylatratzin	0.013	-	-	0.0004	0.011	-	-	0.0002		
	Metoxuron	-	-	0.007	0.623	-	-	0.006	0.622		
	monuron	-	-	0.031	0.037	-	-	0.030	0.035		
	Methabenzthiazuron	-	-	0.024	0.001	-	-	0.023	0.001		
	Linuron	-	-	0.004	-	-	-	0.004	-		
	Aldicarb	-	-	-	0.002	-	-	-	0.002		
	Simazine	-	-	-	0.090	-	-	-	0.089		
	Chlortoluron	-	0.002	-	-	-	0.001	-	-		
	Monolinuron	-	0.002	0.004	-	-	0.001	0.003	-		
	Isoproturon	-	0.006	0.069	-	-	0.001	0.062	-		
	Cyanazine	0.022	0.002	0.200	0.0003	0.022	0.002	0.190	0.0003		
	Propazine	-	0.001	-	-	-	0.001	-	-		
	Terbutryne	-	0.001	0.005	-	-	0.001	0.004	-		
	Crime	-	0.0002	-	0.0003	-	0.004	-	0.0003		
	Metamitron	0.042	0.003	-	-	0.042	0.0001	-	-		
Organophosphates	Parathion-ethyl	-	0.002	-	-	-	0.002	-	-	0.01	0.032
	Vinclozolin	-	-	0.018	-	-	-	0.017	-	0.004	0.012

Table 4: Heavy metal composition of fish

Samples	Lead	Mercury	Cadmium
Headless mackerel	0.10 ± 0.00^{c}	0.125 ± 0.01^{c}	0.023 ± 0.0^{e}
Headless tuna	0.23 ± 0.00^{g}	0.145 ± 0.01^{d}	0.011 ± 0.001^{d}
Headless tilapia	0.003 ± 0.00^{a}	0.068 ± 0.0^{b}	0.001 ± 0.0^{a}
Headless jawfish	0.215 ± 0.001^{f}	0.013 ± 0.0^{a}	0.009 ± 0.0^{c}
Mackerel head	0.085 ± 0.01^{b}	0.13 ± 0.00^{c}	0.027 ± 0.0^{f}
Tuna head	0.155 ± 0.01^{d}	0.085 ± 0.005^{b}	0.006 ± 0.0^{b}
Tilapia head	0.003 ± 0.00^{a}	0.079 ± 0.0^{b}	0.001 ± 0.0^{a}
Jawfish head	0.175 ± 0.01^{e}	0.011 ± 0.01^{a}	0.001 ± 0.0^{a}

Heavy metal content of fish heads and headless parts: A low concentration of heavy metals was obtained in the heads and headless parts of the fish (Table 3). Mean metal concentrations in different tissues, independent of species, increase significantly at the 5% threshold. The variations range from 0.003 to 0.23 mg/kg fresh weight for lead, 0.011 to 0.145 mg/kg fresh weight for mercury, and 0.001 to 0.027 mg/kg fresh weight for cadmium. Maximum concentrations of lead (0.23 mg/kg), mercury (0.145 mg/kg) were determined in headed tuna. Cadmium is higher in heads (0.027 mg/kg) and headless part (0.023± 0.0 mg/kg) of mackerel followed by headless tuna (0.011 mg/kg). The lowest levels are found in the head and headless part of tilapia, as well as in the heads of the jawfish. The lead concentrations in the headless parts (0.003 mg/kg) and heads (0.003 ± 0.00 mg/kg) of tilapia are significantly lower than those found in headed mackerel (0.10 ± 0.00 mg/kg), headless tuna (0.23 ± 0.00), headless jawfish (0.215 ± 0.001), mackerel head (0.085 ± 0.01), tuna head (0.155 ± 0.01) and jawfish head (0.175 ± 0 .01) at the 5% threshold. Mercury concentrations in the heads and decapped parts of catfish, tuna, mackerel and tilapia are similar to the 5% threshold (Table 3).

DISCUSSION

All the values of PAHs (0.0003 to 0.147 µg/kg) and pesticides (0.0001 to 0.623 µg/kg) found in the heads and headless parts of these fish studied are below the maximum permitted concentrations. Indeed, in fish the acceptable doses of PAHs are 0.27 to 3 µg/kg wet weight and 0.34 to 4 µg/kg wet weight for pesticides (EU, 2013). Concerning the organophosphates, only Vinclozolin was really detected in the tilapia at the level of the head (0.017 µg/kg) and in the headless part (0.018 µg/kg). This low contamination could be explained by the fact that the use of these compounds has been banned in the European Union since 2000. In addition, organophosphates would be very little persistent. Most of these bodies are rapidly degraded in the environment and metabolized by organisms. In addition, fish are very sensitive to organophosphates which act rather on an acute mode of toxicity. Fish exposed to organophosphates therefore die very quickly. Organophosphates also quickly disappear from aquatic food webs, thus avoiding chronic contamination. The ingestion of contaminated food, and more particularly aquatic products, is considered to be one of the main sources of persistent organic pollutants in humans (Corsolini *et al.,* 2005). The presence of micropollutants in the heads and headless parts of the fish analyzed does not seem to endanger consumer health.

The cadmium contents in the flesh of mackerel, tuna, jawfish and tilapia (0.001–0.028 mg/kg fresh weight) are very low compared to those of Allinson *et al.* (2002). These authors found values ranging from 0.01 to 0.5 mg/kg of fresh matter in fish from Sri Lankan waters. In their study, Allinson *et al.* 2002 found levels of up to 2.00 mg/kg fresh matter in fish liver. Similarly, the level of contamination of the flesh of the fish analyzed in the present study is lower than that of three species of fish, including the lates, studied in Lake Tanganyika in Tanzania by Chale (2002). The latter determined levels ranging from 0.25 to 0.65 mg/kg of fresh material. In general, the cadnium concentrations measured in our study are lower than those obtained in all the tissues of *L. cephalus cephalus*, in the muscles of *C. carpio*, then in the gills and muscles of L. gibossus (Doganoc, 1995; Andreji *et al.,* 2006; Has-Shön *et al.,* 2006; Mendill and Dogan, 2007). According to Farkas *et al.* (2003), the concentration of metals in the gills reflects the concentration of these metals in the water, while their concentration in the liver indicates the storage of these metals. Considering the standards of the E.U. (regulation 1881/2006), the tolerable weekly doses in mg/kg are 0.3 for lead, 0.51 for mercury and 0.50 for cadmium. The concentrations of these metals in the flesh of the fish analyzed in our study are lower than this 2006 European standard.

CONCLUSION

The mackerel, tuna, jawfish and tilapia collected in Abidjan represent a real source of proteins, lipids and mineral elements, which makes them an essential food for the growth of children. Therefore, apart from the nutritional quality, the evaluation of the micropollutant contents (PAHs, pesticides and heavy metals) was carried out in the heads and headless parts of these fish. The different levels determined are lower than the international standards in force. Thus, the fish studied can be consumed without risk of contamination in children as in adults.

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